

# Isopods in a Changing World

*edited by*

Elisabeth Hornung, Stefano Taiti, Katalin Szlavecz



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ISOPODS IN A CHANGING WORLD

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Cover photo: *Mesoniscus graniger* (Frivaldsky, 1865) – a cave and endogean species described from the Baradla Cave, Hungary (photo credit: Zsolt Ujvári)

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# Contents

## Reviews

- I Preface – 10th ISTIB**  
*Elisabeth Hornung, Stefano Taiti, Katalin Szlavecz*
- 5 Interspecific variation in responses to microclimate by terrestrial isopods: implications in relation to climate change**  
*Mark Hassall, Anna Moss, Bernice Dixie, James J. Gilroy*
- 25 Isopod distribution and climate change**  
*Spyros Sfenthourakis, Elisabeth Hornung*
- 63 Effect of agricultural practices on terrestrial isopods: a review**  
*Catherine Souty-Grosset, Ariel Faberi*
- 97 Terrestrial isopods in urban environments: an overview**  
*Katalin Szlavecz, Ferenc Vilisics, Zsolt Tóth, Elisabeth Hornung*
- 127 Terrestrial isopods as model organisms in soil ecotoxicology: a review**  
*Cornelis A.M. van Gestel, Susana Loureiro, Primož Zidar*

## Taxonomy – Phylogeny – Faunistics

- 163 A molecular phylogeny of Porcellionidae (Isopoda, Oniscidea) reveals inconsistencies with present taxonomy**  
*Andreas C. Dimitriou, Stefano Taiti, Helmut Schmalfuss, Spyros Sfenthourakis*
- 177 Terrestrial isopods of the family Eubelidae Budde-Lund, 1899 from Iran, with description of a new species (Isopoda, Oniscidea)**  
*Ghasem M. Kashani, Abdiyeh Abedini, Giuseppe Montesanto*
- 189 Description of a new species of the genus *Protracheoniscus* Verhoeff, 1917 and redescription of *Protracheoniscus kryszanovskii* Borutzky, 1957 from the southeast of European Russia (Isopoda, Oniscidea, Agnaridae)**  
*Konstantin B. Gongalsky, Ilya S. Turbanov, Dmitry A. Medvedev, Julia S. Volkova*
- 207 A guide to the identification of the terrestrial Isopoda of Maryland, U.S.A. (Crustacea)**  
*Jeffrey W. Shultz*
- 229 The genus *Alpioniscus* Racovitza, 1908 in Sardinia: taxonomy and natural history (Isopoda, Oniscidea, Trichoniscidae)**  
*Stefano Taiti, Roberto Argano, Paolo Marcia, Fabio Scarpa, Daria Sanna, Marco Casu*

**265 Woodlice of Belgium: an annotated checklist and bibliography (Isopoda, Oniscidea)**

*Pallietter De Smedt, Pepijn Boeraeve, Gert Arijs, Stijn Segers*

**305 Terrestrial isopods (Oniscidea) of the White Carpathians (Czech Republic and Slovakia)**

*Karel Tajovský, Jana Štrichelová, Ivan H. Tuf*

## **Ecology**

**323 Terrestrial isopods associated with shallow underground of forested scree slopes in the western Carpathians (Slovakia)**

*Ján Rudy, Michal Rendoš, Peter Luptáčík, Andrej Mock*

**337 Size dependence of offspring production in isopods: a synthesis**

*Andrzej Antol, Marcin Czarnoleski*

**359 Life history characteristics of a cave isopod (*Mesoniscus graniger* Friv.)**

*Dávid Derbák, László Dányi, Elisabeth Hornung*

**371 The role of urban forest patches in maintaining isopod diversity (Oniscidea)**

*Elisabeth Hornung, Andrea Kásler, Zsolt Tóth*

**389 Species compositions of terrestrial isopods in public parks of a commuter town in Japan**

*Takahiro Nasu, Kana Kitagawa, Shigenori Karasawa*

**401 Woodlice and their parasitoid flies: revision of Isopoda (Crustacea, Oniscidea) – Rhinophoridae (Insecta, Diptera) interaction and first record of a parasitized Neotropical woodlouse species**

*Camila T. Wood, Silvio S. Nihei, Paula B. Araujo*

## **Toxicology**

**415 Effect of neem-derived plant protection products on the isopod species *Porcellionides pruinosus* (Brandt, 1833)**

*Pratik Doshi, Anett Mészárosné Póss, Ferenc Tóth, Márk Szalai, György Turóczi*

## **(Eco)Morphology - Physiology**

**427 Comparative ultrastructure of cells and cuticle in the anterior chamber and papillate region of *Porcellio scaber* (Crustacea, Isopoda) hindgut**

*Urban Bogataj, Monika Praznik, Polona Mrak, Jasna Štrus, Magda Tušek-Žnidarič, Nada Žnidarič*

- 459**    **Water vapor absorption allows for volume expansion during molting in *Armadillidium vulgare* and *Porcellio dilatatus* (Crustacea, Isopoda, Oniscidea)**  
*John-David Nako, Nicole S. Lee, Jonathan C. Wright*
- 481**    **Morphological traits – desiccation resistance – habitat characteristics: a possible key for distribution in woodlice (Isopoda, Oniscidea)**  
*Diána Csonka, Katalin Halasy, Krisztina Buczkó, Elisabeth Hornung*
- 501**    **Presence of a stridulatory apparatus in the manca stages of isopods (Crustacea, Isopoda, Oniscidea)**  
*Giuseppe Montesanto*



- |                                  |                            |                                  |
|----------------------------------|----------------------------|----------------------------------|
| 1. Andrezj Antol                 | 35. Andreas Dimitriou      | 52. Ivanklin Soares Campos Filho |
| 2. Sára Ferentő                  | 36. Theodosia Charalambous | 53. Stefano Taiti                |
| 3. Severus-Daniel Covaciu-Marcov | 37. Danil Korobushkin      | 54. Pierre Grève                 |
| 4. Anas Ayari                    | 38. Sándor Farkas          | 55. Elisabeth Hornung            |
| 5. Ivo Karaman                   | 39. Dávid Derbák           | 56. Spyros Sienthourakis         |
| 6. Majed Dalhoumi                | 40. Ferenc Vilisics        | 57. Gábor Bakonyi                |
| 7. Terézia Horváthová            | 41. Máttyás Farkas         | 58. Angéla Zanker                |
| 8. Heikki Setälä                 | 42. Gert Arijjs            | 59. Dána Csonka                  |
| 9. Vladimir Sustr                | 43. Mouna Khila            | 60. Nóra Vili                    |
| 10. Karel Tapovský               | 44. Jana Bedek             | 61. Anett Pöss                   |
| 11. Eva Ilić                     | 45. Sonia Hamaied          | 62. Melinda Mecsóber             |
| 12. Urban Bogataj                | 46. Lamia Medini-Bouaziz   | 63. David Führer                 |
| 13. Miloš Vittori                | 47. Helmut Schmalfuss      | 64. Pallieter De Smedt           |
| 14. Ingrida Satkauskienė         | 48. Isabelle Marcadé       | 65. Pepijn Boeraeve              |
| 15. Ivan Hadrián Tuf             | 49. Didier Bouchon         | 66. Joanne Bertaux               |
| 16. Nuno Ferreira                | 50. Benjamin Herran        | 67. Matty Berg                   |
| 17. Szabolcs Hernadi             | 51. Marius Bredon          |                                  |
| 18. Melinda Babits               |                            |                                  |
| 19. Ján Rudy                     |                            |                                  |
| 20. Konstantin B. Gongalsky      |                            |                                  |
| 21. Yurii Lebedev                |                            |                                  |
| 22. Giuseppe Montesanto          |                            |                                  |
| 23. Mladen Horvathović           |                            |                                  |
| 24. Stijn Segers                 |                            |                                  |
| 25. Chedliya Ghemari             |                            |                                  |
| 26. Jasna Štrus                  |                            |                                  |
| 27. Paula Beatriz Araújo         |                            |                                  |
| 28. Ghaseem Mohammadi Kashani    |                            |                                  |
| 29. Katalin Szilavec             |                            |                                  |
| 30. Andreas Ziegler              |                            |                                  |
| 31. Alexandra Lafitte            |                            |                                  |
| 32. Camila Timm Wood             |                            |                                  |
| 33. Giovanna M. Cardoso          |                            |                                  |
| 34. Andrej Mock                  |                            |                                  |

## Preface – 10<sup>th</sup> ISTIB

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'Even if we have written testimony that the ancient Greek intellectuals were already aware of the existence of woodlice, the history of scientific research on this group of animals starts 2000 years later. ... Today isopodological research includes studies on ecology, behavior, nutritional biology, anatomy, functional morphology, neurology, physiology, biogeography, systematics, and phylogenetic analyses based on morphological and molecular data' (Schmalfuss 2018).

Since the first Symposium on the Biology of Terrestrial Isopods in London, UK (1983), scientists have been meeting every three years to discuss their latest findings on all aspects of terrestrial isopod biology. The 10<sup>th</sup> symposium, held in Budapest, Hungary in 2017 (<http://bio.univet.hu/istib2017/main.html>) brought together over 70 participants from 23 countries representing Europe, North and South America, Africa, and Near East. The meeting was organised by the Department of Ecology (University of Veterinary Medicine, Budapest) and by the Hungarian Biological Society with support from the Hungarian Ecological Society, the Hungarian Natural History Museum, the Budapest Zoo and Pensoft Publishers.

Diverse topics at many spatio-temporal scales were presented, all under the umbrella of our beloved crustaceans: woodlice, slaters, pill bugs, sow-bugs, roly-poly-s, landpissebedden, Asseln, porcellini di terra, ászka, pincebogár, мокрици, γουρουνίτσες, δροσομάμουννα, etc. The 30 oral presentations, seven of which were review type invited



lectures, and 45 lightning talks connecting to posters, covered research on classical and new fields such as taxonomy, biogeography, molecular biology, agroecosystems, sustainable land use, ecosystem services, climate change, human influence, urbanization, structure, and function. Key words given for the presentations included history, phylogeny, taxonomy (new species), biodiversity, species distribution, ecological biogeography, subterranean occurrence, life history, trait approach, habitat fragmentation, parasites, predation, pests, phenology, fluctuating asymmetry, activity, feeding, genetics, ecotoxicology, bioaccumulation, heavy metals, morphology, ultrastructure, physiology, hormones, development, microbiota, symbionts and others.

This ZooKeys special issue is a collection of the presentations of the 10<sup>th</sup> International Symposium on the Biology of Terrestrial Isopods. The title of the volume, 'Isopods in a Changing World', reflects the growing interest of the science community and public in the potential responses of biota, including isopods, to global environmental change. In the first part of the volume five overviews summarize our current knowledge, highlight research needs and provide future directions. Two contributions focus on climate change effects from local to global scale. Specifically, the papers discuss how these effects vary depending on species traits, how these differences might lead to changes in species composition, and how the changing climate might shift distribution boundaries of isopod species. Currently the two major types of land use change are agriculture and urbanization. Two papers discuss the individual, population and community responses of terrestrial isopods to the changing landscape. Based upon their abundance, isopods appear to be a successful group both in agricultural systems and in cities. However, generalist, synanthropic species dominate in both systems, and drastic land conversion may lead to local species extinction. The fifth paper reviews the history of isopods as model animals in ecotoxicology. Although isopods have been used as indicator organisms for decades, given the continuing release of various contaminants to both terrestrial and aquatic environments, the effect of environmental toxins on soil fauna and the role of isopods as indicator organisms for pollution level, remain to be timely. Humans, as a force of nature, are at the center of current environmental change. As a result of human residence and land use change, today less than 25% of Earth's ice free land can be considered as 'wildlands' with minimal or no human influence (Ellis and Ramankutty 2008). At local scale human impact can be direct, for instance via drastically disturbing the physical environment and selectively promoting or removing species, or indirect, e.g., changing local hydrology or altering nutrient cycles. Human actions, whether deliberate or inadvertent, profoundly, and often irreversibly affect local species presence and abundance, and may act as selective forces leading to evolutionary change. It is therefore important to recognize that interpreting community composition, including that of terrestrial isopods, requires knowledge on site history at various temporal scales.

The remaining 18 research papers cover a broad array of disciplines. Contributions to the fields of taxonomy, faunistics and phylogeny indicate that we are far from fully understanding large scale distributions and evolution of Oniscidea. Understanding distributions and abundances at multiple spatial scales requires knowledge on

morphological, physiological and ecological traits. Papers continue exploring water relations of terrestrial isopods, which is a fundamental physiological factor determining their success in terrestrial ecosystems. We are also delighted to see contributions in such less studied topics as isopod-parasite interactions and the importance of stridulatory apparatus in isopod communication. Several oral and poster presentations on cave isopod ecology were given during the symposium. In this special volume one of them reports on growth and reproduction of a cave dwelling species. Cave isopods represent a special group with presumably unique life history characteristics, population dynamics, and evolution. Some troglobitic species might be rare, their habitat threatened, and thus of high conservation value.

We hope that for those just getting started in this field this special issue provides an overview and baseline information. Hopefully, the volume will also stimulate ongoing isopod research taking some projects to new directions.

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# Interspecific variation in responses to microclimate by terrestrial isopods: implications in relation to climate change

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## Abstract

The importance of considering species-specific biotic interactions when predicting feedbacks between the effects of climate change and ecosystem functions is becoming widely recognised. The responses of soil animals to predicted changes in global climate could potentially have far-reaching consequences for fluxes of soil carbon, including climatic feedbacks resulting from increased emissions of carbon dioxide from soils. The responses of soil animals to different microclimates can be summarised as norms of reaction, in order to compare phenotypic differences in traits along environmental gradients. Thermal and moisture reaction norms for physiological, behavioural and life history traits of species of terrestrial isopods differing in their morphological adaptations for reducing water loss are presented. Gradients of moisture reaction norms for respiratory rates and thermal reaction norms for water loss, for a species from the littoral zone were steeper than those for species from mesic environments. Those for mesic species were steeper than for those from xeric habitats. Within mesic species, gradients of thermal reaction norms for aggregation were steeper for *Oniscus asellus* than for *Porcellio scaber* or *Armadillium vulgare*, and moisture reaction norms for sheltering and feeding behaviours were steeper for *Philoscia muscorum* than for either *P. scaber* or *A. vulgare*. These differences reflect differences in body shape, permeability of the cuticle, and development of pleopodal lungs. The implications of differences between different species of soil animals in response to microclimate on the possible influence of the soil fauna on soil carbon dynamics under future climates are discussed. In conclusion a modelling approach to bridging the inter-disciplinary gap between carbon cycling and the biology of soil animals is recommended.

**Keywords**

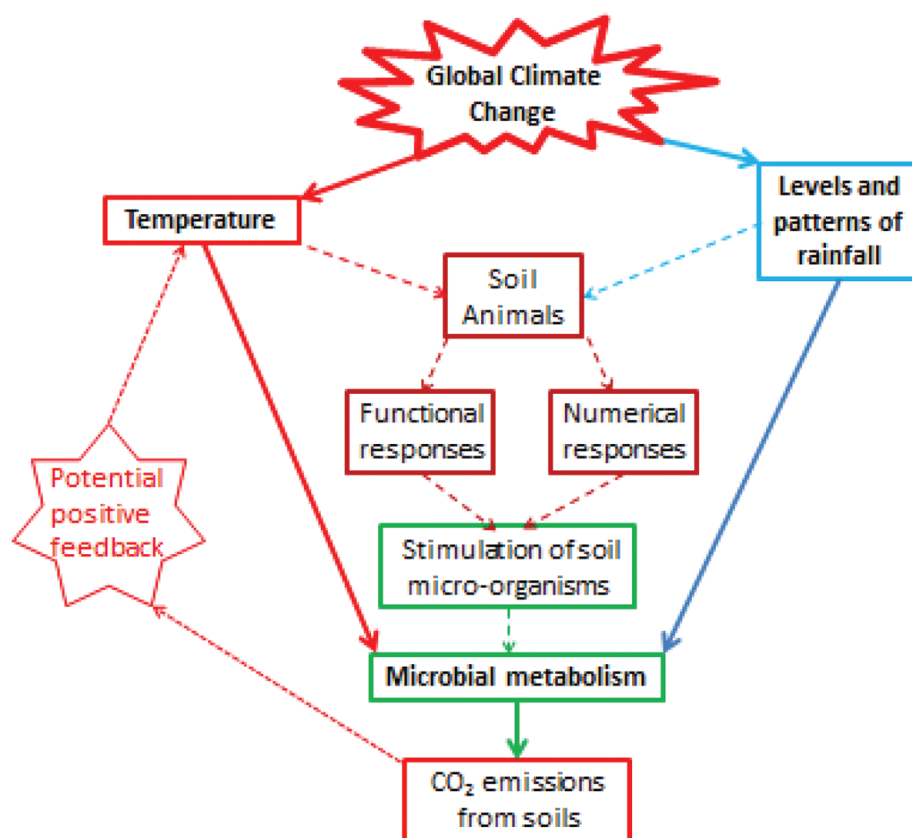
Aggregation, CO<sub>2</sub> emissions from soils, feeding behaviour, future rainfall patterns, life history traits, norms of reaction, response curves, soil animals

**Introduction**

In this review we draw attention to an important contribution that soil biologists, in particular those who study the biology of terrestrial isopods, can potentially make to the current debate as to how global climate change may influence components of the global carbon cycle. Currently the greatest uncertainty in modelling the global carbon cycle is not the fluxes across the ocean atmosphere interface or fluxes relating to net primary production but in modelling carbon fluxes within the soil. Globally ten times more carbon dioxide is emitted from soils than from all anthropogenic sources combined (Adger and Brown 1995, IPCC 2013). Anthropogenic induced climate change has caused shifts in both temperature and rainfall patterns across a range of geographic scales (Rosenzweig et al. 2008). Climate models predict global surface temperatures will rise by 0.3–4.8 °C by 2100 (IPCC 2014). A major challenge of current ecological research is to determine how ecosystem processes will respond to future environmental conditions (Santotja et al. 2017). It is widely acknowledged that food webs play pivotal roles in carbon cycles, including emissions of carbon dioxide from soils under climate change but this is rarely considered in modelling carbon fluxes (Pelini et al. 2015), while understanding of organismic physiological responses under climate change remains very rudimentary (Schmitz 2013). One question that is currently being debated is the extent to which soil animals can be modelled grouped together in broad trophic categories or whether they need to be considered at a finer level of functional trait or taxonomic resolution, depending upon their individual responses to changes in microclimate (Jiguet et al. 2011, Dixie et al. 2015). Terrestrial isopods potentially form a useful model system with which to address this question because their behaviour, physiology, and life histories have been so extensively studied.

Carbon dioxide emissions from soils are mediated predominately by microbial metabolism (Fig. 1), which, when not constrained by moisture availability, is a function of temperature (Schlesinger 1977). There is thus the potential for increased soil temperatures to accelerate emissions of carbon dioxide from soils. The further accumulation of CO<sub>2</sub> in the atmosphere could then lead to further increases in temperature, leading to a positive feedback (Fig. 1) (Crowther et al. 2015, Thakur et al. 2018). This process is of most concern in regions with the highest pools of soil organic matter, such as tundra regions and temperate grasslands (Melillo et al. 2017). However, recent evidence indicates current carbon stores in other soils may also be changing from sinks to sources (Carrera et al. 2011) due to greater increases in soil respiration than in rates of carbon input to the soils.

The possible consequences of this positive feedback cycle could potentially be reduced if soil moisture were to decrease as a result of changes in rainfall patterns (Fig. 1). Climate models predict that globally rainfall will increase but it is predicted to vary strongly both spatially and temporally. Spatially, greater increases are projected



**Figure 1.** A conceptual diagram illustrating some of the pathways by which changes in global climate could potentially impact on rates of carbon dioxide emissions from soils. Both changes in temperature and in the levels and patterns of rainfall have strong direct effects on the metabolism of bacteria and fungi but their ecology and metabolism are also regulated by the extent to which they are stimulated by soil animals. Both functional (e.g., behavioural and physiological) responses and numerical (both life history and population) responses of soil animals are affected by their microclimate. This is in turn affected by larger scale changes in temperature and rainfall. Therefore, as well as their direct effect on microbial metabolism, these climatic variables have a strong indirect effect by influencing the behavioural, physiological, life history, and population processes of soil animals such as isopods.

for high latitudes and mid-latitude wet regions but decreases in subtropical dry regions and many mid-latitude regions (IPCC 2013). Temporally, summer rainfall in temperate regions, such as west and south Europe, is predicted to occur in fewer, but more intense episodes. These will result in longer periods of summer drought which could potentially constrain temperature-induced increases in soil carbon fluxes, including by reducing the stimulation of microbial metabolism by moisture dependent soil animals.

The microbially mediated emissions of carbon dioxide from soils are strongly influenced by soil animals acting as key system regulators (Fig. 1) (Anderson 1991, Lavelle

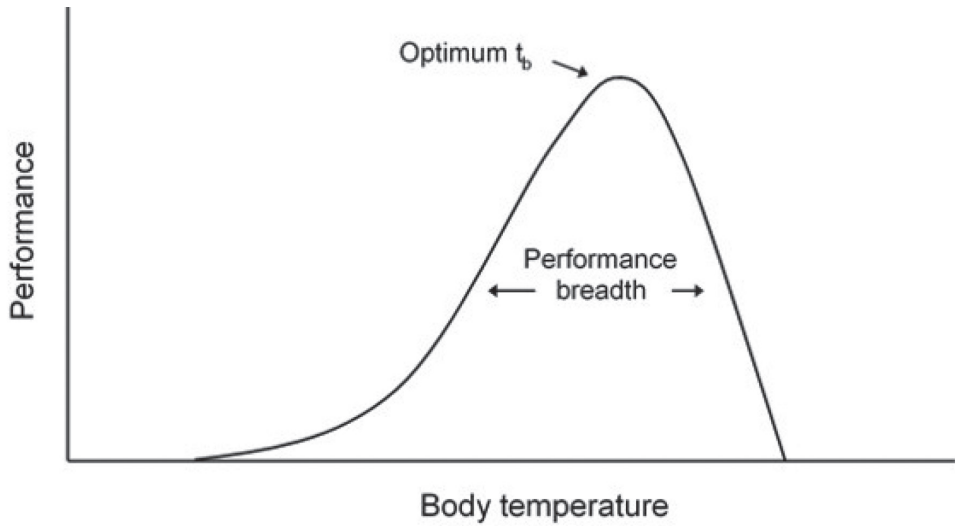
and Spain 2001, Krab et al. 2010, Coleman et al. 2017). The significance of direct and indirect consequences of global climate change on ecosystem processes mediated by invertebrates, including their indirect effects on heterotroph respiration through interactions with microorganisms, are widely recognised (Pelini et al. 2015). However, although they are likely to have a major impact on ecosystems in the future, they are as yet understudied (Blankinship et al. 2011, Carrera et al. 2011, Del Toro et al. 2015).

It is becoming increasingly apparent that the importance of interspecific differences in key traits not only leads to different species having different functional roles (Dias et al. 2013, Zimmer et al. 2002) but also causes different species to be affected differently by climate change (Veldhuis et al. 2017). Because ecosystem science has yet to embrace how principles of evolutionary ecology explain the ways in which organisms mediate ecosystem carbon dynamics (Schmitz 2013), there is an urgent need to increase our understanding of how differential impacts of climate change on basic biological traits of individual species can lead to changes in community structure and function (Jiguet et al. 2011).

Traits of many soil animals are sensitive to relative humidity, making the soil fauna vulnerable to their activities being curtailed by changes in soil moisture (Krab et al. 2010, Crowther et al. 2015). Isopods are the most successful group of crustaceans to have made the transition to the terrestrial environment in terms of their abundance, distribution, and diversity, and have colonised a wide range of terrestrial habitats from the littoral zone to deserts (Edney 1954, Warburg 1987). They have become prominent members of the arthropod macro-decomposer guild in many ecosystems (Sutton 1980). Their morphology, physiology, behaviour, life histories, and ecology are known to be strongly influenced by micro-climates. Isopods therefore form good model soil animals for testing hypotheses concerning how inter-specific differences in their physiological, behavioural and life history traits will lead to interspecific differences in responses to predicted future changes in climate.

In this review we examine the extent to which traits of different species of terrestrial isopods known to have different strategies for reducing water loss in the terrestrial environment, respond to temperature and relative humidity. Phenotypic responses to a gradient in the environment can be summarised as norms of reaction. A reaction norm is the set of phenotypes produced in a range of environments (Schmalhausen 1949, Stearns 1992) and represents either the whole or part of a response curve of an organism to any environmental gradient as for example illustrated by the thermal response curve for enzymes shown in Fig. 2. Differences in the gradients of performance response curves, such as that in Fig. 2, denote how quickly an organism changes its performance for a given change in an environmental gradient. A steep gradient indicates a strong response and a high level of phenotypic plasticity over that region of the environmental gradient. Reaction norms can be very effective in comparing responses not just between different species as has been demonstrated very clearly by Dias et al. (2013) but also to different environmental gradients such as soil moisture and temperature.

We investigated differences in reaction norms of physiological, behavioural, and life history traits in response to differences in temperature and relative humidity for a range of isopod species chosen to reflect differences in their eco-morphology (Schmalfuss 1984,



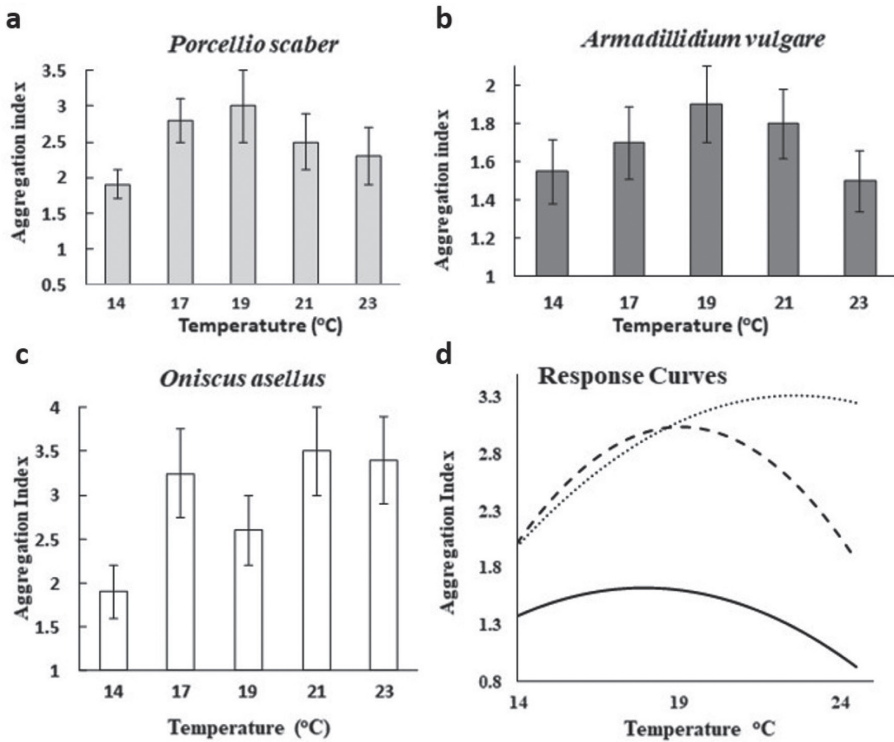
**Figure 2.** A schematic representation of a typical thermal response curve for enzymes (simplified from Huey and Kingsolver 1989). The temperature optimum is the temperature at which performance reaches its maximal level or peak performance. The performance breadth defines how steeply peaked (stenothermal) or broadly plateaued (eurythermal) the response curve is. Any part or the whole of such a curve can be considered to be a reaction norm of a genotype representing a range of phenotypes expressed across an environmental gradient, in this example, of temperature.

Schmidt and Wägele 2001, Broly et al. 2014) and their eco-physiology (Spencer and Edney 1954, Edney 1968, Warburg 1987, 1989, Hornung 2011). We used the selected species to explore possible implications of inter-specific differences in responses to microclimate for the role of soil animals in soil processes, including regulation of microbially mediated soil carbon dynamics (Heemsbergen et al. 2004), under predicted climate change scenarios.

### **Behavioural responses to microclimate: thermal norms of reaction for aggregating behaviour**

*Case study 1.* Here we compare differences in aggregating behaviour of three species of isopod as described by Dixie et al. (2015) who give full details of the methodology including those of the statistical analyses used.

Differences in the degree of aggregation, at 90% relative humidity, between three species of isopods differing in their adaptations to reduce water loss in the terrestrial environment, are shown in Fig. 3. *Oniscus asellus* (Linnaeus, 1758) was most susceptible to water loss, the *Armadillidium vulgare* (Latreille, 1804) is morphologically the best adapted to withstand desiccation while *Porcellio scaber* (Latreille, 1804) has an intermediate level of morphological adaptation but also uses aggregation as a key behavioural tactic in its overall desiccation avoidance strategy.



**Figure 3.** Aggregation of isopod species differing in desiccation resistance at different temperatures. Mean  $\pm$  1 SE aggregation indices (variance:mean ratio) at 90% relative humidity. **a** *P. scaber* ( $F_{4,249} = 3.76$ ,  $p < 0.01$ ) **b** *A. vulgare* ( $F_{4,249} = 1.97$ ,  $P < 0.01$ ) **c** *O. asellus* ( $F_{4,249} = 12.22$ ,  $P < 0.001$ ) **d** thermal reaction norms for aggregation expressed as quadratic response curves for: *P. scaber* (dashed line):  $y = -11.519 + 1.526x - 0.04x^2$ ; *A. vulgare* (solid line):  $y = -3.534 + 0.574x - 0.016x^2$ ; *O. asellus* (dotted line):  $y = -5.890 + 0.814x - 0.018x^2$ .

The temperature response curve of *P. scaber* (Fig. 3a, d) resembles the theoretical one in Fig. 2 in being an upwardly convex curve, with a peak in the aggregation index of 2.93 at 19 °C, a performance breadth at 90% of the peak of 4.7 °C and a gradient of the reaction norm from 14 °C to 17 °C of + 0.8 increase in aggregation index for this 3 °C rise in temperature. At temperatures higher than 19 °C the curve of the aggregation index decreases due to more of the animals leaving the clumps and moving around the arena sometimes climbing up the walls in an attempt to escape to find more favourable lower temperatures (Dixie et al. 2015).

*Armadillidium vulgare* shows a broadly similar pattern of response (Fig. 3b, d), but with a lower peak of 1.9 in the aggregation index, again at 19 °C. The response curve is shallower than for *P. scaber* with a performance breadth of 6 °C at 90% of the peak value and a gradient for the reaction norm for the aggregation index between 14–17 °C of +0.3. As for *P. scaber*, aggregation decreases at temperatures higher than the peak temperature.

The temperature response curve for aggregation of *O. asellus* (Fig. 3c, d) has a higher peak aggregation index of 3.35 at a higher temperature of 21.3 °C, a wide performance breadth of 6.2 °C at 90% of the peak value and a gradient for its thermal norm of reaction between 14–17 °C of 1.5 in the aggregation index over this 3 °C range. This was approximately twice as steep as for *P. scaber* and three times as steep as for *A. vulgare*. The decline following the peak for *O. asellus* was less pronounced than for either of the other two species.

Overall these results show that these three species have substantially different patterns of thermal response curves for aggregating behaviours, reflecting differences in their morphological adaptations to terrestrial life.

### **Behavioural responses to microclimate: moisture norms of reaction for sheltering and feeding behaviours**

Many terrestrial isopods aggregate in shelter sites, particularly during the day, often under stones or pieces of wood where moisture from the soil maintains a more favourable relative humidity than in more open sites. They then emerge to forage at night when temperatures are lower and relative humidity is higher. Sheltering behaviour is thus of central importance in reducing mortality due to desiccation while also reducing the risk of being eaten by diurnal predators, such as insectivorous birds.

*Case study 2.* Effects of substrate moisture content and relative humidity on sheltering and feeding behaviour were investigated by Moss (2007) under controlled temperature conditions in the laboratory. Three species were studied: *A. vulgare*, *Philoscia muscorum* (Scopoli, 1763) and *P. scaber* differing in morphological and physiological adaptations to the terrestrial environment (Dias et al. 2012). The experimental mesocosms (220mm × 150mm × 80mm deep) were lined with plaster of Paris covered by 50mm sand with a feeding tray at one end and a shelter at the other, as described in further detail by Moss (2007). Two simulated rainfall regimes were used: high simulated rainfall, representing current mean summer daily rainfall intensity of 1.65 mm day<sup>-1</sup> calculated from British Atmospheric Data Centre records for Lacock, Wiltshire, England (51°43'N, 2°11'W) during 1996–2006 when rainfall events occurred on average once every 72h. The low simulated rainfall regime was half the intensity of the high rainfall regime, 0.83 mm day<sup>-1</sup>, representing a scenario with 810 ppm concentration of atmospheric CO<sub>2</sub> by 2080 which is predicted to result in a 50% reduction in summer rainfall in south west England (Hulme et al. 2002). For both regimes the simulated rainfall was administered at the start of each 72h period using a seedling watering can with 0.6 mm diameter holes in the rose with the shelter and food trays temporarily covered. Replicate arenas but without animals were used to monitor percentage moisture content of the substrate by taking samples of sand each morning and evening. Differences in the moisture conditions in the mesocosms were caused by both differences in intensity of simu-



lated rainfall and the progressive drying out of mesocosms which occurred with time between rainfall events. Starting moisture level following high rainfall level  $\approx 20\%$  and for low rainfall level  $\approx 10\%$ .

Eight replicate mesocosms were used for each rainfall treatment, with six identical control boxes for monitoring substrate moisture content. Each mesocosm contained twenty individuals of each species. The behaviours of all animals over a 72-hour period were classified according to their location in the mesocosm and activity categories, including sheltering and presence in the feeding area. It was not possible to observe movements of mouthparts so time spent in the feeding area was assumed to be proportional to time spent feeding (Dias et al. 2012). Regression analyses of behavioural traits on substrate moisture were conducted.

The results in Fig. 4 and Table 1 show that *Ph. muscorum* spent significantly less time sheltering than either of the two other species under the highest moisture conditions. It also had the steepest gradient for its moisture reaction norm of  $-2.16$ , compared with values of  $-1.05$  for *A. vulgare* and  $-0.19$  for *P. scaber* (Table 1). *Porcellio scaber* showed the least change in the proportion of time it spent sheltering because it sheltered for more than 95% of the time under all substrate moisture conditions. In contrast *A. vulgare* and *Ph. muscorum* sheltered for 74% and 51% respectively of the time under the moistest conditions (Table 1).

*Ph. muscorum* also had the steepest gradient for its moisture reaction norm for feeding of  $0.55$  (Fig. 4b), which was significantly higher than those for either *P. scaber* ( $0.05$ ) or *A. vulgare* ( $0.03$ ) (Table 1). Time spent feeding decreased from 11.5% when moisture content of the sand was 20%, down to zero under the driest conditions when none of the three species spent a significant amount of time feeding.

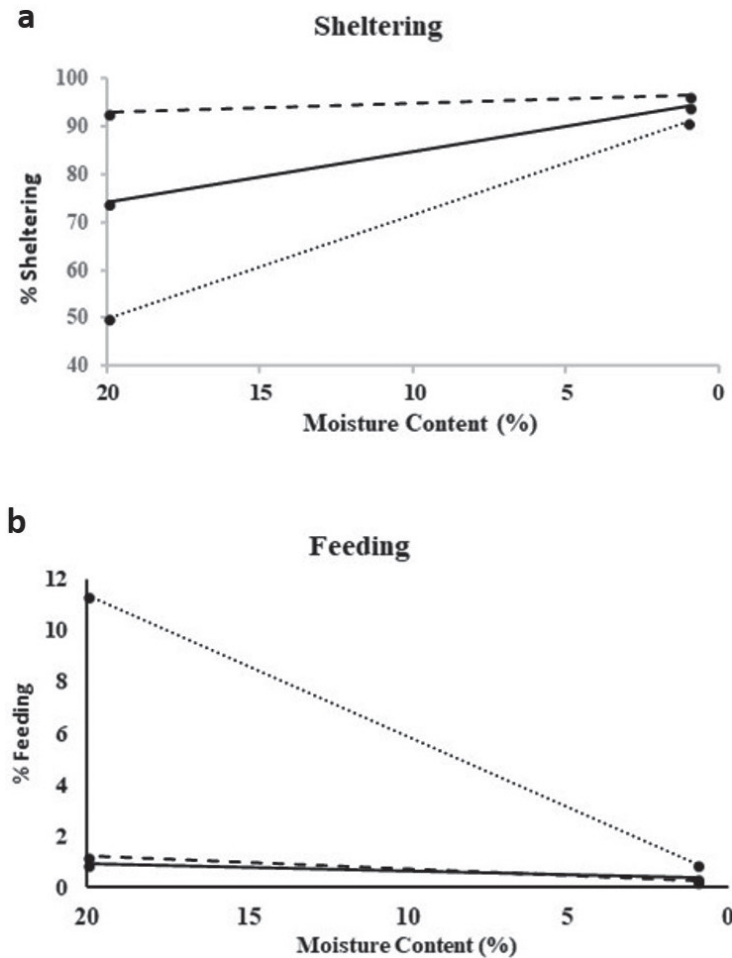
Overall *Ph. muscorum* showed significantly steeper moisture reaction norms than did either of the other species just as *O. asellus*, which similarly lacks pleopodal lungs, had a steeper gradient for its thermal reaction norm for aggregation than did either *P. scaber* or *A. vulgare*.

## Life history responses to microclimate: thermal and moisture norms of reaction for growth and survivorship

*Case study 3.* Both thermal and moisture reaction norms for the key life history traits of growth and mortality were compared for *O. asellus* and *Porcellio dilatatus* Brandt, 1833 by Dixie et al. (2015) who give full details of the methodology and statistical analyses used.

The  $2 \times 2$  factorial experimental design for investigating both relative growth rates and mortality rates permits comparison of thermal and moisture reaction norms simultaneously. Growth rates of *O. asellus* increased significantly at  $5^\circ\text{C}$  higher temperatures under the drier, 70% relative humidity conditions but did not grow significantly faster at the higher temperatures in the moister, 90% humidity (Table 2). Growth of *P. dilatatus* increased with temperature to a similar extent under both experimental relative humidity conditions. All four of the moisture reaction norms for a 20% decrease in moisture are steeper than any





**Figure 4.** Moisture reaction norms for **a**) sheltering and **b**) feeding behaviours with changing sand moisture content (time spent in behaviour as percentages of total observed behaviours). Lines represent linear regression models: *A. vulgare* (solid line) (sheltering:  $y = 95.24 - 1.05x$ ; feeding:  $y = 0.32 + 0.03x$ ), *P. scaber* (dashed line) (sheltering:  $y = 96.72 - 0.19x$ ; feeding:  $y = 0.22 + 0.05x$ ), *Ph. muscorum* (dotted line) (sheltering:  $y = 93.14 - 2.16x$ ; feeding:  $y = 0.36 + 0.55x$ ). Further regression statistics and number of observations (N), are given in Table 1.

of the thermal reaction norms for a 5 °C temperature difference for either species. This indicates a greater sensitivity to a 20% change in relative humidity than to a rise of 5 °C in temperature, suggesting that perhaps these species might respond more to predicted future changes in rainfall than to predicted increases in temperature. The difference in gradients of reaction norms between species (Table 2) reflects the higher rates of growth for *P. dilatatus* under all four combinations of temperature and relative humidity conditions.

Mortality rates (Table 3) were very low at 90% relative humidity; none of the *O. asellus* and very few of the *P. dilatatus* died at this relative humidity. The gradients of thermal reaction norms at 90% relative humidity were therefore not significantly

**Table 1.** Regression statistics for moisture reaction norms for sheltering and feeding behaviours of three species of isopods, *Armadillidium vulgare*, *Porcellio scaber*, and *Philoscia muscorum*, in laboratory arenas. Abbreviations: N: number of observations; *a* and *b*: parameters of regression equation; †: significant differences at  $P < 0.001$  (*t* test).

Regression statistics	N	Sheltering			<i>a</i>	Feeding	
		<i>a</i>	<i>b</i>	<i>t</i>		<i>b</i>	<i>t</i>
<i>A. vulgare</i>	1648	95.24	-1.05	-21.84†	0.32	0.03	3.77†
<i>P. muscorum</i>	824	93.14	-2.16	-25.07†	0.36	0.55	15.32†
<i>P. scaber</i>	1648	96.72	-0.19	-8.39†	0.22	0.05	6.84†
Comparison of gradients		Sheltering			Feeding		
<i>A. vulgare</i> vs. <i>P. muscorum</i>		18.99†			-30.19†		
<i>A. vulgare</i> vs. <i>P. scaber</i>		-25.08†			-3.96†		
<i>P. muscorum</i> vs. <i>P. scaber</i>		-44.43†			28.36†		

**Table 2.** Relative growth rates (mg g<sup>-1</sup> day<sup>-1</sup>) of *Oniscus asellus* and *Porcellio dilatatus*. Three way ANOVA: temperature  $F_{1,72} = 5.15$ ,  $P = 0.026$ ; humidity  $F_{1,72} = 88.62$ ,  $P < 0.001$ ; species  $F_{1,72} = 41.53$ ,  $P < 0.001$ . Reaction norms are derived from the differences in response to the two temperature and two humidity conditions.

Temperature °C		Relative humidity %		Moisture reaction norms
		90%	70%	
<i>O. asellus</i>	13.5 °C	34 ± 6	2 ± 0.1	-32
	18.5 °C	32 ± 2.5	17 ± 9	-15
	Thermal reaction norms	-2	15	
<i>P. dilatatus</i>	13.5 °C	66 ± 0.5	15 ± 4	-51
	18.5 °C	77 ± 8	28 ± 4	-49
	Thermal reaction norms	11	13	

**Table 3.** Mortality rates (numbers dying container<sup>-1</sup> 7days<sup>-1</sup>) of *O. asellus* and *P. dilatatus*. Mann Whitney U test for *O. asellus*: temperature NS, humidity  $U = 1851$   $P < 0.001$ ; for *P. dilatatus*: temperature  $U = 2754$ ,  $P = 0.016$ , humidity  $U = 2277$ ,  $P < 0.001$ . Reaction norms are derived from the differences in response to the two temperature and two humidity conditions.

Temperature °C		Relative humidity %		Moisture reaction norms
		90%	70%	
<i>O. asellus</i>	13.5 °C	0	0.73 ± 0.18	0.73
	18.5 °C	0	0.58 ± 0.12	0.58
	Thermal reaction norms	0	0.46	
<i>P. dilatatus</i>	13.5 °C	0.09 ± 0.04	0.78 ± 0.16	0.69
	18.5 °C	0.03 ± 0.03	0.18 ± 0.08	0.15
	Thermal reaction norms	0.06	0.60	

different to zero. Under drier conditions, at 70% relative humidity, more individuals of both species died at 13.5 °C than at 18.5 °C, leading to steeper thermal reaction norms at 70% relative humidity than at 90% relative humidity.

Overall, 20% lower relative humidity resulted in steeper moisture reaction norms for mortality rates than for thermal reaction norms resulting from a 5 °C rise in temperature, with *O. asellus* being more susceptible to drier conditions than *P. dilatatus*.

## Physiological responses to microclimate: temperature and moisture norms of reaction for respiratory rate and water loss

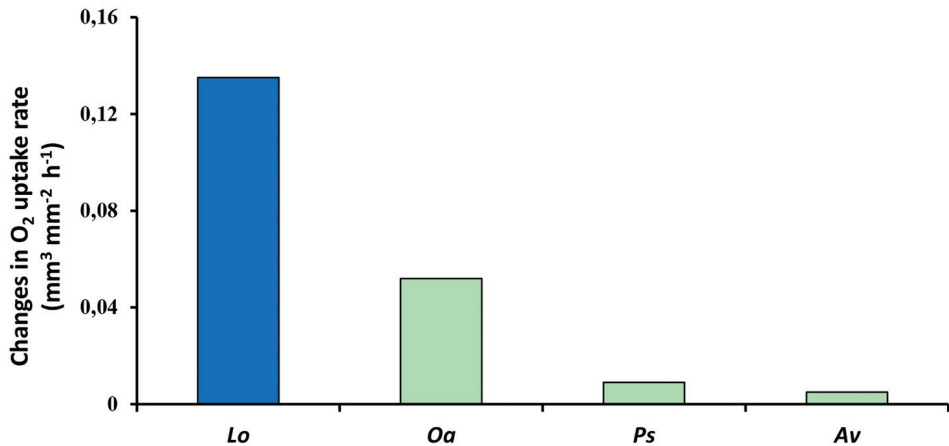
One of the drivers of differences between species of isopods in their behavioural and life history responses to differences in temperature and moisture is their different physiological adaptations to the terrestrial environment. In this section we compare responses of terrestrial isopod species, occurring in a wide range of biomes representing a gradient of moisture conditions.

Moisture reaction norms for differences in respiratory rate for *O. asellus*, *P. scaber*, and *A. vulgare* are shown in Fig. 4 together with that for *Ligia oceanica* (Linnaeus, 1767). The pattern of differences between the physiological reaction norms closely parallels that of behavioural reaction norms (Figs. 3, 4) with the gradient for *O. asellus* over a range of a 50% difference in relative humidity being substantially higher than for *P. scaber* and *A. vulgare*. However the gradient for *L. oceanica*, a littoral species, is more than double that for *O. asellus* reflecting the higher stress that would result from a reduction in relative humidity for a species that lives in a habitat with abundant availability of crevices with saturated micro-climates.

This interspecific comparison is extended for species from a wider range of habitats for thermal reaction norms for the physiological process of water loss in Fig. 6. The pattern for *L. oceanica*, *O. asellus* and *P. scaber* is similar to that for the moisture reaction norms for respiratory rate. *Ph. muscorum* has a higher rate than *O. asellus*, which, together with *P. scaber*, has a slightly steeper moisture reaction norm than either *A. vulgare* or *Armadillidium nasatum* (Budde-Lund, 1885). All five of these species are found in mesic habitats. The three species of *Buddelundia* from semi-arid habitats in South Australia all have lower gradients for their water loss thermal reaction norms than any of the European species from mesic habitats. Least affected by differences in temperature are species from xeric habitats including those found in the Negev and North African deserts such as *Hemilepistus reaumurii* (Milne-Edwards, 1840). The two *Armadillo* species and *Porcellio olivieri* (Audouin, 1826) also occur in desert environments but not so exclusively, as they are also found in other dry Mediterranean habitats. These three species have gradients for their thermal reaction norms that are intermediate between those of the desert specialists (including *Venezillo arizonicus* Mulaik & Mulaik, 1942) and the mesic species.

## Discussion

The range of morphological adaptations in body shape, respiratory surfaces of the pleopods and cuticle of isopods have been comprehensively described (Edney 1968, Warburg 1992, Schmalfuss 1984, Hornung 2011, Csonka et al. 2013, Broly et al. 2014) and reflect a moisture gradient in the habitats in which they are found, from littoral, through mesic to xeric, including desert. It can therefore be predicted that there will be a parallel gradient in the magnitude of physiological responses to changes in microclimate reflected in the gradients of thermal and moisture reaction norms.

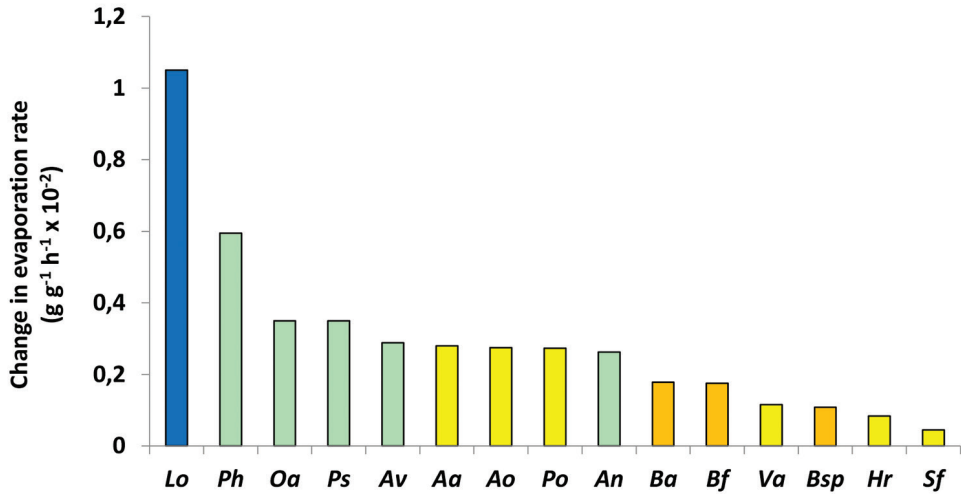


**Figure 5.** Gradients of moisture reaction norms for respiration of isopods differing in their resistance to desiccation. Reaction norms over the range 50–100% relative humidity for respiratory rates measured as rates of oxygen uptake ( $\text{mm}^3 \text{mm}^{-2} \text{body surface h}^{-1}$ ) (Edney 1968). Key to species: *Lo* *Ligia oceanica*, *Oa* *Oniscus asellus*, *Ps* *Porcellio scaber*, *Av* *Armadillidium vulgare*. Key to habitats: littoral (blue), mesic (green).

This hypothesis was supported for respiratory rate moisture reaction norms (Fig. 5), as *L. oceanica*, the species with the most primitive morphological adaptations to the terrestrial environment, had the steepest reaction norm, while *O. asellus*, which does not have pleopodal lungs, had a steeper moisture reaction norm than either *P. scaber* or *A. vulgare*, both of which have well developed pleopodal lungs.

Similarly, gradients of thermal reaction norms for water loss were substantially higher for *L. oceanica* than for the more fully terrestrial species *O. asellus* and *Ph. muscorum*, both of which had steeper gradients for their thermal reaction norms than members of either of the *Porcellio* species or *A. vulgare* (Fig. 6). There was a clear trend of thermal reaction norms for species from semi-arid environments in South Australia and xeric habitats in Europe, including specialist desert species, all having shallower thermal reaction norms for rates of water loss. Thus it can be predicted that those species from mesic environments will be more sensitive to changes in rainfall patterns, including longer periods of summer drought, than those from xeric habitats.

Given that these patterns of inter-specific differences in physiological traits for both thermal and moisture reaction norms reflect differences in morphology (Schmalhuss 1984, Broly et al. 2015), it can be predicted that there would also be a parallel pattern in thermal and moisture reaction norms for behavioural and life history traits. Aggregation in isopods is a behavioural tactic that reduces respiratory rates and water loss (Allee 1926), further elaborated by Friedlander (1965), Cloudsey-Thompson and Constantinou (1987), Caubet et al. (2008), and Broly et al. (2014). This morphologically related hypothesis is supported by both the peak of the thermal response curve and the gradient of the thermal reaction norm for aggregation being higher for *O. asellus* than for either *P. scaber* or *A. vulgare*.



**Figure 6.** Thermal reaction norm gradients for evaporation rate (water loss) for isopods from biomes differing in availability of moisture. Evaporation rate ( $\text{g g}^{-1} \text{h}^{-1} \times 10^{-2}$ ) standardised to a temperature range of  $3.5^\circ\text{C}$  (from Edney 1951; Warburg 1965, 1987, 1989). Key to species: *Lo* *Ligia oceanica*, *Ph* *Philoscia muscorum*, *Oa* *Oniscus asellus*, *Ps* *Porcellio scaber*, *Av* *Armadillidium vulgare*, *Ao* *Armadillo officinalis*, *Aa* *Armadillo albomarginatus*, *Po* *Porcellio olivieri*, *An* *Armadillium nasatum*, *Ba* *Buddelundia albinogrisescens*, *Bf* *Buddelundia frontosa*, *Va* *Venezillo arizonicus*, *Bsp* *Buddelundia* spp. probably *lateralis*, *Hr* *Hemilepistus reaumuri*, *Sf* *Schizidium festai*. Key to habitats: littoral (blue), mesic (green), xeric (yellow), semi-arid (orange) habitats in South Australia.

Sheltering behaviour is another tactic evolved in isopods to help reduce water loss by taking refuge in more humid shelter sites. Again, *Ph. muscorum* had a significantly higher gradient for its sheltering moisture reaction norm than either *P. scaber* or *A. vulgare*. Similarly, feeding behaviour moisture reaction norms of *Ph. muscorum* decreased more steeply than for either of the other two species. Thus within three species, all typical in mesic environments, there was a consistent trend in all of these behavioural traits, with species without pleopodal lungs being more sensitive to changes in microclimate than species better adapted to resist desiccation. This again indicates that there are significant inter-specific differences which suggest those species least adapted to the terrestrial environment might be more susceptible to potential changes in microclimate resulting from changes in future patterns of rainfall.

It would be logical to predict that such a trend might also apply to growth rates but that was not supported by the data because thermal reaction norms were very similar for *O. asellus* and *P. dilatatus* while the gradient for moisture reaction norms at both temperatures were higher for *P. dilatatus*. *P. dilatatus* had higher growth rates under all four combinations of temperature and relative humidity, possibly because the microclimate actually experienced by *P. dilatatus* was moister than that experienced by *O. asellus* due to *P. dilatatus* behavioural trait of burrowing into the sand substrates thereby being subject to a higher relative humidity in its immediate microclimate.

These life history traits of growth and survivorship are important correlates of fitness and have a very strong influence on the population dynamics of isopods. Differences in the gradients of reaction norms of life history traits could thus result in differences in the way abundances of these species might change under future climates.

An important conclusion of this overview is that, due to inter-specific differences in morphological, physiological, behavioural and life history traits, different species of isopods are likely to respond very differently to predicted changes in global climate. Interspecific differences in response to changes in temperature (Hickling et al. 2006) are now well documented for other taxa such as birds (Pearce-Higgins et al. 2015), Lepidoptera (McDermott Long et al. 2017), Orthoptera (Buckley et al. 2015) and Odonata (Stoks et al. 2014). In contrast, much less is known about ecological responses to predicted changes in rainfall patterns (Urban et al. 2016). Such responses are likely to be particularly pronounced for soil animals due to their activity and ecology being so strongly influenced by soil moisture, as shown by Blankinship et al. (2011) who found that precipitation limited all taxa and trophic groups in forest floors.

If the abundance of some species declines, it is not yet known whether other species, less sensitive to changes in microclimate, will respond by expanding their realised niches. It is known that interspecific competition for high quality foods between different species of isopods does occur in the field (Hassall and Dangerfield 1989) so it is possible that total guild densities may change less than species composition of guilds for macro-decomposers. It is known that differential impacts on basic biological parameters of individual species can modify fundamental characteristics of community structure and function (Jiguet et al. 2011). For isopods it is possible that the functional role of the whole isopod guild in stimulating microbial activity may alter less as a result of future climate change than might be expected from effects on particularly sensitive individual species. If other species in the guild, less sensitive to changes in micro-climate, expanded their realised niches in response to the decline of a more sensitive species, this could buffer consequences of climate change for overall decomposition rates. It is yet to be tested whether net community level responses, resulting from differential impacts of climate change on individual species, will impact on the extent to which future changes in rainfall patterns might mitigate potential increased temperature/ induced carbon dioxide emission feedbacks. It is widely agreed however, more focus is required on biotic interactions to clarify the potential feedbacks between climate change and soil carbon dynamics (Carrera et al. 2011).

The importance of species-specific responses to climate change has been highlighted for Collembola by Krab et al. (2010) and Makkonen et al. (2011), who found that interspecific faunal trait variation provided a valuable tool in predicting animal responses to climate change. However, it is noteworthy that Crowther et al. (2015) conclude that the regulatory effects of interspecific interactions are rarely considered in climate feedback studies. Pelini et al. (2015) extend this conclusion further by arguing that without isolating and including the significant impact of invertebrates, climate models will be incomplete, hindering well-informed policy decisions. Because the adaptive value of fundamental biological traits in isopods have been so comprehensively studied and

because isopods form such a prominent component of so many soil macro-arthropod communities, we propose that terrestrial isopods represent excellent model systems for further investigation of species specific responses to predicted climate change and their consequences for soil carbon dynamics.

### **What are the most pressing needs for future research in this field?**

Our understanding of the global carbon cycle is predominantly encapsulated in models. Our understanding of terrestrial isopod biology, in contrast, is mostly based on results of empirical studies. There is a very important need to bridge the gap between these two contrasting approaches and methodologies in these completely different disciplines. Considering the whole series of symposium volumes on the Biology of Terrestrial Isopods from 1984 until 2018, models may qualify as an endangered species.

We know that carbon dynamics in the soil is the least well understood part of the global carbon cycle. Whilst, it is extremely complicated, models by definition are simplifications of reality. Models can never represent the full complexity of the real world, that is not their function, but what they have are both holistic and heuristic properties. Holistic in that it should be easier to appreciate emergent properties of a system as a whole from a model of it, rather than from detailed studies of individual components. Heuristic in that models should generate testable predictions that cannot be made on the basis of studying individual components in isolation.

Terrestrial isopods are soil animals about which we have a wealth of knowledge based on empirical studies on their anatomy, behaviour, physiology, life history, and ecology. What we do not have is integrative models of different aspects of their biology that can then be interfaced with those of other soil animals and ultimately with those of micro-organisms. This integrative approach would significantly contribute to our understanding of how global climate change will affect the soil component of the global carbon cycle.

### **Where can we start?**

Models do not necessarily have to be numerical, at least initially. A topographic map is a model of a landscape. At a glance, that piece of paper only tens of cm wide, shows us where hills, mountains, rivers and bridges are in landscapes at much larger scales. By using a map we can predict how best to travel from A to B. What is very urgently needed in soil biology is some comparably simplified maps of the interactions involving the soil animals under our feet. A starting place could be for the context of many future studies to be introduced using conceptual box and arrow models of the system under study (Fig. 1). Our skills as scientists lie in how effectively we can simplify the real world we are studying by being brave enough to make a whole array of untested assumptions.

The next stage could then be to parameterise these models using the extensive empirical data available in the literature, making assumptions we know to be simplistic



but which enable us to make further quantitative predictions. Progressively testing these predictions experimentally to validate the models further could then increase our understanding of the system.

A problem will always be integrating models representing different levels of organisation which, from previous studies, range from molecules to communities. These may initially appear to be measured in different currencies but ultimately, while we are working within the paradigm of neo-Darwinian evolutionary theory, the answer to this problem may be in using fitness, or at least fitness correlates, to equate different traits at the individual level. Moving from the individual level to the ecosystem function level could then at least be based on a sound theoretical foundation.

The “functional traits” approach (Moretti et al. 2017) may be one way forward. Splitting whole trophic groups, treated as homogeneous entities in some large scale models, into functional groups has been an important step in the right direction. Taking account of interspecific differences in traits within functional groups and incorporating the diversity of responses into systems models could be a further important step. It would appear that the new generation of isopod biologists is now uniquely well placed to do this, thus giving them the potential to fill an extremely important gap in 21<sup>st</sup> Century science in a way that is a meaningful contribution to our understanding of the effects mankind is having by interfering with the global carbon cycle and thus global climate systems. We wish younger colleagues all success with such a challenging venture at such an exciting time and best wishes with carrying the science of terrestrial isopod biology to new horizons.

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# Isopod distribution and climate change

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## Abstract

The unique properties of terrestrial isopods regarding responses to limiting factors such as drought and temperature have led to interesting distributional patterns along climatic and other environmental gradients at both species and community level. This paper will focus on the exploration of isopod distributions in evaluating climate change effects on biodiversity at different scales, geographical regions, and environments, in view of isopods' tolerances to environmental factors, mostly humidity and temperature.

Isopod distribution is tightly connected to available habitats and habitat features at a fine spatial scale, even though different species may exhibit a variety of responses to environmental heterogeneity, reflecting the large interspecific variation within the group.

Furthermore, isopod distributions show some notable deviations from common global patterns, mainly as a result of their ecological features and evolutionary origins. Responses to human disturbance are not always traceable, but a trend towards community homogenisation is often found under strong global urbanisation processes.

In general, even though it is still not clear how predicted climate change will affect isopod distribution, there is evidence that mixed effects are to be expected, depending on the region under study.

We still lack robust and extensive analyses of isopod distributions at different scales and at different biomes, as well as applications of distribution models that might help evaluate future trends.

## Keywords

adaptations, biogeography, community assemblage, diversity, ecology, ecomorphs, ecophysiology, Oniscidea

## Introduction

The present global distribution of terrestrial isopods is the result of historical, palaeogeographical, palaeoecological, and evolutionary processes filtered through more recent effects of climatic (mostly temperature and precipitation regimes), topographic, edaphic, and biotic (mostly vegetation providing shelter and food, and controlling microclimate) factors at different scales. Currently, humans exert strong effects on all terrestrial biomes and biota, mainly through habitat fragmentation, elimination and/or change, urbanisation and pollution. At the same time, humans also provide new kinds of anthropogenic shelter sites, which often favour habitat generalists and result in faunal homogenisation.

Some 15 years after the publication of the world list of all species of terrestrial isopods (Schmalfuss 2003, and the updated electronic version in 2004), we still lack a global scale analysis of distribution data. The list presented each species' distribution at a relatively coarse geographical scale, mostly at country level. Several additional records and taxonomic revisions are published every year (e.g. Campos-Filho et al. 2017), so that today we can estimate a total number somewhat larger than the 3,710 valid isopod species belonging in 527 genera and 37 families given by Sfenthourakis and Taiti (2015) (as per April 2014). It is well known that terrestrial isopods have a worldwide distribution and exploit almost all kinds of terrestrial and coastal habitats, being absent only from polar regions and very high altitudes (> 4,800 m a.s.l. – see Beron 1997).

Oniscidea exhibit a unique feature among terrestrial animal taxa: it is the only monophyletic unit of relatively low taxonomic rank that has extant species representing almost all the range of evolutionary steps made during the transition from water to land. This becomes even more amazing if one also considers that the origin of the taxon is believed to be very old, possibly Palaeozoic (Broly et al. 2013a). Oldest known fossils are much younger (Cretaceous; Perrichot 2004, Broly et al. 2015b), but they belong to differentiated, fully terrestrial forms (Broly et al. 2013a). Genera like *Ligia* Fabricius and *Hemilepistus* Budde-Lund co-exist in time and probably represent the two extremes, from a primary amphibious life to a life under the harsh conditions of a desert. This fortunate fact offers vast opportunities for comparative studies in a wide range of fields, including physiology, morphology, ecology, behaviour, etc. Biogeography can also profit from this, by comparing modern distribution patterns among species and/or higher taxa with very different adaptive syndromes. Environmental change, such as habitat fragmentation, pollution, climate change, etc., may also exert varying effects on different lineages according to their position along this 'water to land to extreme terrestrial habitats' gradient. Furthermore, the study of Oniscidea distribution may provide crucial insight into processes and patterns pertaining to environmental change also due to the fairly well known general effects of relative humidity and temperature on these animals.

Global climate models predict precipitation pattern changes and increase in frequency and severity of droughts by the end of the 21<sup>st</sup> century (IPCC 2014). Such changes are expected to impact ecosystem structure and function, especially where water availability is the major limiting factor for soil organisms. Furthermore, climatic

factors modify the quality and quantity of substrate upon which microbes, a main food source for woodlice, act, bringing about further consequences on soil quality and productivity (Kumar et al. 1992), keeping in mind also that the duration of dry periods between rainfalls is a more important determinant of organic matter decomposition than total precipitation (Austin et al. 2004, Cable et al. 2008). The microbial community is also dependent on the temperature regime, and may change accordingly. Soil temperature directly influences biomass, microbial activity, and community composition with potentially interactive effects on ecosystems' carbon balance (Wall et al. 2012). The general consensus found is that a warmer climate will increase metabolic activity of microbes as well as the rate of litter decomposition (Kirschbaum 1995).

In the present paper we try to overview the current knowledge on isopod distribution at different scales, as well as related issues such as diversity gradients or their role in invasions, etc. We make an attempt to evaluate climate change effects on isopod diversity, especially in view of their tolerance to humidity and temperature. Nevertheless, we should stress from the start that, despite a relatively large literature on this group, we are still far from a comprehensive understanding of their spatial patterns and the underlying processes. There are still many areas of the world that remain unexplored, especially in the tropics. Exact distributions remain poorly known, even in regions from where good species lists exist (with some exceptions, of course).

Taxonomic nomenclature throughout the text follows Schmalzfuss (2004).

## **Distribution patterns at different scales**

Scale is one of the most crucial factors when it comes to distributional patterns. Environmental change too can be perceived quite differently and its effects can vary widely at different scales. Habitat fragmentation, for example, may lead to very different outcomes when it refers to deforestation within a stand of trees compared to the deforestation within a large forest or even, e.g., within the whole Amazon basin. Similarly, climate change at a global scale does not lead to directly analogous changes at local sites or within isopod micro-habitats. Therefore, it is important to study isopod responses to environmental changes at different scales regarding distribution patterns.

### **The global/continental scale**

Available information on terrestrial isopod distribution at a global scale is severely limited, though, due to large inequalities in research activity among different parts of the globe. We do know the general distribution of many species but at a low level of accuracy regarding range limits and site occupancy. During the last decade, at least, there is an increasing trend among isopodologists to provide such detailed information, but we are still very far away from a 'World atlas of Oniscidea'. Nevertheless, even the coarse-grained data at hand can lead to some generalisations.



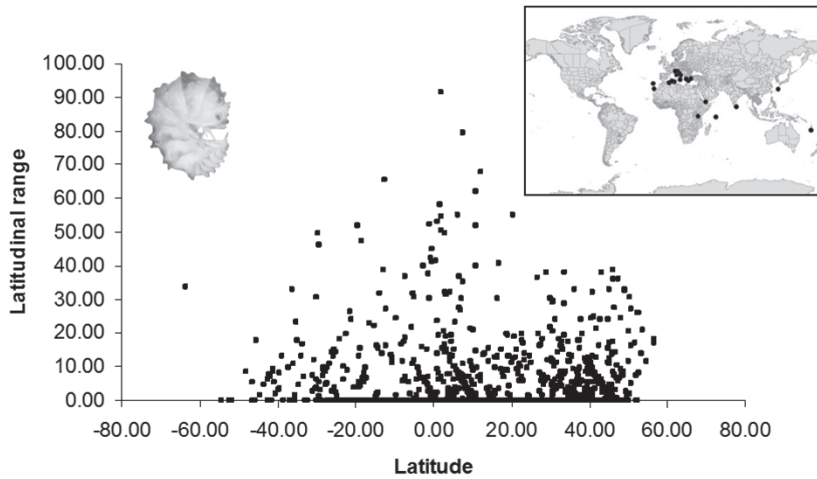
Sfenthourakis et al. (2007) and Kassara (unpublished) in her diploma thesis (Universities of Crete and Patras 2006), attempted an exploration of distribution data at a global scale for the species included in Schmalfuss (2004). They used a relatively coarse-grained mapping of distributions, using a regions-by-country digital map of the world, and the information provided in the above-mentioned paper, further refined by checking the primary literature in many cases where the distribution mentioned was very vague. Specific patterns explored were diversity hotspots, latitudinal trends in species richness and Rapoport's rule (Stevens 1989). Unequal sampling biases notwithstanding, these studies found suggestive evidence in favour of increased diversity in regions with Mediterranean-type ecosystems, as well as in insular areas of the tropics (see Fig. 1). Whether this is related to allopatric speciation patterns in topographically and/or environmentally heterogeneous sites or to other factors, it remains an open question. Latitudinal gradients of species richness are in agreement with this pattern, showing peaks in both northern and southern mid-latitudes.

Furthermore, isopods do not seem to conform to Rapoport's rule (see Fig. 1), indicating that their distribution might not be controlled by competitive interactions, as found also in studies at local scales (e.g., Zimmer 2003). Of course, more detailed data on species occurrences and distribution ranges, especially from tropical regions, are desperately needed, before we can rigidly support any general conclusions. Nevertheless, the patterns exhibited by whole species-rich families, such as Armadillidiidae and Porcellionidae, whose global distribution is relatively well known, are highly indicative for such 'deviating' trends of isopods. These families are restricted in mid-latitudes and contribute to a large percentage of the global species richness, something that is not expected to change much even if groups of a more tropical 'flavor' (e.g., Philosciidae) prove to be much richer than it is known today.

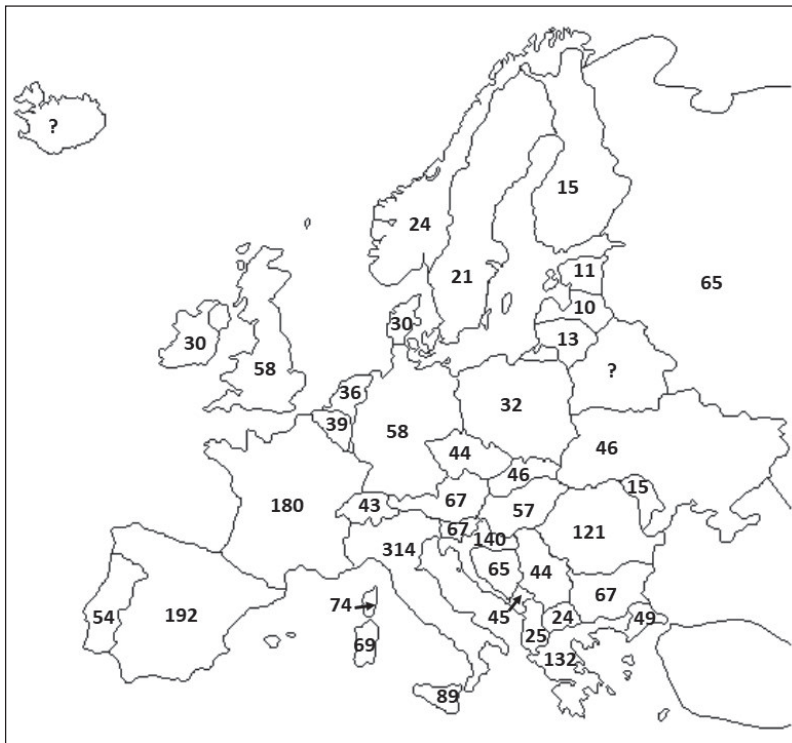
At a narrower latitudinal zone and an intra-continental scale, however, a significant latitudinal gradient of decreasing species richness can be seen. In fact, even though we do not have detailed maps of species richness at a fine geographical grain, when species richness of European countries (Fig. 2) are expressed per area unit (= species density), then one can find an almost perfect linear trend (in logarithmic space) of decreasing density with mean country latitude (Fig. 3). In addition, a study evaluating the latitudinal species richness of ten species-rich (with  $\geq 20$  species) families in Europe (e.g., Philosciidae, Oniscidae, Cylisticidae, Armadillidiidae) identified a gradual decrease of species richness towards north which was consistent in almost all families (except Cylisticidae) (Fig. 4; Hornung and Sólomos 2007). It remains to be seen whether these trends hold also true for other parts of the world. In any case, the European trend is in accordance with the tentative global trend discussed above, given that maximum isopod richness is to be found around the Mediterranean region, at low temperate latitudes.

If this pattern proves actually true and not an artefact of sampling or other biases, what would it imply for possible effects of predicted climate change on isopod distribution? On one hand, if regions around the Mediterranean become drier, thus more hostile to isopods, then we should expect a significant decrease in diversity, especially regarding locally adapted endemic forms. On the other hand, a plausible explanation

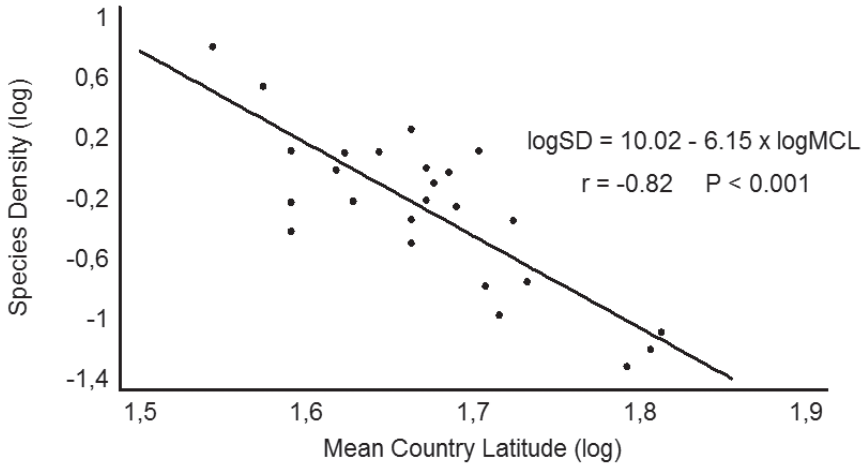




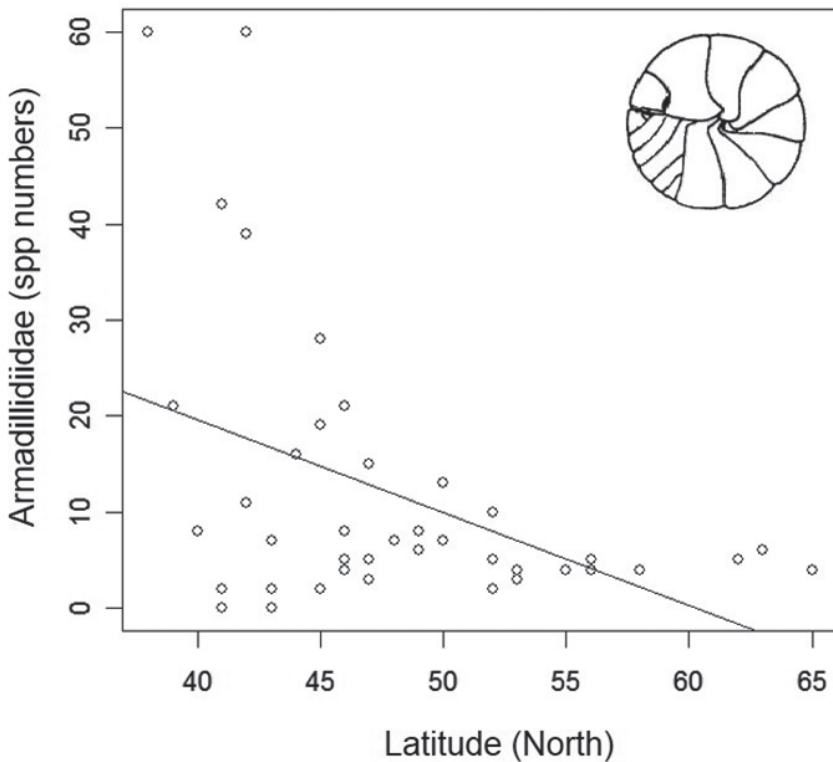
**Figure 1.** The latitudinal range of terrestrial isopod distributions is not related with latitude. The inserted map shows the diversity hotspots of endemic isopods (species with mean distributional range smaller than 1 degree of longitude and latitude) (adapted from data in Sfenthourakis et al. 2007).



**Figure 2.** Approximated species richness for selected European countries (Corsica, Sardinia and Sicily are treated separately; Greece and Italy refer to continental parts only). Data from Fauna Europaea (de Jong et al. 2014) plus some additional country lists, corrected following Schmalfuss (2004). Exact numbers are subject to revision, but with little effect to general trend.



**Figure 3.** The latitudinal gradient of decreasing isopod species density (richness per unit area) with latitude among European countries (mean latitude per country). The trend remains highly significant even after the deletion of Crete and/or Sicily that exhibit very high densities. Mean country latitudes were approximated using Google Earth.

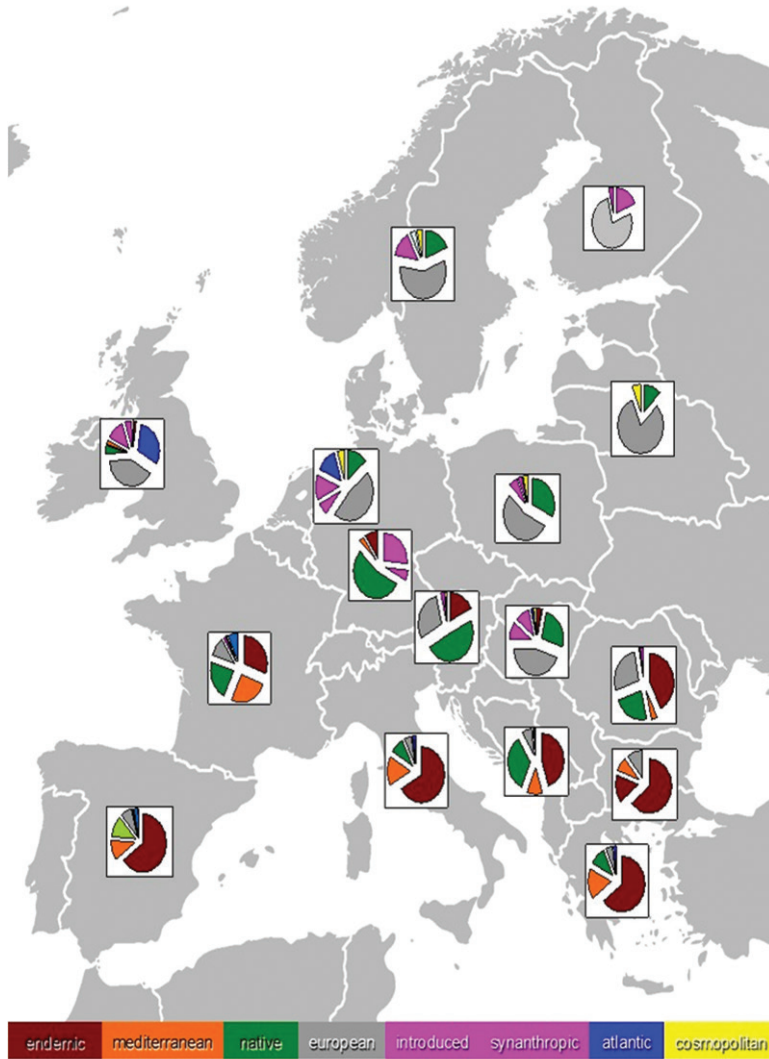


**Figure 4.** Reduction of Armadillidiidae species richness along a latitudinal gradient in Europe (the trend line of a GLM model is shown).

for this diversity pattern could be provided by the high levels of habitat heterogeneity in Mediterranean countries, coupled with the semi-isolated and sparsely distributed favorable humid habitats, conditions that enhance allopatric differentiation hence evolutionary divergence of isopod populations. A further increase of such environmental heterogeneity through climate change might not be fatal for isopod diversity, then, but might even act as a trigger for further diversification. At the same time, positive effects could be expected for central and northern European regions, where increased rainfall and temperature might allow for range expansion of southern species, enriching diversity.

In addition to latitudinal patterns of species richness, there is also a clear cline in the contribution of endemics and other chorological categories (Hornung and Sólýmos 2007). Endemics and Mediterranean elements dominate in the south (Iberian, Apennine and Balkan peninsulas), probably because of high mountains raised barriers to northward dispersal, turning into Atlantic and European elements in the western and central Europe, even to more homogeneous, mostly synanthropic and introduced assemblages in northern Europe (Fig. 5).

A relatively good part of isopod diversity consists of troglobious species, whose distribution patterns might be particularly interesting from the environmental change point of view. In fact, it is believed that climatic changes are among the major selective stresses that lead to adaptation to a life in caves. Manicasteri and Argano (1989) had attempted a global-scale analysis of troglobious species known at the time, and found again that highest diversity is concentrated in circum-Mediterranean countries and at similar latitudes in the New World (southern USA and Mexico), regions near the southern borders of glacial ice cover (Jass and Klausmeier 2006). Of course, the occurrence of troglobious species is strongly related with the availability of proper geological substrate (e.g., karstic systems), so the respective patterns might reflect the global distribution of caves instead. Furthermore, the structure of karstic systems also affects the degree of endemism, as identified by Manicasteri and Argano (1989). For example, troglobious species of Greece are mostly endemic to one or a few neighbouring caves whereas local endemism is much lower for troglobionts of northern Balkans where caves form larger and more inter-connected systems. Nevertheless, this might not always be the case, as shown by the troglobious species of Romania most of which occur in a small number of neighbouring caves (Giurginca et al. 2015). Therefore, explorations on the global-scale distribution of troglobious species should be made among geologically comparable regions. A quarter of a century after this study, though, we still lack data for the isopod fauna of many cave-rich regions of the world (but see Kováč et al. 2014, Giurginca et al. 2015, Souza-Silva and Ferreira 2015, Reboleira et al. 2015), so not much can be said regarding global patterns. Terrestrial isopods, though, are among the most frequent and ubiquitous cavernicolous animal groups, even if the representation of different families in caves is highly skewed. At the same time caves provide an important control system in studies on effects of environmental changes, especially climate driven ones, so intensive studies of caves in all parts of the globe are much needed. If increased drought or lowered temperature, or a



**Figure 5.** The relative contribution of different species' categories in the isopod faunas of selected European countries.

combination of these factors, has been the main selective pressure on isopods towards a cavernicolous life, it remains to be seen how expected climate change might affect them in the future, given that predictions show increased temperature and drought in many regions that are today rich in troglobious or troglphilous forms (e.g., Greece). Given that temperature in caves is usually very close to the mean temperature of their region, if troglobious species are stenothermal, we might expect increased extinction rates with increasing mean temperature, even though troglobious isopods seem to be more sensitive to humidity variation (see below). In any case, troglobious isopods

can provide excellent case studies in modelling cave environments under on-going climate change. In addition, there are also other formations similar to caves, such as sinkholes in karstic regions that provide refugia at a local or regional scale. These habitats provide a 'negative' altitudinal gradient in temperature and humidity that effects species distribution. Rare species can be abundant in such formations, possibly able to rescue a valuable species pool under climate change (Vilisics et al. 2008, Sólýmos et al. 2009).

Another attempt towards identification of global scale patterns has been made by Karagkouni et al. (2016, 2017) who explored trends in isopod body size. These authors used maximum body size of females from several hundred non-halophilous and non-troglobious species and also treated separately certain species-rich families. Body size distribution was found to be under strong phylogenetic control, Bergmann's rule (Bergmann 1847) and the aridity hypothesis (larger sizes favored in more arid areas as a means to avoid desiccation through lower surface/volume ratio) were only weakly supported, while no support for the 'island rule' (Van Valen 1973) could be found. Body size (maybe also body shape; see Broly et al. 2015a) is an important factor for most biological functions (e.g., water loss, respiration, reproduction, brood size). Of course, such effects would be most important at an intraspecific level, since environmental stress towards lower size might lead to decreased reproductive success. Unfortunately, we still lack such studies that would demand exploring links among variation in body size, brood size, reproductive success etc., within the distribution range of the same species. Global trends can provide indications on the mean outcome of the effects of environmental factors on size-related features. The very restricted data at hand indicate an expected pressure towards larger size in regions where temperature and drought are increasing. Nevertheless, it is not very clear whether this means an increased extinction rate of smaller species, a higher reproductive rate of larger individuals within each species, or both. The consequences in each case might be different depending on life history strategies, position along the r-K selection gradient etc.

Based on our experience with these organisms, though, we can assume that increased drought will lead to increased extinction rates for the usually small-sized hygrophilous species through the reduction of available habitat. For example, one of us (SS) has documented the apparent extinction of *Ligidium cycladicum* Matsakis from the island of Kythnos. This is a hygrophilous species endemic on a few Aegean islands where it lives among riparian vegetation. On Kythnos Island it was present at just one freshwater spring till 1990. After a couple of dry years in the '90s, the spring dried up and the species went extinct even after the re-appearance of water during subsequent rainy years. An intensification of such phenomena is expected in the near future. On the other hand, in regions where rainfall is expected to increase, the only gain in diversity might come from range shifts and long-distance dispersal (diffusion), but these are slow processes depending also on many other factors (e.g., isolation, distance to be covered etc.). It is interesting to note that this kind of observations provide a direct link between global and local-scale patterns.

## Elevational gradients

Another link between different scales can be found in the exploration of elevational gradients. These are assumed to reflect large-scale latitudinal patterns, given that changes in environmental conditions along altitudinal zones are very similar to changes encountered when one travels to higher latitudes. As already stated, isopods can be found even at very high elevations (< 4,800 m), exploiting thus a broad range of mountainous environments and providing very useful case studies on the effects of elevation gradients.

It is surprising, then, that just a handful of researchers have addressed this issue. The first such study was made on Mt. Cameroon, by Schmalfuss and Ferrara (1982), who found that isopod diversity sharply declines above the treeline. A few more studies were conducted on Greek mountains, namely by Sfenthourakis (1992) on three mountains of continental Greece, Lymberakis et al. (2003) on mountains of Crete, Sfenthourakis et al. (2005, 2012) on mountains of Peloponnisos (southern Greece) and Sfenthourakis et al. (2008) on five mountains of Greece. Most of these studies reported a general decrease of diversity with altitude (except in Sfenthourakis et al., 2005), and generally similar patterns of species richness on most mountains. The most striking common finding, though, was that on the highest elevations, above the treeline, there is usually one species that dominates in population density reaching high values of total population size. The identity of this species on each mountain-top seems to be determined by historical factors: different species of *Armadillidium* Brandt dominate on most mountain-tops of the long mountain range that is known in palaeogeography as ‘external Hellenides’ (Pindos range plus mountains in Peloponnisos and the mountains of Crete), while a species of *Porcellium* Dahl, is dominant on Mt. Olympos that does not belong to this range. This effect might be partially due to ecological release, in the absence of lizard and/or several invertebrate predators that cannot tolerate alpine environments. Nevertheless, additional factors should be responsible, given that it is exhibited by only one species even though more species often occur at the same elevational zone, albeit in much lower population sizes. It is important to note that species confined to the alpine/sub-alpine zone are very rare, and most species occupy broader elevational ranges. In Greece, at least, the only species that may be confined to high elevations is *Armadillidium lymberakisi* Schmalfuss, Paragamian & Sfenthourakis on Lefka Ori Mt. (Crete), but even for this species there is some evidence for occurrence at lower sites (Lymberakis, personal communication). Evidence for extensive endemism of mountainous isopods, as found in Beron (1997) and Schmalfuss (2003), may not contradict the former finding, since the latter authors do not report elevational ranges of the respective endemic species. Therefore, there might be many endemics on mountains, but these may occur at several altitudes, exploiting a variety of mountainous habitats.

Lopes et al. (2005) explored gradients on elevated sites in Brazil, but the altitudinal range of their study (up to 1000 m a.s.l.) was not large enough to reveal actual effects of elevation on diversity. These authors, though, found a pattern reminding

of a 'mid-domain effect', with species richness increasing in intermediate elevations, usually between 500–800 m. Suggestive evidence for such a phenomenon has been also found by Warburg and Hornung (1999) on similar altitudes in Israel. On the other hand, Giurginca et al. (2015) showed a decrease of cavernicolous species richness with elevation.

Most scenarios of future climate change effects on species distributions indicate a shift towards higher altitudes, especially concerning upper range limits (Parmesan 2006), particularly reducing available habitats for species restricted to mountaintops. In the face of the, admittedly restricted, information on isopods, it is not easy to make safe predictions regarding their responses to such changes, given that most species occurring on high elevations do not seem to be particularly stenoecious (i.e., with narrow tolerance regarding the range of ecological parameters). On the other hand, we might see some reduction of the high population densities of these species, especially if these densities prove to be a result of ecological release, so that more balanced communities are to be expected on high elevations. In addition, if the steep fall in isopod diversity above the tree line reported on tropical mountains (Schmalfuss and Ferrara 1982) is a general pattern, we might even expect an increase of high-altitudes' isopod diversity following an upward shift of the tree line. The actual situation, of course, is much more complex, given the interplay of temperature rise with rainfall and humidity patterns all of which also affect isopod distributions.

### **The 'regional' or other intermediate scales**

Available information on isopod distribution at geographical scales intermediate between the global and the local are mainly in the form of country lists and, more rarely, evaluations of such lists. A seminal work in this regard is the well-known work of Vandel on the isopod fauna of France (Vandel 1960, 1962), where the author, in addition to the per species presentation of taxonomy, ecology and distribution, provided also an account of country-level general patterns. Schmölzer (1971) published a similar work for the Iberian Peninsula, but his biogeographic interpretation was rather elementary. Gruner (1966) also gave ecological and distribution data on the German isopod fauna elements. Giurginca et al. (2015), in their overview of the cavernicolous isopods of Romania, presented also a short account of the species currently known from the country.

Sutton and Harding (1989) provided a more detailed account of British isopods' distributions, which was recently updated by Gregory (2009) taking advantage of a large number of occurrence data from Britain and Ireland. These authors showed that there is a slight northwestern to southeastern trend of increasing species richness in both England and Ireland. Despite of this trend, though, they have concluded that the most crucial factor determining species richness is habitat diversity, or better the availability of certain species-rich habitats, which tend to occur at the southeastern parts of these islands. Published work at this scale, though, is not very useful for evaluating responses to environmental factors, as they are rather phenomenological in nature,



and/or mostly identify the geographical areas that host favourable habitats for isopods within the respective country or region. A more robust analysis at such a large scale was performed by Purse et al. (2012), who showed that habitat use at a fine scale is the main driver of isopod species distribution patterns.

Hornung et al. (2008) analysed distribution data from Transdanubia, the western part of Hungary that hosts the vast majority of Hungarian species, using UTM grids and recording altitude and habitat characteristics. The authors found species richness to decrease from undisturbed wet habitats to disturbed dry ones, as well as a relatively uniform richness pattern but with high compositional turnover among geographical regions and habitat characteristics. Degree of habitat degradation was identified as the main factor shaping the composition of isopod assemblages.

Another useful approach in the frame of this discussion is offered by biogeographical analyses at an intra-country spatial level, like those presented for Mediterranean island groups (Sfenthourakis 1996a, b, Gentile and Argano 2005) or the Canary Islands (Rodríguez 1991). These studies have documented in a robust way the importance of habitat diversity for isopod richness. Further analysis at the same scale (Sfenthourakis and Triantis 2009) has shown that the existence of keystone habitats, like surface freshwater sources, is crucial for the maintenance of isopod diversity. It is not the extent of such habitats that is of importance, but their mere occurrence. This suggests that if we manage to guarantee the persistence of such habitats, we can conserve most of the isopod diversity we have recorded, at least in semi-arid and arid regions.

In another approach, in an analysis of isopod community composition at different scales from local to archipelagic, Sfenthourakis et al. (2004) found that most species occurring at low abundance are local endemics, while the reverse need not be true (i.e., we can find local endemics at a high abundance). Furthermore, local endemics may be sparsely distributed in space, so that a conservation strategy based on a few biodiversity reserves may not be adequate for isopods, or even other invertebrate groups (e.g., see Sfenthourakis and Legakis 2001). These findings should alert us against an increased probability of losing local biodiversity in a wide range of areas, not only those officially designed as protected (usually based on endangered vertebrates and/or plants). It might be relevant to this discussion that the recent Red Data Book of Threatened Animals of Greece (Legakis and Maragou 2009), based on IUCN criteria, includes 47 species of terrestrial isopods whose distribution ranges hardly overlap (Sfenthourakis 2009), even if we exclude the troglobious species that are often endemic to a single cave or to a small local cave system.

Phylogeographic analyses at regional scales have started to appear during the past few years, revealing a strong effect of palaeogeographic history of current distribution of isopod taxa (Poulakakis and Sfenthourakis 2008, Kamilari et al. 2014, Lee et al. 2014) but also effects of environmental factors such as sea water temperature for amphibious species (Eberl et al. 2013) or even evolutionary and dispersal events that may lead to unexpected distribution patterns (Santamaria et al. 2013). There are still just a few such analyses so far, but we should expect a rapid raise of their publication rates given the modern advances in molecular techniques.

## The local scale – isopod communities

Community composition of a certain functional group may depend on local environmental factors and/or on dispersal limitation. Most terrestrial isopods have highly limited dispersal and dispersion abilities. Most of them are ‘prisoners’ of their special demands regarding shelters. The vast majority of ecological studies on isopods focus at the local, community-level scale. There are studies aiming to document the species composition and abundance of isopods at certain biotopes and/or to examine the temporal changes in community structure and dynamics at a seasonal or inter-annual temporal scale. It is not within the scope of this chapter to present an exhaustive review of this body of research, so we shall try to identify general patterns emerging from these studies that might be of interest for the main theme of the present topic.

Isopod communities from different parts of the world and a variety of habitat types seem to exhibit low equitability, due to dominance of one, usually widespread and/or, synanthropic species. The identity of this species, of course, may differ per geographical region and/or habitat. In Itapuã State Park, southern Brazil, for example, Almerão et al. (2006) found *Atlantoscia floridana* (Van Name) to be dominant in all transects studied, while in Europe *Armadillidium vulgare* (Latreille) often contributes to this pattern at certain habitat types (e.g., Hornung et al. 2007, Tajovský et al. 2012). The reasons for this commonly found skewness have not been explored in detail. As already stated, this dominance pattern is very pronounced in alpine communities. At lower sites it is less extreme, but still detectable. Nevertheless, seasonal changes in dominance might also be present (e.g., Souty-Grosset et al. 2008), so the experimental design has to be taken into account before attempting generalisations.

Another aspect of isopod community structure is the moderate to high levels of nestedness exhibited among associated communities (Sfenthourakis et al. 1999, 2004, Triantis et al. 2008, Baini et al. 2014). These, nevertheless, are controlled almost completely by the inclusion of large communities in the data set, while poor communities are more or less heterogeneously structured, reflecting mostly the patchy spatial distribution of habitats. The respective literature on isopods is very restricted, though, but we can cautiously infer that oniscidean communities are principally assembled on the basis of fine-grained, local habitat heterogeneity. Along a related line of study on community assembly, Sfenthourakis et al. (2006), Pitta et al. (2012) and Baini et al. (2014) showed that there is no evidence that interspecific competition plays some significant role in isopod community structure, even among congeneric species. Once more, what repeatedly arises as an important factor across scales is the close association between isopod occurrence patterns and habitat structure (see also Hornung and Warburg 1995, Judas and Hauser 1998, Hornung et al. 2008) suggesting even a role of isopods as indicators of habitat change (e.g., Judd and Horwitz 2003).

A role of biotic interactions with other taxa in shaping isopod communities has not been conclusively shown, but some indirect evidence is suggestive. For example, Antonović et al. (2012) studying isopods in the Dubravica peat bog and surrounding

forest in northwestern Croatia, found that the higher diversity observed near the bog edge could be attributed not only to edge effects, but also to predator pressure by *Myrmica* Latreille ants and lycosid spiders at the bog site.

The crucial role of key habitats for isopod communities has been shown in detail by Sfenthourakis and Triantis (2009) who identified freshwater-related habitats (riparian etc.) as the critical factor allowing for a significant representation of specialist species in Mediterranean island communities. A small core group of generalist species that forms the basis of such communities can be present even on very small dry islets, but the occurrence of freshwater-related habitats enriches insular communities with a wide range of mostly specialist species. In fact, the erratic distribution of such habitats especially on very small islets lies behind the Small Island Effect, at least for terrestrial isopods (Sfenthourakis and Triantis 2009).

Despite this strong association of isopod communities and habitat diversity, there are several studies that have failed to find significant effects of habitat disturbance on isopod diversity. Even if this sounds counter-intuitive, it is not necessarily so, as we'll try to explain below. According to Tajovský et al. (2012), who studied isopods in 13 forest fragments in the Czech Republic ranging in area (0.1 to 254.5 ha), shape and composition of forest vegetation (thermophilous oak, mesophilous oak-hornbeam, thermophilous oak-hornbeam, acidophilous oak, basiphilous oak, beech oak-hornbeam, moist mixed deciduous forest, plantations of deciduous and coniferous trees), highest density and epigeic activity was recorded in the smallest fragments, despite of the fact that larger fragments contained a wider range of habitats. The authors found that forest fragmentation does not necessarily result in a decrease in isopod species richness. Similarly, no support for the hypothesis that diversity decreases in response to habitat disturbance or that species richness is highest in moderately disturbed sites (intermediate disturbance) was found by Hornung et al. (2007) in a study comparing urban, suburban, and rural sites in Hungary, albeit species composition did change along the gradient. According to Vilisics et al. (2007a, b), less than half of the species comprising assemblages in different habitats showed significant association with analysed habitat features. Furthermore, the responses of those that did show some association with certain habitat features were variable, reflecting their more-or-less known ecological characteristics. On the contrary, though, Messina et al. (2014) found a strong association of isopod species with specific plant communities and soil types at a coastal area of Sicily.

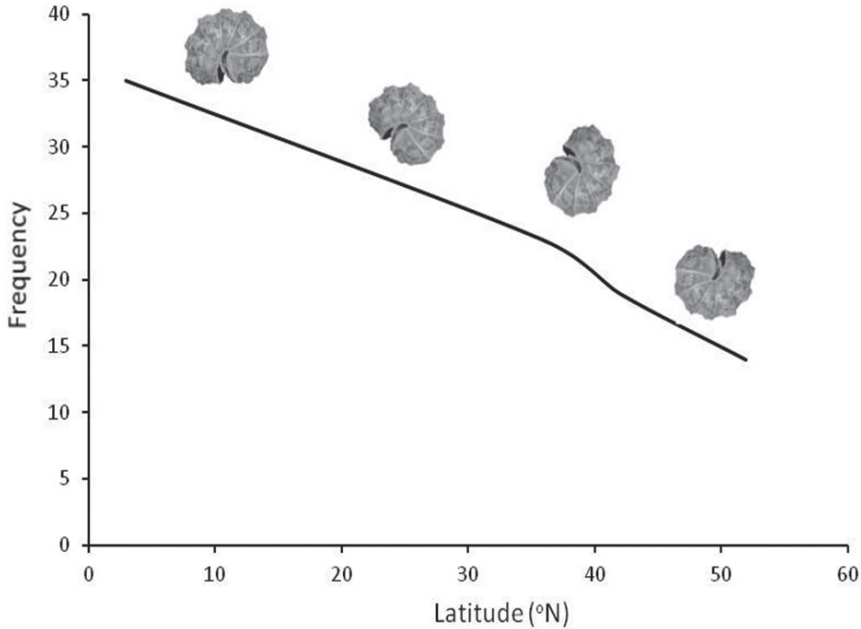
How can we account for this apparent contradiction regarding the role of habitats for isopod diversity? A plausible explanation lies at the scale of environmental heterogeneity exploited by such species of saprophagous animals. There is often significant variation among isopod species encountered at localities separated even by just a few tenths of meters, depending on the mere occurrence of keystone habitats, regardless of the area covered by the latter (Sfenthourakis 1994, Purse et al. 2012). This means that isopods can exploit heterogeneity at very fine scales, so that disturbance and fragmentation may not affect their species richness, as long as keystone habitats are not eliminated.

## Ecomorphological strategies and environmental change

Attention of most lay people that happen to notice terrestrial isopods is mostly attracted by conglobating species, ‘pill-bugs’, large non-conglobating forms, such as several species of *Porcellio* Latreille, *Trachelipus* Budde-Lund and *Oniscus* L., or anthropophilous common species like *Porcellionides pruinosus* (Brandt) that sometimes can be identified as belonging to the same group. It is difficult for most people, though, to see the connection between these forms and those found in Platyarthridae, Haplophthalminae, or even Ligiidae and Philosciidae. The general body design (‘bauplan’) of Oniscidea can be classified into a small number of forms, which are generally believed to be related to their ecological features. If this is true, then these forms might respond differently to environmental change. In this case, we could study responses at a more inclusive interspecific level beyond taxonomic groups.

Schmalfuss (1984) identified five ‘ecomorphological strategies’, namely rollers (able to conglobate), clingers (broad and flat bodies, attaching to the substrate), runners (long legs and narrow bodies), creepers (short appendages, body with ornamentation) and spiny forms, plus ‘non-conformists’, for species not possible to be included in one of the other five, while Hassall et al. (2006) added also ‘jumpers’ for one species of *Burmoniscus* Collinge. These are actually ‘body plans’ presumably showing responses to special ecological roles, and described the general ecology of each. The former author provided also some restricted information on possible latitudinal trends in the relative contribution of each strategy to communities from different geographic regions (see Fig. 6). Even though in the strict sense of the terms we should probably refer to these plans simply as ‘baupläne’ (‘body plans’), we shall maintain the term here to conform to isopod literature. Generalisations regarding possible responses of different ecomorphs to environmental change cannot be easily made, given a certain degree of vagueness in the current description of ecomorphological strategies. In fact, Dias et al. (2013) showed that activity budgets under different relative humidity conditions were mostly related to phylogenetic relatedness than to ecomorphological strategy, since species of Oniscidae following different such strategies were predicted to be more susceptible to desiccation than members of either Porcellionidae or Amadillidiidae. Members of Oniscidae lost mass most quickly and also suffered higher mortality in drier atmosphere. We can actually assume that ‘rollers’ should be better protected from desiccation under warm and dry conditions, but this strategy seems to be related mostly to defense against predators given that it is followed by both xeric species and very hygrophilous species (e.g., several endogean, riparian or humus-living Armadillidiidae). On the other hand, ‘creepers’ seem to exploit a more narrow range of habitats compared to all other ‘strategies’, being found almost exclusively in very humid micro-sites, such as the dense plant litter-layer, wet humus and/or in caves. Therefore, a possible dramatic decrease of humidity might have negative effects for ‘creepers’ at dry regions.

A more detailed ecomorphological analysis of isopods is necessary before we can make trustworthy inferences on varying responses to environment among ecomorphs. The ‘non-conformists’ category of Schmalfuss (1984) could be divided into functional



**Figure 6.** Reduction of the relative contribution of ‘rollers’ (isopods able to conglobate) among the various isopod ecomorphs with increasing latitude (based on Schmalfuss 1984).

morphological categories (e.g., species of *Leptotrichus* Budde-Lund and related forms could be identified as ‘diggers’), ‘rollers’ could be further divided into, let say ‘sluggish endogeans’, ‘vigorous semi-runners’ etc. There is an increased interest in functional morphology and its relation to biodiversity dynamics in the last decade, so it is time to apply relevant approaches to this amazing group that exhibits unique morphological and ecophysiological adaptations.

## Effects of climatic factors on isopod distribution

### General

Detritivore soil invertebrates – among them terrestrial isopods – are responsible for turnover of litter and are effective regulators (litter transformers, ecosystem engineers) of that process (Lavelle 1997). The functional role of isopods is a crucial part of the soil food web. Woodlice, even though they can be omnivorous, mainly feed on decaying plant material. They have a vital role in the fragmentation, fungal and bacterial inoculation of dead plant material that is to make organic matter available for other elements of the decomposer network. In spite of the important ecosystem service of macro-detritivores in the belowground life, studies concerning their function are underrepresented in the scientific literature.

It is already widely acknowledged that climate change has altered the distribution of several invertebrate, vertebrate and plant species. Abiotic environmental factors, first of all humidity and temperature are of basic importance in the life of soil dwelling macro-detritivores, too. Climatic, microclimatic relations strongly influence the abundance, community composition and functioning of soil organisms, including woodlice, and so indirectly decomposition rates and soil macrofauna (Wardle 2002). By certain climate scenarios some soils will get wetter, others drier; the conditions and resources for soil animals are not changing consistently.

### Climatic effects

Oniscidea originate from marine ancestors and there is a distinctive gradient within the taxon of increased adaptation to land conditions so that a variety of climatic components could be identified as determinants of species' occurrence, establishment, survival, or prosperity (Hornung 2011). In addition, the degree of dependence on such climatic factors may vary through different scales (biomes through regions, to habitats and microhabitats). In general, though, the most critical factors determining terrestrial isopod establishment, existence and survival at any given locality are temperature and humidity. Microclimatic tolerance, niche dimensions of single species or species assemblages are central topics of isopod studies since the turnout of woodlice ecology (e.g., see the works of Miller 1938, Edney 1954, 1968, Warburg 1965, 1987, Warburg et al. 1984) in the last century. Certain species are differently sensitive to environmental conditions and this broad range of evolutionary adaptation is coupled with a wide range of ecomorphological and life history strategies (Schmalfuss 1984, Sutton et al. 1984). This variety involves considerable distinct values of desiccation resistance among species (Warburg 1993, Dias et al. 2013). Protection against desiccation evolved along different routes, such as physiological, ecomorphological and/or behavioral pathways. Evolutionary changes in respiration and respiratory organs, changes in cuticle structure and morphology, conglobation mechanisms and tendency towards aggregation, are all parts of terrestrial adaptations promoting fine and large-scale dispersal (Warburg 1987, Hassall et al. 2010, Hornung 2011, Broly et al. 2013b, 2014).

Hygrokinesis and photoreaction of woodlice are of high significance to direct their reactions, movements and demographic strategies, depending on the features of the region where they occur (e.g., in temperate, xeric, mesic, semi-arid or arid environments), but there is an evolutionary trend from strongly water-dependent to more drought-tolerant species (Warburg 1968). Species differ also widely in their tolerance limits regardless of terrestrialsation stages, with habitat specialists (e.g., *Armadillidium zenckeri* Brandt sticking to wet and cool, marshy conditions in Europe, and *Hemilepistus reaumurii* (Milne-Edwards) burrowing in loess deserts) and habitat generalists [like *Armadillidium vulgare* or *Porcellium collicola* (Verhoeff)] being present in the same genera or families, and reasonably, the distribution of species along a habitat scale is in close correlation with their respective environmental requirements (Vilisics et al. 2007a, b, Hornung et al. 2008, Dias et al. 2013).



## Climate and life history

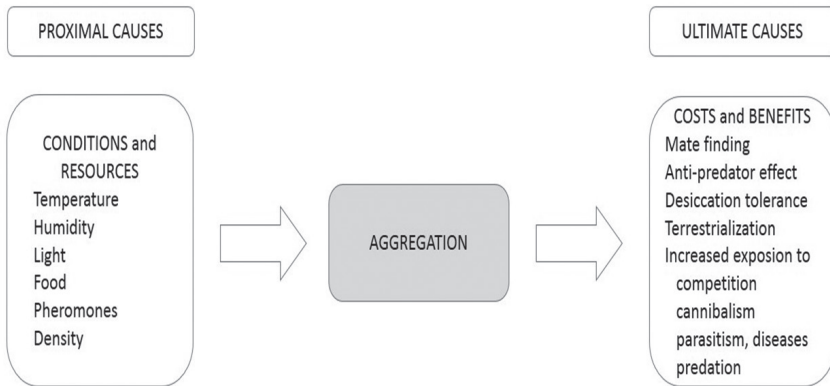
Woodlice, besides physiological and ecomorphological responses, can protect themselves from water loss by behavioural adaptations. They may show varying seasonal and diurnal patterns of activity, increased nocturnal activity and/or vertical and lateral movements towards sites with increased humidity. There is a correlation between precipitation pattern and isopod surface activity. In one study, aboveground abundance was found to increase about one month after the onset of rainy season in the Mediterranean (Warburg et al. 1984). This pattern is conspicuously reflected in seasonal surface appearance, presence or absence of woodlice in mesic and xeric regions (Davis et al. 1977, Warburg 1987, Hornung 1989, 1991, Hassall and Tuck 2007). Temperature fluctuations also can regulate population size (McQueen and Carnio 1974).

In a recent study, Dias et al. (2013) could make a generalised statement on the correspondence of water loss, desiccation resistance, and habitat choice. Based on data from 22 species, they found that differences in body water loss rate are the main mechanism behind interspecific variation in desiccation resistance. There was a low variation in lethal water loss among investigated species, while water loss rate and desiccation resistance (measured as average survival time in hours) had a significant interspecific variation in connection with ventral surface area, the main vaporiser body part. Other morphological traits such as structure of pleopodal lungs, and cuticle (Csonka et al. 2013) are also good indicators for environmental tolerance, influencing habitat choice and distribution. Regional species distribution and habitat moisture levels showed rather clear correspondence evaluating 758 records of 48 isopod species in western Hungary (Hornung et al. 2008).

Conglobating species have the advantage to reduce water loss rate and CO<sub>2</sub> release: e.g. the pill-bug *Armadillidium vulgare* was able to decrease water loss by 34.8%, and CO<sub>2</sub> release by 37.1% in a relevant experiment (Smigel and Gibbs 2008). In the case of non-conglobating isopods, locomotion gives a way for active escape from predators and finding shelter, but also for avoidance of desiccation. *Porcellio laevis* Latreille has been shown to avoid temperatures above 25 °C owing to the danger of increased evaporative water loss. There is a progressive decrease in speed after a weight loss >10 % compared to initial body mass due to desiccation (Dailey et al. 2009). Habitat specialist species are the most sensible against desiccation. For example, *Mesoniscus graniger* (Frivaldsky), a cave dwelling isopod (adapted to a constant 93% relative humidity and around 10 °C average temperature in Baradla cave, Hungary) can tolerate gradual temperature changes on a quite broad scale (0–22 °C) but changes in humidity have a lethal effect (Gere 1970).

Another important behavioural adaptation of isopods towards water loss reduction is the tendency of many species to aggregate (Allee 1926). Water loss is dramatically reduced by crowding, as shown by several studies on aggregation patterns. Several relevant data have been summarised by Broly et al. (2012, 2013b, 2014, 2015c). Aggregation as a behavioural variation, adaptation can buffer also the effects of changes in microclimate (Hassall et al. 2010). Major proximate and ultimate causes of aggregation for woodlice are depicted in Fig. 7.





**Figure 7.** Biotic and abiotic factors ('proximate causes') and cost-benefit relations ('ultimate causes') of aggregation in terrestrial isopods as a behavioral adaptation to avoid desiccation (original idea from Broly et al. 2013b).

Temperature values determine also growth rate (with species-specific minima and maxima) and cohort survival rate (mancas proved to be the most sensitive). In one study, the lower lethal temperature (50% mortality) for *Porcellio scaber* Latreille was below zero, between  $-1.4$  and  $-4.6$  °C, with a super-cooling point at about  $-7$  °C (Tanaka and Udawaga 1993).

Food quality and temperature have important effects on rate of gravid females and on the growth of future offspring. Females become gravid at a smaller size at increased temperature while increased food quality results in larger size (Helden and Hassall 1998).

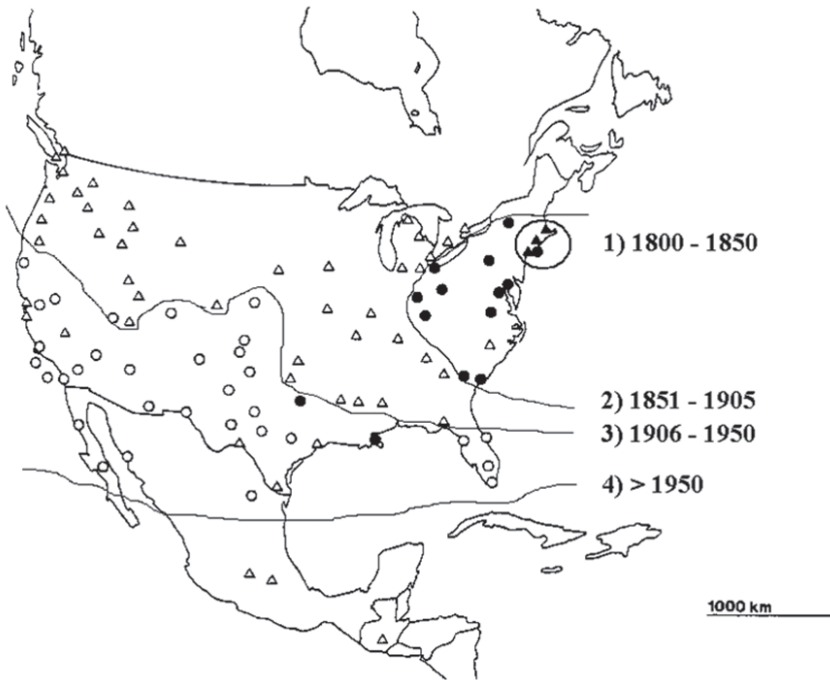
Climatic changes definitively influence population processes, too. Life history traits such as reproduction (timing, parity, number of potential offspring) are highly influenced by humidity, moisture, and temperature (Souty-Grosset et al. 1988). There are life history parameters which help isopod establishment and range expansion. Successful species, in addition to the omnivore and detritivore feeding habits, have developed several advantageous reproductive strategies, such as parthenogenesis, multivoltine iteroparity, sperm storage, and high offspring number. Under stressful weather conditions individuals can modify their reproductive output (phenotypic plasticity) to increase survival probability (Hornung and Warburg 1998). Climate change may influence reproduction through indirect effects, such as food quality (plant growth and quality) (Rushton and Hassall 1983). The current climate change trend could affect such traits. Species with wider tolerance limits, higher reproductive output, and with the ability of sperm storage (Suzuki and Ziegler 2005) have better chances for survival and geographical expansion, and might contribute to the homogenisation of isopod fauna world-wide. Such phenomena have already been documented in urban regions, where habitat generalists and introduced, sometimes even invasive species use the human-made environment as a 'spring-board' for successful establishment (Garthwaite et al. 1995, Hornung and Szlavecz 2003, Tartally et al. 2004). The process is promoted by human-aided dispersal, repeated introductions, and creation of new, favourable

habitats for such species. On the other hand, in species with different life history characteristics, semelparity, univoltine iteroparity, small body and brood size, and habitat specialisation hinder successful dispersal. Sutton et al. (1984) classified life history variations into two strategies, reminiscent of the extremes in the r–K selection continuum: ‘stenodynamic’, similar to r-selected, and ‘eurydynamic’, similar to K-selected life history strategies.

A great number of studies prove that there is a general relationship between female size and clutch number. Stochastic environmental changes modify the actual results on different scales (Hornung and Warburg 1998) and species show significant phenotypic variation (Dangerfield and Hassall 1992). There is intra- and interspecific, annual, and geographic variations in timing and duration of reproduction in sympatric species as well (Zimmer and Brauckmann 1997). Individuals might postpone reproduction and/or decrease output by oosorption, converting energy back when faced with extreme conditions (Hornung and Warburg 1993, 1994). Females of equal size belonging to different species may carry very contrasting offspring numbers according to idiosyncratic life histories and reproductive strategies, or to phylogenetic effects.

Despite these variations, duration of gravidity is generally temperature dependent (Snider and Shaddy 1980, Hornung and Warburg 1994). There is a threshold and an optimal range for all species. For most species, day length (and temperature above threshold values) is the trigger for oogenesis (McQueen and Carnio 1974, Hornung and Warburg 1993, 1994, 1998, Hornung 1996) and increased temperature accelerates marsupial development (Hornung and Warburg 1993, 1994). In the same species, onset of reproduction may depend also on latitudinal day-length shifts (Souty-Grosset et al. 1988).

Woodlice generally are considered as having restricted dispersal abilities. Many species may hardly be able to expand their distribution ranges but some have been rather successful in colonising new territories. At the same time, distribution patterns of isopods reflect ecological tolerance at a macroecological scale. The activity radius of an individual is species specific, depending on certain ecomorphological, and life history characters, humidity requirements. *Hemilepistus reaumurii*, for example, was found to cover several meters per day while foraging (Hoffmann 1984, 1989) whilst other species, mostly small-sized, soil-dwelling ‘creepers’ (Schmalfuss 1984), move only within a few centimeters. On the other side, locomotion is essential to find food, mate, and shelter or to escape predators. In a changing environment, species are forced either to go extinct under unfavorable conditions or to migrate, following the shift of favorable environmental conditions (diffusion-type dispersal). This may have happened repeatedly in the past, e.g., during the succession of glacial and interglacial periods in the Pleistocene. In North America, the species-rich southern parts could have acted as refugia and sources for northward dispersal (Van Name 1936, Jass and Klausmeier 2000, 2006). In addition, several species were introduced from Europe, arriving in soil ballast carried by ship mainly during the 19<sup>th</sup> century (Lindroth 1957), which is a jump-dispersal followed by local dispersion. Lindroth (1957) examined critically the routes of introduction of European fauna into North America, both in



**Figure 8.** The expansion of *Armadillidium vulgare* in North-America (modified after Garthwaite et al. 1995).

recent times and in the late Pleistocene. A good example is the documented historical expansion of the, originally Mediterranean but now cosmopolitan, species *Armadillidium vulgare* (Fig. 8). Dispersal ability is the result of complex features and life-history processes. In fact, the spread of *A. vulgare* in California grassland (Paris 1963) and in North America in general (Garthwaite et al. 1995) is a real ‘success story’. Terrestrial isopods are continuously introduced in the present time. Worldwide trade of living plants, establishing botanic gardens, parks with exotic trees and so on give opportunities to introduce and spread new, mainly tropical, subtropical, or Mediterranean soil-dwelling species all around the globe (Korsós et al. 2002, Hornung and Szlavecz 2003, Hornung et al. 2005, Vilisics and Hornung 2009, Gregory 2014, 2015). In some documented cases isopod swarming was observed in different species and geographical regions. This behaviour implies occasional mass occurrence and migration of great numbers of individuals of the same. The explanation is still not clear, but should be related to demographic factors (Warburg et al. 1984). Similar phenomena occur often also in Myriapoda (Korsós 1998).

Isopods are excellent objects for anthropochorous distribution and jump dispersal. It is known that a fertilised female can reproduce several times without a successive copulation (Lueken 1963, Johnson 1982). As gravid females carry their eggs/larvae in a brood pouch till hatching, they are saved against unfavorable environmental effects. These small-sized animals can easily be propagated with soils and with ornamental plants



**Figure 9.** Distribution of Oniscidea in the European part of the former USSR prove the existence of climatic barrier: no woodlice were found above the line of 120 days/year with a temperature above 10 °C. Black dots – positive samples; grey dots – sample localities without isopods (modified after Kuznetsova and Gongalsky 2012).

(Hornung and Szlavetz 2003). Species introduction, a passive dispersal process today assisted mainly by humans, is usually followed by a rapid population increase and then active dispersion. Isopods might be called ‘silent invaders’: introducing a small number of specimens (in extreme cases even one gravid female) may lead to the establishment of a stable, persistent population (Vilisics and Hornung 2009). We still lack, though, a robust study on isopod propagule size, invasion dynamics, and related processes.

Interesting cases of long-distance dispersal are offered by the small and blind, specialist myrmecophilous isopod species *Platyarthrus schoblii* Budde-Lund. It probably originated from the Mediterranean region but have been successfully introduced in northern parts of Europe, accompanying *Lasius neglectus* van Loon, Boomsma and Andrásfalvy. The ant is an aggressive, invasive species that invaded Europe (Dekoninck et al. 2007). As the ant is distributed by colony budding, isopods can easily disperse attached (Tartally et al. 2004).

At a larger scale, dispersal of isopods may be constrained by climatic and edaphic factors. Annual temperature, amount of daylight and precipitation regimes may condition the existence, dispersal, and activity of isopods. For example, Kuznetsova and Gongalsky (2012) found that woodlice distribution in the former USSR is thus severely constrained by the isotherm of  $> 10^{\circ}\text{C}$  for at least 120 days per year, beyond which no isopod species could be found (Fig. 9).

### Niche shift in isopods

The niche of an isopod is determined first of all by microclimatic conditions. Michael R. Warburg, as a postgraduate student of G. Evelyn Hutchinson, made the first studies on isopods' niches. He partitioned an isopod's niche into two parts (Warburg 1965): habitual (commonly occurring situations) and extreme (extreme situations). Nowadays, the niche might be compiled of the effective values of climate/microclimate, resources, intra- and interspecific relations, and all physical and biological factors influencing reproductive output. Very often chemical, physical characteristics of soil, such as pH and/or carbonate content are limiting factors (Zimmer et al. 2000) and eco-physiological requirements direct distribution of woodlice. The actual microclimatic changes influence the daily activity, diurnal and seasonal rhythm of the populations. Above-ground surface activity and activity density of the populations belonging to different species also depend on their affection to soil temperature, soil moisture, above ground relative humidity and availability of shelter microsites as well. Humid shelters are particularly important during reproduction, egg, embryo development, and manca release to save offspring against dehydration.

Cold adaptation might exist among isopods. *Porcellio scaber*, a species with worldwide although mainly synanthropic distribution, has proved to be tolerant of temperatures below  $0^{\circ}\text{C}$ . Its super-cooling point was approximately  $-7^{\circ}\text{C}$  (Tanaka and Udagawa 1993). Active specimens of different woodlice species occur rather often under snow inside the decomposing litter layer in temperate regions. Such kind of abilities might help both the altitudinal and latitudinal dispersal of cold-tolerating species. The expected effect of climatic changes is the shift in distribution limits at least in the case of habitat generalist and synanthropic species. The potential 'climate change-driven' northward range expansion of certain terrestrial isopods (and other detritivores) might contribute to the increase of litter decomposition rates. Decomposition rate changes depend mostly not on species composition but on the biomass of macro-decomposers. Increased decomposition facilitates carbon emission (van Geffen et al. 2011). In consequence, superdominance of generalist, widely distributed species itself might cause important changes in decomposition rate and carbon release.

On the other extreme, a study on *Armadillidium vulgare* proved that critical thermal maximum decline interrelated with declining oxygen concentration (Klok et al. 2004). A developed 'tracheal' system in certain terrestrial isopods, such as in *A. vulgare* (Csonka et al. 2013) makes oxygen delivery more efficient. Hypoxia

causes a considerable effect decreasing metabolic rate and upper thermal tolerance, compared to insects.

Of course, other factors besides climatic ones may also constraint the ability of isopods to disperse or undergo niche shift. These involve ecophysiological requirements, soil pH, soil calcium content etc. (Zimmer et al. 2000).

## Conclusions, open questions, and future directions

Climate change has always been a trigger for evolution and for changes in the distribution of all species on Earth. Nevertheless, if one of the various scenarios of global climate change presented by IPCC proves to approximate the actual climate of the next few decades, then the speed of the change will not allow for distributional shifts and evolutionary adaptations of most organisms. Different scenarios forecast global temperature and sea-level rise, and more frequent occurrence of extreme climatic phenomena. The extent of climate change effects on different geographical regions, though, is expected to vary widely.

As far as terrestrial isopods are concerned, we still need to gather more detailed data before we can provide reliable estimates of expected changes in distribution patterns. Based on available evidence, as presented in this paper, though, we may attempt some general tentative hypotheses. Most scenarios predict increased drought in circum-Mediterranean regions, which are assumed to become even more arid than today. Since the highest known species richness of isopods is found in this region, it is very probable that a significant part of isopod diversity, primarily those more dependent on humid habitats (such as riparian etc.), will be negatively affected. Such effects will be more extensive in insular assemblages where water availability is even more restricted. Given that Mediterranean islands host many endemic species, the expected problems will be also of qualitative importance. On the other hand, higher latitudes (and, also, altitudes) may experience an increase in isopod diversity, if species will be able to expand their distribution ranges following increasing annual temperatures. Nevertheless, in most regions that will not experience pronounced habitat alterations, we do not expect to see significant effects on isopod assemblages, given that these animals are not particularly sensitive to intermediate levels of disturbance.

An important relevant process that, till today, has led to an increased isopod diversity in some regions of the world (e.g., North America, Britain), is the human-caused introduction of alien species (Jass and Klausmeier 2000, Hornung and Szlavecz 2003, Gregory 2009, 2014, 2015). Coupled with the fact that isopod species do not usually exhibit pronounced interspecific competition, introduction of alien isopod species is not expected to have negative effects on native species, so that the net result will be an increase in species richness. Nevertheless, there are some cases where introduced species may have led to exclusion of natives (Taiti, personal communication). Such effects might be seen also in isolated tropical islands and human-made ‘tropical houses’ after the anthropochorous dispersal of pantropical species, but also in temperate regions



where several species have been introduced mainly of European origin. Temperature increase might provide more opportunities for alien introductions. Of course, this process has its negative side too, since we do not yet know the effects of other alien taxa introductions on native isopod species (e.g., possible predators such as spiders or lizards etc., or competitors such as millipedes, detritivore larvae etc.).

Climate change may also alter vegetation that can lead to changes in litter quality, which in turn may also influence detritivore species like isopods. The processes involved, though, are very complicated, so we cannot make safe predictions at the current state of knowledge.

Climate change, of course, is not the only important factor behind on-going environmental change. Besides chemical and organic pollution that might be important in some places, the most important processes for isopods are the homogenisation of agricultural landscapes and the increased urbanisation in many parts of the world. Such habitats are subject to faunal homogenisation processes that favour a small number of adaptable, habitat generalist, isopod species. Even though we have not been able to document decreased levels of local (alpha) diversity in such habitats, overall diversity is negatively affected by decreasing beta-diversity among such localities. Nevertheless, this process has started to attract the attention of isopod researchers only recently, so we still need more data and also from a wider geographical range, before we can evaluate these processes reliably.

So, which are the most important open questions on the subject that should lead isopod research in the years to come? Herein, we give some answers to this question, but these should not be regarded as a finite list. In fact, we need input from a wide variety of research field in order to be able to draw a good picture of distribution patterns under environmental, including climate, change.

1. The first open question is both the most trivial and the most difficult to answer, but still is the most important: we need to record global isopod diversity, in terms of species occurrence and exact distribution ranges at a habitat and a regional scale, but also in terms of population abundance. How can we evaluate distribution patterns without a solid knowledge of actual distributions? Research on isopod distribution, of course, should be coupled with advancements in isopod taxonomy and phylogenetic analysis, using also molecular data, so that we can get a good picture of genetic diversity and variation patterns, in addition to a more robust natural taxonomy of isopods.
2. Global and regional latitudinal and altitudinal patterns of isopod diversity are still largely unknown. In addition, research on such gradients might also take into account possible trends in life-history parameters or other relevant features, such as body size etc. In addition, explorations of 'species – area – habitat diversity' relationships at different scales and in different systems ('habitat islands', oceanic versus continental islands etc.) could provide crucial information on processes that shape isopod communities.
3. Isopod invasions and long-distance dispersal remain a terra incognita for isopods, with current knowledge based mainly on anecdotal evidence. We need to solidly



document such processes, possibly also using molecular approaches. There are several closely related questions that we also need to address, such as: What constitutes a propagule for different isopod species? To what extent are isopods able to exhibit niche shifts in order to establish populations in new habitats?

4. We need to employ state-of-the-art tools in order to predict distribution changes, taking advantage of all evidence available, at least for those species that have been studied in detail for many years in the past. Such tools include modern Species Distribution Models that are not solely based on species presence and/or absence data, but are also able to incorporate information on life-history parameters, abundance etc. There is a wide array of relevant models that are continuously improved and applied to a vast variety of taxa. We still lack, though, any application on terrestrial isopods, despite the fact that the information needed to apply some of these models is available, at least for a few, mostly European, regions.
5. We need detailed data on the life history (longevity, fecundity etc.) and other autecological parameters of as many species as possible. These data may also include tolerance to environmental factors, reaction norms of different phenotypes etc. Such data are crucial for evaluating population trends, niche shifts, for population viability analyses etc.
6. We need further research on expected changes of soil processes that might affect isopod distribution. Soil dynamics are under intensive research for quite a long time. The complexity of the systems, though, has not allowed researchers to provide sufficient models of soil processes under supposed climate change variations. The rapidly evolving tools of ‘next generation sequencing’, especially those concerning microbiome analysis, might prove of prime importance towards an increased understanding of a variety of relevant processes, especially taking into account the role of micro-organisms as food for isopod species. The effects of temperature increase and changes in local precipitation regimes on soil processes must be studied in detail, because they might play a crucial role in determining isopod distribution, acting synergistically with other factors, such as physiological, behavioural etc.
7. Functional diversity, including ‘ecomorphological strategies’, is tightly connected with species distribution dynamics. We urgently need a classification of isopod features, including aspects of morphology, physiology, behaviour, and several life-history parameters that will provide a meaningful description of functional diversity. Within this framework, it will be possible to make a finer analysis of isopod body plans, and to identify additional features that might be connected to these plans. The analysis of current functional diversity will offer lots of evidence for possible future trends under climate change.
8. We still lack robust data on isopod intra- and interspecific interactions. Available data are both taxonomically and geographically restricted, so we need to study such interactions for more species, coming also from more biomes. Interspecific interactions, of course, should not be restricted to isopods, but should also include possible interactions with predators, parasites, hosts, competitors and/or other species that might interact with isopods, such as other detritivores like diplopods etc. The

examples of myrmecophilous species with the role of relations with ants for their distribution described in this chapter are highly indicative for the importance of this line of research for evaluating distribution dynamics under environmental change.

9. Research on evolutionary processes pertaining to isopod diversification is still lacking today, even though it is crucial in order to evaluate long-termed effects of current changes for isopod species. The role of environmental stresses as selective forces leading to speciation or extinction should be explored also within the framework of adaptive and non-adaptive radiations. The effects of population bottlenecks and population isolation due to habitat fragmentation should be closely studied in different species from a variety of isopod clades. We should also note here that evolutionary studies should particularly address the issue of regressive evolution, related to the path towards a life in caves or in deep soil, a path very commonly followed by isopods.
10. Last, but not least, we have to take full advantage of the unique features of terrestrial isopods among animal taxa, features that make them exceptionally useful models for the study of a wide range of phenomena, including those related to climate change effects on distribution. Isopods provide a unique opportunity for comparative studies among clades that represent almost all stages of evolution from a life in the sea to a life in almost all terrestrial habitats.

As a conclusion to this paper, we have to stress the need for an up to date, revised web-based isopod database, where all new data on species occurrences and, possibly also other data, will be regularly added in a format that will enable communication with GIS analytical tools etc.

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# Effect of agricultural practices on terrestrial isopods: a review

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## Abstract

Terrestrial isopods (approximately 3700 known species in the world) are encountered in temperate and tropical regions, from the seashore to high altitudes and from floodplain forests to deserts. They are known to contribute to soil biodiversity. Environmental factors and anthropogenic actions, particularly land use changes such as primarily agricultural practices, and urbanization affect soil biodiversity and their functions. Human practices, such as soil tillage, pesticide application, chemical pollution, along with soil acidification adversely affect isopod abundance and diversity. It is thus important to recognise the vital contributions of soil biodiversity in support of environmental quality protection through maintaining soil functions and their significance to sustainable land use. This review will also deal with recent studies attempting to evaluate the impact of returning to an environmentally friendly agriculture by restoring refuge habitats such as grass strips, hedges, and woodlands for terrestrial isopods.

## Keywords

agroecosystems, detritivores, ecosystem services, food sources, pests, tillage, woodlice



## Introduction

Among the most important anthropogenic influences on climate are changes in competing land uses such as agriculture. Global croplands, pastures, and plantations have expanded in recent decades, accompanied by large increases in energy, water consumption, and agrochemical consumption, leading to considerable losses of biodiversity.

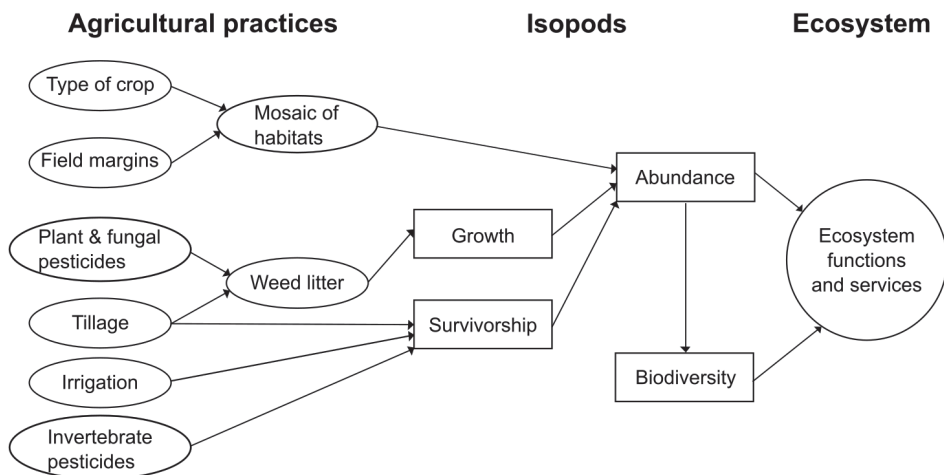
Agriculture is a dominant form of land management and agroecosystems cover ca. 40 % of land surface (Millennium Ecosystem Assessment 2005, Power 2010). Agricultural productivity depends on several ecosystem functions, such as decomposition and nutrient cycling by microbes and soil fauna, pollination by animals, biological control of pests. In agroecosystems, soil organisms actively influence soil fertility (Lavelle et al. 1994). The soil fauna mediates a number of essential ecological processes that are vital to the entire ecosystem, such as the degradation of organic matter, cycling of nutrients, sequestration of carbon, and the development and maintenance of soil structure which influences gas and water transportation. Macrofauna such as terrestrial isopods (woodlice) process dead organic matter and facilitate bacterial and fungal decomposition by mechanically breaking up residues and dispersing microbial propagules (Zimmer 2004; Hattenschwiler et al. 2005; Špaldoňová and Frouz 2014). Additionally, by preferentially feeding on certain fungi, isopods alter microbial community composition, and indirectly the fungal feeding invertebrate community (Crowther et al. 2014). As litter transformers, terrestrial isopods can utilize more than 10 % of the annual litter, increasing fourfold the surface available to micro-organisms (Jambu et al. 1987, Mocquard et al. 1988). Moreover, as ecosystem engineers, they contribute to bioturbation of the soil. Bioturbation allows water concentration and thus the formation of moist microenvironments. Feedback mechanisms linked to bioturbation need to be considered at the geomorphic and ecosystem levels in relation to runoff and erosion processes. In *Hemilepistus reaumuri* (Milne-Edwards, 1840), Shachak et al. (1976) showed that by ingestion and defecation of organic matter and inorganic soil particles, this burrowing species could alter the structure of the decomposition substrate and increase the rate of decomposition in deserts. Bioturbation activity leads to a reduction in salt accumulation and to the preservation of a favourable environment for the engineer species (Yair 1995). Isopods also may move litter deeper into the soil (Hassall et al. 1987). One of the most important contributions of invertebrates to soil structure is their feces. The fine structure of soils, and therefore many of its structural features that contribute to soil fertility, is largely determined directly (topsoil) or indirectly (mineral soil) by macrodetritivore fecal dynamics. Consequently, terrestrial isopods are considered as key system regulators of ecosystem functions such as decomposition and nutrient recycling, and affect physical properties of soil.

Terrestrial isopods play a key role in ecosystems influenced by environmental factors, including climate, and so, by global climatic changes (David and Handa 2010, Berg et al. 2010). Terrestrial isopods were first estimated at more than 3,500 species recorded worldwide (Schmalfuss 2003). Recently Sfenthourakis and Taiti (2015) updated the world list of terrestrial isopods containing 3,710 species belonging to 527 genera and 37 families.

They are thus studied in several countries and ecosystems. In Europe, the most oniscid species-rich areas are found in the circum-Mediterranean region (Sfenthourakis et al. 2007; Hornung 2011), and a latitudinal gradient in species richness has been shown from the Mediterranean to the northern regions in Europe (Vilisics et al. 2007; Souty-Grosset et al. 2008). Few isopods are included in the IUCN Red List (<https://www.iucnredlist.org>). Isopods have recently been represented in studies connected to the biodiversity of some protected areas (Szlávecz 1991, Sallai 1993, Almerão et al. 2006, Vilisics et al. 2008, 2011, Messina et al. 2011, 2014) and they are now considered as reliable bioindicators.

The negative impact of land-use on biodiversity in Europe has been documented since the 1990s (Pimentel et al. 1992; Altieri 1999). Decline of grasslands versus crops is threatening diversity of terrestrial isopods (Moss and Hassall 2006). Direct and indirect effects of agricultural management practices negatively affect the abundance and diversity of isopods and in consequence they are generally very low in cultivated plots (Paoletti and Hassall 1999). Direct effects are associated with increases of mortality rates and lowered fecundity due to tillage operations and insecticide application (Fischer et al. 1997). Indirect effects are related to changes in habitat structure and reduced availability of shelter sites and food sources because of herbicide application or burying of plant residues. These herbicides reduce available food and can change soil pH, an important parameter for isopods (van Straalen and Verhoef 1997). It has been observed that the specific diversity and abundance of terrestrial isopods decrease in intensive agricultural systems, with particularly marked differences between organically and conventionally managed plots.

The conceptual diagram (Figure 1) shows how several common agricultural practices can potentially impact the way in which isopods can influence ecosystems. The type of



**Figure 1.** Conceptual diagram illustrating some of the ways in which agricultural practices can influence various aspects of isopod ecology and how these might then potentially impact on ecosystem functions and ecosystem services.

crop, whether pasture, arable or horticultural, helps to determine the mosaic of habitats in the landscape which in turn affects the abundance and distribution of isopods. These are also influenced by the type of field margin (agro-ecological infrastructure), not just by the width of the verge left uncultivated but also whether the margin of the crop itself is managed for wildlife under an agro-environment scheme. Whether the field boundary is a ditch, hedge or fence also determines the habitats available for isopods to use as refugia which can then form a source for future colonization of the rest of the field.

The amount of dicotyledonous plant leaf litter available as high quality food for isopods and hence influencing their growth rates is determined by the type of crop residue and the species composition and biomass of weeds present. These are affected by herbicide applications and possibly indirectly by fungicides but also very much by the type of tillage practiced. As tillage also impacts on the physical structure of the habitat at the soil surface, determining the abiotic favourableness of the habitat, especially its relative humidity which may also be influenced directly by irrigation, both processes potentially impacting on survivorship of isopods and thus on their relative abundance.

The habitat mosaic and population parameters of growth and survivorship interact to determine abundance of different species in different ways, thus influencing biodiversity of the isopod community which, together with the abundance of all the isopod species combined, is instrumental in affecting arable ecosystem functions and hence the level of ecosystem service that they provide. Because of its worldwide distribution, most studies focus on the common pillbug, *Armadillidium vulgare* (Latreille, 1804).

## **Agricultural land-use and diversity of terrestrial isopods**

### **Preferential habitats of terrestrial isopods**

In order to evaluate the impact of agricultural practices on the diversity of terrestrial isopods the preliminary investigations must first include an inventory of species present and their preferred habitat. Paoletti and Hassall (1999) underlined how terrestrial isopods are very widespread, easily identified and form a dominant component of the soil arthropod macrodecomposer community in many temperate habitats (Hassall and Sutton 1977), reaching densities of up to 3000 m<sup>-2</sup> in calcareous grasslands. Isopods were collected using hand search or pitfall trapping; one of the most commonly used methods of sampling ground-dwelling arthropods (Barber 1931, Duelli et al. 1999, Matalin and Makarov 2011).

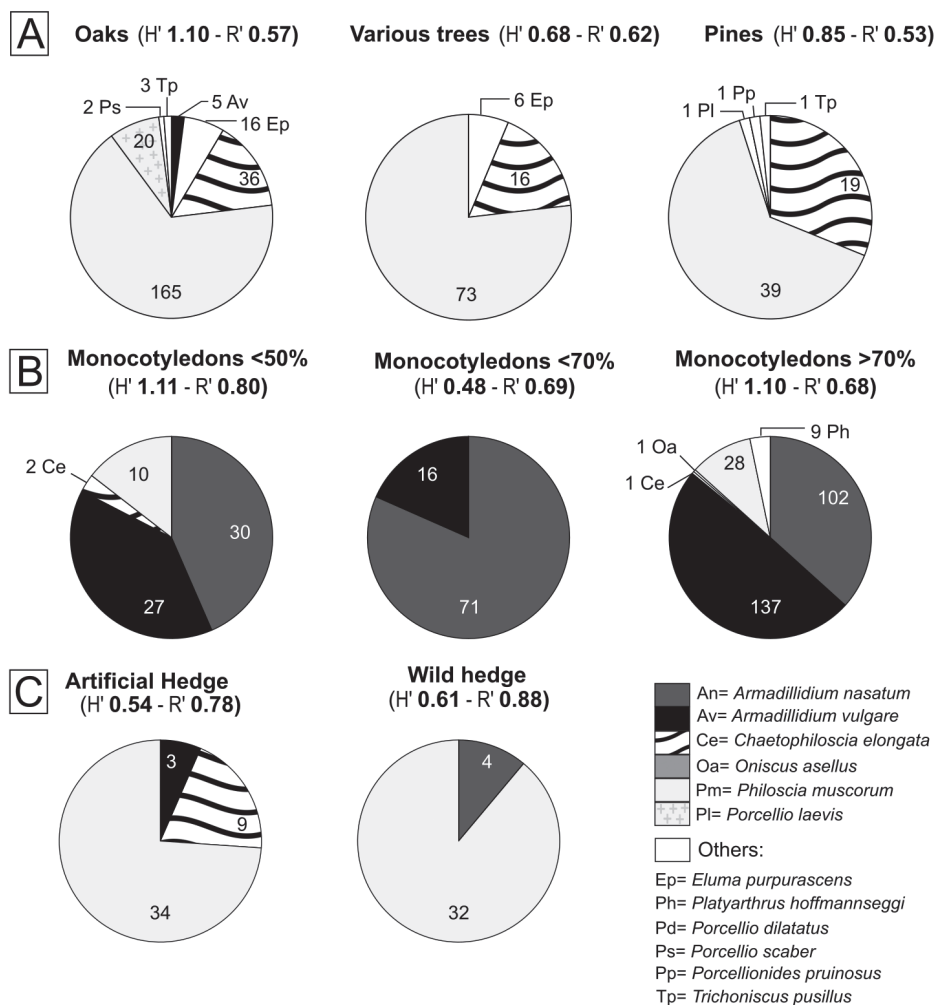
They are used as surrogates for natural habitats and grassland biodiversity. They are important primary consumers and are an important food source for other animals, often because of their richness in calcium that is more readily absorbable than in molluscs. The species exhibit a lack of tolerance to low or high values of pH.

