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COPEPODA FROM A DEEP-GROUNDWATER POROUS AQUIFER
IN CONTACT WITH KARST: DESCRIPTION OF A NEW SPECIES,
PARAMORARIOPSIS BRIGITAE N. SP. (COPEPODA, HARPACTICOIDA)

BY

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ABSTRACT

Fauna from four wells supplying water to a nearby city was collected over one year at approximately two-week intervals. Three of the wells collect water from a depth of 10-26 m and one from a depth of 33-96 m. Representatives of 35 taxa were collected. Some of them are epigeal and were transported from a nearby river. The most abundant group was Copepoda, where 15 taxa were recognized; all but one were stygobionts. There was a noticeable difference between the fauna from the shallow and from the deep wells. In the former, *Elaphoidella charon* Chappuis, 1936 and *Diacyclops languidoides* (Lilljeborg, 1901) prevailed, while in the deep well the most abundant species were *Graeteriella unisetigera* (Graeter, 1908), *Ceuthonectes serbicus* Chappuis, 1924, and *Paramorariopsis brigitae* n. sp. The latter was relatively common, 70 specimens being collected (51 ♀♀; 19 ♂♂), with the maximum number of 10-12 specimens per sampling in September/November.

A detailed description of the new species is presented, together with some information on its ecology and specific morphological adaptations, which are similar to those in the two already known species from the same genus (*P. anae* Brancelj, 1991 and *P. irenae* Brancelj, 2006).

RÉSUMÉ

La faune de quatre puits alimentant une ville proche en eau a été collectée pendant un an à raison d'une fois toutes les deux semaines approximativement. Trois des puits ont été échantillonnés à une profondeur de 10-26 m et le dernier à une profondeur de 33-96 m. Les représentants de 35 taxons ont ainsi été récoltés. Certains sont épigés et proviennent d'une rivière proche. Le groupe le plus abondant est représenté par les Copépodes, dont 15 taxons ont été identifiés, tous sauf un sont des stygobiontes. Une différence notable a été observée entre la faune des puits peu profonds et celle du puits profond. Dans les premiers, *Elaphoidella charon* Chappuis, 1936 et *Diacyclops languidoides* (Lilljeborg, 1901) dominaient, tandis que dans le

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puis profond, les espèces les plus abondantes étaient *Graeteriella unisetigera* (Graeter, 1908), *Ceuthonectes serbicus* Chappuis, 1924 et *Paramorariopsis brigittae* n. sp. Cette dernière espèce était relativement commune, 70 spécimens ayant été collectés (51 ♀♀, 19 ♂♂), avec un nombre maximum de 10-12 spécimens par prélèvement en Septembre/Novembre.

Une description détaillée de la nouvelle espèce est présentée, ainsi que des informations sur son écologie et ses adaptations morphologiques spécifiques, qui sont analogues à celle des deux autres espèces déjà connues du même genre (*P. anae* Brancelj, 1991 et *P. irenae* Brancelj, 2006).

INTRODUCTION

In the second half of the 20th century, “exciting advances” were made in our knowledge of groundwater biodiversity (Gilbert & Culver, 2009). In Europe, the majority of groundwater fauna is represented by Crustacea, with Copepoda, Ostracoda, Isopoda, and Amphipoda as the most characteristic and abundant representatives (Deharveng et al., 2009; Galassi et al., 2009). Similar results were obtained from bore-holes in eastern Australia (Hancock & Boulton, 2009) and Western Australia (Eberhard et al., 2009). Only limited information is available from groundwater of South and North America or Asia (Pesce & Apostolov, 1985; Bruno et al., 2001; Lewis & Reid, 2007; Bork et al., 2008). Most of the stygobitic Copepoda known so far for Europe, have been collected in karstic aquifers, either from the saturated or unsaturated zone, or from the hyporheic or shallow phreatic zone along the rivers (up to about 8 m depth), as reported from the recent pan-European project PASCALIS (Dole-Olivier et al., 2009). In Australia, where alluvial plains prevail and bore-holes constitute the predominant sampling sites, most of the latter in Western Australia were not deeper than 30 m below ground level (b.g.l.), with a maximum depth of 84 m b.g.l. (Eberhard et al., 2009). In eastern Australia most of the sampling sites were >20 m b.g.l., with a maximum depth of 28 m b.g.l. (Hancock & Boulton, 2009).

One of the main constraints in sampling deeper groundwater aquifers (i.e., where the water table is more than 10 m below ground level) is the sampling method, restricted to net hauls and pumping (Pospisil, 1992; Hancock & Boulton, 2009). Net hauls are slow and labour intensive, while pumping is dependent on the power supply and carries a high risk of damaging specimens. As a result, relatively little is known about the stygobitic fauna from deeper porous aquifers, especially from depths greater than 30 m below ground level (regardless of the depth of the water column).

Unconsolidated sediment in porous aquifers is characterized by constant low temperature and lack of light, as well as restricted “vital space” (Galassi

et al., 2009). Food, however, is not necessarily a limiting factor, since the microbial community, consisting of heterotrophs and chemoautotrophs, can reach 10^6 - 10^8 cells cm^{-3} , but is usually distributed patchily on solid substrate (for details see Griebler & Lueders, 2009). Such a microbial population provides enough food for the rather poor population of metazoans, including Copepoda.

In bore holes, where most samplings are usually done, there is a high probability that additional food for stygobionts comes from the surface, especially when bore holes are not capped (Karanovic, 2006). At the same time, larger or more aggressive species/specimens can prey on smaller ones in bore holes, reducing the actual local groundwater biodiversity (Brancelj & Culver, 2005). Thus, sampling bore holes with a Cvetkov phreatobiological net can provide relatively large numbers of specimens, even when relatively small volumes of water are filtered (Hancock & Boulton, 2009) but the results may not reflect the real community structure. Direct pumping of water provides some more realistic estimates of abundance and biodiversity of groundwater fauna, although results can depend greatly on the mobility of animals or their resistance to water flow induced by pumping. In most samplings by means of pumps >1000 l of water was filtered and only in a few the filtered volume was <1000 l (for instance Malard et al., 1997; Hancock & Boulton, 2009).

