

REDESCRIPTION OF THE CAVE-DWELLING
BRACHYDESMUS TROGLOBIUS DADAY, 1889 (DIPLOPODA,
POLYDESMIDA)

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The cavernicolous polydesmid *Brachydesmus troglobius* Daday, 1889 was described from the Hungarian Abaliget Cave (Mecsek Mountains). Although previously known from only its type locality, the species was later found in caves of other European countries. Redescription of the species from museum samples and newly collected material using optical and scanning electron microscopy is here complemented with mitochondrial cytochrome c oxidase subunit I (COI) sequences as barcodes. New distributional data, remarks on the species' ecology and suggestions for its conservation are also given. Using COI sequences of 17 polydesmid taxa from GenBank and our own collections, we delimit interspecific and intergeneric boundaries within the family Polydesmidae.

Key words: Hungary, Western Mecsek Mountains, caves, polydesmid, redescription, phylogeny, troglobiont.

INTRODUCTION

The polydesmid millipede *Brachydesmus troglobius* Daday, 1889 was first found in the Abaliget Cave (South Hungary, Mecsek Mountains) on an unknown date by the Hungarian entomologist János Pável (1842–1901). It was described as a species endemic to the cave (DADAY 1889) by Jenő Daday (1855–1920), curator of invertebrates in the Hungarian National Museum. The rather short description, written in Latin, did not contain any drawings; however, Daday commented on the similarity to *B. subterraneus* Heller, 1858: the two quite similar species can be distinguished by the different shape and structure of the collum and the gonopods. VERHOEFF (1928) described and illustrated the gonopods of *B. troglobius* in a publication on the Hungarian millipede fauna. Later faunistic records from the Abaliget Cave were published by BOKOR (1924), GEBHARDT (1934, 1963, 1967), KORSÓS (2000), KORSÓS *et al.*

(2006) and ANGYAL and KORSÓS (2013). *B. troglobius* was collected in numerous caves in the Dinaric Karst, as well, and there are records from Croatia, Montenegro, Romania, Serbia and Slovenia (STRASSER 1971, MRŠIĆ 1988, 1994, 1998, ČURČIĆ & MAKAROV 1998, MAKAROV 2004, ENGHOFF 2013). CEUCA (1992) published a paper about the variability of the gonopod in Romanian populations of some *Polydesmus* and *Brachydesmus* – including *B. troglobius* – species. ČURČIĆ and MAKAROV (1998) described the postembryonic development of *B. troglobius* in samples from Lazareva Cave (Serbia), and revealed that the species completes its entire life cycle within the cave. However, GEBHARDT (1966) and MRŠIĆ (1988) mention the observation of epigeal populations in Hungary and Serbia. MAKAROV *et al.* (2012) studied the chemical defense of the species and showed that it secretes allomones against predators.

‘Integrative taxonomy’ is the science that aims to delimit the units of life’s diversity from multiple and complementary perspectives, applying comparative morphology, phylogeny, population genetics, ecology, development, behaviour, etc. (DAYRAT 2005). For phylogenetic reconstruction in the order Polydesmida, cladistic analysis based solely on morphological characters has been the traditional method (e.g. SIMONSEN 1990, BUENO-VILLEGAS *et al.* 2008, DJURSVOLL *et al.* 2000). However, recently, application of molecular taxonomic data for the same purpose also started to unfold (e.g. MAREK & BOND 2006, MAREK & BOND 2007, SPELDA *et al.* 2011).

The isolated Mecsek Mountains are situated in South Hungary and surrounded by the Pannonian plains (Fig. 1). The range is populated by a relatively high number of locally endemic species, the origin of which may date back to the Tertiary and therefore may have survived mass extinctions in previous glacial periods (GEBHARDT 1967). The subterranean environment of the Mecsek Mts harbors numerous endemic terrestrial and aquatic invertebrates, known only from one or a few caves (ANGYAL *et al.* 2015). Using preserved museum samples and newly collected material from two caves of the Mecsek Mts (including the type locality), our aim was to contribute to the knowledge of the morphology and molecular genetics of *B. troglobius* with the help of scanning electron microscopy, DNA barcoding and phylogenetic analysis. Our additional aim was to improve understanding of the species’ distribution and ecology and to provide a basis for future conservation management decisions.

Table 1. Basic data of the Abaliget Cave and the Törökpince Cave (both in Mecsek Mts).

Name of cave	Abaliget Cave	Törökpince Cave
Type of cave	outflow cave	inflow cave
Cadastré number	4120-1	4120-13
Entrance's altitude above sea level (m)	218	275
Entrance's coordinates (WGS-84 FI)	46°08'11"N	46°07'57"N
Entrance's coordinates (WGS-84 LA)	18°06'59"E	18°06'35"E
Length of cave (m)	2000	87
Vertical extension of cave (m)	48	7

MATERIAL AND METHODS

Sampling sites and methods

Fourteen caves of Western Mecsek Mts (South Hungary) were regularly visited between 2010 and 2013 to sample their fauna. Among these, *B. troglobius* populations were

