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 synonymysed 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*.


ADDITIONAL KEYWORDS: Dinarides – phylogeny – subterranean.

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 Rupečica spring (Croatia; referred to as Monolistra (Microlistra) sp. – Rupečica hereafter in the text), samples from all listed localities are stored in the invertebrate collection of Oddelel za biologijo, Biotehniška fakulteta, Univerza v Ljubljani (OB BF UL). The Monolistra (Microlistra) sp. – Rupečica 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 toptype 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′-GCCAGCAGCCGGGTATTA-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′-AGGGAAACTTCCG AGGGAACC-3′ and 5′-CAAGTACCGGTAGGGAAGTT-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).

**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.)

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

Sequence names are identical to the ones depicted on the phylogenetic tree: ‘T’ with taxon designation, denotes totopytic 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 $4 \times 10^6$ 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 Stoch, 2000; Monolistra (Microlistra) sketi (Deeleman, 1971); Monolistra (Microlistra) sp. (cf. sketi) (Rupecica) 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 troglobiotic members of the genus Caecosphaeroma, with two species and a subspecies, are West European (Sket, 1986a). Another sphaeromatid genus with troglobiotic species is the ‘antipodean’ Bilistra Sket & Bruce, 2004, from New Zealand. Other Sphaeromatidae are epigean, 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

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. Pleotelson without a pair of terminal caudal processes; its proximal lateral processes, if developed, short and thick, blunt

2.* Pleotelson with pairs of narrowly pointed lateral and caudal processes

3. Along the pereon, a median, and a pair of lateral rows of short and thick, setulose processes, nodules between them much smaller

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. Pleotelson dorsocaudal bulge tapering in caudal direction, with a narrow apex; male pleopod II endopodite with ~20 marginal plumose setae

4.* Pleotelson dorsocaudal bulge broadly rounded apically; male pleopod II endopodite with ~15 marginal plumose setae

5. Anterior pereonites without sculptures; pleotelson without a median process

5.* Some anterior pereonites with spine-like processes

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

6.* Pereon without any processes, pleonal processes longer

7. Pleotelson without a median, only with paired sickle-shaped processes; lateral sickle-shaped processes on pereonites I–III and V–VI, *Microlistra (Microlistra) bolei*

7.* Pleotelson with two pairs of processes plus a median sickle-shaped process; lateral sickle-shaped processes on pereonites I, II, IV, and VI

8. Processes longer; tips of lateral pleonal ones reaching the ventrocaudal border

8.* Processes shorter, tips of lateral pleonal ones (in adults) not reaching ventrocaudal border

9. Pereonal lateral processes shorter than distances between their bases

9.* Pereonal lateral processes longer than distances between their bases

10. Pleotelson with only five major processes; processes strongly curved

10.* Besides five major processes, also some small processes on pleotelson; processes less curved and longer

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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)

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

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


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

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


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. – Kočeve–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.


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

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

<table>
<thead>
<tr>
<th><em>Monolistra</em> (<em>Microlistra</em>) species</th>
<th>Co-occurring <em>Monolistra</em> species</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>M. (Microlistra) bolei brevispinosa</em></td>
<td><em>M. (Monolistra) bolei bolei</em></td>
</tr>
<tr>
<td><em>M. (Microlistra) fongi</em> sp. nov.</td>
<td><em>M. (Monolistra) caeca</em></td>
</tr>
<tr>
<td><em>M. (Microlistra) schottaenderi</em></td>
<td><em>M. (Monolistra) caeca</em></td>
</tr>
<tr>
<td><em>M. (Microlistra) spinosa</em></td>
<td><em>M. (Monolistra) caeca</em></td>
</tr>
<tr>
<td><em>M. (Microlistra) spinosa</em> ssp. (Kočeve–Ribnica)</td>
<td><em>M. (Monolistra) caeca</em></td>
</tr>
<tr>
<td><em>M. (Microlistra) spinosissima</em></td>
<td><em>M. (Monolistra) caeca</em></td>
</tr>
<tr>
<td><em>M. (Typhlosphaeroma) karamani</em></td>
<td><em>M. (Typhlosphaeroma) karamani</em></td>
</tr>
<tr>
<td><em>M. (Microlistra) sketi</em></td>
<td>None</td>
</tr>
<tr>
<td><em>M. (Microlistra) sp.-Rupećica</em></td>
<td>None</td>
</tr>
<tr>
<td><em>M. (Microlistra) pretneri</em></td>
<td>None</td>
</tr>
<tr>
<td><em>M. (Microlistra) jalzici</em> sp. nov.</td>
<td>None</td>
</tr>
</tbody>
</table>

### 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 (Skit, 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 *Monolistra*, 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 *Monolistra* received high support, but the relationships among species belonging to different subgenera (*sensu* Sket, 1965) remains unresolved, possibly because of insufficient sampling in other subgenera. The subgenus *Microlistra* is monophyletic and distinctly separated into two main clades: one including *M. Microlistra pretneri* and *Monolistra (Microlistra) jalzici* sp. nov., and the other including the type species *Monolistra (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: *Monolistra (Microlistra) jalzici* forms a separate basal *Microlistra*
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. – Rupečica 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–spinosisima 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) spinosisima, 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) spinosisima, 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

**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 Jalzic, 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 distally.

