Proceedings of the 16th International Congress of Myriapodology, Olomouc, Czech Republic

Edited by
Ivan H. Tuf & Karel Tajovský

PENSOFT®

Sofia–Moscow
2015

ZooKeys 510 (Special Issue)

Proceedings of the 16th International Congress of Myriapodology, Olomouc, Czech Republic

Edited by Ivan H. Tuf & Karel Tajovský

First published 2015
ISBN 978-954-642-781-6 (paperback)

Pensoft Publishers
12 Prof. Georgi Zlatarski Street, 1700 Sofia, Bulgaria
Fax: +359-2-870-42-82
info@pensoft.net
www.pensoft.net

Printed in Bulgaria, June 2015

Contents

ZooKeys 510: 1–4 (2015)
doi: 10.3897/zookeys.510.10176
http://zookeys.pensoft.net

EDITORIAL

A peer-reviewed open-access journal
ZooKeys
Launched to accelerate biodiversity research

Introduction for Proceedings volume of 16th International Congress of Myriapodology

Karel Tajovský[1], Ivan H. Tuf[2]

[1] *Institute of Soil Biology, Biology Centre CAS, Na Sádkách 7, České Budějovice, Czech Republic* [2] *Department of Ecology and Environmental Sciences, Faculty of Science, Palacký University, Šlechtitelů 27, Olomouc, Czech Republic*

Corresponding author: *Ivan H. Tuf* (ivan.tuf@upol.cz)

Received 19 June 2015 | Accepted 19 June 2015 | Published 30 June 2015

http://zoobank.org/6BC07BC0-E030-4682-9F5A-7FB7976CEABF

Citation: Tajovský K, Tuf IH (2015) Introduction for Proceedings volume of 16th International Congress of Myriapodology. In: Tuf IH, Tajovský K (Eds) Proceedings of the 16th International Congress of Myriapodology, Olomouc, Czech Republic. ZooKeys 510: 1–4. doi: 10.3897/zookeys.510.10176

The papers in this special issue of ZooKeys were presented at the 16th International Congress of Myriapodology (16ICM) held in Olomouc, the Czech Republic on July 20–25, 2014. International Congresses of Myriapodology are scientific meetings of scientists, students and enthusiastic amateurs with specific interest in millipedes, centipedes, symphylans and pauropods, as well as velvet worms. Those are organised under the auspices of the Centre International de Myriapodologie (http://www.myriapodology.org). To date, these congresses have a 46 year tradition and have taken place in 13 countries:

1ICM: 1968 – Paris, France
2ICM: 1972 – Manchester, UK
3ICM: 1975 – Hamburg, Germany
4ICM: 1978 – Gargnano, Italy
5ICM: 1981 – Radford, USA
6ICM: 1984 – Amsterdam, The Netherlands
7ICM: 1987 – Vittorio Veneto, Italy
8ICM: 1990 – Innsbruck, Austria
9ICM: 1993 – Paris, France
10ICM: 1996 – Copenhagen, Denmark
11ICM: 1999 – Bialowieza, Poland
12ICM: 2002 – Mtunzini, South Africa
13ICM: 2005 – Bergen, Norway
14ICM: 2008 – Görlitz, Germany
15ICM: 2011 – Brisbane, Australia
16ICM: 2014 – Olomouc, Czech Republic

Figure 1. Collective photo of 16ICM participants.

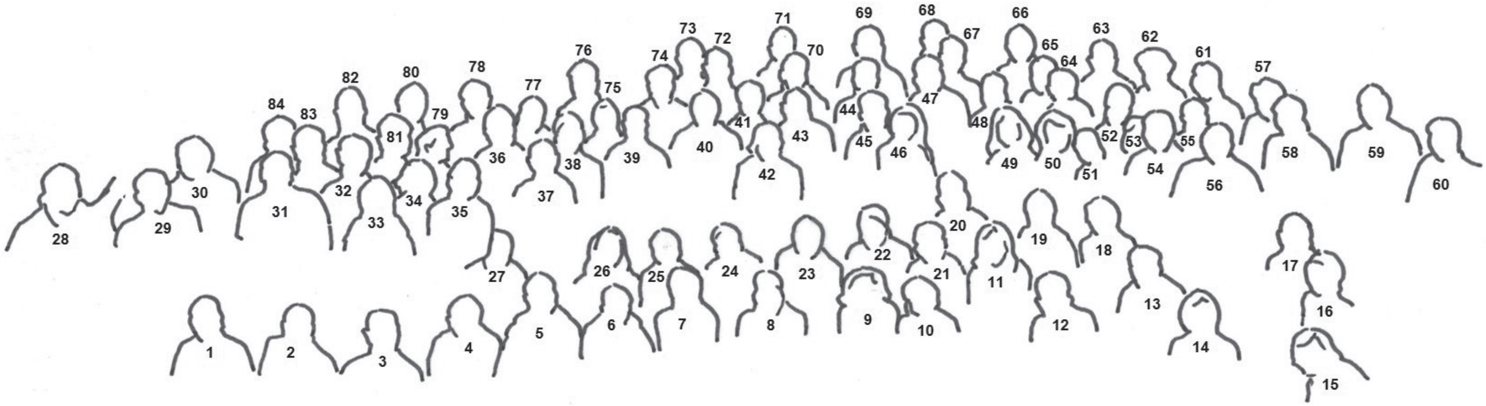


Figure 2. Legend of Fig. 1. **1** Boyan Vagalinski **2** Oliver Macek **3** Lucio Bonato **4** Hsueh-Wen Chang **5** Norman Lindner **6** Laura Del Latte **7** Irina Semenyuk **8** Michaela Bodner **9** Fieng-Lan Sun **10** Chao-chun Chen **11** Piyatida Pimvichai **12** Warut Siriwut **13** Natdanai Likhitrakarn **14** Małgorzata Leśniewska **15** Jolanta Wytwer **16** Michaela Kratochvílová **17** Christian Kronmüller **18** Cuong Huynh **19** Nattarin Wongthamwanich **20** João Paulo Pena-Barbosa **21** Julian Bueno-Villegas **22** Varpu Vahtera **23** Peter Decker **24** Barbara Jäschke **25** Julia Nefedieva **26** Roghaieh Zarei **27** Leszek Jendryszik **28** Ivan H. Tuf **29** Karel Tajovský **30** Sergei Ilyich Golovatch **31** Günther Raspotnig **32** Hans S. Reip **33** Darina Bachvarova **34** Elena Valentinovna Mikhaljova **35** Michalina Kszuk-Jendrysik **36** Pavel Stoev **37** Somsak Panha **38** Nesrine Akkari **39** Pavel Nefediev **40** Jean-Jacques Geoffroy **41** Giuseppe Fusco **42** Karin Voigtländer **43** Jean-Francois David **44** Bjarne Meidell **45** Amazonas Chagas-Jr. **46** Manoela Karam Gemael **47** Carsten H. G. Müller **48** Ivan Kos **49** Blanka Ravnjak **50** Maja Kastelic **51** Branka Vode **52** Megan Short **53** Hilke Ruhberg **54** Pavel Kocourek **55** Timotej Mock **56** Grzegorz Antoni Kania **57** John Lewis **58** Greg Edgecombe **59** Joseph Hannibal **60** Vladimír Šustr **61** Michal Rendoš **62** Andrej Mock **63** László Dányi **64** Ansgar Poloczek **65** Stylianos Simasiakis **66** Andy Sombke **67** Eivind Andreas Baste Undheim **68** Pavel Saska **69** Aleksandr Evsiukov **70** Willi Xylander **71** Bruce A. Snyder **72** Jan Philip Oeyen **73** Petr Dolejš **74** Henrik Enghoff **75** Ana Komerički **76** Markus Koch **77** Jörg Rosenberg **78** Iurii Diachkov **79** Daniela Bartel **80** Nikolaus U. Szucsich **81** Zoltán Korsós **82** Thomas Wesener **83** David Bogyo **84** Per Djursvoll.

Aleksandar Doichinov, Elisavet Georgopoulou, Eszter Lazányi, Gabriella Papastefanou and Irina Zenkova are missing.

16ICM was the first myriapodological congress to be held in the Czech Republic. The 84 participants and 18 accompanying persons came from 24 countries on five continents, making the 16th Congress a truly international and global meeting of myriapod specialists (Figs 1, 2).

The organisers of the 16ICM are very grateful to the Palacký University, Olomouc, which provided us a pleasant area for all scientific as well as social parts of the Congress. We also thank the Biology Centre CAS and partial support from several unnamed colleagues who allowed the participation of young students at this Congress.

Many thanks to Lyubomir Penev and the friendly staff at Pensoft Publishers for making the 16ICM papers freely available to a global online audience.

ZooKeys 510: 5–14 (2015)
doi: 10.3897/zookeys.510.8636
http://zookeys.pensoft.net

CATALOGUE

A peer-reviewed open-access journal
ZooKeys
Launched to accelerate biodiversity research

Type specimens of centipedes (Myriapoda, Chilopoda) in the National Museum, Prague (Czech Republic)

Petr Dolejš[1]

1 *Department of Zoology, National Museum-Natural History Museum, Cirkusová 1740, CZ-193 00, Praha 9-Horní Počernice, Czech Republic*

Corresponding author: *Petr Dolejš* (petr_dolejs@nm.cz)

Academic editor: *Ivan H. Tuf* | Received 22 September 2014 | Accepted 8 May 2015 | Published 30 June 2015

http://zoobank.org/93FC6F2B-4980-4355-AF48-E9EB0A204E92

Citation: Dolejš P (2015) Type specimens of centipedes (Myriapoda, Chilopoda) in the National Museum, Prague (Czech Republic). In: Tuf IH, Tajovský K (Eds) Proceedings of the 16th International Congress of Myriapodology, Olomouc, Czech Republic. ZooKeys 510: 5–14. doi: 10.3897/zookeys.510.8636

Abstract

The centipede collection in the National Museum in Prague contains type material of 16 taxa (14 species and two subspecies), of which 15 were described by Luděk J. Dobroruka and one by Karl W. Verhoeff: *Allothereua wilsonae* Dobroruka, 1979; *Chinobius alenae* Dobroruka, 1980; *Lithobius corrigendus* Dobroruka, 1988; *L. creticus* Dobroruka, 1977; *L. erythrocephalus mohelensis* Dobroruka, 1959; *L. evae* Dobroruka, 1958; *L. magurensis* Dobroruka, 1971; *L. purkynei* Dobroruka, 1957; *L. tatricus* Dobroruka, 1958; *L. tatricus monounguis* Dobroruka, 1958; *Monotarsobius homolaci* Dobroruka, 1971; *M. krali* Dobroruka, 1979; *Pachymerium dilottiae* Dobroruka, 1976; *P. hanzaki* Dobroruka, 1976; *Scolopendra aztecorum* Verhoeff, 1934 and *Strigamia olympica* Dobroruka, 1977. Of these 16 taxa, five were described from the Czech Republic, three from Slovakia and eight from other countries (Greece, Iraq, Kyrgyzstan, Mexico, Nepal, Russia and Uzbekistan). The eight taxa described from the Czech and Slovak Republics are now considered as junior synonyms but the eight taxa described from the other countries are still valid.

Keywords

Zoological collection, Luděk Jindřich Dobroruka, *Allothereua*, *Lithobius*, *Pachymerium*, *Scolopendra*, *Strigamia*

Introduction

The most important part of the zoological collection of National Museum in Prague (NMP) is the type material. Up to now, catalogues of the type material of spiders (Růžička et al. 2005), vertebrates (Mlíkovský et al. 2011) and horsehair worms (Dolejš 2012) were published. The process of cataloguing the zoological type material is ongoing with this catalogue of centipede type material. There are no types of other myriapod classes despite 19 millipede and two symphylan taxa were described from the territory of the Czech Republic (Bezděk 2011). The centipede type material was deposited to the NMP in three distinct periods: 1930–1936 (material collected during expeditions organized by the NMP and people who were collaborating with the NMP), 1975–1978 (material collected by various collectors and provided to L. J. Dobroruka) and 2012–2014 (material found in Dobroruka's personal collection). Majority of the material was collected by Czechs and the new taxa originated from these collections were described by L. J. Dobroruka and K. W. Verhoeff. The latter author described new *Scolopendra* species based on material collected by Czech acarologist Prof. Jaroslav Štorkán (20 April 1890 – 1 June 1942) who was collecting for the NMP in 1920s and 1930s. Thus, it is not surprising that a part of material collected by him came back to the NMP.

The collection of centipedes (containing ca. 1500 specimens representing ca. 150 species and subspecies from about 40 countries) is perfectly organized and catalogued thanks to RNDr. Ing. Luděk Jindřich Dobroruka (20 October 1933 – 4 July 2004) who worked in the NMP as a curator of Invertebrates in 1956 and continued with his work on centipedes in the NMP also in 1970s. His work resulted in publishing of catalogues of non-type Bulgarian (Dobroruka 1977a), Greek (Dobroruka 1977b) and Brazilian (Dobroruka 1979a) centipedes deposited there. Despite collaborating with the NMP, some types described by him remained in his personal collection and a part of them were deposited to the NMP after the work of Tuf et al. (2008). Unfortunately, the destination of several Dobroruka's types is still unknown. More information about L. J. Dobroruka can be found in Felix (2004a, b), Hanák (2004), Kellnerová (2004), Růžička (2005), Tuf (2005) and Bartoš (2006).

Methods

All the specimens are preserved in 80% ethanol. Nomenclatural issues follow the International Code of Zoological Nomenclature (ICZN 1999, 2012). Orig. = original combination (as proposed in the original publication), the first page with descriptions including figures and sex of the illustrated animal. Now = current status of the taxon. For grid squares of Czech and Slovak localities, see Buchar (1982) and Pruner and Míka (1996). Condition of the type material is provided as follows: excellent – good – reasonable – poor.

Results

Order: Scutigeromorpha
Family: Scutigeridae

Allothereua wilsonae Dobroruka, 1979

Orig. *Allothereua wilsonae* Dobroruka, 1979b: 101, figs 1–5 (♀).

Now. *Allothereua wilsonae* Dobroruka, 1979. The status of this species has never been revised since its original description.

Holotype. NMP P6E-1761, ♀ in poor condition (in two pieces, legs detached), collected by Jane M. Wilson on 14 October 1971 in one of the tents of the camp, near Mahendra Cave, Pokhara Valley, Nepal; 28°14.00'N, 83°59.00'E.

Order: Scolopendromorpha
Family: Scolopendridae

Scolopendra aztecorum Verhoeff, 1934

Orig. *Scolopendra aztecorum* Verhoeff, 1934: 49.

Now. *Scolopendra aztecorum* Verhoeff, 1934. Valid species according to Cupul-Magaña (2013).

Syntype. NMP P6E-1303, one adult specimen in good condition, collected by Jaroslav Štorkán in 1930 under decaying cactuses, La Paz, Baja California Sur Region, Mexico; 24°8.28'N, 110°18.58'W.

Note. Other syntypes are deposited in the Zoologisches Museum der Humboldt-Universität, Berlin, Germany under number ZMB 13378 (Moritz and Fischer 1979); in the Bavarian State collection, Munich, Germany under numbers ZSM/Myr-20051044 and ZSM/Myr-20051045 (SysTax 2015); and in the Naturalis Biodiversity Center, Leiden, the Netherlands under number RMNH.CHIL.154 (C. A. Martínez-Muñoz in litt.). Both Shelley (2006) and Cupul-Magaña (2013) erroneously regarded the specimen from Berlin as a holotype despite Verhoeff (1934) did not designate any type specimen and despite Moritz and Fischer (1979) referred to it as “Syntypus”.

Order: Geophilomorpha
Family: Geophilidae

Pachymerium dilottiae Dobroruka, 1976

Orig. *Pachymerium dilottiae* Dobroruka, 1976: 259, figs 1A–D (♂♀).

Now. *Pachymerium dilottiae* Dobroruka, 1976. The status of this species has never been revised since its original description.

Holotype. NMP P6E-1326, ♀ in reasonable condition (in two pieces, head mounted separately on a permanent slide), collected by Vlasta Kálalová-di Lotti in 1929 in Baghdad, Iraq; 33°20.43'N, 44°24.05'E.

Paratypes. NMP P6E-1327, 1♂ in good condition and 1♂ in reasonable condition (missing the caudal part of the body), same data as holotype.

Pachymerium hanzaki Dobroruka, 1976

Orig. *Pachymerium hanzaki* Dobroruka, 1976: 260, figs 2A–E (♂).

Now. *Pachymerium hanzaki* Dobroruka, 1976. The status of this species has never been revised since its original description.

Holotype. NMP P6E-1328, ♂ in reasonable condition (head mounted separately on a permanent slide), collected by Vlasta Kálalová-di Lotti in 1929 in Baghdad, Iraq; 33°20.43'N, 44°24.05'E.

Family: Linotaeniidae

Strigamia olympica Dobroruka, 1977

Orig. *Strigamia* (*Strigamia*) *olympica* Dobroruka, 1977b: 163, figs 5–8 (♀).

Now. *Strigamia olympica* Dobroruka, 1977. Valid species according to Bonato et al. (2012).

Holotype. NMP P6E-1352, ♀ in reasonable condition, collected by Karel Táborský on 5 June 1935 in Óros Ólympos Mt., Greece; 40°3.72'N, 22°20.70'E.

Order: Lithobiomorpha
Family: Lithobiidae

Lithobius (*Chinobius*) *alenae* (Dobroruka, 1980)

Orig. *Chinobius alenae* Dobroruka, 1980: 92, figs 1–6 (♀).

Now. *Lithobius* (*Chinobius*) *alenae* (Dobroruka, 1980). The status of this species has never been revised since its original description.

Holotype. NMP P6E-3912, ♀ in reasonable condition, collected by Alena Čepická on 5 July 1977 under bark of fallen trees in Voronezhskove, 30 km NE from Khabarovsk, Khabarovskiy Kray, Russia; 48°41.67'N, 135°13.20'E.

Paratype. NMP P6E-3913, ♀ in good condition, same data as holotype.

***Lithobius* (*Lithobius*) *corrigendus* Dobroruka, 1988**

Orig. *Lithobius corrgendus* (sic!) Dobroruka, 1988: 6 (*nomen novum* for *Lithobius parvus* Folkmanová, 1946).

Now. Junior synonym of *Lithobius* (*Lithobius*) *lucifugus* L. Koch, 1862 (Tuf et al. 2008).

Neotype. NMP P6E-3901, ♂ in reasonable condition, collected by Luděk J. Dobroruka on 12 August 1997 on the left bank of the Dyje river by Devět mlýnů, Podyjí National Park, Znojmo District, Jihomoravský Region, Czech Republic; 48°49.08'N, 15°58.38'E (7161d); designated by Tuf et al. (2008).

Note. Another (non-type) female specimen in excellent condition (NMP P6E-3902) determined by Dobroruka as *L. corrigendus* was revised as *Lithobius* (*Lithobius*) *latro* Meinert, 1872 by Jolanta Wytwer, Ivan H. Tuf and Karel Tajovský in 2006.

***Lithobius* (*Lithobius*) *creticus* Dobroruka, 1977**

Orig. *Lithobius creticus* Dobroruka, 1977b: 161, figs 1–4 (♀).

Now. *Lithobius* (*Lithobius*) *creticus* Dobroruka, 1977. Valid species according to Zapparoli (2002).

Holotype. NMP P6E-1350, ♀ in good condition, collected by Josef Mařan and Otakar Štěpánek in 1937 in Óros Ídi Mt., Crete, Greece; 35°14.04'N, 24°46.84'E.

Paratype. NMP P6E-1351, ♂ in good condition, same data as holotype.

***Lithobius* (*Lithobius*) *erythrocephalus mohelensis* Dobroruka, 1959**

Orig. *Lithobius erythrocephalus mohelensis* Dobroruka, 1959: 105, figs 3 (♂), 4 (♀).

Now. *Lithobius* (*Lithobius*) *erythrocephalus* C. L. Koch, 1847 (Tuf et al. 2008).

Syntypes. NMP P6E-3078, 2♂♂ and 1♀ in poor condition (legs detached), collected by Luděk J. Dobroruka in April and June 1955 in Serpentine Steppe of Mohelno, Třebíč District, Vysočina Region, Czech Republic; 49°6.47'N, 16°11.25'E (6863c).

***Lithobius* (*Lithobius*) *evae* Dobroruka, 1958**

Orig. *Lithobius* (*Lithobius*) *evae* Dobroruka, 1958b: 26, figs 1 (♂), 2 (♀).

Now. Junior synonym of *Lithobius* (*Lithobius*) *tenebrosus* Meinert, 1872 (Tuf et al. 2008).

Type material. NMP P6E-3079, 2♂♂ and 4♀♀ in poor condition (legs detached), collected by Eva M. Homoláčová and Miloš E. Homoláč on 14 September 1956 among fallen beech leaves in Bukovec Hill near Jizerka, Jizerské hory Mts., Jablonec nad Nisou District, Liberec Region, Czech Republic; 50°48.86'N, 15°21.47'E (5158c).

Note. Male holotype and three female paratypes were not labelled and thus indistinguishable among the type material mentioned.

Lithobius (*Monotarsobius*) *homolaci* Dobroruka, 1971

Orig. *Monotarsobius homolaci* Dobroruka, 1971: 262, fig. 2 (♂).

Now. junior synonym of *Lithobius* (*Sigibius*) *burzenlandicus* Verhoeff, 1931 (Tuf et al. 2008).

Holotype. NMP P6E-1454, ♂ in good condition, collected (sifted from beech leaf litter) by Miloš E. Homoláč in September 1959 in Spišská Magura Mts., Kežmarok District, Prešov Region, Slovakia; 49°17.04'N, 20°22.38'E (6788a).

Paratype. NMP P6E-1455, ♀ in reasonable condition, same data as holotype.

Lithobius (*Monotarsobius*) *krali* (Dobroruka, 1979)

Orig. *Monotarsobius krali* Dobroruka, 1979c: 161, figs 1–3 (♂♀), 4 (♀).

Now. *Lithobius* (*Monotarsobius*) *krali* (Dobroruka, 1979). Valid species according to Dányi and Tuf (2012).

Holotype. NMP P6E-1762, ♂ in good condition, collected by Josef Král on 8 July 1976 in Ala Archa, Chüy Region, Kyrgyzstan; 42°38.70'N, 74°28.73'E.

Paratype. NMP P6E-1763, ♀ in reasonable condition, collected by Josef Král on 3 July 1976 in Gora Chimgan Mt., Toshkent Region, Uzbekistan; 41°40.45'N, 69°42.90'E.

Lithobius (*Lithobius*) *magurensis* Dobroruka, 1971

Orig. *Lithobius magurensis* Dobroruka, 1971: 261, fig. 1 (♂).

Now. Junior synonym of *Lithobius* (*Lithobius*) *muticus* C. L. Koch, 1847 (Tuf et al. 2008).

Holotype. NMP P6E-1452, ♂ in excellent condition, collected by Miloš E. Homoláč in September 1959 in Spišská Magura Mts., Kežmarok District, Prešov Region, Slovakia; 49°17.04'N, 20°22.38'E (6788a).

Paratype. NMP P6E-1453, ♀ in good condition, same data as holotype.

Lithobius (*Lithobius*) *purkynei* Dobroruka, 1957

Orig. *Lithobius* (*Lithobius*) *purkyněi* Dobroruka, 1957: 174, figs 1A–B (♀).

Now. Junior synonym of *Lithobius* (*Lithobius*) *macilentus* L. Koch, 1862 (Tuf et al. 2008).

Type material. NMP P6E-3080, 5♀♀ in poor condition (legs detached), collected by Luděk J. Dobroruka on 17 and 22 June 1956 in Boreč, Litoměřice District, Ústí nad Labem Region, Czech Republic; 50°30.85'N, 13°59.31'E (5449d).

Note. Female holotype and four female paratypes were not labelled and thus indistinguishable among the type material mentioned. Other (non-type) male and female specimens (NMP P6E-1290) determined by Dobroruka as *L. purkynei* were mentioned in Dobroruka (1977c).

***Lithobius* (*Lithobius*) *tatricus* Dobroruka, 1958**

Orig. *Lithobius* (*Archilithobius*) *tatricus* Dobroruka, 1958a: 115, figs 1-2 (♂♀), 3 (♀).

Now. Junior synonym of *Lithobius* (*Lithobius*) *mutabilis* L. Koch, 1862 (Tuf et al. 2008).

Type material. NMP P6E-3081, three specimens in a poor condition (legs detached), collected by Luděk J. Dobroruka on 26 June 1955 from sieved material and under bark of fallen trees in Bielovodská dolina Valley, Belanske Tatry Mts., Poprad District, Prešov Region, Slovakia; 49°13.31'N, 20°6.14'E (6786d).

Note. Male holotype, three male and one female paratypes were not labelled and thus indistinguishable among the type material mentioned. Other (non-type) male and female specimens (NMP P6E-1376, P6E-1380, P6E-1427, P6E-1439 and P6E-3082) determined by Dobroruka as *L. tatricus* were mentioned in Dobroruka (1977c, 1998).

***Lithobius* (*Lithobius*) *tatricus monounguis* Dobroruka, 1958**

Orig. *Lithobius* (*Archilithobius*) *tatricus monounguis* Dobroruka, 1958b: 27.

Now. Junior synonym of *Lithobius* (*Lithobius*) *latro* Meinert, 1872 (Tuf et al. 2008).

Holotype. NMP P6E-3082, ♂ in poor condition (legs detached), collected by Eva M. Homoláčová and Miloš E. Homoláč on 14 September 1956 under moist beech leaves in Bukovec Hill near Jizerka, Jizerské hory Mts., Jablonec nad Nisou District, Liberec Region, Czech Republic; 50°48.86'N, 15°21.47'E (5158c).

Conclusions

The zoological collection of the National Museum in Prague hosts (among others) type material of 16 centipede taxa. Both sctutigeromophs (Scutigeridae) and scolopendromorphs (Scolopendridae) are represented by one species, geophilomorphs (Geophilidae and Linotaenidae) by three species and lithobiomorphs (Lithobiidae) by 11 species and subspecies. Five species from the first three orders (*Allothereua wilsonae*, *Scolopendra aztecorum*, *Pachymerium dilottiae*, *P. hanzaki* and *Strigamia olympica*) and three species from Lithobiomorpha (*Lithobius alenae*, *L. creticus* and *L. krali*) are currently valid. The eight remaining lithobiomorph species and subspecies (*Lithobius corrigendus*, *L. erythrocephalus mohelensis*, *L. evae*, *L. homolaci*, *L. magurensis*, *L. purkynei*, *L. tatricus* and *L. tatricus monounguis*) are now considered as junior synonyms.

Acknowledgments

I thank Ivan H. Tuf and Jana Dobroruková for information about L. J. Dobroruka and for providing me appropriate literature. I am grateful to Carlos A. Martínez-Muñoz for information about *Scolopendra aztecorum*. Finally, I would like to thank two anonymous reviewers for their helpful comments and suggestions improving the earlier version of the manuscript. This study was fully supported by the project NAKI (DF12P01OVV021).

References

Bartoš L (Ed.) (2006) RNDr. Ing. Luděk J. Dobroruka *20. 10. 1933, † 4. 7. 2004. Zprávy ČSEtS 20: 1–30. http://www.csets.sk/soubory/zpravy202.pdf [In Czech]

Bezděk J (2011) Přehled živočišných druhů popsaných z území České republiky [A review of animal species described from the Czech Republic]. Mendelova univerzita v Brně, Brno, 420 pp. [In Czech, English summary]

Bonato L, Dányi L, Socci AA, Minelli A (2012) Species diversity of *Strigamia* Gray, 1843 (Chilopoda: Linotaeniidae): a preliminary synthesis. Zootaxa 3593: 1–39. http://www.mapress.com/zootaxa/2012/f/z03593p039f.pdf

Buchar J (1982) Způsob publikace lokalit živočichů z území Československa. Publication of faunistic data from Czechoslovakia. Věstník Československé společnosti zoologické 46: 317–317. [In Czech, Russian and English summary]

Cupul-Magaña FG (2013) La diversidad de los ciempiés (Chilopoda) de México. Dugesiana 20(1): 17–41. http://dugesiana.cucba.udg.mx/dugesiana_agost2013/17-42.pdf

Dányi L, Tuf IH (2012) *Lithobius* (*Monotarsobius*) *franciscorum* sp. nov., a new lithobiid species from the Altai, with a key to the Central Asian species of the subgenus (Chilopoda: Lithobiomorpha). Zootaxa 3182: 16–28. http://www.mapress.com/zootaxa/2012/f/z03182p028f.pdf

Dobroruka L (1957) Neue Chilopoda aus dem Böhmischen Mittelgebirge. Zoologischer Anzeiger 159(7–8): 174–179.

Dobroruka LJ (1958a) Příspěvek k poznání stonožek z Tater (Chilopoda). Beitrag zur Kenntnis der Tausendfüssler aus Tatra (Chilopoda). Věstník Československé zoologické společnosti 22(2): 113–120. [In Czech, German summary]

Dobroruka LJ (1958b) Neue Chilopoden aus Böhmen. Zoologischer Anzeiger 160(1–2): 25–28.

Dobroruka LJ (1959) Chilopoda státní přírodní rezervace Mohelno. Chilopoden des staatlichen Naturschutzgebietes Mohelno. Ochrana přírody 14(4): 104–106. [In Czech, German summary]

Dobroruka LJ (1971) Chilopoda von Belanské Tatry und Spišská Magura. Věstník Československé společnosti zoologické 35(4): 261–264.

Dobroruka LJ (1976) Einige Chilopoden aus Irak. Věstník Československé společnosti zoologické 40(4): 259–262.

Dobroruka LJ (1977a) Bulgarische Chilopoden in der Sammlung von Nationalmuseum Praha. Věstník Československé společnosti zoologické 41(1): 5–7.

Dobroruka LJ (1977b) Chilopoden aus Griechenland und Kreta in der Sammlung des Nationalmuseums Prag. Věstník Československé společnosti zoologické 41(3): 161–164.

Dobroruka LJ (1977c) Neue Funde seltener Chilopoden-Arten in der Tschechoslowakei (Myriopoda: Chilopoda). Věstník Československé společnosti zoologické 41(4): 245–247.

Dobroruka LJ (1979a) Notes on the chilopods of Brazil in the collection of the National Museum Prague. Věstník Československé společnosti zoologické 43(2): 98–100.

Dobroruka LJ (1979b) *Allothereua wilsonae* sp. n., a new centipede from Nepal (Chilopoda: Scutigeromorpha: Thereuoneminae). Věstník Československé společnosti zoologické 43(2): 101–103.

Dobroruka LJ (1979c) Zur weiteren Kenntnis der zentralasiatischen Chilopoden. Věstník Československé společnosti zoologické 43(3): 161–164.

Dobroruka LJ (1980) *Chinobius alenae* sp. n., eine neue Chilopoden-Art aus dem Fernen Osten (Chilopoda: Lithobiomorpha). Věstník Československé společnosti zoologické 44(2): 92–93.

Dobroruka LJ (1988) Chilopoda of the Białowieża Forest. National parks and nature reserves 9(1): 5–7.

Dobroruka LJ (1998) Annotated preliminary checklist of Chilopoda of the Podyjí National Park. Thayensia (Znojmo) 1: 131–136.

Dolejš P (2012) Type specimens of gordioids (Nematoida: Nematomorpha) in the National Museum, Prague. Journal of the National Museum (Prague), Natural History Series 181(4): 21–25. http://www.nm.cz/publikace/publikace-download.php?name=File1&dir=archiv&table=tabPublikaceArchiv&id=3597

Felix J (2004a) Za významným zoologem Luďkem J. Dobrorukou [After an outstanding zoologist Luděk J. Dobroruka]. Živa 52(4): XLII. http://ziva.avcr.cz/files/ziva/pdf/za-vyznamnym-zoologem-ludkem-j-dobrorukou.pdf [In Czech]

Felix J (2004b) Za RNDr. Ing. Luďkem J. Dobrorukou [After RNDr. Ing. Luděk J. Dobroruka]. TamTamy 7(4): 23–24. [In Czech]

Folkmanová B (1946) Dva nové druhy Lithobiidů z Křivoklátska. Deux nouveaux éspéces de *Lithobius*. Věstník Československé zoologické společnosti 10: 90–100. [In Czech, French summary]

Hanák V (2004) Za RNDr. Ing. Luďkem J. Dobrorukou. In memoriam RNDr. Ing. Luděk J. Dobroruka. Lynx, n. s. 35: 273–280. http://www.nm.cz/publikace/publikace-download.php?name=File1&dir=archiv&table=tabPublikaceArchiv&id=2218 [In Czech]

ICZN (1999) International code of zoological nomenclature. Fourth Edition. The International Trust for Zoological Nomenclature, London.

ICZN (2012) International Commission on Zoological Nomenclature. Amendment of Articles 8, 9, 10, 21 and 78 of the *International Code of Zoological Nomenclature* to expand and refine methods of publication. ZooKeys 219: 1–10. doi: 10.3897/zookeys.219.3944

Kellnerová D (2004) Malá tajemství Luďka J. Dobroruky [Little secrets of Luděk J. Dobroruka]. Bedrník 2(6): 18–20. [In Czech]

Koch CL (1847) System der Myriapoden. In: Herrich-Schäffer L (Ed.) Kritische Revision der Insectenfauna Deutschlands. Pustet, Regensburg, 3: 1–270.

Koch L (1862) Die Myriapodengattung *Lithobius*. JL Lotzbeck, Nürnberg, 94 pp.

Meinert F (1872) Myriapoda Musaei Hauniensis. Bidrag til Myriapoderns Morphologi og Systematik. II. Lithobiini. Naturhistorisk Tidsskrift (3) 8: 281–344. [In Danish]

Mlíkovský J, Benda P, Moravec J, Šanda R (2011) Type specimens of recent vertebrates in the collections of the National Museum, Prague, Czech Republic. Journal of the National Museum (Prague), Natural History Series 180(10): 133–164. http://www.nm.cz/publikace/publikace-download.php?name=File1&dir=archiv&table=tabPublikaceArchiv&id=2997

Moritz M, Fischer S-C (1979) Die Typen der Myriapoden-Sammlung des Zoologischen Museums Berlin. II. Chilopoda. Mitteilungen aus dem Zoologischen Museum in Berlin 55(2): 297–352. doi: 10.1002/mmnz.4830550215

Pruner L, Míka P (1996) Seznam obcí a jejich částí v České republice s čísly mapových polí pro síťové mapování. List of settlements in the Czech Republic with associated map field codes for faunistic grid mapping system. Klapalekiana 32(Suppl.): 1–175. [In Czech, English abstract and summary]

Růžička V (2005) In memoriam RNDr. Ing. Luděk J. Dobroruka, 1933–2004. Arachnologische Mitteilungen 29: 64. doi: 10.5431/aramit2914

Růžička V, Kůrka A, Buchar, Řezáč M (2005) Czech Republic–the type material of spiders (Araneae). Časopis Národního muzea, Řada přírodovědná 174(1–4): 13–64. http://www.nm.cz/publikace/publikace-download.php?name=File1&dir=archiv&table=tabPublikaceArchiv&id=164

Shelley RM (2006) A chronological catalog of the New World species of *Scolopendra* L., 1758 (Chilopoda: Scolopendromorpha: Scolopendridae). Zootaxa 1253: 1–50. http://www.mapress.com/zootaxa/2006f/z01253p050f.pdf

SysTax (2015) A Database System for Systematics and Taxonomy: *Scolopendra aztecorum* Verhoeff, 1934. http://www.biologie.uni-ulm.de/cgi-bin/query_all/details.pl?id=85045&stufe=7&typ=ZOO&sid=T&lang=e&pr=nix

Tuf IH (2005) Luděk Jindřich Dobroruka [1993–2004]. Bulletin du Centre International de Myriapodologie 38: 7–10. http://www.ekologie.upol.cz/ad/tuf/pdf/papers/Tuf_2005b.pdf

Tuf IH, Wytwer J, Tajovský K (2008) On the identity of the species described in the genus *Lithobius* Leach, 1814 by L. J. Dobroruka from the former Czechoslovakia (Czech and Slovak Republics) (Chilopoda: Lithobiomorpha). Zootaxa 1788: 37–46. http://www.mapress.com/zootaxa/2008/f/z01788p046f.pdf

Verhoeff KW (1931) Chilopoden der Insel Elba (Isopoden). Zoologischer Anzeiger 95(11–12): 302–312.

Verhoeff KW (1934) Beiträge zur Systematik und Geographie der Chilopoden. Zoologische Jahrbücher, Abteilung für Systematik 66: 1–112.

Zapparoli M (2002) A catalogue of the centipedes from Greece (Chilopoda). Fragmenta Entomologica 34: 1–146.

ZooKeys 510: 15–29 (2015)
doi: 10.3897/zookeys.510.8852
http://zookeys.pensoft.net

RESEARCH ARTICLE

A peer-reviewed open-access journal
ZooKeys
Launched to accelerate biodiversity research

First results of the German Barcode of Life (GBOL) – Myriapoda project: Cryptic lineages in German *Stenotaenia linearis* (Koch, 1835) (Chilopoda, Geophilomorpha)

Thomas Wesener[1], Karin Voigtländer[2], Peter Decker[2],
Jan Philip Oeyen[1], Jörg Spelda[3], Norman Lindner[4]

1 *Zoologisches Forschungsmuseum Alexander Koenig, Leibniz Institute for Animal Biodiversity, Center for Taxonomy and Evolutionary Research (Section Myriapoda), Adenauerallee 160, 53113 Bonn, Germany* **2** *Senckenberg Museum of Natural History Görlitz, Am Museum 1, 02826 Görlitz, Germany* **3** *Bavarian State Collection of Zoology, Münchhausenstraße 21, 81247 Munich, Germany* **4** *Lazarusstraße 34, 04347 Leipzig, Germany*

Corresponding author: *Thomas Wesener* (t.wesener@zfmk.de)

Academic editor: *Ivan H. Tuf* | Received 28 October 2014 | Accepted 15 May 2015 | Published 30 June 2015

http://zoobank.org/CD9AA03B-A43C-4D0A-B2A2-A6FF690470F9

Citation: Wesener T, Voigtländer K, Decker P, Oeyen JF, Spelda J, Lindner N (2015) First results of the German Barcode of Life (GBOL) - Myriapoda project: Cryptic lineages in German *Stenotaenia linearis* (Koch, 1835) (Chilopoda, Geophilomorpha). In: Tuf IH, Tajovský K (Eds) Proceedings of the 16th International Congress of Myriapodology, Olomouc, Czech Republic. ZooKeys 510: 15–29. doi: 10.3897/zookeys.510.8852

Abstract

As part of the German Barcode of Life (GBOL) Myriapoda program, which aims to sequence the COI barcoding fragment for 2000 specimens of Germany's 200 myriapod species in the near future, 44 sequences of the centipede order Geophilomorpha are analyzed. The analyses are limited to the genera *Geophilus* Leach, 1814 and *Stenotaenia* Koch, 1847 and include a total of six species. A special focus is *Stenotaenia*, of which 19 specimens from southern, western and eastern Germany could be successfully sequenced. The *Stenotaenia* data shows the presence of three to four vastly different (13.7–16.7% p-distance) lineages of the genus in Germany. At least two of the three lineages show a wide distribution across Germany, only the lineage including topotypes of *S. linearis* shows a more restricted distribution in southern Germany. In a maximum likelihood phylogenetic analysis the Italian species *S.* '*sorrentina*' (Attems, 1903) groups with the different German *S. linearis* clades. The strongly different *Stenotaenia linearis* lineages within Germany, independent of geography, are a strong hint for the presence of additional, cryptic *Stenotaenia* species in Germany.

Keywords
Barcode, biodiversity, COI, cryptic diversity

Introduction

The German Barcode of Life – Myriapoda project aims to sequence part of the mitochondrial cytochrome *c* oxidase subunit I gene known as the barcode fragment for all approximately 200 Myriapoda species in Germany (Voigtländer et al. 2011). Introduced species, mainly from greenhouses (Decker et al. 2014), will also be included. Myriapod barcoding is still in its infancy. While some studies incorporate COI data, this is mostly done on the species-level (e.g. Oeyen et al. 2014), and occasionally in genus-level studies (e.g. Stoev et al. 2010, Wesener et al. 2014). In Germany, a study of Bavarian myriapods (Spelda et al. 2011) pioneered research in this field.

Here, we show the preliminary results of one of the largest barcoding datasets compiled for centipedes of the order Geophilomorpha, with a special focus on the recently revised *Stenotaenia* Koch, 1847 (Bonato and Minelli 2008). *Stenotaenia* is distributed in Europe and the adjacent Mediterranean area and now includes 15 valid species. *Stenotaenia linearis* (Koch, 1835) is the type species of the genus, and the only species recorded from Germany (Voigtländer et al. 2011). After the resurrection of the genus in 2008, some redescriptions were undertaken (Dányi 2010), and the species *S. linearis* was recorded from Belgium for the first time (Lock 2009).

The taxonomic situation of the type species of *Stenotaenia*, *S. linearis*, is slightly confused, as the original Koch type specimens from Regensburg, Germany are apparently lost (Bonato and Minelli 2008). Seven species are currently synonymized under the name *S. linearis* (Bonato & Minelli, 2014). Another four valid species, *S. asiaeminoris* (Verhoeff, 1898), *S. giljarovi* (Folkmanova, 1956), *S. naxia* (Verhoeff, 1901), and *S. palaestina* (Verhoeff, 1925), spanning the entire geographical range of the genus, are difficult to distinguish from *S. linearis* (see Bonato and Minelli 2008). A correct definition of *S. linearis* is therefore a crucial necessity for any further taxonomic work in the genus.

Molecularly, little was done in *Stenotaenia*. One specimen of *S. linearis* was used for the Fauna Bavarica project (Spelda et al. 2011). Of other *Stenotaenia* species, only one sequence of *Stenotaenia 'sorrentina'* (Attems, 1903), a putative synonym (ICZN 2014) of *Geophilus forficularius* Fanzago, 1881, which was part of a recent phylogenetic study (Bonato et al. 2014) can be found. The discovery of unusually large genetic distances between different clades in German *S. linearis*, not found in any other German Geophilomorpha, and potentially independent of biogeography, prompted us to focus our attention on this species. In this study, the genetic distances in between German *Stenotaenia linearis* specimens are geographically analyzed and interpreted.

Material and methods

Specimen collection and preparation

Specimens were determined and collected by the authors of the study by hand, and either directly or after a few days transferred to vials containing 95% undenatured ethanol. The vials contain an individual GBOL number with which the specimens can be connected to the accompanying data. After conservation the specimens were either sent to the GBOL facility at the Museum Koenig, Bonn, Germany (ZFMK) or to the corresponding laboratory at the Bavarian State collection of Zoology, Munich, Germany (ZSM). Upon arrival, all specimens were photographed (images will be uploaded to BOLD, http://www.boldsystems.org/), and a tissue sample was removed for DNA extraction. All specimens will later be stored as vouchers in 95% undenatured ethanol, either at the ZFMK, the SMNG (Senckenberg Museum für Naturkunde, Görlitz) or the ZSM (see Table 1). For this specific GBOL subproject, DNA extraction was attempted for more than 35 specimens of *Geophilus* and 24 *Stenotaenia*, all specimens from Germany.

DNA extraction and sequencing

At the ZFMK, DNA was extracted from the tissue samples using the BioSprint96 magnetic bead extractor by Qiagen (Germany). After the extraction, samples were outsourced for PCR and sequencing (BGI China). For PCR and sequencing, HCO/LCO primer pairs (Folmer et al. 1994) were utilized. Because of a low PCR and sequencing success (<50%) for the Myriapoda, the degenerated primer pair HCOJJ/LCOJJ (Astrin and Stüben 2008) was used for further sequencing attempts, resulting in a much higher success rate (>75%). At the ZSM, a tissue sample was removed from each specimen and transferred into 96 well plates for subsequent DNA extraction at the Canadian Center for DNA Barcoding (CCDB) where they were processed using standard barcoding protocols. All protocols for DNA extraction, PCR amplifications and Sanger Sequencing procedures are available online under: http://www.dnabarcoding.ca/pa/ge/research/protocols.for DNA. DNA was extracted from the whole voucher at the CCDB. All samples were PCR amplified with modified Folmer primers CLepFolF, and the same primers were employed for subsequent Sanger sequencing. All voucher information and the DNA barcode sequences, primer pairs and trace files were uploaded to BOLD (http://www.boldsystems.org).

However, for more than five *S. linearis* and more than 10 *Geophilus* specimens no sequences could be obtained. Sequences were obtained for 19 *Stenotaenia* and 25 *Geophilus* specimens. Sequence identities were confirmed with BLAST searches (Altschul et al. 1997). All 44 new sequences were deposited in GenBank (see Table 1 for

Table 1. GBOL numbers, Genbank codes, locality data. GBOL number refers to DNA extraction and BOLD registration. SMNG = Senckenberg Museum für Naturkunde, Görlitz, Germany; ZFMK = Zoological Research Museum A. Koenig, Bonn, Germany; ZSM = Zoologische Staatssammlung München, Germany.

GBOL	GenBank	Voucher	Species	Locality
ZFMK-TIS-1318	KM999124	SMNG VNR016755-1	*Geophilus alpinus*	Saxony, Hirschfelde, Neißetal.
ZFMK-TIS-1449	KM999119	ZFMK MYR3840	*Geophilus alpinus*	Saxony, Leipzig, Leipziger Auwald, Revierort „Die Nonne“
ZFMK-TIS-1520	KM999120	ZFMK MYR3871	*Geophilus alpinus*	Saxony, Jähstadt, Annaberger Ratswald.
ZFMK-TIS-1560	KM999118	ZFMK MYR3875	*Geophilus alpinus*	Bavaria, Donaustauf.
ZFMK-TIS-1647	KM491674	ZFMK MYR3720	*Geophilus alpinus*	Saxony-Anhalt, Ilsenburg, Ilsetal.
ZFMK-TIS-1656	KM491579	ZFMK MYR3725	*Geophilus carpophagus*	Saxony-Anhalt, Ilsenburg, Ilsensteinhang.
ZFMK-TIS-2519834	KM491622	ZFMK MYR3813	*Geophilus carpophagus*	Saxony-Anhalt, Ilsenburg, Ilsensteinhang.
ZFMK-TIS-1413	KM491587	ZFMK MYR3653	*Geophilus electricus*	Saxony-Anhalt, Nordharz, Heudeber.
ZFMK-TIS-1518	KM491687	ZFMK MYR3673	*Geophilus electricus*	Saxony-Anhalt, Halberstadt, Athenstedt.
ZFMK-TIS-1650	KM491673	ZFMK MYR3723	*Geophilus electricus*	Saxony-Anhalt, Ilsenburg, Dreisageblocksberg.
ZFMK-TIS-19414	KM491636	ZFMK MYR2107	*Geophilus electricus*	North Rhine-Westphalia, Windeck, Stromberg.
ZFMK-TIS-1468	KM999123	ZFMK MYR3850	*Geophilus flavus*	Saxony, Zwickau, Brückeberg.
ZFMK-TIS-1525	KM491642	ZFMK MYR3676	*Geophilus flavus*	Saxony-Anhalt, Schönhausen (Elbe).
ZFMK-TIS-1603	KM491670	ZFMK MYR3705	*Geophilus flavus*	Saxony-Anhalt, Gerbstedt, Friedeburg.
ZFMK-TIS-6359	KM491617	ZFMK MYR3536	*Geophilus flavus*	Saxony, Gröditz, Weißenberg.
ZFMK-TIS-15516	KM491627	ZFMK MYR1004	*Geophilus flavus*	North Rhine-Westphalia, Bonn, Oberkassel Steinbruch.
ZFMK-TIS-15764	KM491602	ZFMK MYR1060	*Geophilus flavus*	North Rhine-Westphalia, Wuppertal, NSG ‚Im Hölken‘
ZFMK-TIS-15774	KM491626	ZFMK MYR1070	*Geophilus flavus*	North Rhine-Westphalia, Siebengebirge, Löwenburg.
ZFMK-TIS-15821	KM491693	ZFMK MYR1117	*Geophilus flavus*	North Rhine-Westphalia, Wuppertal, Dolinengelände Krutscheid.
ZFMK-TIS-19577	KM491685	ZFMK MYR1526	*Geophilus flavus*	North Rhine-Westphalia, Bonn, Kottenforst.
ZFMK-TIS-19591	KM491632	ZFMK MYR1543	*Geophilus flavus*	North Rhine-Westphalia, Heimbach, Meuchelberg.
ZFMK-TIS-19602	KM491649	ZFMK MYR1554	*Geophilus flavus*	North Rhine-Westphalia, Königswinter, Südhang Wolkenburg.
ZFMK-DNA-112780112	KM491570	ZSM-JSP100815-007	*Geophilus flavus*	North Rhine-Westphalia, Bielefeld, Brackweder Wald.
ZFMK-DNA-112780116	KM999125	ZSM-JSP120413-004	*Geophilus flavus*	Baden-Württemberg, Bad Urach, St. Johann Fohlenhof.
ZFMK-DNA-112780042	KM999126	ZSM-JSP120413-002	*Geophilus ribauti*	Baden-Württemberg, Bad Urach, St. Johann Fohlenhof.
ZFMK-TIS-19495	KM999122	ZFMK MYR1630	*Stenotaenia linearis*	North Rhine-Westphalia, Bonn, Oberkassel Steinbruch.

GBOL	GenBank	Voucher	Species	Locality
ZFMK-TIS-1450	KM999121	ZFMK-TIS-1450	*Stenotaenia linearis*	Saxony, Leipzig, Leipziger Auwald, Revierort „Die Nonne“.
ZFMK-TIS-15771	KM491663	ZFMK MYR1067	*Stenotaenia linearis*	North Rhine-Westphalia, Wuppertal, NSG ‚Im Hölken‘.
ZFMK-TIS-15861	KM491574	ZFMK MYR1157	*Stenotaenia linearis*	North Rhine-Westphalia, Wuppertal, Dolinengelände Krutscheid.
ZFMK-TIS-19430	KM491573	ZFMK MYR2030	*Stenotaenia linearis*	Rheinland-Pfalz, Altenkirchen, Seelbach bei Hamm.
ZFMK-DNA-112780045	KM491689	ZSM-JSP120412-003	*Stenotaenia linearis*	Baden-Württemberg, Esslingen, St. Bernhard.
ZFMK-DNA-112780062	KM491558	ZSM-JSP100514-021	*Stenotaenia linearis*	Bavaria, Dachau, palace garden.
ZFMK-DNA-112780066	KM491631	ZSM-JSP120411-001	*Stenotaenia linearis*	Baden-Württemberg, Esslingen, St. Bernhard.
ZFMK-DNA-112780069	KM491658	ZSM-JSP120408-007	*Stenotaenia linearis*	Baden-Württemberg, Hegnach, Hardtwald.
ZFMK-DNA-112780093	KM491637	ZSM-JSP120408-002	*Stenotaenia linearis*	Baden-Württemberg, Stuttgart, SW Max-Eyth-See.
GBOL11002	KP698104	ZSM-JSP141102-010	*Stenotaenia linearis*	Bavaria, Regensburg
GBOL10999	KP698105	ZSM-JSP141102-004	*Stenotaenia linearis*	Bavaria, Regensburg
ZFMK-TIS-19423	KR559681	ZFMK MYR2119	*Stenotaenia linearis*	North Rhine-Westphalia, Euskirchen, Bad Münstereifel, Gilsdorf.
ZFMK-TIS-2538216	KR559680	ZFMK MYR3467	*Stenotaenia linearis*	Saxony, Dresden, Gruna
ZFMK-TIS-1645	KR559679	ZFMK MYR3878	*Stenotaenia linearis*	Saxony, Zwickau, Brueckeberg
GBOL12266	KR736251	SMNG-VNR016704-1	*Stenotaenia linear*is	North Rhine-Westphalia. Bochum, Tippelsberg
GBOL12450	KR736248	ZSM-JSP150117-056	*Stenotaenia linearis*	Baden-Württemberg, Breisgau, Badenweiler
GBOL12421	KR736250	SMNG-MYR016705-1	*Stenotaenia linearis*	North Rhine-Westphalia. Unna, Selm
GBOL11224	KR736249	ZSM-JSP141113-005	*Stenotaenia linearis*	Baden-Württemberg, Ulm, Kiesental

accession numbers). The only available COI sequence of *Stenotaenia* (KF569300.1), labelled as *S. sorrentina*, was added to the dataset. In order to rule-out the accidental amplification of nuclear copies of the mitochondrial COI gene, the whole dataset was translated into amino acids following the 'invertebrate' code in MEGA6 (Tamura et al. 2013); internal stop codons were absent in our dataset. There were a total of 658 positions in the final dataset, gaps were absent.

Phylogenetic analysis

Sequences were aligned by hand in Bioedit (Hall 1999). The final dataset included 45 nucleotide sequences with 658 positions (44 newly sequenced and the one of *S.* '*sorrentina*' from GenBank). Phylogenetic analyses were conducted in MEGA6 (Tamura et al. 2013). A Modeltest, as implemented in MEGA6 (Tamura et al. 2013), was performed to find the best fitting maximum likelihood substitution model. Models with the lowest BIC scores (Bayesian Information Criterion) are considered to describe the best substitution pattern. Codon positions included were 1st+2nd+3rd+Noncoding. Modeltest selected the Tamura-Nei model (Tamura and Nei 1993) with gamma distribution and invariant sites as best fitting model (lnL -4245.19958, Invariant 0.55674, Gamma 1.176355, R 3.46, Freq A: 0.288843, T: 0.282885, C: 0.262778, G: 0.16546).

The evolutionary history was inferred by using the maximum likelihood method based on the selected Tamura-Nei model (Tamura and Nei 1993). The tree with the highest log likelihood (-4247.0145) is shown (Nei and Kumar 2000). The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+G, parameter = 1.1347)). The rate variation model allowed for some sites to be evolutionarily invariable ([+I], 55.5093% sites). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site.

Distance analysis

The number of pairwise base differences per site were calculated in MEGA6 (Tamura et al. 2013). Codon positions included were 1st+2nd+3rd+Noncoding. In the distance analysis, all positions containing 'N's were removed for each sequenced pair. There were a total of 658 positions in the final dataset. To further evaluate the divergence within the genera *Geophilus* and *Stenotaenia*, the frequency distribution of the pairwise intra- and inter-specific distances were analysed.

Results

Phylogenetic analysis

Geophilus is not clearly separated from *Stenotaenia* in our analysis (Fig. 1). The basal-most node of the tree supports three monophyletic groups: *G. flavus* (de Geer, 1778), a species formerly separated in a different genus, *Necrophloeophagus* Newport, 1842, all other *Geophilus*, and *Stenotaenia*. However, the other *Geophilus* receive little statistical support (34%). The monophyly of the individual *Geophilus* species, as well as the *Stenotaenia* lineages L1–L3, all receive 100% bootstrap support (Fig. 1).

All 13 specimens of *G. flavus* show little genetic distance (0–2.4%) to one another. Within the group containing the remaining *Geophilus* species, *G. ribauti* Brölemann, 1908, a species formerly treated as a member of the genus *Brachygeophilus* Brölemann, 1908, is in a basal position to a weakly supported clade (64% statistical support) including *G. electricus* (Linné, 1758), *G. carpophagus* Leach, 1814, and *G. alpinus* Meinert, 1870. In this clade, *G. electricus* (100% statistical support) is opposed to the sister-taxa *G. carpophagus* and *G. alpinus* (83% statistical support). Inside *G. electricus*, the one specimen from western Germany is opposed to the three from Saxony-Anhalt (Table 1 and Fig. 1). *G. alpinus* is the only analyzed *Geophilus* species with widely separated intraspecific groups (Fig. 1). A basal trichotomy (Fig. 1) divides the five analyzed specimens into three groups that can not be separated geographically.

Within *Stenotaenia*, a basal trichotomy separates the specimens into (1) *S. linearis* L1, (2) *S. 'sorrentina'*, and (3) the weakly supported (56% bootstrap support) *S. linearis* L2 (including the topotypes) together with *S. linearis* L3 (Fig. 1). *S. linearis* L1 includes three specimens, one from Bonn, another from Euskirchen, both in western Germany and one from Leipzig in eastern Germany. *S. linearis* L2 contains a single specimen from Dachau, one close to Ulm, as well as two topotypes from Regensburg, all in southern Germany, while the majority (12) of analyzed German *S. linearis* specimens are recovered in *S. linearis* L3 (Fig. 1). The L3 group is divided into two clusters (L3a and b), one including seven specimens representing a single haplotype from seven different localities in western and eastern Germany, and the other one including five specimens also representing a single haplotype from four different localities (Esslingen, Hegnach, Badenweiler, and Stuttgart) in south-western Germany.

Distance analysis

The distance analysis shows a first cluster of intraspecific distances ranging from 0–2.8%, with a *G. electricus* outlier at 4.9% (Fig. 2), a second cluster at 9.4–10.2%, and a third cluster, which overlaps with the interspecific distances, at 13.7–16.7%. Interspecific distances inside German *Geophilus* and *Stenotaenia* are high, varying from 16.3–22.0%. The highest observed genetic distance is between *Stenotaenia* and *Geophilus* species (16.6–22.7%), while the *Geophilus* species differ from one another by 17.2–21.7%.

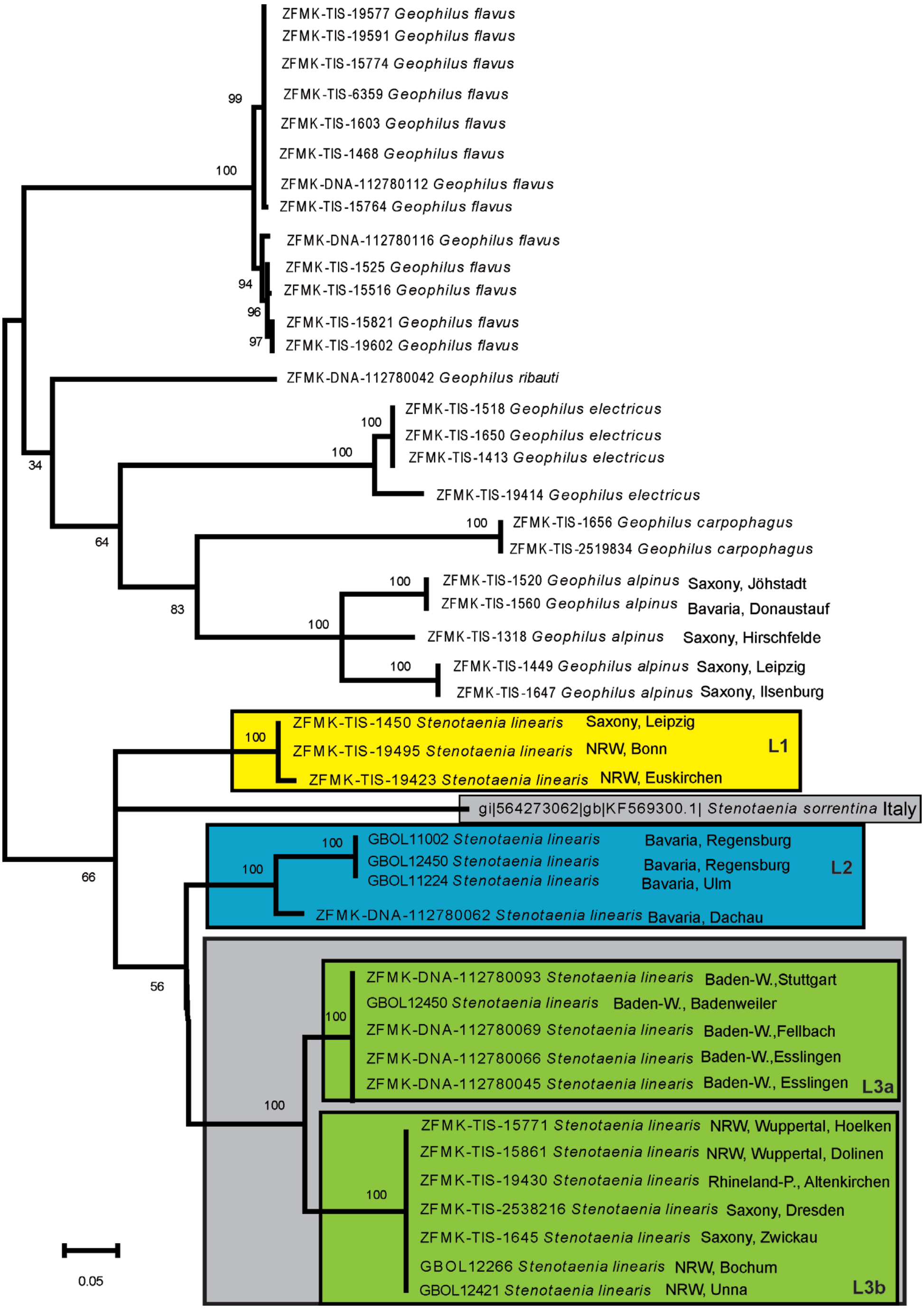


Figure 1. Maximum likelihood tree, 1000 bootstrap replicates. L1–L3 = *S. linearis* lineages 1–3; NRW = North Rhine-Westphalia; Baden-W = Baden-Württemberg. *S.* '*sorrentina*' comes from GenBank and might refer to *S. forficularis*. For exact locality data, see Table 1.

Discussion

Distance analysis

Clear intraspecific distances in German Geophilomorpha range from 0–5% (Fig. 2). A potential barcoding gap, however, is filled by the relatively high intraspecific distances (Fig. 2) of *G. alpinus* and *Stenotaenia* L3 (9.4–10.2% range). The genetic distances (13.7–16.7%) between the different *Stenotaenia* lineages (L1, L2 & L3) fall partly in the interspecific range of variation of the German Geophilomorpha (Fig. 2). The large interspecific distances (16.6–22.7%) observed among German Geophilomorpha are an indication that all species can be easily separated using the COI barcode marker. The distance analysis is partly biased towards interspecific distances because only a few specimens per species were analyzed. To explain the high nucleotide variability, excluding cryptic species, the presence of the maternally inherited endosymbionts (Hurst et al. 2005), as well as the origin of the lineages from different glacial refugia (Babik et al. 2005) followed by a subsequent fusion to a single species, need to be checked.

Three lineages of *Stenotaenia* in Germany

The three German *Stenotaenia* lineages are only weakly geographically separated (Fig. 3). *Stenotaenia* L1 is represented in our dataset with one specimen from Bonn, one from Euskirchen, and a third specimen from Leipzig (Fig. 1), the first two localities are separated from the third by more than 400 km apart (Fig. 3). This clade can be described as the *Stenotaenia* specimen from central Germany. All three specimens show the same haplotype.

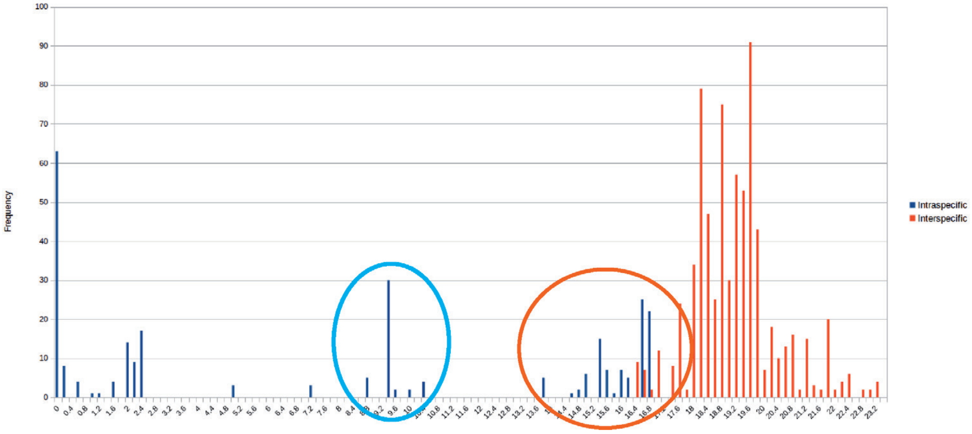


Figure 2. Frequency distribution of pairwise intraspecific (blue) and interspecific (red) distances. Blue circle = intraspecific distances of *G. alpinus* and among *S. linearis* L3; Red circle = interspecific distances and distances between *S. linearis* lineages. Basic table see Suppl. material 1.

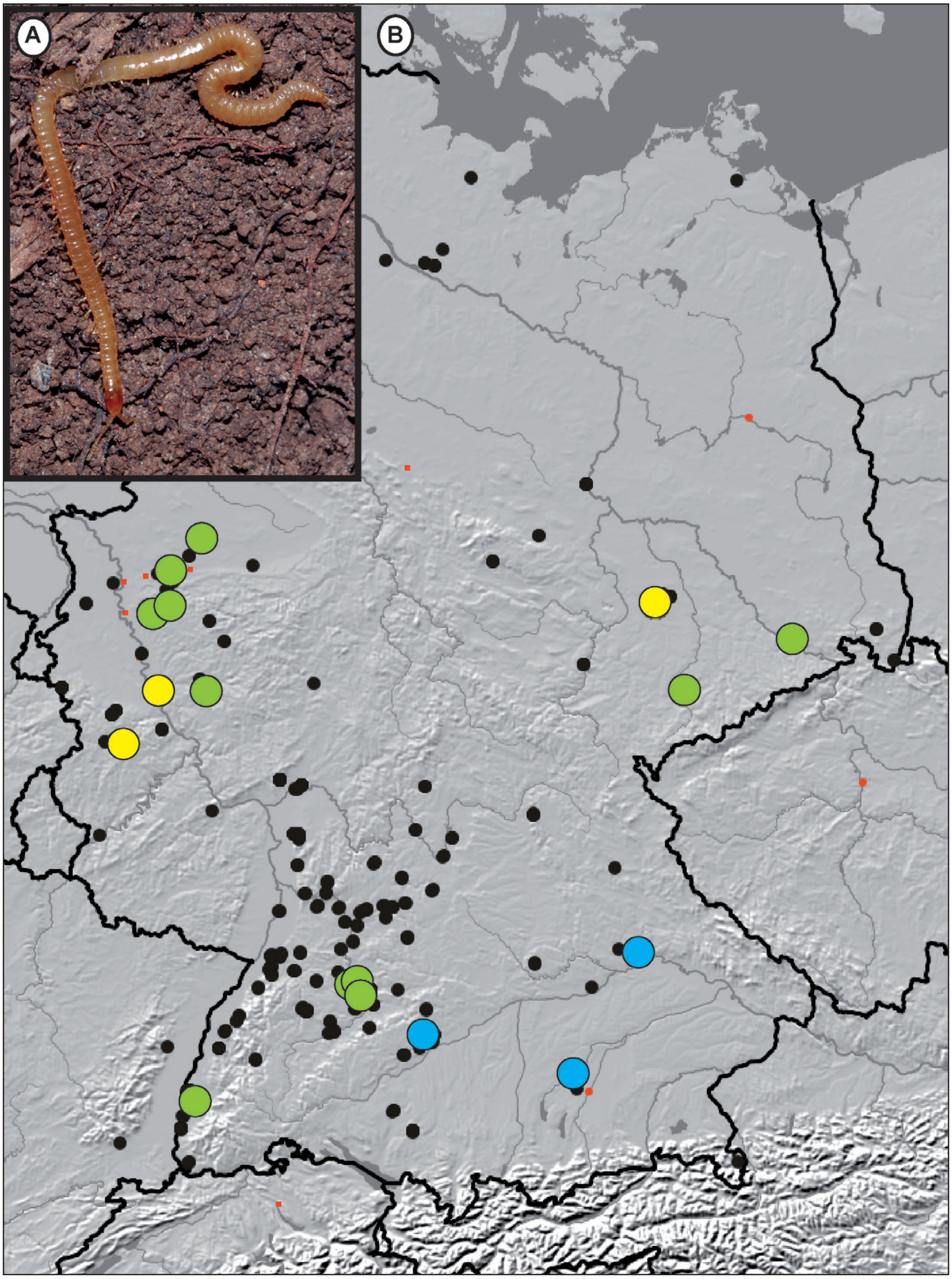


Figure 3. Map of *S. linearis* samples studied during GBOL (large dots), as well as other *S. linearis* records from Edaphobase, the ZSM and ZFMK collection (small dots, status 10.2014). Yellow = *S. linearis* L1; Blue = *S. linearis* L2; Green = *S. linearis* L3. (A) *S. linearis* in the field, photo: J. Spelda, specimen from Stuttgart-Hofen, Zuckerberg.

Stenotaenia L2 represents topotypic material from Regensburg, a specimen from the Kiesental near Ulm, as well as a single specimen from Dachau in southern Germany. All three localities are more than 100 km apart but only the specimen from Dachau differs by 1.4%. *S.* L2 differs significantly (13.7–16.7%) from other German *Stenotaenia*. This clade might be characterised as of southeastern German origin along the Danube river system.

Both clades of lineage 3, one from western and eastern Germany (L3a), the other from SW Germany (L3b) show identical haplotypes, but differ from one another by 9.4% (Fig. 3). The intraspecific difference is similar to the differences observed in some *Geophilus* species (9.4–10.2% in *G. alpinus*), but significantly larger than the differences observed in the widespread *G. flavus* (0.2–2.4%), which often come from the exact same localities as the *Stenotaenia* specimens (Table 1).

Whether or not the apparent sympatric distribution of the three different lineages of *Stenotaenia* in Germany (Fig. 3) might have been influenced by human-induced introduction or dispersal is not known. Virtually all collection localities are close to human habitats, but differ strongly in their current direct exposure to human activities.

Potential analysis problems and what we can learn for future work

Such a large project faces a set of predictable technical problems, which can potentially cause wrong results.

Specimen collections: According to the main aim of the project (get approx. 10 specimens from at least five localities for each species to capture the estimated German-wide COI variation), the different collectors preferred localities where they could find many myriapod specimens easily – a potential collection bias. The amount of successfully sequenced *S. linearis* specimens as well as *G. alpinus* specimens and their different positions and deep splits within the maximum likelihood tree (Fig. 1) tell us that a larger amount of specimens from many more regions in Germany (Fig. 3) would be a desirable object for future taxonomic and/or biogeographical studies on these species. Bergsten et al. (2012) showed that up to 70 individuals are required to sample 95% of the intraspecific variation.

Specimen determination: As done by Bonato et al. (2014) for all Geophilomorpha species, a data matrix of additional morphological characters, presumably morphometric characters, should be created for the detection of usable characters for determining the possible cryptic *S. linearis* taxa. However, it is not feasible to have such morphological studies as part of a large barcoding project like GBOL.

Taxonomic implications

Our analysis shows the importance of COI barcode data in the detection of taxonomic problems inside the centipede order Geophilomorpha. However, it also illustrates that barcode data alone does not clarify taxonomic problems. Only a thorough morphological study of the *Stenotaenia* species, including the types, plus the addition of nuclear markers, may be able to solve the complex picture of this genus.

As a result of the voucher-based barcoding effort, all analyzed specimens, and even their DNA extracts, are available for loan and should be incorporated into any future study of *Stenotaenia*.

Acknowledgements

Collection permits for natural protection areas were thankfully provided by the following people: H. Mölleken (Ressort Umweltschutz, Stadt Wuppertal), W. Wasch (Personal- und Organisationsamt, Bundesstadt Bonn), M. Ehling (Struktur- und Genehmigungsdirektion Nord, Rhineland-Palatine), F. Makiolczyk (Amt für Natur- und Landschaftsschutz, Rhein-Sieg-Kreis). T. Klug (ZFMK) provided assistance in collecting and determining the specimens. B. Rulik, J. Thormann, L. von der Mark form the GBOL-Team in Bonn and photographed, extracted and sequenced the ZFMK specimens, their invaluable help is greatly appreciated. M. Geiger assisted with the upload of the sequence data to GenBank. Special thanks go to M. Balke, F. Glaw, A. Hausmann, O. Hawlitschek, R. Melzer, J. Moriniere, I. Stöger, S. Schmidt for discussion, F. Ceseña, S. Friedrich, T. Lehmann, T. Meier, V. Svara, S. Swoboda and U. Biener-Miller (all ZSM) for sorting and preparing the ZSM samples. H. E. Wesener thankfully corrected the English of the manuscript. Two anonymous reviewers provided numerous comments that improved the quality of the here presented work.

This is a publication of the German Barcode of Life (GBOL) project of the Humboldt Ring, financed by the German Federal Ministry for Education and Research.

References

Altschul SF, Madden TL, Schäffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Research 25: 3389–3402. doi: 10.1093/nar/25.17.3389

Astrin JJ, Stüben PE (2008) Phylogeny in cryptic weevils: molecules, morphology and new genera of western Palaearctic Cryptorhynchinae (Coleoptera: Curculionidae). Invertebrate Systematics 22: 503–522. doi: 10.1071/IS07057

Attems C (1903) Synopsis der Geophiliden. Zoologische Jahrbücher, Abteilung für Systematik, Ökologie und Geographie der Tiere 18(2): 155–302.

Babik W, Branicki W, Crnobrnja-Isailovic J, Cogalniceanu D, Sas I, Olgun K, Poyarkov A, Gracia-Paris M, Arntzen JW (2005) Phylogeography of two European newt species – discordance between mtDNA and morphology. Molecular Ecology 14: 2475–2491. doi: 10.1111/j.1365-294X.2005.02605.x

Bergsten J, Bilton DT, Fujisawa T, Elliott M, Monaghan MT, Balke M, Hendrich L, Geijer J, Herrmann J, Foster GN, Ribera I, Nilsson AN, Barraclough TG, Vogler AP (2012) The effect of geographic scale of sampling on DNA barcoding. Systematic Biology 61(5): 851–869. doi: 10.1093/sysbio/sys037

Bonato L, Minelli A (2008) *Stenotaenia* Koch, 1847: a hitherto unrecognized lineage of western Palaearctic centipedes with unusual diversity in body size and segment number (Chilopoda: Geophilidae). Zoological Journal of the Linnean Society 153: 253–286. doi: 10.1111/j.1096-3642.2008.00394.x

Bonato L, Minelli A (2014) Chilopoda Geophilomorpha of Europe: a revised list of species, with taxonomic and nomenclatorial notes. Zootaxa 3770: 1–136. doi: 10.11646/zootaxa.3770.1.1

Bonato L, Drago L, Murienne J (2014) Phylogeny of Geophilomorpha (Chilopoda) inferred from new morphological and molecular evidence. Cladistics 30(5): 485–507. doi: 10.1111/cla.12060

Brölemann HW (1908) La haute Vallée de la Neste (Myriapodes). Bulletin de la Société d'Histoire Naturelle de Toulouse 41: 57–67.

Dányi L (2010) A Moesian geophilomorph (Myriapoda: Chilopoda) species from the Carpathians: Review and redescription of *Stenotaenia rhodopensis* (Kaczmarek, 1970). Biologia Bratislava 65(6): 1028–1033. doi: 10.2478/s11756-010-0116-5

Decker P, Reip HS, Voigtländer K (2014) Millipedes and Centipedes in German greenhouses (Myriapoda: Diplopoda, Chilopoda). Biodiversity Data Journal 2: e1066. doi: 10.3897/BDJ.2.e1066

de Geer C (1778) Neuvieme Memoire - Des Cloportes, Des Scolopendres & Des Jules. In: Mémoires pour servir à l'histoire des insectes, Tome VII. P. Hesselberg, Stockholm, VI+950 pp.

Fanzago F (1881) Ein neuer italienischer Geophilus. Zoologischer Anzeiger 4: 378–379.

Folkmanova B (1956) On new forms of Geophilomorpha from southern provinces of U.S.S.R. to the knowledge of the Myriapoda of U.S.S.R. Zoologicheskii Zhurnal 35: 1633–1646. [In Russian]

Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Molecular Marine Biology and Biotechnology 3(5): 294–299.

Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acid Science 41: 95–98.

Hurst GDD, Jiggins FM (2005) Problems with mitochondrial DNA as marker in population, phylogeographic and phylogenetic studies: the effects of inherited symbionts. Proceedings of the Royal British Society 272: 1525–1534. doi: 10.1098/rspb.2005.3056

ICZN (2014) Opinion 2335: *Geophilus linearis* CL Koch, 1835 (currently *Stenotaenia linearis*; Chilopoda): specific name conserved and *Geophilus sorrentinus* (currently *Stenotaenia sorrentina*; Chilopoda) Attems, 1903: specific name not conserved. Bulletin of Zoological Nomenclature 71(2): 138–140.

Koch CL (1835) Deutschlands Crustaceen, Myriapoden und Arachniden. In: Herrich-Schäffer GAW (Ed.) Deutschlands Insecten. Pustet, Regensburg, 136–190.

Leach WE (1814) Crustaceology. In: Brewster D (Ed.) The Edinburgh Encyclopaedia. Blackwood, Edinburgh 7(2): 383–437.

Lock K (2009) *Stenotaenia linearis* (C. L. Koch 1835): an expected species new to the Belgian fauna (Myriapoda Chilopoda). Bulletin de la Société Royale Belge des Electriciens 145: 114–116.

Meinert F (1870) Myriapoda Musaei Hauniensis. Bitrag til Myriapodernes morphologi og systematik. I. Geophile. Naturhistorisk Tidsskrift (3) 7: 1–128.

Nei M, Kumar M (2000) Molecular evolution and phylogenetics. Oxford University Press, Oxford.

Oeyen JP, Funke S, Böhme W, Wesener T (2014) The Evolutionary History of the Rediscovered Austrian Population of the Giant Centipede *Scolopendra cingulata* Latreille, 1829 (Chilopoda, Scolopendromorpha). PLoS ONE 9(9): 1–11. doi: 10.1371/journal.pone.0108650

Spelda J, Reip HS, Oliveira Biener U, Melzer RR (2011) Barcoding Fauna Bavarica: Myriapoda – a contribution to DNA sequence-based identifications of centipedes and millipedes (Chilopoda, Diplopoda). ZooKeys 115: 123–139. doi: 10.3897/zookeys.156.2176

Stoev P, Akkari N, Zapparoli M, Porco D, Enghoff H, Edgecombe GD, Georgiev T, Penev L (2010) The centipede genus *Eupolybothrus* Verhoeff, 1907 (Chilopoda: Lithobiomorpha: Lithobiidae) in North Africa, a cybertaxonomic revision, with a key to all species in the genus and the first use of DNA barcoding for the group. ZooKeys 50: 29–77. doi: 10.3897/zookeys.50.504

Tamura K, Nei M (1993) Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Molecular Biology and Evolution 10: 512–526.

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. Molecular Biology and Evolution 30: 2725–2729. doi: 10.1093/molbev/mst197

Verhoeff KW (1898) Beiträge zur Kenntniss paläarktischer Myriopoden. VI. Aufsatz: Ueber paläarktische Geophiliden. Archiv für Naturgeschichte 64(1): 335–362.

Verhoeff KW (1901) Zur vergleichenden Morphologie, Systematik und Geographie der Chilopoden. Beiträge zur Kenntnis paläarktischer Myriopoden XVI. Nova acta Academiae Caesareae Leopoldino-Carolinae Germanicae Naturae Curiosorum 77: 369–465.

Verhoeff KW (1925) Mediterrane Chilopoden und Notiz zur Periodomorphose der Juliden. Zoologischer Anzeiger 64(3-4): 63–80.

Voigtländer K, Reip HS, Decker P, Spelda J (2011) Critical reflections on German Red Lists of endangered myriapod species (Chilopoda, Diplopoda) (with species list for Germany). In: Mesibov R, Short M (Eds) Proceedings of the 15th International Congress of Myriapodology, 18–22 July 2011, Brisbane, Australia. International Journal of Myriapodology 6: 85–105. doi: 10.3897/ijm.6.2175

Wesener T, Le DMT, Loria SF (2014) Integrative revision of the giant pill-millipede genus *Sphaeromimus* from Madagascar, with the description of seven new species (Diplopoda, Sphaerotheriida, Arthrosphaeridae). ZooKeys 414: 67–107. doi: 10.3897/zookeys.414.7730

Supplementary material 1

Table. Estimates of Evolutionary Divergence between Sequences

Authors: Thomas Wesener, Karin Voigtländer, Peter Decker, Jan Philip Oeyen, Jörg Spelda, Norman Lindner

Data type: Measurement

Explanation note: The number of base differences per site from between sequences are shown. The analysis involved 45 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All ambiguous positions were removed for each sequence pair. There were a total of 658 positions in the final dataset. Evolutionary analyses were conducted in MEGA6.

ZooKeys 510: 31–47 (2015)
doi: 10.3897/zookeys.510.8808
http://zookeys.pensoft.net

RESEARCH ARTICLE

A peer-reviewed open-access journal
ZooKeys
Launched to accelerate biodiversity research

Molecular-based estimate of species number, phylogenetic relationships and divergence times for the genus *Stenotaenia* (Chilopoda, Geophilomorpha) in the Italian region

Laura Del Latte[1], Francesca Bortolin[1], Omar Rota-Stabelli[2],
Giuseppe Fusco[1], Lucio Bonato[1]

1 *Department of Biology, University of Padova, via U. Bassi 58/B, 35131 Padova, Italy* **2** *Department of Sustainable Agro-Ecosystems and Bioresources, Edmund Mach Foundation, San Michele all'Adige, 38010 Trento, Italy*

Corresponding author: *Laura Del Latte* (laura.del.latte@gmail.com)

Academic editor: *Ivan H. Tuf* | Received 23 October 2014 | Accepted 9 June 2015 | Published 30 June 2015

http://zoobank.org/EDF390C0-1278-4071-BCFF-FD079A612372

Citation: Del Latte L, Bortolin F, Rota-Stabelli O, Fusco G, Bonato L (2015) Molecular-based estimate of species number, phylogenetic relationships and divergence times for the genus *Stenotaenia* (Chilopoda, Geophilomorpha) in the Italian region. In: Tuf IH, Tajovský K (Eds) Proceedings of the 16th International Congress of Myriapodology, Olomouc, Czech Republic. ZooKeys 510: 31–47. doi: 10.3897/zookeys.510.8808

Abstract

Stenotaenia is one of the largest and most widespread genera of geophilid centipedes in the Western Palearctic, with a very uniform morphology and about fifteen species provisionally recognized. For a better understanding of *Stenotaenia* species-level taxonomy, we have explored the possibility of using molecular data. As a preliminary assay, we sampled twelve populations, mainly from the Italian region, and analyzed partial sequences of the two genes *COI* and *28S*. We employed a DNA-barcoding approach, complemented by a phylogenetic analysis coupled with divergence time estimation. Assuming a barcoding gap of 10–16% K2P pairwise distances, we found evidence for the presence of at least six *Stenotaenia* species in the Italian region, which started diverging about 50 million years ago, only partially matching with previously recognized species. We found that small-sized oligopodous species belong to a single clade that originated about 33 million years ago, and obtained some preliminary evidence of the related genus *Tuoba* being nested within *Stenotaenia*.

Keywords

COI, DNA barcoding, evolution, genetic distances, molecular dating, 28S

Introduction

Stenotaenia Koch, 1847 is one of the largest and most widespread genera of geophilid centipedes occurring in the Western Palearctic. Species of *Stenotaenia* have been recorded mainly from the Italian region, through the Balkan peninsula and the Aegean islands, to Anatolia (Bonato and Minelli 2008). However, there are also sparse records reaching the British isles, to the west (Barber 2009), the Alborz mountains in Iran (R. Zarei, unpublished data), to the east, and the Atlas chain plus Crete, Cyprus and Israel, to the south (Chipman et al. 2013, Simaiakis et al. 2013).

Like in most other centipedes, taxonomic recognition and delimitation of species in *Stenotaenia* have so far been based on scanty morphological evidence. However, all *Stenotaenia* species are remarkably similar in body anatomy and several fine morphological details, including most of the characters that are traditionally considered diagnostic at the species level in other geophilid genera (e.g., details of the labrum and the maxillary complex, shape and denticulation of the forcipules, arrangement of the sternal pore areas along the trunk, structure and shape of the legs of the ultimate pair and the associated metasternite, and arrangement of the coxal pores). On the contrary, *Stenotaenia* exhibits high variability in the number of trunk segments and adult body size, so that species-level current taxonomy is based almost exclusively on these two characters. Extreme morphologies are represented by *S. romana* (Silvestri, 1895), which is reported being less than 17 mm long when fully grown, with some specimens having only 43 leg-bearing segments, and *S. sturanyi* (Attems, 1903), reaching 77 mm in length, with specimens having up to 115 leg-bearing segments (Bonato and Minelli 2008). According to a recent critical reassessment of all available morphological information, no more than fifteen species have been recognized in *Stenotaenia* and more than half of these should be considered valid only provisionally, because supporting evidence is inadequate (Bonato and Minelli 2008).

For a better understanding of *Stenotaenia* species-level taxonomy, we have explored the possibility of using molecular data. As a preliminary assay, we analyzed two genes in a sample of populations from a significant part of the geographic range of the genus. Our aim was to estimate how many species could be recognized on the basis of DNA sequences, especially in comparison with the taxonomic scheme currently in use. We adopted a DNA-barcoding approach, complemented by a phylogenetic analysis coupled with divergence time estimation.

Material and methods

Sampling and DNA extraction

The target of our study was the genus *Stenotaenia* according to the taxonomic concept and circumscription currently in use (Bonato and Minelli 2008).

Our sampling focused on the western part of the known range of the genus. This area is centred on the Italian region s.l., which extends from the Alps and Istria, through

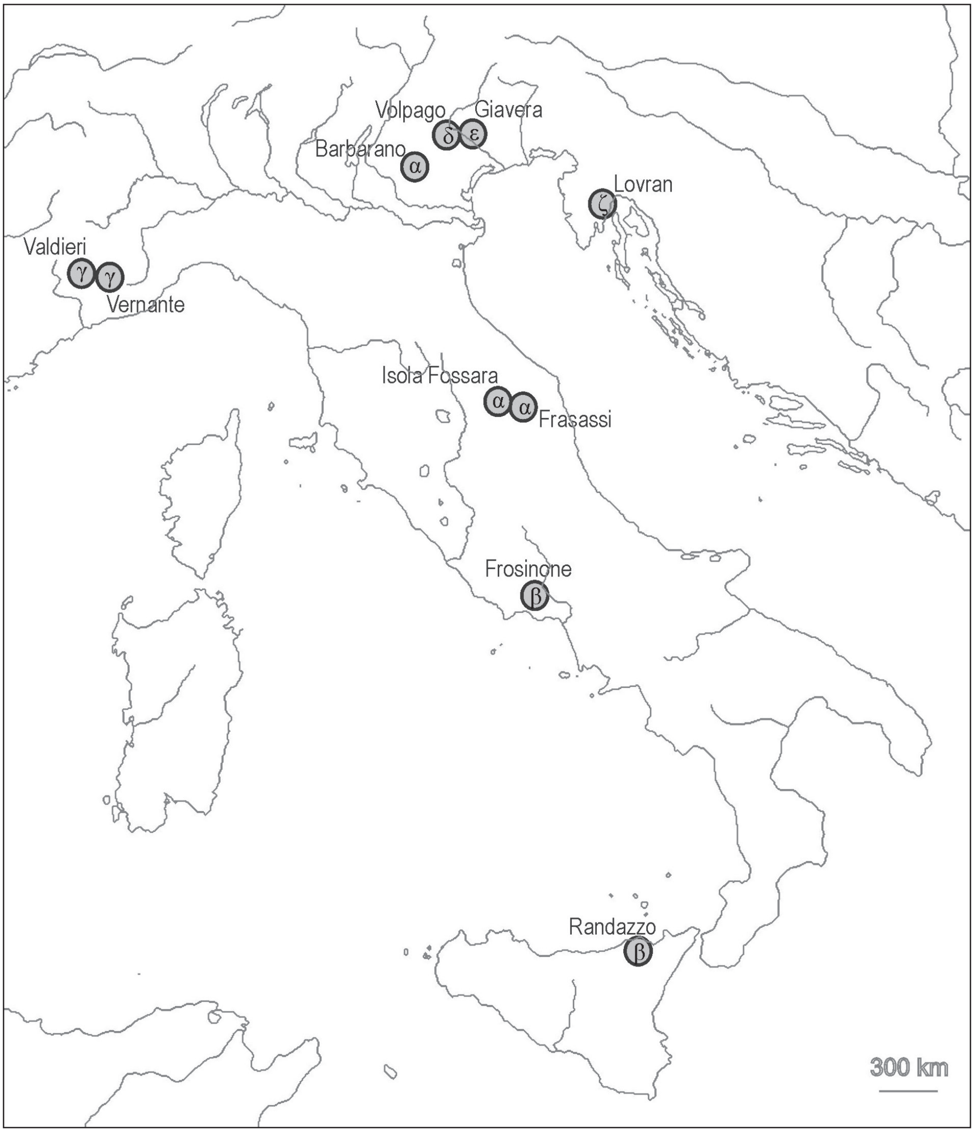


Figure 1. Sampling localities of *Stenotaenia* in the Italian region. Greek letters refer to the species tentatively recognized after the analyses (see text).

the entire Italian peninsula, to the Italian islands (Fig. 1). So far, this area has been investigated much more intensively than the remaining range of the genus (Bonato and Minelli 2008) and it represents the only part of the range for which a consistent taxonomic scheme for *Stenotaenia* has been developed. We sampled ten specimens from ten different populations within this area and two specimens from localities at the eastern borders of the known range of the genus, i.e. from Cyprus and from Iran (Table 1). Most of the specimens were obtained directly by recent sampling in the field, because most of

Table 1. Sampled specimens of *Stenotaenia*, arranged west to east and then north to south. The preliminary identification is just a tentative one, based only on the few morphological characters hitherto proposed as diagnostic at the species-level, including number of legs and geographical provenance (Bonato and Minelli 2008). Abbreviations for collectors: FB = F. Bortolin, GF = G. Fusco, LB = L. Bonato, MZ = M. Zapparoli, RZ = R. Zarei. Abbreviations for repositories of voucher specimens: BM = Bonato-Minelli's collection, Department of Biology, University of Padova; TE = Zoological Museum, University of Tehran.

Label	Region	Locality	Date and collectors	# of leg pairs	Sex	Repository and code	Species identification	
							preliminary	post analyses
Valdieri	Maritime Alps	near Valdieri: Sant'Anna di Valdieri	24.IX.2012. FB, GF, LB lg	61	♂	BM 3876	*Stenotaenia* cf. *sorrentina*	species γ
Vernante	Maritime Alps	near Vernante: Valle Grande	23.IX.2012. FB, GF, LB lg	63	♀	BM 3830	*Stenotaenia* cf. *sorrentina*	species γ
Barbarano	Berici hills	near Barbarano: San Giovanni	22.X.2012. LB lg	49	♀	BM 3570	*Stenotaenia romana*	*Stenotaenia romana*
Volpago	Venetian Prealps	near Volpago del Montello: Valle Padovana	2010. LB lg	63	♀	BM 767	*Stenotaenia* cf. *sorrentina*	species δ
Giavera	Venetian Prealps	near Giavera del Montello: Valle del Cavalletto	2013. FB, GF, LB lg	77	♂	BM 1787	*Stenotaenia linearis*	species ε
Lovran	Istria	near Lovran: Lovranska Draga-Visoče	23.IX.2011. LB lg	55	♂	BM 1816	*Stenotaenia palpiger*	*Stenotaenia palpiger*
Isola Fossara	Umbro-Marchigian Apennines	near Isola Fossara: Costa del Corno	2.XI.2007. LB lg	57	♀	BM 601	*Stenotaenia sorrentina*	*Stenotaenia romana*
Frasassi	Umbro-Marchigian Apennines	near Frasassi	XII.2010. LB lg	49	♀	BM 1453	*Stenotaenia romana*	*Stenotaenia romana*
Frosinone	Ausoni hills	near Frosinone: Falvaterra-Pastena	8.XII.2011. MZ lg.	63	-	BM 3668	*Stenotaenia sorrentina*	*Stenotaenia sorrentina*
Randazzo	Sicily	near Randazzo: Bosco del Flascio	8.IV.2013. FB, GF, LB, RZ lg	61	-	BM 4553	*Stenotaenia sorrentina*	*Stenotaenia sorrentina*
Cyprus	Cyprus	near Neo Chorio: Smigies-Kefalovrysia	2.I.2010. LB lg	75	♂	BM 1478	*Stenotaenia naxia*	*Stenotaenia naxia*
Iran	Alborz Mountains	near Dasht-e Lar	23.V.2012. RZ lg	-	-	TE 4298	*Stenotaenia* sp.	*Stenotaenia* sp.

the material already available in collections has proved unsuitable for DNA extraction, amplification and sequencing; in fact, the DNA of materials stocked in ethanol 70% or in other solutions used for morphological studies (i.e., lactophenol) easily degrades.

After a preliminary species identification based on morphology (Table 1) following Bonato and Minelli (2008), the specimens were fixed in absolute ethanol to preserve DNA from degradation. Total genomic DNA was extracted from a dissected intermediate portion of the trunk of each specimen, using the DNeasy Blood and Tissue kit (Qiagen, Hilden, Germany).

DNA amplification and sequencing

We sequenced a portion of the *cytochrome c oxidase subunit I* (*COI*) using the primer pair LCOI490/HCO2198 (Folmer et al. 1994), which amplifies an approximately 700 bp long fragment, and a portion of *28S rRNA* (*28S*) using the primer pair 28SD1F/28SrD4b (Boyer and Giribet 2007, Edgecombe and Giribet 2006), which amplifies a 1050 bp long fragment. For *COI* amplification we employed the following PCR conditions: an initial denaturation step at 95 °C for 5 min, followed by a variable number of cycles (27–35) including denaturation at 94 °C for 1 min, annealing (ranging from 40 to 47 °C) for 1 min and extension at 72 °C for 1.5 min, then a final step at 72 °C for 7 min. For *28S* amplification we applied the same thermal cycling profile except for the annealing step, ranging from 42 to 50 °C for 1 min. Each PCR product was screened for the potential successful amplification by electrophoresis on 1% agarose gel in 1X TAE and purified using MinElute PCR Purification Kit (Qiagen). Then it was directly sequenced on both strands with the same primer sets as used for amplification, using an ABI 3130 XL automatic capillary sequencer (Applied Biosystems, Branchburg, USA; service provided by BMR Genomics, Padova, Italy).

We obtained *COI* sequences for all 12 specimens of *Stenotaenia* (between 642 bp and 647 bp long) and *28S* sequences from 11 *Stenotaenia* specimens (between 952 and 1005 bp long) (Table 2).

Finch TV 1.4.0 (Geospiza, PerkinElmer) was used to check each chromatogram for nucleotide signal intensity and whole sequence signal strength. Sequences were edited manually to obtain a more accurate reading. For each specimen, forward and reverse sequences of the gene were aligned with default parameters with Clustal W2 (Larkin et al. 2007) and combined in a single sequence. After that, datasets of *COI* and *28S* of *Stenotaenia* specimens were aligned with the same procedure.

Analyses of sequence variation

For delimiting species in our sample through a DNA-barcoding approach, we calculated the pairwise distances of the *COI* sequences between all 12 *Stenotaenia* specimens in two alternative ways, namely by K2P distances (which is a standard for DNA-barcoding;

Table 2. GenBank accession numbers, GC-skew and GC-content of the sequences of all specimens of *Stenotaenia* and the outgroup species included in the phylogenetic analysis.

Label	**Repository and code**	**GenBank accession number**		**GC-skew**		**GC-content (%)**	
		COI	***28S***	***COI***	***28S***	***COI***	***28S***
Valdieri	BM 3876	LN811344	LN810434	-0.189	0.051	40.0	61.0
Vernante	BM 3830	LN811343	LN810433	-0.183	0.053	39.7	60.9
Barbarano	BM 3570	LN811341	LN810431	-0.176	0.054	38.2	59.3
Volpago	BM 767	LN811336	-	-0.246	-	45.9	-
Giavera	BM 1787	LN811339	LN810429	-0.136	0.044	38.6	61.5
Lovran	BM 1816	LN811340	LN810430	-0.201	0.051	41.4	57.5
Isola Fossara	BM 601	KF569300	KF569278	-0.237	0.047	43.0	59.3
Frasassi	BM 1453	LN811337	LN810437	-0.244	0.054	42.5	59.8
Frosinone	BM 3668	LN811342	LN810432	-0.244	0.058	41.4	61.1
Randazzo	BM 4553	LN811346	LN810435	-0.206	0.056	40.6	61.5
Cyprus	BM 1478	LN811338	LN810428	-0.188	0.069	46.8	61.8
Iran	TE 4298	LN811345	LN810436	-0.218	0.040	43.9	62.1
Arctogeophilus glacialis	-	KF569291	KF569268	-0.254	0.049	46.9	55.7
Clinopodes carinthiacus	-	KF569292	KF569269	-0.156	0.068	41.1	61.0
Geophilus alpinus	-	KF569294	KF569271	-0.235	0.080	47.3	57.5
Geophilus electricus	-	AY288750	HM453296	-0.217	0.072	43.4	58.1
Geophilus flavus	-	KF569296	KF569273	-0.232	0.063	42.4	61.5
Tuoba sydneyensis	-	AY288751	HM453297	-0.205	0.055	39.3	59.7

e.g., Hebert et al. 2003, Ratnasingham and Hebert 2007, Chevasco et al. 2014) and p-distances (which is also used in arthropods; e.g., Montagna et al. 2013), treating gaps with partial deletion and estimating standard errors by 500 bootstrap pseudoreplicates. The software MEGA 6 (Tamura et al. 2013) was employed. We analyzed the frequency distribution of both kinds of pairwise distances in order to recognize the putative *barcoding gap*. This is the interval which is expected to separate within-species distances from between-species distances in the distribution of pairwise distances (Hebert et al. 2003, Ratnasingham and Hebert 2007).

Phylogenetic analysis

For the phylogenetic analysis of the *Stenotaenia* sequences, we chose as outgroups six species of Geophilidae (Table 2) for which sequences of *COI* and *28S* were already available. These species were selected as representative of the genera reputed to be the most strictly related to *Stenotaenia* according to previous phylogenetic analyses (Murienne et al. 2010, Bonato et al. 2014) and, as far as possible, including the type species of these genera. Because compositional biases may interfere with the phylogenetic signal of the dataset (Rota-Stabelli and Telford 2008), we checked whether the outgroup sequences were ingroup-like in the GC-content and the GC-skew.

The full set of ingroup and outgroup sequences were aligned using Clustal W2 (with default parameters) and only the positions shared by all sequences were considered for the analyses. The single genes and the concatenated sequences were analyzed by a maximum likelihood (ML) approach with PHYML 3.1 (Guidon et al. 2010) and by a Bayesian approach using BEAST v1.7.2 (Drummond et al. 2012). We estimated the best-fitting replacement model according to the Akaike information criterion (Posada and Buckley 2004) implemented in jModelTest v2.1.1 (Darriba et al. 2012). The ML analyses were bootstrapped using 100 pseudoreplicates. Newick output trees were visualized and manually rooted (assuming, as far as possible, the monophyly of the ingroup) with FigTree v1.4.0 (Rambaut 2009).

Molecular dating

To estimate divergence dates between *Stenotaenia* sequences, we used the Bayesian method implemented in BEAST v1.7.2 with XML input files prepared using BEAUti v1.7.2 (Drummond et al. 2012). Since no fossil record exists for any of the taxa represented in our dataset, we relied on both a root prior and a replacement rate derived from previous date estimates. As root prior we used an estimate of 200 million years (Ma), with a permissive SD=50, for the basal split in the phylogeny obtained, between the most basal outgroup and the remaining taxa, according to the dated phylogeny of Murienne et al. (2010). As replacement rate we assumed a value of 0.0016 substitutions/site/Ma, with SD = 0.0010, which was previously estimated analyzing a *18S+28S* dataset from a wide range of arthropods, including myriapods (Rota-Stabelli et al. 2013). The choice of this rate was motivated by a lack of more taxon-specific rate for centipede *28S*. We modelled the molecular replacement with the best-fitting model selected by the Akaike information criterion, and the rate distribution using a random clock model. All other priors were those of BEAST at default settings.

Results

Pattern of sequence diversity

Considering the pairwise distances of *COI* between the sampled specimens of *Stenotaenia* (Table 3), the minimum distance (about 0.5% for both the K2P and the p-distances) was recorded between two specimens from the Western Alps (Valdieri, Vernante), which had been previously recognized as belonging to a single species based on morphology (Table 1). The maximum distance (27.4% for the K2P distances, 22.6% for the p-distances) was estimated between a specimen from Cyprus and a specimen from the Eastern Alps (Barbarano), previously recognized as belonging to distinct species based on morphology (Table 1). Considering the frequency distributions of the pairwise distances of both models (Fig. 2), two major intervals of zero frequency might

Table 3. Pairwise distances (in percentage, with standard errors in parenthesis) between all *Stenotaenia* specimens, based on *COI* sequences, obtained with the K2P model (top right) and with the p-distances (bottom left).

