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Head of School: Prof. Dr. Angéla Anda

Integrative taxonomic revision of *Niphargus* spp. and other rare and endemic troglobiont macroinvertebrates from the caves of the Western Mecsek Mts. (South Hungary)

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Supervisor: Dr. Előd Kondorosy

Written by: Dorottya Angyal

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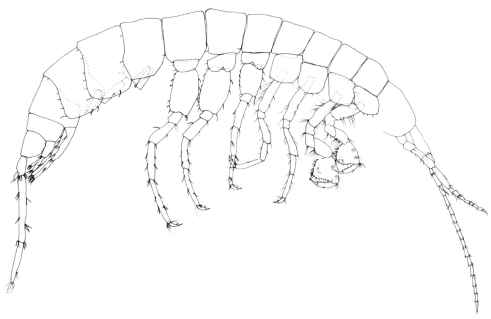

Integrative taxonomic revision of *Niphargus* spp. and other rare and endemic troglobiont macroinvertebrates from the caves of the Western Mecsek Mts. (South Hungary)

Értekezés doktori (PhD) fokozat elnyerése érdekében

Írta:

ANGYAL DOROTTYA

Készült a Pannon Egyetem Festetics Doktori Iskola keretében

Témavezető: Dr. Kondorosy Előd egyetemi docens, CSc

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A jelölt a doktori szigorlaton …….%-ot ért el.

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Szigorlati Bizottság elnöke

Az értekezést bírálóként elfogadásra javaslom:

Bíráló neve: igen/nem

Bíráló neve: igen/nem

A jelölt az értekezés nyilvános vitáján ……%-ot érte el.

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A doktori (PhD) oklevél minősítése

Az EDT elnöke

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ABSTRACT

Integrative taxonomic revision of *Niphargus* spp. and other rare and endemic troglobiont macroinvertebrates from the caves of the Western Mecsek Mts. (South Hungary)

Present thesis focuses on the careful revision of seven rare and endemic obligate cave-dwelling macroinvertebrate taxa, which inhabit some caves of the Western Mecsek Mts. The author provides detailed and richly illustrated redescriptions of *Niphargus molnari* Méhely, 1927 and *Niphargus gebhardti* Schellenberg, 1934 with the addition of cytochrome c oxidase subunit I (COI) barcode sequences, and presents phylogenetic relationships of both species within the genus *Niphargus* using three independent molecular markers. She completes the traditional morphological studies with comparative scanning electron microscopy, applied for the first time on niphargids, *Protelsonia hungarica hungarica* Méhely, 1924, *Protelsonia hungarica robusta* Méhely 1927 and *Brachydesmus troglobius* Daday, 1889. Using further integrative taxonomic methods, like the analysis of COI and 16S rRNA gene sequences as well as shell morphometric studies, the author contributes to the clarification of the taxonomic positions of *Bythiospeum hungaricum* (Soós, 1927) and *Bythiospeum* cf. *gebhardti* (H. Wagner, 1931). Due to the found distinguishing characters of the *Protelsonia* morphotypes, the author verifies the validity of the two separate subspecies. Performing phylogenetic studies, she contributes to the knowledge on the relationships of *B. hungaricum*, *B.* cf. *gebhardti* and *B. troglobius* to the rest of the rissooid and polydesmid genera, and also participates to the delimitation of their interspecific and intergeneric boundaries. Based on the new distributional data of the studied taxa and the evaluation of the present condition of their habitats, suggestions on their conservation planning are also added. A faunalist of 105 macroinvertebrate species and subspecies (apart from the revised taxa) from the 14 studied caves, including 25 and 7 taxa new for the fauna of the Abaligeti Cave and the Mánfai-kőlyuk Cave is also given.

KIVONAT

A Nyugat-Mecsek barlangjaiban élő ritka és endemikus valódi barlanglakó makrogerinctelenek integratív taxonómiai revíziója, különös tekintettel a *Niphargus* fajokra

Jelen disszertáció a Nyugat-Mecsek néhány barlangjában élő hét, ritka és endemikus, kizárólagosan barlangokhoz kötődő makrogerinctelen taxonjának körültekintő revíziójára összpontosít. A szerző a *Niphargus molnari* Méhely, 1927 és *Niphargus gebhardti* Schellenberg, 1934 fajok részletes és gazdagon illusztrált újraleírásait közli, melyeket a citokróm c-oxidáz I (COI) barcode szekvenciák megadásával és a három független marker felhasználásával készült, *Niphargus* genuszon belüli filogenetikai elemzéssel egészít ki. A hagyományos morfológiai módszereket a szerző összehasonlító pásztázó elektronmikroszkópia használatával ötvözi, melyre korábban nem volt példa vakbolharákok, valamint a *Protelsonia hungarica hungarica* Méhely, 1924, a *Protelsonia hungarica robusta* Méhely 1927 és a *Brachydesmus troglobius* Daday, 1889 fajok esetében. További integratív taxonómiai módszerek alkalmazásával - mint a COI és a 16S rRNA génszakaszok analízise, valamint a héjmorfometriai elemzések - a szerző hozzájárul a *Bythiospeum hungaricum* (Soós, 1927) és a *Bythiospeum* cf. *gebhardti* (H. Wagner, 1931) taxonómiai helyzetének tisztázásához. A *Protelsonia* morfotípusok esetében felderített elkülönítő bélyegek segítségével a szerző megerősíti a *P. hungarica robusta* alfaji elkülönítésének érvényességét. Az elvégzett filogenetikai vizsgálatok eredményeként a szerző hozzájárul a *B. hungaricum*, a *B.* cf. *gebhardti* és a *B. troglobius* rissooidea és polydesmida csoportokon belüli rokonsági fokának felderítéséhez és a fajok és génuszok közti határok kijelöléséhez. A szerző javaslatokat tesz a revideált fajok és alfajok védelmét illetően. A 14 vizsgált barlangból egy 105 makrogerinctelen taxonból álló faunalistát is közread, mely többek között 25 illetve 7 fajt és alfajt tartalmaz, melyek az Abaligeti-barlang és a Mánfai-kőlyuk faunájára újak.

RESUMEN

Revisión taxonómica integral de *Niphargus* spp. y otros macroinvertebrados troglobiontes raros y endémicos provenientes de las cuevas de montaña Mecsek occidental (Hungría del sur)

Esta tesis se enfoca en el revisión de siete táxones de macroinvertebrados troglobiontes obligadas raras y endémicas que habitan en algunas cuevas del Mecsek occidental. Se proveen re-descripciones e ilustraciones detalladas de *Niphargus molnari* Méhely, 1927 y *Niphargus gebhardti* Schellenberg, 1934, en conjunto con la secuencia 'código de barras' del Citocromo c oxidasa subunidad I (COI). Además presenta las relaciones filogenéticas de ambas especies dentro del género *Niphargus* utilizando tres marcadores moleculares independientes. Se aplicó el método tradicional de estudio morfológico comparando imágenes de microscopía electrónica de barrido tomadas por primera vez a los niphárgidos, *Protelsonia hungarica hungarica* Méhely, 1924, *Protelsonia hungarica robusta* Méhely 1927 y *Brachydesmus troglobius* Daday, 1889. La autora contribuye a esclarecer las posiciones sistemáticas de *Bythiospeum hungaricum* (Soós, 1927) y *Bythiospeum* cf. *gebhardti* (H. Wagner, 1931) utilizando métodos taxonómicos integrales adicionales como los análisis de COI y la secuenciación genética de 16S rRNA en conjunto con estudios morfométricos del caparazón. Debido a los caracteres distintivos encontrados en los morfotipos de *Protelsonia*, este estudio verifica la validez de la separación de ambas subespecies. Al realizar estudios filogenéticos la autora contribuye al conocimiento de las relaciones de *B. hungaricum*, *B*. cf. *gebhardti* y *B. troglobius* con el resto de los grupos rissooides y polydesmidos, lo que conduce a su vez a la delimitación de las fronteras interespecíficas y intergenéricas. Se agregan además sugerencias para la planeación de la conservación de los taxa estudiados basadas en nuevos datos de distribución y las condiciones actuales de sus hábitats. Se proporciona asimismo una lista de 105 especies y subespecies de macroinverterbrados (además de los táxones revisados) de las 14 cuevas estudiadas, incluidos 25 y 7 táxones nuevos para la fauna de la Cueva de Abaliget y Cueva Mánfai-kőlyuk.

1. INTRODUCTION AND LITERARY OVERVIEW

Over the past decades, the amount of zoospeleological research in Hungary has declined significantly. Due to the special challenges of collecting in caves and the low number of researchers involved in it, only a small proportion of the cavities have been sampled at all. Bajomi (1977) referred only 49 caves that had been studied in zoological aspect until the 1970s. Only 10 of them had been sampled more or less systematically, while from the remaining ones only sporadic data are available. Aside from a few studies (e.g. Korsós 2000, Seres 2000), between the 1980s and 2010, extensive zoospeleological research had not been performed either, which has resulted in a significant fallback compared to neighboring countries' such as Slovenia's or Romania's results and knowledge in this field.

Although, the Hungarian zoospeleology possessed a rather flourishing epoch too. In the 1920s, a productive period had begun, when illustrious zoologists of the era, like Endre Bokor, Lajos Soós, Lajos Méhely, Antal Gebhardt and Endre Dudich had started their intensive faunistic, taxonomic and ecological survey in some of the caves of the Aggtelek Karst and the Mecsek Mts., with special regard to the Baradla Cave, the Abaligeti Cave and the Mánfai-kőlyuk Cave (e.g. Bokor 1921, Méhely 1924, Soós 1927, Dudich 1932, Gebhardt 1934). Endre Dudich is dubbed as the 'father of the Hungarian biospeleology', as the establishment of the biospeleological laboratory in the Baradla Cave and the start of the 'Biologica Hungarica' publication series were also thanked to him (Salamon et al. 2014). From the end of the 1950s, still related to the survey of the Baradla Cave, further - mainly taxonomic - studies had been published dealing with various invertebrate taxa, like Nematoda, Oligochaeta, Palpigradi, Bathynellidae or Copepoda (e.g. Ponyi 1957, Andrássy 1959, Dózsa-Farkas 1970, Dózsa-Farkas & Loksa 1970, Zicsi 1974). Bajomi (1969) had performed the extensive zoological survey of the Meteor Cave and had proved the presence of 90 animal species.

Caves provide unique conditions which affect the development of a highly specific invertebrate fauna. The ecological classification of cave-dwelling animals was rather heterogeneous until the general acceptance of Sket's category system (Sket 2008). According to that, 'trogloxene' is a species that only occurs sporadically in a hypogean habitat, and unable to establish subterranean population. 'Subtroglophiles' are species inclined to perpetually or temporarily inhabit subterranean habitats, but are intimately associated with epigean habitats for some biological functions. 'Eutroglophiles' are essentially epigean species that are able to maintain a permanent subterranean population, while 'troglobiont' is a species which binds solely to hypogean habitats (Sket 2008). The latter group has the greatest importance in biospeleology. Aside from some cases (e.g. the pigmented *Duvalius* species), common features of the aquatic or terrestrial troglobiont animals are reduction of eyes, depigmentation, elongation of appendices and development of sensory and chemical organs (e.g. Culver et al. 1995, Trontelj et al. 2012). The troglobionts often possess a rather restricted distribution area; sometimes they are bound to a single cave or karstic area (Romero, 2009).

Fragmented mountain areas in East-Central Europe have been suggested to be centers of endemism that evolved through a complex geological history including Eocene marine regression-transgression cycles and Pleistocene glacial cycles (Mamos et al. 2014, Meleg et al. 2013, Hou et al. 2013). Mecsek Mountains is one of these isolated mountain ranges that is situated in South Hungary and surrounded by the Pannonian plains (Figure 1). In biological sense, the area is populated by a relatively high number of endemic species the origin of which may date back to Tertiary and which therefore apparently have survived mass extinctions in glacial periods (Gebhardt 1967). The subterranean environment of the Western Mecsek harbours numerous terrestrial and aquatic highly endemic invertebrates, known only from one or a few caves. Discovery and study of these species dates back to the end of the 19th century, when the first description of a troglobiont, endemic invertebrate species was born (Daday 1889a). Although, the real zoospeleological assessment of both the Abaligeti Cave and the Mánfai-kőlyuk Cave is due to the intensive research of Elemér Bokor, Antal Gebhardt, Lajos Méhely and Endre Dudich, carried out between the 1920s and 1930s (e.g. Bokor 1924, Dudich 1929, Gebhardt 1931; 1933; 1934, Méhely 1925). The vast majority of the taxa found in the caves had been collected by Antal Gebhardt, who successfully cooperated with the most relevant specialists of the era such as Karl Verhoeff, which had led to the description of some highly endemic species, like the chordeumatid millipede species *Hungarosoma bokori* Verhoeff, 1928 (Verhoeff 1928). Due to the collectors' and the taxonomists' results, until 1931, the presence of 159 and 190 animal species had been revealed from the Mánfai-kőlyuk Cave and the Abaligeti Cave (e.g. Gebhardt 1933, Kolosváry 1928, Mödlinger 1930, Stach 1929). Later on, further records of additional invertebrate species (e.g. *Bathynella chappuisi* Delachaux, 1920, Farkas 1957) had increased the number of the taxa found in the Abaligeti Cave. According to the last publication of Gebhardt written about the zoological survey of the Mánfai-kőlyuk Cave and the Abaligeti Cave, 159 and 286 animal species, respectively were known from the two caves (Gebhardt 1967), however he included the 92 Protozoa species identified from the Abaligeti Cave by Lajos Varga too (Gebhardt 1964). That time, eight taxa had been treated as endemic of the two caves. The planarians '*Dendrocoelides pannonicus*' (*Dendrocoelum pannonicum* Méhely, 1927) and '*Polycelis tóthi*' (*Polycelis tothi* Méhely, 1927), the aquatic isopod '*Stenasellus hungaricus* v. *robustus*' (*Protelsonia hungarica robusta* Méhely, 1927) and the hydrobiid '*Paladilhiopsis gebhardti*' (*Bythiospeum* cf. *gebhardti* (H. Wagner, 1931)) were known only from the Mánfai-kőlyuk Cave, while '*Stenasellus hungaricus*' (*Protelsonia hungarica hungarica* Méhely, 1924), the amphipod '*Niphargus foreli gebhardti*' (*Niphargus gebhardti* Schellenberg, 1934), and the hydrobiid '*Paladilhiopsis hungarica*' (*Bythiospeum hungaricum* (Soós, 1927)) were mentioned as the endemisms of the Abaligeti Cave. '*Niphargus leopoliensis molnári*' (*Niphargus molnari* Méhely, 1927) was known from both caves. Three millipede species, *Hungarosoma bokori* Verhoeff, 1928, '*Orobainosoma hungaricum*', (*Haasea hungarica* (Verhoeff, 1928)) and *Brachydesmus troglobius* Daday, 1889 were treated as rare fauna elements known only from a few habitats (Gebhardt 1967). Seven of these rare end endemic taxa had been chosen for further studies as objects of present thesis, namely the amphipods *Niphargus molnari* and *Niphargus gebhardti*, the aquatic isopods *Protelsonia hungarica hungarica* and *Protelsonia hungarica robusta*, the hydrobiid snails *Bythiospeum*

hungaricum and *Bythiospeum* cf. *gebhardti* and the polydesmid millipede *Brachydesmus troglobius*. All of them can be classified as troglobionts. Previous knowledge related to these taxa is detailed at the beginning of each chapters of the result part of present thesis.

In case of taxa with uncertain taxonomic positions - like the focal rare and endemic species -, revising all possible sources of data might increase the robustness of taxonomic conclusions (Padial et al. 2010). 'Integrative taxonomy' is defined as 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). Among some hardly identifiable invertebrate groups, like *Niphargus* or Hydrobiidae, the use of molecular studies for supplementing or confirming morphological data, spreads rapidly (e.g. Liu et al. 2001, Fehér et al. 2013, Ntakis et al. 2015). Another great advantage of molecular analysis of closely related taxa is the possibility of detecting cryptic species, which might influence future conservation decisions (Trontelj & Fišer 2009). For phylogenetic reconstruction in the order Polydesmida, the use of cladistic analysis based on morphological characters is the generally recognized method (Bueno-Villegas et al. 2008, Djursvoll et al. 2000), however recently, application of molecular taxonomic data for the same purpose also started to be unfolded (Spelda et al. 2011). Adaptation of shell morphometric studies is extremely helpful in cases of snail taxa with remote visible morphological characters (e.g. Harl et al. 2014a).

Applying the methods of modern integrative taxonomy, my aims were:

i) to revise the seven above mentioned rare and endemic troglobiont macroinvertebrate taxa from the caves of the Western Mecsek and to clarify their taxonomic positions;

ii) to contribute to the knowledge on morphology and molecular genetics of the focal taxa with the help of newly applied methods, like scanning electron microscopy, DNA barcoding or phylogenetic analysis;

iii) to record the new distributional data of the focal species and subspecies and to make suggestions on their conservation planning, based on evaluation of their rarity and the present condition of their habitats;

iv) and to create a faunistic list based on the newly collected aquatic and terrestrial macroinvertebrate material other than the revised species and subspecies.

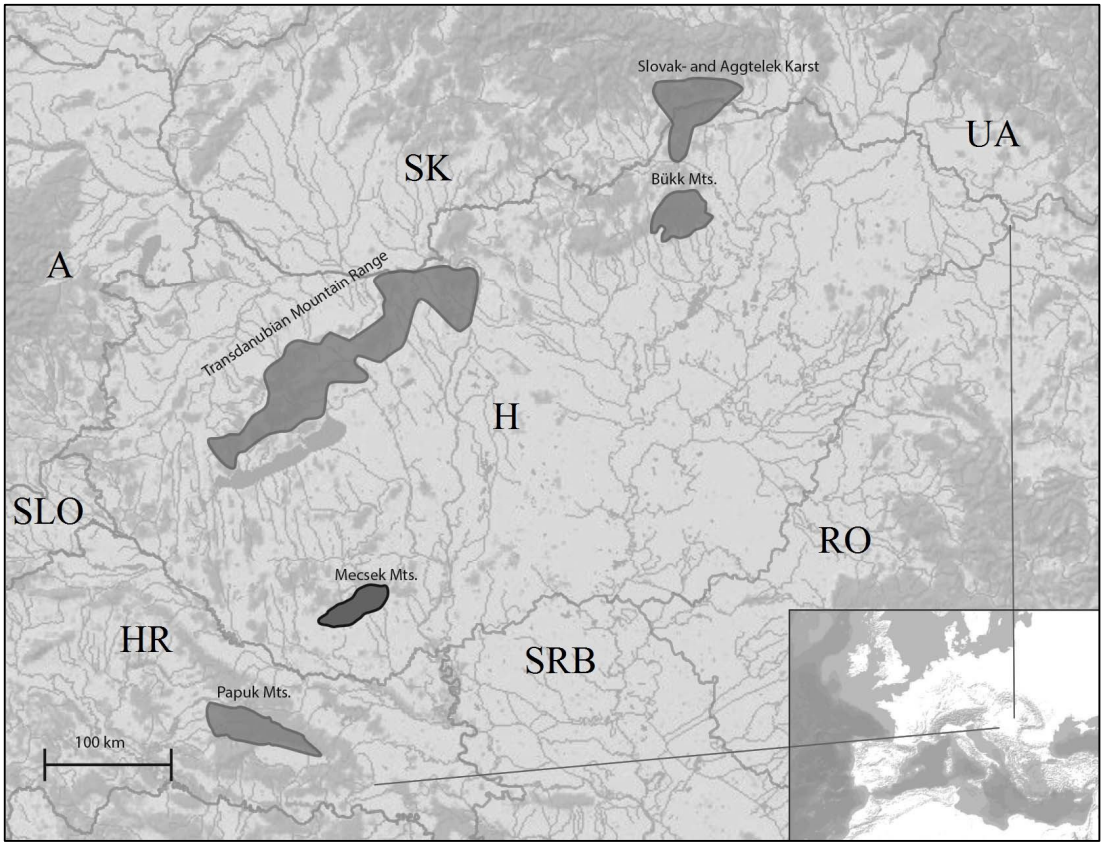


Figure 1: Location of the Mecsek Mts. and other karst areas within the Pannonian biogeographical region (Made by G. Balázs).

2. MATERIAL AND METHODS

2.1 Introduction of the karstic area and the studied caves

The Mecsek Mountains is relatively small with its approximately 545 km² extension. The base of the fragmented, creased-structured block mountain is a structural unit of granitoids and metamorphic rocks, formed in Variscan tectonic phase in Precambrium and early Perm. Until the end of the Paleozoic, due to the repeated and altering oriented crust movements, certain parts of the ancient mountain range descended, while others had eroded. The area belonged to the Pangea ancient continent and had been flooded by the Tethys Sea, which had caused intensive sediment formation. During the Triassic period of the Mesozoic (250-200 million years ago), sedimentary rocks, like sandstone, slaty clay, limestone and dolomite had been stratified on the 'Jakabhegyi Red Limestone' formation (Lehmann 2003). At present, 230 caves are known from the Mecsek Mts., however only 28 of them exceed 50 meters in length (Nyerges & Takácsné Bolner 2011). Mecsek Mts. can be divided into three parts: Western-, Middle- and Eastern Mecsek. Among these, Western Mecsek is the richest in karstic objects. Ten catchment areas are known from there, the most considerable ones are the Abaligeti Cave and the Kispaplika Spring in Abaliget, the Vízfő Spring and the Mészégető Spring in Orfű and the Mánfai-kőlyuk Cave, the Kánya Spring and the Melegmány Spring near Mánfa. The area is rich in surface karst phenomena too, like dolines and springs. Five caves of the area are highly protected by law.

During my investigations conducted between September 2010 and December 2013, 14 caves were studied in biospeleological aspect in the karstic area of the Western Mecsek with the permission of the Duna-Dráva National Park Directorate and the South Transdanubial Environmental Protection and Nature Conservation Inspectorate. Rare and endemic troglobiont macroinvertebrate taxa discussed in present thesis have been found in 8 of them, these caves are introduced below in the text. Basic data of the 14 studied caves are listed in Table 1. The studied caves belong to three different catchment areas. Some of the caves are hydrologically connected with each other according the following model. From the Szajha-felső Cave and the Vadetetős Cave the karstic water flows into the main passage of the Abaligeti Cave somewhere behind the siphon in a yet unknown part of the cave. As a part of the Vízfő catchment area, the karstic water of the Trió Cave, the Gilisztás Cave and the Spirál Cave associates, constituting the same hydrological system. The Mánfai-kőlyuk Cave originally was hydrologically distinct from the other six caves, however from the 1960s to the 1990s it was artificially connected with the Vízfő system for increasing the water supplement of Komló coal-mining city (Rónaki 1972, Tegzes 2012 pers. comm.).

Table 1: Basic data of the studied caves. Caves marked with '*' refer habitats of the focal revised rare and endemic macroinvertebrates.

Name of cave both in English and Hungarian	Type of cave	Cadastre number	Entrance's altitude above sea level (m)	Entrance's coordinates EOV-Y	Entrance's coordinates EOV-X	Length of cave (m)	Vertical extension of cave (m)
Abaligeti Cave * (Abaligeti-barlang)	outflow cave	4120-1	218	578 056.429	88 434.520	2000	48
Mánfai-kőlyuk Cave * (Mánfai-kőlyuk)	outflow cave	4120-2	240	585 324.364	89 720.420	360	11
Vadetetős Cave * (Vadetetős-víznyelőbarlang)	inflow cave	4120-27	320	577 872.842	86 795.058	180	35,7
Trió Cave * (Trió-barlang)	inflow cave	4120-71	301	580 722.262	86 347.182	250	58
Gilisztás Cave * (Gilisztás-víznyelőbarlang)	inflow cave	4120-70	307	580 693.262	86 268.727	134	51.1
Spirál Cave * (Spirál-víznyelő)	inflow cave	4120-130	350	582 719.925	87 242.072	1400	86.4
Szajha-felső Cave * (Szajha-felső-víznyelőbarlang)	inflow cave	4120-16	283	578 056.137	88 041.665	148	42
Törökpince Cave * (Törökpince-víznyelőbarlang)	inflow cave	4120-13	275	577 544.640	88 007.391	87	7
Római Cave (Római-zsomboly)	pothole	4120-222	248	578 465.730	88 298.610	26.6	24
Kispaplika Cave (Kispaplika-forrásbarlang)	outflow cave	4120-22	220	578 537.570	88 409.775	40	9.5
Nyárás-völgyi Cave (Nyárás-völgyi-víznyelő)	inflow cave	4120-31	291	578 760.081	86 896.453	34	19
Orfűi Vízfő Cave (Orfűi Vízfő-barlang)	outflow cave	4120-3	211	581 611.158	88 670.206	330	27
Achilles Cave (Achilles-víznyelőbarlang)	inflow cave	4120-90	288	580957.614	87 510.384	140	28
Akácos Cave (Akácos-víznyelőbarlang)	inflow cave	-	269	577 686.412	88 185.955	?	?

The highly protected Abaligeti Cave is the largest known cave 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 (Havasi et al. 2003). The Western 2 collateral is in connection with the Akácos Cave, which serves as a second entrance of the Abaligeti Cave. Hydrological connection of the Western 2 collateral with the periodically active Törökpince Cave had mentioned in the literature too (e.g. Gebhardt 1963), however the latter cave has been dried out for a long time. The Abaligeti Cave is characterized by both streaming and stagnant water. Streaming water

can be found in the main passage ('Styx Stream') and in the two Western collaterals. Shallow pools of water in the cave are of two types: some are formed by dripping water of the speleothems, whereas other are filled during floods and contain residual water. The most significant nutriment source of the cave is the epigean originated vegetal material aggregated in the stream's alluvium and the decaying wooden remains introduced by human activity. The cave has been opened for the public since 1957. Some of the most attractive speleothem formations are illuminated by lamps, which has caused the evolvement of the 'lamp flora', serving alternative type of energy source for the cave-dwelling invertebrates. Bat guano aggregations are not substantial elements of the ecosystem, as the one-time vast bat colonies have been recently reduced (Szatyor 2004). With its 12.6 ºC, the average temperature of the cave is relatively high. Considerable fluctuation can be detected only until 40-50 m distance from the entrance. The relative humidity is 97%.

The outflow cave, Mánfai-kőlyuk Cave is situated in the Eastern edge of the Western Mecsek, 3 km from Mánfa village and by today, it is in highly protected status. It opens with a remarkable, spacious entrance. The cave possesses an upper and a lower passage, the former had been discovered until its third siphon, and then the research had to be finished because of the artificial utilization of the cave. In 1957 the local waterworks started to use the cave's spring for supporting the water supplement of Komló mining city. In 1969, a 58 m long artificial tunnel was scooped in the upper passage, to carry the water to the dam in the Mély-völgy ('Deep valley') where the outflow water associated with the water of the nearby springs. 163 m from the entrance of the tunnel, a 10.8 m thick concrete dam had been emerged in order to swell the water behind that (Havasi 2003, Kordos 1984). These interventions had caused the change of the cave's hydrological system and the destruction of the natural passages with their original formations. Utilization of the cave's water had stopped in the 1990s, but the artificial pieces, like rusty water pipes and other instruments had been left in the cave (Figure 2). However, the Duna-Dráva National Park took steps towards the rehabilitation of the cave in 2013, it was impossible to set back the original, natural conditions. At present the possibilities for occupying suitable microhabitats in the cave by invertebrates are rather restricted. The water carrier canal in the upper passage and the streaming water in the lower passage serve as aquatic habitats, while the entrance region and the remained small lateral chambers with some organic material (e.g. decaying woods) provide shelter for the terrestrial hypogean fauna. The average temperature of the cave is 10.6 ºC, though, due to the deliberately large entrance, the fluctuation is rather high.

Vadetetős Cave is an inflow cave near Kővágótöttös village. Its present horizontal extension is 180 m, however it is under excavation by the local speleological group, Pro Natura Karst and Cave Research Society. The vast majority of the cave can be characterized by narrow passages, which widen in some certain parts, forming small halls. The cave is relatively rich in calcite and sinter formations. Shallow pools and slowly streaming waters can also be found in the cave. In the entrance region, appreciable amount of plant material (mainly decaying leaves) is deposited.

As parts of the same system, the two inflow caves, Trió Cave and Gilisztás Cave are situated in the Szuadó Valley near Orfű village. Their entrances are quite close to each other. With its 55 m depth, the Trió Cave is the third deepest cave of the area. It is rather rich in speleothem formations and it possesses various combinations of spacious halls, narrow passages and high chimney-stacks. Reminding to a pothole, Gilisztás Cave consists of mainly vertical, descending parts with a siphon at its end. Both caves are built up by steel ladders to ease the movement in the cave. Regarding the water bodies, the two caves can be characterized mainly by small pools formed by dripping water and residual water. Some wooden remains can be found in the caves due to human impact, which could serve as nutrition resource for the cave-dwelling fauna.

The Spirál Cave is situated near Pécs city. With its 86 m depth, it is the deepest cave of the Mecsek Mts. Its horizontal extension is also remarkable, the known length is at present 1400 m and it is still under excavation by the Karst Research Group of Mecsek. The majority of the vertical passages are supplied by ladders, though; some parts of the caves can be visited only by applying rope technique using special equipment. Chimney-stacks, spacious halls, narrow rives and smaller chambers vary in the caves. A streamy branch can also be found at the lowest level, which ends in a siphon. Beautifully developed speleothems and sinter pews can also be observed, the 'Spirálszíve-terem' (Spirál's heart hall) is the richest of them.

The Szajha-felső Cave is situated in the area of a platform right above the Abaligeti Cave and the caves are supposedly connected with each other. The cave entrances are approximately 1 km from each other (Dezső 2011). The present deepest point of the cave is 42 m, though it is still being excavated. The first part of the cave is a vertical shaft, which continues in a narrow horizontal session. The cave is rather poor in formations. A few small, permanent shallow pools can be found; epigean nutrition source is uncharacteristic.

Contrary to the above discussed caves, 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 proved to be dry in all seasons, has not contained any types of water bodies. As the cave directly opens in deciduous woodland, its first few meters contain massive amount of organic matter in all seasons, which causes the appearance of trogloxene invertebrate species in the entrance zone. The cave is periodically occupied by a badger (*Meles meles*).

Figure 2: Detail of the artificial tunnel of the Mánfai-kőlyuk Cave.

2.2 Sampling methods

The focal 8 caves were regularly visited between September 2010 and December 2013. The most frequently visited cave was the Abaligeti Cave, collection trips had been conducted in all seasons, in total 20 occasions. 8 trips in the Mánfai-kőlyuk Cave had been performed, while the other 6 caves had been visited in total 22 times. Kispaplika Cave, Orfűi Vízfő Cave, Achilles Cave, Római Cave, Akácos Cave and Nyárás-völgyi Cave had been visited less frequently; two collecting trips were conducted in each. Except in case of the show-cave Abaligeti Cave, the collecting-, observation- and documentary trips had happened by the assistance of speleologist colleagues from the Pro Natura Karst and Cave Research Society.

The applied collecting methods were heterogeneous, though, the most frequently used method was the 'singling', which means catching the single individual noticed on the spot. This method has an obvious advantage: it reduces the opportunity of 'over collecting' in the sensitive cave ecosystem. Singling can be happened both from aquatic and terrestrial habitats, using entomological (soft) forceps, aspirator or a hand net. Occasionally, pitfall traps and aquatic traps were also used. The former meant 2 dl volume plastic cups filled with ethanol or ethylene glycol. It can be baited or unbaited. As baits, various nutriments can be used, like meat, cheese or beer which should be placed in a vial. Soil traps had been emptied after 5-10 days. Two types of traps for collecting the aquatic fauna were applied. The baited 'bottle trap' is suitable for collecting omnivore invertebrates, while 'leaf litter trap' - developed by the author - attracts mainly detritivores. The former meant a plastic bottle with a reversed open neck placed meat bait inside, while the latter meant perforated nylon pockets filled with

sterilized leaves. Bottle traps had been left in the water for one day, while leaf litter traps had been emptied after two weeks. Four water collectors (0.5 l volume bottles with a funnel and a drain) were placed under stalactites in the Abaligeti Cave in order to collect epikarstic water. Containers were emptied monthly in total three times; water was filtered by plankton net (Figure 3). Collected specimens were fixed and stored in 70 or 96% ethanol.

Figure 3: Sampling methods applied for collecting the aquatic and terrestrial cave-dwelling macroinvertebrate fauna. A: checking of organic material (bat guano), B: singling by forceps, C: singling by aspirator, D: singling by hand net, E: bottle trap (note that the photo was taken in the Baradla Rövid-alsó Cave), F: leaf litter trap, G: collecting of epikarstic water, H: pitfall trap (Photos: D. Angyal, G. Balázs & A. Illés).

2.3 Morphological studies

2.3.1 Morphological studies on *Niphargus molnari* and *N. gebhardti*

Following the instructions of Cene Fišer taxonomist and zoospeleologist, modern *Niphargus* taxonomic processes had been acquired during my study trips in the Department of

Biology, Biotechnical Faculty, University of Ljubljana. Preparation techniques were used after Fišer et al. (2009). Specimens were cooked in 10% KOH solution, rinsed with HCl and washed in distilled water. Cleared exoskeletons were stained with chlorazol black in glycerol, and then dissected in glycerol gelatin under a Leica MZ75 and a Leica M125 stereomicroscope. Two slides were made of each specimens, one contained the left side appendages and the mouth parts, while the other contained the whole body with the right side appendages. The slides were examined using a Leica DM 1000 light microscope (Figure 4). Drawings were made using a drawing tube mounted on the light microscope and were computer graphically edited afterwards. Measurements were made using the AnalySIS Program Package, the computer was connected with a Zeiss Axioscope II light microscope. 230 morphological characters on each specimen were examined according to the characters of the DELTA program package (Fišer et al. 2009) which were recorded in an Excel data matrix. Slides and material preserved in 96% ethanol have been deposited in the Crustacea Collection of the Hungarian Natural History Museum (HNHM).

Figure 4: Leica DM 1000 light microscope with drawing tube used for morphological analysis and for making of drawings.

2.3.2 Morphological studies on *Protelsonia hungarica hungarica* and *P. hungarica robusta*

Same procedure was applied for the dissection and for making of drawings as in case of the *Niphargus* specimens. In some occasions only the pleopods, gnathopods or the uropods were dissected. Slides and material preserved in 96% ethanol have been deposited in the Crustacea Collection of the HNHM.

2.3.3 Morphometric studies on *Bythiospeum hungaricum* and *B.* cf. *gebhardti*

Images of the used snail individuals were made by a camera attached to a Zeiss Axioscope II light microscope. Three independent photos were made of each individual in order to perform a repeatability test. ImageJ scientific image analysing program was used for making the measurements, from where the data were converted into an Excel file. Material preserved in 96% ethanol has been deposited in the Mollusca Collection of the HNHM.

2.3.4 Morphological studies on *Brachydesmus troglobius*

Brachydesmus specimens were examined under a Leica M125 stereomicroscope. In some cases the male's gonopods were dissected and studied under higher magnification. Studied specimens and dissected gonopods have been deposited in the Myriapoda Collection of the HNHM.

2.3.5 Scanning electron microscopy and multilayer photos

Scanning electron micrographs of the main characters of a male and a female specimen of *N. molnari*, *N. gebhardti*, *B. troglobius* and *P. hungarica hungarica* were made with a HITACHI S-2600 N scanning electron microscope in the Department of Botany of the HNHM. Specimens were placed in absolute alcohol for one day, then cleaned in an EMAG Emmi-16 Ultrasonic Cleaner and dried out on air. Dry samples were stuck onto holders and were sputter-coated by gold-palladium. Micrographs were digitally edited.

Multilayer photos of *P. hungarica hungarica* and *B. troglobius* were shot by Tamás Németh in the Department of Zoology, HNHM with Nikon D5200 camera using Mitutoyo M Plan Apo 5X microscope lens. Single flash were diffused with a paper cylinder. Exposures were stacked in Zerene Stacker.

2.4 Molecular studies

2.4.1 Molecular methods applied for studies on *N. molnari* and *N. gebhardti*

DNA extraction of two specimens of *N. molnari* from the Abaligeti Cave and six specimens of *N. gebhardti* from the Abaligeti Cave, Trió Cave, Gilisztás Cave, Vadetetős Cave, Spirál Cave and the Szajha-felső Cave (one specimen of each cave) was performed using QIAamp DNA Microkit® (Qiagen) or Sigma Aldrich GenElute Mammalian Genomic

DNA Miniprep Kit® following the manufactrer's instructions (Figure 5). Only a few pereopods were used for DNA isolation of each animal. The following mitochondrial and nuclear markers were used: cytochrome c oxidase subunit I (COI), two fragmnets of 28S rDNA and histone H3. The primer pairs used for PCR amplifications are as follows: for COI: LCO 1490 - HCO 2198 (Folmer et al. 1994), for 28S rDNA: 28S lev2 - 28S des2 or 28S rtest2 (Verovnik et al. 2005, Zakšek et al., 2007) and H3aF2- H3aR2 (Colgan et al. 2000) for histone (H3). Data of primers applied during the molecular studies on the *Niphargus* spp. are listed in Table 2. Protocols and thermo profiles used in PCR were as follows:

1) cytochrome c oxidase subunit I (COI) - *N. molnari*

Primers: F: LCO 1490, R: HCO 2198

PCR reactions (15 µl) were obtained by mixing 11 µl mQ water, 1.5 µl 10X PCR buffer (with $MgCl_2$) , 1.5 µl dNTP, 0.2 µl of each primers (5µM), 0.07 µl BIOTOOLS DNA Polymerase® (5U/ µl) and 1 µl DNA extract. PCR temperature conditions were as follows: initial denaturation for 4 min at 95°C, denaturation for 1 min at 95°C, hybridization for 1 min at 45°C, and polymerization for 2 min 30 sec at 72°C. After fourty cycles a final extension for 7 min at 72°C was added.

2) cytochrome c oxidase subunit I (COI) - *N. gebhardti*

Primers: F: LCO 1490, R: HCO 2198

PCR reactions (25 µl) were obtained by mixing 13.85 µl mQ water, 2.5 µl 10X PCR buffer, 2.5 µl dNTP mix (2mM), 1.5 µl of each primers (5µM), 0.15 µl Fermentas Dream Taq® (5U/ µl) and 3 µl DNA extract. PCR temperature conditions were as follows: initial denaturation for 3 min at 94°C, denaturation for 45 sec at 94°C, hybridization for 45 sec at 48°C, and polymerization for 1 min at 72°C. After thirty cycles a final extension for 3 min at 72°C was added.

3) 28S rDNA - *N. molnari, N. gebhardti*

Primers: F: 28S lev2, R: 28S des2, 28S rtest2

PCR reactions (15 µl) were obtained by mixing 11 µl mQ water, 1.5 µl 10X PCR buffer (with $MgCl_2$), 1.5 µl dNTP, 0.2 µl of each primers (5µM), 0.07 µl BIOTOOLS DNA Polymerase® (5U/ µl) and 1 µl DNA extract. PCR temperature conditions were as follows: initial denaturation for 3 min at 94°C, denaturation for 30 sec at 94°C, hybridization for 1 min at 45°C, and polymerization for 1 min at 72°C. After fourty cycles a final extension for 5 min at 72°C was added.

4) histone H3- *N. molnari, N. gebhardti*

Primers: F: H3aF2, R: H3aR2

PCR reactions (15 µl) were obtained by mixing 11 µl mQ water, 1.5 µl 10X PCR buffer (with $MgCl_2$) , 1.5 µl dNTP, 0,2 µl of each primers (5µM), 0,07 µl BIOTOOLS DNA Polymerase® (5U/ µl) and 1 µl DNA extract. PCR temperature conditions were as follows: initial denaturation for 3 min at 94°C, denaturation for 45 sec at 94°C, hybridization for 1 min at 46°C, and polymerization for 1 min at 72°C. After fourty cycles a final extension for 3 min at 72°C was added.

PCR products were cleaned using Roche High Pure Purification Kit® or Exonuclease I and Alkaline Phosphatase (Fermentas, Germany) according to the manufacturer's instructions. The fragments were sequenced in both directions using PCR amplification primers using ABI 3130 sequencer in the Laboratory of Molecular Taxonomy of the HNHM or in Macrogen Europe (Amsterdam, The Netherlands). Contigs were assembled and sequences were edited using Geneious Pro 5.5.6. (Biomatters, New Zeland).

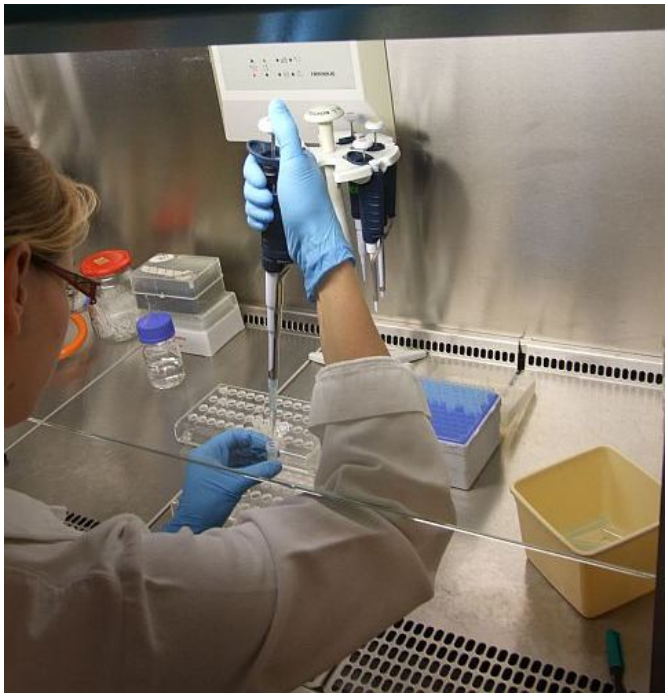


Figure 5: DNA isolation in the Laboratory of Molecular Taxonomy of the HNHM.

Table 2: Data of primers applied during the molecular studies on the *Niphargus* spp.