In 2008, several wells at the water-supply station near Ljubljana were sampled for one year. These wells collect water at 8-96 m depth. Each well is equipped with a pump with a capacity of about 10 l s^{-1} . Several taxa of Nematoda, Oligochaeta, and Crustacea, other than Copepoda were collected, too. Among "non-Copepoda taxa" one taxon of Bathynellacea and two taxa of Amphipoda were collected quite regularly. Several species of Copepoda usually found in low numbers (i.e., *Ceuthonectes serbicus* Chappuis, 1924; *Elaphoidella charon* Chappuis, 1936; and also *Graeteriella unisetigera* (Graeter, 1908)), appeared to be abundant in some wells (for a complete list of taxa and their abundance see table I). Among Copepoda, a rather large number of specimens was identified as members of a new species of *Paramorariopsis* Brancelj, 1991, which is described hereafter.

MATERIAL AND METHODS

Location and sampling methods

The sampling area is located on an alluvial plain 10 km south of Ljubljana and 0.7 km south of the small village of Brest. The coordinates of the sampling

TABLE I

List of Copepoda collected from water supply station (village the Brest, Ljubljana, SLO) and their cumulative abundance in a period 14 February 2008–3 March 2009

	Shallow sub-aquifer* (10-26 m b.g.l.)	Deep sub-aquifer* (31-96 m b.g.l.)	Temporary pool**
CYCLOPOIDA			
<i>Diacyclops languidoides</i> (Lilljeborg, 1901)	149	1	2
<i>Graeteriella unisetigera</i> (Graeter, 1908)	1	31	<300
Cyclopoida — species 1 (cf. <i>Austriocyclops</i>)	1		
Cyclopoida — species 2 (<i>Acanthocyclops</i> cf. <i>sambugarae</i>)		1	2
HARPACTICOIDA			
<i>Bryocamptus pyrenaicus</i> (Chappuis, 1923)		1	1
<i>Ceuthonectes serbicus</i> Chappuis, 1924	1	98	1
<i>Elaphoidella charon</i> Chappuis, 1936	126	2	
<i>Elaphoidella millennii</i> Brancelj, 2009	2	1	
<i>Nitocrella hirta</i> Chappuis, 1923	1		
<i>Paracamptus schmeili</i> (Mrázek, 1893)		4	1
<i>Paramorariopsis brigatae</i> n. sp.		64	6
<i>Parastenocaris gertrudae</i> Kiefer, 1968	1		
<i>Parastenocaris nollii alpina</i> Kiefer, 1969		6	
Harpacticoida — species 1 (cf. <i>Chappuisius</i>)		4	

b.g.l., below ground level; * sub-aquifer, see explanation in text; ** filled with pumped water from a deep sub-aquifer.

area are: 45°57'39"N 14°29'42"E; altitude: 300 m a.s.l. The plain was in the past, after the Mindel glaciation (about 500 000 years ago), filled with alluvial sediment transported by the river Iška (Pavšič, 2009). The alluvial plain is hydro-geologically closely connected to the nearby Krim massif, where the dominant geology is a mixture of limestone and dolomite.

The whole aquifer in the alluvial plane is separated into three sub-units: the upper, middle and lower one. They are separated with two, about 2 m thick impermeable layers of clay, which prevent hydrological communication between sub-units. These layers are situated at a depth of 30-32 and 58-60 m, respectively (Geological Survey of Slovenia, technical report).

On the alluvial plain, several shallow (up to 28 m deep) and deep (up to 100 m deep) wells were drilled, from which drinking water is pumped into the pipe system of the city. The main source for the upper sub-unit of aquifer is the nearby river Iška (800 m from the well), while for the middle and lower

sub-units the main source is percolating (i.e., karstic) water from the nearby Krim massif (Brancelj, 2009).

Four wells were sampled for groundwater fauna from February 2008 to March 2009, at approximately two-week intervals. Three wells collect water from an upper sub-unit of aquifer and one collects water from the middle and lower sub-unit of aquifer. Water from each well was pumped for 30 minutes at a rate of 8-10 l s⁻¹ (i.e., in total 14.4-18 m³ per well on each date) and filtered through a plankton net (mesh size 60 µm).

Most of the sampled specimens of *Paramorariopsis brigittae* n. sp. originated from a well (indicated as 1A), but some animals were collected also from a nearby small temporary pool, filled by water pumped from well 1A. The diameter of the well is 800 mm, with holes in the inox-steel tube of the well with a dimension of 100 × 5 mm. The perforated section of the well is between 33-55 m (middle sub-unit of aquifer) and 62-96 m (lower sub-unit of aquifer) depth.

The average temperature of the water in the well 1A was 11.4°C, conductivity 481 µS cm⁻¹, pH 7.6, and the value of total organic carbon (TOC) was 0.55 mg l⁻¹. Oxygen concentration created by the pumping process was not measured.

Within 4 hours of the time of sampling, the samples were sorted in the laboratory and specimens were preserved in 70% alcohol.

Before dissection, specimens were placed in a mixture of glycerol and 70% alcohol (ratio ~1 : 10 v/v), which was replaced within one hour by pure glycerol. They were dissected at 100× magnification under an Olympus SZH 2 stereomicroscope. All appendages and body ornamentation were examined under a magnification of 1000×. All drawings, except for the female habitus, were made at the same magnification (1000×), with a drawing tube mounted on an Olympus microscope (BHS 40). Final versions of the drawings were made using the CorelDRAW® 12 graphic program. For permanent slides, all body parts were put in a drop of glycerol on a microscope slide, covered by a cover glass and sealed with nail polish.

The following abbreviations are used throughout the text and figures: NHM = Natural History Museum (London); Enp = endopod; Exp = exopod; Exp/Enp-1 = proximal segment; Exp/Enp-2 = middle segment; Exp/Enp-3 = distal segment; P1-P5 = thoracic appendages. Nomenclature and descriptive terminology follow Huys & Boxshall (1991).