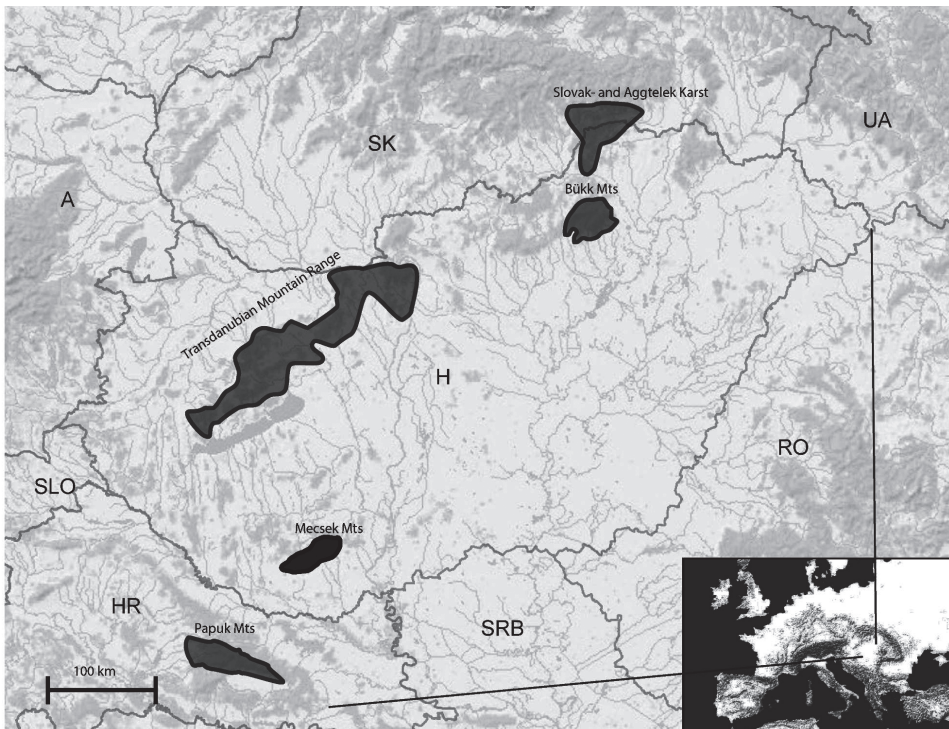


Fig. 1. Location of the Mecsek Mts among the Hungarian karstic mountains and the Croatian Papuk Mts (map by G. Balázs)

found only in the type locality (Abaliget Cave) and the Törökpince Cave. Both of these are situated near Abaliget village (18 km from Pécs city), and their entrances open less than a kilometre from each other. Basic data of the two caves are listed in Table 1. The highly protected Abaliget Cave is the largest cave known in the Mecsek Mts. With its three collaterals (Eastern, Western 1 and Western 2) and the main passage, the total length of the cave is 2000 m. Its lowest point below the entrance is 10 m, while its highest point is 38 m above the entrance (HAVASI *et al.* 2003). The Western 2 collateral is in connection with the Akácos Cave, which serves as a second entrance to the Abaliget Cave. The Abaliget Cave is characterized by both streaming and stagnant water. The most significant nutrient source of the cave is vegetable material of epigeal origin aggregated in the stream's alluvium and the decaying wooden fragments introduced by human activity. The cave has been opened to the public since 1957. Some of the most attractive speleothem formations are illuminated by lamps, which has caused the development of a 'lamp flora' serving as an alternative energy source for the cave-dwelling invertebrates (ANGYAL 2015). Bat guano accumulations are not a substantial element of the ecosystem, as the formerly vast bat colonies have been recently reduced (SZATYOR 2005). At 12.6 °C, the average temperature of the cave is relatively high, and considerable fluctuation can be detected only up to 40–50 m from the entrance. The relative air humidity is 97%.

The Törökpince Cave is formed in conglomerate. The cave opens with an extremely tight entrance aperture, which continues in an 87 m long, narrow horizontal passage. Recently, the cave has proven to be dry in all seasons. As the cave directly opens in deciduous woodland, its first few meters contain a massive amount of organic matter, which supports the existence of troglone invertebrate species in the entrance zone.

In both caves, specimens were collected by hand using entomological (soft) forceps or an aspirator, and by baited or unbaited pitfall traps. Among the different types of baits (meat, dog food, cheese, beer) used for sampling the cave invertebrate fauna, only the beer attracted the millipedes. Specimens were preserved and stored in 70% ethanol for morphological purposes and in 96% ethanol for molecular studies, and are deposited in the Myriapoda Collection of the Hungarian Natural History Museum (HNHM), Budapest.

Morphological studies

Brachydesmus specimens were examined under a Leica M125 stereomicroscope. In some cases the male's gonopods were dissected and studied under higher magnification. A drawing tube mounted on a Leica DM1000 light microscope was used for making drawings. Redescription was made using the characters of Polydesmida character matrix by DJURSVOLL *et al.* (2000) and the characters of ANTIC *et al.* (2013), which follow modern trends in millipede morphological taxonomy. Scanning electron micrographs of the main characters of a male and a female specimen of *B. troglobius* were made with a Hitachi S-2600 N scanning electron microscope in the Department of Botany of the HNHM. Specimens were placed in absolute ethanol for one day, then cleaned in an EMAG Emmi-16 Ultrasonic Cleaner and air-dried. Dry samples were attached to stubs and were sputter-coated by gold-palladium. Micrographs were digitally edited. Multilayer photos of entire specimens were taken in the Department of Zoology of the HNHM with a Nikon D5200 camera using Mitutoyo M Plan Apo 5X microscope lens and single flash diffused with a paper cylinder. Exposures were stacked with Zerene Stacker software.

Table 2. New COI samples used in the phylogenetic analysis. Abbreviations: HU = Hungary, RS = Republic of Serbia; DA = Dorottya Angyal, SM = Slobodan Makarov.