Marker	Primer	Direction	Sequence	Reference
COI	LCO 1490	Forward	5' GGTCAACAAATCATAAAGATATTGG 3'	Folmer et al. 1994
COI	HCO 2198	Reverse	5' TAAACTTCAGGGTGACCAAAAAAT 3'	Folmer et al. 1994
28S rDNA	28S lev2	Forward	5' CAAGTACCGGTGAGGGAAAGTT 3'	Verovnik et al. 2005
28S rDNA	28S des2	Reverse	5' GTTCACCATCTTTCGGGTC 3'	Zakšek et al. 2007
28S rDNA	28S rtest2	Reverse	5' AGGGAAACTTCGGA-GGGAACC 3'	Verovnik et al. 2005
H3	H3aF2	Forward	5' ATGGCTCGGTACCAAGCAGAC 3'	Colgan et al. 2000
H3	H3aR2	Reverse	5' ATTTCCTTGGGCATGATTGTTAC 3'	Colgan et al. 2000

2.4.2 Phylogenetic analysis applied in studies on *N. molnari* and *N. gebhardti*

638 base pair long COI sequences of *N. gebhardti* from 6 caves of the Western Mecsek were compared to study the intraspecific variation. Sequences were edited by BioEdit Sequence Alignment Editor program and were fitted by ClustalW Multiple Sequence Alignments program. In order to recover phylogenetic relationships of *N. molnari* and *N. gebhardti* within the genus *Niphargus*, a dataset of three molecular markers were complied, using available *Niphargus* sequences from previous studies (see references among the supplements) and *Synurella ambulans* as outgroup taxon (Švara et al. 2015, Meleg et al. 2013). Altogether 104 taxa were included in the final dataset. List of taxa and sequences with GenBank accession numbers used in the analyses are listed as supplements at the end of present thesis (chapter 9.1). The sequences were aligned using MAFFT 7 (Katoh & Standley 2013). Each sequence alignment was concatenated to the joint dataset and analysed as a single dataset in phylogenetic analysis. The length of combined dataset, including sequences of COI, 28S rDNA and H3 was 2068bp. A general time-reversible model with a proportion of invariant sites and a gamma distribution of rate heterogeneity (GTR+I+Γ) assuming six discrete gamma categories was chosen as the most appropriate model according to AIC and BIC criteria, using ModelGenerator (Keane et al. 2006). Phylogenetic relationships were reconstructed with Bayesian inference (BA) using MrBayes v3.2 (Ronquist & Huelsenbeck 2003). Two parallel searches with four chains each were run for 20 million generations, sampled every 1000th generation. After discarding the first 25% of the sampled trees, the final tree was constructed according to the 50% majority rule. MrBayes phylogenetic analysis was run on the CIPRES Science Gateway, www.phylo.org (Miller et al. 2012). COI sequences of

one individual of *N. molnari* from the Abaligeti Cave and two specimens of *N. gebhardti* from the Abaligeti Cave and the Szajha-felső Cave (one of each) have been uploaded to the GenBank (http://www.ncbi.nlm.nih.gov/) with the accession numbers KP967552 (*N. molnari*) and KP967553 (*N. gebhardti,* Abaligeti Cave) and KP967554 (*N. gebhardti*, Szajha-felső Cave).

2.4.3 Molecular methods applied for studies on *B. hungaricum* and *B.* cf. *gebhardti*

First stage:

First stage of the molecular studies on *Bythiospeum* taxa performed in the Laboratory of Molecular Taxonomy of the HNHM. One specimen from the Abaligeti Cave (collected in 11. 10. 2010 in the main passage's stream from stones, 230 m from the entrance) and four individuals from the Mánfai-kőlyuk Cave (collected in 21. 10. 2011 in the upper passage from the water carrier canal) were used for the analysis. Samples were dried by vacuum centrifuge, then shells were removed under stereomicroscope and the dry body was smashed using Polycar AT reducer. DNA extraction was performed using QIAamp DNA Microcit® (Qiagen) following the manufactrer's instructions. The following primer pairs were used for PCR amplifications of cytochrome c oxidase subunit I (COI): LCO 1490 (Folmer et al. 1994) and COI-H (Machodrom et al. 2003). PCR reactions (25 µl) were obtained by using the following concentrations: 0.25 mM dNTP, 0.4 uM primer, 2 mM $MgCl_2$, 2.128 ug/ul BSA, 50 ng DNS and 1 U Fermentas Dream Taq DNA Polymerase®. PCR temperature conditions were as follows: initial 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 fourtyone cycles a final extension for 6 min at 72°C was added. PCR products were cleaned using Roche High Pure Purification Kit® according to the manufacturer's instruction. The fragments were sequenced in both directions using PCR amplification primers, by Big-Dye fluorescent sequencing kit on ABI 3130 sequencer.

Second stage:

Second stage of the molecular studies was done in the Laboratory of Molecular Taxonomy of the HNHM and in the Laboratory of Molecular Systematics, Museum of Natural History, Vienna. Data of the involved specimens (10 from the Abaligeti Cave and 11 from the Mánfai-kőlyuk Cave) are listed in Table 3. Shells of the snails were removed under stereomicroscope. Only the soft bodies were used for DNA extraction, except sample BG_Man 12, which individual was smashed together with the shell because of experimental purpose. DNA extraction was performed using QIAamp DNA Microcit® (Qiagen) following the manufactrer's instructions with a single change: instead of 20 µl Protainase K, 25 µl was added. After the overnight lysis, QIAshredder (50) was used in case of the imperfectly lysed samples. For PCR amplifications of cytochrome c oxidase subunit I (COI) LCO 1490 and HCO 2198 (Folmer et al. 1994) primers were used. PCR reactions (25 µl) were obtained by

mixing 13.8 µl mQ water, 2.5 µl 10X PCR buffer (with $MgCl_2$), 2.5 µl dNTP, 1 µl 10 µg/ µl BSA, 1.5 µl of each primers (5µM), 0.2 µl Fermentas Dream Taq DNA Polymerase® (5U/ µl) and 2 µl DNA extract. PCR temperature conditions were as follows: initial denaturation for 1 min at 96°C, denaturation for 1 min at 94°C, hybridization for 1 min at 40°C, and polymerization for 1 min 30 sec at 72°C. After thirtyfive cycles a final extension for 10 min at 72°C was added.

For PCR amplifications of the 16S ribosomal RNA (16S) 16 sar - 16 sbr (Palumbi et al. 1991) and 16SLOrc2_fwd - 16SLOrc_rev (Harl et al. 2014b) primers were used. Data of the primers used during the molecular studies on *Bythiospeum* are listed in Table 4. PCR reactions with the 'Palumbi primers' were obtained for 25 µl reaction volume by mixing 11.9 µl mQ water, 2.5 µl 10X PCR buffer, 2 µl 25 mM $MgCl_2$, 2.5 µl 2 mM dNTP, 2 µl 10 µg/ µl BSA, 1 µl of each primers (5µM), 0.1 µl Fermentas Taq DNA Polymerase® (5U/ µl) and 2 µl DNA extract. PCR temperature conditions were as follows: initial denaturation for 4 min at 92°C, denaturation for 1 min at 92°C, hybridization for 1 min at 52°C, and polymerization for 1 min at 72°C. After thirty-five cycles a final extension for 5 min at 72°C was added. In case of the 'Harl primers', the PCR reactions were obtained for 25 µl reaction volume by mixing 17.875 µl mQ water, 5 µl 10X PCR buffer (with $MgCl_2$), 0.5 µl 2 mM dNTP, 0.25 µl of each primers (5µM), 0.125 µl Fermentas Taq DNA Polymerase® (5U/ µl) and 1 µl DNA extract. PCR temperature conditions were as follows: initial denaturation for 3 min at 94°C, denaturation for 30 sec at 94°C, hybridization for 30 sec at 52°C, and polymerization for 30 sec at 60°C. After thirty-five cycles a final extension for 7 min at 72°C was added. Uncleaned PCR products had been sent to LGC Genomics (Berlin, Germany), where the Microtitre plate sequencing includes the clean-up treatment of all PCR products.

Table 3: Data of 'Hungarian blind snail' samples used for the second stage of the molecular studies.

Sample code	**Cave**	**Locality within cave**	**Date of collection**
BH_Aba_21, BH_Aba_22, BH_Aba_23, BH_Aba_24, BH_Aba_25, BH_Aba_26, BH_Aba_27, BH_Aba_28, BH_Aba_29, BH_Aba_30	Abaligeti Cave	main passage, stream, on stones, 200-300 m from the entrance	14. 04. 2014
BG_Man_01, BG_Man_02, BG_Man_03, BG_Man_04, BG_Man_05, BG_Man_06, BG_Man_07, BG_Man_08, BG_Man_09, BG_Man_10, BG_Man_12	Mánfai-kőlyuk Cave	upper (artificial) tunnel, from the water carrier canal	14. 04. 2014

Table 4: Data of primers used in molecular studies on *Bythiospeum* species.

Marker	Primer	Direction	Sequence	Reference
COI	LCO 1490	Forward	5' GGTCAACAAATCATAAAGATATTGG 3'	Folmer et al. 1994
COI	HCO 2198	Reverse	5' TAAACTTCAGGGTGACCAAAAAAT 3'	Folmer et al. 1994
COI	COI-H	Reverse	5′ TCAGGGTGACCAAAAAATCA 3′	Machodrom et al. 2003
16S	16 sar	Forward	5' CGCCTGTTTATCAAAACAT 3'	Palumbi et al. 1991
16S	16 sbr	Reverse	5' CCGGTCTGAACTCAGATCACG'	Palumbi et al. 1991
16S	16SLOrc2_fwd	Forward	5' TTACCTTTTGCATAATGGTTAAATTA 3'	Harl et al. 2014b
16S	16SLOrc_rev	Reverse	5' CGGTCTGAACTCAGATCATG 3'	Harl et al. 2014b

2.4.4 Phylogenetic analysis applied for studies on *B. hungaricum* and *B.* cf. *gebhardti*

First stage:

COI sequences were edited using Bio Edit Sequence Alignment Editor. Alignments were fitted by ClustalW Multiple Sequence Alignments program. Further sequence analysis was performed by MEGA 6 software (Tamura et al. 2013). 638 bp COI sequences of one individual of *B. hungaricum* and *B*. cf. *gebhardti* have been uploaded to the GenBank with the accession numbers KP296923 (*B. hungaricum*) and KP296922 (*B*. cf. *gebhardti*).

Second stage:

Sequences were edited using Bio Edit Sequence Alignment Editor. Alignments were fitted by ClustalW Multiple Sequence Alignments program. In order to study the phylogenetic relationships within the genus *Bythiospeum* and within the superfamily Rissooidea, a dataset was compiled using the own sequences and sequences downloaded from the GenBank database (http://www.ncbi.nlm.nih.gov/). Data of the downloaded COI sequences are listed in Table 5. In total 43 taxa have been involved in the analysis, including 31 *Bythiospeum* taxa and 12 species from 10 other rissooid genera. The major part of the genera were chosen based on the phylogenetic study of Wilke et al. (2001). *Moitessieria* cf. *puteana* was used as outgroup taxon. Distance estimation of the 43 taxa was performed by variance estimation method, using p-distance model in MEGA6 (Tamura et al. 2013). Bayesian phylogeny analysis was performed too, including the *Bythiospeum* COI sequences and the *M*. cf. *puteana* sequence. Saturation of phylogenetic information was examined using Xia's test (Xia et al. 2003 and Xia & Lemey 2009) employed in DAMBE v5.3.8 (Xia & Xie 2001). The alignments showed only little substitution saturation, with $I_{SS.C}$ values (P= 0.000): $I_{ss.c}$ 0.7377 > I_{ss} 0.1537. The most appropriate model (HKY+I) was selected with jModeltest v.0.1.1

(Posada 2008), under the Bayesian Information Criterion. Baysian inference was calculated with MrBayes 3.2.2 (Huelsenbeck & Ronquist 2001, Ronquist & Huelsenbeck 2003) for 5 x 10^6 generations (samplefreq = 100, nchains = 4, burnin = 10%).

Table 5: Data of rissooid sequences downloaded from National Center for Biotechnology Information homepage used in phylogenetic analysis.

Genbank accession number	Species	Valid name	Length of COI fragment	Author (uploaded by)	Publication	Distribution of species
HM.107133.1	*Bythiospeum francomontanum*	*Bythiospeum francomontanum* Bernasconi, 1973	603 bp	Hirsch et al. 2010	unpublished	France, Switzerland
HM.107121.1	*Bythiospeum acutum*	*Bythiospeum suevicum* (Geyer, 1905)	603 bp	Hirsch et al. 2010	unpublished	Germany
HM107134.1	*Bythiospeum husmanni*	*Bythiospeum husmanni* (C. Boettger, 1963)	603 bp	Hirsch et al. 2010	unpublished	Germany, The Netherlands (doubtful)
HM107127.1	*Bythiospeum* sp. (Wasserflaare)		603 bp	Hirsch et al. 2010	unpublished	
HM107126.1	*Bythiospeum* sp. (Blautopf)		603 bp	Hirsch et al. 2010	unpublished	
HM107125.1	*Bythiospeum saxigenum saxigenum*	*Bythiospeum saxigenum* (Geyer, 1905)	603 bp	Hirsch et al. 2010	unpublished	Germany
HM107123.1	*Bythiospeum pellucidum*	*Bythiospeum pellucidum* (Seckendorf, 1846)	603 bp	Hirsch et al. 2010	unpublished	Germany
HM107118.1	*B. suevicum*	*Bythiospeum suevicum* (Geyer, 1905)	603 bp	Hirsch et al. 2010	unpublished	Germany
HM107115.1	*B. quenstedti quenstedti*	*Bythiospeum quenstedti quenstedti* (Wiedersheim, 1873)	603 bp	Hirsch et al. 2010	unpublished	Germany
AF367634.1	*B*. sp. (France)		638 bp	Wilke 2001	Wilke et al. 2001	
AF367635.1	*Moitessieria* cf. *puteana*	*Spiralix puteana* (Coutagne, 1883)	638 bp	Wilke 2001	Wilke et al. 2001	France
FJ029100.1	*Bythinella carinulata*	*Bythinella carinulata* (Drouet, 1867)	638 bp	Benke et al. 2009	Benke et al. 2009	France
AY222649.1	*Bythinella schmidtii*	*Bythinella schmidtii* (Kuster, 1852)	638 bp	Szarowska & Wilke 2004	Szarowska & Wilke 2004	Slovenia
AF213340.1	*Erhaia jianouensis*	*Erhaia jianouensis* Liu & Zang, 1979	638 bp	Wilke et al. 2000	Wilke et al. 2000	China, India
AF213348.1	*Amnicola limosa*	*Amnicola limosus* (Say, 1817)	638 bp	Wilke et al. 2000	Wilke et al. 2000	Atlantic Ocean, Gulf of Maine

Genbank accession number	Species	Valid name	Length of COI fragment	Author (uploaded by)	Publication	Distribution of species
AF354769.1	*Amnicola dalli*	*Amnicola dalli* Call, 1884	634 bp	Liu 2001	Liu et al. 2001	Nevada
AF322409.1	*Marstoniopsis insubrica*	*Marstoniopsis insubrica* (Kuster, 1853)	638 bp	Wilke & Falinowski 2001	unpublished	Europe (widely distributed)
AF253079.1	*Hydrobia neglecta*	*Hydrobia neglecta* Muus 1963	645 bp	Davis et al. 1998	Davis et al. 1998	France, Germany, Britain, Iceland, Danmark
AF367640.1	*Hauffenia teliinii*	*Hauffenia tellinii* (Pollonera 1898)	638 bp	Wilke 2001	Wilke et al. 2001	Italy, Slovenia
KF193076.1	*Sadleriana robici*	*Sadleriana sadleriana robici* (Clessin 1890)	656 bp	Szarowska & Falinowski 2013	Szarowska & Falinowski 2013	Slovenia
AF520920.1	*Floridobia petrifons*	*Floridobia petrifons* (Thompson, 1968)	609 bp	Hershler et al. 2003	Hershler et al. 2003	Florida
AY676127.1	*Dianella thiesseana*	*Dianella thiesseana* (Kobelt 1878)	638 bp	Wilke 2004	unpublished	Greece

2.4.5 Molecular methods applied for studies on *B. troglobius*

DNA extraction of three *Brachydesmus* and one *Polydesmus* species from 5 different caves of Hungary and Serbia performed in the Laboratory of Molecular Taxonomy of the HNHM, using QIAamp DNA Microcit® (Qiagen) following the manufacturer's instructions. Data of the used samples are listed in Table 6. The following primer pairs were used for PCR amplifications of cytochrome c oxidase subunit I (COI): LCO 1490 - HCO 2198 (Folmer et al. 1994) and LCO 1490 - COI-H (Machodrom et al. 2003). Data of primers are listed in Table 7. Details of PCR conditions are written below. PCR products were cleaned using Roche High Pure Purification Kit® according to manufacturer's instructions. Fragments were sequenced in both directions in case of the two *B. troglobius* samples, and only in forward direction in case of the other polydesmid samples. Sequencing was performed by ABI 3130 sequencer, using PCR amplification primers.

Protocols and thermo profiles used in PCR were as follows:

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

PCR reactions (25 µl) were obtained by mixing 10.775 µl mQ water, 2.5 µl 10X PCR buffer (with $MgCl_2$), 3.125 µl dNTP, 1.75 µl of each primers (5µM), 0.01 µl Fermentas Dream Taq DNA Polymerase® (5U/ µl) and 5 µl DNA extract. PCR temperature conditions were as

follows: 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 fourty cycles a final extension for 6 min at 72°C was added.

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

PCR reactions (25 µl) were obtained by mixing 8.775 µl mQ water, 2.5 µl 10X PCR buffer, 2 µl 25 mM $MgCl_2$, 3.125 µl dNTP mix (2mM), 1.75 µl of each primers (5µM), 0.1 µl Fermentas Taq Polymerase® (5U/ µl) and 5 µl DNA extract. PCR temperature conditions were as follows: 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 fourty cycles a final extension for 6 min at 72°C was added.

Table 6: Basic data of own samples used in phylogenetic analysis.

Genbank accession number	**Species**	**Country**	**Region, town**	**Cave, locality**	**Date of collection**	**Collector**
KT343290 (BR_TRO/Aba)	*B. troglobius*	Hungary	Mecsek, Abaliget	Abaligeti Cave,	13. 06. 2013	D. Angyal
KT343289 (BR_TRO/Ser)	*B. troglobius*	Serbia	West Serbia, Valjevo	Petnička's Cave	21. 05. 2010	S. Makarov
KT343291 (BR_SUP/Alba)	*B. superus*	Hungary	Bakony, Csőszpuszta	Alba Regia Cave	10. 11. 2012	D. Angyal
KT343292 (BR_HER/Ser)	*B. herzogowinensis*	Serbia	West Serbia, Ivanjica	Hadži Prodanova Cave	23. 07. 2012	S. Makarov
KT343288 (PO_DEN/Soly)	*P. denticulatus*	Hungary	Budai Mts., Solymár	Solymári-ördöglyuk Cave	03. 03. 2012	D. Angyal

Table 7: Data of primers used in molecular studies on *Brachydesmus* species.

Marker	**Primer**	**Direction**	**Sequence**	**Reference**
COI	LCO 1490	Forward	5' GGTCAACAAATCATAAAGATATTGG 3'	Folmer et al. 1994
COI	HCO 2198	Reverse	5' TAAACTTCAGGGTGACCAAAAAAT 3'	Folmer et al. 1994
COI	COI-H	Reverse	5′ TCAGGGTGACCAAAAAATCA 3′	Machodrom et al. 2003

2.4.6 Phylogenetic analysis applied for studies on *B. troglobius*

In order to evaluate the intra- and interspecific, as well as the intra- and intergeneric distances within the genus *Brachydesmus* and with other closely related polydesmid genera, a

dataset of COI markers was complied, using own data (Table 6) and sequences downloaded from the GenBank. From the genus *Brachydesmus* Heller, 1858 only the sequence of a single species (*B. superus*) was available. Eight additional species from four genera were also included. *Antichiropus variabilis* (Paradoxosomatidae) were used as outgroup taxon. In some cases, sequences of more than one specimen of the same species were used in order to study the intraspecific variation. Data of the downloaded sequences are listed in Table 8. Altogether 17 taxa were included in the dataset. Sequences were aligned using Bio Edit Sequence Alignment Editor. Alignments were fitted by ClustalW Multiple Sequence Alignments program. Phylogeny reconstruction was estimated by neighbor-joining of amino acid pairwise distance in MEGA 6 (Tamura et al. 2013). COI barcode sequences of our own study were uploaded on GenBank (see Table 6).

Table 8: Data of polydesmid sequences downloaded from National Center for Biotechnology Information homepage used in phylogenetic analysis.

Genbank accession number	**Valid name**	**Length of COI fragment**	**Author (uploaded by)**	**Publication**	**Distribution of species**
HQ966183.1	*Brachydesmus superus* Latzel, 1884	658 bp	Spelda et al. 2011	Spelda et al. 2011	widely distributed in Europe, East Palearctic, N Africa and Australia
JN306630.1	*Polydesmus edentulus* C. L. Koch, 1847	658 bp	Spelda et al. 2011	Spelda et al. 2011	widely distributed in Europe
JN306634.1	*Propolydesmus helveticus* (Verhoeff, 1894)	658 bp	Spelda et al. 2011	Spelda et al. 2011	Austria, France, Germany, Switzerland
HQ966172.1	*Propolydesmus testaceus* (C. L Koch, 1847)	658 bp	Spelda et al. 2011	Spelda et al. 2011	widely distributed in N Europe
HQ966181.1	*Polydesmus denticulatus* C. L. Koch, 1847	600 bp	Spelda et al. 2011	Spelda et al. 2011	widely distributed in Europe and Nearctic region
HQ966182.1	*Polydesmus denticulatus* C. L. Koch, 1847	658 bp	Spelda et al. 2011	Spelda et al. 2011	widely distributed in Europe and Nearctic region
HQ966176.1	*Polydesmus angustus* Latzel 1884	658 bp	Spelda et al. 2011	Spelda et al. 2011	widely distributed in Europe and Nearctic region
HQ966178.1	*Polydesmus complanatus* (Linnaeus 1761)	658 bp	Spelda et al. 2011	Spelda et al. 2011	widely distributed in Europe, Near East and Nearctic region
HQ966177.1	*Polydesmus complanatus* (Linnaeus 1761)	658 bp	Spelda et al. 2011	Spelda et al. 2011	widely distributed in Europe, Near East and Nearctic region
HQ966180.1	*Polydesmus complanatus* (Linnaeus 1761)	658 bp	Spelda et al. 2011	Spelda et al. 2011	widely distributed in Europe, Near East and Nearctic region
HQ966179.1	*Polydesmus complanatus (Linnaeus 1761)*	658 bp	Spelda et al. 2011	Spelda et al. 2011	widely distributed in Europe, Near East and Nearctic region
JN637363.1	*Antichiropus variabilis* (Attems, 1911)	658 bp	Wojcieszek 2012	Wojcieszek & Simmons 2012	Australia

2.5 Statistical methods

Principal Component Analysis, Repeatability Test and General Linear Model Analysis had been performed using the SPSS Statistics software package by Gergely Balázs (Eötvös Loránd University).

3 RESULTS

3.1 Revision of *Niphargus molnari* Méhely, 1927 and *Niphargus gebhardti* Schellenberg, 1934 (Amphipoda, Niphargidae)

3.1.1 Preliminary knowledge related to *N. molnari* and *N. gebhardti*

Niphargus molnari Méhely, 1927 was described from the stream of the Mánfai-kőlyuk Cave (Méhely 1927). The description is not detailed, as it contains only little information about the body length, the pereonits, the pleon segments, the first antenna, the uropods and the telson, and two drawings about the epimeral plates and the pereion segments. Further drawing of the right lacinia mobilis can be found in Méhely's summarizing work (Méhely 1941). At approximately the same period the species was also studied by Schellenberg, who analysed samples from Abaligeti Cave. In his early study he first treated it as *N. leopoliensis molnari* (Schellenberg 1933), but later he acknowledged its species status and supplemented description with data about the seta number of the palpus of the first maxilla (Schellenberg 1935). The species was found in the Mánfai-kőlyuk Cave (Gebhardt 1933, 1934, 1963, 1967) and in the stream of the Abaligeti Cave too (Gebhardt 1934, 1963, 1967).

Niphargus gebhardti Schellenberg, 1934 was described from the pools formed by dripping water of the Abaligeti Cave, originally as *Niphargus foreli gebhardti* (Schellenberg 1934). Brief description reports on only few characters, like the pereopods, the antennae and the mouth parts, and two drawings about the second gnathopod's propodus and the telson. Later the author gave additional data on the body length and the telson (Schellenberg 1935). Gebhardt mentioned the species' distribution from pools of the Abaligeti Cave's main passage in various papers (Gebhardt 1934, 1963, 1967). The species rank was proposed for the first time in Méhely's synthetic work (Méhely 1941), wherein a drawing of the pleopod's retinacles and some data about the lacinia mobilis are also presented. Dudich (1941) mentioned '*Niphargus foreli gebhardti*' from the Abaligeti Cave as a fauna element of the historical Hungary. The holotypes of both species are either in an unknown place or had been destroyed.

3.1.2 Redescription of *N. molnari* and *N. gebhardti*

***Niphargus molnari* Méhely, 1927**

Niphargus molnari: Méhely 1927 (description); *Niphargus leopoliensis molnari*: Schellenberg 1933 (morphological data); *Niphargus molnari*: Schellenberg 1935 (morphological data); *Niphargus leopoliensis molnari*, *Niphargus molnari*: Gebhardt 1933, 1934, 1963, 1967 (distributional data); *Niphargus molnari*: Méhely 1941 (additional morphological data); *Niphargus molnari*: Angyal & Balázs 2013a (morphological data); *Niphargus molnari*: Angyal & Balázs 2013b (distributional data); *Niphargus molnari*: Balázs & Angyal 2013, Angyal & Balázs 2014, Balázs et al. 2015 (evaluation of the Hungarian species); *Niphargus molnari*: Angyal et al. 2015 (redescription)

Material examined for redescription: 7 females and 3 males from the stream of the Western 2 collateral of the Abaligeti Cave, collected in 23 March 2013 (leg. D. Angyal and A. Illés), dissected and mounted on slides; additional 4 specimens not dissected.

Diagnosis

Small to medium-sized niphargid; epimeral plate III postero-ventral corner sharply inclined. Telson with 3-4 apical spines, 1-3 lateral spines, 0-2 lateral plumose setae, 0-2 spines in cleft, dorsal surface with 1-3 spines in mediobasal position. Maxilla I outer lobe with 7 spines, 1.-3. pluri-toothed, 4.-7. variable (uni-, bi-, pluri-toothed). Gnathopod I and gnathopod II dactyls with single seta on outer margin. Gills II-VI ovoid, approximately same size as pereopod VI coxa, posterior margin slightly concave. Pleopods I-III with 2 retinacles on each. Uropod I length of endopodite: length of exopodite ratio as 1.00: (1.00-1.20) on males and 1.00: (1.15-1.18) on females. Uropod III sexually dimorphic, exopodite rod-shaped, distal article of exopodite on males 83-115% of proximal article length and 18-73% on females.

Description

Body and telson

Small to medium sized species, females are 6.4 mm to 9.0 mm, males are 7.8 mm to 10.6 mm. Head length up to 13% of body length; rostrum absent. Pereonites I-IV without setae; pereonite V, VI, VII with 1 postero-ventral seta each. Pleonites I-III with 3-6 setae along dorso-posterior margin (Figure 6). Epimeral plate II ventral and posterior margins straight or sinusoid, ventro-postero-distal corner approximately perpendicular and pointed;

along ventral margin 1-3 spiniform setae; along posterior margin 4-6 thin setae (Figure 8). Epimeral plate III ventral margin convex and posterior margin straight, ventropostero-distal corner sharply inclined, strongly produced; along ventral margin 2-3 spiniform setae; along posterior margin 4-6 thin setae (Figure 6, 7). Urosomite I postero-dorso-laterally with 1-2 spiniform seta; urosomite II postero-dorso-laterally with 2-3 spiniform setae; urosomite III without setae. Near insertion of uropod I 1 spiniform seta (Figure 6).

Telson length: width as 1.0:0.6-0.8; cleft 71-87% of length; lobes apically rounded. Telson spines (per lobe): 3-4 apical spines; lateral margins with 1-3 spine, 0-2 plumose setae; 0-2 in cleft spines, dorsal surface with 1-3 basal spines in mediobasal position (Figure 12). Length of apical spines 20-25% of telson length.

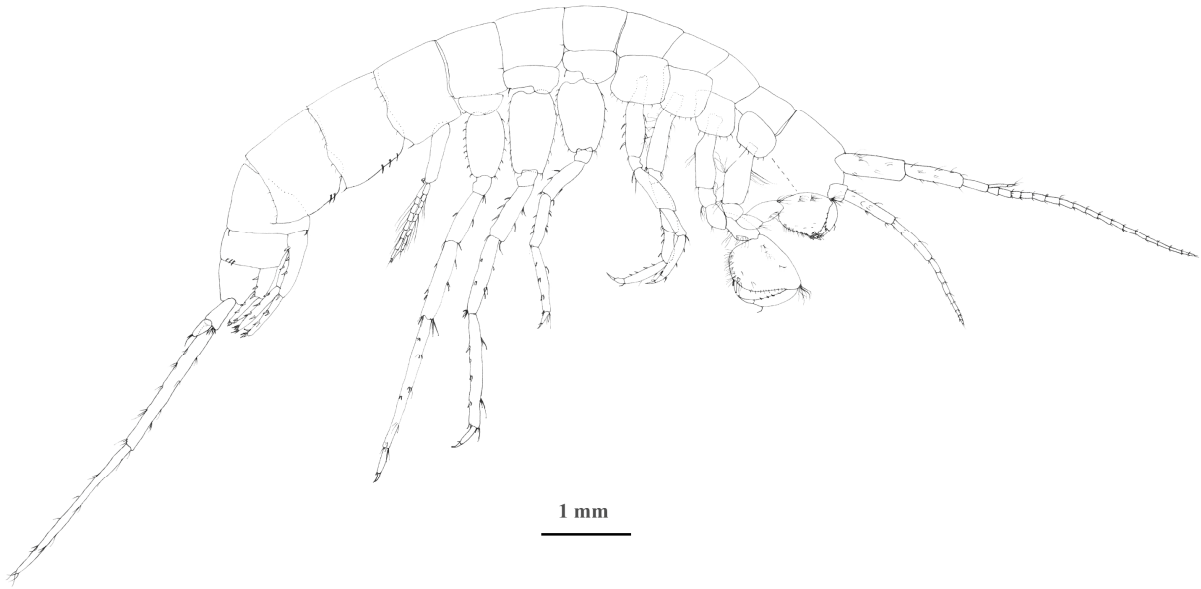


Figure 6: *N. molnari*, male from the Abaligeti Cave, lateral view. Telson and pleopod II-III are not drawn.

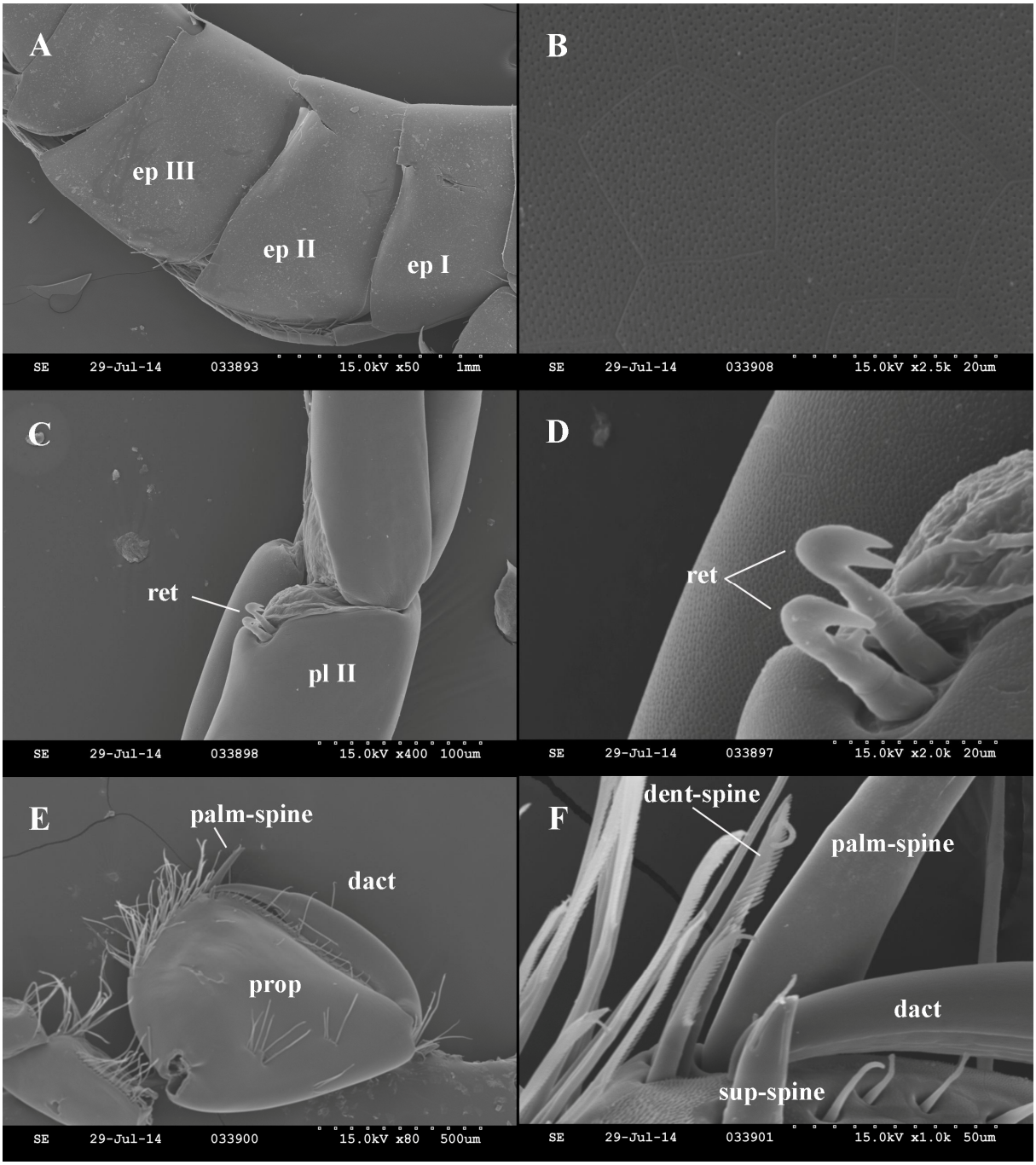


Figure 7: *N. molnari*, scanning electron micrographs. A: epimeral plates (Ep1-3: epimeral plates 1-3), B: honeybee-cell pattern on the exosceleton (tipical feature of amphipods), C: pleopod with two retinacles (pl-r: pleopod ramus, ret: retinaculum), D: retinaculi on the pleopod (ret: retinaculum), E: gnathopod II propodus (dact: dactylus, prop: propodus, sup-spine: supporting spine), F: palmar region of gnathopod II propodus (dent-spine: denticulated spine, n: nail, palm-spine: palmar spine, sup-spine: supporting spine).

Antennae and mouthparts

Antenna I 35-48% of body length. Flagellum with up to 19 articles; each article with 1 long aesthetasc. Peduncle article 1:2:3 proportions 1.0: 0.78 (0.72-0.88): 0.4 (0.36-0.46). Proximal article of peduncle dorso-distally slightly produced. Accessory flagellum biarticulated; distal article shorter than one-half of the proximal article. Lengths of antennae I: II as 1.0: 0.50. Flagellum of antenna II with 6-8 articles. Lengths of peduncle articles 4: 5 as 1.0: (0.84-0.95); flagellum 54-70% of peduncle length (articles 4+5) (Figure 8).

Inner lobes of labium longer than half of outer lobes (Figure 8).

Left mandible: incisor with 5 teeth, lacinia mobilis with 4 teeth; between lacinia and molar 6-9 thick, serrated setae, long seta at base of molar absent (Figure 8).

Right mandible: incisor processus with 4-5 teeth, lacinia mobilis with several small denticles (more then 12), between lacinia and molar 6-7 thick, serrated setae, long seta at base of molar present. Proportions of mandibular palp articles 2: 3 (distal) = 1.0: 1.20 (1.17-1.32). Proximal palp article without setae; second article with 9-11 seta in 5-6 groups; distal article with 1 group of 3-5 ‘A setae;’ 3 groups of ‘B setae’; 16-24 ‘D setae’ and 3-5 ‘E setae’ (Figure 8).

Maxilla I distal palp article with 2-3 apical and subapical setae. Outer lobe of maxilla I with 7 spines, 1-3 spines are always pluri-toothed with 3-6 lateral tooth, while 4-6 spines are uni-, or bitoothed. Inner lobe with 1-2 setae (Figure 8).

Maxilla II inner lobe slightly smaller than outer lobe; both of them setose apically and subapically, number of setae is approximately 13-23 per lobe (Figure 8).

Maxilliped palp article 2 with 11-17 rows of setae along inner margin; distal article with dorsal seta and group of small setae at base of nail. Maxilliped outer lobe with 6-12 flattened, thick setae and 3-8 serrated setae; inner lobe with 2-3 flattened, thick setae apically and 5-9 serrated setae (Figure 8).

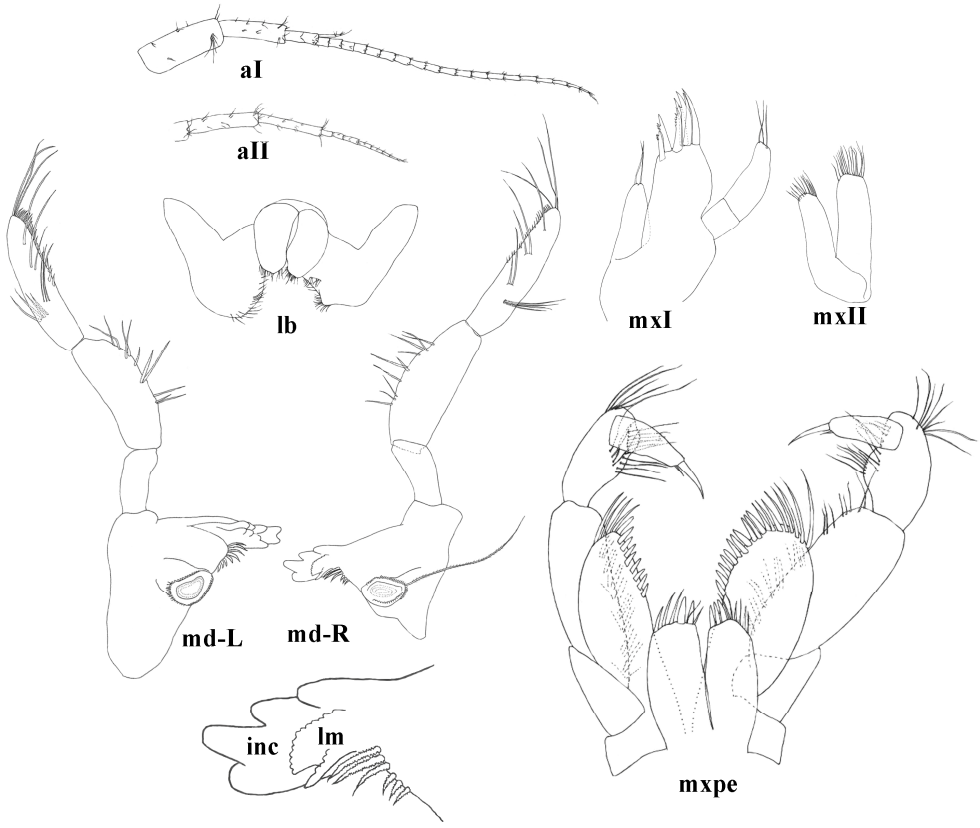


Figure 8: *N. molnari*, aI: antenna I, aII: antenna II, mxI: maxilla I, mxII: maxilla II, md-R: right mandibula, lm: lacinia mobilis, inc: incisor, md-L: left mandibula, lb: labium, mxpe: maxilliped.

Coxal plates

Coxal plate I width: depth as 1.00: 1.03 (0.89-1.16), antero-ventral corner subrounded; anterior and ventral margin of coxa I with 3-6 setae (Figure 9). Coxal plate II width: depth as 1.00: 0.84 (0.76-0.95); anterior and ventral margin with 5-8 setae. Coxal plate III width: depth as 1.00: 0.82 (0.71-1.00); along antero-ventral margin 4-7 setae (Figure 10). Coxal plate IV width: depth as 1.00: 1.03 (1.26-0.88); posteriorly concave; along antero-ventral margin 5-7 setae (Figure 10). Coxal plates V-VI: anterior lobe well developed; along posterior margin 1 seta (Figure 10, 11). Coxal plate VII half-egg shaped, along posterior margin 1 seta (Figure 11). Gills II-VI ovoid, with approximately same size as coxa VI (Figure 10).

Gnathopods

Basis width is 38 (33-45)% of basis length. Gnathopod I ischium with 4-8 posterodistal setae in 1 row. Carpus length 62 (57-75)% of basis length and 87 (80-100)% of propodus length. Anterior margin of carpus only with distal group of setae; carpus posteriorly with transverse rows of setae proximally and a row of lateral setae, posterior enlargment

small. Propodus subquadrate, palm convex. Along posterior margin 6-8 rows of denticulated setae. Anterior margin with 10-17 setae in 2-3 groups, antero-distal group with 6-12 setae. Group of 2-4 facial setae below (proximal of) palmar spine; 2-4 single surface setae present. Palmar corner with palmar spine, single supporting spine on inner surface, and 3 (rarely 4) denticulated, thick spiniform setae on outer side. Nail length 36 (34-37)% of total dactylus length; along anterior margin single seta; along inner margin 4-5 setae (Figure 9).

Gnathopod II basis width: length as 1.0: 0.26 (0.21-0.29). Ischium with 2-6 postero-distal setae. Carpus length 56 (50- 61)% of basis length and 86 (71-94)% of propodus length. Anterior margin of carpus only with distal row of setae; carpus posteriorly with transverse rows of setae proximally, a row of lateral setae; postero-proximal bulge small, positioned proximally. Propodus medium-sized (sum of length, diagonal and palm length measures up to 19 (15-21)% of body length) and larger than propodus of gnathopod I (1.0: 0.57 (0.65-0.85)). Propodus rectangular, palm convex. Posterior margin convex with 6-9 rows of denticulated setae. Anterior margin with 10-20 setae in 3-5 groups; antero-distal group with 7-9 setae. 1 group of 2-3 facial setae below (distal of) palmar spine; 1-4 individual surface setae present. Palmar corner with strong palmar spine, single supporting spine on inner surface, and 1 denticulated, thick spiniform seta on outer side. Nail length 31 (22-36)% of total dactylus length. Along anterior margin a single seta; along inner margin 4-6 short setae (Figures 7, 9).

