Zootaxa 4178 (2): 234–256
http://www.mapress.com/j/zt/

Article

ISSN 1175-5326 (print edition)
ZOOTAXA
ISSN 1175-5334 (online edition)

http://doi.org/10.11646/zootaxa.4178.2.4
http://zoobank.org/urn:lsid:zoobank.org:pub:466B4B88-F8C2-4321-80F8-59E38F8EBAE9

Hungarosoma bokori Verhoeff, 1928 (Diplopoda: Chordeumatida): new insights into its taxonomy, systematics, molecular genetics, biogeography and ecology

ANDREJ MOCK[1,8], KAREL TAJOVSKÝ[2], MARTINA ŽUROVCOVÁ[3], ANDREA JAROŠOVÁ[3],
PAVEL KOCOUREK[4], JÜRGEN GRUBER[5], DOROTTYA ANGYAL[6] & JÖRG SPELDA[7]

[1]*Pavol Jozef Šafárik University, Faculty of Sciences, Institute of Biology and Ecology, Moyzesova 11, SK-041 67 Košice, Slovakia. E-mail: andrej.mock@upjs.sk*
[2]*Institute of Soil Biology, Biology Centre, Czech Academy of Sciences, Na Sádkách 7, 37005 CZ-České Budějovice, Czech Republic. E-mail: tajov@upb.cas.cz*
[3]*Institute of Entomology, Biology Centre, Czech Academy of Sciences, Branišovská 1160/31, 37005 České Budějovice, Czech Republic. E-mail: martina@entu.cas.cz*
[4]*Chyňava 27, 267 07 Chyňava, Czech Republic. E-mail: kocourek.pavel@post.cz*
[5]*Naturhistorisches Museum Wien, Burgring 7, 1010 Vienna, Austria. E-mail: juergen.gruber@nhm-wien.ac.at*
[6]*Hungarian Natural History Museum, Baross utca 13, 1088 Budapest, Hungary. E-mail: angyal.dorottya@zoo.nhmus.hu*
[7]*Bavarian State Collection of Zoology, Münchhausenstrasse 21, 812 47 Munich, Germany. E-mail: spelda@zsm.mwn.de*
[8]*Corresponding author*

Abstract

Hungarosoma bokori Verhoeff, 1928 is a millipede species which was originally classified solely on the basis of a female specimen. Subsequently, a long history of field searching for and surmising about the systematic position of this small, enigmatic species followed. In April 2013, 85 years after its first description, a series of nine specimens were sampled in the type locality, the Abaliget Cave, in southern Hungary. An adult male was collected for the first time, along with females and juveniles. Descriptions of the gonopods and the female vulvae, both important for considerations of the systematic position of the species, are presented for the first time. Revision and re-designation of the type material was made. The cryptic life of the species is connected with its activity in winter, and its known fragmented distribution corresponds with its presence in undisturbed microhabitats having a specific microclimate, often in the soil at cave entrances.

Molecular methods showed a positive detection of the intracellular prokaryotic parasite *Wolbachia* in *H. bokori*, reflecting its highly probable parthenogenetic character in the main part of its known area of occurrence. This is the first demonstration of *Wolbachia* in a millipede.

The legitimacy of the family Hungarosomatidae Ceuca, 1974, as a separate taxon was analysed using morphological and molecular approaches. Results of both methods confirmed the existence of a distinct phyletic line. DNA barcoding has shown its closest position to Attemsiidae Verhoeff, 1899, or Neoatractosomatidae Verhoeff, 1901. Based on records from Austria, the Czech Republic, Hungary and Slovakia, the residual circum-pannonic distribution that the whole genus (family) probably represents is proposed.

Key words: Hungarosomatidae, redescription, gonopods, distribution, cytochrome oxidase 1 gene, DNA barcoding, *Wolbachia*

Introduction and history of recognition of the taxon

Hungarosoma bokori Verhoeff, 1928, has been a great rebus for myriapodologists of several generations. The male of the species remained unknown, and there was no consensus on the family affiliation of the species, its recent area of distribution, history, questionable parthenogenesis as well as its ecology.

Korsós (2000) briefly mentioned the interesting and enigmatic history of the species' discovery, but also the loss of the specimens of the type species, *Hungarosoma bokori* Verhoeff, 1928. The first female of this small chordeumatid millipede was recorded in the Abaliget Cave (Abaligeti-barlang) in the karst area of the Mecsek Mts. in south-western Hungary by the famous entomologist and military officer, Elemér Bokor, in 1924 (12.viii.). The

Accepted by T. Wesener: 27 Aug. 2016; published: 25 Oct. 2016

material was sent to Karl W. Verhoeff, who recognised it as a new taxon and established the new genus and new species (Verhoeff 1928) using a quite detailed description of its external morphology. He mentioned the extruded vulvae and the presence of eggs.

The most important characteristic of *H. bokori* observed by Verhoeff was the shape of the pleurotergites, which have short but wide and easily distinguished lateral processes, with an anterior edge limited by a deep notch. Verhoeff (1928) also made a sufficient drawing of these characteristics. Other important features were the minute grey-white body (ca 5 mm long), 28 body segments and 11 ocelli on both sides of the head ordered as 4-3-2-1-1. The female had well manifested vulvae. A short comment on the morphology of the vulvae was published later (Verhoeff 1932), where the author compared the general anatomy of vulvae between the genera *Macrochaeteuma* Verhoeff, 1914 and *Hungarosoma*. A marked process observed in the vulvae of *Macrochaeteuma* was absent in *H. bokori*. The original description (Verhoeff 1928) was reprinted in the monograph on the Abaliget Cave fauna published by Gebhardt (1934), sometimes wrongly cited as Verhoeff's paper from the year 1934. A specimen deposited in the Natural History Museum (Museum für Naturkunde) in Berlin, Germany (Moritz & Fischer 1978), was stated as being the holotype of *H. bokori*. Another four specimens of probable type value exist in the collection of the Bavarian State Collection of Zoology (Zoologische Staatssammlung München) in Munich, Germany. It is highly probable that they were sampled by Antal Gebhardt, a leading expert on the fauna of the Abaliget Cave.

Verhoeff (1928) also added considerable notes on the systematic position and zoogeography, though prematurely due to the absence of a male specimen, as he himself pointed out. He recognised that *H. bokori* is close to some Brachychaeteumatidae (formerly Brachychaeteumidae), especially to the monotypic genus *Macrochaeteuma* Verhoeff, 1914 (Macrochaeteumatidae), described from Japan. In his monograph (Verhoeff 1932) he repeated his opinion on the relationship of both taxa and united them in the Macrochaeteuminae (now Macrochaeteumatinae), a subfamily of the family Brachychaeteumatidae.

More comments on the occurrence of *H. bokori* in the Abaliget Cave are available in several articles and monographs published by Gebhardt (1933, 1934, 1963, 1967). He reported more specimens collected at the type locality (Gebhardt 1934), mentioning several records of females and larvae (without a number and date), all collected near the siphon at the distant end of the cave, some of them on bait (rotten organic material and mosses). In the following papers (Gebhardt 1963, 1967) he repeated his own older data and concluded that the species was not found in any other caves in the Mecsek Mts. except the Abaliget Cave, where all samples were recorded at the same site. Angyal & Korsós (2013) recently confirmed the absence of this species in the other caves of the Mecsek Mts. Surprisingly, Loksa (1961) found *H. bokori* in another region of western Hungary, far north in the Kovácsi Mts., in a lime-ash forest on basalt bedrock near the village of Vindornyaszőlős, north-west of the town of Keszthely on the south-western edge of Lake Balaton. He also found only females, but they were sampled on the surface, on basalt bedrock in a valley with a climatic inversion. These records brought a new light on the distribution and ecology of *H. bokori*. However, the material collected by Loksa has probably been lost (Korsós 1994). Later, Korsós (2000) added a short description of a newly recorded single juvenile specimen of *H. bokori* that he himself sampled at the type locality: "Although it is a juvenile, its characteristics fit well the description by Verhoeff, i.e. a depigmented body, long macrosetae and the shape of the segment. The completely white body is ornamented only by three distinctly black ocelli on each side of the head. It has 21 body segments (+head and anal ring), length ca 5 mm". Korsós (2000) also added a short note concerning its ecology. The specimen was found under woody debris in the Big Hall, the part most distant from the entrance of the cave. He classified this species, according to Dudich (1932), as a hemitroglobiont. Papáč *et al.* (2014) recently reported the occurrence of females of *Hungarosoma* cf. *bokori* in the Baradla-Domica Cave System, south-western Slovakia (Western Carpathians), hundreds of kilometres from the type locality.

The genus *Hungarosoma* was monotypic until the description of *Hungarosoma inexpectatum* Ceuca, 1967. Two females of this new species were collected in the soil of the garden at the Zoological Institute of Cluj, Romania. Ceuca (1967) distinguished three different characteristics between *H. inexpectatum* and *H. bokori*: body colouration (coffee-clear versus grey-white), numbers of ocelli (9 versus 11) and the line between the prozonite and metazonite (a curve versus a straight-line). The shape of the lateral processes of the pleurotergites, a characteristic feature for the genus *Hungarosoma*, was identical in both species. The original description of *H. inexpectatum* was made again on the female features, but 6 years later, Ceuca (1974) found a single male (ratio of male:females = 1:79) at type locality and published a re-description based on the male gonopods. *H. inexpectatum* was never found again, and its habitat preference and natural distribution is therefore unknown.

Ceuca (1974) proposed the new family Hungarosomidae Ceuca, 1974 (= Hungarosomatidae) for the genus based on the discovered male. Hoffman (1980) probably overlooked this paper and did not mention this family even as a synonym in his classification of the millipedes. He referred the genus *Hungarosoma* to the family Anthroleucosomatidae, subfamily Brachychaeteumatinae. Thereafter, neither Shear (2000, 2011) nor Shelley (2002) or Enghoff *et al.* (2015), in critical revisions of the classification of the order Chordeumatida on the family level, mentioned the family Hungarosomatidae. In the Fauna Europaea scheme, both species are assigned to the family Anthroleucosomatidae (Enghoff 2013). In contrast, Ćurčić *et al.* (2007) did not mention the genus *Hungarosoma* in the global list of genera belonging to this family; thus, the systematic position of the genus has remained unclear.

Parthenogenesis is strongly assumed in some millipedes, but *Hungarosoma* is the only European chordeumatid in which reproduction via non-fertilised females is expected (Ceuca 1974; Minelli & Michalik 2015). Very few examples of parthenogenetic chordeumatids have been found in the world, fewer still recognised in the easternmost Palearctic and North America, like the genus *Underwoodia* Chamberlin, 1925 (Shelley 1993; Mikhaljova 2004).

The incomplete original description of *H. bokori*, the unclear identity of the separated populations, the absence of material for molecular study and the assumption of new distributional data on this millipede were the stimuli for new fieldwork in the type area in the spring of 2013. Nine specimens of *H. bokor*i were collected, featuring adult females and, for the first time, a male specimen. Together with a detailed morphological analysis of the diagnostic characteristics, we have analysed unpublished material from the collections of the authors. Furthermore, material from three different areas was used for molecular analysis and testing for the presence of the intracellular bacterium *Wolbachia*, not previously recognised in the millipedes.

Material and methods

Specimen collecting and illustrations: Material of *H. bo*kori was collected in the type locality, the Abaliget Cave in the Mecsek Mts., Hungary, during an excursion on 18.iv.2013 (leg. A. Mock & D. Angyal). This 1,750 m long horizontal cave is open to the public, with one main and three lateral passages. The entrance is 285 m a.s.l. (N 46° 8' 12", E 18° 6' 58"). All specimens were found at the deepest end of the main corridor, near the so-called Big Hall. The slowly moving specimens were individually sampled from wood debris.

Other specimens were collected in the karst regions of the Western Carpathians; the Slovak-Aggtelek Karst (Slovakia and Hungary), the Revúcka Highland and the Little Carpathians (all in Slovakia), and two localities outside the Carpathians: one non-karst locality in Austria, and in one karst region of the Bohemian Massif, the Moravian Karst (Czech Republic).

Basic morphological observations, body measurements and counting of ocelli were done using an Olympus Stereomicroscope SZ61 (0-45x) and an Olympus Camedia C-5060 Wide Zoom Camera with QuickPHOTO MICRO 2.3 software. Dissected structures were observed using lactic acid as a medium in temporary microscopic slides and documented under a Leica DM2500 light microscope with magnification up to 1000x. Microphotos were arranged using the Helicon Focus 5.3 software. Detailed observation of external morphology was made using a scanning electron microscopy (JEOL JMS-7401F, Biology Centre CAS, České Budějovice).

The counting of ocelli was done on partly dried specimens under the stereomicroscope. The examined material is deposited in the invertebrate collections of the Department of Zoology, Institute of Biology and Ecology, Faculty of Sciences, Pavol Jozef Šafárik University in Košice, Slovakia (material from Hungary and Slovakia), and the Institute of Soil Biology, Biology Centre, Czech Academy of Sciences, České Budějovice, Czech Republic, and in the collection of Pavel Kocourek (material from the Czech Republic). Austrian samples are deposited in the Myriapoda Collection of the Vienna Natural History Museum (NMW). Types and early collected topotypes are in the The Bavarian State Collection of Zoology in Munich, Germany (holotype, topotypoids) and Museum für Naturkunde in Berlin, Germany (topotypoids).

DNA extraction and sequencing. Total genomic DNA was extracted either from the heads or whole individuals (Table 1) using the DNeasy Blood and Tissue Kit from QIAGEN (Valencia, CA, USA) according to the manufacturer's protocol.

