ZooKeys 1225: 1–6 (2025)
DOI: 10.3897/zookeys.1225.147808

Editorial

Looking back at the 12th International Symposium on Terrestrial Isopod Biology

Ivan Hadrián Tuf[1], Karel Tajovský[2], Stefano Taiti[3]

1 Department of Ecology and Environmental Sciences, Faculty of Science, Palacký University Olomouc, Šlechtitelů 27, 77900 Olomouc, Czech Republic
2 Biology Centre CAS, Institute of Soil Biology and Biogeochemistry, Na Sádkách 7, 37005 České Budějovice, Czech Republic
3 Research Institute on Terrestrial Ecosystems, National Research Council (IRET-CNR), Via Madonna del Piano 10, 50019 Sesto Fiorentino (Florence), Italy
Corresponding author: Ivan Hadrián Tuf (ivan.tuf@upol.cz)

It was Professor Warburg who originally suggested the idea of organizing a meeting of those specialists who, in various fields, were engaged in the study of terrestrial isopods (Oniscidea). Thanks to the efforts of Stephen L. Sutton and David M. Holdich, the first symposium devoted to this group of invertebrates was held in 1983 under the auspices of the Zoological Society of London. This symposium was followed by the publication of the first comprehensive collection of papers bringing together new knowledge about the biology, ecology, and behaviour of terrestrial isopods in one place. This initial impulse was subsequently followed by further meetings at irregular intervals, which gave rise to what is now a relatively long tradition of symposia dedicated to terrestrial isopods (Fig. 1). Proceedings based on the symposia were usually published in the year following the meetings, traditionally containing selected published papers. Their number varied as the rules for accepting manuscripts and the practice of publishing results in the wider scientific community changed (Fig. 2). At a certain point in time, the era of monographic proceedings came to an end, and scientists were increasingly expected to publish mainly in prestigious scientific journals. However, this was accompanied by changes in the funding of proceedings, with costs shifting from all participants to individual authors. Starting with the 8th symposium, it became a tradition that the proceedings were published as a special issue of the peer-reviewed open-access journal ZooKeys. We thank the editorial board of the journal for this long-standing favour and support.

A change in the behaviour of scientists was linked to the COVID-19 pandemic, which led to the 11th Symposium being held in Ghent only online. This option, excellently organised by the colleague of the Spinicornis (the Belgian Terrestrial Isopod Group), was used by 117 participants, an absolute record, but only 12 contributions (again a record at the time) were published in the subsequent proceeding volume.

The last ISTIB, the 12th in order, was held in Olomouc, the Czech Republic under the joint auspices of the Biology Centre CAS and Faculty of Science, Palacký University Olomouc (Fig. 3). A total of 30 participants from 11 countries attended the meeting (Fig. 4), which was conducted in a pleasantly intimate atmosphere. Altogether, 21 lectures were presented and 14 posters were displayed. The result of the symposium is published in a proceedings volume that contains nine papers on various aspects of the biology, ecology, taxonomy, and behaviour of terrestrial isopods.

Published: 5 February 2025

ZooBank: https://zoobank.org/E5387FD2-5525-4147-AECE-59EB3BE949E2

Citation: Tuf IH, Tajovský K, Taiti S (2025) Looking back at the 12th International Symposium on Terrestrial Isopod Biology. In: Tuf IH, Tajovský K, Taiti S (Eds) The Biology of Terrestrial Isopods, XII. ZooKeys 1225: 1–6. https://doi.org/10.3897/zookeys.1225.147808

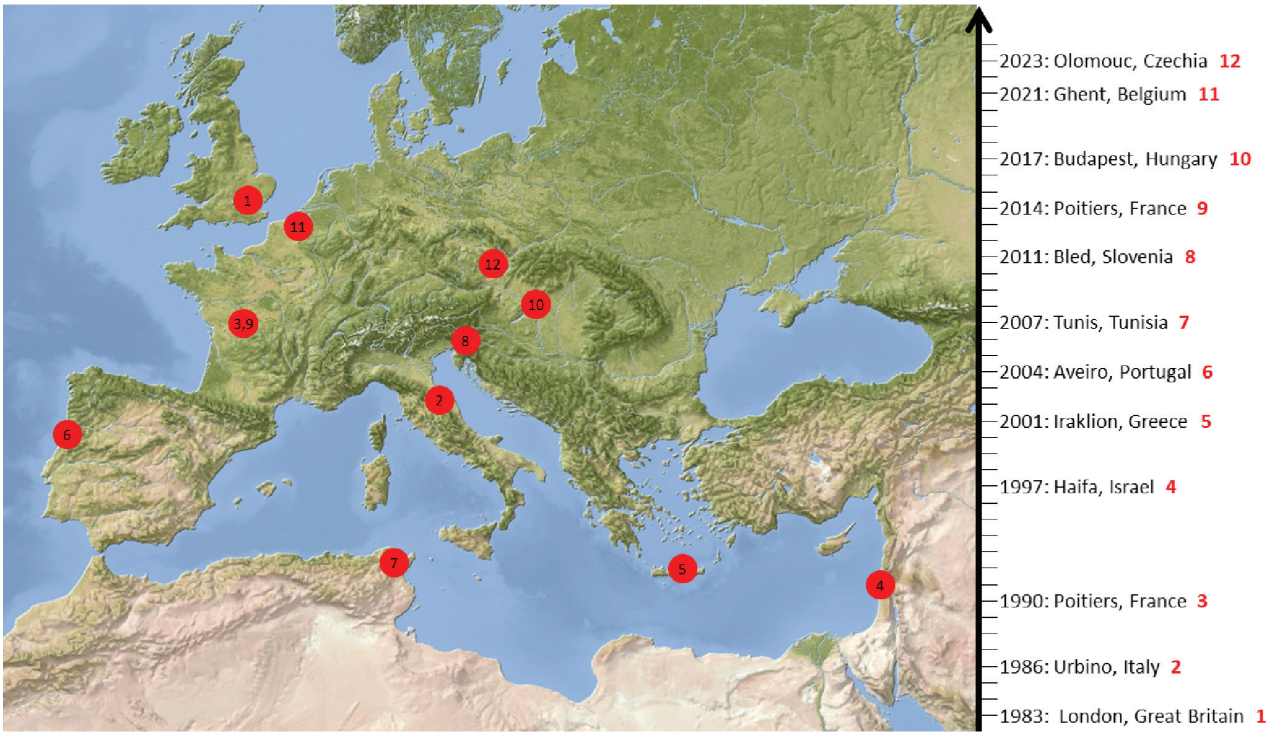

Figure 1. Spatio-temporal distribution of all symposia on terrestrial isopods. The red number following the year and place corresponds to the spots in the map.

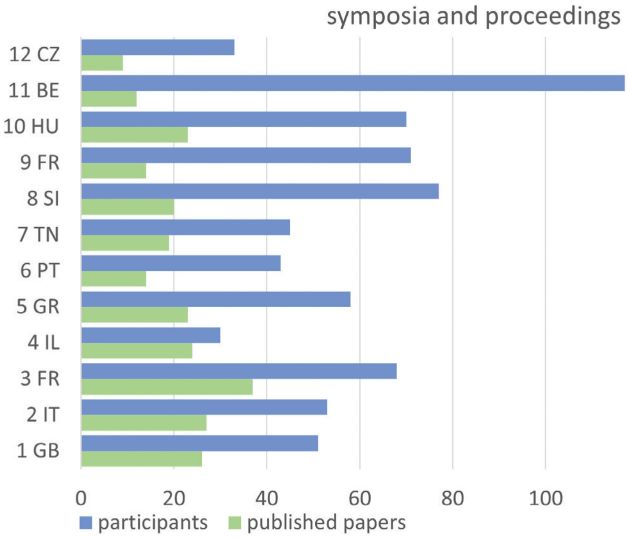

Figure 2. Symposia on terrestrial isopods in numbers. Numbers of participants and published papers in individual proceedings.

Research on the biology of terrestrial isopods is still ongoing as there are many gaps to fill in their taxonomy, phylogeny, physiology, ecology, and behaviour. Currently, more than 4,100 species are known worldwide, with an increase of approximately 500 species during the last 20 years. However, many regions, particularly in the tropics, remain poorly studied, and many new taxa in various museum collections are still awaiting description. We look forward to the next symposium to learn more about this fascinating and important group of soil arthropods, our favourite woodlice, also called sowbugs, pill bugs, slaters, roly polies, butchy boys, or potato bugs.

Proceedings published from the ISTIB symposia:

Sutton SL, Holdich DM (Eds) (1984) The Biology of Terrestrial Isopods. The Proceedings of a Symposium held at the Zoological Society of London on 7th and 8th of July 1983. (Symposia of the Zoological Society of London 53). Clarendon Press, Oxford, 518 pp.

Ferrara F, Argano R, Manicastri C, Schmalfuss H, Taiti S (Eds) (1989) Proceedings of the Second Symposium on the Biology of Terrestrial Isopods (Urbino, Italy, 10–12 September 1986). Monitore Zoologico Italiano, NS Monogr. 4, 512 pp.

Juchault P, Mocquard JP (Eds) (1991) Third Symposium on the Biology of Terrestrial Isopods, Poitiers, France, 10–12 July 1990. Université de Poitiers, France, 222 pp.

Hassall M, Hornung E, Warburg MR (Eds) (1998) Oniscidean Isopods. Proceedings of the 4th Symposium on the Biology of Terrestrial Isopods, Haifa. Israel Journal of Zoology 44: 1–250.

Sfenthourakis S, de Araujo PB, Hornung E, Schmalfuss H, Taiti S, Szlávecz K (Eds) (2003) The biology of terrestrial isopods, V. Oniscidea rolling into the new millennium: Proceedings of the 5th International Symposium on the Biology of Terrestrial Isopods, Irakleio (Iraklion), Crete, Greece, 19–23 May 2001. (Crustaceana Monographs 2). Brill Academic Publisher, Leiden, 386 pp.

Loureiro S, Soares AMVM, Araujo PB, Sfenthourakis S, Hornung E, Zimmer M, Schmalfuss H, Taiti S (Eds) (2005) The biology of terrestrial isopods, VI. Proceedings of the 6th International Symposium on the Biology of Terrestrial Isopods. European Journal of Soil Biology 41(3–4): 55–167.

Zimmer M, Charfi-Cheikhrouha F, Taiti S (Eds) (2008) Proceedings of the international symposium on terrestrial isopod biology: ISTIB-07. Shaker-Verlag, Aachen, 176 pp, 5 appendices.

Štrus J, Taiti S, Sfenthourakis S (Eds) (2012) Advances in Terrestrial Isopod Biology. ZooKeys 176: 1–296.

Taiti S, Hornung E, Štrus J, Bouchon D (Eds) (2015) Trends in Terrestrial Isopod Biology. ZooKeys 515: 1–206.

Hornung E, Taiti S, Szlavecz K (Eds) (2018) Isopods in a Changing World. ZooKeys 801: 1–518.

De Smedt P, Taiti S, Sfenthourakis S, Campos-Filho IS (Eds) (2022) Facets of terrestrial isopod biology. ZooKeys 1101: 1–212.

Tuf IH, Tajovský K, Taiti S (Eds) (2025) The Biology of Terrestrial Isopods, XII. ZooKeys 1225: 1–154.

Figure 3. Congress photo of the 12th International Symposium on Terrestrial Isopod Biology. The logo of this meeting, made by Eva Tajovská, depicted the species *Protracheoniscus politus* (C. Koch, 1841), one of the 18 taxa of terrestrial isopods described from the Czech Republic (photograph Ota Blahoušek).

Figure 4. Participants of the 12th ISTIB. 1 – Ivanklin Soares Campos-Filho; 2 – Urban Bogataj; 3 – Miloš Vittori; 4 – Pietro Gardini; 5 – Jessica Thomas Thorpe; 6 – Tina Petrišič; 7 – Ana Nagode; 8 – Petra Štern; 9 – Martin Martinka; 10 – Barbora Ďurajková; 11 – Karel Tajovský; 12 – Ivan Hadrián Tuf; 13 – Katalin Szlavecz; 14 – Spyros Sfenthourakis; 15 – Gert Arijs; 16 – Pepijn Boeraeve; 17 – Pallieter De Smedt; 18 – Stijn Segers; 19 – Faouzia Charfi-Cheikhrouha; 20 – Polona Mrak; 21 – Katja Kunčič; 22 – Mariana Nunes; 23 – Elisabeth Hornung; 24 – Jana Bedek; 25 – Karima Nasri Ammar; 26 – Stefano Taiti; 27 – Lamia Medini-Bouaziz; 28 – Nermine Laifi; 29 – Imane Benchana; 30 – Salsabil Abidi.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

Funding

No funding was reported.

Author contributions

Conceptualization IHT, KT, ST. Writing – original draft: IHT, KT, ST.

Author ORCIDs

Ivan Hadrián Tuf https://orcid.org/0000-0003-0250-0482
Karel Tajovský https://orcid.org/0000-0002-3619-3815
Stefano Taiti https://orcid.org/0000-0002-4909-6037

Data availability

All of the data that support the findings of this study are available in the main text.

ZooKeys 1225: 7–32 (2025)
DOI: 10.3897/zookeys.1225.116717

Research Article

Architecture of microtubule cytoskeleton in the hindgut cells of *Porcellio scaber*

Urban Bogataj[1], Polona Mrak[1], Jasna Štrus[1], Nada Žnidaršič[1]

[1] *University of Ljubljana, Biotechnical Faculty, Department of Biology, Večna pot 111, 1000 Ljubljana, Slovenia*
Corresponding author: Urban Bogataj (urban.bogataj@bf.uni-lj.si)

Abstract

The distribution and orientation of microtubules were investigated in cells of distinct shapes from different hindgut regions of adult *Porcellio scaber* Latreille, 1804 and during hindgut morphogenesis in late embryonic and early postembryonic development. All hindgut cells of adult *P. scaber* contain abundant apico-basal microtubules organized in extensive bundles, but the architecture of bundles is specific for distinct cells. In the anterior chamber the architecture of microtubule bundles closely coincides with different shapes of the cells in this hindgut region and are most prominent in hindgut cells associated with extensive muscles. The shape of cells that form the typhlosole and typhlosole channels is particularly complex. In the papillate region the microtubule bundles protrude between the infoldings of apical plasma membrane and the mitochondria are closely aligned along the microtubules, thus the microtubule bundles in the papillate region are likely involved in the stabilization of the apical labyrinth and positioning of mitochondria. During hindgut morphogenesis the apico-basal microtubule bundles are established relatively late, mainly during early postembryonic development. Morphogenesis of the typhlosole is characterized by coinciding changes in cell shape and microtubule arrangement.

Key words: Digestive system, hindgut, microtubules, morphogenesis, terrestrial isopods

Academic editor: Karel Tajovsky
Received: 1 December 2023
Accepted: 11 January 2024
Published: 5 February 2025

ZooBank: https://zoobank.org/7BBAF5F9-C644-4524-B568-CB72619CA1B1

Citation: Bogataj U, Mrak P, Štrus J, Žnidaršič N (2025) Architecture of microtubule cytoskeleton in the hindgut cells of *Porcellio scaber*. In: Tuf IH, Tajovský K, Taiti S (Eds) The Biology of Terrestrial Isopods, XII. ZooKeys 1225: 7–32. https://doi.org/10.3897/zookeys.1225.116717

Introduction

Extensive apico-basally oriented bundles of microtubules are a hallmark of the hindgut epithelium of terrestrial isopods (Witkus et al. 1969; Bogataj et al. 2018). The apico-basally oriented arrangement of microtubules is a general characteristic of epithelial cells (Mays et al. 1994; Bartolini and Gundersen 2006), however this general arrangement is modified in different epithelia (Toya and Takeichi 2016).

Arrangement of microtubules in epithelial cells and their reorganization during cell differentiation

Different epithelia in animal organisms provide a barrier between the external and internal environment. As such they are often exposed to mechanical strains, to which they cope using the interconnected system of cell junctions and cytoskeleton (Hagios et al. 1998). The rearrangement of this system is crucial also for the morphogenetic changes during the development of animal organisms (Schöck and Perrimon 2002). The microtubules are the stiffest among the main three cytoskeletal elements in eukaryotic cells and can form linear tracks that span the entire cells. Microtubules are polarized polymers and have complex assembly and disassembly dynamics. In non-polarized cells, the microtubules generally form radial arrays, with their minus ends positioned at the centrosome and their plus ends towards the cell periphery (Bornens 2002). The two best known functions of microtubules are that they form tracks for the directed intracellular transport and that they form the mitotic spindle during cell divisions for the segregation of chromosomes (Fletcher and Mullins 2010). Microtubules have also been proposed to influence cell shape and mechanics due to their abilities to resist the compressive forces (Brangwynne et al. 2006). During the differentiation of epithelial cells, the microtubules usually reorganize from the radial arrangement to the apico-basally polarized arrangement (Bré et al. 1987; Bacallao et al. 1989; Mimori-Kiyosue 2011). In polarized epithelial cells the majority of microtubules is apico-basally oriented, with their minus ends positioned in the apical cytosol and their plus ends in the basal cytosol. This arrangement is crucial for the establishment and maintenance of epithelial cell polarity and for the directed intracellular transport (Mays et al. 1994; Müsch 2004; Bartolini and Gundersen 2006).

The spatial arrangement of microtubules has been extensively investigated in mammalian epithelia, including intestinal cells, cochlear supporting cells of the inner ear and in various epithelial cells in culture. For arthropods, the most extensive data is available for the arrangement of microtubules in tracheal epithelial cells and pupal wing epithelial cells of *Drosophila* (Toya and Takeichi 2016; Muroyama and Lechler 2017). In other arthropods a lot of research has been conducted on extensive arrays of microtubules in tenocytes, which are specialized epidermal cells that serve as attachment sites of muscles to exoskeleton (Nakazawa et al. 1992; Tucker et al. 2004; Criel et al. 2005; Žnidaršič et al. 2012). Extensive arrays of microtubules have also been reported in various ectodermal epithelia with transportive functions, such as the gill epithelium of amphipods (Shires et al. 1994; Shires et al. 1995) and the hindgut epithelium of isopods (Witkus et al. 1969; Holdich and Ratcliffe 1970; Vernon et al. 1974; Wägele et al. 1981; Bogataj et al. 2018). Particularly in various mechanically burdened epithelial cells such as mammalian cochlear epithelial cells and tenocytes of arthropods the microtubules are often bundled and associated with different types of extensive cell junctions (Henderson et al. 1995; Tucker et al. 2004; Criel et al. 2005).

Structure and function of the hindgut of terrestrial isopods

The digestive system of terrestrial isopods is composed of ectodermal foregut and hindgut that together form the entire alimentary canal and blind ending digestive glands which are connected to the stomach and represent the only endodermal part of the digestive system (Vernon et al. 1974; Hassall and Jennings 1975; Bettica et al. 1987; Storch and Štrus 1989; Štrus et al. 2008). Exception are some amphibious species where a narrow ring of endodermal midgut cells is present between the ectodermal stomach and the hindgut (Štrus et al. 1995).

The foregut consists of a tubular esophagus and stomach. Morphologically, the stomach represents the most complex part of the digestive system where the food is triturated and filtrated to the digestive glands by the movement of individual parts of the stomach, including different cuticular masticatory structures and filters, driven by the extensive musculature that surrounds the stomach (Storch 1987; Hames and Hopkin 1989; Storch and Štrus 1989). The main part of nutrient absorption takes place in the blind ending tubules of digestive glands, which receive filtered material from the stomach filters (Hames and Hopkin 1989).

The coarse solid food particles are directed to the hindgut which is the longest part of the digestive tract and consists of the anterior chamber, papillate region and rectum. The anterior chamber is characterized by a longitudinal dorsal fold termed typhlosole forming two parallel typhlosole channels on each side (Hassall and Jennings 1975; Hames and Hopkin 1989). In the anterior chamber the food is digested by the digestive enzymes and the liquid digestion products are transported along the typhlosole channels anteriorly, back to the stomach for further filtration and absorption in the digestive glands (Hames and Hopkin 1989). In the papillate region the dome-shaped basal parts of epithelial cells bulge into the haemocoel between the muscular net surrounding the hindgut, giving this part of the hindgut papillate appearance (Hassall and Jennings 1975; Hames and Hopkin 1989; Štrus et al. 1995). According to its structural characteristics the papillate region is considered a site of ions and water absorption (Vernon et al. 1974; Coruzzi et al. 1982; Palackal et al. 1984), important in terrestrial isopods to prevent excessive water loss. The rectum is separated from the papillate region by a muscular sphincter. Rectal epithelium is extensively folded and involved in the compaction of dry fecal pellets (Hames and Hopkin 1989; Storch and Štrus 1989).

The hindgut epithelium is monolayered and apically lined by a thin chitinous cuticle (Vernon et al. 1974; Palackal et al. 1984; Storch and Štrus 1989; Mrak et al. 2015). The hindgut cells have extensively infolded apical and basal plasma membrane and numerous mitochondria associated with the apical and basal infoldings, to support the active transport processes across the hindgut epithelium. The apical plasma membrane is connected to the hindgut cuticle with apical junctions that structurally resemble similar junctions in tenocytes. On lateral plasma membranes extensive and convoluted septate junctions are present, providing an efficient paracellular diffusion barrier (Vernon et al. 1974; Coruzzi et al. 1982; Storch and Štrus 1989; Bogataj et al. 2018).

Microtubules in the hindgut cells of terrestrial isopods

Witkus et al. (1969) reported extensive apico-basally oriented bundles of microtubules concentrated in the lateral parts of hindgut epithelial cells in terrestrial isopod *Oniscus asellus* Linnaeus, 1758. They have proposed that these extensive bundles of microtubules are involved in several functions, including the structural support of extremely large hindgut cells. Similar apico-basally oriented bundles of microtubules in lateral parts of hindgut epithelial cells were later reported also in terrestrial isopods *Armadillidium vulgare* Latreille, 1804 and *Porcellio scaber* Latreille, 1804 and in marine isopods *Dynamene bidentata* Adams, 1800 and *Cyathura carinata* Krøyer, 1847 (Holdich and Ratcliffe 1970; Vernon et al. 1974; Wägele et al. 1981; Bogataj et al. 2018). Authors of studies performed on *A. vulgare* and *P. scaber* report that the bundles of microtubules in the hindgut cells associate with apical and basal plasma membrane infoldings (Vernon et al. 1974) and with junctions between the apical plasma membrane and cuticle (Bogataj et al. 2018).

Hindgut morphogenesis during development of *Porcellio scaber*

The embryonic and early postembryonic development of *Porcellio scaber* and other terrestrial isopods takes place in an osmotically regulated aqueous environment of marsupium, which is a fluid filled structure at the ventral side of a gravid female body (Surbida and Wright 2001). The eggs and developing embryos are enclosed in two embryonic envelopes: the outer chorion and the inner vitelline membrane (Wolff 2009; Milatovič et al. 2010; Mrak et al. 2012). According to Milatovič et al. (2010), the embryonic development of *P. scaber* lasts approximately 25 days and is divided into 20 developmental stages. Stages S1–S5 comprise the early-stage embryos which contain large amount of yolk and have no visible limb buds. Stages S6–S15 comprise the mid-stage embryos which are characterized by the appearance of the limb buds and are dorsally bent. During mid-embryogenesis, the yolk is gradually enclosed into the developing digestive glands and the formation of foregut and hindgut begins with the invagination of proctodeum and stomodeum. The proctodeum appears in stage S6 embryos and gradually elongates towards the stomodeum during stages S6–S14. Stomodeum and proctodeum fuse into a continuous digestive tube at the end of mid-embryogenesis in stage S15 embryos. Stages S16–S19 comprise late-stage embryos. Stage S16 embryos hatch from the chorion and become ventrally bent. In late-stage embryos the cuticular structures in the filtering regions of the stomach are formed and in S18 embryos the division of the hindgut into the anterior chamber and the papillate region becomes apparent (Štrus et al. 2008; Wolff 2009; Milatovič et al. 2010). Stage S20 designates the marsupial manca after the release from the vitelline membrane (Milatovič et al. 2010).

The term manca denotes all juvenile animals that already closely resemble adult animals but still lack the 7th pair of pereopods. The marsupial mancae develop within the marsupium for approximately ten more days. Mrak et al. (2012) defined early-stage, mid-stage, and late-stage marsupial mancae according to differences in size, pigmentation, motility, and amount of yolk in the digestive glands. Marsupial mancae already have distinct filters, ridges, and folds in the stomach. The typhlosole in the anterior chamber is elaborated and functional and the hindgut cuticle consists of procuticle and thin epicuticle (Štrus et al. 2008; Mrak et al. 2015; Štrus et al. 2019). After the release from the marsupium, the mancae develop as postmarsupial mancae for up to additional 20 days before reaching the juvenile stage with seven pairs of pereopods (Tomescu and Craciun 1987). During the postmarsupial development certain ultrastructural features of hindgut cells, such as the apical and basal labyrinths and the septate junctions, are further elaborated (Bogataj et al. 2019).

Aim

The outstanding ultrastructural feature of isopod hindgut cells are extensive apico-basal bundles of microtubules. In order to better understand the relation between the architecture of microtubule bundles and the shape and function of cells we have investigated for the first time the arrangement of microtubules in cells from different hindgut regions of adult *P. scaber* animals and in hindgut cells of selected developmental stages. For this, we have employed a combination of immunofluorescent labelling of α-tubulin on paraffin and semithin plastic sections and the ultrastructural analysis with transmission electron microscopy. The aim of the current study is primarily to provide a detailed description of microtubule organization in morphologically and functionally different hindgut regions of *P. scaber* and to discuss the obtained results in respect to the functions that the analyzed hindgut regions have been assigned to. Furthermore, we aim to establish how the microtubule arrays in the hindgut form during the embryonic and early postembryonic development.

Materials and methods

Specimens of *Porcellio scaber*

Adult animals as well as embryos and mancae of *Porcellio scaber* were obtained from a laboratory culture that had been maintained in a glass terrarium with ground cover of soil and leaf litter. Animals were bred at 25 °C, high humidity, and 12 h light/12 h dark cycle. Embryos and marsupial mancae were isolated from the marsupia of gravid females. The developmental stages of embryos were determined morphologically according to Milatovič et al. (2010) and the developmental stages of marsupial mancae according to Mrak et al. (2012). Investigated were the late embryos in stage S18, early-stage marsupial mancae and late-stage marsupial mancae. Postmarsupial mancae have been sampled at the day of the release and 1 week after the release from the marsupium. Gravid females were held separately in plastic petri dishes that each contained a moist filter paper and a dry leaf until mancae release.

Fixation and embedding procedures of samples for the immunohistochemistry

To obtain histological paraffin sections of adult animals, the animals were first anesthetized in a petri dish containing a small piece of cotton wool soaked in diethyl ether. Some of the anesthetized animals were transversely cut in half and both halves were processed whole. Some of the anesthetized animals were dissected, and the isolated hindguts were processed. The embryos, marsupial and postmarsupial mancae were processed whole. All samples were fixed in 4% formaldehyde in 0.1 mol/L HEPES buffer (pH 7.2). After the fixation the samples were rinsed with 0.1 mol/L HEPES buffer. The samples were then dehydrated in graded series of ethanol (50%, 70%, 80%, 90%, 96% and 100% ethanol) and cleared in xylene. After the dehydration and clearing the samples were infiltrated with Paraplast at 60 °C (2 changes overnight) and embedded in Paraplast on embedding station HistoCore Arcadia (Leica). Seven μm thick paraffin sections were prepared on a Leica RM 2265 microtome.

To obtain semithin sections of LR-White embedded samples, the hindguts were isolated from the anesthetized adult animals, while the embryos and mancae were processed whole. The samples were fixed either in 0.25% glutaraldehyde and 2% formaldehyde in 0.1 mol/L HEPES buffer (pH 7.2) or in 4% formaldehyde in 0.1 mol/L HEPES buffer (pH 7.2). After the fixation, the samples were rinsed in 0.1 mol/L HEPES buffer and dehydrated in graded series of ethanol (30%, 50%, 70%, 90% and 100% ethanol). Dehydrated samples were infiltrated with acrylic resin LR-White (London Resin Company Ltd). The resin was thermally cured in gelatin capsules at 60 °C for 24 h. 0.5 µm thick semithin sections were prepared using glass knives or a diamond histo knife (Diatome) on an ultramicrotome Reichert Ultracut S (Leica). Semithin sections are considerably thinner than the paraffin sections and thus higher resolution images of fluorescently labelled microtubules can be obtained. Some semithin sections were stained with Azure II – Methylene Blue for histological examinations, while most of the sections were used for immunolabelling.

Immunofluorescent labelling of microtubules on paraffin and LR-White sections

To visualize the microtubules immunolabelling of α-tubulin was performed. The α-tubulin forms dimers with β-tubulin which assemble to form the microtubules. Before the immunolabelling the paraffin sections were deparaffinized in xylene and rehydrated in graded series of alcohol (100% propanol, 96% ethanol, 70% ethanol) and distilled water. The rehydrated paraffin sections and LR-White sections were rinsed in PBS (phosphate buffered saline), blocked with 1% BSA (bovine serum albumin) in PBS and incubated overnight at 4 °C with primary mouse antibodies monoclonal anti-α-tubulin (mouse IgG1 isotype) (Sigma-Aldrich, catalog no.: T9026) diluted 1:500 in 1% BSA in PBS. After the incubation with primary antibodies the sections were rinsed with PBS and incubated with secondary goat antibodies against mouse IgG conjugated with fluorescent dye AlexaFluor 488 (ThermoFisher Scientific, A-11001) and diluted 1:300. Following the incubation with secondary antibodies sections were rinsed with PBS and covered with mounting medium FluoroShield with DAPI (4',6-Diamidine-2'-phenylindole dihydrochloride) (Sigma-Aldrich) to counterstain cell nuclei.

Immunolabelled sections were examined with AxioImager Z.1 microscope (Zeiss) using the fluorescence and differential interference contrast (DIC) imaging modes. For the fluorescence microscopy filter sets appropriate for DAPI (excitation BP 365/12, beam splitter FT 395, emission LP 397) and AlexaFluor 488 (excitation BP 450–490, beam splitter FT 510, emission LP 515) were used. The digital micrographs were acquired with HRc AxioCam camera (Zeiss) using the AxioVision software (Zeiss). The images were processed (brightness and contrast adjusted) and the overlays of epifluorescence and DIC images were prepared in FIJI (ImageJ) software.

Ultrastructural analysis of resin embedded samples with transmission electron microscopy

The ultrastructural information on the arrangement of microtubules in hindgut cells was obtained by transmission electron microscopy of ultrathin sections of resin embedded samples. The adult animals were dissected to isolate the hindguts and the embryos and mancae were processed whole. The samples were chemically fixed in 2.5% glutaraldehyde and 4% paraformaldehyde in 0.1 mol/L HEPES buffer. After the fixation the samples were rinsed with 0.1 mol/L HEPES buffer and postfixed in 1% OsO_4 in 0.1 mol/L HEPES buffer. Then the samples were dehydrated in graded series of ethanol and acetone (50%, 70%, 90%, 100% ethanol, and 100% acetone) and infiltrated with epoxy resin Agar 100 (AgarScientific). The resin was polymerized in silicone molds at

60 °C for 24 h. The semithin sections were prepared as described for LR-White embedded specimens. 70 nm thick ultrathin sections were cut with a diamond ultra knife (Diatome) on the ultramicrotome Reichert Ultracut S (Leica), transferred to copper mesh grids and contrasted with 1% uranyl acetate and 0.1% lead citrate. Prepared ultrathin sections were examined with a Phillips CM100 transmission electron microscope and the electron micrographs acquired with a Bioscan 792 (Gatan) and an Orius SC200 (Gatan) digital camera, using the Digital Micrograph (Gatan) software.

Results

Histological structure of the typhlosole and the shape of hindgut cells in adult *P. scaber*

In the adult hindgut four regions can be distinguished at the level of gross anatomy: anterior chamber, papillate region, sphincter region and rectum (Fig. 1A). The anterior chamber in its anterior part is characterized by a relatively narrow typhlosole and two deep typhlosole channels (Fig. 1B). The dorsal cells that constitute the typhlosole and two typhlosole channels have very complex shapes. Typhlosole cells are highly curved in cross-section. The two cells that enclose the typhlosole channels immediately on each side of the typhlosole are wide and flattened and the two cells further away are curved in cross-section and form two ridges protruding into the hindgut lumen on each side of the typhlosole. The remaining hindgut cells in the anterior chamber, which build the lateral and ventral hindgut wall, are isodiametric to prismatic with dome shaped apical parts protruding into the hindgut lumen. In the posterior part of the anterior chamber the typhlosole widens considerably and becomes anvil shaped, and the two typhlosole channels are very narrow (Fig. 1C). In cross-section, the curvature of the typhlosole cells is even more pronounced than anteriorly. The cells enclosing the typhlosole channels are wider and the two ridges on each side of the typhlosole are absent. Cells that build the lateral hindgut wall are relatively small and isodiametric. The ventral cells are very large, prismatic and with apical parts protruding deep into the hindgut lumen. In the papillate region all the cells of the hindgut epithelium look similar. They are isodiametric with dome shape basal parts protruding into the haemocoel (Fig. 1D). In the sphincter region extensive circular muscles surround the hindgut epithelium, which forms deep folds that narrow the hindgut lumen. The hindgut cells are isodiametric with dome shaped basal parts protruding into the haemocoel (Fig. 1E). In the rectum the hindgut lumen becomes wide again and the musculature surrounding the hindgut is less extensive than in the sphincter region. The shape of the hindgut cells is the same as in the sphincter region (Fig. 1F).

Specific arrangement of microtubules in the epithelial cells in distinct hindgut regions of adult *P. scaber*

We compared the abundance and arrangement of microtubules in hindgut cells of diverse shapes and sizes within the anterior chamber, papillate region, sphincter region and rectum. In the anterior chamber the epithelial cells contain extensive arrays of microtubules (Fig. 2A). The cells of the typhlosole contain long

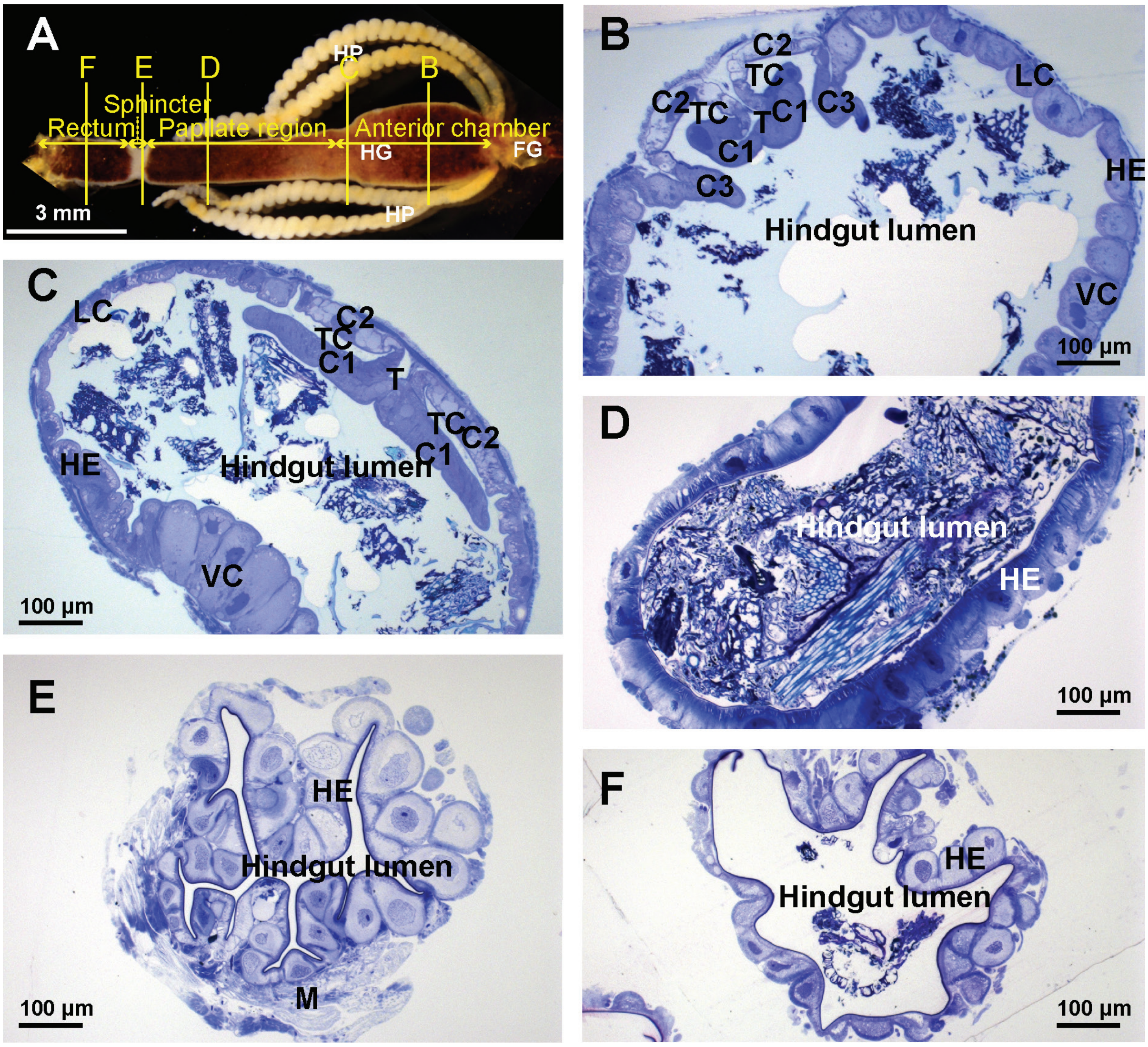

Figure 1. Anatomical and histological structure of the hindgut in adult *P. scaber* **A** anatomical structure and hindgut regions. Lines B, C, D, E, and F denote where the cross-sections depicted in images B, C, D, E and F were made **B** cross-section across the anterior part of the anterior chamber **C** cross-section across the posterior part of the anterior chamber **D** cross-section across the papillate region **E** cross-section across sphincter **F** cross-section across rectum. Abbreviations: C1 – curved cells forming the typhlosole; C2 – wide cells enclosing the typhlosole channels; C3 – curved cells forming ridges on each side of the typhlosole; FG – foregut; HE – hindgut epithelium; HG – hindgut; HP – hepatopancreas; LC – lateral cells; M – muscles; T – typhlosole; TC – typhlosole channels; VC – ventral cells.

transcellular bundles oriented perpendicular to the apical cell surface. The hindgut cells around the two typhlosole channels contain short profiles of densely stacked microtubule bundles present only in the apical cytosol (Fig. 2B). The remaining epithelial cells that form the lateral and ventral hindgut walls contain thick bundles of microtubules concentrated in the lateral cytosol (Fig. 2C, D). The ultrastructural analysis of hindgut epithelial cells in the anterior chamber reveals extensive apico-basal bundles of microtubules associated with lateral borders of cells. These bundles completely fill large areas of lateral cytosol (Fig. 2E, F).

In the posterior part of the anterior chamber close to the papillate region the apico-basally oriented bundles of microtubules are particularly extensive (Fig. 3A). The most prominent transcellular microtubular bundles are present in dorsal hindgut cells of the typhlosole and typhlosole channels (Fig. 3B, C)

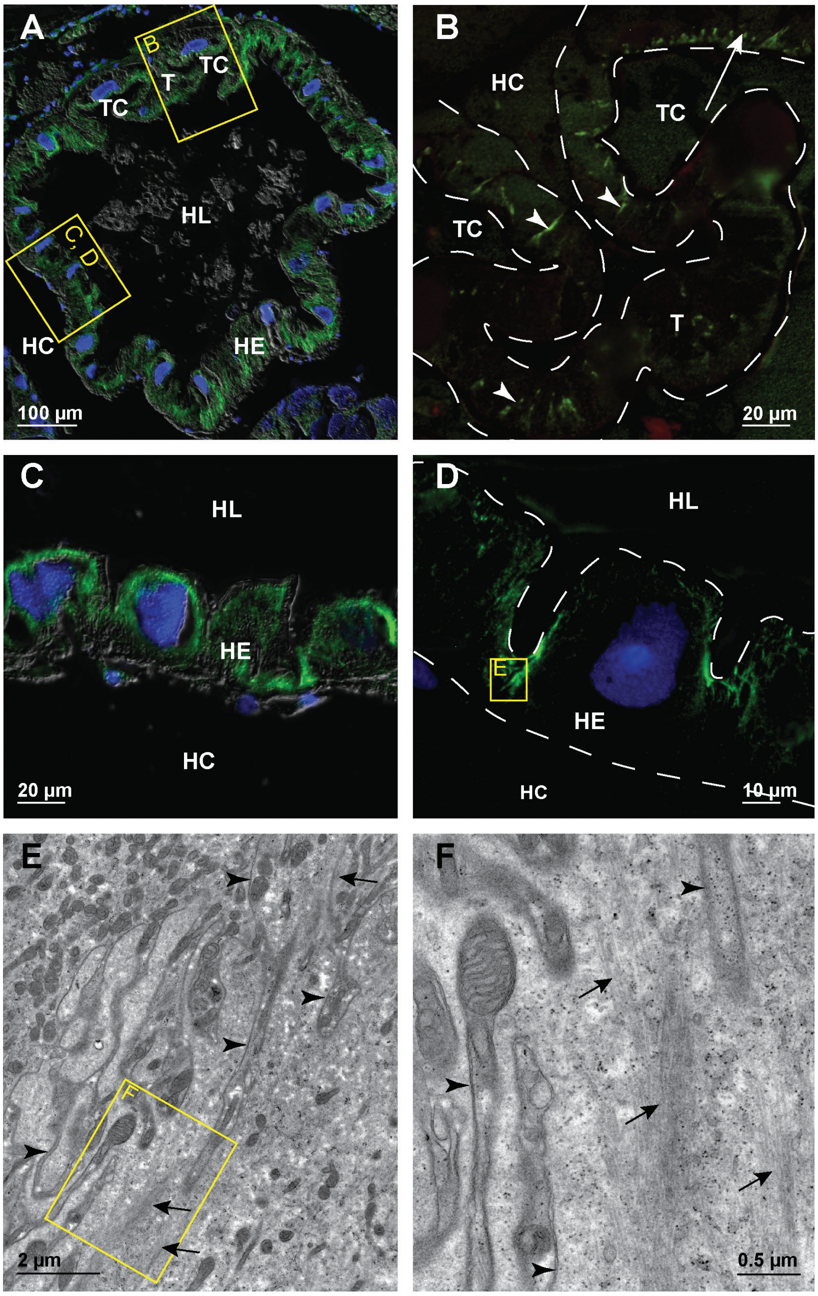

Figure 2. Microtubules in hindgut cells in the anterior chamber of adult *P. scaber* **A** overlay of epifluorescence and DIC images obtained on paraffin section showing microtubules in green (AlexaFluor 488) and cell nuclei in blue (DAPI). Image depicts cross-section across the anterior chamber **B** image of immunofluorescent-labelled microtubules in green on LR-White section. Image depicts equivalent area as denoted by frame B in image A. Arrowheads point to transcellular microtubule bundles in typhlosole cells oriented perpendicular to the apical cell surface. Arrow points to short profiles of microtubule bundles present in the apical cytosol of wide cells around the typhlosole channels. The white dashed lines outline the apical and basal surface of epithelial cells **C** overlay of epifluorescence and DIC images obtained on paraffin section showing microtubules in green (AlexaFluor 488) and cell nuclei in blue (DAPI). Image depicts equivalent area as denoted by the frame C, D in image A **D** overlay of epifluorescence images obtained on semithin LR-White section showing microtubules in green (AlexaFluor 488) and cell nuclei in blue (DAPI). Image depicts equivalent area as denoted by the frame C, D in image A. The white dashed lines outline the apical and basal surface of hindgut epithelium **E** electron micrograph showing equivalent area as denoted by frame E in image D. Arrows point to abundant microtubule bundles near the lateral plasma membrane (arrowheads) **F** higher magnification of an area denoted by frame F in image E. Arrows point to bundled individual microtubules near the lateral plasma membrane (arrowheads). Abbreviations: HC – hemocoel; HE – hindgut epithelium; HL – hindgut lumen; T – typhlosole; TC – typhlosole channels.

and in high dome shaped ventral cells (Fig. 3D). In cells around the typhlosole channels the microtubules occur near the apical and the basal surface of these cells (Fig. 3B). In the dorsal epithelium, both the typhlosole cells and the wide cells around the typhlosole channels contain parallel bundles of microtubules (Fig. 3C). In highly folded typhlosole cells, the microtubule bundles are oriented perpendicular to the apical cell surface.

In the papillate region all cells in the hindgut epithelium contain fine apico-basally oriented bundles of microtubules (Fig. 4A, B). This arrangement of microtubules is general throughout the papillate region epithelium. The bundles of microtubules appear thinner than in the anterior chamber and are evenly distributed throughout the cytosol (Fig. 4C). At the ultrastructural level prominent bundles of microtubules are evident along the narrow cytoplasmic bands between the infoldings of apical plasma membrane (Fig. 4D). These microtubules are also in close contact with numerous mitochondria present in the apical cytosol. Cross-sectioned profiles of microtubules oriented perpendicular to the apico-basal axis of epithelial cells are often observed in the vicinity of septate junctions in the papillate region (Fig. 4E).

In the sphincter region between the papillate region and the rectum the hindgut epithelium is relatively thin (Fig. 5A), and the hindgut cells contain numerous microtubules concentrated in the apical cytosol (Fig. 5B). Fine bundles of microtubules are oriented in the apico-basal direction and limited to the apical cytosol of cells as discernible on semi-thin LR-White sections (Fig. 5C).

The hindgut epithelium is also relatively thin in the rectum (Fig. 6A). The arrangement of microtubule bundles in epithelial cells of the rectum is similar as in the sphincter region, with microtubules concentrated in the apical cytosol (Fig. 6B). In the posterior part of the rectum toward the anal opening, strong dilator muscles are attached to the hindgut epithelium (Fig. 6C) in contrast to the remaining part of the rectum. In this posterior part the epithelial cells are relatively small and almost filled with microtubules (Fig. 6D).

Arrangement of microtubules in the hindgut cells of embryos and mancae

In the paraffin sections of the whole late embryos of stage S18 the immunofluorescent labelling of α-tubulin shows only a weak reaction in the hindgut cells. A strong positive reaction is discernible in the epidermis of the limb buds (Fig. 7A). In the hindgut epithelium along most of the alimentary canal the reaction of fluorescent labelling is weak and diffusely distributed in the cytosol (Fig. 7B). At the ultrastructural level we have observed individual microtubules in the cytosol of hindgut cells (Fig. 7C). The distinct apico-basally oriented bundles are discernible only in the most posterior part of the alimentary canal, i.e., the rectal epithelium (Fig. 7D).

In marsupial mancae the immunofluorescent labelling of microtubules is considerably more pronounced than in the late embryos, on both paraffin and semithin sections. We did not observe any considerable differences in labelling between the early-stage and late-stage marsupial mancae. The most pronounced fluorescent labelling is detectable in certain sites of tergite's epidermis, presumably tenocytes, in the hindgut epithelium and in the cells of the ventral nerve cord (Fig. 8A). In the hindgut epithelium the apico-basal orientation of microtubule bundles is evident in all observed regions of the hindgut. In the anterior chamber there is a distinct difference in the abundance of

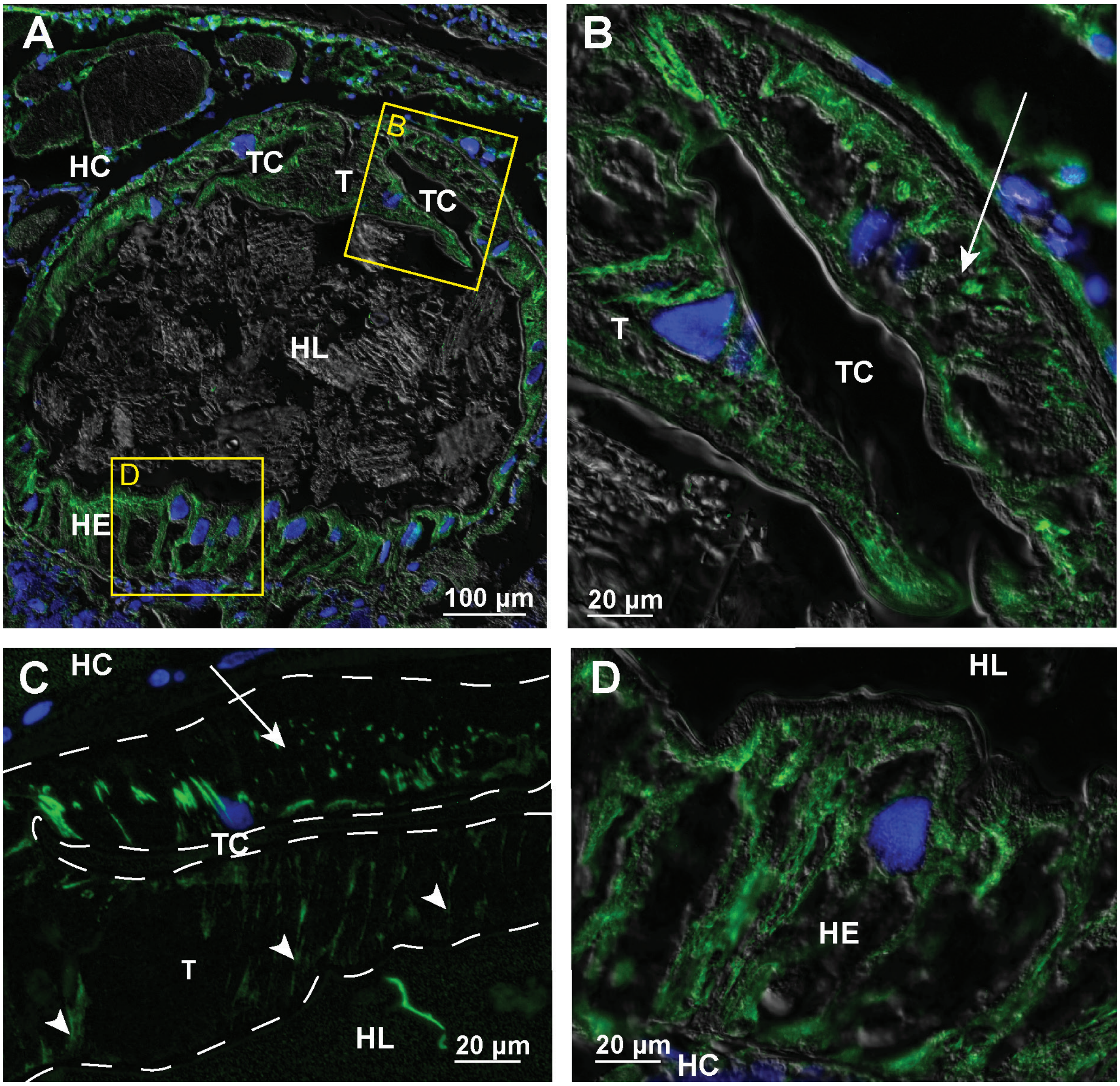

Figure 3. Microtubules in hindgut cells in the posterior part of anterior chamber of adult *P. scaber* **A** overlay of epifluorescence and DIC images obtained on paraffin section showing microtubules in green (AlexaFluor 488) and cell nuclei in blue (DAPI). Image depicts cross-section across the anterior chamber near the transition to the papillate region **B** overlay of epifluorescence and DIC images obtained on paraffin section showing microtubules in green (AlexaFluor 488) and cell nuclei in blue (DAPI). Image depicts area denoted by the frame B in image A **C** overlay of epifluorescence images obtained on semithin LR-White section showing microtubules in green (AlexaFluor 488) and cell nuclei in blue (DAPI). Image depicts equivalent area as image B. Arrow points to large cell that surround the typhlosole channels and contain abundant microtubule bundles. Arrowheads point to parallel bundles of microtubules oriented perpendicular to the apical surface of typhlosole cells. The white dashed lines outline the contour of hindgut epithelium **D** overlay of epifluorescence and DIC images obtained on paraffin section showing microtubules in green (AlexaFluor 488) and cell nuclei in blue (DAPI). Image depicts area denoted by the frame D in image A. Abbreviations: HC – hemocoel; HE – hindgut epithelium; HL – hindgut lumen; T – typhlosole; TC – typhlosole channels.

microtubules between the dorsal part and the ventral part of the hindgut epithelium. The labelling of microtubules is very distinct in the dorsal epithelium, particularly in cells of typhlosole and typhlosole channels. Cells in the ventral epithelium are only weakly labelled (Fig. 8B). The bundles of microtubules are particularly extensive in ventral and dorsal cells at the transition between the

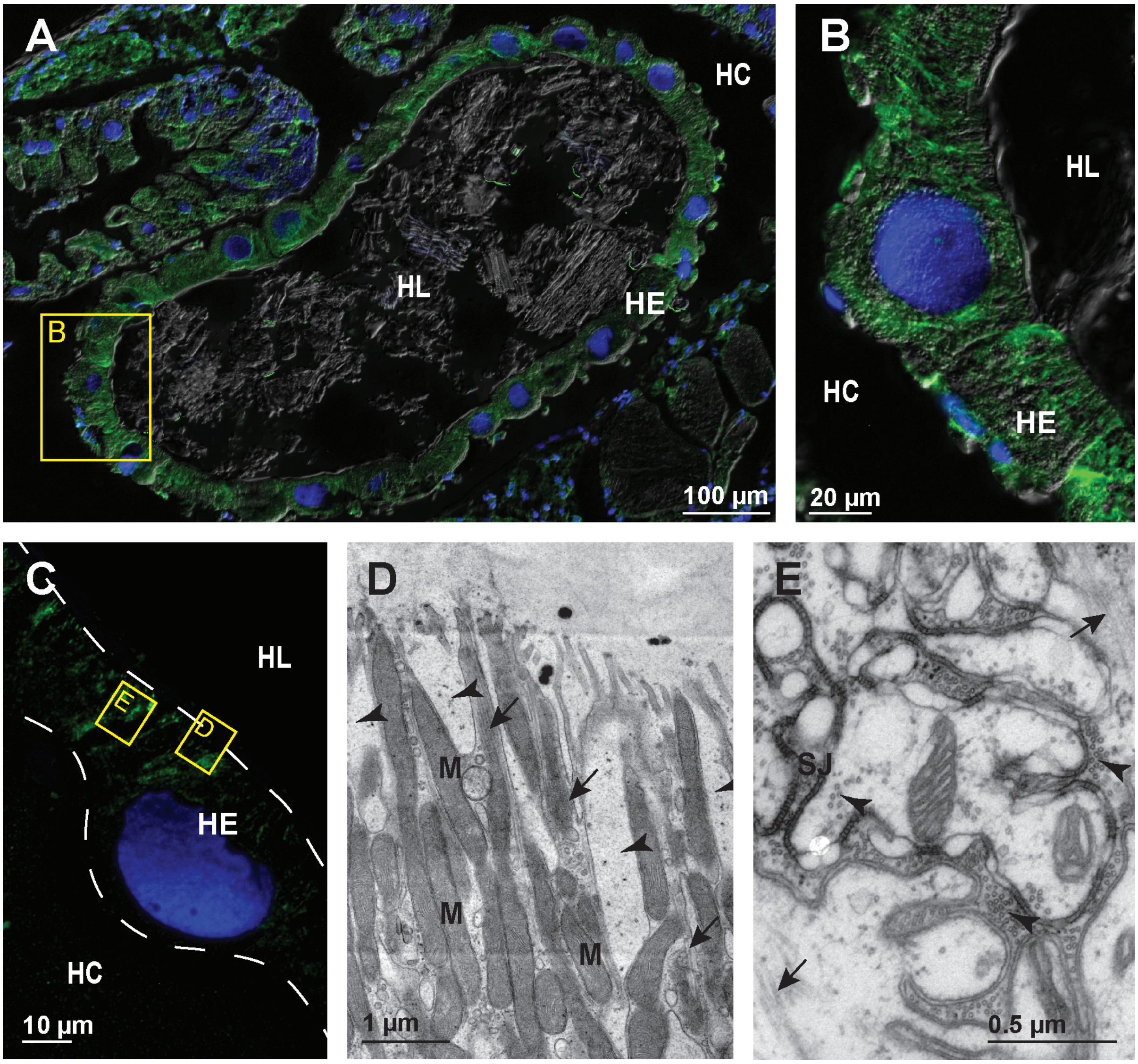

Figure 4. Microtubules in hindgut cells in the papillate region of adult *P. scaber* **A** overlay of epifluorescence and DIC images obtained on paraffin section showing microtubules in green (AlexaFluor 488) and cell nuclei in blue (DAPI). Image depicts cross-section across the papillate region **B** overlay of epifluorescence and DIC images obtained on paraffin sections showing microtubules in green (AlexaFluor 488) and cell nuclei in blue (DAPI). Image depicts area denoted by the frame B in image A **C** overlay of epifluorescence images obtained on semithin LR-White section showing microtubules in green (AlexaFluor 488) and cell nuclei in blue (DAPI). Image depicts equivalent area as image B. The white dashed lines in figure C outline the apical and basal surface of hindgut epithelium **D** electron micrograph showing equivalent area as denoted by frame D in image C. Arrows point to microtubule bundles in the cytosol between the infoldings of apical plasma membrane which are indicated by the arrowheads **E** electron micrograph showing equivalent area as denoted by frame E in image C. Arrowheads point to numerous cross-sectioned microtubules in the vicinity of septate junctions. Arrows point to longitudinally sectioned apico-basal microtubules. Abbreviations: HC – hemocoel; HE – hindgut epithelium; HL – hindgut lumen; M – mitochondria; SJ – septate junctions.

anterior chamber and the papillate region (Fig. 8C). In the papillate region the distribution of the microtubules in epithelial cells differs between anterior part and posterior part of the region. In the anterior part fine bundles of microtubules span the entire cells in the apico-basal direction (Fig. 8D), while in the posterior part the bundles are condensed in the apical cytosol (Fig. 8E). At the ultrastructural level it is evident that microtubules associate laterally with one

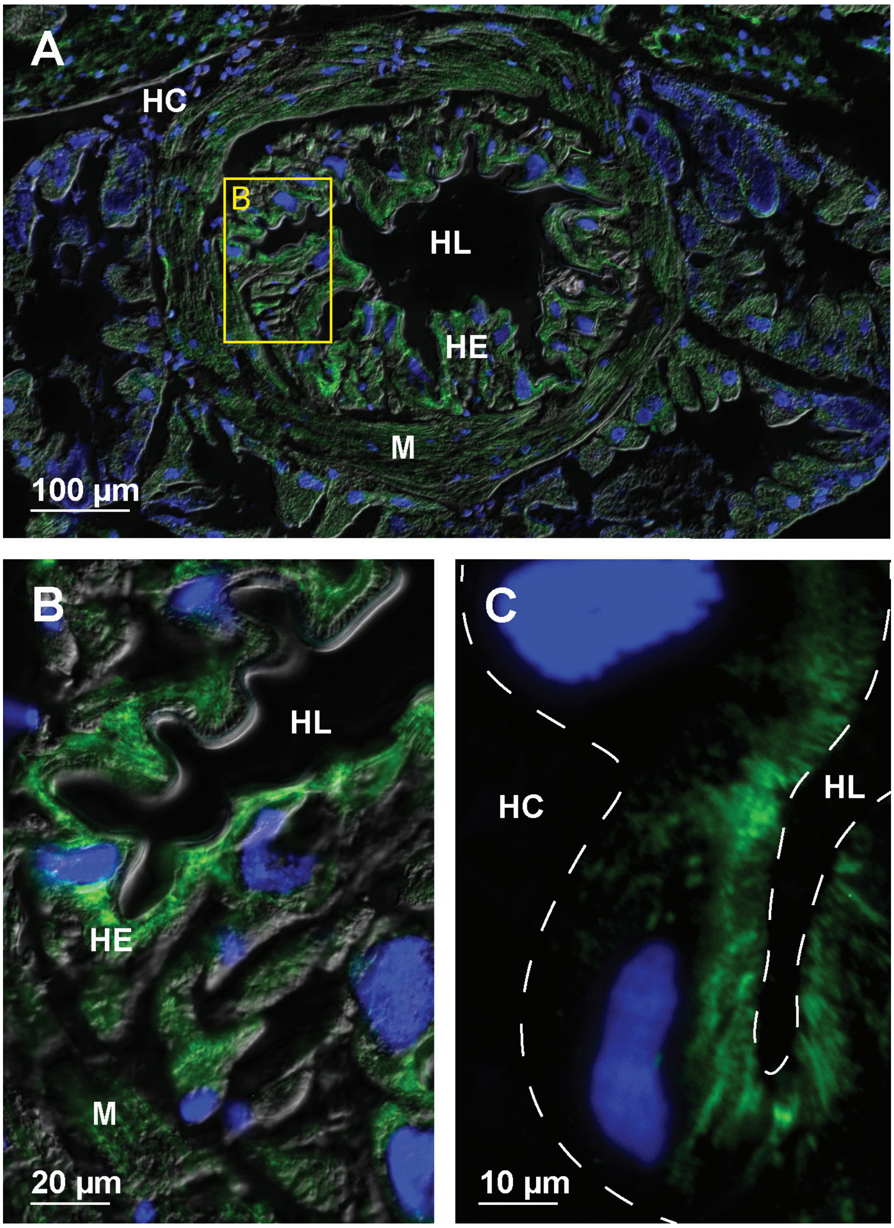

Figure 5. Microtubules in hindgut cells in the sphincter region in adult *P. scaber* **A** overlay of epifluorescence and DIC images obtained on paraffin section showing microtubules in green (AlexaFluor 488) and cell nuclei in blue (DAPI). Image depicts cross-section across the sphincter region **B** overlay of epifluorescence and DIC images obtained on paraffin section showing microtubules in green (AlexaFluor 488) and cell nuclei in blue (DAPI). Image depicts area denoted by frame B in image A **C** overlay of epifluorescence images obtained on semithin LR-White section showing microtubules in green (AlexaFluor 488) and cell nuclei in blue (DAPI). Image depicts cross-section of hindgut epithelium in sphincter region at high magnification. The white dashed lines in figure C outline the contour of hindgut epithelium. Abbreviations: HC – hemocoel; HE – hindgut epithelium; HL – hindgut lumen; M – muscles.

another and form small apico-basally oriented bundles (Fig. 8F). In the terminal part of the rectum near the anal opening the arrangement of apico-basal bundles of microtubules is similar as in the late embryos of stage S18.