	Cyprus	**Volpago**	**Lovran**	**Barbarano**	**Frosinone**	**Isola Fossara**	**Frasassi**	**Randazzo**	**Iran**	**Vernante**	**Valdieri**	**Giavera**
Cyprus		26.6 (2.3)	27.0 (2.2)	27.4 (2.3)	25.1 (2.1)	26.0 (2.2)	25.3 (2.1)	24.5 (2.1)	23.4 (2.0)	24.4 (2.0)	24.4 (2.0)	22.9 (2.0)
Volpago	22.1 (1.5)		22.4 (1.9)	20.5 (1.8)	16.6 (1.7)	21.2 (1.8)	21.2 (1.8)	16.6 (1.6)	19.9 (1.9)	18.9 (1.9)	19.5 (2.0)	23.8 (2.0)
Lovran	22.4 (1.5)	19.2 (1.4)		19.5 (1.8)	23.7 (2.0)	23.0 (2.0)	21.7 (1.9)	23.5 (2.0)	22.6 (1.9)	22.4 (1.8)	23.0 (1.9)	23.0 (1.9)
Barbarano	22.6 (1.5)	17.8 (1.4)	17.0 (1.4)		18.0 (0.17)	10.4 (1.2)	10.2 (1.2)	20.3 (1.8)	21.3 (1.9)	20.4 (1.8)	21.0 (1.9)	24.0 (2.0)
Frosinone	21.2 (1.5)	14.7 (1.3)	20.1 (1.4)	15.9 (1.4)		18.5 (1.7)	18.7 (1.6)	6.5 (1.1)	16.1 (1.6)	17.2 (1.7)	17.4 (1.8)	23.5 (2.0)
Isola Fossara	21.6 (1.5)	18.2 (1.3)	19.5 (1.4)	9.5 (1.0)	16.3 (1.3)		7.6 (1.1)	20.6 (1.9)	22.3 (2.0)	21.3 (1.9)	21.6 (2.0)	23.8 (1.9)
Frasassi	21.2 (1.5)	18.2 (1.3)	18.6 (1.4)	9.5 (1.1)	16.4 (1.3)	7.0 (1.0)		21.0 (1.8)	20.1 (1.8)	20.2 (1.8)	20.5 (1.8)	23.1 (1.9)
Randazzo	20.7 (1.4)	14.7 (1.3)	20.0 (1.4)	17.6 (1.4)	6.2 (0.9)	17.8 (1.3)	18.1 (1.3)		16.5 (1.6)	16.8 (1.8)	16.6 (1.8)	22.3 (1.9)
Iran	19.9 (1.5)	17.2 (1.4)	19.3 (1.4)	18.4 (1.4)	14.4 (1.3)	19.0 (1.4)	18.1 (1.4)	14.6 (1.2)		16.2 (1.6)	16.4 (1.6)	24.7 (2.0)
Vernante	20.6 (1.4)	16.2 (1.4)	19.2 (1.4)	17.8 (1.4)	15.2 (1.4)	18.4 (1.4)	17.6 (1.4)	14.9 (1.4)	14.4 (1.3)		0.5 (0.3)	21.3 (1.9)
Valdieri	20.6 (1.4)	16.7 (1.4)	19.6 (1.4)	18.2 (1.4)	15.3 (1.4)	18.6 (1.3)	17.8 (1.4)	14.7 (1.4)	14.5 (1.3)	0.5 (0.3)		21.9 (1.9)
Giavera	19.5 (1.4)	20.1 (1.4)	19.6 (1.4)	20.3 (1.4)	19.9 (1.4)	20.1 (1.3)	19.6 (1.3)	19.0 (1.4)	20.7 (1.4)	18.2 (1.3)	18.7 (1.3)	

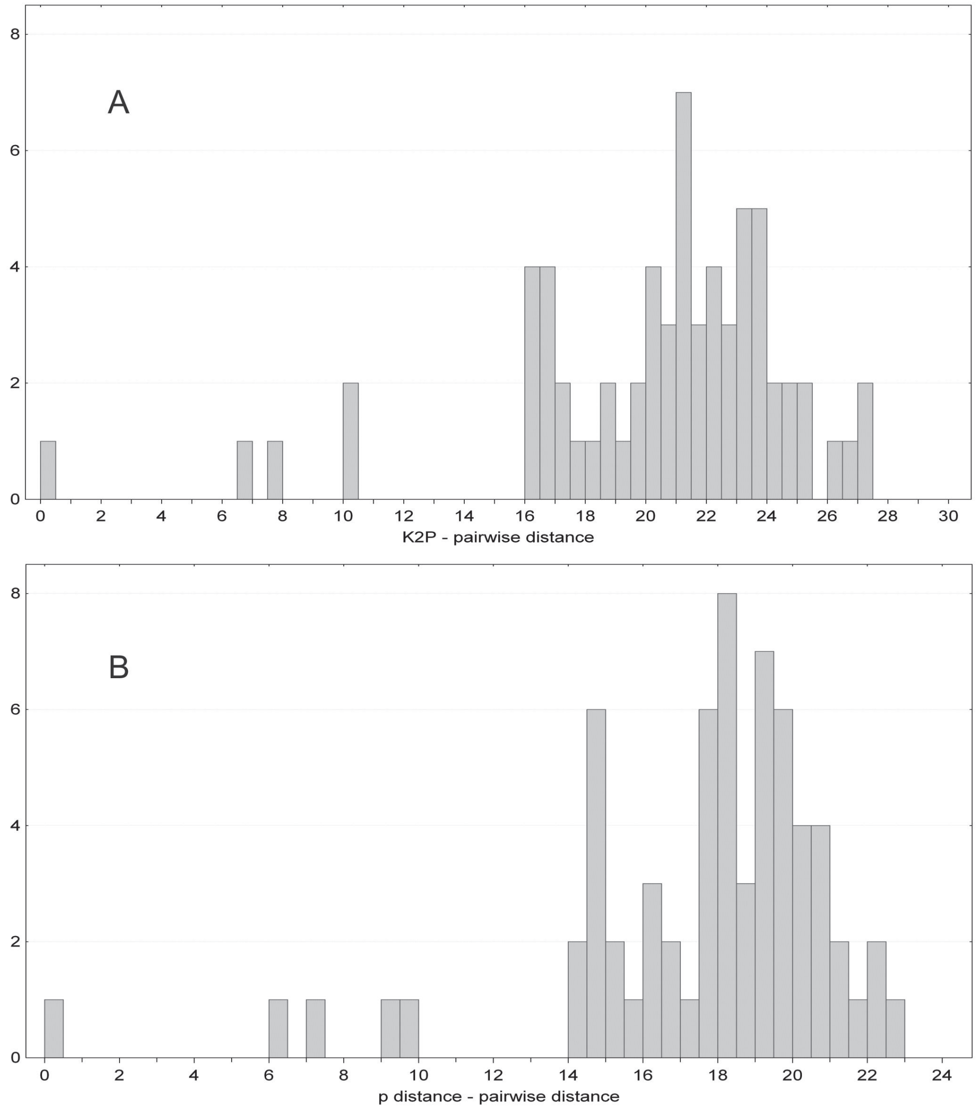


Figure 2. Frequency distribution of *COI* pairwise distances. **A** K2P distances. **B** p-distances.

be recognized: a lower one (0.5–6.5% for K2P, 0.5–6.2% for p-distances) and a higher one (10.4–16.1% for K2P, 9.5–14.4% for p-distances).

Assuming a barcoding gap corresponding to the lower observed gap, we would obtain 11 species of *Stenotaenia* from our sample of 12 specimens, of which nine species in the Italian region (between Alps, Istria and Sicily), with only two specimens from the Western Alps resolved as conspecific. On the contrary, assuming a barcoding gap corresponding to the higher observed gap, we would obtain eight species of *Stenotaenia*, of which six in the Italian region.

Phylogenetic relationships

After the alignment of ingroup and outgroup sequences, the average nucleotide composition of *COI* turned to be A = 0.289, C = 0.248, G = 0.163, T = 0.299, and that of *28S* resulted as A = 0.219, C = 0.283, G = 0.317, T = 0.181. A bias against G-C in the composition of *COI* has been observed in other Chilopoda as well (Spelda et al. 2011, Oeyen et al. 2014). The GC-contents (Table 2) of the sequences of the outgroups turned out being within the range of variation of *Stenotaenia* sequences (38.2–46.8% for *COI*, 57.5–62.1% for *28S*), with minor exceptions for *A. glacialis* and *G. alpinus* (slightly higher for *COI*, slightly lower for *28S*). Also the GC-skew values (Table 2) of most of the outgroup sequences turned out within the range of variation of the *Stenotaenia* sequences (between -0.246 and -0.136 for *COI*, between 0.040 and 0.069 for *28S*), but with some exceptions (slightly lower for *COI* in *A. glacialis*, higher for *28S* in *G. alpinus* and *G. electricus*).

For the ML phylogenetic analysis, the Generalized Time Reversible model with proportion of invariable sites and a Gamma distribution (GTR+I+G) with four discrete categories was selected as the best-fit model for nucleotide substitution with the Akaike information criterion. We applied this model for the datasets of the *COI* sequences and the *28S* sequences, when analyzed separately and when concatenated.

The ML tree, obtained from the concatenated sequences of the 11 *Stenotaenia* specimens from which we got workable sequences for both genes, is shown in Fig. 3. Very similar topologies for the ingroup were found in the ML trees obtained from the single genes separately and in the Bayesian tree obtained from the concatenated sequences (trees not shown, but node supports shown on the ML tree, Fig. 3). Three groups of *Stenotaenia* specimens were found to be strongly supported (at least when analyzing the two genes together) and corresponded to groups obtained by the DNA-barcoding analysis assuming the higher barcoding gap (10.4–16.1% for K2P, 9.5–14.4% for p-distances). These are: a group including three specimens between the Eastern Prealps and the northern part of the Italian peninsula (Barbarano, Frasassi, Isola Fossara), a second group with the two specimens from the southern part of the Italian peninsula and Sicily (Frosinone, Randazzo), and a last group with both specimens from the Western Alps (Vernante, Valdieri). In addition, we found strong support for a clade including the first group listed above and a specimen from Istria (Lovran), and for another clade including the other two groups already listed plus another specimen from the Eastern Prealps (Giavera) and a specimen from Iran.

However, the monophyly of the genus *Stenotaenia*, as currently circumscribed, was not recovered in our analyses: the specimen representative of the genus *Tuoba* was found nested within the clade encompassing all *Stenotaenia* specimens in both ML and Bayesian analyses, and also in the ML analysis of *28S*, even though in different positions (Fig. 3); in the ML analysis of *COI*, instead, the specimen representative of the genus *Clinopodes* was nested within *Stenotaenia*.

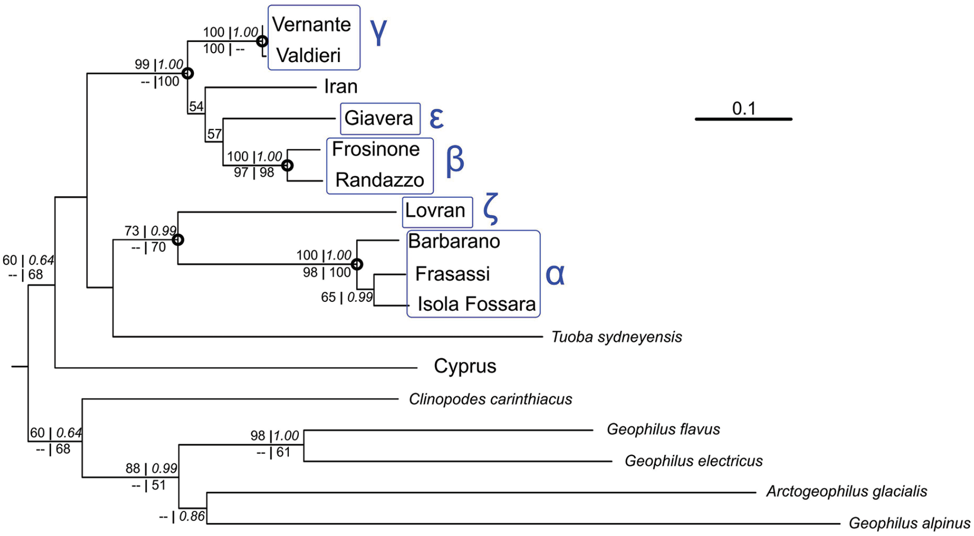


Figure 3. Maximum likelihood phylogeny. ML tree obtained from concatenated *COI* and *28S* sequences, by the GTR+I+G model, and manually rooted. The following support values are indicated at the nodes (only for those present in the topology obtained from the concatenated sequences): ML bootstrap for the analysis of concatenated genes (upper left); Bayesian posterior probabilities (upper right, in italics); ML bootstrap for the analysis of *COI* sequences (lower left); ML bootstrap for the analysis of *28S* sequences (lower right). Bootstrap values < 50% and posterior probabilities < 0.50 are not shown. Circles indicate ingroup nodes that are highly supported in the tree based on concatenated sequences. Terminal node groupings indicated by Greek letters refer to the species tentatively recognized (see text and Fig. 1). The specimen from Volpago (species δ) is absent because its *28S* sequence was not obtained.

Divergence time

By applying a molecular clock on a Bayesian analysis of the *28S* sequences, we obtained a tree topology (Fig. 4) largely consistent with ML and Bayesian trees obtained from the concatenated dataset (Fig. 3). Differences are limited to the unstable position of *Tuoba* and two nodes with weak support.

The common ancestor of all *Stenotaenia* representatives (including *Tuoba* if it comprises a monophyletic group together with *Stenotaenia*) was dated at about 64 Ma. The divergence between the two major groups of *Stenotaenia* present in the Italian region (one represented by specimens from the northern part of the Italian peninsula, besides from the Eastern Prealps and Istria; another represented by specimens from the southern part of Italian peninsula and Sicily, besides from the Western Alps and the Eastern Prealps) was dated at about 51 Ma. Within the first group, the lineage of the specimen from Istria diverged from the remaining lineages around 33 Ma. The estimated age of the subsequent divergences are all younger than 10 Ma. In particular, for the groups obtained by the DNA-barcoding analysis of *COI* sequences assuming a higher barcoding gap and well supported in the phylogenetic analyses (see above), the

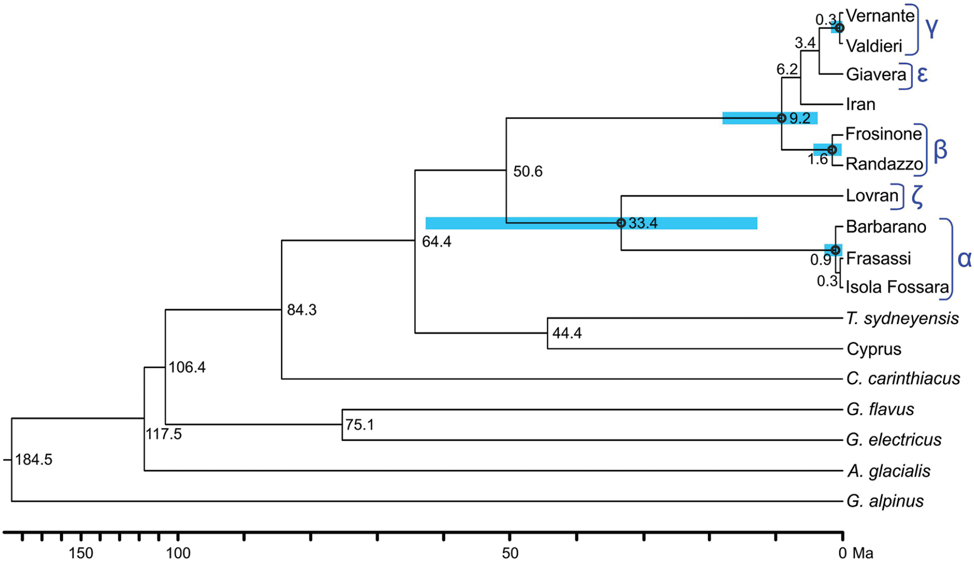


Figure 4. Dated phylogeny. Estimates of divergence time, calculated using *28S* sequences and two priors (age of the root and substitution rate) in the package BEAST v1.7.2 (see text). 95% High Posterior Density intervals are represented by coloured bars for the most robust nodes, emphasized by a circle. Greek letters refer to the species tentatively recognised (see Fig. 1). The tree has the same topology of the concatenated ML tree of Fig. 3, but for the position of *Tuoba sydneyensis* and the relationships within the group formed by species β, ε, γ and the specimen from Iran. The specimen from Volpago (species δ) is absent because its *28S* sequence was not obtained. Time scale is different in the two intervals 0–100 and 100–200 Ma.

common ancestor of each group was dated younger than 1.6 Ma, whereas all divergences between these groups and other specimens of *Stenotaenia* were all dated older than 3.4 Ma.

Putative species of *Stenotaenia*

The results of the DNA-barcoding analysis of *COI* sequences, of the phylogenetic reconstructions on the basis of the two genes, and of the divergence date estimation based on the *28S* sequences, all agree in suggesting that at least six species of *Stenotaenia* are recognizable in our sample from the Italian region (Fig. 1): α (three populations between the Eastern Prealps and the northern part of the Italian peninsula), β (two populations from the southern part of Italian peninsula and Sicily), γ (two populations from Western Alps), δ and ε (each represented by a single population in the Prealps), ζ (one population from Istria). Additionally, the two populations from Cyprus and Iran probably belong to different species.

Discussion

The analysis of the pairwise distances of the *COI* sequences, following our DNA-barcoding approach, suggested two alternative putative barcoding gaps, both consistent with the results of our phylogenetic and dating analyses. However, the two gaps entail different scenarios for the cladogenesis in *Stenotaenia*, including a different estimate of the minimum number of species inhabiting the Italian region.

Assuming a low barcoding gap (1–6% of K2P distances) would be in agreement with a threshold around 1% between intra- and inter-specific distances, as more frequently found and applied in a large assortment of animal taxa, including vertebrates and some groups of arthropods (Ratnasingham and Hebert 2007, Chevasco et al. 2014). However, in this case we should conclude that (i) almost every single specimen in our small sample would actually represent a distinct species, (ii) some of these species separated as recently as a few hundred thousand years ago, and (iii) at least nine species of *Stenotaenia* actually inhabit the Italian region. In this scenario, a remarkable number of species would be expected for the entire geographic range of the genus, in face of the morphological uniformity between species and departing dramatically from the taxonomic scheme in use (Bonato and Minelli 2008).

As an alternative, assuming a higher barcoding gap (10–16% of K2P distances) would be in agreement with gaps estimated in other centipedes, in the very few studies so far published: a gap in p-distances between 9% and 14% for the scolopendromorph genus *Scolopendra* (Oeyen et al. 2014), a gap in K2P distances between 7% and 10% for the lithobiomorph genus *Eupolybothrus* (Stoev et al. 2010, 2013) and average intraspecific vs. interspecific K2P distances of 7% vs. 18%, for a centipede sample from the Bavarian fauna (Spelda et al. 2011). This is also in agreement with the divergence time analysis, as under the higher barcoding gap scenario all interspecific nodes would be older than one Ma, which is a more reasonable figure (Coyne and Orr 2004). In this scenario, we should acknowledge a lower number of species in our sample. On the basis of this more parsimonious assumption, the minimum number of six species we obtain for the Italian region is nonetheless higher than the four species hitherto recognized on the basis of morphology, a number which besides is derived by a far more extensive geographic sampling (Bonato and Minelli 2008).

Despite evidence being very preliminary, species delimitation suggested by our study may be tentatively compared and matched with these four morphospecies.

The putative species α might correspond to *Stenotaenia romana* (Silvestri, 1895), even if it includes also a specimen initially identified as *S. sorrentina*. *S. romana* was customarily distinguished from all other species in the Italian region by its remarkably minute body size (total length not surpassing 17 mm) and a distinctly lower range of variation in the number of trunk segments (43–49 pairs of legs, Bonato and Minelli 2008). *S. romana* inhabits mainly the Tyrrhenian side of the Italian peninsula, from Liguria to Campania, including some minor islands (e.g., Ischia and Elba), and also Sardinia; apparently disjunct populations are present in the Euganei and Berici hills (unpublished data).

Species β confidently corresponds to *S. sorrentina* (Attems, 1903), which is usually circumscribed by having intermediate body size (at least 20 mm when fully grown) and an intermediate number of trunk segments (usually 53–67 leg pairs, Bonato and Minelli 2008). This morphospecies has been frequently recorded in the Italian peninsula from Maritime and Ligurian Alps to Gargano and Calabria, and more rarely and doubtfully also from the Prealps. It is also reported from several islands, including Sardinia and minor Tyrrhenian islands (e.g., Elba, Ponza, Capri and Ischia). Our sample from Sicily (Table 1) confirms the presence of this species in this island, from where only old and dubious records were available so far (Bonato and Minelli 2008).

The species ζ may correspond to *S. palpiger* (Attems, 1903), which was known so far only for the holotype (total length 15 mm and 49 leg pairs), collected from Istria, about 30 km from the locality of our sample. The species name *palpiger* has been most often ignored and only recently resurrected as a potentially valid species (Bonato and Minelli 2008). However, the identity and distribution of *S. palpiger* remain to be clarified, especially with respect to other nominal species of *Stenotaenia* reported from the Dinaric region, including *S. antecribellata* (Verhoeff, 1898) and *S. cribelliger* (Verhoeff, 1898) (Bonato and Minelli 2008).

The remaining three putative species (γ from Western Alps, δ and ε from Eastern Prealps) cannot be assigned confidently to known species. Different number of legs in their specimens had initially suggested different tentative identification (see Table 1). However, according to their origins, it is impossible to tell which of them, if any, actually belongs to *S. linearis*, which is the fourth species traditionally reported for the Italian region. The type locality of *S. linearis* is in Baviera (near Regensburg) and based on morphology the species has been reported also from the Alps, as far south as the Maritime Alps (Bonato and Minelli 2008).

While the specimen from Cyprus is confidently recognizable as a different species, *S. naxia* (Verhoff, 1901) (Simaiakis et al. 2013), which is also recorded from the Aegean area (Bonato and Minelli 2008), nothing can be said for the specimen from Iran. The number of leg-bearing segments and the morphology of the ano-genital region are unknown because the posterior part of the trunk is missing. This makes it very difficult to associate this specimen to any known species, also because no previous records for the genus are known for this geographic area.

Our preliminary evaluation of the phylogenetic relationships and the divergence dates between these species have implications on the evolutionary history of the group which invite some cautious comments.

All specimens in our sample that are representative of species with smaller body size and lower number of legs (*S. romana* and *S. palpiger*) apparently represent a single derived clade within the genus, suggesting that these features are synapomorphic for these two species. Considering the entire clade, as far as known, the body length is less than 20 mm long at full growth and the number of leg pairs ranges from 43 (in *S. romana*; Bonato and Minelli 2008) to 57 (in the *S. romana* specimen from Isola Fossara).

The genus *Tuoba* Chamberlin, 1920, which is currently recognized as distinct from *Stenotaenia* in different respects (morphological features, ecological niche, pattern of distribution; Bonato 2011) turned out to be strictly related to the latter, suggesting also the possibly that *Tuoba* is a derived clade within *Stenotaenia*. The phylogenetic position of *T. sydneyensis* among the *Stenotaenia* representatives in our sample is unstable and weakly supported, and could also be an artefact of our analysis, possibly produced by the attraction among sequences sharing similar GC-content and GC-skew values (Table 2).

This study is a very preliminary attempt to address the diversity of *Stenotaenia* with genetic data. It was limited by a relatively low efficacy in DNA extraction, amplification and sequencing from the available specimens (actually up to 15 specimens from 14 localities had been originally processed). This prevented us from analyzing a larger sample of specimens, and did not permit taking into account variation within populations. Nevertheless, our results could serve as a basis for forthcoming investigations on the genus *Stenotaenia*, not only in the Italian region, but also in the entire distribution range of the genus.

Acknowledgements

We are grateful to R. Zarei (University of Tehran) and M. Zapparoli (University of Tuscia, Viterbo) for providing precious specimens for the analysis. We also thank G.D. Edgecombe and V. Vahtera for providing insightful comment on a previous version of the article. This work has been supported by a grant (CPDA115439/11) from the Italian Ministry of Education, University and Research (MIUR) to LB.

References

Barber AD (2009) Synopses of the British Fauna. The Linnean Society of London 58: 228 pp.

Bonato L (2011) The Chilopoda – Taxonomic overview. Order Geophilomorpha. In: Minelli A (Ed.) The Myriapoda Vol. 1. Brill, Leiden, 407–443.

Bonato L, Drago L, Murienne J (2014) Phylogeny of Geophilomorpha (Chilopoda) inferred from new morphological and molecular evidence. Cladistics 30: 485–507. doi: 10.1111/cla.12060

Bonato L, Minelli S (2008) *Stenotaenia* Koch, 1847: a hitherto unrecognized lineage of western Palaearctic centipedes with unusual diversity in body size and segment number (Chilopoda: Geophilidae). Zoological Journal of the Linnean Society 153: 253–286. doi: 10.1111/j.1096-3642.2008.00394.x

Boyer SL, Giribet G (2007) A new model Gondwanan taxon: systematics and biogeography of the harvestman family Pettalidae (Arachnida, Opiliones, Cyphophthalmi), with a taxonomic revision of genera from Australia and New Zealand. Cladistics 23: 337–361. doi: 10.1111/j.1096-0031.2007.00149.x

Chevasco V, Elzinga JA, Mappes J, Grapputo A (2014) Evaluation of criteria for species delimitation of bagworm moths (Lepidoptera: Psychidae). European Journal of Entomology 111: 121–136. doi: 10.14411/eje.2014.013

Chipman A, Dor N, Bonato L (2013) Diversity and biogeography of Israeli geophilomorph centipedes (Chilopoda: Geophilomorpha). Zootaxa 3652(2): 232–248. doi: 10.11646/zootaxa.3652.2.2

Coyne JA, Orr HA (2004) Speciation. Sinauer Associates, Sunderland, MA, 545 pp.

Darriba D, Taboada GL, Doallo R, Posada D (2012) jModelTest 2: more models, new heuristics and parallel computing. Nature, Methods 9(8): 772. doi: 10.1038/nmeth.2109

Drummond AJ, Suchard MA, Xie D, Rambaut A (2012) Bayesian phylogenetics with BEAUti and the BEAST 1.7. Molecular Biology and Evolution 29: 1969–1973. doi: 10.1093/molbev/mss075

Edgecombe GD, Giribet G (2006) A century later - a total evidence re-evaluation of the phylogeny of scutigeromorph centipedes (Myriapoda, Chilopoda). Invertebrate Systematics 20: 503–525. doi: 10.1071/IS05044

Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Molecular Marine Biology and Biotechnology 3: 294–299. http://www.mbari.org/staff/vrijen/PDFS/Folmer_94MMBB.pdf

Guidon S, Dufayard JF, Lefort V, Anisimova M, Hordijk W, Gascuel O (2010) New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. Systematic Biology 59: 307–321. doi: 10.1093/sysbio/syq010

Hebert PDN, Cywinska A, Ball SL, deWaard JR (2003) Biological identifications through DNA barcodes. Proceedings of the Royal Society B 270: 313–321. doi: 10.1098/rspb.2002.2218

Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, McWilliam H, Valentin F, Wallace IM, Wilm A, Lopez R, Thompson JD, Gibson TJ, Higgins DG (2007) Clustal W and Clustal X version 2.0. Bionformatics 23: 2947–2948. doi: 10.1093/bioinformatics/btm404

Montagna M, Sassi D, Giorgi A (2013) *Pachybrachis holerorum* (Coleoptera: Chrysomelidae: Cryptocephalinae), a new species from Apennines, Italy, identification by integration of morphological and molecular data. Zootaxa 3741(2): 243–253. doi: 10.11646/zootaxa.3741.2.3

Murienne J, Edgecombe GD, Giribet G (2010) Including secondary structure, fossils and molecular dating in the centipede tree of life. Molecular Phylogenetics and Evolution 57: 301–313. doi: 10.1016/j.ympev.2010.06.022

Oeyen JP, Funke S, Böhme W, Wesener T (2014) The evolutionary history of the rediscovered Austrian population of the giant centipede *Scolopendra cingulata* Latreille 1829 (Chilopoda, Scolopendromorpha). PLoS ONE 9(9): e108650. doi: 10.1371/journal.pone.0108650

Posada D, Buckley TR (2004) Model selection and model averaging in phylogenetics: advantages of Akaike information criterion and Bayesian approaches over likelihood ratio tests. Systematic Biology 53: 793–808. doi: 10.1080/10635150490522304

Rambaut A (2009) FigTree, ver. 1.3.1. [Online]. http://tree.bio.ed.ac.uk/software/figtree

Ratnasingham S, Hebert PDN (2007) bold: the Barcode of Life Data System (www.barcodinglife.org). Molecular Ecology Notes 7: 355–364. doi: 10.1111/j.1471-8286.2007.01678.x

Rota-Stabelli O, Daley AC, Pisani D (2013) Molecular time trees reveal a Cambrian colonization of land and a new scenario for ecdysozoan evolution. Current Biology 23: 392–398. doi: 10.1016/j.cub.2013.01.026

Rota-Stabelli O, Telford MJ (2008) A multi criterion approach for the selection of optimal outgroups in phylogeny: recovering some support for Mandibulata over Myriochelata using mitogenomics. Molecular Phylogenetics and Evolution 48: 103–111. doi: 10.1016/j.ympev.2008.03.033

Simaiakis SM, Zapparoli M, Minelli A, Bonato L (2013) The centipede fauna (Chilopoda) of the island of Cyprus, with one new lithobiomorph species. Zootaxa 3647(2): 279–306. doi: 10.11646/zootaxa.3647.2.3

Spelda J, Reip HS, Oliveira-Biener U, Melzer RR (2011) Barcoding Fauna Bavarica: Myriapoda – a contribution to DNA sequence-based identifications of centipedes and millipedes (Chilopoda, Diplopoda). Zookeys 156: 123–139. doi: 10.3897/zookeys.156.2176

Stoev P, Akkari N, Zapparoli M, Porco D, Enghoff H, Edgecombe GD, Georgiev T, Penev L (2010) The centipede genus *Eupolybothrus* Verhoeff, 1907 (Chilopoda: Lithobiomorpha: Lithobiidae) in North Africa, a cybertaxonomic revision, with a key to all species in the genus and the first use of DNA barcoding for the group. Zookeys 77: 29–77. doi: 10.3897/zookeys.50.504

Stoev P, Komericki A, Akkari N, Liu S, Zhou X, Weigand AM, Hostens J, Hunter CI, Edmunds SC, Porco D, Zapparoli M, Georgiev T, Mietchen D, Roberts D, Faulwtter S, Smith V, Penev L (2013) *Eupolybothrus cavernicolus* Komericky and Stoev sp. n. (Chilopoda: Lithobiomorpha: Lithobiidae): the first eukaryotic species description combining transcriptomic, DNA barcoding and micro-CT imaging data. Biodiversity Data Journal 1: e1013. doi: 10.3897/BDJ.1.e1013

Tamura K, Stecher G, Peterson D, Peterson N, Filpski A, Kumar S (2013) MEGA6: molecular evolutionary genetics analysis. Molecular Biology and Evolution 30: 2725–2729. doi: 10.1093/molbev/mst197

ZooKeys 510: 49–64 (2015)
doi: 10.3897/zookeys.510.8675
http://zookeys.pensoft.net

RESEARCH ARTICLE

A peer-reviewed open-access journal
ZooKeys
Launched to accelerate biodiversity research

Steps towards a phylogeny of the pill millipedes: non-monophyly of the family Protoglomeridae, with an integrative redescription of *Eupeyerimhoffia archimedis* (Diplopoda, Glomerida)

Jan Philip Oeyen[1], Thomas Wesener[1]

[1] *Zoologisches Forschungsmuseum Alexander Koenig, Leibniz Institute for Animal Biodiversity, Center for Taxonomy and Evolutionary Research (Section Myriapoda), Adenauerallee 160, D-53113 Bonn, Germany*

Corresponding author: *Jan Philip Oeyen* (janphilipoyen@gmail.com)

Academic editor: *Ivan H. Tuf* | Received 30 September 2014 | Accepted 7 May 2015 | Published 30 June 2015

http://zoobank.org/175DD451-08A4-477B-8A06-9AB5F09F65BA

Citation: Oeyen JP, Wesener T (2015) Steps towards a phylogeny of the pill millipedes: non-monophyly of the family Protoglomeridae, with an integrative redescription of *Eupeyerimhoffia archimedis* (Diplopoda, Glomerida). In: Tuf IH, Tajovský K (Eds) Proceedings of the 16th International Congress of Myriapodology, Olomouc, Czech Republic. ZooKeys 510: 49–64. doi: 10.3897/zookeys.510.8675

Abstract

Eupeyerimhoffia archimedis (Strasser, 1965) is redescribed based on several specimens collected at a number of sites close to the type locality on Sicily, Italy. Scanning electron microscopy is used to illustrate several unusual morphological characters for a member of the Glomerida for the first time. A fragment of the mitochondrial COI gene (668bp) is sequenced for the first time in *Eupeyerimhoffia* to provide a species-specific barcode and to gain first insights into the genetic distances between the genera in the widespread family Protoglomeridae. The novel sequences are compared to representatives of all other genera of the family: *Protoglomeris vasconica* (Brölemann, 1897) from northern Spain, the dwarfed *Glomerellina laurae* Silvestri, 1908 from Italy and *Glomeroides primus* (Silvestri, 1929) from western North America. The addition of COI sequences from the two other families of the Glomerida renders the family Protoglomeridae paraphyletic with *Glomeroides primus* being more closely related to *Glomeridella minima* (Latzel, 1884) than to the other genera in the family. The large genetic distances (13.2–16.8%) between *Eupeyerimhoffia* and the other genera in the order, as well as its unusual morphological characters, including unique morphological adaptations to roll into a ball, are probably an indication of the old age of the group.

Keywords

COI, Glomerida, integrative, taxonomy, redescription, Sicily

Introduction

The pill millipedes of the order Glomerida comprise about 290 species in 34 genera (Mauriès 2005, Golovatch et al. 2010, Wesener 2010, 2012) and exhibit a Holarctic distribution, with species found in North America, Europe and North Africa, and Asia with the exception of India south of the Himalayas (Shelley and Golovatch 2011). The Glomerida are currently divided into three families (Mauriès 1971, 2005), the two species-poor families Glomeridellidae and Protoglomeridae, and the family Glomeridae, which contains the majority of species and genera (~240 species in 27 genera) (Mauriès 2005, Wesener 2012).

While the two genera of the Glomeridellidae are Mediterranean, the four genera and 20 species of the Protoglomeridae show a disjunct distribution, partly European, in Spain, the eastern Mediterranean, Algeria and Sicily, and partly in the New World from Guatemala to California (Mauriès 2005).

Here we redescribe the little-known species *Eupeyerimhoffia archimedis* (Strasser, 1965), and describe the male telopods for the first time. Additionally, we illustrate several unusual (and potentially apomorphic) morphological characters of a member of the family Protoglomeridae for the first time using scanning electron microscopy. To complete our integrative approach, we also analyze the genetic distances between the four genera of the family using the common barcoding fragment, COI.

Material and methods

Samples of *Eupeyerimhoffia archimedis* were collected by hand in July 2013. A single male and several females were collected close to the type locality (Ferla; Fig. 1C) and further samples were collected at a new locality (East of Palazzolo Acreide, Sicily). Exact coordinates are provided in Table 1. All samples were conserved in 98% EtOH for further analyses and deposited in the collection of the Zoological Research Museum Alexander Koenig (ZFMK, Bonn, Germany).

Morphological analysis

A female and the single male from the type locality were dissected under an Olympus SZX12 stereomicroscope with Dumont 5 Inox B forceps. Samples were dehydrated in 100% EtOH for 12 hrs, mounted on aluminum stubs, dried for 12 hrs at 45 °C and sputter coated with 50 nm of pure gold in a Hummer VI sputtering system (Anatech LTD, USA). Samples were observed with a Hitachi S-2460N SEM (Hitachi LTD, Japan) and digital images were captured using DISS5 (point electronic GmbH, Germany).

Figure 1. *Eupeyerimhoffia archimedis* (Strasser, 1965) female in situ and habitat. **A** *E. archimedis* female rolled up, in situ **B** *E. archimedis* female in situ **C** Habitat of *E. archimedis*, close to the type locality Ferla.

Molecular analysis

Muscle tissue was removed from specimens of *Onychoglomeris tyrolensis* (Latzel, 1884), *Protoglomeris vasconica* (Brölemann, 1897), *Glomerellina laurae* Silvestri, 1908, and *Eupeyerimhoffia archimedis* (Strasser, 1965). Sequences of *Glomeroides primus* (Silvestri, 1929) were downloaded from GenBank. Additionally, sequences from GenBank of *Glomeridella minima* (Latzel, 1884), a member of the basal family Glomeridellidae, as well as of *Glomeris marginata* (Villers, 1789), *Geoglomeris subterranea* Verhoeff, 1908 and *Trachysphaera* sp. from the family Glomeridae (Table 1) were also downloaded. Specimens from which DNA was extracted were stored as vouchers at the ZFMK. Accession numbers, locality data and voucher information for all samples included in the study are displayed in Table 1.

Total genomic DNA was extracted using the Qiagen DNAeasy Blood&Tissue kit following the standard protocol. A fragment of the mitochondrial cytochrome *c* oxidase subunit I (COI) gene was amplified via PCR (Saiki et al. 1988) using the Nancy (Simon

Table 1. Sample information with voucher numbers (ZFMK = Zoological Research Museum Alexander Koenig, Bonn, Germany. ZSM = Bavarian State Collection of Zoology. NHMC = Natural History Museum of Crete), GenBank accession numbers (Acc.#) and locality information. Samples where sequences were downloaded from GenBank are marked with an asterisk.

Species	Specimen Voucher	Acc. #	Locality
**Glomeris marginata* (Villers, 1789)	ZFMK MYR0009	FJ409909	Germany, Nordrhein-Westfalen, Bonn, Venusberg, coll. T. Wesener, IX.2007
**Glomeridella minima* (Latzel, 1884)	ZFMK MYR0003	JQ074181	Slovenia, Lower Sava, Brežice, Prilipe, dry creek valley, 45.8773°N, 15.6246°E, 150 m, coll. H. Reip, 17.x.2009.
**Geoglomeris subterranea* Verhoeff, 1908	BC ZSM MYR 00370	JQ350441	Switzerland, Aargau
**Trachysphaera* sp.	ZFMK MYR0006	JQ074180	Italy, Piemonte, Biella, NW Sanctuary of Oropa, Fagus forest with stones, 45.62947°N, 7.98168°E, 1200 m, coll. T. Wesener, 14.iv.2011
**Glomeroides primus* (Silvestri, 1929)	ZFMK MYR0004	JQ074182	U.S.A., California, Mendocino County, between Fort Bragg and Whiskey Springs, 39.3976°N, 123.6946°W, 35 m, coll E. Garcia, C. Richart & A. Schönhofer, 29.iii.2011.
Onychoglomeris tyrolensis (Latzel, 1884)	ZFMK MYR1276	KP205571	Italy, Trentino-Südtirol, Prov. Trient, Madonna di Campiglio, Beech forest, 46.2209528°N, 010.8296250°E, 1553 m, coll. T. Wesener, 04.x.2012.
Protoglomeris vasconica (Brölemann, 1897)	ZFMK MYR0934	KP205572	Spain, Galicia, Ribadeo, Trabada, deep and moist creek valley with deciduous forest, 43.4295°N, 7.2290°E, coll. H. Reip, 29.vii.2012.
Glomerellina laurae Silvestri, 1908	ZFMK MYR2260	KP205573	Europe, Greece, Rhodos, Kapi - Profitis Ilias, coll. NHMC, 01.i.2000.
Eupeyerimhoffia archimedis (Strasser, 1965) 1	ZFMK MYR1876	KP205574	Italy Sicily, Province Syracuse, South of Ferla, Southern slope, deciduous forest, 37.1151333°N, 014.9403667°E, coll. J.P. Oeyen & P. Erkeling, 10.vii.2013.
Eupeyerimhoffia archimedis (Strasser, 1965) 2	ZFMK MYR1965	KP205575	Italy, Sicily, Province Syracuse, East of Palazzolo Acreide, Ravine, deciduous forest, 37.0997667°N, 015.0232000°E, coll. J.P. Oeyen & P. Erkeling, 13.vii.2013.

et al. 1994) and LCO (Folmer et al. 1994) primer pair following previously published protocols (Wesener et al. 2010). Both strands were sequenced by Macrogen (Macrogen Europe Laboratory, Amsterdam, The Netherlands), following the Sanger sequencing method (Sanger et al. 1977). Sequencing reads were assembled and aligned by hand with Bioedit 7.1.3 (Hall 1999) and confirmed with BLAST searches (Altschul et al. 1997). Sequences were uploaded to GenBank (Accession numbers: KP205571 to KP205557).

Mean pairwise distances between terminals (transformed into percentages) were determined using MEGA5.2 (Tamura et al. 2011). To better illustrate relationships between genera, a maximum likelihood phylogenetic analysis was conducted in MEGA5.2 (Tamura et al. 2011). The implemented ModelTest selected the HKY+G+I model (Hasegawa et al. 1985) as best-fitting (BIC = 5783.1, -lnL = -2791.2354,

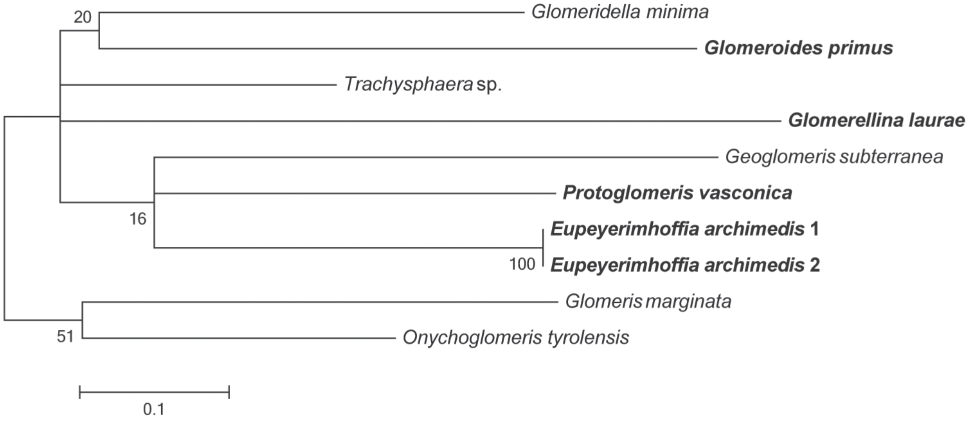


Figure 2. Maximum likelihood bootstrap consensus tree. Members of the family Protoglomeridae are marked in bold.

freqA = 0.2647, freqC = 0.1366, freqG = 0.2014, freqT = 0.3972, gamma shape = 0.3364). The bootstrap consensus tree (Fig. 2), inferred from 1000 replicates (Felsenstein 1985), is used to represent the evolutionary history of the analyzed taxa. All positions containing gaps and missing data were eliminated. There were a total of 668 positions in the final dataset.

While the genetic marker used does not allow a study of the phylogeny of the group, first insights into the separation of the genera are provided.

Results

Distance analysis

The uncorrected pairwise distances between genera included in the present study are relatively high. The genetic distances are not lower between species within the same family than between species of different families. The distances range from 18.8% between *Glomerellina laurae* (Protoglomeridae) and *Glomeris marginata* (Glomeridae) to 12.0% between *Protoglomeris vasconica* (Protoglomeridae) and *Glomeridella minima* (Glomeridellidae). The two *Eupeyerimhoffia archimedis* samples show a 0.2% sequence divergence, but also show both the highest (16.8%: *G. laurae*) and lowest distance (13.2 and 13.4%: *P. vasconica*) to other species within the family.

Tree description

The maximum likelihood tree receives little to no support, most nodes remain unresolved and all taxa are separated by long branches (Fig. 2). The family Protoglomeridae (P)

Table 2. Pair-wise uncorrected p-distances (%) of the COI-fragment.

#	Species	1	2	3	4	5	6	7	8	9
1	*Glomeris marginata*									
2	*Glomeridella minima*	16.0								
3	*Geoglomeris subterranea*	17.4	15.6							
4	*Trachysphaera sp.*	15.0	13.2	15.6						
5	*Glomeroides primus*	16.3	14.2	16.4	15.0					
6	*Onychoglomeris tyrolensis*	14.3	13.2	16.8	13.2	15.3				
7	*Protoglomeris vasconica*	14.8	12.0	15.3	13.5	15.0	13.2			
8	*Glomerellina laurae*	18.8	16.0	18.3	15.3	16.7	16.3	15.8		
9	*Eupeyerimhoffia archimedis 1*	16.1	15.2	15.0	13.1	16.5	15.0	13.2	16.8	
10	*Eupeyerimhoffia archimedis 2*	16.2	15.3	15.0	13.2	16.7	15.2	13.4	16.8	0.2

could not be recovered. All members of the family are recovered within a major polytomy together with species from both Glomeridellidae and Glomeridae (Fig. 2). Within the polytomy *Glomeroides primus* (Protoglomeridae) groups together with *Glomeridella minima* (Glomeridellidae). *Glomerellina laurae* (P) does not cluster with any species within the polytomy and rests on the longest branch within the tree. *Protoglomeris vasconica* (P) and *Eupeyerimhoffia archimedis* (P) are recovered in a polytomy together with *Geoglomeris subterranea* (Glomeridae). Only the subfamily Glomerinae (*Glomeris* & *Onychoglomeris*) could be resolved as monophyletic (Fig. 2) as the sister group to the polytomy, though with weak support.

Family Protoglomeridae Brölemann, 1913

Diagnosis. Simple telopods with four podomeres distal to syncoxite, forming pincers. Telopoditomeres 1–3 lacking trichosteles. Telopoditomere 2 with a non-membranous immovable finger located almost parallel to telopoditomere 3. Here we follow the typological system of Mauriès (2005), despite the fact that no phylogenetic analysis has been undertaken to characterize the families in the order.

Eupeyerimhoffia Brölemann, 1913

Eupeyerimhoffia Brölemann, 1913: 166–174 (first description); Jeekel 1971: 13 (note); Strasser 1976: 581–583 (synonymization *Trinacriomeris*); Hoffann 1980: 67 (list); Foddai et al. 1995: 11 (list); Shelley et al. 2000: 11 (list); Mauriès 2005: 242 (classification); Kime and Enghoff 2011: 39 (atlas).

Trinacriomeris Strasser, 1965: 10–14. syn.

Diagnosis. Tergite 11 fused to anal shield. Telopod simple with four podomeres. Process of telopoditomere 2 of telopod short and stout. Male leg-pair 17 with four podomeres. Coxa of male leg-pair 18 not fused to syncoxite. Lateral palpi of gnathochilarium as large as inner palpi. One of the largest Glomerida, 18–22 mm long. Mandible with large condylus. Members might be mistaken in habitus, size and color with the species of the genus *Onychoglomeris* Verhoeff, 1909, whose species differ greatly in the telopods and many other characteristics.

Type species. *Eupeyerimhoffia algerina* Brölemann, 1913 from Algeria

Other species included. *Eupeyerimhoffia archimedis* (Strasser, 1965) from southern Sicily.

Eupeyerimhoffia archimedis (Strasser, 1965)

Trinacriomeris archimedis Strasser 1965: 10–14 (first description);
Trinacriomeris archimedis Strasser 1970: 153 (list);
Trinacriomeris archimedis Strasser 1976: 581–583 (synonymization *Trinacriomeris*);
Eupeyerimhoffia archimedis Foddai et al. 1995: 11 (list);
Trinacriomeris archimedis Shelley et al. 2000: 11 (list).

Material examined. 1 F, **MHNG**, lectotype (designated herewith), labeled paratype, in 70% ethanol, Italie (Sicile): Siracuse: Avola pr. Siracuse. 1 F, **MHNG 3460**, dried and mounted, Italie (Sicile): Siracuse: Avola pr. Siracuse; 1 F, **ZFMK MYR01879**, 1 M, **ZFMK MYR01875**, Italy, Sicily at type locality, south of Ferla, 37.1151333°N, 014.9403667°E, coll. J.P. Oeyen & P. Erkeling, 10.vii.2013; 1 F, **ZFMK MYR 1965**, Italy, Sicily, Province Syracuse, East of Palazzolo Acreide, Ravine, deciduous forest, 37.0997667°N, 015.0232000°E, coll. J.P. Oeyen & P. Erkeling, 13.vii.2013.

Comment. A second female type specimen from Ferla, Sicily was, according to the first description, stored at the University of Catania, Institute of Zoology, Italy.

Re-diagnosis. Can easily be distinguished from the other Sicilian Glomerida species by size and color. It is the largest and only light brown species on the island. It can be distinguished from its congener *E. algerina* in having: (1) Single continuous anterior stria on collum, posterior stria divided in lateral parts; (2) thoracic shield with single continuous stria reaching the lateral lobes on both sides.

Description. General coloration (living specimen) light brown, almost copper. Collum, head, antennae, posterior margin and lateral speckled fields of tergites lighter, almost golden cream color (Fig. 1A, B).

Head sparsely covered with minute setae, >10 supralabral setae (Fig. 3A, C). Incisura lateralis (IL) directed slightly laterally, not reaching height of organ of Tömösváry (TO) or antennal basis (Fig. 3A–C). Lateral marginal bulge thickest at IL, decreasing gradually dorsally until terminating at height of dorsal-most ocellus (Fig. 3C). A furrow running laterally between ventral-most ocellus and TO, circumventing antennal fossa and terminating at height of IL (Fig. 3C).

Labrum wide, with 19 marginal setae (Fig. 3A, B). Central labral tooth projecting beyond lateral margin.

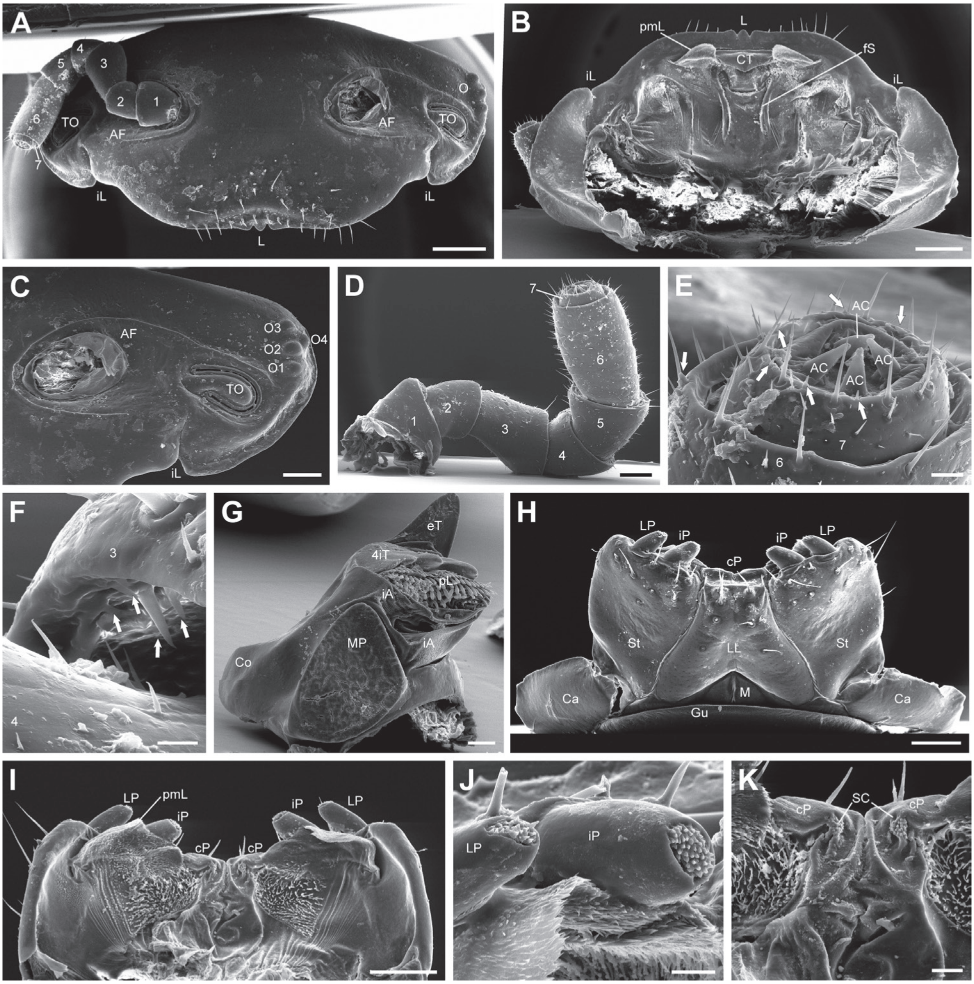


Figure 3. *Eupeyerimhoffia archimedis* (Strasser, 1965) male, SEM. **A** Head, frontal view **B** Head, ventral view **C** Head, detail of lateral area **D** Antenna, posterior view**E** Antenna, antennomere 6 and 7 **F** Antenna, apical edge of antennomere 3 **G** Mandible, mesal view **H** Gnathochilarium, ventral view **I** Endochilarium, dorsal view **J** Gnathochilarium, lateral and inner palpi, dorsal view **K** Endochilarium, detail of median area, dorsal view. Abbreviations: 1-7 = Antennal segments number 1-7; 4iT = 4-combed inner tooth; AC = Apical cone; AF = Antennal fossa; Ca = Cardine; Co = Condylus; cP = Central pads; CT = Central tooth; eT = External tooth; fS = fringed seam; Gu = Gula; iA = Intermediate area; iL = Incisura lateralis; iP = Inner palpi; L = Labrum; LL = Lamella linguales; LP = Lateral palpi; M = Mentum; MP = Molar plate; O = Ocellaria; O1-O4 = Ocelli 1-4; pL = Pectinate lamellae; pmL = paramedian lobe; TO = Organ of Tömösváry; SC = Sensory clusters; St = Stipites. Arrows mark *sensilla basiconica*. Scale bar: 400 µm (**A, B**); 200 µm (**C**); 150 µm (**D**); 25 µm (**E**); 10 µm (**F**); 100 µm (**G**); 250 µm (**H, I**); 40 µm (**J**); 50 µm (**K**).

Epipharynx with pronounced central tooth and two lateral membranous lobes, covered densely in cuticular scales (Fig. 3B). Incisura lateralis clearly visible, reaching margin of head capsule. Two paramedian fringed seams stretching from central tooth posteriorly towards hypopharynx.

Ocellaria black, 3+1 convex lenses (Fig. 3C).

Antennae with four apical cones (Fig. 3E). Antennomere 3 approximately as long as 1 and 2 combined (Fig. 3D). Antennomere 6 approximately 1.9 times longer than wide. Antennomeres 1–5 only sparely setose, 6th more densely setose. Multiple sensilla basiconica on proximal apical edge of antennomere 3 (Fig. 3F) as well as at apical edge of antennomere 7 (Fig. 3E).

Organ of Tömösváry recessed**,** elongate, curved ventrally (Fig. 3C). 1.9 times longer than wide. Bulging cone and slit margins smooth. Cone narrower at midpoint. No internal structures visible in SEM.

Gnathochilarium ventrally with 8 large setae on lamella linguales, 12 large setae on each stipites (Fig. 3H). Remaining ventral surface glabrous. Cardines large. Inner palpi slightly larger than lateral palpi (Fig. 3H–J). Inner palpi with >40 sensory cones standing in single field (Fig. 3J). Lateral palpi also with field of >20 sensory cones (Fig. 3J).

Endochilarium with large anterior membranous paramedian lobes (pmL), densely covered with cuticular scales (Fig. 3I). Fields of long setae posterior to membranous lobes. Central pads with single cluster of sensilla directed towards median furrow (Fig. 3I, K).

Mandible with single large outer tooth and four-combed inner tooth (Fig. 3G). Proximal comb of inner tooth slightly ovoid. Six rows of pectinate lamellae. Lateral areas of intermediate area covered with small cuticular scales, central part smooth with possible pore. Molar plate almost triangular, marginal bulge at anterior edge, no anterior depression and posterior tip slightly curved towards mandibular basis. Condylus pronounced (Fig. 3G).

Collum with one continuous anterior and two posterior lateral striae (Fig. 4I). Uniformly covered with minute setae, recessed into small pits.

Thoracic shield with very small schism (Fig. 4A). 3 median striae. Marginal furrow widest laterally, narrowing medially. Uniformly covered with minute recessed setae. Ventral area of lobe with seven anterior marginal depressions on lobe and a single depression at the posterior margin (Fig. 3B, C; see below for function of depressions).

Tergites 3–10 covered with minute recessed setae, with single complete transverse anterior stria and short lateral striae anteriorly circumventing a depression (Fig. 4E, G). Lateral edges not projecting posteriorly. Stout pegs on ventral areas projecting posteroventrally from lateral most part of anterior edge (Fig. 4D, F).

Ozopore simple, neither with special sutures nor other structures (Fig. 4H).

Tergite 11 and anal shield completely fused but both dorsally and ventrally distinguishable by a pronounced furrow (Fig. 4J–L). Tergite 11 with 3 short lateral striae and a single stria circumventing a lateral depression and stretching across whole tergite (Fig. 4L). Tergite 11 and anal shield dorsally evenly covered with minute setae, with neither any special notches nor structures.

Pleurites evenly covered with small setae, bulge at anterior edge widest medially narrowing towards proximal edge. Pleurite 1.2 times wider than long.

Stigmatic plates reaching around coxa on both anterior and posterior sides. 1.5 times wider than long, almost pentagonal in shape. Plate with regular margin, lacking any projections. Spiracle inconspicuous, protected by small knob.

Midbody legs sparsely covered with minute setae (Fig. 5F). Coxa almost triangular, much wider at base than apically. Coxa mesally elongated to process carrying a single spine.

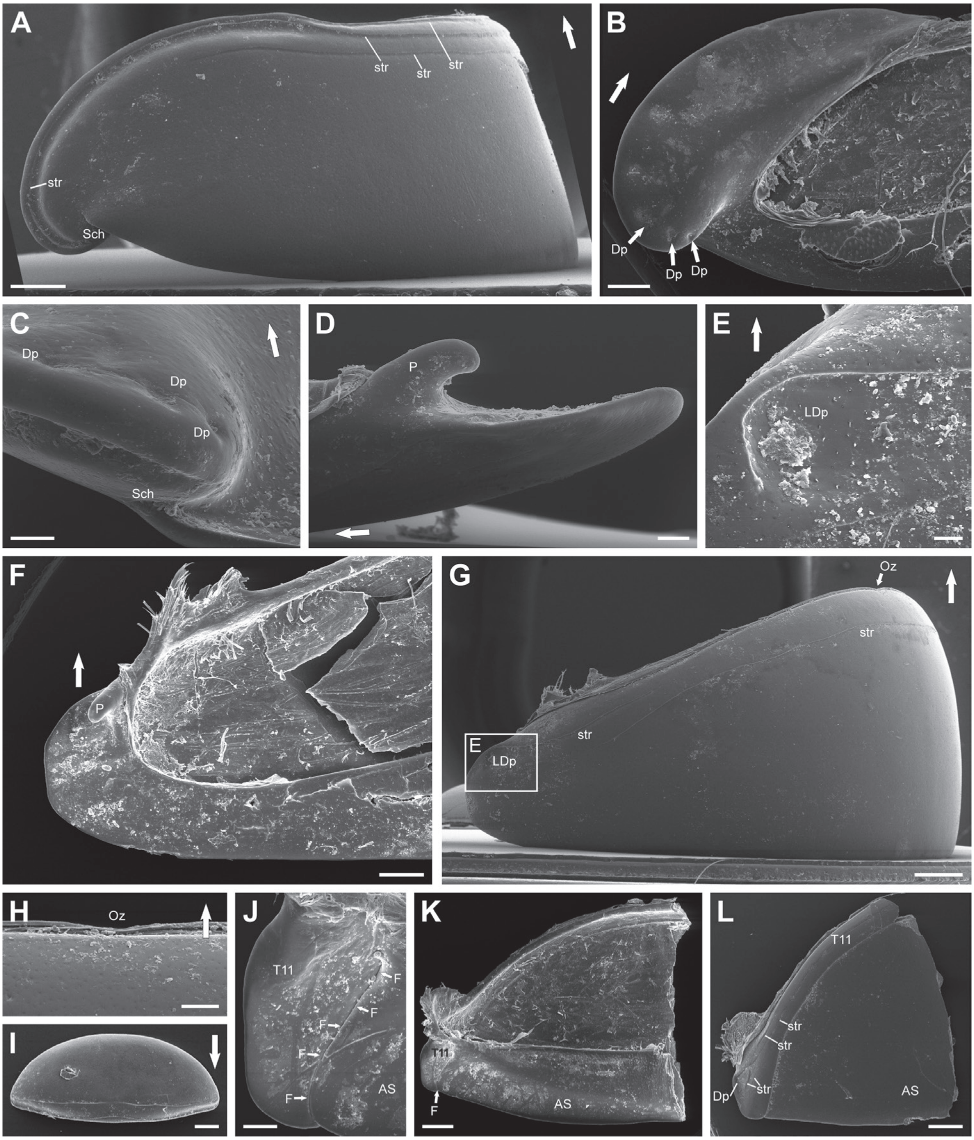


Figure 4. *Eupeyerimhoffia archimedis* (Strasser, 1965) male, SEM. **A** Thoracic shield, dorso-lateral view **B** Thoracic shield, meso-lateral **C** Thoracic shield, schism detail, ventro-lateral view **D** Tergite, detail of peg, antero-lateral view **E** Tergite, detail of depression, lateral view **F** Tergite, ventral view **G** Tergite, dorso-lateral view **H** Tergite, ozopore, dorsal view **I** Collum, dorsal view **J** Tergite 11 and anal shield, detail of furrow, ventro-lateral view **K** Tergite 11 and anal shield, left side, anterior view **L** Tergite 11 and anal shield, right side, posterior view. Abbreviations: AS = Anal shield; Dp = Depression; F = Furrow; Oz = Ozopore; LDp = Lateral depression; P = Peg; Sch = Schisma; stri = striae; T11 = Tergite 11. Arrows point anteriorly. Scale bar: 400 µm (**A, H, I, K, L**); 300 µm (**B, F**); 100 µm (**C, D, J**); 50 µm (**E**); 500 µm (**G**).

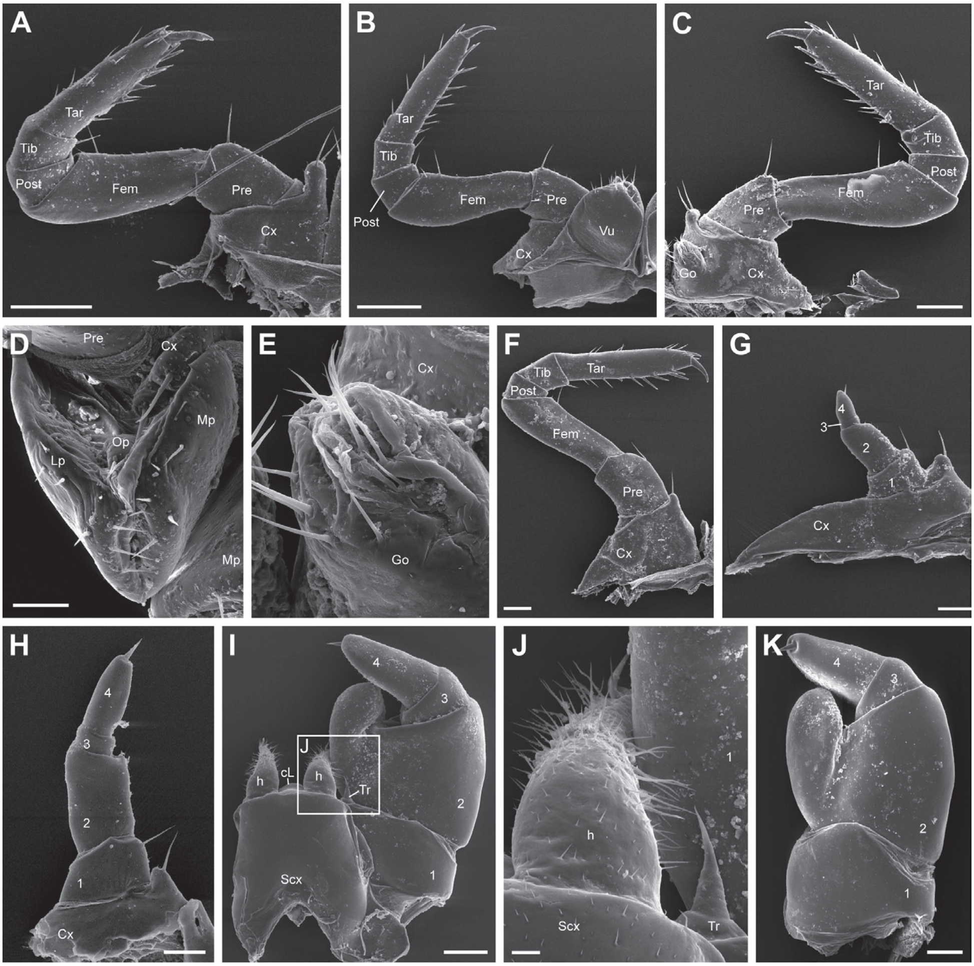


Figure 5. *Eupeyerimhoffia archimedis* (Strasser, 1965) male and female, SEM. **A** Leg-pair 1, male, right side, posterior view **B** Leg-pair 2, female, right side, posterior view **C** Leg-pair 2, male, left side, posterior view **D** Leg-pair 2, female, right vulva, ventral view **E** Leg-pair 2, male gonopore, posterior view **F** Leg-pair 9, male, right side, posterior view **G** Leg-pair 17, left side, anterior view **H** Leg-pair 18, right side, posterior view **I** Telopod with syncoxite, anterior view **J** Telopod, inner horn of syncoxite **K** Telopod, posterior view. Abbreviations: Cx = Coxa; Pre = Prefemur; Fem = Femur; Post = Postfemur; Tib = Tibia; Tar = Tarsus; Vu = Vulva; Go = Gonopore; Mp = Median plate; Lp = Lateral plate; Op = Operculum; 1-4 = Podomere 1-4; cL = Central lobe; h = Inner horn; Scx = Syncoxite; Tr = Trichostele. Scale bar: 400 µm (**A, B**); 250 µm (**C**); 100 µm (**D, H**); 25 µm (**E**); 200 µm (**F, I**); 150 µm (**G, K**); 50 µm (**J**).

Two coxal furrows originating at center of coxal basis, one stretches apically around coxa, the second terminates after 2/3 of coxal height in a meso-apical direction. Tibia, pre- and postfemur with a single mesal spine, femur with two. Apical margin of prefemur with a single small apical protrusion. Femur almost 3 times longer than wide. Tarsus with no apical, 11 dorsal and 8–11 ventral spines. Tarsus 4.5 times longer than wide. Claw elongated.

Male sexual characters.

Male tergite 11 and anal shield do not show any special structures (Fig. 4J–L). See further and more detailed descriptions above.

Male first leg-pair sparsely covered with minute setae (Fig. 5A). Coxa not widened at basis, but mesally elongated to a process carrying two spines. Postfemur and tibia each with single mesal apical spine, prefemur and femur with two. Apical margin of prefemur with a single small protrusion. Tarsus with 7–10 dorsal and 8 ventral spines. Claw elongated but stout at basis. Tarsus almost 4 times longer than wide.

Male second leg-pair similar to midbody legs, but with a bulbous medial coxal protrusion carrying two spines (Fig. 5C), similar to leg 1. Tarsus approximately 3.8 times longer than wide.

Male gonopore clam-shaped and mesally protruding from posterior side of coxa 2 (Fig. 5E). Single elongate membranous opening surrounded by 9 or 10 apical and 4 basal setae. No division into separate plates.

Male leg 17 reduced with 4 podomeres (Fig. 5G). Coxa with small medial process bearing a subapical spine and a wide but narrow, almost triangular, coxal lobe. Apical edge of coxa with small protrusion. Podomere 1 with mesal spine. Second podomere approximately 1.8 times wider than podomere 3. Podomere 3 very short and inconspicuous. Podomere 4 with subapical spine. Complete leg sparsely covered with minute setae.

Male leg 18 reduced, but to a lesser extent than leg-pair 17 (Fig. 5H). Coxa slightly damaged during dissection, but apparently without widened coxal lobe and not fused to syncoxite. Small mesal coxal process with single subapical spine. Single, well-developed medial spine on podomere 1. Apical edge of podomere 1 with apical protrusion. Podomere 2 approximately 1.5 times as wide as podomere 3. Podomere 3 very short, no spines and with very inconspicuous borders to podomere 4. Podomere 4 with apical spine.

Telopod (male leg 19) stout, syncoxite likewise (Fig. 5I–K). Syncoxal lobe small and rounded. Inner horns of syncoxite with numerous hairs of varying length and well-developed subapical spine, which is curved almost 90° (Fig. 5J). Telopoditomere 1 with mesal, highly reduced trichostele (Fig. 5I, J). Telopoditomere 2 mesally elongated into large bulbous process (immovable finger) with knobbed proximal surface. Telopoditomere 3 short, approximately 2 times wider than long, devoid of any peculiarities. Telopoditomere 4 with medial field of knobs juxtaposed to process of telopoditomere 2, and a large posteriorly oriented spine. Telopoditomere 4 forms chela (pincer) against medial process of second telopoditomere.

Female sexual characters.

Female second leg-pair similar to midbody legs, but coxa with two spines on separate medial protrusions which are fused basally (Fig. 5B). Tarsus 4 times longer than wide.

Female vulva large, attached to posterior side of coxa via membranes (Fig. 5D). Operculum recessed between vulva plates. Posterior end of operculum narrower than anterior one, with two spines. Vulva with ventrally symmetrical mesal and lateral plates, carrying altogether 7 or 8 spines. Lateral plate overlaps mesal one apically on posterior side, but both are fused together at their base via a membranous connection.

Intraspecific variation. Not enough samples present to describe morphological variation. The populations from Ferla and Palazzolo Acreide have two different haplotypes, differing at one base pair position.

Volvation. As described by Strasser (1965), the genus has a volvation strategy which differs from what is known from most other Glomerida. When rolling up into a ball the ventral ends of the tergites are not inserted in the schisma of the thoracic shield, unlike in *Glomeris*, but rest on top of it (Fig. 1A). The pegs on the ventral side of the tergites (Fig. 4D, F) rest within the depressions on the ventral side of the thoracic shield (Fig. 4B, C).

Habitat. All of the samples were collected during the day in deciduous forests. Specimens were mainly found in the leaf litter or under small stones. Curiously, some were also found in close proximity to ant nests (Hymenoptera) and under moss growing directly on an exposed rocky surface.

Discussion

Problems during the morphological analysis:

The described position of the vulva operculum might be an artifact, as the structural integrity of membranous structures was not preserved by critical point drying. This should be considered for future studies of glomerid vulvae.

The sampling within the present study did not allow for a description of the morphological variation within the species. However, the 0.2% sequence divergence between the two reported localities shows that there is variability within the species, with at least two haplotypes present on the island.

Unique morphological characters of *Eupeyerimhoffia*

Eupeyerimhoffia archimedis shows several interesting characters. The mandible with a large condylus and flat molar plate lacking a groove (Fig. 3G) is very atypical of Glomerida. Glomerida are generally described as lacking a condylus and always possessing a molar plate with a distinct deep groove (Köhler and Alberti 1990). Furthermore, contrary to previous descriptions, the Protoglomeridae-like telopods possess a trichostele on the first podomere, which represents another special character of the species, if not of the genus. The presence of this trichostele violates the diagnosis of the family as proposed by Mauriès (2005).

Volvation strategy

The volvation strategy of *Eupeyerimhoffia* is another striking and possibly unique feature of the genus inside the order Glomerida. Similar pegs on the tergites have been reported for members of the genera *Epiromeris* (Thaler and Knoflach-Thaler 1998) and *Trachysphaera* (Strasser, 1965). These do not, however, possess the herein described thoracic shield lobe with a reduced schisma in combination with ventral depressions (Fig. 4A–C). Both modifications allow *Eupeyerimhoffia* a unique method of rolling into a ball. To understand this phenomenon, further inquiries into the origin and diversification of glomerid volvation are necessary and jointly represent a very interesting future research topic on its own.

Relationships of the four genera of the Protoglomeridae and impact on Glomerida phylogeny

As stated above, the COI fragment is not well suited to study the group's phylogeny. Therefore it is not surprising that the COI tree lacks resolution and receives little statistical support. Nonetheless, together with the distance analysis, it is sufficient to observe that the members of the family Protoglomeridae are not each other's closest relatives (e.g. *Glomeroides primus* grouping with *Glomeridella minima* from the separate suborder Glomeridelloidea) and possibly that the family does not constitute a monophyletic unit. Similar results have also been reported by Wesener (2012) in a study that did not include all members of the family. This supports the notion that characters based mainly on the telopods are not sufficient to infer relationships within the order Glomerida. This is especially true when considering the close relationship between *Eupeyerimhoffia archimedis* and *Protoglomeris vasconica*, despite the fact that *E. archimedis* does not conform to the diagnosis of the family. Therefore, a phylogenetic analysis based on a much broader dataset, including further molecular markers and morphological characters, is required to illuminate the evolutionary history of the pill millipedes.

Acknowledgements

We thank K. Ulmen (ZFMK: SEM) and C. Etzbauer (ZFMK: Molecular lab) for help in the lab, H. Reip (Jena) for the specimens of *Protoglomeris vasconica*, S. Simaiakis (NHMC) for the specimens of *Glomerellina laurae*, and A. Schönhofer for the specimens of *Glomeroides primus*. We also thank P. Schwendinger (MHNG) for his advice and for hosting one of us (TW) during a stay at Geneva in the summer of 2012. We thank the Alexander-Koenig-Gesellschaft (AKG) for funding the trip to re-collect *Eupeyerimhoffia archimedis* in Sicily and P. Erkeling for his help in the field. Sergei Golovatch and a second reviewer provided useful comments that enhanced the quality of the here presented article. Lastly, we thank P.B. Frandsen (Rutgers University, USA) for proofreading and giving constructive comments on the manuscript.

References

Altschul SF, Madden TL, Schäffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic acids research 25: 3389–402. doi: 10.1093/nar/25.17.3389

Brölemann HW (1913) Eupeyerimhoffia algerina nouvelle forme de Gloméride (Myriapodes). Bulletin de la Société d'Histoire naturelle de l'Afrique du Nord 5: 166–174.

Felsenstein J (1985) Confidence Limits on Phylogenies: An Approach Using the Bootstrap. Evolution 39: 783. doi: 10.2307/2408678

Foddai D, Minelli A, Scheller U, Zapparoli M (1995) Chilopoda, Diplopoda, Pauropoda, Symphyla. In: Minelli A, Ruffo S, La Posta S (Eds) Checklist delle Specie della Fauna Italiana. Bologna, 1–35.

Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Molecular marine biology and biotechnology 3: 294–9.

Golovatch SI, Mikhaljova EV, Chang HW (2010) Pill-millipedes (Glomerida, Diplopoda) in Taiwan. Zootaxa 2477: 1–20.

Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor. Nucleic Acids Symposium Series 41: 95–98.

Hasegawa M, Kishino H, Yano T (1985) Dating of the human-ape splitting by a molecular clock of mitochondrial DNA. Journal of molecular evolution 22: 160–174. doi: 10.1007/BF02101694

Hoffman R (1980) Classification of the Diplopoda. Museum d'Histoire Naturelle, Genève, 1–237.

Jeekel CAW (1971) Nomenclator generum et familiarum Diplopodorum: a list of the genus and family-group names in the class Diplopoda from the 10th edition of Linnaeus, 1758, to the end of 1957. Monografieen van de Nederlandse Entomologische Vereniging 5: 1–412.

Kime RD, Enghoff H (2011) Atlas of European millipedes (Class Diplopoda): Volume 1, orders Polyxenida, Glomerida, Platydesmida, Siphonocryptidae, Polyzoniida, Callipodida, Polydesmida. Pensoft, Sofia-Moscow.

Köhler H-R, Alberti G (1990) Morphology of the mandibles in the millipedes (Diplopoda, Arthropoda). Zoologica Scripta 19: 195–202. doi: 10.1111/j.1463-6409.1990.tb00255.x

Mauriès J-P (1971) Diplopodes épigés et cavernicoles de Pyrénées espagnoles et des monts cantabriques: VII. Glomérides. Essai de classification des Glomeroidea. Bulletin de la Société d'Histoire naturelle de Toulouse 107: 423–453.

Mauriès J-P (2005) Attempt at a classification of Glomerida (Diplopoda), with descriptions of two new genera from the northwest of the Iberian Peninsula. Arthropoda Selecta 14: 241–249.

Saiki R, Gelfand D, Stoffel S, Scharf S, Higuchi R, Horn G, Mullis K, Erlich H (1988) Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. Science 239: 487–491. doi: 10.1126/science.2448875

Sanger F, Nicklen S, Coulson AR (1977) DNA sequencing with chain-terminating inhibitors. Proceedings of the National Academy of Sciences 74: 5463–5467. doi: 10.1073/pnas.74.12.5463

Shelley RM, Golovatch SI (2011) Atlas of Myriapod Biogeography. I. Indigenous Ordinal and Supra-Ordinal Distributions in the Diplopoda: Perspectives on Taxon Origins and Ages, and a Hypothesis on the Origin and Early Evolution of the Class. Insecta Mundi 0158: 1–134.

Shelley RM, Sierwald P, Kiser SB, Golovatch SI (2000) Nomenclator generum et familiarum Diplopodorum II. Sofia.

Simon C, Frati F, Beckenbach A, Crespi B, Liu H, Flook P (1994) Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. Annals of the Entomological Society of America 87: 651–701. doi: 10.1093/aesa/87.6.651

Strasser K (1965) I diplopodi della fauna siciliana. Atti della Accademia gioenia di Scienze naturali in Catania 7: 1–35.

Strasser K (1970) Diplopodi della Sicilia e della Calabria. Memorie del Museo civico di Storia naturale di Verona 17: 151–200.

Strasser K (1976) Über Diplopoda-Chilognatha Griechenlands, II. Revue suisse de Zoologie 83: 579–645.

Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S (2011) MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Molecular Biology and Evolution 28: 2731–9. doi: 10.1093/molbev/msr121

Thaler K, Knoflach-Thaler B (1998) Das Männchen von *Epiromeris aelleni* Strasser (Diplopoda, Glomerida). Entomologische Nachrichten und Berichte 42: 1–4.

Wesener T (2010) Revision of the American Pill Millipedes I: *Onomeris* and *Trichomeris*. Zootaxa 2725: 28–40.

Wesener T (2012) *Nearctomeris*, a new genus of Pill Millipedes from North America, with a comparison of genetic distances of American Pill Millipede Genera (Glomerida, Glomeridae). Zootaxa 68: 58–68.

Wesener T, Raupach MJ, Sierwald P (2010) The origins of the giant pill-millipedes from Madagascar (Diplopoda: Sphaerotheriida: Arthrosphaeridae). Molecular Phylogenetics and Evolution 57: 1184–93. doi: 10.1016/j.ympev.2010.08.023

ZooKeys 510: 65–77 (2015)
doi: 10.3897/zookeys.510.8573
http://zookeys.pensoft.net

RESEARCH ARTICLE

A peer-reviewed open-access journal
ZooKeys
Launched to accelerate biodiversity research

Species limits and phylogeography of *Newportia* (Scolopendromorpha) and implications for widespread morphospecies

Gregory D. Edgecombe[1], Varpu Vahtera[2], Gonzalo Giribet[3], Pipsa Kaunisto[2]

1 *Department of Earth Sciences, The Natural History Museum, Cromwell Road, London SW7 5BD, UK* **2** *Zoological Museum, Department of Biology, University of Turku, Turku FI-20014, Finland* **3** *Museum of Comparative Zoology, Department of Organismic and Evolutionary Biology, Harvard University, 26 Oxford Street, Cambridge, MA 02138, USA*

Corresponding author: *Gregory D. Edgecombe* (g.edgecombe@nhm.ac.uk)

Academic editor: *Ivan H. Tuf* | Received 12 September 2014 | Accepted 15 May 2015 | Published 30 June 2015

http://zoobank.org/C2A99E47-11F6-4F1B-B7BE-2088DCA23573

Citation: Edgecombe GD, Vahtera V, Giribet G, Kaunisto P (2015) Species limits and phylogeography of *Newportia* (Scolopendromorpha) and implications for widespread morphospecies. In: Tuf IH, Tajovský K (Eds) Proceedings of the 16th International Congress of Myriapodology, Olomouc, Czech Republic. ZooKeys 510: 65–77. doi: 10.3897/zookeys.510.8573

Abstract

The genus *Newportia* Gervais, 1847, includes some 60 nominal species distributed in the Caribbean islands and from Mexico to central South America. Modern keys to species and subspecies are available, greatly facilitating identification, but some species are based on few specimens and have incomplete documentation of taxonomically-informative characters. In order to explore genetic variability and evolutionary relationships within geographically-widespread morphospecies, specimens of *N.* (*N.*) *stolli* (Pocock, 1896) and *N.* (*N.*) *divergens* Chamberlin, 1922, two nominal species distinguished principally by differences in suture patterns on T1, were sequenced for mitochondrial 16S rRNA and cytochrome *c* oxidase subunit I (COI) genes from populations in southern Mexico, Guatemala, Honduras and Brazil. *N.* (*N.*) *stolli* is paraphyletic with respect to *N.* (*N.*) *divergens* within a clade from Guatemala, Honduras, and Chiapas (Mexico), most trees being consistent with a single loss of a connection between the anterior transverse suture on T1, whereas specimens of "*N.* (*N.*) *stolli*" from Brazil are not closely allied to those from the Mesomerican type area. The widespread morphospecies *N.* (*N.*) *monticola* Pocock, 1890, was sequenced for the same loci from populations in Costa Rica, Colombia and Brazil, finding that specimens from these areas do not unite as a monophyletic group. Samples of *N.* (*N.*) *oreina* Chamberlin, 1915, from different regions of Mexico form geographic clusters that resolve as each other's closest relatives. These results suggest that some widespread species of *Newportia* may be taxa of convenience more so than natural groupings. In several cases geographic proximity fits the phylogeny better than taxonomy, suggesting that non-monophyletic species do not result from use of inappropriate molecular markers. Molecular identification is possible for specimens missing taxonomically informative morphological characters, notably damaged specimens that lack the ultimate leg pair, a protocol that may also apply to other taxonomically difficult genera that are prone to damage (such as *Cryptops*).

Keywords

Scolopocryptopidae, Newportiinae, Neotropics, phylogeny

Introduction

Newportia Gervais, 1847 is a species-rich Neotropical genus that belongs to the family Scolopocryptopidae, encompassing blind Scolopendromorpha with 23 leg-bearing segments, pectinate second maxillary claws, and kinked and pineapple-shaped processes in the gizzard (Shelley and Mercurio 2005; Koch et al. 2009, 2010). *Newportia* has until recently been classified as one of two genera in the subfamily Newportiinae, distinguished from *Tidops* Chamberlin, 1915, by different forcipular structures (Chagas-Júnior 2011). Phylogenetic analyses based on multi-locus molecular sequence data have, however, indicated that *Tidops* nests within *Newportia* rather than being the sister group, as does another clade that had been assigned to a separate subfamily, the Mesoamerican Ectonocryptopinae (Vahtera et al. 2013).

The geographic distribution of *Newportia* (including *Tidops*, *Ectonocryptops* Crabill, 1977, and *Ectonocryptoides* Shelley & Mercurio, 2005 as subgenera: Vahtera et al. 2013) extends from northern Mexico throughout Central America and the Caribbean islands to Paraguay. Most species of *Newportia* have tarsus 2 of the ultimate leg divided into five to nearly 40 tarsomeres, or with indistinct separation of tarsi 1 and 2. Currently some 60 nominal species or subspecies are recognised (Minelli et al. 2006 and onwards; Schileyko 2013). In many species, diagnostic features involve the spinose processes on the ultimate prefemora and femora and the number of tarsomeres, all inconvenient characters because individuals frequently lose these legs when collected.

We propose a solution to the taxonomic impediment of missing ultimate legs by using mitochondrial sequence data to supplement identifications. We also explore phylogeographic patterns within and between select species of *Newportia* from Mexico and Central America using parsimony and maximum likelihood methods. The resultant phylogenies allow the taxonomic value of purportedly diagnostic morphological characters to be evaluated and for the limits of morphospecies to be tested.

Methods

Thirty-four specimens of *Newportia* from Mexico, Guatemala, Honduras, and Costa Rica were sorted mostly from collections made by the LLAMA (Leaf Litter Survey of

Mesoamerica) project, deposited in the Museum of Comparative Zoology (MCZ), Harvard University, Cambridge Massachusetts, USA and accessible through the dedicated data base MCZbase (http://mczbase.mcz.harvard.edu). All tissues were fixed in absolute ethanol and thus were amendable to DNA sequencing.

Identifications were made using the most recent key for *N.* (*Newportia*) (Schileyko, 2013), supplemented with taxonomic descriptions in modern literature (Schileyko and Minelli 1998; Chagas-Júnior and Shelley 2003), standard monographs (Attems 1930), original descriptions, and examination of type material designated by R. I. Pocock in The Natural History Museum (London) and or by R. V. Chamberlin in the MCZ.

LLAMA specimens keyed to either *N.* (*N.*) *monticola* Pocock, 1890, *N.* (*N.*) *stolli* (Pocock, 1896), *N.* (*N.*) *oreina* Chamberlin, 1915, or *N.* (*N.*) *divergens* Chamberlin, 1922. All LLAMA specimens were sequenced for two mitochondrial loci: 16S rRNA and cytochrome *c* oxidase subunit I (COI). These loci were selected because they vary both within and between species, and even between individuals from geographically close populations. The 34 LLAMA samples were supplemented with *N.* (*Newportia*) and *N.* (*Ectonocryptoides*) sequences from our previous work (Vahtera et al. 2013), nine new *Newportia* specimens from five localities in Amazonas and Roraima, Brazil, and novel sequences for an individual of *N.* (*N.*) *pusilla* Pocock, 1893, from Ecuador (see Table 1 for morphospecies determinations and locality data).

Total DNA was extracted from the legs utilizing the NucleoSpin®Tissue kit (Macherey-Nagel). Samples were incubated overnight. PCR amplifications were performed with illustra TM PuReTaq TM Ready-To-GoTM PCR Beads (GE Healthcare). The COI fragments were amplified using primer pair HCO1490 (Folmer et al. 1994) and HCOout (Carpenter and Wheeler 1999) and the 16S rRNA fragments using primer pair 16Sa/16Sb (Xiong and Kocher 1991; Edgecombe et al. 2002). The normal amplification cycle for COI consisted of an initial denaturation step (2 min at 95 °C), followed by 35 cycles of denaturation (1 min at 95 °C), annealing (1 min at 43 °C) and extension (1.5 min at 72 °C), followed by a final extension step (4 min at 72 °C). For the 16S rRNA fragment the cycle consisted of an initial denaturation step (2 min at 94 °C), followed by 35 cycles of denaturation (30 s at 94 °C), annealing (30 s min at 43 °C) and extension (1 min at 72 °C), followed by a final extension step (7 min at 72 °C). Visualization of the PCR products was done by 1 % agarose electrophoresis using Midori Green Advanced DNA Stain and FastGene® GelPic LED Box (Nippon Genetics, GmbH).

Samples were purified using ExoSAP-IT (Affymetrix) and sent to FIMM (Institute for Molecular Medicine Finland) for sequencing. Chromatograms were visualized and assembled using Sequencer 5.0.1 (Gene Codes Corp., Ann Arbor, Michigan, USA). Sequence alignment editor Se-Al (Rambaut 1996) was used to visualize the sequences simultaneously. GenBank registrations for new sequences are listed in Table 1.

Parsimony analysis was conducted with POY ver. 5.1.1 (Wheeler et al. 2014) run in 16 nodes in the high-performance supercluster Taito at CSC (IT-Center of Science), Finland. A timed search of three hours was first performed on the unaligned data set.

Table 1. Specimens sequenced in this study and their GenBank accession numbers. Institutional abbreviation: MCZ, Museum of Comparative Zoology, Harvard University. Bold font indicates new sequence data.

Species	Voucher ID number	Lab code	Voucher	Country (State)	16S	COI	Lat. (degrees)	Long. (degrees)
Newportia adisi	130770	-	MCZ	Brazil (Amazonas)	KF676465	KF676506	2.93355S	59.96611W
N. (*Ectonocryptoides*) *quadrimeropus*	130826	-	MCZ	Mexico (Jalisco)	HQ402494	HQ402546	-	-
N. collaris	18827	95a	MCZ	Brazil (Roraima)	**KP099547**	**KP099504**	0.99185N	62.15915W
N. divergens	98078	72	MCZ	Honduras	**KP099524**	**KP099481**	14.45748N	89.06819W
N. divergens	99129	75	MCZ	Honduras	**KP099525**	**KP099482**	14.45603N	89.06904W
N. divergens	98978	81	MCZ	Honduras	**KP099526**	**KP099483**	15.69449N	86.86339W
N. divergens	99154	82	MCZ	Honduras	**KP099527**	**KP099484**	14.48139N	87.53225W
N. divergens	88191	85	MCZ	Guatemala	**KP099528**	**KP099485**	14.5357724N	90.69427782W
N. divergens	89343	101	MCZ	Guatemala	**KP099529**	**KP099486**	14.94704N	89.27627W
N. divergens	89474	105	MCZ	Guatemala	**KP099530**	**KP099487**	14.53256659N	90.15252622W
N. ernsti ernsti	18828	94	MCZ	Brazil (Roraima)	**KP099522**	**KP099479**	1.01113S	62.11409W
N. ernsti ernsti	105917	-	MCZ	Dominican Republic	JX422692	JX422669	-	-
N. longitarsis stechowi	130774	LP2871	AMNH	French Guiana	JX422693	JX422670	4.506277N	52.058305W
N. monticola	130778	-	MCZ	Colombia	JX422694	JX422671	5.7095080242N	73.4601469617W
N. monticola	80065	40	MCZ	Costa Rica	**KP099531**	**KP099488**	8.40667N	83.32833W
N. monticola	80743	49	MCZ	Costa Rica	**KP099532**	**KP099489**	8.78658N	82.95987W
N. monticola	81355	55	MCZ	Costa Rica	**KP099533**	**KP099490**	8.94997N	82.83375W
N. monticola	21666	91	MCZ	Brazil (Roraima)	**KP099534**	**KP099491**	1.01113S	62.11409W
N. oreina	94265	57	MCZ	Mexico (Tamaulipas)	**KP099535**	**KP099492**	23.0344N	99.18697W
N. oreina	94726	58	MCZ	Mexico (Oaxaca)	**KP099536**	**KP099493**	17.89844N	96.36253W
N. oreina	94185	59	MCZ	Mexico (Tamaulipas)	**KP099537**	**KP099494**	23.0233N	99.2883W
N. oreina	93765	60	MCZ	Mexico (Tamaulipas)	**KP099538**	**KP099495**	23.0611N	99.21564W
N. oreina	93666	62	MCZ	Mexico (Tamaulipas)	**KP099539**	**KP099496**	23.00835N	99.28511W
N. oreina	95181	66	MCZ	Mexico (Oaxaca)	**KP099541**	**KP099500**	17.65934N	96.33426W
N. oreina	93981	68	MCZ	Mexico (Oaxaca)	**KP099540**	**KP099497**	17.89844N	96.36253W
N. pusilla	18758	86	MCZ	Ecuador	**KP099542**	**KP099498**	0.6083333S	77.8825W
N. pusilla	18824	90	MCZ	Brazil (Amazonas)	**KP099543**	**KP099499**	2.93349S	59.96895W

Species	Voucher ID number	Lab code	Voucher	Country (State)	16S	COI	Lat. (degrees)	Long. (degrees)
N. sp.	81282	54	MCZ	Costa Rica	**KP099544**	**KP099501**	8.94997N	82.83375W
N. sp.	18822	89b	MCZ	Brazil (Roraima)	**KP099545**	**KP099502**	0.99539S	62.15904W
N. sp.	18825	92	MCZ	Brazil (Roraima)	**KP099546**	**KP099503**	1.02897S	62.08722W
N. stolli	106516	37	MCZ	Guatemala	**KP099510**	**KP099467**	14.91852N	91.10458W
N. stolli	81361	42	MCZ	Guatemala	**KP099511**	**KP099468**	15.1144N	89.68046667W
N. stolli	81360	44	MCZ	Mexico (Chiapas)	**KP099505**	**KP099462**	16.13853333N	90.90146667W
N. stolli	79982	47	MCZ	Mexico (Chiapas)	**KP099506**	**KP099463**	16.96385N	91.59313W
N. stolli	80143	48	MCZ	Guatemala	**KP099512**	**KP099469**	15.0583333N	89.676667W
N. stolli	80175	50	MCZ	Mexico (Chiapas)	**KP099507**	**KP099464**	17.17536N	93.14939W
N. stolli	81363	52	MCZ	Mexico (Chiapas)	**KP099508**	**KP099465**	16.97416667N	91.58591667W
N. stolli	80208	53	MCZ	Mexico (Chiapas)	**KP099509**	**KP099466**	16.75181N	92.68267W
N. stolli	99225	71	MCZ	Guatemala	**KP099514**	**KP099471**	15.08405N	89.94991W
N. stolli	99279	78	MCZ	Guatemala	**KP099513**	**KP099472**	15.07708N	89.94795W
N. stolli	18826	88a	MCZ	Brazil (Roraima)	**KP099520**	**KP099477**	1.02897S	62.08722W
N. stolli	18830	93a	MCZ	Brazil (Roraima)	**KP099521**	**KP099478**	1.01113S	62.11409W
N. stolli	18827	95b	MCZ	Brazil (Roraima)	**KP099523**	**KP099480**	0.99185N	62.15915W
N. stolli	89566	99	MCZ	Guatemala	**KP099515**	**KP099470**	15.21241135N	90.21480799W
N. stolli	89321	100	MCZ	Guatemala	**KP099516**	**KP099473**	16.44568931N	89.54981728W
N. stolli	89306	102	MCZ	Guatemala	**KP099517**	**KP099474**	17.24033736N	89.62094017W
N. stolli	89355	103	MCZ	Guatemala	**KP099518**	**KP099475**	15.21318939N	90.21921316W
N. stolli	89606	104	MCZ	Guatemala	**KP099519**	**KP099476**	16.44147064N	89.53447W
N. stolli	130787	-	MCZ	Guatemala	KF676467	KF676508	15.0833333N	89.9441666W
Cryptops punicus	130604	-	MCZ	Italy	KF676461	KF676503	40.01471N	9.22261E
Scolopocryptops mexicanus	105626	-	MCZ	Ecuador	JX422703	JX422679	1.336111N	77.263055W

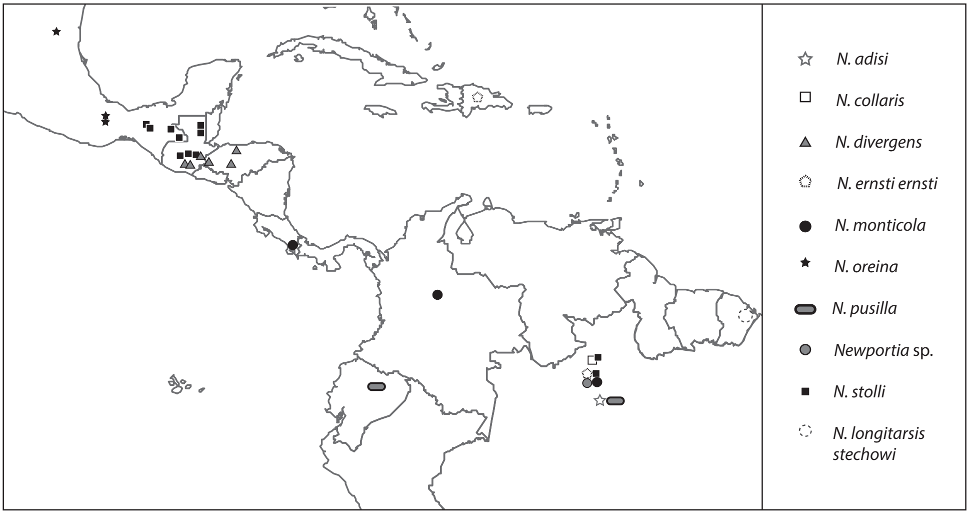


Figure 1. Map of Mesoamerica, the Caribbean and northern South America showing geographic distribution of *Newportia* specimens analyzed herein (see Table 1 for coordinates of samples).

The resulting tree was used as the starting tree for the next round in which an additional timed search of six hours was performed. Parameter set 111 (indel/transversion and transversion/transition costs all equal) was used throughout the searches and branch lengths were reported using the newly implemented command "report ("file_name.tre", trees:(total, branches:true))". Nodal support was calculated using parsimony jackknifing (Farris et al. 1996).

Additional analyses used a probabilistic approach with the maximum likelihood program RAxML ver. 8.0.22 (Stamatakis 2014). For these, multiple sequence alignments (MSA) were first estimated with MUSCLE ver. 3.6 (Edgar 2004) and then trimmed using Gblocks ver. 0.91b (Castresana 2000; Talavera and Castresana 2007) to remove areas of ambiguous alignment. Since COI sequences showed no length variation, they were not trimmed in Gblocks. The amount of 16S rRNA data that remained after trimming was 59% of the original 585 positions. The two data sets were concatenated using SequenceMatrix (Vaidya et al. 2011) and the concatenated data were analyzed with RAxML in the CIPRES Science Gateway (Miller et al. 2010). A unique general time reversible (GTR) model was specified for each partition independently. Nodal support was estimated using the rapid bootstrap algorithm (applying the Majority Rule Criterion) using the GTR-CAT model (Stamatakis et al. 2008).

Results

The combined analysis of both COI and 16S fragments using parsimony as the optimality criterion resulted in two most parsimonious (MP) trees of length 4625 steps.

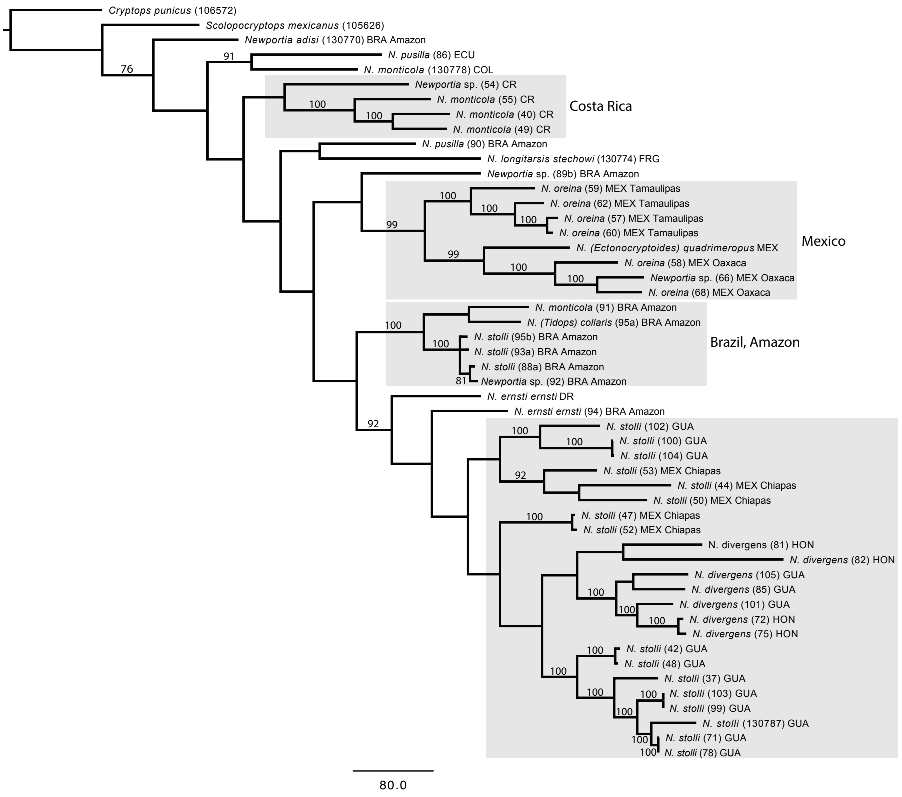


Figure 2. Strict consensus of two optimal cladograms for *Newportia* under parameter set 111 for parsimony (POY) analysis. Abbreviations: BRA, Brazil; COL, Colombia; CR, Costa Rica; DR, Dominican Republic; ECU, Ecuador; FRG, French Guiana; GUA, Guatemala; HON, Honduras; MEX, Mexico.

The strict consensus tree (Fig. 2) shows these two trees are almost identical, differing only in the placement of two Brazilian specimens of *N.* (*N.*) *stolli* in relation to each other. Comparing strongly supported clades, the maximum likelihood tree (ln*L* -14054.372302: Fig. 3) shows the same major geographic and taxonomic groupings as the parsimony tree. This congruence is noteworthy because the data sets analyzed under these two optimality criteria were different (unaligned in POY and analyzed using the concept of dynamic versus static homologies with some regions removed in RAxML), as are the resampling methods (jackknifing and bootstrapping, respectively). Parts of the trees that are incongruent between the two analyses involve nodes that received low resampling supports in both analyses (e.g., the positions of *N.* (*N.*) *adisi* and Brazilian specimen 89b relative to other species). Both analyses depict substantial branch lengths both within and between species, with only a few instances of no (or minimal) variation between specimens from the same or geographically close populations.

As in previous analyses based on sparser sampling for *Newportia* (Vahtera et al. 2013), *Tidops* (*T. collaris*) and *Ectonocryptoides* (*E. quadrimeropus*) nest within *Newportia* in all

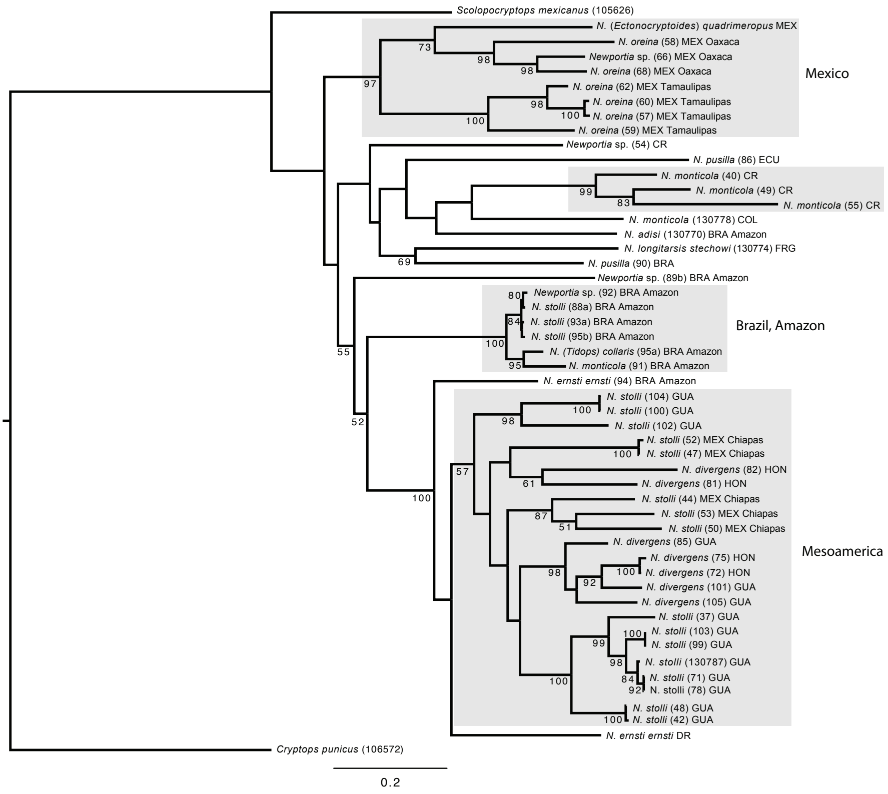


Figure 3. Maximum likelihood tree (ln*L* = -14054.372302). Abbreviations for countries as in Fig. 2.

analyses. Specifically, they unite with *Newportia* (*Newportia*) spp. that inhabit the same geographic region i.e., *N.* (*T.*) *collaris* from the Brazilian Amazon groups within a clade composed of species of *Newportia* (*Newportia*) from there, whereas *N.* (*E.*) *quadrimeropus* from Jalisco, Mexico, groups with the Mexican *N.* (*N.*) *oreina*. These results reinforce proposals to classify *Tidops*, *Ectonocryptoides* and presumably allied *Ectonocryptops* within *Newportia* and to regard Ectonocryptopinae as subordinate to Newportiinae (Vahtera et al. 2013). The traditional classification of *N.* (*Tidops*) and *N.* (*Ectonocryptoides*) as separate genera because of their obvious phenotypic differences from *N* (*Newportia*) might have predicted that they would be markedly different from *N.* (*Newportia*) genetically. However, neither *N.* (*Tidops*) *collaris* nor *N.* (*Ectonocryptoides*) *quadrimeropus* depict long branch divergences from their closest relatives with respect to the studied loci, indeed being shorter than some population-level branches within species.