For example, in Western France, Souty-Grosset et al. (2008) investigated diverse types of habitats in several areas from Poitou-Charentes. The survey was carried out in grasslands (cultivated, temporary or permanent), forests, woods and humid zones, all

components of the territory investigated. Humid zones investigated were the margins of ponds and of the Vienne River. Terrestrial isopods were also sampled in connections – either natural or artificial hedges – in order to describe faunal richness in these valuable junctions. Compost heaps and dunghills were also investigated. Sites were randomly prospected with a minimum distance of around 4 km between any two sites. By the end of the survey, 39 different locations were sampled, some of them having several habitats. The study involved 51 habitats. To obtain a correct estimation of isopod diversity, the protocol was simple and reproducible: a hand search method was applied with random sampling for one hour; in order to obtain the same probability of capture, the same three persons were always involved. *Armadillidium vulgare*, found to be a common species in the region by Legrand (1954), except in forests, was for the first time sampled in oak forests (Figure 2), where the pH is slightly acidic. The species is most prevalent in grasslands rich in monocotyledons, although it has been previously described as preferring dicotyledons (Rushton and Hassall 1983a, b, 1987). No forest harboured *A. nasatum* Budde-Lund, 1885, a species characterized by having a lower tolerance to pH and tannins than *A. vulgare*. Grasslands are the typical habitat of the genus *Armadillidium* by offering suitable pH and humidity. In grasslands, both *A. vulgare* and *A. nasatum* can be present whether or not monocotyledons are dominant. Only grasslands with around 50 % of monocotyledons exhibited a prevalence of *A. nasatum*. The investigation of field boundaries revealed a possible exclusion between *A. vulgare* and *A. nasatum*: wild hedgerows provide *A. nasatum* with higher humidity than do planted hedges; these latter, composed of regularly spaced shrubs with higher temperature and light, harbour particularly *A. vulgare*. Moreover, *A. vulgare* was found in all types of compost, especially from corn and withstanding high temperatures. *A. vulgare* is absent from damp habitats whereas *A. nasatum* was found near ponds as expected. This species is originally a littoral species which colonized inland habitats by following river valleys (Vandel 1960, 1962).

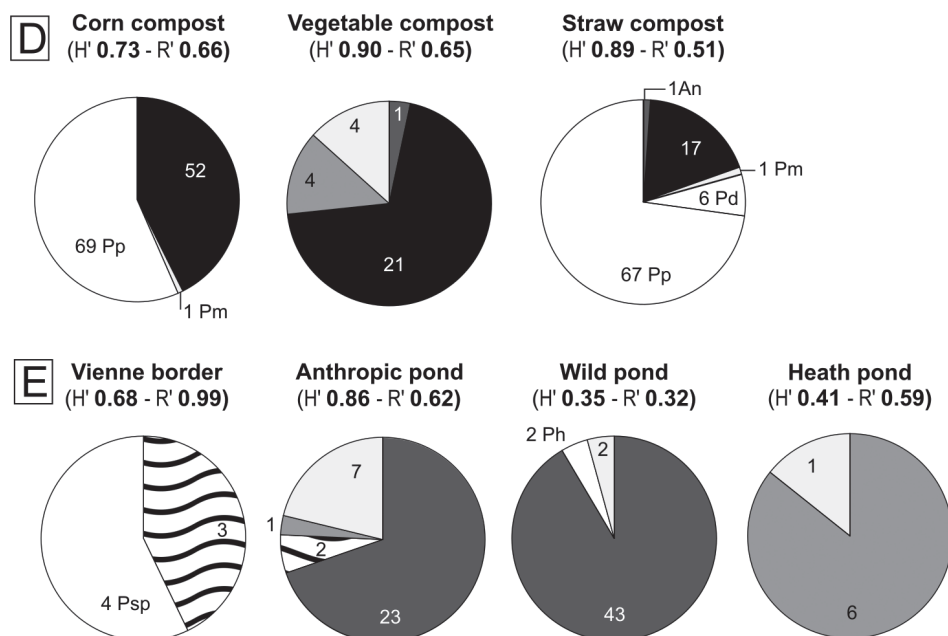
Less abundant species included *Oniscus asellus* Linnaeus, 1758 and *Porcellionides pruinosus* (Brandt, 1833). *Oniscus asellus* is a common species in forests and is a litter feeding macroarthropod, favouring humidity of the soil (Jambu et al. 1987). As the species has no pseudotracheae, it requires a microhabitat with high relative humidity. Moreover, *O. asellus* has a pH preference of around 5.1 (van Straalen and Verhoeff 1997), which explains its occurrence in oak and pine forests. *Oniscus asellus* was also collected near water, where the water availability is high, allowing it to take up water by mouth and anus (Edney and Spencer 1955). Composts were dominated by *P. pruinosus*. This species is capable of adaptation to all types of habitats, except those too cold or too humid (Vandel 1962). However, they were absent in grasslands, field boundaries and pond margins. Grasslands and hedges are not favourable because of the high climatic variation and ponds margins are too humid. Composts provide high temperatures to this polyphagous species, allowing high reproductive activity (Juchault et al. 1985).

By comparison, in the Carei Plain natural reserve of north-western Romania, Ferenti et al. (2012) identified 15 species: *Haplophthalmus mengii* (Zaddach, 1844), *Haplophthalmus danicus* Budde-Lund, 1880, *Hyloniscus riparius* (C. Koch, 1838), *Hyloniscus transsylvanicus* (Verhoeff, 1901), *Platyarthrus hoffmannseggii* (Brandt, 1833),



**Figure 2.** Distribution of isopod species in different types of habitat. **A** Forest **B** Grassland **C** Connections: hedges **D** Compost **E** Humid zones. Shannon ( $H'$ ) and equitability ( $R'$ ) indices are written below each graph (from Souty-Grosset et al. 2008).

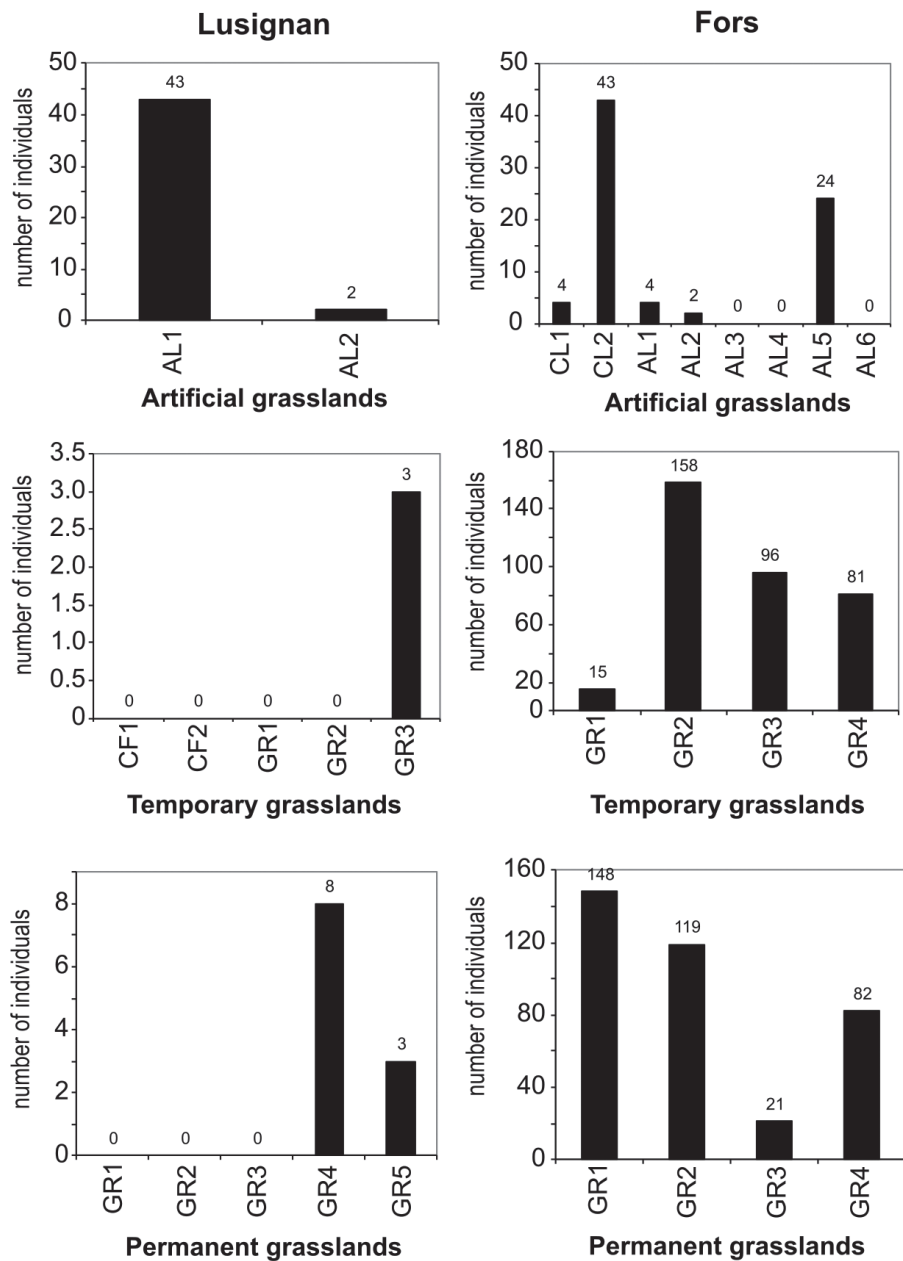
*Cylisticus convexus* (De Geer, 1778), *Porcellionides pruinosus* (Brandt 1833), *Protracheoniscus politus* (C. Koch, 1841), *Trachelipus arcuatus* (Budde-Lund, 1885), *Trachelipus nodulosus* (C. Koch, 1838), *Trachelipus rathkii* (Brandt, 1833), *Porcellium collicola* (Verhoeff, 1907), *Porcellio scaber* (Lamarck, 1818), *Armadillidium vulgare* and *Armadillidium versicolor* Stein 1859. The diversity of the terrestrial isopods in this protected area was high due to the diversity of habitats. The highest species diversity was found in wetlands, with the lowest in plantations and forests. Sylvan species were also present in the open wetlands. Unlike marshes, sand dunes harboured only anthropophilic and invasive species.



**Figure 2.** Continued.

As a result of modern agricultural practices, calcareous grasslands have been declining both in their extent and quality across Europe. As the abundance of terrestrial isopods was described in grasslands Reynolds et al. (2004), Souty-Grosset et al. (2005a) investigated the diversity of isopods in natural and cultivated grasslands of western France, both as grassland detritivores and further considering that their diversity as grassland detritivores could thus be a potential guide to ecosystem activity in natural and cultivated grasslands. Woodlice diversity was studied in different grassland types at two sites: Fors, with mixed farming (crops and livestock), and Lusignan, with intensive farming. Woodlice were collected by hand in plot centres, borders, and field boundaries. Isopod numbers were higher at Fors than at Lusignan. The total numbers of isopods in plots, their borders, and connections (Figure 3) were clearly lower at Lusignan compared to Fors, regardless of the grassland type. Species assemblages were dominated by *Philoscia muscorum* (Scopoli, 1763) at Lusignan whereas this species was less numerous at Fors than *A. vulgare* and *A. nasatum*. These results also differ with grassland type, with high species diversity or number of individuals in temporary and permanent grasslands.

Hedges were important in increasing isopod diversity within plots. The structure of the landscape and its capacity to provide connections between habitats has been found to be important for isopods. The proximity of a suitable habitat for a permanent community of isopods will favour colonization of new habitats. In this study, Souty-Grosset et al. (2005a) showed this influence by the relationship between con-



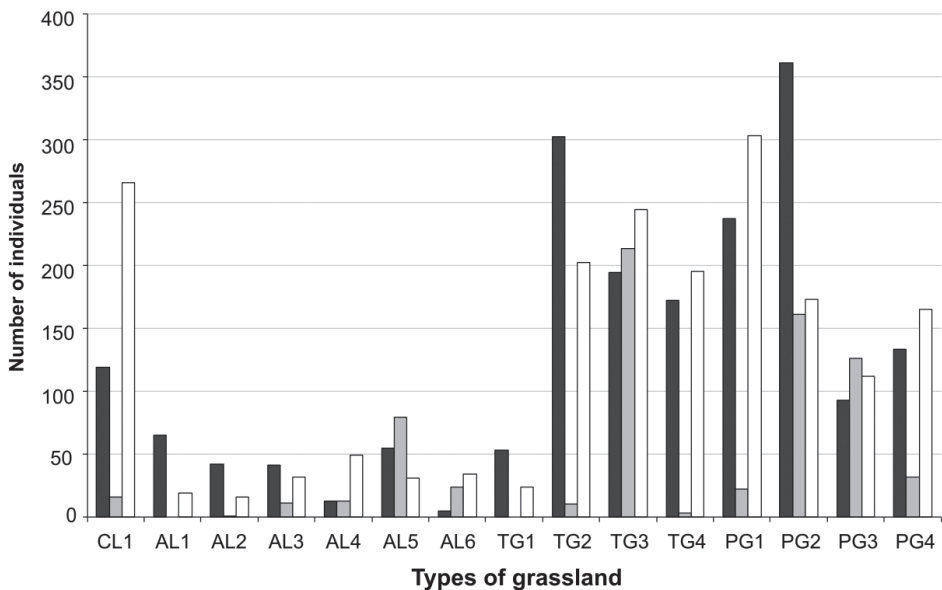
**Figure 3.** Number of isopods collected by hand-searching in three different types of grasslands in western France. The two sites differ in farming intensity: Lusignan has experienced intensive practices over many years, whereas Fors is in a zone of mixed farming, with a more recent history of intensification. Note the different scales in the y axes (from Souty-Grosset et al. 2005a).



nections and plots. Thus, hedges are potentially a source of woodlice for grasslands. Therefore, most species present in a plot occur also in the connections. Isopods are highly affected by variations in habitat structure (Davis 1984); while the presence of some species is linked with the degree of openness of the land with some shrubs still present, other species are most common in closed habitats (David et al. 1999). High density of woodlice indicates high habitat quality, as is the case in permanent grasslands. Consequently, some woodlice species may be characteristic of various Atlantic grasslands, and thus are useful as bioindicators of undisturbed and semi-natural conditions. Souty-Grosset et al. (2005b) studied the diversity of woodlice in three types of grasslands: artificial (established for less than 5 years and sown only with leguminous fodder crops), temporary (less than 5 years old, sown with fodder grasses, pure or mixed with leguminous plants) and permanent (sown 6–10 years earlier), in spring, summer, and autumn.

Relative abundance of isopods was different among habitats and the three sampling periods (Figure 4). Associations were dominated by *A. vulgare*, *A. nasatum*, and *P. muscorum*.

Some species were clearly linked to the degree of openness of the land, agreeing with the conclusions by David et al. (1999). The plot level differences were due to differences in management (cutting or grazing). According to Curry (1994), cutting for silage is a major disturbance for soil arthropods in general. Isopods were most



**Figure 4.** Number of isopods collected at Fors in three seasons: spring (black bars), summer (grey bars) and autumn (white bars). Habitat abbreviations: CL: clover, AL: alfalfa, TG: temporary grasslands less than 5 years old PG: permanent grasslands more than 5 years old (PG) (from Souty-Grosset et al. 2005b).

abundant in five years old grasslands. Climatic conditions affect the isopod abundance, and the species dominance may change according to season. In permanent grasslands, populations of *P. muscorum* are most abundant in autumn, *A. vulgare* in summer and *A. nasatum* in spring (personal observation).

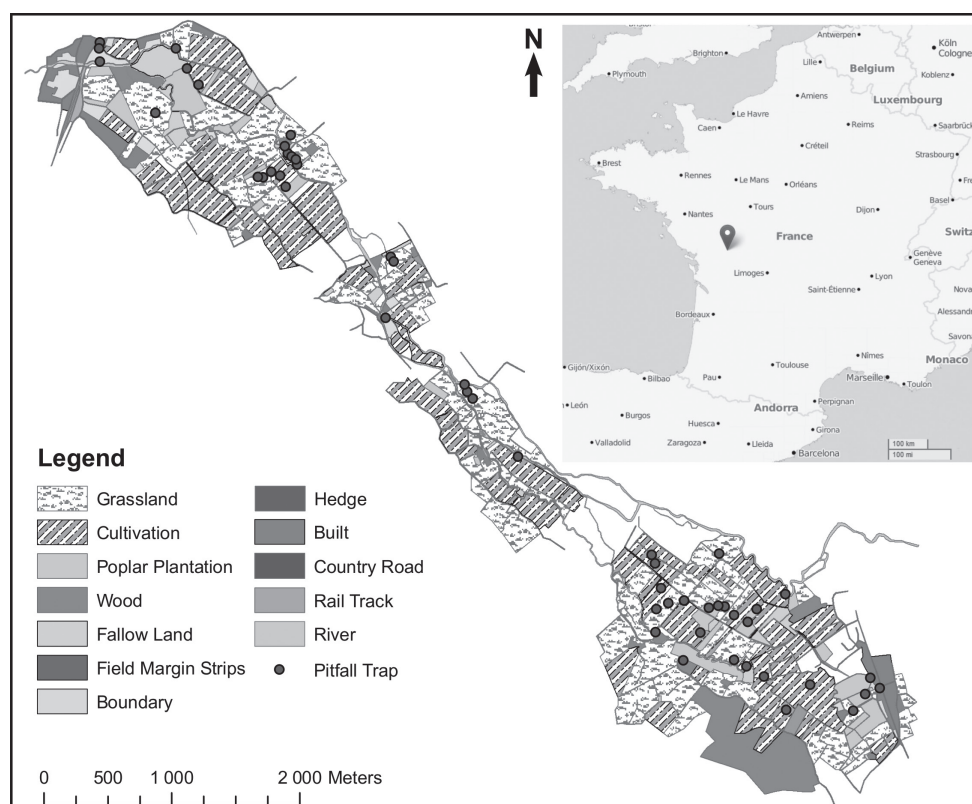
Singer et al. (2012) surveyed isopods in the tallgrass prairie ecosystem in Kansas and found the same species as in Western France. Of the four species known in Kansas thus far, all non-native, *A. vulgare* was the most abundant, accounting for 93 % of all individuals. *Armadillidium nasatum*, *C. convexus*, and *P. pruinosis* were also found and the authors also reported the first record of *Porcellio laevis* Latreille, 1804 in Kansas. There was no relationship between isopod abundance and either fire frequency or grazing treatment. Plum (2005), reviewing information mainly from Western and Central Europe, showed that isopods are rare in flooded grasslands. The most frequent species is *Trachelipus rathkii*; all other species occur only occasionally or locally. The most commonly recorded species only occurred in unflooded reference sites (*A. vulgare*, *P. scaber*) or were totally absent (*O. asellus*).

### Diversity of isopods in cultivated plots from a mosaic landscape

Following the previous study, Souty-Grosset and co-workers (unpublished results) investigated the diversity of terrestrial isopods in a site («Plaine Mothaise»), where changes in agricultural practices led to replacement of more than 25 % of grasslands by crops and poplar plantations within the period 2000–2010 (Figure 5). The approximately 200 ha study area is located between the towns St. Maixent-L'Ecole and La Mothe Saint-Héray (46°22'52"N, 0°06'18"W) in the Poitou Charentes region, central-western France. The localities are connected by the Sèvre Niortaise stream. The area is dominated by croplands (corn), also forest and grassland land uses are represented. This affects habitats, resources and finally the ecosystem services. In order to further integrate restoration management of this area, the impact on terrestrial isopod diversity was first investigated (Table 1).

A total of 5 726 isopods were captured representing 15 species and seven families.

The effect of agricultural management on soil arthropod diversity and functioning is often context dependent, e.g. diversity of functionally important taxa such as decomposers may be enhanced by increasing habitat heterogeneity (Diekötter et al. 2010). The role of landscape connectivity in facilitating dispersal between habitat patches has emerged as a key area of research in conservation ecology (Perovic et al. 2010). Arthropod diversity of adjacent agricultural systems can lead to movements of species between them. Thus, even though higher invertebrates are small organisms with specific habitat requirements, at a higher integrative level, diversity evaluation can be based on landscape parameters (Paoletti and Hassall 1999). Several indicators of landscape composition and structure in Plaine Mothaise were calculated. Land cover features of the study site were determined using aerial photographs (Google Earth) and field inspections. Linear landscape characteristics such as riparian, hedge, continuous



**Figure 5.** Land cover map with sampling sites in Plaine Mothaise, central-western France. Land cover features of the study site were determined using aerial photographs (Google Earth™) and field inspections. Linear characteristics from the landscape were distinguished such as riparian, hedge, continuous and intermittent vegetation. The final categories obtained are Cultivation (crops), Grassland, Poplars, Types of connection, roads and urban. Landscapes were mapped using Arcmap 9.3™ (ESRI, 2004) as a main geographical information system. Black dots indicate sites of pitfall trap sampling.

**Table 1.** Isopod species collected in Plaine Mothaise in different habitat types. Abbreviations: presence of species in grasslands (G), poplars (P), semi-natural habitats (SN) and Crops (C).

Family/Species	Habitat type
<b>Oniscidae</b>	
<i>Oniscus asellus</i> (Linné, 1758)	GPSN
<b>Philosciidae</b>	
<i>Chaetophiloscia elongata</i> (Dollfus, 1884)	GPSNC
<i>Philoscia muscorum</i> (Scopoli, 1763)	GPSNC
<b>Platyarthridae</b>	
<i>Platyarthrus hoffmannseggii</i> (Brandt, 1833)	P
<b>Armadillidiidae</b>	
<i>Armadillidium nasatum</i> (Budde-Lund, 1885)	GPSNC
<i>Armadillidium vulgare</i> (Latreille, 1804)	GPSNC

Family/Species	Habitat type
<b>Porcellionidae</b>	
<i>Porcellio gallicus</i> (Dollfus, 1904)	GPSN
<i>Porcellio monticola</i> (Lereboullet, 1853)	GPSN
<i>Porcellio scaber</i> (Latreille, 1804)	GPSN
<i>Porcellionides cingendus</i> (Kinahan, 1857)	SN
<b>Scleropactidae</b>	
<i>Sphaerobathytropa ribauti</i> (Verhoeff, 1908)	PSN
<b>Trichoniscidae</b>	
<i>Haplophthalmus mengei</i> (Zaddach, 1844)	P
<i>Oritoniscus flavus</i> (Budde-Lund, 1906)	GPSN
<i>Trichoniscoides</i> sp. (Sars, 1899)	GPSN
<i>Trichoniscoides</i> sp. (Sars, 1899)	P

and intermittent vegetation were also distinguished. The following final land use categories were then established: cropland, grassland, forest, connection, roads and urban. Landscapes were mapped using Arcmap 9.3 (ESRI 2004) as a main geographical information system and the database was created.

Table 2 shows the correlation between the degree of response (as measured by the diversity of isopods) and the landscape descriptors. Results show that the riparian vegetation and other connecting elements in the landscape provide higher diversity of terrestrial isopods. Although human modifications of landscape have a negative effect on the diversity, those fragmented landscapes still offering either forest or connections, maintained shelters for isopods.

**Table 2.** Agricultural practices, correlation analyses for landscape metrics and Diversity index of Isopoda in Plaine Mothaise. Field metrics: Total size of site (ha); Length of each type of boundary around focal fields (m). Landscape metrics: Relative area of each land cover type (%) in the landscape; Shannon’s Diversity Index ( $H = -\sum p_i \ln(p_i)$ ) where  $p_i$  = Land cover and  $I$  = Total Land cover categories. Shannon’s diversity equals zero when there is only one land cover and increases with both the number of land covers and the evenness of land covers; Shannon’s Evenness Index ( $HE = H/H_{max} = (-\sum p_i \ln(p_i))/\ln(M)$ ) of the landscape; N: Numbers of land cover types. Evenness equals one when all land-uses cover the same surface and tends to zero when a land-use dominates the landscape. Landscape structure metrics: number of patches: total number of patches in the landscape within each sub site; Mean area-perimeter ratio: sum of the area/perimeter ratio of all patches divided by number of patches in the landscape per sub site; mean patch edge: average amount of edge per patch in the landscape around pairs of fields (m).

Landscape metric	Shannon Index (Isopoda)		
	Pearson Correlation	Sig. (2-tailed)	N
Num habitats	0.424	0.477	5
Num patches	0.675	0.211	5
Total area	-0.795	0.108	5
Total perimeter	0.727	0.164	5
Average area	-0.744	0.149	5
Average perimeter	0.290	0.636	5
Shannon landscape	-0.332	0.585	5
Evenness	-0.630	0.254	5

Landscape metric	Shannon Index (Isopoda)		
	Pearson Correlation	Sig. (2-tailed)	N
% Cropland	0.386	0.521	5
% Refuge	-0.229	0.711	5
% Forest	-0.705	0.184	5
% Road	0.277	0.652	5
% Connection	0.600	0.285	5
% Grassland	-0.083	0.895	5
% Urban	-0.392	0.514	5

## Occurrence of Isopoda in cultivated habitats in other parts of the world

In Greece, generally, organic vineyards and maize were the poorest in Isopoda species, while olive groves, both conventional and organic, were the richest (Hadjicharalampous et al. 2002). *Trachelipus squamuliger* (Verhoeff 1907b), the only representative of the Trachelipodidae, was the dominant species in olive groves. *Armadillidium vulgare*, one of the two Armadillidiidae species found in the studied fields, was the second most abundant species, especially in organic olive groves (Hadjicharalampous et al. 2002).

Studies in other continents show that a drought-tolerant species, such as *A. vulgare*, could have colonised croplands from field margins and boundaries: In Argentina, since the late 1990s *A. vulgare* has been an abundant and frequent species in agricultural land colonizing broad areas (Trumper and Linares 1999, Saluso 2004, Faberi et al. 2011, Villarino et al. 2012, Faberi et al. 2014). *Armadillidium vulgare* is also found in agricultural lands in Illinois and Kansas, USA (Byers et al. 1983; Johnson et al. 2012) and in the Gauteng Province of South Africa (Tribbe and Lube 2010). *Porcellio scaber* and *Balloniscus sellowii* Brandt, 1833 are found although less frequently, the latter recorded only in Entre Rios Province, Argentina (Saluso 2004). Other very common species in agricultural lands are *Australiodillo bifrons* (Budde-Lund, 1885) in New South Wales, Australia (Paoletti et al. 2008), and *Trachelipus rathkii* in Pennsylvania, USA (Byers and Bierlein 1984), respectively.

## Tillage systems, agro-ecological infrastructure, and isopod populations

In general, untilled agricultural soils are similar to grassland soils since the absence of tillage allows the accumulation of litter on the soil surface, reducing erosion, modifying the soil surface and topsoil environmental characteristics by reducing soil aeration, stronger mechanical resistance to root penetration, smaller soil temperature amplitudes and thus creating a more favourable microhabitat for soil organisms (Hendrix et al. 1990). In Argentina, as in other parts of the world, during the 1970's, an intensification of agriculture process took place as agriculture has become more profitable than cattle farming (Studdert 2003, Manuel-Navarrete et al. 2005). Increasing cultivation involved aggressive tillage using moldboard and/or disk ploughs (i.e., conventional tillage - CT). Intense use of CT practices accelerates soil erosion and other degradation processes through its impact on the physical, chemical, and biological factors related to

soil quality. In response to these problems, farmers have adopted a conservation tillage system such as no-tillage (NT) as a soil-protecting measure (Studdert and Echeverría 2000, García-Préchac et al. 2004). Under NT, litter and soil organic matter tend to concentrate in the upper 5 cm layer of soil (Dominguez et al. 2005). Since the soil is less disturbed NT practice improves soil aggregation (Carter 1992; Beare et al. 1994) decreases litter decomposition rate (Sánchez et al. 1996, Creus et al. 1998) and reduces organic matter loss (García-Préchac et al. 2004), is. Additionally, in NT systems soil erosion by water and wind is reduced (Fabrizzi et al. 2005, Hobbs et al. 2008).

The litter layer under NT systems enhances habitat conditions favourable for isopods. These include reducing soil temperature and moisture extremes and provisioning of food and shelter (Stinner and House 1990, Wolters and Ekschmitt 1997). On the other hand, soil disturbance in CT has direct and indirect effects on isopod populations. Direct effects are related to injury or mortality of individuals and indirect effects are related to habitat destruction (Wallwork 1976, House and Parmelee 1985, Hendrix et al. 1990, Stinner and House 1990, Holland and Reynolds 2003, Estrade et al. 2010, Errouissi et al. 2011).

According to Paoletti and Hassall (1999), when NT is adopted, isopod biomass and diversity increases compared to CT. In Argentina *A. vulgare* individuals are found both in CT and NT fields. However, this species has taken on considerable importance in agricultural land under NT systems (Trumper 2001, Faberi et al. 2011). Its abundance is higher in NT with respect to CT in winter-spring and in summer-autumn seasons (Manetti et al. 2013). In addition, this species represents a high proportion of the total abundance of arthropods in NT systems, reaching up to 45.5%, while under CT systems it represents approximately 1–9 % of the total abundance of macro-arthropod decomposers (Manetti et al. 2013). The other isopod species, *P. scaber* and *B. sellowii* colonizing agricultural land, were always found under NT systems.

In France, agriculture has changed much during the past 50 years, with the transition from small farms to large farms and the overuse of pesticides causing decreases in biodiversity. In Western France, Deschamps et al. (2011) investigated the diversity of isopods with the return of more environmentally friendly agriculture. With this aim, a group of farmers compiled practices about Major Economic Crops. From 2008 to 2010, the relevance of these modifications of practices was tested. A study was initiated in collaboration with 18 farmers located in Poitou-Charentes and Indre aiming to evaluate the impact of changing farming practices on macrofaunal diversity. Terrestrial isopods have been sampled as bioindicators of the quality of agroecosystems. The abundance of terrestrial isopods is the highest in wheat and agro-ecological infrastructures (AEI). *Armadillidium vulgare* is dominant in wheat, grass strips, and grasslands. *Philoscia muscorum* is dominant in hedges and secondary in woods. *Armadillidium nasatum* is the most abundant in grassland and generally less often present than *A. vulgare*. The diversity of isopods is also higher in wheat and agro-ecological infrastructures. In AEI, *A. vulgare* made up 99 % of the total sampling in wheat.

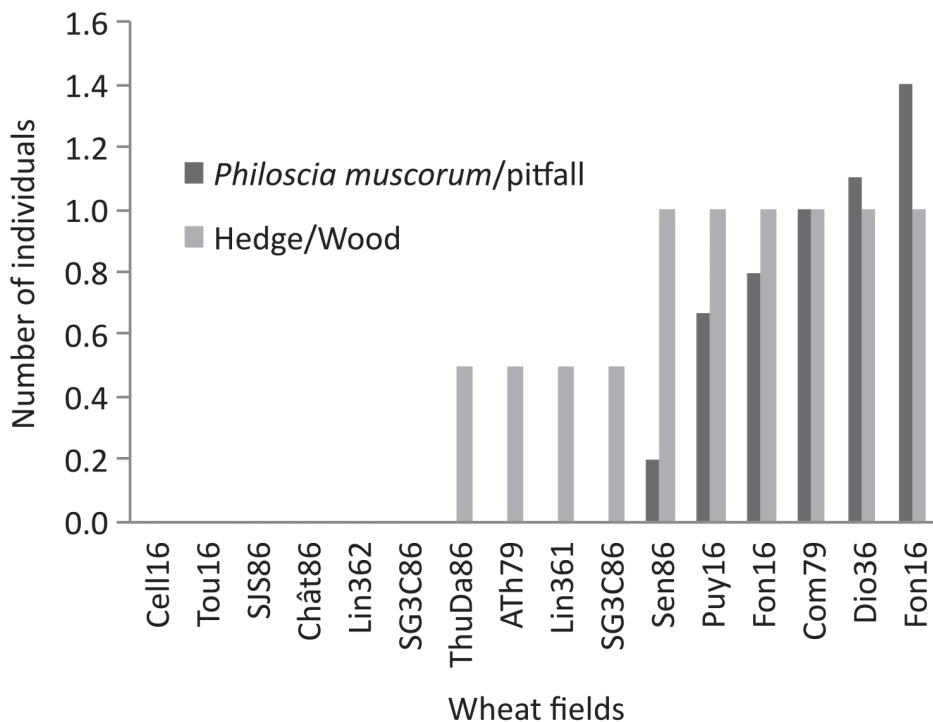
Shannon indices and species evenness (Table 3) showed the highest values in grasslands and hedges, secondly in wheat.

In the case of wheat plots, *Philoscia muscorum* is encountered in the cultivated plot when a hedge and /or a border is present on the side of the plot (Figure 6).

The size of the plots also affects the number of *Philoscia muscorum* ( $R^2 = 0.2954$ , 29 % of the presence of *P. muscorum* in cultivated plots is explained by the size of the plots).

**Table 3.** Diversity of isopods in plots, hedges and woodland. Shannon indices (H) and species evenness (EN) according to the different types of cultivations and agro-ecological infrastructures as hedges and wood.

Iso-pods	Wheat			Maize/sunflower			Grassland			Hedges			Wood		
	H'	Hmax	EN	H'	Hmax	EN	H'	Hmax	EN	H'	Hmax	EN	H'	Hmax	EN
Mean	0.192	0.318	0.198	0.056	0.069	0.0811	0.316	0.448	0.371	0.403	0.645	0.432	0.156	0.311	0.181
Min	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Max	0.926	1.099	1	0.562	0.693	0.811	0.639	1.099	0.902	1.142	1.386	1	0.652	1.099	0.593



**Figure 6.** Sampling *Philoscia muscorum* in wheat (pitfall traps). Importance of hedges and woods for inducing the presence of the species in the studied plots. Key: Dark bar: *P. muscorum* in the plot. Light gray bar: *P. muscorum* in the borders of the plot (hedges/wood). Codes were expressed for each plot: the first three letters corresponded to the name of the location, the two following numbers to the French department (16: Charente; 86: Vienne; 79: Deux Sèvres; 36 (1 and 2): Indre).



The presence of *P. muscorum* in wheat is correlated both with the presence of agro-ecological infrastructures bordering the plot (the higher the numbers in the hedge, the higher the numbers in the plot) and with the size of the plot (the smaller the plot, more species in the center of the plot are from a nearby field margin).

This study shows the importance of refuge habitats like grass strips, hedges and woods for terrestrial isopod populations. Results on *A. vulgare* in wheat were analysed according to tillage, size of the plot, and presence of pebbles (Table 4). PCA analyses showed that the more the soil is tilled, the fewer *A. vulgare* are obtained in samples; also the size and the number of pebbles are not related with the abundance of *A. vulgare* (Souty-Grosset, unpublished data).

Isopod abundance and diversity are related to the type of cultivation, the practices (Tillage and use of phytosanitary products (herbicides, nematocides, and fungicides) expressed by TFI i.e., Treatment Frequency Index, calculated also without Herbicides TFIH-), the size of the plot, the presence of agro-ecological infrastructure (AEI) and its quality AEI+) (Table 5).

**Table 4.** Number of isopods in wheat plots according to tillage, presence of pebbles and the size. Key: *A.v.*: *Armadillidium vulgare*/pitfall; Till: tillage indices (depending upon the number of rotation and depth); Peb: 1: presence; 0: absence of pebbles; Size: plot size (ha). Same abbreviations used for plots than in figure 6.

Field	A.v	Till	Peb	Size (Ha)
Sen86	0	3	0	6.0
Cell16	0	3	0	4.2
Puy16	0	3	0	7.0
Chât86	0	4	0	8.7
Lin36a	0	3	0	8.0
Lin36b	0	2	0	8.1
Sg3c86a	0	3	0	7.5
Sg3c86b	0	3	0	8.0
Thuda86	0.2	1	0	5.9
Ath79	0.2	3	1	6.2
Com79	0.2	3	0	6.7
Sjs86	1.6	2	1	7.8
Fon16	2.2	0	0	3.9
Dio36	5.6	2	1	6.0
Fon16	162	0	0	2.2
Tou16	223.4	0	1	7.5

**Table 5.** Impact of agricultural practices and landscape on the abundance and diversity of terrestrial isopods. (TFI: Treatment Frequency Index; TFIH: Index calculated without herbicides; AEI: agro-ecological infrastructures ; AEI+: agro-ecological infrastructures of good quality): + low significant impact; ++ significant impact; +++ high significant impact).

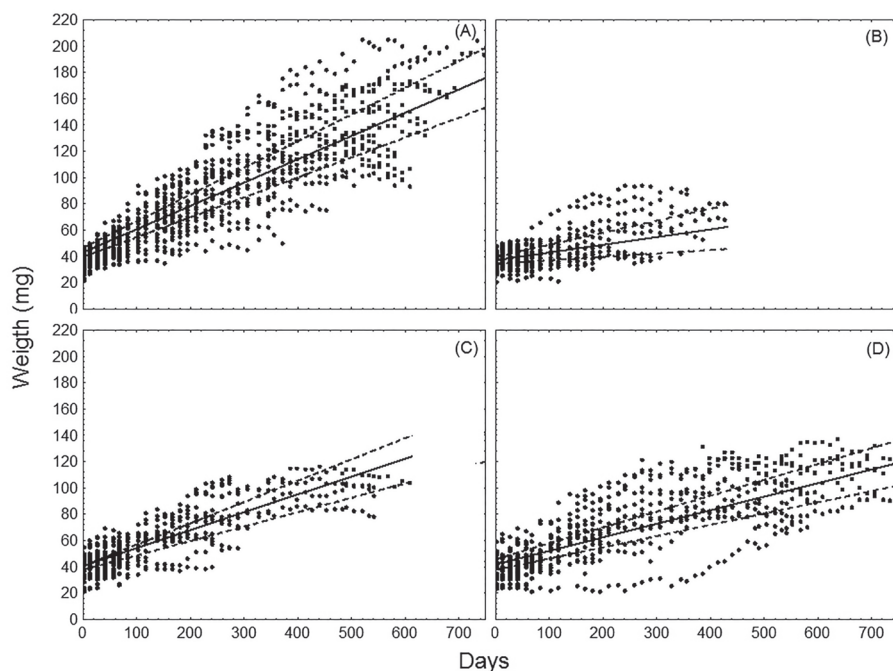
Isopods	Agricultural practices			Landscape		
	Tillage	TFI	TFIH-	Plot size	AEI	AEI+
Abundance	+++	++	+	+++	+	++
Diversity	++	+	++	+++	++	++

## Terrestrial isopods and food sources in agricultural systems

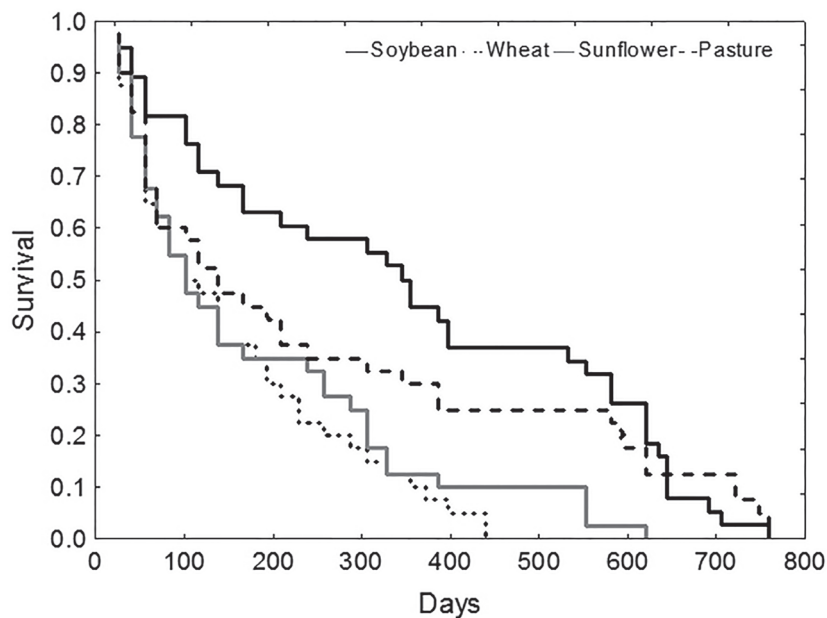
While the effects of isopods on decomposition processes and nutrient cycling are rarely considered in agroecosystems, they are beneficial because they provide ecosystem services, enhancing nutrient cycling by comminuting organic debris and transporting it to moister microsites in the soil (Zimmer 2004). In agroecosystems, these functions are very important to allow the continuation of crop production. In agricultural systems, isopod populations must feed on dead crop residue and weeds (Johnson et al. 2012). Different plant species are cultivated in field crops and these are sown and harvested in different seasons through the year. Then their residues dramatically alter the resource input into the agroecosystem. Consequently, isopods are exposed to food sources of different quality and quantity that is also changing temporally.

As an example of agricultural systems in Argentina, crop rotation principally includes wheat, maize, sunflower and soybean crops. When these crops are harvested, different amounts of residues are left in the field, with highest amounts of residues from wheat and maize (7,500 and 6,000 kg ha<sup>-1</sup> of dry matter), medium amounts from soybean (3,000 kg ha<sup>-1</sup> of dry matter) and the lowest amount from sunflower (2,000 kg ha<sup>-1</sup> of dry matter) (Dominguez et al. 2005). Correspondingly, the wheat litter layer provides 100 % of soil cover, maize provides >90 % and both soybean and sunflower provide 65–80 % of soil cover. At the same time, those crops have different chemical properties, such as e.g. the C:N ratio, therefore different degradation rates. Decomposition is slower in residues of wheat and maize with a high C:N ratio than in residues of soybean and sunflower with low C:N ratio (Sánchez et al. 1996). The monocotyledonous maize and wheat crop residues have high C:N ratios (63 and 60, respectively) while the dicotyledonous soybean and sunflower crops have lower C:N ratios (37 and 45, respectively) (Dominguez et al. 2005). The amounts of residues and soil cover thus decrease faster throughout the decomposition period with residues of soybean and sunflower than of wheat and maize. According to Johnson et al. (2012) the reduction of maize residue levels had minimal impact on *A. vulgare* numbers. The density of *A. vulgare* was similar between fields with a litter layer of wheat and soybean. However, when the amount of soybean residues decreases to 1600 kg ha<sup>-1</sup> of dry matter, the density of *A. vulgare* was severely reduced (Faber 2010). In NT systems, the litter layer is a very important component to ensure isopod development, but if this is in low quantity it can adversely affect the stability of isopod populations in the agroecosystem.

Additionally, the C:N ratio of residues is related to their quality as a food source. Food quality is known to influence the biology of isopods. In general, growth rate and survival are higher when they feed on dicotyledonous leaves than on monocotyledonous leaves (Rushton and Hassall 1983a, 1987, Hassall and Dangerfield 1990, Faber et al. 2011). The same trend was observed with diets with higher N content as in diets with lower N content (Lardies et al. 2004). Both adult and juvenile *A. vulgare* responded to differences in food quality: in a laboratory experiment; growth rate and survival was higher on soybean residues than on wheat residues (Faber et al. 2011) (Figures 7, 8). In agroecosystems with a litter layer of soybean it is expected that *A. vulgare* populations have a better habitat from the nutritional point of view.



**Figure 7.** Growth of adult *Armadillidium vulgare* fed with different types of leaf litter. **A** soybean **B** wheat **C** sunflower **D** pasture. Solid line: Linear growth model, dashed lines: 95% confidence interval of the model (from Faberi et al. 2011).



**Figure 8.** Mortality (Kaplan-Meier method) of adult *Armadillidium vulgare* feeding on different types of leaf litter: soybean, wheat, sunflower, and pasture during development (from Faberi et al. 2011).

## Live plant materials as food source: herbivore isopods

Isopods are omnivorous and they have a tendency to shift their food source (Warburg 1993). The capacity of isopods to switch between feeding on dead and green living tissues, i.e., herbivorous feeding, in the field has been demonstrated for several decades. Different isopod species have been observed feeding on green living tissues of several plants even if they have another food source as a choice (Paris and Sikora 1965, Byers et al. 1983, Szlávecz and Maiorana 1990, Hopkin 1991, Benetti et al. 2002; Morisawa et al. 2002; Paoletti et al. 2008; Farmer and Dubugnon 2009; Tierranegra-Garcia et al. 2010, Faberi et al. 2011, Miller 2011, Johnson et al. 2012, Faberi et al. 2014, 2017). In addition, more recently isopods have been reported to exhibit granivory, i.e., feeding on seeds of weeds and crop plants, of some typical plants in agroecosystems (Saska 2008; Koprdoová et al. 2012, Salvio et al. 2012).

The preference for or selection of green tissues over decayed leaf litter can be related to the higher N content of green tissues (Szlávecz and Maiorana 1990), or the level or lack of chemical anti-herbivore defense compounds on living plants, e.g. the jasmonate signal pathway (Farmer and Dubugnon 2009) and phenolics (Wood et al. 2012). For example, parallel laboratory experiments identified *P. scaber* and *A. vulgare* as being capable of predation on intact plants. Their feeding was strongly facilitated in jasmonate-deficient *Arabidopsis* and rice plants and revealed potentially detritivore-sensitive, jasmonate-protected Achilles' heels in these architecturally different plants (petioles and inflorescence stems in *Arabidopsis*, and lower stem and mesocotyl in rice). The work addresses the question of what stops both species from attacking living plants and provides evidence that it is, in part, the jasmonate signal pathway. Additionally, when isopod populations increase in agroecosystems intra-specific competition among isopods is induced and in consequence may result in consumption of live plant material as is observed in *A. vulgare* (Paris and Sikora 1965, Trumper 2001, Saska 2008, Johnson et al. 2012, Faberi et al. 2014) and in *Australiodillo bifrons* (Paoletti et al. 2008).

Isopod population outbreaks and their diet switching between dead and live plant material have two possible consequences in agroecosystems. On the one hand, Saska (2008) and Koprdoová et al. (2012) suggested that the predation of isopods on weed seeds and seedlings may contribute to biological control of weeds. On the other hand, at really high abundances, isopods themselves can become crop pests.

## Isopods as crop pests

There are reports on terrestrial isopods as a crop pest over several years (Paris 1963, Byers et al. 1983). Commonly synanthropic species have been reported damaging young buds of fruits, vegetables, and flowers. Hopkin (1991) noted that isopods cause significant economic horticultural damage to greenhouse crops. Losses up to 80% have been reported in vegetable crops such as lettuce, tomato, beans, and pepper (Benetti et

al. 2002, Morisawa et al. 2002, Whitworth et al. 2008, Tierranegra-Garcia et al. 2011, Miller 2011).

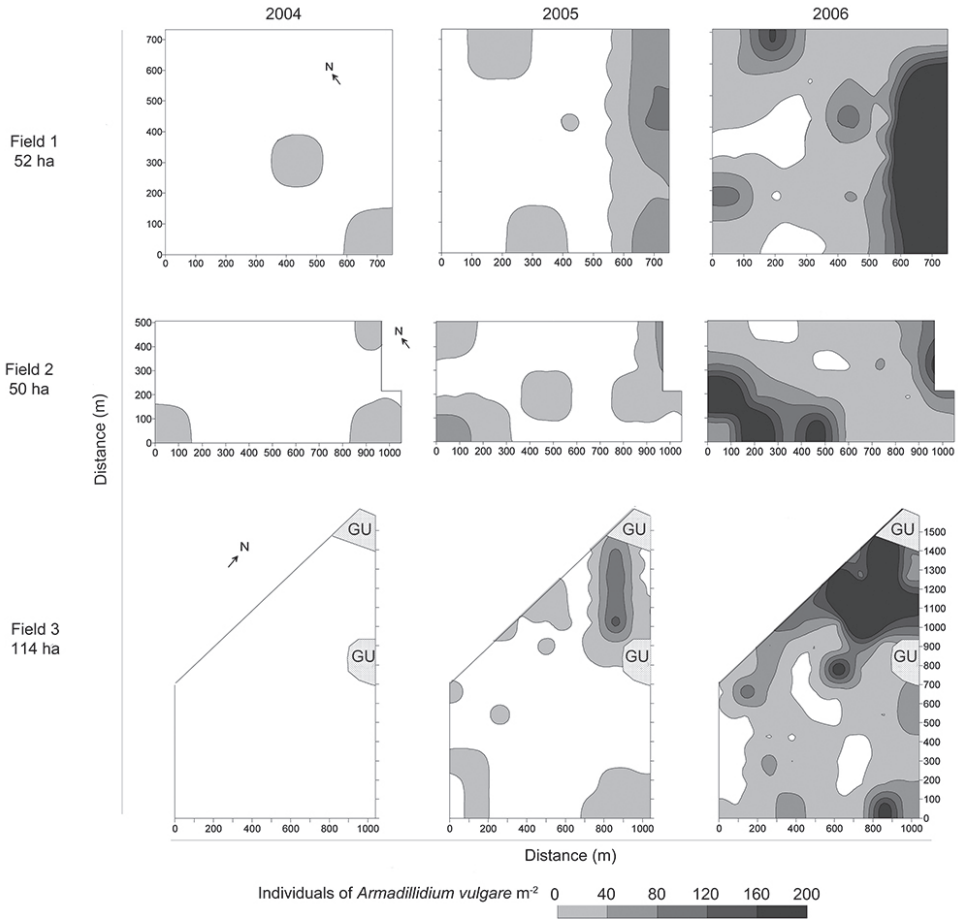
Until the end of the 20<sup>th</sup> century the general belief was that terrestrial isopods play a beneficial role in agroecosystems, and that their impact as possible pests is limited (Paoletti and Hassall 1999). While there were few documented cases of isopods as pests in field crops, they were seen consuming seedlings only in particular situations in which no alternative food was available (Byers et al. 1983, Wolters and Ekschmitt 1997).

At the beginning of the 21<sup>st</sup> century, as a consequence of the adoption and increased utilization of conservation tillage the number of reports in isopods as pests increased. The first cases were reported in Argentina, where *A. vulgare* was found damaging seeds and seedlings of soybean, and they were named “emerging pests” (Trumper 2001). Then, their populations became pests of sunflower and soybean crops (Mastrorardi 2006, Faberi et al. 2014, 2017). This species can quickly colonize field plots within a few years (Figure 9) and reach pest status (Larsen et al. 2007). In South Africa, *A. vulgare* populations are responsible for the loss of canola seedlings (Tribe and Lubbe 2010). A similar situation has been observed in canola crops in Argentina (Villarino et al. 2012) and in soybean fields in the USA (Johnson et al. 2012). *Australiodillo bifrons* populations are a pest of cereal crops in Australia (Paoletti et al. 2008). Today, after more than 10 years’ history of NT, isopods have reached high densities and they are recognized in these places as a key pest of several crops under NT system. Recently in central Kansas observations in soybean under no-tillage management revealed that *A. vulgare* was feeding on succulent stem tissues beneath the cotyledons of seedlings, causing significant stand reductions (Alfaress 2012).

Isopod damage to crops is greatest at the time of sowing and immediately after germination, when plants are most susceptible. Monocotyledonous species such as cereals can sustain a substantial amount of grazing from the ends of the leaves without it significantly reducing yield, because grasses and cereals have basal meristems.

The animals feed both on seeds and seedlings, principally at the hypocotyl level, dramatically reducing plant density. These consumptions are correlated with the density of isopods (Faber et al. 2014) (Table 6). Sometimes crops need to be re-sown due to the extent of feeding damage. In cereal plants isopods crawl up the plant and feed mainly on the tips of the leaves.

In response to this problem, some management practices, such as residue management, planting date and rate, seed treatment, and chemical control have been tested with different efficacy (Tribe and Lubbe 2010, Johnson et al. 2012, Salvio et al. 2012, Villarino et al. 2012). Chemical control alone or with other management practices, is the most effective way to manage isopod populations. An active ingredient (i.e., Carbaryl) is formulated as bait pellets and acts as a neurological poison. The final objective is maintaining the pest population below the level of economic loss, which allows maintaining the plant densities of crops (Faber et al. 2014, 2017).



**Figure 9.** Density of *Armadillidium vulgare* during 3 years in the same field plots. Samples were collected in October of each year before the sowing of summer crops (from Larsen et al. 2007). Abbreviation: GU: ground under.

**Table 6.** *Armadillidium vulgare* damage to plants. Pearson correlation coefficient between variables: severe injury in the hypocotyl (SIH), number of plant  $m^{-2}$  (NP), yield (Y) and *Armadillidium vulgare* density (AvD). \* ( $p < 0.05$ ), \*\* ( $p < 0.01$ ).

	Agricultural cycle (years)								
	1			2			3		
	Severe injury in the hypocotyl	Number of plant $m^{-2}$	Yield	Severe injury in the hypocotyl	Number of plant $m^{-2}$	Yield	Severe injury in the hypocotyl	Number of plant $m^{-2}$	Yield
NP	-0.77 **			-0.94 **			-0.94 **		
Yield	-0.61 **	0.68 **		-0.38 *	0.34		-0.88 **	0.81 **	
Isopod density	0.54 **	-0.57 **	-0.53 **	0.50 **	-0.57 **	-0.25	0.67 **	-0.60 **	-0.62 **

## Terrestrial isopod diversity related to irrigation

To evaluate the effects of irrigation on terrestrial isopod assemblages Fraj et al. (2010) studied species richness, diversity and relative abundance under three types of irrigation (drip, surface mode and sprinkler) in nine types of cultivation: market gardening (artichoke, tomato and melon); vegetable crop (alfalfa, sorghum and maize) and fruit-trees (apple, pear and olive) in the Majerda low plain in north-east Tunisia. Seven species of terrestrial isopods were identified, with highest richness in sprinkler irrigation. The mean relative abundance of *Porcellio laevis* was high when the surface mode of irrigation was used. *Porcellio variabilis* Lucas, 1849 and *Porcellionides sexfasciatus* (Budde-Lund, 1885) showed the highest relative abundance under sprinkler and drip irrigation, respectively. Both Shannon's diversity and equitability indices were higher with the sprinkler mode. Species richness was higher in the alfalfa and maize cultivation, while mean isopod diversity was higher in the sorghum cultivation. The mode of irrigation has an effect on the abundance and distribution of woodlice at different types of cultivation. The sprinkler irrigation appears to be a good system for isopod diversity conservation. The difference in species richness related to the mode of irrigation could be explained by the fact that, in drip and surface systems, soil moisture exceeded the optimum survival of terrestrial isopods (80% of relative humidity). Thus, sprinkler irrigation may be effectively considered the best for maintaining a good isopod diversity.

## Ecosystem services, conservation issues in agroecosystems

Isopods provide important ecosystem services, such as the decomposition of leaf litter. Usually isopods are rarely considered in agricultural studies and most of the reports are from habitats adjacent to these lands (Wolters and Ekschmitt 1996). During the last hundred years, a drastic decline of natural grassland area all across Europe has been reported (van Dijk 1991). The main reason is that grassland farming is more efficient in cultivated stands than in natural permanent grasslands and urbanization is also having an effect.

Soil biodiversity plays an essential role in the regulation of soil processes that underlie important ecosystem services (Bardgett et al. 2005, Bardgett and Wardle 2010). Traditionally, the value of soil biodiversity in agricultural land has been considered from an economic perspective. However, it is equally important to recognise the vital contributions of soil biodiversity to maintain soil functions which is essential for sustainable land use. Soil organisms are the primary driving agents of nutrient cycle, regulate the dynamics of soil organic matter, soil carbon sequestration, and greenhouse gas emissions, modify soil physical structure, and enhance the level and efficiency of nutrient acquisition by the vegetation (Bardgett and Wardle 2010). Some soil organisms such as terrestrial isopods have been shown to be potentially useful indicators of soil health, because they respond to soil management in time scales (months/years) that are relevant to land management. On the other hand, the level of activity of different species depends on particular management practices, as these affect the micro-environmental conditions, including temperature, moisture, pH, and type of food sources.



## Conclusions

Detritivores, constituting the majority of the soil fauna, i.e., species and functional groups, act in different ways (Zimmer et al. 2004), sometimes with synergistic (positive non-additive) effects (Zimmer et al. 2005). To this end, a diverse soil fauna (Heemsen et al. 2004, Hättenschwiler et al. 2005), as well as a diverse vegetation (Zimmer 2002; Hättenschwiler and Gasser 2005), promote decomposition processes and nutrient cycling. On the other hand, soil animals depend on both the composition of the leaf litter (Zimmer and Topp 1997, 2000) and abiotic environmental conditions (Zimmer et al. 2000). Human practices, such as soil tillage and pesticide application, affect soil macrofaunal abundance and biodiversity (Dangerfield 1990, Souty-Grosset et al. 2005a, b, Manetti et al. 2013) and create disequilibrium. Chemical pollution, along with soil acidification, adversely affects soil fauna (Natal da Luz et al. 2004, Zimmer and Topp 1997).

Soil macroinvertebrates have a considerable impact on soil functions important to the restoration process, such as decomposition. Snyder and Hendrix (2008) reviewed how large obligate detritivores (earthworms, millipedes and isopods) have been used to accomplish restoration goals, assess restoration progress, and function as bioindicators. Patterns of detritivore community succession, and how these communities are themselves restored during restoration of perturbed ecosystems, are also discussed. Increasing studies of these taxa are required in ongoing and future restoration projects as well as the outreach activities that should be associated with use of these organisms.

Grassland biodiversity is a function of time among other factors; after disturbance, natural or human-induced, it may take a considerable time for natural communities to re-establish themselves. Plant diversity does not bear a close relationship with faunal diversity, for instance, in fallow land rich in plant species the soil fauna may be poor and unstructured. Isopods as surrogates have considerable potential as indicators of the biodiversity potential of plants in grassland habitats. To develop sampling strategies in order to test community recovery and biodiversity of cultivated grassland plots of different ages in Western France, isopod distribution patterns have been studied (Souty-Grosset et al. 2005a, b). These findings would come into the context of arable systems and help to integrate knowledge on pastoral and arable grasslands and on the influence of management on the grassland fauna (Curry 1994). Following ploughing and reseedling, most soil surface macroinvertebrates must recolonise from adjacent areas. In some long-established grassland plots, species associations within the grassland became similar to those in the boundaries. The permanent ecological corridors such as hedgerows and ditches are important elements of the mosaic of intensively farmed, fine-grained landscapes. Recognising the significance of such landscape features should help in developing guidelines and strategies for conservation management and effective restoration.

Species richness and activity density of woodlice is known to be largely affected by local management and associated habitat characteristics such as soil humidity, pesticide application, or tillage operations (Paoletti and Hassall 1999). The diversity of woodlice was shown to be affected by an interaction of local and regional land use

(Dauber et al. 2005). The cover of arable land in the vicinity was found to have no effect on species richness in arable fields, whereas it had a positive effect in grassland and a negative effect in fallow land. As terrestrial isopods play a fundamental role in the agroecosystems, a change in the spatial and temporal structure of Oniscidea communities caused by anthropogenic disturbance may have a cascade effect on ecological cycles, because terrestrial isopods play a fundamental role in nutrient cycling (Magrini et al. 2011).

### **New modes of investigation to assess the health of agricultural fields**

Rapid biodiversity assessment (RBA): RBA has been proposed by Obrist and Duelli (2010). It is an affordable indicator for monitoring local species richness of arthropods and sustainability of related ecosystem services. The indicator is based on strictly standardised sampling procedures and the identification of parataxonomic units (morphospecies) instead of species identification. Over a period of eight years, annual mean numbers of morphospecies were assessed in Switzerland in 15 agricultural habitats, in 15 managed forests, and in 12 unmanaged habitats ranging from protected lowland wetlands to Alpine meadows. The annual RBA-trend in unmanaged habitats is used for assessing the influence of climate and weather on biodiversity, and as a reference for measuring the relative influences of recent management changes in agriculture and forestry. The average number of morphospecies per sampling station per year depends on temperature, and was only marginally significantly increasing over time in agriculture, but not in forestry or unmanaged areas. Three RBA indices considered to be relevant for maintaining ecosystem services were calculated from the average number of morphospecies per location per year: (1) an indicator for ecological resilience and sustainability (all morphospecies), (2) an indicator for pollinator diversity (taxa with a majority of pollinators) and (3) an indicator for biocontrol diversity (ratio between carnivore and herbivore guilds).

A synthetic index of biological soil quality (IBQS) was developed by Ruiz et al. (2011) studying soil macro-invertebrate community patterns to assess soil quality in 22 sites representing the diversity of agroecosystems encountered in France. Using hierarchical classification, sites could be separated into four homogeneous groups and using the ‘indicator value’ method, 46 indicator taxa characteristic of one or another of these groups were identified. They used a formula that takes into account the abundance of indicator species and their respective indicator values to score soils from 1 to 20. IBQS was able to detect the effects of management practices on soil quality. Soil quality varied from 6 to 20 in forests, 7 to 9 in pastures, and 2 to 9 in crops respectively. Indicator species, such as *T. pusillus* and *O. asellus* had an indicator value of 73 % and 60 %, respectively. Both species are tolerant to soil acidity and their abundances were strongly correlated with soil water-holding capacity (van Straalen and Verhoef 1997).

## Future research

The research must be now conducted in two ways. First, it is necessary to know why isopod population outbreaks occur and what intrinsic or extrinsic factors drive the shift in their feeding behaviour. Second, we need to understand isopod density/crop damage relationships in order to know the lowest population density that each crop can tolerate and then reach agroecological equilibrium. According to Moonen and Barberi (2008), in order to frame the importance of biodiversity in agroecosystems, three main questions were addressed: (1) What does biodiversity mean in natural and agricultural ecosystems; (2) How is the concept of functionality used in relation to biodiversity; and (3) Which biodiversity measures are currently used to express agriculture/biodiversity relationships?

Analysis of the literature was also performed by Moonen and Barberi (2008) and resulted in a framework consisting of three steps. First, the objectives of biodiversity research and policies have to be defined. Three options can be foreseen here: (a) species, community, habitat or overall biodiversity conservation regardless of its functions, (b) biodiversity conservation to attain production and environmental protection services, and (c) use of bio-indicators for agroecosystem monitoring. For example, studying the bioindicator *A. nasatum* at a regional scale, Masson et al. (2014) have developed microsatellite markers as the most efficient markers for studying the influence of landscape features and agricultural practices on genetic structure and demographic history of *A. nasatum* populations. In the second step the appropriate target elements for conservation have to be chosen based on an agroecosystem approach. Finally, the third step involves selection of adequate biodiversity measures of composition, structure and function for each target element. In conclusion, functional biodiversity is important in relation to the provision of specific agroecosystem services. The study of functional biodiversity should start with the definition of agroecosystem functional groups comprising all elements that interact with the desired service, and the subsequent determination of the role of diversity within these functional groups for the fulfilment of the agroecosystem service. Therefore, a more precise definition of 'functional biodiversity' was proposed by Moonen and Barberi (2008) as 'that part of the total biodiversity composed of clusters of elements (at the gene, species or habitat level) providing the same (agro)ecosystem service, that is driven by within-cluster diversity'.

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# Terrestrial isopods in urban environments: an overview

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## Abstract

In an increasingly urbanized world scientific research has shifted towards the understanding of cities as unique ecosystems. Urban land use change results in rapid and drastic changes in physical and biological properties, including that of biodiversity and community composition. Soil biodiversity research often lags behind the more charismatic groups such as vertebrates and plants. This paper attempts to fill this gap and provides an overview on urban isopod research. First, a brief overview on urban land use change is given, specifically on the major alterations on surface soils. Historical studies on urban isopods is summarized, followed by the status of current knowledge on diversity, distribution, and function of urban isopod species and communities. A review of more than 100 publications revealed that worldwide 50 cities and towns have some record of terrestrial isopod species, but only a few of those are city-scale explorations of urban fauna. A total of 110 isopod species has been recorded although the majority of them only once. The ten most frequently occurring isopods are widely distributed synanthropic species. Knowledge gaps and future research needs call for a better global dataset, long term monitoring of urban populations, multi-scale analyses of landscape properties as potential drivers of isopod diversity, and molecular studies to detect evolutionary changes.

## Keywords

Adaptation, anthropogenic habitats, biotic homogenization, ecosystem services, soil fauna



## Introduction

In 2008 humans reached a major milestone: more than 50% of the global population now lives in cities (United Nation Population Division, World Urbanization Prospects 2011). Urbanization has become the major type of land use change in the 21<sup>st</sup> century. Conversion of wild lands or of former agricultural land to urban, suburban and exurban areas fundamentally alter the landscape and its biodiversity. Changes in the soil subsystem are especially dramatic. During construction, physical clearing, removal of upper horizons, filling, and mixing result in the loss of natural soil profiles. Urban soils are often highly compacted, have low hydraulic conductivity and low organic matter content. Soil sealing is one of the most visible surficial changes. Impervious surfaces disrupt the connection between the soil and the atmosphere, altering runoff, infiltration, nutrient input and gas exchange. It restricts or entirely prevents movement of soil organisms between the surface and deeper soil. Additionally, roads, pavements and other built structures lead to an even more heterogeneous landscape, acting as barriers for horizontal movement. At the same time, underground pipe systems can serve as corridors connecting habitat fragments. Chemical alteration of soils is also significant and is related either to accidental events (e.g., leakage of ageing sewer systems, spillage of contaminants), or direct management (e.g., application of road salts, fertilizers, and pesticides) (Pouyat et al. 2007, 2010). Soil degradation and soil sustainability are of national and global concern as described in the recent report by the European Academies (van der Putten et al. 2018).

In the urban-suburban landscape the habitat unit is often the parcel, a piece of land owned by private citizens, organizations (e.g., neighborhood associations), companies, or other groups of landowners. Decisions about land management happen at this scale further increasing spatial heterogeneity. Individual homeowners or small groups decide on planting, mulching, irrigation, and usage of fertilizers and pesticides. Large amounts of soil and other landscaping material are moved around, overcoming natural or man-made barriers. All these accidental or deliberate actions affect distribution and abundance of soil organisms including that of terrestrial isopods.

While urban land conversion often destroys existing habitats, it also creates new ones. Urban habitat patches range from remnants of the 'natural' community through more or less disturbed and/or managed habitats (e.g., parks, backyards, industrial grounds) to entirely novel habitats, such as green roofs, greenhouses, or even soilless environments such as basements and underground pipe systems. This wide spectrum of habitat types may result in overall higher species richness than expected. On the one hand, remnant patches of the native vegetation can sustain populations of the regional soil fauna. On the other hand, the physical environment in the novel habitats often allow the existence of species that otherwise would not survive under the normal climatic conditions. Cities are often viewed as hot-spots for non-native species introduction due to high traffic and trade (Vilisics and Hornung 2009).

Major restructuring of the surface-subsurface affects not only biogeochemical processes, but the biota mediating these processes as well. Soil fauna and microorganisms



are key players in regulating pathways and rates of decomposition, thereby affecting storage and release of carbon, nitrogen and other nutrients. Endogeic fauna can modify porosity, which affects water holding capacity, infiltration, and gas diffusivity. Thus, urban soils provide the same ecosystem services that naturally developing or agricultural soils do (Millennium Ecosystem Assessment 2005, Pavao-Zuckerman 2013).

The relative importance of natural vs. anthropogenic drivers in structuring urban communities and controlling ecosystem functions is variable, but generally the latter is viewed as a dominant force (Alberti 1999, Kaye et al. 2006). According to the Urban Ecosystem Convergence Hypothesis (Pouyat et al. 2003), this leads to more similar soil characteristics across regional or global scales compared to the soils they replaced. Data on soil organic matter and pH from five cities has supported this hypothesis (Pouyat et al. 2015a). Urbanization also results in more similar soil fauna, a process known as Biotic Homogenization (McKinney 2006, Olden et al. 2016). Two major components of biotic homogenization in urban areas are the extinction of local fauna, and the transportation of non-native, usually synanthropic species across geographical boundaries.

In this review we summarize past and current research on terrestrial isopods in urban environments. First, we provide an overview on the history of urban isopod research. We then summarize major findings, and highlight research gaps. For species names we follow the nomenclature by Schmalfuss (2003).

## Research on urban isopods – history

Early studies on urban isopods were mostly zoological surveys in the neighboring parks, backyards or as part of regional fauna assessments (e.g., Flasarová 1995). Specialized habitats such as botanical gardens and greenhouses have always been favored by zoologists; papers with species records go back to the turn of the 20<sup>th</sup> century (e.g., Bagnall 1909, Holthuis 1945, for full list see Schmalfuss 2002). Greenhouses and botanical gardens with many exotic plants and introduced soil were promising habitats to discover species new to the region or even to science (Korsós et al. 2002, Kontschán 2004, De Smedt et al. 2017). Exploration of these green spaces still continues (Montesanto 2015, De Smedt et al. 2017). The early records of species occurrences are extremely valuable, as we can use this historical information to document changes in the local fauna. For instance Montesanto (2015) reexamined isopod fauna from the botanical garden in Pisa, Italy. While total number of species did not change much (seven species between 1914–16, and eight species in 2014–15), species composition did: only one species, *Armadillidium depressum* Brandt, 1833, was recorded in both time periods.

Ecological studies on urban isopods coincide with the rise of urban ecology as a discipline in the 1980–90s (e.g., Klausnitzer 1987, Trepl 1995, Sukopp 1998), and were predominantly conducted in Europe (e.g., Tischler 1980, Kühnelt 1989, Schulte et al. 1989). In the US, the establishment of two urban Long Term Ecological Research (LTER) sites, Phoenix AZ, and Baltimore MD, launched urban ecosystem research in 1997. Information on isopod species composition and abundance is often published

as part of a broader study specifically focusing on urban soil fauna (e.g., Schaefer 1982, Fründ et al. 1989, Smith et al. 2006a, Jordan and Jones 2007, Bolger et al. 2000). Finally, a body of literature exists on the effects of pollutants, especially heavy metals on isopods. These organisms are known to accumulate heavy metals, and thus have been used as indicators of pollution levels in cities (Dallinger et al. 1992, Hopkin et al. 1993, Komarnicki 2005, Pedrini-Martha et al. 2012).

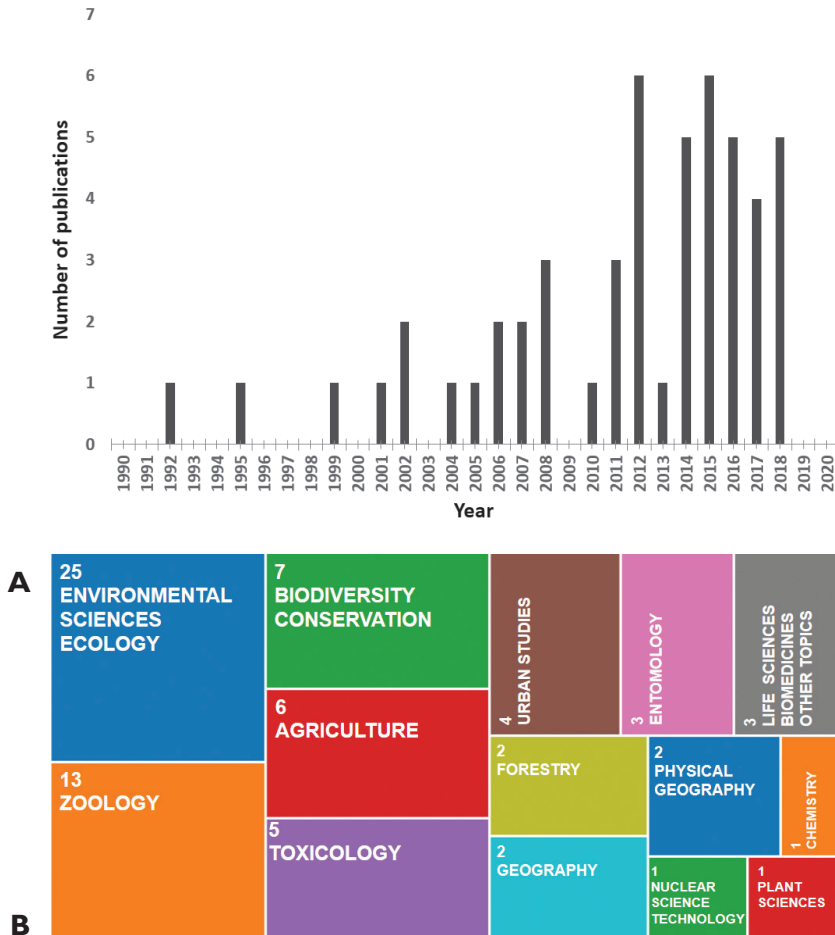
The development of better theoretical framework and methodological approaches (Grimm et al. 2000, Pickett et al. 2008) shifted urban ecology from descriptive to more mechanistic (Shochat et al. 2006). Attempts to explain biodiversity in urban habitat patches include the theory of island biogeography (Fründ and Ruszkowsky 1989), the intermediate disturbance hypothesis (Niemelä et al. 2000), and more recently, the metacommunity theory (Swan et al. 2011). One influential concept in early urban ecosystem research is the urban-rural gradient approach (McDonnell et al. 1993), which was adopted in various urban research programs including the GLOBENET Project (Niemelä et al. 2000), an international research initiative. In this project replicates of urban, suburban and rural forest patches were established to compare soil fauna and test the hypotheses about the connection between disturbance and diversity, and the effects of urbanization on morphological traits. Originally designed to study carabid beetles, the project extended to other epigeic arthropods, including isopods (Magura et al. 2008a, 2008b, Vilisics et al. 2007a, Hornung et al. 2007b).

With the growing interest in cities as ecological systems, the number of publications focusing in urban soil fauna grew, and isopod literature followed this trend (Figure 1A). More and more cities have conducted isopod surveys although the global distribution of these studies is heavily biased towards European cities (Fig 2). The research topics further diversified (Figure 1B) and today include observations on altered behavior of isopods in urban environments (Houghtaling and Kight 2006, Cividini and Montesanto 2018a, 2018b). To date a total of 50 cities have some records of isopod species (Suppl. material 1: Table S1). These cities vary in size, age, geographical location, major landscape features (e.g., types and coverage of green spaces), and land use type of the surrounding matrix.

## **Terrestrial isopods in cities**

### **Diversity and distribution**

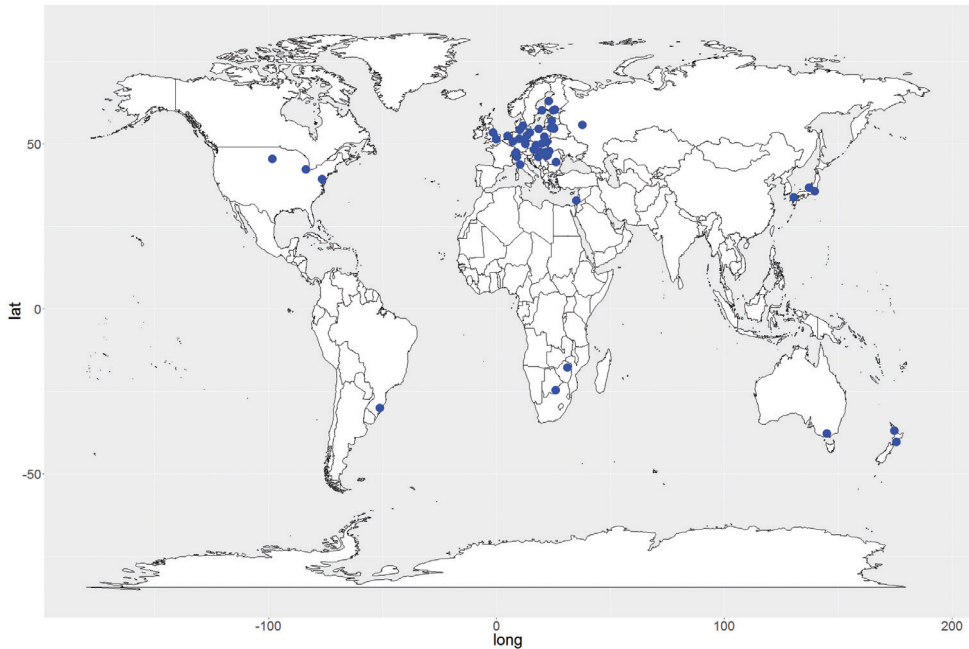
In any urban area species richness consists of two components: a subset of the regional pool of native species, and a group of accidentally or intentionally introduced species. Oniscidea form a diverse group with regard to size, mobility (thus dispersal ability), and tolerance to environmental extremes (Schmalfuss 1984, Warburg 1993, Hornung 2011). Therefore, the highly fragmented urban landscape will affect local fauna (and thus alpha diversity) differently. In general, local endemic species may disappear, and can be replaced by synanthropic, widely distributed species. Native habitat specialists (e.g., species requir-



**Figure 1.** Publications on urban isopods in the past three decades. Source: Web of Science using the following keywords: terrestrial isopods, woodlice, oniscid, urban, anthropogenic **A** Number of publications per year **B** Frequency of publications by subdisciplines.

ing coarse woody debris, bogs or ravines) are especially vulnerable and thus are often extirpated. However, this is not always the case: even local endemics can survive in small pockets of natural remnants within the city matrix (Vilics and Hornung 2009) similarly to isopods in forest fragments embedded in an agricultural landscape (Tajovský et al. 2012). Unmanaged gardens and yards can also serve as refuges for native species (Hornung et al. 2007, Vilics and Hornung 2009, Vilics et al. 2012, Hornung et al. 2018).

Local microhabitat characteristics are one major determinant in the survival of species. For instance, ruins of medieval castles or other historical buildings, stone walls or large slabs of concrete provide suitable shelter for *Porcellio spinicornis* Say, 1818 even in close proximity to the city core (Vilics 2007). In general, neglected areas that often lack tall vegetation, but are abundant in hard surfaces, abandoned brownfield and construction sites and crumbling medieval ruins share similar site characteristics for isopods. These areas



**Figure 2.** Cities with records on urban isopod diversity. Names of cities with references are listed in Suppl. material 1: Table S1. Publications with isopod abundance but without species composition are not included in the map, but referenced elsewhere in the text.

often support less common, drought tolerant species (e.g., *Porcellio dilatatus* Brandt, 1833, *Porcellio spinicornis* and *Porcellionides pruinosus* (Brandt, 1833)) (Vilisics and Hornung 2009, 2011). Native species may appear remarkably resistant to disturbances brought about by urbanization and successfully colonize many urban habitats. Examples are *Porcellium collicola* (Verhoeff, 1907) in Europe, and *Balloniscus glaber* Araujo & Zardo, 1995 in Brazil. The latter species has been found at high abundances in the city of Porto Alegre, RS, despite being categorized as a habitat specialist and K-strategist (Kenne and Araujo 2015). These species can be examples of 'urban adapters' a term coined by Blair (1996) and McKinney (2006), although it is unclear if adaptation in the evolutionary sense has occurred.

The review of urban isopod records revealed a list of over 100 species belonging to 15 families (Suppl. material 2: Table S2). The majority of these species (70 in total) has been recorded only once. *Porcellio scaber* Latreille, 1804, *Trachelipus rathkii* (Brandt, 1833) and *Armadillidium vulgare* Latreille, 1804, occur in more than 50% of the cities listed in Suppl. material 1: Table S1, with the latter being the most frequent. The other, frequently occurring species are also well known, widely distributed synanthropic species, including *Oniscus asellus* Linnaeus, 1758, *Philoscia muscorum* (Scopoli, 1763), *Porcellio scaber*, *Porcellionides pruinosus*, *Hyloniscus riparius* (C. Koch, 1838) and *Cylisticus convexus* (De Geer, 1778) (Table 1). These species can also be common in more 'natural' areas far from the urban core. For instance, *Trachelipus rathkii* can dominate on floodplains (Farkas 1998, Tajovský 1998), and *Armadillidium vulgare* is common

**Table 1.** List of the ten most common Isopoda in urban environments. References not older than 70 years were used for this list. Full list of species is in Suppl. material 2: Table S2, and references are listed in Suppl. material 1: Table S1.

Species	Family	Percentage of records (N = 50)
<i>Armadillidium nasatum</i>	Armadillidiidae	38
<i>Armadillidium vulgare</i>	Armadillidiidae	72
<i>Cylisticus convexus</i>	Cylisticidae	44
<i>Haplophthalmus danicus</i>	Trichoniscidae	38
<i>Hyloniscus riparius</i>	Trichoniscidae	42
<i>Oniscus asellus</i>	Oniscidae	42
<i>Porcellionides pruinosus</i>	Porcellionidae	48
<i>Porcellio scaber</i>	Porcellionidae	68
<i>Porcellio spinicornis</i>	Porcellionidae	38
<i>Trachelipus rathkii</i>	Trachelipodidae	56

on sandy shores (Hassall and Sutton 1978) and in forest remnants of the Hungarian Great Plains (Szlavec 1991). The repeated presence of these species leads to greater similarity among urban isopod faunas. However, we need to emphasize that urban species records are often incomplete. Surveys are often restricted for a particular habitat type (parks, remnant forest patches, riparian areas or vacant lots), and/or are short term studies. Large scale surveys are needed to explore how many of these common, synanthropic species have been established in climatic regions and biomes outside of their more natural range, and how many rare species persist in less studied, unique habitats.

What fraction of the regional species pool survives in a city depends on the type and strength of environmental filtering, and tolerance and adaptability of the native species. Both are related to geological and land use history of the region. Cities can harbor a large fraction of the regional native Oniscidea fauna. In Budapest, Hungary, Korsós et al. (2002) recorded 38% of the then known isopod fauna of Hungary; since then several new species have been added to the fauna list (Vilisics 2007) elevating this number. In Warsaw, Poland, 60% of the regional fauna was recovered in urban-suburban areas (Jedryczkowski 1981). Out of the 30 species recorded in rural habitats in NE Bohemia, Czech Republic, 17 occurred also in urban parks and gardens (Flasarová 1995). Similar total species richness numbers were reported from Bucharest, Romania (Giurginca 2006), Kiel, Germany (Tischler 1980), Leipzig, Germany (Arndt and Mattern 1996), and Olomutz, Czech Republic (Riedel et al. 2009). Broken down to main habitat types, gardens have been shown to support high diversity in Budapest, Hungary (Vilisics and Hornung 2009), Warsaw, Poland (Jedryczkowski 1981) and London, England (Smith et al. 2006b). At the other end of the spectrum are regions with low or zero native species richness. In North America, although approximately two thirds of the 115 listed species are endemic, most native species are restricted to the southern states, coastal areas and caves (Jass and Klausmeier 2000). Inland isopod faunas in the Atlantic, Midwest, and in Canada consist almost entirely of exotic, cosmopolitan, synanthropic isopods (Leistikow and Wägele 1999, Jass and Klausmeier 2000). Most likely they were introduced from Europe when ships carried soil as ballast material and in plant containers brought along

by immigrants (Lindroth 1957). Lack of competition with native counterparts likely enhanced successful colonization and spread of these species. They became widespread and abundant in wildland areas, such as forests, grasslands, or wetlands, as well as in agricultural fields and cities (e.g., Hatchett 1947, Sorensen and Burkett 1977, Hornung et al. 2015). In this situation the regional and local species composition overlap (Hornung and Szlavecz 2003). Reconstructing the origin and spread of these non-native species is challenging, but molecular techniques greatly enhanced our ability to reveal the past (Lee et al. 2014). For instance, combining historical records with molecular studies on European and North American populations of *Armadillidium vulgare*, Garthwaite et al. (1995) suggested that the species first was introduced to greenhouses, and later spread throughout the United States. Molecular studies also revealed that the East Coast and West Coast were independently colonized by founder populations from different latitudes in Europe, which corresponds to the known history of European settlement in North America.

Many exotic species do not establish successfully in the new environment, although this is difficult to demonstrate. Others sustain populations on the long-term, but are still restricted to man-made environments. *Trichorhina tomentosa* (Budde-Lund, 1893), *Buddeundiella cataractae* Verhoeff, 1930, and *Armadillidium nasatum* Budde-Lund, 1885 are common in greenhouses across Europe, although the latter is somewhat different. *Armadillidium nasatum*, often called ‘greenhouse pillbug’, has adapted to outdoor conditions and occurs in many areas north of its native range (Allspach 1987, Berg et al. 2008, Vilisics et al. 2012). It is also common in the Mid-Atlantic region in North America, where it was found in suburban gardens, parks, forests, and cropfields (Hornung and Szlavecz 2003, Szlavecz unpubl.). Interestingly, even populations of synanthropic species can decline over time. Harding (2016) speculated that the recent decrease in *Porcellio laevis* (Latreille, 1804) localities in Britain might be associated with the decrease of suitable synanthropic sites.

Many cities have been built in regions where the climatic conditions are outside of the tolerance limit of most isopods. These settlements may still harbor high abundance of organisms because the way humans modify the landscape and microclimate, shifts habitat conditions towards more optimal. In metropolitan Phoenix, Arizona, where the natural biome is desert, changes in vegetation cover and regular irrigation shifted residential yards from xeric to mesic. The abundance of isopods increased by a factor of 50, and they became the second most abundant macroarthropod group following ants (Cook and Faeth 2006). At high latitudes, where persistent cold temperatures limit isopod distribution in wildlands (e.g., Kuznetsova and Gongalsky 2012), urban areas provide favorable microhabitats. In Finland, isopods were found in urban forests and parks but not in rural forests (Vilisics and Terhiuvo 2009). Urban woodlands have more broadleaf tree species providing food and shelter for these detritivores. Heated buildings have been shown to be essential for winter survival of several mesic isopod populations in South Dakota, USA (Wright 1997). The isopods have only modest cold tolerance and suffer high mortality in the winter unless they migrate to great depth, or overwinter in the cracks and crevices of heated houses. In such extreme cold climates isopods exhibit extreme synanthropy. Future climate and land use change may open up new areas for colonization from these urban centers expanding the boundaries of distribution.



## Abundance of isopods in urban environments

Regardless of diversity, urban habitats support large abundances of isopods. Isopods have been shown one of the most numerous group compared to other epigeic arthropods (Bolger et al 2000, Cook and Faeth 2006, Smith et al. 2006a, Vilisics et al. 2007a, Philpott et al. 2014) although opposite examples also exist (Norton et al. 2014) (Table 2). Higher abundance of isopods has been reported from urban parks and forests than in rural habitat types (Tischler 1980, Walton et al. 2006, Hornung et al. 2015).

In the above mentioned GLOBENET project, pitfall trap material was analyzed in two cities: Sorø, Denmark, and Debrecen, Hungary. Isopod abundance was consistently higher in the urban core than in the rural habitats (Hornung et al. 2007, Magura et al. 2008a). Due to low overall species richness (5–6) there was no pattern regarding species composition. However, the relative abundance of species was different along the urban-rural gradient (Figure 3), indicating a species level response to urbanization. In Sorø, abundance of *Porcellio scaber* was an order of magnitude higher in urban parks than in rural forests, *Armadillidium vulgare* and *Ligidium hypnorum* (Cuvier, 1792) reached their maxima in suburban patches, and *Philoscia muscorum* was evenly distributed along the gradient (Vilisics et al. 2007a). Similar, species-specific response was detected in Debrecen, Hungary, as well (Magura et al. 2008a). It is important to note that pitfall trap sampling is selective, and small, slow moving, rare species, or species occupying special microhabitats (e.g., endogean species), can be missed.

## Isopods as environmental indicators

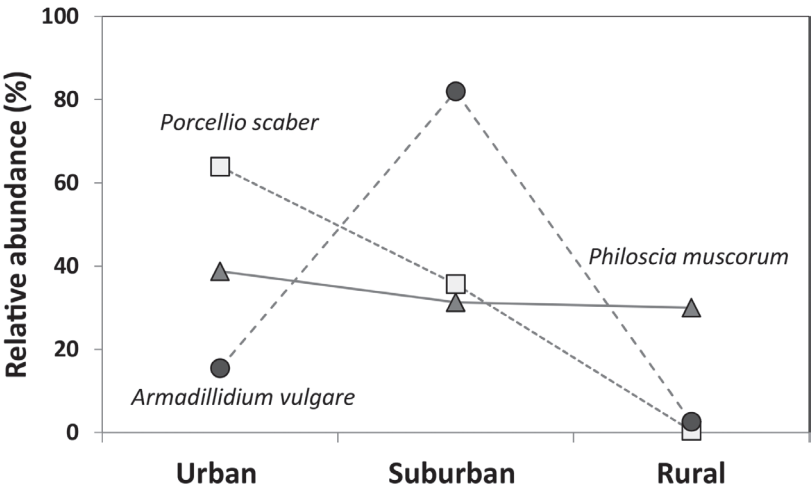
In the 1980–90s the main focus of urban research was contamination, especially heavy metal pollution (van Gestel 2012). Due to heavy industry near cities, emissions from vehicular traffic and other fossil fuel burning, and intensive use or accidental spills of organic compounds, the general view was that urban soils have high concentrations of lead (Pb), cadmium (Cd), copper (Cu), zinc (Zn) and other metals as well as organic contaminants. A wealth of papers has been published about how exposure to contaminated sites and food affect morphology (Godet et al. 2012, Mazzei et al. 2014), physiology (Farkas et al. 1996; Hornung et al. 1998a, Zidar et al. 1998) life history (Eckwert and Köhler 1997, Kammenga et al. 2001, Calh  a et al. 2012) and behavior (Drahokoupilov   and Tuf 2012) of isopods. These organisms are known to accumulate heavy metals, often to a greater degree than other invertebrates (Heikens et al. 2001). The ability of isopods to respond to environmental stress has made them ideal test organisms to evaluate environmental quality (Drobne and Hopkin 1995, Drobne 1997, Hornung et al. 1998b, Cortet et al. 1999, Caseiro et al. 2000), and to this day isopods are standard organisms in laboratory ecotoxicology tests (van Gestel 2012). Isopods were proposed to use as indicators of pollution levels in cities (Dallinger et al. 1992, Komarnicki 2005) and even at global scale (Hopkin et al. 1993). Recently Pedrini-Martha et al. (2012) showed that isopods are good indicators of mercury (Hg)



**Table 2.** Relative abundance of terrestrial isopods in the urban and suburban landscape. Numbers are expressed as percentage of isopods in pitfall trap materials, thus reflecting their abundance in relation to epigeic arthropods.

Location†	Land use/cover type	Percentage of isopods	Reference
San Diego CA, USA	Various suburban	48	Bolger et al. 2000
Toledo OH, USA	Various	59	Philpott et al. 2014
Sheffield, UK	Gardens	45	Smith et al. 2006
Yorkshire, UK	Urban agriculture	51	Turnbull 2012
Baltimore MD, USA	Vacant lots	52	Szlavec, unpubl.
Chicago IL, USA	Woodland fragments	34/55‡	McCary et al. 2017
Melbourne, Australia	Grass with variable cover	<1	Norton et al. 2014
Osaka, Japan	Variable	88	Lee and Kwon 2015
Phoenix, AZ, USA	Variable, mesic-xeric	1.9/12‡	Cook and Faeth 2006
Phoenix, AZ, USA	Irrigated residential yards	7/37‡	Cook and Faeth 2006

† City might include greater metropolitan area  
‡ First number was reported in publication and includes all arthropods. Second number is isopod percentage after removing Collembola and Acari. Removal of microarthropods allows more realistic comparison of macrofauna relative abundances.



**Figure 3.** Responses of three synanthropic isopod species to urbanization gradient. Each data point is percentage of total number of individuals (N) of a given species caught in pitfall traps. *Philoscia muscorum*: N = 7473, *Porcellio scaber*: N = 12314, *Armadillidium vulgare*: N = 816. The study was carried out in urban, suburban, and rural forest patches and parks in Sorø, Denmark. Data from Vilisics et al. (2007); original figure.

contamination, while Papp et al. (2018) reported significant differences in Ba and Cu concentration of *Armadillidium vulgare* individuals along an urbanization gradient. Heavy metal concentration in isopod and other invertebrate tissue can be significantly affected by sampling and sample preparation protocols, which is important to keep in

mind when making between-city comparisons (Zödl and Wittmann 2003). A growing number of studies focus on organic pollutants (e.g., van Brummelen et al. 1996, Loureiro et al. 2005) which is a particularly serious environmental problem in urban soils. Agodi et al. (2015) has shown *Armadillo officinalis* Duméril, 1816 to be a promising indicator species to benzene exposure including the effect of this carcinogenic air pollutant on mitochondrial DNA. Since isopods are food source of vertebrate and invertebrate predators, one threat is the transfer and potential accumulation of these contaminants at higher trophic levels. In a regional assessment of the Greater Washington-Baltimore Metropolitan area, Pouyat et al. (2015b) found a positive correlation between lead concentration of soil, isopod body, and blood of American robin (*Turdus migratorius* Linnaeus, 1766) nestlings from the same residential yards.

Fluctuating asymmetry (FA) has been proposed as a method to assess environmental quality (Clarke 1993). Developmental perturbations, due to environmental stress or genetic causes, results in deviations from bilateral symmetry and can be used as an indicator of levels of contamination. So far studies on isopod populations produced contradictory results. Both higher (Peters et al. 2001) and lower (Godet et al. 2012) levels of FA were reported from contaminated sites compared to control sites. Papp et al. (2018) found no difference along an urban-rural gradient. Exploring a different type of environmental stress, Vilisics et al. (2005) reported higher asymmetry at a locality with dramatic changes in soil moisture conditions, than from a more stable habitat.

Isopod assemblages can also reflect the level of disturbance and/or 'naturalness' of an area. Simple species numbers or diversity indices do not inform us about community composition. In cities, where non-native, synanthropic, and/or common species mix with native and/or rare ones, species diversity can be high, yet from a conservation point of view, the quality of the habitat is poor (Hornung et al. 2007, 2008, 2009, Vilisics and Hornung 2009). The 'Terrestrial Isopod Naturalness Index' (TINI) proposes a scoring system to characterize the isopod species. Adding species scores of a community and divided by species number ( $\Sigma \text{TINI}/S$ , where  $S$  is the number of species) gives Average Rarity Index (ARI), which then indicates local environmental quality. Basic components of the scoring system include whether the species is (1) introduced, (2) well established, (3) synanthropic, (4) disturbance tolerant and (5) member of the fauna in the region under study. Each component of the index receives a score with a maximum value of 20 (Hornung et al. 2008, 2009, Hornung et al. 2018).

## Terrestrial isopods are a successful group in urban environments

The fact that isopods reach high population densities indicates that while species level differences clearly exists, as a group they can successfully colonize urban and suburban habitats. Multiple factors contribute to their success. First, as detritivores, they are food generalists capable of living on leaf litter, garden and kitchen refuse, and even on pet food. In some countries, it is common landscaping practice to cover tree bases,

planting beds and bare soil surfaces with a thick layer of mulch. Mulch is often shredded woody material that retains moisture and, by slowly decomposing, adds organic matter to the soil surface (Byrne et al. 2008). This is both ideal sheltering habitat and food resource to isopods even in relatively open areas. Second, the built environment is rich in calcium (Ca) that isopods need. Calcium is present in the form of concrete dust, pavements, and landscaping limestone rocks, and gravel. Isopods are sensitive to soil acidity (van Straalen and Verhoef 1997), but land management can overcome this limitation: soil, too acidic for grass to grow, is amended with lime as part of the annual lawn care packages in the US. Third, another management practice, irrigation allows isopods to cope with their most significant abiotic limiting factors: low humidity and soil moisture, as demonstrated by the dramatic increase of isopod abundance in irrigated residential yards in a desert biome (Cook and Faeth 2006). Finally, the fourth component of their successful urban existence is that many species can live in soil-less substrates, primarily buildings. They can use the built environment, cellars, cisterns, and tool sheds temporarily in extreme weather conditions (Wright 1997), or permanently (e.g., Klausnitzer and Herr 1988). The Mediterranean species *Chaetophiloscia cellaria* (Dollfus, 1884) and *Protracheoniscus major* (Dollfus, 1903) of Central-Asian origin are examples of domicole species in Central Europe (Vilicsics and Hornung 2009). Isopods are moderate dispersers, but can be easily moved with plants, topsoil, compost, mulch, and even trash. Isopods are also used as pet food which further increases the chance of being transported to new areas. For instance, juvenile *Trichorhina tomentosa* are favored for certain amphibians (McMonigle 2013). This tropical South-American isopod survives in indoor conditions, and its establishment is also plausible under milder climates.

## Ecosystem services and disservices of terrestrial isopods in the urban landscape

### Regulating ecosystem services

Isopods are macro-decomposers feeding primarily on plant detritus but complementing it with other nitrogen rich resources, such as roots and tubers, vegetables, occasional green leaves, dead animal tissue and animal droppings. Together with other soil invertebrates, they accelerate plant matter decomposition and promote microbial access to organic carbon, thereby affecting nutrient turnover and soil formation. In urban parks and remnant forests they function similarly to wildland ecosystems. However, in residential areas they may be viewed differently. Some species, such as *Porcellionides pruinosus*, *Porcellionides sexfasciatus* (Budde-Lund, 1885), and *Armadillidium vulgare*, are common inhabitants of compost heaps and manure piles (Sousa et al. 2000, Wijnhoven 2000, Achouri et al. 2008), and considered to be beneficial organisms. Tropical species can also be found in compost; an example is *Venezillo parvus* (Budde-Lund, 1885) (*V. evergladensis* in reference) in Ft. Lauderdale, USA

(Johnson 1985). At the same time, because isopods occur in cellars, garages, and occasionally wander into houses, many residents view them as nuisance. In the US, pest control companies include woodlice in their list of household pests and recommend their extermination.

Urban agriculture is a fast growing phenomenon worldwide with over 800 million people practicing some form of farming or animal husbandry (FAO 2017). Small community gardens, farms spanning over several blocks, rooftop farms, and other forms of farming provide access to better quality food for inner city residents, build social cohesion in the neighborhood, and utilize abandoned land. Urban farms use large amounts of organic mulch and produce green refuse for composting, both of which are resources for terrestrial isopods, leading to them becoming permanent features in community and residential gardens. As detritivores, the commonly held view was that they cause only minor damage in vegetable and other crops (Paoletti and Hassall 1999). However, a growing number of studies indicate that under special circumstances isopod populations can grow out of control which may lead to attacking crops (Plate and Frömming 1953, Byers and Anderson 1985, Honek et al. 2009). Souty-Grosset and Faberi (in press) report that several species, for instance *Armadillidium vulgare* and *Australiodillo bifrons* (Budde-Lund, 1885) are now considered emerging pests causing significant economic damage in cereal, soybean, canola and other fields. In greenhouses isopod populations can grow exponentially especially if fresh compost is present. The woodlice then shift to and damage the organically grown vegetables as alternative food source (Messelink and Bloemhard 2007). It remains to be seen if in urban farms isopod populations reach the pest status and if their populations need to be controlled.

Isopods are food source for many invertebrate and vertebrate predators. Ground feeding birds, such as the European blackbird (*Turdus merula* Linnaeus, 1758) especially benefit from the abundance of soil macrofauna, such as earthworms, insect larvae, isopods, and other invertebrates (e.g., Török and Ludwig 1988). In Nacogdoches, Texas, USA, isopods were one of the three major prey items in the diet of the Mediterranean house gecko, *Hemidactylus turcicus* (Linnaeus, 1758) (Saenz 1996). Even more interesting is the behavior of the ant *Leptogenys propefalcigera* Roger, in the urban areas of Sao Vicente, Brazil. Colonies nest in building wall cracks and fissures, and feed exclusively on oniscid isopods, rejecting other invertebrates and honey as food source (Freitas 1995).

### Cultural ecosystem services

Neighborhood parks, schoolyards, university campuses community gardens and other green spaces serve as ‘living classrooms’ for children and adults. In the shrinking cities of US, low income inner city residents may not have the means to ‘venture out to nature’, they experience plant and animal life through green spaces near their homes. Urban soil biodiversity can be surprisingly high in cities, providing opportunity to demonstrate the variety of life forms, to talk about their functions, and to

connect soil health and human health. Isopods are part of this conversation, because most people played with roly-polies as children, and because they are easy to observe, culture and experiment with. Citizen scientists can be actively involved in isopod surveys, add new records of species and localities, or make observations on the life cycle of their local populations, while they themselves learn about global change or conservation issues. For instance, the British Myriapod & Isopod Group (<http://www.bmig.org.uk/>) trains volunteers to identify and record centipedes, millipedes, pauropods, symphylans, woodlice and waterlice. The objective of the Spinicornis Project (<https://www.spinicornis.be>) is to build an ecological atlas of Belgian terrestrial isopods. To engage volunteers, the project organizes collecting excursions, provides simple identification key, and guidelines on how to take high quality photographs on invertebrates. Another example of citizen involvement is the Bioblitz Program, overseen by the National Geographic Society (<https://www.nationalgeographic.org/projects/bioblitz/>), also local environmental organizations, and agencies. Bioblitz events record local biodiversity in a short period of time, usually 24–48 hours, with the active participation of the public. In 2016, as part of the celebration of the USA National Park Service, a large scale Bioblitz was organized in Washington DC. To keep citizens engaged the group organizes regular field meetings, publishes a newsletter and bulletin, and maintains a webpage with distribution maps, images, and helpful comments for identification. Involving citizen scientists to collect scientific data is not without challenges, but utilizing this valuable resource can be very rewarding (Cohn 2008, Bonney et al. 2009).

## Research needs on urban isopod ecology and evolution

### Greater geographical coverage

Research questions in this area

- 1) What is the fraction of regional species pool persisting in urban/suburban areas?
- 2) Does urban isopod species richness exhibit a latitudinal gradient, and if so, how does it compare to trends in natural habitats?
- 3) What is the rate of species turnover of urban isopod fauna as a function of distance?

Huge gaps exist in regional and global scale distribution of isopods not only in cities but on the regional species pool, as well (Figure 2). Most European cities are old, with hundreds or even thousands years of history and major disturbances such as land use change, migration, industrialization and wars. Interpreting fauna data today is challenging in light of such long term changes. However, currently most urban land conversion takes place in the developing world, especially in coastal areas, tropical dry and moist forests, deserts, and tropical grasslands (Elmquist et al 2013). Many of these areas are biodiversity hotspots, thus the proper assessment of local extinction

and colonization of species including that of terrestrial isopods in these areas is an urgent necessity.

Extending geographical scale results in examination of a broader range of climatic conditions, biomes and soil types, different cultures, economies, and human perception and value systems. Only a sufficiently large dataset enables us to examine large scale biogeographical patterns. Sfenthourakis and Hornung (2018) have shown that in Europe a latitudinal gradient exists with decreasing species numbers to the North. Moreover, biogeographical and ecological species characters shift with latitude. Endemic species, dominating in the Mediterranean, gradually disappear at higher latitudes and are replaced by habitat generalist species. Testing general hypotheses such as Biotic Homogenization in urban settings also requires large scale datasets. We do not know whether different zoogeographical/climatic regions have specific subsets of homogenizing species, or the same 8–10 species (Table 1) dominate everywhere. Preliminary surveys indicate that in the Mediterranean region *Agabiformius lentus* (Budde-Lund, 1885) regularly occur in cities (Vilisics et al. 2012). Combining distribution records with genetic data can reveal origin, dispersal patterns, and timing of introduction of non-native species worldwide.

### City and neighborhood scale surveys

Research questions in this area

- 1) What is the relationship between city size, age, area of green spaces, and other landscape features in isopod diversity?
- 2) What is the human perception of soil macroarthropods in general, and specifically on isopods in different regions and cultures?

To answer the questions in this and the previous section, we need reliable data on isopod species richness and composition at city scale. Most studies reviewed here targeted a particular set of urban habitats, such as forest fragments, gardens or urban parks, and their species lists are likely incomplete. Many species are undocumented because they are rare, i.e. their abundance is low, and/or present only in a few, specialized landscape patches, such nearby historical ruins, greenhouses, or sewer drains (Kontschán 2004, Vilisics 2007). Assessments of epigeic arthropod communities usually rely on pitfall trap material. Smaller isopod species rarely fall into those, and thus will be underestimated. It is very important to complement pitfall trap sampling with other methods such as leaf litter sifting and traditional hand collecting that targets unusual habitats. Recently in Manaus, Brazil, Ogawa (2008) found isopods in the nest of the urban pigeon, *Columba livia* Gmelin, 1789. More sampling points increases the coverage on the landscape, and even though unless standardized, hand sampling usually provides only presence-absence data, the true species richness of the city and suburban areas will be better captured.

## Diversity and stability of isopod assemblages

### Research questions in this area

- 1) What is the relative contribution of local environmental factors (soil type, vegetation, microclimate) and management (irrigation, amendments, pesticide use, litter collection) in determining composition and abundance of isopod assemblages?
- 2) What is the role of corridors in dispersal and exchange of individuals among local populations? Do isopods use grey infrastructure (buildings, underground conduits) to disperse?
- 3) What are the key landscape properties ensuring long term persistence of isopod populations, and how do these vary with climatic conditions?

The urbanized landscape is highly fragmented leading to isolation of communities. Species richness of these isolated patches can be highly variable from one species to ten or more. Priority effect can play a major role in which species get established. Isopods are being moved around with soil, plants, mulch and other landscaping materials. Consequently, species presence in a local patch might be determined by ‘who gets there first’. Soil invertebrate surveys in cities are often campaign-like, such as the above mentioned Bioblitz efforts, which provide a snapshot of the local community. We know essentially nothing about the persistence of these populations. Long term field monitoring is needed to reveal how patterns of alpha and beta diversity change over time and how stable urban isopod communities are. For arthropods in general, local landscape features have been shown to drive local diversity, although the specific drivers are taxon dependent (Smith et al. 2006a, Philpott et al. 2014). A complex habitat creates more diverse resources and microhabitat conditions allowing coexistence of more species (Byrne 2007, Ossola et al. 2016). Research is needed to explore the role of habitat structure and the defining components of the local landscape specifically on terrestrial isopod assemblages. Experimental data show that the probability of success to increase biodiversity varies by the type of design changes as well as by the targeted taxa (Gaston et al. 2005). This knowledge is also important for practitioners when making decisions about urban green spaces for promoting local diversity, protecting rare species or enhancing particular ecosystem services (Snyder and Hendrix 2008, Szlavecz et al. 2018).

## Function of isopods in novel ecosystems

### Research questions in this area

- 1) Given their often high abundances, what is the role of terrestrial isopods in nutrient turnover, especially in regions lacking earthworms?
- 2) Under what circumstances can isopod become pests in urban gardens, local crop fields, and greenhouses?



The urban landscape is dominated by highly manipulated, engineered, and built components designed by humans to serve a given purpose. Soils are often engineered to support buildings and roads, to plant street trees, to establish green roofs, or to intercept and retain water. A particular example of the latter is natural water treatment systems such as rain gardens and bioswales. These novel ecosystems are readily colonized by soil fauna, including isopods (Ayers 2009, Mehring and Levin 2015) but we do not know if their presence and activity enhance or reduce such functions as nutrient release and retention, organic matter processing, infiltration, contaminant and pathogen removal, and plant growth. Woodlice might also have an important role in distributing mycorrhizal propagules (Rabatin and Stinner 1988, 1989). The role of soil fauna in the efficiency in these engineered systems needs to be quantified, and, combined with ecological theory, living organisms should be included in the design process (Levin and Mehring 2015).

In general, the urban soil food web is highly altered, because the resource base is altered. On the one hand, leaf litter, a major food source for terrestrial isopods, is removed from lawns and impervious surfaces. On the other hand compost, mulch, manure and other landscaping material create a concentrated abundant resource supply. How does this affect spatio-temporal abundance of terrestrial isopods? A related issue is the relative role of urban isopods in decomposition and nutrient turnover. Grass/lawn is the dominating land cover type in temperate urban/suburban areas. Pieper and Weigmann (2008) have shown that the presence of *Porcellio scaber* and two Collembola species resulted in faster decomposition of grass litter, greater losses of most cations, but higher retention of organic carbon in urban soil, but such mesocosm studies need to be extended to long-term field investigations.

## Adaptation and evolution in the urban environment

Research questions in this area

- 1) What environmental factors in an urban environment can act as selective forces?
- 2) What are the key biological traits contributing to the success of some urban isopod species?
- 3) Are life history characteristics of urban and corresponding rural populations of isopods different?
- 4) Are urban and rural isopod populations genetically different?

Humans are major drivers of both adaptive and non-adaptive evolutionary change. Urban evolution is an emerging field focusing on individual and population responses to urban selective forces, and the underlying micro-evolutionary changes. So far research has been heavily biased towards vertebrates, and plants (McDonnell and Hahs 2015), with invertebrate studies lagging behind (Johnson and Munshi-South 2017). Isopods are excellent model organisms to study mechanisms of evolutionary change because of their ubiquitous occurrence, somewhat limited dispersal ability, and dem-

onstrated responses to known urban stressors, such as pollution and the altered urban climate. Moreover, their reproductive cycle is strongly driven by light regimes (e.g., McQueen and Steel 1980, Warburg et al. 1984, Juchault et al. 1985), and they have been shown to alter their behavior to increased vibration, a much less studied urban effect (Houghtaling and Kight 2006, Cividini and Montesanto 2018a, 2018b). Successful establishment and range expansion of terrestrial isopods depends on their reproductive strategies, phenotypic plasticity, and omnivore feeding habits (Rushton and Hassall 1983, Hornung and Warburg 1998, Hornung 2011). Species with wider tolerance limits, higher reproductive output, and with the ability of sperm storage (Suzuki and Ziegler 2005) or being parthenogenetic have a better chance for colonization and geographical range expansion. To separate true evolutionary response from phenotypic plasticity, which is commonly exhibited in isopods, molecular studies are needed.

## Conclusions

Terrestrial isopods are ubiquitous members of the epigeic soil fauna in cities. They are well established in the built environment and in all types of urban green spaces including remnant habitat patches, parks, residential yards, vacant lots, and greenhouses. Urban isopod assemblages are a mixture of a few cosmopolitan species that thrive in human dominated landscapes, a subset of the native fauna, and more recently introduced species. The urbanized landscape is highly fragmented, leading to isolation of communities. Alpha diversity of these isolated patches varies, but comparable to species richness in more natural areas. At the same time, species turnover among the habitat patches can be high resulting in high species richness at city scale. These observations are highly biased, because the overwhelming majority of data were collected in the temperate zone. Globally, distribution of isopods is limited at higher latitudes due to cold temperatures. However, current warming trends coupled with urbanization that provides refuges from extreme conditions, are pushing these boundaries northward.

Synanthropic species thrive in the city, often dominating the detritivore macrofauna, but special habitats can be refuges for native species, as well. The urban setting provides an excellent opportunity to study the dynamics of spatially isolated communities, the underlying mechanisms of local extinction, colonization, and dispersal, and the role of human perception, disturbance and management plays in these processes. As abundant, often dominant detritivores, their role in decomposition and nutrient release needs to be studied especially in engineered ecosystems. Future research should also include eco-evolutionary changes, preferably in the rapidly urbanizing regions in the world.

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## Supplementary material 1

### List of cities with terrestrial isopod records

Authors: Katalin Szlavecz, Ferenc Vilisics, Zsolt Tóth, Elisabeth Hornung

Data type: occurrence

Explanation note: Publications older than 70 years are not included. Localities listed here are mapped on Fig. 2 in the text. Source of geographical coordinates and population data: <https://latitude.to>

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Link: <https://doi.org/10.3897/zookeys.801.29580.suppl1>

## Supplementary material 2

### List of terrestrial isopod species records in urban areas

Authors: Katalin Szlavecz, Ferenc Vilisics, Zsolt Tóth, Elisabeth Hornung

Data type: occurrence

Explanation note: Species name have been cross-checked with the world catalog by Schmalzfuss (2003). Synonyms have been changed to valid species names. In some cases the species was not listed in the catalog. Notes regarding uncertainties are also from Schmalzfuss (2003).

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# Terrestrial isopods as model organisms in soil ecotoxicology: a review

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## Abstract

Isopods play an important role in the decomposition of leaf litter and therefore are making a significant contribution to nutrient cycling and soil ecosystem services. As a consequence, isopods are relevant models in soil ecotoxicology, both in laboratory toxicity tests and in field monitoring and bioindication studies. This paper aims at reviewing the use of isopods as test organisms in soil ecotoxicology. It provides an overview of the use of isopods in laboratory toxicity tests, with special focus on comparing different exposure methods, test durations, and ecotoxicological endpoints. A brief overview of toxicity data suggests that chemicals are more toxic to isopods when exposed through soil compared to food. The potential of isopods to be used in bioindication and biomonitoring is discussed. Based on the overview of toxicity data and test methods, recommendations are given for the use of isopods in standardized laboratory toxicity tests as well as in situ monitoring studies.

## Keywords

Bioaccumulation, biomonitoring, indicator organisms, Isopoda, toxicity tests

## Introduction

Increasing human activities have caused serious effects on man and the environment. Since the industrial revolution in the 19<sup>th</sup> century, pollution from industries and metal contamination from mining activities has increased. In the 20<sup>th</sup> century, after the Second World War, the massive use of pesticides resulted in widespread environmental pollution. The first evidence of chemical pollution was shown by air pollution, for instance leading to smog episodes in cities. Air pollution was a major problem already in the 19<sup>th</sup> century and still is one of the major factors threatening human health, especially in rapidly developing industrial regions like China (see e.g., Kan et al. 2012). The quality of surface waters also became seriously affected, with many incidences of fish killings all over the world (see e.g., Langer 1964); but reports on soil pollution for some time seemed to lag behind those on air and water pollution. Since the 1980s, however, soil pollution has also become highlighted as a major issue. It was realised that polluted soils are generally more difficult and considerably more expensive to remediate than cleaning or preventing water and air pollution (see e.g., Swartjes 2011).

Apart from threatening human health, air, water and soil pollution may also affect ecosystems. By affecting major functions of natural systems, pollution can alter the so-called ecosystem services (Hunt and Wall 2002; Faber and Van Wensem 2012), which include 1. provisioning services: material outputs from ecosystems, such as food, water, and other resources, 2. regulating services: ecosystems acting as regulators e.g., of the quality of air and soil or by providing flood and disease control, 3. habitat or supporting services: providing living space for plants or animals, maintaining a diversity of different breeds of plants and animals, etc., and 4. cultural services: non-material benefits people obtain from contact with ecosystems, such as aesthetic, spiritual and psychological benefits (The Millennium Ecosystem Assessment 2005; TEEB 2010). For maintaining ecosystem services, a high biodiversity is essential, as was recently shown by Soliveres et al. (2016).

Pollution may damage ecosystems and by that the support ecosystems provide to (the quality of) human life. Protection of ecosystem services therefore is essential not only for safeguarding the health of our ecosystems but also for our own benefit (Faber and Van Wensem 2012; TEEB 2010), and should thus receive more attention in the risk assessment of chemicals (see e.g., Nienstedt et al. 2012; Forbes and Calow 2013; Maltby 2013). For that reason, ecotoxicologists and ecologists are aiming at protecting ecosystems, including all key-organisms that together contribute to its functioning.

Isopods play an important role in the functioning of soil ecosystems and therefore also in the ecosystem services provided by soils. They act mainly on the first processes of litter fragmentation, contributing to the input of high quality organic matter, and increasing the microbiome for further nutrient cycling in soil (Hassall et al. 1987). This paper will discuss the possible role of isopods as model organisms in ecotoxicology. For that purpose, before focusing on isopods, we will first briefly describe some ecotoxicological concepts and the general principles of ecotoxicological risk assessment. Next we will discuss the use of isopods as model organisms in predictive (and diagnostic)

ecotoxicological approaches. This will include the use of different endpoints, the relevance of different routes of exposure and the importance of bioavailability, and will be supported by a review of the literature on the toxicity and bioaccumulation of different chemicals in isopods. Finally we will discuss the use of isopods as diagnostic tools in monitoring soil quality.

## Ecotoxicology and ecotoxicological risk assessment

After the publication of the book *Silent Spring* by Rachel Carson (1962), awareness of the side effects of pesticides and other chemicals on the environment increased. The term ecotoxicology was coined only in 1977 by Truhaut. The aim of ecotoxicology is to protect ecosystems from adverse effects of chemical pollution, alone or in combination with other anthropogenic stressors. To realise its aims, ecotoxicology makes use of knowledge from different disciplines, like environmental chemistry, toxicology, and ecology (Van Leeuwen and Vermeire 2007).

Environmental chemistry provides insight into the way chemicals interact with components of the environment, determining their fate, and therefore also the exposure of organisms and ecosystems. From knowledge of persistence and partitioning, environmental chemistry enables estimating Predicted Environmental Concentrations (PEC), which are the starting point for the risk assessment of chemicals. One important issue is bioavailability: the notion that total concentration of a chemical in the environment is not indicative of its risk, because only a fraction of it may be available for uptake and therefore causing effects (Van Gestel 2008, 2012). Especially in soils, due to their large capacity of complexing chemicals, bioavailability is an important issue. Therefore, and besides the distribution of chemicals in environmental compartments, bioavailability will focus also on the fate of chemicals in the organisms, bridging the gap between what is in the environment and the compartmentalisation of chemicals inside biota (external versus internal bioavailability) (Vijver et al. 2004).

Toxicology provides insight into the interaction of chemical pollutants with molecules, tissues, and organs resulting in effects at the molecular and individual level (Van Leeuwen and Vermeire 2007). From (laboratory) tests, dose-response relationships are derived to provide quantitative measures of the toxicity of chemicals, like  $LC_{50}$  (lethal concentration killing 50 % of the tested population),  $EC_{10}$  or  $EC_{50}$  (concentrations causing 10 % or 50 % reduction in a measured parameter, e.g., growth or reproduction, compared with the response in the untreated control) or NOEC (No-Observed Effect Concentration, the highest concentration tested causing no significant reduction in a measured parameter compared to the untreated control).  $LC_{50}$  values usually are determined in short-term or acute toxicity tests, with mortality as the main endpoint.  $EC_{10}$ ,  $EC_{50}$ , and NOEC values are derived in sublethal toxicity tests, using growth, reproduction or another sublethal endpoint. Such tests usually require longer exposure times, and are therefore sometimes indicated as chronic tests. The term chronic however, should only be used when test duration includes a substantial part of the life span

of the test organism. From the available toxicity data, a safe concentration of a pollutant can be derived, as a so-called Predicted No-Effect Concentration (PNEC). Different approaches may be followed to derive a PNEC, extrapolating effects from acute ( $LC_{50}$ ) to sublethal effects ( $EC_{10}$ ,  $EC_{50}$  or NOEC), from one or few species to many species and from the laboratory to the field. The resulting PNEC should be protective of effects not only at the individual level but at higher levels of biological organisation, like populations and communities.

Ecology provides the knowledge for the extrapolation from individual-level effects to effects on populations, communities, and ecosystems. Ecology adds knowledge about life histories of organisms, their functioning in different processes or their interactions with other species or the abiotic environment. Knowledge on life histories and other ecological properties or traits of species also helps understanding how organisms will be exposed to chemicals in the environment and how this may affect populations. The behaviour of an organism may for instance differ depending on its life stage, while it may have great influence on exposure. Again, this seems even more important in soils, where pollution is only rarely distributed homogeneously. Finally, knowledge about ecological interactions between species may help translating effects to the community and ecosystem level.

Ecotoxicology may follow predictive/prospective and diagnostic/retrospective approaches (Van Gestel 2012). Predictive approaches aim at preventing possible effects of chemicals before they are introduced onto the market or to limit side effects after introduction. Predictive ecotoxicological risk assessment of chemicals is based on a comparison of the hazard of a chemical, expressed as its toxicity or safe levels derived from that (PNEC values), with predicted or measured exposure levels (PEC or MEC). This will provide an assessment of the possible risk predicted or diagnosed for exposed ecosystems. Standards or risk limits for chemicals in the environment generally are based on PNEC values, sometimes lowered by application of a safety or uncertainty factor, depending for instance on the availability of toxicity data (number of data, representation of different species (see also below), endpoints etc.).

Diagnostic approaches are applied to monitor possible effects of chemicals after introduction onto the market, e.g., as post-registration monitoring of pesticides, or to assess the actual risk of contaminated soils. Diagnostic risk assessment, for instance of contaminated soils, relies on a combination of ecological, toxicological and chemical approaches. Basically, toxicity tests (in this case usually called bioassays) are used as diagnosis tools to assess the toxicity of soil samples from the contaminated site. Results of the bioassays are considered together with those of measurements of total or available concentrations of a selected number of chemicals and ecological field observations. The added value of bioassays is that they provide information on the actual risk of bioavailable concentrations of all chemicals present in the contaminated samples; such information cannot be obtained from chemical analyses (see e.g., van Gestel et al. 2001; Loureiro et al. 2005, 2006). Together these approaches result in an assessment of the potential risk of soil contamination, for instance in the TRIAD

approach, which combines three lines of evidence: chemistry (total or available concentrations of pollutants), toxicity of the polluted soil and on site ecological observations (Jensen and Mesman 2006).

## **Toxicity tests**

One key issue in ecotoxicological risk assessment is the selection of test species for generating the required toxicity data. For a proper risk assessment it is crucial that test species are representative of the community or ecosystem to be protected. Criteria for selection of tests and therefore also for organisms to be used in toxicity tests have been summarised by Van Gestel et al. (1997). They include 1. Practical arguments, including issues like feasibility, cost-effectiveness and rapidity of the test, 2. Acceptability of tests, like the need to be reproducible and standardised, and 3. Ecological significance, including sensitivity, biological validity etc.

There is no species that is most sensitive to all pollutants. Which species is most sensitive depends on the mode of action and possibly also other properties of the chemical, and the properties of the organism (e.g., presence of specific targets, physiology, etc.). It is therefore important to always test a number of species, with different life traits, functions, and position within a trophic chain. Such a battery of test species should be (according to Van Gestel et al. 1997):

1. Representative of the ecosystem to protect, so including organisms having different life-histories, representing different functional groups, different taxonomic groups and different routes of exposure;
2. Representative of responses relevant for the protection of populations and communities; and
3. Uniform, so all tests in a battery should be applicable to the same test media and applying the same test conditions.

Once tests have been developed, accepted, and validated, they may be standardised by international organisations like the International Standardization Organization (ISO) or the Organization for Economic Co-operation and Development (OECD). Chemical registration authorities usually only accept results of tests standardised by these organisations. For the soil environment different OECD and ISO standardised tests are available (for an overview see e.g., Van Gestel 2012), including earthworms, enchytraeids, springtails, insect larvae and molluscs as test species. ISO tests are primarily focusing on diagnostic/retrospective risk assessment while OECD tests are required for predictive/prospective risk assessment. Some tests determine short-term or acute exposures, usually with mortality as the endpoint, while others focus on sublethal endpoints like reproduction. Also tests on avoidance behaviour with earthworms (ISO 2008) and springtails (ISO 2011) have been developed as a fast method of assessing another relevant sublethal endpoint.

The ecological relevance of isopods, their typical routes of exposure (soil, food) and life history characteristics, the possibility to determine different endpoints (see below), and the fact that they have already been used for testing for more than 30 years, make them highly suitable test organisms (Drobne 1997; Van Gestel 2012). Unfortunately, so far, no toxicity tests on isopods have reached the level of standardisation, although several methods have been applied in the literature. One reason for the lack of a standardised test with isopods could be their relatively long and more complex life cycle compared to springtails or earthworms. This makes it more difficult, at least for a number of species, to culture them in the lab. For many isopod species it takes several months or more from hatching to reproductive age. Another problem is the rather large variation in test results, which could in part be attributed to the difficulty of obtaining age-synchronised groups of test animals but could also be an intrinsic property of isopods, like the moulting process or their daily excretion process within the hepatopancreas. Both the long life span and the large variation, together with the ability of females to store sperm, make it more difficult to standardise a reproduction toxicity test. Another point which may hamper the standardisation of toxicity tests with isopods may be related to the uncertainty about the main route of exposure (soil vs. food; see below). Nevertheless, there are possibilities of getting around these problems, as will become clear from the following sections.

## Main sources of isopod exposure in soil

In terrestrial systems, several pollutants can reach the soils from diverse sources and can also be found in decaying organic matter. These sources can be considered as point and/or diffuse (non-point) and they will include different forms of contamination: gaseous (atmospheric), solid or liquid hazardous substances that will be mixed with soil.

Industry and commerce are two of the major economic sectors responsible for soil contamination (36 %), either by negligence or by accident (European Environment Agency (EEA); [www.eea.europa.eu](http://www.eea.europa.eu)). This includes atmospheric emissions from production, spilling or burying chemical substances directly in the soil or through runoff from surrounding areas. Such polluted sites are common in Europe and worldwide, and are commonly named as historically-contaminated sites, where metals (37.3 %) and mineral oils (33.7 %) are the main harmful substances to be considered, followed by Polycyclic Aromatic Hydrocarbons (PAHs) (13.3 %), Aromatic Hydrocarbons (BTEX) (6 %), phenols (3.6 %), chlorinated hydrocarbons (2.4 %), and other chemical compounds (3.6 %) (EIONET priority data, EEA). Along with industrial and commercial sources, waste treatment and disposal is another main source of soil contamination (EEA; [www.eea.europa.eu](http://www.eea.europa.eu)). Sewage sludge is often applied to agricultural fields as fertiliser. This provides a considerable input of hazardous substances to soils, some of them considered as emerging pollutants, like those in daily care products, pharmaceuticals, or nanomaterials. In addition, Phthalates (e.g., diethylhexylphthalate

(DEHP), dibutylphthalate (DBP)), Octylphenols, Nonylphenols, Linear alkylbenzene sulfonates (LAS), Polychlorinated biphenyls (PCBs), PAHs and metals are amongst the substances most commonly found in sewage sludge that is applied to soils for agricultural purposes. Considering this cocktail of chemicals along with pesticides and fertilisers, agricultural soils are a major sink for contaminants. Pesticides have been introduced by man in ecosystems initially as natural compounds, by using poisonous plants or extracting chemical substances from other natural sources. Later in the 20<sup>th</sup> century, especially after the 2<sup>nd</sup> World war, anthropogenically modified or synthesised compounds have been introduced at a large scale, that nowadays represent a wide range of organic chemical groups, including organophosphates, carbamates, triazines, organochlorines, pyrethroids, neonicotinoids, sulfonylurea and biopesticides (EEA; [www.eea.europa.eu](http://www.eea.europa.eu)).

Along with agricultural areas, urban areas have been identified as hotspots for soil contamination (Santorufu et al. 2012), with sources like petrol stations and mechanical workshops where PAHs can be found (Din et al. 2013), traffic and urban roads as sources of metals (e.g., copper, zinc, lead) or changing soil pH (see e.g., Falkengren-Grerup 1986; Løkke et al. 1996; Blake et al. 1999), and agricultural practices in small gardens leading to increasing levels of several contaminants like metals and organic chemical substances. One of these case studies was performed in China where agricultural plastic film was spotted as a possible important source of soil phthalate ester contamination in a suburban area (Wang et al. 2013).

Lately emerging chemicals like nanomaterials (e.g., nanoparticles) are showing a potential risk to aquatic and terrestrial organisms as they are being used for different applications and are expected to appear in the environment, mainly through sewage sludge discharges. Recently also microplastics have been added to this list of pollutants potentially threatening the soil environment (Rillig et al. 2017), although some biodegradable plastics can also be used by isopods as a food source facilitating the degradation of these materials (Wood and Zimmer 2014).

In addition to chemical stress, natural stress can also affect the performance of soil organisms, including isopods. Considering their evolution from water to land, terrestrial isopods have acquired several features to succeed their appearance in terrestrial environments. One limiting habitat property is moisture content, while temperature, salinity, and UV radiation increase are also of importance. These factors can become stressors on their own when tending to extremes but they can also act as joint stressors in addition to chemicals. This joint effect can be caused by the interaction of stressors in exposure media but most interestingly also inside the organisms. It is known that several environmental conditions like temperature fluctuation patterns will influence an organism's physiology and behaviour, changing therefore the metabolism of chemicals upon exposure or affecting its behaviour, e.g., its aggregation behaviour (Hassall et al. 2012). Donker et al. (1998) showed that zinc was highly toxic to *Porcellio scaber* at higher temperatures mainly due to an increase in isopod metabolism rather than due to the increase of zinc body burdens. UV radiation was also reported to harm isopods (*Porcellionides pruinosus*) by itself, showing that juveniles and pre-adults were



more affected than adults, mainly for energy-related parameters (Morgado et al. 2013). Besides temperature, there are only few studies investigating the effects of exposure conditions on the toxicity of chemicals to terrestrial isopods.

## Routes of exposure

In soil, chemicals may be distributed over different compartments, the soil solid phase, pore water, and air. Isopods living on and in the soil may be exposed to all three compartments, with food acting as another compartment from which chemicals may be taken up. Very little data is available on the way isopods are exposed and on what the relative importance of each route of exposure is. Likely, the relative importance of either route of exposure is dependent on the properties of the chemical, including its volatility, water solubility and sorption or soil/water partition constant, and the properties of the soil, like organic matter content, clay content, and pH. As a consequence of these factors, bioavailability and therefore exposure may be quite different for soil and food. For volatile chemicals inhalation may present another route of uptake, which again will be hard to quantify. It therefore remains hard to predict the role of either route of exposure, and this will also require knowledge of several factors related to isopod behaviour (see Løkke and Van Gestel 1998 for some considerations on routes of exposure).

In the toxicity tests described in the literature, generally two routes of exposure have been tested, usually separately: exposure to food only or to soil only (see Table 1 for a summary of literature data on the toxicity of selected chemicals to isopods; Suppl. material 1: Table S1 in the Supporting Information provides a more detailed but not exhaustive overview of toxicity data). In few cases, however, animals were exposed to both treated food and treated soil. In the latter case, it should be noted that it will be very hard to realise the same exposure in terms of bioavailable concentrations in food and soil as properties may also be quite distinct with food generally containing much higher organic matter contents than soil (but with different properties). As a consequence, Fischer et al. (1997) found that dimethoate was more toxic for *Porcellio scaber* when mixed in with soil than with food. This finding was supported by Hornung et al. (1998b) with data for copper and LAS, using the same species, by Sousa et al. (2000) studying the accumulation of lindane in *Porcellionides pruinosus* and by Vink et al. (1995) determining toxicity of benomyl, carbofuran, and diazinon to the latter species.

Food can be an important route of exposure, especially in case of input from the air with freshly fallen decaying leaves containing high levels of pollution. Food exposure may also be more uncertain and more difficult to quantify due to the possibility of isopods avoiding contaminated food. It is known that isopods may be able to survive for very long time without food (Donker 1992), but starvation will of course affect sublethal endpoints like reproduction by affecting energy reserves.

Vijver et al. (2005) demonstrated that surface adsorption of metals is negligible compared to uptake, so dermal uptake seems less relevant than oral uptake unless metals may pass the skin to be internalised rapidly. As mentioned above, soil exposure was

**Table 1.** Summary of data on the toxicity of chemicals to isopods in different tests with exposures in soil or through food. For each chemical, species and endpoint, the lowest value is reported. For a more complete overview of data, it is referred to the Supporting Information.

Test compound	Species	Soil/food	Time (d)	Criterion	Endpoint	Result (mg/kg dry soil or food)	Reference
2-phenyl-ethyl isothiocyanate	<i>Porcellio scaber</i>	food	28	LC50	survival	>1000	Van Ommen Kloeke et al. 2012
		Lufa2.2	28	LC50	survival	65.3	
3-phenylpropionitrile	<i>Porcellio scaber</i>	food	28	LC50	survival	>1000	Van Ommen Kloeke et al. 2012
		Lufa2.2	28	LC50	survival	155	
abamectin	<i>Porcellio scaber</i>	Lufa 2.2	21	LC50	survival	69	Kolar et al. 2008
		Lufa 2.2	21	NOEC	weight loss	3	Kolar et al. 2010
AgNO <sub>3</sub>	<i>Porcellionides pruinosus</i>	food	14	EC50	growth	233	Tourinho et al. 2015
			14	LC50	survival	396	
		Lufa 2.2	14	EC50	consumption	56.7	
			2	EC50	avoidance	13.9	
AgNPs	<i>Porcellionides pruinosus</i>	food	14	EC50	growth	>1500	Tourinho et al. 2015
			14	LC50	survival	>455	
		Lufa 2.2	14	EC50	growth	114	
			2	EC50	avoidance	15.8	
benomyl	<i>Porcellionides pruinosus</i>	2 Soils	14	LC50	survival	>1000	Jänsch et al. 2005
benzo[a]anthracene	<i>Oniscus asellus</i>	food	329	NOEC	growth	3	Van Brummelen et al. 1996a
	<i>Porcellio scaber</i>	food	112	NOEC	growth	>9.6	
benzo[a]pyrene	<i>Oniscus asellus</i>	food	63	NOEC	growth	10.6	Van Brummelen et al. 1996a
	<i>Porcellio scaber</i>	food	63	NOEC	growth	10.6	
bisphenol A	<i>Porcellio scaber</i>	sandy soil	112	NOEC	growth	≤10	Lemos et al. 2010
			70	LC50	survival	910	Lemos et al. 2009
carbendazim	<i>Porcellionides pruinosus</i>	2 Soils	14	LC50	survival	>1000	Jänsch et al. 2005
Cd	<i>Armadillidium vulgare</i>	food	21	NOEC	MT/ HSP70 expression	43.14	Mazzei et al. 2015
	<i>Oniscus asellus</i>	food	91	LC50	survival	~1600	Crommentuijn et al. 1994
	<i>Porcellio scaber</i>	food	308	LC50	survival	86	Crommentuijn et al. 1995
			70	EC10	growth/biomass	1.35	Abdel-Lateif et al. 1998
			21	LOEC	food selection	20	Zidar et al. 2005
			21	NOEC	moulting/survival	>200	
	<i>Porcellionides pruinosus</i>	food	28	EC50	egestion ratio	370	Loureiro et al. 2006
			28	LOEC	assimilation efficiency	19850	
chloranthraniliprole	<i>Porcellio scaber</i>	Lufa 2.2	32	LC50	survival	>1000	Lavtizar et al. 2016
			32	NOEC	growth	≥1000	

Test compound	Species	Soil/ food	Time (d)	Criterion	Endpoint	Result (mg/kg dry soil or food)	Reference
chlorpyrifos	<i>Porcellionides pruinosus</i>	Lufa 2.2	14	NOEC	biomass	≥3	Morgado et al. 2016
Cu	<i>Porcellio scaber</i>	food	28	EC10	growth	45	Farkas et al. 1996
			28	LC50	survival	1117	
		Lufa 2.2	28	NOEC	growth	500	Hornung et al. 1998a
			28	LC50	survival	3755	
	<i>Porcellionides pruinosus</i>	food	28	EC50	consumption ratio	1038	Loureiro et al. 2006
			28	EC50	egestion ratio	483	
			28	LOEC	assimilation efficiency	>10500	
		Lufa 2.2	2	EC50	avoidance behavior	802	Loureiro et al. 2005
dimethoate	<i>Porcellio scaber</i>	food	28	LC50	survival	>75	Fischer et al. 1997
			28	NOEC	growth	>75	Hornung et al. 1998
		2 soils	28	EC10	female gravidity	3.8	Fischer et al. 1997
		Lufa 2.2	28	NOEC	growth	10	Hornung et al. 1998a
			28	NOEC	food consumption	10	
	<i>Porcellio dilatatus</i>	black silt	2	NOEC	active time	<5	Engenheiro et al. 2005
	<i>Porcellionides pruinosus</i>	Lufa 2.2	2	EC50	avoidance behavior	28.7-39.7	Loureiro et al. 2009; Santos et al. 2010
doramectin	<i>Porcellio scaber</i>	Lufa 2.2	21	LC50	survival	>300	Kolar et al. 2008
endosulfan	<i>Porcellio dilatatus</i>	food	21	NOEC	glycogen / lipids	<0.1	Ribeiro et al. 2001
fluoranthene	<i>Oniscus asellus</i>	food	329	NOEC	growth, reproduction	>267	Van Brummelen et al. 1996a
fluorene	<i>Oniscus asellus</i>	food	329	NOEC	protein (females)	7	Van Brummelen et al. 1996a
	<i>Porcellio scaber</i>		112	NOEC	growth	>219	
glyphosate	<i>Porcellionides pruinosus</i>	Lufa2.2	2	EC50	avoidance behavior	39.7	Santos et al. 2010
imidacloprid	<i>Porcellio scaber</i>	food	14	NOEC	growth	5	Drobne et al. 2008
		Lufa 2.2	28	LC50	survival	7.6	De Lima e Silva et al. 2017
lambda-cyhalothrin	<i>Porcellionides pruinosus</i>	2 Soils	14	LC50	survival	0.5-1.4	Jänsch et al. 2005
			14	EC50	reproduction	0.13-0.4	
lasalocid	<i>Porcellio scaber</i>	Lufa 2.2	28	NOEC	growth	202	Žižek and Zidar 2013
			2	NOEC	avoidance behavior	<4.51	

Test compound	Species	Soil/ food	Time (d)	Criterion	Endpoint	Result (mg/kg dry soil or food)	Reference
mancozeb	<i>Porcellionides pruinosus</i>	Lufa 2.2	14	NOEC	biomass	176	Morgado et al. 2016
Ni	<i>Porcellionides pruinosus</i>	Lufa2.2	1-8	NOEC	integrated biomarkers	50	Ferreira et al. 2015
parathion	<i>Porcellio dilatatus</i>	food	21	NOEC	AChE	<0.1	Ribeiro et al. 1999
Pb	<i>Armadillidium vulgare</i>	food	21	NOEC	MT/ HSP70 expression	478	Mazzei et al. 2015
	<i>Porcellio scaber</i>	food	80	NOEC	oxygen consumption	1178	Knigge and Köhler 2000
	<i>Porcellionides pruinosus</i>	food	28	EC50	egestion ratio	14050	Loureiro et al. 2006
			28	LOEC	assimilation efficiency	>42070	
			28	LOEC	growth efficiency	>31790	
phenanthrene	<i>Oniscus asellus</i>	food	329	NOEC	growth, reproduction	>235	Van Brummelen et al. 1996a
	<i>Porcellionides pruinosus</i>	Lufa 2.2	14	LC50	survival	110-143	Tourinho et al. 2015
			14	EC50	biomass	16.6-31.6	
spirodiclofen	<i>Porcellionides pruinosus</i>	Lufa2.2	2	EC50	avoidance behavior	0.9	Santos et al. 2010
thiacloprid	<i>Porcellio scaber</i>	Lufa 2.2	28	LC50	survival	>32	De Lima e Silva et al. 2017
			28	EC50	consumption	>32	
TiO <sub>2</sub> NPs	<i>Porcellio scaber</i>	food	3	NOEC	CAT/GST	>3000	Jemec et al. 2008
tributyltin	<i>Porcellionides pruinosus</i>	food	14	NOEC	consumption rate	1	Silva et al. 2014
		soil	14	LC50	survival	99.2	
			2	EC50	avoidance behavior	<0.2	
vinclozolin	<i>Porcellio scaber</i>	sandy soil	70	LC50	survival	298	Lemos et al. 2009
			35	NOEC	molt delay	10	
Zn	<i>Porcellio scaber</i>	food	72	EC50	growth	1916	Van Straalen et al. 2005
			35	NOEC	fecal production	1000	Drobne and Hopkin 1995
	<i>Porcellionides pruinosus</i>	food	28	EC50	consumption ratio	11100	Loureiro et al. 2006
			28	EC50	assimilation efficiency	3650	
			28	EC50	egestion ratio	3520	
		4 Soils	14	LC50	survival	1792-2352	Tourinho et al. 2013
			14	EC50	biomass	312-1400	
	<i>Porcellionides pruinosus</i>	4 Soils	14	LC50	survival	2169-2894	Tourinho et al. 2013
			14	EC50	biomass	119-1951	
ZnO NPs (3–8 nm)	<i>Porcellionides pruinosus</i>	4 Soils	14	LC50	survival	1757->3369	Tourinho et al. 2013
			14	EC50	biomass	713-1479	

shown to be more effective in causing toxicity and bioaccumulation of chemicals in isopods, so it seems that in spite of the low surface sorption, dermal uptake may be an important route of exposure of isopods.

In addition, it should be noted that organisms may affect exposure by their behaviour. Feeding behaviour affects the dietary exposure, while mobility may play a role in determining the degree of contact with soil and therefore may affect soil exposure.

It will be difficult to construct an experiment that completely separates the different exposure routes. This also is not necessary when the focus is not on the mechanisms behind uptake and accumulation but rather on the consequences of exposure in terms of the toxicity. The amount of chemical accumulated instead of the concentration in the environment might provide a suitable measure of exposure and integrates aspects of bioavailability and route of exposure (Escher and Hermens 2004).

It is also not self-evident that the route of exposure will be the same under laboratory and field test conditions. In most standard laboratory tests, the test animal is kept on a relatively thin, homogeneous soil layer and is (if food is provided) forced to feed on a single food item (Hornung et al. 1998a). The only possibility to avoid the polluted food item is to stop feeding, which will only occur when the pollutant makes the food item distasteful or when it is affecting the health of the organism (see e.g., Zidar et al. 2004). In field or mesocosm studies, various food items may be available and test animals may have the ability to escape to less contaminated, deeper layers or to safe micro sites in the heterogeneous soil environment, or simply switch to less toxic food.

### Toxicity testing with isopods: species selection

The first record on relating isopods to terrestrial contamination was a study from Martin et al., in 1976, where the availability and uptake of several metals from woodland litter were recorded and described in the woodlouse *Oniscus asellus*. In 1977, this species was mentioned as a biomonitor of environmental cadmium (Coughtrey et al. 1977) and later, in 1982, *Oniscus asellus* was used by Hopkin and Martin to study the distribution of several metals present in soil. Isopods were mentioned as bioindicators of zinc pollution in England (Hopkin et al. 1986), using species like *Porcellio scaber* and *Oniscus asellus* (Hopkin et al. 1989). *Porcellio scaber* was used as test species in ecotoxicology for the first time in 1978, by Beeby, where the combined uptake of lead and calcium was studied and discussed, continuing later studying the effects of lead assimilation on the brood size of this species (Beeby 1980).

In 1991, *Armadillidium vulgare* appeared as a test species in a study on the sequestration of copper and zinc in the hepatopancreas, and its relation with previous exposure to lead (Tomita et al. 1991). Vink et al. (1995) discussed the importance of exposure routes and for that used the saprotrophic species *Porcellionides pruinosus*. In 1998, *Porcellio laevis* appeared as a test species to be used in laboratory experiments where mortality, body mass and behaviour were recorded upon lead exposure

(Odendaal and Reinecke 1998). One year later, Ribeiro et al. (1999) described the effects of parathion-ethyl and endosulfan-sulfate on enzyme activities (AChE and LDH) of *Porcellio dilatatus*, also showing that this species was a good candidate for biomarkers in ecotoxicity tests.

*Porcellionides pruinosus* has more recently been proposed as a suitable test species for ecotoxicity testing. The advantage of this species is its somewhat shorter life cycle making it easier to culture, and therefore it is more suitable for performing reproduction toxicity tests (e.g., Jänsch et al. 2005). Although this species seems less abundant in temperate zones, and is more representative of Mediterranean and tropical regions, it can easily be tested at 20 °C, which is the standard temperature for most soil toxicity tests.

