



Biogeography and phylogenetic relations within the Dinaric subgenus *Monolistra* (*Microlistra*) (Crustacea: Isopoda: Sphaeromatidae), with a description of two new species

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A phylogenetic review of *Monolistra* (*Microlistra*), a freshwater cavernicolous subgenus of isopod crustaceans, distributed in the north-western part of the Dinaric karst, is presented. The distribution data and an identification key are provided for known taxa. Seven species are reviewed and two new species are described: *Monolistra* (*Microlistra*) **fongi** sp. nov. and *Monolistra* (*Microlistra*) **jalzici** sp. nov. *Monolistra* (*Microlistra*) *pretneri spinulosa* Sket is synonymised with the nominate subspecies because of the morphological variability in the type subspecies and the genetic uniformity of the species. Two major, geographically vicariant and morphologically different clades have been identified by molecular analysis. Low genetic differentiation within the subgenus, as well as conspicuous dorsal sculpturing of animals, indicate their apparently recent colonization of the hypogean realm. These indications are confirmed by the distribution of *Microlistra* species within the current river systems, rather than palaeo-hydrographically defined basins, as is the case of other subterranean aquatic groups of crustaceans, including other members of the genus *Monolistra*.

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INTRODUCTION

In 1929, Racovitza described *Microlistra spinosa*, an extraordinarily shaped subterranean sphaeromatid, from the collections of ‘Društvo za raziskovanje jam’ (Cave Research Society in Ljubljana). Soon afterwards *Microlistra spinosissima* was described, a species with an even more bizarre shape (Racovitza, 1929a, b). Although Racovitza recognized their affinities with the genus *Monolistra* Gerstaecker, 1856, he established a new genus *Microlistra* for both new species. He distinguished the new genus from *Monolistra* by the absence of grasping ‘pincers’ (subchelae)

in male pereopods II, but ignored their very showy, long tergal processes, anticipating their occurrence as taxonomically irrelevant. In 1930 Stammer described *Monolistra* (*Typhlosphaeroma*) *schottlaenderi* with numerous, but very short dorsal processes, but moved it to *Microlistra* in 1932, as the third species of the genus. Some additional species of *Microlistra* have been described since then, one even with an entirely smooth dorsum (Sket, 1960, 1964, 1965, 1982). Karaman (1954), Stoch (1984), Deeleman-Reinhold (1971), and Sket (1982) contributed some new taxonomic and/or distribution data, but the latest taxonomic and distribution reviews of the entire genus *Monolistra* and the related genus *Caecosphaeroma* Dollfus, 1896, were provided by Sket (1967, 1986a).

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In his review of the 'Monolistrini', Sket (1964) showed that the adequate position of Racovitza's genus *Microlistra* would be as a subgenus within the genus *Monolistra*: namely, considering morphology, *Microlistra* relates to *M. (Monolistra) s.str.*, the same way as do the subgenera *Typhlosphaeroma* Racovitza, 1910, *Pseudomonolistra* Hubault, 1937, and the newly established *Monolistrella* Sket, 1964. To avoid the identification problems in samples with no males, he proposed considering all these taxa as subgenera of *Monolistra*.

In our study, we investigate the affinities between species within the subgenus *Microlistra*, which we prove to be a monophyletic clade nested within the genus *Monolistra*. We list all known taxa within the subgenus, and investigate their phylogenetic and biogeographic relations within the 'north-western merodinaric biogeographic area' (*sensu* Sket, 1994). We use morphological characteristics to provide an easy-to-use identification key. Two new species are described. We consider this subgenus worth presenting because: it is a clearly established monophylum, with an apparent morphological diversity and high molecular homogeneity; it is biogeographically well defined as a typical north-western Dinaric element; it differs from some other cave-dwelling groups by its species being distributed within recent drainages (rather than by palaeo-drainages). We succeeded to sample nearly all taxa for DNA analysis. We do not, however, discuss the phylogenetic values of other nominal subgenera here; they will be discussed when the complete phylogenetic tree for the genus *Monolistra* is provided.

MATERIAL AND METHODS

MATERIAL

We collected all known taxa and some undescribed forms of *Microlistra* (and of other *Monolistra* groups). All localities were georeferenced (see the Appendix) and distribution maps have been produced for biogeographic studies. Most of the localities were sampled repeatedly.

With the exception of samples of *Monolistra (Microlistra) schottlaenderi* and undescribed *Monolistra (Microlistra) sp.* (cf. *sketi*) from the Rupećica spring [Croatia; referred to as *Monolistra (Microlistra) sp.* – Rupećica hereafter in the text], samples from all listed localities are stored in the invertebrate collection of Oddelek za biologijo, Biotehniška fakulteta, Univerza v Ljubljani (OB BF UL). The *Monolistra (Microlistra) sp.* – Rupećica sample is kept in the collection of Hrvatsko biospeleološko društvo (Croatian Biospeleological Society), Zagreb; a description of this possibly new species is in the domain of colleagues from Croatia. *Monolistra (Microlistra)*

schottlaenderi localities are listed in the checklist of Italian fauna (Ruffo & Stoch, 2000).

We included a total of 24 *Monolistra* specimens belonging to 12 species (Table 1) in our DNA analyses. Two species of the related genus *Caecosphaeroma* were used as out-groups. The in-group taxa were all the available *Microlistra* spp. and the type species of all other subgenera. To ensure the taxonomic identity, we mainly used specimens from topotype populations: this was not possible for *Monolistra (Microlistra) spinosissima*, and no material was available for DNA analysis for *Monolistra (Microlistra) calopyge*.

DNA EXTRACTION, AMPLIFICATION, AND SEQUENCE ANALYSIS

Genomic DNA was extracted from specimens preserved in 96% ethanol using a modified Mouse Tail protocol of the Nucleospin Tissue kit (Macherey-Nagel, Düren, Germany), as described in Verovnik *et al.* (2003). For each of the 26 specimens (including the out-group taxa) an approximately 550-bp fragment of the mitochondrial *12S* rDNA gene was amplified using the primers 5'-CCTACTTTGTTAC GACTTAT-3' and 5'-GCCAGCAGCCGCGGTTA-3', designed by comparing the available invertebrate *12S* rDNA sequences. Approximately 500 bp of the mitochondrial *16S* rDNA were amplified using the universal primers 16Sar and 16Sbr (Simon, 1991). Amplification of an approximately 1280–1420-bp-long fragment of the nuclear *28S* rRNA gene was performed using the primers 5'-AGGGAACTTCGG AGGGAACC-3' and 5'-CAAGTACCGGTGAGGGAA AGTT-3' that were designed by comparing available invertebrate *28S* rDNA sequences. Purified PCR products were sequenced on an Applied Biosystems 3730xl sequencer by Macrogen (Seoul, Korea). Sequences were aligned using MUSCLE (Edgar, 2004).

PHYLOGENETIC ANALYSIS

The *12S*, *16S*, and *28S* sequence alignments were analyzed separately using the neighbour-joining algorithm as implemented in MEGA4 (Kumar *et al.*, 2007). As all three data sets produced almost identical trees (results not shown, see below), sequences were concatenated for the final analysis.

The alignment of concatenated sequences is 2452-bp long. Sequence data were analyzed using Bayesian inference. The program MrBayes 3.1 (Ronquist & Huelsenbeck, 2003) was used. Hierarchical likelihood tests (Posada & Crandall, 1998) were employed in order to test alternative models of evolution, using MrModeltest 2.2 (Nylander, 2004).

Table 1. List of sequenced samples of *Monolistra* and *Caecosphaeroma*, with corresponding information about nucleotide sequence accession numbers (Acc. N.)

Taxon name	Designation in phylogenetic tree	Location (in short)	Acc. N. 12S	Acc. N. 16S	Acc. N. 28S
<i>Monolistra (Microlistra) spinosa</i>	spinosa T	Tominčev studenec, SLO	FJ842006	FJ842007	FJ842008
<i>Monolistra (Microlistra) spinosa</i>	spinosa Kočevje	Slovenska vas, SLO	FJ842059	FJ842060	FJ842061
<i>Monolistra (Microlistra) spinosa</i>	spinosa Ribnica	Mobišaht, SLO	FJ842029	FJ842030	FJ842031
<i>Monolistra (Microlistra) schottlaenderi</i>	Schottlaenderi	Doberdo/Doberdob, ITA	FJ842062	FJ842063	–
<i>Monolistra (Microlistra) spinosissima</i>	spinosissima Vrhnika	Retovje, SLO	FJ842035	FJ842036	FJ842037
<i>Monolistra (Microlistra) spinosissima</i>	spinosissima Vrhnika & Logatec	Gašpinova jama, SLO	FJ842026	FJ842027	FJ842028
<i>Monolistra (Microlistra) sp.</i>	sp. Rupečica	Rupečica, CRO	FJ842048	FJ842049	FJ842050
<i>Monolistra (Microlistra) sketi</i>	sketi T	Pećina špilja, CRO	FJ842045	FJ842046	FJ842047
<i>Monolistra (Microlistra) fongi</i> sp. nov.	fongi T	Kuruzovića pećina, CRO	FJ842032	FJ842033	FJ842034
<i>Monolistra (Microlistra) bolei bolei</i>	b bolei T	Stobe, SLO	FJ842012	FJ842013	FJ842014
<i>Monolistra (Microlistra) bolei brevispinosa</i>	b brevispinosa T	Vinica, SLO	FJ851101	FJ851102	–
<i>Monolistra (Microlistra) pretneri cf. spinulosa</i>	p cf spinulosa Obrovac	Kusa, CRO	FJ842043	FJ842044	–
<i>Monolistra (Microlistra) pretneri pretneri</i>	p pretneri T	Pećina kod Vrane, CRO	FJ842015	FJ842016	FJ842017
<i>Monolistra (Microlistra) pretneri cf. spinulosa</i>	p cf spinulosa Žegar	Milića špilja, CRO	FJ842051	FJ842052	–
<i>Monolistra (Microlistra) pretneri spinulosa</i>	p spinulosa T	Špilja kod mlina na Miljacki, CRO	FJ842041	FJ842042	–
<i>Monolistra (Microlistra) jalzici</i> sp. nov.	jalzici T	Čepić tunnel, CRO	FJ842009	FJ842010	FJ842011
<i>Monolistra (Typhlosphaeroma) racovitzai racovitzai</i>	r racovitzai Postojna	Postojnska jama, SLO	FJ842023	FJ842024	FJ842025
<i>Monolistra (Monolistra) caeca caeca</i>	c caeca T	Podpeška jama, SLO	FJ842053	FJ842054	FJ842055
<i>Monolistra (Pseudomonolistra) h. hercegoviniensis</i>	h hercegoviniensis T	Vjetrenica, BiH	FJ842038	FJ842039	FJ842040
<i>Monolistra (Monolistrella) velkovrhi</i>	velkovrhi T	Stobe, SLO	FJ842018	FJ842019	FJ842020
<i>Caecosphaeroma virei</i> Dollfus	<i>C. virei</i> FRA-Doubs	Moullin des Iles, FRA	FJ842021	FJ842022	–
<i>Caecosphaeroma b. burgundum</i> Dollfus	<i>C. burgundum</i> FRA-Moselle	Gorze, FRA	FJ842056	FJ842057	FJ842058

Sequence names are identical to the ones depicted on the phylogenetic tree: ‘T’ with taxon designation, denotes topotypic population; BiH, Bosnia and Herzegovina; CRO, Croatia; FRA, France; ITA, Italy; SLO, Slovenia; for the additional data and for a list of all localities see the Appendix.