In postmarsupial mancae an intense immunofluorescent labelling of microtubules is visible in epidermal cells, presumably tenocytes, the ventral nerve cord and the hindgut epithelium. The labelling does not differ considerably between postmarsupial mancae at the time of the release from the marsupium and postmarsupial

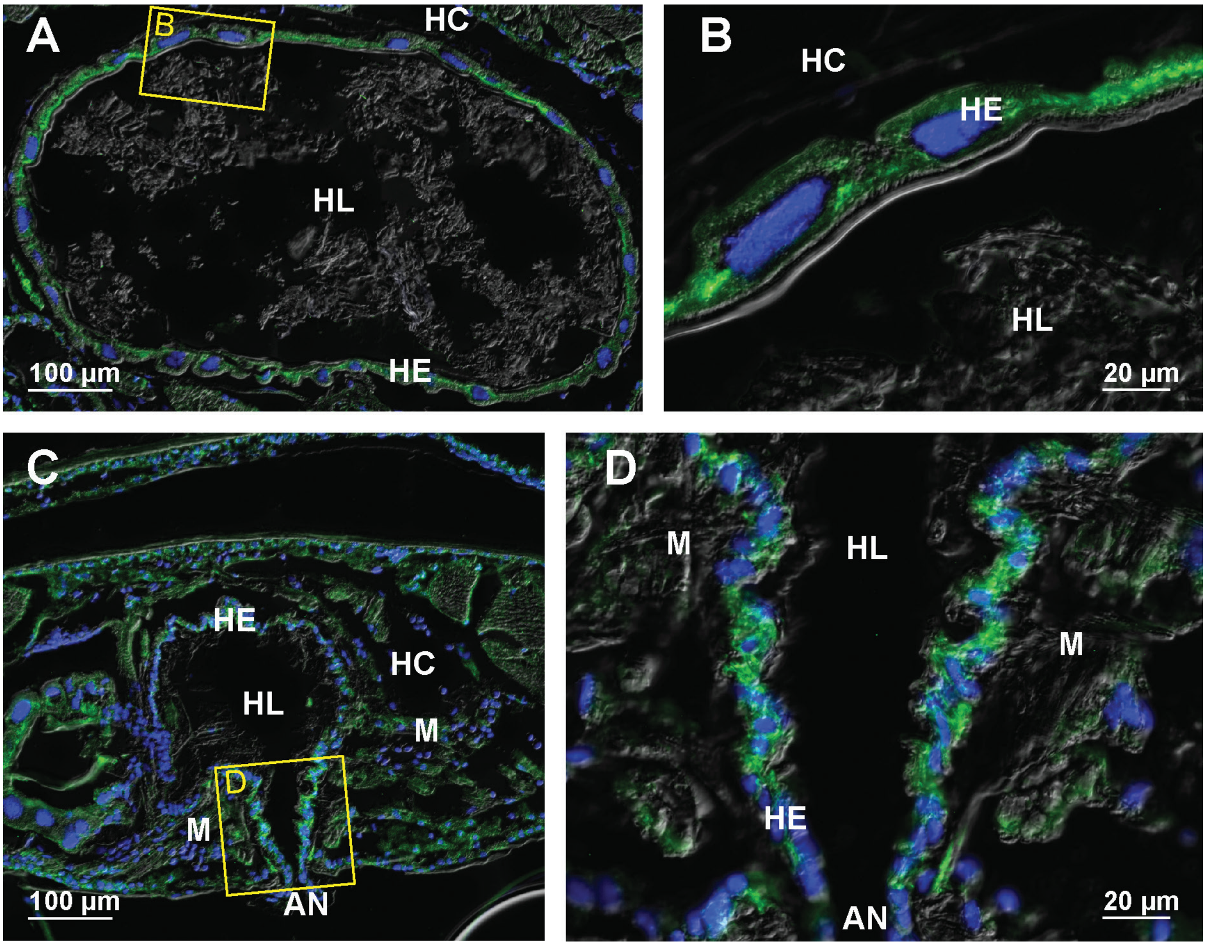

Figure 6. Microtubules in hindgut cells in the rectum in adult *P. scaber* **A** overlay of epifluorescence and DIC images obtained on paraffin section showing microtubules in green (AlexaFluor 488) and cell nuclei in blue (DAPI). Image depicts cross-section across the rectum **B** overlay of epifluorescence and DIC images obtained on paraffin section showing microtubules in green (AlexaFluor 488) and cell nuclei in blue (DAPI). Image depicts area denoted by frame B in image A **C** overlay of epifluorescence and DIC images obtained on paraffin section showing microtubules in green (AlexaFluor 488) and cell nuclei in blue (DAPI). Image depicts cross-section across the rectum near the anal opening **D** overlay of epifluorescence and DIC images obtained on paraffin section showing microtubules in green (AlexaFluor 488) and cell nuclei in blue (DAPI). Image depicts area denoted by frame D in image C. Abbreviations: AN – anal opening; HC – hemocoel; HE – hindgut epithelium; HL – hindgut lumen; M – dilator muscles.

mancae one week after the release from the marsupium. In the anterior chamber all hindgut cells display strong fluorescent labelling (Fig. 9A, B). As in earlier stages the most extensive fluorescent labelling is present in the hindgut epithelium at the transition between the anterior chamber and the papillate region (Fig. 9C). In postmarsupial mancae the typhlosole in this hindgut section becomes anvil shaped and contains particularly large bundles of microtubules. In the anterior part of the papillate region fine bundles of microtubules are distributed throughout the cytosol of hindgut cells (Fig. 9D). In the most posterior part of the papillate region the microtubules are concentrated in the apical part of hindgut cells (Fig. 9E). At the ultrastructural level, the apico-basal microtubule bundles become extensive in postmarsupial mancae and are concentrated in the lateral cytosol near the lateral plasma membranes in the anterior chamber (Fig. 9F). In the papillate region of postmarsupial mancae we observed cross-sectioned microtubules in the vicinity of septate junctions which are not apparent in the earlier stages (Fig. 9G).

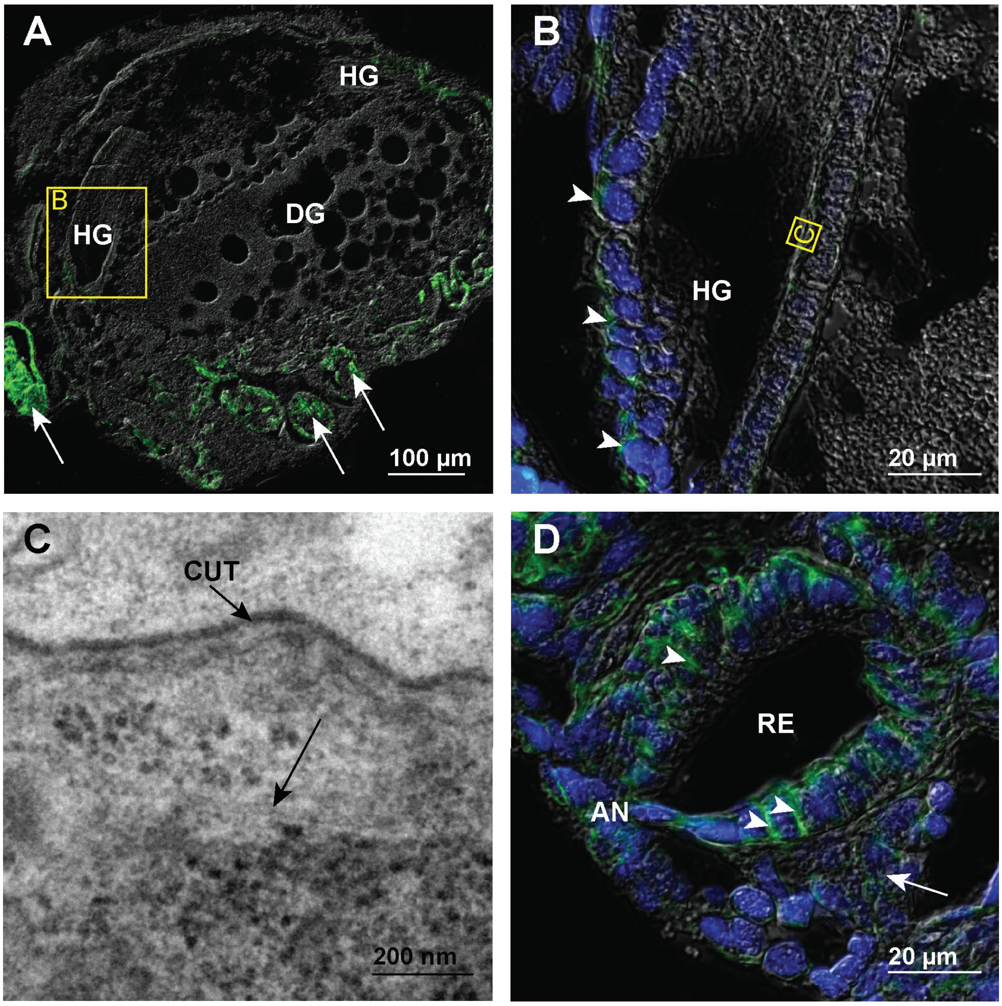

Figure 7. Microtubules in hindgut cells of late embryos of stage S18 **A** overlay of epifluorescence and DIC images obtained on paraffin section showing microtubules in green (AlexaFluor 488). Image depicts longitudinal section of the embryo. Arrows point to distinct fluorescent labelling of microtubules in epidermis of the limb buds **B** overlay of epifluorescence and DIC images obtained on paraffin section showing microtubules in green (AlexaFluor 488) and cell nuclei in blue (DAPI). Image depicts area denoted by frame B in image A. Arrowheads point to diffuse fluorescent labelling in the hindgut **C** electron micrograph of equivalent area as denoted by frame C in image B. Arrow points to an individual microtubule in the apical cytosol **D** overlay of epifluorescence and DIC images obtained on paraffin section showing microtubules in green (AlexaFluor 488) and cell nuclei in blue (DAPI). Image depicts cross-section of the rectum near the anal opening. Arrowheads point to apico-basal bundles of microtubules in the rectum. An arrow points to distinct dilator muscles surrounding the rectum. Abbreviations: AN – anal opening; CUT – cuticle; DG – digestive glands; HG – hindgut; RE – rectum.

Typhlosole morphogenesis

We have also investigated the morphogenesis of the typhlosole since it is a major remodeling process of the hindgut epithelium and prominent microtubule bundles are present in typhlosole cells. In late embryos a primordium of the typhlosole is established and appears in cross-section as two large dorsal cells bulged into the hindgut lumen (Fig. 10A). Later in marsupial mancae the dorsal hindgut cells already form distinct folds of the typhlosole and two typhlosole channels (Fig. 10B). The morphology of the typhlosole in marsupial mancae is similar along the entire length of the alimentary canal. In postmarsupial mancae the typhlosole at the transition between the anterior chamber and the papillate region widens and becomes anvil shaped (Fig. 10C, D) as in adults (Fig. 10E, F).

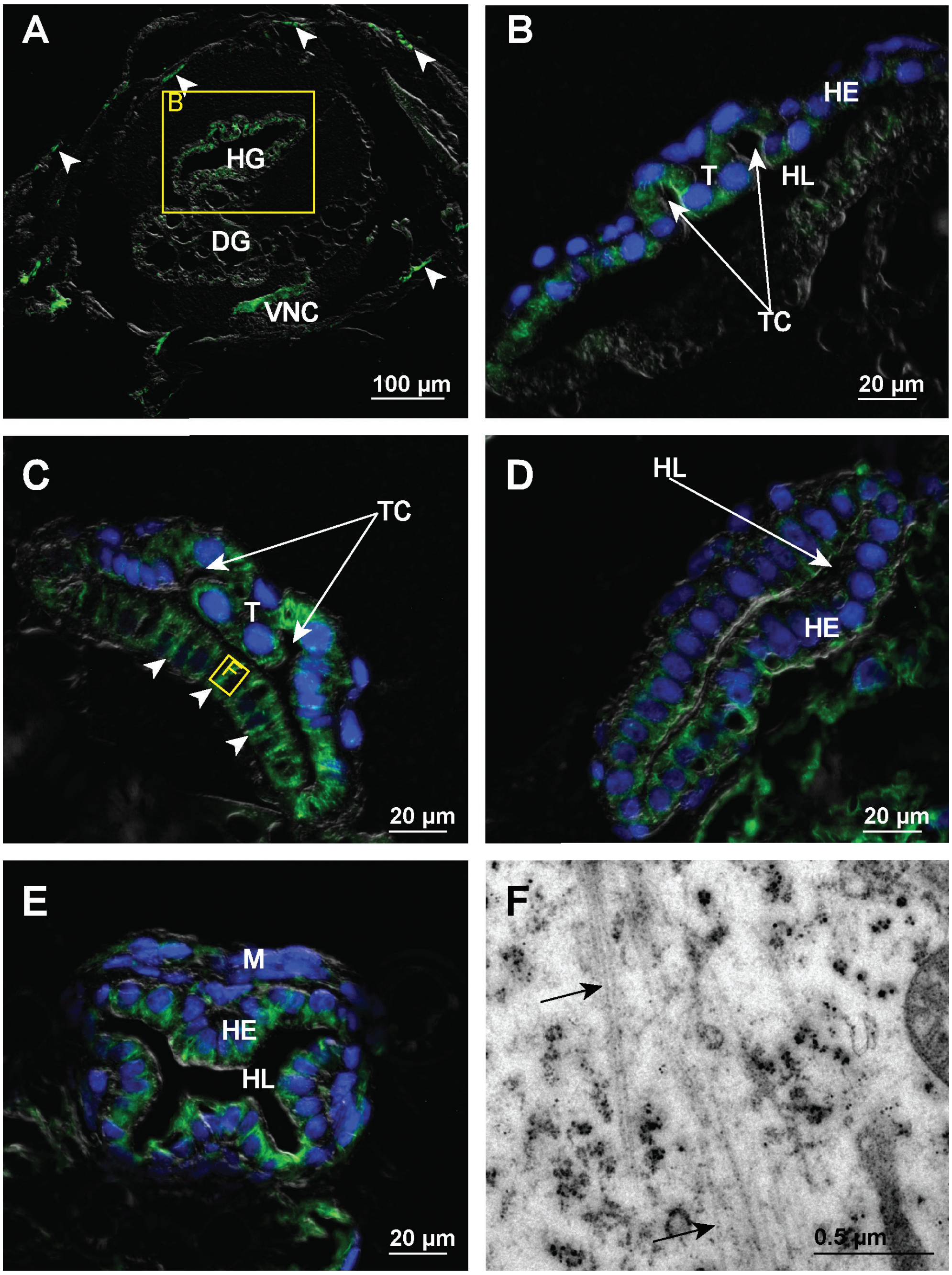

Figure 8. Microtubules in hindgut cells of early-stage marsupial mancae **A** overlay of epifluorescence and DIC images obtained on paraffin section showing microtubules in green (AlexaFluor 488). Image depicts a transverse section across the anterior part of manca. Arrowheads point to microtubules in dorsal tergites **B** overlay of epifluorescence and DIC images obtained on paraffin section showing microtubules in green (AlexaFluor 488) and cell nuclei in blue (DAPI). Image depicts area denoted by frame B in image A **C** overlay of epifluorescence and DIC images obtained on paraffin section showing microtubules in green (AlexaFluor 488) and cell nuclei in blue (DAPI). Image depicts hindgut epithelium at the transition between the anterior chamber and the papillate region. Arrowheads point to cells containing distinct apico-basal bundles of microtubules **D** overlay of epifluorescence and DIC images obtained on paraffin section showing microtubules in green (AlexaFluor 488) and cell nuclei in blue (DAPI). Image depicts hindgut epithelium from the anterior part of the papillate region **E** overlay of epifluorescence and DIC images obtained on paraffin section showing microtubules in green (AlexaFluor 488) and cell nuclei in blue (DAPI). Image depicts hindgut epithelium from the posterior part of the papillate region **F** electron micrograph of equivalent area as denoted by frame F in image C showing the cell in the ventral hindgut epithelium. Arrows point to small bundles of microtubules in the cytosol. Abbreviations: DG – digestive glands; HE – hindgut epithelium; HG – hindgut; HL – hindgut lumen; M – muscles; T – typhlosole; TC – typhlosole channels; VNC – ventral nerve cord.

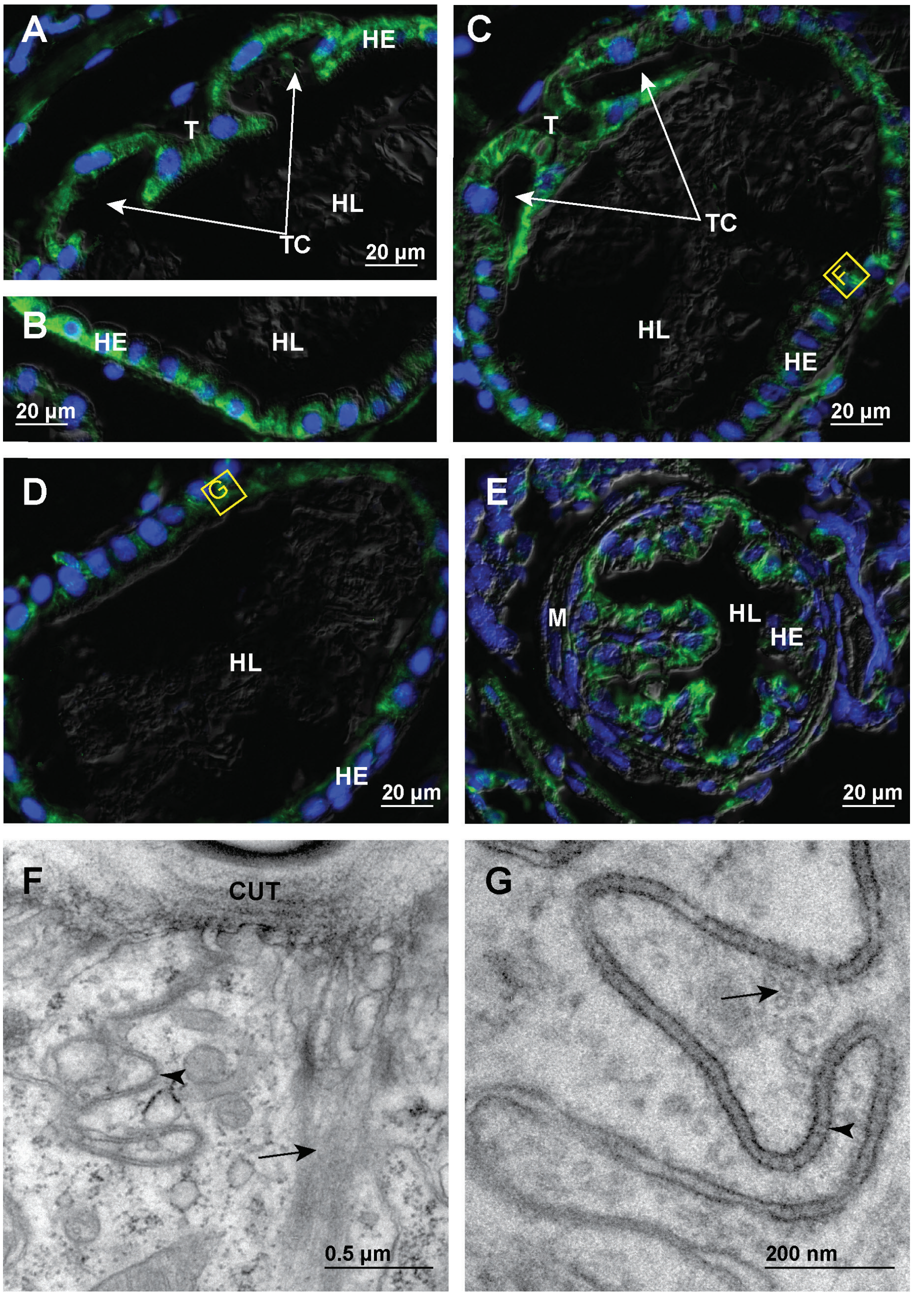

Figure 9. Microtubules in hindgut cells of postmarsupial mancae 1 week after their release from the marsupium **A** overlay of epifluorescence and DIC images obtained on paraffin section showing microtubules in green (AlexaFluor 488) and cell nuclei in blue (DAPI). Image depicts dorsal hindgut epithelium in the anterior chamber **B** overlay of epifluorescence and DIC images obtained on paraffin section showing microtubules in green (AlexaFluor 488) and cell nuclei in blue (DAPI). Image depicts hindgut epithelium of the lateral hindgut wall in the anterior chamber **C** overlay of epifluorescence and DIC images obtained on paraffin section showing microtubules in green (AlexaFluor 488) and cell nuclei in blue (DAPI). Image depicts hindgut epithelium at the transition between the anterior chamber and the papillate region **D** overlay of epifluorescence and DIC images obtained on paraffin section showing microtubules in green (AlexaFluor 488) and cell nuclei in blue (DAPI). Image depicts hindgut epithelium in the anterior part of the papillate region **E** overlay of epifluorescence and DIC images obtained on paraffin section showing microtubules in green (AlexaFluor 488) and cell nuclei in blue (DAPI). Image depicts hindgut epithelium in the posterior part of the papillate region **F** electron micrograph of equivalent area as denoted by frame F in image C. Arrow points to a large bundle of microtubules near the lateral plasma membrane which is indicated by arrowhead **G** electron micrograph of equivalent area as denoted by frame G in image D. Arrowhead points to septate junctions. Arrow points to cross-sectioned microtubules. Abbreviations: CUT – cuticle; HE – hindgut epithelium; HL – hindgut lumen; M – muscles; T – typhlosole; TC – typhlosole channels.

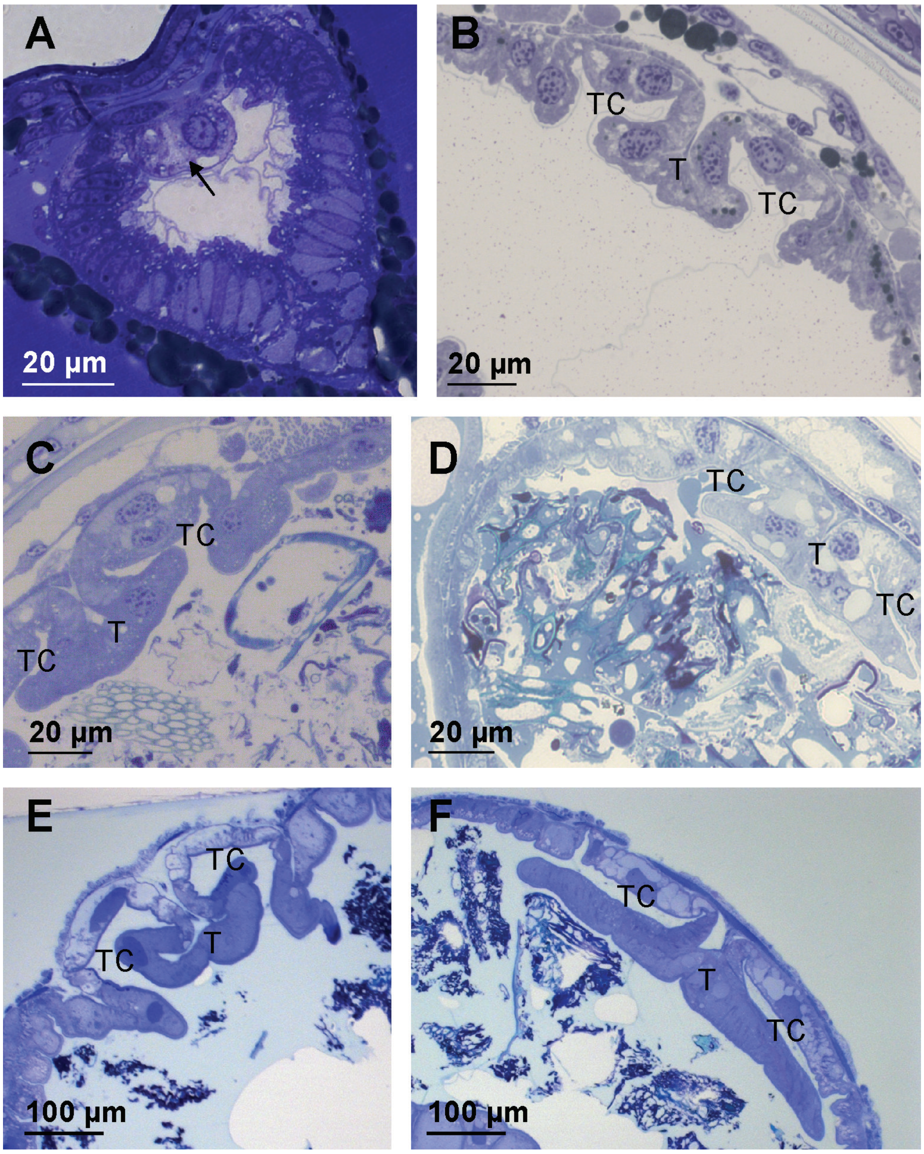

Figure 10. Typhlosole morphogenesis in the hindgut of *P. scaber* **A** typhlosole primordium (arrow) in hindgut of late embryo of stage S18 **B** typhlosole in the anterior chamber of marsupial manca **C** typhlosole in the anterior part of the anterior chamber in postmarsupial manca **D** typhlosole in the posterior part of the anterior chamber near the transition to the papillate region in postmarsupial manca **E** typhlosole in the anterior part of the anterior chamber in adult animal **F** typhlosole in the posterior part of the anterior chamber near the transition to the papillate region in adult animal. Abbreviations: T – typhlosole; TC – typhlosole channels.

Discussion

Architecture of microtubule bundles in the hindgut cells of adult *P. scaber*

The microtubules are the stiffest of the three cytoskeletal filaments and form almost linear tracks that primarily serve as pathways for the directed intracellular transport (Fletcher and Mullins 2010) and in differentiated cells the microtubules are also important for the maintenance of cell shape (Gelfand and Bershadsky 1991). In combination with other cytoskeletal components, the microtubules can bear considerable compressive forces (Brangwynne et al. 2006), which is particularly important in the regulation of cell height of epithelial cells (Burnside 1973; Picone et al. 2010). Hindgut cells of isopods contain extremely

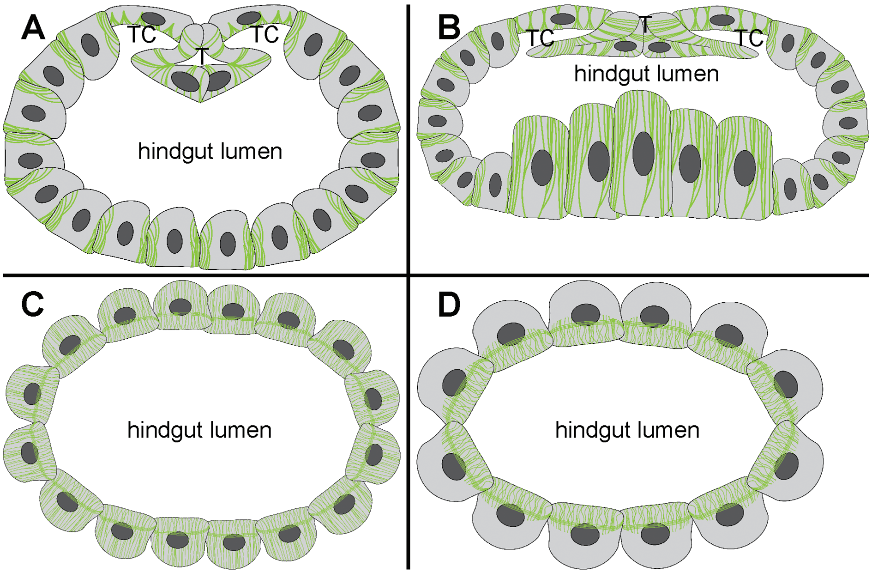

Figure 11. Illustration of microtubule architecture in hindgut cells from different regions of adult *P. scaber* hindgut **A** anterior chamber **B** posterior part of the anterior chamber at the transition to the papillate region **C** papillate region **D** sphincter and rectum.

abundant microtubule bundles as reported in *P. scaber* (Bogataj et al. 2018), *O. asellus* (Witkus et al. 1969), *A. vulgare* (Vernon et al. 1974) and two species of marine isopods *D. bidentata* (Holdich and Ratcliffe 1970) and *C. carinata* (Wägele et al. 1981), while microtubule arrangement in the cells of morphologically and functionally distinct hindgut regions has not been studied before.

We show here that the cells in all hindgut regions of adult *P. scaber* contain abundant microtubules which organize into extensive apico-basally oriented bundles. Our results demonstrate that the spatial arrangement of these apico-basal bundles varies considerably in cells from distinct parts of the hindgut that differ markedly in their shape as summarized in the schematic representation (Fig. 11). In the anterior chamber the cells that form the ventral and lateral walls of the hindgut contain relatively thick microtubule bundles concentrated in the vicinity of lateral plasma membranes that are highly interdigitated between the neighboring cells (Fig. 11A). Similar association of microtubule bundles with lateral plasma membranes have been reported in hindgut cells of other isopods (Witkus et al. 1969; Holdich and Ratcliffe 1970; Vernon et al. 1974). In cells that form the typhlosole a specific microtubule arrangement is evident. Long transcellular apico-basal bundles of microtubules are oriented perpendicular to the apical cell surface and distributed along the highly curved outline of typhlosole cells (Fig. 11A). The wide cells around the typhlosole channels contain relatively short densely stacked microtubule bundles in the apical cytosol that ramify in the apical direction (Fig. 11A). In the posterior part of the anterior chamber at the transition to the papillate region the microtubule bundles are the most extensive. The largest microtubule bundles were observed in anvil shaped typhlosole and cells around the typhlosole channels in this transition region as well as in high dome shaped ventral cells (Fig. 11B). In the papillate region all hindgut cells contain fine apico-basal microtubule bundles which appear thinner than in the anterior chamber (Fig. 11C). The microtubule bundles in the papillate region apically protrude into the thin cytoplasmic projections between the infoldings of apical labyrinth and are closely associated with numerous mitochondria. The presence of microtubules in the cytoplasmic projections between the apical infoldings and the association with mitochondria have been reported also in *O. asellus* and *A. vulgare* (Witkus et al. 1969; Vernon et al. 1974). In the papillate region numerous cross-sectioned microtubules near the septate junctions are evident, which likely form a subapical circumferential ring of microtubules perpendicular to the apico-basal ones, but further 3D characterization is needed to verify this assumption. In the sphincter region and rectum, the apico basal microtubule bundles are concentrated in the apical cytosol (Fig. 11D).

In the anterior chamber the most prominent bundles of microtubules are observed in parts of the hindgut epithelium that are associated with extensive visceral muscles. One example are the typhlosole cells, where distinct muscles insert into the typhlosole fold from the basal side (Hames and Hopkin 1989). Another example is the posterior part of the anterior chamber at the transition to the papillate region where extensive network of circular and longitudinal muscles surrounds the hindgut (Vernon et al. 1974; Palackal et al. 1984; Hames and Hopkin 1989). Together with other cytoskeletal elements the microtubules can resist mechanical forces acting on cells and are thus important for the maintenance of cell shape (Wang et al. 2001; Brangwynne et al. 2006; Picone et al. 2010; Sato et al. 2013; Kubitschke et al. 2017). The microtubules are particularly abundant in the tenocytes of arthropods (Nakazawa et al. 1992; Tucker et al. 2004; Criel et al. 2005; Žnidaršič et al. 2012) and cochlear supporting cells of mammals (Tucker et al. 1993; Henderson et al. 1995), which are, similar as the hindgut cells in the anterior chamber of terrestrial isopods, under constant mechanical stress. The extensive microtubule bundles in the typhlosole cells and cells around the typhlosole channels might be involved in the maintenance of the very complex shape of these cells that form the typhlosole fold. This together with the contractions of muscles that insert into the typhlosole fold enables the opening and closing of the typhlosole channels, which is important for the transport of partly digested liquid food during the feeding cycle of terrestrial isopods (Hames and Hopkin 1989). In cells of the lateral and ventral hindgut wall and particularly in large ventral cells in the posterior part of the anterior chamber the apico-basal bundles of microtubules are likely important for the support of the apically dome shaped cells. Accordingly, the hindgut cells can maintain their shape during the peristaltic contractions of extensive musculature surrounding the hindgut, which squeezes the partly digested liquid food into the typhlosole channels and then pushes it anteriorly into the stomach for the filtration and transport into the digestive glands where the absorption takes place (Hames and Hopkin 1989).

In the papillate region the bundles of microtubules protrude deep into the cytoplasmic projections between the infoldings of the apical plasma membrane. In the more posterior sphincter region and rectum the microtubule bundles are concentrated in the apical cytosol where the deep infoldings of apical labyrinth are present in these cells. Thus, the microtubule bundles in the papillate region, sphincter region and rectum could play a role in the stabilization of the deep infoldings of the apical plasma membrane labyrinth, which is important for the function of the papillate region in ion and water transport (Vernon et al. 1974; Coruzzi et al. 1982; Palackal et al. 1984). It is known from studies in different organisms that microtubules with associated molecular motors can deform membranes and are thus important for the positioning and shaping of different cell membranes (Stephens 2012). In cultured mammalian cells it has been shown that the microtubules together with the associated molecular motors also drive the formation of tubular invaginations in the plasma membrane during clathrin independent endocytosis (Day et al. 2015). Microtubules in hindgut cells in the papillate region are in close contact with numerous mitochondria present in the apical cytosol of these cells, which are also aligned in the same apico-basal direction as the microtubules. This is consistent with the role of microtubules in the transport, positioning and shaping of various cell organelles (Tolić-Nørrelykke 2008; Stephens 2012) including the mitochondria (Fujita et al. 2007; Fernández Casafuz et al. 2023).

Reorganization of microtubules in relation to hindgut morphogenesis in late embryonic and early postembryonic development

We have characterized the arrangement of microtubules in the hindgut of late-embryos and postembryonic developmental stages and thus provided new data of microtubule arrangement during hindgut morphogenesis in crustaceans. We have observed that the microtubules in the hindgut cells of *P. scaber* organize into distinct apico-basal bundles relatively late during development. In late embryos in stage S18 distinct apico-basal bundles of microtubules are present only in the most posterior part of the hindgut epithelium, in the rectum near the anal opening. Distinct apico-basal bundles of microtubules are evident in the cells of all hindgut regions in the early postembryonic stages of marsupial mancae. The microtubule bundles are gradually increased in length and thickness in postmarsupial mancae. One of the crucial aspects associated with epithelial differentiation and morphogenesis is the rearrangement of the cytoskeletal system in combination with the remodeling of cell junctions. Particularly well understood is the involvement of the actin-myosin cytoskeleton and its contractility in epithelial cell shape change and morphogenetic processes (Lecuit and Lenne 2007). More recent studies have shown that the microtubules also play an important role in cell shape changes and tissue remodeling (Matis 2020), and the involvement of microtubular cytoskeleton in the morphogenesis of invertebrate epithelia is currently an active research topic (Röper 2020). Recent research in *Drosophila melanogaster* Meigen, 1830 has elucidated that the reorganization of microtubules plays important roles in remodeling of epithelial sheets during morphogenesis. The microtubules promote or directly cause various cell shape changes needed for epithelial folding (Takeda et al. 2018; Ko et al. 2019).

Our data on morphogenesis show that the formation of typhlosole encompass drastic cell shape changes. In late embryos when only a primordium of the typhlosole is present in the anterior hindgut just thin microtubule bundles or possibly single microtubules are present in the cytosol of hindgut cells. Later in marsupial mancae apico-basal microtubule bundles become apparent in dorsal cells that already form distinct folds of the typhlosole and two typhlosole channels. In postmarsupial mancae the apico-basal bundles of microtubules are further expanded and elaborated when the posterior section of the typhlosole widens and becomes anvil shaped. Our results show that the reorganization of microtubules closely coincides with shape changes of typhlosole cells.

Conclusions

All cells in adult *P. scaber* hindgut contain abundant apico-basally oriented microtubules. The architecture of these microtubule bundles varies considerably in different parts of the hindgut epithelium along the anterior-posterior axis. In the anterior chamber the thick microtubule bundles are probably involved in the maintenance of cell shape and resistance to the compressive mechanical forces during peristaltic contractions of the hindgut muscles. Specific arrangement of microtubule bundles is evident in the morphologically complex typhlosole fold. In the papillate region the microtubule bundles protrude into the cytoplasmic projections between the deep apical plasma membrane infoldings and might be involved in the stabilization of the apical plasma membrane infoldings and serve in the transport and tethering of mitochondria. During hindgut morphogenesis the apico-basal microtubule bundles are established relatively late, mainly during early postembryonic development. Morphogenesis of the typhlosole is characterized by coinciding changes in cell shape and microtubule arrangement.

Acknowledgments

This work was financed by the Slovenian Research Agency (ARRS), programme Integrative Zoology and Speleobiology P1-0184. The equipment of the infrastructural centers ‘Microscopy of biological samples’ MRIC I0-0022 (Biotechnical faculty, University of Ljubljana) and I0-0004 IC NIB was used.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

Funding

This work was financed by the Slovenian Research Agency (ARRS), programme Integrative Zoology and Speleobiology P1-0184.

Author contributions

UB and NZ designed the study. UB reared the animals in a laboratory culture. UB and PM prepared the samples for electron and light microscopy. UB performed imaging with light and transmission electron microscopy. PM, JS and NZ contributed to the interpretation of data. UB wrote the first draft of the manuscript. PM, JS and NZ contributed to the text and made numerous comments and recommendations. All authors revised the manuscript and read and approved the final version.

Author ORCIDs

Polona Mrak https://orcid.org/0000-0003-3642-8191
Nada Žnidaršič https://orcid.org/0000-0002-0164-9050

Data availability

All of the data that support the findings of this study are available in the main text.

References

Bacallao R, Antony C, Dotti C, Karsenti E, Stelzer EH, Simons K (1989) The subcellular organization of Madin-Darby canine kidney cells during the formation of a polarized epithelium. The Journal of Cell Biology 109(6): 2817–2832. https://doi.org/10.1083/jcb.109.6.2817

Bartolini F, Gundersen GG (2006) Generation of noncentrosomal microtubule arrays. Journal of Cell Science 119(20): 4155–4163. https://doi.org/10.1242/jcs.03227

Bettica A, Witkus R, Vernon GM (1987) Ultrastructure of the foregut-hindgut junction in *Porcellio scaber* Latreille. Journal of Crustacean Biology 7(4): 619–623. https://doi.org/10.2307/1548647

Bogataj U, Praznik M, Mrak P, Štrus J, Tušek-Žnidarič M, Žnidaršič N (2018) Comparative ultrastructure of cells and cuticle in the anterior chamber and papillate region of *Porcellio scaber* (Crustacea, Isopoda) hindgut. ZooKeys 801: 427–458. https://doi.org/10.3897/zookeys.801.22395

Bogataj U, Mrak P, Štrus J, Žnidaršič N (2019) Ultrastructural differentiation of plasma membrane and cell junctions in the hindgut cells is synchronized with key developmental transitions in *Porcellio scaber*. Arthropod Structure & Development 50: 78–93. https://doi.org/10.1016/j.asd.2019.04.004

Bornens M (2002) Centrosome composition and microtubule anchoring mechanisms. Current Opinion in Cell Biology 14(1): 25–34. https://doi.org/10.1016/S0955-0674(01)00290-3

Brangwynne CP, MacKintosh FC, Kumar S, Geisse NA, Talbot J, Mahadevan L, Parker KK, Ingber DE, Weitz DA (2006) Microtubules can bear enhanced compressive loads in living cells because of lateral reinforcement. The Journal of Cell Biology 173(5): 733–741. https://doi.org/10.1083/jcb.200601060

Bré MH, Kreis TE, Karsenti E (1987) Control of microtubule nucleation and stability in Madin-Darby canine kidney cells: The occurrence of noncentrosomal, stable detyrosinated microtubules. The Journal of Cell Biology 105(3): 1283–1296. https://doi.org/10.1083/jcb.105.3.1283

Burnside B (1973) Microtubules and microfilaments in amphibian neurulation. American Zoologist 13(4): 989–1006. https://doi.org/10.1093/icb/13.4.989

Coruzzi L, Witkus R, Vernon GM (1982) Function-related structural characters and their modifications in the hindgut epithelium of two terrestrial isopods, *Armadillidium vulgare* and *Oniscus asellus*. Pathobiology 50(4): 229–240. https://doi.org/10.1159/000163151

Criel GR, Van Oostveldt P, MacRae TH (2005) Spatial organization and isotubulin composition of microtubules in epidermal tendon cells of *Artemia franciscana*. Journal of Morphology 263(2): 203–215. https://doi.org/10.1002/jmor.10298

Day CA, Baetz NW, Copeland CA, Kraft LJ, Han B, Tiwari A, Drake KR, De Luca H, Chinnapen DJ, Davidson MW, Holmes RK, Jobling MG, Schroer TA, Lencer WI, Kenworthy AK (2015) Microtubule motors power plasma membrane tubulation in clathrin-independent endocytosis. Traffic (Copenhagen, Denmark) 16(6): 572–590. https://doi.org/10.1111/tra.12269

Fernández Casafuz AB, De Rossi MC, Bruno L (2023) Mitochondrial cellular organization and shape fluctuations are differentially modulated by cytoskeletal networks. Scientific Reports 13(1): 4065. https://doi.org/10.1038/s41598-023-31121-w

Fletcher DA, Mullins RD (2010) Cell mechanics and the cytoskeleton. Nature 463(7280): 485–492. https://doi.org/10.1038/nature08908

Fujita T, Maturana AD, Ikuta J, Hamada J, Walchli S, Suzuki T, Sawa H, Wooten MW, Okajima T, Tatematsu K, Tanizawa K, Kuroda S (2007) Axonal guidance protein FEZ1 associates with tubulin and kinesin motor protein to transport mitochondria in neurites of NGF-stimulated PC12 cells. Biochemical and Biophysical Research Communications 361(3): 605–610. https://doi.org/10.1016/j.bbrc.2007.07.050

Gelfand VI, Bershadsky AD (1991) Microtubule dynamics: Mechanism, regulation, and function. Annual Review of Cell Biology 7(1): 93–116. https://doi.org/10.1146/annurev.cb.07.110191.000521

Hagios C, Lochter A, Bissell MJ (1998) Tissue architecture: The ultimate regulator of epithelial function? Philosophical Transactions of the Royal Society of London, Series B, Biological Sciences 353(1370): 857–870. https://doi.org/10.1098/rstb.1998.0250

Hames CAC, Hopkin SP (1989) The structure and function of the digestive system of terrestrial isopods. Journal of Zoology (London, England) 217(4): 599–627. https://doi.org/10.1111/j.1469-7998.1989.tb02513.x

Hassall M, Jennings JB (1975) Adaptive features of gut structure and digestive physiology in the terrestrial isopod *Philoscia muscorum* (Scopoli) 1763. The Biological Bulletin 149(2): 348–364. https://doi.org/10.2307/1540531

Henderson CG, Tucker JB, Mogensen MM, Mackie JB, Chaplin MA, Slepecky NB, Leckie LM (1995) Three microtubule-organizing centres collaborate in a mouse cochlear epithelial cell during supracellularly coordinated control of microtubule positioning. Journal of Cell Science 108(1): 37–50. https://doi.org/10.1242/jcs.108.1.37

Holdich DM, Ratcliffe NA (1970) A light and electron microscope study of the hindgut of the herbivorous isopod, *Dynamene bidentata* (Crustacea: Peracarida). Cell and Tissue Research 111(2): 209–227. https://doi.org/10.1007/BF00339786

Ko CS, Tserunyan V, Martin AC (2019) Microtubules promote intercellular contractile force transmission during tissue folding. The Journal of Cell Biology 218(8): 2726–2742. https://doi.org/10.1083/jcb.201902011

Kubitschke H, Schnauss J, Nnetu KD, Warmt E, Stange R, Kaes J (2017) Actin and microtubule networks contribute differently to cell response for small and large strains. New Journal of Physics 19(9): 093003. https://doi.org/10.1088/1367-2630/aa7658

Lecuit T, Lenne PF (2007) Cell surface mechanics and the control of cell shape, tissue patterns and morphogenesis. Nature Reviews Molecular Cell Biology 8(8): 633–644. https://doi.org/10.1038/nrm2222

Matis M (2020) The mechanical role of microtubules in tissue remodeling. BioEssays 42(5): e1900244. https://doi.org/10.1002/bies.201900244

Mays RW, Beck KA, Nelson WJ (1994) Organization and function of the cytoskeleton in polarized epithelial cells: A component of the protein sorting machinery. Current Opinion in Cell Biology 6(1): 16–24. https://doi.org/10.1016/0955-0674(94)90111-2

Milatovič M, Kostanjšek R, Štrus J (2010) Ontogenetic development of *Porcellio scaber*: Staging based on microscopic anatomy. Journal of Crustacean Biology 30(2): 225–235. https://doi.org/10.1651/09-3189.1

Mimori-Kiyosue Y (2011) Shaping microtubules into diverse patterns: Molecular connections for setting up both ends. Cytoskeleton 68(11): 603–618. https://doi.org/10.1002/cm.20540

Mrak P, Znidaršič N, Tušek-Žnidarič M, Klepal W, Gruber D, Strus J (2012) Egg envelopes and cuticle renewal in *Porcellio* embryos and marsupial mancas. ZooKeys 176: 55–72. https://doi.org/10.3897/zookeys.176.2418

Mrak P, Bogataj U, Štrus J, Žnidaršič N (2015) Formation of the hindgut cuticular lining during embryonic development of *Porcellio scaber* (Crustacea, Isopoda). ZooKeys 515: 93–109. https://doi.org/10.3897/zookeys.515.9468

Muroyama A, Lechler T (2017) Microtubule organization, dynamics and functions in differentiated cells. Development (Cambridge, England) 144(17): 3012–3021. https://doi.org/10.1242/dev.153171

Müsch A (2004) Microtubule organization and function in epithelial cells. Traffic (Copenhagen, Denmark) 5(1): 1–9. https://doi.org/10.1111/j.1600-0854.2003.00149.x

Nakazawa E, Katoh K, Ishikawa H (1992) The association of microtubules with the plasmalemma in epidermal tendon cells of the river crab. Biology of the Cell 75(2): 111–119. https://doi.org/10.1016/0248-4900(92)90130-S

Palackal T, Faso L, Zung JL, Vernon G, Witkus R (1984) The ultrastructure of the hindgut epithelium of terrestrial isopods and its role in osmoregulation. Symposia of the Zoological Society of London 53: 185–198.

Picone R, Ren X, Ivanovitch KD, Clarke JD, McKendry RA, Baum B (2010) A polarised population of dynamic microtubules mediates homeostatic length control in animal cells. PLoS Biology 8(11): e1000542. https://doi.org/10.1371/journal.pbio.1000542

Röper K (2020) Microtubules enter centre stage for morphogenesis. Philosophical Transactions of the Royal Society B Biological Sciences 375(1809): 20190557. https://doi.org/10.1098/rstb.2019.0557

Sato Y, Akitsu M, Amano Y, Yamashita K, Ide M, Shimada K, Yamashita A, Hirano H, Arakawa N, Maki T, Hayashi I, Ohno S, Suzuki A (2013) The novel PAR-1-binding protein MTCL1 has crucial roles in organizing microtubules in polarizing epithelial cells. Journal of Cell Science 126(20): 4671–4683. https://doi.org/10.1242/jcs.127845

Schöck F, Perrimon N (2002) Molecular mechanisms of epithelial morphogenesis. Annual Review of Cell and Developmental Biology 18(1): 463–493. https://doi.org/10.1146/annurev.cellbio.18.022602.131838

Shires R, Lane NJ, Inman CB, Lockwood AP (1994) Structural changes in the gill cells of *Gammarus duebeni* (Crustacea, Amphipoda) under osmotic stress; with notes on microtubules in association with the septate junctions. Tissue & Cell 26(5): 767–778. https://doi.org/10.1016/0040-8166(94)90059-0

Shires R, Lane NJ, Inman CB, Lockwood AP (1995) Microtubule systems associated with the septate junctions of the gill cells of four gammarid amphipods. Tissue & Cell 27(1): 3–12. https://doi.org/10.1016/S0040-8166(95)80003-4

Stephens DJ (2012) Functional coupling of microtubules to membranes - implications for membrane structure and dynamics. Journal of Cell Science 125(12): 2795–2804. https://doi.org/10.1242/jcs.097675

Storch V (1987) Microscopic anatomy and ultrastructure of the stomach of *Porcellio scaber* (Crustacea, Isopoda). Zoomorphology 106(5): 301–311. https://doi.org/10.1007/BF00312004

Storch V, Štrus J (1989) Microscopic anatomy and ultrastructure of the alimentary canal in terrestrial isopods. In: Ferrara F, et al. (Eds) Monitore Zoologico Italiano. Monografia 4: 105–126.

Štrus J, Drobne D, Ličar P (1995) Comparative anatomy and functional aspects of the digestive system in amphibious and terrestrial isopods (Isopoda: Oniscidea). In: Alikhan MA (Ed.) Terrestrial isopod biology. A.A. Balkema, Rotterdam, 15–23.

Štrus J, Klepal W, Repina J, Tusek-Znidaric M, Milatovic M, Pipan Z (2008) Ultrastructure of the digestive system and the fate of midgut during embryonic development in *Porcellio scaber* (Crustacea: Isopoda). Arthropod Structure & Development 37(4): 287–298. https://doi.org/10.1016/j.asd.2007.11.004

Štrus J, Žnidaršič N, Mrak P, Bogataj U, Vogt G (2019) Structure, function and development of the digestive system in malacostracan crustaceans and adaptation to different lifestyles. Cell and Tissue Research 377(3): 415–443. https://doi.org/10.1007/s00441-019-03056-0

Surbida KL, Wright JC (2001) Embryo tolerance and maternal control of the marsupial environment in *Armadillidium vulgare* (Isopoda: Oniscidea). Physiological and Biochemical Zoology 74(6): 894–906. https://doi.org/10.1086/324474

Takeda M, Sami MM, Wang YC (2018) A homeostatic apical microtubule network shortens cells for epithelial folding via a basal polarity shift. Nature Cell Biology 20(1): 36–45. https://doi.org/10.1038/s41556-017-0001-3

Tolić-Nørrelykke IM (2008) Push-me-pull-you: How microtubules organize the cell interior. European Biophysics Journal 37(7): 1271–1278. https://doi.org/10.1007/s00249-008-0321-0

Tomescu N, Craciun C (1987) Postembryonic ontogenetic development in *Porcellio scaber* (Crustacea: Isopoda). Pedobiologia 30(5): 345–350. https://doi.org/10.1016/S0031-4056(23)00385-2

Toya M, Takeichi M (2016) Organization of non-centrosomal microtubules in epithelial cells. Cell Structure and Function 41(2): 127–135. https://doi.org/10.1247/csf.16015

Tucker JB, Paton CC, Henderson CG, Mogensen MM (1993) Microtubule rearrangement and bending during assembly of large curved microtubule bundles in mouse cochlear epithelial cells. Cell Motility and the Cytoskeleton 25(1): 49–58. https://doi.org/10.1002/cm.970250107

Tucker JB, Mackie JB, Cottam DM, Rogers-Bald MM, Macintyre J, Scarborough JA, Milner MJ (2004) Positioning and capture of cell surface-associated microtubules in epithelial tendon cells that differentiate in primary embryonic *Drosophila* cell cultures. Cell Motility and the Cytoskeleton 57(3): 175–185. https://doi.org/10.1002/cm.10167

Vernon GM, Herold L, Witkus ER (1974) Fine structure of the digestive tract epithelium in the terrestrial isopod *Armadillidium vulgare*. Journal of Morphology 144(3): 337–359. https://doi.org/10.1002/jmor.1051440307

Wägele JW, Welsch U, Müller W (1981) Fine structure and function of the digestive tract of *Cyathura carinata* (Krøyer) (Crustacea, Isopoda). Zoomorphology 98: 69–88. https://doi.org/10.1007/BF00310321

Wang N, Naruse K, Stamenović D, Fredberg JJ, Mijailovich SM, Tolić-Nørrelykke IM, Polte T, Mannix R, Ingber DE (2001) Mechanical behavior in living cells consistent with the tensegrity model. Proceedings of the National Academy of Sciences of the United States of America 98(14): 7765–7770. https://doi.org/10.1073/pnas.141199598

Witkus ER, Grillo RS, Smith WJ (1969) Microtubule bundles in the hindgut epithelium of the woodlouse *Oniscus asellus*. Journal of Ultrastructure Research 29(3): 182–190. https://doi.org/10.1016/S0022-5320(69)90099-9

Wolff C (2009) The embryonic development of the malacostracan crustacean *Porcellio scaber* (Isopoda, Oniscidea). Development Genes and Evolution 219(11–12): 545–564. https://doi.org/10.1007/s00427-010-0316-6

Žnidaršič N, Mrak P, Tušek-Žnidarič M, Strus J (2012) Exoskeleton anchoring to tendon cells and muscles in molting isopod crustaceans. ZooKeys 176: 39–53. https://doi.org/10.3897/zookeys.176.2445

ZooKeys 1225: 33–47 (2025)
DOI: 10.3897/zookeys.1225.118414

Research Article

Morphological analysis of regenerated antennae in the isopod *Porcellio scaber* (Isopoda, Crustacea), with emphasis on the main sensory structures

Primož Zidar[1], Primož Mihelič[1]

[1] *Department of Biology, Biotechnical Faculty, University of Ljubljana, Večna pot 111, SI-1000 Ljubljana, Slovenia*
Corresponding author: Primož Zidar (primoz.zidar@bf.uni-lj.si)

Abstract

The second antennae, an important sensory organ of terrestrial isopods, are often attacked and amputated by predators or members of the same species. If an amputation does happen, the antenna usually regenerates after the very first or the next moult that follows. However, the new regenerated antenna is smaller than the original one. This raises the question of whether a smaller regenerated antenna is potentially also less functional as the sensory elements of the antenna undergo regeneration as well. In this study, the regeneration process of the second antenna of *Porcellio scaber* was followed after two consecutive amputations. The original antennae were compared with the regenerated ones under light and scanning electron microscopes in view of the dimensions of segments and the size and number of sensory elements on them. As expected, all regenerated antennae were reduced in size, however, the reduction of different antennal segments was different. The second segment of the flagellum was reduced by almost half as much as the first segment of the flagellum or the last segment of the peduncle. After the next consecutive amputation, the reduction of the regenerated antennae does not increase further. The size and shape of the apical organ and lateral seta did not change during the regeneration process, neither after the first nor after the next amputation. In contrast, the number of plural receptor elements, such as aesthetascs and tricorn sensilla, decreases in accordance with reduced segment size. Therefore, since only the number of the most common sensory structures is reduced during regeneration, the regenerated antenna probably retains its sensory functionality.

Key words: Aesthetascs, apical organ, autotomy, moulting cycle, regeneration, SEM, tricorn sensilla, woodlice

Academic editor: Ivan H. Tuf
Received: 8 January 2024
Accepted: 27 February 2024
Published: 5 February 2025

ZooBank: https://zoobank.org/37E163AD-3648-4BF4-8E70-F935EF629C74

Citation: Zidar P, Mihelič P (2025) Morphological analysis of regenerated antennae in the isopod *Porcellio scaber* (Isopoda, Crustacea), with emphasis on the main sensory structures. In: Tuf IH, Tajovský K, Taiti S (Eds) The Biology of Terrestrial Isopods, XII. ZooKeys 1225: 33–47. https://doi.org/10.3897/zookeys.1225.118414

Introduction

Epimorphic regeneration, by which an animal restores damaged or lost body parts, is a characteristic of many arthropods (reviewed in Hopkins and Das 2015). Many of them can regenerate limbs that were lost after injury or amputation, but not many can do that as adults (reviewed in Maruzzo and Bortolin 2013). Namely, a limb cannot be regenerated into a functional structure without undergoing one or more moults and many crustaceans, like most decapods, amphipods and isopods, moult throughout their life, however, the frequency of moulting may change with age (Skinner 1985).

Porcellio scaber (Latreille, 1804) is a common terrestrial isopod (Isopoda, Oniscidea) native to continental Europe, but distributed on all continents except Antarctica (Riggio 2013). In its environment, it may be attacked by predators or members of its own species and their second antennae can easily be damaged or lost (Schmalfuss 1998). Namely, as in other members of suborder Oniscidea, the second antenna of *P. scaber* is much larger than the extremely diminished first antenna and is also the most exposed limb (Schmalfuss 1998). The second antenna of *P. scaber*, as in most terrestrial isopods, consists of a five-jointed peduncle and a two-jointed flagellum and is considered the main sensory organ (Hoese 1989; Schmalfuss 1998). They possess several different sensory structures: an apical organ with a mechano- and chemoreceptory function (Mead et al. 1976), several aesthetascs with a chemoreceptory function (Hoese 1989) and many tricorn sensilla with a potentially hygroreceptory (Price and Holdich 1980) and/or mechanoreceptory function (Friedlander 1964; Ziegler and Altner 1995). The loss of the second antenna is undoubtedly a handicap for the animal due to its importance; therefore, the successful and rapid regeneration of the antenna is extremely important. Madhavan and Madhavan (1981) demonstrated that the amputation of one or both antennae of *Armadillidium vulgare* (Latreille, 1804) in an earlier intermoult phase leads to a shortening of the moult cycle. Namely, moulting and regeneration are regulated by the same steroid hormones called ecdysteroids. Low levels of ecdysteroids observed after limb loss are thought to facilitate the initial stages of regeneration, while high levels inhibit it (Madhavan and Schneiderman 1969; Maruzzo and Bortolin 2013).

Regeneration of a damaged limb often begins with an autotomy of an injured part of the limb at a predetermined breakage point (PBP) proximal to the injury (Maruzzo et al. 2005; Hopkins and Das 2015). Autotomy minimises blood loss and ensures faster regeneration with fewer irregularities (Bliss 1960; Maruzzo et al. 2005). Klintz (1907) documented two such PBPs on the second antenna of *P. scaber*: one between the first and second peduncular segment and the other between the fourth and fifth peduncular segment. Which of the two PBPs was active depended on which segment the antenna was originally cut off. However, the regeneration of the last segment of the flagellum takes place without any triggered autotomy (Klintz 1907). In terrestrial isopods the regenerating structure develops within the antenna stub (Klintz 1907; Madhavan and Madhavan 1981), similarly as described in cockroach legs (Goss 1969; Maruzzo and Bortolin 2013). The regenerated limb first appears outside the stub after the very first or the next moult after amputation, all depending on the period of the intermoult cycle in which the limb was amputated (Madhavan and Madhavan 1981; Hopkins and Das 2015). According to Klintz (1907), the regenerated antenna is smaller, only 2/3 the length of the original one. This raises the question of whether a smaller regenerated antenna is potentially also less functional as sensory elements of the antenna undergo regeneration as well.

In this study, we aimed to determine the degree of reduction of the second antenna length and the number and size of antennal sensory elements in *P. scaber* after multiple amputations. We hypothesised that: (1) the regenerated antenna will be shorter after each successive amputation as limb regeneration requires a significant investment of energy (Hopkins and Das 2015); and (2) the number and size of antennal sensory elements will be reduced with the reduction of antennal segments as was reduced on regenerated legs in the amphipod *Parhyale hawaiensis* (Almazán et al. 2022). At the same time, by measuring the length of the moulting cycle, we tried to find out whether the length of the moulting cycle changes after limb amputation as reported for *A. vulgare* (Madhavan and Madhavan 1981).

Materials and methods

Experimental animals

Adult males of *P. scaber* of comparable size (10 ± 0.5 mm) were used in the study. All animals originated from the same laboratory-bred population. Due to sexual dimorphism, which is also reflected in the dimensions of the antennae (Lefebvre et al. 2000), females were excluded. The selected animals were kept in an incubator under the following conditions: 14 h day and 10 h night, 20 ± 0.5 °C, with prior gradual acclimatisation to these conditions. The humidity in the containers was maintained by hydrating the plaster that covered the bottom of the containers. Animals were fed with maple leaves and carrots. The animals were dorsally marked with a coloured permanent marker, which allowed us to easily notice when animals were moulted. The day after a successful moulting, the animals were marked again.

One day after moulting, the left second antennae of the animals were amputated in the middle of the third segment of the peduncle (P3) and fixed in 70% ethanol. In animals that successfully regenerated the amputated antennae, the regenerated antennae were re-amputated the day after moulting and fixed in the aforementioned manner. In animals that successfully regenerated the amputated antennae for a second time, the regenerated antennae were amputated again and fixed. In total, 11 original antennae (orig), 11 regenerated antennae of the first generation (reg1) and 9 regenerated antennae of the second generation (reg2) were analysed.

Measurements

The animals were monitored for several months and the time between two successful moultings was recorded. The length of the moulting cycles of control animals, whose antennae were not amputated, were compared with the length of the moulting cycles of animals with amputated antennae. Some animals with amputated antennae were observed daily with a stereo microscope (Leica EZ4, Leica Microsystems, Germany) and all morphological changes at the amputation site were documented.

All amputated antennae were first analysed with a stereo microscope (Leica MZ FLIII, Leica Microsystems, Germany) using the Leica application suite (LAS) software (Leica Microsystems, Germany) for image capture. The maximum length and width of the last three segments of the antennae, that is, the 5th segment of the peduncle (P5) and the first (F1) and second segment of the flagellum (F2), were measured using the Fiji image processing package of ImageJ2

(Schindelin et al. 2012). The length of P5 and F1 was measured between two joints, and the length of F2 between the joint and the base of the apical organ. The width was measured at the widest part of the segment. The 4th segment of the peduncle (P4) was used for manipulation of the antenna and to attach it on a holder for SEM analysis.

The amputated antennae (orig, reg1 and reg2) of seven males were dried with hexamethyldisilazane (HMDS, Merck, Germany). The individual antenna was attached to a metal holder (SPI supplies, USA) using adhesive aluminium foil (Fig. 1). This allows a greater rotation of the sample in the microscope. The samples were coated with platinum (up to 14 nm) with a sputter coating machine (SCD 050, BAL-TEC, USA). Such prepared samples were analysed with SEM (JEOL JSM-7500F, Japan). Images made with SEM were analysed in the ImageJ program with the Fiji package. The length of the apical organ (Fig. 1A), the number of aesthetascs (Fig. 1B) and the maximum density of tricorn sensilla on the lateral side of F2 (Fig. 1C) were analysed. The number of tricorn sensilla was determined by fitting the maximum number of sensilla bases into a square with sides of 100 μm (0.01 mm^2). Additionally, the length of the seta on the lateral side of the fifth segment of the peduncle (P5 seta) (Fig. 1D) was analysed as well.

Statistics

The Wilcoxon signed ranks test for dependent samples was used to compare the parameters measured on the original and regenerated antennae. Namely, we assumed that in an animal with a relatively shorter and narrower original antennae, the regenerated antennae are probably relatively shorter and narrower as well. We assumed the same for the sensory structures on the antennae. The length of the moulting cycle of control animals and the length of the moulting cycle after antennal amputation were compared using the Mann-Whitney test. The correlation between the parameters was determined using the Spearman correlation coefficient. All analyses were performed with IBM SPSS Statistics for Windows, v. 28.0.

Results

Pattern of antennae regeneration and the length of the moult cycle

In less than a minute after amputation of the antenna, the flow of hemolymph from the wound had stopped. In the next 24 h, autotomy occurred in 100% and 80% of the cases after the first and second consecutive amputation, respectively (Fig. 2). The PBP was at the junction between the first (basis) and second (coxa) segment of the peduncle. After autotomy, the wound on the basis segment was closed by a pigmented scab. In two animals (out of nine) autotomy did not occur after the second consecutive amputation, and a damaged third peduncular segment remained on the antenna residue until moulting. In all cases, with or without autotomy, no other morphological changes of the antenna stump were noticed until moulting. The regenerated antenna, in all cases, contained all segments but was less pigmented and visibly smaller compared to the other, undamaged antenna.

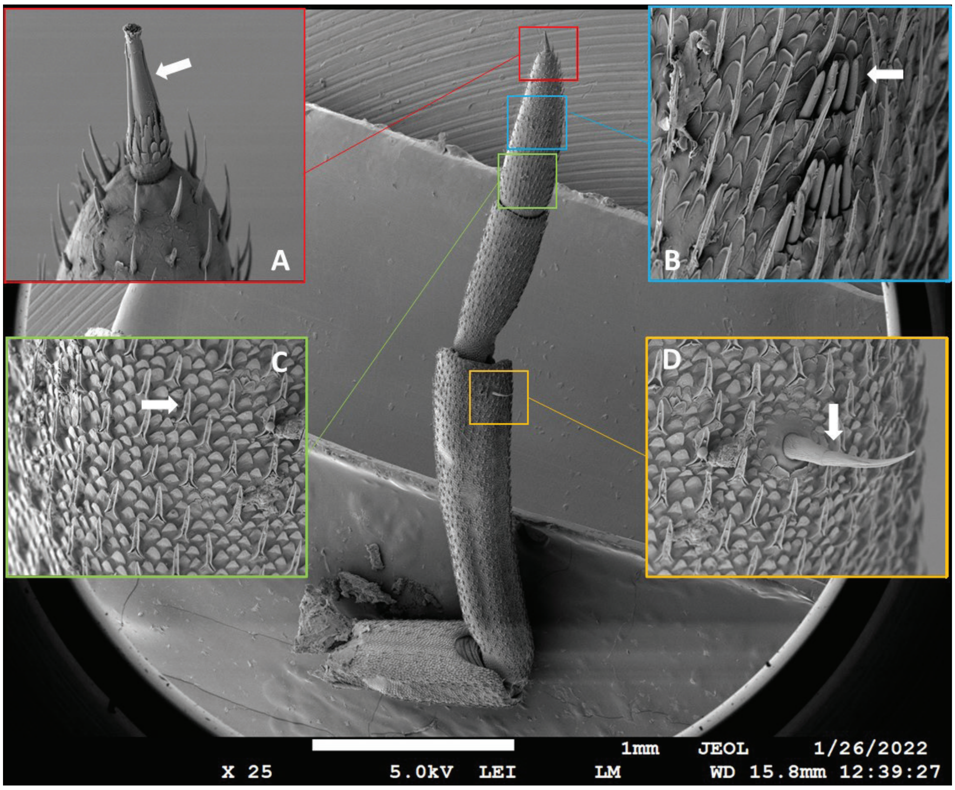

Figure 1. Sense organs on the second antenna in *Porcellio scaber* **A** apical organ (arrow) **B** aesthetascs (arrow) **C** tricorn sensillum (arrow) **D** lateral seta on the fifth peduncular segment (arrow).