Based on the above, it remains difficult to recommend one or the other species for toxicity isopod testing. A toxicity test with isopods may therefore use different species, but its duration and design may differ depending on the species chosen.

### **Toxicity testing with isopods: exposure set-up and endpoints**

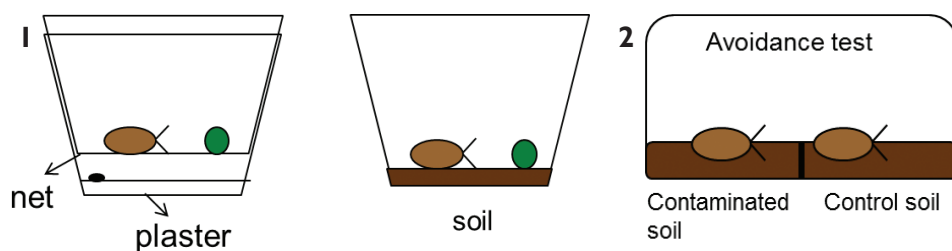
Although no standard test guidelines are available for assessing chemical toxicity to isopods, they are used as test organisms, applying different routes of exposure (food, soil), different test durations, and different endpoints. Table 2 provides an overview of different isopod toxicity tests and the endpoints determined as described in the literature. Figures 1–2 show some typical experimental setups employed for toxicity testing with isopods.

Toxicity tests with isopods in soil use either artificial soil, prepared following the methods described by OECD (1984), or natural soils. Since the work of Hornung et al. (1998a), the use of the German standard soil Lufa 2.2 seems most preferred. This soil has a fairly constant composition, with properties appearing less variable than those of artificial soils prepared by different laboratories (see e.g., Bielska et al. 2012). Generally chemicals are mixed in with the soil at different concentrations to assess toxicity. Food exposure uses different matrices, ranging from intact tree leaves (or pieces of leaf) to finely ground leaf material or pellets prepared from tree leaves, sometimes mixed with some other materials. In case intact pieces of leaf are used, they are either soaked, smeared or sprayed with solutions containing different concentrations of the test chemical to achieve a range of exposure. Ground leaves of pelleted food may be spiked by mixing in the test chemicals at different concentrations. In all cases, both soil and food, a control without test chemical is included. In case the chemical has low water solubility and spiking the chemical required a solvent, a solvent control is included as well. Another methodology has been also applied by mixing chemical powder directly in soil and afterwards adjusting its moisture content, which has proved to be a good and reliable technique (Loureiro et al. 2005).

**Table 2.** Overview of isopod toxicity and bioaccumulation test methods described in the literature. References are just given as an example; in many cases several papers are available describing a more or less similar method of testing. For an overview of toxicity data generated using these methods, see Table 1 and also Table S1 in the supporting information.

Toxicity test	Species	Age	Exposure time (days)	Route of exposure	Endpoints	Test validity criteria	References
acute toxicity	<i>Porcellionides pruinosus</i>	adult	14	soil	survival		Tourinho et al. 2013
growth toxicity	<i>Porcellio scaber</i>	juvenile	28	soil (artificial and natural)	survival, biomass change	control mortality <20%	Hornung et al. 1998a
				food pellets, leaf litter	survival, biomass change	control mortality <20%	
reproduction toxicity	<i>Porcellio scaber</i>	adult	up to 70	soil (artificial and natural)	survival, oosorption, gravid females, offspring	control mortality <20%	Hornung et al. 1998a
	<i>Porcellio scaber</i>	adult	up to 70	food pellets, leaf litter	survival, oosorption, gravid females, offspring	control mortality <20%	
	<i>Porcellio dilatatus</i>	adult	54	food (lettuce incorporated in gelatine)	survival, time to pregnancy, pregnancy duration, abortions, juveniles		Calh��a et al. 2012
feeding inhibition	<i>Porcellio scaber</i>	adult	21	food (pellets)	food consumption rate, chemical assimilation, growth, moulting and survival		Zidar et al. 2005
	<i>Porcellionides pruinosus</i>	pre-adult	14	soil	consumption rate, assimilation rate, biomass change		Tourinho et al. 2013; Silva et al. 2014
				food (leaf litter)	consumption rate, assimilation rate, excretion rate, biomass change		Loureiro et al. 2006
feeding inhibition	<i>Porcellio scaber</i> <i>Oniscus asellus</i>	adult	35	food (leaf litter)	feeding rate, excretion rate, assimilation efficiency, accumulation, chemical ingestion		Drobne and Hopkin 1995
	<i>Porcellio scaber</i>	adult	28	food (leaf litter or pellets)	body mass gain, food consumption, gravid females, juveniles		Farkas et al. 1996
			84	food (leaf litter or pellets)	survival		
avoidance behaviour	<i>Porcellionides pruinosus</i>	adult	2	soil	% avoidance, habitat function	no avoidance in control vs control	Loureiro et al. 2005
foraging behaviour	<i>Porcellio scaber</i>	adult	2	food	preference (video tracking)		Zidar et al. 2005
bioaccumulation	<i>Porcellionides pruinosus</i>	pre-adult	40 (21 uptake; 19 elimination)	soil	bioaccumulation, kinetics		Sousa et al. 2000
		pre-adult	41 (21 uptake; 20 elimination)	food (leaf litter)	bioaccumulation, kinetics		
bioaccumulation	<i>Porcellio scaber</i>	adult	32 (16 uptake; 16 elimination)	Leaf powder	bioaccumulation kinetics		Kampe and Schlechtriem 2016





**Figures 1–2.** **1** Design of feeding inhibition tests with isopods, applying exposure through food (left) or to contaminated soil with contaminated or uncontaminated food (right). In the test with contaminated food only, the animals are kept on a net or gauze allowing also for collecting faeces produced; this will enable estimating food assimilation efficiency. By offering the animals pre-weighed disks or pieces of leaf, food consumption can easily be determined. **2** Design of an avoidance test with isopods. The test uses containers with two compartments. One compartment is filled with contaminated soil, the other one with clean soil. After two days of exposure, the position of the animals in the container is checked. By testing a range of concentrations, including a control (clean soil in both compartments), a dose-response relationship for avoidance may be obtained. The test may also be used to assess avoidance responses to field-contaminated soils, but in that case it might be more difficult to find a proper control soil. Drawing made by Paula Tourinho.

Toxicity tests with isopods have been carried out with several endpoints (Tables 1, 2). Along with the systems biology approach, exposures have been carried out using endpoints at the individual, organ, tissue, and cell or even at the molecular level. Tests focusing on whole organism responses (as shown in Table 2) usually range from 7 to 28 days and evaluate parameters like growth, feeding activity, moulting, mortality or behaviour and > 28 days when reproduction is the endpoint (e.g., Hornung et al. 1998a; Drobne and Hopkin 1995; Drobne and Štrus 1996; Loureiro et al. 2005). These parameters are considered ecologically relevant and usually related to the specific ecological properties of isopods or/and their role in soil function. As examples, feeding inhibition tests are closely related with the isopods' function as detritivores, and reduced feeding rate as a consequence of either avoidance or intoxication by ingestion of contaminated food is related to the soil habitat function. Feeding inhibition tests were first developed using contaminated food as exposure route. This could be somehow difficult to transpose to real contamination scenarios and therefore new strategies for using this ecologically relevant parameter were developed. New studies on the effects of soil contamination on the feeding activity of isopods have been carried out, along with effects on their biomass (Tourinho et al. 2013).

Avoidance response behaviour to contaminated food or soil is also the fastest endpoint, with test durations of no more than two days. No standard test guideline for avoidance tests with isopods is available, but such tests have been done by e.g., Loureiro et al. (2005) and Zidar et al. (2005) using *Porcellionides pruinosus* and *Porcellio scaber*, respectively. The latter test did not only focus on avoidance but also included foraging behaviour as an endpoint. Avoidance tests may be as sensitive as reproduction

for some chemicals, while for others it is at least as sensitive as survival. Behavioural response studies with isopods have been developed due to organisms' ability to detect chemicals, by using their chemoreceptors located on the second antenna, as a major advantage for establishing avoidance behaviour tests (Loureiro et al. 2005). The methodology proposed by Loureiro et al. (2005) was adapted from the earthworm avoidance behaviour test (ISO 2008). It is easy to perform and suitable to evaluate laboratory soils spiked with organic chemicals, metals, single and as binary mixtures (Loureiro et al. 2009), but also with natural contaminated soils from an abandoned mine (Loureiro et al. 2005).

Another interesting endpoint that is directly related to decomposition is the microbiome in the isopod gut (Drobne et al. 2002). This may also provide information on how colonisation and further decomposition of isopod faeces in the environment is influenced by the presence of chemicals.

In addition, mortality, growth, and reproduction are more related to the population level and bridge the gap to higher levels of biological organisation. These studies are more difficult to perform, as mentioned above, due to the life-span of isopods and their moulting behaviour. Reproduction tests with isopod have been performed in a 48-week exposure test via food contaminated with PAHs using *Oniscus asellus* (Van Brummelen et al. 1996a), and by Lemos et al. (2010) using *Porcellio scaber* exposed to bisphenol A and vinclozolin.

Looking at lower organisational levels, biomarkers are defined as any measurable biochemical, cellular, histological, physiological or behavioural change that can provide evidence of exposure and/or effects from one to more contaminants (Van Gestel and Van Brummelen 1996). Different kinds of biomarkers have been studied in isopods and some were successfully implemented in field studies. The isopods' hepatopancreas has been used as a key organ to evaluate deleterious effects due to chemical bioaccumulation. The hepatopancreas is a storage organ where detoxified, sequestered chemicals (metals) can be stored but it also plays an important role in the animal's metabolism. The hepatopancreas contains two cell types, the Big cells (B cells) and the Small (S cells), that differ in their excretion behaviour. The S cells are known to accumulate large amounts of metals, mainly related to metal storage, and B cells are renewed frequently, therefore playing the main role in excretion (Hopkin and Martin 1982). The histopathological changes of isopods' digestive glands have also been characterised and studied (Drobne and Strus 1996; Odendaal and Reinecke 2003, 2004; Lapanje et al. 2008; Lešer et al. 2008). The neonicotinoid insecticide imidacloprid showed to affect the digestive gland epithelial thickness in *Porcellio scaber* (Drobne et al. 2008). In addition, and for the species *Porcellionides pruinosus* the mean percentage of cellular area (thickness) of digestive gland epithelia was also significantly lower in animals from the smelting work compared to animals from unpolluted sites. Later studies revealed that epithelial thickness can be significantly affected by starvation (Lešer et al. 2008).

In order to study endocrine disruptor effects, Lemos et al. (2009) studied moulting behaviour and total ecdysteroid (20E) concentration of *Porcellio scaber* upon exposure to bisphenol A and vinclozolin. At the molecular or enzymatic level, isopods

have also been used to evaluate the response to oxidative stress and neurologic effects. Several biomarker methodologies have been adapted from other organisms' protocols to be used in isopods. Stress proteins (e.g., heat-shock proteins; HSPs) were studied in *Oniscus asellus* as a molecular marker of multiple metal exposure, while cholinesterase activity was measured in *Porcellio dilatatus* upon exposure to dimethoate and related with locomotor activity (Engenheiro et al. 2005). Other biomarkers have also been used to evaluate chemical exposure, such as lysosomal membrane stability (Nolde et al. 2006), glutathione S-transferase (GST) or catalase (Jemec et al. 2008), or energy reserve contents, by studying the effects of chemicals on the lipid, protein and glycogen contents (Donker 1992). To improve biomarker methodologies in isopods and also to use basal levels as foundations for isopod exposure or just for isopod health status in cultures, Ferreira et al. (2010) characterised the basal levels of several biomarkers and energy reserves in *Porcellionides pruinosus*.

These biomarkers have been used to detect effects of individual chemicals but also to unravel modes of actions of chemicals and explain their effects when present in the environment as mixtures. In the study of Santos et al. (2010a), molluscicide baits induced extreme effects to the isopod *Porcellionides pruinosus*. In this study glutathione S-transferase (GST), acetylcholinesterase (AChE) and catalase (CAT) were analysed upon exposure to single chemicals and to binary mixtures. Although the carbamate methiocarb significantly inhibited AChE activity, no oxidative stress was detected (by using CAT and GST levels). On the other hand, metaldehyde showed a completely different mode of action with no effects on AChE, but inducing a decrease in GST activity as well as a general increase in CAT activity. The combined exposure to the two molluscicides resulted in a general decrease in AChE and CAT activity, but no visible effects were observed in terms of GST activity.

HSPs, initially discovered in salivary glands of *Drosophila* exposed to heat (Ritossa 1962), were intensively investigated in isopods in the 1990s (Köhler et al. 1992, 2000; Köhler and Eckwert 1997; Eckwert and Köhler 1997; Knigge and Köhler 2000; Arts et al. 2004; Weeks et al. 2004). HSPs in *Porcellio scaber* and *Oniscus asellus* from metal-polluted and unpolluted sites were analysed and compared with semi-field (microcosm) and laboratory studies. In field populations a high inter-site variability of the hsp70 level was detected. The stress response level was positively correlated with metal solubility, C/N ratio and negatively correlated with soil pH and with site-specific pollution history-adaptation (Köhler et al. 2000; Arts et al. 2004; Weeks et al. 2004). Potential adaptation in HSP responses was demonstrated previously in a laboratory study where *Oniscus asellus* from unpolluted and polluted sites were exposed to metals. In non-adapted populations the level of stress proteins increased after exposure to metals, while in metal adapted populations the induction of stress proteins was less prominent or even decreased (Eckwert and Köhler 1997). Some interspecies variation in HSP level was reported as well (Arts et al. 2004; Weeks et al. 2004). In *Porcellio scaber* collected from the vicinity of smelter, the HSP level was comparable to control animals while in *Oniscus asellus* from the same location it was much higher compared to *Porcellio scaber* and control animals (Arts et al. 2004). Arts et al. (2004) concluded

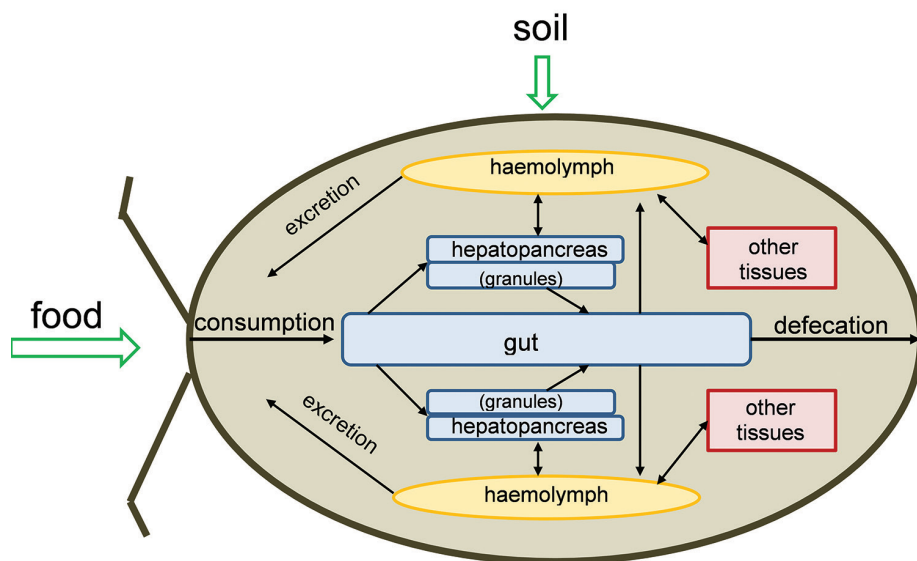
that hsp70 level is a suitable biomarker of effect in non-adapted and adapted populations but of exposure only in non-adapted individuals.

In addition to HSPs, several other biomarkers were studied *in situ* in relation to predominantly metal(s) contamination. Metal storage granules and energy reserves were investigated in *Oniscus asellus* and *Porcellio scaber* in relation to distance to the smelter at Avonmount, UK (Schill and Köhler 2004). *Porcellio scaber* and *Oniscus asellus* showed different response to metal pollution, which was in accordance to what was achieved by Arts et al. (2004). In *Oniscus asellus* the number and size of metal granules increased with decreasing distance to the smelter while the amount of lipids and glycogen decreased. None of this was found in *Porcellio scaber*. Nolde et al. (2006) and Lapanje et al. (2008) investigated lysosomal membrane stability (LMS) of digestive gland cells in *Porcellio scaber* as a biomarker of effect. LMS in animals from highly mercury polluted environments was less affected compared to animals from moderately or unpolluted environments when exposed to mercury. Besides LMS, gut bacterial structure was less affected as well (Lapanje et al. 2008).

## Bioaccumulation of chemicals in isopods

Considering the extremely high metal concentrations found in animals from contaminated areas (see e.g., Hopkin 1989), metal bioaccumulation in isopods has been studied to a great extent. These studies focused on several elements. Initial studies were mainly restricted to assessing whole body metal concentrations in isopods exposed for a fixed time to contaminated soil or food. Later studies also included metal uptake and elimination kinetics, sometimes also in relation to the internal distribution of metals inside the isopod's body (see e.g., Figure 3). Finally, several studies focused more in detail on the mechanisms of metal sequestration in hepatopancreas tissues.

Isopods have been shown to have a tremendous capacity of storing metals in the hepatopancreas. As mentioned above, the hepatopancreas plays an important role in metal sequestration. Due to the high storage capacity of the hepatopancreas, metal uptake kinetics tends to be fairly slow in isopods. Basically, this means that it takes a long time to reach equilibrium, as was shown for cadmium (Vijver et al. 2006). This high capability of sequestering metals is strongly related to storage of the metal in inert fractions, as was shown for zinc and cadmium (Vijver et al. 2006). As a consequence, the lethal body concentration for cadmium is very high in isopods (*Porcellio scaber*, *Oniscus asellus*) compared to other soil arthropods (Crommentuijn et al. 1994). This also explains why metal concentrations in isopods from contaminated sites can reach very high levels without causing the population to go extinct. But it also means that static tests with fixed exposure times will never be able to provide insight into body concentrations to be expected in field-exposed animals. At best, such tests may provide Bioaccumulation Factors (BAFs), relating body concentrations to soil or litter concentrations. Since kinetics is slow, BAFs for metals in isopods will be time-dependent. For that reason, it is preferred to estimate BAFs on the basis of uptake and elimination kinetics parameters



**Figure 3.** Schematic overview of the routes of uptake and internal processing of chemical pollutants in isopods. Adapted from Donker et al. (1996).

derived from toxicokinetics tests rather than from static tests. In addition, it is well known that BAFs for metals are also concentration-dependent (McGeer et al. 2003).

Metal speciation in food may have an effect on metal uptake in isopods, as was for instance shown by Calh  a et al. (2006) and Monteiro et al. (2008). These studies demonstrate that Cd speciation and subcellular distribution in plants influence the assimilation efficiency of Cd in the isopod *Porcellio dilatatus*. Only few studies have addressed the potential for food chain transfer of the inert metal fractions in the hepatopancreas of isopods. Bioaccumulation of organic compounds in isopods has been determined in several studies, but only few studies were performed to assess uptake and elimination kinetics of organic compounds in isopods (e.g., Van Brummelen and Van Straalen 1996; Sousa et al. 2000; Loureiro et al. 2002; Santos et al. 2003; Stroomberg et al. 2004; Kampe and Schlechtriem 2016). From the available literature, it is clear that isopods do show high uptake of metals like Cd, but less of Pb and uptake of organic chemicals may be quite variable. The studies of Van Brummelen and Van Straalen (1996) and Stroomberg et al. (2004) demonstrated that isopods (*Porcellio scaber*) have a high capacity of biotransforming PAHs. As a result these compounds are rapidly eliminated from the isopod's body and PAH concentrations tend to be fairly low in animals from polluted sites when compared to other soil invertebrates like earthworms (Van Brummelen et al. 1996b).

Test methods for determining the uptake and elimination kinetics of organic chemicals in isopods are summarised in Table 2. The methods use either soil or food exposure, and include an uptake phase during which the animals are exposed to contaminated media followed by an elimination phase on clean media. In addition to the

species mentioned in Table 2, *Porcellionides pruinosus*, also other isopod species could be used. Bioaccumulation kinetics approaches may also be used to assess the bioavailability of contaminants in soils. As proposed by Van Straalen et al. (2005) the uptake rates (or initial slope of the uptake curve) determined in uptake and elimination tests may provide an indication of metal bioavailability. Animals can be exposed to soil from the field under controlled laboratory conditions, but such an approach may also be applied to freshly spiked soils. A similar approach was used by Udovič et al. (2013) to assess the remediation efficiency. The disadvantage of such approach is that it neglects the impact of natural environmental conditions. This disadvantage could however, also be seen as an advantage: the bioassay provides an integrated assessment of the situation at the time of sampling, but it could be much more expensive, time- and labour-consuming than just performing chemical measurements.

### The use of isopods in microcosm/mesocosm-based toxicity tests

Single species toxicity tests have several shortcomings (adapted from Van Leeuwen and Vermeire 2007) among others:

1. they are performed under stable and controlled laboratory conditions that are not similar to natural conditions;
2. interactions between different species and interaction with natural stressors are not taken into account;
3. the distribution and degradation of chemicals is ignored;
4. usually genetically homogeneous laboratory raised animals are used.

In contrast to single species toxicity tests microcosms, mesocosms or macrocosms are small, medium, or large multispecies systems that simulate natural situations to a certain degree (Walker et al. 2012). They are more ecologically relevant and compared to full-scale field tests less complex and less expensive. They can be performed in a controlled laboratory environment or under the field conditions. Microcosms with isopods were designed from small containers filled with soil (Eckwert and Köhler 1997, Köhler et al. 2000; Arts et al. 2004; Engenheiro et al. 2005) or sand (Van Wensem 1989) with partly decayed leaves on the surface to complex systems with several plant and animal species (Gunderson et al. 1997; Foerster et al. 2006; Domene et al. 2010; Santos et al. 2011). Uncontaminated and artificially contaminated soils (Santos et al. 2011) or field-contaminated soils (Köhler and Eckwert 1997; Köhler et al. 2000; Arts et al. 2004) were used. Microcosms were exposed to the field conditions (Eckwert and Köhler 1997, Köhler et al. 2000; Arts et al. 2004), to greenhouse semi-field conditions (Gunderson et al. 1997; Engenheiro et al. 2005) or performed in the laboratory (Foerster et al. 2006; Santos et al. 2011). Chemicals were applied by spraying (Santos et al. 2011) or introduced into the soil (Domene et al. 2010). Besides mortality, growth, and reproduction (Gunderson et al. 1997; Foerster et al. 2006), carbon dioxide pro-



duction (Van Wensem 1989), locomotor behaviour (Engenheiro et al. 2005) and diverse molecular biomarkers (Köhler and Eckwert 1997; Köhler et al. 2000; Arts et al. 2004; Engenheiro et al. 2005; Santos et al. 2011) were measured.

The main disadvantage of such multispecies tests is that the more they imitate a natural environment the more difficult they are to replicate and to standardise. Microcosm tests are higher tier tests, usually designed to test a specific hypothesis, and not to be used routinely.

## Isopods in field studies and biomonitoring

Field studies may take both a predictive and a diagnostic approach. In case of a predictive approach, field studies are just the next step after micro- or mesocosm-based toxicity tests described above. Such tests have rarely been done with isopods. Diagnostic field studies are mainly performed within the framework of monitoring, in order to assess the occurrence of effects at (contaminated) field sites. This section will mainly focus on the latter approaches.

Field (*in situ*) studies on the effects of pollutants on biota bridge the gap between laboratory-conducted toxicity studies and abiotic measuring of pollution. An increased concentration of a pollutant in the environment does not necessarily mean disruptive effects to biota. To cause toxic effects, a chemical needs to be sensed or taken up by the organism; therefore, bioavailability is crucial for toxicity. Moreover, in the field organisms simultaneously respond to a variety of anthropogenic and also natural stressors with antagonistic and synergistic actions among them. Therefore, biological monitoring is important to measure the disruptive effects of pollutants to biota. There are four main approaches to biological monitoring of pollution (adapted from Hopkin 1993; see also Walker et al. 2012):

1. Monitoring the changes in community structure; absence or presence of a particular species indicates particular pollution.
2. Measuring the concentration of pollutants in a tolerant indicator species; body concentrations indicate bioavailability of pollutants and also indicate the toxicity level to other more sensitive related organisms.-
3. Measuring the effects of pollutants on organisms; physiological, biochemical, cellular and other markers at the organism level can be used as a screening tool in monitoring.
4. The detection of genetically different populations of species that have evolved resistance in response to a pollutant.

In 1975 a marine monitoring scheme ‘The mussel watch’ was proposed to follow the level of marine contamination with metals, artificial radionuclides, petroleum and chlorinated hydrocarbons (Goldberg 1975). This has led to a considerable effort to find a terrestrial invertebrate group equivalent to mussels to monitor contamination and bioavailability of metals on land. Attributes of mussels that also terrestrial invertebrates had to fulfil were:



common and widespread, large populations, resistant to pollutants, bioaccumulation, and long half-lives of pollutants once accumulated in the body. Isopods, already known at that time by their remarkable ability to accumulate Cu (Wieser 1961, 1968; Wieser and Markart 1961), were soon suggested as a useful tool for monitoring available Cu (Wieser et al. 1976), as well as Cd and Pb (Martin et al. 1976) in contaminated ecosystems. This suggestion was supported by further work of Wieser et al. (1977) and Coughtrey et al. (1977) on Cu and Cd, respectively. The authors analysed soil and litter metal concentrations in several polluted regions (around smelters and mines) in Austria and England and compared them with concentrations in isopods from the same locations. Mainly three species of isopods were examined: *Tracheoniscus ratkei*, *Oniscus asellus* and *Porcellio scaber*. Total body concentrations of metals and concentrations in different body parts (hepatopancreas, gut, ovaries, and exoskeleton) were analysed. The main findings were:

1. concentrations of Cu and Cd in isopods increase with increasing metal concentrations in litter (Martin et al. 1976; Wieser et al. 1976, 1977; Coughtrey et al. 1977);
2. the Bioaccumulation Factor isopods/litter (BAF) for Cu (Wieser et al. 1977) and Cd (Martin et al. 1976) is around 6, the highest among terrestrial invertebrates;
3. mean Cu content in isopods is highly correlated with Cu concentration in litter but less with Cu concentration in soil (Wieser et al. 1977);
4. Cu and Cd content in isopods is related to body weight (Wieser et al. 1976, 1977; Coughtrey et al. 1977); and
5. Cu content in isopods fluctuates with season and it is temperature-dependent (Wieser et al. 1977).

Almost ten years later, results from a large field study were published (Hopkin et al. 1986). Concentrations of Zn, Cd, Pb and Cu in litter from 89 sites around smelting works in the Avonmouth area, south-west England were mapped together with metal concentrations in *Porcellio scaber* (hepatopancreas and whole animal concentrations) (Hopkin et al. 1986). In the next study by Hopkin et al. (1993) Zn, Cd, Pb, and Cu concentrations in *Porcellio scaber* were compared with concentrations in *Oniscus asellus* from the same sites and correlated with soil concentrations. Main findings of these studies were:

1. concentrations in isopods also correlate with soil or litter concentrations of Zn and Pb, like Cd and Cu (Hopkin et al. 1986, 1993);
2. Zn, Pb, Cd, and Cu concentrations in the hepatopancreas correlate with whole animal concentrations at all sites (Hopkin et al. 1986), thus analyses of separate body parts are not necessary;
3. BAFs for Zn and Pb are much lower compared to Cu and Cd (Hopkin et al. 1986);
4. BAFs related to litter are much higher compared to soil and vary greatly between locations; and
5. correlation between *Porcellio scaber* and *Oniscus asellus* is closer than between isopods and soil, therefore concentrations in *Oniscus asellus* and probably also in other invertebrate groups can be predicted from those in *Porcellio scaber* (Hopkin et al. 1993).

Isopods were also studied in urban areas as bioindicators for Zn (Hopkin et al. 1989) as well as Pb and Cd pollution (Dallinger et al. 1992). *Porcellio scaber* and *Oniscus asellus* were sampled from 63 sites in Reading, England (Hopkin et al. 1989) and 356 points over the city of Innsbruck, Austria (Dallinger et al. 1992). As leaves were not present in most of the urban sites, soil was sampled at the same locations. The main findings were:

1. Zn concentrations in *Porcellio scaber* were about two times higher than those in *Oniscus asellus* at each site (Hopkin et al. 1989);
2. sources of Zn pollution can be identified according to soil or isopod samples on a small scale (0.5 km apart) but not on a large scale (5 km apart) (Hopkin et al. 1989);
3. Pb concentrations in isopods correlate with traffic density in individual districts of the city (Dallinger et al. 1992).

Field studies where isopods were used in monitoring of contamination with organic chemicals are very rare compared to metal contamination. Some laboratory studies showed that isopods may also accumulate organic chemicals, like veterinary pharmaceuticals (Kolar et al. 2010) or rodenticides (Brooke et al. 2013). The antiparasitic abamectin accumulated in *Porcellio scaber* in a dose-dependent manner (Kolar et al. 2010) but no data about its retention time were provided. The anticoagulant rodenticide brodifacoum that accumulates in isopods (different species) was measurable even after 45 days after exposure (Brooke et al. 2013). Bioaccumulation of PAHs was also shown (Van Brummelen et al. 1996b), but concentrations were relatively low compared to e.g., earthworms, which might be attributed to the high capability of isopods to biotransform and eliminate these compounds (Van Brummelen and Van Straalen 1996; Stroomberg et al. 2004).

All these studies showed that isopods, particularly *Porcellio scaber*, have favourable attributes to become a leading organism in terrestrial biomonitoring (see e.g., Paoletti and Hassall 1999), especially of metal contamination. Nonetheless, they never reached the status of *Mytilus edulis* in marine biomonitoring, just as no other terrestrial invertebrate group has. There are several reasons for that. All the studies mentioned above showed a certain discrepancy between metal concentrations in isopods and in soil or litter from different locations with comparable metal contamination. As the digestive system is the main route for metal intake in terrestrial isopods (reviewed by Hopkin 1989), their internal metal concentration is actually a measure of metal availability in their food source and not in the soil. Metal concentrations in isopods were in fact more significantly correlated with metal concentrations in litter than in soil. In the field, the isopod diet consists mainly of leaf litter, but the exact food source is often hard to define and in some urban locations also hard to collect. Besides, animals are mobile and their resting locations are not necessarily their feeding locations. To make things even more complex, isopods also discriminate food sources due to different plant species, plant defences (e.g., tannins) and state of microbial decay (Hassall and Rushton 1984; Gunnarsson 1987; Szlávecz and Maiorana 1990) and can probably also differentiate certain contaminants (Loureiro et al. 2005; Zidar et al. 2012; Žižek and Zidar 2013), including metals (Zidar et al. 2005).

The previously mentioned studies also demonstrated a strong correlation between body metal concentrations and isopod body mass. It was therefore suggested to compare animals from the same weight class (Wieser et al. 1977) or to use the slope of regression lines (weight – metal concentration) rather than mean metal concentration for comparison between locations, as population-size structure from different locations may vary (Coughtrey et al. 1977). But size and weight are no reliable markers for age, which indicates time of exposure. The overlap of age classes could occur when the weight of younger but better fed isopods exceeds the weight of individuals born in the previous year (Hopkin 1989). Overlap of age classes might also explain the seasonal fluctuation of copper content in isopods with the maximum in winter and minimum in summer (Wieser et al. 1977), when this year's generation of isopods already starts to reproduce. Witzel (1998) reported that accumulation of Pb and Cd in *Porcellio scaber* shows two different phases. Up to the age of 2–3 months assimilation exceeds the rate of growth and leads to rapidly increasing concentrations. After 3 months (at a mass of around 5 mg fresh weight) the rate of accumulation is proportional to the rate of growth and the heavy metal concentrations remain on a stabilised level. Besides, size of isopods varies between polluted and unpolluted sites. Donker et al. (1993) reported that females from *Porcellio scaber* populations near a Pb mine and a Zn smelter reproduced at lower weight. This might indicate slower growth or earlier maturity compared to females from clean environments. A significant difference in the mean and maximum size of *Porcellio scaber* has also been reported among sites in the Avonmouth area in England (Jones and Hopkin 1998). At the most polluted sites populations with the smallest maximum size were found, which might be related to higher energy expenditure for detoxification but also to a genetic differentiation of populations in polluted environments.

In the terrestrial environment metal contamination on one side and isopod distribution on the other are influenced more prominently by local environmental conditions compared to marine ecosystems. Amount and distribution of rainfall during the year, wind directions and relief of the landscape together with soil chemistry and vegetation influence the deposition, retention, and availability of metals to biomonitoring organisms on one side and appropriate conditions for their living on the other. All this makes 'a global woodlouse watch scheme' (Hopkin et al. 1993) even more difficult. Nevertheless, attempts to use isopods as indicators of metal pollution are still being made, as for instance shown by a study on the use of isopods as an indicator of mercury pollution (Pedrini-Martha et al. 2012; Longo et al. 2013).

Faber et al. (2013) proposed a conceptual approach for implementing ecosystem services in monitoring frameworks. They showed that isopods can also be included in monitoring soil quality and in that way contribute to the assessment of possible effects of land use and other human activities on ecosystem services.

## Conclusions

Isopods are important organisms in terrestrial ecosystems. For that reason they should be considered as test organisms in soil ecotoxicology. A standardised test with isopods

could be a relevant and important addition to the existing battery of toxicity tests with soil invertebrates. The difficulties in culturing and testing could be overcome by selecting species with shorter life cycles, like *Porcellionides pruinosus*, and by putting more effort in optimising culture conditions for species like *Porcellio scaber*. This may also help developing standardised toxicity tests that include more relevant endpoints like reproduction, in addition to growth and feeding activity. Little insight exists in the difference in sensitivity of isopod species to different chemicals. The harmonisation between exposure time, the existence of validation criteria based on basal levels for optimum exposure (considering temperature and time) and common endpoints could be a step forward for the accuracy improvement and comparison between studies. Isopods are also relevant and useful organisms for use in field monitoring approaches, for instance to assess the bioavailability of metals, possible (post-registration) effects of pesticide use, exposure to (mixtures of) chemicals and in biological soil quality networks aimed at protecting ecosystem services.

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## Supplementary material I

### Supporting information

Authors: van Gestel CAM, Loureiro S, Zidar P

Data type: references

Explanation note: Table S1: Overview of literature data on the toxicity of selected chemicals to isopods exposed through food or soil.

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# A molecular phylogeny of Porcellionidae (Isopoda, Oniscidea) reveals inconsistencies with present taxonomy

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## Abstract

Porcellionidae is one of the richest families of Oniscidea, globally distributed, but we still lack a comprehensive and robust phylogeny of the taxa that are assigned to it. Employing five genetic markers (two mitochondrial and three nuclear) we inferred phylogenetic relationships among the majority of Porcellionidae genera. Phylogenetic analyses conducted via Maximum Likelihood and Bayesian Inference resulted in similar tree topologies. The mtDNA genes cytochrome oxidase I (COI) and 16s rRNA (16s) were used for clade dating using previously published mutation rates. Our results provide evidence against the monophyly of both Porcellionidae and the largest genus of the family, *Porcellio*. These results are compared to previous published work based on morphological evidence. The genera *Leptotrichus* and *Brevurus* are not grouped with the rest of Porcellionidae whereas Agnaridae are grouped with part of Porcellionidae. *Armadillidium* and *Schizidium* (Armadillidiidae) occupy a basal position on the phylogenetic tree. Even though the African genera *Tura* and *Uramba* (distributed in East Africa) are grouped together, there is no general geographical pattern in other sub-clades. Additional taxonomic issues that arise in this work, such

as the assignment of the recently described genus *Levantonicus*, are also discussed. The status of Porcellionidae should be further revised and morphological characters traditionally used in Oniscidea taxonomy should be reconsidered in view of molecular evidence. The origin of the monophyletic clade within Porcellionidae, as indicated in the present work, is dated back to the Oligocene (~32 mya).

### Keywords

Crinocheta, genetic markers, monophyly, node dating, taxonomic revision, woodlice

## Introduction

The Oniscidea family Porcellionidae is one of the richest in species, with 333 species, belonging to 19 genera, currently assigned to it (Sfenthourakis and Taiti 2015). Family members are unable to conglobate, with the exception of the genus *Atlantidium* Arcangeli, 1936. There is remarkable morphological variation among Porcellionidae species and genera, especially in head structure, pleotelson, and body shape. Familial assignment of taxa is based mostly on the combination of two character states, namely an antennal flagellum with two articles and the presence of monospiracular, covered lungs on the first two pairs of pleopods (Schmidt 2003). However, certain authors, based on morphological and recent molecular work, suggest that these characters could be symplesiomorphies, as they are not exclusively found in Porcellionidae (Schmalfuss and Ferrara 1978, Schmidt 2003).

Different authors have found Porcellionidae to be closely related with Oniscidae, Trachelipodidae, Cylisticidae, Agnaridae or Armadillidiidae (Michel-Salzat and Bouchon 2000, Mattern 2003, Schmidt 2008, Lins et al. 2017). Furthermore, monophyly of the most species-rich genera, *Porcellio* Latreille, 1804 and *Porcellionides* Miers, 1877, has been debated on the basis of both morphology (Vandel 1962, Schmalfuss 1992, 1998) and molecular evidence (Michel-Salzat and Bouchon 2000, Mattern 2003). More specifically, some *Porcellionides* species appear to be more closely related to the genus *Porcellio* (Michel-Salzat and Bouchon 2000, Mattern 2003) or even to the genus *Cylisticus* Schnitzler, 1853 that belongs to another family (Cylisticidae), than to other congeneric species (Michel-Salzat and Bouchon 2000). Hence, also the monophyly of the family has been repeatedly questioned on the basis of both morphological and genetic data (Schmalfuss 1989, Michel-Salzat and Bouchon 2000, Mattern and Schlegel 2001, Schmidt 2003, 2008).

Members of Porcellionidae were originally reported from the circum-Mediterranean region, Atlantic islands, Arabian Peninsula and East Africa. Nowadays they are known from all over the world, being introduced into many regions by human activities (Schmidt 2003). Porcellionidae are considered to be among the isopod species that are better adapted to terrestrial environments, and they can be found in a wide range of habitats, from tropical rainforests to deserts (Schmidt 2003, Medini-Bouaziz et al. 2017).

The present study aims to a more detailed investigation of phylogenetic relationships among genera of Porcellionidae, using two mitochondrial and three nuclear genes that allow estimation of divergence times among extant taxa.

## Materials and methods

### Sampling

Isopod specimens belonging to five Porcellionidae genera, one to Trachelipodidae (*Levantonicus* Cardoso, Taiti & Sfenthourakis, 2015) and two to Armadillidiidae (*Armadillidium* Brandt, 1831 and *Schizidium* Verhoeff, 1901) were collected on Cyprus between 2014 and 2016. Additional specimens came from the collection of the Istituto per lo Studio degli Ecosistemi, deposited in the Museum of Natural History of the University of Florence, and from the personal collection of one of the authors (H.S.). Members of the families Armadillidiidae, Agnaridae and Trachelipodidae that are assumed to be closely related to Porcellionidae were included in the analyses to test the monophyly of the latter, whilst specimens of the more distant families Scyphacidae (*Actaecia euchroa* Dana, 1853) and Philosciidae (*Chaetophiloscia elongata* (Dollfus, 1884) were included as outgroups. More details about specimens used are given in Table 1.

We were not able to include specimens of five Porcellionidae genera, namely the monotypic *Congocellio* Arcangeli, 1950 and *Tropicocellio* Arcangeli, 1950, both distributed in the Democratic Republic of the Congo, *Dorypoditius* Verhoeff, 1942 from Mozambique, *Atlantidium* Arcangeli, 1936 from Madeira, and *Pondo* Barnard, 1937 from South Africa (Pondoland and Natal).

### Molecular analyses

Fresh specimens were placed in 96% alcohol immediately after collection and stored at -20 °C. The majority of samples from museums and private collections had been preserved in 70% alcohol. Whole animals or legs of larger specimens were used for extraction of total genomic DNA using DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany) following manufacturer's instructions. NanoDrop 2000/200c (Thermo Fisher Scientific Inc., USA) was used to determine the final concentration and purity (A260/A280nm absorption rate) of DNA extractions.

### DNA extraction amplification and sequencing

The following mitochondrial and nuclear genetic loci were targeted using common PCR procedures: partial mitochondrial cytochrome *c* oxidase subunit 1 (COI), ribosomal 16S rRNA (16s), the nuclear, non-coding 18S ribosomal RNA (18s) and

**Table 1.** Species, locality of origin, available sequence data from targeted genes, and GenBank accession numbers of individuals used in the molecular phylogenetic analyses.

Species ( <i>code</i> )	Locality	Genes					Acc. No
		COI	16s	18s	28s	NAK	
Porcellionidae							
<i>Proporcellio vulcanius</i> (Verhoeff, 1908) (1)	Cyprus (Larnaca)	√	√		√	√	MG887933/MG887948/-/MG887988/MG887906
<i>Agabiformius excavatus</i> Verhoeff, 1941 (2)	Cyprus (Paphos)		√	√	√	√	-/MG887955/MG887969/MG888009/MG887921
<i>A. excavatus</i> (3)	Cyprus (Paphos)		√			√	-/MG887956/-/-/MG887922
<i>Porcellio laevis</i> Latreille, 1804 (4)	Cyprus (Lemesos)	√	√	√	√	√	MG887936/MG887957/MG887986/MG887993/MG887913
<i>P. laevis</i> (5)	Cyprus (Lemesos)	√	√	√	√	√	MG887937/MG887958/MG887987/ MG887994/MG887914
<i>Porcellionides pruinosis</i> (Brandt, 1833) (6)	Cyprus (Larnaca)	√	√		√	√	MG887934/MG887949/-/MG888010/MG887907
<i>P. pruinosis</i> (7)	Cyprus (Larnaca)	√	√		√	√	MG887935/ MG887950/-/MG887989/MG887908
<i>Leptotrichus kosswigi</i> Strouhal, 1960 (8)	Cyprus (Paphos)				√	√	-/-/-MG888013/MG887915
<i>L. kosswigi</i> (9)	Cyprus (Paphos)		√	√	√	√	-/MG887963/MG887970/MG888014/MG887916
<i>Porcellio nasutus</i> Strouhal, 1936 (10)	Greece (Parnon)	√	√		√	√	MG887944/ MG887953/-/MG887998/MG887910
<i>P. nasutus</i> (11)	Greece (Parnon)		√	√	√	√	-/MG887954/MG887980/MG887999/MG887911
<i>Tura</i> sp. (12)	Kenya (Mombasa)	√	√	√	√	√	MG887946/ MG887966/MG887983/MG888001/MG887920
<i>Caeroplastes porphyrivagus</i> (Verhoeff, 1918) (13)	France (Toulon)	√		√	√		MG887932/-/ MG887981/MG887990/ -
<i>Uramba triangulifera</i> Budde-Lund, 1910 (14)	Kenya (Aberdare National Park )		√		√	√	-/ MG887961/-/MG888002/MG887923
<i>Thermocellio</i> sp. (15)	Tanzania (Dar es Salaam)		√		√		-/ MG887962/-/MG887995/-
<i>Lucasius pallidus</i> (Budde-Lund, 1885) (16)	Italy (Sardinia)			√	√	√	-/-/MG887974/MG887992/MG887917
<i>Mica tardus</i> (Budde-Lund, 1885) (17)	Italy (Sardinia)		√		√		-/ MG887959/-/MG887996/-
<i>Acaeroplastes melanurus melanurus</i> (Budde-Lund, 1885) (18)	Italy (Sardinia)	√	√	√	√	√	MG887945/ G887960/MG887982/ MG887991/MG887912
<i>Soteriscus laouensis</i> Taiti & Rossano, 2015 (19)	Morocco (Tirinese)	√	√	√	√	√	MG887931/MG887964/MG887975/MG887997/MG887918
<i>Brevurus masandaranus</i> Schmalzfuss, 1986 (20)	Iran				√	√	-/-/-MG888008/MG887919
<i>Porcellionides cilius</i> (Verhoeff, 1918) (21)	Cyprus (Nicosia)					√	-/-/-/MG887909

Species ( <i>code</i> )	Locality	Genes					Acc. No
		COI	16s	18s	28s	NAK	
Trachelipodidae							
<i>Levantoniciscus bicostulatus</i> Cardoso, Taiti & Sfenthourakis, 2015 (22)	Cyprus (Paphos)			√	√	√	-/-/MG887976 / MG888000/MG887928
<i>Trachelipus aegaeus</i> (Verhoeff, 1907) (26)	Greece (Naxos)	√	√	√		√	EF659961/KF891440/ MG887984 -/-/MG887925
Agnaridae							
<i>Hemilepistus klugii</i> (Brandt, 1933 (23)	Iran (Isfahan)	√	√	√	√	√	MG887938/MG887951/ MG887978 /MG888011/ MG887926
<i>H. schirazi</i> Lincoln, 1970 (24)	Iran (Shahreza)	√	√	√	√	√	MG887939/MG887952/ MG887979 /MG888012/ MG887927
<i>Agnara madagascariensis</i> (Budde-Lund, 1885) (25)	U.A.E.			√	√	√	-/-/MG887977 / MG888003/MG887924
Armadillidiidae							
<i>Armadillidium vulgare</i> (Latraille, 1904) (27)	Cyprus (Limassol)	√	√	√	√		KR424609/AJ419997/ MG887972/MG888006/-
<i>Schizidium fissum</i> (Budde-Lund, 1885) (28)	Cyprus (Paphos)			√	√		-/-/MG887973/ MG888005/-
Philosciidae							
<i>Chaetophiloscia elongata</i> (Dollfus, 1884) (29)	Italy (Sardinia)	√	√	√	√	√	KJ668161/AJ388091/ MG887971/MG888004/-/ MG887929
Scyphacidae							
<i>Actaecia euchroa</i> Dana, 1853 (30)	New Zealand	√	√	√	√	√	GQ302701/AJ388093/ MG887985/MG888007/ MG887930

28S ribosomal RNA (28s), and the protein coding Sodium-Potassium Pump (NAK). Mitochondrial COI and 16s genes were successfully amplified using the universal LCO1490/HCO2198 (Folmer et al. 1994) and the widely used 16sar/16sbr and 16sar-intsf (Palumbi 1996, Parmakelis et al. 2008) primers, respectively. The primer pairs 18sai/18sbi and 18Aimod/700R (Hermann and Wägele 2001, Raupach et al. 2009) were used for the amplification of 18s, and the 28sa/28sb pair (Whiting et al. 1997) was used successfully for all available samples. Finally, the protein coding NAK amplicons were targeted with NAK for-b/NAK rev 2 (Tsang et al. 2008) and the newly designed reverse primer NAK 638R: 5'-GGD RGR TCR ATC ATD GAC AT -3'.

All PCR reactions were performed in a Veriti thermal cycler (Applied Biosystems, USA) with the following common steps: a) initial denaturation for 5 min at 94 °C, followed by b) 5 cycles of 3 minutes equally separated at 94 °C/60 °C/72 °C, c) 5 cycles of 3 minutes equally separated at 94 °C/55 °C/72 °C, d) 10 cycles of 3 minutes equally separated at 94 °C/50 °C/72 °C, e) 10 cycles of 3 minutes equally separated at 94 °C/47 °C/72 °C, f) 10 cycles of 3 minutes equally separated at 94 °C/42 °C/72 °C, and g) a final extension step of 72 °C for 10 min. Beyond fresh specimens, this touch-

down PCR approach with 50 cycles in total allowed us to successfully amplify genes from ill-preserved samples increasing specificity, sensitivity and yield, eliminating aspecific products (Korbie and Mattick 2008).

The final reaction volume in all cases was 20  $\mu\text{L}$ , and consisted of 0.1  $\mu\text{L}$  of Kapa *Taq* DNA Polymerase (5U/ $\mu\text{L}$ ), 1.2  $\mu\text{L}$  of 25 mM  $\text{MgCl}_2$ , 2  $\mu\text{L}$  of Kapa PCR buffer A, 0.6  $\mu\text{L}$  of 10 mM dNTP (Kapa) 0.6  $\mu\text{L}$  of each primer (10  $\mu\text{M}$ ) and >10 ng of DNA template. PCR product purification was made using Qiaquick Purification Kit (Qia-gen, Germany) under manufactures protocol instructions. Both DNA strands of purified products were sequenced at Macrogen facilities (Amsterdam, The Netherlands).

### Alignments and genetic divergence

Sequence chromatograms were manually edited and assembled with CodonCode Aligner (v. 3.7.1; CodonCode Corp., USA). Separate multiple alignments for each gene/data set were performed using MAFFT v.7 (Katoh et al. 2002). Our data were further enriched by a limited number of publicly available NCBI GenBank mtDNA sequences (Table 1). The final concatenated data set was partitioned by gene into five distinct data blocks. The optimal nucleotide substitution models were identified using PartitionFinder v.1.1.1 (Lanfear et al. 2012). Three independent runs in PartitionFinder were applied, using the greedy search algorithm with linked branch lengths in calculations of likelihood scores under the Bayesian Information Criterion (BIC). The difference between these three runs was the restriction of candidate models to only those that are implemented in MRBAYES v.3.2.6 (Ronquist et al. 2012), BEAST v. 2.3.0 (Bouckaert et al. 2014) or RAxML v. 8.1.21 (Stamatakis 2014). Models that included both gamma distribution and invariable sites were neglected (Yang 2006).

### Phylogenetic analyses

Construction of phylogenetic trees was conducted using Bayesian inference (BI) and Maximum Likelihood (ML) methods. The analysis of BI was implemented in MRBAYES v. 3.2.6 (Ronquist et al. 2012) with four independent runs and eight chains per run for  $3 \times 10^7$  generations, with a sampling frequency of 100. Consequently, the summaries of BI were based on  $3 \times 10^5$  sampled trees from each run. The convergence and stationarity of each run was evaluated by monitoring the average standard deviation of split frequencies of the four simultaneous and independent runs in MRBAYES, and further by inspection of generation versus log probability of the data plot viewed in TRACER v.1.5.0 (Rambaut and Drummond 2007). The  $-\ln$  value reached stationarity well before pre-requested  $10^7$  generations. From the sampled trees, 25% were discarded as burn-in phase. Therefore a majority rule consensus tree relied on 300,004 trees and posterior probabilities were calculated as the percentage of samples recovering any particular clade (Huelsenbeck and Ronquist 2001).



RAxML (v. 8.1.21) (Stamatakis 2014) was recruited for Maximum Likelihood analyses which were conducted using the RAxMLGUI v.1.5 platform (Silvestro and Michalak 2012). The GTR+G model of evolution was used for the estimation of parameters for each partition. The optimum ML tree was selected after 500 iterations and the reliability of the branches was assessed by 1,000 thorough bootstrap replicates (Felsenstein 1985).

### Clock calibration and divergence time estimation

Molecular dating of clades was inferred using BEAST v. 2.3.0 (Bouckaert et al. 2014). The appropriate model of nucleotide substitution, as indicated by PartitionFinder under the BIC criterion was implemented for each marker in our partitioned analysis. Due to the absence of reliable geological or fossil data related to taxa included in our analyses, time of divergence was calibrated based on available gene-specific substitution rates. More specifically, the substitution rates of the mitochondrial genes 16s and COI were used as reported from previous studies for isopods (Held 2001, Poulakakis and Sfenthourakis 2008, Kamilari et al. 2014). Clock rate was set at 0.0007 (substitutions per site per Myr) for 16s and 0.0082 (min rate 0.0078, max 0.0086) for COI.

Four independent runs were performed for 100 million generations, each sampling every 5,000<sup>th</sup> generation. An uncorrelated lognormal relaxed clock under a Yule tree prior and the default options for all other prior and operator settings, were used in each case. Trace plots were inspected in order to compare the divergence estimates across runs and ensure the convergence of Markov Chain Monte Carlo chains using TRACER v. 1.5 (Rambaut and Drummond 2007). Resulting log files were combined, after removing 10% as burn-in, using LOGCOMBINER v.2.3.0 (Bouckaert et al. 2014). A maximum clade credibility tree exhibiting the means of node heights was constructed with TREEANNOTATOR v.2.3.0 (Bouckaert et al. 2014).

### Results

At least four out of five targeted genes were successfully amplified and sequenced for the great majority of available individuals, with final DNA extraction yield over 20 ng/ $\mu$ l and A260/A280 purity rate over 1.5. Since some important samples were old (collected more than two decades ago, mainly from Africa) or ill-preserved for a long time (i.e., in 70% alcohol) we didn't manage to retrieve sequences from all targeted genes. However, specimens not represented by all gene fragments were also included in the analyses. The final concatenated alignment obtained consisted of 3,841 base pairs (bp). More details about the aligned sequences length, conserved, variable and parsimony-informative sites for each gene are given in Table 2.

Available sequences were separated in different groups at the genus level except for *Porcellio* species which were treated as different groups due to the alleged non-monophyly of the genus. Between groups p-genetic distances for each gene are given in Suppl. material 1.

**Table 2.** Aligned bases length, conserved, variable, and parsimony-informative sites for each gene used in the present analysis.

Gene	Alignment length (bp)	Conserved sites	Variable sites	Parsimony informative sites
COI	655	214	434	302
16S	454	151	277	211
18S	863	417	332	177
28S	1167	314	827	567
NAK	702	512	188	109

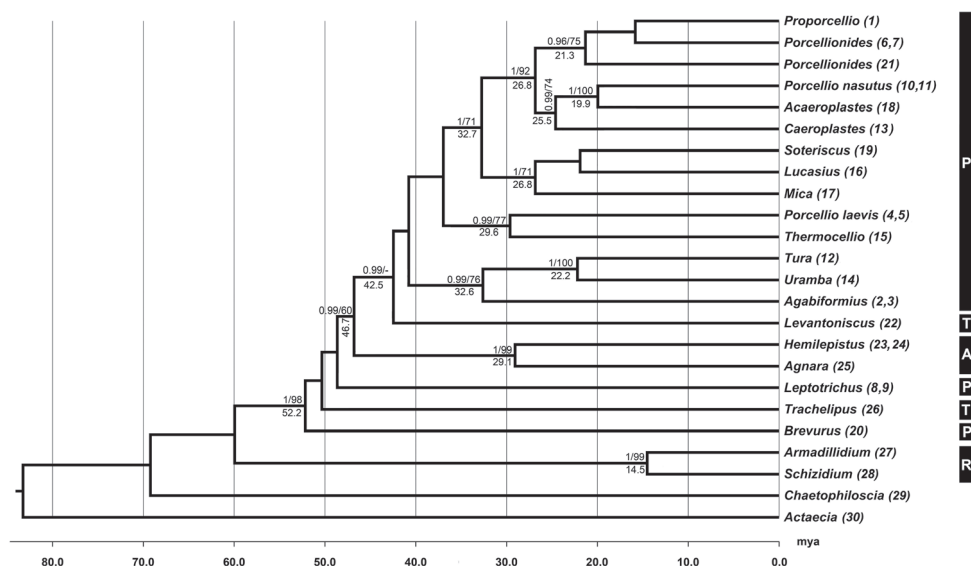
The best-fit nucleotide substitution models for each partition/gene selected under the BIC criterion were (for both MRBAYES and BEAST) the HKY+G+X, HKY+G+X, TRNEF+G, TRN+G, TRN+G+X and GTR+G+X for COI, 16s, 18s, 28s and NAK genes, respectively. The selected model under --raxml commandline option at Partion-Finder was the GTR+G (-ln =26511.0556641) for all genes.

Maximum Likelihood and Bayesian Inference analyses (implemented both in BEAST and MRBAYES) resulted into phylogenetic trees with similar, well-supported topologies. Given the congruence among the results of the two methods, only the Bayesian tree is presented herein (Figure 1). The ML tree is given in Suppl. material 1: (Figure S1). The separate analysis of different gene markers showed that the concatenated tree topology is mainly determined by nuclear genes. Missing data, and possibly also the depth of the phylogeny, led to largely unresolved trees for mtDNA markers. Nevertheless, these were used mainly to estimate node dates based on published mutation rates. The poor mtDNA-based resolution did not affect the final tree, given that the tree based solely on nuclear genes (see Suppl. material 1) has identical topology.

Our results provide evidence against the monophyly of both the family Porcellionidae and the genus *Porcellio*. *Brevurus* appears to belong to a supported distant clade, external to that formed by the remaining Porcellionidae+Trachelipodidae+Agnaridae. *Leptotrichus* is an external branch to Agnaridae + part of Porcellionidae. Monophyly of Agnaridae is supported. *Levantoniscus* forms the sister clade of all monophyletic Porcellionidae. Finally Armadillidiidae branches early in the tree, not showing any close relationship to Porcellionidae.

The African genera *Tura* and *Uramba* are sister taxa sharing a common ancestor at around 22.2 mya (95% HPD 12.1 – 33.5 mya) and are grouped with *Agabiformius*. On the other hand, *Thermocellio*, also distributed in Kenya and the neighboring Tanzania, appears to be more closely related to *Porcellio laevis*, native to Europe and North Africa. Another African/Atlantic genus, *Soteriscus*, forms a well-supported clade with *Lucasius* and *Mica* that are distributed in Africa and on some Mediterranean islands. The Mediterranean genera *Acaeroplastes*, *Caeroplastes*, *Porcellionides* and *Proporcellio*, together with part of *Porcellio*, are grouped in the most derived clade that diverged at around 27 mya.

The genus *Porcellio* as currently perceived is represented in two well-supported separate clades. *P. laevis* groups with *Thermocellio* while *P. nasutus* with *Acaeroplastes* in a clade also including *Caeroplastes*.



**Figure 1.** Dated phylogram based on concatenated data set including five genes (COI, 16s, 18s, 28s, NAK), generated using a relaxed lognormal clock in BEAST. BI posterior probabilities (>0.9) and ML bootstrap values (>60) are presented above the nodes. Estimated mean divergence time is given below the nodes only where nodes are statistically supported or the topology was identical between BI, ML and BEAST analyses. Subclades including individuals from more than one species have been collapsed to genus level, since all (except *Porcellio*) were monophyletic. Abbreviations: P. Porcellionidae, T. Trachelipodidae, A. Agnaridae, R. Armadillidiidae. Numbers in parentheses after each taxon name refer to numbering of taxa in Table 1.

Genetic distances between Porcellionidae genera (or species in the case of the non-monophyletic *Porcellio*) varied significantly among genes. The range of variation per gene is: COI: 16.9–50.3 %; 16s: 16.9–36.5 %; 18s: 3.6–28.5 %; 28s: 0.4–44.2%; NAK: 2.3–9.1%. The p-distances between *Trachelipus* and *Agnara* for NAK, and *P. laevis* and *Lucasius* for 18s, could be artifacts due to the comparatively shorter sequence length in *Agnara* and *P. laevis*, respectively (see Suppl. material 1).

It is worth noticing also that minimum and maximum distances are not exhibited by the same taxa for all genes. More specifically, highest / lowest genetic divergence is found between the following groups: *Tura* - *Porcellio nasutus* / *Soteriscus* - *Leptotrichus* (16s), *Porcellio laevis* - *Lucasius* / *Proporcellio* - *Porcellionides* (COI), *Agabiformius* - *Porcellio nasutus* / *Caeroplastes* - *Acaeroplastes* (18s), *Brevurus* - *Thermocellio* / *Porcellio laevis* - *Thermocellio* (28s) and *Uramba* - *Brevurus* / *Proporcellio* - *Porcellionides* (NAK). The allegedly congeneric *Porcellio* species never exhibit a minimum genetic distance.

## Discussion

This is the first comprehensive study aiming to resolve phylogenetic relationships among Porcellionidae genera using a multi-locus approach, thus increasing reliability

of results. Our findings undermine the monophyly of both the family Porcellionidae and the genus *Porcellio*, in line with suggestions by previous authors (Schmalfuss 1989, Mattern 2003, Michel-Salzat and Bouchon 2000, Schmidt 2003, 2008).

The extremely high genetic distances, which reached up to 50.3 in mtDNA and 44.2 in nDNA, are confirming the vast divergence among taxa within Porcellionidae. Observed inconsistencies of group distances among different genes highlight the usefulness of the multi-locus approach followed herein for a reliable phylogenetic reconstruction of the taxa examined.

In view of the herein estimated phylogeny, a monophyletic Porcellionidae should exclude *Brevurus* and *Leptotrichus*. Moreover, the supposedly subtle morphological differences between *Leptotrichus* and *Agabiformius* that had led to a presumed sister-group relationship between these genera, are misleading, since they are found to be very distant (Schmalfuss 2000, Verhoeff 1908). *Brevurus* has been proposed as a possible synonym of *Porcellium* Dahl, 1916 (a genus of Trachelipodidae) (Khisametidonova and Schmalfuss 2012), an hypothesis that cannot be evaluated in view of our results.

The genus *Levantoniscus*, tentatively assigned to Trachelipodidae (Cardoso et al. 2015), has been found to be closer to the monophyletic subgroup of Porcellionidae. Given that the genus appears as the sister clade of all remaining monophyletic Porcellionidae, we cannot propose the assignment of this taxon into the same family, given that no known morphological characters can be used as synapomorphies of such a taxon. The characters considered as autapomorphies of *Levantoniscus* by Cardoso et al. (2015) could as well define a separate new family. A more inclusive phylogeny is required before we can decide on its familial status, given also the lack of robust synapomorphies defining Trachelipodidae, a family in need of a sound revision.

As indicated by the tree topology, Porcellionidae is more closely related to Trachelipodidae and Agnaridae rather than Armadillidiidae. A similar result has been found by Lins et al. (2017), even though these authors had included only two species in two genera (*Porcellio* and *Porcellionides*) of Porcellionidae in their analysis. It is evident that morphological characters traditionally used in Oniscidea systematics, such as the structure of pleopodal lungs, the number of flagellar segments and the head structure, do not seem to provide adequate evidence that support a robust taxonomy, at least not in all cases.

In conclusion, the monophyly of Porcellionidae as currently perceived cannot be supported by molecular evidence. Of course, we still need to identify phenotypic synapomorphies defining the family, since the characters used so far cannot be considered as valid. In addition, the genus *Porcellio* needs to be revised, as it appears to be polyphyletic, comprising of at least two separate groups.

The monophyletic subgroup of Porcellionidae seems to have an African origin, diverging at the end of the Palaeogene (Oligocene) and then differentiating further during the Miocene. Based on the cladochronology estimated herein, more basal cladogenetic events, leading to the branching of other related families, happened in the Eocene. This chronology is compatible with the very old (Mesozoic) origin of Oniscidea suggested by Broly et al. (2013, 2015).

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## **Supplementary material I**

### **Percentage sequence divergence among the main clades of Porcellionidae and Maximum Likelihood phylogenetic tree.**

Authors: Dimitriou AC, Taiti S, Schmalzfuss H, Sfenthourakis S

Explanation note: Each genus comprised a different group, except for Porcellio whose species were treated as different groups because they don't form a monophyletic group.

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Link: <https://doi.org/10.3897/zookeys.801.23566.suppl1>

# Terrestrial isopods of the family Eubelidae Budde-Lund, 1899 from Iran, with description of a new species (Isopoda, Oniscidea)

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## Abstract

In the present work, terrestrial isopods of the family Eubelidae are investigated in Iran. The genera *Periscyphis* and *Somalodillo* are reported for the first time. More localities are presented for *Koweitoniscus tamei* (Omer-Cooper, 1923) while *K. shafeii* Kashani, **sp. n.** is described and figured. A map indicating the sampling localities for the species is presented.

## Keywords

Eubelidae, Iran, new species, new records, Oniscidea

## Introduction

The family Eubelidae Budde-Lund, 1899 includes 50 genera distributed mostly in the tropical areas of Africa, and partly in south-eastern Asia and in the Arabian Peninsula (Taiti et al. 1991). Schmidt (2003) considered the occurrence of some eubelid species in the Neotropics due to human activities. Uropods with flattened sympodites and reduced exopodites inserting medially on the distal margin of the sympodites, presence of *sulcus arcuatus* on the lateral margins of the first coxal plates along with the conglobation ability are the most significant diagnostic characters of the family (Taiti et al. 1991;

Schmidt 2003). *Koweitoniscus tamei* (Omer-Cooper, 1923) was the first eubelid species reported from Iran (Kashani 2014). In the present study, more localities are reported for the species and two more species are identified: *Periscyphis vittatus* Omer-Cooper, 1926 and *Koweitoniscus shafiei* Kashani, sp. n. The genus *Somalodillo* is also reported for the first time based on one female specimen. Sampling localities are presented on a map.

## Material and methods

The material examined in the present study was collected in Iran since 2008 (Figure 1). The specimens were collected by hand and preserved in 96% ethanol. The specimens were dissected and body parts were slide-mounted using Euparal (Carl Roth, Karlsruhe). Drawings were made using a drawing tube on a Nikon Y-IDT compound microscope. Color images were taken using an Olympus DP71 digital camera on an Olympus SZH10 stereomicroscope. Type material of the newly described species is deposited in the Zoological Museum, University of Tehran (ZUTC), the Iranian Research Institute of Plant Protection (IRIPP) and in the personal collection of the first author (PCGMK). Drawing and plates were arranged with the methods described in Montesanto (2015, 2016).

## Systematic account

**Order Isopoda Latreille, 1817**

**Suborder Oniscidea Latreille, 1802**

**Family Eubelidae Budde-Lund, 1899**

**Genus *Periscyphis* Gerstaecker, 1873**

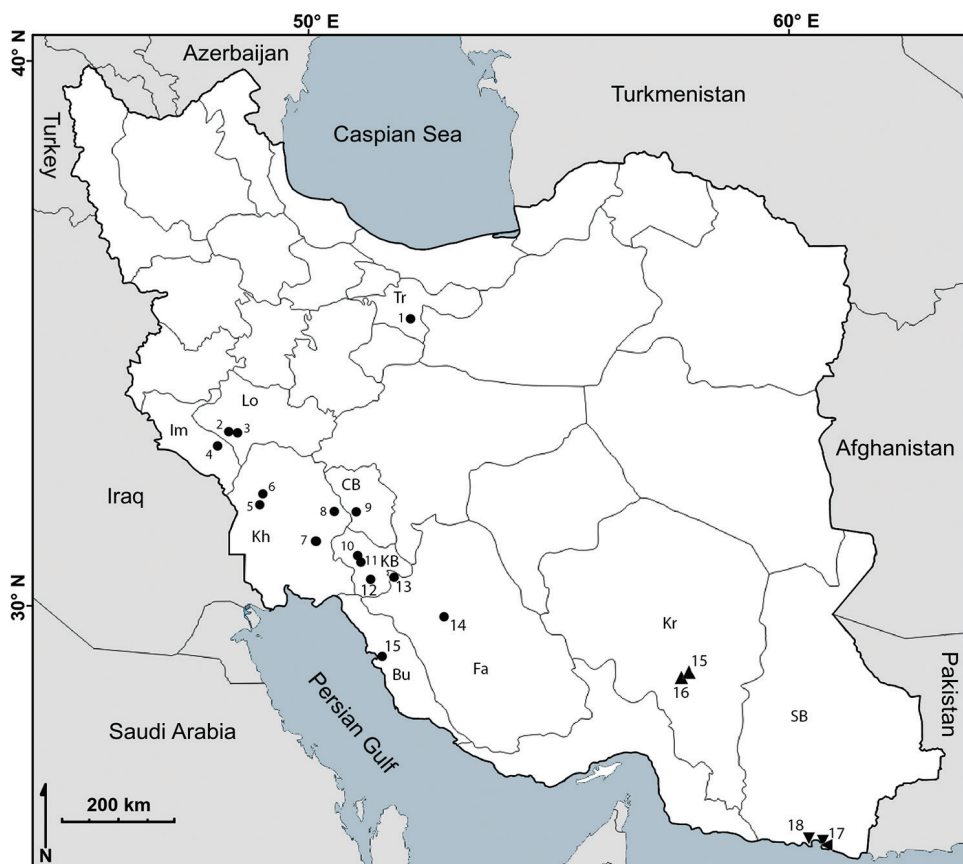
**Type species.** *Periscyphis trivialis* Gerstaecker, 1873 by monotypy.

### ***Periscyphis vittatus* Omer-Cooper, 1926**

Figure 2A

**Material examined.** Sistan va Balouchistan, [17] 6 ♀♀, Chabahar, 4 Dec. 2008, leg. H. Salehi (PCGMK 1385); [18] 3 ♂♂, 10 ♀♀, 15 Km to Konarak, 25°26.3'N, 60°29.5'E, 9 Feb. 2009, leg. E. Entezari (PCGMK 1714); [18] 1 ♂, 1 ♀, 15 Km E Konarak, 25°26.3'N, 60°29.5'E, 9 Feb. 2009, leg. E. Entezari (IRIPP Iso.1066).

**Remarks.** The genus *Periscyphis* comprises 46 species, mostly present in eastern Africa and the Arabian Peninsula (Schmalfluss 2003; Taiti and Checcucci 2011; Taiti and Schotte 2016; Taiti and Montesanto 2018). *Periscyphis vittatus* is reported here for the first time from two localities in southern Iran (Figure 1). As reported for Pakistan (Schmalfluss 2003), this species is most probably introduced to Iran. The identification of



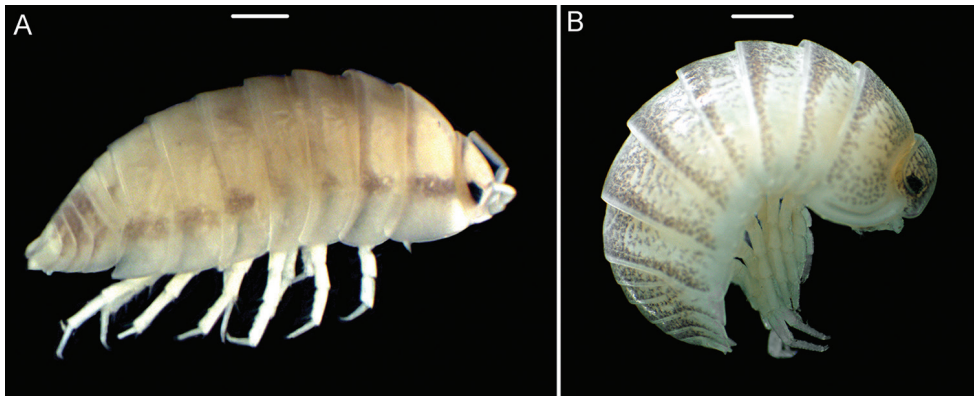
**Figure 1.** Map of Iran indicating the sampling localities of *Periscyphis vittatus* (▼), *Koweitoniscus tamei* (●), *K. shafiei* (▲), and *Somalodillo* sp. (◄). Numbers refer to the sampling localities indicated in brackets in the material examined section for each species. Abbreviations: Bu: Bushehr; CB: Chehar–Mahal va Bakhtiari; Fa: Fars; Im: Ilam; KB: Kohgiluyeh va Boyer–Ahmad; Kh: Khuzestan; Kr: Kerman; Lo: Lorestan; SB: Sistan va Balouchistan; Tr: Tehran.

the species was based on the comparison of the characters of the specimens (Figure 2A) with the description and illustration presented by Ferrara & Taiti (1986: 96; fig. 6).

**Distribution.** Eritrea; Djibouti; Mozambique; Somalia; Arabian Peninsula; Socotra Island; Pakistan; Iran.

### Genus *Koweitoniscus* Vandel, 1975

**Type species.** *Koweitoniscus ahmadii* Vandel, 1975 (= *Periscyphis tamei* Omer-Cooper, 1923) by original designation and monotypy.



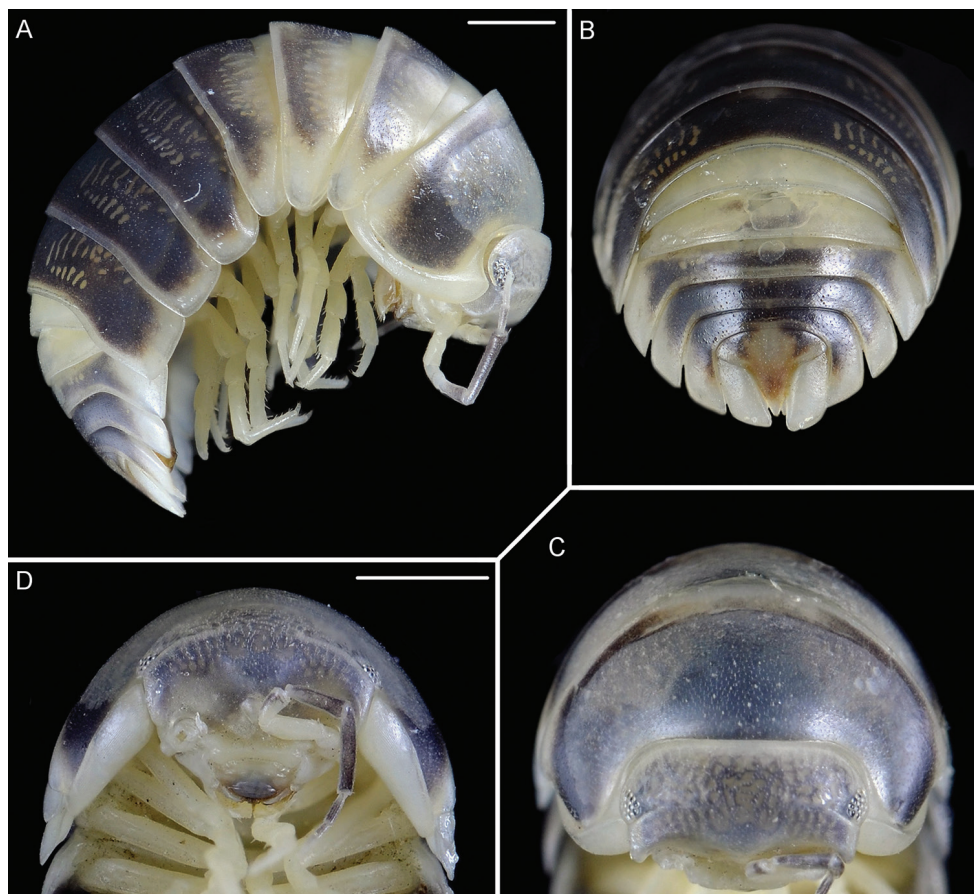
**Figure 2.** **A** *Periscyphis vittatus* **B** *Koweitoniscus tamei*. Scale bar: 1 mm.

### *Koweitoniscus tamei* (Omer-Cooper, 1923)

Figure 2B

**Material examined.** Boushehr, [15] 1 ♂, 1 ♀, Borazjan to Boushehr, 20 Km to Boushehr, 29°02.7'N, 51°03.0'E, 16 May 2008, leg. G.M. Kashani, (PCGMK 1224); Kohgiluyeh-va-Boyerahmad, [10] 1 ♂, Sough, 2 May 2003, leg. M. Rezaei (PCGMK 1271); 1 ♀, Sough, 2 May 2003, leg. M. Rezaei (IRIPP Iso-1006); [11] 1 ♀, Dehdasht, 2 May 2003, leg. M. Rezaei (PCGMK 1272); [12] 1 ♀, Gachsaran, Tappe-Aqgrab, 3 May 2003, leg. M. Rezaei (PCGMK 1268); Cheharmahal-va-Bakhtiari, [9] 1 ♂, Sarkhoun to Lordegan, 31°40.3'N, 50°44.4'E, 18 June 2016, leg. G.M. Kashani, A. Abedini & Z. Hatami (PCGMK 2328); Lorestan, [2] 1 ♂, 1 ♀, Poldokhtar, 33°07.0'N, 47°43.4'E, 12 Nov. 2008, leg. G.M. Kashani (IRIPP Iso-1007); [3] 15 ♂♂, 20 ♀♀, Poldokhtar to Andimeshk, 80 Km to Andimeshk, 32°56.2'N, 47°52.5'E, 12 Nov. 2008, leg. G.M. Kashani (PCGMK 1395); [3] 1 ♂, 7 ♀♀, Poldokhtar to Andimeshk, 80 Km to Andimeshk, 32°56.2'N, 47°52.5'E, 12 Nov. 2008, leg. G.M. Kashani (IRIPP Iso-1008); Ilam, [4] 1 ♂, 1 ♀, Dehloran to Abdanan, 32°39.9'N, 47°32.0'E, 13 Nove. 2008, leg. G.M. Kashani (PCGMK 1399); Tehran, [1] 1 ♂, Varamin, Pishva, 35°12.4'N, 51°48.4'E, 26 Apr. 2009, leg. G.M. Kashani (PCGMK 1436); Khuzestan, [5] 5 ♂♂, 1 ♀, Shoush, Karkhe national park, 5 Aug. 2017, leg. H. Maddahi (PCGMK 2642); [6] 2 ♀♀, Shoush to Andimeshk, Safar Abad village, by the Dez river, 32°16.1'N, 48°24.0'E, 6 May 2009, leg. H. Salehi (IRIPP Iso-1010); [7] 9 ♂♂, 9 ♀♀, Ramhormoz, Bony village, 23 Mar. 2015, leg. M. Larti (PCGMK 2078); [8] 3 ♂♂, 9 ♀♀, Dehdez to Izeh, 22 Km to Izeh, 31°49.0'N, 50°03.0'E, 19 Jun. 2016, leg. G.M. Kashani, A. Abedini & Z. Hatami (PCGMK 2350); Fars, [13] 1 ♀, Kopen to Masiri, 2 Km to Dozak village, 30°18.5'N, 51°22.7'E, 19 Jul. 2015, leg. G.M. Kashani, Z. Hatami & A. Abedini (PCGMK 2207); [14] 1 ♂, 1 ♀, Shiraz, by the Parishan lake, 31 Oct. 2012, leg. S. Hosseini (IRIPP Iso-1011).

**Remarks.** The broad distribution of *Koweitoniscus tamei* in south and south-western Iran was reported by Kashani (2014). Here, more localities are introduced for the



**Figure 3.** *Koweitoniscus shafiei* sp. n., female, paratype. **A** lateral view **B** pleon in dorsal view **C** cephalothorax and pereonite 1 in dorsal view **D** cephalothorax in frontal view. Scale bar: 1 mm.

species (Figure 1). The presence of the species in the town of Tehran might be due to human activities. The identification of the species was based on the comparison of the species (Figure 2B) with the original descriptions and illustrations presented by Omer-Cooper (1923: 96, figs 1–16) and the figures by Ferrara & Taiti (1986: 100, fig. 15).

**Distribution.** Syria; Iraq; Kuwait; Iran.

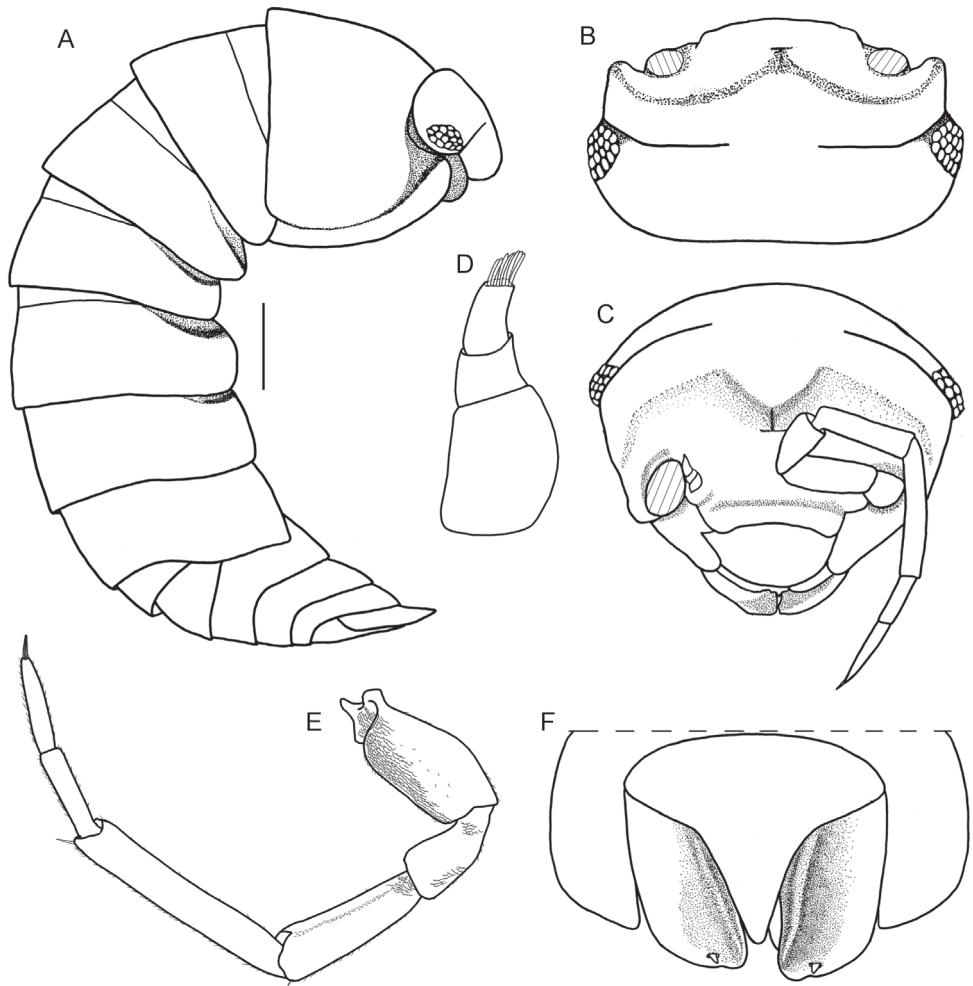
***Koweitoniscus shafiei* Kashani, sp. n.**

<http://zoobank.org/DC0328B2-6868-4361-B85D-D7FF7C49E55E>

Figs 3–5

**Material examined. Holotype:** ♂, 10 mm, [15] Kerman, Jebalbarez to Jiroft, 10 Km to Jiroft, 28°45.8'N, 57°45.5'E, elev. 835m, 25 Feb. 2009, leg. G.M. Kashani (ZUTC 6747). **Paratypes:** 1 ♀, same data as holotype (ZUTC 6748); 1 ♀, same data as holo-





**Figure 4.** *Koweitoniscus shafiei* sp. n., paratype, female. **A** lateral view **B** cephalothorax in dorsal view **C** cephalothorax in frontal view **D** antennula **E** antenna **F** telson and uropods. Scale bar: 1 mm.

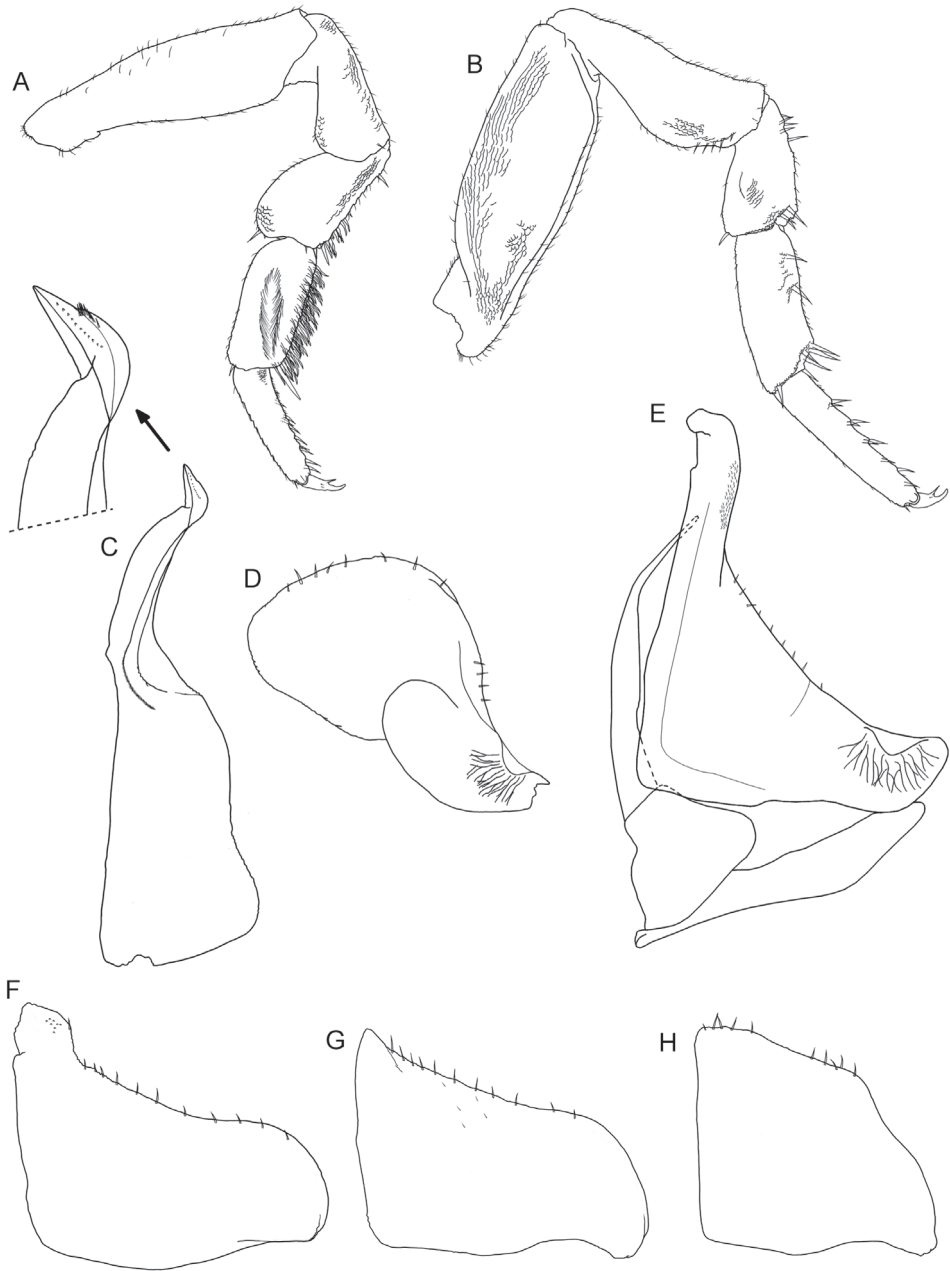
type (IRIPP Iso.1009); 2 ♂♂, 4 ♀♀, same data as holotype (PCGMK 1450); [16] 1 ♂, Jiroft, 6 Apr. 2009, leg. E. Entezari (PCGMK 1440).

**Diagnosis.** Head with an interrupted frontal ridge; inner lobe of schisma longer than outer one; telson with pointed distal part; male pleopod 1 endopodite bent inward with pointed apex.

**Description.** Maximum length: male 10 mm; female 11 mm.

**Coloration:** vertex, pereon-tergites, pleon-tergites 3–5 and telson blackish; posterior margins of pereon-tergites II–III, pereon and pleon epimera, uropods, pleonites I–II and pereopods yellowish (Figure 3A). **Body** smooth, convex, able to roll up into a ball (Figs 3A, 4A).





**Figure 5.** *Koweitoniscus shafiei* sp. n., male, paratype. **A** pereopod 1 **B** pereopod 7 **C** pleopod 1 endopodite with enlarged apex **D** pleopod 1 exopodite **E** pleopod 2 **F** pleopod 3 exopodite **G** pleopod 4 exopodite **H** pleopod 5 exopodite.

*Cephalothorax* (Figs 3C, D; 4B, C) with frontal ridge broadly open in the middle; eyes with 16–18 ommatidia. Antennula (Figure 4D) with second article shortest; third article bearing a tuft of aesthetascs at apex. Antenna (Figure 4E) with fifth article of peduncle longer than flagellum; flagellum with two articles, proximal article as long as the distal one.

*Pereonite 1* (Figs 3A, 4A) with a wide *sulcus arcuatus* along the lateral margin; posterolateral corner with a schisma, inner and outer lobes rounded, inner lobe more protruding backwards than outer one; posterior margin straight.

*Telson* (Figs 3B, 4F) approximately 1.3 times as wide as long, with concave sides and distal part triangular with pointed apex. Uropod (Figs 3B, 4F) with subquad-rangular protopodite, posterior margin slightly sinuous but not indented; exopodite minute inserted dorsally near the posterior margin.

Male: Pereopods 1–4 carpus with a brush of pointed setae (Figure 5A). Pereopod 7 (Figure 5B) ischium narrow with concave ventral margin; merus and carpus elongated, without distinct specializations. Pleopod 1 endopodite (Figure 5C) with medial part bent outward and distal part bent inward with pointed apex bearing a row of fine setae on outer margin; exopodite (Figure 5D) short, with widely rounded hind lobe equipped with a row of pointed setae. Pleopod 2 (Figure 5E) exopodite longer than wide with numerous small scales and a line of setae on the outer margin. Pleopod 3–5 exopodites as in Figs 5F–H.

**Etymology.** The species is named after Dr. Soheila Shafiei, Ph.D. classmate of GMK, now a herpetologist in Shahid Bahonar University of Kerman, Iran.

**Remarks.** The genus *Koweitoniscus* presently embraces five species (Schmalfuss 2003; Taiti and Checcucci 2011; Kashani 2014, Taiti and Montesanto 2018): *K. tamei* (Omer-Cooper, 1923) from Syria, Iraq, Kuwait and Iran, *K. rostratus* Ferrara & Taiti, 1986 from south-western Saudi Arabia, *K. vanharteni* Ferrara & Taiti, 1996 from Yemen, *K. korshunovi* Taiti & Checcucci, 2011 from the United Arab Emirates, and *K. agnellii* Taiti & Montesanto, 2018 from Djibouti. The new species differs from *K. rostratus* and *K. vanharteni* in having an interrupted frontal margin in the cephalothorax, and from *K. tamei*, *K. korshunovi*, and *K. agnellii* in having the inner lobe of schisma longer than outer one and the distal part of the male pleopod 1 endopodite bent inward with pointed apex. *Koweitoniscus shafieii* was found in southern Iran and according to current knowledge appears to be endemic to this region.

**Distribution.** Iran (endemic): Jiroft district.

## Genus *Somalodillo* Taiti & Ferrara, 1982

**Type species.** *Somalodillo squamatus* Taiti & Ferrara, 1982 by original designation.

### *Somalodillo* sp.

Figure 6

**Material examined.** Sistan va Balouchistan, [17] 1 ♀, Chabahar, 9 Feb. 2009, leg. E. Entezari (PCGMK 2111).



**Figure 6.** *Somalodillo* sp. **A** lateral view **B** cephalothorax and pereonite 1–2 in lateral view **C** cephalothorax in frontal view **D** pleon in dorsal view. Scale bar: 1 mm.

**Remarks.** This is the first record of the genus *Somalodillo* reported from Iran. According to Taiti & Ferrara (1982; 2004), pereonite 1 with a schisma and sulcus arcuatus (Figure 6A–C), the telson with a rectangular distal part, uropod with minute exopodite inserted dorsally close to distal margin (Figure 6D), and pleopod exopodite 2 with monospiracular lungs clearly identify the specimen as a member of the genus *Somalodillo*. With no available male specimens, it was not possible to identify this female to specific level.

## Discussion

Despite several contributions on the terrestrial isopod fauna of Iran, especially in recent years, the knowledge on this taxon is relatively poor. In the present work, four eubelid

species were found restricted to south and south-western Iran. The broad distribution of *Koweitoniscus tamei* can be explained by the range expansion of the species to the south-western part of Iran while *Periscyphus vittatus* and *Somalodillo* sp. are most probably introduced to Iran by human activities. The new species, *Koweitoniscus shafiei*, is endemic to southern Iran. It seems that southern Iran represents the northernmost border for distribution of eubelid terrestrial isopods in the region. This work expands our knowledge on the oniscidean fauna of Iran, adding two genera and three species to the fauna of the country. Prior to the present study, 41 species were reported from Iran and this contribution raised the number to 44, which is still far from the real number of species probably present in the country.

## Acknowledgments

We cordially thank Hasan Salehi, Ehsan Entezari, Hasan Maddahi, and Mehrdad Rezaei for donating their specimens to PCGMK. We would like to acknowledge Dr. Helmut Schmalfuss and Dr. Stefano Taiti who made invaluable suggestions and useful comments. Dr. Nathalie Yonow is appreciated for improving the English. This study was supported in part by a grant from INSF (project no: 96003230).

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# Description of a new species of the genus *Protracheoniscus* Verhoeff, 1917 and redescription of *Protracheoniscus kryszanovskii* Borutzky, 1957 from the southeast of European Russia (Isopoda, Oniscidea, Agnaridae)

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<http://zoobank.org/E9C39357-8030-4CAD-B918-6D174DBAE71>

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## Abstract

A new species of woodlice, *Protracheoniscus pokarzhevskii* Gongalsky & Turbanov, **sp. n.** is described from Kalmykia, NE Pre-Caspian region, Russia. *Protracheoniscus kryszanovskii* Borutzky, 1957 from the same area is also redescribed. Diagnostic features of these species as well as affinities within the genus are provided and discussed.

## Keywords

COI, *Desertoniscus*, Hungary, Isopoda, Kalmykia, new species, Oniscidea, *Protracheoniscus*, phylogenetic analysis, woodlouse



## Introduction

The highest diversity of terrestrial isopods in Russia is recorded in the Black Sea area and in the Caucasus, depending mostly on the availability of temperature sufficient to complete their life cycle and on appropriate soil moisture. Within the former USSR, these regions as well as the Central Asia are relatively well surveyed with many records of terrestrial isopods (Borutzky 1945, 1957, Eshaghi et al. 2015). However, there are gaps with very few isopod records across this territory, such as in the north of the Caspian region (Kuznetsova and Gongalsky 2012). The low Volga River basin attracts attention due to a unique position in Europe with natural steppes and sometimes with semi-deserts.

During a study of soil fauna in the surroundings of rice paddies in NE Kalmykia, a number of isopod species were recorded. Some of them were species new to Russia, and some have not been described yet. One new species of *Protracheoniscus* Verhoeff, 1917 is described. A species in the same genus described by EV Borutzky (1957) was collected as well. It has not been recorded for 60 years since description, and only sketch drawings of the diagnostic features of this species were available by now. A re-description of this species is provided below.

## Materials and methods

Sampling was done by the first author and his colleagues in the steppe ecosystems of Kalmykia in 2016–2017. Woodlice were collected by hand and fixed in 96% ethanol. Terminology used in the species description is mainly based on Vandel (1960). Processing and dissections were done by using a Leica MZ8 binocular microscope. Micro preparations of diagnostic body appendages were done in euparal (Carl Roth GmbH). Line drawings were executed with the help of an Olympus BX41 microscope supplied with an Olympus U-DA camera lucida. The material is deposited in the collection of the Zoological Museum of Moscow University, Russia (ZMMU), and partly retained in the private collection of the author (AN Severtsov Institute of Ecology and Evolution of Russian Academy of Sciences, Moscow, Russia), as indicated below.

*Phylogenetic analysis.* To confirm the validity of *P. pokarzhevskii* sp. n., a phylogenetic analysis was undertaken. In the molecular analysis, we used also specimens of *P. politus* from Budapest, Hungary, *P. pokarzhevskii* sp. n., *P. kryszanovskii*, *P. nogaicus*, *P. major* and *Desertoniscus zaitsevi* from Kalmykia, Russia. In the phylogenetic construction, a number of species from GenBank (Table 1) were used as outgroup taxa. To isolate total DNA, pereopods 4 or 5 were used from individuals fixed in 96% ethanol. Total cell DNA was isolated using a QIAamp DNA Investigator Kit (Qiagen, Germany). For the analysis of genetic variability, fragments of the mtDNA COI loci were used. The study was performed using full-length fragments obtained by amplification of mtDNA with primers HCO2198 / LCO1490 (Folmer et al. 1994).

**Table 1.** List of specimens, sampling sites and accession numbers of the sequences for COI mt DNA included in this study. References are given for sequences obtained from GenBank.

Specimen	Locality	Reference / or year of sampling	
		COI mtDNA	Reference
<i>Desertioniscus zaitsevi</i> Gongalsky, 2017	Russia, Republic of Kalmykia, 1 km N of Bolshoy Tsaryn (47.9040N, 45.3929E) 29.04.2017, K. Gongalsky leg.	MH400725	This study / 2018
<i>Protracheoniscus</i> <i>kryszanovskii</i> Borutzky, 1957	Russia, Republic of Kalmykia, 1 km W of Tsagan Nur, bank of lake Sarpa (47.362N, 45.201E), 27.04.2017 K. Gongalsky leg.	MH400727	This study / 2018
<i>Protracheoniscus major</i> (Dollfus, 1903)	Russia, Republic of Kalmykia, 1 km N of Bolshoy Tsaryn (47.9040N, 45.3929E) 29.04.2017, K. Gongalsky leg.	MH400726	This study / 2018
<i>Protracheoniscus nogaicus</i> Demianowicz, 1932	Russia, Republic of Kalmykia, 1 km N of Bolshoy Tsaryn (47.9040N, 45.3929E) 29.04.2017, K. Gongalsky leg.	MH400724	This study / 2018
<i>Protracheoniscus</i> <i>pokarzhevskii</i> sp. n.	Russia, Republic of Kalmykia, 1 km N of Bolshoy Tsaryn, (47.9040N, 45.3929E) 29.04.2017, K. Gongalsky leg.	MG696253, MH400723	This study / 2017–18
<i>Protracheoniscus politus</i> (C. Koch, 1841)	Hungary, Budapest, Janos Hegy Mt. (47.5158N, 18.9602E) 29.08.2017, K. Gongalsky leg.	MG696252	This study / 2017
<i>Burmoniscus kathmandius</i> (Schmalfuss, 1983)	Nepal	LC075192	Karasawa 2016
<i>Ligia baudiniana</i> Milne- Edwards, 1840	Colombia	KF555872	Santamaria et al. 2014

The polymerase chain reaction was carried out on a Bio-Rad T 100 thermocycler (Bio-Rad, USA) in a specially selected temperature regime: the initial denudation of 95 °C was 5 min; annealing of 95 °C for 35 seconds, 48 °C for 40 seconds, 72 °C for 40 seconds (35 cycles), the final elongation of 72 °C lasted for 7 minutes. For the PCR, a set of reagents for the amplification of “5× Mas Mix-2025” manufactured by Dialat Ltd (Moscow, Russia) was used. The 15 µl reaction mixture contained 1 µl total DNA, 3 µl mix and 1 µl of each primer. The amplification products were separated by electrophoresis in 1.5% agarose gel in 1× TBE and visualized with ethidium bromide. The DNA sequence was determined with a forward and reverse primer using the Big Dye 3.1 kits on an ABI 3500 genetic analyzer from Applied Biosystems, USA, in a POP7 polymer environment.

The obtained sequences were aligned with the help of BioEdit v. 5.0.9 software. The obtained fragments of sections of COI mtDNA genes were used in phylogenetic analysis. The phylogenetic tree was built in MEGA 6.0.

In the species assessment, the Neighbor Joining (NJ) method was used, based on all the sequences obtained and with the calculation of bootstrap support of branch nodes (1000 replicates). The loci of COI mtDNA *Burmoniscus kathmandius* (Karasawa, 2016) and *Ligia baudiniana* (Santamaria et al., 2014) were used as outgroup taxa. The results obtained are presented in the form of a phylogenetic tree (Figure 11).

## Taxonomy

**Class Malacostraca Latreille, 1802**

**Order Isopoda Latreille, 1817**

**Family Agnaridae Schmidt, 2003**

**Genus *Protracheoniscus* Verhoeff, 1917**

***Protracheoniscus pokarzhevskii* Gongalsky & Turbanov, sp. n.**

<http://zoobank.org/C2AC4DE5-A416-4637-8AF7-47B8E25601C5>

**Type material. Holotype:** ♂ (ZMMU), Russia, Republic of Kalmykia, 1 km N of Bolshoy Tsaryn (47.9040N, 45.3929E), dry steppes (*Artemisia austriaca*, *Festuca valesiaca*, *Tanacetum achilleifolium*), 29.04.2017, K. Gongalsky leg. **Paratypes:** 2 ♂♂, 2 ♀♀ (ZMMU), 3 ♂♂, 4 ♀♀ (private collection of K. Gongalsky), same date, location and collector.

**Other material examined.** *Protracheoniscus politus* (C. Koch, 1841): 3 ♂♂, 6 ♀♀, Hungary, Budapest, János-Hegy (47.5158N, 18.9602E), 29.08.2017, K. Gongalsky leg. *Protracheoniscus nogaicus* Demianowicz, 1932: 2 ♂♂, Russia, Republic of Kalmykia, 1 km N of Bolshoy Tsaryn (47.9040N, 45.3929E), 29.04.2017, K. Gongalsky leg. *Protracheoniscus major* (Dollfus, 1903): 6 ♂♂, 15 ♀♀, same date, location, and collector. *Desertoniscus zaitsevi* Gongalsky, 2017: 3 ♂♂, 5 ♀♀, same date, location, and collector.

**Diagnosis.** A species of *Protracheoniscus* characterized by the antennal flagellum with the articles of a ratio close to 1:1; male exopod of pleopod 1 with almost rounded apex; telson with distal part elongated and distal corner forming triangle; and one of four medial spines of outer endite of maxillula is twice as small as the others.

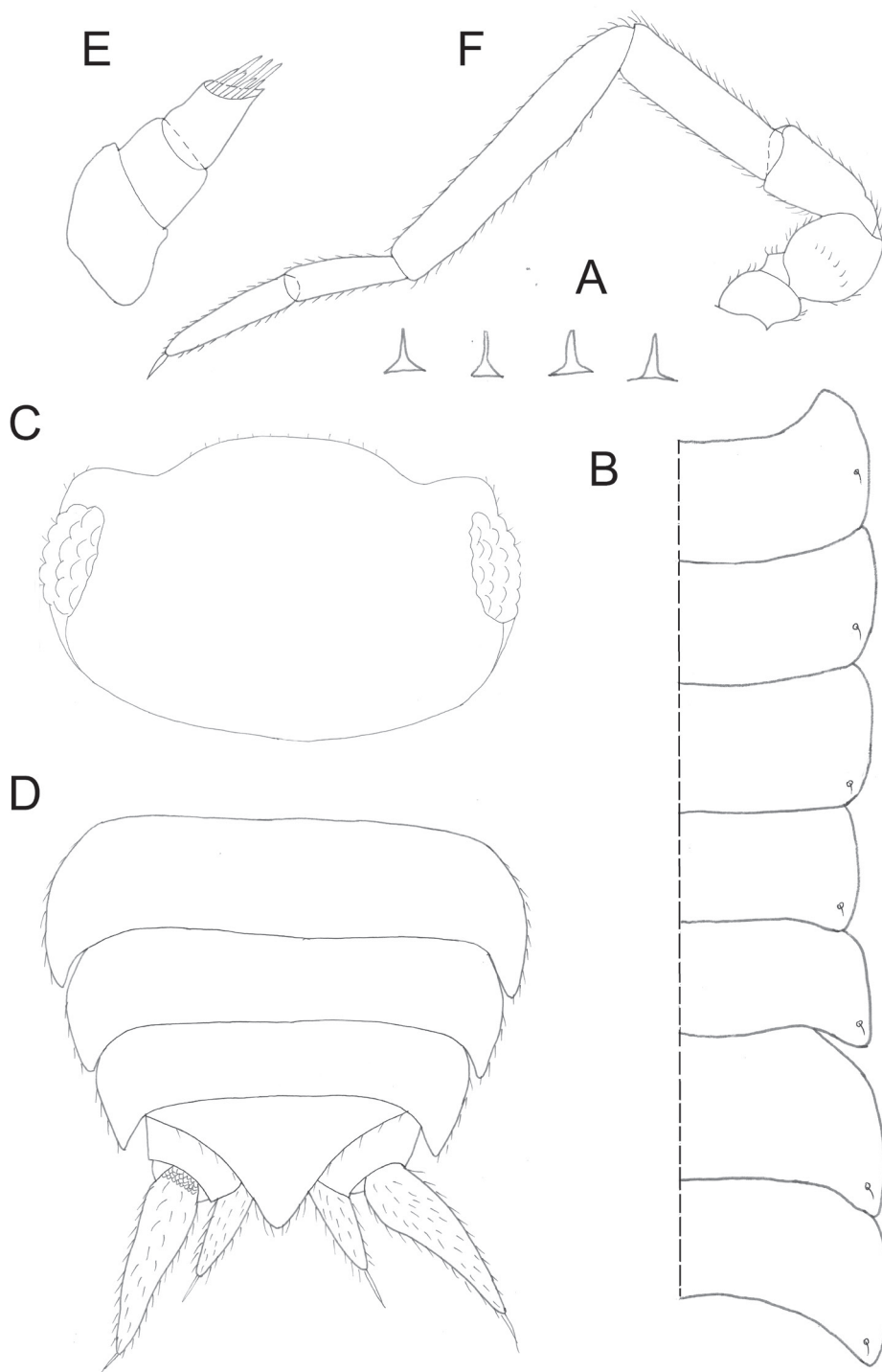
**Description.** *Somatic characters.* Maximum body length: male 5.0 mm; female 5.5 mm. Holotype body length 4.7 mm. Body color dark grey-brown; frontal part of head much darker than rest of the body; light grey-brown spots at base of coxal plates of pereon segments 2–7 (Figure 1A). Dorsal surface of tergites smooth. Posterior edges of coxal plates of pereonites straight (Figs 1A, 2B). Distal part of head covered with scattered sharp triangular dorsal setae (Figure 2A). Noduli laterales on pereonites located close to coxal plates edges (Figure 2B). Body relatively elongated; pleon not continuous with pereon outline (Figure 1A). Cephalic lobes poorly developed; distal edge of median lobe rounded (Figure 2C). Telson with distal part elongated and distal corner forming triangle (Figure 2D).

*Appendages.* Uropods (Figure 2D) colored as dorsal body surface; exopods elongated. Antennula of three articles (Figure 2E); first article wide and relatively long, second article slightly shorter than first, third article almost as long as first and narrow, bearing a tuft of aesthetascs at apex. Antenna reaching pereonite 3 (Figure 1A); flagellum of two articles, proximal article slightly shorter than distal one (Figure 2F). Left mandible (Figure 3A); pars incisiva with two teeth and lacinia mobilis with straight edge; molar penicil consisting of ca. ten setae. Right mandible smaller than left mandible, pars incisiva with three teeth and lacinia mobilis with two teeth, molar penicil consisting of ca. ten setae (Figure 3B).

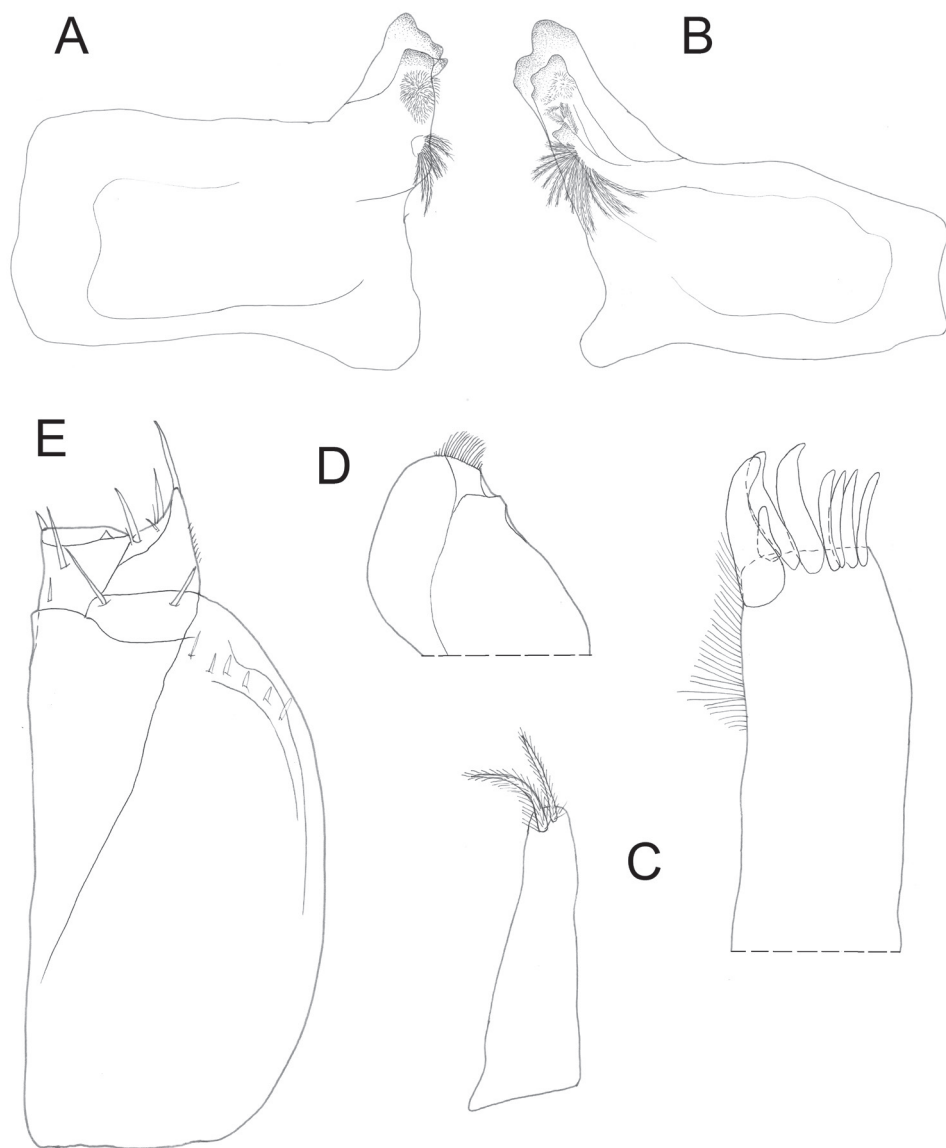


**Figure 1.** Dorsal view of male paratype of *Protracheoniscus pokarzhevskii* sp. n., 4 mm (**A**), and male *Protracheoniscus kryzhanovskii* Borutzky 1957, 9 mm (**B**), from Kalmykia, SE of European Russia.

Maxillula (Figure 3C): medial corner of inner endite with two strong penicils; apical edge of outer endite bearing 4 + 4 teeth with simple tips, with one tooth in medial group twice smaller than other three. Maxilla with bilobate edge, medial half of apical edge of outer lobe with dense brush of short setae (Figure 3D); inner margin with subapical tubercle. Maxilliped with outer corner of endite with two acute tips and large spine near inner corner (Figure 3E). Pleopods (Figure 5). All exopods with monospiracular covered lungs.



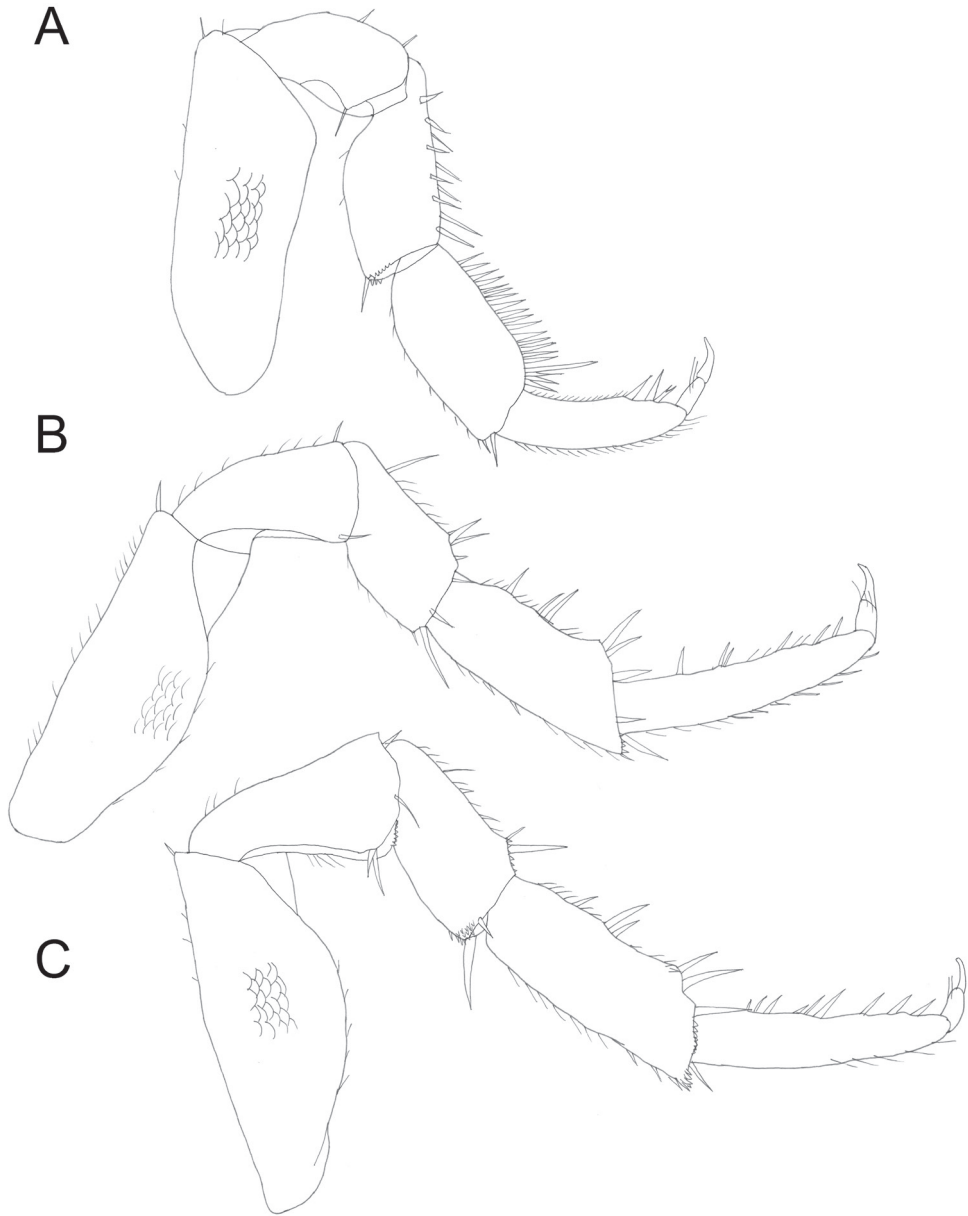
**Figure 2.** *Protracheoniscus pokarzhevskii* sp. n.: **A** dorsal scale-setae **B** pereon edge **C** head **D** pleonites 3–5, telson and uropods **E** antennula **F** antenna (female, paratype).



**Figure 3.** *Protracheoniscus pokarzhevskii* sp. n.: **A** left mandible **B** right mandible **C** maxillula **D** maxilla **E** maxilliped (female, paratype).

Male: Pereopods (Figure 4A–C): pereopod 1 with dactylus slightly bent ventrally; carpus with brush of setae with split tips. Pereopod 6 and 7 ischia with sternal margin straight. Genital papilla slightly extended at tip (Figure 5H). Exopod of pleopod 1 (Figure 5A) with almost rounded tip and ca. ten setae at apex, outer margin slightly concave. Endopod of pleopod 1 with split distal part: straight sharp triangular tip bearing row of spines and lateral bulb (Figure 5B). Pleopod 2: exopod triangular with concave outer margin bearing two setae with split tips (Figure 5C); endopod much



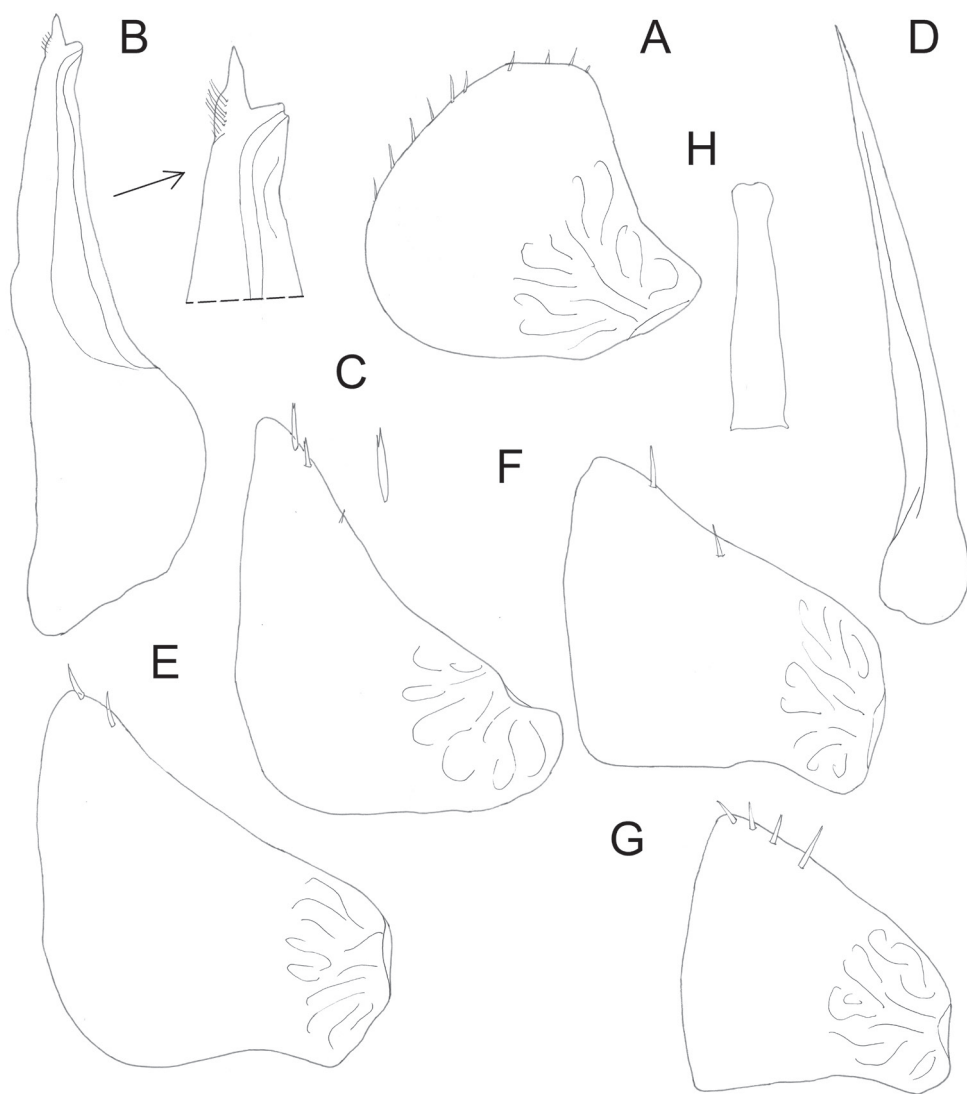


**Figure 4.** *Protracheoniscus pokarzhevskii* sp. n.: **A** pereopod 1 **B** pereopod 6 **C** pereopod 7 (male, paratype).

longer than exopod, narrow, with parallel sides (Figure 5D). Pleopod 3–5: exopods (Figure 3E–F) trapezoidal, slightly decreasing in size from 3 to 5. Pleopod 5 exopod with sharp medial corner (Figure 5G).

**Remarks.** This species is morphologically closest to *Protracheoniscus politus* (C Koch, 1841) in the similar shape of the endopodite of the male pleopod 1. However, the new species differs from *P. politus* in the following: (i) exopod of the male





**Figure 5.** *Protracheoniscus pokarzhevskii* sp. n.: **A** exopod of pleopod 1 **B** endopod of pleopod 1 **C** exopod of pleopod 2 **D** endopod of pleopod 2 **E** exopod of pleopod 3 **F** exopod of pleopod 4 **G** exopod of pleopod 5 **H** genital papilla (male, paratype).

pleopod 1 with shorter posterior lobe; (ii) tip of endopod of pleopod 1 not bent laterally; (iii) telson with less concave sides and shorter and less acute tip; (iii) ratio of articles of flagellum is close to 1:1 (almost close to 1:2 in *P. politus*) (Gruner 1966; Tomescu et al. 2016).

Recently, two close species of *Protracheoniscus* were described from north Iran (Kashani and Hamidnia 2016). *Protracheoniscus pokarzhevskii* sp. n. differs from *P. kiabii* Kashani & Hamidnia, 2016 in (i) the noduli laterales located much closer to the pereonites' margins; (ii) much shorter uropods; (iii) straight instead of concave sternal

margin of the male pereopod 7 ischium; (iv) sharp tip of endopod of the male pleopod 1. From *P. golestanicus* Kashani & Hamidnia, 2016 it differs in (i) different shape of both exopod and endopod of the male pleopod 1; (ii) different position of noduli laterales. Evidently, a molecular analysis is needed for the complex of these small species of *Protracheoniscus*.

**Distribution.** The new species has only been found between the Volga and Vostochnyi Manych Rivers. It occupies the steppes of Kalmykia (*Artemisia austriaca*, *Festuca valesiaca*, *Tanacetum achilleifolium*).

**Etymology.** The species is named after Prof Dr Andrey D Pokarzhevskii (1946–2006), a prominent Russian soil zoologist who encouraged the first author to study terrestrial isopods.

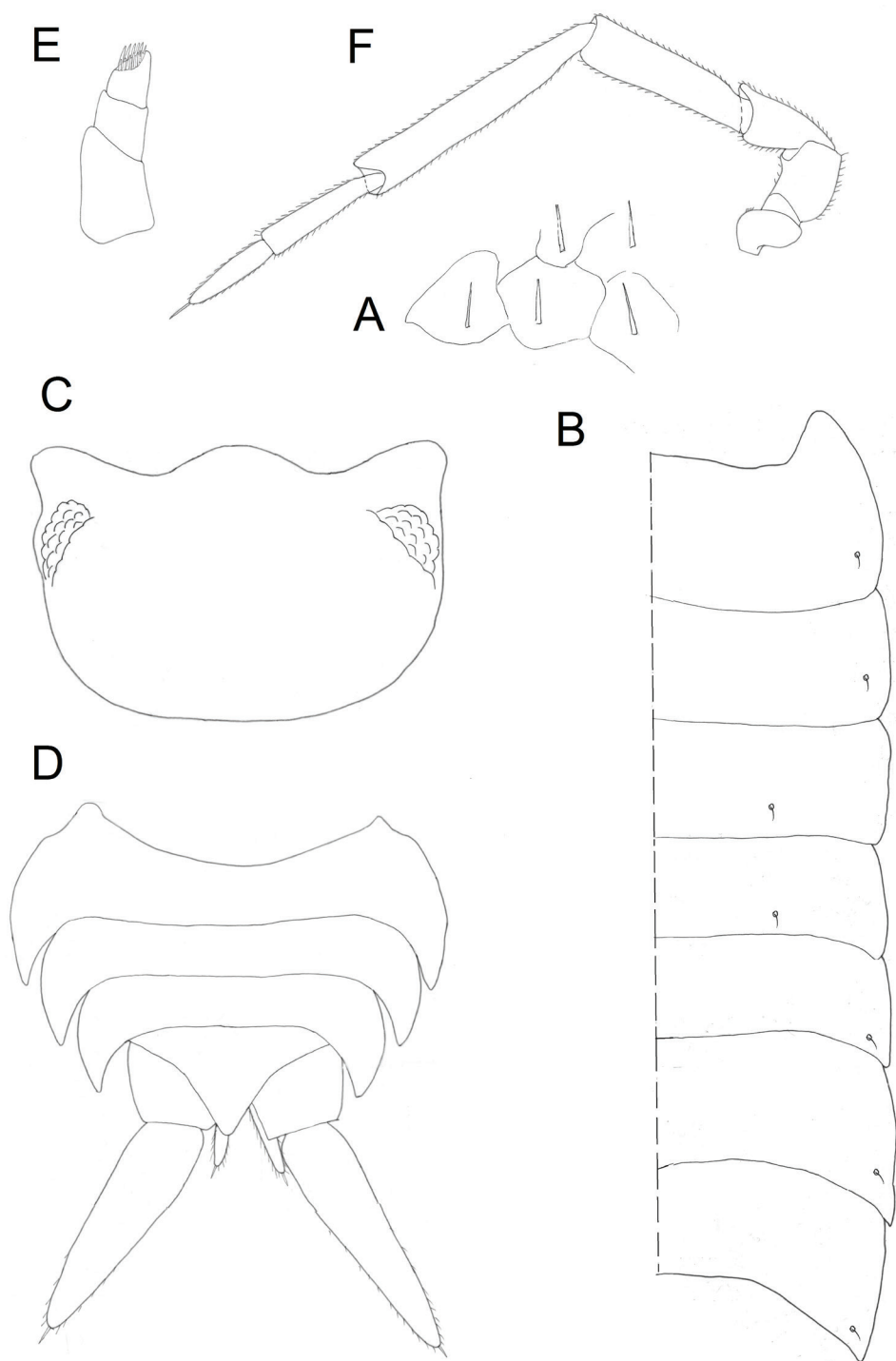
### *Protracheoniscus kryszanovskii* Borutzky, 1957

**Material examined.** 1 ♂, 1 ♀, 6 juveniles (ZMMU Mc-627); [USSR, Stavropol Region, bank of lake] Manych, 4 km NE of Divnoe, 20.05.1950. O. Kryzhanovsky leg. 2 ♂♂, 5 ♀♀ (ZMMU); Russia, Republic of Kalmykia, 1 km W of Tsagan Nur, bank of lake Sarpa. 27.04.2017 (47.362, 45.201), K. Gongalsky leg. 4 ♂♂, 8 ♀♀ (private collection of K. Gongalsky), same date and location.

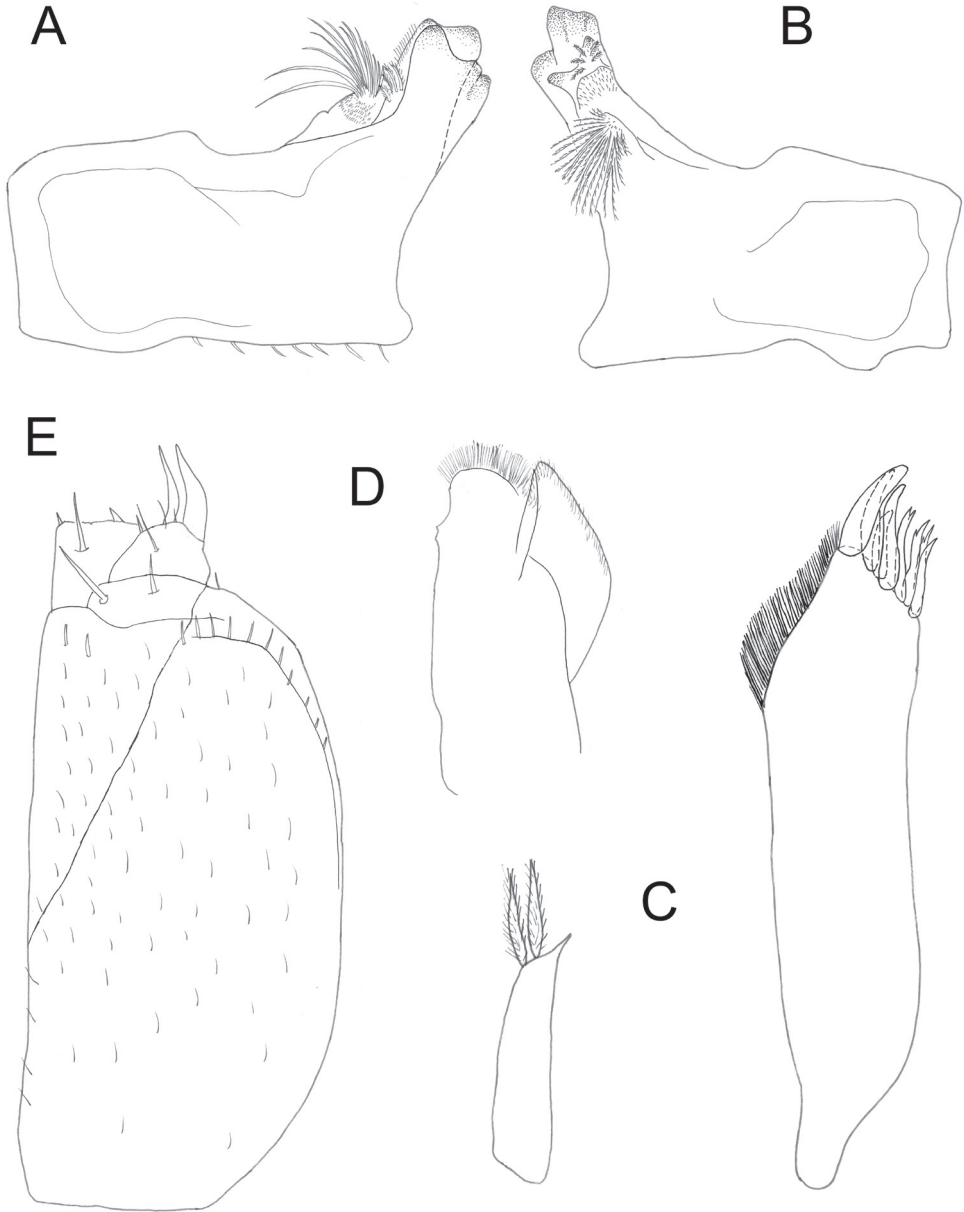
**Diagnosis.** *Protracheoniscus kryszanovskii* is characterized by the shape of cephalic lobes; exopod of male pleopod 1; and dactylus of male pereopods 6 and 7 widened in the middle (Borutzky 1957).

**Re-description.** *Somatic characters.* Maximum body length: male 21 mm; female 14 mm (Borutzky 1957). Body color dark grey-brown white or yellow spots at base of coxal plates of pereonites 2–7 (Figure 1B). Dorsal surface of tergites smooth. Posterior edges of coxal plates of pereonites straight (Figure 6B). Distal part of head covered with scattered sharp triangular dorsal scale-setae (Figure 6A). Noduli laterales on pereonites 3–4 located distinctly more distant from coxal plates lateral edges (Figure 6B). Body relatively elongated; pleon outline not continuous with pereon (Figure 1B). Cephalic lobes well developed; median lobe rounded (Figure 6C). Telson with distal part elongated and distal corner forming sharp triangle (Figure 6D).

*Appendages.* Uropods (Figure 6D) colored as dorsal surface of body; exopods elongated, widened in the middle. Telson not reaching endopods of uropods. Antennula with three articles (Figure 6E); first article wide and relatively long; second article 1.5–2 times shorter than first; third article almost as long as second and narrow, bearing a tuft of setae at apex. Antenna long, reaching pereonite 3 (Figure 1B); flagellum with proximal article 1.5 times longer than distal one (Figure 6F). Left mandible (Figure 7A) with pars incisiva with two teeth and lacinia mobilis with straight edge; molar penicil with ca. 12–15 setae. Right mandible smaller than left with pars incisiva with three teeth and lacinia mobilis with two teeth bearing five penicil setae; molar penicil with 15–18 setae (Figure 7B). Maxillula (Figure 7C): medial corner of inner endite with two strong penicils and sharp tip; apical edge of outer endite with 4 + 4

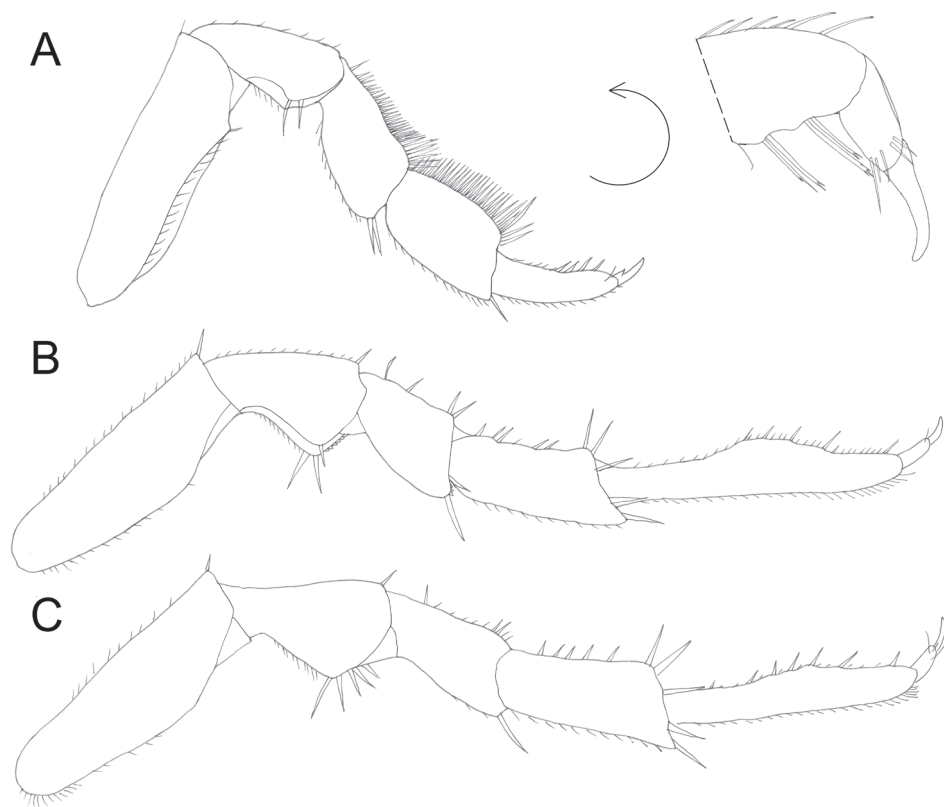


**Figure 6.** *Protracheoniscus kryszanovskii* Borutzky, 1957: **A** dorsal scale-setae **B** perceon edge **C** head **D** telson **E** antennula **F** antenna (female).



**Figure 7.** *Protracheoniscus kryszanovskii* Borutzky, 1957: **A** left mandible **B** right mandible **C** maxillula **D** maxilla **E** maxilliped (female).

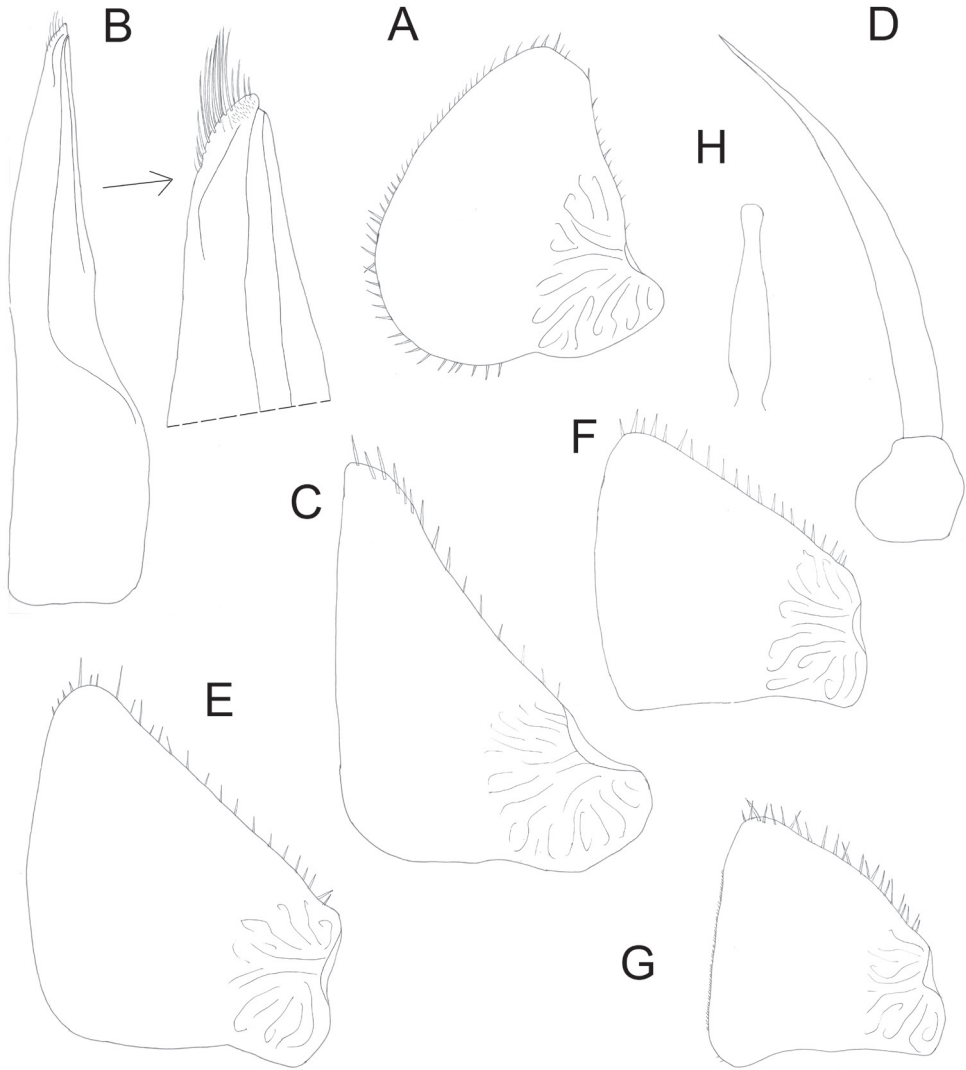
teeth, four of which apically cleft. Maxilla with bilobate edge, medial half of apical edge of inner lobe with a dense brush of short setae (Figure 7D); inner margin with subapical tubercle. Maxilliped with outer corner of endite with two acute tips and large spine near the inner corner (Figure 7E). All exopods of pleopods with monospiracular covered lungs.



**Figure 8.** *Protracheoniscus kryszanovskii* Borutzky, 1957: **A** pereopod 1 **B** pereopod 6 **C** pereopod 7 (male).

Male: Pereopods (Figure 8A–C): Pereopod 1 (Figure 8A) with a brush of setae with split tips on merus and carpus; dactylus of pereopods 6 and 7 widened in the middle (Figure 8C). Genital papilla slightly inflated at tip (Figure 8H). Exopod of pleopod 1 (Figure 9A) with almost rounded tip and numerous small setae at apex, outer margin slightly concave with minute setae; endopod of pleopod 1 with triangular apical part with tuft of long setae on inner margin (Figure 9B). Pleopod 2: exopod triangular with straight outer margin bearing more than 15 setae (Figure 9C); endopod much longer than exopod, narrow, with parallel sides (Figure 9D). Pleopod 3–4 exopods (Figure 9E–F) trapezoidal, slightly decreasing in size. Pleopod 5 exopod triangular, with sharply rounded corners (Figure 9G).

**Remarks.** This species belongs to the central Asian group of *Protracheoniscus* characterized by the position of noduli laterales on pereonites 3 and 4 at a distance from the lateral edge (Borutzky, 1957). The distinctive feature of the genus is the male dactylus of pereopod 6 and 7 widened in the middle. This species is the morphologically closest to *P. major* (Dollfus, 1903), from which it differs in lacking the enlargement of dactyli of male pereopods 6 and 7 (see Gruner 1966; Tomescu et al. 2016). *Protracheoniscus major* is one of the dominant woodlice species in Kalmykia and broadly distributed



**Figure 9.** *Protracheoniscus kryszanovskii* Borutzky, 1957: **A** exopod of pleopod 1 **B** endopod of pleopod 1 **C** exopod of pleopod 2 **D** endopod of pleopod 2 **E** exopod of pleopod 3 **F** exopod of pleopod 4 **G** exopod of pleopod 5 **H** genital papilla (male).

around the Caspian Sea (Kashani and Hamidnia 2016). The study of several specimens from the type locality confirmed the identity of our specimens with the type series designated by Borutzky (1957). Both the old collection and recent one of *P. kryszanovskii* showed substantial variability in the endopodite of the male pleopod 1.

**Distribution.** The species has been found between the Volga and Vostochnyi Manych Rivers so far (Figure 10). It occupies steppes of Kalmykia (*Artemisia austriaca*, *Festuca valesiaca*, *Tanacetum achilleifolium*) and is common in various biotopes being one of the dominant species in the region. The species prefers salted soils,



**Figure 10.** Distribution in the SE of European Russia of *Protracheoniscus kryszanovskii* from the original description by Borutzky (1957) (circles) and new location on Sarpa Lake, Kalmykia (square), and location of *Protracheoniscus pokarzhevskii* sp. n. in Bolshoi Tsaryn (diamond).

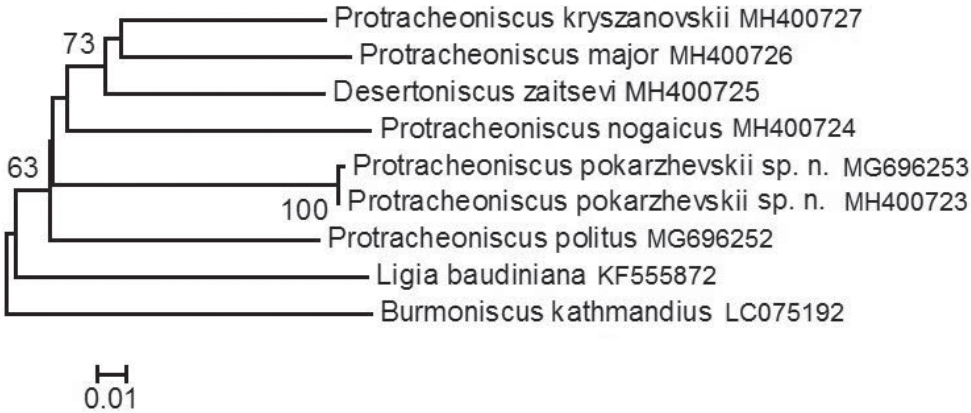
namely the banks of lakes with salted water. In the surroundings of Kamyshin (Volgograd Region) it was found in the burrows of *Spermophilus* sp. and in leaf litter (Borutzky 1957).

**Phylogenetic analysis.** The results of the pairwise distance analysis based on the analysis of the mtDNA COI gene (Table 2) show that the difference between *P. politus* and *P. pokarzhevskii* sp. n. by mtDNA COI is 23.0%. The differences between *P. pokarzhevskii* sp. n. from the types of outgroup taxa by mtDNA COI range from 24.0% to 30.0%. The results obtained indicate the species' independence of *P. pokarzhevskii* sp. n. and a considerable divergence from the morphologically close *P. politus*.