A general time reversible (GTR) model of nucleotide substitution, with gamma-distributed rate heterogeneity and a significant proportion of invariable sites, was selected. Uniform or fixed default prior settings were used. A Markov chain Monte Carlo search was run with four chains for 4×10^6 generations, taking samples every 100 generations. The approximate number of generations needed to obtain stationarity of the likelihood values ('burn-in') of the sampled trees was estimated graphically, and was set to 5000. From the resulting trees, posterior probabilities were assessed for individual clades based on their observed frequencies.

MORPHOLOGICAL EXAMINATION AND DESCRIPTION OF NEW SPECIES

Specimens preserved in 70% ethanol were transferred to glycerol, partly dissected, and the appendages were mounted in glycerol on slides, for examination and drawing. Measurements were made under an Olympus SZX12 stereoscope equipped with a Sony® DXC-390P colour video camera, by means of the Windows-supported program analySIS®. Original drawings were made with a camera lucida. The appendages were transferred from the slides and deposited in 70% ethanol, together with the specimens. For SEM, the whole specimen was dehydrated in a graded ethanol series, followed by acetone and 1,1,1,3,3,3-hexamethyldisilazane (HMDS), coated with gold, and then observed under a scanning electron microscope (JEOL® JSM-840A).

The terminology of setae (non-cuticular structures, articulating), spines (cuticular structures, articulating), setules (cuticular in origin, being derived from cuticular scales), and setulose fringe (a dense mass of setules on the posterior margin of the pereopod articles), on the appendages, follows that of Bruce (1994). Particular terms have been coined to describe the dorsal sculpturing of the species. The prominent large structures on tergites and pleotelson are referred to as processes, which may be 'conical', 'sickle-shaped', 'spine-like', and other. Small dorsal embossments on dorsum are termed nodules, which may be 'rounded', 'conical', or of another shape.

USE OF TAXONOMIC CATEGORIES

We tried to implement the 'biological species concept' and 'polytypic species concept' of Mayr & Ashlock (1991) and Mayden (1997), taking into consideration the cogent arguments of the authors. As all studied taxa at the rank of species are allopatric, there is no direct evidence about their interfertility/intersterility. Their generally low molecular differences, however,

do not indicate clear reproductive isolation. On the other hand, the evident differences in dorsal sculpturing can certainly represent effective pre-mating barriers. As we suppose that populations with low morphological differences (like *Monolistra (Microlistra) bolei bolei* and *Monolistra (Microlistra) bolei brevispinosa*) might potentially be interfertile, we consider such populations as morphologically distinct subspecies.

Out of convenience we also use the subgenus category. From the phylogenetic point of view, there is no 'natural' criterion for the distinction of categories. Also, the ICZN (1999) provides no criteria. It is, however, very useful for the user of the classification (an ecologist, faunist, etc.) to be able to identify the lowest possible taxonomic rank for collected biota. So, even though in samples containing only females, specimens could not be identified to the species level, they could at least be identified to the genus level (as *Monolistra* sp., containing ~40 species). On the other hand, splitting of the genus *Monolistra* into five new genera (the present subgenera) would result in the identification of females as 'Sphaeromatidae gen. sp.', i.e. as one of the innumerable and ecologically diverse species.

RESULTS

REVIEW OF SPECIES AND SUBSPECIES

Within the subgenus *Microlistra* seven species with two additional subspecies have already been described. Two new species are described herein, whereas some further taxa have not yet been studied in detail.

Genus *Monolistra* Gerstaecker, 1856

Subgenus *Microlistra* Racovitza, 1929

Diagnosis: A *Monolistra* group of species with body smaller in males than in females. Male pereopod II without subchela, its distal articles only slightly shortened and swollen; mature male pleopod II with appendix masculina as long as endopodite or longer, strongly curved in its distal part; pleopod IV with large area respirans ('aire respiratoire' of Racovitza, respiratory area hereafter in the text); uropod reduced to tiny tubercle on a shallow elevation ('socle') of pleotelson surface; pleotelson caudal bulge projecting beyond the ventrocaudal border.

Remarks: Within the genus *Monolistra*, most of the characters described above are diagnostic (Sket, 1965). In the secondary characters of sexual dimorphism (size relationships, development of male pereopods II), specimens of the subgenus *Microlistra* resemble, to some degree, specimens of the subgenera

Pseudomonolistra and *Monolistrella*. In both later subgenera, however, males are only slightly smaller or are of equal size as females, and in *Monolistrella* male pereopods are more differentiated. Apomorphic for *Microlistra*, and probably unique within the family, are the specifically positioned and, to differing extents, reduced uropods.

Type species, by original designation: Microlistra spinosa Racovitza, 1929.

Other taxa: Monolistra (Microlistra) bolei bolei Sket, 1960; *Monolistra (Microlistra) bolei brevispinosa* Sket, 1982; *Monolistra (Microlistra) fongi* sp. nov.; *Monolistra (Microlistra) jalzici* sp. nov.; *Monolistra (Microlistra) pretneri pretneri* Sket, 1964; *Monolistra (Microlistra) pretneri spinulosa* Sket, 1965; *Monolistra (Microlistra) schottlaenderi schottlaenderi* (Stammer, 1930); *Monolistra (Microlistra) schottlaenderi* ssp. (Trieste/Trst) Stoch, 2000; *Monolistra (Microlistra) sketi* (Deeleman, 1971); *Monolistra (Microlistra) sp.* (cf. *sketi*) (Rupećica) Bedek; *Monolistra (Microlistra) spinosissima* (Racovitza, 1929).

Besides the characters mentioned above, which are not present in females, the sculpturing of tergites is characteristic for the subgenus, and distinguishes between species and subspecies. As a rule, the head and pereonal tergites are armed with transversal rows of up to nine nodules or spine-like processes, and, in the majority of species and subspecies, such rugosities are also present on the pleotelson. In some species, some of the nodules are absent (probably reduced), whereas in others they are elongated into processes, the length of which may reach the trunk width. The position of processes differs among species. The most stable is the position of both processes placed just in front of the vestigial uropods.

The only exception from these trends is the dwarfish (~6-mm long) *Monolistra (Microlistra) calopyge*, which has a smooth tergum, a dorsally smooth pleotelson, and uropods that do not jut out. On the other hand, its caudal bulge on the pleotelson is particularly apparent (projecting). As molecular data are not available, the position of this species remains ambiguous, but its classification to the subgenus was evidently erroneous (see below). Some specimens of the morphologically variable *Monolistra (Microlistra) pretneri* are also nearly smooth, but with uropods of the *Microlistra*-type.

DISTRIBUTION

The members of the genus *Monolistra* are distributed in the European continental karst groundwaters: along the Southern Calcareous Alps and Dinarides in northern Italy, southern Switzerland, Slovenia,

Croatia, Bosnia and Herzegovina, and Montenegro, i.e. between Como (Italy) and Podgorica (Montenegro). The troglotic members of the genus *Caeosphaeroma*, with two species and a subspecies, are West European (Sket, 1986a). Another sphaeromatid genus with troglotic species is the 'antipodean' *Bilistra* Sket & Bruce, 2004, from New Zealand. Other Sphaeromatidae are epigeal, and are entirely or predominantly marine, with the exception of the thermophilic genus *Thermosphaeroma* Cole & Bane, 1978, from the southern North America.

The distribution of the members of the subgenus *Microlistra* (see the exact localities in the Appendix) is limited to the so-called north-western Dinaric area (Sket, 1994). Species are known from approximately 40 localities, spread from the extreme east of Italy, through southern (Dinaric) Slovenia, to south-western Croatia. The most south-eastern localities are along the Dalmatian river Krka north of Šibenik, the most northern localities are along the Slovenian river Krka, and in the western distribution area they are found in the Italian part of the region Kras/Carso. The largest extension of the known distribution area is approximately 300 km. All species inhabit fresh karst-groundwater, and may sometimes be sampled in springs or resurgences.

1. *Monolistra (Microlistra) bolei* Sket, 1960

Distribution: Localities of both subspecies within the river Kolpa/Kupa drainage.

2. *Monolistra (Microlistra) bolei bolei* Sket, 1960

Distribution: South-eastern Slovenia, Bela Krajina, Črnomelj–Otovec.

3. *Monolistra (Microlistra) bolei brevispinosa* Sket, 1982

Distribution: South-eastern Slovenia, Bela Krajina; Dragatuš–Vinica, south of the type subspecies.

4. *Monolistra (Microlistra) fongi* sp. nov.

Distribution: Croatia, Kordun. Probably the river Korana drainage.

Remarks: See below for the description of the species.

5. *Monolistra (Microlistra) jalzici* sp. nov.

Distribution: Croatia, south-eastern Istra (Istria). Drainage of the brook Boljunščica, close to the Adriatic.

6. *Monolistra (Microlistra) pretneri* Sket, 1964

IDENTIFICATION KEY

Species and subspecies of *Microlistra* are easily distinguished by their dorsal ornamentation.

1. Caudal pleotelson bulge longer than 45% total ventral pleotelson length or 75% pleotelson height; tergites smooth; pleopods without respiratory areas.....*Monolistra* (?*Microlistra*) *calopyge*
- 1.* Length of caudal pleotelson bulge (spine-like processes not included) shorter than 30% of total ventral pleotelson length, and never surpassing half pleotelson height.....2
2. Pleotelson without a pair of terminal caudal processes; its proximal lateral processes, if developed, short and thick, blunt.....3
- 2.* Pleotelson with pairs of narrowly pointed lateral and caudal processes.....5
3. Along the pereon, a median, and a pair of lateral rows of short and thick, setulose processes, nodules between them much smaller.....*Monolistra* (*Microlistra*) *jalzici* sp. nov.
- 3.* On pereonites, only ± equal nodules in transversal lines, the lateral ones at the most twice as long as the others (exceptionally with smooth body).....4
4. Pleotelson dorsocaudal bulge tapering in caudal direction, with a narrow apex; male pleopod II endopodite with ~20 marginal plumose setae.....*Monolistra* (*Microlistra*) *schottlaenderi*
- 4.* Pleotelson dorsocaudal bulge broadly rounded apically; male pleopod II endopodite with ~15 marginal plumose setae.....*Monolistra* (*Microlistra*) *pretneri*
5. Anterior pereonites without sculptures; pleotelson without a median process.....6
- 5.* Some anterior pereonites with spine-like processes.....7
6. Pereonite VI with a lateral pair of short, sickle-shaped processes; two pairs of similar processes on pleotelson a little longer than wide at base.....*Monolistra* (*Microlistra*) *sketi*
- 6.* Pereon without any processes, pleonal processes longer.....*Monolistra* (*Microlistra*) sp. – Rupećica
7. Pleotelson without a median, only with paired sickle-shaped processes; lateral sickle-shaped processes on pereonites I–III and V–VI, *Monolistra* (*Microlistra*) *bolei*.....8
- 7.* Pleotelson with two pairs of processes plus a median sickle-shaped process; lateral sickle-shaped processes on pereonites I, II, IV, and VI.....9
8. Processes longer, tips of lateral pleonal ones reaching the ventrocaudal border.....*Monolistra* (*Microlistra*) *bolei bolei*
- 8.* Processes shorter, tips of lateral pleonal ones (in adults) not reaching ventrocaudal border.....*Monolistra* (*Microlistra*) *bolei brevispinosa*
9. Pereonal lateral processes shorter than distances between their bases....*Monolistra* (*Microlistra*) *fongi* sp. nov.
- 9.* Pereonal lateral processes longer than distances between their bases.....10
10. Pleotelson with only five major processes; processes strongly curved.....*Monolistra* (*Microlistra*) *spinosa*
- 10.* Besides five major processes, also some small processes on pleotelson; processes less curved and longer.....*Monolistra* (*Microlistra*) *spinosissima*

Syn. *Monolistra* (*Microlistra*) *pretneri spinulosa* Sket, 1965, new synonymy

Distribution: Croatia, northern Dalmacija–Kvarner (Quarnero). Drainages of the (Dalmatian) rivers Krka, Zrmanja, and the lake Vransko jezero; southern tip of Cres Island.