Newportia oreina consists of two geographical clades and this division is found in both parsimony and likelihood analyses; one clade consists of all specimens from Tamaulipas (JK, BS 100) and the other of ones from Oaxaca (JK 100, BS 98). Interestingly, *N.* (*Ectonocryptoides*) *quadrimeropus* forms a well-supported (JK 99, BS 73) clade with the *N.* (*N.*) *oreina* populations from Oaxaca, rendering *N.* (*N.*) *oreina* paraphyletic with respect to *Ectonocryptoides* (and presumably *Ectonocryptops*). A previous scolopendromorph phylogeny (Vahtera et al. 2013) had also indicated affinity between *N.* (*N.*) *oreina* and *N.* (*Ectonocryptoides*) *quadrimeropus*; analyses based on combined molecular and morphological data resolved them as sister-groups, although only one individual of each was then available. We note that *N. oreina* possesses a shorter tarsus than most congeners. The phylogeny interprets the ancestral condition of the ultimate leg tarsi of *Newportia* as being elongate and divided into tarsomeres, with the relatively short tarsus 2 of *N.* (*N.*) *oreina* being a possible precursor to the stout tarsi of the submerged taxon, "Ectonocryptopinae". This transformation series increases the plausibility of the subclavate "ectonocryptopine" ultimate legs being derived from an ancestor with flagelliform tarsi, a result that was already strongly signaled by molecular phylogenies (Vahtera et al. 2013) and is reinforced by the current trees.

A Mesoamerican clade uniting *N.* (*N.*) *stolli* and *N.* (*N.*) *divergens* from Mexico (Chiapas), Guatemala and Honduras is recovered in both parsimony and likelihood analyses (Figs 2, 3), though resampling methods did not strongly support it (JK <50, BS 57). *N.* (*N.*) *divergens* is resolved as monophyletic in the POY analyses but is nested within a paraphyletic *N.* (*N.*) *stolli*, implying a single loss of the median part of the anterior transverse suture on T1 (Fig. 2). However, there is no jackknife support for the *divergens* clade. In contrast, the likelihood analysis did not support monophyly of *N.* (*N.*) *divergens*; six individuals from Guatemala and Honduras resolve as a well-supported clade (BS 98), but two others from Honduras (81, 82) are grouped with two Mexican *N.* (*N.*) *stolli* specimens, albeit with weak nodal support.

Specimens identified as *N.* (*N.*) *stolli* from the Brazilian Amazon do not unite with supposed congeners from Mesoamerica but are instead most closely related to other taxa from the same region, i.e., a specimen identified as *N.* (*N.*) *monticola* (91) and *N.* (*Tidops*) *collaris*. This result implies that *N.* (*N.*) *stolli* is polyphyletic and an indistinct segmentation of ultimate tarsus 2 has multiple (convergent) origins. This character had once served as the basis for recognising a subgenus *N.* (*Scolopendrides*), e.g., in the classification of Bücherl (1974), but this taxon is not used in current classifications (Schileyko and Minelli 1998). We re-examined the *N.* (*N.*) *stolli* specimens again in light of the signal for non-monophyly in the phylogenetic analysis, attempting to recognize any morphological character(s) that would separate the specimens from Brazil from those from Mesoamerica. However, we found no distinctive characters between the samples; the specimens appear to be morphologically indistinguishable and using the existing keys they would all be identified as *N.* (*N.*) *stolli* with confidence.

Costa Rican specimens of *N.* (*N.*) *monticol*a unite as a monophyletic group (JK 100, BS 99) in both analyses. In the maximum likelihood tree (Fig. 3) a Colombian specimen of *N.* (*N.*) *monticola* (103974) is resolved as a sister taxon to the Costa Rican clade but this relationship is not found in the parsimony tree (Fig. 2). In neither analysis did a Brazilian specimen identified as *N.* (*N.*) *monticola* unite with the other supposed conspecifics.

The two included specimens of *N.* (*N.*) *pusilla*, one from Ecuador (specimen 86) and the other from Brazilian Amazonas (specimen 90), likewise do not form a clade but instead are situated in different parts of the tree. The Brazilian specimen conforms to "Amazonian type *pusilla*" of Schileyko and Minelli (1998), characterized by rudimentary paramedian sutures on T1 (in contrast to their complete absence in other populations). Both analyses group this Brazilian specimen together with *N.* (*N.*) *longitarsis stechowi* but since there is no strong resampling support in either analysis (JK <50, BS 69), the question about its identity and closest relative remains unclear.

We also included a few *Newportia* specimens that could not be identified morphologically since they lacked ultimate legs, were juveniles, or did not key out to any known species. A specimen (54) from Costa Rica has a unique character combination and is apparently a distinct species but lacks its ultimate legs. In the POY analysis it groups together, although with weak support, with the Costa Rican *N.* (*N.*) *monticola* clade. A very distinctive Brazilian specimen (89b) with all tarsi bipartite and tarsus 2 of the ultimate leg undivided groups at the base of the Mexican *N.* (*N.*) *oreina*/*N.* (*E.*) *quadrimeropus* clade in the parsimony analysis. However, there is poor resampling support for this grouping and it is instead allied to species with indistinctly segmented ultimate tarsus 2 and the Brazilian clade in the likelihood tree. The poor support values and topological instability under different analytical conditions render the affinities of this undescribed species uncertain.

Discussion

Some of the specimens used in this study were either of small size because of the collection methods employed (and thus may not have been appropriate for keying using traditional criteria formulated for mature specimens) or were missing their taxonomically-informative ultimate legs. Nonetheless, several such specimens could be identified with a high degree of accuracy because their sequence data placed them within clades whose nomenclature could be established based on standard external morphological characters. An example is provided by a juvenile from Brazil (92) that is in poor condition and cannot be identified to species. However, the analysis shows it to be a juvenile of a Brazilian clade assigned to *N.* (*N.*) *stolli*. This approach is likely to be valuable in other groups of taxonomically-difficult centipedes that rely heavily on characters of the ultimate leg pair but often lack those legs in fixed specimens, such as *Cryptops*, where the numbers of tibial and tarsal saw teeth are fundamental taxonomic characters. The identification of developmental stages or adults without key taxonomic characters is becoming standard for many groups of animals, including other arthropod groups, such as insects (Monaghan et al. 2009; Gattolliat and Monaghan 2010) and arachnids (Fernández et al. 2014).

Some morphologically delimited species were found to be monophyletic groups, like *N.* (*N.*) *divergens* in the parsimony analysis, but others were paraphyletic or polyphyletic. This could be interpreted as a failure of the taxonomic characters traditionally used to delimit species or a failure in reconstructing an accurate tree by the markers selected. The second option is unlikely for the reasons outlined below, especially the biogeographical patterns exhibited in many clades where "distinct" species from the same regions tend to cluster together and not with their supposed conspecifics from other geographical regions. In particular *N.* (*N.*) *stolli* formed a series of geographic groupings that in part were paraphyletic with respect to sympatric species (specifically, to *N.* (*N.*) *divergens* in Mesoamerica) or in other cases were found to be distantly related (Brazilian "*N.* (*N.*) *stolli*"). The first pattern is consistent with *N.* (*N.*) *stolli* being a grade united by a plesiomorphy (a continuous anterior transverse suture on T1), some parts of which are most closely related to a species defined by an apomorphic state (i.e., loss of the median extent of the anterior transverse suture). The tree topology, however, suggests that the Brazilian specimens identified as *N.* (*N.*) *stolli* are misidentified. *Newportia* (*N.*) *monticola* is likewise a questionable taxon, the monophyletic Costa Rican group never uniting with a specimen of the same putative species from Brazil and only variably so with one from Colombia. Brazilian *N.* (*N.*) *monticola* and *N.* (*N.*) *stolli* unite in a well-supported clade (JF and BS 100), indicating that, in this instance, geography is a better predictor of relationships than taxonomy. It is noteworthy that *N.* (*N.*) *stolli* and *N.* (*N.*) *monticola* are among the most geographically widespread "species" of *Newportia*, but our results suggest that the wide distribution is partly an artifact of morphologically-based identifications. The same evidently applies to *N.* (*N.*) *pusilla*, a morphospecies that is regarded as ranging from St. Vincent through Colombia to the Brazilian Amazon (Schileyko and Minelli 1998; Chagas-Júnior et al. 2014). Polyphyly of this species in the molecular trees suggests that its diagnostic characters (absent or rudimentary paramedian sutures on T1 and a lack of ventral spinose processes on the ultimate leg femora) evolved convergently in different regions.

Centipede systematics, still strongly influenced by mid 20th Century conceptualisations of species (see Edgecombe 2007), primarily assumes polymorphic and geographically widespread entities. The existing concepts that *N.* (*N.*) *monticola* and *N.* (*N.*) *stolli* are widespread throughout much of Central and South America exemplify where morphospecies do not appear to correspond to clades but rather are classes defined by combinations of characters. In these instances, molecular tools may prove to be invaluable for species delimitations, and novel morphological characters will need to be identified to rediagnose polyphyletic species.

Acknowledgements

Most samples were collected from the LLAMA (Leaf Litter Survey of Mesoamerica) survey, a collecting program supported by NSF grant DEB-0640015 to John Longino. Laboratory expenses were covered by a grant from the Finnish Entomological Society. Funding for GG's fieldwork in Brazil was provided by the National Geographic Society to the Amazon (2012), and by US National Science Foundation grant #1144417 (Collaborative Research: ARTS: Taxonomy and systematics of selected Neotropical clades of arachnids) to GG and G. Hormiga. We thank the University of Turku for supporting GDE's visit in January 2014, and CSC – IT Center for Science Ltd. for the allocation of computational resources. Emil Vahtera and Kari Kaunisto assisted with figures. Input from Arkady Schileyko and Rowland Shelley as referees improved the manuscript.

References

Attems C (1930) Myriapoda 2. Scolopendromorpha. In: Schulze FE, Kükenthal W (Eds) Das Tierreich, 54. Walter de Gruyter, Berlin, 1–308.

Bücherl W (1974) Die Scolopendromorpha der Neotropischen Region. In: Blower JG (Ed.) Myriapoda. Sysmposia of the Zoological Society of London 32. Academic Press, 99–133.

Carpenter JM, Wheeler WC (1999) Towards simultaneous analysis of morphological and molecular data in Hymenoptera. Zoologica Scripta 28: 251–260. doi: 10.1046/j.1463-6409.1999.00009.x

Castresana J (2000) Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. Molecular Biology and Evolution 17: 540–552. doi: 10.1093/oxfordjournals.molbev.a026334

Chagas-Júnior A (2011) A review of the centipede genus *Tidops* Chamberlin (Scolopendromorpha, Scolopocryptopidae, Newportiinae). International Journal of Myriapodology 5: 63–82. doi: 10.3897/ijm.5.1649

Chagas-Júnior A, Chaparro E, Galvis Jiménez S, Triana Triana HD, Flórez E, Sícoli Seoane JC (2014) The centipedes (Arthropoda, Myriapoda, Chilopoda) from Colombia: Part 1. Scutigeromorpha and Scolopendromorpha. Zootaxa 3779: 133–156. doi: 10.11646/zootaxa.3779.2.2

Chagas-Júnior A, Shelley RM (2003) The centipede genus *Newportia* Gevais, 1847, in Mexico: description of a new troglomorphic species; redescription of *N. sabina* Chamberlin, 1942; revival of *N. azteca* Humbert & Saussure, 1869; and a summary of the fauna (Scolopendromorpha: Scolopocryptopidae: Newportiinae). Zootaxa 379: 1–20.

Crabill RE Jr. (1977) A new cryptopid genus, with key to the genera known to occur in North America including Mexico (Chilopoda: Scolopendromorpha: Cryptopidae). Proceedings of the Entomological Society of Washington 79: 346–349.

Edgar RC (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res. 32(5): 1792–1797. doi: 10.1093/nar/gkh340

Edgecombe GD (2007) Centipede systematics: progress and problems. Zootaxa 1668: 327–341.

Edgecombe GD, Giribet G, Wheeler WC (2002) Phylogeny of Henicopidae (Chilopoda: Lithobiomorpha): a combined analysis of morphology and five molecular loci. Systematic Entomology 27: 31–64. doi: 10.1046/j.0307-6970.2001.00163.x

Farris JS, Albert VA, Källersjö M, Lipscomb D, Kluge AG (1996) Parsimony jackknifing outperforms neighbor-joining. Cladistics 12: 99–124. doi: 10.1111/j.1096-0031.1996.tb00196.x

Fernández R, Vélez S, Giribet G (2014) Linking genetic diversity and morphological disparity: biodiversity assessment of a highly unexplored family of harvestmen (Arachnida : Opiliones : Neopilionidae) in New Zealand. Invertebrate Systematics 28: 590–604. doi: 10.1071/IS14029

Gattolliat JL, Monaghan MT (2010) DNA-based association of adults and larvae in Baetidae (Ephemeroptera) with the description of a new genus *Adnoptilum* in Madagascar. Journal of the North American Benthological Society 29: 1042–1057. doi: 10.1899/09-119.1

Koch M, Edgecombe GD, Shelley RM (2010) Anatomy of *Ectonocryptoides* (Scolopocryptopidae: Ectonocryptopinae) and the phylogeny of blind Scolopendromorpha (Chilopoda). International Journal of Myriapodology 3:51–81. doi: 10.1163/187525410X12578602960344

Koch M, Pärschke S, Edgecombe GD (2009) Phylogenetic implications of gizzard morphology in scolopendromorph centipedes (Chilopoda). Zoologica Scripta 38: 269–288. doi: 10.1111/j.1463-6409.2008.00372.x

Miller MA, Pfeiffer W, Schwartz T (2010) Creating the CIPRES Science Gateway for inference of large phylogenetic trees. In: Proceedings of the Gateway Computing Environments Workshop (GCE), 14 Nov. 2010, New Orleans, LA, 1–8. doi: 10.1109/GCE.2010.5676129

Minelli A, Bonato L, Dioguardi R, Chagas-Júnior A, Edgecombe GD, Lewis JGE, Pereira LA, Shelley RM, Stoev P, Uliana M, Zapparoli M (2006 and onwards) CHILOBASE. A web resource for Chilopoda taxonomy. http://chilobase.bio.unipd.it

Monaghan MT, Wild R, Elliot M, Fujisawa T, Balke M, Inward DJ, Lees DC, Ranaivosolo R, Eggleton P, Barraclough TG, Vogler AP (2009) Accelerated species inventory on Madagascar using coalescent-based models of species delineation. Systematic Biology 58: 298–311. doi: 10.1093/sysbio/syp027

Schileyko AA (2013) A new species of *Newportia* Gervais, 1847 from Puerto Rico, with a revised key to the species of the genus (Chilopoda, Scolopendromorpha, Scolopocryptopidae). ZooKeys 276: 39–54. doi: 10.3897/zookeys.276.4876

Schileyko AA, Minelli A (1998) On the genus *Newportia* Gervais, 1847 (Chilopoda: Scolopendromorpha: Newportiidae). Arthropoda Selecta 7: 265–299.

Shelley RM, Mercurio R (2005) *Ectonocryptoides quadrimeropus*, a new centipede genus and species from Jalisco, Mexico; proposal of Ectonocryptopinae, analysis of subfamilial relationships, and a key to subfamilies and genera of the Scolopocryptopidae (Scolopendromorpha). Zootaxa 1094: 25–40.

Stamatakis A, Hoover P, Rougemont J (2008) A rapid bootstrap algorithm for the RAxML web servers. Systematic Biology 57: 758–771. doi: 10.1080/10635150802429642

Stamatakis A (2014) RAxML Version 8: A tool for Phylogenetic Analysis and Post-Analysis of Large Phylogenies. Bioinformatics (2014) 30(9): 1312–1313. doi: 10.1093/bioinformatics/btu033

Talavera G, Castresana J (2007) Improvement of phylogenies after removing divergent and ambiguously aligned blocks from protein sequence alignments. Systematic Biology 56: 564–577. doi: 10.1080/10635150701472164

Vahtera V, Edgecombe GD, Giribet G (2013) Phylogenetics of scolopendromorph centipedes: Can denser taxon sampling improve an artificial classification? Invertebrate Systematics 27: 578–602. doi: 10.1071/IS13035

Vaidya G, Lohman DJ, Meier R (2011) SequenceMatrix: concatenation software for the fast assembly of multi-gene datasets with character set and codon information. Cladistics 27: 171–180. doi: 10.1111/j.1096-0031.2010.00329.x

Wheeler WC, Lucaroni N, Hong L, Crowley LM, Varón A (2014) POY version 5: phylogenetic analysis using dynamic homologies under multiple optimality criteria. Cladistics 31: 189–196. doi: 10.1111/cla.12083

Xiong B, Kocher TD (1991) Comparison of mitochondrial DNA sequences of seven morphospecies of black flies (Diptera: Simuliidae). Genome 34: 306–311. doi: 10.1139/g91-050

ZooKeys 510: 79–94 (2015)
doi: 10.3897/zookeys.510.8640
http://zookeys.pensoft.net

RESEARCH ARTICLE

A peer-reviewed open-access journal
ZooKeys
Launched to accelerate biodiversity research

Cave Diplopoda of southern China with reference to millipede diversity in Southeast Asia

Sergei I. Golovatch[1]

[1] *Institute for Problems of Ecology and Evolution, Russian Academy of Sciences, Leninsky pr. 33, Moscow 119071, Russia*

Corresponding author: *Sergei I. Golovatch* (sgolovatch@yandex.ru)

Academic editor: *Ivan H. Tuf* | Received 23 September 2014 | Accepted 30 April 2015 | Published 30 June 2015

http://zoobank.org/8CF61E59-8A36-4F41-AFA9-6C19978B2C09

Citation: Golovatch SI (2015) Cave Diplopoda of southern China with reference to millipede diversity in Southeast Asia. In: Tuf IH, Tajovský K (Eds) Proceedings of the 16th International Congress of Myriapodology, Olomouc, Czech Republic. ZooKeys 510: 79–94. doi: 10.3897/zookeys.510.8640

Abstract

The diversity of Diplopoda in caves of southern China is remarkably high, often 5–6 species per cave, consisting mostly of local endemics and presumed troglobionts. These are evidently biased to just a few lineages, mainly members of the orders Chordeumatida and Callipodida, the families Cambalopsidae (Spirostreptida) and Haplodesmidae (Polydesmida) or the genera *Pacidesmus*, *Epanerchodus* and *Glenniea* (all Polydesmida, Polydesmidae), *Trichopeltis* (Polydesmida, Cryptodesmidae), *Dexmoxytes* (Polydesmida, Paradoxosomatidae) and *Hyleoglomeris* (Glomerida, Glomeridae). All these taxa, especially the Paradoxosomatidae and Cambalopsidae (usually amounting to about 60% and 10% of the total species diversity in the Oriental fauna, respectively), are moderately to highly speciose across Southeast Asia, being largely epigean. However, the epigean Diplopoda of southern China are yet badly understudied, since much of the collecting and taxonomic exploration efforts still focus on cavernicoles. The Oriental Region is the only biogeographic realm globally that harbours all 16 orders of Diplopoda, of which 14 have already been encountered in China and/or the immediately adjacent parts of Indochina. Thus, China may actually prove to support no less than 1,000 millipede species of various origins, mainly Oriental and Palaearctic.

Keywords

Millipede, fauna, richness, cavernicoly, China, Oriental realm

Introduction

The class Diplopoda, or millipedes, is among the largest of the terrestrial arthropod groups globally, with about 8,000 described species in nearly 1,900 genera, 147 families and 16 orders (Shear 2011). However, the diversity of the Diplopoda is sometimes estimated at up to 80,000 species (Hoffman 1980), currently between 15,000–20,000 using some modern statistics (Brewer et al. 2012). The earliest millipedes are known from the Silurian (early Palaeozoic) and show remarkable ordinal-level diversity, with six orders recorded so far since the Carboniferous (late Palaeozoic) (e.g. Shear and Edgecombe 2010). Being so ancient and diverse taxonomically, widespread (present on all continents except Antarctica), virtually fully terrestrial (even fossils show spiracles), poorly vagile (with highly limited dispersal capacities) and highly limited in compensatory ecological faculties (strongly restricted by a single limiting ecological factor even if the others are favourable), millipedes have long been considered as an exemplary group for biogeographical studies and reconstructions (e.g. Shelley and Golovatch 2011).

Diplopods are largely detritivores, only a few species can be considered omnivores, even fewer as carnivores. The environment that can be postulated as the most typical of the Diplopoda as a whole is temperate (especially deciduous), subtropical or tropical forest (in particular, humid ones). The most typical habitats are leaf litter, the litter/soil interface, the uppermost soil, and dead wood. Being mainly hygro- to mesophilous, millipedes tend to be absent from or only marginal in most of the extreme habitats such as tundra or desert (Golovatch and Kime 2009).

Several basic millipede morphotypes are known: polyxenoid (Polyxenida), glomeroid (Glomerida and Sphaerotheriida), juloid (virtually all Juliformia), polydesmoid (Polydesmida, some Chordeumatida) and platydesmoid (Colobognatha). Similarly, five life-forms, or ecomorphotypes, have been delimited in millipedes (Kime and Golovatch 2000, Golovatch and Kime 2009). Thus, stratobionts, restricted to litter and the uppermost soil, are dominant in the Diplopoda and represented by all five morphotypes. Pedobionts, or geobionts, mainly restricted to mineral soil and represented by the smaller juloid, glomeroid and polydesmoid morphotypes, usually show body miniaturization or elongation, the shortening of appendages, often also decoloration of the teguments and the loss of eyes. Troglobionts, likewise represented almost entirely by the juloid, glomeroid and polydesmoid morphotypes, usually demonstrate a drastic elongation of the extremities, depigmentation of the teguments, blindness, sometimes mouthpart modifications and often also “cave gigantism”. Under-bark xylobionts, or subcorticoles, are also represented by all five morphotypes, but tend to be either particularly flat-bodied (polydesmoids, platydesmoids) or miniature (polyxenoids, glomeroids), often also especially thin (juloids). Finally, epiphytobionts, again with all five morphotypes involved, seem to be characteristic of suspended soil in warm humid forests and are characterized by very small body sizes. The life-form of epiphytobionts is still too poorly delimited to be sure. Moreover, since life-forms of arboricoles (= dendrobionts), symbionts of ants or termites, deserticoles etc. are habitually even less conspicuous, none of them seems to warrant the recognition of a separate life-form.

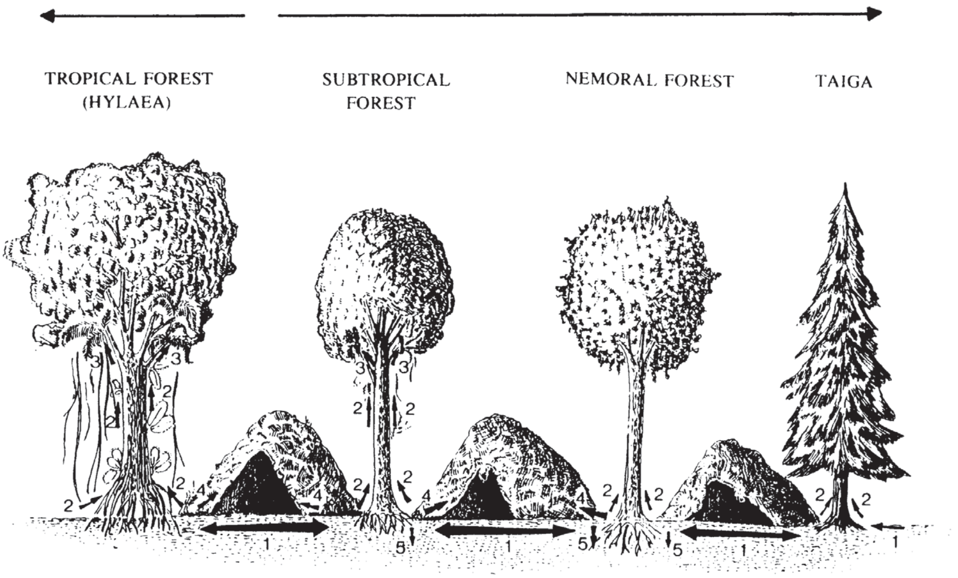


Figure 1. Main trends in the ecological evolution of Diplopoda. All are life forms except for arboricoles. **1** stratobionts **2** trunk and crown arboricoles, as well as subcorticolous xylobionts **3** epiphytobionts **4** troglobionts **5** geobionts. **NB:** The thickness of numbered arrows roughly corresponds to the share of the respective ecological grouping along a gradient of biome succession with age (uppermost arrows, the gap between them indicates the primary subtropical biome whence developed all the main extant biomes). After Kime and Golovatch (2000). Drawing courtesy S. Dashdamirov.

Figure 1 schematically depicts the main trends in, and pathways of, diplopod ecological evolution (Kime and Golovatch 2000). The biomes are arranged according to their age along two vectors of past climatic/biotic change, one showing deterioration from poor to worse conditions (overcooling from subtropical to boreal forest), and the other from warm to hot conditions (overheating, from subtropical to tropical forest) since the end of the so-called "warm Earth", i.e. terminal Oligocene. Generally speaking, all derivative life-forms, i.e. subcorticoles, epiphyto-, troglo- and geobionts, may have evolved from the main stratobionts more or less simultaneously, relatively recently and perhaps since the onset of the Plio-Pleistocene glaciations. The few and rather indistinctly defined life-forms in Diplopoda seem to support this opinion. Yet one must distinguish the age of a taxon from that of a derivative life-form, because there are quite a number of relictual high-level diplopod taxa scattered across the world, including cavernicoles, whose origins seem to date back to the early Cenozoic or even Mesozoic times. We can assume that early in their evolution Diplopoda were generally detritivores living on the forest floor, and that this still applies to the majority. Cylindrical burrowers (juloids), flat-backed litter-splitters (polydesmoids) and rollers (glomeroids) are known already since the Palaeozoic. These were mainly large, spiny or crested forms, unequivocally stratobionts (Kime and Golovatch 2000).

To summarize, only a few millipede life-forms can be distinguished. Diplopoda as a group of soil/litter macrofauna are somewhat to markedly sensitive to water deficit and are often calciphilous as well. In general, they appear to have failed, both morpho-physiologically and ecologically, to conquer environments and habitats significantly deviating from a forest floor (Kime and Golovatch 2000, Golovatch and Kime 2009).

The fauna of China in the context of that of Southeast Asia

Holt et al. (2012), based on the modern distributions and phylogenies of amphibians, birds and non-marine mammals (altogether, 21,000+ species), have recently advanced a new terrestrial zoogeographical regionalization of the world, recognizing 20 major zoogeographical regions grouped into 11 larger realms. Among other novelties, a new, independent, Sino-Japanese region has been discriminated which also covers southern China and which appears to show closer phylogenetic affinities to the Palaearctic than to the Oriental realm. Eventually, that paper represents one of the most consistent, but no less unsuccessful attempts at uncritically combining the landscape-typological (= zonal) and faunogenetic approaches to biogeography which must be clearly separated at least as regards the biotas of older biomes (e.g. Chernov 1975). In addition, past distributions and the fossil record have been totally neglected, whereas some rivaling phylogenies preferred over others. As a result, based on the present-day borders of the tundra, steppe (= grassland) and desert zones, Holt et al. (2012) incorporated most of the tundra-clad northern North America into the Palaearctic and also distinguished a separate, arid, Saharo-Arabian region.

Heiser and Schmitt (2013), based on the distribution of the insect order Odonata in Eurasia and using various statistics, have recently provided an attempt at drawing a refined boundary between the Palaearctic and Oriental realms. The result was predictably clear and fully agreeing with common wisdom, as it shows a broad transition zone between these realms. Hardly surprisingly, this zone roughly corresponds to the Sino-Japanese region as delimited by Holt et al. (2012), naturally with southern China considered as its part.

Speaking of the Diplopoda, Southeast Asia is the only biogeographic region globally that supports all of its 16 orders (Shelley and Golovatch 2011). Because China together with the adjacent parts of Indochina alone encompasses at least 14 of these, its southern and central parts not only represent the northern periphery of the Oriental realm bordering on and intermingling with the Palaearctic one, but also a huge refuge harbouring numerous relict elements at various levels.

The following examples illustrate the declining millipede orders Siphoniulida and Siphonocryptida, neither reported from continental China yet.

All 2–3 species of Siphoniulida are only known from Sumatra (1) and Mexico+Guatemala (1–2). The pattern demonstrated by the Siphonocryptida is also quite peculiar. This small order contains two genera with three species in each. *Hiru-*

dicryptus Enghoff & Golovatch, 1985 has a species living on Madeira and the Canaries, where it is largely confined to the relict laurisilva biome, one species from a 2500 m elevation in Nepal, and one from Taiwan. *Siphonocryptus* Pocock, 1894 contains one species from Sumatra, Indonesia, and two from the southern half of Malay Peninsula. Such patterns seem to date back at least to the Oligocene times of the so-called "Warm Earth" and have firm causal explanations (Golovatch 1997, Zherikhin 2003). Being so vastly disjunct, they are best accounted for by extinction events, extinction being as an integral part of evolution, both phylogenetic and spatial, as speciation. In contrast, most if not all of the remaining orders of Diplopoda are currently in an expansive stage of their evolution (Hoffman 1980, Shelley 2011).

Troglobionts versus other life-forms

Faunistic records in southern China (not only of Diplopoda, but of many other arthropod groups) appear to be strongly biased towards caves; many if not most of the species are suspected or confirmed troglobionts, often with up to 5 or 6 troglomorphs per cave, for example in the Mulun Karst in Guangxi which hosts perhaps the richest cave fauna at least in China (Deharveng et al. 2008). We can easily predict that actually a far richer millipede diversity is represented by the other life-forms combined, both endogean, i.e. pedobionts, and above-ground, i.e. strato-, xylo-, epiphytobionts and, especially, stratobionts. Troglobionts, however common in the so cave-rich, karst-dominated, southern provinces of China, still constitute there only a subordinate fraction of the region's overall diplopod diversity. This must never be forgotten when collecting anywhere, including China.

The cave millipedes of Southeast Asia, including the adjacent areas of southern China, in contrast to their epigean faunas, appear to be strongly biased and restricted to rather few lineages. In other words, even though the Oriental Region does support perhaps the richest and most diverse diplopod fauna globally (Table 1), the cavernicolous millipedes, however common in terms of abundance (quite often) and species richness, are represented by surprisingly few families and genera. Like elsewhere in the world, most/all of the troglobionts as a distinct life-form actually belong to the orders Spirostreptida, Glomerida, Chordeumatida, Callipodida and Polydesmida. The proportions of the few troglobitic species of Polyxenida, Glomeridesmida or Spirobolida known at the present are negligible (currently zero in the entire Oriental realm).

Biogeographically, the millipede fauna of southern China, including cavernicoles, is clearly dominated by Oriental elements, whereas the influence of the Palaearctic is low. Such a pattern fully agrees with common wisdom. The same concerns the obvious preponderance of troglobitic Diplopoda to particularly local endemism, mostly restricted to a single cave or cave system, even as compared to the low-vagile and also mostly highly locally distributed epigean counterparts (Golovatch 1997).

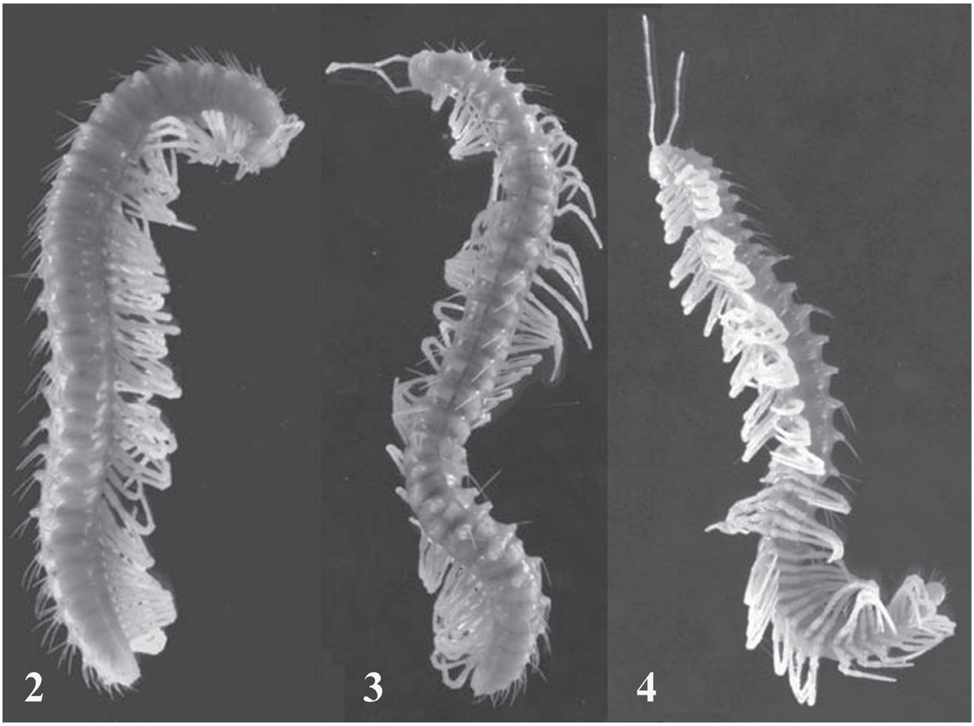
Table 1. Millipedes of Southeast Asia (and the world) versus those in southern China.

Orders	Distribution pattern	Troglobionts
Polyxenida	Cosmopolitan	very few troglobionts, but none in E & SE Asia
Glomeridesmida	Pantropical	very few troglobionts, but none in E & SE Asia
Glomerida	Holarctic + Oriental	numerous troglobionts, including E & SE Asia
Sphaerotheriida	Old World	no troglobionts
Siphoniulida	Neotropical + Oriental	no troglobionts
Siphonophorida	Pantropical	no troglobionts
Siphonocryptida	Palaearctic + Oriental	no troglobionts
Polyzoniida Platydesmida	Subcosmopolitan	no troglobionts
Chordeumatida	Holarctic + Neotropical + Oriental	no troglobionts
Callipodida	Subcosmopolitan, but mainly Holarctic	numerous troglobionts, including E & SE Asia
Stemmiulida	mainly Holarctic + Oriental	rather few troglophiles, but hardly any true troglobionts, which are mostly restricted to SE Asia
Julida	Pantropical	no troglobionts
	mainly Holarctic + Oriental	numerous troglobionts, including E Asia, but excluding SE Asia
Spirostreptida	Pantropical	numerous troglobionts, including E & SE Asia
Spirobolida	Pantropical	very few troglobionts, but none in E & SE Asia
Polydesmida	Cosmopolitan	numerous troglobionts, including E & SE Asia

The main lineages of cave-dwelling Diplopoda in the Oriental Region and southern China

The most common, often also highly abundant group clearly dominating the cave millipede fauna of Southeast (and partly South) Asia is the family Cambalopsidae (Spirostreptida). The most speciose genera are *Glyphiulus* Gervais, 1847 (Golovatch et al. 2007a, 2007b, 2011b, 2011c, Golovatch, Geoffroy et al. 2012b) and *Plusioglyphiulus* Silvestri, 1923 (Golovatch et al. 2009a, 2011a), followed by *Trachyjulus* Peters, 1864 (Golovatch, Geoffroy et al. 2012a) and *Hypocambala* Silvestri, 1895 (Golovatch et al. 2011d). All of them largely contain epigean species, however at least two dozen *Glyphiulus* species (e.g. Fig. 10), plus one *Hypocambala*, are presumed troglobionts in southern China. The real diversity of Cambalopsidae in Chinese caves is difficult to estimate, but the number of likely troglobitic species of *Glyphiulus* alone may well amount to a hundred.

The huge, Eurasian, basically warm-temperate to tropical genus *Hyleoglomeri*s Verhoeff, 1910 (Glomeridae, Glomerida) currently contains nearly a hundred species, including at least two dozen cavernicoles. Unlike the glomerid fauna of the adjacent Indochina which harbours a considerable proportion of endemic genera (60% in Vietnam, see Golovatch, Geoffroy and VandenSpiegel 2013), continental China currently supports only 23 species of *Hyleoglomeris*, of which over a dozen occur in caves alone (Golovatch, Geoffroy and VandenSpiegel 2012). According to our estimates, this figure may easily double or even triple with further studies on the cave Diplopoda of China.



Figures 2–4. Habitus of *Nepalella grandoides* Golovatch, Geoffroy & Mauriès, 2006, a completely unpigmented, blind, long-legged and long-antenned troglobiont from Sichuan, length nearly 38 mm. After Golovatch et al. (2006b). Photos courtesy L. Albenga.

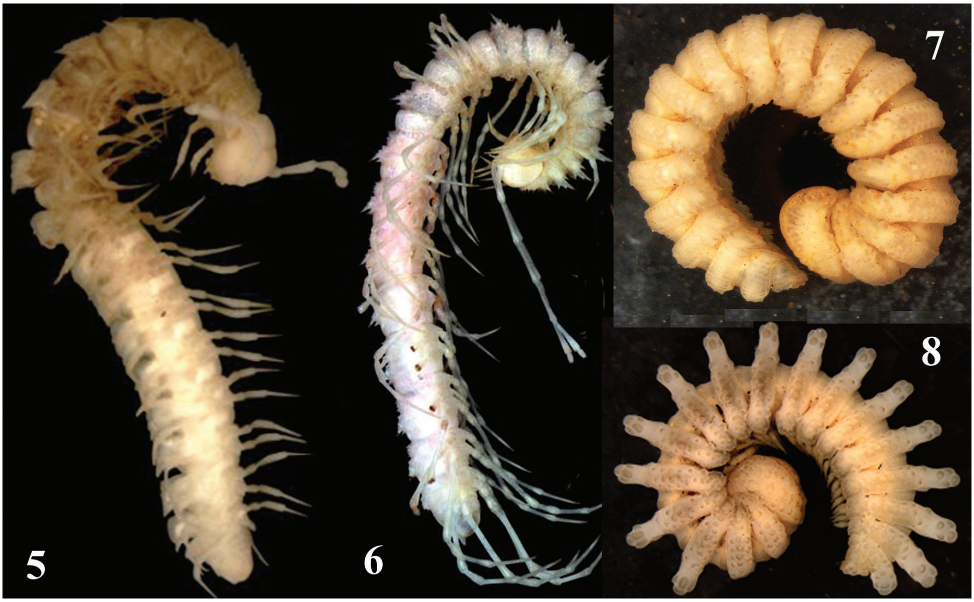
Species of the large order Chordeumatida dominate the Holarctic, being much more subordinate in Australasia (including southern India and Sri Lanka in the West, through Malay Peninsula and Indonesia, to tropical and subtropical eastern Australia and New Zealand), Madagascar (absent from the remaining Afrotropical areas), Central America (only north of Panama) and South America (only Chile). In Southeast Asia together with the adjacent areas of southern China, the fauna is restricted to a few genera only. The most important is *Nepalella* Shear, 1979 (Megalotylidae), with 23 species or subspecies from Nepal (10), Thailand (2), Myanmar (2), Vietnam (1) and southern China (8, several presumed troglobionts, e.g. Figs 2–4) (Golovatch et al. 2006a, 2006b). The oligotypic genera *Vieteuma* Golovatch, 1984 and *Lipseuma* Golovatch, Geoffroy & Mauriès, 2006 (both Kashmireumatidae), include one and two presumed troglobionts in southern China, respectively (Mauriès and Nguyen Duy-Jacquemin 1997, Golovatch et al. 2006a, 2006b). The small Oriental family Heterochordeumatidae (two genera and four species) is as yet unknown from southern China and contains no cavernicoles (Shear 2000). The same can be stated as regards the closely related, but much larger Metopidiotrichidae, with seven genera and over 50 species ranging from Japan, Taiwan, Indochina, Myanmar, Malaysia and the Philippines, through Indonesia, to Papua New Guinea, eastern Australia and New Zealand (Shear

2002). The only family-level endemic of southern China is the troglobitic monobasic genus *Guizhousoma* Mauriès, 2005 (Guizhousomatidae). Finally, also of relevance is the small family Pygmaeosomatidae which hosts only a few species in southern India, Sri Lanka and Madagascar, albeit none of them is cave-dwelling.

The rather small, mostly Holarctic order Callipodida is represented in the Oriental realm by 3–4 genera and three families. Perhaps the most interesting is *Sinocallipus* Zhang, 1993, with six described species, largely cavernicolous, from Indochina and the adjacent parts of southern China (Stoev and Enghoff 2011). This genus forms a distinct family and suborder, i.e. Sinocallipodidae and Sinocallipodidea, respectively, possibly the basalmost component in the entire order. Similarly, the genus *Paracortina* Wang & Zhang, 1993 (= ? *Angulifer* Zhang, 1997) is tropical or subtropical. At the moment it harbours 12 species, mostly cave-dwelling, in southern China and northern Vietnam, and it represents still another Oriental family of its own, Paracortinidae (review: Stoev and Geoffroy 2004). In contrast, the genus *Bollmania* Silvestri, 1896 (Caspiopetalidae) is chiefly Central Asian (south to Punjab in Pakistan). It contains only eight described species, mainly epigean, including a single troglobiont from Yunnan, southern China (Stoev and Enghoff 2005).

Hardly surprisingly, the order Polydesmida, which is the largest globally, is also the most diverse in the Oriental Region. However, only a few families are represented in caves while even fewer seem to comprise troglobionts. The most common is the principally Holarctic family Polydesmidae only marginally represented in tropical Asia, reaching Indochina in the South. Two polydesmid genera dominate the fauna of China and adjacent areas, showing lots of cavernicoles as well. Thus, *Epanerchodus* Attems, 1901 is the largest genus of Polydesmida in Central to East Asia, including southern China. Altogether it contains 70+ species, mainly in Japan from where numerous troglobionts are known. Only 17 species of *Epanerchodus* have hitherto been recorded in mainland China (Golovatch 2014a, 2014b, Golovatch and Geoffroy 2014); at least 6 of them are presumed troglobionts which are all encountered only in southern China (Geoffroy and Golovatch 2004, Golovatch, Liu et al. 2012, Golovatch and Geoffroy 2014). The distribution of *Pacidesmus* Golovatch, 1991 (Figs 5 and 9) is even more spectacular: one species has been found at 2200–2500 m elevations in northern Thailand, whereas the remaining eight known congeners are troglobionts in southern China (Golovatch, Geoffroy and Mauriès 2010, Golovatch and Geoffroy 2014). Most of the few species of the basically Himalayan genus *Glenniea* Turk, 1945 are epigean, including one congener found in Guangxi (Golovatch, Liu et al. 2012); however, two species of *Glenniea*, one of which is fairly troglomorphic, have only been encountered in caves of Sichuan (Golovatch and Geoffroy 2014).

The family Paradoxosomatidae (Polydesmida) is among the largest in the entire class Diplopoda (nearly 200 genera and >950 species, amounting to about 60% of the total species diversity in the Oriental fauna), but it is highly uncharacteristic of caves. Like the Cambalopsidae (probably another 10% of the total species richness of Oriental Diplopoda), the Paradoxosomatidae are largely epigean, in part because much of the collecting and taxonomic exploration efforts still focus on cavernicoles alone. Only



Figures 5–8. Habitus photos of *Pacidesmus armatus* Golovatch, Geoffroy & Mauriès, 2010, *Desmoxytes scolopendroides* Golovatch, Geoffroy & Mauriès, 2010, *Eutrichodesmus filisetiger* Golovatch, Geoffroy, Mauriès & VandenSpiegel, 2009, and *E. aster* Golovatch, Geoffroy, Mauriès & VandenSpiegel, 2009, all presumed trogloionts from Guangxi, Guangxi, Vietnam, and Vietnam, respectively. After Golovatch et al. (2009c, 2010a, 2010b). Photos courtesy L. Deharveng & A. Bedos.

the large (35 species, mainly epigean), basically Southeast Asian genus *Desmoxytes* Chamberlin, 1923, often referred to as "dragon millipedes", encompasses 11 unquestioned troglobionts (e.g. Fig. 6), all confined to southern China (Golovatch, Geoffroy and Mauriès 2010a, Golovatch, Li et al. 2012, Liu et al. 2014). The small Indochinese genus *Piccola* Attems, 1953 is represented in southern China by one troglomorphic, likely troglobitic species (Liu and Tian, in preparation). Similarly, the rather small, Australasian family Haplodesmidae is largely represented by epigean species as well, however several presumed troglobionts (Figs 7, 8), especially from the largest genus *Eutrichodesmus* Silvestri, 1910 (with 45 described species), are known from Indochina and southern China (Golovatch et al. 2009b, 2009c, 2015, Golovatch, Mikhaljova et al. 2010, Liu and Tian 2013). The quite large, mainly tropical family Cryptodesmidae is also dominated by epigean species, but a few are presumed troglobionts both in the Neotropical and Oriental regions, including a species of *Trichopeltis* Pocock, 1894 in southern China (Golovatch et al. 2010).

In contrast, species of the small, generally Oriental family Opisotretidae (Polydesmida) also occur epigeically in the karst regions of southern China, but the few likely troglobionts have only been encountered in Sulawesi, Indonesia and in Papua New Guinea (Golovatch, Geoffroy et al. 2013). Furthermore, apparently none of the very few, partly still unidentified species of the family Trichopolydesmidae (Polydesmida),

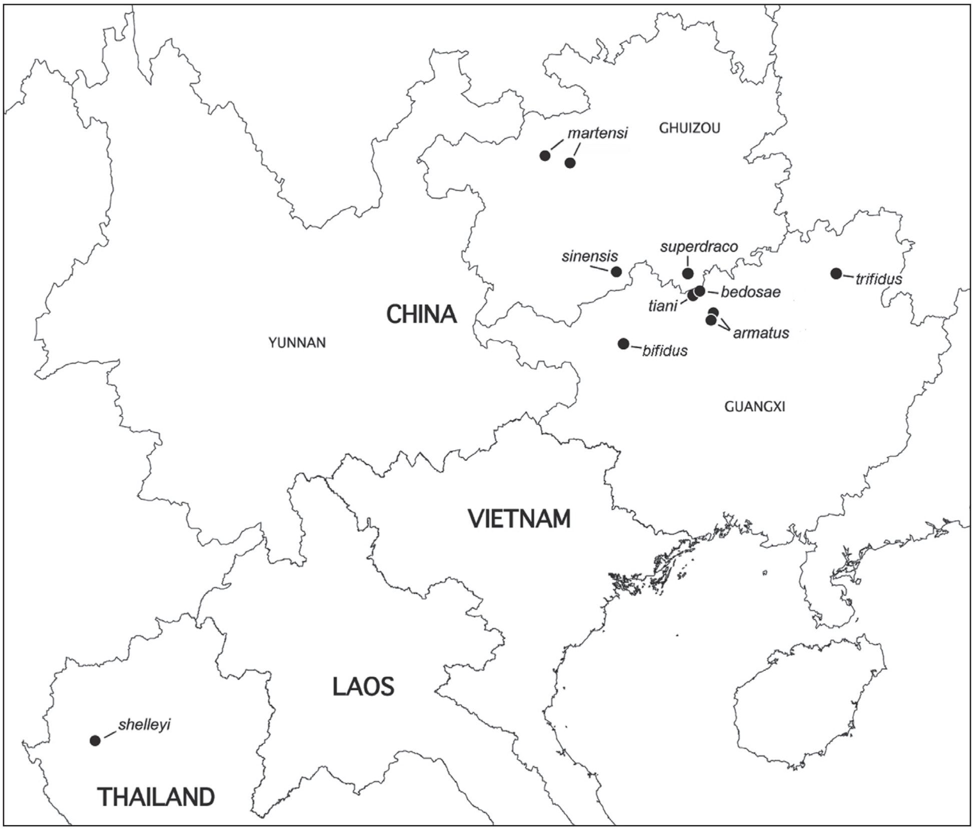


Figure 9. Distribution of *Pacidesmus* species. After Golovatch and Geoffroy (2014). Map courtesy L. Deharveng & A. Bedos.

the order Glomeridesmida (both these groups pantropical), or the order Julida (basically Holarctic), which have occasionally been encountered in caves in Southeast Asia, seem to represent troglobionts.

Not only is the fauna of cavernicolous diplopods of China clearly biased to rather few lineages, but also the morphotypes they represent appear to be fewer compared to epigean counterparts. Thus, only juloid, glomeroid and polydesmoid morphotype millipedes occur among troglobionts in southern China.

The representation of millipede orders in the fauna of southern China, also roughly showing the proportion of presumed troglobionts, is summarized in Table 1.

Conclusions

Generally speaking, southern China harbours a very rich and diverse fauna of Diplopoda, probably numbering several hundred species. It consists of not only clearly dominating Oriental elements, but also a proportion of Palaearctic ones. Yet only a highly lim-

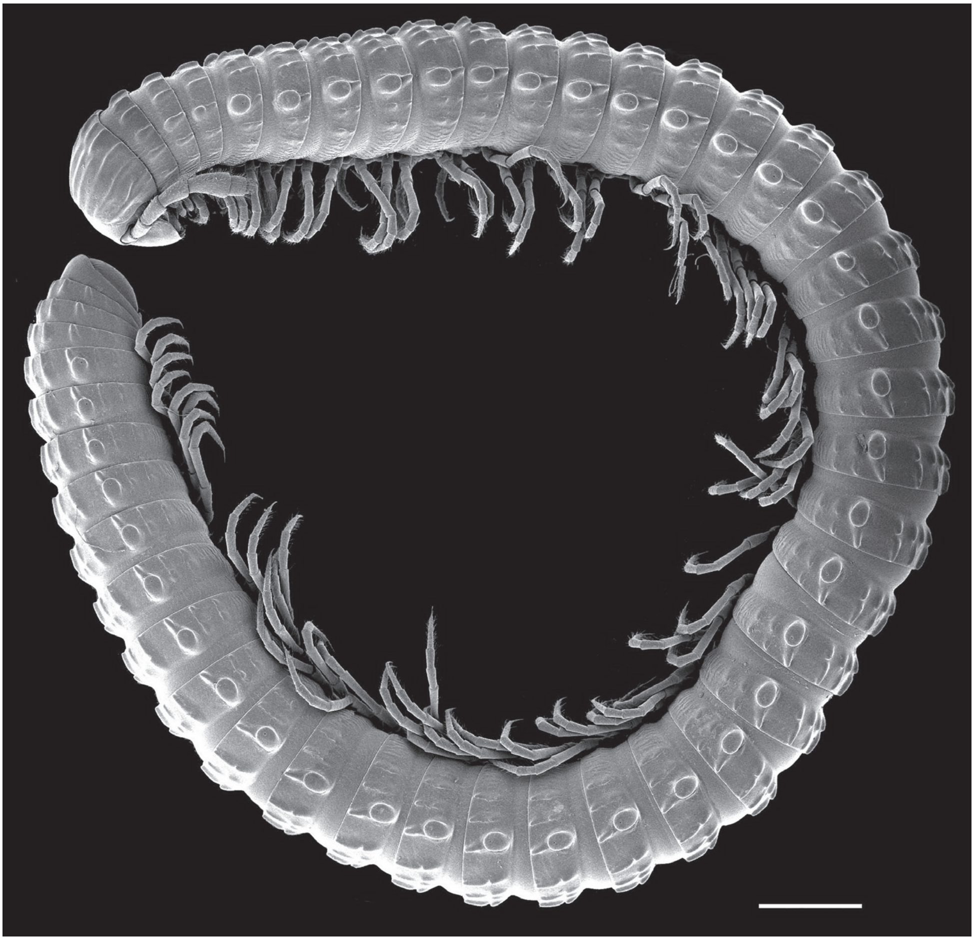


Figure 10. Habitus of *Glyphiulus difficilis* Golovatch, Geoffroy, Mauriès & VandenSpiegel, 2011, a presumed troglobiont from Guangxi. Scale bar: 1.5 mm. After Golovatch et al. (2011b). SEM micrograph courtesy D. VandenSpiegel.

ited number of lineages appear to have successfully colonized the cave environment, not only in China, but in the entire Oriental realm. Biogeographically, these lineages, however few, also demonstrate the dominance of presumably Oriental groups (Kashmireumatidae, Megalotylidae, Cambalopsidae, Sinocallipodidae, Paracortinidae, *Hyleoglomeris*, *Desmoxytes*, *Pacidesmus*, *Glenniea*, *Trichopeltis*, *Piccola*) over the clearly Palaearctic ones (*Bollmania*, *Epanerchodus*). In full agreement with common wisdom, the fauna is actually a mixture of components from both these realms, definitely with numerous further troglobitic species still to be revealed. Because truly cave-dwelling genera, tribes or families are nearly absent among the Chinese or even entire Oriental Diplopoda, future explorations seem far more likely to yield lots of further new species, but barely anything else of a higher taxonomic rank. The only remarkable exceptions in southern China are the endemic family Guizhousomatidae, monobasic, and the oligotypic genus

Lipseuma, both these taxa likely highly relictual troglobionts. Continental China may well prove to support about 1,000 millipede species of various origins, mainly Oriental and Palaearctic. Most of this impressive diversity is expectedly confined to the warmer, highly montane, humid tropical and subtropical parts of the country where numerous karst massifs are also known to often dominate the landscapes.

The exceptional biotic richness and abounding local endemism of the karsts and their caves in southern China (Deharveng et al. 2008), where millipedes are among the dominant terrestrial groups showing particularly high rates of diversification (e.g. Deharveng and Bedos 2012), are certainly among the most vulnerable elements of biodiversity from a conservation point of view. Global change, coupled with an increasingly powerful anthropogenic pressure such as the removal or fragmentation of native woods often replaced by timber eucalypt or pine plantations (both inevitably followed by soil and water acidification), land development, woodworking, mining/cement and similarly detrimental industries, environmental pollution, water removal etc., must be regarded as the main targets and concerns of nature conservation in the region. Because we still know too little, it is highly difficult to impossible to provide meaningful estimates of the current threats faced by cave biodiversity in southern China, but the problems are certainly quite acute and the stakes pretty high. Not just millipedes, albeit one of the major components of cave and karst environments, but rather the karsts themselves deserve conservation, especially those where aboriginal woody and shrub vegetation still dominates, through a network of national or regional nature reserves and parks. This work is being planned or already underway, in Guangxi under the supervision and with the ongoing support of the Biodiversity Conservation Office, Department of Environmental Protection, Guangxi Regional Government, Nanning, China (e.g. Liu et al. 2014, Tian et al. 2014).

Acknowledgements

I am most grateful to the Organizing Committee of the International "Guangxi Karst and Cave Biodiversity and Conservation Symposium", held in Nanning, Guangxi, China on 1–5 December 2012, for the kind invitation to participate in the conference and to present a lecture on the same topic. Louis Deharveng and Anne Bedos (Paris, France) have generously rendered their technical assistance. Helen Read (Burnham Beeches, UK), Robert Mesibov (Penguin, Tasmania, Australia) and Pavel Stoev (Sofia, Bulgaria) kindly edited advanced drafts and made several important suggestions.

References

Brewer MS, Sierwald P, Bond JE (2012) Millipede taxonomy after 250 years: Classification and taxonomic practices in a mega-diverse yet understudied arthropod group. PLoS ONE 7(5): e37240. doi: 10.1371/journal.pone.0037240

Chernov YI (1975) [Nature zonation and terrestrial animal life]. Mysl, Moscow, 222 pp. [In Russian]

Deharveng L, Bedos A (2012) Diversity patterns in the tropics. In: White WB, Culver DC (Eds) Encyclopedia of Caves. Academic Press, Chennai, 238–250. doi: 10.1016/B978-0-12-383832-2.00032-3

Deharveng L, Bréhier F, Bedos A, Tian My, Li Yb, Feng Z, Wengeng Q, Xuefeng T (2008) Mulun and surrounding karsts (Guangxi) host the richest cave fauna of China. Subterranean Biology 6: 75–79.

Geoffroy JJ, Golovatch SI (2004) Some polydesmidan millipedes from caves in southern China (Diplopoda: Polydesmida), with descriptions of four new species. Arthropoda Selecta 13(1/2): 19–28.

Golovatch SI (1997) On the main patterns of millipede distribution and faunogenesis in Eurasia (Diplopoda). Entomologica Scandinavica, Supplement 51: 199–208.

Golovatch SI (2014a) Review of the millipede genus *Epanerchodus* Attems, 1901 in continental China, with descriptions of new species (Diplopoda: Polydesmidae). Zootaxa 3760(2): 275–288. doi: 10.11646/zootaxa.3760.2.7

Golovatch SI (2014b) Two new and one little-known species of the millipede genus *Epanerchodus* Attems, 1901 from southern China (Diplopoda, Polydesmida, Polydesmidae). Fragmenta Faunistica 56(2): 157–166 (for 2013). doi 10.3161/00159301FF2013.56.2.157

Golovatch SI, Geoffroy JJ (2014) On some new or poorly-known species of the millipede family Polydesmidae from southern China (Diplopoda: Polydesmida). Russian Entomological Journal 23(2): 91–105.

Golovatch SI, Kime RD (2009) Millipede (Diplopoda) distributions: A review. Soil Organisms 81(3): 565–597.

Golovatch SI, Geoffroy JJ, Mauriès JP (2006a) Four new Chordeumatida (Diplopoda) from caves in China. Zoosystema 28(1): 75–92.

Golovatch SI, Geoffroy JJ, Mauriès JP (2006b) Several new or poorly-known cavernicolous millipedes (Diplopoda) from southern China. Arthropoda Selecta 15(2): 81–89.

Golovatch SI, Geoffroy JJ, Mauriès JP (2010a) Two new species of the millipede genus *Desmoxytes* Chamberlin, 1923 (Diplopoda: Polydesmida: Paradoxosomatidae) from caves in southern China. Arthropoda Selecta 19(2): 57–61.

Golovatch SI, Geoffroy JJ, Mauriès JP (2010b) Review of the millipede genus *Pacidesmus* Golovatch, 1991, with descriptions of three new species from caves in southern China (Diplopoda: Polydesmida: Polydesmidae). Tropical Natural History 10(2): 159–169.

Golovatch SI, Geoffroy JJ, VandenSpiegel D (2013) On several new species of the millipede family Glomeridae from Vietnam (Diplopoda: Glomerida). Arthropoda Selecta 22(3): 201–206.

Golovatch SI, Liu Wx, Geoffroy JJ (2012) Review of the millipede genus *Hyleoglomeris* Verhoeff, 1910 in China, with descriptions of new species (Diplopoda, Glomerida, Glomeridae). Zootaxa 3358: 1–27.

Golovatch SI, Geoffroy JJ, Mauriès JP, VandenSpiegel D (2007a) Review of the millipede genus *Glyphiulus* Gervais, 1847, with descriptions of new species from Southeast Asia (Diplopoda: Spirostreptida: Cambalopsidae). Part 1. The *granulatus*-group. Zoosystema 29(1): 7–49.

Golovatch SI, Geoffroy JJ, Mauriès JP, VandenSpiegel D (2007b) Review of the millipede genus *Glyphiulus* Gervais, 1847, with descriptions of new species from Southeast Asia (Diplopoda: Spirostreptida: Cambalopsidae). Part 2. The *javanicus*-group. Zoosystema 29(3): 417–456.

Golovatch SI, Geoffroy JJ, Mauriès JP, VandenSpiegel D (2009a) Review of the millipede genus *Plusioglyphiulus* Silvestri, 1923, with descriptions of new species from Southeast Asia (Diplopoda: Spirostreptida: Cambalopsidae). Zoosystema 31(1): 71–116. doi: 10.5252/z2009n1a5

Golovatch SI, Geoffroy JJ, Mauriès JP, VandenSpiegel D (2009b) Review of the millipede family Haplodesmidae Cook, 1895, with descriptions of some new or poorly-known species (Diplopoda, Polydesmida). ZooKeys 12: 1–53. doi: 10.3897/zookeys.7.117

Golovatch SI, Geoffroy JJ, Mauriès JP, VandenSpiegel D (2009c) Review of the millipede genus *Eutrichodesmus* Silvestri, 1910 (Diplopoda, Polydesmida, Haplodesmidae), with descriptions of new species. ZooKeys 7: 1–46. doi: 10.3897/zookeys.12.167

Golovatch SI, Geoffroy JJ, Mauriès JP, VandenSpiegel D (2010) Two new species of the millipede genus *Trichopeltis* Pocock, 1894 (Diplopoda: Polydesmida: Cryptodesmidae) from Vietnam and China. Arthropoda Selecta 19(2): 63–72.

Golovatch SI, Geoffroy JJ, Mauriès JP, VandenSpiegel D (2011a) The millipede genus *Plusioglyphiulus* Silvestri, 1923 in Thailand (Diplopoda: Spirostreptida: Cambalopsidae). Zootaxa 2840: 1–63.

Golovatch SI, Geoffroy JJ, Mauriès JP, VandenSpiegel D (2011b) New species of the millipede genus *Glyphiulus* Gervais, 1847 from the *granulatus*-group (Diplopoda: Spirostreptida: Cambalopsidae). Arthropoda Selecta 20(2): 65–114.

Golovatch SI, Geoffroy JJ, Mauriès JP, VandenSpiegel D (2011c) New species of the millipede genus *Glyphiulus* Gervais, 1847 from the *javanicus*-group (Diplopoda: Spirostreptida: Cambalopsidae). Arthropoda Selecta 20(3): 149–165.

Golovatch SI, Geoffroy JJ, Mauriès JP, VandenSpiegel D (2011d) Two new species of the millipede genus *Hypocambala* Silvestri, 1895 from China and Vietnam (Diplopoda: Spirostreptida: Cambalopsidae). Arthropoda Selecta 20(3): 167–174.

Golovatch SI, Geoffroy JJ, Mauriès JP, VandenSpiegel D (2012a) New or poorly-known species of the millipede genus *Trachyjulus* Peters, 1864 (Diplopoda: Spirostreptida: Cambalopsidae). Arthropoda Selecta 21(2): 103–129.

Golovatch SI, Geoffroy JJ, Mauriès JP, VandenSpiegel D (2012b) An unusual new species of the millipede genus *Glyphiulus* Gervais, 1847 from Borneo (Diplopoda: Spirostreptida: Cambalopsidae). Russian Entomological Journal 21(2): 133–137.

Golovatch SI, Geoffroy JJ, Stoev P, VandenSpiegel D (2013) Review of the millipede family Opisotretidae (Diplopoda, Polydesmida), with descriptions of new species. ZooKeys 302: 13–77. doi: 10.3897/zookeys.302.5357

Golovatch SI, Geoffroy J-J, Mauriès J-P, VandenSpiegel D (2015) Review of the millipede genus Eutrichodesmus Silvestri, 1910, in China, with descriptions of new cavernicolous species (Diplopoda, Polydesmida, Haplodesmidae). ZooKeys 505: 1–34. doi: 10.3897/zookeys.505.9862

Golovatch SI, Li Youbang, Liu Weixin, Geoffroy JJ (2012) Three new cavernicolous species of dragon millipedes, genus *Desmoxytes* Chamberlin, 1923, from southern China, with notes on a formal congener from the Philippines (Diplopoda, Polydesmida, Paradoxosomatidae). ZooKeys 185: 1–17. doi: 10.3897/zookeys.185.3082

Golovatch SI, Liu Weixin, Li Youbang, Geoffroy JJ (2012) One new and two little-known species of the millipede family Polydesmidae from southern China (Diplopoda: Polydesmida). Arthropoda Selecta 21(2): 131–136.

Golovatch SI, Mikhaljova EV, Korsós Z, Chang HW (2010) The millipede family Haplodesmidae (Diplopoda, Polydesmida) recorded in Taiwan for the first time, with the description of a new species. Tropical Natural History 10(1): 27–36.

Heiser M, Schmitt T (2013) Tracking the boundary between the Palaearctic and the Oriental region: new insights from dragonflies and damselflies (Odonata). Journal of Biogeography 40: 2047–2058. doi: 10.1111/jbi.12133

Hoffman RL (1980) Classification of the Diplopoda. Muséum d'histoire naturelle, Genève, 237 pp.

Holt BG, Lessard JP, Borregaard MK, Fritz SA, Araújo MB, Dimitrov D, Fabre PH, Graham CH, Graves GR, Jønsson KA, Nogués-Bravo D, Wang ZH, Whittaker RJ, Fjeldså J, Rahbek C (2012) An update of Wallace's zoogeographic regions of the world. Science Express: 1–191. doi: 10.1126/science.1228282

Kime RD, Golovatch SI (2000) Trends in the ecological strategies and evolution of millipedes (Diplopoda). Biological Journal of the Linnean Society of London 69: 333–349. doi: 10.1111/j.1095-8312.2000.tb01209.x

Liu Weixin, Tian Mingyi (2013) Four new cavernicolous species of the millipede genus *Eutrichodesmus* Silvestri, 1910 from southern China (Diplopoda: Polydesmida: Haplodesmidae). Zootaxa 3734(2): 281–291. doi: 10.11646/zootaxa.3734.2.11

Liu Weixin, Golovatch SI, Tian Mingyi (2014) A review of the dragon millipede genus *Desmoxytes* Chamberlin, 1923 in China, with descriptions of four new species (Diplopoda, Polydesmida, Paradoxosomatidae). ZooKeys 448: 9–26. doi: 10.3897/zookeys.448.8081

Mauriès JP (2005) *Guizhousoma latellai* gen.n., sp.n., de Chine continentale, type d'une nouvelle famille de la superfamille des Neoatractosomatoidea (Diplopoda: Chordeumatida). Arthropoda Selecta 14(1): 11–17.

Mauriès JP, Nguyen Duy-Jacquemin M (1997) Nouveaux craspedosomides et glyphiulides cavernicoles de Chine (Diplopoda). Mémoires de Biospéologie 24: 49–62.

Shear WA (2000) On the milliped family Heterochordeumatidae, with comments on the higher classification of the order Chordeumatida (Diplopoda). Invertebrate Taxonomy 14: 363–376. doi: 10.1071/IT99016

Shear WA (2002) The milliped genus *Metopidiothrix* Attems (Diplopoda: Chordeumatida: Metopidiotrichidae). Invertebrate Systematics 16: 849–892. doi: 10.1071/IS02005

Shear WA (2011) Class Diplopoda de Blainville in Gervais, 1844. In: Zhang Z-Q (Ed.) Animal biodiversity: An outline of higher-level classification and survey of taxonomic richness. Zootaxa 3148: 159–164.

Shear WA, Edgecombe GD (2010) The geological record and phylogeny of the Myriapoda. Arthropod Structure & Development 39: 174–190. doi: 10.1016/j.asd.2009.11.002

Shelley RM (2011) The milliped order Glomeridesmida (Diplopoda: Pentazonia: Limacomorpha) in Oceania, the East Indies, and southeastern Asia; first records from Palau, the Philippines, Vanuatu, New Britain, the Island of New Guinea, Cambodia, Thailand, and Borneo and Sulawesi, Indonesia. Insecta Mundi 0196: 1–11.

Shelley RM, Golovatch SI (2011) Atlas of myriapod biogeography. I. Indigenous ordinal and supra-ordinal distributions in the Diplopoda: Perspectives on taxon origins and ages, and a hypothesis on the origin and early evolution of the class. Insecta Mundi 0158: 1–134.

Stoev P, Enghoff H (2005) A new cave-dwelling millipede of the genus *Bollmania* Silvestri, 1896 from Yunnan, China, with remarks on the reduction of the second female leg-pair (Diplopoda: Callipodida: Caspiopetalidae). Journal of Natural History 39(21): 1875–1891. doi: 10.1080/00222930400025896

Stoev P, Enghoff H (2011) A review of the millipede genus *Sinocallipus* Zhang, 1993 (Diplopoda, Callipodida, Sinocallipodidae), with notes on gonopods monotony *vs.* peripheral diversity in millipedes. ZooKeys 90: 13–34. doi: 10.3897/zookeys.90.1291

Stoev P, Geoffroy JJ (2004) Review of the millipede family Paracortinidae Wang & Zhang 1993 (Diplopoda: Callipodida). Acta Arachnologica 53(2): 93–103. doi: 10.2476/asjaa.53.93

Tian Mingyi, Yin Haomin, Huang Sunbin (2014)_Du'an Karst of Guangxi: a kingdom of the cavernicolous genus *Dongodytes* Deuve (Coleoptera, Carabidae, Trechinae). ZooKeys 454: 69–107. doi: 10.3897/zookeys.454.7269

Zherikhin VV (2003) [A history of biomes]. In: Zherikhin VV, Selected Works. KMK Scientific Press, Moscow: 98–188 [In Russian]

ZooKeys 510: 95–114 (2015)
doi: 10.3897/zookeys.510.9614
http://zookeys.pensoft.net

RESEARCH ARTICLE

A peer-reviewed open-access journal
ZooKeys
Launched to accelerate biodiversity research

At the end of the rope: *Geophilus hadesi* sp. n. – the world's deepest cave-dwelling centipede (Chilopoda, Geophilomorpha, Geophilidae)

Pavel Stoev[1], Nesrine Akkari[2], Ana Komerički[3],
Gregory D. Edgecombe[4], Lucio Bonato[5]

1 *Pensoft Publishers and National Museum of Natural History, Sofia, Bulgaria* **2** *Naturrhistorisches Museum Wien, Burgring 7, 1010 Wien, Austria* **3** *Croatian Biospeleological Society, Zagreb, Croatia* **4** *Department of Earth Sciences, Natural History Museum, Cromwell Road, London SW7 5BD, UK* **5** *Department of Biology, University of Padova, via U. Bassi 58b, I-35131 Padova, Italy*

Corresponding author: *Pavel Stoev* (pavel.e.stoev@gmail.com)

Academic editor: *Ivan H. Tuf* | Received 18 March 2015 | Accepted 6 May 2015 | Published 30 June 2015

http://zoobank.org/B29F488D-8748-45A5-8D9A-7A83F37CDCFB

Citation: Stoev P, Akkari N, Komerički A, Edgecombe GD, Bonato L (2015) At the end of the rope: *Geophilus hadesi* sp. n. – the world's deepest cave-dwelling centipede (Chilopoda, Geophilomorpha, Geophilidae). In: Tuf IH, Tajovský K (Eds) Proceedings of the 16th International Congress of Myriapodology, Olomouc, Czech Republic. ZooKeys 510: 95–114. doi: 10.3897/zookeys.510.9614

Abstract

A new geophilomorph centipede, *Geophilus hadesi* sp. n., is described from caves in the Velebit Mountain, central Croatia. Together with *Geophilus persephones* Foddai & Minelli, 1999, described from Pierre Saint-Martin cave in France, they are the only two remarkably troglomorphic geophilomorphs hitherto known. The new species apparently belongs to a group of *Geophilus* species inhabiting mainly Western and Southern Europe, with a uniquely modified pretarsus in the second maxillae. *G. hadesi* **sp. n.** shows unusual traits, some of which commonly found in troglobitic arthropods, including exceptionally elongated antennae, trunk segments and leg claws. The species is described upon specimens found in two caves at a depth below -250 m. Another two specimens apparently belonging to the same species have been recorded in another deep vertical cave at -980 m and -1100 m. The latter represents the world's deepest record of Chilopoda as a whole.

Keywords

Geophilus, troglomorphism, cave-dwelling species, extreme habitats, Velebit Mountain, Croatia

Introduction

Centipedes are common cave inhabitants. However, most species find shelter there only occasionally, thus representing trogloxenes or troglophiles at most. True troglobites – species with an entire life cycle confined to cave environments – are much rarer in the group (see review of cave myriapods by Culver and Shear (2012)). Such species are usually pallid, eyeless (or with reduced numbers of ocelli) and with long appendages that bear elongated setae, characters which have evolved in relation to the underground lifestyle. Other troglomorphic characters known for lithobiomorph centipedes include enlargement of Tömösváry's organ and coxal pores.

Troglobites are currently described only in three out of the five extant orders of Chilopoda, namely Lithobiomorpha, Scolopendromorpha and Geophilomorpha, and an instance has been recorded in Scutigeromorpha as well (Howarth and Stone 1990; GDE, pers. obs.). However, they are not equally represented in these orders: lithobiomorphs seem much more prone to penetrate and adapt to this environment than the members of the other two orders, where only a handful of species are known (most of which belong to the blind scolopendromorph genus *Cryptops* Leach, 1815). Troglobitic centipedes are mainly known from the Northern Hemisphere, in particular southern Europe (the Balkan peninsula, the Pyrenees and Sardinia), in areas with vast coverage of limestone and high number of caves. Few troglobites are known from elsewhere, with only isolated examples south of the equator (Negrea and Minelli 1994; Edgecombe 2005, 2006; Ázara and Ferreira 2013, 2014a, b).

More than twenty years have elapsed since Negrea and Minelli (1994) provided a global overview of the cave-inhabiting centipedes. During that period, active biospeleological work has been carried out in several poorly explored regions of the world, delivering remarkable novelties. To name a few, the first troglomorphic geophilomorph was discovered in the Pyrenees (Foddai and Minelli 1999). Highly cave-adapted species of the genus *Cryptops* – the first descriptions of troglobitic centipedes on their respective continents – were found from caves in Australia (Edgecombe 2005, 2006) and Brazil (Ázara and Ferreira 2013, 2014a). New troglobitic species of Scolopocryptopidae (Scolopendromorpha) from the genera *Newportia* Gervais, 1847 and *Scolopocryptops* Newport, 1844 were described from Mexico (Chagas-Júnior and Shelley 2003), Puerto Rico (Schileyko 2013) and Brazil (Ázara and Ferreira 2014b, Chagas-Júnior and Bichuette 2015). New troglobitic species of Lithobiomorpha were found recently in Sardinia (Zapparoli 1997), Corsica (Iorio 2010), and North Africa (Stoev et al. 2010). Furthermore, the unique Movile cave in Romania was reported to harbor a troglobitic population of the common European centipede *Cryptops anomalans* (Negrea, 1993), a phenomenon which still needs to be further explored, taking into account the high number of endemic taxa reported from this underground ecosystem.

The centipede order Geophilomorpha is known to encompass about 1000–1250 extant species distributed on all continents except for Antarctica (Bonato et al. 2011, Bonato et al. 2014a). All species lack sight, have a more or less distinctly dorsoventrally flattened trunk, and are well adapted to an underground mode of life. Burrowing locomotion is typical for geophilomorphs but species that dwell on the surface are also known in the group. Some 'extreme' habitat adaptations are demonstrated, for example, by species that inhabit surge zones of seashores, periodically inundated forests in Amazonia or deserts.

Although geophilomorphs can occasionally be found in caves, very few of them seem to be highly adapted to this environment. Members of the genus *Thracophilus* Verhoeff, 1926 (Himantariidae) have been for example most often recorded from caves in Europe but none of the species exhibit particularly troglomorphic traits. *Ityphilus cavernicolus* (Matic, Negrea & Fundora Martínez, 1977) (Ballophilidae), found in several caves in Cuba, was speculatively presumed to be a troglobite or a regular troglophile (Matic et al. 1977, Lewis 1981), though no sound evidence for this has yet been presented. True troglobites were unknown for the order until 1999, when *Geophilus persephones* Foddai & Minelli, 1999 was described from the large cave system of Pierre Saint-Martin in southern France.

Here we describe a new troglobitic species of *Geophilus* Leach, 1814, *G. hadesi* sp. n., characterized by relatively elongated trunk segments and appendages, including unusually long claws of the legs. The new species was recently found by Croatian biospeleologists at a great depth in some vertical caves in Velebit Mountain, central Croatia (Fig. 1). Specimens apparently belonging to the same species have been found also in the 15th deepest cave in the world, Lukina jama – Trojama system (-1431 m) (Fig. 2), where one was collected in a large chamber at -980 m and another has been observed at an unreachable spot at -1100 m, the latter representing the world's deepest record of a centipede known to date.

Material and methods

Four specimens have been encountered during the exploration of three caves: Muda labudova, Munižaba and Lukina jama – Trojama cave system. With the exception of a specimen that was not reachable, the other three specimens were collected by hand, one from each cave, and preserved in 70% or 96% ethanol. Before collection, one of them was photographed *in situ* using a Canon 400D camera, with a 65 mm macro objective. All three specimens were compared by P.S. and A.K. using respectively a Zeiss and a Leica MZ16A stereomicroscopes. Additionally one specimen was documented by N.A. using scanning electron microscopy: all body parts were cleaned with ultrasound, transferred to 96% ethanol then to acetone, air-dried, mounted on aluminium stubs, coated with platinum/palladium and studied in a JEOL JSM-6335F scanning electron microscope. Images were edited in Adobe Lightroom 5 and Adobe Photoshop CS6. The specimens are shared between the Croatian Biospeleological Society Collection which is a part of Natural History Museum, Zagreb (CBSS) and the Naturrhistorisches Museum Wien (NHMW). All images included in this publication have been deposited in MorphBank (http://www.morphbank.net). Terminology for external anatomy follows Bonato et al. (2010).

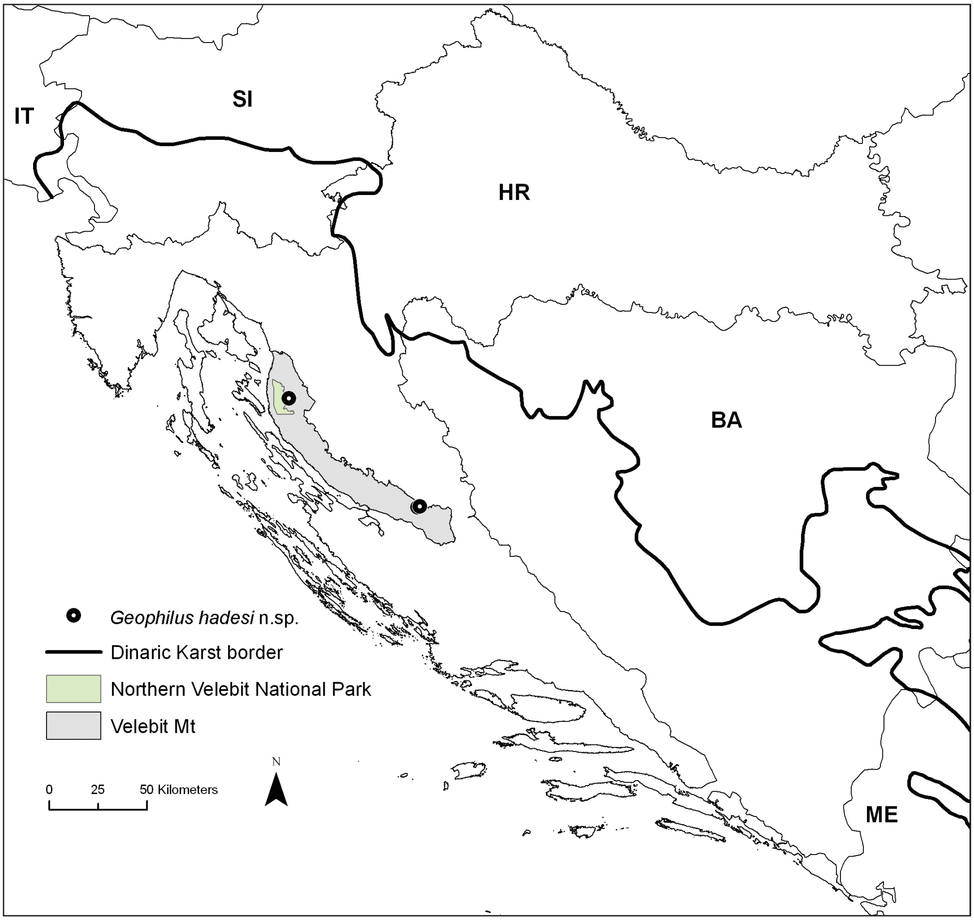


Figure 1. Map of Croatia showing the localities of *Geophilus hadesi* sp. n.

Results

Taxonomy

Family Geophilidae Leach, 1815
Genus *Geophilus* Leach, 1814

Geophilus hadesi **Stoev, Akkari, Komerički, Edgecombe & Bonato, sp. n.**
http://zoobank.org/1D7108A5-11D9-49C0-8D4E-EF39DBD80957
Figs 2–19

Material examined. Holotype: female, 28 mm long, with 33 pairs of legs; Croatia, Zadarska županija (Zadar County), Southern Velebit Mountain, Crnopac massif, city Gračac, Munižaba cave, N44°15'57.4", E 15°52'09"; *circa* -250 m below the surface (cave entrance at 915 m a.s.l.); hand collected, 19 February 2011, leg. B. Jalžić (CBSS, collection code: CHP 532).

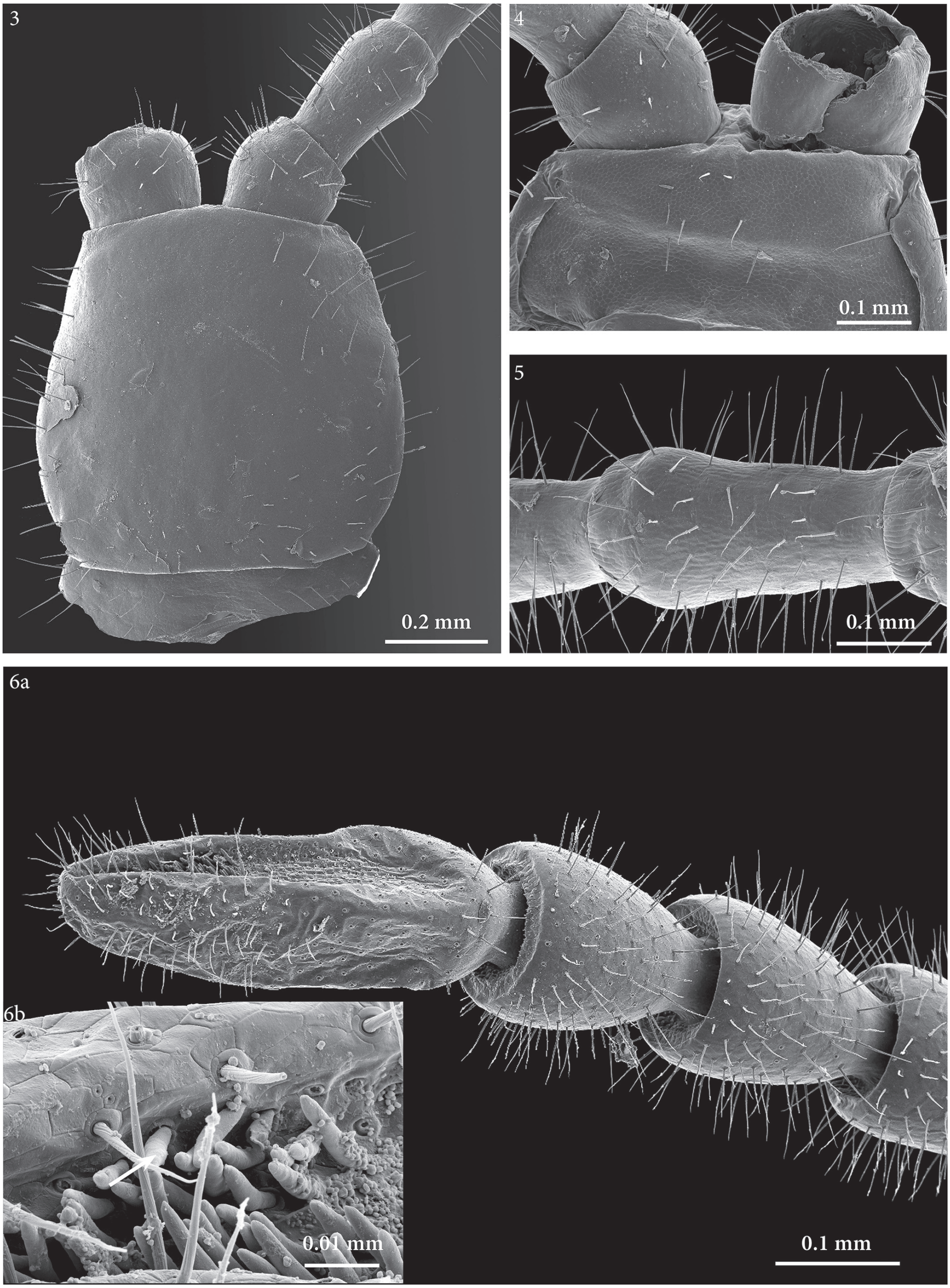
Figure 2. *Geophilus hadesi* sp. n. Habitus of male specimen (CBSS: CHP515). Photo taken *in situ* in the Lukina jama – Trojama cave system, at -980 m below the surface (Fig. 22), 1–3 August 2011. Photo credit: J. Bedek.

Paratype: female (damaged), approx. 22 mm long, with 33 pairs of legs; same area as the holotype, Muda labudova cave, N44°15'38.5", E15°51'18.2"; -500 m below the surface (cave entrance at 1020 m a.s.l.); hand collected, 2-3 July 2011, leg. J. Bedek (NHMW, collection code: NHMW8363, 3 SEM stubs)

Other specimen examined: male, approx. 27 mm long, with 33 pairs of legs; Croatia, Ličko-senjska županija (Lika-Senj County), Northern Velebit Mountains, Hajdučki i Rožanski kukovi Strict Reserve, Lukina jama – Trojama cave system, N44°46'01.6", E15°01'52.7"; -980 m below the surface (cave entrace at 1475 m a.s.l.); hand collected on boulder, 1–3 August 2011, leg. J. Bedek (CBSS: CHP515).

Origin of name. The specific epithet derives from Hades, god of the underworld in Greek mythology and husband of Persephone, in analogy with the name of the only other known troglobite in the genus.

Diagnosis. A species of *Geophilus* with a slender body, adult *circa* 2.2–2.8 cm in length; antennae *circa* 4.5–5 times as long as the head; second maxillary pretarsus very small, tubercle-like with a short tip; exposed part of the forcipular coxosternite more than 1.8 times as wide as long, coxopleural sutures strongly diverging forwards all along their length, chitin-lines incomplete; trunk metasternites elongate, with carpophagus pit; sternal pore-fields on both anterior and posterior parts of trunk; legs elongate, 33 pairs, with long claws; metasternite of the ultimate leg-bearing segment wider than long; coxal pores only on the ventral side, most of them close



Figures 3–6. *Geophilus hadesi* sp. n. **3** cephalic capsule, dorsal **4** clypeus, ventral **5** right antenna, article IV, dorsal **6a** right antenna, ultimate articles, dorsal **6b** right antenna, close up of sensilla basiconica cluster, mesal. SEM micrographs taken from female paratype NHMW8363 from cave Muda labudova.

to the margin of the metasternite, also a single one isolated posteriorly; legs of the ultimate pair with claws.

From the other European species of *Geophilus* with a similarly low number of legs, it can be readily distinguished by a number of traits (see Table 1 and Discussion below).

Description of the holotype. Length 28 mm (damaged paratype: *circa* 22 mm).

Cephalic plate. Cephalic plate as long as wide (also in paratype: Fig. 3), anterior margin slightly angulated, lateral margins evidently convex and convergent forward for their anterior two thirds, posterior margin almost straight; transverse suture absent. Most setae 100–120 µm long.

Antennae. *Circa* 4.6 times as long as the maximum width of the head (also in paratype: Figs 5, 6); all but first article longer than broad, proportions between articles: V=VI>VII=IV>VIII>IX>III>II=X>XI>I>XIV>XIII>XII. Ultimate article twice as long as penultimate (also in paratype: Fig. 6a). Antennae densely covered with setae, which are nearly as long as antennal breadth on the basal articles, gradually shorter and more dense from basal towards the distal articles. Apical sensilla slender, spear-like, *circa* 14 µm long, slightly narrowing towards the tip. Club-like sensilla (sensilla basiconica) in two groups, one on the internal side and the other on the external side on article XIV (also in paratype: Fig. 6b).

Clypeus. Uniformly areolate, without finely areolate clypeal areas; three pairs of setae including a paramedian pair close to the anterior margin and two pairs of larger setae in the middle of clypeus (also in paratype: Fig. 4).

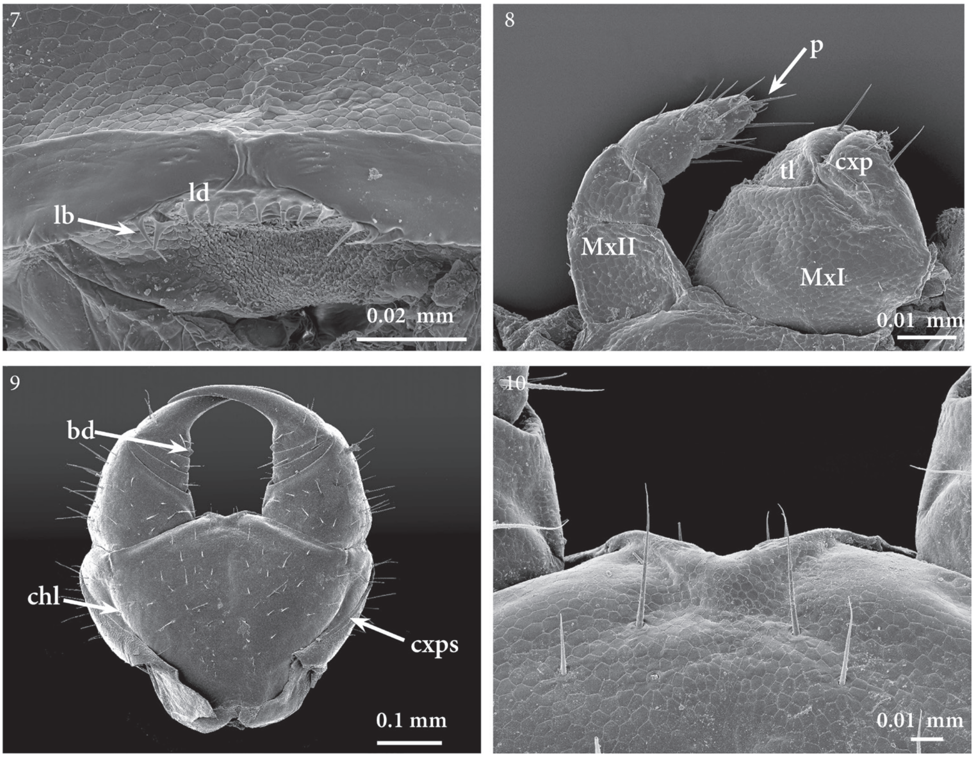
Labrum. About eight triangular denticles on the intermediate part of the margin, and some longer and thinner bristles on the lateral parts (also in paratype: Fig. 7).

Maxillary complex. First maxillae: coxosternite entire; coxal projections subtriangular, bearing four large setae and one subapical spine-like seta; telopodites almost the size of the coxal projections, with the intermediate articulation weakly distinct; lappets apparently absent or very short (also in paratype: Fig. 8). Second maxillae: coxosternite entire, uniformly areolate, the anterior margin widely concave, with setae close to the anterior margin; telopodite composed of three articles, gradually narrowing towards the tip; basal article with three long setae close to the inner margin, article 2 with one seta, article 3 with 13–15 setae; pretarsus small, tubercle-like with a small apical tip (also in paratype: Fig. 8).

Forcipular segment. Forcipules, when closed, not exceeding the anterior margin of the head. Tergite wider than long, lateral margins evidently convex. Ventrally exposed part of the coxosternite 1.8–1.9 times as wide as long, anterior margin without denticles (also in paratype: Figs 9, 10); coxosternal denticles replaced by a prominent coxosternal ridge, with a pair of long setae exceeding the coxosternal margin (Fig. 10); coxopleural sutures strongly diverging forwards all along their length; chitin-lines incomplete and pointing lateral from the condyles. Trochanteroprefemur with external side almost twice as long as the internal side. Tarsungulum with a small denticle at the base, abruptly narrowing and bent basad, then gradually tapering. No denticles on the other forcipular articles.

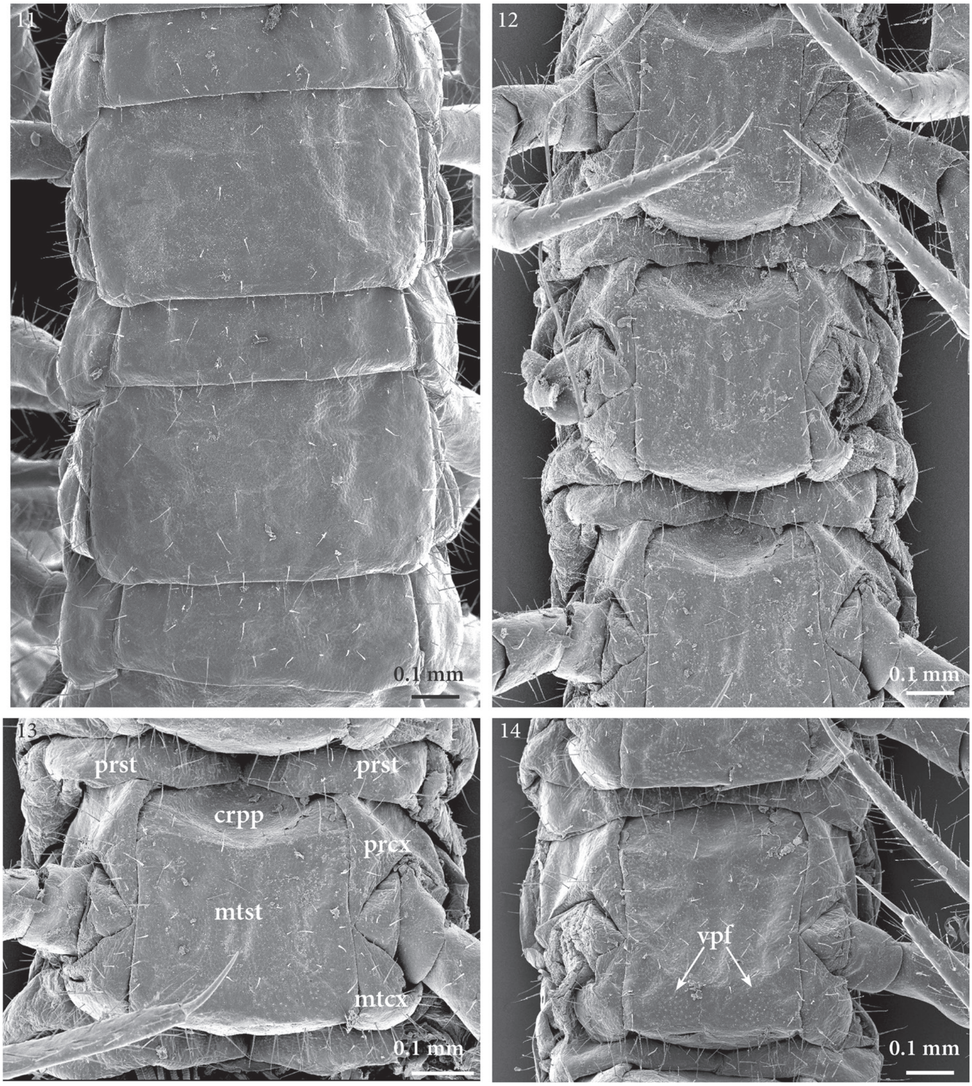
Table 1. Comparison between *Geophilus hadesi* sp. n. and all other European congeners that have fewer than 37 pairs of legs. Data mainly from Bonato et al. (2014b). * The morphology of *G. guanophilus* and *G. minimus* is poorly known.

	***G. hadesi* sp. n.**	***G. guanophilus* Verhoeff, 1939**	***G. minimus* Verhoeff, 1928**	***G. persephones* Foddai & Minelli, 1999**	***G. piae* Minelli, 1983**	***G. ribauti* Brölemann, 1908**	***G. richardi* Brölemann, 1904**	***G. truncorum* Bergsøe & Meinert, 1866**
Distribution	Velebit	Salento peninsula	SW Alps, Sardinia	Pyrenees	Sardinia, Sicily	Pyrenees, Massif Central, SW Alps	W Alps, Sardinia, Italian peninsula, Sicily, Ionian islands	central and northern Europe
Recorded in caves	yes	yes	no	yes	no	no	no	no
Body length (maximum recorded)	28 mm	18 mm	9.5 mm	16.2 mm	11 mm	<20 mm	10 mm	20 mm
Antennae length / head width	4–5	?	<3	6–7	<3	<3	<3	<3
Second maxillae: pretarsus: shape	stout tubercle with small tip	gradually tapering, curved	stout tubercle with small tip?	stout tubercle with small tip	stout tubercle with small tip	gradually tapering, curved	gradually tapering, curved	gradually tapering, curved
Forcipular coxosternite: width / length of exposed part	>1.8	?	<1.8	<1.8	<1.8	<1.8	<1.8	<1.8
Legs: number of pairs (recorded values)	33	35, 37	33, 35, 37	29	35, 37	33, 35, 37	29, 31, 33	37, 39, 41
Carpophagus pit	yes	yes	?	yes	yes	yes	no	yes
Pore-fields	present (also in posterior part)	absent	present (only in anterior part)	present (also in posterior part)	present (also in posterior part)	absent	absent	absent
Coxal pores: number on each side (maximum recorded)	6	3	4	4	2	4	2	2
Coxal pores: isolated posterior pore	yes	?	?	no	no	no	no	no



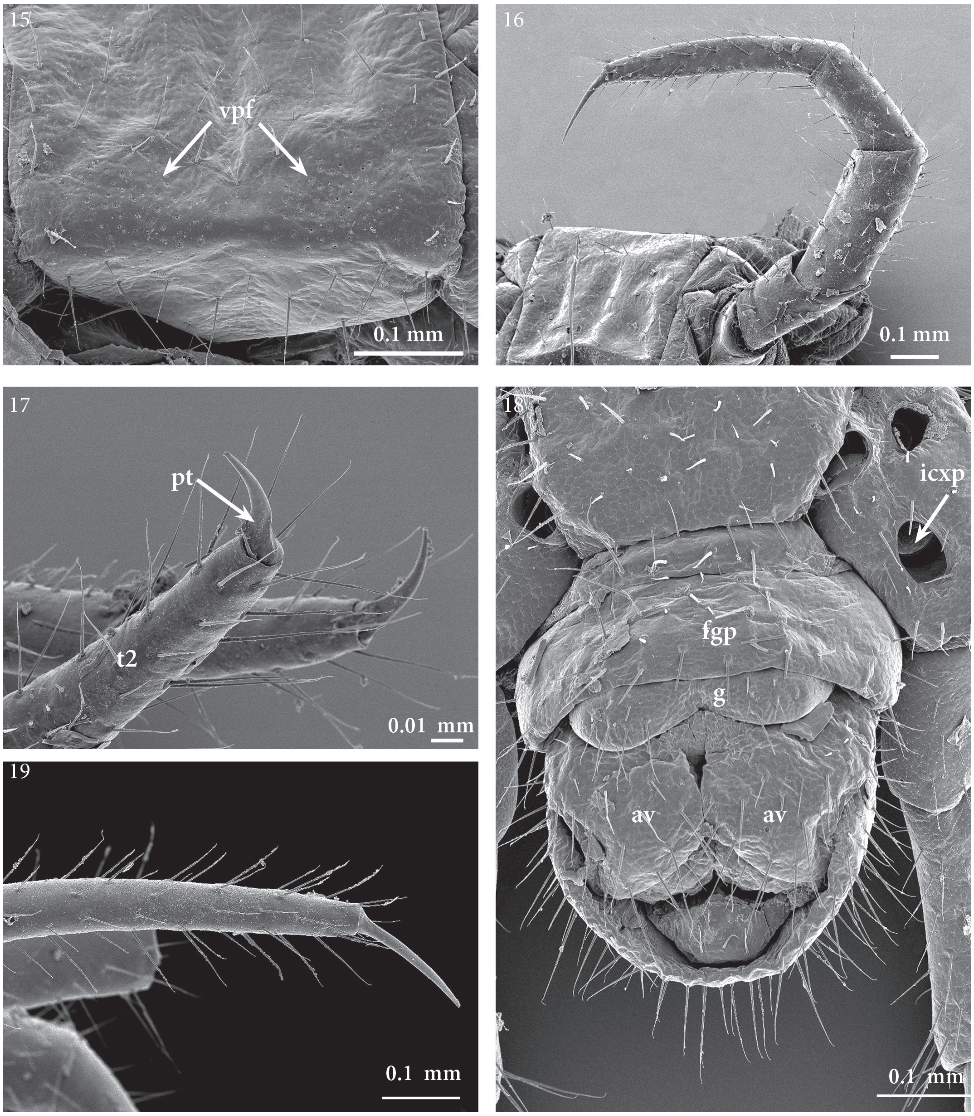
Figures 7–10. *Geophilus hadesi* sp. n. **7** intermediate part of labrum, ventral **8** right half of the maxillary complex, ventral **9** forcipular segment, ventral **10** close up of forcipular coxosternite, ventral. SEM micrographs taken from female paratype NHMW8363 from cave Muda labudova. Abbreviations: labral bristle (*lb*), labral denticle (*ld*), first maxillae (*MxI*), second maxillae (*MxII*), first maxillary telopodite (*tl*), first maxillary coxal projection (*cxp*), second maxillary pretarsus (*p*), coxopleural suture (*cxps*), chitin-line (*chl*), basal denticle of tarsungulum (*bd*).