**Table 2.** Estimates of pairwise sequence divergence (uncorrected p-distances) of gene COI mtDNA haplotypes.

	1	2	3	4	5	6	7	8	9
1 <i>Protracheoniscus kryszanovskii</i> MH400727	–								
2 <i>Protracheoniscus major</i> MH400726	0.15	–							
3 <i>Protracheoniscus nogaicus</i> MH400724	0.21	0.19	–						
4 <i>Protracheoniscus politus</i> MG696252	0.19	0.21	0.20	–					
5 <i>Protracheoniscus pokarzhevskii</i> sp.n. MG696253	0.20	0.21	0.21	0.20	–				
6 <i>Protracheoniscus pokarzhevskii</i> sp.n. MH400723	0.20	0.21	0.21	0.19	0.00	–			
7 <i>Desertoniscus zaitsevi</i> MH400725	0.16	0.16	0.20	0.19	0.19	0.19	–		
8 <i>Burmoniscus kathmandius</i> LC075192	0.23	0.25	0.25	0.23	0.24	0.24	0.24	–	
9 <i>Ligia baudiniana</i> KF555872	0.21	0.24	0.25	0.24	0.24	0.24	0.23	0.25	–



**Figure 11.** Phylogeny of six species of the genera *Protracheoniscus* and *Desertoniscus* with outgroup taxa based on the analysis of the mtDNA COI gene by the Neighbor Joining method (NJ) with the calculation of bootstrap support of branch sites (1000 replications).

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# A guide to the identification of the terrestrial Isopoda of Maryland, U.S.A. (Crustacea)

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## Abstract

The terrestrial isopod fauna of Maryland is inferred using the taxonomic literature, internet-based citizen science projects, and original collecting. Twenty-two species are either known or are likely to occur in the state. This includes 17 mostly-European adventive species that comprise the vast majority of records. Of the five expected native species, three occur in or near marine or estuarine littoral habitats and each has yet to be recorded or recorded from only a single locality. This situation likely reflects the long history of systematic work on the European fauna and the availability of keys for the identification of these taxa, which contrasts with the limited taxonomic work on native species. A taxonomic key, illustrations, and brief descriptions of species known or expected to occur in Maryland are provided.

## Keywords

Oniscidea, taxonomy, taxonomic key

## Introduction

There are no up-to-date, comprehensive taxonomic keys to the terrestrial isopod species of the eastern United States, the most recent being that of Muchmore (1990). A standard solution to the identification problem has been to send specimens to taxonomic experts, but such expertise is increasingly rare (Sfenthourakis and Taiti 2015). Alternatively, the internet offers several citizen-science projects: BugGuide

(<https://bugguide.net/>), iNaturalist (<https://www.inaturalist.org/>), and Maryland Biodiversity Project (<https://www.marylandbiodiversity.com/>) where photos of specimens can be posted for identification by the internet community. Unfortunately, the photos often do not show key diagnostic features and the specimens thus remain unidentified or even misidentified. Given such problems, it has become common in ecological studies to circumvent the issue by grouping unidentified specimens into look-alike morphospecies, a practice known as “taxonomic minimalism” (Beattie and Oliver 1994), which limits comparisons across studies and may inadvertently inflate or reduce the actual number of species in a study (Krell 2004). This “solution” hampers the ability to associate taxonomic species with particular localities or habitats, to understand the dynamics of communities, and to recognize new native, adventive, or invasive species. The goal of this contribution is to provide a taxonomic key to the 22 species of terrestrial isopods that are known or expected to occur in Maryland as a step toward reducing the need for taxonomic minimalism in ecological studies in the state and adjacent areas.

Most terrestrial isopod species in Maryland are widely distributed and common European adventives (e.g., Hornung and Szilávecz 2003, Hornung et al. 2015, Norden 2008). Consequently, identification tools designed for use in Europe (e.g., Hopkin 2014) may be useful in the eastern United States and can potentially identify native forms to genus (e.g., *Ligidium*, *Miktoniscus*). Still, because such keys deal with a more diverse fauna than exists in Maryland and may omit some European species, such as *Chaetophiloscia sicula* (Hornung and Szilávecz 2003), the potential for misidentification is increased.

Maryland’s native terrestrial isopod fauna is small and probably consists of five species. Three marine or estuarine littoral species are either known from the state, *Miktoniscus spinosus* (Say, 1818) (Trichoniscidae) and *Scyphacella arenicola* Smith, 1873 (Scyphacidae), or are expected to occur based on established distributions, *Littorophiloscia vittata* (Say, 1818) (Halophilosciidae). Two inland terrestrial species are also expected, *Miktoniscus medcofi* (Van Name, 1940) (Trichoniscidae) and *Ligidium elrodii* (Packard, 1873) (Ligiidae), with the first state record of *L. elrodii* reported here. In addition, *M. spinosus* has been found in moist inland habitats in North Carolina (Schultz 1976) and this could be the case in Maryland. In fact, a recent photo taken at a suburban park in the District of Columbia, which is essentially encompassed geographically by Maryland, appears to show a specimen of *Miktoniscus* sp. (BugGuide: <https://bugguide.net/node/view/1469429>). The paucity of state records for native terrestrial isopods likely reflects several factors, including the lack of taxonomic work on the group, substantial reduction in native habitat, and the limited accessibility of taxonomic keys and other methods of identification.

## Basic external anatomy of terrestrial isopods

The isopod body has three main regions or tagmata: head (cephalothorax), pereon (thorax), and pleon (abdomen) (Figure 1C). The anterior part of the head is typically

divided transversely by a raised frontal margin (fm, Figure 1A) that begins on the lateral surface, passes anteriorly ventral to the eye region and dorsal to the base of the antennae, and traverses the anterior surface of the head. It separates the cephalic dorsum (or vertex) (cd, Figure 1A) from the frontal lamina (frl, Figure 1A), a sclerotized region bordered laterally by the bases of the two large antennae. In some cases the frontal margin is interrupted medially or is absent, with the latter obscuring the distinction between the cephalic dorsum and frontal lamina. The frontal margin is often produced into a pair of anterolateral lobes (al, Figure 1A) between the eye region and base of the antenna. The frontal margin may also be produced medially in various ways (e.g., Figs 1A, 4A, C–F).

Each eye consists of either a compound eye (ce, Figure 1A) or one to three ocelli and is absent in some species (e.g., Figure 1C). Antennules (= antennae I) are very small and positioned medial to the base of the large antennae (antenna II). The five proximal articles (antennomeres) of the antennae constitute the peduncle (pe, Figure 1D), with the distal-most article bearing a terminal flagellum (fl, Figure 1D). Most species have only two or three distinct flagellar articles (Figs 3E, 4A, B, 5A, B) but some have 10 or more (Figure 5F, G). Species in the family Trichoniscidae have what can appear to be a thin, tapering undivided flagellum (Figure 2A–C), but inspection with high magnification will reveal multiple articles, the number of which can be useful for species identification.

The pereon consists of seven broad segments (Figure 1C), each bearing a pair of legs (pereopods) ventrally. The dorsal surface of each segment, the tergite (tg, Figure 1D), may be smooth or armed with tubercles or other sculpture (e.g., Figure 2B, C). The tergites bear ventrolateral extensions (epimera) (ep, Figure 1D) that collectively form the protected ventral space within which the legs operate.

The pleon has five free segments. The lateral margins of the first two lack epimera. The remaining three may be abruptly narrower than the last pereonal tergite (b, Figure 1E), often due to the absence or reduction of epimera. However, when large epimera are present, the pleonal margin may appear as a smooth continuation of the pereonal margin (a, Figure 1D).

Each free pleonal segment has a pair of ventral appendages, the pleopods, with a large plate-like part, the exopodite, and a medial part, the endopodite (Figure 1B). The endopodites of the first two pairs are enlarged and elongated in males (en, Figure 1B) and bear species-specific modifications (e.g., Figure 2D–G). The exopodites may have air-filled respiratory tubules called pseudotracheae that function as a lung. The lungs are visible in live specimens as thickened white patches on the lateral part of the exopodites. The white color typically disappears through loss of air when the animal is preserved. In species found in Maryland, the lungs may be absent, present in the first two pairs of pleopods (lg, Figure 1B) or present in all pleopods.

The last apparent segment of the pleon is the pleotelson, a combination of a terminal segment and the telson (pt, Figs 1B, D, 3B, D, 5C–E). The pleotelson has a pair of uropods, each comprising a basal protopodite that articulates distally with a

medial endopodite and lateral exopodite (Figure 1B–D). The relative sizes, lengths, and shapes of these elements and their position with respect to the pleotelson are often useful in identification.

### Key to adult terrestrial isopods of Maryland

- 1 Cuticle translucent to white. Eyes absent. Antenna with antennomere V much wider than the other antennomeres (Figure 1C). Associated with ants. (Platyarthridae) ..... ***Platyarthrus hoffmannseggii* Brandt, 1833**
- Cuticle color variable. Eyes present, each usually compound or with one or three ocelli. Antenna variable, but antennomere V not significantly wider than the others. Not associated with ants or associated with ants only incidentally ..... **2**
- 2 Antennal flagellum comprising two to many distinct articles (Figs 1D, 3E, 4A, B, 5A, B, F, G) ..... **3**
- Antennal flagellum superficially appearing to be one article, either robust (fl, Figure 1E) or thin, tapering and elongate (Figure 2A–C), but comprising up to six articles visible with high magnification ..... **4**
- 3 Antenna with two distinct flagellar articles (Figs 3E, 4A, B). Uropods may extend beyond elliptical perimeter of the body in dorsal view (Figure 4A, B) or may not (Figure 3B, D) ..... **9**
- Antenna with three distinct flagellar articles (Figure 5A, B). Uropods extending beyond elliptical perimeter of body in dorsal view; protopodite usually not extending beyond pleotelson; exopodite large, conical or spear-head shaped; endopodite thin (Figure 5A, B, D) ..... **16**
- Antenna with four distinct flagellar articles (Figure 1D). Uropods completing posterior elliptical perimeter of body in dorsal view, protopodite large, dorsoventrally flattened; exopodite small, endopodite elongate (Figure 1D). (Detonidae) ..... ***Armadilloniscus ellipticus* (Harger, 1878)**
- Antenna with flagellum long, comprising 10 or more flagellar articles (Figure 5F, G). Uropods extending beyond elliptical perimeter of body in dorsal view; with protopodite robust, projecting posteriorly beyond pleotelson; endopodite and exopodite thin, elongate (Figure 5F, G). (Ligiidae) ..... **19**
- 4 Compound eye present. Antennal flagellum approx. the same width basally as antennomere V and comprising four articles that decrease in size distally (fl, Figure 1E). Sandy marine beaches. (Scyphacidae) ..... ***Scyphacella arenicola* Smith, 1873**
- Compound eye absent, but with one or three ocelli. Antennal flagellum abruptly narrower than preceding article, a narrow tapering cone (Figure 2A–C) comprising up to six articles visible with high magnification. Not usually associated with sandy marine beaches. (Trichoniscidae) ..... **5**



- 5 Surface of pereon smooth; without tubercles, denticles or other sculpture (Figure 2A) ..... **6**
- Surface of pereon sculptured, with tubercles and/or denticles (Figure 2B, C).... **7**
- 6 Each eye with three ocelli. Antenna with four or five flagellar articles visible with high magnification. Body length up to 5 mm (Figure 2A) .....  
..... *Trichoniscus pusillus* Brandt, 1833
- Each eye with one ocellus. Antenna with six flagellar articles visible with high magnification. Body similar to Figure 2A, length up to 7 mm .....  
..... *Hyloniscus riparius* (C. L. Koch, 1838)
- 7 Pereon with tubercles or denticles on longitudinal ridges (Figure 2B). Pleon not abruptly narrower than pereon; pleonal tergites with prominent epimera. Cuticle translucent or white, without dark pigments. Antenna with three flagellar articles visible with high magnification .....  
..... *Haplophthalmus danicus* Budde-Lund, 1880
- Pereon with tubercles in transverse or roughly transverse rows (Figure 2C). Pleon abruptly narrower than pereon. Cuticle pigmented. Antenna with four flagellar articles visible with high magnification. (*Miktoniscus*) ..... **8**
- 8 Male pleopod I (Figure 2D) with exopodite long, almost as long as endopodite, ending in point; tip of endopodite long, round in cross section. Near marine or estuarine grasses, debris, etc. but may also occur in moist inland habitats ..... *Miktoniscus spinosus* (Say, 1818)
- Male pleopod I (Figure 2F) with exopodite shorter, approx. half the length of endopodite, tapering distally but terminus broadly rounded; endopodite long, flattened in cross section. Moist terrestrial habitats .....  
..... *Miktoniscus medcoffi* (Van Name, 1940)
- 9 Exopodite of uropod not extending beyond elliptical perimeter of body in dorsal view; broad, dorsoventrally flattened; protopodite and endopodite largely hidden in dorsal view (Figure 3B, D, E, cf. 1B). In life, able to roll into a ball with antennae hidden. (Armadillidiidae: *Armadillidium*) ..... **10**
- Exopodite of uropod extending beyond elliptical perimeter of body in dorsal view; protopodite visible in dorsal view; exopodite prominent, attaching at terminus of protopodite, broad basally, tapering distally; endopodite thin, attaching at medial base of protopodite (Figure 4A, B). In life, unable to roll into a ball or, if able to enroll, antennae remain exposed ..... **11**
- 10 Head with median frontal projection extending dorsally, with dorsal margin overlapping anterior edge of cephalic dorsum (fp, Figure 3A). Pleotelson subtrapezoidal, with posterior margin nearly straight (pt, Figure 3B) .....  
..... *Armadillidium vulgare* (Latreille, 1804)
- Head with prominent, median frontal projection extending dorsoanteriorly and not overlapping anterior edge of cephalic dorsum (fp, Figure 3C). Pleotelson subtriangular with posterior apex variably produced, but typically with rounded terminus (pt, Figure 3D) ..... *Armadillidium nasatum* Budde-Lund, 1885

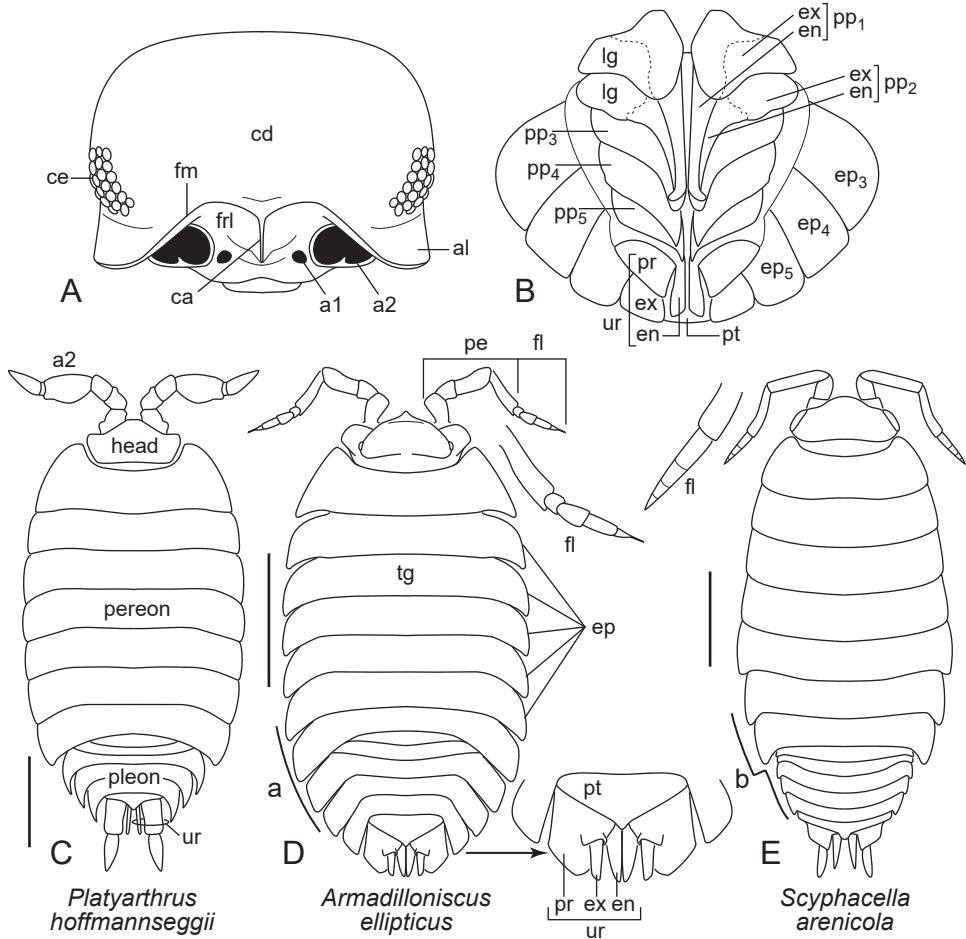
- 11 Pleon abruptly narrower than pereon (Figure 4B). Anterolateral lobes very small. Cuticle often with frosted-gray or dusty appearance, but this may be absent. Antennomeres IV and V usually with white terminal band. Frontal margin without evident median projection. (Porcellionidae, in part).....  
..... ***Porcellionides pruinus* (Brandt, 1833)**
- Pleon not abruptly narrower than pereon (Figure 4A). Anterolateral lobes prominent (Figure 4A, C–F). Cuticle without frosted-gray appearance. Antenna color variable but antennomeres IV and V usually without terminal white bands. Frontal margin with some form of median projection (Figure 4A, C–F) ..... **12**
- 12 Dorsal surface of pereon essentially smooth ..... **13**
- Dorsal surface of pereon with numerous bumps or tubercles..... **14**
- 13 Posterolateral margin of first pereonal tergite produced posteriorly into pointed angle (arrow, Figure 4F). Five pairs of lungs present. Frontal margin of head with small median triangular projection (Figure 4F) corresponding to vertical median carina of frontal lamina (ca, Figure 1A). In life, capable of rolling into a ball with antennae exposed. (Cylisticidae).....  
..... ***Cylisticus convexus* (De Geer, 1778)**
- Posterolateral angle of first pereonal tergite bluntly rounded, not produced posteriorly into a pointed angle (arrow, Figure 4E). Lungs restricted to pleopods I and II. Frontal margin with broad, convex median projection (Figure 4E). In life, not capable of rolling into a ball. (Porcellionidae, in part).....  
..... ***Porcellio laevis* Latreille, 1804**
- 14 Anterolateral lobes very prominent and broad, width of each approx. one-third width of head; in dorsal view, junction between median projection and anterolateral lobes V-shaped or nearly so (Figure 4C). Head dark brown to black, contrasting with base color of pereon. Pereon with dark mid-dorsal stripe (sometimes broken), usually bordered laterally by bright yellow markings that may be lost in preservative. (Porcellionidae, in part) .....  
..... ***Porcellio spinicornis* Say, 1818**
- Anterolateral lobes not so wide, joining convex median projection via curved margin, not V-shaped notches (Figure 4A, D). Head color not usually contrasting with base color of pereon. Pereon with dorsal color variable; if dark median line present, then not bordered by bright yellow markings ..... **15**
- 15 Five pairs of lungs. Dorsum of pereon with low, irregular bumps and tubercles; surface usually with pattern of dark brown, reddish brown and tan; lateral surface at base of epimera with tan to nearly-white patches creating a pair of broken lines. Frontal margin with broad convex median projection (Figure 4D). (Trachelipodidae) ..... ***Trachelipus rathkii* (Brandt, 1833)**
- Lungs restricted to pleopods I and II (as in Figure 1B). Dorsum of pereon tuberculate; color variable, ranging from solid brown or gray to various patterns, sometimes similar to *Trachelipus* but usually without a pair of broken light lines at base of epimera. Frontal margin with prominent triangular to subtriangular median projection with rounded apex (Figure 4A). (Porcellionidae, in part) ..... ***Porcellio scaber* Latreille, 1804**

- 16 Head with prominent anterolateral lobes. Pleon not abruptly narrower than pereon, body broad and distinctly elliptical in dorsal view (Figure 5A). (Oniscidae)..... ***Oniscus asellus* Linnaeus, 1758**
- Head without anterolateral lobes. Pleon abruptly narrower than pereon, body more elongate, oblong in dorsal view (Figure 5B). ..... **17**
- 17 Found in vegetation or under objects near marine or brackish water. Pleotelson with lateral margins weakly concave, posterior apex bluntly rounded to truncate (pt, Figure 5E). Head with color similar to that of pereon; pereon often with dark mid-dorsal line bordered laterally by bright yellow splotches. (Halophilosciidae) ..... ***Littorophiloscia vittata* (Say, 1818)**
- Found in terrestrial environments. Pleotelson with lateral margins essentially straight (pt, Figure 5D) or distinctly concave (pt, Figure 5C). Coloration differing from above. (Philosciidae) ..... **18**
- 18 Pleotelson triangular, with lateral margins straight or nearly so in dorsal view, posterior apex blunt (pt, Figure 5D). Pleon rather elongate, sides straight in dorsal view, epimera not forming lateral serration. Dorsal coloration of pereon purple-brown with small, light longitudinal markings (lineoles), typically without distinct mid-dorsal stripe; head color similar to pereon. Thus far known in Maryland only from forests in Baltimore..... ***Chaetophiloscia sicula* Verhoeff, 1908**
- Pleotelson with lateral margins distinctly concave, posterior apex pointed (pt, Figure 5B,C). Pleon more compact, epimera giving sides a serrate appearance in dorsal view. Dorsal coloration of pereon highly variable, but often brown with pattern of lighter markings, usually with very dark mid-dorsal line; head dark brown to black, often contrasting with lighter pereon. Widespread. (Philosciidae) ..... ***Philoscia muscorum* (Scopoli, 1763)**
- 19 Uropod with protopodite very long, surpassing posterior terminus of pleotelson by more than the length of pleotelson; endopodite and exopodite long and thin, about equal in length, both arising from tip of protopodite (Figure 5F). Marine or brackish shorelines, splash zone, and directly adjacent areas. Larger, up to 4.8 cm ..... ***Ligia exotica* Roux, 1828**
- Uropod with protopodite exceeding posterior tip of pleotelson by about one length of the pleotelson or less; endopodite about 1.5 times the length of exopodite, endopodite arising from protopodite proximal to exopodite (Figure 5G). Wet litter in woodlands, wetlands, near streams, etc. in mountains. Smaller, up to 1 cm ..... ***Ligidium elrodii* (Packard, 1873)**

## Family and species summaries

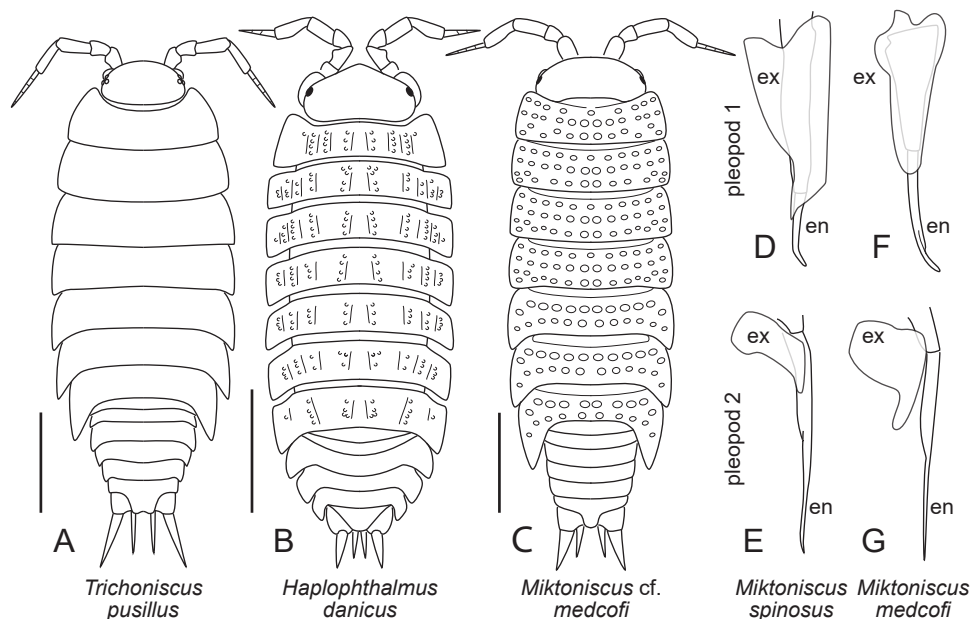
### Family Armadillidiidae (*Armadillidium*) (Figs 1B, 3A–E)

Length up to 15 mm. Compound eyes present. Frontal lamina with broad projection [scutellum] (fp, Figure 3A, C). Frontal margin interrupted medially (Figure 3C) but this is hidden by scutellum in *A. vulgare* (Figure 3A). Anterolateral lobes prominent. An-



**Figure 1.** **A** *Cylisticus convexus*, head, dorsoanterior view, antennae removed (based on Schmidt 2008: figure 32) **B** *Armadillidium vulgare*, male pleon, ventral view **C** *Platyarthrus hoffmannseggii* (after Hopkin 2014: figure 17) **D** *Armadilloniscus ellipticus*, insets highlight uropods and antennal flagellum **E** *Scyphacella arenicola*, inset highlights antennal flagellum. Abbreviations: **a** lateral perimeter narrows gradually from pereon to pleon; **a1** socket of antenna I; **a2** antenna II or socket of antenna II; **al** anterolateral lobe; **b** lateral perimeter narrows abruptly from pereon to pleon; **ca** carina; **cd** cephalic dorsum; **ce** compound eye; **en** endopodite; **ex** exopodite; **fl** flagellum; **fm** frontal margin; **frl** frontal lamina; **lg** lung; **tg** tergite; **pe** peduncle of antenna II; **pp** pleopod; **pr** protopodite; **pt** pleotelson; **ur** uropod. Scale bars: 1 mm.

tenna with two distinct flagellar articles (Figure 3E). Pleon not abruptly narrower than pereon (Figure 3E). Lungs limited to first two pairs of pleopods (lg, Figure 1B). Uropod with exopodite broad, dorsoventrally flattened, completing rounded posterior outline of body in dorsal view (ex, Figure 3B, D, E); protopodite and endopodite, largely hidden in dorsal view (Figs 1B, 3B, D). In life, capable of rolling into a ball with antennae hidden.



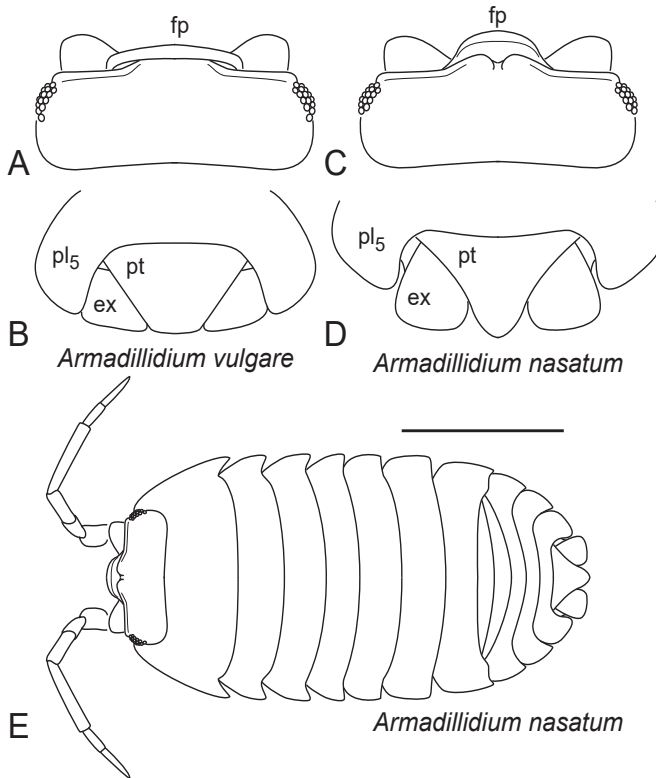
**Figure 2.** Trichoniscidae. **A** *Trichoniscus pusillus* **B** *Haplophthalmus danicus*, highlighting cuticular sculpture of pereon **C** *Miktoniscus cf. medcofi*, highlighting cuticular sculpture of pereon **D–E** *Miktoniscus spinosus*, male **D** right pleopod I, ventral view **E** right pleopod II, ventral view (based on Schultz 1976: figs 8, 9, as *M. halophilus*) **F–G** *Miktoniscus medcofi*, male: **F** right pleopod I, ventral view **G** right pleopod II, ventral view (based on Schultz 1976: figs 40, 41). Abbreviations: **en** endopodite; **ex** exopodite. Scale bars: 1 mm.

### *Armadillidium vulgare* (Latreille, 1804) (Figs 1B, 3A, B)

With features of the family and genus, also the following: Projection of frontal lamina triangular, with broad, transverse dorsal margin overlapping frontal margin (fp, Figure 3A). Pleotelson subtrapezoidal, broadly truncated posteriorly (pt, Figure 3B). Sources: Richardson (1905), Schultz (1982) and Hopkin (2014). U.S. Distribution: Introduced. Throughout contiguous 48 states (Jass and Klausmeier 2000, 2001). Recorded in Maryland by Richardson (1905), Hornung and Szlávéc (2003), Norden (2008), BugGuide, iNaturalist, and Maryland Biodiversity Project. Habitat: Synanthropic. Litter and under stones or other objects, occurs in somewhat drier conditions than most terrestrial isopods.

### *Armadillidium nasatum* Budde-Lund, 1885 (Figure 3C)

With features of the family and genus, also the following: Projection of frontal lamina extending dorsoanteriorly, not overlapping anterior margin of cephalic dorsum (Figure 3C). Pleotelson subtriangular, with rounded to somewhat pointed posterior apex (pt, Figure 3D). Sources: Richardson (1905), Schultz (1982), and Hopkin (2014).



**Figure 3.** Armadillidiidae. **A–B** *Armadillidium vulgare* **A** head, dorsal view **B** posterior end of pleon, dorsal view **C–E** *Armadillidium nasatum* **C** head, dorsal view **D** posterior end of pleon, dorsal view **E** Dorsal view. Abbreviations: **ex** exopodite of uropod; **fp** frontal projection; **pl<sub>5</sub>** pleonal tergite V; **pt** pleon-telson. Scale bar: 5 mm.

U.S. Distribution: Introduced. Eastern and central states, also Idaho and California (Jass and Klausmeier 2000, 2001). Recorded in Maryland by Smith and Goodhue (1945), Hornung and Szlávecz (2003), Norden (2008), Hornung et al. (2015), BugGuide, iNaturalist, and Maryland Biodiversity Project. Habitat: Synanthropic. Litter and under stones or other objects, often in drier habitats than most terrestrial isopods.

### Family Cylisticidae (*Cylisticus*)

*Cylisticus convexus* (De Geer, 1778) (Figs 1A, 4F)

Body length up to 15 mm. Compound eyes present. Antenna with two distinct flagellar articles. Frontal lamina divided by median vertical ridge (carina) that terminates dorsally as a small, triangular median projection at frontal margin (ca, Figure 1A). Anterolateral lobes prominent (al, Figs 1A, 4F). Posterolateral margin

of first pereonal tergite produced into broad, posteriorly pointed angle (arrow, Figure 4F), thus distinguishing it from *Porcellio laevis* (arrow, Figure 4E). Surface of pereon smooth. Pleon not abruptly narrower than pereon (as in Figure 4A). Five pairs of lungs. In life, capable of rolling into a ball, with antennae exposed. Sources: Richardson (1905), Schultz (1982) and Hopkin (2014). U.S. Distribution: Introduced. Throughout most of contiguous 48 states (Jass and Klausmeier 2000, 2001). Recorded in Maryland by Hornung and Szlávecz (2003), Norden (2008), Hornung et al. (2015), BugGuide, and Maryland Biodiversity Project. Habitat: A variety of moist litter and soil habitats.

### **Family Detonidae (*Armadilloniscus*)**

*Armadilloniscus ellipticus* (Harger, 1878) (Figure 1D)

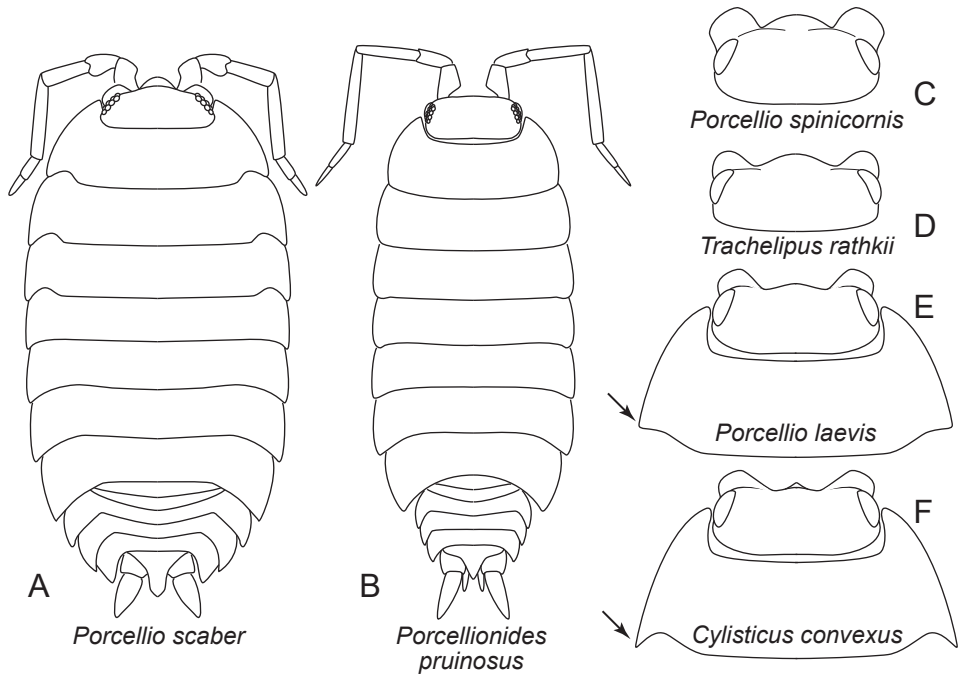
Body length up to 4 mm. Compound eye present, each with five to ten facets. Antenna with four distinct flagellar articles (fl, Figure 1D), an additional minute terminal article may be present. Pleon not abruptly narrower than pereon (a, Figure 1D). Lungs absent. Uropods with protopodite greatly enlarged, flattened, projecting posteriad far beyond pleotelson, resembling adjacent tergal extension; exopodite short, arising from dorsomedial margin of protopodite; endopodite long, arising from base of protopodite; protopodite, exopodite (ex) and endopodite (en) all ending posterior to pleotelson (pt) (Figure 1D). Sources: Harger (1880), Richardson (1905) and Schultz (1982). U.S. Distribution: Probably introduced. Massachusetts, New York, North Carolina, Florida (Jass and Klausmeier 2000, 2001), and Alabama (BugGuide). Not yet recorded from Maryland. Scattered, worldwide distribution (Schmalfuss 2003). Habitat: Drift line on marine shores, especially under planks, stones, vegetation, etc. Clings to under-surface of objects, usually does not run when disturbed (Schultz 1982).

### **Family Halophilosciidae (*Littorophiloscia*)**

*Littorophiloscia vittata* (Say, 1818) (Figure 5E)

Body length up to 5 mm. Compound eyes present. Head without anterolateral lobes. Antenna with three distinct flagellar articles (as in Figure 5B). Pleon abruptly narrower than pereon (as in Figure 5B). Lungs absent. Unable to roll into a ball. Head color usually similar to that of pereon. Pereon and pleon usually with dark median stripe bordered by bright yellow patches, also a series of lateral submarginal yellow markings. Pleotelson with lateral margins weakly concave, posterior end rounded to almost truncate (pt, Figure 5E). Sources: Schultz (1963, 1974). U.S. Distribution: Native. Coasts of Atlantic Ocean and Gulf of Mexico, New York to Texas (Jass and Klausmeier 2000, 2001), not yet recorded from Maryland. Habitat: Marine and brackish shores





**Figure 4.** Porcellionidae, Trachelipodidae, Cylisticidae. **A** *Porcellio scaber* **B** *Porcellionides pruinosus* **C** *Porcellio spinicornis*, head, dorsal view **D** *Trachelipus rathkii*, head, dorsal view **E** *Porcellio laevis*, head and first pereon, dorsal view **F** *Cylisticus convexus*, head and first pereon, dorsal view.

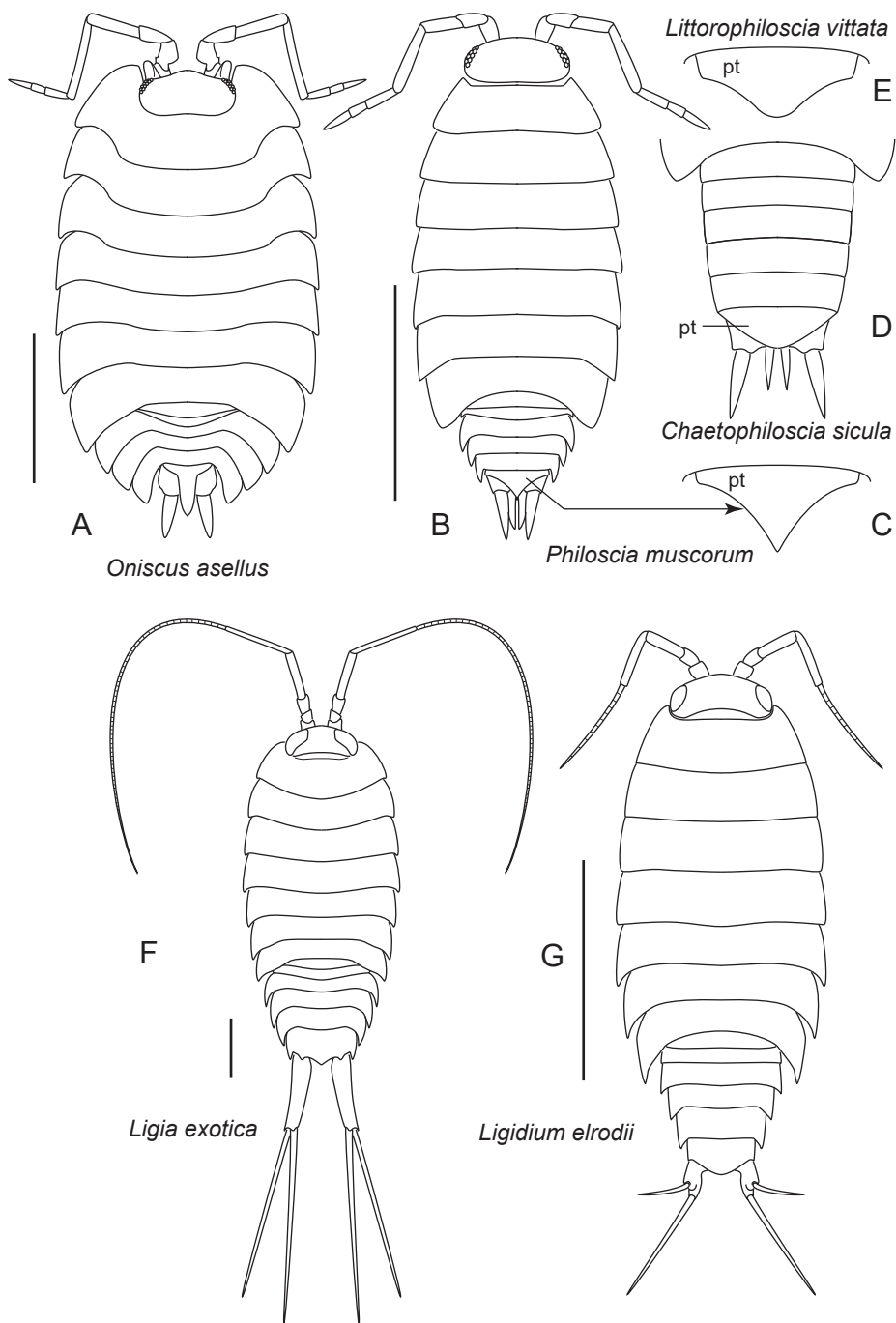
between water and drift line, but also somewhat farther away from shore under objects, in marsh grass, debris, etc. (Schultz 1974).

#### Family Ligiidae (*Ligia*, *Ligidium*) (Figure 5F, G)

Compound eyes present, very large with many facets. Antenna with many (> 10) flagellar articles. Uropod with protopodite projecting posteriorly beyond tip of pleotelson; endopodite and exopodite long and thin. Lungs absent. Rapid runners.

*Ligia exotica* Roux, 1828 (Figure 5F)

Only marine littoral species known in Maryland with features of the family, also the following: Body length (excluding uropods) up to 30 mm. Antenna, especially flagellum, very long with more than 20 flagellar articles. Pleon not abruptly narrower than pereon, outline of body fusiform, tapering posteriorly. Uropod with protopodite very long, cylindrical; endopodite and exopodite nearly equal in length, attaching to protopodite at its terminus, total length of uropod up to 18 mm. Sources: Richardson (1905) and Schultz



**Figure 5.** Oniscidae, Philosciidae, Halophilosciidae, Ligiidae. **A** Oniscidae: *Oniscus asellus* **B–E** Philosciidae **B–C** *Philoscia muscorum* **B** dorsal view **C** pleotelson **D** *Chaetophiloscia sicula*: dorsal view of pleon (after Vandel 1962: figure 247) **E** *Littorophiloscia vittata*: pleotelson, dorsal view **F–G** Ligiidae **F** *Ligia exotica* **G** *Ligidium elrodii*. Scale bars: 5 mm.

(1982). U.S. Distribution: Introduced. Known from New Jersey, North and South Carolina, Texas, Florida and California (Jass and Klausmeier 2000, 2001). Recorded from Maryland (Chesapeake Bay) by BugGuide, iNaturalist, and Maryland Biodiversity Project. Habitat: Marine to brackish shoreline, especially adjacent surfaces (pilings, rocks, etc.)

*Ligidium elrodii* (Packard, 1873) (Figure 5G)

Only terrestrial species to occur in Maryland with features of the family, also the following: Body length up to 10 mm. Antenna with approx. 11 flagellar articles. Uropod with protopodite extending distad to pleotelson; endopodite articulating with protopodite at a point distad to articulation with exopodite, endopodite almost 1.5 times longer than exopodite. Sources: Richardson (1905) and Schultz (1970, 1982). U.S. Distribution: Native. Known from localities throughout the eastern states, also California [?] (Jass and Klausmeier 2000, 2001). Recently recorded from forests in Garrett County (J. Shultz, original observation). Habitat: Moist to wet litter and moss especially near swamps, marshes, springs, ponds, streams, also caves.

### Family Oniscidae (*Oniscus*)

*Oniscus asellus* Linnaeus, 1758 (Figure 5A)

Body length up to 16 mm. Perimeter a wide ellipse in dorsal view. Compound eyes present. Antenna with three distinct flagellar articles. Head with prominent anterolateral lobes. Pleon not abruptly narrower than pereon. Lungs absent. Pleotelson elongate, pointed. Dorsoventrally flattened, not capable of rolling into a ball. Sources: Richardson (1905), Schultz (1982), and Hopkin (2014). U.S. Distribution: Introduced. Scattered eastern and central states, also Washington and Oregon (Jass and Klausmeier 2000, 2001). Recorded from Maryland by Norden (2008), BugGuide, iNaturalist, and Maryland Biodiversity Project. Habitat: Leaf litter, under bark and similar situations.

### Family Philosciidae (*Chaetophiloscia*, *Philoscia*) (Figure 5B–D)

Compound eyes present. Antenna with three distinct flagellar articles (Figure 5B). Head without anterolateral lobes. Pleon abruptly narrower than pereon (Figure 5B, D). Lungs absent. Unable to roll into a ball.

*Chaetophiloscia sicula* Verhoeff, 1908 (Figure 5D)

With features of the family, also the following: Body length up to 7.5 mm. Body outline similar to Figure 5B, but pleon more elongate and lateral margins smooth, not

serrated (Figure 5D). Pleotelson broad, triangular (width about double the length) with lateral margins straight or nearly so, posterior apex bluntly rounded (pt, Figure 5D). Dorsum with purplish-brown pigment interrupted by median series of small light markings and over much of surface by light longitudinal markings (lineoles); epimera pigmented except for distal light marginal line and proximal light mark that widens anteriorly; head with markings similar to pereon. Source: Vandel (1962). U.S. Distribution: Introduced. Known thus far only from Baltimore, Maryland (Hornung and Szlávecz 2003). Habitat: In Baltimore, litter in mature temperate deciduous forest.

*Philoscia muscorum* (Scopoli, 1763) (Figure 5B, C)

With features of the family, also the following: Body length up to 12 mm. Pleon compact with epimera forming serrated lateral margin (Figure 5B). Pleotelson with lateral margins concave, posterior apex pointed (pt, Figure 5C). Head typically dark, often contrasting with lighter pereon. Pereon usually with dark mid-dorsal stripe. Sources: Schultz (1974) and Hopkin (2014). U.S. Distribution: Introduced. New England, New York, New Jersey; also Washington State (Jass and Klausmeier 2000, 2001). Recorded from Maryland by Schultz (1974), Hornung and Szlávecz (2003), Norden (2008), Hornung et al. (2015), BugGuide, iNaturalist, and Maryland Biodiversity Project. Habitat: A variety of mesic terrestrial habitats.

### **Family Platyarthridae (*Platyarthrus*)**

*Platyarthrus hoffmannseggii* Brandt, 1833 (Figure 1C)

Body length up to 5 mm. Cuticle white to translucent. Eyes absent. Antenna with pre-flagellar antennomere very robust, wider than other peduncular antennomeres; flagellum superficially appearing to be undivided but actually with two articles, basal article very short. Anterolateral lobes prominent. Pleon not abruptly narrower than pereon. Lungs absent. Source: Hopkin (2014). U.S. Distribution: Introduced. Recorded from New York, Connecticut (Jass and Klausmeier 2000, 2001) and Philadelphia, Pennsylvania (BugGuide). Not yet reported from Maryland, but the record from Philadelphia indicates that it may occur in the state. Habitat: Soil, associated with ants.

### **Family Porcellionidae (*Porcellio*, *Porcellionides*) (Figure 4A–C, E)**

Compound eyes present. Antennae with two distinct flagellar articles (Figure 4A, B). Lungs restricted to pleopods I and II. Uropods extending posteriad beyond end of pleotelson (Figure 4A, B).

*Porcellio laevis* Latreille, 1804 (Figure 4E)

With features of the family, also the following: Body length up to 18 mm. Large, broad species, body outline similar to Figure 4A. Medial frontal margin projecting anteriorly as a broad weakly convex edge. Anterolateral lobes prominent, each less than third the width of head. Pereon smooth, without tubercles or other significant sculpture. Pleon not abruptly narrower than pereon (as in Figure 4A). Posterolateral angle of first tergite bluntly rounded, not produced posteriorly (arrow, Figure 4E). Source: Hopkin (2014). U.S. Distribution: Introduced. Widely distributed in the contiguous 48 states (Jass and Klausmeier 2000, 2001). First records from Maryland are vacant lots in Baltimore City (K. Szlávecz, unpublished observation) as well as agricultural fields in Anne Arundel and Prince Georges Counties and forests in Garrett County (J. Shultz, original observation). Habitat: Diverse environments, especially synanthropic habitats.

*Porcellio scaber* Latreille, 1804 (Figure 4A)

With features of the family, also the following: Body length up to 17 mm. Head with frontal margin produced medially to form a prominent triangular to subtriangular projection. Dorsal surface of head and pereon with numerous tubercles. Pleon not abruptly narrower than pereon. U.S. Distribution: Introduced. Widely distributed in the northeastern, north-central, and western states, also Florida (Jass and Klausmeier 2000, 2001). Recorded from Maryland by Richardson (1905), Hornung and Szlávecz (2003), Norden (2008) and Maryland Biodiversity Project. Habitat: Diverse mesic terrestrial habitats.

*Porcellio spinicornis* Say, 1818 (Figure 4C)

With features of the family, also the following: Body length up to 12 mm. Body outline similar to Figure 4A. Head with frontal margin produced medially into broad convex to rectangular projection that extends laterally almost to the medial edge of large anterolateral lobes; each anterolateral lobe about a third the width of head; in dorsal view, the median and anterolateral lobes join at V-shaped notch. Head dark, pereon generally lighter than head but with dark median stripe; in life, dark stripe bordered laterally by bright yellow markings. Source: Hopkin (2014). U.S. Distribution: Introduced. Northeastern and northern Great Lake states, Nebraska, Tennessee, Arkansas (Jass and Klausmeier 2000, 2001); also North Dakota, Iowa (BugGuide), and Ohio (iNaturalist). Not yet recorded from Maryland. Habitat: Moist substrates and associated vertical surfaces.

*Porcellionides pruinosus* (Brandt, 1833) (Figure 4B)

With features of the family, also the following: Body length up to 12 mm. Body often with frosted gray-white appearance due to layer of microscopic beads, structural color that varies with lighting and timing of molt cycle (Hadley and Hendricks 1985). When coating absent, color varying but often red-brown, sometimes with light wavy

markings. Head with frontal margin lacking median projection. Anterolateral lobes weakly developed. Antennae with articles IV and V with terminal white bands; first flagellar article about twice as long as second. Pleon abruptly narrower than pereon. Sources: Richardson (1905), Schultz (1982) and Hopkin (2014). U.S. Distribution: Introduced. Recorded throughout the contiguous 48 US states. Recorded in Maryland by Richardson (1905) (as *Metoponorthus pruinosus*); *Porcellionides* sp. recorded by Maryland Biodiversity Project. Habitat: Rotting logs, dung, etc.

### **Family Scyphacidae**

*Scyphacella arenicola* Smith, 1873 (Figure 1E)

Body length up to 5 mm. Compound eyes present. Antennae with four flagellar articles that decrease in length distad, base of flagellum only slightly narrower than preceding article. Head without frontal margin, no clear border between cephalic dorsum and frontal lamina. Pereon with numerous scales; cuticle tuberculate, each tubercle tipped with small spine. Source: Richardson (1905), Schultz (1972). U.S. Distribution: Native. Massachusetts, Rhode Island, Connecticut, New Jersey, Delaware, and Florida (Jass and Klausmeier 2000, 2001). Reported from Maryland (Dorchester Co.) by Richardson (1905) and Schultz (1972) at Choptank River (not “Cleoptauk River”). Habitat: Littoral; marine sand beaches above high water mark.

### **Family Trachelipodidae**

*Trachelipus rathkii* (Brandt, 1833) (Figure 4D)

Body length up to 15 mm. Body outline similar to Figure 4A in dorsal perspective. Compound eyes present. Antennae with two distinct flagellar articles. Pereon with low bumps or tubercles; surface usually mottled dark brown, red brown and tan, with distinct lateral tan patches at base of epimera creating a pair of broken lateral light lines. Five pairs of lungs. Uropods projecting beyond pleotelson and general body outline. Sources: Richardson (1905) and Hopkin (2014). U.S. Distribution: Introduced. Maine south to North Carolina and west to Wisconsin and Arkansas; also Washington State (Jass and Klausmeier 2001). Recorded in Maryland by Norden (2008), Hornung et al. (2015), BugGuide, iNaturalist, and Maryland Biodiversity Project. Habitat: Moist soil, leaf litter, etc.

### **Family Trichoniscidae (*Haplophthalmus*, *Hyloniscus*, *Miktoniscus*, *Trichoniscus*) (Figure 2)**

Body length < 8 mm. Each eye with one ocellus (*Haplophthalmus*, *Hyloniscus*, *Miktoniscus*) or three ocelli (*Trichoniscus*). Antenna ending in a narrow, tapering, pointed

flagellum (Figure 2A–C) comprising up to six articles when observed using high magnification. Lungs absent. Pleotelson with terminus truncate or with median notch or concavity (emarginate), not pointed (Figure 2A–C).

*Haplophthalmus danicus* Budde-Lund, 1880 (Figure 2B)

With features of the family, also the following: Body length up to 4 mm. Each eye with one ocellus. Antennae with three flagellar articles observable with high magnification. Cuticle lacking dark pigments; translucent, white or cream. Head densely tuberculate dorsally, tubercles conical. Tergites of pereon armed with low longitudinal crests with roughened and/or tuberculate dorsal surfaces. Pleon segments III–V with prominent epimera; outline of pleon not abruptly narrower than pereon. Sources: Richardson (1905), Schultz (1982) and Hopkin (2014). U.S. Distribution: Introduced. Known from the eastern and southwestern states (Jass and Klausmeier 2000, 2001). Recorded in Maryland by Lohmander (1927), Hornung and Szlávecz (2003), Norden (2008) and Hornung et al. (2015). Habitat: Moist litter and debris.

*Hyloniscus riparius* (C. L. Koch, 1838)

With features of the family, also the following: Body length up to 7 mm. Body outline similar to Figure 2A. Each eye with one ocellus. Antennae with six flagellar articles observable with high magnification. Dorsum smooth, without tubercles or other sculpture. Pleon abruptly narrower than pereon. Male with hook on third segment (merus) of pereopod 7. U.S. Source: Richardson (1905) and Schultz (1965). U.S. Distribution: Introduced. Recorded from Maryland by Hornung and Szlávecz (2003), Norden (2008) and Hornung et al. (2015). Habitat: Moist to wet soil or litter, flood plains.

*Miktoniscus spinosus* (Say, 1818) (= *M. halophilus* Blake, 1931) (Figure 2C–E)

With features of the family, also the following: Body length up to 5 mm. Each eye with one ocellus. Antennae with four flagellar articles observable with high magnification. Dorsum of pereon with transverse to roughly transverse rows of tubercles. Pleon abruptly narrower than pereon. In male: Pleopod I with exopodite an elongate plate narrowing distally to a terminal point, exopodite only slightly shorter than endopodite; endopodite cylindrical, terminating with a distinct medial bend (Figure 2D). Pleopod II with plate-like endopodite short, with small distomedial lobe; terminus of endopodite spatulate with square apical border (Figure 2E). Source: Schultz (1976). U.S. Distribution: Native. Coastal reed marshes from Massachusetts south to Georgia (Schultz 1976, 2001); unidentified *Miktoniscus* in DC (BugGuide). Recorded from “edge of an estuary” in Calvert County, Maryland by Schultz (1976) as *M. halophilus*. Habitat: Brackish and estuarine marshes along the Atlantic Coast,



also moist leaf litter of inland forests in river bottoms and near streams in piedmont of North Carolina (Schultz 1976).

*Miktoniscus medcofi* (Van Name, 1940) (Figure 2C, F, G)

With features of the family, also the following: Body length up to 5 mm. Each eye with one ocellus. Antennae with four flagellar articles visible with high magnification. Dorsum of pereon with transverse to roughly transverse rows of tubercles. Pleon abruptly narrower than pereon. In male: Pleopod I with plate-like exopodite tapering distally to broadly rounded apex; endopodite tapering and flattened distally but resulting lamella with central longitudinal “mid-rib”, terminus with file-like striations under high magnification (Figure 2F). Pleopod II with plate-like exopodite comparatively long with long distomedial lobe; terminus of endopodite not spatulate (Figure 2G). Sources: Van Name (1940) and Schultz (1976). U.S. Distribution: Native. New York south to Florida west to central states (Jass and Klausmeier 2000, 2001), including Texas (Hutchins and Drukker 2016); unidentified *Miktoniscus* in DC (BugGuide). Not yet recorded from Maryland, but its presence in adjacent areas suggests that it occurs in the state. Habitat: Moist soil, under logs, caves.

*Trichoniscus pusillus* Brandt, 1833 (Figure 2A)

With features of the family, also the following: Body length up to 5 mm. Each eye with three ocelli. Antenna with four or five flagellar articles visible using high magnification. Dorsal cuticle smooth, with reddish to purplish pigments. Pleon abruptly narrower than pereon. Posterior margin of pleotelson with median concavity (emarginate). Sources: Richardson (1905), Schultz (1982) and Hopkin (2014). U.S. Distribution: Introduced. Northeastern states from Maine south to North Carolina and west to Wisconsin and Arkansas; also Washington State (Jass and Klausmeier 2001). Recorded in Maryland by Hornung and Szlávecz (2003) and Norden (2008). Habitat: Moist soil and litter.

## Acknowledgements

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# The genus *Alpioniscus* Racovitza, 1908 in Sardinia: taxonomy and natural history (Isopoda, Oniscidea, Trichoniscidae)

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## Abstract

The genus *Alpioniscus* Racovitza, 1908 (Trichoniscidae) from Sardinia is revised. Three new cave-dwelling species are described: *A. onnisi* Taiti & Argano, **sp. n.**, *A. stochi* Taiti & Argano, **sp. n.**, and *A. sideralis* Taiti & Argano, **sp. n.** The genus *Utopioniscus* Schmalfuss, 2005 is considered to be a junior synonym of *Alpioniscus*, after morphological and molecular analyses. *Alpioniscus fragilis* (Budde-Lund, 1909) and *A. kuehni* from Grotta del Bue Marino are illustrated. With the new species, the genus *Alpioniscus* in Sardinia comprises six species: two troglobionts (*A. fragilis* and *A. onnisi*), one endogean and troglobiont (*A. thanit* Taiti & Argano, 2009), and three stygobionts (*A. kuehni*, *A. stochi*, and *A. sideralis*). All the species occur in karstic areas in the central-eastern and south-eastern part of the island. A key to all the Sardinian species of *Alpioniscus* is provided.

## Keywords

*Alpioniscus*, caves, Crustacea, new species, phylogeny, Sardinia

## Introduction

At present, 92 species of terrestrial isopods are known from Sardinia (Taiti and Argano 2009, 2011), many of which strictly endemic. Several new species already identified during field investigations in the last years are waiting to be described. The aim of this study is to attempt a reconstruction of the biogeographic history of the genus *Alpioniscus* Racovitza, 1908 in Sardinia on the basis of new data.

The geographical range of the genus *Alpioniscus* is discontinuous. To date, the genus comprises 31 subterranean species in two subgenera (see Tabacaru 1966): the nominal subgenus with 14 species living in the caves of the Western Alps and the southern Balkans reaching Greece, and the subgenus *Illyrionethes* Verhoeff, 1927, with 17 species populating caves of Catalonia, Sardinia and the Dinaric Alps (Bedek and Taiti 2011, Bedek et al. 2017). In Sardinia two species are known in the subgenus *Illyrionethes* (Taiti and Argano 2009, 2011): *A. fragilis* (Budde-Lund, 1909), widely distributed in the karst caves of central-eastern and south-eastern areas of the island, and *A. thanit* Taiti & Argano, 2009, from endogean environments and some caves in the central-eastern area.

A large number of specimens have been recently collected from many new localities (mainly caves) on Sardinia, revealing a more complex taxonomic scenario. In the same part of Sardinia, Schmalfuss (2005) described a very interesting new species and genus of trichoniscids, the aquatic *Utopioniscus kuehni* Schmalfuss, 2005, occurring in two submarine caves. This species was considered among the most archaic forms of the family Trichoniscidae. New populations of this species have recently been examined from subterranean freshwaters in coastal and inland caves, and other stygobiotic species with intermediate characters between *Alpioniscus* and *Utopioniscus* have also been identified. In this paper three new species of *Alpioniscus* are described and the synonymy between *Alpioniscus* and *Utopioniscus* proposed, on the basis of both morphological and molecular analyses. Analyses performed on both morphological and molecular techniques allow an exhaustive integrative taxonomic approach, which has been effectively used in several case studies on small-sized faunal taxa (see e.g., Casu et al. 2011, 2014, Scarpa et al. 2016, 2017a, b).

## Materials and methods

### The study area

Sardinia is the second largest island of the Mediterranean (24,090 km<sup>2</sup>) with a complex geology. Karsts cover 9% of the total surface and are divided in 219 distinct areas of different ages, ranging from sea level up to 1,500 m altitude. These areas are separated from each other by non-karstic rocks, so they evolve and behave independently to one another (De Waele 2003, 2009). *Alpioniscus* species in Sardinia are limited to the central-eastern and south-eastern main karst groups (Gulf of Orosei, Supramonte, Taccchi, Quirra, and Sarrabus).

## Collectors of materials

The specimens examined were collected by some of the authors, several biospeleologists, and the astronauts from Europe, USA, Russia, Canada, Japan, and China, participating in the ESA CAVES training courses.

## Morphological analysis

All material collected for morphological analysis was stored in 75% ethanol. The species were illustrated with the aid of a *camera lucida* mounted on Wild M5 and M20 microscopes. Figures were digitally drawn following the methods described in Montesanto (2015, 2016). The World Geodetic System 1984 (WGS84) was the datum used for all geographic coordinates.

## Molecular analysis

Several specimens of each Sardinian species from the type localities have been tested for molecular analysis (Table 1), including one specimen of *Utopioniscus kuehni* studied by Schmalfuss (2005). Moreover, in order to test the assignment of the Sardinian species of *Alpioniscus* to the subgenus *Illyrionethes* we included also specimens of *Alpioniscus strasseri* (Verhoeff, 1927), type species of the subgenus *Illyrionethes* from Friuli Venezia Giulia (Italy), and of *A. feneriensis* (Parona, 1880), type species of the subgenus *Alpioniscus* Racovitza, 1908, from Piedmont (Italy). A specimen of *Androniscus dentiger* Verhoeff, 1908 (fam. Trichoniscidae) from Tuscany (Italy) was used as outgroup.

Molecular analyses have been performed using the COI gene (Cytochrome c Oxidase subunit I) with either the universal COI primers by Folmer et al. (1994) or new specific primers designed by the authors (H: grgatgaycaratytayaatgt, L: ctaggrrtcaaaaaarcawgtgtt). DNA extraction and PCR have been performed following Sanna et al. (2014). Annealing temperature was set at 44° C for both of primers pairs; positive and negative controls were also used for PCR.

PCR products were purified by ExoSAP-IT (USB Corporation) and sequenced using an external sequencing core service (Macrogen Inc., Europe). The sequencing runs were performed both for forward and reverse strands. Sequences were aligned using Clustal W (Thompson et al. 1994), implemented in BioEdit 7.0.5.2 software (Hall 1999). The best probabilistic model of sequence evolution was determined after evaluation by jModeltest 2.1.1 (Posada 2008), with a maximum likelihood optimized search, using the Akaike Information Criterion (AIC) and the Bayesian Information Criterion (BIC). The model TPM2uf + G has been chosen as the best fitting both AIC and BIC. Phylogenetic relationships were investigated using the Bayesian Inference (BI) and the Maximum Likelihood (ML) methods. BI was carried out using the software MrBayes 3.2.2 (Ronquist et al. 2012), setting as model parameters:  $N_{ST} = 3$ , rates = gamma, ngam-



**Table 1.** Specimens used for molecular analysis.

Species	Collection data	GenBank number
<i>Alpioniscus feneriensis</i> (Parona, 1880)	Piedmont: Buco della Bondaccia, c.n. 2517 Pi/VC, Borgosesia, Monte Fenera, 30.IX.2000, leg. F. Stoch, T. Pascutto and S. Bugalla	MH092992
<i>Alpioniscus strasseri</i> (Verhoeff, 1927)	Friuli Venezia Giulia: Grotta del Bosco dei Pini, c.n.16 VG/TS, Basovizza, 2.VI. 2011, leg. F. Gasparo and F. Stoch	MH092986
<i>Alpioniscus fragilis</i> (Budde-Lund, 1909)	Sardinia: Grotta del Bue Marino, 25.IV.2012, leg. S. Taiti, P. Dore and S. Dessena	MH092990
<i>Alpioniscus kuehni</i> (Schmalfuss, 2005)	Sardinia: Grotta del Bel Torrente (Paratype), 22.VII, 2004, leg. A. Oertel	MH092988
<i>Alpioniscus thanit</i> Taiti & Argano, 2009	Sardinia: Cala Fuili, 25.IV.2008, leg. R. Argano and S. Taiti	MH092993
<i>Alpioniscus onnisi</i> sp. n.	Sardinia: Grotta Giuanniccu Mene, 20.IV.2012, leg. C. Onnis, S. Taiti and R. Argano	MH092987
<i>Alpioniscus stochi</i> sp. n.	Sardinia: Grotta Su Palu, 1.V.2009, leg. F. Stoch and G. Tomasin	MH092991
<i>Alpioniscus sideralis</i> sp. n.	Sardinia: Grotta Su Bentu, 11-14.IX.2012, leg. P. Marcia and ESA astronauts	MH092989
<i>Androniscus dentiger</i> Verhoeff, 1908 (outgroup)	Tuscany: Tana di Magnano, 162 To/LU, Villa Collemantina, 11.VI.2012, leg. S. Taiti	MH092985

macat = 4. Two independent runs each consisting of four Metropolis-coupled MCMC chains (one cold and three heated chains) were run simultaneously for 5,000,000 generations, sampling trees every 1,000 generations. The first 25% of sampled trees were discarded. Run was executed by means of the Cipres Phylogenetic Portal (Miller et al. 2010). Convergence of chains was checked following the procedures described by Ronquist et al. (2012) and Gelman and Rubin (1992). ML analysis were conducted using the software RAXMLGUI version 1.3 (Silvestro and Michalak 2011) setting the default setting for the “ML + thorough bootstrap” analysis option. Analysis was carried out with 100 runs and 1,000 bootstrapping replicates. Consensus trees were visualized by means of the FigTree 1.4.0 software (<http://tree.bio.ed.ac.uk/software/figtree/>).

## Abbreviations

<b>c.n.</b>	Cadastral number;
<b>ESA CAVES</b>	European Space Agency, Cooperative Adventure for Valuing and Exercising human behaviour and performance Skills;
<b>MZUF</b>	Museo di Storia Naturale dell'Università di Firenze, Sezione di Zoologia “La Specola”, Florence, Italy;
<b>n.c.n.</b>	No cadastral number;
<b>SMNS</b>	Staatliches Museum für Naturkunde, Stuttgart, Germany.

## Taxonomic results

### Family Trichoniscidae Sars, 1899

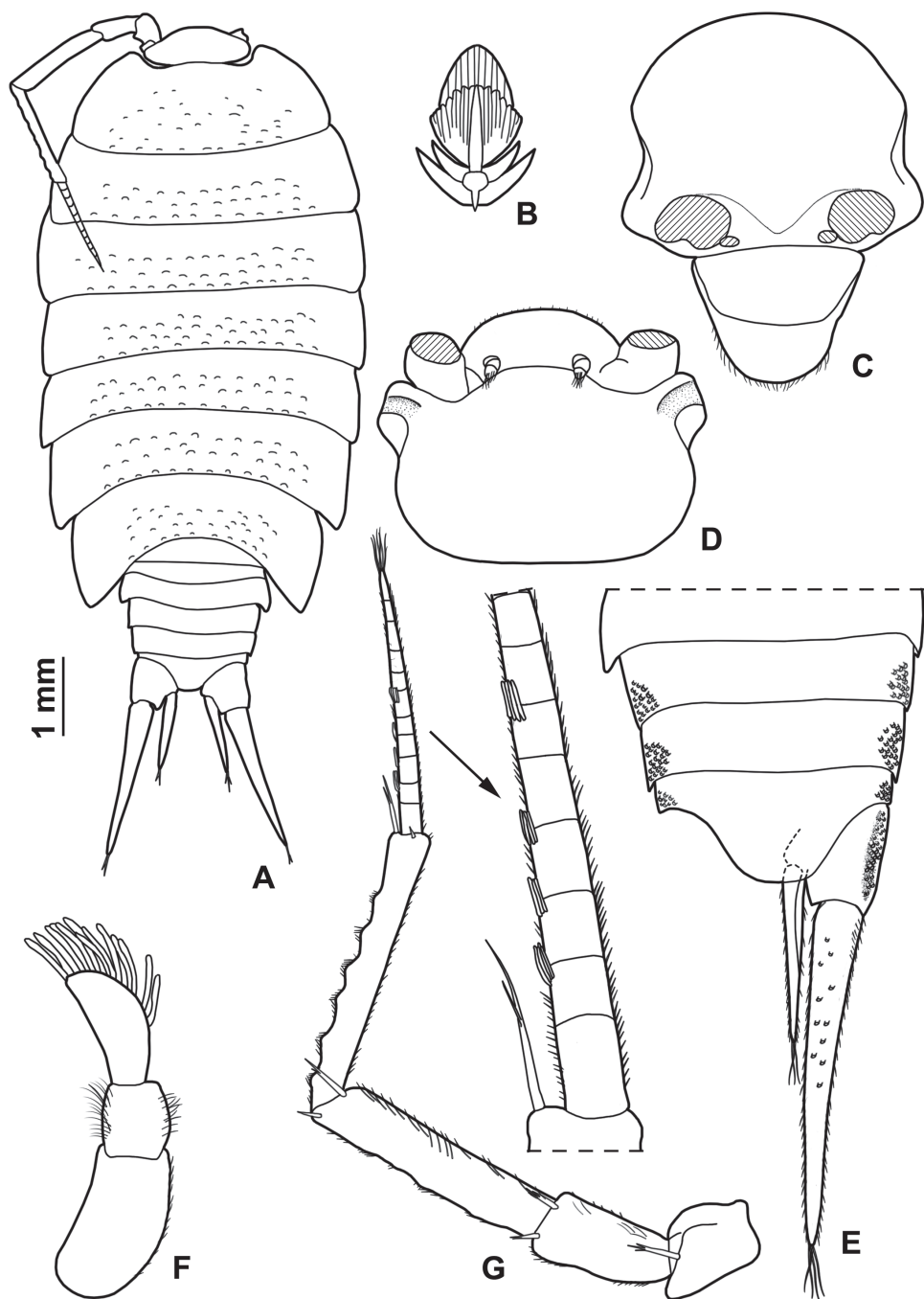
### Genus *Alphoniscus* Racovitza, 1908

#### *Alphoniscus fragilis* (Budde-Lund, 1909)

Figs 1–4, 18, 19

*Alphoniscus fragilis*; Taiti and Argano 2011: 166 (for previous records and references; nec Grotta del Caprone Tyson, p. 167).

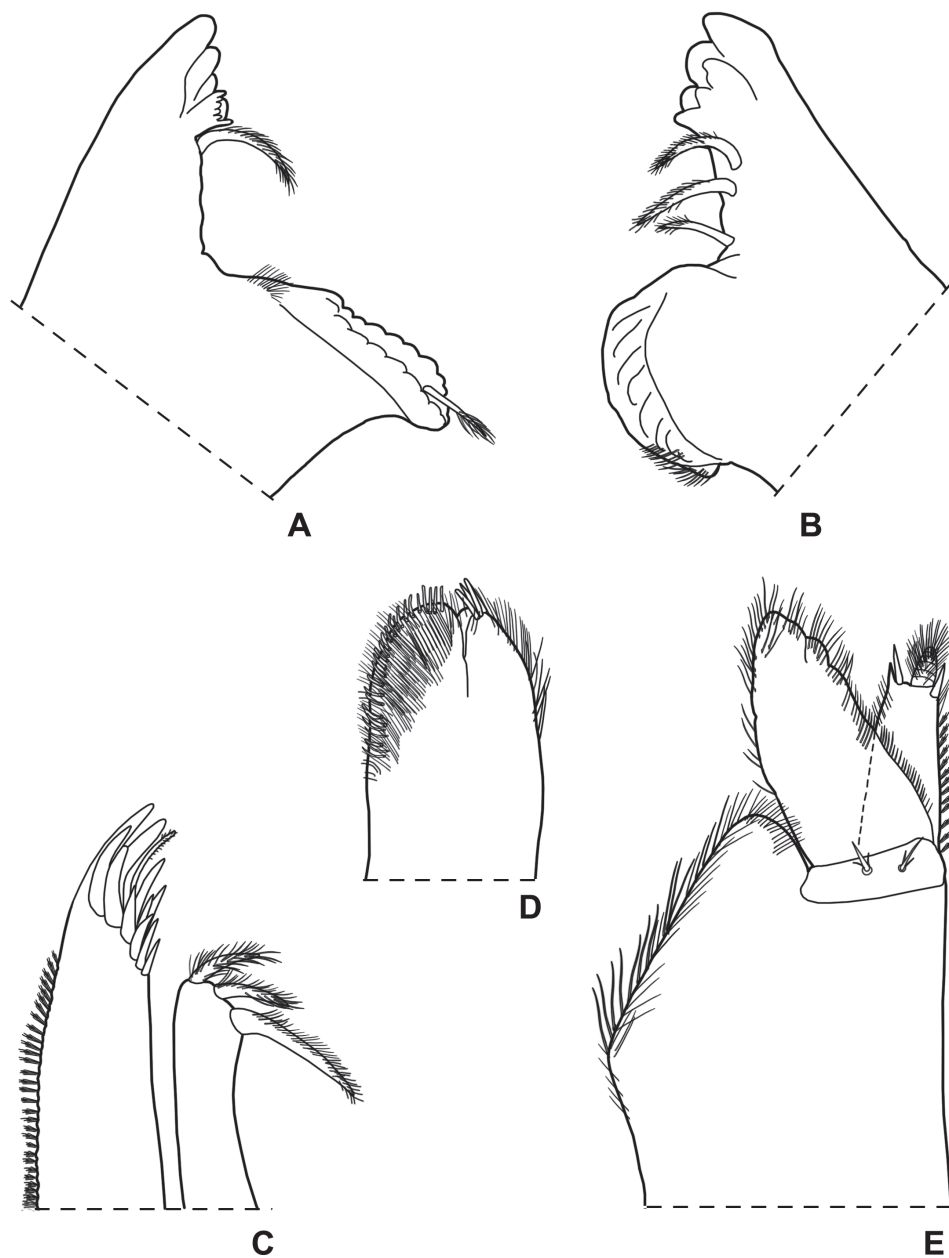
**Material examined. Prov. Nuoro:** 5 ♂♂, 16 ♀♀, 1 juv. (MZUF 9770), Grotta del Bue Marino, c.n. 12 Sa/NU, 40°14'55.72"N, 9°37'24.80"E, Cala Gonone, Dorgali, on cave walls, 25.IV.2012, leg. S. Taiti, P. Dore and S. Dessena; 1 ♂, 4 ♀♀ (MZUF 9826), same locality, date and collectors, under submerged stones; 1 ♀ (MZUF 9774), Grotta Pisanu or Gurenoro, c.n. 215 Sa/NU, 40°17'56.40"N, 9°33'05.30"E, 142 m, Gurenoro, Dorgali, 17.III.2014, leg. P. Magrini; 2 ♂♂, 1 ♀, 15 juvs (MZUF 9777), Grotta Elighes Artas, c.n. 907 Sa/NU, 40°14'20.3"N, 9°28'49.8"E, 360 m, Oliena, 25.IV.2013, leg. P. Marcia and S. Taiti; 1 ♂, 2 juvs (MZUF 9778), same locality, 15.I.2012, leg. P. Marcia; 1 ♂, 2 ♀♀, 3 juvs (MZUF 9779), same locality, 25.XII.2012, leg. G. Mulas; 2 ♂♂, 4 ♀♀, 2 juvs (MZUF 9780), Grotta Su Bentu, c.n. 105 Sa/NU, 40°15'18.23"N, 9°29'6.52"E, Lanaittu, Oliena, 3.XII.2011, leg. P. Marcia; 5 ♂♂, 3 ♀♀ (MZUF 9781), same locality, 6.I.2013, leg. P. Marcia; 2 ♀♀, 1 juv. (MZUF 9782), same locality 11–14.IX.2012, leg. P. Marcia and Astronauts; 1 ♂, 2 ♀♀ (MZUF 9783), same locality, 4.XII.2011, leg. P. Marcia; 4 ♂♂, 6 ♀♀, 9 juvs (MZUF 9784), Grotta S'Istampu de Sas Ballas, c.n. 106 Sa/NU, 40°15'20.82"N, 9°29'13.72"E, Oliena, 30.XII.2012, leg. P. Marcia; 4 ♀♀ (MZUF 9785), Grotta sa Seneppida, n.c.n., sa Seneppida, Orgosolo, 13.I.2013, leg. E. Dallochio; 4 ♂♂, 8 ♀♀, 6 juvs (MZUF 9823), Voragine di Tiscali, c.n. 88 Sa/NU, 40°14'12.23"N, 9°29'6.52"E, Oliena, 23.X.2011, leg. P. Marcia. **Prov. Ogliastra:** 2 ♀♀ (MZUF 9772), Grotta Lovettecannas, c.n. 2642 Sa/OG, 40°08'33.72"N, 9°34'35.35"E, Baunei, 1.IV.2013, leg. P. Marcia; 1 ♂, 1 ♀ (MZUF 9773), Grotta di Baccherutta, c.n. 1008 Sa/OG, 40°04'5.22"N, 9°37'34.54"E, Baunei, 14.III.2009, leg. C. Onnis and N. Ibbia; 1 ♂ (MZUF 9786), Grotta Piggios de Jana, n.c.n., Tauledda, Codula del Flumineddu, Urzulei, 28.X.2012, leg. C. Corongiu; 1 ♂ (MZUF 9787), Grotta Sa rutta e Mannaresuru, c.n. 2267 Sa/OG, 40°07'44.64"N, 9°26'49.99"E, Urzulei, 15.VII.2012, leg. P. Marcia; 4 ♂♂, 14 ♀♀ (MZUF 9788), same locality, 7.X.2012, leg. P. Marcia; many ♂♂ and ♀♀ (MZUF 9789), Grotta Sa Rutta 'e s'Edera, c.n. 588 Sa/OG, 40°05'51.3"N, 9°27'22.5"E, 950 m, Fennau, Urzulei, 23.IV.2012, leg. R. Argano and S. Taiti; 1 ♂, 6 ♀♀, 5 juvs (MZUF 9790), Grotta Su Palu, c.n. 1988 Sa/OG, 40°10'38.23"N, 9°33'50.53"E, 185 m, Codula Ilune, Urzulei, 8.XII.2012, leg. P. Marcia; 1 ♀ (MZUF 9775), Grotta Su Molente, c.n. 966 Sa/OG, 40°13'00.90"N, 9°36'10.95"E, Codula Ilune, Dorgali, 9.XI.2013, leg. M. Marrosu; 1 ♀ (MZUF 9807),



**Figure 1.** *Alpiniscus fragilis* (Budde-Lund, 1909) from Grotta del Bue Marino, ♂: **A** adult specimen, dorsal **B** dorsal scale-seta **C** cephalon, frontal **D** cephalon, dorsal **E** pleonites 3-5, telson and right uropod **F** antennula **G** antenna.

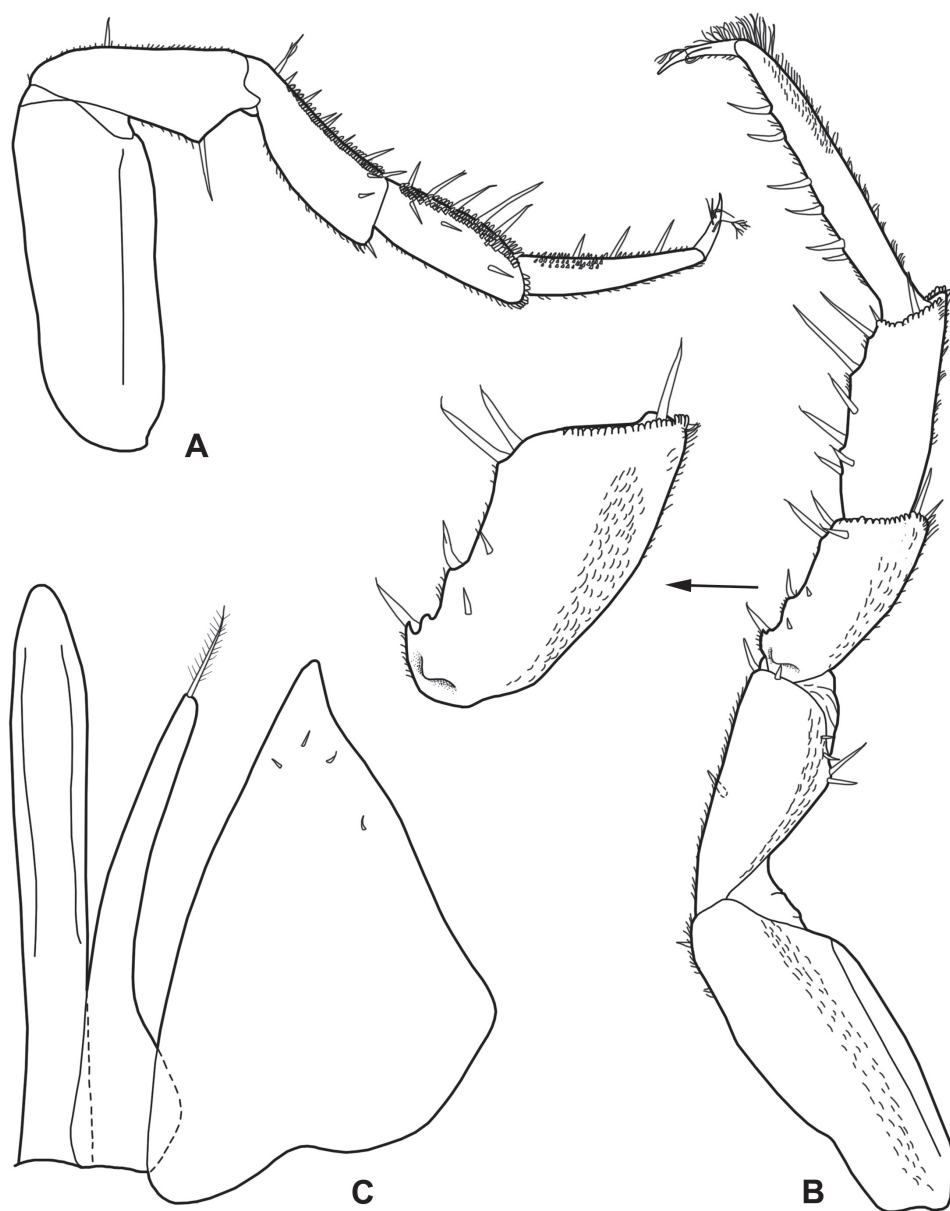
same locality, 10.XI.2013, leg. E. Seddone; 4 ♂♂, 7 ♀♀ (MZUF 9771), Grotta Sos Cicinderos, n.c.n., Baunei, 7.VII.2013, leg. C. Onnis and M. Papacoda; 1 ♀ (MZUF 9791), Voragine Tesulali, c.n. 2681 Sa/OG, 40°07'42.14"N, 9°34'50.82"E, Baunei, 26.II.2012, leg. C. Onnis; 7 ♂♂, 19 ♀♀ (MZUF 9792), same locality, 10.II.2013, leg. C. Onnis; 1 ♂, 1 juv. (MZUF 9793), Grotta Su Tufu de Mangalistru, c.n. 422 Sa/OG, 40°06'17.28"N, 9°39'09.03"E, Baunei, 25.III.2012, leg. C. Corongiu; 1 ♀ (MZUF 9794), Grutta 'e S'Arena, c.n. 673 Sa/OG, 39°51'04.29"N, 9°27'44.43"E, Taquisara, 22.V.2013, leg. C. Onnis and P. Marcia; 1 ♂, 1 ♀ (MZUF 9798), same locality, 15.IX.2013, leg. C. Onnis and J. Costantino; 4 ♂♂, 5 ♀♀ (MZUF 9795), Grotta Istirzili, c.n. 50 Sa/NU, 40°04'49.50"N, 9°37'13.40"E, II..2013, leg. C. Onnis; 4 ♂♂, 7 ♀♀, 2 juvs (MZUF 9796), same locality, 12.V.2013, leg. C. Onnis; 3 ♂♂, 8 ♀♀ (MZUF 9797), Grotta S'erriu Mortu, n.c.n., Punta Giradili, Baunei, 7.VII.2013, leg. C. Onnis and M. Papacoda; 1 ♀ (MZUF 9799), Sa Grutta de su Coloru, c.n. 670 Sa/OG, 39°50'54.20"N, 9°27'34.68"E, Gairo Taquisara, 15.IX.2013, leg. C. Onnis and J. Costantino; 1 ♀ (MZUF 9801), same locality, 21.VIII.2013, leg. C. Onnis; 2 ♂♂, 21 ♀♀ (MZUF 9800), Lequarci or Lecorci Falls, Santa Barbara, Ulassai, 39°47'29.2"N, 9°27'11.6"E (WGS84), 560 m, under big stones outside cave, 28.III.2016, leg. R. Argano and S. Taiti. **Prov. Cagliari:** 1 ♂, 1 ♀, 2 juvs (MZUF 9824), Grotta Gospuru, c.n. 148 Sa/CA, 39°31'48.31"N, 9°26'13.21"E, 100 m, Baccu Gospuru, Armungia, IV.2012, leg. C. Onnis; 1 ♀, 2 juvs (MZUF 9776), Grotta Su Pittiolu de Gospuru, c.n. 1865 Sa/CA, 39°31'59.65"N, 9°26'04.41"E, 125 m, Baccu Gospuru, Armungia, 5.X.2008, leg. C. Onnis and N. Ibba; 2 ♂♂, 2 ♀♀ (MZUF 9825), same locality, 6.III.2011, leg. P. Marcia; 8 ♂♂, 15 ♀♀, 7 juv. (MZUF 9802), Grotta Su Fummu, n.c.n., San Nicolò Gerrei, 1.V.2012, leg. C. Onnis; 1 ♂, 12 ♀♀ (MZUF 9803), same locality, 17.III.2013, leg. C. Onnis; 2 ♂♂, 5 ♀♀ (MZUF 9804), Risorgenza Sa Gistera, n.c.n., 39°30'08.4"N, 9°19'05.2"E, San Nicolò Gerrei, IX.2012, leg. C. Onnis; 2 ♀♀ (MZUF 9805), same locality, 20.IV.2013, leg. C. Onnis, R. Argano and S. Taiti; 3 ♂♂, 9 ♀♀ (MZUF 9806), Sa Rutt'e Scusi, c.n. 602 Sa/CA, 39°29'42.20"N, 9°24'18.00"E, Villasalto, V.2013, leg. C. Onnis.

**Redescription.** Maximum length: ♂, 9 mm; ♀, 14 mm. Colourless body, pleon narrower than pereon (Fig. 1A). Dorsal surface distinctly granulated with ovoid scale-setae as in Fig. 1B. Many gland pores on lateral margins of pleonites 4 and 5, telson, lateral surface of uropodal protopods, and some scattered pores on dorsal surface of uropodal exopods (Fig. 1E). Cephalon (Fig. 1C, D) with suprantennal line V-shaped with rounded middle part; antennal lobes quadrangular, obliquely directed outwards with concave dorsal surface. Eyes absent. Posterior margin of pereonite 1 and 2 straight, and of pereonites 3–7 progressively more concave (Fig. 1A). Pleonites 3–5 with very short posterior points (Fig. 1A, E). Telson (Fig. 1E) approx. twice as wide as long; distal part with concave sides and very broadly rounded apex. Antennula (Fig. 1F) with second article distinctly shorter than first and third; third article distally enlarged and bearing 12–13 apical aesthetascs. Antenna (Fig. 1G) with fifth article as long as flagellum; flagellum of 10–13 articles with four groups of aesthetascs.



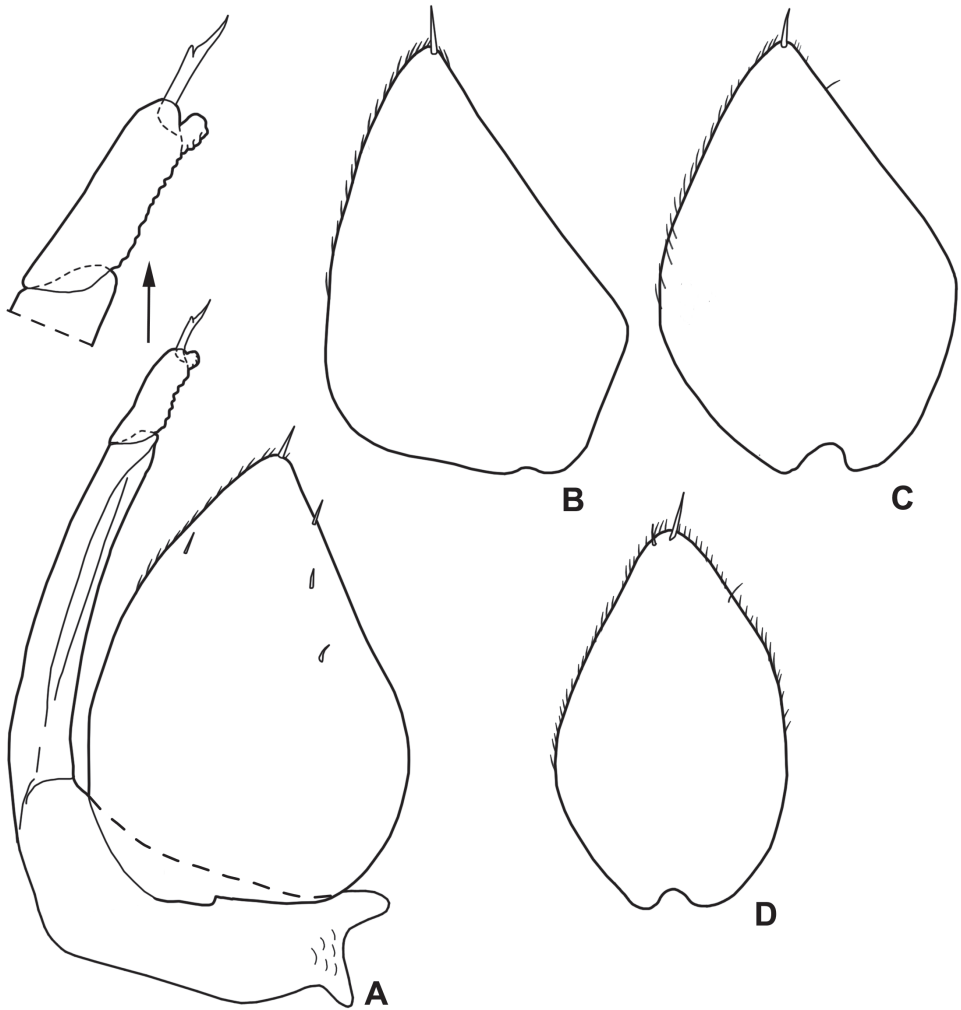
**Figure 2.** *Alpioniscus fragilis* (Budde-Lund, 1909) from Grotta del Bue Marino, ♂: **A** right mandible **B** left mandible **C** maxillula **D** maxilla **E** maxilliped.

Mandibles with one free penicil and one short molar penicil in the right (Fig. 2A) and three free penicils in the left (Fig. 2B). Maxillula (Fig. 2C) outer branch with 4 + 7 teeth, apically entire, and one slender setose stalk; inner branch with three long penicils. Maxilla (Fig. 2D) with setose and bilobate apex, inner lobe smaller. Maxil-



**Figure 3.** *Alpioniscus fragilis* (Budde-Lund, 1909) from Grotta del Bue Marino, ♂: **A** pereopod 1 **B** pereopod 7 **C** genital papilla and pleopod 1.

lipid (Fig. 2E) basis with outer margin distally oblique and setose; palp stout, apically with tuft of setae and three tufts of setae on medial margin, basal article with two setae; endite narrow, with two apical stout setae and a large penicil. Pereopods with setose dactylar seta distally bifid (Fig. 3A), pereopod 7 with water conducting system on basis, ischium and merus. Uropod (Fig. 1E) with endopod distinctly shorter than exopod, endopod inserted proximally to exopod.



**Figure 4.** *Alphoniscus fragilis* (Budde-Lund, 1909) from Grotta del Bue Marino, ♂: **A** pleopod 2 **B** pleopod 3 exopod **C** pleopod 4 exopod **D** pleopod 5 exopod.

*Male.* Pereopod 1–4 (Fig. 3A) with carpus and merus bearing numerous short scales on sternal margin. Pereopod 7 (Fig. 3B) ischium with straight sternal margin covered with short setae; merus with three lobes proximally. Genital papilla (Fig. 3C) with a rounded tip. Pleopod 1 (Fig. 3C) exopod triangular with acute apex; endopod enlarged at base, distal part narrow with almost parallel sides and bearing an apical seta. Pleopod 2 (Fig. 4A) exopod triangular with convex outer margin and a small apical seta; endopod of two articles, longer than exopod, first article approx. three times longer than second, second article bearing distally rounded lobe and strong seta sub-apically cleft. Pleopod 3–5 exopods subtriangular with apical seta (Fig. 4B–D).



**Distribution.** The species seems to be widely distributed in the central-eastern and south-eastern karstic areas of Sardinia. The species is mainly terrestrial even if it occasionally occurs also in water.

**Remarks.** This species was described by Budde-Lund (1909) as *Titanethes (Alphoniscus) fragilis* from a Sardinian cave on material collected by Forsyth Major. No name for a specific cave was mentioned. However, considering that Forsyth Major discovered some troglobiotic species of different invertebrates during a digging campaign in caves of the Orosei Gulf, Casale et al. (2009) suggest that the cave explored by Forsyth Major most likely was the Grotta dell'Arciprete (= Grotta di Toddeitto) or one cave nearby in the same karstic area., e.g., Grotta del Bue Marino. The species is here redescribed on specimens from this last cave.

### *Alphoniscus thanit* Taiti & Argano, 2009

*Alphoniscus thanit* Taiti & Argano, 2009: 39, figs 1–3; Taiti and Argano 2011: 169.

**Type material re-examined. Prov. Nuoro:** 13 ♂♂, 29 ♀♀ paratypes (MZUF 9281), Dorgali, Cala Fuili, 40°15'27.8"N, 9°36'56.2"E, 25.IV.2008, leg. R. Argano and S. Taiti.

**Material examined. Prov. Nuoro:** 3 ♂♂, 1 ♀ (MZUF 9812), Pozzo N.1 di Tres Puntas, c.n. 1150 Sa/NU, 40°22'44.76"N, 9°38'25.85"E, Monte Tuttavista, Galtelli, 2.VI.2013, leg. P. Marcia; 1 ♀ (MZUF 9813), same locality, 10.II.2013, leg. P. Marcia; 1 ♂, 4 ♀♀ (MZUF 9814), Grotta di Sos Jocos (or Grotta Taramelli), c.n. 344 Sa/NU, 40°19'04.30"N, 9°36'58.70"E, Su Anzu, Dorgali, 18.IX.2013, leg. P. Marcia and P. Nespoli; 1 ♀ (MZUF 9815), stessa località, 5.VI.2012, leg. P. Marcia; many ♂♂ and ♀♀ (MZUF 9816), same locality, 19.IV.2014, leg. P. Marcia, R. Argano and S. Taiti. **Prov. Ogliastra:** many ♂♂ and ♀♀ (MZUF 9808), Pedra Longa, Baunei, 40°01'35.4"N, 9°42'20.9"E, 20.V.2011, leg. R. Argano and S. Taiti; 1 ♂, 3 ♀♀ (MZUF 9809), same locality, 21.IV.2012, leg. R. Argano and S. Taiti; 1 ♂, 8 ♀♀ (MZUF 9810), Funtana Bausu, near Grotta S'erriu Mortu, Punta Giradili, Baunei, VI.2013, leg. C. Onnis; 1 ♂, 2 ♀♀ (MZUF 9811), Bacu Stirzili, Baunei, 23.II.2014, leg. C. Onnis.

**Distribution.** The species is endemic to the area of Orosei Gulf, central-eastern Sardinia, where it occurs in both endogean and cave habitats.

### *Alphoniscus onnisi* Taiti & Argano, sp. n.

<http://zoobank.org/A37ECC77-62C7-4650-B6D7-1D170683E4F8>

Figs 5–8, 18, 19

*Alphoniscus fragilis*; Taiti and Argano 2011: 167 (partim: Grotta del Caprone Tyson).

**Material examined. Prov. Cagliari:** *Holotype:* ♂ (MZUF 9817), Grotta Giuanniccu Mene, c.n. 735 Sa/CA, 39°31'32.3"N, 9°36'08.9"E, Monte Castello di Quirra, Villaputzu 20.IV.2012, leg. C. Onnis, S. Taiti, R. Argano. *Paratypes:* many ♂♂

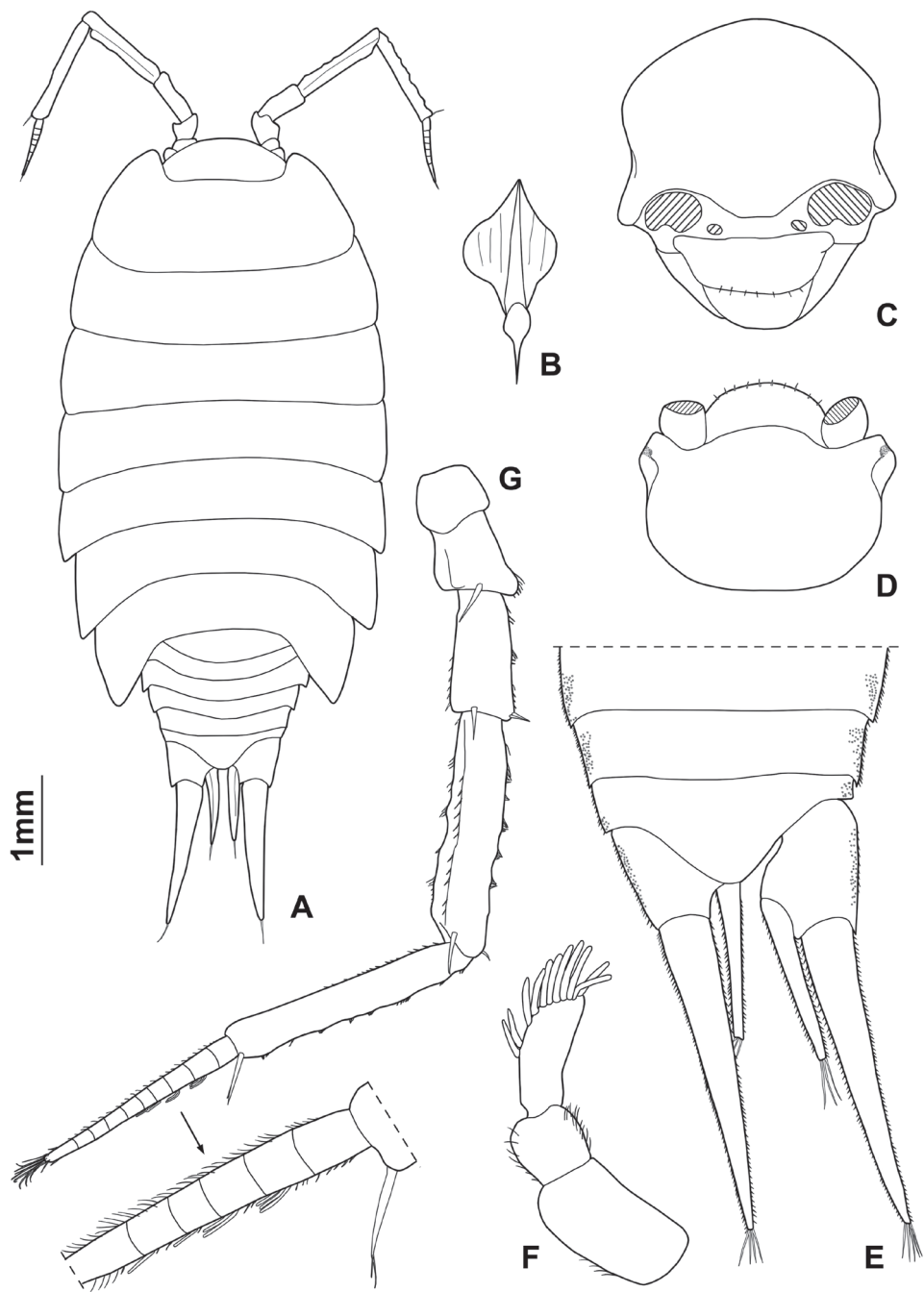
and ♀♀ (MZUF 9817), same data as holotype; 1 ♀ (MZUF 9818), same locality, 8.I.2011, leg. C. Onnis; 4 ♂♂, 15 ♀♀, 2 juvs (MZUF 9819), same locality, 20.IV.2013, leg. C. Onnis, R. Argano and S. Taiti; 2 ♂♂, 4 juvs (MZUF 9820), same locality, 16.III.2012, leg. C. Onnis; 2 ♂♂, 7 ♀♀ (MZUF 9821), Grotta del Caprone Tyson, n.c.n., Monte del Castello di Quirra, Villaputzu, I.1999, leg. G. Marini; 1 ♂ juv. (MZUF 9822), “Prisoni” Ipogean Tomb, 39°31'24"N, 9°36'26"E, 15 m, Monte del Castello di Quirra, Villaputzu, 20.IV.2012, leg. C. Onnis, R. Argano and S. Taiti.

**Description.** Maximum length: ♂, 7.5 mm; ♀, 9 mm. Colourless body, outline as in Fig. 5A. Dorsal surface smooth with lanceolate scale-setae as in Fig. 5B. Many gland pores on lateral margins of pleonites 4 and 5, telson and lateral surface of uropodal protopod (Fig. 5E). Cephalon (Fig. 5C, D) with suprantennal line medially blunt; antennal lobes quadrangular obliquely directed outwards with concave dorsal surface. Eyes absent. Posterior margin of pereonite 1–3 straight, and of pereonites 4–7 progressively more concave (Fig. 5A). Pleonites 3–5 with reduced posterior points (Fig. 5A, E). Telson (Fig. 5E) more than twice as wide as long; distal part triangular with concave sides and rounded apex. Antennula (Fig. 5F) with second article distinctly shorter than first and third; third article distally enlarged and bearing approx. ten apical aesthetascs. Antenna (Fig. 5G) with fifth article slightly longer than flagellum; flagellum of ten articles with four groups of aesthetascs. Mouth parts (Fig. 6A–E) as in *A. fragilis*. Pereopods with setose dactylar seta distally bifid, pereopod 7 with well developed water conducting system consisting of a groove with scales on basis, and lines of scales on ischium and merus. Uropod (Fig. 5E) with endopod distinctly shorter than exopod, endopod inserted proximally to exopod.

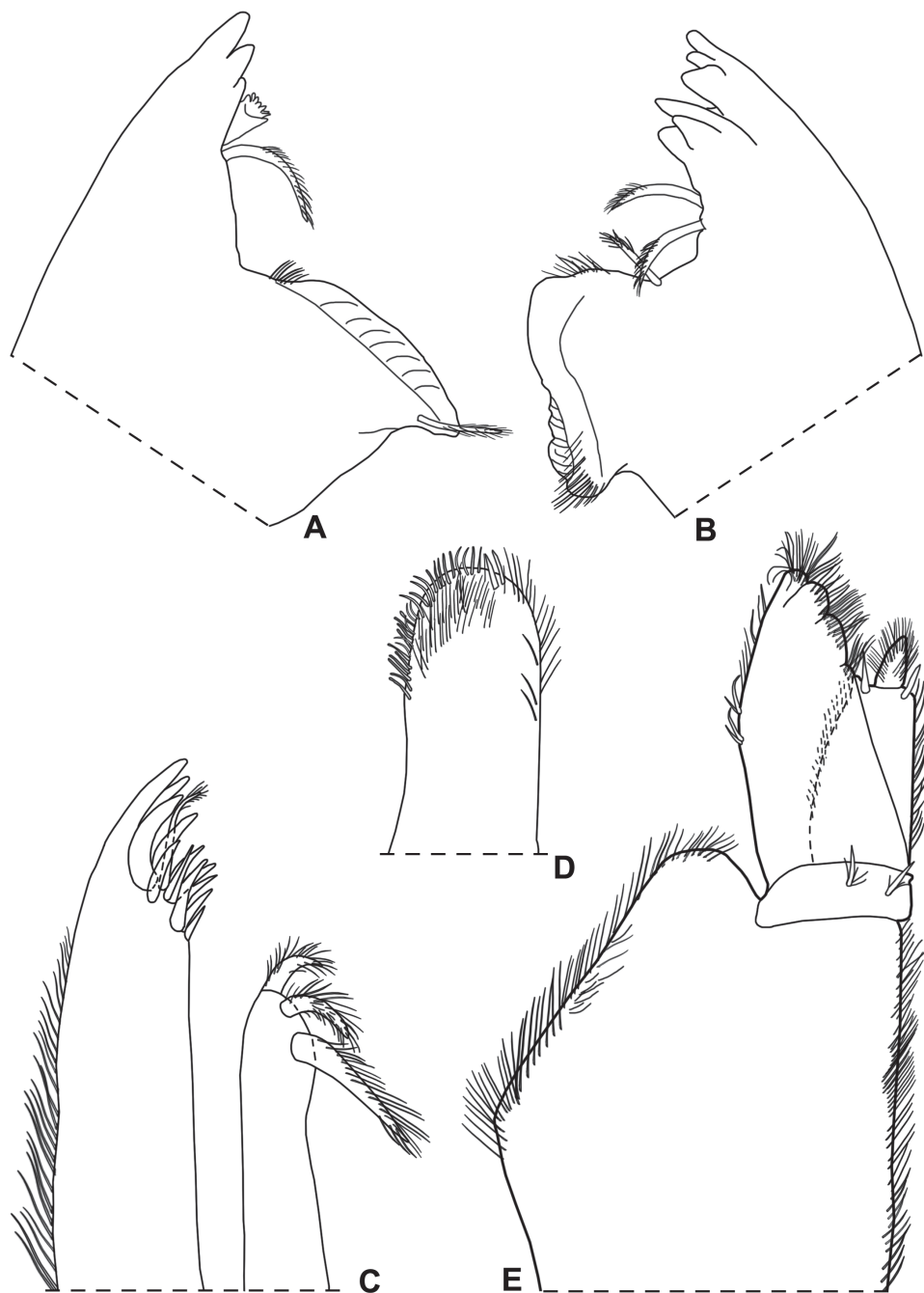
**Male.** Pereopod 1–4 (Fig. 7A) with carpus and merus bearing short scales on sternal margin. Pereopod 7 (Fig. 7B) ischium with straight sternal margin covered with short setae; merus with three lobes proximally. Genital papilla (Fig. 7C) with a rounded tip. Pleopod 1 (Fig. 7C) exopod triangular with acute apex; endopod enlarged at base, distal part narrow with almost parallel sides and bearing an apical seta. Pleopod 2 (Fig. 7D) exopod triangular with convex outer margin and a small apical seta; endopod with first article nearly twice as long as second, second article with a strong seta subapically cleft. Pleopod 3–5 exopods with a short apical seta (Fig. 8A–C).

**Etymology.** The new species is named after our Sardinian friend Carlo Onnis for his enthusiastic and efficient activity in collecting subterranean fauna, including part of the material treated here.

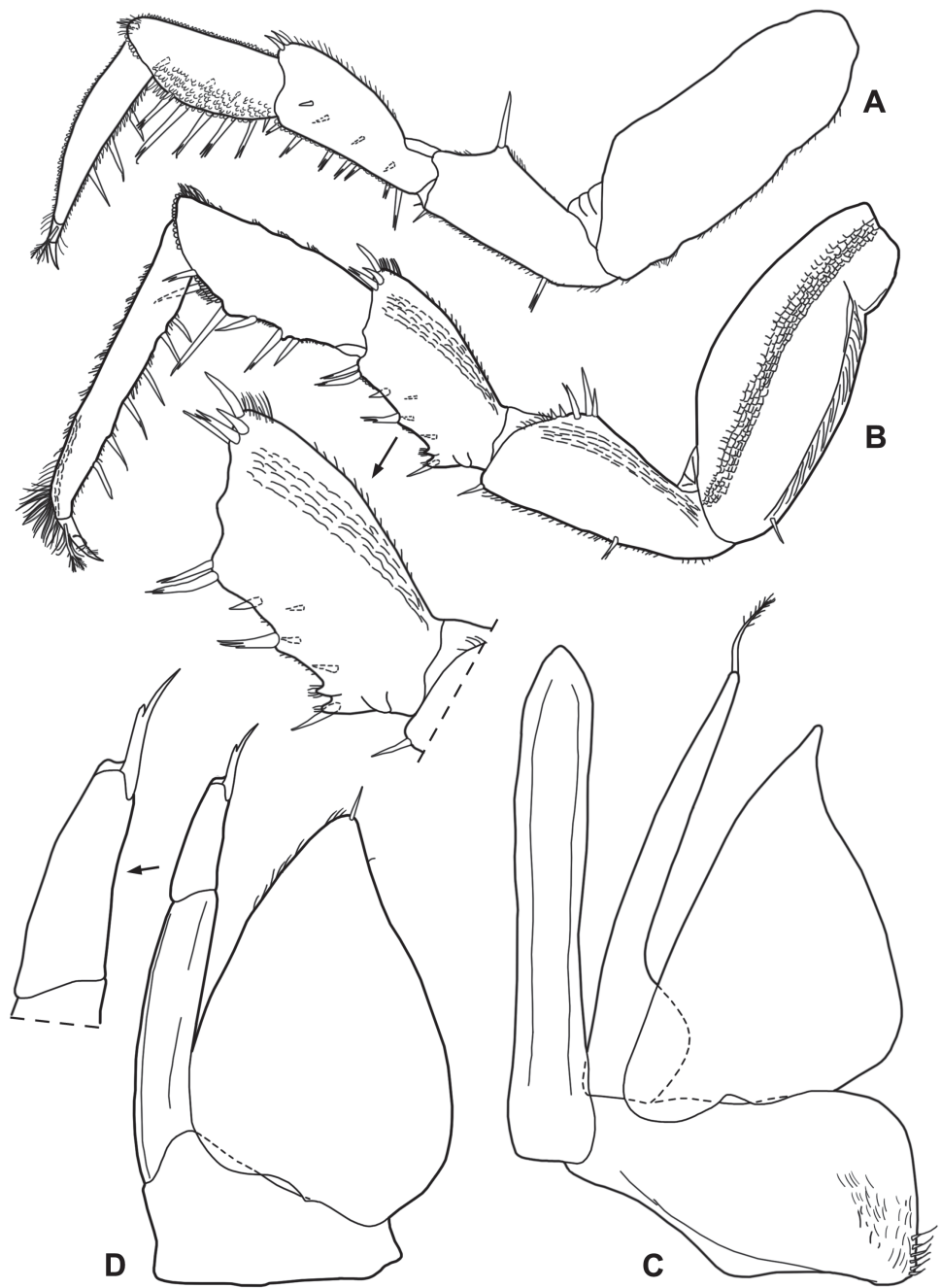
**Remarks.** *Alpioniscus onnisi* sp. n. is very similar to *A. fragilis* and *A. thanit*. It differs from the former in the smooth instead of granulated dorsal surface, the shape of the dorsal scale-setae, telson with triangular instead of trapezoidal distal part, and the male pleopod 2 endopod lacking a subapical lobe; from the latter mainly in the larger body shape and the male pleopod 1 exopod with slightly convex, instead of concave, medial margin, and shorter distal part.



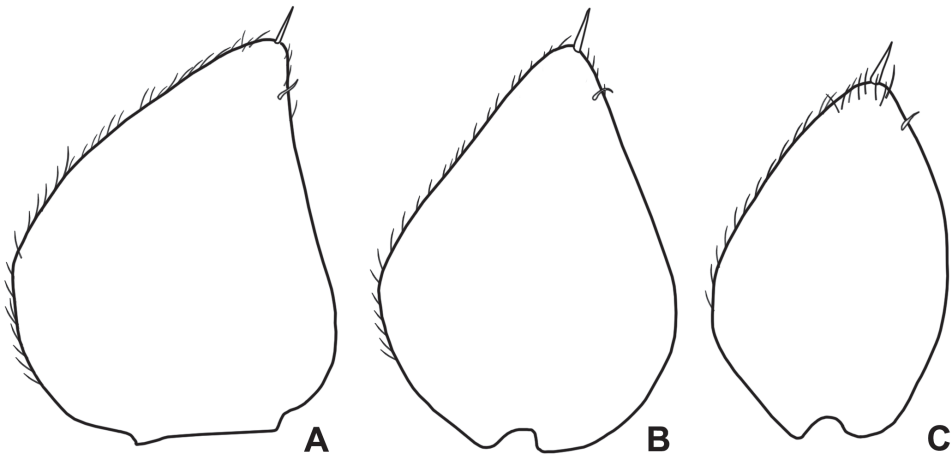
**Figure 5.** *Alpioniscus onnisi* Taiti & Argano, sp. n. from Grotta Giuanniccu Mene, ♂ paratype: **A** adult specimen, dorsal **B** dorsal scale-seta **C** cephalon, frontal **D** cephalon, dorsal **E** pleonites 4, 5, telson and uropods **F** antennula **G** antenna.



**Figure 6.** *Alpioniscus onnisi* Taiti & Argano, sp. n. from Grotta Giuanniccu Mene, ♂ paratype: **A** right mandible **B** left mandible **C** maxillula **D** maxilla **E** maxilliped.



**Figure 7.** *Alpioniscus onnisi* Taiti & Argano, sp. n. from Grotta Giuanniccu Mene, ♂ paratype: **A** pereopod 1 **B** pereopod 7 **C** genital papilla and pleopod 1 **D** pleopod 2.



**Figure 8.** *Alpioniscus onnisi* Taiti & Argano, sp. n. from Grotta Giuanniccu Mene, ♂ paratype: **A** pleopod 3 exopod **B** pleopod 4 exopod **C** pleopod 5 exopod.

***Alpioniscus kuehni* (Schmalfuss, 2005), comb. n.**

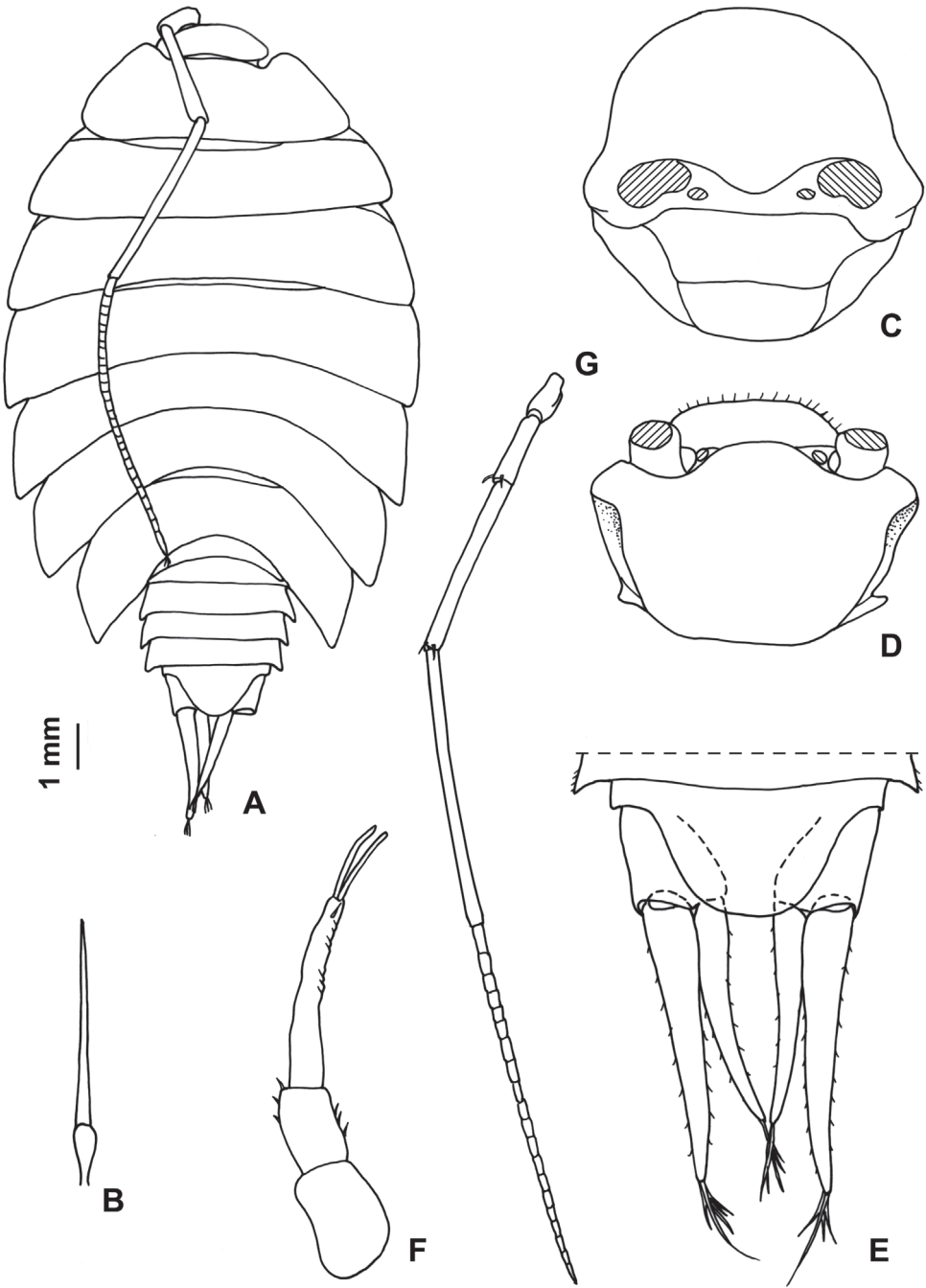
Figs 9–11, 18, 19

*Utopioniscus kuehni*; Schmalfuss 2005: 2, figs 1–5, 7–35; Oertel and Patzner 2007: 62, 64, fig. 4; Taiti and Argano 2011: 166.

**Material examined.** **Prov. Nuoro:** 4 ♂♂, 3 ♀♀ (MZUF 9827), Grotta del Bue Marino, c.n. 12 Sa/NU, 40°14'55.72"N, 9°37'24.80"E, Cala Gonone, Dorgali, 25.IV.2012, leg. E. Dallochio and P. Marcia; 1 ♂, 1 juv. (MZUF 9828), same locality, 10.IX.2006, P. Marcia and F. Stoch; 11 ♂♂, 4 ♀♀ (MZUF 9829), Grotta Su Bentu, c.n. 105 Sa/NU, 40°15'18.23"N, 9°29'6.52"E, Lanaittu, Oliena, 6.I.2013, leg. P. Marcia; 2 ♂♂ (MZUF 9830), same locality, 11–14.IX.2012, leg. P. Marcia and Astronauts; 1 ♀ (MZUF 9831), same locality, 21.IX.2013, leg. Astronauts; 1 ♂, 2 ♀♀ (MZUF 9832), same locality, 15.IX.2014, leg. Astronauts.

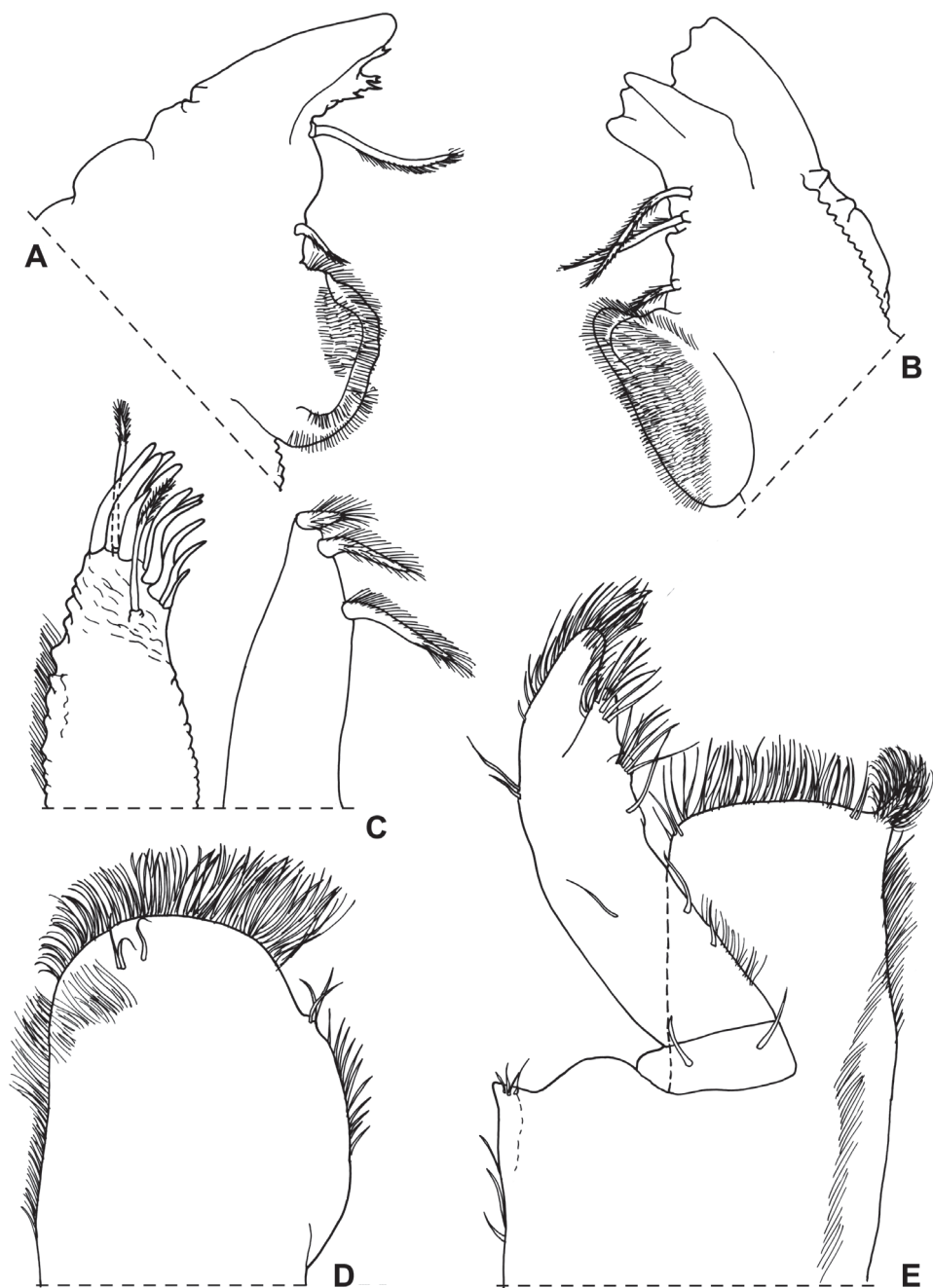
**Distribution.** The species is endemic to karstic areas of Supramonte, central-eastern Sardinia.

**Remarks.** *Alpioniscus kuehni* was originally described as *Utopioniscus kuehni* gen. n., sp. n. by Schmalfuss (2005) on specimens collected in underground waters from two caves on the central-eastern coast of Sardinia, Grotta dell'Utopia and Grotta del Bel Torrente, with entrances at 30 m and 16 m below sea level, respectively. These caves are the estuaries of subterranean streams which open up in the sea (De Waele and Forti 2003). In the Grotta del Bel Torrente the specimens were collected 700 m inland, where water salinity was 1‰ (Schmalfuss 2005; Oertel and Patzner 2007). In the Grotta dell'Utopia they were collected 2000 m inland together with

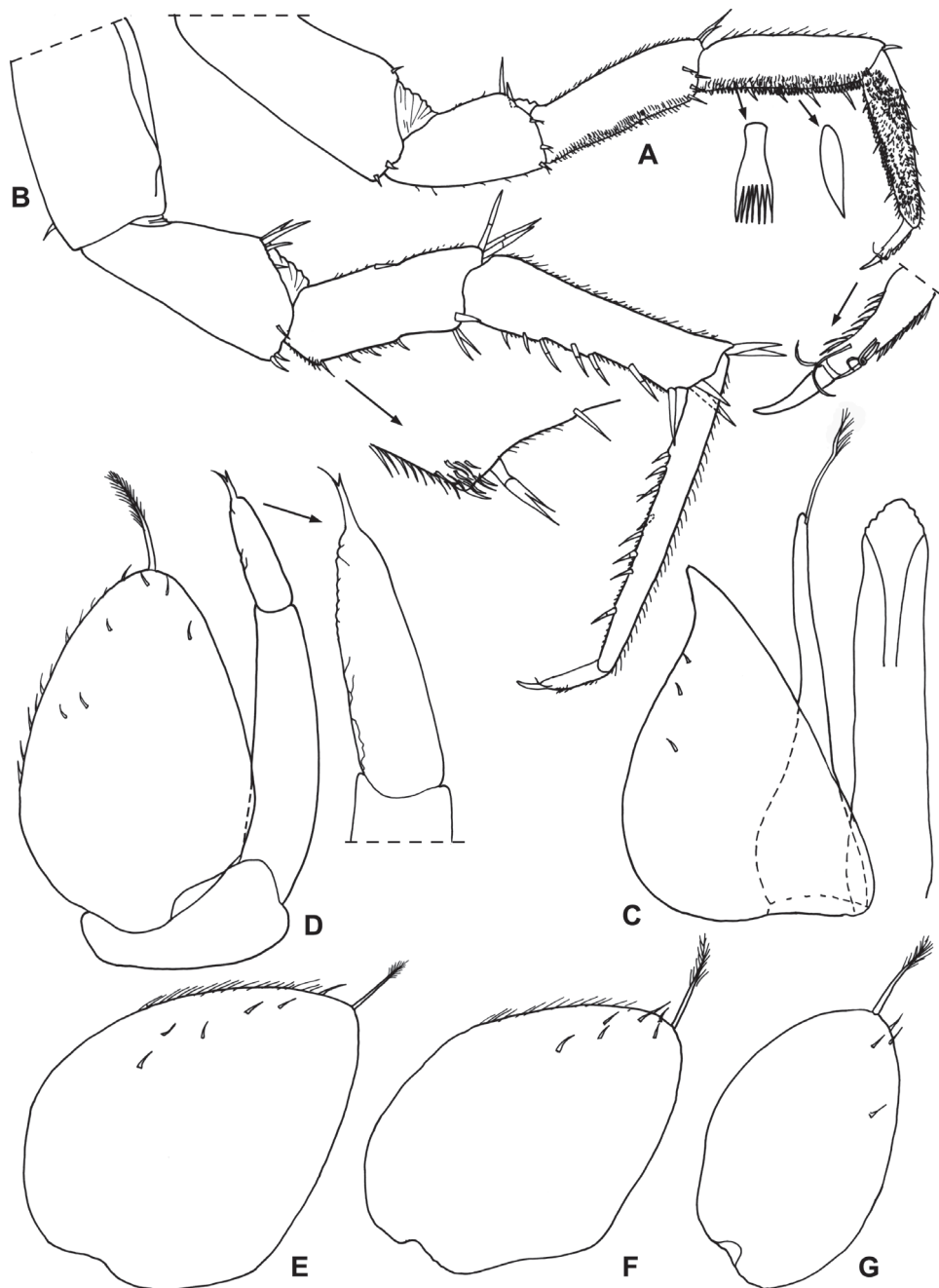


**Figure 9.** *Alpioniscus kuebni* (Schmalfuss, 2005), comb. n. from Grotta del Bue Marino, ♀: **A** adult specimen, dorsal **B** dorsal scale-seta **C** cephalon, frontal **D** cephalon, dorsal **E** telson and uropods **F** antennula **G** antenna.





**Figure 10.** *Alphoniscus kuehni* (Schmalfuss, 2005), comb. n. from Grotta del Bue Marino, ♀: **A** right mandible **B** left mandible **C** maxillula **D** maxilla **E** maxilliped.



**Figure 11.** *Alpioniscus kuehni* (Schmalfuss, 2005), comb. n. from Grotta del Bue Marino, ♂: **A** pereopod 1 **B** pereopod 7 **C** genital papilla and pleopod 1 **D** pleopod 2 **E** pleopod 3 exopod **F** pleopod 4 exopod **G** pleopod 5 exopod.

a species of the freshwater stygobiotic *Stenasellus* Dollfus, 1897 (Asellota, Stenasellidae). The specimens from Grotta del Bue Marino and Grotta Su Bentu here examined were collected in fresh water lakes and are morphologically identical to the specimens from the type localities. This fact confirms that *A. kuehni* is a freshwater stygobiotic species.

The species is well described by Schmalfuss (2005) and is here fully illustrated on specimens from Grotta del Bue Marino (Figs 9–11). As confirmed by molecular data (see below), the genus *Utopioniscus* must be considered to be a junior synonym of *Alpioniscus*. In fact, all the diagnostic morphological characters of the genus *Alpioniscus* mentioned by Vandel (1960) are present also in *A. kuehni*, in particular the shape of the male pleopods 1 and 2, as already pointed out by Schmalfuss (2005). *Alpioniscus kuehni* is mainly characterized by the enlarged shape of the pereon, the reduction of the number of aesthetascs of the antennula, the very thin antenna with the flagellum of 20 to 30 articles, the enlargement of the maxillipedal endite, and the lack of groove with scales on the pereopod 7 for the water conducting system. The last two characters are certainly adaptive for aquatic life. The enlarged maxillipedal endite is, in fact, present also in the following two new species which are both aquatic.

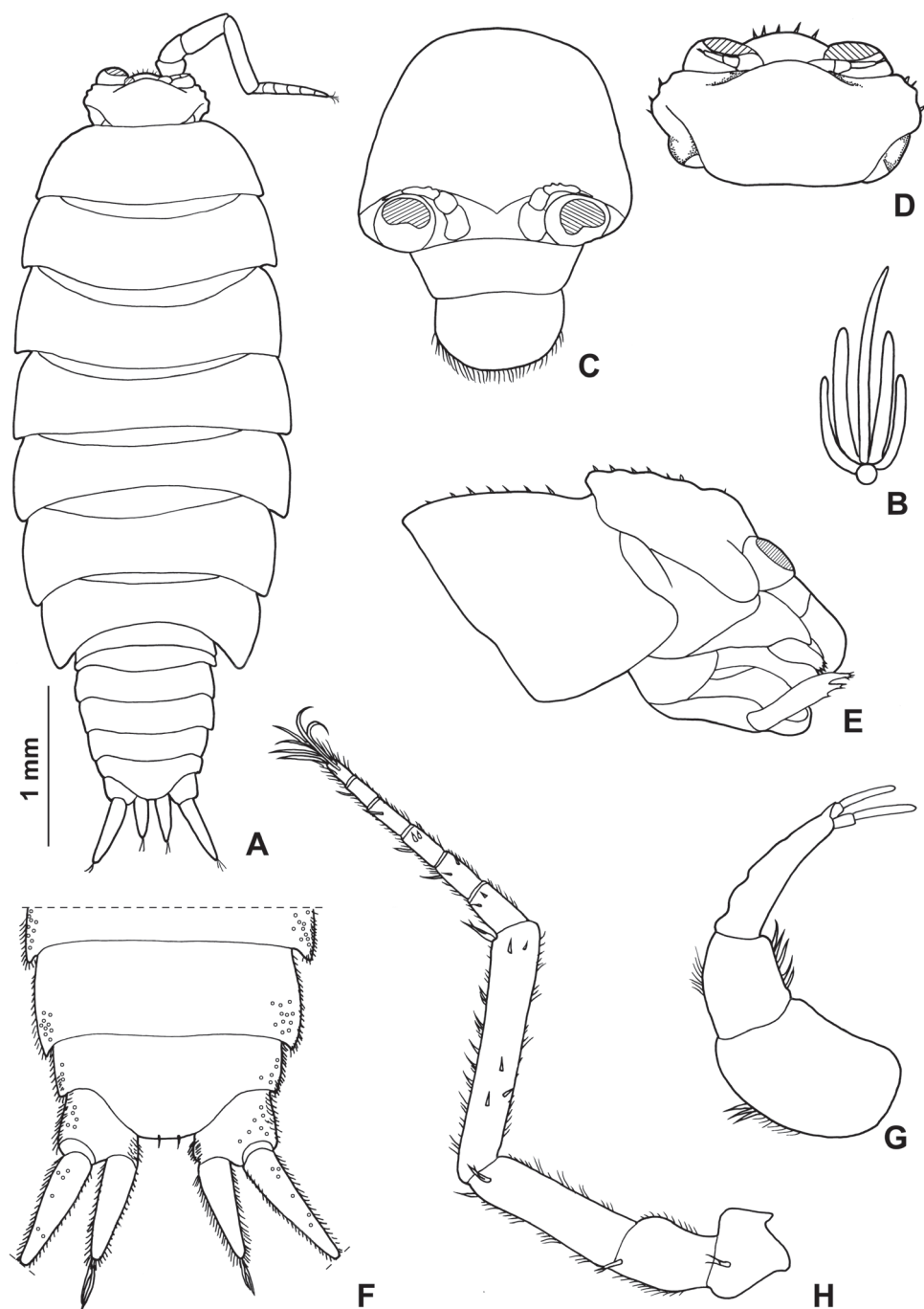
***Alpioniscus stochi* Taiti & Argano, sp. n.**

<http://zoobank.org/E76BDBF8-0FE5-4DEF-BAF1-7CA0C3F92F34>

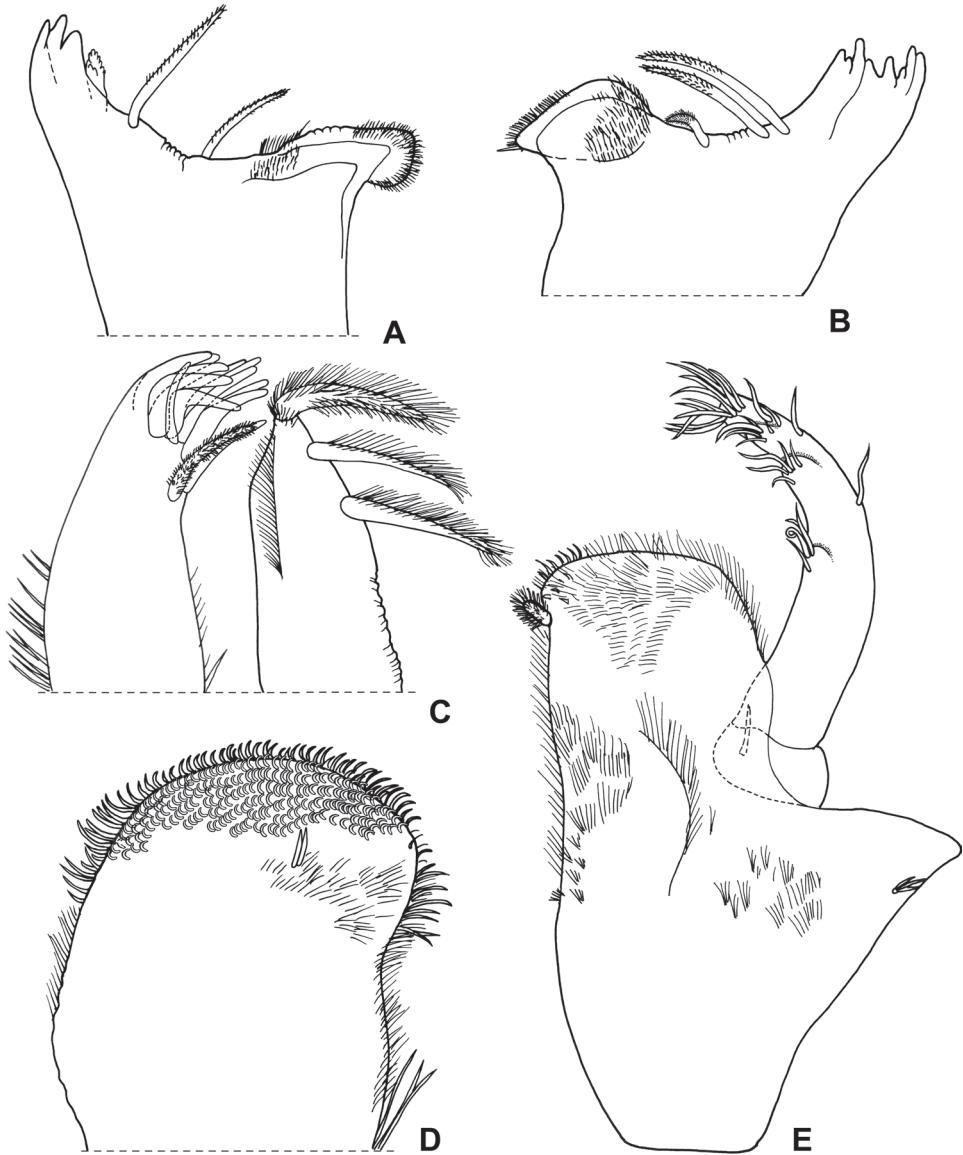
Figs 12–14, 18, 19

**Material examined.** **Prov. Ogliastra:** *Holotype:* ♂ (MZUF 9833), Grotta Su Palu, c.n. 1988 Sa/OG, 40°10'38.23"N, 9°33'50.53"E, 185 m, Codula Ilune, Urzulei, 1.V.2009, leg. F. Stoch and G. Tomasini. *Paratypes:* 3 ♂♂, 4 ♀♀ (MZUF 9833), same data as holotype; 4 ♂♂, 5 ♀♀ (MZUF 9834), same locality, 18.IX.2010, leg. G. Tomasini.

**Description.** Maximum length: ♂ and ♀, 4.5 mm. Colourless body, pleon slightly narrower than pereon (Fig. 12A). Dorsal surface smooth with some scattered scale-setae as in Fig. 12B. Some gland pores on lateral margins of pleonites 4 and 5, telson and on dorsal surface of uropodal protopod and exopod (Fig. 12F). Cephalon (Fig. 12C–E) with suprantennal line V-shaped; antennal lobes quadrangular. Eyes absent. Posterior margins of pereonite 1–4 straight, of pereonites 5–7 progressively more concave (Fig. 12A). Pleonites 3–5 with very short posterior points (Fig. 12F). Distal part of telson with concave sides and very broadly rounded apex (Fig. 12F). Antennula (Fig. 12G) with distal article narrow and bearing two apical aesthetascs. Antenna (Fig. 12H) with fifth article as long as flagellum; flagellum of five to seven articles. Mandibles with two penicils in the right (Fig. 13A) and three penicils in the left (Fig. 13B). Outer branch of maxillula with 5 + 6 teeth, apically entire, three or four of the outer group strongly bent inwards, two slender stalks, one setose; inner branch with three long penicils (Fig. 13C). Maxilla with setose apex (Fig. 13D). Maxilliped (Fig. 13E) endite quadrangular with a setose distal margin and a distinct subapical short penicil

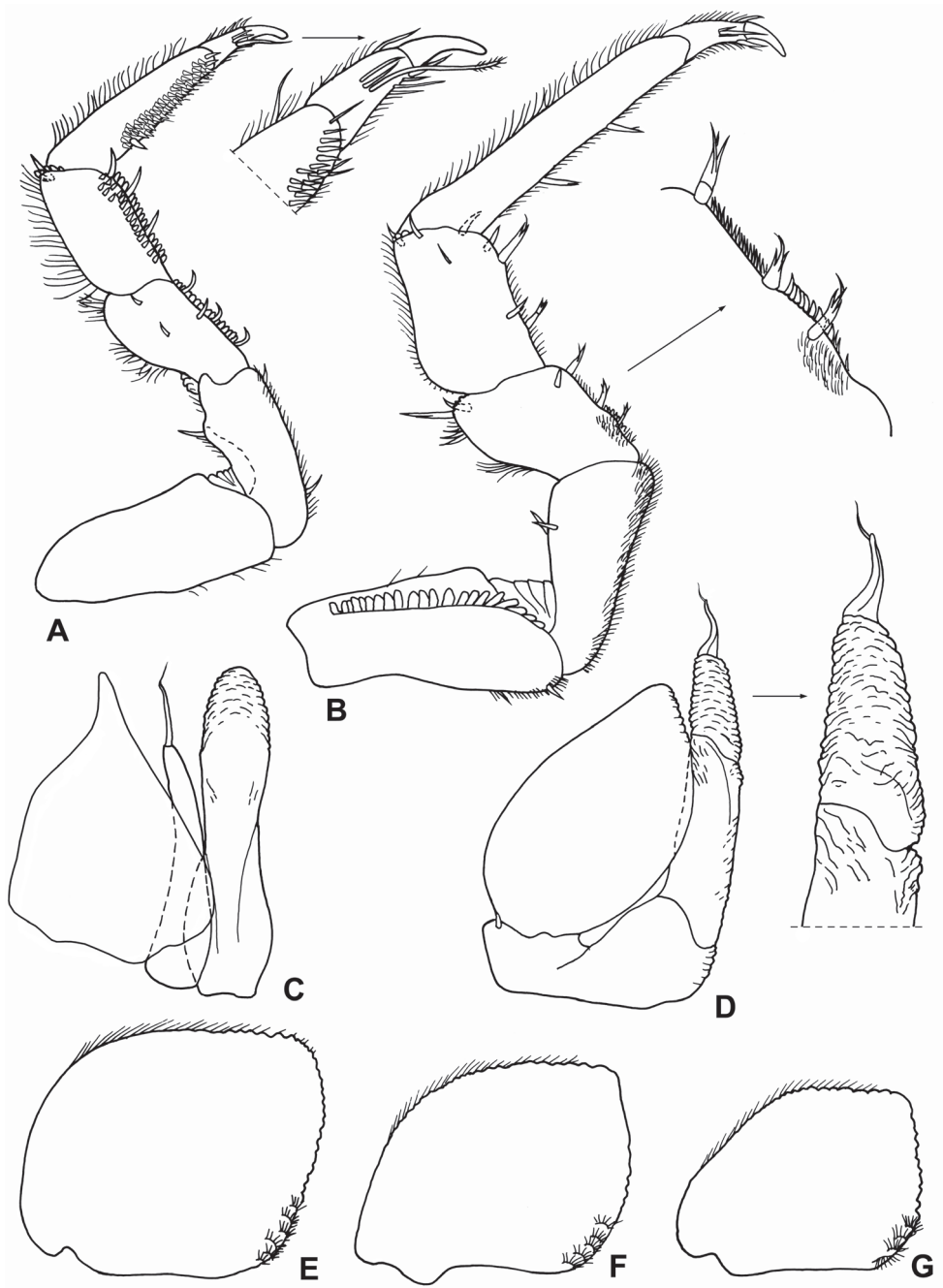


**Figure 12.** *Alpioniscus stochi* Taiti & Argano, sp. n. from Grotta Su Palu, ♂ paratype: **A** adult specimen, dorsal **B** dorsal scale-seta **C** cephalon, frontal **D** cephalon, dorsal **E** cephalon and pereonite 1, lateral **F** pleonites 4, 5, telson and uropods **G** antennula **H** antenna.