PCR amplification was carried out using the Unis Taq system (TopBio s.r.o., Prague, Czech Republic) and the universal primers LCO1490 and HCO2198 (Folmer *et al.* 1994) to amplify ca 590 base pairs (bp) of the 5' end of mitochondrial cytochrome oxidase I (*cox1*). Reaction volumes (12.1 µl) consisted of 7.25 µl of ddH_2O, 1 µl of template DNA (not quantified), 1.25 µl of 10 × reaction buffer, 0.5 µl of BSA (bovine serum albumin) 1 µl of dNTPs mixture, 0.75 µl of each primer (5pmol/ µl), 0.1 µl of UNIS *Taq* polymerase and 1 µl of the unquantified DNA extract. The PCR reactions were carried out in a Mastercycler ep gradient S thermocycler (Eppendorf AG, Hamburg, Germany) with the following profile: 94°C for 1 min followed by 35 cycles of 94°C for 30 sec, 47°C for 35 sec, 72°C for 1 min 30 sec, and a final extension at 72°C for 1 min 30 sec. If no PCR product was obtained, the annealing temperature was lowered to 40°C and the elongation temperature to 68°C.

TABLE 1. A list of *H. bokori* material sequenced for barcoding and the presence of *Wolbachia*. Dates and collectors: **Hbok1-6**, 28.xi.2013, leg. K. Tajovský & A. Mock; **Hbok9**, 18.iv.2013, leg. A. Mock; **Hbok13**, 15.x.2011, leg. K. Tajovský & P. Kocourek.

Voucher # Isolate	Genbank #	Locality	Haplo-type	*Wolbachia*
Hbok1-SK	KX467614	Čertova diera (SK)	1	-
Hbok2-SK	KX467615	Čertova diera (SK)	1	-
Hbok3-SK	KX467616	Líščia diera (SK)	1	-
Hbok4-SK	KX467617	Líščia diera (SK)	1	-
Hbok5-SK	KX467618	Líščia diera (SK)	1	-
Hbok6-SK	KX467619	Líščia diera (SK)	2	-
Hbok9-MM	KX467620	Abaliget Cave (HU)	2	-
Hbok13-MK	KX467621	Hostěnice (CZ)	1	Presumably type B

Bacteria of the genus *Wolbachia* were detected with specific primers for 16S RNA (Werren & Wilson 2000), WspecF and WspecR, and using the same Unis Taq system and subsequent profile: 94°C for 2 min followed by 30 cycles of 94°C for 30 sec, 55°C for 45 sec, 72°C for 1 min, and a final extension at 72°C for 1 min 30 sec.

PCR products were cleaned enzymatically with ExoSAP-IT (USB Corporation, Cleveland, Ohio, USA) before direct sequencing. Sequencing using a forward primer was performed by the SEQme Company (Dobříš, Czech Republic). The reverse primer did not provide very good chromatograms. For the following analysis only high quality portion of the chromatograms were used, the ambivalent 5´and 3´ ends were trimmed off. Table 1 shows a list of material, sampled into 96% ethanol, analysed for DNA barcoding and the presence of *Wolbachia* agents. Voucher specimens could not be deposited as they were destroyed during the DNA extraction process.

Molecular data analyses. Sequences were edited with Bioedit v.7 (Hall 2013) and confirmed as being an adequate DNA taxon using the GenBank BLASTn search and BOLD databases. This data on European chordeumatid millipedes is based on the studies of Spelda *et al.* (2011). Since none of them contained stop codons or insertions/deletions, all were considered to be true genes and not pseudogenes.

A ca. 590 bp fragment of the *cox1* gene was sequenced from 8 individuals of *H. bokori*. An alignment of the sequences is available from the authors upon request. All of the sequences are deposited in GenBank (Table 1). The overall averaged nucleotide composition showed a slight A+T bias, a feature common to insect mitochondrial DNA (Simon *et al.* 1994).

The evolutionary history was inferred using the Maximum Likelihood method based on the best-fitting model (with the lowest Bayesian Information Criterion), selected by the Modeltest as implemented in MEGA6 (Tamura *et al.* 2013). Included codon positions were 1st+2nd+3rd+Noncoding. Modeltest selected the General Time Reversible model (Nei and Kumar 2000) with gamma distribution and invariant sites (lnL -4551.4, Invariant 0.32, Gamma 0.36, R 3.15, Freq A: 0.2767, T: 0.3758, C: 0.1846, G: 0.1629). The tree with the highest log likelihood (-4682.7057) is shown. The initial tree(s) for the heuristic search were obtained by applying the Neighbor-Joining method to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories). The rate variation model allowed for some sites to be evolutionarily invariable. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The robustness of the nodes was evaluated by the bootstrap test (1000 replicates) (Kimura 1980; Felsenstein 1985). In this analysis, additional sequences from the millipedes *Polyxenus lagurus* (Linnaeus, 1758) (Polyxenida, Polyxenidae), *Polydesmus angustus* Latzel, 1884

(Polydesmida, Polydesmidae) and additional representatives of the Attemsiidae, Brachychaeteumatidae, Craspedosomatidae and Neoatractosomatidae accessible in the BOLD or GenBank databases were used. The aligned data set was trimmed to keep the ORF of the invertebrate mitochondrial genetic code. All positions with less than 95% site coverage were eliminated. There were 588 positions in the final dataset.

Haplotypes were analyzed with the DnaSP v5 software (Librado & Rozas 2009).

Results

Taxonomy

Family Hungarosomatidae Ceuca, 1974

Hungarosomatidae Ceuca 1974: 91–93

Genus *Hungarosoma* Verhoeff, 1928

Two species.

Type species: *Hungarosoma bokori* Verhoeff, 1928

Hungarosoma bokori Verhoeff, 1928

(Table 2, Figs 1–17)

Hungarosoma bokori: Verhoeff, 1928: 192–195 (description from a female); Verhoeff 1932: 1485–6, 1893 (diagnosis, notes on systematics and distribution); Gebhardt 1933; 1963; 1967; (all faunistic notes from type locality); Gebhardt 1934: 203–207 (reprint of the original description); Moritz & Fischer 1978 (data on type material); Loksa 1961 (first record outside the type locality); Korsós 2000 (new record on the type locality); Papáč *et al.* 2014 (first record in the Carpathians).

Ochogona moravica nomen nudum: Skoumalová, 2010: 229–230, 324 (preliminary diagnosis of a presupposed new species, drawings orig. P. Kocourek, faunistic data from the Moravian Karst).

Material examined. Holotype: Hungary: Abaliget Höhle (=cave), **ZSM-A-20032282**: 1♀, 12.viii.1924, leg. E. Bokor, det. K. W. Verhoeff, mounted on permanent microscopic slide, dissected. Deposited in The Bavarian State Collection of Zoology in Munich, Germany.

Topotypoid material. Hungary: Abaliget (cave?), **ZMB13596**: 1♀, without date and collector, det. K. W. Verhoeff, complete body mounted on a permanent slide, deposited in the Museum für Naturkunde in Berlin; Abaliget Cave, material determined by K. W. Verhoeff: **ZSM-A-200322811**: 1 juvenile, permanent slide, without date and collector; **ZSM-A-20034336**: 1 juvenile; **ZSM-A-20060246**: 1 subadult male and 3 juveniles in a vial with ethanol, all deposited in The Bavarian State Collection of Zoology in Munich, Germany.

New material from type locality. The cave at the end of main corridor (at siphon), sampled on the old cave-timber from oak wood, 18.iv.2013, 1♂, 3♀, 5 juveniles (stages III, VI, VII), leg. A. Mock.

Other examined material. **Austria, 2** ♀: Lower Austria: Waldviertel (Southern Bohemian Massif), Albrechtsberg an der Großen Krems, Sommerbachtal N of church, montane beech forest on metamorphites as bedrock, on right hand slope (NW exposition) between a footpath higher up and the creek, collected from valley floor a few meters upwards, 28.x.1999, hand collecting, 1 ♀, leg. J. Gruber; **NMW 8831**; 15.xi.2000, leaf litter sifting, 1 ♀, leg. J. Gruber, **NMW 8832**.

Czech Republic, 47 ♀: Moravian Karst: Hostěnice near Ochoz u Brna, litter of *Melampyro nemorosi-Carpinetum* (mesophilous oak-hornbeam forest "Mokerský les" and forest/meadow ecotone), 13.x.2005, 1 ♀, leg. P. Kocourek; 28.x.2005, 7 ♀, leg. P. Kocourek; 15.iv.2006, 1 ♀, leg. P. Kocourek; 23.iii.–19.x.2007, pitfall traps, 2 ♀, leg. P. Kocourek; 19.x.2007, 20 ♀, leg. P. Kocourek; 19.x.2008, 2 ♀, leg. P. Kocourek; 3.x.2009, 4 ♀, leg. P. Kocourek; 15.x.2011, 7 ♀, leg. K. Tajovský & P. Kocourek; 15.x.2011–7.v.2012, pitfall traps, 1 ♀, leg. K. Tajovský; 5.xi.2012–31.v.2013, pitfall traps, 2 ♀, leg. K. Tajovský; 16.ix.2013–28.iv.2014, pitfall traps, 2 ♀, leg.

K. Tajovský. Note: the samples from the years 2005–2008 are presented in the diploma thesis of Skoumalová (2010) under the name (nomen nudum) "*Ochogona moravica"*.

Hungary, 2 ♀, 1 juv.: Aggtelek Karst: Aggtelek, litter of *Corneto-Quercetum* at the foothills of reefs at the main entrance of Baradla, 21.iii.2013, 2 ♀, leg. A. Mock; Vass Imre Cave, leaf litter of hornbeam-oak forest concentrated at the lower entrance to the corridor of discoverers, extracted organic material, 5.ix.2013, 1 juv. (IV st.), leg. A. Mock.

Slovakia, 36 ♀: Slovak Karst: Čertova diera Cave, litter of *Corneto-Quercetum* in the entrance hole, 23.x.1997, 1 ♀, leg. A. Mock; 22.x.1999, 1 ♀, leg. A. Mock; 1.x.2000, 2 ♀, leg. A. Mock; 8.iii.2001, 1 ♀, leg. A. Mock; 24.ix.2003, 3 ♀, leg. K. Tajovský; 28.xi.2013, 2 ♀, leg. K. Tajovský & A. Mock; Líščia diera Cave, leaf litter of *Corneto-Quercetum* at the entrance, 1.xii.2000, 1 ♀, leg. A. Mock; 13.iii.2012, 3 ♀, leg. A. Mock; 28.xi.2013, 4 ♀, leg. K. Tajovský & A. Mock; Domica Cave (artificial corridor to the Styx river), 10 m from the entrance, on wood, 9.x.2003, 1 ♀, leg. A. Mock; Domica Cave, 30 m from the entrance of an artificial corridor, remainder of mine-timber on the ceiling, 3.x.2001, 1 ♀, leg. A. Mock; Silická Plateau, sifting of leaf litter under bushes at the edge of Jašteričie jazierko sinkhole, 1.x.2010, 1 ♀, leg. A. Mock; Gombasek Cave, Čierna vyvieračka Spring, litter and humus layers at the spring, 24.ix.2003, 2 ♀, leg. K. Tajovský; New Brzotínska Cave, litter of oak-hornbeam forest nearby the cave entrance, 3.x.2005, 2 ♀, leg. K. Tajovský; Brzotínska Spring, litter of oak-hornbeam forest nearby the spring, 3.x.2005, 1 ♀, leg. K. Tajovský; forested scree slope at the Ardovská Cave, subterranean traps with water solution of ethylene glycol, 5 cm and 45 cm under the surface, 27.x.2014–29.iv.2015, 2 ♀, leg. P. Ľuptáčik; Revúcka vrchovina (Drienčanský Karst): Blh River valley, alluvium and foot of the slopes with Malá Drienčanská Cave, forest, sifted litter and soil, 18.x.2013, 1 ♀, leg. A. Jászayová; Malé Karpaty: Driny Cave, 2001–2003, pitfall traps, 1 ♀, leg. O. Majzlan, P. Zvonár; small corridor at the site "Chodba spolupracovníkov", sampled on wood, 29.iv.2005, 2 ♀, leg. P. Ľuptáčik; same place, 29.iv.–13.ix.2005, pitfall traps, 3 ♀; litter at the entrance, 11.xi.2009, 3 ♀, leg. A. Mock.

Re-diagnosis. Body length 6.0–6.6 mm, width 0.5–0.6 mm. The same habitus in both sexes, but females overgrow males moderately. Number of ocelli in the adults (11) differs from that in *H. inexpectatum* (9). Pale brownish colouration. Rounded paranota with notch prior to the front line of them (Fig. 3). Macrosetae long. In males two pairs of legs are modified as diversified copulatory organs, the 8th and 9th. Compared to its congener, *H. inexpectatum*, the cheirites of gonopods in *H. bokori* are elongated, with a flattened and rewound central part and the tail in a shape like "two fingers" (Figs 13–16), while in *H. inexpectatum* the gonopod cheirites are drawn short with a simple tail.

General morphology. Selected morphological characteristics of *Hungarosoma bokori* are summarised in Table 2. *Coloration:* The small body of the earlier postembryonic stadia is without colouration. The subadults (stadium VII) have a poorly pigmented head; only the frons is light brown in colour. The adult stadium (VIII) is light brown, with a darker head, antennae and dorsal part of trunk segments; the metazonites are darker than the prozonites; without sexual dimorphism (Figs 1, 2, 6, 7).

TABLE 2. Some characteristics of the anamorphosis of *H. bokori* from the type locality. The counting of pleurotergites was done according to Blower (1985). *—the first smaller (semi)ocellus separated from triangular field of ocelli to more posterior location on the head. Stadia I–II and IV–V were not found. Colour of cuticle: ○○○—without colour; ●○○—light-coloured frons of the head, trunk white; ●●●—finely coloured head and trunk. Body length, height and width in mm.