Genbank accession number	Species	Country	Region, town	Cave name	Date of collection	Collector
KT343290 (BR_TRO/Aba)	<i>B. troglobius</i> Daday, 1889	HU	Mecsek, Abaliget	Abaligeti	13. 06. 2013	DA
KT343289 (BR_TRO/Ser)	<i>B. troglobius</i> Daday, 1889	RS	West Serbia, Valjevo	Petnička	21. 05. 2010	SM
KT343291 (BR_SUP/Alba)	<i>B. superus</i> Latzel, 1884	HU	Bakony, Csőszpuszta	Alba Regia	10. 11. 2012	DA
KT343292 (BR_HER/Ser)	<i>B. herzogowinensis</i> Verhoeff, 1897	RS	West Serbia, Ivanjica	Hadži Prodanova	23. 07. 2012	SM
KT343288 (PO_DEN/Soly)	<i>P. denticulatus</i> C. L. Koch, 1847	HU	Budai Mts, Solyvár	Solyvári- ördöglyuk	03. 03. 2012	DA

Molecular studies

DNA extraction of three *Brachydesmus* and one *Polydesmus* species from five different caves in Hungary and Serbia was performed in the Laboratory of Molecular Taxonomy of the HNHM, using QIAamp DNA Microkit® (QIAGEN) following the manufacturer's instructions. Data for the samples used are listed in Table 2. The primer pairs were used for PCR amplifications of cytochrome c oxidase subunit I (COI) were LCO 1490 – HCO 2198 (FOLMER *et al.* 1994) and LCO 1490 – COI-H (MACHODROM *et al.* 2003) (Table 3). PCR products were cleaned using Roche High Pure Purification Kit® according to manufacturer's instructions. Fragments were sequenced in an ABI 3130 sequencer, using PCR amplification primers.

Protocols and thermo profiles used in PCR were as follows:

Primers: LCO 1490 (forward), HCO 2198 (reverse)

PCR reactions (ca 25 µl total) were obtained by mixing 10.775 µl mQ water, 2.5 µl 10× PCR buffer (with MgCl₂), 3.125 µl dNTP, 1.75 µl of each primer (5 µM), 0.01 µl Fermentas Dream Taq DNA Polymerase® (5U/ µl) and 5 µl DNA extract. PCR cycling: initial denaturation for 1 min at 95 °C, denaturation for 1 min at 94 °C, hybridization for 1 min 30 sec at 42.9 °C, and polymerization for 1 min 30 sec at 72 °C. After 40 cycles the sample was left for 6 min 72 °C.

Table 3. Primers used with *Brachydesmus* and *Polydesmus* species.

Marker	Primer	Direction	Sequence	Reference
COI	LCO 1490	Forward	5' GGTCAACAAATCAT- AAAGATATTGG 3'	FOLMER <i>et al.</i> 1994
COI	HCO 2198	Reverse	5' TAAACTTCAGGGT- GACCAAAAAAT 3'	FOLMER <i>et al.</i> 1994
COI	COI-H	Reverse	5' TCAGGGTGAC- CAAAAAATCA 3'	MACHODROM <i>et al.</i> 2003

Table 4. GenBank sequences used in phylogenetic analysis.

GenBank accession number	Valid name	Publication	Distribution of species
HQ966183.1	<i>Brachydesmus superus</i> Latzel, 1884	SPELDA <i>et al.</i> 2011	widely distributed in Europe, East Palaearctic, N Africa and Australia
JN306630.1	<i>Polydesmus edentulus</i> C. L. Koch, 1847	SPELDA <i>et al.</i> 2011	widely distributed in Europe
JN306634.1	<i>Propolydesmus helveticus</i> (Verhoeff, 1894)	SPELDA <i>et al.</i> 2011	Austria, France, Germany, Switzerland
HQ966172.1	<i>Propolydesmus testaceus</i> (C. L. Koch, 1847)	SPELDA <i>et al.</i> 2011	widely distributed in N Europe
HQ966181.1	<i>Polydesmus denticulatus</i> C. L. Koch, 1847	SPELDA <i>et al.</i> 2011	widely distributed in Europe and Nearctic Region
HQ966182.1	<i>Polydesmus denticulatus</i> C. L. Koch, 1847	SPELDA <i>et al.</i> 2011	widely distributed in Europe and Nearctic Region
HQ966176.1	<i>Polydesmus angustus</i> Latzel, 1884	SPELDA <i>et al.</i> 2011	widely distributed in Europe and Nearctic Region
HQ966178.1	<i>Polydesmus complanatus</i> (Linnaeus, 1761)	SPELDA <i>et al.</i> 2011	widely distributed in Europe, Near East and Nearctic Region
HQ966177.1	<i>Polydesmus complanatus</i> (Linnaeus, 1761)	SPELDA <i>et al.</i> 2011	widely distributed in Europe, Near East and Nearctic Region
HQ966180.1	<i>Polydesmus complanatus</i> (Linnaeus, 1761)	SPELDA <i>et al.</i> 2011	widely distributed in Europe, Near East and Nearctic Region
HQ966179.1	<i>Polydesmus complanatus</i> (Linnaeus, 1761)	SPELDA <i>et al.</i> 2011	widely distributed in Europe, Near East and Nearctic Region
JN637363.1	<i>Antichtropus variabilis</i> (Attems, 1911)	WOJCIESZEK & SIMMONS 2012	Australia

Primers: LCO 1490 (forward), COI-H (reverse)

PCR reactions (ca 25 µl total) were obtained by mixing 8.775 µl mQ water, 2.5 µl 10× PCR buffer, 2 µl 25 mM MgCl₂, 3.125 µl dNTP mix (2 mM), 1.75 µl of each primer (5 µM), 0.1 µl Fermentas Taq Polymerase® (5 U/µl) and 5 µl DNA extract. PCR cycling: initial denaturation for 1 min at 94 °C, denaturation for 1 min at 94 °C, hybridization for 1 min 30 sec at 40 °C, and polymerization for 1 min 30 sec at 72 °C. After 40 cycles the sample was left for 6 min at 72 °C.