TAXONOMIC ACCOUNT

Order HARPACTICOIDA Sars, 1903

Family CANTHOCAMPTIDAE Sars, 1906

Genus *Paramorariopsis* Brancelj, 1991***Paramorariopsis brigitae* n. sp.**

(figs. 1-2 (female), 3-4 (male))

Material examined. — Holotype: adult female, completely dissected and mounted on a slide in glycerol and sealed with nail polish; collected on 21 April 2008 in well 1A (village Brest, Ljubljana, Slovenia); deposited in NHM, registration no.: NHM 2009.245. Allotype: adult male, completely dissected and mounted on a slide in glycerol and sealed with nail polish; collected on 21 April 2008 in well 1A (village Brest, Ljubljana, Slovenia); deposited in NHM, registration no.: NHM 2009.246. Paratypes: 4 ♂♂ and 11 ♀♀ (collected on 13 October 2008); deposited in NHM; registration no.: NHM 2009.247-256. Supplementary material: 1 ♀ (collected on 14 February 2008; completely dissected, on a slide), 4 ♂♂ and 3 ♀♀ (21 April 2008), 1 ♀ (collected on 10 June 2008; completely dissected; on a slide), 1 ♀ (collected on 7 July 2008), 1 ♀ (collected on 28 July 2008), 1 ♀ (collected on 22 September 2008), 3 ♂♂ and 9 ♀♀ (collected on 2 October 2008), 3 ♂♂ and 10 ♀♀ (collected on 23 October 2008), 3 ♂♂ and 5 ♀♀ (collected on 14 November 2008), 1 ♂ and 5 ♀♀ (collected on 23 November 2008; male completely dissected, on a slide), 2 ♀♀ (collected on 16 January 2009); deposited in the author's collection; non-dissected material stored in 70% alcohol. All the material collected by the author.

Description. — Female: body length, measured from tip of rostrum to posterior margin of caudal rami, 443-466 μm (mean: 450; $n = 10$); elongated, with cephalothorax as wide as the rest of the body, colourless (fig. 1A). Naupliar eye not discernible. Rostrum small. Cephalothorax with 3, and following thoracic somites with 1 or 2 pairs of sensilla laterally; integumental window (Nackenorgan, nucal organ) not present (fig. 1A). Posterior margins of thoracic and abdominal somites dorsally finely serrated (fig. 1B). Genital double-somite ventro-laterally with a row of 12-15 robust spinules with a wide gap medially (fig. 1C), spinules not extending on dorsal side. Dorsal side with 3-4 longitudinal rows of small spinules, with a gap medially, except the posterior one. Genital complex (fig. 1C) with single, large, sclerotized and bell-shaped copulatory pore; seminal receptacles relatively large and rigidly sclerotized. Fused plate with reduced P6 positioned just above seminal receptacles. Ventral distal margin of third and fourth abdominal somite with a row of strong spinules (fig. 1C). Anal somite ventrally with three strong spinules near base of caudal ramus, median one the strongest; five strong spines laterally; short row of very small spinules medially (fig. 1C); dorsally with three transversal rows of small spinules; distal one just above base of operculum with small sensillum at each end (fig. 1B).

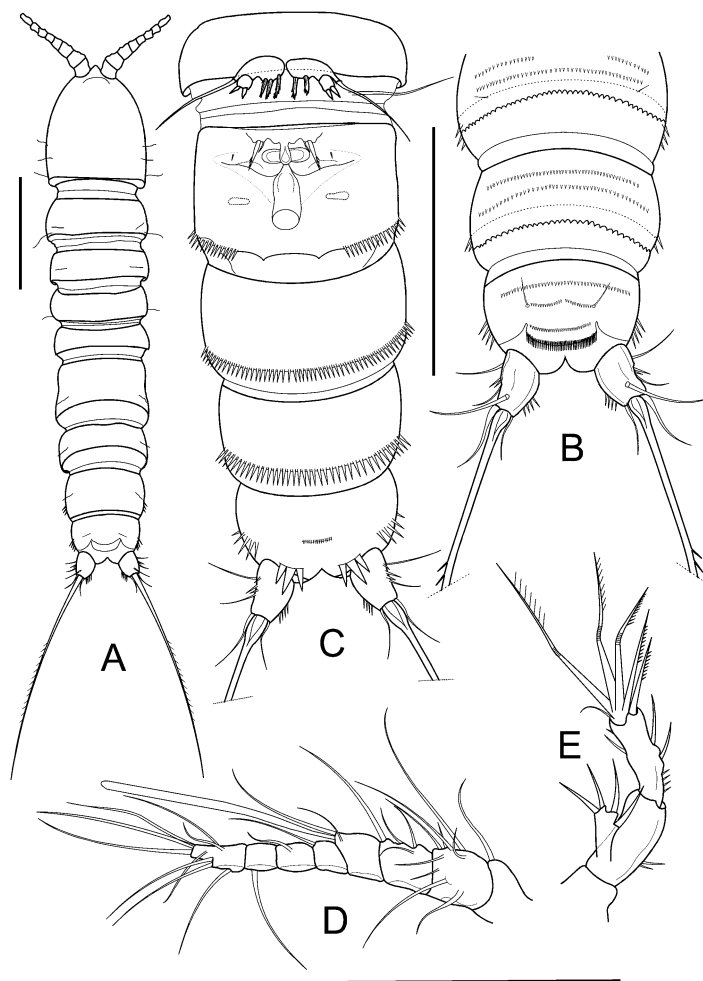


Fig. 1. *Paramorariopsis brigatae* n. sp. A, B, C, female paratype; D, E, female holotype. A, habitus, dorsal view (variability); B, abdominal somites and anal somite, dorsal view (variability); C, genital double-somite, abdominal somites and anal somite, ventral view (variability); D, antenna; E, antenna. Scale bars: 100 μ m.