Character	Stadium III	VI	VII	VIII (male)	VIII (female)
Pleurotergites	11	23	26	28	28
Leg pairs	10	32	40	44	46
Ocelli	0	4+1*	6+1*	10+1*	10+1*
Body length	1.9	4.2	5.2	6.0	6.2–6.6
Body height	0.2	0.3	0.4	0.4	0.5
Body width	0.3	0.4	0.4	0.6	0.6–0.7
Colour	○○○	○○○	●○○	●●●	●●●

FIGURE 1. *Hungarosoma bokori* Verhoeff, 1928, female, sampled at the entrance of the Baradla Cave, Hungary, 21.iii.2013. Photo: Ľubomír Kováč & Andrej Mock.

Setation and paranota: Typical rounded paranota (pleurotergite processes) with triplets of long setae are present in both sexes during all postembryonic development (Figs 3, 7–9). There is a characteristic notch prior to the front line of the paranota. Setae are quite long (up to 0.18 mm) and slightly curved, sticking up from the body segments. Two setae are on the paranota, one after another. There is almost a right angle between the connecting line of both setae and the third, the short median seta (96–99°).

Surface: The surface of the body is smooth, but the edges of the paranota are toothed due to outgoing scales of the cuticle (Figs 3, 10, 11).

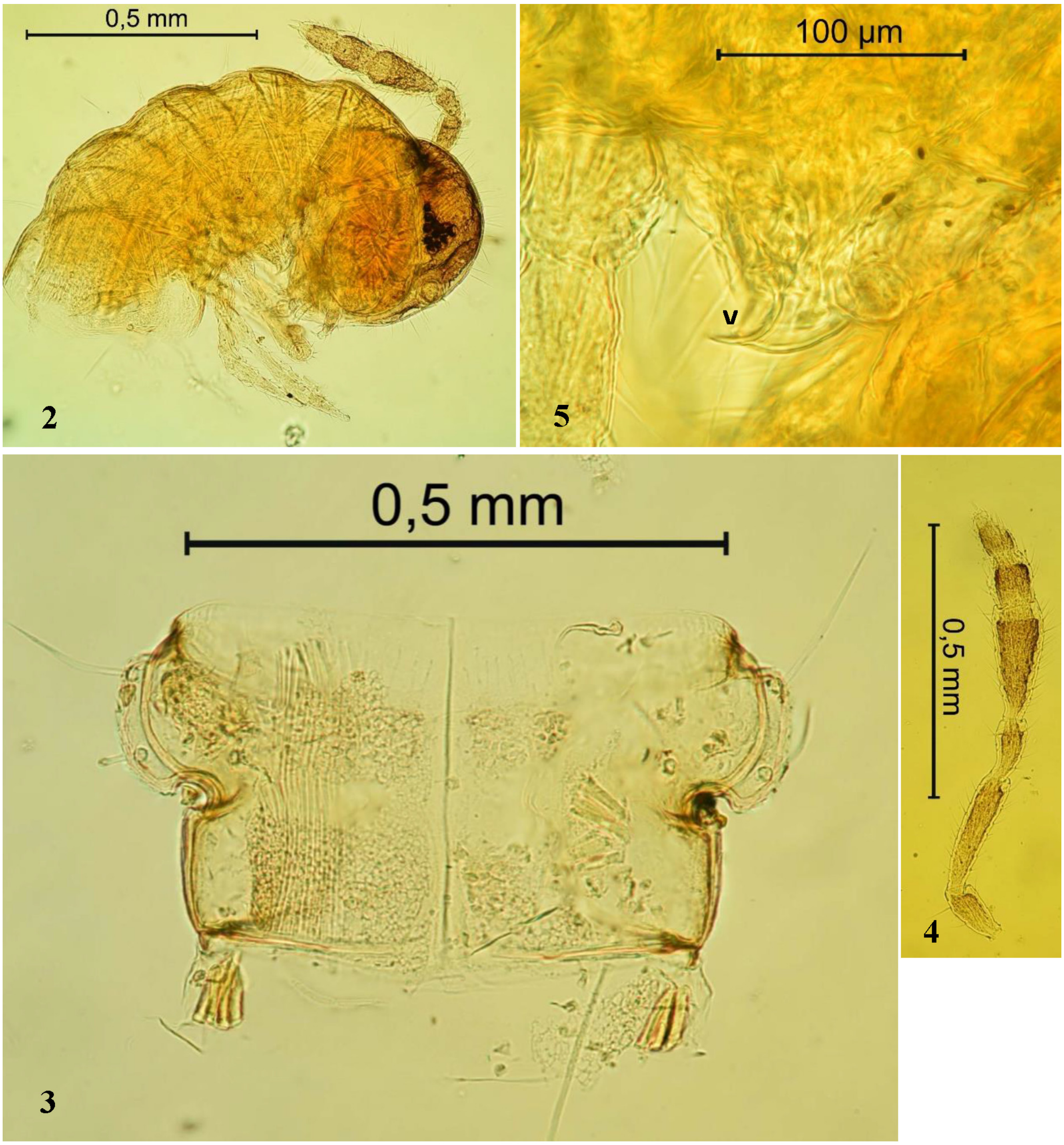
Eyes: Juveniles up to the stadium III are eyeless; then the number of ocelli increases from 5 (stadium IV) to 11 (stadium VIII, adults), forming a triangular field on both sides of the head. The first ocellus is separated from the triangular field of ocelli more behind and often smaller ("semi-oculus"), apparently rudimentary.

Extremities: No other specific characteristics on the antennae (Figs 4, 12) or legs, except the ones found in the male mating apparatus, were observed.

Male characters. The male is less robust than the female, with a body length of 6.0 mm, a body width of 0.5 mm.

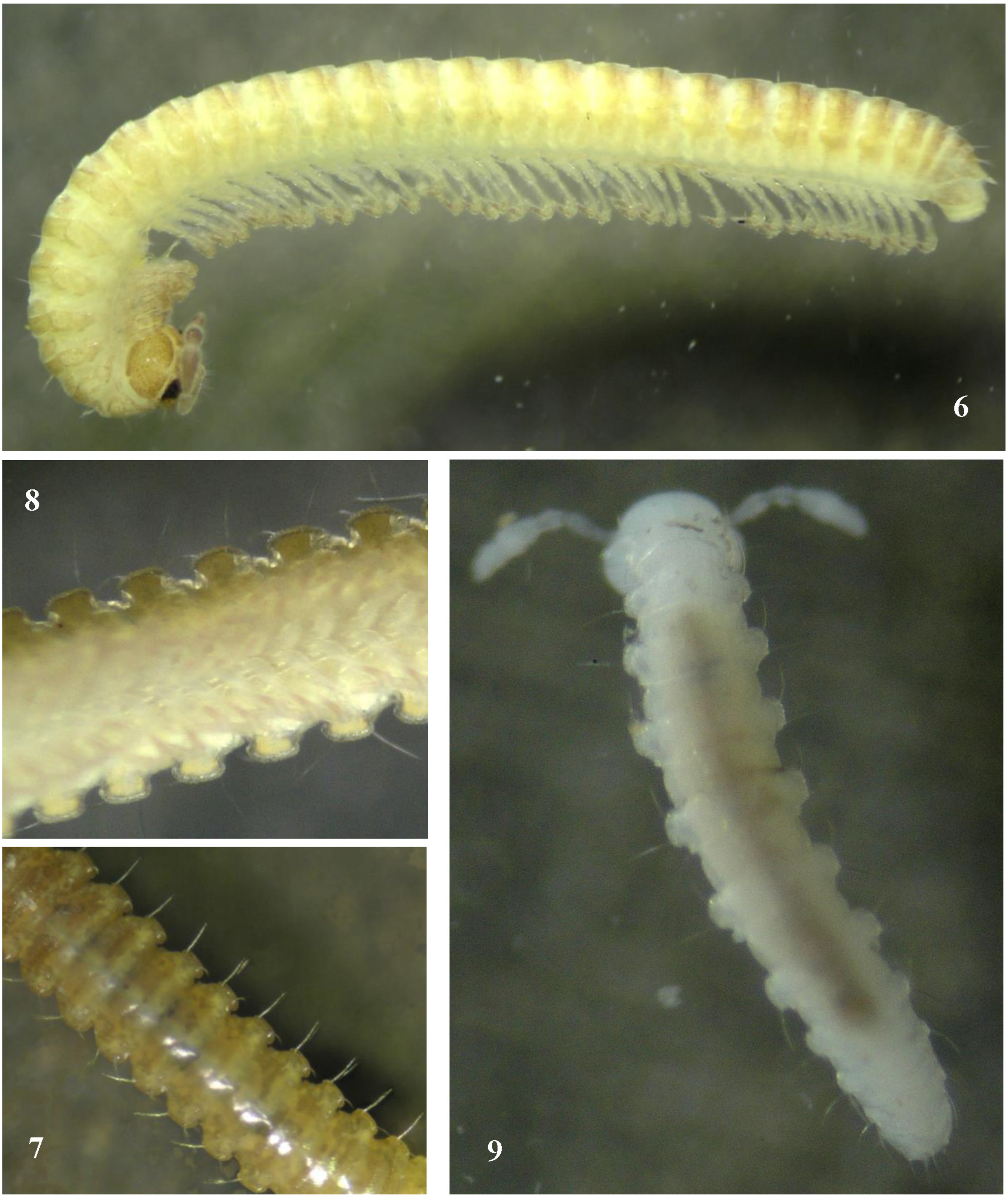
Anterior legs 1–7 are slightly shorter and more robust than the legs following the gonopods, but without other species-specific characteristics. The tarsus carries a chain of short, erect setae, ordered in a moderate spiral (in both sexes).

Legs 8 modified to anterior or 'proper' gonopods (Figs 13–16). This apparatus consists of a short frontal median process followed by long and thick cheirites growing from the robust proximal parts. The cheirites are curved twice toward the posterior gonopods, similar to the shape of the neck of the common heron or duck, with long beak-like apices that are better visible laterally (Figs 14, 16). The ends of the cheirites are flattened with divided tails like fingers and/or the pedipalps of scorpions or nippers of crayfish (Figs 15a, 16a). Medially, behind the cheirites, are paired delicate brush-like arms with dense fimbricated setae on their slightly extended flat top (Fig. 16b), followed by another pair of stronger arms. Laterally the stronger arms seem to be completed in a simple projection, but the anterior view shows they are curved toward the median plane and divided into two "teeth" like a repair spanner (Fig. 15c). These arms extend to half the length of the cheirites. In the median zone between the spanner-like arms and close to the posterior gonopods (Fig. 15-e) there are two hyaline processes with rounded tips equipped apically with short spines (Fig. 15d).

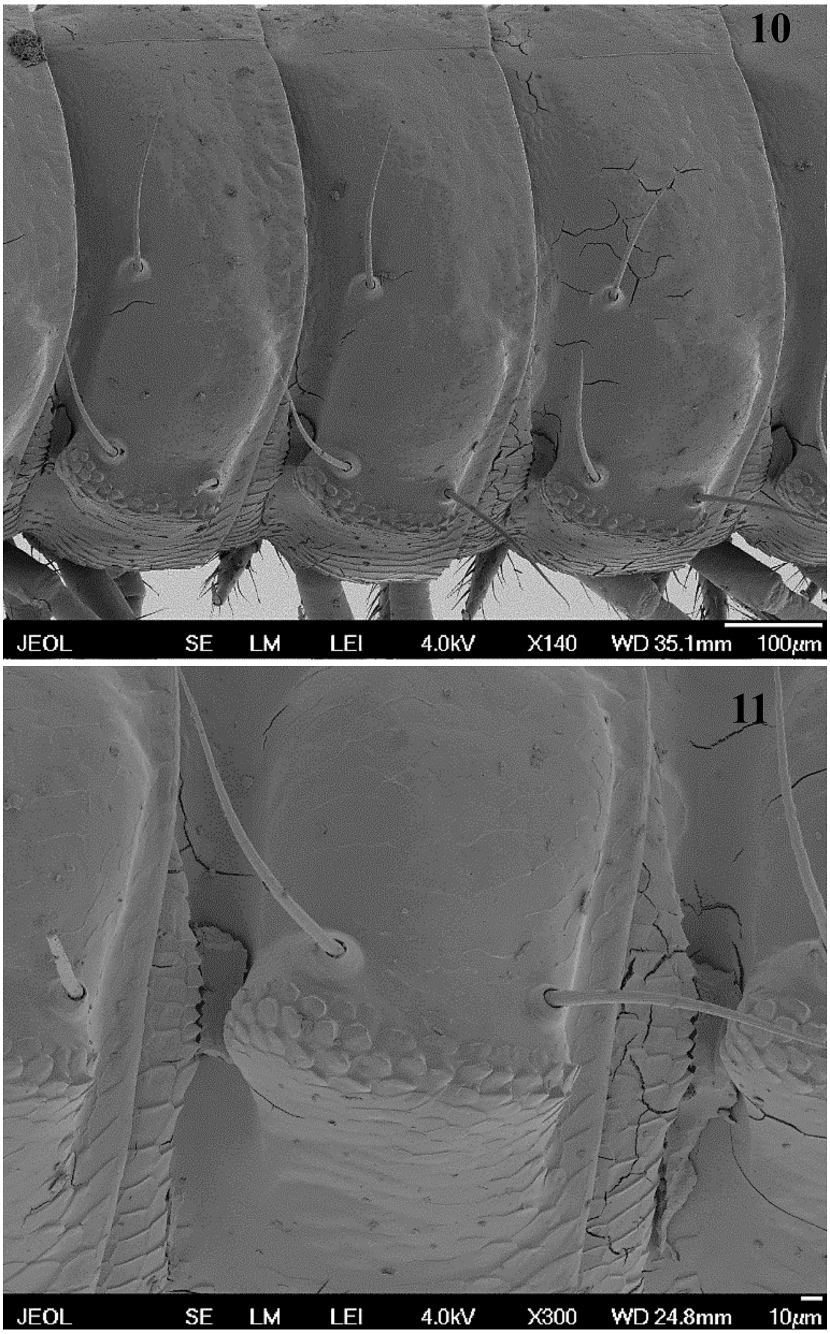


FIGURES 2–5. *Hungarosoma bokori* Verhoeff, 1928, female, holotype (Abaliget Cave). **2:** Head end of the body, right lateral view. **3**: Tergite 15, dorsal view. **4**: Antenna, lateral view. **5**: Discernable vulvae *in situ* (v), right lateral view. Photos: Jörg Spelda.