Remarks: Specimens of the type population (cave Pećina, Vrana, Zadar) are dorsally either completely smooth or of the *spinulosa* type. All other populations are of the *spinulosa* type, and the one with the most prominent, cone-shaped spines (cave Kusa, Obrovac) is molecularly closest to the type population. Bearing in mind the morphological variability, we do not consider the named subspecies to be valid.

7. *Monolistra* (*Microlistra*) *schottlaenderi* (Stammer, 1930)

Monolistra (*Microlistra*) *schottlaenderi schottlaenderi* (Stammer, 1930)

Syn. *Monolistra* (*Typhlosphaeroma*) *schottlaenderi* Stammer, 1930

Microlistra schottlaenderi Stammer, 1932

Distribution: North-eastern Italy, Carso/Kras. Springs and caves north-east and south-east of Monfalcone. All localities are parts of a coastal karst aquifer at the Adriatic.

8. *Monolistra* (*Microlistra*) *schottlaenderi* ssp. (Trieste/Trst) Stoch, 2000

Distribution: North-eastern Italy, Kras/Carso. Spring Fonte Oppia/Klinčica, Bagnoli/Boljunc, Trieste/Trst. Probably a hydrographically separated part of the coastal aquifer.

Remarks: Only one specimen found (F. Stoch, pers. comm.), not yet studied.

9. *Monolistra (Microlistra) sketi* (Deeleman-Reinhold, 1971)

Syn. *Microlistra sketi* Deeleman-Reinhold, 1971

Distribution: Croatia, Lika. Drainage of the river Gacka.

10. *Monolistra (Microlistra) sp.* (cf. *sketi*) – Rupećica

Distribution: Croatia, Kordun. Rupećica spring, Ivanac, Ogulin.

Remarks: Molecularly almost identical to *M. (Microlistra) sketi*; morphologically similar to it, but nevertheless distinct, without any projections on pereon, and with longer anterior pleonal processes. Collected by B. Jalžić. Headwaters of the river Dobra.

11. *Monolistra (Microlistra) spinosa* (Racovitza, 1929)

Syn. *Microlistra spinosa* Racovitza, 1929

Distribution: South-eastern Slovenia. Springs and caves in a belt along the river Krka (Dolenjska Krka).

12. *Monolistra (Microlistra) spinosa* ssp. – Kočevje-Ribnica

Distribution: Southern Slovenia. Two localities within a syncline, parallel with the river Krka (Dolenjska Krka), but 15 km from it: both probably draining separately towards the river.

Remarks: Morphologically very similar, and molecularly nearly identical with *M. spinosa*, but with slightly longer and less curved spines.

13. *Monolistra (Microlistra) spinosissima* (Racovitza, 1929)

Syn. *Microlistra spinosissima* Racovitza, 1929

Monolistra spinosa spinosissima S. Karaman, 1954

Distribution: South-western Slovenia. An aquifer including the subterranean parts of the river Ljubljanica between Postojna and Vrhnika.

14. *Monolistra (?Microlistra) calopyge* Sket, 1982

Distribution: South-eastern Slovenia. Boreholes in Družinska vas, near Kronovo, Novo mesto. One locality in the drainage of the river Krka (Dolenjska Krka); hypothermic water (16–18 °C).

Remarks: Because of the complete absence of sculpturing, the reduction of pleopod respiratory areas, and very short article 6 of male pereopod II, the

Table 2. List of species co-occurring with *Monolistra (Microlistra)*

<i>Monolistra (Microlistra)</i> species	Co-occurring <i>Monolistra</i> species
<i>M. (Microlistra) bolei brevispinosa</i>	<i>M. (Monolistra) caeca</i> Gerstaecker
<i>M. (Microlistra) bolei bolei</i>	<i>M. (Monolistrella) velkovrhi</i> Sket
<i>M. (Microlistra) fongi</i> sp. nov.	<i>M. (Monolistrella)</i> sp. and <i>M. (Monolistra) caeca</i> Gerstaecker
<i>M. (Microlistra) schottlaenderi</i>	<i>M. (Typhlosphaeroma) racovitzae</i> Stammer
<i>M. (Microlistra) spinosa</i>	<i>M. (Monolistra) caeca</i> Gerstaecker
<i>M. (Microlistra) spinosa</i> ssp. (Kočevje–Ribnica)	<i>M. (Monolistra) caeca</i> Gerstaecker
<i>M. (Microlistra) spinosissima</i>	<i>M. (Typhlosphaeroma) racovitzae</i> Stammer
<i>M. (?Microlistra) calopyge</i>	<i>M. (Typhlosphaeroma) karamani</i> Sket
<i>M. (Microlistra) sketi</i>	None
<i>M. (Microlistra) sp.</i> -Rupećica	None
<i>M. (Microlistra) pretneri</i>	None
<i>M. (Microlistra) jalzici</i> sp. nov.	None

assignment to the subgenus is doubtful. No specimen could be obtained recently for DNA analysis.

CO-OCCURRENCE

All *Microlistra* species within Slovenia and Italy were found to share the same locality with (at least) one *Monolistra* species from another phyletic group (Table 2). This was surprisingly not the case in most (with one exception) Croatian species or localities. On the other hand, all *Microlistra* species are strictly allopatric. Also, the co-occurrence of species from other subgenera is extremely rare (Sket, 1965).

PHYLOGENETIC RELATIONSHIPS

In addition to the tree presented here (Fig. 1), we calculated phylogenies including 75 *Monolistra s.l.* samples, both *Caecosphaeroma* spp., and some marine Sphaeromatidae. The result corroborated the position of *Caecosphaeroma* as a sister clade to *Monolistra s.l.* To investigate the phylogeny of just the species within the *Microlistra* clade, we used a smaller set of taxa. The phylogeny of *Monolistra s.l.* is not yet ready for publication, as the samples of some important species are still missing.

Of 24 examined specimens of the genus *Monolistr*, we found 21 with differences in the concatenated alignment. Of 768 (31.2%) variable characters, 318 (12.9%) were parsimony informative. The obtained Bayesian tree (Fig. 1) is highly resolved, with high posterior probability support values. The monophyly of the genus *Monolistr* received high support, but the relationships among species belonging to different subgenera (*sensu* Sket, 1965) remains unresolved, possibly because of insufficient sampling in other sub-

genera. The subgenus *Microlistra* is monophyletic and distinctly separated into two main clades: one including *M. Microlistra pretneri* and *Monolistr (Microlistra) jalzici* sp. nov., and the other including the type species *Monolistr (Microlistra) spinosa* with all other species.

Only two differences exist between the single gene trees (data not shown) and the Bayesian tree (Fig. 1), both with low bootstrap support: *Monolistr (Microlistra) jalzici* forms a separate basal *Microlistra*

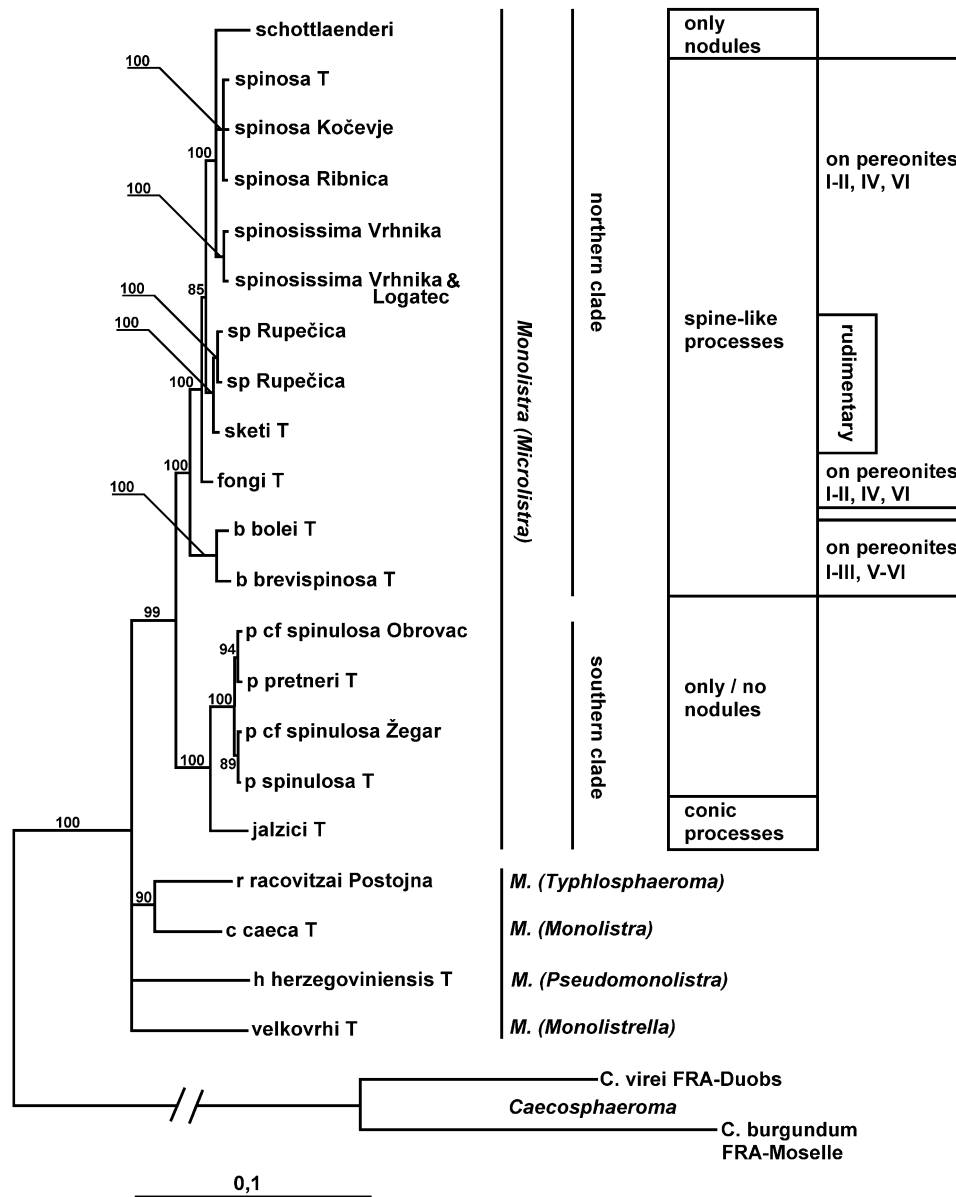


Figure 1. Phylogenetic relationships of *Monolistr (Microlistra)* species derived from 35 000 Bayesian trees in comparison to their tergal sculpturing. Values on major branches are Bayesian posterior probabilities. The concatenated sequences of 12S, 16S and 28S rDNA are named as in Table 1: b = *bolei*; c = *caeca*; h = *herzegoviniensis*; p = *pretneri*; r = *racovitjai*; M. = *Monolistr*. ‘T’ after the taxon name denotes that specimen was collected at type locality.