Anal operculum large, rounded, not reaching distal end of anal somite, with about 40 fine spinules along distal margin (fig. 1B).

Caudal rami conical, divergent, about 1.5 times as long as wide, without dorsal keel (fig. 1B, C). Inner margin smooth. Anterolateral seta (I) inserted at about 1/3 of caudal ramus; slightly shorter than caudal ramus, with a small spinule near its insertion. Mediolateral accessory seta (II) very short and thin, with additional small spinule near its insertion. Posterolateral seta (III) slightly shorter than anterolateral seta (I). Outer terminal seta (IV) as long as caudal

ramus, weak; fracture plane not visible. Inner terminal seta (VI) slightly shorter than caudal ramus, weak. Dorsal seta (VII) about 1.5 times as long as caudal ramus, inserted laterally at about 3/4 length of caudal ramus. Medial terminal seta (V) as long as abdomen, distal two-thirds with a row of spinules on both margins; fracture plane not visible.

Antennule (fig. 1A, D) relatively short, eight-segmented. Aesthetasc on fourth segment cylindrical, straight, with rounded tip and well over-reaching end of antennule. Second aesthetasc, on terminal segment, shorter and weaker than the first one. Setal formula: 1.9.4.2 + ae.1.1.1.8 + ae.

Antenna (fig. 1E) with allobasis, and robust one-segmented Exp and Enp. Enp with two strong spines on outer margin accompanied by several strong shorter spinules of different lengths; terminal armature consisting of one short spine, and one normal and three geniculate setae. Exp with four spiniform setae.

Mandible (fig. 2A1, A2) short and robust, with three strongly chitinized teeth on gnathobase. One dorsal seta near gnathobase. Mandibular palp short, two-segmented; Exp reduced to one seta and Enp to three setae, subequal in length.

Maxillule (fig. 2C): Praecoxal arthrite with one seta on anterior surface, distal margin with six spines. Coxa without endites, carrying one long transformed seta. Basis with one strong bipinnate spine and two setae; Enp represented by four setae.

Maxilla (fig. 2B): Praecoxa naked, with one coxal endite carrying three thin spines and one seta. Coxa drawn out into two slightly curved, thin claws, the inner one bearing one thin seta, outer one a row of fine spinules. Basis drawn into two slightly curved claws, the inner one bearing one thin seta. Enp represented by two setae of subequal length.

Maxilliped (fig. 2D) comprising syncoxa, basis, and one-segmented Enp. Syncoxa without ornamentation. Basis three times as long as wide with about 30 spinules in two rows positioned near palmar margin dorsally, decreasing in length distally. Enp drawn out into strong, acutely curved claw; slightly longer than basis and armed with several spinules in distal half; accessory armature represented by short seta.

P1 (fig. 2E) with three-segmented Exp and two-segmented Enp. Enp as long as Exp. Basis with inner spine and relatively weak outer basal spine. Exp-1 and Exp-2 with one strong outer spine and several small spinules on each segment. Exp-3 with one strong spine on the outer distal corner, and one spine and two long geniculate setae apically, the inner one as long as Exp. Enp-1 with

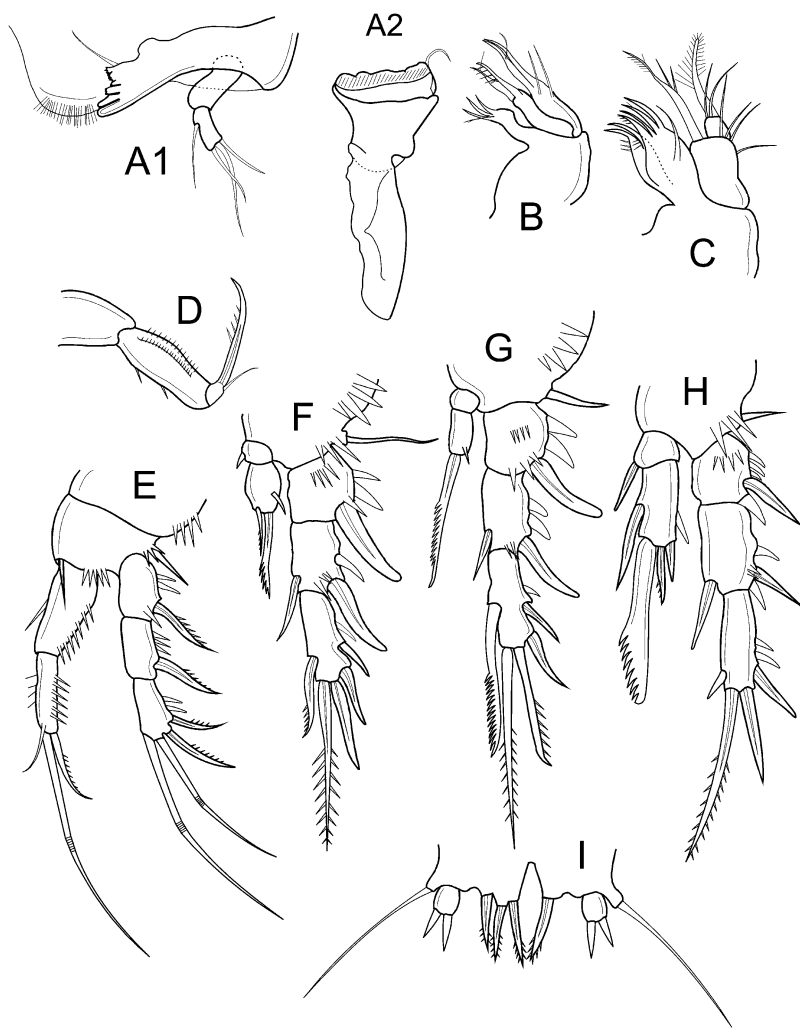


Fig. 2. *Paramorariopsis brigatae* n. sp., female holotype. A1, mandible, ventral view; A2, mandible, inner side; B, maxilla; C, maxillule; D, maxilliped; E, P1; F, P2; G, P3; H, P4; I, P5. Scale bar: 100 μ m.

several strong spinules along outer margin; short seta at half length of inner margin. Enp-2 with three setae; innermost thin and short, terminal seta long and geniculate, outer one spiniform, with spinules on outer margin.