Leg 9 or posterior gonopods (Figs 15e, 16e) are strongly reduced, formed by a uniformly wide basal (coxal?) segment, with long setae, the length of which increases towards the top of the gonopods. The tops of the posterior gonopods are armed by a prominent claw-shaped process, with the tip pointed medially and with long seta on a lateral base (Figs 15g, 16g). This claw-shaped process seems to be a rudiment of the following podomeres of the original walking leg. Next-to this structure, a short hyaline prominence occurs in front of the ventral edge (Figs 15f, 16f). The surface of the posterior gonopods is nodulated, especially on the anterior and posterior sides (Fig. 16).



FIGURES 6–9. *Hungarosoma bokori* Verhoeff, 1928, specimens from the Abaliget Cave, preserved in alcohol (not scaled). **6**: Habitus of adult male in lateral view; the cheirites of anterior gonopods are visible. **7**: Details of the dorsal part of the male trunk. **8**: Ventral side of mid-body segments in detail. **9**: Dorsal side of a juvenile of stadium III with the shape of the pleurotergites typical for the genus (all material from the Abaliget Cave, Hungary). Photos: Andrej Mock.

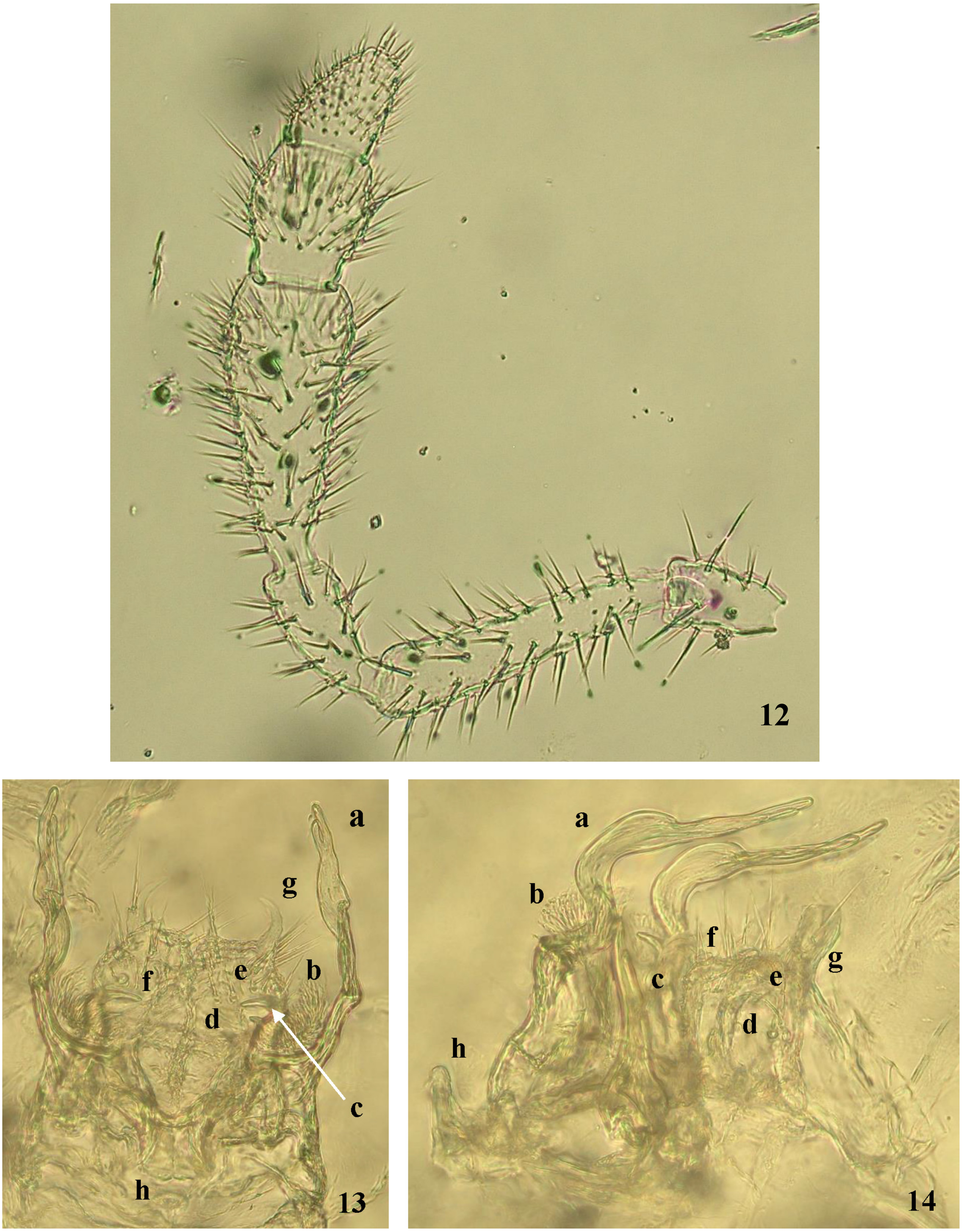


FIGURES 10–11. *Hungarosoma bokori* Verhoeff, 1928, female from the Driny Cave, scanning electronic microscopy of details of the shape and surface of mid-body segments. **10**: Dorsolateral view (left side). **11**: A pleurotergite, dorsolateral view in detail. Photos: Andrej Mock & Karel Tajovský.

Legs 10 and 11 are of full length and differ from the other legs only by the presence of coxal sacs for sperm material.

Female characters. The females are more robust; their body length reaches up to 6.6 mm, and body width is about 0.6 mm. No modifications on the legs were observed.

Vulvae fused to form one complex and are free of any postvulval structures (Figs 5, 17). Each bursa has an oval shape, with a roof-like structure on the top. Setation is present on the inner side of the bursae: 4 long setae consecutively one after another, with the longest one on the top. A short setation (5 or 6 in number) is located on the lateral edge of each bursa. At the lateral base of the bursae are 2 long setae. The receptaculum is a branch-free, pipe-shaped tube. The opercula are coupled together. Their distal edge is curved toward the bursae. Four very short setae are present on the external (anterior) surface of the opercula. They are ordered diagonally.



FIGURES 12–14. *Hungarosoma bokori* Verhoeff, 1928, male (Abaliget Cave). **12**: Antenna. **13**: Gonopod complex, anterior view. The right side of pair structures is slightly turned laterally. **14**: Gonopods in right lateral view. **Abbreviations**: Letters a–h signal equivalent structures in both views. Anterior gonopods (legs 8): a = cheirite, b = brush-like arm, c = additive divided arm, d = hyaline process. Posterior gonopods (legs 9): e = gonopod, f = ventral hyaline prominence, g = claw shape process with long seta. Not scaled. Photos: Andrej Mock.

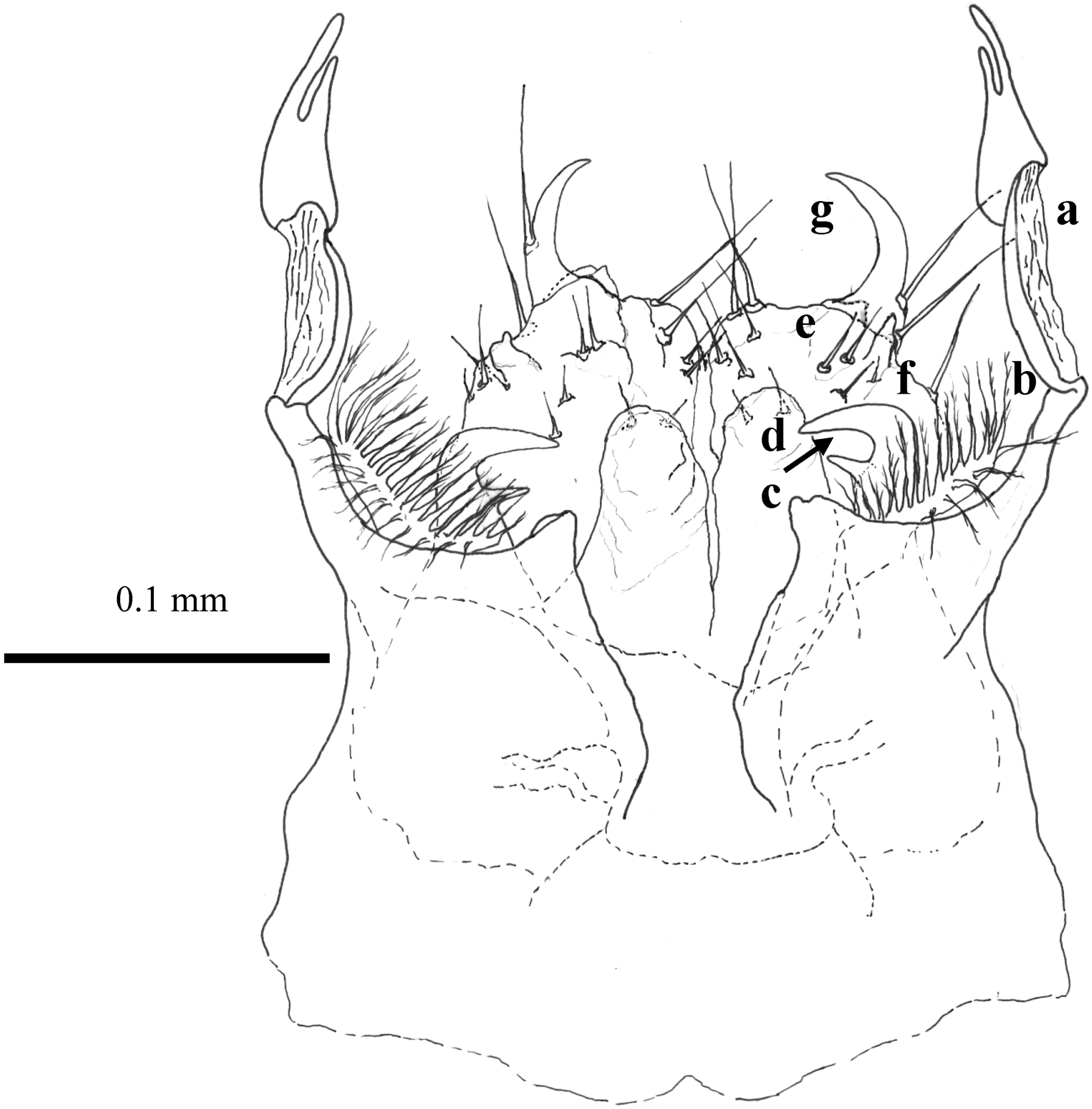


FIGURE 15. *Hungarosoma bokori* Verhoeff, 1928, male, gonopods (Abaliget Cave). Anterior view (right side of pair structures is slightly turned laterally). **Abbreviations**: Letters a–h signal equivalent structures in both views. Anterior gonopods (legs 8): a = cheirite, b = brush-like arm, c = additive divided arm, d = hyaline process. Posterior gonopods (legs 9): e = gonopod, f = ventral hyaline prominence, g = claw shape process with long seta.

Molecular analysis. Analysis in BOLD (Barcoding of Life Database) via the "species identification" module revealed that *H. bokori* was thus far not registered; therefore, the three top matches are reported below. In *H. bokori*, two haplotypes were found among the 8 sequences, one in the samples from the Slovak/Aggtelek Karst and the Moravian Karst, while the second was found both in south-western Hungary (Abaliget) and the Slovak/ Aggtelek Karst, thus, not in clear dependence on geography (Table 3).

The intraspecific distance (Kimura 2 parameter) of *H. bokori* was rather low, 0–0.4%, with an overall average of 0.2% (SE =0.1%). The optimal neighbor-joining tree, with the sum of the branch length =1.67893986, is presented (Fig. 18). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches (values >45% are shown). The tree is drawn to scale, with branch lengths in the same units (the number of base substitutions per site) as those of the genetic distances

(Kimura 2 parameter) used to infer the dendrogram. The analysis involved 64 nucleotide sequences, and the tree was rooted with *Polyxenus lagurus*.

The presence of the two haplotypes is also visible on the dendrogram (Fig. 18), where all the samples with the same haplotype clustered together, although the cluster of haplotype 2 is not strongly supported. Within the dendrogram *Hungarosoma* represents a separate cluster. The proximity of *H. bokori* to some representatives of the families Attemsiidae (*Dendromonomeron oribates* (Latzel, 1884), *Syngonopodium aceris* Verhoeff, 1913, *S. cornutum* (Verhoeff, 1929)) and Neoatractosomatidae (*Pseudocraspedosoma grypischium* (Rothenbühler, 1900)) is suggested rather than to the other Craspedosomatidae genera (species) or even to the *Brachychaeteuma*-species (Brachychaeteumatidae). However, the boostrap support is too low to draw more strict conclusions and more thorough analysis is needed.

TABLE 3. Identification of the sequenced haplotypes with the BOLD Species Identification module (May 2016). All sequences private without voucher or unique BOLD indentifier. Top ten matches. Dates for samples 1 and 6 are in the Table 1.