Phylogenetic analysis

In order to evaluate the phylogenetic relationships within the genus *Brachydesmus* Heller, 1858, as well as the intergeneric distances between *Brachydesmus* and other polydesmid genera, a dataset of COI markers was compiled, using our own data and sequences downloaded from GenBank. Accession numbers for our own samples are listed in Table 2. Another five polydesmid species were included in the dataset (Table 4), summing up a total of nine polydesmid species in three genera. For *Brachydesmus*, only *B. superus* sequence was available from GenBank at the time of our analysis. *Antichiropus variabilis* (Paradoxosomatidae) was included in the dataset as outgroup taxon.

The DNA sequences were aligned with ClustalW (THOMPSON *et al.* 1994) implemented in MEGA 6.06 (TAMURA *et al.* 2013). Nucleotide substitution model selection carried out with MEGA V6.0 using the Bayesian information criterion (BIC) (SCHWARZ 1978) revealed that the best fitting model for COI is GTR + G + I. Phylogenetic tree was constructed using Bayesian analysis with BEAST 1.8.0 (DRUMMOND & RAMBAUT 2007) using Metropolis coupled Markov chain Monte Carlo simulations for 10 million generations, sampling a tree in every 1000 generations. After removing the first 2000 trees as burn-in, the remaining 8000 sampled trees were analyzed with TreeAnnotator v1.8.0 and visualized by FigTree 1.4.0 (RAMBAUT 2012). Pairwise genetic distances were calculated in MEGA 6.06 using the Kimura 2-Parameter model (KIMURA 1980).

RESULTS

Redescription of *Brachydesmus troglobius* Daday, 1889 (Figs 2–7)

Brachydesmus troglobius: DADAY 1889 (description), VERHOEFF 1928 (additional morphological data), BOKOR 1924 (faunistic data), GEBHARDT 1934, 1963, 1967 (faunistic data), STRASSER 1971 (distributional data), MRŠIĆ 1988 (distributional data), MRŠIĆ 1994, 1998 (distributional data), CEUCA 1992 (morphological data), ČURČIĆ & MAKAROV 1998 (morphological data), KORSÓS 2000 (faunistic data), MAKAROV *et al.* 2004, (distributional data), KORSÓS *et al.* 2006 (faunistic data), MAKAROV *et al.* 2012 (physiological data), ANGYAL & KORSÓS 2013 (additional distributional data), ENGHOFF 2013 (distributional data).

Material examined – Historic museum samples: **830/1888**, 205/253, Abaliget Cave, leg. ?, det. J. Daday, revid. D. Angyal, 2015: 2 ♀, broken, in bad condition, labeled as **SYNTYPES**; **1722/1928**, Abaliget Cave, 21/10/1922, leg. E. Bokor, det. K. W. Verhoeff, revid. E. Loksa, 245/1953, revid. D. Angyal, 2015: 5 ♀, 3 ♂; **1720/1928**, Abaliget Cave, 10/1923, leg. E. Bokor, det. K. W. Verhoeff, revid. E. Loksa, 240/1953, revid. D. Angyal, 2015: 2 ♀; **1721/1928**,

Abaligeti Cave, 12/08/1924, leg. E. Dudich, det. K. W. Verhoeff, revid. E. Loksa, 234/1953, revid. D. Angyal, 2015: 2 ♀, 1 ♂, 2 juv.; **1719/1928**, Abaligeti Cave, 15/03/1925, leg. E. Bokor, det. K. W. Verhoeff, revid. Loksa 257/1953, revid. D. Angyal, 2015: 1 ♂, 1 ♀; **40**, Abaligeti Cave, 19/05/1930, leg.?, det.?, revid. D. Angyal, 2015: 25 ♀, 4 ♂, 11 juv.; **3571**, Abaligeti Cave, 12/09/1991, leg. Z. Korsós & H. Read, det. Z. Korsós, revid. D. Angyal, 2015: 2 ♀, 1 juv.

Newly collected material: **BRT-01**, Abaligeti Cave, Western 2 collateral, 25/11/2010, leg. D. Angyal, det. Z. Korsós, 1 ♂; **BRT-02**, Abaligeti Cave, main passage, 22./09/2010, leg. D. Angyal, det. Z. Korsós, 1 ♂; **BRT-03**, Abaligeti Cave, entrance of Western 2 collateral, 09/12/2010, leg. D. Angyal, det. D. Angyal, 2 ♂; **BRT-04**, Abaligeti Cave, main passage, 350 m from entrance, on lamp flora, 04/11/2010, leg. D. Angyal, det. Z. Korsós, 1 ♀; **BRT-05**, Abaligeti Cave, main passage, 'Nagyterem', on lamp flora, 23/11/2010, leg. D. Angyal, det. D. Angyal, 1 ♀, 1 juv; **BRT-06**, Törökpince Cave, 30 m from entrance, 27/10/2010, leg. D. Angyal, det. Z. Korsós, 1 ♀; **BRT-07**, Abaligeti Cave, Western 2 collateral, 25/11/2010, leg. D. Angyal, det. Z. Korsós, 1 juv.; **BRT-08**, Abaligeti Cave, main passage, before the siphon, 10/11/2010, leg. D. Angyal, det. Z. Korsós, 1 ♀; **BRT-09**, Abaligeti Cave, Eastern collateral, 23/11/2010, leg. D. Angyal, det. D. Angyal, 1 ♀, 1 ♂; **BRT-10**, Abaligeti Cave, Eastern collateral, 22/09/2010, leg. D. Angyal, det. D. Angyal, 1 juv.; **BRT-11**, Abaligeti Cave, entrance of Western 2 collateral, 19/04/2011, leg. D. Angyal, det. D. Angyal, 1 juv.; **BRT-12**, Abaligeti Cave, main passage, 350 m from entrance, on lamp flora, 22/09/2010, leg. D. Angyal, det. D. Angyal, 1 ♂; **BRT-13**, Abaligeti Cave, main passage, upper passage before the siphon, 23/03/2013, leg. D. Angyal, A. Mock & P. Luptačik, det. D. Angyal, 1 ♂; **Abaligeti Cave**,