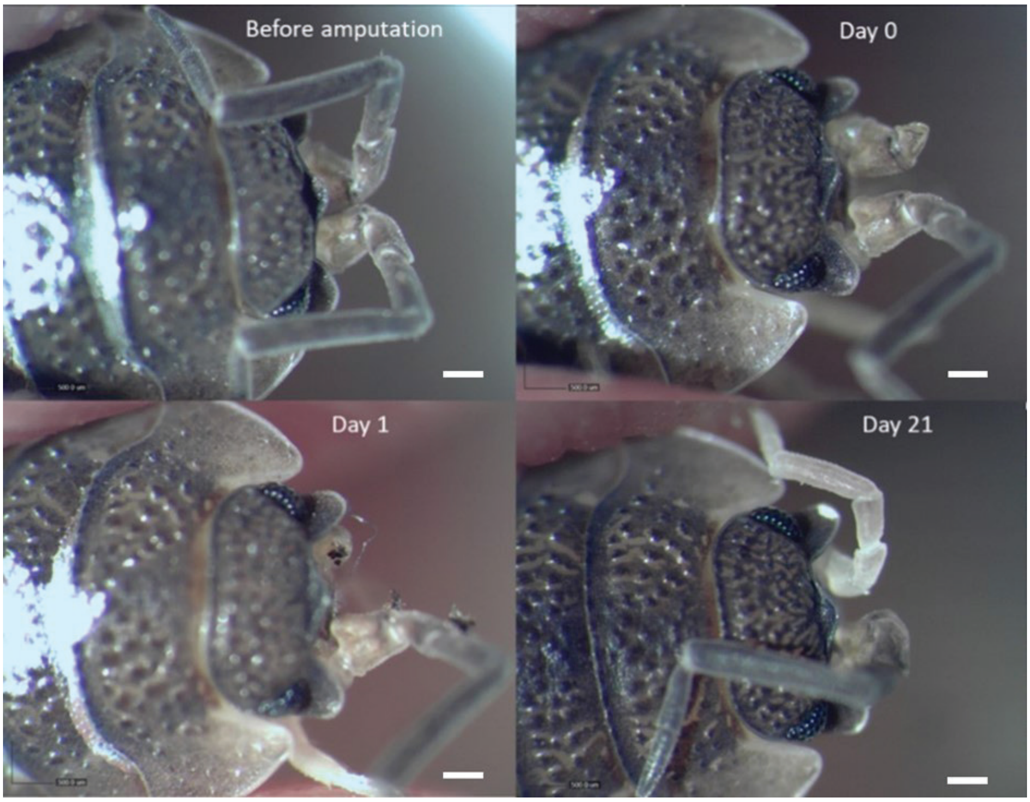

Figure 2. The regeneration process of the second antennae after amputation in *Porcellio scaber*. Before amputation: The animal with undamaged antennae; Day 0: The left antenna amputated in the middle of the third segment; Day 1: The left antenna after autotomy; Day 21: The regenerated left antenna. Scale bar: 0.5 mm.

The length of the moult cycle did not differ between control animals and amputees (Mann-Whitney test, $p = 0.321$). The moult cycle lasted 16–32 days in animals without amputation (AVR = 24 days, SD = 5, $N = 10$) and 17–36 days in amputees (AVR = 26 days, SD = 5, $N = 25$).

Dimensions of the second antennae

The measured part of the regenerated antennae was compared to the original ones, on average 32% and 37% shorter after the first and second regeneration, respectively. All three measured antennal segments were significantly shorter in all regenerated antennae (Wilcoxon signed ranks test, $p < 0.01$) (Fig. 3A). There were no significant differences in the length of the segments between the regenerated antennae of the first and second regeneration (reg1 and reg2; Wilcoxon signed ranks test, $p > 0.069$).

The reduction of the seventh flagellar segment (F2) was significantly (Wilcoxon signed ranks test, $p < 0.01$) smaller compared to the fifth and sixth segments (P5 and F1). After the first regeneration, P5 and F1 shortened by about 35%, while F2 only shortened by about 20%. After the second regeneration, P5 and F1 shortened by about 40%, while F2 only shortened by about 24%.

Individual segments of the regenerated antennae were significantly narrower than the original ones (Wilcoxon signed ranks test, $p < 0.01$) (Fig. 4A). There were no differences in segments' width between the regenerated antennae of first and second regeneration.

The width reduction was the highest in P5 after the second regeneration (Fig. 4B). The width reduction of P5 was significantly higher only compared to that of F2 (Wilcoxon signed ranks test, $p < 0.05$).

Analysis of sense organs

Apical organ

The apical organ of all regenerated antennae had a characteristic structure (Fig. 1A): the basal part arising from the indentation of the distal part of the last flagellar segment and continuing into the conical apical part with a tuft of short terminal hairs and two lateral sensilla lying close to the apical part.

After the first regeneration, the length of the apical organ on the regenerated antennae (reg1) did not differ from the original length of the organ (Fig. 5). In three animals, the apical organs were a few percent longer (Fig. 5B). After the second regeneration (reg2), the newly formed apical organs were shorter than the originals in all animals (Wilcoxon signed ranks test, $p < 0.05$). On average, the length of the apical organs after the second regeneration decreased by 9% (Fig. 5B).

Aesthetascs

Aesthetascs on the last flagellar segment of the antennae (F2) were present in 2–4 groups of 2–6 units (12 in one case) and individually, up to 4 units per segment. The number and spatial distribution of aesthetascs on F2 differed between animals as well as between original and regenerated antennae of the same animal (Fig. 6A). On the regenerated antennae there were significantly

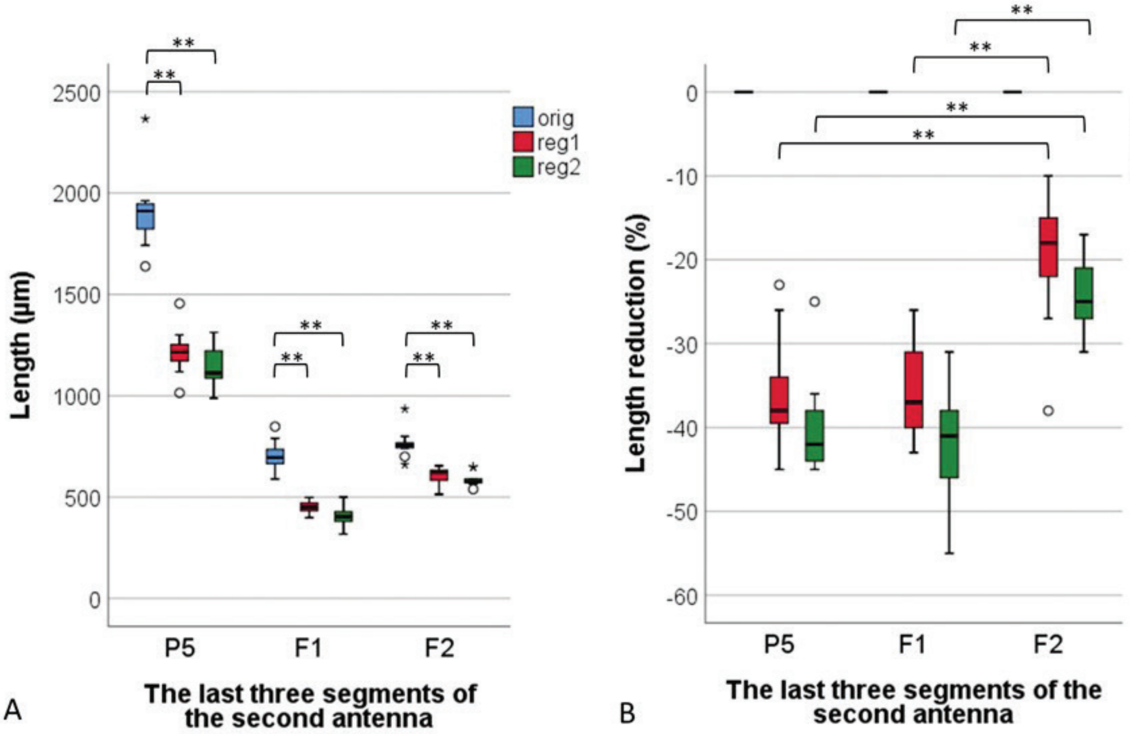

Figure 3. Length (**A**) and length reduction (**B**) of the last three segments (P5, F1 in F2) of the original antennae (orig), regenerated antennae after first regeneration (reg 1) and regenerated antennae after the second regeneration (reg2) of *Porcellio scaber*. Key: box: 25th, 50th, and 75th percentile; whiskers: value ≤ 1.5 IQR (interquartile range); o – outlier: 3 IQR ≤ value > 1.5 IQR; * – extreme: value > 3 IQR; ** – significantly different, $p < 0.01$.

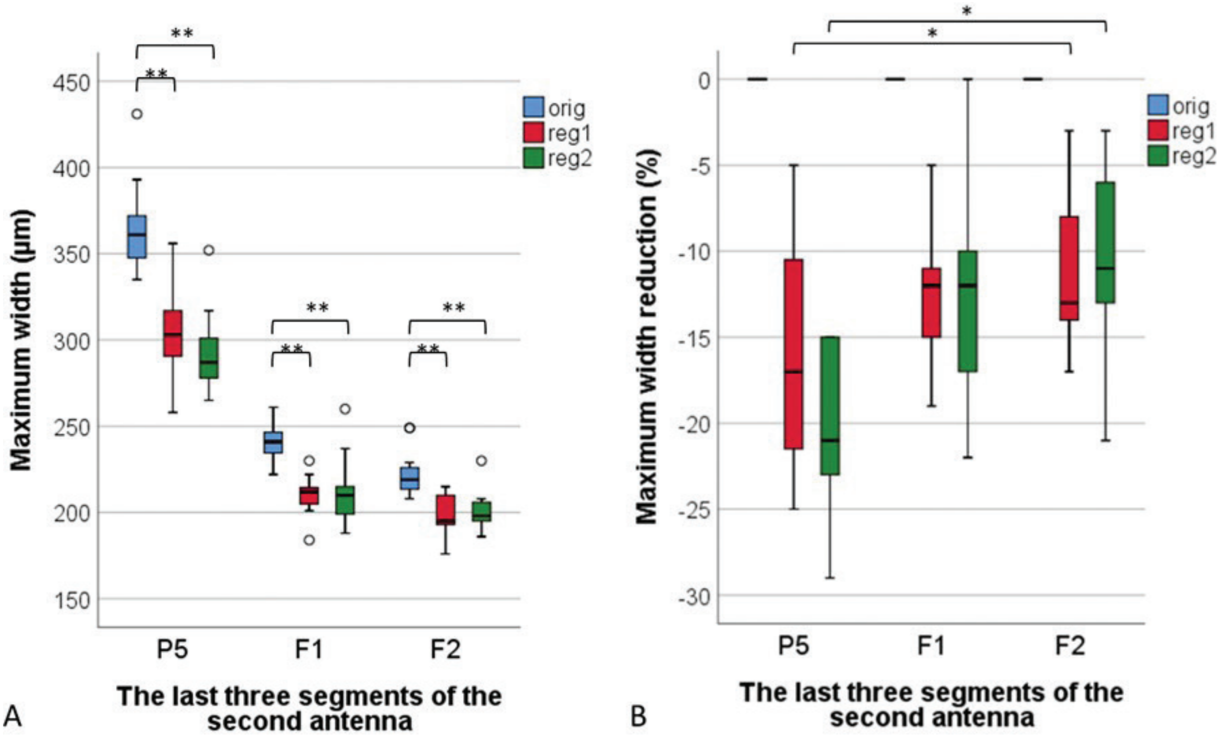

Figure 4. Width (**A**) and reduction of width (**B**) of the last three segments (P5, F1 in F2) of the original antennae (orig), regenerated antennae after first regeneration (reg 1) and regenerated antennae after the second regeneration (reg2) of *Porcellio scaber*. Key: box: 25th, 50th, and 75th percentile; whiskers: value ≤ 1.5 IQR; o – outlier: 3 IQR ≤ value > 1.5 IQR; * – significantly different, $p < 0.05$; ** – as previous, but $p < 0.01$.

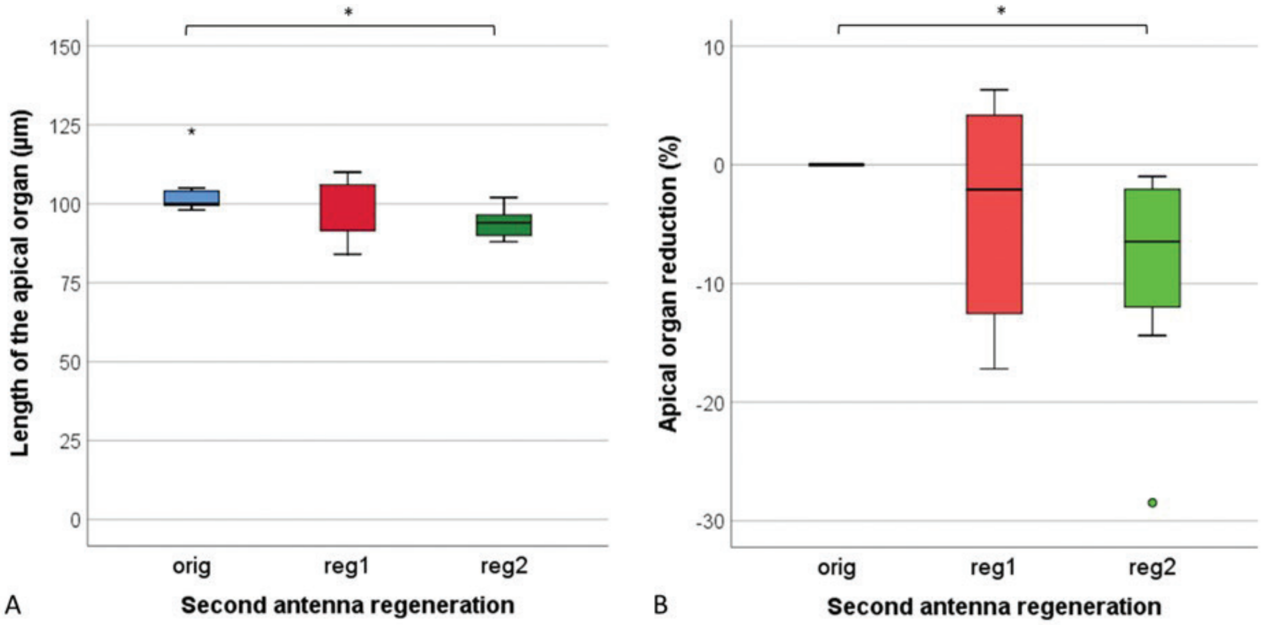

Figure 5. Length (**A**) and length reduction (**B**) of the apical organ on the last segment of the original antennae (orig), regenerated antennae after first regeneration (reg 1) and regenerated antennae after second regeneration (reg2) in *Porcellio scaber*. Key: box: 25th, 50th, and 75th percentile; whiskers: value ≤ 1.5 IQR; o – outlier: 3 IQR ≤ value > 1.5 IQR; * – extreme: value > 3 IQR; * – significantly different, $p < 0.05$).

(Wilcoxon signed ranks test, $p < 0.05$) fewer aesthetascs than on the original antennae (Fig. 6B). On average, the number of aesthetascs after the first and second regeneration decreased by 21% and 28%, respectively. However, in two cases the number of aesthetascs increased after regeneration.

Tricorn sensilla

The maximal density of tricorn sensilla on the last segment of the second antenna was 13 sensilla per 0.01 mm^2 (Fig. 7A, orig). The density of sensilla on the regenerated antennae (reg1 and reg2) was significantly lower (Wilcoxon signed ranks test, $p < 0.05$) (Fig. 7A). The density of tricorn sensilla after the first and second regeneration did not differ (Fig. 7A, B). On regenerates the density decreased on average by 25% (Fig. 7B).

Lateral seta

The length of the lateral seta on the fifth segment of the antennal peduncle of control animals was 81–101 µm (Fig. 8A). The seta has an articulated base, a surface with longitudinal ridges (looks striated), a curved posterior end and an annulus on an anterior third of the length (on Fig. 1D visible near the arrow). After the first regeneration the lateral seta did not differ from that of the original antenna neither by size nor by shape (Fig. 8A). In most of the regenerates, the seta was a few percent larger (Fig. 8B). In one animal, after the first regeneration, two setae were present: in addition to the lateral seta of normal dimensions, a similar but somewhat shorter seta was present. After the second regeneration, this animal, like the others, had only one lateral seta once again. After the second regeneration, the seta was shorter in 5 out of 9 animals, for 7% up to 19%.

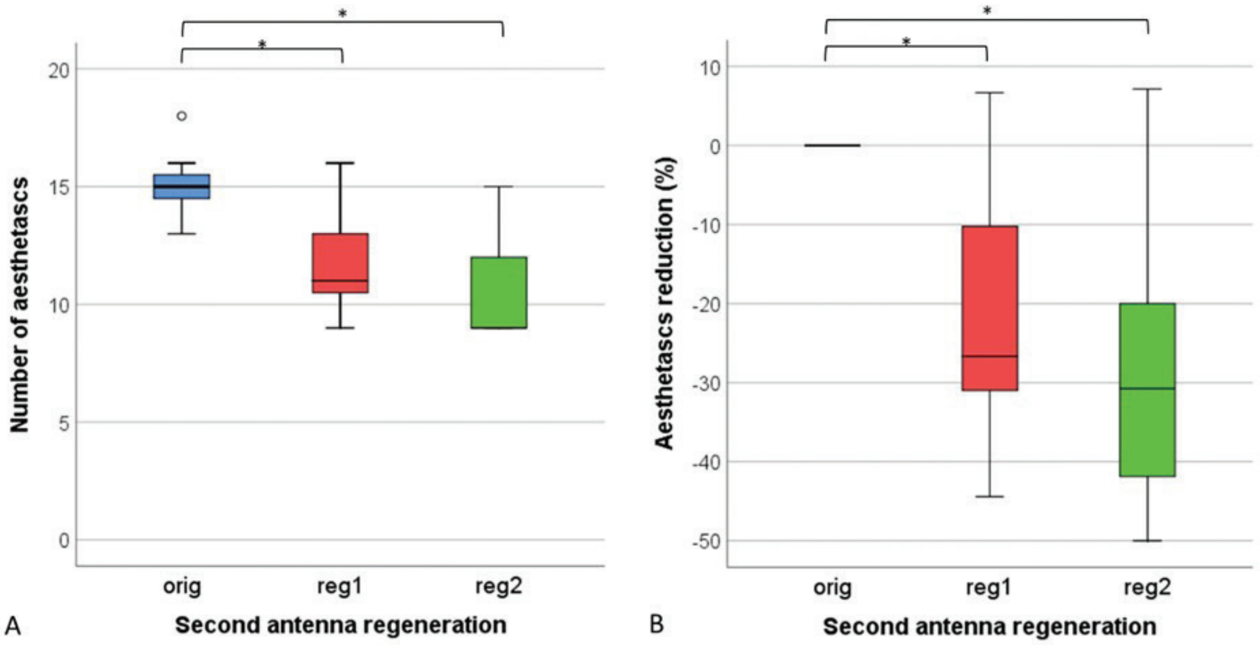

Figure 6. Number of aesthetascs (**A**) and aesthetascs number reduction (**B**) on the last segment of the original antennae (orig), regenerated antennae after first regeneration (reg 1) and regenerated antennae after second regeneration (reg2) in *Porcellio scaber*. Key: box: 25th, 50th, and 75th percentile; whiskers: value ≤ 1.5 IQR; o – outlier: 3 IQR ≤ value > 1.5 IQR; * – significantly different, $p < 0.05$).

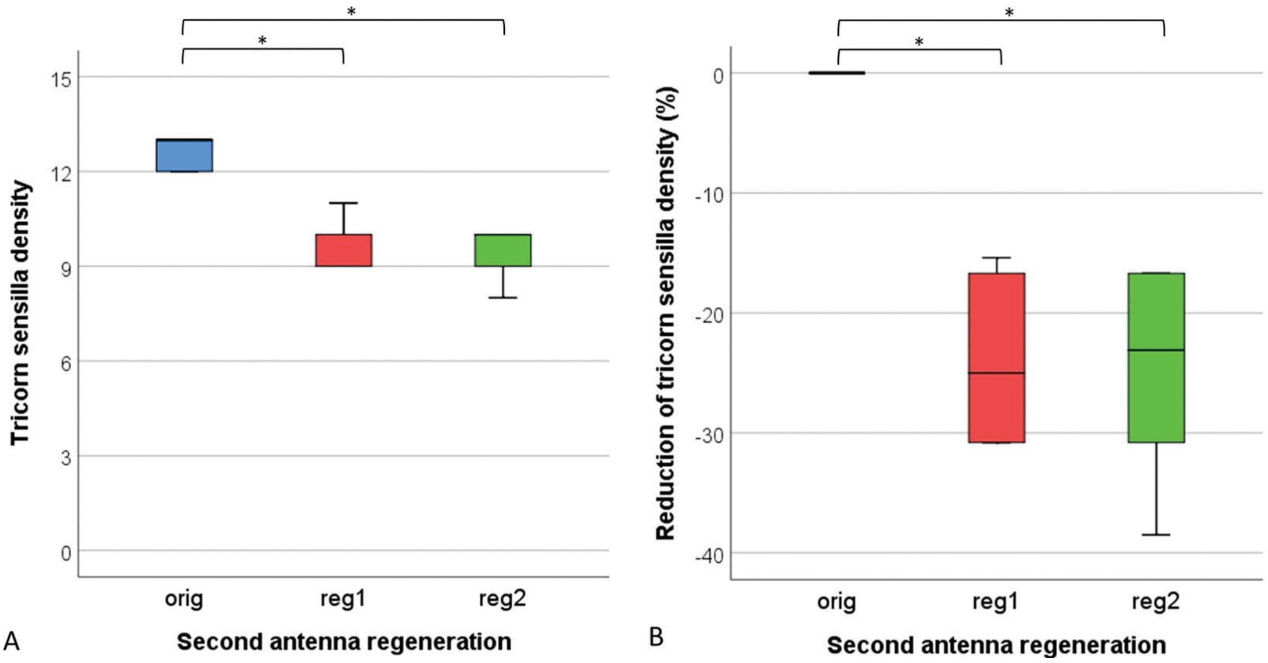

Figure 7. Tricorn sensilla density as number per 0.01mm^2 (**A**) and reduction of tricorn sensilla density (**B**) on the last segment of the original antennae (orig), regenerated antennae after first regeneration (reg 1) and regenerated antennae after second regeneration (reg2) in *Porcellio scaber*. Key: box: 25th, 50th, and 75th percentile; whiskers: value ≤ 1.5 IQR; o – outlier: 3 IQR ≤ value > 1.5 IQR; * – significantly different, $p < 0.05$).

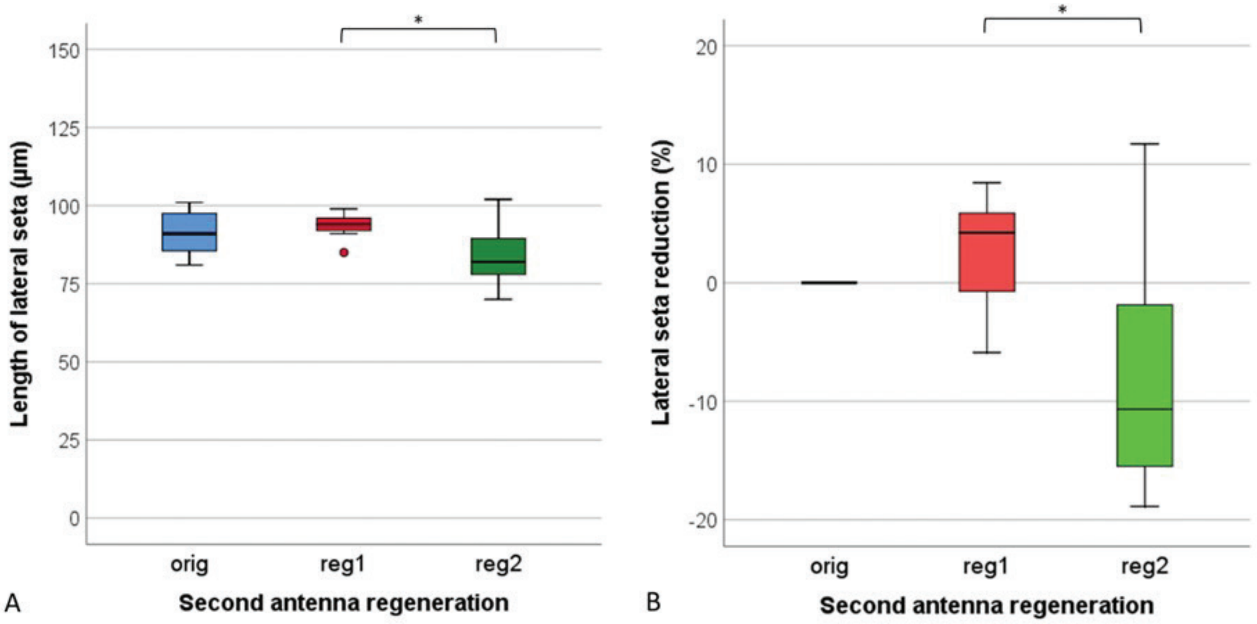

Figure 8. Length (**A**) and length reduction (**B**) of the lateral seta on the fifth peduncular segment of the original antennae (orig), regenerated antennae after first regeneration (reg 1) and regenerated antennae after second regeneration (reg2) in *Porcellio scaber*. Key: box: 25th, 50th, and 75th percentile; whiskers: value ≤ 1.5 IQR; o – outlier: 3 IQR ≤ value > 1.5 IQR); * – significantly different, $p < 0.05$).

Discussion

Pattern of antennae regeneration and the length of moult cycle

The autonomy of the part of the damaged antenna was the first noticeable change in the process of regeneration. Autonomy occurs within 24 hours after amputation between the first and second segment of the antenna (in 90% of cases). This PBP site was first documented by Klintz (1907). The process of autotomy happened overnight, so we don't know if other limbs may be involved in this process, as in autotilly (Maruzzo et al. 2005). After removing a similar number of segments, autotomy at the same PBP also occurred in *A. vulgare*, but in a smaller proportion (Madhavan and Madhavan 1981). As mentioned in the review article of Maruzzo et al. (2005), the PBP in the legs of *Porcellio* sp. contains a connective tissue septum called the autotomy membrane, which acts as a valve that closes the hole left after autotomy, minimising the wound trauma (Hopkins and Das 2015). Whether the PBPs of the antennae of *Porcellio* also contain the autotomy membrane is still to be investigated. Namely, in the genus *Asellus*, the autotomy membrane is present in the PBP of the legs but not in the antennae (Needham 1965; Maruzzo et al. 2005). A day after the autotomy, we noticed rather a large, pigmented scab covering the wound. The pigmented scab likely resulted from the action of the immune system: degranulation of hemocytes (Hopkins 1993) and activation of the phenoloxidase system, which initiates melanization (Theopold et al. 2004). We observed no other changes in the limb stub, while Madhavan and Madhavan (1981) reported that in *A. vulgare*, the distal surface of the wound becomes whitish due to the withdrawal of the pigmented epithelium. In *P. scaber* and *A. vulgare* a regenerating antenna was produced entirely inside the limb stub (Madhavan and Madhavan 1981), in our case solely in the small first segment - basis. Therefore, the space for a regenerating antenna was extremely small. A cuticular sac at the tip of the stub, where a regenerating limb is produced, was reported for decapod crustaceans (Maruzzo and Bortolin 2013) as well as for *Asellus* (Needham, 1965 in Maruzzo et al. 2005) but not for terrestrial isopods. The regenerated antennae were smaller and whitish compared to contralateral antennae as observed also by Klintz (1907) and Madhavan and Madhavan (1981). However, the regenerated antennae show the same properties as the original one in terms of the ability to repeat autotomy and regenerate as described for legs in fiddler crab *Uca pugilator* (Hopkins 1993).

After amputation of the antenna, the length of the moulting cycle was, on average, two days longer than that of control specimens, but the difference was not statistically significant. In contrast, in *A. vulgare* the moulting cycle was shortened by an average of three days when one antenna was amputated two days after moulting (Madhavan and Madhavan 1981). According to Madhavan and Schneiderman (1969), arthropods require some basic amount of ecdysteroid hormones for regeneration to begin. As reported by Hoarau and Hirn (1981) amputation of a limb causes a decline in ecdysteroids in the hemolymph and takes time to return to the baseline, causing the moulting cycle to lengthen. It seems that the low level of ecdysteroids in the hemolymph of our animals the day after moulting was further reduced by the amputation, which caused a delay in moulting.

Reduction of the antennae and sensory structures during the regeneration process

The measured part of the regenerated antennae of our animals was about one-third shorter than the same part of the original antennae, which agrees with the findings of Klintz (1907). The reduction of the regenerated antenna could be explained by the additional expenditure of energy and material for the regeneration process, but more likely by the limited space in which the regenerated limb is formed (Hopkins and Das 2015). We found that even if the antennae are amputated several times in a row, there are no major differences between the dimensions of the regenerated antennae. However, neither energy expenditure nor limited space can explain why the reduction of individual segments of the antennae in the regeneration process is different. Namely, the shortening of the most distal antennal segment was almost half that of the fifth or sixth segment. It can be assumed that the last flagellar segment is functionally more important than the other segments of the antenna, as it carries many sensory organs.

The morphology of the regenerated apical organs did not differ from the original ones and, in all cases, corresponded to the description of the organ given by Hoese (1989). Besides, no length reduction of the apical organ was observed until the second regeneration (Fig. 5B, 'reg2'). Although the last segment of the flagellum shortened during the second regeneration on average by a quarter (Fig. 3B, 'reg2'), the shortening of the apical organ did not exceed 10%. Apical organ length showed only a weak and insignificant correlation with the length (r_s = 0.399, p = 0.073) or width (r_s = 0.337, p = 0.136) of the last flagellar segment. This suggests a key role of the apical organ in isopod chemo- and mechanoreception. Namely, the apical organ may contain tens of receptor cells (Seelinger 1977) that respond to mechanical, olfactory, and gustatory stimuli and thus play an important role in surface screening (Hoese 1989), social interactions (Linsenmair and Linsenmair 1971) and food detection (Seelinger 1977).

Similar to the apical organ, no significant reduction in length or change in shape of the lateral seta was noticed. The up to 100 µm long seta was located on the lateral side of the last segment of the peduncle on all antennae, original and regenerated. A similar seta with two segments and a striated surface was described on the antennal peduncle of *A. vulgare* by Rife (1993). This seta probably has a mechanoreceptory function (Crouau, 1995, 1997). Despite around 40% reduction in the length of the last peduncle segment and around 20% reduction in its width, the seta length reduced insignificantly. Its length showed only a weak and insignificant correlation with the length (r_s = 0.268, p = 0.241) and with the width (r_s = 0.390, p = 0.080) of the last peduncular segment.

In accordance with the reduction of the antennal segments' surface, the number of plural sensory organs, such as aesthetascs and tricorn sensilla, is reduced. The number of aesthetascs decreased mainly due to the decrease in the number of aesthetascs per group and not by reduction of the number of groups. Aaesthetascs were mainly in 3–4 groups as reported by Hoese (1989). However, up to 4 aesthetascs were frequently located also individually outside these groups. Reduction of aesthetascs coincides with the reduction of the length and width of the last flagellar segment. Spearman's correlation coefficient showed a moderate to strong positive correlation between the number of aesthetascs and the length (r_s = 0.671, p = 0.001) and width of the segment (r_s = 0.735, p = 0.000). Aesthetascs have an important olfactory role (Hoese 1989; Schmalfuss 1998).

During the regeneration, the density of tricorn sensilla on the last segment of the flagellum decreased by around 25%. Spearman's correlation coefficient showed a strong positive correlation between the density of tricorns and the length of the segment (r_s = 0.784, p = 0.000) and a moderate correlation with its width (r_s = 0.557, p = 0.009). Tricorn sensilla are characteristic for terrestrial isopods and are the most numerous sensilla on the second antennae of terrestrial isopods (Holdich 1984; Schmalfuss 1998). These triangular-shaped structures are known to contain sensory cells (Ziegler and Altner 1995), but their role is still unknown. It was suggested that they may have a hygroreceptory (Price and Holdich 1980), mechanoreceptory (Holdich and Lincoln 1974) or even chemoreceptory function (Tsuneo 1989).

Conclusions

This study revealed that:

- The length of the moult cycle did not differ between amputated and control individuals.
- The regenerated antennae are reduced in size compared to the original ones, however, the reduction does not increase with multiple consecutive amputations.
- The reduction of individual antenna segments in the regeneration process is different. The most distal segment of the flagellum receives the smallest share of the reduction.

- The size and shape of the apical organ and lateral seta do not change during the regeneration process, although the size of the segment on which they are located decreases. In contrast, the number of plural receptor elements, such as aesthetascs and tricorn sensilla, decreases in accordance with the segment size reduction.

Acknowledgments

The authors thank the Slovenian Research Agency for financial support through the Research Core Funding P1-0184 and Dr Miloš Vittori for help in preparing SEM samples. The authors also thank the editor Ivan H. Tuf and the reviewers Raymond Bauer and Régis Augusto Pescinelli for their helpful comments and corrections.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

Funding

The authors thank the Slovenian Research Agency for financial support through the Research Core Funding P1-0184.

Author contributions

All authors have contributed equally.

Author ORCIDs

Primož Zidar https://orcid.org/0000-0002-9876-621X

Data availability

All of the data that support the findings of this study are available in the main text.

References

Almazán A, Çevrim Ç, Musser JM, Averof M, Paris M (2022) Crustacean leg regeneration restores complex microanatomy and cell diversity. Science Advances 8(34): eabn9823. https://doi.org/10.1126/sciadv.abn9823

Bliss DE (1960) Autotomy and regeneration. In: Waterman TH (Ed.) The physiology of Crustacea Metabolism and growth (Vol. 1). Academic Press, New York-London, 561–589. https://doi.org/10.1016/B978-0-12-395628-6.50023-3

Crouau Y (1995) Association in a crustacean sensory organ of two usually mutually exclusive mechanosensory cells. Biology of the Cell 85(2–3): 191–195. https://doi.org/10.1016/0248-4900(96)85280-4

Crouau Y (1997) Comparison of crustacean and insect mechanoreceptive setae. International Journal of Insect Morphology & Embryology 26(3–4): 181–190. https://doi.org/10.1016/S0020-7322(97)00020-2

Friedlander CP (1964) Thigmokinesis in woodlice. Animal Behaviour 12(1): 164–174. https://doi.org/10.1016/0003-3472(64)90118-6

Goss RJ (1969) Principles of regeneration. Academic Press, New York and London, 300 pp.

Hoarau F, Hirn M (1981) Effects of amputation and subsequent regeneration of a leg on the duration of the intermoult period and the level of circulating ecdysteroids in *Helleria brevicornis* Ebner (ground isopod). General and Comparative Endocrinology 43(1): 96–104. https://doi.org/10.1016/0016-6480(81)90036-8

Hoese B (1989) Morphological and comparative studies on the second antennae of terrestrial isopods. In: Ferrara F, Argano R, Manicastri C, Schmalfuss H, Taiti S (Eds) Monitore Zoologico Italiano. Second Symposium on the Biology of Terrestrial Isopods, Urbino (Italy) September 1986. Florence. Monographia 4: 127–152.

Holdich DM (1984) The cuticular surface of woodlice: a search for receptors. In: Sutton SL, Holdich DM (Eds) The Biology of Terrestrial Isopods. Symposia of the Zoological Society of London. Cambridge University Press, Cambridge 53, 97–107.

Holdich DM, Lincoln RJ (1974) An investigation of the surface of the cuticle and associated sensory structures of the terrestrial isopod, *Porcellio scaber*. Journal of Zoology (London, England) 172(4): 469–482. https://doi.org/10.1111/j.1469-7998.1974.tb04379.x

Hopkins PM (1993) Regeneration of walking legs in the fiddler crab *Uca pugilator*. American Zoologist 33(3): 348–356. https://doi.org/10.1093/icb/33.3.348

Hopkins PM, Das S (2015) Regeneration in crustaceans. In: The Natural History of the Crustacea. Oxford University Press, New York 4: 168–198.

Klintz JH (1907) Regeneration der Antenne bei der Kellerassel (*Porcellio scaber* Latr.). Development Genes and Evolution 23(4): 552–559. https://doi.org/10.1007/BF02288310

Lefebvre F, Limousin M, Caubet Y (2000) Sexual dimorphism in the antennae of terrestrial isopods: A result of male contests or scramble competition? Canadian Journal of Zoology 78(11): 1987–1993. https://doi.org/10.1139/z00-128

Linsenmair KE, Linsenmair C (1971) Paarbildung und Paarzusammenhalt bei der monogamen Wüstenassel Hemilepistus reaumuri (Crustacea, Isopoda, Oniscoidea). Zeitschrift für Tierpsychologie 29: 134–155. https://doi.org/10.1111/j.1439-0310.1971.tb01728.x

Madhavan K, Madhavan MM (1981) Regeneration of antennae and its control of molting in the terrestrial isopod, *Armadillidium vulgare*. The Journal of Experimental Zoology 216(2): 277–284. https://doi.org/10.1002/jez.1402160208

Madhavan K, Schneiderman HA (1969) Hormonal control of imaginal disc regeneration in *Galleria mellonella* (Lepidoptera). The Biological Bulletin 137(2): 321–331. https://doi.org/10.2307/1540104

Maruzzo D, Bortolin F (2013) Arthropod regeneration. In: Minelli A, Boxshall G, Fusco G (Eds) Arthropod Biology and Evolution: Molecules, Development, Morphology. Springer Verlag, Berlin-Heidelberg, 149–169. https://doi.org/10.1007/978-3-642-36160-9_7

Maruzzo D, Bonato L, Brena C, Fusco G, Minelli A (2005) Appendage loss and regeneration in arthropods: a comparative view. In: *Crustacea and arthropod relationships*. CRC PRESS, Boca Raton, 16: 215–245. https://doi.org/10.1201/9781420037548.ch9

Mead F, Gabouriaut D, Corbière-Tichané G (1976) Structure of the apical sensory organ of the antenna in a terrestrial isopod, *Metoponorthus sexfasciatus* Budde-lund (Crustacea, Isopoda). Zoomorphologie 83: 253–269. https://doi.org/10.1007/BF00993512

Needham AE (1965) Regeneration in the Arthropoda and its endocrine control. Regeneration in animals and related problems, 283–323.

Price JB, Holdich DM (1980) The formation of the epicuticle and associated structures in *Oniscus asellus* (Crustacea, Isopoda). Zoomorphologie 94(3): 321–332. https://doi.org/10.1007/BF00998208

Rife GS (1993) A scanning electron microscopy study of cuticular structure in some Ohio terrestrial isopods (Crustacea: Isopoda: Oniscoidea). PhD Dissertation, Bowling Green State University, Bowling Green, United States of America.

Riggio C (2013) Porcellio scaber, Animal Diversity Web. https://animaldiversity.org/accounts/Porcellio_scaber/ [August 24, 2023]

Schindelin J, Arganda-Carreras I, Frise E, Kaynig V, Longair M, Pietzsch T, Preibisch S, Rueden C, Saalfeld S, Schmid B, Tinevez JY, White DJ, Hartenstein V, Eliceiri K, Tomancak P, Cardona A (2012) Fiji: An open-source platform for biological-image analysis. Nature Methods 9(7): 676–682. https://doi.org/10.1038/nmeth.2019

Schmalfuss H (1998) Evolutionary strategies of the antennae in terrestrial isopods. Journal of Crustacean Biology 18(1): 10–24. https://doi.org/10.2307/1549516

Seelinger G (1977) The apical cone on the antenna of the desert woodlouse *Hemilepistus reaumuri*, a complex sensory organ (Crustacea, Isopoda). Journal of Comparative Physiology. A, Neuroethology, Sensory, Neural, and Behavioral Physiology 113(1): 95–103. https://doi.org/10.1007/BF00610455

Skinner DM (1985) Molting and regeneration. In: Bliss DE, Mantel LH (Eds) The Biology of Crustacea, Volume 9. Academic Press, Orlando, 43–146. https://doi.org/10.1016/B978-0-12-106409-9.50013-0

Theopold U, Schmidt O, Söderhäll K, Dushay MS (2004) Coagulation in arthropods: Defence, wound closure and healing. Trends in Immunology 25(6): 289–294. https://doi.org/10.1016/j.it.2004.03.004

Tsuneo H (1989) Responses of dorsal tricorn-type sensilla on *Ligia exotica*. Comparative Biochemistry and Physiology. A. Comparative Physiology 92(4): 513–519. https://doi.org/10.1016/0300-9629(89)90358-7

Ziegler A, Altner H (1995) Are the most numerous sensilla of terrestrial isopods hygroreceptors? Ultrastructure of the dorsal tricorn sensilla of *Porcellio scaber*. Cell and Tissue Research 282(1): 135–145. https://doi.org/10.1007/BF00319140

ZooKeys 1225: 49–63 (2025)
DOI: 10.3897/zookeys.1225.124521

Research Article

A new species of woodlouse (Isopoda, Oniscidea) from the Canarian laurel forest

Raúl Orihuela-Rivero[1,2], Carmen Balibrea[1], Víctor Noguerales[1], Heriberto López[1], Pedro Oromí[3]

1 *Instituto de Productos Naturales y Agrobiología (IPNA), CSIC, C/Astrofísico Francisco Sánchez 3, 38206 La Laguna, Santa Cruz de Tenerife, Canary Islands, Spain*
2 *School of Doctoral and Graduate Studies, University of La Laguna, C/Astrofísico Francisco Sánchez s/n, 38200 La Laguna, Santa Cruz de Tenerife, Canary Islands, Spain*
3 *Department of Animal Biology, Edaphology and Geology, University of La Laguna, C/Astrofísico Francisco Sánchez s/n, 38200 La Laguna, Santa Cruz de Tenerife, Canary Islands, Spain*

Corresponding author: Raúl Orihuela-Rivero (rorihuela@ipna.csic.es)

Abstract

A new species of terrestrial isopod, *Porcellio aguerensis* Orihuela-Rivero, **sp. nov.** of the family Porcellionidae (Oniscidea), is described from the laurel forest of Tenerife, Canary Islands. This new species belongs to the Atlantic group ("*scaber*") as defined by Vandel due to the structure of the male pleopod 1 and its "primitive" glandular system. Some diagnostic characters that allow it to be differentiated from other species are revealed, such as (i) the smooth dorsal surface, (ii) the sinuosity of the posterior margin of the first pereonites, (iii) the configuration of the glandular system, and (iv) the structure of the male pleopod 1 exopod. The affinity of *Porcellio aguerensis* Orihuela-Rivero, **sp. nov.** with the morphologically closest members of the genus is discussed, both with continental and insular species, hypothesizing a relationship between the Canarian species of *Porcellio* and the "primitive" continental lineages of the genus. A key of the *Porcellio* species occurring in Tenerife is included. The conservation of *Porcellio aguerensis* Orihuela-Rivero, **sp. nov.** within a scenario of increasing dominance of invasive species is discussed.

Key words: Canary Islands, Porcellionidae, taxonomy, Tenerife, woodlice

OPEN ACCESS

Academic editor: Ivan H. Tuf
Received: 2 April 2024
Accepted: 5 July 2024
Published: 5 February 2025

ZooBank: https://zoobank.org/DBC3F2FB-8205-478A-AE67-3F4109BBC7ED

Citation: Orihuela-Rivero R, Balibrea C, Noguerales V, López H, Oromí P (2025) A new species of woodlouse (Isopoda, Oniscidea) from the Canarian laurel forest. In: Tuf IH, Tajovský K, Taiti S (Eds) The Biology of Terrestrial Isopods, XII. ZooKeys 1225: 49–63. https://doi.org/10.3897/zookeys.1225.124521

Introduction

The terrestrial isopod fauna of the Canary Islands remains notoriously understudied. Until the recent publication of the description of *Soteriscus jandiensis* Cifuentes & Prieto, 2023, no new taxon of the suborder Oniscidea had been described for the archipelago for 16 years (Taiti and López 2008). The Canary Islands host a high diversity of terrestrial isopods, which has already been demonstrated in 1991 by Rafael Rodríguez Santana in his detailed but unfortunately unpublished doctoral thesis. The archipelago harbours at least 35 native species, 29 of which are endemic (Gobierno de Canarias 2024). Of these,17 species belong to the genus *Porcellio* Latreille, 1804.

The family Porcellionidae is composed of 19 genera, making it the fourth most diverse family (over 330 species) of Oniscidea (Sfenthourakis and Taiti 2015). The family is characterized by (i) the flagellum of second antenna having two joints, (ii) the absence of conglobational ability (except *Atlandidium* Arcangeli, 1936), and (iii) the presence of monospiracular covered lungs on pleopod 1 and 2 exopods (Schmidt 2003). Despite these shared morphological characters, recent molecular studies question the monophyly of Porcellionidae and the monophyly of several of its constituent genera, such as *Porcellio* and *Porcellionides* Miers, 1877 (Dimitriou et al. 2018).

In the Canary Islands, Porcellionidae is represented by 32 species, of which 22 are endemic, with half of them described in the last 40 years (Dalens 1984; Hoese 1985; Rodríguez Santana 1990; Rodríguez and Vicente 1992a, 1992b, 1992c; Rodríguez and Barrientos 1993; Cifuentes and Prieto 2023). Within this family, *Porcellio* is the genus with the highest species richness, with 22 species representing the halophilic ("*lamellatus*"), North African ("*laevis*"), and Atlantic ("*scaber*") groups following the group-classification of Vandel (1951, 1956, 1962). This classification, which encompasses several morphological characters and homogeneous distributions, although it is accepted by most experts as a very useful tool to deal with this diverse genus, it still needs to be tested with molecular analysis to rule out that these similarities are due to evolutionary convergence phenomena.

The aim of this study is to describe a new species of the genus *Porcellio* from Tenerife (Canary Islands) and to discuss its similarities and differences with what appear to be closely related species, both insular and continental.

Study area

Tenerife is situated in an almost central position within the archipelago of the Canary Islands (Fig. 1) located off the northwest coast of Africa in the Atlantic Ocean. Like all of the Canary Islands, Tenerife has a volcanic origin, although it is thought to have originally formed from three paleoislands (Roque del Conde, Teno, and Anaga) that later merged as a consequence of more recent volcanic episodes (Carracedo and Pérez-Torrado 2013). Our sampling took place in the Anaga massif (Fig. 1), with an estimated age of 3.9–4.9 million years (My) (Guillou et al. 2004). Its age and high geological stability (Ancochea et al. 1990) have promoted long periods of intense erosion producing landscapes with complex topographies that have stimulated geographical isolation of populations and, ultimately, processes of speciation (Fernández-Palacios et al. 2017; Salces-Castellano et al. 2020). Due to an east-west orientation of the dorsal ridge of the peninsula, and its altitude of up to 1024 m above sea level (a.s.l.), orographic cloud formations are a feature of the massif, supplying up to 1200 mm of water per year by horizontal precipitation (Fernández-Palacios et al. 2017; del Arco and Rodríguez-Delgado 2018). This cloud formation sustains the presence of the laurel forest, a relict arboreal plant formation rich in laurophilous trees comparable to the humid subtropical evergreen rainforests that spread over the Mediterranean during the Palaeogene and Neogene (but see Kondraskov et al. 2015). On spurs and ridges between 600 and 1100 m a.s.l., there is a dense, low to medium-altitude formation dominated by the Macaronesian endemic *Erica platycodon* (Webb & Berthel.) Rivas-Mart. & al. and other typical laurel forest species such as *Ilex canariensis* Poir. in Lamarck, *Laurus novocanariensis* Rivas-Mart., Lousa, Fern. Prieto, E. Días, J.C. Costa & C. Aguiar, and *Morella faya* (Aiton) Wilbur. Areas of forest with greater exposure to cloud formation and more limited exposure to solar radiation are rich in lichens,

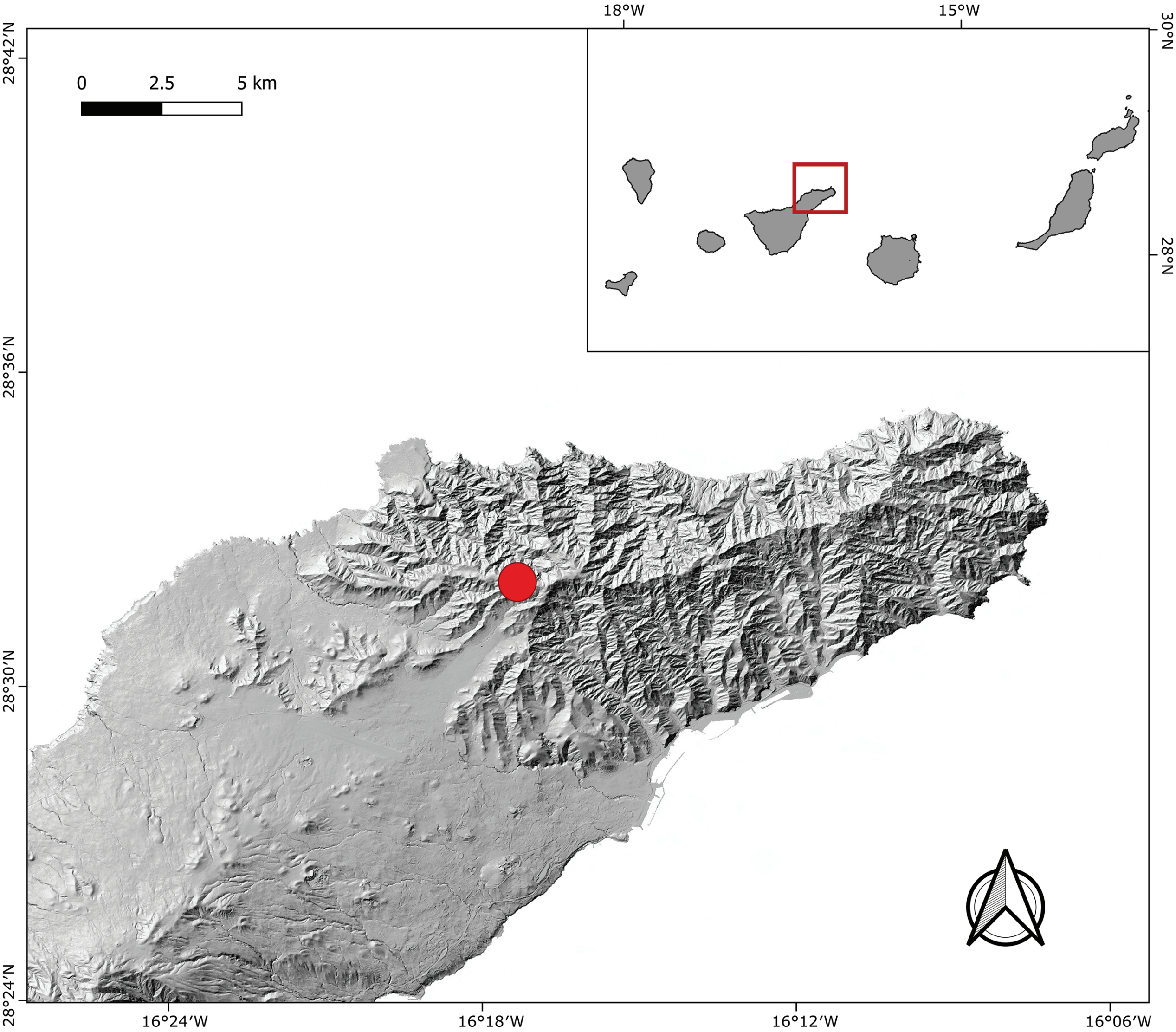

Figure 1. Geographic location of Tenerife within the Canary Islands and sampling sites in Anaga massif (red point).

mosses and ferns, but there are also periods of desiccation due to near-continuous wind in the upper canopy (del Arco and Rodríguez-Delgado 2018). This is the case of the sampling area, located above 900 m a.s.l. on the western summits of Anaga (Monte de Las Mercedes, Fig. 1), with an average temperature of 15.2 °C and a rainfall of 1050 mm per year (Fernández-Palacios et al. 2017).

Methods

The studied specimens were collected within the laurel forest by pitfall traps, with a subset of specimens preserved in absolute ethanol for future molecular analysis. Terminology used for the description is based on Vandel (1960). For the examination of anatomical parts, slides for microscopy were prepared with dimethyl hydantoin formaldehyde and temporary preparations in glycerol, as appropriate. Preserved specimens were examined with a Zeiss Stemi SV6 stereomicroscope and a Zeiss Axioskop 40 binocular microscope. Images were obtained using the photo stacking technique, attaching a Canon EOS 750D digital camera to these microscopes. Each final image was obtained processing their stack of photographs with the program Zerene Stacker v. 1.04 (Zerene Systems, LLC, Richland, WA), combining them into a single image using pmax and dmap methods. The software Photoshop was used for final retouching. Drawings were made with graphic editor Inkscape v. 1.3. (https://inkscape.org). The type specimens of this new species have been deposited into the Collection of the Institute of Natural Products and Agrobiology, La Laguna, Tenerife (**IPNA**); the Collection of the Department of Animal Biology (Zoology) of the University of La Laguna (**DZUL**); and the Collection of the Museum of Nature and Archaeology of Tenerife (**TFMC**).

Results

Taxonomy

Order Isopoda Latreille, 1816
Suborder Oniscidea Latreille, 1802
Family Porcellionidae Brandt in Brandt & Ratzeburg, 1831
Genus *Porcellio* Latreille, 1804

***Porcellio aguerensis* Orihuela-Rivero, sp. nov.**
https://zoobank.org/FF7E5C1A-085B-4785-8387-187AD0D4316B

Type material. ***Holotype*:** Spain • ♂; Canary Islands, Tenerife, Zapata; 28°32.06'N, 16°17.63'W; 915 m a.s.l.; May 2023; Carmen Balibrea and Víctor Noguerales leg.; pitfall trap; IPNA BC2649. ***Paratypes*:** Spain • 1 ♂; Canary Islands, Tenerife, Zapata; 28°31.93'N, 16°17.53'W; 930 m a.s.l.; May 2023; Carmen Balibrea and Víctor Noguerales leg.; pitfall trap; DZUL 36998 • 1 ♀ ovigerous; Canary Islands, Tenerife, Zapata; 28°31.93'N, 16°17.13'W; 960 m a.s.l.; May 2023; Carmen Balibrea and Víctor Noguerales leg.; pitfall trap; DZUL 36999 • 1 ♂; same collection data as for preceding; BC2650 • 1 ♀ ovigerous; Canary Islands, Tenerife, Zapata; 28°31.93'N, 16°17.19'W; 955 m a.s.l.; May 2023; Carmen Balibrea and Víctor Noguerales leg.; pitfall trap; IPNA BC2651 • 1 ♀; Canary Islands, Tenerife, Zapata; 28°31.99'N, 16°17.55'W; 925 m a.s.l.; May 2023; Carmen Balibrea and Víctor Noguerales leg.; pitfall trap; TFMCEN-2381.

Diagnosis. Teguments smooth; glandular fields associated to lateral margins located in widening of marginal groove of pereonites; d/c coordinate values of noduli laterales with a peak on pereonite 4; pereonites 1–2 with posterior margins slightly sinuous; telson triangular with apex rounded; second antenna not reaching posterior margin of second pereonite; uropods with protopods posterior margin very oblique; exopods narrow. Male pereopod 7 ischium with concave ventral margin and slight distal depression on its rostral face covered with numerous setae; male pleopod 1 exopod with short posterior lobe.

Description. Maximum length: male 8.5 mm, female 9.8 mm. Body strongly convex with epimera oriented obliquely, ovoid (Fig. 2A, B); outline between pereon and pleon continuous. Colour (Fig. 2A–C) mottled brown with irregular broad dark midline reaching posterior margin of cephalon; pereonites 4–7 usually with pairs of paramedian diffuse yellow spots; pigmented epimera, presenting small depigmented area around noduli laterales and band of depigmentation at base of epimera; pereopods and pleopods pigmented; dark maxilliped; uropod exopods often reddish or brown. Cephalon, pleon and telson smooth. Dorsal surface covered with large Y-shaped scale-setae (Fig. 3H) and imbricated rounded scales. Pereonites 1–7 epimera with distinct groove along entire lateral margin with one widening concentrating glandular pores ranging from 6 to 20, number of pores gradually decreasing towards posterior pereonites; widening located at anterior or in middle portion of epimera, in the first pereonite at anterior corner of epimeron (Fig. 2D). One line of large noduli laterales per side on pereonites 1–7, far from lateral margins, with high d/c ratio as in Fig. 3C. Cephalon (Fig. 3A) without supra-antennal line; frontal-line arches slightly forming rounded small median lobe; lateral lobes rounded or slightly quadrangular; eyes with 24–28 ommatidia. Pereonites 1–2 with posterior margins slightly sinuous (Fig. 2C); 3–6 straight, posterior corners slightly directed backwards; 7, regularly concave. Pleonites 3–5 with well-developed epimera; posterior corners bending backwards; pleonite 5 epimera not surpassing uropod protopods or telson apex (Fig. 3B). Telson (Fig. 3B) wider than long, triangular, with concave margins, sides of distal part of some specimens almost parallel; apex rounded. First antenna (Fig. 3F) tri-articulated; basal and distal article subequal in length; distal article with tuft of about 30 subapical aesthetascs. Second antenna (Fig. 3D, E) not reaching posterior margin of second pereonite when extended backwards; finely setose; flagellum bi-articulated, slightly shorter than fifth article of peduncle; second article about 1.5 times longer than first, bearing several rows of aesthetascs (Fig. 3E). Mandibles with dichotomized molar penicil; left mandible (Fig. 4B) with 2 + 9 penicils; right mandible (Fig. 4A) with 1 + 4 penicils. Maxillule

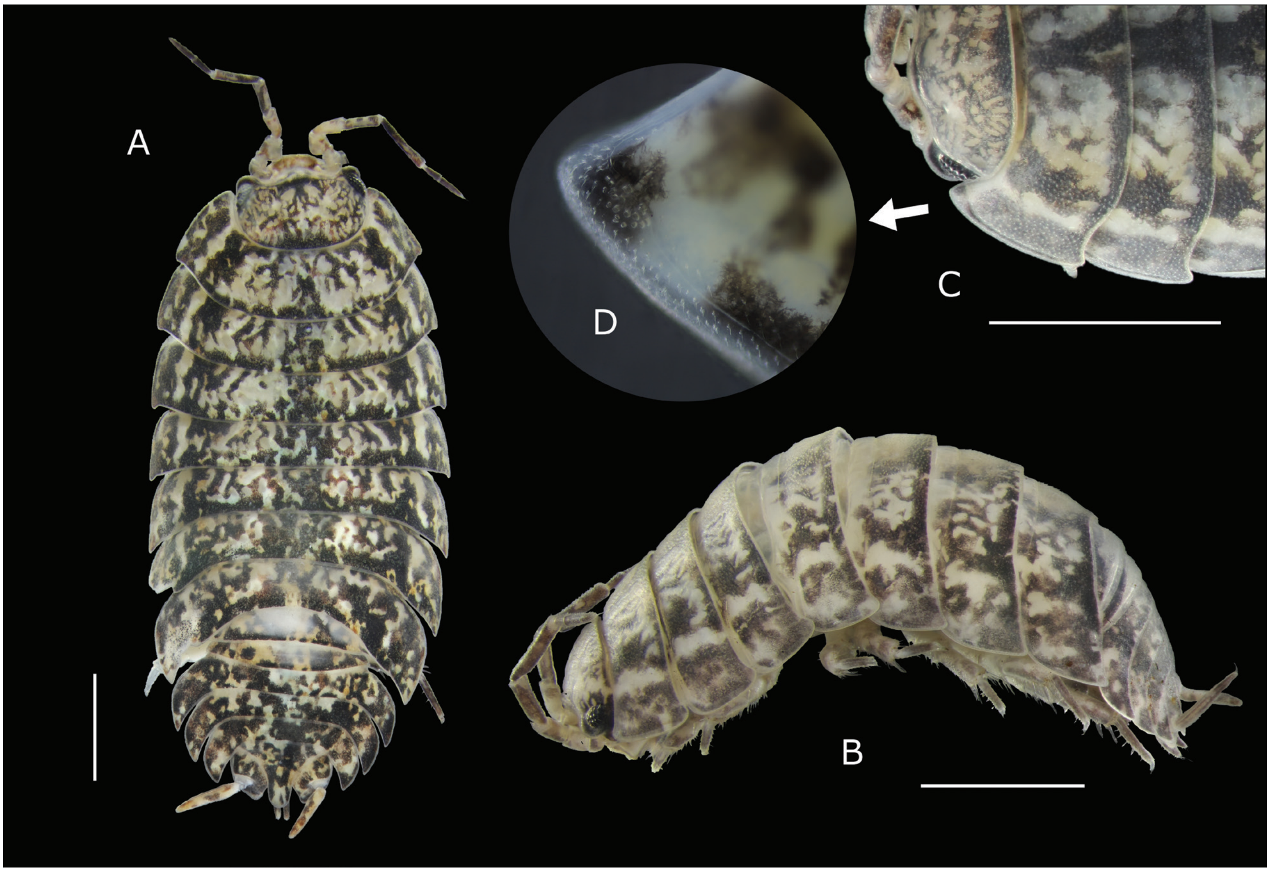

Figure 2. *Porcellio aguerensis* sp. nov. **A** paratype ♀, habitus, dorsal view **B** holotype ♂, habitus, lateral view **C** paratype ♂, details of the first pereonites **D** details of configuration of the glandular field of the first pereonite. Scale bars: 2 mm (**A, B, C**).

external branch (Fig. 4C) with 4 + 6 teeth, four of them subapically cleft; internal branch (Fig. 4D) with two thick penicils and one short sharp posterior corner. Maxilla inner lobe quadrangular (Fig. 4E), wider than outer lobe, apex covered fine and thick setae; outer lobe (Fig. 4E) with three long incurved setae on margin between lobes; covered with thin setae. Maxilliped (Fig. 4F) palp with two

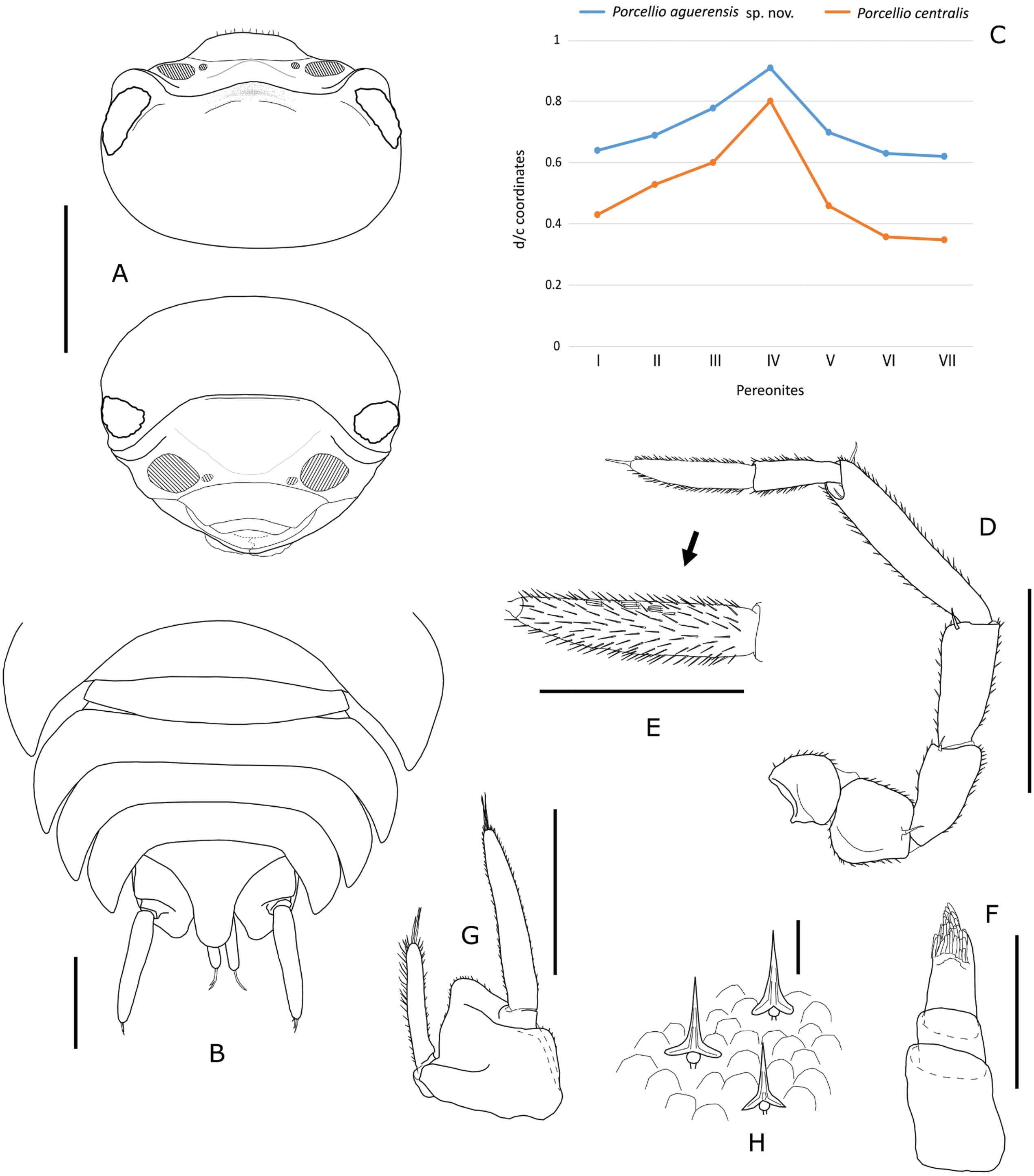

Figure 3. *Porcellio aguerensis* sp. nov. **A** paratype ♀, cephalon, dorsal and frontal view **B** paratype ♀, details of pleon, telson and uropods **C** paratype ♀, noduli laterales d/c coordinates and comparison with *Porcellio centralis* Vandel, 1954 **D** paratype ♂, second antenna **E** paratype ♂, details of the distal article of antennal flagellum **F** paratype ♂, first antenna **G** paratype ♀, uropod **H** paratype ♀, dorsal cuticular scales and scale-setae. Scale bars: 1 mm (**A, B, D, G**); 0.5 mm (**E**); 0.2 mm (**F**); 0.02 mm (**H**).

strong setae on first article, distal article with one tuft of setae; endite quadrangular, with one strong seta and four triangular teeth, three on distal margin and one on lateral margin. Pereopods short and stout. Pleopod 1 and 2 exopods with monospiracular covered lungs with indented outer margin. Uropodal protopods (Fig. 3G) with ribbed outer margin and very oblique posterior margin, with its inner end almost reaching tip of telson (Fig. 3B); endopod and exopod (Fig. 3G) inserted at different level; exopods narrow, styliform, about 1.5 times longer than endopods; endopods surpassing telson apex (Fig. 3B).