Order	Family	Genus	Species	Similarity (%)
Hungarosoma bokori - haplotype 1				
Chordeumatida	Attemsiidae	*Syngonopodium*	*cornutum*	85.74
Chordeumatida	Chordeumatidae	*Chordeuma*	*sylvestre*	84.46
Chordeumatida	Chordeumatidae	*Chordeuma*	*sylvestre*	84.19
Chordeumatida	Craspedosomatidae	not identified	not identified	84.19
Chordeumatida	Attemsiidae	*Schubartia*	*lohmanderi*	84.19
Chordeumatida	Neoatractosomatidae	*Pseudocraspedosoma*	*brentanum*	84.19
Chordeumatida	Attemsiidae	not identified	not identified	84.02
Chordeumatida	Chordeumatidae	*Chordeuma*	*sylvestre*	84.02
Chordeumatida	Craspedosomatidae	*Craspedosoma*	*rawlinsii transsilvanicum*	83.85
Chordeumatida	Craspedosomatidae	*Listrocheiritium*	*noricum*	83.85
Hungarosoma bokori - haplotype 2				
Chordeumatida	Attemsiidae	*Syngonopodium*	*cornutum*	85.57
Chordeumatida	Chordeumatidae	*Chordeuma*	*sylvestre*	84.46
Chordeumatida	Attemsiidae	*Schubartia*	*lohmanderi*	84.36
Chordeumatida	Attemsiidae	not identified	not identified	84.19
Chordeumatida	Chordeumatidae	*Chordeuma*	*sylvestre*	84.19
Chordeumatida	Craspedosomatidae	not identified	not identified	84.19
Chordeumatida	Chordeumatidae	*Chordeuma*	*sylvestre*	84.02
Chordeumatida	Neoatractosomatidae	*Pseudocraspedosoma*	*brentanum*	84.02
Chordeumatida	Chordeumatidae	*Chordeuma*	*sylvestre*	83.68
Chordeumatida	Chordeumatidae	*Chordeuma*	*sylvestre*	83.68

Hungarosoma inexpectatum **Ceuca, 1967**

Hungarosoma inexpectata: Ceuca T. 1967: 113 (description from a female); Ceuca T. 1974: 91–93 (re-description based on male, assignation of the family Hungarosomatidae, formerly as Hungarosomidae); Ceuca D. 2008: 49 (deposition of females collected 25.xi.1968, leg. et det. T. Ceuca, in The Zoological Museum of the Babeş-Bolyai University, Cluj-Napoca, Collections Scientifiques Bocal nr. 1A), 55 (habit preference).

Hungarosoma inexpectatum: Ceuca T. 1992 (review of distribution of all Balkan millipedes).

Type locality. Romania, Cluj-Napoca Town, garden of the Institute of Zoology. Other localities unknown.

Not examined.

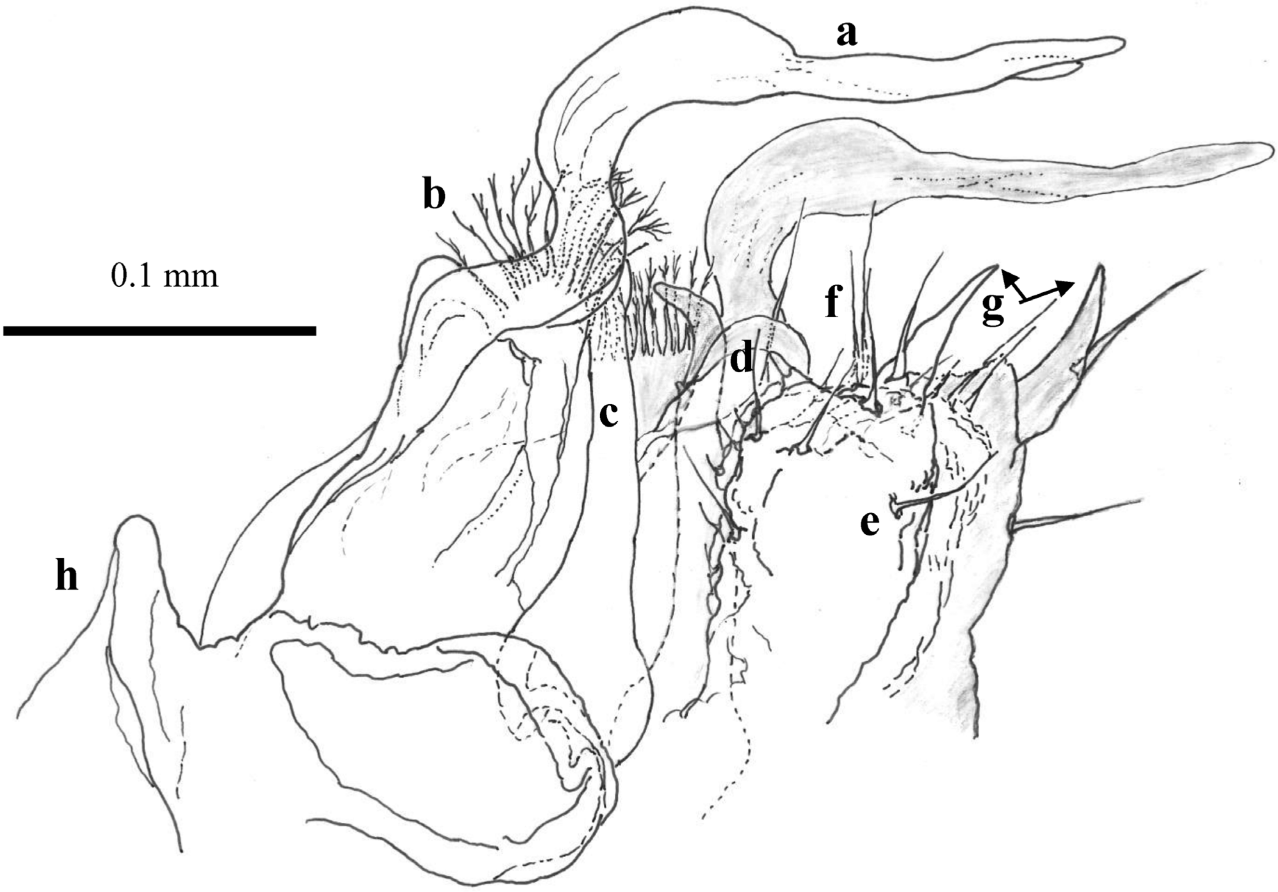


FIGURE 16. *Hungarosoma bokori* Verhoeff, 1928, male, gonopods (Abaliget Cave). Right lateral view. Letters a–h signal equivalent structures in both views. **Abbreviations**: Letters a–h signal equivalent structures in both views. Anterior gonopods (legs 8): a = cheirite, b = brush-like arm, c = additive divided arm, d = hyaline process. Posterior gonopods (legs 9): e = gonopod, f = ventral hyaline prominence, g = claw shape process with long seta.

Discussion

Disputes on type material of *H. bokori*: Verhoeff (1928) stated in his original publication that he had only a single female at his disposal, which was characterised by well discernible vulvae ("…schauen die Cyphopoden deutlich etwas heraus." p. 195). The only specimen fitting this description is the one registered in the ZSM as ZSM-A-20032282 (Figs 2–5), because: a) it is partly dissected, but all parts are mounted on the same slide; b) it shows a well discernable vulva in side view; c) it is the only preparation where some tergites are mounted in dorsal view, and they correspond to the figure in the original description by Verhoeff (1928, p. 193, illustration 4); d) it shows eggs inside the body; and e) the slide is designated with Abaliget Cave (Abaliget-Höhle) and female on the label; all other slides are located only to Abaliget. Of these other samples of *Hungarosoma bokori* in the collections of the ZSM (see above, type material), some are marked by a red cross, often used as a type marker by Verhoeff, but represent all juvenile individuals,

Due to the courtesy of Jason Dunlop (Museum für Naturkunde) digital photos of the Berlin slide (ZMB13596) were studied. This preparation is signed as being the holotype, but there are clear contradictions: a) the whole specimen is mounted non-dissected in side view; b) vulvae are not discernable; c) it is probably a juvenile (the photo is quite good, but even modern digital photography has its limits); d) generally, Verhoeff's types described before 1905 are usually found in Berlin, while later types are usually found in Munich.

For these reasons we identify item ZSM-A-20032282 as the holotype and item ZMB13596 deposited in the Museum für Naturkunde in Berlin as a topotypoid. Other items in the ZSM (ZSM-A-20032281, ZSM-A-20034336, ZSM-A-20060246) are probably a series of topotypic material.

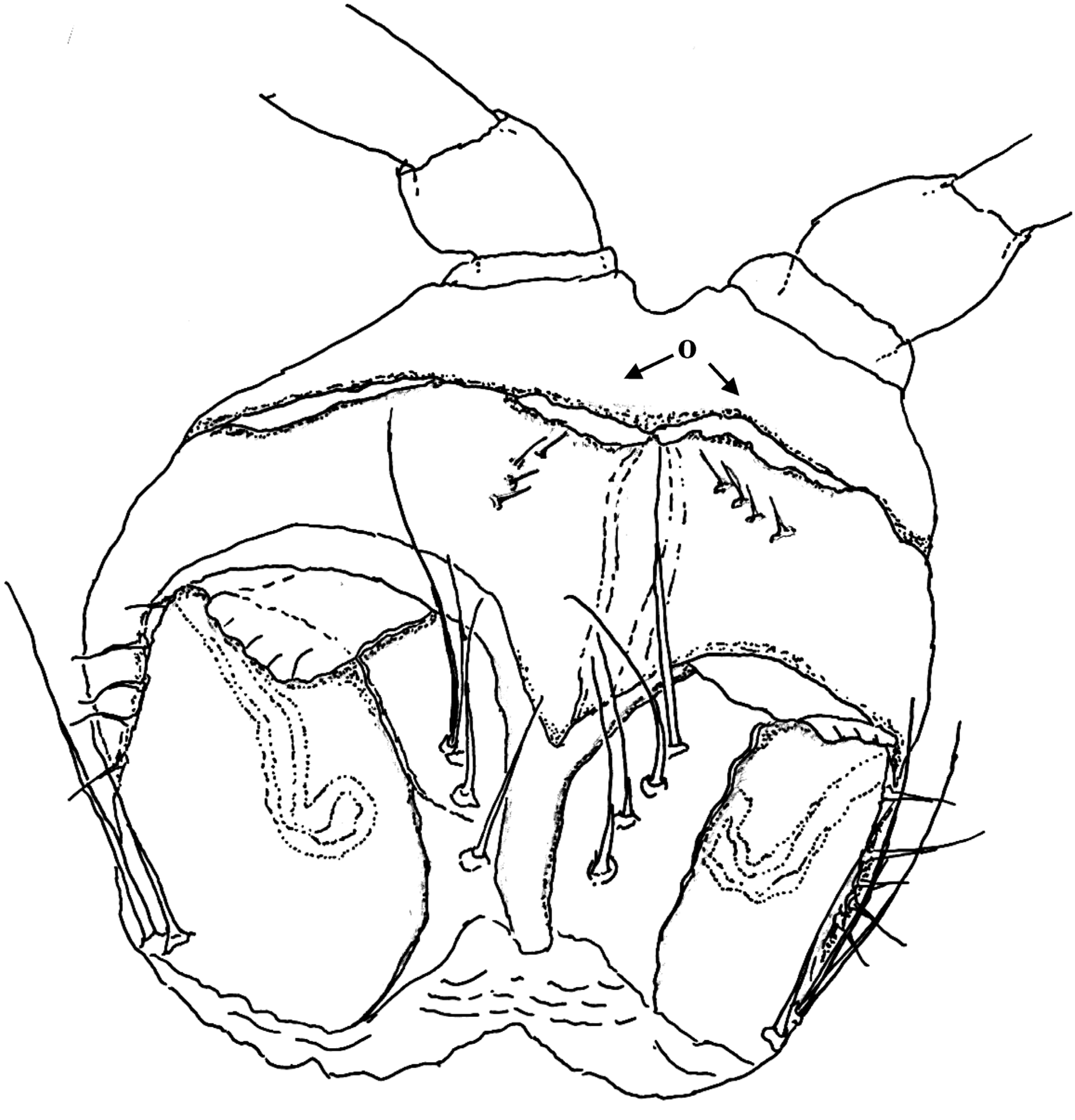


FIGURE 17. *Hungarosoma bokori* Verhoeff, 1928, female, vulvae (Driny Cave). Vulvae in posterior-ventral view (o = opercula) Not scaled.

All material determined by Verhoeff was with high probability collected in the Abaliget Cave and not outside at the village of Abaliget. In his book (Verhoeff 1932, p. 1486) Verhoeff briefly characterised the distribution as "one species in a Hungarian cave". The surroundings of the cave were visited several times by myriapodologists, but without positive records of *Hungarosoma*, even in neighbouring caves (Korsós 2000, Angyal & Korsós 2013, Angyal & Mock unpubl.).

Distribution of the genus *Hungarosoma*: The Abaliget Cave, the type locality, is probably the only refuge of *H. bokori* in the Mecsek Mts. as the type area. Recent investigations in the caves and surrounding above-ground habitats in this region did not bring any new records, even in the other "siphon part" of the cave (Korsós 2000; Angyal & Korsós 2013; Mock & Angyal unpubl.). This locality is the southernmost spot within the entire area of the genus (family) distribution. Nevertheless, some millipede specimens generally similar in character were recently found in Bosnia (D. Antič pers. com.); thus, other Mediterranean refugia are expected to be found.