Leg-bearing segments. A total of 33 leg-bearing segments (also in paratype). Tergites wider than long (Fig. 11), tergite 1 slightly wider than metatergite 2. Metasternites longer than wide, both in the anterior and the posterior part of trunk. Those in the anterior part of the trunk, with the exception of the first segment, with anterior carpophagus pits taking up one third of the length of the metasternite (also in paratype: Figs 12, 13); pits decreasing in size towards the body end to almost vanishing in the last five segments (Fig. 14). Pore-fields present on most part of the metasternites (also in paratype: Figs 12–15): a single transverse band along the posterior margin of each metasternite, appearing as two paramedian groups on the metasternites of the posterior half of the trunk, and poorly visible posterior to leg-bearing segment 26. Legs distinctly longer than the breadth of the body; their length increases towards leg pairs 25–26, then gradually decreasing until pair 32; leg 1 shortest. Leg claws very slender (Figs 16, 17, 19), more than 6 times as long as broad at the basis, with two long and pointed accessory spines.



Figures 11–14. *Geophilus hadesi* sp. n. **11** midbody tergites, dorsal **12** sternites of leg-bearing segments 6–8, ventral **13** leg-bearing segment 8, ventral **14** leg-bearing segment 25, ventral. SEM micrographs taken from female paratype NHMW8363 from cave Muda labudova. Abbreviations: presternites (*prst*); carpophagus pit (*crpp*), procoxa (*prcx*), metacoxa (*mtcx*), metasternite (*mtst*), ventral pore-field (*vpf*).

Ultimate leg-bearing segment. Metasternite sub-trapezoidal, *circa* 1.3 times as wide as long, lateral margins converging posteriorly; posterior margin narrower than anterior; setae uniformly scattered. Coxopleura moderately swollen, reaching backward approximately two thirds of first genital pleurosternite. Coxal organs opening through distinct pores, all on the ventral side, mostly close to the lateral margins of the metasternite, 5+6 (5+5 in paratype). Coxal pores generally large, one lying in the middle of the ventral side of the posterior part of the coxopleuron, somewhat apart from the rest (also in paratype:



Figures 15–19. *Geophilus hadesi* sp. n. **15** close up of metasternite of leg-bearing segment 26, ventral **16** left leg 8, anterio-lateral **17** close up of the tip of the right ultimate leg, external view **18** postpedal segments, ventral **19** close up of pretarsus and tarsus of midbody leg, lateral. SEM micrographs taken from female paratype NHMW8363 from cave Muda labudova. Abbreviations: tarsus 2 (*t2*), pretarsus (*pt*), isolated coxal pore (*icxp*), anal valve (*av*), first genital pleurosternite (*fgp*), gonopods (*g*).

Figs 18, 20). Telopodites of the ultimate pair almost as long as legs 25-26. Proportions between the lengths of the leg articles: trochanter<prefemur<coxa=tarsus2<femur=tibia =tarsus1; claws shorter than those of preceding legs, accessory spines distinctly shorter.

Postpedal segments. First genital pleurosternite entire. Gonopodal lamina distinctly bilobed. Anal pores present (also in paratype: Fig. 18).

Figure 20. Ultimate leg-bearing segment and postpedal segments, ventral. Photo taken from female paratype NHMW8363 from cave Muda labudova.

Discussion

On the taxonomic position of *G. hadesi* sp. n.

Although relationships between the species in the genus *Geophilus* are still largely unclear, *G. hadesi* sp. n. is probably related to a subgroup of species sharing a putatively modified shape of the second maxillary pretarsus, which resembles a stout tubercle with a short spine rather than an elongate curved claw. Three genus group names are available for this group, i.e. *Orinomus* Attems, 1895, *Orinophilus* Cook, 1896, and *Cyphonychius* Verhoeff, 1928, but their nomenclatural status is yet to be evaluated. Most of these species inhabit western and southern Europe, but *G. alpinus* Meinert, 1870 and *G. oligopus* (Attems, 1895) have been recorded also from the Balkan peninsula (Bonato et al. 2014b). Among all these species, *G. oligopus*, *G. persephones* and *G. piae* especially resemble *G. hadesi* sp. n. in the reduced number of segments, with their leg pairs varying mostly from 29 (recorded in *G. persephones*) to 39 (recorded in *G. oligopus*), but they are smaller than *G. hadesi* sp. n., with a length not reaching 2 cm. The other known troglobitic species *G. persephones* shares with the new species a number of distinctive traits (including elongate antennae, walking and ultimate legs), but this may be due to convergence to a troglomorphic habitus. Another shared trait is the shape of the labrum devoid of separation between mid and lateral parts.

On the habitat of *G. hadesi* sp. n.

Besides its troglomorphic appearance, the fact that all the four specimens (three collected) were recorded in the same type of habitat suggests that *G. hadesi* sp. n. is a highly adapted cave animal.

The holotype and paratype were found in two caves, Munižaba and Muda labudova, both situated in Crnopac massif, Southeastern Velebit Mountain. Munižaba cave is the most voluminous cave in Croatia, with the entrance dimensions of 30 × 35 m and a vertical drop of 200 meters (Fig. 21). The cave has been explored to the depth of -510 m and up to 9715 m in horizontal length. A specimen of *G. hadesi* was found under the entrance shaft at a depth of *circa* -250 m, but further details are not known. Muda labudova is a 680 m deep cave with low temperatures and a lot of snow in the first 200 m of depth. There a specimen was found at *circa* -500 m while moving on the cave wall. Detailed description of the morphology, genesis and climatic conditions of the Munižaba and Muda labudova caves, along with their maps and associated fauna, can be found in Casale et al. (2012).

Another two specimens apparently belonging to the same species were found in the Lukina jama – Trojama cave system, which is 1431 meters deep and is currently the deepest cave in the Dinaric Karst and the 15th deepest cave in the world (see http://www.caverbob.com/wdeep.htm and Bakšić et. al. 2007) (Fig. 22). The cave is situated inside the Hajdučki i Rožanski kukovi Strict Reserve, Velebit National Park, and has two entrances, which connect at 558 m of depth, the upper one (Trojama) being at 1475 m above sea level (Fig. 23). Both entrances are blocked with snow and ice blocks during most of the year. In the cave system two temperature gradients can be determined; one from the entrance to a depth of -200 m, and a second from -200 m to the bottom of the cave. The entrance part down to -200 m is under influence of outside conditions and the ice and the temperature gradually lowers to -5.8 °C, while from -200 m to the bottom it slowly rises, reaching 3.3–4.4 °C in the chamber at -980 m and 5.0 °C at the bottom (Paar et al. 2013). There is a constant water flow from a depth of -550 meters to the bottom and measurements have confirmed that the water level in the cave rises over 100 meters above the bottom during high water stands (Bedek et al. 2012). A specimen was found in Lukina jama – Trojama cave system in 2011 in a vast chamber at -980 m while moving slowly across a large boulder close to a water flow (Fig. 2). A second specimen was observed at *circa* -1100 m while moving on the wall and was out of reach of the collector. The latter represents the world's deepest record of Chilopoda as a whole.

A total of 54 animal taxa have been recorded in Lukina jama – Trojama cave system until 2013, including four new genera and seven new species (Čuković et al. 2014, present study). Terrestrial taxa are dominant (37 species), and include 19 troglobites, while out of 17 aquatic taxa 13 are stygobites. Certain taxa, i.e. *Velebitodromus smidai* (Casale, Giachino & Jalžić, 2004) (Coleoptera), *Congeria jalzici* Bilandžija & Morton, 2013 (Bivalvia) and *Zospeum tholossum* Weigand, 2013 (Gastropoda) exhibit high level of adaptation to the cave environment. A tentative list of all associated fauna is presented in Table 2.

Figure 21. The entrance of cave Munižaba. Photo: D. Bakšić.

The three caves have been biospeleologically investigated on numerous occasions during the past 10 years, but only three centipede specimens have been collected so far. One of the reasons is the difficulty of collecting in vast cave systems with large chambers and passages which provide an enormous habitat surface that is impossible to examine thouroughly. Collecting effort is also to be taken into consideration. In 2010 and 2013 caving expeditions in the Lukina jama – Trojama cave system included teams of biologists spending a minimum of two days in the chamber at -980 m, with more than 36 man-hours in 2010 (Bedek et al. 2012) and over 128 man-hours in 2013. The result was only one observed specimen at -1100 m. However in 2011, when a specimen was collected, only one biologist was in the chamber collecting for 18 hours. Unlike in 2011, in 2010 and 2013 baited pitfall traps were placed throughout the cave system and the area around them was examined in case a predator such as a centipede or beetle was attracted to the possibility of nearby prey, but no centipede was found. Such were also the cases with some Araneae and Opiliones species recorded in the cave system and can only be explained by “collectors’ luck”.

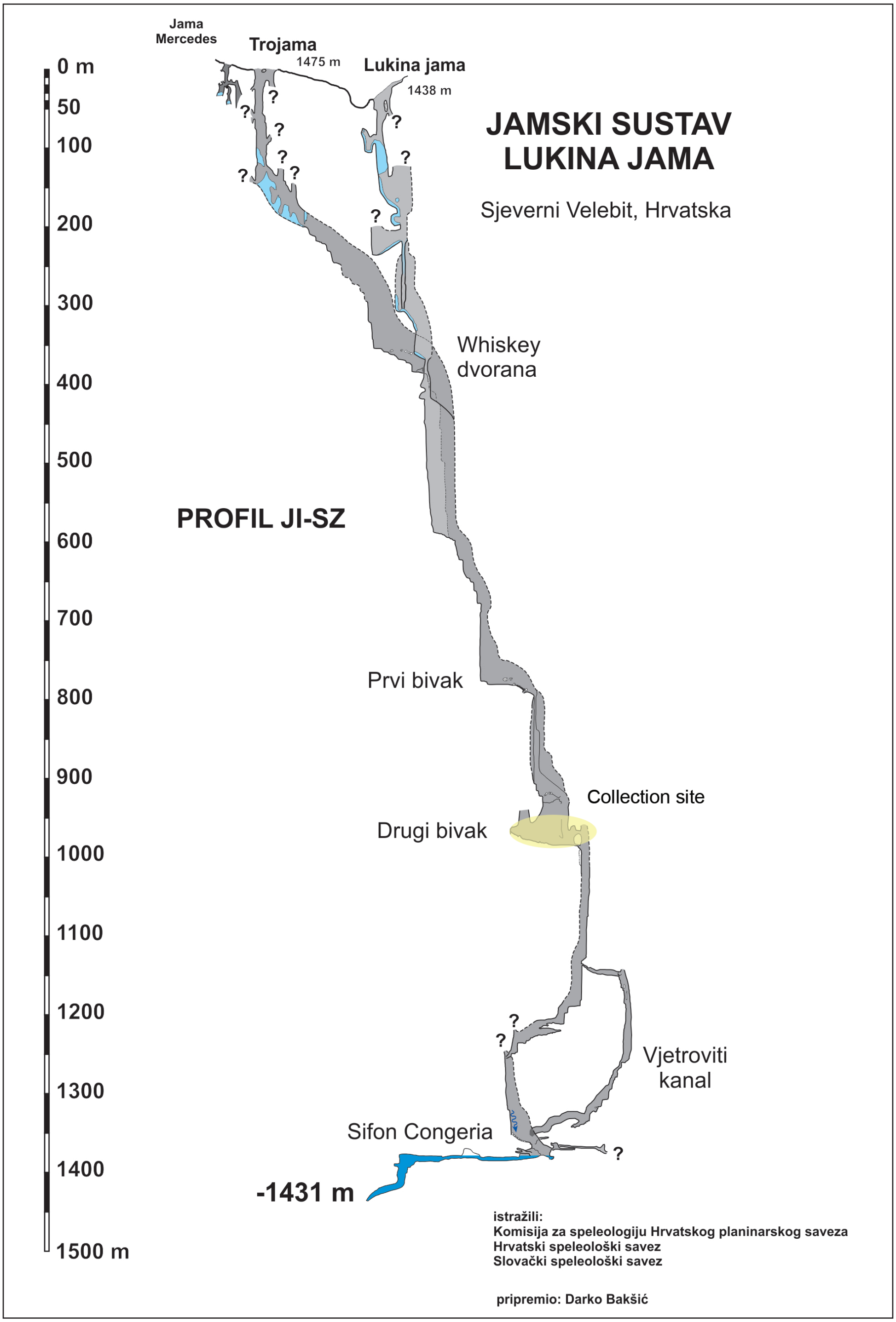


Figure 22. A map of Lukina Jama – Trojama cave system. Collection site marked in yellow.

Table 2. List of animal taxa found in Lukina jama – Trojama cave system (Čuković et al. 2014, present study).

Taxonomic group	Taxa
Porifera	*Eunapius subterraneus* Sket & Velikonja, 1984
Rotifera	*Keratella quadrata* (Müller, 1786)
Gastropoda	*Lanzaia* sp., *Hauffenia* sp., *Zospeum subobesum* Bole, 1974, *Zospeum tholossum* Weigand, 2013
Bivalvia	*Congeria jalzici* Bilandžija & Morton, 2013
Polychaeta	*Marifugia cavatica* Absolon & Hrabe, 1930
Hirudinea	*Croatobranchus mestrovi* Kerovec, Kučinić & Jalžić, 1999
Acari	*Rhagidia* sp., *Nicoletiella* sp.
Pseudoscorpiones	*Neobisium svetovidi* Ćurćić, 1988
Isopoda	*Proasellus* sp., *Alpioniscus* sp. n.
Amphipoda	*Niphargus arbiter* G. Karaman, 1985*, N. brevirostris* Sket, 1971*, N. croaticus* (Jurinac, 1888), *Niphargobates* sp.
Decapoda	*Troglocaris* cf. *kapelana, Troglocaris* sp.
Diplopoda	*Brachydesmus* sp., *Haasia stenopodium* (Strasser, 1966)
Chilopoda	*Geophilus hadesi* sp. n.
Collembola	Isotomidae gen. n., sp. n., *Disparrhopalites* sp. n., Tullbergiidae gen. sp.
Diplura	*Plusiocampa* (*Stygiocampa*) sp. n.
Coleoptera	*Astagobius angustatus* Schmidt, 1852, *Spelaeodromus pluto* (Reitter, 1881), *Velebitodromus smidai* (Casale, Giachino & Jalžić, 2004), *Laemostenus* sp.
Diptera	Chironomidae gen. n., sp. n., Mycetophilidae gen. sp.
Chiroptera	*Myotis mystacinus* (Kühl, 1817)

Figure 23. Entrance of cave Lukina jama. Photo: M. Jekić

On the subterranean biodiversity of Velebit Mountain

Velebit stretches over 145 km and is situated in the Croatian Dinaric Karst area (Paar et al. 2013), which is considered a remarkable hot spot of subterranean diversity (Casale et al. 2012). The mountain is divided into four regions: Northern, Middle, Southern and Southeastern (Bognar 1994). There is a significant difference in karst morphology between the regions. Northern Velebit hosts a large number of extremely vertical and deep caves (Paar et al. 2013), including all the three caves in Croatia deeper than 1000 m. Towards the south, caves are more horizontal. Southeastern Velebit is an area of Crnopac massif, where caves reach depths of over 700 m but create complex systems. The longest cave in the Dinaric Arc, the Kita Gačešina – Draženova puhaljka cave system, over 27 km in length, is surrounded by more caves and systems awaiting investigation. Currently there is a perspective of connecting this system with Munižaba into a unique system over 37 km long (Bakšić and Rakovac 2014). Despite the differences in karst morphology, the conditions in the caves in Velebit are similar, with stable low temperatures and an average of 90–100% humidity.

The lack of finds in Middle and South Velebit is possibly due to undersampling in these areas, which have been much less investigated speleologically and biologically than the caves in Northern Velebit and the Crnopac area.

Some subterranean species are endemic to caves on Velebit Mountain (e.g., *Croatobranchus mestrovi* Kerovec, Kučinić & Jalžić, 1999) while for others (e.g., *Neobisium* (*Pennobisium*) *stribogi* Ćurčić, 1988) it is only a part of their distribution range. Certain areas of Croatian Dinaric Karst are still poorly investigated, e.g. Biokovo Mountain in the south of Croatia, where caves have so far been explored to -831 m of depth. Future research in these undersampled areas will provide a better insight into the distribution area and possible habitat preferences of the species.

While several highly specialized cave invertebrates are known to inhabit both caves, Muda labudova and Munižaba, thus showing similar distribution patterns (Casale et al. 2012, Antić et al. 2014), still little is known about the faunal links between caves in the northern part of Velebit, such as the Lukina jama – Trojama cave system, and those in the south. *G. hadesi* sp. n. offers an example for investigating the history of underground colonization in these two areas.

Acknowledgements

Part of this study was carried out with financial support to PS by the European Commission's (FP 6) Integrated Infrastructure Program SYNTHESYS (DK-TAF). Many thanks are due to J. Bedek and B. Jalžić for collecting and providing us with this interesting material. J. Bedek also supplied details on the collecting sites and photographed the new species *in situ*. M. Jekić and D. Bakšić kindly provided photos of the Lukina jama and Munižaba caves. The map of the Lukina jama-Trojama cave system was contributed by D. Bakšić. Further information can be found at the Croatian Speleological Server, http://www.speleologija.hr.

References

Antić DŽ, Reip HS, Dražina T, Rađa T, Makarov SE (2014) Three new monotypic genera of Trichopolydesmidae from Croatia, Balkan Peninsula (Diplopoda, Polydesmida). Zootaxa 3884(2): 101–121.

Ázara LN, Ferreira RL (2013) The first troglobitic *Cryptops* (*Trigonocryptops*) (Chilopoda: Scolopendromorpha) from South America and the description of a non-troglobitic species from Brazil. Zootaxa 3709: 432–444. doi: 10.11646/zootaxa.3709.5.2

Ázara LN, Ferreira RL (2014a) *Cryptops* (*Cryptops*) *spelaeoraptor* n. sp. a remarkable troglobitic species (Chilopoda: Scolopendromorpha) from Brazil. Zootaxa 3826: 291–300. doi: 10.11646/zootaxa.3826.1.10

Ázara LN, Ferreira RL (2014b) Two new troglobitic *Newportia* (*Newportia*) from Brazil (Chilopoda: Scolopendromorpha). Zootaxa 3881(3): 267–278. doi: 10.11646/zootaxa.3881.3.5

Bakšić A, Rakovac M (2014) Munižaba u 2014. godini. Croatian Speleological Server. http://www.speleologija.hr, http://www.speleologija.hr/lukinajama

Bakšić D, Barišić T, Božić V, Buzjak S, Čaplar A, Jalžić B, Lacković D, Stroj A, Smida B, Vrbek B, Vrbek M (2007) Jamski sustav Lukina jama – Trojama. Hrvatski planinarski savez, Zagreb, 139.

Bedek J, Lukić M, Jalžić B, Ozimec R, Bilandžija H, Dražina T, Hamidović D, Pavlek M, Patarčić I, Komerički A (2012) Subterranean community from Lukina jama – Trojama cave system, the deepest cave in Dinaric Karst (Northern Velebit Mt.,Croatia). 21st International Conference on Subterranean Biology, Book of Abstracts. Košice, Slovakia, 28–29.

Bognar A (1994) Temeljna skica geoekoloških osobina Velebita. Senjski zbornik 21: 1–8.

Bonato L, Drago L, Murienne J (2014a) Phylogeny of Geophilomorpha (Chilopoda) inferred from new morphological and molecular evidence. Cladistics 30: 485–507. doi: 10.1111/cla.12060

Bonato L, Edgecombe GD, Lewis JGE, Minelli A, Pereira LA, Shelley RM, Zapparoli M (2010) A common terminology for the external anatomy of centipedes (Chilopoda). ZooKeys 69: 17–51. doi: 10.3897/zookeys.69.737

Bonato L, Edgecombe GD, Zapparoli M (2011) Chilopoda-Taxonomic overview. In: Minelli A (Ed.) Treatise on Zoology - The Myriapoda, vol. 1. Brill, Leiden, 363–443.

Bonato L, Minelli A (2010) The geophilomorph centipedes of the Seychelles islands (Chilopoda: Geophilomorpha). In: Gerlach J, Marusik Y (Eds) Arachnida and Myriapoda of the Seychelles islands. Siri Scientific Press, 360–375.

Bonato L, Minelli A, Lopresti M, Cerretti P (2014b) ChiloKey, an interactive identification tool for the geophilomorph centipedes of Europe (Chilopoda, Geophilomorpha). ZooKeys 443: 1–9. doi: 10.3897/zookeys.443.7530

Casale A, Jalžić B, Lohaj R, Mlejnek R (2012) Two new highly specialized subterranean beetles from the Velebit massif (Croatia): *Velebitaphaenops* (new genus) *giganteus* Casale & Jalžić, new species (Coleoptera: Carabidae: Trechini) and *Velebitodromus ozrenlukici* Lohaj, Mlejnek & Jalžić, new species (Coleoptera: Cholevidae: Leptodirini). Natura Croatica 21(1): 129–153.

Chagas-Júnior A, Bichuette ME (2015) A new species of *Scolopocryptops* Newport: a troglobitic scolopocryptopine centipede from a remarkable siliciclastic area of eastern Brazil (Scolopendromorpha, Scolopocryptopidae, Scolopocryptopinae). ZooKeys 487: 97–110. doi: 10.3897/zookeys.487.9262

Chagas-Júnior A, Shelley RM (2003) The centipede genus *Newportia* Gervais, 1847, in Mexico: description of a new troglomorphic species; redescription of *N. sabina* Chamberlin, 1942; revival of *N. azteca* Humbert & Saussure, 1869; and a summary of the fauna (Scolopendromorpha: Scolopocryptopidae: Newportiinae). Zootaxa 379: 1–20.

Čuković T, Jalžić B, Bedek J, Komerički A, Bilandžija A, Dražina T, Pavlek M, Lukić M, Miculinić K, Ozimec R (2014) Biospeleološka istraživanja na ekspediciji Lukina jama – sifon 2013. Subterranea croatica 16: 13–15.

Culver DC, Shear WA (2012) Myriapods. In: White WB, Culver DC (Eds) Encyclopedia of caves. Academic Press, Chennai, 538–542. doi: 10.1016/B978-0-12-383832-2.00078-5

Edgecombe GD (2005) A troglomorphic species of the centipede *Cryptops* (*Trigonocryptops*) (Chilopoda: Scolopendromorpha) from Western Australia. Records of the Western Australian Museum 22: 31–323.

Edgecombe GD (2006) A troglobitic cryptopid centipede (Chilopoda: Scolopendromorpha) from western Queensland. Records of the Western Australian Museum 23: 193–198.

Foddai D, Minelli A (1999) A troglomorphic geophilomorph centipede from southern France (Chilopoda: Geophilomorpha: Geophilida). Journal of Natural History 33: 267–287. doi: 10.1080/002229399300416

Howarth FG, Stone FD (1990) Elevated Carbon dioxide levels in Bayliss Cave, Australia: implications for the evolution of obligate cave species. Pacific Science 44: 207–218.

Iorio E (2010) Les Lithobies et genres voisins de France. Revue de l'Association Roussillonnaise d'Entomologie, suppl. 19: 1–104.

Lewis JGE (1981) The biology of centipedes. Cambridge University Press, New York, 1–476. doi: 10.1017/cbo9780511565649

Matic Z, Negrea SG, Fundora Martinez C (1977) Recherches sur les Chilopodes hypogés de Cuba. In: Résultats des Expéditions Biospéléologiques Cubano-Roumaines à Cuba. II. Editura Academiei Republicii Socialiste Romania, Bucuresti 2: 277–301.

Negrea Ş (1993) Sur une population troglobionte de *Cryptops anomalans* Newport, 1844 (Chilopoda, Scolopendromorpha) trouvée dans la grotte "Pestera de la Movile", (Dobrogea, Roumanie). Travaux de l'Institut de Spéologie "Émile Racovitza" 32: 87–94.

Negrea S, Minelli A (1994) Chilopoda. In: Juberthie Ch, Decu V (Eds) Encyclopaedia biospeologica, 1. Société de Biospéologie, Moulis & Bucarest, 249–254.

Paar D, Buzjak N, Bakši D, Radoli V (2013) Physical research in Croatia's deepest cave system: Lukina jama-Trojama, Mt. Velebit. Proceedings of the 16th International Congress of Speleology 2: 442–446.

Schileyko A (2013) A new species of *Newportia* Gervais, 1847 from Puerto Rico, with a revised key to the species of the genus (Chilopoda, Scolopendromorpha, Scolopocryptopidae). ZooKeys 276: 39–54. doi: 10.3897/zookeys.276.4876

Stoev P, Akkari N, Zapparoli M, Porco D, Enghoff H, Edgecombe GD, Georgiev T, Penev L (2010) The centipede genus *Eupolybothrus* Verhoeff, 1907 (Chilopoda: Lithobiomorpha:

Lithobiidae) in North Africa, a cybertaxonomic revision, with a key to all species in the genus and the first use of DNA barcoding for the group. ZooKeys 50: 29–77. doi: 10.3897/zookeys.50.504

Zapparoli M (1997) *Lithobius nuragicus* n. sp., a new *Lithobius* from a Sardinian cave (Chilopoda Lithobiomorpha). International Journal of Speleology 25 (1996): 59–66.

ZooKeys 510: 115–124 (2015)
doi: 10.3897/zookeys.510.8838
http://zookeys.pensoft.net

RESEARCH ARTICLE

A peer-reviewed open-access journal
ZooKeys
Launched to accelerate biodiversity research

Millipedes faced with drought: the life cycle of a Mediterranean population of *Ommatoiulus sabulosus* (Linnaeus) (Diplopoda, Julida, Julidae)

Jean-François David[1], Mathieu Coulis[1]

[1] *Centre d'Ecologie Fonctionnelle & Evolutive, UMR 5175, CNRS–Université de Montpellier, 1919 route de Mende, F–34170 Montpellier cedex 5, France*

Corresponding author: *Jean-François David* (jean-francois.david@cefe.cnrs.fr)

Academic editor: *Ivan H. Tuf* | Received 27 October 2014 | Accepted 4 May 2015 | Published 30 June 2015

http://zoobank.org/B7BBC82C-84CB-47C8-BBF4-AD8D8C3C31E9

Citation: David J-F, Coulis M (2015) Millipedes faced with drought: the life cycle of a Mediterranean population of *Ommatoiulus sabulosus* (Linnaeus) (Diplopoda, Julida, Julidae). In: Tuf IH, Tajovský K (Eds) Proceedings of the 16th International Congress of Myriapodology, Olomouc, Czech Republic. ZooKeys 510: 115–124. doi: 10.3897/zookeys.510.8838

Abstract

Growth, development and life-cycle duration of the millipede *Ommatoiulus sabulosus* (f. *aimatopodus*) were studied in a Mediterranean shrubland of southern France and compared with previous data from northwest Europe. Changes in the proportions of stadia during the course of the year were analysed in several generations. The results show that stadia VII and VIII are consistently reached after the first year of growth, and stadia IX and X after the second year. First reproduction may occur at the age of two years in males reaching maturity at stadium X, but not until the age of three in those reaching maturity at stadia XI and XII. Reproduction cannot occur until at least the age of three in females, which carry mature eggs from stadium XI onwards. In comparison with more northern populations, life-cycle duration is not shorter in the Mediterranean population but there are marked differences in its phenology: the breeding period is in autumn, so that juveniles of stadia II to VI are never faced with the summer drought, and larger individuals are mostly inactive in summer; moreover, all individuals moult once every winter. The results illustrate how julid millipedes of humid temperate regions could respond to higher temperatures and drier summer conditions in the context of climate change.

Keywords

Millipedes, life cycle, phenology, climate change

Introduction

In many organisms, ongoing climate change affects the timing of life-cycle events such as activity, growth and reproduction (Parmesan 2006). When no long-term data sets are available to analyse trends in local populations, potential phenological responses to climate change can be studied by examining intraspecific variation in widespread species that live in a wide range of conditions.

In millipedes (Diplopoda), geographic variation in life-cycle characteristics has been documented for some species of European julids (Fairhurst 1974, David 1982), but there is little precise information for populations living in the Mediterranean region. A number of widespread species such as *Cylindroiulus caeruleocinctus* (Wood), *Cylindroiulus punctatus* (Leach), *Leptoiulus belgicus* (Latzel), *Ommatoiulus rutilans* (C. L. Koch) and *Ommatoiulus sabulosus* (Linnaeus) have populations in this area, which is typified by cool winters and hot, dry summers. The study of phenological characteristics in Mediterranean populations of these species is particularly interesting, because climate change scenarios predict warmer and drier summer conditions over large parts of western Europe for the end of the 21st century (IPCC 2013). The purpose of the present study is to describe the life cycle of a Mediterranean population of *O. sabulosus* and to compare the results with those previously obtained further north in Europe, so as to highlight differences between populations from the two climatic zones. The species has a wide distribution, from Finland and Scotland to Albania and Spain, in contrast to most *Ommatoiulus* species that are confined to the Iberian peninsula and north Africa (Akkari and Enghoff 2012). Two forms occur in southern France: that in which adults have two orange-yellow bands on the back, and a typically Mediterranean form, the so-called *O. sabulosus aimatopodus* (Risso), in which adults are black dorsally. The latter form is the most common in southern France and often occurs at high population densities in shrubland ecosystems on limestone (Coulis et al. 2013).

The post-embryonic growth and development of *O. sabulosus* were described in detail by Halkka (1958) and Sahli (1969). The life cycle, i.e. the calendar of events between birth and reproduction, was studied under field conditions by Halkka (1958) in Finland, Sahli (1968) in Germany, Biernaux (1972) in Belgium and Fairhurst (1974) in Great Britain. Reproduction occurs in late spring and summer in all these regions. Biernaux (1972) tentatively suggested that both males and females mature in two years in Belgium, but Fairhurst (1974) concluded that males take two or three years, and females three or four years, to reach maturity in Great Britain. As regards Mediterranean populations, Sahli (1991a, 1992) studied in detail the timing of male maturation and adult–intercalary male successions (periodomorphosis) in *O. sabulosus aimatopodus* from the Alpes-Maritimes and Provence, southern France. This author mentioned that egg-laying occurs in late summer–early autumn (Sahli 1991a), but provided very limited information on the growth, development and age at reproduction of females. Sahli (1991b) suggested that females could breed only once before dying in Mediterranean populations (semelparity), in contrast to Biernaux (1972) who concluded, based on his own study of egg development in populations from Belgium, that females can breed in successive years (iteroparity). The presence of an abundant population of *O. sabulosus aimatopodus* in a garrigue ecosystem of Provence provided the opportunity to clarify some aspects of the species' biology in the field, with particular attention to how this julid adjusts its phenology under warmer and drier conditions.

Methods

This study was conducted at the Massif de l'Etoile near Marseille, southern France (5°25'E; 43°22'N), in a shrubland dominated by rockrose (*Cistus albidus* L.), kermes oak (*Quercus coccifera* L.), rosemary (*Rosmarinus officinalis* L.) and gorse (*Ulex parviflorus* Pourr.). The soil is shallow rendzina on limestone, in which rock fragments and stones represent about 60% of the soil volume in the top 20 cm. The mean annual temperature in the area is 15.1 °C, mean monthly temperatures ranging from 7.1 °C in January to 24.1 °C in July, and the mean annual rainfall is 555 mm (Marseille 1981–2010 climate normals). The driest months are June, July and August, during which the soil becomes very dry. The millipede community, heavily dominated by *O. sabulosus aimatopodus*, also comprises an abundant population of *Polyxenus lagurus* (Linnaeus) (Polyxenidae) and rare specimens of *Leptoiulus* sp. (Julidae) and *Trichoblaniulus* sp. (Trichoblaniulidae).

Collections of millipedes were made using different methods. (1) Twenty three pitfall traps were set on the site in late March 2010 (8 days) and late April 2010 (10 days). (2) Leaf litter and topsoil samples were taken within 25 × 25 cm quadrats in May 2010 (31 sampling units), November 2010 (12 s.u.), May 2012 (31 s.u.), October 2013 (15 s.u.), November 2013 (11 s.u.), March 2014 (12 s.u.), April 2014 (31 s.u.) and September 2014 (13 s.u.). Millipedes were extracted using Tullgren funnels. (3) Large individuals were also collected by hand in leaf litter to determine their reproductive status.

Individuals were assigned to a stadium by counting the rows of ocelli (R.O.) on each side of the head (1 R.O. = stadium II, 2 R.O. = stadium III, etc.) (Enghoff et al. 1993). The method, however, was often difficult to apply from stadium XI onwards. The numbers of podous rings (including the collum) and apodous rings (excluding the telson) were counted. Intercalary males were distinguished from other males by a much smaller first pair of legs than in immature males, but not modified into hooks as in copulatory males (Halkka 1958). Forty-two females of stadia X and higher were dissected to determine whether mature eggs (i.e. brownish, subspherical eggs about 0.6 mm long) were present in the ovitube.

The growth of several cohorts in the field was studied by examining changes in the proportions of stadia in successive samples (Blower 1970). In addition, 40 individuals of various stadia were reared in the laboratory for periods ranging from a few months to two years. They were kept in transparent plastic boxes containing sieved soil and moist leaf litter. The boxes were placed in incubators fitted with a glass door, in which temperature followed the long-term monthly mean temperatures of Marseille, with a daily thermoperiod of low amplitude. All millipedes received a pinch of powder yeast every month and, occasionally, rabbit faeces as supplementary food.

Results

Post-embryonic growth and development in the field

The stadia identified using the numbers of R.O., and the numbers of body rings counted in each stadium, are indicated in Table 1. In terms of ring numbers, growth was slightly different from that reported in more northern populations, with an extra apodous ring in stadia III and IV. This was confirmed by the higher numbers of podous rings from stadium IV onwards. The maximum number of stadia is uncertain because it was generally impossible to decipher the exact number of R.O. in the largest individuals. However, a few males had at least 12 R.O. (stadium XIII) and a female with 57 podous rings had at least 13 R.O. (stadium XIV).

Sexual dimorphism was apparent at stadium VI. Although two males reared in the laboratory reached maturity at stadium IX, the smallest adult males found in the field were in stadium X (Table 1). Immature males were numerous up to and including stadium XI, indicating that many males mature for the first time in stadia XI or XII. Intercalary males were found from stadium XI onwards. Dissection of females in late summer–early autumn, just before the breeding period (see below), showed that ovigerous females carrying mature eggs were present in any stadium from stadium XI onwards. None of the stadium X females that were dissected (n = 6) were ovigerous.

Phenology

Juveniles were active in leaf litter in late October as stadium II, in mid-November as stadia II and III (Fig. 1b), and in mid-December as stadia III and IV. Also, a female kept for

Table 1. Growth and development of *O. sabulosus* in Provence. The number of rows of ocelli (R.O.), the range of podous rings (collum included) and the numbers of apodous rings (telson excluded) are given for each stadium. Male stages: Im. = Immature; Ad. = adult; Int. = Intercalary.

Stadium	R.O.	Podous rings / Apodous rings			Male development
		Juveniles	Females	Males	
II	1	6 / 5			
III	2	11 / 5,6			
IV	3	16–17 / 6,7			
V	4	22–24 / 6,7			
VI	5		29–32 / 6,7,8	29–32 / 6,7	Im.
VII	6		35–38 / 5,6	36–39 / 5,6	Im.
VIII	7		42–45 / 3,4	41–45 / 2,3,4	Im.
IX	8		45–49 / 1,2,3	44–49 / 1,2,3	Im.
X	9		47–50 / 1,2	47–50 / 1,2	Im., Ad.
XI	10		48–53 / 1	48–50 / 1	Im., Ad., Int.
XII+	≥ 11		49–57 / 0,1	50–55 / 0,1	Ad., Int.

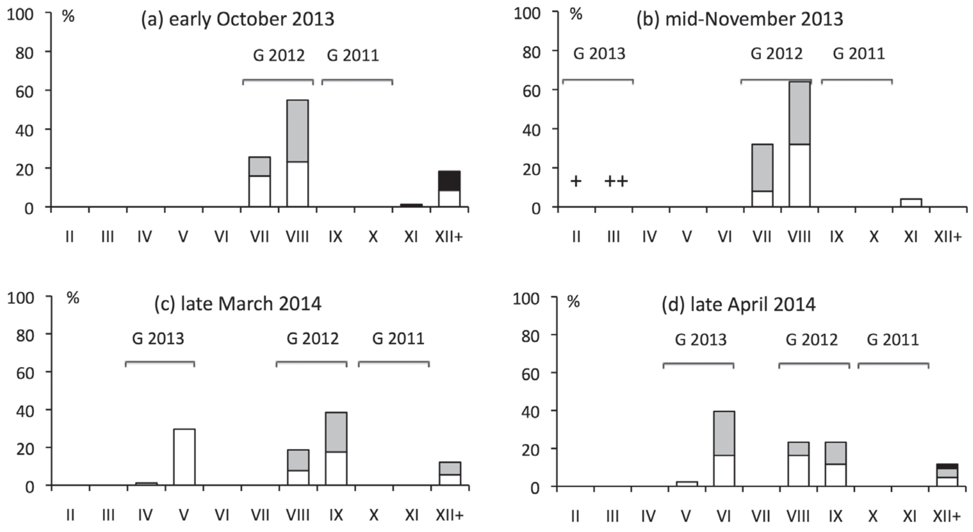


Figure 1. Phenology of *O. sabulosus* in Provence (October 2013–April 2014). Stadia are indicated on the horizontal axis and those of three identifiable generations (G 2011 without any individuals, G 2012 and G 2013) are grouped together. White bars = undifferentiated juveniles and females; grey bars = immature and intercalary males; black bars = adult males. Abundant (+) or very abundant (++) juveniles of stadia II and III were not included in the calculation of percentages.

months in the laboratory produced stadium II juveniles in late October. Samples taken in late March showed that the new generation was mainly in stadium V by the end of winter (Figs 1c, 2a), which implies moulting during the winter. This result was confirmed in laboratory rearings, in which juveniles that had hatched in October emerged from the soil as stadium V in March. The rearings further showed that the new generation continued to grow rapidly in spring, from stadium V in March (rearing temperature: 10 ± 2 °C) to stadium VI in April (13 ± 2 °C) and to stadium VII in May (17 ± 2 °C), exactly as in the field (Fig. 2b). Sexual differentiation at stadium VI thus occurs at the age of about 6 months. The pace of growth slowed markedly around the summer. Litter and topsoil samples taken in early October showed that the smallest individuals, born in autumn of the preceding year, were in stadia VII and VIII (Fig. 1a), indicating that only one moult had occurred since May. All those one-year old millipedes were immature in both sexes.

During the second year of growth, no moult occurred from October to mid-November (Fig. 1b). At this time of year, the population becomes progressively inactive in the soil, both in the field and in the laboratory. One-year old millipedes moulted once during the winter and emerged from the soil in late March as stadia VIII and IX (Figs 1c, 2a). This was confirmed in laboratory rearings, in which six males and females of stadium VIII collected in the field in October burrowed into the soil in October–November and emerged as stadium IX in March. Field samples taken in 2010 (Fig. 2) showed that the generation that was in stadia VIII and IX at the end of winter remained in these stadia until May, moulted in mid-May (as shown by the large

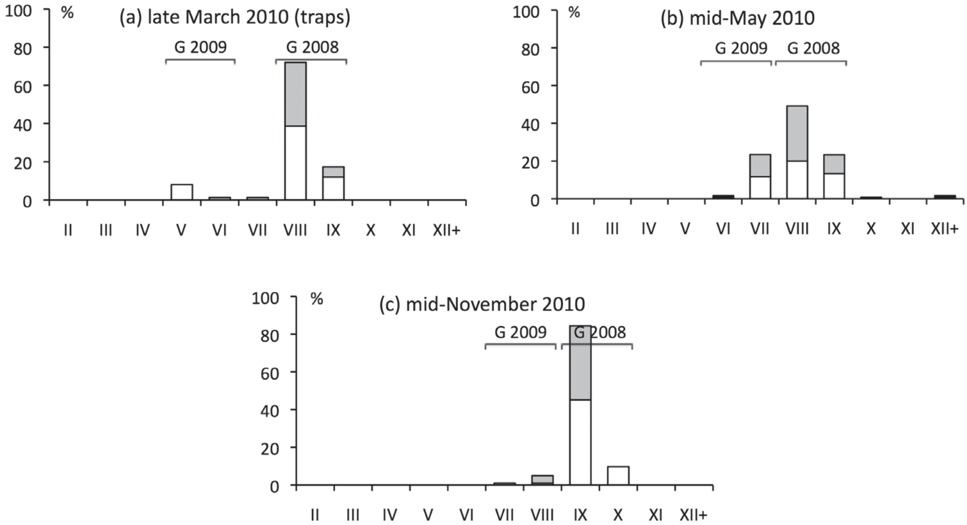


Figure 2. Phenology of *O. sabulosus* in Provence (March 2010–November 2010). See explanations in the legend to Fig. 1.

proportion of millipedes that were moulting at the time of sampling), and was still in stadia IX and X in mid-November, at the age of two. The complete absence of stadia IX and X in the autumn of 2013 (Fig. 1a, b) indirectly confirms that these stadia are reached in two years, since, for unexplained reasons, there was no recruitment in the autumn of 2011. Similarly, samples taken in the autumn of 2010 (Fig. 2c) suggest there was no or little recruitment in that year, which was confirmed in subsequent samples (data not shown).

The number of moults during the third year of growth cannot be deduced from the field data. Assuming that there are two further moults — one in winter and one in spring, as in the second year of growth — most individuals of stadia XI and XII found in the autumn of 2013 (Fig. 1a) would be three years old. However, this would be inconsistent with the lack of juvenile recruitment in 2010, and it is likely that most individuals in stadia XI and XII collected in the autumn of 2013 were actually born before 2010.

Life-cycle duration

By combining the results on individual development and phenology, one may infer that a small proportion of males that reach maturity at stadium X reproduce at the age of two. However, many males that mature for the first time at stadia XI and XII cannot reproduce until the age of three. There is no evidence that some females breed at the age of two, since no ovigerous females were found in stadia IX and X in late summer. Females need at least three years to reach stadium XI and lay eggs in early autumn.

The continuation of the life cycle was observed in a few adults reared in the laboratory. Three adult males collected in autumn moulted during the winter and emerged from the soil in March as intercalary males. They became mature again after a further moult in spring and remained in the adult stage until the following autumn. Two large females collected in autumn also moulted during the winter but, in contrast to males, they did not moult again in spring or summer. One of these females bred in October, overwintered a second time in the laboratory, and survived until the following September but without moulting. Post-mortem inspection showed that this female had no apodous ring and contained no eggs.

Discussion

The present study provides the first estimate of life-cycle duration for *O. sabulosus* in southern France. The interpretation of our field data was made easy by the generally high abundance of juveniles and also by gaps between successive generations, possibly due to reproduction failures and/or high juvenile mortality rates in some years. In the population studied near Marseille, stadia VII and VIII are consistently reached after the first year of growth, and stadia IX and X after the second year. This pattern was observed in three generations born between 2008 and 2012, despite some variation from one year to another (e.g. the cohort born in 2008 was mainly in stadium VIII in late March 2010, while that born in 2012 was mainly in stadium IX in late March 2014). Our results differ from those of Sahli (1992), who assumed that stadia X and XI were reached at the age of three in the region of Provence.

As adult males were found from stadium X onwards in our samples, some males may reproduce at the age of two. However, males that reach maturity in stadia XI or XII cannot reproduce until the age of three at the earliest. Also, ovigerous females, which were found from stadium XI onwards in our samples, cannot breed until the age of three at the earliest. Moreover, the presence of some stadium XI females without any mature eggs in early autumn suggests they may start breeding at the age of four. Therefore, the duration of the life cycle, which corresponds to the age of females at first reproduction, is three or possibly four years in this Mediterranean population, i.e. the same as in populations studied by Fairhurst (1974) in Great Britain.

It remains unclear whether each female breeds only once during its lifetime (semelparity) or can breed over several years (iteroparity). Sahli (1991a) assumed that *O. sabulosus* females might be semelparous in Mediterranean populations, reproduction being spread over different stadia and different years in each generation. In the present study, the single female that bred in the laboratory survived for a further year but died without breeding again, so that there is still no direct evidence for iteroparity. Moreover, we did not find clusters of small oocytes at the same time as mature eggs in ovigerous females, which Biernaux (1972) mentioned as evidence for iteroparity in *O. sabulosus*. On the other hand, dissection of females in late summer–early autumn revealed that the proportion of those not carrying mature eggs in stadia XI and higher was rather low (22%), and the question is whether this is sufficiently high to be consistent with semelparity. Semelparity would imply that many females in stadia XI, XII and even XIII postpone reproduction until the next year(s), which should result in a substantial proportion of females without eggs in early autumn. This topic requires further research.

Although Mediterranean conditions do not modify the length of the life cycle in *O. sabulosus*, several phenological characteristics are very different between the population of Marseille and more northern populations. First, there is a shift of the breeding season. In northwestern Europe, the species generally breeds in summer (Sahli 1968, Biernaux 1972, Fairhurst 1974). Under milder climate conditions, as on the island of Jersey, the species tends to breed earlier (Fairhurst 1974). However, our study confirms that, in the Mediterranean region, the breeding period of *O. sabulosus* is delayed until the autumn (Halkka 1958, Sahli 1991a). Juveniles of stadia II to IV were collected only in this season. They grow rapidly from autumn to the following spring and the first part of the life cycle is similar to that of *Ommatoiulus moreleti* (Lucas) in southern Portugal, which breeds in late autumn–early winter (Baker 1984). In both species, the earliest active stadia (stadia II to VI) are never faced with the hot and dry conditions of the summer, which may be an adaptation of the Mediterranean populations of *Ommatoiulus*. It should be noted that, in other millipedes, the youngest stadia are by far the least resistant to desiccation (David and Vannier 2001).

The seasonal patterns of activity and growth also differ between the two climatic areas. In northern populations, there is generally a single period of activity and growth from spring to autumn and the species is active in summer (Halkka 1958, Fairhurst 1974). The duration of the active season clearly increases with increasing temperatures in areas where the risk of summer drought is low (cf. Halkka 1958, Fairhurst 1974, Meyer 1985). In the Mediterranean population, however, activity stops during the summer months, and our study has shown that there is at most one moult between May and September. Similarly, in *O. moreleti* living at low altitudes in Madeira, Read (1985) reported that growth slows down during the summer, presumably due to dry conditions. The presence of two long periods of inactivity, in summer as well as winter, largely explains why the life cycle of *O. sabulosus* is not shorter under Mediterranean conditions, as would have been expected for millipedes living in a warmer climate (David and Handa 2010).

Conclusion

The life cycle of *O. sabulosus* in the Mediterranean region appears to be influenced mainly by the summer drought. The dry season especially impacts phenology, i.e. the timing of activity, growth and reproduction. Contrary to many organisms that breed earlier in spring under warmer conditions, this julid breeds in autumn under Mediterranean conditions, so that juveniles are unlikely to be exposed to severe drought. Moreover, larger stadia become inactive in summer and the total duration of activity over a year is roughly the same as in northern populations. As a result, the life cycle is not shorter in the Mediterranean region than in Great Britain. Although it is too soon to generalize, the life cycle of *O. sabulosus* in southern France is quite similar to that of *O. moreleti* in southern Portugal, suggesting ways in which a number of julids could respond to drier summer conditions in the context of climate change.

Acknowledgements

We thank Anais Rancon, Anne Gorgeon, Mathieu Santonja, and all the members of the Bioflux team who assisted in field sampling. This study was conducted as part of the CLIMED project funded by the French National Agency for Research (ANR-09-CEP-007).

References

Akkari N, Enghoff H (2012) Review of the genus *Ommatoiulus* in Andalusia, Spain (Diplopoda: Julida) with description of ten new species and notes on a remarkable gonopod structure, the fovea. Zootaxa 3538: 1–53.

Baker GH (1984) Distribution, morphology and life history of the millipede *Ommatoiulus moreletii* (Diplopoda: Iulidae) in Portugal and comparisons with Australian populations. Australian Journal of Zoology 32: 811–822. doi: 10.1071/ZO9840811

Biernaux J (1972) Chorologie et étude biologique comparée de deux familles de Myriapodes Diplopodes belges: les Blaniulidae et les Iulidae. PhD Thesis, Faculté des Sciences Agronomiques de l'Etat, Gembloux, Belgium.

Blower JG (1970) The millipedes of a Cheshire wood. Journal of Zoology (London) 160: 455–496. doi: 10.1111/j.1469-7998.1970.tb03092.x

Coulis M, Hättenschwiler S, Fromin N, David JF (2013) Macroarthropod–microorganism interactions during the decomposition of Mediterranean shrub litter at different moisture levels. Soil Biology & Biochemistry 64: 114–121. doi: 10.1016/j.soilbio.2013.04.012

David JF (1982) Variabilité dans l'espace et dans le temps des cycles de vie de deux populations de *Cylindroiulus nitidus* (Verhoeff) (Iulida). Revue d'Ecologie et de Biologie du Sol 19: 411–425.

David JF, Handa IT (2010) The ecology of saprophagous macroarthropods (millipedes, woodlice) in the context of global change. Biological Reviews 85: 881–895. doi: 10.1111/j.1469-185X.2010.00138.x

David JF, Vannier G (2001) Changes in desiccation resistance during development in the millipede *Polydesmus angustus*. Physiological Entomology 26: 135–141. doi: 10.1046/j.1365-3032.2001.00226.x

Enghoff H, Dohle W, Blower JG (1993) Anamorphosis in millipedes (Diplopoda) — the present state of knowledge with some developmental and phylogenetic considerations. Zoological Journal of the Linnean Society 109: 103–234. doi: 10.1111/j.1096-3642.1993.tb00305.x

Fairhurst C (1974) The adaptive significance of variations in the life cycles of Schizophylline millipedes. Symposia of the Zoological Society of London 32: 575–587.

Halkka R (1958) Life-history of *Schizophyllum sabulosum* (L.) (Diplopoda, Iulidae). Annales Zoologici Societatis Zoologicae-Botanicae Fennicae Vanamo 19: 1–71.

IPCC (2013) Climate Change 2013: The Physical Science Basis. Cambridge University Press, Cambridge, 1535 pp.

Meyer E (1985) Distribution, activity, life-history and standing crop of Julidae (Diplopoda, Myriapoda) in the Central High Alps (Tyrol, Austria). Holarctic Ecology 8: 141–150. doi: 10.1111/j.1600-0587.1985.tb01164.x

Parmesan C (2006) Ecological and evolutionary responses to recent climate change. Annual Review of Ecology Evolution and Systematics 37: 637–669. doi: 10.1146/annurev.ecolsys.37.091305.110100

Read HJ (1985) Stadial distributions of *Ommatoiulus moreleti* at different altitudes in Madeira with reference to life history phenomena (Diplopoda, Julidae). Bijdragen tot de Dierkunde 55: 177–180.

Sahli F (1968) Observations sur la biologie et la périodomorphose chez le Diplopode *Schizophyllum sabulosum* (L.) en Allemagne. Bulletin Scientifique de Bourgogne 25: 333–346.

Sahli F (1969) Contribution à l'étude du développement post-embryonnaire des Diplopodes Iulides. Annales Universitatis Saraviensis, Mathematisch-Naturwissenschaftliche Fakultät 7: 1–154.

Sahli F (1991a) Recherches sur le cycle des mâles d'*Ommatoiulus sabulosus* (L.) (Myriapoda, Diplopoda, Julidae) dans les Alpes-Maritimes et en Provence. Bulletin Scientifique de Bourgogne 44: 33–39.

Sahli F (1991b) Fréquence de la périodomorphose chez le Diplopode Julide *Ommatoiulus sabulosus* (L.) dans les Alpes-Maritimes et en Provence II. Passages males intercalaires-males adultes. Bulletin Scientifique de Bourgogne 44: 41–47.

Sahli F (1992) On male reproduction strategies in *Ommatoiulus sabulosus* (L.) in the Maritime Alps and Provence (France): juvenile to adult maturation moults. Berichte des Naturwissenschaftlich-Medizinischen Vereins in Innsbruck Supplementum 10: 167–176.

ZooKeys 510: 125–139 (2015)
doi: 10.3897/zookeys.510.8763
http://zookeys.pensoft.net

RESEARCH ARTICLE

A peer-reviewed open-access journal
ZooKeys
Launched to accelerate biodiversity research

Centipede (Chilopoda) richness and diversity in the Bug River valley (Eastern Poland)

Małgorzata Leśniewska[1], Piotr Jastrzębski[2],
Marzena Stańska[3], Izabela Hajdamowicz[3]

1 *Department of General Zoology, Adam Mickiewicz University ul. Umultowska 89, 61-614 Poznań, Poland* **2** *"Natura" Ecology Research Laboratory Marek Wierzba, ul. Kubusia Puchatka 78, Żabokliki 08-110 Siedlce, Poland* **3** *Siedlce University of Natural Science and Humanities, Faculty of Natural Sciences, Department of Zoology, ul. B. Prusa 12, 08-110 Siedlce, Poland*

Corresponding author: *Małgorzata Leśniewska* (malgorzata.lesniewska@amu.edu.pl)

Academic editor: *Ivan H. Tuf* | Received 16 October 2014 | Accepted 20 May 2015 | Published 30 June 2015

http://zoobank.org/FEB80A12-80DE-406B-A270-8D70FCEC32AE

Citation: Leśniewska M, Jastrzębski P, Stańska M, Hajdamowicz I (2015) Centipede (Chilopoda) richness and diversity in the Bug River valley (Eastern Poland). In: Tuf IH, Tajovský K (Eds) Proceedings of the 16th International Congress of Myriapodology, Olomouc, Czech Republic. ZooKeys 510: 125–139. doi: 10.3897/zookeys.510.8763

Abstract

The main aim of the survey was to describe the diversity and richness of Chilopoda in the selected area of the Bug River valley. The study sites were located in two regions differing in the shape of the valley, the presence of thermophilous habitats and the size of riparian forests. Pitfall traps were used as a sampling method. As a result, 444 specimens belonging to 12 centipede species of two orders – Geophilomorpha (four species) and Lithobiomorpha (eight species) were caught. *Lithobius* (*Monotarsobius*) *curtipes* C.L.Koch, 1847, *Pachymerium ferrugineum* (C.L.Koch, 1835), *Lamyctes* (*Lamyctes*) *emarginatus* (Newport, 1844) and *Lithobius* (*Monotarsobius*) *dudichi* Loksa, 1947 were the most common and the most numerous species. Of particular note is *L. dudichi* found in Poland for the first time and previously known based on a single specimen. Two to 10 Chilopoda species were found in each habitat under investigation. The greatest species richness was found in thermophilous thickets (10 species), sandy grasslands (eight), xerothermic grasslands (eight) and mesic meadows (six). The fewest number of species (two) was found in rushes at oxbows and in wet meadows. We found differences in the species composition and the number of Chilopoda between the lower (102 specimens, six species) and the middle (324 specimens, 11 species) section of the river valley. Our results confirm the need to protect xerothermic habitats, unique almost throughout entire Central Europe, which due to their distribution and their small area covered are fairly easily subject to the process of destruction.

Keywords

Big river valley, centipedes, species richness, habitat preferences

Introduction

Habitats in valleys of European rivers are relatively poorly known in terms of species diversity, habitat selection and the dynamics of Chilopoda communities. The very few studies in this field include, for instance, Zerm (1997a,b, 1999). In Poland, such studies have not been conducted so far.

Centipedes from river valleys have been studied mainly in the context of changes in the communities as a result of seasonal flooding (Zulka 1991, Pižl and Tajovský 1998, Tajovský 1999, Tuf 2000, 2003, Tufová and Tuf 2005, Marx et al. 2009), and in the context of life strategies enabling survival in periodically flooded habitats (Adis et al. 1996, Adis and Junk 2002). Xerothermic environments (often naturally occurring in the river valleys) have rarely been the subject of research on Chilopoda (Voigtländer 2003).

River valleys, especially the natural ones, only slightly changed – unregulated, are extremely valuable areas with habitats found more and more rarely, which already start to disappear across the continent. Studies on these habitats provides an opportunity not only to learn about the biodiversity but also to develop appropriate management and protection schemes. The Bug River is one of the few rivers of such a size in Europe, which still remain almost unregulated (Dombrowski et al. 2002). Its length in Poland amounts to 587 km.

The aim of this study is to describe the diversity and richness of Chilopoda in the selected area of the Bug valley through:

- determination of the species composition and structure of Chilopoda communities in different habitats of the Bug valley;
- distinction of the most numerous species in a given habitat and those that are most flexible in many environments;
- determination of the habitat preferences of centipede species in the studied areas.

Materials and methods

Research in the Bug river valley was conducted since March to November in 2007 and 2008.

Study area

The study sites were located in two regions differing in the shape of the valley, the presence of thermophilous habitats and the size of riparian forests (Figure 1):

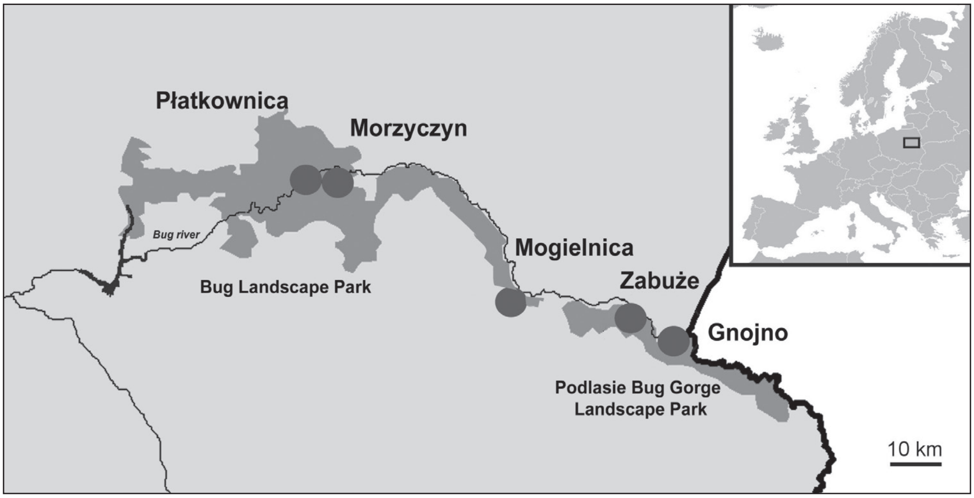


Figure 1. Location of the study area in Poland. Study area. Morzyczyn, Płatkownica – lower section of the Bug valley: Mogielnica, Zabuże, Gnojno – central section of the Bug valley.

(1) In the middle section of the river, as it meanders and cuts through glacial uplands at the depth of 30 m creating a unique landscape in Europe, where the characteristic feature is the presence of thermophilous habitats and well-preserved riparian forests (near localities: Gnojno, Zabuże in the protected area 'Podlasie Bug Gorge Landscape Park', and near locality Mogielnica in the protected area 'Bug Landscape Park');

(2) In the lower course of the river, where the valley is much wider (it stretches up to several kilometers wide) with an overgrown flood terrace at its bottom. In this section, the Bug river slowly meanders and sometimes changes its course (near localities: Morzyczyn, Płatkownica, in the protected area 'Bug Landscape Park').

In the Bug valley we can come across habitats that vary in terms of moisture content and structure – two important parameters from the point of view of Chilopoda biology. On the side of the river – from flooded and very humid habitats through medium moist ones to grasslands. Riparian forests within the floodplain are closest to the river, then there are meadows of lower flooded terrace – submerged during river floods. Mesic meadows are found in higher terraces, while slopes of terraces feature xerothermic grasslands, and thermophilous thickets. Sandy grasslands and rushes at oxbows are located in the mosaic of meadows, sometimes closer to the river bed, and sometimes closer to the edge of the valley (Głowacki et al. 2002). In terms of the structure, according to the classification by Voigtländer (2005) one can distinguish high (riparian forests), middle (thermophilous thickets, rushes at oxbows) and low (meadows, grasslands) vegetation cover.

Below, the data about the habitat (along with an abbreviation used throughout the study), coordinates, phytocoenosis, location, substrate, at every site are given.

The coordinates of the sites were determined using a GPS device by Garmin.

Sandy grassland (sgr):

1) Gnojno, sandy grassland; 52°28'13"N, 23°13'64"E; *Diantho-Armerietum*; raised, flat flooded terrace, several meters from the clearly visible edge of the meadow terrace; desiccated river alluvial soils;
2) Morzyczyn, sandy grassland; 52°68'43"N, 21°91'99"E; *Sclerantho-Herniarietum glabre*; the community inhabits the upper flooded terrace in the vicinity of the Bug riverbed; desiccated river alluvial soils;
3) Płatkownica, sandy grassland; 52°69'02"N, 21°84'53"E; *Diantho-Armerietum*; the phytocenosis occupies the raised, flat flooded terrace at the foot of the southern slopes of the flood embankment, within the base of the Bug valley cut off from the inundation area; desiccated river alluvial soils.

Xerothermic grassland (xgr):

4) Gnojno, xerothermic grassland; 52°27'59"N, 23°13'88"E; impoverished form of the *Adonido-Brachypodietum arrhenatheretosum*; slope of the upper terrace, southern exposure and inclination of approx. 30°; proper parendzina;
5) Mogielnica, xerothermic grassland; 52°40'08"N, 22°57'04"E; *Adonido-Brachypodietum*; slope of the upper terrace, eastern exposure and inclination of approx. 30°; proper pararendzina;
6) Morzyczyn, xerothermic grassland; 52°68'43"N, 21°91'50"E; *Tunico-Poetum compresse*; the analyzed patch of the phytocoenosis evolved in an anthropogenic habitat, with a slightly alkaline pH. It is the southern slope of the flood embankment with an inclination of about 30°; anthropogenic pararendzinas;
7) Płatkownica, xerothermic grassland; 52°69'00"N, 21°84'43"E; grassland with *Carex praecox* of the *Agropyretea intermedio-repentis* class; southern slope of the flood embankment with an inclination of about 35 degrees; anthropogenic pararendzinas.

Rushes at oxbows (rux):

8) Gnojno, rushes at oxbows; 52°28'20"N, 23°13'64"E; *Phalaridetum arundinaceae*; periphery of the Bug river, between club-rushes – on the water side – and flooded grasslands (*Agropyro-Rumicion*) on the mainland side; alluvial soils;
9) Mogielnica, rushes at oxbows; 52°40'06"N, 22°57'40"E; *Glycerietum maximae*; in the final, very shallow stretch of the Bug river, surrounded by tall herb communities and meadow communities on the land side; alluvial soils;
10) Morzyczyn, rushes at oxbows; 52°68'17"N, 21°91'24"E; *Glycerietum maximae*; located in a very shallow, bank stretch of the Bug river, surrounded by meadow communities on the land side and by *Caricetum gracilis* reed on the water surface side; alluvial soils;
11) Płatkownica, rushes at oxbows; 52°69'21"N, 21°84'54"E; *Glycerietum maximae*; located on the edge of the shallow old riverbed of the Bug river; alluvial soils.

Thermophilous thickets (tht):

12) Gnojno, thermophilous thickets; 52°28'19"N, 23°13'47"E; *Rhamno-Cornetum sanguinei*; on the edge of the Bug valley. The phytocoenosis habitat is a moraine slope with eastern exposure and inclination of approx. 30°; leached brown soils;

13) Mogielnica, thermophilous thickets; 52°40'02"N, 22°57'08"E; *Rhamno-Cornetum sanguinei*; on the edge of the Bug valley. The phytocoenosis habitat is a moraine slope with eastern exposure and inclination of approx. 30°; brown soils.

Mesic meadow (mm):

14) Zabuże, mesic meadow; 52°33'32"N, 23°00'00"E; *Poo-Festucetum*; raised upper flooded terraces of the Bug valley; river alluvial soils;
15) Mogielnica, mesic meadow; 52°40'08"N, 22°57'22"E; *Poo-Festucetum*; raised upper flooded terrace of the Bug valley; river alluvial soils;
16) Morzyczyn, mesic meadow; 52°68'51"N, 21°91'54"E; *Poo-Festucetum*; raised upper flooded terraces of the Bug valley; river alluvial soils;
17) Płatkownica, mesic meadow; 52°69'06"N, 21°84'53"E; *Poo-Festucetum*; raised upper flooded terraces of the Bug valley; river alluvial soils.

Riparian forest (rfo):

18) Zabuże, riparian forest; 52°33'33"N, 23°00'86"E; *Salicetum albae-fragilis*; N slope of the low flooded terrace in the patch of the riparian willow; alluvial processes accumulate coarse-grained material of sand and river sediments. During periods of low water the initial alluvial soil may undergo significant desiccation;
19) Mogielnica, riparian forest; 52°40'32"N, 22°57'21"E; *Salicetum albae-fragilis*; the flooded terrace; proper alluvial soil;
20) Morzyczyn, riparian forest; 52°69'05"N, 21°91'36"E; *Salicetum albae-fragilis*; slightly elevated riverbed bank, within the flooded terrace; initial alluvial soil;
21) Płatkownica, riparian forest; 52°69'19"N, 21°84'06"E; *Salicetum albae-fragilis*; flooded terrace; proper alluvial soils.

Wet meadow (wm):

22) Zabuże, wet meadow; 52°33'23"N, 22°99'95"E; *Violo-Cnidietum*; upper flooded terrace of the Bug valley, submerged regularly during the floods of the river; river alluvial soils;
23) Morzyczyn, wet meadow; 52°68'84"N, 21°91'61"E; a meadow with *Carex praecox* and *Poa angustifolia* (formed from the disturbed *Cnidion* meadow); lower flooded terrace, currently submerged only occasionally during high floods of the river; desiccated river alluvial soils;
24) Płatkownica, wet meadow; 52°69'16"N, 21°84'42"E; *Violo-Cnidietum*; upper flooded terrace of the Bug valley, submerged regularly during the floods of the river; river alluvial soils.

Pitfall traps were used as a sampling method. An aqueous solution of propylene glycol (about 50%), containing a few drops of a detergent per 1 liter to reduce the surface tension of the fluid, was used as a preservation liquid. In each of the sites ten pitfall traps were placed in one straight line, at a distance of two meters one from another. The beginning of the trapping period was in the middle of March and the end was in the middle of November. The traps were replaced every two weeks.

The material analyzed in the current work was obtained during studies related to different groups of arthropods – including primarily spiders, carabids, diplopods and butterflies – under the project titled "The diversity of habitats and the biological diversity of selected groups of Arthropoda in the Bug valley" (Oleszczuk et al. 2011, Jastrzębski 2012, Hajdamowicz et al. 2014). Since Chilopoda were not taken into account during the planning of the study, the specificity of this group of animals was not accounted for in the applied methodology.

In this work, standard methods and analysis indicators were applied: The Shannon-Weaver diversity index (H), Pielou's measure of species evenness (J), Morisita index values as modified by Horn, the cluster analysis – distance/similarity measure Bray and Curtis; cluster method: nearest neighbor.

Results

Species

444 specimens belonging to 12 centipede species of two orders – Geophilomorpha (four species) and Lithobiomorpha (eight species) were caught (Table 1).

In the area under investigation, the following four centipede species were most numerous and most common:

1.) *Lithobius* (*Monotarsobius*) *curtipes* – present in six habitats, with the highest number in riparian forests (77% of specimens);
2.) *Pachymerium ferrugineum* – found in five habitats, most abundant in the mesic meadows (53% of specimens);
3.) *Lamyctes* (*Lamyctes*) *emarginatus* – caught in six habitats, most numerous in very wet habitats – in rushes at oxbows (33% of specimens), in the wet meadows (25%) and in riparian forests (20%);
4.) *Lithobius* (*Monotarsobius*) *dudichi* – present in five habitats, prevalent in sandy grasslands (35% of specimens) and in xerothermic grasslands (32%).

These species constitute 88% of all centipedes caught during the study, thus establishing themselves as the most typical ones of almost all habitats of the study area. It is only in thermophilous thickets that a species from outside this group of four dominates – *Lithobius* (*Lithobius*) *mutabilis* (Table 1).

Interspecies occurrence similarity (Figure 2) [the analysis does not include *Lithobius* (*Lithobius*) *tenebrosus*, as regrettably the information about the site on the label describing the specimen was completely obscured].

Three distinct groups can be identified in the dendrogram. The first group includes species associated with dry habitats, which are rarely found in the Bug valley (*S. nemorensis*, *G. proximus* and *L. forficatus* related to these species). The second cluster consists of species occurring mainly in thermophilous thickets (*L. melanops*, *L. erythrocephalus* and *S. acuminata*). *L. mutabilis* – which is dominant in thermophilous thickets, but also found in

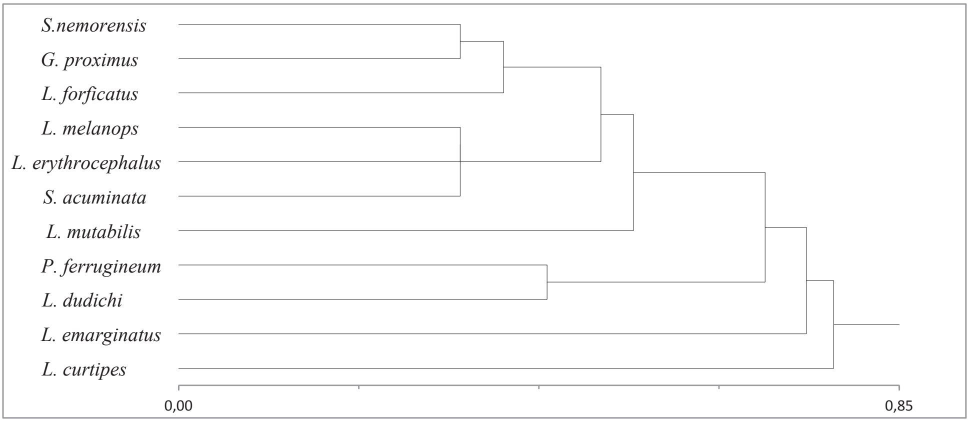


Figure 2. Similarity of species (distance/similarity measure Bray and Curtis; cluster method: nearest neighbor). Species nomenclature in Table 1.

sandy and xerothermic grasslands, is related to these two groups. The last cluster consists of numerous and frequently found species of *P. ferrugineum* and *L. dudichi*, which occur together in four habitats. In sandy grasslands, xerothermic grasslands and in thermophilous thickets their number is similar. The most distinctive species are *L. curtipes* – found most frequently in the Bug valley and reported from the great number of habitats, although clearly predominant in riparian forests and *L. emarginatus* – also caught in many habitats, although predominantly in wetlands and in regularly flooded areas.

In terms of ecology and zoogeography European eurytopic species prevail (Table 1).

Chilopoda were collected throughout all the months of the study, although one can observe certain tendencies in particular species – especially with regard to four, most frequent species. *L. curtipes* was active throughout all the months, though predominantly in October and November. *L. emarginatus* occurred from June to November, while it was most numerous in September. *P. ferrugineum* was reported from April until November, especially in May and June, but also in August and September. Finally, *L. dudichi* was most active in May and November.

Habitats

Two to ten Chilopoda species were found in each habitat under investigation. The greatest species richness was found in thermophilous thickets (ten species), sandy grasslands (eight species), xerothermic grasslands (eight species) and mesic meadows (six species). The fewest number of species was found in rushes of reed mannagrass (*Glyceria maxima*) and in wet meadows (two species at each location) (Table 1).

The greatest number of specimens was found in the following habitats: riparian forests, mesic meadows and xerothermic grasslands (Table1).

Table 1. List, the number (N), dominance (D%) of Chilopoda species found in the Bug valley; Values of the index of species diversity by Shannon-Weaver (H'), the highest (H max) value of the Shannon-Weaver index for individual habitats and values of Pielou's measure of species evenness (J); *– species new for Poland. Ecological and zoogeografical classification of species – Af – African, Austras – Australasian, e – eurytopic; E – European; f – forest; H – Holarctic; i – introduced; NA – North American; Naf – North African; s – synanthropic; WP – West Palearctic; ? – unknown.

	Species/habitats	**Ecol. and zoog. classif.**	**thermophilous thickets (tht)**		**sandy grassland (sgr)**		**xerothermic grass-land (xgr)**		**mesic meadow (mm)**	
			N	**D**	**N**	**D**	**N**	**D**	**N**	**D**
1	*Lamyctes* (*Lamyctes*) *emarginatus* (Newport, 1844)	e, Austras, E, Am, Af			1	2.4	1	1.6	15	17.6
2	*Lithobius* (*Monotarsobius*) *curtipes* C.L.Koch,1847	f, P	4	9.8	1	2.4	11	18	5	5.9
3	*Lithobius* (*Monotarsobius*) *dudichi* Loksa, 1947 *	?	5	12.2	22	52.4	20	32.8	9	10.6
4	*Lithobius* (*Lithobius*) *erythrocephalus erythrocephalus* C.L.Koch,1847	e, E	1	2.4	1	2.4				
5	*Lithobius* (*Lithobius*) *forficatus forficatus* (Linnaeus, 1758)	e, WP	4	9.8	1	2.4	1	1.6		
6	*Lithobius* (*Lithobius*) *melanops melanops* Newport, 1845	e, s, E	1	2.4						
7	*Lithobius* (*Lithobius*) *mutabilis mutabilis* L.Koch, 1862	f, E	17	41.5	1	2.4	2	3.3		
8	*Lithobius* (*Lithobius*) *tenebrosus tenebrosus* Meinert, 1872	f, E								
	Lithobius species		5	-	1	-	5	-	1	-
9	*Geophilus proximus* C.L.Koch,1847	e, E, iNA	2	4.9	1	2.4	1	1.6	3	3.5
10	*Pachymerium ferrugineum* (C.L. Koch, 1835)	e, H	1	2.4	14	33.3	22	36.1	49	57.6
11	*Schendyla nemorensis* (C.L. Koch, 1837)	e, s, E, Naf, iNA	4	9.8			3	4.9	4	4.7
12	*Strigamia acuminata* (Leach, 1815)	f, E, iNA	2	4.9						
	number of specimens		46		43		66		86	
	number of species		10		8		8		6	
	number of sites		2		3		4		4	
	H'		0.81		0.54		0.65		0.56	
	H max		1.0		0.90		0.90		0.78	
	J		81.2		59.6		72.4		72.0	

Table 1. Continued.

	Species/habitats	riparian forest (rfo)		rushes at oxbows (rux)		wet meadow (wm)		no data	total		number of habitats
		N	**D**	**N**	**D**	**N**	**D**	**N**	**N**	**D**	
1	*Lamyctes* (*Lamyctes*) *emarginatus* (Newport, 1844)	12	8.6	20	95.2	11	84.6	1	61	14.3	6
2	*Lithobius* (*Monotarsobius*) *curtipes* C.L.Koch,1847	122	87.8	1	4.8			14	158	37.0	6
3	*Lithobius* (*Monotarsobius*) *dudichi* Loksa, 1947 *	5	3.6					2	63	14.8	5
4	*Lithobius* (*Lithobius*) *erythrocephalus erythrocephalus* C.L.Koch,1847								2	0.5	2
5	*Lithobius* (*Lithobius*) *forficatus forficatus* (Linnaeus, 1758)							1	7	1.6	3
6	*Lithobius* (*Lithobius*) *melanops melanops* Newport, 1845								1	0.2	1
7	*Lithobius* (*Lithobius*) *mutabilis mutabilis* L.Koch, 1862								20	4.7	3
8	*Lithobius* (*Lithobius*) *tenebrosus tenebrosus* Meinert, 1872							1	1	0.2	
	Lithobius species	5	-						17	-	
9	*Geophilus proximus* C.L.Koch,1847								7	1.6	4
10	*Pachymerium ferrugineum* (C.L. Koch, 1835)					2	15.4	5	93	21.8	5
11	*Schendyla nemorensis* (C.L. Koch, 1837)							1	12	2.8	3
12	*Strigamia acuminata* (Leach, 1815)								2	0.5	1
	number of specimens	144		21		13		25	444		7
	number of species	3		2		2			12		
	number of sites	4		4		3					
	H’	0.19		0.08		0.19					
	H max	0.48		0.30		0.30					
	J	40.6		27.6		61.9					

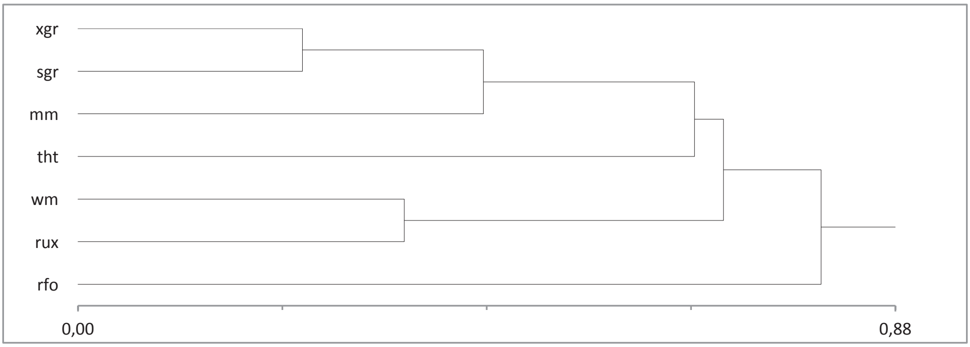


Figure 3. Dendrogram of the similarities of centipede composition in different habitats (distance/similarity measure – Bray and Curtis, cluster method –nearest neighbor) For designations see Table 1.

Table 2. Similarity of dominance structures – Morisita index values as modified by Horn. For designations see Table 1.

tht	1						
sgr	0.29	1					
xgr	0.35	0.90	1				
rfo	0.18	0.07	0.33	1			
rux	0.01	0.04	0.04	0.15	1		
wm	0.01	0.13	0.14	0.10	0.98	1	
mm	0.13	0.66	0.79	0.12	0.26	0.42	1
	tht	sgr	xgr	rfo	rux	wm	mm

In the majority of habitats one species was overwhelmingly dominant (from 41.5% to 95.2%). In rushes at oxbows and in the wet meadows it was *L. emarginatus*, in riparian forests *L. curtipes*, in thermophilous thickets *L. mutabilis*, in sandy grasslands *L. dudichi*, and in the mesic meadows *P. ferrugineum*. It was only in xerothermic grasslands that two species co-dominated (*L. dudichi* and *P. ferrugineum*) (Table 1).

The Shannon-Weaver diversity index (H) and Pielou's measure of species evenness (J) reached their highest values in thermophilous thickets, xerothermic grasslands, mesic meadows, while the lowest values – in rushes at oxbows and riparian forests (Table 1).

The cluster analysis conducted on the basis of the species composition and dominance structure demonstrated the greatest similarity between communities of warm and dry habitats on the one hand, and wet and flooded – on the other (Figure 3, Table 2).

We note differences in the species composition and the number of Chilopoda between the lower (102 specimens from 6 species) and the middle section of the river valley (324 specimens from 11 species). This result reflects the differences in the structure and the vegetation of the two regions of the Bug valley – especially the presence of thermophilous habitats in the middle section of the valley.

Discussion

Although several studies from European river valley areas have been conducted, this habitat is still poorly explored in terms of Chilopoda. Meanwhile natural and seminatural habitats associated with valleys of big rivers are already disappearing throughout the continent (Głowacki et al. 2002).

This study is based on the materials obtained in studies on groups of arthropods other than Chilopoda. This should explain the applied methodology, which does not take into account the specificity of Chilopoda. This undoubtedly affected the results. Pitfall traps limited the set of species to active epigeic forms. The expansion of the method to include quantitative soil samples and direct qualitative capture would contribute to a more complete picture of this group of Chilopoda.

The data about the preferences of the majority of species with regard to the habitats was confirmed in the investigated area. The wettest and flooded habitats feature the lowest number of Chilopoda species. This is understandable, as the colonization of periodically flooded habitats requires special adaptations from animals in morphology, physiology or life cycle (Adis and Junk 2002, Zulka 1999, Voigtländer 2011). The species that definitely prevails in rushes at oxbows is the introduced *L. emarginatus* – a parthenogenetic species, commonly found not only in floodplains, but also in disturbed upland areas (Eason 1964, Dunger and Voigtländer 1990, Zulka 1991, Adis and Junk 2002, Leśniewska 2004). It is known as a one-year 'autumn species' (Barber and Keay 1988, Zulka 1991). *L. emarginatus* has a preference for humid and very wet habitats with low vegetation cover (Voigtländer 2005). In this species flood resistance of inactive stages (eggs) was found (Zulka 1991). Eggs survive inundation between winter and early spring in dormancy. Immatures hatch shortly thereafter and reach maturity already 6–12 weeks later, in contrast to most lithobiomorph species which need a few years until they are mature. *L. emarginatus* actively dives, walks under water and hunts for prey near the water surface (Adis and Junk 2002).

As noted by Zulka (1999) and Voigtländer (2011), the one-year life cycle of *L. emarginatus* is a strategy that allows this species to populate the same habitats as *L. curtipes* – a species of a similar body size and probably very similar ecological requirements. Adult specimens of the annual species *L. emarginatus* appear in the environment in the summer and fall, when there is enough food to suffice for perennial species, such as *L. curtipes*.

The riparian forest habitat is dominated by *L. curtipes* – a species that prefers wet and humid habitats with high vegetation cover. This species, was found alive after 34 days of inundation (Adis and Junk 2002). In Central Europe it inhabits primarily riparian forests, alder swamp forests, river and brook sides, wet meadows with flooding and more rarely humid deciduous forests (Voigtländer 2005). In the investigated area a small number of the specimens of this species was also collected from dry and xerothermic habitats (Table 1). It can be assumed that these specimens have only immigrated from surrounding habitats, and they do not form stable populations in these habitats.

In the Bug valley greater species diversity is found in sandy and xerothermic grasslands, where *P. ferrugineum* and *L. dudichi* prevail. *P. ferrugineum* is one of the most widely spread Chilopoda species. It is found throughout the entire Holarctic. This species appears to be less sensitive to changes in moisture and temperature than other species of Chilopoda and it is often found in the littoral zone, and also in open environments – in meadows, grasslands, cultivated fields, on open rocks etc. (Palmén and Rantala 1954, Eason 1964, Barber 2009). Suomalainen (1939) reports that *P. ferrugineum* can survive under water up to 178 days, which explains its presence on numerous islands, for example in the Faroe Is., Azores, Madeira, the Canary Is. In the Bug valley *P. ferrugineum* is mainly found in habitats with a low vegetation cover and featuring various levels of moisture – both in flooded terraces and grassland slopes, which confirms the preferences of the species reported from other areas.

Most species in the Bug river valley inhabit thermophilous thickets – an environment that is already similar to forest habitats. This is clearly manifested by the composition of Chilopoda community, in which we can find typically forest-dwellers – such as *L. mutabilis* or *S. acuminata*.

As one of the most important results obtained in the present study is the reported high number of specimens of *L. dudichi*, the species new to the Polish fauna. This species has so far been known to science only on the basis of one reported specimen (Loksa 1947, Matic 1966). This finding shows that river valley habitats are still poorly investigated and one should pay more attention to them in order to gain more comprehensive knowledge about the diversity of Chilopoda. Loksa (1947) reports *L. dudichi* from one site in Romania. The fact that these two sites are so far away from each other may suggest that the range of the species is probably very large. This may be the Eastern European or the Pontic range. The Bug river gorge is the gateway for migratory species from the widely interpreted East-Ukrainian and Russian steppes and the Pontic region (along the eastern Carpathian belt and further north). These migrations have enriched Polish fauna in south-eastern species: for exemple xerothermophilous weevils (Wanat and Gosik 2003), thermophilous and higrophilous spiders (Hajdamowicz and Jastrzębski 2007, Oleszczuk et al. 2011, Hajdamowicz et al. 2014) and diplopods (Jastrzębski 2012).

Our results confirm the need to protect xerothermic habitats, unique almost throughout entire Central Europe, which due to their dispersion and their small area covered are fairly easily subject to the process of destruction. These environments are refuges for rare species of animals – including centipedes, as our research shows. *Lithobius dudichi* presumably belongs to the relict xerothermic species of steppe provenance and is presumably in danger of extinction.

The results from our research in the Bug Valley also show that centipedes are a valuable indicator group for the assessment of habitat conditions. The information about the species composition of Chilopoda communities, the dominance structure and their dynamics may thus be useful in characterizing specific location types.

Acknowledgements

We wish to extend our gratitude to Karin Voigtländer and Ivan Tuf for the extremely valuable comments to the first version of the manuscript.

M. Stańska and I. Hajdamowicz were supported by grant of Siedlce University of Sciences and Humanities 222/05/S.

References

Adis J, Minelli A, Morais JW de, Pereira LA, Barbieri F, Rodrigues JMG (1996) On abundance and phenology of Geophilomorpha (Chilopoda) from central Amazonian upland forests. Ecotropica 2: 165–175.

Adis J, Junk WJ (2002) Terrestrial invertebrates inhabiting lowland river floodplains of Central Amazonia and Central Europe: a review. Freshwater Biology 47: 711–731. doi: 10.1046/j.1365-2427.2002.00892.x

Barber AD (2009) Centipedes. Keys and Notes for the Identification of the Species. Field Studies Council Shrewsbury, 228 pp.

Barber AD, Keay AN (1988) Provisional atlas of the centipedes of the British Isles. Biological Records Center, Huntington, 127 pp.

Dombrowski A, Głowacki Z, Jakubowski W, Kovalchuk I, Michalczyk Z, Nikiforov M, Szwajgier W, Wojciechowski KH (Eds) (2002) Bug River Valley as the Ecological Corridor. State – Threats – Protection. Foundation IUCN Poland, Warsaw, 145 pp.

Dunger W, Voigtländer K (1990) Succession of Myriapoda in primary colonization of reclaimed land. In: Minelli A (Ed.) Proceedings of the 7th International Congress of Myriapodology, Leiden, 219–227.

Eason EH (1964) Centipedes of the British Isles. London-Warne, 294 pp.

Głowacki Z, Marciniuk P, Wierzba M (2002) Szata roślinna doliny Bugu w Polsce – odcinek dolny. In: Dombrowski A, Głowacki Z, Jakubowski W, Kovalchuk I, Michalczyk Z, Nikiforov M, Szwajgier W, Wojciechowski KH (Eds) Korytarz ekologiczny doliny Bugu. Stan – Zagrożenia – Ochrona. Fundacja IUCN Poland, Warszawa, 122–141.

Hajdamowicz I, Jastrzębski P (2007) Threats to a rare European spider species, *Tetragnathare imoseri* (Araneae: Tetragnathidae), and fishponds as an alternate habitat. Nature Conservation 64: 31–37.

Hajdamowicz I, Stańska M, Rutkowski T (2014) *Walckenaeria incisa* (O.P.- Cambridge) – a rare European species, new to Poland (Araneae: Linyphiidae). Genus 25: 357–363.

Jastrzębski P (2012) *Brachyiulus jawlowskii* Lohmander, 1928, a millipede species new to the fauna of Poland (Myriapoda: Diplopoda). Fragmenta Faunistica 55(1): 1–3. doi: 10.3161/00159301FF2012.55.1.001

Leśniewska M (2004) *Lamyctes emarginatus* (Newport, 1844) (Chilopoda: Lithobiomorpha) w Wielkopolsce. Badania Fizjograficzne nad Polską Zachodnią, Seria C – zoologia 50: 45–51.

Loksa I (1947) Beiträge zur Kenntnis der Steinläufer – Lithobiiden-Fauna des Karpatenbeckens. I. Fragmenta Faunistica Hungarica 10: 73–85.

Marx MT, Guhmann P, Lessel T, Decker P, Eisenbeis G (2009) Die Anpassungen verschiedener Arthropoden (Araneae [Webspinnen]; Coleoptera: Carabidae [Laufkäfer]; Collembola [Springschwänze]; Diplopoda und Chilopoda [Tausend- und Hundertfüßer]) an Trockenheit und Überflutung. – Mitt. Pollichia 94: 139–160.

Matic Z (1966) Fauna Republicii Socialiste Romania. Clasa Chilopoda Subclasa Anamorpha. Academia Republicii Socialiste România, Bucureşti 6(1): 1–266.

Oleszczuk M, Hajdamowicz I, Stańska M (2011) The distribution and habitat preferences of an extremely rare European spider, *Glyphesis taoplesius* (Araneae: Linyphiidae). Entomologica Fennica 22: 15–20.

Palmén E, Rantala M (1954) On the life-history and ecology of *Pachymerium ferrugineum* (C.L.Koch) (Chilopoda, Geophilidae). Annals Zoologici Societatis Zoologicae Botanicae Fennicae 'Vanamo' 16, 3: 44.

Pižl V, Tajovský K (1998) Impact of summer flood on soil macrofauna on floodplain forest in Litovelské Pomoravi. In: Krajina, voda, povodeň. Správa CHKO ČR, Prague, 47–54.

Suomalainen P (1939) Zur Verbreitungsökologie von *Pachymerium ferrugineum* C. Koch in finnischen Schärenhof. Annals Zoologici Societatis Zoologicae Botanicae Fennicae 'Vanamo' 7(4): 10–14.

Tajovský K (1999) Impact of inundations on terrestrial arthropod assemblages in southern Moravian floodplain forests, The Czech Republic. Ekológia 18 (Supplement): 177–184.

Tuf IH (2000) Communities of centipedes (Chilopoda) in three floodplain forests of various age in Litovelské Pomoraví (Czech Republic) In: Wytwer J, Golovatch S (Eds) Progress in studies on Myriapoda and Onychophora. Fragmenta Faunistica Supplement 43: 327–332.

Tuf IH (2003) Four-year development of a centipede (Chilopoda) community after a summer flood. African Invertebrates 44(1): 265–276.

Tufová J, Tuf IH (2005) Survival under water – comparative study of millipedes (Diplopoda), centipedes (Chilopoda) and terrestrial isopods (Oniscidea). In: Tajovský K, Schlaghamerský J, Pižl V (Eds) Contributions to Soil Zoology in Central Europe I. ISB AS CR, České Budějovice, 195–198.

Voigtländer K (2003) Species distribution and assemblages of centipedes (Chilopoda) in open xeric sites of Saxony-Anhalt (Germany). African Invertebrates 44(1): 283–291.

Voigtländer K (2005) Habitat preferences of selected Central European centipedes. Peckiana 4:163–179.

Voigtländer K (2011) Chilopoda – ecology. In: Minelli A (Ed.) The Myriapoda. Treatise on zoology – anatomy, taxonomy, biology. Volume 1. Chilopoda. Brill: 309–325. doi: 10.1163/9789004188266_016

Wanat M, Gosik R (2003) Materiały do znajomości ryjkowców (Insecta, Coleoptera: Curculionoidea) doliny Bugu. Nowy Pamiętnik Fizjograficzny 2: 31–52.

Zerm M (1997a) Distribution and phenology of *Lamyctes fulvicornis* and other lithobiomorph centipedes in the floodplain of the Lower Oder Valley, Germany (Chilopoda, Henicopidae, Lithobiidae). In: Enghoff H (Ed.) A collection of papers on Myriapoda and Onychophora. Entomologica scandinavica, Supplement 51: 125–132.

Zerm M (1997b) Die Fauna der Tausend-, Hundert- und Zwergfüsser (Myriapoda: Diplopoda, Chilopoda, Symphyla) sowie der Landasseln (Isopoda: Oniscidea) im Unteren Odertal, unter besonderer Berücksichtigung des Standortfaktors Überschwemmung. Zoologische Beiträge 38: 97–134.

Zerm M (1999) Vorkommen und Verteilung von Tausendfüßern, Hundertfüßern, Zwergfüßern (Myriapoda: Diplopoda, Chilopoda, Symphyla) und Landasseln (Isopoda: Oniscidea) in den Auen des Unteren Odertals. In: Dohle W, Bornkamm R, Weigmann G (Hrsg.) Das Untere Odertal. E. Schweizerbart'sche Verlagsbuchhandlung (Nägele u. Obermiller) Limnologie aktuell, Band/Vol. 9. Stuttgart, 197–210.

Zulka KP (1991) Überflutung als ökologischer Faktor: Verteilung, Phänologie und Anpassungen der Diplopoda, Lithobiomorpha und Isopoda in den Flußauen der March. PhD thesis, University of Wien, Wien, Austria.

Zulka KP (1999) Terrestriche Arthropoden. In: Kelemen J, Oberleitner I (Eds) Fließende Grenzen. Lebensraum March Thaya-Auen. Umweltbundesamt, Wien, 259–271.

ZooKeys 510: 141–161 (2015)
doi: 10.3897/zookeys.510.8855
http://zookeys.pensoft.net

RESEARCH ARTICLE

A peer-reviewed open-access journal
ZooKeys
Launched to accelerate biodiversity research

Distribution of millipedes along an altitudinal gradient in the south of Lake Teletskoye, Altai Mts, Russia (Diplopoda)

Julia S. Nefedieva[1], Pavel S. Nefediev[2],
Miroslava B. Sakhnevich[3], Yuri V. Dyachkov[2]

1 *Barnaul Branch of OJSC "GIPRODORNII", Papanintsev street 105, Barnaul, 656000, Russia* **2** *Department of Ecology, Biochemistry and Biotechnology, Altai State University, Lenina avenue 61, Barnaul,656049, Russia* **3** *Altai State Nature Biosphere Reserve, Naberezhnyi lane 1, Gorno-Altaisk, 649000, Russia*

Corresponding author: *Julia S. Nefedieva* (j.nefedieva@mail.ru)

Academic editor: *Ivan H. Tuf* | Received 28 October 2014 | Accepted 26 May 2015 | Published 30 June 2015

http://zoobank.org/9A4EB171-797E-415A-8872-0F0182099AFA

Citation: Nefedieva JS, Nefediev PS, Sakhnevich MB, Dyachkov YV (2015) Distribution of millipedes along an altitudinal gradient in the south of Lake Teletskoye, Altai Mts, Russia (Diplopoda). In: Tuf IH, Tajovský K (Eds) Proceedings of the 16th International Congress of Myriapodology, Olomouc, Czech Republic. ZooKeys 510: 141–161. doi: 10.3897/zookeys.510.8855

Abstract

The distribution of millipedes along an altitudinal gradient in the south of Lake Teletskoye, Altai, Russia based on new samples from the Kyga Profile sites, as well as on partly published and freshly revised material (Mikhaljova et al. 2007, 2008, 2014, Nefedieva and Nefediev 2008, Nefediev and Nefedieva 2013, Nefedieva et al. 2014), is established. The millipede diversity is estimated to be at least 15 species and subspecies from 10 genera, 6 families and three orders. The bulk of species diversity is confined both to low- and mid-mountain chern taiga forests and high-mountain shrub tundras, whereas the highest numbers, reaching up to 130 ind./m^2, is shown in subalpine *Pinus sibirica* sparse growths. Based on clustering studied localities on species diversity similarity two groups of sites are defined: low-mountain sites and subalpine sparse growths of *P. sibirica* ones.

Keywords

Millipedes, Diplopoda, altitudinal gradient, Lake Teletskoye, Altai, Siberia

Introduction

This paper continues ecological researches on the Altai millipede fauna in the south of Lake Teletskoye, Russian Altai (Nefedieva and Nefediev 2008, Nefediev and Nefedieva 2013). Some faunistic records of *Sibiriulus altaicus* Gulička, 1972 and specimens of the family Diplomaragnidae from the study localities have been made earlier (Mikhaljova et al. 2007, 2008, 2014). A brief historical account of Altai millipede fauna research can be obtained from the publication of Mikhaljova et al. (2008) and Nefediev and Nefedieva (2013).

Since 1998 the Altai State Nature Biosphere Reserve and a buffer zone around Lake Teletskoye are inscribed as one of three separate areas of UNESCO World Natural Heritage Site under the name of «Golden Mountains of Altai». Lake Teletskoye, being the deepest and the largest body of freshwater in southwest Siberia, exerts a great warming influence on local climate, in its southern part especially. Situated at a height of 435 m above sea level, the lake lies between the mountain ridges of Altyntu and Korbu, and the Chulyshman river highlands in the south.

The aim of our present paper is to explore the distribution of millipedes along an altitudinal gradient in the south of the Teletskoye Lake in the Kyga Biogeocenosis Profile.

Material and methods

Material was collected by the first and the second authors of this article in August 2005 using hand sampling from the litter and standard technique of soil sampling (Ghilarov 1987): 8 soil samples per each studied numbered site, sample area ¼ m^2, depth 10 cm. The total amount of studied millipedes is 968 specimens.

The Kyga Biogeocenosis Profile was laid in 1959–1961 in the territory of watershed of the Kyga and Bayas rivers in the south of the Teletskoye Lake near the cordon of Chiri, Ulagan District, Republic of Altai, Siberia, Russia. The profile encompasses 22 numbered sites at different altitudes, and comprising relic *Pinus sibirica* forests from the Tertiary period. Its length is about 12 km, and its altitude ranges from 443 to 2267 m a.s.l. It is begun at the mouth of the Kyga river and ends on the top of the Malaya Koliushta mountain. The vertical vegetation zonation here is characterized by the presence of forest and high-mountain belts. There are widespread dark coniferous forests with *Betula pendula* and *Populus tremula*, also called as chern taiga, and sparse growths of *P. sibirica* in the former belt, whereas alpine meadows do not occur almost at all in the latter. Above the timberline at a height of 2100 m above sea level, all hilltops are occupied by shrub, moss-lichen and rocky tundras.

All sites we collected are listed and described below according to the following standard: site number (bold): GPS (WGS84) position, altitude, habitat, sampling date, sampling methods.

1: 51°20'47,3"N, 87°51'14,2"E, 443 m a.s.l., *Pinus sylvestris* and *Betula pendula* forest with *Larix sibirica*, *Abies sibirica* and *P. sibirica*, 12.08.2005, soil sampling.

2: 51°20'29,3"N, 87°51'40,0"E, 494 m a.s.l., *A. sibirica* and *P. sibirica* forest with ferns, 12.08.2005, soil sampling.

4: 51°19'53,3"N, 87°51'78,0"E, 675 m a.s.l., *A. sibirica* forest with *P. sibirica* and *B. pendula*, 18.08.2005, soil sampling.

5: 51°19'28,5"N, 87°52'4,8"E, 853 m a.s.l., *A. sibirica* forest with *P. sibirica*, *Populus tremula* and *B. pendula*, 18.08.2005, soil sampling and hand sampling.

6: 51°19'31,6"N, 87°52'16,1"E, 942 m a.s.l., *P. tremula* forest with *A. sibirica* and *P. sibirica*, 18.08.2005, soil sampling and hand sampling.

7: 51°19'31,2"N, 87°52'21,1"E, 960 m a.s.l., *A. sibirica*, *P. sibirica* and *P. tremula* forest, 18.08.2005, soil sampling and hand sampling.

8: 51°19'30,4"N, 87°52'50,0"E, 1100 m a.s.l., *P. sibirica* forest with *A. sibirica*, 17.08.2005, soil sampling.

8a: 51°19'23,6"N, 87°53'2,1"E, 1191 m a.s.l., *P. sibirica* forest with *A. sibirica*, 17.08.2005, soil sampling and hand sampling.

9: 51°19'07,5"N, 87°53'15,0"E, 1414 m a.s.l., *P. sibirica* forest with *A. sibirica*, 17.08.2005, soil sampling.

10: 51°18'58,5"N, 87°53'33,3"E, 1468 m a.s.l., *P. sibirica* forest with *A. sibirica*, 17.08.2005, soil sampling.

10a: 51°18'43,7"N, 87°54'23,7"E, 1699 m a.s.l., sparse growths of *P. sibirica* with *B. pendula* and *A. sibirica*, 16.08.2005, soil sampling.

11: 51°18'41,3"N, 87°55'34,7"E, 1736 m a.s.l., old fire-site, *B. rotundifolia* and *Salix glauca* bushes with *P. sibirica* and *A. sibirica* sparse growths, 16.08.2005, soil sampling.

12: 51°18'27,8"N, 87°54'57,4"E, 1847 m a.s.l., old fire-site, *B. rotundifolia* and *S. glauca* bushes with *P. sibirica* and *A. sibirica* sparse growths, 16.08.2005, soil sampling and hand sampling.

13: 51°18'09,4"N, 87°55'43"E, 1861 m a.s.l., subalpine *P. sibirica* forest, 14.08.2005, hand sampling.

13a: 51°18'24,1"N, 87°55'06,9"E, 1877 m a.s.l., subalpine sparse growths of *P. sibirica*, 16.08.2005, soil sampling and hand sampling.

14: 51°18'23,0"N, 87°55'22,1"E, 1903 m a.s.l., subalpine sparse growths of *P. sibirica*, 14.08.2005, soil sampling.

15: 51°18'24,5"N, 87°55'31,0"E, 1962 m a.s.l., subalpine sparse growths of *P. sibirica* with *A. sibirica*, 14.08.2005, soil sampling and hand sampling under stones.

16: 51°18'33,6"N, 87°55'32,9"E, 2028 m a.s.l., subgoltsy sparse growths of *P. sibirica* with *B. rotundifolia* and *S. glauca* bushes, 15.08.2005, soil sampling and hand sampling.

18: 51°18'30,0"N, 87°56'10,7"E, 2194 m a.s.l., *B. rotundifolia* and *S. glauca* mountain tundra with *Dryas* and lichens, 15.08.2005, soil sampling and hand sampling under stones.

19: 51°18'30,5"N, 87°56'21,7"E, 2267 m a.s.l., summit of Malaya Koliushta Mt., *B. rotundifolia* and *S. glauca* rocky mountain tundra with *Dryas*, *Festuca* and lichens, 15.08.2005, soil sampling and hand sampling under stones.

Also we collected some material from two additional sampling sites (not included to official list of profile's sites):

A: 51°20'16,8"N, 87°51'47,6"E, about 500 m a.s.l., *Duschekia fruticosa* forest on the bank of the river Bayas, 12.08.2005, hand sampling.
B: 51°18'05,4"N, 87°55'48,3"E, about 1900 m a.s.l., *P. sibirica* sparse growths, 14.08.2005, hand sampling.

The cluster analysis was performed using Statistica 10 (StatSoft 2011).