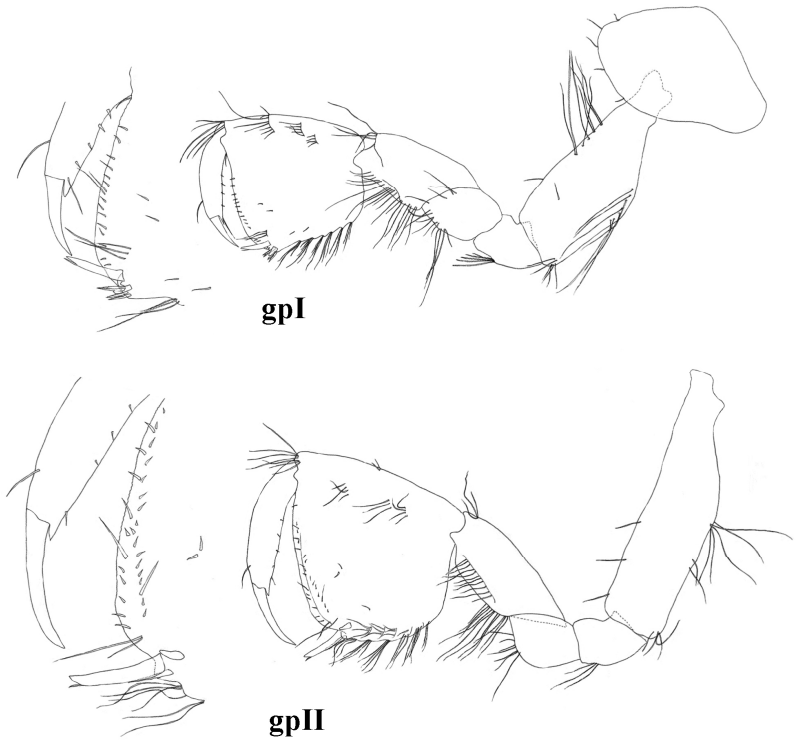


Figure 9: *N. molnari*, gpI: gnathopod I, gpII: gnathopod II.

Pereopods III-IV

Proportions of pereopods III: IV as 1: 0.95 (0.93-0.97). Dactylus IV 45 (39-51)% of propodus IV; nail length 47 (39-52)% of total dactylus length. Dactyli III-IV with one dorsal plumose seta, one spine-like seta at the base of the nail, and tiny seta near the spine-like seta (sometimes not visible or absent). Additional spiniform setae on posterior margin are absent (Figure 10).

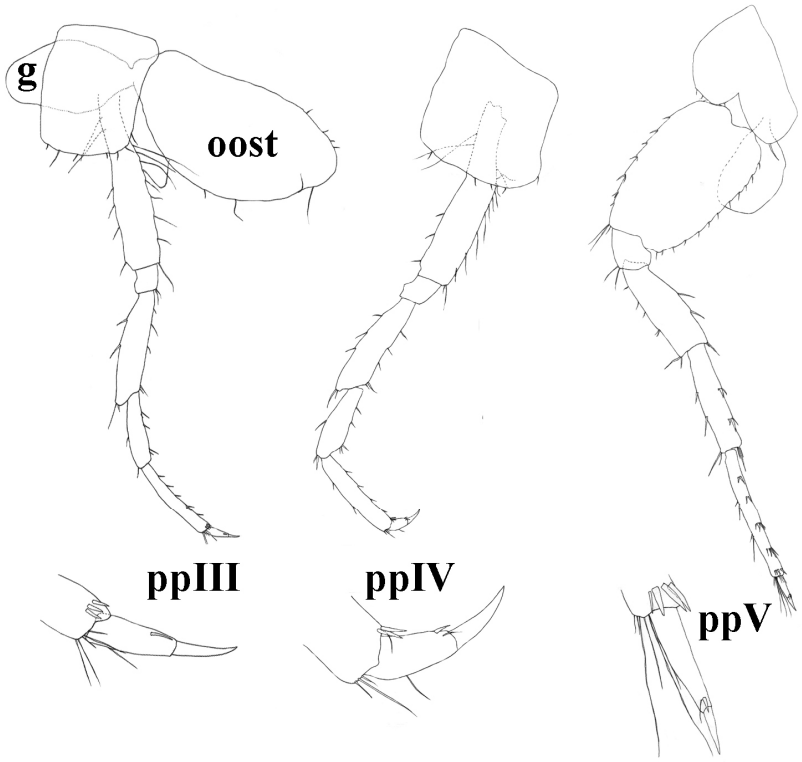


Figure 10: *N. molnari,* ppIII: pereopod III, ppIV: pereopod IV, ppV: pereopod V, g: gill, oost: oostegit.

Pereopods V-VII

Proportions of pereopods V: VI: VII as 1.00: 1.4 (1.37-1.54): 1.5 (1.42-1.61). Pereopod VII length 47 (42-52)% of body length. Basis V-VII narrow with convex posterior margins. Basis V width is 70 (60-78)% of length, basis VI is 67 (59-76)% of length and basis VII is 66 (56-76)% of length. Basis V with small posterodistal lobe, posterior margin with 8-13 setae, anterior margin with 6-8 groups of setae. Dactylus V with one dorsal plumose seta, one spine-like seta at the base of the nail, and tiny seta near the spine-like seta (sometimes not visible or absent). Additional spiniform setae on posterior margin are absent (Figure 11). Basis VI with small posteriodistal lobe, posterior margin with 9-14 setae, anterior margin with 6-10 setae. Dactylus VI with one dorsal plumose seta (sometimes not visible or absent), one spine-like seta at the base of the nail, and a tiny seta near the spine-like seta (sometimes not

visible or absent). Additional spiniform setae on posterior margin are absent (Figure 11). Basis VII posterior margin with 6-13 setae, anterior margin with 6-11 groups of setae. Total number of basis setae is 15-21. Dactylus VII length 26 (24-29)% of propodus VII length; nail length 26 (16-33)% of total dactylus length. Dactylus VII with one spine-like seta at the base of the nail. Additional spiniform setae on posterior margin are absent (Figure 11).

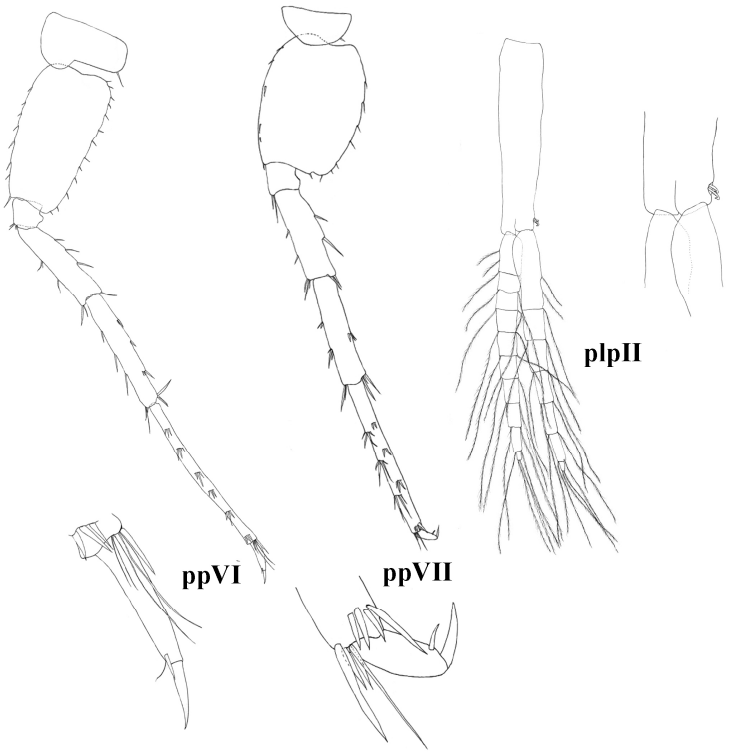


Figure 11: *N. molnari*, ppVI: pereopod VI, ppVII: pereopod VII, plpII: pleopod II.

Pleopods

Pleopods I-III with 2-hooked retinacles. Pleopod II rami of 16-20 articles each (Figures 7, 11).

Uropods

Uropod I basipodite with 6 dorso-lateral and 6 dorsomedial spinifom setae. Length ratio endopodite: exopodite as 1.00: 0.89 (0.83-1); rami slightly curved. Endopodite total

setae number 2-4 in 2-3 groups, apically 5 spinifom setae. Exopodite with 2-7 spines; apically 5 spinifom setae (Figure 12).

Uropod II endopodite: exopodite length as 1.00: 0.81 (0.77-0.9) (Figure 12).

Uropod III up to 38-46% (males) and 12-42% (females) of body length. Basipodite without lateral seta and with 3-6 apical spiniform and thin setae. Endopodite 58-61% (males) and 48-70% (females) of basipodite length, endopodite apically with 1-2 thin-flexible and spiniform setae; laterally 0-1 seta. Exopodite of uropod III rod-shaped, distal article of exopodite 83-115% (males) and 18-73% (females) of proximal exopodite article length. Proximal article with 4-5 groups of plumose, thin-flexible and spiniform setae along inner margin and 4 groups of thin-flexible and spiniform setae along outer margin. Distal article with 3-6 apical setae; lateral setae only on males. (Figure 12).

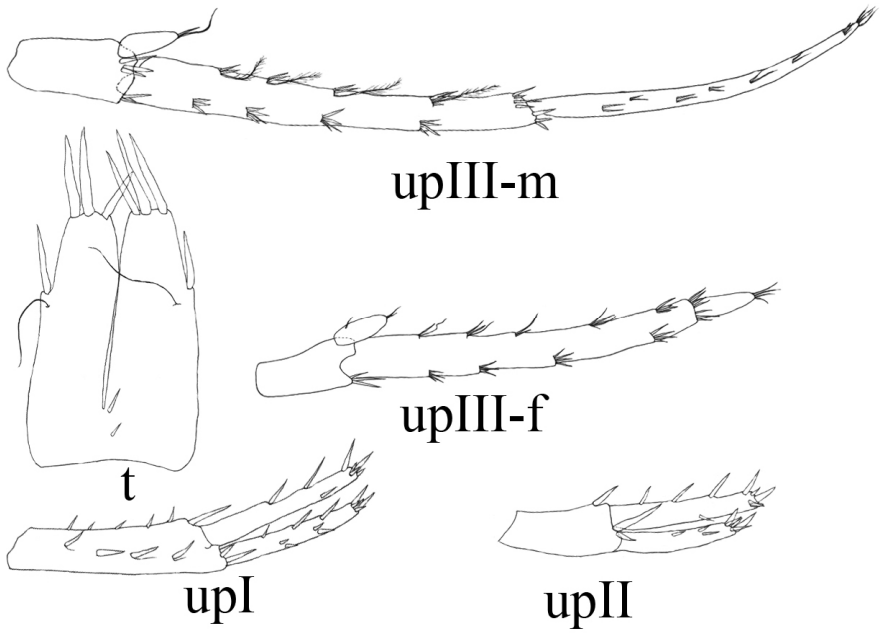


Figure 12: *N. molnari*, t: telson, upI: uropod I, upII: uropod II, upIII-f: female's uropod III, upIII-m: male's uropod III.

Niphargus gebhardti Schellenberg, 1934

Niphargus gebhardti: Schellenberg 1934 (description); *Niphargus foreli gebhardti*: Schellenberg 1935 (additional morphological data); *Niphargus foreli gebhardti*, *Niphargus gebhardti*: Gebhardt 1934, 1963, 1967 (distributional data); *Niphargus molnari*: Angyal & Balázs 2013a (morphological data); *Niphargus molnari*: Angyal & Balázs 2013b (distributional data); *Niphargus molnari*: Balázs & Angyal 2013, Angyal & Balázs 2014, Balázs et al. 2015 (evaluation of the Hungarian species); *Niphargus molnari*: Angyal et al. 2015 (redescription).

Material examined for redescription: 7 females and 4 males from a permanent pool in the main passage near 'Karthago's Ruins' hall of the Abaligeti Cave, collected in 23 March 2013 (leg. D. Angyal & A. Illés), dissected and mounted on slides; additional 4 specimens not dissected.

Description

Body and telson

Small sized niphargid species, females 4.9-5.9 mm, males 5.9-7.0 mm. Head length up to 9% of body length; rostrum absent. Pereonites I-VI without setae; pereonite V, VI, VII with 1 postero-ventral seta each. Pleonites I-III with 1-2 setae along dorso-posterior margin. Epimeral plate II posterior and ventral margins convex, ventro-postero-distal corner rounded. Along ventral margin 1-3 spiniform setae; along posterior margin 3-4 thin setae. Epimeral plate III ventral and posterior margins convex, ventropostero-distal corner rounded; along ventral margin 2-3 spiniform setae; along posterior margin 4 thin setae. Urosomite I postero-dorso-laterally with 1 seta; urosomite II postero-dorso-laterally with 1 spiniform seta; urosomite III without setae. Near insertion of uropod I 1 spiniform seta (Figures 13, 14).

Telson length: width as 1.0: 0.88 (0.84-0.91 1.09-1.19); cleft 74 (70-79)% of length; lobes apically widely rounded. Telson spines (per lobe): 2-4 apical spines of 33.5 (28-39)% telson length; lateral margins with 0-2 spines and 0-1 plumose seta; 0-1 in cleft spines, 0 or 1 dorsal surface spine and 1 basal spine (Figures 14, 19).

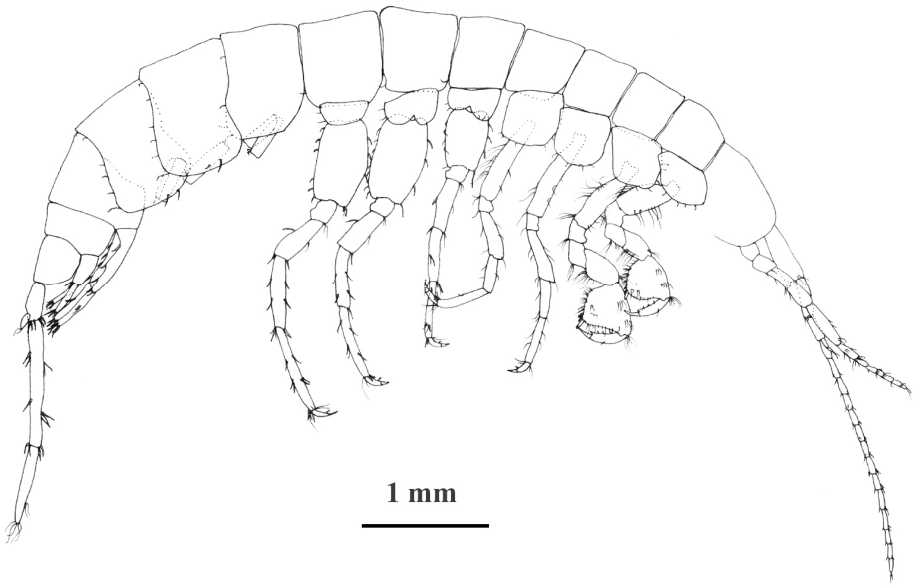


Figure 13: *N. gebhardti*, female from the Abaligeti Cave, lateral view. Mouthparts, rami of pleopods and telson are not drawn.

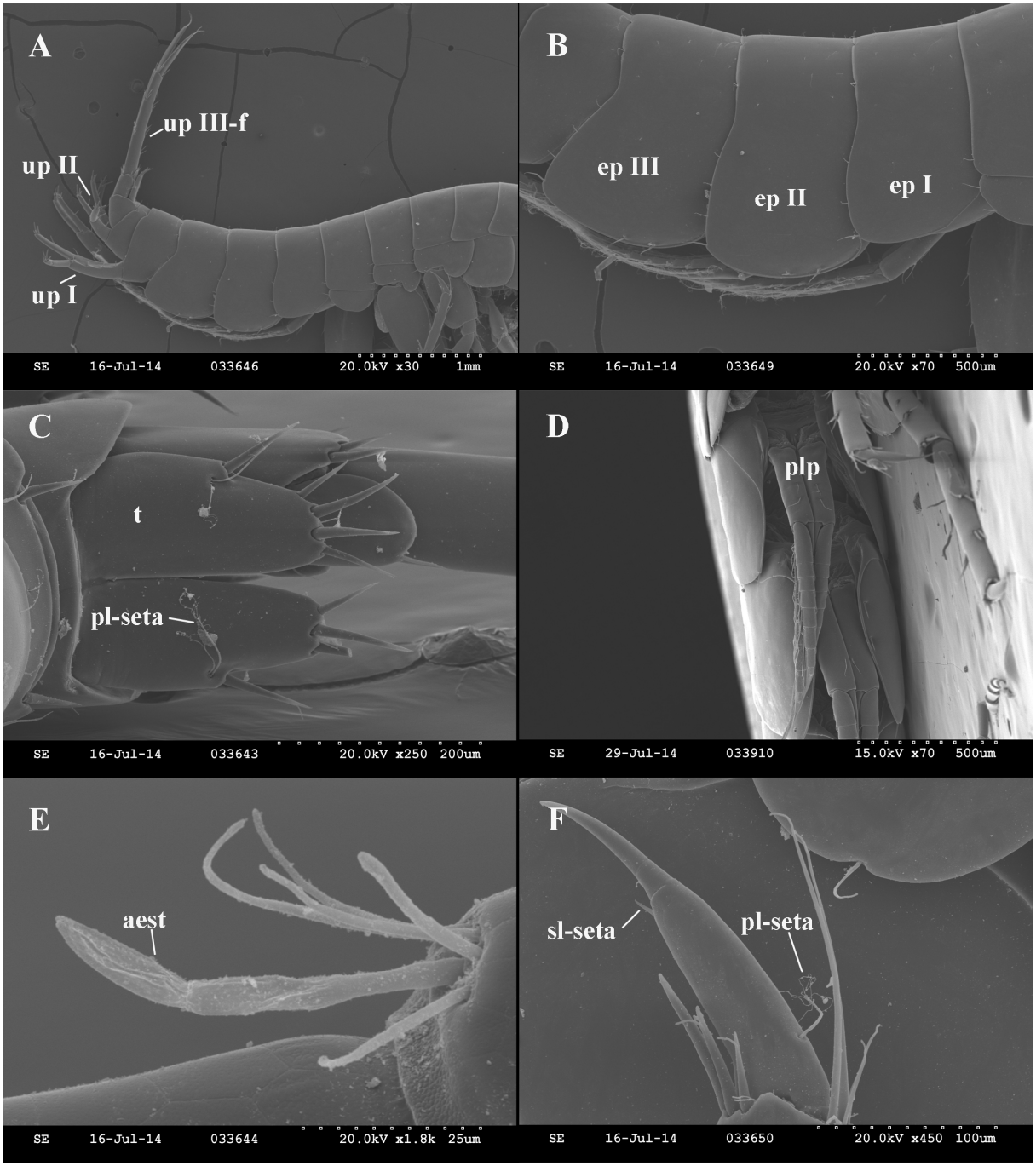


Figure 14: *N. gebhardti* scanning electron micrographs. A: epimeral plates with uropods (Ep1-3: epimeral plates 1-3, upI: uropod I, upII: uropod II, upIII-f: female's uropod III), B: epimeral plates (Ep1-3: epimeral plates 1-3), C: telson (pl-seta: plumose seta, t: telson,), D: pleopods (plp: pleopod), E: aesthetasc on antenna I (aest: aesthetasc), F: pereopod VI dactylus (pl-seta: plumose seta, sl-seta: spine-like seta at the base of the nail).

Antennae and mouthparts

Antenna I 37 (34-41)% of body length. Flagellum with up to 13-16 articles; each

article with 1 long aesthetasc (Figure 14). Peduncle article 1: 2: 3 as 1.0: 0.69 (0.60-0.76) : 0.37 (0.30-0.4). Proximal article of peduncle dorso-distally slightly produced. Accessory flagellum biarticulated; distal article 52 (38-67)% of proximal article. Lengths of antennae I: II as 1.0: 0.48 (0.42-0.52). Flagellum of antenna II with 6-8 articles. Lengths of peduncle articles 4:5 as 1.0: 0.85 (0.81-0.91); flagellum 73 (57-81)% of peduncle length (articles 4+5) (Figure 15).

Inner lobes of labium longer than half of outer lobes (Figure 16).

Left mandible: incisor with 5 teeth, lacinia mobilis with 4 teeth; between lacinia and molar 5-7 thick, serrated setae, long seta at base of molar absent (Figure 15).

Right mandible: incisor processus with 4 teeth, lacinia mobilis with 5-6 denticles, between lacinia and molar 6-8 thick, serrated setae, 1 long seta at base of molar present. Proportions of mandibular palp articles 2:3 (middle: distal) as 1.0: 1.1 (1.00-1.21). Proximal palp article without setae; second article with 4-6 seta in 3-4 groups; distal article with 1 group of 3-4 ‘A setae’; 2-4 of ‘B setae’ (single or in groups); 9-13 ‘D setae’ and 3-5 ‘E setae’ (Figure 15).

Maxilla I distal palp article with 3-6 apical and subapical setae. Outer lobe of maxilla I with 7 spines, pluri-, uni-, bi-toothed spines alternating. Inner lobe with 1 seta (Figure 15).

Maxilla II inner lobe slightly smaller than outer lobe; both of them setose apically and subapically, number of setae is approximately 6-11 on inner lobe and 8-12 on outer lobe (Figure 15).

Maxilliped palp article 2 with 8-11 rows of setae along inner margin; distal article with dorsal seta and group of small setae at base of nail. Maxilliped outer lobe with 6-8 flattened, thick setae and 3-5 serrated setae; inner lobe with 2-3 flattened, thick setae apically and 2-4 serrated setae (Figure 15).

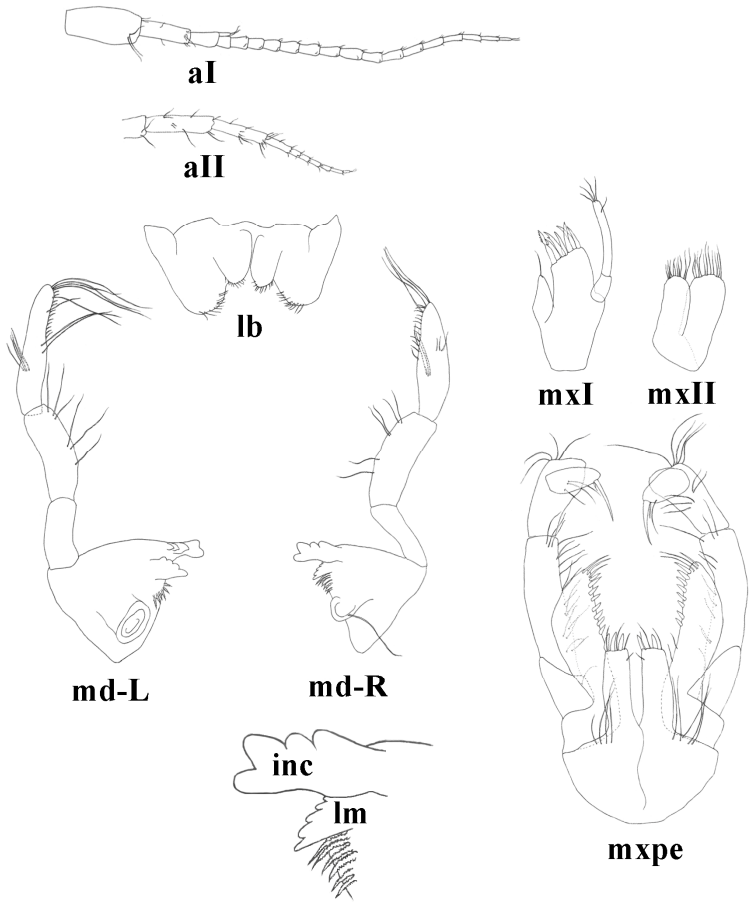


Figure 15: *N. gebhardti*, aI: antenna I, aII: antenna II, mxI: maxilla I, mxII: maxilla II, md-R: right mandibula, inc: incisor, lm: lacinia mobilis, md-L: left mandibula, lb: labium, mxpe: maxilliped.

Coxal plates

Coxal plate I width: depth as 1.00: 0.76 (0.6-0.9), antero-ventral corner subrounded; anterior and ventral margin of coxa I with 4-6 setae. Coxal plate II width: depth as 1.00: 9.7 (0.83-1.21); anterior and ventral margin with 3-6 setae (Figure 16). Coxal plate III width: depth as 1.00: 1.12 (1.05-1.2); along antero-ventral margin 4-6 setae. Coxal plate IV width: depth as 1.00: 1.04 (0.97-1.12); posteriorly concave; along antero-ventral margin 4-5 setae (Figure 17). Coxal plates V-VI with well developed anterior lobe, and smaller posterior lobe with usually 2 setae (occasionally with 1 or 3) in posteroventral corner. Coxal plate VII half-egg shaped, along posterior margin 2 setae. Gills II-VI ovoid, of approximately similar size as coxa VI (Figure 17).

Gnathopods

Gnathopod I basis width 42 (38-47)% of basis length. Ischium with 3-4 posterodistal setae in 1 row. Carpus length 61 (52-82)% of basis length and 98 (87-110)% of propodus length. Anterior margin of carpus only with distal group of setae; carpus posteriorly with transverse rows of setae proximally and a row of lateral setae, posterior enlargment small. Propodus subquadrate, palm and posterior margin convex. Along posterior margin 3-4 rows of denticulated setae. Anterior margin with 6-11 setae in 2-3 groups, antero-distal group with 4-8 setae. Group of 2-3 facial setae below (proximal of) palmar spine; 1-4 surface setae in 1-2 groups present. Palmar corner with palmar spine, single supporting spine on inner surface, and 2-3 denticulated, thick spiniform setae on outer side. Nail length 33 (30-39)% of total dactylus length; along anterior margin single seta; along inner margin 3-4 setae (Figure 16).

Gnathopod II basis width: length as 1.0: 0.34 (0.27-0.45). Ischium with 3-4 postero-distal setae in 1 row. Carpus length 59 (48-69)% of basis length and 106 (96-111)% of propodus length. Anterior margin of carpus only with distal row of setae; carpus posteriorly with transverse rows of setae, proximally a row of lateral setae; postero-proximal bulge small and positioned proximally. Propodus small to medium-sized (sum of length, diagonal and palm length measures up to 12-15% of body length) and larger than propodus of gnathopod I (1.0: 0.87 (0.78-0.96)). Propodus rectangular, palm convex. Posterior margin straight or convex with 4-5 rows of denticulated setae. Anterior margin with 3-9 setae in 1-2 groups; antero-distal group with 4-8 setae. Group of 2-4 facial setae below (proximal of) palmar spine; 2-3 surface setae in 1-2 groups present. Palmar corner with strong palmar spine, single supporting spine on inner surface, and 2-3 denticulated, thick spiniform setae on outer side. Nail length 34 (29-42)% of total dactylus length. Along anterior margin a single seta; along inner margin 3 short setae (Figure 16).

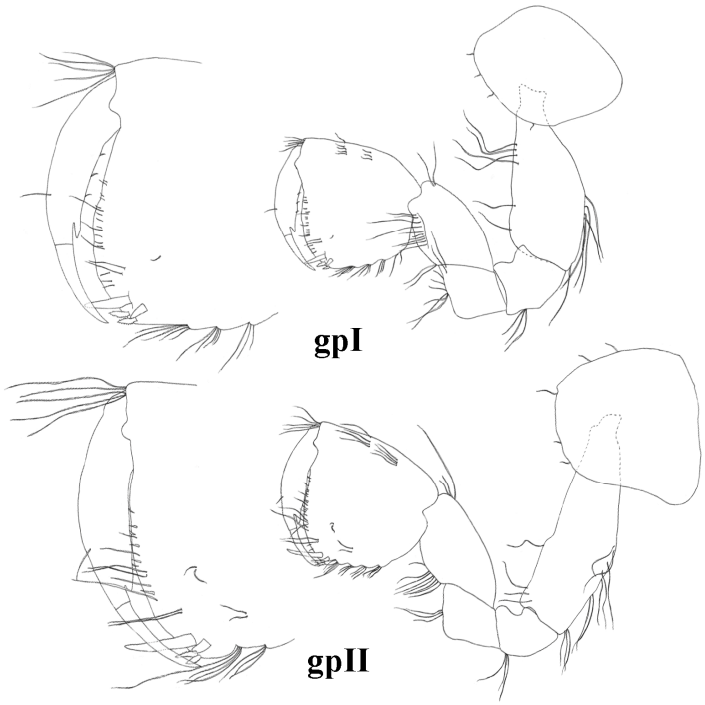


Figure 16: *N. gebhardti*, gpI: gnathopod I, gpII: gnathopod II.

Pereopods III-IV

Proportions of pereopods III: IV as 1: 0.96 (0.89-1). Dactylus IV 51 (46-57)% of propodus IV length; nail length 53 (44-61)% of total dactylus length. Dactyli III-IV with one dorsal plumose seta (sometimes not visible or absent), one spine-like seta at the base of the nail, and a tiny seta near the spine-like seta (sometimes not visible or absent). Additional spiniform setae on posterior margin are absent (Figure 17).

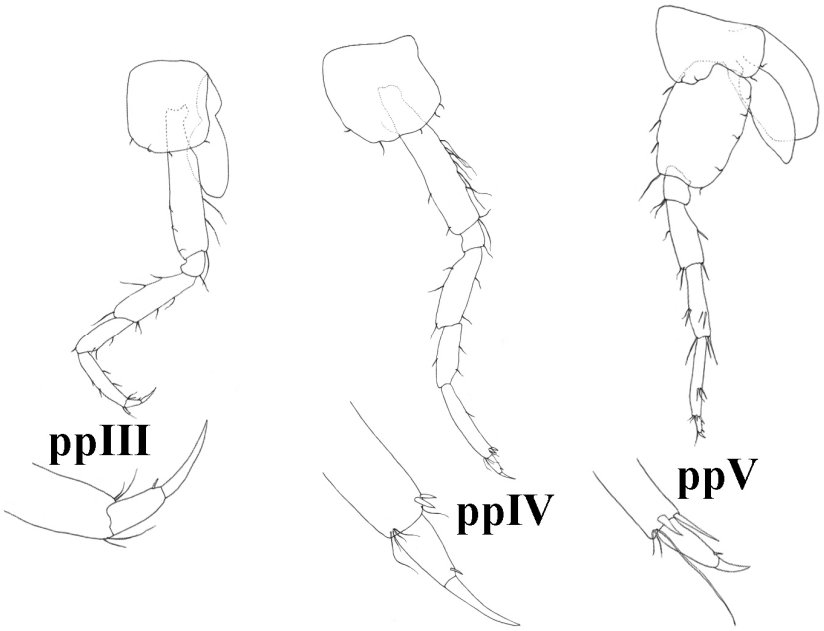


Figure 17: *N. gebhardti*, ppIII: pereopod III, ppIV: pereopod IV, ppV: pereopod V.

Pereopods V-VII

Proportions of pereopods V: VI: VII as 1.00: 1.3 (1.27-1.49): 1.5 (1.46-1.58). Pereopod VII length 42-45% of body length. Basis V-VII with convex posterior margins. Basis V width is 71 (66-80)% of length, basis VI is 68 (64-73)% of length, and basis VII is 66 (63-69)% of length. Basis V with small posterodistal lobe, posterior margin with 4-6 setae, anterior margin with 4-9 setae in 3+1 groups (Figure 17). Pereopod dactylus V with one dorsal plumose seta (sometimes not visible or absent), and one spine-like seta at the base of the nail (Figure 17). Basis VI with small posterodistal lobe, posterior margin with 6-7 setae, anterior margin with 5-8 setae in 3-4 groups. Dactylus VI with one spine-like seta at the base of the nail, and a tiny seta near the spine-like seta (sometimes not visible or absent). Additional spiniform setae on posterior margin are absent (Figure 14, 18). Basis VII posterior margin with 5-8 setae, anterior margin with 3-5 groups of setae. Total number of basis setae is 11-15. Dactylus VII length 26 (23-35)% of propodus VII length; nail length 28.5 (25-38)% of total dactylus length. Dactyli VII with one dorsal plumose seta (sometimes not visible or absent), one spine-like seta at the base of the nail, and a tiny seta near the spine-like seta

(sometimes not visible or absent). Additional spiniform setae on posterior margin are absent (Figure 18).

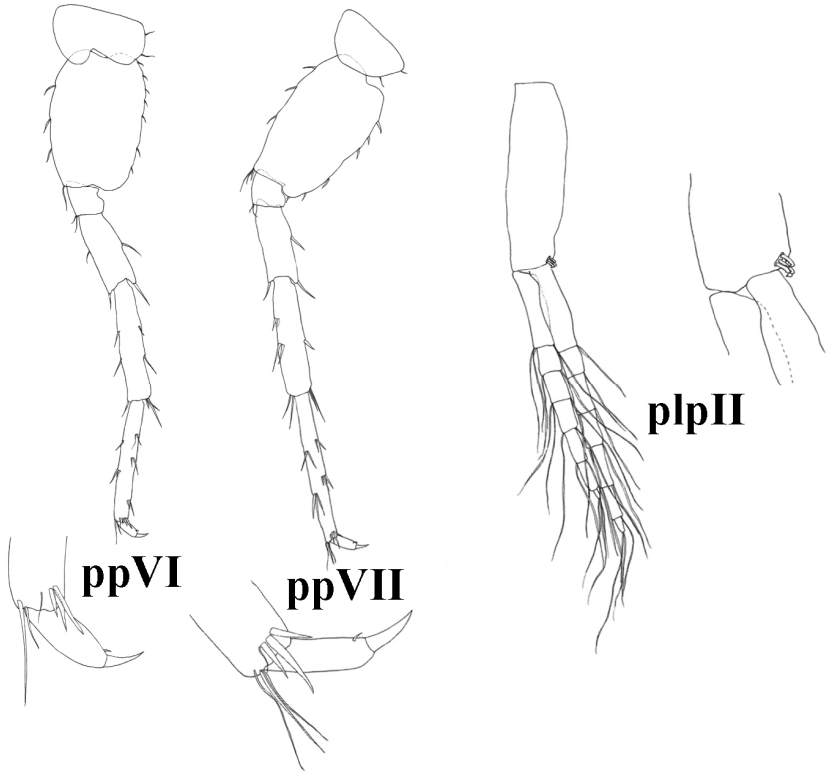


Figure 18: *N. gebhardti* ppVI: pereopod VI, ppVII: pereopod VII, plpII: pleopod II.

Pleopods

Pleopods I-III with 3, rarely 4 hooked retinacles. Pleopod II rami of 11-13 articles each (Figsures 14, 18).

Uropods

Uropod I basipododite with 4-5 dorso-lateral and 1-3 dorsomedial spiniform setae including spiniform setae in distal position. Length ratio endopodite: exopodite as 1.00: 0.91 (0.87-0.97); rami slightly curved. Endopodite with 1-2 setae, apically 5 spinifom setae. Exopodite with 1-4 setae or spines in 1-2 groups; apically 5 spinifom setae (Figure 14, 19).

Uropod II endopodite: exopodite length as 1.00: 0.84 (0.77-0.951.05-1.32) (Figures

14, 19).

Uropod III 38 (37-39)% (males) and 26 (24-30)% (females) of body length. Basipodite with 0-1 lateral setae and 5-6 apical spiniform and thin setae. Endopodite 41 (39-44)%

(males) and 48 (41-54)% (females) of basipodite length; endopodite apically with 0-2 thin-flexible and spiniform setae; laterally with 0-1 seta. Exopodite of uropod III rod-shaped, distal article of exopodite 100 (95-105)% (males) 60 (52-78)% (females) of proximal article length. Proximal article with 3-4 groups of plumose, thin-flexible and spiniform setae along inner margin and 2-4 groups of thin-flexible and spiniform setae along outer margin. Distal article without lateral seta (males) or with 3 setae in 1 group (females); apically 4-7 setae (Figure 14, 19).

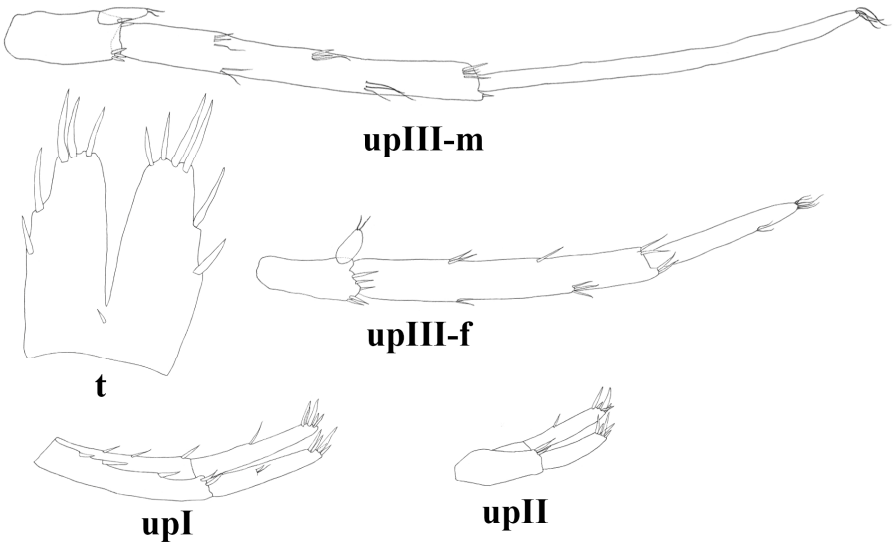


Figure 19: *N. gebhardti*, t: telson, upI: uropod I, upII: uropod II, upIII-m: male's uropod III, upIII-f: female's uropod III.

Comparison with phylogenetically related and morphologically similar species

N. molnari and *N. gebhardti* share few main traits (the same body size class, slender body, sexually dimorphic uropod III but not uropod I), but differ from each other in the shape of epimeral plates, the size of gnathopod propodi, in denticulation of spines on outer lobe of maxilla I and in the number of retinacles (Angyal & Balázs 2013). Keeping these differences in mind, both species were compared to the species that are either closely related according to molecular phylogeny, or to the species that live in the same geographic area.

***Niphargus vadimi* Birstein, 1961** is known from Crimea. Despite it's close position suggested by the presented molecular tree, this species differs from phylogenetically related *N. gebhardti* and non-related *N. molnari* in considerably larger body size and much larger gnathopods.

High morphological similarity to the focal pair of species reveal another four species phylogenetically related to *N. gebhardti*, namely ***Niphargus bihorensis* Schellenberg, 1940**, ***Niphargus fongi* Fišer & Zagmajster, 2009**, ***Niphargus carniolicus* Sket, 1960** and ***Niphargus dobati* Sket, 1999**. Epikarstic *N. bihorensis* is known from Romania and Italy, whereas the latter three are known from epikarst and karst river beds from Slovenian caves. All four species share with the focal species' main traits (body size, slender body, sexually dimorphic uropod III but not uropod I).

N. bihorensis and *N. fongi* differ from the focal species in the shape of gills (being narrow instead of ovoid as in focal species) and in higher number of retinacles on pleopods. In addition, *N. fongi* differs from *N. molnari* and *N. gebhardti* by (i) the elevated number of setae along posterior margin of epimeral plate III, (ii) the longer apical telson spines, (iii) and the reduced number of denticulated spines in palmar corners of both gnathopods. *N. bihorensis*, which is a complex of at least two morphologically indistinguishable species (Meleg et al. 2013), differs from the focal species by (i) reduced number of spines on maxilla I outer lobe (only 6), (ii) more numerous setae on maxilla I palpus (7-8), (iii) and by more numerous retinacles.

N. carniolicus and *N. dobati* differ from the focal pair of species in the length of rami of uropod I (expopodite equal to or slightly longer than endopodite versus exopodite consistently shorter to endopodite in focal species). In addition, *N. carniolicus* differs from *N. molnari* and *N. gebhardti* by (i) shorter apical spines on telson, and (ii) fewer denticulated spines on palmar corner of gnathopods. *N. dobati* differs from the two focal species by (i) the elevated number of spines on uropod I basipodite, (ii) the length of pereopod V and VI (which are longer comparing with pereopod VII), and the (iii) elevated number of mandibular palp 'D seta'.

Phylogenetic relationship of *N. molnari* to the rest of *Niphargus* species is not clear, however a few morphologically similar species, like ***Niphargus schellenbergi* Karaman S., 1932** are known. It differs from *N. molnari* and *N. gebhardti* by (i) the differently ornamented telson (5-7 long apical spines and 2-5 lateral spines in *N. schellenbergi*), (ii) more numerous apical setae on uropod III endopodite, (iii) elevated number of pleopod retinaculi, (iv) by the length of uropod I exopodite, which is slightly longer than endopodite, (v) by several setae along outer margin of gnathopod dactyli, and (vi) by bigger body size (>10 mm).

The following inland subterranean species are compared with *N. molnari* and *N. gebhardti* due to their geographical vicinity. ***Niphargus forroi* Karaman G., 1986** was described from Northeast Hungary, and is known from only a couple of caves from the Bükk Mts. Beside the close body size, *N. forroi* agree with *N. molnari* by the similar seta numbers and arrangement on the gnathopods, by the telson spine-pattern, as well as by the number of different spine and seta types on pereopod dactyls. *N. forroi* differs from *N. molnari* by (i) the subrounded posteroventral corner of the epimeral plates, (ii) the lower number of mandibular palp 'D setae' and by (iii) the reduced number of maxilla distal article apical seta. *N. forroi* differs from both *N. molnari* and *N. gebhardti* by the number of posterior margin setae on

pereopods V-VII. The description of ***Niphargus hungaricus*** **Méhely, 1937** (endemic species of the Kőszegi Mts.) contains no drawings and not enough characters that would be needed for proper comparison. A later work of Méhely (1941) is only partially filling this gap by containing a drawing on the first gnathopod and some additional data on its seta arrangement. According to the available information, *N. hungaricus* differs from *N. molnari* and *N. gebhardti* by (i) the setae number of gnathopods dactyli outer margin (always more than 1 seta of *N. hungaricus*) and by (ii) the length of male's uropod I endopodite (inner ramus is elongated and two times long as outer ramus in *N. hungaricus*). There are different *Niphargus* populations in the Bükk Mts. and in the Aggtelek Karst belonging to the ***Niphargus tatrensis*** **Wrzesniowsky, 1888** species group including ***Niphargus aggtelekiensis*** **Dudich, 1932**. Although the taxonomic status of these populations is not clear, the complex shares several distinct morphological characters that can be compared with focal species. Populations of *N. tatrensis* - *N. aggtelekiensis* complex differ from *N. molnari* and *N. gebhardti* by (i) larger body size (>15 mm), (ii) the elevated number of setae along outer margin of gnathopods dactyli (there are more than one), (iii) the lower mandibular 'A' and 'D seta' number and (iv) the elongated distal article of uropod III of both sexes. Main diagnostic characters are presented in Table 9.

Table 9: Main diagnostic characters of the *Niphargus* species involved in the comparison.