P2 (fig. 2F) basis with outer seta. Three-segmented Exp and two-segmented Enp. Enp as long as Exp-1. Exp-1 as long as wide, with one strong blunt spine at distal corner. Exp-2 1.5 times as long as wide, with one strong outer spine with rounded tip, one thin inner spiniform seta. Exp-3 two times as long as

wide, with two outer spines, one terminal spine and one terminal seta, and one spiniform inner seta. Outer terminal spine half the length of inner terminal seta. Enp-1 shorter than wide, with one short spine on inner margin. Enp-2 1.5 times as long as wide, with one short spine subapically on outer margin; one outer terminal seta longer than terminal segment, unilaterally feather-like; one inner terminal seta half the length of outer one, with several strong spinules unilaterally at the tip.

P3 (fig. 2G) basis with spiniform outer seta. Exp-1 and 2 similar to that of P2; Exp-3 with outer terminal seta with several strong spinules unilaterally on the distal half, inner terminal seta 1.5 times as long as outer one. Inner seta long, unilaterally feather-like. Enp two-segmented; proximal segment shorter than wide, bare. Terminal segment twice as long as wide, with one short subapical spine; a long terminal seta, unilaterally feather-like.

P4 (fig. 2H) basis with short outer spine. Three-segmented Exp and two-segmented Enp. Exp-1 and Exp-2 with distal outer spine. Exp-3 2.5 times as long as wide, with one small outer spine, one robust blade-like outer terminal and one inner terminal spiniform seta arcuated inward, robust, with several strong spinules on distal half. Inner seta very reduced, spine-like. Outer terminal seta as long as segment bearing it, inner terminal seta twice as long as inner one. Proximal segment of Enp short, with one long spine on inner margin. Enp-2 twice as long as wide, with one long, spiniform seta on inner margin and one sub-terminal spiniform seta on outer margin, two short, spiniform terminal setae. And one transformed inner terminal seta, longer than Enp, very flat and unilaterally feather-like.

Additional ornamentation of P1–P4 as in fig. 2E–H.

P5 (figs. 1C, 2I): with separate Exp and baseoendopod; baseoendopod poorly-developed, with 1-3 strong pinnate spines, sub-equal in length. Exp small, as long as wide, with two short spiniform apical setae. Outer lateral seta on baseoendopod very long and bare.

Egg sac: not present.

Male: slightly smaller than female; body shape similar to that of female; body length, measured from tip of rostrum to posterior margin of caudal rami, 415-438 μm (mean 429; $n = 6$). Posterior margins of thoracic and abdominal somites dorsally smooth. Abdomen dorsally with many sensilla (fig. 3A). Ventral distal margin of second, third, and fourth abdominal somite with continuous row of small spinules each. Anal somite ventrally with three strong spines at base of caudal ramus. Laterally four strong spinules on each side, positioned on middle of somite (fig. 3B). Anal operculum large, rounded,

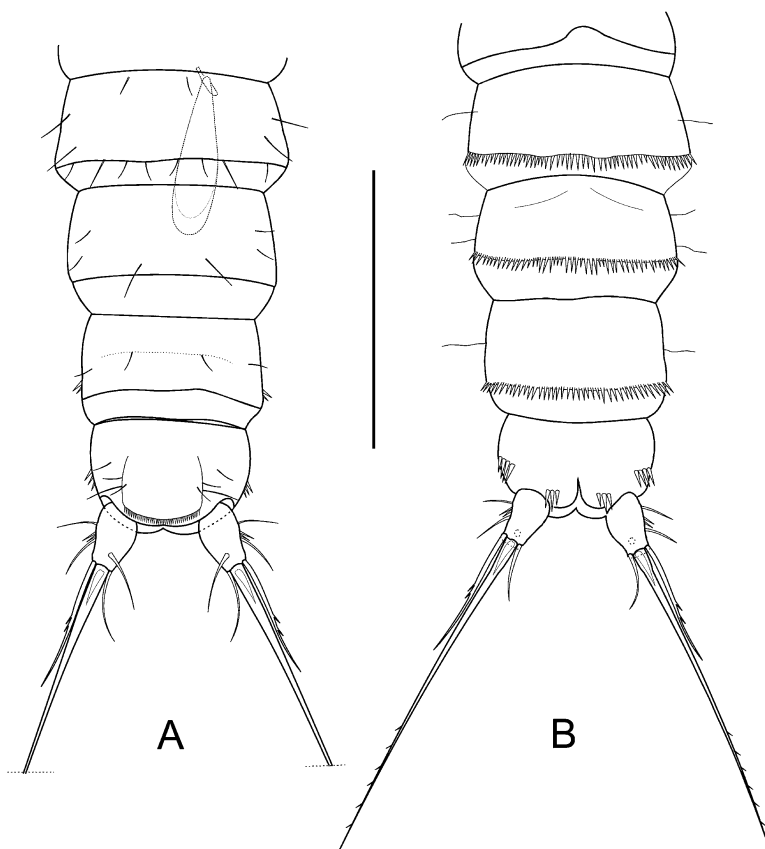


Fig. 3. *Paramorariopsis brigittae* n. sp., male allotype. A, abdominal somites 2-4 and anal somite, dorsal view; B, abdominal somites 1-4 and anal somite, ventral view. Scale bar: 100 μm .

not reaching to distal end of anal somite, with many small spinules along distal margin (fig. 3A). Caudal rami similar to those of female, but outer terminal seta twice as long as in female, robust, with 2-3 small spinules laterally (fig. 3A, B).

Antenna (including setal formula) and mouthparts similar to those of female.

Antennule (fig. 4A) eight-segmented. Aesthetasc on fourth segment cylindrical, curved, with rounded tip and slightly longer than antennule. Second aesthetasc on terminal segment small, seta-like.