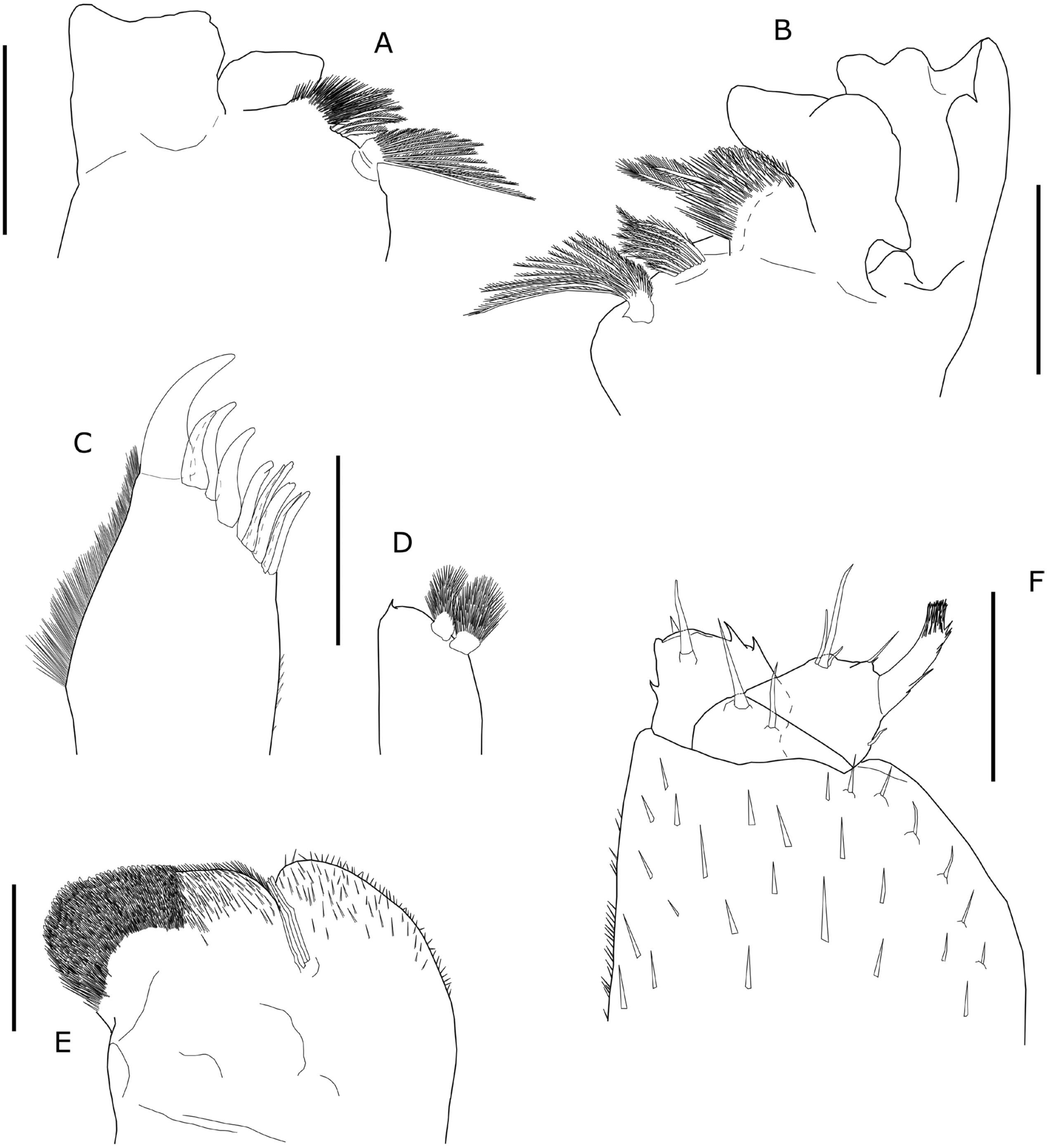

Figure 4. *Porcellio aguerensis* sp. nov., paratype ♂ **A** right mandible **B** left mandible **C** maxillule, external branch **D** maxillule, internal branch **E** maxilla **F** maxilliped. Scale bars: 0.2 mm (**A, B, C, D, F**); 0.1 mm (**E**).

Male: Pereopod 1 (Fig. 5A) merus and carpus with strong setae on sternal margin, more numerous than in female. Pereopod 2–3, also with these modifications, but less noticeable. Pereopod 7 (Fig. 5B) ischium with slightly concave ventral margin and slight distal depression on its rostral face covered with numerous setae. Pleopod 1 exopod (Fig. 5C, E) with short posterior lobe ending in rounded apex or with small protruding tip (Fig. 5E), inner margin with strong setae; endopod (Fig. 5C) about twice as long as exopod, with straight or slightly curved distal margin, apex with row of spines (Fig. 5D). Pleopod 2 (Fig. 5F) exopod triangular, with straight inner margin; distal part of outer margin with some setae; endopod styliform, about 1.3 times longer than exopod. Pleopods 3–5 exopods (Fig. 5G–I) triangular.

Etymology. The species name is derived from “Aguere”, the aboriginal term of the geographic area where currently the municipality of La Laguna is situated and where the species was found.

Ecology. Epigean species. *Porcellio aguerensis* Orihuela-Rivero, sp. nov. has only been recorded so far from the higher altitude laurel-forest areas of the western sector of Anaga, appearing only occasionally in pitfall traps. This species is associated with *Erica platycodon* communities, although some traps were also positioned in more humid laurel forest characterized by the presence of *Laurus novocanariensis*. Several co-occurring Canary Island endemics were also sampled: very abundantly *Porcellio anagae* Hoese, 1985 (family Porcellionidae), and more rarely *Ctenorillo ausseli* (Dollfus, 1893) (family Armadillidae). *Armadillidium vulgare* (Latreille, 1804), an invasive Mediterranean species of the family Armadillidiidae (Arndt and Mattern 2005) was also commonly sampled together with *P. aguerensis* Orihuela-Rivero, sp. nov.

Identification key of the *Porcellio* species from Tenerife (Canary Islands)

1 Male pleopod 1 exopod with elongated posterior lobe. Glandular fields not associated with the margin of the epimera **2**
– Male pleopod 1 exopod with short posterior lobe. Glandular fields, when present, associated with the margin of the epimera **3**
2 Dorsal surface without or with some flattened granules; scales absent except on the anterior corner of the tergites .... ***P. laevis***
– Dorsal surface with conspicuous, small, rounded granules, especially remarkable on cephalon; scales present on almost the entire surface .... ***P. alluaudi***
3 Dorsal surface without granules **4**
– Dorsal surface with granules **5**
4 Epimera with few pores distributed in an inconspicuous marginal groove. Male pereopod 7 without noticeable sexual dimorphism. Troglobitic species .... ***P. martini***
– Epimera with pores arranged in a glandular field located in a widening of the marginal groove. Male pereopod 7 ischium with concave ventral margin. Epigean species .... ***P. aguerensis* sp. nov.**
5 Glandular field in a marginal groove occupying all or almost the entire length of the lateral margin of the epimeron **6**
– Glandular field located in a limited semi-elliptical region associated to the lateral margin of the epimeron **8**

6 Uropod endopods not surpassing the telson apex. Noduli laterales in the 4th pleonite with d/c coordinate value > 1 .... ***P. medinae***

– Uropod endopods surpassing the telson apex. Noduli laterales in the 4th pleonite with d/c coordinate value < 0.85... **7**

7 Rounded telson tip. Male pleopod 1 exopod with respiratory field deeply indented. Male pereopod 7 ischium with concave ventral margin....... ***P. anagae***

– Acute telson tip. Male pleopod 1 exopod with respiratory field not indented. Male pereopod 7 ischium without noticeable sexual dimorphism....... .... ***P. septentrionalis***[1]

8 Glandular fields present only in the first 4 pereonites. Cephalon with large semicircular median lobe .... ***P. canariensis***

– Glandular fields present in all pereonites. Cephalon with triangular median lobe.. ***P. scaber***

Discussion

Porcellio aguerensis Orihuela-Rivero, sp. nov. fits into the Atlantic group (“*scaber*”) as defined by Vandel (1951), sharing the male pleopod 1 exopod with a respiratory field located in a markedly lateral position and with a short posterior lobe. Also in common with the group, it has a “primitive” glandular system (plesiomorphic character state not yet tested by molecular analysis) associated with the margin of the epimera, in this case enclosed in a sector of a marginal groove. Within this group, the described species is quite characteristic for its somatic, integumentary and sexual characters. However, we can find similarities with other species, both continental and insular.

Among the continental species, the closest one is found in the Betic-Rifian subgroup as defined by Vandel (1958) (not to be confused with the Betic-Rifian or “*hoffmannseggii*” group). This group includes three species, *Porcellio debueni* Dollfus, 1892, *Porcellio colasi* Vandel, 1958, and *Porcellio humberti* Paulian de Félice, 1939, exclusive to the Iberian Peninsula and Morocco.

Porcellio humberti, which is native from Morocco and the southern Iberian Peninsula, shares a convex body with *P. aguerensis* Orihuela-Rivero, sp. nov., together with a glandular system with the same configuration, with the glandular pores confined to a region of a marginal groove; dorsal surface without granules, with conspicuous noduli laterales; a poorly developed cephalic median lobe; and the male pleopod 1 exopod with similar characteristics (see Vandel 1958; Taiti and Rossano 2015). However, *P. aguerensis* Orihuela-Rivero, sp. nov. primarily differs from *P. humberti* in having: higher d/c coordinate values, with a peak on pereonite 4; the posterior margin of the first pereonites sinuous; telson with a distinctly rounded tip; and second antennae without sexual dimorphism. It should also be mentioned that, as Paulian de Félice (1939) commented with respect to *P. humberti*, *P. aguerensis* Orihuela-Rivero, sp. nov. has superficial similarities with *Porcellio gallicus* Dollfus, 1904; however, it differs in having a completely different glandular system and configuration of the male pleopod 1 exopod, which explains its inclusion in a different group (western Mediterranean or “*provincialis*” group) as proposed by Vandel (1956, 1962).

---

[1] The validity of *Porcellio septentrionalis* has been questioned by Hoese (1984, 1985). We hope that further comprehensive studies will solve this issue.

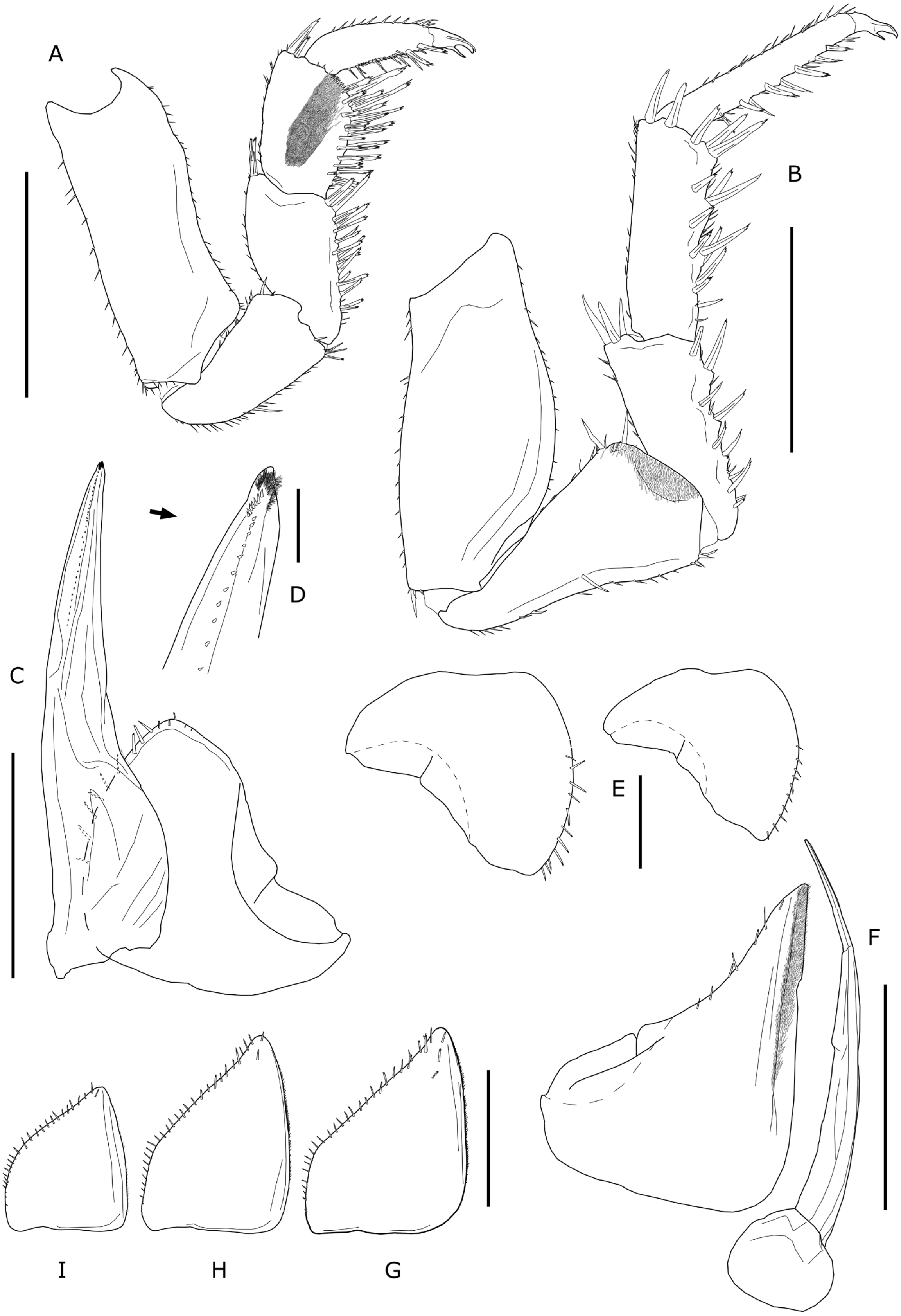

Figure 5. *Porcellio aguerensis* sp. nov., paratype ♂ **A** first male pereopod **B** seventh male pereopod **C** first male pleopod, ventral view **D** distal portion of the first male pleopod endopod, ventral view **E** variation of the first male pleopod, frontal view **F** second male pleopod, frontal view **G** third male pleopod, frontal view **H** fourth male pleopod, frontal view **I** fifth male pleopod, frontal view. Scale bars: 1 mm (**A, B, C, F, G, H, I**); 0.5 mm (**E**); 0.1 mm (**D**).

The other two species of the Betic-Rifian subgroup, *P. colasi* and *P. debueni*, are endemic to the Iberian Peninsula, and Vandel (1958) considers them slightly more derived forms than *P. humberti*. *Porcellio aguerensis* Orihuela-Rivero, sp. nov. shares some characteristics with both, such as a strongly convex body; smooth dorsal surface (although in the continental species there are flattened granules); poorly developed cephalic lobes; posterior margin of the first pereonites sinuous; telson with rounded tip, variable in *P. debueni* (see Vandel 1946, 1958); and male pereopod 7 ischium with concave ventral margin. In addition, with *P. colasi* it also shares a peak on d/c coordinates on pereonite 4. However, *P. aguerensis* Orihuela-Rivero, sp. nov. is easily differentiated from both species by the morphology of the male pleopod 1 exopod, with respiratory field indented, and by the configuration of the glandular system (see Vandel 1946, 1958).

Similarities with continental taxa can also be found in other group of species formerly called "*dispar*" by Vandel (1946), later integrated into the Atlantic group (Vandel 1951, 1956, 1962). Among them, *Porcellio herminiensis* Vandel, 1946, from Iberian Peninsula, undoubtedly stands out, sharing the following with the new species: a convex body; glandular system with a similar configuration (see Vandel 1946); dorsal surface with conspicuous noduli laterales; poorly developed cephalic median lobe; and posterior margin of the first pereonites sinuous. *Porcellio aguerensis* Orihuela-Rivero, sp. nov. differs from this species by its completely smooth dorsal surface; its higher d/c coordinates; its telson with rounded tip and lateral margins without marked angles; the absence of antennal sexual dimorphism; the presence of pereopod 7 sexual dimorphism; and the male pleopod 1 exopod without such a developed tip.

In addition to the continental species mentioned above, there are also several Canary Islands species with which *P. aguerensis* Orihuela-Rivero, sp. nov. shares characteristics. Among these, *Porcellio centralis* Vandel, 1954, endemic to the laurel forest of Gran Canaria island, shares a markedly convex body; a glandular system with the same configuration; similar d/c profile coordinates, although lower in *P. centralis* (Fig. 3C); poorly developed and rounded cephalic lobes; posterior margin of the first pereonites sinuous; and uropods of similar morphology with very oblique protopods. *Porcellio aguerensis* Orihuela-Rivero, sp. nov. differs from *P. centralis* mainly by its completely smooth dorsal surface (in contrast, *P. centralis* has granules, although not very apparent); by the morphology of the posterior lobe of the male pleopod 1 exopod, which in *P. centralis* is totally truncated; and by the presence of sexual dimorphism in the pereopod 7.

Porcellio aguerensis Orihuela-Rivero, sp. nov. also shares similarities to *Porcellio meridionalis* Vandel 1954, a species from the laurel forest of La Gomera and El Hierro: a glandular system with the same configuration; a poorly protruding cephalic median lobe; the posterior margin of the first pereonites sinuous; and a male pleopod 1 exopod with a short but individualized posterior lobe (although with different morphology). However, *P. aguerensis* Orihuela-Rivero, sp. nov. differs from this species by its smaller size (more than 10 mm in *P. meridionalis*); by its totally smooth dorsal surface, with higher d/c coordinates; the lateral cephalic lobes less developed and rounded; and by the presence of sexual dimorphism in the pereopod 7.

On the island of Tenerife, *P. aguerensis* Orihuela-Rivero, sp. nov. also presents similarities, although to a lesser extent, with *Porcellio martini* Dalens, 1984 with which it has in common a convex body, the absence of granules and the configuration of the male pleopod 1 exopod. However, the glandular system configuration and the presence of sexual dimorphism in the pereopod 7 clearly distinguish it (see Dalens 1984). Moreover, the morphology of the male pleopod 1 exopod of the new species is similar to that of *Porcellio septentrionalis* Vandel, 1954 and *Porcellio anagae*, but the rest of characteristics are very different.

In line with the suggestion of Vandel (1958), these morphological similarities argue for a possible relationship between the hypothetical most "primitive" continental species of *Porcellio* of the Atlantic group ("*scaber*"), represented by the Betic-Rifian subgroup, with some of the more "primitive" species of the Canary Islands, such as *Porcellio centralis*, *Porcellio meridionalis*, and *Porcellio aguerensis* Orihuela-Rivero, sp. nov. We also propose a close relationship among these three Canary Island species, which are typical of laurel forests. Both hypotheses can be verified in the future using molecular techniques.

Regarding its conservation status, there remains little understanding of the chorology of the Canary Islands woodlice so the extent to which *P. aguerensis* Orihuela-Rivero, sp. nov. is endemic to the laurel forest of the Anaga is unclear. However, we can assert that in the sampled area, the species would appear to be under intense pressure due to the presence of *Armadillidium vulgare*, an invasive species associated to warmer environments at lower altitudes (Arndt and Mattern 2005). Our sampling at different altitudes indicates that it may be displacing native species, such as *Porcellio anagae*, towards higher altitudes (unpublished data), a phenomenon already studied on other islands by Arndt and Mattern (2005). This situation is especially alarming for already scarce species such as *Porcellio aguerensis* Orihuela-Rivero, sp. nov.

The new species is currently known only from the protected natural area of Anaga Rural Park, so habitat destruction does not pose a threat. However, being in an area highly frequented by visitors, a thorough assessment is recommended.

Conclusion

This study increases the species richness of isopods in the Canary Islands to a total of 36 native species, 30 which are considered endemic. The morphological characteristics of the new species described, *Porcellio aguerensis* Orihuela-Rivero, sp. nov., support the connections between the continental and insular fauna established by previous authors. In the same way, a possible process of radiation between endemic laurel forest species of the archipelago are evidenced. Future studies based on molecular techniques are expected to test these hypotheses.

Acknowledgments

The authors thank Dr Nuria Esther Macías Hernández (University of La Laguna) and Dr Manuel Nogales Hidalgo (IPNA-CSIC) for their support to this study through the loan and cession of dissecting equipment; Dr Daniel Basilio Suárez Ramos and Dr Brent C. Emerson (IPNA-CSIC) for help in the fieldwork; Dr Daniel Basilio Suárez Ramos for his guidance in the use of the graphic editor Inkscape; Dr Lluc García Socias (Museu Balear de Ciències Naturals) and Dr Julio Cifuentes Colmenero (Autonomous University of Madrid) for their technical advice in working with the material examined; Dr Brent C. Emerson (IPNA-CSIC) for reviewing the English text and providing valuable comments; and Ivanklin Campos-Filho and two anonymous reviewers for their constructive comments and suggestions. Fieldwork was supported by the collecting permits Ref. No. Expte. 2022/9790 and Ref. Sigma 133-23 AFF 17-23 provided by the Biodiversity Administration of the Canary Islands Government and the Cabildo of Tenerife, respectively. We acknowledge support for the publication fee by the CSIC Open Access Publication Support Initiative through its Unit of Information Resources for Research (URICI).

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

Funding

Sampling was funded by the project Arthropod Dispersal and Environmental Niche in a changing climate: past and present consequences (ArthroDEN - PID2020-116788GB-I00) supported by the Ministry of Economy and Competitiveness (MINECO) and led by Brent C. Emerson. ROR was supported by University Teacher Training (FPU) program (FPU21/02585) funded by the Ministry of Universities, and VN by a Juan de la Cierva-Formación postdoctoral fellowship (FJC2018-035611-I) funded by MCIN/AEI/10.13039/501100011033.

Author contributions

ROR conceived the original idea with support from HL. CB and VN performed fieldwork and specimen sorting. CB contributed to sample preparation. ROR and CB carried out the experiment. ROR wrote the manuscript with support and inputs from CB, VN, HL and PO. All authors provided critical feedback to the final manuscript.

Author ORCIDs

Raúl Orihuela-Rivero https://orcid.org/0000-0001-6682-0106
Carmen Balibrea https://orcid.org/0000-0002-3143-9329
Víctor Noguerales https://orcid.org/0000-0003-3185-778X
Heriberto López https://orcid.org/0000-0001-6988-5204
Pedro Oromí https://orcid.org/0000-0003-4855-5542

Data availability

All of the data that support the findings of this study are available in the main text.

References

Ancochea E, Fuster J, Ibarrola E, Cendrero A, Coello J, Hernan F, Cantagrel JM, Jamond C (1990) Volcanic evolution of the island of Tenerife (Canary Islands) in the light of new K-Ar data. Journal of Volcanology and Geothermal Research 44(3): 231–249. https://doi.org/10.1016/0377-0273(90)90019-C

Arndt E, Mattern D (2005) Ecological data of isopods (Crustacea: Oniscidea) in laurel forests from the Western Canary Islands. Vieraea 33(Vieraea 33): 41–50. https://doi.org/10.31939/vieraea.2005.33.05

Carracedo JC, Pérez-Torrado FJ (2013) Geological and geodynamic context of the Teide volcanic complex. In: Carracedo J, Troll V (Eds) Teide Volcano. Active Volcanoes of the World. Springer, Berlin, 23–36. https://doi.org/10.1007/978-3-642-25893-0_2

Cifuentes J, Prieto C (2023) Una nueva especie del género *Soteriscus* Taiti & Rossano, 2015 de Fuerteventura (islas Canarias, España): *Soteriscus jandiensis* n. sp. (Isopoda, Oniscidea, Porcellionidae). Boletin de la Asociacion Espanola de Entomologia 47(1–2): 23–33. https://www.entomologica.es/publicaciones-boletin/art1983

Dalens H (1984) Isopodes terrestres rencontrés dans les cavités volcaniques de l'île de Tenerife. Travaux du Laboratoire d'Écobiologie des Arthropodes Édaphiques, Toulouse 5: 12–19.

del Arco MJ, Rodríguez-Delgado O (2018) Vegetation of the Canary Islands. Springer, Cham, 429 pp. https://doi.org/10.1007/978-3-319-77255-4

Dimitriou AC, Taiti S, Schmalfuss H, Sfenthourakis S (2018) A molecular phylogeny of Porcellionidae (Isopoda, Oniscidea) reveals inconsistencies with present taxonomy. ZooKeys 801: 163–176. https://doi.org/10.3897/zookeys.801.23566

Fernández-Palacios JM, Arévalo JR, Balguerías E, Barone R, de Nascimento L, Delgado JD, Elias RB, Fernández-Lugo S, Méndez J, Naranjo A, Sequeira M, Otto R (2017) La Laurisilva: Canarias, Madeira y Azores. Macaronesia Editorial, Santa Cruz de Tenerife, 417 pp.

Gobierno de Canarias (2024) Banco de datos de Biodiversidad de Canarias. https://www.biodiversidadcanarias.es/biota/ [Accessed on 06.03.2024]

Guillou H, Carracedo JC, Paris R, Torrado FJP (2004) Implications for the early shield-stage evolution of Tenerife from K/Ar ages and magnetic stratigraphy. Earth and Planetary Science Letters 222(2): 599–614. https://doi.org/10.1016/j.epsl.2004.03.012

Hoese B (1984) Checkliste der terrestrischen Isopoden der Kanarischen Inseln. Courier Forschungsinstitut Senckenberg 71: 27–37.

Hoese B (1985) Three new species of *Porcellio* (Isopoda: Oniscidea) from the western Canary Islands. Stuttgarter Beitrage zur Naturkunde, Serie A, Biologie 381: 1–12.

Kondraskov P, Schütz N, Schüßler C, de Sequeira MM, Guerra AS, Caujapé-Castells J, Jaén-Molina R, Marrero-Rodríguez Á, Koch MA, Linder P, Kovar-Eder J, Thiv M (2015) Biogeography of Mediterranean hotspot biodiversity: Re-evaluating the 'Tertiary Relict' hypothesis of Macaronesian Laurel forests. PLOS ONE 10(7): e0132091. https://doi.org/10.1371/journal.pone.0132091

Paulian de Félice L (1939) Récoltes de R. Paulian et A. Villiers dans le Haut Atlas Marocain, 1938. (Septième Note). Isopodes terrestres. Bulletin de la Société des Sciences naturelles du Maroc 19: 191–213.

Rodríguez R, Barrientos JA (1993) Dos nuevas especies de isópodos terrestres de las Islas Canarias: *Porcellio babilonus* n. sp. y *Porcellio medinae* n. sp. (Oniscidea: Porcellionidae). Vieraea 22: 79–87.

Rodríguez R, Vicente MC (1992a) Las especies de *Porcellio* Latreillei, 1804 del grupo *laevis* en las Islas Canarias con la descripción de *Porcellio ancararum* n. sp. (Oniscidea, Porcellionidae). Boletin de la Asociacion Espanola de Entomologia 16: 227–241.

Rodríguez R, Vicente MC (1992b) *Porcellio eserensis* sp. n. of terrestrial isopod from the Canary Islands (Isopoda: Oniscidea: Porcellionidae). Bocagiana 162: 1–7.

Rodríguez R, Vicente MC (1992c) *Soteriscus trilineatus* n. sp. de isópodo terrestre de las Islas Canarias (Oniscidea, Porcellionidae). Zoologica Baetica 3: 191–198.

Rodríguez Santana R (1990) Algunas consideraciones sobre el género *Soteriscus* en las Islas Canarias con la descripción de una nueva especie: *Soteriscus dissimilis* n. sp. (Crustacea: Isopoda: Oniscidea). Boletin de la Asociacion Espanola de Entomologia 14: 115–126.

Salces-Castellano A, Patiño J, Alvarez N, Andújar C, Arribas P, Braojos-Ruiz JJ, Arco-Aguilar M, García-Olivares V, Karger DN, López H, Manolopoulou I, Oromí P, Pérez-Delgado AJ, Peterman WE, Rijsdijk KF, Emerson BC (2020) Climate drives community-wide divergence within species over a limited spatial scale: Evidence from an oceanic Island. Ecology Letters 23(2): 305–315. https://doi.org/10.1111/ele.13433

Schmidt C (2003) Contribution to the phylogenetic system of the Crinocheta (Crustacea, Isopoda). Part 2 (Oniscoidea to Armadillidiidae). Zoosystematics and Evolution 79(1): 3–179. https://doi.org/10.1002/mmnz.20030790102

Sfenthourakis S, Taiti S (2015) Patterns of taxonomic diversity among terrestrial isopods. ZooKeys 515: 13–25. https://doi.org/10.3897/zookeys.515.9332

Taiti S, López H (2008) New records and species of Halophilosciidae (Crustacea, Isopoda, Oniscidea) from the Canary Islands (Spain). In: Zimmer M, Charfi Cheikhrouha F, Taiti S (Eds) Proceedings of the International Symposium of Terrestrial Isopod Biology, ISTIB-07. Shaker-Verlag, Aachen, 43–58.

Taiti S, Rossano C (2015) Terrestrial isopods from the Oued Laou basin, north-eastern Morocco (Crustacea: Oniscidea), with descriptions of two new genera and seven new species. Journal of Natural History 49(33–34): 2067–2138. https://doi.org/10.1080/00222933.2015.1009512

Vandel A (1946) Crustacés isopodes terrestres (Oniscoidea) épigés et cavernicoles du Portugal; études des récoltes de Monsieur A. de Barros Machado. Anais da Faculdade de Ciencias do Porto 30: 135–427.

Vandel A (1951) Le genre *Porcellio* (crustacés; isopodes: Oniscoidea). Évolution et systématique. Mémoires du Muséum National d’Histoire Naturelle, Série A, Zoologie 3(2): 81–192.

Vandel A (1954) Étude des isopodes terrestres recueillis aux îles Canaries par J. Mateu en Mars–Avril 1952. Mémoires du Muséum National d’Histoire Naturelle, Série A, Zoologie 8(1): 1–60.

Vandel A (1956) Une nouvelle classification du genre *Porcellio* (crustacés; isopodes terrestres). Bulletin du Muséum National d’Histoire Naturelle, 2^{e} Série 28: 124–128.

Vandel A (1958) Les porcellions du groupe atlantique et du sous-groupe bético-rifain. Bulletin du Muséum National d’Histoire Naturelle (Paris), 2^{e} Série 30: 443–453.

Vandel A (1960) Isopodes terrestres (première partie). Faune de France 64: 1–416.

Vandel A (1962) Isopodes terrestres (deuxième partie). Faune de France 66: 417–931.

ZooKeys 1225: 65–75 (2025)
DOI: 10.3897/zookeys.1225.120937

Short Communication

Chaetophiloscia sicula Verhoeff, 1908 (Isopoda, Oniscidea), an invasive isopod currently spreading in North America

Katalin Szlavecz[1], Nathan T. Jones[2], Franck Noël[3], Pepijn Boeraeve[4], Pallieter De Smedt[4,5]

1 *Department of Earth and Planetary Sciences, Johns Hopkins University, Baltimore, MD, USA*
2 *American Isopod and Myriapod Group, Virginia, USA*
3 *Saint-Martin-de-Connée, France*
4 *Spinicornis, Bonheiden, Belgium*
5 *Forest & Nature Lab, Ghent University, Gontrode, Belgium*
Corresponding author: Katalin Szlavecz (szlavecz@jhu.edu)

Abstract

Chaetophiloscia sicula Verhoeff, 1908 (Philosciidae) is a small terrestrial isopod of Mediterranean origin which was first reported in North America in 2000 in an urban forest in Baltimore, Maryland, and it was thought to be a recent introduction, with a restricted range. Here we report the current state of knowledge of *C. sicula* distribution in North America. Since the original observation, the species has been reported by citizen scientists from eight additional states. Standardized field surveys in Maryland and Washington D.C. revealed a strong habitat preference towards anthropogenic and coastal areas. The affinity of *C. sicula* to urban environments, including residential areas and urban parks, is reinforced by citizen-science data and is most likely key to its fast spread throughout North America. Keeping isopods as pets and trading them among hobbyists may also play a role especially in establishing core populations in urban centers. The species is likely to expand in the USA in the coming decade. This study highlights that thorough, systematic surveys, using a variety of collecting techniques, are essential to address existing knowledge gaps on terrestrial isopod distribution and spread in North America and elsewhere.

Key words: Introduction, non-native species, pet trade, urban habitats, woodlice

Academic editor: Ivan H. Tuf
Received: 16 February 2024
Accepted: 20 May 2024
Published: 5 February 2025

ZooBank: https://zoobank.org/5607E112-E3D9-471E-B99B-E8EB8036B27B

Citation: Szlavecz K, Jones NT, Noël F, Boeraeve P, De Smedt P (2025) *Chaetophiloscia sicula* Verhoeff, 1908 (Isopoda, Oniscidea), an invasive isopod currently spreading in North America. In: Tuf IH, Tajovský K, Taiti S (Eds) The Biology of Terrestrial Isopods, XII. ZooKeys 1225: 65–75. https://doi.org/10.3897/zookeys.1225.120937

Introduction

Humans have been transporting species beyond their historical ranges for centuries, and these intentional and unintentional introductions often negatively affected native biodiversity and ecosystem functions. In North America, many soil invertebrates that were introduced and established a long time ago (Lindroth 1957) have been invading wildland habitats where they potentially alter soil-microbial community structure, understory vegetation, and decomposition and nutrient cycling pathways. Extensive research has shown this to be the case for earthworm-invaded areas (e.g., Szlavecz et al. 2011; Ferlian et al. 2018; Chang et al. 2021). However, only a handful of studies, such as by Frouz et al. (2008) on the isopod *Armadillidium vulgare* and MacLeod et al. (2024) on the larvae of the invasive Japanese beetle *Popillia japonica*, have demonstrated that other invasive soil organisms can also significantly affect ecosystem properties. Proper assessment of potential risks by species invasions requires 1) accurate species identification, 2) sound knowledge on native and introduced distribution ranges, and 3) systematic monitoring to estimate spread (Pagad et al. 2018). In North America, huge data gaps exist in all three requirements for most non-native saprophagous soil macro- and mesofauna.

The terrestrial isopod fauna of North America is dominated by non-native species, especially at mid- to high latitudes. Native species are restricted to coastal and wetland areas, caves, and arid regions at lower latitudes (Jass and Klausmeier 2000; Shultz 2018). The most common non-native species had arrived with the early European settlers (Lindroth 1957) and spread throughout the continent (e.g., Garthwaite et al. 1995). Many species have reached extremely high abundances in a variety of habitats, including mesic and hydric forests (Frouz et al. 2004; Hornung et al. 2015), grasslands (Paris and Pitelka 1962), lake shorelines (Hatchett 1947), and residential areas (Philpott et al. 2014; Szlavecz et al. 2018).

Two decades ago, *Chaetophiloscia sicula* Verhoeff, 1908 (Philosciidae, Fig. 1) was reported in Baltimore, Maryland, for the first time from North America (Hornung and Szlavecz 2003). It was found in an urban forest patch near a housing complex and later on the Homewood campus of Johns Hopkins University. *Chaetophiloscia sicula* was thought to be a recent introduction with a restricted range. Since 2017, an increasing number of observations on the citizen-science platform iNaturalist (2024) has indicated that the species might be more common and widespread than previously thought. Using data from a recent, extensive field survey in the state of Maryland, where it was first recorded, and verified records of iNaturalist (2024), we map the current distribution of the species. The goal is to assess habitat preferences and invasion potential of this recent introduction into North America.

Methods

To assess the current distribution of *C. sicula* we (1) collected data during a large field survey campaign to assess soil arthropod distribution in the state of Maryland and Washington D.C. and (2) extracted community science data from iNaturalist (2024).

The field campaign was conducted across the state of Maryland and Washington D.C between July and November 2022, with additional surveys in May 2023. We selected this area due to its topographic, physiographic, and ecological diversity, with flat coastal plains in the south-east towards more mountainous forested areas in the west. Surveying a variety of ecosystems allowed us to gain insight into the habitat preferences of the species. Following the methodology of Boeraeve et al. (2022), the study area was divided into 10 km × 10 km UTM-squares, 367 squares in total (Fig. 2). To get a good representation of all ecological regions, every square in the western and eastern part of the state was sampled, while in the middle region every third square was sampled (Fig. 2). Within each square, at least two out of the following four habitat types were visited: forest, open habitat, anthropogenic habitat, and coastal habitat. If present, cave habitats were also visited. At each location isopods

Figure 1. *Chaetophiloscia sicula* Verhoeff, 1908 collected in Baltimore, Maryland, in a pile of wood chips in May 2023. The specimen in the picture is about 6 mm long. © Spinicornis – Gert Arijs.

were searched for 1 h by sifting leaf litter, compost, or mulch or turning over dead wood, stones and other cover objects. We also recorded environmental variables such as canopy cover and the vicinity of water bodies. In total, 374 localities were searched for terrestrial isopods of which 148 forests, 34 open landscapes, 130 anthropogenic habitats, 53 coastal habitats, and nine caves. Individuals of *C. sicula* were preserved in 70% ethanol and taken to the laboratory for verification and sex determination.

iNaturalist observations (iNaturalist 2024) of *C. sicula* were verified by the authors based on “Research Grade” and “Needs ID” quality grade observations through habitus photos of live individuals between November 2017 and February 2024. Location data was exported using the iNaturalist website’s built-in export tool on February 11, 2024.

Maps were produced in QGis version 3.4.6. Locality information is provided in the Suppl. material 1.

Results

During the structured field surveys, we collected *Chaetophiloscia sicula* at 32 locations (8.5% of all locations). In all cases it was collected with other cosmopolitan species, including *Armadillidium nasatum* (25 out of 32 locations), *Philoscia muscorum* (24 locations), *Hyloniscus riparius* (21 locations), *Armadilliddium vulgare* (18 locations), and *Haplophthalmus danicus* (14 locations). Where the species was present, we collected between one and 26 individuals (median 5.5) for a total of 240 individuals. Of these, 214 individuals were adults, with 71 individuals (33.2%) being males. Geographically, the species was only found in the Piedmont Plateau Province and the Atlantic Coastal Plain Province (Fig. 2). The species shows a pronounced habitat preference (Table 1), with 56% percent of the observations made in anthropogenic habitat, 34% in

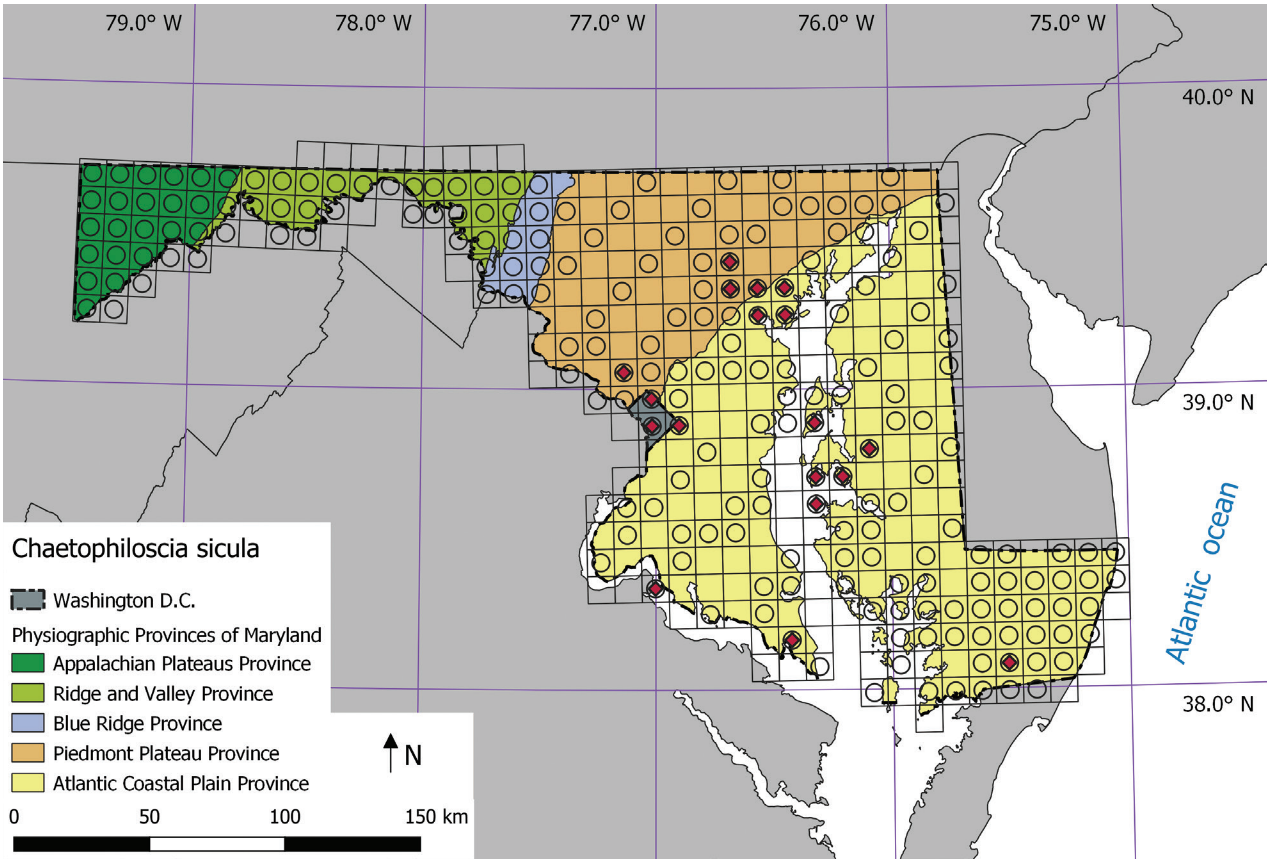

Figure 2. Distribution map of *Chaetophiloscia sicula* in Maryland and Washington D.C, USA. Squares are 10 km × 10 km. Circles represent the visited squares and red diamonds indicate the presence of *C. sicula*. Physiographic provinces follow Reger and Cleaves (2008).

Table 1. Distribution of *Chaetophiloscia sicula* records by habitat types. Data from the structured survey in Maryland and Washington D.C. The species was not recorded in any of the nine caves included in the survey.

	Forest	Anthropogenic	Coast	Open landscape
Number of locations with *C. sicula*	3	18	11	0
Number of locations sampled	139	132	53	34
Percentage of locations with *C. sicula*	2.2	13.6	20.8	0

coastal habitat (seashores), and 9% (only three observations) in forests. We did not find *C. sicula* in either open landscape habitat or caves. *Chaetophiloscia sicula* was found in 13.6% of all sampled anthropogenic locations and in 20.8% of all coastal locations. The majority of anthropogenic records were from gardens and public parks with no or an open canopy. The coastal locations were almost always the upper parts of sandy beaches under driftwood and other debris. A few locations were under debris between stones of jetties. Coastal locations were open with little or no shade from trees. Most coastal locations were close to anthropogenic environments. Abundances in anthropogenic and coastal habitat were often high with tens of individuals collected while sieving one litter sample. All coastal locations where *C. sicula* was recorded were in the

Chesapeake Bay while no records were done at the coast of the Atlantic Ocean. The three observations in forested habitats were in urban forests in Baltimore or Washington D.C. These deciduous forests were relatively open with a maximum of 75% canopy cover. Only a few individuals were found in each forest and all of them were collected close to a stream.

We identified 140 records of *C. sicula* from iNaturalist from nine states and Washington D.C. (Fig. 3). The majority of the records were from Virginia (80) and Maryland (37). We document the species for the first time in Virginia (first record in 2017), Texas (2018), Tennessee (2021), New Jersey (2021), California (2022), North Carolina (2022), Alabama (2023), and Pennsylvania (2023), and present multiple records from Washington D.C. (earliest from 2017). The sighting in California is interesting and indicates that the distribution of the species has also expanded to the US west coast.

In addition to the identification based on photographs, several Alabama, Virginia, and Texas specimens were verified based on the dissection of male sexual characteristics. All but one records are from anthropogenic habitat. For two observations, no habitat data could be retrieved, as the observer had obscured it. As for the structured field surveys, most anthropogenic records are from private gardens and public parks. At least 58 of the 140 locations are close to a stream or water body. The record from North Carolina represents the only coastal record along the Atlantic coast so far.

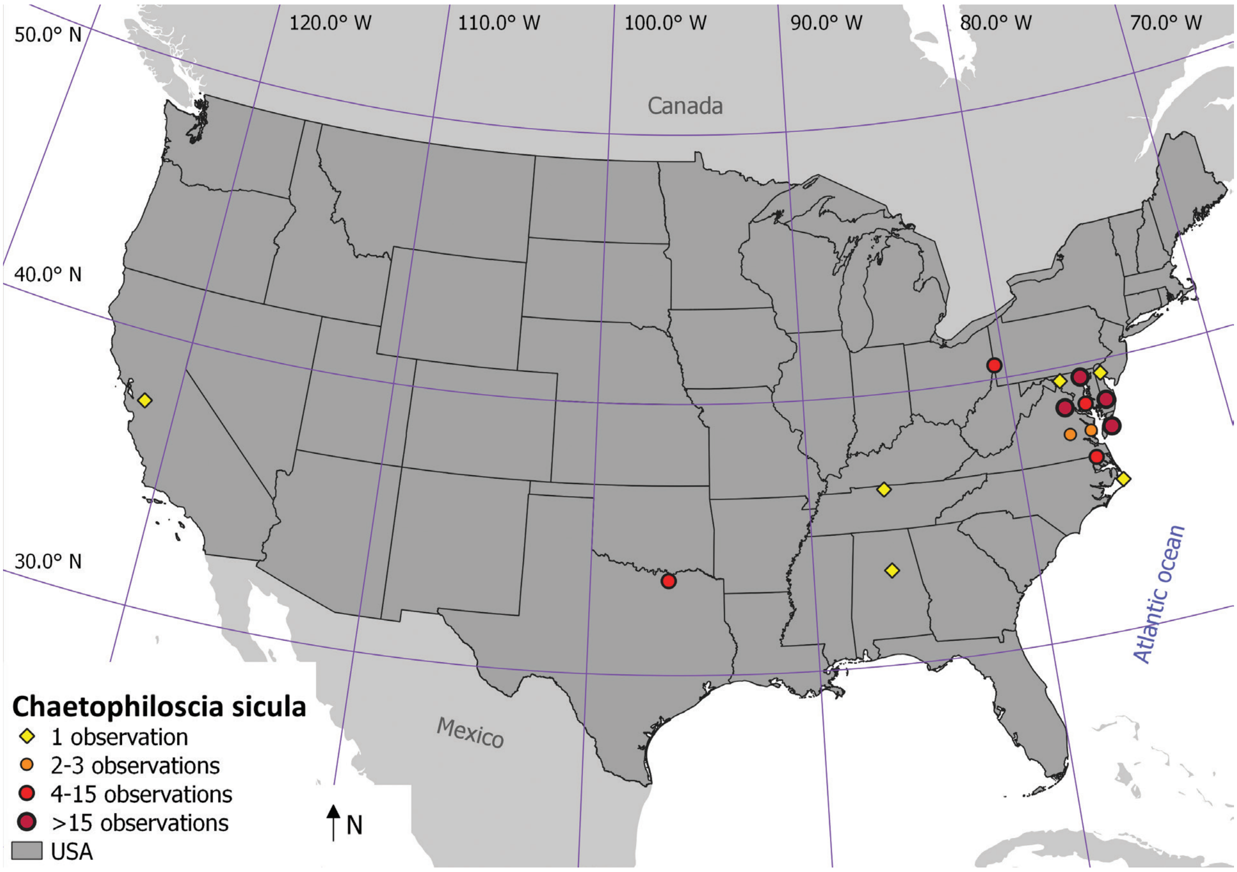

Figure 3. Distribution of *Chaetophiloscia sicula* in the USA as of February 2024. Symbols indicate the number of observations (both iNaturalist and field survey observations) in 1 degree by 1 degree grid cells.

Discussion

Chaetophiloscia sicula is clearly spreading across North America. It appears to be most common around Baltimore where it was first recorded (Hornung and Szlavecz 2003). It has become widespread in the states of Maryland and Virginia, but there are recent records from at least seven other US states. This is probably an underestimation since the over 113,000 terrestrial isopod records on the iNaturalist platform, many still not validated, may yield new records in additional states (iNaturalist 2024). Although most of the records in the seven states are isolated, the distribution and habitat preference in Maryland indicate that the species can establish and spread from isolated localities. With a first record from the west coast, it can be expected that the species will colonize the entire United States. During the last three years, the species was recorded in two additional states every year.

We are aware of issues regarding misidentification and the pitfalls using iNaturalist data for scientific research (Barbato et al. 2021; Munzi et al. 2023). The problem is tangible especially for taxonomically difficult groups as is the case for many soil invertebrate taxa. For instance, the California observation of *C. sicula* was originally misidentified as the genus *Ligidium*. Expert validation is crucial here, which is what we provided by only examining high quality photographs and by additional dissections of the male specimen.

Most locations are in or nearby populated areas. While this may indicate human bias, i.e., people preferentially search in green spaces near their residences, we can still safely assume that human-aided transport is the most important way of spreading. Our records also suggest that *C. sicula* can establish populations in more natural systems such as coastal areas and (urban) forests. The species tolerates salt spray well; humid conditions along coastlines and forested habitats can facilitate natural spread of the species. *Chaetophiloscia sicula* is small and, like other philosciid isopods, probably drought sensitive (Dias et al. 2013); thus they depend on moist conditions to spread. Many observations from riparian areas appear to confirm this and also suggest that stream banks could act as natural corridors for active dispersal.

Interestingly, the species is also rapidly spreading in Europe outside its native range. *Chaetophiloscia sicula* originates from the northern Mediterranean region and occurs from northeastern Spain to Greece (Schmalfuss 2003). Recent records suggest that it is expanding not only within its native range, but beyond it. In Spain it appears to spread southwards with new observations in the central regions up to Madrid. In France it is rapidly expanding westward, from the Mediterranean coast towards the Atlantic coast, and north-east, with recent records from Paris, Dijon and Châlons-en-Champagne (Noël et al. 2014; Noël in press). New records indicating eastward expansion have also been reported. While *C. sicula* has not been found in Slovenia (Vittori et al. 2023), Giurginca and Vănoaica (2000) and Giurginca (2022) reported it from Romania. Turbanov and Gongalsky (2014) reported the species in Sevastopol, the Crimean Peninsula. Two recent observations were also reported in Sevastopol and Simferopol (iNaturalist 2024). In Great Britain, *C. sicula* was recently discovered but appears to be restricted to heated greenhouses (Gregory 2014). While naturally occurring in alluvial plains, meadows, and other open habitats (Cruz 1991; Taiti and Argano 2011; Noël et al. 2014), records from newly colonized locations are often from garden centers and nurseries. These new and isolated anthropogenic records in Europe underpin the importance of human-aided dispersal for *C. sicula*, highlighting similar mechanisms on both continents. In Europe a similar expansion is occurring by *Armadillidium arcangelii*, another Mediterranean species that is rapidly spreading to new areas via potted plants (Noël et al. 2022; De Smedt and Van Dijck 2023). These examples illustrate how fast soil invertebrates can spread in just a few years when facilitated by human activities.

In addition to unintentional transport in North America and elsewhere, increasing national and international pet trade might further facilitate the rapid spread of *C. sicula*. In recent decades, keeping isopods as pets has gained significant popularity with tens of thousands of breeders in the US alone looking for colorful and rare species to breed (De Smedt et al. submitted). A few years ago, a supposedly native American isopod was collected from Alabama and sold nationally under the name *Rhyscotus texensis*. The individuals were later correctly identified as *C. sicula*. When this came to light, vendors culled their populations to prevent its spread; as a result, the species virtually disappeared from the trade (personal observations). However, intentional and accidental release of *C. sicula* colonies might have followed contributing to the increased sightings in other states.

Despite strict restrictions on live specimen import and tens of thousands of interceptions at the US borders annually (Turner et al. 2021), species introductions are still happening as the current cases for Emerald ash borer (*Agrilus planipennis* Fairmaire, 1888) and the agricultural pest *Thrips parvispinus* (Thysanoptera, Thripidae) indicate (Poland and McCullough 2006; Ataide et al. 2024). Small organisms are unnoticed and/or illegally smuggled into the country. Soil organisms as potential invasive species might be overlooked as well, because soil import is heavily regulated, which, among other things, requires that only designated laboratories can receive foreign soil and strict protocols are in place for processing and discarding samples.

In conclusion, *C. sicula* successfully established in North America, and it is currently spreading. The species might have been around for longer than originally thought but was undetected due to its smaller size and lack of effort to systematically assess soil fauna in various ecosystems. As for consequences of new species invasions, in the absence of information on specific traits, one can only speculate. *Chaetophiloscia sicula* is common and abundant in residential areas, potentially becoming a nuisance species. Another philosciid species of similar size, *Pulmoniscus turbanaensis*, is currently invading Colombia, South America, and causing problems in buildings and in small family farms (López-Orozco et al. 2017; De Smedt et al. submitted). Hornung and Szlavecz (2003) provided some characteristics of the Baltimore population. *Chaetophiloscia sicula* exhibited highly clumped dispersion with a mean density of 22.7 individuals m^{-2}. Median body lengths of females and males are 5.9 and 4.3 mm, respectively. Reproductive peak is in June and July with 7–18 eggs in the marsupium depending on female size. Comparison of characteristics well as genetic similarity of these disjunct populations both in North America and in Europe may shed light on the mechanisms of introduction and spread.

Based upon our data, we propose to add *C. sicula* to the list of synanthropic species (Jass and Klausmeier 2000) that dominate the isopod fauna of North America. This study highlights the need for more systematic faunal surveys in the US which will provide a clearer picture on the shifting ranges of native and introduced species.

Acknowledgements

We are grateful to Gert Arijs for his photo of *Chaetophiloscia sicula*. We thank the residents in Maryland and Washington D.C. for permission to sample in their properties. The contribution of the iNaturalist community was essential to have a better picture on the distribution of this terrestrial isopod. We appreciate the time and effort of two reviewers who provided constructive comments on the earlier version of the manuscript.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

Funding

Pallieter De Smedt is funded by the Research Foundation Flanders (FWO).

Author contributions

KS: Conceptualization, investigation, writing, NTJ: Investigation, validation, writing, FN: Writing, PB: Visualization, writing, PDS: conceptualization, methodology, investigation, writing

Author ORCIDs

Katalin Szlavecz https://orcid.org/0000-0003-2504-0298
Nathan T. Jones https://orcid.org/0000-0002-2365-6980
Pepijn Boeraeve https://orcid.org/0000-0002-3752-6407
Pallieter De Smedt https://orcid.org/0000-0002-3073-6751

Data availability

All of the data that support the findings of this study are available in the main text or Supplementary Information.

References

Ataide LM, Vargas G, Velazquez-Hernandez Y, Reyes-Arauz I, Villamarin P, Canon MA, Yang X, Riley SS, Revynthi AM (2024) Efficacy of conventional and biorational insecticides against the invasive pest *Thrips parvispinus* (Thysanoptera: Thripidae) under containment conditions. Insects 15(1): 48. https://doi.org/10.3390/insects15010048

Barbato D, Benocci A, Guasconi M, Manganelli G (2021) Light and shade of citizen science for less charismatic invertebrate groups: Quality assessment of iNaturalist nonmarine mollusc observations in central Italy. The Journal of Molluscan Studies 87(4): eyab033. https://doi.org/10.1093/mollus/eyab033

Boeraeve P, Arijs G, Segers S, Brosens D, Desmet P, Swinnen K, Lambrechts J, De Smedt P (2022) Inventory of the terrestrial isopods in Belgium (2011–2020). ZooKeys 1101: 57–69. https://doi.org/10.3897/zookeys.1101.65810

Chang C-H, Bartz ML, Brown G, Callaham Jr MA, Cameron E, Dávalos A, Dobson A, Görres JH, Herrick BH, Ikeda H, James SW, Johnston MR, McCay TS, McHugh D, Minamiya Y, Nouri-Aiin M, Novo M, Ortiz-Pachar J, Pinder RA, Ransom T, Richardson JB, Snyder BA, Szlavecz K (2021) The second wave of earthworm invasions in North America: Biology, environmental impacts, management and control of invasive "jumping worms". Biological Invasions 23(11): 3291–3322. https://doi.org/10.1007/s10530-021-02598-1

Cruz A, (1991) Especies nuevas o poco conocidas de isópodos terrestres de la Península Ibérica. II. Isópodos epigeos de España y Portugal (Crustacea, Oniscidea). Bulletin de la Société d'histoire naturelle de Toulouse 127: 71–75.

De Smedt P, Van Dijck T (2023) The first record of the terrestrial isopod *Armadillidium arcangelii* Strouhal, 1929 (Isopoda: Armadillidiidae) from Belgium, on an extensive green roof. Bulletin de la Société royale belge d'Entomologie/Bulletin van de Koninklijke Belgische Vereniging voor Entomologie 159: 124–129.

De Smedt P, Jones NT, Kästle B, Robla J, Campos Filho IS, Sfendourakis S, Taiti S, Verheyen K (submitted). Szlavecz. [An urgent call for policy on the worldwide terrestrial isopod trade.]

Dias AT, Krab EJ, Mariën J, Zimmer M, Cornelissen JH, Ellers J, Wardle DA, Berg MP (2013) Traits underpinning desiccation resistance explain distribution patterns of terrestrial isopods. Oecologia 172(3): 667–677. https://doi.org/10.1007/s00442-012-2541-3

Ferlian O, Eisenhauer N, Aguirrebengoa M, Camara M, Ramirez-Rojas I, Santos F, Tanalgo K, Thakur MP (2018) Invasive earthworms erode soil biodiversity: A meta-analysis. Journal of Animal Ecology 87(1): 162–172. https://doi.org/10.1111/1365-2656.12746

Frouz J, Ali A, Frouzova J, Lobinske RJ (2004) Horizontal and vertical distribution of soil macroarthropods along a spatio-temporal moisture gradient in subtropical Central Florida. Environmental Entomology 33(5): 1282–1295. https://doi.org/10.1603/0046-225X-33.5.1282

Frouz J, Lobinske R, Kalčík J, Ali A (2008) Effects of the exotic crustacean, *Armadillidium vulgare* (Isopoda), and other macrofauna on organic matter dynamics in soil microcosms in a hardwood forest in central Florida. The Florida Entomologist 91(2): 328–331. https://doi.org/10.1653/0015-4040(2008)91[328:EOTECA]2.0.CO;2

Garthwaite RL, Lawson R, Sassaman C (1995) Population genetics of *Armadillidium vulgare* in Europe and North America. Crustacean Issues 9: 145–199.

Giurginca A (2022) Oniscidea of Romania. Editura Transversal, Târgovişte, 240 pp.

Giurginca A, Vănoaica L (2000) *Chaetophiloscia sicula* Verhoeff, 1908 (Isopoda, Oniscidea: Philosciidae), a new species for the Romanian fauna. Travaux de l'Institut de Spéologie. Emile Racovitza 37–38: 159–166.

Gregory SJ (2014) Woodlice (Isopoda: Oniscidea) from the Eden project, Cornwall, with descriptions of species new to Britain and poorly known British species. Bulletin of the British Myriapod and Isopod Group 27: 3–26.