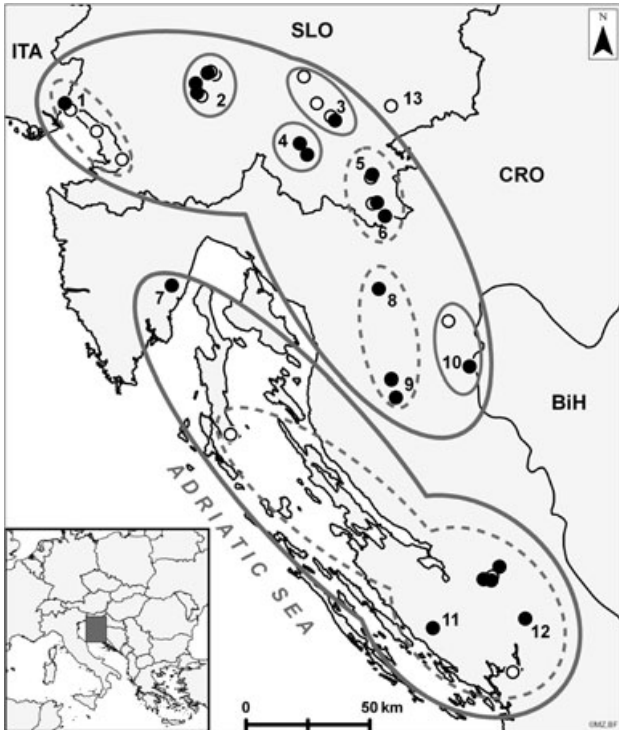


Figure 2. Geographical distribution of species of *Monolistra (Microlistra)*. Black circles denote localities of sequenced samples, the others are open. Solid lines encircle the southern and the northern clade, respectively; smaller ovals encircle single uniform species (solid lines) or pairs of closely related species or subspecies (dashed lines). Numbers are close to the type localities (see text) and denote: *M.* – *Monolistra (Microlistra)*, 1 – *M. schottlaenderi*, 2 – *M. spinosissima*, 3 – *M. spinosa*, 4 – *M. spinosa* spp., 5 – *M. bolei bolei*, 6 – *M. bolei brevispinosa*, 7 – *M. jalzici*, 8 – *M. sp.-Rupećica*, 9 – *M. sketi*, 10 – *M. fongi*, 11 – *M. pretneri*, 12 – *M. pretneri* ‘*spinulosa*’, 13 – *M. calopyge*; BiH, CRO, ITA, SLO, – Bosnia-Herzegovina, Croatia, Italy, Slovenia.

clade in the 12S tree, and *Monolistra (Microlistra) fongi* sp. nov. is the sister taxon to the *Monolistra (Microlistra)* sp. – Rupećica clade in the 16S tree.

The distinction of subspecies in *Monolistra (Microlistra) pretneri* is not supported, as the sequences of both morphotypes are almost identical. The assignment of the insular (Cres Island) population to the mentioned species could not be verified because of the lack of an appropriate sample. *Monolistra (Microlistra) jalzici* sp. nov. is positioned as a sister taxon to *Monolistra (Microlistra) pretneri* in our molecular analysis. This is in accordance with the (southern) distribution of both species along the Dinaric syncline, and across the gulf of Kvarner (Quarnero), which was submerged in the Pleistocene (Marković-Marjanović, 1971; Sket, 1988).

In the northern clade (Fig. 2), the expected sister relationship between the two species with longest processes (*Monolistra (Microlistra) spinosa* and *Monolistra (Microlistra) spinosissima*) is confirmed. The unnamed variety of *Monolistra (Microlistra) spinosa* from the cave Mobi šaht near Ribnica (morphologically between both species mentioned above) is nearly identical to *Monolistra (Microlistra) spinosa* from the type locality according to our molecular analysis: they both occur in two parallel valleys ~15 km apart. The *Monolistra (Microlistra)* sp. – Rupećica species group and the newly described *M. (Microlistra) fongi* sp. nov. are positioned as sister groups to the type-species group, but their geographic distribution does not correspond entirely with the phylogenetic relationships. Namely, they geographically encircle the *Monolistra (Microlistra) bolei* species group positioned basally within the northern clade. Despite geographic relations and morphological similarities with the southern clade, *Monolistra (Microlistra) schottlaenderi* clearly belongs to the northern *spinosa*–*spinosissima* clade in our molecular phylogeny.

Both newly described species are genetically distinct from all the rest, and their separation is confirmed by high posterior probabilities. *Monolistra (Microlistra) pretneri*, the sister species of *Monolistra (Microlistra) jalzici* sp. nov., was separated by an average Kimura’s two-parameter (K2P) genetic distance of 3.4%. The sister relationship of *Monolistra (Microlistra) fongi* sp. nov. is less clear, however, as its position as a sister clade to the clade including *Monolistra (Microlistra) sketi*, *Monolistra (Microlistra) spinosa*, and *Monolistra (Microlistra) spinosissima*, is poorly resolved. The average K2P genetic distance between these species and *Monolistra (Microlistra) fongi* sp. nov. was 1%.

Based on the two available molecular clock calibrations for 16S rDNA in Malacostraca (Crustacea) (Sturmbauer, Levinton & Christy, 1996; Schubart, Diesel & Hedges, 1998), the estimated time of the split between the subgenus *Microlistra* and the rest of the genus was 1.61 ± 0.35 or 2.23 ± 0.48 Mya, and the split between the northern and the southern clades of *Microlistra* dates to 1.11 ± 0.17 or 1.54 ± 0.23 Mya. The timing of the final speciation events could be represented by the split between the morphologically very different, and geographically and hydrographically unrelated, species *Monolistra (Microlistra) schottlaenderi* and its sister clade *Monolistra (Microlistra) spinosa* – *Monolistra (Microlistra) spinosissima*, which dates to 0.55 ± 0.25 or 0.76 ± 0.35 Mya.

DESCRIPTIONS OF THE NEW SPECIES

Genus *Monolistra* Gerstaecker, 1856

Subgenus *Microlistra* Racovitza, 1929

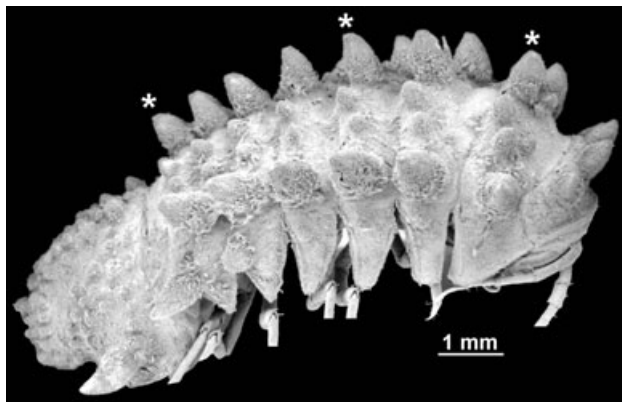


Figure 3. *Monolistra (Microlistra) jalzici* sp. nov. tunnel Čepić, Istra, Croatia, female 11.6 mm, oblique-lateral view (SEM JEOL JSM-840A by K. Drašlar). * – *, median row of dorsal spines.

***MONOLISTRA (MICROLISTRA) JALZICI* SP. NOV.**
(FIGS 3–5)

Holotype: Adult male, 7.9 mm, Croatia, Istra Peninsula, springs in the drainage tunnel Čepić, near Kršan, Labin; leg. B. Sket and S. Legović. Inv. No. 'Malacostraca 2240 ht', partly dissected, preserved in ethanol, deposited in the collection of the OB BF UL.

Paratypes: Same data as holotype. Three adult females (one ovigerous, two non-ovigerous, 11.6–6.4 mm). Collection of OB BF UL Inv. No. Malacostraca 2240 pt.

One paratype deposited in the Hrvatski prirodoslovni muzej (Croatian Natural History Museum), Zagreb, Croatia.

Etymology: The species is named after our good colleague Branko Jalzić, a deserving Croatian caver and cave-fauna researcher.

Diagnosis: *Microlistra* species with short, but stout, densely setulose massive cones on its dorsal surface. Pleotelson irregularly nodular, with large lateral protuberances and uropod rudiments elevated on small elevations ('socles'), projecting over pleotelson outline (Fig. 4). Anterior coxae truncated; posterior ones blunt and slightly turned up distally.

Description of holotype: Male, 7.9-mm long. Body width 44% of body length. Head (cephalothorax) dorsally with transverse row of four conical processes. Pereonites I–VI each with one median and two lateral conical processes. Pereonite I with three, and pereonites II–VI each with two conical paramedian nodules on each side; protuberances on epimeral apex larger, gradually increasing in size from epimeron II–VI;

protuberances on epimeron V–VI similar to median and lateral conical processes. Pereonite VII without lateral and epimeral processes. Pleonite I without lateral and epimeral nodules. Pleotelson width 150% of pleotelson length, highly vaulted, and its surface densely and irregularly covered with conical and rounded nodules. Only paired lateral conical processes (resembling those on epimeron VI) protruding remarkably over pleotelson outline, and followed by raised socles carrying uropod rudiments. Pleotelson ventro-caudal border without a groove, with a comparatively narrow dorsocaudal bulge reaching far beyond it. All large dorsal protuberances covered with thick mat of very long setules, except at the very base and apex.

Antenna I length 32% of body length; peduncle article 1 bent perpendicularly; 6 flagellar articles, articles 3–6 each with single, long aestethasc. Antenna II length about 40% of body length; 11 flagellar articles. Length ratio of peduncular articles and flagellum in antenna I 100 : 73 : 80 : 135, in antenna II, 68 : 100 : 123 : 191 : 653.

Left mandible stout, incisor and lacinia mobilis both unicuspidate, bluntly rounded (spatulate); apical spine row of 13 spiniform processes on a long stalk; molar process with prominent serrations around smooth mesial surface. Right mandible without lacinia mobilis. Other mouth appendages and maxilliped as in type species, *Monolistra (Monolistra) caeca* Garstaecker (Racovitza, 1910).