The material treated herein has been deposited mainly in the collection of the Altai State University, Barnaul, Russia (ASU), and partly shared also with the collection of the Institute of Biology and Soil Science, Far Eastern Branch, Russian Academy of Sciences, Vladivostok, Russia (IBSS), as indicated in the text. The species names include the literature references concerning Asian Russia only.

Taxonomic part

Order Julida Brandt, 1833
Family Julidae Leach, 1814
Genus *Julus* Linnaeus, 1758

Julus ghilarovi ghilarovi Gulička, 1963

Julus ghilarovi Gulička, 1963: 521, 520: figs.
Julus ghilarovi – Mikhaljova 2002: 206; Nefediev 2002b: 35; Mikhaljova and Nefediev 2003: 84.
Julus ghilarovi ghilarovi – Lokšina and Golovatch 1979: 386; Mikhaljova 1993: 11: figs; 2004: 59–61, 60: figs, 61: map; 2013a: 8; Mikhaljova and Golovatch 2001: 104; Nefediev 2005a: 41; 2005b: 8; Nefediev and Nefedieva 2006: 98; 2007a: 139; 2007b: 161; 2008a: 117; 2008b: 62; 2013: 87; Nefedieva and Nefediev 2008: 123; Babenko et al. 2009: 183; Nefediev et al. 2014: 63; Nefedieva et al. 2014: 65.

Material examined. 1 male, 7 females, 10 juv. (ASU), site 1; 1 male, 4 females, 3 juv. (ASU), site A; 1 female (IBSS), site 4; 5 males, 12 females, 4 juv. (ASU), site 5; 4 males, 6 females, 5 juv. (ASU), site 6; 1 female, 3 juv. (ASU), site 7; 3 juv. (ASU), site 8; 1 male, 1 female (IBSS), 3 males, 8 females, 11 juv. (ASU), site 19.

Distribution. This species appears to be widespread in the south of Siberia, Russia: Novosibirsk Area, Kemerovo Area, Altai Province, Republic of Altai, Republic of Khakassia, southern part of Krasnoyarsk Province. It is very likely it also occurs in the adjacent part of the Republic of Tyva.

Remarks. High ecological plasticity of this species allows it to inhabit different habitats like small-leaved, mixed and dark coniferous forests, herbaceous and alpine meadows, and montane moss-stony tundras. In the Kyga Biogeocenosis Profile the animal prefers forest litter in low- and mid-mountain chern taiga forests up to about 1200 m a.s.l., and also recorded in rocky mountain tundra on the summit of Malaya Koliushta Mt. at 2267 m a.s.l. It is very likely that the species is displaced from subalpine sparse growths of *P. sibirica* by the congener of *Julus insolitus*. The numbers range from 0.5 to 18 ind./m^2.

Julus insolitus **Mikhaljova, 2009**

non *Julus ghilarovi brachydactylus* – Nefedieva and Nefediev 2008: 123.
Julus insolitus Mikhaljova, 2009b: 66–67, 64: figs.
Julus insolitus – Nefediev and Nefedieva 2013: 87; Nefedieva et al. 2014: 65.

Material examined. 4 males, 5 females, 4 juv. (ASU), site 9; 4 males, 1 female (IBSS), 6 males, 9 females, 10 juv. (ASU), site 10; 2 males, 6 females, 17 juv. (ASU), site 10a; 7 males, 17 females, 25 juv. (ASU), site 11; 20 males, 9 females, 49 juv., 1 fragm. (ASU), site 12; 1 male, 1 female, 1 juv. (ASU), site 13; 2 males, 4 females, 7 juv. (ASU), site 13a; 3 males, 8 females, 10 juv. (ASU), site 14; 1 female, 2 juv. (ASU), site 15; 14 males, 15 females, 22 juv. (ASU), site B; 10 males, 12 females, 15 juv. (ASU), site 16; 2 females, 6 juv. (ASU), site 18.

Distribution. The species appears to be spread only in the south of Siberia, Russia: Republic of Altai.

Remarks. This species has been described by Mikhaljova (2009b) on two male specimens, which are known to occur in forest-tundra and rocky tundra. In the Kyga Biogeocenosis Profile the species is collected from the upper line of mid-mountain chern taiga forests through subalpine sparse growths of *P. sibirica* to mountain tundra with *B. rotundifolia* and *S. glauca*. The maximum abundance is about 98 ind./m^2 registered in the old fire-site of sparse growths of *P. sibirica* now occupied with a succession of dwarf trees of *B. rotundifolia* and *S. glauca*. The above female specimens are the first records in this species.

Genus ***Pacifiiulus*** **Mikhaljova, 1982**

Pacifiiulus amurensis **(Gerstfeldt, 1859)**

Julus amurensis Gerstfeldt, 1859: 271.
Julus amurensis – Lokšina and Golovatch 1979: 387; Mikhaljova 1993: 34.
Pacifiiulus imbricatus Mikhaljova, 1982: 211, 212: figs.
Pacifiiulus imbricatus – Mikhaljova 1983: 87; 1988: 70; Mikhaljova and Petukhova 1983: 53; Ganin 1988: 7; 1989: 145; 1994: 60; 1995: 370; 1997: 10; Ryabinin et

al. 1988: 31; Mikhaljova and Bakurov 1989: 40; Gromyko 1990: 66; Mikhaljova 1993: 12: map; 1997: 145; Enghoff 1994: 27; Shelley et al. 2000: 50.

Pacifiiulus amurensis – Mikhaljova 1998a: 5; 1998b: 64: figs, 65: map; 2004: 66–69, 67: figs, 68: map; 2009a: 603; 2009c: 3; 2012a: 23; 2012b: 112; Mikhaljova and Golovatch 2001: 105; Mikhaljova and Nefediev 2003: 84; Mikhaljova and Marusik 2004: 3; Nefediev 2005a: 48; 2005b: 8; Nefediev and Nefedieva 2006: 98; 2007a: 139; 2007b: 160; 2008b: 62; 2013: 87; Nefedieva et al. 2014: 65.

Material examined. 1 male (IBSS), site 9; 3 males (ASU), site 16; 1 male (ASU), site 18.

Distribution. This species is characterized by disjunctive area. The first distribution area is in the south of Siberia (Republic of Altai, Republic of Khakassia, southern part of Krasnoyarsk Province, Republic of Tyva) and the second one spreads in the Russian Far East (Maritime Province, southern part of Khabarovsk Province, Amur Area, Jewish Autonomous Area) and North-Eastern China.

Remarks. This species is characterized by euryoky, dwelling in Siberia in herbaceous meadows, small-leaved, mixed and dark coniferous forests, and subalpine meadows and montane tundras, up to 2500 m a.s.l. (Mikhaljova and Nefediev 2003). In the Kyga Biogeocenosis Profile the species is very rare (0.5–1.5 ind./m^2), collected from the mid-mountain dark coniferous forest, and also from subgoltsy sparse growths of *P. sibirica* and mountain tundra with dwarf bushes of *B. rotundifolia* and *S. glauca*, with the maximum altitude registered is about 2194 m a.s.l.

Genus *Sibiriulus* Gulička, 1963

Sibiriulus altaicus Gulička, 1972

Cylindroiulus (*Sibiriulus*) *altaicus* Gulička 1972: 43–44, 44: fig.

Sibiriulus altaicus – Lokšina and Golovatch 1979: 387; Mikhaljova 1993: 13; 2004: 75: fig, 74: map; Mikhaljova and Golovatch 2001: 106; Mikhaljova et al. 2007: 57–59, 62, 58: figs; 2014: 45–47, 46: figs; Nefediev and Nefedieva 2007b: 162; 2008a: 117; 2008b: 62; 2013: 86–87; Nefedieva and Nefediev 2008: 123–124; Nefedieva et al. 2014: 65.

Material re-examined (specimens published by Mikhaljova et al. 2007, 2014)**.** 1 male (ASU), site 1; 3 males, 2 juv. (IBSS), site 4; 1 male, 1 female, 2 juv. (IBSS), site 7; 1 male (ASU), site 8; 1 female (IBSS), 1 female (ASU), site 8A; 1 male (ASU), site 9; 1 male (IBSS), site 14.

Distribution. The species is known only in the south of Lake Teletskoye, Republic of Altai, its terra typica.

Remarks. In the Kyga Biogeocenosis Profile this species dwells in low- and mid-mountain chern taiga forests, and also subalpine sparse growths of *P. sibirica*, with the maximum altitude registered is about 1903 m a.s.l.

Family Nemasomatidae Bollman, 1893
Genus *Orinisobates* Lohmander, 1933

Orinisobates sibiricus (Gulička, 1963)

Isobates sibiricus Gulička, 1963: 522: figs.

Isobates (*Orinisobates*) *sibiricus* – Gulička 1972: 45: figs; Nefediev and Nefedieva 2008a: 117.

Orinisobates sibiricus – Lokšina and Golovatch 1979: 387; Enghoff 1985: 53, 54: figs; Mikhaljova 1993: 16; 2002: 206; 2004: 96–97, 96: figs, 94: map; Mikhaljova and Golovatch 2001: 107; Mikhaljova and Nefediev 2003: 83; Nefediev 2005a: 39; 2005b: 8; Nefediev and Nefedieva 2006: 98; 2007a: 139; 2007b: 160; 2008a: 117; 2008b: 62; 2013: 87; Nefedieva and Nefediev 2008: 123; Nefediev et al. 2014: 63; Nefedieva et al. 2014: 65.

Material examined. 1 male (ASU), site 1; 1 female (ASU), site 8a.

Distribution. The species appears to be quite widespread in the south of Siberia, Russia: Kemerovo Area, Republic of Khakassia, Altai Province, Republic of Altai, southern part of Krasnoyarsk Province, Republic of Tyva, Chita Area. Also it has been recorded in Eastern Kazakhstan and Kyrgyzstan.

Remarks. The species inhabits forest litter of small-leaved, mixed and dark coniferous forests, under bark of logs and trees, and in mosses and mushrooms. The maximum altitude registered is about 1700 m a.s.l. (Mikhaljova and Golovatch 2001). In the Kyga Biogeocenosis Profile the species is very rare collected from low- and mid-mountain chern taiga forest, with the maximum abundance registered is about 1 ind./m^2.

Julidae gen. sp.

Material examined. 1 juv. (ASU), site 2; 2 females, 1 juv. (ASU), site 9; 1 female, 2 juv. (ASU), site 10.

Remarks. It is very likely these females and juveniles appear to belong to *S. altaicus* or *P. amurensis*.

Order Chordeumatida C. L. Koch, 1847
Family Diplomaragnidae Attems, 1907
Genus *Altajosoma* Gulička, 1972

Altajosoma bakurovi bakurovi (Shear, 1990)

Diplomaragna bakurovi Shear, 1990: 22, 23: figs.

Diplomaragna bakurovi – Mikhaljova 1993: 18.

Altajosoma bakurovi – Mikhaljova 2000: 161: fig; 2004: 178–179, 178: figs, 116: map; Mikhaljova and Golovatch 2001:108; Nefediev 2002: 30; Mikhaljova and Nefediev 2003: 86; Mikhaljova et al. 2008: 51; Nefedieva and Nefediev 2008: 123–124; 2013: 87; Nefedieva et al. 2014: 65.

Material re-examined (specimen published by Mikhaljova et al. 2008)**.** 1 male (ASU), site A.

Distribution. The species is known to occur in the south of Siberia, Russia: Tomsk, Novosibirsk and Kemerovo areas, Krasnoyarsk Province and Republic of Altai.

Remarks. This species dwells in various forest habitats like small-leaved, mixed and dark coniferous forests, and also mesophytous meadow and mountain tundra, with the maximum altitude registered is about 2500 m a.s.l (Mikhaljova and Nefediev 2003). In the Kyga Biogeocenosis Profile a single male is collected only by hand sampling from *D. fruticosa* forest on the bank of the river Bayas at about 500 m a.s.l.

Altajosoma deplanatum (Stuxberg, 1876)

Craspedosoma deplanatum Stuxberg, 1876a: 34, figs.

Craspedosoma deplanatum – Stuxberg 1876b: 317; Lokšina and Golovatch 1979: 382; Nefediev and Nefedieva 2008a: 117.

Altajosoma pinetorum Gulička, 1972: 37: figs.

Altajosoma pinetorum – Lokšina and Golovatch 1979: 382; Shelley et al. 2000: 62; Nefediev and Nefedieva 2008a: 117.

Diplomaragna deplanata – Shear 1990: 19, 20: figs; Mikhaljova 1993: 22.

Diplomaragna pinetorum – Shear 1990: 38; Mikhaljova 1993: 25.

Altajosoma deplanatum – Mikhaljova 2000: 160; 2004: 170–171, 171: figs, 162: map; 2013a: 7; Mikhaljova and Golovatch 2001: 108; Nefediev 2002b: 35; 2002d: 30; Mikhaljova and Nefediev 2003: 86; Nefediev 2005a: 50; 2005b: 9; Nefediev and Nefedieva 2005: 177; 2006: 98; 2007a: 139; 2007b: 161; 2007c: 99; 2008b: 62; 2011: 100; 2012a: 51; 2012b: 47; 2013: 87; Nefedieva and Nefediev 2008: 123; Mikhaljova et al. 2008: 51; Nefedieva et al. 2014: 65.

Material examined. 2 females (ASU), site 14.

Material re-examined (specimens published by Mikhaljova et al. 2008)**.** 1 male (ASU), site 6; 1 male, 6 juv. (ASU), site 14.

Distribution. The species appears to be quite widespread in the south of Siberia, Russia: Tomsk, Novosibirsk and Kemerovo areas, Republic of Altai and Republic of Khakassia, and originally described from between the city of Achinsk (Krasnoyarsk Province) and the city of Mariinsk (Kemerovo Area).

Remarks. This species lives mainly in various forest habitats like small-leaved, mixed and dark coniferous forests, forest- and shrub tundra, and also mesophytous meadow. The maximum altitude registered is about 2080 m a.s.l (Mikhaljova et al.

2008). In the Kyga Biogeocenosis Profile the species quite rare found in the mid-mountain mixed forest and in the subalpine sparse growths of *P. sibirica*, with the maximum abundance registered is about 6 ind./m^2.

Altajosoma katunicum **Mikhaljova, 2000**

Altajosoma katunicum Mikhaljova, 2000: 161–162, 162: figs.
Altajosoma katunicum – Mikhaljova and Golovatch 2001: 108; Mikhaljova 2004: 176–177, 177: figs, 112: map; Mikhaljova et al. 2008: 52; Nefedieva et al. 2014: 65.

Material re-examined (specimens published by Mikhaljova et al. 2008). 1 male (ASU), site 1.

Distribution. This species in known to occur only in the Republic of Altai, Russia.

Remarks. This species originally described from the Katunskii Mt. Range, Central Altai at 1600–2200 m a.s.l. (Mikhaljova 2000). In the Kyga Biogeocenosis Profile the species is very rare (1 ind./m^2), collected only from the mixed forest in the lowest study site at 443 m a.s.l.

Altajosoma kemerovo **(Shear, 1990)**

Diplomaragna kemerovo Shear, 1990: 21, 20: figs.
Diplomaragna kemerovo – Mikhaljova 1993: 25; Nefediev and Nefedieva 2008a: 117.
Altajosoma kemerovo – Mikhaljova 2000: 161; 2004: 180–181, 180: figs, 173: map; 2013a: 7; Mikhaljova and Golovatch 2001: 108; Vorobiova et al. 2002: 60; Mikhaljova and Nefediev 2003: 86; Nefediev 2005a: 53; 2005b: 9; Nefediev and Nefedieva 2006: 98; 2007a: 139; 2007b: 161; 2008b: 62; 2013: 87; Nefedieva and Nefediev 2008: 123; Mikhaljova et al. 2008: 51; Nefedieva et al. 2014: 65.

Material examined. 4 females (ASU), site 7; 1 juv. (ASU), site 8; 7 juv. (ASU), site 13a; 1 female, 3 juv. (ASU), site 16.

Material re-examined (specimens published by Mikhaljova et al. 2008)**.** 1 male (ASU), site A; 1 male (ASU), site 5; 1 male (ASU), site 6; 5 males (ASU), site 7; 1 male (ASU), site 8; 1 male (ASU), site 13a; 1 male (ASU), site 16.

Distribution. This species appears to be spread in the south of Siberia, Russia: Kemerovo and Novosibirsk areas, Republic of Altai, Republic of Khakassia, southern part of Krasnoyarsk Province.

Remarks. The species dwells in different forest habitats like small-leaved, mixed and dark coniferous forests. In the Kyga Biogeocenosis Profile it is very rare (0.5–5 ind./m^2), mainly collected in low- and mid-mountain chern taiga at 853–1100 m a.s.l., but also found in subalpine sparse growths of *P. sibirica* and subgoltsy sparse growths of *P. sibirica* with *B. rotundifolia* and *S. glauca* bushes, with the maximum altitude registered is about 2028 m a.s.l.

Shearia teletskaya Mikhaljova, 2000

Shearia teletskaya Mikhaljova, 2000: 167–168, 167: figs.

Shearia teletskaya – Mikhaljova and Golovatch 2001: 111; Mikhaljova 2004: 160–161, 160: figs, 112: map; Mikhaljova et al. 2008: 54; Nefedieva et al. 2014: 65.

Material examined. 1 juv. (ASU), site 16.

Material re-examined (specimens published by Mikhaljova et al. 2008)**.** 1 male, 4 juv. (ASU), site B; 1 male, 8 juv. (ASU), site 12; 1 male (IBSS), 1 male (ASU), site 16.

Distribution. The species is known to occur only in the south of Lake Teletskoye, Republic of Altai, Russia.

Remarks. This species inhabits dark coniferous taiga forests at 1350–1750 m a.s.l. and the subalpine belt (= goltsy) at 1750–2000 m a.s.l. (Mikhaljova 2004). In the Kyga Biogeocenosis Profile sites investigated the species is very rare (3 ind./m^2), mainly collected by hand sampling in subalpine sparse growths of *P. sibirica* at 1847–2028 m a.s.l.

Diplomaragnidae gen. sp.

Material examined. 1 female (ASU), site A; 1 female, 1 juv. (ASU), site 5; 5 juv. (ASU), site 8a; 4 juv. (ASU), site 9; 1 female, 14 juv. (ASU), site 10; 5 juv. (ASU), site 10a; 1 female, 9 juv. (ASU), site 11; 1 female, 1 fragm. (ASU), site 13; 1 female, 1 juv. (ASU), site 18; 1 female, 1 juv. (ASU), site 19.

Remarks. The above specimens appear to belong to some species of *Altajosoma* or *S. teletskaya*.

Family Anthroleucosomatidae Verhoeff, 1899
Genus *Ghilarovia* Gulička, 1972

Ghilarovia kygae Gulička, 1972

Ghilarovia kygae Gulička, 1972: 39, 40: figs.

Ghilarovia kygae – Lokšina and Golovatch 1979: 383; Shear 1988: 55: figs; Mikhaljova 1993: 16; 2002: 203, 202: figs; 2004: 188–190,188: figs, 189: map; 2013a: 8; Shelley et al. 2000: 68; Mikhaljova and Golovatch 2001: 107; Nefediev 2005a: 58; 2005b: 9; Nefediev and Nefedieva 2007b: 161; 2008a: 117; 2008b: 62; 2013: 87; Nefedieva and Nefediev 2008: 123; Nefedieva et al. 2014: 65.

Material examined. 18 males, 16 females (ASU), site 1; 9 males, 6 females (ASU), site 2; 2 males, 1 female (ASU), site A; 3 males, 9 females, 2 juv. (ASU), site 4; 24 males, 18 females, 2 juv. (ASU), site 5; 15 males, 15 females, 2 juv. (ASU), site 6; 21 males, 15 females, 1 juv., 1 fragm. (ASU), site 7; 5 males, 3 females (ASU), site 8; 6 males, 6 females, 14 juv. (ASU), site 8a; 2 females, 1 juv. (ASU), site 9; 2 males (ASU), site 10a; 3 males, 3 females (ASU), site 11; 3 males, 1 female, 8 juv. (ASU), site 12; 3 males, 1 female (ASU), site 12; 6 males, 8 females (ASU), site 13; 2 males, 5 females (ASU), site 13a; 5 males, 16 females (ASU), site 14; 1 male, 1 female, 1 fragm. (ASU), site 15; 2 males, 8 females (ASU), site B; 1 male (ASU), site 16.

Distribution. The species is known to occur in the Republic of Altai, Siberia, Russia only.

Remarks. This species prefers to live in mixed and dark coniferous forests, and also known from subalpine habitats like golsty, with the maximum altitude registered is about 2000 m a.s.l. (Mikhaljova and Golovatch 2001). In the Kyga Biogeocenosis Profile the species shows the highest ecological plasticity, dwelling in low- and mid-mountain chern taiga forests, and subalpine sparse growths of *P. sibirica* up to sub-goltsy, with the maximum altitude registered is about 2028 m a.s.l.

Family Kirkayakidae Özdikmen, 2008

(syn. Altajellidae Mikhaljova & Golovatch, 2001)

Genus *Kirkayakus* Özdikmen, 2008

(syn. *Altajella* Gulička, 1972)

Kirkayakus pallidus (Gulička, 1972)

(syn. *Altajella pallida* Gulička, 1972)

Altajella pallida Gulička, 1972: 42, 43: figs.

Altajella pallida – Lokšina and Golovatch 1979: 383; Shear 1988: 51; Mikhaljova 1993: 34; 2004: 196–199, 197: figs, 105: map; Shelley et al. 2000: 61; Mikhaljova and Golovatch 2001: 111, 112: figs; Nefediev 2005a: 58; 2005b: 9; Nefediev and Nefedieva 2007b: 161; 2008a: 117; 2008b: 62; Nefedieva and Nefediev 2008: 123.

Kirkayakus pallidus – Özdikmen 2008: 342; Nefediev and Nefedieva 2013: 87; Nefedieva et al. 2014: 65.

Material examined. 1 male (ASU), site 8; 1 female (ASU), site 9.

Distribution. This species is an endemic in the south of Lake Teletskoye, Republic of Altai, Siberia, Russia.

Remarks. The species lives in dark coniferous forests up to 1350 m a.s.l. (Mikhaljova and Golovatch 2001). In the Kyga Biogeocenosis Profile the animal prefers mid-mountain dark coniferous forests at 1100–1414 m a.s.l., when it is very rare, and the numbers range from 0.5 to 1 ind./m². The above material appears to belong to topotypes, and this is the first record of the female specimen in this species.

Genus *Teleckophoron* Gulička, 1972

Teleckophoron montanum Gulička, 1972

Teleckophoron montanum Gulička, 1972: 41: figs.
Teleckophoron montanum – Lokšina and Golovatch 1979: 383; Mikhaljova 1993: 35; 2004: 193–196, 195: figs, 107: map; Shelley et al. 2000: 79; Mikhaljova and Golovatch 2001: 113, 114: figs; Nefediev 2005a: 59; 2005b: 9; Nefediev and Nefedieva 2006: 98; 2007b: 161; 2008a: 117; 2008b: 62; 2013: 87; Nefedieva and Nefediev 2008: 123; Nefedieva et al. 2014: 65.

Material examined. 3 males, 1 female, 4 juv. (ASU), site 8; 1 female, 3 juv. (ASU), site 8a.

Distribution. The area of this species appears to encompass the Republic of Altai and the southern part of the Krasnoyarsk Province, both Siberia, Russia.

Remarks. This species inhabits dark coniferous forests and montane tundras. The maximum altitude registered is about 1000 m a.s.l. (Gulička 1972). In the Kyga Biogeocenosis Profile the species prefers mid-mountain dark coniferous forests up to 1191 m a.s.l., where the numbers range from 3 to 8 ind./m².

Order Polydesmida Leach, 1815
Family Polydesmidae Leach, 1815
Genus *Schizoturanius* Verhoeff, 1931

Schizoturanius clavatipes (Stuxberg, 1876)

Polydesmus clavatipes Stuxberg, 1876a: 34, figs.
Polydesmus clavatipes – Stuxberg 1876b: 316; Nefediev and Nefedieva 2008a: 117.
Schizoturanius clavatipes – Lohmander 1933: 27; Hoffman 1975: 81, 82: figs; Lokšina and Golovatch 1979: 384; Mikhaljova 1993: 31, 32: figs; 2002: 206; 2004: 238–240, 239: figs, 228: map; 2013a: 9; 2013b: 221; Nefediev 2001: 84; 2002c: 139; 2002d: 30; Mikhaljova and Golovatch 2001: 116; Vorobiova et al. 2002: 60; Mikhaljova and Nefediev 2003: 81; Nefediev 2005a: 61; 2005b: 9; Nefediev and Nefedieva 2005: 178; 2006: 98; 2007a: 139; 2007b: 161; 2007c: 102; 2008b: 62; 2011: 100; 2012a: 51; 2012b: 47; 2013: 87;

Nefedieva and Nefediev 2008: 123; Nefediev et al. 2014: 63; Nefedieva et al. 2014: 65.

Material examined. 4 males, 2 females, 4 juv. (ASU), site 1; 2 males, 2 females, 3 juv. (ASU), site 2; 2 males, 1 female, 1 juv. (ASU), site 4; 4 males, 2 females, 1 juv. (ASU), site 5; 2 males, 1 females (ASU), site 6; 6 males, 2 females, 11 juv. (ASU), site 7; 1 male, 1 female (ASU), site 8a; 1 female (ASU), site 9; 7 juv. (ASU), site 14; 3 juv. (ASU), site B.

Distribution. This species appears to be quite widespread in the south of Siberia, Russia: Tomsk, Novosibirsk and Kemerovo areas, Altai Province, Republic of Altai, Republic of Khakassia, southern part of Krasnoyarsk Province.

Remarks. Being highly euryoecic, the species populates various forest habitats (small-leaved, mixed and dark coniferous forests), and also meadows and glades. In the Kyga Biogeocenosis Profile the species prefers low- and mid-mountain chern taiga forests, where its numbers range from 0.5 to 10 ind./m^2, but also collected from subalpine sparse growths of *P. sibirica* at about 1903 m a.s.l.

Schizoturanius tabescens (Stuxberg, 1876)

Polydesmus tabescens Stuxberg, 1876a: 35, figs.
Polydesmus tabescens – Stuxberg 1876b: 316; Lokšina and Golovatch 1979: 385.
Turanodesmus salairicus Gulička, 1963: 523, 522: figs; Nefediev and Nefedieva 2008a: 117.
Schizoturanius salairicus – Lokšina and Golovatch 1979: 384; Mikhaljova 1993: 31; Nefediev 2001: 84, 2002a: 40; 2002b: 35; 2002c: 139; 2002d: 30; Mikhaljova and Golovatch 2001: 116; Mikhaljova and Nefediev 2003: 83.
Schizoturanius tabescens – Mikhaljova 1993: 31, 32: figs; 2004: 240–242, 241: figs, 242: map; 2013b: 221; Vorobiova 1999: 33; Mikhaljova and Golovatch 2001: 116; Vorobiova et al. 2002: 60; Rybalov 2002; Mikhaljova and Marusik 2004: 8, 7: figs; Nefediev 2005a: 64; 2005b: 9; Nefediev and Nefedieva 2005: 178; 2006: 98; 2007a: 139; 2007b: 161; 2007c: 102; 2008a: 117; 2008b: 62; 2011: 100; 2012a: 51; 2012b: 47; 2013: 87; Nefedieva and Nefediev 2008: 123; Babenko et al. 2009: 183; Nefedieva et al. 2014: 65.

Material examined. 3 juv. (ASU), site 2; 2 females, 2 juv., 1 fragm. (ASU), site 4; 4 juv. (ASU), site 5; 17 juv. (ASU), site 7; 2 females, 1 juv. (ASU), site 8a; 1 female (ASU), site 9; 2 females (ASU), site B.

Distribution. Being rather widespread, the species is known to occur in the south of Siberia, Russia: Tomsk, Novosibirsk and Kemerovo areas, Altai Province, Republic of Altai, Republic of Khakassia, southern part of Krasnoyarsk Province.

Remarks. This species lives in different forests like small-leaved, mixed and dark coniferous ones, and also populates meadows. In the Kyga Biogeocenosis Profile the animal prefers low- and mid-mountain chern taiga forests, where its numbers range from 0.5 to 8 ind./m^2, but also collected from subalpine sparse growths of *P. sibirica* at about 1900 m a.s.l.

Results

The millipede diversity in the south of Lake Teletskoye is estimated to be at least 15 species and subspecies from 10 genera, 6 families and three orders: *Julus ghilarovi ghilarovi* Gulička, 1963, *J. insolitus* Mikhaljova, 2009, *Orinisobates sibiricus* (Gulička, 1963), *Pacifiiulus amurensis* (Gertsfeldt, 1859), *Sibiriulus altaicus* (Gulička, 1972), *Ghilarovia kygae* Gulička, 1972, *Kirkayakus pallidus* (Gulička, 1972) (synonym of *Altajella pallida* Gulička, 1972), *Teleckophoron montanum* Gulička, 1972, *Altajosoma bakurovi bakurovi* (Shear, 1990), *A. deplanatum* (Stuxberg, 1876), *A. katunicum* Mikhaljova, 2000, *A. kemerovo* (Shear, 1990), *Shearia teletskaya* Mikhaljova, 2000, *Schizoturanius clavatipes* (Stuxberg, 1876) and *S. tabescens* (Stuxberg, 1876).

The bulk of species diversity is confined both to low- and mid-mountain chern taiga forests and high-mountain shrub tundras with *Betula rotundifolia* and *Salix glauca*, achieving from 5 to 9 species, whereas subalpine sparse growths of *Pinus sibirica* are characterized by the lowest millipede diversity, with 3 the most widespread species (Table 1). One of them, *G. kygae*, has the maximum of ecological plasticity. This species is recorded in almost all study sites, dwelling in low- and mid-mountain chern taiga forests, and subalpine sparse growths of *P. sibirica* up to subgoltsy, with the maximum altitude registered is about 2028 m a.s.l.

The numbers of diplopods range from 14.5 to 65 ind./m^2 in subzones of low- and mid-mountain chern taiga forests, and from 5.5 to 8 ind./m^2 in high-mountain shrub tundras (Table 1). Despite of the lowest species diversity in subalpine sparse growths of *P. sibirica*, millipedes show the maximum numbers, reaching up to 130 ind./m^2 in the old fire-site (site 11), evidently caused by the abundance of plant debris of dwarf vegetation of *Betula rotundifolia* and *Salix glauca* that appear to be more suitable for feeding of millipedes than pine litter.

Clustering of investigated sites in the Kyga Biogeocenosis Profile on species diversity allows to grouping at least two obvious sets of sites (Figure 1). The first group unites low-mountain chern taiga forests (sites from 2 to 7), with altitudes range from 494 to 960 m a.s.l. The second group includes subalpine sparse growths of *P. sibirica* localities (sites from 10a to 15), and also the highest locality of chern taiga forest (site 10) and a transition locality from subalpine sparse growths to shrub mountain tundra (site 16), with altitudes range from 1468 to 2028 m a.s.l. Some localities of mid-mountain chern taiga forests and mountain tundras are less similar both to each other and to other groups.

The very interesting situation is observed with two congeners of *Julus* as regards to altitude distribution, showing competitive relationships to each other. Thus, *J. g. ghilarovi* is mainly recorded in low-mountain and in the beginning of mid-mountain chern taiga forests, while it almost disappears in subalpine sparse growths of *P. sibirica*, but it emerges again in rocky mountain tundra on the top of the investigated biogeocenosis profile. At the same time, *J. insolitus* populates mainly subalpine sparse growths of *P. sibirica* and lifts up to shrub mountain tundra at 2194 m a.s.l. Taking into account that *J. insolitus* is very abundant here, we assume it wins the competition and

Table 1. Millipede abundance (ind./m^2) and hand sampling (+) in the Kyga Biogeocenosis Profile sites investigated.

Species	Sites																				
	1	**2**	**A**	**4**	**5**	**6**	**7**	**8**	**8a**	**9**	**10**	**10a**	**11**	**12**	**13**	**13a**	**B**	**14**	**15**	**16**	**18**	**19**
Julida																						
J. g. ghilarovi	18		+	0.5	3	5	2	3														6
J. insolitus										6.5	15	50	98	20	+	13	+	14	3	15.5	4	
S. altaicus	1			2.5			2	1	2	0.5								1				
O. sibiricus	1								1													
P. amurensis										0.5										1.5	0.5	
Julidae gen. sp.		1								1.5	1.5											
Chordeumatida																						
A. b. bakurovi			+																			
A. deplanatum						+												6				
A. katunicum	1																					
A. kemerovo			+		1.5	0.5	3	2								5				2.5		
S. teletskaya														3			+			+		
Diplomaragnidae gen. sp.			+						4	2	7.5	10	20		+						1	2
G. kygae	34	15	+	7	16.5	14.5	14.5	8	22	1.5		4	12	2	+	4	+	14	2	0.5		
K. pallidus								1		0.5												
T. montanum								8	3													
Polydesmida																						
S. clavatipes	10	7		2	1.5	1	8		2	0.5							+	4.5				
S. tabescens		3		2.5	2		8		2	0.5							+					
Total abundance	**65**	**26**	**-**	**14.5**	**24.5**	**21**	**37.5**	**23**	**36**	**14**	**24**	**64**	**130**	**25**	**-**	**22**	**-**	**39.5**	**5**	**20**	**5.5**	**8**
Numbers of species	6	4	4	5	5	5	6	6	7	9	3	3	3	3	3	3	5	5	2	5	3	2

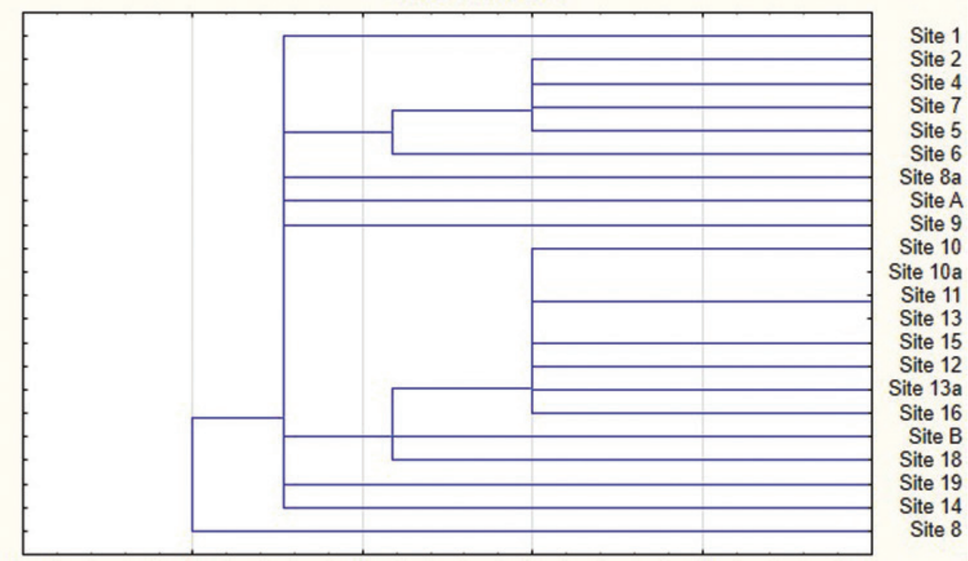


Figure 1. Neighbour-joining tree of similarity / dissimilarity of studied sites on species diversity.

displaces *J. g. ghilarovi* from subalpine *P. sibirica* sparse growths. The record of female specimens of *J. insolitus* is the first one in this species.

Both members of Kirkayakidae, *K. pallidus* and *T. montanum*, are reported in chern mid-mountain taiga at the highest altitudes for the first time. Also this is the first record of the female specimen in the former species.

Acknowledgements

We are thankful to D.A. Demidko (Krasnoyarsk, Russia) and V.S. Bogdanov (Chiri, Russia) for their great help with the organization of the collecting trip in Altai for the first and second authors. Special thanks are also addressed to anonymous reviewers and section editor for comments.

References

Babenko AS, Nefediev PS, Nefedieva JS (2009) [The fauna and population dynamics of millipedes (Diplopoda) of chern taiga of Salair]. Vestnik Tomskogo gosudarstvennogo universiteta, Seriya biologiya 319: 182–185. [In Russian]

Enghoff H (1985) The millipede family Nemasomatidae with the description of a new genus and a revision of *Orinisobates* (Diplopoda, Julida). Entomologica Scandinavica 16: 27–67. doi: 10.1163/187631285X00045

Enghoff H (1994) Geographical parthenogenesis in millipedes (Diplopoda). Biogeographica 70(1): 25–31.

Ganin GN (1988) [Estimation of the role of millipedes of Siberian pine-broadleaved forests of Priamurye in litter destruction]. Izvestiya Sibirskogo otdeleniya AN SSSR, Biology 20(3): 7–10. [In Russian, with English summary]

Ganin GN (1989) [The role of millipedes in the decay and transformation of forest litter in the southern Khabarovsk Province]. Zoologicheskii Zhurnal 68(1): 145–149. [In Russian, with English summary]

Ganin GN (1994) [Role of soil mesofauna in the biogenic turnover of elements in forest and meadow ecosystems of the Amur region]. Ekologiya 5–6: 59–67. [In Russian]

Ganin GN (1995) [Heavy metals in soil invertebrates in nature reserves of the Russian Far East]. Ekologiya 5: 368–372. [In Russian]

Ganin GN (1997) [Soil animals of the Ussuri region]. Dalnauka Publ., Vladivostok-Khabarovsk, 159 pp. [In Russian, with English summary]

Gerstfeldt G (1859) Ueber einige zum Theil neue Arten Platoden, Anneliden, Myriapoden und Crustaceen Sibiriens, namentlich seines östlichen Theiles und des Amur-Gebietes. Mém. savants étrangers Acad. Imp. Sci. St. Pétersbourg 8: 1–36.

Ghilarov MS (1987) [Recording of large invertebrates (mesofauna)]. In: Quantitative methods in soil zoology. Nauka Publ., Moscow, 9–26. [In Russian]

Gromyko MN (1990) [Peculiarities in the structure of soil populations in oakwood ecosystems of the Sikhote-Alin Biosphere Reserve]. In: Ecological investigations in the Sikhote-Alin Nature Reserve. TSNIL Glavokhoty RSFSR Publ., Moscow, 57–72. [In Russian]

Gulička J (1963) [New millipedes (Diplopoda) from the USSR. Part 1]. Zoologicheskii Zhurnal 42(4): 518–524. [In Russian, with English summary]

Gulička J (1972) [New millipedes (Diplopoda) from the USSR. Part 2]. Zoologicheskii Zhurnal 51(1): 36–45. [In Russian, with English summary]

Hoffman RL (1975) A note on the status of *Polydesmus clavatipes* and some related species from Central Asia (Diplopoda, Polydesmidae). Zoologischer Anzeiger 194: 79–83.

Lohmander H (1933) Über Diplopoden aus Zentralasien. Arkiv för Zoologi 25A(6): 1–71. [for 1932]

Lokšina IE, Golovatch SI (1979) Diplopoda of the USSR fauna. Pedobiologia 19(6): 381–389.

Mikhaljova EV (1982) [New millipedes of the family Julidae (Diplopoda) from the USSR Far East]. Zoologicheskii Zhurnal 61(2): 210–216. [In Russian, with English summary]

Mikhaljova EV (1983) [Distribution of millipedes (Diplopoda) along the soil profile in the forests of the Primorsky Province]. In: Fauna and ecology of arthropods in the Far East. Vladivostok, 77–90. [In Russian]

Mikhaljova EV (1988) [Millipedes (Diplopoda) of the Ussuriyskii and «Kedrovaya Pad» nature reserves]. In: A role of insects in biocenoses of the Far East. Vladivostok, 114–126. [In Russian]

Mikhaljova EV (1993) The millipedes (Diplopoda) of Siberia and the Far East of Russia. Arthropoda Selecta 2(2): 3–36.

Mikhaljova EV (1998a) On new and poorly-known millipedes (Diplopoda) from the Far East of Russia. Far Eastern Entomologist 60: 1–8.

Mikhaljova EV (1998b) The millipedes of the Far East of Russia (Diplopoda). Arthropoda Selecta 7(1): 1–77.

Mikhaljova EV (2000) Review of the millipede family Diplomaragnidae (Diplopoda: Chordeumatida). Arthropoda Selecta 8(3): 153–181. [for 1999]

Mikhaljova EV (2002) On some poorly-known millipedes from Siberia (Diplopoda). Arthropoda Selecta 10(3): 201–207. [for 2001]

Mikhaljova EV (2004) The millipedes (Diplopoda) of the Asian part of Russia. Pensoft Publishers, Sofia-Moscow, Series Faunistica 39, 292 pp. doi: 10.11646/zootaxa.3866.1.2

Mikhaljova EV (2009a) The millipedes (Diplopoda) of the Russian Far East islands and the Kamchatka Peninsula. Soil Organisms 81(3): 599–616.

Mikhaljova EV (2009b) New species of the family Julidae Leach 1814 from Altai, Russia (Diplopoda, Julida). Zootaxa 2235: 59–68.

Mikhaljova EV (2009c) A list of the millipedes (Diplopoda) of the Russian Far East. Far Eastern Entomologist 197: 1–8.

Mikhaljova EV (2012a) [Class Diplopoda – Millipedes]. Wildlife of Bastak Nature Reserve. Blagoveshchensk State Pedagogical University Publ., Blagoveshchensk, 22–25. [In Russian]

Mikhaljova EV (2012b) [Millipede (Diplopoda) taxonomic diversity in the Asian part of Russia]. In: Regions of a new exploration: theoretical and practical questions of biodiversity and landscape investigations and conservation. Institute for Water and Environmental Problems Publ., Khabarovsk, 111–114. [In Russian, abstract in English]

Mikhaljova EV (2013a) New data on the millipede fauna (Diplopoda) of Altai, Russia. Far Eastern Entomologist 265: 1–10.

Mikhaljova EV (2013b) [Endemism of millipedes (Diplopoda) in the Asian part of Russia]. Investigation, conservation and restoration of natural landscapes. In: Proceedings of III International Scientific Conference, Volgograd. Planeta Publ., Moscow, 220–223. [In Russian]

Mikhaljova EV, Bakurov VD (1989) [On millipedes (Diplopoda) of the Siniy Mountain Ridge (Sikhote-Alin)]. In: Soil invertebrates in the south of the Far East. Khabarovsk, 38–47. [In Russian]

Mikhaljova EV, Golovatch SI (2001) A review of the millipede fauna of Siberia (Diplopoda). Arthropoda Selecta 9(2): 103–118. [for 2000]

Mikhaljova EV, Marusik YM (2004) New data on taxonomy and fauna of the millipedes from the Russian Far East, Siberia and Mongolia (Diplopoda). Far Eastern Entomologist 133: 1–12.

Mikhaljova EV, Nefediev PS (2003) A contribution to the millipede fauna of Siberia (Diplopoda). Arthropoda Selecta 11(1): 81–87. [for 2002]

Mikhaljova EV, Nefediev PS, Nefedieva JS (2007) New data on millipedes of the family Julidae (Diplopoda, Julida) from Altai, Siberia. Zootaxa 1541: 57–63.

Mikhaljova EV, Nefediev PS, Nefedieva JS (2008) A new species and new records of millipedes of the family Diplomaragnidae (Diplopoda, Chordeumatida) from Altai. Zootaxa 1931: 49–56.

Mikhaljova EV, Nefediev PS, Nefedieva JS, Sakhnevich MB, Dyachkov YuV (2014) Review of the millipede genus *Sibiriulus* Gulička, 1972, with descriptions of three new species from Altai, Siberia, Russia (Diplopoda, Julida, Julidae). Zootaxa 3866(1): 30–52.

Mikhaljova EV, Petukhova EL (1983) [A comparative analysis of the millipede faunas (Diplopoda) of Primorsky Province's forests with the help of inclusion and similarity indices]. In: Graph theoretic methods in biogeographical investigations. Vladivostok, 48–66. [In Russian]

Nefediev PS (2001) [On the fauna and ecology of myriapods (Myriapoda) in the environs of the village of Smolenskoe, Altai Province]. In: Landscapes of Western Siberia: Investigation problems, ecology and rational use. Proceedings of 7 International Conference, devoting to the International Day of the Earth. Biysk Pedagogical State University Publ., Biysk, 84–86. [In Russian]

Nefediev PS (2002a) [Eco-faunistic investigations of myriapods in the Teguldet District, Tomsk Area]. In: Proceedings of International Conference of Students and Young Researchers «Lomonosov–2002». Moscow State University Publ., Moscow, 7: 40–41. [In Russian]

Nefediev PS (2002b) [The fauna and ecology of myriapods (Myriapoda) of a relict lime grove (village of Kuzedeyevo)]. In: Proceedings of 40 International Scientific Student Conference «Students and Scientific and Technical Progress». Novosibirsk State University Publ., Novosibirsk, 35. [In Russian]

Nefediev PS (2002c) [Populations and some ecological peculiarities of myriapods of gray forest soils in the southern of the Tomsk Area]. In: Biology – Science of the XXI Century. Abstracts of 6 School Conference of Young Researchers. Pushchino Scientific Centre RAS Publ., Pushchino-on-Oka, 138–139. [In Russian]

Nefediev PS (2002d) On the Diplopoda fauna of South-West Siberia. In: 12th International Congress of Myriapodology. Book of Abstracts. Pietermaritzburg, 30.

Nefediev PS (2005a) [Millipedes (Myriapoda, Diplopoda) of the Southeast of Western Siberia (fauna, zoogeography, ecology)]. Thesis of Candidate (Ph.D.) of Biological Sci. Degree. Tomsk, 175 pp. [In Russian]

Nefediev PS (2005b) [Millipedes (Myriapoda, Diplopoda) of the Southeast of Western Siberia (fauna, zoogeography, ecology)]. Autoreferate of the Thesis of Candidate (Ph.D.) of Biological Sci. Degree. UFO-print Publ., Tomsk, 21 pp. [In Russian]

Nefediev PS, Dyachkov YuV, Nefedieva JS (2014) Fauna and ecology of millipedes (Diplopoda) in the Tigirek State Nature Reserve, Russian Altai. In: Tuf IH, Tajovský K (Eds) 16th International Congress of Myriapodology. Book of Abstracts. Institute of Soil Biology, BC ASCR & Faculty of Science, Palacký University, Olomouc, 63.

Nefediev PS, Nefedieva JS (2005) [Seasonal fluctuations of diplopod density in some forests of Western Siberia]. In: Ecological diversity of soil biota and biological productivity of soils. Proceedings of IV (XIV) All-Russian Conference of Soil Zoology. Tyumen State University Publ., Tyumen, 177–178. [In Russian]

Nefediev PS, Nefedieva JS (2006) [Regional peculiarities of millipede fauna (Diplopoda) in the south-east of Western Siberia]. In: Ecology of South Siberia and adjacent territories. Proceedings of X International School Conference of Students and Young Researchers. Khakassian State University Publ., Abakan 1: 98. [In Russian]

Nefediev PS, Nefedieva JS (2007a) A brief analysis of the biotopic distribution of millipedes (Diplopoda) in the south-east of Western Siberia. In: Forest Soils: Research Results, Problems and Future Outlook. Abstracts of International Conference. Institute of Biology, Komi Scientific Center, Ural Division of RAS Publ., Syktyvkar, 139–140. [In Russian and in English]

Nefediev PS, Nefedieva JS (2007b) [Biogeographical characteristic of millipede fauna in the south-east of Western Siberia]. In: Biodiversity of invertebrate animals. Proceedings of II All-Russian School Conference «Conceptual and applied aspects of scientific investigations and education in invertebrate zoology». Deltaplan Publ., Tomsk, 133–138. [In Russian]

Nefediev PS, Nefedieva JS (2007c) [Seasonal dynamics of locomotor activity of millipedes (Diplopoda) in forests of Western Siberia]. In: Ecological problems of unique natural and anthropogenic landscapes. Proceedings of All-Russian Conference. Yaroslavl State University, Yaroslavl, 98–103. [In Russian]

Nefediev PS, Nefedieva JS (2008a) [A historical review of faunistic investigations of millipedes (Diplopoda) in Western Siberia]. In: Altai: ecology and environmental management. Proceedings of VII Russian-Mongolian Conference of Students and Young Researchers. Biysk Pedagogical State University Publ., Biysk 1: 117–120. [In Russian]

Nefediev PS, Nefedieva JS (2008b) Zoogeographical analysis of the millipede fauna (Diplopoda) in the south-east of Western Siberia. In: Myriapoda and Onychophora of the World Diversity, Biology and Importance. Abstracts of 14th International Congress of Myriapodology. Staatliches Museum für Naturkunde, Görlitz. Peckiana 6: 62.

Nefediev PS, Nefedieva JS (2011) [Millipedes (Diplopoda) of green plantations of Tomsk City and its suburbs]. In: Conceptual and applied aspects of scientific investigations in invertebrate zoology. Proceedings of III All-Russian School Conference. Agraf-Press Publ., Tomsk, 100–102. [In Russian]

Nefediev PS, Nefedieva JS (2012a) [Some peculiarities of distribution of millipedes (Diplopoda) along the soil profile in the south-taiga and small-leaved forests of Western Siberia]. Izvestia Altaiskogo gosudarstvennogo universiteta, Biologicheskie nauki 3(75),1: 49–54. [In Russian]

Nefediev PS, Nefedieva JS (2012b) [Some peculiarities of seasonal dynamics of sex-age structure of milliped populations (Diplopoda) in the southern taiga and small-leaved forests of Western Siberia]. Izvestia Altaiskogo gosudarstvennogo universiteta, Biologicheskie nauki 3(75),2: 46–48. [In Russian]

Nefediev PS, Nefedieva JS (2013) [Biodiversity and ecology of millipedes in the environs of Lake Teletskoe (Diplopoda)]. Izvestia Altaiskogo gosudarstvennogo universiteta, Biologicheskie nauki 3(79),1: 86–87. [In Russian]

Nefedieva JS, Nefediev PS (2008) Ecofaunistical investigations of millipedes (Diplopoda) in the environs of Lake Teletskoe. In: Myriapoda and Onychophora of the World Diversity, Biology and Importance. Abstracts of 14th International Congress of Myriapodology. Staatliches Museum für Naturkunde, Görlitz. Peckiana 6: 123–124.

Nefedieva JS, Nefediev PS, Sakhnevich MB, Dyachkov YuV (2014) Distribution of millipedes (Diplopoda) along an altitudinal gradient in the south of Lake Teletskoye, Altai Mts. In: Tuf IH, Tajovský K (Eds) 16th International Congress of Myriapodology. Book of Abstracts. Institute of Soil Biology, BC ASCR & Faculty of Science, Palacký University, Olomouc, 65.

Özdikmen H (2008) New family and genus names, Kirkayakidae nom. nov. and *Kirkayakus* nom. nov., for the millipedes (Diplopoda: Chordeumatida). Munis Entomology and Zoology 3(1): 342–344.

Ryabinin NA, Ganin GN, Pankov AN (1988) [Effect of sulfuric acid industry wastes on soil invertebrate complexes]. Ekologiya 6: 29–37. [In Russian]

Rybalov LB (2002) [Zonal-landscape change of soil invertebrate population in Yenisei Region of Middle Siberia and role of temperature adaptation in meridional (zonal) distribution of invertebrates]. Russian Entomological Journal 11(1): 77–86. [In Russian, with English summary]

Shear WA (1988) Systematic position of the milliped species *Alloiopus solitaries* Attems and the genus *Ghilarovia* Gulička (Chordeumatida, Anthroleucosomatidae). Myriapodologica 2(8): 51–58.

Shear WA (1990) On the Central and East Asian milliped family Diplomaragnidae (Diplopoda, Chordeumatida, Diplomaragnoidea). American Museum Novitates 2977: 1–40.

Shelley RM, Sierwald P, Kiser SB, Golovatch SI (2000) Nomenclator generum et familiarum Diplopodorum II. A list of the genus and family-group names in the class Diplopoda from 1958 through 1999. Pensoft Publishers, Sofia-Moscow, 167 pp.

StatSoft (2011) STATISTICA (data analysis software system), version 10. www.statsoft.com

Stuxberg A (1876a) Myriopoder från Sibirien och Waigatsch on samlade under Nordenskiöldska expeditionen 1875. Öfversigt k. Vetensk.-Akad. Förhandl. 33(2): 11–38.

Stuxberg A (1876b) On the Myriopoda, from Siberia and Waigatsch Island, collected during the expedition of Prof. Nordenskiöld, 1875. Annals and Magazine of Natural History 4(17): 306–318. doi: 10.1080/00222937608681955

Vorobiova IG (1999) [An ecofaunistic characteristic of the myriapod population (Myriapoda) in a midflow region of the Yenisey River]. In: Biodiversity and life of soil system. Abstracts of XII All-Union Conference of Soil Zoology. Moscow, 33. [In Russian]

Vorobiova IG, Rybalov LB, Rossolimo TE, Zalesskaya NT (2002) [Zonal and landscape distribution of the myriapod fauna and populations (Myriapoda) in the Yenisey River basin]. In: Study, conservation and reconstruction of ecosystem biodiversity in the Yenisey ecological transect: Wildlife, etno-ecological investigations. Moscow 2: 60–71. [In Russian]

ZooKeys 510: 163–179 (2015)
doi: 10.3897/zookeys.510.8414
http://zookeys.pensoft.net

RESEARCH ARTICLE

A peer-reviewed open-access journal
ZooKeys
Launched to accelerate biodiversity research

Centipede assemblages along an urbanization gradient in the city of Heraklion, Crete (Greece)

Gabriella Papastefanou[1], Eleni Panayiotou[2],
Moisis Mylonas[1,2], Stylianos Michail Simaiakis[2]

1 *Department of Biology, University of Crete, GR-71100 Heraklion, Crete, Greece* **2** *Natural History Museum of Crete, University of Crete, Knossos Av., PO Box 2208, GR-71409 Heraklion, Crete, Greece*

Corresponding author: *Stylianos Michail Simaiakis* (ssimaiakis@yahoo.com)

Academic editor: *Ivan H. Tuf* | Received 9 August 2014 | Accepted 2 June 2015 | Published 30 June 2015

http://zoobank.org/AB394815-9EF7-45E9-B6D0-5268B4B2DF6C

Citation: Papastefanou G, Panayiotou E, Mylonas M, Simaiakis SM (2015) Centipede assemblages along an urbanization gradient in the city of Heraklion, Crete (Greece). In: Tuf IH, Tajovský K (Eds) Proceedings of the 16th International Congress of Myriapodology, Olomouc, Czech Republic. ZooKeys 510: 163–179. doi: 10.3897/zookeys.510.8414

Abstract

Global urbanization is a major force that causes alteration and loss of natural habitats. Urban ecosystems are strongly affected by humans and there is a gradient of decreasing human influence from city centers to natural habitats. To study ecological changes along this continuum, researchers introduced the urban-rural gradient approach. The responses of centipedes to an urbanization gradient (urban-suburban-rural areas) were studied using pitfall traps in and near the city of Heraklion, in the island of Crete, Greece, from November 2010 to November 2011. Our results do not support the intermediate disturbance hypothesis, in which suburban areas located in the transitional zone between urban and rural habitats failed to indicate significant increase in terms of species richness and diversity.

Keywords

Abundance, activity density, *Eupolybothrus litoralis*, *Lithobius nigripalpis*, pitfall traps, *Scolopendra cretica*, *Scutigera coleoptrata*, spatial distribution, species diversity, temporal distribution

Introduction

With respect to global threats, some scientists believe that our planet is facing a new biodiversity crisis, frequently called as the sixth mass extinction, which is a human-caused phenomenon (Eldredge 1998). Regarding biodiversity, over the past few decades there has been a growing interest to explore biological components of cities in order to understand their ecology at different geographical scales (McDonnell et al. 2009). At the same time, urban areas are increasing worldwide (Sorace 2001), while the rapid and worldwide urbanization of human population (Niemelä 1999) raises concerns about the sustainability of cities (Andersson 2006).

A city represents an ideal terrestrial ecosystem to investigate fauna composition. It retains specific microclimatic and hydrological parameters and is sensitive to human activities and climate change (Ricklefs 1990). In addition, many invertebrate species form a biodiversity component critical for the persistence of the ecosystem (Oliver and Beattie 1996), and are well-adapted to live in this environment (Wallwork 1976). However, soil invertebrates are marginalized from conservation works, mainly because of lacking data (Ward and Lariviere 2004). Consequently, without information on species distribution, community ecology and on the evolutionary processes that influence them, terrestrial invertebrates may remain threatened, leading to loss of species. In fact, habitat fragmentation, habitat loss, accumulation of pollutants to soil, water and atmosphere affect negatively native biodiversity (Clark et al. 2007) and are primary causes of species extinctions (e.g., Wilcove et al. 1998, McKinney 2002).

Nowadays thousands of species are characterized as nationally extinct, threatened, or near threatened in broad habitats, particularly in urban areas, as a result of the significant declining areas of natural patches. These habitats represent an important patchily distributed environment for thousands of species (Hanski 2004). For example, it is well known that in highly fragmented landscapes (e.g., city), one decaying tree trunk or a flower-bed may support local soil populations for many generations. Soil arthropods such as ground beetles, centipedes, millipedes, spiders, and scorpions can be readily surveyed. Regarding urban areas, species richness is often determined by climatic changes, solar radiation, and the availability of host plants. Thus, soil arthropods are potentially useful ecological indicators of urbanization (Clark et al. 2007).

The effect of urbanization on biodiversity has focused primarily on vertebrates (e.g., Germaine et al. 2001). Several works have also investigated terrestrial invertebrates (e.g., ground beetles) along a gradient from a highly disturbed urban environment to a less disturbed rural environment (e.g., Alaruikka et al. 2002, Niemelä et al. 2002, Ishitani et al. 2003, Ulrich et al. 2008). In general, in northwestern parts of Europe such focused studies are numerous, but in southeastern Mediterranean region the knowledge is scanty.

Because of the relatively high diversity and the quite high availability of species occurrence records, centipedes provide a suitable taxonomic group for studying ecological aspects. However, only few studies have focused on the impact of urbanization on centipede species assemblages. In this study we performed several comprehensive analyses to investigate the responses of centipede species in the city of Heraklion, on Crete. In particular:

a) we quantify species richness, abundance and diversity along an urban-rural gradient,
b) we study centipede species structure emphasizing on the spatial and temporal distribution along the three urbanization zones, and,
c) we explore patterns of distribution of the identified generalist species.

Methods

Study area

The city of Heraklion (35°20'0"N; 25°8'0"E) is the largest city of Crete and the fourth largest in Greece located in the centre of the northern coast of the island. It covers an area of approximately 24 km^2 with an estimated population of 173,450 (according to the General Population Census 2011) at a density of 7,227 residents per square kilometer. Heraklion is mainly flat with several prominent hills, characterized by numerous floral species, mainly introduced, such as *Hirschefeldia incana* and *Conyza albida* (common species along the roadsides), *Petromarula pinnata* (endemic plant of Crete), *Ailanthus altissima* (dominant plant at the archaeological site of Knossos), *Hyoscyamus aureus* (common in the Venetian city walls) (for further habitat description see also Vogiatzakis and Rackham 2008 and Kaltsas et al. 2014).

We selected three sampling areas along an urbanization gradient, as proposed by the Globenet protocol (Niemelä et al. 2000), from north to south and from west to east of Heraklion: a) a highly disturbed habitat – urban, b) a moderate disturbed habitat – suburban, and, c) a less disturbed habitat – rural. Within each disturbance area we settled three replicate sites covering the west (1), the south (2) and the east (3) side of the city (Fig. 1). In particular, nine sampling sites were selected: i) three urban sites within the city of Heraklion, ii) three suburban sites on the boarders of the city, and iii) three rural sites in natural environment. The selection of the sampling sites was made on the basis of the similarity of vegetation and the percentage of built-up area (Ishitani et al. 2003, Kaltsas et al. 2014). Further details on the environment of each site can be found in Fig. 1. The urban habitats, at the western part of the Venetian city walls (U1), at the southern side of the Venetian city walls (U2), and at the eastern part of the city, not far from the main port (U3), were characterized by the dominance of nonnative herbaceous vegetation. The suburban habitats were located at the western end of Ammoudara district (S1), at the southern part of the city near the road to Moires (S2), and at the eastern side of the city close to the industrial area (S3), and were dominated by indigenous herbaceous vegetation and *Nerium oleander*. Rural habitats were located in the west, at the Palaiokastro bridge next to the national road (R1), in the south district of Heraklion (R2), and in the east near Kokkini Chani close to the national road (R3). The Euclidian distances between the sampling sites were: U1-U2 = 1.22 km, U2-U3 =

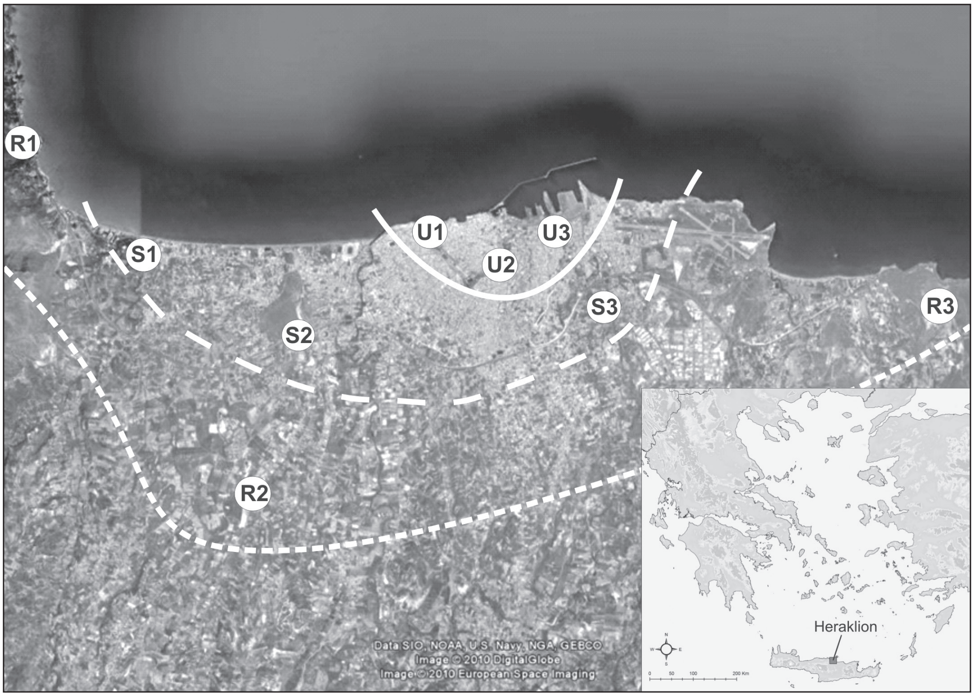


Figure 1. Map of the study area and sampling sites in and near Heraklion city. **U** urban sites (**U1** athletic centre, roads, parking area, buildings and gardening activities **U2** roads, parking area, dense buildings and gardening activities **U3** Heraklion port, roads, numerous buildings and gardening activities) **S** suburban sites (**S1** hotels, roads, sandy substrate **S2** hotels, electricity power factory, roads **S3** industrial area, roads, numerous buildings) **R** rural sites (**R1–R3** roads and little grazing).

1.2 km, S1-S2 = 4.0 km, S2-S3 = 6.4 km, R1-R2 = 6.99 km, R2-R3= 13.21 km. All rural habitats were dominated by typical phrygana, such as *Sarcopoterium spinosum*, *Thymbra capitata* and *Genista acanthoclada* (see also Kaltsas et al. 2014). We could not settle any sampling site in the north side of Heraklion because of its proximity to the Aegean Sea.

Sampling design

Centipedes were collected with pitfall traps along the aforementioned urban–rural gradient in accordance to the GLOBENET program protocol for capturing soil arthropods (Niemelä et al. 2000, 2002). At each site we placed 10 traps along a transect line positioned at a distance of 10 m from one another and specimens were collected monthly. Each trap was a plastic container with a diameter of about 10 cm placed into the ground in a depth of almost 12 cm. Overall we placed 90 traps across the urban – rural gradient. Pitfall traps contained ethylene glycol as a preservative liquid. The collection of material took place over a year, from November 2010 to November 2011.

We determined geographic coordinates for each sampling site using a GPS apparatus. All captured centipedes are preserved in 95% alcohol and are deposited in the Myriapod collection of the Natural History Museum of Crete (hereafter NHMC).

Data analyses

Centipede species richness for each site was estimated using two nonparametric richness estimators, in particular Chao1 and Bootstrap (see Colwell et al. 2012 for review), based on species-by-sample data. We calculated survey completeness for each sampling site as the observed number of captured species divided by the average estimated number of species. In agreement with Meijer et al. (2011), a value of sampling completeness above 0.75 is generally accepted. In our case, none of the nine mentioned sites were excluded from analyses. We also prepared species accumulation curves, as a function of sampling completeness, of the rate at which new species were found at each site along the urbanization gradient.

In terms of richness the number of species and genera was counted at each site. We also calculated the diversity of centipedes using: (i) the Shannon diversity index *H'* (for further details see Magurran 1988), and (ii) the evenness diversity index *J'* (for further details see also Magurran 1988).

We calculated activity density in terms of number of individuals per 100 trap-days at each habitat along the gradient. The temporal distribution of centipede assemblages was analysed in terms of the average number of species per sampling period, i.e., one month, called $\bar{\alpha}$ diversity, and the proportion of cumulative *a* diversity, known as a measure of temporal turnover (Romanuk and Kolasa 2001):

$$\alpha\,\% = \left(\frac{\bar{\alpha}}{S}\right) x$$

where *S* is the total number of species captured in a site (Zamora et al. 2007). We also measured the temporal beta diversity (*β*t), known as the temporal change of species structure at each site. To find temporal beta diversity, we first calculated the complementarity index for each sampling period, i.e., one month (see Colwell and Coddington (1994):

$$C_{jk} = [(S_j + S_k - 2V_{jk}) / (S_j + S_k - V_{jk})] \text{ x } 100$$

where S_j is the number of species captured in sampling period *j*, S_k the number of species captured in consecutive sampling period *k* (k=j+1) and V_{jk} the common species captured in periods *j* and *k*. After these calculations, temporal beta diversity (*β*t) was measured as the average for each site assemblage.

Among others, we performed one-way ANOVA tests for differences in richness, abundance, activity density, and diversity (Shannon *H'*, temporal diversity, *β*t and proportion of cumulative *a* represented by average *a*, α %) along the urbanization gradient.

We also performed a non-metric multi-dimensional scaling (NMDS) ordination plot based on Bray-Curtis dissimilarities of square-root transformed centipede abundance data, to find out whether there are structural differences in centipede assemblages along the urban–rural gradient. Structural differences may concern species composition and species activity density. NMDS analysis was performed in PAST 2.16 (Hammer et al. 2001). Furthermore, we tested if temporal beta diversity (βt) increases as α % decreases using a simple linear regression.

Results

Overall, 993 individuals (36.3 individuals per site / 100 trap-days) were collected and identified, belonging to 18 centipede species (8.0 ± 1.7 per site) and 11 genera (5.9 ± 0.9 per site) (Table S1 in Suppl. material 1). Seven species were collected at all three urbanization levels (*Clinopodes flavidus*, *Eupolybothrus litoralis*, *Lithobius creticus*, *L. erythrocephalus*, *L. nigripalpis*, *Scolopendra cretica*, *Scutigera coleoptrata*). Among these, *Lithobius creticus* and *Scolopendra cretica* are endemic in Crete. Six species were characterized as single habitat species (e.g., *Cryptops trisulcatus*, *Lithobius aeruginosus*, *L. lapidicola*, *L. pamukkalensis*, *Pachymerium ferrugineum*, *Schendyla nemorensis*), out of which four, namely *C. trisulcatus*, *L. aeruginosus*, *L. lapidicola*, and *Schendyla nemorensis*, were represented by single individuals (singletons) (see Table S1 in Suppl. material 1). The most abundant species were *Scolopendra cretica*, *Lithobius nigripalpis*, *Scutigera coleoptrata*, and *Eupolybothrus litoralis*, with approximately 37.7%, 21.0%, 17.9%, and 17.3% of the total centipede catch, respectively.

The total and average abundance were maximal in suburban sites, minimal in urban sites, and intermediate in the rural sites (Fig. 2). As for the species richness, 12 species were identified in both rural and urban habitats, while 13 species were collected from the suburban sites (Fig. 2). In detail, most of the individuals were captured in suburban (499 individuals, 50.3% of the total) and rural habitats (300 individuals, 30.2% of the total), while in the city centre, only 194 centipede individuals were collected (19.5% of the total) (Table 1). Shannon and evenness diversity indices were slightly different along the urbanization gradient (Table 1). The species accumulation curves of the rate at which new species are found within each site along the urbanization gradient are presented in Fig. 3. Accumulation curve data showed that completeness of samplings was relatively high for all nine sites, ranging between 0.79 and 1.00, with an average value of 0.91 ± 0.06 per site (Table 1).

The activity density (individuals/100 trap-days) of centipede assemblages ranged from ca 12.1 to 25.7 in urban habitats (mean value of 21.2 ± 7.8), from ca 43.9 to 66.3 in suburban habitats (mean value of 54.5 ± 11.3), and from ca 31.6 to 34.0 in rural sites (mean value of 33.1 ± 1.3) (see Table 1 and Table S2 in Suppl. material 1). The average species richness ($\bar{\alpha}$) varied from ca 1.2 to 3.3 among the sampling habitats (Table 1). Moreover, the proportion of cumulative *a* represented by average *a* diversity (α %) varied from 25% to about 43% and the temporal beta diversity (βt) varied from about 42.9 to 74.4 among the sampling sites (Table 1).

Table 1. Summary results of centipede species diversity for each site (R: rural, S: Suburban, U: urban). Richness is shown in terms of captured genera (G), species (S), total number of individuals in samples (Sa). Species diversity is estimated based on Shannon-Wiener (*H'*) and Evenness (*J'*) diversity indices. Sampling effort is shown in terms of expected number of species (Se) based on Chao 1 and Bootstrap estimators (mean ± SD) and completeness of samplings (Sc). Temporal diversity is shown in terms of activity density (A), average species richness ($\bar{\alpha}$), proportion of cumulative *a* represented by average *a* diversity (α%), and temporal beta diversity (βt).

Site	G	S	Sa	H'	*J'*	Se Chao 1 (mean ± SD)	Se Bootstrap (mean ± SD)	Sc	A	$\bar{\alpha}$	α%	βt
U1	4	4	37	0.72	0.51	4.12 ± 0.72	4.48 ± 0.29	0.93	12.14	1.17	29.17	62.96
U2	6	9	74	1.61	0.56	9.77 ± 2.16	9.98 ± 0.05	0.91	25.68	2.25	25.00	60.15
U3	7	8	83	1.55	0.59	8.54 ± 1.38	8.85 ± 0.28	0.92	25.66	2.67	33.33	57.27
S1	6	9	132	1.51	0.50	10.00 ± 2.26	9.85 ± 0.02	0.91	43.93	3.25	36.11	49.85
S2	5	8	162	0.75	0.27	9.00 ± 2.25	9.05 ± 0.03	1.00	53.30	2.33	29.17	60.30
S3	6	7	205	1.17	0.46	7.35 ± 1.22	7.68 ± 0.09	0.93	66.35	3.00	42.86	45.61
R1	6	9	98	1.62	0.56	12.00 ± 4.48	10.07 ± 0.33	0.82	31.58	3.00	33.33	71.04
R2	7	9	106	1.31	0.41	12.51 ± 4.74	10.56 ± 0.27	0.79	33.95	2.42	26.85	74.39
R3	6	9	96	1.68	0.60	9.37 ± 2.09	9.51 ± 0.27	0.95	33.69	3.17	35.18	42.88

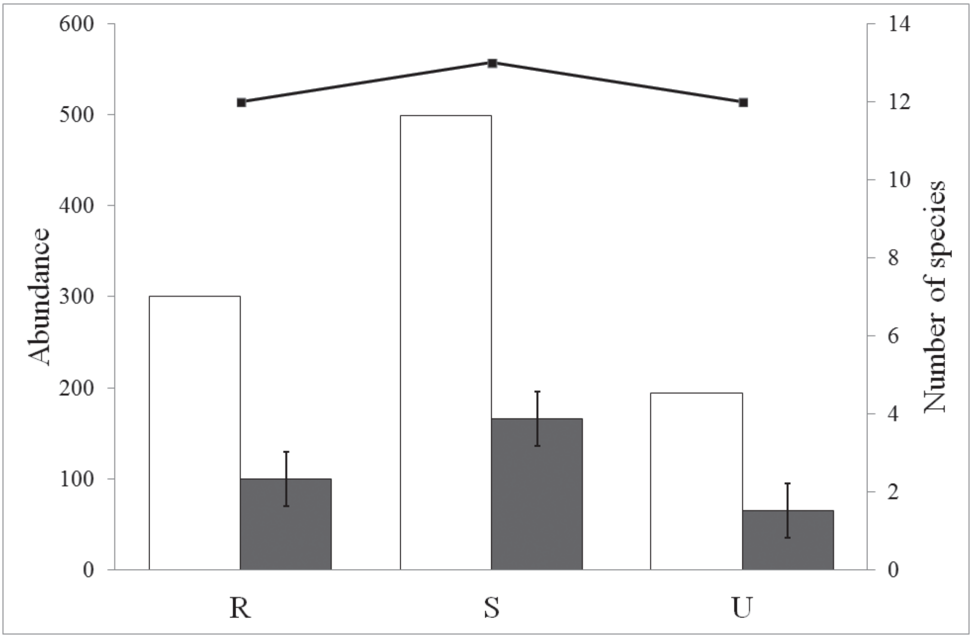


Figure 2. Total and average abundance of centipedes as well as total species richness along the urban-rural gradient. White columns show total abundance, grey columns show average abundance with bars with standard deviation, dark line shows species richness.

Table 2. One-way ANOVA results showing statistical differences of species richness, abundance, activity density (A density), and diversity (Shannon *H'*, temporal diversity, *β*t and proportion of cumulative *a* represented by average *a*, α%), along the urbanization gradient in Heraklion. Degrees of freedom (DF), sum of squares (SS), mean square (MS), F values (*F*). The last column shows the significant differences between the gradient levels (p < 0.05) based on the Tukey test. R: rural, S: suburban, U: urban.

	DF	SS	MS	F	p	Tukey test
Species richness	2	12.39	6.19	2.39	0.11	
Abundance	2	2664.11	1332.06	0.91	0.41	
Shannon *H'*	2	2.70	1.35	0.27	0.77	
A density	2	47.65	23.82	4.47	0.02	U < R, S
*β*t	2	653.45	326.72	0.96	0.39	
a%	2	5.56	2.78	3.09	0.03	U < R, S

The difference in species richness along the urbanization gradient was not statistically significant (Table 2). Likewise, although the average abundance of centipedes in suburban sites was about 1.7 and 2.6 times higher than in rural and urban sites respectively, we did not find any statistical significance (Table 2). Though the mean value of Shannon diversity index (*H'*) was higher in the rural sites (1.53 ± 0.2) than in the urban (1.29 ± 0.5) and suburban habitats (1.15 ± 0.4), it did not differ significantly among the three areas ($F = 0.27$, $p = 0.77$). In contrast, activity density differed significantly along

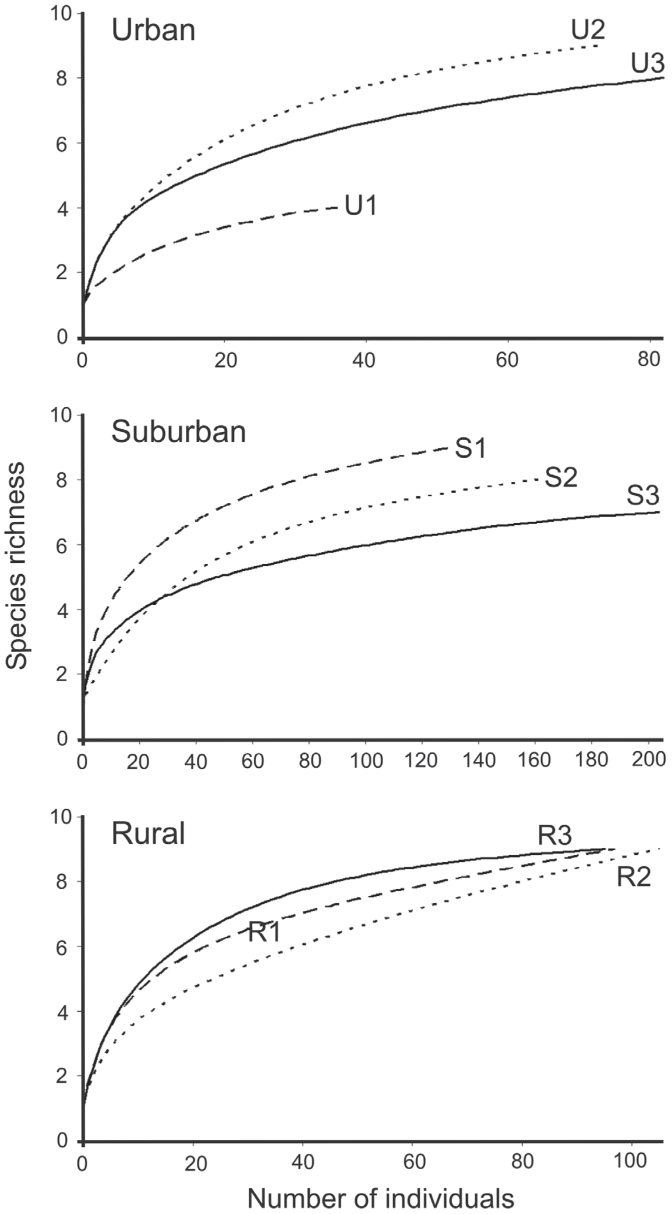


Figure 3. Species accumulation curves (recorded as a function of sampling effort) of the rate at which new species are found within each site along the urbanization gradient.

the gradient ($F = 4.47$, $p = 0.02$), as a result of the significantly lower values of A density in urban sites (21.2 in average) compared to suburban (54.5 in average) and rural sites (33.1 in average). Moreover, as shown in Table 2, the proportion of cumulative *a* represented by average *a* diversity (*a* %) is significantly smaller in urban sites compared to

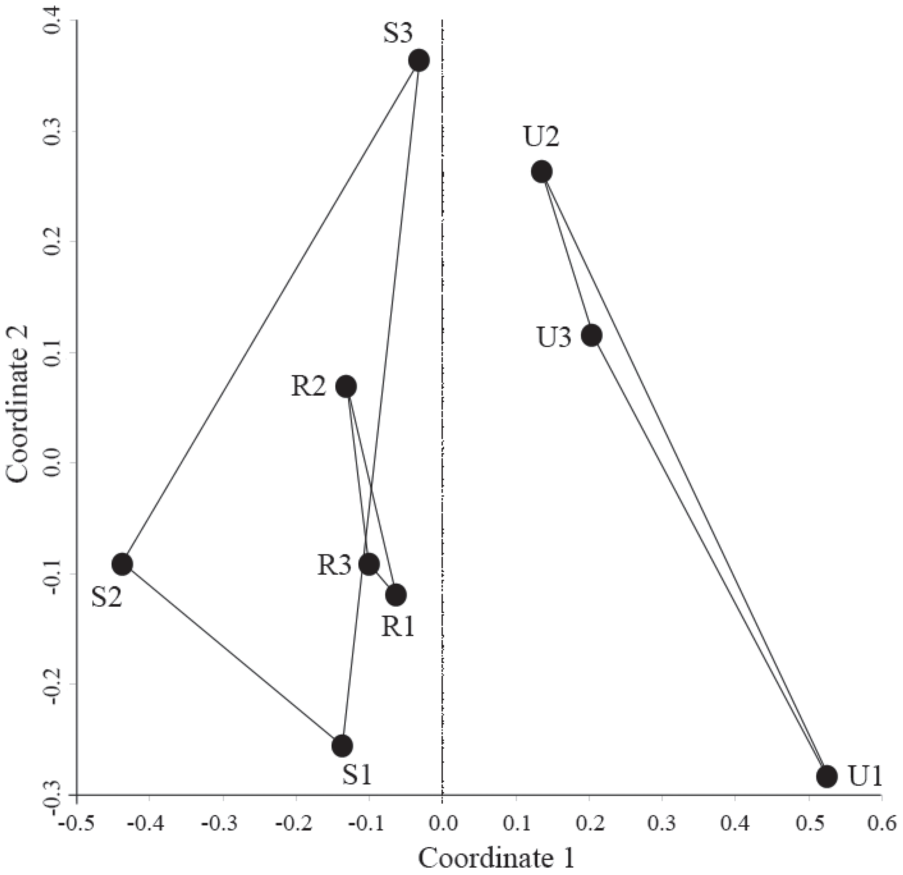


Figure 4. NMDS two-dimensional ordination plot based on the Bray-Curtis dissimilarity matrix of the nine sites along the urbanization gradient.

rural and suburban areas ($F = 3.09$, $p = 0.03$). However, the difference in temporal beta diversity (βt) along the gradient level was statistically insignificant (Table 2).

The structural difference of urban centipede assemblages compared to the other two habitat zones was apparent when we performed NMDS. The two-dimensional (2D) ordination plot explained 90% of the variance in the distance matrix (Axis 1: 72 %, Axis 2: 18 %, stress = 0.12; see Fig. 4). As expected, temporal beta diversity (βt) was independent of species richness (Spearman's $r_s = 0.05$, $p = 0.89$). Furthermore, βt decreases significantly ($r^2 = 0.47$, $p = 0.04$) as α% increases (that is, the difference between cumulative and average α decreases) (Fig. 5).

Scolopendra cretica was the dominant species in rural and suburban sites covering 50.6% and 86.9% of the total captures in these sites respectively. However, in urban sites its capturing coverage lowered to 2.1%, while the dominant species in urban sites

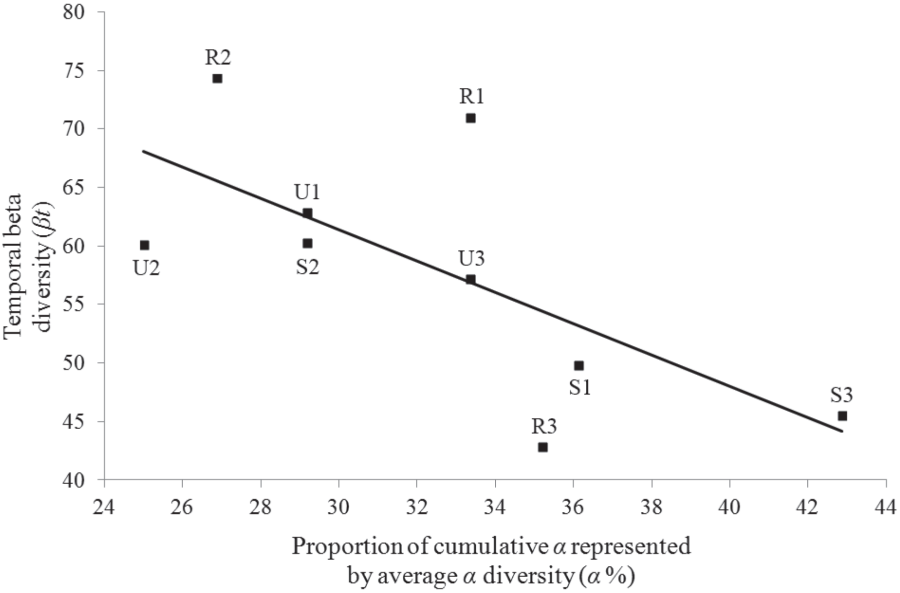


Figure 5. Simple linear regression between temporal beta diversity (βt) and the proportion of cumulative a represented by average a diversity (a%). Model: (βt) = 101.65 – 1.34(a%), r^2 = 0.47, p = 0.04. U: urban sites, S: suburban sites, R: rural sites.

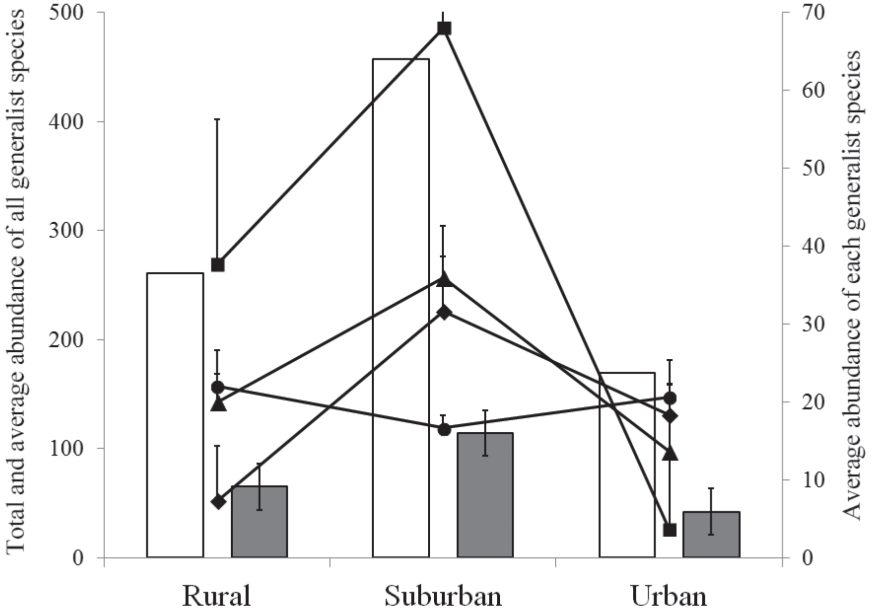


Figure 6. Total and average abundance of all four generalist species as well as average abundance of each generalist species along the urban-rural gradient, *Eupolybothrus litoralis* (♦), *Lithobius nigripalpis* (▲), *Scolopendra cretica* (■), *Scutigera coleoptrata* (●). White columns show total abundance, grey columns show average abundance with bars with standard deviation.

was *Scutigera coleoptrata* (72.7%). *S. coleoptrata* also covered a large portion of captures in rural areas (21%). Two more species could be considered dominant, namely *Lithobius nigripalpis* that covers 14.1% and 18.5% of the total captures in urban and suburban sites respectively, and, *Eupolybothrus litoralis* that covers 25.5% and 19.8% of the total captures in urban and suburban sites respectively. *L. nigripalpis* was also dominant in rural areas covering 12.8% of the total captures. The percentage of individuals of opportunistic centipede species to the total individuals proved to differ significantly along the urbanization gradient. The abundance of opportunistic species in the suburban sites was 1.75 and 2.7 times higher than their abundance in rural and urban sites respectively (Fig. 6). Generalist species covered 91.6% of the total captures in suburban areas, whereas the respective percentages in rural and urban sites were 87% and 55%. Specifically, all generalist species were mostly abundant in suburban sites, except for *Scutigera coleoptrata* which in this area appeared to be less abundant.

Discussion

According to several studies, urbanization reduces species richness in many animal groups owing to the impoverished flora, in terms of habitat loss (McKinney 2002) and habitat fragmentation leading to isolated populations (Collins et al. 2000). In addition, studies have shown that the urban soil content in nitrogen and carbon is profoundly lower compared to the rural soil either owing to their cycle alteration (Lorenz and Lal 2009) or as a result of the impenetrable surfaces in urban areas (Raciti et al. 2012).

Unlike the aforementioned examples, our results failed to indicate negative urbanization effect on centipede species richness and diversity. Alaruikka et al. (2002) studied carabid and spider species in Finland only to reach the same conclusion on the abundance and species richness, suggesting that, in particular, spiders might be more sensitive to small-scale habitat changes rather than to large-scale changes. Likewise, urbanization has not reduced overall centipede species richness and diversity in the city of Poznaǹ (Leśniewska et al. 2008), nor has reduced the ground beetles species richness in several cases where data from Globenet Project were used (Magura et al. 2010b). Even though abundance in our results, differed along the urbanization gradient with the highest abundance found in suburban areas, followed by the second higher abundance in rural areas, the difference was not confirmed statistically.

Additionally, in terms of species richness and diversity, our results are not statistically significant to support the suburban peak (McKinney 2002). Similar results were also reported about isopods (Hornung et al. 2007) and ground-dwelling spiders (Horvath et al. 2012). In general, suburban environments are considered as transitional zones between natural and urban habitats and show characteristically high environmental heterogeneity, since diverse habitats occur together alongside one another (McKinney 2002). This pattern is well-documented in numerous urban-to-rural gradient studies that examine changes in diversity on plants (Kowarik 1995), butterflies (Blair 1999, Konvicka and Kadlec 2011), mammals, birds, lizards, bumblebees ants (McKinney 2002 and references therein) and carabid beetles (Tothmeresz et al. 2011). On the other hand, Ishitani et al. (2003) found that species richness in carabid beetles increases from urban to rural environments in Japan, similar to the case of Magura et al. (2010a) who studied ground-dwelling spiders along an urban-rural forest gradient in Hungary.

As for temporal beta diversity (βt), it showed no difference among the three zones indicating no variance in species richness between sampling periods. The high (βt) values found, in most cases above 50, are attributed to nomadic assemblages or degraded habitats, under fast environmental alterations or intense perturbations (Romanuk and Kolasa 2001). In addition, temporal turnover of species assemblages barely changed along the urbanization gradient in the city of Heraklion. However, our results have validated α% as a quantitative metric of temporal turnover as it was inversely proportional to βt. These results are in accordance with Moreno and Halffter (2001). With α% values relatively low (see Table 1) and statistically higher in rural and suburban areas (see Table 2), we assume that temporal turnover is greater in urban areas. Moreover, statistically significant lower activity density in the suburban and rural sites compared to the urban sites shows both higher centipede activity and possibly higher population density.

The structure of centipede assemblages differed substantially along the urban-rural gradient. We also observed great similarity in centipede diversity between rural and suburban sites. The distribution from rural to suburban areas is not impossible since in suburban sites human constructions retain green areas as centipede habitats. Within the city of Heraklion though, species composition is significantly different from both rural and suburban due to great habitat loss and fragmentation. The four generalist species (*Eupolybothrus litoralis*, *Lithobius nigripalpis*, *Scolopendra cretica*, *Scutigera coleoptrata*) were found in great abundance in all zones. However, highest centipede abundance was found in suburban areas, followed by the rural areas and fewer individuals were caught in the centre of the city, showing that mild human pressure can promote the abundance of these species. Three species, namely *Lithobius aeruginosus*, *L. lapidicola*, and *Schendyla nemorensis* were found exclusively in the city centre, suggesting that human activities such as gardening and landscaping introduce new species in cities through transferred soil. On the other hand, *Cryptops trisulcatus* and *Pachymerium ferrugineum* occurred only in suburban sites indicating specific habitat preferences under large stones and sand soil substrate, respectively. Although *Scolopendra cretica* was the dominant species in rural and suburban habitats, its capturing coverage was extremely low in urban environments showing low tolerance to the intense human activity. In contrast, *Scutigera coleoptrata* with a capturing coverage of about 73% showed a highly opportunistic character in the city habitats. Finally, only one representative of the species *Lithobius pamukkalensis* was collected in rural sites, which is so far the most western distribution of this species in Crete.

Conclusion

Different studies consistently highlight alternative animal responses to urbanization. Even same animal groups differed in their reaction to the increasing human activity in different cities. Our study failed to indicate negative urbanization effect on centipede species richness and diversity in the city of Heraklion. It is noteworthy that even though there is a trend of increasing abundance towards the suburban habitats, the difference was not confirmed statistically. Furthermore, our results are not consistent with those that have supported the intermediate disturbance hypothesis. This means that the suburban environment of Heraklion may not be considered as transitional zone between natural and urban habitats.

Acknowledgements

We thank Aris Nersessian and Nikos Tsirigotakis for their contribution to set pitfall traps in the field and collecting specimens. We are grateful to Manolis Nikolakakis for mapping sampling sites. We also thank two anonymous referees for their critical comments on the manuscript and Marc Rossello for improving the clarity of the manuscript.

References

Alaruikka D, Kotze DJ, Matveinen K, Niemel J (2002) Carabid Beetle and Spider Assemblages Along a Forested Urban – Rural Gradient in Southern Finland. Journal of Insect Conservation 6: 195–206. doi: 10.1023/A:1024432830064

Andersson E (2006) Urban Landscapes and Sustainable Cities. Ecology and Society 11(1), art. 34.

Blair RB (1999) Birds and butterflies along an urban gradient: Surrogate taxa for assessing biodiversity? Ecological Applications 9: 164–170. doi: 10.1890/1051-0761(1999)009[0164:BABAAU]2.0.CO;2

Clark PJ, Reed JM, Chew FS (2007) Effects of urbanization on buttterfly species richness, guild structure, and rarity. Urban Ecosystems 10(3): 321–337. doi: 10.1007/s11252-007-0029-4

Collins JP, Kinzig A, Grimm N-B, Fagan WF, Hope D, Wu J, Borer WT (2000) A new urban ecology. American Scientist 88: 416–425. doi: 10.1511/2000.5.416

Colwell RK, Chao A, Gotelli NJ, Lin S-Y, Mao CX, Chazdon RL, Longino JT (2012) Models and estimators linking individual-based and sample-based rarefaction, extrapolation, and comparison of assemblages. Journal of Plant Ecology 5: 3–21. doi: 10.1093/jpe/rtr044

Colwell RK, Coddington JA (1994) Estimating terrestrial biodiversity through extrapolation. Philosophical Transactions of the Royal Society B 345: 101–118. doi: 10.1098/rstb.1994.0091

Eldredge N (1998) Life in the Balance. Humanity and the Biodiversity Crisis. Princeton University Press, Princeton.

Germaine SS, Schweinsburg RE, Germaine HL (2001) Effects of residential density on sonoran desert nocturnal rodents. Urban Ecosystems 5(3): 179–185. doi: 10.1023/A:1024041903822

Hammer Ø, Harper DAT, Ryan PD (2001) PAST: paleontological statistics software package for education and data analysis. Palaeontol Electron 4(1): 9. http://palaeo-electronica.org/2001_1/past/issue1_01.htm

Hanski I (2004) Metapopulation theory, its use and misuse. Basic and Applied Ecology 5: 225–229. doi: 10.1016/j.baae.2004.03.002

Hornung E, Tóthmérész B, Magura T, Vilisics F (2007) Changes of Isopod Assemblages Along an Urban–suburban–rural Gradient in Hungary. European Journal of Soil Biology 43(3): 158–165. doi: 10.1016/j.ejsobi.2007.01.001

Horváth R, Magura T, Tóthmérész B (2012) Ignoring Ecological Demands Masks the Real Effect of Urbanization: a Case Study of Ground-Dwelling Spiders Along a Rural–urban Gradient in a Lowland Forest in Hungary. Ecological Research 27(6): 1069–1077. doi: 10.1007/s11284-012-0988-7

Ishitani MD, Kotze J, Niemelä J (2003) Changes in Carabid Beetle Assemblages Across an Urban-Rural Gradient in Japan. Ecography 26(4): 481–489. doi: 10.1034/j.1600-0587.2003.03436.x

Kaltsas D, Panayiotou E, Chatzaki M, Mylonas M (2014) Ground spider assemblages (Araneae: Cnaphosidae) along an urban-rural gradient in the city of Heraklion, Greece. European Journal of Entomology 111(1): 59–67. doi: 10.14411/eje.2014.007

Konvicka M, Kadlec T (2011) How to increase the value of urban areas for butterfly conservation? A lesson from Prague nature reserves and parks. European Journal of Entomology 108: 219–229. doi: 10.14411/eje.2011.030

Kowarik L (1995) On the role of alien species in urban flora and vegetation. In: Pysek PK, Parch M, Rejmanek M, Wade PM (Eds) Plant Invasions: General Aspects and Special Problems. SPB Academic, Amsterdam, The Netherlands, 85–103.

Leśniewska M, Leśniewski P, Szybiak K (2008) Effect of urbanization on centipede (Chilopoda) diversity in the Wielkopolska-Kujawy Lowlands of western Poland. Biologia 63(5): 711–719. doi: 10.2478/s11756-008-0121-0

Lorenz K, Lal R (2009) Biogeochemical C and N cycles in urban soils. Environment International 35: 1–8. doi: 10.1016/j.envint.2008.05.006

Magura T, Horváth R, Tóthmérész B (2010a) Effects of Urbanization on Ground-Dwelling Spiders in Forest Patches, in Hungary. Landscape Ecology 25(4): 621–629. doi: 10.1007/s10980-009-9445-6

Magura T, Lövei GL, Tóthmérész B (2010b) Does Urbanization Decrease Diversity in Ground Beetle (Carabidae) Assemblages? Global Ecology and Biogeography 19(1): 16–26. doi: 10.1111/j.1466-8238.2009.00499.x

Magurran AE (1988) Ecological diversity and its measurement. Princeton University Press, Princeton. doi: 10.1007/978-94-015-7358-0

McDonnell MJ, Breuste J, Hahs AK (2009) Ecology of Cities and Towns: A Comparative Approach. Cambridge University Press. doi: 10.1017/CBO9780511609763

McKinney ML (2002) Urbanisation, Biodiversity, and Conservation. Bioscience 52(10): 883–890. doi: 10.1641/0006-3568(2002)052[0883:UBAC]2.0.CO;2

Meijer SS, Whittaker RJ, Borges PAV (2011) The effects of land-use change on arthropod richness and abundance on Santa Maria Island (Azores): unmanaged plantations favour endemic beetles. Journal of Insect Conservation 15: 505–522. doi: 10.1007/s10841-010-9330-2

Moreno CE, Halffter G (2001) Spatial and Temporal Analysis of α, β and γ Diversities of Bats in a Fragmented Landscape. Biodiversity & Conservation 10(3): 367–382. doi: 10.1023/A:1016614510040

Niemelä J (1999) Is there a need for a theory of urban ecology? Urban Ecosystems 3: 57–65. doi: 10.1023/A:1009595932440

Niemelä J, Kotze J, Ashworth A, Brandmayr P, desender K, New T, Penev L, Samways M, Spence J (2000) The search for common anthropogenic impacts on biodiversity: a global network. Journal of Insect Conservation 4: 3–9. doi: 10.1023/A:1009655127440

Niemelä J, Kotze DJ, Venn S, Penev L, Stoyanov I, Hartley D, Montes De Oca E (2002) Carabid Beetle Assemblages (Coleoptera, Carabidae) Across Urban-Rural Gradients: an International Comparison. Landscape Ecology 17(5): 387–401. doi: 10.1023/A:1021270121630

Oliver I, Beattie AJ (1996) Designing a cost-effective invertebrate survey: a test of methods for rapid assessment of biodiversity. Ecological Applications 6: 594–607. doi: 10.2307/2269394

Raciti SM, Hutyra LR, Finzi AC (2012) Depleted soil carbon and nitrogen pools beneath impervious surfaces. Environmental Pollution 164: 248–251. doi: 10.1016/j.envpol.2012.01.046

Ricklefs RE (1990) Ecology. W. H. Freeman and Company, New York, 896 pp.

Romanuk TN, Kolasa J (2001) Simplifying the complexity of temporal diversity dynamics: a differentiation approach. Ecoscience 8: 259–263.

Simaiakis S, Minelli A, Mylonas M (2004) The centipede fauna (Chilopoda) of Crete and its satellite islands (Greece, Eastern Mediterrenean). Israel Journal of Zoology 50: 367–418. doi: 10.1560/HBE1-QJER-YDKF-BD8Q

Sorace A (2001) Value to Wildlife of Urban-Agricultural Parks: A Case Study from Rome Urban Area. Environmental Management 28(4): 547–560. doi: 10.1007/s002670010243

Tóthmérész B, Máthé I, Balázs E, Magura T (2011) Responses of Carabid Beetles to Urbanization in Transylvania (Romania). Landscape and Urban Planning 101(4): 330–337. doi: 10.1016/j.landurbplan.2011.02.038

Ulrich W, Komosinski K, Zalewski M (2008) Body size and biomass distributions of carrion visiting beetles: do cities host smaller species? Ecological Research 23(2): 241–248. doi: 10.1007/s11284-007-0369-9

Vogiatzakis IN, Rackham O (2008) Chapter 11: Crete. In: Vogiatzakis IN, Pungetti G, Mannion A (Eds) Mediterranean Island Landscapes: Natural and Cultural Approaches. Landscape Series Vol. 9. Springer, Dordrecht, 245–270. doi: 10.1007/978-1-4020-5064-0_11

Wallwork JA (1976) The distribution and diversity of soil fauna. Academic Press, London, 355 pp.

Ward DF, Lariviere MC (2004) Terrestrial invertebrate surveys and rapid biodiversity assessment in New Zealand: lessons from Australia. New Zealand. Journal of Ecology 28(1): 151–159.

Wilcove DS, Rothstein D, Dubow J, Phillips A, Losos E (1998) Quantifying threats to imperiled species in the United States. Bioscience 48: 607–615. doi: 10.2307/1313420

Zamora J, Verdú JR, Galante E (2007) Species richness in Mediterranean agroecosystems: spatial and temporal analysis for biodiversity conservation. Biological Conservation 134: 113–121. doi: 10.1016/j.biocon.2006.08.011

Supplementary material 1

Abundance and activity density of centipede species per sampling site.

Authors: Gabriella Papastefanou, Eleni Panayiotou, Moisis Mylonas, Stylianos Michail Simaiakis

Data type: occurrence

Explanation note: List of species and individuals captured at each sampling site.