**Figure 13.** *Alphoniscus stochi* Taiti & Argano, sp. n. from Grotta Su Palu, ♂ paratype: **A** right mandible **B** left mandible **C** maxillula **D** maxilla **E** maxilliped.

on medial margin; palp narrow and bent in medial direction, three tufts of setae on medial margin and one apically, basal article with a single seta; basis distally enlarged with a triangular lobe on outer margin. Pereopods with setose dactylar seta (Fig. 14A). Pereopod 7 (Fig. 14B) basis with a groove covered with scales on rostral surface (water



**Figure 14.** *Alpioniscus stochi* Taiti & Argano, sp. n. from Grotta Su Palu, ♂ paratype: **A** pereopod 1 **B** pereopod 2 **C** genital papilla and pleopod 1 **D** pleopod 2 **E** pleopod 3 exopod **F** pleopod 4 exopod **G** pleopod 5 exopod.

conducting system). Uropod (Fig. 12F) with protopod not grooved on outer margin; endopod slightly shorter than exopod, exopod and endopod inserted at the same level.

**Male.** Pereopod 1–4 (Fig. 14A) with carpus and merus bearing numerous short scales on sternal margin. Pereopod 7 (Fig. 14B) ischium with straight sternal margin covered with short setae; merus with scales on sternal margin, carpus enlarged proximally. Genital papilla (Fig. 14C) with a rounded tip. Pleopod 1 (Fig. 14C) exopod triangular with narrow posterior point; endopod narrow with almost parallel sides, armed with an apical seta. Pleopod 2 (Fig. 14D) exopod subovoidal, with no apical seta; endopod with first article nearly twice as long as second, strong terminal seta ending with thinner seta. Pleopods 3–5 exopods (Fig. 14E–G) quadrangular with no apical seta.

**Etymology.** The new species is named after our colleague and friend Dr. Fabio Stoch, who greatly contributed to the knowledge of Italian stygobiotic fauna and collected part of the material.

**Remarks.** The specimens were collected under stones on the bottom of a subterranean stream in the Grotta Su Palu, in the eastern part of the karstic area of Supramonte. *Alpioniscus stochi* sp. n. is similar to *A. kuehni* in having a thin third article of the antennula bearing a small number of aesthetascs, and the maxilliped with a quadrangular endite and a narrow palp bent medially. It is readily distinguishable from *A. kuehni* by the smaller size (4.5 mm vs. 12.0 mm of the latter), narrower body shape, shorter and more thickset antenna with smaller number of flagellar articles, uropods with shorter branches, the presence of a water conducting system on pereopod 7 ischium, a more thickset merus of the male pereopod 7, a stouter male pleopod 2 endopod, and no apical seta on the exopod of the male pleopods 2–5.

***Alpioniscus sideralis* Taiti & Argano, sp. n.**

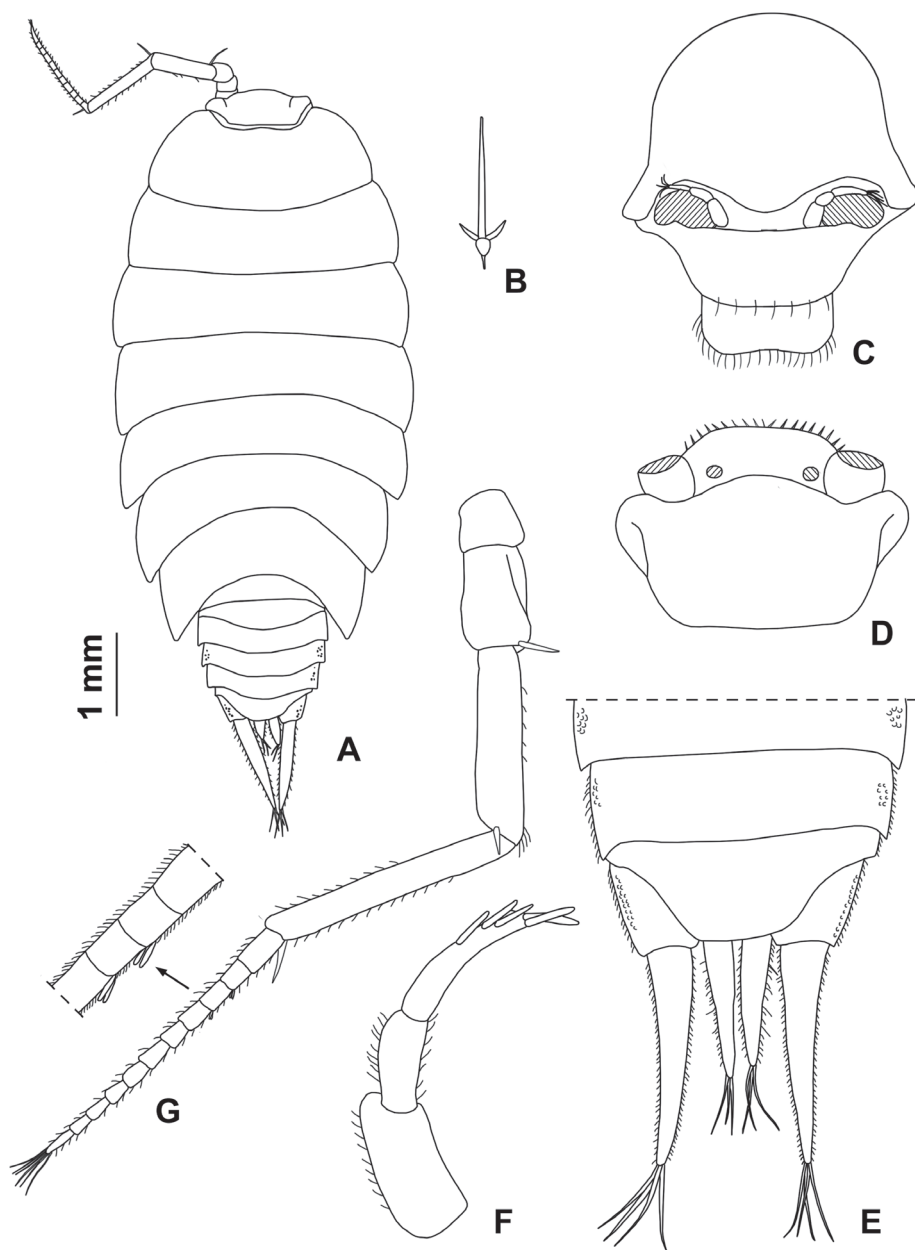
<http://zoobank.org/06FC60AA-B680-4684-B5A3-1FF995C44254>

Figs 15–19

*Alpioniscus* n. sp.; Bessone et al. 2013: 325, fig. 11; 2016: 218, fig. 8.

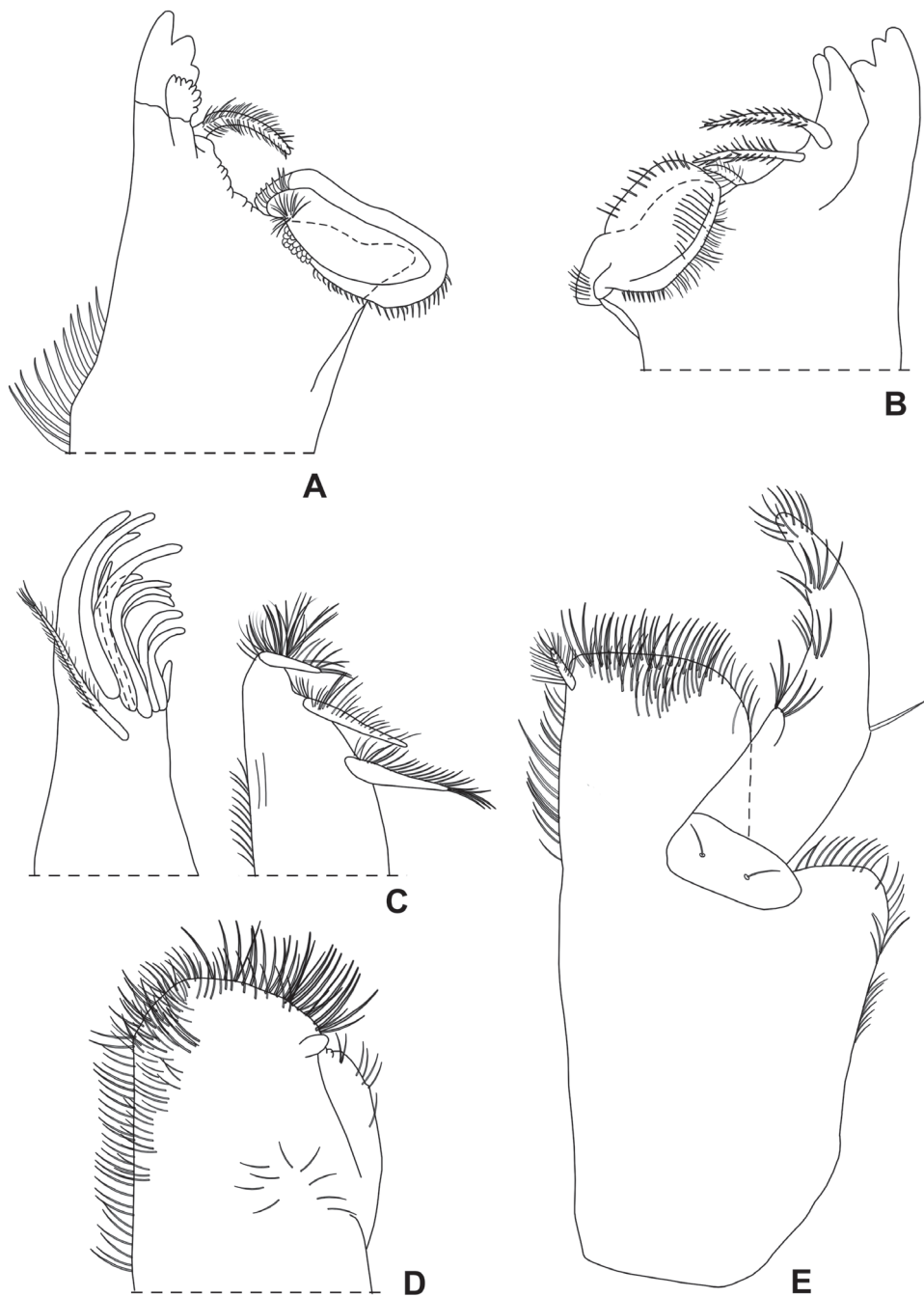
**Material examined. Prov. Nuoro:** *Holotype:* ♂ (MZUF 9835), Grotta Su Bentu, c.n. 105 Sa/NU, 40°15'18.23"N, 9°29'6.52"E, Lanaittu, Oliena, 21.IX.2013, leg. Astronauts. *Paratypes:* 1 ♂, 1 ♀ (MZUF 9836), same data as holotype; 1 ♂, 3 ♀♀ (MZUF 9836), same locality, 11–14.IX.2012, leg. P. Marcia and Astronauts; 1 ♂, 2 ♀♀ (MZUF 9837), same locality, 6.I.2013, leg. P. Marcia; 2 ♂♂, 1 ♀ (MZUF 9838), Grotta Sas Venas, c.n. 3064 Sa/NU, 40°07'32.33"N, 9°26'27.04"E, Orgosolo, VI.2013, leg. P. Marcia; 3 ♀♀ (MZUF 9839), Grotta Piggio de Janas, c.n. 3236 Sa/NU, 40°08'32.70"N, 9°27'18.60"E, Tauledda, Codula del Flumineddu, Orgosolo, 28.X.2012, leg. C. Corongiu. **Prov. Ogliastra:** 2 ♂♂, 1 ♀ (MZUF 9840), Grotta Lovettecannas, c.n. 2642 Sa/OG, 40°08'33.72"N, 9°34'35.35"E, Baunei, 5.I.2013, leg. L. Sanna; 7 ♂♂, 7 ♀♀ (MZUF 9841), same locality, 1.IV.2013, leg. P. Marcia; 1 ♂ (MZUF 9842), Grotta Su Palu, c.n. 1988 Sa/OG, 40°10'38.23"N,



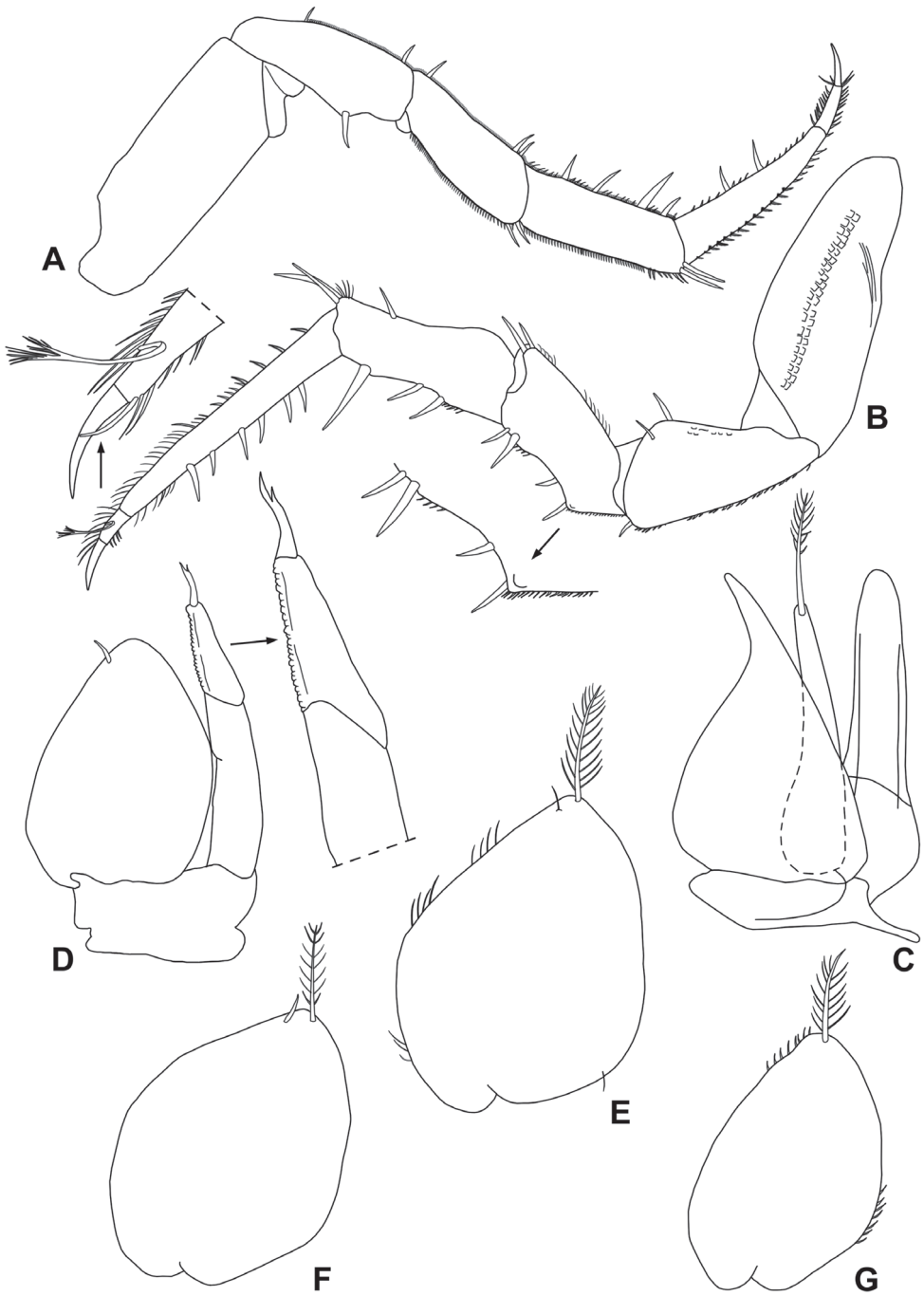


**Figure 15.** *Alpioniscus sideralis* Taiti & Argano, sp. n. from Grotta Su Bentu, ♂ paratype: **A** adult specimen, dorsal **B** dorsal scale-seta **C** cephalon, frontal **D** cephalon, dorsal **E** pleonites 4, 5, telson and uropods **F** antennula **G** antenna.

9°33'50.53"E, 185 m, Codula Ilune, Urzulei, 8.XII.2012, leg. P. Marcia; 5 ♂♂, 9 ♀♀ (MZUF 9843), Grotta Istirzili, c.n. 50 Sa/OG, 40°04'49.50"N, 9°37'13.40"E, Baunei, 12.V.2013, leg. C. Onnis.



**Figure 16.** *Alphoniscus sideralis* Taiti & Argano, sp. n. from Grotta Su Bentu, ♂ paratype: **A** right mandible **B** left mandible **C** maxillula **D** maxilla **E** maxilliped.



**Figure 17.** *Alpioniscus sideralis* Taiti & Argano, sp. n. from Grotta Su Bentu, ♂ paratype: **A** pereopod 1 **B** pereopod 7 **C** genital papilla and pleopod 1 **D** pleopod 2 **E** pleopod 3 exopod **F** pleopod 4 exopod **G** pleopod 5 exopod.

**Description.** Maximum length: ♂, 7 mm; ♀, 7.5 mm. Body colourless, ovoidal, with pleon narrower than pereon (Fig. 15A). Dorsal surface smooth with some scattered scale-setae as in Fig. 15B. Some gland pores on lateral margins of pleonites 4 and 5 and on uropodal protopods (Fig. 15E). Cephalon (Fig. 15C, D) with suprantennal line sinuous; antennal lobes quadrangular. Eyes absent. Posterior margin of pereonite 1–3 straight, of pereonites 4–7 progressively more concave (Fig. 15A). Pleonites 3–5 with very short posterior points (Fig. 15E). Distal part of telson with slightly concave sides and truncate apex (Fig. 15E). Antennula (Fig. 15F) with distal article narrow and bearing two apical and three subapical aesthetascs. Antenna (Fig. 15G) with fifth article of peduncle shorter than flagellum; flagellum of 10–11 articles with couple of aesthetascs on second and third article. Mandibles with one penicil in the right (Fig. 16A) and three penicils in the left (Fig. 16B). Outer branch of maxillula with 5 + 6 teeth, apically entire, and two slender setose stalks; inner branch with three penicils increasing in length from distal to proximal (Fig. 16C). Maxilla with setose and bilobed apex, inner lobe very small (Fig. 16D). Maxilliped (Fig. 16E) endite quadrangular with setose distal margin and distinct subapical penicil on medial margin; palp narrow and bent in medial direction, with three tufts of setae on medial margin, tuft at apex and single seta on outer margin, basal article with two setae; basis distally enlarged with a rounded lobe on outer margin. Pereopods with bifid setose dactylar seta (Fig. 17A). Pereopod 7 (Fig. 17B) basis with water conducting system. Uropod (Fig. 15E) with protopod not grooved on outer margin; endopod distinctly shorter than exopod, exopod inserted slightly distally to endopod.

**Male.** Pereopod 1–4 (Fig. 17A) with ischium, carpus and merus bearing numerous short scales on sternal margin. Pereopod 7 (Fig. 17B) ischium with straight sternal margin covered with short setae; merus with triangular lobe at base of sternal margin, more or less protruding according to size; carpus enlarged proximally. Genital papilla (Fig. 17C) with a rounded tip. Pleopod 1 (Fig. 17C) exopod triangular with long narrow distal point slightly bent outwards; endopod narrow with almost parallel sides and proximally enlarged, armed with an apical plumose seta. Pleopod 2 (Fig. 17D) exopod subovoidal with no apical plumose seta; endopod with first article nearly twice as long as second, a strong terminal bifid seta. Pleopods 3–5 exopods (Fig. 17E–G) ovoidal with a long plumose apical seta.

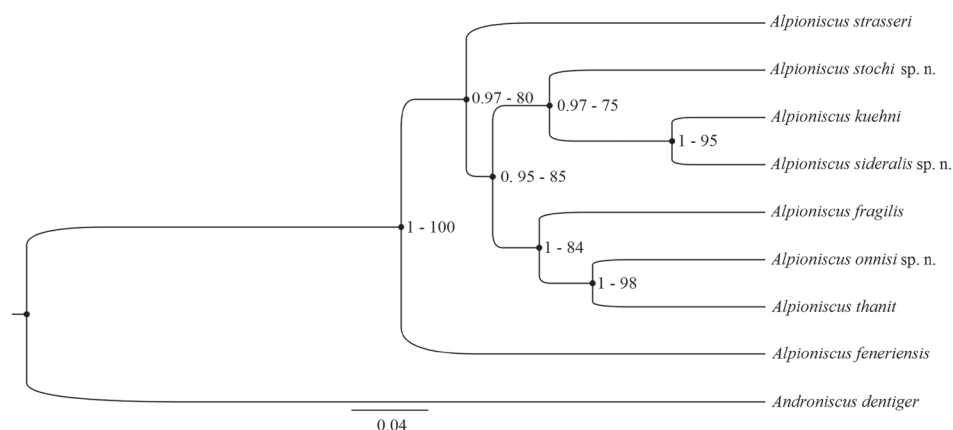
**Etymology.** Latin: *sideralis* meaning of or with respect to the distant stars. The name refers to the international team of astronauts, taking part in training courses (ESA CAVES) in Sardinia organized by the European Space Agency, who first collected this new species.

**Remarks.** The species occurs in subterranean freshwater ponds and streams in the karstic areas of Supramonte. It is closely related to the other two Sardinian stygobiotic species of *Alpioniscus* (*A. kuehni* and *A. stochi* sp. n.). It is readily distinguishable from *A. kuehni* by the smaller size (7.7 mm vs 12 mm), narrower body shape, shorter and more thickset antenna with smaller number of flagellar articles, presence on pereopod 7 ischium of a water conducting system, male pereopod 7 carpus

shorter with more enlarged proximal part, male pleopod 1 exopod with narrower distal part, endopod shorter than exopod, male pleopod 2 exopod without setose apical seta. *Alpioniscus sideralis* differs from *A. stochi* in its larger size (7.5 mm vs. 4.5 mm of the latter), antennula with a group of subapical aesthetascs, more numerous flagellar articles of the antenna (10-11 vs. 5-7), maxillipedal basis with a rounded instead of triangular lobe on outer margin, uropods with longer exopod and endopod, male pereopod 7 with thinner merus and carpus, merus with a distinct lobe on sternal margin.

## Molecular results

After alignment, a 405 bp-long sequence dataset was obtained (see Table 1 for GenBank accession numbers). Since BI and ML analyses generated trees with identical topologies, only the BI tree is reported (Fig. 18 for more details). Each node is highly supported by both bootstrap values (BV) and posterior probability (PP). Among the species belonging to the genus *Alpioniscus*, *A. (Alpioniscus) feneriensis* from Piedmont is placed in the tree as the most external taxon. Its sister clade groups both *A. (Illyri-onethes) strasseri* from Friuli Venezia Giulia and Sardinian species. All Sardinian species are clustered together in one clade. Sardinian terrestrial species (*Alpioniscus fragilis*, *A. thanit*, and *A. onnisi* sp. n.) set in a monophyletic group, showing a sister-taxon relationship with the clade including Sardinian aquatic species (*A. stochi* sp. n., *A. sideralis* sp. n., and *A. kuehni*). Within the terrestrial clade, *A. onnisi* sp. n. is the sister species of *A. thanit*. Within the aquatic clade, *A. kuehni* is the sister species of *A. sideralis* sp. n.



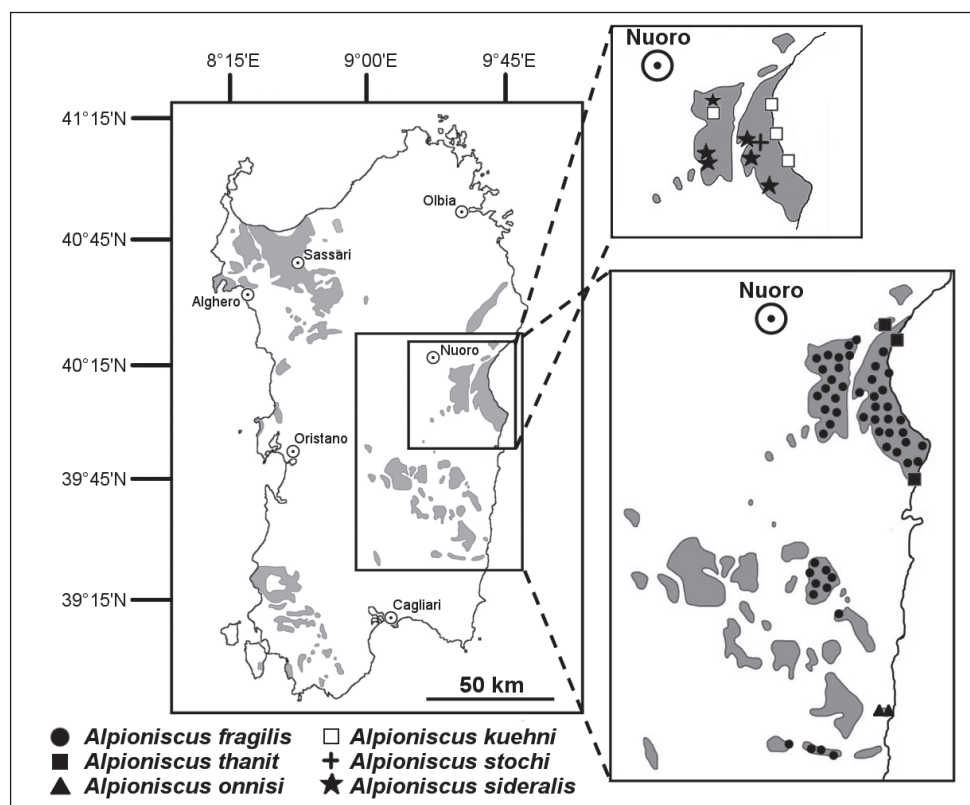
**Figure 18.** Gene tree according to BI showing the interrelationships among species based on COI. The branch length scale refers to the number of substitutions per site. Nodal supports are BI posterior probabilities (the first value), and ML bootstrap values (the second value).

### Key to species of *Alpioniscus* from Sardinia

- 1 Body dorsal surface distinctly granulated (Fig. 1A) ..... **2**
- Body dorsal surface smooth (Figs 5A, 9A, 12A, 15A) ..... **3**
- 2 Maximum length 14 mm; male pleopod 1 exopod triangular with outer margin almost straight (Fig. 3C); male pleopod 2 endopod with a subapical rounded lobe on distal article (Fig. 4A) ..... *Alpioniscus fragilis*
- Maximum length 6–7 mm; male pleopod 1 exopod triangular with outer margin distinctly sinuous; male pleopod 2 endopod with no subapical rounded lobe on distal article ..... *Alpioniscus thanit*
- 3 Telson with distal part triangular (Fig. 5E); maxillipedal endite narrow with thickset apical penicil (Fig. 6E) ..... *Alpioniscus onnisi*
- Telson with distal part trapezoidal (Figs 9E, 12E, 15E); maxillipedal endite enlarged, rectangular with small penicil near distal-medial corner (Fig. 10E, 13E, 16E) ..... **4**
- 4 Maximum length 15 mm; antenna thin and long, reaching pereonite 7 (Fig. 9A); antennal flagellum with 20–30 articles (Fig. 9G); pereopod 7 ischium with no trace of water conducting system (Fig. 11B) ..... *Alpioniscus kuehni*
- Maximum length 7.5 mm; antenna thicker and much shorter than pereon (Figs 12A, 15A); antennal flagellum with up to 11 articles (Figs 12H, 15G); pereopod 7 ischium with distinct water conducting system (Figs 14B, 17B) ..... **5**
- 5 Maximum length 4.5 mm; antennula with two apical aesthetascs (Fig. 12G); antennal flagellum with 5 to 7 articles; male pereopod 7 merus with no lobe on sternal margin and thickset carpus (Fig. 14B); pleopod 3–5 exopods with no setose apical seta (Fig. 14E–F) ..... *Alpioniscus stochi*
- Maximum length 7.5 mm; antennula with two apical and 3 subapical aesthetascs (Fig. 15F); antennal flagellum with 10 or 11 articles; male pereopod 7 merus with triangular lobe on sternal margin and slender carpus (Fig. 17B); pleopod 3–5 exopods with setose apical seta (Fig. 17E–F) ..... *Alpioniscus sideralis*

### Discussion

According to the data of the present study, the genus *Alpioniscus* is represented in Sardinia by six different species inhabiting various underground environments within a fragmented karst area of limited extension in the central-eastern and south-eastern part of the island (Fig. 19). All six species have morphological characters corresponding to the diagnosis of the subgenus *Illyrionethes* proposed by Verhoeff (1927), i.e., the distal article of the male pleopod 2 endopod shorter than or of equal length as the second article. This conclusion based on morphology is also supported by the molecular comparison with *Alpioniscus* (*Illyrionethes*) *strasseri*, type species of the subgenus *Illyrionethes*, and *A. (Alpioniscus) feneriensis*. However, a definite assessment of the taxonomic status within the genus *Alpioniscus* can be reached only after



**Figure 19.** Distribution map of *Alpioniscus* species in Sardinia (after Taiti and Argano 2011 and new data).

the comparison of all, or most of the species belonging to the genus from its entire distribution area.

According to the molecular phylogeny, the six Sardinian species are grouped into two distinct clades, one including the three terrestrial species (*A. fragilis*, *A. thanit*, and *A. onnisi*) and the other the three aquatic species (*A. stochi*, *A. sideralis*, *A. kuehni*). It seems most probable that the aquatic mode of life evolved once and then the aquatic species split into other species either by vicariant events related to the karst areas or by dispersal along subterranean aquifers with subsequent isolation. The dispersal in subterranean waters seems to be possible as demonstrated by the presence of *A. sideralis* and *A. kuehni* in two separated, even if close, karst areas (see Fig. 19). Dispersal of aquatic species across terrestrial habitats is unlikely. The presence of both terrestrial and aquatic species in the same genus is uncommon among Oniscidea. The only other case known so far is represented by the genus *Trogloniscus* Taiti & Xue, 2012, which includes three terrestrial and two aquatic species from caves in southern China (Taiti and Xue 2012).

*Alpioniscus fragilis* is a terrestrial species distributed along an area covering that of the whole genus in Sardinia. This species is mainly troglobiotic, but it can be found also in endogean habitats (e.g., under big stones near Lecorci Falls, Ogliastra) and in an aquatic environment (e.g., under submerged stones in Bue Marino Cave). So, the



species shows a remarkable adaptive plasticity which allowed it to be distributed along a large and discontinuous area in suitable ecological conditions. All the other species have more restricted distributions. In some caves both terrestrial and aquatic species occur sympatrically. *Alpioniscus fragilis* coexists with two stygobiotic species, i.e., *A. kuehni* in the Grotta del Bue Marino, and *A. sideralis* in the Grotta Lovettecannas and Grotta Istirzili. In the Grotta Su Bentu *A. fragilis* occurs together with the two stygobiotic *A. kuehni* and *A. sideralis*, while in the Grotta Su Palu with the stygobiotic *A. stochi* and *A. sideralis*. The last two species share adaptive traits linked to the aquatic environment with *A. kuehni*, e.g., the quadrangular endite of the maxilliped, but also aspects that recall a previous existence in terrestrial environments, i.e., the water conducting system on the ischium of the pereopod 7. It is interesting to notice that some aquatic species co-occur in the same cave (i.e., *A. kuehni* and *A. sideralis* in the Grotta Su Bentu, and *A. stochi* and *A. sideralis* in the Grotta Su Palu), while this does not happen with the terrestrial species.

This richness in Sardinian *Alpioniscus* species is probably due to the complex geological and palaeoecological events that affected Sardinia, such as marine ingression or strong ecological variations that isolated the small karst islands (Zattin et al. 2008; Lichter et al. 2010), interrupting gene flow among populations and promoting speciation. Migratory events of underground fauna of the archipelago among the various geographical units may have occurred during the emersion periods with suitable environmental conditions. The co-occurrence of two aquatic species in the same cave may be due to subsequent invasions or different habitat preference. This last condition might be true for *A. stochi*, which occurs under stones on the bottom of the subterranean stream in the Grotta Su Palu, and *A. sideralis*, which is found on the submerged walls of the same stream. Two or more stygobiotic oniscidean species (genus *Haloniscus* Chilton, 1920) in the same subterranean aquifer are known also from some calcretes in Western Australian (Taiti and Humphreys 2001; Cooper et al. 2008).

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# Woodlice of Belgium: an annotated checklist and bibliography (Isopoda, Oniscidea)

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## Abstract

Woodlice are key organisms for nutrient cycling in many terrestrial ecosystems; however, knowledge on this invertebrate group is limited as for other soil fauna taxa. Here, we present an annotated checklist of the woodlice of Belgium, a small but densely populated country in Western Europe. We reviewed all 142 publications on Belgian woodlice, the oldest dating back to 1831 and re-identified all doubtful specimens from the Royal Belgian Institute of Natural Sciences (RBINS) collection. These data is complemented with observations from extensive field surveys dating from March 2014 until December 2017. We report 36 species of woodlice with free-living populations for Belgium. Nine species can be added compared to the latest checklist published in 2000 being *Hyloniscus riparius* (C. Koch, 1838), *Miktoniscus patiencei* Vandel, 1946, *Trichoniscoides sarsi* Patience, 1908, *Haplophthalmus montivagus* Verhoeff, 1941, *Porcellio monticola* Lereboullet, 1853, *Metatrachoniscoides leydigii* (Weber, 1880), *Trichoniscus alemannicus* Verhoeff, 1917, *Eluma caelata* (Miers, 1877) and *Philoscia affinis* Verhoeff, 1908. Two species are deleted from the checklist (*Ligidium germanicum* Verhoeff, 1901 and *Armadillidium depressum* Brandt, 1833) because records are doubtful and no material has been preserved. Additionally the data of the field surveys is used to determine a species status of occurrence in Belgium. For each species, a short overview of their first records is provided and their confirmation as part of the Belgian fauna, their current status, as well as a complete bibliography of the species in Belgium.

## Keywords

Belgium, macro-detritivores, species distribution, terrestrial isopods

## Introduction

Woodlice (Isopoda: Oniscidea) are amongst the largest representatives of the soil invertebrate community in European terrestrial ecosystems (Jeffery et al. 2010). They fragment dead organic material on the forest floor (Anderson 1988; Grelle et al. 2000) and their activity significantly contributes to nutrient cycling in many terrestrial ecosystems (see e.g. David 2014). However, despite their functional importance, they are still poorly studied (David and Handa 2010). In Belgium, distribution data on many species are very scarce (Wouters et al. 2000) in contrast to extensive work in neighbouring countries like the Netherlands (Berg et al. 2008), Great Britain (Gregory 2009), Germany (Grüner 1965) and France (Vandel 1960, 1962, Sechet and Noël 2015). The latest Belgian checklist only reported 27 species with confirmed free-living populations (Wouters et al. 2000) and by comparing this with neighbouring countries it can be assumed that many species could be added to this list (see e.g. Lock and Durwael 2000, De Smedt et al. 2015, Boeraeve et al. 2017).

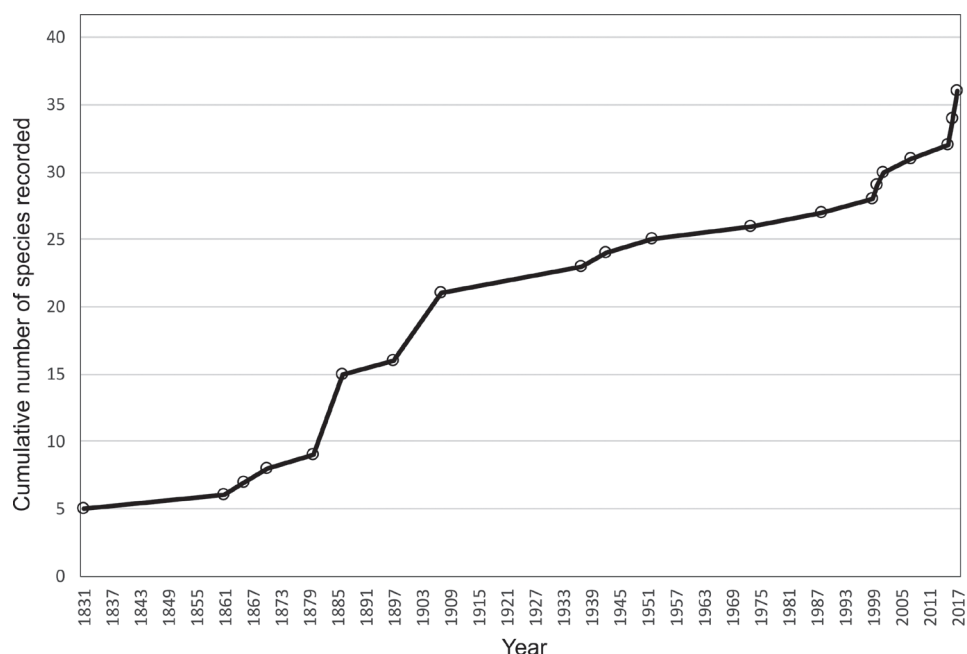
A complete overview of the history of woodlice research in Belgium is provided, by checking all existing literature on Belgian woodlice and re-identifying all doubtful or difficult to recognise species present in the collections from the Royal Belgian Institute of Natural Sciences (RBINS). These data are complemented with extensive field surveys carried out from March 2014 until December 2017 in order to produce a new checklist of Belgian woodlice. Additionally the data of the field surveys is used to determine a status of occurrence in Belgium for all species.

The oldest record of woodlice species in Belgium dates back to 1831 (Carlier 1831) (Fig. 1) and was published in a geographical monograph including all animal species from the province of Liège. This book mentions six species of which one was a synonym of *Armadillidium vulgare*, which was also mentioned in the list. Carlier (1831) mentions besides the latter species also the three common species being *Oniscus asellus*, *Philoscia muscorum*, and *Porcellio scaber*. The fifth species is *Porcellio laevis*, which is surprising since the species is extremely rare nowadays in Belgium as in the UK (Harding 2016). Apparently, this species was much more common in previous centuries (see Harding 2016).

During the second half of the 19<sup>th</sup> century, there was a slow increase in the number of publications and recorded species with nine species in 1870 and the first checklist for Belgium (Plateau 1870) (Fig. 1). From the mid 1880's until 1910 there was a first peak in woodlouse interest and publications, mostly because of work published by A. Preudhomme de Borre (1886b) and R.S. Bagnall (1907). Preudhomme de Borre (1886b) published a second checklist with 15 species (Fig. 1). In 1910, 21 species were recorded (Fig. 1).

From the 1910's to the 1970's, most woodlouse research in Belgium focused on caves (see e.g. all publications by Leruth in the 1930's and Kersmaekers in the 1970's). Capart (1942) produced a third checklist but excluded *Ligia oceanica*, since this species was seen as a marine species by some authors (see e.g., the comments by Pelseneer in 1886). At the time of Capart (1942), 24 species were recorded from Belgium (Fig. 1). In the mid





**Figure 1.** Cumulative number of species published as part of the native fauna of Belgium between 1831 (five species) and 2017 (36 species). Exotic species exclusively living in greenhouses were omitted.

1950's and 1960's, Ph. Polk (see e.g. Polk and Van Oye (1956)) undertook extensive field surveys and identifications and published the first distribution maps for eleven native species (Polk 1957). Despite the new observations, the maps were far from complete and only for a limited number of species. He published a fourth checklist (Polk 1959b) in 1959 but did not really add confirmed species to the list since he claimed *Haplophthalmus danicus* and *Metatrichoniscoides leydigii* as new species. Capart (1942) did not mention the first, as he did not cite the publication by Bagnall (1907) when the species was recorded for the first time in Belgium. *M. leydigii* could only be confirmed in 2015 (De Smedt et al. 2016a). Nevertheless, Ph. Polk made an important contribution to the knowledge of Belgian woodlice and compiled the first more extensive bibliography with 33 papers (Polk 1959b). Towards the end of the 20<sup>th</sup> century a lot of work was summarized under impulse of J.M. Tavernier and K. Wouters who published a fifth checklist, together with a bibliography (Tavernier and Wouters 1989), reporting 27 species that could be validated (Fig. 1). They produced a sixth (Tavernier and Wouters 1991) and seventh (Wouters et al. 2000) checklist but they did not add confirmed species. Wouters et al. (2000) produced an extensive bibliography of 81 papers. After Polk (1957), they were also the second authors to publish distribution maps and this for the 27 native species. However, the distribution data were mostly collected from literature, especially from the extensive but geographically limited field survey by Boon et al. (1993), and from the RBINS collection. Therefore, only few new observations were added, resulting in insufficient data to assess the status of occurrence of woodlice species in Belgium.

At the start of the 21<sup>st</sup> century, there was a renewed interest in woodlouse research with the discovery of four new species for Belgium by K. Lock (Fig. 1). In 2014, a national terrestrial isopod group “Spinicornis” was founded by the authors of this paper. They aimed to survey the entire territory of Belgium at 10 km × 10 km square resolution by 2020. This led to the discovery of four new species for the fauna of Belgium and finally the confirmation of *M. leydigii* (De Smedt et al. 2016a). This brings the current number of woodlouse species for Belgium at 36 (Fig. 1). Additionally the field surveys undertaken by Spinicornis since 2014 resulted in many new records for almost all Belgian woodlice species. This enables to assess the status and countrywide distribution of all Belgian species for the first time.

## Materials and methods

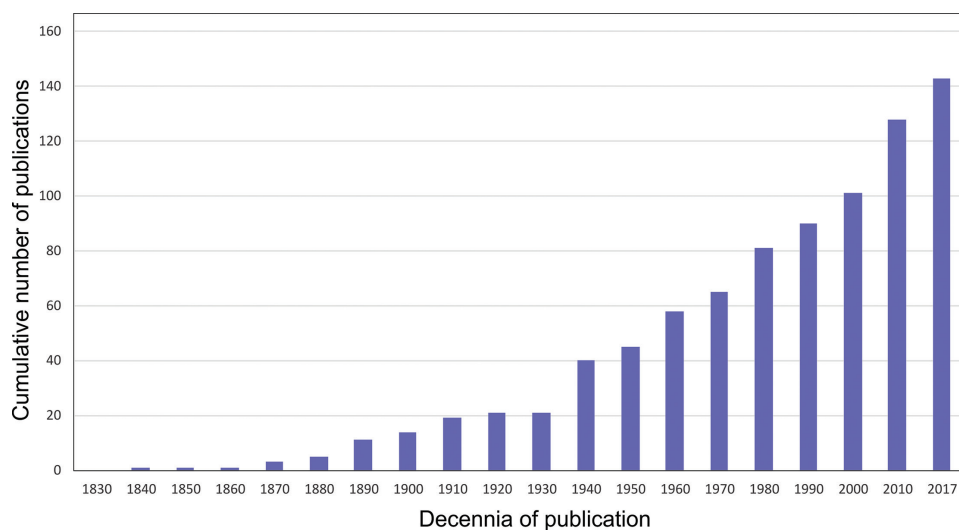
### Study area

Belgium is a rather small country (ca. 30.500 km<sup>2</sup>) in Western Europe, but despite its small size, the country shows a rich geology (Pirson et al. 2008). There is a small shoreline (approx. 65 km) and its territory penetrates up to 290 km inland. Along this gradient, the country changes from a largely flat Atlantic region in the north with Holocene and Pleistocene deposits, towards a more continental hilly landscape (up to 694 m elevation) in the east and south with much older (Mesozoic and Palaeozoic) deposits. This varied gradient across such a small country gives the opportunity for many species to establish. This is also the case for woodlice, finding coastal species (Kersmaekers 1988, Lock and Durwael 2000) up to alpine ones (De Smedt et al. 2016b) within this small country.

### Checklist

#### *Literature*

All existing literature published or accepted about Belgian woodlice was reviewed if containing distribution data, descriptions, and ecology up to the end of 2017. Our search was based on old bibliographies from Belgium (Polk 1959b, Wouters et al. 2000), all volumes from journals produced by the Royal Belgian Entomological Society (SRBE/KBVE) and through Web of Science and Google Scholar using the keywords [“Belgium” AND “Woodlice”/“Isopod(a)”]. The same searches were carried out for Dutch and French translations [“België” AND “Pissebed(den)”/“Isopod(a)”] and [“Belgique” AND “Cloporte(s)”/“Isopod(a)”]. Subsequently, all articles were scanned on the citing of Belgian woodlice species. Relevant MSc-theses were also included. The retrieved papers are the base for the checklist used to confirm species records by checking original descriptions. All used manuscripts are listed in the bibliography at the end of this paper. Our search resulted in 142 publications of any scientific significance published on Belgian woodlice from 1831 until 2017 (Fig. 2). There has been



**Figure 2.** Cumulative number of publications on Belgian woodlice from 1831 until 2017.

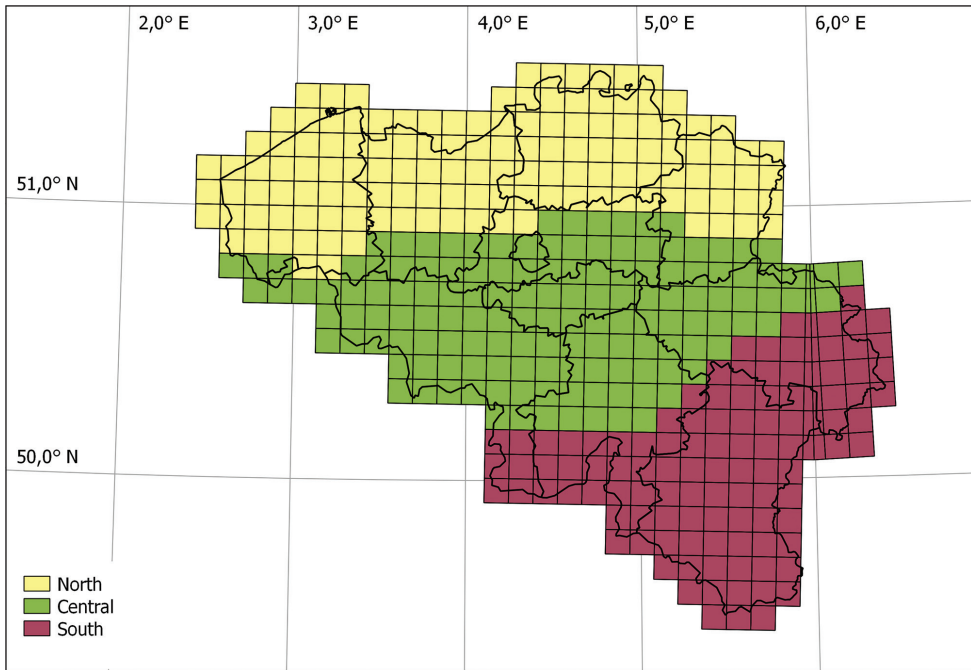
a steady increase in number of publications since 1830, with only a small dip around World War I. While the first literature records of Belgian woodlice were done in 1831, it increased to 14 publications by the beginning of the 20<sup>th</sup> century and to 101 at the beginning of the 21<sup>st</sup> century. Currently, there are 142 publications dealing with Belgian woodlice (Fig. 2).

### ***Museum collections***

All individuals of 18 species present in the collections of the RBINS were re-identified. *Armadillidium nasatum*, *A. opacum*, *A. pictum*, *A. pulchellum*, *Cylisticus convexus*, *Haplophthalmus danicus*, *H. mengii*, *Philoscia muscorum*, *Porcellium conspersum*, *Trachelipus rathkii*, *Trichoniscus pusillus*, *T. pygmaeus*, and *Trichoniscoides helveticus* were checked because these species can easily be misidentified or because closely resembling species were only discovered many years later. *Androniscus dentiger*, *Porcellio dilatatus*, *P. laevis*, *Porcellionides pruinosus*, and *Trichoniscoides albidus* were checked because only very limited knowledge is available for these species on both the historical and current distribution and ecology. Records labelled with *Armadillidium album* and *Trichoniscus provisorius* were not present in the collections. Records of *Ligia oceanica*, *Ligidium hypnorum*, *Oniscus asellus*, *Platyarthrus hoffmannseggii*, *Armadillidium vulgare*, *Porcellio scaber*, and *P. spinicornis* are widespread and easy to recognise therefore they are expected to be correct. This re-identification enabled us to check the presence of all species and to verify literature references.

### ***Field survey and status***

Field surveys were carried out over a four-year period from March 2014 until December 2017 by the authors together with other volunteers from “Spinicornis”. During



**Figure 3.** Map of Belgium with the UTM 10×10 km grid. The different colours indicate the different topographical regions used to determine the species status.

these surveys, firstly searches for all known Belgian species on locations of old records were carried out, as well as for species that could be expected to occur in Belgium based on their preferred habitat in neighbouring countries.

Secondly, systematic searches of squares of the UTM 10 × 10 km grid were carried out in order to map species distribution patterns across Belgium. Main woodlice biotopes were visited in every square. The biotopes visited were (1) an (ancient) forest, (2) a wetland/rivers edge or swamp forest and (3) synantropic habitat (e.g., public park, garden, graveyard...) if present in the 10 km square. These three biotopes cover the habitat niches of most woodlouse species. Additionally, 10 km squares containing a shoreline were surveyed for coastal habitats (e.g., dunes) as well. In some regions, old farms or old quarries have also been visited when present. Woodlice were hand collected by turning stones/dead wood and by sieving the litter layer.

By the end of 2017, the field survey campaign has not been completed but enough data has already been collected to assess the current status of occurrence for all species. In order to give a first indication of the distribution pattern this status is not only determined for the complete territory but also for three different topographical regions. The three regions are roughly based on the Belgian topography with lowlands in the north, hilly landscape in the centre and uplands (up to 694 m) in the south (Fig. 3).

Data of the field surveys were used to assess the status of all species but only observations made in sufficiently surveyed squares were withheld. The criterion for a square

**Table 1.** Number of squares of the UTM 10×10 km grid per region and number and percentage of squares surveyed between March 2014 and December 2017.

Region	squares in region	squares surveyed	
		#	%
North	127	89	70.1
Centre	140	97	69.3
South	114	68	59.6
Total	381	254	66.7

**Table 2.** Number of records per region and per three-month period, corresponding with the seasons of the year.

	North	Centre	South	Total
December – February (Winter)	525	617	174	1316
March – May (Spring)	472	395	164	1031
June – August (Summer)	237	469	428	1034
September – November (Autumn)	727	624	378	1729
Total	1961	2005	1144	5110

**Table 3.** Status categories for the Belgian woodlice, together with the lower and upper limits for the percentage of squares where a species was recorded between March 2014 and December 2017 in a certain region.

Status	No. of squares	Rel. no. of squares
Not present	0	0%
Very rare	1–5	< 1.3%
Rare	6–15	1.3–3.9%
Rather common	16–40	3.9–10.5%
Common	41–120	10.5–31.5%
Very common	> 120	> 31.5%

to be sufficiently surveyed was at least five species recorded in the square. In some parts of Belgium this is about the maximum number of species that can be found so a higher lower-limit would exclude well-searched squares in those parts of Belgium. Records from heated greenhouses and of specimens only identified to genus-level were removed from the dataset. The resulting dataset contains 5110 records from March 2014 until December 2017.

For every region, at least 59.6% of the squares have been sufficiently surveyed, with a total of 254 visited squares out of 381 (66.7%) (Table 1). The field surveys took place across the seasons in every region (Table 2).

The status was assessed based on the number of squares of the UTM 10×10 km grid in which the species was recorded compared to the number of squares that have been surveyed. Six different categories are distinguished from “not present” (0% of the squares) to “very common” (more than 31.5% of the squares) (Table 3).

## Results

### Checklist

Since 1831, seven checklists have been published about Belgian woodlice (Plateau 1870, Preudhomme de Borre 1886b, Capart 1942, Polk 1959b, Tavernier and Wouters 1989, 1991, Wouters et al. 2000). The last checklist includes 27 confirmed native species. This checklist adds nine species being *Hyloniscus riparius* (C. Koch, 1838), *Miktoniscus patiencei* Vandel, 1946, *Trichoniscoides sarsi* Patience, 1908, *Haplophthalmus montivagus* Verhoeff, 1941, *Porcellio monticola* Lereboullet, 1853, *Metatrachoniscoides leydigii* (Weber, 1880), *Trichoniscus alemannicus* Verhoeff, 1917, *Eluma caelata* (Miers, 1877), and *Philoscia affinis* Verhoeff, 1908. The new checklist below reports 36 species from 19 genera and nine families. Exotic species that were exclusively found in greenhouses are mentioned with an asterisk (\*) but are not counted as Belgian species. New species in bold.

#### Family Ligiidae

1. *Ligia oceanica* (Linnaeus, 1767)
2. *Ligidium hypnorum* (Cuvier, 1792)

#### Family Trichoniscidae

3. *Androniscus dentiger* Verhoeff, 1908
4. *Haplophthalmus danicus* Budde-Lund, 1880
5. *Haplophthalmus mengii* (Zaddach, 1844)
6. ***Haplophthalmus montivagus* Verhoeff, 1941**
7. ***Hyloniscus riparius* (C. Koch, 1838)**
8. ***Metatrachoniscoides leydigii* (Weber, 1880)**
9. ***Miktoniscus patiencei* Vandel, 1946**
10. *Trichoniscoides albidus* (Budde-Lund, 1880)
11. *Trichoniscoides helveticus* (Carl, 1908)
12. ***Trichoniscoides sarsi* Patience, 1908**
13. ***Trichoniscus alemannicus* Verhoeff, 1917**
14. *Trichoniscus provisorius* Racovitza, 1908
15. *Trichoniscus pusillus* Brandt, 1833
16. *Trichoniscus pygmaeus* Sars, 1898

#### Family Styloniscidae

- \* *Cordioniscus stebbingi* (Patience, 1907)

#### Family Oniscidae

17. *Oniscus asellus* Linnaeus, 1758

#### Family Philosciidae

18. ***Philoscia affinis* Verhoeff, 1908**
19. *Philoscia muscorum* (Scopoli, 1763)

#### Family Platyarthridae

20. *Platyarthrus hoffmannseggii* Brandt, 1833
- \* *Trichorhina tomentosa* (Budde-Lund, 1893)

## Family Armadillidiidae

21. *Armadillidium album* Dollfus, 1877
22. *Armadillidium nasatum* Budde-Lund, 1885
23. *Armadillidium opacum* (C. Koch, 1841)
24. *Armadillidium pictum* Brandt, 1833
25. *Armadillidium pulchellum* (Zencker, 1798)
26. *Armadillidium vulgare* (Latreille, 1804)
27. ***Eluma caelata* (Miers, 1877)**

## Family Armadillidae

- \* *Reductoniscus costulatus* Kesselyák, 1930

## Family Cylisticidae

28. *Cylisticus convexus* (De Geer, 1778)

## Family Porcellionidae

29. *Porcellio dilatatus* Brandt, 1833
30. *Porcellio laevis* Latreille, 1804
31. ***Porcellio monticola* Lereboullet, 1853**
32. *Porcellio scaber* Latreille, 1804
33. *Porcellio spinicornis* Say, 1818
34. *Porcellionides pruinosus* (Brandt, 1833)

## Family Trachelipodidae

- \* *Nagurus cristatus* (Dollfus, 1889)
- 35. *Porcellium conspersum* (C. Koch, 1841)
- 36. *Trachelipus rathkii* (Brandt, 1833)

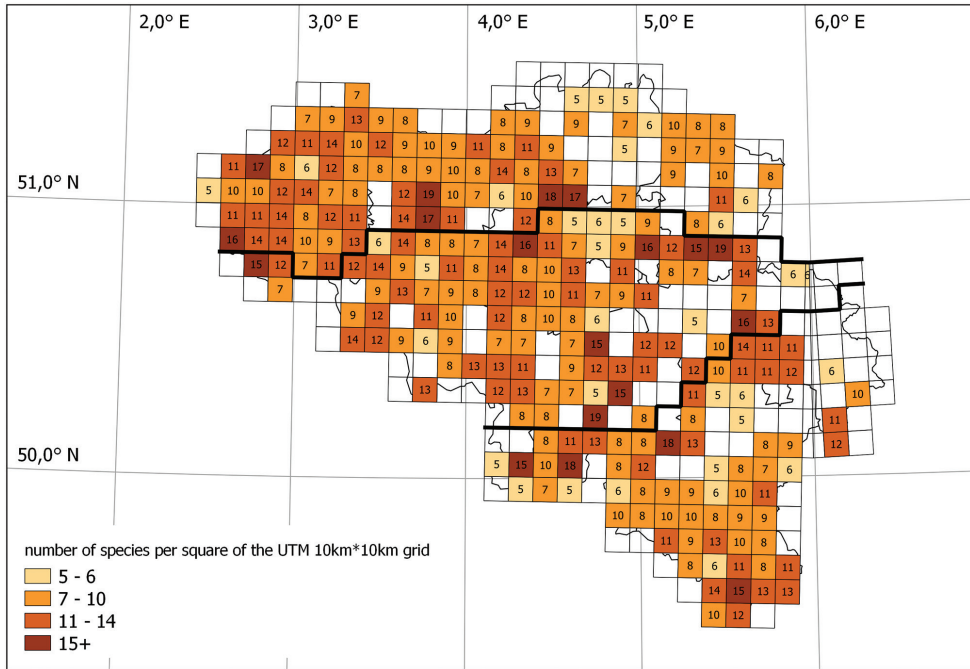
## Field survey and status

During the field surveys, 5110 records of woodlice in Belgium are collected between March 2014 and December 2017. For 35 of the 36 Belgian species there is at least one record in the database (Table 4). Only the species *Miktoniscus patiencei* was not found during the field surveys. The number of species per square of the UTM 10 × 10 km grid ranged between five and 19 (Fig. 4). At national level, one species was not recorded, six species are very rare, two are rare, three are rather common, fourteen are common and nine are very common (Table 4). Based on percentage occurrence in the number of visited UTM-squares (Table 3) the status of each species per region is given in the discussion.

## Discussion

Although many papers have been published on woodlice, many records remained doubtful and the reference collection at the RBINS contained a considerable number of identification errors. Additionally, the number of species recorded in Belgium was relatively low compared to neighbouring countries. This new checklist adds nine species to the last





**Figure 4.** Map of Belgium with the number of species per square of the UTM 10×10 km grid.

checklist published only 17 years ago (Wouters et al. 2000). In this section, the first record of all species with free-living populations in Belgium is discussed, their current status and a complete bibliography per species is given. The bibliography reports all papers mentioning the particular species. Papers in bold represent the first confirmed Belgian records. Papers in *italic* include information about the species ecology or distribution.

Certain exotic species are in Belgium only recorded from greenhouses and do not have free-living populations. These species are discussed in a recent paper dedicated to greenhouse species in Belgium (De Smedt et al. 2017a) and only briefly in a separate section of this discussion as they are not considered as part of the Belgian fauna.

## Order Isopoda

### Suborder Oniscidea

### Section Diplocheta

### Family Ligiidae

### Genus *LIGIA* Fabricius, 1798

#### 1. *Ligia oceanica* (Linnaeus, 1767)

Van Beneden (1861) first mentioned this species in 1861 as being abundant between stones where they reach the seawater. Since this is a strictly littoral species, certain authors

**Table 4.** Number of visited squares where a certain species is recorded and their relative occurrence per region and countrywide.

Species	North		Centre		South		Belgium	
	#	%	#	%	#	%	#	%
<i>Androniscus dentiger</i>	9	10.1	44	45.4	18	26.5	71	28.0
<i>Armadillidium album</i>	1	1.1	0	0.0	0	0.0	1	0.4
<i>Armadillidium nasatum</i>	27	30.3	46	47.4	31	45.6	104	40.9
<i>Armadillidium opacum</i>	0	0.0	7	7.2	22	32.4	29	11.4
<i>Armadillidium pictum</i>	0	0.0	11	11.3	20	29.4	31	12.2
<i>Armadillidium pulchellum</i>	2	2.2	13	13.4	12	17.6	27	10.6
<i>Armadillidium vulgare</i>	62	69.7	53	54.6	16	23.5	131	51.6
<i>Cylisticus convexus</i>	2	2.2	0	0.0	4	5.9	6	2.4
<i>Eluma caelata</i>	3	3.4	0	0.0	0	0.0	3	1.2
<i>Haplophthalmus danicus</i>	44	49.4	29	29.9	7	10.3	80	31.5
<i>Haplophthalmus mengii</i>	30	33.7	15	15.5	4	5.9	49	19.3
<i>Haplophthalmus montivagus</i>	1	1.1	34	35.1	37	54.4	72	28.3
<i>Hyloniscus riparius</i>	11	12.4	26	26.8	10	14.7	47	18.5
<i>Ligia oceanica</i>	2	2.2	0	0.0	0	0.0	2	0.8
<i>Ligidium hypnorum</i>	37	41.6	63	64.9	63	92.6	163	64.2
<i>Metatrachoniscoides leydigii</i>	9	10.1	2	2.1	0	0.0	11	4.3
<i>Miktoniscus patiencei</i>	0	0.0	0	0.0	0	0.0	0	0.0
<i>Oniscus asellus</i>	89	100.0	92	94.8	68	100.0	249	98.0
<i>Philoscia affinis</i>	3	3.4	34	35.1	10	14.7	47	18.5
<i>Philoscia muscorum</i>	87	97.8	90	92.8	62	91.2	239	94.1
<i>Platyarthrus hoffmannseggii</i>	38	42.7	26	26.8	18	26.5	82	32.3
<i>Porcellio dilatatus</i>	2	2.2	4	4.1	0	0.0	6	2.4
<i>Porcellio laevis</i>	0	0.0	1	1.0	0	0.0	1	0.4
<i>Porcellio monticola</i>	0	0.0	0	0.0	3	4.4	3	1.2
<i>Porcellio spinicornis</i>	88	98.9	93	95.9	65	95.6	246	96.9
<i>Porcellio scaber</i>	65	73.0	77	79.4	62	91.2	204	80.3
<i>Porcellionides pruinosus</i>	13	14.6	7	7.2	2	2.9	22	8.7
<i>Porcellium conspersum</i>	0	0.0	4	4.1	29	42.6	33	13.0
<i>Trachelipus rathkii</i>	37	41.6	29	29.9	8	11.8	74	29.1
<i>Trichoniscoides albidus</i>	40	44.9	20	20.6	0	0.0	60	23.6
<i>Trichoniscoides helveticus</i>	0	0.0	10	10.3	7	10.3	17	6.7
<i>Trichoniscoides sarsi</i>	32	36.0	15	15.5	0	0.0	47	18.5
<i>Trichoniscus alemannicus</i>	0	0.0	1	1.0	2	2.9	3	1.2
<i>Trichoniscus provisorius</i>	55	61.8	36	37.1	7	10.3	98	38.6
<i>Trichoniscus pusillus</i>	69	77.5	64	66.0	55	80.9	188	74.0
<i>Trichoniscus pygmaeus</i>	23	25.8	39	40.2	14	20.6	76	29.9

(e.g., Plateau (1870) and Capart (1942)) did not consider it as part of the terrestrial isopod fauna (see e.g., Pelseneer (1886) for a discussion about this). Nevertheless, it is nowadays fully considered as a terrestrial isopod because it can inhabit higher littoral zones and within this genus, there are a few species that are not bound to coastal conditions (Schmalfuss 2003).

**Status:** Coastal species, rare in the north of the country.

**Bibliography:** *Van Beneden (1861)*, *Bellynck (1865)*, *Pelseneer (1886)*, *Preudhomme de Borre (1886b)*, *Lameere (1895)*, *Maitland (1897)*, *Gilson (1900)*, *Bagnall (1907)*, *Lameere (1909, 1913, 1931, 1938)*, *Leloup and Miller (1940)*, *Gils (1947)*, *Holthuis (1950)*, *Kesteloot (1956)*, *Lefèvere et al. (1956)*, *Leloup and Konietzko (1956)*, *Polk and Van Oye (1956)*, *Leloup (1957)*, *Polk (1959a,b)*, *Leloup et al. (1963)*, *Polk (1963)*, *Lefèvere (1965)*, *Polk (1965)*, *Leloup and Polk (1967)*, *Daro (1969)*, *Jocqué and Van Damme (1971)*, *Polk (1976)*, *Van Gompel and Rabaut (1976)*, *Rappé (1977)*, *Eneman (1984)*, *Tavernier and Wouters (1986)*, *Rappé (1989a,b)*, *Tavernier and Wouters (1989, 1991)*, *Boon et al. (1993)*, *Mares (1994)*, *Lock and Durwael (2000)*, *Wouters et al. (2000)*, *Engledow et al. (2001)*, *Jonckheere and Van Rillaer (2001)*, *Huwaë and Rappé (2003)*, *Maelfait et al. (2004)*, *Vandepitte et al. (2010)*, *Segers (2015)*, *De Smedt et al. (2017b)*.

## Genus *LIGIDIUM* Brandt, 1833

### 2. *Ligidium hypnorum* (Cuvier, 1792)

Plateau (1870, 1873) was the first to mention the species from Belgium in the 1870's as *Ligidium persoonii* (Brandt) (Plateau 1870) and *Ligidium agile* (Plateau, 1873). Since this publication, the species has been mentioned in many papers.

**Status:** Very common across the country.

**Bibliography:** *Plateau (1870, 1873)*, *Pelseneer (1886)*, *Preudhomme de Borre (1886b)*, *Lameere (1895)*, *Maitland (1897)*, *Bagnall (1907)*, *Leruth (1937f)*, *Lameere (1938)*, *Leruth (1939)*, *Capart (1942)*, *Polk and Van Oye (1956)*, *Polk (1957)*, *Leloup and Van Meel (1958)*, *Polk (1959a,b)*, *Delhez and Kersmaekers (1973)*, *Kersmaekers and Deroeck (1973)*, *Kersmaekers (1973d)*, *Gysels et al. (1976)*, *Tavernier (1981)*, *Tavernier and Kerwyn (1982)*, *Tavernier and Wouters (1989, 1991)*, *Boon et al. (1993)*, *Branquart et al. (1995)*, *Delhez et al. (1999)*, *Devaere (1999)*, *De Bakker et al. (2000)*, *Scholten (2000)*, *Wouters et al. (2000)*, *Baeté et al. (2003a)*, *Vandekerckhove et al. (2003)*, *Baeté et al. (2004)*, *Dekoninck et al. (2005)*, *Baeté et al. (2006a,b)*, *Van De Vyver (2009)*, *Dethier and Hubart (2010)*, *Segers (2015)*, *De Smedt et al. (2016b,c)*, *Nijs et al. (2016)*, *Boeraeve et al. (2017)*, *De Smedt et al. 2018a,b*.

## Section Synocheta

### Superfamily Trichoniscoidea

### Family Trichoniscidae

### Genus *ANDRONISCUS* Verhoeff, 1908

### 3. *Androniscus dentiger* Verhoeff, 1908

Expected to occur in Belgium by Preudhomme de Borre (1886b), but mentioned as *Trichoniscus roseus*. First recorded by Lameere (1897) near Thon-Samson (Namur). Vandel (1933) is the first author to mention the name *A. dentiger*. In the following years, both names are

used by different authors. Capart (1942) is the first one to mention both species on his checklist, but indicates that the record of *Trichoniscus roseus* by Lameere (1897) is doubtful and could be *A. dentiger*. Finally, Polk (1957) indicates that the species identified as *T. roseus* is probably *A. dentiger*, and removes *T. roseus* from his checklist. *A. dentiger* specimens from the RBINS were re-identified and all specimens belonged to *A. dentiger* of which the oldest ones dated back to 1916 from Jemelle (Namur) and Schaerbeek (Brussels).

**Status:** Very common in the centre of the country, common in the south and rather common in the north.

**Bibliography:** Moniez (1886), Preudhomme de Borre (1886b), **Lameere (1897)**, Maitland (1897), *Bagnall (1907, 1908)*, *Vandel (1933)*, *Leruth (1936a,b,c,d,e, 1937b,d,f)*, *Lameere (1938)*, *Leruth (1939)*, Capart (1942), *Polk and Van Oye (1956)*, *Polk (1957, 1959a,b)*, *Delhez and Houssa (1969)*, *Delhez et al. (1973)*, *Delhez and Kersmaekers (1973)*, *Gilson and Hubart (1973)*, *Kersmaekers and Deroeck (1973)*, *Holthuis (1983)*, *Tavernier and Wouters (1989, 1991)*, *Boon et al. (1993)*, *Delhez et al. (1999)*, *Wouters et al. (2000)*, *Dethier and Hubart (2010)*, *Segers (2015)*, *De Smedt et al. (2017a)*.

## Genus *HAPLOPHTHALMUS* Schöbl, 1860

### 4. *Haplophthalmus danicus* Budde-Lund, 1880

First mentioned from greenhouses by Bagnall (1907, 1908), but the species is not incorporated in the checklists from Lameere (1938) and Capart (1942). In 1956, Polk and Van Oye (1956) discovers the species in Ghent and claims the first record, despite citing Bagnall (1907, 1908). The species was discovered in a medieval basement in Brussels (Kersmaekers 1974), but it took until the 21<sup>st</sup> century for the first confirmed records from wild populations. *H. mengii* samples from the collections of RBINS were re-identified and the oldest samples of *H. danicus* dated back from 2002 (Ramioul, Liège) and 2004 (Cheratte, Liège). However, numerous observations after 2010 proved that the species is much more common than previously thought.

**Status:** Very common in the north and common to rather common in the rest of the country.

**Bibliography:** *Bagnall (1907, 1908)*, *Polk and Van Oye (1956)*, *Polk (1957, 1959a,b)*, *Kersmaekers (1974)*, *Tavernier and Wouters (1989, 1991)*, *Boon et al. (1993)*, *Wouters et al. (2000)*, *Lock (2007)*, *Segers (2015)*, *De Smedt et al. (2016c, 2017a)*.

### 5. *Haplophthalmus mengii* (Zaddach, 1844)

First mentioned by Maitland (1897) but unclear if the species was already recorded from Belgium or only from the Netherlands, therefore, the species is mentioned as new for the Belgian fauna by Bagnall (1907). He collected one specimen in a greenhouse in Antwerp. After investigating all museum specimens of *H. mengii*, a specimen collected in 1899 in Han-sur-Lesse (Namur) was discovered. The record consists of one male

and one female specimen and is the first record of the species in Belgium. Specimens belonging to *Haplophthalmus mengii/montivagus* were also present in the collections from 1897 and 1898 but it was impossible to identify the species.

**Status:** Very common in the north and common to rather common in the rest of the country.

**Bibliography:** MaiMaitland (1897), *Bagnall (1907)*, *Leruth (1937a,b,c,e,f, 1939)*, *Capart (1942)*, *Leleup (1948)*, *Polk and Van Oye (1956)*, *Leclercq (1957)*, *Polk (1957, 1959a,b)*, *Delhez et al. (1973)*, *Delhez and Kersmaekers (1973)*, *Kersmaekers and Deroeck (1973)*, *Tavernier and Wouters (1989, 1991)*, *Boon et al. (1993)*, *Branquart et al. (1995)*, *Delhez et al. (1999)*, *Wouters et al. (2000)*, *Lock (2007)*, *Dethier and Hubart (2010)*, *Segers (2015)*, *Nijs et al. (2016)*, *De Smedt et al. 2017a*.

## 6. *Haplophthalmus montivagus* Verhoeff, 1941

First reported record of the species was done by Lock (2007) in 2006. This species closely resembles *H. mengii* and has probably been overlooked for a long time. After checking specimens of *H. mengii* from the collection of the RBINS, *H. montivagus* appeared to be collected in 1998 (Comblain-au-Pont, Liège) and 2002 (Stoumont, Liège).

**Status:** Very common in the centre and south of the country, very rare in the north.

**Bibliography:** *Lock (2007)*, *Segers (2015)*, *De Smedt et al. (2016b)*.

## Genus *HYLONISCUS* Verhoeff, 1908

### 7. *Hyloniscus riparius* (C. Koch, 1838)

Discovered in Belgium through pitfall trap research in 1998 (Lock and Vanacker 1999). Recent observations indicated that the species is not rare in the country (Fig. 5c). All specimens of *Trichoniscus pusillus* s.l. from the RBINS were re-identified, because *H. riparius* could be easily confused with this species. However, no historical records from *H. riparius* could be discovered.

**Status:** Common across the country.

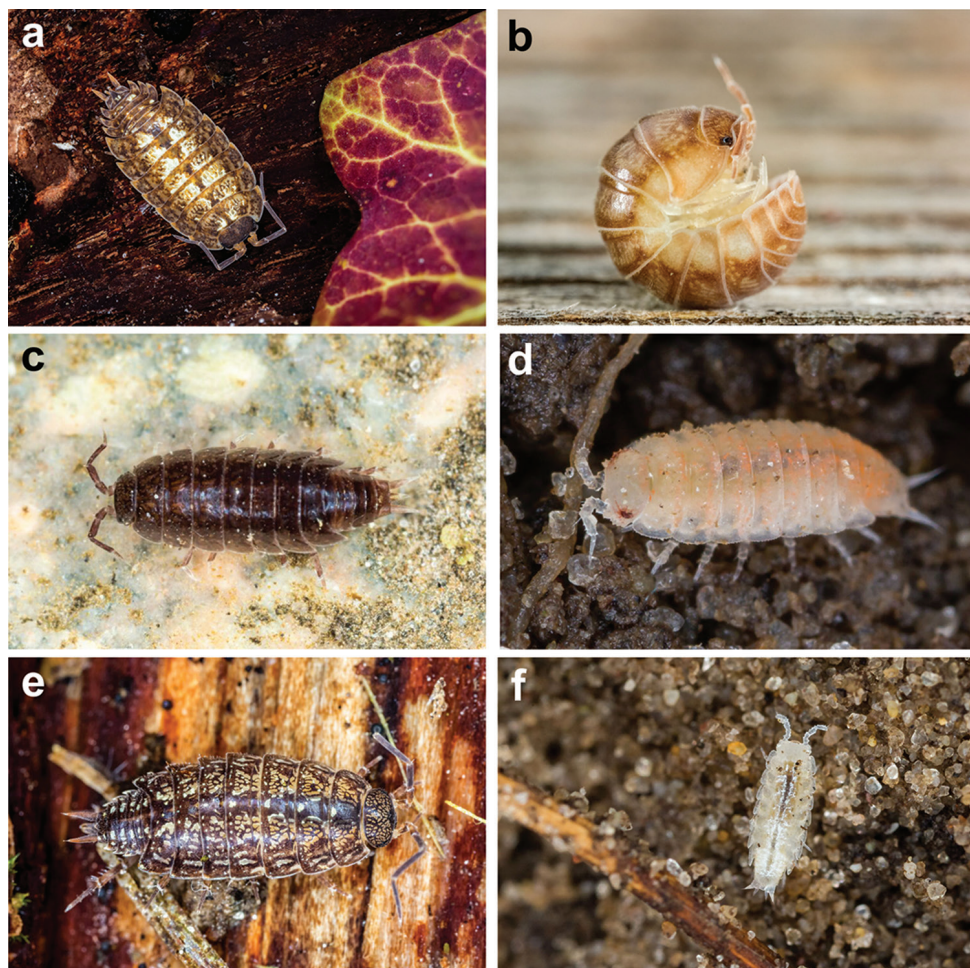
**Bibliography:** *Lock and Vanacker (1999)*, *Lock and Durwael (2000)*, *Lock (2001)*, *Huwaë and Rappé (2003)*, *Lock (2007)*, *Segers (2015)*.

## Genus *METATRICHONISCOIDES* Vandel, 1942

### 8. *Metatriconiscoides leydigii* (Weber, 1880)

Reported by Maitland (1897), but probably this is based on a record from the Netherlands. Polk and Van Oye (1956) found the first individuals of this genus, but the





**Figure 5.** Six of the nine species added to this new checklist, **a** *Porcellio monticola* **b** *Eluma caelata* **c** *Hylo-niscus riparius* **d** *Trichoniscoides sarsi* **e** *Philoscia affinis* and **f** *Metatrachoniscoides leydigii*; photos: Gert Arijs.

individuals were all females. Identification is only possible by checking male pleopods. Nevertheless, the species was mentioned on all subsequent checklists. A second observation was done in 2009, but it took until 2015 before the first males were observed and the species could be confirmed for the Belgian fauna (De Smedt et al. 2016a) (Fig. 5f).

**Status:** Rather common in the north of the country, very rare in the centre and absent from the south.

**Bibliography:** Maitland (1897), Polk and Van Oye (1956), Polk (1957, 1959a,b), Tavernier and Wouters (1989,1991), Boon et al. (1993), Wouters et al. (2000), Segers (2015), **De Smedt et al. (2016a).**

## Genus *MIKTONISCUS* Kesselyák, 1930

### 9. *Miktoniscus patiencei* Vandel, 1946

Only two sightings of this species are known in Belgium. After its discovery in 1999 (Lock and Durwael 2000) a second record was done by Lock (2001). In 2015–2016 searches at the same locations where the first two sightings were done could not re-discover the species. The area where the species was found strongly changed through restoration works. It is unclear if the species could be found on other locations in Belgium since the lack of suitable habitat (for details on habitat see Lock and Durwael 2000, Berg et al. 2008).

**Status:** Coastal species, not recorded during the recent field surveys.

**Bibliography:** *Lock and Durwael (2000)*, *Lock (2001, 2007)*, *Huwaé and Rappé (2003)*, *Segers (2015)*, *De Smedt et al. (2017b)*.

## Genus *TRICHONISCOIDES* Sars, 1898

### 10. *Trichoniscoides albidus* (Budde-Lund, 1880)

Expected to occur in Belgium by Preudhomme de Borre (1886b) and first recorded from Durbuy (Luxembourg) in 1933 by Leruth (1937f). In the collection of the RBINS a male from Rochefort (Namur) in 1929 was discovered, this is probably the first collected individual of this species in Belgium. Records of this species remained extremely scarce until the 21<sup>st</sup> century.

**Status:** Very common in the north of the country, common in the centre but absent from the south.

**Bibliography:** Preudhomme de Borre (1886b), *Leruth (1937f, 1939)*, Capart (1942), *Vandel (1952)*, *Polk and Van Oye (1956)*, *Leclercq (1957)*, *Polk (1957, 1959a,b)*, Tavernier and Wouters (1989, 1991), *Boon et al. (1993)*, *Delhez et al. (1999)*, *Wouters et al. (2000)*, *Segers (2015)*, *De Smedt et al. (2017b, 2018a,b)*.

### 11. *Trichoniscoides helveticus* (Carl, 1908)

First individuals identified by Vandel (1933) from Jemelle (Namur), but the exact date is unknown. Records of this species before 2010 are very scarce.

**Status:** Absent in the north, rather common in the rest of the country.

**Bibliography:** *Vandel (1933, 1952)*, *Polk and Van Oye (1956)*, *Polk (1957, 1959a,b)*, *Delhez and Kersmaekers (1973)*, *Kersmaekers (1973a)*, *Kersmaekers and Deroeck (1973)*, Tavernier and Wouters (1989, 1991), *Boon et al. (1993)*, *Delhez et al. (1999)*, *Wouters et al. (2000)*, *Lock (2001)*, *Segers (2015)*.



## 12. *Trichoniscoides sarsi* Patience, 1908

First recorded by Lock (2001) (Fig. 5d). Probably, this species had been overlooked for a long time because of its close resemblance to *T. helveticus*.

**Status:** Very common in the north of the country, common in the centre but absent from the south.

**Bibliography:** Lock and Durwael (2000), **Lock (2001, 2007)**, *Huwaé and Rappé (2003)*, Segers (2015), *De Smedt et al. (2017b)*.

## Genus TRICHONISCUS Brandt, 1833

## 13. *Trichoniscus alemannicus* Verhoeff, 1917

Discovered in 2015 (De Smedt et al. 2016b), but probably overlooked for a long time because of its close resemblance to *T. pusillus* and *T. provisorius*.

**Status:** Rare in the south of the country, very rare in the centre and absent from the north.

**Bibliography:** *De Smedt et al. (2016b)*.

## 14. *Trichoniscus provisorius* Racovitza, 1908

First recorded by Kersmaekers (1973c) as a subspecies of *T. pusillus*. Nowadays, no longer considered as a subspecies (Schmalfuss 2003) and can be distinguished from *T. pusillus* by the different shape of the male first pleopod (see e.g. Vandel 1960, De Smedt et al. 2016b). It was not mentioned on the checklists of Tavernier and Wouters (1989, 1991) and only as a subspecies by Wouters et al. (2000). Recordings of this species are extremely scarce in Belgium, since the species was considered a subspecies for a long time. Therefore, all specimens (945 individuals) of *Trichoniscus pusillus* s.l. present at the RBINS were re-identified of which 15 males and 930 females. All males belonged to *T. provisorius*. Interestingly, all male specimens were recorded after 1980. Vandel (1960) reports the species as being expansive and comparing the historical data with the recent surveys it can be assumed that the species is nowadays much more widespread. Historical data from the RBINS collections until 1970 recorded 0% of males across the country while this is 0.04% between 1970 and 2000 and about 1% after 2010.

**Status:** Very common in the north and centre of the country, common in the south.

**Bibliography:** *Kersmaekers (1973c)*, *Wouters et al. (2000)*, De Smedt et al. (2015), Segers (2015), *De Smedt et al. (2016b, 2018a,b)*.

### 15. *Trichoniscus pusillus* Brandt, 1833

First mentioned by Preudhomme de Borre (1886b), but later on the species appeared to be two species: *T. pusillus* and *T. provisorius*. Except for Kersmaekers (1973c), no author distinguished between the two species. For a sure identification the first male pleopod needs to be examined, but males are extremely rare (about 1.6%) of the population in *T. pusillus* (Vandel 1960). Therefore, identification of this species is often done based on the sex ratio of a large sample of the population (see Fussey 1984, De Smedt et al. 2016b). All *Trichoniscus pusillus* s.l. present in the RBINS collections were re-identified (see *Trichoniscus provisorius*). No males of *T. pusillus* were detected, but from three localities populations with more than 30 female individuals were recorded and no males were present. These are from Brussels in 1941 (166 ind.), Wanze (Liège) in 1979 (70 ind.) and from Ethe (Luxembourg) in 1981 (109 ind.).

The bibliography presented below should be considered as a bibliography for the species complex *T. alemannicus/pusillus/provisorius*, except for references from 2015 onwards.

**Status:** Very common across the country.

**Bibliography:** Pelseneer (1886), *Preudhomme de Borre (1886b)*, Lameere (1895, 1897), Maitland (1897), Bagnall (1907), Leruth (1937a,b,d,e,f, 1939), Capart (1942), Leleup (1948), Leloup et al. (1954), Polk and Van Oye (1956), Polk (1957), Leloup and Van Meel (1958), Polk (1959a,b), Delhez and Kersmaekers (1973), Kersmaekers and Deroeck (1973), Kersmaekers (1973c), Tavernier (1981), Tavernier and Kerwyn (1982), Holthuis (1983), Tavernier and Wouters (1989, 1991), Boon et al. (1993), Branquart et al. (1995), Delhez et al. (1999), Devaere (1999), Lock and Vanacker (1999), Lock and Durwael (2000), Schollen (2000), Wouters et al. (2000), Lock (2001), Baeté et al. (2002, 2003a,b, 2004), Dekoninck et al. (2005), Baeté et al. (2006a), Loones et al. (2008), Dethier and Hubart (2010), De Smedt et al. (2015), Segers (2015), De Smedt et al. (2016b,c, 2017a,b, 2018a,b).

### 16. *Trichoniscus pygmaeus* Sars, 1898

Bagnall (1907) recorded the first specimens in greenhouses of the Botanical Gardens in Antwerp (Antwerp) and Brussels. A year later, the same author reported free-living populations in Brussels (Bagnall 1908).

**Status:** Very common in the centre of the country, common in the north and the south.

**Bibliography:** *Bagnall (1907, 1908)*, Vandel (1933), Capart (1942), Polk and Van Oye (1956), Polk (1957, 1959a,b), Kersmaekers (1973c), Kersmaekers and Deroeck (1973), Tavernier and Wouters (1989,1991), Boon et al. (1993), Delhez et al. (1999), Wouters et al. (2000), Segers (2015), De Smedt et al. (2016b, 2017a,b).

**Section Crinocheta****Superfamily Oniscoidea****Family Oniscidae****Genus *ONISCUS* Linnaeus, 1758****17. *Oniscus asellus* Linnaeus, 1758**

One of the first five species mentioned for the fauna of Belgium by Carlier (1831). From Bellynck (1865) until Preudhomme de Borre (1886b) referred to as *Oniscus murarius* (Cuvier). No less than 61 publications deal with this species, making it the third most cited species in Belgian literature references.

**Status:** Very common across the country.

**Bibliography:** *Carlier (1831)*, *Bellynck (1865)*, *Plateau (1870)*, *Pelseneer (1886)*, *Plateau (1886)*, *Preudhomme de Borre (1886b)*, *Lameere (1895, 1897)*, *Maitland (1897)*, *Schouteden (1901)*, *Bagnall (1907)*, *Leruth (1937f)*, *Lameere (1938)*, *Leruth (1939)*, *Capart (1942)*, *Leleup (1948)*, *Leloup et al. (1954)*, *Polk and Van Oye (1956)*, *Leclercq (1957)*, *Polk (1957, 1959a,b)*, *Kersmaekers and Deroeck (1973)*, *Kersmaekers (1973c)*, *Gysels et al. (1976)*, *Tavernier (1981)*, *Tavernier and Kerwyn (1982)*, *Holthuis (1983)*, *Tavernier and Wouters (1989, 1991)*, *Boon et al. (1993)*, *Branquart et al. (1995)*, *Lambrechts (1997)*, *Delhez et al. (1999)*, *Devaere (1999)*, *Boon and Wijns (2000)*, *De Bakker et al. (2000)*, *Schollen (2000)*, *Wouters et al. (2000)*, *Baeté et al. (2002, 2003a,b)*, *Huwaé and Rappé (2003)*, *Hendrickx et al. (2003)*, *Vandekerckhove et al. (2003)*, *Baeté et al. (2004)*, *Dekoninck et al. (2005)*, *Baeté et al. (2006a,b)*, *Loones et al. (2008)*, *Van De Vyver (2009)*, *Dethier and Hubart (2010)*, *Segers (2015)*, *De Smedt et al. (2016b,c)*, *Nijs et al. (2016)*, *Boeraeve et al. (2017)*, *De Smedt et al. (2017a,b, 2018a,b)*.

**Family Philosciidae****Genus *PHILOSCIA* Latreille, 1804****18. *Philoscia affinis* Verhoeff, 1908**

Expected to occur in Belgium by De Smedt et al. (2015) and shortly afterwards discovered in 2014 (Boeraeve et al. 2017) (Fig. 5e). Boeraeve et al. (2017) checked all individuals present in the collection of the RBINS and discovered that the species was already collected in Belgium in 1938 but misidentified as *P. muscorum*. In total, they discovered eight historic records. The species proved to be widespread in Belgium and was recorded in eight out of ten provinces after 2014.

**Status:** Very common in the centre of the country, common in the south and rare in the north.

**Bibliography:** *De Smedt et al. (2015)*, *Segers (2015)*, *Boeraeve et al. (2017)*.

## 19. *Philoscia muscorum* (Scopoli, 1763)

One of the five first species mentioned for the fauna of Belgium by Carlier (1831) as *Philoscia sylvestris* (Latr.). This is the second most cited species in Belgian woodlouse literature with 63 publications mentioning the species.

**Status:** Very common across the country.

**Bibliography:** *Carlier (1831)*, Bellynck (1865), Plateau (1870, 1873), Pelse-neer (1886), Plateau (1886), *Preudhomme de Borre (1886b)*, *Lameere (1895, 1897)*, Maitland (1897), *Schouteden (1901)*, Bagnall (1907), *Leruth (1937f)*, *Lameere (1938)*, *Leruth (1939)*, Capart (1942), *Leleup (1948)*, *Leloup et al. (1954)*, *Leloup and Konietzko (1956)*, *Polk and Van Oye (1956)*, *Polk (1957, 1959a,b)*, *Dumont and Gysels (1971)*, *Kersmaekers and Deroeck (1973)*, *Kersmaekers (1973c)*, *Gysels et al. (1976)*, *Tavernier (1981)*, *Tavernier and Kerwyn (1982)*, *Holthuis (1983)*, *Tavernier and Wouters (1989, 1991)*, *Boon et al. (1993)*, *Branquart et al. (1995)*, *Lambrechts (1997)*, *Devaere (1999)*, *De Bakker et al. (2000)*, *Lock and Durvael (2000)*, *Schollen (2000)*, *Wouters et al. (2000)*, *Lock (2001)*, *Baeté et al. (2002, 2003a,b)*, *Huwaé and Rappé (2003)*, *Hendrickx et al. (2003)*, *Vandekerckhove et al. (2003)*, *Baeté et al. (2004)*, *Maelfait et al. (2004)*, *Dekoninck et al. (2005)*, *Baeté et al. (2006a)*, *Loones et al. (2008)*, *Van De Vyver (2009)*, *Dethier and Hubart (2010)*, *Segers (2015)*, *De Smedt et al. (2016b,c)*, *Nijs et al. (2016)*, *Boeraeve et al. (2017)*, *De Smedt et al. (2017a,b, 2018a,b)*.

## Family Platyarthridae

### Genus *PLATYARTHURUS* Brandt, 1833

## 20. *Platyarthrus hoffmannseggii* Brandt, 1833

First recorded by Mac Leod (1880), and appeared to be common but undersampled (*Lameere 1897*, *Adam and Leloup 1940*) because the unusual habitat (ant nests) for a woodlouse. This is the only myrmecophilous woodlouse species in Belgium.

**Status:** Very common in the north of the country, common in the centre and south.

**Bibliography:** *Mac Leod (1880)*, *Moniez (1886)*, *Pelseneer (1886)*, *Preudhomme de Borre (1886b)*, *Lameere (1895, 1897)*, *Maitland (1897)*, *Schouteden (1901)*, *Bagnall (1907)*, *Massart (1912)*, *Collart (1936)*, *Lameere (1938)*, *Adam and Leloup (1940)*, *Capart (1942)*, *Polk and Van Oye (1956)*, *Polk (1957, 1959a,b)*, *Kersmaekers and Deroeck (1973)*, *Tavernier (1981)*, *Tavernier and Wouters (1989, 1991)*, *Boon et al. (1993)*, *Wouters et al. (2000)*, *Dekoninck et al. (2007)*, *Segers (2015)*, *De Smedt et al. (2017a)*, *Parmentier et al. (2017)*.

**Superfamily Armadilloidea****Family Armadillidiidae****Genus *ARMADILLIDIUM* Brandt, 1833****21. *Armadillidium album* Dollfus, 1877**

Discovered by Kersmaekers (1988), which is the only published faunistical record so far, but it was also recorded during our field surveys. The species is both mentioned on the marine and brackish water isopod checklist (Rappé 1989a) as on terrestrial isopod checklists (Tavernier and Wouters 1989, 1991, Wouters et al. 2000), because its restriction to coastal habitat.

**Status:** Coastal species, very rare in the north of the country.

**Bibliography:** *Kersmaekers (1988)*, *Rappé (1989a)*, Tavernier and Wouters (1989, 1991), Boon et al. (1993), Lock and Durwael (2000), *Wouters et al. (2000)*, *Huwaé and Rappé (2003)*, *Maelfait et al. (2004)*, Hoffmann (2006), Segers (2015), De Smedt et al. (2017b).

**22. *Armadillidium nasatum* Budde-Lund, 1885**

Expected to occur in Belgium by Preudhomme de Borre (1886b) and first discovered by Bagnall (1907) in greenhouses in Brussels and Antwerp. In the collections of the RBINS records from 1941 and 1943 from the museum gardens and on a roadside verge are present, both anthropogenic environments. It took until 1972 before the first non-anthropogenic populations were discovered in the southern part of the country (Kersmaekers 1972).

**Status:** Very common in the centre and the south of the country, common in the north.

**Bibliography:** Preudhomme de Borre (1886b), Maitland (1897), *Bagnall (1907, 1908)*, *Capart (1942)*, *Polk and Van Oye (1956)*, *Polk (1957, 1959a,b)*, *Kersmaekers (1972)*, *Kersmaekers and Deroeck (1973)*, Tavernier and Wouters (1989, 1991), *Boon et al. (1993)*, *Wouters et al. (2000)*, *Huwaé and Rappé (2003)*, Segers (2015), *De Smedt et al. (2017a)*.

**23. *Armadillidium opacum* (C. Koch, 1841)**

First mentioned by Preudhomme de Borre (1886b) as *A. sulcatum*, but he corrected the identification later on to *A. opacum* (Preudhomme de Borre 1886a, Capart 1942). Nevertheless, *A. sulcatum* instead of *A. opacum* was reported on the checklist of Maitland (1897) and by Bagnall (1907). The oldest individuals that could be re-identified from the RBINS collections were collected by A. Capart in the 1940's.

**Status:** Very common in the south of the country, rather common in the centre and absent in the north.

**Bibliography:** Pelseneer (1886), *Preudhomme de Borre* (1886a,b), Maitland (1897), Bagnall (1907), Capart (1942), *Polk and Van Oye* (1956), *Polk* (1957, 1959a,b), *Gysels et al.* (1976), Tavernier and Wouters (1989, 1991), *Boon et al.* (1993), *Devaere* (1999), *Wouters et al.* (2000), *Vandekerckhove et al.* (2003), *Dekoninck et al.* (2005), Segers (2015), *Nijs et al.* (2016), *De Smedt et al.* (2018a,b).

#### 24. *Armadillidium pictum* Brandt, 1833

Mentioned for the first time for Belgium by Plateau (1870) but the species was re-identified by Preudhomme de Borre (1886) as being *A. pulchellum*. Additionally, Bagnall (1907) mentioned the species as occurring in Belgium but without any reference. Leruth (1937f) could therefore be the first one to record the species from Belgium. Belgian specimens from the RBINS were re-identified, which mostly originated from the surveys done by Capart (1942), and found both *A. pictum* and *A. pulchellum* in the samples. Both species are easily confused and historical records without preserved animals should be treated with caution.

**Status:** Common in the centre and south of the country, absent from the north.

**Bibliography:** Plateau (1870), Preudhomme de Borre (1886b), Bagnall (1907), *Leruth* (1937f, 1939), Capart (1942), *Polk and Van Oye* (1956), *Polk* (1957, 1959a,b), *Kersmaekers and Deroeck* (1973), *Gysels et al.* (1976), *Holthuis* (1983), Tavernier and Wouters (1989, 1991), *Boon et al.* (1993), *Wouters et al.* (2000), *Dekoninck et al.* (2005), *Dethier and Willems* (2005), Segers (2015), *De Smedt et al.* (2016b).

#### 25. *Armadillidium pulchellum* (Zencker, 1798)

Preudhomme de Borre (1886b) re-identified the specimens collected by Plateau (1870) and concluded that the species under consideration was *A. pulchellum* and not *A. pictum*. This is the first record of the species for Belgium. However, the species is easily confused with *A. pictum* (see section on *A. pictum* for additional information).

**Status:** Common in the centre and south of the country, and rare in the north.

**Bibliography:** *Preudhomme de Borre* (1886a,b), Pelseneer (1886), *Lameere* (1895, 1897), Maitland (1897), Bagnall (1907), Capart (1942), *Polk and Van Oye* (1956), *Polk* (1957, 1959a,b), *Gysels et al.* (1976), Tavernier and Wouters (1989, 1991), *Boon et al.* (1993), *Devaere* (1999), *De Bakker et al.* (2000), *Wouters et al.* (2000), *Vandekerckhove et al.* (2003), *Dekoninck et al.* (2005), Segers (2015)



## 26. *Armadillidium vulgare* (Latreille, 1804)

One of the five first species on the Belgian list (Carlier 1831), Carlier (1831) mentions two species (*Armadillo vulgaris* Latr. and *Armadillo variegatus* Latr.) that eventually proved to be the same species (Schmalfuss 2003). Belynck (1865) mentions *Armadillo triviale*, which also proves to be a synonym of *A. vulgare* (Schmalfuss 2003). Plateau (1870) reports both *Armadillidium vulgare* and *Armadillidium triviale*. Preudhomme de Borre (1886b) and Maitland (1897) mention *A. triviale* or *A. trivialis* as a subspecies of *A. vulgare*. This was also supported by Capart (1942). Afterwards, only *A. vulgare* has been mentioned in the Belgian literature. Interesting is the record by Troubleyn et al. (2009) from the remains of two woodlice, one unidentified woodlouse and the other one being *A. vulgare*, that were found in cesspits of an old prison at the main square of Malines dating back to the 14<sup>th</sup> century. This is the oldest record of a woodlouse in Belgium.

**Status:** Very common in the north and the centre of the country, common in the south.

**Bibliography:** *Carlier (1831)*, Belynck (1865), *Plateau (1870)*, Pelseneer (1886), *Plateau (1886)*, *Preudhomme de Borre (1886a,b)*, *Lameere (1895, 1897)*, Maitland (1897), *Schouteden (1901)*, Bagnall (1907), *Senden (1936)*, *Lameere (1938)*, Capart (1942), *Leleup (1948)*, *Leloup and Konietzko (1956)*, *Polk and Van Oye (1956)*, *Polk (1957, 1959a,b)*, *Kersmaekers and Deroeck (1973)*, *Gysels et al. (1976)*, *Tavernier (1981)*, *Tavernier and Wouters (1989, 1991)*, *Boon et al. (1993)*, *Branquart et al. (1995)*, *Lambrechts (1997)*, *Lock and Vanacker (1999)*, *Lock and Durwael (2000)*, *Schollen (2000)*, *Wouters et al. (2000)*, *Lock (2001)*, *Baeté et al. (2003a)*, *Huwaé and Rappé (2003)*, *Vandekerckhove et al. (2003)*, *Maelfait et al. (2004)*, *Troubleyn et al. (2009)*, *Van De Vyver (2009)*, *Dethier and Hubart (2010)*, Segers (2015), Nijs et al. (2016), *De Smedt et al. (2017a,b, 2018a,b)*.

## Genus *ELUMA* Budde-Lund, 1885

### 27. *Eluma caelata* (Miers, 1877)

Discovered for the first time in Belgium in 2016 (De Smedt et al. 2017b) (Fig. 5b). The species was expected to occur in Belgium since its discovery in the Netherlands close to the Belgian border (Lock and Durwael 2000), but it took more than 20 years since its first sighting in the Netherlands, to find the first Belgian specimens. It is still unclear if the species is truly native or naturalised in Belgium after colonisation from the Netherlands, where it could be accidentally introduced (De Smedt et al. 2017b). Berg et al. (2008) mentions the species from Belgium based on a reference of Lock in 2000, but this publication does not exist and can be classified as a typo.

**Status:** Rare in the north of the country, absent from the centre and the south.

**Bibliography:** Lock and Durwael (2000), *Huwaé and Rappé (2003)*, *De Smedt et al. (2017b)*, Boeraeve et al. (2017).



**Family Cylisticidae****Genus *CYLISTICUS* Schnitzler, 1853****28. *Cylisticus convexus* (De Geer, 1778)**

Expected to occur in Belgium by Preudhomme de Borre (1886b) and recorded for the first time in the same year by Moniez (1886). Records of this species have always been scarce with a peak during field research from Capart (1942); he collected specimens from at least six locations in the south of the country. Our recent observations indicate that the species is still scarce in the south of the country but was discovered as some isolated populations in the north as well.

**Status:** Rather common in the south of the country, rare in the north and absent from the centre.

**Bibliography:** Preudhomme de Borre (1886b), **Moniez (1886)**, Bagnall (1907), Leruth (1937f, 1939), Capart (1942), Polk and Van Oye (1956), Polk (1957, 1959a,b), Kersmaekers and Deroeck (1973), Tavernier and Wouters (1989,1991), Boon et al. (1993), Wouters et al. (2000), Segers (2015), De Smedt et al. (2017a).

**Family Porcellionidae****Genus *PORCELLIO* Latreille, 1804****29. *Porcellio dilatatus* Brandt, 1833**

First mentioned by Plateau (1870), but according to Preudhomme de Borre (1886b), the identifications by Plateau (1870) were not correct and appeared to be *P. scaber* (see also Plateau 1886). Therefore, Preudhomme de Borre (1886) does the first record in 1886. Re-identification of specimens from the RBINS dated back to 1898 from Charleroi (Hainaut). Sightings of the species are very rare and mostly associated to manmade structures like old horse and cow stables.

**Status:** Rather common in the centre of the country, rare in the north and absent from the south.

**Bibliography:** Plateau (1870), Pelseneer (1886), Plateau (1886), **Preudhomme de Borre (1886b)**, Lameere (1895), Maitland (1897), Bagnall (1907), Leruth (1937f, 1939), Capart (1942), Polk and Van Oye (1956), Polk (1957, 1959a,b), Kersmaekers and Deroeck (1973), Holthuis (1983), Tavernier and Wouters (1989, 1991), Boon et al. (1993), Delhez et al. (1999), Wouters et al. (2000), Segers (2015), De Smedt et al. (2017a).

**30. *Porcellio laevis* Latreille, 1804**

One of the first five species mentioned for the country by Carlier (1831). He mentions that the species could be found frequently under stones. Records from the 20<sup>th</sup> century are extremely rare and the only literature records are from Schouteden (1901), Polk

and Van Oye (1956) and Boon et al. (1993). In the collection of the RBINS some individuals collected in Belgium in 1916 in Leuven (Flemish-Brabant) and the 1940's in the Museum Garden (a zoo at that time) (Brussels) were found. Since 2000, the species has only been recorded from Wellen (Limburg) in 2015 in an old horse stable. However, despite an intensive search in 2017, after the buildings at the site were renovated, the species could not be rediscovered.

**Status:** Very rare in the centre, absent from the rest of the country.

**Bibliography:** *Carlier (1831)*, *Plateau (1870)*, *Pelseneer (1886)*, *Preudhomme de Borre (1886a,b)*, *Lameere (1895,1897)*, *Maitland (1897)*, *Schouteden (1901)*, *Bagnall (1907)*, *Capart (1942)*, *Polk and Van Oye (1956)*, *Polk (1957, 1959a,b)*, *Tavernier and Wouters (1989,1991)*, *Boon et al. (1993)*, *Wouters et al. (2000)*, *Segers (2015)*.

### 31. *Porcellio monticola* Lereboullet, 1853

Expected to occur in Belgium by Preudhomme de Borre (1886b) (mentioned as *Porcellio lugubris*), but only recently discovered in Belgium in 2014 (De Smedt et al. 2015) (Fig. 5a).

**Status:** Rather common in the south, absent from the rest of the country.

**Bibliography:** *Preudhomme de Borre (1886b)*, *De Smedt et al. (2015)*, *Segers (2015)*, *Boeraeve et al. (2017)*.

### 32. *Porcellio scaber* Latreille, 1804

One of the first five species mentioned for the fauna of Belgium by Carlier (1831). This species is mentioned in 64 publications on Belgian woodlice, making it the most cited species.

**Status:** Very common across the country.

**Bibliography:** *Carlier (1831)*, *Bellynck (1865)*, *Plateau (1870)*, *Pelseneer (1886)*, *Plateau (1886)*, *Preudhomme de Borre (1886b)*, *Lameere (1895, 1897)*, *Maitland (1897)*, *Schouteden (1901)*, *Bagnall (1907)*, *Senden (1936)*, *Leruth (1937f)*, *Lameere (1938)*, *Leruth (1939)*, *Capart (1942)*, *Leleup (1948)*, *Leloup et al. (1954)*, *Leloup and Konietzko (1956)*, *Polk and Van Oye (1956)*, *Polk (1957, 1959a,b)*, *Delhez and Kersmaekers (1973)*, *Kersmaekers and Deroeck (1973)*, *Gysels et al. (1976)*, *Tavernier (1981)*, *Holthuis (1983)*, *Tavernier and Wouters (1989, 1991)*, *Donker and Bogert (1991)*, *Donker (1992)*, *Boon et al. (1993)*, *Donker et al. (1993)*, *Branquart et al. (1995)*, *Lambrechts (1997)*, *Delhez et al. (1999)*, *Devaere (1999)*, *Lock and Vanacker (1999)*, *Lock and Durwael (2000)*, *Schollen (2000)*, *Wouters et al. (2000)*, *Lock (2001)*, *Baeté et al. (2003a)*, *Huwaé and Rappé (2003)*, *Hendrickx et al. (2003)*, *Vandekerckhove et al. (2003)*, *Baeté et al. (2004)*, *Maelfait et al. (2004)*, *Dekoninck et al. (2005)*, *Dethier and Willems (2005)*, *Baeté et al. (2006a)*, *Swiecicka and Mahillon (2006)*, *Loones et al. (2008)*, *Van De Vyver (2009)*, *Dethier and Hubart (2010)*, *De Smedt et al. (2015)*, *Segers (2015)*, *De Smedt et al. (2016b,c)*, *Nijs et al. (2016)*, *Boeraeve et al. (2017)*, *De Smedt et al. (2017a, b, 2018a, b)*.

### 33. *Porcellio spinicornis* Say, 1818

First mentioned by Bellynck (1865) with the French name “Porcellion peint”. The first checklist by Plateau (1870) refers to the publication of Bellynck (1865) as the only observation up to that date. Afterwards, almost exclusively recorded from anthropogenic habitats.

**Status:** Very common across the country.

**Bibliography:** *Bellynck (1865)*, *Plateau (1870)*, *Pelseneer (1886)*, *Preudhomme de Borre (1886b)*, *Lameere (1895)*, *Maitland (1897)*, *Bagnall (1907)*, *Leruth (1937f, 1939)*, *Capart (1942)*, *Polk and Van Oye (1956)*, *Polk (1957, 1959a,b)*, *Kersmaekers and Deroeck (1973)*, *Gysels et al. (1976)*, *Holthuis (1983)*, *Tavernier and Wouters (1989, 1991)*, *Tavernier and Wouters (1991)*, *Boon et al. (1993)*, *Wouters et al. (2000)*, *Dethier and Willems (2005)*, *Segers (2015)*, *De Smedt et al. (2015, 2017a)*.

## Genus *PORCELLIONIDES* Miers, 1877

### 34. *Porcellionides pruinosus* (Brandt, 1833)

First observations from the 1870's and first mentioned by Preudhomme de Borre (1886b). Observations are scattered and Boon et al. (1993) carried out the bulk of the observations during an intensive field survey. They found the species in most of the old stables and compost heaps they visited. The species is always associated with anthropogenic environments (compost heaps, graveyards, old stables...).

**Status:** Common in the north of the country, rather common in the centre, and rare in the south.

**Bibliography:** *Preudhomme de Borre (1886b)*, *Pelseneer (1886)*, *Lameere (1895, 1897)*, *Maitland (1897)*, *Schouteden (1901)*, *Bagnall (1907)*, *Capart (1942)*, *Polk and Van Oye (1956)*, *Polk (1957, 1959a,b)*, *Holthuis (1983)*, *Tavernier and Wouters (1989, 1991)*, *Boon et al. (1993)*, *Wouters et al. (2000)*, *Segers (2015)*, *De Smedt et al. (2017a)*.

## Family Trachelipodidae

## Genus *PORCELLIUM* Dahl, 1916

### 35. *Porcellium conspersum* (C. Koch, 1841)

First recorded by Capart (1942) in 1941 and confirmed based on individuals stored in the RBINS collections. Records remain very scarce until 2014, but targeted research shows that the species is more common than observed from the few records.

**Status:** Very common in the south of the country, rather common in the centre and absent from the north.

**Bibliography:** *Capart (1942)*, *Polk and Van Oye (1956)*, *Polk (1957, 1959a,b)*, *Kersmaekers (1974)*, *Tavernier and Wouters (1989, 1991)*, *Boon et al. (1993)*, *Branquart et al. (1995)*, *Wouters et al. (2000)*, *Dekoninck et al. (2005)*, *Segers (2015)*.

## Genus *TRACHELIPUS* Budde-Lund, 1908

### 36. *Trachelipus rathkii* (Brandt, 1833)

First mentioned by Preudhomme de Borre (1886b) as being common in the country. Since the species can easily be confused with e.g. different *Porcellio* species, all material present at the RBINS collections was re-examined (286 individuals from 78 records). However, no misidentifications could be detected. The oldest individuals were from Leuven (Flemish-Brabant) in 1916.

**Status:** Very common in the north of the country, common in the centre and in the south.

**Bibliography:** *Pelseneer (1886)*, *Preudhomme de Borre (1886b)*, *Lameere (1895)*, *Maitland (1897)*, *Bagnall (1907)*, *Capart (1942)*, *Leleup (1948)*, *Polk and Van Oye (1956)*, *Polk (1957, 1959a,b)*, *Kersmaekers and Deroeck (1973)*, *Tavernier (1981)*, *Tavernier and Kerwyn (1982)*, *Tavernier and Wouters (1989, 1991)*, *Boon et al. (1993)*, *Devaere (1999)*, *Lock and Vanacker (1999)*, *Wouters et al. (2000)*, *Huwaé and Rappé (2003)*, *Dekoninck et al. (2005)*, *Van De Vyver (2009)*, *De Smedt et al. (2015)*, *Segers (2015)*, *Nijs et al. (2016)*, *De Smedt et al. (2017b, 2018a,b)*.

## Deleted species

Six species were mentioned on at least one of the previous checklists, but are not present anymore on the current checklist. Most species appeared to be misidentifications or could not be confirmed because material was not preserved and literature citings are incomplete.

*Androniscus roseus* (C. Koch, 1838) was first mentioned by Lameere (1897), but after much of confusion between this species and *Androniscus dentiger* by different authors mentioning one of the two species, it became clear that only *A. dentiger* was recorded from Belgium (Polk 1957) (see *Androniscus dentiger* above).

*Armadillidium depressum* Brandt, 1833 was first mentioned by Tavernier and Wouters (1989). The species was apparently collected on a graveyard in the province of East-Flanders near Ninove. However, the species could not be verified and even if the identification is correct the species can be assumed as imported e.g. from Great Britain where the species is common in the south (Gregory 2009) and the species has no current free-living populations in Belgium. Extensive searches for woodlice on Belgian graveyards did not reveal the presence of the species. The species was included in the checklists from Tavernier and Wouters (Tavernier and Wouters 1989, 1991, Wout-

ers et al. 2000) and Huwaé and Rappé (2003) mentioned the species based on the same references. Baeté et al. (2003b) found the species in the nature reserve Walenbos (Flemish-Brabant), but later on, this appeared to be *A. opacum*. Finally, De Smedt et al. (2015) propose to remove the species from the Belgian list.

*Armadillidium sulcatum* Milne-Edwards, 1840 is a species from northern Algeria (Schmalfuss 2003) and was mentioned by Preudhomme de Borre (1886b) as found in Belgium. However, after re-identification this specimen proved to be *A. opacum* (Preudhomme de Borre 1886a, Capart 1942).

*Armadillidium triviale* Schöbl, 1861 mentioned by Bellynck (1865) and Plateau (1870) appeared to be *A. vulgare* (Preudhomme de Borre 1886b, Capart 1942). This species proved to be a synonym of *A. vulgare* (Schmalfuss 2003).

*Ligidium germanicum* Verhoeff, 1901 was mentioned by Gysels et al. (1976), but was not mentioned on the checklists of Tavernier and Wouters (1989, 1991). However, the species appears on the checklist of Wouters et al. (2000) and is cited by Schmalfuss (2003). Wouters et al. (2000) already mentions the species as doubtful since no material has been preserved. *Ligidium germanicum* was deleted from this new checklist because its presence could not be confirmed.

*Eoniscus simplicissimus* Arcangeli was a specimen collected by Leruth (1937) and described as a new species to science in a new genus and family by Arcangeli (1935). Verhoeff (1937) re-examined the individual and concluded that it was a larva of a species from the millipede genus *Polydesmus* (Polk and Van Oye 1956, Polk 1957).

## Species from greenhouses

Literature on Belgian woodlice in greenhouses is very limited. Only five papers deal with inventories carried out in Belgian greenhouses and they are all from the northern part of the country. Up to date only four exotic species could be confirmed in Belgian greenhouses. They cannot be considered as part of the Belgian woodlice fauna, because of the lack of wild populations, and are not included in this checklist as Belgian species. However, they were included in previous checklists (see e.g. Capart 1942, Polk 1959a, Wouters and Tavernier 1989, 1991, Wouters et al. 2000).

The first exotic species recorded from Belgian greenhouses is *Cordioniscus stebbingi* (Patience, 1907) by Bagnall in 1908 from a greenhouse in Brussels. Polk and Van Oye (1956) mention *Trichorhina tomentosa* (Budde-Lund, 1893) from Ghent. De Smedt et al. (2017a) mention *Nagurus cristatus* (Dollfus, 1889) and *Reductoniscus costulatus* Kesselyák, 1930 both from greenhouses in Ghent (East-Flanders) and the first species also from Meise (Flemish-Brabant). In addition, Polk and Van Oye (1956) mention an individual of the genus *Rhyscotus* Budde-Lund, 1885 and De Smedt et al. (2017a) mention an individual of the genus *Synarmadillo* Dollfus, 1892. However, both specimens were lost and could not be verified.

**Greenhouse literature:** Bagnall (1907, 1908), Polk and Van Oye (1956), Kersmaekers (1973b), De Smedt et al. (2017a).

## Species to be expected

Twenty-five percent of the Belgian woodlice species were added on this new checklist and all were discovered the last 20 years, therefore it is still possible that even more species can be discovered in Belgium. Below, some species recorded in neighbouring countries and relatively close to the Belgian border are listed:

- *Porcellio gallicus* Dollfus, 1904. This species is found to be abundant in small deciduous forest fragments in agricultural areas in the north of France (Landifay-et-Bertaignemont) only 45 km from the Belgian border (De Smedt et al. 2018b). Similar habitats are also present in the southern and central part of Belgium.
- *Porcellio montanus* Budde-Lund, 1885. Found in Germany (Wiesbaden) around 100 km from the Belgian border (edaphobase.org). Also reported from Grand Duchy of Luxembourg (Weber 2013) at only 18 km from the Belgian border. However, the latter record is not well documented. According to Grüner (1965) the species occurs in forest edges, under bark of trees and stone heaps. The species could therefore be expected in the south of Belgium.
- *Androniscus roseus* (C. Koch, 1838). A species closely resembling *A. dentiger* and recorded about 160 km from the Belgian border in Frankfurt (Germany) (edaphobase.org). There the species is reported from riparian habitat and forest fringe communities. The species could be overlooked since its close resemblance to *A. dentiger* and can be expected in the eastern part of the country.
- *Trachelipus ratzeburgi* (Brandt, 1833). Another species that could be overlooked in Belgium because of its close resemblance to *T. rathkii*. The species occurs in all kinds of woodland and the closest records are from Herborn in Germany at 140 km from the Belgian border (edaphobase.org). Therefore, the species could be expected in the east of the country.
- *Chaetophiloscia cellaria* (Dollfus, 1884). This species has recently been discovered in northern France at three localities of which two at 35 km from the Belgian border (Delasalle and Séchet 2014). The species was recorded in association with anthropogenic environments, like cemeteries. Therefore it is very likely that the species can also be found in similar habitat in Belgium.

Three of the last five new species on the Belgian list are large to medium-sized and therefore it is possible that the above-mentioned species are present and awaiting discovery.

## Conclusions

With 36 species Belgium now has a comparable amount of species, relative to its size, to neighbouring countries like the Netherlands (33 species see Berg et al. (2008) and Berg and Krediet (2017)), Great Britain (41 species see Gregory (2009) and Segers et



al. (2017)) and Germany (about 50 species see edaphobase.org). France (218 species including greenhouse species see Séchet and Noël (2015)) has far more species but this is due to the additional southern species and many endemics. Despite the large amount of published papers, Belgium lagged behind in number of species recorded, probably because of the lack of an interest group, as exists for Great Britain and the Netherlands. Belgium has caught up with its neighbouring countries, although there are still some species that may be present in Belgium. Future field surveys should fill the last “blank spots” in the distribution maps and will form the base of a first distribution atlas of woodlice in Belgium. This will be a valuable way forward to understand the ecology and habitat-preference of many Western European woodlouse species.

## Acknowledgements

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# Terrestrial isopods (Oniscidea) of the White Carpathians (Czech Republic and Slovakia)

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## Abstract

This paper summarizes data regarding the terrestrial isopods of the White Carpathians range in the Western Outer Carpathians, based on field research undertaken during the past several decades in natural meadow, pasture, and forest localities. Using a combination of four collection methods, 19 species belonging to nine families were recorded. The most common representatives were *Protracheoniscus politus*, *Trachelipus rathkii*, and *Ligidium hypnorum*. In general, the biodiversity of isopod communities in the studied area was considerable, with half of the localities explored inhabited by six to ten species. The composition of the isopod assemblages was determined by the character of the biotope and its geographical location. Forest habitats were considerably richer in species than the meadow and pasture ecosystems. Some xerothermic localities in the Slovak part of the area were inhabited by *Trachelipus nodulosus* and *Orthometopon planum*, thermophilic species typically associated with warmer parts of Europe. Two relic species (*Hyloniscus mariae* and *Ligidium germanicum*) were confirmed for this area. Except for only one finding of *Porcellio scaber*, no other evidently introduced or synanthropic species were recorded. Based on the data analyzed, the high nature conservancy value of the given area is emphasised.

## Keywords

Czech Republic, Isopoda, Oniscidea, Slovakia, terrestrial isopods, Western Carpathians, White Carpathians

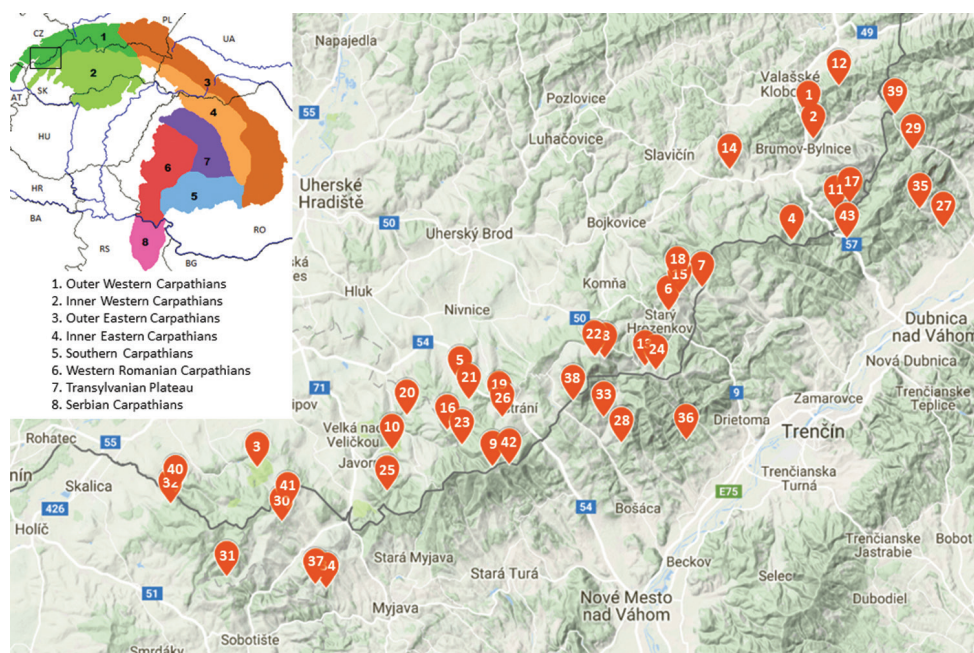
## Introduction

The Carpathian range measures approximately 1,500 km and covers ca. 203,000 km<sup>2</sup>. The entire Carpathian chain is usually divided into three major parts: the Western Carpathians (Austria, the Czech Republic, southwestern Poland, Slovakia and Hungary), the Eastern Carpathians (southeastern Poland, eastern Slovakia, Ukraine and Romania), and the Southern Carpathians (Romania and Serbia). The Western Carpathians comprise ca. 70,000 km<sup>2</sup> and are divided into the four geological zones: 1) an outer flysch zone; 2) a zone with isolated limestone outcrops; 3) a central zone with transformed and underground igneous rocks; 4) a zone with limestone sediments as well as an inner zone with overground igneous rocks. In the Czech Republic, only a part of the Outer Western Carpathians (Figure 1) is situated in the south-eastern Moravia, constituted from west to east by the South-Moravian Carpathians, Central Moravian Carpathians, Slovak-Moravian Carpathians, West-Beskidian Piedmont and, in part, the Western Beskids. Due to its geological and geographic development, this area is (with the exception of other parts of the Central European Hercynian Mountains) distinctive in its vegetation as well as faunal composition.

Research regarding isopod fauna in the Czech part of the Carpathians was initiated by Frankenberger (1941, 1942, 1944, 1954, 1959). He subsequently published data about several species from the Pálava Hills (South-Moravian Carpathians), Chřibý Hills (Central Moravian Carpathians), Vsetínské vrchy Hills, the surroundings of the town of Vizovice, the White Carpathians (all within the Slovak-Moravian Carpathians) and the Moravskoslezské Beskydy Mountains (Western Beskids). One of the most interesting findings was the record of *Hyloniscus mariae*, on the Solánec peak (located in the Vsetínské vrchy Hills), a Carpathian species that at the time was known only in Slovakia. Later, Frankenberger (1944) identified *Trachelipus difficilis* in the Beskydy Mountains (mentioned as *T. waechtleri*). Flasarová (1958) investigated isopod fauna in the Vsetínské vrchy Hills and the Chřibý Hills and announced 10 species, including the species *Hyloniscus mariae*. Spitzer et al. (2007), who investigated soil fauna in fir-beech forests of the Vsetínske vrchy Hills through the sole use of pitfall trapping, found four isopod species.

The White Carpathians are geographically located along the border between the Czech Republic and Slovakia, and constitute one of the westernmost parts of the entire mountain range, with a relatively high altitude that reaches above 900 m a.s.l. in the peaks. A large part of the territory of the White Carpathians on both the Czech and Slovak sides is designated a Protected Landscape Area (PLA).

In Slovakia, the isopod fauna of the White Carpathians has yet to be studied. Only in the 1990s, selected localities of importance to conservation in the Slovak part of the White Carpathians, were sampled for terrestrial isopods by †Pavel Deván. These were submitted to the first author of this contribution for study, but have not been elaborated. The Little Carpathians, which lie along the southern part of the White Carpathians but are orographically linked to the Inner Carpathians, were surveyed by Flasarová (1980, 1986) and Flasar and Flasarová (1989) via intensive sampling at more than 50



**Figure 1.** The zonation of Carpathians, with enlarged inset part of position of studied localities in CZ/SK White Carpathians. Source of the map of the Carpathian zones: <https://commons.wikimedia.org/wiki/File:Mapcarpat2.png> accessed 24.7.2018; the map with position of studied localities according to Google Maps.

localities. Flasarová recorded a total of 27 species from both natural and synanthropic habitats, and reported noteworthy species *Hyloniscus transsilvanicus* (Verhoeff, 1901) at a single locality in Slovakia, as well as *Armadillidium zenckeri* Brandt, 1833. Moreover, Kuracina and Kabátová (2005) investigated the locality Devínska Kobyla, which also belongs to the Little Carpathians. Unfortunately, their data regarding 12 species are rather dubious owing to their apparently inaccurate determination and the researchers' inability to verify missing material (A. Mock pers. comm.). Other research (Tuf and Tufová 2005) primarily targeted isopod communities in oak-hornbeam forests in this area. Štrichelová and Tuf (2012) recorded 10 species in the city of Bratislava and its surrounding whilst investigating localities belonging to the Little Carpathians (except for two urban ones). To date, 30 species have been recorded in the Little Carpathians.

The Czech part of the White Carpathians PLA was explored for terrestrial isopods by Tajovský (2008). He studied meadow and grassland habitats, focusing on the effects of grazing on soil biodiversity. He recorded 14 species, and demonstrated that intensive grazing had a negative impact on the abundance and species richness of soil fauna. Recently, the last author and his students explored predominantly forest localities in the White Carpathians within a series of faunistic inventories of soil fauna in protected areas, but this work has yet to be published.

In this paper, we summarize data from a wide spectrum of biotopes in both the Czech and Slovak parts of the White Carpathians, based on the published records and



elaboration of all available material regarding terrestrial isopods. Our results provide basic information about the isopod fauna of this part of the Western Carpathian, facilitating comparison with other areas of the Carpathian mountain range as a whole.

## Materials and methods

The target area, which is protected as the bilateral White Carpathians PLA in both the Czech Republic and Slovakia, is situated along the border of these countries. The Czech part is 70 km long, with a northeast-southwest orientation and an altitude ranging from 175 to 970 m a.s.l. The PLA was established in 1980 on a territory measuring 747 km<sup>2</sup>. Typical of the southern part is a vast complex of species-rich calcareous meadows with dispersed, solitary trees. The landscape in the central part of PLA was created between the 17<sup>th</sup> and 18<sup>th</sup> centuries during the Wallachian colonization. It is characterized by scattered houses, alternating forest and non-forest areas, with a mozaic of wetlands, small forests, shrubs and patches of grassland. The northeastern part is situated at a higher altitude and is mainly covered by old-growth beech forests (Mackovčín et al. 2002).

In the present contribution, we surveyed terrestrial isopod fauna in 26 localities representing different natural habitats of the White Carpathians in the Czech Republic between 2002 and 2009, as well as 17 localities with meadow and forest habitats in the Slovak part of the range (Figure 1). The investigations were undertaken during a series of research and monitoring projects with a large variety of methodological approaches. Here we only briefly summarize the four main methods used for the collection of isopods: 1) repeated individual sampling at favorable microsites; 2) pitfall trapping (different numbers of traps per locality, different time of exposition); 3) heat extraction of isopods from soil samples (usually 3–5 samples taken several times per year); 4) heat extraction of isopods from sieved litter sampled in selected (primarily forest) localities. The majority of the localities were intensively studied for one or two years. Most are under nature protection as National Nature Reserves (NNR), Nature Reserves (NR) and Nature Monuments (NM). Short descriptions of the sites are provided below and are distinguished into either Czech or Slovak subgroups. The localities are listed alphabetically. For more detailed characteristics, see Kuča et al. (1992), Mackovčín et al. (2002) and Jongepierová (2008).

Localities in the Czech part of the White Carpathians:

- 1 Bílé potoky NR – 49°06'56"N, 18°01'39"E, 380–500 m a.s.l., two meadow enclaves surrounded by mixed deciduous forests, 120 years old.
- 2 Brumov – 49°05'58"N, 18°01'59"E, 400 m a.s.l., meadow with traditional pasture management.
- 3 Čertoryje NNR – 48°51'31"N, 17°24'42"E, 350–445 m a.s.l., meadow (*Cirsio-Brachypodium pinnati*) with solitary oak and lime trees.
- 4 Chladný vrch NM – 49°01'31"N, 18°00'32"E, 550–575 m a.s.l., beech forest (*Carici pilosae-Fagetum*), 150–170 years old.



- 5 Drahy NR – 48°55'16"N, 17°38'16"E, 400–513 m a.s.l., meadow (*Cirsio-Brachypodium pinnati*).
- 6 Hrozenkovský lom – 48°58'24"N, 17°52'15"E, 500–520 m a.s.l., abandoned basalt quarry with mixture of grassland and forest vegetation.
- 7 Hutě NR – 48°59'26"N, 17°54'30"E, 450–535 m a.s.l., meadows and pastures (*Anthoxantho-Agrostietum*) with beech forest fragments.
- 8 Lopenické sedlo – 48°56'20"N, 17°48'00"E, 700 m a.s.l., pasture.
- 9 Javořina NNR – 48°51'34"N, 17°40'27"E, 835–970 m a.s.l., beech forest (*Dentario enneaphylli-Fagetum*, *Lunario-Aceretum*).
- 10 Jazevčí NNR – 48°52'18"N, 17°33'45"E, 340–473 m a.s.l., meadow (*Cirsio-Brachypodium pinnati*) and pasture.
- 11 Okrouhlá NR – 49°02'48"N, 18°03'27"E, 620–655 m a.s.l., mixed beech forest (predominantly *Fagus sylvatica*, as well as other deciduous tree species), 130 years old.
- 12 Ploštiny NR – 49°08'18"N, 18°03'40"E, 670–739 m a.s.l., meadow with dispersed trees (*Carpinus betulus*, *Juniperus communis*, *Fagus sylvatica*, *Abies alba*).
- 13 Pod Hribovňou NM – 48°55'58"N, 17°50'43"E, 550–640 m a.s.l., meadows and pastures (*Anthoxantho-Agrostietum*) with solitary trees.
- 14 Pod Vrchy NM – 49°04'37"N, 17°56'21"E, 330–370 m a.s.l., hornbeam forest (*Carici pilosae-Carpinetum*), 70 years old.
- 15 Pod Žitkovským vrchem NR – 48°59'11"N, 17°52'59"E, 480–620 m a.s.l., meadows and pastures (*Violion caninae*, *Calthion*) with forest fragments.
- 16 Porážky NNR – 48°53'08"N, 17°37'26"E, 540–610 m a.s.l., meadow (*Cirsio-Brachypodium pinnati*, *Angelico-Cirsietum oleracei*).
- 17 Sidonie NR – 49°03'09"N, 18°04'24"E, 425–560 m a.s.l., old and well-preserved beech forest with a predominance of *Fagus sylvatica*, 170 years old.
- 18 Skaličí – 48°59'40"N, 17°52'53"E, 600–630 m a.s.l., limestone block outcrop with forest growth (*Fagus sylvatica*).
- 19 Strání – 48°54'10"N, 17°40'55"E, 490–500 m a.s.l., intensively grazed pasture.
- 20 Trnovský mlýn – 48°53'47"N, 17°34'44"E, 450 m a.s.l., pasture.
- 21 Uvezené NM – 48°54'30"N, 17°38'53"E, 490–570 m a.s.l., hornbeam forest (*Carici pilosae-Carpinetum*).
- 22 U Zvonice NM – 48°56'23"N, 17°47'20"E, 630–670 m a.s.l., meadow (*Anthoxantho-Agrostietum*, *Filipendulenion*).
- 23 Vápenky NM – 48°52'31"N, 17°38'27"E, 470–570 m a.s.l., beech forest (*Carici pilosae-Fagetum*).
- 24 Ve Vlčí NR – 48°55'47"N, 17°51'24"E, 580–720 m a.s.l., pastures (*Anthoxantho-Agrostietum*) with forest fragments (*Fagus sylvatica*).
- 25 Výzkum – 48°50'27"N, 17°33'25"E 400–425 m a.s.l., meadow, an experimental area for the monitoring of successional development of herbaceous-rich grasslands in the area.
- 26 Záhumenice NM – 48°53'42"N, 17°41'09"E, 500 m a.s.l., mosaic of meadow habitats (*Calthion*, *Sparganio-Glycerion fluitantis*, *Cirsio-Brachypodium pinnati*).

## Localities in the Slovak part of the White Carpathians:

- 27 Babiná NM – 49°02'05"N, 18°10'40"E, 310–400 m a.s.l., xerothermic forest-steppe habitats on slopes with southwestern aspect.
- 28 Blažejová NM – 48°52'34"N, 17°49'07"E, 400–440 m a.s.l., typical meadows with orchids on western slopes with local springs.
- 29 Brezovská dolina NM – 49°05'28"N, 18°08'36"E, 440–470 m a.s.l., meadow locality with solitary trees, lime tufa and landslide springs.
- 30 Bučkova Jama NM – 48°49'07"N, 17°26'23"E, 480–550 m a.s.l., mosaic complex of preserved White Carpathian meadows and forests.
- 31 Chvojníca NM – 48°46'42"N, 17°22'42"E, 300–550 m a.s.l., narrow alluvium of the Chvojníca brook, in summer represented only by a set of puddles.
- 32 Fráterka – 48°49'55"N, 17°18'56"E, 375 m a.s.l., hornbeam forest (*Carici pilosae-Carpinetum*) near a hunting lodge of the same name at Skalica.
- 33 Grúň NM – 48°53'42"N, 17°47'56"E, 390–490 m a.s.l., mosaic of mesophilous and wet meadows with solitary trees.
- 34 Kožíkov vrch NM – 48°46'11"N, 17°29'21"E, 390–420 m a.s.l., old abandoned field, currently a mowed meadow.
- 35 Krivoklátska Tiesňava NM – 49°02'53"N, 18°09'05"E, 350–450 m a.s.l., limestone outcrops with beech and mixed forest growth.
- 36 Kurinov vrch NM – 48°52'43"N, 17°53'26"E, 425 m a.s.l., meadows on tufa terraces with characteristic vegetation, surrounded by forests.
- 37 Malejov NM – 48°46'19"N, 17°28'36"E, 420–430 m a.s.l., fragments of White Carpathian wet and dry meadows.
- 38 Mravcové NM – 48°54'26"N, 17°45'53"E, 475–500 m a.s.l., wet meadows with tufa and solitary trees.
- 39 Nebrová NR – 49°07'03"N, 18°07'27"E, 500–520 m a.s.l., alluvial alder growth (*Alnetum*) along small brooks.
- 40 Šmatlavé Uhlisko NR – 48°50'29"N, 17°19'14"E, 400 m a.s.l., hornbeam forest (*Carici pilosae-Carpinetum*)
- 41 Štefanová NM – 48°49'44"N, 17°26'44"E, 520–560 m a.s.l., herbaceous-rich meadows irregularly mowed.
- 42 Veľká Javorina NR – 48°51'39"N, 17°41'37"E, 860–870 m a.s.l., beech and maple forests (*Acero-Fagetum*, *Acero-Fagetum Lunarietosum*, *Fagetum pauper*) on the southeastern slopes.
- 43 Zábava – 49°01'35"N, 18°04'11"E, 280 m a.s.l., riparian vegetation along the Vlára Brook near Zábava-Horné Srnie village.

Given that the data were attained in different years using different methods, it was not possible to compare all parameters of isopod assemblages in detail. Therefore, this paper presents a general overview of the fauna of terrestrial isopods in the study area. For analysis of isopod assemblages according to their presence or absence, the programme CANOCO 5, unconstrained analysis, DCA (Lepš and Šmilauer 2014) was used.

## Results

In total, 19 species of terrestrial isopods belonging to nine families (see Appendix 1) were recorded for the whole area of the White Carpathians. For the region belonging to the Czech Republic, 16 species were found (Table 1), and in the Slovak region, 14 species were found (Table 2). Assemblages of isopods at individual localities consisted of one to ten species. The species with the highest frequency of occurrence within the whole study area was *Protracheoniscus politus* (22 and 12 localities in the Czech and Slovakian parts, respectively), *Trachelipus rathkii* (22 and 8 localities), *Ligidium hypnorum* (19 and 12 localities) and *Porcellium collicola* (13 and 10 localities). These species appear to be typical of the White Carpathians. Two species categorized as relic species (i.e., species that exclusively inhabit undisturbed, nature closest habitats with low level of human impact, cf. Tuf and Tufová 2008) were recorded: the Carpathian endemic *Hyloniscus mariae* at the locality Javořina, and *Ligidium germanicum* in eleven forest localities. In the Czech part, the community with the highest degree of species richness was found at the locality Pod Hřibovňou (locality 13 with 10 species), containing a mosaic of meadows, pastures and solitary trees. In addition, other species-rich communities, with nine isopod species, were recorded in the localities with a mosaic vegetation structure, Pod Žitkovským vrchem (locality 15) and Čertoryje (locality 3), or in the well-preserved beech forest of the Javořina (locality 9). In the Slovak part of the territory, the highest species richness with ten species was recorded in the meadow restored from an abandoned field at Kožíkov vrch (locality 34). High number of species was also found in the narrow alluvium of the Chvojníka brook and in the mosaic of mesophilous and wet meadows at Grůň (localities 31 and 33, both with eight species). Evidently, in the study area, well-preserved natural habitats support a relatively rich isopod fauna.

## Similarity of assemblages of the White Carpathians

Given that the analyzed data came from different studies, we compared the assemblages of terrestrial isopods at individual localities according to the presence or absence of the species only. Frequently occurring species were clustered in the first and second quadrat, primarily along the y-axis (Figure 2). A more isolated position was typical of relatively rare species with a small number of records, such as *Orthometopon planum*, *Cylisticus convexus*, *Trachelipus nodulosus*, and *H. mariae*.

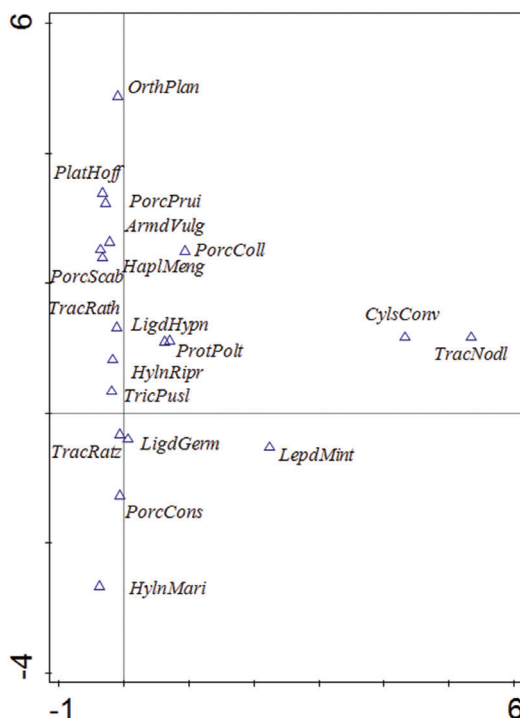
The dense clustering of localities (Figure 3) corresponds with relatively high rates of similarity of isopod assemblages in most of the studied meadow and forest sites. Nevertheless, a certain gradient from meadow to forest localities can be distinguished. Isopod assemblages in herbaceous-rich natural meadow localities (numbers 2, 3, 5, 8, 10, 19, 20, 25, 28) are isolated and situated in the upper part of the biplot. Their position corresponds with the species *O. planum*, *Platyarthrus hoffmannseggii* (obtained only by soil sampling) and *Porcellionides priuosus* or *Armadillidium vulgare* (Figure 2).

**Table 1.** Terrestrial isopods recorded in individual localities of the White Carpathians, in the Czech Republic, their presence (+), absence (–) and frequency of occurrence (F%). For numbers and description of localities, see Materials and methods.

Localities	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	Total	F(%)
<i>Ligidium germanicum</i>	–	–	–	+	–	–	+	–	+	–	+	–	+	+	+	+	+	+	–	–	–	–	–	+	–	–	11	42.3
<i>Ligidium hypnorum</i>	–	–	+	+	–	+	+	–	+	+	+	+	+	+	+	+	+	+	–	–	+	+	+	+	+	–	19	73.1
<i>Haplophthalmus mengii</i>	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	2	7.7
<i>Hyloniscus mariae</i>	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	3.8
<i>Hyloniscus riparius</i>	–	–	+	–	+	–	+	–	+	–	–	–	+	+	+	–	–	+	–	–	+	–	+	+	+	–	12	46.2
<i>Trichoniscus pusillus</i>	+	–	+	–	–	–	–	–	+	–	–	–	+	+	+	–	–	–	–	–	+	–	+	+	+	–	11	42.3
<i>Platyarthrus hoffmannseggii</i>	–	–	+	–	+	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	4	15.4
<i>Lepidoniscus minutus</i>	–	–	–	+	–	+	–	–	–	–	+	–	+	+	–	+	+	+	–	–	–	–	–	–	–	–	8	30.8
<i>Cylistiscus convexus</i>	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	3.8
<i>Orthometopon planum</i>	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0	–
<i>Protracheoniscus politus</i>	+	–	+	+	+	+	+	–	+	–	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	22	84.6
<i>Porcellium collicola</i>	–	+	+	–	+	+	+	+	–	+	–	–	+	–	+	–	–	–	–	+	+	+	–	+	–	–	13	50.0
<i>Porcellium conspersum</i>	–	–	–	–	–	–	–	–	+	–	–	–	+	–	+	+	–	–	–	–	–	–	–	–	–	–	4	15.4
<i>Trachelipus nodulosus</i>	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0	–
<i>Trachelipus rathkii</i>	+	+	+	+	+	–	+	–	+	+	+	+	+	+	+	+	+	+	+	–	–	+	+	+	+	+	22	84.6
<i>Trachelipus ratzeburgii</i>	+	–	–	+	–	–	+	–	+	–	+	+	+	+	+	–	+	+	–	–	+	–	–	–	–	–	14	53.8
<i>Porcellio scaber</i>	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0	–
<i>Porcellionides pruinosus</i>	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	2	7.7
<i>Armadillidium vulgare</i>	–	+	+	–	+	–	–	–	–	+	–	–	–	–	–	+	–	–	+	+	–	–	+	–	–	+	10	38.5
Total number of species	4	3	9	6	7	5	7	1	9	5	6	4	10	8	9	7	7	7	3	2	6	4	7	8	8	4		

**Table 2.** Terrestrial isopods recorded in individual localities of the White Carpathians, in Slovakia, with their presence (+), absence (–), and frequency of occurrence (F%). Numbers of localities, see Materials and methods.