**Description of holotype:** Male, 7.9-mm long. Body width 44% of body length. Head (cephalo thorax) 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 V–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 ventrocaudal 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 aesthetasc. 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 maxillipeds 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 postero distal angle; propodus with three biserrate spines on posterior margin and two at postero distal 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 postero distal angle, and one on distal margin; propodus with two short serrate spines on posterior margin, one serrate spine at postero distal 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 postero distal angle; carpus with one serrate spine at postero distal 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.

Pereopod 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. Pereopod 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. Pereopod 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 proximal internal margin. Pereopod IV shape similar; respiratory area length 55% of exopodite length, surface 35% of exopodite surface. Pereopod 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 Monolista (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 aesthetasc. 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 bisserrate 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; merus with one or two bisserrate spines at anterodistal angle, and one or two bisserrate 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 bisserrate 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 : 61/100 : 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.

Pereopods 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 cirolanid 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 Monolista 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 reproductve 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.

**Monolista (Microlistra) fongi sp. nov.**
(Figs 6, 7)

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

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. *Monolistema (Microlistema) 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 sockets 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.


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 bisserrate spines on posterodistal margin, and one at posterostral angle; propodus with seven bisserrate spines on posterior margin and 2 (1) at posterostral angle; telulose 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; telulose 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 posterostral angle, and one on anterostral angle; propodus with 4 (3) short serrate spines on posterior margin; telulose 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 anterostral angle; ischium with one short plumose seta on anterior margin; merus with one serrate and one bisserrate spine at anterostral, angle and one short plumose seta on posterostral angle; carpus with two serrate spines on posterior margin, one at posterostral angle, one bisserrate spine at anterostral angle, and two on distal margin; propodus with three serrate spines on posterior margin, one at posterostral angle and one short plumose seta at anterostral angle; telulose fringe present from posterostral 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 : 56 : 67 : 37), in pereopod VII 100 : 95 : 41 : 67 : 81 : 35 (100 : 93 : 43 : 64 : 78 : 32).

Pereopod I protopodite with many fine long setules along external margin, fewer and shorter setules along internal margin, and 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. Pereopod 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 sicle-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. Pereopod III exopodite elongate subvoid, without respiratory area, with long transverse suture at its external margin and short suture at the internal margin. Pereopod IV of similar shape, transverse suture at the lateral margin almost reaching respiratory area; area length 71% (76%) of exopodite length, surface 50%...
respiratory area surface 42 and 40% of exopodite length, respectively; pleopods IV and V respiratory area length 71 and 28% of pleopods III and IV shapes as in males. Pleopods IV (25) plumose setae along distal endopodite margin. 73 : 30/100 : 90 : 42 : 64 : 83 : 32. 53 : 64 : 34 in pereopod VII 100 : 77 : 35 : 57 : 58 : 35/100 : 58 : 37 : 100 : 45 : 35 : 17 : 61 : 35, in their articles (coxa excluded) in pereopod I 50–54% of body length, respectively; length ratio of length of pereopods I, II, and VII 27–29, 38–41, and short plumose seta at anterodistal angle. Relative margin, none or one at posterodistal angle, and one between one and three serrate spines on posterior biserrate spines at anterodistal angle; propodus with one serrate spine at posterodistal angle and two with one serrate spine at anterodistal angle; carpus spines and setulose fringe as in males, but merus with one or two at posterodistal angle. Pereopod II spines and four biserrate spines on posterior margin and one or biserrate spines at posterodistal angle; propodus with setulose fringe as in males, but carpus with two flagellum of 14 or 15 articles. Pereopod I spines and aesthetasc. Antenna II length 34–38% of body length, lar articles, articles 3–6 and 8, each with single long males.

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 scarely in the residual pools in deeper parts of the cave Kuruzovića pećina (= Kukuruzovićeva 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. (Monolistrælla) 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 troglobioticler Proasellus sp. (Isopoda) and Niphargus steueri Schellenberg (Amphipoda). Besides crustaceans, numerous snails, Hydrobioidea (Gastropoda), few Oligochaeta and Nematoda, and only three specimens of certainly trogloxene 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 (Skeč, 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 (Sket, 1964). In contrast, pleopod respiratory areas absent respiratory areas on pleopods are encountered.