Species	No. apical telson spines	No. lateral telson spines	Pleopod I. no. hooks in retinacle	Pleopod II. no. hooks in retinacle	Pleopod III. no. hooks in retinacle	Uropod I endopodite/ exopodite length	Gnathopod dactylus anterior margin seta no.	Shape of gills II-IV	Epimeral plates postero-ventral corner shape	Source of data
***N. molnari* Méhely, 1927**	3-4	1-3	2	2	2	endopodite slightly longer	single	ovoid	sharply inclined	own slides
***N. gebhardti* Schellenberg, 1934**	3-6	0-2	3 (rarely 4)	3 (rarely 4)	3 (rarely 4)	endopodite slightly longer	single	ovoid	subrounded	own slides
***N. carniolicus* Sket, 1960**	4-5	1-2	4-5	4-5	4-5	exopodite slightly longer	single	?	subrounded	Sket 1960, G. Karaman 1989
***N. dobati* Sket, 1999**	3+1	2	3-4	3-4	3-4	nearly equal	single	narrow	subrounded	Sket 1999
***N. vadimi* Birstein, 1961**		3	?	?	?	?	?	?	sharply inclined	Birstein 1961
***N. fongi* Fišer & Zagmajster, 2009**	3-5	1-2	4-7	3-5	4-5	equal	single	narrow	subrounded	Fišer & Zagmajster 2009
***N. bihorensis* Schellenberg, 1940**	5-7	1 pair, plumose	4-6	4-6	4-6	exopodite slightly longer	single	long and recurved	I., II. subrounded, III. angular	G. Karaman 1980
***N. schellenbergi* S. Karaman, 1932**	5-7	2-5	4-6	3-5	3-6	exopodite slightly longer	more than 1	?	subrounded	S. Karaman 1932
***N. forroi* G. Karaman, 1986**	2	2	2	2	2	endopodite longer	single	narrow	subrounded	G. Karaman 1986
***N. hungaricus* Méhely, 1937**	3-5	1-2	?	?	?	endopodite 2x longer	more than 1	?	subrounded	Méhely 1937, 1941
***N. tatrensis* Wrzesniowsky, 1888**	3-4	0-3	2	2	2	nearly equal	more than 1	large, irregula-rely ovoid	III. sharply inclined	Fišer et al. 2010

3.1.3 Phylogenetic studies on *N. molnari* and *N. gebhardti*

Analysis of the 638 bp COI sequences of *N. gebhardti* from 6 distinct caves of the Western Mecsek revealed one haplotype with only two single nucleotide polymorphisms (Figure 20).

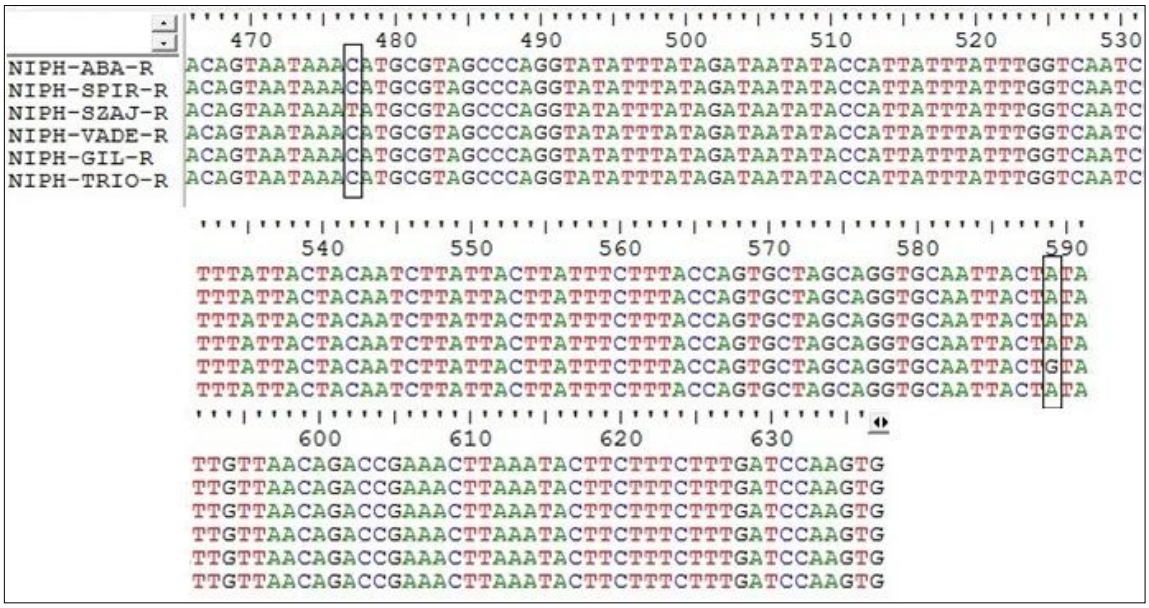


Figure 20: Detail of the compared *N. gebhardti* COI sequences. Framed parts contain polymorphisms.

Phylogenetic relationships within the genus *Niphargus* (Figure 21) showed that the two redescribed species of *Niphargus* from Hungary are not phylogenetically closely related. Phylogenetic relationship of *N. molnari* to the rest of *Niphargus* species is unclear; species is nested within basal polytomy. *N. gebhardti* belongs to the clade of Central to Eastern European species. The focal species is in sister relationship with a pair of morphologically cryptic species endemic to Western Carpathian (*N. bihorensis*, see Meleg et al. 2013). Other closely related species include *N. vadimi* from Crimea, *Pontoniphargus racovitzai* from Eastern Romania and a clade of epikarstic and interstitial species from Southern Slovenia (*N. fongi*, *N. carniolicus*, *N. wolfi* and *N. dobati*).

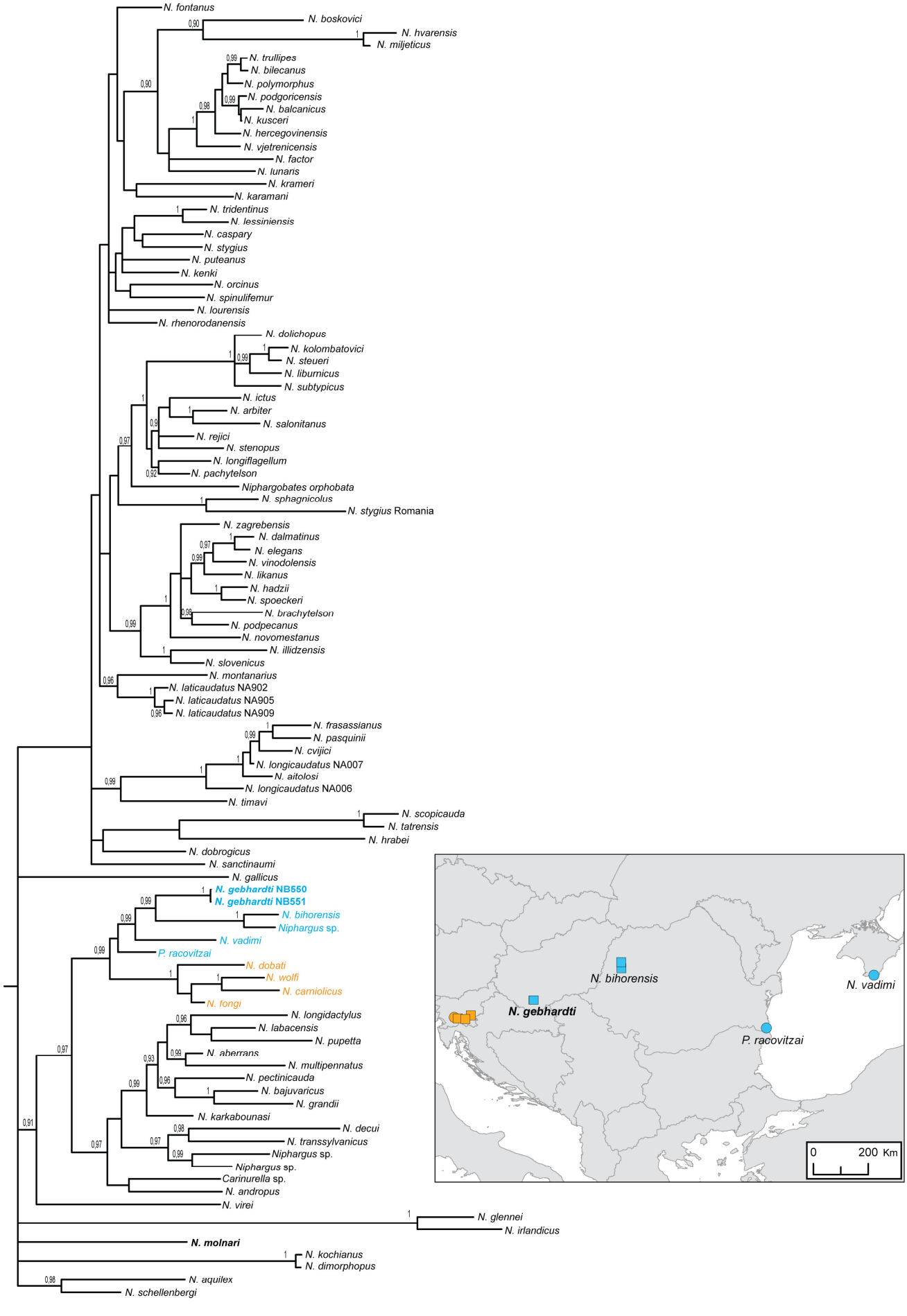

N. fontanus
N. boskovici
N. hvarensis
N. miljeticus
N. trullipes
N. bilecanus
N. polymorphus
N. podgoricensis
N. balcanicus
N. kusceri
N. hercegovinensis
N. vjetrenicensis
N. factor
N. lunaris
N. krameri
N. karamani
N. tridentinus
N. lessiniensis
N. caspary
N. stygius
N. puteanus
N. kenki
N. orcinus
N. spinulifemur
N. lourensis
N. rhenorodanensis
N. dolichopus
N. kolombatovici
N. steueri
N. liburnicus
N. subtypicus
N. ictus
N. arbiter
N. salonitanus
N. rejici
N. stenopus
N. longiflagellum
N. pachytelson
Niphargobates orphobata
N. sphagnicolus
N. stygius Romania
N. zagrebensis
N. dalmatinus
N. elegans
N. vinodolensis
N. likanus
N. hadzii
N. spoeckeri
N. brachytelson
N. podpecanus
N. novomestanus
N. illidzensis
N. slovenicus
N. montanarius
N. laticaudatus NA902
N. laticaudatus NA905
N. laticaudatus NA909
N. frasassianus
N. pasquinii
N. cvijici
N. longicaudatus NA007
N. aitolosi
N. longicaudatus NA006
N. timavi
N. scopicauda
N. tatrensis
N. hrabei
N. dobrogicus
N. sanctinaumi
N. gallicus
N. gebhardti NB550
N. gebhardti NB551
N. bihorensis
Niphargus sp.
N. vadimi
P. racovitzai
N. dobati
N. wolfi
N. carniolicus
N. fongi
N. longidactylus
N. labacensis
N. pupetta
N. aberrans
N. multipennatus
N. pectinicauda
N. bajuvaricus
N. grandii
N. karkabounasi
N. decui
N. transsylvanicus
Niphargus sp.
Niphargus sp.
Carinurella sp.
N. andropus
N. virei
N. glennei
N. irlandicus
N. molnari
N. kochianus
N. dimorphopus
N. aquilex
N. schellenbergi
0.1
N. bihorensis
N. vadimi
N. gebhardti
P. racovitzai
0
200 Km

Figure 21: Bayesian phylogenetic tree of 104 amphipod taxa (including *N. molnari* and *N. gebhardti*) based on COI, 28S and histone (H3) sequences. Map represents distributions of species belong to the same clade with *N. gebhardti*. Squeres represent epikarstic species and circles species from other subterranean habitats.

3.1.4 New distributional data for *N. molnari* and *N. gebhardti* and remarks on their ecology

In the Abaligeti Cave *N. molnari* was found in the stream of the Western 2 collateral. *N. gebhardti* was collected from a permanent pool in a lateral chamber of 'Karthago romjai' hall in the main passage and from a pool at the end of Western 2 collateral, near Akácos Cave's entrance (Figure 22). Among the studied two species, *N. gebhardti* was the more frequently found, as it was observed in five other caves of the Western Mecsek in addition to the type locality, namely Trió Cave, Gilisztás Cave, Szajha-felső Cave, Vadetetős Cave and Spirál Cave (Angyal & Balázs 2013) (Figure 23). In most of these, small pools and streaming water also exist. Amount of water in the caves is dependent on the rainfall in the surface. In all six caves, *N. gebhardti* specimens were found in isolated, shallow pools in limestone, sinter or clay, most likely formed by dripping water (Figure 24). Specimens were never observed in streams or any other streaming waters. During my repeated visits between 2010 and 2013 (in total 24 occasions altogether in the 6 caves), the same pools were checked every time and some specimens were always found (except when the pools dried out). *N. gebhardti* specimens were never found in the water samples of the epikarstic water collectors. Once it was observed as a group of *N. gebhardti* (approximately 20 specimens) were fed upon a dead *Oxychilus* snail in a pool. In the Vadetetős Cave in a shallow sinter basin (called 'Kút') *N. gebhardti* coexisted with the blind aquatic isopod *Protelsonia hungarica hungarica* Méhely, 1924. Same observation was made in 'Spirál szíve-terem' of Spirál Cave in a small limestone basin. Localities of *N. gebhardti* in Gilisztás Cave, Szajha-felső Cave, Trió Cave, Vadetetős Cave and Spirál Cave are represented in Figures 25-29.

N. molnari was observed in the Abaligeti Cave and in two sinkholes that the other species (*N. gebhardti*) was also inhabited (Angyal & Balázs 2013) (Figures 24, 29, 30). Density of *N. molnari* was high in the stream of the Western 2 collateral of the Abaligeti Cave, however in the other two caves only a few specimens were found in streaming water, always in deeper parts of the caves. The two species were always spatially well segregated. In the Abaligeti Cave *N. molnari* coexisted with *P. hungarica hungarica* and with the troglomorphic specimens of *Gammarus fossarum* Koch, 1836. In one occasion coexistence of *N. molnari* and *P. hungarica hungarica* was also observed at the beginning of the streamy branch of the Spirál Cave. Individuals of both species were collected by singling, using soft forceps or a hand net; using of bottle traps was not successful.

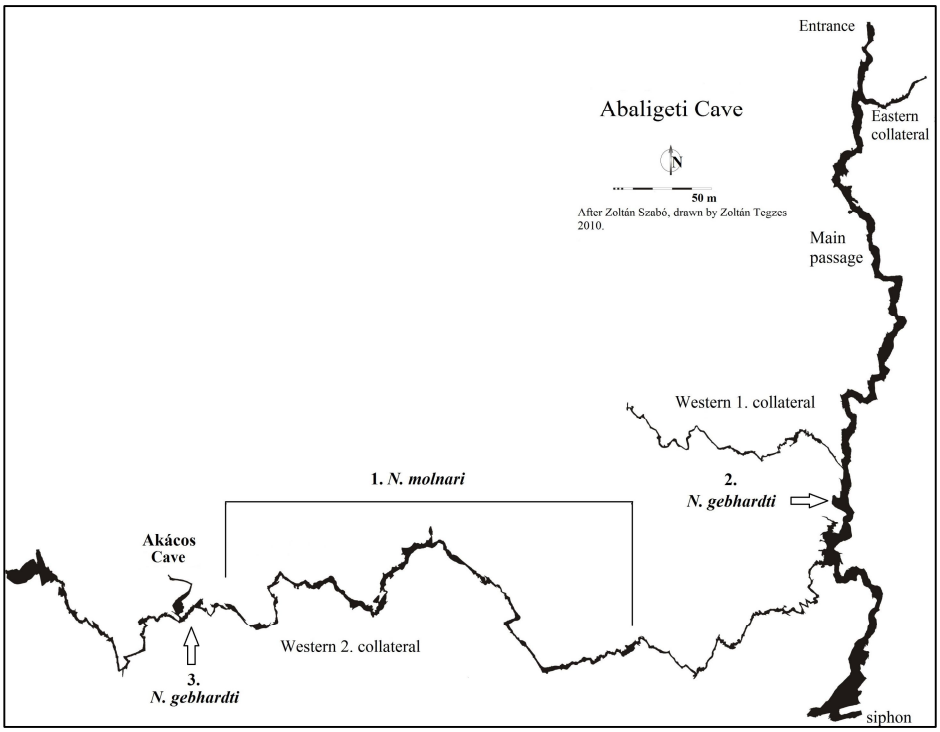


Figure 22: Distribution of *N. molnari* and *N. gebhardti* within the Abaligeti Cave. 1: *N. molnari* along the stream of the Western 2 collateral, 2: *N. gebhardti* in a permanent pool of 'Karthago romjai', 3: *N. gebhardti* in a permanent pool near the Akácos Cave's entrance.

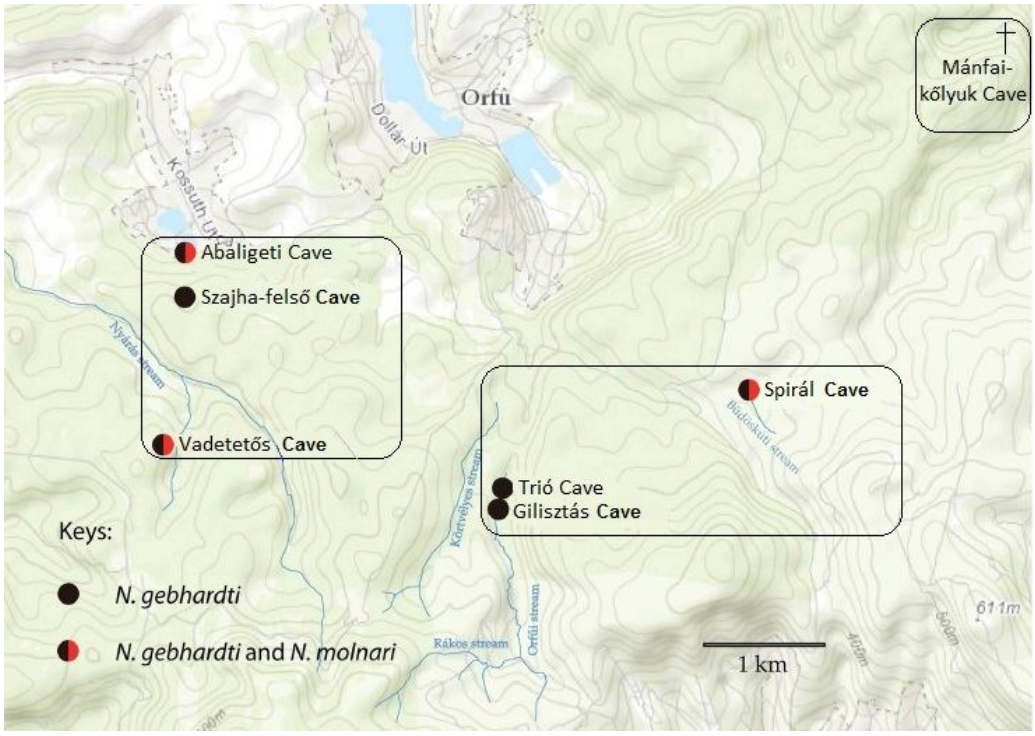


Figure 23: Distribution of *N. molnari* and *N. gebhardti* within the Western Mecsek. Hydrologically connected caves are in quadrats.

Figure 24: One of the habitats of *N. gebhardti* within the Trió Cave.

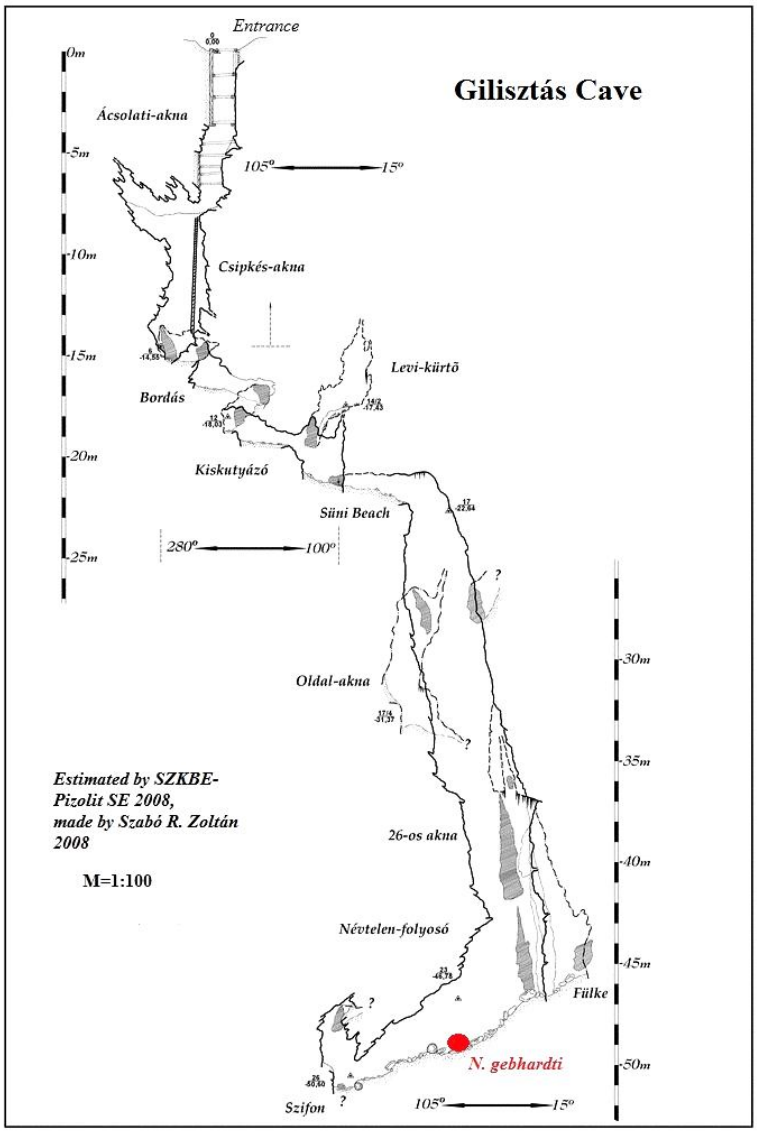


Figure 25: Locality of *N. gebhardti* within the Gilisztás Cave.

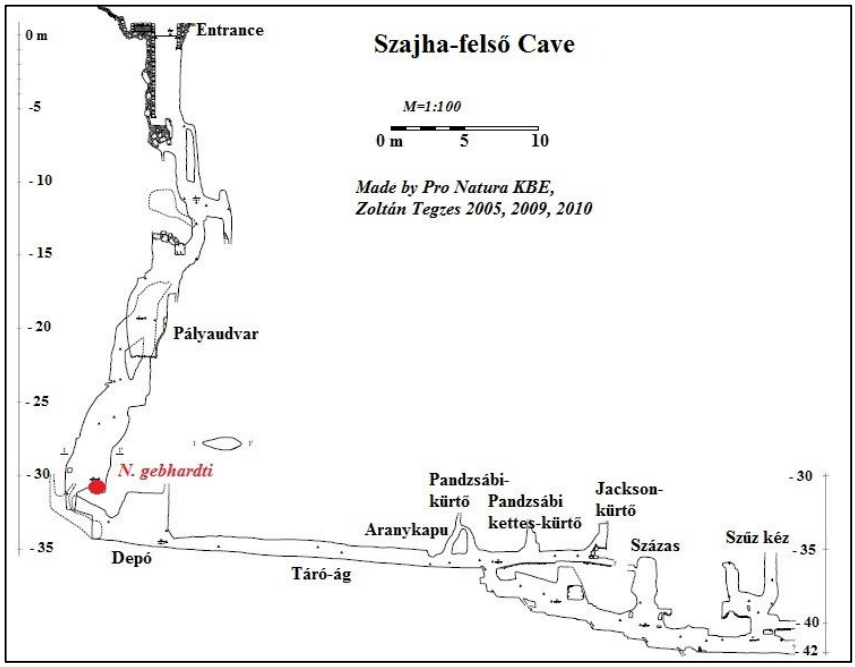


Figure 26: Locality of *N. gebhardti* within the Szajha-felső Cave.

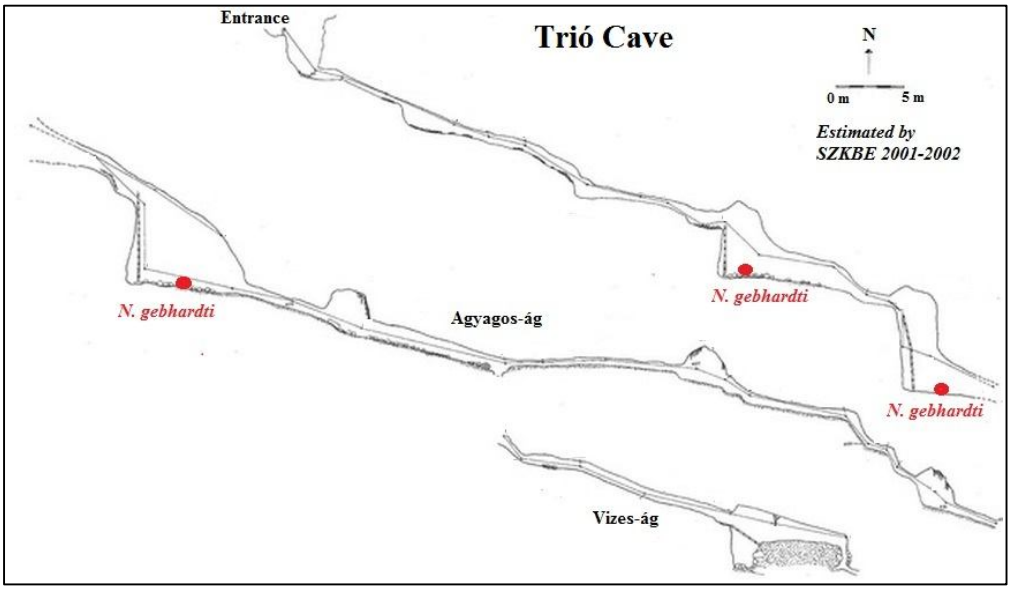


Figure 27: Localities of *N. gebhardti* within the Trió Cave.

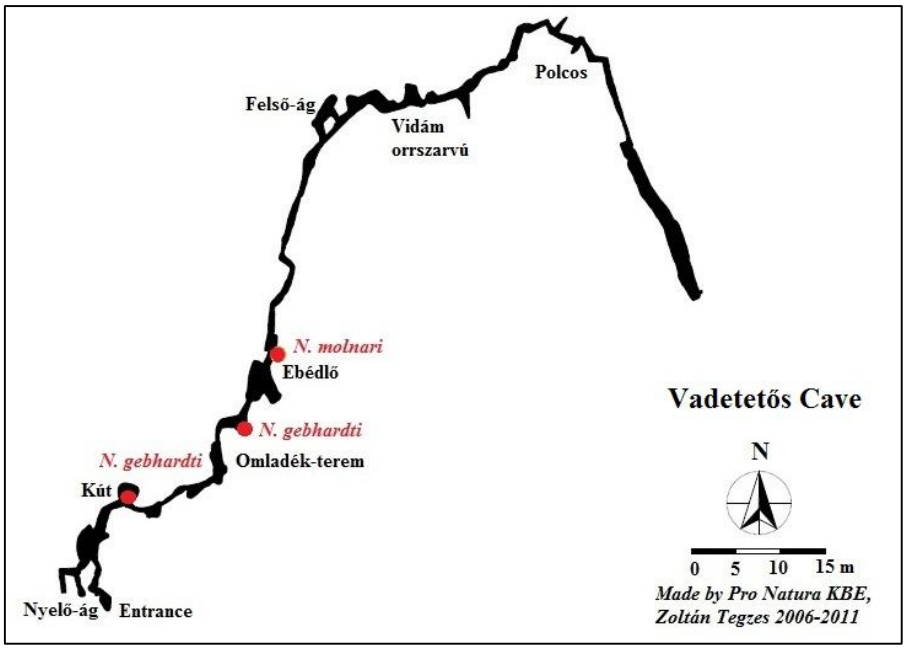


Figure 28: Localities of *N. gebhardti* and *N. molnari* within the Vadetetős Cave.

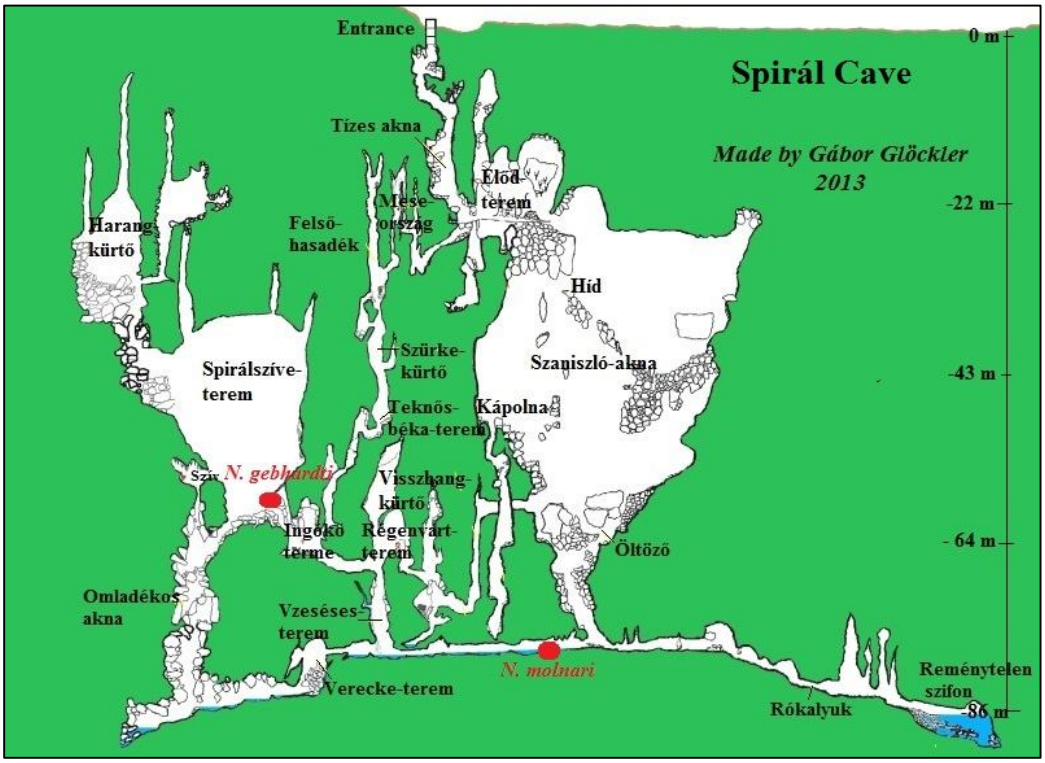


Figure 29: Localities of *N. gebhardti* and *N. molnari* within the Spirál Cave.

3.2 Revision of *Protelsonia hungarica hungarica* Méhely, 1924 and *Protelsonia hungarica robusta* Méhely, 1927 (Isopoda, Stenasellidae)

3.2.1 Preliminary knowledge related to *P. hungarica hungarica* and *P. hungarica robusta* and to the genera *Protelsonia* Méhely, 1924 and *Stenasellus* Dollfus, 1897

The aquatic isopod genus *Protelsonia* and the species *Protelsonia hungarica* was described by Lajos Méhely in 1924, based on the specimens that were collected from the stream of the Abaligeti Cave by the author in October 1924 (Méhely 1924). However, Bokor (1924, 1925) stated in his publications written about the faunistic investigation of the Abaligeti Cave that the first two female specimens of the new asellid had been found by him in 15 October 1923, 416 m far from the entrance. Méhely (1924) explained the necessity of the description of the new species by the incomplete and controversial publications of two French colleagues about a rather similar cave-dwelling Asellota. In 1896 Armand Viré discovered a single specimen of a blind aquatic isopod in the Padirac Cave, France. Two years later, Adrien Dollfus described that as a new species, *Stenasellus virei* Dollfus, 1898. In his study, among the main characters of the new species, he mentioned the three free (memberless) segments between the last pereion segment and the pleotelson. He also stated that pereopods II-IV originate on the middle of the ventral margin of the pereion segments, while pereopods V-VII originate on the lateral corners of the ventral margin of the pereion segments (Dollfus 1898). Four years later, Viré revised the description. Although the two authors had studied the same specimen, they added controversial data about that. Viré had stated two -instead of three- free segments between the last pereion segment and the pleotelson, and had drawn the pereopods all of them with medioventral origin (Viré 1902). In the description of *P. hungarica* written in German, Méhely (1924) agreed with Viré in the number of free segments and named the new genus after this character '*Protelsonia*' ('pro telson'). However, he disagreed with Viré regarding the position of the origins of pereopods, as the first four pairs of pereopods of *P. hungarica* have lateroventral-, while the last three pairs have medioventral origins. In that same paper, detailed information about the pleopods of males and females was also given, as well as some observations related to the species' ecology. However, drawings or any illustrations had not been added. One year later, Méhely (1925) published the same explanation about the necessity of his description in Hungarian language too. At the same time, Dudich (1924, 1925) revised the *Stenasellus virei* topotype material of Viré's collection (Padirac Cave, 1913), which was preserved in the Hungarian National Museum. He compared these specimens with the new species from the Abaligeti Cave and he found that all the generic characters of *S. virei* agree with the characters of *P. hungarica*, therefore the genus *Protelsonia* Méhely, 1924 is the synonym of the genus *Stenasellus* Dollfus, 1898. Based on the five consequent characters found between *S. virei* and *P. hungarica*, Dudich stated that the two species are assuredly different and the name of the new Hungarian species should be *Stenasellus hungaricus* (Méhely, 1924). A new family, Stenasellidae had also been established by Dudich in that same paper. In his comparative

study, Racovitza (1924) also treated the genus *Protelsonia* as a synonym of *Stenasellus* and described a new species from a Serbian cave, *Stenasellus gjorgjevici* Rakovitza, 1924. Three years after the discovery of the blind asellid species from the Abaligeti Cave, Méhely (1927) described a new subspecies, *Protelsonia hungarica robusta* Méhely, 1927 based on the specimens collected from the Mánfai-kőlyuk Cave by István Molnár in 1 November 1926. He found two consequently different characters between the specimens of the two population: i) the posteroventral corner of the exopodite of male's first pleopod bears only two long setae and one shorter, spiniform seta, ii) exopodite and endopodite of male's second pleopod is larger (the name 'robusta' may refers to this feature) and the riffle-shaped sinus of endopodite that is visible in the specimens from the Abaligeti Cave cannot be found in the new subspecies. A drawing illustration was also given about the latter character. Rémy (1948), in his publication about the cavernicolous crustaceans of Europe, added further drawings about the pleopods (I-V) of *P. hungarica* from the Abaligeti Cave. Later on, two new species of *Stenasellus* were described from Bulgaria (Racovitza, 1950, Buresch & Guéorguiev 1962), namely *Stenasellus bureschi* Racovitza, 1950 and *Stenasellus lakatnicensis* Buresch & Guéorguiev, 1962. Mestrov (1960) described a new subspecies of the Hungarian species, *Stenasellus hungaricus thermalis* Mestrov, 1960 from a thermal spring near Zagreb. Four decades later, Magniez (2000) validated the genus *Protelsonia,* and redescribed the nominal subspecies *P. hungarica hungarica* Méhely, 1924 with the addition of drawings about the mouthparts, pleopod I and II, and a habitus drawing. Based on the four differing generic characters found as a result of his revision of Stenasellidae, he stated that without the knowledge of other closely relative species, the creation of the genus *Protelsonia* was a 'brave prediction' from Méhely, however he was right. In that same paper, Magniez (2000) transposed the stenasellid species and subspecies from Bulgaria and the former Yugoslavia into the genus *Protelsonia* as *P. bureschi* (Rakovitza, 1950), *P. gjorgjevici* (Racovitza, 1924), *P. hungarica thermalis* (Mestrov, 1960) and *P. lakatnicensis* (Buresch & Gueorguiev, 1962). He added that the revision of *P. hungarica robusta* Méhely, 1927 -that had not been happened until that time- would also be required. One year later he added a more detailed drawing of the male's second pleopod of *P. hungarica hungarica* in his paper written about the recent data on stygobiotic Stenasellidae and assessed that *Protelsonia* species appear more related to West and Northwest African lineages than the widely spread genus *Stenasellus* Dollfus, 1897 (Magniez 2001).

3.2.2 Revision of the Stenasellidae material preserved in the Hungarian Natural History Museum

Fourteen vials of *Stenasellus* and *Protelsonia* species and subspecies, preserved in the Crustacea Collection of the HNHM have been revised, including the type seria of *P. hungarica robusta*. Sexes, body length data and notes on condition of the specimens were also registered. Holotype of *Protelsonia hungarica* Méhely, 1924 is most likely to be destroyed.

Stenasellus virei

Isop. No. 1664, Puits de Padirac, 1913, leg. Dr. A. Viré, det. Dr. A. Viré ('*Stenasellus virei* Dolf.'), revid. D. Angyal, 2015: 2 ♀ (10 mm, 11 mm)

Isop. No. 1665, Puits de Padirac, 12/1925, leg. Dr. A. Viré, det. Dr. A. Viré ('*Stenasellus viréi* Dfs.'), revid. D. Angyal, 2015: 5 ♀ (6.5 mm, 7 mm, 7 mm, 7 mm, 8 mm)

Protelsonia hungarica hungarica

Isop. No. 1656, Abaligeti Cave, 12/08/1924, leg. Dr. E. Bokor, det. Dr. E. Dudich ('*Stenasellus hungaricus*'), revid. D. Angyal, 2015: 2 ♀ (4.5 mm, 6 mm)

Isop. No. 1657, Abaligeti Cave, 18/07/1927, leg. Dr. E. Dudich & Dr. A. Gebhardt, det. Dr. E. Dudich ('*Stenasellus hungaricus* Méhely'), revid. D. Angyal, 2015: 2 ♀ (3.5 mm, 4 mm)

Isop. No. 1653, Abaligeti Cave, 19/09/1930, leg. Dr. E. Dudich, det. Dr. E. Dudich ('*Stenasellus hungaricus* Méhely'), revid. D. Angyal, 2015: 2 ♂ (4 mm, 5 mm)

Isop. No. 1654, Abaligeti Cave, stream, 21/11/1923, leg. Dr. E. Dudich, det. Dr. E. Dudich ('*Stenasellus hungaricus* My'), revid. D. Angyal, 2015: 1 ♂ (3.5 mm)

Isop. No. 1655, Abaligeti Cave 26/09/1926, leg. Dr. L. Méhely, det. Dr. L. Méhely ('*Protelsonia hungarica* Méh.'), revid. D. Angyal, 2015: 16 ♀ (4-6.5 mm), 10 ♂ (4-6 mm)

Isop. No. 1658, Abaligeti Cave, 19/30/1930, leg. Dr. A. Gebhardt, det. ? ('*Stenasellus* (*Protelsonia*) *hungaricus* Méhely'), revid. D. Angyal, 2015: 1 ♂ (4.5 mm), 1 ♀ (5 mm)

Isop. No. 1652, Abaligeti Cave, 15/03/1925, leg. Dr. E. Bokor, det. Dr. E. Dudich (' *Stenasellus hungaricus* Méh.') revid. D. Angyal, 2015: 1 ♀ (6 mm), 1 ♂ (5 mm)

3257/1953, **Amphipoda 1988**, Abaligeti Cave, 24/10/1936, leg. Dr. Kesselyák, det. ? ('*Protelsonia hungarica*?'), revid. D. Angyal, 2015: 15 ♀ (3-4.5 mm), 7 ♂ (2.5-4 mm), 6 juv.(2-3 mm), 2 broken

Protelsonia hungarica robusta

Isop. No. 1663, Mánfai-kőlyuk Cave, 1/11/1926, leg. I. Molnár, det. Dr. L. Méhely ('*Protelsonia* sp.'), revid. D. Angyal, 2015: 1 ♂ (4 mm, in bad condition), 4 ♀ (3 mm, 3 mm, 3.5 mm, 3.5 mm, head is missing). However it was not labeled, the **type seria** was most likely to be found now, as the exact date, locality and the collector's name agree with the data published in the subspecies' description (Méhely 1927). Méhely labeled '6 ♀, Bouin: fix.', which means that slides had also been made by him, though, those cannot be found in the present Crustacea Collection.

Isop. No. 1659, Mánfai-kőlyuk Cave, 30/03/1931, leg. Dr. A. Gebhardt, det. Dr. E. Dudich ('*Stenasellus hungaricus robustus* Méh.'), revid. D. Angyal, 2015: 1 ♂ (6 mm, in bad condition), 1 juv. (3 mm, in bad condition)

Isop. No. 1660, Mánfai-kőlyuk Cave, stream, 30/05/1931, leg. Dr. A. Gebhardt, det. Dr. E. Dudich ('*Stenasellus hungaricus robustus* Méh.'), revid. D. Angyal, 2015:1 ♀ (6 mm)

Isop. No. 1661, Mánfai-kőlyuk Cave, 30/08/1931, leg. Dr. A. Gebhardt, det. Dr. E. Dudich ('*Stenasellus hungaricus robustus* Méhely'), 1 ♀ (6 mm), 1 ♂ (5 mm)

3.2.3 Morphological studies on the newly collected *P. hungarica hungarica* and *P. hungarica robusta* material

Newly collected material (62 specimens) from three caves (Abaligeti Cave, Vadetetős Cave, Mánfai-kőlyuk Cave) have been examined and 12 of them have been dissected on slides. Beside the body length (measured from the origin of the antennae to the base of the uropods), distinguishing characters of *P. hungarica hungarica* and *P. hungarica robusta* suggested by Méhely (1924, 1927) were studied. Appendages and mouthparts of a female specimen from the Western 2 collateral of the Abaligeti Cave have been studied by scanning electron microscopy.

Protelsonia hungarica hungarica Méhely, 1924

Protelsonia hungarica: Méhely 1924 (description); *Protelsonia hungarica*: Bokor 1924, 1925 (faunistic data); *Stenasellus hungaricus*: Dudich 1924, 1925 (systematic stand); *Stenasellus hungaricus*: Gebhardt 1933, 1963, 1967 (faunistic data); *Protelsonia hungarica hungarica*: Magniez 2000 (redescription); *Protelsonia hungarica hungarica*: Magniez 2001 (additional morphological data); *Protelsonia hungarica*: Angyal & Balázs 2013b (distributional data).