ZooKeys 510: 181–195 (2015)
doi: 10.3897/zookeys.510.8657
http://zookeys.pensoft.net

RESEARCH ARTICLE

A peer-reviewed open-access journal
ZooKeys
Launched to accelerate biodiversity research

Distribution of millipedes (Myriapoda, Diplopoda) along a forest interior – forest edge – grassland habitat complex

Dávid Bogyó[1,2], Tibor Magura[1], Dávid D. Nagy[3], Béla Tóthmérész[3]

1 *Department of Ecology, University of Debrecen, P.O. Box 71, Debrecen H-4010, Hungary* **2** *Hortobágy National Park Directorate, P.O. Box 216, Debrecen H-4002, Hungary* **3** *MTA-DE Biodiversity and Ecosystem Services Research Group, P.O. Box 71, Debrecen H-4010, Hungary*

Corresponding author: *Dávid Bogyó* (davidbogyo@yahoo.co.uk)

Academic editor: *Ivan H. Tuf* | Received 27 September 2014 | Accepted 4 May 2015 | Published 30 June 2015

http://zoobank.org/47B52B34-E4BB-4F13-B5BE-162C2CD2AE7B

Citation: Bogyó D, Magura T, Nagy DD, Tóthmérész B (2015) Distribution of millipedes (Myriapoda, Diplopoda) along a forest interior – forest edge – grassland habitat complex. In: Tuf IH, Tajovský K (Eds) Proceedings of the 16th International Congress of Myriapodology, Olomouc, Czech Republic. ZooKeys 510: 181–195. doi: 10.3897/zookeys.510.8657

Abstract

We studied the distribution of millipedes in a forest interior-forest edge-grassland habitat complex in the Hajdúság Landscape Protection Area (NE Hungary). The habitat types were as follows: (1) lowland oak forest, (2) forest edge with increased ground vegetation and shrub cover, and (3) mesophilous grassland. We collected millipedes by litter and soil sifting. There were overall 30 sifted litter and soil samples: 3 habitat types × 2 replicates × 5 soil and litter samples per habitats. We collected 9 millipede species; the most abundant species was *Glomeris tetrasticha*, which was the most abundant species in the forest edge as well. The most abundant species in the forest interior was *Kryphioiulus occultus*, while the most abundant species in the grassland was *Megaphyllum unilineatum*. Our result showed that the number of millipede species was significantly lower in the grassland than in the forest or in the edge, however there were no significant difference in the number of species between the forest interior and the forest edge. We found significantly the highest number of millipede individuals in the forest edge. There were differences in the composition of the millipede assemblages of the three habitats. The results of the DCCA showed that forest edge and forest interior habitats were clearly separated from the grassland habitats. The forest edge habitat was characterized by high air temperature, high soil moisture, high soil pH, high soil enzyme activity, high shrub cover and low canopy cover. The IndVal and the DCCA methods revealed the following character species of the forest edge habitats: *Glomeris tetrasticha* and *Leptoiulus cibdellus*. Changes in millipede abundance and composition were highly correlated with the vegetation structure.

Keywords

Edge effect, soil arthropod, biodiversity, forest ecosystem

Introduction

Millipedes (Myriapoda, Diplopoda) are detrivores, feeding mainly on decaying plant material and they found usually under leaf litter (Hopkin and Read 1992). The typical and suitable habitats for millipedes are the deciduous temperate, subtropical and tropical forests where the population density of these animals can reach 1000 individuals per square metre (Golovatch and Kime 2009). Millipedes are also found in caves, deserts, grasslands, and in boreal (taiga) forests (Hopkin and Read 1992, Golovatch and Kime 2009). Changes in millipede's assemblage composition, species diversity and population density correlate with vegetation structure (David et al. 1999). In habitat mosaics saprophagous macroarthropods have high species richness (David and Handa 2010). During recent destruction and fragmentation of forested habitats around the world, the percentage of forest edges rapidly grows (Saunders et al. 1991, David and Handa 2010). To monitor these processes, forest edges are in focus of the ecological and conservation researches (Ries et al. 2004, Tóthmérész et al. 2014).

Forest edges have distinctive structure with highly variable environmental conditions. Ries and Sisk (2004) defined edges as the boundary between patches with differing qualities. Edge effects create differences in biotic and abiotic factors on the borders of two habitats. At forest edges, changes in microclimate (radiation fluxes, wind, water flux) and decomposition processes can support changes in vegetation compared to the forest interior (Saunders et al. 1991, Gehlhausen et al. 2000, Riutta et al. 2012). A complex interaction of microclimate and biotic factors drives the changes in the vegetation across the forest edge to the forest interior (Gehlhausen et al. 2000). On the other hand, changes in the spatial distribution of animal species near habitat edges are also reported (Ries and Sisk 2004, Wirth et al. 2008). Classically, an increased species richness and density is predicted at habitat edges (Odum 1971, Magura et al. 2001, Wirth et al. 2008).

The edge effect on different arthropod taxa was frequently studied during the last decades (Coleoptera: Magura 2000, Lövei et al. 2006; Ewers and Didham 2008; Isopoda: Antonovic et al. 2012; multi-taxa approaches: Frainer and Duarte 2009, Biereinger et al. 2013). Millipedes were relatively little studied in the context of the edge effect, in spite of the rapid changes in temperature, soil structure and soil water content at forest edges (Camargo and Kapos 1995, Gehlhausen et al. 2000) and its major influence on litter decomposition processes in forests. But a few papers are available discussing the edge effect on millipedes (Gulička 1957, David et al. 1999, Tracz 2000, Weiermans and van Aarde 2003, Didham et al. 2009, Riutta et al. 2012).

The aim of our study was to test the edge effect hypothesis on millipedes, that predicts higher species diversity and population density in forest edges than in the adjacent habitats (Odum 1971, Magura et al 2001). Our study provided data on millipede abundance, diversity and assemblage composition in a habitat complex of forest interior, forest edge and grassland habitats. We also studied the relationships between saprophagous millipedes and selected environmental factors.

Methods

We tested the edge effect hypothesis on millipedes in forest interior – forest edge – grassland habitat complex in Northeast Hungary. The study area is located in the Hajdúság Landscape Protection Area, about 3.5 km north-east from the centre of Vámospércs, 130–135 m above sea level (47°33'09"N, 21°56'18"E). The Hajdúság Landscape Protection Area is a mosaic of grasslands, forests and wetlands. Average annual temperature of this region is 9.6–9.8 °C, while average annual rainfall is 550–575 mm. Brown forest soils and sandy soils are the main soil types of the study area.

The studied habitat types were as follows: (1) a closed forest dominated by English oak (*Quercus robur*) and associated with narrow-leafed ash (*Fraxinus angustifolia* ssp. *pannonica*), field elm (*Ulmus minor*) and common alder (*Alnus glutinosa*) with shrubs and herbs and high percentage of canopy cover, (2) a forest edge with increased ground vegetation and dense shrub cover (dominated by *Crataegus monogyna*, *Prunus spinosa* and *Rhamnus catharticus*) as well as some invasive tree species, like *Robinia pseudoacacia*, and (3) a mesophilous grassland with dense herbaceous vegetation, dominated by *Festuca pratensis*, *Poa pratensis*, *Deschampsia caespitosa* and *Carex* species, together with *Orchis laxiflora* ssp. *elegans* and *Dactylorrhiza incarnata*.

This area was a forested area during the last decades. The mesophilous grassland is utilized for grazing (sheep and cattle). The age of trees in the forest interior and forest edge was 45–50 years, while the forest was unmanaged during the last 30 years. The density of trees was 250–300 trees/ha. The width of the forest edge was 6-14m. Non-native species occurred sparsely with single specimens in the forest edge.

We collected millipedes monthly (7 times from April to October in 2009; the year of 2009 was free from extreme weather conditions) during the vegetation period. Sampling of millipedes followed David et al. (1999) by litter and soil sampling using a metal frame (25 cm × 25 cm, and a depth of 5 cm). The material was sifted at the study site. The samples were sorted in the laboratory by hand within the next 24–48 hours. We collected samples along 100m long transects (parallel to the forest edge) per habitat type using 5 plots in each habitat. Along the 100 m long transects we signed 5 plot centers (25 m from each other), with 2.5 m radius around, from where every time a sample was taken at random. We collected 30 samples altogether in two spatial replicates, having a distance of 150m from each other (3 habitat types × 2 replicates × 5 samples). For identification we have used the works of Hauser and Voigtländer (2009) and Dziadosz (1968). Valid nomenclature was applied according to Enghoff (2013).

We selected 13 environmental variables to test the influence on millipede assemblages. We measured soil pH value, soil dehydrogenase activity, soil moisture, soil temperature (in a depth of 2 cm), air temperature and relative humidity on the surface

Table 1. Average values of the environmental variables in the studied habitats.

	Grassland	Edge	Forest
Air temperature (°C)	26.60	24.20	21.61
Canopy cover (%)	0.00	50.30	69.80
Cover of decaying wood (%)	0.00	23.50	23.30
Cover of herbs (%)	93.80	25.30	23.50
Cover of leaf litter (%)	0.00	83.40	92.60
Dehydrogenase enzyme activity	0.37	0.31	0.29
Depth of leaf litter (cm)	0.00	2.47	2.88
Humidity (%)	69.88	66.08	71.30
Number of woody plant species	0.00	5.40	4.90
pH	8.54	7.83	7.40
Shrub cover (%)	0.00	66.10	34.80
Soil moisture (%)	33.28	28.17	16.97
Soil temperature (°C)	22.30	17.35	16.77

in the study plots. For pH measurement soil solution was prepared from 6.0 g wet soil. Soil samples were put into plastic beakers and after it filled with 50 ml deionized water. The pH was measured with a digital measurement type Testo 206 (Testo AG, Germany). Soil dehydrogenase enzyme activity (indicator of microbiological activity through the oxidative metabolisms in soil) was determined using triphenyltetrazolium chloride method (Alef 1995).We measured soil moisture in the laboratory, comparing fresh and dried (at 105 °C for 24 hours) samples. Soil temperature, air temperature, and relative humidity were measured with field instrument Voltcraft DT-8820. We used the average of measurements (spring, summer, autumn). We also estimated the percentage cover of leaf litter, decaying wood materials, herbs, shrubs and canopy and measured the depth of the leaf litter within a circle of 250 cm radius around the plot centers. In addition we counted the number of woody plant species within a circle of 250 cm radius around the plot centers (Table 1).

Data analysis

Mixed Generalized Linear Model (GLMM) was used to test differences in the millipede abundance, species richness and Shannon diversity among the three habitat types (forest interior, forest edge, grassland). Factorial design was used; habitats and spatial replicates were regarded as factors. The response variables (millipede abundance, species richness, and Shannon diversity) were defined as a quasi-Poisson distribution with log link function (Zuur et al. 2009). When GLM revealed a significant difference between the means, a Tukey's HSD test was performed for multiple comparisons among means.

The composition of the millipede assemblages along the forest interior – forest edge – grassland habitats was compared by hierarchical cluster analysis based on the abundance of millipedes using the Hellinger distance and the Ward fusion algorithm (Legendre and Legendre 1998). Quantitative character species of the studied habitats were identified using indicator value method (IndVal). This method quantifies the fidelity and specificity of the species in relation to groups of sites in a user-specified classification of sites, and tests for the statistical significance of the associations by permutation (Dufrene and Legendre 1997, Elek et al. 2001). Relationships between the studied environmental factors and the abundance of millipedes were examined using detrended canonical correspondence analysis (DCCA) by second order polynomials using the Canoco software package. Triplot scaling in the ordination was focused on the inter-species distances.

Results

There were 999 specimens of millipedes (Diplopoda) identified to species level. In total 9 species of 4 families (Glomeridae, Julidae, Mastigophorophyllidae, Polydesmidae) were recorded from the study area (Table 2).

The forest edge and forest interior habitats were more species rich (9 species) than the grassland. 5 species of millipedes was found in the grassland habitat. *Glomeris tetrasticha* , *Julus terrestris*, *Kryphioiulus occultus*, *Leptoiulus cibdellus* and *Megaphyllum unilineatum* were recorded in all studied habitats. The following millipede species were the most frequent: *Glomeris tetrasticha* (416 individuals), *Kryphioiulus occultus* (269 individuals) and *Mastigona bosniensis* (142 individuals), while *Brachyiulus bagnalli* (8 individuals) and *Brachydesmus superus* (11 individuals) had the lowest total abundance in the studied habitats. The most abundant millipede species, *Glomeris tetrasticha* represented 41.6% of the total millipede catch. The same species was also the most frequent millipede (54.9% of the millipede individuals) in the edge habitat. The highest total abundance of millipedes was found in the edge habitat (696 individuals), while the lowest number of millipede individuals (31) was found in the grassland habitat.

Table 2. List of millipede species with their abundances recorded in the studied habitats.

	Grassland	Edge	Forest
Brachyiulus bagnalli (Broelemann, 1924)		3	5
Brachydesmus superus Latzel, 1884		1	10
Glomeris tetrasticha Brandt, 1833	1	382	33
Julus terrestris Linnaeus, 1758	6	8	8
Kryphioiulus occultus (C.L. Koch, 1847)	7	121	141
Leptoiulus cibdellus (Chamberlin, 1921)	1	34	2
Mastigona bosniensis (Verhoeff, 1897)		104	38
Megaphyllum unilineatum (C.L. Koch, 1838)	16	21	12
Polydesmus complanatus (Linnaeus, 1761)		22	23
Total	31	696	272

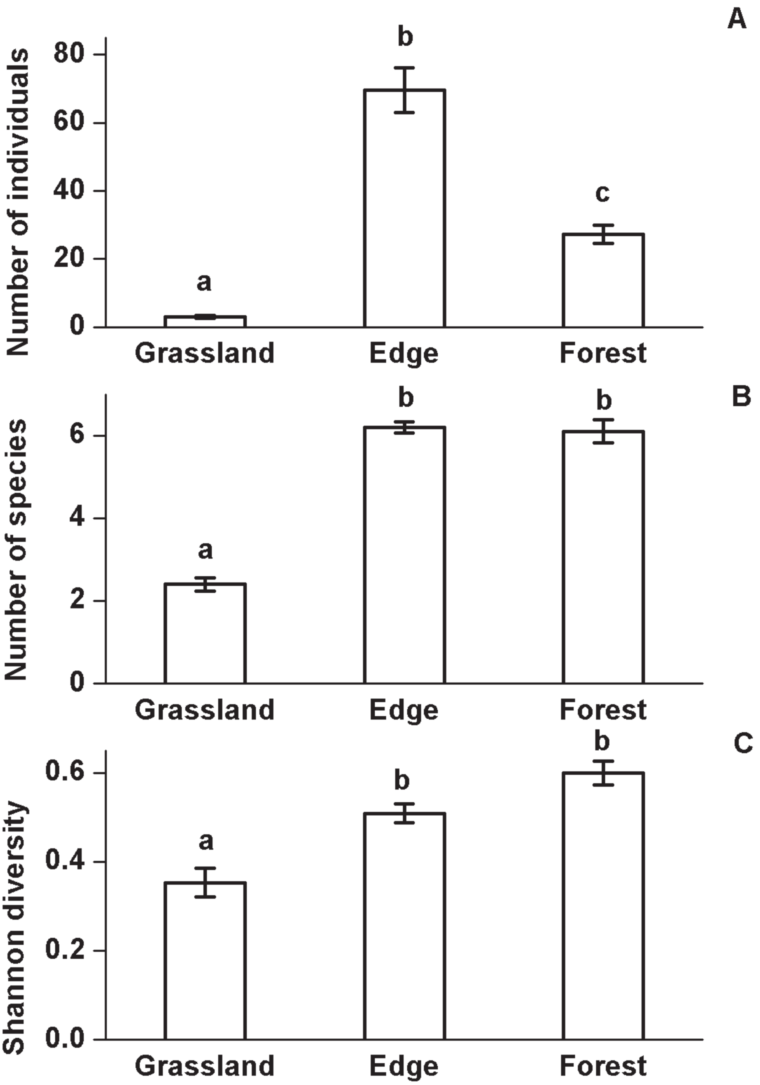


Figure 1. Millipede abundance, species richness and Shannon diversity at the studied habitats. Mean values (±SD) of the overall millipede abundance (**A**), species richness (**B**) and Shannon diversity (**C**) per samples at the studied habitats. Different letters indicate significant differences by Tukey test.

The number of millipede individuals was significantly higher in the forest edge than in the grassland and forest interior. Furthermore, the number of millipede individuals was significantly higher in the forest interior than in the grassland ($Chi^2=179.275$; df=2; $p<0.0001$; Figure 1a). The number of millipede species was significantly lower in the grassland than in the forest edge and forest interior ($Chi^2=231.974$; df=2; $p<0.0001$; Figure 1b), while there was no difference between the number of millipede species in the forest edge and forest interior. A similar result was found for the Shannon diversity

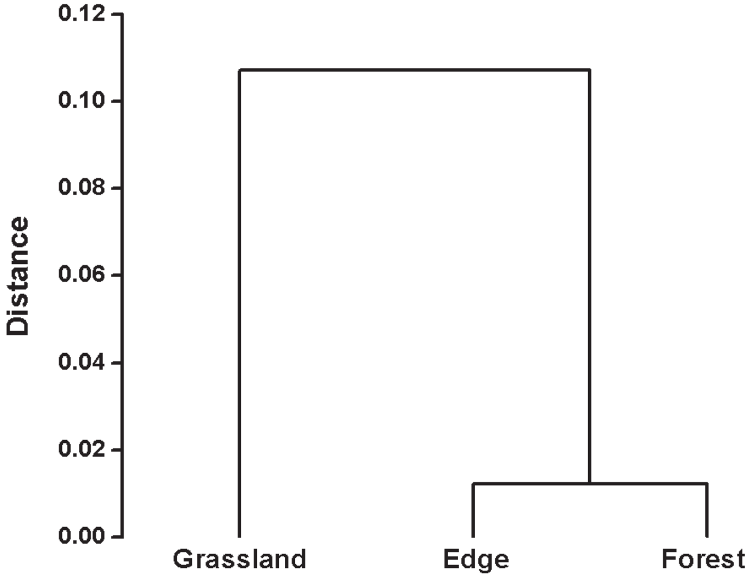


Figure 2. Hierarchical cluster analysis of millipede assemblages of the studied habitats using Hellinger distance and Ward fusion method.

Table 3. Habitat preference and quantitative character values of the millipede species presented with more than 30 individuals altogether. The IndVal column shows the species character value for the corresponding cluster level. Notations: * - p<0.05. A: the number of specimens present, B: the number of samples where the species is present in the sample group.

Species	IndVal	p	Grassland		Edge		Forest	
			A	B	A	B	A	B
Forest edge								
Leptoiulus cibdellus	82.7	*	1	1	34	9	2	2
Forest edge and forest interior								
Glomeris tetrasticha	99.5	*	1	1	382	10	33	10
Mastigona bosniensis	95	*	0	0	104	10	38	9
Kryphioiulus occultus	90.2	*	7	7	121	9	141	10
Polydesmus complanatus	85	*	0	0	22	8	23	9

of the millipede assemblage which was significantly lower in the grassland habitat than in the forest edge and forest interior ($Chi^2=40.849$; df=2; $p<0.0001$; Figure 1c). There was no significant difference between the diversity of millipede assemblage in the forest edge and forest interior habitats.

The millipede assemblages of the studied habitats formed two separated groups by hierarchical cluster analysis (Figure 2). The first group included the grassland plots, while the second one included the forest edge's and forest interior's plots.

We identified two groups of quantitative character species by the IndVal method for the studied habitats (Table 3): (1) species characteristic of the forest edge habitat

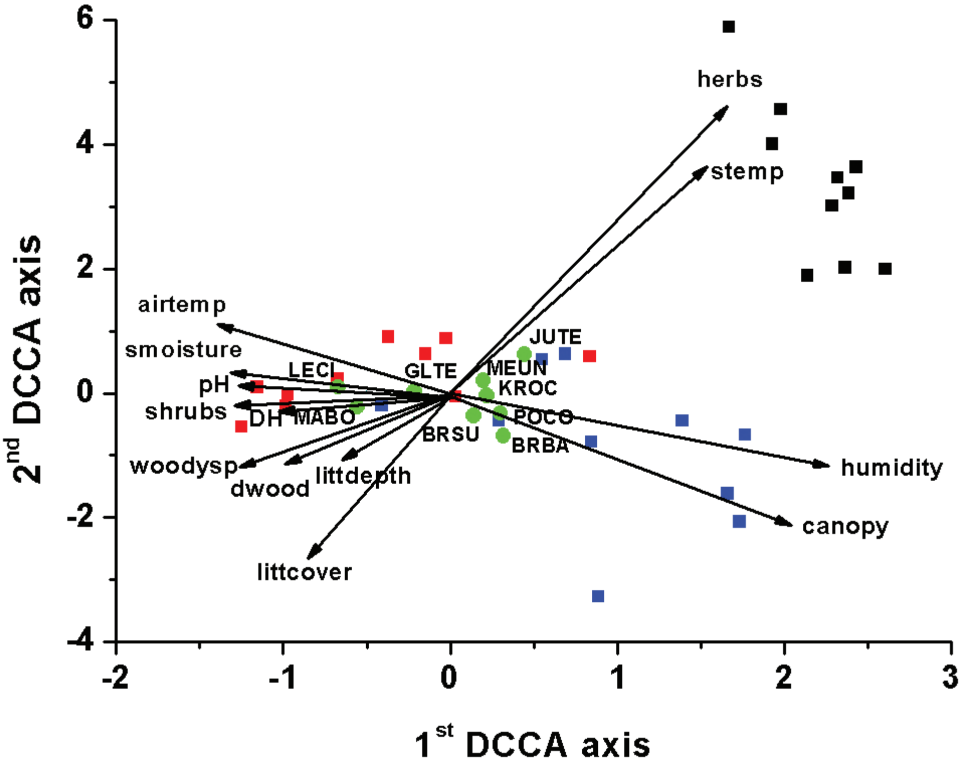


Figure 3. DCCA analysis for the millipede species of the study area. Squares represent the sampled habitats (blue squares: samples from the forest interior habitat; red squares: samples from the forest edge habitat; black squares: samples from the grassland habitat).The arrows denote the increase of the value of the environmental variables (airtemp: air temperature on the surface; canopy: canopy cover; DH: soil dehydrogenase enzyme activity; dwood: cover of decaying wood material; herbs: cover of herbs; littcover: cover of leaf litter; littdepth: depth of leaf litter; humidity: relative humidity on the surface; pH: soil pH; shrubs: cover of shrubs; stemp: soil temperature at 2cm depth; smoisture: soil moisture; woodysp: number of woody plant species). Green circles and the four-letter abbreviations indicate the millipede species (BRBA: *B. bagnalli*; BRSU: *B. superus*; GLTE: *G. tetrasticha*; JUTE: *J. terrestris*; KROC: *K. occultus*; LECI: *L. cibdellus*; MABO: *M. bosniensis*; MEUN: *M. unilineatum*; POCO: *P. complanatus*).

(*L. cibdellus*); (2) species characteristic of the forest edge and forest interior habitats (*G. tetrasticha*, *K. occultus*, *M. bosniensis*, *P. complanatus* Linnaeus, 1761). The result of the DCCA showed a marked separation of the three studied habitats (Figure 3). The forest interior habitat was characterized by high canopy cover, high relative humidity and low air temperature, while *B. superus* was associated with this habitat. The forest edge habitat was characterized by high air temperature, high soil moisture, high soil pH, high soil dehydrogenase activity, high shrub cover and low canopy cover. According to the DCCA results, *G. tetrasticha* and *L. cibdellus* were associated with the forest edge. The grassland habitat differed from the forest edge and forest interior habitats, which were more similar to each other. The grassland habitat plots were located on the right upper region, whereas the forest edge plots on the left center region, and the forest interior plots on the right lower part of the ordination plot. The grassland habitat was characterized by high soil temperature and high cover of herbs as well as the absence of the leaf litter and dead wood. Similarly to the results of the IndVal method, no species was associated with this habitat. *M. unilineatum* and *J. terrestris* were situated near the origin, indicating no clear preference for any of the studied habitats. The remaining four species (*M. bosniensis*, *K. occultus*, *P. complanatus*, *B. bagnalli*), similarly to the IndVal results, associated with the forest edge and forest interior habitats.

Discussion

We found a relatively low number of millipede species in the complex of three habitat types in a protected area of Hungary, which represents approximately 9% of the Hungarian millipede fauna (Bogyó et al. 2012). The majority of the millipede species were Central-European species preferring forested habitats (Korsós 1994, Voigtländer 2011, Bogyó et al. 2012, Enghoff 2013), which confirmed the relatively undisturbed conditions in the Hajdúság Landscape Protection Area. The most abundant species in the study and in the forest edge habitat was *G. tetrasticha*. This is a widespread montane species in eastern part of Central Europe, preferring humid conditions, while the occurrences in Hungary were formerly considered as glacial relicts (Korsós 1994). However it is not uncommon that the species occurs in different lowland forests of Northeastern Hungary (Szlávecz and Loksa 1991, Bogyó et al. 2012). The dominance of glomerid species in forested habitats was pointed out by other authors in contrast with the dominance of julid species in grasslands (Tajovský 1990, David et al. 1999). The second and third most abundant species (*K. occultus* and *M. bosniensis*) are found in a wide range of wooded and more or less opened habitats across Central-Europe (Korsós 1994, Voigtländer 2011, Bogyó et al. 2012).

The aim of the study was to reveal a relationship between millipedes (abundance, species richness, diversity and assemblage composition) and the vegetation structure along a forest interior-forest edge-grassland gradient. We found that the total millipede abundance was highest in the forest edge which supports the classical edge effect hypothesis (Odum 1971, Magura et al. 2001, Wirth et al. 2008). Such pattern was expected, but distribution of some species is slightly different from the pattern described in the literature. Our results are also coherent with the predictions of Ries and Sisk (2004). In the forest edge habitat millipedes can benefit from higher temperatures and higher openness compared to the forest interior, but they also benefit from higher percentage of leaf litter cover and thicker leaf litter layer, as well as the higher amount of dead wood and soil moisture than in the adjacent habitats. A review on edge effect (Wirth et al. 2008) reported that the majority of the studies showed a positive edge effect on the abundance of herbivorous arthropods. This work highlighted that edges can have a positive effect on the palatability of resources by affecting fluxes of nutrients, while on the other hand edges represent high-resource environments for forest plants (according to higher light and nutrient availability) which leads to an increase of leaf productivity. However, millipedes are not herbivores, but saprophages, and they are strongly related to the quality and quantity of decaying plant material (Hopkin and Read 1992, Stašiov et al. 2012). Previous studies of edge effect on millipede abundance also reported a positive effect. In a Mediterranean landscape of France, David et al. (1999) found higher millipede and woodlouse population densities and biomass in semi-opened sites (covered by shrubs within the height of 0.25m and 2m) than in forested areas (oak forests with more than 2m high trees). Similarly, high millipede abundance was reported from ecotones of Poland (Tracz 2000). Tracz (2000) studied three sites, where the core forest was a beech-oak mixed forest (*Fago-Quercetum petreae*) and the ecotone was represented by high dominance of the (1) common broom (*Cytisus scopariius*) or (2) common aspen (*Populus tremula*) or (3) beech (*Fagus silvatica*). In the edge habitats of tawa (*Beilschmiedia tawa*) forests of New Zeeland a positive edge effect on millipede abundance was also demonstrated. In contrary, other studies showed no difference in millipede abundance between forest and forest edge habitats in Brasil (Frainer and Duarte 2009), in South-Africa (Weiermans and van Aarde 2003) and in the United Kingdom (Riutta et al. 2012).

Our study showed no significant edge effect on the species richness and diversity of millipedes. Millipede species richness and diversity was higher in the forest interior and forest edge than in the grassland, but there was no difference between the two forested habitats (forest interior, and forest edge). A positive edge effect on species richness and/or diversity in forest edges was reported in previous studies on arthropod taxa (Magura et al. 2000, 2001, Magura 2002, Wirth et al. 2008, Antonovic et al. 2012). However, other studies showed an opposite trend or reported no significant edge effect on species richness and/or diversity of arthropods (Dangerfield et al. 2003, Ries et al. 2004, Wirth et al. 2008). In case of millipedes, David et al. (1999) found significantly higher species diversity in semi-open sites compared with forested and open habitats, which was interpreted as an edge effect. High diversity of millipedes was reported from ecotonal zones of forests (Tracz 2000) and plant borders of city gardens (Smith et al. 2006). On the other hand, Riutta et al. (2012) showed significantly higher millipede species richness in the forest interior than in the forest edge in a temperate mixed deciduous forest. No clear trend was found in millipede species richness of coastal dune forests and its edge habitats in South-Africa (Weyermans and van Aarde 2003). Former studies (David and Handa 2010) predicted higher millipede diversity in habitat mosaics with higher heterogeneity, because of the presence of different habitat patches. In our study, we do not detected higher millipede diversity in the forest edge habitat compared to the forest interior.

The millipede assemblages of the studied habitats clearly separated from each other, based on the abundance of the millipede species. The millipede assemblages formed two main clusters: the first included the grassland habitat, the second included the two forested habitats. However, forested habitats (forest edge and forest interior) also showed a clear separation from each other. It is known, that saprophagous macroarthropod assemblages are changing with the change of vegetation structure on a landscape scale, or on a smaller scale (David et al. 1999, Wytwer et al. 2009, David and Handa 2010, Foster and Claeson 2011). Moreover, even the tree species composition can significantly affect millipede assemblage composition (Stašiov et al. 2012). The influence of the habitat type on millipede assemblages can override the effect of the successional stage also (Schreiner et al. 2012).

Using the IndVal method we found significant character species for the studied habitats. The grassland habitat had no significant character species. The forest edge was characterized by *L. cibdellus*, a species preferring humid, woodland habitats with a Northern and Central-East European distribution (Korsós 1994, Bogyó et al. 2012, Enghoff 2013). Species associated with the forest edge and forest interior habitats (*G. tetrasticha, K. occultus*, *M. bosniensis*, *P. complanatus*) are more or less natural woodland species. According to the literature (Korsós 1994, Voigtländer 2011, Bogyó et al. 2012, Enghoff 2013), some of these species are described from opened and xeric woodland habitats (*K. occultus*, *M. bosniensis*), while others prefer more closed and humid forests (*G. tetrasticha*, *P. complanatus*). Other works in Europe also listed characteristic species of edge habitats (David et al. 1999, Tracz 2000). David et al. (1999) found that semi-open sites were dominated by a *Glomeris* species, while the dominance of the family Julidae was pointed out by Tracz (2000), supporting the hypothesis that juloid morphotype is the best adaptation to various adverse environments. In our study, the most abundant millipede species in the edge habitat was *G. tetrasticha.* In the forest edge the abundance of this species was twice as much than the total abundance of millipedes of the Julidae family.

The results of the DCCA showed that forest edge and forest interior habitats are clearly separated from the grassland habitats. Forest edge and forest interior offer more suitable habitat for millipedes with high amount of leaf litter (both cover and depth of leaf litter), dead wood, canopy cover, as well as with more humid microclimate. The studied forest edge habitat was characterized by high air temperature, high soil moisture, high soil pH and low canopy cover (higher openness) which are key factors affecting millipede assemblages in forested habitats (Hopkin and Read 1992, David 1999, Stašiov 2005, 2009). Lower abundance of saprophagous macroarthropods in forested sites and decreasing abundance by increasing oak cover was observed by David et al. (1999) in France. In our study the edge habitat was also characterized by high percentage of shrub cover as well as high number of woody plant species. Even if the edge habitat has lower percentage of canopy cover and higher air temperature, the saplings (as well as the shrubs) can protect the forest edge from desiccation, leading to high soil moisture (Camargo and Kapos 1995). Lindsay and French (2006) reported that the natural shrublands infested with a dense population of the non-native bitou bush in Australia resulted in an increase in millipede abundance, possibly as a result of a changing microclimate with moister environment. The moister microhabitats could be favorable for millipede species preferring humid conditions, like the two characteristic species of the forest edge, *G. tetraticha* and *L. cibdellus*. In our study, the changes in canopy and shrub cover (as well as light conditions) combined with high soil moisture and diverse leaf litter could lead to the significantly higher millipede abundance in the forest edge compared to the adjacent habitats. High values of dehydrogenase activity were also found in the forest edge which is usually has a positive correlation with millipede abundance (Tripathi et al. 2013, Vasconcellos et al. 2013).

Conclusion

The results of the study revealed that millipede assemblages altered in a short distance along the grassland-forest edge-forest habitat complex. Our study showed a positive edge effect on millipede abundance and assemblage composition in the studied natural habitats. We found significant edge-associated millipede species by IndVal method. On the other hand there was no edge effect on millipede species richness and diversity. Our results support the former findings that some millipede species may be specialized to natural forest edge habitats. Forest edges may have key habitats in the conservation of millipedes during the next decades.

Acknowledgements

The authors are thankful for the assistance of Roland Horváth, Levente Lality, Szabolcs Mizser, Edina Simon and Gyula Szabó. We thank the help of the Hortobágy National Park Directorate, especially the help of László Demeter and László Szél. This work was partially supported by the European Union and the European Social Fund through project (grant no.: TAMOP-4.2.2.C-11/1/KONV-2012-0010). Furthermore, the work is supported by the TAMOP 4.2.1/B-09/1/KONV-2010-0007, and TAMOP-4.2.2/B-10/1-2010-0024 projects.The research of Dávid Bogyó was supported by the European Union and the State of Hungary, co-financed by the European Social Fund in the framework of TÁMOP 4.2.4.A/2-11-1-2012-0001 'National Excellence Program'.

References

Alef K (1995) Estimation of microbial activities. Dehydrogenase activity. In: Alef K, Nannipieri P (Eds) Methods in Applied Soil Microbiology and Biochemistry. Academic Press, London, UK, 228–231.

Antonović I, Brigić A, Sedlar Z, Bedek J, Šoštarić R (2012) Terrestrial isopod community as indicator of succession in a peat bog. ZooKeys 176: 171–188. doi: 10.3897/zookeys.176.2379

Bieringer G, Zulka KP, Milasowszky N, Sauberer N (2013) Edge effect of a pine plantation reduces dry grassland invertebrate species richness. Biodiversity and Conservation 22: 2269–2283. doi: 10.1007/s10531-013-0435-2

Bogyó D, Korsós Z, Lazányi E, Hegyessy G (2012) Millipedes (Diplopoda) from the Zemplén Mountains, Northeast Hungary, with two julid species new to the Hungarian fauna. Opuscula Zoologica Budapest 43: 131–145.

Camargo JLC, Kapos V (1995) Complex edge effects on soil moisture and microclimate in central Amazonian forest. Journal of Tropical Ecology 11: 205–221. doi: 10.1017/S026646740000866X

Dangerfield JM, Pik AJ, Britton D, Holmes A, Gillings M, Oliver I, Briscoe D, Beattie AJ (2003) Patterns of invertebrate biodiversity across a natural edge. Austral Ecology 28: 227–236. doi: 10.1046/j.1442-9993.2003.01240.x

David JF, Devernay S, Loucougaray G, Le Floc'h E (1999) Belowground biodiversity in a Mediterranean landscape: relationships between saprophagous macroarthropod communities and vegetation structure. Biodiversity and Conservation 8: 753–767. doi: 10.1023/A:1008842313832

David JF, Handa IT (2010) The ecology of saprophagous macroarthropods (millipedes, woodlice) in the context of global change. Biological Reviews 85: 881–895. doi: 10.1111/j.1469-185X.2010.00138.x.

Didham RK, Barker GM, Costall JA, Denmead LH, Floyd CG, Watts CH (2009) The interactive effects of livestock exclusion and mammalian pest control on the restoration of invertebrate communities in small forest remnants. New Zealand Journal of Zoology 36: 135–163. doi: 10.1080/03014220909510148

Dufrêne M, Legendre P (1997) Species assemblages and indicator species: The need for a flexible asymmetrical approach. Ecological Monographs 67: 345–366. doi: 10.2307/2963459

Dziadosz C (1964) Eine für Polen neue Art von Tausendfüsslern, Brachyiulus bagnalli (Brölemann), (Diplopoda, Iulidae). Bulletin de l'Academie Polonaise des Sciences 12: 207–209.

Elek Z, Magura T, Tóthmérész B (2001) Impacts of non-native Norway spruce plantation on abundance and species richness of ground beetles (Coleoptera: Carabidae).Web Ecology 2: 32–37. doi: 10.5194/we-2-32-2001

Enghoff H (2013) Diplopoda, Millipedes. Fauna Europaea version 2.6. http://www.faunaeur.org

Ewers RM, Didham RK (2008) Pervasive impact of large-scale edge effects on a beetle community. Proceedings of the National Academy of Sciences 105: 5426–5429. doi: 10.1073/pnas.0800460105

Foster AD, Claeson SM (2011) Habitats and seasonality of riparian-associated millipedes in southwest Washington, USA. Terrestrial Arthropod Reviews 4: 203–220. doi: 10.1163/187498311X591102

Frainer A, Duarte MM (2009) Soil invertebrates in southern Brazilian Araucaria forest-grassland mosaic: differences between disturbed and undisturbed areas. Iheringia Série Zoologia 99: 307–312. doi: 10.1590/S0073-47212009000300013

Golovatch SI, Kime RD (2009) Millipede (Diplopoda) distributions: a review. Soil Organisms 81: 565–597.

Gehlhausen SM, Schwartz MW, Augspurger CK (2000) Vegetation and microclimatic edge effects in two mixed-mesophytic forest fragments. Plant Ecology 147: 21–35. doi: 10.1023/A:1009846507652

Gulicka J (1957) Kvalitatívno-kvantitatívny rozbor pdnej fauny Cierneho lesa (Ostrov) (Myriapoda, Isopoda, Opiliones). Acta Facultatis rerum naturalium Universitatis Comenianae Zoologia 2: 119–139.

Hauser H, Voigtländer K (2009) Doppelfüßer (Diplopoda) Ostdeutschlands: Bestimmung, Biologie und Verbreitung. Göttingen, Jugendbund für Naturbeobachtung, Göttingen, 112 pp.

Hopkin SP, Read HJ (1992) The biology of millipedes. Oxford University Press, Oxford, 233 pp.

Korsós Z (1994) Checklist, preliminary distribution maps, and bibliography of millipedes in Hungary (Diplopoda). Miscellanea Zoologica Hungarica 9: 29–82.

Legendre PL, Legendre L (1998) Numerical ecology. Second English Edition. Amsterdam Elsevier Science, Amsterdam.

Lindsay EA, French K (2006) The impact of the weed Chrysanthemoides monilifera ssp. rotundata on coastal leaf litter invertebrates. Biological Invasions 8: 177–192. doi: 10.1007/s10530-004-5856-0

Lövei GL, Magura T, Tóthmérész B, Ködöböcz V (2006) The influence of matrix and edges on species richness patterns of ground beetles (Coleoptera: Carabidae) in habitat islands. Global Ecology and Biogeography 15: 283–289. doi: 10.1111/j.1466-8238.2005.00221.x

Magura T (2002) Carabids and forest edge: spatial pattern and edge effect. Forest Ecology and Management 157: 23–37. doi: 10.1016/S0378-1127(00)00654-X

Magura T, Tóthmérész B, Molnár T (2001) Edge effect on carabid assemblages along forest-grass transects. Web Ecology 2: 7–13. doi: 10.5194/we-2-7-2001

Magura T, Tóthmérész B, Molnár T (2000) Spatial distribution of carabids along grass-forest transects. Acta Zoologica Academiae Scientiarum Hungaricae 46: 1–17.

Odum EP (1971) Fundamentals of ecology. Saunders, Philadelphia, 574 pp.

Ries L, Fletcher RJ, Battin J, Sisk TD (2004) Ecological responses to habitat edges: mechanisms, models, and variability explained. Annual Review of Ecology, Evolution, and Systematics 35: 491–522. doi: 10.1146/annurev.ecolsys.35.112202.130148

Ries L, Sisk TD (2004) A predictive model of edge effects. Ecology 85: 2917–2926. doi: 10.1890/03-8021

Riutta T, Slade EM, Bebber DP, Taylor ME, Malhi Y, Riordan P, Macdonald DW, Morecroft MD (2012) Experimental evidence for the interacting effects of forest edge, moisture and soil macrofauna on leaf litter decomposition. Soil Biology and Biochemistry 49: 124–131. doi: 10.1016/j.soilbio.2012.02.028

Saunders DA, Hobbs RJ, Margules CR (1991) Biological consequences of ecosystem fragmentation - a Review. Conservation Biology 5: 18–32. doi: 10.1111/j.1523-1739.1991.tb00384.x

Schreiner A, Decker P, Hannig K, Schwerk A (2012) Millipede and centipede (Myriapoda: Diplopoda, Chilopoda) assemblages in secondary succession: variance and abundance in Western German beech and coniferous forests as compared to fallow ground. Web Ecology 12: 9–17. doi: 10.5194/we-12-9-2012

Smith J, Chapman A, Eggleton P (2006) Baseline biodiversity surveys of the soil macrofauna of London's green spaces. Urban Ecosystems 9: 337–349. doi: 10.1007/s11252-006-0001-8

Stašiov S (2005) Millipede communities (Diplopoda) of oak-hornbeam ecosystems (the Malé Karpaty Mts, Trnavská pahorkatina hills, SW Slovakia). Ekológia (Bratislava) 24: 143–151.

Stašiov S (2009) Millipede (Diplopoda) communities in mixed oak-hornbeam forest stands–effect of selected site factors. Polish Journal of Ecology 57: 785–792.

Stašiov S, Stašiová A, Svitok M, Michalková E, Slobodník B, Lukáčik I (2012) Millipede (Diplopoda) communities in an arboretum: Influence of tree species and soil properties. Biologia 67: 945–952. doi: 10.2478/s11756-012-0097-7

Szlávecz K, Loksa I (1991) Diversity of soil arthropods in the Bátorliget Nature Reserve, Hungary. In: Zombori L, Peregovits L (Eds) Proceedings of the 4th ECE/XIII. SIEEC, Gödöllő, Hungary, 801–807.

Tajovský K (1990) Diplopoda in a secondary soil successional row. In: Minelli A (Ed.) Proceedings of the 7th International Congress of Myriapodology. Brill, Leiden, 229–234.

Tóthmérész B, Nagy DD, Mizser Sz, Bogyó D, Magura T (2014) Edge effects on ground-dwelling beetles (Carabidae and Staphylinidae) in oak forest-forest edge-grassland habitats in Hungary. European Journal of Entomology 111: 686–691. doi: 10.14411/eje.2014.091

Tracz H (2000) The Diplopoda and Chilopoda of selected ecotones in northwestern Poland. Fragmenta Faunistica 43: 351–360.

Tripathi G, Deora R, Singh G (2013) The influence of litter quality and micro-habitat on litter decomposition and soil properties in a silvopasture system. Acta Oecologica 50: 40–50. doi: 10.1016/j.actao.2013.01.013

Vasconcellos RL, Segat JC, Bonfim JA, Baretta D, Cardoso EJ (2013) Soil macrofauna as an indicator of soil quality in an undisturbed riparian forest and recovering sites of different ages. European Journal of Soil Biology 58: 105–112. doi: 10.1016/j.ejsobi.2013.07.001

Voigtländer K (2011) Preferences of common central European millipedes for different biotope types (Myriapoda, Diplopoda) in Saxony-Anhalt (Germany). International Journal of Myriapodology 6: 61–83. doi: 10.3897/ijm.6.2172

Weiermans J, Van Aarde RJ (2003) Roads as ecological edges for rehabilitating coastal dune assemblages in Northern KwaZulu-Natal, South Africa. Restoration Ecology 11: 43–49. doi: 10.1046/j.1526-100X.2003.00026.x

Wirth R, Meyer ST, Leal IR, Tabarelli M (2008) Plant herbivore interactions at the forest edge. Progress in Botany 69: 423–448. doi: 10.1007/978-3-540-72954-9_17

Wytwer J, Golovatch SI, Penev L (2009) Variation in millipede (Diplopoda) assemblages in oak woodlands of the Eastern European Plain. Soil Organisms 81: 791–813.

Zuur A, Ieno EN, Walker N, Saveiliev AA, Smith GM (2009) Mixed effects models and extensions in ecology with R. Springer, New York, 574 pp. doi: 10.1007/978-0-387-87458-6

ZooKeys 510: 197–207 (2015)
doi: 10.3897/zookeys.510.9020
http://zookeys.pensoft.net

RESEARCH ARTICLE

A peer-reviewed open-access journal
ZooKeys
Launched to accelerate biodiversity research

Hay-bait traps are a useful tool for sampling of soil dwelling millipedes and centipedes

Ivan H. Tuf[1], Vojtěch Chmelík[1], Igor Dobroruka[1], Lucie Hábová[1],
Petra Hudcová[1], Jan Šipoš[1,2,3], Slavomír Stašiov[4]

1 *Department of Ecology and Environmental Sciences, Faculty of Science, Palacký University, Šlechtitelů 11, 77900 Olomouc, Czech Republic* **2** *Department of Biology and Ecology, Faculty of Science, University of Ostrava, Chittusiho 10, 71000 Slezská Ostrava, Czech Republic* **3** *Global Change Research Centre, Academy of Sciences of the Czech Republic, Bělidla 986/4a, 603 00 Brno, Czech Republic* **4** *Department of Biology and General Ecology, Faculty of Ecology and Environmental Sciences, Technical University in Zvolen, T. G. Masaryka 24, 96053 Zvolen, Slovakia*

Corresponding author: *Ivan H. Tuf* (ivan.tuf@upol.cz)

Academic editor: *Karel Tajovský* | Received 26 November 2014 | Accepted 30 April 2015 | Published 30 June 2015

http://zoobank.org/E2458727-A81B-4645-934F-42F23A718E09

Citation: Tuf IH, Chmelík V, Dobroruka I, Hábová L, Hudcová P, Šipoš J, Stašiov S (2015) Hay-bait traps are a useful tool for sampling of soil dwelling millipedes and centipedes. In: Tuf IH, Tajovský K (Eds) Proceedings of the 16th International Congress of Myriapodology, Olomouc, Czech Republic. ZooKeys 510: 197–207. doi: 10.3897/zookeys.510.9020

Abstract

Some species of centipedes and millipedes inhabit upper soil layers exclusively and are not recorded by pitfall trapping. Because of their sensitivity to soil conditions, they can be sampled quantitatively for evaluation of soil conditions. Soil samples are heavy to transport and their processing is time consuming, and such sampling leads to disturbance of the soil surface which land-owners do not like. We evaluated the use of hay-bait traps to sample soil dwelling millipedes and centipedes. The effectiveness of this method was found to be similar to the effectiveness of soil sampling. Hay-bait traps installed for 8–10 weeks can substitute for direct soil sampling in ecological and inventory studies.

Keywords

Diplopoda, Chilopoda, soil sampling, agroecosystem, soil fauna

Introduction

Soil macrofauna is commonly used for monitoring or evaluation of sites. Besides ground beetles (e.g. Hůrka et al. 1996, Kotze et al. 2011), spiders (e.g. Buchar and Růžička 2002, Maelfait et al. 2004) or woodlice (e.g. Souty-Grosset et al. 2005, Tuf and Tufová 2008), centipedes and millipedes are sampled frequently too (Tuf and Tufová 2008, Dunger and Voigtländer 2009). Nevertheless, not all species of centipedes and millipedes are ground-dwelling with many species inhabiting the upper soil layer exclusively (Lee 2006, Barber and Keay 1988). Soil dwelling animals can be sampled using litter/soil sieving, soil sampling or hand-collecting. Sieved material and soil samples have to be hand-sorted or processed using heat extractors, e.g. Tullgren funnel or Kempson apparatus (Tuf and Tvardík 2005). Handling of soil samples can be difficult due to the higher weight of samples (one sample of size 25 × 25 × 10 cm weighs around 6 kg). Litter/soil sieving can reduce the weight of samples, nevertheless as with hand-collecting, it is time consuming and attention-intensive. Moreover, soil sampling can cause damage to the site; pot-holes created by a soil corer can endanger people passing the site and can increase water erosion on slopes. These pot-holes are definitely not popular among land-owners of the sampled sites. For these reasons (severity of sampling, damage of ground), we have attempted to evaluate the effectiveness of sampling centipedes and millipedes using hay-bait traps. The aims of this research were 1) comparison of the efficiency of hay-bait trapping, soil sampling and pitfall trapping and 2) to find the optimal length of exposure of hay-bait traps for maximum efficiency.

Material and methods

Field study

The research was done at three sites in the Czech Republic from May to July 2013. The first site was an alfalfa field (49°34.41'N, 17°17.17'E) on the border of the town of Olomouc. This large field of ca 250 square metres is surrounded by other fields (with cereals) and a railway embankment. In the previous year it had also grown alfalfa. The field is under conventional management including use of herbicides and ploughing.

The second site was an old meadow (50°26.85'N, 15°0.00'E) being mown once to twice per year for the last 30 years. This meadow of ca 500 square metres is surrounded by fields and gardens with mixed wood across the road and is ca 6 km north-east of the town of Mladá Boleslav. The third site studied was a mixed forest (49°15.66'N, 17°17.72'E) 6 km south-west of the town of Kroměříž. The forest is classified as *Fageto-Quercetum illimerosum trophicum*; dominant trees are oaks, hornbeams and some pines, with *Rubus fruticosus*, *Galium odoratum* and *Galium aparine* as dominants of undergrowth. The soil surface of this forest is covered by a rather thick layer of oak leaf litter.

In the Czech Republic generally, the weather conditions during the study period were characterised by average or slightly increased temperatures and higher than average precipitation in May-June, and a very hot July in contrast to long-term average values. The previous winter season was rather warmer and with higher precipitation (ref. historical territorial data at www.chmi.cz).

Soil macrofauna, including millipedes and centipedes, was sampled using three methods at each site. Pitfall traps (10 traps consisting of glass jars with inserted plastic pots of diameter 7.5 cm filled with 2 dl of 4% formaldehyde in water with some detergent, metal covers) were arranged in 2 lines of 5 traps with a span of 10 m, and inspected at 2-week intervals. Five soil samples (25 × 25 × 10 cm including litter layer) were obtained using a spatula, three times per study (i.e. 15 soil samples per site) and transported to the laboratory in plastic bags. Hay-bait traps were made from a wire gauze (2 cm mesh) shaped as a simple pocket of size 25 × 25 cm. Each pocket was marked by a code written on the band. These pockets were filled with hay (commercial hay mixture for feeding rodent pets) and submerged into water for 2 hours before installation. Altogether, 60 hay-bait traps were placed horizontally at each site in a following scheme: 5 lines of 12 traps (2-5 cm under soil surface) over a length of 2 m with 10 m between lines. All traps were installed at the same time and 5 traps were taken away each week during the course of the study lasting for 12 weeks. Hay-traps were transported into the laboratory inside separate plastic bags.

Sample processing

Soil samples and hay-traps were heat-extracted immediately in the laboratory using simple Kempson devices (Tuf and Tvardík 2005). Hay-traps were extracted for a week, soil samples for 2 weeks, both under electric 60W-bulbs. Extracted animals from both soil samples and hay-traps were sorted to higher taxonomic groups and millipedes and centipedes were identified to the species level.

Data analyses

We tested the effects of trapping time and methods on species richness by repeated-measures on traps with nested design. The traps were nested in each of the three study sites (field, meadow, forest). Explanatory variables in the model were trapping time and trapping methods. The response variable was defined as a number of species per trap for particular time and place. Habitat type was used as random variable. We used a mixed model to estimate the correct error term and degrees of freedom. To test this effect, a generalized linear mixed model (glmmPQL, part of R package MASS) was used with negative binomial error distribution and log link function (Bates et al. 2014).

To test if one level of a particular factor (trapping method and study site) is more variable than other levels of the same factor, a permutation test was used (permutest.betadisper, part of R package vegan). This permutation based method tests pairwise comparisons of group mean dispersions. It is based on the t-statistic computed on pairwise group dispersions. A distance matrix was computed based on "Bray-Curtis" index of dissimilarity (vegdist, part of R package vegan). Then the function "betadisper" (part of vegan package in R) was used to calculate variance for each group of samples. Variance was computed as average distance of group members to the group centroid.

Rarefaction curves were constructed to show how the species richness varies for the same sample size between the three trapping methods. Function "rarefy" (part of vegan package in R software) computed the expected species richness and standard deviation in random subsamples of a particular sample size from the community. Data were analysed using R software (R Development Core Team 2011).

Results

Altogether, we obtained 541 millipedes from 17 species and 435 centipedes from 13 species (Table 1). Based on the number of recorded animals, the richest site was the forest (553 myriapods) and poorest site was the field (100 myriapods). Number of species showed the same pattern: 21 myriapod species in the forest and 6 in the field. Soil sampling was the least efficient for sampling species (9 millipede and 7 centipede species) as well as individuals (36 and 100 individuals respectively), whereas pitfall traps and hay-bait traps were similar in their efficiency: 14–15 millipede and 9–10 centipede species; for number of individuals, see Table 1.

Methods at individual sites were evaluated according to their efficiency using rarefactions (Fig. 1). Bait traps sampled higher numbers of species in contrast to other methods in the field site, meanwhile increasing sampling effort (number of sampled animals) was connected with a bigger species list in bait traps as well as in pitfall traps in forest.

Differences between species lists at all sites and lists sampled by individual methods were compared by pairwise comparisons and differences confirmed between all pairs of sites (Table 2a). Nevertheless, the same analysis revealed there was no statistically significant difference between the suite of species sampled by hay-bait traps and soil samples (Table 2b).

Evaluation of colonization of hay-bait traps (Fig. 2) showed that the highest diversity as well as abundance of collected myriapods in these traps is after 7 weeks following installation in field, or 9-10 weeks following installation in forest or meadow. A longer period of exposure leads to a decrease of both parameters of myriapod communities. Generalized linear mixed models reveal that changes in abundance during exposure was significantly influenced by the second power of time (LRT = 6.43, p = 0.040, AIC = 667.83). The analogous model for diversity confirmed significant changes during time (LRT = 5.81, p = 0.042, AIC = 543.38) which were site dependent too (LRT = 6.74, p = 0.034, AIC = 544.12).

Table 1. List of millipedes obtained using three methods from three biotopes (ind./10 pitfall traps/12 weeks, ind./60 bait traps and ind./0.94m^2 respectively.

	Pitfall traps			Hay-bait traps			Soil samples			Total pitfall traps	Total hay-bait traps	Total soil samples
	field	meadow	forest	field	meadow	forest	field	meadow	forest			
Glomeris connexa C. L. Koch, 1847	-	9	1	-	-	1	-	-	-	10	1	0
Blaniulus guttulatus (Fabricius, 1798)	-	2	-	-	31	1	-	2	-	2	32	2
Brachyiulus bagnalli (Curtis, 1845)	2	-	-	5	-	-	-	-	-	2	5	0
Cylindroiulus boleti (C.L. Koch, 1847)	-	-	3	-	-	-	-	-	-	3	0	0
Cylindroiulus caeruleocinctus (Wood, 1864)	1	-	-	-	-	-	-	-	-	1	0	0
Enantiulus nanus (Latzel, 1884)	-	-	64	-	-	32	-	-	4	64	32	4
Julus scandinavius Latzel, 1884	-	-	-	-	-	1	-	-	-	0	1	0
Leptoiulus proximus (Němec, 1896)	-	-	2	-	-	1	-	-	-	2	1	0
Megaphyllum projectus Verhoeff, 1894	-	-	2	-	-	2	-	-	-	2	2	0
Ommatoiulus sabulosus (Linnaeus, 1758)	-	-	10	-	-	11	-	-	4	10	11	4
Ophiulus pilosus (Newport, 1842)	27	-	-	59	-	-	2	-	-	27	59	2
Unciger foetidus (C.L. Koch, 1838)	-	9	36	-	30	26	-	3	2	45	56	5
Brachydesmus superus Latzel, 1884	-	-	-	-	-	-	-	3	-	0	0	3
Polydesmus complanatus (Linnaeus, 1761)	-	3	1	-	2	3	-	-	1	4	5	1
Polydesmus denticulatus C.L. Koch, 1847	-	8	-	-	7	-	-	-	-	8	7	0
Polydesmus inconstans Latzel, 1884	-	1	-	-	39	-	-	6	-	1	39	6
Strongylosoma stigmatosum (Eichwald, 1830)	-	-	47	-	-	26	-	-	9	47	26	9
Diplopoda	30	32	166	64	109	104	2	14	20	228	277	36
Clinopodes flavidus C.L. Koch, 1847	-	-	2	1	-	9	-	-	-	2	10	0
Geophilus electricus (Linnaeus, 1758)	-	-	-	-	-	-	-	9	-	0	0	9
Geophilus flavus (DeGeer, 1778)	-	-	10	-	9	20	1	30	-	10	29	31
Geophilus truncorum Bergsoe & Meinert, 1866	-	-	-	-	-	-	-	1	-	0	0	1
Schendyla nemorensis (C.L. Koch, 1836)	-	-	23	-	11	60	-	26	-	23	71	26
Strigamia transsilvanica (Verhoeff, 1928)	-	-	4	-	-	2	-	-	-	4	2	0
Lithobius aerugineus L. Koch, 1862	-	-	41	-	-	39	-	-	-	41	39	0
Lithobius austriacus (Verhoeff, 1937)	-	-	-	-	-	2	-	-	-	0	2	0
Lithobius dentatus C.L. Koch, 1844	-	-	2	-	-	1	-	-	-	2	1	0
Lithobius erythrocephalus C.L. Koch, 1847	-	-	1	-	-	-	-	-	-	1	0	0
Lithobius forficatus (Linnaeus, 1758)	-	-	-	-	-	1	-	-	1	0	1	1
Lithobius microps Meinert, 1868	-	4	-	-	47	-	-	31	-	4	47	31
Lithobius mutabilis L. Koch, 1862	-	-	3	-	-	3	-	-	1	3	3	1
Lithobius spp.	-	-	14	2	-	24	-	-	-	14	26	0
Chilopoda	0	4	100	3	67	161	1	97	2	104	231	100

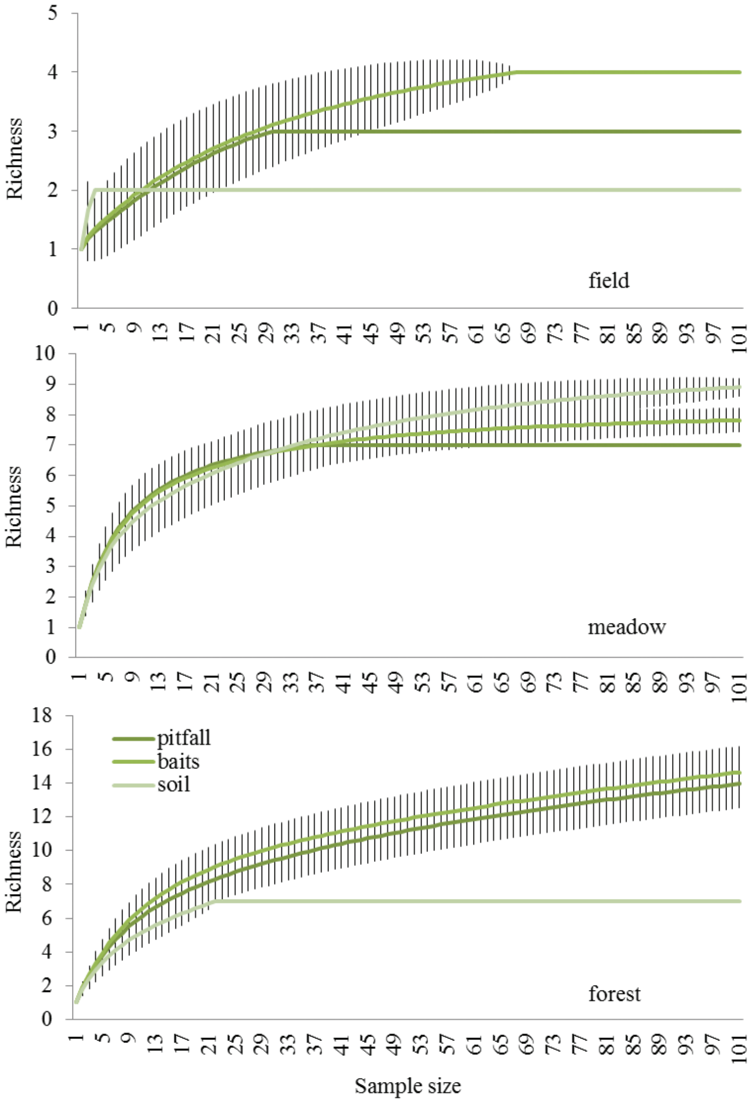


Figure 1. Rarefactions of estimated species richness (i.e. number of species) in increasing size of random samples (i.e. number of individuals), comparison of effectiveness of sampling by individual methods at different sites. Vertical lines represent standard errors.

Table 2. Pairwise comparisons of species lists collected (a) at different sites and (b) by different methods. (Observed p-value below diagonal, permuted p-value above diagonal).

a)	field	forest	meadow	b)	hay-bait	pitfall	soil
field	-	0.001	0.048	hay-bait	-	0.003	0.917
forest	0.000	-	0.001	pitfall	0.003	-	0.043
meadow	0.041	0.004	-	soil	0.911	0.052	-

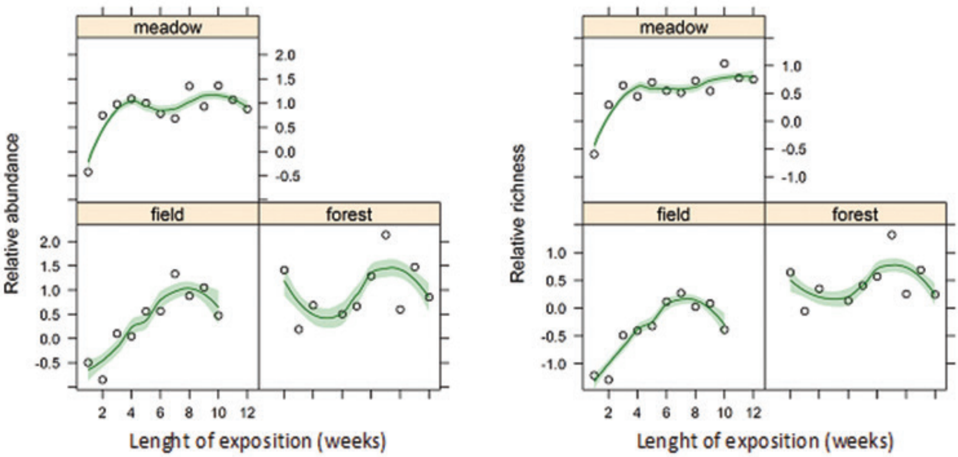


Figure 2. Changes in myriapod communities inside hay-bait traps installed in three biotopes during the 12 week trapping period. Qualitative as well as quantitative parameters are shown for these communities. Open dots are observed parameters, whereas solid lines represent models of succession including standard errors (green shading).

Discussion

Centipedes and millipedes live on the soil surface and inside soil. We can find them through the whole soil gradient to a depth of one meter (e.g. Ilie 2003) although they are abundant in upper layers predominantly. This is the reason why pitfall traps are not sufficient for sampling the whole community adequately. We evaluated efficiency of hay-bait traps for sampling soil-dwelling millipedes and centipedes with the time consuming soil sampling (connected with destruction of the soil surface and transport of heavy samples to the laboratory).

Bait traps were used for sampling invertebrates, mainly beetles, in caves originally (Barber 1931). Bait traps are much more common for carnivorous or necrophagous species; baits are represented by pieces of flesh, fish or cheese above a fixation solution surface, or direct addition of beer to a solution. Straw, wood or yeast is placed in caves occasionally as the baits for detritivores (Mock pers. comm.). Nevertheless, baits are not working there as traps, as they need to be visited and inspected continuously to collect attracted animals to avoid them to leave baits.

The first documented version of bait traps for millipedes was a shingle trap by Barber (1997) filled with kitchen tissue and potatoes. He used this trap to sample millipedes and isopods at a shingle beach in England. Similar kinds of bait traps, containing sweet potatoes or corn, were used by Brunke et al. (2012) for sampling *Cylindroiulus caeruleocinctus* in Canadian fields. Almost the same traps are used with the name litter bags for studying decomposition of different kinds of litter and/or by different size groups of decomposers (according to diameters of holes in the traps). Litter bags are also used for sampling soil mesofauna or microarthropods (e.g. Wiegert 1974).

Prasifka et al. (2007) used litter bags to sample ground dwelling invertebrates; they installed litter bags at the soil surface as well as below the soil surface in a corn field. Above-ground bait traps were attractive for centipedes (millipedes were not recorded in this research). Apart from these publications, we did not found any records of the use of bait traps for sampling millipedes or centipedes.

Hay-bait traps vs. soil samples

If we are interested in using hay-bait traps as an adequate (or even better) substitute for soil sampling, we have to compare species lists of millipedes and centipedes trapped by these methods. There were only three species recorded exclusively from soil sampling, i.e. missing in hay-bait traps: millipede *Brachydesmus superus* and centipedes *Geophilus electricus* and *G. truncorum*. The minute millipede species lives preferably in clay soils with litter (Lee 2006) usually in huge quantities. This species is a dominant species recorded by pitfall traps in cities (Riedel et al. 2009), so its absence in pitfall traps at the meadow site is probably caused by its low abundance. *Geophilus truncorum* was recorded once only, so it is hard to evaluate effectiveness of sampling of so "rare" a species. Nevertheless, both geophilomorphs (*G. electricus* and *G. truncorum*) are known as predators of earthworms (Sergeeva et al. 1985, Keay 1986); for this reason, hay-bait may be not attractive for them as they follow earthworms into their corridors in soil. So, to collect *G. electricus*, soil sampling or direct hand collecting seems to be necessary. Other geophilomorphs (*C. flavidus*, *S. nemorensis*, *S. acuminata*, *G. flavus*) are common species, which are frequently found by individual hand-collecting; they live in soil near the surface, under logs, bark and stones (e.g. Barber and Keay 1988). Their presence in shallow hay-bait traps is not surprising as these species were sampled by pitfall trapping too.

One millipede species, *Julus scandinavius* was recorded exclusively in a hay-bait trap, but as one specimen only was found no generalization can be made. Many more species were found in both hay-bait traps and pitfall traps but not in soil samples. Nevertheless, hay-bait traps are not a substitute method to pitfall trapping as there were significant differences between species lists recorded by these methods (see Tab. 2), but it can definitely substitute the soil samples.

Colonisation of hay-bait traps

Centipedes, and especially millipedes, are attracted into the hay-bait traps. The possible reason can be as a food source and/or sustainable shelter with higher humidity. At least for millipedes, food source seems to be the more probable explanation; wet cloth method (offering higher humidity) did not record any millipedes in African savannah ecosystems (Druce et al. 2004). More probably, millipedes and centipedes are attracted by food availability, as it can be associated with hay decomposition and colonization of the traps. Smaller decomposers colonising baits are welcomed food for carnivorous centipedes (e.g. Perry et al. 1997).

Eight to ten weeks seems to be the optimal exposure time for hay-bait traps in Central European conditions. A similar result was found by Ožanová (2001) using grass traps (a small heap of mowed grass on the surface of meadow), with a much higher number of species after 7 weeks than for a shorter exposure time. Although Prasifka et al. (2007) did not evaluate the effect of length of exposure time of bait traps, it is evident from their results that below-ground traps were more effective after 8 weeks than after 6 weeks. It supports our results that the best length of exposure of bait traps is from 8 to 10 weeks, although we aware of difficulties with this generalization. The best length of exposure is not dependent only on a type of habitat, but also on climate conditions (rainy or dry weather) and time of year when exposed. Traps installed in Central European conditions in late autumn or in winter or during dry hot summer can be colonised in different ways as invertebrates change their activity and position in the soil profile during the year (David 1984, Geoffroy 1985, David et al. 1996, Tuf 2002). The general recommendation for using these traps when installed in spring is to use them for 8–10 weeks. Timing of installation and the length of time exposed in field sites will need to coordinate with agricultural activities such as sowing and harvest times.

Conclusion

Centipedes and millipedes inhabit the soil surface as well as the soil profile. For a complete knowledge of myriapod fauna, pitfall trapping needs to be combined with a method to collect soil dwelling species, e.g. soil sampling. Hay-bait traps were tested for their ability to replace soil sampling. Our results showed that hay-bait traps are attractive to myriapods and can have a similar sampling effort as soil sampling. The optimal length of exposure of hay-bait traps in soil seems to be ca 2 months (8–10 weeks).

Acknowledgement

This research was supported by grant of Ministry of Agriculture, No. QJ1230066 and European Project supported by the Operational Programme Cross Border Co-operation CZ-PL No. CZ.3.22/1.2.00/12.03445. We are also grateful for support of project CzechGlobe – Centre for Global Climate Change Impacts Studies, Reg. No. CZ.1.05/1.1.00/02.0073 and LC06073. We are grateful to Megan Short (Deakin University, Australia) for her English improvement as well as to Petr Dolejš and Jolanta Wytwer for their valuable comments and suggestions.

References

Barber AD (1997) A simple shingle trap for Myriapods. Bulletin of the British Myriapod Group 13: 27–28. http://www.bmig.org.uk/sites/www.bmig.org.uk/files/bulletin_bmg/BullBMG13-1997.pdf

Barber AD, Keay AN (1988) Provisional Atlas of the Centipedes of the British Isles. Institute of Terrestrial Ecology Merlewood Research station, Grangeover-Sands, 127 pp. http://nora.nerc.ac.uk/7275/1/Centipedes.pdf

Barber HS (1931) Traps for cave-inhabiting insects. Journal of the Elisha Mitchell Society 46: 259–266.

Bates D, Maechler M, Bolker B, Walker S (2014) lme4: Linear mixed-effects models using Eigen and S4. R package version 1. 1–7. http://CRAN.R-project.org/package=lme4

Brunke AJ, O'Keefe L, Bahlai CA, Sears MK, Hallett RH (2012) Guilty by association: an evaluation of millipedes as pests of carrot and sweet potato. Journal of Applied Entomology 136: 772–780. doi: 10.1111/j.1439-0418.2012.01708.x

Buchar J, Růžička V (2002) Catalogue of Spiders of the Czech Republic. Peres, Praha, 351 pp. http://arachnology.cz/cas/app_contents/downloads/bibliography/ARA681.pdf

David J-F (1984) Le cycle annuel du diplopode *Microchordeuma gallicum* (Latzel, 1884). Bulletin de la Société zoologique de France 104: 61–70.

David J-F, Célérier M-L, Vannier G (1996) Overwintering with a low level of cold-hardiness in the temperate millipede *Polydesmus angustus*. Acta Oecologica 17: 393–404.

Druce D, Hamer M, Slotow R (2004) Sampling strategies for millipedes (Diplopoda), centipedes (Chilopoda) and scorpions (Scorpionida) in savanna habitats. African Zoology 39: 293–304. http://africanzoology.journals.ac.za/pub/article/view/299/280

Dunger W, Voigtländer K (2009) Soil fauna (Lumbricidae, Collembola, Diplopoda and Chilopoda) as indicators of soil eco-subsystem development in post-mining sites of eastern Germany – a review. Soil Organisms 81: 1–51. http://www.senckenberg.de/files/content/forschung/publikationen/soilorganisms/volume_81_1/01_dunger.pdf

Geoffroy J-J (1985) Les Diplopodes d'un écosystème forestier tempéré, répartition spatio-temporelle des populations. In: Ellis WN, Jeekel CAW, Pieters FFJM (Eds) Proceedings of the 6th International Congress of Myriapodology. Bijdragen tot de Dierkunde 55: 78–87.

Hůrka K, Veselý P, Farkač J (1996): Využití střevlíkovitých (Coleoptera: Carabidae) k indikaci kvality prostředí (The use of carabid communities (Coleoptera, Carabidaae) for the indication of the environment quality). Klapalekiana 32(1-2): 15–26. [In Czech with English abstract and German summary]

Ilie V (2003) Chilopoda species from the edaphic and subterranean environments of the Cloşani karstic area (Romania). Travaux du Muséum National d´ histoire Naturelle «Grigore Antipa» 45: 129–137.

Keay AN (1986) Predation and prey in *Henia* (*Chaetechelyne*) *vesuviana* (Newport) (Chilopoda, Geophilomorpha). Bulletin of the British Myriapod Group 3: 21–25. http://www.bmig.org.uk/sites/www.bmig.org.uk/files/bulletin_bmg/BullBMG03-1986.pdf

Kotze DJ, Brandmayr P, Casale A, Dauffy-Richard E, Dekoninck W, Koivula MJ, Lövei GL, Mossakowski D, Noordijk J, Paarmann W, Pizzolotto R, Saska P, Schwerk A, Serrano J, Szyszko J, Taboada A, Turin H, Venn S, Vermeulen R, Zetto T (2011) Forty years of carabid beetle research in Europe – from taxonomy, biology, ecology and population studies to bioindication, habitat assessment and conservation. ZooKeys 100: 55–148. doi: 10.3897/zookeys.100.1523

Lee P (2006) Atlas of the millipedes (Diplopoda) of Britain and Ireland. Pensoft, Sofia, 216 pp.

Maelfait J-P, Baert L, Bonte D, De Bakker D, Gurdebeke S, Hendrickx F (2004) The use of spiders as indicators of habitat quality and anthropogenic disturbance in Flanders, Belgium. In: Samu F, Szinetár C (Eds) European Arachnology 2002. Plant Protection Institute, Budapest & Berzsenyi College, Szombathely, 129–141. http://www.european-arachnology.org/proceedings/20th/Maelfait.pdf

Ožanová J (2001) Utilization of grass traps for the study of millipedes. Myriapodologica Czecho - Slovaca 1: 69–71. [In Czech with English abstract]

Perry WB, Christiansen TA, Perry SA (1997) Response of soil and leaf litter microarthropods to forest application of diflubenzuron. Ecotoxicology 6: 87–99. doi: 10.1023/A:1018658223388

Prasifka JR, Lopez MD, Hellmich RL, Lewis LC, Dively GP (2007) Comparison of pitfall traps and litter bags for sampling ground-dwelling arthropods. Journal of Applied Entomology 131: 115–120. doi: 10.1111/j.1439-0418.2006.01141.x

R Development Core Team (2011) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/

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. http://www.ekologie.upol.cz/ad/tuf/pdf/papers/Riedel+Navratil+Tuf+Tufova2009.pdf

Sergeeva TK, Kudryasheva IV, Titova LP (1985) Seasonal aspects of feeding of geophilids (Chilopoda, Geophilomorpha) in the oak forests of the southern forest-steppe. Zoologicheskij zhurnal 64(9): 1377–1383. [In Russian]

Souty-Grosset C, Badenhausser I, Reynolds JD, Morel A (2005) Investigations on the potential of woodlice as bioindicators of grassland habitat quality. European Journal of Soil Biology 41: 109–116. doi: 10.1016/j.ejsobi.2005.09.009

Tuf IH (2002) Contribution to the knowledge of vertical distribution of soil macrofauna (Chilopoda, Oniscidea) in floodplain forest. In: Tajovský K, Balík V, Pižl V (Eds) Studies on Soil Fauna in Central Europe. Institute of Soil Biology, ASCR, České Budějovice, 241–246. http://ekologie.upol.cz/ad/tuf/pdf/papers/Tuf_2002a.pdf

Tuf IH, Tvardík D (2005) Heat-extractor – indispensable tool for soil zoological studies. In: Tajovský K, Schlaghamerský J, Pižl V (Eds) Contributions to Soil Zoology in Central Europe I. Institute of Soil Biology, ASCR, České Budějovice, 191–194. http://ekologie.upol.cz/ad/tuf/pdf/papers/Tuf_Tvardik_2005.pdf

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 Opava (A) 57: 37–44. http://www.ekologie.upol.cz/ad/tuf/pdf/papers/Tuf+Tufova2008.pdf

Wiegert RG (1974) Litterbag studies of microarthropod populations in three South Carolina old fields. Ecology 55: 94–102. doi: 10.2307/1934621, http://www.jstor.org/stable/1934621

ZooKeys 510: 197–207 (2015)
doi: 10.3897/zookeys.510.9020
http://zookeys.pensoft.net

RESEARCH ARTICLE

A peer-reviewed open-access journal
ZooKeys
Launched to accelerate biodiversity research

Hay-bait traps are a useful tool for sampling of soil dwelling millipedes and centipedes

Ivan H. Tuf[1], Vojtěch Chmelík[1], Igor Dobroruka[1], Lucie Hábová[1],
Petra Hudcová[1], Jan Šipoš[1,2,3], Slavomír Stašiov[4]

1 *Department of Ecology and Environmental Sciences, Faculty of Science, Palacký University, Šlechtitelů 11, 77900 Olomouc, Czech Republic* **2** *Department of Biology and Ecology, Faculty of Science, University of Ostrava, Chittusiho 10, 71000 Slezská Ostrava, Czech Republic* **3** *Global Change Research Centre, Academy of Sciences of the Czech Republic, Bělidla 986/4a, 603 00 Brno, Czech Republic* **4** *Department of Biology and General Ecology, Faculty of Ecology and Environmental Sciences, Technical University in Zvolen, T. G. Masaryka 24, 96053 Zvolen, Slovakia*

Corresponding author: *Ivan H. Tuf* (ivan.tuf@upol.cz)

Academic editor: *Karel Tajovský* | Received 26 November 2014 | Accepted 30 April 2015 | Published 30 June 2015

http://zoobank.org/E2458727-A81B-4645-934F-42F23A718E09

Citation: Tuf IH, Chmelík V, Dobroruka I, Hábová L, Hudcová P, Šipoš J, Stašiov S (2015) Hay-bait traps are a useful tool for sampling of soil dwelling millipedes and centipedes. In: Tuf IH, Tajovský K (Eds) Proceedings of the 16th International Congress of Myriapodology, Olomouc, Czech Republic. ZooKeys 510: 197–207. doi: 10.3897/zookeys.510.9020

Abstract

Some species of centipedes and millipedes inhabit upper soil layers exclusively and are not recorded by pitfall trapping. Because of their sensitivity to soil conditions, they can be sampled quantitatively for evaluation of soil conditions. Soil samples are heavy to transport and their processing is time consuming, and such sampling leads to disturbance of the soil surface which land-owners do not like. We evaluated the use of hay-bait traps to sample soil dwelling millipedes and centipedes. The effectiveness of this method was found to be similar to the effectiveness of soil sampling. Hay-bait traps installed for 8–10 weeks can substitute for direct soil sampling in ecological and inventory studies.

Keywords

Diplopoda, Chilopoda, soil sampling, agroecosystem, soil fauna

Introduction

Soil macrofauna is commonly used for monitoring or evaluation of sites. Besides ground beetles (e.g. Hůrka et al. 1996, Kotze et al. 2011), spiders (e.g. Buchar and Růžička 2002, Maelfait et al. 2004) or woodlice (e.g. Souty-Grosset et al. 2005, Tuf and Tufová 2008), centipedes and millipedes are sampled frequently too (Tuf and Tufová 2008, Dunger and Voigtländer 2009). Nevertheless, not all species of centipedes and millipedes are ground-dwelling with many species inhabiting the upper soil layer exclusively (Lee 2006, Barber and Keay 1988). Soil dwelling animals can be sampled using litter/soil sieving, soil sampling or hand-collecting. Sieved material and soil samples have to be hand-sorted or processed using heat extractors, e.g. Tullgren funnel or Kempson apparatus (Tuf and Tvardík 2005). Handling of soil samples can be difficult due to the higher weight of samples (one sample of size 25 × 25 × 10 cm weighs around 6 kg). Litter/soil sieving can reduce the weight of samples, nevertheless as with hand-collecting, it is time consuming and attention-intensive. Moreover, soil sampling can cause damage to the site; pot-holes created by a soil corer can endanger people passing the site and can increase water erosion on slopes. These pot-holes are definitely not popular among land-owners of the sampled sites. For these reasons (severity of sampling, damage of ground), we have attempted to evaluate the effectiveness of sampling centipedes and millipedes using hay-bait traps. The aims of this research were 1) comparison of the efficiency of hay-bait trapping, soil sampling and pitfall trapping and 2) to find the optimal length of exposure of hay-bait traps for maximum efficiency.

Material and Methods

Field study

The research was done at three sites in the Czech Republic from May to July 2013. The first site was an alfalfa field (49°34.41'N, 17°17.17'E) on the border of the town of Olomouc. This large field of ca 250 square metres is surrounded by other fields (with cereals) and a railway embankment. In the previous year it had also grown alfalfa. The field is under conventional management including use of herbicides and ploughing.

The second site was an old meadow (50°26.85'N, 15°0.00'E) being mown once to twice per year for the last 30 years. This meadow of ca 500 square metres is surrounded by fields and gardens with mixed wood across the road and is ca 6 km north-east of the town of Mladá Boleslav. The third site studied was a mixed forest (49°15.66'N, 17°17.72'E) 6 km south-west of the town of Kroměříž. The forest is classified as *Fageto-Quercetum illimerosum trophicum*; dominant trees are oaks, hornbeams and some pines, with *Rubus fruticosus*, *Galium odoratum* and *Galium aparine* as dominants of undergrowth. The soil surface of this forest is covered by a rather thick layer of oak leaf litter.

In the Czech Republic generally, the weather conditions during the study period were characterised by average or slightly increased temperatures and higher than average precipitation in May-June, and a very hot July in contrast to long-term average values. The previous winter season was rather warmer and with higher precipitation (ref. historical territorial data at www.chmi.cz).

Soil macrofauna, including millipedes and centipedes, was sampled using three methods at each site. Pitfall traps (10 traps consisting of glass jars with inserted plastic pots of diameter 7.5 cm filled with 2 dl of 4% formaldehyde in water with some detergent, metal covers) were arranged in 2 lines of 5 traps with a span of 10 m, and inspected at 2-week intervals. Five soil samples (25 × 25 × 10 cm including litter layer) were obtained using a spatula, three times per study (i.e. 15 soil samples per site) and transported to the laboratory in plastic bags. Hay-bait traps were made from a wire gauze (2 cm mesh) shaped as a simple pocket of size 25 × 25 cm. Each pocket was marked by a code written on the band. These pockets were filled with hay (commercial hay mixture for feeding rodent pets) and submerged into water for 2 hours before installation. Altogether, 60 hay-bait traps were placed horizontally at each site in a following scheme: 5 lines of 12 traps (2-5 cm under soil surface) over a length of 2 m with 10 m between lines. All traps were installed at the same time and 5 traps were taken away each week during the course of the study lasting for 12 weeks. Hay-traps were transported into the laboratory inside separate plastic bags.

Sample processing

Soil samples and hay-traps were heat-extracted immediately in the laboratory using simple Kempson devices (Tuf and Tvardík 2005). Hay-traps were extracted for a week, soil samples for 2 weeks, both under electric 60W-bulbs. Extracted animals from both soil samples and hay-traps were sorted to higher taxonomic groups and millipedes and centipedes were identified to the species level.

Data analyses

We tested the effects of trapping time and methods on species richness by repeated-measures on traps with nested design. The traps were nested in each of the three study sites (field, meadow, forest). Explanatory variables in the model were trapping time and trapping methods. The response variable was defined as a number of species per trap for particular time and place. Habitat type was used as random variable. We used a mixed model to estimate the correct error term and degrees of freedom. To test this effect, a generalized linear mixed model (glmmPQL, part of R package MASS) was used with negative binomial error distribution and log link function (Bates et al. 2014).

To test if one level of a particular factor (trapping method and study site) is more variable than other levels of the same factor, a permutation test was used (permutest.betadisper, part of R package vegan). This permutation based method tests pairwise comparisons of group mean dispersions. It is based on the t-statistic computed on pairwise group dispersions. A distance matrix was computed based on "Bray-Curtis" index of dissimilarity (vegdist, part of R package vegan). Then the function "betadisper" (part of vegan package in R) was used to calculate variance for each group of samples. Variance was computed as average distance of group members to the group centroid.

Rarefaction curves were constructed to show how the species richness varies for the same sample size between the three trapping methods. Function "rarefy" (part of vegan package in R software) computed the expected species richness and standard deviation in random subsamples of a particular sample size from the community. Data were analysed using R software (R Development Core Team 2011).

Results

Altogether, we obtained 541 millipedes from 17 species and 435 centipedes from 13 species (Table 1). Based on the number of recorded animals, the richest site was the forest (553 myriapods) and poorest site was the field (100 myriapods). Number of species showed the same pattern: 21 myriapod species in the forest and 6 in the field. Soil sampling was the least efficient for sampling species (9 millipede and 7 centipede species) as well as individuals (36 and 100 individuals respectively), whereas pitfall traps and hay-bait traps were similar in their efficiency: 14–15 millipede and 9–10 centipede species; for number of individuals, see Table 1.

Methods at individual sites were evaluated according to their efficiency using rarefactions (Fig. 1). Bait traps sampled higher numbers of species in contrast to other methods in the field site, meanwhile increasing sampling effort (number of sampled animals) was connected with a bigger species list in bait traps as well as in pitfall traps in forest.

Differences between species lists at all sites and lists sampled by individual methods were compared by pairwise comparisons and differences confirmed between all pairs of sites (Table 2a). Nevertheless, the same analysis revealed there was no statistically significant difference between the suite of species sampled by hay-bait traps and soil samples (Table 2b).

Evaluation of colonization of hay-bait traps (Fig. 2) showed that the highest diversity as well as abundance of collected myriapods in these traps is after 7 weeks following installation in field, or 9-10 weeks following installation in forest or meadow. A longer period of exposure leads to a decrease of both parameters of myriapod communities. Generalized linear mixed models reveal that changes in abundance during exposure was significantly influenced by the second power of time (LRT = 6.43, p = 0.040, AIC = 667.83). The analogous model for diversity confirmed significant changes during time (LRT = 5.81, p = 0.042, AIC = 543.38) which were site dependent too (LRT = 6.74, p = 0.034, AIC = 544.12).

Table 1. List of millipedes obtained using three methods from three biotopes (ind./10 pitfall traps/12 weeks, ind./60 bait traps and ind./0.94m^2 respectively.

	Pitfall traps			**Hay-bait traps**			**Soil samples**			**Total pitfall traps**	**Total hay-bait traps**	**Total soil samples**
	field	**meadow**	**forest**	**field**	**meadow**	**forest**	**field**	**meadow**	**forest**			
Glomeris connexa C. L. Koch, 1847	-	9	1	-	-	1	-	-	-	10	1	0
Blaniulus guttulatus (Fabricius, 1798)	-	2	-	-	31	1	-	2	-	2	32	2
Brachyiulus bagnalli (Curtis, 1845)	2	-	-	5	-	-	-	-	-	2	5	0
Cylindroiulus boleti (C.L. Koch, 1847)	-	-	3	-	-	-	-	-	-	3	0	0
Cylindroiulus caeruleocinctus (Wood, 1864)	1	-	-	-	-	-	-	-	-	1	0	0
Enantiulus nanus (Latzel, 1884)	-	-	64	-	-	32	-	-	4	64	32	4
Julus scandinavius Latzel, 1884	-	-	-	-	-	1	-	-	-	0	1	0
Leptoiulus proximus (Němec, 1896)	-	-	2	-	-	1	-	-	-	2	1	0
Megaphyllum projectus Verhoeff, 1894	-	-	2	-	-	2	-	-	-	2	2	0
Ommatoiulus sabulosus (Linnaeus, 1758)	-	-	10	-	-	11	-	-	4	10	11	4
Ophiulus pilosus (Newport, 1842)	27	-	-	59	-	-	2	-	-	27	59	2
Unciger foetidus (C.L. Koch, 1838)	-	9	36	-	30	26	-	3	2	45	56	5
Brachydesmus superus Latzel, 1884	-	-	-	-	-	-	-	3	-	0	0	3
Polydesmus complanatus (Linnaeus, 1761)	-	3	1	-	2	3	-	-	1	4	5	1
Polydesmus denticulatus C.L. Koch, 1847	-	8	-	-	7	-	-	-	-	8	7	0
Polydesmus inconstans Latzel, 1884	-	1	-	-	39	-	-	6	-	1	39	6
Strongylosoma stigmatosum (Eichwald, 1830)	-	-	47	-	-	26	-	-	9	47	26	9
Diplopoda	30	32	166	64	109	104	2	14	20	228	277	36
Clinopodes flavidus C.L. Koch, 1847	-	-	2	1	-	9	-	-	-	2	10	0

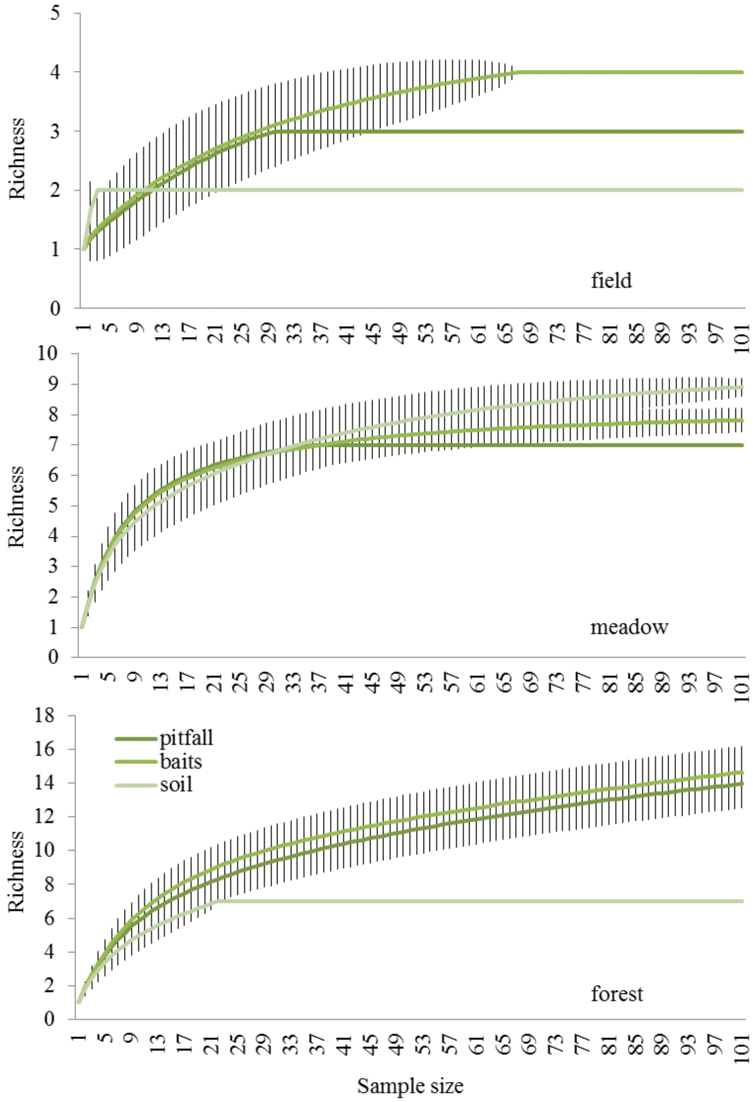


Figure 1. Rarefactions of estimated species richness (i.e. number of species) in increasing size of random samples (i.e. number of individuals), comparison of effectiveness of sampling by individual methods at different sites. Vertical lines represent standard errors.

Table 2. Pairwise comparisons of species lists collected (a) at different sites and (b) by different methods. (Observed p-value below diagonal, permuted p-value above diagonal).

a)	field	forest	meadow	b)	hay-bait	pitfall	soil
field	-	0.001	0.048	hay-bait	-	0.003	0.917
forest	0.000	-	0.001	pitfall	0.003	-	0.043
meadow	0.041	0.004	-	soil	0.911	0.052	-

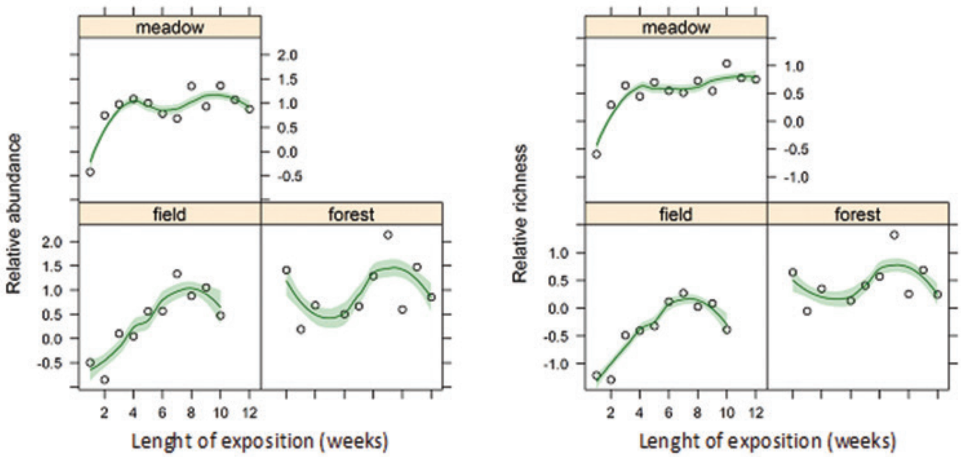


Figure 2. Changes in myriapod communities inside hay-bait traps installed in three biotopes during the 12 week trapping period. Qualitative as well as quantitative parameters are shown for these communities. Open dots are observed parameters, whereas solid lines represent models of succession including standard errors (green shading).

Discussion

Centipedes and millipedes live on the soil surface and inside soil. We can find them through the whole soil gradient to a depth of one meter (e.g. Ilie 2003) although they are abundant in upper layers predominantly. This is the reason why pitfall traps are not sufficient for sampling the whole community adequately. We evaluated efficiency of hay-bait traps for sampling soil-dwelling millipedes and centipedes with the time consuming soil sampling (connected with destruction of the soil surface and transport of heavy samples to the laboratory).

Bait traps were used for sampling invertebrates, mainly beetles, in caves originally (Barber 1931). Bait traps are much more common for carnivorous or necrophagous species; baits are represented by pieces of flesh, fish or cheese above a fixation solution surface, or direct addition of beer to a solution. Straw, wood or yeast is placed in caves occasionally as the baits for detritivores (Mock pers. comm.). Nevertheless, baits are not working there as traps, as they need to be visited and inspected continuously to collect attracted animals to avoid them to leave baits.

The first documented version of bait traps for millipedes was a shingle trap by Barber (1997) filled with kitchen tissue and potatoes. He used this trap to sample millipedes and isopods at a shingle beach in England. Similar kinds of bait traps, containing sweet potatoes or corn, were used by Brunke et al. (2012) for sampling *Cylindroiulus caeruleocinctus* in Canadian fields. Almost the same traps are used with the name litter bags for studying decomposition of different kinds of litter and/or by different size groups of decomposers (according to diameters of holes in the traps). Litter bags are also used for sampling soil mesofauna or microarthropods (e.g. Wiegert 1974).

Prasifka et al. (2007) used litter bags to sample ground dwelling invertebrates; they installed litter bags at the soil surface as well as below the soil surface in a corn field. Above-ground bait traps were attractive for centipedes (millipedes were not recorded in this research). Apart from these publications, we did not found any records of the use of bait traps for sampling millipedes or centipedes.

Hay-bait traps vs. soil samples

If we are interested in using hay-bait traps as an adequate (or even better) substitute for soil sampling, we have to compare species lists of millipedes and centipedes trapped by these methods. There were only three species recorded exclusively from soil sampling, i.e. missing in hay-bait traps: millipede *Brachydesmus superus* and centipedes *Geophilus electricus* and *G. truncorum*. The minute millipede species lives preferably in clay soils with litter (Lee 2006) usually in huge quantities. This species is a dominant species recorded by pitfall traps in cities (Riedel et al. 2009), so its absence in pitfall traps at the meadow site is probably caused by its low abundance. *Geophilus truncorum* was recorded once only, so it is hard to evaluate effectiveness of sampling of so “rare” a species. Nevertheless, both geophilomorphs (*G. electricus* and *G. truncorum*) are known as predators of earthworms (Sergeeva et al. 1985, Keay 1986); for this reason, hay-bait may be not attractive for them as they follow earthworms into their corridors in soil. So, to collect *G. electricus*, soil sampling or direct hand collecting seems to be necessary. Other geophilomorphs (*C. flavidus*, *S. nemorensis*, *S. acuminata*, *G. flavus*) are common species, which are frequently found by individual hand-collecting; they live in soil near the surface, under logs, bark and stones (e.g. Barber and Keay 1988). Their presence in shallow hay-bait traps is not surprising as these species were sampled by pitfall trapping too.

One millipede species, *Julus scandinavius* was recorded exclusively in a hay-bait trap, but as one specimen only was found no generalization can be made. Many more species were found in both hay-bait traps and pitfall traps but not in soil samples. Nevertheless, hay-bait traps are not a substitute method to pitfall trapping as there were significant differences between species lists recorded by these methods (see Tab. 3), but it can definitely substitute the soil samples.

Colonisation of hay-bait traps

Centipedes, and especially millipedes, are attracted into the hay-bait traps. The possible reason can be as a food source and/or sustainable shelter with higher humidity. At least for millipedes, food source seems to be the more probable explanation; wet cloth method (offering higher humidity) did not record any millipedes in African savannah ecosystems (Druce et al. 2004). More probably, millipedes and centipedes are attracted by food availability, as it can be associated with hay decomposition and colonization of the traps. Smaller decomposers colonising baits are welcomed food for carnivorous centipedes (e.g. Perry et al. 1997).

Eight to ten weeks seems to be the optimal exposure time for hay-bait traps in Central European conditions. A similar result was found by Ožanová (2001) using grass traps (a small heap of mowed grass on the surface of meadow), with a much higher number of species after 7 weeks than for a shorter exposure time. Although Prasifka et al. (2007) did not evaluate the effect of length of exposure time of bait traps, it is evident from their results that below-ground traps were more effective after 8 weeks than after 6 weeks. It supports our results that the best length of exposure of bait traps is from 8 to 10 weeks, although we aware of difficulties with this generalization. The best length of exposure is not dependent only on a type of habitat, but also on climate conditions (rainy or dry weather) and time of year when exposed. Traps installed in Central European conditions in late autumn or in winter or during dry hot summer can be colonised in different ways as invertebrates change their activity and position in the soil profile during the year (David 1984, Geoffroy 1985, David et al. 1996, Tuf 2002). The general recommendation for using these traps when installed in spring is to use them for 8–10 weeks. Timing of installation and the length of time exposed in field sites will need to coordinate with agricultural activities such as sowing and harvest times.

Conclusion

Centipedes and millipedes inhabit the soil surface as well as the soil profile. For a complete knowledge of myriapod fauna, pitfall trapping needs to be combined with a method to collect soil dwelling species, e.g. soil sampling. Hay-bait traps were tested for their ability to replace soil sampling. Our results showed that hay-bait traps are attractive to myriapods and can have a similar sampling effort as soil sampling. The optimal length of exposure of hay-bait traps in soil seems to be ca 2 months (8–10 weeks).

Acknowledgement

This research was supported by grant of Ministry of Agriculture, No. QJ1230066 and European Project supported by the Operational Programme Cross Border Co-operation CZ-PL No. CZ.3.22/1.2.00/12.03445. We are also grateful for support of project CzechGlobe – Centre for Global Climate Change Impacts Studies, Reg. No. CZ.1.05/1.1.00/02.0073 and LC06073. We are grateful to Megan Short (Deakin University, Australia) for her English improvement as well as to Petr Dolejš and Jolanta Wytwer for their valuable comments and suggestions.

References

Barber AD (1997) A simple shingle trap for Myriapods. Bulletin of the British Myriapod Group 13: 27–28. http://www.bmig.org.uk/sites/www.bmig.org.uk/files/bulletin_bmg/BullBMG13-1997.pdf

Barber AD, Keay AN (1988) Provisional Atlas of the Centipedes of the British Isles. Grange-over-Sands, Institute of Terrestrial Ecology Merlewood Research station, 127 pp. http://nora.nerc.ac.uk/7275/1/Centipedes.pdf

Barber HS (1931) Traps for cave-inhabiting insects. Journal of the Elisha Mitchell Society 46: 259–266.

Bates D, Maechler M, Bolker B, Walker S (2014) lme4: Linear mixed-effects models using Eigen and S4. R package version 1. 1–7. http://CRAN.R-project.org/package=lme4

Brunke AJ, O'Keefe L, Bahlai CA, Sears MK, Hallett RH (2012) Guilty by association: an evaluation of millipedes as pests of carrot and sweet potato. Journal of Applied Entomology 136: 772–780. doi: 10.1111/j.1439-0418.2012.01708.x

Buchar J, Růžička V (2002) Catalogue of Spiders of the Czech Republic. Peres, Praha, 351 pp. http://arachnology.cz/cas/app_contents/downloads/bibliography/ARA681.pdf

David J-F (1984) Le cycle annuel du diplopode *Microchordeuma gallicum* (Latzel, 1884). Bulletin de la Société zoologique de France 104: 61–70.

David J-F, Célérier M-L, Vannier G (1996) Overwintering with a low level of cold-hardiness in the temperate millipede *Polydesmus angustus*. Acta Oecologica 17: 393–404.

Druce D, Hamer M, Slotow R (2004) Sampling strategies for millipedes (Diplopoda), centipedes (Chilopoda) and scorpions (Scorpionida) in savanna habitats. African Zoology 39: 293–304. http://africanzoology.journals.ac.za/pub/article/view/299/280

Dunger W, Voigtländer K (2009) Soil fauna (Lumbricidae, Collembola, Diplopoda and Chilopoda) as indicators of soil eco-subsystem development in post-mining sites of eastern Germany – a review. Soil Organisms 81: 1–51. http://www.senckenberg.de/files/content/forschung/publikationen/soilorganisms/volume_81_1/01_dunger.pdf

Geoffroy J-J (1985) Les Diplopodes d'un écosystème forestier tempéré, répartition spatio-temporelle des populations. In: Ellis WN, Jeekel CAW, Pieters FFJM (Eds) Proceedings of the 6th International Congress of Myriapodology. Bijdragen tot de Dierkunde 55: 78–87.

Hůrka K, Veselý P, Farkač J (1996): Využití střevlíkovitých (Coleoptera: Carabidae) k indikaci kvality prostředí (The use of carabid communities (Coleoptera, Carabidaae) for the indication of the environment quality). Klapalekiana 32(1-2): 15–26. [In Czech with English abstract and German summary]

Ilie V (2003) Chilopoda species from the edaphic and subterranean environments of the Cloşani karstic area (Romania). Travaux du Muséum National d´ histoire Naturelle «Grigore Antipa» 45: 129–137.

Keay AN (1986) Predation and prey in *Henia* (*Chaetechelyne*) *vesuviana* (Newport) (Chilopoda, Geophilomorpha). Bulletin of the British Myriapod Group 3: 21–25. http://www.bmig.org.uk/sites/www.bmig.org.uk/files/bulletin_bmg/BullBMG03-1986.pdf

Kotze DJ, Brandmayr P, Casale A, Dauffy-Richard E, Dekoninck W, Koivula MJ, Lövei GL, Mossakowski D, Noordijk J, Paarmann W, Pizzolotto R, Saska P, Schwerk A, Serrano J, Szyszko J, Taboada A, Turin H, Venn S, Vermeulen R, Zetto T (2011) Forty years of carabid beetle research in Europe – from taxonomy, biology, ecology and population studies to bioindication, habitat assessment and conservation. ZooKeys 100: 55–148. doi: 10.3897/zookeys.100.1523

Lee P (2006) Atlas of the millipedes (Diplopoda) of Britain and Ireland. Pensoft, Sofia, 216 pp.