Hatchett SP (1947) Biology of the Isopoda of Michigan. Ecological Monographs 17(1): 48–79. https://doi.org/10.2307/1948613

Hornung E, Szlavecz K (2003) Establishment of a Mediterranean Isopod (*Chaetophiloscia sicula* Verhoeff, 1908) in a North American Temperate Forest. Crustaceana Monographs 2: 181–189. https://doi.org/10.1163/9789047412854_015

Hornung E, Szlavecz K, Dombos M (2015) Demography of some non-native isopods (Crustacea, Isopoda, Oniscidea) in a Mid-Atlantic forest, USA. ZooKeys 515: 127–143. https://doi.org/10.3897/zookeys.515.9403

iNaturalist (2024) iNaturalist. https://www.inaturalist.org [Accessed on 11/02/2024]

Jass J, Klausmeier B (2000) Endemics and immigrants: North American terrestrial isopods (Isopoda, Oniscidea) north of Mexico. Crustaceana 73(7): 771–799. https://doi.org/10.1163/156854000504804

Lindroth CH (1957) The Faunal Connections Between Europe and North America. John Wiley & Sons, Inc., New York, 344 pp. https://doi.org/10.5962/bhl.title.6759

López-Orozco CM, Carpio-Díaz YM, Navas SGR, Campos-Filho IS (2017) A new species and first record of *Pulmoniscus* Leistikow, 2001 (Isopoda, Oniscidea, Philosciidae) from Colombia. Nauplius 25(0): e2017014. https://doi.org/10.1590/2358-2936e2017014

MacLeod GR, Richmond DS, Filley TR (2024) Invasive Japanese beetle (*Popillia japonica* Newman) larvae alter structure and carbon distribution in infested surface soil. The Science of the Total Environment 918: 170687. https://doi.org/10.1016/j.scitotenv.2024.170687

Munzi S, Isocrono D, Ravera S (2023) Can we trust iNaturalist in lichenology? Evaluating the effectiveness and reliability of artificial intelligence in lichen identification. Lichenologist (London, England) 55(5): 93–201. https://doi.org/10.1017/S0024282923000403

Noël F (in press) *Orthometopon dalmatinum* (Verhoeff, 1901) (Crustacea: Isopoda: Oniscidea), nouvelle espèce pour la faune de France. Revue scientifique Bourgogne-Franche-Comté Nature 39

Noël F, Séchet E, Mouquet C, Bécheau F (2014) Découverte de *Chaetophiloscia sicula* Verhoeff, 1908 (Philosciidae) et de *Stenophiloscia glarearum* Verhoeff, 1908 (Halophilosciidae) dans l'Ouest de la France (Crustacea, Isopoda, Oniscidea). Invertébrés Armoricains 10: 47–53.

Noël F, Gregory SJ, Agapakis G (2022) *Armadillidium arcangelii* Strouhal, 1929 (Isopoda: Oniscidea): a step towards the conquest of Europe? Bulletin of the British Myriapod and Isopod Group 34: 72–82.

Pagad S, Genovesi P, Carnevali L, Schigel D, McGeoch MA (2018) Introducing the global register of introduced and invasive species. Scientific Data 5(1): 170202. https://doi.org/10.1038/sdata.2017.202

Paris OH, Pitelka FA (1962) Population characteristics of the terrestrial isopod *Armadillidium vulgare* in California grassland. Ecology 43(2): 229–248. https://doi.org/10.2307/1931979

Philpott SM, Cotton J, Bichier P, Friedrich RL, Moorhead LC, Uno S, Valdez M (2014) Local and landscape drivers of arthropod abundance, richness, and trophic composition in urban habitats. Urban Ecosystems 17(2): 513–532. https://doi.org/10.1007/s11252-013-0333-0

Poland TM, McCullough DG (2006) Emerald ash borer: Invasion of the urban forest and the threat to North America's ash resource. Journal of Forestry 104(3): 118–124. https://doi.org/10.1093/jof/104.3.118

Reger JP, Cleaves ET (2008) Physiographic map of Maryland: Baltimore, MD. Maryland Geological Survey. http://www.mgs.md.gov/publications/data_pages/phys_prov_gis.html [Accessed on: 2024-5-29]

Schmalfuss H (2003) World catalog of terrestrial isopods (Isopoda: Oniscidea). Stuttgarter Beitrage zur Naturkunde. Serie A, Biologie 654: 1–341.

Shultz JW (2018) A guide to the identification of the terrestrial Isopoda of Maryland, USA (Crustacea). ZooKeys 801: 207–228. https://doi.org/10.3897/zookeys.801.24146

Szlavecz K, McCormick M, Xia L, Saunders J, Morcol T, Whigham D, Filley T, Csuzdi C (2011) Ecosystem effects of non-native earthworms in Mid-Atlantic deciduous forests. Biological Invasions 15(5): 1165–1182. https://doi.org/10.1007/s10530-011-9959-0

Szlavecz K, Vilisics F, Toth Zs, Hornung E (2018) Terrestrial isopods in urban environments: An overview. ZooKeys 801: 97–126. https://doi.org/10.3897/zookeys.801.29580

Taiti S, Argano R (2011) Oniscidea di Sardegna (Crustacea, Isopoda). In: Nardi G, Whitmore D, Bardiani M, Birtele D, Mason F, Spada L, Cerretti P (Eds) Biodiversity of Marganai and Montimannu (Sardinia). Research in the framework of the ICP Forests network. Conservazione Habitat Invertebrati 5: 163–222.

Turbanov IS, Gongalsky KB (2014) Finding of a new to the Crimea woodlouse species — *Chaetophiloscia sicula* (Isopoda, Oniscidea, Philosciidae). Vestnik Zoologii 48: 475–478. https://doi.org/10.2478/vzoo-2014-0057

Turner RM, Brockerhoff EG, Bertelsmeier C, Blake RE, Caton B, James A, MacLeod A, Nahrung HF, Pawson SM, Plank MJ, Pureswaran DS, Seebens H, Yamanaka T, Liebhold AM (2021) Worldwide border interceptions provide a window into human-mediated global insect movement. Ecological Applications 31(7): e02412. https://doi.org/10.1002/eap.2412

Vittori M, Mavrič B, Pekolj A, Govedič M, Zagmajster M (2023) A checklist of isopods (Crustacea: Isopoda) in Slovenia. Natura Sloveniae 25(2): 27–52. https://doi.org/10.14720/ns.25.2.27-52

Supplementary material 1

Localities of *Chaetophiloscia sicula* in North America

Authors: Katalin Szlavecz, Nathan T. Jones, Franck Noël, Pepijn Boeraeve, Pallieter De Smedt

Data type: xlsx

Link: https://doi.org/10.3897/zookeys.1225.120937.suppl1

ZooKeys 1225: 77–102 (2025)
DOI: 10.3897/zookeys.1225.125027

Checklist

Terrestrial isopods (Isopoda, Oniscidea) of Slovakia: an annotated checklist, new records, and bibliography

Andrej Mock[1], Karel Tajovský[2], Adrián Purkart[3], Ivan Hadrián Tuf[4]

1 *Department of Zoology, Faculty of Science, Institute of Biology and Ecology, Pavol Jozef Šafárik University, Šrobárova 2, SK-04154 Košice, Slovakia*
2 *Biology Centre CAS, Institute of Soil Biology and Biogeochemistry, Na Sádkách 7, CZ-37005 České Budějovice, Czech Republic*
3 *Department of Zoology, Faculty of Natural Sciences, Comenius University, Mlynská dolina, Ilkovičova 6, SK-84215 Bratislava 4, Slovakia*
4 *Department of Ecology and Environmental Sciences, Faculty of Science, Palacký University Olomouc, Šlechtitelů 27, CZ-77900 Olomouc, Czech Republic*
Corresponding author: Andrej Mock (andrej.mock@upjs.sk)

Abstract

Woodlice (terrestrial isopods) represent an unmissable, often very numerous components of macrosaprophages inhabiting soil, rocky environments, rotting wood and subterranean habitats, as well as the dwellings of various organisms, including humans. The paper provides a comprehensive summary of the literature on woodlice in Slovakia, including a list of species with unpublished data on two exotic species first recorded in Slovakia. Research on Slovakian terrestrial isopods has been documented in 213 papers (from 1794 to 2024) and 25 theses. To date, 48 species from 14 families have been recorded in Slovakia. Of these, 30 species are autochthonous, while 18 species have been introduced into anthropogenic habitats, with eight species found exclusively in greenhouses. The list also includes an analysis of habitat preferences, bioindicator potential, and biogeographic comments. Notably, two species, *Buddelundiella cataractae* and *Reductoniscus costulatus*, are recorded in Slovakia for the first time. Carpathian elements are sparsely distributed within the mosaic of well-preserved natural forests. *Hyloniscus mariae* is likely the only surface-dwelling endemic species in the northern parts of the Carpathian range. Other species, such as *Hyloniscus transsilvanicus*, *Trichoniscus carpaticus* and *Trachelipus difficilis*, have a broader distribution within the Carpathians. Additionally, *Mesoniscus graniger*, *Orthometopon planum* and a few other taxa reach their northern distribution limits in Slovakia. Current research is predominantly focused on the fauna of subterranean habitats and urbanized landscapes.

Key words: Carpathian-Pannonian region, chorotypes, distribution, ecological classification, list of species, woodlice

OPEN ACCESS

Academic editor: Stefano Taiti
Received: 8 April 2024
Accepted: 26 July 2024
Published: 5 February 2025

ZooBank: https://zoobank.org/88E6C6C7-C121-4047-B806-5A9702570672

Citation: Mock A, Tajovský K, Purkart A, Tuf IH (2025) Terrestrial isopods (Isopoda, Oniscidea) of Slovakia: an annotated checklist, new records, and bibliography. In: Tuf IH, Tajovský K, Taiti S (Eds) The Biology of Terrestrial Isopods, XII. ZooKeys 1225: 77–102. https://doi.org/10.3897/zookeys.1225.125027

Introduction

Slovakia (the Slovak Republic) is a small, landlocked country situated in Central Europe. Historically, it has been part of significant state entities, such as the Kingdom of Hungary and later the Austro-Hungarian Monarchy. In 1918, Slovakia joined with Czechia to form the Czechoslovak Republic. During World War II (1938–1945), the southern parts of Slovakia were annexed by the Kingdom of Hungary, while an independent Slovak Republic was established. After the war, Slovakia became part of Czechoslovakia until 1993, when it became an independent country.

This historical context is crucial when examining faunistic data. Faunistic publications from the region have been authored in various languages, including Hungarian, Slovak, German, Czech, Polish, and English. Historically, the rugged and less urbanized landscape attracted zoologists, including isopodologists. Researchers from neighbouring countries such as Hungary, Austria, Czechia, and Germany, with local authors, have contributed to the exploration of Slovak isopod fauna.

The country's geography is diverse, with the flattened and mostly deforested landscape of the Pannonian (Danube) Lowland in the south bordered by the forested Carpathian Mountains in the north. The Carpathians are a significant extra-Mediterranean European biodiversity hotspot, often acting as a barrier for thermophilous species distribution and a crossroads for woodland faunas of different origins.

Rapid environmental changes, habitat destruction and the spread of allochthonous species (neobiota) underscore the importance of studying sensitive biota, such as soil arthropods like woodlice. Despite numerous references to terrestrial isopods in the literature, these soil arthropods have historically received inadequate attention. The lack of recent faunistic surveys, questionable historical data and ambiguities regarding species origins and endemism in Slovakia (covering the northern parts of the Carpathians and the northernmost tips of the Pannonian Lowland), with a paucity of ecological data on species, have resulted in an incomplete understanding of woodlouse fauna. During the past two decades, knowledge of isopods in Slovakia has significantly increased, but a comprehensive synthesis is still lacking. This gap in knowledge prompted the creation of this paper.

History

There are only minimal records of terrestrial isopods from the territory of present-day Slovakia before the 20th century. The first information on woodlice in the broader region of what is now Slovakia dates to the 18th century, mentioning taxa from the genera "*Oniscus*" and "*Porcellio*" (Grossinger 1794: 353). Significant faunistic publications began appearing in the mid-19th century. One of the earliest was by medical doctor Gustáv M. Reuss in 1853–1854, who documented the fauna of the town of Revúca in a large manuscript. This unpublished work was partly reviewed by Uhrin and Panigaj (2017), listing two common synanthropic species, *Porcellio scaber* (as *Porcellio asellus*) and *Oniscus asellus* (as *Oniscus murarius*).

Cornelius Chyzer (1858), an expert on the natural history of present-day Eastern Slovakia, identified seven species in former Hungary, but only *Porcellio scaber* was localised to the town of Bardejov (Bartfeld) in northeast Slovakia (Chyzer 1858). Other sporadic data from the period came from the Tatra Mountains (Nowicki 1867) and regions bordering Poland (Dyduch 1903, 1904), as well as small towns such as Lučenec (= Losoncz; Malesevics 1892) and Banská Štiavnica (= Selmeczbánya; Petricskó 1892).

Little additional information was provided until the end of World War I and the Austro-Hungarian Monarchy. Dollfus (1901) reviewed terrestrial isopods in the National Museum in Budapest, documenting four common species from regions now in Slovakia. Ortvay (1902) reviewed the fauna of the former Pozsony (Pressburg or Bratislava) County, noting terrestrial isopods collected by Rudolf Szép. Karl Mergl, a professor at the lyceum in Bratislava, listed four species in his extensive manuscript focused on the flora and fauna of the city of Bratislava

(Mergl 1940): *Porcellio scaber*, *P. laevis*, *Oniscus asellus* (as *O. murarius*) and *Armadillidium vulgare* (as *Amadillo vulgaris*).

The first synthesis of isopod fauna knowledge, including data from Slovakia, was conducted by Csiki (1926). He summarised data from the Hungarian Monarchy, reviewing samples from fifteen Slovak localities. During the first half of the 20th century, faunistic data were collected by Dudich (1925, 1928, 1942, 1957), Méhelÿ (1929), Pongrácz (1936), and Kesselyák (1936). However, the most significant contributions to the taxonomy and biogeography of local isopod fauna were made by Verhoeff (1907, 1908, 1927a, b, 1937), Strouhal (1929a, b, 1939a, b, 1940a, b, 1947, 1948, 1951, 1953, 1964, 1965), and Frankenberger (1939, 1940, 1954, 1959, 1964). Strouhal (1940a, b) and Frankenberger (1940) provided the first comprehensive overviews of the Slovakian isopod fauna, briefly repeated by Babor (1943). Strouhal (1951) summarised woodlouse fauna knowledge in Central Europe and Frankenberger completed the work on Czech and Slovak fauna with a precise identification key (Frankenberger 1954) and monograph (Frankenberger 1959). Zdeněk Frankenberger was a decisive person defining modern faunistic and taxonomic research of terrestrial isopods in the former Czechoslovakia (Flasarová 1967).

Walter Černý, a prominent ornithologist and university teacher, also contributed to the study of terrestrial isopods. He provided collections to Frankenberger and reviewed his monograph (Frankenberger 1959). Černý's unpublished collection, preserved in the National Museum in Prague, includes approximately 30 items (tubes) from Slovakia, containing 21 isopod species (nearly 2500 individuals) collected between 1931–1956 by various collectors. This material has been recently examined and the data were prepared for detailed publication (see Mock and Dolejš 2023).

After World War II, the study of isopods in Slovakia was continued by Ján Gulička, Miroslav Krumpál, and Marie Flasarová. Flasarová conducted faunistic and ecological studies (Flasarová 1980, 1986, 1994, 1998; Flasar and Flasarová 1989) and compiled an annotated bibliography and checklist of Slovak isopod fauna, published as an addition to her paper on the fauna of the Danube Lowland (Podunajská nížina) (Flasarová 1998). Her work includes short notes on findings in Slovakia, mainly focusing on the Czech fauna (Flasarová 1960, 1976, 1988, 1995). She was supported by influential Slovak zoologists Ján Gulička (Little Carpathians region), Ján Brtek (karst and lowlands of Slovakia) and Pavel Deván (Biele Karpaty Mts. and surroundings), who provided their collections. While Brtek and Deván apparently gave Flasarová their entire collections (Flasarová 1994), much of Gulička's collection remains unprocessed in his estate, located in the Natural History Museums in Bratislava and Liptovský Mikuláš and the Department of Zoology, Faculty of Natural Sciences, Comenius University in Bratislava. Rudy et al. (2021) analysed these items, with the other material from isopods sampled in Slovak caves, stored at the affiliated laboratory of the first author of this study. A small collection of isopods from the Košel estate in Bratislava was analysed by Mock (2023).

In the 21st century, terrestrial isopods in Slovakia have been frequently studied as part of cave arthropod assemblages. Comprehensive summaries of these studies have been provided by Košel (2007, 2009, 2012) and Kováč et al. (2014a). The unique invertebrate communities found in underground scree slopes have also been a source of valuable information on terrestrial isopods (Rendoš and Mock 2010, 2012; Rendoš 2012, 2016; Rendoš et al. 2016, 2019;

Rudy et al. 2018). Tóth (2019) studied community isopods within the assemblage of macrofauna of solifluction volcanic debris.

The cave isopod *Mesoniscus graniger* Frivaldszky, 1965, is particularly well studied in Slovakia. Extensive research covers its distribution, taxonomy, food preferences, gut content, cuticle anatomy, and life history, incorporating data from Slovak localities (Staněk 1932a, b; Kettner 1936; Balthasar and Frankenberger 1937; Arcangeli 1939; Gruner and Tabacaru 1963; Gulička 1975, 1978, 1982; Košel 1975, 1976, 1994, 2006; Gaál 1978, 1987, 2000; Hůrka and Pulpán 1978; Pomichal 1982; Mlejnek 1999, 2000, 2002; Gaál et al. 2000; Giurginca 2000; Franc and Mlejnek 2000; Mlejnek and Ducháč 2001, 2003; Elhottová et al. 2003, 2004; Giurginca 2005; Krištůfek et al. 2005; Nováková et al. 2005; Šustr et al. 2005, 2009; Balciar 2007; Piksa and Farkas 2007; Papáč 2008; Lukešová and Nováková 2009; Višnovská and Barlog 2009; Giurginca et al. 2012, 2015, 2016; Smrž et al. 2015; Tajovský and Mock 2015; Bella et al. 2016, 2022; Derbák et al. 2018; Ratkovský et al. 2019; Gaál et al. 2020). *Mesoniscus graniger* and its associated species are also noted in biodiversity studies of caves (Košel 1984; Kováč et al. 2001, 2002a, b, 2004, 2005a, b, c, 2006, 2008, 2010b; Mock et al. 2002, 2004a, b, 2009; Lukáň et al. 2004; Papáč et al. 2006, 2007, 2009, 2014, 2019, 2020). Although *M. graniger* is absent in some regions with karst or non-karst underground habitats, common epigeic terrestrial isopods are sometimes found in shallow underground areas (Kováč et al. 2003, 2014b; Mock et al. 2003, 2005; Ľuptáčik et al. 2005; Papáč 2006, 2018; Papáč et al. 2015; Višňovská et al. 2017; Galabová 2022; Pribišová 2022).

The diversity and distribution of terrestrial isopods in Slovak caves were summarized by Rudy et al. (2021) and updated by Melega et al. (2022), incorporating new data from various collectors, including the estates of Gulička and Václav Ducháč. Studies on the phenology of *M. graniger* and co-occurring species, as well as their distribution along the depth gradient in limestone talus habitats, have been conducted by Mock et al. (2015), Rendoš et al. (2016) and Rudy et al. (2018).

Terrestrial isopods have also been found associated with the rhizosphere of plants in spring fens (Axamská 2021).

Woodlice assemblages have also been used to indicate environmental changes (Gulička 1957, 1960; Krumpál 1973, 1983; Čarnogurský et al. 1994; Majzlan and Hošták 1996; Jedlička et al. 1999; Jurík 2001; Snopková 2001; Stašiov 2001; Tajovský 2002; Majzlan et al. 2004; Dubovský 2005; Tuf and Tufová 2005; Holecová et al. 2005, 2012; Drinková 2006; Štrichelová and Tuf 2012; Martinka 2022; Martinka et al. 2022, 2023a, b). Haľková-Valkay et al. (2022) investigated isopods in leaf litter during winter.

Krumpál (1976, 1977) studied the reproductive biology and development of woodlice, proposing a method for evaluating biomass and length (Krumpál and Žitňanský 1977). Šustr et al. (2005) and Hámorský (2010) conducted eco-physiological experiments on some autochthonous isopod species.

Detailed faunistic studies have been conducted in specific regions, such as Tekov County (Comitat Bars) (Kesselyák 1936; Dudich 1957), the Malé Karpaty Mts. (Little Carpathians) (Mišík et al. 1974; Flasarová 1986; Flasar and Flasarová 1989; Kuracina and Kabátová 2005; Tuf and Tufová 2005; Majzlan et al. 2011; Štrichelová and Tuf 2012), the Nízke Beskydy Mts. (Krumpál 1975; Mock 2020), the Slovak Karst and Muránska Plateau (Gulička 1985), the Danube Lowland (Flasarová 1998; Gogová 2004), the Burda Mts. (Flasarová 1998; Mock 2017; Mock et al. 2020), the Pieniny Mts. (Hudáková and Mock 2006) and the

Cerová vrchovina Highland (Tajovský and Mock 2015). Other notable studies include those on the isopod fauna of the Biele Karpaty Mts. (Tajovský et al. 2018), the Eastern Slovak Plain and Zemplínske vrchy Mts. (Mock et al. 2021), and the Čierna hora Mts. (Timková 2008; Timková and Mock 2008). Topp et al. (2006) studied isopod diversity in central Slovakia's primeval forest. Turis and Vidlička (2013) noted *Protracheoniscus politus* in invertebrate assemblage associated with rare endemic plant, *Cyclamen fatrense*. Some species were mentioned in the general characteristics of protected areas (Čaputa 1987, 1988, 1991; Hudec 2002; Kováč et al. 2010a; Lantos et al. 2010) or Slovakia overall (Ferianc et al. 1972; Vilček and Hudec 2019). Mock et al. (2007) compared isopod biodiversity between faunistically well-studied regions in Poland and Slovakia.

Woodlice in synanthropic sites such as urban soils (Krumpál 1993), forest fragments in cities (Čemesová 2001; Jakabová 2001; Remešicová 2011; Štrichelová and Tuf 2012; Plutinská 2015), house interiors (Krumpál and Krištofík 1982, 1985; Krumpál et al. 1985), compost heaps (Rudy 2016; Rudy and Mock 2016) and greenhouses (Krumpál et al. 1997, 1999; Palkovičová and Mock 2008; Droběnová and Mock 2009) have also been noted. Additionally, studies on fauna in bird and mammal nests and burrows have included terrestrial isopods (Nosek and Lichard 1962; Fenďa et al. 1998; Cyprich et al. 2000; Vaníková 2009; Mock et al. 2008, 2021).

Historical data have been recently published, such as the collection of Czech arachnologist František Miller, revised by Dolejš and Tuf (2018), documenting twelve common species in northwest Slovakia and the Slovak Karst. Many other papers list terrestrial isopods faunistically or mention a few species marginally.

Data on terrestrial isopods of Slovakia have also been extracted from unpublished student theses defended at various universities, including Pavol Jozef Šafárik University in Košice, Comenius University in Bratislava, Technical University in Zvolen, Palacký University Olomouc, Technical University of Ostrava, and Prešov University in Prešov. All theses have been checked by the authors of this paper.

Secondary data on terrestrial isopods in Slovakia can also be found in monographs or papers focused on isopod fauna of other European regions (e.g., Wächtler 1937; Urbański 1950; Dominiak 1962; Gruner 1966; Schmölzer 1965; Radu 1983, 1985; Flasarová 1995; Tomescu et al. 2015, 2016 and Giurginca 2022), in the worldwide catalogue of terrestrial isopods (Schmalfuss 2003) or in the determination key of invertebrates from the Czech and Slovak republics (Buchar et al. 1995). Štrichelová (2010) tried to outline the characteristics of the isopodofauna of the Western Carpathians, while Kseňáková (2011) collected published data on the occurrence of representatives of the Trichoniscidae family in Slovakia.

Since 2000, a small informal working group of isopodologists from the Czech and Slovak republics has been meeting together with myriapodologists with a frequency of approximately one and a half years at workshops that include presentations with abstract proceedings, discussions, and field mapping alternately at locations in the Czech Republic and Slovakia. The last, thirteenth, meeting of the isopologic community took place in Hostomice, Czech Republic in 2003 (for proceedings and history of meetings see Dolejš 2023).

This study presents a critical list of the fauna with an analysis of habitat preferences, bioindication potential and biogeography, with notes on the occurrence of rare species, dubious literature data and first recorded species.

Methods

We thoroughly reviewed all taxonomic, faunistic and ecological literature from 1794 to the end of 2023 to compile a comprehensive bibliography of terrestrial isopods identified to the species level within present-day Slovakia. This bibliography includes more than 200 publications and more than 20 unpublished university theses. We excluded abstracts from scientific congresses and meetings if their topics were later published in regular articles. The resulting checklist was critically reviewed based on current taxonomy, ecology, and distribution knowledge, as well as field experiences. The nomenclature aligns with the views of authorities like Schmalfuss (2003) and Boyko et al. (2024).

Collections of terrestrial isopods from Slovakia are housed in various museums:

- The Natural History Museum in London, United Kingdom
- The Hungarian National History Museum in Budapest, Hungary
- The National Museum in Prague, Czech Republic
- The Natural History Museum in Vienna, Austria
- The East Slovak Museum in Košice, Slovakia
- The Slovak Museum of Nature Protection and Speleology in Liptovský Mikuláš, Slovakia

The most extensive unpublished collection is in the National Museum in Prague, primarily due to the estates of Z. Frankenberger, W. Černý, and M. Flasarová. Additionally, significant collections are maintained in the authors' institutions.

Results and discussion

We found 213 publications documenting terrestrial isopod species in Slovakia, authored by 191 individuals. Of these, 127 authors contributed to just one publication each, while 29 authors had five or more publications (Fig. 1). Notably, five of the most prolific authors were speleologists who extensively sampled cave fauna throughout Slovakia. The interest in Slovakian terrestrial isopods peaked at three distinct times (Fig. 2): around the turn of the 19th and 20th centuries, just before World War II and at the start of the 21st century. The most recent peak reflects a surge in speleological research and university-driven studies, with 25 theses defended primarily at the universities in Košice, Bratislava (Slovakia), and Olomouc (Czech Republic).

Summarizing all published and unpublished data, we compiled a list of 48 woodlice species from 14 families in Slovakia (Table 1). When comparing woodlouse fauna of neighbouring countries, species counts vary: 57 in Hungary (Forró and Farkas 1998; Farkas and Vilisics 2013), 43 in the Czech Republic (Orsavová and Tuf 2018), 34 in Poland (Jażdżewski and Konopacka 1993) and 96 in Romania (Giurginca 2022). These differences are influenced by several factors, including the extent of faunistic knowledge, country dimensions and heterogeneity, landscape naturalness, latitude position and proximity to main biorefugia like the Balkan Peninsula the Alps and the Carpathians. The presence of introduced species correlates with the urbanization of the environment and trade traffic (Sfenthourakis and Hornung 2018).

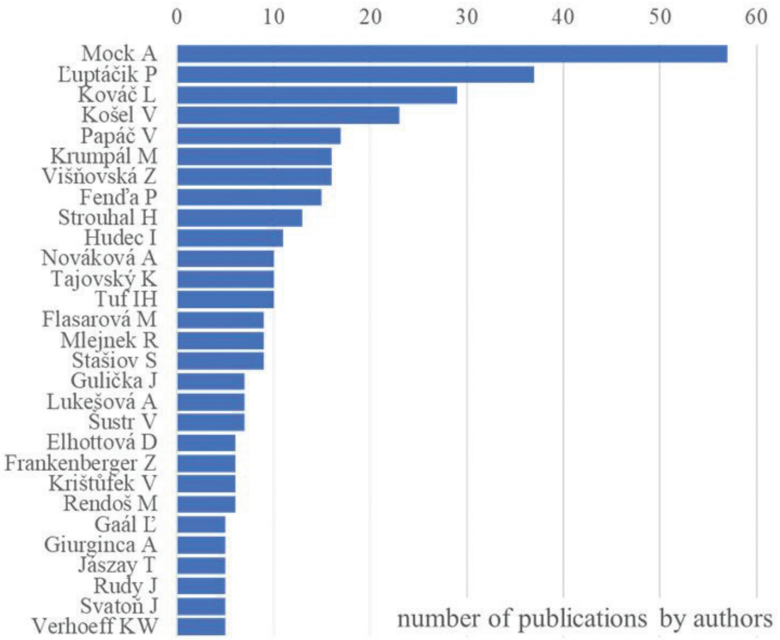

Figure 1. List of the most active authors publishing five or more publications on Slovak terrestrial isopods (1794–2023).

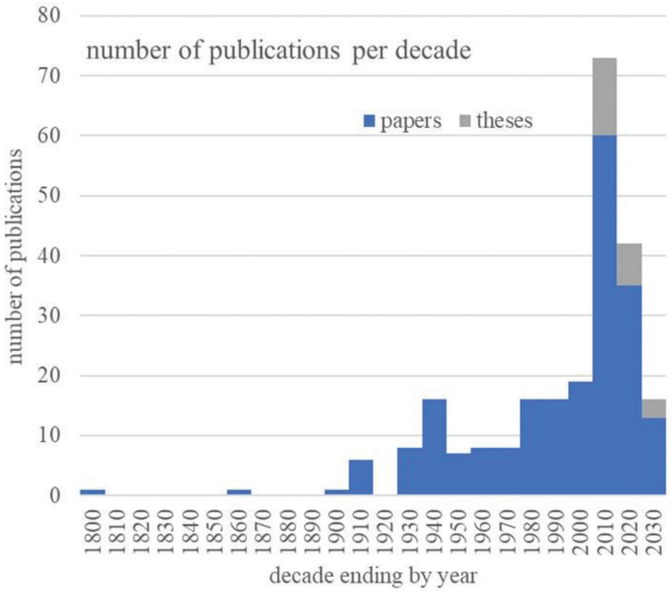

Figure 2. Development of interest about Slovak terrestrial isopod fauna represented by the number of papers published. Counts of papers are summarised in decades, complete list contains 221 papers and 25 theses.

Mountainous areas in Slovakia are particularly diverse in isopod species, hosting 24 species, half of which are exclusive to these regions and likely autochthonous. In contrast, lowland areas are less diverse, with 16 species, among which *Armadillidium zenckeri* is native and exclusive to these areas, where it prefers wetlands. Lowlands are more affected by human activities, resulting in higher proportions of both eurytopic and non-native species. Urban environments support a significant number of isopod species, with a notable presence of alien, thermophilic, and xerotolerant species.

Table 1. List of terrestrial isopods (Oniscidea) documented in Slovakia with geographical and ecological characteristics.

Families	List of species	Regional chorotypes	Global chorotypes	Relictness	Habitat
LIGIDIIDAE	_Ligidium germanicum_ Verhoeff 1901	M	C+SE Europe	R	forest (leaf litter)
	Ligidium hypnorum (Cuvier, 1792)	M	W Palearctic	A	forest (leaf litter)
	Ligidium intermedium Radu, 1950	M	**Carpathian**	R	forest (leaf litter)
MESONISCIDAE	_Mesoniscus graniger_ (Frivaldszky, 1865)	S	**Carpathian +Dinaric**	R	cave, scree
STYLONISCIDAE	*_Cordioniscus stebbingi_ (Patience, 1907)	U	SW Europe	E	greenhouse
TRICHONISCIDAE	_Androniscus roseus_ (C. Koch, 1838)	U	S Europe	E	rotten wood
	Haplophthalmus danicus Budde-Lund, 1880	U/L	Europe	E	rotten wood
	Haplophthalmus mengii (Zaddach, 1844)	M	W Palearctic	A	forest (rotten wood)
	Hyloniscus mariae Verhoeff, 1908	M	**Carpathian**	R	forest (leaf litter)
	Hyloniscus riparius (C. Koch, 1838)	L/M/U	C+E Europe	E	forest (leaf litter)
	Hyloniscus transsilvanicus (Verhoeff, 1901)	L/M	**Carpathian**	R	forest (leaf litter)
	*_Miktoniscus linearis_ (Patience, 1908)	U	unknown	E	greenhouse
	*_Trichoniscoides albidus_ (Budde-Lund, 1880)	U	W Europe	E	greenhouse
	Trichoniscus carpaticus Tabacaru, 1974	M/L	**Carpathian**	R	forest (leaf litter)
	Trichoniscus noricus Verhoeff, 1917	U	C+S Europe	R	forest (leaf litter)
	Trichoniscus provisiorus Racovitza, 1908	U	W Palearctic	R	forest (leaf litter)
	Trichoniscus pusillus Brandt, 1833	U/L/M	E Europe	E	forest (leaf litter)
	Trichoniscus pygmaeus Sars, 1898	S	W Palearctic	R	cave. soil
BUDDELUNDIELLIDAE	[1] *_Buddelundiella cataractae_ Verhoeff, 1930	U	S Europe	E	greenhouse
ONISCIDAE	_Oniscus asellus_ Linnaeus, 1758	U	N+W Europe	A	rock
PHILOSCIIDAE	_Lepidoniscus minutus_ (C. Koch, 1838)	M	C+S Europe	R	forest (leaf litter)
PLATYARTHRIDAE	_Platyarthrus hoffmannseggii_ Brandt, 1833	U/L	W Palearctic	E	grassland
	*_Trichorhina tomentosa_ (Budde-Lund, 1893)	U	Tropical America	E	greenhouse
PORCELLIONIDAE	_Porcellio dilatatus_ Brandt, 1833	U	S Europe	E	grassland, rock
	Porcellio laevis Latreille, 1804	U	Mediterranean	E	grassland. shrub
	[2]_Porcellio montanus_ Budde-Lund, 1885	M	Alp's surroundings	R	forest (leaf litter)
	Porcellio scaber Latreille, 1804	U	SW Europe	E	rock (walls)
	Porcellio spinicornis Say, 1818	U	Europe	E	rock (walls)
	Porcellionides pruinosus (Brandt, 1833)	U/L	S Europe	E	grasslands
ARMADILLIDIIDAE	*_Armadillidium nasatum_ Budde-Lund, 1885	U	S Europe	E	grassland
	Armadillidium opacum (C. Koch, 1841)	M	Europe	R	forest (leaf litter)
	[2]_Armadillidium pictum_ Brandt, 1833	M	C+W Europe	R	rocky grassland
	Armadillidium versicolor Stein, 1859	U/L/M	C+S Europe	E	rock
	Armadillidium vulgare (Latreille, 1804)	U/L/M	S Europe	E	rock
	Armadillidium zenckeri Brandt, 1833	L	E+C Europe	R	marshland
ARMADILLIDAE	[1] *_Reductoniscus costulatus_ Kesselyák, 1930	U	Paleotropical	E	greenhouse
TRACHELIPODIDAE	*_Nagurus cristatus_ (Dollfus, 1889)	U	Pantropical	E	greenhouse
	Porcellium collicola (Verhoeff, 1907)	L/M	C+SE Europe	E	grassland, shrubs
	Porcellium conspersum (C. Koch, 1841)	L/M	C Europe	A	forest (leaf litter)
	[2]_Trachelipus arcuatus_ (Budde-Lund, 1885)	M?	Mediterranean	R	forest (leaf litter)
	Trachelipus difficilis (Radu, 1950)	M	**Carpathian**	R	rock
	Trachelipus nodulosus (C. Koch, 1838)	L/M	C+S Europe	A	grassland
	Trachelipus rathkii (Brandt, 1833)	U/L/M	Europe excl. Mediterranean	E	forest (leaf litter)
	Trachelipus ratzeburgii (Brandt, 1833)	M/L	C+E Europe	R	forest (litter, barks)
AGNARIDAE	_Orthometopon planum_ (Budde-Lund, 1885)	L/M	NW Mediterranean + Pannonian	R	shrubs, rocks
	*_Protracheoniscus major_ (Dollfus, 1903)	U	C+W Asia	E	rock (walls)
	Protracheoniscus politus (C. Koch, 1841)	M	C+S Europe	R	forest (leaf litter)
CYLISTICIDAE	_Cylisticus convexus_ (De Geer, 1778)	U/L/M/S	W Palearctic	E	rock (walls)

Notes: [1] the first record, [2] revision of the occurrence needed, *exotic species (originated in different zoogeographic area and/or in the other climate zone).
Regional chorotypes (following definition by Fattorini (2015)) distinguished species occurring in Slovakia into four groups, three with natural proposal distribution, subterranean (S), lowland landscape as cultural steppe, fragment of woods and marshlands (L), and mountainous landscape with zonation of vegetation and predominancy of woodland (M) and urbanized environment, created or strongly influenced by human communities (built-up areas, urban greenery, brown field) (U). Urban environment and, to a lesser extent, lowland environments are the open doors for non-native (alien) species to establish.
Global chorotypes (species distribution area) refer the natural area of species mainly according to Schmalfuss (2003) and Forró and Farkas (1998).
Relictness means the rate of nature of the occurrence and relation the habitat type (see Tuf and Tufová 2008): R = relic species (exclusive dweller of natural sites), A = adaptable species (preference of natural sites, colonising connected sites moderately arranged by humans), E = eurytopic, opportunistic species (able to colonise or even prefer various sites including these strongly modified by humans).

The natural subterranean environment has lower species diversity. Central region caves and screes host the cavernicolous species *Mesoniscus graniger* (Rudy et al. 2018, 2021) and an unidentified blind subterranean species from the family Trichoniscidae (Košel et al. 2007; Melega et al. 2022), although the latter is not included in Table 1 due to the absence of the male specimens needed for proper identification.

New records

Two new terrestrial isopod species were documented in Slovakia for the first time, collected during a visit to the Pavol Jozef Šafárik Botanic Garden in Košice on 7 February 2017. Numerous females and juveniles of both species were found in a greenhouse with tropical vegetation, in humus soil and under rotten wood. These species are *Buddelundiella cataractae* Verhoeff, 1930 (Fig. 3; autochthonous in the Mediterranean region) and *Reductoniscus costulatus* Kesselyák, 1930 (Fig. 4; originally from tropical regions of the Oriental zone), both introduced in other parts of Europe, especially under the Atlantic climate. Both species are tiny (2–3 mm), pale, hemispherical and roll into a sphere when disturbed. Their introduction is likely recent, as they were not recorded in previous inspections (Droběnová and Mock 2009). Subsequent inspections confirmed their establishment in the greenhouse.

Taxa described from Slovakia

Several taxa have been described from Slovakia, primarily by K. W. Verhoeff and H. Strouhal. Many of these are now considered junior synonyms (see Schmalfuss 2003):

- ***Ligidium carpathicum* Verhoeff, 1937**: Type locality: Strečno, Váh River Valley. Junior synonym of *Ligidium hypnorum* (Cuvier, 1792) (Bonnefoy and Marchal 1943).
- ***Haplophthalmus verhoeffi* Strouhal, 1948**: Type locality: Kováčov, SW Slovakia. Junior synonym of *Haplophthalmus mengii* (Zaddach, 1844). Legrand et al. (1950) compared the type specimens of *H. verhoeffi* with *Haplophthalmus perezi* Legrand, 1943, finding slight morphological differences. They observed the similarity but not the identity of both taxa. Recently, both names have been considered junior synonyms of *Haplophthalmus mengii* (Zaddach, 1844).
- ***Hyloniscus mariae* Verhoeff, 1908**: Type locality: Belianske Tatry Mts. Accepted species. The syntype (male) is deposited in the Natural History Museum in London (Britain) as a part of Verhoeff's collection of isopods (Ellis and Lincoln 1975).
- ***Protracheoniscus saxonicus slovakius* Strouhal, 1940**: Type locality: Krompachy. Accepted as *Protracheoniscus politus slovakius* Strouhal, 1940.
- ***Lepidoniscus carpathicus* Strouhal, 1940**: Type locality: Krompachy. Junior synonym of *Lepidoniscus minutus* (C. Koch, 1838).
- ***Lepidoniscus germanicus roubali* Strouhal, 1940**: Type locality: middle section of the Hron River, Central Slovakia. Taxonomic status uncertain (Boyko et al. 2024).

Dubious and questionable data

Some species in Table 1 are marked as questionable due to their location outside a continuous species range, lack of repeated findings, or single-specimen documentation. Notable examples include (see Frankenberger 1959):

- ***Porcellio montanus***: One male from the town Žilina, possibly mislocated due to confusion with the village Žilina in the Czech Republic.
- ***Trachelipus arcuatus***: A questionable find in the Slovak Karst, with incomplete specimens making identification uncertain.

Expected species

Additional woodlice species are expected in Slovakia, particularly in the family Trichoniscidae. Unidentified juveniles from the genera *Androniscus* and *Trichoniscus* suggest the presence of potentially new species. The genus *Haplophthalmus* needs revision due to the complexity of the *H. mengii* species complex. For example, *H. hungaricus* Kesselyák, 1930, was recognised in the material collected near the southern border of Slovakia in Hungary (Vilisics et al. 2008). The myrmecophilous species *Platyarthrus schoblii* Budde-Lund, 1885 may also occur, spreading with the garden ant *Lasius neglectus* Van Loon, Boomsma & Andrasfalvy, 1990 (Hornung et al. 2005).

Zoogeographic notes

Most woodlice species in Slovakia have a Central European to European distribution, with some species considered Carpathian endemics. The Carpathians' status as a biodiversity hotspot is noted, but endemic species are fewer compared to regions like the Romanian Carpathians. Future research, including molecular taxonomy, is needed to clarify the true extent of endemism and species diversity.

Figure 3. *Buddelundiella cataractae*, Botanical Garden of the Pavol Jozef Šafárik University in Košice (photograph Ľ. Kováč and A. Mock).

Figure 4. *Reductoniscus costulatus*, Botanical Garden of the Pavol Jozef Šafárik University in Košice (photograph Ľ. Kováč and A. Mock).

Ecological classification

Slovak isopod fauna is classified into relict, adaptable and eurytopic species (Table 1). Eurytopic species dominate (24 species), followed by relict (19 species) and adaptable (5 species). Native relict species predominantly inhabit montane forests (Hudáková and Mock 2016), wetlands (Mock et al. 2021) and natural subterranean habitats (Rudy et al. 2018, 2021). Urban and transformed landscapes host mainly adaptable and eurytopic species, often non-native (Kseňáková 2014). Poor isopod fauna characterizes agrocenoses (Flasarová 1998; Mock et al. 2021), coniferous forests, and high-altitude Carpathian habitats (Mock 2008; Timková 2008; Válentová 2013). Relict species are vulnerable and are included in the forthcoming Red Book of Slovak Invertebrates (Mock et al. 2023; Kokavec et al. 2024).

Conclusions

The current study comprehensively analyses the bibliography of terrestrial isopods in Slovakia, culminating in a list of 48 species, including two species documented in Slovakia for the first time based on the authors' collections. The checklist is critically reviewed and updated in accordance with current taxonomic standards and field experience. The study highlights species with dubious records due to inconsistencies in location data or identification, emphasising the need for further verification through precise field research. This exhaustive bibliography and species list of terrestrial isopods in Slovakia holds significant potential for enhancing both taxonomic clarity and ecological insights, thereby aiding in the conservation and study of Slovakia's natural habitats.

Acknowledgements

The authors thank Petr Dolejš, curator of the collection of isopods at the National Museum in Prague, for providing collection material. We would also like to thank Sándor Farkas, Zuzana Krumpálová, and Ivan Országh for their consultations on the creation of the isopod bibliography. The valuable suggestions of two reviewers, Elisabeth Hornung and Spyros Sfenthourakis, improved the quality of the text.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

Funding

The study was financially supported by grants VEGA 1/0438/22 (Slovak Scientific Grant Agency), APVV-21-0379 (Slovak Research and Development Agency), and the Erasmus+ programme.

Author contributions

Design of study, bibliography, list of species and classification (AM), data and linguisitic correcction (AP, KT), data and design correction, and figures with graphs (IT)

Author ORCIDs

Andrej Mock https://orcid.org/0000-0002-6541-9633
Karel Tajovský https://orcid.org/0000-0002-3619-3815
Adrián Purkart https://orcid.org/0000-0002-8471-5083
Ivan Hadrián Tuf https://orcid.org/0000-0003-0250-0482

Data availability

All of the data that support the findings of this study are available in the main text.

References

Arcangeli A (1939) Per una migliore conoscenza del genere *Mesoniscus* Carl e della sua posizione sistematica (Isopodi terrestri). Bolletino dei Musei di Zoologia et di Anatomia comparata della reale Universita di Torinoser. 3, 47: 7–36.

Axamská G (2021) Rhizosféra v krasových prameniskách – neznámy mikrohabitát bezstavovcov. Diploma thesis. Faculty of Science, Pavol Jozef Šafárik University in Košice, 92 pp. (supervisor A. Mock)

Babor JF (1943) Slovenská fauna [Slovak fauna]. In: Novák Ľ (Ed.) Slovenská vlastiveda I. Vydavateľstvo Slovenskej akadémie vied a umení, Bratislava, 403–463.

Balciar I (2007) Šlosiarova jaskyňa. Spravodaj Slovenskej speleologickej spoločnosti 38(2): 27–29.

Balthasar V, Frankenberger Z (1937) Exkurze Slovenského vlastivědného musea do Východoslovenského krasu. Bratislava, časopis pro výskum Slovenska a Podkarpatské Rusi 9: 312.

Bella P, Haviarová D, Višňovská Z, Kunáková L, Zelinka J, Kudla M, Labaška P (2016) Brestovská jaskyňa, ďalšia sprístupnená jaskyňa na Slovensku. Aragonit, Liptovský Mikuláš 21(1–2): 3–10.

Bella P, Haviarová D, Littva J, Papáč V, Smetanová I, Višňovská Z, Zelinka J (2022) Važecká jaskyňa na hornom Liptove. Národná prírodná pamiatka v podzemí doliny Bieleho Váhu. Aragonit, Liptovský Mikuláš 27(1): 5–10.

Bonnefoy F, Marchal J-L (1943) Contribution a l'étude des *Ligidium* (Isopodes terrestres, Ligiidae). Bulletin du Muséum national d'histoire naturelle 15: 103–107.

Boyko CB, Bruce NL, Hadfield KA, Merrin KL, Ota Y, Poore GCB, Taiti S (Eds) (2024) World Marine, Freshwater and Terrestrial Isopod Crustaceans database. [Accessed at https://www.marinespecies.org/isopoda] [accessed 5 March 2024] https://doi.org/10.14284/365

Buchar J, Ducháč V, Hůrka K, Lellák J (1995) Klíč k určování bezobratlých. Scientia, Praha, 287 pp.

Čaputa A (1987) Bezstavovce. In: Vološčuk I, Terray J (Eds) Vihorlat, chránená krajinná oblasť. Príroda, Bratislava, 95–101.

Čaputa A (1988) Bezstavovce. In: Vološčuk I (Ed.) Východné Karpaty, chránená krajinná oblasť. Príroda, Bratislava, 106–112.

Čaputa A (1991) Bezstavovce. In: Vološčuk I, Pelikán V (Eds) Muránska planina, chránená krajinná oblasť. Obzor, Bratislava, 122–129.

Čarnogurský J, Krumpálová Z, Kalúz S, Wirthová M (1994) Soil arthropods of forest and adjacent agrocoenozes in certain localities of the Danube region in Southwestern Slovakia. Biologia, Bratislava 49/2: 173–183.

Čemesová V (2001) Taxocenózy hygrofilných Isopoda Oniscoidea vybraných lokalít chráneného areálu Horský park (Bratislava). Diploma thesis. Faculty of Education, Comenius University in Bratislava, 118 pp. [supervisor D. Kuracina]

Chyzer C (1858) Ueber die Crustaceen-Fauna Ungarns. Verhandlungen der Zoologisch-Botanischen Gesellschaft in Wien 8: 505–518.

Csiki E (1926) Magyarország szárazföldi Isopodái (Isopoda terrestria Hungariae). Annales historico-naturales Musei nationalis hungarici 23: 1–79.

Cyprich D, Fenďa P, Krumpál M, Lukáš J, Ambruš B (2000) Hniezdna fauna zebričky červenozobej (*Taenopygia guttata castanotis*). Tichodroma 13: 189–199.

Derbák D, Dányi L, Hornung E (2018) Life history characteristics of a cave isopod (*Mesoniscus graniger* Friv.). In: Hornung E, Taiti S, Szlavecz K (Eds) Isopods in a Changing World. ZooKeys 801: 359–370. https://doi.org/10.3897/zookeys.801.23391

Dolejš P (Ed.) (2023) Sborník abstraktů z 13. česko-slovenského myriapodologického a isopodologického semináře. National Musem, Prague, 23 pp. https://publikace.nm.cz/neperiodicke-publikace/sbornik-abstraktu-z-13-cesko-slovenskeho-myriapodologickeho-a-isopodologickeho-seminare

Dolejš P, Tuf IH (2018) Catalogue of the woodlice (Isopoda: Oniscidea) in Miller's collection in the Department of Zoology, National Museum, Prague Acta Societatis Zoologicae Bohemicae 82: 9–16.

Dollfus A (1901) Catalogue des Isopodes terrestres de Hongrie, appartenant au Museum National de Budapest. Természetrajzi Füzetek 24: 143–151.

Dominiak B (1962) Materiały do fauny równonogow Polski: I. Isopoda terrestria (Materialen zur Isopoden-Fauna Polens. I. Isopoda terrestria). Fragmenta faunistica (Warszawa) 10: 227–249. https://doi.org/10.3161/00159301FF1962.10.16.227

Drinková K (2006) Taxocenózy terestrických Isopoda – Oniscoidea vybraných lokalít Chránenej krajinnej oblasti Záhorie (Gajary). Diploma thesis. Depon in: Faculty of Education, Comenius University in Bratislava, 79 pp. [supervisor D. Kuracina]

Droběnová S, Mock A (2009) Spoločenstvá mnohonôžok (Diplopoda) a suchozemských rovnakonôžok (Oniscidea) ako súčasť epigeickej makrofauny Botanickej záhrady UPJŠ v Košiciach. In: Baláž I, Cápay M, Jakab M, Palmárová V, Petluš P, Vanková V (Eds) Mladí vedci 2009. Proceedings. Fakulta prírodných vied UKF, Nitra, 413–421.

Dubovský M (2005) Štruktúra a dynamika spoločenstiev stonožičiek (Symphyla) a vidličiarok (Diplura) v indikácii kvality ekologických systémov Martinského lesa. Diploma thesis. Prírodovedecká fakulta Univerzity Komenského, Bratislava, 99 pp. [supervisor P. Fedor]

Dudich E (1925) Faunisztikai jegyzetek. I. Állattani Közlemények (Budapest) 12: 39–46.

Dudich E (1928) Faunisztikai jegyzetek III. Állattani Közlemények (Budapest) 25: 38–45.

Dudich E (1942) Nachträge und Berichtigungen zum Crustaceen-Teil des ungarischen Faunenkataloges. Fragmenta Faunistica Hungarica 5(1): 17–29.

Dudich E (1957) Die Grundlagen der Krebstierfauna des Komitates Bars. Opuscula zoologica 2: 27–41.

Dyduch T (1903) Materialy do fauny krajowych rownonogow (Isopoda). Sprawozdanie Komisyi Fizjograficznej, Krakau 36: 3–10.

Dyduch T (1904) Materialien zu einer Isopodenfauna Galiziens. Bulletin international de l'Academie des Sciences et des Lettres de Cracovie (1903): 61–64.

Elhottová D, Krištůfek V, Nováková A, Kováč Ľ, Mock A, Ľuptáčik P (2003) Domica a Ardovská jaskyňa – štúdium interakcií medzi faunou a mikroflórou. Aragonit, Liptovský Mikuláš 8: 38–40.

Elhottová D, Krištůfek V, Nováková A, Lukešová A, Tříska J, Kováč Ľ, Mock A, Ľuptáčik P (2004) Zdroje polynenasycených mastných kyselin v jeskyních Slovenského krasu. In: Bella P (Ed.) Výskum, využívanie a ochrana jaskýň. 4. vedecká konferencia s medzinárodnou účasťou, Tále, 5.-8. októbra 2003. Proceedings, Liptovský Mikuláš, 155–161.

Ellis JP, Lincoln RJ (1975) Catalogue of the types of terrestrial isopods (Oniscoidea) in the collections of the British Museum (Natural History). II. Oniscoidea, excluding Pseudotracheata. Bulletin of the British Museum (Natural History), Zoology 28/2: 63–100. https://doi.org/10.5962/p.241844

Farkas S, Vilisics F (2013) Magyarország szárazföldi ászkarák faunájának határozója (Isopoda: Oniscidea). Natura Somogyiensis 23: 89–123. https://doi.org/10.24394/NatSom.2013.23.89

Fattorini S (2015) On the concept of chorotype. Journal of Biogeography 42: 2246–2251. https://doi.org/10.1111/jbi.12589

Fenďa P, Krumpál M, Cyprich D (1998) The soil fauna in the birds´ nests in Slovakia. In: Pižl V, Tajovský K (Eds) Soil Zoological Problems in Central Europe. Proceedings of the 4th Central European Workshop on Soil Zoology, Ceske Budejovice, Czech Republic, April 1997, 23–30.

Ferianc O, Korbel L, Vilček F (1972) Živočíšne spoločenstvá. In: Lukniš M (Ed.) Slovensko 2. Príroda. Obzor, Bratislava, 652–745.

Flasar I, Flasarová M (1989) Ergänzungen zur Monographie "The Soil Fauna of the Little Carpathians" (Mollusca et Isopoda) – Faunistische Abhandlungen. Staatliches Museum für Tierkunde in Dresden 17(1): 1–18.

Flasarová M (1967) Zdenek Frankenberger (1892–1966). Crustaceana 13(3): 329–331. https://doi.org/10.1163/156854067X00503

Flasarová M (1976) O našich svinkách (Isopoda, Oniscidea). Živa (Praha) 24[62]/1: 23–24.

Flasarová M (1980) *Hyloniscus transsilvanicus* (Verhoeff 1901) im Gebirge Malé Karpaty in der Westslowakei (Isopoda: Oniscoidea). Faunistische Abhandlungen. Staatliches Museum für Tierkunde in Dresden 7(30): 273–278. https://doi.org/10.1515/9783112578643-020

Flasarová M (1986) Isopoda (Asellota, Oniscidea) of the Little Carpathians. In: Nosek J (Ed.) The Soil Fauna of the Little Carpathians. Vydavateľstvo SAV, Bratislava, 183–216.

Flasarová M (1988) Zur Kenntnis der Isopoden Mittelböhmens (Crustacea, Isopoda, Asellota et Oniscidea). Faunistische Abhandlungen. Staatliches Museum für Tierkunde in Dresden 15/11: 119–130.

Flasarová M (1994) Über einige Landasseln aus der Slowakei, gesammelt von Herrn Dr. Ján Brtek (Crustacea: Isopoda: Oniscoidea). Faunistische Abhandlungen. Staatliches Museum für Tierkunde in Dresden 19/18: 135–140.

Flasarová M (1995): Die Isopoden Nordwestbohmens (Crustacea: Isopoda: Asellota et Oniscidea). Acta scientiarum naturalium Academiae Scientiarum Bohemicae, Brno, 29(2–4): 1–156.

Flasarová M (1998) Bericht über Isopoden (Asellota et Oniscidea) im slowakischen Donaugebiet. Acta Musei Nationalis Prague, Series B, Historia Naturalis 54(3–4), Praha, 6–78.

Flasarová M (1960) K revisi *Haplophthalmus mengei* (Zaddach) v Čechách a na Moravě. Acta musei Silesiae, Series A 9: 115–122.

Forró L, Farkas S (1998) Checklist, preliminary distribution maps, and bibliography of woodlice in Hungary (Isopoda: Oniscidea). Miscellanea Zoologica Hungarica 12: 21–44.

Franc V, Mlejnek R (2000) O záhadném sekáči podzemních biotopů. Speleofórum, Česká speleologická společnost 19: 49–51.

Frankenberger Z (1939) O jeskynním Isopodu *Mesoniscus graniger* Friv. z Domice. Věstník Královské české společnosti nauk. Třída mathematicko-přírodovědecká 20: 1–12.

Frankenberger Z (1940) Oniscoidea Slovakiae. Sborník Entomologického oddělení Národního musea v Praze 18: 60–69.

Frankenberger Z (1954) Řád: Stejnonožci – Isopoda. In: Hrabě S (Ed.) Klíč zvířeny ČSR, díl I. Nakladatelství Československé akademie věd, Praha, 499–507.

Frankenberger Z (1959) Stejnonožci suchozemští – Oniscoidea. Fauna ČSR, svazek 14, NČSAV, Praha, 212 pp.

Frankenberger Z (1964) *Orthometopon planum* B.-L. (Isopoda-Oniscoidea), zajímavý příslušník slovenské fauny. Biológia (Bratislava) 19: 792–799.

Gaál Ľ (1978) Prieskum jaskyne Podbanište v Drienčanskom krase Spravodaj slovenskej speleologickej spoločnosti 9(3): 4–8.

Gaál Ľ (1987) Kras Rimavskej kotliny. Spravodaj slovenskej speleologickej spoločnosti 25: 5–28.

Gaál Ľ (2000) Kras a jaskyne Drienčanského krasu. In: Kliment J (Ed.) Príroda Drienčanského krasu. Štátna ochrana prírody, Banská Bystrica, 29–96.

Gaál Ľ, Grego J, Holúbek P, Mlejnek R (2000) Jaskyne pod Skalkou v Kremnických vrchoch. Slovenský Kras (Acta Carsologica Slovaca) 38: 141–154.

Galabová B (2022) Jaskynná fauna Slovenska s dôrazom na parietálne taxóny. Bachelor thesis. Archived in: Comenius University in Bratislava, 42 pp. [supervisor B. Stančeková]

Giurginca A (2000) The spreading of the genus *Mesoniscus* in the Romanian Carpathians. Travaux de l'Institut de Spéologie "Émile Racovitza" 39: 11–22.

Giurginca A (2005) Contribution to the historical biogeography of the oniscidean lineage *Mesoniscus* (Microcheta, Isopoda, Crustacea). Travaux de l'Institut de Spéologie "Émile Racovitza" 43–44 (2004–2005): 75–89.

Giurginca A (2022) Oniscidea of Romania. "Emil Racoviţă" Institute of Speleology Romanian Academy, Transversal, Târgovişte, 240 pp.

Giurginca A, Šustr V, Tajovský K (2012) Feeding ecology of *Mesoniscus graniger* – experimental and anatomical evidence. In: Murariu D, Adam C, Chisamera G, Iorgu E, Popa LO, Popa OP (Eds) The Third Annual Zoological Congress of “Grigore Antipa” Museum, 21–23 November 2012, Bucharest, Romania, Abstract Book, 172–173.

Giurginca A, Šustr V, Tajovský K, Giurginca M, Matei I (2015) Spectroscopic parameters of the cuticle and ethanol extracts of the fluorescent cave isopod *Mesoniscus graniger* (Isopoda, Oniscidea). In: Taiti S, Hornung E, Štrus J, Bouchon D (Eds) Trends in Terrestrial Isopod Biology. ZooKeys 515: 111–125. https://doi.org/10.3897/zookeys.515.9395

Giurginca A, Šustr V, Tajovský K (2016) Morphological structures on the integument of *Mesoniscus graniger*. Travaux de L`Institut de Spéologie “Emile Racovitza” 55: 129–133.

Gogová Z (2004) Taxocenózy hygrofilných Isopoda vybraných lokalít povodia Dunaja. Diplomová práca, PdF UK Bratislava. [supervisor D. Kuracina]

Grossinger JB (1794) Universa Historia Physica Regni Hungariae. Tomus IV. Regni animalis. Pars IV. Entomologia, sive Historia insectorum. Simonis Petri Weber, Posonii et Comaromii, 416 pp. + 12 pp. [register]

Gruner HE (1966) Die Tierwelt Deutschlands. 53. Teil. Krebstiere oder Crustacea. V. Isopoda, 2. Lieferung. Gustav Fischer, Jena, 151–380.

Gruner HE, Tabacaru I (1963) Revision der Familie Mesoniscidae Verhoeff, 1908 (Isopoda, Oniscoidea). Crustaceana 6: 15–34. https://doi.org/10.1163/156854063X00318

Gulička J (1957) Kvalitatívno-kvanititatívny rozbor pôdnej fauny Čierneho lesa (Ostrov) (Myriapoda, Isopoda, Opiliones). Acta facultatis rerurm naturalium Universitatis Comenianae, Zoologia 2/4: 119–139.

Gulička J (1960) Vplyv kolísania vodného režimu na pôdnu makrofaunu Svätojurského šúru (Diplopoda, Chilopoda, Isopoda). Acta facultatis rerurm naturalium Universitatis Comenianae, Zoologia 4/6–8: 437–481.

Gulička J (1975) Fauna slovenských jaskýň. Slovenský kras 13: 37–85.

Gulička J (1978) K otázke výskytu pravých troglobiontov a klasifikácie jaskynných spoločenstiev. Slovenský kras 16: 69–93.

Gulička J (1982) Biospelológia. In: Jakál J (Eds) Praktická speleológia. Osveta, Martin, 159–189.

Gulička J (1985) Pôdna a jaskynná makrofauna krasových pohorí Západných Karpát (I). Slovenský kras 23: 89–129.

Haľková-Valkay B, Rudy J, Mock A (2022) Diversity and structure of litter-dwelling macrofauna (Oniscidea and Diplopoda) in upland forest fragments in the winter period. North-Western Journal of Zoology 18(1): 95–99.

Hámorský J (2010) Termopreferencia mnohonôžok (Diplopoda) – experimentálna štúdia. Diploma thesis. Faculty of Science, Pavol Jozef Šafárik University in Košice, 57 pp. [supervisor A. Mock]

Holecová M, Krumpál M, Országh I, Krumpálová Z, Stašiov S, Fedor P (2005) Biodiversity of selected invertebrate groups in oak-hornbeam forest ecosystems in SW Slovakia. Ekológia (Bratislava) 24, Supplement 2/2005: 205–222.

Holecová M, Christophoryová J, Mrva M, Roháčová M, Stašiov S, Štrichelová J, Šustek Z, Tirjaková E, Tuf IH, Vďačný P, Zlinská J (2012) Faunal richness of selected invertebrate groups in epigeon of oak-hornbeam forests on the territory of Bratislava. In: Holecová M, Christophoryová J, Mrva M, Roháčová M, Stašiov S, Štrichelová J, Šustek Z, Tirjaková E, Tuf IH, Vďačný P, Zlinská J (Eds) Biodiversity of soil micro- and macrofauna in oak-hornbeam forest ecosystem on the territory of Bratislava. Comenius University in Bratislava, Bratislava, 129–137.

Hornung E, Vilisics F, Tartally A (2005) Occurrence of *Platyarthrus schoblii* (Isopoda, Oniscidea) and its ant hosts in Hungary. European Journal of Soil Biology 41, 129–133. https://doi.org/10.1016/j.ejsobi.2005.11.001

Hudáková J, Mock A (2006) Suchozemské rovnakonôžky (Isopoda: Oniscidea) Pieninského národného parku. Entomofauna Carpathica 18: 47–55.

Hudec I (2002) Kôrovce (Crustacea). In: Panigaj Ľ (Ed.) Pieniny, Príroda a človek I., Fauna a flóra Pienin. Vivit, Kežmarok, 43–46.

Hůrka K, Pulpán J (1978) Revision der Arten-Gruppe *Duvalius* (*Duvalius*) *micropthalmus* (Col. Carabidae). 1978, Praha, 297–255.

Jakabová A (2001) Taxocenózy terestrických Isopoda vybraných lokalít intravilánu Bratislavy. Diploma thesis. Faculty of Education, Comenius University in Bratislava, 86 pp. [supervisor D. Kuracina]

Jażdżewski K, Konopacka A (1993) Survey and Distribution of Crustacea Malacostraca in Poland. Crustaceana 65: 176–191. https://doi.org/10.1163/156854093X00540

Jedlička L, Országh I, Čejka T, Darolová A, Kulfan M, Mikulíček P, Šustek Z, Žiak D (1999) Terrestrial fauna. In: Mucha I (Ed.) Gabčíkovo Part of the Hydroelectric Power Project. Environmental Impact Review. Ground water Consulting, Ltd., Bratislava, 323–382.

Jurík R (2001) Štruktúra a dynamika epigeickej makrofauny v CHKO Štiavnické vrchy. Diploma thesis. Faculty of Ecology and Environmental Sciences, Technical University in Zvolen, 52 pp. [supervisor S. Stašiov]

Kesselyák A (1936) Bars vármegye szárazföldi ászkarákjai (Die Landasselfauna des Komitates Bars). Állatani Közlemények 33: 142–148.

Kettner R (1936) Přehled dosavadní výzkumné činnosti Krasové komise KČST. Krásy Slovenska 15: 71–79.