Both major clades revealed by our molecular study are well separated geographically, and we can roughly designate them as the southern and the northern races. 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 (palaeo-) river drainages. Therefore, distributions of Monolistra 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 Monolistra 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

Our study indicates that the previous calculations (Trontelj et al., 2007) regarding the timing of splits within the subgenus might have been inaccurate. The Monolistra 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 Monolistra 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 Monolistra, 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 Monolistra 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 Monolistra 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).
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.

<table>
<thead>
<tr>
<th>Name</th>
<th>DNA</th>
<th>Loc. typ.</th>
<th>Locality name</th>
<th>Nearby settlement</th>
<th>Country</th>
<th>WGS_Xdd</th>
<th>WGS_Ydd</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>M. (Mic.) fongi</em></td>
<td>D</td>
<td>T</td>
<td>cave Kuruzoviča pečina</td>
<td>Pašina Luka, Drenžnik Grad, Rakovica</td>
<td>CRO</td>
<td>15,7025507</td>
<td>44,91472811</td>
</tr>
<tr>
<td><em>M. (Mic.) jalzici</em></td>
<td>D</td>
<td>T</td>
<td>spring in Tunnel Čepić</td>
<td>Kožljuk, Čepičko polje, Podpičan</td>
<td>CRO</td>
<td>15,1598545</td>
<td>45,17021515</td>
</tr>
<tr>
<td><em>M. (Mic.) pretneri</em></td>
<td>D</td>
<td>T</td>
<td>cave Pečina kod Vrane</td>
<td>Pečina, Vrana, Pakšćane</td>
<td>CRO</td>
<td>15,55492234</td>
<td>43,95979217</td>
</tr>
<tr>
<td><em>M. (Mic.) pretneri (spinulosa)</em></td>
<td>D</td>
<td>T</td>
<td>cave Milčica spilja</td>
<td>Milčica, Bogatnik, Kaštel Žegarski</td>
<td>CRO</td>
<td>16,01861835</td>
<td>44,00313313</td>
</tr>
<tr>
<td><em>M. (Mic.) pretneri</em></td>
<td>D</td>
<td>T</td>
<td>spring 1 km east Žegar</td>
<td>Žegar, Kaštel Žegarski, Obrovac</td>
<td>CRO</td>
<td>15,84712464</td>
<td>44,15461241</td>
</tr>
<tr>
<td><em>M. (Mic.) pretneri</em></td>
<td>D</td>
<td>T</td>
<td>spring below cave Miljacka spilja</td>
<td>Miljacka mlin, Kistanje, Knin</td>
<td>CRO</td>
<td>16,01861835</td>
<td>44,00313313</td>
</tr>
<tr>
<td><em>M. (Mic.) pretneri</em></td>
<td>D</td>
<td>T</td>
<td>spring at power plant Jaruga</td>
<td>Skradin, Šibenik</td>
<td>CRO</td>
<td>15,96267831</td>
<td>43,80726946</td>
</tr>
<tr>
<td><em>M. (Mic.) sketi</em></td>
<td>D</td>
<td>T</td>
<td>cave Kusa nad Manastirskom lukom</td>
<td>Manastir Krupa, Pirevište, Obrovac</td>
<td>CRO</td>
<td>15,88169165</td>
<td>44,19049677</td>
</tr>
<tr>
<td><em>M. (Mic.) sketi</em></td>
<td>D</td>
<td>T</td>
<td>cave Kusaca jama</td>
<td>Žegar, Kaštel Žegarski</td>
<td>CRO</td>
<td>15,84249204</td>
<td>44,13953176</td>
</tr>
<tr>
<td><em>M. (Mic.) pretneri</em></td>
<td>D</td>
<td>T</td>
<td>well in Punta Križa Cres (island)</td>
<td></td>
<td>CRO</td>
<td>14,49492522</td>
<td>44,639318</td>
</tr>
<tr>
<td><em>M. (Mic.) pretneri</em></td>
<td>D</td>
<td>T</td>
<td>cave Kusa na Manastirskom lukom</td>
<td>Manastir Krupa, Pirevište, Obrovac</td>
<td>CRO</td>
<td>15,88169165</td>