Pereopod I propodus (article 6) width 36% of propodus length; merus (article 4) with two serrate spines at anterodistal angle; carpus (article 5) with two biserrate spines at posterodistal angle; propodus with three biserrate spines on posterior margin and two at posterodistal angle; setulose fringe near continuous on posterior margin of basis (article 2) to beginning of unguis (distal part of article 7, claw); setules lengths on ischium (article 3) about 50% of article width, diminishing towards unguis; setulose fringe also present on anterior margin of basis to merus, length of setules increasing towards merus; secondary unguis finely serrate. Pereopod II basis with three long plumose setae on anterior margin; merus with two serrate spines at anterodistal angle; carpus with one biserrate spine at posterodistal angle, and one on distal margin; propodus with two short serrate spines on posterior margin, one serrate spine at posterodistal angle, and one long plumose seta at anterodistal angle; setulose fringe present on posterior margin of distal third of ischium to beginning of unguis, nearly continuous on anterior margin of basis to unguis; secondary unguis finely serrate. Pereopod VII basis with one long plumose seta on anterior margin; merus with two serrate spines at posterodistal angle; carpus with one serrate spine at posterodistal angle, one biserrate spine at

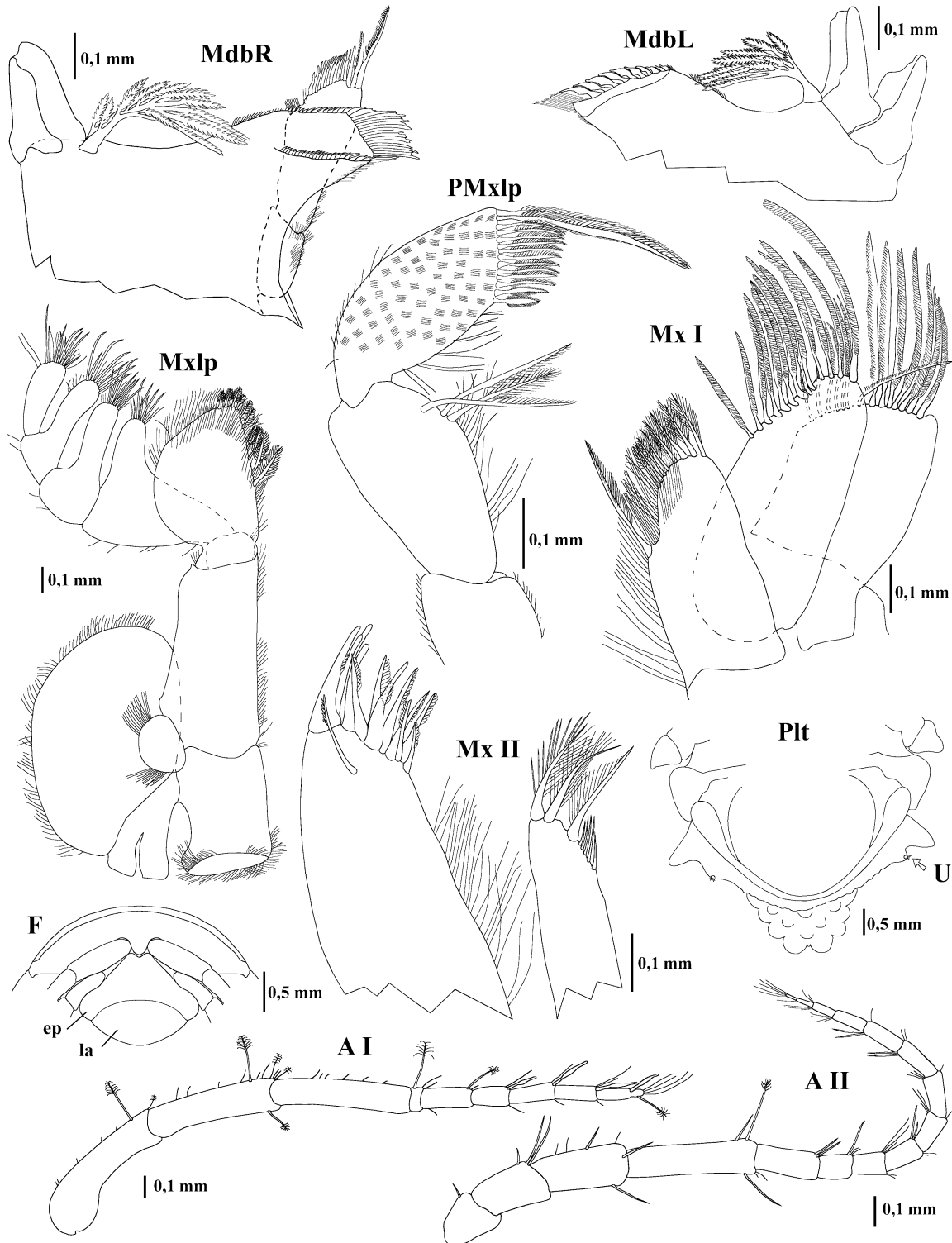


Figure 4. *Monolistra (Microlistra) jalzici* sp. nov., tunnel Čepić, Istra, Croatia, dissected ovigerous female: MdbL, left mandible (apical part); MdbR, right mandible with palp (apical parts); Mx I, II, maxilla I and II (apical parts); Mxlp, maxilliped; PMxlp, maxilliped palp. Holotype male, 7.9 mm: A I, II, antennae I and II; F, frons: with ep, epistome and la, labrum; Plt, pleotelson, ventral view: with U, uropod rudiment on its socle.

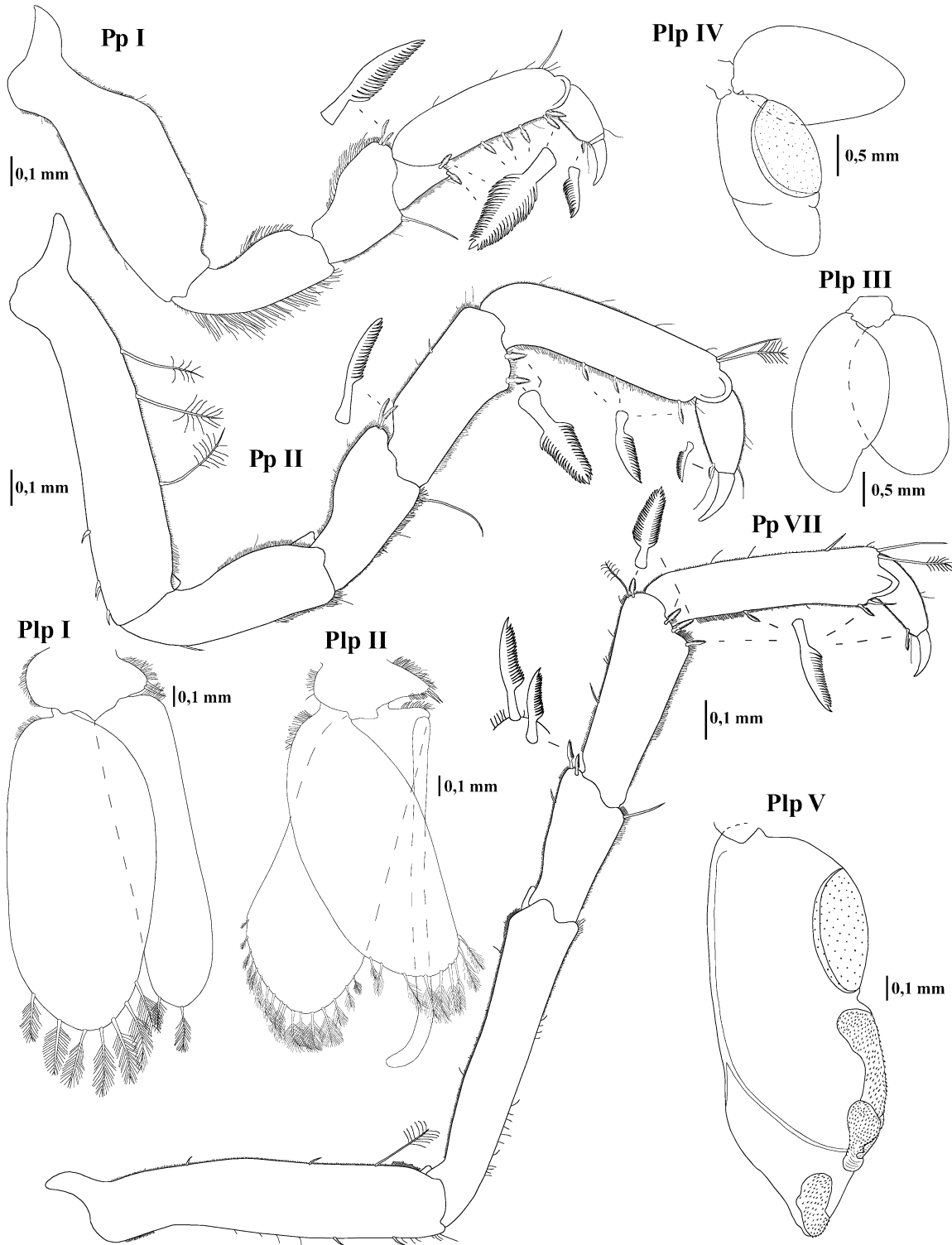


Figure 5. *Monolistra (Microlistra) jalzici* sp. nov., tunnel Čepić, Istra, Croatia, holotype male, 7.9 mm: Plp I–V, pleopods I–V; Pp I, II, VII, pereopods I, II, VII.

anterodistal angle, and two on distal margin; propodus with one serrate spine on posterior margin, one at posterodistal angle, and one long plumose seta at anterodistal angle; setulose fringe as in pereopod II, except setules shorter; secondary unguis finely serrate. Relative lengths of pereopods I, II, and VII: 33, 41, 48% of body length; length ratio of articles (coxa excluded) in pereopod I 100 : 49 : 31 : 19 : 54 : 40, in pereopod II 100 : 51 : 32 : 48 : 53 : 38 in pereopod VII 100 : 91 : 34 : 53 : 63 : 33.

Pleopod I protopodite with fine long setules along internal and external margins, and two strong spines at internal angle; exopodite elliptical, with 7 plumose setae along terminal margin, and setules at proximo-external angle; endopodite about half as wide and nearly as long as exopodite, parallel sided, with two plumose setae on rounded terminal margin and setules at proximo-internal angle. Pleopod II similar to I, but with more numerous plumose setae; endopodite as wide as exopodite, slightly widened distally, with 17 setae; appendix masculina distally curved and terminally blunt, its length 120% of endopodite length; exopodite with 9 plumose setae. Pleopod III exopodite elongate, subovoid, without respiratory area, with short transverse suture at the external margin, and with a trace of the same suture at the internal margin. Pleopod IV shape similar; respiratory area length 55% of exopodite length, surface 35% of exopodite surface. Pleopod V exopodite irregularly elliptical with thick sclerotized ridge along proximal half of external margin; three patches on intero-distal half densely scaled; respiratory area on intero-proximal half, its length 30% of exopodite length. Uropods vestigial, as in the subgenus type species *Monolistra (Microlistra) spinosa* Racovitza (1929).

Description of paratypes: Females of 11.6, 8.2, and 6.4 mm in length; larger than male if adult; body width 67% of body length. Pleotelson dorsocaudal vault surpassing ventrocaudal pleotelson border to greater extent than in male.

Antenna I length 29–39% of body length; of between six and nine flagellar articles, articles 4–6 and 8, or 3–5 and 7, each with single, long aestethasc. Antenna II length 38–43% of body length, flagellum of between 10 and 12 articles.