Kokavec I, Devánová A, Kyška-Pipík R, Rendoš M, Mock A (2024) Kôrovce. In: Fedor P (Ed.) Červená kniha bezstavovcov Slovenska [Red book of invertebrates in Slovakia]. SNC SR, Banská Bystrica. [in press]

Košel V (1976) Fauna Medvedej jaskyne v Slovenskom raji (Západné Karpaty). Slovenský kras 14: 105–113.

Košel V (1984) Súčasný stav poznania fauny v jaskyniach Slovenského raja. Spravodaj Slovenskej speleologickej spoločnosti 15(1): 38–40.

Košel V (1994) Živočíšstvo jaskýň. In: Rozložník M, Karasová E (Eds) Slovenský kras – chránená krajinná oblasť, biosférická rezervácia. Osveta, Martin, 240–245.

Košel V (2006) *Mesoniscus graniger* (Crustacea, Isopoda) – história poznávania. In: Tajovský K (Ed.) 5. Česko–Slovenský myriapodologický seminář, Hrubá Vrbka, 20.–22.9.2006. Abstracts. Ústav pudní biologie, Biologické centrum Akademie věd České republiky, České Budějovice, 5 pp.

Košel V (2007) História výskumu subteránnej fauny v Západných Karpatoch (1841–1990). Univerzita Komenského, Bratislava, 1–84.

Košel V (2009) Subteránna fauna Západných Karpát. Biologické centrum AVČR, České Budějovice, 204 pp.

Košel V (2012) Subterranean fauna of the Western Carpathians. Tribun EU, Brno, 210 pp.

Košel V, Papáč V, Fenďa P, Ľuptáčik P, Mock A (2007) Zoologický výskum v jaskyni Ľudmila – Leontína po 48 rokoch (Národný park Slovenský kras). Slovenský kras (Acta carsologica Slovaca) 45: 159–168.

Košel V (1975) Faunistický prieskum v priepasti Brázda (Barazdaláš) v Slovenskom krase. Slovenský kras 13: 181–185.

Kováč Ľ, Hudec I, Ľuptáčik P, Mock A (2001) Demänovské jaskyne – biospeleologická lokalita európskeho významu. Aragonit 6: 25–28.

Kováč Ľ, Hudec I, Ľuptáčik P, Mock A, Košel V, Fenďa P (2002a) Spoločenstvá kavernikolných článkonožcov (Arthropoda) Demänovských jaskýň. In: Bella P (Ed.) Výskum, využívanie a ochrana jaskýň, 14–16 November 2001. Proceedings. Liptovský Mikuláš, 155–164.

Kováč Ľ, Ľuptáčik P, Mock A (2002b) Článkonožce (Arthropoda) jaskyne Bobačka (Muránska planina). Výskum a ochrana Muránskej planiny 3: 141–145.

Kováč Ľ, Mock A, Ľuptáčik P, Hudec I, Višňovská Z, Svatoň J, Košel V (2003) Bezstavovce Harmaneckej jaskyne (Veľká Fatra). Aragonit 8: 31–34.

Kováč Ľ, Mock A, Ľuptáčik P, Košel V (2004) Terestrické článkonožce Domického jaskynného systému a Ardovskej jaskyne (Slovenský kras) – mikrohabitaty a diverzita. In: Bella P (Ed) Výskum, využívanie a ochrana jaskýň. 4. vedecká konferencia s medzinárodnou účasťou, Tále, 5.–8. októbra 2003. Proceedings. SNC SR, Liptovský Mikuláš, 138–144.

Kováč Ľ, Mock A, Ľuptáčik P, Hudec I, Nováková A, Košel V, Fenďa P, Višňovská Z (2005a) Živé organizmy. In: Stankovič J, Cílek V (Eds) Krásnohorská jaskyňa Buzgó. Regionálna rozvojová agentúra, Rožňava, 88–95.

Kováč Ľ, Mock A, Ľuptáčik P, Košel V, Fenďa P, Svatoň J, Mašán P (2005b) Terrestrial arthropods of the Domica Cave System and the Ardovská Cave (Slovak Karst) – principal microhabitats and diversity. In: Tajovský K, Schlaghamerský J, Pižl V (Eds) Contributions to Soil Zoology in Central Europe I, 61–70.

Kováč Ľ, Mock A, Ľuptáčik P, Višňovská Z (2005c) Terestrické a vodné bezstavovce Diviačej priepasti (Slovenský kras). Aragonit, Liptovský Mikuláš 10: 16–19.

Kováč Ľ, Mock A, Ľuptáčik P, Višňovská Z, Fenďa P (2006) Bestavovce (Evertebrata) Dobšinskej jaskyne (Slovenský raj). In: Bella P (Ed.) Výskum, využívanie a ochrana jaskýň 5. Proceedings. Liptovský Mikuláš, 179–186.

Kováč L, Mock A, Višňovská Z, Ľuptáčik P (2008) Spoločenstvá fauny Brestovskej jaskyne (Západne Tatry). Slovenský kras 46(1): 97–110.

Kováč Ľ, Mock, A, Ľuptáčik P (2010a) Pôdna fauna. In: In: Koutná A, Chovancová B (Eds) Tatry – Príroda. Baset, Praha, 437–442.

Kováč Ľ, Mock A, Ľuptáčik P, Nováková A, Papáč V, Višňovská Z, Hudec I, Stankovič J (2010b) Jaskynné mikroorganizmy a bezstavovce. In: Stankovič J, Cílek V, Schmelzová R (Eds) Plešivecká planina. Speleoklub Minotaurus, Rožňava, 143–149.

Kováč Ľ, Elhottová D, Mock A, Nováková A, Krištůfek V, Chroňáková A, Lukešová A, Mulec J, Košel V, Papáč V, Ľuptáčik P, Uhrin M, Višňovská Z, Hudec I, Gaál Ľ, Bella P (2014a) The Cave Biota of Slovakia. Slovak Cave Administration, SNC SR, Liptovský Mikuláš, 192 pp.

Kováč Ľ, Mock A, Ľuptáčik P, Parimuchová A (2014b) Článkonožce (Arthropoda) jaskýň Strážovských vrchov. Spravodaj slovenskej speleologickej spoločnosti 45(4): 22–27.

Krištůfek V, Elhottová D, Šustr V, Lasák R, Kováč Ľ, Mock A, Ľuptáčik P (2005) Does the cellular fatty acid and enzyme content of cave bacteria affect the feeding preferences of *Enchytraeus crypticus* (Oligochaeta, Enchytraeidae)? In: Tajovský K, Schlaghamerský J, Pižl V (Eds) Contributions to Soil Zoology in Central Europe I: 71–75.

Krumpál M (1973) Seasonal dynamics of terrestrial isopod populations (Isopoda, Oniscidea) in Jurský Šúr Nature Reserve. Biológia, Bratislava 28(11): 879–884.

Krumpál M (1975) Príspevok k poznaniu Oniscoidei okolia Sniny. Acta Rerum Naturalium Musei Nationalis Slovaci Bratislava 21: 133–142.

Krumpál M (1976) Knowledge from Biology of Isopode Reproduction in Jurský šúr. Acta Facultatis Rerum Naturalium Universitatis Comenianae, Zoologia 20: 63–67

Krumpál M (1977) Growth of *Hyloniscus riparius* (Crustacea, Oniscoidea). Biológia (Bratislava) 32(5): 363–365.

Krumpál M (1983) Sezónna dynamika pôdnej makrofauny v ŠPR Jurský Šúr a jej príčiny. Práce Slovenskej entomologickej spoločnosti, Bratislava 5: 141–146.

Krumpál M (1993) Bezstavovce. In: Bertová L (Ed.) Karlova Ves. Vlastivedná monografia. Alfa, Bratislava, 57–61.

Krumpál M, Krištofík J (1982) Fauna článkonožcov ľudských obydlí v Malackách. In: Halgoš J, Kalivodová E (Eds) Živočíšstvo ako indikátor zmien životného prostredia. Bratislava, 205–210.

Krumpál M, Krištofík J (1985) Fauna článkonožcov (Arthropoda) v bytoch Bratislavy. In: Feriancové-Masárová Z, Halgoš J (Eds) Zoocenózy urbánnych a suburbánnych celkov so zvláštnyrn akcentom na podmienky Bratislavy. Slovenská zoologická spoločnosť pri SAV, Bratislava, 106–108.

Krumpál M, Žitňanský B (1977) Method of evaluation of relationships between dry mater mass of *Hyloniscus riparius* (Oniscoidea) and its length and between dry matter mass and that in living state. Biologia (Bratislava) 32 (2): 133–139.

Krumpál M, Krištofík J, Ondrejková Z (1985) Fauna článkonožcov (Arthropoda) ľudských obydlí v Malackách. Biológia (Bratislava) 40(2): 169–179.

Krumpál M, Krumpálová Z, Cyprich D (1997) Bezstavovce (Evertebrata) skleníkov Bratislavy (Slovensko). Entomofauna carpathica 9: 102–106.

Krumpál M, Krumpálová Z, Cyprich D (1999) Skleníky ako biotopy umožňujúce invázie bezstavovcov (Evertebrata). In: Invázie a invázne organizmy 2. Príspevky z vedeckej konferencie. Sekos, Nitra, 72–75.

Kseňáková D (2011) Suchozemské rovnakonôžky (Crustacea: Oniscidea) čeľade Trichoniscidae na Slovensku. Bachelor thesis. Faculty of Science, Pavol Jozef Šafárik University in Košice, 31 pp. (supervisor A. Mock)

Kseňáková D (2014) Suchozemské rovnakonôžky (Oniscidea) v urbánnom prostredí. Diploma thesis. Faculty of Science, Pavol Jozef Šafárik University in Košice, 70 pp. (supervisor A. Mock)

Kuracina D, Kabátová A (2005) Terestrické rovnakonôžky (Crustacea: Isopoda: Oniscoidea). In: Majzlan O (Ed.) Fauna Devínskej Kobyly. APOP, Bratislava, 56–57.

Lantos I, Krištín A, Svatoň J (2010) Živočíšstvo, hodnoty živočíšnej ríše – bezstavovce (Evertebrata). In: Gaálová K (Ed.) Chránená krajinná oblasť Karancs-Medves a Chránená krajinná oblasť Cerová vrchovina. Bükk Nemzeti Park Igazgatóság, Eger – Rimavská Sobota, 43–156.

Legrand JJ, Strouhal H, Vandel A (1950) Remarques critiques sur quelques Trichoniscidae. Bulletin de la Société Zoologique de France 75: 307–312.

Lukáň M, Rajecová K, Kováč Ľ, Ľuptáčik P, Mock A (2004) Predbežné výsledky prieskumu spoločenstiev terestrických článkonožcov (Arthropoda) Jasovskej jaskyne. In: Bella P (Ed.) Výskum, využívanie a ochrana jaskýň. 4. vedecká konferencia s medzinárodnou účasťou, Tále, 5.–8. októbra 2003. Proceedings. SNC SR, Liptovský Mikuláš, 169–173.

Lukešová A, Nováková A (2009) Interactions between the soil microflora and invertebrates in Slovak and Moravian caves. In: Tajovský K, Schlaghamerský J, Pižl V (Eds) Contributions to Soil Zoology in Central Europe III. ISB BC AS CR, České Budějovice, 89–96.

Ľuptáčik P, Mock A, Kováč Ľ (2005) Príspevok k poznaniu jaskynnej fauny Beskydského predhoria. Telekia, Spravodaj CHKO Vihorlat 3: 30–32.

Majzlan O, Hošták P (1996) Bioindikačný význam Oniscoidea a Curculionidea v pôde dubového lesa NPR Dubník pri Seredi. Ochrana prírody 14: 83–87.

Majzlan O, Rychlík I, Lendvay M (2004) Chrobáky (Coleoptera) a rovnakonôžky (Isopoda) vybraných slanísk na južnom Slovensku. Biosozologia, Bratislava 2: 71–88.

Majzlan O, Zápražný B, Fedor P (2011) Suchozemské rovnakonôžky (Crustacea: Oniscidea) v NPR Nad Šenkárkou v Malých Karpatoch. Acta facultatis ecologiae, Zvolen 24–25: 91–98.

Malesevics E (1892) Losoncz faunája vagyis az 1876. év őszétől, az 1891. év végeig talált és maghatározott állatfajok rendszeres és a fauna jellemzése. A Losonczi magy. [Fauna of Losoncz or from autumn 1876 to the end of 1891 collected, determinated and classified animal species and description of the fauna. In Hungarian]. Királyi Állami főgymnasium értesírője. 1891–92 (Losoncz): 3–47.

Martinka M (2022) Trvalý lesný priesek ako bariéra pre pôdne článkonožce. Diploma thesis. Faculty of Science, Palacký University Olomouc, 63 pp. [supervisor I. H. Tuf]

Martinka M, Tuf IH, Stašiov S, Machač O (2022) The power line clearing as a barrier for soil invertebrates. In: Mašová Š, Košulič O (Eds) 8th Conference of the Czech Society for Ecology, 7—9 September 2022, Book of abstracts. Czech Society for Ecology & Faculty of Forestry and Wood Technology of Mendel University in Brno, 114 pp.

Martinka M, Stašiov S, Machač O, Tuf IH (2023a) Význam krovín v prerušenej kontinuite lesných biotopov z pohľadu pôdnych bezstavovcov. In: Bryja J, Horsák M, Horsáková V (Eds) Zoologické dny Brno 2023. Sborník abstraktů z konference 9.–10. února 2023. Ústav biologie obratlovců AV ČR, v.v.i., Brno: 136–137.

Martinka M, Stašiov S, Machač O, Tuf IH (2023b) Where to live in degraded land: Shrub refuges for terrestrial isopod community under transmission power lines. In: Tuf IH, Tajovský K (Eds) 12th International Symposium on Terrestrial Isopod Biology. Olomouc: Faculty of Science, Palacký University Olomouc & Institute of Soil Biology and Biogeochemistry, BC CAS, 23 pp.

Méhelÿ L (1929) Species generis *Hyloniscus*. Studia zoologica Regiæ scientiarum universitatis hungaricæ budapestiensis fundatæ a Petro Pázmány (Budapest) 1: 1–75. [1–9 pls]

Melega M, Parimuchová A, Ľuptáčik P, Jászay T, Košel V, Čech V, Šestáková A, Mock A (2022) Fauna bezstavovcov jaskýň planiny Galmus (Volovské vrchy). Slovenský kras – Acta carsologica Slovaca, Liptovský Mikuláš 60(1): 63–92.

Mergl K (1940) Fauna Posoniensis. Unattended and non-paged manuscript written in German. Archived in: Archív hlavného mesta Bratislava (The Archive of the Capital City of Bratislava). [Note: The year of creation of the manuscript is unknown, it is estimated to be the beginning of the 1940s.]

Mišík V, Gulička J, Urvichiarová E (1974) Devínska Kobyla. Geologické pomery, kvetena a fauna. Obzor, Bratislava, 107 pp.

Mlejnek R (1999) Nález kořenových útvarů v Nyáryho jaskyni (CHKO Cerová vrchovina). Pseudokrasový sborník 1, Knihovna Československej speleologickej společnosti 35: 68–70.

Mlejnek R (2000) Objav koreňových útvarov na Slovensku. Sinter 8: 18–19.

Mlejnek R (2002) Dokumentační údaje ze sledování kořenových útvarů v Nyáryho jaskyni (Západní Karpaty, Cerová vrchovina). Speleo 35: 19–22.

Mlejnek R, Ducháč V (2003) Troglobiontní a endogenní výskyt druhu *Mesoniscus graniger* (Crustacea: Isopoda: Oniscoidea) na území Západních Karpat. Acta Musei Reginaehradecensis, serie A 29: 71–79.

Mlejnek R, Ducháč V (2001) Rozšíření *Mesoniscus graniger* (Crustacea: Isopoda: Oniscoidea) v Západních Karpatech. Natura Carpatica, Košice 42: 75–88.

Mock A (2008) Oniscidea a Myriapoda Tatier – aktuálny stav poznania. In 6. česko-slovenský myriapodologický seminár, Opátka, Slovensko, 15.-17.10.2008. Proceedings. Pavol Jozef Šafárik University in Košice, 7 pp.

Mock A (2017) Burda – zem ešte stále neznáma? In: 10. česko-slovenský myriapodologický a isopodologický seminár, 4.-7.10.2017, Burda-Kováčov, Slovensko. Abstracts, 10 pp.

Mock A (2020) Suchozemské rovnakonôžky (Oniscidea) a mnohonôžky (Diplopoda) v širšom okolí obce Regetovka. In: Fulín M (Ed) 44. Východoslovenský tabor ochrancov prírody, Regetovka. Proceedings. ZO SZOPK Trebišov a ŠOP SR RCOP Prešov, 95–98.

Mock A (2023) Biospeleológ Vlado Košel a jeho prínos k poznaniu rovnakonôžok (Isopoda) a viacnôžok (Myriapoda) Západných Karpát. In: Dolejš P (Ed.) Sborník abstraktů z 13. česko-slovenského myriapodologického a isopodologického semináře, Hostomice, 23.-23. května 2023. Národní muzeum, Praha, 10–11.

Mock A, Dolejš P (2023) Walter Černý, známy ornitológ a (takmer) neznámy isopodológ. In: Dolejš P (Ed.) Sborník abstraktů z 13. česko-slovenského myriapodologického a isopodologického semináře, Hostomice, 23.–26. května 2023. Národní muzeum, Praha, 12 pp.

Mock A, Kováč Ľ, Ľuptáčik P, Košel V, Hudec I, Fenďa P (2002) Bezstavovce Važeckej jaskyne a vyvieračky Teplica. Aragonit, Liptovský Mikuláš 7: 30–32.

Mock A, Kováč Ľ, Ľuptáčik P, Višňovská Z, Hudec I, Košel V (2003) Bezstavovce Bystrianskej jaskyne (Horehronské podolie). Aragonit, Liptovský Mikuláš 8: 35–38.

Mock A, Kováč Ľ, Ľuptáčik P, Mlejnek R, Višňovská Z, Košel V, Fenďa P (2004a) Kavernikolné článkonožce (Arthropoda) Važeckého krasu. In: Bella P (Ed.) Výskum, využívanie a ochrana jaskýň. 4. vedecká konferencia s medzinárodnou účasťou, Tále, 5.-8. októbra 2003. Proceedings. SNC SR, Liptovský Mikuláš, 145–154.

Mock A, Ľuptáčik P, Fenďa P, Papáč V (2004b) Biologická charakteristika jaskýň Bujanovských vrchov (Čierna hora). Aragonit, Liptovský Mikuláš 9: 35–40.

Mock A, Ľuptáčik P, Fenďa P, Svatoň I, Országh I, Krumpál M (2005) Terrestrial arthropods inhabiting caves near Veľký Folkmar (Čierna hora Mts., Slovakia). In: Tajovský K, Schlaghamerský J, Pižl V (Eds) Contributions to Soil Zoology in Central Europe I: 95–101.

Mock A, Kania G, Miklisová D (2007) Terrestrial isopods (Oniscidea) of the Ojców National Park (Poland). In: Tajovský K, Schlaghamerský J, Pižl V (Eds) Contributions to Soil Zoology in Central Europe II, 97–102.

Mock A, Stanko M, Čanády A (2008) Hniezda myši kopčiarky (*Mus spicilegus*) ako rezervoár pôdnej makrofauny (Oniscidea, Diplopoda). In 6. česko-slovenský myriapodologický seminár, Opátka, Slovensko, 15.-17.10.2008. Proceedings. Pavol Jozef Šafárik University in Košice, 8 pp.

Mock A, Jászay T, Svatoň J, Christophoryová J, Stašiov S (2009) Suchozemské článkonožce (Arthropoda) jaskýň Čiernej hory (Západné Karpaty). Slovenský kras 47/2: 259–274.

Mock A, Šašková T, Raschmanová N, Jászay T, Ľuptáčik P, Rendoš M, Tajovský K, Jászayová A (2015) An introductory study of subterranean communities of invertebrates in forested talus habitats in southern Slovakia. Acta Societatis Zoologicae Bohemicae 79: 237–250.

Mock A, Stašiov S, Ľuptáčik P, Fenďa P, Tuf IH (2020) Suchozemské rovnakonôžky (Oniscidea) a viacnôžky (Myriapoda) pohoria Burda a okolia – predbežná správa. In: Fenďa P (Ed) 18. Arachnologická konferencia. Zborník abstraktov. Slovenská arachnologická spoločnosť o.z, Bratislava, 20–21.

Mock A, Škovranová S, Tajovský K (2021) Terrestrial and aquatic isopods (Isopoda: Oniscidea, Asellota) of the Latorica Protected Landscape Area. In: Panigaj Ľ, Tajovský K, Mock A (Eds) Invertebraes of the Latorica Protected Landscape Area. State Natural Conservancy of the Slovak Republic, Banská Bystrica and Administration of the Latorica PLA Trebišov, 170–189.

Mock A, Stašiov S, Tuf IH, Tajovský K (2023) Suchozemské rovnakonôžky (Oniscidea) a viacnôžky (Myriapoda) – príprava zoznamu vzácnych a ohrozených druhov do „červenej knihy“ bioty Slovenska. In: Dolejš P (Ed) Sborník abstraktů z 13. česko-slovenského myriapodologického a isopodologického semináře. Hostomice, 23.–26. května 2023. Národní muzeum, Praha, 13–14.

Nosek J, Lichard M (1962) Beitrag zur Kenntnis der Vogelnestfauna. Biologické práce SAV, VIII/6. Entomologické problémy 2: 29–51.

Nováková A, Elhottová D, Krištůfek V, Lukešová A, Hill P, Kováč Ľ, Mock A, Ľuptáčik P (2005) Feeding sources of invertebrates in Ardovská Cave and Domica Cave system – preliminary results. In: Tajovský K, Schlaghamerský J, Pižl V (Eds) Contributions to Soil Zoology in Central Europe I: 107–112.

Nowicki M (1867) Zapiski z fauny tarzańskiéj. Sprawozdań Komisyi Fizyograficznej, Kraków 1: 179–206.

Orsavová J, Tuf IH (2018) Suchozemští stejnonožci: atlas rozšíření v České republice a bibliografie 1840–2018 [Woodlice: Distribution Atlas in the Czech Republic and bibliography 1840–2018]. Acta Carpathica Occidentalis, Supplementum 1/2018: 1–124.

Ortvay T (1902) Poszonyvármegye és a területén fekvő Pozsony, Nagyszombat, Bazin, Modor s Szentgyőrgy várasok állatvilága. Kiadja Pozsony város anyagy hozzájárulásával Pozsonyvár-megye kőzőnsége, Pozsony, 648 pp.

Palkovičová S, Mock A (2008) Epigeická makrofauna (Oniscidea, Diplopoda) Botanickej záhrady Univerzity P. J. Šafárika v Košiciach. In: Littera P, Budzáková M (Eds) Študentská vedecká konferencia, 23. apríl 2008 Bratislava. Proccedings. 1. zväzok – biologická sekcia. PF UK, Bratislava, 242–244.

Papáč V (2006) Príspevok k poznaniu fauny Ponickej jaskyne. Aragonit, Liptovský Mikuláš 11: 42–43.

Papáč V (2008) Bezstavovce (Evertebrata) jaskyne Podbanište (Revúcka vrchovina, Drienčanský kras). Aragonit, Liptovský Mikuláš 13(1): 25–29.

Papáč V (2018) Bezstavovce (Evertebrata) Jaskyne nad kameňolomom (Čachtický kras, Malé Karpaty). Slovenský kras (Acta carsologica Slovaca) 56(2): 191–204.

Papáč V, Kováč Ľ, Mock A, Košel V, Fenďa P (2006) Terestrické článkonožce (Arthropoda) vybraných jaskýň Silickej planiny. In: Bella P (Ed.) Výskum, využívanie a ochrana jaskýň 5. Proceedings. SNC SR, Liptovský Mikuláš, 187–199.

Papáč V, Ľuptáčik P, Fenďa P, Košel V, Christophoryová J (2007) Spoločenstvá terestrických článkonožcov NPP Snežná diera (Slovenský kras, Horný vrch). Slovenský kras (Acta carsologica Slovaca) 45: 151–157.

Papáč V, Fenďa P, Ľuptáčik P, Mock A, Svatoň J, Christophoryová J (2009) Terestrické bezstavovce (Evertebrata) jaskýň vo vulkanitoch Cerovej vrchoviny. Aragonit, Liptovský Mikuláš 14(1): 32–42.

Papáč V, Hudec I, Kováč Ľ, Ľuptáčik P, Mock A (2014) Bezstavovce. In: Gaál Ľ, Gruber P (Eds) Jaskynný systém Domica-Baradla. Jaskyňa, ktorá nás spája. Aggteleki Nemzeti Park Igazgatóság, Jósvafő, 267–279.

Papáč V, Višňovská Z, Gresch A (2015) Poznatky o výskyte bezstavovcov a netopierov v Malužinskej jaskyni (Nízke Tatry). Aragonit, Liptovský Mikuláš 20(2): 107–113.

Papáč V, Jászay T, Ľuptáčik P, Mock A (2019) Spoločenstvá terestrických bezstavovcov (Evertebrata) Moldavskej jaskyne (Medzevská pahorkatina). Aragonit, Liptovský Mikuláš 24: 22–30.

Papáč V, Košel V, Kováč Ľ, Ľuptáčik P, Mock A, Parimuchová A, Raschmanová N, Višňovská Z (2020) Invertebrates of the Dobšinská Ice Cave and Stratenská Cave System, Slovak Paradise, Slovakia. Slovenský kras (Acta Carsologica Slovaca) 58: 5–36.

Petricskó J (1892) Selmeczbánya vidéke állatani tekintetben. Kiadja saját kőltségén a selmeczbányai gyógyászati és természettudományi egyesűlet. Selmeczbánya, 133 pp.

Piksa K, Farkas S (2007) The first records of the cave isopod *Mesoniscus graniger* (Frivaldszky, 1865) (Crustacea, Isopoda, Oniscidea) in Poland. Fragmenta faunistica 50(2): 87–90. https://doi.org/10.3161/00159301FF2007.50.2.087

Plutinská K (2015) Pôdna makrofauna mesta Martin. Diploma thesis. Faculty of Science, Pavol Jozef Šafárik University in Košice, 83 pp. (supervisor A. Mock)

Pomichal R (1982) Faunistický prieskum bezstavovcov v jaskyniach Drienčanského krasu. Spravodaj Slovenskej speleologickej spoločnosti 13(2): 16–21.

Pongrácz S (1936) Helyesbítesek a magyar fauna jegyzékében. Állattani Közlemények 33: 181–193.

Pribišová D (2022) Spoločenstvá vodných bezstavovcov (Evertebrata) v podzemnom toku jaskyne Diablova diera. Diploma thesis. Archived in: Prešov University in Prešov, 65 pp. (supervisor M. Rendoš)

Radu VG (1983) Ordinul Isopoda, Oniscoidee inferioare. In: Fauna Republicii Socialiste România 4 (13), 168 pp.

Radu VG (1985) Ordinul Isopoda, Oniscoidea, Crinocheta. In: Fauna Republicii Socialiste România 4 (14), 155 pp.

Ratkovský Š, Višňovská Z, Haviarová D, Bella P, Gažík P, Vlček L, Zelinka J (2019) Geoekologické mapovanie riečneho úseku Drienovskej jaskyne a priestorové rozloženie fauny. Aragonit, Liptovský Mikuláš 24(1): 3–17.

Remešicová E (2011) Ekologicko-faunistická charakteristika stejnonožců (Isopoda) ve městě. Bachelor thesis. Archived in: VSB – Technical University of Ostrava, 52 pp. [+figs (supervisor J. Kupka)]

Rendoš M (2012) Arthropod communities within the scree slope in NNR Sivec. Diploma thesis. Faculty of Science, Pavol Jozef Šafárik University in Košice, 80 pp. [supervisor A. Mock]

Rendoš M (2016) Invertebrate communities in forested scree slopes of the Western Carpathians. PhD thesis. Faculty of Science, Pavol Jozef Šafárik University in Košice, 111 pp. [supervisors Ľ. Kováč, A. Mock]

Rendoš M, Mock A (2010) Aktivita viacnôžok (Myriapoda) a rovnakonôžok (Isopoda) v podzemí zalesneného suťového svahu NPR Sivec (Čierna hora, Slovensko). In: 7. česko – slovenský myriapodologický seminář, České Budějovice, 8.-9.4.2010. UPB CAS, České Budějovice, 9 pp.

Rendoš M, Mock A (2012) Oniscidea a Diplopoda v sutinovom svahu: skúsenosť s etylénglykolom. In: Viacnôžky: od faunistiky k ekológii. 8. česko-slovenský myriapodologický seminár, 19– 21.4.2012, Železná Breznica. Technická univerzita vo Zvolene, 9 pp.

Rendoš M, Mock A, Miklisová D (2016) Terrestrial isopods and myriapods in a forested scree slope: subterranean biodiversity, depth gradient and annual dynamics. Journal of Natural History 50(33–34): 2129–2140. https://doi.org/10.1080/00222933.2016.1193642

Rendoš M, Červená M, Haľková B, Jakšová P, Jureková N, Rudy J, Jászay T, Raschmanová N, Mock A, Kováč Ľ, Ľuptáčik P (2019) Zalesnené sutinové svahy – pozoruhodný podzemný biotop Západných Karpát. Slovenský kras (Acta Carsologica Slovaca) 57(2): 231–247.

Rudy J (2016) Pôdna makrofauna kompostovísk. Diploma thesis. Faculty of Science, Pavol Jozef Šafárik University in Košice, 61 pp. [supervisor A. Mock]

Rudy J, Mock A, (2016) Makrosaprofágy (Oniscidea, Diplopoda) ako súčasť fauny kompostovísk. In: Krumpálová Z, Zigová M, Tulis F (Eds) Proceedings of the scientific congress „Zoológia 2016“, Nitra (Slovakia). Constantine the Philosopher University, Nitra, 184–186.

Rudy J, Rendoš M, Ľuptáčik P, Mock A (2018) Terrestrial isopods associated with shallow underground of forested scree slopes in the Western Carpathians (Slovakia). In: Hornung E, Taiti S, Szlavecz K (Eds) Isopods in a Changing World. ZooKeys 801: 323–335. https://doi.org/10.3897/zookeys.801.24113

Rudy J, Mlejnek R, Papáč V, Mock A (2021) Terrestrial isopods (Oniscidea) in Slovak caves: species diversity and distribution along regional and geographical gradients. Acta Carpathica Occidentalis 12: 12–42. https://doi.org/10.62317/aco.2021.002

Schmalfuss H (2003) World catalog of terrestrial isopods (Isopoda: Oniscidea). Stuttgarter Beiträge zur Naturkunde, Serie A, Nr. 654, 341 pp.

Schmölzer K (1965) Ordnung Isopoda (Landasseln). In: Franz H (Ed.) Bestimmungsbücher zur Bodenfauna Europas. Akademie-Verlag, Berlin, 468 pp.

Sfenthourakis S, Hornung E (2018) Isopod distribution and climate change. ZooKeys 801: 25–61. https://doi.org/10.3897/zookeys.801.23533

Smrž J, Kováč Ľ, Mikeš J, Šustr V, Lukešová A, Tajovský K, Nováková A, Režňáková P (2015) Food sources of selected terrestrial cave arthropods. Subterranean Biology 16: 37–46. https://doi.org/10.3897/subtbiol.16.8609

Snopková E (2001) Štruktúra epigeickej makrofauny a jej dynamika v NPR Príboj. Diploma thesis Faculty of Ecology and Environmental Sciences, Technical University in Zvolen, 48 pp. [supervisor S. Stašiov]

Staněk VJ (1932a) Nové přírůstky zoologických sbírek Národního Musea z Hosúsovských jeskyň na Slovensku. Časopis Národního Musea, Praha 106: 55–59.

Staněk VJ (1932b) Zpráva zoologa V. J. Staňka o prohlídce jeskyně za fotografické spolupráce MUDr. W. Heinricha v lednu 1932. Časopis turistů, Praha 44: 16–18.

Stašiov S (2001) Vybrané skupiny epigeickej makrofauny (Opilionida, Diplopoda a Chilopoda) ako indikátory stavu vrchnej pôdnej vrstvy v podhorskej bučine. Technická univerzita vo Zvolene. Vedecké štúdie 8/2001/A, 88 pp.

Štrichelová J (2010) Terrestrial isopods (Oniscidea) of Western Carpathians. Diploma thesis, Faculty of Science, Palacký University Olomouc, 47 pp. [supervisor I. H. Tuf]

Štrichelová J, Tuf IH (2012) Terrestrial isopods (Crustacea: Isopoda, Oniscidea). In: Holecová M, Christophoryová J, Mrva M, Roháčová M, Stašiov S, Štrichelová J, Šustek Z, Tirjaková E, Tuf IH, Vďačný P, Zlinská J (Eds) Biodiversity of soil micro- and macrofauna in oak-hornbeam forest ecosystem on the territory of Bratislava. Comenius University in Bratislava, Bratislava, 43–48.

Strouhal H (1929a) Über einige Arten der Gattung *Protracheoniscus* Verhoeff (Isop. terr.). Annalen des Naturhistorischen Museums in Wien, Serie B 43: 1–12.

Strouhal H (1929b) Über einige mitteleuropäische Landisopoden. Zoologischer Anzeiger, Jena, 80 (7–9): 205–214.

Strouhal H (1939a) Einige bemerkenswerte Vorkommnisse von Wirbellosen, besonders Isopoden, in der Ostmark. Festschrift zum 60. Geburtstage von Professor Dr. Embrik Strand (Riga) 5: 68–80. https://doi.org/10.1111/j.1365-3032.1939.tb00030.x

Strouhal H (1939b) Landasseln aus Balkanhöhlen, gesammelt von Prof. Dr. Karl Absolon. Studien aus dem Gebiete der allgemeinen Karsforschung, der wissenschaftlichen Höhlenkunde, der Aiszeitforschung und den Nachbargebieten, Brünn, B. Biologische Serie 7: 1–35.

Strouhal H (1940a) Über Landisopoden der Slowakei. I. Ost-Slowakei. Zoologischer Anzeiger 129: 80–95.

Strouhal H (1940b) Über Land-Isopoden der Slowakei. II. Mittelslowakei. Zoologischer Anzeiger 139: 207–213.

Strouhal H (1947) *Protracheoniscus amoenus* C. L. Koch (= *politus* Verh.) und *P. politus* C. L. Koch (= *saxonicus* Verh.). Fragmenta faunistica hungarica 10: 50–55.

Strouhal H (1948) Die Landasseln Kärntens und Osttirols. Carinthia (II) 137–138: 103–152.

Strouhal H (1951) Die österreichischen Landisopoden, ihre Herkunft und ihre Beziehungen zu den Nachbarlädern. Verhandlungen der zoologisch-botanischen Gesellschaft Wien 92: 116–142.

Strouhal H (1953) Mitteilungen über einige paläarktische Isopoda terrestria. Zoologischer Anzeiger (Jena), 151(11–12): 281–288.

Strouhal H (1964) Die österreichischen *Haplophthalmus*-Arten der *mengii*-Gruppe (Isopod. Terr.). Annalen des Naturhistorischen Museums in Wien, Serie B 67: 499–558.

Strouhal H (1965) Die *Haplophthalmus*-Arten Ungarns (Isopoda Terrestria). Acta zoologica Academiae Scientarum Hungaricae 11(3–4): 465–473.

Šustr V, Elhottová D, Krištůfek V, Lukešová A, Nováková A, Tajovský K, Tříska J (2005) Ecophysiology of the cave isopod *Mesoniscus graniger* (Frivaldszky, 1856) (Crustacea: Isopoda). European Journal of Soil Biology 41: 69–75. https://doi.org/10.1016/j.ejsobi.2005.09.008

Šustr V, Lukešová A, Nováková A, Vošta O (2009) Potravní preference jeskynního stejnonožce *Mesoniscus graniger* (Isopoda, Oniscidea) v laboratorních testech. Slovenský kras (Acta carsologica Slovaca), 47/2: 275–281.

Tajovský K (2002) Soil macrofauna (Diplopoda, Chilopoda, Oniscidea) in a pine forest disturbed by wildfire. In: Tajovský K, Balík V, Pižl V (Eds) Studies on Soil Fauna in Central Europe. AS CR, České Budějovice: 227–232.

Tajovský K, Mock A (2015) Millipedes (Diplopoda), centipedes (Chilopoda) and terrestrial isopods (Oniscidea) of the Cerová vrchovina Protected Landscape Area, Slovakia. In: Tajovský K (Ed.) 13th Central European Workshop on Soil Zoology. Abstract book with programme and list of participants. April 13–15, 2015, ISB BC CAS, České Budějovice, Czech Republic, 51 pp.

Tajovský K, Štrichelová J, Tuf IH (2018) Terrestrial isopods (Oniscidea) of the White Carpathians (Czech Republic and Slovakia) In: Hornung E, Taiti S, Szlavecz K (Eds) Isopods in a Changing World. ZooKeys 801: 305–321. https://doi.org/10.3897/zookeys.801.24133

Timková G (2008) Oniscidea Čiernej hory. Diploma thesis. Faculty of Science, Pavol Jozef Šafárik University in Košice, 94 pp. [supervisor A. Mock]

Timková G, Mock A (2008) Čo vieme o faune a ekológii suchozemských rovnakonôžok (Crustacea: Oniscidea) orografického celku Čierna hora? In 6. česko-slovenský myriapodologický seminár, Opátka, Slovensko, 15.–17.10.2008. Proceedings. Pavol Jozef Šafárik University in Košice, 14 pp.

Tomescu N, Teodor LA, Ferenţi S, Covaciu-Marcov S-D (2015) *Trachelipus* species (Crustacea, Isopoda, Oniscidea) in Romanian fauna: morphology, ecology, and geographic distribution. North-Western Journal of Zoology 11 (Supplement 1): e150301 (S1-S106).

Tomescu N, Teodor LA, Ferenţi S, Covaciu-Marcov S-D (2016) Two *Protracheoniscus* Species (Crustacea, Isopoda, Oniscidea) in Romanian Fauna: Morphology, Ecology and Distribution. Studia Universitatis Babeş-Bolyai Biologia 40: 147–166.

Topp W, Kappes H, Kulfan J, Zach P (2006) Distribution pattern of woodlice (Isopoda) and millipedes (Diplopoda) in four primeval forests of the Western Carpathians (Central Slovakia). Soil Biology and Biochemistry 38: 43–50. https://doi.org/10.1016/j.soilbio.2005.04.012

Tóth P (2019) Pôdna makrofauna v soliflukčných kamenných moriach Slanských vrchov. Diploma thesis. Faculty of Science, Pavol Jozef Šafárik University in Košice, 65 pp. [supervisor A. Mock]

Tuf IH, Tufová J (2005) Communities of terrestrial isopods (Crustacea: Isopoda: Oniscidea) in epigeon of oak-hornbeam forests of SW Slovakia. Ekológia (Bratislava), 24, Supplement 2/2005: 113–123.

Tuf IH, Tufová J (2008) Proposal of ecological classification of centipede, millipede and terrestrial isopod faunas for evaluation of habitat quality in Czech Republic. Časopis Slezského Muzea v Opavě (A) 57: 37–44.

Turis P, Vidlička Ľ (2013) Relationship of animals to the cyclamen *Cyclamen fatrense* Halda et Soják: pollinators, consumers, and occasional visitors. Biologia 68(3): 517–524. https://doi.org/10.2478/s11756-013-0178-2

Uhrin M, Panigaj Ľ (2017) Zoological records in Gustáv M. Reuss' manuscript on Revúca from years 1853 and 1854. Folia faunistica Slovaca 22: 41–56. https://doi.org/10.1111/j.1469-7998.1854.tb07226.x

Urbański J (1950) Równonogi (Isopoda, Crust.) Pomorza. Badania Fizjograficzne nad Polska Zachodnia, Poznań 4: 225–437.

Válentová Z (2013) Sekundárna sukcesia spoločenstiev suchozemských rovnakonôžok (Oniscidea) a mnohonôžok (Diplopoda) v smrečinách Vysokých Tatier po veternej kalamite. Diploma thesis. Faculty of Science, Pavol Jozef Šafárik University in Košice, 100 pp. [supervisor A. Mock]

Vaníková S (2009) Neparazitické článkonožce (Arthropoda) v hniezdach vtákov. Bachelor thesis. Faculty of Science, Pavol Jozef Šafárik University in Košice, 31 pp. [supervisor A. Mock]

Verhoeff KW (1907) Über Isopoden, 10. Aufsatz: Zur Kenntnis der Porcellioniden. Sitzungsberichte der Gesellschaft naturforschender Freunde zu Berlin 8: 229–281.

Verhoeff KW (1908) Über Isopoden: 15. Aufsatz. Archiv für Biontologie 2: 335–387.

Verhoeff KW (1927a) Über einige zoogeographisch bedeutsame Isopoden-Arten. Zoologischer Anzeiger 73: 323–333.

Verhoeff KW (1927b) Über einige südosteuropäischen Trichonisciden. Zoologischer Anzeiger 70: 200–223.

Verhoeff KW (1937) Die Isopoden des alemannischen Gaues und ihre geographisch-ökologische Bedeutung. Beiträge zur naturkundlichen Forschung in Südwestdeutschland 2: 22–39.

Vilček F, Hudec I (2019) Kôrovce. In: Krumpál M, Krejča J (Eds) Veľká kniha živočíchov, Príroda, Bratislava, 6th edn, 70–77.

Vilisics F, Nagy N, Sólymos P, Farkas R, Kemencei Z, Páll-Gergely P, Kisfali M, Hornung E (2008) Data on the terrestrial isopoda fauna of the Alsó-Hegy, Aggtelek National Park, Hungary. Folia faunistica Slovaca 13(4): 19–22.

Višňovská Z, Barlog M (2009) Príspevok k poznaniu fauny pseudokrasovej Jaskyne pod Jankovcom 2 v Levočských vrchoch. Aragonit, Liptovský Mikuláš 14(1): 63–66.

Višňovská Z, Papáč V, Kováč Ľ, Mock A, Košel V, Parimuchová A, Ľuptáčik P (2017) Spoločenstvá bezstavovcov (Evertebrata) jaskyňe Zápoľná (Invertebrates of the Zápoľná Cave, Kozie Chrbty Mts., Slovakia). Slovenská kras (Acta Carsologica Slovaca) 55/2: 203–219.

Wächtler W (1937) Isopoda, Asseln. In: Brohmer P, Ehrmann P, Ulmer G (Eds) Die Tierwelt Mitteleuropas, Band II, Lief. 2b. Verlag Quelle & Meyer, Leipzig, 225–317.

ZooKeys 1225: 103–113 (2025)
DOI: 10.3897/zookeys.1225.121048

Research Article

The terrestrial isopod fauna (Isopoda, Oniscidea) of Abrau Peninsula, north-west Caucasus, Russia

Daria M. Kuznetsova[1,2], Konstantin B. Gongalsky[1]

1 *Severtsov Institute of Ecology and Evolution of the Russian Academy of Sciences, Moscow 119071, Russia*
2 *Present address: Edinburgh, UK*
Corresponding author: Konstantin B. Gongalsky (gongalsky@gmail.com)

Abstract

From 2001 to 2022, the woodlice fauna of Abrau Peninsula, north-west Caucasus, Russia was examined. The collections yielded 5,581 specimens, which belong to 25 species, 19 genera, and 15 families. The most diverse families are Cylisticidae, Plathyarthridae, Trachelipodidae, and Trichoniscidae, each with three species. The most diverse genera are *Platyarthrus* and *Trachelipus*. Five species have been noticed to the Russian fauna for the first time: *Tylos europaeus*, *Acaeroplastes melanurus*, *Platyarthrus caudatus*, *Buddelundiella cataractae*, and *Armadillidium* cf. *marmoratum*. The fauna of the Abrau Peninsula is predominantly Mediterranean and with a high percentage of endemics (12%). Further records are expected with more detailed studies of especially the family Trichoniscidae.

Key words: Checklist, database, inventory, soil fauna, soil macrofauna

Academic editor: Ivan H. Tuf
Received: 19 February 2024
Accepted: 6 May 2024
Published: 5 February 2025

ZooBank: https://zoobank.org/37280F5E-76C1-45D3-8601-148CBA2D33DA

Citation: Kuznetsova DM, Gongalsky KB (2025) The terrestrial isopod fauna (Isopoda, Oniscidea) of Abrau Peninsula, north-west Caucasus, Russia. In: Tuf IH, Tajovský K, Taiti S (Eds) The Biology of Terrestrial Isopods, XII. ZooKeys 1225: 103–113. https://doi.org/10.3897/zookeys.1225.121048

Introduction

Mediterranean ecosystems are among the most disturbed in the world (Mouillot et al. 2002), but they are also one of the possible centers of origin of woodlice, which have a high diversity here (Taiti et al.1998; Sfenthourakis and Taiti 2015). Due to this, the Mediterranean ecosystems need both research and conservation. In the territory of Russia, sub-Mediterranean ecosystems are widespread in the north-west Caucasus. Abrau Peninsula is a unique well-preserved area of sub-Mediterranean ecosystems on the Black Sea coast, and the Navagir Ridge separates it from the main transportation lines, making it almost inaccessible to tourists. It serves as a reference for comparable regions around the Black Sea coast. The peninsula is currently protected by a nature reserve, making understanding of its wildlife particularly important.

The soil fauna have been intensively explored in Russia's Mediterranean ecosystems. K.V. Arnoldi and M.S. Gilyarov began exploring forest ecosystems between Novorossiysk and Dzhankhot beginning in the 1950s (Arnoldi and Gilyarov 1958; Gilyarov 1965, 1972). Later, owing to the efforts of A.D. Pokarzhevskii, thorough research was conducted in the Utrish State Nature Reserve, which was established on the Abrau Peninsula in 2010 (Gongalsky et al. 2004, 2006; Korobushkin 2014).

Woodlice (Isopoda, Oniscidea) are among the most common soil biota in these ecosystems. Our initial attempts to identify the material were rather unsuccessful due to the lack of proper keys, but with the assistance of major specialists in woodlice taxonomy (see Acknowledgements), we were able to produce an up-to-date identification of species. Based on this, both faunal (Gongalsky 2017, 2022; Gongalsky et al. 2024) and ecological publications (Gongalsky and Kuznetsova 2011) on woodlice in the region have been published. The advancement of molecular genetic technologies has lately allowed for the clarification of the status of some dubious species, and now this work has some horizons that bring us closer to understanding the woodlice fauna of the Abrau Peninsula. However, there are still plenty of opportunities for work in this region, and the proposed list is far from complete. Our goal is to compile all woodlice findings made on Abrau Peninsula to this time and conduct a preliminary analysis of its fauna.

Materials and methods

Study area

Studies of the woodlice fauna on Abrau Peninsula were conducted between 2001 and 2022. For sampling, we used the most diverse biotopes and the maximal diversity of microhabitats within each of them (Figs 1, 2).

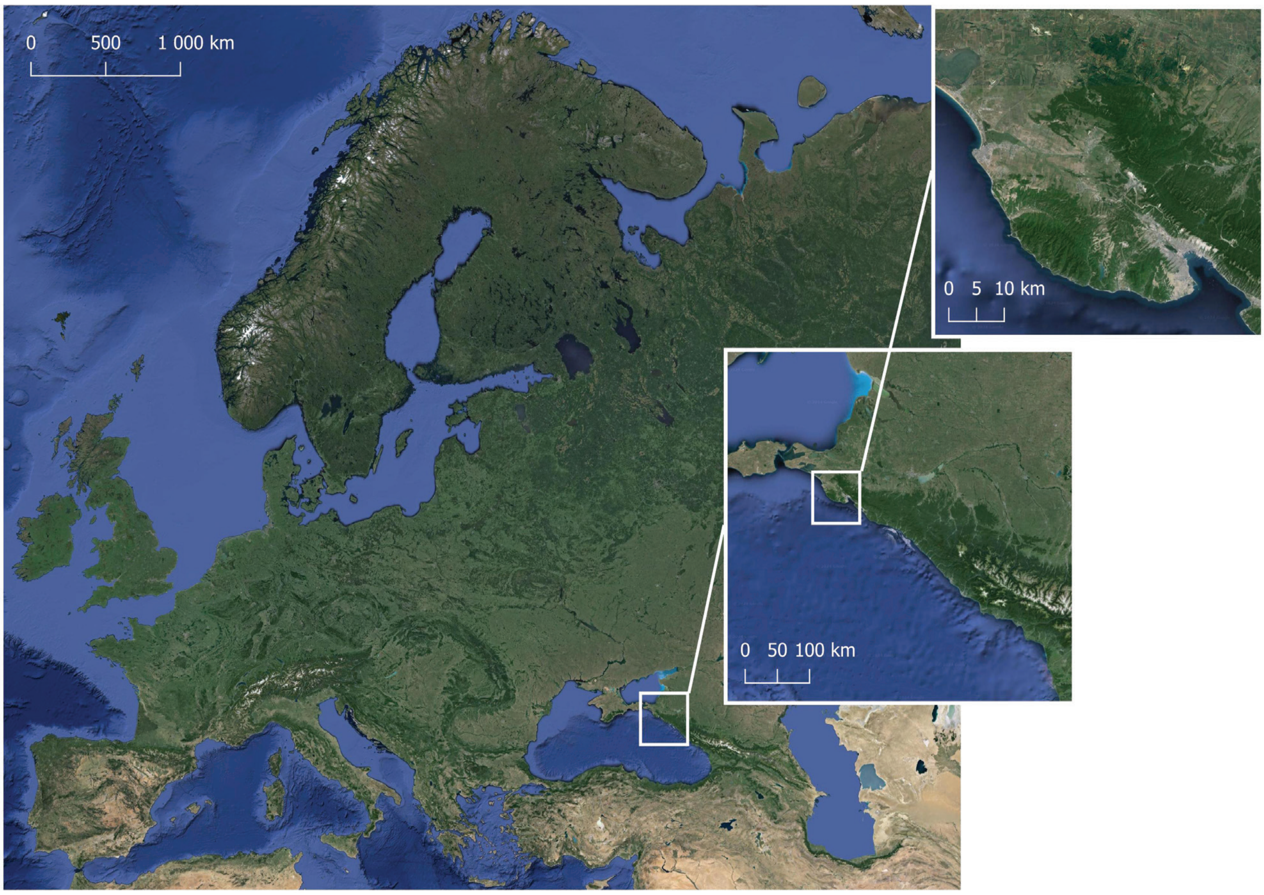

Figure 1. Map of the location of the study area (Abrau Peninsula).

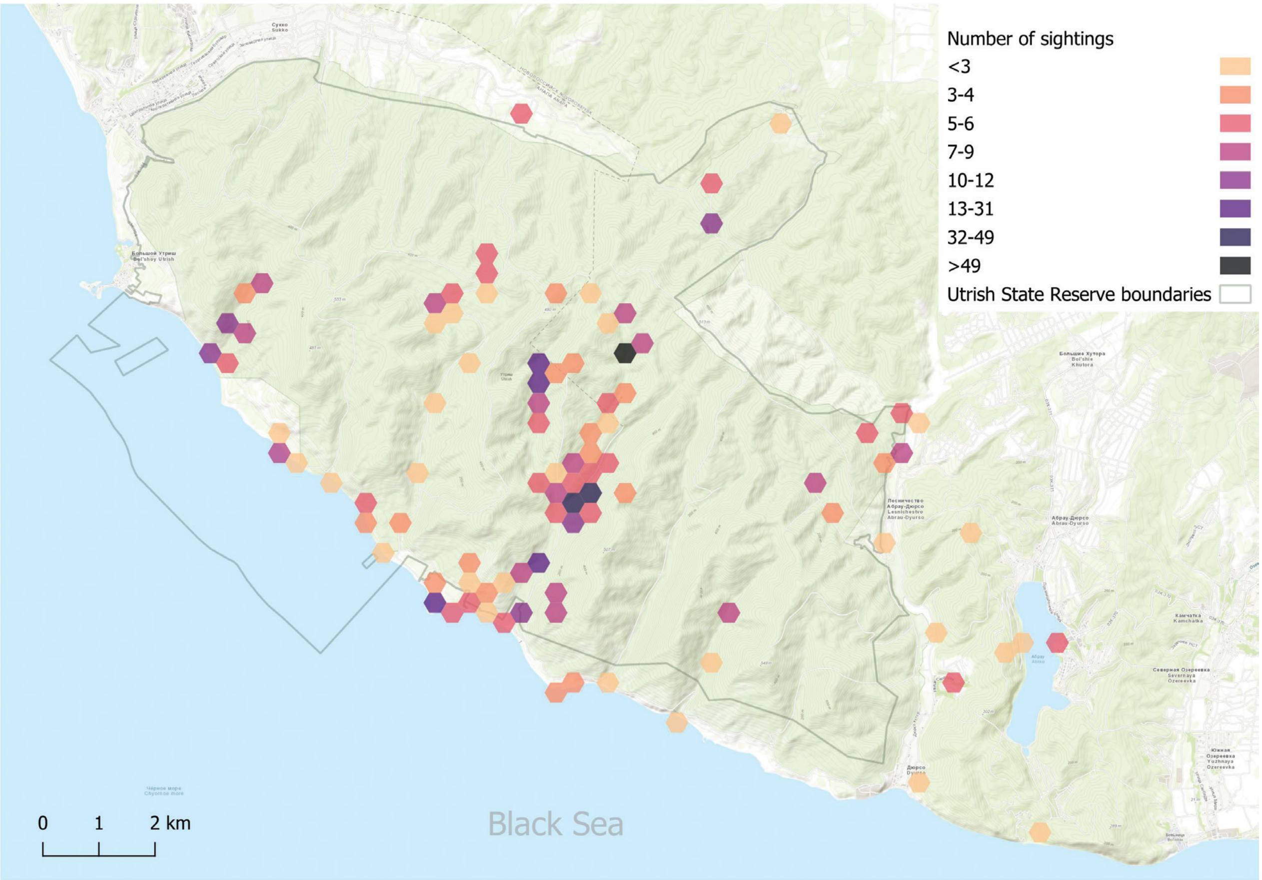

Figure 2. Map of observations.

Sampling methods

The resulting database of isopods location records comprised both individual faunal discoveries and finds from soil macrofauna surveys. All species were collected using hand-sorting from the litter and upper soil layer (down to a depth of 10–15 cm).

All collected animals were fixed in alcohol for subsequent laboratory identification. The biotope parameters, location, and date of sample were documented. Isopod species were identified using original descriptions (Borutzky 1948, 1977) and monographs on terrestrial isopods in Europe (Vandel 1960, 1962; Gruner 1966; Schmolzer 1965; Schmidt 1997; Sutton 2013). The taxonomy follows Schmalfuss (2003).

Description of the database

A database of Abrau Peninsula isopod faunistic sightings was published in GBIF (https://doi.org/10.15468/zdypk6). It has 639 occurrences of 5,581 specimens from 25 species. The dataset covers the period from 2001 to 2022. Each entry includes the species, the names of the collector(s) and identifiers, geographical coordinates, the date (at least the month and year), and the number of specimens collected.

Analysis of the fauna

We used the range classification proposed by Schmalfuss (2003). Since many range types are more detailed, they are grouped under these five categories: (i) Abrau endemics, (ii) Caucasus (Caucasus; North Caucasus), (iii) Mediterranean (Black Sea coast; Mediterranean and Black Sea coasts; Mediterranean, Black Sea costs and Middle Asia; Mediterranean, Atlantic and Black Sea coasts; Mediterranean and Atlantic coasts; European Mediterranean and Atlantic coasts; Macaronesian, Mediterranean and Black Sea coasts; Mediterranean), (iv) Europe (Europe, North Africa, Asia Minor; Europe, Asia Minor; Europe), (v) Cosmopolitan (Coastal cosmopolitan).

Results

The database contains 639 records for 5,581 individuals of woodlice. The woodlice fauna of the Abrau Peninsula includes 25 species from 19 genera and 15 families.

A commented list of terrestrial isopods of the Abrau Peninsula

First record for the Russian fauna is indicated by an asterisk (*).

Family Ligiidae Leach, 1814

1. *Ligidium fragile* Budde-Lund, 1885 – Western Caucasus. Hygrophilic. Widely distributed in the western Caucasus. Occurs along the banks of streams and freshwater reservoirs, not more than a few meters from the water. Number of records: 50.

Family Tylidae Dana, 1852

2. **Tylos europaeus* Arcangeli, 1938 – East Atlantic and Mediterranean. Coastal halophilic. The species has a wide eastern Mediterranean distribution, but was overlooked in the material from the Abrau Peninsula. It prefers stony seashores. Number of records: 2.

Family Buddelundiellidae Verhoeff, 1930

3. **Buddelundiella cataractae* Verhoeff, 1930 – European. Mesophytic forests. The species inhabits deep moist litter, mainly in broadleaf forests. There is high diversity within this genus (Gardini and Taiti 2023), so molecular analysis is welcomed to check the presence of cryptic species on the Abrau Peninsula. Number of records: 6.

Family Trichoniscidae G.O. Sars, 1899

4. *Caucasocyphoniscus taitii* Gongalsky, 2022 – Abrau Peninsula (endemic). Hygrophilic. This species was once recorded in 2004 as single individual in thick leaf litter of broadleaf forests and then found again in high numbers in

2017–2020 in water oozing from cracks of cliffs. This presumes that this is an inhabitant of MSS (milieu souterrain superficiel). Number of records: 3.

5. *Haplophthalmus danicus* Budde-Lund, 1880 – European, now cosmopolitan. Hygrophilic, this species inhabits deep moist litter, mainly in broadleaf forests. Number of records: 28.
6. *Trichoniscus pygmaeus* Sars, 1898 – Atlantic–Mediterranean. Hygrophilic. The species inhabits the banks of streams and freshwater reservoirs and no more than a few metres from the water's edge. Given the high diversity within the genus in Europe, as well as the presence of other species of the genus on the Black Sea coast of the Caucasus, molecular genetic analyses are needed to identify potential cryptic species in this genus on the Abrau Peninsula. Number of records: 68.

Family Philosciidae Kinahan, 1857

7. *Chaetophiloscia hastata* Verhoeff, 1928 – Eastern Mediterranean–Central Asian. Xerophilic. One of the main isopod species of Mediterranean shrublands (consisting of several species of juniper, pistachio, and Jerusalem thorn). This species was included in the regional Red Data Book of Krasnodar Krai as an indicator species of Mediterranean shrublands forests. Number of records: 40.

Family Halophilosciidae Verhoeff, 1908

8. *Halophiloscia couchii* (Kinahan, 1858) – Mediterranean–Atlantic–Black Sea coasts by origin, but now almost cosmopolitan. Coastal halophilic. A terrestrial isopod that lives on the sea coast within a few meters of the water's edge. Number of records: 7.

Family Platyarthridae Verhoeff, 1949

9. **Platyarthrus caudatus* Aubert & Dollfus, 1890 – Mediterranean. Myrmecophilic. Detected only in recent faunal studies due to the mass survey of anthills. The species corresponds to the type description. Number of records: 3.
10. *Platyarthrus hoffmannseggii* Brandt, 1833 – Europe–North Africa–Asia Minor by origin, but now Holarctic. Myrmecophilic. The species is widespread throughout the peninsula, both in anthills located in Mediterranean vegetation and a few kilometres from the sea in the belt of broadleaf forests. Number of records: 11.
11. *Platyarthrus schoblii* Budde-Lund, 1885 – Macaronesian–Mediterranean–Black Sea coasts. Myrmecophilic. Despite the wide distribution of the species along the Black Sea coast, it was found in only one locality, in an anthill near a settlement. Probably spread by humans. Number of records: 1.

Family Agnaridae Schmidt, 2003

12. *Protracheoniscus krivolutskyi* Gongalsky, 2024 – Abrau Peninsula (endemic). Eurytopic. A common and numerous species on the peninsula. It dominates in many ecosystems, in particular, *Quercus petraea* oak forests.

This species inhabits lowlands, slopes, and tops of gorges, and it may be the only species in upland communities. Until recently it was attributed to *Protracheoniscus fossuliger* (Verhoeff, 1901), but closer examination of morphology and application of molecular genetic markers revealed the authenticity of this species (Gongalsky et al. 2024). Number of records: 105.

Family Cylisticidae Verhoeff, 1949

13. *Cylisticus convexus* (De Geer, 1778) – Almost cosmopolitan. Mesophitic forests. This species is mainly distributed in the settlements of Abrau and Maly Utrish, but it also occurs in forests. Molecular genetic data confirm that our material belongs to this species (data not published). Number of records: 13.
14. *Cylisticus giljarovi* Borutzky, 1977 – Northern Caucasus. Mesophitic forests. This species occurs exclusively beyond the Navagir Ridge, on the northern macro-slope of the ridge separating these ecosystems from the sea. It inhabits oak and hornbeam forests. The present finding is the second mentionioning of the species since its description (Borutzky 1977). Number of records: 2.
15. *Parcylisticus dentifrons* (Budde-Lund, 1885) – Northern Caucasus and northern Pre-Caspian. The species is probably at the edge of its range; it inhabits mainly the Caucasus. In addition to the Abrau Peninsula, it also occurs in Novorossiysk. Number of records: 1.

Family Porcellionidae Brandt, 1831

16. **Acaeroplastes melanurus* (Budde-Lund, 1885) – Mediterranean and East Atlantic distribution. Mesophitic forests. Found exclusively behind Navagir Ridge, on the northern macro-slope of the ridge separating these ecosystems from the sea. Occurs in oak and oriental hornbeam forests. Fully corresponds to the type description. Number of records: 2.
17. *Porcellionides pruinosus* (Brandt, 1833) – Mediterranean, but now cosmopolitan. Synanthropic. The species prefers forest-steppe and steppe territories. Where detected on the Abrau Peninsula, the sites have mainly synanthropic habitats. Number of records: 5.

Family Trachelipodidae Strouhal, 1953

18. *Trachelipus lutshnikii* (Verhoeff, 1933) – Western-Caucasus. Mesophitic forests. The species has been described from the environs of Sochi, and the present find is the westernmost point, considerably extending its range. Fully corresponds to the type description. Number of records: 2.
19. *Trachelipus razzautii* Arcangeli, 1913 – Mediterranean. Mesophitic forests. One of the most widespread species of isopods on the Abrau Peninsula and is found almost everywhere. Probably, may be a cryptic species; therefore, a comparison using molecular genetic markers with material from the topotype of *T. razzautii* is necessary. Number of records: 102.
20. *Trachelipus utrishensis* Gongalsky, 2017 – Abrau Peninsula (endemic). Mesophitic forests. It is a recently described species, which inhabits only broadleaf forests on the peninsula. Number of records: 41.

Family Detonidae Budde-Lund, 1904

21. *Armadilloniscus ellipticus* (Harger, 1878) – Almost cosmopolitan. Coastal halophilic. Occurs in the littoral areas. Prefers rocks in the tidal zone. Number of records: 15.

Family Armadillidiidae Brandt, 1833

22. **Armadillidium* cf. *marmoratum* Strouhal, 1929 – Mediterranean. Coastal halophilic. This species was found only on beaches. It is probably a new species, as identification in collaboration with S. Taiti resulted in an unclear diagnosis. Molecular genetic analysis and thorough morphological study are required, which is our planned. Number of records: 7.
23. *Armadillidium vulgare* (Latreille, 1804) – Mediterranean, but now cosmopolitan. Euryoecious. This is one of the most common and largest species on the peninsula. Number of records: 103.

Family Armadillidae Brandt, 1831

24. *Armadillo officinalis* Duméril, 1816 – Mediterranean. Xerophilic. One of the main inhabitants of Mediterranean shrublands (consisting of several species of juniper, pistachio, and Jerusalem thorn). This species was Included in the regional Red Data Book of Krasnodar Krai as an indicator species of Mediterranean shrublands. It has been found in the settlement of Malyi Utrish. Number of records: 20.

Incertae sedis

25. *Buchnerillo littoralis* (Verhoeff, 1942) – Mediterranean. Hygrophilic. The species was captured between stones in a vertical wall at the Zhemchuzhnyi waterfall, the place where the watercourse pours out of the crevasse into the sea. The waterfall is located tens of meters from the tide line. Due to the displacement of the waterfall in the last few years, findings have ceased. Perhaps the species was introduced here, survived for a few years, and then disappeared. Number of records: 2.