<td>44,19049677</td>
</tr>
<tr>
<td><em>M. (Mic.) bolei bolei</em></td>
<td>D</td>
<td>T</td>
<td>cave Sobe</td>
<td>Petrova vas, Črnomelj</td>
<td>SLO</td>
<td>15,17073562</td>
<td>45,69420565</td>
</tr>
<tr>
<td><em>M. (Mic.) bolei brevispinosa</em></td>
<td>D</td>
<td>T</td>
<td>cave Džud Belčji Vrh</td>
<td>Belčji Vrh, Črnomelj</td>
<td>SLO</td>
<td>15,24430696</td>
<td>45,5443663</td>
</tr>
<tr>
<td><em>M. (Mic.) spinosa</em></td>
<td>D</td>
<td>T</td>
<td>spring Tominc'ev studenec</td>
<td>Žužemberk</td>
<td>SLO</td>
<td>14,96855801</td>
<td>45,79628929</td>
</tr>
<tr>
<td><em>M. (Mic.) spinosa</em></td>
<td>D</td>
<td>T</td>
<td>cave Bobnova jama</td>
<td>Stavča vas, Novo mesto</td>
<td>SLO</td>
<td>15,17073562</td>
<td>45,69420565</td>
</tr>
<tr>
<td><em>M. (Mic.) spinosa</em></td>
<td>D</td>
<td>T</td>
<td>spring below Rivčja jama</td>
<td>Male Brbee, Šmihel pri Žužemberku</td>
<td>SLO</td>
<td>14,70053432</td>
<td>45,85604346</td>
</tr>
<tr>
<td><em>M. (Mic.) spinosa</em></td>
<td>D</td>
<td>T</td>
<td>cave Stobe</td>
<td>Žužemberk</td>
<td>SLO</td>
<td>15,20062456</td>
<td>45,50234957</td>
</tr>
<tr>
<td><em>M. (Mic.) spinosa</em></td>
<td>D</td>
<td>T</td>
<td>cave Logarček</td>
<td>Laze, Logatec</td>
<td>SLO</td>
<td>14,28521292</td>
<td>45,86491437</td>
</tr>
<tr>
<td><em>M. (Mic.) spinoidea</em></td>
<td>D</td>
<td>T</td>
<td>spring Bistra</td>
<td>Vrhnika</td>
<td>SLO</td>
<td>14,33310623</td>
<td>45,94603575</td>
</tr>
<tr>
<td><em>M. (Mic.) spinoidea</em></td>
<td>D</td>
<td>T</td>
<td>cave Gašpinova jama</td>
<td>Logatec</td>
<td>SLO</td>
<td>14,23573699</td>
<td>45,91070877</td>
</tr>
<tr>
<td><em>M. (Mic.) spinosissima</em></td>
<td>D</td>
<td>T</td>
<td>resurgence-cave Izvir pod orehom</td>
<td>Verd</td>
<td>SLO</td>
<td>14,29960828</td>
<td>45,95250727</td>
</tr>
<tr>
<td><em>M. (Mic.) spinosissima</em></td>
<td>D</td>
<td>T</td>
<td>resurgence-cave Malo Okence</td>
<td>Verd, Vrhnika</td>
<td>SLO</td>
<td>14,29541385</td>
<td>45,95442877</td>
</tr>
<tr>
<td><em>M. (Mic.) spinosissima</em></td>
<td>D</td>
<td>T</td>
<td>cave near Mirke</td>
<td>Verd, Vrhnika</td>
<td>SLO</td>
<td>14,29541385</td>
<td>45,95442877</td>
</tr>
<tr>
<td><em>M. (Mic.) spinosissima</em></td>
<td>D</td>
<td>T</td>
<td>cave Najdena jama</td>
<td>Laze, Planina</td>
<td>SLO</td>
<td>14,24541819</td>
<td>45,86730616</td>
</tr>
<tr>
<td><em>M. (Mic.) spinosissima</em></td>
<td>D</td>
<td>T</td>
<td>resurgence-cave Retorje</td>
<td>Verd, Vrhnika</td>
<td>SLO</td>
<td>14,2958184</td>
<td>45,94969846</td>
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<td><em>M. (Mic.) spinosissima</em></td>
<td>D</td>
<td>T</td>
<td>resurgence-cave Veliko okence</td>
<td>Verd, Vrhnika</td>
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<td>14,2958284</td>
<td>45,94969446</td>
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<td><em>M. (Mic.) spinosissima</em></td>
<td>D</td>
<td>T</td>
<td>resurgence-cave in D9Verd</td>
<td>Vrhnika</td>
<td>SLO</td>
<td>14,31112931</td>
<td>45,95589294</td>
</tr>
<tr>
<td><em>M. (Mic.) calopyge</em></td>
<td>T</td>
<td></td>
<td>well in Kronovo</td>
<td>Šmarške toplice</td>
<td>SLO</td>
<td>15,25622146</td>
<td>45,85633622</td>
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