Localities	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	Total	F(%)
<i>Ligidium germanicum</i>	–	–	+	–	–	–	+	–	–	–	–	+	–	–	–	–	–	3	17.6
<i>Ligidium hypnorum</i>	–	–	+	+	+	–	–	+	–	–	–	+	+	–	+	+	–	12	70.6
<i>Haplophthalmus mengii</i>	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0	–
<i>Hyloniscus mariae</i>	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0	–
<i>Hyloniscus riparius</i>	–	–	–	+	+	–	+	–	–	–	–	–	–	–	–	–	–	5	29.4
<i>Trichoniscus pusillus</i>	–	–	–	–	+	–	+	–	–	–	–	–	–	+	+	–	–	6	35.3
<i>Platyarthrus hoffmannseggii</i>	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0	–
<i>Lepidoniscus minutus</i>	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0	–
<i>Cylisiscus convexus</i>	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	5.9
<i>Orthometopon planum</i>	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	5.9
<i>Protracheoniscus politus</i>	–	+	+	+	+	–	+	+	–	–	–	+	–	+	+	–	–	12	70.6
<i>Porcellium collicola</i>	–	+	+	+	+	–	+	+	+	–	+	+	–	–	–	–	–	10	58.8
<i>Porcellium conspersum</i>	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0	–
<i>Trachelipus nodulosus</i>	+	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	2	11.8
<i>Trachelipus rathkii</i>	–	+	–	+	+	–	+	+	–	–	+	+	–	–	+	–	–	8	47.1
<i>Trachelipus ratzeburgii</i>	–	–	–	–	+	–	–	+	–	–	+	–	–	–	–	–	–	3	17.6
<i>Porcellio scaber</i>	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	1	5.9
<i>Porcellionides pruinosus</i>	–	+	–	–	–	–	+	+	–	–	–	–	–	–	–	–	–	3	17.6
<i>Armadillidium vulgare</i>	–	–	–	+	+	–	–	+	–	–	+	+	–	–	+	–	+	7	41.2
Number of species	2	6	4	6	8	1	8	10	2	1	6	6	1	2	6	2	3		

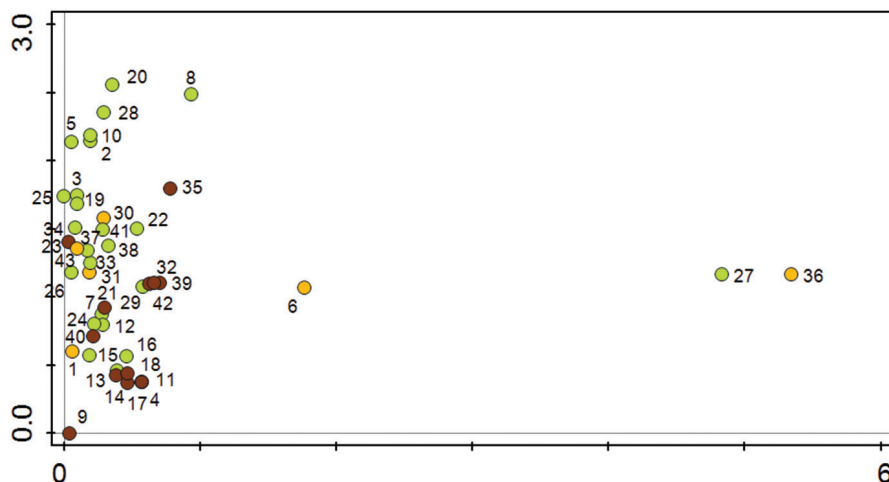


**Figure 2.** The ordination analysis of isopod species recorded at individual study localities in the White Carpathians (CANOCO 5, unconstrained analysis, DCA). For abbreviation of species' names, see Table 2.

Further down (closer to the intersection of the axes) are clustered many sites of forest and non-forest character; forest localities are generally situated in the lower half of the whole biplot as a whole. The analysis separated out several localities, which probably represent most xerothermic and open habitats. Locality 6, Hrozenkovský lom quarry, was partly separated to the right of the others owing to the presence of *Cylisticus convexus*. A distinct separation of two localities, xerothermic forest-steppe habitats at Babinná (locality 27) and meadow on tufa terraces at Kurinov vrch (locality 36), corresponds only with the finding of individual species of *T. nodulosus* (at both localities) and *C. convexus* (locality 27).

## Discussion

A total of 43 species of terrestrial isopods are currently known in the Czech Republic, hence our material pertaining to the Czech part of the White Carpathians represents 37 % of Czech fauna. Similarly, in the Slovak part of the White Carpathians, the 14 recorded species represent approximately 31 % of total known Slovak fauna (45 species). Given that in half of the localities, isopod communities were composed of six to 10



**Figure 3.** The ordination analysis (CANOCO 5, unconstrained analysis, DCA) of individual localities (1–43) in the White Carpathians according to present terrestrial isopod assemblages. For numbers of individual localities, see Materials and methods. Key: brown spots, forest localities; light green spots, meadows and pastures; yellow spots, localities of mixed meadows and woods.

species, we can consider the White Carpathians rich in woodlice fauna. The data from localities with only three or fewer species should be considered an underestimation due to the sampling method and effort. Additional surveys would certainly increase total numbers through other frequently occurring species.

It must be mentioned that our study summarizes data only from natural and not synanthropic habitats. In comparison with other areas heretofore explored in the Western Carpathians, this represents another rich area after the Little Carpathians (30 species, Flasar and Flasarová 1989, Flasarová 1980, 1986), Bükk Mts (24 species, Allspach 1996, Forró and Farkas 1998, Kontschán 2004), Aggtelek and Slovak Karst (both 20 species, Forró and Farkas 1998, Kontschán 2004, Vilisics et al. 2008, Frankenberger 1940, Flasarová 1994, 1998) and Pieniny (19 species, Hudáková and Mock 2006). The Little Carpathians constitute the neighboring area, and so we can expect more species to be present in the White Carpathians. Thus the White Carpathians, especially their Slovak part, deserve further attention. However, it should be acknowledged that the large number of species in the Little Carpathians can also be linked to the fact that Flasarová (1986) collected material both in natural and anthropogenic habitats, whereas in all other species-rich regions isopods were collected in more or less natural biotopes. The urban environment offers higher microhabitat diversity and favorable conditions for synanthropic species, illustrated by the fact that more species-rich communities can be found in the cities (Riedel et al. 2009). Given that the access to calcium represents an important factor influencing the distribution of terrestrial isopods (Sutton 1972), karstic regions are richer in species than others (Vilisics et al. 2008). In forest habitats,



the number of species at one locality usually varies from three to seven (Farkas et al. 1999, Tajovský 2002), hence the forest localities of the White Carpathians with species ranges of six to 10 are very rich in isopod fauna.

In the present study, we surveyed a relatively wide spectrum of biotopes in the White Carpathians. In total, we sampled a range of forest, meadow and pasture sites as well as sites with a mixture of habitats. Differences in isopod species composition were observed, including between forest biotopes, cultivated sites and pastures (e.g., Paoletti 1987). However, some species in Central Europe are eurytopic, very common and widely distributed. For example, *Trachelipus rathkii* is typical of disturbed and open habitats in the initial phases of succession on colliery heaps (Tajovský 2001), as well as being common in wetlands and the floodplain forests in Hungary (Farkas 1998) and the Czech Republic (Tajovský 1998), yet in other areas it can avoid forests (Schmidt 1997). In our study area, it was present in nearly all forest sites. It has been acknowledged that other species typical of forests (*Lepidoniscus minutus*, *Trachelipus ratzeburgii*, *Protracheoniscus politus*) rarely penetrate open habitats. This statement was confirmed in our study, with the exception of *P. politus*, which was found in almost all localities. Typical inhabitants of the White Carpathian meadows and pastures include *Armadillidium vulgare* and *T. rathkii*. Both are ubiquitous and able to colonise forest habitats. *Armadillidium vulgare*, a species introduced in numerous parts of the world by human activities, is often connected with stony habitats (Schmalfuss 2003) and is viewed as less common in forest stands (Allspach 1996). Given that it was only present in one forest locality, Vápenky (protected as a Nature Monument, albeit somewhat influenced by human activities), we consider the White Carpathian forest localities more or less undisturbed and thus of high conservation value. Species with high levels of affinity to woodlands with moist and shady sites (*Hyloniscus riparius*, *Trichoniscus pusillus*) can also be found in grasslands (Sutton 1968). Their occurrence in the meadows and pastures studied corresponds with wet patches typical of some White Carpathian grasslands (Mackovčín et al. 2002). We can conclude that despite being open ecosystems the meadows in highland areas support hygrophilous and forest species similar to those in forest sites (Tomescu et al. 2005).

From a zoogeographical point of view, European and Central European species predominated (Schmalfuss 2003). *Lepidoniscus minutus*, *P. politus*, *H. riparius*, *Porcellium collicola*, and *Ligidium germanicum* form a group that is distributed from Central Europe to the Balkan Peninsula. Their common occurrence may be explained by the fact that following the last glaciation, a significant proportion of contemporary Central European fauna migrated from the Balkans to the Carpathian Basin (Farkas 2007).

The meadows and pastures of the White Carpathians have in fact been formed and influenced by humans for numerous centuries (cf. Mackovčín et al. 2002). Nevertheless, a lack of introduced and synanthropic species reveals a weak influence on present-day isopod fauna. Only one species, *Porcellionides pruinosus*, which can be

considered introduced, was recorded. Nevertheless, its presence is faunistically interesting because the White Carpathians appear to represent the northernmost limit of its apparently original South European or Mediterranean distribution. Further north, this species is known only in synanthropic sites (Frankenberger 1959). The presence of the synanthropic species *Porcellio scaber* at the locality Kožíkov vrch can be related to this habitat, representing an old, abandoned field that had been transformed into a regularly mown meadow.

The record of the Carpathian endemic *Hyloniscus mariae* is very important. It was found at the Javořina National Nature Reserve, a locality with great biodiversity, predominantly including old and partly krummholz-like beech forest at the peak of the highest mountain. According to the current Red List of Threatened Species in the Czech Republic (Tajovský and Tuf 2017), this species is categorized as endangered. The presence of *Orthometopon planum* and *Trachelipus nodulosus* confirmed the spread and penetration of termophilous or xerothermic species to this area from the South.

When evaluating the (dis)similarity of communities of the White Carpathians, a northeast-southwest geographical as well as ecological gradient (meadow – pasture – forest) was observed. The analysis divided the localities into herbaceous-rich meadow sites and other meadows and forests with relatively rich isopod fauna, and distinguished several specific (and mostly xerothermic) sites (Figs 2 and 3). Indeed, the White Carpathians were deemed a valuable area due to their considerable biodiversity (Webster et al. 2001).

In conclusion, the recorded number of species, their distribution within meadows, pastures and forests, the occurrence of species-rich communities (especially in forest habitats), and the presence of the relic species, *Hyloniscus mariae* and *Ligidium germanicum*, together with the absence of introduced and ubiquitous species, indicate the high nature conservancy value of the whole area. The diversity of habitats in the White Carpathians presents a favorable environment for rich communities of terrestrial isopods in the Central European region.

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## Appendix I

### Systematic list of the species of terrestrial isopods recorded in the White Carpathians, the Czech Republic, and Slovakia

#### Suborder Oniscidea

##### Family Ligiidae

*Ligidium germanicum* Verhoeff, 1901

*Ligidium hypnorum* (Cuvier, 1792)

##### Family Trichoniscidae

*Haplophthalmus mengii* (Zaddach, 1844)

*Hyloniscus mariae* Verhoeff, 1908

*Hyloniscus riparius* (C. Koch, 1838)

*Trichoniscus pusillus* Brandt, 1833

Family Platyarthridae

*Platyarthrus hoffmannseggii* Brandt, 1833

Family Philosciidae

*Lepidoniscus minutus* (C. Koch, 1838)

Family Cylisticidae

*Cylisticus convexus* (De Geer, 1778)

Family Agnaridae

*Orthometopon planum* (Budde-Lund, 1885)

*Protracheoniscus politus* (C. Koch, 1841)

Family Trachelipodidae

*Porcellium collicola* (Verhoeff, 1907)

*Porcellium conspersum* (C. Koch, 1841)

*Trachelipus nodulosus* (C. Koch, 1838)

*Trachelipus rathkii* (Brandt, 1833)

*Trachelipus ratzeburgii* (Brandt, 1833)

Family Porcellionidae

*Porcellio scaber* Latreille, 1804

*Porcellionides pruinosus* (Brandt, 1833)

Family Armadillidiidae

*Armadillidium vulgare* (Latreille, 1804)





# Terrestrial isopods associated with shallow underground of forested scree slopes in the Western Carpathians (Slovakia)

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## Abstract

The shallow underground of forested scree slopes represents a little-studied subterranean biotope. In this paper, species diversity and depth distribution of terrestrial isopod communities studied in the depth profile (5–95 cm from the surface) of eight forested scree slopes in the Western Carpathians (Slovakia) is discussed. The southern edge of the Western Carpathians where the study sites are located represents the northernmost limit of distribution of obligate subterranean fauna in Europe. The sites differ from each other in type of bedrock, forest composition, slope aspect, and altitude. To sample isopods, a set of three subterranean pitfall traps consisting of a plastic cylinder (Ø 110 mm) and ten plastic cups was buried in each studied scree slope. In total, 252 isopods belonging to eleven species were sampled (1–5 species per site). Of the species found, the blind and depigmented *Mesoniscus graniger* was the sole species closely associated with deeper parts of the depth profile and was present in most of the sites studied. Another ten species were represented by a small number of individuals and their occurrence deeper in the scree slope profile was rather accidental. A comparison between winter and summer periods indicates apparent differences in seasonal activities of isopods. Ethylene glycol seems to be more appropriate fixative solution for trapping isopods than formaldehyde.

## Keywords

depth distribution, MSS, Oniscidea, shallow subterranean habitat, species diversity, subterranean traps

## Introduction

An extensive labyrinth of air-filled dark voids among the rocky fragments found inside the forested scree slopes represents a peculiar type of shallow subterranean habitat (Culver and Pipan 2014). The mesovoid shallow substratum, as this habitat is ordinarily referred to, lies immediately below the soil, ranges from a depth of several centimeters up to several dozen meters and in the karst areas, it is interconnected with caves and narrow cracks situated deep below the ground surface (Juberthie et al. 1980, Giachino and Vailati 2010, Mam-mola et al. 2016). The soil and the forest growing above ameliorate fluctuations in temperature and humidity throughout the depth profile of scree slope. Leaves falling from trees onto the scree slope surface during autumn represent a rich and easily accessible source of nutrients that are brought to the scree slope interior either passively by percolating rainwater or actively by the migration of soil macrofauna (Gers 1998, Pipan et al. 2010). Due to environmental conditions that are intermediate between stable caves and variable surface, the mesovoid shallow substratum is populated, beside common soil dwelling species of invertebrates, by rare subterranean species, most of which possess morphological adaptations towards life in constant darkness such as anophthalmia, depigmentation, and elongation of appendages (Sket 2008, Nitzu et al. 2014, Jiménez-Valverde et al. 2015).

Communities of terrestrial isopods dwelling shallowly underground in forested scree slopes have been explored minutely so far only in the Czech Republic (Tuf et al. 2008), Romania (Nitzu et al. 2010, 2011) and Spain (Jiménez-Valverde et al. 2015). Comparing the species richness of mesovoid shallow substratum with the adjacent subterranean habitats, these studies found that the number of isopod species tend to decline in the gradient between soil and cave and some isopod species, particularly troglaphiles, show high affinity to mesovoid shallow substratum.

Previous research in subterranean biology in the Western Carpathians has favored caves (Košel 2012, Kováč et al. 2014). Mock et al. (2015) and Rendoš et al. (2012, 2016a) conducted the initial thorough survey focused on diversity and depth distribution of subterranean invertebrate communities, including terrestrial isopods, at three model sites. In this paper, we summarize existing knowledge, both published and unpublished, of terrestrial isopod communities sampled intensely over the past years in the forested scree slopes along the southern edge of the Western Carpathians. Our aim was to (1) evaluate  $\alpha$  and  $\gamma$  diversity of terrestrial isopod communities inhabiting interior of forested scree slopes, and (2) to describe the depth distribution of particular species with a special emphasis on subterranean species. Some methodological aspects are also discussed.

## Materials and methods

### Study sites

Our study was carried out successively from November 2008 to January 2016 on 8 forested scree slopes situated in 5 geomorphological units of the Western Carpathians

(Slovakia). The studied scree slopes were predominately formed during the Pleistocene to Holocene by frost weathering and they differ in the type of bedrock (Figure 1 and Table 1), forest composition, structure of depth profile.

- 1 Scree slope in the Strmina Natural Reserve (Malé Karpaty Mountains) overgrown with beech forest (*Fagus*). This study site lies on acidic granitoid bedrock but in its immediate vicinity, there is a karst area (Borinka Karst) characterized by several karst formations such as caves and sinkholes. The scree slope profile has four distinct layers: litter and humus (0–5 cm), organo-mineral layer (5–20 cm), a layer consisting of mixture of mineralized soil and rocks (20–75 cm) and scree partially clogged with soil (75–110 cm).
- 2 Scree slope on basalt bedrock in the Belinské skaly National Nature Monument belonging to the Cerová vrchovina Highlands, an area known for the presence of a number of shallow pseudokarst caves. The scree slope profile is covered with a xerophilous oak-hornbeam forest (*Quercus-Carpinetum*) and its profile consists of three different layers: litter and humus (0–5 cm), organo-mineral layer (5–30 cm) and scree with spaces filled substantially with mineralized soils (30–110).
- 3 Limestone scree slope in the Drienok Valley (Revúcka vrchovina Highlands) situated a few meters below the entrance to the Špaňopolská Cave. The site is surrounded by beech-hornbeam forest (*Fago-Carpinetum*) and in the scree slope profile, there are four distinct layers: leaf litter and humus (0–5 cm), organo-mineral layer (5–25 cm), mixture of rocks and soil (25–70 cm) and scree partly filled with soil (70–110 cm).
- 4 Limestone scree directly at the entrance to the Malá drienčanská Cave without apparent stratification of the profile. The interior of the scree includes a mixture of humus, soil and rocky fragments up to a depth of 30 cm. Below this, large compact boulders predominate.
- 5 Scree slope within the collapse above the Veľká drienčanská Cave. The slope profile without evident stratification consists predominately of rocky fragments originating from the previously collapsed cave ceiling. The spaces among the large limestone boulders are slightly filled with the mixture of soil and humus. Both sites (4 and 5) are located approximately 10 km west of the Drienok Valley (site 3) and are overgrown with beech hornbeam forest (*Fago-Carpinetum*).
- 6 Limestone scree slope in the Slovak Karst National Park situated about 30 m west of the entrance to the Ardovská Cave. The slope is overgrown with dogwood-maple forest (*Corno-Carpinetum*). On the scree slope surface, there are several large boulders, most of them covered by bryophytes. Three clearly separated layers are recognizable inside the scree slope: a layer consisting of litter and humus (0–15 cm) is followed by organo-mineral layer with admixtures of tiny rocks (15–75 cm) and scree formed by large rock fragments (75–110 cm).
- 7 Limestone scree slope along the doline near the entrance to the ice cave Silická ladnica situated in the Slovak Karst National Park. The site is forested by linden-hornbeam, and maple (*Tilio-Aceretum* with *Carpinus betulus*) trees and the scree

slope profile is divided into three layers: leaf litter and humus (0–5 cm), thick organo-mineral layer (5–30 cm) and scree (30–110 cm).

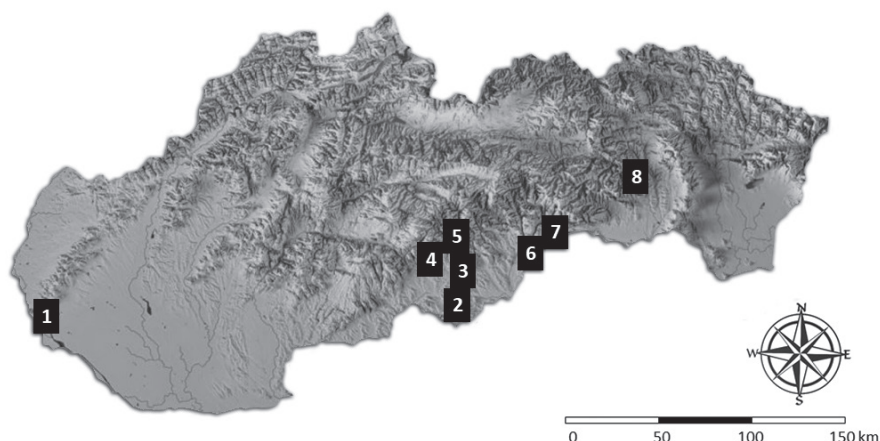
- 8 Limestone scree slope in the Sivec National Reserve (Čierna Hora Mountains) including a massive limestone cliff with several shallow caves. The vertical profile of the scree slope covered with linden-maple forest (*Tilio-Aceretum*) is composed of the following layers: leaf litter and humus (0–15 cm), organo-mineral layer (15–45 cm) clearly separated from scree (45–110).

## Sampling

To sample isopods, non-baited subterranean pitfall traps designed by Schlick-Steiner and Steiner (2000) and subsequently modified by Rendoš et al. (2016a) were used. Each trap consists of a plastic cylinder (length 110 cm, diameter 10.5 cm) circumferentially perforated with openings (Ø0.8 cm) in ten regular horizontal levels (5, 15, 25...95 cm). A removable set of ten plastic cups (volume 500 ml) filled with 4% formaldehyde or 50% ethylene glycol fixative solution is inserted into the plastic cylinder interior. The cups are placed right under the openings on the cylinder surface, enabling animals to be trapped at that particular level. At each studied scree slope, a triad of subterranean pitfall traps were placed 50 cm apart in a previously excavated pit over a meter deep. On the scree slope next to the Malá drienčanská Cave, and on the collapse above the Veľká drienčanská Cave (sites 4 and 5), we buried shorter (30 cm long) subterranean pitfall traps due to the presence of large boulders that prevented us from digging a deeper pit. Afterwards, the pit was backfilled to the maximum possible extent with the dugout soil and rocks in the original order of the layers and the cylinders were tightly closed by a plastic lid and covered by rocks and leaves found around the study site. To empty the traps and retrieve the sampled specimens, the set of plastic cups was pulled out of the cylinder; the content of each cup was poured into a plastic bottle and transported to the laboratory of soil biology (Institute of Biology and Ecology, PJ Šafárik University in Košice). The isopod material was later fixed in 75% ethyl alcohol and determined to the species level using several determination keys, such as Frankenberger (1959) and Radu (1985). Systematics and nomenclature of Isopoda species found follow the World Catalogue of Terrestrial Isopods by Schmalfuss (2003). The subterranean pitfall traps were emptied for the first time approximately a month after burial. This is, according to our previous experience, the period needed for regeneration of excavated soil layers and revival the locomotor activity of some sensitive species (Mock et al. 2015). Thereafter, the isopod sampling was timed so as to include at least one “cold” (winter) and one “warm” (summer) period of the year. The cold period refers to the months between October and April / May while warm period refers to the months between May and October. Sampling periods for each scree slope site studied are stated precisely in Table 1. In total, 195 traps were used.

**Table 1.** Characteristics of scree slope sites studied.  $\text{pH}_{(\text{H}_2\text{O})}$  and  $\text{C}_{(\text{ox})}$  values, both after Rendoš et al. (2016b), were measured at four depths (5, 35, 55, and 95 cm). The numbers indicating particular studied scree slopes are stated in Figure 1.

Locality	Bedrock	GPS coordinates	Altitude (m)	Exposition	Sampling period	Sampling days	$\text{pH}_{(\text{H}_2\text{O})}$ (5–95 cm)	$\text{C}_{(\text{ox})}$ (5–95 cm)
1	Granitoid	49°77'N, 17°66'E	410	southwest	15 Jan 2015–16 Jan 2016	365	4.1–4.7	10.8–1.1
2	Basalt	48°13'N, 19°52'E	460	southwest	15 May 2012–17 Oct 2013	520	5–6.4	3.2–0.5
3	Limestone	48°32'N, 20°07'E	315	north	15 May 2012–17 Oct 2013	520	6.6–8.3	7.3–1.7
4	Limestone	48°29'N, 20°04'E	248	southwest	18 Oct 2013–4 Jun 2014	229	–	–
5	Limestone	48°29'N, 20°03'E	246	southwest	18 Oct 2013–4 Jun 2014	251	–	–
6	Limestone	48°31'N, 20°25'E	328	southwest	11 Jun 2014–29 Apr 2015	322	7.3–8.3	12.5–2.3
7	Limestone	48°33'N, 20°30'E	489	west	11 Jun 2014–29 Apr 2015	322	6.8–7.9	10–3.7
8	Limestone	48°50'N, 21°06'E	530	northeast	15 Nov 2008–15 Jul 2010	607	7.7–8.3	15.5–8.8



**Figure 1.** Location of the study sites. 1/ Borinka (Malé Karpaty Mts.), 2/ Belinské skaly (Cerová vrchovina Highlands), 3/ Drienok Valley, 4/ Slope next to the Malá drienčanská Cave, 5/ Collapse above the Veľká drienčanská Cave (three sites are in the Revúcka vrchovina Highlands), 6/ Vysoký vrch Hill, 7/ Doline next to Silická ľadnica Ice Cave (both sites in Slovak Karst), 8/ Malý Ružinok Valley (Čierna hora Mts.).

## Community characteristics

To describe quantitative and qualitative characteristics of isopod communities, we calculated dominance, constancy, Shannon's diversity index, and Pielou's evenness index. The last two indices were first calculated for the material from each scree slope site separately and then collectively for the material from all sites. Due to the low number of isopods sampled, we were not able to perform more complex statistical analysis of our results. Dominance ( $D$ ) was calculated by the formula  $D = 100 \cdot n/N$  where  $n$  is the number of individuals belonging to the specific species and  $N$  is the number of all

individuals sampled. Constancy ( $C$ ) was calculated using the formula  $C=100 \cdot pA/P$  where  $pA$  is the number of study sites on which, the specific species were sampled and  $P$  is the total number of study sites. Then after calculations, species were subdivided into the categories reflecting their dominance: subrecedent ( $D < 1\%$ ), recedent ( $D = 1\text{--}2\%$ ), subdominant ( $D = 2\text{--}5\%$ ), dominant ( $D = 5\text{--}10\%$ ), eudominant ( $D > 10\%$ ) and constancy: rare ( $C < 25\%$ ), widespread ( $C = 25\text{--}50\%$ ), constant ( $C = 50\text{--}75\%$ ), common ( $C > 75\%$ ). Shannon's diversity index ( $H'$ ) was calculated by the formula  $H' = -\sum (P_i \cdot \ln P_i)$ , where  $P_i$  is the fraction of the entire population made up of species  $i$  (proportion of a species is relative to a total number of species present, not encountered). Pielou's evenness index ( $J'$ ) was computed by the formula  $J' = H'/H'_{\max} = H'/\ln S$  where  $S$  is the number of species encountered (Heip et al. 1998).

## Results

In total, 252 isopod specimens belonging to eleven species and six families were sampled from eight investigated scree slope sites (Table 2). The number of species sampled on individual study site varied from 1 to 5, with 2.8 species sampled on average per site. On the scree slopes near the Malá drienčanská and Veľká drienčanská caves, the values of Shannon's diversity index equalled 0. The highest value of both Shannon diversity index and Pielou evenness index ( $H'=1.35$  and  $J'=0.32$ ) was recorded on the scree slope near the entrance to the Ardovská Cave. Among the captured Isopoda, *Mesoniscus graniger* was characterized as common, because of its highest constancy ( $C = 75.0\%$ , the species occurred at 6 of 8 sites); one species was classified as constant, three other species as widespread, and six species as rare. At the same time, *M. graniger* represented the only eudominant isopod species ( $D = 54.4\%$ ). The remaining species were classified as dominant (2 spp.), subdominant (3 spp.), subrecedent (3 spp.), and recedent (2 spp.) (Table 2).

Looking at depth distribution, the vast majority of species were exclusively sampled in the uppermost levels of the depth profile (5–15 cm). Only three species were distributed deeper in higher numbers, *M. graniger*, *Porcellium collicola*, and *Hyloniscus riparius*. The occurrence of other species in the lower parts of the scree slope profile was rather infrequent (Table 3). The individuals of *M. graniger* occurred abundantly in two parts of the depth profile: at the depths between 15–45 cm and 65–85 cm (Figure 2).

Assessment of isopod seasonal activity was based on *M. graniger* sampled on two pairs of sites with the same duration of samplings: the first pair is represented by the scree slopes in the Belinské skaly and Drienok Valley (sites 2 and 3, respectively) while the second pair by the scree slopes next to the Ardovská Cave and ice cave Silická ľadnica (sites 6 and 7, respectively). On the sites 2 and 6, warm/cold (= May–October/November–April) season ratio was almost 50:50 but on the sites 3 and 7, markedly more specimens were collected during the warm sampling period. Taking into account all 4 study sites together, 70% of specimens were collected during the warmer sampling periods (Figure 3). Chi square test confirmed warm period to be a season of higher

**Table 2.** List of sampled isopod species and community characteristics. Abbreviations: C-Constancy: com-common, con-constant, wi-widespread, ra-rare; D-Dominance: ed-eudominant, do-dominant, sd-subdominant, re-recedent, sr-subrecedent. The numbers indicating particular studied scree slopes are stated in Figure 1.

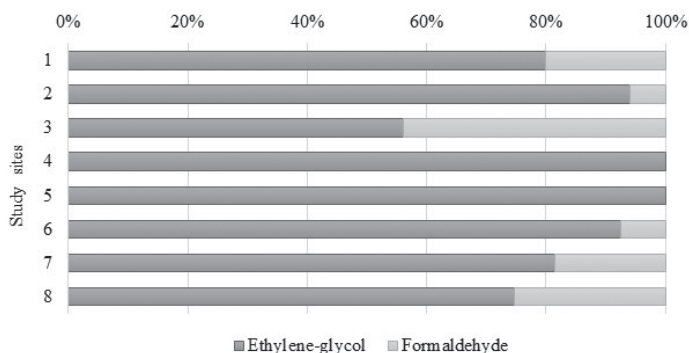
Species	Study sites / ex									C		D	
	1	2	3	4	5	6	7	8	Σ	(%)	degree	(%)	degree
<i>Hyloniscus riparius</i> (C Koch, 1838)	7	–	1	–	1	2	–	–	11	50	con	4.4	sd
<i>Lepidoniscus minutus</i> (C Koch, 1838)	–	–	–	–	–	25	–	2	27	25	wi	10.7	do
<i>Ligidium germanicum</i> Verhoeff, 1901	–	–	–	–	–	–	–	37	37	12.5	ra	14.7	do
<i>Ligidium hypnorum</i> (Cuvier, 1792)	–	–	–	–	–	–	–	2	2	12.5	ra	0.8	sr
<i>Mesoniscus graniger</i> (Frivaldsky, 1865)	–	7	15	40	–	16	14	45	137	75	com	54.4	ed
<i>Orthometopon planum</i> (Budde-Lund, 1885)	–	–	–	–	–	4	–	–	4	12.5	ra	1.6	re
<i>Porcellium collicola</i> (Verhoeff, 1907)	–	–	–	–	–	17	–	–	17	12.5	ra	6.8	sd
<i>Porcellium conspersum</i> (C Koch, 1841)	–	–	–	–	–	–	1	–	1	12.5	ra	0.4	sr
<i>Protracheoniscus politus</i> (C Koch, 1841)	–	2	1	–	–	–	1	–	4	37.5	wi	1.6	re
<i>Trachelipus ratzeburgii</i> (Brandt, 1833)	2	–	–	–	–	–	–	–	2	12.3	ra	0.8	sr
<i>Trichoniscus carpaticus</i> Tabacaru, 1974	–	–	1	–	–	–	–	9	10	25	wi	4.0	sd
Σ ex.	9	9	18	40	1	64	16	95	252	100	–	100	–
Σ spp.	2	2	4	1	1	5	3	5	11	–	–	–	–
Shannon's diversity index	0.53	0.53	0.63	0	0	1.35	0.46	1.11	–	–	–	–	–
Pielou's evenness index	0.2	0.24	0.22	0	0	0.32	0.17	0.24	–	–	–	–	–

**Table 3.** A summary overview of the isopod depth distribution in the eight Western Carpathian scree slopes.

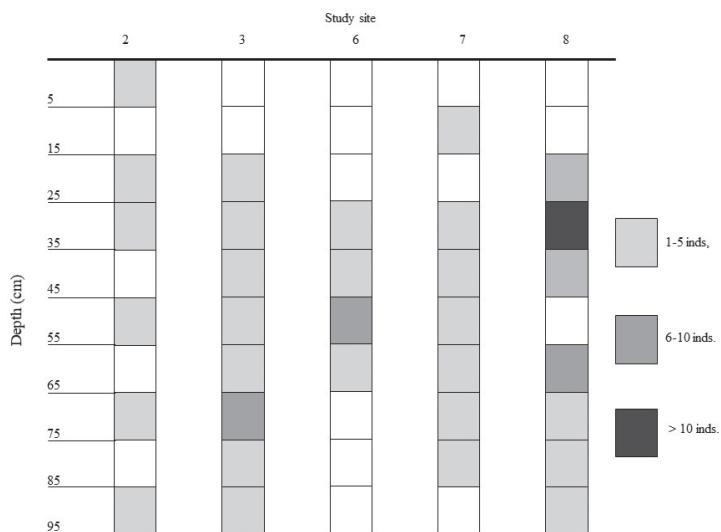
Depth (cm)	<i>H. riparius</i>	<i>M. graniger</i>	<i>L. minutus</i>	<i>Li. germanicum</i>	<i>Li. hypnorum</i>	<i>O. planum</i>	<i>P. collicola</i>	<i>P. conspersum</i>	<i>Pr. politus</i>	<i>Tra. ratzeburgii</i>	<i>T. carpaticus</i>	Σ
5	2	1	26	36	2	4	7	–	2	2	10	92
15	3	14	–	1	–	–	4	–	2	–	–	24
25	–	25	–	–	–	–	1	–	–	–	–	26
35	–	29	–	–	–	–	1	1	–	–	–	31
45	–	18	–	–	–	–	1	–	–	–	–	19
55	3	7	1	–	–	–	–	–	–	–	–	11
65	–	11	–	–	–	–	–	–	–	–	–	11
75	1	11	–	–	–	–	1	–	–	–	–	13
85	–	14	–	–	–	–	1	–	–	–	–	15
95	2	7	–	–	–	–	1	–	–	–	–	10
Σ	11	137	27	37	2	4	17	1	4	2	10	252

activity (Chi square test p-value 0.0009). As regards the comparison of effectiveness of two types of fixative solutions used in this study, ethylene glycol appears to be significantly more attractive (or less repellent) for isopods than formaldehyde (Figure 4).  
Regarding pH, more study sites are located on limestone, thus soil pH is alkaline. Only two study sites have acidic soil pH. In comparison of abundance, on alkaline study sites 193 specimens were sampled, but on acidic sites only 18 (soil pH was not

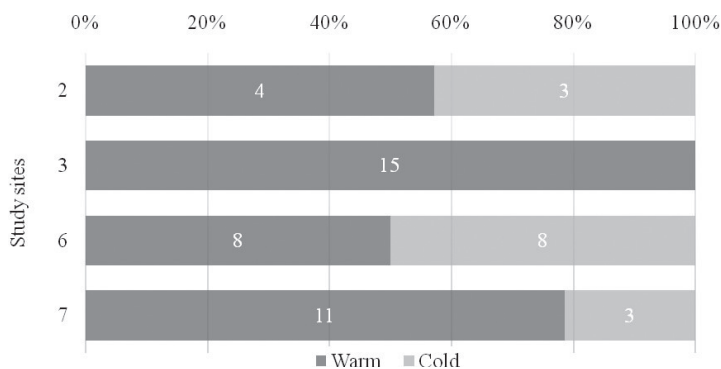




**Figure 2.** Ethylene glycol to formaldehyde ratio of sampled specimens from all study sites, where both fixating solutions were used.



**Figure 3.** Depth distribution of *Mesoniscus graniger*. Site 1 is out of the species range. Study sites 4 and 5 were not depicted, because whole depth gradient was not represented (see locality description).



**Figure 4.** Comparison of warm and cold period sampling of *Mesoniscus graniger* on study sites 2, 3, 6, 7.

measured on study sites 3 and 4). Alkaline study sites are more diverse with average of four species on one study site, against two species on both acidic study sites. Total number of species on alkaline study sites is ten, on acidic study sites it is only four.

## Discussion

Rather scarce communities of terrestrial isopods, in terms of species number, were found to inhabit shallow depths on forested scree slopes in the Western Carpathians. This finding reflects the distributional pattern of terrestrial isopods in Europe with the most important hotspots situated in the Mediterranean regions (Manicasteri and Argano 1989, Sfenthourakis and Taiti 2015). Eleven species documented within the studied forested screes represent approximately 20% of the entire Isopod fauna known from the territory of the W. Carpathians. All species can be considered as autochthonous and indicate the undisturbed state of the sites. They account for more than one third of all autochthonous isopod fauna in Slovakia (Frankenberger 1959, upgraded by our own unpublished data). Only two cavernicolous isopods are known from Slovakia: *Mesoniscus graniger* (Mesoniscidae) and an undescribed blind species from the family Trichoniscidae (Kováč et al. 2014).

In the case of Isopoda, the typical number of species sampled in various surface habitats, such as forests, groves or pastures, ranges from 3 to 7 (Farkas et al. 1999). The number of species sampled in the depth profile of forested scree slopes varied between 1 and 5 with average count of almost 3 species per study site. The lowest counts of species were recorded on the scree slopes built on basalt and quartzite rocks (two species). The most diverse isopod communities were observed on limestone bedrock (five species captured on a single site at the most). Two sites where shortened traps were buried revealed very low species diversity (one species captured per site). This was probably caused by the short and unsuitable period of subterranean pitfall traps exposition. A considerable similarity of the results was confirmed between the forested scree slopes and the caves from the same region. Some large-body forms of terrestrial isopods, frequently found in the entrance zone of caves, were missing in the screes (Papáč et al. 2009, Rendoš et al. 2016a). Differences in depth distribution of isopods among the studied scree slopes are ambiguous due to the low count of specimens sampled on the quartzite and basalt sites. What all studied scree slopes have in common is that the highest counts of specimens were sampled at depths of 5 and 15 cm. Associations of some isopods with basalt or quartzite bedrock is not assumed, since all species sampled on these two types of bedrocks were also found on the limestone scree slopes.

Relative abundance and depth distribution of isopods sampled inside the forested scree slopes clearly reflect the amount of organic residues along the depth profile, which tends to be the highest in the uppermost layers and to decrease downwards the depth profile as observed by Rendoš et al. (2016b). The vast majority of species were sampled in the nutrient-rich topsoil layers (5–15 cm) characterized by a high content of leaf litter and humus. Based on Sket's (2008) classification of subterranean organisms, the isopods inhabiting these uppermost levels can be divided into two groups. The first

group includes well pigmented troglomenes: *Lepidoniscus minutus*, *Ligidium germanicum*, *Ligidium hypnorum*, *Orthometopon planum*, *Protracheoniscus politus*, *Trachelipus ratzeburgii*, and *Trichoniscus carpaticus* which penetrate cave and other subterranean habitats very occasionally. The smallest species, *T. carpaticus*, only recently documented in Slovakia (Rendoš et al. 2016a), is probably a hemiedaphic woodlouse. It does not penetrate any deeper underground in screes or caves in the Western Carpathians. It was described in Romania and is often present in caves and is considered to be troglophilous (Tabacaru and Giurginca 2013). The second group is represented by subtroglophiles *Hyloniscus riparius*, *Porcellium collicola*, and *Porcellium conspersum* possessing no morphological adaptations to life in dark subterranean environment. These mostly surface dwelling species often use deeper soil horizons to overcome drought. Deeper parts of the scree slope profiles (from 15 cm downwards), characterized by a stable microclimate and much higher organic matter content than in deep caves, are almost exclusively inhabited by *Mesoniscus graniger* – an eyeless and depigmented eutroglophile often found in subterranean habitats of the Carpathian and Dinaric Mountains (Mock et al. 2005, Giurginca 2009, Bedek et al. 2011, Tabacaru and Giurginca 2013)

As regards seasonal activity of *M. graniger*, our results prove the warm period of the year (vegetation period) to be the seasons with higher activity of *M. graniger*. Seasonal activity of other species was not assessed, due to their low abundance. This indirectly suggests that there is no massive seasonal vertical migration of the isopods into deeper layers of the forest scree slopes. Migration of terrestrial isopods into the deep soil is not necessary, since they can spend winter periods at shallow depths. Fallen leaves and snow cover provide a sufficient isolating layer to prevent lower lethal temperatures to isopods from being reached. Avoiding the uppermost layer of substrate, where the temperature has fallen below freezing point for several winter months, as overwintering isopod strategy could be minimized to move to a depth of a few centimeters under appropriate conditions, when the surface of soil is covered by fallen leaves and snow (Tanaka and Udagawa 1993).

Comparison of fixative solutions ended up more positively for ethylene glycol in which, more than 50% individuals of Isopoda were sampled. This was probably caused by the repelling effect of formaldehyde, since ethylene glycol was confirmed to show neither strong repellent nor strong attractive effect on arthropods (Gerlach et al. 2009). Another possible cause of lower number of Isopoda caught by formaldehyde traps could be also the non-mixing of detergent into formaldehyde solution. Using attractive bait in the traps, highly favored by coleopterists, will bring more faunistic knowledge, including unique findings (Mammola et al. 2016), but without the possibility to interpret the depth preferences of invertebrates.

It is not clear from our research, how pH is affecting terrestrial isopods assemblages, since our species and specimens counts are very low. If we take a look at the dominant *M. graniger*, we can see that this species is not affected by pH, due to the presence of this species on both alkaline and acidic study sites. The reason why this species is missing from study site 1 is that it is outside the distribution area of this species (Košel 2012). Terrestrial isopods need calcium, because of their exoskeleton structure (Zimmer et al. 2000). Limestone bedrock is a very good source of calcium ions, so this

could be a reason why alkaline study sites (on limestone bedrock) are more abundant and diverse. Soil pH is not the sole reason of this phenomenon. We assume that it is a combination of soil pH, temperature, humidity and other externalities.

## Conclusions

The shallow underground of forested scree slopes in Slovakia is not inhabited by unique terrestrial isopods, but is uncommonly visited by surface (forest) species or by the sole subterranean species sampled (*Mesoniscus graniger*). The interior of forested scree slope can be considered as a part of subterranean environment inhabited in the long term by fauna specialized to live in permanent darkness. Eleven species were sampled in total, which is more than one third of the autochthonous isopod fauna in Slovakia. Well-preserved status of scree habitats is supported by the presence of the rare species, *Mesoniscus graniger*, *Orthometopon planum*, and minute Carpathian endemic *Trichoniscus carpathicus*. Subterranean pitfall traps with ethylene glycol proved themselves as suitable apparatuses to collect macrofauna from this environment.

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# Size dependence of offspring production in isopods: a synthesis

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## Abstract

In isopods, parental care takes the form of offspring brooding in marsupial pouches. Marsupial brooding was an important step towards the origin of terrestrial lifestyles among isopods, but its potential role in shaping isopod life histories remains unknown. It is here considered that marsupial brooding imposes costs and creates a temporary association between the survival of mothers and that of their offspring. Integrating findings from different life history models, we predicted that the effects of marsupial brooding set selective conditions for the continuation of growth after maturation, which leads to indeterminate growth, and the production of larger offspring by larger females. Based on this perspective, a study on the size dependence of offspring production in the woodlouse *Porcellio scaber* was performed and the generality of the results was tested by reviewing the literature on offspring production in other isopods. In *P. scaber* and almost all the other studied isopods, clutch size is positively related to female size. Such dependence is a necessary pre-condition for the evolution of indeterminate growth. The body mass of *P. scaber* differed six-fold between the largest and smallest brooding females, indicating a high potential for post-maturation growth. Our review showed that offspring size is a rarely studied trait in isopods and that it correlates negatively with offspring number but positively with female size in nearly half of the studied species. Our study of *P. scaber* revealed similar patterns, but the positive effect of female size on offspring size occurred only in smaller broods, and the negative relation between clutch size and offspring size occurred only in larger females. We conclude that the intraspecific patterns of offspring production in isopods agree with theoretical predictions regarding the role of offspring brooding in shaping the adaptive patterns of female investment in growth, reproduction, and the parental care provided to individual offspring.

**Keywords**

clutch size, female size, indeterminate growth, life history evolution, offspring brooding, offspring size, parental care, trade-off

**Introduction**

Most crustaceans engage in different types of parental care, which, in isopods, takes the form of offspring brooding in marsupia (Thiel 2000, Vogt 2016). During the moult preceding reproduction, the isopod female produces exoskeletal extrusions that form a marsupial pouch, which is used as a chamber for egg laying and carrying developing larvae (Hornung 2011, Appel et al. 2011). At the end of brooding, the female releases the offspring into the environment. In terrestrial species, individuals inside the marsupium undergo the change from the aqueous to the gaseous environment (Horváthová et al. 2015). Marsupial brooding was crucial for the origin of terrestrial lifestyles in isopods (Hornung 2011, Appel et al. 2011, Horváthová et al. 2017). Interestingly, land colonisation occurred independently at least twice in the evolutionary history of this group (Lins et al. 2017). Here, we consider that marsupial brooding plays a role in the evolution of life history strategies, especially by affecting adaptive patterns of female investment in growth, reproduction, and the parental care provided to individual offspring. To the best of our knowledge, this perspective remains largely unexplored in isopods.

The theory of life history evolution predicts that resource availability limits imposed by physiological and ecological circumstances forces organisms to optimise the lifetime allocation of investment among growth, reproduction and other competing demands to ensure the highest expected fitness under given mortality and production conditions (Stearns 1992). Adopting this basic principle, life history modelling has demonstrated that somatic growth is beneficial as long as one calorie invested in increasing body mass increases the future expected reproductive output by more than one calorie (Kozłowski 1992). Likewise, organisms are expected to optimise the amount of resources retained over unfavourable periods to fuel activities in favourable periods (Ejsmond et al. 2015); the timing of reproductive activity during a season, compromising the future prospects of offspring (Ejsmond et al. 2010); and the amount of resources invested in single offspring, compromising offspring number (Smith and Fretwell 1974). Developments in life history theory have led to an important conclusion: there is a wealth of distant optima with similar fitness consequences, which explains why life histories are so enormously diverse in nature (Stearns 1992, Czarnoleski et al. 2003, Kozłowski 2006).

A range of life history models predict the evolution of a bang-bang resource allocation strategy, which is associated with the complete cessation of growth after maturation and the so-called determinate growth pattern (Kozłowski and Wiegert 1987, Stearns 1992, Kozłowski 2006). In contrast, many isopods continue to moult after maturation, combining their capacity for reproduction with the capacity for somatic

growth. This ability results in the potential for continuation of growth for the entire life span and the so-called indeterminate growth pattern. Beside isopods and some other crustaceans, indeterminate growth has evolved in annelids, molluscs, fish, amphibians, and reptiles (Kozłowski 1996). The prevalence of indeterminate growth in nature awaits explanation, but life history theory predicts that this growth strategy provides fitness advantages if the capacity to produce new tissue and/or survive strongly increases with body mass and if these capacities change discontinuously through time (Stearns 1992, Perrin and Sibly 1993, Kozłowski 2006). Modelling of optimal allocation has shown that discontinuities driven by either seasonal changes in mortality/productive capacity (Kozłowski and Teriokhin 1999) or unequal future prospects of offspring released into the environment at different times of the year (Ejsmond et al. 2010) lead to the evolution of alternating shifts between the investment in somatic growth and that in reproduction, resulting in the indeterminate growth pattern. For many organisms, including isopods, seasonality is the primary selective force responsible for the evolution of indeterminate growth. Nevertheless, specific characteristics of species biology, such as the reproduction via clutches instead of via a series of single offspring, can elicit discontinuous changes in mortality/production capacity, similar in principle to the effects of seasonality (Czarnoleski and Kozłowski 1998). Such characteristics can help explain why indeterminate growth originated among annuals or perennials living in non-seasonal environments. Heino and Kaitala (1996) designed a life history model for gill-brooding unionid mussels (e.g., *Sinanodonta woodiana* Labecka and Domagala 2018) and demonstrated that an indeterminate growth pattern can evolve in non-seasonal environments if carrying the offspring is associated with costs, either decreased physiological performance or increased mortality and with a temporary association between the fate of the offspring and the survival of the parent. Importantly, using a different model to explore the role of parental care in the evolution of offspring size among fish, Jørgensen et al. (2011) concluded that offspring brooding selects for the increased investment of larger females in individual offspring. For indeterminately growing animals, such a strategy involves constant changes in the optimal size of offspring as females increase their body mass. Under this strategy, the production of larger offspring is expected to require prolonged brooding, which temporarily links the fate of the offspring with that of the mother. If larger females have higher survival probability than do smaller females, then the increased investment in individual offspring becomes more beneficial for larger females. Overall, these theoretical considerations suggest that marsupial brooding might be an important driver of growth strategy and offspring size in isopods. To investigate this hypothesis, we performed a study on the common rough woodlouse (*Porcellio scaber*) and evaluated the generality of our results by analysing data from the literature on other isopods. We aimed at integrating information on intraspecific patterns of size dependence in offspring production over as wide a range of isopod species as possible. In particular, we focused on the relationships between female size and the number and size of offspring in broods and on evidence of an allocation trade-off between the number and size of the offspring in broods. Generally, we expected reproductive capacity to increase as females grow in size, which is the

fundamental condition favouring the strategy of indeterminate growth (see above). Therefore, we expected a positive relationship between female body size and clutch mass/clutch size (hypothesis i). We also tested this relationship for non-linearity, assuming that a negative allometry would indicate an increased relative space limitation in larger females, whereas a positive allometry would indicate a decreased relative space limitation in larger females. Next, we examined whether the investment of females in individual offspring increased with the size of females, which should produce a positive correlation between the average offspring mass in a brood and female body mass (hypothesis ii). Finally, we analysed data on the mean mass of offspring in relation to the number of offspring per brood, looking for an allocation trade-off between offspring size and number (hypothesis iii).

## Materials and methods

### A case study of *Porcellio scaber*

In June–July 2014, individuals of *P. scaber* were collected in an old backyard in Kraków, Poland. In our study, we used females in the 3<sup>rd</sup> and 4<sup>th</sup> stages of brood development (classified according to Lardies et al. 2004a). Each gravid female was placed in a plastic box (100 ml). The boxes were perforated to provide aeration, lined with paper towel and supplied with a piece of moist sponge (water source), a piece of clay pot (shelter) and the dry leaves of the alder (*Alnus glutinosa*) and ash (*Fraxinus excelsior*), which served as *ad libitum* food source. For additional control of humidity, the boxes were placed inside a larger plastic container with wet sand in the bottom. The container with boxes was placed in a shaded patio of the Institute of Environmental Sciences, Jagiellonian University in Kraków. Each day, the boxes were assessed for the presence of new offspring. Emerging offspring were collected, and the female was weighed to the nearest 0.001 mg (Mettler Toledo XP26, Greifensee, Switzerland). The clutches were dried for one hour at 60 °C in an oven (UFE 400, Memmert GmbH + Co. KG, Germany), and the dry mass of each clutch was measured to the nearest 0.001 mg (Mettler Toledo XP26, Greifensee). The offspring in each clutch were counted under a stereoscopic microscope. To calculate the mean dry mass of a single offspring, we divided the clutch dry mass by the number of offspring.

All statistical analyses were performed with R 3.4.1 software (R Core Team 2017), and the *rgl* package of R (Adler and Duncan Murdoch 2017) was used to create graphs. To test whether larger females produced heavier and larger clutches (hypothesis i) and larger offspring (hypothesis ii), we correlated clutch dry mass, clutch size, and mean offspring dry mass with female body mass. To evaluate the nature of these relations, we fitted linear and power regression models to our data and selected the best model using AIC. In this way, we did not a priori assume any particular relationship between the studied variables. When fitting our regression functions, we used either an ordinary least square (OLS) method or the weighted least square (WLS) method, which

allowed us to account for the observed increase in the variance of dependent variables at higher values of an independent variable. Note that the OLS method assumes homogeneity in the variances of the independent variables. According to Knaub (2009), the issue of non-homogeneity can be overcome by using the WLS method, which assigns decreasing weights to observations with increasing levels of variance. Following Knaub's (2009) procedure, we first ordered our data according to an increasing value of an independent variable to identify four quartiles. For data from the first quartile, the weights were calculated as an inverse of the highest value of the independent variable in this quartile (56.328 mg). For data from the other quartiles, the weights were calculated as the inverse of the actual value of the independent variable. To examine whether larger offspring emerged from smaller clutches (hypothesis iii), we used a multiple regression analysis with the mean offspring mass as a dependent variable and clutch size and female body mass as two independent variables. The use of a multiple regression allowed us to dissect the independent effects of each of the two independent variables. Thus, we also re-examined hypothesis (ii) regarding the link between female size and offspring size, with a control for the potential links between clutch size and offspring size. We allowed our model to consider an interaction between our two independent variables. Therefore, to assess the independent effects of each variable (partial regression), we estimated and tested this effect after centring the whole model in either the minimum or maximum value of each independent variable (Quinn and Keough 2002). The multiple regression analysis was performed with the use of either OLS or WLS, and the best model was chosen based on AIC.

### Intraspecific patterns in isopods

To evaluate the generality of our hypotheses (i–iii) and the empirical results for *P. scaber*, we reviewed the published literature on isopods for intraspecific information on at least one of the following relationships: clutch size with female size, offspring size with female size, and clutch size with offspring size. Relevant publications were identified by an extensive search of keywords in scientific databases, the review of reference lists of available publications and by personal communication with specialists in the field. Whenever we found relevant information regarding one of the three relationships, we classified the relationship as either statistically significant or non-significant; we also identified significant relationships as either positive or negative. If available, correlation coefficient ( $r$ ) values were also assigned to each relationship. Traits used to study the relationship between female size and either clutch size or mass varied substantially among authors and species; therefore, we additionally recorded information regarding the types of measured traits. For each type of relationship, each species was classified according to the nature of this relationship, integrating all the results on a species reported in the literature. If a relationship for a given species was consistently reported to be significantly positive, significantly negative, or non-significant, the species was regarded as exhibiting a positive (+) or negative (-) relationship or no relationship (NS).

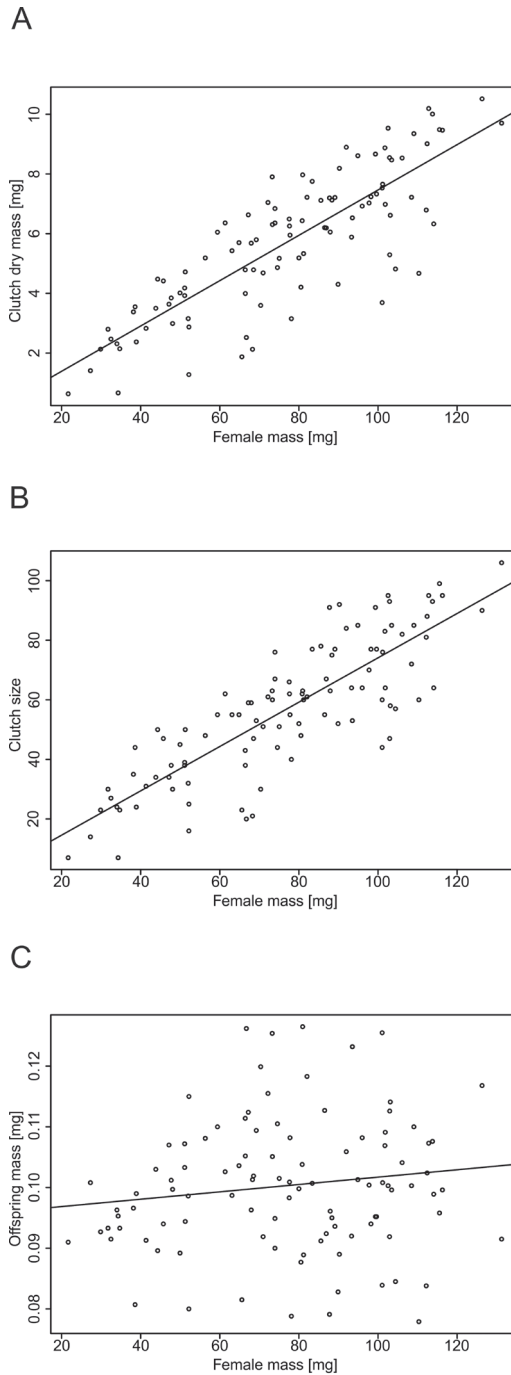
Species for which mixed results were reported, showing either non-significant/significantly positive relationships or non-significant/significantly negative relationships were classified as NS/+ or NS/-, respectively. Ultimately, we used this integrated species information to calculate how frequently among the studied isopods a given pattern (+, -, NS, NS/+ and NS/-) of each relationship occurred. In addition, we used a 1–4 scale to evaluate the confidence in the support for each pattern (+, -, NS, NS/+ and NS/-) to predict the directions of the studied relationships (hypotheses i–iii). Consistently positive/negative relationships (+/-) were treated as providing reliable evidence to support or oppose a hypothesis. Non-significant patterns (NS) were regarded as not supporting a hypothesis, but we also considered the possibility that they might represent false negatives due to low statistical power. The level of support given by inconsistent results (NS/+ and NS/-) was dependent on the context. If among the non-significant and significant results, the significant results were consistent with our predictions, we treated the mixed results as weakly supporting our hypothesis. However, if the significant results were in conflict with the predictions, we regarded the mixed results as strongly opposing the hypothesis.

## Results

### A case study of *Porcellio scaber*

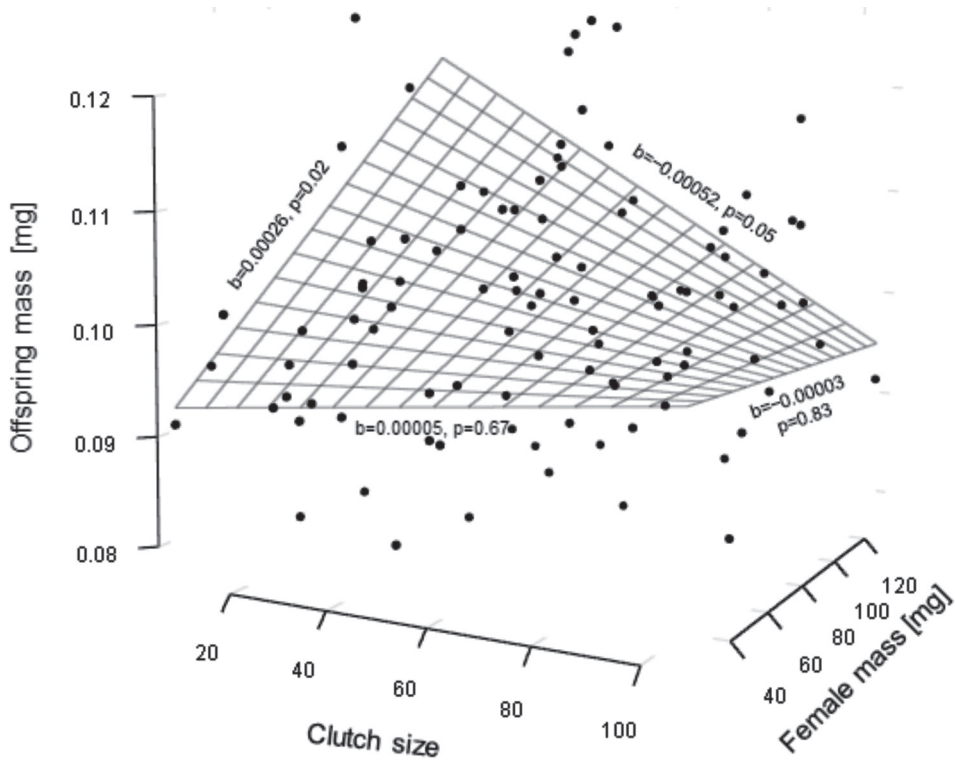
Among 101 brooding females of *P. scaber*, body mass ranged from 21.682 to 131.236 mg, clutch sizes ranged from 7 to 106 juveniles, and the mean dry body mass of offspring ranged from 0.078 to 0.126 mg between clutches. Larger females produced heavier ( $r = 0.83$ ,  $t_{1,99} = 14.9$ ,  $p < 0.001$ , Fig. 1A) and larger clutches ( $r = 0.83$ ,  $t_{1,99} = 15.09$ ,  $p < 0.001$ , Fig. 1B), but the mean offspring mass did not show a consistent relationship with female mass ( $r = 0.14$ ,  $t_{1,99} = 1.44$ ,  $p = 0.15$ , Fig. 1C). Comparison of AIC between the alternative regression models showed that a linear weighted regression produced the best fit to our data (Fig. 1). Therefore, we concluded that clutch size and clutch mass increased linearly with female body mass, which is consistent with our finding that the dry body mass of offspring did not change systematically with female body mass.

The results of the multiple regression analysis (Fig. 2) showed no effect of clutch size ( $t_{1,97} = 0.74$ ,  $p = 0.46$ ) and a positive effect of female mass ( $t_{1,97} = 2.38$ ,  $p = 0.02$ ) on the mean dry body mass of offspring. The interaction between the two independent variables was non-significant ( $t_{1,97} = -1.60$ ,  $p = 0.11$ ). When we centred the model at the value of the smallest broods (7 offspring), the positive link between offspring dry mass and female body mass was still significant ( $t_{1,97} = 2.39$ ,  $p = 0.02$ ), but the significance disappeared when we centred the model at the value of the largest clutches (107 offspring) ( $t_{1,97} = -0.22$ ,  $p = 0.83$ ). When we centred the model at the minimum female body mass (21.682 mg.), clutch size and offspring body mass appeared to be unrelated ( $t_{1,97} = 0.44$ ,  $p = 0.66$ ), but centring at the maximum body mass (131.236 mg) revealed



**Figure 1.** In *Porcellio scaber*, the dry mass of clutches (**A**) and clutch size (**B**) increased linearly with female body mass, but the mean dry mass of offspring did not depend on female mass in a consistent way (**C**). Lines represent fitted regressions **A**  $y = -0.13 + 0.08x$  ( $r = 0.83$ ,  $p < 0.001$ ) **B**  $y = -0.32 + 0.74x$  ( $r = 0.83$ ,  $p < 0.001$ ) **C**  $y = 0.1 + 0.00006x$  ( $r = 0.14$ ,  $p = 0.15$ ).



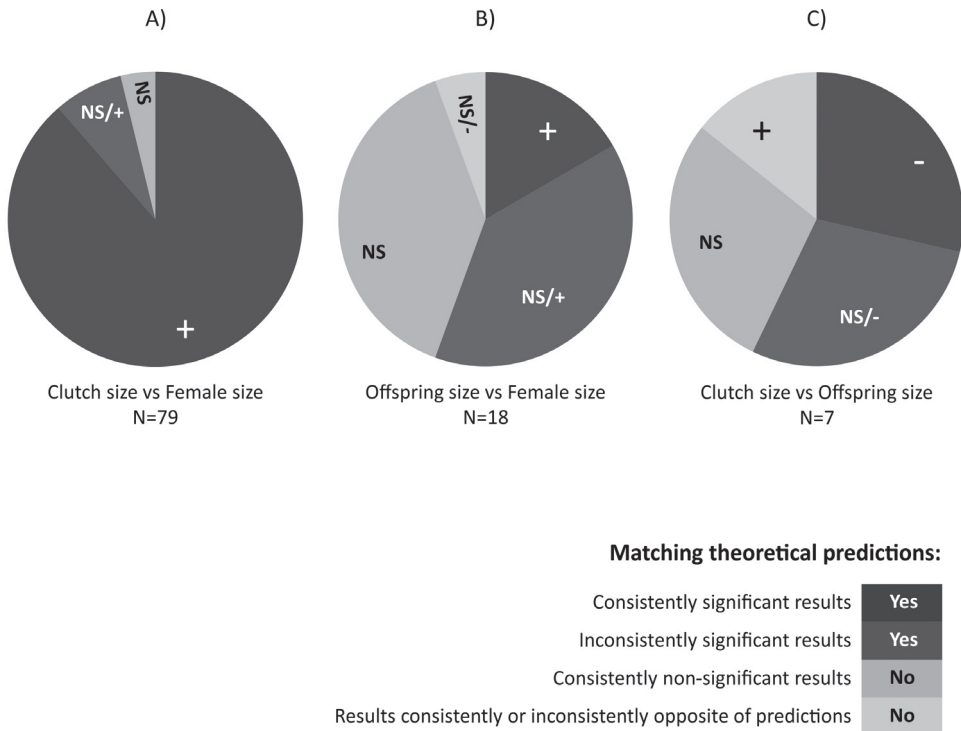


**Figure 2.** In *Porcellio scaber*, the heaviest offspring were released by large females that produced small clutches. The plane represents a multiple regression model fitted to the data; the partial slopes depicted on the edges were calculated by setting the other predictor value to its minimum and maximum values.

a negative relationship between clutch size and offspring body mass, though the effect was marginally significant ( $t_{1,97} = -1.98$ ,  $p = 0.05$ ). Overall, this analysis indicated that the largest offspring were produced by large females with small clutches.

### Intraspecific patterns in isopods

Our literature search identified a total of 79 species of isopods that were studied with respect to at least one of the following relationships: clutch size with female size (Fig. 3A), offspring size with female size (Fig. 3B), and clutch size with offspring size (Fig. 3C). Detailed results of the review are provided in Table 1S (Suppl. material 1). The effect of female size on clutch size was the most frequently studied relationship (79 species), while the relationships between female size and offspring size and between offspring size and clutch size were studied in only 18 and 7 species, respectively, including *P. scaber* as reported in this study. For the vast majority of the studied isopods (Fig. 3A), we found evidence that supports a positive relationship between female size and clutch size (hypothesis i). Importantly, we found no reports of the opposite pattern and only



**Figure 3.** The literature search identified 79 species of isopods that were studied with respect to at least one of the following relationships: clutch size with female size (**A**), offspring size with female size (**B**), and clutch size with offspring size (**C**). Each graph shows how frequently a given nature of each relationship was found among the studied isopod species. The exact number of species for which the relationships **A**, **B**, **C** were evaluated is given by N. For each type of the relationships **A**, **B**, **C** each species was classified according to the nature of this relationship. If a relationship for a given species was consistently reported to be significantly positive, negative, or non-significant, the species was marked by a positive (+) or negative (-) symbol or by NS. Species for which mixed results were reported in the literature, showing either non-significant/significantly positive relationships or non-significant/significantly negative relationships, were marked by NS/+ or NS/-, respectively. Colour intensity indicates values along a 1–4 scale of confidence to the support provided by each relationship pattern (+, -, NS, NS/+ and NS/-) to hypotheses (i–iii). Relationship **A**: a positive relationship predicted between female body size and clutch mass/clutch size (hypothesis i). Relationship **B**: a positive correlation predicted between the average offspring mass in a brood and female body mass (hypothesis ii). Relationship **C**: a negative correlation predicted between the mean mass of offspring and the number of offspring per brood (hypothesis iii).

occasional reports of a non-significant pattern. However, the non-significant reports were typically found along with reports of significantly positive patterns, suggesting that many of the non-significant results might be false negatives. For nearly half of the species (Fig. 3B, C), we found evidence that supports a positive relationship between female size and offspring size (hypothesis ii) and a trade-off between offspring size and clutch size (hypothesis iii).

## Discussion

Growth patterns vary considerably in nature (Stearns 1992, Czarnoleski et al. 2003, 2005, Ejsmond et al. 2010), but understanding the origin of this variance is more challenging than it might initially appear. Our data on *P. scaber* suggest that this species of woodlouse has evolved a life history strategy with intense resource allocation to somatic growth in the reproductively mature stages. We found up to six-fold differences in body mass between the largest and the smallest brooding females, which suggests that only 20% of the body mass of a fully-grown female might be achieved before maturation, with the majority of growth potentially co-occurring with reproduction in such instances. Consistent with the idea that species with indeterminate growth should be characterized by a strong dependence of reproductive capacity on body size (hypothesis i), we found that larger females of *P. scaber* carried larger and heavier broods. This evidence clearly shows that mature females can gain reproductive capacity by further increasing body mass. The results of our literature search indicate that such size dependence is widespread among other isopod species. Interestingly, we found no reports of a negative pattern of this relationship and few reports of non-significant effects of female size on clutch size, which are likely to be false negatives. A strong size dependence of reproductive capacity promotes the evolution of iteroparous breeding with indeterminate growth, but alone, it is not sufficient to explain such evolution (Heino and Kaitala 1996, Czarnoleski and Kozłowski 1998). In fact, some isopods, such as *Ligia oceanica*, have evolved a semelparous breeding strategy with determinate growth, despite the size dependence of reproductive capacity (Sutton et al. 1984, Willows 1987). Given this, what might be the ultimate drivers of the evolution of indeterminate growth in isopods? A life history theory calls attention to the pattern of resource allocation among growth, reproduction and other competing demands, which should be optimised to ensure the highest expected lifetime fitness in given mortality and production conditions (Stearns 1992, Kozłowski 2006). Considering this idea, the alternating allocations between growth and reproduction that lead to indeterminate growth reflect changes in allocation optima, with temporal shifts in the capacity to survive and/or reproduce. The woodlouse *P. scaber* and many other isopod species inhabit seasonal environments, and life history models have demonstrated that seasonal alternations of the periods suitable for survival, offspring production, and growth with less favourable periods establish the selective forces that favour the continuation of somatic growth after maturation (Kozłowski and Teriokhin 1996, Czarnoleski and Kozłowski 1998, Ejsmond et al. 2010). However, as suggested by Heino and Kaitala (1996) and Czarnoleski and Kozłowski (1998), the strategy of indeterminate growth might also bring additional fitness benefits if organisms engage in offspring brooding. Carrying offspring creates temporary changes in mortality/physiological performance and links between the fate of the offspring and that of the mother, leading to shifts in the optimality of growth and reproduction through time. Unfortunately, the costs associated with offspring brooding are poorly studied in isopods, but we might expect them in the form of increased vulnerability to predation and/or increased energetic costs associated with locomotion and supplementation of offspring. For example, Kight and Ozga (2001) observed that gravid

females of *Porcellio laevis* were less mobile than were non-gravid females. In addition, female isopods are postulated to regulate the pH and osmolality of their marsupial fluids and provision their broods with necessary resources via the so-called cotyledon (Lardies et al. 2004a). Furthermore, Lardies et al. (2004a) showed that gravid females had lower ingestion rates and digestibility and higher metabolic rates than did non-gravid ones. Interestingly, Perrin and Sibly (1993) suggested another mechanism that favours indeterminate growth among offspring brooders, which is non-exclusive of the hypothesis of a role of discontinuities in mortality/physiological capacity. If current offspring production is limited by the space provided by the brooding cavities rather than by the physiological capacity to produce new tissue, organisms are selected to direct surplus resources to further somatic growth, thereby increasing their fertility at the following reproductive event. There is some evidence to suggest that the maximal reproductive performance of isopods might be restricted by the volume of the marsupial pouches (Lardies et al. 2004a, Appel et al. 2011). Nevertheless, we found no indication that such limitations change with body size in females of *P. scaber*. The relationship between clutch size and female size did not deviate from linearity. In addition, we detected substantial variance in the mass of clutches produced by females of a given body size, which suggests that reproductive capacity might not be entirely dependent on the space limitation of the marsupium, unless the volume of the marsupium is highly variable at a given body mass.

Our data on *P. scaber* show that the dry body mass of offspring differed between broods by as much as 62%. A significant part of this variance was linked to differences in clutch size and female body mass, but the pattern of this dependence was complex. Supporting hypothesis ii, the size of offspring was positively related to female size, but this pattern existed only if we considered small clutches. Focusing on larger clutches, we found no apparent relationship between offspring size and female size. In accord with hypothesis iii, the size and number of offspring were inversely related, but this pattern existed only among larger females. In broods produced by smaller females, the two traits were not correlated. To date, studies of isopods have only occasionally addressed the question of whether offspring size changes with either female size or clutch size. According to our literature search, the relationships between female size and offspring size and between offspring size and clutch size have only been studied in 18 and 7 species, respectively. For nearly half of these species, we found evidence that supports a positive relationship between female size and offspring size (hypothesis ii) and a trade-off between offspring size and clutch size (hypothesis iii). It is suggestive that all studies that failed to find evidence of such a trade-off (Fig. 3C) overlooked the potential effects of female size in the statistical analysis of offspring size and clutch size data. In effect, many of these results might represent false negatives because differences in clutch size driven by female size are not primarily generated by the trade-off between offspring size and number but rather by the higher capacity of larger animals to produce new tissue (as shown in Figs 1A, 3A). Furthermore, the positive effects of female size on offspring size can lead to a positive correlation between offspring size and clutch size. Apparently, this is the case in the isopod *Bethalus pretoriensis* (Telford and Dangerfield 1995), which was the only species we found for which a positive association

between clutch size and offspring size was reported; furthermore, a positive association between offspring size and female size was found in this species (see Suppl. material 1).

Examples of life history strategies in which offspring size is a function of parent size are rare in nature, and their evolutionary origins are puzzling (Rollinson and Rowe 2016). Apart from isopods, positive relationships between offspring size and female size have previously been reported in some other arthropods (Fox and Czesak 2000) and some species of snakes (Ford and Seigel 2011) and fish (Hendry et al. 2001, Hendry and Day 2003). Interestingly, in the pipefish (Syngnathidae), the positive relationship between offspring size and female size characterized pouch-brooding species but not ventral-brooding species (Braga Goncalves et al. 2011). In isopods, the positive correlation between female size and offspring size was also demonstrated on the interspecific level (Sutton et al. 1984). Different phenomena have been invoked to understand why larger females might produce larger offspring, including competition between siblings (Parker and Begon 1986), unequal benefits from increased fecundity in small vs large females (McGinley 1989), varying efficiency of resource acquisition from parents (Sakai and Harada 2001), increased parental mortality during reproduction (Kindsvater and Otto 2014), and an increased capacity of larger females to meet the overhead costs of reproduction (Filin 2015). With different degrees of relevance, each of these phenomena might apply to isopods. Nevertheless, here we consider that in live-bearing organisms such as isopods, the survival of offspring during brooding is tightly linked to the survival of the parent, a concept that has helped explain the evolution of indeterminate growth pattern in isopods. According to the life history model of Jørgensen et al. (2011), this tight association promotes increased investment in individual offspring by larger females if larger females have improved survival compared to smaller females. If the development of larger offspring requires longer brooding and if brooding is costly, then the production of larger offspring should be more beneficial to larger females because brooding is relatively less costly for them. Importantly, this scenario can help to rationalise the complex pattern found in our data on *P. scaber*. It is suggestive that larger females produced larger offspring only if we considered small broods. We can expect that a small brood (several offspring in our case) is relatively more costly for small females than for large females, which have much higher reproductive potential (more than 100 offspring in our case). If the cost of brooding corresponds to the risk of mortality, then larger brooding females with small broods should suffer relatively lower costs, which should select them for increased investment in individual offspring. Certainly, before drawing firm conclusions regarding this phenomenon, future studies should better identify how the costs of marsupial brooding change with clutch size and female size.

## Conclusions

Based on the integrated findings reported here, we can attempt to form conclusions about the most common patterns in the size dependence of isopod reproduction and

the significance of these patterns for understanding the evolution of isopod life histories. In nearly all the studied species, we found a strong size dependence of female reproductive capacity. Such a dependence is important for explaining the evolution of an indeterminate growth strategy in many species of isopods. Data from nearly half of the isopod species revealed a negative relationship between offspring size and offspring number and a positive relationship between mother size and offspring size. Importantly, our case study of *P. scaber* suggests that the emergence of each pattern is context-dependent: a positive effect of female size on offspring size was observed only in smaller broods, and a negative relationship between clutch size and offspring size was observed only for larger females. We propose that these patterns be viewed as different elements of a single phenomenon: a lifetime strategy of investment in growth, reproduction and the parental care provided to single offspring that is shaped by selective conditions. The key message of this study is that to gain a better understanding of this strategy in isopods, we must consider the effects of marsupial brooding, especially its costs and the linkage between the survival of mothers and that of their offspring. We hope that our synthesis of theoretical ideas and data on isopods will increase the intersection of life history theory and empirical research in isopods and that this work will stimulate further theory development and lead to an improved understanding of the ecology and evolution of isopods.

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## **Supplementary material I**

### **Table S1**

Authors: Andrzej Antoł, Marcin Czarnoleski

Data type: species data

Explanation note: Results of the literature search for reports of at least one of the following relationships in isopods: clutch size vs female size, offspring size vs female size, offspring size vs clutch size.

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Link: <https://doi.org/10.3897/zookeys.801.23677.suppl1>





# Life history characteristics of a cave isopod (*Mesoniscus graniger* Friv.)

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## Abstract

The special environmental conditions of caves provide habitat for several endemic and relict species, among them terrestrial isopods. The Baradla Cave system (north-eastern Hungary) hosts *Mesoniscus graniger* (Frivaldszky, 1865) (Oniscidea, Microcheta, Mesoniscidae), a pygmy, blind, fragile troglophile woodlice species. Its stable environment can be characterised by the lack of light, high relative humidity (96%), low and constant temperature (about 10 °C). We explored the population characteristics (sex ratio, size distribution) and life history traits of the species (e.g. longevity, reproductive strategy, offspring number, and size). Sex ratio and size distribution of the individuals (head-width measurements) were estimated based on a yearly pooled pitfall-trap data set (N = 677). We studied the species' reproductive strategy under natural conditions (Baradla Cave, Aggtelek National Park). Model populations were set up in the cave and checked monthly between March and October, 2016 (15 replicates, each with 12 randomly chosen adult individuals;  $\Sigma N = 180$ ). Digital photos were taken of the live animals and their length was estimated based on the photos by using ImageJ software (average body length:  $6.56 \pm 0.79$  mm). The results showed female dominance in the population [(male:female = 0.43:0.57);  $p < 0.001$  (GLM)]. Female head width ( $0.87 \pm 0.18$  mm) was significantly greater than that of males [ $0.79 \pm 0.08$  mm;  $p < 0.001$  (t-test)]. Based on our present data we assume that the offspring number per single female is low (3–5), and new-borns have a relatively large size (body length:  $4.22 \pm 0.53$  mm) compared to the adults. The probability of reproduction was continuous by monthly intervals (binomial test) and longevity exceeds one year. Our results suggest that the species follows a stenodynamic life history.

**Keywords**

continuous breeding, large offspring, K-strategy, small clutch size, sexual size dimorphism

**Introduction**

Life history strategies in terrestrial isopods were first reviewed and divided into stenodynamic and eurodynamic traits by Sutton et al. (1984). These strategies can be matched to endogeic that is soil-active (stenodynamic) and epigeic or surface-active (eurodynamic) species by their characters. A stenodynamic species shows low growth rate, long time to maturity, and produces few and relatively large offspring, while the eurodynamic species have an opposite character. They grow faster, mature earlier, reproduce more frequently, and produce more, but smaller offspring (Achouri et al. 2008, Sutton et al. 1984, Warburg 1994, Brody and Lawlor 1984, Warburg and Cohen 1991, Grundy and Sutton 1989). These strategies are similar to r-K life history strategies (Stearns 1977): stenodynamic species tend to be 'K', while eurodynamic species can be best compared to the 'r' life history strategy. In reproduction terrestrial isopods show mainly uni-, bi-, or multivoltine iteroparity, sparsely semelparity, and some species are parthenogenetic (Hornung 2011).

The known number of troglobiotic, troglophile terrestrial isopods is over 330 and is increasing as a result of intensive cave faunal surveys worldwide (e.g. Campos-Filho et al. 2011, 2014, 2016, Kováč 2014, Reboleira et al. 2015, Tabacaru and Giurginca 2013, 2014, Taiti 2014, Taiti and Gruber 2008, Taiti and Wynne 2015). The cave environment can be diverse, as abiotic and biotic characteristics depend on parent rock, geological formation, climatic zone, or biome and ecosystem types.

*Mesoniscus graniger* (Frivaldszky, 1865) was described from Baradla cave, Hungary, and it is one of the two known species of the genus *Mesoniscus* Carl, 1906, Mesoniscidae, Microchaeta (Giurginca 2003, 2005, Gruner and Tabacaru 1963, Schmalfuss 2003). Its known distribution covers the Carpathian Mountains from the Northwest Carpathians (Poland, Slovakia, Hungary), the Western Transylvanian Mountains (Romania, Serbia), the Dinaric Mountains (Serbia, Bosnia and Herzegovina) and the Julian Alps (Slovenia), occurring mainly in caves (Schmalfuss 2003, Giurginca 2003, 2005, Šustr et al. 2005, Piksa and Farkas 2007; Tabacaru and Giurginca 2014). The species was found also endogeic in Poland, Romania, Serbia and Croatia far from caves, in moist and isothermal surface habitats (Mlejnek and Ducháč 2003, Gruner and Tabacaru 1963, J. Bedek, S. Ferentí, I. Karaman personal communication). Its occurrence is also expected in Ukraine (Šustr et al. 2005). It is the only and abundant terrestrial isopod in the Baradla-Domica cave system in Hungary and Slovakia (Frivaldszky 1865, Šustr et al. 2005). The environmental tolerance and food preference of the species are known from several studies (Gere 1964, Giurginca et al. 2012, Šustr et al. 2005, 2009). The species consumes a mixture of organic and inorganic substrates, e.g. bat guano, rotting wood rests, macroscopic fungi, and algae, with a clear preference for grazing on cave sediment (Giurginca et al. 2012). Its temperature tolerance ranges from -1.5 to 18.5 °C (Gere 1964, Šustr et al. 2005, Šustr et al. 2009, Smrž et al. 2015).

In our study we aimed to define the population characteristics as well as to clarify the life history traits of the species. Our goal was to reveal sex ratio and size distribution of the *M. graniger* population. We aimed to define the average number per female and the average size of offspring. We were particularly interested in the possible ‘trigger’ and any other factors affecting timing of the reproduction of this species under the constant cave conditions, as well as in longevity of the individuals.

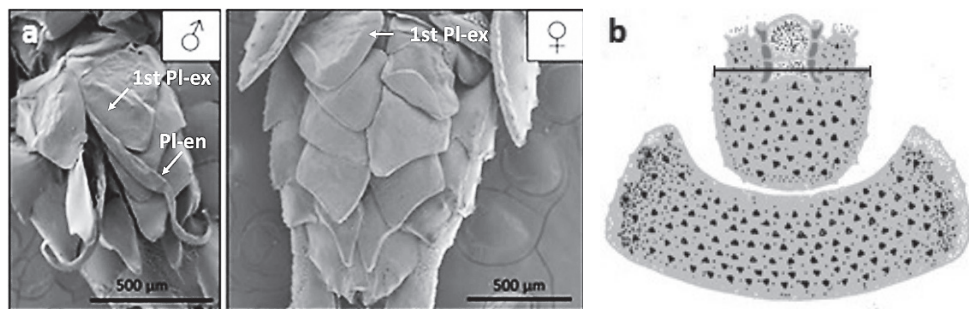
## Materials and methods

### Collection of individuals

To estimate sex ratio and size distribution of the population composite pitfall-trap material was used. Traps were placed at 16 plots, in several arms of the Baradla – Domica cave system (in Hungary and Slovakia) in 2012–2013 [Research program: „Management of caves of the World Heritage in the Aggtelek and Slovak Karst“, (HUSK/1101/2.2.1./0180)]. The traps worked through different time periods (mainly four months long). The collected individuals were stored in 70% ethanol.

### Sex and size identification

Sex was determined by the presence (♂) or lack (♀) of pleopodite-exopodite extensions (Fig. 1a). The size of individuals was estimated on the basis of head width (Sutton 1968). Digital images were taken under a stereomicroscope (Nikon SMZ800; Nikon E4500 camera) about the head region. The distance between two fixed points of the frontal edge of head capsule was taken as head width (Fig. 1b). The distance was calculated on the images using ImageJ software (<https://imagej.nih.gov/ij/>). Juvenile category was determined by size smaller than the minimum value of males.



**Figure 1.** **a** Sexual dimorphism of *Mesoniscus graniger*. Scale bar 500 µm. Key: 1<sup>st</sup> Pl-ex – first pleopodite-exopodite, Pl-en – pleopodite-endopodite **b** black line – head-width measurement.

## Model populations

To explore components of life-history characteristics, such as growth, reproductive events, offspring number, and size, individuals were collected and model populations ( $N = 15$ ; 12 randomly selected adults in each container; a total of 180 individuals) were set up under natural conditions in a side branch of the Baradla cave (Róka-branch; Aggtelek National Park, Hungary) in March 2016. We used a modified method of Vilisics et al. (2012) to prepare microcosm dishes. Transparent polyethylene containers of 8 cm in height and 9 cm in diameter, without lids were covered by gauze net to ensure ambient humidity but to prevent accidental escape of individuals. The bottom of the containers were filled with a layer of plaster of Paris. Solid plaster has been shown to be suitable in laboratory experiments (e.g., Hassall et al. 1987, van Vliet et al. 1993). We mixed gypsum with activated charcoal powder to detect tiny, depigmented, white animals on a dark background. The bottom of dishes was in contact with the cave floor to ensure the usual wet conditions (Vilisics et al. 2012; Fig. 2).

The source of nutrition was plant debris, bat guano, and loam collected in the cave, placed in small Petri dishes sunk in the plaster of Paris in a central position. Air temperature and relative humidity were measured continuously by a Voltcraft DL-121TH USB data logger. The background factors were constant: 24 hrs dark, temperature 10 °C, and humidity 97%.



**Figure 2.** Microcosm model populations in the Baradla cave (north-eastern Hungary). **a** *Mesoniscus graniger* individuals aggregating on decaying wood **b** plan view of a microcosm container with food in the middle **c** schematic cross section of a container.

Growth, reproduction, and offspring data

The status and number of individuals and the appearance of new-borns were monitored on a monthly basis (March to October, 2016). The changes were followed at the model populations’ level. In order to minimize external disturbance, we took digital images of each model population with a Nikon D5100 camera. Offspring release could be stated by changes in individual numbers and by size differences (Fig. 4a). The length of individuals (the distance between the medial lobe of the head to the end of the pleotelson) was measured on the photos applying ImageJ software. The camera was placed at a fixed, constant height at each occasion and a calibration scale was added into the container (Fig. 4a).

Statistics

All statistics were performed with R 3.2.5 RStudio software.

Assuming that the sex ratio for both sexes was 50%, our statistical null hypothesis was that the difference between the two ratios was zero. A binomial test was used to estimate the sex ratio, and the ratios obtained were tested by a generalized linear model (GLM, family = ‘binomial’). The difference between the male and female mean head width was tested by a Welch test.

In the case of each model population temporal changes in the minimum body length values were compared in monthly intervals. Numbers of 0 (no or positive change) or 1 (decreased minimum value) were added depending on results for each microcosm in each consecutive month. We used a 15-element vector for each month based on summed 0 and 1 values. Our statistical null hypothesis was that 50% was the probability of finding smaller sized individuals in any container compared to the previous month. The hypothesis was tested by a binomial test.

The normality of head width and body length variables was tested before the statistical analysis (QQNORM in R 3.2.5).

Results

Sex ratio and size distribution

Isopods collected in the pitfall traps were sorted into females, males and juveniles by their sexual characters and size, respectively (Table 1). Based on the binomial test (95% confidence interval) male ratio ranged from 0.39 to 0.47, while that of females

Table 1. Distribution of pit-fall trapped individuals by gender and age.

	Males	Females	Juveniles	ΣN
number	280	375	22	677
rate	0.41	0.55	0.04	

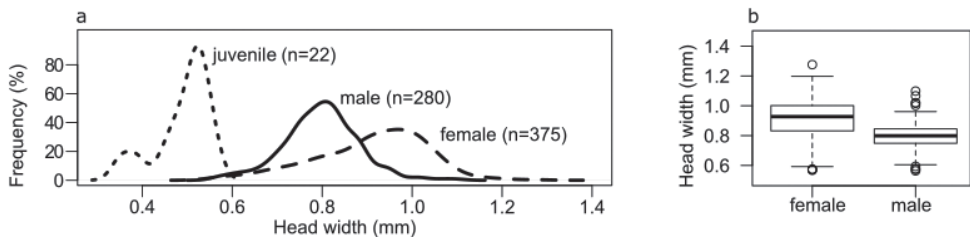


from 0.53 to 0.61. Sex ratio was significantly overweighed for females (GLM test,  $p$ -value  $< 0.001$ ).

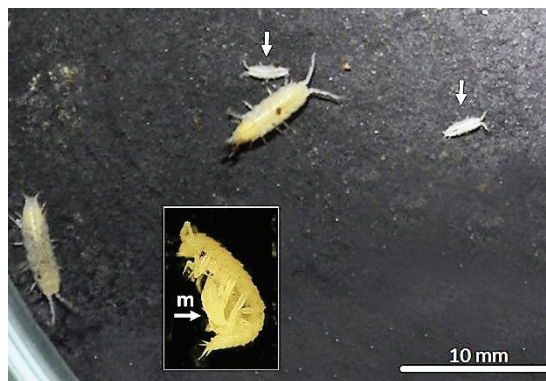
While females had an average head width of 0.87 mm ( $SE \pm 0.18$  mm), the value for males was 0.79 mm ( $SE \pm 0.08$  mm) (Fig. 3a, b). Distribution of both male and female head width data was near to normal (Fig. 3). The mean head width of females was significantly higher than that of males (Welch test;  $p$ -value  $< 0.001$ ). There were two markedly separable peaks in the case of juveniles; one between 0.3–0.4 mm and the other at approximately 0.5 mm.

### Reproduction events, number, and size of offspring

During the study period (8 months) we observed juveniles in 8 containers. Offspring could be identified by their size (Fig. 4). Their number was low: 3–5 individuals per breeding occasion (female)  $4.2 \pm 0.5$  specimens on the average. Mancas were approx. 1/3 of the size of females; average adult size and manca size is  $6.65 \text{ mm} \pm 0.68$ ;  $4.22 \pm 0.53$  mm respectively (Fig. 4 insertion). Based on the binomial tests performed, the probability of reproduction, on the model population's level, was between 30% and 40% in each month.



**Figure 3.** a Frequency of size distribution of the studied *Mesoniscus graniger* population by gender and age b Significant differences in head width distribution (average  $\pm$  SE) of the sexes (Welch test;  $p$ -value  $< 0.001$ ).



**Figure 4.** Adults and juveniles (juv – white arrows); insertion: Pregnant female giving birth to a manca (m).



## Discussion

Assuming that males and females appeared in the traps with equal probability over the sampling period, the sex ratio of *Mesoniscus graniger* was significantly shifted towards females ( $\sigma:\varphi = 0.41:0.55$ ). Similarly, the annual distribution of sexes in *Porcellionides pruinosus* (Brandt, 1833) species ( $\sigma:\varphi = 0.35:0.65$ ) was shifted to females (Achouri et al. 2002). Male to female ratio was found to be 1:5 in *Chetophilosciasicula* Verhoeff, 1908 (Hornung and Szlavecz 2003). Warburg and Cohen (1991) found a fixed male:female ratio of 1:6 in *Schizidium tiberianum* Verhoeff, 1923, a semelparous species, while Hornung and Warburg (1994) mentioned a constant sex ratio for *Porcellio ficulneus* Budde-Lund, 1885, a facultative iteroparous species ( $\sigma:\varphi = 1:4$ ). In published case studies authors found either equal (1:1) sex ratio (e.g., Carefoot 1973, Tomescu et al. 1992, Warburg and Cohen 1992, Accola et al. 1993, Warburg 2007) or female dominance (Dangerfield and Telford 1994), or sometimes male dominance (Tomescu et al. 1992) for the different species. In iteroparous species females usually prevail over males (Williams and Franks, 1988). The difference in sex ratio might reflect real values characteristic for the population, but in most cases it means a temporal pattern caused by the different surface activity of the sexes (Dangerfield and Hassall 1994). The data of Oberfrank et al. (2011) on *Protracheoniscus politus* (C. Koch, 1841) supports the hypothesis of Dangerfield and Hassall (1994): an early male activity was followed by extremely low male presence after onset of reproduction. Hornung et al. (2015) found that male ratio varied over time in the populations of *Trachelipus rathkii* (Brandt, 1833) and *Cylisticus convexus* (De Geer, 1778) as well. The highest and lowest male : total ratio was found to be 0.57 and 0.26 for *T. rathkii*, 0.61 and 0.27 for *C. convexus*, respectively. This pattern can be explained by the different surface activity of sexes in time: first by the mate search of males and later by the ideal shelter search of gravid females (Dangerfield and Hassall 1994).

Year-round reproduction was reported for *P. pruinosus* (Dangerfield and Telford 1990) and for *Atlantosciana floridana* (van Name, 1940) (Araujo and Bond-Buckup 2005) under seasonal climate but with relatively favourable microclimate. While *P. pruinosus* was collected in synanthropic habitats which were partially buffered from extreme environmental conditions, *A. floridana* lived in habitats with soil temperature varying from 7.5 to 24.6 °C and litter temperature oscillating between 7.0 and 28.0 °C but little variation in air relative humidity (68.9% to 88.8%). Continuous breeding was also shown in *Porcellio olivieri* Aud. et Sav., and *Agabiformius obtusus* (Budde-Lund, 1909) (Warburg 1995) under laboratory conditions.

Relative to the adults, few juvenile specimens were collected by the pit-fall traps (Table 1). This might be caused by the selectivity of the method (Topping and Sunderland 1992) and by the low dispersion ability of small juveniles. The bimodal size distribution of immature isopods (Fig. 3a) probably reflects the presence of both manca (head width between 0.3–0.4 mm) dropped out from the marsupium of trapped pregnant females and larger juveniles (head width 0.5 mm).

Apparent sexual dimorphism in size is not common in terrestrial isopods. One example is in *P. ficulneus* populations where males are significantly smaller in size than females (Hornung, unpubl). Similarly, females were found significantly larger than males both in body length, and in head width in *C. sicula* in a North-American established introduced population (Hornung and Szlavecz 2003).

From our observations we conclude that *Mesoniscus graniger* is able of continuous reproduction on population level at least from early spring to late autumn, during our study period. Under the constant conditions of the cave environment (10 °C, 97% RH, complete lack of light), reproduction has no abiotic triggers. It is assumed that sexual maturation of females is controlled by their critical mass (Caubet 1998). Female size might be the trigger of reproduction under these conditions. This assumption would be consistent with the constant presence of males but further field studies have to verify this speculation. The low number of offspring per female (in our experiments 3–5), and the large size of the mancas indicate “K” (stenodynamic) life history strategy.

Based on laboratory observations we cannot state but we can assume that the life expectancy of individuals is relatively long, at least 1–1.5 year. By the features found (number of offspring, size of mancas), *M. graniger* can be classified into the stenodynamic life history group together with other endogeic species (Sutton et al. 1984).

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# The role of urban forest patches in maintaining isopod diversity (Oniscidea)

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## Abstract

Compositional changes in natural communities associated with anthropogenic influence often lead to localised extinctions and biodiversity loss. Soil invertebrates are also threatened by urbanisation due to habitat fragmentation, vegetation changes and management, soil alteration, degradation, and disappearing shelter sites. The aim was to assess terrestrial isopod (Oniscidea) assemblages in differently degraded urban forest patches of a metropolitan area (Budapest, Hungary). Study sites were compared by their species richness, composition and the relevant background factors (soil properties, dead wood, litter characteristics, and canopy closure). The degree of urban disturbance was expressed using an urbanisation index (UI) based on built-up density and vegetation cover. The isopods were identified to species level, and were qualified by their habitat preference and naturalness index (TINI). Average Rarity Index (ARI), derived from TINIs provided information on the degree of naturalness/disturbance of each habitat. Altogether 14 isopod species were collected from 23 sample sites. Urbanisation indirectly affected on the composition of isopod assemblages through the quantity of dead wood and soil plasticity. ARIs and UIs of sample sites were negatively correlated. Urban patches harboured habitat generalist, synanthropic and established introduced species with low naturalness value of assemblages. Areas with no or low anthropogenic disturbance maintained stable native, autochthonous assemblages that were characteristic of rural sites in the region. Transitional zones between rural and urban habitats usually maintained a mixed isopod fauna consisting of both urban and rural elements.



**Keywords**

biotic homogenisation, disturbance tolerance, ecological character, habitat specialist, urbanisation index, woodlice

**Introduction**

Currently increasing number of studies explore the effects of urbanisation on biological communities at a global level (Niemelä et al. 2000, McKinney 2008, Richter and Weiland 2012, Wang et al. 2012a). The alteration and fragmentation of natural habitats generally leads to a shift in species composition, resulting in biotic homogenisation and changes in ecosystem services as well (McPherson 1998, Whitford et al. 2001, McKinney 2006, Tratalos et al. 2007). Human activity, such as construction industry, air pollution and pollutant emissions of vehicles and the use of chemicals, contributes to urban soil degradation (Pouyat et al. 2008).

The majority of soil invertebrates are highly sensitive to disturbances (Barbercheck et al. 2009) and environmental changes (Santorufu et al. 2012). This includes the macrodetritivore fauna, which has an important role in the ecosystems' nutrient cycling. These invertebrates fragment dead plant material through their feeding activity increasing its surface area and promoting microbial decomposition (Hanlon and Anderson 1980, Bardgett 2005). Woodlice (Isopoda: Oniscidea) are one of the major invertebrate group contributing to these processes (Anderson 1988, Paoletti and Hassall 1999).

Terrestrial isopods can be used as ecological indicators of habitat qualification. They are widespread, limited in their dispersal abilities, and relatively easy to collect and identify. Based on a single species' ecological needs and tolerances, species composition informs us about habitat characteristics including habitat disturbance/naturalness (Vilisics et al. 2007, Tuf and Tufová 2008, Hornung et al. 2008, 2009). Urban areas are hot spots for species introduction (Vilisics and Hornung 2009) threatening natural communities. Successfully established introduced species are usually eurytopic and/or cosmopolitan ones leading to global urban soil fauna homogenisation and convergence (McKinney 2006, Pouyat et al. 2015).

To study the effects of urbanisation on oniscidean fauna differently urbanised woodland habitat patches were compared. We expected that our data would be in accordance with the following hypotheses:

- (1) the 'Intermediate Disturbance Hypothesis' (IDH; Connell 1978) that predicts diversity being the highest in habitat with moderate levels of disturbance;
- (2) the 'Habitat specialist hypothesis' that indicates that the abundance and species richness of forest specialist species will decline along a rural–suburban–urban gradient (Magura et al. 2008) and
- (3) the 'Synanthropic species hypothesis' that predicts that abundance and species richness of synanthropic species will increase along a rural–suburban–urban gradient (Magura et al. 2008).

## **Materials and methods**

### **Study sites and design**

The Budapest metropolitan area is divided by the Danube River, which separates the two major parts of the city, Buda and Pest. Buda can be characterised by a uniform parent rock (primarily limestone and dolomite). The area included in this study is in the urbanised area of the Buda Hills. Historically, Buda was covered by continuous natural forest that was fragmented by the growing city. Rural and differently degraded urban forests and other woody patches (e.g. planted forests, parks, gardens, and cemeteries) were selected in Buda (Fig. 1). Rural forests, situated in the Buda Landscape-Protection Area, represented semi-natural woodlands.

### **Landscape structure characteristics**

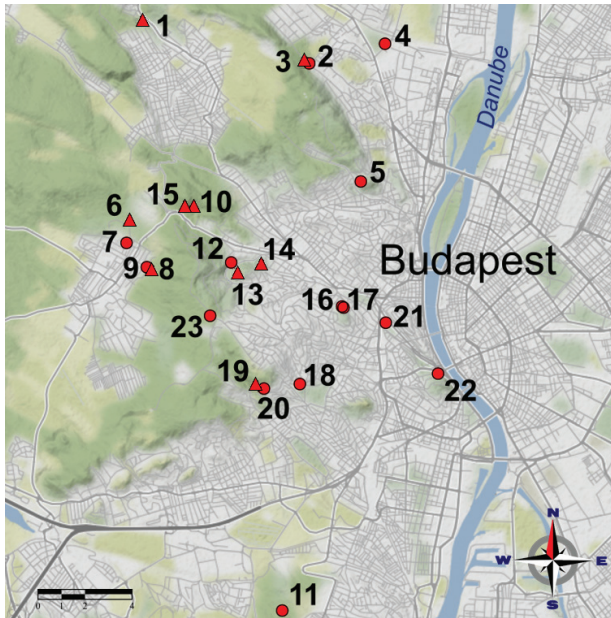
To quantify urbanisation intensity an urbanisation index (UI) was applied as proposed in Seress et al. (2014). Vegetation cover, building density, and the presence of sealed surface (roads) were scored for 100 cells of 1 km<sup>2</sup> area around each study site using the QGIS software (version: 2.16). For each site, the urbanisation index was calculated by extracting the first principal component (PCA1) from a principal component analysis (PCA) of five urbanisation variables (mean building density, number of cells with high building density, number of cells with road, mean vegetation density, number of cells with high vegetation density).

### **Soil sampling and analyses**

Composite samples were taken from 0–15 cm of the topsoil layer of each study site. Soil physicochemical properties were determined at the Soil Conservation Laboratory of National Food Chain Safety Office (Velence, Hungary). Soil pH (H<sub>2</sub>O) was measured in 1:2.5 soil:water suspensions for 12 h after mixing. Soil organic matter (SOM, m/m %) was determined by the standard ignition method. Total soluble salt content of the soil (m/m %) was measured with a conductometre (Radelkis OK-102/1). To characterise soil texture, the soil plasticity index ( $K_A$ ) that refers to the soil clay content, was applied (MSZ-08-0205 1978). Soil CaCO<sub>3</sub> (m/m %) was determined with a Labor MIM calcimetre (MSZ-08-0206-2 1978).

### **Vegetation characteristics**

Structural attributes of vegetation important for isopods were recorded using a 10 × 10 m quadrat at each site, in May and October, 2016. Percentage cover of dead wood



**Figure 1.** The arrangement of sample sites (23) in Buda, the western, hilly side of Budapest. The numbers indicate the sample sites (see Suppl. material 1). Symbols indicate rural (▲) and disturbed (●) habitats.

and litter, canopy closure was estimated visually (Jennings et al. 1999, Humphrey and Bailey 2012), while litter depth was measured with a ruler. For data analysis, variables were classified into the following categories:

- amount of dead wood: 1 (0 %), 2 (0–20 %), 3 (> 20 %)
- litter cover: 1 (0–35 %), 2 (36–65 %), 3 (66–100 %)
- canopy closure: 1 (0–35 %), 2 (36–65 %), 3 (66–100 %)
- litter depth: 1 (0 cm), 2 (0–1.5 cm), 3 (> 1.5 cm)

### Isopod sampling and species/habitat qualification

Terrestrial isopods were collected by time-restricted hand sorting (60 minutes per site) during their main activity seasons, in May and October, 2016. To ensure that rare or habitat specialist species were not missed, special attention was paid to favourable microhabitats, such as leaf litter, fallen tree trunks or branches, and shelter sites under bark and stones. Individuals were preserved in 70 % ethanol and later identified to species level using the key by Gruner (1966). Species nomenclature follows Schmalzfuss (2003).

Isopod species were categorised using the terminology by Williamson and Fitter (1996). Introduced species with a self-sustaining population were called ‘established introduced’. Species distributed in most continents were considered ‘cosmopolitans’.

Synanthropic label was applied for species that are connected with built-up areas and do not occur in the wild. Non-native species were those that went through adaptation and occur in suburban – semi-natural fringe areas. Native species are autochthonous and/or have been established by dispersal/dispersion presumably before historical times.

In species qualification we utilised the Terrestrial Isopod Naturalness Index (TINI) under development. This additive index is based on the following attributes of the single woodlouse species: global (cosmopolitan – endemic), regional (frequent – rare) distribution, ecological (habitat generalist – specialist) and disturbance tolerance (Hornung et al. 2007a, 2008, 2009). In this ranking system introduced and/or common species are assigned 0 or low while endemic and/or rare species receive high scores (max. 20). Species found in this study were ranked based on the nomenclature of Hornung et al. (2007a, 2008).

The ecological tolerance of woodlice sets the limits of their occurrence. Considering their poor dispersal abilities (philopatry) and the ecological features (TINI) of the species present at a location makes them available for the characterisation of the habitats in question: species composition reflects habitat quality. By applying TINI scores of species to an assemblage one can compile scores assigned to the habitat. The summed TINI indices of species standardised by the number of species present gave Average Rarity Index ( $ARI = \Sigma TINI/N$  where  $N$  is the number of species in the assemblage). This index results in a novel way to compare different localities involving a single species' naturalness – disturbance tolerance. This way it gives a more realistic, qualitative biodiversity indicator than simply species richness.

## Statistical analyses

All statistical analyses were performed in R software version 3.2.5., using the R packages 'lme4' (Bates et al. 2015) and 'mvabund' (Wang et al. 2012b). Hierarchical cluster analysis was carried out with the software PAST 3.10 (Hammer et al. 2001). Relationships between species richness, composition of isopods and environmental variables (soil properties, vegetation characteristics, and urbanisation index) were tested by generalised linear mixed models (GLMMs). After fitting the full models for each dependent variable, Akaike Information Criterion (AIC) was used to select the most parsimonious model. For species composition analyses, GLMMs with a multivariate approach were applied. Since we had presence-absence data, the 'manyglm' method (family = binomial) was used. The sampling time (spring, autumn) was considered as a random factor in the models. To compare isopod assemblages of study sites, a hierarchical cluster analysis with the Jaccard similarity index was carried out. The myrmecophilous *Platyarthrus hoffmannseggii* was excluded from this analysis because its accidental occurrence connected to ant nests. Spearman rank correlation tests were performed to examine relationships between soil properties, ARI and UI. Assumptions of normality and homoscedasticity of the residuals were verified visually using diagnostic plots. Statistical significance was determined at the level:  $\alpha = 0.05$ .

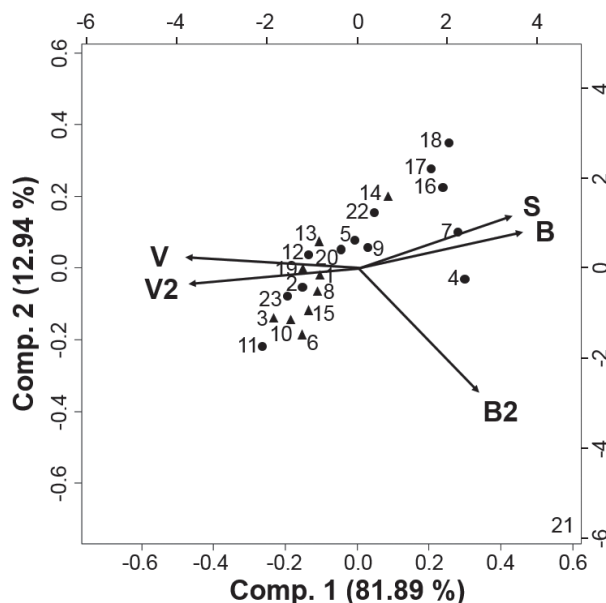
## Results

### Urbanisation intensity of sample sites

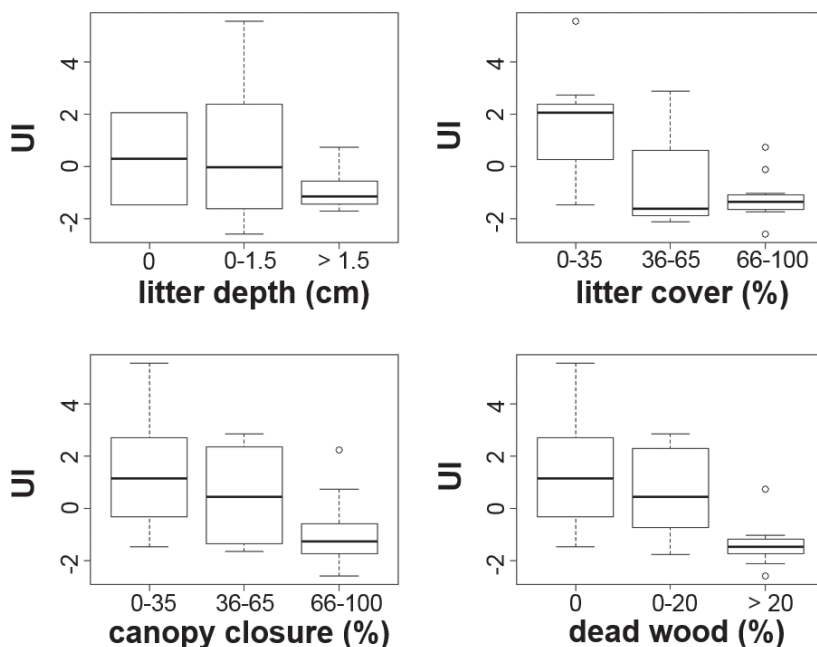
The 23 sampling locations in this study had the same parent material, but differed in the amount of woody vegetation cover that varied from planted trees through isolated forest remnants to rural forests. Sampling plots represented differently urbanised habitat fragments. Intensity of anthropogenic disturbance was expressed in urbanisation indices (UI). Urbanisation indices ranged from -2.58 to 5.56 (Suppl. material 1) with higher values indicating more urbanised habitats. According to the results of PCA, sample sites were arranged along a gradient that reflects the intensity of urbanisation (Fig. 2). The pattern showed a more or less continuous transition from rural to the most disturbed habitats. The first two principal components explained 94.83 % of the total variance of the dataset.

### Relationship between soil, vegetation characteristics, and urbanisation intensity

The soils of the sample sites did not show high variability, with the exception of the  $\text{CaCO}_3$  content (Suppl. material 1), which had a positive correlation with the urbanisa-



**Figure 2.** PCA biplot of the sample sites according to the urbanisation variables. Abbreviations: B - mean building density, B2 - number of cells with high building density, S: number of cells with road, V: mean vegetation density, V2: number of cells with high vegetation density. Numbers are sample site (same as in Fig. 1). Symbols indicate rural (▲) and disturbed (●) habitats.



**Figure 3.** Box plots of urbanisation intensity (UI) according to vegetation characteristics. Horizontal line within a box indicates the median.

tion index ( $r_s = 0.377$ ,  $p = 0.001$ ). In contrast, SOM content ( $r_s = -0.363$ ,  $p = 0.013$ ) and soil plasticity ( $K_A$ ) ( $r_s = -0.321$ ,  $p = 0.029$ ) were negatively influenced by urbanisation intensity. Soil pH and total soluble salt content showed marginally significant positive relationships ( $r_s = 0.256$ ,  $p = 0.086$  and  $r_s = 0.250$ ,  $p = 0.095$  respectively) with urbanisation. More natural habitats (with lower UIs) had higher litter depth and cover, canopy closure and amount of dead wood compared to the more urbanised ones (Fig. 3).

### Isopod diversity and species scores

Altogether 14 isopod species were recorded during the survey (Table 1). Eight of them were native, but differed in habitat preference and frequency of occurrence. Four species were categorised as cosmopolitan, one as established introduced, and three were synanthropic (Table 1). *Armadillidium vulgare*, *Orthometopon planum*, and *Protracheoniscus politus* were the most widespread ones among the sampled spots. The distribution of *A. vulgare* differed from that of the other two species: the presence of the latter two indicated low or no human disturbance (low or negative UI indices, high ARI values of sampling localities). *Armadillidium vulgare* showed up as a constant element of urbanised neighbourhood, sometimes being the only representative of the isopod fauna (site 11). It occurred in 13 out of the 23 sampled habitats. These plots proved to

**Table 1.** The collected Oniscidea species, their naturalness scores (TINI), and frequency of occurrence. (Categories are given according to Hornung et al. 2008 and are valid for the Pannonian region).

Family	Species	Species category	TINI	Number of sites of occurrence
Agnaridae	<i>Orthometopon planum</i> (Budde-Lund, 1885)	native frequent	19	11
	<i>Protracheoniscus politus</i> (C. L. Koch, 1841)	native frequent	17	9
Armadillidiidae	<i>Armadillidium vulgare</i> (Latreille, 1804)	cosmopolitan, widely distributed	9	13
Cylisticidae	<i>Cylisticus convexus</i> (De Geer, 1778)	established introduced	10	3
Platyarthridae	<i>Platyarthrus hoffmannseggii</i> Brandt, 1833	cosmopolitan widely distributed	12	5
Porcellionidae	<i>Porcellio scaber</i> Latreille, 1804	cosmopolitan synanthropic	3	8
	<i>Porcellio spinicornis</i> Say, 1818	established introduced synanthropic	8	3
	<i>Porcellionides pruinosus</i> (Brandt, 1833)	cosmopolitan synanthropic	7	3
Trachelipodidae	<i>Porcellium collicola</i> (Verhoeff, 1907)	native widely distributed	10	4
	<i>Trachelipus nodulosus</i> (C. L. Koch, 1838)	native widely distributed	11	1
Trichoniscidae	<i>Androniscus roseus</i> (C. L. Koch, 1838)	native	13	1
	<i>Haplophthalmus mengii</i> (Zaddach, 1844)	native	13	1
	<i>Hyloniscus riparius</i> (C. L. Koch, 1838)	native widely distributed	10	4
	<i>Trichoniscus pusillus agg</i> Brandt, 1833	native widely distributed	12	3

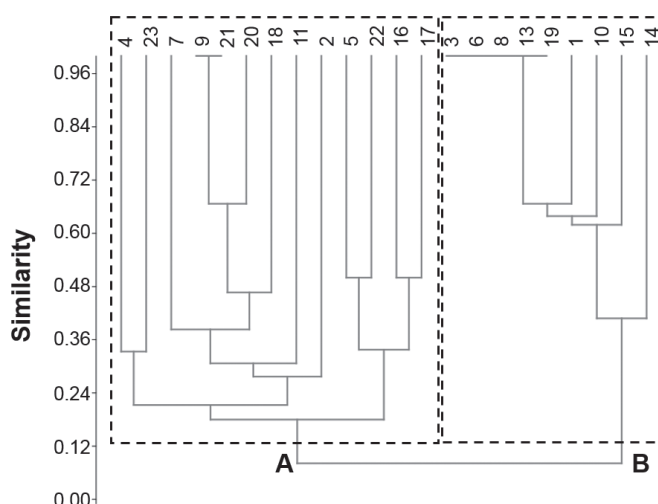
be highly urbanised, or in some cases transitional areas. No rare (either native rare or disturbed rare) species were found.

The native species (*O. planum*, *Pr. politus*, *Porcellium collicola*, *Androniscus roseus*, *Haplophthalmus mengii*, *Hyloniscus riparius*, and *Trichoniscus pusillus*) were restricted to rural and/or fringe areas. Cosmopolitan, introduced established and synanthropic species included *A. vulgare*, *Cylisticus convexus*, *Porcellio scaber*, *P. spinicornis*, and *Porcellionides pruinosus* and occurred in disturbed, human dominated places. The typical urban isopod assemblage consisted of *A. vulgare*, *P. scaber*, *Ps. pruinosus* (in order of prevalence), and occasionally *P. spinicornis* and *C. convexus*. These species were not found in rural woodlands.

Widely distributed, hygrophilic, mainly endogeic species were: *A. roseus*, *H. mengii*, and *Hy. riparius* (sites 2, 4, 16, 18 and 23). A highly managed urban park (site 21) and green verges along pavements (sites 7, 9) still harbour one or two woodlouse species, most often *A. vulgare* and/or *P. scaber*. Fringe areas, that is, rural – urban transition zones or ecotones (sites 2, 4, 14, 16, 17, and 18) had a mixed isopod fauna, resulting the highest species richness (Suppl. material 2). High species dissimilarity (85–100%) occurred within ten metres (sites 2, 3 and 8, 9) in localities where built-in area sharply turns into quasi-natural forest.

The hierarchical cluster analysis clearly separates the isopod assemblages into two groups (Fig. 4). Group 'A' consists of urbanised or transitional habitats dominated by the previously mentioned urban species assemblage, while group 'B' refers to rural areas predominated by the presence of *O. planum* and *Pr. politus*. Site 14 seemed to be separated within group B.





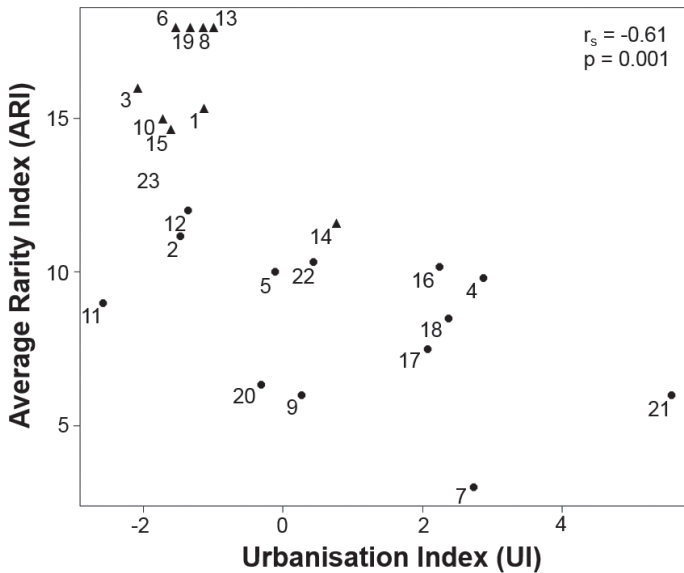
**Figure 4.** Hierarchical cluster analysis dendrogram showing two main groups (A and B) based on the species composition of isopod assemblages. Numbers on the top are the numbers (ID-s) of the sample sites (for IDs see Suppl. material 1).

### Effects of habitat characteristics on woodlice assemblages

According to the results of the GLMMs, the quantity of dead wood (Dev = 108.27,  $p < 0.001$ ) and soil plasticity (Dev = 49.36,  $p = 0.002$ ) were the most significant environmental variables affecting species composition. *Orthometopon planum* and *Pr. politus* (native species with high TINIs) preferred sample sites with high amount of dead wood (Dev = 13.88,  $p = 0.005$  and Dev = 23.23,  $p = 0.001$ , respectively), while *P. scaber*, a cosmopolitan species with low TINI showed the opposite trend (Dev = 22.03,  $p = 0.001$ ). The presence of *A. vulgare*, a cosmopolitan species with medium TINI, was primarily determined by soil texture (Dev = 8.49,  $p = 0.05$ ): it was mainly found in habitats where soil clay content (expressed in  $K_A$ ) was low. While individual species exhibited preference towards certain habitat parameters, total species richness of isopods was not affected.

### Relationship between Average Rarity Index (ARI) and urbanisation intensity (UI)

The Spearman rank correlation test showed negative correlation between ARI based on species qualification of isopods and UI (Fig. 5). The ARI values significantly decreased with the urbanisation level of habitats. Rural, semi-natural habitats form a group (see ▲ symbols on Fig. 5) with the highest ARI scores (13 – 18) and low UIs (-2.11 – -1.03, except site 14 with UI = 0.74). ARIs of urban and fringe habitats are under these values (6 – 13), while UI scores of this group are highly variable (-2.58 – +5.56).



**Figure 5.** Average Rarity Index (ARI) decreased with higher urbanisation (UI). Higher value of UI means increasing urbanisation. Numbers are habitat identifiers, see Fig. 1 and Suppl. material 1 (▲ – rural, ● – differently disturbed habitats).

## Discussion

Urban soils are overwhelmed by strong human physical effects (e.g. grading and irrigation) and tend to lack the effects of native factors (e.g. topography and drainage) that formed development of soil characteristics during a long time period. These soils vary widely in their characteristics and are dependent on both direct and indirect effects resulting from urban land use and cover change (Pouyat et al. 2010). The various plots differed slightly in soil parameters, mainly in  $\text{CaCO}_3$  content. In accordance with results of previous urban studies (Craul and Klein 1980, Short et al. 1986) higher  $\text{CaCO}_3$  content, higher pH, but lower humus content and soil plasticity ( $K_A$ ) was experienced in habitats with high urbanisation index compared to rural sites. Building materials, for example concrete, asphalt and bricks can be the sources of increased  $\text{CaCO}_3$  level (Alexandrovs-kaya and Alexandrovskiy 2000), which can lead to higher soil pH (Pouyat et al. 2015).

The 14 isopod species found in our survey represent 50% and 25% of the recorded fauna of Budapest and Hungary, respectively (Hornung et al. 2007a, 2008, Vilisics and Hornung 2008, 2009). Korsós et al. (2002) and later Vilisics and Hornung (2009) summarised data on the oniscidean fauna of the entire metropolitan area of Budapest, including different kinds of man-made habitats, e.g. gardens, courtyards, densely built-up areas and botanical gardens. The present study focused only on a subset of the diverse urban habitat types on the Buda side of the city and thus fewer species were detected.

Although average species richness ( $\alpha$  diversity) is usually low in Hungary, three species per location on average (Hornung et al. 2008), in the present study 6 locations out

of the 23 (26%) resulted 4–6 species. These habitats could be referred to fringe areas. The species number and assemblage composition of some other European cities are also known. In an urban study of Olomouc, Czech Republic, sampling city parks, built up areas, gardens, ruderal and natural habitats altogether 17 woodlice species were collected (Riedel et al. 2007). In relation to habitat naturalness the species spectrum was dominated by adaptable and eurytopic species (sensu Tuf and Tufova 2008) meaning habitat generalist species. Ferenți et al. (2015) published eleven species from 19 localities within Salonta town (western Romania) with the highest (6) species richness from a wetland habitat and most sampling sites harbouring only one or two species. In Bucharest (Romania) 17 species were sampled by Giurginca et al. (2017). In three Swiss cities 17 species were mentioned by Vilisics et al. (2012). The highest species richness was found in Lucerne (13), while Zurich had eleven and Lugano nine species. The data mentioned above are influenced by difference in sampling methods, but give a tentative picture of the species richness of different geographic regions. The natural species pools surrounding urban areas in the different (bio)geographical areas may also differ within Europe. In addition to the basic European fauna they may contain different regional biogeographical elements.

The results of the present study failed to fulfil the requirements of the Intermediate Disturbance Hypothesis. There was no consistent pattern in species richness distribution. However, urban habitats harboured more species on average, but without any statistically significant correlation. In a rural – urban gradient study (Debrecen, Eastern Hungary) IDH was also not proved for woodlice, while it was valid for millipedes (Hornung et al. 2007b, Bogyó et al. 2015). In another extensive urban study (Pécs/Hungary; Farkas and Vilisics 2006) either habitat generalists (*A. vulgare*, *Po. collicola*, *Trachelipus rathkii*) or synanthropic species (*P. scaber*, *C. convexus*, *Ps. pruinus*) dominated densely built-in areas while in the city edge ecotone zones the more habitat specialists also joined the assemblages (*T. nodulosus* and *Pr. politus*). Rural – urban transition zones and ecotones also had a mixed isopod fauna in the present study.

The Habitat specialist and the Synanthropic species hypotheses (Magura et al. 2008) were confirmed by the present study. We experienced a species exchange between habitat specialists and generalists and/or synanthropic species along the rural – urban gradient. Typical forest species (*Pr. politus*, *O. planum*) were constant elements of rural habitats while habitat generalist and/or synanthropic species (porcellionids, *A. vulgare*, *C. convexus*) constituted the species assemblages of urbanised forest patches.

The apparent negative association of *P. scaber*, a typical urban faunal element, with dead wood can be explained by its high tendency for aggregation (Broly et al. 2012). Hornung et al. (2008) found that species in the family Porcellionidae show a clear preference for human settlements in Hungary. *Porcellio scaber*, *C. convexus* and *Ps. pruinus* are typical elements of urban ecosystems and farmlands. They are common also in many other parts of the world (Hornung et al. 2008). The unusual appearance of species with a low desiccation tolerance (Trichoniscidae species with hygrophilic and/or endogeic nature) was always connected with some kind of constant water supply, e.g. public artesian wells (sites 4, 18, 16). The significant correlation of *O. planum* and

*Pr. politus* with the amount of wood debris can be attributed to their typical sylvicolic nature (Hornung et al. 2008, Tomescu et al. 2008). Geographical distance may also be important. The inner city is fairly isolated from rural areas preventing species dispersal and colonisation.

Isopods are indicators of the naturalness of vegetation, and the quality and quantity of dead wood and litter in their habitats, which are used by them both as food and shelter (Rushton and Hassall 1983). Habitats with a lot of dead wood were favourable for many natural species. Dead wood was the main driver of species composition in isopod assemblages, probably because they largely contribute to the supply of organic matter and affect microclimatic variables, e.g. humidity. Preventing removal of dead wood from urban green spaces could enhance the survival of the epigeic invertebrate fauna (Bang and Faeth 2011). This indicates the importance of small-scale management decisions on local biodiversity. Urban planning and changes in the management of public areas, e.g. retaining leaf litter, might be advantageous for soil fauna survival which in turn provide food for various vertebrates and may increase biodiversity as a whole (Stagoll et al. 2010, Threlfall et al. 2016).

However, the  $1 \times 1$  km area units used for determination of UIs seems to be a too broad a scale compared to the small-scale heteromorphic sensibility of the investigated flightless epigeic macrodetritivore fauna. Their habitat preference may be controlled on a much finer scale (see also McCary et al. 2017). UI might be also misleading in some cases as e.g. forests with high hiking traffic but without any buildings and sealed surfaces (sites 11 and 23 in our case). These showed low urbanisation (negative UI scores) and naturalness values (ARI) but impact of anthropogenic disturbance is hidden. Similarly, sharp habitat boundaries with mixtures of urban and rural species explains the exceptional position of sample site 14 on Figs. 4 and 5. The UIs and the total naturalness index of isopod species ( $\Sigma$ TINI) at the sample sites did not show significant relatedness, but the habitats' ARIs did, which confirms previous statements (Hornung et al. 2009): ARI is a good tool in estimating habitats' naturalness as it involves the species' ecological features and enables a more refined evaluation. High species number does not always mean high naturalness from a conservation biological point of view.

Urbanisation often leads to changes in species richness and community composition. New landscapes and habitats are formed that do not occur elsewhere (Niemelä 1999, Mabelis 2005). Species have different responses to anthropogenic habitat modification, depending on their ecological needs and tolerance. Urbanisation worldwide is accompanied by the occurrence and dominance of habitat generalist species with broad tolerances and the establishment of introduced, mainly synanthropic species. These changes lead to homogenisation and convergence of urban faunas on both local and global scales.

## Conclusions

Woody habitats within the urban matrix can still support biodiversity to varying degrees. As species have different responses to anthropogenic impacts, the species composition of

urban areas can depend greatly on the habitat characteristics of the local and surrounding areas and their distances from natural species pools. Urban patches harbour assemblages that are relatively modest in species richness and have low naturalness values. The composition usually consists of typical homogenising urban species. Transitional zones (fringe areas) between rural and urban habitats might maintain an assemblage of rural, habitat specialist elements with high naturalness value mixed with urban ones. Areas with no or low disturbance maintain species poor but stable native, autochthonous assemblages, with high naturalness value, characteristic for rural sites in the region.

The Terrestrial Isopod Naturalness Index (TINI) and the Average Rarity Index (ARI) give good possibilities to assess urban effects on habitats and serve as potential tools for habitat qualification. Our study demonstrates that maintaining litter layer with dead wood in urban habitats is an essential factor for favouring natural/unique oniscidean assemblages and we suggest that remnants of natural habitats within cities receive further attention in urban planning.

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## **Supplementary material I**

### **Geographical location and habitat characterization (urbanization intensity, soil and vegetation characteristics) of study sites in Buda, Hungary**

Authors: Elisabeth Hornung, Andrea Kásler, Zsolt Tóth

Data type: geographic location

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Link: <https://doi.org/10.3897/zookeys.801.22829.suppl1>

## **Supplementary material 2**

### **Species occurrence, richness and Average Rarity Index of study sites in Buda, Hungary**

Authors: Elisabeth Hornung, Andrea Kásler, Zsolt Tóth

Data type: species data

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Link: <https://doi.org/10.3897/zookeys.801.22829.suppl2>

# Species compositions of terrestrial isopods in public parks of a commuter town in Japan

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## Abstract

The species compositions of terrestrial isopod assemblages in 150 public parks in a commuter town in Japan were investigated. Eleven species of terrestrial isopods were present, of which seven were considered native or oriental species, and four were exotic species originally distributed in the Mediterranean and European regions. An exotic species *Armadillidium vulgare* Latreille, 1804 was found in all parks. Logistic model analysis indicated that the surrounding land use affected the distributions of three native species, *Burmoniscus kathmandius* (Schmalfuss, 1983), *Ligidium koreanum* Flasarova, 1972, and *Mongoloniscus koreanus* Verhoeff, 1930, indicating that landscape properties are important factors that limit the distributions of terrestrial isopods. The present study also showed that the public parks surrounded by forests or semi-natural environments in a commuter town provide habitats for native terrestrial isopods.

## Keywords

GIS, GLM, landscape environments, local environments, logistic distributions

## Introduction

Urbanization has rapidly spread throughout the world and has changed species compositions of regions through decreased diversity of native species (McKinney 2008) and increased numbers of exotics and/or generalist species (Niemelä 1999, Kotze et al. 2011), but the patterns of diversity changes are substantially different depending on biological group and climatic region (Faeth et al. 2011). Thus, understanding and protecting the biodiversity of urban regions is a major ecological concern. Many researchers have contributed to our knowledge of urban biodiversity in recent decades (e.g., McKinney 2008, Jones and Leather 2012, Nielsen et al. 2014), and some suggest that terrestrial isopods are the dominant macro-arthropods in soils of urban regions (Bolger et al. 2000, Smith et al. 2006, Magura et al. 2008a, b, Lee and Kwon 2015). Moreover, it has been observed that urban green spaces in parks provided important habitats that conserve biodiversity in urban environments (Kotze et al. 2011, Barratt et al. 2015). In Japan, many researchers have studied diversity of vegetation, birds, and insects in public parks (e.g., Ishii et al. 1991, Hata et al. 2003, Imai and Nakashizuka 2010, Hattori 2015). However, there has been no comprehensive study of the terrestrial isopods in public parks of Japan.

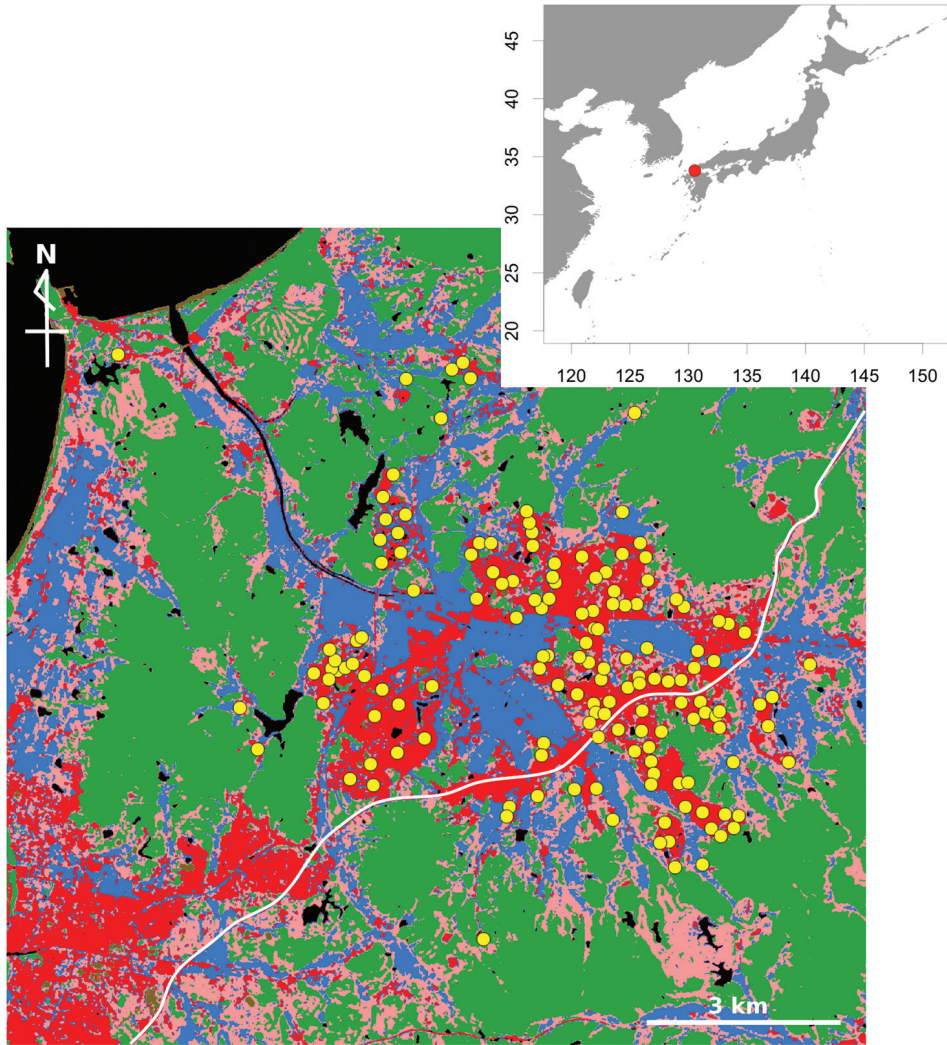
The Mediterranean region is considered a hotspot of terrestrial isopod diversity (Sfenthourakis and Taiti 2015) and some species that were originally distributed in the Mediterranean and European regions have become dispersed as exotic species throughout the world (e.g., Schmalfuss 2003, Cochard et al. 2010, Hornung et al. 2015). In Japan, seven species that are considered exotic have distribution areas with a potential for expansion (Nunomura 2007, Karasawa and Nakata 2018). These facts suggest that exotic species might already have occupied urban habitats and displaced native species in Japan.

The aims of this study were: 1) to describe the terrestrial isopod fauna in public parks of a commuter town in Japan; 2) to evaluate environments that limit species distributions; and 3) to determine whether public parks in the town provide habitats for native terrestrial isopods.

## Materials and methods

### Study area and sampling method

One hundred fifty public parks were selected in Munakata City, Fukuoka, Japan (Figure 1). Munakata City has been urbanized as a commuter town over several decades. The areas of the parks studied ranged from 74 m<sup>2</sup> to 17,089 m<sup>2</sup>. Two researchers walked around each park and searched for sites where terrestrial isopods were distributed, before collecting animals. To equalize the sampling effort among parks, the total time for animal collection was standardized as 7 min 30 s. All specimens found were collected, except for the highly abundant *Armadillidium vulgare* Latreille, 1804, for which only three or four voucher specimens were collected. Sampling was carried out



**Figure 1.** Location of Munakata city (red circle in the top right map) and map of the study area. Yellow circles represent sampling sites (parks). Blue denotes rice paddy; red denotes residential region; pink denotes grass; green denotes forest; the white line is Japan National Route 3. The map was created by modifying the high resolution land-use and land-cover map ver16.02 ([http://www.eorc.jaxa.jp/ALOS/lulc/jlulc\\_jpn.htm](http://www.eorc.jaxa.jp/ALOS/lulc/jlulc_jpn.htm)).

from 27 March to 27 August 2015. In addition, species lists were constructed for forests and a grassland area of the same city to determine the possible source of species in parks. To develop the species list for the forests, five sites (diameter ca. 5 m) were set up inside and at the edge of 18 forests (i.e., 10 sites per forest). In the survey, one researcher collected all terrestrial isopods for 10 min at each site. The species list for grassland was based on that of Tanaka and Karasawa (2018), which was carried out on a grass field in the same city.



Environmental variables were recorded at two levels: local and landscape scales. For local environments, the presence or absence of flower beds and woods in the parks were recorded during sampling. In addition, the percentage of the area of grass in parks was estimated using Google Map (<https://www.google.co.jp/maps>). For landscape environments, the areas of land use around the parks were measured using geographical information system (GIS) data. A raster data set for a high-resolution land-use and land-cover map (ver. 16.02) was obtained from the advanced land-observing satellite ([http://www.eorc.jaxa.jp/ALOS/lulc/jlulc\\_jpn.htm](http://www.eorc.jaxa.jp/ALOS/lulc/jlulc_jpn.htm); Hashimoto et al. 2014). For categorizing land use, grassland and crops were combined as ‘grass’, and four types of forest (broad-leaved, deciduous, evergreen and conifer) were combined as ‘forest’. Thus, four types of landscape environments were considered: ‘rice paddy’, ‘residential region’, ‘grass’, and ‘forest’. To evaluate suitable areas for analysis of landscape environments, buffer zones at five scales were created around each park (widths 50 m, 100 m, 200 m, 500 m, and 1000 m) and the areas of land use in each of the buffers were measured. GIS analyses were carried out using QGIS 2.14 (QGIS Development Team 2009).

### Statistical analyses

To evaluate the appropriate areas to use for the landscape environments, the Akaike information criteria (AIC) of the generalized linear models (GLMs) were compared at the five buffer widths for each species. The GLM was also used to evaluate relationships between the presence of 10 species and the species richness of native and exotic species (as response variables) with respect to environmental variables (as explanatory variables). Logistic (binary) and Poisson distributions were applied to the response variables of presence and species richness (of native and exotic species), respectively. Environmental variables were composed of three local variables (area percentage of grass, presence of flower beds and woods), and four landscape variables (areas of rice paddy, residential region, grass and forest). Using this procedure, perfect or quasi-perfect separations were found in the logistic models for some species. Thus, Firth’s bias-reduced logistic regression (Firth 1993; Heinze and Schemper 2002) was applied to the analysis of the presence of species. The proposed models were compared with the null model by the Likelihood Ratio Test. Significances of coefficient values in the proposed models were evaluated using the Chi-squared test (for presence of species) and the Wald test (for species richness). Multicollinearity among explanatory variables was assessed using variance inflation factors (VIF) for each model: variables with values > 10 were excluded. In addition, spatial autocorrelation of each species and species richness was evaluated by Moran’s *I*. To calculate the Moran’s *I*, distance weight was based on the distances among sampling sites: pairs of sites that are close together have higher values than pairs of sites that are far apart. All statistical analyses were carried out using the functions `glm`, `logistf`, `vif`, `lrtest`, and `Moran.I` in R version 3.4.1 (R Core Team 2017). *Armadillidium vulgare* was excluded from the latter analysis because this species was present in all parks (see Results section).



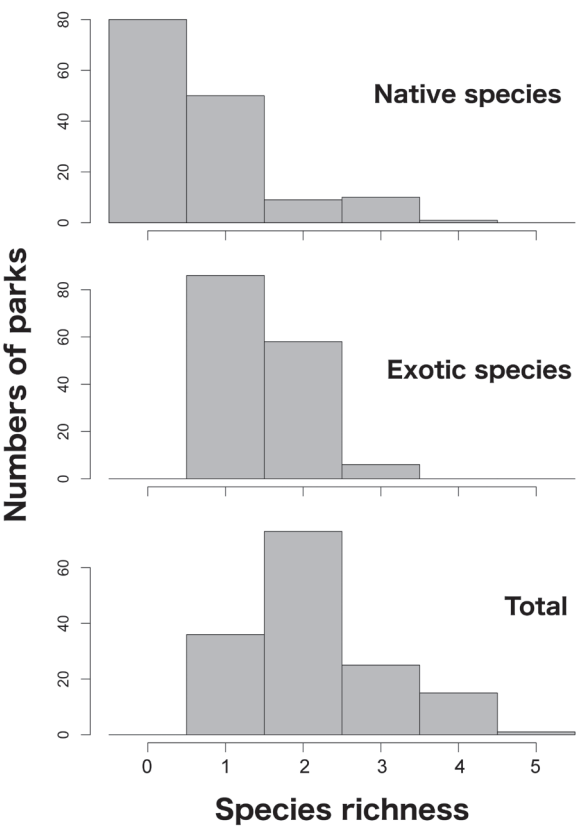
**Table 1.** Species, numbers of individuals collected and collection sites in the urban parks, grassland and forest in Munakata City, Japan.

Species	Urban park		Grassland <sup>a</sup>	Forest
	No. of ind.	Sites	No. of ind.	No. of ind.
<b>Native species</b>				
<i>Agnara pannuosa</i>	18	9	a few <sup>b</sup>	1089
Armadillidae sp. 1 <sup>c</sup>	9	5	—	1955
Armadillidae sp. 2	—	—	—	60
Armadillidae sp. 3	—	—	—	1
<i>Burmoniscus dasystylus</i>	—	—	—	52
<i>Burmoniscus kathmandius</i>	17	5	—	—
<i>Exalloniscus cortii</i>	—	—	—	1
<i>Ligidium koreanum</i>	8	3	—	683
<i>Lucasiodides</i> spp.	15	8	—	853
<i>Mongoloniscus koreanus</i>	172	36	3781	2
<i>Mongoloniscus vannamei</i>	110	36	a few	13
<i>Mongoloniscus</i> sp.	—	—	a few	—
<b>Exotic species</b>				
<i>Armadillidium vulgare</i> <sup>d</sup>	372	150	4526	2220
<i>Haplophthalmus danicus</i>	—	—	—	1
<i>Porcellio laevis</i>	60	34	—	—
<i>Porcellio scaber</i>	264	26	—	21
<i>Porcellionides pruinosus</i>	51	10	—	1

<sup>a</sup> The data from Tanaka and Karasawa (2018)<sup>b</sup> This species was identified as Agnaridae in Tanaka and Karasawa (2018)<sup>c</sup> This species was identified as *Spherillo* sp. in Karasawa et al. (2014)<sup>d</sup> Three or four specimens only were collected from each park.

## Results

A total of 17 isopod species was recorded in the city, eleven of which were collected in the public parks. Five species, *A. vulgare*, *Haplophthalmus danicus* Budde-Lund, 1880, *Porcellio laevis* Latreille, 1804, *P. scaber* Latreille, 1804 and *Porcellionides pruinosus* (Brandt, 1833), are exotic species in Japan (Karasawa and Nakata 2018). *Armadillidium vulgare* was found in all parks and has also become the dominant species in the forests. Seven native species were collected in the parks but five of them were found in nine or fewer parks. In contrast, *Agnara pannuosa* (Nunomura, 1987), Armadillidae sp. 1, *Ligidium koreanum* Flasarova, 1972, *Lucasiodides* spp. were abundant in the forests. *Mongoloniscus koreanus* Verhoeff, 1930, *M. vannamei* (Arcangeli, 1927), *P. laevis*, and *P. scaber* were widely distributed in the parks (> 25 parks) but their abundances were low in the forests. *Mongoloniscus koreanus* was dominant in grassland, as well as *A. vulgare* (Table 1). Overall, species richness in each park ranged from one to five (Figure 2). At least one exotic species was found in each park, but 80 parks lacked native species. The maximum species richness in a park was four for the native species and three for the exotic species.