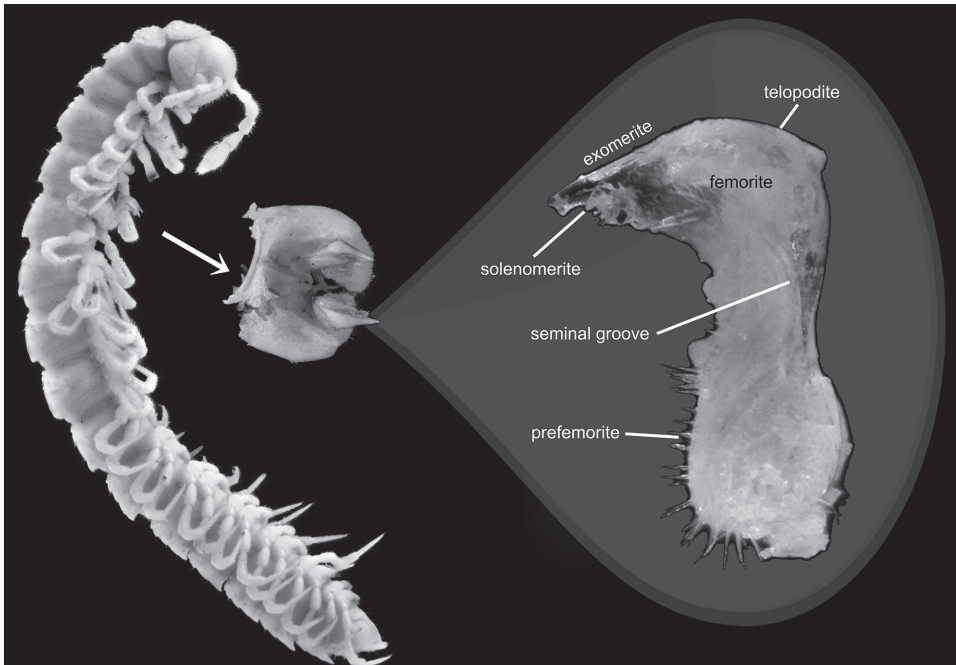


Fig. 2. *Brachydesmus troglobius*, male from the Abaligeti Cave, habitus and gonopods (photo by T. Németh)

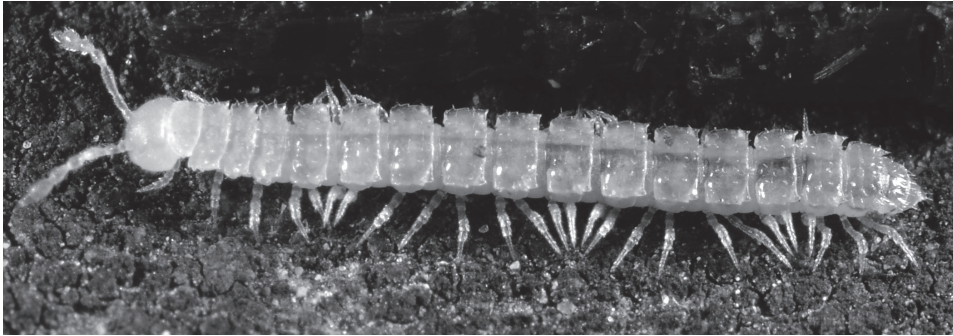
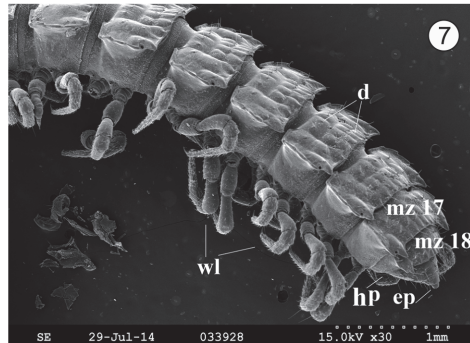
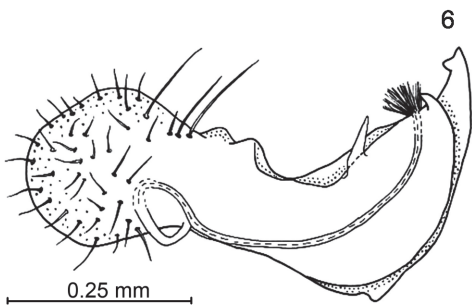
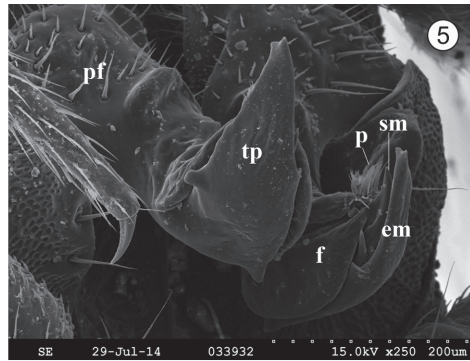
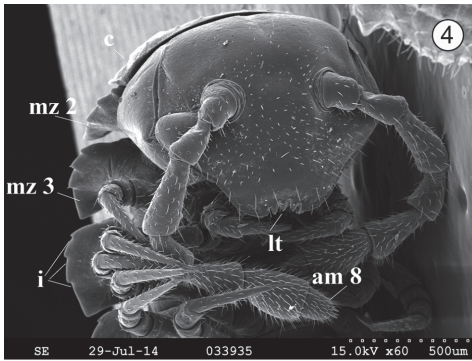