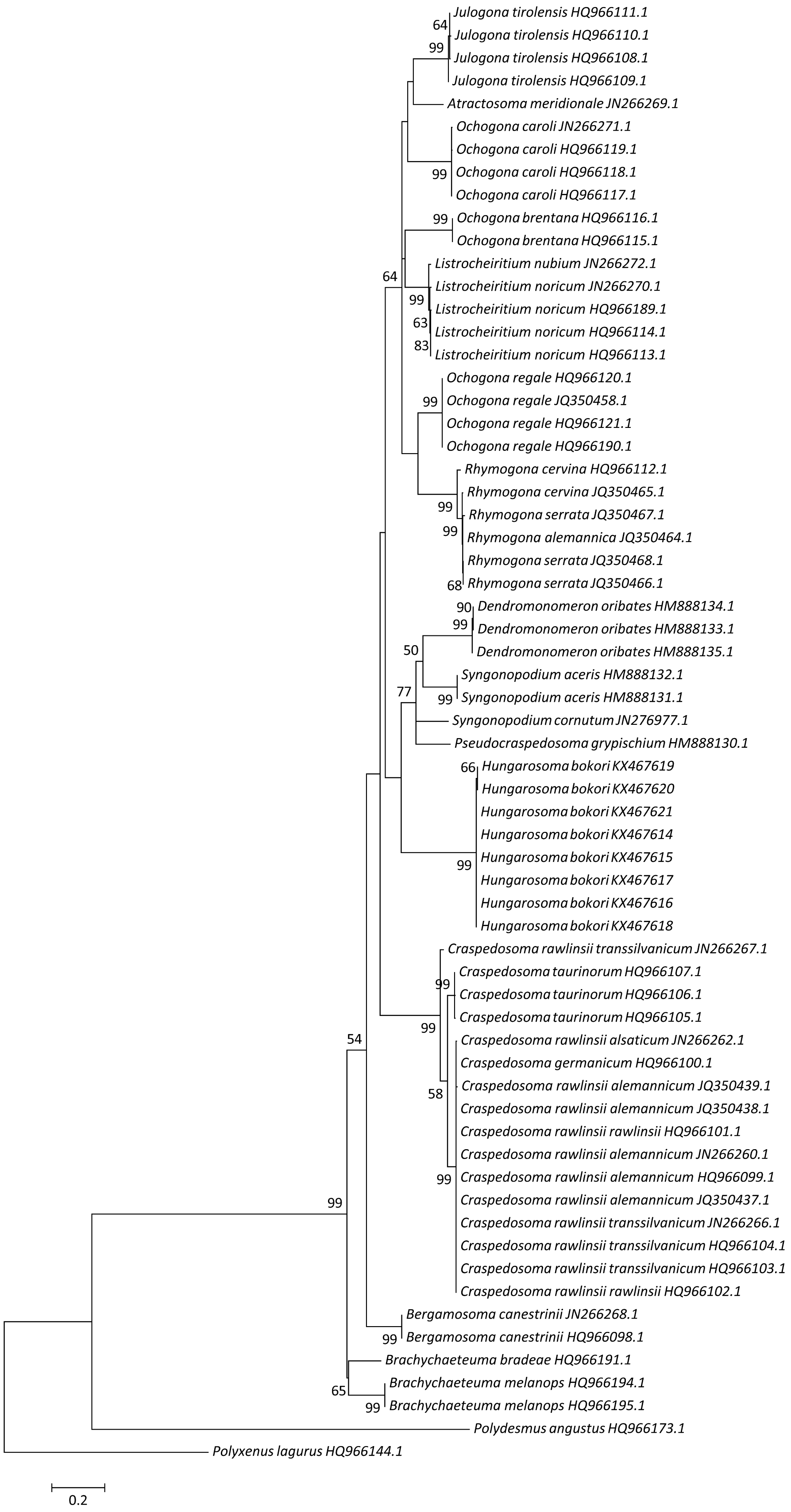


FIGURE 18. A Maximum-Likelihood tree (GTR+G+I model) based on the COI gene and rooted with *Polyxenus lagurus*. All data—except from *H. bokori*—were obtained from Genbank. Numbers refer to bootstrap values (1000 replicates). Scale bar = 0.02 substitutions/site. For origin of the *H. bokori* material, see Table 1.

Although for a long period *H. bokori* was supposed to be endemic to the Abaliget Cave, such is now doubtful due to the lack of reliable faunistic data caused by the minute body, absence of males and cryptic life of the species (Verhoeff 1932, p. 1893). *H. bokori* is distributed in the wider circum-pannonic landscape in Central Europe. The presence of *H. bokori* in the northern parts of its actually known distribution range was overlooked for decades because of its small body size and generally cryptic occurrence. Multiple isolated localities of this species were recently found beyond the northern limits of the Pannonian Lowland (Carpathian Basin), most frequently at the entrances of limestone caves or in soil on Mesozoic limestone bedrock in the Western Carpathians and in similar conditions outside the Carpathians in the Moravian Karst (Fig. 19). The latter site on non-carbonic (basalt) bedrock was mentioned by Loksa (1961) from a location with forested rocky debris and a temperature inversion in south-western Hungary. The other non-carbonic locality is the one mentioned in this paper. The known localities are in marginal highlands or mountains connected or situated closely to the Pannonian Lowland and its foreland basins from the west and north, with prevailing elevations between 250–400 m a.s.l., and a whole altitudinal range of 230–610 m a.s.l. (Fig. 20).

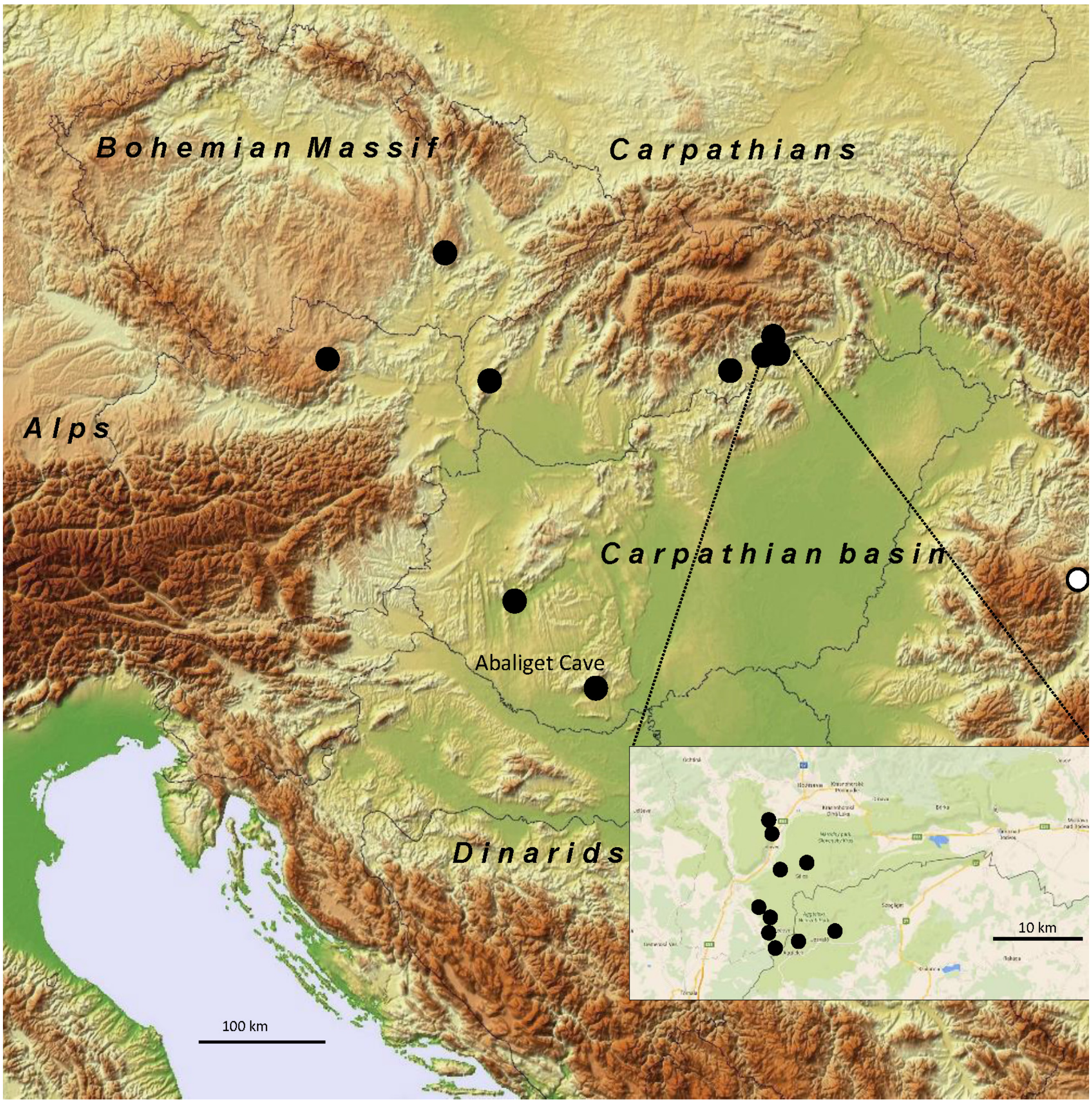


FIGURE 19. Distribution of the genus *Hungarosoma* Verhoeff, 1928. Empty dot: *H. inexpectatum*, solid dots: *H. bokori*. Distribution of *H. bokori* in Slovak-Aggtelek Karst drawn in higher scale.

The recent circum-pannonic distribution is known for several soil invertebrate groups, like earthworms (Csuzdi *et al.* 2011), uropodid mites or springtails (Mahunka *et al.* 2013). The distributional pattern of the genus *Hungarosoma* corresponds with the hypothesis that it belongs to the relict terrestrial fauna of microplates (mega-blocks or mega-units) initially situated in the area of present-day Hungary (Alcapa, Tisia = Tisza) during the

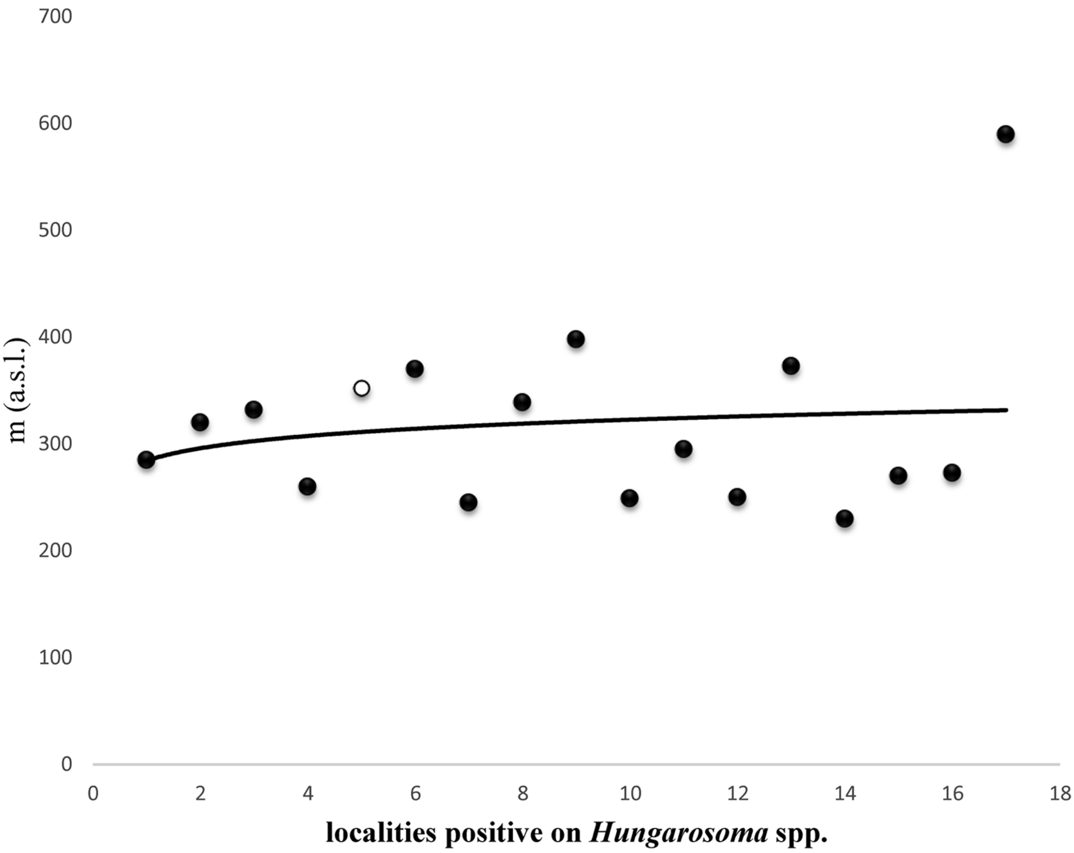
Tertiary period, connecting the Alps, the Dinarids and the Carpathians, and later almost completely slumped under the sediments of the Carpathian Basin (Košel 2012; Szederkényi *et al.* 2013). The former lands of the Pannonian Lowland not flooded by the Tertiary Paratethys Sea could have hosted extinct (?) bisexual ancestors of *H. bokori*. Thelytokous derivates of *H. bokori* (and *H. inexpectatum*) are probably residual at the peripheries of the former area, where they have survived up to the present time, while the hypothetical central area of their distribution range with a bisexual population came to an end (Golovatch & Kime 2009). Regardless, the recent distribution of *Hungarosoma* spp. avoids or closely frames the area flooded by the ancient Pannonian Sea during the Miocene – Pliocene (Kázmér 1990).

The high rate of inter- and intrapopulation genetic identity of asexual clones appears to form a monophyletic lineage. Similar results were obtained for the thelytokous derivates of the millipede *Nemasoma varicorne* C. L. Koch, 1847 (Hoy Jensen *et al.* 2002).