**Figure 2.** Frequencies of species richness of native, exotic, and total species in parks

**Table 2.** Coefficients of explanatory variables and *p* values of the Likelihood ratio test.

Species	Local environments			Landscape environments			Intercept	Likelihood ratio test <i>p</i> -value
	Grass	Flower bed	Wood	Residential	Rice paddy	Grass	Forest	
<i>Agnara panmuosa</i>	0.181	1.493	-0.634	<b>-0.105</b>	-0.083	–	0.074	0.177
<i>Armadillidae</i> sp. 1	1.756	-0.026	-1.340	–	-0.068	0.027	0.194	0.399
<i>Burmoniscus kathmandius</i>	18.912	-0.472	0.745	<b>-0.047</b>	-0.036	–	-0.011	<b>0.045</b>
<i>Ligidium koreanum</i>	-2.809	-1.331	-1.137	<b>-0.001</b>	-0.001	0.0001	–	<b>0.048</b>
<i>Lucasioides</i> spp.	-0.210	<b>-1.906</b>	0.148	–	0.001	0.001	-0.002	<b>0.015</b>
<i>Mongoloniscus koreanus</i>	-0.035	-0.031	0.063	–	<b>0.009</b>	<b>0.012</b>	<b>0.011</b>	<b>0.00001</b>
<i>Mongoloniscus vannamei</i>	0.893	1.103	-0.729	–	-0.001	0.001	-0.0002	0.243
<i>Porcellio laevis</i>	-0.010	0.250	0.632	–	-0.010	-0.018	-0.036	0.158
<i>Porcellio scaber</i>	1.200	0.452	0.398	–	-0.0004	<b>0.0000001</b>	-0.00002	0.272
<i>Porcellionides pruinosus</i>	0.102	-0.636	0.961	-0.079	0.032	–	-0.150	0.395
Number of species								
Native species	0.374	0.061	0.109	–	0.002	<b>0.006</b>	<b>0.004</b>	<b>-1.647</b> <b>0.0002</b>
Exotic species	0.107	0.079	0.327	–	-0.0001	-0.001	-0.001	0.002 0.906

Bold represents *p* < 0.05 by the Chi-squared test.

Values of Moran's  $I$  revealed that distribution of *P. scaber* showed significant spatial autocorrelation ( $I = 0.027$ ,  $p < 0.05$ ), while other species and species richness showed no significant spatial autocorrelation. There were differences among species in the spatial scales having the lowest AIC values: *A. pannuosa*, 25 m; Armadillidae sp. 1, 25 m; *B. kathmandius* (Schmalfuss, 1983), 50 m; *L. koreanum*, 500 m; *Lucasioides* spp., 250 m; *M. koreanus*, 100 m; *M. vannamei*, 250 m; *P. laevis*, 50 m; *P. scaber*, 500 m; *P. pruinus*, 25 m (Supplementary material 1). In addition, the spatial scale of 100 m showed the lowest AIC for the species richness of native and exotic species. Coefficients of variables for the models fitted are described in Table 2. The likelihood ratio test significantly rejected the null model for *B. kathmandius*, *L. koreanum*, *Lucasioides* spp., and *M. koreanus*, and for the species richness of native species. Conversely, there was no significant difference between the proposal and null models for another species and species richness of exotic species, indicating that their distributions were not explained by the seven environmental variables. Local environment significantly affected the distribution of *Lucasioides* spp. only: the presence of flower beds may have a negative effect on the presence of *Lucasioides* spp. The distributions of four native species, *A. pannuosa*, *B. kathmandius*, *L. koreanum*, and *M. koreanus* were significantly affected by landscape properties, although there was no significant difference between the proposed and null models for *A. pannuosa*. *Agnara pannuosa*, *B. kathmandius*, and *L. koreanum* were negatively affected by the surrounding residential region, while natural or semi-natural environments (rice paddy, grass and forest) had positive effects on the distribution of *M. koreanus*. In addition, the model for the species richness of native species showed significant positive values for grass and forest areas, indicating that species richness of native species was high at sites surrounded by natural conditions. However, the model for *P. scaber* indicated a significant positive value for rice paddy only, although *P. scaber* showed spatial autocorrelation and the null model was not rejected.

## Discussion

Lee and Kwon (2015) reported that terrestrial isopods comprised approximately 90% of ground arthropods in urban parks of Osaka, a Japanese mega-city, but they did not identify them at species level. The present study found a total of eleven terrestrial isopod species in the public parks of a commuter town, four of which were exotic species. At least one terrestrial isopod species was found in the parks and the maximum species richness was five. These results imply that urban parks in Japan provide important habitats for terrestrial isopods, as observed in another country (Giurginca et al. 2017).

The species compositions in patchily distributed areas such as parks are known to be affected by local environments within the areas and by landscape environments around the parks (Jokimäki 1999, Germaine and Wakeling 2001, Smith et al. 2005). This study indicated that landscape environments play a more important role in determination of species distributions than do local environments; three native species

(*B. kathmandius*, *L. koreanum* and *M. koreanus*) preferred parks surrounded by forest or semi-natural environments (rice paddy and grass) to parks located in residential regions. *Ligidium koreanum* and *M. koreanus* have become abundant in forests and grassland within the city. In addition, *B. kathmandius* is widely distributed in East Asia and some Pacific Islands (Schmalfuss 1983, Taiti and Ferrara 1991, Karasawa 2016), and is usually found in grasslands of southern regions in Japan (SK, personal observations). Thus, these species are considered grass or forest specialists that may migrate to parks from surrounding semi-natural environments or forests. This finding suggests a hypothesis that the species richness of native species may increase in the parks surrounded by semi-natural and forest environments. The model analysis supported this hypothesis and these observations also indicate that an understanding of the distribution of terrestrial isopods requires evaluation of the surrounding landscape (Dauber et al. 2005).

Four exotic species with original distributions in the Mediterranean and European regions (Schmalfuss 2003, Cochard et al. 2010) were widely distributed in the public parks of the commuter town, but the present study failed to clarify the environmental factors that determined the distribution of exotic species; the null models were not rejected, and *P. scaber* also tended to be geographically concentrated. Three exotic species, *A. vulgare*, *P. scaber*, and *P. pruinosus*, are considered to be a habitat generalist, an urban specialist and a synanthropic species, respectively (Dangerfield and Telford 1990; Magura et al. 2008b), and they have been commonly found in urban regions (Vilisics et al. 2007, 2012, Vilisics and Hornung 2009, Giurginca et al. 2017). This may explain why human activity plays a more important role than environmental factors for their distributions in the city. Moreover, *A. vulgare* was distributed in all parks. This species is able to walk 24 m d<sup>-1</sup> and is a potentially able to walk 50 m h<sup>-1</sup> (Furukawa et al. 2017). This high dispersal ability could facilitate the distribution of *A. vulgare* via roads and residential regions and could be an important factor in the establishment and success of populations over a wide range. The establishment of populations of exotic species in public parks has the potential to be a source of exotic species in the future.

## Conclusions

Eleven terrestrial isopod species were found in the urban parks of a commuter town. Native species tended to be distributed in the parks located adjacent to natural environments, while their distributions had little relationship with the local environments within the parks. It is proposed that the location of parks is an important factor to consider in their design to protect the largest number of species of native terrestrial isopods.

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## Supplementary material I

### AIC values of GLMs at the five spatial scales

Authors: Takahiro Nasu, Kana Kitagawa, Shigenori Karasawa

Data type: species data

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Link: <https://doi.org/10.3897/zookeys.801.21875.suppl1>





# Woodlice and their parasitoid flies: revision of Isopoda (Crustacea, Oniscidea) – Rhinophoridae (Insecta, Diptera) interaction and first record of a parasitized Neotropical woodlouse species

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## Abstract

Terrestrial isopods are soil macroarthropods that have few known parasites and parasitoids. All known parasitoids are from the family Rhinophoridae (Insecta: Diptera). The present article reviews the known biology of Rhinophoridae flies and presents the first record of Rhinophoridae larvae on a Neotropical woodlouse species. We also compile and update all published interaction records. The Neotropical woodlouse *Balloniscus glaber* was parasitized by two different larval morphotypes of Rhinophoridae. Including this new record, there are 18 Isopoda species known to be parasitized and 13 Rhinophoridae species with known hosts, resulting in 35 interactions. There are a total of 53 interaction records from Holarctic and Neotropical countries. Of the 18 known isopod hosts, only five species have more than one parasitoid, including the new Neotropical host record presented in this work.

## Keywords

Diptera, Isopoda, Oniscidea, parasitoids, Rhinophoridae

## Introduction

Terrestrial isopods are soil macroarthropods involved in decomposition processes and nutrient cycling (Zimmer 2002). This group has many predators within the soil but few known parasites and parasitoids. Among parasitoids, all known species belong to the family Rhinophoridae (Insecta: Diptera) (Sutton 1980). This family of flies comprises about 150 species worldwide that mainly parasitize woodlice (Pape and Arnaud 2001, Nihei 2016). Despite their numbers, not many papers discuss the woodlouse-parasitoid interaction. Studies regarding the interaction and fly's larval stages are scarce and difficult to find and the taxonomy and phylogeny of both groups have been considerably modified since those studies were published. Hence, there is no current list of recorded interactions and a need to update them taxonomically. Information from immature stages and their biology is crucial for evaluating the systematic position of many aberrant oestroid flies such as the rhinophorids (Pape and Arnaud 2001), so knowledge of the morphology of larval stages may help phylogenetic analysis and classification (Cerretti et al. 2014), as well as to understand its evolutionary history in association with the woodlice hosts. Therefore, this work aims to (1) review the known biology of Rhinophoridae larvae focusing on the woodlouse-larva interaction, (2) present the first record of Rhinophoridae larvae on a Neotropical woodlouse species and (3) update the recorded interactions according to current taxonomy of both groups.

## Material and methods

Bibliographic searches in the platforms Web of Science, Science Direct, Biodiversity Heritage Library and Google Scholar were performed using the following keywords: Rhinophoridae, woodlouse flies, Tachinidae, Rhinophorinae. All the subsequent references from obtained papers were searched in available databases and scientific libraries.

Regarding the new woodlouse host record, infected individuals of *Balloniscus glaber* Araujo & Zardo, 1995 that had been collected in Morro Santana, Porto Alegre, Southern Brazil (30°4'4"S, 51°7'22"W) were discovered from laboratory culture. The location is at 100 m of elevation and the vegetation consists of a mosaic of Atlantic forest and grassland (Overbeck et al. 2006). Hosts were carefully dissected, photographed, and preserved in ethanol 70 %. Larvae were heated in water at 60 °C before being transferred to ethanol whenever possible. The material used in this study is deposited in Museu de Zoologia, Universidade de São Paulo, São Paulo, Brazil (MZUSP).

Taxonomy of isopod species was updated according to Schmalfuss (2003) and recent revisions. Taxonomy and name validity of Rhinophoridae species were based on regional catalogues and recent generic revisions, when available (Herting 1993, Cerretti and Pape 2007, 2009).

## Results and discussion

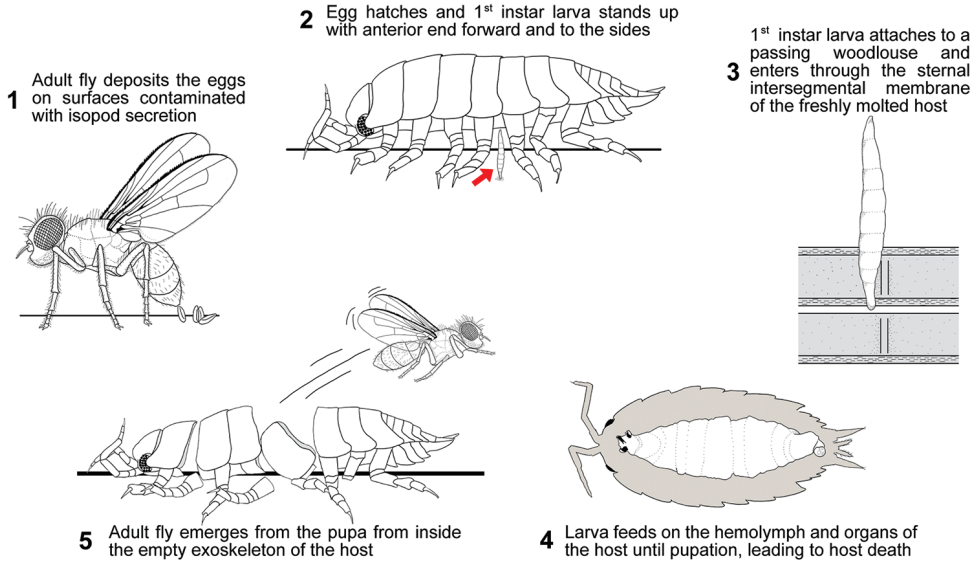
### Biology of larval stages: Isopoda-Rhinophoridae interaction

Very few studies regard the biology of the larva and its effect on the woodlouse host. These studies usually demand a long period of time due to the difficulty of obtaining the parasitoids (Thompson 1934, Bedding 1965, 1973). This difficulty is partially explained by the low prevalence of this parasitoid on natural populations and for the apparent specificity of host species (Bedding 1965). Prevalence on natural populations is usually lower than 2% and seems to be associated with the infection method.

Adult Rhinophoridae flies copulate and the female deposits the eggs on substrates (Bedding 1965, Wijnoven 2001) contaminated by uropod gland secretion of isopods rather than on the host itself (Bedding 1965) which may be a derived character in this group of parasitoids (Wood 1987). This secretion is not commonly observed in all woodlice species but it is rather easily obtained from *Porcellio scaber* Latreille, 1804 (Gorvett 1946, Deslippe et al. 1996) which might explain why this species has the highest number of known parasitoids and highest prevalence on natural populations (Bedding 1965, Sassaman and Pratt 1992).

The eggs deposited on the soil hatch and the 1<sup>st</sup> instar larva attaches itself to the body of a passing woodlouse. The larva may wave its anterior end slowly forward and sideward in an attempt to attach itself to the body of a passing woodlouse (Pape and Arnaud 2001). This method of infection is affected by host size since the larva cannot reach the sternites of bigger (taller) animals. It was also observed the suitability of the host relates to a specific period of the molting cycle of the isopod. Differently from insects, crustaceans present a highly calcified cuticle (Roer et al. 2015). Within crustaceans, isopods have developed specific strategies to recycle calcium from the old cuticle such as a biphasic molting (they first molt the posterior half and then the anterior half of the body) and accumulation of amorphous calcium carbonate in the anterior sternites prior ecdysis (Greenaway 1985, Steel 1993, Ziegler 1994). The fly larva attaches itself to isopods with calcium plates (i.e., during premolt or intramolt) and penetrates through the intersegmental membrane of the sternites of the freshly molted host (Bedding 1965), since they present a softer cuticle at this stage. Nonetheless, there is a high rate of cannibalism of freshly molted isopods (Bedding 1965) thus reducing the chances of survival of the fly larva inside the host and possibly explaining the low prevalence among natural populations.

After the larva has entered the host, it then molts to its 2<sup>nd</sup> instar and starts feeding, first on the hemolymph, and then on the organs of the host. The 3<sup>rd</sup> instar larva fills most of the body cavity leading to isopod death. Pupation occurs inside the empty exoskeleton of the host (Thompson 1934, Bedding 1965) (Figure 1).



**Figure 1.** Schematic representation of the infection cycle of a Rhinophoridae fly in a woodlouse host. 3 is modified from Thompson (1934).

### First Neotropical woodlouse host record

Almost all records from Rhinophoridae hosts are from the Palearctic region. Outside the Palearctic, there is only mention of *Porcellio scaber*, *Oniscus asellus* Linnaeus, 1758 and *Porcellionides pruinosus* (Brandt, 1833) in the Nearctic (Brues 1903, Jones 1948, Sassaman and Garthwaite 1984, Sassaman and Pratt 1992) and *Armadillidium* sp. (probably *Armadillidium vulgare* (Latreille, 1804)) in the Neotropic (Parker 1953). All of these woodlice species were parasitized by *Melanophora roralis* (Linnaeus, 1758). Nonetheless, all the aforementioned oniscidean and rhinophorid species are introduced from the Palearctic on these locations. Some authors hypothesize that transportation of infected woodlice can explain the occurrence of Palearctic Rhinophoridae in the Nearctic and Neotropic (Mulieri et al. 2010, O'Hara et al. 2015) provided that introduced woodlice are common in these regions (Jass and Klausmeier 2000). The lack of native woodlouse hosts in the Nearctic region is thought to be associated with the low diversity of native woodlice species there (*c.f.* Schmalfuss 2003), but the same is not true for the Neotropic. In fact, in Brazil alone there is circa 200 described species, most of them native (Cardoso et al. 2018).

In the Neotropic, 19 native species of Rhinophoridae have been described (Cerretti et al. 2014, Nihei et al. 2016), but there is no information regarding parasitoid-host interaction so far. Of these, only the 1<sup>st</sup> instar larva of *Bezzimyia yepesi* Pape & Arnaud, 2001 (Venezuela) is known (Pape and Arnaud 2001) and no host record has been made before, even for the two introduced species, *Melanophora roralis* (L.) and *Stevenia deceptor* (Loew, 1847) (Mulieri et al. 2010) (Figure 2).



**Figure 2.** Distribution map of native and introduced Rhinophoridae species in the Neotropical region (area in gray) including the new larvae record. *Apomorphyto inbio* records from Pape (2010) and Cerretti et al. (2014); *Bezzimyia* spp. records from Pape and Arnaud (2001); *Shannoniella* spp. records from Nihei et al. (2016); *Trypetidomima* spp. records from Nihei and Andrade (2014); *Melanophora roralis* records from Parker (1953), Guimarães (1977), González (1998), Cerretti and Pape (2009) and Mulieri et al. (2010); *Stevenia deceptoria* records from Mulieri et al. (2010). Base map modified from: commons.wikimedia.org/

Here we observed that the Neotropical isopod *Balloniscus glaber* is a host for the dipterous larvae in southern Brazil (Figure 2), and the two observed 3<sup>rd</sup> instar larvae morphotypes are different from the nine Palearctic species with previously described 3<sup>rd</sup> instar larval forms (Thompson 1934, Bedding 1965), including the introduced *Melanophora roralis*.

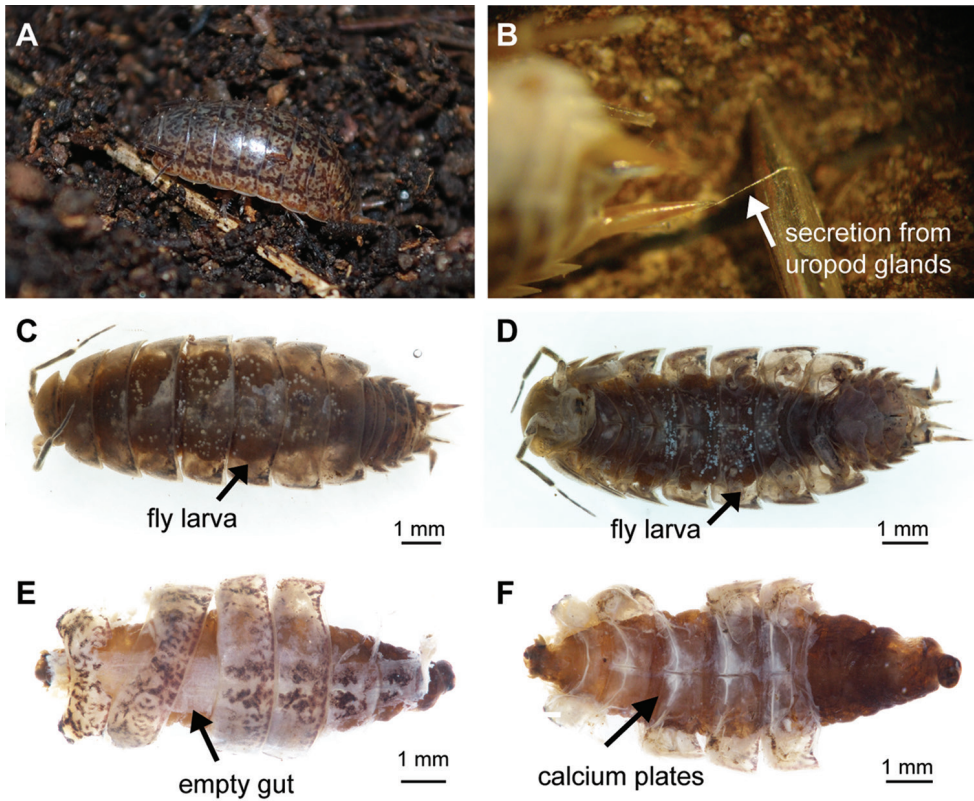
*Balloniscus glaber* shares many characteristics with clingers (Wood et al. 2017) although it does not present a typical clinger eco-morphological body type like *Porcellio scaber* (*sensu* Schmalfuss 1984). However, it presents clinging behavior (Figure 3A) for predator avoidance (Quadros et al. 2012, Wood et al. 2017) and its legs are shorter than in runner type animals of similar size. These morphological and behavioral characteristics might facilitate larva infection due to reduced distance of sternites to the substrate. Furthermore, like *Porcellio scaber*, this species also frequently discharges a sticky secretion from their uropod glands upon stimulation (Figure 3B), secretion that is recognized by adult fly females and might stimulate oviposition (Bedding 1965). Five infected individuals have been recorded in the same location (Figure 3C–F). The larvae (one per host) occupied the full body cavity, reaching up to 7 mm in length and resulted in death of all woodlice hosts (Suppl. material 2). Hosts lacked discernible internal reproductive system and the empty gut was the only remaining organ (Figure 3E). No host presented any signs of alteration in overall appearance. The parasitoids could only be identified at the family level due to the lack of larval descriptions for the native species and lack of adults to get a more precise identification. The larvae were identified as Rhinophoridae based on comparative examination of descriptions and illustrations available on the literature; both collected morphotypes presented elongate body shape, anterior and posterior spiracles, and cephaloskeleton as characterized by rhinophorid species. The two 3<sup>rd</sup> instar larvae morphotypes are conspicuously different on body shapes, posterior ends, cephaloskeleton, and anterior and posterior spiracles (Figs 4, 5). These forms differ from the known larval stages described by Thompson (1934) and Bedding (1965, 1973). Given the apparent specificity of host records (see next topic) we believe they are Neotropical species (and none of the introduced species). They may be larvae of the described Neotropical species of *Shannoniella* Townsend, 1939 or *Trypetidomima* Townsend, 1935, or they may even belong to undescribed species, since the distribution of *Balloniscus glaber* (Lopes et al. 2005) does not extend to the locations where these native Rhinophoridae have been found, namely, the southeastern portion of Brazilian Atlantic Forest (Nihei and Andrade 2014, Nihei et al. 2016). Furthermore, the location of the new Rhinophoridae record is at a low altitude and Neotropical woodlouse flies seem to be rare in the lowlands, being usually found at elevations of 600–1200 meters in Brazil (Nihei and Andrade 2014, Nihei et al. 2016). Nonetheless, *Balloniscus glaber* can be found in altitudes up to 1000 meters in southern latitudes (Lopes et al. 2005) while another species from the genus, *Balloniscus sellowii* (Brandt, 1833), presents a broader latitudinal distribution (Schmalfuss 2003).

A further publication will describe in detail the morphology of the two 3<sup>rd</sup> instar morphotypes, and DNA sequencing will be performed trying to obtain a more precise identification.

### Reviewed interactions records following current taxonomy

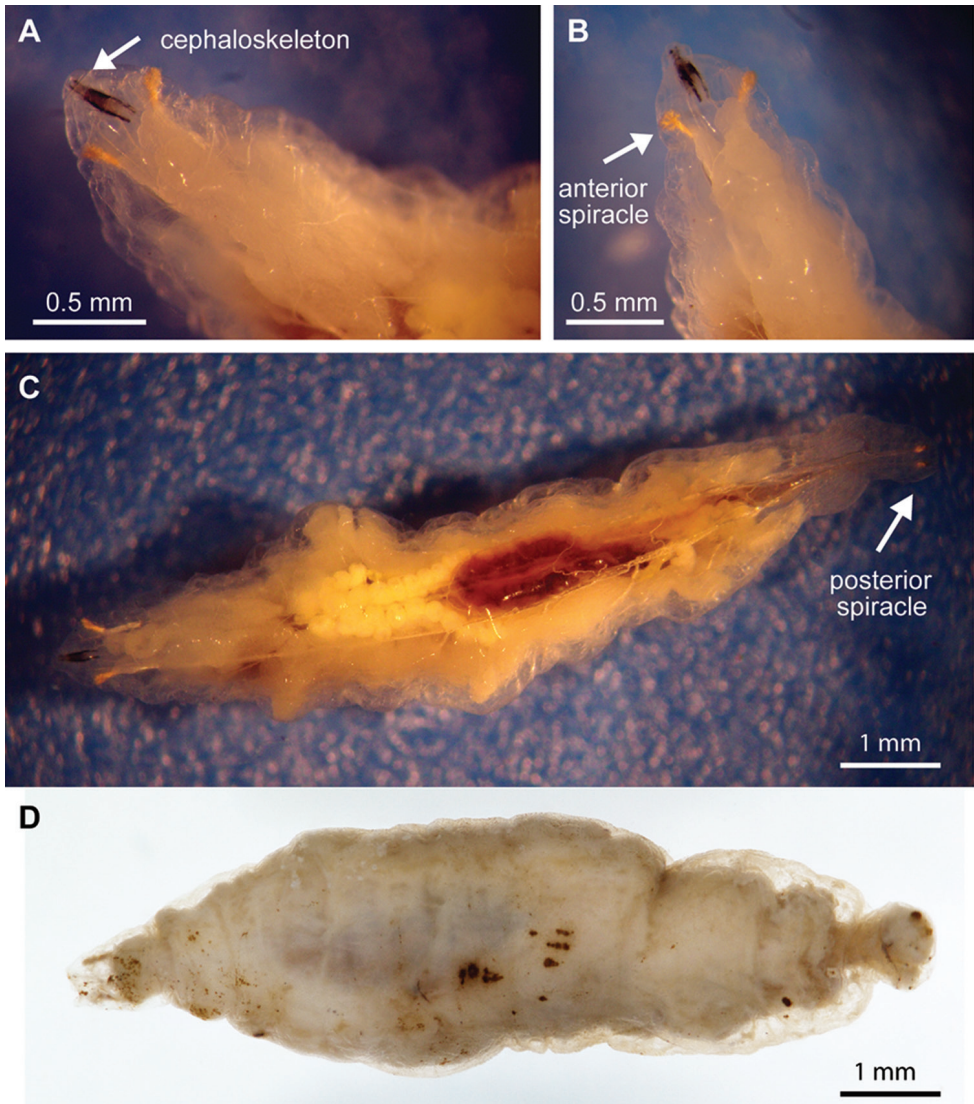
The earliest reference to a Rhinophoridae parasitoid of woodlice appears to be from von Roser (1840 *apud* Thompson 1934) that created some confusion in the literature in





**Figure 3.** *Balloniscus glaber* infected with 3<sup>rd</sup> instar Rhinophoridae larva **A** alive *B. glaber* clinging to the substrate **B** secretion discharged from uropod glands **C–D** dorsal (**C**) and ventral (**D**) views of *Balloniscus glaber* with a third instar Rhinophoridae larva inside **E–F** partially dissected *Balloniscus glaber* infected with Rhinophoridae larva showing the empty gut on dorsal view (**E**) and the calcium plates on ventral view (**F**).

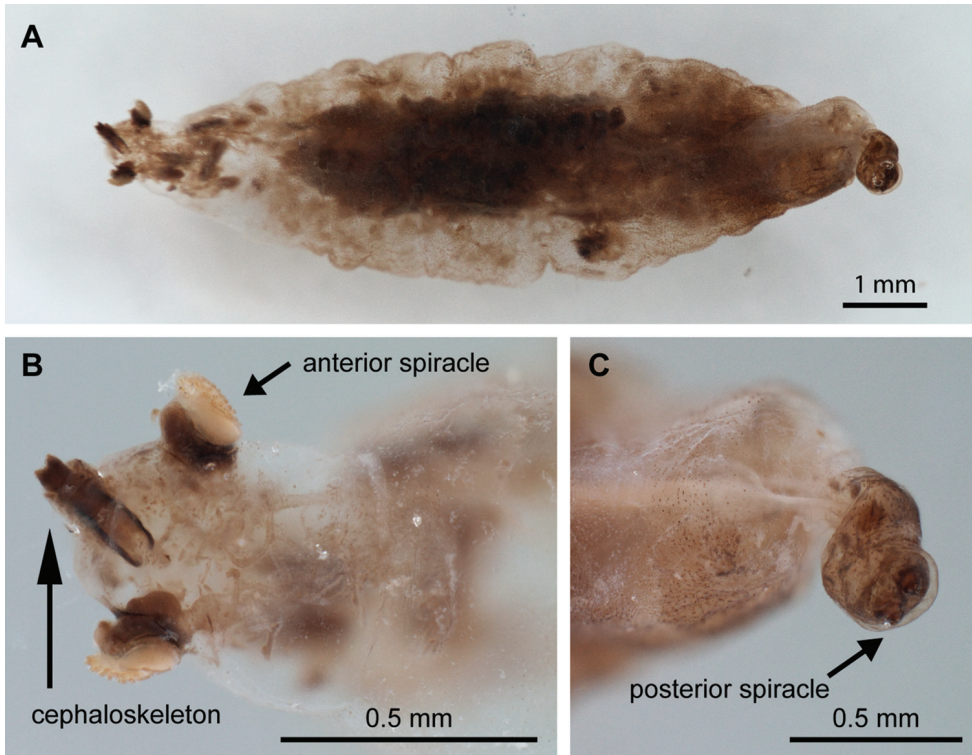
later years. In his paper, the dipteran “*Tachinia atramentaria*” (currently *Stevenia atramentaria* (Meigen, 1824)) is mentioned as a parasite of a woodlouse, possibly *Oniscus asellus*. Thompson (1934), Herting (1961), Bedding (1965) and Verves and Khrokalo (2010) mentioned that *Oniscus asellus* was probably a wrong identification while Cerretti and Pape (2007) mention *Oniscus asellus* as a possible host for *Stevenia atramentaria*. The doubtful record was finally resolved in Kugler (1978) where the author states the record was based on a misidentification of *Trachelipus rathkii* (Brandt, 1833) according to a personal communication from Herting, that apparently had already been corrected in Sutton’s book (1980). Rognes (1986) and Dubiel and Bystrowski (2016) still list *Oniscus asellus* as a host or possible host of *Stevenia atramentaria* but referencing articles that mention the species as a possible host, probably following von Roser’s reference from 1840. Therefore, we could not find any reliable record of *Oniscus asellus* as a host of *Stevenia atramentaria*. Dubiel and Bystrowski (2016) list *Trachelipus rathkii* as a host from *Stevenia atramentaria* for the first time, but it should be the third record



**Figure 4.** Rhinophoridae larva obtained from the Neotropical isopod *Balloniscus glaber*. Morphotype 1, 3<sup>rd</sup> instar **A** detail view of the cephaloskeleton on dorsal view **B** detail view of the anterior spiracle **C** alive larva with transparent integument **D** fixed larva, dorsal view.

of this interaction if the identification correction from von Roser's article is taken into account as well as the thesis from Bedding (1965).

The following record on the literature is from Brues (1903) indicating *Melanophora roralis* as a parasitoid of *Porcellio* sp., probably *Porcellio scaber* in Massachusetts, USA. Besides *Porcellio scaber* as a host, this dipteran species was also recorded as a parasitoid of *Oniscus asellus* (Jones 1948, Sassaman and Garthwaite 1984, Sassaman and Pratt 1992) and *Porcellionides pruinosus* (Sassaman and Garthwaite 1984) in the



**Figure 5.** Rhinophoridae larva obtained from the Neotropical isopod *Balloniscus glaber*. Morphotype 2, 3<sup>rd</sup> instar **A** dorsal view **B** detail from anterior part showing the cephaloskeleton and anterior spiracles **C** detail of the posterior spiracle.

United States. In the Palearctic region, besides the aforementioned isopods, *Porcellio spinicornis* Say, 1818 (Irwin 1983) is also listed as host for *Melanophora roralis*. This species of fly shows the highest plasticity of hosts as well as largest geographical distribution that is not restricted to the Palearctic region. It is found in the U.S.A. (Brues 1903, Jones 1948, Sassaman and Garthwaite 1984, Cerretti and Pape 2009), Chile (González 1998), Argentina, Brazil and Jamaica (Guimarães 1977, Mulieri et al. 2010).

Records of interactions are available in von Roser (1840 *apud* Thompson 1934), Brues (1903), Thompson (1934), Jones (1948), Parker (1953) Herting (1961) Bedding (1965), Kugler (1978), Irwin (1983), Sassaman and Garthwaite (1984), Nash (1985), Bürgis (1991, 1992), Cordaux et al. (2001), Wijnhoven (2001), Cerretti et al. (2014) and Dubiel and Bystrowski (2016) although most of this literature is not taxonomically updated. Other works like Arnaud (1978), Draber-Monko (1981), Rognes (1986), Wijnhoven and Zeegers (1999), Cerretti and Pape (2007), and Ziegler (2008) report known hosts from the literature (some of them with current species' names), but don't present new records. Therefore, the number of interactions is certainly higher than it has been recorded so far. Currently, there are 18 Isopoda species known to be parasitized (one with an undetermined Rhinophoridae species), and 13 Rhinophoridae species with known



**Table 1.** Records of Isopoda-Rhinophoridae (host-parasitoid) interactions from the literature with updated taxonomy. The records from Bedding 1965 are also presented in Sutton (1980). Supplementary dataset presents, additionally, the name of the species of both Rhinophoridae and Isopoda in the original record publication, country, and biogeographical region Suppl. material 1.

Isopod species	Rhinophoridae species	Source
<b>Family Armadillidae</b>		
<i>Armadillidium frontirostre</i>	<i>Stevenia signata</i>	Bürgis 1992
<i>Armadillidium nasatum</i>	<i>Phyto melanocephala</i>	Herting 1961 (after Legrand)
	Rhinophoridae sp.	Cordaux et al. 2001
<i>Armadillidium silvestrii</i>	<i>Phyto melanocephala</i>	Herting 1961 (Verhoeff after Séguéy 1941)
<i>Armadillidium versicolor</i>	<i>Phyto melanocephala</i>	Herting 1961 (Verhoeff after Séguéy 1941)
<i>Armadillidium vulgare</i>	<i>Phyto melanocephala</i>	Thompson 1934 (found by Donisthorpe); Bedding 1965
	<i>Stevenia signata</i>	Bürgis 1991, 1992
<i>Armadillidium</i> sp. (probably <i>A. vulgare</i> )	<i>Melanophora roralis</i>	Parker 1953
<i>Armadillo officinalis</i>	<i>Phyto armadillonis</i>	Kugler 1978
<b>Family Balloniscidae</b>		
<i>Balloniscus glaber</i>	Rhinophoridae sp.	Wood et al. 2018 (present study)
<b>Family Cylisticidae</b>		
<i>Cylisticus convexus</i>	<i>Rhinomorinia sarcophagina</i>	Dubiel and Bystrowski 2016
<b>Family Oniscidae</b>		
<i>Oniscus asellus</i>	<i>Melanophora roralis</i>	Jones 1948
	<i>Paykullia maculata</i>	Thompson 1934, Bedding 1965
	<i>Phyto discrepans</i>	Thompson 1934, Bedding 1965
	<i>Phyto melanocephala</i>	Thompson 1934 (found by Donisthorpe)
<b>Family Philosciidae</b>		
<i>Philoscia affinis</i>	<i>Stevenia atramentaria</i>	Herting 1961 (Verhoeff after Séguéy 1941)
<b>Family Porcellionidae</b>		
<i>Porcellio laevis</i>	<i>Phyto luteisquama</i>	Kugler 1978
<i>Porcellio scaber</i>	<i>Melanophora roralis</i>	Thompson 1934, Jones 1948, Bedding 1965, Sassaman and Garthwaite 1984
	<i>Paykullia maculata</i>	Thompson 1934, Bedding 1965
	<i>Phyto discrepans</i>	Thompson 1934, Bedding 1965
	<i>Phyto melanocephala</i>	Thompson 1934, Bedding 1965
	<i>Rhinophora lepida</i>	Thompson 1934, Bedding 1965
	<i>Stevenia atramentaria</i>	Thompson 1934
	<i>Tricogena rubricosa</i>	Thompson 1934, Bedding 1965, Nash 1985, Dubiel and Bystrowski 2016
	<i>Stevenia</i> sp.	Cordaux et al. 2001
<i>Porcellio</i> sp. (probably <i>Porcellio scaber</i> )	<i>Melanophora roralis</i>	Brues 1903
	<i>Stevenia deceptoria</i>	Cerretti et al. 2014
<i>Porcellio spinicornis</i>	<i>Melanophora roralis</i>	Irwin 1983
	<i>Phyto melanocephala</i>	Herting 1961 (Legrand after Seguy 1941)
<i>Porcellionides pruinosus</i>	<i>Melanophora roralis</i>	Sassaman and Garthwaite 1984
	<i>Phyto angustifrons</i>	Thompson 1934
<i>Protracheoniscus politus</i>	<i>Paykullia maculata</i>	Herting 1961 (Verhoeff after Séguéy 1941)
<b>Family Trachelopodidae</b>		
<i>Trachelipus arcuatus</i>	<i>Stevenia atramentaria</i>	Herting 1961 (Verhoeff after Séguéy 1941)
<i>Trachelipus rathkii</i>	<i>Paykullia maculata</i>	Wijnhoven 2001
	<i>Stevenia atramentaria</i>	von Roser 1840 (corrected in Kugler 1978 by Herting as personal communication), Bedding 1965, Dubiel and Bystrowski 2016
	<i>Tricogena rubricosa</i>	Dubiel and Bystrowski 2016
<i>Trachelipus ratzeburgii</i>	<i>Paykullia maculata</i>	Herting 1961

hosts, resulting in 35 known interactions (and two others lacking host species identification) and a total of 53 records from 12 countries. Out of the 18 known isopod hosts, only five species have more than one parasitoid: *Porcellio scaber* (seven or eight rhinophorid species), *Oniscus asellus* (four spp.), *Trachelipus rathkii* (three spp.), *Armadillidium vulgare* (two or three spp.), *Porcellio spinicornis* and *Porcellionides pruinosus* (two spp.), and *Balloniscus glaber* (with two undetermined morphotypes recorded here) (Table 1).

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## Supplementary material I

### Compilation of Isopoda-Rhinophoridae records (Dataset)

Authors: Camila T Wood, Silvio S Nihei, Paula B Araujo

Data type: species data

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Link: <https://doi.org/10.3897/zookeys.801.26052.suppl1>

## Supplementary material 2

### Rhinophoridae larva from a Neotropical woodlouse species

Authors: Camila T Wood, Silvio S Nihei, Paula B Araujo

Data type: multimedia

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Link: <https://doi.org/10.3897/zookeys.801.26052.suppl2>

# Effect of neem-derived plant protection products on the isopod species *Porcellionides pruinosus* (Brandt, 1833)

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## Abstract

Neem-based products have gained major attention over the last few years due to their wide range of applications in pest management, and have been in the focus of biological plant protection research in the past decade. Yet, there is limited information available to understand the side effects of these neem-derived pesticides on non-target species in soil. Therefore, *Porcellionides pruinosus*, a terrestrial isopod, was chosen as a non-target species to investigate such possible effects. Two different experiments were conducted to study two different neem-derived plant protection products, i.e., NeemAzal T/S (1% azadirachtin) which is a commercial product registered in the EU, and neem leaf extract from dried neem leaves (1%). The latter simulates the plant protection product, is domestically produced, and widely used by farmers in India and other tropical and subtropical countries. Findings are consistent with previous results obtained with other non-target organisms, i.e., neither of the tested neem products have adverse effects on the mortality of *P. pruinosus*. However, further research on a wider range of soil organisms is needed to prove the safety of neem-based products as biological control agents and to be part of integrated pest management.

## Keywords

azadirachtin, biological pest control, isopod, neem leaf extract, non-target organism

## Key message

The safe use of neem (*Azadirachta indica*)-based biological insecticides requires more information about their possible side effects on non-target organisms. Such organisms are the woodlice species, important decomposers of organic material in agricultural areas. It was demonstrated that neither a commercial product of neem nor a domestic neem leaf extract had any adverse effect on a ubiquitous woodlice species, *Porcellionides pruinosus*.

## Introduction

Different environmental conditions and chemical stressors may interact and can have a negative impact on the soil biota (Morgado et al. 2016). The long-term effects of indiscriminate and excessive use of synthetic pesticides induced research to develop alternative biological control strategies (Gurjar et al. 2012) which needs to be cost effective, non-toxic, biodegradable, eco-friendly (Girish and Bhat 2008). Such non-chemical and biological pest control methods are not only fundamental to organic farming but they are also involved in the basic principles of sustainable integrated pest management (Barzman et al. 2015). Amongst the wide variety of biological control agents available, plant-derived crude products or formulated pesticides are very popular because their use can be both efficient and economically rational (Gahukar 2014). This interest in plant derived products has brought attention to the neem tree (*Azadirachta indica* A. Juss) which has been known in the Indian sub-continent for more than 4000 years (Boursier et al. 2011).

The neem tree, *Azadirachta indica* is also referred to as *Melia azadirachta* L., Indian lilac or Margosa (Koul et al. 1990). The extracts of neem tree have been studied for their diverse properties and have been used extensively against wide range of pest species (Gahukar 2014). Different biologically active compounds are found in all the parts of neem tree (Faheem et al. 2014) such as nimbin, nimbinin, meliatriol, azadirachtol, azadirone, azadirachtin, salannin, nimolcinic acid, etc. (Koul et al. 1990) of which azadirachtin is the most widely studied compound.

Azadirachtin is a tetranortriterpenoid plant limonoid which possess anti-feedant and growth-disrupting properties. It was first isolated from seeds of *Azadirachta indica* by Butterworth and Morgan in 1968 and its detailed structure was given by Broughton and his team in 1987 (Mordue and Blackwell 1993). Azadirachtin, the most active phytochemical component found in neem, is known to be effective against 550 insect species, many of them being major pests of cultivated crops (Mondal and Mondal 2012). Due to worldwide demand of organic cotton, azadirachtin-based insecticides have been gaining popularity in plant protection of this plant (Gahukar 2000).

Neem extracts and products have been used against different orders of insects such as Coleoptera (for example Jilani and Saxena (1990), Reed et al. (1982), Zehnder and Warthen (1988)) and Hemiptera (for example Abudulai et al. (2003), dos Santos-Cividanes et al. (2004)). Klemm and Schmutterer (1993) studied the effects of neem preparation on *Plutella xylostella* (Linnaeus) (Lepidoptera), one of the most important cab-

bage pests in agriculture. Extensive research is being conducted with neem and its derived products against target pests. Abudulai et al. (2003) found that a commercial product of neem had an antifeedant effect as well, as it affected development and molting. Dos Santos-Cividanes et al. (2004) conducted an experiment to check the effect of neem seed powder on *Aphis gossypii* (Glover) and found that the aqueous neem seed extract is efficient in nymph mortality and reducing the survival period and fecundity of the cotton aphid.

The effect of neem-derived products on non-target organisms has also been studied. However, information on the effect of neem-based pesticides and formulations on non-target organisms of soil biota are still limited. Scott and Kaushik (1998) found that neem products have a threshold chronic toxicity on non-target organisms like the crustaceans *Daphnia magna* (Straus, 1820) and *Hyaella azteca* (Saussure, 1858). Wagenhoff et al. (2013) tested the effects of NeemAzal T/S on reproductive output of *Nicrophorus vespilloides* (Herbst 1783), a common burying beetle, feeding on a variety of animal carcasses. *Nicrophorus vespilloides* were fed with cockchafer which were previously fed with neem-treated leaves. They found that it had no negative impact on the reproduction nor they found any impacts on the morphology of *N. vespilloides*.

Woodlice species (Isopoda, Oniscidae) are ubiquitous saprophagous members of the soil fauna (Paoletti and Hassall 1999). They are present in various densities both in conventional and organic farming systems (ibid), and as such exposed to any pesticide treatment. Isopods can also be used for biomonitoring, both in contaminated or remediated areas (Loureiro et al. 2006). Isopods inhabit littoral zone, beach, grassland, woodland, desert, and more special habitats (Warburg 1987). Adaptations to these environments are thought to be largely behavioral but it now appears that there are also well-established physiological adaptations, based on anatomical structures. Certain terrestrial isopod genera are able to detect chemical cues using their second antenna pair (Harzsch et al. 2011). This can explain the results of Santos et al. (2011) where the binary combinations of dimethoate, glyphosate and spirodiclofen, an insecticide and an herbicide and an acaricide respectively, resulted a dose related avoidance response of *P. pruinosus*.

In another study, single and combined toxicity of atrazine, dimethoate, lindane, zinc and cadmium were tested in *Porcellionides pruinosus* (Brandt, 1833) and *Enchytraeus albidus* (Henle, 1837) an annelid, commonly known as white worm, using avoidance as test parameter. For both the species, patterns of antagonism were found when exposed to dimethoate and atrazine, synergism for lindane, dimethoate, and atrazine, synergism for lindane and dimethoate exposures and concentration addition for cadmium and zinc occurred, while the exposure to cadmium and dimethoate showed dissimilar patterns (Loureiro et al. 2009).

This soil 'cleaning' result can be defined as a positive ecosystem service (ES) (MA 2005). ES can provide numerous goods and services by the organisms, guilds, and ecological communities to society (Garbach et al. 2014). Soil functions are strictly dependent on structure and biodiversity. They exposed to several physical, chemical, and biological stressors, which are directly or indirectly related to anthropogenic activities (Morgado et al. 2018). If the biodiversity is not too affected by these it can provide

other important roles like decomposition (Jia et al. 2015; El-Wakeil 2015), or heavy metal accumulation (Mazzei et al. 2014) that especially isopods execute. Moreover just with their presence the biodiversity becomes more complete and more balanced (Pokarzhevskii et al. 2003; Smeding and de Snoo 2003).

A small-scale terrestrial ecosystem containing soil collected from an agricultural field in Central Portugal was used to evaluate the effects of the combination of the herbicide glyphosate and the insecticide dimethoate in another study. The application of dimethoate led to a decrease in feeding activity in all concentrations tested. The mortality of isopods exposed to dimethoate in single and binary exposures was high. Exposure to dimethoate decreased the acetylcholinesterase activity of isopods (Santos et al. 2011).

In this paper we present results on the side-effect of NeemAzal T/S and neem leaf extract on the terrestrial isopod species *Porcellionides pruinosus*. We selected *P. pruinosus* as a non-target organism, because it is ubiquitous and it occurs in anthropogenic environments, where pest control is applied. They play a vital role in the fragmentation and decomposition process of leaf litter, thereby causing changes in soil quality and soil services (Ferreira et al. 2016).

## Materials and methods

The methodology of Akca et al. (2015) was followed with modifications. Experiments were carried out with six different concentrations of both NeemAzal T/S and neem leaf extract with a control, each replicated ten times under laboratory conditions.

*Collection of isopod species:* *Porcellionides pruinosus* adults were collected from Regional Waste Management Center Pusztázámor, Hungary, by hand sorting. Isopods were bred and maintained at the Institute of Plant Protection of Szent István University, Gödöllő, Hungary. Species level identification was based on the taxonomic key developed by Brandt (1833) (Farkas and Vilisics 2013).

*Preparation of neem leaf extract:* For neem leaf extract, air-dried neem leaves were obtained from local growers in India, Maharashtra, Konkan Division, Mumbai Suburban area. A stock concentration of 1% was prepared by soaking 1g of crushed dried neem leaves in 100 ml distilled water overnight and then filtered using a non-sterile filter paper. Different working concentrations (0.05, 0.1, 0.25, 0.5, 0.75, and 1%) of neem leaf extract were prepared from 1% stock solution using distilled water in the laboratory and were used on the same day. Generally the applied dosage used by local growers in India has a maximum concentration of 1%. In this experiment, we tried to model the concentration used by the local growers in the field conditions.

*Preparation of azadirachtin:* NeemAzal T/S (Trifolio-M GmbH), a commercial product containing 1% azadirachtin, registered in the EU, was used. A stock concentration of 1% azadirachtin was prepared (from NeemAzal T/S which is 1% azadirachtin) by diluting 1 ml NeemAzal T/S in 100 ml of distilled water which equals to 0.01%

azadirachtin. It was further diluted to get the 0.0005, 0.001, 0.0025, 0.005, 0.0075 and 0.01% azadirachtin concentrations respectively and was used on the same day. The registered dosage of azadirachtin ranges from 0.0025 to 0.005%, depending on the plant culture in the EU.

A control with only distilled water was used for both experiments. The working concentrations and distilled water were sprayed using a hand sprayer under laboratory conditions.

*Experimental design:* Five adults of *Porcellionides pruinosus* were placed in glass Petri dishes (13 cm in diameter), with 1 g of commercial horticultural soil (pH = 7.0) and approximately 1 g of fresh potato as a food source. Two milliliters of different working concentrations of neem leaf extract and azadirachtin were sprayed using a hand sprayer. After spraying, the Petri dishes were kept in the dark, checked after time periods of 1, 24, 48, 72, 96, and 120 hours post-application of neem leaf extract and azadirachtin respectively, and mortality data was recorded. The mortality data obtained after 120 hrs was subjected to statistical analysis using R software (R Core Team 2017). Logistic regressions were fitted (as the response was binary, i.e., the isopods were either dead or alive) to check the effect of the two different products on isopod mortality. To test whether the concentrations have significant effect on mortality, chi-squared tests were performed on model deviances. Prior to running the tests model diagnostic plots were investigated to assess homoscedasticity and residual normality (Faraway 2002).

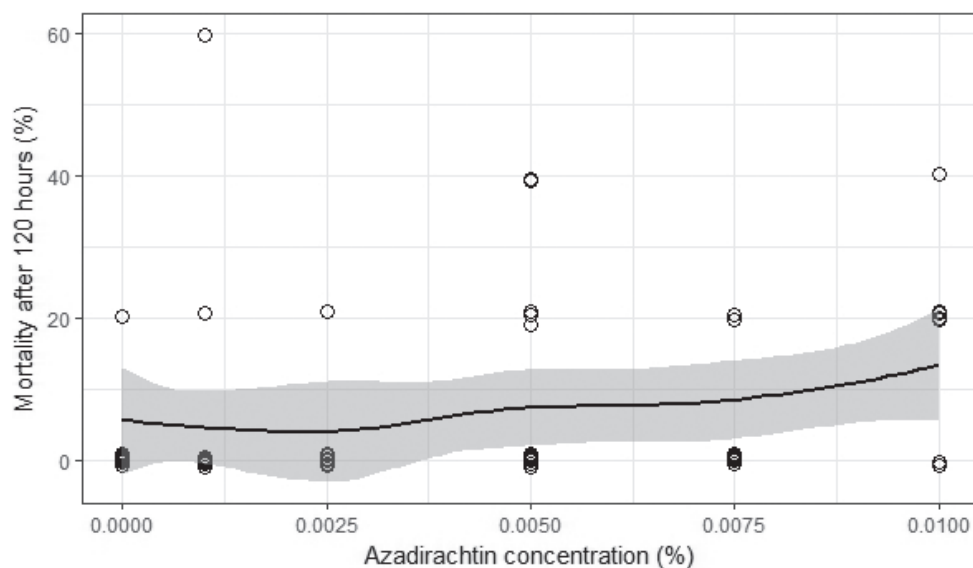
## Results

The mortality of *P. pruinosus* was generally low in all treatments. In case of azadirachtin, even after 120 hours zero mortality was observed in seven replicates of 0.0005% concentration, eight replicates at 0.001%, nine replicates of 0.0025%, seven replicates of 0.005 and 0.0075% each, and four replicates of 0.01%.

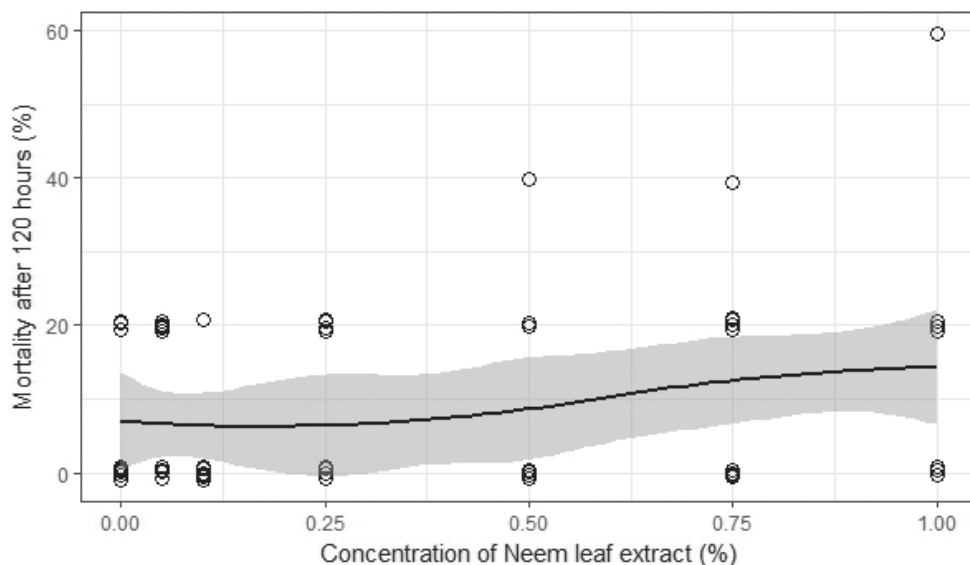
The same was observed in the case of neem leaf extract, after the time period of 120 hours: zero mortality in case of five replicates of 0.05% concentration, nine replicates of 0.1%, six replicates of 0.25%, seven of 0.5%, four replicates of 0.75%, and five replicates of 1% (see Table 2).

The mortality slightly increased with the concentration but this observed increment was not statistically significant (Table 2). Unusually high values (i.e., higher mortality) were occasionally observed both in NeemAzal T/S and neem-leaf extract treatments. These can be attributed either to the juvenile mortality of *P. pruinosus* (Dangerfield and Telford 1995) or suboptimal conditions.

Different concentrations of NeemAzal T/S and neem leaf extracts were compared to check their respective effects on the mortality of the isopods. Neither azadirachtin nor neem leaf extract affected the observed isopod mortality (p-values are 0.43 and 0.39 and McFadden's pseudo  $R^2$ : 0.04 for azadirachtin, 0.05 for neem leaf extract respectively; Figs 1, 2).



**Figure 1.** Mortality rate of the isopod *Porcellionides pruinosus* after 120 hours at different concentrations of NeemAzal T/S (1% azadirachtin). The vertically jittered circles (to avoid perfect overlapping) indicate the individual isopods whereas the line indicates the trend of the mortality with respect to increasing concentrations and the grey area represents the 95% confidence level.



**Figure 2.** Mortality rate of the isopod *Porcellionides pruinosus* after 120 hours to at different concentrations of neem leaf extract. The vertically jittered circles (to avoid perfect overlapping) indicate the individual isopods whereas the line indicates the trend of the mortality with respect to increasing concentrations and the grey area being the 95% confidence level.



**Table 1.** Effect of NeemAzal on the mortality of *Porcellionides pruinosus* expressed as cumulative mean for different time intervals.

Treatment	conc (%)		Mean mortality rate after time interval					
			1 hr	24 hrs	48 hrs	72 hrs	96 hrs	120 hrs
azadirachtin (NeemAzal T/S)	0	mean	0.1	0.1	0.1	0.1	0.1	0.2
		SD	0.32	0.32	0.32	0.32	0.32	0.42
	0.0005	mean	0.2	0.3	0.3	0.3	0.3	0.5
		SD	0.42	0.67	0.67	0.67	0.67	0.85
	0.001	mean	0	0.3	0.3	0.3	0.4	0.4
		SD	0	0.95	0.95	0.95	0.97	0.97
	0.0025	mean	0	0	0	0	0	0.1
		SD	0	0	0	0	0	0.32
	0.005	mean	0.1	0.2	0.2	0.2	0.2	0.3
		SD	0.32	0.42	0.42	0.42	0.42	0.48
	0.0075	mean	0.2	0.2	0.2	0.2	0.2	0.3
		SD	0.42	0.42	0.42	0.42	0.42	0.48
	0.01	mean	0	0.1	0.5	0.5	0.5	0.7
		SD	0	0.42	0.71	0.71	0.71	0.67

Key: conc = concentration, SD= Standard deviation. Each value is an average of ten replicates.

**Table 2.** Effect of neem leaf extract on the mortality of *Porcellionides pruinosus* expressed as cumulative mean for different time intervals.

Treatment	conc (%)		Mean mortality rate after time interval					
			1 hr	24 hrs	48 hrs	72 hrs	96 hrs	120 hrs
neem leaf extract	0	mean	0	0	0	0	0.1	0.33
		SD	0	0	0	0	0.33	0.5
	0.05	mean	0	0	0	0.1	0.2	0.527
		SD	0	0	0	0.32	0.42	0.52
	0.1	mean	0	0	0	0	0	0.1
		SD	0	0	0	0	0	0.32
	0.25	mean	0	0	0	0	0.2	0.4
		SD	0	0	0	0	0.42	0.52
	0.5	mean	0	0	0	0	0	0.4
		SD	0	0	0	0	0	0.7
	0.75	mean	0	0.1	0.2	0.2	0.2	0.7
		SD	0	0.32	0.42	0.42	0.42	0.67
	1	mean	0	0.1	0.2	0.3	0.4	0.7
		SD	0	0.32	0.42	0.67	0.7	0.95

Key: conc = concentration, SD= Standard deviation. Each value is an average of ten replicates.

## Discussion

While there are numerous literature references available on the effect of neem and neem-derived products on target organisms, some of the studies reported data on non-target organisms as well. For instance, Goktepe et al. (2004) carried out an ecological

risk assessment of neem-based products on six aquatic animals through short-term acute toxicity tests and concluded that the risk values did not exceed the criteria and were safe for use. In contrast, it has been noted that neem components do have adverse effects on non-target aquatic organisms such as *Daphnia* species (Straus, 1820) as studied by Stark (1997) and fish (Tangtong and Wattanasirmit 1997). Scott and Kaushik (1998) assessed the effect of Margosan-O (a product of neem seeds) on non-target aquatic invertebrates. Their investigation revealed that there can be some effects of the product on non-target organisms at higher concentration but if applied in agricultural systems, Margosan-O may not reduce the survival or reproduction of the non-target aquatic organisms. Wagenhoff et al. (2013) studied the effects of NeemAzal T/S on the burying beetle *Nicrophorus vespilloides*, which co-occurs with the forest cockchafer *Melolontha hippocastani* (Fabricius, 1801) and also feeds on the carcasses of *M. hippocastani*. In their study, they fed *N. vespilloides* with dead *M. hippocastani* which were previously fed with neem-treated leaves. They neither observed any impact on the mean larval weights nor on the morphology of *N. vespilloides*. Still, they authors did not dismiss the possibility of azadirachtin passing through the food chain and affecting other non-target organisms.

Akca et al. (2015) investigated the effect of azadirachtin (NeemAzal T/S) on terrestrial isopod *Philoscia muscorum* (Scopoli, 1763) and did not find any negative effects on *P. muscorum*. The results of our experiments were found to be similar and this experiment for the first time investigated the effects of two different neem products on this non-target isopod species, i.e., *Porcellionides pruinosus*.

From our results it can be concluded that neither NeemAzal T/S nor neem leaf extracts pose any risk to the terrestrial isopod species studied in the tested concentrations. However, further research is needed to test the possible effect of various neem products on the members of the soil fauna. Also, it can be concluded that NeemAzal T/S and domestic neem leaf extract do not differ in respect to their mortality effects on *P. pruinosus*.

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## Author contribution statement

PD and AMP designed and conducted the experiments. MS analyzed the data. PD wrote the manuscript. AMP, FT, GT, and MS gave their feedback. All authors read and approved the manuscript.

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# Comparative ultrastructure of cells and cuticle in the anterior chamber and papillate region of *Porcellio scaber* (Crustacea, Isopoda) hindgut

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## Abstract

Isopod hindgut consists of two anatomical and functional parts, the anterior chamber, and the papillate region. This study provides a detailed ultrastructural comparison of epithelial cells in the anterior chamber and the papillate region with focus on cuticle ultrastructure, apical and basal plasma membrane labyrinths, and cell junctions. Na<sup>+</sup>/K<sup>+</sup>-ATPase activity in the hindgut epithelial cells was demonstrated by cytochemical localisation. The main difference in cuticle ultrastructure is in the thickness of epicuticle which is almost as thick as the procuticle in the papillate region and only about one sixth of the thickness of procuticle in the anterior chamber. The apical plasma membrane in both hindgut regions forms an apical plasma membrane labyrinth of cytoplasmic strands and extracellular spaces. In the papillate region the membranous infoldings are deeper and the extracellular spaces are wider. The basal plasma membrane is extensively infolded and associated with numerous mitochondria in the papillate region, while it forms relatively scarce basal infoldings in the anterior chamber. The junctional complex in both hindgut regions consists of adherens and septate junctions. Septate junctions are more extensive in the papillate region. Na<sup>+</sup>/K<sup>+</sup>-ATPase was located mostly in the apical plasma membranes in both hindgut regions. The ultrastructural features of hindgut cuticle are discussed in comparison to exoskeletal cuticle and to cuticles of other arthropod transporting epithelia from the perspective of their mechanical properties and permeability. The morphology of apical and basal plasma membranes and localisation of Na<sup>+</sup>/K<sup>+</sup>-ATPase are compared with other arthropod-transporting epithelia according to different functions of the anterior chamber and the papillate region.



**Keywords**

cell junctions, digestive system, extracellular matrix, ion transporting epithelium, plasma membrane labyrinth

**Introduction**

The digestive system in terrestrial isopods is composed of a foregut, hindgut, and hepatopancreas (midgut glands). The foregut and hindgut are of ectodermal origin and form the entire alimentary canal, thus the blind-ending tubules of hepatopancreas connected to the foregut represent the only endodermal part of digestive system (Vernon et al. 1974, Hassall and Jennings 1975, Bettica et al. 1987, Storch and Štrus 1989, Štrus et al. 2008). In some amphibious species, a short segment of endodermal midgut is located between the foregut and the hindgut (Štrus et al. 1995). The hindgut is divided into an anterior chamber, papillate region and short rectum separated from the papillate region by a muscular sphincter. In the anterior chamber of terrestrial isopods, the folded dorsal hindgut wall forms a dorsal longitudinal fold termed typhlosole, together with two typhlosole channels. In the papillate region the dome-shaped basal parts of epithelial cells bulge into haemocoel between the longitudinal and circular muscles surrounding the hindgut (Hassall and Jennings 1975, Hames and Hopkin 1989, Štrus et al. 1995). Hames and Hopkin (1989) reported that the anterior chamber is the site where digestion of food mixed with digestive enzymes takes place, while the papillate region is involved in compaction of faecal pellets and removal of water.

The hindgut epithelium is mono-layered and lined by a chitinous cuticle on the luminal side. Basal parts of the epithelial cells are supported by a basal lamina and are exposed to haemolymph. The hindgut cuticle is thin and consists of two distinct layers, the electron dense epicuticle facing the lumen and the electron lucent procuticle beneath. Posteriorly directed cuticular spines are present at the surface of epicuticle (Vernon et al. 1974, Palackal et al. 1984, Storch and Štrus 1989, Mrak et al. 2015). The main ultrastructural characteristics of hindgut epithelial cells are the extensively infolded apical and basal plasma membranes, numerous mitochondria associated with membrane infoldings, abundant apico-basally oriented microtubules, and extensive septate junctions (Vernon et al. 1974, Coruzzi et al. 1982, Palackal et al. 1984, Storch and Štrus 1989). Previous ultrastructural studies have provided knowledge concerning the general ultrastructure of hindgut epithelial cells in isopods. The specific gaps to be addressed are the differences in the ultrastructure of hindgut epithelial cells in the two main hindgut regions, the anterior chamber, and the papillate region.

Notwithstanding the presence of apical chitinous cuticle, the ultrastructural characteristics indicate that the hindgut epithelium is involved in various transport processes. The epithelium of the anterior chamber was reported to function in the absorption of food material in addition to the hepatopancreas, which represents the main site of food absorption (Hryniewiecka-Szyfter and Storch 1986, Hames and Hopkin 1989).

Extensively infolded apical and basal plasma membranes associated with numerous mitochondria, abundant microtubular network and extensive septate junctions indicate the involvement of papillate region epithelium in ion and water transport and thus in osmoregulation (Vernon et al. 1974, Coruzzi et al. 1982, Palackal et al. 1984). Ultracytochemical localisation of  $\text{Na}^+/\text{K}^+$ -ATPase activity in the hindgut of *Armadillo officinalis* has shown  $\text{Na}^+/\text{K}^+$ -ATPase activity in the apical or basolateral plasma membrane infoldings (Warburg and Rosenberg 1989).

The aim of the present study is ultrastructural characterisation, quantification of the selected characters and comparison of the hindgut epithelial cells in the anterior chamber and the papillate region of terrestrial isopod *Porcellio scaber* to upgrade previous knowledge and to get additional insight into the hindgut functional morphology. The selected ultrastructural features for a detailed analysis were: (i) cuticle, (ii) apical and basal plasma membrane labyrinths, and (iii) cell junctions. A comparative investigation of the selected ultrastructural features in the anterior chamber and papillate region epithelium is presented, including quantitative evaluation of the selected morphological characteristics, and the possible functional implications are discussed. A method for cytochemical localisation of  $\text{Na}^+/\text{K}^+$ -ATPase activity in the hindgut of strictly terrestrial isopod *P. scaber* was used to demonstrate ion fluxes in the hindgut epithelium of intermoult and postmoult specimens.

## Materials and methods

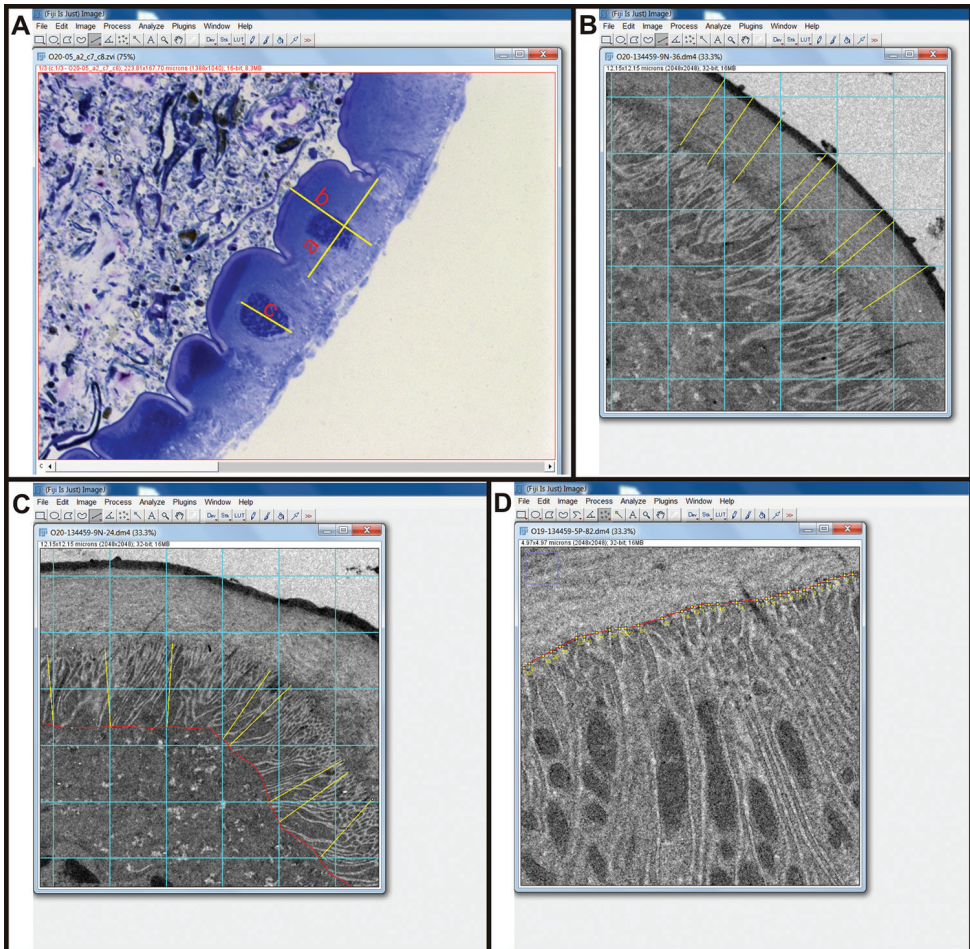
### Sample preparation and imaging

A laboratory culture of *P. scaber* was maintained in a glass terrarium with a ground cover of soil and leaf litter. Animals were bred at 25 °C, in high humidity and a 12h light/12h dark cycle. In this study the hindgut samples of seven adult intermoult animals were analysed at the levels of light and electron microscopy. Three animals were anaesthetised by cooling and dissected in a physiological solution (0.9% NaCl). The hindguts were isolated and fixed in 2.5 % glutaraldehyde in 0.1 M HEPES buffer (pH 7.2). Four animals were anaesthetised with diethyl ether, dissected in a solution of 2 % paraformaldehyde and 2.5% glutaraldehyde in 0.1 M HEPES buffer (pH 7.2) and isolated hindguts were fixed in the same fixative solution. After fixation, all samples were rinsed with 0.1 M HEPES buffer and post-fixed in 1 %  $\text{OsO}_4$ . Subsequently the samples were rinsed again with 0.1 M HEPES buffer. Before the embedding samples were dehydrated in a graded series of ethanol (50 %, 70 %, 80 %, 90 %, and 100 % ethanol) and transferred to absolute acetone. After the dehydration, samples were infiltrated and embedded in Agar 100 resin. Resin-embedded samples were polymerised in embedding moulds for 48 h at 60 °C. Semithin and ultrathin sections were cut with glass and diamond knives on Reichert Ultracut S ultramicrotome (Leica). Semithin sections were stained with Azure II – Methylene Blue and imaged with AxioImager Z.1 light microscope (Zeiss). Microscopic images were acquired with a HRc AxioCam

camera using Axiovision software. Ultrathin sections were contrasted with 4 % uranyl acetate and 10 % lead citrate and examined with a Philips CM100 transmission electron microscope. Microscopic images were acquired with Bioscan 792 and Orius 200 (Gatan) cameras using Digital Micrograph software.

### Measurements of selected morphological characteristics

Measurements of cell size and cell nuclei diameter, cuticle and basal lamina thicknesses, depth of membrane labyrinths and spatial density of membrane infoldings were carried out with Fiji/ImageJ software for processing and analysis of digital micrographs. Cell height and width and largest cell nucleus diameter were measured on light micrographs with a straight-line tool in Fiji (Fig. 1A). Measurements were done on seven individual animals. In each animal we measured 7–29 cells from the anterior chamber and from the papillate region. Only cells sectioned across their cell nucleus were measured. In the Results section, measurements are presented graphically with a scatter plot depicting the median values of cell width, cell height and cell nucleus diameter in the anterior chamber against median values in the papillate region of seven animals. In the supplementary information (SI), individual measurements from the two hindgut regions of individual animals are presented graphically with boxplots accompanied by stripcharts. Measurements of cuticle and basal lamina thicknesses and membrane labyrinths depths were done on 3 individual animals. For each animal 7–71 measurements were made on 1–4 cells from the anterior chamber and from the papillate region. For measurements of cuticle and basal lamina thicknesses overlays of multipurpose grid on the electron micrographs were made in Fiji. The multipurpose grid is available as macro at: [https://imagej.nih.gov/ij/macros/Multipurpose\\_grid.txt](https://imagej.nih.gov/ij/macros/Multipurpose_grid.txt). The thicknesses of the cuticle and of the basal lamina were measured at the intersections of grid lines with the cuticle/basal lamina surface. Measurements were made perpendicular to the cuticle/basal lamina surface with a straight-line tool (Fig. 1B). In the Results section, measurements are presented graphically with stripcharts depicting median values of cuticle thickness and basal lamina thickness in cells from the anterior chamber and the papillate region of 3 animals. In the SI, individual measurements on cells from the two hindgut regions of each animal are presented graphically with boxplots accompanied by stripcharts. For measurements of apical and basal membrane labyrinth depths the edges of the membrane labyrinths were outlined on electron micrographs with segmented line tool in Fiji and overlays of multipurpose grid on micrographs were made. Membrane labyrinth depth was measured at intersections of grid lines with the outline of membrane labyrinth edge. Measurements were made perpendicular to the apical/basal cell surface with a straight-line tool (Fig. 1C). In the Results section, measurements are presented graphically with scatter plots depicting the median values of apical labyrinth depth against median values of basal labyrinth depth in cells from the anterior chamber and the papillate region of three animals. In the SI, individual measurements on cells from the two hindgut regions of each animal are presented graphically with



**Figure 1.** Measurements in ImageJ/Fiji. **A** Measurements of cell width (line a), height (line b) and nucleus diameter (line c) **B** Measurements of cuticle and basal lamina thickness. Cuticle and basal lamina thickness (yellow lines) were measured at intersections of grid lines (blue grid) with cuticle/basal lamina surface **C** Measurements of membrane labyrinths depth. Membrane labyrinth depths (yellow lines) were measured at intersections of grid lines (blue grid) with the outline of membrane labyrinth edge (red line) **D** Measurements of spatial density of membrane infoldings. The length of apical/basal surface outline (red line) was measured and the infoldings along the outline (yellow points) were counted.

boxplots accompanied by stripcharts. Measurements of spatial density of membrane infoldings were done on three individual animals. For each animal 1–6 measurements were made on 1–4 cells from the anterior chamber and from the papillate region. In Fiji the apical/basal cell surface were outlined with a segmented line tool. The length of outline was measured and the number of membrane infoldings along the outline was counted (Fig. 1D). The density of membrane infoldings per 1  $\mu\text{m}$  of apical/basal cell surface was calculated. In the Results section, measurements are presented graphically with scatter plots depicting median values of apical infolding density against median

values of basal infolding density in cells from the anterior chamber and the papillate region of three animals. In the SI, individual measurements on cells from the two hindgut regions of each animal are presented graphically with stripcharts. All plots were done in RStudio.

### Na<sup>+</sup>/K<sup>+</sup>-ATPase activity

Ultracytochemical localisation of ouabain-sensitive Na<sup>+</sup>/K<sup>+</sup>-ATPase activity (K<sup>+</sup>-NPPase) in the hindgut of *P. scaber* was performed in postmoult and intermoult adults, using the method of Mayahara et al. (1980). Adult postmoult and intermoult specimens of *P. scaber* were dissected and the hindguts were isolated and cut into small pieces of the anterior and papillate parts. Tissue samples were fixed in 0.5 % glutaraldehyde and 2 % paraformaldehyde in 0.1 M sodium cacodylate buffer (pH 7.4). After fixation tissue samples were incubated for 20 min at 25 °C in a medium containing 250 mM glycine/KOH buffer (pH 9), 4 mM lead citrate, 25 % DMSO, 10 mM p-nitrophenylphosphate (p-NPP), and 2.5 mM levamisole. Tissue samples for controls were incubated in 1) a substrate-free (p-NPP) medium, 2) a medium in which K<sup>+</sup> ions were replaced by Na<sup>+</sup> ions and 3) a medium containing 10 mM ouabain, an inhibitor of K<sup>+</sup>-NPPase activity. Tissue samples were then postfixed in 1 % osmium tetroxide and embedded in Spurr resin. Unstained ultrathin sections were examined with a Gatan Bioscan 972 camera attached to a TEM Philips CM 100.

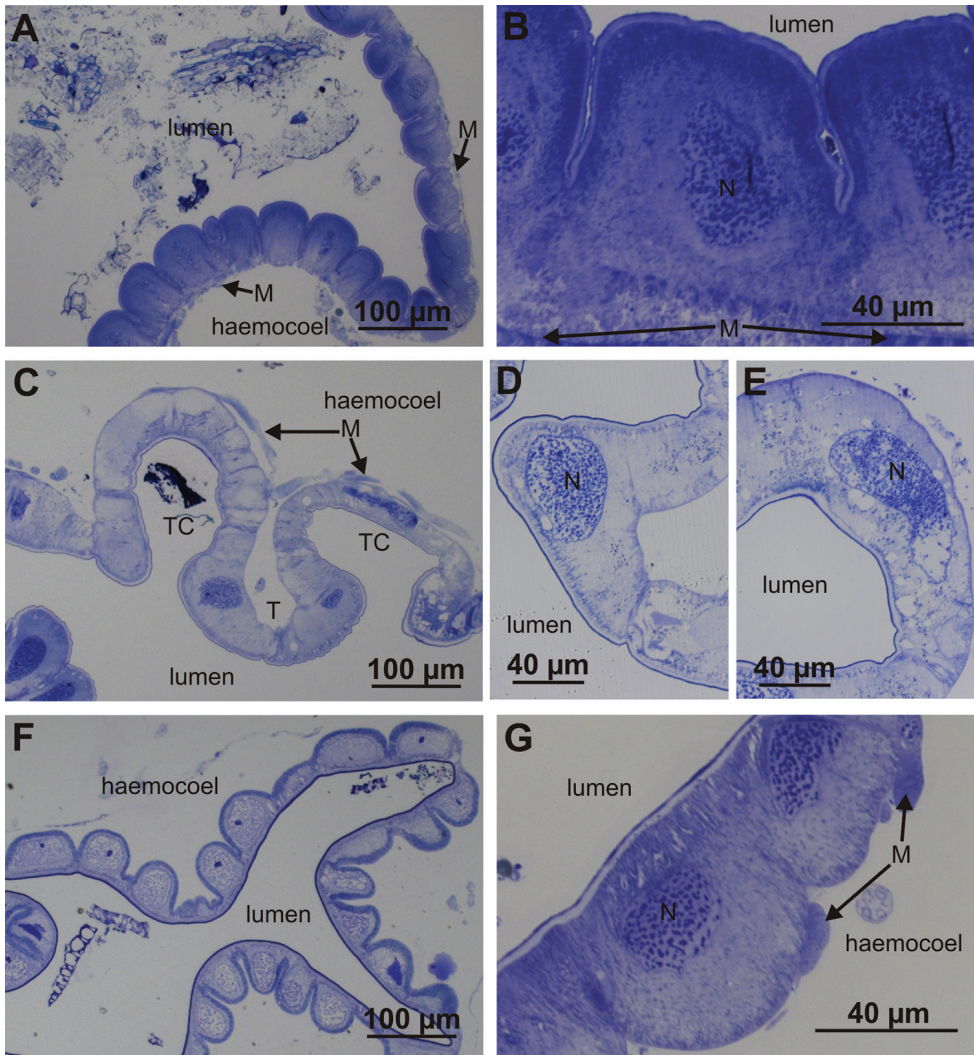
## Results

### Histological structure

Cells forming the ventral and lateral hindgut wall of anterior chamber are isodiametric and dome-shaped, their apical parts protruding into the lumen of the anterior chamber (Fig. 2A, B). The dorsal epithelial cells that build the typhlosole and typhlosole channels are flatter and wider than cells of the ventral and lateral walls (Fig. 2C, D, E). In the papillate region, epithelial cells are isodiametric, and in contrast to those in the anterior chamber, display dome-shaped basal parts, which protrude into the haemocoel (Fig. 2F, G). Muscle layers are present on the basal side of epithelium in both hindgut regions and are more abundant in the anterior chamber.

Our measurements of cell width, cell height and cell nuclei size do not reveal difference in the size of the cells and their nuclei between the two hindgut regions (Fig. 3). Cells in both regions are large and the majority of the cells in the anterior chamber are 40 to 80 µm wide and 40 to 100 µm high. In the papillate region the majority of the cells are 60 to 90 µm wide and 50 to 80 µm high. All hindgut cells have large cell nuclei, which in a majority of the cells are between 20 and 50 µm in diameter (Suppl. material 1).

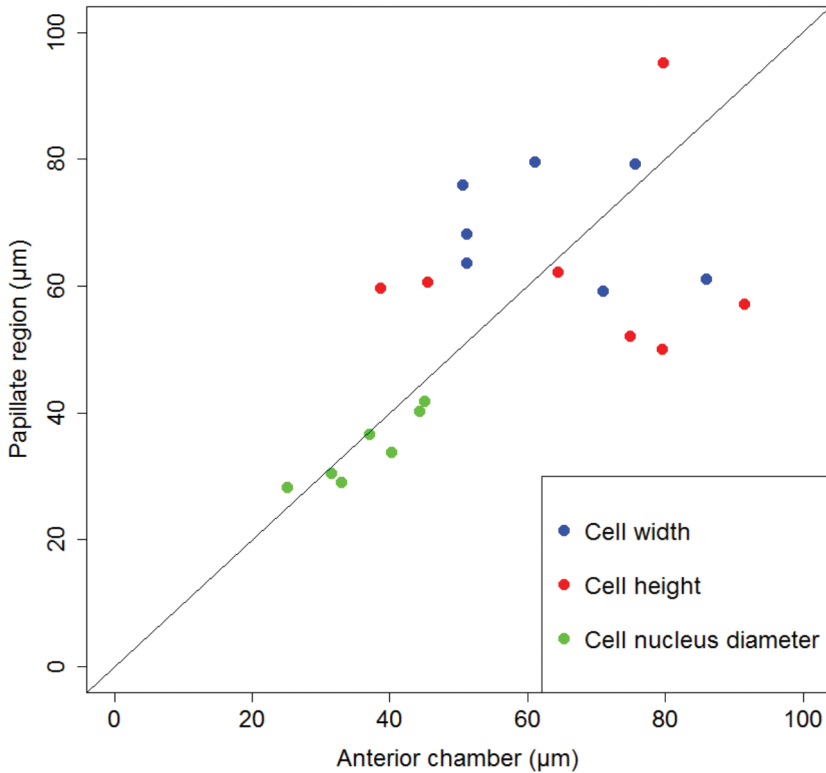




**Figure 2.** Histological structure of the hindgut epithelium in the anterior chamber and papillate region. **A** Ventral-lateral epithelium in the anterior chamber **B** Apical parts of ventral and lateral epithelial cells are bulging into the hindgut lumen **C** Dorsal epithelium in the anterior chamber forms typhlosole (T) and two typhlosole channels (TC) **D** Epithelial cell of typhlosole **E** Epithelial cell of typhlosole channel **F** Hindgut epithelium in the papillate region **G** Basal parts of epithelial cells in the papillate region are bulging into haemocoel. Abbreviations: M – muscles, N – cell nucleus.

### Cuticle ultrastructure

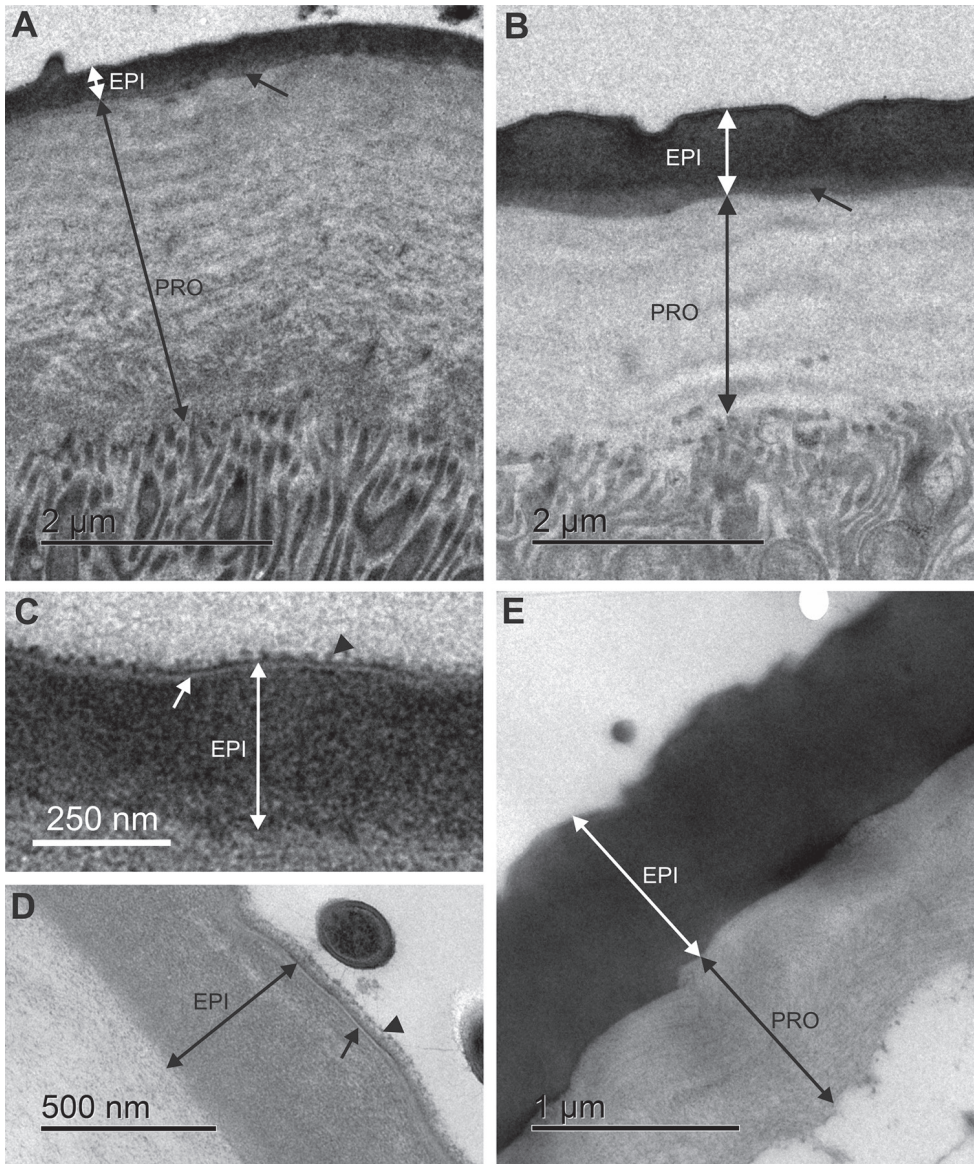
The apical side of the epithelium in both hindgut regions is lined by a cuticle, which consists of an outer electron dense epicuticle and an inner electron lucent procuticle (Fig. 4A, B). The epicuticle consists of thin three-layered outer part and thicker electron dense inner part. The outer part of epicuticle is about 10 nm thick



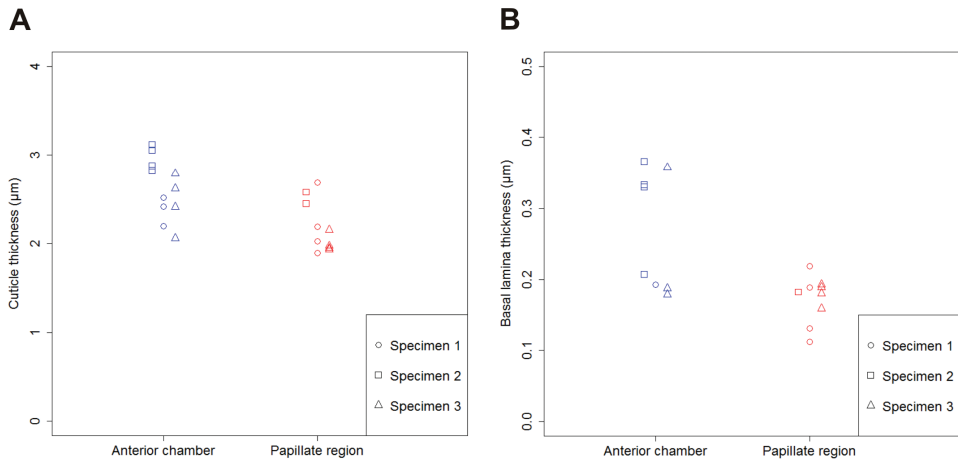
**Figure 3.** Cell width, cell height and cell nucleus diameter in the two hindgut regions. Scatter plot depicting median values of cell width, cell height and cell nucleus diameter in the anterior chamber against median values of same parameters in the papillate region of seven specimens. The central line is the line of equality. Points lying on the line indicate that the median value of measured parameter is equal in both hindgut regions. Points below the line indicate that the median value of measured parameter is larger in the anterior chamber than in the papillate region. Points above the line indicate that the median value of measured parameter is larger in the papillate region than in the anterior chamber.

and consists of two electron dense sheets enclosing a central electron lucent sheet. The inner part of epicuticle is homogenous and 300–500 nm thick in the anterior chamber and 700–900 nm thick in the papillate region. The outer part of epicuticle is covered with a fuzzy layer on the luminal surface (Fig. 4C, D). Between the epicuticle and the procuticle a thin layer of intermediate electron density is present (Fig. 4A, B). No pore canals were observed in the hindgut cuticle. The thickness of the entire cuticle in both hindgut regions is in the range between 1.5 and 3 μm (Fig. 5A, Suppl. material 2). A notable difference exists in the ratio of epicuticle to procuticle thickness. In the anterior chamber the procuticle is significantly thicker than the epicuticle and the ratio of epicuticle to procuticle thickness is approximately 1:5 (Fig. 4A). The ratio of epicuticle to procuticle thickness in the papillate region





**Figure 4.** Ultrastructure of the cuticle in the anterior chamber and the papillate region. **A** The cuticle in the anterior chamber consists of thin epicuticle (EPI) and thick procuticle (PRO) with distinct sublayers. Between the epicuticle and the procuticle, a thin layer of intermediate electron density is present (arrow) **B** Cuticle in the papillate region consists of epicuticle (EPI) which is proportionally thicker according to the procuticle (PRO). Procuticle sublayers are less pronounced than in the anterior chamber. Between the epicuticle and the procuticle a thin layer of intermediate electron density is present (arrow) **C, D** The outer part of the epicuticle (EPI) in the anterior chamber (**C**) and in the papillate region (**D**) is three-layered (arrow) and covered with a fuzzy layer (arrowhead) **E** Procuticle (PRO) in the papillate region can be of approximately the same thickness as epicuticle (EPI) and without apparent sublayers.



**Figure 5.** Cuticle and basal lamina thickness in the two hindgut regions. Median values of cuticle thickness (**A**) and basal lamina thickness (**B**) in cells from the anterior chamber (blue) and the papillate region (red) of three specimens.

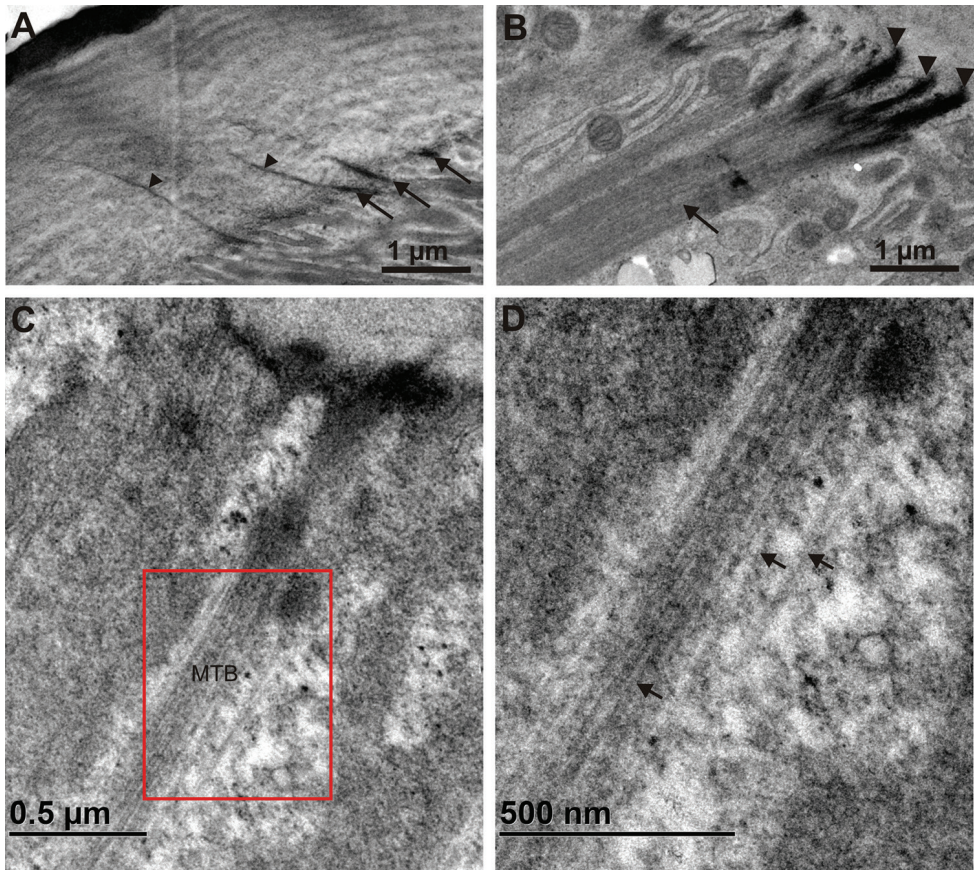
is between 1:3 and 1:1 (Fig. 4B, E). In the papillate region the epicuticle is thicker than in the anterior chamber. The difference was also observed in the procuticle sublayers that appear due to helicoidally arranged chitin protein fibres. In the anterior chamber the procuticle sublayers can be distinguished (Fig. 4A). The procuticle sublayers in the papillate region are either less pronounced and thicker than in the anterior chamber (Fig. 4B) or not visible (Fig. 4E). In addition, the ultrastructure of connections between apical plasma membrane and cuticle is shown. Connections are present in individual epithelial cells in both hindgut regions and consist of electron dense fibres, which extend from the electron dense plaques at the apical cell surface into the procuticle. These fibres are more prominent in the anterior chamber (Fig. 6A). Connections in both hindgut regions are associated with apico-basally oriented bundles of microtubule-like filaments. These bundles are more abundant in the papillate region (Fig. 6B, C, D).

In the papillate region of one specimen, bacteria were observed near the cuticle or directly attached to the fuzzy layer at the cuticle surface (Fig. 7A). Spherical or rod-shaped profiles of sectioned bacteria were noted. A dense cytoplasm is enveloped by two electron dense sheets enclosing the middle electron lucent sheet. Bacteria are connected to the fuzzy layer of epicuticle by thin filamentous structures (Fig. 7B). The thin filamentous connections between bacterial cells are also clearly resolved (Fig. 7A).

### Apical and basal plasma membrane labyrinths

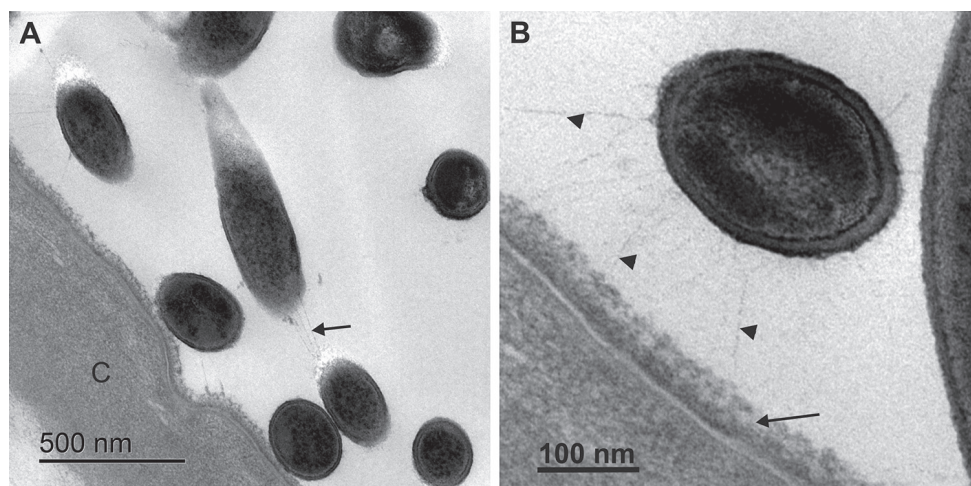
The general characteristic of the apical and basal plasma membranes of epithelial cells in both hindgut regions is that they are infolded, and form apical and basal plasma mem-



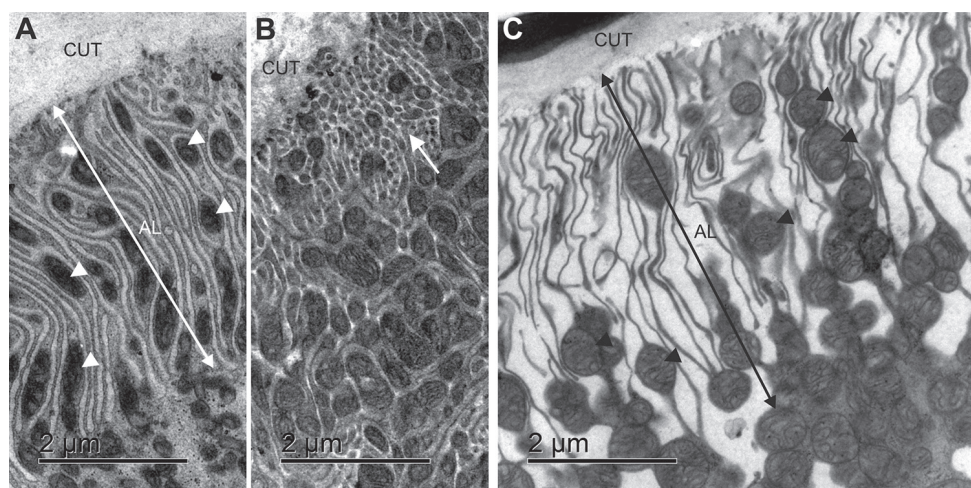


**Figure 6.** Ultrastructure of junctions between cuticle and apical plasma membrane. **A** Junctions between cuticle and apical plasma membrane in the anterior chamber visible as electron dense structures (arrows), from which fibers (arrowheads) extend into the procuticle **B** Junctions between cuticle and apical plasma membrane in the papillate region (arrowheads) associated with abundant bundles of microtubules inside the cell (arrow) **C, D** Higher resolution images of bundles of microtubules (MTB) associated with junctions between cuticle and apical plasma membrane. Image **D** displays the area denoted on image **C** (red rectangle) where individual microtubules (arrows) can be discerned.

brane labyrinths. However, considerable differences between the two hindgut regions were observed in the depth of the membrane infoldings, in the spatial density of membrane infoldings and in the abundance of mitochondria associated with membrane infoldings. The apical plasma membrane in cells of both hindgut regions forms extensive and complex labyrinth of cytoplasmic strands and extracellular spaces. Numerous mitochondria are present inside the cytoplasmic strands (Figs 8A, B, C). In the anterior chamber, the cytoplasmic strands are separated by extracellular spaces that are of approximately the same width as the cytoplasmic strands (Fig. 8A). Certain sections indicate that cytoplasmic strands are tubular structures rather than leaflets (Fig. 8B). In the papillate region the cytoplasmic strands are narrow and separated by wide extracellular spaces (Fig. 8C).



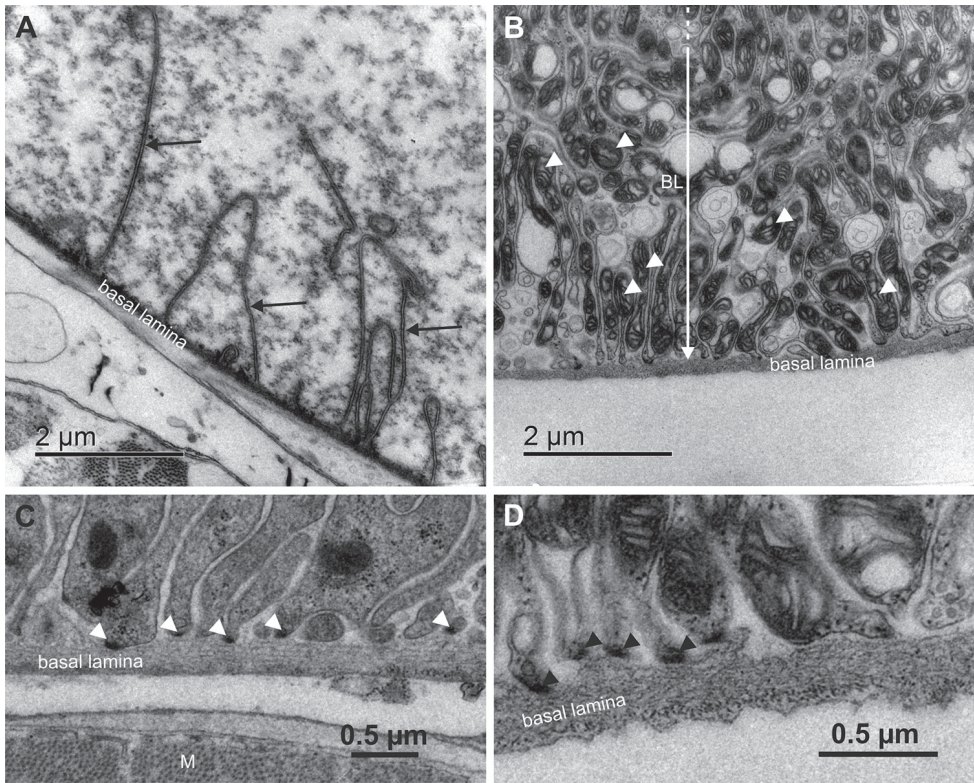
**Figure 7.** Bacteria associated with hindgut cuticle in the papillate region of one specimen. **A** Bacteria are visible near or at the cuticle (C) surface. Bacterial cells are connected to each other with thin filamentous structures (arrow) **B** Bacteria are connected to the fuzzy layer of epicuticle (arrow) by thin filamentous structures (arrowheads).



**Figure 8.** Ultrastructure of the apical plasma membrane labyrinth in the anterior chamber and the papillate region. **A** Apical membrane labyrinth (AL) in the anterior chamber. In the cytoplasmic strands mitochondria (arrowheads) are present **B** A tubular appearance of cytoplasmic strands is evident in certain sections (arrow) **C** Apical membrane labyrinth (AL) in the papillate region. Key: arrowheads – mitochondria, CUT – cuticle.

The differences in the basal membrane labyrinths of epithelial cells in the two hindgut regions are even more prominent. The basal plasma membrane in the anterior chamber forms relatively sparse narrow infoldings which are accompanied by scant mitochondria (Fig. 9A). The abundance of mitochondria in the vicinity of basal infoldings

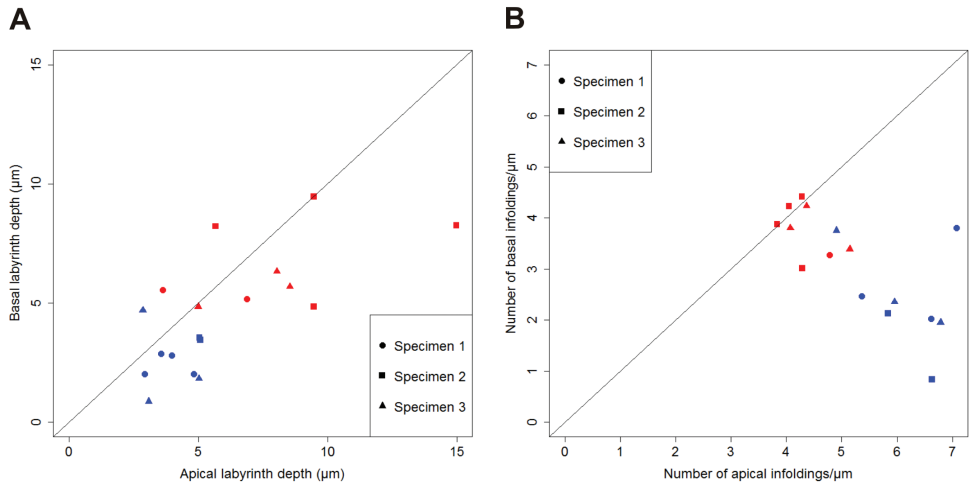




**Figure 9.** Ultrastructure of the basal plasma membrane labyrinth in the anterior chamber and the papillate region. **A** Basal plasma membrane in the anterior chamber forms sparse narrow infoldings (arrows) **B** Extensive basal membrane labyrinth (BL) in the papillate region is associated with numerous mitochondria (arrowheads) **C** Basal lamina in the anterior chamber. Hemidesmosome-like junctions are visible as small electron dense plaques (arrowheads) **D** Basal lamina in the papillate region. Hemidesmosome-like junctions are visible as small electron dense plaques (arrowheads). Abbreviation: M – muscle.

is no greater than elsewhere in the cytoplasm. In the papillate region the infoldings of basal plasma membrane are closely spaced and form a deep basal labyrinth. Mitochondria in the papillate region are concentrated in the area of basal labyrinth and associate with the basal membrane infoldings (Fig. 9B). In both hindgut regions the basal plasma membrane is supported by an outstandingly thick basal lamina, measuring typically between 100 and 300 nm. In the anterior chamber in particular, the basal lamina can reach up to 400 nm in thickness (Fig. 5B, Suppl. material 3). Hemidesmosome-like junctions, visible as small electron dense plaques (Fig. 9C, D), were observed at sites where the basal plasma membrane is in contact with the basal lamina.

Measurements of the membrane labyrinths depth suggest that the apical and the basal labyrinths are both deeper in the papillate region in comparison to the anterior chamber and that in both hindgut regions the apical membrane labyrinth is slightly deeper than the basal membrane labyrinth (Fig. 10A). The apical labyrinth is mainly

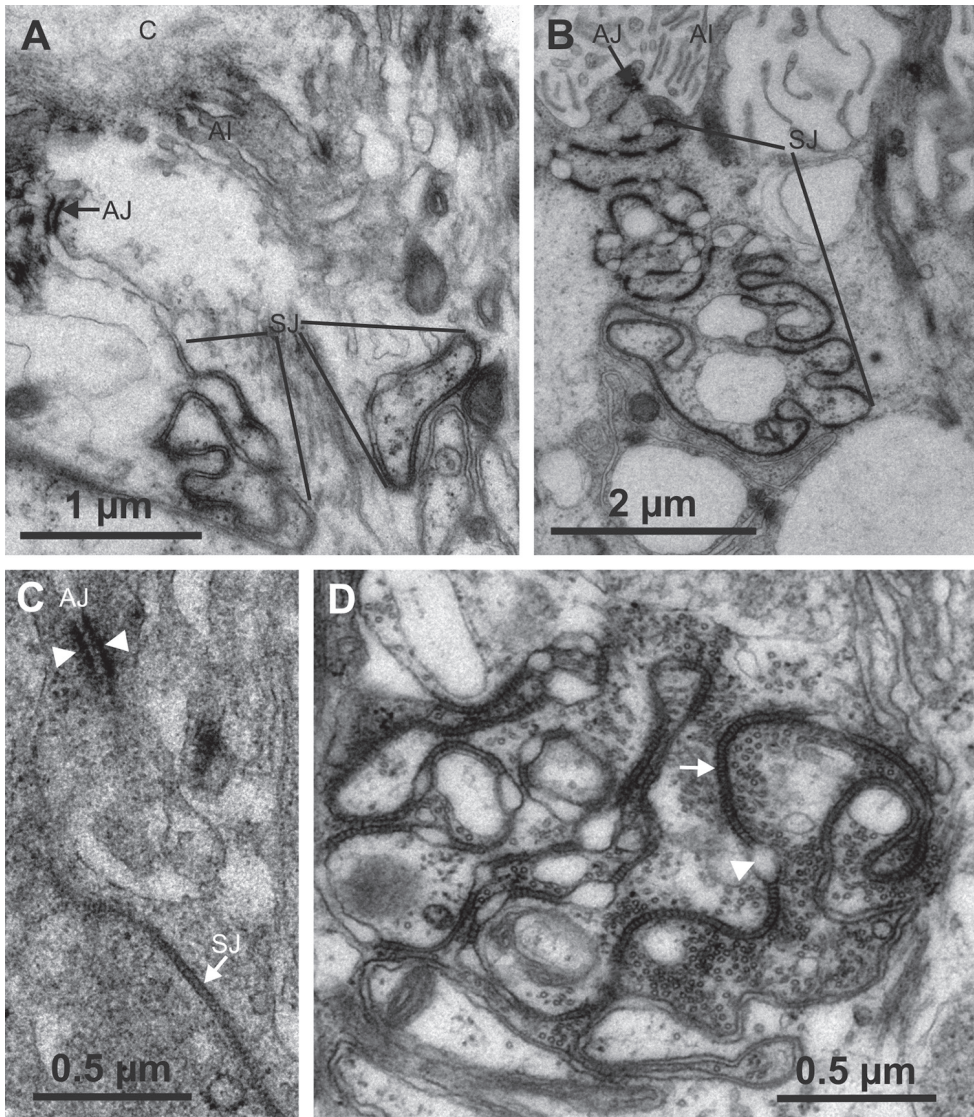


**Figure 10.** Apical and basal labyrinth depth and density of membrane infoldings in the two hindgut regions. Scatter plots depict: **A** Median values of apical labyrinth depth versus the median values of basal labyrinth depth in cells from the anterior chamber (blue) and the papillate region (red) of three specimens **B** Median values of apical infoldings density against the median values of basal infoldings density in cells from the anterior chamber (blue) and the papillate region (red) of three specimens. The central line in both plots is the line of equality. Points lying on the line indicate that median value of depth/density is equal, apically and basally. Points below the line indicate that the median value of depth/density is larger apically than basally. Points above the line indicate that the median value is larger basally than apically.

between 2 and 5  $\mu\text{m}$  deep in the anterior chamber and between 3 and 15  $\mu\text{m}$  deep in the papillate region (Suppl. material 4). The basal labyrinth is mainly between 1 and 4  $\mu\text{m}$  deep in the anterior chamber and between 3 and 10  $\mu\text{m}$  deep in the papillate region (Suppl. material 5). Measurements of the spatial density of membrane infoldings suggest that in the anterior chamber the apical infoldings are more closely spaced than the basal infoldings, while in the papillate region the spatial densities of apical and basal infoldings are more equal (Fig. 10B). The apical membrane infoldings are more closely spaced in the anterior chamber (5–8 infoldings/ $\mu\text{m}$ ) than in the papillate region (3–6 infoldings/ $\mu\text{m}$ ) (Suppl. material 6). The basal membrane infoldings are more closely spaced in the papillate region (3–5 infoldings/ $\mu\text{m}$ ) than in the anterior chamber (1–4 infoldings/ $\mu\text{m}$ ) (Suppl. material 7).

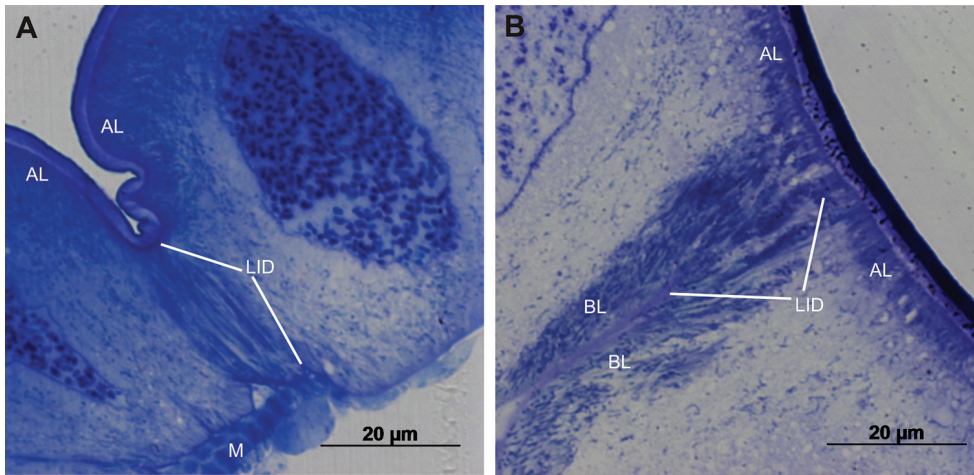
## Cell junctions

The junctional complexes between epithelial cells in both hindgut regions consist of subapically located adherens junctions and septate junctions which are located beneath the adherens junctions (Fig. 11A, B). Ultrastructurally, the adherens junctions appear to be alike in both hindgut regions. They consist of two electron dense plaques at the



**Figure 11.** Ultrastructure of cell junctions in the anterior chamber and the papillate region. **A** The junctional complex in the anterior chamber consists of subapically located adherens junctions (AJ) and septate junctions (SJ) located beneath the adherens junctions **B** The junctional complex in the papillate region consists of subapically located adherens junctions (AJ) and extremely long and convoluted septate junctions (SJ) located beneath the adherens junctions **C** Adherens junctions consist of two electron dense plaques (arrowheads) at the cytoplasmic sides of lateral plasma membranes of two neighbouring cells and electron dense material in the intercellular space between the membranes **D** Septate junctions are visible as electron dense septa arranged in strings (arrow). Dilated intercellular spaces are visible where septate junctions are locally interrupted (arrowhead). Abbreviations: C – cuticle, AI – apical infoldings, SJ – septate junction.





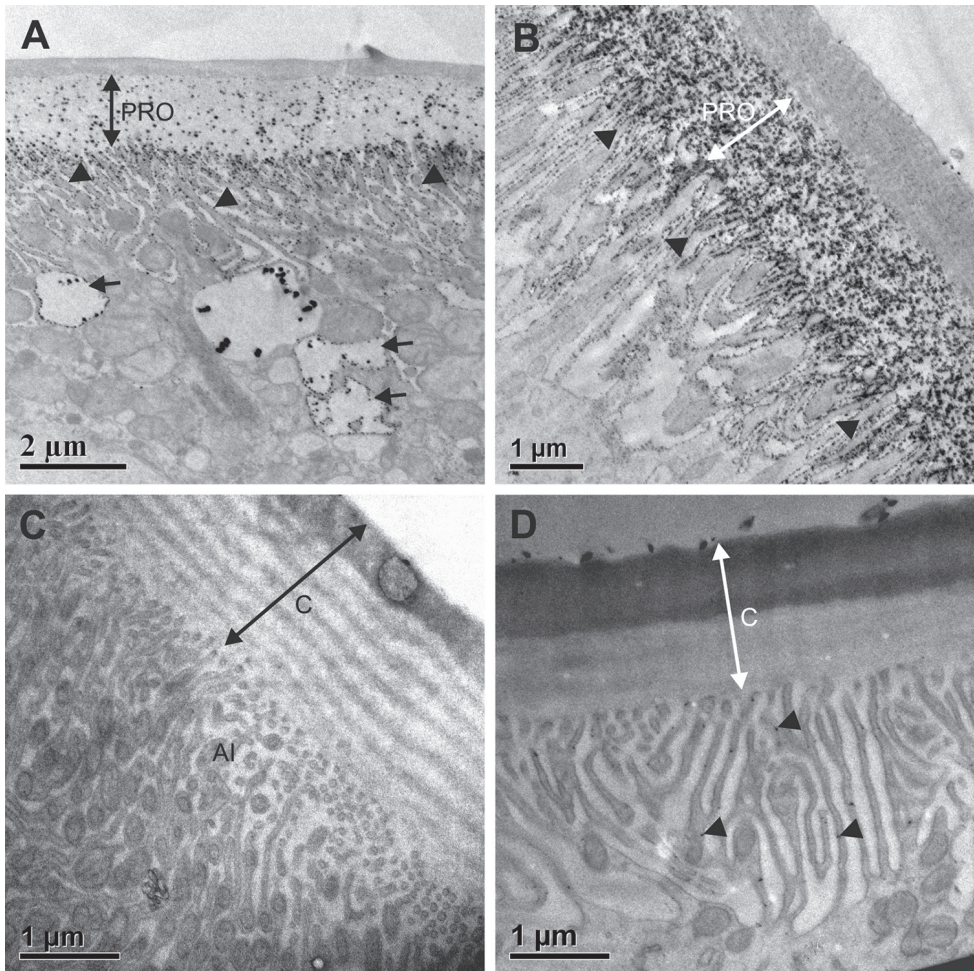
**Figure 12.** Lateral parts of epithelial cells in the anterior chamber and the papillate region. **A** Two neighbouring cells in the anterior chamber **B** Two neighbouring cells in the papillate region. Abbreviations: LID – area of lateral interdigitations, AL – apical labyrinth, BL – basal labyrinth, M – muscle.

cytoplasmic sides of lateral plasma membranes of the two neighbouring cells and electron dense material in the intercellular space between the membranes (Fig. 11C). The ladder-like septate junctions are visible as strings of electron dense septa in the intercellular space between lateral plasma membranes of the two neighbouring cells. Strings of electron dense septa are interrupted at some sites by dilated intercellular spaces. Abundant microtubules are present in the vicinity of septate junctions (Fig. 11D).

Septate junctions in both hindgut regions occupy considerable portions of the lateral membranes. In the papillate region the membrane area with septate junctions is intensely convoluted and the overall length of septate junctions is larger than that of the anterior chamber (Fig. 11). Large parts of lateral plasma membranes, which lie basally of the septate junctions, are heavily interdigitated between neighbouring cells (Fig. 12).

### **Na<sup>+</sup>/K<sup>+</sup>-ATPase activity localisation**

Na<sup>+</sup>/K<sup>+</sup>-ATPase activity was localised indirectly through lead phosphate deposits, which were present in the procuticle and along apical membranous invaginations of the anterior hindgut in postmoult and intermoult animals. The deposits were observed in tubular and dilated vacuolar infoldings (Fig. 13A) and were not localised in apical mitochondria. In the hindgut papillate region Na<sup>+</sup>/K<sup>+</sup>-ATPase activity was very intense in postmoult animals (Fig. 13B) with abundant reaction product in newly secreted procuticle and along deep tubular apical infoldings. Some reaction product was present also along basal membrane infoldings. In control sections where K<sup>+</sup> ions were replaced by Na<sup>+</sup> ions, deposits were not detected (Fig. 13C), but some deposits were present in controls treated with ouabain (Fig. 13D).



**Figure 13.** Localization of  $\text{Na}^+/\text{K}^+$ -ATPase activity in hindguts of intermolt and postmolt animals. **A**  $\text{Na}^+/\text{K}^+$ -ATPase activity in the anterior chamber of intermolt *P. scaber*. Electron dense deposits are present along tubular (arrowheads) and dilated (arrows) apical membranous infoldings and in the procuticle (PRO) **B**  $\text{Na}^+/\text{K}^+$ -ATPase activity in the papillate region of postmolt *P. scaber*. Abundant deposits are present along the apical infoldings (arrowheads) and in the procuticle (PRO) **C** Control section in the anterior chamber of intermolt animal where  $\text{K}^+$  ions were replaced by  $\text{Na}^+$  ions. No reaction product is present along apical infoldings (AI) or in the cuticle (C) **D** Control section in the papillate region of postmolt animal treated with ouabain. Some deposits (arrowheads) are present along the apical infoldings. Abbreviations: C – cuticle.

## Discussion

The present study complements and upgrades the previous knowledge of the hindgut epithelial cell ultrastructure in isopods by providing a detailed description and quantification of ultrastructural characters of these cells and their comparison between the main two hindgut regions. We also report on connections between the epithelial cells

and the hindgut cuticle, which were not described in previous works. Our results show clear evidence of prominent ultrastructural differences between the epithelia in the anterior chamber and in the papillate region. Cuticle structure and the thickness of epicuticle and procuticle differ significantly between the two hindgut regions, which suggests different mechanical and functional aspects of the cuticle, such as permeability, stiffness, and compactness. Another feature that differentiates epithelial cells in the two hindgut regions is the intensity of apical and basal membrane infolding. This is likely related to transport of nutrients, water and ions through epithelia between the hindgut lumen and haemocoel. Epithelial cells differ also in the extent of septate junctions and the interdigitations of lateral plasma membranes, which are important for the epithelial paracellular permeability in the two hindgut regions.

Epithelial cells in the hindgut of *P. scaber* are in general columnar to isodiametric and large, measuring between 40 and 100  $\mu\text{m}$  in diameter. We consider that the cell width and height varies also due to contractions of an extensive neuromuscular network and due to distension of epithelium in guts filled with food. Measured epithelial cells in the papillate region were flatter since the papillate region in majority of specimens was completely full and epithelium was more distended.

Both the hindgut epithelium and the epidermis in crustaceans are of ectodermal origin and are apically lined by a chitinous cuticle. A structural comparison between these two different cuticles in the same species is helpful in the evaluation of the observed cuticle characteristics in relation to their specific functions. While the epidermis with the mineralised exoskeleton provides protection against predators, infections and desiccation, communicates with external environment and provides mechanical support of the body crucial for locomotion, the hindgut epithelium is involved in transport of nutrients, water and ions (Hryniewiecka-Szyfter and Storch 1986, Hames and Hopkin 1989). The ultrastructure and composition of exoskeletal cuticle in isopods, including *P. scaber*, have been described precisely (Compere 1991, Štrus and Compere 1996, Ziegler 1997a, Štrus and Blejec 2001, Hild et al. 2008, 2009, Seidl et al. 2011, Seidl and Ziegler 2012, Vittori and Štrus 2014) and here the ultrastructure of the *P. scaber* hindgut cuticle is discussed in respect to the exoskeleton of this animal. The hindgut cuticle of *P. scaber* is about ten times thinner than the exoskeletal cuticle, which is likely a modification for passage of molecules and ions. But on the other hand, the hindgut epicuticle is between one and four-times thicker than that of the exoskeleton (~150 to 250 nm). Accordingly, the ratio of epicuticle thickness to procuticle thickness is considerably higher in hindgut cuticle than in exoskeletal cuticle of *P. scaber*. The hindgut cuticle is connected to individual epithelial cells by cuticle–epithelium attachments, which ultrastructurally resemble connections between the exoskeletal cuticle and specialised epidermal cells (tendon cells) in arthropods (Buchholz and Buchholz 1989, Mellon 1992, Lai-Fook and Beaton 1998, Bitsch and Bitsch 2002, Žnidaršič et al. 2012).

The hindgut epicuticle of *P. scaber* structurally resembles the homogenous inner and trilayered outer epicuticle of the animals' exoskeleton. The detailed description of the outer epicuticle in isopod exoskeletons revealed that it usually consists of several



thin layers (Ziegler 1997a, Compere 1991, Vittori and Štrus 2014). The additional electron dense layer, deposited inside the outer epicuticle of exoskeleton, has been referred to as the 'waxy layer' and is presumably composed of lipids and acts as a surface waterproofing barrier (Compere 1991). This layer was not distinguished in *P. scaber* hindgut epicuticle and thus may not be present in this case. This could imply better permeability of the hindgut cuticle. The fuzzy layer covering the epicuticle surface has already been reported in *P. scaber* hindgut by Holdich and Mayes (1975) as a diffuse outer layer of epicuticle. It resembles the exoskeletal 'surface coat', which has been reported to be more pronounced in aquatic crustaceans (Compere 1995) and thinner in isopods (Compere 1991, Vittori and Štrus 2014). The function and composition of the surface coat in the crustacean exoskeleton is not fully understood. More data are available for the similar fuzzy surface coat overlying the trilayered epicuticle in nematode integument, but it must be stressed here that the nematode cuticle is composed largely of collagen and is in this concern not directly comparable to the chitinous cuticle of arthropods. The main constituents of the nematode surface coat appear to be glycoproteins and proteins and its biological role is mainly assigned to the interactions between nematodes and microorganisms, as well as between parasitic nematodes and their hosts (Jansson et al. 1986, Wright 1987, Blaxter et al. 1992, Spiegel and McClure 1995). In the papillate region of one specimen we observed bacteria attached to the fuzzy layer of epicuticle with thin filamentous structures. In terrestrial isopods it is known that some bacteria are able to attach to the hindgut cuticle. This has been reported for the rod-like bacteria *Candidatus* Bacilloplasma, which is known to attach to the tips of cuticular spines in the papillate region of *P. scaber* with spherical attachment structures (Kostanjšek et al. 2003, Kostanjšek et al. 2006, Kostanjšek et al. 2007). The inner epicuticle is thicker in the papillate region than in the anterior chamber. This may influence different epithelial permeability and mechanical properties. Zimmer (1999) reported that the cuticle in the anterior chamber of *P. scaber* is permeable to the molecules up to the size of the *tri*-galloyl-glucose, while in the papillate region the cuticle is impermeable for the molecules of the size of glucose. The cuticle in the anterior chamber thus allows the absorption of digestive products up to the size of 1.9 nm (Zimmer 2002). Thin vertical canals in the inner epicuticle described in insect and crustacean exoskeletons (Locke 1961, Compere and Goffinet 1987a, b, Compere 1991, 1995, Ziegler 1997a), were not observed in our study of the hindgut cuticle. Epicuticular canals in exoskeleton serve as access routes for lipids in insects (Locke 1961) and it has been suggested that they may be related to the waxy layer formation in the isopod exoskeleton (Compere 1991).

The procuticle in the hindgut displays distinctive sublayers, which are more obvious in the anterior chamber. The hindgut cuticle has thinner procuticle relative to the entire cuticle thickness, and structurally displays less pronounced organisation of the chitin-protein lamellae than the exoskeletal cuticle. The lamellar organisation contributes to exoskeletal cuticle stiffness and mechanical resistance as a consequence of the complex hierarchical structure at all levels of organisation, from molecules to helicoidally stacked chitin-protein planes (Raabe et al. 2005, 2006, Fabritius et al. 2009, Dil-

laman et al. 2013). The exoskeletal cuticle is in addition hardened by mineralisation (Compere et al. 2004, Luquet 2012, Dillaman et al. 2013). The difference between the hindgut and exoskeletal procuticles suggests different mechanical properties and indicates lower stiffness of the hindgut cuticle in comparison to that of the exoskeleton. As it is proposed for decapod exoskeleton, a higher stacking density of the chitin-protein planes leads to greater cuticle stiffness and hardness (Raabe et al. 2005). In this regard, a closer resemblance of the procuticle in the anterior chamber to that of the exoskeleton, with more numerous and thinner lamellae, implies higher stiffness of the cuticle in this region than in the papillate region. This is in favour of active digestion of coarse food particles and intensive fluxes in relation to backflow of digested material through typhlosolis channels in the anterior chamber. These processes are caused by mechanical compression of the gut content performed by the surrounding muscular network, which is more pronounced in this region than in the papillate region. Pore canals traversing the cuticular matrix, generally characteristic of the exoskeletal cuticle, were not observed in the hindgut cuticle. Absence of pore canals is also characteristic of some flexible and elastic cuticles, such as cuticles of locust wing hinges and the pleural arch of fleas (Neville 1975), both of which contain the elastic protein resilin (Weis-Fogh 1960, Burrows et al. 2008). Structurally similar procuticle as in the *P. scaber* papillate region, i.e. without discernible division between exocuticle and endocuticle and no clearly distinct sublayers, is present in cuticles of some other transporting epithelia of arthropods. In the marine isopod *Jaera nordmanni*, the cuticle of ion transporting gill epithelium consists of epicuticle and amorphous flocculent material underneath (Bubel and Jones 1974). The very thin cuticle of gills in dragonfly nymphs consists of epicuticle and procuticle in up to two sublayers (Neville 1975). The tracheal cuticle of insects also consists of epicuticle and conspicuous procuticle with amorphous organisation of chitin (Moussian 2013). Cuticles of ion transporting epithelia often display epicuticular depressions as in the rectal chloride epithelium of larval dragonflies (Kukulies and Komnick 1983) and the rectal papillae of *Musca domestica* (Flower and Walker 1979) or porous plates as in mayfly larval gills (Komnick and Stockem 1973, Neville 1975). Pores of different diameters have been reported at the cuticle surface in the hindgut of locust *Locusta migratoria*. Smaller pores in the cuticle covering the rectal pads are probably involved in water resorption (Klein and Applebaum 1975). We did not observe any such specialisations in the hindgut cuticle of *P. scaber*. Overall, our results suggest that the hindgut cuticle of *P. scaber* shares some structural characteristics with some other cuticles in transporting epithelia and with some cuticles displaying a pronounced elasticity.

Infolded plasma membranes associated with mitochondria, as reported here for the hindgut epithelium, are a general characteristic of transporting epithelial cells. The infolded apical, lateral or basal plasma membranes enlarge the surface across which the transport can take place and provide enclosed extracellular spaces, where ion gradients can build up. This in turn can drive secondary transport and the flow of water. Associated mitochondria are important in providing energy for the active transport of ions (Cioffi 1984, Fukudome 2001, Weihrauch and O'Donnell 2015). The differences in

the extent of apical and basal surface amplification and in the association of mitochondria with plasma membrane infoldings indicate differences in function. In the anterior chamber the apical surface of epithelial cells is amplified and associated with mitochondria in particular. This reflects intensive material exchange between epithelial cells and the hindgut lumen, which supports the proposed role of anterior chamber in the absorption of nutrients (Hryniewiecka-Szyfter and Storch 1986, Hames and Hopkin 1989). In the papillate region both the apical and the basal surfaces of epithelial cells are greatly amplified and associated with mitochondria. This indicates intensive material exchange with both the hindgut lumen and the haemocoel, which is consistent with transepithelial transport of ions and water in the papillate region (Vernon et al. 1974, Coruzzi et al. 1982, Palackal et al. 1984). Transepithelial transport of ions and water in the papillate region can drive osmoregulatory processes, such as ion sequestration in the hindgut during dehydration (Wright et al. 1997) or salt excretion with faeces in supralittoral and littoral genera *Ligia* and *Tylos* (Wright et al. 2003).

Epithelial cells in the anterior chamber of *P. scaber* have their apical surfaces amplified by apical membrane infoldings. More commonly, the absorptive cells in digestive systems of arthropods are of endodermal origin and have apical surfaces amplified by brush border of microvilli (Cioffi 1984, Icely and Nott 1992, Vogt 1994, Shanbhag and Tripathi 2009). In terrestrial isopods, which lack endodermal midgut, such cells are present in hepatopancreas and probably fulfil the main part of nutrient absorption (Hames and Hopkin 1989). Epithelial cells in the papillate region of *P. scaber* are structurally similar to various epithelial cells involved in ion transport. Ion transporting cells in arthropods often have amplified apical membrane in the form of leaflets (Cioffi 1984). Examples of ion transporting epithelial cells with extensively infolded apical membranes are cells in the rectal papillae of *Calliphora* (Berridge and Gupta 1967), the salt transporting cells in crustacean gills (Bubel and Jones 1974, Taylor and Taylor 1992, Kikuchi and Matsumasa 1993, Luquet et al. 2000, Luquet et al. 2002) and distal duct cells in salivary glands of cockroach *Periplaneta americana* which modify the primary saliva by absorption of  $\text{Na}^+$  ions and secretion of  $\text{K}^+$  ions (Just and Walz 1994). Besides the apical infoldings, extensive basal labyrinths associated with numerous mitochondria are also common in the salt transporting epithelial cells in crustacean gills and the distal duct cells in cockroach salivary glands. In the epidermis of *P. scaber* the anterior sternal epithelium is involved in  $\text{Ca}^{2+}$  transepithelial transport during premoult and intramoult, when the formation and resorption of sternal  $\text{CaCO}_3$  deposits take place. Anterior sternal epithelial cells in premoult and intramoult animals have amplified basolateral plasma membranes in the form of interstitial dilations and ramifying channels. In intramoult animals, the apical plasma membranes are also amplified by subcuticular folds. Such elaborations of basolateral and apical plasma membranes are absent in the posterior sternal epithelium (Ziegler 1996).

Two important ion pumps in ion transporting epithelia are the  $\text{Na}^+/\text{K}^+$ -ATPase and the vacuolar  $\text{H}^+$ -ATPase (Weihrauch and O'Donnell 2015). In the hindgut of *P. scaber*  $\text{Na}^+/\text{K}^+$ -ATPase activity is localised primarily in the apical membranes of epithelial cells and scantily in the basal membranes. The intense ATPase activity in the apical



membranous labyrinth of papillate region is probably due to extensive transepithelial transport of the water and ions necessary for osmoregulation. This apical localisation of  $\text{Na}^+/\text{K}^+$ -ATPase is rather unusual, since in general it is more common that the  $\text{Na}^+/\text{K}^+$ -ATPase is localised in the basolateral membranes of epithelial cells. In decapod Crustacea the  $\text{Na}^+/\text{K}^+$ -ATPase activity was cytochemically localised in the basolateral membranes of ion transporting cells in gill epithelia of crabs *Callinectes sapidus* and *Carcinus maenas* (Towle and Kays 1986).  $\text{Na}^+/\text{K}^+$ -ATPase was also immunolocalised in the basal membranes of branchiostegite epithelial cells in lobster *Homarus gammarus* (Lignot et al. 1999) and shrimp *Macrobrachium amazonicum* (Boudour-Boucheker et al. 2014). Just and Walz (1994) immunolocalised  $\text{Na}^+/\text{K}^+$ -ATPase in the salivary gland of cockroach *P. Americana*. In this case ATPase is localised in the apical membranes in peripheral cells for secretion of NaCl rich primary saliva and in the basolateral membranes of distal duct cells which modify the saliva by absorption of  $\text{Na}^+$  and secretion of  $\text{K}^+$ . In terrestrial isopods  $\text{Na}^+/\text{K}^+$ -ATPase activity was cytochemically localised in the basolateral or apical membrane infoldings in the posterior hindgut of *A. officinalis* (Warburg and Rosenberg 1989).  $\text{Na}^+/\text{K}^+$ -ATPase was also immunolocalised in the basolateral membranes of the anterior and posterior sternal epithelial cells of premoult and intramoult *P. scaber* (Ziegler 1997b).

The outstanding ultrastructural hallmarks of the hindgut epithelium are extensive septate junctions (SJ) located basal to adherens junctions. In different arthropod epithelia, two types of septate junctions have been described: pleated SJ and smooth SJ, which both typically form circumferential belts around the apicolateral regions of epithelial cells (Izumi and Furuse 2014, Jonusaite et al. 2016). Pleated SJ are generally observed in ectodermal epithelia, including epidermis, foregut and hindgut and their septa form regular undulating rows. Smooth SJ are present in endodermal epithelia and their septa are arranged in parallel lines. Data on morphology of arthropod SJ are extensive, but their functional aspects, molecular architecture and regulation are still not well characterised and these studies are limited mostly to *Drosophila*. Septate junctions are considered occluding junctions, restricting the passage of material through the paracellular pathway, but detailed properties of their barrier function are not clearly understood. The extensive SJ documented in our study imply that the paracellular transport is tightly regulated in the whole hindgut and in particular in the papillate region, where the SJ area is enlarged due to convoluted plasma membrane and where the cumulative number of septa spanning the intercellular space is higher. In the vicinity of septate junctions numerous microtubules were observed. As clusters of cross-sectioned microtubules were evident, we consider that these microtubules run parallel to lateral plasma membranes. A similar organisation of microtubules associated with septate junctions was observed in gill epithelium of some gammarid amphipods (Shires et al. 1995), which was interpreted as a protection against expansion and contraction of cells in the lateral plane necessary for the integrity of the permeability barrier.

The basal lamina beneath the basal plasma membrane of epithelial cells in both hindgut regions is outstandingly thick (100 – 300 nm). The typical thickness of basal laminae in vertebrates is between 50 and 100  $\mu\text{m}$  (Ghadially 1997). Analyses of ver-

tebrate basal laminae revealed their heterogeneity in molecular composition and organisation and elucidated diversity in their biological roles (LeBleu et al. 2007). Basal laminae fulfil many biological functions, among others they provide structural support for cells and serve as selective barriers. In recent years, valuable data on biomechanical properties of basal laminae became available, which importantly improved the understanding of their complex properties and dynamics (Halfter et al. 2015, Miller 2017). Data on basal laminae structure and properties in invertebrates are scarce. Reddy and Locke (1990) investigated the penetration of gold nanoparticles through different insect basal laminae. Basal laminae of tissues that synthesise and secrete haemolymph proteins, such as fat body, epidermis and pericardial cells, have thin basal laminae, which are permeable for gold nanoparticles, up to 15 nm in diameter. The basal laminae of tissues such as silk glands, Malpighian tubules, heart and muscles, which are not involved in the synthesis of haemolymph proteins, have thick basal laminae, which are impermeable for gold nanoparticles 6 nm in diameter. The thick basal lamina in *P. scaber* hindgut thus might be a modification related to the mechanical integrity of the hindgut that has to be adapted to a changing volume and has to withstand forces due to muscles contractions. As basal lamina is in general an integrative part of tissue permeability/barrier properties, additional data on the molecular composition would be necessary to evaluate further this issue.

## Conclusions

Epithelial cells in both hindgut regions have ultrastructural features typical of transporting epithelia. The observed ultrastructural differences indicate different transport roles in the two hindgut regions and are consistent with the proposed nutrient absorptive function of anterior chamber and transepithelial ion and water fluxes of the papillate region.

Thicker procuticle with more numerous thinner lamellae implies higher stiffness of cuticle in the anterior chamber in comparison to the papillate region. This could be linked to the intensive food processing in the anterior chamber by the contraction of the surrounding muscle layers.

The hindgut cuticle is connected to individual epithelial cells by anchoring junctions on the apical plasma membrane and fibres that protrude into the cuticular matrix, which is a similar architecture as reported for exoskeletal cuticle connections to tendon cells.

Epithelial cells in the anterior chamber have an extended apical surface in particular. This indicates extensive exchange of material between cells and the hindgut lumen, which supports the proposed nutrient absorptive function of the anterior chamber. In the papillate region, both the apical and the basal surfaces are greatly increased. This indicates extensive exchange of material with both the hindgut lumen and haemocoel, which is consistent with transepithelial transport of ions and water in the papillate region involved in osmoregulation.

The intense  $\text{Na}^+/\text{K}^+$ -ATPase activity at infoldings of apical plasma membrane may represent the driving force for transepithelial transport of ions and water in the papillate region.

Extensive septate junctions in *P. scaber* hindgut indicate that the paracellular transport is tightly regulated in the entire hindgut and especially in the papillate region.

The basal lamina is relatively thick in comparison to basal laminae in other tissues and probably provides mechanical support for the hindgut epithelium.

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## Supplementary material 1

### SI Figure 1. Boxplots depicting individual measurements of cell size and cell nuclei diameter

Authors: Urban Bogataj, Monika Praznik, Polona Mrak, Jasna Štrus, Magda Tušek-Žnidarič, Nada Žnidaršič

Data type: boxplot

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Link: <https://doi.org/10.3897/zookeys.801.22395.suppl1>

## Supplementary material 2

### SI Figure 2. Boxplots depicting individual measurements of cuticle thickness

Authors: Urban Bogataj, Monika Praznik, Polona Mrak, Jasna Štrus, Magda Tušek-Žnidarič, Nada Žnidaršič

Data type: boxplot

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### **Supplementary material 3**

#### **SI Figure 3. Boxplots depicting individual measurements of basal lamina thickness**

Authors: Urban Bogataj, Monika Praznik, Polona Mrak, Jasna Štrus, Magda Tušek-Žnidarič, Nada Žnidaršič

Data type: boxplot

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Link: <https://doi.org/10.3897/zookeys.801.22395.suppl3>

### **Supplementary material 4**

#### **SI Figure 4. Boxplots depicting individual measurements of apical membrane labyrinth depth**

Authors: Urban Bogataj, Monika Praznik, Polona Mrak, Jasna Štrus, Magda Tušek-Žnidarič, Nada Žnidaršič

Data type: boxplot

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### **Supplementary material 5**

#### **SI Figure 5. Boxplots depicting individual measurements of basal membrane labyrinth depth**

Authors: Urban Bogataj, Monika Praznik, Polona Mrak, Jasna Štrus, Magda Tušek-Žnidarič, Nada Žnidaršič

Data type: boxplot

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## Supplementary material 6

### **SI Figure 6. Stripcharts depicting individual measurements of the spatial density of apical membrane infoldings**

Authors: Urban Bogataj, Monika Praznik, Polona Mrak, Jasna Štrus, Magda Tušek-Žnidarič, Nada Žnidaršič

Data type: stripchart

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Link: <https://doi.org/10.3897/zookeys.801.22395.suppl6>

## Supplementary material 7

### **SI Figure 7. Stripcharts depicting individual measurements of the spatial density of basal membrane infoldings**

Authors: Urban Bogataj, Monika Praznik, Polona Mrak, Jasna Štrus, Magda Tušek-Žnidarič, Nada Žnidaršič

Data type: stripchart

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Link: <https://doi.org/10.3897/zookeys.801.22395.suppl7>

# Water vapor absorption allows for volume expansion during molting in *Armadillidium vulgare* and *Porcellio dilatatus* (Crustacea, Isopoda, Oniscidea)

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## Abstract

Arthropods require periodic molting in order to grow which presents a number of challenges to terrestrial taxa. Following ecdysis, the pliant new cuticle is susceptible to buckling under gravity and requires elevated hydrostatic pressure for support. Terrestrial species also require a mechanism of volume expansion and stretching of the integument prior to sclerotization, a need that is readily met in aquatic arthropods by drinking. Options for land arthropods include drinking of dew, swallowing of air, or using muscular contractions to inflate air sacs in tracheate taxa. In this study we tested the hypothesis that crinochete terrestrial isopods (Isopoda: Oniscidea: Crinocheta) exploit their capacity for active water vapor absorption (WVA) to increase volume during molting. Two crinochete species, *Armadillidium vulgare* and *Porcellio dilatatus*, were studied and compared with the non-absorbing species *Ligidium lapetum* (Oniscidea: Ligiamorpha). Pre-molting animals were identified by sternal CaCO<sub>3</sub> deposits and exposed to 100% or 97% relative humidity (RH). Mass-changes were monitored by daily weighing and the timing of the posterior and anterior ecdyses was used to categorize time (days premolt and days post-molt) over the molt cycle. In each treatment RH, *A. vulgare* and *P. dilatatus* showed a progressive mass increase from 5 days premolt until the posterior or anterior ecdysis, followed abruptly by period of mass-loss lasting 3–4 days post-molt. The fact that the initial mass-gain is seen in 97 % RH, a humidity below the water activity of the hemolymph, confirms the role of WVA. Similarly, since the post-molt mass-loss is seen in 100 % RH, this must be due to active expulsion of water, possibly via maxillary urine. Concurrent changes in hemolymph



osmolality were monitored in a separate batch of *A. vulgare* and show sustained osmolality during premolt and an abrupt decrease between the anterior and posterior ecdysis. These patterns indicate a mobilization of sequestered electrolytes during premolt, and a loss of electrolytes during the post-molt mass-loss, amounting to approximately 8.6 % of total hemolymph solutes. WVA, in conjunction with pulses of elevated hemolymph pressure, provides an efficient mechanism of pre-molt volume expansion prior to and during the biphasic molt in these species. Premolt *Ligidium lapetum* exposed to same treatments failed to molt successfully and no premolt animals survived to day 3 (72 h) even in 100 % RH. The apparent dependence of this species on liquid water for successful molting could explain its obligatory association with riparian fringe habitats.