Fig. 3. *Brachydesmus troglobius*, male from Törökpince Cave (in situ photo by Z. Korsós)



Figs 4-7. *Brachydesmus troglobius*, male from the Abaliget Cave: 4 = head, frontal view, scanning electron micrograph. am 8 = antennomere VIII, c = collum, i = incisions, lt = labral teeth, mz 2 = metazonite II, mz 3 = metazonite III; 5 = gonopods in situ, scanning electron micrograph. em = exomerite, f = femorite, p = pulsilla, pf = prefemorite, sm = solenomerite, tp = telopodite; 6 = right gonopod, mesal view; 7 = posterior body segments, dorsolateral view, scanning electron micrograph. d = dentate metazonite, ep = epiproct, hp = hypoproct, mz 17 = metazonite XVII, mz 18 = metazonite XVIII, wl = walking legs

Eastern collateral, lamp flora, 13/06/2012, leg. D. Angyal, det. Z. Korsós, 3 ♀, 1 ♂; **Török-pince Cave**, 50 m from entrance, 11/06/2012, leg. D. Angyal, det. Z. Korsós, 1 ♀, 1 ♂.

Total body length 10–12 mm, eyeless, depigmented (from white to light brown). Adult males and females with 19 body rings (17 + 1 + telson).

Head (Figs 2–4): Broader than collum, densely covered by minute setae. Three well developed labral teeth visible. Occipital sulcus visible. Antennae long, surpassing somite 3. Antennomere I length is 2/3 of antennomere II. Antennomeres II, IV and V approximately equally long. Antennomere III longest among all. Antennomere VI slightly shorter than antennomere III. Antennomere VII length is 1/3 of antennomere VI. One C-shaped sensitive seta on antennomere VII visible. All antennomeres densely covered with setae. Antennomeres IV–VII with 1–3 long sensitive setae. Subapically, antennomere VII with knob-supporting field of few sensitive microsetae. Apical antennomere with 4 large cones.

Collum (Figs 2, 4): Convex, anterior and posterior edge both semicircular without caudal incisions of lateral sides.

Body (Figs 2, 3, 7): Body segments gently broadening until segment VII, parallel-sided from segment VIII to XV, and from segment XVI rapidly tapering toward body end. Metazonite II, III, IV, VI, VIII and XI with 3 incisions, while metazonite V, VII, IX, X, XII–XVIII with 4 incisions. Yellow colored ozopores laterally clearly visible on living specimens. Ozopores situated near caudal corner of paraterga on metazonite V, VII, IX, X, XII–XVIII. Posterior edges of metazonites dentate. Epiproct medium sized, subtriangular in dorsal view, slightly flattened dorsoventrally. Tip of epiproct rounded. Paraprocts semicircular, each with a knob-supporting seta. Hypoproct subtrapezoid. Male leg length medium, between 1.5–2.0 times as long as midbody height.

Gonopods (Figs 2, 5, 6): Telopodite a little longer than coxite. Prefemur shorter than femorite. Prefemoral setation normal, dense. Femorite slightly elongated and simple with a single exomerite. Distal loop of seminal groove relatively long. Accessory seminal chambers absent. Setose pulvillus large with armature. Solenomerite small. Exomerite small, delicately curved. Position of its base lateral, starts near recurved point of seminal groove. Exomerite slightly longer than femorite at best and separated from it by a sulcus.

Molecular studies on B. troglobius and other polydesmids

In the COI genetic distance matrix (Table 5) it was found that *B. troglobius* from the Abaliget Cave differed only by 0.9% from *B. troglobius* collected in the Serbian Petnička Cave, and all substitutions proved to be synonymous. The two Hungarian *Brachydesmus* species (*B. troglobius* and *B. superus*) collected in caves from two different karstic areas, differed by 9.8%. As it can be seen in the Bayesian tree (Fig. 8), among the studied taxa, the closest relative of *B. troglobius* is *B. herzogowinensis*, however, further analysis will be necessary, because the length of the single available *B. herzogowinensis* COI sequence was only 430 bp long. Identification of a juvenile female specimen of *Polydesmus denticulatus* collected in the Solymári-ördöglyuk Cave was possible by comparison of its COI sequence with the *P. denticulatus* sequences available in GenBank. This *P. denticulatus* individual differed from the Hungarian

Table 5. Pairwise nucleotide K2P differences (in %) between the investigated polydesmid species/lineages.

species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1 <i>A. variabilis</i>																
2 <i>P. cf. edentulus</i>	0.225															
3 <i>B. herzogovinensis</i>	0.233	0.105														
4 <i>B. superus_Ger</i>	0.242	0.106	0.079													
5 <i>B. superus_Hun</i>	0.243	0.122	0.071	0.051												
6 <i>B. troglobius_Hun</i>	0.233	0.117	0.076	0.086	0.098											
7 <i>B. troglobius_Ser</i>	0.234	0.115	0.071	0.081	0.092	0.009										
8 <i>P. angustus</i>	0.249	0.156	0.155	0.155	0.155	0.154	0.161									
9 <i>P. complanatus_1</i>	0.261	0.159	0.149	0.138	0.143	0.162	0.159	0.154								
10 <i>P. complanatus_2</i>	0.263	0.157	0.155	0.135	0.137	0.160	0.157	0.148	0.008							
11 <i>P. complanatus_3</i>	0.261	0.159	0.149	0.138	0.143	0.162	0.159	0.154	0.000	0.008						
12 <i>P. complanatus_4</i>	0.263	0.157	0.155	0.135	0.137	0.160	0.157	0.148	0.008	0.000	0.008					
13 <i>P. denticulatus_Ger_1</i>	0.257	0.150	0.162	0.142	0.160	0.153	0.153	0.173	0.163	0.157	0.163	0.157				
14 <i>P. denticulatus_Hun</i>	0.258	0.156	0.180	0.150	0.168	0.166	0.166	0.180	0.170	0.164	0.170	0.164	0.013			
15 <i>P. denticulatus_Ger_2</i>	0.266	0.160	0.162	0.147	0.164	0.154	0.152	0.171	0.166	0.160	0.166	0.160	0.000	0.011		
16 <i>Pr. helveticus</i>	0.259	0.161	0.133	0.137	0.145	0.133	0.128	0.190	0.194	0.188	0.194	0.188	0.194	0.199	0.188	
17 <i>Pr. testaceus</i>	0.230	0.148	0.127	0.124	0.145	0.126	0.121	0.180	0.186	0.182	0.186	0.182	0.161	0.174	0.168	0.099