Pereopod I spines and setulose fringe as in male, but propodus with between three and five biserrate spines on posterior margin, and two at posterodistal angle. Pereopod II spines and setulose fringe as in male, but basis with one or two long plumose setae on anterior margin; carpus with one or two biserrate spines at anterodistal angle, and one or two biserrate spines on distal margin; propodus with one or two short serrate spines on posterior margin. Pereopod VII spines and setulose fringe as in male, but basis

with between two and five long plumose setae on anterior margin; merus with one or two biserrate spines at anterodistal angle; carpus with between one and three biserrate spines at anterodistal angle, one on distal margin and two at posterodistal angle; propodus with one or two short serrate spines on posterior margin. Relative length of pereopods I, II, and VII, 30–34, 36–40, and 43–50% of body length, respectively; length ratio of their articles (coxa excluded) in pereopod I 100 : 47 : 28 : 13 : 48 : 35/100 : 54 : 37 : 16 : 57 : 49/100 : 54 : 39 : 19 : 58 : 48, in pereopod II 100 : 49 : 31 : 45 : 49 : 34/100 : 55 : 34 : 53 : 61 : 35/100 : 79 : 46 : 67 : 81 : 60, in pereopod VII 100 : 94 : 39 : 57 : 64 : 33/100 : 93 : 35 : 58 : 61 : 32/100 : 101 : 39 : 62 : 64 : 38.

Pleopods I and II as in male, but with different number of plumose setae: pleopod I with one or two and six or seven plumose setae; pleopod II with 13–18 and 8–11 plumose setae. Pleopods III and IV shapes as in male. Pleopods IV and V respiratory area length 54–63 and 32–42% of exopodite length, pleopod V respiratory area surface 26–41% of exopodite surface.

Distribution and ecology: Numerous specimens were found in small freshwater springs, appearing in the tunnel draining the karst polje (a large depression within karst) Čepičko polje, north of Labin, eastern Istra Peninsula, Croatia. They were accompanied by the large cirrolanid isopod *Sphaeromides virei virei* (Brian), and by an atyid cave shrimp *Troglocaris* sp. In the past, numerous cave salamanders, *Proteus anguinus* Laurenti (Amphibia: Proteidae), used to be washed up from the springs into the tunnel (Sket, 1997). Some specimens of the new *Monolistra* were dorsally nearly black, presumably as a result of bacterial iron deposition.

Remarks: The pleopods of *M. (Microlistra) jalzici* sp. nov. are nearly identical to those of *M. (Microlistra) pretneri* Sket, 1964. In most populations of the latter species, dorsal protuberances are present on pereonites; however, they are all of equal length, and are conical and smooth. We believe that prominent differences in dorsal sculpturing might play the role of a reproductive barrier, and therefore we consider *M. (Microlistra) jalzici* sp. nov. to be a true species, in accordance with the molecular tree and with the biological species concept.

***MONOLISTRA (MICROLISTRA) FONGI* SP. NOV.**

(FIGS 6, 7)

Holotype: Adult male of 13.5 mm in length, Croatia, Kordun, cave Kuruzovića pećina, Vaganac, near Rakovica; leg. B. Sket. Inv. Collection of OB BF UL, Inv. No. 'Malacostraca 2245 ht', partly dissected, preserved in ethanol.

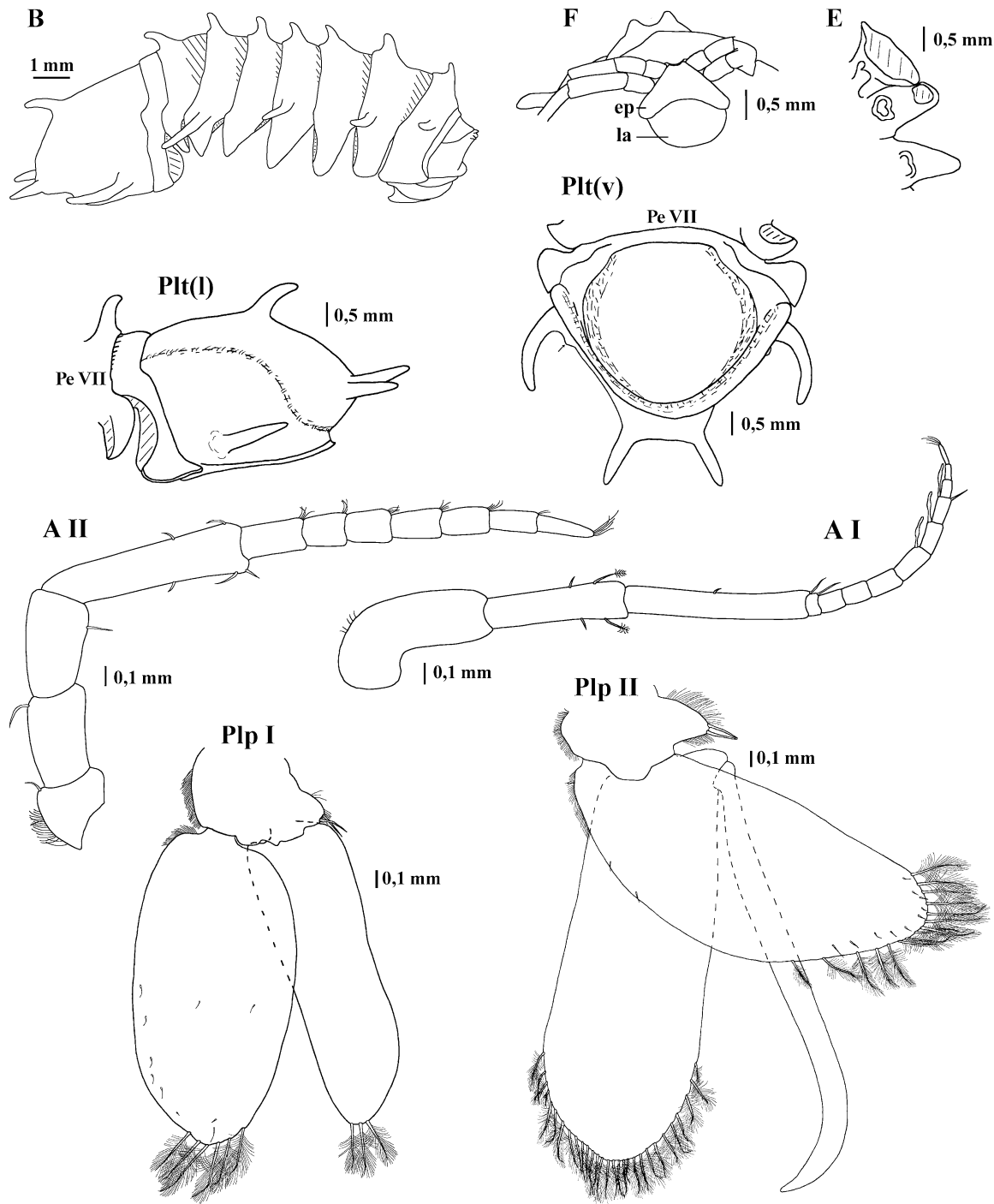


Figure 6. *Monolistra (Microlistra) fongi* sp. nov., cave Koruzovića pećina, Croatia, holotype male, 13.5 mm: A I, II, antennae I and II; B, body oblique-lateral view; E, epimera; F, frons: with ep, epistome and la, labrum; Pe VII, pereomere VII; Plp I–II, pleopods I–II; Plt(1), pleotelson, lateral view; Plt(v), pleotelson, ventral view: with U, uropod rudiment.

Paratypes: Same data as for holotype, two adult males (11.2- and 10.9-mm long) and two adult females (non-ovigerous, 13.3- and 11.9-mm long), Inv. No. 'Malacostraca 2245 pt'. One paratype deposited in the Hrvatsko prirodoslovni muzej (Croatian Natural History Museum), Zagreb, Croatia.

Other material: Croatia, Kordun, spring of the river Slunjčica, Slunj, one ex. leg. B. Jalžić, deposited in collection of Hrvatski prirodoslovni muzej, Zagreb.

Etymology: The species is named after our colleague Daniel W. Fong, a renowned American speleobiologist.

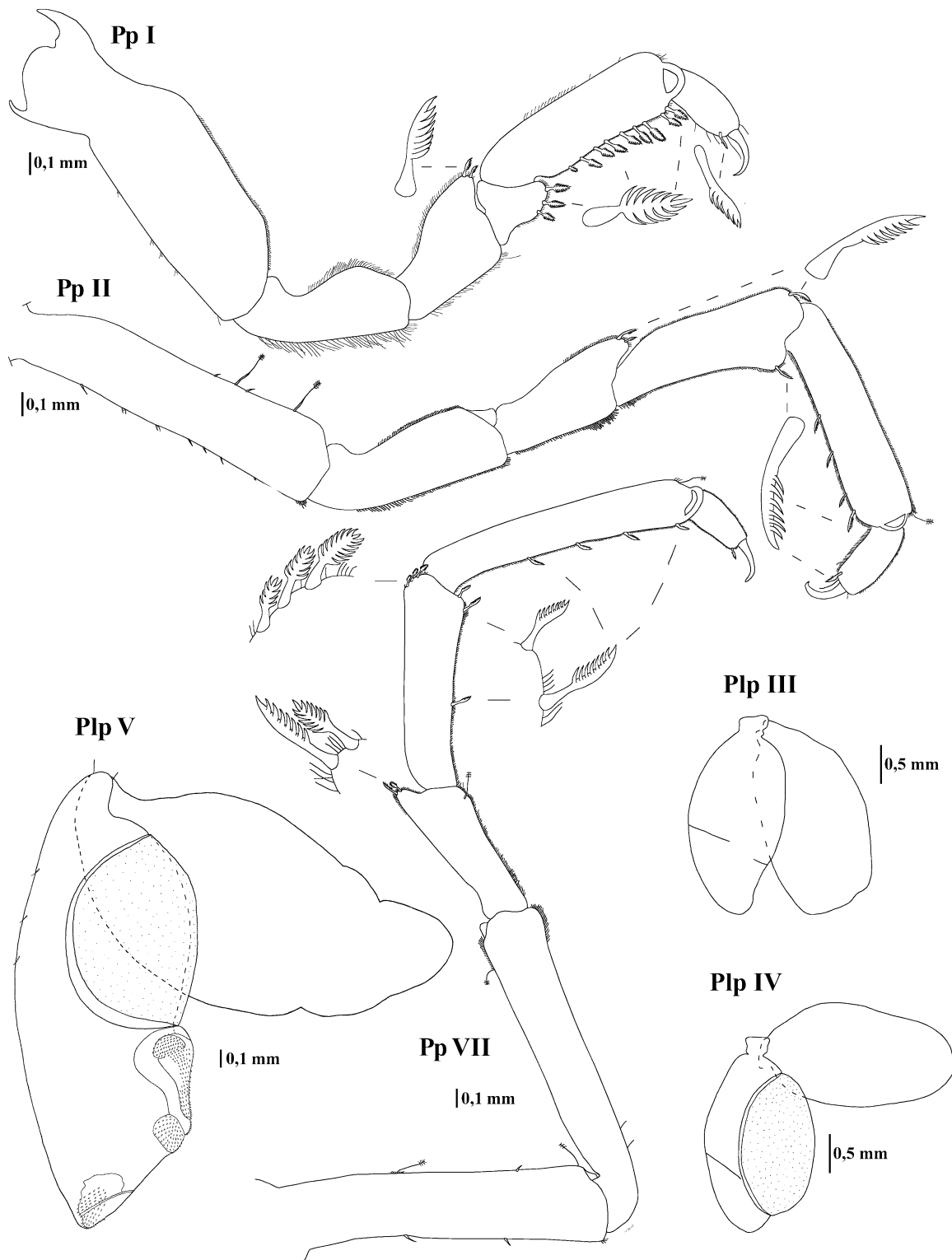


Figure 7. *Monolistra (Microlistra) fongi* sp. nov., cave Koruzovića pećina, Croatia, holotype male, 13.5 mm: Plp III–V, pleopods III–V; Pp I, II, VII, pereopods I, II, VII.