P1 (fig. 4B) with Exp similar to that of female. Enp as long as Exp. Enp-1 with several strong spinules along outer margin; relatively long, unilaterally feather-like seta at half length of inner margin. Enp-2 with three setae; innermost soft and short; terminal seta long and geniculate; outer one half the length of terminal one, spiniform, with spinules on outer margin.

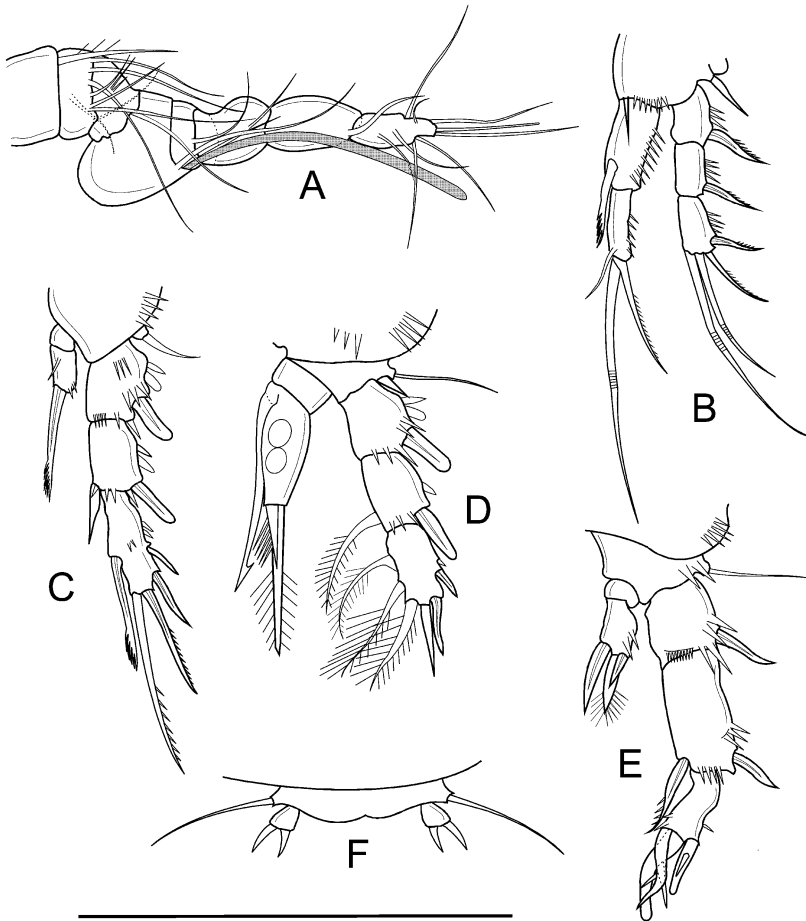


Fig. 4. *Paramorariopsis brigatae* n. sp., male allotype. A, antennule; B, P1; C, P2; D, P3; E, P4; F, P5. Scale bar: 100 μ m.

P2 (fig. 4C) basis with outer spiniform seta. Three-segmented Exp and two-segmented Enp. Enp shorter than Exp-1. Exp-1 as long as wide, with one strong, round-tipped spine at distal corner. Exp-2 with one strong round-tipped outer spine and short, robust inner spiniform seta. Exp-3 2.5 times as long as wide, with two outer spines, one apical outer spine, one long apical inner seta, and one transformed unilaterally feather-like inner seta. Outer apical spine about 2/3 the length of inner apical seta. Inner seta as long as outer terminal seta. Enp-1 shorter than wide, bare. Enp-2 1.5 times as long as wide, with two short spines along outer margin, one short seta on inner margin and one unilaterally feather-like terminal seta, 2.5 times as long as terminal segment.

P3 (fig. 4D) with long, bare seta on outer margin of basis. Exp-1 and 2 similar to those of P2 but inner seta of Exp-2 long, with several long spinules on distal half. Exp-3 with two relatively weak spines on outer margin. One short terminal spine and short seta; two setae on inner margin. Enp two-segmented; proximal segment shorter than wide, bare. Enp-2 with long proximal inner spine, with hook-like tip; with short, unilaterally spinulose inner apical spine and robust outer terminal seta, as long as Enp-2.

P4 (fig. 4E) with long, bare outer basal seta. Three-segmented Exp and two-segmented Enp. Exp-1 as long as wide, one robust spine on distal outer corner. Exp-2 twice as long as wide, with robust spine on distal outer corner and short, robust spiniform seta on distal inner corner. Exp-3 2 times as long as wide; outer spine and two terminal setae heavily modified. Outer spine slightly shorter than Exp-3, very robust, with rounded tip and strong spinule laterally. Outer terminal seta as long as Exp-3, curved, with rounded tip and strong spinule laterally. Inner terminal seta slightly shorter than outer one, with sharp tip and strong spinule laterally. Proximal segment of Enp short, bare. Enp-2 twice as long as wide, with one sub-terminal seta shorter than Enp-2, and two terminal robust, blade-like setae equal in length, as long as Enp-2, the inner one bare, outer with spinules distally.

Additional ornamentation of P1–P4 as in fig. 4B–E.

P5 (fig. 4F): baseoendopod without spines/setae. Exp small, pear-like, with two short, robust, spiniform apical setae. Outer lateral seta on baseoendopod very long and bare.

P6: not present.

Spermatophore: elongated, drop-like (fig. 3A).

Variability. — No variability was observed in males except for the number of spinules along Exp-1 of P4: normally three but sometimes four. In females variability was observed in the ornamentation of the caudal rami, where about 10% of the females have three or four spines on the inner margin (fig. 1A, B); combinations with only one or two spines were not observed. However, females with or without spines have identical armature of P1–P4 as well as other characters. Additional variability was observed in number of pinnate spines on baseoendopod of P5 of females, which varied from one to three in the same specimen, with the most common combination being 2 + 3 spines, followed by 3 + 3 and 3 + 1.