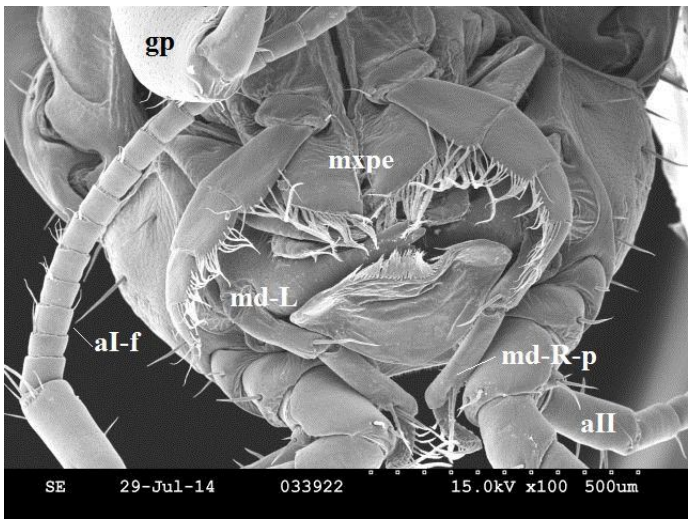
Examined material: P_ABA_01,02: Abaligeti Cave, Western 2 collateral, stream, 23/03/2013, 2 ♀ (6.5 mm, 5.5 mm); P_ABA_03: Abaligeti Cave, main passage, stream, 23/03/2013, ♀ (5 mm); P_ABA_04: Abaligeti Cave, Western 2 collateral, stream, ♂ (6 mm, dissected on slide); P_ABA_05-06: Abaligeti Cave, main passage, stream, 23/03/2013, 2 ♀ (5.5 mm, 5.5 mm); P_ABA_07-16: Abaligeti Cave, main passage, stream, 14/04/2014, 4 ♀ (7 mm, 7 mm, 5.5 mm, 6 mm), ♂ (5 mm, dissected on slide), 5 juveniles (3.5 mm, 3.5 mm, 2.5 mm, 2.5 mm, 2.5 mm); P_ABA_17-18: Abaligeti Cave, end of Western 2 collateral, pool, 23/03/2013, ♀ (7 mm), ♂ (5 mm, dissected on slide); P_VADE_01: Vadetetős Cave, 'Ebédlő-terem', streaming water, ♂ (5 mm, dissected on slide); P_VADE_02-05: Vadetetős Cave, after 'Kút' in a pool, 04/07/2012, 2 ♀ (6 mm, 6 mm), 2 ♂ (5.5 mm, 4.5 mm); P_VADE_06: Vadetetős Cave, 'Kút', sinter pew, 02/04/2013, ♀ (5.5 mm); P_VADE_07-11: Vadetetős Cave, 'Kút', sinter pew, 04/07/2012, 2 ♀ (6 mm, 5 mm), 2 ♂ (4.5 mm, dissected on slide, 4.5 mm), juvenile (3 mm).

White, eyeless, elongated, vermicular asellid, body size up to 7 mm (Figures 30, 31). Females are larger than males. Average length of females is 6.05 mm (5.5-7 mm) of specimens from the Abaligeti Cave and 5.9 mm (5.5-6 mm) of specimens from the Vadetetős Cave. Average length of males is 5.33 mm (5-6 mm) of specimens from the Abaligeti Cave and 4.8 mm (4.5-5.5) of specimens from the Vadetetős Cave. Antenna I with 18-25 articles, antenna II with 5-7 articles (Figure 32). Incisor of mandibles small, left mandible with 4 teeth, right mandible with 12-13 teeth, near pars molaris a raw of fine setae. Mandibular palp with 3 articles (Figure 32). Maxilliped palpus large, with 5 articles (Figure 32). Two memberless segments before the pleotelson (Figures 31, 33). Propodus of gnathopod elongated, posterior margin with 6 denticulated spines and 3-4 setae. Anterior margin of propodus with 1-2 single setae. At the base of the dactylus, 2 setae. Inner margin of dactlyus with 3 thick denticulated spines. In front of the nail 0-4 setae with central (not marginal) origin. Posterior margin of

carpus with 5-7 strong spines usually uni-toothed, sometimes bi-toothed (Figure 34). Pereopods II-VII equal, dactyli with single nails (Figures 31, 35). On pereion segment VII at the base of pereopod VII of males a pair of chitinized tube (penis). Males with 5, females with 4 pairs of pleopods. Female's first pair of pleopod is missing, pleopod II is a simple, triangle-shaped lamina (Figures 33, 35). Protopodite of males's pleopod I convex, without setae, ventral margin of exopodite with 3 long setae and 1-2 short, spiniform setae (Table 10, Figure 36). Proximal article of male's pleopod II exopodite with 1, distal article with 4-5 long setae. Endopodite with riffle-shaped sinus (Table 10, Figure 37). Pleopod III (operculum) a large, oval lamina (Figures 31, 33). Pleopods IV-V endopodite sack-like, serve as gills, exopodites finger-shaped, biarticulated, pleopod IV with 2-3 long apical setae on the distal article of exopodite. Uropod with massive basipodite, endopodite slightly longer than exopodite, both rami apically with long plumose setae. Adult females with oostegites.

Figure 30: Multilayer habitus photo of a male specimen of *P. hungarica hungarica* from the Vadetetős Cave.

Figure 31: Multilayer habitus photo of a female specimen of *P. hungarica hungarica* from the Abaligeti Cave.



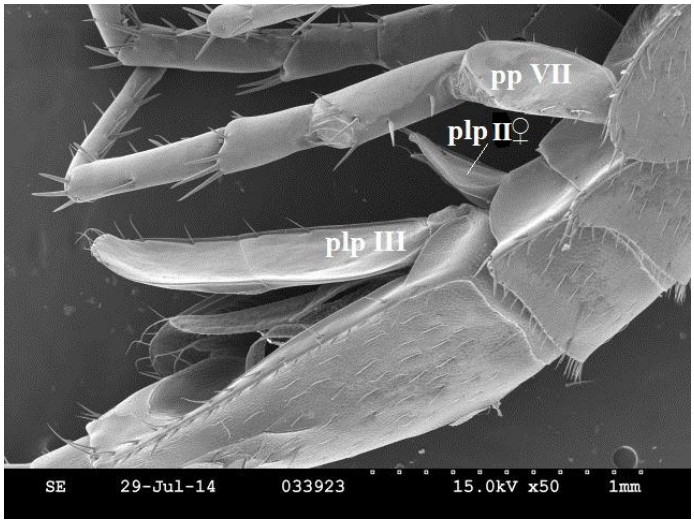
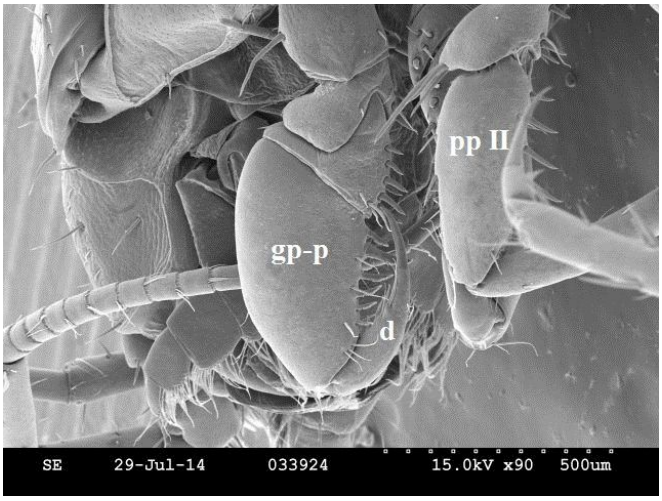


Figure 32 (left): *P. hungarica hungarica*, head ventral view, female from the Abaligeti Cave, scanning electron micrograph. aI-f: antenna I flagellum, aII: antenna II, gp: gnathopod, md-L: left mandible, md-R-p: palpus of right mandible, mxpe: maxilliped.

Figure 33 (right): *P. hungarica hungarica*, posterior segments, lateral view, female from the Abaligeti Cave, scanning electron micrograph. plp II ♀: pleopod II of female, plp III: pleopod III, pp VII: pereopod VII.



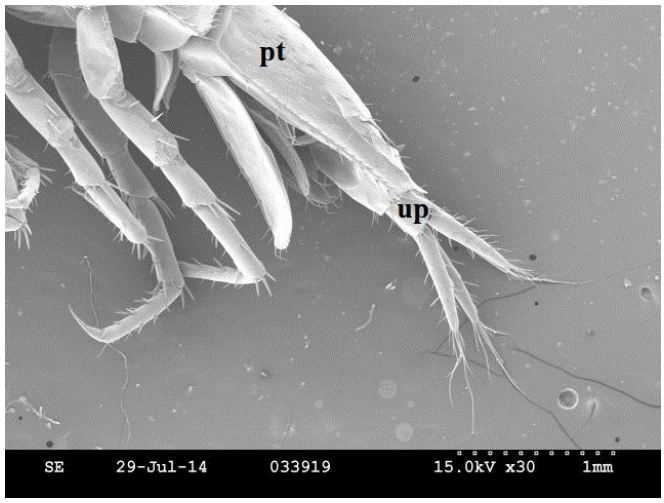


Figure 34 (left): *P. hungarica hungarica*, anterior body part, lateral view, female from the Abaligeti Cave, scanning electron micrograph. d: gnathopod dactylus, gp-p: gnathopod propodus, pp II: pereopod II.

Figure 35 (right): *P. hungarica hungarica*, posterior body part, lateral view, female from the Abaligeti Cave, scanning electron micrograph. pt: pleotelson, up: uropod.

Table 10: Comparison of main distinguishing characters of *P. hungarica hungarica* of the original description (Méhely 1924) with the characters of the newly collected material

	***P. hungarica hungarica*, original description**	***P. hungarica hungarica*, Abaligeti Cave (new collection)**	***P. hungarica hungarica*, Vadetetős Cave (new collection)**
Body length	up to 7.5 mm	5-7 mm	4.5-6 mm
Pleopod I exopodite setae number	3 long setae + few short, spiniform seta	3 long setae + 1 short, spiniform seta	3 long setae + 2 short, spiniform setae
Pleopod II exopodite distal article setae number	4-5	4-5	4-5
Pleopod II exopodite proximal article seta number	1	1	1
Presence of riffle-shaped sinus of pleopod II endopodite	present	present	present

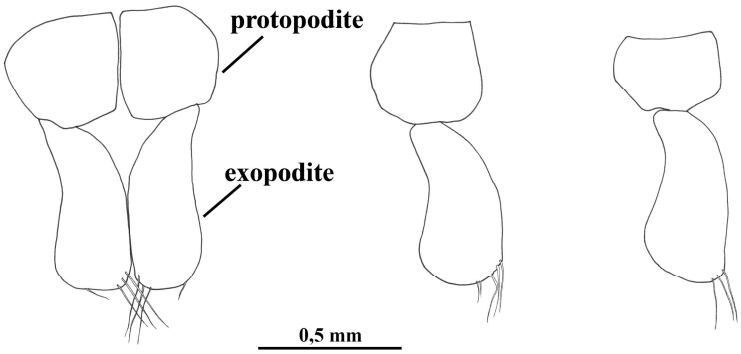


Figure 36: Comparison of male's pleopod I of *P. hungarica hungarica* and *P. hungarica robusta*. (Left: male from the Abaligeti Cave, middle: male from the Vadetetős Cave, right: male from the Mánfai-kőlyuk Cave.

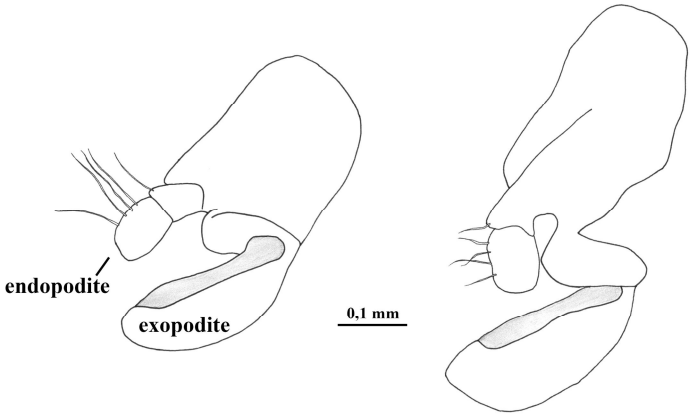


Figure 37: Male's pleopod II of *P. hungarica hungarica*. (Left: male from the Abaligeti Cave, right: male from the Vadetetős Cave, ventrally elongated (deformed) under coverslip.

Protelsonia hungarica robusta Méhely, 1927

Protelsonia hungarica robusta: Méhely 1927 (description); *Stenasellus hungaricus* v. *robustus*: Gebhardt 1933, 1963, 1967 (faunistic data); *Protelsonia hungarica robusta*: Magniez 2000 (evaluation of stenasellids); *Protelsonia hungarica robusta*: Magniez 2001 (evaluation of stenasellids); *Protelsonia hungarica*: Angyal & Balázs 2013b (distributional data).

Examined material: P_MAN_01-33: Mánfai-kőlyuk Cave, upper passage, artificial tunnel, streaming water, 22/12/2010, 24 ♀ (5-7.5 mm, P_MAN_01 dissected on slide), 8 ♂ (4.5-5.5 mm, P_MAN_09, 11, 30, 33 dissected on slides).

White, eyeless, elongated, vermicular asellid, body size up to 7.5 mm. Females are larger than males. Average length of females is 5.83 mm (5-7.5 mm). Average length of males is 4.85 mm (4.5-5.5 mm). Antenna I with 18-24 articles, antenna II with 5-7 articles. Incisor of mandibles small, left mandible with 4 teeth, right mandible with 12-13 teeth, near pars molaris a raw of fine setae. Mandibular palp with 3 articles. Maxilliped palpus large, with 5 articles. Two memberless segments before the pleotelson. Propodus of gnathopod elongated, posterior margin with 6 denticulated spines and 6 setae. Anterior margin of propodus with 2 single setae. At the base of the dactylus, 2 setae. Inner margin of dactlyus with 3 thick denticulated spines. In front of the nail 4 setae with central (not marginal) origin. Posterior margin of carpus with 5 strong spines usually uni-toothed, sometimes bi-toothed, and 2 additional setae. Pereopods II-VII equal, dactyli with single nails. On pereion segment VII at the base of pereopod VII of males a pair of chitinized tube (penis). Males with 5, females with 4 pairs of pleopods. Female's first pair of pleopod is missing, pleopod II is a simple, triangle-shaped lamina (Figure 37). During the examination of male's pleopod I and pleopod II, two categories (forms) of specimens were found. Characters of 70% of the specimens -called 'form Mánfa'- agreed with the original description (Méhely, 1927), however 30% - called 'form Abaliget' were nearer to the characters of *P. hungarica hungarica*. Protopodite of males's pleopod I of 'form Mánfa' convex, without setae, ventral margin of exopodite with 3 long setae and without spiniform setae (Table 11, Figure 36). Proximal article of males's pleopod I of 'form Abaliget' convex, without setae, ventral margin of distal article with 3 long setae and with 1-3 short, spiniform setae (Table 11). Proximal article of male's pleopod II exopodite of 'form Mánfa' without setae, distal article with 3 long setae. Endopodite long, simple, tongue-shaped, without sinus (Table 11, Figure 39). Proximal article of male's pleopod II exopodite of 'form Abaliget' with 0-1 seta, distal article with 3-5 long setae. Endopodite with riffle-shaped sinus (Table 11, Figure 39). Pleopod III (operculum) a large, oval lamina. Pleopods IV-V endopodite sack-like, serve as gills, exopodites finger-shaped, biarticulated, pleopod IV with 2-3 long apical setae on the distal article of exopodite (Figure 38). Uropod with massive basipodite, endopodite slightly longer than exopodite, both rami apically with long plumose setae. Adult females with oostegites.

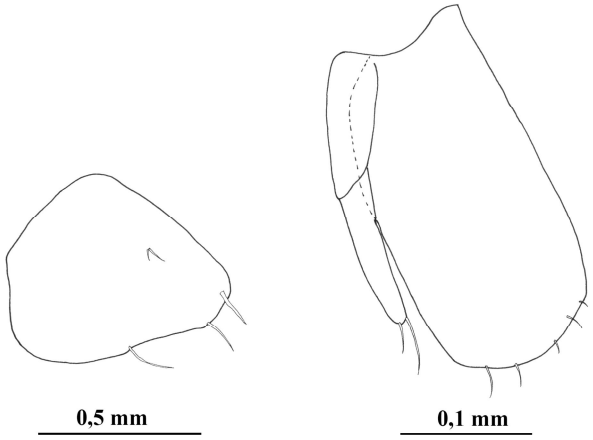


Figure 38: Female's pleopod II and male's pleopod IV of *P. hungarica robusta*. (Left: female from the Mánfai-kőlyuk Cave, right: male from the same cave.)

Table 11: Comparison of main distinguishing characters of *P. hungarica robusta* of the original description (Méhely 1927) with the characters of the newly collected material.

	***P. hungarica robusta*, original description**	***P. hungarica robusta*, Mánfai-kőlyuk Cave (new collection), 'form Mánfa'**	***P. hungarica robusta*, Mánfai-kőlyuk Cave (new collection), 'form Abaliget'**
Body length	up to 7.5 mm	4.5-7.5 mm	4.5-7.5 mm
Pleopod I exopodite setae number	2 long setae + 1 short, spiniform seta	3 long setae + 0 short, spiniform seta	1-3 long setae + 1-3 short, spiniform setae
Pleopod II exopodite distal article setae number	4	3	3-5
Pleopod II exopodite proximal article seta number	1	0	0-1
Presence of riffle-shaped sinus of pleopod II endopodite	absence (simple, tongue-shaped)	absence (simple, tongue-shaped)	presence

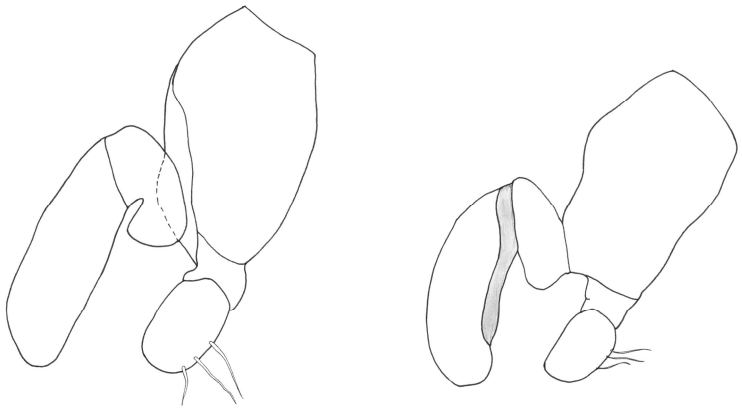


Figure 39: Male's pleopod II of *P. hungarica robusta*. (Left: 'form Mánfa', right: 'form Abaliget'. Both specimens were collected in the Mánfai-kőlyuk Cave.)

In total five differing characters have been found between the examined *P. hungarica hungarica* specimens and the 'form Mánfa' individuals of *P. hungarica robusta*. i) **Posterior margin of gnathopod** bears 6 denticulated spines and 3-4 setae in case of *P. hungarica hungarica*, while *P. hungarica robusta* has 6 denticulated spines and **6 setae**. ii) **Posterior margin of carpus** of *P. hungarica hungarica* with 5-7 strong spines (usually uni-toothed, sometimes bi-toothed), while apart the spines, *P. hungarica robusta* bears **2 additional setae**.

iii) On **ventral margin** of **male's pleopod I exopodite** of *P. hungarica hungarica* there are 3 long setae and 1-2 short, spiniform setae, while **spiniform setae** of *P. hungarica robusta* are **missing**. iv) Proximal article of **male's pleopod II exopodite** of *P. hungarica hungarica* with 1, distal article with 4-5 long setae, while in case of *P. hungarica robusta* **seta of proximal article is missing** and **distal article** bears **3 setae**. v) **Endopodite** of **male's pleopod II** of *P. hungarica hungarica* with riffle-shaped sinus, while in case of *P. hungarica robusta* endopodite is **simple, long, tongue-shaped**, without sinus.

3.2.4 New distributional data for *P. hungarica hungarica* and *P. hungarica robusta* and remarks on their ecology

During my regular visits conducted between 2000 and 2013, *P. hungarica hungarica* was found in two caves. In the Abaligeti Cave the subspecies was present in high abundance after the first 100 m from the entrance to approximately 400 m from the entrance in the stream of the main passage (Figure 40), where specimens could be found on flat stones lying in the streambed. Swimming individuals were never seen, they were most likely only able to crawl around the food resources and inside the fine particled sediment of the streambed. Lattinger-Penko (1972) 'in vitro' recorded the hiding movement of *P. hungarica thermalis* and found that the subspecies built narrow channels in the sediment as shelters. Within the Abaligeti Cave, the subspecies was also found in the Western 2 collateral in streaming water and in stagnant water. The latter meant a small pool near the entrance of the Akácos Cave, where the subspecies coexisted with *Niphargus* specimens (Figure 40). Similarly, in the Vadetetős Cave individuals of the subspecies were collected from both streaming water and small pools formed by dripping water (Figure 41) and coexistence with niphargids was not a rare phenomenon. Observations were recorded in 'Kút' about feeding specimens assembling around decaying vegetal debris in shallow water. *P. hungarica hungarica* specimens were collected by singling and by leaf litter traps. Single specimens of *P. hungarica* were found in the Spirál Cave in a limestone basin and at the beginning of the streamy branch (Figure 42), however the two collected specimens were not identified until subspecies rank as they were not involved in morphological studies.

P. hungarica robusta was collected by singling and by water (leaf litter) traps in the artificial tunnel of the Mánfai-kőlyuk Cave (Figure 43). However, so far, the only available habitat for the aquatic invertebrates is the water carrier canal made of concrete, specimens in all stages and in both sexes could be found in relatively high abundance. Individuals were not found in the lower passage of the cave.

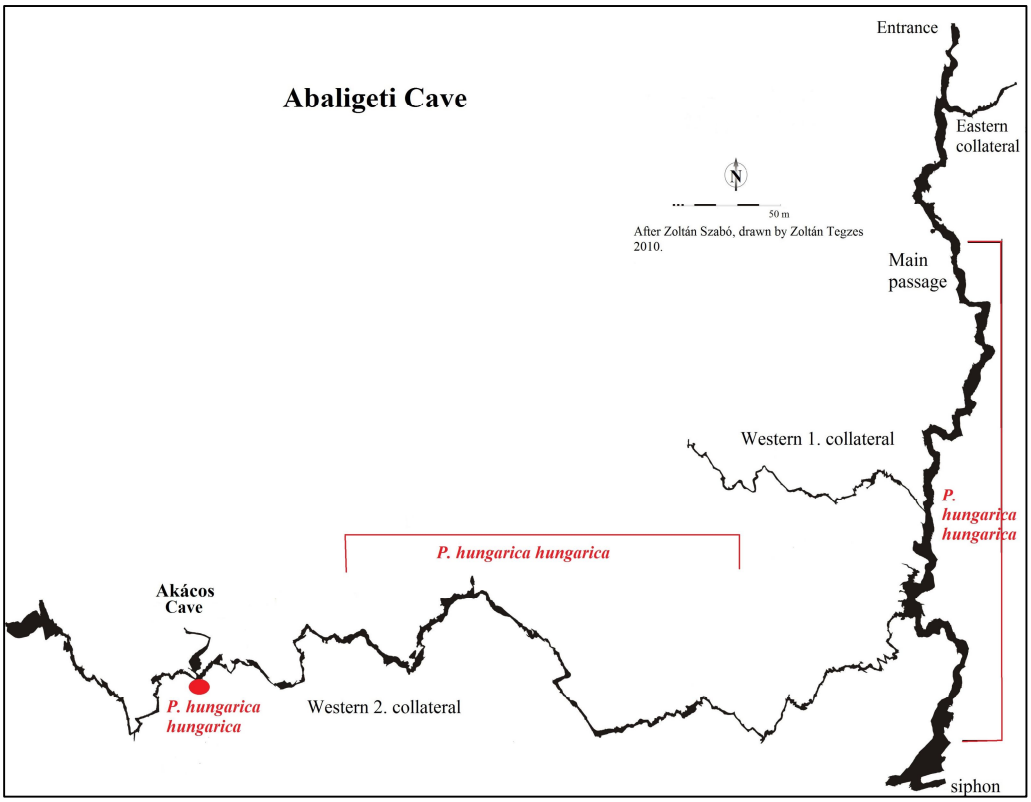


Figure 40: Localities of *P. hungarica hungarica* within the Abaligeti Cave.

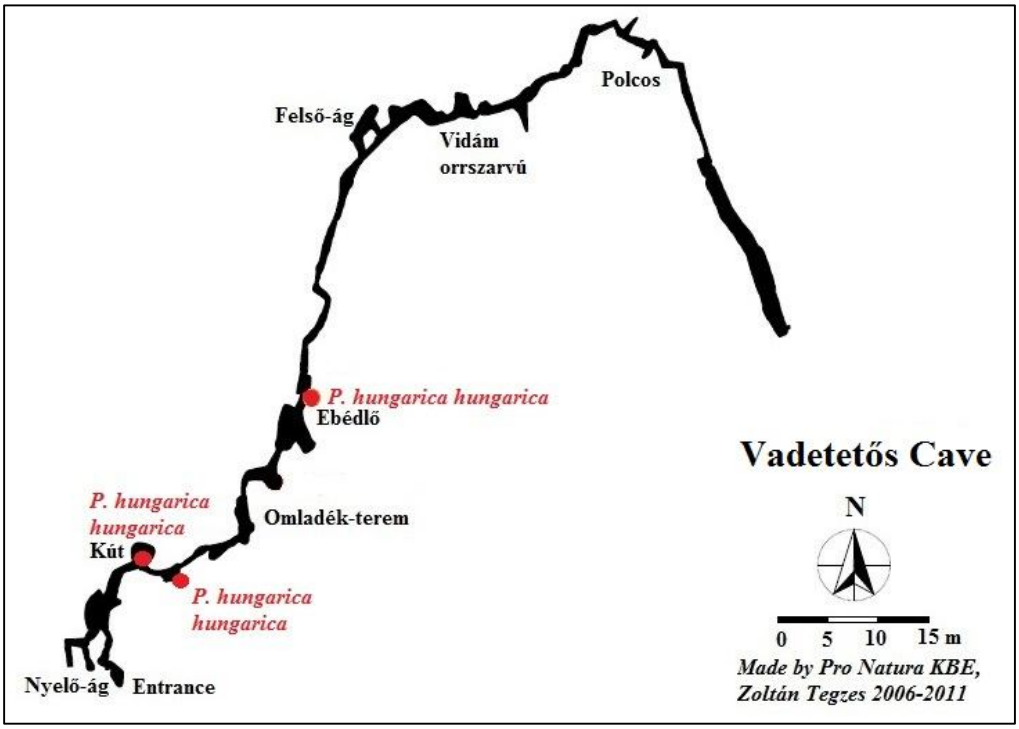


Figure 41: Localities of *P. hungarica hungarica* within the Vadetetős Cave.

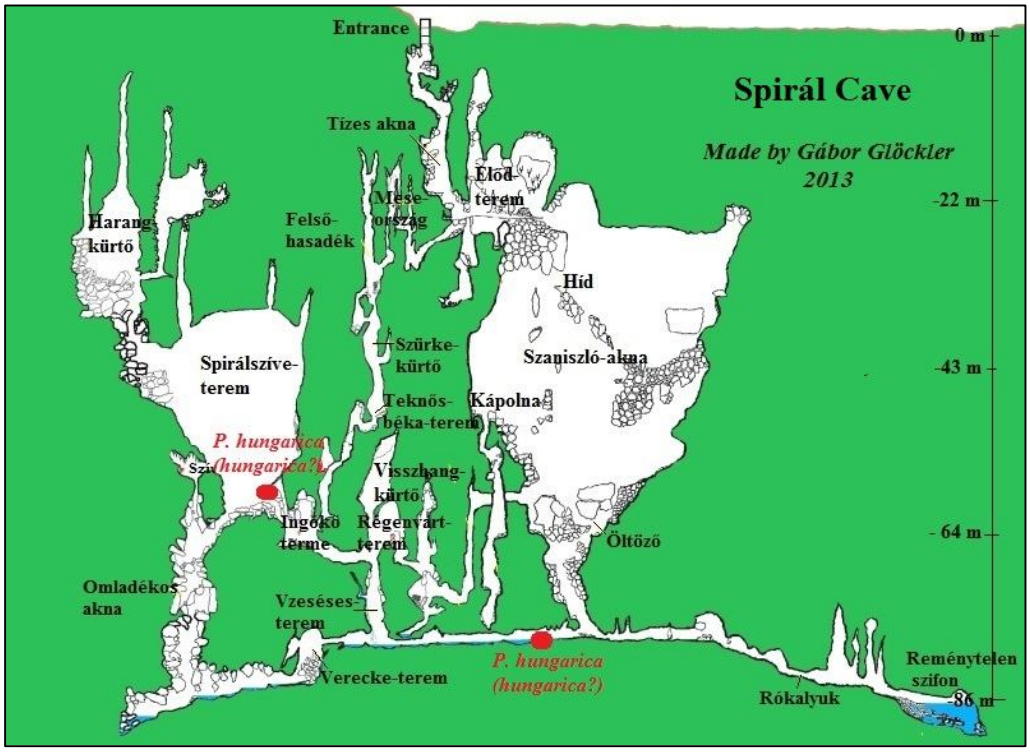


Figure 42: Localities of *P. hungarica* within the Spirál Cave.

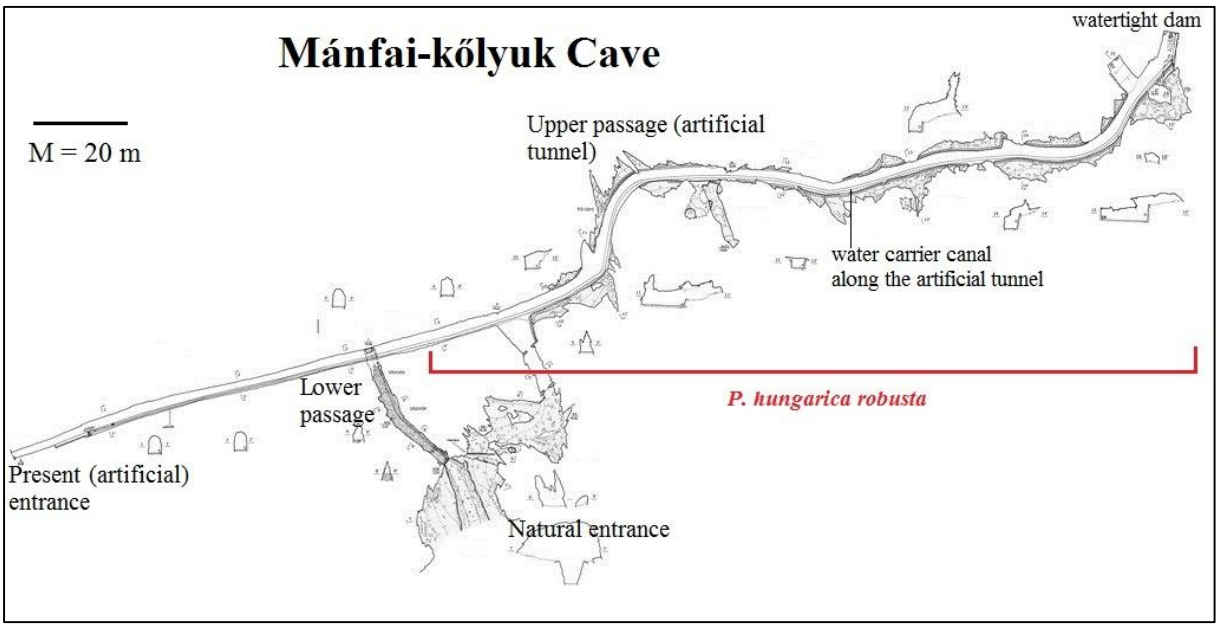


Figure 43: Localities of *P. hungarica robusta* within the Mánfai-kőlyuk Cave.

3.3 Revision of *Bythiospeum hungaricum* (Soós, 1927) and *Bythiospeum* cf. *gebhardti* (H. Wagner, 1931) (Littorinimorpha, Hydrobiidae)

3.3.1 Preliminary knowledge related to *B. hungaricum* and *B.* cf. *gebhardti*

The 'Hungarian blind snail', *Bythiospeum hungaricum* (Soós, 1927) was first found in the stream in the Abaligeti Cave in 1927 by Endre Dudich and Antal Gebhardt and was described in that same year by Lajos Soós, originally as *Lartetia hungarica* Soós, 1927 (Soós 1927). Four years later, based on the specimens collected from the Mánfai-kőlyuk Cave, Hans Wagner considered it reasonable to define a new species, which was named in honour of the collector *Paladilhiposis gebhardti* H. Wagner, 1931 (H. Wagner 1931). According to the description, the main morphological difference between the two species was the broader shell of the specimens collected from the Mánfai-kőlyuk Cave. Furthermore, he considered that the two caves are not hydrologically connected with each other, resulting in divergent evolution in the isolated biotopes. Later on, records of *P. gebhardti* were published from springs situated near by the Mánfai-kőlyuk Cave (H. Wagner 1942, Gebhardt 1958). During the revolution in 1956, type material of the two species preserved in the Hungarian Natural History Museum had perished. Pintér (1968) revised the two taxa based on neotypes designated by him, and had shown that there was no significant morphological difference between the specimens collected from the two caves and considered that *P. gebhardti* and *P. hungarica* are conspecific. He disapproved the slant of hydrological isolation too (Fehér et al. 2006). According to the actually accepted state point, the Fauna Europea database handles *Bythiospeum gebhardti* (H. Wagner, 1931) as the synonym of *Bythiospeum hungaricum* (Soós, 1927) (Bank 2013). During the latest Red List evaluation, Sólymos & Fehér (2011) suggested that the Hungarian blind snail should be placed into the 'Vulnerable' category, being endemic species with highly restricted distribution area (Sólymos et al. 2006). Confirming the uncertain taxonomic state of the *Bythiospeum* species within the genus, *B. gebhardti* can also be found on the IUCN Red List of Threatened Species as the synonym of the 'Endangered' German species, *Bythiospeum labiatum* (Geyer, 1904) (Falkner & Niederhöfer 2011). Sólymos et al. (2007), in their publication written about the conservation priorities of the Hungarian mollusc fauna, found the state of *B. hungaricum* satisfactory, being protected species inhabiting protected habitats. Although, Angyal (2012a, 2012b) published data about the negative impact of the industrial utilization of the Mánfai-kőlyuk Cave, which has caused habitat loss of endemic species and changing of the cave's hydrology.

3.3.2 Molecular taxonomic revision of *B. hungaricum* and *B.* cf. *gebhardti*

Molecular taxonomic analyses were performed in two stages. During the first stage, our aim was to discover the degree of genetic divergence between the specimens collected from the two isolated caves (Abaligeti Cave and Mánfai-kőlyuk Cave), applying the mitochondrial cytochrome c oxidase subunit I (COI) marker (Angyal et al. 2013). In the second stage of the analysis, we were going to involve small populations of each cave, still using mitochondrial markers, like COI and 16S ribosomal RNA (16S). The main goals in this stage were i) to test whether there are further haplotypes of intermediate position, ii) to examine the potential of gene flow between the two populations, iii) and to study the focal species' phylogenetic relationships within the genus *Bythiospeum* and within the superfamily Rissooidea.

1) First stage

After the analysis of 638 base pair region of COI sequences, 45 bp (7.05%) difference was found, as shown below. Mutations are marked with colours.

LOCUS KP296923 (GenBank accession number) 638 bp

DEFINITION ***Bythiospeum hungaricum*** isolate ABA1 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial.

1 ATTTTGTTCG CTATATGATC TGGGCTGGTC GGAACTGCTT TGAGTTTGCT GATTCGAGCT
61 GAATTAGGAC AGCCTGGTGC TTTACTGGGG GATGATCAGC TTTATAATGT TATTGTTACT
121 GCACATGCAT TCGTAATGAT TTTTTTTATA GTAATACCAA TGATAATAGG GGGGTTTGGG
181 AATTGATTGC TCCCATTAAT ATTGGGAGCT CCTGATATAG CGTTTCCGCG CTTAAATAAT
241 ATAAGTTTTT GACTTTTGCC TCCTGCTTTA TTATTGTTGT TATCATCCGC TGCAGTTGAA
301 AATGGGGCCG GAACAGGATG AACGGTATAC CCTCCTTTGG CGGGTAATTT AGCTCATGCT
361 GGAGGCTCAG TAGACTTGGC TATTTTTTCT TTACATTTAG CTGGTGCATC TTCTATTCTA
421 GGGTCTGTAA ATTTTATTAC TACTGTTATT AATATACGAT GACGAGGTAT ACAATTTGAG
481 CGACTTCCCC TATTTGTGTG ATCTGTAAAA ATTACGGCCA TTTTACTTGT ATTATCTTTA
541 CCAGTTTTAG CCGGGGGCAT TACTATGCTT TTAACTGATC GAAATTTTAA TACAACTTTT
601 TTTGACCCGG CTGGGGGCGG AGATCCCGTT CTTTATCA

LOCUS KP296922 (GenBank accession number) 638 bp
DEFINITION ***Bythiospeum* cf. *gebhardti*** isolate ABA1 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial.

1 ATTTTGTTTG CTATGTGATC TGGGTTAGTT GGAACTGCTT TGAGCTTATT GATTCGAGCT
61 GAGCTAGGAC AACCTGGTGC TTTATTAGGG GATGATCAGC TTTATAATGT TATTGTTACT
121 GCACATGCAT TCGTAATAAT TTTTTTTATA GTGATACCAA TAATAATGGG AGGGTTTGGA
181 AATTGACTAC TTCCTTTGAT ATTGGGGGCT CCTGATATAG CGTTTCCGCG CTTAAATAAT
241 ATAAGTTTTT GACTTTTACC TCCTGCTTTA TTATTGTTGT TATCATCCGC CGCAGTTGAA
301 AATGGGGCGG GAACAGGATG AACTGTATAC CCTCCTTTGG CAGGTAATTT AGCTCATGCT
361 GGAGGCTCAG TAGACTTGGC TATTTTTTCT TTACACTTAG CTGGTGCGTC TTCTATTTTA
421 GGGTCTGTAA ATTTTATTAC TACTGTTATC AACATACGAT GACGAGGTAT GCAATTTGAG
481 CGGCTTCCTT TATTTGTGTG ATCTGTAAAA ATTACGGCTA TTTTACTTGT ATTGTCTTTA
541 CCAGTCTTAG CAGGGGGTAT TACTATGCTT TTAACTGATC GAAATTTTAA TACAACTTTT
601 TTTGACCCGG CTGGAGGCGG AGATCCCGTT CTTTATCA

All the mutations were synonymous, 8 of them located in the first codon position, while 37 on the third codon position. Transition: transversion rate was 41: 4. Without a sufficient calibration point, estimation of divergence time is difficult. However, if we estimate the mutation rate of COI between 1.15 (Albrecht et al. 2006) and 8.6 (Pons et al. 2010), divergence may have happened 3.000.000 to 400.000 years ago in the Upper Pliocene or the Pleistocene (Angyal et al. 2013). The four individuals from the Mánfai-kőlyuk Cave belong to the same COI haplotype. Although, Fehér et al. (2013) found 4.5% intraspecific variability in case of the hydrobiid *Bythinella pannonica* (Frauenfeld, 1865), for the second stage of the study, we were going to involve more specimens of each population to test the possibility of further haplotypes in intermediate position and the chance of genflow.

2) Second stage

Agreeing with the results of the first stage of the experiment, COI sequence analysis of the 10 and 11 specimens of *B. hungaricum* and *B.* cf. *gebhardti* respectively, resulted two haplotypes. The analysed COI sequences of the specimens from the Abaligeti Cave were identical in 100%. Unexpectedly, three of the specimens collected from the Mánfai-kőlyuk Cave (BG_Man 01, BG_Man 07 and BG_Man 08) belonged to the 'Abaliget haplotype', their examined gene sequences proved to be completely identical with the ones from the Abaligeti Cave. The rest of the samples from the Mánfai-kőlyuk Cave belonged to the 'Mánfa haplotype' and comparing in 638 bp, differed by 45 bp (7.05%) from the 'Abaliget haplotype'. The examined COI sequences of five individuals of the 'Mánfa haplotype' (BG_Man 02, 03, 05, 06 and 12) were identical in 100%, while three individuals (BG Man 04, 09 and 10) differed from the other four by 4 bp.

16S rRNA PCR with the primer pair 16 sar - 16 sbr (Palumbi et al. 1991) was successfull only in case of three of the amplified 21 *B. hungaricum* and *B.* cf. *gebhardti* samples. These three samples were BG_Man 05, 06 and 07. Analysis of a 501 bp region of the gene has supported the existence of two haplotypes in the Mánfai-kőlyuk Cave: sample BG_Man 07 differed in 13 bp (2.59%) from the other two, completely identical samples. By the successful PCR amplification of the samples BG_Man 04 and 05 using the primer pair 16SLOrc2_fwd - 16SLOrc_rev (Harl et al. 2014b), it was possible to study the operation of the two different 16S primer pairs. Amplification using the 'Palumbi primer pairs' resulted an appoximately 500 bp long fragment, while 'Harl primer pair' resulted a 996 bp long fragment of the 16S rRNA gene. The 'Palumbi fragment' was overlapping with the 'Harl fragment'. 5' directed binding region of 16sar primer can be seen in Figure 44. There were no differences in the 3' ends of the fragments amplified by the two different primer pairs, however, in the 5' end there's a single base difference.

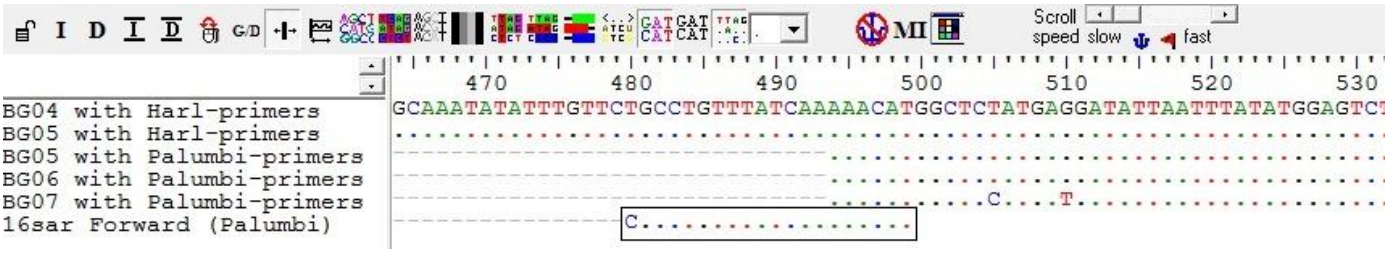


Figure 44: A fragment of *B*. cf. *gebhardti* 16S rRNA gene, amplified by two different primer pairs and the binding region of 16sar on the 5' end. BG07 belongs to the 'Abaliget haplotype'.

The Bayesian phylogenetic tree (Figure 45) has shown the unambiguous separation of the 'Abaliget haplotype' (including samples BG_Man 01, 07 and 08) and the 'Mánfa haplotype'. It has also been revealed that the German *Bythiospeum* taxa *B. quenstedti quenstedti*, *B.* sp. (from Blautopf), *B. acutum*, *B. saxigenum saxigenum*, *B.* sp. (from Wasserflaare) and *B. suevicum* belong to the same haplotype. *Moitessiera* cf. *puteana* is closer to the Alpine *Bythiospeum* species, than to the species from the Mecsek Mts. The distance between the two taxa in the Mecsek Mts. corresponds with the distance between the known most disctint Alpine *Bythiospeum* species. P-distances between our two endemic taxa and the Alpine *Bythiospeum* species are 17-20%, while p-distances between the Alpine species are only 6-9%. The genera *Bythinella*, *Amnicola*, *Erhaia*, *Marstoniopsis*, *Hauffenia*, *Sadleriana*, *Floridobia* and *Dinella* differ in 18-23% from the *Bythiospeum* species. In most cases these genera are situated more distant from the two taxa from the Mecsek Mts., than from the other *Bythiospeum* species. For instance, p-distances between *Erhaia jianonensis* and *B. hungaricum* are 23%, while *E. jianonensis* differ only in 19-20% from the other Alpine *Bythiospeum* species. Among the studied taxa, the closest genera to *B. hungaricum* and *B.* cf. *gebhardti* are *Hauffenia* (p-distance = 19%) and *Floridobia* (p-distances = 19-20%).