Diagnosis: *Microlistra* species with pairs of sickle-shaped lateral processes on pereonites I, II, IV, and VI, and median sickle-shaped process on pereonites I–VII; lateral processes length shorter than distances between their bases. Epimera apically narrowly rounded to bluntly pointed. Pleotelson with pair of long sickle-shaped lateral processes, a pair of shorter, straight, narrowly pointed terminal caudal processes, and a sickle-shaped median process. Uropod rudiments elevated on small bulges, projecting over pleotelson outline.

Description of holotype and paratype males (data in parentheses): Holotype male 13.5-mm long (paratype males 11.2- and 10.9-mm long). Body width 45% (48–53%) of body length. Head (cephalothorax) dorsally with two conical nodules. All pereonites with one median sickle-shaped process; pereonites I, II, IV, and VI each with a pair of longer sickle-shaped lateral processes, gradually increasing in size in caudal direction. Pleonite I without dorsal structures. Pleotelson width 140% (150–157%) of pleotelson length, highly vaulted, with pair of sickle-shaped lateral processes and pair of shorter straight terminal processes; lateral process length 152% (160–182%) of terminal process length. Lateral processes followed by two raised socles with uropod rudiments. Pleotelson ventrocaudal border without a groove, comparatively narrow dorsocaudal bulge projecting far beyond it. All processes remarkably shorter than distances between them. Anterior epimera apically narrowly rounded, posterior ones bluntly pointed.

Antenna I length 27% (26–28%) of body length; peduncle article 1 bent perpendicularly; ten (9) flagellar articles, articles 5–7 (3–6 and 8) with single long aestethasc each. Antenna II length about 30% (41%) of body length, seven (13–15) flagellar articles. Length ratio of peduncular articles and flagellum in antenna I 100 : 92 : 114 : 155 (100 : 26 : 93 : 113 : 167), in antenna II 100 : 110 : 113 : 233 : 453 (100 : 114 : 140 : 211 : 703).

Mouth parts as in *Monolistra (Microlistra) jalzici* sp. nov. Pereopod I propodus (article 6) width 33% (39–42%) of propodus length; merus (article 4) with two serrate spines at anterodistal angle; carpus (article 5) with two biserrate spines on posterodistal margin, and one at posterodistal angle; propodus with seven biserrate spines on posterior margin and 2 (1) at posterodistal angle; setulose fringe near continuous on posterior margin of ischium (article 3) to beginning of dactylus (article 7); setules length on ischium only one third article width, diminishing towards dactylus; setulose fringe also present on anterior margin of basis to merus, setules longest on ischium; secondary unguis finely serrate. Pereopod II basis with two medium long plumose setae on anterior margin;

merus with two serrate spines at anterodistal angle; carpus with one serrate spine at posterodistal angle, and one on anterodistal angle; propodus with 4 (3) short serrate spines on posterior margin; setulose fringe present on posterior margin of ischium to beginning of unguis (distal part of article 7, claw), near continuous on anterior margin of ischium to unguis; secondary unguis finely serrate. Pereopod VII basis with one short plumose seta on anterior margin and one at anterodistal angle; ischium with one short plumose seta on anterior margin; merus with one serrate and one biserrate spine at anterodistal, angle and one short plumose seta on posterodistal angle; carpus with two serrate spines on posterior margin, one at posterodistal angle, one biserrate spine at anterodistal angle, and two on distal margin; propodus with three serrate spines on posterior margin, one at posterodistal angle and one short plumose seta at anterodistal angle; setulose fringe present from posterodistal angle of ischium to beginning of unguis, anterior margins mainly without setules, except for distalmost parts of ischium, merus, and carpus, setules extremely short; secondary unguis hidden within thick mat of unresolved structure. Relative length of pereopods I, II, and VII: 29, 40, and 55% (30, 52, and 55%) of body length; length ratio of articles (coxa excluded) in pereopod I 100 : 63 : 45 : 20 : 76 : 46 (100 : 61 : 41 : 23 : 64 : 35), in pereopod II 100 : 56 : 38 : 57 : 62 : 36 (100 : 62 : 40 : 56 : 67 : 37), in pereopod VII 100 : 95 : 41 : 67 : 81 : 35 (100 : 93 : 43 : 64 : 78 : 32).

Pleopod I protopodite with many fine long setules along external margin, fewer and shorter setules along internal margin, and two strong spines at internal angle; exopodite elliptical, with six (7) plumose setae along terminal margin, scarce slender short spines on upper surface, and setules at proximo-external angle; endopodite about half as wide and nearly as long as exopodite, proximally almost parallel sided, distally slightly tapering, with three plumose setae on rounded terminal margin, without setules at proximo-internal angle. Pleopod II similar to I, but with more numerous plumose setae; endopodite as wide as exopodite, slightly widened distally, with 29 (28) plumose setae on terminal margin; appendix masculina distally sickle-shaped, apically pointed, its length 120% (116–119%) of endopodite length; exopodite with 15 (13) plumose setae on terminal margin, and slender short spines on the upper distal surface. Pleopod III exopodite elongate subovoid, without respiratory area, with long transverse suture at its external margin and short suture at the internal margin. Pleopod IV of similar shape, transverse suture at the lateral margin almost reaching respiratory area; area length 71% (76%) of exopodite length, surface 50%

(54%) exopodite surface. Pleopod V exopodite irregularly elliptical without thick sclerotized ridge, but with three slender spines along proximal half of external margin; three sclerotized patches on medio-distal half differently shaped and less densely scaled than in *M. (Microlistra) jalzici* sp. nov.; respiratory area on interproximal half, its length 39% (48%) of exopodite length, surface 30% (36%) of exopodite surface. Vestigial uropods as in *M. (Microlistra) jalzici* sp. nov.

Description of paratype females: Females of 11.9 and 13.3 mm in length; larger than males; body width 43–44% of body length. Pleotelson width 126–128% of pleotelson length, dorsocaudal vault surpassing pleotelson ventrocaudal border to greater extent than in males.

Antenna I length 25% of body length; of ten flagellar articles, articles 3–6 and 8, each with single long aestethasc. Antenna II length 34–38% of body length, flagellum of 14 or 15 articles. Pereopod I spines and setulose fringe as in males, but carpus with two biserrate spines at posterodistal angle; propodus with four biserrate spines on posterior margin and one or two at posterodistal angle. Pereopod II spines and setulose fringe as in males, but merus with one or two serrate spine at anterodistal angle. Pereopod VII spines and setulose fringe as in males, but merus with one serrate spine at anterodistal angle; carpus with one serrate spine at posterodistal angle and two biserrate spines at anterodistal angle; propodus with between one and three serrate spines on posterior margin, none or one at posterodistal angle, and one short plumose seta at anterodistal angle. Relative length of pereopods I, II, and VII 27–29, 38–41, and 50–54% of body length, respectively; length ratio of their articles (coxa excluded) in pereopod I 100 : 45 : 35 : 17 : 61 : 35/100 : 52 : 34 : 17 : 58 : 35, in pereopod II 100 : 57 : 34 : 51 : 62 : 35/100 : 58 : 37 : 53 : 64 : 34 in pereopod VII 100 : 77 : 35 : 57 : 73 : 30/100 : 90 : 42 : 64 : 83 : 32.

Pleopod I and II as in males, but pleopod II with 24 (25) plumose setae along distal endopodite margin. Pleopods III and IV shapes as in males. Pleopods IV and V respiratory area length 71 and 28% of exopodite length, respectively; pleopods IV and V respiratory area surface 42 and 40% of exopodite surface, respectively.

Distribution and ecology: Specimens were found scarcely in the residual pools in deeper parts of the cave Kuruzovića pećina (= Kukuruzovićeve pećina, K. špilja), functioning periodically as a boiling spring, near Vaganac, Kordun, Croatia. They were accompanied by a few specimens of two additional

species, *M. (Monolistra) caeca* and *M. (Monolistrella)* sp., some shrimps (*Troglocaris* sp., Decapoda), slightly troglomorphic specimens of *Synurella ambulans* O.F. Müller (Amphipoda), more numerous unidentified Cyclopoidea (Copepoda), and single specimens of troglobiotic *Proasellus* sp. (Isopoda) and *Niphargus steueri* Schellenberg (Amphipoda). Besides crustaceans, numerous snails, Hydrobioidea (Gastropoda), few Oligochaeta and Nematoda, and only three specimens of certainly troglaxene Chironomidae larvae (Diptera) were present in the pools. The other locality is the big karst spring of Slunjčica, ~20 km in the north-western, i.e. 'Dinaric', direction.

Remarks: The anterior and the posterior margins of the pereopod articles are covered with an unidentified layer of varying thickness, considerably hindering the observation and illustration of the pereopods; the lining may represent an extremely dense crust of the interlaced short setulae, and/or mats of bacteria attached to setae, or of something else.

Monolistra (Microlistra) fongi sp. nov. is most similar to the related type species *Monolistra (Microlistra) spinosa* by its appearance: its dorsal processes are similarly arranged, but remarkably shorter. Its epimera are apically blunt, whereas they are sharply pointed in *Monolistra spinosa* and some other species with long processes. The huge respiratory areas on exopodites of pleopods IV and V are even larger than in *Monolistra (Microlistra) spinosa*, *Monolistra (Microlistra) spinosissima*, and *Monolistra (Microlistra) sketi*.

DISCUSSION

TAXONOMY, BIOGEOGRAPHY

Monolistra calopyge was included in the subgenus *Microlistra* (Sket, 1982) because of its pleotelson shape, the absence of subchelae on male pereopods, reduced uropods, and presence of a long and apically curved appendix masculina, but its assignment to *Microlistra* is dubious. It differs from all other *Microlistra* spp. because males are larger than females, male pereopod II is more differentiated, pleopods IV and V have reduced respiratory areas, and there is no sculpturing on tergites, including those surrounding the uropods. Molecular data for this species are not available.

Excluding *Monolistra* (? *Microlistra*) *calopyge*, the subgenus *Microlistra* appears quite homogeneous in less obvious morphological characters, i.e. pereopod and pleopod morphology. This is in strong contrast with the differentiation seen in *M. (Monolistra) caeca*.

In its area of distribution, less than 150 km in linear extension, races with moderately large to reduced or absent respiratory areas on pleopods are encountered (Sket, 1964). In contrast, pleopod respiratory areas remain virtually unchanged within more than 300 km of the distribution range of *Microlistra* species. *Microlistra* species exhibit extreme diversity in their overall appearance and dorsal sculpturing; the sculpturing may be absent, present in the form of small tubercles (nodules), or present as large/long processes. Nodules are rare among nearly 30 other species and subspecies of the genus *Monolistra*, occurring only in *Monolistra (Monolistra) monstrosa* Sket, 1970.