Etymology. — The new species is named after Brigita Jamnik, Ph.D., who granted permission to the author to sample the wells at the water-supply pumping station. The name thus is a noun in the genitive singular.

PHYLOGENETIC RELATIONSHIPS

The number of segments of Enp P1–P4 in females was the character that allowed establishing the genus *Paramorariopsis* Brancelj, 1991 (cf. Brancelj, 1991). The first described species, *Paramorariopsis anae* Brancelj, 1991, has a reduced number of Enp segments on P2 and P3 (endopodal formula 2/1/1/2), distinguishing it from the genus *Moraria* T. & A. Scott, 1893, which has the Enp P1–P4 formula 2/2/2/2, and from the genus *Morariopsis* Borutzky, 1930, which has the segmentation formula 2/1/1/1. The additional two species described for the genus, *P. irenae* Brancelj, 2006 and *P. brigittae* n. sp., have the same segmentation of Enp P1–P4 as *Moraria*, which sets the genus *Paramorariopsis* closer to *Moraria* than to *Morariopsis*. At the same time, the male's Enp P3 in the new species is 2-segmented, as opposed to 3-segmented in *P. anae*. However, the new characters recorded in *P. brigittae* n. sp. do not change the diagnosis of the genus *Paramorariopsis*, but modify it as follows:

Female: small and slender harpacticoid with short antennule. Exopodites of P1–P4 three-segmented, Enp of P1 and P4 two-segmented, Enp P2 and P3 one- or two-segmented. Enp of P1 as long as Exp. Basipodite of P5 with 3 (exceptionally 2 or 1) spines, Exp short, with two spines. Caudal rami short and divergent.

Male: in habitus similar to female. Exp of P1–P4 three-segmented, Enp of P1, P2, and P4 two-segmented; Enp P3 two- or three-segmented. Spines on distal segment of Exp P4 antler-like. Basipodite of P5 spineless, Exp short, with two spines.

The most probable phylogenetic relation between the genera *Moraria*, *Morariopsis*, and *Paramorariopsis* is that the predominantly stygophilic genus *Moraria* is an ancestor of both later stygobitic genera, one being distributed in the pre-Alpine and Alpine regions (i.e., *Paramorariopsis*), where several undescribed species were collected (F. Stoch, pers. comm.). The second one (i.e., *Morariopsis*) is present in the Dinaric region (Kiefer, 1930; Petkovski, 1959; Brancelj, 2000), with a disjunct location around Lake Baikal (Borutzky, 1964).

Both stygobitic genera fit the biogeographical pattern already described for the stygobitic Cladocera (cf. Brancelj & Dumont, 2007), where eastern (i.e., the Dinaric) taxa have more reductions of some body parts than western ones (i.e., the Alpine). In Copepoda, the eastern taxa (genus *Morariopsis*) have a more reduced number of segments on Enp P2–P4 in females than do taxa belonging to the genera *Moraria* and *Paramorariopsis*. However, in *Morariopsis* there is a similar transformation of the terminal spine on the

male's Enp-2 P4 (spiral-like) as in *Moraria*. In *Paramorariopsis*, the number of segments on P2–P4 is similar to that of *Moraria*, but the transformation of the terminal spines of P4 in males (antler-like) is different from that of *Moraria*. Reductions of P5 in males and females of *Paramorariopsis* are greater than those of *Morariopsis* and *Moraria*. Differences in segmentation and ornamentation of P1–P4 presumably are a result of the specific habitat occupied by a particular genus (Galassi, 2001; Galassi et al., 2009).

A unique feature in *P. brigatae* n. sp. is the variable number of pinnate spines on the baseoendopod of P5 in females, which is here recorded for the first time in Canthocamptidae. We hypothesize that such variability, along with the presence/absence of additional spines on the inner margins of the caudal rami in females, indicates that some characters are not constant and are actually a reflection of environmental conditions (for details see discussion below).

In *Paramorariopsis brigatae* n. sp., the body shape, the short and divergent caudal rami, and the characteristic structure of P5 in both sexes lead to its classification as a member of the genus *Paramorariopsis*. Although the new species shares all the general characteristics with the other two members of the genus, there are clear differences between them (table II). Females of all three species differ in the shape and form of the caudal rami, which is shortest and most divergent in *P. anae* and longest and least divergent in *P. brigatae* n. sp. Females can also be well distinguished on the basis of the anal operculum, which is smooth and rounded in *P. anae*, with numerous fine spinules in *P. brigatae* n. sp., and with several strong spinules in *P. irenae*. The P5 in both sexes has a characteristic structure but with a number of spines on the baseoendopodite in females. Of the three species, *P. anae* shows the greatest reduction in segmentation of Enp P1–P4, with 1-segmented Enp P2 and P3, while the other two species have all Enp P1–P4 2-segmented. Females of *P. irenae* and *P. brigatae* n. sp. have spines on the inner margin of the caudal rami, although these are absent in part of the population of *P. brigatae* n. sp. In *P. anae* there are no additional spines on the inner side of the caudal ramus and no spines on the operculum, which are present in *P. irenae* and *P. brigatae* n. sp.