In Abrau Peninsula, there are three endemic species: *Caucasocyphoniscus taitii*, *Trachelipus utrishensis*, and *Protracheoniscus krivolutskyi*.Four other species were defined as the Caucasus endemics: northern Caucasus *Cylisticus giljarovi* and *Parcylisticus dentifrons*, and western Caucasus *Ligidium fragile* and *Trachelipus lutshnikii*. For last species, the Abrau Peninsula record is the westernmost and, thus, extend this species' range substantially.

Almost a half of the woodlice species on the Abrau Peninsula are characteristic of Mediterranean ecosystems (Fig. 3). The most widely distributed among Mediterranean species are the four eastern Atlantic species, *Buchnerillo littoralis*, *Trichoniscus pygmaeus*, *Tylos europaeus*, and *Halophiloscia couchii*, and also an eastern Mediterranean–Central Asian species, *Chaetophiloscia hastata*. Another six species are endemic to Mediterranean ecosystems:

Figure 3. Types of woodlice species original distribution of Abrau Peninsula based on number of species. Captions: 1 – Black Sea coast; 2 – Mediterranean and Black Sea coasts; 3 – Mediterranean, Black Sea costs, and Central Asia; 4 – Mediterranean, Atlantic, and Black Sea coasts; 5 – Mediterranean and Atlantic coasts; 6 – European Mediterranean and Atlantic coasts; 7 – Macaronesian, Mediterranean, and Black Sea coasts; 8 – Mediterranean.

Acaeroplastes melanurus, *Armadillidium vulgare*, *Armadillo officinalis*, *Platyarthrus caudatus*, *Porcellionides pruinosus*, and *Trachelipus razzautii*. *Halophiloscia couchii*, *A.vulgare* and *P. pruinosus* originated in the Mediterranean region but are now cosmopolitan.

Another four species in the fauna are widespread: one European (*Buddelundiella cataractae*), one originally European–Asia Minorian but now cosmopolitan (*Cylisticus convexus*), one originally eastern Palearctic but now Holarctic (*Platyarthrus hoffmannseggii*) and one cosmopolitan (*Armadilloniscus ellipticus*).

Some of the species are recorded for the first time from the Russian woodlice fauna: *B. cataractae*, *A. melanurus*, *P. caudatus*, *A.* cf. *marmoratum*, and *T. europeus*. For two species, the westernmost extent of their ranges are reported from the Abrau Peninsula: *C. giljarovi*, previously known from the northern Caucasus, and *T. lutshniki*, which had been found only in the environs of Sochi.

According to the ecological needs, the 25 species of Abrau Peninsula may be classified into seven groups: hydrophilic, mesophytic, xerophilic, halophilic, myrmecophilic, synanthropic, and euryoecious. *Ligidium fragile*, *T. pygmaeus*, *C. taitii*, *H. danicus*, *T. pygmaeus*, and *B. littoralis* can be described as hydrophilic inhabitants of freshwater surroundings. *Armadilloniscus ellipticus*, *A.* cf. *marmoratum*, *H. couchii*, and *T. europeus* are coastal halophilic species. Mesophytic *B. cataractae*, *C. convexus*, *C. giljarovi*, *P. dentifrons*, and all trachelipodids are found solely in wooded biotopes on the peninsula. *Chaetophiloscia hastata* and *A. officinalis* are xerophilic, and all three platyarthrid species are myrmecophilic. *Armadillidium vulgare* and *P. krivolutskyi* are euryoecious. The only synanthropic species is *P. pruinosus*.

Three species were recorded from more than 100 observations each: *Armadillidium vulgare*, *P. krivolutskyi*, and *T. razzautii*. *C. hastata*, *L. fragile*, *T. utrishensis*, and *T. pygmaeus* were less common but still found in over 40 locations were. *Armadillo officinalis*, *A. ellipticus*, and *H. danicus* were discovered at 15–20 locations. All myrmecophilic species and 75% of halophilic species were found to be uncommon in our surveys. Other ecological groupings are well represented, ranging from rare to common species.

Discussion

The terrestrial isopod fauna of the Abrau Peninsula is quite diverse on a national scale. In our study area, 25 species were found, while the fauna of the former USSR has been reported to have as many as 192 species (Kuznetsova and Gongalsky 2012).

Compared to terrestrial isopod faunas in other Mediterranean and sub-Mediterranean regions, the species diversity on the Abrau Peninsula is not very high. For example, the fauna of Liguria is 97 species (Gardini and Taiti 2023); of the Mediterranean islands, 176 species (Gentile and Argano 2005); and of the Aegean islands, 69 species (Sfenthourakis 1996). Despite the relatively low species diversity, the Abrau Peninsula woodlice fauna is quite rich in endemics of the Caucasus and the peninsula.

The majority of the faunal finds were in June and July. These are the most popular months for soil zoology investigations, when the peninsula is still wet but also warm enough. However, species of isopods can reach their population peak at different times of the year (Warburg 1987). Thus, it seems reasonable that future studies be undertaken in other months. Every few years, one or more species are added to the fauna, indicating that species saturation has not yet been reached.

There is a bias towards natural habitats in the dataset. Many species common in settlements were seldom recorded (e.g. *P. pruinosus*). So, another approach to studying terrestrial isopods of the Abrau Peninsula is to examine buildings and cellars in local settlements.

Acknowledgements

We cordially thank Helmut Schmalfuss, Christian Schmidt, and Stefano Taiti for their help with identifications during various stages of our study, Andrei D. Pokarzhevskii, Olga A. Leontyeva (both late) for initiating studies of soil fauna on the Abrau Peninsula, Daniil I. Korobushkin, Ruslan Saifutdinov, Philipp S. Byzov, and Ivan N. Marin for providing materials for examination, students of various years at the Department of Biogeography, Moscow State University, for their help in collecting, staff of the Utrish Marine Biological Station of Severtsov Institute of Ecology and Evolution, Russian Academy of Sciences, and staff of the Utrish State Nature Reserve (especially, deputy head, Olga N. Bykhalova). We thank Alexei D. Elagin for editing the English language of the manuscript.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

Funding

No funding was reported.

Author contributions

Conceptualization: KBG. Data curation: DMK. Formal analysis: DMK. Investigation: KBG. Methodology: KBG. Validation: KBG. Writing – original draft: DMK. Writing – review and editing: KBG.

Author ORCIDs

Daria M. Kuznetsova https://orcid.org/0000-0002-1237-1066
Konstantin B. Gongalsky https://orcid.org/0000-0003-1600-8448

Data availability

All of the data that support the findings of this study are available in the main text.

References

Arnoldi KV, Gilyarov MS (1958) Soil fauna of Mediterranean habitats of the North-West Caucasus and its significance for their characterization. Zoologicheskii jurnal [Зоологический журнал] 37(6): 801–819. [In Russian]

Borutzky EV (1948) Terrestrial Isopods of caves of the Crimea and the Caucasus. II. Vestnik Moskovskogo Gosudarstvennogo Universiteta 5: 137–146. [In Russian]

Borutzky EV (1977) New species of the genus *Cylisticus* (Isopoda, Oniscoidea, Cylisticidae). Zoologicheskii jurnal [Зоологический журнал] 56: 28–37. [In Russian with English summary]

Gardini P, Taiti S (2023) New species of terrestrial isopods (Crustacea, Isopoda, Oniscidea) from Liguria and surrounding regions, northern Italy. Diversity 15(1): 68. https://doi.org/10.3390/d15010068

Gentile G, Argano R (2005) Island biogeography of the Mediterranean Sea: The species–area relationship for terrestrial isopods. Journal of Biogeography 32(10): 1715–1726. https://doi.org/10.1111/j.1365-2699.2005.01329.x

Gilyarov MS (1965) Zoological Method of Soil Diagnostics. Nauka Publ., Moscow, 279 pp. [In Russian]

Gilyarov MS (1972) Soil fauna of brown soils of the Main Caucasian Range. In: Gilyarov MS (Ed.) Problems of soil zoology. Abstracts of IV All-Union Conference. Nauka Publ., Moscow, 37–38. [In Russian]

Gongalsky KB (2017) A new species of *Trachelipus* Budde-Lund, 1908 (Isopoda: Oniscidea: Trachelipodidae) from the Utrish Nature Reserve, northwestern Caucasus. Arthropoda Selecta 26(1): 35–40. https://doi.org/10.15298/arthsel.26.1.05

Gongalsky KB (2022) First terrestrial species of the troglobiont genus *Caucasocyphoniscus* Borutzky, 1948 (Isopoda, Oniscidea, Trichoniscidae) from northwestern Caucasus. Arthropoda Selecta 31(4): 387–391. https://doi.org/10.15298/arthsel.31.4.03

Gongalsky KB, Kuznetsova DM (2011) Fauna and population of woodlice (Isopoda: Oniscidea) of Abrau Peninsula (North-Western Caucasus). Zoologicheskii jurnal [Зоологический журнал] 90(8): 916–922. [In Russian, with English summary]

Gongalsky KB, Pokarzhevskii AD, Filimonova ZV, Savin FA (2004) Stratification and dynamics of bait-lamina test in three forest soils along a North-South gradient in Russia. Applied Soil Ecology 25(2): 111–122. https://doi.org/10.1016/j.apsoil.2003.09.001

Gongalsky KB, Pokarzhevskii AD, Savin FA (2006) Soil macrofauna of submediterranean ecosystems in the Abrau Peninsula, the Northwestern Caucasus. Entomological Review 86(S2): S165–S171. https://doi.org/10.1134/S0013873806110078

Gongalsky KB, Byzov PS, Medvedev DA (2024) A new species of the genus *Protracheoniscus* Verhoeff, 1917 (Isopoda, Oniscidea, Agnaridae) from the northwestern Caucasus. Arthropoda Selecta 33(1): 65–75. https://doi.org/10.15298/arthsel.33.1.06

Gruner H (1966) Die Tierwelt Deutschlands. 53. Teil. Krebstiere oder Crustacea. V. Isopoda, 2. Lieferung, Jena, 151–380.

Korobushkin DI (2014) Role of allochthonous carbon in the energy of terrestrial invertebrate communities at different distances from the Black Sea and a freshwater lake (isotopic evidence). Russian Journal of Ecology 45(3): 223–230. https://doi.org/10.1134/S1067413614030060

Kuznetsova DM, Gongalsky KB (2012) Cartographic analysis of woodlice fauna of the former USSR. ZooKeys 176: 1–11. https://doi.org/10.3897/zookeys.176.2372

Mouillot F, Rambal S, Joffre R (2002) Simulating climate change impacts on fire frequency and vegetation dynamics in a Mediterranean-type ecosystem. Global Change Biology 8(5): 423–437. https://doi.org/10.1046/j.1365-2486.2002.00494.x

Schmalfuss H (2003) World catalog of terrestrial isopods (Isopoda: Oniscidea). Stuttgarter Beiträge zur Naturkunde, Serie A 654: 1–341.

Schmidt C (1997) Revision of the European species of the genus *Trachelipus* Budde-Lund, 1908 (Crustacea: Isopoda: Oniscidea). Zoological Journal of the Linnean Society 121(2): 129–244. https://doi.org/10.1111/j.1096-3642.1997.tb00337.x

Schmolzer K (1965) Bestimmungsbucher zur Bodenfauna Europas. Ordnung Isopoda (Landasseln). Lieferung 4 and 5: Berlin, 468 pp.

Sfenthourakis S (1996) A biogeographical analysis of terrestrial isopods (Isopoda, Oniscidea) from the central Aegean islands (Greece). Journal of Biogeography 23(5): 687–698. https://doi.org/10.1111/j.1365-2699.1996.tb00029.x

Sfenthourakis S, Taiti S (2015) Patterns of taxonomic diversity among terrestrial isopods. ZooKeys 515: 13–25. https://doi.org/10.3897/zookeys.515.9332

Sutton S (2013) Woodlice. Elsevier, London, 144 pp.

Taiti S, Paoli P, Ferrara F (1998) Morphology, biogeography, and ecology of the family Armadillidae (Crustacea, Oniscoidea). Israel Journal of Zoology 44(3–4): 291–301.

Vandel A (1960) Faune de France. Vol. 64, Isopodes terrestres (première partie). Lechevalier, Paris, 1–416.

Vandel A (1962) Faune de France. Vol. 66, Isopodes terrestres (deuxieme partie). Lechevalier, Paris, 417–931.

Warburg MR (1987) Isopods and their terrestrial environment. Advances in Ecological Research 17: 187–242. https://doi.org/10.1016/S0065-2504(08)60246-9

ZooKeys 1225: 115–125 (2025)
DOI: 10.3897/zookeys.1225.123647

Research Article

The effect of formaldehyde solution in pitfall traps on the probability of catching woodlice (Isopoda, Oniscidea)

Ivan Hadrián Tuf[1], Lucie Novotná[1], Pavel Fryčka[1]

1 *Department of Ecology and Environmental Sciences, Faculty of Science, Palacký University Olomouc, Šlechtitelů 27, Olomouc, CZ-77900, Czech Republic*
Corresponding author: Ivan Hadrián Tuf (ivan.tuf@upol.cz)

Abstract

Pitfall traps containing a fixative solution are commonly used by ecologists to study ground-dwelling invertebrates. The effect of the solution on the animals being caught is a frequent topic of studies. Our study compares the effect of formaldehyde solution, water, and the random probability of catch simulated by using dry traps. Ninety pitfall traps were placed in a floodplain forest ground: one-third used 4% formaldehyde solution as a fixative, one-third used water, and one-third was left without any liquid to simulate the random probability of a catch. A layer of dry wood chips was used in the dry traps to reduce predation between the caught animals. The traps were placed in the field between April and May 2022 and emptied twice a week. Both the numbers of animals and the species caught were found to be significantly affected by the fixative solution in use. Significantly more woodlice were caught in traps filled with water; these traps also attracted significantly more *Porcellium conspersum* and *Trachelipus rathkii* compared to the dry traps. Average day temperature and the order the traps were checked (i.e. date) had an influence on the animals caught as well. Additional laboratory experiments with *Porcellio scaber* confirmed that terrestrial isopods avoid formaldehyde pitfall traps more than those with water.

Key words: Barber traps, epigeic invertebrates, epigeon, model species, terrestrial isopods, trapability

Academic editor: Karel Tajovsk
Received: 21 March 2024
Accepted: 1 November 2024
Published: 5 February 2025

ZooBank: https://zoobank.org/6DC523F8-F745-47B8-9266-FFE3D970A87F

Citation: Tuf IH, Novotná L, Fryčka P (2025) The effect of formaldehyde solution in pitfall traps on the probability of catching woodlice (Isopoda, Oniscidea). In: Tuf IH, Tajovský K, Taiti S (Eds) The Biology of Terrestrial Isopods, XII. ZooKeys 1225: 115–125. https://doi.org/10.3897/zookeys.1225.123647

Introduction

Although the use of pitfall traps was first mentioned more than a hundred years ago, it was not described in detail until the publication by Herbert Spencer Barber, who used traps to study cave invertebrates (Barber 1931). Pitfall traps, now also called Barber´s traps, are considered to be the most widely used method for studying epigeic invertebrates (Bater 1996). This method is popular not only because of its affordability and ease of construction (Hohbein and Conway 2018) but also its effectiveness (Topping and Sunderland 1992).

The basic form of a pitfall trap is an open container buried into the ground, with the edge of the trap exposed on the ground level. The original traps constructed by Barber used glass tubes with rotting meat as bait and ethanol as a fixative solution. Since then, a large number of studies and reviews tried to find the best universal design for pitfall traps (e.g., Adis 1979; Knapp et al. 2016).

Various forms of bait have been tested, from rotting meat or smelly cheese to rotting fruit (Silva et al. 2012), cattle dung or blood (Gatty and Grández 2020) or human faeces (Filgueiras et al. 2009). Bait can be used to massively increase the number of caught invertebrates, especially in the case of predatory and scavenging species. Bait in liquid form, such as beer (Santalla et al. 2002) or wine (Baini et al. 2016), can also serve as a fixative solution. It is important to note that the fixative solution (Gerlach et al. 2009) or trapped animals can also act as unintentional bait.

Fixative solution can be used to quickly kill and prevent decomposition of the captured animals (Knapp and Růžička 2012). Pitfall traps without preservative solution are occasionally used to catch epigeon for breeding, feeding pet reptiles or amphibians, experiments or educational purposes. In that case, it is recommended to fill them partly with dry leaves, crumpled wet newspaper, moss or wire mesh to reduce predation between the caught specimens (Skuhravý 1957; Hatten et al. 2007).

One of the disadvantages of using a fixative solution is its possible effect as an attractant or repellent for different species (Adis 1979). Many scientists have evaluated the effects of water, salt solution, ethanol, ethylene glycol, propylene glycol, formaldehyde, paraffin, vinegar, and others (Knapp 2007), primarily focusing on maximizing the number of caught animals. In contrast, our study aims to understand how the fixative solution (formaldehyde or water) affects the catch size of terrestrial isopods in general. The behaviour of a model species of woodlouse was tested in a natural as well as a controlled laboratory environment.

Material and methods

Field experiment

The site chosen for the experiment was the floodplain forest in the Litovelské Pomoraví Protected Landscape Area near Olomouc, Czech Republic (49°39'11.1"N, 17°12'42.3"E, total size 179 ha). The tree floor in the selected part of the forest was dominated by hornbeam (*Carpinus betulus*), linden (*Tilia platyphyllos*) and oak (*Quercus robur*) with an admixture of maples (*Acer campestre*, *Acer pseudoplatanus*). Mean temperatures during the study period (April and May 2022) were comparable to the long-term average, with minimum daily temperatures ranging between -3.1 and 16.4 °C and maximum daily temperatures between 3.2 and 28.1 °C. Precipitation was strongly above the long-term average (higher by c. 33%).

The sampling of ground-dwelling invertebrates was carried out continuously from April 2 to May 28 using 90 pitfall traps. Each trap was made from a glass jar with an inserted plastic cup of inner diameter 6.5 cm and depth 10.5 cm and covered by a metal hood 2 cm above ground. The traps were placed in nine lines of ten at regular 12 m intervals between each. Thirty pitfall traps contained a 4% formaldehyde fixative solution, 30 traps were filled with plain water (without added detergent) and 30 were left dry. The distribution of traps by their content was not random, but regular by lines. Dry traps were partially filled by dry wood shavings to prevent predation between caught animals. The traps were collected each Tuesday and Friday for a period of 9 weeks (i.e. 17 inspections), and the collected material was stored in a freezer box. Subsequently, the captured terrestrial isopods were identified.

Laboratory experiment

A design similar to the experiment by Gerlach et al. (2009) was used to monitor the animal behaviour. A hole was cut in the middle of the bottom of a 17 × 17 × 11 cm plastic container to insert a glass jar (height 15 cm, neck width approximately 7.7 cm, volume 0.7 L) so that the top of the jar protruded approximately 4 cm inside the container. The container was then filled with a layer of gypsum up to the neck of the jar. Gypsum was used to retain moisture and was covered by a thin layer of soil. The portion of the glass jar below the container was then wrapped in black foil to prevent light from coming into the trap. Shelters (rocks and brick fragments) were placed in each of the four corners of the apparatus, along with pieces of decaying herbs.

A plastic cup with a neck diameter of approximately 7.7 cm and a height of 11 cm was placed into the jar. A plastic strainer was fixed c. 5 cm above the bottom of the cup to prevent animals from falling into the fixative solution poured just below the strainer. In total, two identical apparatuses were assembled. One with a trap containing water and one with a trap containing a 4% formaldehyde solution (Fig. 1). A Genius FaceCam 311 webcam, connected to a laptop, was mounted above the trap to record animal behaviour.

Porcellio scaber Latreille, 1804, easily available in sufficient numbers, was chosen as a model species for laboratory experiments. A group of 10 adult individuals, collected in parks in Olomouc, Czech Republic, was placed in the trap container each day at 17:00 h. The trap was covered for 15 minutes to prevent immediate capture of the animals. Recording ran until 14:00 the following day. Because the cameras were unable to record during the twilight or darkness, each container was illuminated with a 40W red bulb throughout the experiment; isopods are at least sensitive to longer wavelengths (Hariyama et al. 2001). The experiment was run three times with water and three times with formaldehyde at room temperature (c. 20 °C) and with a natural light regime. A new group of animals was used for each recording.

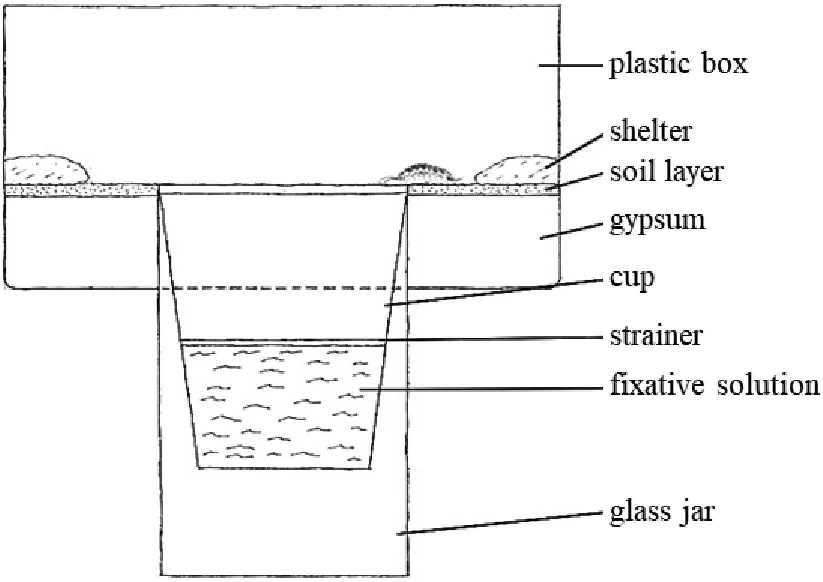

Figure 1. Laboratory apparatus imitating pitfall trap with surrounding soil surface.

Statistical analyses

Field experiment

Mean catches as a function of fixation solution were compared using univariate ANOVAs, and Tukey's tests were used to test the significance of differences between pairs of trap types. Data analysis was performed using CANOCO for Windows 5.0. At the beginning of the analysis, it was necessary to determine the dependent (species data) and independent (environmental data) variables. Species data represented the number of trapped individuals for each species. The environmental data represented trap type (categorical variables "water", "formaldehyde" and "dry"), average daily temperature one (*t-1*), two (*t-2*), and three (*t-3*) days prior to trap inspection, average temperature for those three days ("*t* mean"), and number of collecting occasion ("sample").

The length of the gradient in the species data (3.58) allowed the use of a direct linear gradient analysis (RDA) that plots multivariate relationships between species and environmental data. To express the relationships between temperature and the number of individuals of captured species, generalized linear models (GLMs) were used. Statistical significance and model power were tested using a Monte Carlo permutation test (499 repetitions). All the above diagrams generated by the linear method were created in CanoDraw for Windows.

Laboratory experiment

The activity of the animals during the experiment was read from the recordings obtained. The movement of an individual in a 2 cm band around the edge of the trap was taken as "one behavioural act". The total length of the recording was divided into 2-hour segments to which activity counts were assigned. Activity values obtained during the first two hours of observation (i.e., 17:00 to 19:00) were omitted due to the strikingly high movement of animals associated with their placement in a new environment. Animal behaviour observed was classified (cf., Gerlach et al. 2009) into these categories:

1. "nearby trap" activity - passing through the observed area without contact with the trap and without a change of direction or passing through the observed area without contact with the trap with a change of direction;
2. "in contact" activity - changing of direction after contact with the trap or going over the edge of the trap with part of the body and backing out;
3. "watching inside" activity - going over the edge with more than half of the body and backing out;
4. "trapped" activity - getting caught in the trap.

The number of occurrences for each category was recorded, with each next category including the previous ones, i.e., each "trapped" individual was "watching inside" before, which was the result of "in contact", etc. The mean values of the above categories were calculated from the three repetitions with the given fixative solution. The "Rate of Self-Rescue" (Gerlach et al. 2009), i.e., the percentage of animals that were able to climb out of the trap after overstepping its edge by more than half of their body to the number of animals that overstepped the trap edge by more than half of their body that was trapped, was used to represent the ability of a species to rescue itself from falling into the trap. Line graphs were used to graphically represent the values in the categories. The statistical significance of differences between these categories for water traps and formaldehyde traps within species was determined using a goodness-of-fit test.

Results

Field experiment

During the two-month study in the Litovelské Pomoraví Protected Landscape Area, a total of 2910 terrestrial isopods of 8 species were captured using 90 pitfall traps. On average, 26.9 ± 15.9 individuals were caught in a single dry trap, 25.4 ± 15.6 individuals were caught in a trap with formaldehyde, but significantly more individuals were caught in a trap with water (44.7 ± 23.0 ind.; $F = 10.17$, $p < 0.001$).

It is evident (Fig. 2), that using formaldehyde fixative solution did not significantly affect trap efficiency compared to the dry pitfall traps. The most abundant trapped species was *Trachelipus rathkii* (43% of the catch, Fig. 2). This species was significantly more likely to be caught in water traps than in dry traps or formaldehyde traps, similar to *Porcellium conspersum*. The other species did not show significant differences in catch size for a particular type of trap, although *Armadillidium vulgare* was insignificantly more numerous in traps with water and *Ligidium hypnorum* was more numerous in traps with formaldehyde solution.

A direct linear gradient analysis (RDA) looked at the effect of the trap fill (dry/water/formaldehyde), average daily temperature between trap inspections, and the effect of season (expressed as number of trapping occasion) on the abundance of trapped woodlouse (Fig. 3). The first axis of the model explains 8.2% of the variability (*pseudo-F* = 24.0; $p = 0.002$), and the model is significant (*pseudo-F* = 27.2; $p = 0.002$). Simple term effect was significant for all tested factors ($p < 0.05$) but conditional term effect highlighted changes associated with season ("sample", explains 6.24%, *pseudo-F* = 108, $p = 0.002$), temperature 2 days before trap inspection (*t-2*, explains 0.53%, *pseudo-F* = 9.3, $p = 0.002$) and fixative solution – against "formaldehyde", "water" explains 1.9% (*pseudo-F* = 33.5, $p = 0.002$) and "dry" traps explain 0.27% of variability

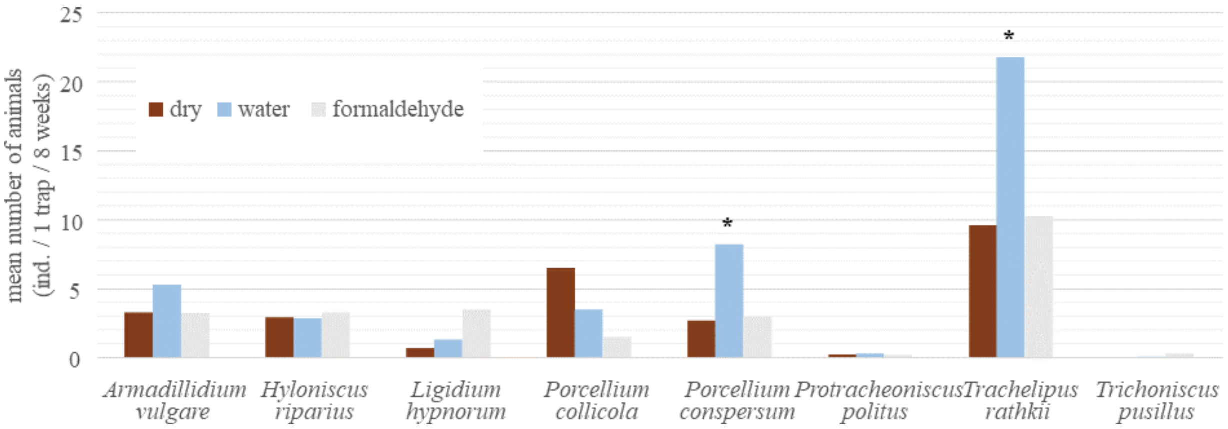

Figure 2. Mean numbers of trapped terrestrial isopods in traps differing in fixative solution during spring months in floodplain forest. Asterisk marks significantly different caught of particular species suggesting relative attractivity of marked solution in comparison with other treatments.

in species abundances (*pseudo-F* = 4.8, *p* = 0.002). The last valuable environmental factor for RDA was mean temperature ("*t* mean", explains 0.18%, *pseudo-F* = 3.1, *p* = 0.028).

Fitted generalized linear models (GLM) revealed a significant positive effect between temperature two days before trap inspection and the number of trapped animals of all terrestrial isopod species, except two less abundant species (Table 1, Fig. 4).

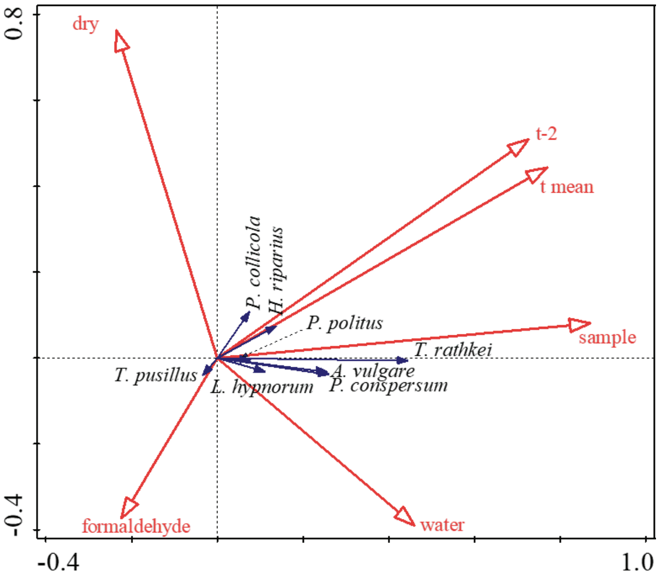

Figure 3. RDA biplot illustrating the effect of environmental factors (red) on trapping probability of terrestrial isopods. Only significant factors are illustrated: "*t mean*" – mean temperature three days before trap inspection, *t-2* – mean temperature during the second day before trap inspection, "*sample*" – order of trap inspection, "*formaldehyde/water/dry*" – types of fixative solution in pitfall traps.

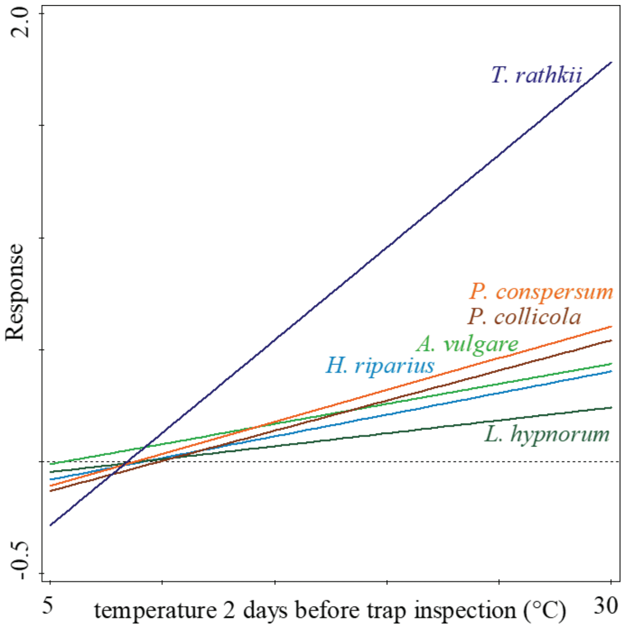

Figure 4. Generalised linear model for the effect of daytime mean temperature two days before trap inspection on terrestrial isopods´ probability to be trapped. Only species with significant predictions are illustrated (see Table 1).

Table 1. Summary of fitted Generalised Linear Model of mean day temperature two days before trap inspection predicting number of trapped terrestrial isopods. R-squared represents the proportion of the deviance explained by the model, F value is a value on the F distribution (power of result) and the p-value compares the fitted model to a null model.

Response	R2[%]	F	p
Trachelipus rathkii (Brandt, 1833)	10.4	188.4	<0.00001
Hyloniscus riparius (C. Koch, 1381)	3.1	51.1	<0.00001
Porcellium conspersum (C. Koch, 1841)	2.7	45.3	<0.00001
Armadillidium vulgare (Latreille, 1804)	1.8	29.4	<0.00001
Ligidium hypnorum (Cuvier, 1792)	1.1	18.6	0.00002
Porcellium collicola (Verhoeff, 1907)	1.1	17.4	0.00003
Protracheoniscus politus (C. Koch, 1841)	0.2	3.8	0.05084
*Trichoniscus pusillus*Brandt, 1833	0.0	0.5	0.50501

Laboratory experiment

Porcellio scaber activity was highest between 23:00 and 7:00 (Fig. 5). Higher activity was recorded for *P. scaber* in the water-filled trap apparatus; this difference in overall "nearby trap" count was statistically significant ($\chi^2 = 198.88$, $p < 0.001$, Fig. 6). When walking near the trap (at a distance of 2 cm or less), approx-

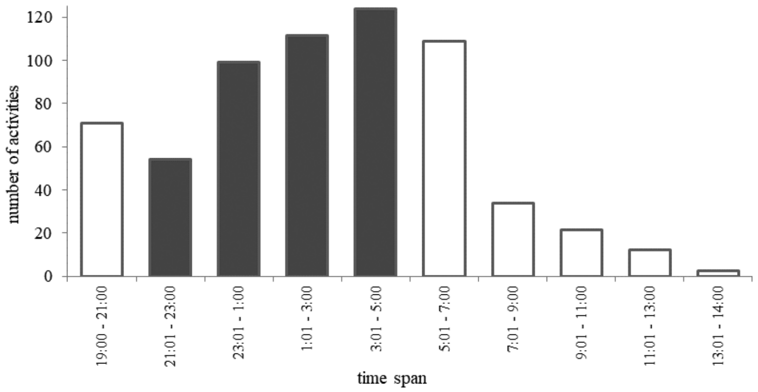

Figure 5. Pattern of activity of *Porcellio scaber* in experimental apparatuses with pitfall trap, expressed as the number of behavioural acts counted during particular two-hour intervals. Dark columns correspond to night hours.

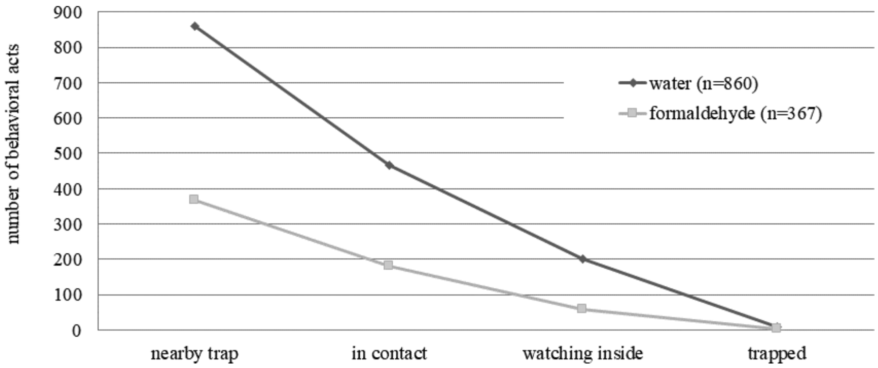

Figure 6. Behavioural acts of *Porcellio scaber* counted in experimental apparatuses with pitfall traps filled with water and formaldehyde, respectively. For explanation of categories see Material and methods.

imately half of the behavioural acts represented "contact" with the trap (54% for the water-filled trap and 50% for the formaldehyde-filled trap, cf., Fig. 6) the difference between the formaldehyde and water trap also being significant in this case ($\chi^2 = 124.47$, $p < 0.001$, Fig. 6). Only 23% and 16% of isopods being "nearby trap" (the water- and formaldehyde-filled traps, respectively) were "watching inside" the trap, i.e., inserting more than half of the body beyond the trap edge. This difference was also statistically significant ($\chi^2 = 198.88$, $p < 0.001$, Fig. 6). Regardless of the type of the fixative solution, falling into the trap was very rare, with only a few "trapped" individuals, and there was no difference between the two groups ($\chi^2 = 1.33$, $p = 0.85$). The "Rate of Self-Rescue" of *P. scaber* in the water trap apparatus was 67% (only 8 "trapped" individuals out of 24 that overstepped more than half of their body behind the edge of the trap), while in the formaldehyde trap, the rate was 78% (4 individuals of 18).

Discussion

Numerous modifications of pitfall traps used by field ecologists make it impossible to easily compare data between different studies. In this study, we tested the effect of formaldehyde and plain water on the probability of trapping terrestrial isopods, using the dry pitfall trap results as a baseline probability of capture. The dry traps were used as the baseline because we assume they do not contain anything that could attract the animals. There still could be one factor in play, the tendency of woodlice to actively avoid falling into the trap (see laboratory experiment below), but this would affect all the pitfall traps regardless of the fixative solution used.

We believe that water traps were more attractive to terrestrial isopods, as they need to visit shelters with high atmospheric humidity to compensate for their own loss of water (Wright and Machin 1990). Studies have shown that the most preferred level of humidity for terrestrial isopods is between 77 and 94% (Snyder 1959). Woodlice, in general, are hygrophilous (Hornung 2011), and *T. rathkii* and *P. conspersum* have similarly low desiccation resistance (around 25 hours) (Dias et al. 2012) and, because of that, have a similar need to seek humidity. We found these two dominant species significantly more likely to be captured in water traps. We also assume that the ambient humidity under the metal hoods above the water traps was higher, although we did not measure this factor.

Predation may have also influenced the results for traps without killing fixative solutions (Bouget 2001). Despite frequent inspections of traps and lining their bottom with wood shavings, remains of body parts of smaller species (not only woodlice) were found. We sought to minimize this effect by collecting the trapped animals every three to four days. It seems that the random chance of capture by dry traps was somewhat underestimated.

Formaldehyde, as the fixative solution used, acts as an attractant for ground beetles (Luff 1975) but as a repellent to the harvestmen (Pekár 2002), where a decrease in the number of individuals caught can be observed with the increasing concentration of formaldehyde solution. On the contrary, spiders were not significantly affected by the changing concentration (Pekár 2002). Although Gerlach et al. (2009), among others, did not observe any attraction or repulsion effect of water for ground-dwelling arthropods under laboratory conditions, significantly more terrestrial isopods were caught in water traps in this study. Nevertheless, the number of trapped isopods in formaldehyde traps was similar to the number of isopods in dry traps, which gave random (i.e., not affected by attraction nor repulsion effect) trapping probability value.

In our laboratory experiment, formaldehyde was confirmed to have a repellent effect on *P. scaber*. The specimens near the formaldehyde trap were less active and had a higher “Rate of Self-Rescue” compared to the traps filled with water. Similar results were found by Gerlach et al. (2009) for *Armadillidium opacum* (Koch, 1844) and *Oniscus asellus* Linnaeus, 1758.

In addition to the effect of the fixative solution, the effect of average daily temperature between trap inspections on the trap catch size was also observed. The catch size in pitfall traps increased with increasing temperature. Although high temperatures were observed to decrease the activity of woodlice in a laboratory setting (Ďurajková et al. 2022), the temperature in the forest was much lower than in a laboratory and its increase may have led to an increased woodlice activity. A similar relationship between soil temperature (and humidity) and the activity of terrestrial isopods during spring was observed by Hornung et al. (2015). Since formaldehyde is volatile, it likely evaporated more as the temperature increased during the time of our study, which may have altered the strength of the fixative solution effect. At the same time, ground-dwelling species bound to moist environments may have searched for water as the temperature increased and thus fell into the pitfall traps with clear water more often. Rendoš (2012) found that terrestrial isopods significantly avoided formaldehyde traps even in subterranean environments where sufficient moisture is expected throughout the year.

It does not seem necessary to change a proven fixative solution (formaldehyde) in further ecological field studies, apart from its toxicity. It can be assumed that its use in traps will continue to provide valuable results. However, it should be kept in mind that the data obtained by this method may be skewed, especially when studying a community structure and dominant species in an area – some species are more sensitive to formaldehyde and may actively avoid it. Obtained community characteristics are comparable with another similarly skewed, but such comparison is based on unmonitored changes in the abundances of sensitive species.

Acknowledgements

We would like to thank the students of *Ecological practices* course (academic year 2022/23) for their help with installation and inspections of the pitfall traps. This study was partly supported by an internal grant of the Faculty of Science of Palacký University Olomouc (IGA_PrF_2022_013 and IGA_PrF_2024_014). We are grateful to three anonymous reviewers for their valuable comments and suggestions, highly improving the quality of this text.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

Funding

This work was supported by Internal Grant Agency of the Faculty of Science of Palacký University Olomouc.

Author contributions

Conceptualization: IHT. Data curation: LN, PF. Formal analysis: PF, IHT. Funding acquisition: IHT. Investigation: LN, PF. Methodology: IHT, PF. Supervision: IHT. Writing - original draft: LN, PF. Writing - review and editing: IHT.

Author ORCIDs

Ivan Hadrián Tuf https://orcid.org/0000-0003-0250-0482

Data availability

All of the data that support the findings of this study are available in the main text.

References

Adis J (1979) Problems of interpreting arthropod sampling with pitfall traps. Zoologischer Anzeiger 202: 177–184.

Baini F, Del Vecchio M, Vizzari L, Zapparoli M (2016) Can the efficiency of pitfall traps in collecting vary according to the used mixtures as bait? Rendiconti Lincei 27: 495–499. https://doi.org/10.1007/s12210-016-0504-z

Barber HS (1931) Traps for cave-inhabiting insects. Journal of the Elisha Mitchell Scientific Society 46: 259–266.

Bater JE (1996) Micro- and macro-arthropods. In: Hall GS (Ed.) Methods for the Examination of Organismal Diversity in Soils and Sediments. CAB International, Oxford, 163–174.

Bouget C (2001) Echantillonnage des communautés de Coléoptères Carabiques en milieu forestier. Relation espèces – milieu et variations d'efficacité du piège à fosse. Symbioses 4: 55–64.

Dias ATC, Krab EJ, Mariën J, Zimmer M, Cornelissen JHC, Ellers J, Wardle DA, Berg MP (2012) Traits underpinning desiccation resistance explain distribution patterns of terrestrial isopods. Oecologia 172: 667–677. https://doi.org/10.1007/s00442-012-2541-3

Ďurajková B, Hladký R, Tuf IH (2022) Higher temperature and substrate vibrations as stress factors for terrestrial isopods – model species matter. ZooKeys 1101: 71–85. https://doi.org/10.3897/zookeys.1101.77549

Filgueiras BK, Liberal CN, Aguiar CM, Hernández MI, Iannuzzi L (2009) Attractivity of omnivore, carnivore and herbivore mammalian dung to Scarabaeinae (Coleoptera, Scarabaeidae) in a tropical Atlantic rainforest remnant. Revista Brasileira De Entomologia 53: 422–427.https://doi.org/10.1590/S0085-56262009000300017

Gatty C, Grández R (2020) Efectividad de cebos en la captura de escarabajos saprófagos (Insecta: Coleoptera) en Allpahuayo Mishana, Amazonía peruana. Ciencia Amazónica (Iquitos) 8: 71–84. https://doi.org/10.22386/ca.v8i1.281

Gerlach A, Voigtländer K, Heidger CM (2009) Behavioural response of selected epigeic arthropods on pitfall traps (Diplopoda, Chilopoda, Oniscidea, Carabidae, Staphylinidae). In: Tajovský K, Schlaghamerský J, Pižl V (Eds) Contributions to Soil Zoology in Central Europe III. Institute of Soil Biology, Biology Centre, ASCR, v.v.i., České Budějovice, 41–46.

Hariyama T, Meyer-Rochow VB, Kawauchi T, Takaku Y, Tsukahara Y (2001) Diurnal changes in retinula cell sensitivities and receptive fields (two-dimensional angular sensitivity functions) in the apposition eyes of *Ligia exotica* (Crustacea, Isopoda). Journal of Experimental Biology 204: 239–248. https://doi.org/10.1242/jeb.204.2.239

Hatten TD, Bosque-Pérez NA, Labonte JR, Guy SO, Eigenbrode SD (2007) Effects of tillage on the activity density and biological diversity of carabid beetles in spring and winter crops. Environmental Entomology 36: 356–368. https://doi.org/10.1093/ee/36.2.356

Hohbein R, Conway CJ (2018) Pitfall traps: A review of methods for estimating arthropod abundance. Wildlife Society Bulletin 42: 597–606. https://doi.org/10.1002/wsb.928

Hornung E (2011) Evolutionary adaptation of oniscidean isopods to terrestrial life: Structure, physiology and behavior. Terrestrial Arthropod Reviews 4: 95–130. https://doi.org/10.1163/187498311X576262

Hornung E, Szlavecz, K Dombos M (2015) Demography of some non-native isopods (Crustacea, Isopoda,Oniscidea) in a Mid-Atlantic forest, USA. ZooKeys 515: 127–143. https://doi.org/10.3897/zookeys.515.9403

Knapp M (2007) Metoda zemních pastí. [Pitfall Trapping.] Master thesis. Faculty of Forestry and Environmental Sciences, Czech University of Life Sciences, Prague. Ms., 69 pp. [in Czech]

Knapp M, Růžička J (2012) The effect of pitfall trap construction and preservative on catch size, species richness and species composition of ground beetles (Coleoptera: Carabidae). European Journal of Entomology 109: 419–426.https://doi.org/10.14411/eje.2012.054

Knapp M, Baranovská E, Jakubec P (2016) Effects of bait presence and type of preservative solution on ground and carrion beetle samples collected by pitfall trapping. Environmental Entomology 45: 1022–1028.https://doi.org/10.1093/ee/nvw047

Luff ML (1975) Some features influencing the efficiency of pitfall traps. Oecologia 19: 345–357. https://doi.org/10.1007/BF00348110

Pekár S (2002) Differential effects of formaldehyde concentration and detergent on the catching efficacy of surface active arthropods by pitfall traps. Pedobiologia 46: 539–547.https://doi.org/10.1078/0031-4056-00158

Rendoš M (2012) Arthropod communities within the scree slope in NNR Sivec. Master thesis. Faculty of Science, Pavol Josef Šafárik University in Košice. Ms., 81 pp.

Santalla S, Salgado JM, Calvo L, Fernandez MMF (2002) Changes in the Carabidae community after a large fire in a *Pinus pinaster* stand. In: Trabaud L, Pradon R (Eds) Fire and Biological Processes. Backhuys Publishers, Leiden, The Netherlands, 215–231.

Silva PG, Vaz-de-Mello FZ, Di Mare RA (2012) Attractiveness of different bait to the Scarabaeinea (Coleoptera: Scarabaeidae) in forest fragments in extreme southern Brazil. Zoological Studies 51: 429–441.

Skuhravý V (1957) Metoda zemních pastí. [Pitfall Trapping.] Časopis Československé Společnosti Entomologické 54: 27–40. [in Czech]

Snyder GG (1959) Relative humidity and survival in isopods. Bios 30: 208–211.

Topping CJ, Sunderland KD (1992) Limitations to the use of pitfall traps in ecological studies exemplified by a study of spiders in a field of winter wheat. Journal of Applied Ecology 29: 485–491.https://doi.org/10.2307/2404516

Wright JC, Machin J (1990) Water vapor absorption in terrestrial isopods. Journal of Experimental Biology 154: 13–30. https://doi.org/10.1242/jeb.154.1.13

ZooKeys 1225: 127–139 (2025)
DOI: 10.3897/zookeys.1225.125030

Research Article

Spatial activity and sheltering behaviour of terrestrial isopods (Isopoda, Oniscidea): a field experiment

Romana Fialová[1], Ivan Hadrián Tuf[1]

[1] Department of Ecology and Environmental Sciences, Faculty of Science, Palacký University Olomouc, Olomouc, Czech Republic
Corresponding author: Ivan Hadrián Tuf (ivan.tuf@upol.cz)

Abstract

A field study of spatial activity and sheltering behaviour of terrestrial isopods was carried out using *Porcellio scaber* and *Armadillidium versicolor*, two model species of woodlice. Individuals of both species were colour-marked and released into an area with artificial shelters, and their behaviour was then observed for several days. Vagility of both species was found to be similar; their dispersal ability was measured to be at least 1.1 m/h. The number of animals recaptured declined over time as they left the field-experiment area. The provided shelters were found to be unevenly inhabited both in relation to their position and the time elapsed since the beginning of the experiment. Nocturnal activity (night and dawn) of both species was confirmed.

Key words: *Armadillidium versicolor*, circadian activity, locomotion, marking, *Porcellio scaber*

Academic editor: Karel Tajovsky
Received: 8 April 2024
Accepted: 17 December 2024
Published: 5 February 2025

ZooBank: https://zoobank.org/B6D140BB-4920-4FDE-B401-D0218437FAF5

Citation: Fialová R, Tuf IH (2025) Spatial activity and sheltering behaviour of terrestrial isopods (Isopoda, Oniscidea): a field experiment. In: Tuf IH, Tajovský K, Taiti S (Eds) The Biology of Terrestrial Isopods, XII. ZooKeys 1225: 127–139. https://doi.org/10.3897/zookeys.1225.125030

Introduction

Hornung (2011) and Tuf and Ďurajková (2022) comprehensively reviewed isopod adaptations contributing to colonisation of land. As water balance has great importance to woodlice, their physiological, morphological, and behavioural adaptive strategies have been extensively studied. Behavioural reactions are thought to contribute to the range of adaptations, both equally to morphological and physiological. Terrestrial isopods have developed various behavioural strategies to maintain water balance, such as pattern of activity, sheltering, and aggregation.

The avoidance of unfavourable environmental factors during the daytime via nocturnal activity was described by Cloudsley-Thompson (1952, 1956). It was suggested that conditions are more suitable at night due to lower temperatures, and, thereby, higher relative air humidity is favourable. The intensity of nocturnal activity is negatively correlated with the terrestriality of species, those better adapted to terrestrial conditions. *Armadillidium vulgare* (Latreille, 1804) was found to be less active at night, with 60% of individuals nocturnally active, whereas *Philoscia muscorum* (Scopoli, 1763), which is thought to be less adapted, had 92% of individuals nocturnally active. During the daylight period, the isopods are expected to stay hidden in shelters to avoid desiccation.

Horizontal as well as vertical movements (Brereton 1957; Den Boer 1961; Tuf and Weissová 2022) enable finding suitable microhabitats. Behavioural responses to the environment, such as phototaxis, thigmokinesis, or hygrokinesis, are thought to stimulate movement. Photonegative orientation is associated with searching for dark habitats (shelters), which provide higher humidity and lower temperatures in comparison to the surrounding environment (Cloudsley-Thompson 1977).

Thigmokinetic reactions were linked with sheltering and aggregative behaviour by Allee (1926) and Broly et al. (2012). Thigmokinesis was characterised by Friedlander (1964) as a cessation of movement in response to contact stimuli. The fact that thigmokinetic reactions vary by individual was pointed out by Friedlander (1964) and Warburg (1968). This behaviour is supposed to strengthen or weaken spatial movement in response to environmental conditions.

Sheltering

Hiding in shelters during the day to avoid predators or unfavourable environmental conditions such as low humidity has been extensively studied (Hornung and Warburg 1996; Dias et al. 2012), and a trade-off between time spent by foraging and sheltering was described by Dias et al. (2012). Woodlice species spend more time sheltering at a lower relative humidity (Dias et al. 2012; Dixie et al. 2015; Leclercq-Dransart et al. 2019; Delhoumi et al. 2023). There were also differences found in their main period of the year when they shelter. *Armadillidium vulgare* preferred spring (March and April), whereas in *P. scaber* sheltering peaked during a pre-breeding period (May and June). Dangerfield and Hassall (1994) observed that males were more active and used artificial shelters less frequently than females, which possibly maximized foraging time and increased mating chance in May and June. Females were more often observed in shelters during the breeding season (June and July). For females, the shelters likely offered protection against desiccation and predation, and the higher average temperatures enabled shorter brood development time.

Kuenen and Nooteboom (1963) studied how three species, *Oniscus asellus* Linnaeus, 1758, *P. scaber*, and *A. vulgare*, react to external stimuli when searching for shelters at the end of their nocturnal activity. These authors suggested that olfactory signals influenced the individuals in addition to thigmotaxis and negative phototaxis, and they hypothesised that olfactory aggregation signals were supported by the presence of pheromones in faecal pellets, as known in several woodlouse species, including *P. scaber* and *A. vulgare* (Takeda 1980; Ebisuno et al. 1982).

Aggregations

Aggregation, which usually occurs in shelters, enables animals to significantly reduce water loss. Increased aggregation was observed during dry conditions, and aggregated animals were found to evaporate 50% less water (Warburg 1968). Friedlander (1965) suggested that the aggregation stimuli could be influenced by contact (thigmokinesis) combined with special movements or a chemical signal. This social aspect of aggregation, probably related to pheromones, was studied in detail by Broly et al. (2012) who encountered a maximum aggregate of 70 woodlice. Despite the number and density of individuals of *P. scaber* in this experiment, the location of aggregates was determined by individual preferences. The dynamics of aggregation and collective choice were found to be controlled by social interaction between conspecifics.

Direct connections between water balance, temperature, light, and other factors have been examined through the lens of various behaviours, such as circadian activity (Cloudsley-Thompson 1956), sheltering behaviour (Den Boer 1961), aggregation (Allee 1926; Friedlander 1964; Broly et al. 2012), and movement activity (including phototaxes, thigmokinesis, hygrokonesis; Warburg 1993). On the other hand, Cloudsley-Thompson (1977) recommended a holistic approach, because various factors might be unknown, interacting in unexpected ways, and individuals could respond to them in various ways (Cloudsley-Thompson and Constantinou 1987). Since spatial movements are considered a significant behavioural adaptation to survive in terrestrial environment, a field study to explore locomotion and sheltering activity of terrestrial isopods was carried out.

We aim to evaluate (1) vagility of isopods by measuring the distance of woodlice from the releasing point, (2) sheltering behaviour of aggregations under artificial shelters, and (3) activity patterns, or numbers of isopods outside the shelters. The behaviour of two model species was compared.

Materials and methods

Model species, experimental site and design

Field experiments were carried out in 2011. The main part of the experiment was the monitoring of sheltering and movement of 1,000 marked individuals in the field, where a grid of artificial shelters was arranged. A pilot study had been conducted at the beginning of June. The main experiment was then redesigned based on the findings of the pilot study and run twice, once in June 2011 (Experiment 1) and once in September 2011 (Experiment 2). As a model species, *Armadillidium versicolor* Stein, 1859 (Variegated Pill Isopod) and *Porcellio scaber* Latreille, 1804 (Common Rough Woodlice) were selected. Several comparative studies have examined abilities of woodlice species to survive in terrestrial conditions (Edney 1951; Schmidt and Wägele 2001). According to these studies, *A. vulgare*, which is considered comparable to *A. versicolor* (Csonka et al. 2013), was evaluated to be better adapted to the dry environment.

Both species were collected in Olomouc City (Czech Republic) and kept in captivity for a few days before the experiment in large plastic boxes with soil and leaves, at a natural temperature, and natural light regime. Acetone-based mother-bee marking polish was used to identify five different colour groups (Fig. 1). These colour markings were used for quick counting of the animals during night inspections to reduce disturbance. White and green colours were used for *P. scaber*, and red, yellow, and blue colours were used for *A. versicolor*. There were 200 individuals marked for each colour group. Marking was done according to the method described by Drahokoupilová and Tuf (2012), who recommended making small, but clearly visible dots on dorsal sheet of the first segment of the pereion (Fig. 2). These markings had no negative effect on survival or feeding behaviour (Kenne et al. 2019) and is recommended for short-time field studies of dispersal of terrestrial isopods. Manipulation with individuals was quick and gentle to minimize stress, and specimens were marked 48 h prior to release.

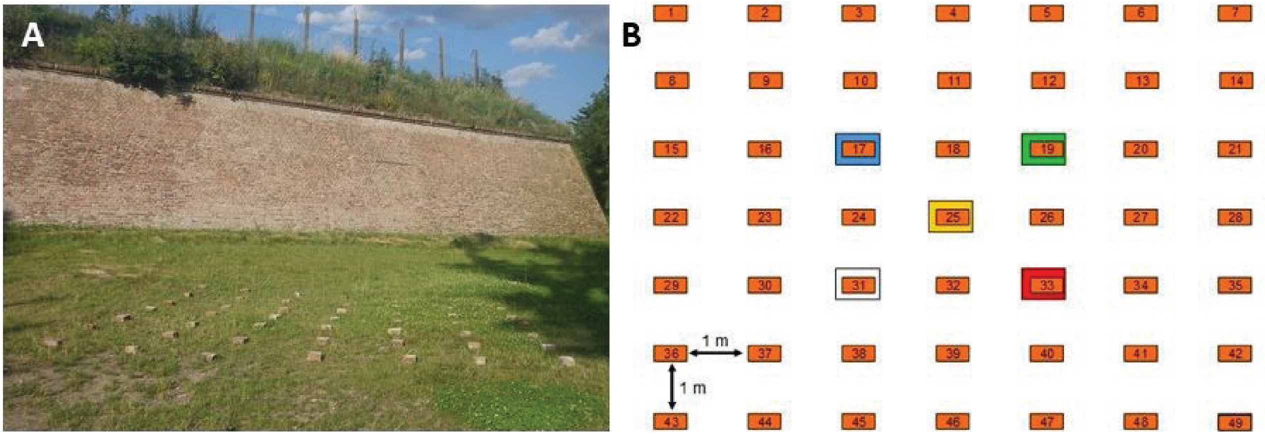

Figure 1. A grid of artificial brick shelters at experiment site nearby Korunní pevnůstka fortress (photograph RF) and **B** experimental design scheme with numbered orange bricks and releasing points of 200 coloured woodlice marked by larger boxes of relevant colours.

Figure 2. Marked individuals of **A** *Armadillidium versicolor* and **B** *Porcellio scaber* (photographs RF).

The field study took place in a 36 m^2 flat, square area in front of the Korunní Pevnůstka fortress in Olomouc, near a large park (49.5919°N, 17.2585°E). The study area consisted of a small grassy area with a regularly mowed lawn in front of the brick walls of the fortress. The site was selected as an optimal place for various terrestrial isopods, including *A. versicolor* and *P. scaber*, following Riedel et al. (2009), who described such a site as a natural habitat for various species of terrestrial isopods. The presence of bricks was also a consideration.

A grid of 49 shelters (7 × 7), each 1 m apart, was established to observe sheltering behaviour of 1,000 labelled animals. Bricks (29 × 15 × 6.5 cm) were used as shelters. Beneath each brick, a small space was dug out (15 × 10 × 1 cm) to avoid killing animals when manipulating the brick. Shelters had been put in place 10 days before the release of animals. Five shelters were used as release points when the experiment started. Each colour group (200 specimens) was released under one shelter (Fig. 1).

The study site was visited regularly three times each night: at dusk, in the middle of the night, and at dawn. In Experiment 1, isopods were monitored for three nights (June 24–27, 2011, checks at 1:00, 5:00, 21:00) and in Experiment 2 for seven nights (September 22–29, 2011, checks at 0:00, 7:00, 19:00). Each shelter was briefly opened (the brick was lifted), and the number and identity (colour group) of individuals present were recorded by a camera using weak, white illumination. Cole's comments (Cole 1946) on the counting procedure, that is, count animals on the ground surface and on the underside of the brick, do not count animals hidden in soil crevices, were following.

Data processing and analysis

Data sets from each monitoring check were processed according to the colour groups and species. The individual records were then matched with the distances achieved from the release point, distances achieved for each colour group, and then the distance for each species was calculated.

The Wilcoxon nonparametric paired test was used to compare vagility of two species and, therefore, two equal groups were needed. This meant comparing only 400 individuals of *A. versicolor* with the 400 individuals of *P. scaber*. The *Armadillidium* colour group selection was carried out according to the sum of the average distances (in meters) of two colour groups, the middle sum was chosen as a representative. Blue and red colour groups were selected by this method.

Firstly, it was necessary to count subtractions of mean achieved distances for each night ("dusk distance mean" minus "dawn distance mean"). The limit of 10 individuals was set to decrease random effects. These figures were counted for each species. Secondly, obtained figures were used for the Wilcox nonparametric paired test. Experiments 1 and 2 were tested separately. Prism 6 software by GraphPad Software Inc was used to compute the test.

The effect of activity pattern of woodlice was tested by evaluating the abundances in the three periods during the night (dusk, night, and dawn). Because time was expected to significantly affect the abundances, the generalized linear mixed model (GLMM) was used to justify effect of two fixed variables, such as time (quantitative: 1–3 in Experiment 1, 1–7 in Experiment 2) and day period (categorical: dusk, night, or dawn). It was set Poisson error distribution and logarithmic link function to model the effect of both predictors. While creating the model, we considered predictor time for replication because of the repeated measurements. SAS v. 9.2 (SAS Institute Inc. 2007) was used for the statistical analysis.

To enable comparison, blue and red groups for *A. versicolor* were used to create graphs based on similar process, as described above for vagility comparison.

Results

Movement

In both experiments, shelters at distances of 1–5 m from the release point were inhabited by specimens of both species after 20 h. The maximum recorded distance was 5.56 m travelled by *P. scaber* in 5 h and the same distance was travelled by *A. versicolor* in 12 h in Experiment 2 (Table 1).

Vagility

There was no significant difference in vagility between the two species (Fig. 3). In Experiment 1, vagility of both species was similar ($\text{mean}_{\text{P.scaber}} = 0.54$ m, $\text{mean}_{\text{A.versicolor}} = 0.57$ m; $p_{\text{Exp1}} > 0.9999$). Non-significantly higher vagility of *A. versicolor* was observed in experiment 2 ($\text{mean}_{\text{P.scaber}} = 0.007$ m, $\text{mean}_{\text{A.versicolor}} = 0\ .5140$ m; $p_{\text{Exp2}} = 0.0938$).

Table 1. Maximum observed distance (m) measured from releasing point achieved by isopods during start of experiment.

Experiment 1			Experiment 2		
Time	***P. scaber***	***A. versicolor***	**Time**	***P. scaber***	***A. versicolor***
4 h	4.12 m	4.47 m	5 h	5.56 m	3.16 m
20 h	4.47 m	4.47 m	12 h	5.56 m	5.56 m
24 h	5.56 m	4.47 m			
28 h	5.56 m	5.56 m			

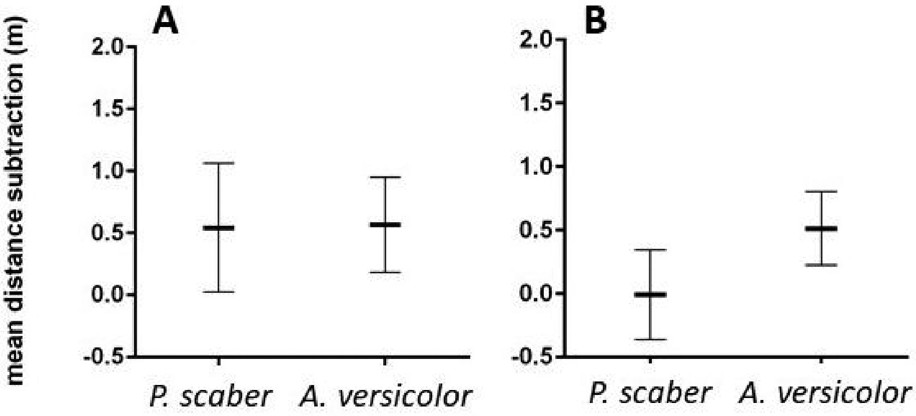

Figure 3. Species vagility comparison of results represented by mean distance subtractions (mean ± SE) during **A** Experiment 1 and **B** Experiment 2.

Sheltering pattern

Abundance of sheltering individuals differed in each experiment, as well between the species (Figs 4, 5). Individuals were more abundant in Experiment 1 (289 pillbugs and 179 woodlice recorded in the 1st and 2nd evening), compared to 111 and 102 individuals in Experiment 2. Individuals of *A. versicolor* were more frequently detected than *P. scaber* (Fig. 6).