Maelfait J-P, Baert L, Bonte D, De Bakker D, Gurdebeke S, Hendrickx F (2004) The use of spiders as indicators of habitat quality and anthropogenic disturbance in Flanders, Belgium. In: Samu F, Szinetár C (Eds) European Arachnology 2002. Plant Protection Institute, Budapest & Berzsenyi College, Szombathely, 129–141. http://www.european-arachnology.org/proceedings/20th/Maelfait.pdf

Ožanová J (2001) Utilization of grass traps for the study of millipedes. Myriapodologica Czecho - Slovaca 1: 69–71. [In Czech with English abstract]

Perry WB, Christiansen TA, Perry SA (1997) Response of soil and leaf litter microarthropods to forest application of diflubenzuron. Ecotoxicology 6: 87–99. doi: 10.1023/A:1018658223388

Prasifka JR, Lopez MD, Hellmich RL, Lewis LC, Dively GP (2007) Comparison of pitfall traps and litter bags for sampling ground-dwelling arthropods. Journal of Applied Entomology 131: 115–120. doi: 10.1111/j.1439-0418.2006.01141.x

R Development Core Team (2011) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/

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. http://www.ekologie.upol.cz/ad/tuf/pdf/papers/Riedel+Navratil+Tuf+Tufova2009.pdf

Sergeeva TK, Kudryasheva IV, Titova LP (1985) Seasonal aspects of feeding of geophilids (Chilopoda, Geophilomorpha) in the oak forests of the southern forest-steppe. Zoologicheskij zhurnal 64(9): 1377–1383. [In Russian]

Souty-Grosset C, Badenhausser I, Reynolds JD, Morel A (2005) Investigations on the potential of woodlice as bioindicators of grassland habitat quality. European Journal of Soil Biology 41: 109–116. doi: 10.1016/j.ejsobi.2005.09.009

Tuf IH (2002) Contribution to the knowledge of vertical distribution of soil macrofauna (Chilopoda, Oniscidea) in floodplain forest. In: Tajovský K, Balík V, Pižl V (Eds) Studies on Soil Fauna in Central Europe. Institute of Soil Biology, ASCR, České Budějovice, 241–246. http://ekologie.upol.cz/ad/tuf/pdf/papers/Tuf_2002a.pdf

Tuf IH, Tvardík D (2005) Heat-extractor – indispensable tool for soil zoological studies. In: Tajovský K, Schlaghamerský J, Pižl V (Eds) Contributions to Soil Zoology in Central Europe I. Institute of Soil Biology, ASCR, České Budějovice, 191–194. http://ekologie.upol.cz/ad/tuf/pdf/papers/Tuf_Tvardik_2005.pdf

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 Opava (A) 57: 37–44. http://www.ekologie.upol.cz/ad/tuf/pdf/papers/Tuf+Tufova2008.pdf

Wiegert RG (1974) Litterbag studies of microarthropod populations in three South Carolina old fields. Ecology 55: 94–102. doi: 10.2307/193462,1 http://www.jstor.org/stable/1934621

ZooKeys 510: 209–222 (2015)
doi: 10.3897/zookeys.510.8668
http://zookeys.pensoft.net

RESEARCH ARTICLE

A peer-reviewed open-access journal
ZooKeys
Launched to accelerate biodiversity research

New records of *Lophoproctus coecus* Pocock, 1894 (Diplopoda, Polyxenida, Lophoproctidae) extend the range of the genus *Lophoproctus*

Megan Short[1]

[1] *Deakin University, 221 Burwood Highway, Burwood, Melbourne, Australia*

Corresponding author: *Megan Short* (mshort@deakin.edu.au)

Academic editor: *Ivan H. Tuf* | Received 29 September 2014 | Accepted 5 May 2015 | Published 30 June 2015

http://zoobank.org/4FF544AC-67B8-413A-A544-38A3F299FCF1

Citation: Short M (2015) New records of *Lophoproctus coecus* Pocock, 1894 (Diplopoda, Polyxenida, Lophoproctidae) extend the range of the genus *Lophoproctus*. In: Tuf IH, Tajovský K (Eds) Proceedings of the 16th International Congress of Myriapodology, Olomouc, Czech Republic. ZooKeys 510: 209–222. doi: 10.3897/zookeys.510.8668

Abstract

The geographic distribution of the genus *Lophoproctus* Pocock, 1894 has greatly expanded with new records of the species *Lophoproctus coecus* Pocock, 1894, together with the reassignment of a number of millipedes formerly identified as *Lophoproctus lucidus* (Chalande, 1888). *L. coecus* was found to be the sole representative of the family Lophoproctidae in collections examined from Crimea and the Caucasian region. The species was also identified from Iran and Kyrgyzstan. *Lophoproctus* specimens collected in Italy by Verhoeff were reassigned as *L. coecus* with the exception of one specimen of *L. jeanneli* (Brölemann, 1910) from Capri. These data were combined with all available information from the literature to look at the pattern of distribution of the four species in the genus. The range of the genus *Lophoproctus* extends from Portugal to Central Asia. *Lophoproctus coecus* is widespread from Italy eastward, while the morphologically very similar species *L. lucidus* is confined to France and northern Africa. The two species have a narrow overlap in the Alpes Maritimes region of France. *L. jeanneli* has a scattered coastal distribution around the Mediterranean Sea. The troglobitic species *L. pagesi* (Condé, 1982) has only been recorded from a cave on Majorca, Spain.

Keywords

Penicillata, millipedes, geographic distribution, Caucasus, Crimea, Italy

Introduction

Genera and species in the family Lophoproctidae Silvestri, 1897 have very similar morphology, with species adapted to an endogenous mode of life, being found in soil, caves and under stones. All Lophoproctidae lack ocelli, their integument lacks pigmentation and the 8th antennal article is elongate. Species in the family also share the same arrangement of caudal trichomes and similar organisation of tergal trichomes (Fig. 1).

Characters used to determine genus and species of the family can be difficult to observe and include number and arrangement of antennal sensilla, number and arrangement of linguiform processes along the anterior margin of the labrum, structure of the telotarsus, leg setae, and tarsal spine. There are currently 5 genera: *Lophoproctus* Pocock, 1894, *Lophoturus* Brolemann, 1931, *Ancistroxenus* Schubart, 1947, *Lophoproctinus* Silvestri, 1948, and *Alloproctoides* Marquet & Condé, 1950. Two further genera *Barroxenus* Chamberlin, 1940 and *Trichoproctus* Silvestri, 1899, known only from single collections, are of uncertain status as they are inadequately described.

Pocock (1894) established the genus *Lophoproctus* for a species collected from soil at Nervi in Liguria, Italy. As the species lacked ocelli he called it *coecus* ('blind' in latin). Previously in 1888, Chalande had described the species *Pollyxenus* (sic) *lucidus* from Palalda, Eastern Pyrenees, France, which he initially described as having ocelli. In 1894 Silvestri identified *Polyxenus lucidus* from Italy, then later the same year recognising that the specimens had no ocelli he moved the species into the genus *Lophoproctus*. He further suggested that *L. coecus* and *L. lucidus* were synonymous (Silvestri 1894b) as did Verhoeff some years later (Verhoeff 1921). Both Silvestri and Verhoeff collected widely throughout Italy (Silvestri 1894a, 1894b, Verhoeff 1921, 1952) and identified all lophoproctids they found as *L. lucidus* with the exception of those from Capri that Verhoeff mistakenly described as a new species *Lophoproctus litoralis* (Verhoeff, 1952). *L. litoralis* was later determined to be *Lophoproctus jeanneli* (Brölemann, 1910) (Condé 1969). Condé (1978) re-examined material from Verhoeff's collection from Isernia and Teramo, Zannone (Pontine Islands) and Sardinia, Italy, and noted that they differed from *L. lucidus* in that they had a different arrangement of sensilla on antennal article VI which was also more elongate. On the basis of Condé's description, Nguyen Duy-Jacquemin (1993) confirmed that these specimens were *L. coecus*.

In the Caucasian and Crimean regions the previous records of *Lophoproctus* are by Lignau. He collected *Lophoproctus* in Krasnodar Polyanna (Lignau 1903), Crimea (Lignau 1905) and in Gagri (Lignau 1914) and although in his earlier papers he had identified the specimens incorrectly, in his 1911 paper he identified all as *Polyxenus* (*Lophoproctus*) *lucidus* (Lignau 1911). Subsequent species lists published all include *Lophoproctus lucidus* (Lohmander 1936, Kobakhidze 1965, Talikadze 1984) presumably based on Lignau's early collections.

In 1993, Nguyen Duy-Jacquemin redescribed *L. coecus* from syntypes from Nervi, Italy that together with specimens collected in Zannone, Italy by Verhoeff and from Rome, Italy by Silvestri, confirmed that *L. lucidus* and *L. coecus* were not synonymous.

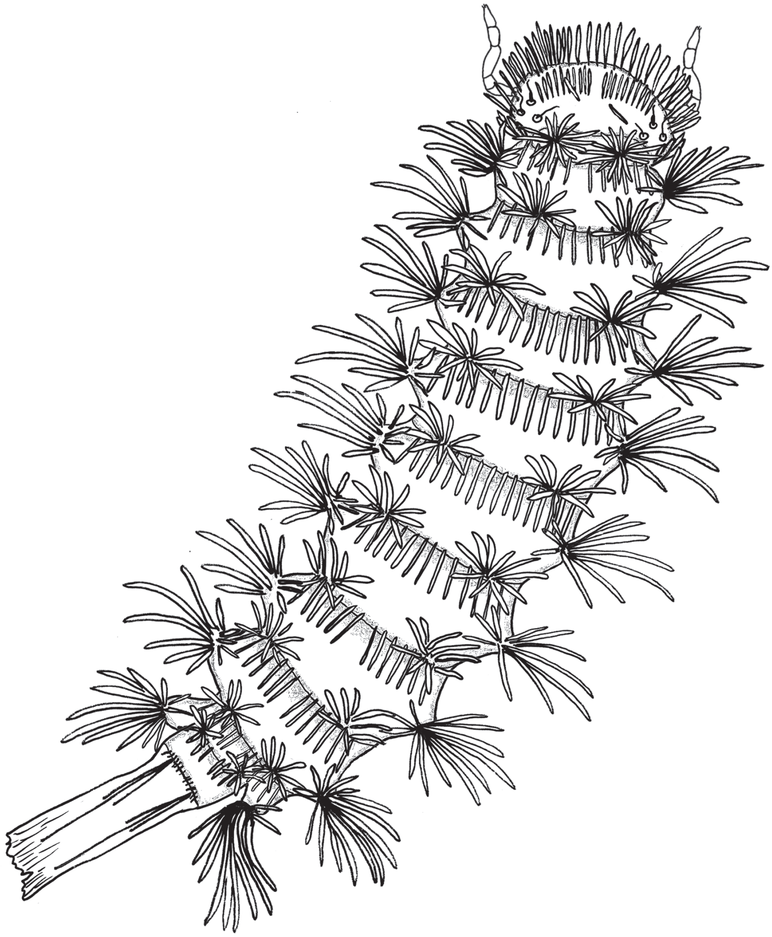


Figure 1. Habitus drawing of *Lophoproctus coecus* Pocock, 1894 showing typical morphology of the family Lophoproctidae.

Her paper clearly illustrates that the two species differ in arrangement of sensilla on antennal article VI (Fig. 2), structure of the median lobe on the anterior edge of the labrum, the number of ridges on the leg setae and the ratio of the length of the tarsal spine to length of the claw (Fig. 4). Initially described as a subspecies of *L. lucidus*, *L. jeanneli* is also found in the Mediterranean region of Europe but is easily distinguishable from other species of *Lophoproctus* by the presence of a denticle on the claw of the telotarsus.

The most recently described species *Lophoproctus pagesi* Condé, 1982, is a troglobitic species collected in caves on the island of Majorca, Spain. Specimens similar to *L. pagesi* have also been collected from caves in Portugal. *L. pagesi* differs from other species of *Lophoproctus* in details of the labrum, antennal sensilla and telotarsus. As well it has elongate antennae and legs typical of troglobitic species (Condé 1982, Nguyen Duy-Jacquemin 1993).

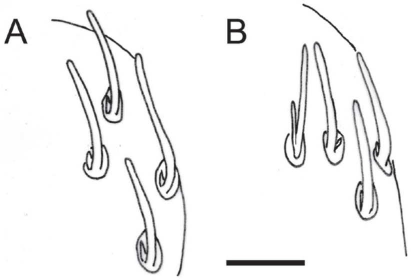


Figure 2. Typical pattern of basiconic sensilla on the right 6th antennal article. **A** *Lophoproctus coecus* **B** *Lophoproctus lucidus*. The coeloconic sensilla are not visible due to angle of view. Scale bar: 20 µm (**A, B**).

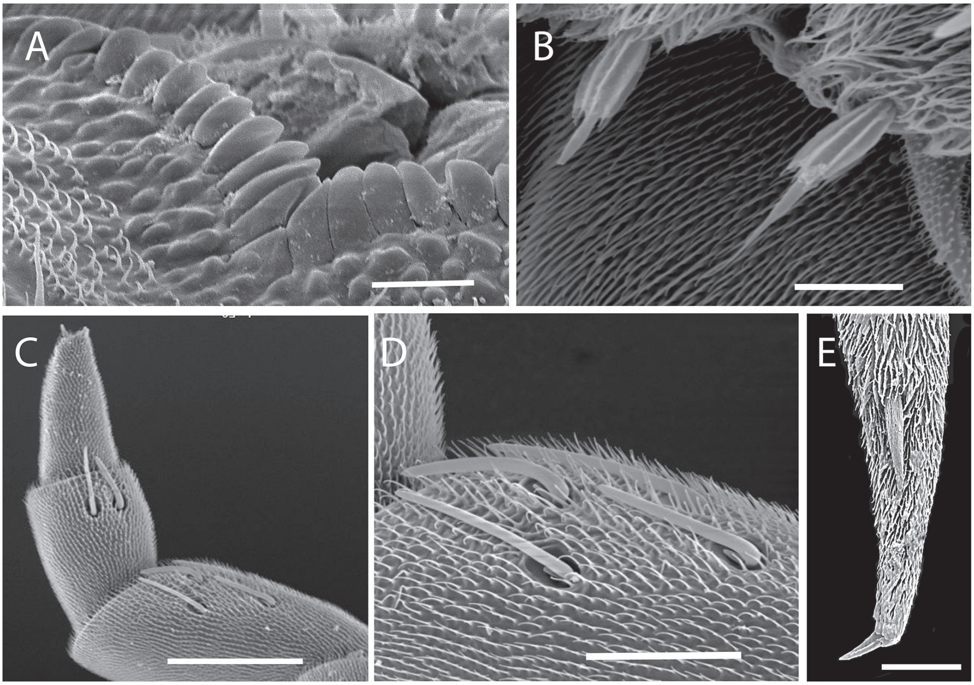


Figure 3. *Lophoproctus coecus* (Pocock, 1888), Krasnodar Province, Russia. Illustration of diagnostic features. **A** labrum showing triangular median linguiform process **B** typical setae on coxa **C** right antenna showing articles VI–VIII with sensilla **D** right antennal article VI showing arrangement of sensilla **E** tarsus 2 and telotarsus. Scale bars: 10 µm (**A, B**); 50 µm (**C**); 20 µm (**D, E**).

In this study I re-assessed specimens from The Zoological State Collection, Munich collected by Verhoeff in Italy and identified as *L. lucidus*. I also identified Lophoproctidae from Crimea, Caucasus, Iran and Kyrgyzstan in the collection of the Zoological Museum of Moscow. These data were then combined with details obtained from the published literature on *Lophoproctus* species to determine distribution of species in the genus.

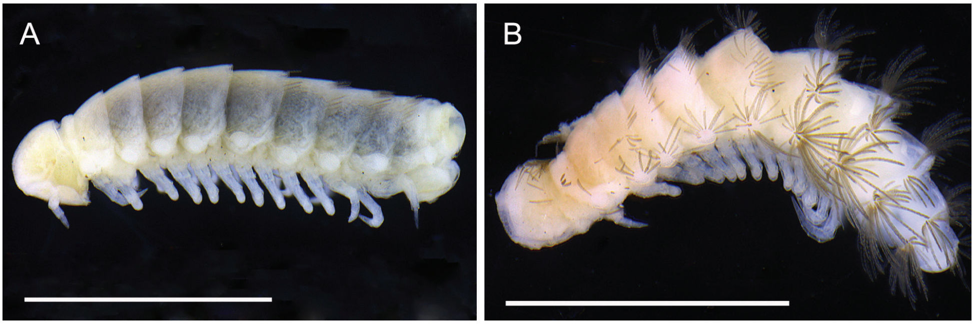


Figure 4. *Lophoproctus coecus* (Pocock, 1888) Krasnodar Province, Russia. **A** Lateral view showing antenna **B** Dorso-lateral view showing tergal trichomes. Scale bars: 1 mm.

Methods

The material examined for this study is lodged in the Zoological Museum of Moscow and the Zoological State Collection in Munich, Germany.

Specimens from the Zoological Museum of Moscow were examined and identified. All specimens were preserved in ethanol. These specimens were examined by light microscopy and scanning electron microscopy. For light microscopy, specimens were mounted on slides in Hoyer's medium, dried at 60 °C and examined with an Olympus CX 41 compound microscope. Scanning electron micrographs were obtained of selected whole specimens that were dehydrated in a graded series of ethanol, 80%, 90% and 100%, then air-dried. Specimens were then mounted on stubs using adhesive tabs, sputter-coated with gold and examined with a Philips XL20 scanning electron microscope. Photographs of whole specimens were taken with a Leica Integrated Stereomicroscope System comprising a Leica 205C microscope with a DFC425 camera and 5000HDI dome illuminator. Images were stacked using Leica Application Suite and enhanced using Adobe Photoshop CS6.

Specimens from the Zoological State Collection, Munich, are slide mounts in Canada Balsam made by KW. Verhoeff. The slides lack both date of collection and site habitat details. The slides were examined by light microscopy using an Olympus CX41 compound microscope. Due to the thickness of the slide mounts, they could not be examined at magnifications higher than 400×.

As no coordinates were available for most of the material examined, Google Earth was used to provide an estimate of geographical position for mapping purposes (a table of localities with coordinates is available in supplementary material). A map of the distribution of all species in the genus *Lophoproctus* was generated using SimpleMappr (Shorthouse 2010). New records determined in this study were included together with all known published records. Many records in the literature, especially those by Verhoeff, Silvestri and Tabacaru are questionable and these have been treated separately.

Results

Sixty collections of Polyxenida in the Zoological Museum of Moscow were examined and *L. coecus* identified in 15. In most cases less than 5 specimens were collected at a site. No other species of Lophoproctidae were found.

Twenty slides from the Zoological State Collection, Munich (ZSM/Myr. 20031594–612, 615) all labelled as *L. lucidus* were examined and 19 found to be *L. coecus*. Slide ZSM/Myr. 20031615 contained a whole mount of *L. jeanneli*.

The geographic distribution of all known localities of the genus *Lophoproctus* was plotted using the data listed below (Fig. 5).

Systematics

Order Polyxenida Lucas, 1840
Family Lophoproctidae Silvestri, 1897
Lophoproctus Pocock, 1894

1. *Lophoproctus coecus* Pocock, 1894

Records from literature. Nervi, suburb of Genova, Italy, on the open hill-side behind the town, beneath stones, alt. 400–500 ft. (Pocock 1894); Grotta di San Antonino, Finale Ligure, Italy, leg. Ascenso 1950 (Nguyen Duy-Jacquemin 1993); Grotta di San Antonio =Antonino, Finale Ligure, Italy, leg. Comotti Baldan 13 Aug 1986 (Nguyen Duy-Jacquemin 1993); Grotta di Arma do Rian, Finalborgo, Italy, leg. Franciscolo 16 Mar 1952 (Nguyen Duy-Jacquemin 1993); Zannone, Ponziane Islands, Italy, leg. Condé 28–29 Jan 1966, 26–27 Feb 1966, 17 Jan 1967 (Nguyen Duy-Jacquemin 1993); Grotta di Nettuno, Porto Conte, Alghero, Sardinia, Italy, near the pier, 4 Oct

Figure 5. Map indicating geographic distribution of *Lophoproctus* species. Legend: red star = *L. coecus*; yellow circle = *L. lucidus*; white star = *L. coecus*/*L. lucidus*? (many in similar localities as *L. coecus* and hence hidden); aqua triangle = *L. jeanneli*; purple square = *L. pagesi*; purple hexagon = *L.* cf. *pagesi*; white circle = *Lophoproctus* sp. indet. Map created using SimpleMappr, http://www.simplemappr.net, (Shorthouse 2010).

1955, leg. Condé (Nguyen Duy-Jacquemin 1993); Isernia, Italy, leg. Verhoeff (Condé 1982), ? same specimen as listed below; Teramo, Italy, leg. Verhoeff (Condé 1982), ? same specimen as listed below; Villa Pamphyli, Rome, Italy, leg. Silvestri Nov 1893 (Silvestri 1894a, reclassified Nguyen Duy-Jacquemin 1993).

Unpublished record. Nice (pers. comm. Nguyen Duy-Jacquemin 2014).

Re-examined material collected in Italy by Verhoeff (date of collection known for only 2 specimens). ZSM/Myr-20031594, Tivoli, Lazio; ZSM/Myr 20031595, Isernia, Molis ; ZSM/Myr-20031596,Teramo, Abruzzo; ZSM/Myr-20031597, Portofino, Genova, Liguria; ZSM/Myr-20031598, Portofino, Genova, Liguria, molt; ZSM/Myr-20031599, Italy, molt; ZSM/Myr-20031600, Elba, Livorno, Toscana; ZSM/Myr-0031601, Chiesetal, Vestone, Brescia, Lombardia; ZSM/Myr-20031602, Isernia, Molise; ZSM/Myr-20031603, Ferrania, Liguria, 01.07.1933; ZSM/Myr-20031604, Mele, Genova, Liguria; ZSM/Myr-20031605, Monte Cimino, Soriano, Viterbo, Lazio; ZSM/Myr-20031606, Porto Santo Stefano, Grosseto, Toscana; ZSM/Myr-20031607, Veneto, Vicenza; ZSM/Myr-20031608, Ferrania, Liguria; ZSM/Myr-20031609, Santuario, Savona, Liguria; ZSM/Myr-20031610, Frigido, Toscana, from under stones at a mill ruin, April 1907; ZSM/Myr-20031611, illegible labelling; ZSM/Myr-20031612 Capri (No 27), Napoli, Campania.

New material from Zoological Museum of Moscow. Nikita Botanical Garden, Cape Martyan, near Yalta, Crimea, 4 Nov 1947, leg. M.S. Ghilarov; Gurzuf, Yalta, Crimea, Jun–Sep 1947, leg. M.S. Ghilarov (2 vials); Utrish Nature Reserve, Krasnodar province, Russia, oak hornbeam forest, 15 Jun 2013, leg. I. Tuf; Utrish Nature Reserve, Krasnodar province, Russia, hornbeam forest, 14 Jun 2013, leg. I. Tuf; Goryachy Klyuch, Mtn ridge, Markotkh plateau, Krasnodar province, Russia, 3 Jul 1956, leg. M.S. Ghilarov; Dagomys, Sochi, Krasnodar province, Russia, *Quercus* shrub, *Carpinus, Fagus* etc., 18 May 1983, leg. S. Golovatch; on road 2 km N of Dagomys, Krasnodar province, Russia, 2 Jun 2014, leg. M. Potapov; Cave “Our Lady”, ca 8 km from Khosta Sochi, Krasnodar province, Russia, *Buxus, Fagus, Acer* etc., forest near entrance, litter and under stones, 16 May 1985, leg. S. Golovatch; Khosta, Sochi region, Krasnodar province, Russia, *Sambucus*, 26 Jun 1956, leg. M.S. Ghilarov; Ris Forest, Bobcai east, Gumista River, Abkhazia, Russia, litter, 5 Jun 1982, leg. J. Bohàc; environs of Keda, Adjaria, Georgia, *Picea* and deciduous forest, 1 Oct 1975, A. Druk; Nedzura River valley 8km SE of Akhaldaba, Borzhomi district, Georgia, *Picea, Carpinus* and *Fagus* forest, litter, logs ,12 May 1983, leg. S. Golovatch; Arslanbob, Fergana mountain range, environs of Yarodar, Kyrgyzstan, dry limestone slopes with grass, under stones, 28 Sep 1983, leg. K. Eskov; Sari, Mazanderan province, Iran, *Quercus* and *Carpinus* forest, 11 Apr 2013, leg. M. Mehrafrooz.

Distribution. South-East France, Italy, Russia, Georgia, Iran and Kyrgyzstan.

2. *Lophoproctus lucidus* (Chalande, 1888)

Records from literature. Palalda (now Amélie-les-Bains-Palalda), Pyrénées-Orientales, France, in soil under litter layer in oak woods, leg. Chalande (Chalande 1888);

cave Gourgue,Canton Aspet, No. 229, Haute-Garonne, France, (Nguyen Duy-Jacquemin 1993); Baumo de las Fadas, Canton du Barjac, Dept du Gard, France, 26 Aug 1909, leg. Brölemann (Brölemann 1910); Albères, France, 1926, leg. Brolemann (Condé 1950); Banyuls sur Mer, France, leg. Brölemann (Nguyen Duy-Jacquemin 1993); Hyères, France leg, A Dollfus (Nguyen Duy-Jacquemin 1993); Ariège, France, leg. Nguyen Duy-Jacquemin (Nguyen Duy-Jacquemin 2000); Souk el Arba, Jendouba district, Tunisia, 30 Mar 1896, leg. Silvestri (Silvestri 1896); La Pérouse (now known as Tamentfoust), Dar El Beïda district of Algiers, Algeria, in wave washed and dry plant material (Seurat 1930); Marrakech, Morocco, 10 Dec 1950, (Condé 1954); Marrakech, Morocco, Jardin de l' Aguedal, near the Mechouar (Condé 1954); Marrakech, Morocco, Jardin de la Bahia (Condé 1954); Marrakech, Morocco, Parc de la Villa Majorelle, under flower pots and stones, 10 Dec 1950 (Condé 1954).

Unpublished records. El Ghazalaf Ariana, nr. Tunis, Tunisia, garden of private house in earth, beneath *Cydonia* tree (pers. comm. N. Akkari, 2014).

New material. La Parc Phoenix, Nice, France, in cold greenhouse, Jan 2014, leg. JM Lemaire.

Distribution. France, Tunisia, Algeria, Morocco.

3. *Lophoproctus jeanneli* (Brölemann, 1010)

Re-examined material collected by Verhoeff (date not given). ZSM/Myr-20031615: Corsica, France.

Records from literature. Baume (grotto) du Colombier, Alpes-Maritimes, commune de Roquefort-les-Pins, canton de Bar-sur-Loup, France, 17 Sep 1905, 27 Sep 1908 (Brölemann 1910); Grotte de la Chèvre d'Or, canton de Bar-sur-le-Loup, France 25 Nov 1987, leg. V. Aellen, (Condé 1989); City park, Barcelona, Spain, Sep 1950 leg. Condé (Condé 1954); Lower Gravona River, left bank of western arm of river, opposite Canapajolo, Corsica, France (Condé 1953); Pointe de Porticcio, sur la côte S. du golfe d'Ajaccio, near houses, 1 km to south west of Fallaccioli, Corsica, France (Condé 1953); Togna, commune de Sari-di-Porto Vecchio, at the edge of a ravine and a garden well, Corsica, France, (Condé 1953); Cueva de la Moriguilla (Vilacarrilo), Andalucia, Spain (Golovatch and Mauries 2013); Esporlas near Palma, Majorca, Balaeric Islands, Spain, at irrigation canal overgrown with dry compact rootlets 17 Aug–23 Sept 1954, leg. J. Pagès (Condé 1955); near Bagno di Tiberio, beach, Capri, Italy, leg. Verhoeff (Condé 1969); Malta leg. Silvestri (Nguyen Duy-Jacquemin 1993, with reservation); near Dékouané, 7 Km to east of Beirut, Lebanon April 1952, leg. PJ. Corset, (Condé 1954, Nguyen Duy-Jacquemin 1993 with reservation); Tel Dan, Israel, 26 Dec 1963 , leg. G. Levy (Condé and Nguyen Duy-Jacquemin 1970).

Distribution. France (mainland and Corsica), Spain (mainland and Majorca), Italy (Capri), Malta, Lebanon, Israel.

4. *Lophoproctus lucidus/coecus*?

Specimens identified as *L. lucidus* but likely to be *L. coecus* as Silvestri and Verhoeff thought the two species were synonymous.

Records from literature. Bevagna, Umbria, Italy, in meadow and forest, late October 1893, leg. Silvestri (Silvestri 1894a); Woods at Madama and Acquacetosa, near Rome, Italy, on ground in plant debris, in forest and open places, leg. Silvestri, November 1893 (Silvestri 1894a); at Colle Pezzo, Mt. Martano, Umbria, Italy 15 Oct 1893, leg. Silvestri (Silvestri 1894a); Medol Casello, Lombardy, Italy, on floor of cave 1935 –1940, leg. various unnamed (Manfredi 1940): Sicily, Italy, leg. Silvestri (Silvestri 1898); Mt. Schignano, Italy, in soil among plant debris, not only in forests, but also in open places, 15 Oct 1893, leg. Silvestri (Silvestri 1894a); Syracuse, Sicily, Italy, 1962–1968, leg. Institute of Zoology of Catania (Strasser 1970); Ciminà, Aspromonte, Calabria, Italy 25 Oct 1966, leg. G. Osella (Strasser 1970); St Remo, Italy under stones in an olive terrace 7–21 April 1907, leg. Verhoeff (Verhoeff 1921); Pegli, Italy in creek valley 7–21 April 1907, leg. Verhoeff (Verhoeff 1921); Massa, Carrara, Italy, in sandstone gorge 7–21 April 1907, leg. Verhoeff (Verhoeff 1921); St. Margherita, Italy, in chestnut wood 7–21 April 1907, leg. Verhoeff (Verhoeff 1921).

5. *Lophoproctus* sp. indet., reported as *Lophoproctus lucidus*

Records from literature never formally identified (pers. comm. Nguyen Duy-Jacquemin, 2014): Pădurea Comorova, Romania (Tabacaru 1966); Mangalia, Romania, 20 Nov 1963 (Tabacaru 1966); Pădurea Hagieni, Romania, 17 May 1963, (Tabacaru 1966); Canaraua de pe Graniţă - comuna Băneasa, Romania 2 Aug 1962, leg. Dumitrescu et al. (Tabacaru 1966); Cave Gura Dobrogii litoclazic, Romania 17 Jun 1963 leg. Dumitrescu et al. (Tabacaru 1966); Cave Gura Dobrogii, Romania, at entrance 17 Sep 1963 leg. Dumitrescu et al. (Tabacaru 1966); Casian, Romania 27 Jul 1962, 7 May 1963, 30 Aug 1964 leg. Dumitrescu et al. (Tabacaru 1966).

Further record from literature, identification uncertain. Marine de Sisco, Corsica, France, 3 Sept 1942, leg. P. Remy (Verhoeff 1943).

6. *Lophoproctus* sp. indet.

Record from literature. Kalimantsi, South Pirin Mountains,Bulgaria, in ant nests 1 Mar 2003, leg. Lapeva-Gjonova (Stoev and Lapeva-Gjonova 2006)

7. *Lophoproctus pagesi* (Condé, 1982)

Records from literature. Cueva de Genova near to Palma, Majorca, Balearic Islands, Spain (Condé 1982); Cueva de Bellver, Palma, Majorca, Balearic Islands, Spain, unpublished, Condé det. (Nguyen Duy-Jacquemin 1993);

Lophoproctus cf. *pagesi* : "Gruta do Fumo", Parque Natural da Arrábida, Portugal (Cardoso et al. 2008, Nguyen Duy-Jacquemin 2014).

Distribution. Caves on Majorca (*L.* cf. *pagesi* – cave in Portugal).

Discussion

Lophoproctus coecus has previously been considered to occupy a scattered range within the Central Mediterranean region, but the results of this study indicate that the species is widespread throughout Europe particularly in Eastern Europe with its distribution extending into Central Asia. *L. lucidus* in comparison seems limited to the Southern France, as well as Morocco, Algeria and Tunesia in Northern Africa. The identification of *L. jeanneli* from Capri, Italy reinforces the Mediterranean coastal distribution previously noted by Kime and Enghoff (2011). It is of interest that species within the genus may overlap in their geographic distribution with both *L. jeanneli* and *L. coecus* being found in Capri and in the Alpes Maritimes region of France. *Lophoproctus pagesi* and *L. jeanneli* both occur on the island of Majorca, with *L. pagesi* restricted to caves while *L. jeanneli* was found in humid, sunny hilly areas.

A number of identifications were unable to be checked. In the case of the *Lophoproctus* identified by Lignau as *L. lucidus*, specimens identified as *L. coecus* in this study were found at all 3 of Lignau's collection areas, indicating that it is most probable that the specimens collected by Lignau were in fact *L. coecus*. Prior to publication of Nguyen Duy-Jacquemin (1993), the difference between *L. coecus* and *L. lucidus* was not understood with Silvestri (1894b) considering the two species synonymous. Hence, until it can be confirmed, the identification of specimens of *Lophoproctus* as *L. lucidus,* from Romania (Tabacaru 1966) and Sicily (Silvestri 1903, Strasser 1970) must remain questionable. *Lophoproctus* has also been collected from Bulgaria but has not yet been identified to species (Stoev and Lapeva-Gjonova 2006).

The distribution map (Fig. 5) indicates very clearly that there is a big gap in our knowledge of *Lophoproctus* in Greece, the Balkans and Turkey. It is predicted that *L. coecus* does occur in these three locations, and that *L. jeanneli* may also occur in coastal regions. All species except the troglobitic *L. pagesi* have the ability to live in many of the habitats in these areas of Europe and Asia. Unfortunately there is limited information available to guide collection of these tiny millipedes. In most cases millipedes in this study were collected by hand collecting in the field, or by sieving of litter and/or soil followed by direct collection from a tray of sieved material (Fig. 6). Recently in Dagomys, Russia, *L. coecus* was collected from forest litter by funnel extraction (M. Potapov pers comm. 2014). Habitats from which *Lophoproctus* has been collected vary from maquis and forest litter, top layer of soil, cave floors, to under stones and logs, and in

Figure 6. Ivan H. Tuf and Daria Kuznetsova collecting invertebrates including *Lophoproctus coecus* from sieved soil and litter, Utrish Nature Reserve, Krasnodar province, Russia.

ants nests (Stoev and Lapeva-Gjonova 2006). In the case of *L. coecus* from Kyrgyzstan, the single specimen was collected from under stones on a dry grassy limestone slope, a similar habitat to that of the type collected by Pocock (1894), 'open hillside beneath stones'. As well as the above mentioned habitats, *L. lucidus* has been found in North African cities in city parks, in gardens and under pot plants and stones (N Akkari pers comm. 2014, Condé 1954), and near Algiers on the beach in accretions of both dry and damp marine plant material (Condé 1954). *Lophoproctus jeanneli* has been collected in abundance from cracks in bricks and under dry stones on the ground in heavy shade in the Barcelona City Park (Condé 1954) as well as on the sea shore (Verhoeff 1952).

Methods of dispersal have not been studied, but it is probable that the presence of *L. lucidus* in North African cities is due to anthropogenic activities dating back to the French colonial period as millipedes confirmed to be *L. lucidus* appear limited to Southern France and centres of French colonial activity in North Africa. Polyxenida are thought to passively disperse via the wind and incidental attachment to the feathers of birds. It is likely in the case of soil and litter dwelling lophoproctids that dispersal via wind is less common as they do not appear to be living in elevated situations in trees and bushes in contrast to Polyxenida from the families Synxenidae and Polyxenidae. However, limited methods of dispersal do not seem to have restricted the geographic ranges of species of the genus *Lophoproctus*, especially *L. coecus*.

Acknowledgements

I am grateful to Sergei Golovatch for providing me with the opportunity to study the Crimean and Caucasian Polyxenida from the Zoological Musum of Moscow and to Mikhail Potapov and Ivan Tuf for some very recent specimens from the same region. Thanks also to Cuong Huynh and Stefan Friedrich for access to Verhoeff's *Lophoproctus* slides, to Khanh Tran and Nick Porch for assistance with SEM and photomontage imaging. I greatly appreciated the improvements to the manuscript suggested by Jean-Francois David and an anonymous reviewer. Finally many thanks to Monique Nguyen Duy-Jacquemin for her continuing advice and support. This study was funded by the author.

References

Brölemann HW (1910) Biospeologica. XVII. Symphyles, Pselaphognathes, Polydesmoides et Lysiopetaloides (Myriapodes) (première série). Archives de Zoologie Expérimentale et Générale, 5e série 5(7): 339–378.

Cardoso P, Nguyen Duy-Jacquemin M, Rasteiro F (2008) New and first records of Polyxenida (Diplopoda: Penicillata) from Portugal. Boletin de la Sociedad Entomologica Aragonesa 42: 360.

Chalande J (1888) Les Pollyxenidae de France; révision du genre *Pollyxenus*. Bulletin de la Société d'Histoire Naturelle de Toulouse 22: 56–90.

Condé B (1950) Un Diplopode nouveau pour la France. L'entomologiste 6(4–5): 109–116.

Condé B (1953) Diplopodes Pénicillates de Corse. Bulletin de la Société Zoologique de France 52: 144–146.

Condé B (1954) Sur la faune endogée de Majorque (Penicillates, Protures, Diploures Campodéidés, Palpigrades). Bulletin du Muséum National d'Histoire Naturelle, 2e série 26: 674–677.

Condé B (1969) Le statut de Lophoproctus litoralis Verhoeff (Diplopode Pénicillate). Bulletin du Muséum National d'Histoire Naturelle, 2e série 40(3): 497–499.

Condé B (1978) Diplopodes Pénicillates, Diploures Campodéidés et Palpigrades des îles Ponziane. Revue d'Ecologie et de Biologie du Sol 15(2): 273–271.

Condé B (1982) Un Pénicillate cavernicole de Majorque (Diplopoda, Penicillata). Archives des Sciences de Genève 34(3): 313–318.

Condé B (1989) Découverte, dans une seconde grotte, du Diplopode Pénicillate *Lophoproctus jeanneli* (Brölemann). Revue Suisse de Zoologie 96(1): 91–97

Condé B, Nguyen Duy-Jacquemin M (1970) Pénicillates d'Israël rassemblés par G. Levy. Bulletin du Muséum National d'Histoire Naturelle, 2e série 42(6): 1251–1258.

Golovatch S, Mauries J-P (2013) Myriapoda, Diplopoda. In: Pérez Fernández T, Pérez Ruiz A (Eds) Los invertebrados de hábitats subterráneos de Jaén. Grupo de Espeleología de Villacarrillo, Jaén, 87–93.

Kime RD, Enghoff H (2011) Atlas of European Millipedes (Class Diplopoda). Volume 1. Orders Polyxenida, Glomerida, Platydesmida, Siphonocryptida, Polyzoniida, Callipodida, Polydesmida. Co-published by Pensoft Publishers, Sofia-Moscow & European Invertebrate Survey, Leiden.

Lignau NG (1903) Mnogonojki Cernomorskogo poberejîia Kavkaza Die Myriopoden der Pontus-Küsten von Caucasus. Mémoires de la Société des Naturalistes de la Nouvelle-Russie 25: 82–125.

Lignau (1905) Die Myriapoden der Krim (Mnogonojki Krîma). Mémoires de la Société des Naturalistes de la Nouvelle-Russie 28: 197–199.

Lignau NG (1911) Neue Beiträge zur Myriopodenfauna des Kaukasus. Annuaire du Musée Zoologique de l'Académie Impériale des Sciences de St. Pétersbourg 16: 37–60.

Lignau NG (1914) Vielfüsser aus Abchasien. Annuaire du Musée Zoologique de l'Académie Impériale des Sciences de St. Pétersbourg 39: 23, 295–296, 391.

Kobadakhidze DN (1965) Verzeichnis der Tausendfüssler (Diplopoda) der Grusinischen SSR. Fragmenta Faunistica 11(21): 390–398.

Lohmander H (1936) Über die Diplopoden des Kaukasusgebiets. Goteborg. Vetensk. Samh. Handl. Goteborg, Serie B, 5(1): 165–191.

Manfredi P (1940) VI. contributo alla conoscenza dei Miriapodi cavernicoli Italiani. Atti della Società italiana di scienze naturali, e del Museo civile di storia naturale 79: 221–252.

Nguyen Duy-Jacquemin M (1993) Convergences évolutives entre Diplopodes Pénicillates vivant dans des grottes. Mémoires de Biospéologie 20: 147–155

Nguyen Duy-Jacquemin M (2000) Redescription of *Polyxenus albus* POCOCK (Diplopoda, Polyxenida), an addition to the French fauna. Fragmenta Faunistica 43(supp): 251–258.

Nguyen Duy-Jacquemin M (2014) Two new species of *Lophoturus* (Diplopoda, Penicillata, Lophoproctidae) from caves in Christmas Island, Australia, including the second troglomorph in Penicillata. Zoosystema 36(1): 29–39. doi: 10.5252/z2014n1a2

Pocock RI (1894) Res ligusticae. XXI. Contributions to the knowledge of the Diplopoda of Liguria. Annali del Museo Civico di Storia Naturale di Genova, serie 2 14: 505–525.

Seurat LG (1930) Exploration zoologique de l'Algérie de 1830 à 1930. Collection du Centenaire de l'Algérie, Paris (Masson): 1–708.

Shorthouse DP (2010) SimpleMappr, an online tool to produce publication-quality point maps. http://www.simplemappr.net [accessed 25 August 2014]

Silvestri F (1894a) Sulla presensa del *Polyxenus lucidus* Chalande in Italia. Bollettino della Società Romana per gli Studi Zoologici 3: 1–3.

Silvestri F (1894b) Contribuzione alla conoscenza dei Chilopodi, Symphyli, Pauropodi e Diplopodi dell' Umbria e del Lazio. Bollettino della Società Romana per gli Studi Zoologici 3(5-6): 191–201.

Silvestri F (1896) Una escursione in Tunisia (Symphyla, Chilopoda, Diplopoda). Naturalista Siciliano, Nuova Serie 1(8–12): 143–161

Silvestri F (1897) Systema Diplopodum. Annali del Museo Civico di Storia Naturale di Genova, serie 2 18: 644–651.

Silvestri F (1898) Contributo alla conosceza dei Chilopodi e Diplopodi della Sicilia. Bollettino della Società entomologica Italiana 29(4): 233–261.

Silvestri F (1903) Note Diplopodologiche. Bollettino del Musei di Zoologia e di Anatomia comparata della Reale Università di Torino 18(433): 1–21.

Stoev P, Lapeva-Gjonova A (2006) Myriapods from ant nests in Bulgaria (Chilopoda, Diplopoda). Peckiana 4: 131–142

Strasser (1970) Diplopodi della Sicilia e della Calabria. Memorie del Museo Civico di Storia Naturale di Verona 17: 151–200.

Tabacaru I (1966) Contribuţii la cunoaşterea faunei de diplopode din Dobrogea. Lucrarile Institutului de Speologie 'Emil Racovita' Bucharest 5: 169–183

Talikadze DA (1984) On the fauna of millipedes (Diplopoda) of the Colchida Province of Caucasus. In: Zoologicheskii Zhurnal, Rossijskaja Akademija Nauk, Otdelenie Obscej Biologii 63(1): 142–145.

Verhoeff KW (1921) Über Diplopoden der Riviera und einige alpenländische Chilognathen Diplopoden-Aufsatz). Archiv für Naturgeschichte 87A(2): 1–110.

Verhoeff KW (1943) Über Diplopoden der Insel Korsika. Zoologischer Anzeiger 142(11–12): 215–226.

Verhoeff KW (1952) Weitere Beiträge zur Kenntnis der Isopoden und Diplopodenfauna von Ischia und Capri. Bonner Zoologische Beiträge 3: 125–150.

Supplementary material 1

Record details of all *Lophoproctus* collections.

Author: Megan Short

Data type: Comma-separated values files (CSV)

Explanation note: A record of all specimens of *Lophoproctus* examined, or mentioned in the literature, is available.

ZooKeys 510: 223–231 (2015)
doi: 10.3897/zookeys.510.8672
http://zookeys.pensoft.net

RESEARCH ARTICLE

A peer-reviewed open-access journal
ZooKeys
Launched to accelerate biodiversity research

The current knowledge on centipedes (Chilopoda) in Slovenia: faunistic and ecological records from a national database

Blanka Ravnjak[1], Ivan Kos[1]

[1] *University of Ljubljana, Biotechnical faculty, Department of Biology, Večna pot 111, 1000 Ljubljana, Slovenia*

Corresponding author: *Blanka Ravnjak* (blanka.ravnjak@gmail.com)

Academic editor: *Ivan H. Tuf* | Received 30 September 2014 | Accepted 22 May 2015 | Published 30 June 2015

http://zoobank.org/C668F0CD-CB5B-4187-8CAF-CC9763DC32EF

Citation: Ravnjak B, Kos I (2015) The current knowledge on centipedes (Chilopoda) in Slovenia: faunistic and ecological records from a national database. In: Tuf IH, Tajovský K (Eds) Proceedings of the 16th International Congress of Myriapodology, Olomouc, Czech Republic. ZooKeys 510: 223–231. doi: 10.3897/zookeys.510.8672

Abstract

In spite of Slovenia's very high biodiversity, it has only a few animal groups that have been significantly investigated and are well known in this area. Slovenian researchers have studied only about half of the species known to be living in the country (Mršić 1997), but among well investigated species are centipedes. All available data about centipedes in Slovenia collected from 1921 to 2014 have been consolidated and constitute a general electronic database called "CHILOBIO", which was created to provide an easy overview of the Slovenian centipede fauna and to allow entry and interpretation of new data collected in future research. The level of investigation has been studied with this database, in conjunction with a geographic information system (GIS). In the study period, 109 species were identified from 350 localities in 109 of the 236 UTM 10 × 10 km quadrants which cover the study area. The south-central part of the country has been the subject of the best investigations, whereas there is an absence of data from the south-eastern, eastern and north-eastern regions The highest number of species (52) has been recorded near the Iška valley (Central Slovenia, quadrant VL68). In 48% of the UTM quadrants investigated fewer than 10 species were recorded and just 5 species were found in one locality. Seventeen species were reported only in the Dinaric region, 4 in the Prealpine-subpannonian region and 7 in the Primorska-submediterranean region.

Keywords

Centipede fauna, biodiversity maps

Introduction

Centipede research has a very long tradition in Slovenia. In the 18th century the Italian naturalist Giovanni Antonio Scopoli gathered specimens in the surroundings of the mining town of Idrija. Some of these specimens were subsequently studied by C. L. Koch (Eason 1970). Therefore, records concerning centipedes in the Slovenian area have been collected for more than 200 years (Valvasor 2004). Systematic and particularly intensive research has been performed during the last thirty years. In this period, new findings on Lithobiomorpha has been published (Kos 1988). The knowledge on Geophilomorpha also increased (Lesar 2002), especially on *Stenotaenia* C. L. Koch, 1847 (Huzimec 2009) and *Strigamia* Gray, 1843 (Schoss 1991). In addition, the centipede diversity of some areas in Slovenia has been studied in detail (e.g. Kos 1988, 1995, Kos and Praprotnik 2000, Grgič and Kos 2003, Ravnjak 2006, Pagon 2006, Kohek 2012, Bagola 1997). The data gathered allows the conclusion that the centipede fauna in Slovenia is very rich. By 2011, the presence of 98 species, out of 538 in Europe (Engoff 2013), was confirmed in Slovenia. Among the 98, 35 species are endemics to the study area (Kos 2001), and 41.8% are evaluated in the Red List of Endangered Species in Slovenia (Official Gazette of RS No. 82/2002).

The reasons underlying such species diversity include the country's biogeographic features, as well as paleogographic and paleoclimatic events. A factor that most probably enabled species diversity, is the tectonic evolution of Slovenia, which influenced geology, relief features and edaphic conditions of the country (Mršić 1997). Additionally, climate, resulting from geographical position, and microclimate factors should be noted (Mršić 1997). Slovenia is one of the southernmost European countries. With an area of 20,256 km^2, it lies at the junction of four geographical macroregions: the Alps, the Pannonian basin, the Dinarides and the Mediterranean. Besides its biogeographic articulation, three climate types interweave: Mountain, Mediterranean and Continental (Ogrin 2004). The annual amount of precipitation decreases from west to the east, and the maximum precipitation in a year can be as much as 3.500 mm. Temperature extremes are also typical, with winter values falling to -30 °C and summer values as high as +40 °C. A total of 514 habitat types have been described in the area and the dominant ecosystem consists of forests, which cover some 56% of the territory (Jogan et al. 2004). Mixed forests predominate and the most important habitat types are colinian and submountainean beech forests, and acidophilic and fir beech forests (Agencija Republike Slovenije za okolje 2001).

Despite various investigations in different areas and habitat types have been carried out, no comprehensive review on which parts are well investigated, from which areas data are still lacking and what the quantity and quality of data about centipedes is in Slovenia have been done. Also no clear overview about the state of centipede research in individual habitat types was available. In this report, all available data concerning centipedes have been collected and maps allowing evaluation of the state of research on centipede fauna in Slovenia are presented.

Methods

The base for research presented here was the data about centipede findings in the whole area of Slovenia. This data consist from centipede findings between the years 1921 and 2014. In terms of geographical distribution the findings were associated with the 10 × 10 km UTM grid, where the area of Slovenia then is divided into 263 quadrants. The research included all the quadrants also those which only partial cover the Slovenian area. Then a “CHILOBIO” database, in form of a MS Excel table, has been created. The number of species and specimens found in a single UTM quadrant was used as a basis for mapping the state of research on centipedes. The data in this database result from random findings as well as from targeted research. All the specimens gathered within this research are stored in the collection of microscopic and ethanol-preserved preparates at the Department of Biology at Biotechnical Faculty of the University of Ljubljana. Each data record contains the name of the species from a certain finding location along with the number of specimens found and all the accompanying data. Some of the species found are even new for centipede fauna and their descriptions are not published yet. A basic database entry provides species, inventory number of the specimen from this species and the location of the finding. Besides the basic data about each species the database also contains information (elevation above s.l., nearby town) about localities in which species were encountered along with some basic environmental data, date of the collection, data about the collector and the sampling methods. Detailed data was incomplete or missing in most of the random centipede findings, while target research included abovmentioned data. Among the location data, the UTM quadrant where the species was found is included. Subsequently, these quadrants were used as basic cartographic units for representation of the state of research on centipedes in Slovenia. For the majority of the older data, besides the collecting site, only the respective UTM quadrant is used to define the geolocation. The vector layer of the UTM grid was made with the geographical information system software tools (ArcGIS ver. 9.2, ESRI). The state of research on centipedes was represented by the number of species and specimens found within each individual quadrant. The result is a map of Slovenia in which the number of species and specimens per quadrant is presented.

For the evaluation of the state of research within each habitat types, we compared the number of specimens and species recorded. Only that kind of data where the habitat type of finding site was also recorded, where included in this comparison. Such data were mostly collected by quantitative sampling methods, where the area of individual sampling unit and the number of units were known.

Results

The total number of records stored specimens recorded in the “CHILOBIO” database is 14.835. Records refers to 25,651 specimens describing 109 species. One percent of specimens were not identified to the species level, due to damages of morphological features.

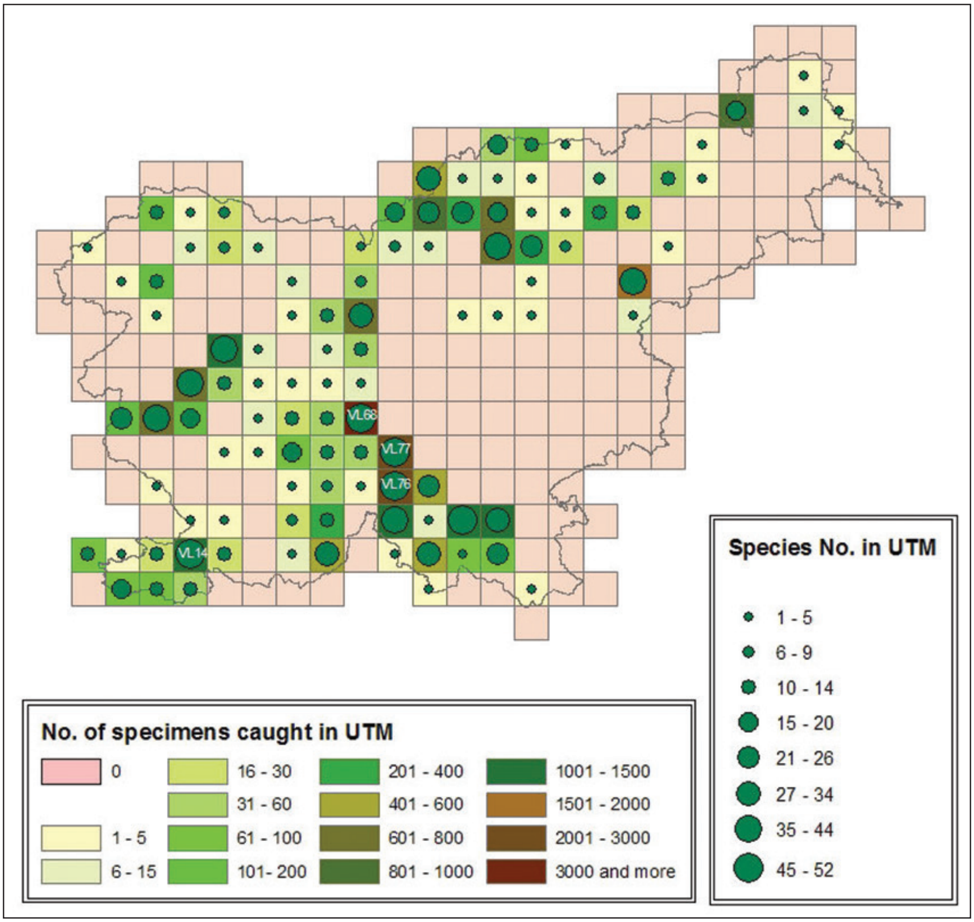


Figure 1. Number of centipede species and specimens caught in individual UTM quadrants from 1921 till 2014 in Slovenia.

To date, species have been found in 350 localities within 105 UTM quadrants, representing 41% of all quadrants in the Slovenian UTM grid. The highest number of species (52) was found in in the quadrant VL68 that lies to the south of the capital, Ljubljana. In terms of the number of species identified, this quadrant is followed by its neighbouring quadrants (VL76 and VL77) with 48 and 43 species found, respectively. In the area south of Ljubljana (including 12 UTM quadrants), which consists mostly of dense Dinaric forests, the number of quadrants with more than 20 species is highest. In terms of number of centipede species found per quadrant one area in western Slovenia (Trnovski gozd area) and one in northern Slovenia (Mountain chain in Upper Savinjska valley along Austrian border) are prominent. In 48% of 105 investigated UTM quadrants, the recorded number of species was fewer than 10.

In terms of number of specimens identified, the quadrant VL68, with 6.479 recorded specimens, leads again. A high number of specimens, but less than 50% of the

Table 1. Number of species and specimens recorded per habitat types.

Habitat type	No. of species	No. of specimens
Beech forest	79	9582
Mixed forest	48	1298
Oak forest	44	799
Thermophilic forest	39	563
Grassland	37	374
Frost hollow forest	32	563
Coniferous forest	32	290
Overgrowing meadow	23	214
Hornbeam forest	22	126
Bush	21	325
Dwarf pine stands	19	339
Meadow	19	87
Frost hollow spruce stands	16	337
Floodplain forest	13	40
Alder stands	11	60
Juniper stands	9	30
Anthropogenic habitats	3	333

total, were found in quadrants VL76 (2.264), VL75 (1.479) and VL77 (1.312). These quadrants mostly are those with higher number of species found. All of this four are in the Dinaric geographic region.

Comparing the number of specimens caught in different habitats, the majority (63%) were found in beech forests. Just 8% were found in mixed forests, while 5% and less were found in other habitats. The highest number of species (79) was found in beech forests, while 48 were caught in mixed and 44 in thermophilic forests (Table 1).

Discussion

With such a volume of data there emerged a need for a universal electronic database in which all researchers could store and access the gathered data as necessary. The “CHILOBIO” database will be a source for faunistic and systemized research of various researchers, who will be able to perform different spatial analyses even without additional sampling. Till now this database is placed at the Department of Biology of Biotechnical faculty of Ljubljana and there is not yet a free access to data for anyone, except for the researchers of this faculty. The “CHILOBIO” database is a useful source for spatial research of centipede fauna because it contains some basic parameters, including collecting site coordinates, altitude above sea level, UTM quadrant, habitat type, of each individual specimen and species. By using the basic tools from the geographical information system (GIS) it is possible to create maps of distribution of individual species that support planning of further research (Wadsworth and Treweek

1999). Distribution maps of this sort had been created previously by Simaiakis and Mylonas (2006) for 14 centipede species in the Aegean archipelago. For Slovenia, no such cartographic representation of centipede biodiversity has been made to date and the map presented here is the first of its kind. In this representation the state of research in the study area can easily be seen. However, the high number of registered species and specimens found in certain quadrants is primarily a consequence of sampling effort of targeted research and investigations. Most of this research was performed in the area of Dinarides between Ljubljana, Velike Lašče and Kočevje (Kos 1988, 1995, Grgič 2005, Kohek 2012). Other research was carried out in western Slovenia (Pagon 2006), eastern Slovenia (Ravnjak 2006), Carinthia (Kos et al 2000), NE part of Slovenia (Bagola 1997) or in the Primorska region as far as Snežnik (Kos 2010). Based on this, it can be concluded that low number of specimens and species recorded, does not represent the actual state of art but only the low sampling effort to date invested. Continued research is needed in the future, especially in the SE and NE parts of Slovenia, to establish a comprehensive evaluation of species diversity of centipede fauna. These two areas represent the major part of the Pannonian biogeographical region where findings of species with Pannonian distribution are expected and where knowledge about the distribution of species can be improved. It would be reasonable to supplement the existing cartographic representation with the representation for some species, as was done by Tuf and Laška (2005) for the Czech Republic and by Barber and Key (1988) for the British Isles. The same was done also by Zapparoli and Minelli (2007) for Italy and by Simaiakis et al (2013) for Cyprus. For Slovenia, investigation of the areas along the borders with neighbouring countries is also reasonable as species already found in those countries can be expected there.

Although only about a half of Slovenia has been investigated, that 109 species have already been recorded confirms that centipede fauna of this area is very rich. According to the European Atlas of Soil Biodiversity for the millipede group (Diplopoda Myriapoda), Slovenia is at the top (Jeffrey et al. 2010). These authors estimate that the number of millipede species in Slovenia is expected to be between 151 and 622. Among recorded species, eight await scientific description. Based on the available data (Kos 1992, 1988) the majority of centipede species found in Slovenia (52 species, 48%) have an in-Dinaric distribution, according to biogeographical distribution models proposed by Matvejev and Puncer (1989), while somewhat fewer have ex-Dinaric distribution (41 species, 37%). In addition, only one species has a Pannonian distribution. With respect to general distribution, the majority of species found in Slovenia (19) have a Palearctic distribution, while seven have a mid-European and six a European distribution. For two species the distribution is South-European and for four Mediterranean (Kos 1992). Due to geographical position of Slovenia, the presence of such distribution patterns is expected. Among all species found, 17 were endemic for the Dinaric region, seven for the Mediterranean and four for the Prealpine-subpannonian regions. Several species, including *Lithobius illyricus* Latzel, 1880, *L. peregrinus* Latzel, 1880, *Cryptops umbricus* Verhoeff, 1931, *Geophilus promontorii* Verhoeff, 1898, and a still unidentified *Brachygeophilus* sp., were found only at one site.

Comparing the proportions of centipede specimens found in individual habitat types, we established that beech forests are the most investigated habitats. In part, this can be explained by the fact that research often was targeted at investigations of beech forest fauna (Grgič 2005), but it should be noted that several entries in "CHILOBIO" lack information about habitat type, and the representation of centipede community in individual habitat type is therefore deficient. In future, it would be reasonable to perform sampling in habitat types such as *Acer* forests, pine forests, ash forests, and alder forests where, so far, little research has been done.

Conclusions

Within this research a general electronic database 'CHILOBIO' with data about centipede findings from 1921 until 2014 in Slovenia was made. The data included in this database are a result of random findings as well as from targeted research. This database represents the first collection of data about centipede fauna in Slovenia and also a basis for a cartographic representation of state of research of centipede fauna in this area. Because one of the main data included in database is also a locality, where centipede species were found, based on data in 'CHILOBIO', the map with state of research presentation was created. A base for evaluation of well and poorly investigated areas in Slovenia was the number of species and specimens found in 10 × 10 km UTM quadrant. Based on the UTM grid with centipede species and specimens per UTM quadrant, we established that more than one-half of the country is still poorly investigated. Whereas especially Pannonian and Alpine regions are poorly investigated, the area of Dinarides between Ljubljana, Velike Lašče and Kočevje is well investigated. Till now all-together 109 centipede species were found in Slovenia. Some among them are new for centipede fauna and their descriptions are not published yet. As well the database as the cartographic representation can be used for designing future targeted investigations, creation of distribution models for individual species and processing of data gathered during sampling and stored in the database.

References

Agencija Republike Slovenije za okolje (2001) Pregled stanja biotske raznovrstnosti in krajinske pestrosti v Sloveniji. Agencija Republike Slovenije za okolje, Ljubljana.

Bagola M (1997) Primerjava med združbami strig (Chilopoda) v gozdni združbi hrasta in gabra (*Querco-Carpinetum*) in koruzne njive v zahodnem Prekmurju. Bachelor's degree, University of Ljubljana, Biotehnical faculty, Ljubljana, Slovenia.

Barber DA, Key AN (1988) Provisional Atlas of the Centipedes of the British isles. Institute of terrestrial Ecology, Huntingdon, 127 pp.

Eason EH (1970) A redescription of the species of *Eupolybothrus* Verhoeff s. str. preserved in the British Museum (Natural History) and the Hope departement of Zoology Oxford

(Chilopoda Lithobiomorpha), Bulletin of the British Museum (Natural History), Zoology 19: 289–310.

Enghoff H (2013) Fauna Europaea: Chilopoda. Fauna Europaea version 2.6. http://www.faunaeur.org

Grgič T (2005) Dynamics of centipede (Chilopoda) communities in unevenly aged beech in Southern Slovenia (Dinamika združb strig (Chilopoda) raznomernega bukovega sestoja v južni Sloveniji). PhD thesis, University of Ljubljana, Biotehnical faculty, Ljubljana, Slovenia.

Grgič T, Kos I (2003) Centipede diversity in patches of different development phases in an unevenly-aged beech stand in Slovenia. African Invertebrates 44(1): 237–252.

Huzimec D (2009) Morphological and taxonomical characteristics of some species from genus *Stenotaenia* (Chilopoda: Geophilomorpha) (Morfološke in taksonomske značilnosti nekaterih vrst rodu *Stenotaenia* (Chilopoda: Geophilomorpha)). Bachelor's degree, University of Ljubljana, Biotehnical faculty, Ljubljana, Slovenia.

Jeffrey S, Gardi C, Jones A, Montanarella L, Marmo L, Milko L, Ritz K, Peres G, Römbke J, van der Putten WH (2010) European Atlas of Soil Biodiversity. European Commison, Publications Office of the European Union, Luxembourg, 128 pp.

Jogan N, Kaligarič M, Leskovar I, Seliškar A, Dobravec J (2004) Habitat types in Slovenia – tipology (Habitatni tipi Slovenije – tipologija). Ministrstvo za okolje, prostor in energijo – Agencija RS za okolje, Ljubljana, 64 pp.

Kohek B (2012) Characteristics of centipede (Chilopoda) assemblies in some frost hollows in Slovenian Dinaric karst (Značilnosti združbe strig (Chilopoda) v nekaterih mraziščih slovenskega Dinarskega krasa). Bachelor's degree, University of Ljubljana, Biotehnical faculty, Ljubljana, Slovenia.

Kos I (1988) Problemi kvalitativnega in kvantitativnega vzorčenja skupine strig (Chilopoda). Master's degree, University of Ljubljana, Biotehnical faculty, Ljubljana, Slovenia.

Kos I (1992) A Review of the Taxonomy, Geographical Distribution and Ecology of the Centipedes of Yugoslavia. Berichte des naturwissenschaftlich-medizinischen. Vereins in Innsbruck 10: 353–360.

Kos I (1995) The role of centipedes (Myriapoda: Chilopoda) in the biocenosis of a xerophilic meadow. (Vloga predatorske skupine strige (Chilopoda) v biocenozi kserofilnega travnika). PhD thesis, University of Ljubljana, Biotehnical faculty, Ljubljana, Slovenia.

Kos I (2001) Stanje biotske raznovrstnosti za področje strig. In: Gregori J, Hlad B, Skoberne P (Eds) Ekspertne študije za Pregled stanja biotske raznovrstnosti in krajinske pestrosti v Sloveniji, Agencija Republike Slovenije za okolje, Ljubljana, 109–115.

Kos I (2010) Prostorsko-populacijska dinamika prostoživečih živali v slovenskih gozdovih kot posledica klimatskih sprememb: Zaključno poročilo o rezultatih raziskovalnega dela na projektu v okviru ciljnega raziskovalnega programa (CRP) "Konkurenčnost Slovenije 2006–2013". Univerza v Ljubljani, Biotehniška fakulteta, Ljubljana, 20 pp.

Kos I, Praprotnik L (2000) Talna gozdna favna na različni geološki podlagi v okolici Kočevske Reke. In: Karigher H, Smolej I (Eds) Raziskave gozdnih tal in rizosfere ter njihov vpliv na nekatere fiziološke parametre gozdnega drevja v izbranih gozdnih ekosistemih, sestojnih tipih in razvojnih fazah gozda, Slovenian Forestry Institute, Ljubljana, 207–220.

Kos I, Grgič T, Potočnik H, Kljun F, Čas M (2000) Pestrost pedofavne -strig (Chilopoda) in deževnikov (Lumbricidae) na vzorčnih ploskvah na rastiščih divjega petelina (*Tetrao urogallus* L.) v gozdnih tipih vzhodnih Karavank in vzhodnih Kamniško-Savinjskih Alp na Koroškem in Štajerskem: Končno poročilo o raziskovalnem delu, segment: Projekt: Ohranjanje habitatov ogroženih vrst divjadi in drugih prostoživečih živali v gozdnih ekosistemih in krajinah, gozdne kure – divji petelin. Slovenian Forestry Institute, University of Ljubljana, Biotehnical faculty Department of biology, Ljubljana, 14 pp.

Lesar H (2002) Taxonomic analysis of some centipedes species (Chilopoda) of genus *Geophilus* (Taksonomska analiza nekaterih vrst strig (Chilopoda) iz rodu *Geophilus*). Bachelor's degree, University of Ljubljana, Biotehnical faculty, Ljubljana, Slovenia.

Matvejev S, Puncer I (1989) Map of Biomes-Landscapes of Yugoslavia. Natural History Museum, Belgrade, 1–77.

Mršić N (1997) Biotska raznovrstnost v Sloveniji, Slovenija "vroča točka" Evrope. Ministrstvo za okolje in prostor, Uprava RS za varstvo narave, Ljubljana, 129 pp.

Ogrin D (2004) Vreme in podnebje. In: Bat M (Ed.) Narava Slovenije. Mladinska knjiga, Ljubljana, 73–101.

Pagon N (2006) Biodiversity of centipedes (Chilopoda), millipedes (Diplopoda) and isopods (Isopoda) in Idria region (Slovenia) (Biodiverziteta strig (Chilopoda), dvojnonog (Diplopoda) in mokric (Isopoda) na Idrijskem). Bachelor's degree, University of Ljubljana, Biotehnical faculty, Ljubljana, Slovenia.

Ravnjak B (2006) Land use and biodiversity of centipedes (Chilopoda) millipedes (Diplopoda) and isopods (Isopoda) in Voglajnsko-sotelska region (Slovenia) (Raba prostora in biodiverziteta strig (Chilopoda), dvojnonog (Diplopoda) in mokric (Isopoda) na območju Voglajnsko-sotelske regije). Bachelor's degree, University of Ljubljana, Biotehnical faculty, Ljubljana, Slovenia.

Schoss M (1991) Poznavanje rodu *Strigamia* (Chilopoda, družina Dignathodontidae) v Sloveniji. Bachelor's degree, University of Ljubljana, Biotehnical faculty, Ljubljana, Slovenia.

Simaiakis S, Mylonas M (2006) Recent knowledge on the centipede fauna (Chilopoda) of south Aegean archipelago (Greece, Eastern Mediterranean). Norwegian Journal of Entomology 53: 281–298.

Simaiakis SM, Zapparoli M, Minelli A, Bonato L (2013) The centipede fauna (Chilopoda) of the island of Cyprus, with one new lithobiomorph species. Zootaxa 3647(2): 279–306. doi: 10.11646/zootaxa.3647.2.3

Tuf IH, Laška V (2005) Present knowledge on the centipedes in the Czech Republic – a zoogeographic analysis and bibliography 1820–2003. Peckiana 4: 143–161.

Valvasor JV (2004) Rastline in živali na Kranjskem. Poseben dotis 18. zvezka zbirke Iconoteca Valvasoriana. Fundacija Janez Vajkard Valvasor pri Slovenski akademiji znanosti in umetnosti, Ljubljana, 108 pp.

Wadsworth R, Treweek J (1999) Geographical Information Systems for Ecology – an introduction. Addison Wesley Longman Limited, Edinburgh, 184 pp.

Zapparoli M, Minelli A (2007) Chilopoda. In: Ruffo S, Stoch F (Eds) Checklist and distribution of the Italian fauna. Memorie del Museo Civico di Storia Naturale di Verona, 2 serie, Sezione Scienze della Vita, 17 (2006): 123–125+data in CD-ROM.

ZooKeys 510: 233–242 (2015)
doi: 10.3897/zookeys.510.8644
http://zookeys.pensoft.net

RESEARCH ARTICLE

A peer-reviewed open-access journal
ZooKeys
Launched to accelerate biodiversity research

Interaction of the tracheal tubules of *Scutigera coleoptrata* (Chilopoda, Notostigmophora) with glandular structures of the pericardial septum

Gero Hilken[1], Gregory D. Edgecombe[2], Carsten H.G. Müller[3,4],
Andy Sombke[3], Christian S. Wirkner[5], Jörg Rosenberg[6]

1 *Central Animal Laboratory, University Clinic of Essen, Hufelandstraße 55, D-45122 Essen, Germany* **2** *The Natural History Museum, Cromwell Road, London SW7 5BD, UK* **3** *Zoological Institute and Museum, University of Greifswald, Soldmannstrasse 23, D-17489 Greifswald, Germany* **4** *Department of Neuroscience, University of Arizona, 1040 E 4th Street, PO Box 210077, Tucson, AZ 85721, USA* **5** *Allgemeine & Spezielle Zoologie, Institut für Biowissenschaften, Universität Rostock, Universitätsplatz 2, D-18055 Rostock, Germany* **6** *Am Kützelbach 3, D-59494 Soest, Germany*

Corresponding author: *Gero Hilken* (gero.hilken@uk-essen.de)

Academic editor: *Ivan H. Tuf* | Received 24 September 2014 | Accepted 4 May 2015 | Published 30 June 2015

http://zoobank.org/51879761-FCD7-4DFB-B62A-D62DBD6BF2C5

Citation: Hilken G, Edgecombe GD, Müller CHG, Sombke A, Wirkner CS, Rosenberg J (2015) Interaction of the tracheal tubules of *Scutigera coleoptrata* (Chilopoda, Notostigmophora) with glandular structures of the pericardial septum. In: Tuf IH, Tajovský K (Eds) Proceedings of the 16th International Congress of Myriapodology, Olomouc, Czech Republic. ZooKeys 510: 233–242. doi: 10.3897/zookeys.510.8644

Abstract

Notostigmophora (Scutigeromorpha) exhibit a special tracheal system compared to other Chilopoda. The unpaired spiracles are localized medially on the long tergites and open into a wide atrium from which hundreds of tracheal tubules originate and extend into the pericardial sinus. Previous investigators reported that the tracheal tubules float freely in the hemolymph. However, here we show for the first time that the tracheal tubules are anchored to a part of the pericardial septum. Another novel finding is this part of the pericardial septum is structured as an aggregated gland on the basis of its specialized epithelium being formed by hundreds of oligocellular glands. It remains unclear whether the pericardial septum has a differently structure in areas that lack a connection with tracheal tubules. The tracheal tubules come into direct contact with the canal cells of the glands that presumably secrete mucous substances covering the entire luminal cuticle of the tracheal tubules. Connections between tracheae and glands have not been observed in any other arthropods.

Keywords

Centipedes, electron microscopy, tracheal system, aggregated recto-canal epidermal glands, respiration

Introduction

The history of research on tracheae of *Scutigera coleoptrata* extends back to the 19th century. Meinert (1868, 1883) denied the existence of tracheae and thought the dorsally located spiracles (Haase 1884: "stomata") are the openings of sticky glands. Pagenstecher (1878), Tömösváry (1881, 1883a, b), Sinclair (1891, 1892), Voges (1882, 1916), Haase (1883, 1884, 1885), and Chalande (1885) were the first to recognize the dorsally situated tracheal systems as the real respiratory organs. Fine structural investigations on the respiratory system and the tracheal tubules were conducted by Prunescu and Prunescu (1996) and Hilken (1997, 1998). The entity of tracheal tubules has often been termed "lungs", tracing back to Chalande (1885: "appareil pulmonaire"). Dubuisson (1928) studied the interaction of both tracheal and circulatory systems with regard to active ventilation. He called the space around the tracheal system – bounded by a septum – a 'cavité péricardique'. The tracheal system of Notostigmophora consists of an unpaired, non-closable spiracle that opens into a wide atrium. From there, hundreds of short tracheal tubules originate and extend into the pericardial sinus. Up to now, it has been thought that the tracheal tubules end blindly and float freely in the hemolymph space that is surrounded by the pericardial septum (e.g. Sinclair 1892, Dubuisson 1928, Hilken 1997, 1998). However, more recent illustrations by Wirkner and Pass (2002: Fig. 3A, p. 198) and Wirkner et al. (2013: Fig. 14.9b, p. 358) indicated that, even though not further addressed in their anatomical descriptions, the tracheal tubules may indeed be in close contact with the pericardial septum.

During recent years, we focused on tracheal systems (Hilken 1997, 1998, Rosenberg 2009) and on comparative investigations of epidermal glands in Chilopoda with regards to their structure and phylogenetic significance (e.g. Hilken et al. 2005, 2011, Hilken and Rosenberg 2006, 2009, Müller et al. 2003, 2009, 2014). In the present study on *Scutigera coleoptrata*, we found an unexpected connection between the tracheal tubules and associated glands using TEM techniques. Here, we describe for the first time that in *Scutigera coleoptrata* (1) the distal tips of the tracheal tubules are anchored in a part of the pericardial septum, (2) this specialized epithelium of the pericardial septum consists exclusively of glandular units, and (3) there are profound interactions between tracheae and the glandular units.

Material and methods

Specimens of *Scutigera coleoptrata* (Linnaeus, 1758) were reared in glass boxes filled with 1 cm soil substrate and moistened tissues and fed with *Drosophila* sp. and *Musca* sp.

Animals were anesthetized with CO_2, subsequently fixed, cut along the tergite edges, and preserved as described below.

For scanning electron microscopic (SEM) investigations of the tracheal tubules, whole specimens were fixed in ethanol (70%). Tracheae were macerated using pepsin. Optimal results were obtained with a solution of 1–2 g pepsin in 100 ml HCl (37%, Hilken 1994, 1998). The complex of tracheal tubules was dehydrated through a graded series of ethanol, critical-point dried, coated with gold, and studied with a CAMSCAN DV4.

For light microscopic (LM) and transmission electron microscopic (TEM) investigations, segments of *Scutigera coleoptrata* were fixed in phosphate buffered paraformaldehyde (4%, pH 7.2), containing 15% saturated picric acid and 0.08% glutaraldehyde. They were postfixed with 1% OsO_4 in the same buffer and, after alcohol dehydration, embedded in Epon. Semithin sections (0.5–1 µm) were stained with 1% toluidine blue in a solution of 1% sodium tetraborate. Sections were studied using a DMSL-Leica microscope. Ultrathin sections were stained with uranyl acetate and lead citrate and studied using a ZEISS EM 902 A electron microscope.

Results

The unpaired openings or spiracles of the tracheal system of *Scutigera coleoptrata* are located dorsomedially on each of the seven long tergites (Fig. 1A). Each of the non-closeable spiracles (Fig. 1B) opens into a wide atrium (Fig. 1B–C). From the wall of the atrium, hundreds of short tracheal tubules originate and extend into the pericardial sinus (Fig. 1B–C). The tracheal tubules traverse the hemolymphatic space up to the epithelium of the distinct pericardial septum (Fig. 1C–E). Here, each tracheal tubule is anchored to the specialized epithelium of the pericardial septum (Figs 1E, 2A–D).

In *Scutigera coleoptrata* the length of the tracheal tubules ranges from 150–200 µm (Fig. 1B). The flat and single-layered epithelium of the tracheal tubule is thin (about 0.2–1.8 µm). A basal lamina is developed (Fig. 2F). The epithelium is covered by a specialized, extremely thin cuticle of about 0.1 µm (Fig. 2E–G). An endocuticle is covered by helically arranged chitin fibers, forming a one- or two-layered fiber network (Fig. 2E–G). Taenidia are not developed over the whole length of the tracheal tubules.

The pericardial septum arises from the atrium, partly accompanies the tergal epidermis, and delimits the hemolymph sinus against the body muscles (Fig. 1C, D). We can only provide light microscopic images from this area and the description is thus preliminary. This epithelium forms a hose-like sinus that houses all tracheal tubules. The heart lies ventral to this hemolymph sinus (Fig. 1C).

The polarity of the pericardial septum is characterized by the development of a distinct basal lamina against the body cavity (Fig. 1E). The opposite apical side of the epithelium is characterized by interactions with the tracheal tubules. Cellular junctions are not found, the contact being developed mainly as a small epithelial layer. In the areas in contact with the tips of the tracheal tubules, the specialized, apparently pseudostratified epithelium consists exclusively of hundreds of glandular units (Fig. 1E).

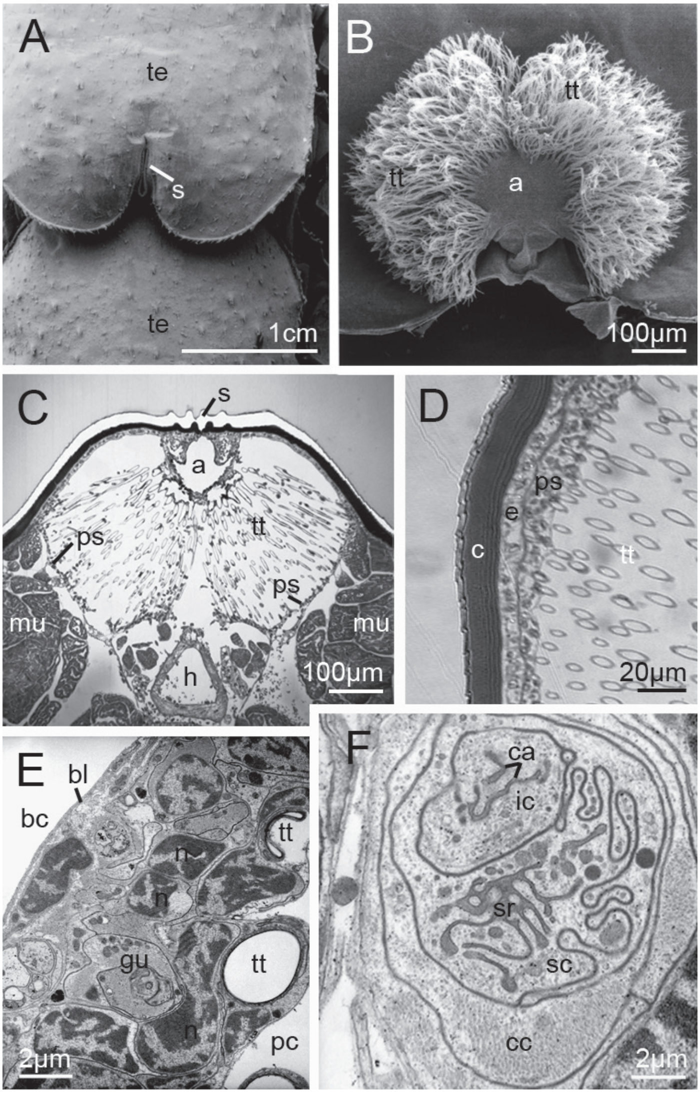


Figure 1. Tracheal system and pericardial septum of *Scutigera coleoptrata*. **A** Spiracle (stomata) situated dorsomedially on long tergite 4 (SEM) **B** Tracheal system with its atrium and hundreds of tracheal tubules (SEM) **C** Cross-section of tracheal system with its tracheal tubules within the pericardial sinus. The sinus is surrounded by the specialized part of the pericardial septum (LM) **D** Inset showing details of the tergal cuticle with epidermis and the pericardial septum. The tracheal tubules extend into the epithelium of the pericardial septum (LM) **E** Overview of the epithelium of the pericardial septum with endings of two tracheal tubules and nuclei of glandular units (TEM) **F** Detail of a glandular unit within the pericardial septum consisting of a secretory cell, an intermediary cell, and a canal cell. In the secretory cell, parts of the microvilli of the secretory reservoir are visible, as are parts of the canaliculi system in the intermediary cell. *a* atrium, *bc* body cavity; *bl* basal lamina, *c*, cuticle, *ca* canaliculi system, *cc* canal cell, *ic* intermediary cell, *e* epidermis, *gu* glandular unit, *h* dorsal heart, *mu* body muscle; *n* nuclei of glandular unit cells, *pc* pericardial cavity; *ps* pericardial septum, *s* spiracle, *sc* secretory cell, *sr* reservoir of the secretory cell, *te* tergite, *tt* tracheal tubules

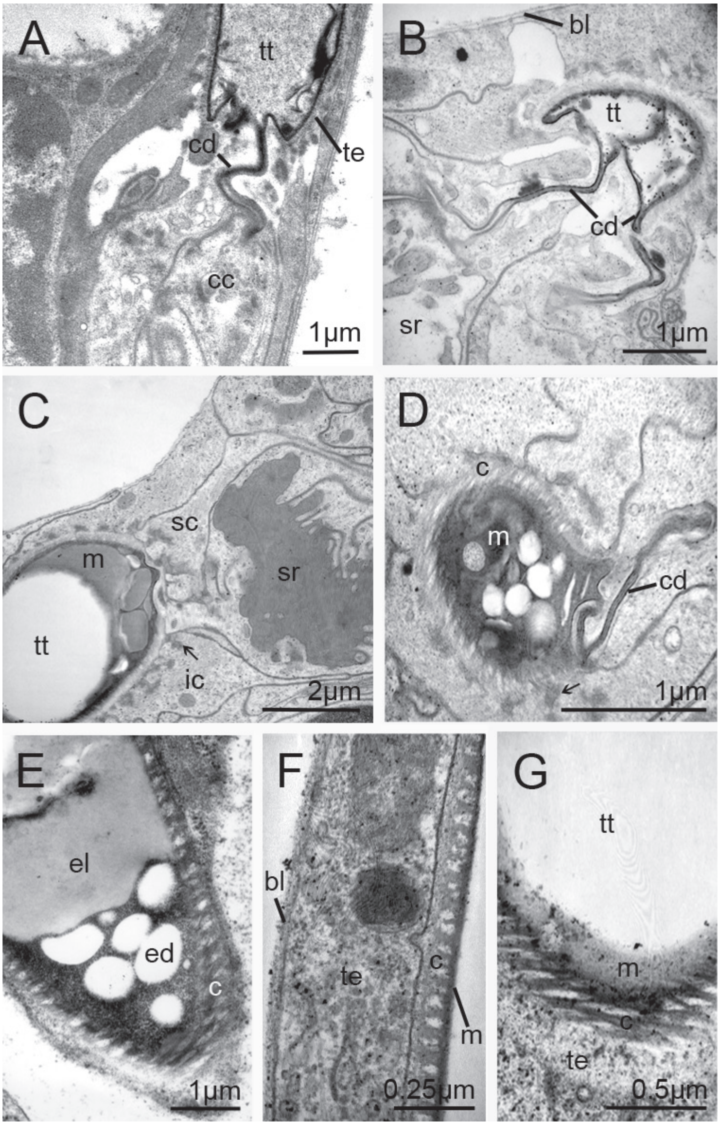


Figure 2. Pericardial glands and mucoid substances. **A** Longitudinal section of a connection of the tip of a tracheal tubule and a canal cell of a glandular unit **B** Cross section of a connection between a tracheal tubule and a canal cell of a glandular unit **C** Tracheal tubule in the vicinity of a glandular unit. The tracheal tubule is filled with mucoid substances **D** Cross section of the ending of a tracheal tubule surrounded by the epithelium of a glandular unit. The tracheal lumen is filled with different mucoid substances **E** Longitudinal section of a tracheal tubule near the pericardial septum filled with electron-dense and electron-lucent mucoid substances **F** Part of a longitudinal section of a tracheal tubule. The specialized cuticle is covered by mucoid substances **G** Oblique section of a tracheal tubule. The tracheal cuticle is covered by a distinct mucoid substance. *bl* basal lamina; *c* cuticle; *cc* canal cell; *cd* cuticular duct of the canal cell; *ed* electron-dense fraction of mucous; *el* electron-lucent fraction of mucus; *ic* intermediary cell; *m* mucoid substance; *sc* secretory cell, *sr* secretory reservoir; *te* tracheal epithelium; *tt* tracheal tubules; arrows, presumed interdigitations between tracheae and glandular compartment.

Each glandular unit is composed of a secretory cell, an intermediary cell, and a canal cell (Fig. 1F). The tip of each tracheal tubule is anchored to the epithelium of the pericardial septum and comes into direct contact with the canal cell of each glandular unit (Fig. 2A–B). Thus, substances are secreted directly via the canal cells into the tracheal tubules. At the point of contact, presumed cellular interdigitations are formed by the surrounding cells (arrows, Fig. 2C). The lumen of the terminations of the tracheal tubules is often completely filled by mucoid substances (Fig. 2C–G). Two mucoid substances are discernible at the outermost tips of the tracheal tubules. An electron-dense fraction that contains several electron-lucent droplets is covered by an electron-lucent fraction (Figs 2C–E). A more or less dense layer of electron-lucent mucoid substances covers the cuticle along the tracheal tubules (Fig. 2F–G).

Discussion

We are now able to resolve previous confusion on the length and connectivity of tracheal tubules of scutigeromorph centipedes. Whereas the majority of reports thus far considered tracheal tubules to end freely in the tracheal sinus compartments (e.g. Sinclair 1892, Dubuisson 1928, Prunescu and Prunescu 1996, Hilken 1997, 1998), we can now show that in *Scutigera coleoptrata* the tracheal tubules are anchored in a part of the pericardial septum. Our observations add further evidence to conclusions drawn from illustrations by Wirkner and Pass (2002) and Wirkner et al. (2013). Other findings described herein are also new, such as (1) the epithelium of the pericardial septum consists of hundreds of glandular units, and (2) the glands of the pericardial septum open into the tracheal tubules. It could be shown that the tips of the tracheal tubules are pierced by the conducting canals of numerous specialized glands. These glandular units are aggregated in a fashion similar to the class of aggregated epidermal glands (see below).

The structure and position of scutigermorph (notostigmophoran) tracheal systems are unique among Chilopoda (Hilken 1997, 1998, Hilken et al. 2011) and have been proposed as constitutive features (apomorphies) of this group (e.g., Borucki 1996, Edgecombe and Giribet 2004). In comparison to their sister group, the Pleurostigmophora, representatives of Notostigmophora possess unpaired, dorsal spiracles localized at the posterior edges of the seven long tergites. The respiratory organ is formed by hundreds of short tracheal tubules which formerly have been described as not having any contact with the organs of oxygen consumption, i.e. muscles or the nervous system (Prunescu and Prunescu 1996, Hilken 1997, 1998). The tracheal tubules are situated within dorsal compartments of the pericardial sinus. Therefore, they are surrounded by hemolymph. Interactions between tracheal tubules and hemocytes have previously been observed (Hilken et al. 2003). The hemolymph is enriched with the respiratory pigment hemocyanin (e.g., Mangum et al. 1985, Kusche et al. 2003). The tracheal tubules are strengthened by helically arranged chitin fibers, forming a one- or two-layered fiber network, whereas taenidia are not developed (Prunescu and Prunescu 1996, Hilken 1997, 1998). Because of these findings and the different position of the stigmata, it has frequently been inferred that the tracheae of notostigmophorans are not homologous to those of Pleurostigmophora and that they might be of an independent origin (Haase 1884, Dohle 1988, 1997, Minelli 1993, Hilken 1997, 1998).

Recently, there has been a particular increase in knowledge of epidermal glands of various degrees of organization in Chilopoda. It is possible to distinguish four classes of epidermal glands: 2-cell-glands (composed of a single secretory cell and a single canal cell), 3-cell-glands (composed of a proximal secretory cell, an intermediary cell, and a distal canal cell), 4-cell-glands (composed of a proximal secretory cell, an intermediary cell, a distal canal cell, and a proximal canal cell), and 5-cell glands (composed of two secretory cells, an intermediary cell, a distal canal cell, and a proximal canal cell) (see Table 2 in Müller et al. 2014). According to the terminology introduced by Hilken et al. (2005), Rosenberg (2009), Müller et al. (2009), and Rosenberg et al. (2011), three classes of epidermal glands can be distinguished depending on their structural complexity: (1) solitary epidermal glands, (2) aggregated epidermal glands (e.g. maxillary organ gland, vesicular gland; Hilken and Rosenberg 2009, Hilken et al. 2005), and (3) compound epidermal glands (maxilla-I-gland, accessory glands; Carcupino 1996, Hilken et al. 2005, Hilken and Rosenberg 2006, Rosenberg 2009, Rosenberg et al. 2011). The diversity and classification of oligocellular epidermal glands were modified, including a classification into recto-canal and flexo-canal epidermal glands, by Müller et al. (2009, 2014).

Many features indicate that the glands in question strongly resemble aggregated recto-canal glands in the epidermis. These include the following: (1) the structure of the glands associated with the tracheae; (2) the universal tricellular construction of the glandular units (consisting of a canal cell, an intermediary cell, and a secretory cell); (3) the presence of an intermediary cell surrounding an almost non-cuticularized duct; and (4) the independent release of a secretion of every glandular unit into the tracheal tubules via its own duct. Thus, an epidermal origin of the tracheae-associated glands cannot be excluded. In no other arthropods are connections between tracheae and multicellular glands observed. Future studies are needed to unravel the cellular diversity, functional complexity, distribution, and organization of the pericardial septum as a whole.

The closely adjoined glandular units release their secretion via canal cells into the connected tracheal tubules. It is likely that each secretory gland secretes mucous substances into the tracheal tubules which cover the luminal side of the cuticle of each tracheal tubule along its entire extension. Sundara Rajulu (1971) detected acid mucopolysaccharides and collagen in the tracheal tubules of *Thereuopoda longicornis* (Scutigeridae, Thereuoneminae) by histological staining. It is possible that these mucopolysaccharides correspond to the mucoid substances observed here on the cuticle of the tracheal tubules in *Scutigera coleoptrata*. It can be assumed that the mucoid layer might support the uptake and/or diffusion of oxygen over the tracheal cuticle and the epithelium into the hemolymph. Here, the respiratory protein hemocyanin serves as a transport molecule of oxygen into the body fluid. Hemocyanins are present in the hemolymph of various myriapod species and have been identified in *Thereuopoda longicornis* (Sundara Rajulu 1969) and *S. coleoptrata* (Mangum et al. 1985), as well as in various Spirostreptidae (Diplopoda) (Jaenicke et al. 1999, Kusche and Burmester 2001, Hagner-Holler 2004). The hemocyanin of *Scutigera coleoptrata* displays a low oxygen affinity and allosteric behavior with very high cooperativity. It is thus an efficient oxygen carrier with considerable functional plasticity (Mangum 1985, Mangum and Goddet 1986, Burmester 2001, 2002, Kusche et al. 2003). Facilitated uptake and/or diffusion of oxygen into the body and subsequent uptake and transport within the hemolymph provide for a passive exhaust ventilation of the tracheal tubules. Thus, we assume that the mucoid substances secreted by the aggregated epidermal glands of the pericardial septum are important in notostigmophoran respiratory physiology.

References

Borucki H (1996) Evolution und Phylogenetisches System der Chilopoda (Mandibulata, Tracheata). Verhandlungen des Naturwissenschaftlichen Vereins in Hamburg (NF) 35: 95–226.

Burmester T (2001) Molecular evolution of the arthropod hemocyanin superfamily. Molecular Biology and Evolution 18(2): 184–195. doi: 10.1093/oxfordjournals.molbev.a003792

Burmester T (2002) Origin and evolution of arthropod hemocyanins and related proteins. Journal of Comparative Physiology (B) 172: 95–107. doi: 10.1007/s00360-001-0247-7

Chalande J (1885) Recherches anatomiques sur l'appareil respiratoire chez les Chilopodes de France. Bulletin de la Société d'Histoire Naturelle de Toulouse 19: 39–66.

Carcupino M (1996) Morphological characterization of female accessory sex glands of *Eupolybothrus fasciatus* (Newport) (Chilopoda: Lithobiomorpha). Journal of Morphology 228: 61–75. doi: 10.1002/(SICI)1097-4687(199604)228:1<61::AID-JMOR5>3.0.CO;2-S

Dohle W (1988) Myriapoda and the ancestry of insects. Meeting British Myriapod Group. Manchester Polytechnic, Manchester, 28 pp.

Dohle W (1997) Are the insects more closely related to the crustaceans than to the myriapods? Entomologica scandinavica Supplement 51: 7–16.

Dubuisson M (1928) Recherches sur la ventilation trachéenne chez les chilopodes et sur la circulation sanguine chez les Scutigères. Archives de zoologie expérimentale et générale 67: 49–63.

Edgecombe GD, Giribet G (2004) Adding mitochondrial sequence data (16S rRNA and cytochrome *c* oxidase subunit I) to the phylogeny of centipedes (Myriapoda Chilopoda): an analysis of morphology and four molecular loci. Journal of Zoological Systematics and Evolutionary Research 42: 89–134. doi: 10.1111/j.1439-0469.2004.00245.x

Haase E (1883) Das Respirationssystem der Symphylen und Chilopoden. Zoologischer Anzeiger 6: 15–17.

Haase E (1884) Das Respirationssystem der Symphylen und Chilopoden. Zoologische Beiträge 1: 65–96.

Haase E (1885) Zur Morphologie der Chilopoden. Zoologischer Anzeiger 8: 693–696.

Hagner-Holler S (2004) Charakterisierung von Arthropodenhämocyaninen. Dissertation Fachbereich Biologie Johannes Gutenberg-Universität Mainz, 180 pp.

Hilken G (1994) Pepsin-Mazeration: Eine Methode zur Herstellung von Präparaten für die Rasterelektronenmikroskopie. Mikrokosmos 83: 207–209.

Hilken G (1997) Tracheal systems in Chilopoda: A comparison under phylogenetic aspects. Entomologica scandinavica Supplement 51: 49–60.

Hilken G (1998) Vergleich von Tracheensystemen unter phylogenetischem Aspekt. Verhandlungen des Naturwissenschaftlichen Vereins in Hamburg 37: 1–91.

Hilken G, Brockmann C, Nevermann L (2003) The hemocytes of the centipede *Scutigera coleoptrata* (Chilopoda, Notostigmophora) with notes on their interactions with the tracheae. Journal of Morphology 257(2): 181–189. doi: 10.1002/jmor.10108

Hilken G, Rosenberg J, Brockmann C (2005) Ultrastructure of the epidermal maxilla II-gland of *Scutigera coleoptrata* (Chilopoda, Notostigmophora) and the ground pattern of epidermal gland organs in Myriapoda. Journal of Morphology 264: 53–61. doi: 10.1002/jmor.10317

Hilken G, Rosenberg J (2006) Ultrastructural investigation of a salivary gland in a centipede: structure and origin of the maxilla-I-gland of *Scutigera coleoptrata* (Chilopoda, Notostigmophora). Journal of Morphology 267: 375–381. doi: 10.1002/jmor.10410

Hilken G, Rosenberg J (2009) Ultrastructural investigation of the vesicular glands in *Scutigera coleoptrata* (Chilopoda, Notostigmophora). Journal of Morphology 270: 451–458. doi: 10.1002/jmor.10700

Hilken G, Müller CHG, Sombke A, Wirkner CS, Rosenberg J (2011) Tracheal system. In: Minelli A (Ed.) Treatise on Zoology – Anatomy, Taxonomy, Biology, The Myriapoda 1. Brill, Leiden, 137–155. doi: 10.1163/9789004188266_008

Jaenicke E, Decker H, Gebauer W, Markl J, Burmester T (1999) Identification, structure, and properties of hemocyanin from diplopod Myriapoda. The Journal of Biological Chemistry 274(41): 29071–29074. doi: 10.1074/jbc.274.41.29071

Kusche K, Burmester T (2001) Diplopod hemocyanin sequence and the phylogenetic position of the Myriapoda. Molecular Biology and Evolution 18: 1566–1573. doi: 10.1093/oxfordjournals.molbev.a003943

Kusche K, Hembach A, Hagner-Holler S, Gebauer W, Burmester T (2003) Complete subunit sequences, structure and evolution of the 6 × 6-mer hemocyanin from the common house centipede, *Scutigera coleoptrata*. European Journal of Biochemistry 270: 2860–2868. doi: 10.1046/j.1432-1033.2003.03664.x

Mangum CP (1985) Oxygen transport in invertebrates. American Journal of Physiology 248: 505–514.

Mangum CP, Goddet G (1986) The hemocyanin of the uniramous Arthropods. In: Linzen B (Ed.) Invertebrate Oxygen Carriers. Springer Verlag, Berlin, 277–280. doi: 10.1007/978-3-642-71481-8_49

Mangum CP, Scott JL, Black REL, Miller KI, van Holde KE (1985) Centipedal hemocyanins: Its structure and implication for arthropod phylogeny. Proceedings of the National Academy of Sciences USA 82: 3721–3725. doi: 10.1073/pnas.82.11.3721

Meinert F (1868) Danmarks Scolopender og Lithobier. Naturhistorisk Tidsskrift 3. Serie (5): 241–268.

Meinert F (1883) De formeentlige Aandedratsredskaber og deres Mundinger (Stomata) hos Slægten *Scutigera*. Videnskabelige meddelelser fra den Naturhistoriske Forening i Kjöbenhavn 4. Aarti (4): 88–107.

Minelli A (1993) Chilopoda. In: Harrison FW (Ed.) Microscopic Anatomy of Invertebrates, Vol. 12: Onychophora, Chilopoda and Lesser Protostomia. Wiley-Liss, 57–114.

Müller CHG, Rosenberg J, Meyer-Rochow VB (2003) Hitherto undescribed interommatidial exocrine glands in Chilopoda. African Invertebrates 44: 185–197.

Müller CHG, Rosenberg J, Hilken G (2009): Fine structure and phylogenetic significance of 'flexo-canal epidermal glands'. In: Xylander W, Voigtländer K (Eds) Myriapoda and Onychophora of the world – Diversity, Biology and Importance – Proceedings of the 14th International Congress of Myriapodology. Soil Organisms 81(3): 269–294.

Müller CHG, Rosenberg J, Hilken G (2014) Ultrastructure, functional morphology and evolution of recto-canal epidermal glands in Myriapoda. Arthropod Structure & Development 43(1): 43–61. doi: 10.1016/j.asd.2013.08.001

Pagenstecher HA (1878) Allgemeine Zoologie oder Grundgesetze des thierischen Baus und Lebens. Wiegandt, Hempel & Parey, Berlin, 347 pp.

Prunescu P, Prunescu CC (1996) The ultrastructure of the tracheae of the tracheal lungs in *Scutigera coleoptrata* (Notostigmophora, Chilopoda). Travaux de l'Institut de Spéologie «Émile Racovitza» 35: 63–67.

Rosenberg J (2009) Die Hundertfüßer (Chilopoda). Neue Brehm-Bücherei 285. Westarp Wissenschaften, Hohenwarsleben, 524 pp.

Rosenberg J, Hilken G (2006) Fine structural organization of the poison gland of *Lithobius forficatus* (Chilopoda, Lithobiomorpha). Norwegian Journal of Entomology 53: 119–127.

Rosenberg J, Müller CHG, Hilken G (2011) Aggregated and compound epidermal glands. In: Minelli A (Ed.) Chilopoda – Integument and Associated Organs, Treatise on Zoology – Anatomy, Taxonomy, Biology, The Myriapoda 1. Brill, Leiden, 84–97.

Sinclair FG (1891) A new mode of respiration in the Myriapoda. Proceedings of the Royal Society London 100(303): 200–201. doi: 10.1098/rspl.1891.0027

Sinclair FG (1892) A new mode of respiration in the Myriapoda. Philosophical Transactions of the Royal Society London B 183: 61–72. doi: 10.1098/rstb.1892.0004

Sundara Rajulu GS (1969) Presence of haemocyanin in the blood of a centipede *Scutigera longicornis* (Chilopoda: Myriapoda). Current Science Bangalore 7: 168–169.

Sundara Rajulu GS (1971) X-ray diffraction and EM studies on the fine structure of the tracheae of the centipede *Scutigera longicornis*, together with observations on their chemical composition. Current Science (Bangalore) 17: 467–468.