The males, known only from *P. anae* and *P. brigatae* n. sp., differ mainly in the morphology of P3 and P4. In *P. brigatae* n. sp. the setae are short and robust on Enp and Exp P3 but long and thin in *P. anae*. The main difference is in P4, both in the armature and in its “robustness”. Enp-2 P4 in *P. brigatae* n. sp. has three robust spiniform setae on the distal end, compared with *P. anae* that has one short spine with rounded tip and one long, fine seta. The antler-like transformed terminal setae on Exp-3 are more robust in *P. brigatae* n. sp.,

TABLE II
Differential characteristics between three *Paramorariopsis* species from Slovenia (females only)

Character	<i>P. anae</i>	<i>P. irenae</i>	<i>P. brigittae</i>
	Brancelj, 1991	Brancelj, 2006	n. sp.
Ornamentation of free margin of operculum	No spinules (smooth)	~20 strong spinules	~40 fine spinules
Divergency of furcal rami	<90°	~60°	~45°
Shape of furcal ramus (width/length)	1 : 1	1 : 1.5	1 : 1.5
Spinules on inner margin of furca (number)	Absent	Present (about 10)	Absent or present (4)
Spinules at the base of furcal ramus	5	4	3
Length of outer terminal spine/length of Fu* (form)	~2-times (robust)	~0.8-times (spiniiform)	~1-time (soft)
Length of inner terminal spine/length of Fu (form)	~1.5-times (soft)	~0.2-times (spiniiform)	~0.8-times (soft)
Ventral row of spinules on 3 rd abdominal somite	Interrupted	Interrupted	Continuous
Number of segments on End P1–P4	2/1/1/2	2/2/2/2	2/2/2/2
Inner terminal seta on End-1 P2	Absent	Absent	Present
Inner terminal seta on End-1 P3	Absent	Present	Absent
Length of Exp-1 P4/End P4	1 : 1.2	1 : 1	1 : 1.5
Inner terminal seta on End-2 P4	Short, spiniiform	Medium, robust	Large, feather-like

Fu* , furcal ramus.

two of them with rounded tips, but sharp in *P. anae*. The length of the caudal rami also differs, being longer in *P. brigatae* n. sp., and the armature of the operculum is smooth in *P. anae* but with a row of fine spinules on its distal margin in *P. brigatae* n. sp.

DISCUSSION

The genus *Paramorariopsis* is considered, along with *Morariopsis*, a typical representative of (epi-)karstic habitats (Brancelj & Culver, 2005; Brancelj, 2006), and the genus *Paramorariopsis* has also been reported from other karstic and/or alpine regions in western Europe (F. Stoch, pers. comm.). The new location sheds a slightly different light on habitat occupation by *Paramorariopsis*. The well where the new species was collected is about 800 m from the Krim Massif, where the dominant geology is dolomite (Brancelj, 2009; Pavšič, 2009), which also continues in the direction of the well. At the location of the well, the dolomite bedrock is covered by about 100 m of alluvium, which is completely filled with water (i.e., a porous aquifer) with a water table at about 8 m b.g.l. (Brenčič, 2009). There are two layers of fine clay at the location, which separate the aquifer hydrologically in vertical direction into three sub-units. Well 1A collects water from the middle (31-55 m b.g.l.) and the lowest sub-unit of the aquifer (60-104 m b.g.l.), while other wells collect water from the upper sub-unit of aquifer (10-26 m b.g.l.) (table I). From table I it is obvious that the aquifer is not only divided hydrologically, but also in terms of faunal composition. Omitting singletons and doubletons (i.e., species represented by only one or two specimens during the whole sampling campaign) (Eberhard et al., 2009), five of the most abundant species provide an additional indication of different aquifers, too. The shallow sub-aquifer is dominated by *Diacyclops languidoides* and *Elaphoidella charon*, while the middle and lower sub-aquifers are dominated by *Ceuthonectes serbicus*, *Graeteriella unisetigera*, and *P. brigatae* n. sp. Since the bottom of well 1A comes close to the dolomite bedrock, it is possible that the collected population of *P. brigatae* n. sp. has actually drifted from the Krim Massif, which is a karstic plateau with no surface run-off. In the nearby cave Velika Pasica, random drift of copepods was observed from epikarst with more intensive transport of specimens after intensive precipitation (i.e., catastrophic drift) (Brancelj, 2000, 2002, 2009; pers. observ.). The massif is actually a large rain-water and melt-water collector. Water from the massif appears in some small permanent springs along the foothills and in a deep siphon spring nearby. The

siphon has its lowest (known) point some 50 m b.g.l. (Brenčič, 2009). A siphon of this kind, completely filled with unconsolidated sediment, could be expected also in the area of the water supply station. Water flow from the siphon is probably sufficiently intensive to transport copepods from the karstic into the porous aquifer. This hypothesis is supported by the fact that the specimens of *P. brigatae* n. sp. (and *C. serbicus*, too) were collected only after the rainy season in autumn, when more intensive drift is expected to occur (details will be published in a separate article, discussing the fauna and hydro-geochemical conditions in the aquifer in more detail; Brancelj et al., in prep.).

The hypothesis of intensive water transport from the karstic aquifer into the porous one also explains the robust and specific armature of the swimming legs in the new species, which is more suitable for life in small fissures where such morphological adaptations (strong armature), along with behavioural adaptations (positive rheotaxis) are crucial for survival (Brancelj, 2006). From this point of view, *P. brigatae* n. sp. could be considered an inhabitant of a karstic aquifer that has drifted into the adjacent porous aquifer, where it probably constitutes a sink population that is bound to die, due either to an unfavourable physical environment, or to predation or competition (Brancelj & Culver, 2005; Brancelj, 2006). In any case, the relatively large number of collected specimens of *P. brigatae* n. sp. indicates that there is a large population in the Krim Massif that provides a constant and/or catastrophic drift into the porous aquifer.

The variable number of pinnate spines on the baseoendopod of P5 in females of *P. brigatae* n. sp. as well as the presence/absence of spines on the inner margins of the furcal rami support a hypothesis that porous aquifers are not the original habitat of the species. Additional support for this hypothesis is the complete absence of juveniles and the absence of spermatophores (but one) or egg sacs in 51 collected females. The main reason for the absence of reproduction as well as the variability in some characters could be quality and quantity of food. Scarce and/or unfavourable food can support survival of specimens but result in incomplete formation of some characters (i.e., spines) and lack of reproduction (although no such changes could be expected once the adult stage has been reached).

ACKNOWLEDGEMENTS

The author is grateful to Brigita Jamnik, who organized the permits to sample the wells; to my colleagues, Andrej Kapla and Tadej Mezek, for their assis-

tance during field work; and to Roger Pain for language improvement. Special thanks to both referees for their valuable comments and recommendations.

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First received 2 September 2009.

Final version accepted 25 March 2010.