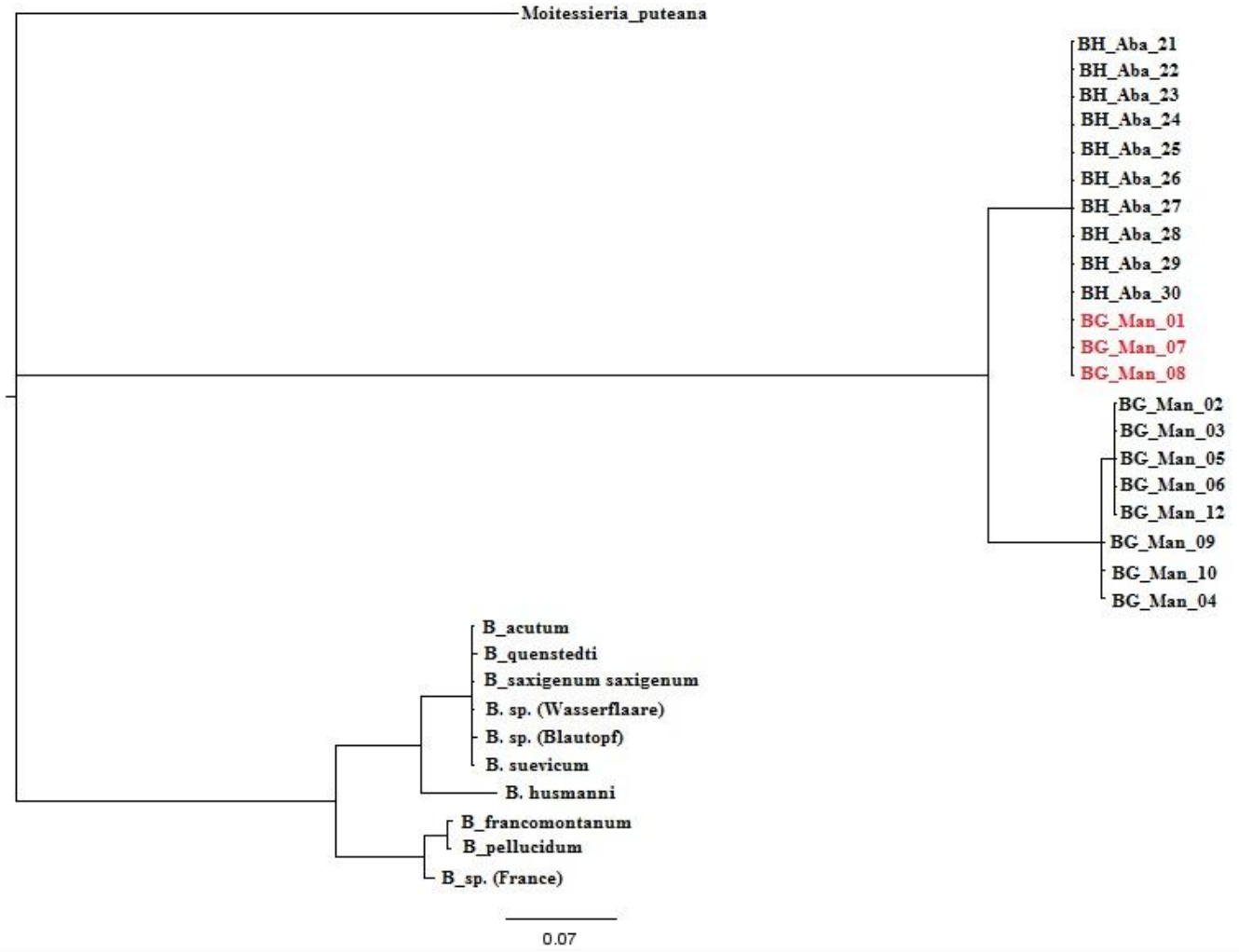


Figure 45: Bayesian phylogenetic tree of 31 *Bythiospeum* taxa based on COI sequences (see detailes in the text).

3.3.3 Shell morphometric studies on *B. hungaricum* and *B.* cf. *gebhardti*

Morphometric studies on shells of 30 *B. hungaricum* and 20 *B.* cf *gebhardti* individuals (Table 12) have been conducted to study the possible morphological differences that may confirm the existence of two distinct species in the caves of the Western Mecsek.

Table 12: List of *B. hungaricum* and *B.* cf. *gebhardti* samples used in morphometric studies. Boldfaced sample codes refer specimens involved in molecular analysis too.

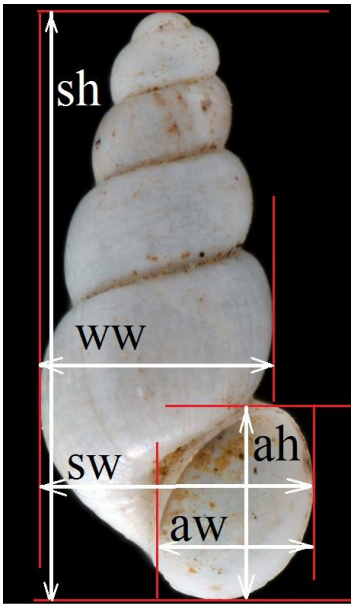
Sample code	**Cave**	**Shell/whole body**	**Collector, collecting method**
BH_Aba 01, BH_Aba 02, BH_Aba 03, BH_Aba 04, BH_Aba 05, BH_Aba 06, BH_Aba 07, BH_Aba 08, BH_Aba 09, BH_Aba 10, BH_Aba 11, BH_Aba 12, BH_Aba 13, BH_Aba 14, BH_Aba 15, BH_Aba 16, BH_Aba 17, BH_Aba 18, BH_Aba 19, BH_Aba 20	Abaligeti Cave (main passage, stream)	empty shells	G. Majoros, elutriation of stream bed sediment material
BH_Aba_21, BH_Aba_22, BH_Aba_23, BH_Aba_24, BH_Aba_25, BH_Aba_26, BH_Aba_27, BH_Aba_28, BH_Aba_29, BH_Aba_30	Abaligeti Cave (main passage, stream, on stones, 200-300 m from the entrance)	whole body preserved in 96% ethanol	D. Angyal, singling with soft forceps
BG_Man_01, BG_Man_02, BG_Man_03, BG_Man_04, BG_Man_05, BG_Man_06, BG_Man_07, BG_Man_08, BG_Man_09, BG_Man_10, BG_Man 11, **BG_Man_12**, BG_Man 13, BG_Man 14, BG_Man 15, BG_Man 16, BG_Man 17, BG_Man 18, BG_Man 19, BG_Man 20	Mánfai-kőlyuk Cave (upper passage, from the water carrier canal)	whole body preserved in 96% ethanol BH_Man 19, 20 are empty shells	D. Angyal, singling with soft forceps

Two types of measurements had been performed. As part of the 'Type 1 measurement', the following variables were measured (Figure 46): total height of shell from apex to aperture ('shell height'), total width of shell ('shell width'), width of the widest (first) whorl ('whorl width'), maximal height of the aperture ('aperture height') and maximal width of the aperture ('aperture width'). Number of the whorls was also registered, those varied between 3.25 and 5.0. Means and standard deviations of shell heights, shell widths and whorl widths were recorded (Table 13). Assuming that the whorl number increases with age, individuals with less than 4.5 whorles had been excluded from the mean-analysis, as well as samples BG_Man 01, 07 and 08, which proved to belong to the 'Abaliget haplotype' according to the molecular results. Due to the low number of 'Abaliget haplotype' samples from the Mánfai-kőlyuk Cave (only two, after the exclusion of BG_Man 01, which had 4 whorls), comparison of their 'mean data' with the data of the two populations was not possible.

Table 13: Means of shell height, shell width and whorl width of samples from the two caves. M: mean, SD: standard deviation.

	Specimens from the Abaligeti Cave	**Specimens from the Mánfai-kőlyuk Cave**
M shell height (mm)	2.20	1.98
SD shell height	*0.10*	*0.12*
M shell width (mm)	0.95	0.83
SD shell width	*0.06*	*0.07*
M whorl width (mm)	0.80	0.70
SD whorl width	*0.05*	*0.07*

A Principal Component Analysis had been conducted too, using the five variables: shell height, shell width, whorl width, aperture height and aperture width. All specimens were included in this analysis, except sample BG_Man 15 because of its broken aperture. Two components were found, which had explained 84% of the variance in the data. The first component (PC1) was the **robustness of the shell**. It came off by the shell height, shell width and whorl width. The second component (PC2) was the **relative aperture size**, came off by the height and width of the aperture. It meant that the population from the Abaligeti Cave could be characterized by more robust shell and smaller aperture, while in the contrary; **population inhabiting the Mánfai-kőlyuk Cave possessed less robust shell with bigger aperture**.



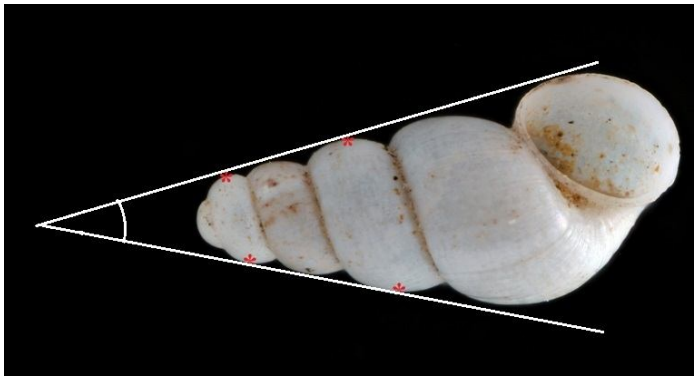


Figure 46 (left): 'Type 1 measurement' applied during the morphometric studies on *B. hungaricum* and *B.* cf. *gebhardti*. ah: aperture height, aw: aperture width, sh: shell height, sw: shell width, ww: whorl width.

Figure 47 (right): Added lines and points, and the mapped angle used in 'Type 2 measurement'.

To eliminate the problem of the differing numbers of whorls which may obscure the real shell morphological differences, we applied a new variable: the 'angle', which mapped the tapering of the shell. Adaptation of added lines and points for the 'Type 2 measurement' can be seen in Figure 47. Angles of all the shells had been measured, except the sample BG_Man 08, because of its thick mineral granule layer (most likely to be manganese) that had covered the whole shell.

In order to test the reliability of the measures, a Repeatability Test had been performed (Becker 1992) using three independent measurements of each individuals. The Repeatability Test revealed reliable exactness (R=0.91), as it can be seen in Table 14.

Table 14: Repeatability Test performed for testing the reliability of shell angle measurements.

Tests of Between-Subjects Effects

Dependent Variable:angle

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	2123,769[a]	49	43,342	33,074	,000
Intercept	73979,729	1	73979,729	56453,125	,000
id	2123,769	49	43,342	33,074	,000
Error	129,736	99	1,310		
Total	76563,503	149			
Corrected Total	2253,505	148			

ME	0,085509138
R	0,914490862
SE	0,020158649

Then, a General Linear Model (GLM) analysis was performed, the correspondence was based on the collection sites (the two caves), disregarding the haplotypes (Table 15). Means of the three measurement seria used for repeatability test were applied during the GLM analysis. The difference was significant: specimens collected in **the Mánfai-kőlyuk Cave showed smaller (more acute) angles**. Furthermore, using 95% confidence interval, the two populations did not overlap. Graphically visualizing the distribution, two outliers were excelled, namely the two 'Abaliget haplotype' samples (BG_Man 01 and 07) from the Mánfai-kőlyuk Cave (Figure 48).

Table 15: General Linear Model (GLM) analysis of 'mean angles', regarding the collection localities. Locality 1.00: Abaligeti Cave, locality 2.00: Mánfai-kőlyuk Cave.

Between-Subjects Factors

		N
locality	1,00	30
	2,00	19

Tests of Between-Subjects Effects

Dependent Variable:mean_angle

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	585,381[a]	1	585,381	229,569	,000	,830
Intercept	21635,176	1	21635,176	8484,672	,000	,994
locality	585,381	1	585,381	229,569	,000	,830
Error	119,846	47	2,550			
Total	25202,261	49				
Corrected Total	705,226	48				

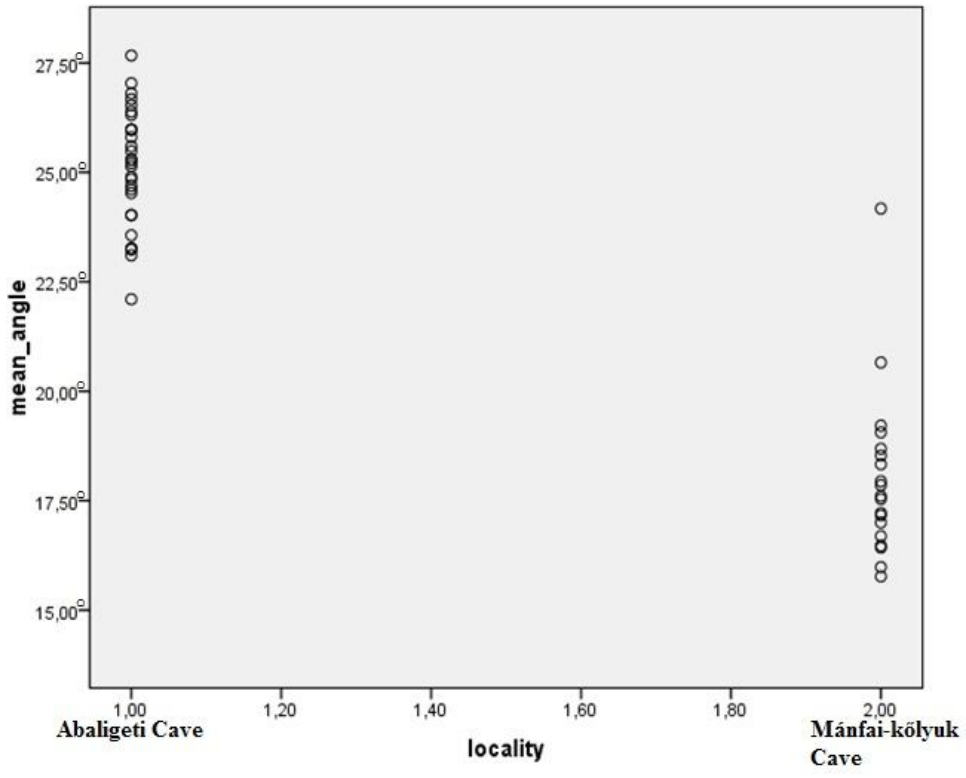


Figure 48: Graphical visualization of the result of GLM analysis for 'mean angles'. Two outliers are visible in the population from the Mánfai-kőlyuk Cave (locality 2.00).

3.3.4 New distributional data for *B. hungaricum* and *B.* cf. *gebhardti*, and remarks on their ecology

Inspite of my repeated visits and intensive search in 14 of the caves in the Western Mecsek - vast majority of them contained streaming or stagnant water -, no hydrobiid snails were found in other caves than the Abaligeti Cave and the Mánfai-kőlyuk Cave. In the former cave, living specimens could be found by careful examination on flat stones lying in the streambed, supposedly feeding on the algae layer. Colours of the shells varied from transparent to dark brown or even black, which phenomenon was caused by mineral granule coating. Empty shells had also been collected from the main passage by checking the fine particled sediment of the streambed. Living specimens on rocks presented after the first 200 m from the entrance to approximately 440 m from the entrance, however in relatively low abundance (Figure 49). Varga (2013) mentioned high number of empty shells collected from the sediment of the Western 2 collateral. In the Mánfai-kőlyuk Cave hydrobiids were relatively abundant in the water carrier canal of the artificial tunnel, where it could be collected by soft forceps or using a water trap. Individuals were observed in the lower passage of the cave too, moving on the wet, clayey residuum at the bank of the streaming water (Figure 50). Sediment sample had been collected from the same place, and after elutriation, plenty of empty shells were found. There are some records of the species drifting out from typical karstic springs, like Kánya Spring, Kantavári Spring, Vízfő Spring, Nagy-Mély-völgyi Spring, however those were only shells in all cases (Demeter 1994, Varga 2013).

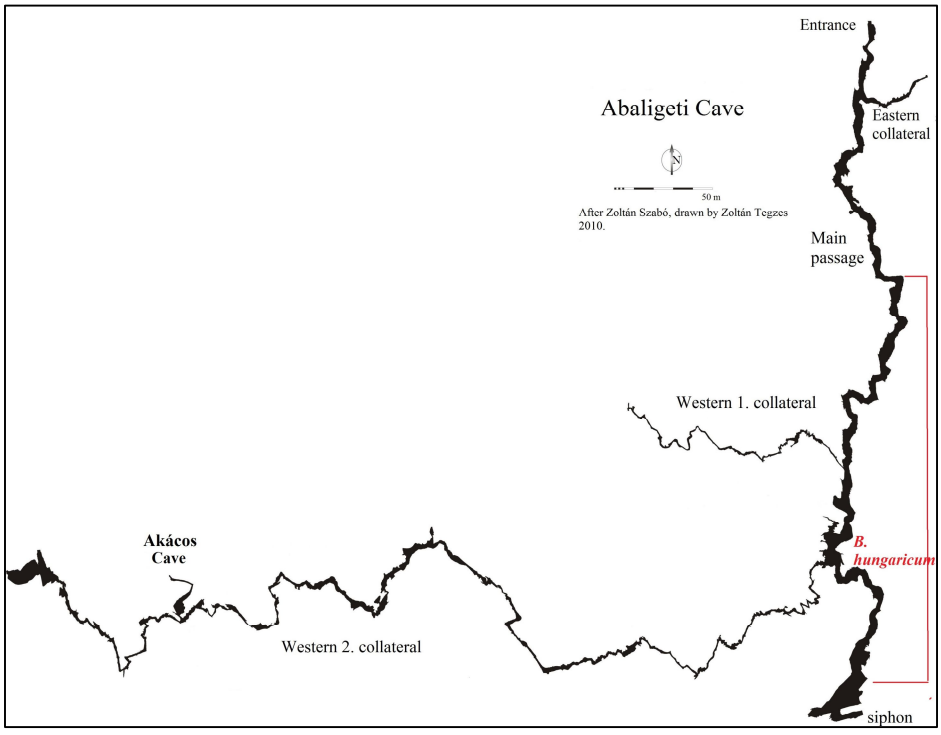


Figure 49: Distribution of *B. hungaricum* within the Abaligeti Cave.

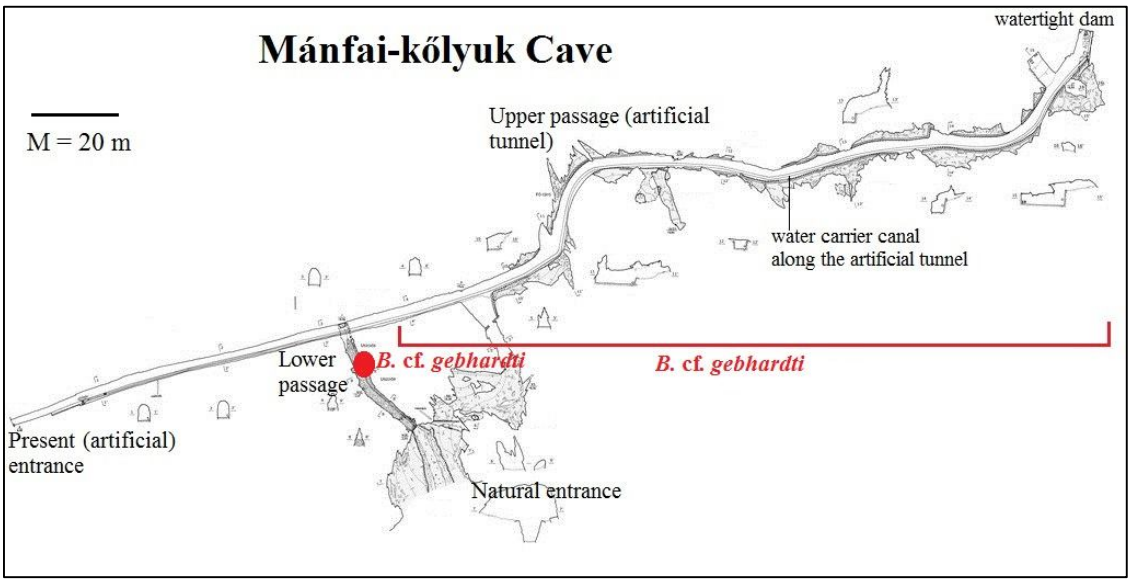


Figure 50: Distribution of *B.* cf. *gebhardti* within the Mánfai-kőlyuk Cave.

3.4 Revision of *Brachydesmus troglobius* Daday, 1889 (Polydesmida, Polydesmidae)

3.4.1 Preliminary knowledge related to *B. troglobius*

The polydesmid millipede, *Brachydesmus troglobius* Daday, 1889 was first found in the Abaligeti Cave by János Pável (date of collection is unknown), and then it was described by Jenő Daday as an endemic species of the cave (Daday 1889a). The rather short description, written in Latin, did not contain any drawings; however a comment on the similarity with *B. subterraneus* Heller, 1858 is mentioned: the two quite similar species can be distinguished by the different shape and structure of the collum and the gonopods. In the same year, descriptions of two other *Brachydesmus* species, *B. chyzeri* from the Recsina valley near Fiume and *B. hungaricus* from Transylvania were also published by Daday (Daday 1889a, 1889b). In the publication of Verhoeff (1928) about the Hungarian millipede fauna, exact data and drawing about the gonopods of *B. troglobius* can be found. Later faunistic records from the Abaligeti Cave were published by Bokor (1924), Gebhardt (1934, 1963, 1967), Korsós (2000), Korsós et al. (2006) and Angyal & Korsós (2013). *B. troglobius* was collected in numerous caves from the Dinaric Karst, too; there are distributional data from Croatia, Montenegro, Romania, Serbia and Slovenia (Strasser 1971, Mršić 1988, 1994, 1998, Ćurčić & Makarov 1998, Makarov 2004, Enghoff 2013). Ćurčić & Makarov (1998) described the postembryonic development of *B. troglobius* in the samples from the Lazareva's Cave (Serbia), and revealed that the species completes its entire life cycle in the cave. However, Gebhardt (1966) and Mršić (1988) mentioned the observation of epigean populations in Hungary and Serbia. Makarov et al. (2012) studied the chemical defense of the species and have revealed that it secretes allomones against predators.

3.4.2 Morphological studies on old museum samples and newly collected material of *B. troglobius*

Redescription of *Brachydesmus troglobius* Daday, 1889

Old museum samples including the type material, and freshly collected individuals preserved in the Myriapoda Collection of the HNHM were revised under stereomicroscope. In some cases male gonopods were dissected. Illustrative photos and scanning electron micrographs were also made. Detailed 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.

Bracydesmus troglobius: Daday 1889a (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), Ćurčić & 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:

Old museum samples

830/1888, 205/253, Abaligeti Cave, leg. ?, det. Dr. J. Daday, revid. D. Angyal, 2015: 2 ♀, broken, in bad condition, labeled as SYNTYPES.

1722/1928, Abaligeti Cave, 21/10/1922, leg. Dr. E. Bokor, det. Dr. K.W. Verhoeff, revid. E. Loksa, 245/1953, revid. D. Angyal, 2015: 5 ♀, 3 ♂.

1720/1928, Abaligeti Cave, 10/1923, leg. Dr. E. Bokor, det. Dr. K.W. Verhoeff, revid. E. Loksa, 240/1953, revid. D. Angyal, 2015: 2 ♀

1721/1928, Abaligeti Cave, 12/08/1924, leg. Dr. E. Dudich, det. Dr. K.W. Verhoeff, revid. E. Loksa, 234/1953, revid. D. Angyal, 2015: 2 ♀, 1 ♂, 2 juv.

1719/1928, Abaligeti Cave, 15/03/1925, leg. Dr. E. Bokor, det. Dr. 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. Dr. Z. Korsós & H. Read, det. Dr. 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. Dr. Z. Korsós, 1 ♂

BRT-02, Abaligeti Cave, main passage, 22/09/2010, leg. D. Angyal, det. Dr. 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. Dr. 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. Dr. Z. Korsós, 1 ♀

BRT-07, Abaligeti Cave, Western 2 collateral, 25/11/2010, leg. D. Angyal, det. Dr. Z. Korsós, 1 juv.

BRT-08, Abaligeti Cave, main passage, before the siphon, 10/11/2010, leg. D. Angyal, det. Dr. 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

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, Eastern collateral, lamp flora, 13/06/2012, leg. D. Angyal, det. Dr. Z. Korsós, 3 ♀, 1 ♂

Törökpince Cave, 50 m from entrance, 11/06/2012, leg. D. Angyal, det. Dr. 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 (Figures 51-53): Broader than collum, densely covered by minute setae. Three well developed labral teeth visible. Occipital sulcus well 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 part with 4 large cones.

Collum (Figures 51, 53): Convex, anterior and posterior edge both semicircular without caudal incisions of lateral sides.

Body (Figures 51, 52, 55): Body segments gently broadening until segment VII, than parallel-sided from segment VIII to XV, and from segment XVI rapidly tapering toward body end. Metazonae II, III, IV, VI, VIII and XI with 3 incisions, while metazonae 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 metazonae V, VII, IX, X, XII-XVIII. Posterior edges of metazonae 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 (Figures 52, 54): 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 recurvatore point of seminal groove. Exomerite slightly longer than femorite at best and separated from femorite by a sulcus.

Figure 51: *B. troglobius*, male from the Törökpince Cave (Photo: Z. Korsós).

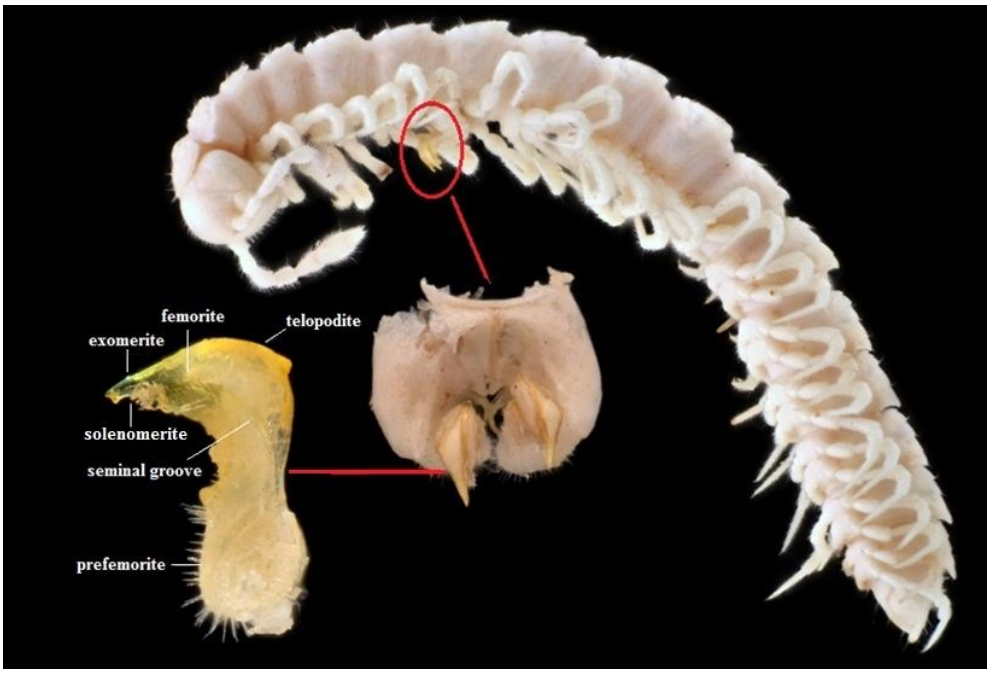
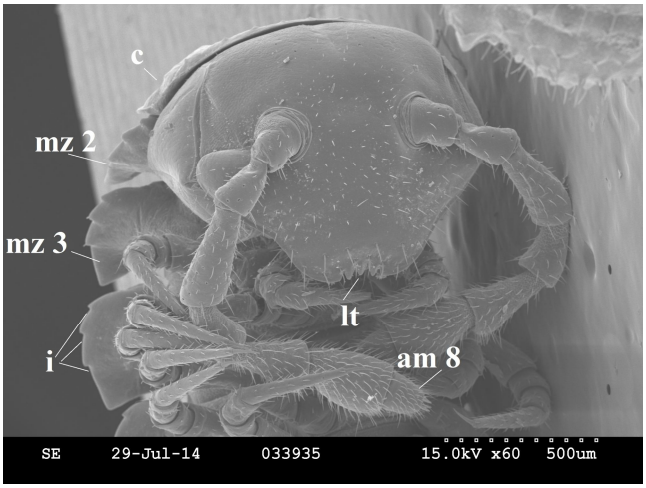


Figure 52: *B. troglobius*, male from the Abaligeti Cave, habitus and gonopods.



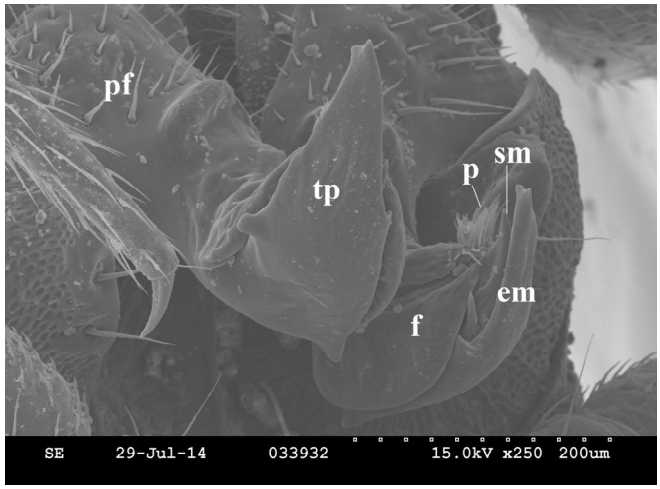


Figure 53 (left): *B. troglobius*, male from the Abaligeti Cave, head, ventral view, scanning electron micrograph. am 8: antennomere VIII, c: collum, i: incisions, lt: labral teeth, mz 2: metazone II, mz 3: metazone III.

Figure 54 (right): *B. troglobius*, male from the Abaligeti Cave, gonopod, scanning electron micrograph. em: exomerite, f: femorite, p: pulsilla, pf: prefemorite, sm: solenomerite, tp: telopodite.

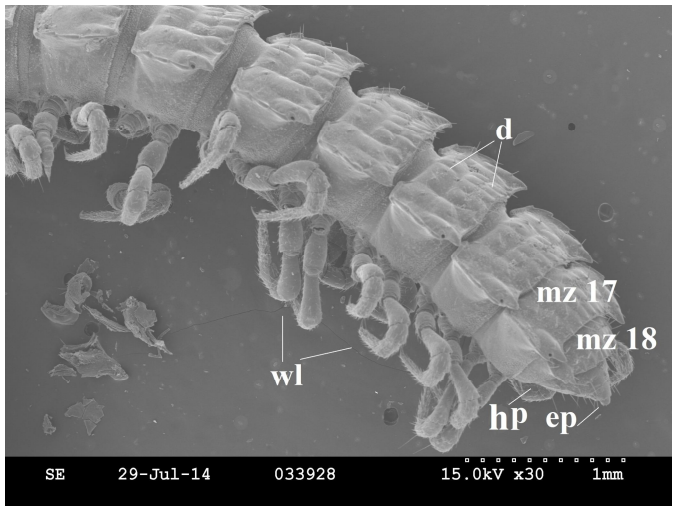


Figure 55: *B. troglobius*, male from the Abaligeti Cave, posterior body segments, lateral view, scanning electron micrograph. d: dentate metazonum, ep: epiproct, hp: hypoproct, mz 17: metazone XVII, mz 18: metazone XVIII, wl: walking legs.

3.4.3 Molecular studies on *B. troglobius* and other polydesmids

Comparing 634 base pair region COI sequences of *B. troglobius* from the Abaligeti Cave and from the Serbian Petnička's Cave, only 5 base pair (0.79%) difference was found, as shown below. Mutations are marked with colours. Four of the mutations located in the third codon position, all of them were synonymous mutations.

LOCUS **KT343290** (BR_TRO/Aba) 634 bp
DEFINITION *B. troglobius* Hungary, Abaligeti Cave, cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial.

1 ATAAAGATAT TGGGACTTTA TATTTAATTT TAGGGGCTTG AGCTGGCTTA AGAGGTTCAG
61 CAATAAGAGG TTTTGTGCGG TTGGAGTTAG GTGTTCCTGG TAGATTTATA GGAGATGATC
121 ATATTTTTAA TGTGGTTGTT ACTGCTCATG CTTTTGTAAT AATTTTTTTT ATGGTAATGC
181 CTATTATAAT TGGAGGTTTT GGAAATTGAT TAGTTCCTAT TATAATTGGT GCCCCTGATA
241 TGGCTTTTCC TCGAATAAAT AATTTGAGTT TTTGATTACT TCCTCCTTCT TTATTGTTAT
301 TGTTAATGTC TTCTTTAGTG GAAATTGGGG TTGGAACAGG GTGAACTGTT TATCCTCCGT
361 TAGCTAGAGG GTTATTTCAT AGTGGAAGAG CTGTGGACTT AGCTATTTTT TCATTACATT
421 TAGCTGGGGC TTCTTCTATT TTAGGGGCTA TTAATTTTAT TACTACTGTA ATTAATATAC
481 GTAGTTGTGG AATAATTTAT GAGCGTTTAC CTTTATTTGT TTGATCTGTT ATTGTAACTG
541 TGGTCTTATT ACTTTTATCT TTACCTGTTC TTGCTGGTGC AATTACTATA CTTTTAAGTG
601 ATCGAAATTT TAACTCTAGT TTTTTTGACC CGGC

LOCUS **KT343289** (BR_TRO/Ser) 634 bp
DEFINITION *B. troglobius* Serbia, Petnička's Cave, cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial.

1 ATAAAGATAT TGGGACTTTA TATTTAATTT TAGGGGCTTG AGCTGGCTTA AGAGGTTCAG
61 CAATAAGAGG TTTTGTGCGG TTGGAGTTAG GTGTTCCTGG TAGATTTATA GGAGATGATC
121 ATATTTTTAA TGTGGTTGTT ACTGCTCATG CTTTTGTAAT AATTTTTTTT ATGGTAATGC
181 CTATTATAAT TGGAGGTTTT GGAAATTGAT TAGTTCCTAT TATAATTGGT GCCCCTGATA
241 TGGCTTTTCC TCGAATAAAT AATTTGAGTT TTTGATTACT TCCTCCTTCT TTATTGTTAT
301 TATTAATGTC TTCTTTAGTG GAAATTGGGG TTGGAACAGG GTGAACTGTT TATCCTCCGT
361 TAGCTAGAGG GTTATTTCAT AGCGGAAGAG CTGTGGATTT AGCTATTTTT TCATTACATT
421 TAGCTGGGGC TTCTTCTATT TTAGGGGCTA TTAATTTTAT TACTACTGTA ATTAATATAC
481 GTAGTTGTGG AATAATTTAT GAGCGTTTAC CTTTATTTGT TTGATCTGTT GTTGTAACTG
541 TGGTCTTATT ACTTTTATCA TTACCTGTTC TTGCTGGTGC AATTACTATA CTTTTAAGTG
601 ATCGAAATTT TAACTCTAGT TTTTTTGACC CGGC

The two Hungarian *Brachydesmus* species (*B. troglobius* and *B. superus*) collected in caves from two different karst areas, differed in 57 bp (9.29%) in a 613 bp COI region. As can be seen in the neighbor-joining tree (Figure 56), among the studied taxa, the closest relative of *B. troglobius* is *B. herzogowinensis*. Identification of a juvenile female specimen of *Polydesmus denticulatus* collected in the Solymári-ördöglyuk Cave was possible by the comparison of its COI sequence with the *P. denticulatus* sequences available in GenBank. The COI sequence of this *P. denticulatus* individual differed from the Hungarian *B. troglobius* in 92 bp (15%) in a 613 bp fragment. In the distance matrix, the intraspecific distance was up

to 4% within the genus *Brachydesmus* and 2% within the genus *Polydesmus*. Distinct species within *Brachydesmus* had at least 7% difference. The maximum intrageneric distance was 11% within *Brachydesmus* and intrageneric distance varied between 12-17% within *Polydesmus*. Intergeneric distance within the two genera varied between 13-16%. In *Propolydesmus-Brachydesmus* relation 10-14%, while in *Propolydesmus-Polydesmus* relation 15-18% differences were found. *Polydesmus* cf. *edentulus* was found to be closer related to *Brachydesmus* species (differed by 10-11%) than to other *Polydesmus* species (14-16%), which raises the question of generic placement of this species. The outgroup taxon, *A. variabilis* differed by 19-22% from the other 16 taxa.

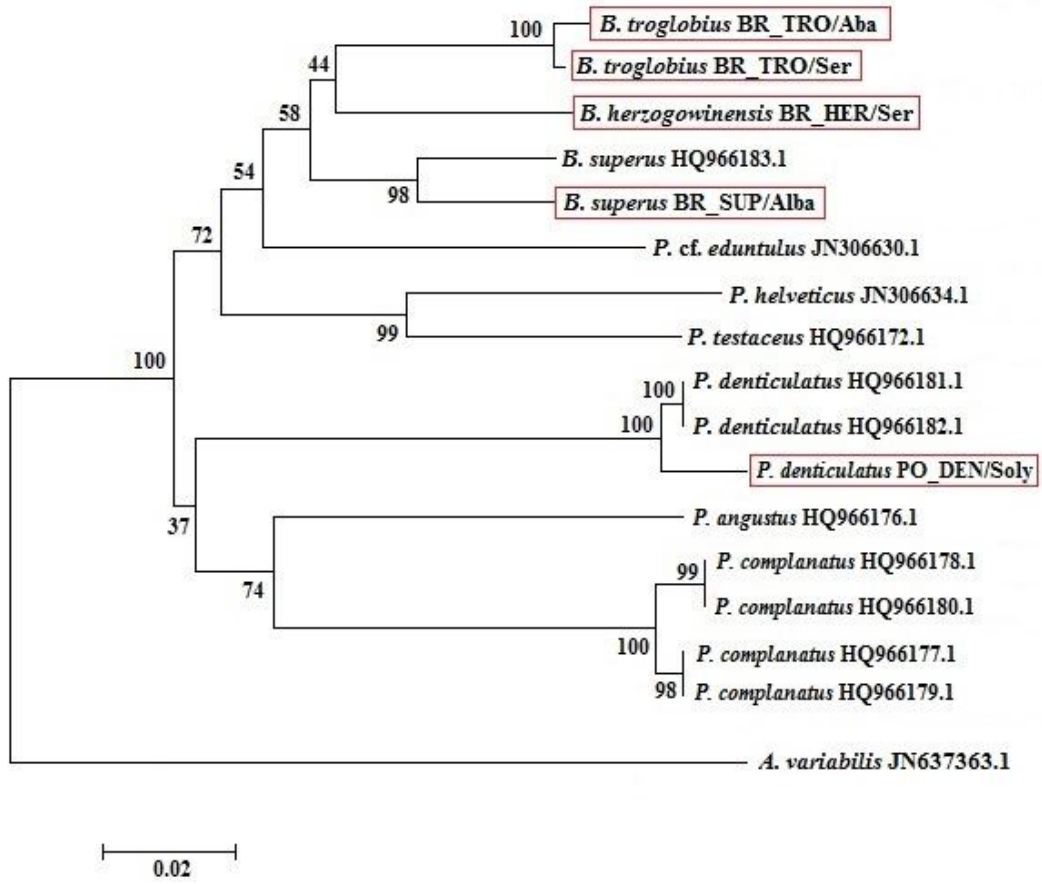


Figure 56: Neighbor-joining tree of 17 polydesmid taxa based on COI. Numbers represent percentage bootstrap support (1000 replicates). Framed taxa represent own data.

3.4.4 New distributional data for *B. troglobius* and remarks on its ecology

Despite my repeated visits to 14 caves in the Western Mecsek, *B. troglobius* was found only in a single cave apart from its type locality. In the Abaligeti Cave they were distributed in the main passage, the Eastern collateral, and the Western 2 collateral (Figure

57), feeding on the lamp flora and decaying wood, or walking on the sediment, and rarely on speleothem formations. Coexistence with the eutroglophile diplopod *Trachysphaera schmidtii* Heller, 1858 and the oniscoid isopod *Haplophthalmus mengei* (Zaddach, 1844) was observed in some occasions, especially on the vegetation developed on illuminated speleothems. The species was also found in the Törökpince Cave. In that cave, specimens from both sexes were collected at 30 m from the entrance and from the deeper zone of the cave (Figure 58), usually close to decaying material. Individuals were sampled by singling applying soft forceps and aspirator, and by pitfall traps in a few occasions.

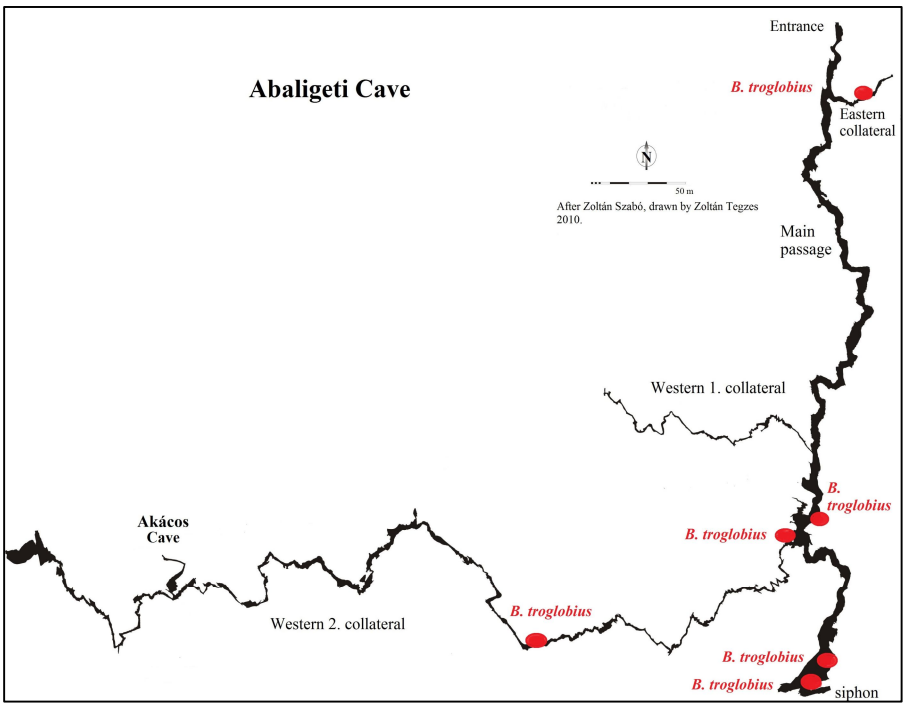


Figure 57: Localities of *B. troglobius* in the Abaligeti Cave.