B. troglobius by 16.6%. Distinct species within *Brachydesmus* had at least 7% and maximum 9% difference. *Polydesmus* cf. *edentulus* was found to be closer related to the *Brachydesmus* species (differed by 10.5–11.6%) than to other *Polydesmus* species (differed by 15.5–15.8%), which raises the question of the generic placement of this species. The maximum intrageneric distance was 17.5% within the genus *Polydesmus*. The average intergeneric distance within the genera *Brachydesmus* and *Polydesmus* was 15.5%. *Propolydesmus* species differed in an average of 13.2% from *Brachydesmus* species and – not counting *P.* cf. *edentulus* –, 18.4% from *Polydesmus* species. The outgroup taxon *A. variabilis* differed by at least 22.5% from the other 16 taxa.

New distribution data for B. troglobius and remarks on its ecology

Despite our repeated visits to 14 caves of the Western Mecsek Mts, *Brachydesmus troglobius* was found only in a single cave apart from its type locality. In the Abaliget Cave they were distributed in the main passage, the Eastern collateral, and the Western 2 collateral (Fig. 9), feeding on the lamp flora and on decaying wood, or walking on the sediment, and rarely on speleothem forma-

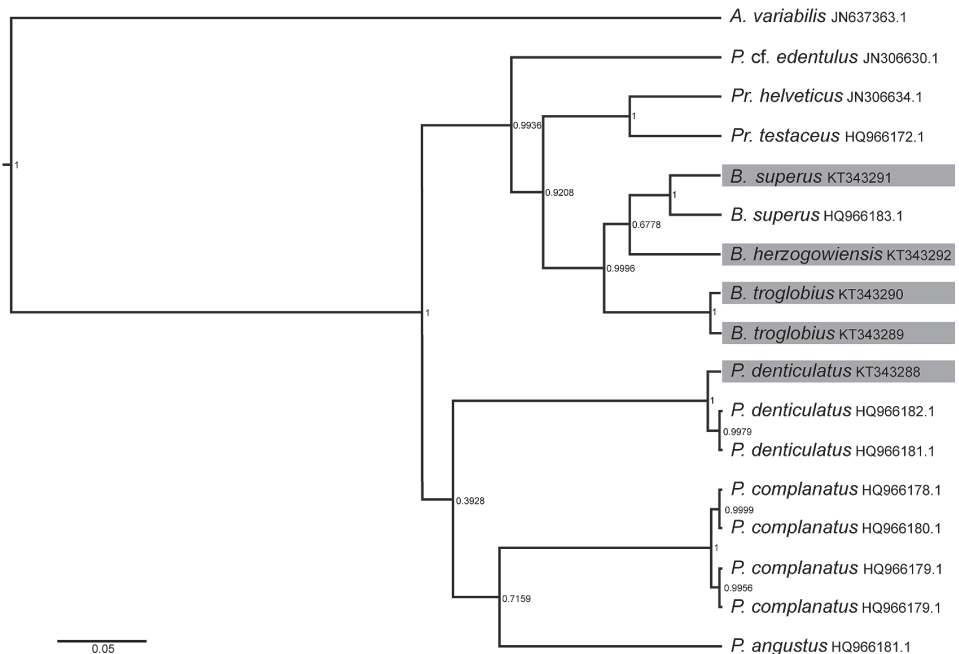


Fig. 8. Bayesian phylogenetic tree of 17 polydesmid taxa based on COI. Framed taxa represent own data