## Keywords

Isopoda, Oniscidea, water vapor absorption, molting, ecdysis

## Introduction

The cuticle of arthropods is an organ of extraordinary adaptive versatility, allowing for articulation and movement via complex joints, sensory transduction using a remarkable variety of permeable or deformable sensilla, variable morphology and coloration from impregnated pigments or refractory laminae (physical coloration), and extreme resistance to water loss from intrinsic or superficial lipids in many terrestrial taxa (Locke 1965, 1974, Chapman 2012). The adaptive plasticity of the cuticle has doubtless contributed to the spectacular adaptive radiations of the insects, arachnids and myriapods in terrestrial habitats. However, the requirement that the cuticle be periodically molted and renewed to allow for growth presents a number of challenges to land colonization.

Molting of the arthropod exoskeleton is preceded by apolysis – the separation of the old cuticle from the underlying epidermis – and the secretion of inactive molting fluid via dermal glands prior to the secretion of the cuticulin layer in the presumptive new cuticle (see Locke 1974, Chapman 2012). Subsequent activation of the molting fluid results in degradation and resorption of the old endocuticle (Samuels and Reynolds 1993). At this stage, the new cuticle is soft and flexible to allow for expansion and growth. An increase in hemolymph hydrostatic pressure precedes ecdysis during which the old cuticle splits along well-defined ecdysial lines and is sloughed. Volume expansion of the newly molted animal typically continues for a few hours to a few days until distension of the new cuticle is inhibited by progressive quinone tanning of the endocuticle proteins and/or mineralization (Fraenkel and Rudall 1947, Vincent and Hillerton 1979). During the intervening period, the soft, pliant cuticle renders the animal vulnerable, limits mobility, and requires that terrestrial species generate elevated hydrostatic pressure for structural support (DeFur et al. 1985, Chapman 2012). Pliant intersegmental cuticle may continue to expand between molts, allowing for a sustained size increase, but mineralized or sclerotized cuticle does not expand. This was well illustrated by Clarke (1957) who showed that intermolt tibia length remains constant in *Locusta*, then increases abruptly at ecdysis, despite a steady increase in whole-animal

mass. Volume increase and growth of the whole animal thus depends, in part, on the expansion of the cuticle during molting.

Details of the hemolymph volume and localized pressure increases accompanying ecdysis have been studied in several insects and aquatic crustaceans. Molting flies and locusts are thought to swallow air to bring about volume expansion (Ewer 1954, Clarke 1957, Cottrell 1962a,b, Cottrell 1964, Lee 1961). Following pupal eclosion, the blowflies *Calliphora* and *Sarcophaga* swallow air which distends the gut, producing a steady increase in hemolymph hydrostatic pressure. Simultaneous contractions of specialized ptilinal and abdominal muscles generate pressure pulses involved in wing expansion (Cottrell 1962a,b). These muscles degenerate a few days following ecdysis. Specialized muscles functioning to create localized pressure increases during molting have been identified in several insects, some of them associated with specific instars (Ewer 1954, Miyan 1989). The hemolymph volume at emergence of adult blowflies is comparable to that of the larvae, and hydrostatic pressure provides structural support to the pharate adult. Hemolymph volume falls dramatically (by about 80 %) over the succeeding 30 hours, as the cuticle is stiffened by tanning. This is accompanied by compensatory intake of air into the thoracic air sacs and some fluid release from the anus (Wigglesworth 1963).

Aquatic crustaceans drink water to increase hemolymph volume prior to and following ecdysis (Dall and Smith 1978, Hartnoll 1982, 1988, DeFur et al. 1985, 1988). In marine decapods, drinking of seawater begins about 1 hour prior to ecdysis and continues for 4–7 hours after, depending on species (Travis 1954, Robertson 1960, Mykles 1980). In juvenile *Homarus americanus*, ingested seawater is absorbed across the midgut and brings about an increase in hemolymph volume of 46 % prior to ecdysis, and 167 % 2.5 h following ecdysis (Mykles 1980). During the succeeding 2 weeks, hemolymph volume falls steadily while intracellular water and dry mass increase. The net mass-gain at completion of the molt is about 30 %. A similar process is seen in the euryhaline blue crab *Callinectes sapidus* (Neufeld and Cameron 1994) and in the amphipod *Gammarus duebeni* (Lockwood and Inman 1973), and the fraction of branchial water uptake (ca. 30 %) is similar in *Callinectes* acclimated to 2 ‰ and 28 ‰; decreased osmotic permeability restricts water uptake in the lower salinity. As with *Homarus*, imbibed water in *Callinectes* (Neufeld and Cameron 1994), and in *Carcinus maenas* (Robertson 1960), moves into the hemolymph across the midgut cecae.

Oral uptake of water similarly provides the main mechanism of volume expansion during molting in freshwater and athassohaline crustaceans but imposes an osmotic challenge. Hemolymph osmolality and specific ion concentrations decline post-molt relative to intermolt in the athassohaline Chinese crab *Eriocheir sinensis* (DeLeersnyder 1967), whiteleg shrimp *Litopenaeus vannamei* (Cheng et al. 2002), and the freshwater crayfish *Cherax destructor* (Zare and Greenaway 1998). Compensatory up-regulation of branchial ion transport during molting occurs in *Callinectes* acclimated to low salinities (Towle and Mangum 1985), and in the freshwater crayfish *Cherax destructor* (Zare and Greenaway 1998), and involves increased activity of both the basolateral Na<sup>+</sup>/K<sup>+</sup>-ATPase and increased apical membrane potentiation via up-regulation of the H<sup>+</sup> V-ATPases.

Mechanisms of volume expansion during molting in terrestrial arthropods other than insects remain under-investigated. Some groups may drink like aquatic crusta-

ceans, but a dependable liquid water source is often not available. Semi-arid grasslands, mountain rain shadows and continental deserts are just three examples of habitats that frequently remain above dew-point temperatures for weeks or months at a time (Cloudsley Thompson 1991, Edney 1977, Hadley 1994). Furthermore, feeding is generally precluded between apolysis and a variable period following ecdysis by the renewal of the cuticular structures overlying the ectodermally derived foregut and hindgut epithelia (Ellis 1951, Chapman 2012). Food intake is therefore probably not a viable means of volume expansion, except perhaps for some fluid-feeders. The present study was undertaken to examine the possibility that active water vapor absorption serves as a mechanism of volume expansion in the terrestrial isopods (Suborder Oniscidea).

Together with a few species of talitrid amphipods (Duncan 1994, Hou and Li 2003), the oniscidean isopods are the only crustaceans that can live truly independently of liquid water (Little 1983, 1991). Both of these groups brood the eggs in a fluid-filled marsupium (Hoesel 1984, Hoesel and Janssen 1989, Pandian 2016). Like other isopods, the Oniscidea have a biphasic molt in which the posterior half of the cuticle is molted first, followed after 1–2 days by the anterior half (George and Sheard 1954); the intervening period constitutes the intramolt. Biphasic molting confers advantages including improved mobility during the molt period and a limit on newly exposed permeable surface area (Price and Holdich 1980a,b). Formation of the marsupium in gravid females involves a specialized parturial molt (George and Sheard 1954, Moreau and Rigaud 2002). Prior to ecdysis, oniscideans reabsorb calcium carbonate from the old cuticle and sequester it as amorphous calcium carbonate spherules in the subcuticular space of the first four pereopodal sternites (Steel 1993, Ziegler 1996, Ziegler and Miller 1997). Sequestered calcium is re-mobilized following ecdysis, allowing for rapid mineralization of the new endocuticle. The calcium deposits are conspicuous and allow for identification of pre-molt animals as well as differentiation of parturial molt females (Moreau and Rigaud 2002).

Changes in hemolymph pressure, volume and ion composition during molting in oniscideans have been studied by a few workers. Price and Holdich (1980b) measured whole-animal mass and hemolymph osmolality during molting in *Oniscus asellus*, and did not identify significant changes between 7 days prior and 7 days following ecdysis. They concluded that localized increases in blood pressure brought about by contractions of somatic musculature, rather than changes in hemolymph volume, generate the requisite pressure for the posterior and anterior ecdyses. Heeley (1941) described rhythmic inter-segmental contractions in the anterior segments preceding the posterior ecdysis, and subsequent contractions in the posterior segments preceding the anterior molt. Later measurements of hemolymph blood pressure in *Porcellio spinicornis* and *Armadillidium vulgare* by Alikhan (1983, 1984) showed that regular pulses of elevated pressure appear a few minutes in advance of the posterior molt, persist intermittently during the intramolt period, and resume for 2–7 h after the anterior ecdysis. However, changes in blood pressure without accompanying increases in volume cannot explain net growth. Measurements of hemolymph electrolytes during the molt cycle in *Porcellio scaber* (Ziegler and Scholz 1997) showed that concentrations of  $K^+$ ,  $Na^+$ ,  $Mg^{2+}$  and  $Cl^-$  all decrease significantly following the posterior molt, consistent with the uptake of water. Furthermore, direct measurements of hemolymph volume

in the supra-littoral oniscidean *Ligia pallasii* show a sharp increase in volume following the posterior ecdysis, apparently due to direct uptake of seawater (Ziegler and Pennings 2000).

Active water vapor absorption (WVA) provides a potential mechanism for volume expansion in a few families of terrestrial arthropods (Machin 1983, O'Donnell and Machin 1988). WVA is defined as any energy-dependent process allowing an animal to absorb water vapor from a vapor pressure below the equilibrium vapor pressure of the animal's body fluids – or from a relative humidity (RH) below about 99 % for practical purposes. The capacity for WVA has evolved independently in several arthropod lineages, including lepidoptera, tenebrionid beetles, fleas, corydiid cockroaches, booklice and biting lice, oniscidean isopods, mites and penicillate millipedes (see Machin 1983, Knülle 1984, O'Donnell and Machin 1988, Gaede 1991, Wright and Westh 2006). Whether WVA plays a role during the molt cycle in any of these groups is unknown, although some species lose the capacity for WVA shortly before ecdysis (Edney 1966, Noble-Nesbitt 1978, Coutchié and Crowe 1979). The sites and mechanisms of vapor absorption vary widely but all involve the depression of water vapor pressure (and hence water activity and free energy) at a specialized absorption site (O'Donnell and Machin 1988). In the oniscidean isopods, uptake involves the secretion of strongly hyperosmotic fluid into the pleoventral (branchial) cavity which drives colligative condensation of water above an absorption threshold of 86–93 % RH depending on species (Wright and O'Donnell 1992, Wright and Machin 1993).

In this study, we set out to test whether WVA serves in volume expansion during molting in two species of oniscidean isopods, *Armadillidium vulgare* (Latreille, 1804) (Armadillidiidae) and *Porcellio dilatatus* Brandt, 1833 (Porcellionidae). Both belong to the section Crinocheta, a well-defined monophyletic group (Erhardt 1997, Mattern 2003, Lins et al. 2017). We compare these two species with a non-crinochete oniscidean, *Ligidium lapetum* Mulaik & Mulaik, 1942 (Ligiidae), belonging to the section Ligiamorpha and incapable of WVA (Wright, personal observation). *Armadillidium vulgare* and *P. dilatatus* are common species in Southern California, originally introduced from Europe (Miller 1936, Arcangeli 1932, Garthwaite and Lawson 1992). *Ligidium lapetum* is a native species in Southern California. Although scantily recorded in the literature since its original description from Central California (Mulaik and Mulaik 1942), *L. lapetum* is common in riparian oak woodlands on the south slope of the San Gabriel Mountains.

## Methods

*Armadillidium vulgare* and *Porcellio dilatatus* were collected from the Pomona College campus and vicinity, Claremont, CA, and *Ligidium lapetum* was collected from local foothill canyons in the San Gabriel Mountains. Animals were maintained in the lab at 22 °C in covered glass bowls with oak litter and shell fragments as a calcium source. Carrot and potato were provided *ad libitum* as supplementary food.

Isopods were examined daily for signs of molting. Pre-molting animals were identified by the appearance of the sternal calcium deposits and separated into individual 20 mL glass vials. The top of each vial was covered with 1-mm fiberglass screen mesh. Ani-

imals were maintained in controlled humidity (100 % or 97 %) by standing the inverted vials on a 4-mm steel grid of a nested sieve set (Wards, Rochester, NY). This 4-mm sieve was inserted into the bottom pan which, in turn, was filled to within 1 cm of the overlying grid with water or with saturated aqueous  $K_2SO_4$  to establish a relative humidity of 100 % or 97 % respectively (Winston and Bates 1960). The top of the upper sieve chamber was then covered with a sheet of Plexiglas ringed with silicone vacuum grease. This experimental design ensured that any animal sitting on the screen mesh of a vial was no more than 1 cm from the liquid surface. Controls using a digital hygrometer probe (VWR International, San Dimas, CA) inserted through a substitute, drilled, Plexiglas cover and sealed with Parafilm showed that the relative humidity immediately above the metal grid came to within 1% of the equilibrium humidity after 15 minutes ( $N = 9$ ).

Each batch of animals was weighed daily at the same time using an Ohaus digital microbalance with a resolution of 10  $\mu$ g. Any fecal pellets produced were weighed separately and then discarded. Total fecal pellet mass-losses in any given 24-h period were usually less than 1 mg and few pellets were produced after animals had been isolated for 3 days. The molt stage of each animal was recorded as follows:

Premolt – sternal calcium deposits visible; recorded as days prior to the posterior ecdysis  
 Posterior ecdysis (PE) – posterior cuticle shed, resulting in a distinct 2-tone appearance  
 Anterior ecdysis (AE) – anterior cuticle shed; sternal deposits no longer visible  
 Postmolt – recorded as days following anterior ecdysis

The number of days pre-molt for each weighing was determined *post-facto* according to the timing of the posterior ecdysis. Following PE and AE, most animals consumed the sloughed exuvium within 2 days. Fragments of uneaten exuvia were left in the chamber and not included in mass measurements.

Since preliminary observations indicated the presence of WVA, we conducted separate trials to examine the impact of WVA on hemolymph osmolality in molting *A. vulgare*. Females undergoing parturial molts were excluded. Procedures were identical to those described above, except that animals were sampled daily for blood by puncturing the thin cuticle at the base of the 7<sup>th</sup> pereopod using a pulled glass micropipette. By holding the tip in place for a few seconds prior to withdrawal, bleeding from the sample location was minimized or (in most cases) eliminated. Each sample (<20 nl) was expelled into mineral oil held in the silver sample plate of a Otago nanoliter osmometer (Otago Instruments, Dunedin, NZ), and the osmolality determined from the freezing point depression ( $\Delta T_f$ ):

$$\text{Osmolality (Osm. kg}^{-1}\text{)} = \Delta T_f / K_f$$

where  $K_f$  is the colligative freezing point depression constant ( $-1.858\text{ }^{\circ}\text{C Osmol}^{-1}$ )

Although the impact of blood sampling on animal masses was small, the mass data from these animals were used solely to calculate predicted changes in osmolality (see below) and not combined with the independently collected mass-change data.

## Results

Mass changes by day (%) for *Armadillidium vulgare* and *Porcellio dilatatus* in 100 % and 97 % RH are shown in Figures 1–4. Several animals initiated the posterior ecdysis (PE) within 6 days of the first weighing, and a few animals died during experimentation, so sample sizes for each day are variable. In 100 % RH, both species showed a similar pattern of progressive mass increase from 5–6 days premolt, reaching a maximum of approximately 3 % d<sup>-1</sup> on the day of PE. In 97 % RH, the pattern was similar but the mass increases smaller, reaching maxima of approximately 2 % d<sup>-1</sup> in *A. vulgare* and 1 % d<sup>-1</sup> in *P. dilatatus*, and peaking 1–2 days prior to PE. Mass gain declined sharply or reversed 1–2 days after PE and was followed by a period of water loss. This was quite variable among animals in 97 % RH, but more clearly defined in 100 % RH with the largest loss fluxes occurring in the day following anterior ecdysis (AE) and declining progressively thereafter. In a few trials with *A. vulgare*, AE occurred more than 24 h after PE; masses recorded on the second day following PE but prior to AE are denoted as PE2.

Estimates of net mass changes over the molt period were derived by summing the daily mass changes and are presented in Table 1. Both species showed a net mass gain over the 12 to 13-day molt period. Although daily uptake fluxes were markedly smaller in 97 % RH for both species, the post-molt losses were also smaller and *A. vulgare* actually showed a larger cumulative mass gain in 97 % than in 100 % RH.

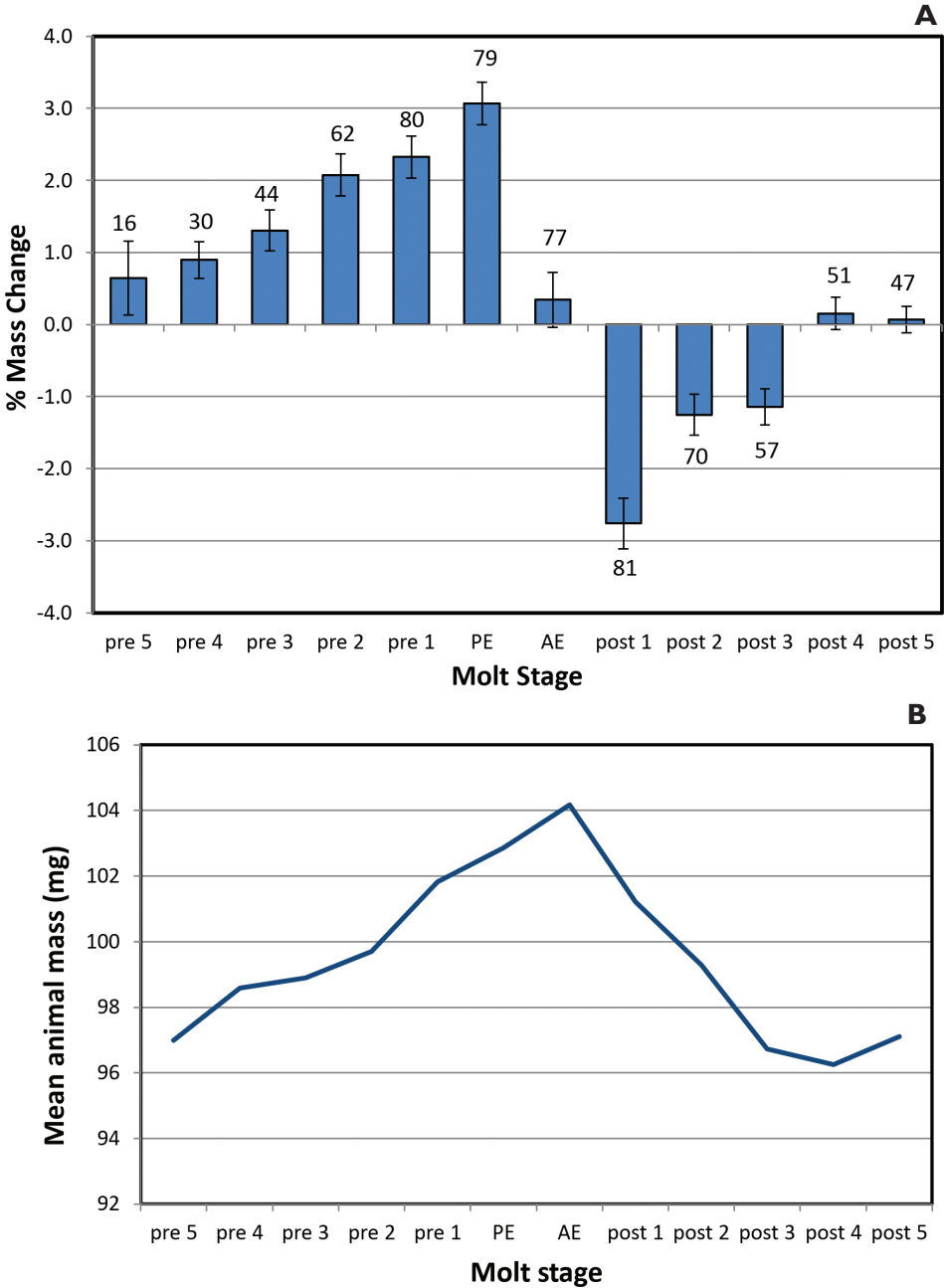
In contrast to the crinochete species, *Ligidium lapetum* failed to show any mass gain in either 100 % or 97 % RH and no animals initiated molting. No specimen survived to Day 3 in 100 % RH (n = 12) and all animals died within 24 h in 97 % RH (n = 11). The mean mass losses after Day 1 were 15.4 % in 100% RH and 43.1 % in 97 % RH. Possible explanations for the significant mass losses in 100 %, despite the rapid equilibration time of the chamber, are considered in the Discussion.

Hemolymph osmolality in *A. vulgare* underwent a pronounced decline following PE2 (Fig. 6) and remained below intermolt values for the 5 days post-molt. Observed osmolalities were compared with predicted values assuming the mean osmolality as a baseline, a hemolymph mass (and volume) of 33.4 % of the hydrated intermolt animal mass (Koh and Wright 2011), and assuming that all mass changes during the molt period represent water moving into/out of the hemolymph:

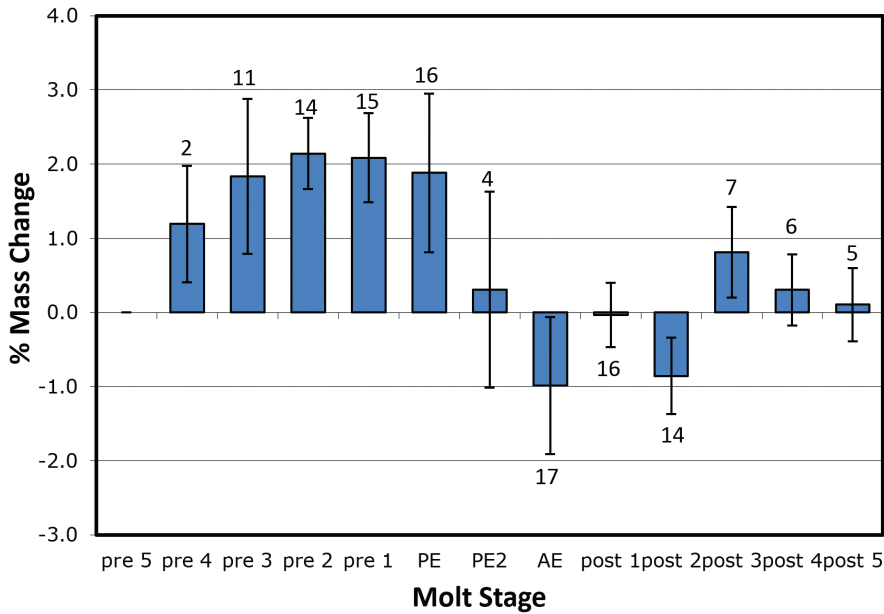
$$\text{Osm}_p = \text{Osm}_i \cdot 33.4 / (\Delta M_x + 33.4)$$

Where  $\text{Osm}_p$  = predicted osmolality (mOsm.kg<sup>-1</sup>),  $\text{Osm}_i$  = measured intermolt osmolality (mOsm.kg<sup>-1</sup>),  $\Delta M_x$  = proportional change in animal mass relative to intermolt mass (%), and 33.4 is the proportional volume of the hemolymph (%). The predicted variation contrasts sharply with the measured values, showing in particular markedly higher values (by 50–60 mOsm.kg<sup>-1</sup>) over the 6-day period following PE2. Measured osmolality is significantly elevated above predicted values at 3 days prior to PE, and significantly depressed below predicted values from AE throughout the post-molt period.

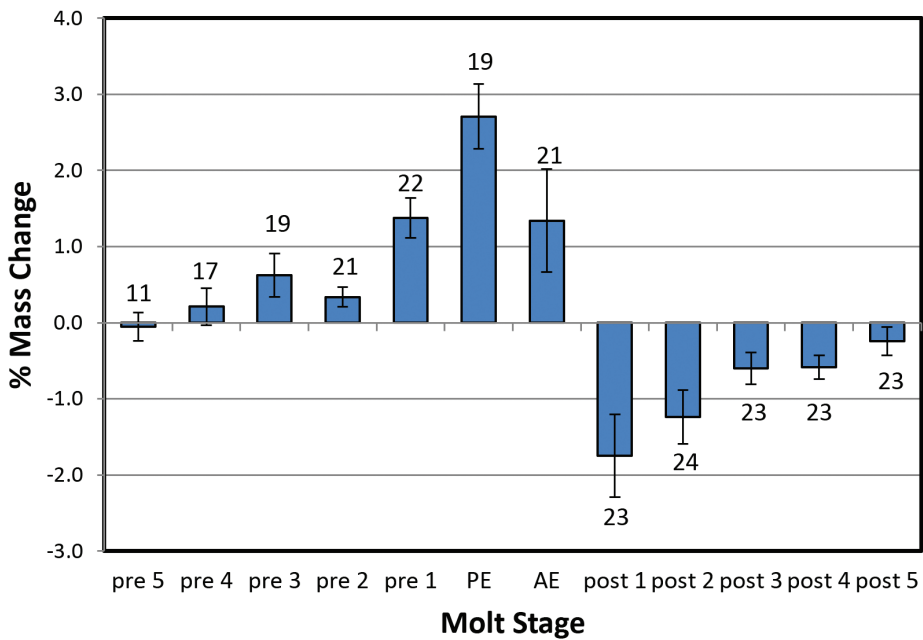




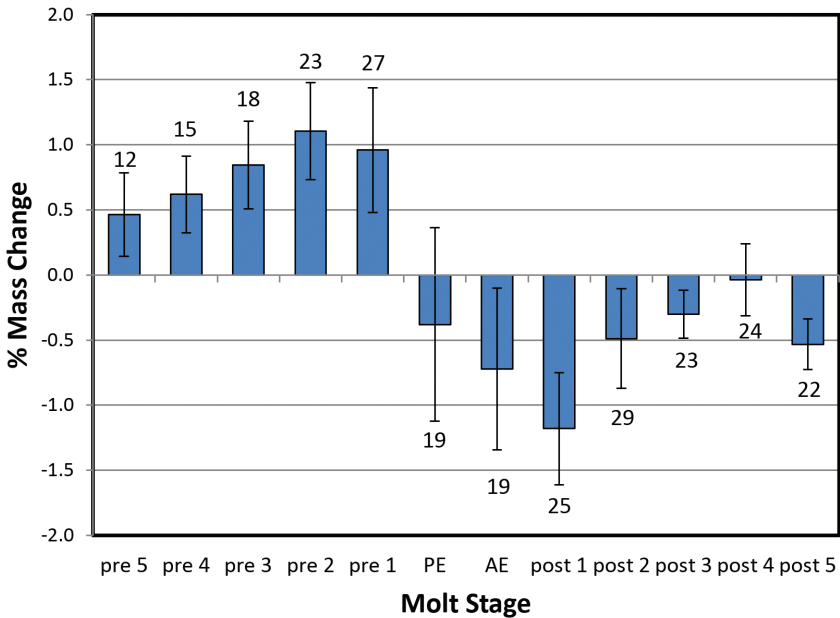
**Figure 1. A** Mass changes of *Armadillidium vulgare* during molting at 100 % RH, without access to food. Pre- and post- labels refer to the number of days before/after ecdysis with data showing the % mass change over the prior 24-h period. PE = posterior ecdysis; AE = anterior ecdysis. Bars show  $\pm$  SEM with sample sizes **B** Mean masses of 4 of these animals, showing the characteristic pattern of mass gain, peaking between PE and AE, followed by loss over the 3 to 4-day post-molt period.



**Figure 2.** Mass changes of *Armadillidium vulgare* during molting in 97 % RH, without access to food. Details as in Figure 1. PE2 refers to the small number of animals reaching a second day after PE without completing the anterior ecdysis.



**Figure 3.** Mass changes of *Porcellio dilatatus* during molting at 100 % RH, without access to food. Bars show  $\pm$  SEM with sample sizes.



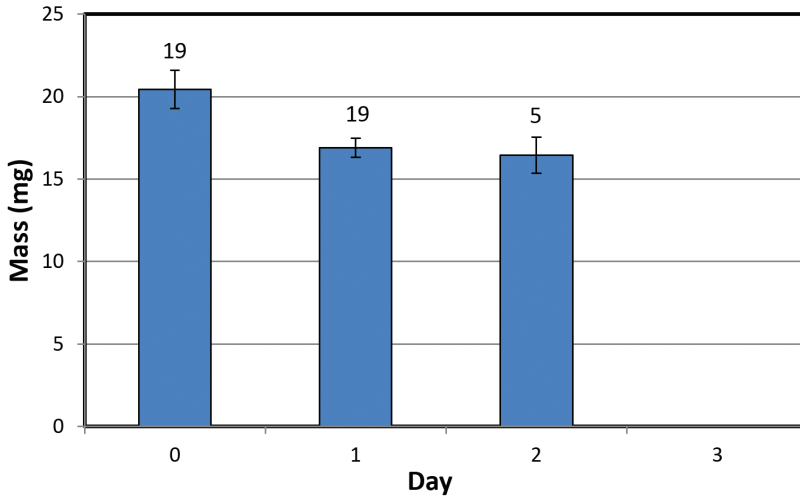
**Figure 4.** Mass changes of *Porcellio dilatatus* during molting at 97 % RH and without food. Data labels and other details as for Fig. 1.

**Table 1.** Cumulative mass changes (%) over the molt cycle derived from the data plotted in Figs 1–4. Data show means changes during the premolt period (6 days premolt to PE), the intramolt period (PE to AE), and the postmolt period (AE to 5 days postmolt), and the net mass gain over the total 12- to 13-day molt period. Asterisks denote a significant mass change during the respective interval (\*  $p < 0.05$ ; \*\*  $p < 0.005$ ; \*\*\*  $p < 0.0005$ ; 2-sample t-test).

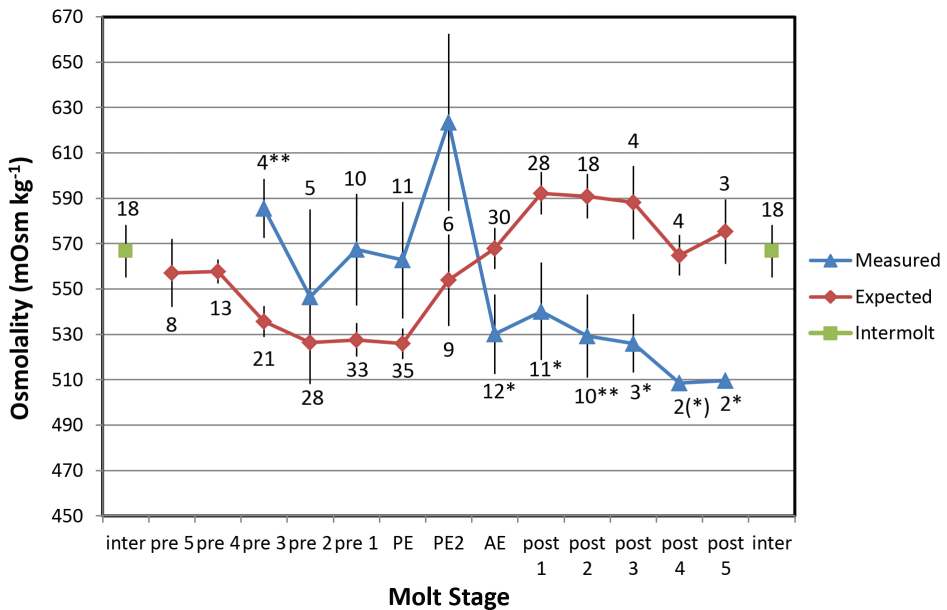
	6-days premolt to PE	PE to AE	AE to 5-days postmolt	Net mass gain
<i>A. vulgare</i>				
100% RH	10.31***	2.72***	-4.93***	5.72
97% RH	9.13**	2.87*	0.33	8.77
<i>P. dilatatus</i>				
100% RH	5.20***	1.37*	-4.41***	2.13
97%	3.61*	0.34	2.53*	0.36

The mean osmolality measured from 3–5 days post-molt is 517 mOsm.kg<sup>-1</sup>, representing a decrease of 8.6 % from the mean intermolt value of 567 mOsm.kg<sup>-1</sup>.

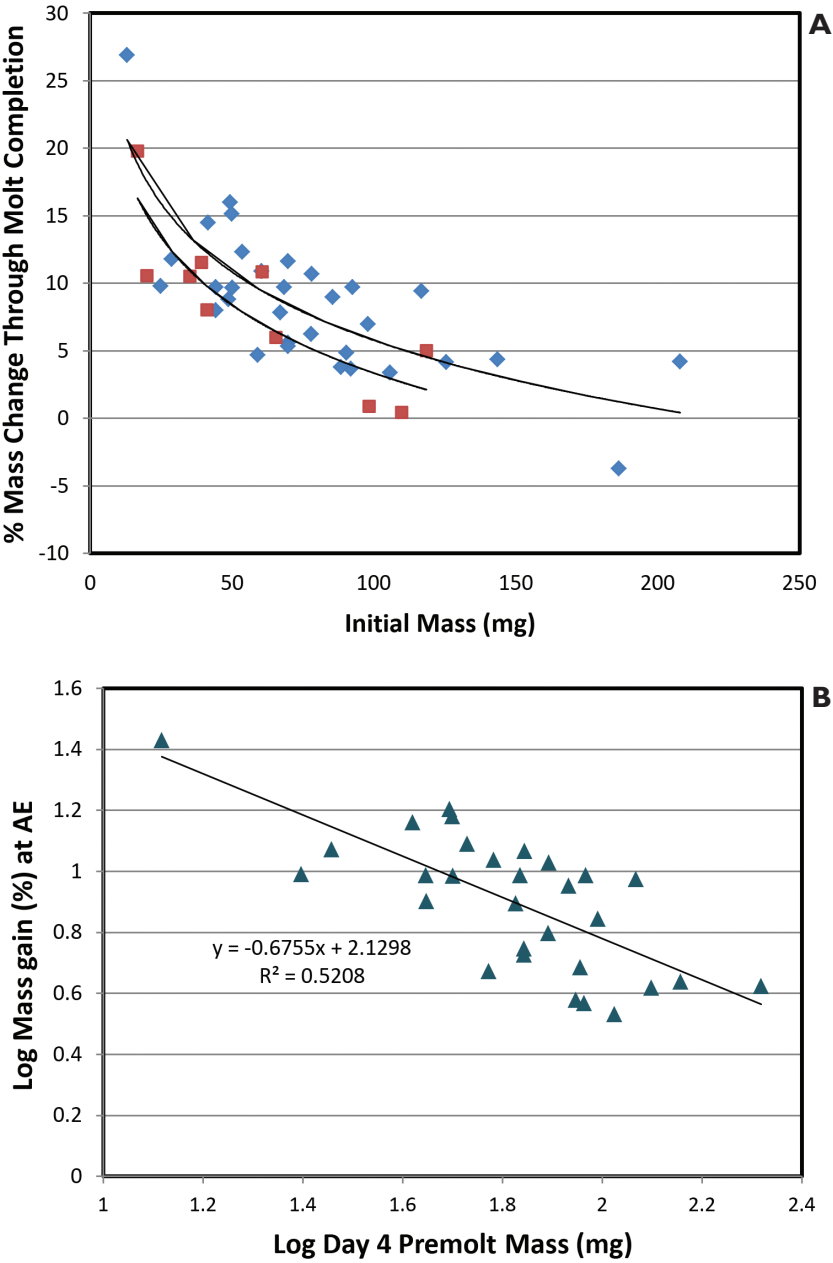
Fractional mass changes of *A. vulgare* through the molting period showed an inverse logarithmic relationship to pre-molt animal mass in both 100 % and 97 % RH (Fig. 7a,b). The scaling exponent for the 100 % RH data is -0.676, indicating an area-limited process. This is considered further in the Discussion.



**Figure 5.** Mean masses of *Ligidium lapetum* in 100% RH. The mean mass of the surviving animals on Day 2 is the mean % mass loss of those animals, subtracted from the mean of all animals at Day 0. No animal survived to Day 3 or initiated ecdysis.



**Figure 6.** Mean measured (blue) and expected (red) values for hemolymph osmolality in *Armadillidium vulgare* during molting in 100 % RH. Expected values are derived from the product of the mean intermolt osmolality (green symbols) and the proportional changes in blood volume over the molt cycle (see text). Bars show  $\pm 1$  SEM with sample sizes. Molt stages as in Figs 1–4. Asterisks denote significant differences between measured and expected means (2-sample t-test). \*  $P < 0.05$ ; \*\*  $P < 0.01$ . (\*)  $P = 0.056$ .



**Figure 7. A** Percentage mass change between 5 days premolt and anterior ecdysis for *Armadillidium vulgare* maintained in 100 % RH (blue) and 97 % RH (red) and plotted as a function of premolt mass. Trendlines show best-fit logarithmic curves. Animals in 97 % RH achieve slightly smaller proportional mass changes to those in saturated air, consistent with the reduced vapor pressure gradient for WVA **B** Log-log plot showing the relationship between fractional mass-gain and pre-molt mass in 100 % RH ( $\% \text{ mass gain} \propto M^{-0.676}$ ).

## Discussion

This study shows for the first time that crinichete oniscideans utilize active water vapor absorption (WVA) to increase body mass prior to ecdysis. In both 100 % and 97 % RH, *A. vulgare* and *P. dilatatus* showed a progressive increase in mass from 5 days prior to ecdysis, typically peaking on the day of ecdysis, and followed by a variable period of mass loss commencing between the posterior and anterior ecdyses. The water uptake in the first few days of weighing will actually be slightly larger than calculated here because most animals lost a small amount of mass (0.3–2.2 mg, or ca. 1–1.5 %) over this period (usually Pre5 to Pre2) in the form of fecal pellets.

The fact that water uptake is seen in 97 % RH ( $a_w = 0.97$ ), a humidity below the equilibrium water activity of the hemolymph in *A. vulgare* and *P. dilatatus* (ca. 0.990; Wright et al. 1997), confirms the role of active WVA. Both species attained a mean water uptake of about 3 % d<sup>-1</sup> in the day of PE, about 3.5 times larger than the estimated passive flux, and larger gains (up to 8.7 % d<sup>-1</sup>) were seen in some animals with masses in the 60–100 mg range. The increase in mass and volume due to WVA likely serves to supplement muscle contraction in generating the pulses of hydrostatic pressure that precede the pre-ecdysial transverse split in the old cuticle and the subsequent posterior and anterior ecdyses (Alikhan 1983, 1984). Critically, WVA provides a mechanism for expansion of the new cuticle and net volume increase during the molt cycle (Table 1), allowing for succeeding tissue growth during intermolt.

Notwithstanding the considerable variation among animals in maximum WVA rates, calculated values are considerably smaller than the fluxes reported by Wright and Machin (1993) in flowing air. WVA in crinichete oniscideans is potentially quite rapid, despite the high absorption thresholds, and in an RH >95 % animals can replenish significant water losses (>10 %) in a few hours. The standardized uptake fluxes for *A. vulgare* (6.7  $\mu\text{g h}^{-1} \text{Pa}^{-1}$ ) and *P. dilatatus* (7.6  $\mu\text{g h}^{-1} \text{Pa}^{-1}$ ), would enable proportional mass-gains of approximately 50 % in 24 h (Wright and Machin 1993). The smaller uptake rates documented here may simply reflect intermittent WVA and the need for controlled rates of cuticle expansion during the premolt. However, it is also likely that uptake fluxes are limited by the lack of air flow and regional lowering of the water vapor pressure adjacent to the condensing pleopodal surface. In that event, we would expect mass-gains under natural conditions often to occur more rapidly, and perhaps take place over fewer days, since animals frequently rest with the pleopods immediately above a saturated surface such as damp wood.

The cessation of WVA following the intramolt (between PE and AE) may be due to an inability to absorb water vapor during the period of new cuticle formation, as apparent also in *Tenebrio* larvae (Coutchié and Crowe 1979), the polyphagid cockroach *Arenivaga* sp. (Edney 1966), and the lepismatid *Thermobia domestica* (Noble-Nesbitt 1978, 2010). In each of these insects, the capacity for WVA ceases prior to ecdysis, then resumes shortly after. In *Thermobia*, WVA resumes about 3h following ecdysis (Noble-Nesbitt 1978) while in *Tenebrio* and *Arenivaga*, the resumption takes about 2 days after ecdysis (Coutchie and Crowe 1979, Edney 1966). It is not known whether



the interruption of absorption capacity is due to a need for the renewal of critical structural components of the new cuticle following apolysis or to a temporary interruption of epithelial transport processes. It is interesting, however, that WVA in *A. vulgare* and *P. dilatatus* ceases only following ecdysis, and this may be facultative rather than reflecting a loss of absorption capacity. The clear period of water loss following ecdysis is seen in both 97 % and 100 % RH and presumably serves to provide a controlled reduction of the elevated hemolymph volume and pressure generated during the premolt period. Immediately following PE, the soft posterior cuticle is expanded by the elevated hemolymph pressure and the distension of the posterior half is often striking (see also Hornung 2011). After this time, contraction of somatic muscles in the posterior segments sustains the elevated hemolymph pressure pulses and assists in the anterior ecdysis and expansion of the new anterior cuticle (Heeley 1941, Alikhan 1983, 1984), even as the animal's net volume shows a modest decline. Calcification of the new posterior and anterior cuticle takes place rapidly, mostly occurring within 24 hours of the respective ecdyses (Steel 1993). Whether intermolt animals show significant mass and volume increase, enabled by extension of pleural and intersegmental cuticle, appears not to have been investigated.

The mass-gain prior to PE and mass-loss following AE seen in both *A. vulgare* and *P. dilatatus* differ from the post-molt volume increase described for the supralittoral ligiid *Ligia pallasii* by Ziegler and Pennings (2000) based on hemolymph volume measurements. During the initial premolt period, hemolymph osmolality in *A. vulgare* remains elevated and higher than predicted based on the mass increase from WVA (Fig. 6), indicating that WVA is initially accompanied by compensatory mobilization of sequestered electrolytes into the hemolymph (Wright et al. 1997, Koh and Wright 2011). Hemolymph osmolality shows a sharp decrease following PE, and concentrations of specific electrolytes ( $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Mg}^{2+}$ ,  $\text{Cl}^-$ ) have been shown to decrease in a similar pattern in *Porcellio scaber* (Ziegler and Scholz 1997). This coincides, curiously, with the onset of the mass and volume decrease and indicates a reversal of the earlier ion mobilization and re-sequestering of electrolytes following PE. The sharp divergence between observed and predicted osmolality following AE indicates that the post-molt water losses are due not to evaporation (which would be precluded in 100 % RH anyway) but to the expulsion of iso-osmotic fluid, possibly maxillary urine. This would have no net impact on hemolymph osmolality which would thus be predicted to remain unchanged following PE when vapor absorption ceases. Indeed, the expected osmolality at PE closely approximates the measured values thereafter (Fig. 6). The post-molt water losses in *A. vulgare* amount to approximately 5 % of total mass (Fig. 1, Table 1), equivalent to 15 % of the hemolymph volume. The initial water gain from WVA followed by removal of isosmotic fluid explains the net loss of solutes and decrease in hemolymph osmolality over the molt cycle; these solutes will need to be replaced during the succeeding intermolt period.

*Ligidium lapetum* presents a clear contrast to the crinochete species, with pre-molt animals unable to molt successfully when isolated in 97 % or 100 % RH and suffering significant mass-losses even in the 100 % RH chamber. This is consistent with the ab-

sence of any WVA capacity in this species. Although some modest water loss is inevitable during chamber equilibration, an outward vapor pressure gradient would persist only for about 15 minutes in 100 % RH chamber and explains the lack of significant mass-loss between Day 1 and Day 2. It is unclear why animals did not survive beyond Day 2 in 100 % RH; this could result from prolonged effects of the initial dehydration, or additional ensuing dehydration from obligatory intermittent production of maxillary urine (Hoese 1981).

The question remains as to how *Ligidium* spp. and other non-crinochete terrestrial oniscideans achieve volume expansion to enable molting in the absence of WVA. We have found *L. lapetum* only in close proximity to liquid water, inhabiting litter and humic soil in the riparian fringe. Here it has ready access to freshwater and could drink or possibly take up water via the uropods and rectum, as documented for the Crinocheta (Spencer and Edney 1955, Drobne and Fajgelj 1993), to bring about pre-molt expansion. The Ligiidae also depend on an external freshwater source to provision the marsupial fluid (Hoese 1981, Yoshizawa and Wright 2011). The other major radiation of terrestrial oniscideans is the family Trichoniscidae, mostly small animals with little resistance to water loss. Trichoniscids are primarily endogean in habit, inhabiting damp soil, decomposing wood and litter (see Sutton 1980, and references therein). Standen (1970) showed that *Trichoniscus pusillus* was unable to gain mass during the molt in the absence of liquid water. Trichoniscids may imbibe soil capillary water as a means of volume expansion during the molt. However, their high permeability and small size would also allow them to gain water quite rapidly through passive diffusion in saturated conditions. Proportional water fluxes measured for 3 species range from 44 to 59 % h<sup>-1</sup> a<sub>w</sub><sup>-1</sup> (Wright and Machin 1993) which translate to approximately 10–15 % mass gain per day in saturated air. Trichoniscids may be able to regulate mass gain and loss during the molt cycle by exploiting passive diffusional gain if essential water losses from maxillary urine can be limited accordingly.

*A. vulgare* females attain reproductive maturity within the first year and may live for up to 4 years (Lawlor 1976). Animal masses in the current study varied appreciably and will account for some of the variance among the mass exchange data. Mass-change of oniscideans during growth is approximately linear as a function of time (Hubbell 1971, Helden and Hassall 1998), so proportional mass gain ( $\Delta M/M$ , %) will vary over time as the function  $M^{-1}$ . This function is the product of the mass-gain over one molt cycle and the intermolt period. To explore this relationship further, we analyzed the proportional mass gain ( $\Delta M/M$ ) at anterior ecdysis as a function of the preceding (intermolt) animal mass ( $M$ , mg) for *A. vulgare*. We used the mass gain at AE rather than the net mass gain over the complete molt cycle owing to the much larger sample size. Results are shown in Figure 7 (a, b). In both 100 % and 97 % RH,  $\Delta M/M$  decreases exponentially as a function of mass, and log transformation (Fig. 7b) yields the following relationships:

100 % RH	$\Delta M/M = 135.M^{0.676}$	(n = 30; r <sup>2</sup> = 0.52)
97 % RH	$\Delta M/M = 78.M^{0.564}$	(n = 8; r <sup>2</sup> = 0.71)

Although the sample size for 97 % RH is small, the reasonable congruence of the two exponents supports the assertion that the relative mass gain prior to molt scales with an exponent of -0.67, and mass-gain ( $\Delta M$ ) scales as  $M^{0.33}$  (or  $L^1$  where  $L$  is length). This indicates that oniscideans follow the Brooks-Dyar Law (Dyar 1890, Daly 1985), showing a regular, geometric increase in the linear dimensions of sclerotized parts of the integument at each molt ( $\Delta L$ ). It further shows that intermolt period must scale as  $M^{0.67}$  ( $\Delta L^2$ ) in order for mass to increase linearly.

To our knowledge, these crinochete isopods represent the first demonstrated instance of WVA functioning in volume increase during molting. Given the limited means of volume expansion available to terrestrial taxa, however, this may be a widespread function of WVA. Further work examining such a role in other vapor-absorbing groups would be revealing, as would studies of volume regulation during molting in arachnids and myriapods where the mechanisms remain largely elusive.

## Conclusion

*Armadillidium vulgare* and *P. dilatatus* show a progressive increase in mass in the absence of food or liquid water from 5–6 days prior to the posterior ecdysis. This mass-gain is seen in 100 % RH or 97 % RH, confirming the role of active water vapor absorption. Following the anterior ecdysis, both species show a variable period (3–4 days) of mass-loss accompanied by loss of ions from the hemolymph. The net mass and volume gain over the premolt period could supplement pulses of hemolymph pressure to bring about the anterior and posterior ecdyses and, critically, will allow for volume expansion and growth of normally sclerotized and/or mineralized cuticle. The ligiid, *Ligidium lapetum*, lacks the capacity for WVA and lost mass over the molt cycle, even in 100%. This species presumably depends on liquid water uptake for volume expansion.

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# Morphological traits – desiccation resistance – habitat characteristics: a possible key for distribution in woodlice (Isopoda, Oniscidea)

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## Abstract

Terrestrial isopods, as successful colonizers of land habitats, show a great variety in species distribution patterns on a global, continental, or regional scale. On a local, within-habitat level these patterns reflect the species' tolerance limits and the presence of suitable hiding places (shelter sites, refugia). Humidity preference reflects a species' capability for water retention which, in turn, depends on the integumental barrier. Desiccation resistance is a key feature in isopod survival under different environmental conditions. The present study shows a correlation between cuticle thickness and desiccation resistance under three relative humidity (RH) ranges (about 30, 75 and 100% RH) in nine species, relating these to the species' differences in meso- and microhabitat choices. Habitat preferences are also associated with differences in cuticle surface morphology. The results support our hypothesis that species distribution and desiccation resistance are associated with particular cuticular morphological traits. Phylogenetic relations seem to be less important in desiccation resistance than cuticle thickness and external morphology.

## Keywords

Eco-morphology, habitat preference, intrageneric comparison, mortality, sympatric species, water loss

## Introduction

Terrestrial isopods (Isopoda, Oniscidea) are successful colonizers of land habitats with over 3700 described species (Sfenthourakis and Taiti 2015). The group has developed various morphological, physiological, and behavioral traits to survive the challenges of terrestrial life (e.g., desiccation, respiration, and reproduction) (Edney 1954, Schmalfuss 1984, Hornung 2011).

The sclerotized cuticle is the main barrier between oniscidean individuals and their environment. The crustacean exoskeleton is composed of four layers: the epicuticle, the procuticle (exo- and endocuticle), and the membranous layer (Travis 1955, Compère 1990). The epicuticular layer is important in inhibiting water loss (Cloudsley-Thompson 1977). It is divided into sublayers: the cement layer, the surface coat, the waxy layer, and the inner epicuticle. The waxy layer located within the cuticulin layer is not homologous with the external wax layer of insects (Compère 1990). However, as in insects, the waxy layer of oniscideans probably acts as a waterproofing barrier which reduces the tegumental water loss in terrestrial environments (Hadley 1982). Hadley and Quinlan (1984) detected cuticular lipids in the mesic *Porcellio laevis*, although the amount of lipid present was not effectively reducing the transcuticular water loss.

It is well-known that terrestrial crustaceans lose water more rapidly than most other land arthropods due to their tegumental transpiration (Edney 1951, 1954, Powers and Bliss 1983, Lillywhite and Maderson 1988, Little 1990, Dias et al. 2012). The rate of water loss is affected by the environmental temperature and humidity (Edney 1951, 1968). Nevertheless, terrestrial isopods can take up water directly (Hoese 1981) and from water vapour (Wright and Machin 1990, 1993), which allows rapid recovery of water loss. The high cuticular permeabilities might explain the capacity for fast replacement of evaporative water loss in woodlice species (Wright and Machin 1993). Moreover, they can obtain preformed and metabolic water from their food and imbibe water through their mouth, anus (Spencer and Edney 1954, Cloudsley-Thompson 1977, Drobne and Fajgelj 1993) and water conducting system (Horiguchi et al. 2007). Greenaway and Warburg (1998) measured the water-flux in 16 terrestrial isopod species originating either from mesic or xeric habitats. Flux data were used to predict threshold water activities for water vapor absorption. They showed a trend of decreasing permeability to water with increasing aridity of the habitat, but no significant differences in the changes of mean water fluxes were found.

In terrestrial isopods, the epicuticle forms several surface structures such as tubercles, micro-ridges, setae, tricornes, plaques and pits (Holdich and Lincoln 1974, Schmalfuss 1978, Powell and Halcrow 1982, Holdich 1984). Some of these are sensory structures (Powell and Halcrow 1982, Price and Holdich 1980, Holdich 1984). Several studies have shown that tricorn receptors are common in terrestrial woodlice species (Sutton 1969, Schmalfuss 1978, Holdich 1984, Fernandes et al. 2016) but are absent in aquatic Crustaceans (Holdich 1976, Schmalfuss 1978). Therefore, these structures are presumably playing a role in terrestrial adaptation. Tsuneto (1989) showed that tricorn-type sensilla can receive mechanical, gustatory, and olfactory stim-

uli. They might be important in the perception of humidity, too. According to Holdich (1984) setae are exteroceptors. The various plaques (micro-scales) have diverse functions, e.g., to conduct water in the cuticle in the water capillary system (Schmalfuss 1978, Hoesle 1981). Powell and Halcrow (1982) showed that micro-scales are a common feature in isopods from different habitats. The dorsal surface of the exoskeleton is adapted to the microhabitat type e.g., the diverse tubercles and micro-ridges may provide anti-adhesive potential for small, endogeic species (Schmalfuss 1978, 1984).

We assumed that differences in preferred habitat type correlate with differences in desiccation resistance. We hypothesized that a key mechanism by which selection has generated the increased resistance was by increased thickness of the cuticle. We explored connections among species distribution, desiccation resistance, and morphological traits focusing on the following questions:

- (i) What is the relationship between distribution and desiccation stress?
- (ii) What is the relationship between microhabitat desiccation stress and species' desiccation resistance?
- (iii) What is the relationship between desiccation resistance and cuticle morphological traits?

We hypothesized that there is a higher similarity among morphological traits in epigeic species with similar ecological needs (that is sharing the same habitat) than among closely related species living under quite different environmental conditions. To test these hypotheses, we measured interspecific and intrageneric desiccation resistance and compared exoskeleton properties in a selected group of species from Central and Eastern Europe. While numerous studies have compared desiccation resistance of terrestrial isopod species (Hadley and Warburg 1986, Davis 1989, Greenaway and Warburg 1998, Tsai et al. 1998, Dias et al. 2012), our study is the first to relate ecological tolerance to morphological traits of cuticle.

## Materials and methods

For interspecific comparison six surface-active isopod species were tested. The species belong to different families and/or genera occurring in the same habitat: *Armadillidium vulgare* (Latreille, 1804), *Cylisticus convexus* (De Geer, 1778), *Orthometopon planum* (Budde-Lund, 1885), *Protracheoniscus politus* (C. Koch, 1841), *Porcellionides pruinosus* (Brandt, 1933), and *Trachelipus rathkii* (C. Koch, 1841). *Armadillidium vulgare* and *T. rathkii* are among the most frequent terrestrial isopod species in Hungary. The generalist *A. vulgare* is a frequent and widely distributed species in diverse habitat types (Hornung et al. 2007, Vilisics and Hornung 2008, Farkas and Vilisics 2013). The Central- and Eastern European species, *P. politus* and *O. planum* are connected to native, undisturbed deciduous forests in Central Europe (Tuf and Tufová 2005, Mock et al. 2007). *Cylisticus convexus* and *P. pruinosus* are synanthropic species, in-



dicating strong human influence (Korsós et al. 2002). We also examined four species belonging to the *Armadillidium* genus, showing allopatric distribution patterns. Their tolerance limits against environmental conditions are different, too. *Armadillidium zenckeri* Brandt, 1833 is a habitat specialist, living exclusively in European swamps and marshlands (Sallai 1993, Farkas and Vilisics 2006). The atlanto-mediterranean *Armadillidium nasatum* Budde-Lund, 1885 is an introduced species in Hungary. It is known from botanical gardens and greenhouses (Farkas and Vadkerti 2002, Korsós et al. 2002, Hornung et al. 2007). The future outdoor spread and establishment of *A. nasatum* is expected as it is widely distributed in the wild in other regions (Schultz 1961, Hornung and Szlavec 2003, Giurginca 2006). *Armadillidium versicolor* Stein, 1859 is widespread in Southeastern Europe. In Hungary it is most abundant along rivers (e.g., along the Danube) and lake shores in the Transdanubian region and the Mátra Mountains (Farkas 2007, Vona-Túri and Szmátóna-Túri 2012). It occurs also in drier habitats, although not in Hungary (Ferenčí et al. 2012).

### Sampling and habitat characteristics

The studied sympatric species (*A. vulgare*, *C. convexus*, *O. planum*, *P. politus*, *P. pruinosus*, *T. rathkii*) were hand collected in a deciduous forest of the Buda Mountains, next to the western part of Budapest, Hungary (at Solymár; 47°35.094'N, 18°57.164'E). Two mesohabitat types, different in humidity and temperature, within the sampling area were searched for woodlice: a valley (Alsó-Jegenye) along a stream (Paprikás patak) accompanied by a trail and an elevated area with an ancient deciduous forest (Felső-patak Hill). Individuals of *A. zenckeri* came from a marshland (Ócsa, Hungary; 47°17'39.5"N, 19°12'27.9"E), and the specimens were collected on the waterside or directly above the water, under the bark of wooden duck-boards. Specimens of *A. nasatum* originated from the tropical glass house of the Botanical Garden of Eötvös Loránd University (Budapest, Hungary; 47°29'05.3"N, 19°05'01.6"E) and specimens of *A. versicolor* were collected in the Margaret Island surrounded by the Danube river (Budapest, Hungary; 47°31'44.4"N, 19°03'06.5"E).

### Experimental design

The collected individuals were kept in the lab in plastic boxes containing moist soil and litter for 14 days to ensure acclimatization. The specimens of each species were kept in 100% relative air humidity (RH) overnight to standardize the initial experimental conditions. This procedure ensured that animals replenished any possible water deficit. The isopods were without food for 24 hours, meanwhile they defecated their gut content, so this did not affect subsequent changes in body mass (Dias et al. 2012).

Water loss rate and mortality were studied in three different RH values in glass desiccators: an extremely dry (~30%), a relatively dry (~75%) and a humid one, nearly 100%. The humidity levels were acquired using silica gel (RH <30%), saturated sodium-chloride (RH 75%) and water (RH 100%) (Winston and Bates 1960). The experimental setup did not allow air to circulate. All experiments took 6 hours. We measured 20 specimens per species individually to avoid the water loss decreasing effect of aggregation of specimens (Broly et al. 2014). In the case of *A. vulgare* different sets of individuals (N = 20 in each experiment) were tested for the inter- and intrageneric comparisons. We used both sexes based on the results of a previous study (Dias et al. 2012) where no differences were found between the desiccation resistances of sexes. At the start of the experiment, the fresh body mass of each individual was measured by an analytical balance (Sartorius AG, Göttingen, Germany). Specimens were re-weighed at the end of the 6-h experiment and the weight-loss of each individual was standardized by its body weight. At the end of each experiment individuals were checked for mortality. In the present study 'desiccation resistance' was defined as the rate of mass-specific water loss ( $\text{g g}^{-1}$ ).

### Microscopic methods

To reveal the characteristics of the exoskeleton, light microscopic investigations (LM) were applied. For this purpose, we fixed two intermolt adult specimens from each species in 4% paraformaldehyde for 7 days (we chose the greatest size category in the sampled population). The fixation was followed by rinsing in distilled water ( $3 \times 1 \text{ h}$ ). We decalcified the tissues overnight in 8% ethylenediamine-tetraacetic acid disodium salt (EDTA). After the tissues became pliable they were dehydrated through an ascending series of ethanol (50% – 1 h, 70% – overnight, 80%,  $2 \times 90\%$ ,  $2 \times 96\%$ , and  $2 \times 100\%$  – 1 h). After dehydration, the samples were kept in xylene ( $2 \times 1 \text{ h}$ ). Thereafter the samples were infiltrated with paraffin wax at 60 °C overnight, and embedded afterward. Histological sections (7  $\mu\text{m}$ ) were cut with a Reichert 2040 microtome and stained with Weighert's hematoxylin-eosin (HE) and Periodic Acid-Schiff (PAS) reagent. With PAS reagent, we aimed to detect possible polysaccharides in tissues and on the integumental surface. The histological sections were studied and photographed with a Leica DM750 microscope.

The surface tergal ornaments were examined with a Hitachi S-2600N scanning electron microscope (SEM). For SEM we used alcohol preserved (70% ethanol) intermolt adult males and females from each studied species. The samples were dehydrated through an ascending series of ethanol (50% – 1 h, 70% – overnight, 80%,  $2 \times 90\%$ ,  $2 \times 96\%$ ,  $2 \times 100\%$  – 1 h) and were air dried (Schmalfuss 1978). Specimens were attached to aluminum holders. Coating with gold-palladium was with a XC7620 Mini Sputter Coater.

## Statistical analysis

To quantify the thickness of the tergites, 100 measurements were taken for each species using light microscope (LM) cross-sections (2 specimens, 5 slides, 10 measurements/slide; Image J software) (Csonka et al. 2013). In the case of *A. vulgare* different sets of specimens (N=2 for each) were tested for the inter- and intrageneric comparisons. We performed a one-way ANOVA followed by a post-hoc Tukey-test to compare cuticle thickness and water loss rates using R 3.2.3 software ('Rcmdr' Package). The assumptions of ANOVA were tested.

The relationship between mass-specific water loss, initial weight, and cuticle thickness was tested by Pearson correlation analysis (R 3.2.3 software). We analyzed the relationship between mass-specific water loss and the thickness of the epi- and procuticle separately. We used one-way ANOVA to analyze whether the variation in desiccation resistance as a function of cuticle thickness differs in the inter- and intrageneric groups (R 3.2.3 software). The comparison of the two groups was made based on the F value of the individual experiment's ANOVA tests. An alpha value of  $p = 0.01$  was used throughout.

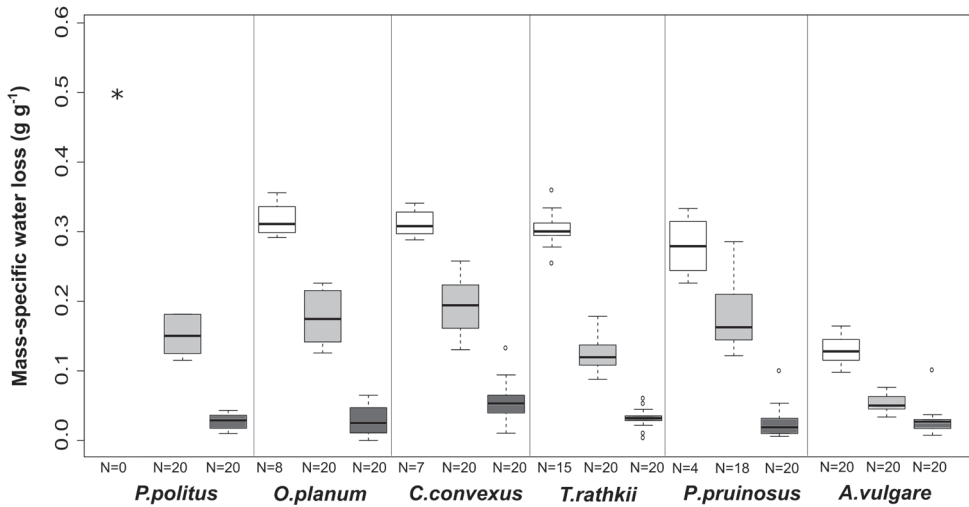
## Results

### Desiccation resistance

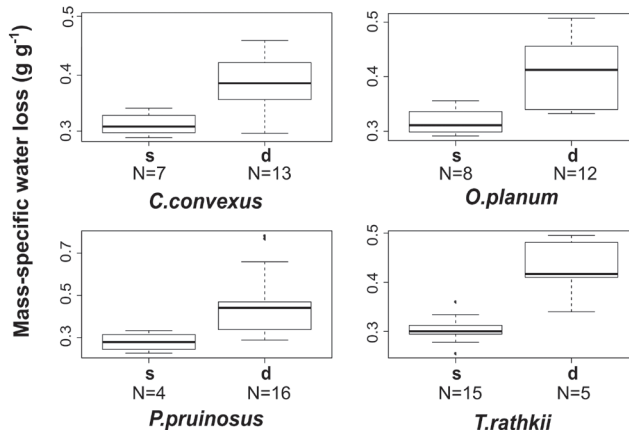
Under extreme dry conditions (RH ~30%) we found high mass-specific water loss at each investigated species (Figure 1). The specimens which died during the experiment lost significantly more water than the surviving ones (Figure 2). *Protracheoniscus politus* had the highest and *A. vulgare* had the lowest water loss ( $P. politus > O. planum > C. convexus > T. rathkii > P. pruinusos > A. vulgare$ ). The ANOVA showed that *A. vulgare* lost significantly less water than the other species ( $p < 0.001$ ; F value: 30.72). Mortality appeared in each species group except *A. vulgare* (*P. politus* – 100%, *P. pruinusos* – 80%, *C. convexus* – 65%, *O. planum* – 60%, *T. rathkii* – 25%).

At 75% relative humidity, the mass-specific water loss rates decreased in the order: *C. convexus > P. pruinusos > O. planum > P. politus > T. rathkii > A. vulgare* (Figure 1). Water loss differed significantly between *A. vulgare* and the other studied species groups ( $p < 0.001$ ; F value: 30.98). Besides that, *T. rathkii* lost significantly less water than *C. convexus*, *O. planum* and *P. pruinusos*. Mortality was seen only in *P. pruinusos* (10%).

We detected the lowest water loss (Figure 1) without significant interspecific differences at the highest humidity level (~100%) (*C. convexus > T. rathkii > P. politus > A. vulgare > O. planum > P. pruinusos*). The mass-specific water loss showed negative correlation with the initial weight (correlation coefficient (R): -0.2663) and the tergal cuticle thickness (R: -0.3067). The thicker epi- (R: -0.2866) and procuticle (R: -0.3261) decreased the mass-specific water loss.

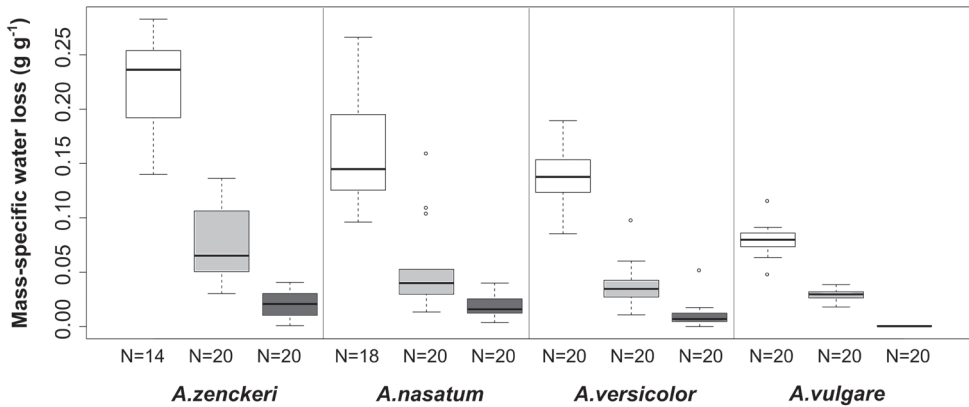


**Figure 1.** Mass-specific water loss of the survived individuals (\*all individuals died) at three different relative humidity values (white: ~30%, medium gray: ~75%, dark grey: ~100%). The experiment took 6 hours. [Measures: median  $\pm$  first quartile and max/min; species initials: *P.politus* – *Protracheoniscus politus*, *O.planum* – *Orthometopon planum*, *C.convexus* – *Cylisticus convexus*, *T.rathkii* – *Trachelipus rathkii*, *P.pruinosus* – *Porcellionides pruinus*, *A.vulgare* – *Armadillidium vulgare*].



**Figure 2.** Mass-specific water loss at ~30% relative humidity. s – surviving individuals, d – dead individuals. [Measures: median  $\pm$  first quartile and max/min (species names as in Figure 1)].

Within the genus *Armadillidium*, *A. vulgare* had the lowest and *A. zenckeri* had the highest water loss rates at 30% RH (Figure 3). Difference between *A. nasatum* and *A. versicolor* was not significant ( $p=0.16$ ; F value: 23.21). Mortality occurred in *A. zenckeri* (30%) and *A. nasatum* (10%).



**Figure 3.** Mass-specific water loss of the survived individuals at the three different relative humidity values (white: ~30%, medium gray: ~75%, dark grey: ~100%). The experiment took 6 hours. [Measures: median  $\pm$  first quartile and max/min; in species names *A.* means *Armadillidium*]

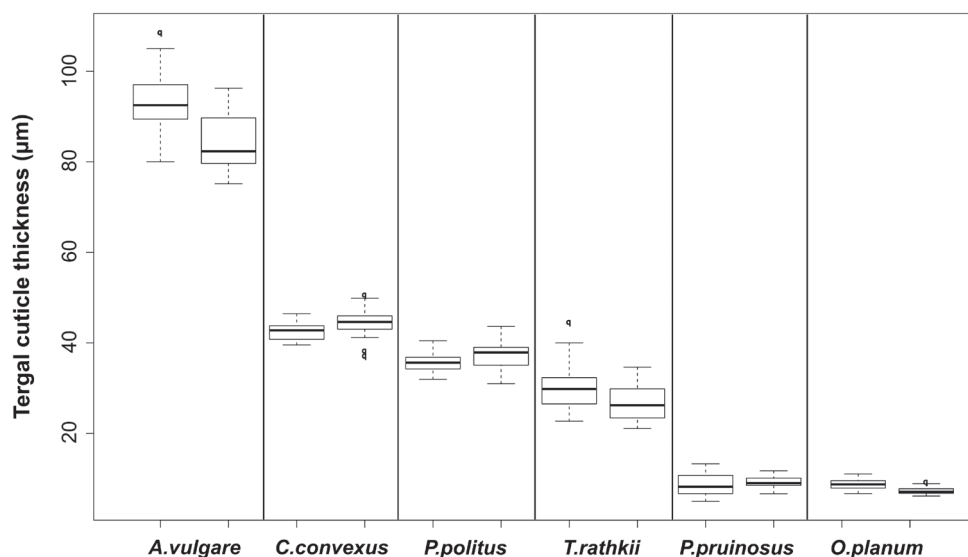
Under higher humidity (75%) the order of mass-specific water loss was the same (Figure 3). *Armadillidium zenckeri* lost significantly more water than *A. versicolor* and *A. vulgare* at this humidity ( $p < 0.001$ ; F value: 7.793). At nearly 100% RH, the water loss rates were the lowest (Figure 3) but the ranking was the same (*A. zenckeri* > *A. nasatum* > *A. versicolor* > *A. vulgare*). Similarly to the interspecific study, within the *Armadillidium* genus, that is body size (R: -0.1020) and thicker cuticle (R: -0.3228) negatively correlated with mass-specific water loss rates. The mass-specific water loss showed negative correlation with the thickness of epi- (R: -0.3001) and procuticle (R: -0.3461).

The comparison of the desiccation resistance of the intrageneric and intergeneric groups has resulted in a lower F value for the former group, which means that there is a smaller relative variance among this group in comparison to the intergeneric group.

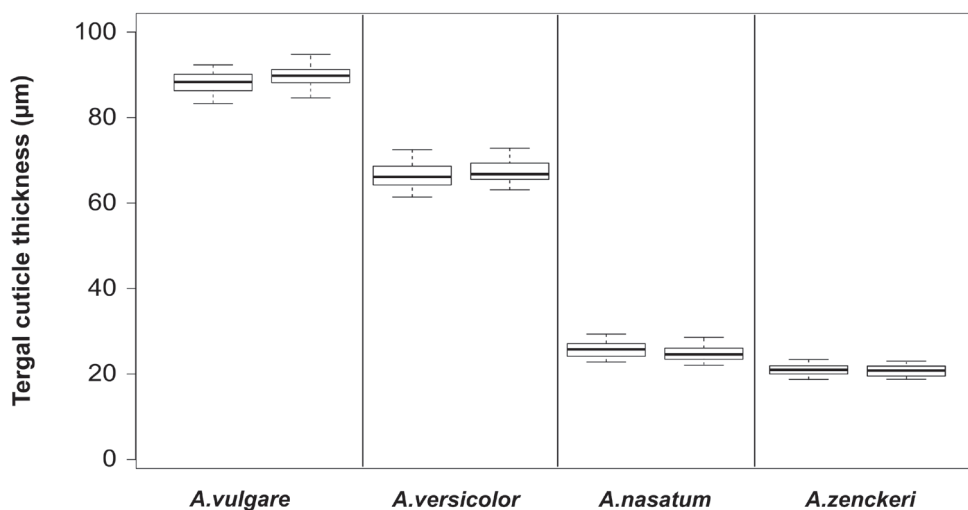
### Tergal cuticle thickness

Based on the average cuticle thickness the species can be sorted in decreasing order: *A. vulgare* > *C. convexus* > *P. politus* > *T. rathkii* > *P. pruinosus* > *O. planum* (Figure 4). Tergal cuticle thickness values were not significantly different between *P. pruinosus* and *O. planum* ( $p=1.000$ ). For all species, thickness values were not significantly different intraspecifically (between the two specimens of the same species) ( $p > 0.59$ ), but they differed significantly among species, that is interspecifically ( $p < 0.001$ ).

Within the *Armadillidium* genus, *A. vulgare* had the thickest tergal cuticle, while *A. zenckeri* had the thinnest cuticle (Figure 5). Our analysis revealed no significant difference intraspecifically (between the two specimens of the same species) ( $p > 0.69$ ), but thickness differed significantly interspecifically ( $p < 0.001$ ).



**Figure 4.** The tergal cuticle thickness in intra-, and interspecific relations (2 specimens/species, 5 slides/specimen, 10 measurements/slide). [Measures: median  $\pm$  first quartile and max/min (species names as in Figure 1)].

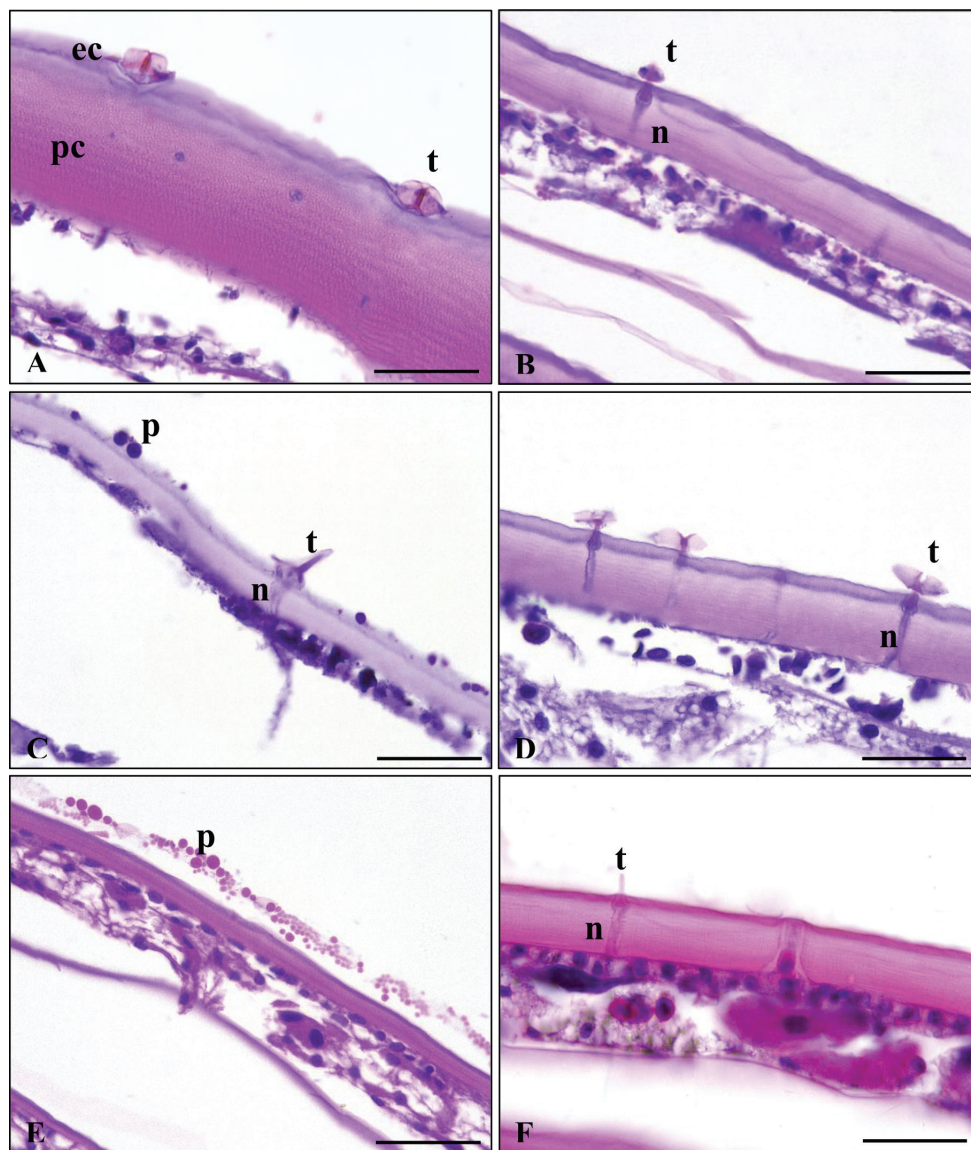


**Figure 5.** Intrageneric (between the two specimens per species) comparison of tergal cuticle thickness in four *Armadillidium* species (2 specimens/species, 5 slides/specimen, 10 measurements/slide). [Measures: median  $\pm$  first quartile and max/min (Species names as in Figure 3)].

### Tergal surface structures

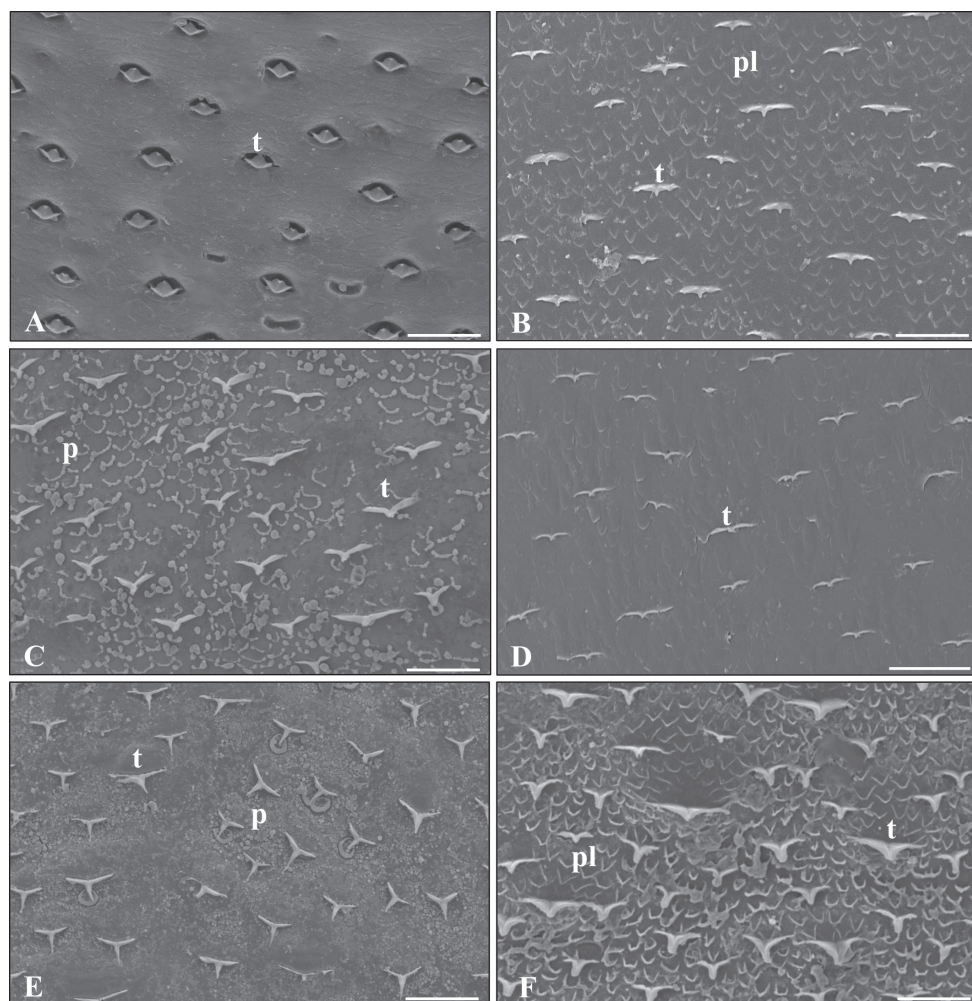
On the LM micrographs we found tricorn exteroceptors in connection with neural processes (Figure 6B, C, D, F). The SEM micrographs showed variously shaped





**Figure 6.** Light microscope micrographs of the studied species' tergites. *Armadillidium vulgare* (A), *Cylisticus convexus* (B), *Orthometopon planum* (C), *Protracheoniscus politus* (D), *Porcellionides pruinosus* (E), *Trachelipus rathkii* (F). Abbreviations: ec – epicuticle, pc – procuticle, p – polysaccharide spheres, t – tricorn receptor, n – nerve; x 63. Staining: hematoxylin-eosin (HE) – A, E, F; Periodic Acid-Schiff (PAS) – B, C, D. Scale bars: 50  $\mu$ m.

tricorns on the cuticular surface of each examined species (Figure 7). Closed, squat tricorns covered the surface of *A. vulgare* (Figures 7A, 8A) while *C. convexus*, *O. planum* and *P. politus* had elongated ones (Figure 7B–D). In the case of *O. planum*

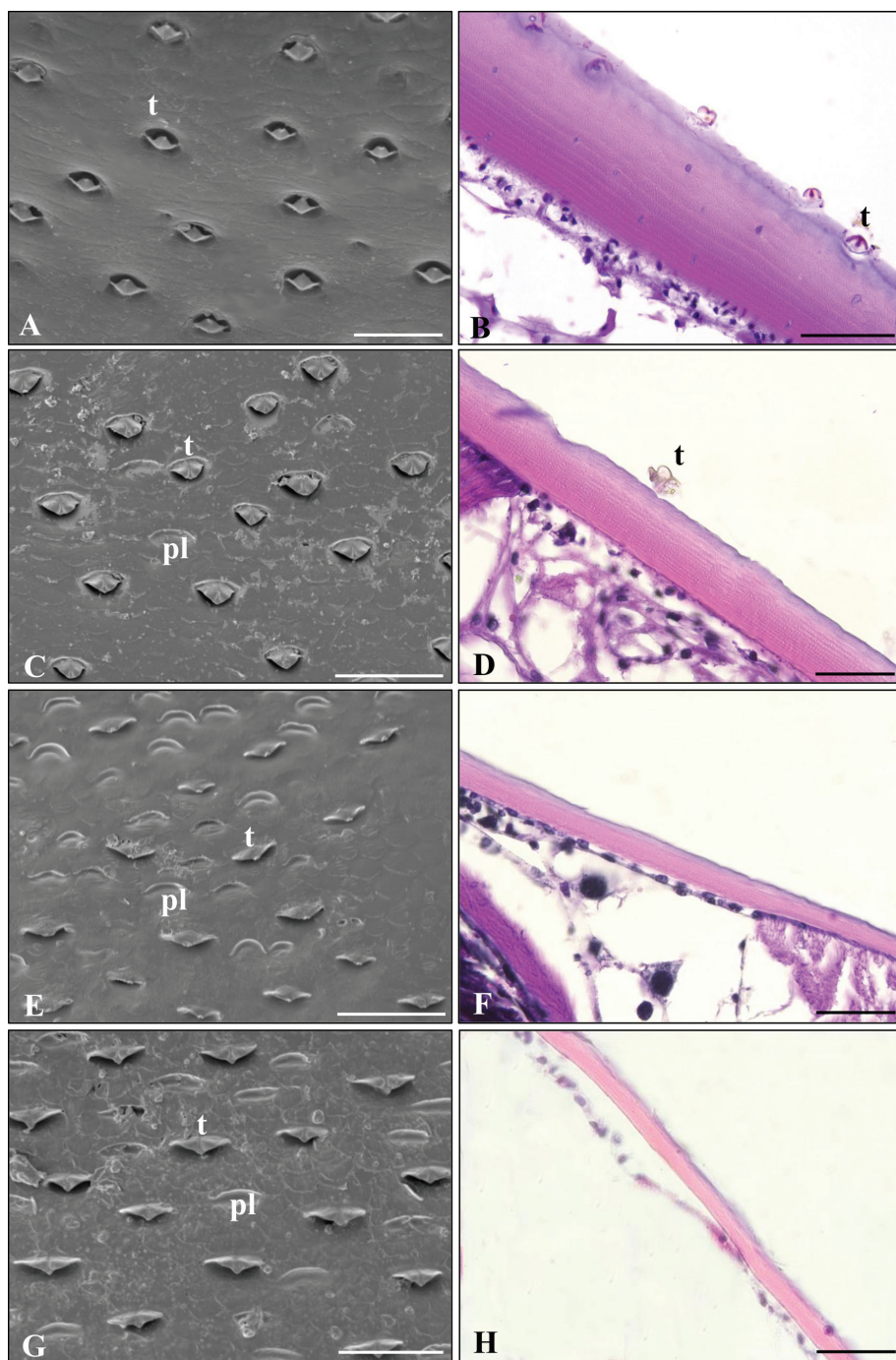


**Figure 7.** The dorsal surface of the studied sympatric terrestrial isopod species. *Armadillidium vulgare* (A), *Cylisticus convexus* (B), *Orthometopon planum* (C), *Protracheoniscus politus* (D), *Porcellionides pruinosus* (E), *Trachelipus rathkii* (F). Abbreviations: pl – plaques, p – polysaccharide spheres, t – tricorn receptor. Scale bars: 50  $\mu$ m.

and *P. pruinosus* PAS-positive spheres covered the tergites (Figs 6C, E, 7C, E). The exoskeleton of *C. convexus* and *T. rathkii* was densely covered by plaques (Figure 7B, F).

Within the *Armadillidium* genus the SEM micrograph showed interspecific differences. There were squat tricorns on the tergites of *A. versicolor* (Figure 8C), but these were not as closed as in *A. vulgare* (Figure 8A). The tergal cuticle surface of *A. zenckeri* and *A. versicolor* were covered by similar tricorns and plaques (Figure 8).





**Figure 8.** Scanning electron (A, C, E, G) and light microscope (B, D, F, H) micrographs on the studied *Armadillidium* species' tergites. *Armadillidium vulgare* (A, B), *A. versicolor* (C, D), *A. nasatum* (E, F), *A. zenckeri* (G, H). Abbreviations: pl – plaques, t – tricorn receptor. Staining: hematoxylin-eosin (HE) – B, D, F, H. Scale bars: 50 μm.

## Discussion

In the present study we compared intergeneric (*A. vulgare*, *C. convexus*, *O. planum*, *P. politus*, *P. pruinus*, *T. rathkii*) and intrageneric (*A. vulgare*, *A. versicolor*, *A. nasatum*, *A. zenckeri*) desiccation resistance of terrestrial isopods under three humidity ranges. As the applied experimental setup did not allow air to circulate, the calculated water loss might be underestimated. We assumed that differences in tolerance limits are connected to morphological characters such as tergal thickness and surface ornaments of the cuticle, which might be related to their habitat preferences.

Dias et al. (2012) showed that differences in body water loss rate serves as the main mechanism behind interspecific variation in mass-specific loss rate. Tsai et al. (1998) demonstrated on two *Ligia* species that intrageneric variation in desiccation resistance could be explained primarily by water loss rate, which is determined by body size. Body size affected desiccation resistance only indirectly, through changes in the water loss rate. Our results confirmed these previous findings: the larger initial weight decreased the mass-specific water loss rate at both intra- and intergeneric levels.

Several studies showed that body shape and cuticle permeability are also significant factors in water loss rate. Broly et al. (2015) studied the effect of surface area and mass on the desiccation rates in three different terrestrial isopod species: *Oniscus asellus*, *Porcellio scaber*, and *A. vulgare*. They found that the lower the individual surface area/volume ratio, the lower the individual water loss rate, both intra- and interspecific levels. According to their results body shape, rather than permeability, appears to explain the difference in mass-specific water loss rates between *A. vulgare* and *P. scaber*. In contrast, Warburg (1965) showed differences in permeability between isopod species with similar shape (*A. vulgare*, *Venezillo arizoniscus*).

In the present study individuals which died during the experiment lost more water than the surviving ones. Dias et al. (2012) observed that the water loss rate was nearly constant over time for all species both before and after the animals died. They suggested that water loss in terrestrial isopods is a passive process.

The cuticle of isopods is more permeable than that of the most terrestrial arthropods, and transpiration through the exoskeleton is a major part of water loss (Warburg 1993, Greenaway and Warburg 1998). Previous studies also suggested that the thickness of the exoskeleton was one of the morphological properties which determine the species' distribution at different spatial scales (Csonka et al. 2013, Vittori and Štrus 2014). The experimental results also supported our expectations that the relatively thick cuticle offered an effective protection to the globally wide-spread, habitat generalist *A. vulgare*. We found that thicker epi- and procuticle separately also decreased the mass-specific water loss. The epicuticle has been proposed as a possible barrier against water loss by other authors (Cloudsley-Thompson 1977, Compère 1990).

Despite the relatively thick tergal cuticle *P. politus* did not survive under extreme dry conditions. The survival of oniscideans in natural habitats is critically dependent not only on habitat but also on daily activity patterns (Cloudsley-Thompson 1956, Edney 1968). According to Ilosvay (1982) and Tuf and Jeřábková (2008) *P. politus* is active

mainly during twilight and early morning when humidity is higher, which minimizes desiccation. Activity peaks were at dusk and at midnight at a humidity of 75–80 %. In contrast, the common pill bug (*A. vulgare*) is active in the morning hours (Cloudsley-Thompson 1951), and very often also during the day (personal observations).

In the case of *O. planum* and *P. pruinosis* we found rather thin exoskeleton covered by polysaccharide spheres that might also reduce water loss. The composition and function of these structures is unknown. Hadley and Hendricks (1985) suggested that they were not composed of lipid as previously suggested (Ermin 1945, Schmalfuss 1978). According to Compère and Goffinet (1995) the glycoproteinaceous surface coat at *Carcinus maenas* (Crustacea, Malacostraca, Brachyura) might be a hydrophilic layer protecting the cuticulin layer and/or reducing the surface tension between the hydrophobic cuticulin layer and the water. Tricorn receptors are common on the tergal surface, but according to Holdich (1984) there was a difference in their form in relation to the habitat. His results suggested that closed tricorns, as on the surface of *A. vulgare*, occur only in species living in drier environments. Besides these receptors, in some species (*C. convexus*, *T. rathkii*) we found plaques on the dorsal surface. According to Holdich (1984) these structures may be involved in the formation of tricorns.

Previous studies showed that ancestral terrestrial isopod species had lower desiccation resistance. Dias et al. (2012) suggested that the resistance against desiccation in Oniscidea differs among major phylogenetic groups. In accordance with this, the F values of our ANOVA analysis indicate smaller relative variance among the group means in intrageneric desiccation resistance.

Nevertheless, the desiccation resistance could not be explained by only phylogenetic relationship. This is further supported in the present study by the different water loss rate of *Armadillidium* species under dry conditions. Desiccation resistance of the four investigated species is in accordance with their cuticle thickness and habitat preference.

## Conclusions

Resistance against desiccation in terrestrial isopod species was significantly associated with the two investigated morphological traits: body mass (size) and thickness of tergal cuticle. Species with the smallest mass-specific water loss rate were larger and possessed thicker tergal cuticle. Significant variation in both desiccation resistance and morphological traits was observed among the four *Armadillidium* species, despite their close phylogenetic relatedness.

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## Supplementary material I

### The non-significant results of the ANOVA tests

Authors: Diána Csonka, Katalin Halasy, Krisztina Buczkó, Elisabeth Hornung

Data type: (The non-significant results of the ANOVA test: Inter- and intrageneric comparisons of tergal cuticle thickness)

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# Presence of a stridulatory apparatus in the manca stages of isopods (Crustacea, Isopoda, Oniscidea)

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## Abstract

*Armadillo officinalis* Duméril, 1816 (Armadillidae) is a widespread terrestrial isopod species in the Mediterranean basin and on the western coasts of the Black Sea. The species is adapted to live in xeric environments and has mainly nocturnal habits. This species is capable of producing stridulations, which is nowadays recognized as a synapomorphy of the genus. In both sexes, these vibrations are produced by a line of scales on the propodus of pereopod 4 and 5. The main goals of this study are: to describe the manca stages of *Armadillo officinalis*; to detect the presence of the stridulatory apparatus in the manca stages; to evaluate the differences of such apparatus in the various manca stages. The manca stages (I, II, III) of *Armadillo officinalis* are described for the first time showing: i, the shortest duration (known in literature) of the manca stage I (approximately 30 minutes); ii, the presence of a rudimental stridulatory organ that may be of great importance in terms of evolutionary aspects and adaptation to terrestrial life. Notes on the reproductive biology are also reported. Furthermore, some considerations on future perspectives for *A. officinalis* as a model species in biotremology are also discussed.

## Keywords

*Armadillo officinalis*, biotremology, crustaceans, manca stages, terrestrial isopods



## Introduction

*Armadillo officinalis* Duméril, 1816 is a species of terrestrial isopod (Crustacea, Isopoda, Oniscidea) belonging to the family Armadillidae. The genus *Armadillo* Latreille, 1802 is restricted to the Mediterranean basin and western Asia (Schmalfuss 1996, 2003). As defined by Schmalfuss (1996), the species of this genus bear, in both sexes, a line of scales on the propodus of the fourth and fifth pereopod, used for stridulation. This stridulatory apparatus was first observed by Verhoeff (1908) on specimens of *A. officinalis*, but only 70 years later it was described by Caruso and Costa (1976). In addition to this line of scales, the *plectrum*, there are also areas, the so-called *pars stridens*, located on the internal surface of the pereon epimera, consisting of several slightly rounded ridges (approx. 70–80 µm long) (Caruso and Costa 1976). The animal produces the sound when it is rolled up, rubbing the *plectrum* against the *pars stridens*. The structures described above have only been recorded in the Mediterranean species of *Armadillo sensu stricto* and certainly represent a synapomorphy of this genus (Schmalfuss 1996). The presence of a similar stridulatory organ was also reported by Taiti et al. (1998) in *Cubaris everesti* Vandel, 1973 (Armadillidae) from Nepal, and in two new still undescribed species in the same genus (S. Taiti pers. comm.). The above-mentioned studies do not report whether the stridulatory apparatus appears in the first stages of the post-embryonic development, or later in the juveniles, or if the character is present only in the adults.

In the last years, *A. officinalis* has been the object of several studies in different fields of ethology, ecology, and reproductive biology (Nair et al. 1989, Warburg and Cohen 1992, AlJetlawi and Nair 1994, Warburg 2012, 2013). This species occurs in xeric environments, with mainly nocturnal habits (Vandel 1962), and reproduces several times during its life (iteroparous species). Its reproductive period varies in different geographic areas: in France from June to August (Vandel 1962), in Sicily from May to July (Messina et al. 2011, 2012), and in Israel in October (Warburg 2013). *Armadillo officinalis* usually lives on several kinds of substrates such as sand, silty-clay substrates, and rocks, as well as in environments populated by different plant communities (Messina et al. 2014).

The feeding preferences and the duration of the stages and substages of the moult cycle of this species was recently studied in detail by Montesanto and Cividini (2017, 2018). Furthermore, a possible use of this species as bioindicator for the exposure to benzene was also examined (Agodi et al. 2015).

Although many of its biological features are well known, the post marsupial manca stages have never been described. The aims of this study are: to describe the manca stages of *Armadillo officinalis*; to detect the presence of the stridulatory apparatus in the manca stages and to describe it in these different stages.

## Materials and methods

With the aid of forceps, numerous specimens of *Armadillo officinalis* were collected, under the stones of Catania University campus, eastern Sicily (DMS: 37°31'39"N 15°04'20"E);

they were then bred in Pisa (western Tuscany), in a climate room at 20°C, with a natural photoperiod. Ovigerous females were separated from the main livestock and bred separately. Each ovigerous female was kept in a periodically moistened Petri dish (Falcon® 351029, 100×15mm, with plaster of Paris substrate), and fed with slices of potatoes and plane-tree leaves (as in Montesanto and Cividini 2017).

Once the mancas were released from the marsupium they were counted, separated from the female, and raised individually in Petri dishes. The ovigerous females were daily monitored, in order to record the time of manca release. In the same way the post-marsupial mancas were observed every day, so that the moulting process and the time of each manca stage could be monitored and recorded. Twenty-five ovigerous females were dissected in order to study the first manca stage in its intramarsupial development.

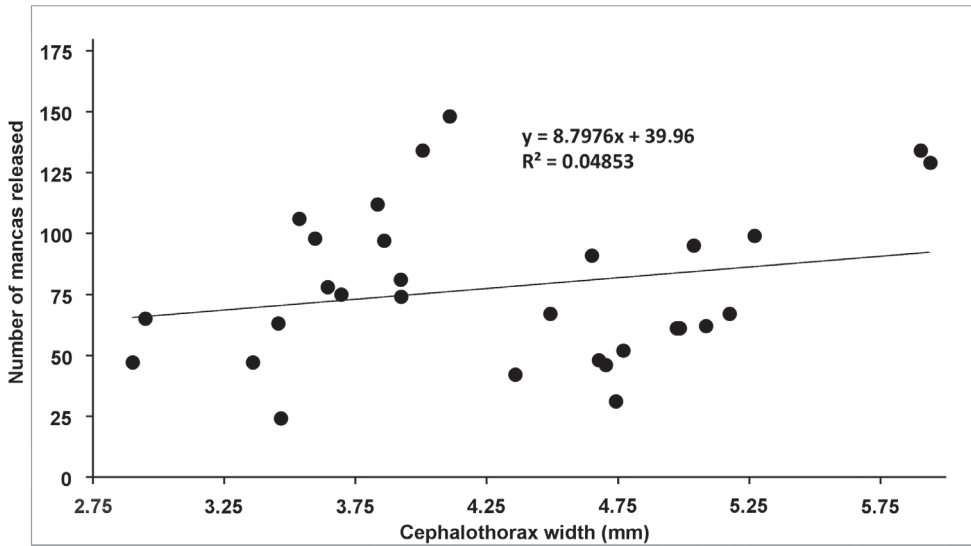
Throughout postmarsupial development, twenty individuals, representing each postmarsupial manca stage, were fixed in 70% ethanol for a later analysis. The manca stages were described in accordance with the previously described procedures (Araujo et al. 2004, Brum and Araujo 2007, Sokolowicz et al. 2008, Montesanto et al. 2012), including the appendages morphological study. The parts were mounted on slides, and then pencil drawings were made using a Carl Zeiss Standard 14 microscope equipped with a *camera lucida* (drawing tube). Final illustrations were prepared using the software GIMP ver. 2.8.14 as in Montesanto (2015, 2016). For the SEM (scanning electron microscope), some samples were dried out at room temperature and then covered with a golden film (Edwards Sputter Coater S150B). They were then observed using a Jeol JSM-5410 with a tension of 15 kV; digital images were taken with the Jeol SemAfore system.

## Results

In order to evaluate the number of mancas released from the marsupium, thirty-two ovigerous females of *Armadillo officinalis* were considered. Fig. 1 reports the number of mancas released in relation to the female size (cephalothorax width); the values ranged between 25 and 150 mancas.

*Armadillo officinalis* showed three postmarsupial stages (called manca stages M I, M II, and M III), separated by ecdysis and generally characterized by the absence of the first pleopods and non-functional seventh pereopods. The following three main characteristics can be used to distinguish each stage: the length of the antennal flagellum articles, the number of ommatidia, the developmental level of the pereopod 7, the presence of the epimera of pereonite 7 (Fig. 2).

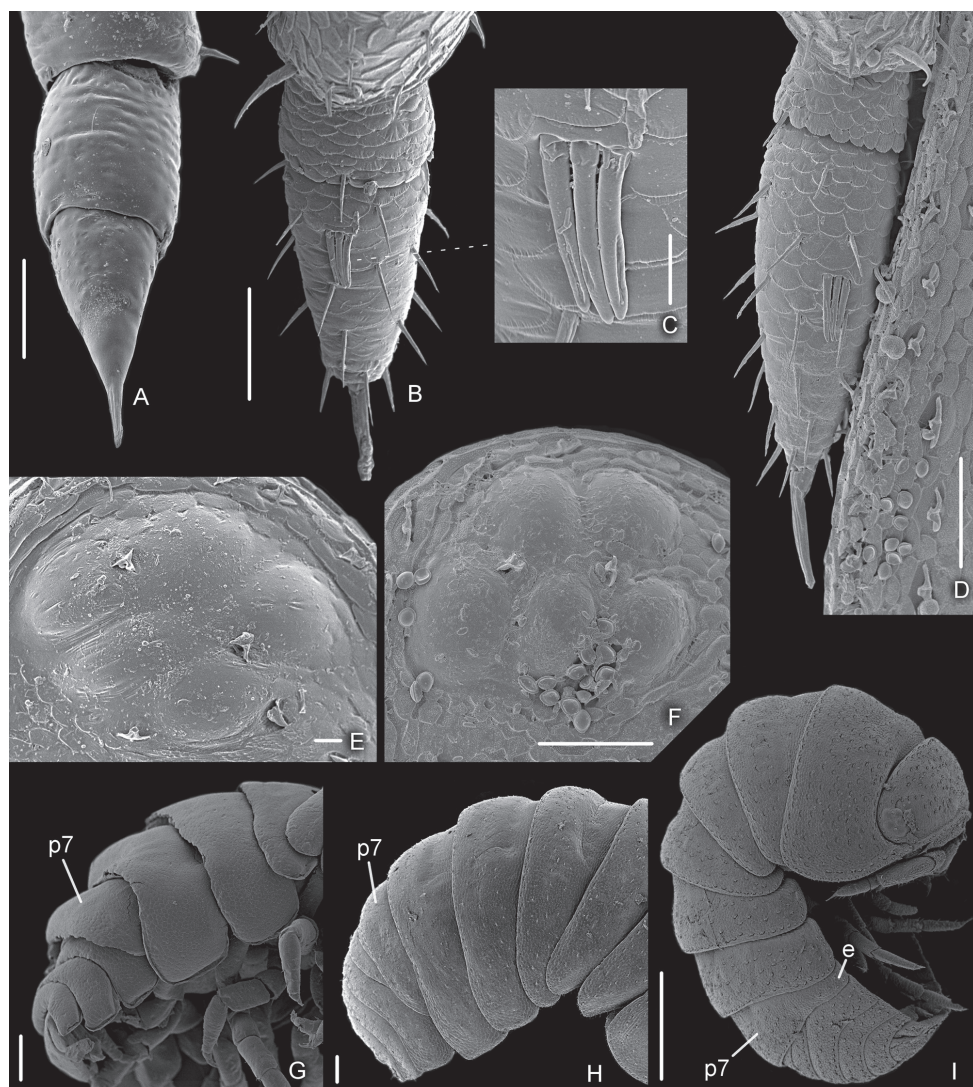
*Manca stage M I.* The duration of this stage varied from a minimum of 21 to a maximum of 43 min, with a mean value of 28 min. The mean body length was 1.76 mm (SD:  $\pm 0.10$ ), with a range from 1.69 to 1.89 mm. The mean cephalothorax width was 0.40 mm (SD:  $\pm 0.07$ ), with a minimum of 0.39 mm and a maximum of 0.46 mm. All the mancas of the first larval stage emerge from the marsupium during the anterior ecdysis process (number of females observed: N = 16) or during the posterior ecdysis



**Figure 1.** Correlation between cephalothorax widths of the ovigerous females with the number of mancas released.

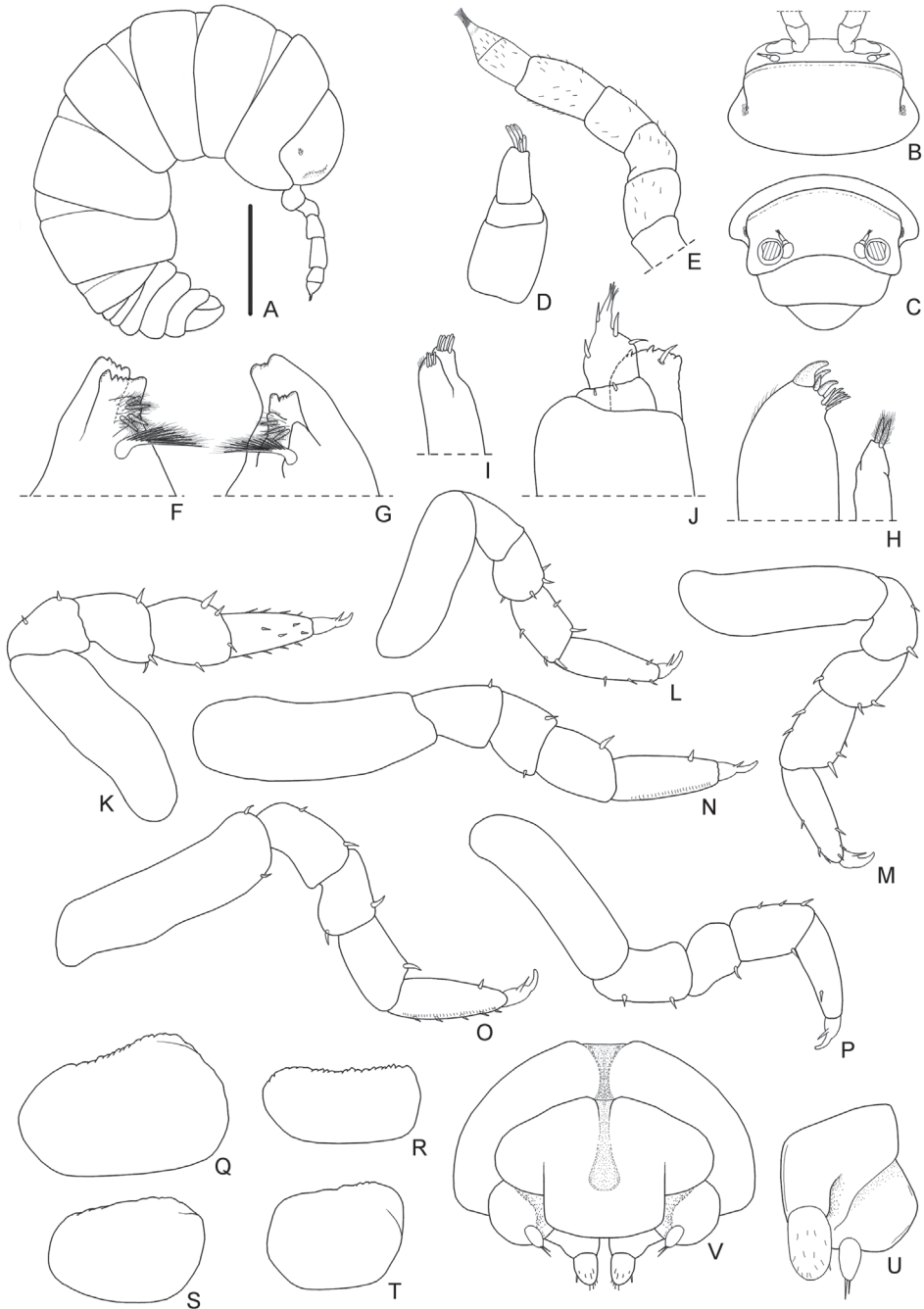
(N = 4). No females were observed while giving birth of mancas M I during premoult or intermoult stages. During the ecdysis the mancas remain under their mother's body and quickly eat both the posterior and anterior exuviae. These mancas had no pigmentation, except for the ommatidia and little brown spots on the pereonite margins; the calcification of the cuticle seems to be incomplete. Because of their body transparency it was possible to observe the exuviae inside the gut. Dorsal surface without scale-setae (see also Fig. 2G). Eyes with 4-5 small pigmented spots, visible under the cuticle and with no external typical structures of ommatidia (Fig. 3A–C). Pereonite 7 incomplete and with no epimera (Figs 2G, 3A). Cephalothorax (Fig. 3B, C) with incomplete frontal line. Antennula (Fig. 3D) of three articles and five apical aesthetascs. Antennal flagellum (Figs 2A, 3E) as long as the fifth article of peduncle, bi-articulated and with the proximal article slightly longer than the apical; no aesthetascs on the flagellum. Mandibles (Fig. 3F, G) with molar penicil semidichotomized, 2+2 free penicils on the left and 1+2 on the right mandible. Maxillula (Fig. 3H) with 4+6 teeth, endite with two long penicils. Maxilla (Fig. 3I) with few setae on the apical part of the lateral and medial lobes. Maxilliped (Fig. 3J) palp with apical setal tuft, and two setae in the basal article; endite with three teeth and one apical seta. Pereopods 1–6 (Fig. 3K–P) with few setae; pereopods 4 and 5 propodus with a line of scales. Pereopods 7 and pleopods 1 absent. Pleopod 2–5 exopods (Fig. 3Q–T) without setae on the margins. Uropods and telson as in Fig. 3U, V.

*Stridulatory apparatus of Manca stage M I.* Presence of a line of 28–30 scales (*plectrum*) of approx. 100  $\mu$ m on sternal margin of pereopod 4 and 5 propodus (Fig. 4A–C). Scales of triangular shape (Fig. 4D). No particular modifications of the ventral side of the pereonite 4–6 epimera, no evidence of a specific *pars stridens*.



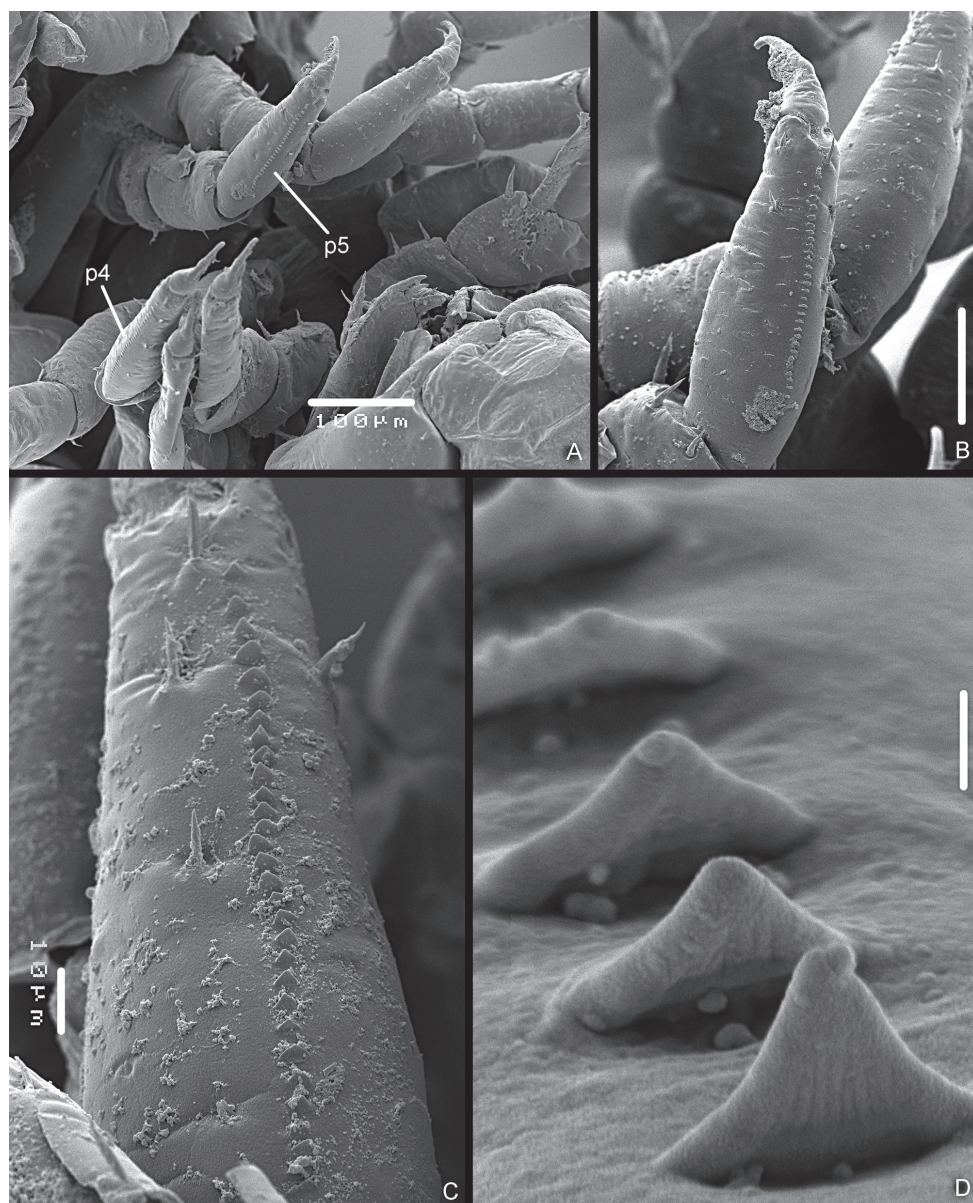
**Figure 2.** *Armadillo officinalis* Duméril, 1816. **A** manca stage M I, antennal flagellum **B** manca stage M II, antennal flagellum **C** manca stage M II, aesthetascs on the second article of antennal flagellum **D** manca stage M III, antennal flagellum **E** manca stage M II, eye **F** manca stage M III, eye **G** manca stage M I, p7: pereonite 7 **H** manca stage M II, p7: pereonite 7 **I** manca stage M III, p7: pereonite 7, e: epimeron. Scale bars: 10  $\mu\text{m}$  (**C**, **E**); 50  $\mu\text{m}$  (**A**, **B**, **D**, **F**); 100  $\mu\text{m}$  (**G**, **H**); 500  $\mu\text{m}$  (**I**).

*Manca stage M II.* The duration of this stage varied from a minimum of six to a maximum of seven days, with a mean value of 6.5 days. The mean body length was 2.01 mm (SD:  $\pm 0.04$ ), with a range from 1.92 to 2.11 mm. The mean cephalothorax width was 0.43 mm (SD:  $\pm 0.06$ ), with a minimum of 0.42 mm and a maximum of 0.45 mm. These larvae showed pigmentation on the cephalothorax and the posterior



**Figure 3.** *Armadillo officinalis* Duméril, 1816. Manca stage M I. **A** body, lateral view (scale bar 0.5 mm) **B** cephalothorax, dorsal view **C** cephalothorax, frontal view **D** antennula **E** antenna **F** left mandible **G** right mandible **H** maxillula **I** maxilla **J** maxilliped **K** pereopod 1 **L** pereopod 2 **M** pereopod 3 **N** pereopod 4 **O** pereopod 5 **P** pereopod 6 **Q** pleopod 2 exopod **R** pleopod 3 exopod **S** pleopod 4 exopod **T** pleopod 5 exopod **U** left uropod **V** telson.





**Figure 4.** *Armadillo officinalis* Duméril, 1816. Manca stage M I. **A** pereopod 4 (p4) and pereopod 5 (p5) showing the line of scales on the propodus **B** pereopod 5 propodus, sternal view **C** the line of scales on the pereopod 4 propodus **D** scales of the plectrum on the pereopod 5 propodus. Scale bars: 1 µm (**D**); 10 µm (**C**); 50 µm (**B**); 100 µm (**A**).

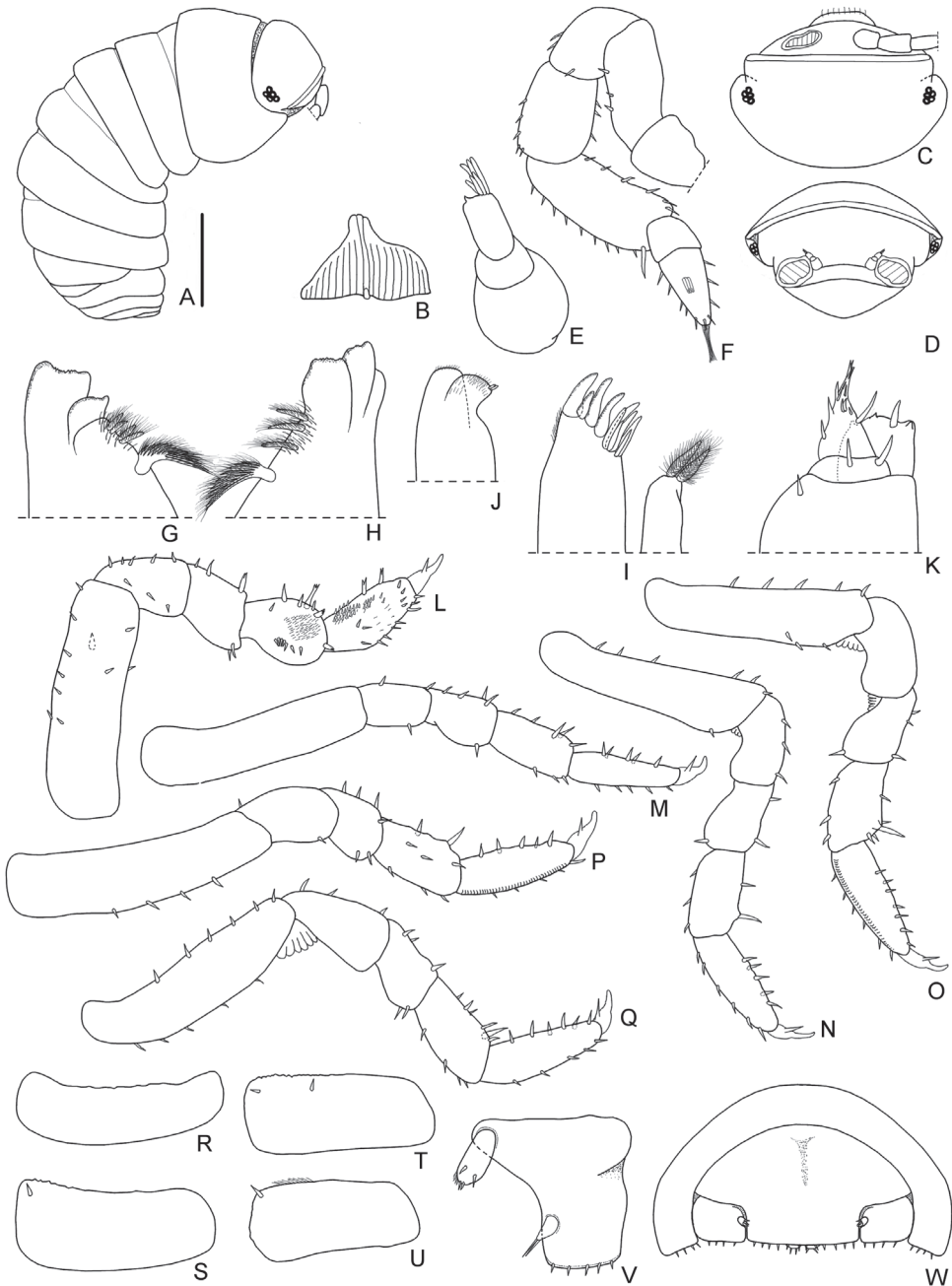
margins of pereonites and pleonites; the calcification of the cuticle seemed to be complete after the previous ecdysis. Even if the body was not completely transparent, a food presence could be observed in the gut as a dark area. At this stage, the mancas left the mother in search of food. Presence of dorsal scale-setae on the body surface (see also



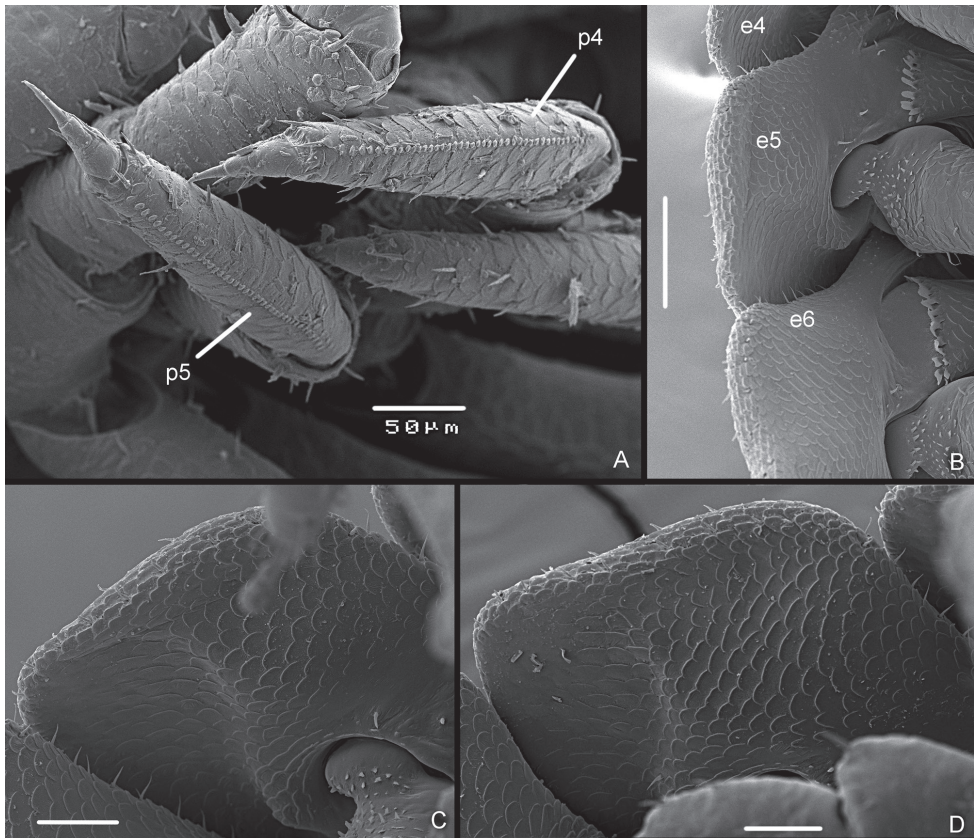
Figs 2H, 5B). Eyes with external typical structures of 4–5 ommatidia (Figs 2E, 5A–D). Pereonite 7 still incomplete and with no epimera (Figs 2H, 5A). Cephalothorax (Fig. 5C, D) with a complete frontal line. Antennula (Fig. 5E) of three articles and six apical aesthetascs. Antennal flagellum (Figs 2B, 5F) slightly shorter than the fifth article of peduncle, bi-articulated and with the proximal article shorter than the apical; three aesthetascs on the second article (Figs 2C, 5F). Mandibles (Fig. 5G, H) with molar penicil semidichotomized, 2+2 free penicils on the left and 1+2 on the right mandible. Maxillula (Fig. 5I) with 4+6 teeth, endite with two long apical penicils. Maxilla (Fig. 5J) with few setae on the apical part of the lateral and medial lobes. Maxilliped (Fig. 5K) palp with apical setal tuft, and two setae in the basal article; the endite had three teeth and one apical seta. Pereopods 1–6 (Fig. 5L–Q) with no particular modification but with more setae, in comparison with the previous stage; pereopods 1 with a hairy area on carpus, pereopods 4 and 5 propodus with a line of scales. Pereopods 7 is present although not completely developed. Pleopods 1 absent. Pleopod 2–5 exopods (Fig. 5R–U) with few setae on margins. Uropod and telson as in Fig. 5V, W.

*Stridulatory apparatus of Manca stage M II.* The presence of a line of 38–40 scales (*plectrum*) of approx. 165  $\mu\text{m}$  on sternal margin of pereopod 4 and 5 propodus could be observed (Fig. 6A, C). Scales had a circular shape. No particular modifications of the ventral side of the pereonite 4–6 epimera (Fig. 6B–D), no evidence of the presence of a specific *pars stridens*.

*Manca stage M III.* The duration of this stage varied from a minimum of 20 to a maximum of 24 days, with a mean value of 22 days. The mean body length was 2.35 mm (SD:  $\pm 0.09$ ), with a range from 2.16 to 2.60 mm. The mean cephalothorax width was 0.56 mm (SD:  $\pm 0.08$ ), with a minimum of 0.51 mm and a maximum of 0.59 mm. These larval stage showed more pigmentation than in the previous stage, on the cephalothorax, pereonites, and pleonites; the calcification of the cuticle is complete after the previous ecdysis. Higher presence of dorsal scale-setae on the body surface (see also Fig. 2I). Eyes with external typical structures of 5 ommatidia (Figs 2F, 7A–C). Pereonite 7 completely developed with the presence of epimera (Figs 2I, 7A). Cephalothorax (Fig. 7B, C) with a complete frontal line. Antennula (Fig. 7D) with 3 articles and 7 apical aesthetascs. Antennal flagellum (Figs 2D, 7E) shorter than fifth article of peduncle, bi-articulated and with the proximal article shorter than the apical; 3 aesthetascs on the second article (Figs 2D, 7E). Mandibles (Fig. 7F, G) with molar penicil semi-dichotomized, 2+2 free penicils on the left and 1+2 on the right mandible. Maxillula (Fig. 7H) with 4+6 teeth, endite with two long penicils and one apical seta. Maxilla (Fig. 7I) with few setae on the apical part of lateral and medial lobes. Maxilliped (Fig. 7J) palp with apical setal tuft, and two setae in the basal article; endite with three teeth and one apical seta. Pereopods 1–6 (Fig. 7K–P) with no particular modification but with more setae, if compared with the previous stage; pereopods 1 with a hairy area and tricorn setae on carpus, pereopods 4 and 5 propodus with a line of scales. Pereopods 7 completely developed, showing articles but folded on the pereonal sternites. Pleopods 1 not fully developed. Pleopod 2–5 exopods (Fig. 7Q–T) with few setae on the margins, tracheal fields present but not completely developed. Uropod and telson as in Fig. 7U, V.



**Figure 5.** *Armadillo officinalis* Duméril, 1816. Manca stage M II. **A** body, lateral view (scale bar 0.5 mm) **B** dorsal scale-seta **C** cephalothorax, dorsal view **D** cephalothorax, frontal view **E** antennula **F** antenna **G** left mandible **H** right mandible **I** maxillula **J** maxilla **K** maxilliped **L** pereopod 1 **M** pereopod 2 **N** pereopod 3 **O** pereopod 4 **P** pereopod 5 **Q** pereopod 6 **R** pleopod 2 exopod **S** pleopod 3 exopod **T** pleopod 4 exopod **U** pleopod 5 exopod **V** left uropod **W** telson.

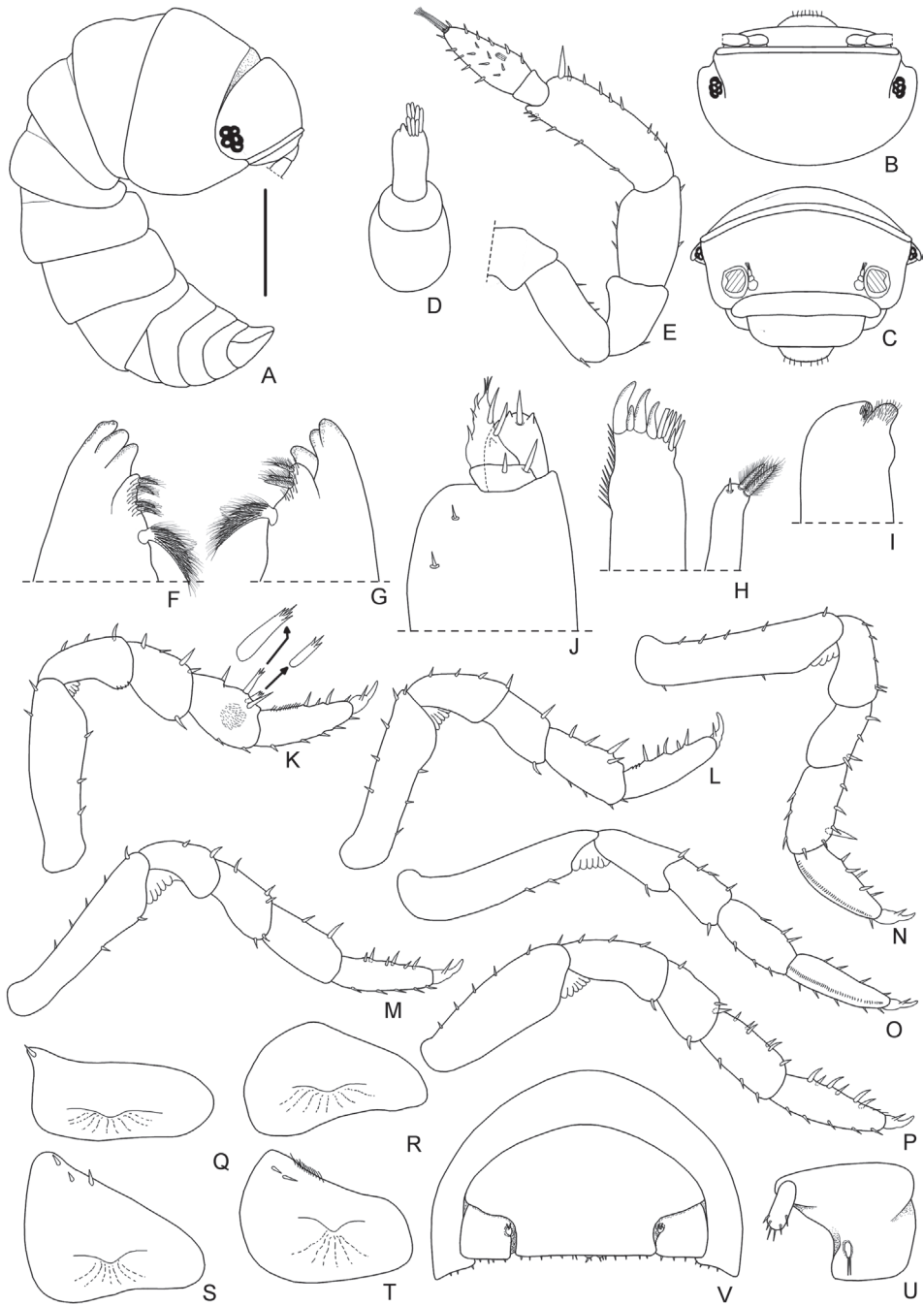


**Figure 6.** *Armadillo officinalis* Duméril, 1816. Manca stage M II. **A** pereopod 4 (p4) and pereopod 5 (p5) showing the line of scales on the propodus **B** epimera of pereonite 4 (e4), pereonite 5 (e5), and pereonite 6 (e6), ventral view **C** epimera of pereonite 5, ventral view **D** epimera of pereonite 6, ventral view. Scale bars: 50 µm (**A**, **C**, **D**); 100 µm (**B**).

*Stridulatory apparatus of Manca III stage.* Also at this stage it was possible to observe the presence of a line of approx. 40 scales (*plectrum*) of 160 µm on sternal margin of pereopod 4 and 5 propodus (Fig. 8A, B). Scales with a circular shape (Fig. 8B). No particular modifications of the ventral side of the pereonite 4–6 epimera (Figs 8C), no evidence of a specific *pars stridens*.

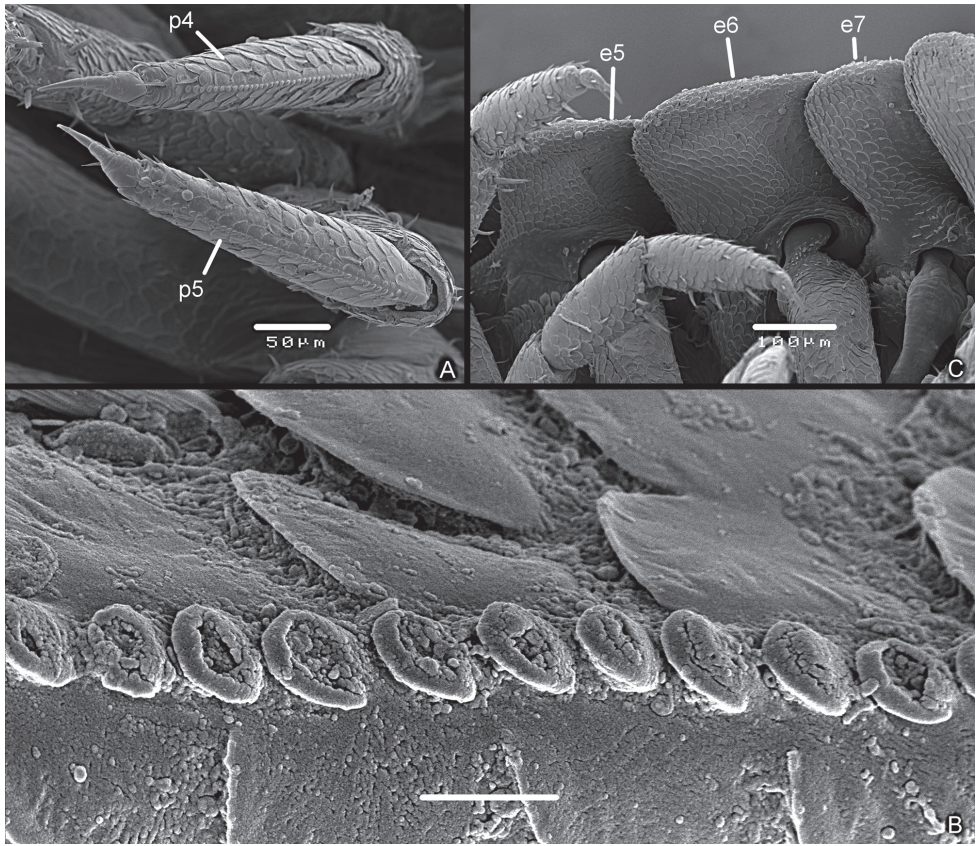
## Discussion

This detailed study of the morphology of the manca stages of *Armadillo officinalis* has highlighted their differences. The cephalothorax develops a complete frontal line, passing from M I to manca stage M II. In the antennula the number of apical aesthetascs varies from five on M I to seven on M III. Antennal flagellum changes the proportion of the two articles from M I to M II; the aesthetascs appear on the flagellum in M II



**Figure 7.** *Armadillo officinalis* Duméril, 1816. Manca stage M III. **A** body, lateral view (scale bar 0.5 mm) **B** cephalothorax, dorsal view **C** cephalothorax, frontal view **D** antennula **E** antenna **F** left mandible **G** right mandible **H** maxillula **I** maxilla **J** maxilliped **K** pereopod 1 **L** pereopod 2 **M** pereopod 3 **N** pereopod 4 **O** pereopod 5 **P** pereopod 6 **Q** pleopod 2 exopod **R** pleopod 3 exopod **S** pleopod 4 exopod **T** pleopod 5 exopod **U** left uropod **V** telson.





**Figure 8.** *Armadillo officinalis* Duméril, 1816. Manca stage M II. **A** pereopod 4 (p4) and pereopod 5 (p5) showing the line of scales on the propodus **B** scales of plectrum of pereopod 4 propodus **C** epimera of pereonite 5 (e5), pereonite 6 (e6), and pereonite 7 (e7), ventral view. Scale bars: 5 µm (**B**); 50 µm (**A**); 100 µm (**C**).

and remain the same in M III. Buccal appendages do not modify their main structure in the three manca stages. The number of setae in pereopods increased from M I to M III; pereopod 1 shows a well-defined hairy area on carpus from manca stage M II, pereopods 4 and 5 propodus show a line of scales in the three manca stages, but the number of the scales and their distance on the sternal margin of propodus varies distinctly; pereopods 7 are absent in the first manca stage, there are hints in the second stage, and they are fully developed but ventrally folded in the third manca stage. Pleopods 1 are absent in manca stages M I and M II, but appear in the third manca stage as a hint. Setae on pleopod 2–5 exopods appear in the manca stages M II and they do not considerably increase their number in the next stage. In the three manca stages, the uropod and telson change substantially in shape and proportion.

As for the comparison with other larval stages of terrestrial isopods, the mancass of *A. officinalis* show some significant differences. First of all, as reported for *Atlantoscia floridana* (Van Name, 1940) (Araujo et al. 2004), *Benthana cairensis* Sokolowicz, Araujo

& Boelter, 2008 (Sokolowicz and Araujo 2008), and *Porcellio siculoccidentalis* Viglianisi, Lombardo & Caruso, 1992 (Montesanto et al. 2012) the manca stage M I represents the last marsupial stage, when the mancas do not eat or moult. In the case here showed, the first manca stage begins its moulting process inside the marsupium. The duration of the manca stage is: 48 hours (Kacem-Lachkar 1997) in *Hemilepistus reaumurii* (Milne Edwards, 1840); 19 hours (Brum and Araujo 2007) in *Porcellio dilatatus* Brandt, 1831; 12 hours (Araujo et al. 2004) in *A. floridana*; approx. 6 hours (Zecchini and Montesanto in press), in *Armadillidium granulatum* Brandt, 1833; just one hour (Montesanto et al. 2012), in *P. siculoccidentalis*. This duration is even shorter in *A. officinalis*, just half of an hour outside the marsupium, which is the shortest duration among the postmarsupial mancas previously described. This could be considered as an adaptation to the xeric environment, where this species normally lives in the Mediterranean area.

The main body development follows the same rules of the already known oniscidean manca stages: the proportion inversion of the antennal flagellum articles, the development of the seventh pereonite, the appearance of pereopod 7 and pleopod 1. Other minor differences are in the number of ommatidia, and in the number of setae on the margins of pereopods and pleopods (Araujo et al. 2004, Brum and Araujo 2007, Milatović et al. 2010, Montesanto et al. 2012). The number of mancas released resulted similar to other data reported for *A. officinalis* from Libya (AlJetlawi and Nair 1994), as well as for some neotropical species, such as *A. floridana* (Araujo et al. 2004), *B. cairensis* (Sokolowicz et al. 2008), *Balloniscus glaber* (Araujo & Zardo, 1996), and *Balloniscus sellowii* (Brandt, 1833) (Quadros et al. 2008).

The present study has also shown the presence of the stridulatory apparatus, even in *A. officinalis* early stages of development, which is an absolutely new issue for the taxon Oniscidea. The presence of similar apparatus in different taxa of Arthropoda (especially in Insecta) was widely known, even in larval or juvenile stages (Johnstone 1964). The structures such as those on the pereopods of *A. officinalis* are barely known, to the best of my knowledge, in the taxon Crustacea. The *plectrum* morphology could be well described in the larval stages of *A. officinalis*, but the presence of the *pars stridens* (*sensu* Caruso and Costa 1976) was not detected in the three larval stages. This may indicate that the stridulatory apparatus is not yet functional at these developmental stages; however, during the past four years I have never heard in the studied mancae the typical vibrational sound of the adults.

The presence of a such stridulatory apparatus definitely is a synapomorphic character of the genus *Armadillo*. The identification of the typical stridulatory structure of adults in the mancas, even in an early stage development, represents a relevant discovery. Its presence must be, without doubt, the result of a long evolutionary process. It leads to believe that this character, defining the genus *Armadillo* (*sensu* Schmalfuss 1996), might also be present in other genera (Taiti et al. 1998).

It still remains an open question the purpose of a stridulatory apparatus in larval stages, even in biological circumstances in which it could not be used (e.g. inside the maternal brood pouch). I have observed that adults of *A. officinalis* produce stridulation only when their body is rolled up in a ball: this should be a further step in an



hypothetic line of a defense strategy or aggregation phenomena. Further studies on these aspects are currently underway. The presence of stridulatory apparatus in larvae of other group of arthropods is well known. Grandi (1951) reported some cases of sound production in preimaginal stages of insects. For stridulation, beside the case of passalid larvae, it is noteworthy the behaviour of other beetle larvae exhibiting stridulatory organs on legs: geotrupid, lucanid, and hybosorid larvae (Palestrini et al. 1990) for instance. Among insects that produce sounds by beating or scrubbing an area of their body against surrounding surfaces, Grandi (1951) reported larvae of the lepidopteran genus *Chimabacche* Zeller, 1839 (gelechiid moths) and pupae of the lepidopteran genus *Eligma* Hübner, 1819 (noctuid moths). The stridulation is also common in *Hydropsyche* Pictet, 1834 larvae (Trichoptera) (see also Johnstone 1964). It could be hypothesized that acoustic recalls are audible by the co-specific larvae. So, it is possible that acoustic recalls of larvae that reached a favourable environment may be useful to stimulate other larvae to reach the same environment (Jansson and Vuoristo 1979).

Moreover, other fields of research investigate the possibility of *A. officinalis* to produce substrate-borne vibrations instead of air-borne sounds, as defined in Hill (2009). Recently, some novel aspects have been published, proving the existence of a significant association between alternating turn behaviour and substrate-borne vibrations in *A. officinalis* (Cividini and Montesanto 2018a, 2018b). It has been found that different species of terrestrial isopods, *A. officinalis* and *Armadillidium vulgare* (Latreille, 1804), show a different reactivity to substrate-borne vibrations, responding with a lower or higher number of turn alternations. This feature might also be involved in the use of substrate-borne vibrations as a means of communication, as in insects. For a better understanding of these so complex phenomena, further research is needed, and *A. officinalis* represents an excellent experimental model to investigate the behaviour of terrestrial isopods in a newly-named discipline known as biotremology (Hill and Wessel 2016, Cividini and Montesanto 2018c).

## Conclusions

Science already knows approximately 3,800 species of terrestrial isopod (Schmalfuss 2003), but larval stages were described only in very few species. Due to many difficulties and technical problems (e.g., related with small larval body size), the study of crustacean larval biology is limited, nevertheless, these fields of research have greatly contributed to improve the knowledge of the life-history of different taxa (ontogeny of species-specific traits), and may have an important and wide appeal in the reconstruction of the phylogeny of higher taxa (“Evo-Devo” perspective) (Anger 2006). A remarkable character that needs to be further studied is the duration of the three post-marsupial manca stages in terrestrial isopods. It highly ranges among the species, especially in the first manca stage, and this should be kept in high consideration in future studies. More data are surely needed, especially in different families aside from the common families Porcellionidae and Armadillidiidae.

With regard to the findings in the present study, the presence of a stridulatory apparatus in a terrestrial crustacean surely constitute an important issue on the terrestrialisation of this taxon (see also Hornung 2011) and may have future implications on the knowledge of the evolutionary biology of the family Armadillidae and the suborder Oniscidea. In the near future, it would be important to understand at what stage of the subsequent development of juveniles stages the stridulatory apparatus becomes functional. These studies are currently underway.

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