Time (each night was considered to be a category of time) was confirmed to negatively affect the abundance of individuals observed under the shelters (Fig. 4) as the individuals of both species dispersed out of the experimental area (Experiment 1: $F_{P.scaber} = 333.93$, $p_{P.scaber} < 0.001$; $F_{A.versicolor} = 500.64$, $p_{A.versicolor} < 0.001$; Experiment 2: $F_{P.scaber} = 477.14$, $p_{P.scaber} < 0.001$; $F_{A.versicolor} = 411.49$, $p_{A.versicolor} < 0.001$). In Experiment 1, 1,000 individuals were released and 198 individuals were observed 24 h later; in Experiment 2 only 111 remained after the same time. The small number of marked individuals found (25 and 28 in Experiments 1 and 2, respectively) limited the duration of the experiment.

Significant differences in abundances between the day periods (dusk, night, and dawn) were found (Experiment 1: $F_{P.scaber} = 77.27$, $p_{P.scaber} < 0.001$; $F_{A.versicolor} = 128.54$, $p_{A.versicolor} < 0.001$; Experiment 2: $F_{P.scaber} = 195.35$, $p_{P.scaber} < 0.001$; $F_{A.versicolor} = 240.80$, $p_{A.versicolor} < 0.001$). In comparing abundances according to day period, the results between experiments differed (Fig. 5). The results also varied between species in Experiment 1. Dusk was found to be the main sheltering period for *A. versicolor* in Experiment 1 and for both species in Experiment 2. Night was least popular for sheltering for both species in Experiment 2.

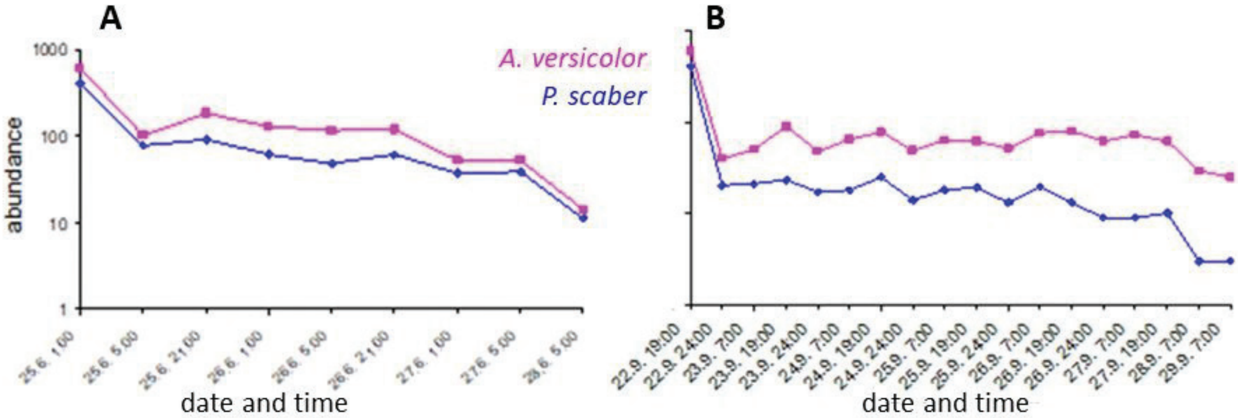

Figure 4. Number of re-detected individuals according to monitoring time controls (note the logarithmic scale of the Y-axis) in **A** Experiment 1 and **B** Experiment 2.

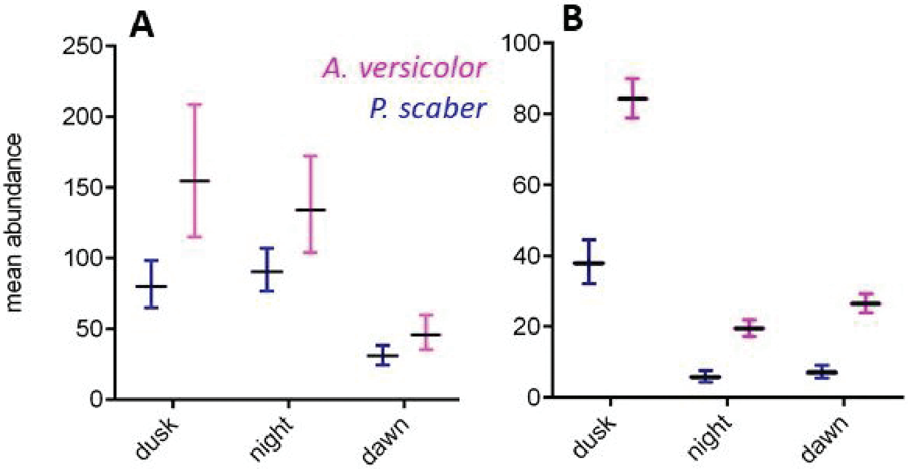

Figure 5. Influence of day period (dusk, night, and dawn) on number of woodlice (mean ± SE) in shelters during **A** Experiment 1 and **B** Experiment 2.

This contrasted with the behaviour of *P. scaber* in Experiment 1, where night was most popular for sheltering. Dawn was the least frequent sheltering period in Experiment 1 for both species.

Shelters were not inhabited equally, and their use varied in both time and in aggregation size. Some shelters were never inhabited (Experiment 1: bricks 11, 21, 36; Experiment 2: bricks 6, 45, 47), whereas individuals of all five colour groups were observed at the same time under some shelters (Experiment 1: bricks 6, 16, 20, 27, 36; Experiment 2: bricks 15, 24).

The sum of individuals under each shelter (aggregation size) varied among shelters and according to time. The aggregation size was categorised according to the sum of individuals (categories: 0, 1, 2–5, 6–10, 11–25, 26 and more; Fig. 7). The frequency of observing empty shelters increased as time progressed. Aggregations of 2–5 individuals were more frequent than one individual, mainly in Experiment 1. Aggregations of 6–10 individuals were observed in almost every check, except the last one. The very large aggregations of 11–25 individuals were found under some shelters (Experiment 1: bricks 12, 19, 20, 25, 31; Experiment 2: bricks 10, 19, 24).

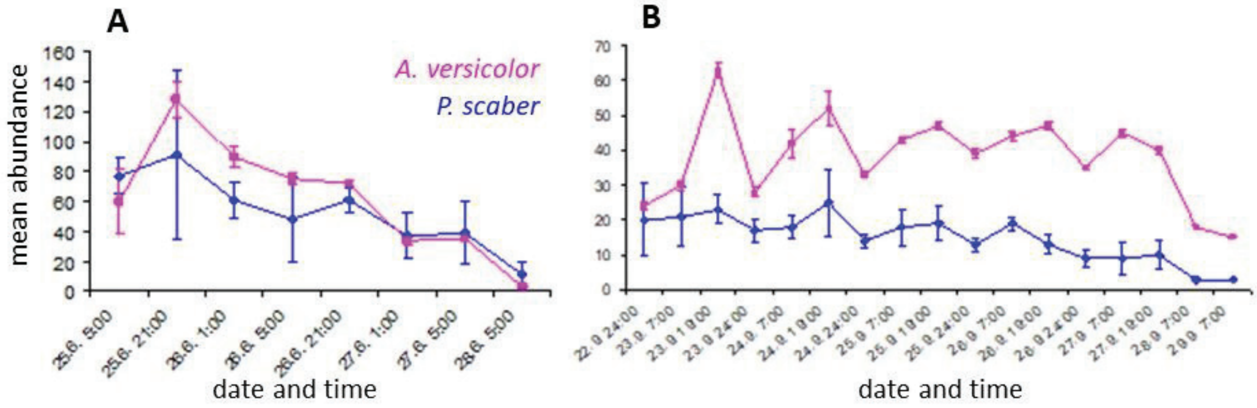

Figure 6. Comparison of species abundance (mean ± SE) based on number of individuals aggregated under one brick in **A** Experiment 1 and **B** Experiment 2.

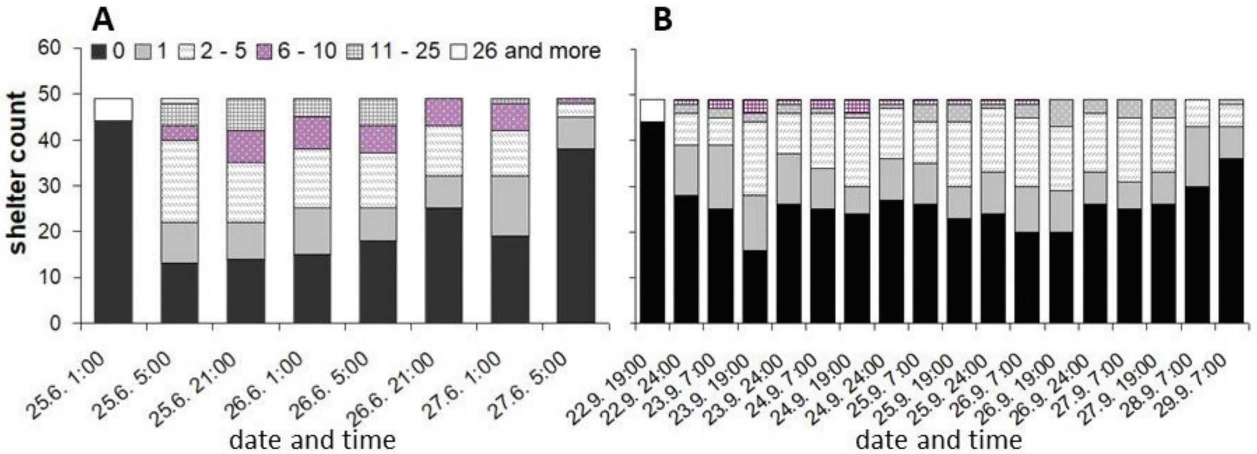

Figure 7. Frequency of aggregation of different size categories (according to sum of individuals of both woodlouse species aggregated) observed under each shelter in **A** Experiment 1 and **B** Experiment 2.

Discussion

We studied the movement and sheltering behaviour of *Porcellio scaber* and *Armadillidium versicolor* in a field experiment. Changes in distribution of marked individuals in artificial shelters were monitored during three nights in June and eight nights in September.

Dispersal activity

Brereton (1957) noted that *P. scaber* was not nomadic and the same individuals were found on the same tree for a long time. On the other hand, he also recorded some immigrants in his study. Paris and Pitelka (1962) stated that the population of *A. vulgare* was very mobile; they released 243 marked animals but only eight animals (3.3%) were recaptured 24 hours later within a circular area (12.5 m^2) with radius of 2 m. In our study, the recapture rates were higher; of the 1,000 individuals released in an area of 36 m^2, 19.8% were observed 24 h later in Experiment 1 and 11.1% in Experiment 2 . However, our study is not entirely comparable because the isopods were sampled only under artificial shelters. In another study, Paris (1965) labelled 1,000 *A. vulgare* by using the P^{32} isotope and released them in a circular grid of artificial shelters. He detected them at a distance of 1 m within 4 min and 3 m within 50 min. The maximum dispersal rate was 13 m in 12 h and maximum distance of 25 m was observed on day 20; greater dispersal rates were observed during a dry season. Our observed distances of 4.4 m/4 h (*A. versicolor*) and 5.6 m/5 h (*P. scaber*) were consistent with Paris's (1965) field experiment.

Other factors influencing natural behaviour, such as colour marking, should be also considered, as they might negatively affect woodlice activity (Drahokoupilová and Tuf 2012; Kenne et al. 2019). Translocation and handling might also negatively influence movement behaviour. Heidinger et al. (2009) found that animals dispersed over longer distances in the first day after release and tended to move further after release in general. Therefore, it is highly probable that woodlice do not usually move as far as observed in our study unless disturbed.

Vagility

We found no significant differences in vagility between the two species, although we observed slightly a higher vagility rate for *A. versicolor* in Experiment 2. These results do not support the hypothesis by Dailey et al. (2009), who suggested that *A. vulgare*, as a conglobating isopod (Schmalfuss 1984), relies on passive defence, whereas *P. laevis* uses active escape strategies for avoiding harsh conditions and predators. The hypothesis of higher vagility in *P. scaber* might be consistent with its lower abundance in our experiments, as compared to *A. versicolor*. In our experiments a greater number of *P. scaber* individuals had run away from the experiment area and, thus, they were not recorded. It is possible that a decline in the abundance of *P. scaber*, probably caused by migration away from the site, is indirect evidence of the higher vagility of this species, which was underestimated in the design of the study area's of size.

Sheltering behaviour

Abundances of shelter inhabitants were uneven, similar to the other studies (Davis and Sutton 1977; Hornung and Warburg 1996). Den Boer (1961) suggested that good hiding sites were occupied by many individuals every night because of better conditions in the shelters and due to active searching behaviour of woodlice for exactly such conditions. Hornung and Warburg (1996) proposed that moisture conditions in the shelters can be important in the uneven distribution. This explanation is not supported by recent studies, where the presence of conspecifics is much more important for sheltering of *P. scaber* than the quality of shelter alone (Devigne et al. 2011; Broly et al. 2012; Zidar and Fišer 2022). This explanation is consistent with olfactory effects of an aggregation pheromone (Kuenen and Nooteboom 1963; Takeda 1980; Ebisuno et al. 1982). Moreover, the aggregation pheromone attracted individuals of woodlice of the same species as well as other terrestrial isopods. This likely means that the empty shelters did not offer attractive conditions and were observed more often than the shelters with at least one individual. The individuals tend to join others in already inhabited hiding sites (Hassall and Tuck 2007) in accordance with the hypothesis that the risk of poor conditions at the site where many other conspecifics have already aggregated is lower. Therefore, the shelter that offered suitable conditions and had been found was more likely to be the one used throughout the whole monitoring period.

Broly et al. (2012) discovered the social aspect of aggregation behaviour of *P. scaber*, noting 60–70 individuals per aggregation as the maximum. The number of individuals found in shelters in our field study was usually much lower, but we counted isopods only during the night when locomotory activity outside the shelters usually occurs.

Temporal pattern of sheltering behaviour

Our woodlice were outside shelters mainly during the night and dawn in Experiment 2, which is in accordance with other studies on *P. scaber* (Den Boer 1961), *A. vulgare* (Cloudsley-Thompson 1952, 1956; Paris 1963; Refinetti 2009), and *A. versicolor* (Tuf and Weissová 2022). Temperature and humidity were not recorded during our study, but it is expected that, by dawn, the humidity of the air increased as the temperature dropped; these are conditions favourable for woodlice foraging. The results of Experiment 1 do not fit the described pattern of night activity, as the experiment was short and, therefore, more affected by the high woodlice activity after their release (Heidinger et al. 2009).

The results of this study suggest that terrestrial isopods can use artificial shelters in natural conditions at least for short research periods. Over the course of several nights, the number of hiding woodlice was observed, and this pattern may be due to increased locomotor activity outside of the shelters, as well as to the animals moving away from the experiment area or using natural shelters in the soil. Our study confirms the temporal activity patterns of woodlice, the abundance of individuals in the shelters, and the simultaneous use of shelters by the two species. For more detailed studies of shelter fidelity and experiments with individual marking of animals will be necessary.

Acknowledgements

We are grateful to two anonymous reviewers for their in-depth comments, suggestions, and corrections, which have greatly improved the manuscript. We also thank Pavel Frycka for his great linguistic help.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

Funding

Publication of this study was partly supported by an internal grant of the Faculty of Science of Palacký University Olomouc (IGA_PrF_2024_014).

Author contributions

Romana Fialová made experiments and wrote the first version of manuscript as her M.Sc. thesis under supervision of Ivan H. Tuf

Author ORCIDs

Ivan Hadrián Tuf https://orcid.org/0000-0003-0250-0482

Data availability

All of the data that support the findings of this study are available in the main text.

References

Allee WC (1926) Studies in animal aggregations: Causes and effects of bunching in land isopods. Journal of Experimental Zoology 45: 255–277. https://doi.org/10.1002/jez.1400450108

Brereton J (1957) The distribution of woodland isopods. Oikos 8: 85–106. https://doi.org/10.2307/3564994

Broly P, Mullier R, Deneubourg JL, Devigne C (2012) Aggregation in woodlice: Social interaction and density effects. ZooKeys 176: 133–144. https://doi.org/10.3897/zookeys.176.2258

Cloudsley-Thompson JL (1952) Studies in diurnal rhythms II. Changes in the physiological responses of the woodlouse *Oniscus asellus* to environmental stimuli. Journal of Experimental Biology 29: 295–303. https://doi.org/10.1242/jeb.29.2.295

Cloudsley-Thompson JL (1956) Studies in diurnal rhythms VII. Humidity responses and nocturnal activity in woodlice (Isopoda). Journal of Experimental Biology 33: 576–582. https://doi.org/10.1242/jeb.33.3.576

Cloudsley-Thompson JL (1977) The Water and Temperature Relations of Woodlice. Meadowfield Press, Durham, 84 pp.

Cloudsley-Thompson JL, Constantinou C (1987) Humidity reactions and aggregation in woodlice (Isopoda, Oniscoidea). Crustaceana 53: 43–48. https://doi.org/10.1163/156854087X00600

Cole L (1946) A study of the cryptozoa of an Illinois woodland. Ecological Monographs 16: 49–86. https://doi.org/10.2307/1943574

Csonka D, Halasy K, Szabó P, Mrak P, Štrus J, Hornung E (2013) Eco-morphological studies on pleopodal lungs and cuticle in *Armadillidium* species (Crustacea, Isopoda, Oniscidea). Arthropod Structure & Development 42: 229–235. https://doi.org/10.1016/j.asd.2013.01.002

Dailey M, Claussen D, Ladd G, Buckner S (2009) The effects of temperature, desiccation, and body mass on the locomotion of the terrestrial isopod, *Porcellio laevis*. Comparative Biochemistry and Physiology Part A 153: 162–166. https://doi.org/10.1016/j.cbpa.2009.02.005

Dangerfield M, Hassall M (1994) Shelter site use and secondary sex ratios in the woodlice *Armadillidium vulgare* and *Porcellio scaber* (Crustacea: Isopoda). Journal of Zoology 233: 1–7. https://doi.org/10.1111/j.1469-7998.1994.tb05257.x

Davis RC, Sutton SL (1977) Spatial distribution and niche separation of woodlice and millipedes in a dune grassland ecosystem. In: Lohm U, Persson T (Eds) Soil Organisms as Component of Ecosystems. Ecological Bulletin (Stockholm) 25: 45–55.

Delhoumi M, Bouslama MF, Achouri MS (2023) Effects of moisture decrease on behavioural and life history traits in three species of terrestrial isopods. The European Zoological Journal 90: 832–839. https://doi.org/10.1080/24750263.2023.2273969

Den Boer PJ (1961) The ecological significance of activity patterns in the woodlouse *Porcellio scaber* Latr. (Isopoda). Archives Néerlandaises de Zoologie 14: 283–409. https://doi.org/10.1163/036551661X00070

Devigne C, Broly P, Deneubourg JL (2011) Individual preferences and social interactions determine the aggregation of woodlice. PLoS ONE 6: e17389. https://doi.org/10.1371/journal.pone.0017389

Dias N, Hassall M, Waite T (2012) The influence of microclimate on foraging and sheltering behaviours of terrestrial isopods: Implications for soil carbon dynamics under climate change. Pedobiologia 55: 137–144. https://doi.org/10.1016/j.pedobi.2011.10.003

Dixie B, White H, Hassall M (2015) Effects of microclimate on behavioural and life history traits of terrestrial isopods: implications for responses to climate change. ZooKeys 515: 145–157. https://doi.org/10.3897/zookeys.515.9399

Drahokoupilová T, Tuf IH (2012) The effect of external marking on the behaviour of the common pill woodlouse *Armadillidium vulgare*. ZooKeys 176: 145–154. https://doi.org/10.3897/zookeys.176.2375

Ebisuno T, Takimoto M, Takeda N (1982) Preliminary characterization of the aggregation pheromone in the sow bug, *Porcellionides pruinosus* (Brandt) (Isopoda: Oniscoidea). Applied Entomology and Zoology 17: 584–586. https://doi.org/10.1303/aez.17.584

Edney EB (1951) The evaporation of water from woodlice and the millipede *Glomeris*. Journal of Experimental Biology 28: 91–115. https://doi.org/10.1242/jeb.28.1.91

Friedlander CP (1964) Thigmokinesis in woodlice. Animal Behaviour 12: 164–174. https://doi.org/10.1016/0003-3472(64)90118-6

Friedlander CP (1965) Aggregation in *Oniscus asellus* Linn. Animal Behaviour 13: 342–346. https://doi.org/10.1016/0003-3472(65)90054-0

Hassall M, Tuck J (2007) Sheltering behavior of terrestrial isopods in grasslands. Invertebrate Biology 126: 46–56. https://doi.org/10.1111/j.1744-7410.2007.00075.x

Heidinger M, Poethke HJ, Bonte D, Hein S (2009) The effect of translocation on movement behaviour: a test of the assumptions of behavioural studies. Behavioural Processes 82: 12–17. https://doi.org/10.1016/j.beproc.2009.03.001

Hornung E (2011) Evolutionary adaptation of oniscidean isopods to terrestrial life: structure, physiology and behavior. Terrestrial Arthropod Reviews 4: 95–130. https://doi.org/10.1163/187498311X576262

Hornung E, Warburg M (1996) Intra-habitat distribution of terrestrial isopods. European Journal of Soil Biology 32: 179–185.

Kenne DC, Araujo PB, Soares GLG (2019) External marking of terrestrial isopods (Isopoda, Oniscidea): efficiency of materials and influence on feeding behavior. Nauplius 27: e2019018. https://doi.org/10.1590/2358-2936e2019018

Kuenen DJ, Nooteboom HP (1963) Olfactory orientation in some land-isopods (Oniscoidea, Crustacea). Entomologia Experimentalis et Applicata 6: 133–142. https://doi.org/10.1111/j.1570-7458.1963.tb00610.x

Leclercq-Dransart J, Pernin C, Demuynck S, Grumiaux F, Lemière S, Leprêtre A (2019) Isopod physiological and behavioral responses to drier conditions: an experiment with four species in the context of global warming. European Journal of Soil Biology 90: 22–30. https://doi.org/10.1016/j.ejsobi.2018.11.005

Paris O (1963) The ecology of *Armadillidium vulgare* (Isopoda: Oniscidea) in Californian grassland: Food, enemies, and weather. Ecological Monographs 33: 1–22. https://doi.org/10.2307/1948475

Paris O (1965) Vagility of P^{32}-labeled isopods in grassland. Ecology 46: 635–648. https://doi.org/10.2307/1935003

Paris O, Pitelka F (1962) Population characteristics of the terrestrial isopod *Armadillidium vulgare* in California grassland. Ecology 43: 229–248. https://doi.org/10.2307/1931979

Refinetti R (2009) Circadian rhythm of locomotor activity in the pill bug, *Armadillidium vulgare* (Isopoda). Crustaceana 73: 575–583. https://doi.org/10.1163/156854000504679

Riedel P, Navrátil M, Tuf IH, Tufová J (2009) Terrestrial isopods (Isopoda: Oniscidea) and millipedes (Diplopoda) of the city of Olomouc. In: Tajovský K, Schlaghamerský J, Pižl V (Eds) Contributions to Soil Zoology in Central Europe III. Institute of Soil Biology, Biology Centre, ASCR, v.v.i., České Budějovice, 125–132.

SAS Institute Inc. (2007) SAS/ STAT User's Guide. Cary (NC), SAS Institute Inc.

Schmalfuss H (1984) Eco-morphological strategies in terrestrial isopods. In: Sutton SL, Holdich D (Eds) The Biology of Terrestrial Isopods (Proceedings of the Zoological Society of London Symposium 53). Clarendon Press, Oxford, 49–63.

Schmidt CH, Wägele J (2001) Morphology and evolution of respiratory structures in the pleopod exopodites of terrestrial Isopoda (Crustacea, Isopoda, Oniscidea). Acta Zoologica (Stockholm) 82: 315–330. https://doi.org/10.1046/j.1463-6395.2001.00092.x

Takeda N (1980) The aggregation pheromone of some terrestrial isopod crustaceans. Experientia 36: 1296–1297. https://doi.org/10.1007/BF01969598

Tuf IH, Ďurajková B (2022) Antipredatory strategies of terrestrial isopods. ZooKeys 1101: 109–129. https://doi.org/10.3897/zookeys.1101.76266

Tuf IH, Weissová N (2022) Bug cemetery: a case study of terrestrial isopod distribution on a brick wall in the Czech Republic. ZooKeys 1101: 191–201. https://doi.org/10.3897/zookeys.1101.76132

Warburg M (1968) Behavioral adaptations of terrestrial isopods. American Zoologist 8: 545–559. https://doi.org/10.1093/icb/8.3.545

Warburg M (1993) Evolutionary Biology of Land Isopods. Springer-Verlag, Berlin, 159 pp. https://doi.org/10.1007/978-3-662-21889-1

Zidar P, Fišer Ž (2022) Avoidance behaviour toxicity tests should account for animal gregariousness: a case study on the terrestrial isopod *Porcellio scaber*. ZooKeys 1101: 87–108. https://doi.org/10.3897/zookeys.1101.76711

ZooKeys 1225: 141–153 (2025)
DOI: 10.3897/zookeys.1225.121574

Research Article

Predator responses to artificial aposematic and cryptic colouration in terrestrial isopods (Isopoda, Oniscidea)

Lenka Skočková[1], Barbora Ďurajková[1], Ivan Hadrián Tuf[1]

[1] *Department of Ecology and Environmental Sciences, Faculty of Science, Palacký University, Olomouc, Czech Republic*
Corresponding author: Ivan Hadrián Tuf (ivan.tuf@upol.cz)

Abstract

Aposematism is a distinctive or warning signal that provides the animal with protection against a potential predator. Aposematic colouration is easier for a predator to remember and to avoid a dangerous and/or unpalatable prey in the future. We investigated whether distinctive colouration has an aposematic function in terrestrial isopods. The common rough woodlice (*Porcellio scaber*) were used as a model species of terrestrial isopods and the Italian wall lizard (*Podarcis siculus*) as a predatory species. To imitate the distinctive colouration on isopods we marked their dorsal plates with yellow dots. The control group of the woodlice were marked with grey spots. Differences in behaviour (observation, manipulation and consummation) and the lizards' behaviour changes towards aposematically and cryptically coloured prey were analysed. Differences were found in prey observation both between sexes and between prey colours.

Key words: *Podarcis siculus*, *Porcellio scaber*, terrestrial isopods

Academic editor: Karel Tajovsky
Received: 23 February 2024
Accepted: 19 September 2024
Published: 5 February 2025

ZooBank: https://zoobank.org/7CE73C2E-7149-499B-AFF9-2A3BF6AB129D

Citation: Skočková L, Ďurajková B, Tuf IH (2025) Predator responses to artificial aposematic and cryptic colouration in terrestrial isopods (Isopoda, Oniscidea). In: Tuf IH, Tajovský K, Taiti S (Eds) The Biology of Terrestrial Isopods, XII. ZooKeys 1225: 141–153. https://doi.org/10.3897/zookeys.1225.121574

Introduction

To leave the sea and fully transition to a terrestrial way of life, terrestrial isopods had to develop many adaptations (Hornung 2011). Different ecomorphological types can be distinguished in this group (Schmalfuss 1984). The first type are the "clingers", which have strong, short pereopods and their body is broad and flat, so they move relatively slowly (genera *Trachelipus*, *Porcellio*). Another type is the "runners", which, on the other hand, move very fast because they have long pereopods and slender bodies (families Ligiidae, Philosciidae). The species that inhabit the soil belong frequently to the group of "creepers", they are smaller, have an elongated body and ribs on the dorsal surface. These species need high ambient humidity. The fourth group are the "rollers", which use conglobation, have a convex body and can roll up (families Armadillidae, Eubelidae). In tropical and subtropical areas, "spiny forms" of isopods are found. They have spikes or thorns on their bodies to protect them from predators and live outside the topsoil (genera *Panningillo*, *Echinodillo*).

Terrestrial isopods have a large number of natural predators. The main predators include centipedes, spiders, various insects, but also insectivorous vertebrates. Predation pressure has helped the isopods to develop various defence and protection mechanisms, whether it be morphological adaptations or behavioural changes (Tuf and Ďurajková 2022). Increased consumption by birds and reptiles is due to an increased need for calcium necessary for eggshell formation (Bureš 1986; Krištín 1992).

Colouration of terrestrial isopods

The body colouration of animals has three main purposes; these are thermoregulation, intraspecific communication, and reduction of predation risk. Body colour and colour pattern play a major role in intraspecific communication, e.g., mate recognition or courtship. Colouration is also important in relation to predation; the animal wants to avoid, deter, or confuse the predator (Endler 1978).

Crustacean chromatophores usually contain a variety of pigments. There are different forms of chromatophores (polychromatic, monochromatic, bichromatic) (Knowles and Carlisle 1956). The brown-black pigment in the melanophores of crabs is melanin, but the dark chromatophore pigments in other crustaceans are ommochromes (Fingerman 1965). The pigments in erythrophores and xanthophores are carotenoids, but there are some exceptions; the most common carotenoid in chromatophores is astaxanthin (Fingerman 1965). Pigments may be distributed freely in the exoskeleton or in chromatophores (Chang and Thiel 2015). Chromatophores are often clustered into larger groups, these multicellular clusters are called chromatosomes. Crustacean chromatophores are asymmetric and mononuclear (Chang and Thiel 2015).

The purpose of cryptic colouration in animals is to reduce the possible detection by predators. In many cases, this colouration can indicate that the animal is trying to blend in with its surroundings (Merilaita 1998). Within terrestrial isopods, such colouration is typical for epigeic species with dusk or daytime activity (e.g., *Porcellio scaber*, *Porcellionides pruinosus*, *Trachelipus rathkei*, *Hemilepistus reaumurii*). During the day, cryptic species usually hide in shelters in or on the soil surface (Davies et al. 2012). Some species are capable of colour-change or pattern transformation on the body. Colour-change, which is based on chromatophores (melanophores and leucophores), is triggered by sensory detection from the surrounding environment (Nery and de Lauro Castrucci 1997). The littoral isopod *Ligia oceanica* uses melanophores to change colour within a circadian rhythm (Hultgren and Mittelstaedt 2015).

In cryptic polymorphism, different colour forms occur within the same species (Veselý et al. 2024). This is because the predator has a certain image in memory (the search image) by which it searches for its prey (Punzalan et al. 2005). Thus, if a predator detects one morph, other individuals with a different pattern are in relative safety (Hughes and Mather 1986; Veselý et al. 2024). This principle protects individuals with rarer colouration, as the predator mainly targets the more numerous colouration pattern of prey. Thus, polymorphism is maintained in the population by predation, as the preference for a particular pattern is determined by the current commonness or rarity of the colouration. However, this principle does not apply to morphs with a high degree of divergence in conspicuousness. Albinos are always conspicuous, even if they are rarer. Within crustaceans, albinism is a relatively rare phenomenon (Geiser 1932). In the isopods, this phenomenon is common in species that live in caves or deeper in the soil. In some species, albinos are also rarely found in natural conditions, but they are regulated by a higher level of predation (Achauri 2009).

Aposematism is a conspicuous warning colouration or other type of warning signal by which an individual alerts potential predator to its (real or perceived) inedibility or toxicity. There are colour combinations that are typical: black or dark brown in combination with yellow, red or orange, or sometimes even white. Stripes or spots on the bodies of aposematically coloured individuals are also common (Davies et al. 2012). Aposematic colouration uses colour contrast, where we can observe as differences in shades or saturations of colour between a given organism and the environment in which it lives. It can also make use of luminance contrast, where the amount of light reflected from the organism and its surroundings varies. Colour contrast is considered important for the effectiveness of aposematism, especially in the case of avian predators and diurnal lizards (e.g., Lacertidae), which have tetrachromatic vision. Conversely, for colour-blind predators, luminance contrast is an important factor that enables them to detect aposematic prey (Prudic et al. 2007). Aposematic colouration is very conspicuous to predators, they can easily spot and recognise it, but also remember it very easily (Prudic et al. 2007). Predators avoid such distinctive individuals, either through innate neophobia (individual avoids things it does not already know) or learned avoidance of a particular pattern or colour (Vickers et al. 2021).

The light spots on the bodies of woodlice are typical for some species. They are often found on species that are active on vertical surfaces, such as rocks or tree trunks, and are typical of the morphotype “clingers” (*Oniscus asellus*, *Porcellio spinicornis*), but also “rollers” (*Armadillidium pictum*, *A. opacum*). Vividly coloured are also the vegetation-dwelling “spiny forms” (e.g. *Pseudolaureola atlantica*).

One theory of the origin of aposematic colouration is that the cryptically coloured toxic species was accidentally consumed by predators who mistook it for a harmless species. They preferentially avoided the more conspicuous specimens that they could readily identify. Through this selection, the conspicuous pattern gradually dominated the prey population (Davies et al. 2012). This theory appears to be applicable to Mediterranean terrestrial isopods, where a similar colour pattern also occurs in unrelated species. The shared colour pattern in syntopic species of millipede, pillbug, and spider was pointed out by Levi (1965) as Müllerian mimicry. If several species have the same predators, therefore the more similar species there are, the more likely the predator will learn to recognize them and not attack them (Davies et al. 2012).

Evidence that distinctive colouration has an aposematic function in terrestrial isopods is still lacking (Tuf and Ďurajková 2022). Yellow and white patches are relatively common in the genera *Armadillidium* and *Porcellio*, but a pattern of a combination of black (dark purple-brown) and white has been observed in related species in West Africa (Schmalfuss and Ferrara 1982) too.

Cognitive abilities of vertebrate predators

The cognition encompasses a set of mental processes that include perception, learning, long-term memory, working memory, attention and, last but not least, decision making (Dukas 2004; Shettleworth 2010). By learning and then remembering aposematic prey, predators can avoid it in the future (Shettleworth 2001).

Birds and diurnal lizards are primarily visual creatures. Both groups have tetrachromatic vision, with four types of cones (Chen and Goldsmith 1986; Pérez i de Lanuza and Font 2016). Taste receptors are relatively poorly developed in birds, with taste buds located at the root of the tongue, in the posterior palate, and in the pharyngeal mucosa (Bill 2007). Olfaction in reptiles is provided by the vomeronasal organ (via the forked tongue) and the olfactory mucosa in the paired nasal cavity (Vitt and Caldwell 2014).

The main aim of this work is to find out whether the colour of the woodlice has an aposematic meaning. We wanted to find out how reptilian predators would react to presented prey and whether colouration would play a role in prey selection. We also investigated whether predatory behaviour and foraging motivation would change in a model predator species over the course of the experiment. We tested the following hypotheses: 1) there is difference in lizards' behaviour towards aposematic and cryptic prey, 2) isopods with aposematic colouration are less consumed than isopods with cryptic colouration, 3) there is no difference in prey consumption between males and females of lizards, and 4) predatory behaviour and foraging motivation of lizards can change throughout the experiment.

Material and methods

Model species

The model prey species was the common rough woodlouse (*Porcellio scaber*). This species has a cosmopolitan distribution (Capinera 2001). It is coloured in shades of brown and grey but may show small patches of colour (black, red, orange, yellow) (Capinera 2001). A number of colour forms have been bred in hobby breeding. Birds, lizards, newts, spiders, beetles, centipedes, and shrews are considered to be the main predators of woodlice.

The collection of woodlice took place in the autumn of 2022 in Olomouc. They were subsequently kept in plastic boxes with lids, inside there was soil, leaves, and shelters (bark, stones), the substrate was kept moist in places, with a constant temperature (18–22 °C). Individuals of 8–10 mm in length were used in the experiments to make them attractive to the predators of interest.

The model reptile predator species was the Italian wall lizard (*Podarcis siculus*). Adults can reach lengths of up to 25 cm and weights of 15 g. The original distribution area is thought to be the Apennine Peninsula (Rivera et al. 2011), but it is now also widespread in the Iberian Peninsula and North Africa. This species is popular among breeders, and the lizards are fed mainly by mealworms and crickets.

Experiments were conducted with four young immature lizards (2 males and 2 females). These lizards were naïve and kept in captivity from their birth. Animals were housed in a terrarium (120 cm × 50 cm) with an 8:16 light regime at the time of the experiment, and the ambient room temperature, but the terrarium also contained a heating pad. Inside the terrarium was a sandy lignocel mixture as a substrate, a water bowl, and bark pieces as a shelter. Before the start of the experiment, the animals were fed by crickets. During the experiment, the lizards were fed only during individual trials to see if their feeding behaviour would change.

Experiment

The experiments were conducted from 24 October to 30 November 2022 according to standard procedures (Exnerová et al. 2006; Veselý et al. 2006; Dolenská et al. 2009). Prior to the experiment, predators had to be placed in empty transparent plastic boxes (57 × 39 × 28 cm) at least two hours before the experiment to ensure habituation and that the experiment was not influenced by fear of the unfamiliar environment (Exnerová et al. 2006). The predators had access to water throughout the two hours. Furthermore, it was necessary to paint the woodlice. Six yellow dots were made on the dorsal plates with nail polish (reminiscent of the colouration of *Porcellio haasi*). The control group consisted of isopods with the same number of spots, painted with grey nail polish (Fig. 1). Thus, both groups were equally altered and differed only in vivid (aposematic) and cryptic colouration. After drawing dots on the isopods, the polish was allowed to dry for at least an hour so that its odour would not interfere with the experiment. As polish nail can affect activity of isopods for more than week (Drahokoupilová and Tuf 2012), fresh new isopods were dotted before each experiment. Its activity one hour after dotting resembled that one of unpainted isopods.

We used selection tests to test whether the isopods' vivid colour has an aposematic function. The selection tests consisted of releasing five aposematically (grey–yellow experimental group) simultaneously with five cryptically (grey–grey control group) painted isopods into the box where one predator was placed, and then observing and recording the predator's behaviour. Before the insertion of the painted prey, the predator was presented with a mealworm to control feeding motivation. In one experiment each lizard experienced a series of ten tests in the following order: mealworm – painted (grey and yellow) isopods – mealworm – painted isopods, etc. Unpainted prey (*P. scaber* without drawn dots) was also presented to predators, always at the beginning and end

Figure 1. Individuals of common rough woodlouse (*Porcellio scaber*) grey painted as cryptic (**A**) and yellow painted as aposematic prey (**B**).

of the experiment. One test lasted for seven minutes. Each predator was tested in this way twice a week for five weeks. Altogether 10 experiments consisting of 10 tests were performed with each lizard giving a total of four hundred observations for each behaviour. Experiments were done during day hours in laboratory with ambient light and temperature. All four lizards were tested on the same days with a time interval of 1.5 hours. Entire experiments were recorded on camera (Niceboy VEGA X PRO) for control possibility.

Predator behaviour was divided into three categories: 1) prey consummation, which involved the direct eating of prey 2) prey manipulation, which involved biting the isopod or devouring it and then spitting it out, and 3) prey observation, which involved turning the head to follow the prey or chasing the isopod. Unless consummation was preceded by a prolonged examination of the prey, this behaviour was not considered as observation. The trials with mealworms were not analysed, they were just used to control for foraging motivation during the whole session.

Data analysis

Graphical representations of behavioural changes (consummation, manipulation, observation) in predators over the course of the experiment were made using MS Excel.

The aim of the two-factor analysis of variance (ANOVA) was to determine whether there were differences between the behavioural factors (consummation and observation). The significance level for the analysis of variance was set at 5%. Predator sex (♂, ♀) and prey colour (A, C) were designated as factors. A separate ANOVA was conducted for each behavioural type. A normality check was performed using graphical display and Levene's test to verify that the assumptions of the ANOVA were met. Due to the failure of the data to follow a normal distribution for one type of behaviour (manipulation), a Kruskal–Wallis test (non-parametric one-factor ANOVA) was performed. The Kruskal–Wallis test tests one factor at a time and compares whether there are differences in variances between the selected groups. The factors tested were predator sex (M, F) and prey colour (A-aposematic, C-cryptic). Two such tests were performed on each factor, and the significance level was set at 5%.

Tukey's test (multiple comparisons test) was performed to determine which factors (and combinations of factors) may influence predator behaviour change. R-Studio software was used to analyses of variance and Kruskal–Wallis tests, followed by graphical representations.

Results

We presented the naïve lizards with yellow painted (aposematic colouring), grey painted (cryptic colouring) as well as with unpainted (*P. scaber* without drawn markings) isopods. During the observed period (i.e., five weeks), the consumption rate of unpainted prey decreased from 65% to 5%.

The most frequent type of lizards' behaviour was the observation of prey regardless of its colouration (Fig. 2), while manipulation of the presented isopods was the least frequent. Consumption of cryptic prey was higher if it

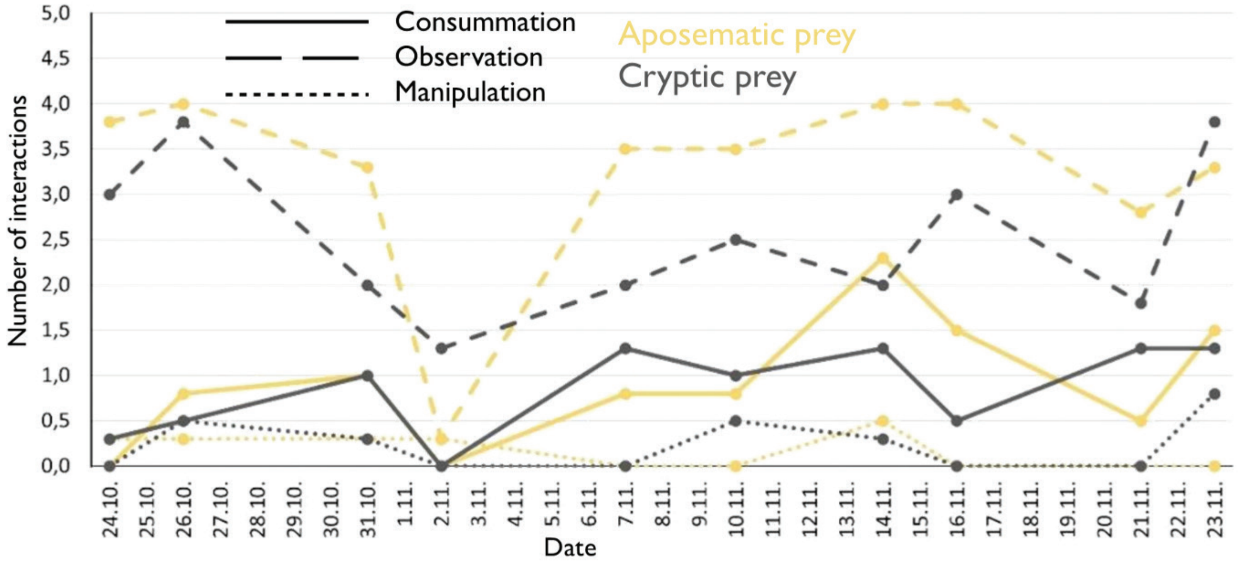

Figure 2. Average number of measured interactions of all tested predatory behaviours of Italian wall lizards (*Podarcis siculus*) towards aposematically or cryptically coloured common rough woodlice (*Porcellio scaber*).

followed a longer time interval (four or more days between experiments) (Fig. 2). For aposematic prey, this relationship was not as apparent. Due to an injury to one female, two of her last trials (24.11. and 30.11.) were not included in the behavioural display.

Differences in lizard behaviour

Two hundred of tests for both males and females (two individuals of each sex, 10 experiments of 10 tests) presented to both types of prey simultaneously were compared. Normality of the data was not confirmed by graphical display, which is not a major obstacle in ANOVA. The analysis of variance is robust to a small failure to meet this assumption, especially if the samples have a size of at least 20, which samples met with exception of data about prey manipulations.

There were statistically significant differences between predator sexes in consummation (ANOVA, $F = 15.72$, $p < 0.001$) but no differences between aposematic and cryptic prey. Significant differences in behaviour are only within predator sex (Fig. 3). Females consumed more prey, both cryptic (significantly) and aposematic (unsignificantly).

The manipulation was relatively rare category thus frequency was tested by the Kruskal–Wallis test, nevertheless, there were no differences between males and females manipulating prey, as well as between aposematic and cryptic prey manipulated ($p > 0.05$).

On the other hand, observation of prey differs significantly between male and female lizards (ANOVA, $F = 5.65$, $p = 0.020$), when females were more interested in observing potential prey as well as between cryptic and aposematic prey ($F = 7.10$, $p = 0.009$), when aposematic prey was more focused. Males observed less cryptic prey than aposematic prey (Fig. 4, $p = 0.047$). Furthermore, there was also a significant difference between females more observed aposematic prey than males observed cryptically coloured prey ($p = 0.004$).

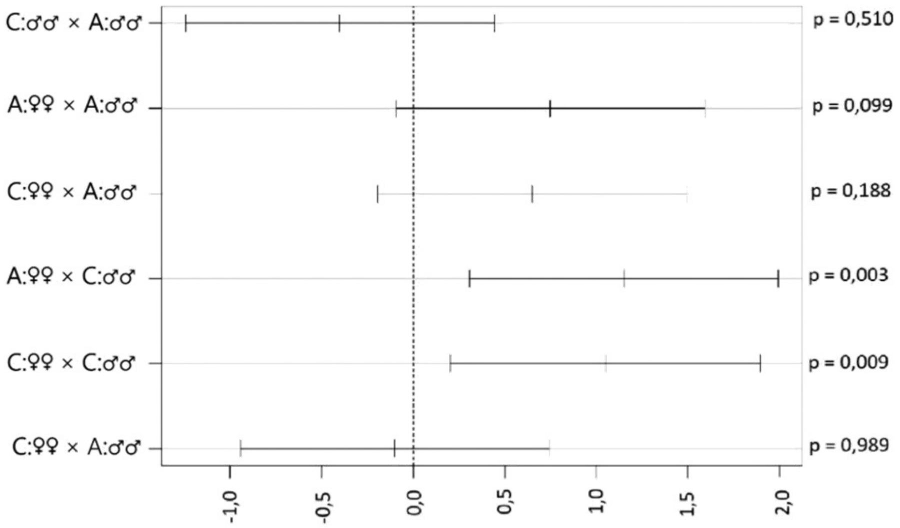

Figure 3. Visualisation Tukey's post hoc tests of consummation of painted common rough woodlice (*Porcellio scaber*) by males and females of Italian wall lizard (*Podarcis siculus*). The mean difference between means of both categories with 95% CI and p-values are presented. Treatment of prey: A – aposematic, C – cryptic.

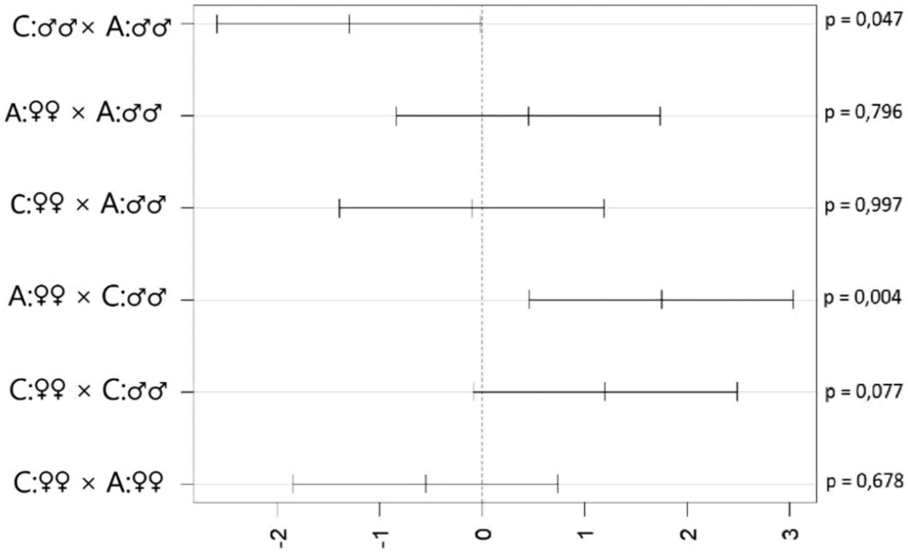

Figure 4. Visualisation Tukey's post hoc tests of observation of painted common rough woodlice (*Porcellio scaber*) by males and females of Italian wall lizard (*Podarcis siculus*). The mean difference between means of both categories with 95% CI and p-values are presented. Treatment of prey: A – aposematic, C – cryptic.

Discussion

We tested the effect of prey colouration on predator behaviour by using choice tests in which lizards were presented with yellow painted woodlice to determine whether it would have an aposematic function.

Already during the interaction with the unpainted prey, it was evident that the predators found the isopods unpalatable; the lizards spat out the prey and chewed it for a long time. This behaviour was also observed in the anole (*Anolis carolinensis*) after consuming the bugs *Neacoryphus bicrucis* and *Lopidea instabilis* (McLain 1984). Woodlice are themselves disgusting, as they produce ammonia, which they excrete from the body via vapours (Sutton 1972) and also a smelly sticky secretion from repugnatory glands. Many animals that exhibit aposematic signals also aid in deterring predators with chemical compounds such as alkaloids (Holloway et al. 1991) or cardenolides (Reichstein et al. 1968). When presented with unpainted prey at the beginning of an experiment, predators consumed more of it than at the end of the experiment. This could have been due to being hungry or curious at the beginning, they certainly did not show the neophobia typical of avian predators (Veselý et al. 2024). In contrast, by the end of the experiment, they had experienced large amounts of presented isopods as distateful prey, and although their foraging motivation (tested on mealworms) was often proven, they were mostly no longer willing to consume the isopods. In experiments with reptilian predators, they orient themselves not only by sight but also by smell, so they may perceive different aposematic signals than birds (Reznick et al. 1981; Hasegawa and Taniguchi 1994; Bonacci et al. 2008; Lee et al. 2018).

For all lizard individuals, the most common behavioural type was observation, with aposematically coloured and also cryptically coloured prey. Less frequently, manipulation or direct consummation of isopod occurred. Prey must move to get their attention, hence the high number of sightings (Sexton 1964). During actual consumption, the odour of the prey plays a large role. Prey odour may be one of the components of aposematic signals in some species (Tseng et al. 2014).

Throughout the experiment, there was no increase in food motivation with the intensity we expected. McLain (1984) conducted experiments with anoles (*Anolis carolinensis*) that he starved for three days before each subexperiment to induce increased appetite. In this condition, the lizards could disregard the odour of prey and attend more to its colouration. Although the rate of interaction with prey increased slightly over the course of the experiments, we did not observe any major changes in their behaviour. On average, males showed less interest in prey than females, which may be due to the different metabolic energy requirements of males and females. Namely, the size of females during the breeding season may affect the number of clutches per year (Galan 1997).

One female lizard had a hind limb injury, so she missed two partial trials (31.10. and 2.11.). At that time the female was fed mealworms to give her enough energy to recover. In subsequent trials she showed a marked increase in interest in the presented isopods, which was probably due to the increased energy required for full recovery.

In experiments with Taiwan japalure (*Diploderma swinhonis*), researchers found that there was a difference in prey generalization between males and females (Ko et al. 2020). The individuals used in the experiments were from the wild. As part of the experiments, they were presented with crickets that were dyed red and green, and were infused with chemicals that ensured that the prey was unpalatable. They were also presented with control prey (black). The females were more cautious after exposure to the toxic prey, and avoided the control group of crickets as well. Males, on the other hand, took more risks and tried the prey even assuming that it might be unpalatable. The researchers explain these differences in behaviour between males and females by the fact that males living in the wild have to defend their territory, which is energetically demanding for them; they are also restricted to hunting only in their territory, so they have to take more risks when choosing food because of limited resources. Females may move across territories of different males, so may be more conservative in their food choices (Ko et al. 2020). In contrast, we observed greater activity by females within as well as outside of sub-trials. Females observed and consumed presented prey to a greater extent than males. The feeding motivation of our female lizards seemed to be more influenced by their young age. These young males had yet to defend any territory. Although the difference in the observation of cryptic and aposematic prey was significant, it was significant only for males.

The predator's size influences feeding behaviour too. Exnerová et al. (2003) found in experiments with *Pyrhocoris apterus* bugs that smaller insectivorous birds (*Parus major*, *Cyanistes caeruleus*, *Erithacus rubecula*, *Sylvia atricapilla*) discriminate well between aposematically and cryptically coloured prey, in contrast to larger (*Turdus merula*) or granivorous birds (*Fringilla coelebs*, *Chloris chloris*, *Emberiza citrinella*). The smaller birds showed nausea when consuming large quantities of unpalatable prey. Perhaps even lizards, after reaching a larger size, will stop being picky and will consume or even discriminate more readily.

In conclusion, the results show that there are differences in prey consumption between the sexes of lizards. Differences were also found in prey observation both between sexes and between prey colour.

In future experiments on this topic, it would be useful to conduct more experiments with different sized lizards. It would also be useful to combine painted prey and preferred prey, such as cockroach nymphs, in selection tests. It would be appropriate to use a different type of prey colouring, such as mixtures of tapioca starch and food colouring, which are free of distinctive taste and odour (Ko et al. 2020).

Acknowledgements

We are grateful to Primož Zidar (University of Ljubljana, Slovenia) and one anonymous reviewer for their valuable comments and suggestions improving significantly quality of this paper.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

Funding

This study was partly supported by an internal grant of the Faculty of Science of Palacký University Olomouc (IGA_PrF_2023_013 and IGA_PrF_2024_013).

Author contributions

Conceptualization: IHT, BĎ. Data curation: BĎ, LS. Formal analysis: LS. Methodology: BĎ, IHT. Resources: BĎ. Supervision: IHT. Validation: LS. Writing – original draft: LS. Writing – review and editing: IHT.

Author ORCIDs

Ivan Hadrián Tuf https://orcid.org/0000-0003-0250-0482
Barbora Ďurajková https://orcid.org/0009-0004-3269-7540

Data availability

All of the data that support the findings of this study are available in the main text.

References

Achauri MS (2009) Albinism and polychromatism in some species of woodlice from Mediterranean regions (Isopoda, Oniscidea). Crustaceana 82: 1045–1056. https://doi.org/10.1163/156854009X452759

Bill FG (2007) Ornithology (3rd edition). W. H. Freeman and Company, New York, 758 pp.

Bonacci T, Aloise G, Brandmayr P, Brandmayr T, Capula M (2008) Testing the predatory behaviour of *Podarcis sicula* (Reptilia: Lacertidae) towards aposematic and non-aposematic preys. Amphibia-Reptilia 29: 449–453. https://doi.org/10.1163/156853808785111986

Bureš S (1986) Composition of the diet and trophic ecology of the collared flycatcher (*Ficedula albicollis albicollis*) in three segments of groups of types of forest geobiocenoses in Central Moravia (Czechoslovakia). Folia Zoologica 35: 143–155.

Capinera JL (2001) Other Invertebrate Pests. In Capinera JL (Ed.) Handbook of Vegetable Pests. Academic Press, Cambridge, 551–576. https://doi.org/10.1016/B978-012158861-8/50014-4

Chang ES, Thiel M (2015) The Natural History of the Crustacea, Physiology. Oxford University Press, Oxford, 532 pp.

Chen D-M, Goldsmith TH (1986) Four spectral classes of cone in the retinas of birds. Journal of Comparative Physiology A 159: 473–479. https://doi.org/10.1007/BF00604167

Davies NB, Krebs JB, West SA (2012) An Introduction to Behavioral Ecology (4th edition). Wiley-Blackwell, Oxford, 528 pp.

Dolenská M, Nedvěd O, Veselý P, Tesařová M, Fuchs R (2009) What constitutes optical warning signals of ladybirds (Coleoptera: Coccinellidae) towards bird predators: colour, pattern or general look? Biological Journal of the Linnean Society 98: 234–242. https://doi.org/10.1111/j.1095-8312.2009.01277.x

Drahokoupilová T, Tuf IH (2012) The effect of external marking on the behaviour of the common pill woodlouse *Armadillidium vulgare*. ZooKeys 176: 145–154. https://doi.org/10.3897/zookeys.176.2375

Dukas R (2004) Evolutionary biology of animal cognition. Annual Review of Ecology, Evolution, and Systematics 35: 347–374. https://doi.org/10.1146/annurev.ecolsys.35.112202.130152

Endler JA (1978) A Predator's View of Animal Color Patterns. In: Hecht MK, Steere WC, Wallace B (Eds) Evolutionary Biology. Springer US, 319–364. https://doi.org/10.1007/978-1-4615-6956-5_5

Exnerová A, Landová E, Štys P, Fuchs R, Prokopová M, Cehláriková P (2003) Reactions of passerine birds to aposematic and nonaposematic firebugs (*Pyrhocoris apterus*; Heteroptera). Biological Journal of the Linnean Society 78: 517–525. https://doi.org/10.1046/j.0024-4066.2002.00161.x

Exnerová A, Svádová KH, Štys P, Barcalová S, Landová E, Prokopová M, Fuchs R, Socha R (2006) Importance of colour in the reaction of passerine predators to aposematic prey: experiments with mutants of *Pyrhocoris apterus* (Heteroptera). Biological Journal of the Linnean Society 88: 143–153. https://doi.org/10.1111/j.1095-8312.2006.00611.x

Fingerman M (1965) Chromatophores. Physiological Reviews 45: 296–339. https://doi.org/10.1152/physrev.1965.45.2.296

Galan P (1997) Reproductive ecology of the lacertid lizard *Podarcis bocagei*. Ecography 20: 197–209. https://doi.org/10.1111/j.1600-0587.1997.tb00362.x

Geiser SW (1932) The frequency of occurrence of albinism in terrestrial isopods. Field and Laboratory 1: 4–7. https://scholar.smu.edu/fieldandlab/vol1/iss1/2

Hasegawa M, Taniguchi Y (1994) Visual avoidance of a conspicuously colored carabid beetle *Dischissus mirandus* by the Lizard *Eumeces okadae*. Journal of Ethology 12: 9–14. https://doi.org/10.1007/BF02350074

Holloway G, de Jong P, Brakefield P, Vos H (1991) Chemical defence in ladybird beetles (Coccinellidae). I. Distribution of coccinelline and individual variation in defence in 7-spot ladybirds (*Coccinella septempunctata*). Chemoecology 2: 7–14. https://doi.org/10.1007/BF01240660

Hornung E (2011) Evolutionary adaptation of oniscidean isopods to terrestrial life: structural – physiological – behavioural aspects. Terrestrial Arthropod Reviews 4: 95–130. https://doi.org/10.1163/187498311X576262

Hughes JM, Mather PB (1986) Evidence for predation as a factor in determining shell color frequencies in a mangrove snail *Littorina* sp. (Prosobranchia: Littorinidae). Evolution 40: 68–77. https://doi.org/10.2307/2408604

Hultgren KM, Mittelstaedt H (2015) Color change in a marine isopod is adaptive in reducing predation. Current Zoology 61: 739–748. https://doi.org/10.1093/czoolo/61.4.739

Knowles SFGW, Carlisle DB (1956) Endocrine control in the Crustacea. Biological Reviews 31: 396–467. https://doi.org/10.1111/j.1469-185X.1956.tb01556.x

Ko Y, Liao C, Clark R, Hsu J, Tseng H, Huang W (2020) Aposematic coloration of prey enhances memory retention in an agamid lizard. Animal Behaviour 161: 1–13. https://doi.org/10.1016/j.anbehav.2019.12.015

Krištín A (1992) Trophische Beziehungen zwischen Singvögeln und Wirbellosen im EichenBuchenwald zur Brutzeit. Der Ornitologische Beobachter 89: 157–169.

Lee C, Yo S, Clark R, Hsu J, Liao C, Tseng H, Huang W (2018) The role of different visual characters of weevils signalling aposematism to sympatric lizard predators. Journal of Zoology 306: 36–47. https://doi.org/10.1111/jzo.12567

Levi H (1965) An unusual case of mimicry. Evolution 19: 261–262. https://doi.org/10.2307/2406381

McLain DK (1984) Coevolution: Müllerian mimicry between a plant bug (Miridae) and a seed bug (Lygaeidae) and the relationship between host plant choice and unpalatability. Oikos 43: 143–148. https://doi.org/10.2307/3544761

Merilaita S (1998) Crypsis through disruptive coloration in an isopod. Proceedings of the Royal Society of London, Series B, Biological Sciences 265: 1059–1064. https://doi.org/10.1098/rspb.1998.0399

Nery LEM, de Lauro Castrucci AM (1997) Pigment cell signalling for physiological color change. Comparative Biochemistry and Physiology Part A, Physiology 118: 1135–1144. https://doi.org/10.1016/S0300-9629(97)00045-5

Pérez i de Lanuza G, Font E (2016) The evolution of colour pattern complexity: selection for conspicuousness favours contrasting within-body colour combinations in lizards. Journal of Evolutionary Biology 29: 942–951. https://doi.org/10.1111/jeb.12835

Prudic KL, Skemp AK, Papaj DR (2007) Aposematic coloration, luminance contrast, and the benefits of conspicuousness. Behavioral Ecology 18: 41–46. https://doi.org/10.1093/beheco/arl046

Punzalan D, Rodd FH, Hughes KA (2005) Perceptual processes and the maintenance of polymorphism through frequency-dependent predation. Evolutionary Ecology 19: 303–320. https://doi.org/10.1007/s10682-005-2777-z

Reichstein T, von Euw J, Parsons JA, Rothschild M (1968) Heart poisons in the Monarch Butterfly. Science 161: 861–866. https://doi.org/10.1126/science.161.3844.861

Reznick D, Sexton OJ, Mantis C (1981) Initial prey preferences in the lizard *Sceloporus malachiticus*. Copeia 1981: 681–686. https://doi.org/10.2307/1444574

Rivera X, Arribas O, Carranza S, Maluquer-Margalef J (2011) An introduction of *Podarcis sicula* in Catalonia (NE Iberian Peninsula) on imported olive trees. Butlletí de la Societat Catalana d'Herpetologia 19: 79–85.

Schmalfuss H (1984) Eco-morphological strategies in terrestrial isopods. Symposia of the Zoological Society of London 53: 49–63.

Schmalfuss H, Ferrara F (1982) Observations on the distribution and ecology of terrestrial isopods (Oniscoidea) in south-west Cameroon. Monitore Zoologico Italiano, Supplemento 17: 243–265. https://doi.org/10.1080/03749444.1982.10736668

Sexton OJ (1964) Differential predation by the lizard, *Anolis carolinensis*, upon unicoloured and polycoloured insects after an interval of no contact. Animal Behaviour 12: 101–110. https://doi.org/10.1016/0003-3472(64)90110-1

Shettleworth SJ (2001) Animal cognition and animal behaviour. Animal Behaviour 61: 277–286. https://doi.org/10.1006/anbe.2000.1606

Shettleworth SJ (2010) Cognition, Evolution, and Behavior (2nd edition). Oxford University Press, Oxford, 720 pp.

Sutton S (1972) Invertebrate Types. Woodlice. Ginn and Co., London, 144 pp.

Tseng H-Y, Lin C-P, Hsu J-Y, Pike D, Huang W-S (2014) The functional significance of aposematic signals: Geographic variation in the responses of widespread lizard predators to colourful invertebrate prey. PloS ONE 9: e91777. https://doi.org/10.1371/journal.pone.0091777

Tuf IH, Ďurajková B (2022) Antipredatory strategies of terrestrial isopods. ZooKeys 1101: 109–129. https://doi.org/10.3897/zookeys.1101.76266

Veselý P, Veselá S, Fuchs R, Zrzavý J (2006) Are gregarious red-black shieldbugs, *Graphosoma lineatum* (Hemiptera: Pentatomidae), really aposematic? An experimental approach. Evolutionary Ecology Research 8: 881–890.

Veselý P, Nedvěd O, Borovička M, Adamovská M, Průchová A, Fuch R (2024) Responses of avian predators to the polymorphic harlequin lady bird (*Harmonia axyridis*). Ecological Entomology 49. https://doi.org/10.1111/een.13310

Vickers M, Heisey M, Taylor L (2021) Lack of neophobic responses to color in a jumping spider that uses color cues when foraging (*Habronattus pyrrithrix*). PloS ONE 16: e0254865. https://doi.org/10.1371/journal.pone.0254865

Vitt LJ, Caldwell JP (2014) Herpetology: an Introductory Biology of Amphibians and Reptiles (4th edition). Academic Press, New York, 776 pp. https://doi.org/10.1016/B978-0-12-386919-7.00002-2