Tömösváry Ö (1881) A *Scutigera coleoptrata* legzöszervéröl (in Hungarian) (On the organ of respiration of *Scutigera coleoptrata*). Dissertation, Kolozsvàr, 25 pp.

Tömösváry Ö (1883a) Über das Respirationsorgan der Scutigeriden. Mathematisch Naturkundliche Berichte Ungarn 1: 175–180.

Tömösváry Ö (1883b) A scutigera-félék légzószervéról (in Hungarian) (On the organ of respiration in the genus *Scutigera*). Mathematikai és Természettudományi Értesító 1: 145–150.

Voges E (1882) Das Respirationssystem der Scutigeriden. Zoologischer Anzeiger 5: 67–69.

Voges E (1916) Myriapodenstudien. Zeitschrift für wissenschaftliche Zoologie 116: 75–135.

Wirkner CS, Pass G (2002) The circulatory system in Chilopoda: functional morphology and phylogenetic aspects. Acta Zoologica (Stockholm) 83: 193–202. doi: 10.1046/j.1463-6395.2002.00112.x

Wirkner CS, Tögel M, Pass G (2013) The arthropod circulatory system. In: Minelli A, Boxshall G, Fusco G (Eds) Arthropod Biology and Evolution. Springer, Berlin, Heidelberg, 343–391. doi: 10.1007/978-3-642-36160-9_14

ZooKeys 510: 243–267 (2015)
doi: 10.3897/zookeys.510.8840
http://zookeys.pensoft.net

RESEARCH ARTICLE

A peer-reviewed open-access journal
ZooKeys
Launched to accelerate biodiversity research

Tentorial mobility in centipedes (Chilopoda) revisited: 3D reconstruction of the mandibulo-tentorial musculature of Geophilomorpha

Markus Koch[1], Johannes Schulz[2], Gregory D. Edgecombe[3]

1 *Institute of Evolutionary Biology and Ecology, University of Bonn, An der Immenburg 1, 53121 Bonn, Germany* **2** *Department of Entomology, Biocentre Grindel and Zoological Museum, Martin-Luther-King-Platz 3, 20146 Hamburg, Germany* **3** *Department of Earth Sciences, The Natural History Museum, Cromwell Road, London SW7 5BD, UK*

Corresponding author: *Markus Koch* (mkoch@evolution.uni-bonn.de)

Academic editor: *Ivan H. Tuf* | Received 27 October 2014 | Accepted 2 June 2015 | Published 30 June 2015

http://zoobank.org/D79285F5-4436-460B-A4E1-17BEADC9972E

Citation: Koch M, Schulz J, Edgecombe GD (2015) Tentorial mobility in centipedes (Chilopoda) revisited: 3D reconstruction of the mandibulo-tentorial musculature of Geophilomorpha. In: Tuf IH, Tajovský K (Eds) Proceedings of the 16th International Congress of Myriapodology, Olomouc, Czech Republic. ZooKeys 510: 243–267. doi: 10.3897/zookeys.510.8840

Abstract

Mandibular mechanisms in Geophilomorpha are revised based on three-dimensional reconstructions of the mandibulo-tentorial complex and its muscular equipment in *Dicellophilus carniolensis* (Placodesmata) and *Hydroschendyla submarina* (Adesmata). Tentorial structure compares closely in the two species and homologies can be proposed for the 14/17 muscles that attach to the tentorium. Both species retain homologues of muscles that in other Pleurostigmophora are traditionally thought to cause swinging movements of the tentorium that complement the mobility of the mandibles. Although the original set of tentorial muscles is simplified in Geophilomorpha, the arrangement of the preserved homologues conforms to a system of six degrees of freedom of movement, as in non-geophilomorph Pleurostigmophora. A simplification of the mandibular muscles is confirmed for Geophilomorpha, but our results reject absence of muscles that in other Pleurostigmophora primarily support see-saw movements of the mandibles. In the construction of the tentorium, paralabial sclerites seem to be involved in neither Placodesmata nor Adesmata, and we propose their loss in Geophilomorpha as a whole. Current insights on the tentorial skeleton and its musculature permit two alternative conclusions on their transformation in Geophilomorpha: either tentorial mobility is primarily maintained in both Placodesmata and Adesmata (contrary to Manton's arguments for immobility), or the traditional assumption of the tentorium as being mobile is a misinterpretation for Pleurostigmophora as a whole.

Keywords
Evolutionary morphology, head endoskeleton, Myriapoda, skeleto-muscular system, histology

Introduction

The tentorium of myriapods is a cuticular formation of the head with a distinct composition of exoskeletal bars around the mouth opening and endoskeletal processes (Koch 2003). Since Manton's (1964) comparative studies of the head morphology in mandibulate arthropods, structural correspondences of the tentorium and its participation in movements of the mandibles are considered as the most compelling morphological evidence for the monophyly of Myriapoda (Edgecombe 2004, Shear and Edgecombe 2010). In the context of myriapod phylogeny (e.g., Edgecombe 2011), however, ambiguity remains with regard to Manton's (1964) conclusion that the myriapod tentorium is primarily mobile, and that its "swinging" movements correlate with the presence of a mobile mandibular gnathal lobe that lacks a mandibular muscle for its abduction. This uncertainty results in particular from the presence of an apparently immobile tentorium in the basalmost offshoot among centipedes, the Scutigeromorpha, and from apparent differences among pleurostigmophoran centipedes, symphylans, and millipedes as to how tentorial movements are transferred onto the mandibles, including eventual absence of tentorial mobility in subordinate taxa within each of these groups (Verhoeff 1918, Attems 1926, 1929, Manton 1964, 1965, Desbalmes 1992).

An immobile tentorium was assumed by Manton (1965) for geophilomorph centipedes among Pleurostigmophora, based on her studies of a few representatives of the clade Adesmata. Recent comparative studies of the peristomatic structures in a broader sampling of geophilomorphs by Koch and Edgecombe (2012) challenged this assumption. They revealed anatomical evidence in support of the view that at least in the sister group to all other geophilomorphs, the Placodesmata, the mobility of the tentorium is maintained, its loss accordingly being potentially synapomorphic for adesmatan Geophilomorpha only. This preliminary conclusion still required more comprehensive anatomical studies of the mandibulo-tentorial complex, including its muscular equipment. Some of the evolutionary transformations of the tentorium in Adesmata as advocated by Koch and Edgecombe (2012) were contradicted by Bonato et al. (2014) within the scope of their analyses of geophilomorph phylogeny.

In order to contribute to a clarification of the mobility versus immobility of the tentorium in geophilomorphs, we here present anatomical 3D-reconstructions of the mandibulo-tentorial complex and its muscular system based on histological studies of two species, one representing each of the clades Placodesmata and Adesmata, respectively. Insights obtained demand a more general revision of whether the tentorium in pleurostigmophoran centipedes is mobile at all.

Materials

This study is based on histological sections of the head of *Dicellophilus carniolensis* (Koch, 1847) (Placodesmata, Mecistocephalidae) and *Hydroschendyla submarina* (Grube, 1792) (Adesmata, Schendylidae). The heads were fixed in alcoholic Bouin solution (modified according to Duboscq-Brazil) after removal of the forcipules, dehydrated in a graded ethanol series, and transferred via propylene oxide into epoxy resin (Araldite). Series of semithin transverse sections (0.5–1 μm thickness) were performed with a Jumbo-Diatome diamond knife on an Ultracut E microtome (Fa. Reichert) and stained with 1% Toluidine blue. Digital images (tiff-format) of the sections were made with an Olympus BX51dotSlide microscope and semi-automatically aligned into a digital image stack with the open source software imodalign (http://www.evolution.uni-bonn.de/mitarbeiter/bquast/software). The image stacks used for 3D-reconstructions (Fig. 1) are provided as supplementary files (Suppl. materials 1 and 2).

For three-dimensional reconstructions the software Amira 5.4.5 (FEI Visualization Sciences Group) was used for segmentation. As recommended by Friedrich and Beutel (2008), the segmented volume data were imported as bmp-files into the software Bitplane Imaris 5.7 (Bitplane AG, Zürich, Switzerland) for surface rendering, smoothing and downsampling. The surfaces (inventor format) were then converted with the freeware Transform2 (Heiko Stark, Jena, Germany; http://starkrats.de) into object format for final processing with the Autodesk Maya software (Students & Educators version 2013; Alias Wavefront, Toronto/Ontario, Canada), applying the Cleanup, Average Vertices, Poly Reduce, and Smooth options to remove minor segmentation artifacts. The surface models were then exported from Maya as u3d-files (Universal 3D format) and processed with the Adobe 3D-Reviewer plug-in in Adobe Acrobat 9 Pro Extended (Adobe Systems, San Jose, California, USA) to generate an interactive pdf-file. Images and plates were edited with the Adobe Illustrator and Adobe Photoshop CS4 software.

The interactive 3D-mode can be activated by clicking on the images of Figures 2C and 3C. A tool bar then opens that allows the user to rotate, move and magnify the model, to isolate elements, and to change the background and light settings. Elements of our models comprise the head capsule, the tentorium, the mandible, and all muscles related to the tentorio-mandibular complex. Single elements can be displayed by opening the "model hierarchy" and clicking off/on the respective elements according to their label. Clicking on any element in the model hierarchy highlights the respective element in red. The "initial configuration" shows all elements in ventral view. The views of our models shown in Figures 1–3 can be displayed by choosing the respective configuration in the toolbar under "views".

Results

In both *D. carniolensis* and *H. submarina* the tentorium forms a clasp-like structure dorsally around the mandible (Figs 1–3: *tt*). Its external sclerotisation comprises a

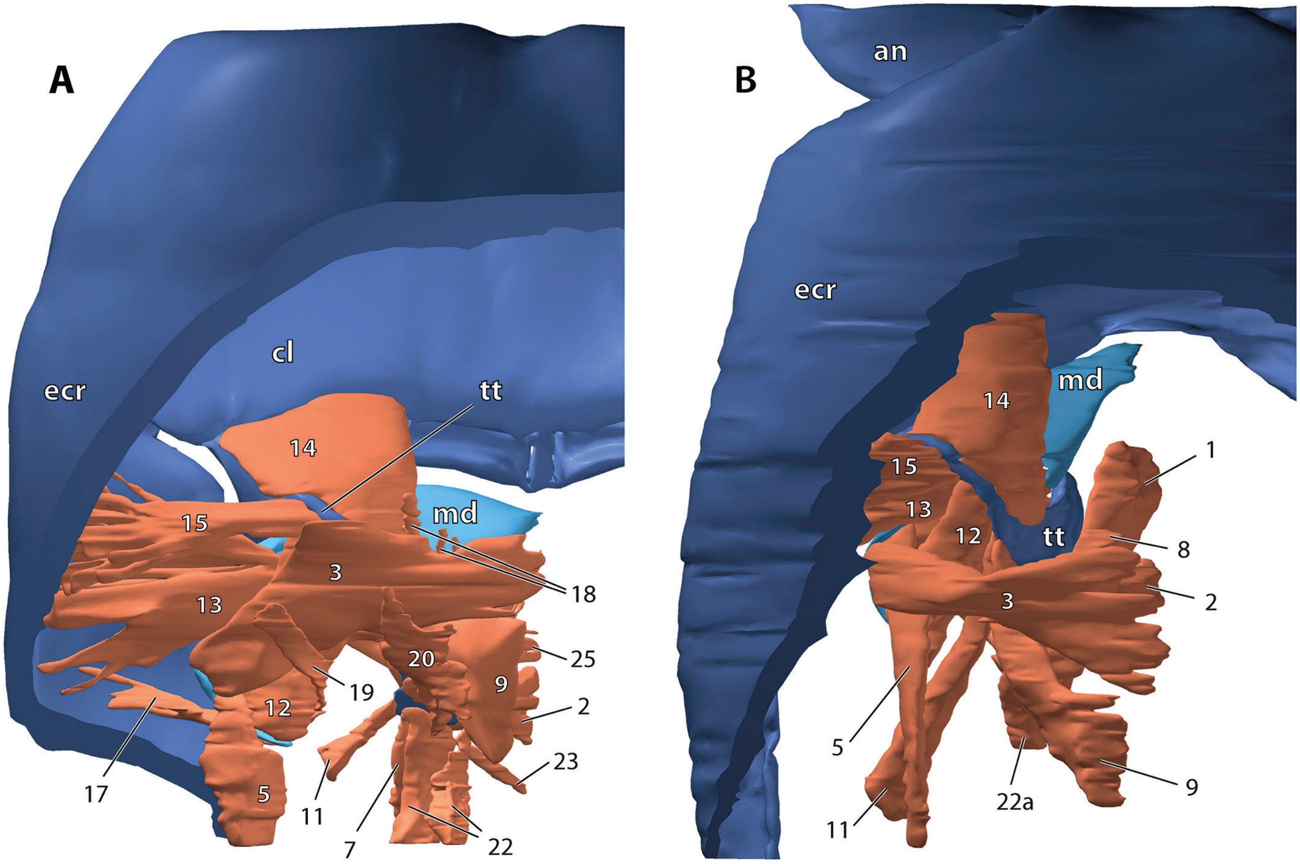


Figure 1. Surface model of the mandibulo-tentorial complex, dorsal view onto the left complex within the head capsule (anterior is top). **A** *Dicellophilus carniolensis* **B** *Hydroschendyla submarina*, tentorial muscles 18 and 22b removed. Numbers refer to muscles as listed in Table 1. Abbreviations: **an** antenna **cl** clypeus, **ecr** epicranium, **md** mandible, **tt** tentorium.

short, slender epipharyngeal bar to which the mandibular gnathal lobe is attached via a condyle on its dorsal surface (arrow in Figs 2A, 3A); a slender transverse bar that is laterally jointed to the cephalic pleurite; and a supramandibular arch that behind the mouth opening continues into a hypopharyngeal bar (Fig. 4C). The configuration of these exoskeletal components in ventral view is Y-shaped in *D. carniolensis* and T-shaped in *H. submarina*, with the epipharyngeal bar being in either a submarginal (*D.c.*) or marginal (*H.s.*) position relative to the labral sidepieces. Endoskeletal processes of the tentorium comprise a massive ridge (including a frontal process fide Koch 2003) formed by the supramandibular arch, and a flat, wing-like apodeme (posterior process fide Koch 2003) arising at the junction with the hypopharyngeal bar and protruding into the head in a curved, almost vertical orientation. A collagenous bridge interconnecting left and right posterior processes is absent in both species.

Tentorial muscles

The muscular equipment of the tentorium largely corresponds in the two species studied (Figs 1–3). A total of 17 (*D.c.*) or 14 (*H.s.*) muscles attach at the tentorium, including extrinsic muscles of the mandibles and of the first and second maxillae, two hypopharyngeal muscles, a dilator of the pharynx, muscles arising from the clypeus and the epicranium, and ventral longitudinal muscles (see Table 1). The muscles attach either at the supramandibular arch or at the posterior process; no muscles insert at or originate from the epipharyngeal bar, the transverse bar, or the hypopharyngeal bar. In *D. carniolensis* the respective muscles of the first and second maxillae (muscles 2, 7, 11, 16, and 23) partly or entirely arise from collagenous tendons attached to the posterior tip of the tentorial posterior process (Fig. 4D). The homologous muscles in *H. submarina* originate directly from the posterior process (Fig. 5D); this species entirely lacks collagenous tendons associated with the tentorium. Antennal muscles do not arise from the tentorium in either species, but from the clypeus and epicranial wall.

Muscles that in Lithobiomorpha and Scolopendromorpha are thought to move the tentorium are represented in *D. carniolensis* and *H. submarina* by the following set:

M.9 is a fan-shaped vertical muscle that originates medio-dorsally from the epicranial wall (Figs 1–3). Its insertion on the tentorium extends from the posterior dorsal edge of the supramandibular arch to the anterior dorsal edge of the posterior process, gradually expanding backwards onto its mesial surface. Relative to the posterior process the origin of this muscle in *H. submarina* lies more posteriorly than in *D. carniolensis*.

M.14 is a broad, fan-shaped muscle passing from the clypeus towards the supramandibular arch of the tentorium to insert on its frontal process and mesial surface along its entire length (Figs 1, 2B, 3B, 4A–C, 5A, B).

M.15 passes almost transversely from the lateral epicranial wall towards the supramandibular arch, on which it inserts along its dorsal rim on its lateral face. In *H. submarina* (Figs 3, 5B) the insertion is direct at the tentorium, whereas it is mediated

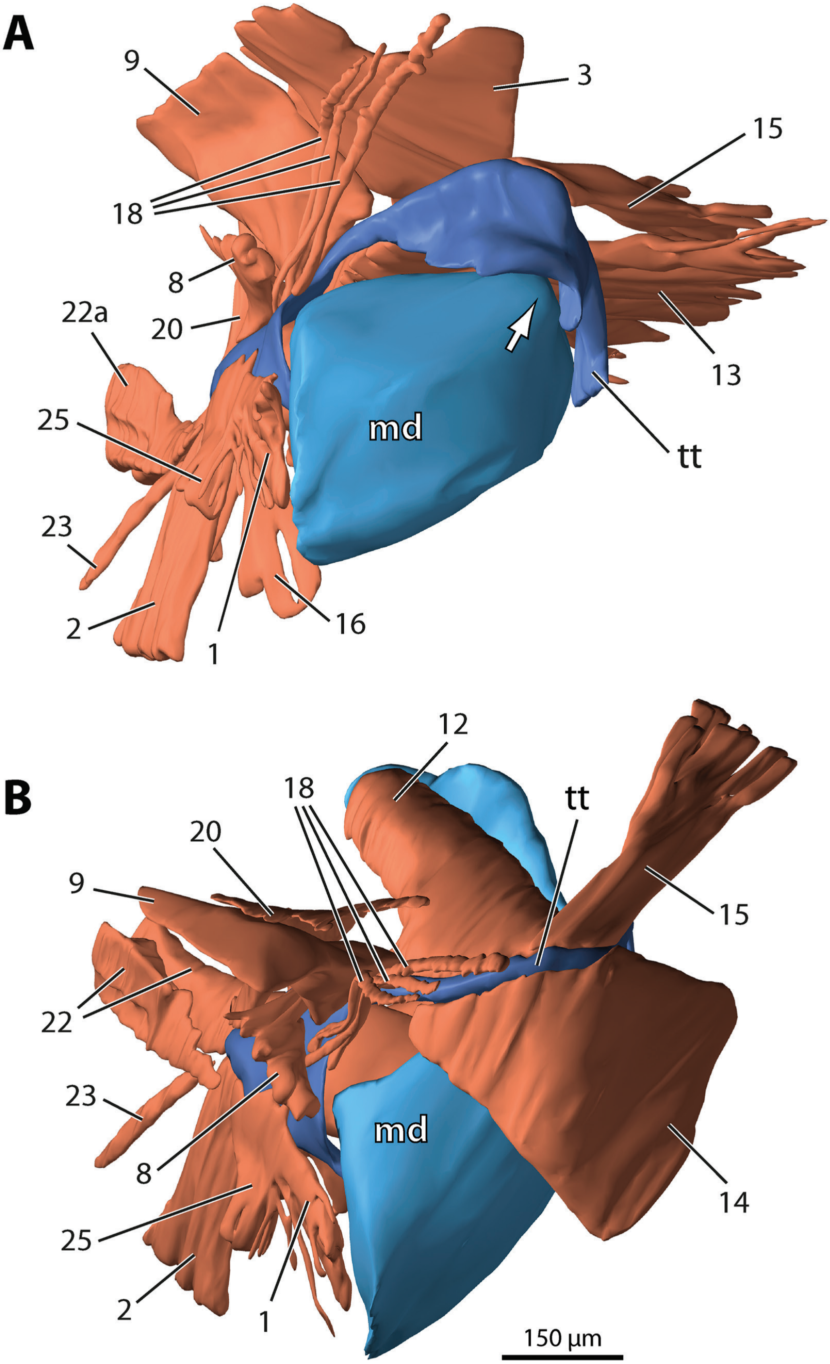


Figure 2. *Dicellophilus carniolensis*, surface model of the left mandibulo-tentorial complex. **A** Medio-frontal view, tentorial muscle 14 removed; arrow points to condyle of mandibular gnathal lobe **B** Oblique dorso-frontal view, extrinsic mandibular muscles removed. Numbers refer to muscles as listed in Table 1. Abbreviations: **md** mandible **tt** tentorium.

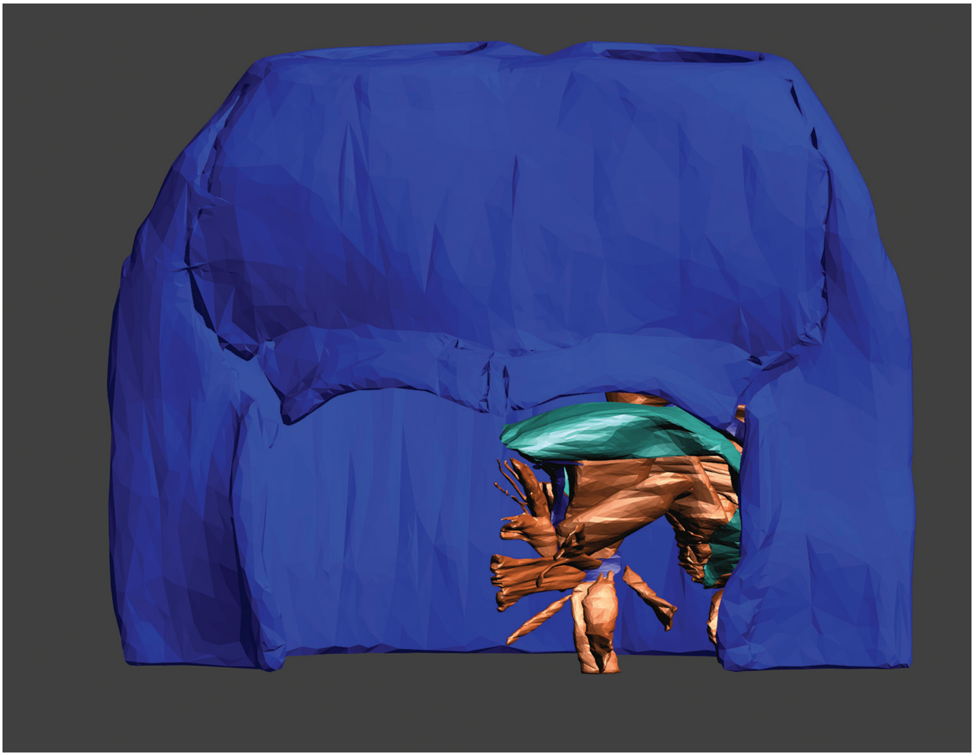


Figure 2C. *Dicellophilus carniolensis*, surface model of the left mandibulo-tentorial complex in situ. Click on the image to activate the interactive 3D-mode.

by a collagenous tendon in *D. carniolensis* (Figs 2, 4C). In both species the origin of this muscle lies immediately above mandibular muscle 13.

M.18 arises medio-dorsally from the epicranial wall and inserts anteriorly on the mesial surface of the posterior process, in front of the insertion of pharyngeal muscle 8 and below the insertion of tentorial muscle 9 (Figs 1A, 2). The relative positions of these three muscles (8, 9, and 18) are the same in the two species studied, although in *H. submarina* tentorial muscle 18 is far larger than in *D. carniolensis* (Fig. 5C, D).

M.22 is a ventral longitudinal muscle attached to the posterior tip of the tentorial posterior process. In both species the muscle consists of two strands. In *D. carniolensis* (Figs 1A, 2B, 4D) they insert adjacent to each other at collagenous tendons connected to the posterior process. In *H. submarina* (Figs 1B, 3) the two strands insert directly on the tentorium: a smaller strand (22a) on the dorso-lateral edge of the posterior process above the origin of maxillary muscle 2, and a larger strand (22b) on the ventro-lateral edge of the posterior process below the origin of maxillary muscle 2 (Fig. 5D).

In *D. carniolensis* an additional fan-shaped muscle (M.20) passes from the back of the posterior process anteriorly towards the dorsal epicranium (Figs 1A, 2B, 4D). Its attachment at the epicranium lies immediately adjacent (lateral) to the origin of tentorial muscle 9.

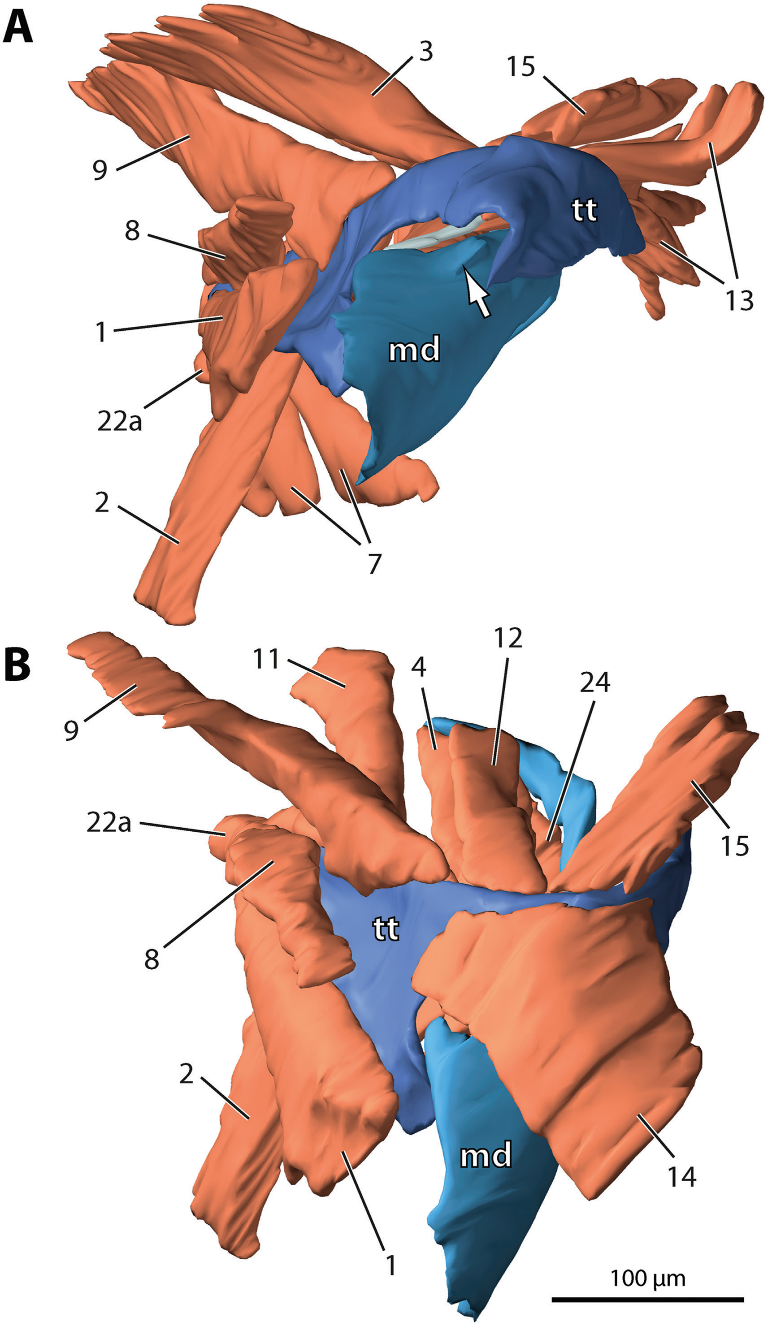


Figure 3. *Hydroschendyla submarina*, surface model of the left mandibulo-tentorial complex. **A** Medio-frontal view, tentorial muscles 14, 18, and 22b removed as well as hypopharyngeal muscle 25; arrow points to condyle of mandibular gnathal lobe **B** Oblique dorso-frontal view, extrinsic mandibular muscles 3, 5, and 13 removed as well as tentorial muscles 18 and 22b. Numbers refer to muscles as listed in Table 1. Abbreviations: **md** mandible **tt** tentorium.

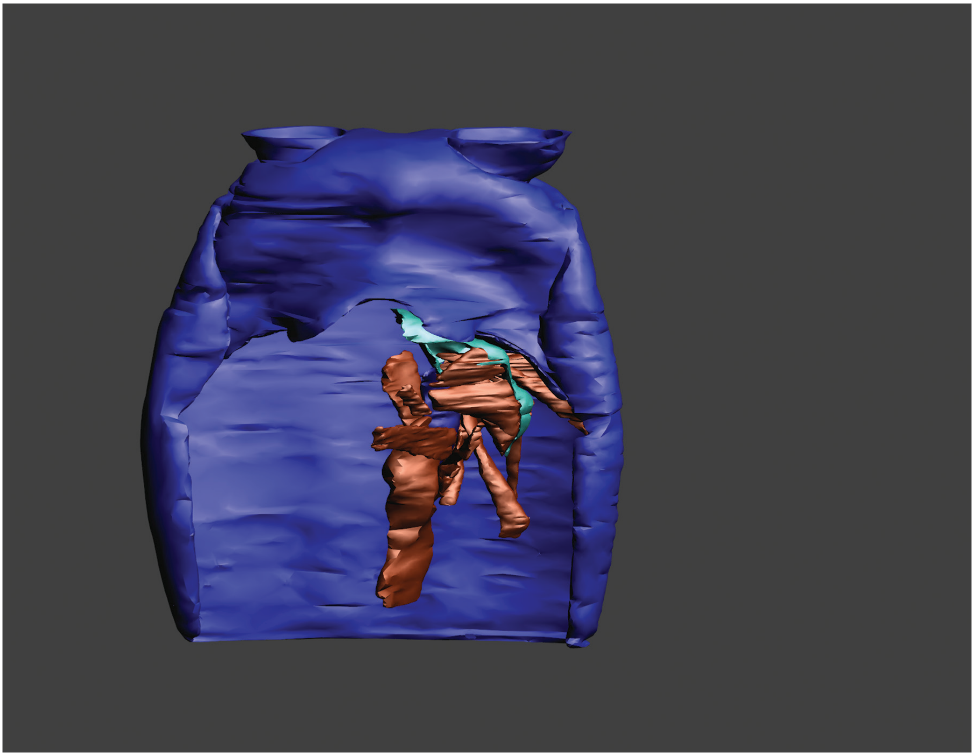


Figure 3C. *Hydroschendyla submarina*, surface model of the left mandibulo-tentorial complex in situ. Click on the image to activate the interactive 3D-mode.

Mandibular muscles

The mandibles of *D. carniolensis* and *H. submarina* each consist of a rod-shaped base, somewhat twisted at half length, and a gnathal lobe (for SEM-illustration of the latter see Koch and Edgecombe 2012, their figs 7C and 17C). Both species lack a suture delimiting the two components, but in *D. carniolensis* a narrow, circumferential stripe of flexibility within the endocuticle (i.e., an area showing the same histological staining properties as the articular membranes between sclerites) indicates their borders (Fig. 4B, inset). The mandibular musculature differs among the two species in some details.

Extrinsic mandibular muscles:

M.3 basically corresponds in the two species in its large extent and transverse orientation. It originates dorso-medially from the epicranial wall and inserts on the dorsal margin of the mandibular base at about half its length (Figs 1, 2A, 3A, 5D).

M.5 is a longitudinal muscle originating dorsally from the posterior wall of the epicranium (Fig. 1). In *D. carniolensis* it consists of about 13 smaller bundles; most

Table 1. Compilation of all mandibular and tentorial muscles, numbered in accordance to their illustration in Figs 1–5 and the 3D-reconstructions. Potential homologues in *Orya barbarica* are inferred from Manton (1965) under citation of her muscle numbering; muscles not mentioned or depicted in her work are indicated by a question mark.

Muscles (this study)	Origin	Insertion	*Dicellophilus carniolensis*	*Hydroschendyla submarina*	*Orya barbarica* (Manton 1965)
Tentorium (tt)					
9	epicranium	posterior process	+	+	? (T10)
14	clypeus	supramandibular arch	+	+	+ (T4/T5, or T2-T6)
15	epicranium	supramandibular arch	+*	+	? (T7)
18	epicranium	supramandibular arch	+	+	? (T9)
20	epicranium	posterior process	+	–	? (T8)
22	forcipular tendon	posterior process	+*	+	slm / vlm
Mandible (md)					
3	epicranium	base	+	+	26
4	base	gnathal lobe	+	+	31
5	epicranium	base	+	+	– (25, or 24+25)
6	tentorium	gnathal lobe	+	+	27
10	gnathal lobe	gnathal lobe	–	+	29
12	tentorium	base	+	+	22/23
13	epicranium	gnathal lobe	+	+	20
17	epicranium	base	+	–	19 (25 fide Manton)
19	epicranium	base	+	– (21?)	–
21	epicranium	gnathal lobe	– (19?)	+**	21
24	tentorium	base	+	+	– (30, unified with 23 fide Manton)
26	mesial interconnection	base	–	+**	– (32)
Hypopharynx (hy)					
1	tentorium	front side along mesial lips	+	+	+
25	tentorium	back side near opening of hypoph. gland	+	+	+
Pharynx (ph)					
8	tentorium	ventral pharyngeal wall	+*	+	?

Muscles (this study)	**Origin**	**Insertion**	***Dicellophilus carniolensis***	***Hydroschendyla submarina***	***Orya barbarica* (Manton 1965)**
Maxilla I (mxI)					
2	tentorium	coxosternite (paramedial)	+*	+	(+)***
16	tentorium	coxosternite (lateral to 2)	+*	–	
23	tentorium	coxosternite (paramedial, posterior to 2)	+*	–	
Maxilla II (mxII)					
7	tentorium	coxosternite (medial at base of telopodite)	+*	+	(+)***
11	tentorium	coxosternite (lateral)	+*	+	

* insertion via collagenous tendon

** not shown in the 3D-reconstruction

*** depicted in Manton's Fig. 81a but no unambiguous homologisation possible

of them insert one by one on the posterior dorsal margin of the mandibular base, except for three bundles that pass into the concavity of the mandibular base to insert on its internal wall, one far anteriorly to the other two (Fig. 4D). In *H. submarina* this muscle comprises only 6 bundles (Fig. 5D), all of which insert within the mandibular concavity on its internal wall.

M.6 arises from the tentorium and passes towards the gnathal lobe. In *D. carniolensis* this muscle is larger than in *H. submarina* and shows a more longitudinal course; it originates from the posterior part of the posterior process, where it covers its entire lateral surface, and inserts on the frontal wall of the gnathal lobe (Fig. 4B,C). In *H. submarina* the origin of this muscle lies anteriorly on the posterior process close to the junction of the hypopharyngeal bar; its insertion extends from the latero-ventral, proximal part of the gnathal lobe onto the anteriormost part of the mandibular base. The course of this muscle accordingly is almost transverse in *H. submarina*.

M.12 passes from the posterior dorsal margin of the mandibular base towards the supramandibular arch of the tentorium. In *D. carniolensis* this muscle is larger than in *H. submarina*, its insertion covering the entire lateral surface of the supramandibular arch from the frontal process towards the origin of the posterior process (Figs 1A, 2B, 4B–D). In *H. submarina* the insertion of this muscle on the dorsal rim of the supramandibular arch is restricted to its posterior part while it expands onto the anteriormost dorsal rim of the posterior process (Figs 1B, 3B, 5C).

M.13 is a transverse muscle passing from the cranial wall towards the gnathal lobe. In both species it consists of two sections, a larger fan-shaped dorsal section (13a) and a few bundles below it (13b). In *D. carniolensis* (Fig. 1A, 2A) both sections arise dorso-laterally from the epicranial wall; the bundles of the dorsal section (13a) insert on a short, inconspicuous cuticular tendon (Fig. 4C, inset) formed by the

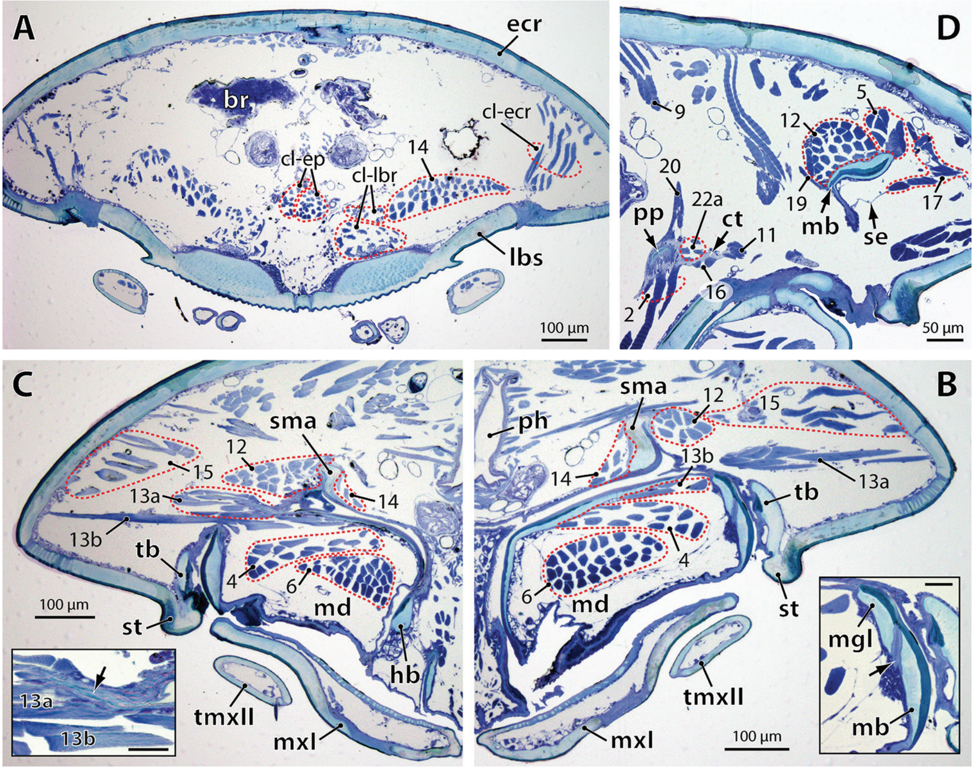


Figure 4. *Dicellophilus carniolensis*, selection of micrographs of transverse sections through the head from anterior to posterior. **A** Section through anterior head part in front of the mandibulo-tentorial complex, highlighting muscles arising from the clypeus **B** Section through the mandibulo-tentorial complex (left side of head) slightly anterior to C; in the inset (scale: 25 µm) the area of flexibility (arrow) between mandibular gnathal lobe and base is magnified. **C** Section through the mandibulo-tentorial complex (right side of head) at the level of the cuticular tendon (arrow in inset; scale: 25 µm) of the mandibular gnathal lobe. **D** Section through posterior part of tentorium and mandible (left side of head), showing collagenous tendon system and mandibular septum. Numbers refer to muscles as listed in Table 1. Abbreviations: **br** brain **cl-ecr** clypeo-epricranial muscle **cl-ep** clypeo-epipharyngeal muscle **cl-lbr** clypeo-labral muscle **ct** collagenous tendon, **ecr** epicranium **hb** hypopharyngeal bar of tentorium **lbs** labral sidepiece **mb** mandibular base **md** mandible **mgl** mandibular gnathal lobe, **mxl** first maxilla **ph** pharynx **pp** posterior process of tentorium, **se** septum of mandibular gnathal pouch **sma** supramandibular arch of tentorium **st** stilus **tb** transverse bar of tentorium **tmxII** telopodite of second maxilla.

dorsal margin of the gnathal lobe, whereas all bundles of the lower section (13b) pass into the concavity of the gnathal lobe to insert directly on its dorsal wall (Fig. 4B,C). In *H. submarina* (Fig. 1B, 3A), the cuticular tendon of the gnathal lobe is larger and more elongate, and all muscle bundles of both sections insert on it. In this species, however, only the bundles of the dorsal section (13a) arise from the latero-dorsal wall of the epicranium, whereas the bundles of the lower section (13b) originate from the ventro-lateral part of the head capsule (Fig. 5B,C).

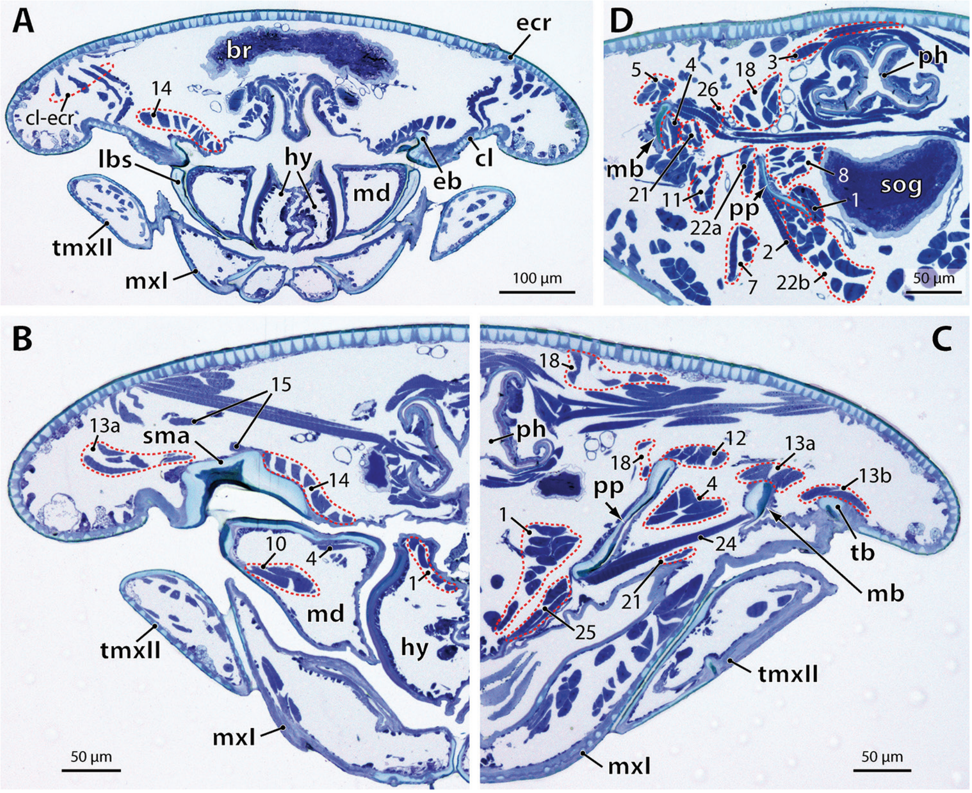


Figure 5. *Hydroschendyla submarina*, selection of micrographs of transverse sections through the head from anterior to posterior. **A** Section through the anterior head part, showing the mandibulo-tentorial complex at the level of the epipharyngeal bar **B** Section through the mandibulo-tentorial complex (right side of head) at the level of the junction between epipharyngeal bar and supramandibular arch. **C** Section through the mandibulo-tentorial complex (left side of head) between the levels shown in B and D. **D** Section through posterior part of tentorium and mandible (right side of head) at the level of the mesial interconnection of the mandibles by muscle 26. Numbers refer to muscles as listed in Table 1. Abbreviations: **br** brain **cl** clypeus, **cl-ecr** clypeo-epicranial muscle **eb** epipharyngeal bar of tentorium, **ecr** epicranium **hy** hypopharynx **lbs** labral sidepiece **mb** mandibular base **md** mandible **mxI** first maxilla **ph** pharynx **pp** posterior process of tentorium **sma** supramandibular arch of tentorium **sog** subesophageal ganglion **tb** transverse bar of tentorium **tmxII** telopodite of second maxilla.

M.17 interconnects the posteriormost end of the mandibular base with the dorsolateral wall of the epicranium along a (collagenous?) septum connected to the posterior cuticular, non-sclerotized wall of the mandibular gnathal pouch (Figs 1A, 4D). Although this septum is present in both species, the muscular bundles connected to it in *D. carniolensis* are absent in *H. submarina*.

M.19 is an almost vertical muscle in *D. carniolensis* (Figs 1A, 4D). It arises from the dorsal epicranial wall and inserts on the ventral margin of the mandibular base at its posterior half. A corresponding muscle seems to be absent in *H. submarina* (but see discussion of its potential homology to mandibular muscle 21)

M.21 is a slender longitudinal muscle composed of four or five small bundles in *H. submarina* (Fig. 5C). It extends from the posterior dorsal wall of the epicranium between the posterior process of the tentorium and the mandibular base towards the gnathal lobe where it inserts proximally at its soft ventral (posterior) wall. A corresponding muscle is absent in *D. carniolensis* (but see discussion of its potential homology to mandibular muscle 19).

M.24 interconnects the ventral margin of the mandibular base with the ventral margin of the posterior process. The course of this muscle is almost transverse in *H. submarina* (Figs 3B, 5C) but more oblique in *D. carniolensis.* In the latter its insertion at the mandibular base lies more anteriorly than its origin on the posterior process, and it does not attach at the sclerotic part of the mandibular base but on its ventral arthrodial membrane.

M.26 is a transverse muscle in *H. submarina* that interconnects the posterior ends of the left and right mandibular bases (Fig. 5D). The strands of the left and right muscle overlap mesially between the pharynx and the subesophageal ganglion, where they seem to be directly interconnected without any contribution by collagenous tendon. *D. carniolensis* lacks this muscle entirely and shares with *H. submarina* the absence of a transverse mandibular tendon.

Intrinsic mandibular muscles:

M.4 is a large muscle in both species, passing from the posterior end of the mandibular base to the dorsal (anterior) wall of the gnathal lobe (Fig. 3B). In *D. carniolensis* this muscle comprises about 15 smaller bundles, all of which extend below mandibular muscle 13 into the gnathal lobe to insert on its proximal, dorsal wall (Fig. 4B,C). In *H. submarina* this muscle instead consists of five larger bundles, three of which extend below the cuticular tendon of mandibular muscle 13 into the gnathal lobe while the other two insert on the base of this tendon (Fig. 5B-D).

M.10 is an intrinsic muscle of the gnathal lobe. In *H. submarina* it connects the lateral distal wall of the gnathal lobe with its proximal, soft mesial wall, immediately in front of mandibular muscle 6 (Fig. 5B). The gnathal lobe of *D. carniolensis* lacks any intrinsic muscles.

Discussion

Verhoeff (1918) was the first to put forth the view that the tentorium of lithobiomorphs and scolopendromorphs is mobile and actively involved in movements of the mandibles. He inferred tentorial mobility in *Lithobius* and *Scolopendra* from dissected heads after removal of all mouthparts behind the mandibles, from which he could 'observe' a rotation of the tentorium along the articulation of its transverse bar with the cephalic pleurite by manually pulling on the loosened mandibles and the tentorial posterior process. For geophilomorphs he concluded that movements of the tentorium are primarily maintained, as exemplified for *Himantarium gabrielis* (Adesmata, Himantariidae), albeit in an altered manner and no longer directly accompanying movements of the mandibles due to transformations of the tentorium and mandible morphology correlated with suctorial feeding habits. Attems (1926, 1929) largely adopted Verhoeff's interpretations.

Manton (1965) basically corroborated Verhoeff's conclusions with comparative studies on the skeleto-muscular system of the head in centipedes, but she denied any tentorial mobility for geophilomorphs. While including direct observations of mandible movements in live *Lithobius* and *Scolopendra* specimens, she could not directly observe movements of the tentorium but, like Verhoeff, inferred them from the presence and positions of muscles attached at the tentorium, and from manipulation of its skeleton (Manton 1964: 87). Her main reasons for considering the tentorium of geophilomorphs as immobile were based on the view that their heads are primarily adapted to burrowing life habits. Anatomical transformations considered by her as to correlate with burrowing life habits include (i) alteration of the tentorial skeleton, especially absence of a transverse bar; (ii) absence of a "coclypeus" (i.e., paralabial sclerites), "*whose movements are not needed*" (p. 340); (iii) absence of all tentorial muscles (T2-T10 fide Manton) effecting the swing of the tentorium in non-geophilomorph representatives of the Pleurostigmophora; and (iv) simplification of the mandibular musculature, including absence of a transverse mandibular tendon, under restriction of mandible movements to those affected by the mandibular muscles alone. In the light of the current results all these assumptions must be reconsidered.

Derivation of the tentorial skeleton

The entire construction of the tentorium surprisingly shows no basic difference between *D. carniolensis* and *H. submarina*. They indeed seem to differ only in the spatial extension of the tentorium in correspondence to the different shape of the head, in being relatively broad and short in *D. carniolensis* versus narrower and more expanded in the longitudinal plane in *H. submarina*. Previous descriptions of the posterior process ("anterior tentorial apodeme" fide Manton 1964, 1965) as rod-shaped proved to be misleading. In both Placodesmata and Adesmata it primarily forms a curved, wing-like apodeme in a similar manner as in non-geophilomorph centipedes. The condition found in *D. carniolensis* seems to be plesiomorphic relative to that in *H. submarina* in that remnants of the collagenous, formerly transverse tendon system attached to the posterior processes are preserved in the former.

Placodesmata

As inferred from *D. carniolensis*, the original composition of the tentorium and ancestral arrangement of its cuticular components in Pleurostigmophora are maintained in Placodesmata. A main deviation in Placodesmata concerns the sclerotization of the exoskeletal components that are reduced to slender strips (Koch and Edgecombe 2012, Bonato et al. 2014), as well as a shortening of the epipharyngeal bar. The latter seems to be characteristic for all geophilomorphs. The manner in which the sclerotized lateral end of the transverse bar in placodesmatans bends anteriorly between the cephalic pleurite and the labral sidepiece is reminiscent particularly of the state in scolopendromorphs, in which the lateral end of the transverse bar similarly bends anteriorly to form a hook-shaped point of articulation with the cephalic pleurite (Desbalmes 1992). While the hook-shaped end of the transverse bar in scolopendromorphs borders anterio-mesially against the paralabial sclerite, the antero-mesial border in Placodesmata seems to be formed by the labral sidepieces; paralabial sclerites are not distinct in Placodesmata (see Koch and Edgecombe 2012, their Fig. 2B). Their apparent absence is traditionally interpreted as a loss correlated with a lateral expansion of the labral sidepieces (e.g. Bonato et al. 2014). The alternative interpretation that the paralabial sclerites may be fused with the labral sidepieces has never been taken into consideration, but may be favoured by the labral muscles in *D. carniolensis* not showing any marked lateral expansion but instead maintaining a paramedian origin and insertion (Fig. 4A), as in lithobiomorphs (Applegarth 1952, Rilling 1968) and scolopendromorphs (Manton 1964, Desbalmes 1992). This argument, however, is not necessarily conclusive because the clypeo-tentorial muscle (M.14) prevents a lateral expansion of the labral muscles. Since no muscles seem to attach at the paralabial sclerites in any pleurostigmophoran, neither of the two interpretations can unambiguously be favoured by the muscular equipment of the head. Another potential marker to recognise paralabial sclerites is their lateral articulation with the cephalic pleurite in lithobiomorphs and scolopendromorphs. We would expect this articulation to be preserved in placodesmatans were the paralabial sclerites fused with the labral sidepieces. The absence of an articulation between the lateral margins of the sclerite interpreted as a broadened labral sidepiece and the cephalic pleurites in Placodesmata instead supports the traditional interpretation that paralabial sclerites are entirely reduced in Placodesmata.

Adesmata

There is general agreement on Verhoeff's (1918) and Manton's (1965) view that the T-shaped configuration of the exoskeletal components in Adesmata is due to a lateral shift of the entire tentorial complex. The coincident transformation of the tentorial transverse bar and of the paralabial sclerites has, however, been contentious. Three hypotheses have been discussed thus far:

(i) The original articulation between the tentorium (i.e., its transverse bar) and the cephalic pleurite is maintained in adesmatans but has been transformed into a hinge for the gain of an additional articulation between the tentorium and the labral sidepieces; paralabial sclerites are exceptionally identified in adesmatan species such as *Himantarium gabrielis*, in which they are no longer articulated to the labral sidepieces but are anteriorly displaced into a 'non-functional' position between the cephalic pleurite and the clypeus (Verhoeff 1918).

(ii) The lateral shift of the tentorium correlates with a loss of both the tentorial transverse bar and the paralabial sclerites as a result of loss of any tentorial mobility (Manton 1965).

(iii) The transverse bar (and its articulation with the cephalic pleurite) is maintained in adesmatans, but it extends straight in line with the epipharyngeal bar, such that the original bifurcation point between these two bars (and their distinct identities) is no longer recognizable. Unified in this manner into a single oblique bar, it positionally replaces the paralabial sclerites, which are lost (Koch and Edgecombe 2012).

Based on light microscopical studies of whole-mounts, Bonato et al. (2014, their Table S2) followed Verhoeff's assumption in considering paralabial sclerites as "*recognizable in the Adesmata*" (coded as present in all sampled adesmatan species), in contrast to Koch and Edgecombe (2012) who could not confirm presence of distinct paralabial sclerites in the position indicated by Verhoeff (1918) in any of the species sampled by them, including *Himantarium gabrielis* (see Koch and Edgecombe 2012, their Fig. 2G). These contrasting views raise the question whether in whole-mounts paralabial sclerites can reliably be distinguished from parts of the tentorium (L. Bonato, pers. comm.). Since the paralabial sclerites in non-geophilomorphs are also articulated to the cephalic pleurites via a hinge-line (e.g., Manton 1965), the difficulty of unambiguously recognizing paralabial sclerites in adesmatan geophilomorphs may indeed be due to the possibility that

(iv) the paralabial sclerites are fused with the tentorium. Loss of the transverse bar would result in the articulation of the tentorium with the cephalic pleurite via its original articulation with the paralabial sclerites.

The cephalic musculature of *H. submarina* does not unambiguously allow a choice of any of the four hypotheses (i-iv) on the transformation of the tentorium in Adesmata. The original muscular equipment of the transverse bar in Pleurostigmophora is absent in this species, which may support the view that the transverse bar is absent as well. Absence of these muscles, however, remains inconclusive, because they are also absent in *D. carniolensis*, in which the transverse bar is unambiguously maintained. The tentorial exoskeleton itself in *H. submarina* still raises doubts that the tentorium is coalesced with the paralabial sclerite. This is because the sclerotized oblique bar passing from the cephalic pleurite towards the labral sidepiece proved to merely represent the sharp lateral margin of the tentorium, its external surface being mostly inclined into the depth of the preoral cavity (see Koch & Edgecombe, their Fig. 1F). If the oblique bar – which we consider to comprise both the former transverse and epipharyngeal bars – were to include the paralabial sclerite, one would expect it to form a broader surface onto the ventral head wall posterior to the clypeal sclerotization (i.e., outside the preoral cavity).

We accordingly propose that geophilomorphs overall lack any marker to unambiguously recognize paralabial sclerites. Our data favour a loss of paralabial sclerites in the geophilomorph stem species as a simpler assumption than assuming different transformations of these sclerites in Placodesmata and Adesmata. This view implies that the transverse bar and its articulation with the cephalic pleurite are (primarily) maintained in Adesmata.

Derivation of the tentorial muscles

The basic set of muscles that in non-geophilomorph Pleurostigmophora are thought to move the tentorium proved to be present in *D. carniolensis* and *H. submarina*. Among them, Manton (1965) only recorded the ventral longitudinal muscles for *Orya barbarica* (M.22 in *D. carniolensis* and *H. submarina*), as well as a large unlabeled muscle passing from the clypeus to the tentorium "*as in no other chilopod*" (p. 339). This latter muscle corresponds to tentorial muscle 14 in *D. carniolensis* and *H. submarina*. Judging from its origin and insertion this muscle can straightforwardly be homologized with tentorial muscles arising in a corresponding position from the clypeus in lithobiomorphs and scolopendromorphs. These comprise a set of muscles (T2-T6 fide Manton) whose insertions are distributed from the anterior tip of the epipharyngeal bar over the supramandibular arch to the lateral tip of the transverse bar. In geophilomorphs they seem to be either unified into a single muscle, or restricted to those muscles formerly inserting close to and on the supramandibular arch at the bifurcation point of the epipharyngeal and transverse bars (i.e., T4 and/or T5). The latter interpretation may be favoured due to the presence of several muscle bundles passing almost vertically from the clypeus to the dorsal epicranial wall in geophilomorphs (Figs 4A, 5A). For these muscle bundles no homologue seems to exist in non-geophilomorph centipedes; with respect to their origin we suspect that they might be derived from tentorial muscles formerly inserting on the epipharyngeal and/or transverse bars. Either transformation of the original set of clypeo-tentorial muscles implies an alignment and expansion of their insertion onto the supramandibular arch and its frontal process.

The set of tentorial muscles arising from the dorsal and dorso-lateral wall of the epicranium in lithobiomorphs and scolopendromorphs (T7-T10 fide Manton) is basically maintained in both *D. carniolensis* and *H. submarina*, except for muscle T8 (M.20 in this study), which seems to be lost in *H. submarina*. In both species tentorial muscle 15 deviates from its homologue (T7) in non-geophilomorphs in the shift of its origin towards the lateral epicranial wall, thus acquiring a transverse orientation. Tentorial muscles 9 and 18 deviate from their homologues in non-geophilomorphs (T9 and T10) in the shift of their origin towards a more posterior position, while keeping their distance relative to each other. Compared to *D. carniolensis*, their greater distance in *H. submarina* seems to correlate with the stronger longitudinal expansion of the tentorium in this species. The insertion of tentorial muscle 18 (T9) is more derived than in non-geophilomorphs in its expansion onto the mesial surface of the tentorial posterior process. In non-geophilomorphs the corresponding surface of the posterior process mainly serves for insertion of antennal muscles, the insertion of T9 being restricted to the dorsal edge of the posterior process. Its expansion onto the mesial surface in geophilomorphs correlates with the shift of the origin of the respective antennal muscles from the tentorium onto the clypeus.

The arrangement of the clypeo-tentorial, epicranio-tentorial, and ventral longitudinal muscles relative to each other in both *D. carniolensis* and *H. submarina* is comparable to a system of six degrees of freedom of movement – its three axes being indicated each by muscles 14 and 22, the set of muscles 9, 18, and 20, and muscle 15, respectively – and basically corresponds to the relative arrangement of their homologues in lithobiomorphs and scolopendromorphs. In applying Manton's (1964) and Desbalmes's (1992) functional interpretations of the homologous muscles in scolopendromorphs to *D. carniolensis* and *H. submarina*, one would consider muscles 14 and 22 as antagonists for tilting the tentorium forwards and backwards, respectively, whereas muscle 15 and the set of muscles 9, 18, and 20 would be antagonists that tilt the tentorium around its longitudinal axis.

A mobile tentorium was already inferred for Placodesmata by Koch and Edgecombe (2012), but their assumption of a shift of tentorial movements from a longitudinal to a transverse plane in this taxon can no longer be upheld. This assumption was based on consideration of only a few muscles of the mandibulo-tentorial complex, especially tentorial muscles 14 (Koch and Edgecombe 2012: t1) and 15 (t2). The entire set of tentorial muscles revealed in the present study instead questions basic differences in the mobility of the tentorium in Placodesmata and Scolopendromorpha. Adesmata as well does not seem to differ in the mobility of the tentorium, as *H. submarina* proved to largely correspond to *D. carniolensis* in the equipment of muscles that are traditionally considered as to primarily cause tentorial movements in other Pleurostigmophora. Koch and Edgecombe (2012) considered the tentorium of adesmatans as immobile because of its transformation correlated with its shift into a more lateral position, with immediate contact to the labrum. This shift and coincident T-shaped configuration of the exoskeletal components necessarily alter the freedom of movements of the tentorium. If this caused the tentorium of adesmatans to be immobile, one would expect the tentorial muscles to be reduced because they are no longer needed. Their presence, however, allows two alternative conclusions: either the tentorium of adesmatans primarily maintains some restricted mobility along a hinge determined by its oblique bar, as advocated by Verhoeff (1918); or the respective tentorial muscles have been misinterpreted as 'movers' of the tentorium – not only in Placodesmata, but in Pleurostigmophora as a whole.

Arguments in support of the former interpretation mainly relate to the strength of the muscles and their apparent arrangement as antagonists for each other. Alternatively, if the tentorial muscles are not used to move the tentorium, their main function may rather be to stabilize the tentorium in suspending it firmly at the cranial wall and to keep it in optimal position for its articulation with the mandibles. This new alternative interpretation gains support from current insights on the tentorium in Adesmata, movements of which have traditionally been denied. A case can further be made to question the view that the tentorium performs swinging movements in Pleurostigmophora as a whole. A considerable bulk of muscles originate from the tentorium: apart from extrinsic mandibular muscles these primarily also include antennal muscles, extrinsic muscles of the first and second maxillae, hypopharyngeal muscles, dilators of the foregut, and suspensory muscles of the collagenous transverse tendon system. These muscles drastically reduce the space available for elaborate movements of the tentorium and, because of their origin from the tentorium, its swinging movements would necessarily have an impact on movements of all these head structures. We further suspect that the original interconnection of the posterior processes of the tentorium by the collagenous transverse tendon system in Pleurostigmophora impedes independent movements of the left and right tentorium, and thus also independent movements of the left and right mandible, if swinging movements of the tentorium are really required for the mandibles. We do not deny that the tentorium is flexible to variable degrees in pleurostigmophorans. This flexibility, however, might not be caused by the need to actively contribute to mandibular movements. To allow the mandibles to act against the tentorium, the latter's flexibility may rather be attributed to a need to stabilize it.

Derivation of the mandibular muscles

Our current results for *D. carniolensis* and *H. submarina* basically confirm Manton's (1965) view that the mandibular muscles are simplified in geophilomorphs. Her homologies with mandible muscles in non-geophilomorph centipedes, however, are revised as follows.

Among the 14–16 mandibular muscles described for scolopendromorphs (Manton 1964, for *Cormocephalus nitidus*; Desbalmes 1992, for *Scolopendra cingulata* and *Theatops erythrocephalus*), nine homologues are identified in *D. carniolensis*, 10 in *H. submarina*. The most remarkable findings pertain to mandibular muscles 17 and 26.

Mandibular muscle 17 was considered by Manton (1965, her muscle 19) to be absent in geophilomorphs, in correlation with the absence of a suspensory sclerite that in Pleurostigmophora primarily connects the posterior tip of the mandibular base along the mandibular gnathal pouch with the epicranial wall. Our results confirm absence of both a suspensory sclerite and associated muscle only for *H. submarina*. Although the suspensory sclerite is also absent in *D. carniolensis*, its mandibular gnathal pouch is connected to a septum along which muscle 17 passes backwards to insert on the posteriormost parts of both gnathal pouch and the mandibular base (Fig. 4D). A corresponding muscle was depicted by Manton (1965) for *Orya barbarica*, but she homologized it with a muscle (25) that in scolopendromorphs differs both in origin and insertion. The respective muscle of scolopendromorphs is represented in a corresponding position in both *D. carniolensis* and *H. submarina* by mandibular muscle 5. Our results accordingly favour the view that a homologue to mandibular muscle 5 (25 fide Manton) is absent in *Orya barbarica* but instead showing a homologue to mandibular muscle 17 (19 fide Manton in Scolopendromorpha).

Mandibular muscle 26 is remarkable as it seems to represent a remnant of a muscle (32 fide Manton) that in other Pleurostigmophora primarily fills almost the entire concavity of the mandible and interconnects left and right mandible via the transverse mandibular tendon. Its presence in *H. submarina* (Fig. 5D) – not known for any other geophilomorph thus far – provides the first evidence that this muscle is primarily maintained in Geophilomorpha.

The remaining mandibular muscles vary to a lesser degree among geophilomorphs. The strongest adductor of the mandibular gnathal lobe (M.13 in this study; 20 fide Manton) is very much alike in geophilomorphs. As pointed out by Koch and Edgecombe (2012), it deviates from its homologue in lithobiomorphs and scolopendromorphs in its almost transverse orientation, based on the shift of its origin from the dorsal towards the lateral epicranial wall. Its insertion on a cuticular tendon of the mandibular gnathal lobe is more derived in *D. carniolensis* (Fig. 4C) than in adesmatans in the reduced size of this tendon and the coincident shift of the insertion of some bundles directly onto the wall of the gnathal lobe. The gnathal lobe of *D. carniolensis* is further derived in entirely lacking intrinsic muscles, whereas at least one of the original set in Pleurostigmophora is maintained in Adesmata (M.10 in *H. submarina*, Fig. 5B; 29 fide Manton in *Orya barbarica* and scolopendromorphs). Its absence in *D. carniolensis* may correlate with the enormous enlargement of a muscle (M.6 in this study; 27 fide Manton) that passes from the tentorium into the gnathal lobe. As pointed out by Manton (1965), this muscle is generally enlarged in geophilomorphs, but in adesmatans apparently to a lesser degree than in *D. carniolensis*. The same holds true for an intrinsic muscle (M.4 in this study; 31 fide Manton) that passes from the mandibular base into the gnathal lobe (Fig. 4B,C). Our results confirm that this muscle is relatively larger and longer in geophilomorphs than in scolopendromorphs, correlated with the shift of its origin to the posterior tip of the mandibular base. Again *D. carniolensis* seems to be more derived than adesmatans in the relatively larger size of this muscle and its strongly broadened insertion on the gnathal lobe.

A muscle of the gnathal lobe arising from the dorsal epicranial wall (M.21 in this study and fide Manton) was considered by Manton (1965) to be also enlarged in geophilomorphs, but in *H. submarina* this muscle is similarly slender as in scolopendromorphs, whereas in *D. carniolensis* this muscle may be lost. We suspect though that it rather shifted its insertion onto the mandibular base, because in this species a muscle arises from the epicranial wall (M.19 in this study) that attaches to the ventral margin of the mandibular base, a condition not known from any other pleurostigmophoran. Another muscle that inserts on the ventral margin of the mandibular base commonly arises from the tentorium in pleurostigmophorans and is also maintained in *D. carniolensis* and *H. submarina* (M.24 in this study; 30 fide Manton). Manton (1965) regarded this muscle as "*indistinguishable*" from longitudinal muscles in *Orya barbarica* (M.12 in this study; 22 and 23 fide Manton) passing from the tentorium to the posterior end of the mandibular base. This view is rejected because these muscles (M.12 and M.24 in this study) have clearly different origins and insertions in both *D. carniolensis* and *H. submarina*, as in scolopendromorphs. This rather supports the view that muscle 24 (30 fide Manton) is entirely reduced in *Orya barbarica*.

The longitudinal muscle (M.12) passing from the tentorium to the posterior end of the mandibular base is remarkably larger in *D. carniolensis* and *O. barbarica* than in *H. submarina*. This may support Manton's view that originally separate muscles (22, 23, and 33 fide Manton) are primarily unified in geophilomorphs but are partly reduced in *H. submarina*. The single muscle arising posteriorly from the dorsal epicranial wall to insert at the dorsal margin of the mandibular base (M.5 in this study) may likewise comprise two formerly separate muscles (24 and 25 fide Manton). These assumptions on unifications of muscles, however, remain uncertain, since in scolopendromorphs some of them seem to be variably reduced (according to Desbalmes 1992: 24 fide Manton in *Scolopendra* and *Theatops*, 33 fide Manton in *Theatops*) and may be convergently reduced in geophilomorphs. The only additional muscle attached to the dorsal margin of the mandibular base (M.3 in this study) seems to be enlarged in *D. carniolensis* and *H. submarina* relative to the state of its homologue (26 fide Manton) in *Orya barbarica* and scolopendromorphs.

Whether these transformations of the mandibular musculature in geophilomorphs alter the mandible mechanism to a degree that movements of the tentorium are dispensable remains unclear. This also considers Manton's (1965) view that the mandibles of geophilomorphs no longer perform see-saw movements. She inferred this mainly from the absence of the transverse mandibular tendon and of the strong mandibular muscle (32 fide Manton) primarily arising from it in Pleurostigmophora. This muscle was interpreted by Manton (1964: 90) to form the "*fulcrum of the see-saw mandibular movements*" in scolopendromorphs. The detection of remnants of it (M.26) in *H. submarina*, albeit devoid of the transverse mandibular tendon, raises doubts on whether abandonment of mandibular see-saw-movements can be generalized for geophilomorphs. Another argument against this view is the presence of a muscle (M.17 in *D. carniolensis*, 19 fide Manton) that Manton considered in scolopendromorphs as to be involved in the "recovery movement" of the mandible (Manton 1964: 91), while being absent in geophilomorphs. The differences in the muscular equipment in geophilomorphs overall seem to mainly correlate with the smaller size of the mandibular base that does not provide enough space for all the muscles present in scolopendromorphs. However, all main "functional groups" fide Manton (1964: 91) nevertheless still seem to be represented in geophilomorphs by at least one muscle, albeit in variably altered manners.

Summary and Perspective

Previous arguments for considering the tentorium of geophilomorphs to be immobile are revised as follows:

(1) Derivation of the tentorial skeleton. – Absence of the transverse bar of the tentorium – until now presumed to determine the axis of tentorial swing in Lithobiomorpha and Scolopendromorpha – is rejected for both Placodesmata and Adesmata. In the former, remnants of the collagenous tendon system proved to be preserved. The muscular system of the tentorium remains ambiguous with regards to the fate of the paralabial sclerites. They may be fused to either the labral sidepiece (Placo-

desmata), or to the transverse bar of the tentorium (Adesmata), but unambiguous evidence for their presence is lacking. Accordingly, paralabial sclerites may have been lost across Geophilomorpha as a whole.

(2) Derivation of the tentorial muscles. – Entire absence of the muscles that are presumed to effect swinging movements of the tentorium in Lithobiomorpha and Scolopendromorpha is rejected for Geophilomorpha. The respective set of muscles is simplified (either by unification or loss), but the main functional groups fide Manton (1965) are primarily preserved in both Placodesmata and Adesmata. The tentorium of geophilomorphs still serves as the origin for muscles of the mandibles, first and second maxillae as well as both the hypopharynx and pharynx, but no longer for antennal muscles.

(3) Derivation of the mandibular muscles. – A simplification of the mandibular musculature is basically confirmed for Geophilomorpha, but Manton's (1965) homologisations are partly refuted. The transformation of the mandibular musculature proved to be variable in Placodesmata and Adesmata but in neither instance can a participation of the tentorium in movements of the mandibles be excluded.

As such, our current insights on the morphology of the mandibulo-tentorial complex in geophilomorphs accordingly cause us to doubt that the mandible mechanism differs among pleurostigmophoran centipedes in terms of whether or not movements of the tentorium are required to abduct the mandibular gnathal lobe. With respect to the apparently immobile tentorium in Scutigeromorpha, the size of the mandible and its armature are inconclusive for this problem. The complexity of the muscles involved renders it difficult to unambiguously reveal their interplay and individual function. We therefore think that kinematic studies of living specimens are required to decisively elucidate the functional role of the tentorium during feeding. For this purpose, current advances in 4D in-vivo microtomography (see, e.g., Santos Rolo et al. 2014) seem to provide the most promising technique.

Acknowledgements

Histological sections for *Hydroschendyla submarina* were contributed by Liesa Rütjes (Univ. Bonn, Germany). Technical assistance with the digital imagery by Frank Friedrich (Univ. Hamburg, Germany) and Björn Quast (Univ. Bonn, Germany) is gratefully acknowledged. We furthermore thank Lucio Bonato (Univ. Padova, Italy) for discussion of his interpretation of geophilomorph head morphology and three referees for input.

References

Applegarth (1952) The anatomy of the cephalic region of a centipede *Pseudolithobius megaloporus* (Stuxberg) (Chilopoda). Microentomology 17: 127–171.

Attems CG (1926) Chilopoda. In: Kükenthal W, Krumbach T (Eds) Handbuch der Zoologie, Vol. 4. Walter De Gruyter, Berlin, 239–402.

Attems CG (1929) Myriapoda I. Geophilomorpha. In: Schultze FE, Kükenthal W (Eds) Das Tierreich, Vol. 52. Walter De Gruyter, Berlin, 388 pp.

Bonato L, Drago L, Murienne J (2014) Phylogeny of Geophilomorpha (Chilopoda) inferred from new morphological and molecular evidence. Cladistics 30: 485–507. doi: 10.1111/cla.12060

Desbalmes G (1992) Funktionsanatomie des Fressapparates der Chilopoda: Die Kopfregion von *Theatops erythrocephala* (C.L. Koch) sowie deren Kauapparat im funktionellen Vergleich mit *Scolopendra cingulata* und *Scutigera coleoptrata*. PhD thesis, University of Vienna, Vienna, Austria.

Edgecombe GD (2004) Morphological data, extant Myriapoda, and the myriapod stem-group. Contributions to Zoology 73: 207–252.

Edgecombe GD (2011) Phylogenetic relationships of Myriapoda. In: Minelli A (Ed) Treatise on Zoology – Anatomy, Taxonomy Biology. The Myriapoda. Volume 1. Brill, Leiden, Boston, 1–20. doi: 10.1163/9789004188266_002

Friedrich F, Beutel RG (2008) Micro-computer tomography and a renaissance of insect morphology. In: Stock R (Ed.) Proceedings of SPIE 7078, Developments in X-Ray Tomography VI: 70781U1-6. doi: 10.1117/12.794057

Koch M (2003) Monophyly of the Myriapoda? Reliability of current arguments. African Invertebrates 44:137–153.

Koch M, Edgecombe GD (2012) The preoral chamber in geophilomorph centipedes: comparative morphology, phylogeny, and the evolution of centipede feeding structures. Zoological Journal of the Linnean Society 165: 1–62. doi: 10.1111/j.1096-3642.2011.00803.x

Manton SM (1964) Mandibular mechanisms and the evolution of arthropods. Philosophical Transactions of the Royal Society of London Series B 247: 1–183. doi: 10.1098/rstb.1964.0001

Manton SM (1965) The evolution of arthopodan locomotory mechanisms, Part 8. Functional requirements and body design in Chilopoda, together with a comparative account of their skeleto-muscular systems and an Appendix on a comparison between burrowing forces of annelids and chilopods and its bearing upon the evolution of the arthropodan haemocoel. Journal of the Linnean Society (Zoology) 46: 252–483.

Rilling G (1968) *Lithobius forficatus*. Grosses Zoologisches Praktikum, Heft 13b. Gustav Fischer Verlag, Stuttgart, 136 pp.

Santos Rolo Td, Ershov A, van de Kamp T, Baumbach T (2014) In vivo X-ray cine-tomography for tracking morphological dynamics. Proceedings of the National Academy of Sciences of the United States of America 111(11): 3921–3926. doi: 10.1073/pnas.1308650111

Shear WA, Edgecombe GD (2010) The geological record and phylogeny of the Myriapoda. Arthropod Structure & Development 39: 174–190. doi: 10.1016/j.asd.2009.11.002

Verhoeff KW (1918) Vergleichende Morphologie und Phylogenie. In: Bronn HG (Ed), Klassen und Ordnungen des Tierreichs, Band 5, Abteilung 2, Gliederfüssler: Arthropoda, Klasse Chilopoda. Lieferungen 63-101, 1902–1925. Akademische Verlagsgesellschaft, Leipzig, 395–538.

Supplementary material 1

Digital image stack used for 3D-reconstruction of the mandibulo-tentorial complex of *Dicellophilus carniolensis*

Authors: Markus Koch, Johannes Schulz, Gregory D. Edgecombe

Data type: QuickTime mov file

Explanation note: Series of transverse histological sections from anterior to posterior, provided as movie.

Supplementary material 2

Digital image stack used for 3D-reconstruction of the mandibulo-tentorial complex of *Hydroschendyla submarina*

Authors: Markus Koch, Johannes Schulz, Gregory D. Edgecombe

Data type: QuickTime mov file

Explanation note: Series of transverse histological sections from anterior to posterior, provided as movie.

ZooKeys 510: 269–278 (2015)
doi: 10.3897/zookeys.510.8674
http://zookeys.pensoft.net

RESEARCH ARTICLE

A peer-reviewed open-access journal
ZooKeys
Launched to accelerate biodiversity research

On the function of the ultimate legs of some Scolopendridae (Chilopoda, Scolopendromorpha)

Christian Kronmüller[1], John G. E. Lewis[2]

1 *Bavarian State Collection of Zoology, Münchhausenstr. 21, D-81247 Munich* **2** *Manor Mill Farm, Halse, Taunton, Somerset TA4 3AQ, UK*

Corresponding author: *Christian Kronmüller* (ck@scolopendromorpha.com)

Academic editor: *Ivan H. Tuf* | Received 30 September 2014 | Accepted 18 June 2015 | Published 30 June 2015

http://zoobank.org/63EEB826-FC41-44D4-AF30-C9C55B16B15B

Citation: Kronmüller C, Lewis JGE (2015) On the function of the ultimate legs of some Scolopendridae (Chilopoda, Scolopendromorpha). In: Tuf IH, Tajovský K (Eds) Proceedings of the 16th International Congress of Myriapodology, Olomouc, Czech Republic. ZooKeys 510: 269–278. doi: 10.3897/zookeys.510.8674

Abstract

The function of the variously shaped ultimate legs of Scolopendridae is briefly reviewed. Their function in *Scolopendra heros* Girard, 1853, *Scolopendra subspinipes* Leach, 1815, *Scolopendra morsitans* (Linnaeus, 1758), *Scolopendra galapagoensis* Bollman, 1889, *Scolopendra hainanum* Kronmüller, 2012, *Scolopendra spinosissima* Kraepelin, 1903 *Cormocephalus aurantiipes* (Newport, 1844) and *Ethmostigmus trigonopodus* (Leach, 1817), in which they are least specialised has been investigated. Specimens were tapped with forceps on different parts of the trunk to simulate the attack of a predator. When tapped on the first third of the trunk (near the head), the centipedes attacked the forceps with their forcipules. When tapped on the last third or the ultimate legs, they adopted a warning position, raising the ultimate legs to display the ventral and medial prefemoral spines as well as the spined coxopleural processes. In some cases the centipedes attacked the forceps with the claws of the ultimate legs by chopping down on them after lifting the legs high into the warning position. When tapped in the mid part of the trunk, the centipedes curled sideways to reach the forceps with their forcipules and ultimate legs simultaneously. *Scolopendra galapagoensis* not only lifted the ultimate legs into the warning position but also the last 3-4 pairs of locomotory legs, presenting their distodorsal prefemoral spines. This resembles the warning posture of some spiders. In addition to their function in warning behaviour, defensive stabbing, ritualised meeting reactions and during courtship behaviour, the ultimate legs may in addition act as hooks and perhaps be involved in species recognition. No evidence was found that the ultimate legs are used to catch prey, nor of prey or predators being held between the prefemora.

Keywords

Chilopoda, Scolopendromorpha, ultimate legs, prey capture, defence reaction, courtship behaviour, mating

Introduction

The ultimate legs of scolopendrids exhibit a variety of shapes, the majority being what Schileyko (2009) called the common shape, the least specialised and most like locomotory legs (Figure 1A). They are pincer-shaped in *Scolopendropsis*, *Edentistoma*, and *Arthrorhabdus* and this type Schileyko stated are adapted for capturing prey (Figure 1B). The African scolopendrid *Asanada socotrana* Pocock, 1899, also has pincer-like legs. These lack prefemoral spines and are probably involved not in the capture of prey but in the distraction of a would-be predator as they are readily autotomised and when detached perform wriggling movements (Lewis, 1981). Species of the genus *Alipes* have large leaf-like ultimate legs (Figure 1C). *Alipes grandidieri* Lucas, 1864 when irritated swings these from side to side and stridulates perhaps to frighten potential mammalian predators (Skovmand and Enghoff 1980). The legs are sometimes autotomised and when this happens the legs continue to stridulate. Cloudsley-Thompson (1961) reported that the long ultimate legs of the West African *Rhysida nuda togoensis* Kraepelin, 1903 (now *R. immarginata togoensis*) (Figure 1D) slowly bend and straighten when detached and emit a faint creaking sound.

In this paper we review the functions of ultimate legs in those species that have the least modified ultimate legs and report the results of laboratory experiments on species of *Scolopendra*, *Cormocephalus* and *Ethmostigmus*.

Review of the function of the ultimate legs in the Scolopendridae

Anchoring

Field observations have shown that the ultimate legs of scolopendrids are used as hooks from which the animal can hang. Thus Remington (1950) reported that *S. subspinipes* Leach, 1815, fastened onto a tent near the ventilator hole with their ultimate legs and swung their bodies to one side or the other to seize insects that had alighted nearby. On 4.xi.95 Peter Daszak and Janet Cottingham found a female *Scolopendra abnormis* Lewis & Daszak, 1996, hanging by her ultimate legs in a hollowed out cavity beneath a slab of tuff on Serpent Island, Mauritius. The anterior part of the body was curled around 40 oval yellow eggs (Lewis unpublished data).

Molinari et al (2005) observed two specimens of *Scolopendra gigantea* L., 1758, feeding on bats whilst hanging from the roof of a Venezuelan cave to which they anchored using the last five or eight pairs of legs.

Kronmüller (2013, unpublished data) reported and photographed a *Scolopendra dehaani* that escaped the plastic container in the laboratory and was later found hanging on the camera tripod just using the ultimate legs (Figure 3A).

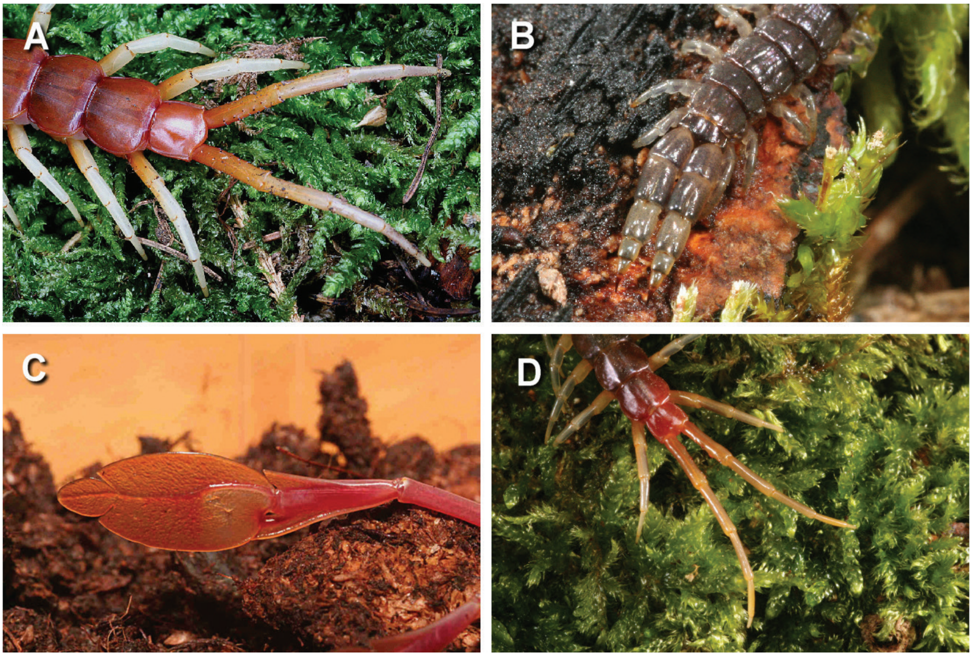


Figure 1. Different shape of ultimate legs: **A** The least specialised ultimate legs of *Scolopendra gigantea* **B** pincer-like ultimate legs in *Asanada akashii* **C** leaf-like ultimate legs of *Alipes grandidieri* **D** the long and slender ultimate legs of *Rhysida longipes* that can be autotomised.

Defence reactions

Warning posture

Lewis (unpublished data) noted in Nigeria that a small *Scolopendra morsitans* L., 1758, raised and splayed its ultimate legs when approached from behind by a large *Mecistocephalus* (Geophilomorpha). Neck (1985) described such threat display in *Scolopendra viridis* Gervais, 1847, the ultimate legs being spread apart and in *Scolopendra heros* Girard, 1853, where the legs were raised and waved back and forth, active defence involving biting or "holding" with the ultimate legs. A photo of an *Ethmostigmus* sp. sent to one of us (J G E L) by Chris Pennington from Krambach, New South Wales, Australia, showed the ultimate legs lifted and splayed revealing the spines on the median surface of the prefemora and on the coxopleural processes. A predator approaching from behind would come into contact with a battery of spines.

Autotomy and sound production

As noted above, in case of danger the members of the genus *Alipes* swing the ultimate legs from side to side and stridulate perhaps to distract or frighten potential predators..

They also use autotomy to detract potential predators like birds, lizards or mammals. In case of an attack, the centipede starts to stridulate which directs the attention of the predator to the ultimate legs which can be autotomised to make the escape easier. When detached with forceps the leaf-like ultimate legs of an *Alipes grandidieri* from Tanzania continued to stridulate for more than half a minute.

Cloudsley-Thompson's (1961) observed that the long ultimate legs of the West African *Rhysida nuda togoensis* (now *R. immarginata togoensis*) slowly bend and straighten when detached and emit a faint creaking sound. This observations were repeated in Northern Nigeria in 1967 by Lewis (unpublished data): an ultimate leg of the same species detached with forceps produced very quiet squeaking for about 75 seconds, flexing at 2–3 second intervals and Kronmüller (2009, unpublished data) observed that the long slender ultimate legs of *Rhysida immarginata immarginata* (Porat, 1876) from the Philippines (Figure 1D) as well as *Rhysida longipes* (Newport, 1845) from various countries in Africa bend and straighten for a few minutes presumably to enable the centipede to flee while potential predators become distracted. In our experiments, when the ultimate legs were detached with forceps,they did not squeak as did those of *Rhysida immarginata togoensis*..

Video clips of *Alipes* sp. showing the typical defence behaviour including the sound of the stridulation as well as the bending movements of the detached ultimate legs of *Rhysida* sp. can be seen on the website http://www.scolopendromorpha.com.

Intraspecific reactions

Meeting reactions

Ritualised meeting reactions have been described for *Scolopendra cingulata* Latreille, 1829 (Klingel 1960), *Cormocephalus anceps anceps* Porat, 1871 (Brunhuber 1969) and observed in *Scolopendra galapagoensis* Bollman, 1899 (Kronmüller 2010, unpublished data). When two specimens meet, each attempts to grasp the posterior region of the trunk of the other with its last pair of legs (Figure 2C).

In head to head contact both rapidly swing round the posterior part of body and attempts to grip the other with the ultimate, followed by the more posterior legs. Brunhuber's plate 1A shows that the legs are parted and hooked over the body of the other animal. In this reaction the median spines on the ultimate legs seem to aid the grip. Kronmüller (unpublished data) observed that when two *Scolopendra dehaani* meet and they grasp each other in the previous described way, they hold this position for several minutes and up to more than half an hour. This ritual may be to avoid any aggressive behaviour.

A short video clip of this meeting reaction can also be seen on the website http://www.scolopendromorpha.com.

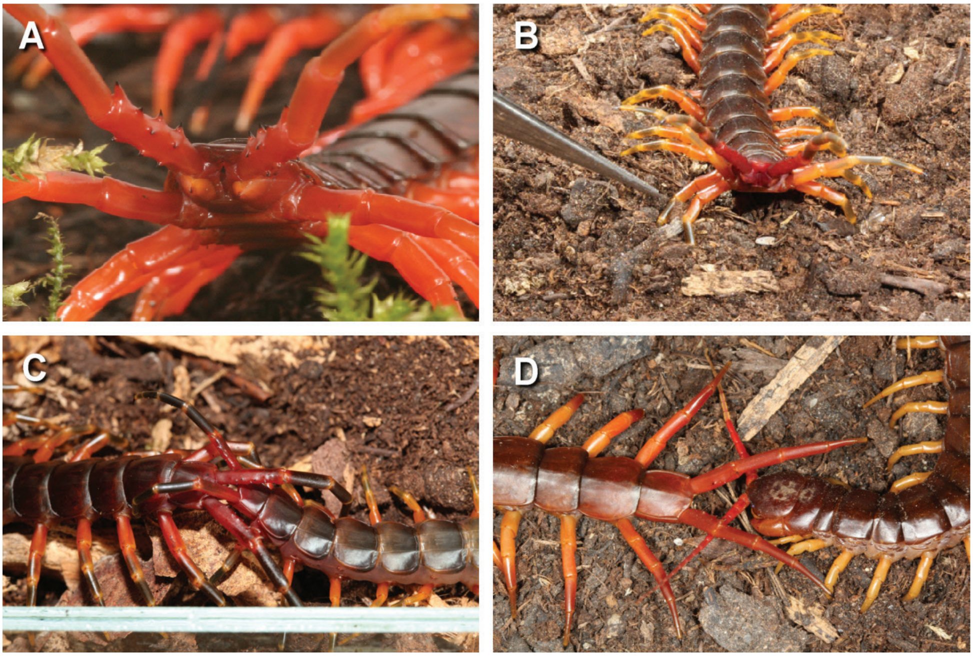


Figure 2. A Warning posture of *Scolopendra spinosissima* **B** Warning posture with lifted ultimate legs as well as the last pairs of locomotory legs in *Scolopendra galapagoensis* **C** Interlocked ultimate legs in a pair of *Scolopendra galapagoensis* just before mating **D** antennae tapping under the lifted ultimate legs in *Scolopendra dehaani* in courtship behaviour.

Courtship behaviour

During courtship behaviour in *Cormocephalus anceps anceps* the "defence posture" is adopted both partners use their antennae to tap the posterior part of the body especially the ultimate legs of the opposite sex (Figure 2D). Tapping and/or stroking with the ultimate legs is carried out by both sexes. The ultimate legs are waved sideways and tapped irregularly up and down and quite quickly (Brunhuber 1969) [for a recent review see Rosenberg (2009)].

Species recognition

Lewis (1985) argued that as the prefemoral spines on the ultimate legs are directed backwards (posteriorly) it is unlikely that they are used to facilitate gripping for which they should be directed forwards (anteriorly). He suggested that the function of the spines was that of specific discrimination during courtship prior to sperm transfer. If this were the case then one would not expect sympatric species to have a similar spine arrangement. There followed a lengthy discussion to justify this hypothesis. However no evidence has been found in this investigation to support this suggestion.

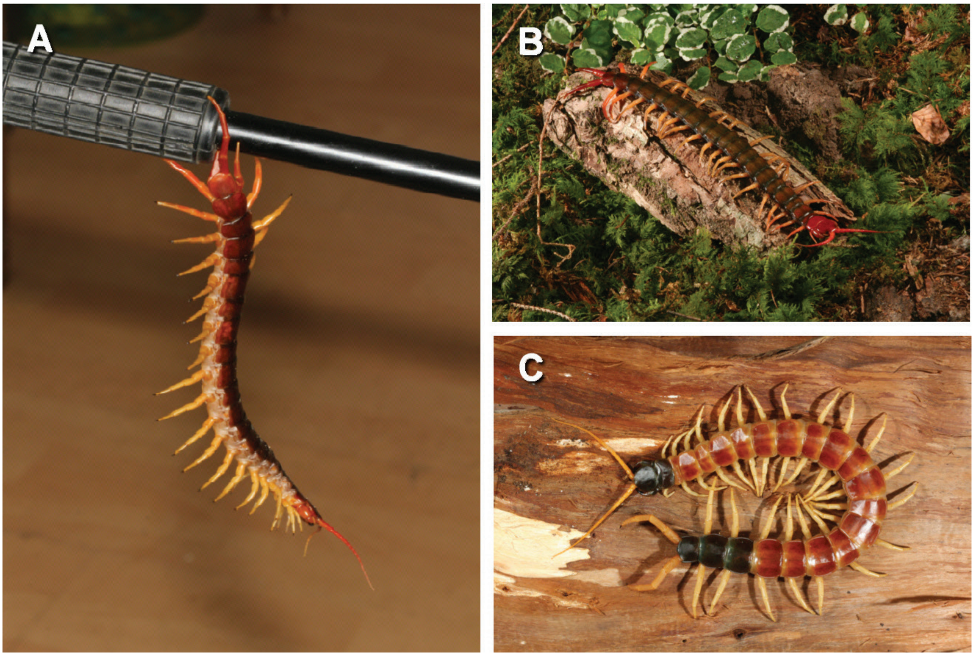


Figure 3. A *Scolopendra dehaani* hanging from a camera tripod just using the ultimate legs **B** *Scolopendra multidens* and **C** *Scolopendra heros* showing possible auto mimicry as the ultimate legs and last segments of the trunk mimic the head and antennae.

Laboratory experiments

Material and methods

Laboratory experiments were carried out to further investigate behaviour in prey capture as well as possible defence reactions in case of a predator attack. To simulate a the attack of mammal or bird predator, a piece of foam held with 25cm forceps was used to tap the centipedes from above.

The centipedes that were used for the experiments were either adult wild caught specimens (*Scolopendra heros*, *Scolopendra subspinipes*, *Ethmostigmus trigonopodus*, *Scolopendra morsitans*, *Scolopendra hainanum*, *Cormocephalus aurantiipes*) or, in one case, an adult captive bred specimen (*Scolopendra galapagoensis*).

The specimens were kept for at least 12 weeks before the experiments started in plastic containers of different sizes depending on the size of the specimen, with an air temperature of 25 degrees Celsius in day-time (around 10 hours illumination with a neon lamp) and 20–22 degrees Celsius at night-time. The average air humidity was around 70%. All plastic containers had a layer of humus and a piece of bark as a hiding-place. Once a week, the humus was sprayed with water to keep it moist and the specimen was fed one adult cricket (*Gryllus assimilis*).

For the experiments, the centipedes were moved to a 20 × 20 cm plastic container with a layer of about 2cm humus and left for at least one hour without disturbance before the experiments started. After each experiment, the centipedes were left for a further hour to recover before the next experiment.

The first experiment involved tapping the centipedes at different parts along the length of the body (anterior third, middle third and posterior third) to observe possible differences in defense reactions and to compare the responses of the investigated species.

In a second experiment a piece of bark was placed in the container as a refuge and the layer of humus was increased to 10cm. A centipede was placed in the container and as soon as the first third of the trunk was hidden either by the centipede crawling under the bark or by it digging into the humus, the tapping experiments described above were repeated.

Photos and short video clips are made with a Canon EOS 60D and a Canon EOS 6D, connected either to a Canon standard lens (18-55mm) or a Canon macro lens (100mm), three Canon Speedlight EX550/EX430 flashes (photos only), a tripod and a remote control (video clips).

Results of the laboratory experiments

Initial experiments were carried out on an adult *Ethmostigmus trigonopodus* (Leach, 1817) from Tanzania, length 13 cm. When tapped on the first third of the trunk (near the head), the centipede attacked the forceps with the forcipules. When tapped on the posterior third, or even at the ultimate legs, it adopted the warning position, raising the ultimate legs to display the ventral and medial prefemoral spines as well as the coxopleural processes which are also equipped with spines. In the most cases the centipede directed the warning position towards the forceps by turning the posterior segments. In some cases, depending on the strength and speed of the simulated attack, the centipede counter-attacked the forceps with the claws of the ultimate legs by chopping down on them after lifting them high into the warning position.

When the *E. trigonopodus* was tapped in the mid part of the trunk, the centipede curled sideways to reach the forceps with the forcipules and the ultimate legs simultaneously. These observations were repeated in experiments with *Scolopendra heros* var. *arizonensis* Kraepelin, 1903 (USA), *Scolopendra galapagoensis* (Peru), *Scolopendra morsitans* (Pakistan), *Scolopendra cingulata* (Spain), *Scolopendra subspinipes* (Indonesia), *Scolopendra hainanum* Kronmüller, 2012 (China), *Scolopendra spinosissima* Kraepelin, 1903 (Philippines), and *Cormocephalus aurantiipes* (Newport, 1844) (Australia). All showed the same warning posture raising the ultimate legs (Figure 2A).

In addition *Scolopendra galapagoensis* lifted not only the ultimate legs into the warning position but also the last 3-4 pairs of locomotory legs (Figure 2B). This may be to present the distodorsal prefemoral spines on these legs. This behaviour was not seen in *S. heros* var. *arizonensis*, the other New World species investigated which lacks these spines.

In the experiments with bark and a thicker layer of humus, no difference was observed in the behaviour depending on whether the specimen was tapped on the mid part of the trunk or on the last third. The result was the same when the centipede was fed (*Schistocerca* sp.) and the forcipules as well as the anterior pairs of locomotory legs were involved in feeding. In this situation the centipede could not use the forcipules to carry out a warning bite and the only defence behaviour was hitting with the ultimate legs and stabbing with the ultimate leg pretarsi (claws). The experiment was repeated with *Ethmostigmus trigonopodus*, *Scolopendra galapagoensis*, *Cormocephalus aurantiipes* and *Scolopendra subspinipes*, which all showed the same results.

Discussion

The scolopendrid species investigated here have unmodified and relatively robust ultimate legs. Species with very long thin ultimate legs such as some *Otostigmus* (*Otostigmus*) and *Rhysida* species, have not been investigated.

Bücherl (1971) stated that "The specimens of *Scolopendra* and *Otostigmus*, *Cryptops* and others attack their prey with the last prehensorial anal legs, then the head is rapidly curved behind and the venom claws deeply and firmly buried in the body of the prey." Lewis (2010) concluded that there was no evidence to support the suggestion that the ultimate legs of *Cryptops* were involved in the capture of prey, instead suggesting that they are defensive; trapping some part of a potential predator and then being autotomised as the centipede makes good its escape. Dugon and Arthur (2012) stated that several species of scolopendrids had been observed seizing prey with the terminal pair of legs and then flicking the anterior half of the body to inject the prey with venom but in experiments with *Scolopendra subspinipes mutilans* Koch, 1878 (now *S. subspinipes* Leach, 1815) prey was only attacked with the forcipules.

We conclude that in the case of the species investigated the use of the ultimate legs is primarily for defence, however, should potential prey make contact with the centipede, the defensive response and defensive biting will lead to feeding whereas in the case of a predator, the centipede will try to escape after one or more warning bites.

Rowland Shelley (email dated 03 January 2011) notes "that there are frequently spines on the medial surface of the scolopendrid (ultimate leg) prefemora and I've always presumed that these must function to hold something steady I had always thought this would be prey ... " We were unable to confirm that scolopendrid centipedes hold prey or predators between the ultimate leg prefemoral, although in the ritualised meeting reaction in some species, the median spines on the ultimate legs seem to aid the grip.

The four main functions in species in which the ultimate legs are little specialised are:

1) Acting as hooks to suspend the animal for example from cave ceilings.
2) Warning position, when the legs are raised and splayed to reveal the prefemoral and coxopleural spines.
3) Stabbing as a defence, especially when the front part of the trunk and the head are unable to carry out any defensive action.

4) Grasping, the legs being flexed as when two animals assume the defence posture or as part of the mating ritual. In this case posteriorly directed spines would aid the grip. No evidence was found of objects being held between the prefemora.

The investigated centipedes (*S. heros*, *S. subspinipes*, *S. morsitans*, *S. hainanum*, *S. galapagoensis*, *S. spinosissima*, *C. aurantiipes*, *E. trigonopodus*) are from different genera, different continents and different types of habitat. Nevertheless, their behaviour in prey capture and defense reaction do not differ. Also the meeting reaction seems to be very similar the same in all observed species.

In addition to the behavioural functions of the ultimate legs, two visual signals should be considered:

Auto mimicry

Some scolopendrid centipedes, especially the giant *Scolopendra* sp. have striking colour patterns. In several species, the colouration of the last trunk segments and the ultimate legs is the same as the colouration of the head and antennae (Figure 3 B and C). Predators may not be able to differentiate between the head and the posterior part of the trunk.

Possible signal co-evolution

The warning posture, especially that of *Scolopendra galapagoensis*, where the last three or four pairs of locomotory legs are raised in addition to the ultimate pair, resembles that of spiders such as *Phoneutria* sp. or tarantulas and may be a case of possible signal co-evolution.

References

Brunhuber BS (1969) The mode of sperm transfer in the scolopendromorph centipede *Cormocephalus anceps anceps* Porat. Zoological Journal of the Linnean Society 48: 409–420. doi: 10.1111/j.1096-3642.1969.tb00720.x

Bücherl W (1971) Venomous chilopods or centipedes. In: Bücherl W, Buckley EE (Eds) Venomous animals and their venoms 3. Academic Press, New York, 169–196. doi: 10.1016/B978-0-12-138903-1.50014-4

Cloudsley-Thompson JL(1961) A new sound-producing mechanism in centipedes. Entomologists Monthly Magazine 96: 110–113.

Dugon MM, Arthur W (2012) Prey orientation and the role of venom availability in the predatory behaviour of the centipede *Scolopendra subspinipes mutilans* (Arthropoda: Chilopoda). Journal of Insect Physiology 58: 874–880. doi: 10.1016/j.jinsphys.2012.03.014

Klingel H (1960) Vergleichende Verhaltensbiologie der Chilopoden *Scutigera coleoptrata* L. (Spinnenassel') und *Scolopendra cingulata* Latreille (Skolopender). Zeitschrift für Tierpsychologie 17: 11–30. doi: 10.1111/j.1439-0310.1960.tb00191.x

Lewis JGE (1981) The biology of centipedes. Cambridge University Press, Cambridge, 476 pp. doi: 10.1017/CBO9780511565649

Lewis JGE (1985) Possible species isolation mechanisms in some scolopendrid centipedes (Chilopoda; Scolopendridae). Bijdragen tot de Dierkunde 55: 125–130.

Lewis JGE (2010) On the function of the ultimate legs in *Cryptops* and *Theatops* (Chilopoda: Scolopendromorpha). International Journal of Myriapodology 3: 145–151. doi: 10.1163/187525410X12578602960542

Molinari J, Gutiérrez EE, De Ascenção AA, Nasar JM, Arends A, Márquez RJ (2005) Predation by giant centipedes, *Scolopendra gigantea* on three species of bats in a Venezuelan cave. Caribbean Journal of Science. 41: 340–346.

Neck RW (1985) Comparative behavior and external color patterns of two sympatric centipedes (Chilopoda: *Scolopendra*) from Central Texas. The Texas Journal of Science 37: 253–255.

Remington CL (1950) The bite and habits of the giant centipede (*Scolopendra subspinipes*) in the Philippine Islands. American Journal of Tropical Medicine 30: 453–435.

Rosenberg J (2009) Die Hundertfüsser. Die Neue Brehm-Bücherei 285. Westarp Wissenschaften, Hohenwarsleben, 524 pp.

Scovmand O, Enghoff H (1980) Stridulation in *Alipes grandidieri* (Lucas) a scolopendromorph centipede. Videnskabelige Meddelelser fra Dansk naturhorisk Forening 142: 151–160.

Schileyko AA (2009) *Ectonocryptopoides sandrops* – a new scolopendromorph centipede from Belize. Soil Organisms 81: 519–530.