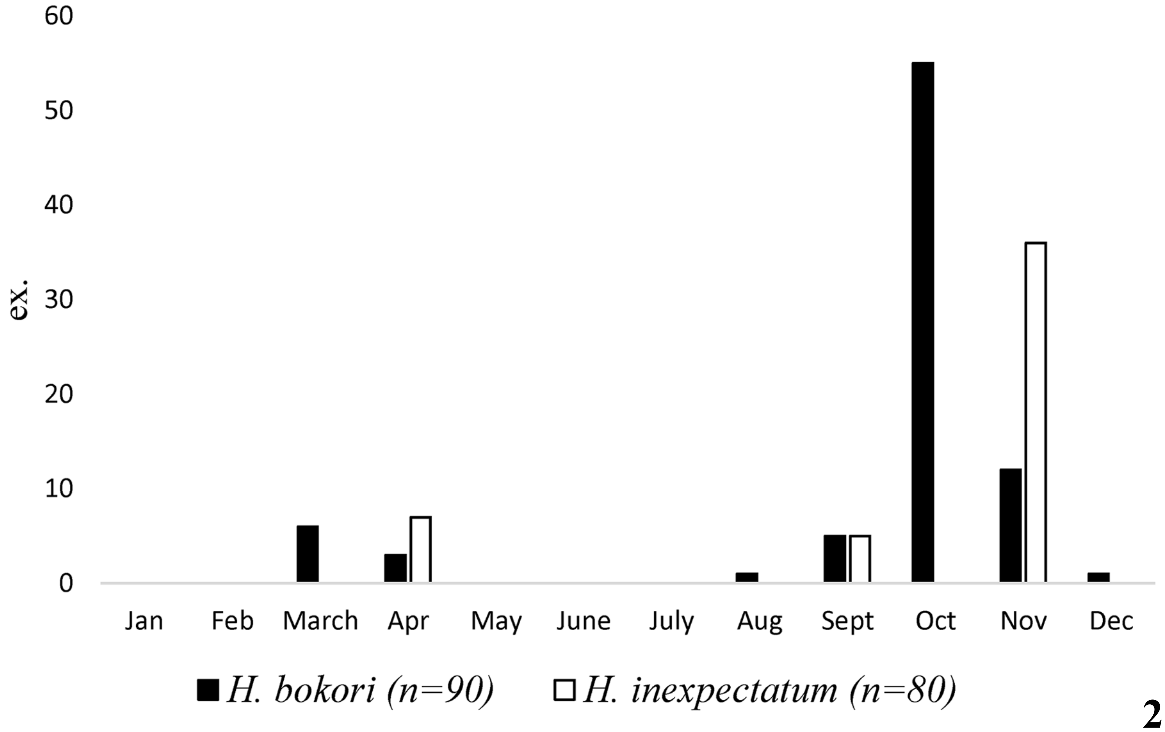
Ecology. According to Golovatch & Kime (2009), parthenogenesis in millipedes is linked with life in suboptimal to extreme environments. Summarising the faunistic data, we suspect that *H. bokori* is a stenotopic and stenothermophilous low-dispersing millipede that avoids habitats with a dry or too dynamic climate, instead preferring shady and moist sites in leaf litter or a cave environment. At the big entrance of the Líščia diera Cave, Slovak Karst, or under the reefs at the Baradla Cave, Aggtelek Karst, we observed the occurrence of this millipede limited to a narrow zone among the underground and surface environments and outside the caves, but not more than few metres from the entrance portal or rock reefs. Despite the long-term detailed study of invertebrates in the Domica-Baradla Cave System, only two specimens were found separately inside the caves; all others were outside. All of the specimens were rarely detected, even with targeted collecting in a suitable season and climate, indicating that their abundances are very low. The scarce data available on the phenology of both species have shown that they prefer cold and humid periods, when the air temperature varies narrowly above 0°C (Fig. 21). The specimens were usually found below the leaf litter of deciduous trees, in the fermentation layer of litter and in caves on dead-wood material. It is interesting that *H. bokori* populations locally dwell exclusively in a cave environment (the Abaliget Cave), while in other places they were found in both cave and soil habitats (the Driny Cave and surroundings) or exclusively in an underground – aboveground ecotone (cave entrances). In the Abaliget Cave *H. bokori* specimens selectively dwell in the decaying wood of deciduous trees and avoid the wood of coniferous trees, which is otherwise most abundant in the cave due to the former caving works. Their close relationship to the wood of deciduous trees corresponds to the predominating beech forest above-ground around the cave at the village of Abaliget.

Loksa (1961) referred to the finding of *H. bokori* in a volcanic forested basalt rocky gap, in leaf litter in a narrow gorge with a climate inversion. The second non-karstic locality was found in Austria. In the Abaliget Cave it is highly probable that the activity and life-cycle is independent of the seasons and the population is well adapted to the present stable conditions. The second species, *H. inexpectatum*, is known only from garden soil in an urban habitat (town of Cluj, Romania) and apparently the natural habitats of its occurrence have not yet been discovered.

Taxonomy and systematics. The description of the gonopods in *H. bokori* confirmed its clear difference to other representatives of the order Chordeumatida but also its close relationship to *H. inexpectatum*. The general pattern of the gonopod structure of both species is similar, though there are doubts about the drawings of *H. inexpectatum* published by Ceuca (1974), which seem to be slightly simplified (or possibly some structures were lost or missed during gonopod preparation). The here conducted study of the gonopod structure has confirmed that both species are close congeners. Males of both species can be easily recognised by the different character of the anterior gonopods. Except for the locality of known distribution, it is still impossible to distinguish juveniles or even adult females of both species, because the general habitus and body size is equal, such as in the same number of body segments or pairs of legs. Characteristics like colouration are not stable during ontogeny and in addition there could be some differences in particular populations. Nevertheless, both species differ in the number of ocelli in adults: 9 in *H. inexpectatum* (Ceuca 1967) and 11 (10+1) in *H. bokori* (Verhoeff 1928; this study). However, this characteristic may not be constant, and there could be confusion due to difficulties in observation (cf. Blower 1985, p. 76). Some specimens of *Hungarosoma bokori*, based on their similar overall habitus and size, were preliminary designated as a member of the genus *Ochogona* Cook, 1895 (see *Ochogona moravica* in Skoumalová 2010). Both genera are found in similar habitats. In the Austrian and Czech localities the co-existence of *H. bokori* and *Ochogona caroli* (Rothenbühler, 1900) was observed (J. Gruber, P. Kocourek orig. data).



20



21

FIGURES 20–21. **20**. Distribution of localities with *Hungarosoma* spp. along the altitudinal gradient*. H. inexpectatum* (empty dot) was found in the town of Cluj (Romania, 350 m a.s.l.), other values belong to the localities with records of *H. bokori* (solid dots). **21**. Seasonality of records of *Hungarosoma* spp. (based on original and available published data).

Variability in vulva morphology among and within the higher taxa in the order Chordeumatida is quite well known and well documented, but the use of separate features of vulvae as a marker of phylogenetic value in these millipedes is problematic (Kurnik 1988). The character of the vulvae of *H. bokori* fits into the general frame of morphology of craspedosomatid vulvae. In general, its shape is close to some Craspedosomatidae or Neoatractosomatidae taxa, like those in the genera *Craspedosoma* Leach, 1814 or *Pseudocraspedosoma* Silvestri, 1898 (cf. Kurnik & Thaler 1985; Kurnik 1988), and more so those in the genus *Brachychaeteuma* Verhoeff, 1911 (Brolemann 1935). The vulvae of *H. inexpectatum* have not yet been described.

The family position of *Hungarosoma*. Verhoeff (1928) assigned the genus *Hungarosoma* to the family Brachychaeteumatidae, subfamily Macrochaeteumatinae, due to the small body with only 28 rings and with specific paranota and rather long dorsal setae. Hoffman (1980) assigned the genus *Hungarosoma* to the family Anthroleucosomatidae, subfamily Brachychaeteumatinae, together with the genera *Brachychaeteuma* (ca 8 species distributed mainly in Western Europe) and *Verhoeffeuma* Strasser, 1937 (with 2 species distributed in the Dinaric region of Bosnia and Italy). Korsós (2000) summarised that the genus belongs to the family Anthroleucosomatidae according to Hoffman (1980), with a close position to the Japan genus *Macrochaeteuma* Verhoeff, 1914. This opinion was recently corrected: Fauna Europaea (Enghoff 2013) listed all of the above-mentioned genera in separate families: the genus *Hungarosoma* in the family Anthroleucosomatidae (with ca 20 other genera), *Brachychaeteuma* in the monotypic family Brachychaeteumidae and *Verhoeffeuma* in the family Chamaesomatidae (with other 9 genera). Mauriès *et al.* (1997) and Ćurčić *et al.* (2007) reviewed the generic composition of the family Anthroleucosomatidae without featuring the genus *Hungarosoma*. Spelda (2016) assigned the genus to the family Brachychaeteumatidae, however, the morphology of the gonopods strongly differs between *Hungarosoma* and *Brachychaeteuma*, as well as between the former and *Macrochaeteuma*. Ceuca (1974) perceived this fact when he re-described *H. inexpectatum* based on the male characteristics. He concluded that the genus belongs to a separate family, Hungarosomidae (or Hungarosomatidae after recent nomenclature). He defined the family as follows (translated from Romanian language): "The small-body forms of ascospermofor-carrying millipedes, which have short but evident wing-like processes on the metazonites, with a gently serrated edge and with both the anterior and posterior corners rounded. Three macrochets on the sides of the rings are located in the apices of the orthogonal triangle. The anterior gonopods consist of two arms (anterior and posterior), which are the telopodite and coxite; between them is an external hyaline structure (orig. "…*proeminează cite o lamă hialină*"). The posterior gonopods consist of two articles, one anterior and one posterior. The proximal parts are well developed and connected to each-other on the medial side. The distal articles are very narrow and sharp."

A comparison with the BOLD database (www.barcodinglife.com) indicates the position of *H. bokori* within the suborder Craspedosomatidea (cf. Shelley 2002), with the closest relation to representatives of the families Attemsiidae and Neoatractosomatidae, though the latter families have a greatly different morphology, slightly more than the family Brachychaeteumatidae or Craspedosomatidae (Fig. 18, Table 3). There was no close relation to a member of the family Anthroleucosomatidae (*Anamastigona pulchella*). The results support the opinion of Ceuca (1974), who erected the separate family Hungarosomatidae.

Parthenogenesis and the presence of *Wolbachia*. Due to the perseverance of Ceuca (1974), who found and described a male of the second species, *H. inexpectata*, 6 years after continuing recording, we recognise that the males of both species are probably extremely rare (1–2% of recorded specimens), or they are completely missing in some localities, which represents a spanandry pattern of population structure.

The presence of the bacterial endosymbiont *Wolbachia* was tested as an agent that impacts the breeding system and implies parthenogenesis; the specimen from the Moravian Karst in the Czech Republic was positive. According to the BLASTn (Basic Local Alignment Search Tool Search for nucleotide sequence), it was 93% identical to the *Wolbachia* type B of hymenopteran *Trichogramma ostriniae* Pang et Cheng. However, the exact type remains to be verified by a multilocus approach.

This is the first finding of the *Wolbachia* endosymbiont in millipedes (cf. Nyirő *et al.* 2002; Witzel *et al.* 2003; Golovatch & Kime 2009).

Conclusions

Based on the male morphology, the re-description of *Hungarosoma bokori* is completed. The described parameters of male morphology clearly distinguish both species of this genus, *H. bokori* and *H. inexpectatum*.

Morphological and molecular analyses confirmed the population of *H. bokori* from the type locality in the Mecsek Mts. with those in the Western Carpathians and Moravian Karst regions are identical.

Data about the known distribution and knowledge of the ecology of *H. bokori* showed that the species inhabits inverse refuges mainly in karst regions, and its actual occurrence has a relict character. Isolated parthenogenetic assemblages could indicate the survival of the rest of the ancestral population at the edges of former area, situated formerly in the Pannonian Lowland. Hypothetically, the centre of previous species was in the Tertiary microplates, recently eroded or plunged under the sediments of the Danube River basin.

The molecular methods employed support to the opinion proposed by Ceuca (1974) that the genus *Hungarosoma* belongs to a separate lineage among the craspedosomatid Chordeumatida millipedes and corroborates the validity of the separate family Hungarosomatidae.

The intracellular prokaryotic parasite *Wolbachia* was recorded in *H. bokori,* and thus for the first time in the Diplopoda. This single positive piece of evidence corresponds with the parthenogenetic character of this millipede; nevertheless, this lone positive finding needs to be further investigated.

Acknowledgements

The authors thank Peter Ľuptáčik (Pavol Jozef Šafárik University, Košice) and Anett Hegedűs, a young enthusiastic caver (Pécs), for their help in the field research and for technical support. The administrators of the Abaliget Cave allowed us to explore the cave. Alexandra Jászayová (Pavol Jozef Šafárik University, Košice), Peter Zvonár (Driny Cave Administration) and Peter Ľuptáčik collected important material. Igor Hudec (Pavol Jozef Šafárik University, Košice) advised the first author during the taking of photos and making of drawings. Ľubomír Kováč (Pavol Jozef Šafárik University, Košice) took photos of a living specimen and Jason Dunlop (Museum für Naturkunde, Berlin) initiated photo-documentation of slide material under his curation. SEM photography was realised in the Laboratory of Electron Microscopy, Institute of Parasitology, Biological Centre of the Czech Academy of Sciences, České Budějovice, under general assistance of Jana Nebesářová. David McLean made language corrections of the text. The study was supported by the grants VEGA 1/0139/09 and VEGA 1/0199/14. We express special thanks to Thomas Wesener, editor of this paper, and the anonymous reviewers who kindly provided very important critiques and suggestions for improving the paper.

References

Angyal, D. & Korsós, Z. (2013) Millipedes (Diplopoda) of twelve caves in Western Mecsek, Southwest Hungary. *Opuscula Zoologica, Budapest*, 44, 99–106.

Blower, G.J. (1985) *Millipedes. Keys and notes for the identification of the species*. Linnean Society of London and The Estuarine and Brackish-Water Sciences Association by E.J.Brill/Dr.W. Backhuys, London, 242 pp.

Brolemann, H.W. (1935) *Myriapodes diplopodes* (*Chilognathes I*). Faune de France, 29. P. Lechevalier, Paris, 369 pp.

Ceuca, D. (2008) Des diplopodes des zones de Cluj. *Oltenia - Studii şi comunicări Ştiinţele Naturii,* 24, 48–55.

Ceuca, T. (1967) Quelques autres Diplopodes nouveaux de la faune de la Roumanie. *Studia Universitatis Babeş-Bolyai. Series Biologia*, 1, 107–117.