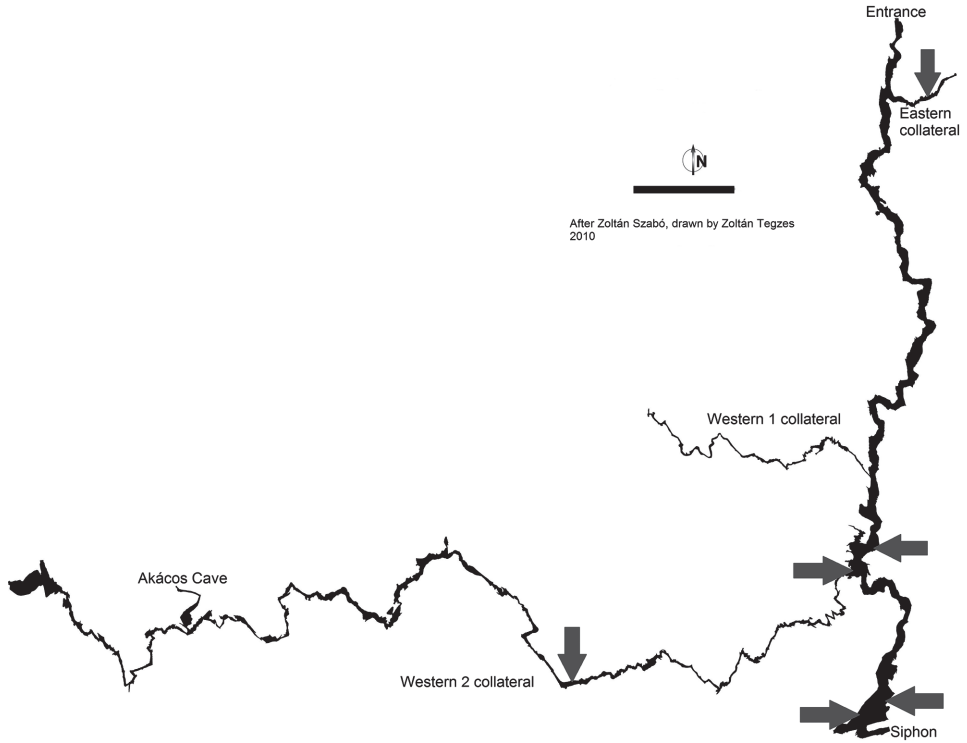


Fig. 9. Localities of *Brachydesmus troglobius* in the Abaliget Cave

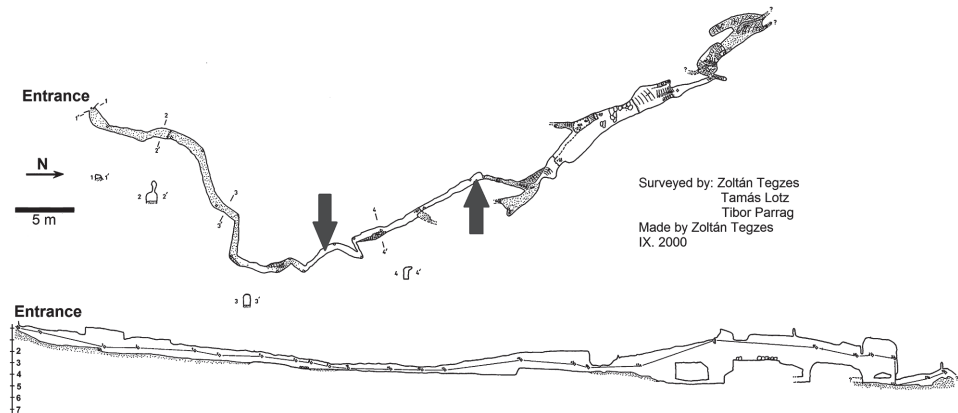


Fig. 10. Localities of *Brachydesmus troglobius* in the Törökpince Cave

tions. Coexistence with the eutroglophile *Trachysphaera schmidtii* Heller, 1858 and the oniscoid isopod *Haplophthalmus mengei* (Zaddach, 1844) was observed on some occasions, especially on the vegetation developed on illuminated speleothems. *B. troglobius* was also found in the Törökpince Cave, where male and female specimens were collected at 30 m from the entrance and from the deeper zone of the cave (Fig. 10), usually close to decaying material.

DISCUSSION

Application of modern molecular phylogenetic methods and morphological studies on the polydesmid *Brachydesmus troglobius* enabled understanding its relationship with other taxa. COI sequences of individuals from the Serbian Petnička Cave and from the Abaliget Cave showed only 0.9% K2P difference, which may suggest that the epigeal ancestor, which had been distributed in the Carpathian Mts and the Dinaric Alps, started to colonize the underground habitats and to evolve in isolation during the recent geological past.

Identification of polydesmid diplopods in the absence of mature males is circumstantial in some cases. The example of *Polydesmus denticulatus* identified by comparison of COI sequences shows the value of complementing traditional morphology with molecular systematics. The results of the phylogenetic study of polydesmid species in this article may help in future delimitation of intrageneric and intergeneric boundaries of European Polydesmidae.

Due to the isolation of hypogean habitats of the Mecsek area, a high degree of endemism likely developed in cave millipedes. VERHOEFF (1928) found very interesting the mixed zoogeographical character of the diplopod fauna of the Abaliget Cave. He noted the relationship of *Brachydesmus troglobius* with the Croatian–Illyrian fauna, while he considered *Hungarosoma bokori* (Verhoeff, 1928), also found in the Abaliget Cave (KORSÓS 2000), to be related to the Asian millipede fauna. The circum-Pannonian distribution of *H. bokori* corresponds with the hypothesis that *Hungarosoma* belongs to relict fauna of the microplates (mega-blocks) in the area of the present Hungary during the Tertiary period, which have recently slumped under sediments of the Pannonian Lowland (MOCK *et al.* 2014, 2016). VERHOEFF (1928) presumed the common lineages of *Haasea hungarica* (Verhoeff, 1928), another species from the Abaliget Cave (KORSÓS 2000), and the Central European millipede species, thereby indicating a European instead of Asian affiliation.

Predation and competition for resources are less intensive in subterranean habitats than in epigeal ones, due to the absence of higher trophic levels, to the low abundance of the species, and to the relatively constant environmental factors (CULVER & PIPAN 2009). *Brachydesmus troglobius* seems to maintain a stable population in the Abaliget Cave, using all types of vegetable organic

material. Although the appearance of the lamp flora is both an aesthetic and conservational problem in public caves like the Abaliget Cave, the vegetation confined to them seemed to be a regular source of energy not only for *B. troglobius*, but also for other detritivores (ANGYAL 2015). For this reason, the lamp flora should not be removed without considering the associated invertebrates. Live invertebrates could be recovered from manually removed vegetation by sifting or Berlese extraction on the spot, and could be transported to an unperturbed part of the cave, near to another potential nutrition source. Although *B. troglobius* is known from some other European countries, in Hungary the species possesses only one known locality apart from its type locality. Owing to their extremely narrow Hungarian distribution, the two local populations (one in the Abaliget Cave and another in the Törökpince Cave) are rare and of potential conservation value.

*

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