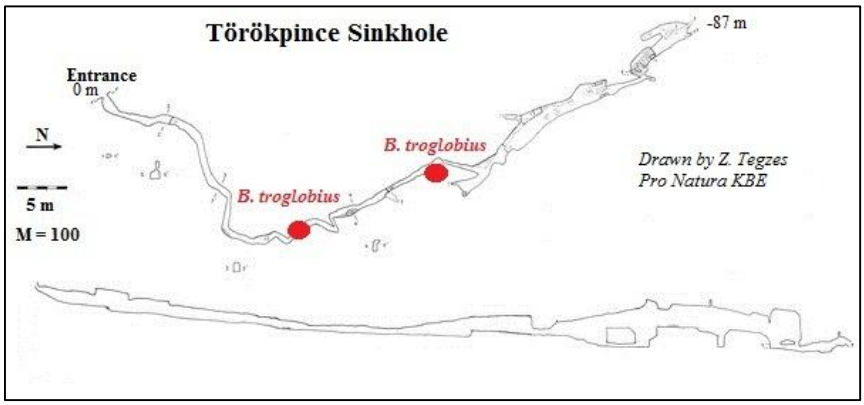


Figure 58: Localities of *B. troglobius* in the Törökpince Cave.

3.5 Invertebrate diversity of the sampled caves - further faunistic results

105 further aquatic and terrestrial macroinvertebrate species or subspecies, collected from 14 caves of the Western Mecsek have been identified by taxonomists. In some cases, identification until species rank was not possible. Up to know, only 70% of the whole collected material has been processed. Comparing with the latest list of Antal Gebhardt on the fauna of the Abaligeti Cave and the Mánfai-kőlyuk Cave (Gebhardt 1963) and with the list of the Hungarian cave-dwelling springtails (Dányi 2011), 25 and 7 new macroinvertebrate records were now made from the two caves, respectively (Table 16). Apart from the revised taxa, 3 further troglobiont species were found. An oligochaete species found in the Abaligeti Cave and the Spirál Cave proved to be new for the Hungarian fauna. Short comments in cases of the most remarkable records, and attempts for ecological classification of the revealed taxa are also given. Apart of the revised species and subspecies (all of them are troglobionts), 3% of the collected taxa belonged to the troglobiont category, 24% was eutroglophile, 26% proved to be subtroglophile, while 47% belonged to the trogloxene group.

Table 16: List of invertebrate species collected in the 14 examined caves. '+^{A}': new for the fauna of the Abaligeti Cave, '+^{M}': new for the fauna of the Mánfai-kőlyuk Cave, '*': troglobiont. Caves: ABA: Abaligeti, ACH: Achilles, AKA: Akácos, GIL: Gilisztás, KIS: Kispaplika, MAN: Mánfai-kőlyuk, NYA: Nyárás-völgyi, ORF: Orfűi Vízfő, ROM: Római, SPI: Spirál, SZA: Szajha-felső, TOR: Törökpince, TRI: Trió, VAD: Vadetetős.

Phylum/ Subphylum	Cl/Subcl/ Ordo	Species/taxon	Cave	Ecological group	Determined by	Notes
Platyhelminthes	Turbellaria	*Polycelis felina* (Dalyell, 1814)	ABA	subtroglophile	T. Fülep	
Platyhelminthes	Turbellaria	*Polycelis* sp. (*Polycelis tothi* Méhely 1927?)	MAN	subtroglophile	T. Fülep	Further integrative taxonomic analysis would be necessary to clarify its position. The endemic *Dendrocoelum pannonicum* Méhely, 1927 supposedly had gone extinct from the cave (Angyal 2012b).
Nematomorpha	Gordioidea	*Gordius* sp.	TOR, TRI, GIL	trogloxene	D. Murányi	
Mollusca	Gastropoda	*Helicodonta obvulata* (O.F. Müller, 1774)	ROM	trogloxene	Z. Fehér	
Mollusca	Gastropoda	*Perforatella incarnata* (O.F. Müller, 1774)	ACH	trogloxene	Z. Fehér	
Mollusca	Gastropoda	*Alinda biplicata* (Montagu, 1803)	ROM, ABA	trogloxene	Z. Fehér	
Mollusca	Gastropoda	*Oxychilus* sp.	TOR, MAN	trogloxene	Z. Fehér	
Mollusca	Gastropoda	*Pupilla muscorum* (Linnaeus, 1758)	ABA	trogloxene	Z. Fehér	fossil
Mollusca	Gastropoda	+^{A}*Trochulus hispidus* (Linnaeus, 1758)	ABA	trogloxene	Z. Fehér	
Mollusca	Gastropoda	*Aegopinella ressmanni* (Westerlund, 1883)	NYA	trogloxene	Z. Fehér	
Mollusca	Gastropoda	*Vitrea diaphana* (Studer, 1820)	TOR, GIL	trogloxene	Z. Fehér	

Phylum/ Subphylum	Cl/Subcl/ Ordo	Species/taxon	Cave	Ecological group	Determined by	Notes
Mollusca	Gastropoda	+[A]*Truncatellina* sp.	ABA	trogloxene	Z. Fehér	fossil
Mollusca	Gastropoda	+[A]*Clausilia* sp.	ABA	trogloxene	Z. Fehér	fossil
Mollusca	Gastropoda	*Oxychilus glaber* (Rossmässler, 1835)	TOR, AKA	trogloxene	Z. Fehér	
Mollusca	Gastropoda	+[M]*Oxychilus draparnaudi* (Beck, 1873)	MAN, ORF	trogloxene?	Z. Fehér	Generally occupies perturbed, urban areas, its appearance in caves may indicate negative effects of artificial utilization (Angyal 2012b).
Annelida	Oligochaeta	*Eiseniella tetraedra* (Savigny, 1826)	ABA, MAN	trogloxene	T. Szederjesi	
Annelida	Oligochaeta	*Aporrectodea sineporis* (Omodeo, 1952)	ROM	trogloxene	T. Szederjesi	
Annelida	Oligochaeta	+[A]*Dendrodrilus rubidus* (Savigny, 1826)	ABA, NYA	subtroglophile?	T. Szederjesi	
Annelida	Oligochaeta	+[A]*Dendrodrilus rubidus rubidus* (Savigny, 1826)	AKA, ABA	subtroglophile?	T. Szederjesi	
Annelida	Oligochaeta	+[A]*Helodrilus oculatus* Hoffmeister, 1845	ABA, SPI	eutroglophile	T. Szederjesi	First Hungarian records of the species. More detailes in Szederjesi et al. 2014. Closely related to the endemic species of the Baradla Rövid-Alsó Cave, *Helodrilus mozsaryorum* (Zicsy, 1947) (see in Salamon et al. 2014).
Annelida	Oligochaeta	Naididae sp.	ABA	trogloxene?	T. Szederjesi	
Myriapoda	Chilopoda	*Lithobius forficatus* (Linnaeus, 1758)	TOR, NYA	subtroglophile	L. Dányi	
Myriapoda	Chilopoda	*Lithobius validus* Meinert, 1872	ROM	subtroglophile	L. Dányi	
Myriapoda	Diplopoda	*Trachysphaera schmidtii* Heller, 1858	ABA, TOR	eutroglophile	Z. Korsós & D. Angyal	
Myriapoda	Diplopoda	*Haasea hungarica* (Verhoeff, 1928)	KIS, TRI	eutroglophile	Z. Korsós & D. Angyal	First records in the Mecsek Mts. from other caves than the Abaligeti Cave.
Myriapoda	Diplopoda	Chordeumatida sp.	NYA, ABA	eutroglophile?	Z. Korsós & D. Angyal	
Myriapoda	Diplopoda	*Hungarosoma bokori* Verhoeff, 1928	ABA	eutroglophile	Z. Korsós, A. Mock & D. Angyal	First male record of the species! Gonopods have been dissected, redescription have been written (Mock et al. 2014).
Myriapoda	Diplopoda	*Mastigona bosniensis* (Verhoeff, 1897)	NYA	trogloxene	Z. Korsós & D. Angyal	
Myriapoda	Diplopoda	*Unciger foetidus* (C. L. Koch, 1838)	TOR	trogloxene	Z. Korsós & D. Angyal	
Myriapoda	Diplopoda	*Cylindroiulus luridus* (C. L. Koch, 1847)	TOR	trogloxene	Z. Korsós & D. Angyal	
Myriapoda	Diplopoda	*Blaniulus guttulatus* (Fabricius, 1798)	TOR	trogloxene	Z. Korsós & D. Angyal	
Myriapoda	Diplopoda	*Boreoiulus tenuis* (Bigler, 1913)	TOR	subtroglophile	Z. Korsós & D. Angyal	First record from the Mecsek Mts.
Myriapoda	Diplopoda	*Polydesmus collaris* C. L. Koch, 1847	VAD, TOR, NYA, MAN, SZA, ABA	trogloxene	Z. Korsós & D. Angyal	
Myriapoda	Diplopoda	+[M] *Polydesmus complanatus* (Linnaeus, 1761)	TOR, MAN	trogloxene	Z. Korsós & D. Angyal	

Phylum/ Subphylum	Cl/Subcl/ Ordo	Species/taxon	Cave	Ecological group	Determined by	Notes
Myriapoda	Opiliones	+[A] *Mitostoma chrysomelas* (Herman, 1804)	ABA	eutroglophile	D. Murányi	Cavernicolous population was previously known only in the Baradla Cave (Aggtelek Karst)
Chelicerata	Opiliones	*Nemastoma bidentatum sparsum* (Gruber & Martens, 1968)	NYA	subtroglophile	D. Murányi	
Chelicerata	Acari	*Ixodes vespertilionis* Koch, 1844	VAD, TOR, ABA	subtroglophile? (bat parasite)	S. Hornok	Collected specimens were involved in the morphological and molecular genetic analysis that resulted the description of a new tick species from the caves of the Pilis Mts. (Hornok et al. 2014).
Chelicerata	Acari	Oribatida sp.	ABA, TOR	trogloxene	J. Kontschán	
Chelicerata	Acari	+[A] Galuminidae sp.	ABA	trogloxene	J. Kontschán	
Chelicerata	Acari	Parasitidae sp.	VAD, ABA, TOR, NYA	subtroglophile? (parasite)	J. Kontschán	
Chelicerata	Araneae	*Meta menardi* (Latreille, 1804)	TOR, AKA	subtroglophile	B. Zalai	
Chelicerata	Araneae	*Meta* sp.	ABA	subtroglophile	B. Zalai	
Chelicerata	Araneae	*Metallina* sp.	TOR, NYA	subtroglophile	B. Zalai	
Chelicerata	Araneae	*Nesticus cellulanus* (Clerck, 1757)	TOR, AKA, ABA	subtroglophile	B. Zalai	
Chelicerata	Araneae	*Urocoras longispinus* (Kulczynski, 1897)	TOR	subtroglophile?	B. Zalai	
Chelicerata	Araneae	+[M] Linyphiidae sp.	MAN	subtroglophile?	B. Zalai	
Chelicerata	Araneae	+[A] *Porrhomma convexum* (Westring, 1861)	ROM, ABA	eutroglophile	B. Zalai	
Chelicerata	Araneae	Theridiidae sp.	TOR, AKA	subtroglophile?	B. Zalai	
Crustacea	Isopoda	*Trachelipus rathkii* (Brandt, 1833)	TOR	trogloxene	J. Kontschán	
Crustacea	Isopoda	+[A M] *Haplophthalmus mengei* (Zaddach 1844)	ABA, MAN	eutroglophile	J. Kontschán	
Crustacea	Isopoda	+[M] *Cylisticus convexus* (De Geer, 1778)	MAN	trogloxene	J. Kontschán	Generally occupies perturbed, urban areas, its appearance in caves may indicate negative effects of artificial utilization (Angyal 2012b).
Crustacea	Amphipoda	*Gammarus fossarum* Koch, 1836	ABA, KIS, MAN	subtroglophile?	J. Kontschán & D. Angyal	Troglomorphic individuals were found in the deeper zones of the Abaligeti Cave.
Crustacea	Amphipoda	*Gammarus roeseli* Gervais, 1835	KIS	trogloxene	J. Kontschán & D. Angyal	
Crustacea	Decapoda	+[A] *Astacus astacus* Linnaeus, 1758	ABA	trogloxene	L. Forró & J. Kontschán	During the autumn of 2011, a small population (13 specimens) was observed in the main passage's stream in the first 100 m. In January 2012 only a dead specimen was found.
Crustacea	Copepoda	*Megacyclops viridis* (Jurine, 1820)	MAN	trogloxene	L. Forró	
Hexapoda	Collembola	*Ceratophysella sp.	TOR	troglobiont	L. Dányi	

Phylum/ Subphylum	Cl/Subcl/ Ordo	Species/taxon	Cave	Ecological group	Determined by	Notes
Hexapoda	Collembola	+[M] *Ceratophysella denticulata* (Bagnall, 1941)	NYA, MAN, TOR	trogloxene	L. Dányi	
Hexapoda	Collembola	*Deuteraphorura inermis* (Tullberg, 1869)	NYA	eutroglophile	L. Dányi	
Hexapoda	Collembola	+[A] *Folsomia* sp.	ABA	eutroglophile	L. Dányi	
Hexapoda	Collembola	+[A] *Folsomia candida* Willem, 1902	TRI, ABA	eutroglophile	L. Dányi	
Hexapoda	Collembola	*Heteromurus nitidus* (Templeton, 1836)	TOR, TRI, MAN,ABA, VAD,SZA, NYA	eutroglophile	L. Dányi	
Hexapoda	Collembola	*Heteromurus*/ (*Verhoeffiella*?) sp.	ABA	eutroglophile	L. Dányi	
Hexapoda	Collembola	*Lepidocyrtus* sp.	ABA, TOR	eutroglophile	L. Dányi	
Hexapoda	Collembola	*Megalothorax* cf. *minimus* Willem, 1900	TRI	eutroglophile	L. Dányi	
Hexapoda	Collembola	*Megalothorax* sp.	TRI, ABA, TOR	eutroglophile?	L. Dányi	
Hexapoda	Collembola	*Neelus murinus* Folsom, 1896	ABA, NYA	trogloxene	L. Dányi	
Hexapoda	Collembola	*Oncopodura crassicornis* Shoebotham, 1911	TRI	eutroglophile	L. Dányi	
Hexapoda	Collembola	*Proisotoma minuta* (Tullberg, 1871)	TRI	trogloxene	L. Dányi	
Hexapoda	Collembola	+[A] **Pygmarrhopalites* cf. *bifidus* (Stach, 1945)	ABA, NYA, TRI	troglobiont	L. Dányi	
Hexapoda	Collembola	**Pygmarrhopalites* cf. *pygmaeus* (Wankel,1860)	NYA	troglobiont	L. Dányi	
Hexapoda	Diplura	+[A] *Campodea* sp.	ABA	eutroglophile	D. Angyal	
Hexapoda	Ephemerop-tera	+[A] Heptageniidae sp.	ABA	subtroglophile	D. Murányi	
Hexapoda	Plecoptera	+[A,M] *Nemoura cinerea* (Retzius, 1783)	ABA, MAN	subtroglophile?	D. Murányi	Its appearance in caves is strange, no other data is known from underground habitats.
Hexapoda	Plecoptera	+[A] *Capnia bifrons* (Newmann, 1839)	ABA, ROM NYA	subtroglophile?	D. Murányi	First records from caves.
Hexapoda	Plecoptera	+[A] *Nemoura* sp.	ABA, ROM NYA	subtroglophile?	D. Murányi	
Hexapoda	Heteroptera	*Velia caprai* (Tamanini, 1947)	KIS	trogloxene	E. Kondorosy	
Hexapoda	Coleoptera	*Agabus guttatus* (Paykull, 1798)	NYA	trogloxene	A. Lőkkös	
Hexapoda	Coleoptera	*Carabus coriaceus coriaceus* Linnaeus, 1759	NYA	trogloxene	Gy. Szél	
Hexapoda	Coleoptera	*Carabus nemoralis* O.F. Müller, 1764	ROM	trogloxene	Gy. Szél	
Hexapoda	Coleoptera	*Carabus scheidleri praescheidleri* Mandl, 1965	SZA	trogloxene	Gy. Szél	

Phylum/ Subphylum	Cl/Subcl/ Ordo	Species/taxon	Cave	Ecological group	Determined by	Notes
Hexapoda	Coleoptera	*Carabus ullrichii ullrichii* Germar, 1824	VAD	trogloxene	Gy. Szél	
Hexapoda	Coleoptera	+[A] *Abax parallelus* (Duftschmid, 1812)	ROM, ABA	trogloxene	Gy. Szél	
Hexapoda	Coleoptera	*Platynus assimilis* (Paykull, 1790)	SZA	trogloxene	Gy. Szél	
Hexapoda	Coleoptera	*Leistus rufomarginatus* (Duftschmid, 1812)	KIS, ROM	trogloxene	Gy. Szél	
Hexapoda	Coleoptera	+[A] *Trechus pilisensis* Csiki, 1918	ABA	trogloxene	Gy. Szél	
Hexapoda	Coleoptera	*Trechoblemus micros* (Herbst, 1784)	ABA, TRI	eutroglophile	Gy. Szél	
Hexapoda	Coleoptera	+[A] *Choleva spadicea* (Sturm, 1839)	ABA	eutroglophile	O. Merkl	
Hexapoda	Coleoptera	*Choleva angustata* (Fabricius, 1781)	KIS, ROM	subtroglophile	O. Merkl	
Hexapoda	Coleoptera	+[A] *Leiodes cinnamomea* (Panzer, 1793)	ABA	trogloxene	O. Merkl	
Hexapoda	Coleoptera	+[A] *Leptinus testaceus* P.W.J. Müller, 1817	TOR, ABA	trogloxene	O. Merkl	
Hexapoda	Coleoptera	+[A] *Phosphuga atrata* (Linnaeus, 1785)	TOR, ABA	trogloxene	O. Merkl	
Hexapoda	Coleoptera	*Anotylus mendus* Herman, 1970	TOR	eutroglophile?	Gy. Makranczy	
Hexapoda	Coleoptera	*Proteinus ovalis* Stephens, 1834	KIS, TOR	trogloxene	Gy. Makranczy	
Hexapoda	Coleoptera	*Atheta spelaea* (Erichson, 1839)	ABA	eutroglophile	Gy. Makranczy	
Hexapoda	Coleoptera	*Aloconota mihoki* (Bernhauer, 1913)	GIL	trogloxene	Gy. Makranczy	
Hexapoda	Coleoptera	*Aloconota sulcifrons* (Stephens, 1832)	TRI	trogloxene	Gy. Makranczy	
Hexapoda	Coleoptera	*Quedius mesomelinus skoraszewskyi* Korge, 1961	TOR, ABA, VAD, NYA	eutroglophile	Gy. Makranczy	
Hexapoda	Coleoptera	*Anoplotrupes stercorosus* (Scriba, 1791)	SZA, TOR	trogloxene	O. Merkl	
Hexapoda	Coleoptera	*Ancistronycha erichsonii erichsonii* (Bach, 1852)	GIL	trogloxene	D. Szalóki	
Hexapoda	Coleoptera	*Cryptophagus nitidulus* Miller, 1858	TOR	eutroglophile	O. Merkl	
Hexapoda	Coleoptera	*Meloe violaceus* Marsham, 1802	SZA	trogloxene	D. Szalóki	
Hexapoda	Trichoptera	*Plecopterina* sp.	AKA	subtroglophile?	D. Murányi	
Hexapoda	Lepidoptera	*Scoliopteryx libatrix* (Linnaeus, 1758)	AKA, TOR, KIS	subtroglophile	D. Angyal	
Hexapoda	Lepidoptera	*Triphosa dubitata* (Linnaeus, 1758)	ABA	subtroglophile	D. Angyal	
Hexapoda	Diptera	Mycetophilidae sp.	ABA	subtroglophile?	E. Lazányi	

4. DISCUSSION

4.1 *Niphargus* studies

Morphology of *Niphargus molnari* and *Niphargus gebhardti* was insufficiently known up to now and could not be used in a broader comparative research of *Niphargus*. To fill this gap, completion of a detailed and richly illustrated redesription of the two species - applying comparative scanning electron micrographs for the first time on *Niphargus* - was highly reasonable. Although the two species share few main traits, they differ from each other in numerous significant characters, like the shape of the epimeral plates, the number of retinacles or the size of gnathopod propodi.

The two niphargids are spatially segregated within the same caves. *N. gebhardti* inhabits isolated pools of stagnant water, which is fed by percolating water from the limestone fissures, so called epikarst. On the contrary, *N. molnari* was always found in streaming waters. Trontelj et al. (2012) had revealed that the morphological diversity in caves is niche based, existence of different microhabitats within a cave correlates with variances of morphological trates. Distinctness of *N. molnari* and *N. gebhardti* was confirmed by our phylogenetic results too. Phylogenetic relationship of *N. molnari* to the rest of *Niphargus* species is for the present not clear, molecular studies being in progress on the Hungarian niphargids may help to approximate the solution of the problem. Agreeing with our hypothesis on its habitat preference, it is now revealed that *N. gebhardti* is closely related to a clade of epikarstic and interstitial species from Southern Slovenia (*N. fongi*, *N. carniolicus*, *N. wolfi* and *N. dobati*). Intrestingly, *N. gebhardti* is also closely related to cryptic species endemic to the Western Carpathians and to *Pontoniphargus racovitzai* from Movile Cave, the chemoautotrophic system in Eastern Romania. If we presume that fissures in limestone constitute an extended, combined system comprehending caves with traceable or even untraceable hydrological connections, the existence of the same *N. gebhardti* haplotypes in six different caves may indicate to recent migration, which supports the concept of epikarstic colonization. On the contrary, if we accept that the focal six caves are devided into two hydrologically distinct groups (see Figure 24) without possible *Niphargus* migration for the last couple of hundred thousand years, same haplotypes in isolated populations may refer slow rate of molecular evolution.

Due to its protected geographical situation, since the Tertiary, the area of Mecsek may played refugial role during the alternating warmer and colder eras, preserving old lineages of Crustaceans. According to our phylogenetic results, *N. molnari* and *N. gebhardti* represent completely distinct lineages, which colonized the Mecsek area independently. The distribution range of the two endemic species is small; a maximum distance between caves is seven kilometers.

Despite of my repeated visits and careful searching, *Niphargus* specimens were not found in the Mánfai-kőlyuk Cave. *N. molnari* is supposedly had gone extinct in its type

locality, due to its industrial utilization in the past (Angyal 2012b). Moreover, the type locality of *N. gebhardti* - which is a public cave with 80.000 annual visitors - may be also endangered. Considering the extremely narrow distributional range of the two species and the vulnerability of their populations, *N. molnari* and *N. gebhardti* are suggested to be placed into the 'Vulnerable (VU)' IUCN Red List of Threatened Species category according to the following criteria: i) number of locations is ≤ 10 ('B2') and ii) area of occupancy is less than 20 km^2('D2').

4.2 *Protelsonia* studies

Magniez (2000) reported on the necessity of revising *Protelsonia hungarica robusta*, which had not been happened until that time. Now, redescription of the subspecies is provided, and comparison of the main morphological traits of the two subspecies using comparative scanning electon micrographs for first time on the genus was also made. Distinguishing characters suggested by Méhely (1924) were compared with characters of newly collected *P. hungarica hungarica* material of two caves, and aside from a minute difference, the characters agreed. Intrestingly, 30% of the *P. hungarica robusta* specimens collected in the Mánfai-kőlyuk Cave showed some peculiar characters of the other subspecies, like the existence of a riffle-shaped sinus on endopodite of male's pleopod II. The rest of the studied individuals' main diagnostic characters more or less agreed with the description of Méhely (1927) and it was found that the division into two subspecies by Méhely was reasonable; however future molecular studies involving the third subspecies, *P. hungarica thermalis* may help to securely clarify their positions. With the addition of new diagnostic characters too, in total five distinguishing characters of the two subspecies was now described.

Existence of the 'Abaliget morphotype' in the Mánfai-kőlyuk Cave raises some questions. Between the 1960s and 1990s, karstic water of the Vízfő Cave (near Orfű village) was in connection with the hydrological system of the Mánfai-kőlyuk Cave in order to increase the volume of exploitable water. Vízfő Cave and the surrounding springs have not been thoroughly examined in faunistic aspect up to now. i) Assuming that the found morphological differences are strong enough for distinguishing the two forms, the possibility of one directional migration towards the Mánfai-kőlyuk Cave is feasible. Future study of the Vízfő system is essential to unravel the question. ii) According to another explanation, the found morphological characters are variable and are may influenced by features of the different microhabitats. Though, regarding the uniformity of the single remained microhabitat in the artificial tunnel of the Mánfai-kőlyuk Cave, it is not probable. iii) Considering that summer speleological expeditions in the past happened simultaneously in different caves of the Western Mecsek, accidental human mediated introduction of Abaliget specimens to Mánfa can not be excluded.

According to Bokor (1924), *P. hungarica hungarica* supposedly inhabits fissure system too. Agreeing with this hypothesis, individuals were found in several occasions in

small pools unconnected with any source of streaming water. In the Vadetetős Cave, specimens were found almost exlusively in shallow sinter basins and pools. Intrestingly, they possessed minor body size than the stream-dwelling ones from the Abaligeti Cave, which could be related to the epikarstic origin.

Magniez (1999) stated that the morphological features of the Stenasellidae indicate that they represent phylogenetically ancient clade, apparently more directly related to marine Asellota (Stenetrioidea, Gnathostenetroidoidea, part of the Microcerberidae) than to the modern family Asellidae. Méhely (1925) presumed that *P. hungarica* ensconced into subterranean aquatic habitats from searing creaks of the Paratethys Sea that encompassed the islands of the Mecsek. Then, by degress, they had been adapted to the subterranean conditions in both physiological and morphological features. He confirmed the Tertiary origin of *Protelsonia* by describing some plesiomorphic characters, like the vermiculous, homogenous, segmented body or the existence of nephrocytes in head, which imply the transition between Annelida and Isopoda.

Referring to the extremely narrow distributional range of the two subspecies and the vulnerability of their populations, *P. hungarica hungarica* and *P. hungarica robusta* are suggested to be placed into the 'Vulnerable (VU)' IUCN category according to the following criteria: i) number of locations is ≤ 10 ('B2') and ii) area of occupancy is less than 20 km^2 ('D2').

4.3 *Bythiospeum* studies

During the first stage of the molecular studies on 'Hungarian blind snail' from the Abaligeti Cave and the Mánfai-kőlyuk Cave, 7.05% mt COI difference was found between the two haplotypes. It may means that the originally epigean snails started to colonize underground refugia and to evolve independently 3-0.4 my years ago in the Upper Pliocene or in the Pleistocene (Angyal et al. 2013). Morphological distinctness detected by shell morphometric methods supports the molecular genetic results. To reveal the haplotype-network structure in more detail, to calculate the genetic distance and to estimate the gene flow between the two populations, larger samples and a new molecular marker (16S rRNA) were involved in the second stage of this study, which resulted the unexpected recovery of the 'Abaliget haplotype' in the Mánfai-kőlyuk Cave population in approximately 30% of the examined individuals. Further haplotypes in intermediate positions have not been found, which supports that not the two distant haplotypes of a polymorphic starburst phylogeny had been sampled. However further studies on even larger samples will be necessary for the certain clarification, referring to the consequent divergent shell morphological characters and the high percentage of mt COI and 16S differences, in my opinion *B. hungaricum* s. str. and *B*. cf. *gebhardti* can be tentatively treated as two distinct species.

According to the most relevant results, the Abaligeti Cave and the Mánfai-kőlyuk Cave are not hidrologically interconnected (Rónaki 1972, Dezső 2011). The possible

explanations for the coexistence of two genetically distant haplogroups in the Mánfai-kőlyuk Cave are as follows. i) Like in case of *Protelsonia*, one directional migration towards the Mánfai-kőlyuk Cave from the Vízfő system is possible. ii) The studied caves are formated in the Lapisi Limestone Formation with the average thickness of 200 m, which means that under the karstic water level there is an approximately 100 m thick karstic rock zone with its own fissure system, totally filled with water. Although, the two caves belong to two distinct catchment areas and are not either interconnected according to the recent water tracing studies, this, so-called deepkarstic zone could be suitable for the locomotion of some aquatic troglobiont invertebrate species (Tegzes 2014, pers. comm.). It would explain the existence of a restricted, but not non-zero gene flow, that is also presumed by Fehér et al. (2013) in case of *Bythinella pannonica* (Frauenfeld, 1865) populations of the Bükk Mts. (North Hungary) and the Gömör-Torna Karst (North Hungary, South Slovakia), which showed two genetically distant haplogroups coexisted within a geographically low range. If we accept this phenomenon, two directional migrations of the snails through the deep karstic fissure system would be expected. In the contrary, our data indicated asymmetric if not completely unidirectional migration. Further molecular studies on larger samples are needed to reveal whether the migration is unidirectional or asymmetric. iii) Application of fluorescent dyes for vadose zone's karstic water tracing in the recent past might have not been suitable for the certain verification of the hydrological distinctness of two caves. Micropassages without active water movement could be in permeable connection to minute invertebrates, like hydrobiids (Varga 2013). It corresponds with the fact of the previously found shell aggregations in the sediment of the Western 2 collateral in the Abaligeti Cave, which shells might be washed away from the epikarst by percolating water and were accumulated in a suitable part of the passage (Varga 2013). iv) Similarly to *Protelsonia*, accidental human mediated introduction of Abaliget specimens to Mánfa can not be excluded.

Assuming the possibility of the two directional migrations, it may be hypothesized that the different effective population sizes cause different rates of sorting. To test this statement, application of a population genetic study would be necessary.

Phylogenetic results based on mitochondrial markers showed that *B. hungaricum* and *B*. cf. *gebhardti* are not closely related to the rest of the *Bythiospeum* species with available sequences (mainly Alpine species). In most cases, species of various rissooid genera proved to be less closely related to the two taxa from the Mecsek Mts., than to the Alpine *Bythiospeum* species. Further studies involving more sequences of more distant epigean groups could be suitable for the reconstruction of the origin and phylogenetic position of *B. hungaricum* and *B*. cf. *gebhardti* among rissooids.

Cryptic diversity, defined as two or more distinct species that were classified as a single one due to morphological similarity is believed to be a potentially important factor, influencing future conservation decisions (Trontelj & Fišer 2009). *B. hungaricum* is already protected by law; its present IUCN state is 'Vulnerable'. However further study on the 'Hungarian blind snail' populations from hypogean waters of the Western Mecsek is necessary for the certain clarification of their taxonomic positions, regarding the vulnerability

of their habitats, managing the two currently known populations as two separate conservation biological units is highly recommanded.

4.4 *Brachydesmus* studies

Application of modern methods in morphological studies on the polydesmid *Brachydesmus troglobius* enabled the analysis and illustrating of fine-scale characters. Analysed COI sequences of individuals from the Serbian Petnicka's Cave and from the Abaligeti Cave showed only 0.76% difference, which may suggest that the originally epigean species, 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 Pleistocene.

Identification of polydesmid diplopods in the absence of mature males is circumstantial in some cases. The example of *Polydesmus denticulatus* C. L. Koch, 1847 identified by comparison of COI sequences shows the value of complementing traditional morphology with molecular systematics. Present results on phylogenetic studies of some of the polydesmid species and genera may help in future delimitation of interspecific and intergeneric boundaries.

Shear (1984) considered that many cave-dwelling millipede species are relics of old taxa searching for a better microhabitat during the last glacial period. Furthermore, due to the isolation of such habitats, a high degree of endemism could have developed in cave millipedes. Verhoeff (1928) found very interesting the mixed zoogeographical character of the diplopod fauna of the Abaligeti Cave and he stated the relationship of *B. troglobius* with the Croatian-Illyrian fauna, while he considered *Hungarosoma bokori* (Verhoeff, 1928) to be related to the Asian millipede fauna. However, the recently discovered circum-Pannonian distribution of *H. bokori* corresponds with the hypothesis that *Hungarosoma* belongs to a 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). Verhoeff (1928) presumed the common lineages of *Haasea hungarica* (Verhoeff, 1928) and the Central European millipede species.

Predation and competition for resources are less intensive in subterranean habitats than in epigean 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). *B. troglobius* seemingly maintains a stable population in the Abaligeti Cave, using all types of vegetal organic material. Although the appearance of the lamp flora is both aesthetic and conservational problem in public caves like the Abaligeti Cave, the vegetation confined to them seemed to be a regular source of energy not only for *B. troglobius*, but also for other detritivores. 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 caves of Slovenia, Croatia, Serbia and Montenegro too, in Hungary, the species possesses only one known locality apart from its type locality. Given this extremely narrow Hungarian distribution, the two local populations (one in the Abaligeti Cave and another in the Törökpince Cave) would be suggested for legal protection.

4.5 Further faunistic data

The 25 and 7 newly revealed species and subspecies from the Abaligeti Cave and the Mánfai-kőlyuk Cave, respectively, suggest that repeated sampling is reasonable to complete our knowledge on the macroinvertebrate fauna of these caves. Visiting of earlier undiscovered sampling sites and application of new collecting methods revealed further species that were previously not known from the caves. Discovery of the Oligochaeta *Helodrilus oculatus* for first time in Hungary in two of the studied caves is quite a remarkable record. Until now only one species of the genus was known from subterranean habitats, the endemic *Helodrilus mozsaryorum* (Zicsi, 1974) from the Baradla Rövid-Alsó Cave in the Aggtelek Karst (Szederjesi et al. 2014). Appearance of populations of epigean species in caves - as in case of *Astacus astacus* in the Abaligeti Cave - may related to short therm effects on climate change events. Artificial utilization of the Mánfai-kőlyuk Cave seemed to contribute to the disappearance of endemic fauna elements and the introduction of perturbed, urban habitat-dwelling species (Angyal 2012b).

The 74 new aquatic and terrestrial records from 12 further caves of the Western Mecsek contribute to get a better knowledge about the distribution of the cave-dwelling macroinvertebrates of the area. Including the new data, still only 6% of the caves of the Mecsek Mts. were investigated in invertebrate zoological aspect, which means that extending the research on more and more caves would be essential. It was revealed that the most abundant invertebrate groups in the studied 12 caves were millipedes, springtails and beetles. In 58% of the newly investigated caves, at least one, or more than one troglobiont taxa were found.

However the vast majority of the collected taxa (47%) belonged to the trogloxene category, the percentage of subtroglophile and eutroglophile species and subspecies (24 and 26%, respectively) is quite remarkable. The high number of endemic troglobiont taxa supports the fact that Mecsek Mts. is extremely rich in endemic relics comparing with other Hungarian karst areas. Together with the 7 revised taxa, in the 14 studied caves, almost 10% of the found species and subspecies belonged to the troglobiont category. Groups with remarkable numbers of eutroglophile and troglobiont taxa (like Gastropoda, Diplopoda, Isopoda, Amphipoda, Collembola or Coleoptera) deserve even more attention.

4.6 Conclusion

Detailed and richly illustrated redesriptions of *Niphargus molnari* and *Niphargus gebhardti* were provided, with the addition of cytochrome c oxidase subunit I barcode sequences. Phylogenetic relationships of both species within the genus *Niphargus* were studied, using three independent molecular markers.

Traditional morphological studies were completed with comparative scanning electron microscopy, applied for the first time on niphargids, *Protelsonia hungarica hungarica*, *Protelsonia hungarica robusta* and *Brachydesmus troglobius*.

Clarification of the distinctness of *Bythiospeum hungaricum* and *Bythiospeum* cf. *gebhardti* was also made, using further integrative taxonomic methods, like the analysis of COI and 16S rRNA gene sequences as well as shell morphometric studies.

Due to the found distinguishing characters of the *Protelsonia* morphotypes, validity of the two separate subspecies was verified. Performing phylogenetic studies, contribution to the knowledge on the relationships of *B. hungaricum*, *B.* cf. *gebhardti* and *B. troglobius* to the rest of the rissooid and polydesmid genera was also performed. Attempts for delimitation of their interspecific and intergeneric boundaries were made too.

As a global conclusion, it can be said that the newly applied integrative taxonomic methods proved to be rather efficient completions of traditional morphology. It has also been revealed that limitation of caves as parts which are passable by humans is not an appropriate conception, as both the epikarstic and the deepkarstic zones seem to be suitable for transport of minute invertebrates. In order to understand the colonization mechanisms of the focal taxa, thinking in systems is essential. Comparing the aquatic and terrestrial cave-dwelling macroinvertebrates, it seemed that though, the terrestrial ones (like millipedes) are globally wider spreaded; locally they possess rather restricted distributional area, known from one or a few caves. It may points to the fact that migration of terrestrial invertebrates through the karstic fissure system is not efficient. In the contrary, the studied aquatic invertebrate taxa are highly endemic, known exclusively from the Mecsek Mts., however they showed wider local distributions. Despite of the increase of their known local distributional range by newly revealed localities, most of the examined taxa are recommended for legal protection. As the hypogean and epigean waters are in close contact, the protection is unimaginable without the careful monitoring of the surface environment of the caves.

5. SUMMARY

Fragmented mountain areas in East-Central Europe had been suggested to be centers of endemism. Mecsek Mts. is one of these isolated mountain ranges. One of its three main parts is Western Mecsek, from where more than 200 caves are currently known. Two of them, the Abaligeti Cave and the Mánfai-kőlyuk Cave had been previously studied in zoospeleological aspect. Due to these studies carried out between the 1920s and 1930s, relatively high number of rare and endemic troglobiont (exclusively cave-dwelling) macroinvertebrate species and subspecies had been revealed. However, due to the insufficient descriptions and inconsequences regarding their systematics, vast majority of them proved to be in rather uncertain taxonomic position.

Seven of these taxa have been chosen by the author for careful revision, namely the amphipods *Niphargus molnari* Méhely, 1927 and *Niphargus gebhardti* Schellenberg 1934, the aquatic isopods *Protelsonia hungarica hungarica* Méhely, 1924 and *Protelsonia hungarica robusta* Méhely, 1927, the hydrobiid snails *Bythiospeum hungaricum* (Soós, 1927) and *Bythiospeum* cf. *gebhardti* (H. Wagner, 1931) and the polydesmid millipede *Brachydesmus troglobius* Daday, 1889. Fourteen caves, including Abaligeti Cave and Mánfai-kőlyuk Cave had been regularly visited between 2010 and 2013; populations of the focal taxa have been found in eight of them. Several sampling methods have been tested, but the most frequently applied was singling (sampling of single individuals noticed on the spot), which allowed the lowest disturbance towards the sensitive ecosystem.

Introducing in Hungary the acquired modern *Niphargus* taxonomic methodology, detailed and richly illustrated redescriptions of *N. molnari* and *N. gebhardti* have been made. Comparative scanning electron micrographs have been also made for the first time on *Niphargus*. The performed phylogenetic studies have shown that the two species - which are spatially segregated in caves where they coexist - represent completely distinct lineages and may have colonized the Mecsek area independently. Phylogenetic relationship of *N. molnari* to the rest of *Niphargus* species is for the present not clear. *N. gebhardti* is closely related to a clade of epikarstic (vadose zone fissure system dwelling) species from Southern Slovenia and to cryptic species (species under the same name because of the lack of visible morphological differences) endemic to Western Carpathians. New localities of both species have been found. The use of mitochondrial marker on six localities of *N. gebhardti* supported the idea of its epikarstic origin. *N. molnari* is supposedly had gone extinct in its type locality due to the industrial utilization in the past. Both species had been suggested for legal protection. *N. gebhardti* and *N. molnari* have been recommended to be listed in 'Vulnerable' category of the IUCN Red List of Threatened Species.

Contribution to the knowledge on morphology of *P. hungarica hungarica* and *P. hungarica robusta* had happened, using comparative scanning electron micrographs and newly applied characters for first time on the genus. Consequent morphological distinguishing characters found between the studied populations have supported the validity of the

distinctness of two subspecies; however, further molecular study is necessary for its certain decision involving the third subspecies (*P. hungarica thermalis*). Interestingly, specimens with *P. hungarica hungarica* characteristics have been found in 30% of the examined individuals from the Mánfai-kőlyuk Cave, which can be explained by i) one directional migration towards the Mánfai-kőlyuk Cave from the Vízfő system during the artificial connection of the two systems, ii) the variability and microhabitat dependent pattern of the studied characters, or by iii) human inducated accidental transfer. Morphological features of *Protelsonia* indicate that they represent phylogenetically ancient clade with marine origin date back to the Tertiary. Present study has revealed three further localities of *P. hungarica hungarica*. Referring to the extremely narrow distributional range of the two subspecies and the vulnerability of their populations, *P. hungarica hungarica* and *P. hungarica robusta* has been suggested to be listed in 'Vulnerable' IUCN category.

The use of mitochondrial markers on the 'Hungarian blind snail' has shown the unambiguous distinctness of *B. hungaricum* and *B.* cf. *gebhardti*, which have been supported by shell morphometric analysis too. Similarly to the *Protelsonia*, in approximately 30% of the sequenced individuals from the Mánfai-kőlyuk Cave, the 'Abaliget haplotype' has been found. The possible explanations are as follows. i) One directional migration towards the Mánfai-kőlyuk Cave from the Vízfő system, ii) two directional migration through the deep karstic zone's fissure system, iii) one or two directional migration through the untraceable epikarstic micropassages, or iv) human inducated accidental transfer. Phylogenetic results showed that *B. hungaricum* and *B.* cf. *gebhardti* are not closely related to the Alpine *Bythiospeum* species and to some of the other rissooid genera with available COI sequences. Among the fourteen visited caves, *B. hungaricum* and *B.* cf. *gebhardti* have been found only in the Abaligeti Cave and the Mánfai-kőlyuk Cave. Regarding the vulnerability of these habitats, managing the two currently known populations as two separate conservation biological units has been highly recommanded.

The use of scanning electron microscopy in morphological studies on the polydesmid *B. troglobius* enabled the analysis and illustrating of barely visible characters. The applied molecular methods have shown only small differences in the COI gene sequences of a Serbian and a Hungarian individual of *B. troglobius*, which might refer to the species' recent colonization of hypogean habitats. Phylogenetic studies of some of the polydesmid species and genera may help in future delimitation of interspecific and intergeneric boundaries. *B. troglobious* is known from Slovenia, Montenegro, Croatia and Serbia too, though, only from one or a few caves of each country. The extremely rare local distribution of the cavernicolous millipedes suggests their ineffective migration through the karstic fissure system. Apart of its type locality, *B. troglobius* has been found only in a single cave in this study. Referring to their rather narrow Hungarian distribution, the two local populations have been suggested for legal protection.