Both major clades revealed by our molecular study are well separated geographically, and we can roughly designate them as the southern and the northern clades. The species belonging to the southern clade are distributed along the outermost Dinaric mountain chains, draining towards the Adriatic. *Monolistra (Microlistra) pretneri* is found near Šibenik, crossing to Cres Island, whereas *Monolistra (Microlistra) jalzici* sp. nov. inhabits the Istra Peninsula. The distribution of *Monolistra (Microlistra) schottlaenderi* could represent an extension of the southern area, although slightly displaced. Nevertheless, in our molecular phylogeny, this species belongs to the *spinosa*–*spinosissima* species group in the northern clade. The latter clade is orographically less defined, covering a long, wide area from Italy, through South Slovenia, to Croatia, between its borders with Slovenia and Bosnia. *Monolistra (Microlistra) bolei* is positioned centrally, partly surrounded by related taxa. The area inhabited by the species belonging to the northern clade is partly drained towards the Adriatic [*M. (Microlistra) schottlaenderi* and *Monolistra (Microlistra) sketi*], but mainly towards the Black Sea. Each species of the subgenus is related to the subterranean parts of present river systems; this is characteristically not the case in some other crustacean species, including some other taxa within the genus *Monolistra* (Sket, 1986b, 2002; Trontelj *et al.*, 2007).

Both main clades are also morphologically well-defined. The bulk of species of the northern clade possess smooth processes, gradually tapering towards their tips, and extended pereonal epimera, the elongation of their pointed tips progressing simultaneously with the progressive elongation of processes. Members of the southern clade never have smooth processes: ornamentation elements are either small nodules or large warts, widened in the middle and not narrowly pointed, and pereonal epimera are short and wide, always obtuse. The exception is the ‘northern’ species *Monolistra (Microlistra) schottlaenderi*, which is morphologically of the ‘southern’ type.

PHYLOGENY

Our study indicates that the previous calculations (Trontelj *et al.*, 2007) regarding the timing of splits within the subgenus might have been inaccurate. The *Microlistra* group had already separated from its sister clades in the beginning of the Pleistocene (see above), but persisted without further splitting for a comparatively long time afterwards. The first split within the group most probably occurred within the period of 1.5–1.1 Mya, which means that it might even be slightly younger than the split between the putative subspecies of the related *M. (Monolistra) caeca* (2.0–1.5 Mya; Trontelj *et al.*, 2007). Such a late split may perhaps explain the great similarity of pleopods within the *Microlistra* clade, in contrast with the pleopods within *M. (Monolistra) caeca*. On the other hand, the habitus of the latter is very homogeneous because of the lack of any prominent ornamentation.

The youngest speciations within the subgenus *Microlistra*, resulting in a weak molecular divergence and little variability in pleopod morphology, are corroborated further by the restriction of species to recent river drainages. The persistence of both the genetic and morphological characters in the *Microlistra* clade, despite its occurrence in a geographically extremely heterogeneous area, can only mean that its predecessors lived and survived in surface waters even after the formation of recent drainages. Namely, areas of most other troglobiotic species and even subspecies are bound to past (palaeo-) river drainages. Therefore, distributions of *Microlistra* species are in strong contrast to the palaeo-hydrographically defined distributions of *M. (Monolistra) caeca* races. These races are not only morphologically and/or molecularly distinct, but they occur in a number of separated current drainages (Sket, 2002).

Different dorsal sculpturing (see above) of both the main *Microlistra* clades (the southern and the northern clades) could only have evolved after the first split. An extreme case of different sculpturing is the difference between the sister species *Monolistra (Microlistra) schottlaenderi* and *Monolistra (Microlistra) spinosa*. Furthermore, *Monolistra (Microlistra) bolei* has its processes positioned as no other species within its sister lineage, which also indicates independent evolution of its dorsal structures. All this shows that the development of the sculpturing is a result of convergent evolution, which resulted in patterns differing in details. Less revealing are the absence of sculpturing (in some *Monolistra (Microlistra) pretneri*) and a weak development of processes [in *Monolistra (Microlistra) sketi*] in some terminal branches, as these might be the results of secondary reductions of an originally richer ornamentation. As

all species are allopatric, we do not consider the development of dorsal structures to be enforced by the need for reproductive isolation (i.e. by a selective reinforcement). Nevertheless, as sphaeromatids are copulating animals, such differences in body shapes could certainly prevent interbreeding. This is the reason why we consider the named taxa as 'biological' species, regardless of their molecular similarities.

It is a fact that dating with a molecular clock results in quite broad potential time-spans for speciations. Nevertheless, with the possible exception of the first intrageneric split, all divergence events clearly fall within the geologically (particularly hydrographically) active and climatically highly fluctuating Pleistocene. Taking into consideration our previous statements about the speciation in *Monolistra* (Sket, 1986b), these late speciation events could only mean that some *Monolistra* populations had to survive glaciations in surface freshwaters, comparatively close to the Alpine glacier.

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APPENDIX 1

List of known localities of *Monolistra* (*Microlistra*) spp. with coordinates. Abbreviations: M., *Monolistra*; Mic., *Microlistra*; D, sequences for phylogenetic analysis; T, type locality; CRO, Croatia; ITA, Italy; SLO, Slovenia.

Name	DNA	Loc. typ.	Locality name	Nearby settlement	Country	WGS_Xdd	WGS_Ydd
<i>M. (Mic.) forigi</i>	D	T	cave Kuruzovica pećina	Pašina Luka, Drežnik Grad, Rakovica	CRO	15,7025507	44,91472811
<i>M. (Mic.) jalzici</i>	D	T	spring in Tunnel Čepić	Kožljak, Čepićko polje, Podpićan	CRO	14,15998546	45,17021515
<i>M. (Mic.) pretneri</i>	D	T	cave Pećina kod Vrane	Pećina, Vrana, Pakoštane	CRO	15,55492234	43,95979217
<i>M. (Mic.) pretneri (spinulosa)</i>	D	(T)?	cave Miljacka spilja 1	Miljacka min, Kistanje, Knin	CRO	16,01861835	44,00313313
<i>M. (Mic.) pretneri</i>	D		cave Milica spilja	Milići, Bogatnik, Kaštel Žegarski	CRO	15,80311235	44,14297815
<i>M. (Mic.) pretneri</i>			spring 1 km east Žegar	Žegar, kaštel Žegarski, Obrovac	CRO	15,84712646	44,15461241
<i>M. (Mic.) pretneri</i>			spring below cave Miljacka spilja 1	Miljacka min, Kistanje, Knin	CRO	16,01861835	44,00313313
<i>M. (Mic.) pretneri</i>	D		spring at power plant Jaruga	Skradin, Šibenik	CRO	15,96267831	43,80726946
<i>M. (Mic.) pretneri</i>	D		cave Kusa nad Manastirskom lukom	Manastir Krupa, Pirevište, Obrovac	CRO	15,88169165	44,1904967
<i>M. (Mic.) pretneri</i>	D		cave Kusaca jama	Žegar, Kaštel Žegarski	CRO	15,84249204	44,13953176
<i>M. (Mic.) pretneri</i>			well in Punta Križa	Cres (island)	CRO	14,49492522	44,6394318
<i>M. (Mic.) sketi</i>	D	T	cave Markovac jama	Pećina, Ličko Lešće	CRO	15,33076608	44,79670227
<i>M. (Mic.) sp. (cf. sketi)</i>	D		spring of Rupećica	Kangrge, Podum, Otočac	CRO	15,30447971	44,86137889
<i>M. (Mic.) schottlaenderi</i>		T	spring of Rupećica	Ivanci, Ogulin	CRO	15,22480539	45,18782454
<i>M. (Mic.) schottlaenderi</i>		T	spring of Rupećica	San Giovanni/Šivan, Monfalcone	ITA	13,58426023	45,80086511
<i>M. (Mic.) schottlaenderi</i>			spring of Rupećica	Sgonico/Zgonik, Prosecco/Prosek	ITA	13,73284909	45,71827308
<i>M. (Mic.) schottlaenderi</i>			cave Abisso Massimo	Monfalcone/Trižič	ITA	13,56025	45,81481
<i>M. (Mic.) schottlaenderi</i>			spring at Lago di Pietrarossa/Laško jezero	San Giovanni/Šivan, Monfalcone	ITA	13,59154	45,79123
<i>M. (Mic.) schottlaenderi</i>			spring at Monfalcone	San Giovanni/Bojunec, Trieste/Trst	ITA	13,87227759	45,62050931
<i>M. (Mic.) bolei bolei</i>			spring Fonte Oppia/Klinčica	Otovec, Črnomelj	SLO	15,16412441	45,59227634
<i>M. (Mic.) bolei bolei</i>	D	T	spring Otovski zdenec	Petrova vas, Črnomelj	SLO	15,17073562	45,60426056
<i>M. (Mic.) bolei brevispinosa</i>	D		cave Stobe	Beljji Vrh, Črnomelj	SLO	15,20062456	45,50239495
<i>M. (Mic.) bolei brevispinosa</i>	D	T	cave Džud	Beljji Vrh, Črnomelj	SLO	15,24430696	45,45443663
<i>M. (Mic.) bolei brevispinosa</i>	D		cave jama v kammolomu – 2950	Vintca	SLO	15,17706432	45,49778204
<i>M. (Mic.) spinosa</i>	D	T	cave Lobešnica	Stara Lipa, G. Suhor pri Vinici, Črnomelj	SLO	14,96855801	45,79628292
<i>M. (Mic.) spinosa</i>			spring Tomincev studenec	Žužemberk	SLO	14,94481174	45,81039298
<i>M. (Mic.) spinosa</i>			cave Bobnova jama	Stavča vas, Novo mesto	SLO	14,87005342	45,85604346
<i>M. (Mic.) spinosa ssp.</i>			spring below Rivčja jama	Male Rebrce, Šmihel pri Žužemberku	SLO	14,79747913	45,95186554
<i>M. (Mic.) spinosa ssp.</i>	D		spring Rupnica	Rupe, Stična	SLO	14,83022311	45,6676524
<i>M. (Mic.) spinosissima</i>	D		water works in Slovenska vas	Slovenska vas, Kočevje	SLO	14,78954216	45,70822136
<i>M. (Mic.) spinosissima</i>	D	T	cave Mobašht	Dolenja vas, Ribnica	SLO	14,26821292	45,86494137
<i>M. (Mic.) spinosissima</i>			cave Logarček	Laze, Logatec	SLO	14,33310623	45,94603575
<i>M. (Mic.) spinosissima</i>	D		spring Gaišnova jama	Vrhnika	SLO	14,23573699	45,91070877
<i>M. (Mic.) spinosissima</i>	D		resurgence-cave Izvir pod orehom	Logatec	SLO	14,29966281	45,95250727
<i>M. (Mic.) spinosissima</i>	D		resurgence-cave Malo Okence	Verd	SLO	14,29541835	45,95144627
<i>M. (Mic.) spinosissima</i>	D		cave near Mirke	Verd, Vrhnika	SLO	14,29309948	45,95429292
<i>M. (Mic.) spinosissima</i>	D		cave Najdena jama	Verd, Vrhnika	SLO	14,24548199	45,87630166
<i>M. (Mic.) spinosissima</i>	D		resurgence-cave Retovje	Laze, Planina	SLO	14,2958184	45,94969846
<i>M. (Mic.) spinosissima</i>	D		resurgence-cave Veliko okence	Verd, Vrhnika	SLO	14,2958284	45,94969446
<i>M. (Mic.) spinosissima</i>			resurgence-cave in D9Verd	Verd, Vrhnika	SLO	14,31112931	45,95558924
<i>M. (Mic.) calopyge</i>		T	well in Kronovo	Šmarješke toplice	SLO	15,25622146	45,85633622