Ceuca, T. (1974) Noi contribuţii la cunoaşterea diplopodelor din fauna României. *Studia Universitatis Babeş-Bolyai. Series Biologia*, 1, 91–97.

Ceuca, T. (1992) Quelques aspects sur la faunistique, l'écologie et la zoogeographie des Diplopodes de la Région Balkanique. *Berichte des Naturwissenschaftlich-Medizinischen Vereins in Innsbruck*, Supplementum, 10, 411–429.

Cook, O.F. (1895) Introductory note on the families of Diplopoda. *In*: Cook, O.F. & Collins, G.N. (Eds.), *The Craspedosomatidae of North America. Annals of the New York Academy of Sciences*, 9, 1–7. http://dx.doi.org/10.1111/j.1749-6632.1896.tb55430.x

Csuzdi, C., Pop, V.V. & Pop, A.A. (2011) The earthworm fauna of the Carpathian Basin with new records and description of three new species (Oligochaeta: Lumbricidae). *Zoologischer Anzeiger*, 250, 2–18. http://dx.doi.org/10.1016/j.jcz.2010.10.001

Ćurčić, B.P.M., Makarov, S.E., Tomić, V.T. & Mitić, B.M. (2007) Global review of some Anthroleucosomatidae (Diplopoda: Chordeumatida) with description of a new millipede genus from Balkan Peninsula. *Canadian Entomologist*, 139, 478–488. http://dx.doi.org/10.4039/n06-062

Dudich, E. (1932) *Biologie der Aggteleker Tropfsteinhöhle "Baradla" in Ungarn*. Speläologische Monographien, 13, Wien, 246 pp.

Latzel, R. (1884) *Die Myriapoden der Österreichisch-Ungarischen Monarchie*. Band 2. Wien, 414 pp.

Enghoff, H. (2013) Diplopoda. Fauna Europaea version 2.6.2 – last update 29 August 2013. Available from: http://www.faunaeur.org (accessed 19 August 2015)

Enghoff, H. & Golovatch, S.I. (2015) Order Chordeumatida Pocock, 1894. *In*: Minelli, A. (Ed.) *The Myriapoda. Treatise on Zoology –Anatomy, Taxonomy, Biology. Volume 2*. Brill, Leiden – Boston, pp. 408–420.

Felsenstein, J. (1985) Confidence limits on phylogenies: An approach using the bootstrap. *Evolution*, 39, 783–791. http://dx.doi.org/10.2307/2408678

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 Biotechnol*ogy, 3, 294–299.

Gebhardt, A. (1933) Az Abaligeti és a Mánfai barlang állatvilágának összehasonlítása (Vergleichung der Tierwelt der Abaligeter- und Mánfaer Höhlen). *Állatani Közlemények*, 30, 36–44.

Gebhardt, A. (1934) Az abaligeti barlang élővilága (The fauna of the Abaliget Cave). *Mathemathikai és Természettudományi Közlemények*, 37, 1–264 + map.

Gebhardt, A. (1963) A Mecsek-hegység barlangjainak biológiai vizsgálata. *Janus Pannonius Múzeum Évkönyve*, 8, 5–32.

Gebhardt, A. (1967) A Mecsek hegység állatvilágának térbeli elterjedése élőhelyek szerint. *Janus Pannonius Múzeum Évkönyve*, 12, 7–14.

Golovatch, S.I. & Kime, R.D. (2009) Millipede (Diplopoda) distribution: A review. *Soil Organisms*, 81, 565–597.

Hall, T. (2013) BioEdit v7.2.5. Available from: http://www.mbio.ncsu.edu/bioedit/page2.html (Accessed 12 Nov. 2013)

Hoffman, R.L. (1980) *Classification of the Diplopoda*. Muséum d´Histoire Naturelle, Genève, 238 pp.

Hoy Jensen, L., Enghoff, H., Frydenberg, J. & Parker, E.D. (2002) Genetic diversity and the phylogeography of parthenogenesis: comparing bisexual and thelytokous populations of *Nemasoma varicorne* (Diplopoda: Nemasomatidae) in Denmark. *Hereditas*, 136, 184–194.
http://dx.doi.org/10.1034/j.1601-5223.2002.1360302.x

Kázmér, M. (1990) Birth, life and death of the Pannonian Lake. *Palaeogeography*, *Palaeoclimatology*, *Palaeoecology*, 79, 171–188.
http://dx.doi.org/10.1016/0031-0182(90)90111-j

Kimura, M. (1980) A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution*, 16, 111–120.
http://dx.doi.org/10.1007/bf01731581

Korsós, Z. (1994) Checklist, preliminary distribution maps, and bibliography of millipedes in Hungary (Diplopoda). *Miscellanea Zoologica Hungarica*, 9, 29–82.

Korsós, Z. (2000) Rare and endemic millipedes (Diplopoda) of the Abaliget Cave. *In*: Mock, A., Kováč, L. & Fulín, M. (Eds.), *Fauna jaskýň (Cave Fauna)*. East Slovak Museum, Košice, pp. 61–66.

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

Kurnik, I. (1988) Zur Taxonomie ostalpiner Chordeumatida: Vulvenmorphologie und Identifikation der Weibchen. *Zoologische Jahrbücher, Abteilung für Systematik, Ökologie und Geographie der Tiere*, 115, 229–302.

Kurnik, I. & Thaler, K. (1985) Die Vulven der Chordeumatida: Merkmale von taxonomischer Bedeutung (Diplopoda, Helminthomorpha). *Bijdragen tot de Dierkunde*, 55, 116–124.

Librado, P. & Rozas, J. (2009) DnaSP v5: A software for comprehensive analysis of DNA polymorphism data. *Bioinformatics*, 25, 1451–1452.
http://dx.doi.org/10.1093/bioinformatics/btp187

Leach, W.E. (1815) A tabular view of the external characters of four classes of animals, which Linné arranged under Insecta; with the distribution of the genera composing three of these classes into orders, &c. and descriptions of several new genera. *Transactions of the Linnean Society of London*, 11, 306–400.
http://dx.doi.org/10.1111/j.1096-3642.1813.tb00065.x

Loksa, I. (1961) A Kovácsi-hegy izéltlábúiról (Die Arthropoden des Kovácsi Berges). *Állatani Közlemények*, 46, 65–80.

Mahunka, S., Murányi, D. & Kontchán, J. (2013) The role of the Balkan Peninsula in the origin and genesis of the soil fauna of the Carpathian Basin: history, aims and results. *Opuscula Zoologica Budapest*, 44 (Supplement 1), 5–10.

Mauriès, J.-P., Golovatch, S.I. & Stoev, P. (1997) The millipedes of Albania: recent data, new taxa; systematical, nomenclatural and faunistical review (Myriapoda, Diplopoda). *Zoosystema*, 19 (2–3), 255–292.

Mikhaljova, E.V. (2004) *The millipedes (Diplopoda) of the Asian part of Russia*. Pensoft, Sofia-Moscow, 292 pp.

Minelli, A. & Michalik, P. (2015) Diplopoda – reproduction. *In:* Minelli, A. (Ed.), *Treatise of Zoology – Anatomy, Taxonomy, Biology. The Myriapoda, volume 2*. Leiden, Koninklijke Brill NV, pp. 237–265.
http://dx.doi.org/10.1163/9789004188273_011

Moritz, M. & Fischer, S.-C. (1978) Die Typen der Myriapoden-Sammlung des Zoologischen Museums Berlin. I. Diplopoda. Teil 6: Nachtrag zu den Teilen 1 bis 4. *Mitteilungen aus dem Zoologischen Museum in Berlin*, 54 (2), 333–343.
http://dx.doi.org/10.1002/mmnz.4830540207

Nei, M. & Kumar, S. (2000) *Molecular Evolution and Phylogenetics*. Oxford University Press, Oxford, 333 pp.

Nyirő, G., Oravecz, O. & Márialigeti, K. (2002) Detection of *Wolbachia pipientis* infection in arthropods in Hungary. *European Journal of Soil Biology*, 38 (1), 63–66.

http://dx.doi.org/10.1016/s1164-5563(01)01127-x

Papáč, V., Hudec, I., Kováč, Ľ., Ľuptáčik, P. & Mock, A. (2014) Bezstavovce (Invertebrates of the Domica Cave). *In*: Gaál, L. & Gruber, P. (Eds.), *Jaskynný systém Domica – Baradla, jaskyňa, ktorá nás spája*. Aggtelek Nemzeti Park, Jósvafő, pp. 269–279.

Ratnasingham, S. & Hebert, P.D.N. (2007) The Barcode of Life Data System. *Molecular Ecology Notes*, 7, 355–364. http://dx.doi.org/10.1111/j.1471-8286.2007.01678.x

Rothenbühler, H. (1900) Ein Beitrag zur Kenntnis der Myriapodenfauna der Schweitz. *Revue suisse de Zoologie*, 8, 167–192. http://dx.doi.org/10.5962/bhl.part.17668

Saitou, N. & Nei, M. (1987) The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution*, 4, 406–425.

Shear, W.A. (2000) On the millipede family Heterochordeumatidae, with comments on the higher classification of the order Chordeumatida (Diplopoda). *Invertebrate Taxonomy*, 14, 363–376. http://dx.doi.org/10.1071/it99016

Shear, W. (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.

Shelley, R.M. (1993) The millipede genus *Underwoodia* (Chordeumatida: Caseyidae). Canadian Journal of Zoology, 71, 168–176. http://dx.doi.org/10.1139/z93-023

Shelley, R.M. (2002) A revised, annotated, family-level classification of the Diplopoda. *Arthropoda Selecta*, 11, 187–207.

Silvestri, F. (1898) Contributo alla conosceza dei Chilopodi e Diplopodi della Sicilia. *Bollettino della Società entomologica Italiana*, 29 (4), 233–261.

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

Skoumalová, I. (2010) *Mnohonožky Českého a Moravského krasu* (*Diplopods of Czech and Moravian karst*). Diploma thesis. Faculty of Education, Charles University, Prague, 356 pp. Available from: https://is.cuni.cz/webapps/zzp/detail/86982?lang=en (accessed 2 May 2016)

Spelda, J. (2016) *Hungarosoma bokori*. *In*: Roskov, Y., Abucay, L., Orrell, T., Nicoson, D., Flann, C., Bailly, N., Kirk, P., Bourgoin, T., DeWalt, R.E., Decock, W. & De Wever, A. (Eds.), *Species 2000 & ITIS Catalogue of Life, 2016 Annual Checklist*. Available from: www.catalogueoflife.org/annual-checklist/2016/. Species 2000 (accessed 25 July 2016)

Spelda, J., Reip, H.S., Oliveira-Biener, U. & Melzer, R.R. (2011) Barcoding Fauna Bavarica: Myriapoda – a contribution to DNA sequence-based identification of centipedes and millipedes. *Zookeys*, 156, 123–139.

Strasser, K. (1937) *Verhoeffeuma* n. g., südostalpiner Höhlendiplopoden. *Zoologischer Anzeiger*, 118, 225–234.

Szederkényi, T., Kovács, S., Haas, J. & Nagymarosy, A. (2013) Geology and History of Evolution of the ALCAPA Mega-Unit. *In*: Haas, J. (Ed.), *Geology of Hungary*. Springer- Verlag, Berlin–Heidelberg, pp. 1–102. http://dx.doi.org/10.1007/978-3-642-21910-8_1

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. http://dx.doi.org/10.1093/molbev/mst197

Verhoeff, K.W. (1899) Zur vergleichenden Morphologie, Phylogenie, Gruppen- und Art-Systematik der Chordeumiden. *Archiv für Naturgeschichte*, 65 (1), 95–154.

Verhoeff, K.W. (1901) Diplopoden aus Herzegowina, Ungarn und Baiern. *Archiv für Naturgeschichte*, 67, 221–240.

Verhoeff, K.W. (1911) Über *Brachychaeteuma* n. g. und *Titanosoma jurassicum* aus England. *Zoologischer Anzeiger*, 38, 455–458.

Verhoeff, K.W. (1913) Über Diplopoden. 63. Aufsatz: *Syngonopodium* n. g. *Sitzungsberichte der Gesellschaft Naturforschender Freunde zu Berlin*, 204, 269–280.

Verhoeff, K.W. (1914) Über Diplopoden. 69. Aufsatz: Ascospermophoren aus Japan. *Zoologischer Anzeiger*, 43, 342–370.

Verhoeff, K.W. (1929) Arthropoden aus Südostalpine Höhlen, Heft 7. *Mitteilungen über Höhlen- und Karstforschung*, 1–21.

Verhoeff, K.W. (1928) Zur Kenntnis der Diplopodenfauna Ungarns. 109. Diplopoden-Aufsatz (Chilopoden). *Állatani Közlemények*, 25, 124–126; 182–199.

Verhoeff, K.W. (1932) Dr. Bronn, H.G.'s Klassen und Ordnungen des Tier-Reichs wissenschaftlich dargestellt in Wort und Bild. 5 (II). Gliederfüssler: Arthropoda. Klasse Diplopoda, 2. Akademische Verlagsgesellschaft m.b.H., Leipzig, pp. 1073–2084.

Werren J.H. & Windsor D.M. (2000) *Wolbachia* infection frequencies in insects: evidence of a global equilibrium? *Proceedings of the Royal Society, B-Biological Sciences,* 267 (1450), 1277–1285. http://dx.doi.org/10.1098/rspb.2000.1139

Witzel, K.-P., Zakharov, I.A., Goryacheva, I.I., Adis, J. & Golovatch, S.I. (2003) Two parthenogenetic millipede species/lines of the genus *Poratia* Cook & Cook, 1894 (Diplopoda, Polydesmida, Pyrgodesmidae) found free from *Wolbachia* bacteria. *African Invertebrates*, 44, 331–338.