Apart from the revised taxa, 105 further aquatic and terrestrial macroinvertebrate species or subspecies have been revealed from the 14 studied caves. Comparing with the latest checklists on the fauna of the Abaligeti Cave and the Mánfai-kőlyuk Cave, 25 and 7 new

records were now made from the two caves, respectively. 3 further troglobiont species (among springtails) and an oligochaete new for the Hungarian fauna were also found.

Careful monitoring of the surface environment of the caves inhabited by rare and endemic macroinvertebrates has been suggested, as the hypogean and epigean habitats are in close contact.

6. NEW SCIENTIFIC RESULTS (THESIS POINTS)

1. Redescription, phylogenetic analysis and detailed illustration of the amphipod species *Niphargus molnari* Méhely, 1927 and *Niphargus gebhardti* Schellenberg, 1934, using molecular genetic and morphological methods.

2. Clarification the degree of relationship of the hydrobiid species *Bythiospeum hungaricum* (Soós, 1927) and *Bythiospeum gebhardti* (H. Wagner, 1931) by comparison of mithocondrial gene fragments and by application of shell morphometric studies. Phylogenetic analysis of the two species within the Rissooidea superfamily.

3. Clarification of the taxonomic state of the asellid subspecies *Protelsonia hungarica hungarica* Méhely, 1924 and *Protelsonia hungarica robusta* Méhely, 1927 by the integration of modern and traditional morphologic methods, based on old museum samples and newly collected material.

4. Redescription, phylogenetic analysis and detailed illustration of the polydesmid diplopod *Brachydesmus troglobius* Daday, 1889, using molecular genetic and morphological methods.

5. Providing new data for conservation management regarding the seven focal rare and endemic species and subspecies.

6. Expansion the faunistic lists of the Abaligeti Cave and the Mánfai-kőlyuk Cave by newly revealed 25 and 7 species, respectively. Contribution to the knowledge of the hypogean invertebrate diversity of the Western Mecsek by sampling in 12 further caves.

7. Contribution to the better knowledge of the invertebrate fauna of the Abaligeti Cave and the Mánfai-kőlyuk Cave by visiting of earlier undiscovered sampling sites and by application of new collecting methods.

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8. ACKNOWLEDGEMENT

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9. SUPPLEMENTS

9.1 List of taxa and sequence data used in phylogenetic analysis of *Niphargus molnari* and *Niphargus gebhardti*.

Species	28S rDNA	Histone H3	COI	Locality
Niphargus gebhardti	KP967556 (NB550)	KP967550 (NB550)	KP967553 (NC041)	Abaligeti Cave, Mecsek Mts., HU
Niphargus gebhardti	KP967557 (NB551)	KP967551 (NB551)	KP967554 (NC037)	Szajha-felső Cave, Mecsek Mts., HU
Niphargus molnari	KP967555	KP967549	KP967552	Abaligeti Cave, Mecsek Mts., HU; NB555
Carinurella paradoxa	-	KR905901	KR905829	Torre, Ruda, Monfalcone, ITA; NA738
Pontoniphargus racovitzai	KF290023	-	-	Pestera de la Movile, Mangalia, Dobrogea, ROM
Niphargus dolichopus	EU693297	JQ815490	KT007331	Suvaja pećina, Lušci palanka, Sanski most, BIH; NA076
Niphargus fontanus	EF617304	-	KC315635	Little stour river, Littlebourn, Littlebourn, / Amersham, Buckinghamshire, BG
Niphargus frasassianus	GU973411	-	GU973034	Grotte di Frasaasi, Fabriano, Perugia, IT
Niphargus gallicus	KF290033	-	KF290225	str. Bisericii 39, Dulceşti, Mangalia, ROM
Niphargus glennei	KC315617	-	KC315644	Plympton farm catchpit, Plymouth, Devon, GB
Niphargus ictus	GU973415	-	GU973012	Grotte di Frasaasi, Fabriano, Perugia, IT
Niphargus irlandicus	KC315618	-	KC315647	Carrigacrump Quarry, Coyne, Cork, GB
Niphargus kolombatovici	JQ815553	JQ815522	-	Žira, Turkovići, Ravno, BIH; NA964
Niphargus liburnicus	KT007478	-	KT007418	Grotta Andrea, Iamiano, Doberdob, IT, NB018

Niphargus montanarius	GU973419	-	GU973003	Grotte di Frasaasi, Fabriano, Perugia, IT
Niphargus steueri	JQ815551	JQ815519	KT007358	Jama pod krogom, Sočerga, Koper, SI
Synurella ambulans	EF617236	-	KR905770	forest ditch near Dept. Of. Biology, Ljubljana, Ljubljana, SI, NA002
Niphargus virei	EF617237	JQ815467	KR905771	Dorpstraat 7 (well), Reijmerstok, Limburg, NED, NA003
Niphargus longicaudatus [Cres]	EF617240	KJ566705	KR905772	Retec (source), Lubenice, Island of Cres, HR, NA006
Niphargus longicaudatus [Gragnano]	EF617241	JQ815469	-	Stream near the road Monte Faito-Vico Equense, Casola, Napoli, IT; NA007
Niphargus pasquinii	EF617244	JQ815471	KR905773	Sorgenti San Vittorino, San Vittorino, Castel Sant'Angelo, IT; NA010
Niphargus dobati	EF617247	JQ815499	KR905774	Rakov Škocjan, Zelše, Cerknica, SI; NA013
Niphargus wolfi	EF617250	JQ815500	KR905775	Križna jama, Bločice, Lož, SI; NA015
Niphargus carniolicus	EF617252	JQ815501	KR905776	Jama pod gradom Luknja, Prečna, Novo mesto, SI; NA017
Niphargus fongi	EF617253	JQ815472	-	Dolga jama pri Koblarjih, Koblarji, Kočevje, SI; NA018
Niphargus longidactylus	EF617256	JQ815473	-	Sneberje (Sava freatic waters), Ljubljana, Ljubljana, SI; NA021
Niphargus labacensis	EF617257	JQ815474	KR905777	Tomačevo (intersticial waters), Ljubljana, Ljubljana, SI; NA022
Niphargus pectinicauda	EF617258	JQ815475	KR905778	Tomačevo (intersticial waters), Ljubljana, Ljubljana, SI; NA023
Niphargus bajuvaricus	EF617259	JQ815476	KT027378	Well A96, Lobau, Wien, AUT; NA024
Niphargus aberrans	EF617260	-	-	Planinska jama, Kačja vas, Planina, SI; NA025
Niphargus scopicauda	EF617261	JQ815477	KR905779	Huda luknja pri Gornjem Doliču, Završe, Slovenj Gradec, SI; NA026
Niphargus tatrensis	EF617263	-	-	Lodowe zrodlo (Icy spring), POL; NA028
Niphargus aquilex	EF617264	KP300936	KC315626	Marden, Marden, Wes Sussex, GB; NA029

Niphargus schellenbergi	EF617267	JQ815478	KR905780	Well near road 800 m SE from Heyd, Heyd, Durbuy, BEL; NA032
Niphargus sphagnicolus	EF617270	-	KR858495	Mostec, Rožnik, Ljubljana, SI; NA035
Niphargus boskovici	EF617271	JQ815502	KR905781	Bjelušica, Zavala, Popovo polje, BIH; NA036
Niphargus hvarensis	EF617273	JQ815479	KR905782	Trsteno, Dubrovnik, Dubrovnik, HR; NA038
Niphargus krameri	EF617275	JQ815503	-	Fojba, Šestani, Pazin, HR; NA040
Niphargus trullipes	EF617281	JQ815504	KR905783	Vjetrenica, Zavala, Popovo polje, BIH; NA046
Niphargus polymorphus	EF617282	JQ815505	KR905784	Obodska pećina, Rijeka Crnojevića, Cetinje, MNE; NA047
Niphargus rejici	EF617283	JQ815481	KR905785	Podpeško jezero, Jezero, Ig, SI; NA048
Niphargus stenopus	EF617284	JQ815506	-	Jama pod gradom Luknja, Prečna, Novo mesto, SI; NA049
Niphargus arbiter [Krk]	EF617286	KR905885	KR905786	Spring in port Vrbnik, Vrbnik, Krk, CRO; NA050
Niphargus salonitanus	EF617289	JQ815483	KR905788	Gospa od Stomorije spring, Kaštel Stari, Split, HR; NA053
Niphargus zagrebensis [Gadina]	EF617295	KR905886	KR905789	Gadina, Loka, Črnomelj, SI; NA059
Niphargus dalmatinus	EF617296	JQ815484	KR905790	Biba spring, Vrana, Pakoštane, HR; NA060
Niphargus elegans	EF617297	JQ815485	KR905791	San Pancrazio, San Pancrazio, Verona, IT; NA061
Niphargus vinodolensis	EF617298	JQ815486	KR905792	Ceovići, Bačići, Novi Vinodolski, HR; NA062
Niphargus tridentinus	EF617299	JQ815487	KR905793	Grotta Bus Pursi, Lumezzane, Brescia, IT; NA063
Niphargus lessiniensis	EF617300	JQ815488	-	Grotta del Aqua, Ponte de Veja, Monte Lessini, IT; NA064
Niphargus puteanus	EF617302	KJ566709	KR905795	Gasthof Zur Walba, Pentling, Pentling, GER; NA066
Niphargus brachytelson	EU693293	JQ815489	KR905797	Lukova jama pri Zdihovem, Suhor,

				Kočevje, SI; NA071
Niphargus caspary	EU693291	KJ566712	-	Tuebingen, Tuebingen, Tuebingen: GER; NA073
Niphargus factor	EU693298	JQ815508	KR905798	Vjetrenica, Zavala, Popovo polje, BIH; NA078
Niphargus grandii	EU693300	KJ566715	KR905799	Torre, Ruda, Monfalcone, IT; NA080
Niphargus longicaudatus [Lisimachia]	EU693310	KP133142	-	Lisimachia, Klisorevmata, Agrinio, GRE; NA081
Niphargus hadzii	EU693301	KR905887	KR905800	Izvir pod orehom, Verd, Vrhnika, SI; NA082
Niphargus hrabei	EU693302	KJ566716	KR905801	Stream near the road W from Lupoglav, Lupoglav, Zagreb, HR; NA083
Niphargus illidzensis	EU693304	JQ815491	KR905802	Vrelo Bosne, Ilidža, Sarajevo, BIH; NA084
Niphargus karamani	EU693305	KR905888	KR905803	Fram 119 (well), Fram, Maribor, SI; NA085
Niphargus kenki	KR905869	-	KR905804	Spring near to Sodna vas 25, Sodna vas, Podčetrtek, SI; NA086
Niphargus kochianus	EU693308	JQ815492	-	Saint Albans, Hertfordshire, Hertfordshire, GB, NA090
Niphargus longiflagellum	EU693311	JQ815520	KR905805	Podpeška jama, Videm, Grosuplje, SI; NA093
Niphargus lourensis	EU693312	-	KR905806	Louros spring, Vouliasta, Ioannina, GRE; NA094
Niphargus lunaris	EU693313	KR905889	KR905807	Bubanj vrelo, Dolac Donji, Trilj, HR; NA095
Niphargus novomestanus	EU693314	JQ815509	KR858496	Tominčev studenec, Žužemberk, Žužemberk, SI; NA096
Niphargus orcinus	EU693315	JQ815510	KR905808	Križna jama, Bločice, Lož, SI; NA099
Niphargus pachytelson	EU693316	JQ815511	KR905809	Podpeška jama, Videm, Grosuplje, SI; NA100
Niphargus podpecanus	EU693317	JQ815512	KR905810	Podpeška jama, Videm, Grosuplje, SI; NA101
Niphargus pupetta	EU693318	KJ566717	-	Tomačevo (intersticial waters), Ljubljana,

				Ljubljana, SI; NA102
Niphargus rhenorodanensis	EU693319	KJ566719	KR905811	Grotte Cormoran, Torcieu, Lyon, FR; NA104
Niphargus sanctinaumi	EU693320	KP133144	KR905812	Sveti Naum spring, Sv. Naum, Ohrid, MAC; NA105
Niphargus slovenicus	EU693322	JQ815493	KR905813	Stražišče, Kranj, Kranj, SI; NA106
Niphargus spinulifemur	EU693323	JQ815494	KR858500	Stream NE to Hrastovlje, Hrastovlje, Koper, SI; NA107
Niphargus spoeckeri	EU693324	JQ815513	KR905814	Pivka jama, Veliki otok, Postojna, SI; NA108
Niphargus stygius	KR905870	KR905890	KR905815	Jelenska jama, Borovnica, Vrhnika, SI; NA110
Niphargus subtypicus	EU693326	JQ815514	KT007433	Jama pod gradom Luknja, Prečna, Novo mesto, SI; NA112
Niphargus timavi	EU693327	JQ815495	KR858497	Labodnica, Trebiciano, Trieste, IT; NA114
Niphargus vjetrenicensis	EU693329	JQ815521	KR858499	Vjetrenica, Zavala, Popovo polje, BIH; NA116
Niphargus dimorphopus	EU693296	JQ815496	-	Gulpen, Gulpen, Limburg, NED; NA125
Niphargus dobrogicus	KR905871	KR905891	KR905816	Well N to Limanu, Mangalia, Dobrogea, ROM; NA140
Niphargus vadimi	KR905872	KR905892	KR905817	Skelska peščera, Rodnikovo, Krym, UKR; NA144
Niphargus cvijici	JQ815554	JQ815516	KR905819	Popovo polje, Ravno, Ravno, BIH; NA147
Niphargus hercegovinensis	JQ815549	JQ815517	KR905820	Žira, Turkovići, Ravno, BIH; NA151
Niphargus stygius [Romania]	KJ566693	KJ566720	KR905821	Valeni (wells), Ploiesti, Prahova, ROM; NA152
Niphargus decui	KF719272	KR905894	KR905822	Limanu springs, Mangalia, Dobrogea, ROM; NA154
Niphargus podgoricensis	KR905875	KR905896	KR905824	Spring at Dobro polje, Dobro polje, Podgorica, MNE; NA166
Niphargus multipennatus	KJ566700	KJ566721	KR905825	Tomačevo (intersticial waters), Ljubljana, Ljubljana, SI; NA169

Niphargus bilecanus	JQ815550	-	KR905826	Ljelješnica, Poraslica, Dabarsko polje, BIH; NA182
Niphargus karkabounasi	KR905877	KR905898	-	Agios Theodoridi, Agios Theodoridi, Korinthos, GRE; NA217
Niphargus miljeticus	KR905878	KR905899	-	Vodice, Babino polje, island of Mljet, HR; NA500
Niphargus likanus	JQ815441	JQ815498	KR905828	Jama v kamnolomu, Vinica, Črnomelj, SLO; NA523
Niphargobates orophobata	KR905879	KR905900	-	Planinska jama, Planina, SI; NA546
Niphargus balcanicus	EF617280	JQ815507	KR905796	Vjetrenica, Zavala, Popovo polje, BIH; NA070
Niphargus kusceri	JQ815443	KR905929	KR905767	Obodska pećina, Rijeka Crnojevića, Cetinje, / Njegoševa pećina, Njeguši, Kotor, MNE; NB422
Niphargus bihorensis	KF218727	KF218657	-	Meziad cave; Meziad, Pădurea Craiului, ROM; NA792
Niphargus sp. 4	KF218731	KF218731	KF218667	Vadu cave; Pădurea Craiului, ROM; NA794
Niphargus laticaudatus	KF218730	KF218659	KF218712	Ungurului cave, Şuncuiuş, Pădurea Craiului, ROM; NA902
Niphargus laticaudatus	KF218722	KF218658	KF218687	Grueţ cave, Roşia, Pădurea Craiului, ROM; NA905
Niphargus laticaudatus	KF218717	KF218660	KF218699	Corbasca cave, Sighiştel, ROM; NA909
Niphargus transsylvanicus	KF218733	-	KF218715	Osoi cave, Vârciorog, ROM; NA904
Niphargus andropus	KF218725	KF218655	-	Măgura cave , Sighiştel, ROM; NA942
Niphargus sp.3	KF218719	KF218719	KF218713	Drăcoaia cave, Sighiştel, ROM; NA943
Niphargus sp. 4	KF218716	KF218653	KF218714	Ciur Izbuc cave , Roşia, ROM; NA944

Sequences were compiled from the following studies:

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9.2 Faunistic lists of the 14 examined caves

Boldfaced species refer new species for the cave's fauna comparing with previous data. Troglobiont species are marked with '*'.

1. ABALIGETI CAVE:

Platyhelminthes, Turbellaria:

Polycelis felina (Dalyell, 1814) - 09/12/2010, main passage, 'Flórián kút', bottle trap; 08/09/2010, main passage, 20 m from entrance, in the stream; 12/01/2010, Western 1 collateral, in streaming water; 07/08/2011, main passage, 20 m from entrance, in the stream.

Mollusca, Gastropoda:

Pupilla muscorum (Linnaeus, 1758) - 26/09/2010, main passage, in the stream; 26/09/2010, main passage, from clayey sediment, fossil sample.

***Trochulus hispidus* (Linnaeus, 1758)** - 26/09/2010, main passage, in the stream.

***Truncatellina* sp.** - 26/09/2010, main passage, from clayey sediment, fossil sample.

***Clausilia* sp.** - 26/09/2010, main passage, from clayey sediment, fossil sample.

Annelida, Oligochaeta:

Eiseniella tetraedra (Savigny, 1826) - 14/01/2012, main passage, chamber before the siphon, from decaying wood.

***Dendrodrilus rubidus* (Savigny, 1826)** - 22/09/2010, in clayey sediment.

***Dendrodrilus rubidus rubidus* (Savigny, 1826) -** 22/09/2010, main passage, in clayey sediment; 14/01/2012, main passage, chamber before the siphon, from decaying wood.

***Helodrilus oculatus* Hoffmeister, 1845** - 14/01/2012, main passage, 'Nagyterem', in a small puddle on the top of a large rock, 470 m from the entrance.

Naididae sp. - 11/10/2010, main passage, in leaf litter trap placed in the siphon.

Myriapoda, Diplopoda:

Trachysphaera schmidtii Heller, 1858 - 23/11/2010, Eastern collateral, 40 m from entrance, on decaying wood; 23/11/2010, entrance of Western 2 collateral, 380 m from entrance, on lamp flora (scant moss).

Chordeumatida sp. - 23/11/2011, main passage, 'Nagyterem', 490 m from entrance, on lamp flora (scant moss).

Hungarosoma bokori Verhoeff, 1928 - 28/04/2013, main passage, upper chamber opens from 'Nagyterem', 490 m from entrance, on decaying wood.

Polydesmus collaris C. L. Koch, 1847 - 10/04/2013, Eastern collateral, 40 m from entrance. *Trogloxene.*

Chelicerata, Opiliones:

***Mitostoma chrysomelas* (Herman, 1804) -** 23/11/2010, main passage, 'Nagyterem', 490 m from entrance, on lamp flora (scant moss).

Chelicerata, Acari:

Ixodes vespertilionis Koch, 1844 - 09/12/2010, Eastern collateral, 40 m from entrance, from cave wall.

Oribatida sp. - 13/01/2011, main passage, 300 m from entrance, in epikarstic water collector bottle.

Galuminidae sp. - 23/12/2010, main passage, 20 m from entrance, in pitfall trap (with glycol).

Parasitidae sp. - 12/10/2010, Western 2 collateral, on soil.

Chelicerata, Araneae:

Meta sp. - 21/09/2010, entrance.

Nesticus cellulanus (Clerck, 1757) - 21/09/2010, entrance.

***Porrhomma convexum* (Westring, 1861)** - 21/09/2010, Abaligeti Cave, 300 m from entrance.

Crustacea, Isopoda:

***Haplophthalmus mengei* (Zaddach 1844)** - 22/09/2010, main passage, at the entrance of Western 2 collateral (380 m from entrance), on lamp flora; 25/09/2010, main passage, at the entrance of Western 2 collateral (380 m from entrance), on lamp flora; 22/09/2010, main passage, 'Karthago romjai', 370 m from entrance, on a rock; 25/09/2010, main passage, at the entrance of Western 2 collateral (380 m from entrance), on lamp flora.

Crustacea, Amphipoda:

Gammarus fossarum Koch, 1836 - 09/12/2010, main passage, 20 m from entrance, in leaf litter trap in the stream; 09/12/2010, main passage, 'Flórián-kút', stream; 12/10/2010, main passage, 'Flórián-kút', stream; 25/11/2010, main passage, 'Flórián-kút', stream; 23/12/2010, main passage, 20 m from entrance, in leaf litter trap in the stream; 23/12/2010, main passage, 380 m from entrance, in leaf litter trap in the stream; 25/11/2010, Western 2 collateral, in streaming water.

Crustacea, Decapoda:

***Astacus astacus* Linnaeus, 1758** - 10/11/2011, main passage, 50 m from the entrance, in the stream, under a flat stone.

Hexapoda, Collembola:

***Folsomia* sp. -** 14/01/2012, entrance of Western 2 collateral, on lamp flora (scant moss).

***Folsomia candida* Willem, 1902** - 14/01/2012, 40 m from entrance, from the stream.

Heteromurus nitidus (Templeton, 1836) - 22/09/2010, 230 m from entrance (after 'Gyógyterem'); 22/09/2010, Eastern collateral, 40 m from entrance; 23/12/2010, 'Nagyterem', 490 m from entrance, pitfall trap (with glycol); 14/01/2012, entrance of Western 2 collateral, on lamp flora (scant moss); 14/01/2012, 'Nagyterem', 490 m from entrance, on lamp flora; 14/01/2012, before siphon, from decaying wood.

Heteromurus/(*Verhoeffiella*?) sp. - 23/12/2010, main passage, before the siphon, 466 m from entrance, pitfall trap (with glycol).

Lepidocyrtus sp. - 25/09/2010, Eastern collateral, 40 m from entrance, baited (sausage + cheese) pitfall trap; 27/10/2010, Eastern collateral, 40 m from entrance, baited (beer) pitfall trap; 14/01/2012, entrance of Western 2 collateral, on lamp flora (scant moss).

Megalothorax sp. - 14/01/2012, entrance of Western 2 collateral, on lamp flora (scant moss).

Neelus murinus Folsom, 1896 - 14/01/2012, main passage, 40 m from entrance, from the stream.

****Pygmarrhopalites* cf. *bifidus* (Stach, 1945) -** 23/11/2010, entrance of Western 2 collateral (380 m from entrance), on a pool.

Hexapoda, Diplura:

***Campodea* sp. -** 12/10/2010, Western 2 collateral, 5. chamber, fallen into the water; 14/03/2011, Western 1 collateral, 30 m before its end; 14/03/2010, Western 1 collateral, baited (water with glucose) pitfall trap; 25/11/2010, Western 2 collateral, after 'Meseterem', fallen into the water.

Hexapoda, Ephemeroptera:

Heptageniidae sp. - 11/10/2010, main passage, after 'Gyógyterem', 240 m from entrance, from the stream, larvae.

Hexapoda, Plecoptera:

***Nemoura cinerea* (Retzius, 1783)** - 09/12/2010, main passage, 'Flórián-kút', 220 m from entrance, under rocks in the stream, larvae; 14/03/2011, main passage, siphon, 466 m from entrance, in leaf litter trap, larvae.

***Capnia bifrons* (Newmann, 1839)** - 23/12/2010, main passage, 370 m from the entrance, in leaf litter trap placed in the stream, larvae.

***Nemoura* sp.** - 23/12/2010, main passage, 370 m from the entrance, in leaf litter trap placed in the stream, larvae.

Hexapoda, Coleoptera:

***Abax parallelus* (Duftschmid, 1812)** - 24/10/2010, main passage, 40 m from entrance, in baited (beer) pitfall trap.

***Trechus pilisensis* Csiki, 1918** - 25/11/2010, Western 2 collateral, after 'Meseterem'.

Trechoblemus micros (Herbst, 1784) - 27/10/2010, main passage, 450 m from entrance, in baited (beer) pitfall trap; 23/11/2010, main passage, 450 m from entrance, in an empty pitfall trap; 14/01/2012, Amain passage, chamber before the siphon, on decaying wood.

***Choleva spadicea* (Sturm, 1839) -** 24/10/2010, main passage, 380 m from entrance, in baited (beer) pitfall trap.

***Leiodes cinnamomea* (Panzer, 1793)** - 14/01/2012, Eastern Collateral, 40 m from entrance.

***Leptinus testaceus* P.W.J. Müller, 1817 -** 07/08/2010, main passage, 20 m from entrance.

***Phosphuga atrata* (Linnaeus, 1785) -** 07/08/2010, main passage, 20 m from entrance.

Atheta spelaea (Erichson, 1839) - 25/09/2010, main passage, 100 m from entrance, in baited (sausage+cheese) pitfall trap; 22/09/2010, main passage, 150 m from entrance, on cave's floor.

Quedius mesomelinus skoraszewskyi Korge, 1961 - 23/12/2010, main passage, 450 m from entrance, in pitfall trap (with glycol); 24/10/2010, main passage, 380 m from entrance, in baited (beer) pitfall trap; 08/11/2010, main passage, 450 m from entrance, in pitfall trap (with glycol); 25/11/2010, Western 2 collateral, 'Lyukas-kő'.

Hexapoda, Lepidoptera:

Triphosa dubitata (Linnaeus, 1758) - 22/09/2010, entrance, on wall.

Hexapoda, Diptera:

Mycetophilidae sp. - 21/12/2014, beginning of Western 2 collateral, larva from the clayey wall; 14/01/2012, main passage, 'Nagyterem', in decaying wood.

2. MÁNFAI-KŐLYUK CAVE:

Platyhelminthes, Turbellaria:

Polycelis sp. (*Polycelis tothi* Méhely 1927?) - 21/10/2011, upper passage, artificial tunnel, water carrier canal, on calcite membrane in streaming water; 21/12/2011, upper passage, artificial tunnel, water carrier canal, on calcite membrane in streaming water.

Mollusca, Gastropoda:

Oxychilus sp. - 11/12/2010, upper passage, artificial tunnel, pitfall trap (with glycol).

***Oxychilus draparnaudi* (Beck, 1873)** - 22/12/2010, upper passage, artificial tunnel, pitfall trap (with glycol); 11/12/2010, upper passage, artificial tunnel, 2. pitfall trap (with glycol), 11/12/2010, upper passage, artificial tunnel, 3. pitfall trap (with glycol).

Annelida, Oligochaeta:

Eiseniella tetraedra (Savigny, 1826) - 21/10/11, upper passage, in a small lateral chamber on decaying wood.

Myriapoda, Diplopoda:

Polydesmus collaris C. L. Koch, 1847 - 11/12/2010, upper passage, artificial tunnel, pitfall trap (with glycol).

***Polydesmus complanatus* (Linnaeus, 1761) -** 20/11/2011, upper passage, beginning of the artificial tunnel.

Chelicerata, Araneae:

Linyphiidae sp. - 22/12/2010, upper passage, artificial tunnel, in a small lateral chamber on decaying wood.

Crustacea, Isopoda:

***Haplophthalmus mengei* (Zaddach 1844)** - 22/12/2010, upper passage, artificial tunnel, lateral chamber, on decaying wood.

***Cylisticus convexus* (De Geer, 1778)** - 21/10/2011, lower passage, on cave's wall.

Crustacea, Amphipoda:

Gammarus fossarum Koch, 1836 - 22/12/2010, upper passage, artificial tunnel, in leaf litter trap placed in the water carrier canal.

Hexapoda, Collembola:

***Megacyclops viridis* (Jurine, 1820)** - 22/12/2010, upper passage, beginning of artificial tunnel, in leaf litter trap placed in a pool.

***Ceratophysella denticulata* (Bagnall, 1941)** - 22/12/2010, Mánfai-kőlyuk Cave, entrance of artificial tunnel, in a pool (leaf litter trap).

Heteromurus nitidus (Templeton, 1836) - 21/10/2011, upper passage, end of artificial tunnel, on a pool; 22/12/2010, upper passage, end of artificial tunnel, pitfall trap (with glycol).

Hexapoda, Plecoptera:

***Nemoura cinerea* (Retzius, 1783)** - 22/12/2010, Mánfai-kőlyuk, upper passage, end of artificial tunnel, in leaf litter trap placed in the water carrier canal, larvae.

3. VADETETŐS CAVE:

Myriapoda, Diplopoda:

Polydesmus collaris C. L. Koch, 1847 - 08/12/2012, entrance, on decaying vegetal material.

Chelicerata, Acari:

Ixodes vespertilionis Koch, 1844 - 08/12/2010, 'Létrás-terem', from the cave wall.

Parasitidae sp. - 08/12/2010, 140 m from entrance, in pitfall trap (with glycol).

Hexapoda, Collembola:

Heteromurus nitidus (Templeton, 1836) - 08/01/2011, 80 m from entrance, pitfall trap (with glycol); 08/12/2010, between 4. and 5. halls, from water surface; 08/01/2011, 130 m from entrance, pitfall trap (with glycol).

Hexapoda, Coleoptera:

Carabus ullrichii ullrichii Germar, 1824 - 08/12/2010, 'Létrás-terem', on the wall.

Quedius mesomelinus skoraszewskyi Korge, 1961 - 08/12/2010, Vadetetős Cave, after 'Létrás-terem', on clay.

4. TRIÓ CAVE:

Nematomorpha, Gordioidea:

Gordius sp. - 15/01/2012, in the first hall, in a pool.

Myriapoda, Diplopoda:

Haasea hungarica (Verhoeff, 1928) - 29/04/2013, 210 m from the entrance, 50 m deep, in a small chamber on decaying wood.

Hexapoda, Collembola:

Folsomia candida Willem, 1902 - 15/01/2012, hall in 'Vizes-ág'.

Heteromurus nitidus (Templeton, 1836) - 05/08/2011, 'Agyagos terem', 15/01/2012, hall in 'Vizes-ág', under decaying wood; 15/01/2012, bottom of 3. pit, on a pool; 15/01/2015, bottom of 'Tamás akna', from a pool.

Megalothorax cf. *minimus* Willem, 1900 - 15/01/2012, 10 m before ending point, under decaying wood.

Megalothorax sp. - 15/01/2012, 10 m before ending point, under decaying wood.

Oncopodura crassicornis Shoebotham, 1911 - 15/01/2015, bottom of 'Tamás akna', from a pool; 15/01/2012, 10 m before ending point, under decaying wood.

Proisotoma minuta (Tullberg, 1871) - 15/01/2012, 10 m before ending point, under decaying wood.

**Pygmarrhopalites* cf. *bifidus* (Stach, 1945) - 05/08/2011, 30 m from entrance, on a pool; 15/01/2015, bottom of 'Tamás akna', from a pool; 05/08/2011, excavated crevice after 'Őrszem-terem'.

Hexapoda, Coleoptera:

Trechoblemus micros (Herbst, 1784) - 15/01/2012, chamber before 'Vizes-ág' (10 m before ending point), under decaying wood.

Aloconota sulcifrons (Stephens, 1832) - 05/08/2011, hall at the end of 'Agyagos-ág', fallen into a pool.

5. GILISZTÁS CAVE:

Nematomorpha, Gordioidea:

Gordius sp. - 30/10/2010, 20 m deep in a shallow pool, 15/01/2012.

Mollusca, Gastropoda:

Vitrea diaphana (Studer, 1820) - 24/10/2010, 15 m from entrance; 05/08/2011, Gilisztás Cave, entrance pit.

Hexapoda, Coleoptera:

Aloconota mihoki (Bernhauer, 1913) - 05/08/2011, 10 m deep, on cave's floor.

Ancistronycha erichsonii erichsonii (Bach, 1852) - 05/08/2011, 10 m deep, on cave's floor.

6. SPIRÁL CAVE:

Annelida, Oligochaeta:

Helodrilus oculatus Hoffmeister, 1845 - 23/02/2013, on clayey sediment in wet environment, 70 m deep.

7. SZAJHA-FELSŐ CAVE:

Myriapoda, Diplopoda:

Polydesmus collaris C. L. Koch, 1847 - 24/11/2010, entrance, on decaying leaves.

Hexapoda, Collembola:

Heteromurus nitidus (Templeton, 1836) - 25/09/2010, 80 m from entrance, 40 m deep, on a pool.

Hexapoda, Coleoptera:

Carabus scheidleri praescheidleri Mandl, 1965 - 09/05/2010, entrance region.

Platynus assimilis (Paykull, 1790) - 07/05/2010, 15 m from entrance.

Anoplotrupes stercorosus (Scriba, 1791) - 09/05/2010, entrance region.

Meloe violaceus Marsham, 1802 - 09/05/2010, entrance region.

8. TÖRÖKPINCE CAVE:

Nematomorpha, Gordioidea:

Gordius sp. - 21/08/2010, 15-20 m from the entrance; 24/10/2010, 30 m from the entrance, in a pool; 27/10/2010, 30 m from the entrance, in a pool.

Mollusca, Gastropoda:

Oxychilus sp. - 24/10/2010, 3 m from entrance.

Vitrea diaphana (Studer, 1820) - 24/10/2010, 15 m from entrance.

Oxychilus glaber (Rossmässler, 1835) - 21/09/2010, entrance region; 24/10/2010, 1-3 m from entrance.

Myriapoda, Chilopoda:

Lithobius forficatus (Linnaeus, 1758) - 21/08/2010, entrance region.

Myriapoda, Diplopoda:

Trachysphaera schmidtii Heller, 1858 - 4/10/2010, 86 m deep on soil.

Unciger foetidus (C. L. Koch, 1838) - 21/08/2010, entrance region.

Cylindroiulus luridus (C. L. Koch, 1847) - 21/08/2010, entrance region.

Blaniulus guttulatus (Fabricius, 1798) - 27/10/2010, 86 m from entrance, in baited (beer) pitfall trap.

Boreoiulus tenuis (Bigler, 1913) - 27/10/2010, 86 m from entrance, in baited (beer) pitfall trap.

Polydesmus collaris C. L. Koch, 1847 - 21/08/2010, entrance region.

Polydesmus complanatus (Linnaeus, 1761) - 21/08/2010, entrance; 24/10/2010, 7 m from entrance.

Chelicerata, Acari:

Ixodes vespertilionis Koch, 1844 - 21/08/2010, 40 m from entrance, from the cave wall.

Chelicerata, Acari:

Oribatida sp. - 24/10/2010, 7 m from entrance on cave's floor.

Parasitidae sp. - 24/10/2010, 3 m from entrance.

Chelicerata, Araneae:

Meta menardi (Latreille, 1804) - 21/08/2010, entrance region; 24/10/2010, 9 m from entrance.

Metallina sp. - 21/08/2010, entrance, on wall; 24/10/2010, entrance, on wall..

Nesticus cellulanus (Clerck, 1757) - 24/10/2010, entrance, on wall; 21/08/2010, entrance, on wall.

Urocoras longispinus (Kulczynski, 1897) - 24/10/2010, entrance, on wall.

Theridiidae sp. - 21/08/2010, entrance, on wall.

Crustacea, Isopoda:

Trachelipus rathkii (Brandt, 1833) - 24/10/2010, 7 m from entrance, on decaying wood.

Hexapoda, Collembola:

**Ceratophysella sp.* - 27/10/2010, 89 m from entrance, baited (beer) pitfall trap; 27/10/2010, 40 m from entrance, pitfall trap (with glycol); 07/08/2011, 30 m from entrance.

Ceratophysella denticulata (Bagnall, 1941) - 27/10/2010, 40 m from entrance, pitfall trap (with glycol).

Heteromurus nitidus (Templeton, 1836) - 27/10/2010, 89 m from entrance, baited (beer) pitfall trap; 15/01/2012, 30 m from entrance, from soil mixed with old and fresh badger guano; 14/01/2015, 8 m from entrance, on leaves.

Lepidocyrtus sp. - 14/01/2012, 8 m from entrance, on leaves.

Megalothorax sp. - 14/01/2012, 8 m from entrance, on leaves.

Hexapoda, Coleoptera:

Leptinus testaceus P.W.J. Müller, 1817 - 14/01/2012, 8 m from entrance, on leaves; 14/01/2012, 30 m from entrance, from soil mixed with old and fresh badger guano.

Phosphuga atrata (Linnaeus, 1785) - 07/08/2011, 20 m from entrance, in pitfall trap (with glycol).

Anotylus mendus Herman, 1970 - 14/01/2012, 8 m from entrance, on leaves.

Proteinus ovalis Stephens, 1834 - 24/10/2010, 3 m from entrance.

Quedius mesomelinus skoraszewskyi Korge, 1961 - 24/10/2010, 10 m from entrance, 27/10/2010, 5 m from entrance, in baited (beer) pitfall trap; 27/10/2010, 60 m from entrance; 27/10/2010, 30 m from entrance, in baited (beer) pitfall trap; 24/10/2010, 10 m from entrance; 24/10/2010, 3 m from entrance, on cave's floor.

Anoplotrupes stercorosus (Scriba, 1791) - 07/08/2011, 20 m from entrance, in pitfall trap (with glycol).

Cryptophagus nitidulus Miller, 1858 - 27/10/2010, 60 m from entrance, in baited (beer) pitfall trap; 27/10/2010, 80 m from entrance, in baited (beer) pitfall trap; 27/10/2010, 5 m from entrance, in baited (beer) pitfall trap; 07/08/2010, 20 m from entrance, in pitfall trap (with glycol).

Hexapoda, Lepidoptera:

Scoliopteryx libatrix (Linnaeus, 1758) - 21/08/2010, entrance; 23/11/2010, 12 m from entrance; 24/10/2010, 3 m from entrance.

9. RÓMAI CAVE:

Mollusca, Gastropoda:

Helicodonta obvulata (O.F. Müller, 1774) - 07/10/2010, entrance region.

Alinda biplicata (Montagu, 1803) - 07/10/2010, entrance region.

Annelida, Oligochaeta:

Aporrectodea sineporis (Omodeo, 1952) - 07/10/2010, entrance region.

Myriapoda, Chilopoda:

Lithobius validus Meinert, 1872 - 07/10/2010, 14 m deep on wall.

Chelicerata, Araneae:

Porrhomma convexum (Westring, 1861) - 23/10/2010, 19 m deep, on wall.

Hexapoda, Plecoptera:

Capnia bifrons (Newmann, 1839) - 23/12/2010, main passage, 370 m from the entrance, in leaf litter trap placed in the stream, larvae; 23/10/2010, 16 m deep in a pool, larvae.

Nemoura sp. - 23/10/2010, 16 m deep in a pool, larvae.

Hexapoda, Coleoptera:

Carabus nemoralis O.F. Müller, 1764 - 23/10/2010, 24 m deep.

Abax parallelus (Duftschmid, 1812) - 23/10/2010, 24 m deep.

Leistus rufomarginatus (Duftschmid, 1812) - 23/10/2010, 24 m deep.

Choleva angustata (Fabricius, 1781) - 23/10/2010, 24 m deep.

10. KISPAPLIKA CAVE:

Hexapoda, Coleoptera:

Haasea hungarica (Verhoeff, 1928) - 07/2010/2010, entrance pit on the wall.

Crustacea, Amphipoda:

Gammarus fossarum Koch, 1836 - 07/10/2010, entrance pit in water.

Gammarus roeseli Gervais, 1835 - 07/10/2010, entrance pit in water.

Hexapoda, Heteroptera:

Velia caprai (Tamanini, 1947) - 07/10/2010, on surface of water in entrance pit.

Hexapoda, Coleoptera:

Leistus rufomarginatus (Duftschmid, 1812) - 07/10/2010, entrance pit on water surface.

Choleva angustata (Fabricius, 1781) - 07/10/2010, entrance pit on water surface.

Proteinus ovalis Stephens, 1834 - 07/10/2010, entrance pit on water surface.

Hexapoda, Lepidoptera:

Scoliopteryx libatrix (Linnaeus, 1758) - 07/10/2010, entrance pit, on the wall.

11. NYÁRÁS-VÖLGYI CAVE:

Annelida, Oligochaeta:

Aegopinella ressmanni (Westerlund, 1883) - 23/11/2010 at the bottom of entrance pit (-10 m).

Dendrodrilus rubidus (Savigny, 1826) - 23/10/10, 14 m deep.

Myriapoda, Diplopoda:

Chordeumatida sp. - 23/11/2010, 20 m deep on humid clay.

Chelicerata, Opiliones:

Nemastoma bidentatum sparsum (Gruber & Martens, 1968) - 24/10/2010, at the bottom of the entrance pit (10 m deep).

Chelicerata, Acari:

Parasitidae sp. - 23/11/2010, 15 m deep, on soil.

Hexapoda, Collembola:

Ceratophysella denticulata (Bagnall, 1941) - 23/11/2010, 19 m deep; 14/01/2012, 25 m deep, on decaying wood and on a pool; 23/11/2010, 'Csigatemető', 13 m deep.

Deuteraphorura inermis (Tullberg, 1869) - 23/11/2010, 20 m deep on a pool; 14/01/2012, 25 m deep on decaying wood and 30 m deep from a pool.

Neelus murinus Folsom, 1896 - 14/01/2012, 10 m deep, from decaying wood.

**Pygmarrhopalites* cf. *bifidus* (Stach, 1945) - 23/11/2010, 19 m deep.

**Pygmarrhopalites* cf. *pygmaeus* (Wankel,1860) - 14/01/2012, 30 m deep, on a pool.

Hexapoda, Plecoptera:

Capnia bifrons (Newmann, 1839) - 06/11/2010, 28 m from the entrance, 16 m deep, larvae.

Nemoura sp. - 06/11/2010, 28 m from the entrance, 14 m deep, larvae.

Hexapoda, Coleoptera:

Agabus guttatus (Paykull, 1798) - 23/11/2010, 16 m deep, from a pool.

Carabus coriaceus coriaceus Linnaeus, 1759 - 23/11/2010, 10 m deep.

Quedius mesomelinus skoraszewskyi Korge, 1961 - 23/11/2010, 'Csigatemető', 13 m deep.

12. ORFŰI VÍZFŐ CAVE:

Mollusca, Gastropoda:

Oxychilus draparnaudi (Beck, 1873) - 05/08/2011, between the entrance and 'Paxit-terem', on the wall.

13. ACHILLES CAVE:

Mollusca, Gastropoda:

Perforatella incarnata (O.F. Müller, 1774) - 05/08/2011, entrance region.

14. AKÁCOS CAVE:

Mollusca, Gastropoda:

Oxychilus glaber (Rossmässler, 1835) - 12/10/2010, narrow passage after the entrance pit.

Annelida, Oligochaeta:

Dendrodrilus rubidus rubidus (Savigny, 1826) - 12/10/10, at the bottom of the entrance pit.

Chelicerata, Araneae:

Meta menardi (Latreille, 1804) - 12/10/2010, entrance pit.

Nesticus cellulanus (Clerck, 1757) - 12/10/2010, entrance pit.

Crustacea, Isopoda:

Theridiidae sp. - 12/10/2010, entrance pit.

Hexapoda, Ephemeroptera:

Plecopterina sp. - 12/10/2010, narrow passage after entrance pit, larvae.

Hexapoda, Lepidoptera:

Scoliopteryx libatrix (Linnaeus, 1758) - 12/10/2010, entrance pit.