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First Record of the Harpacticoid Genus *Morariopsis* (Crustacea: Copepoda: Canthocamptidae) in Japan, and its Zoogeographic Implications

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Morariopsis grygieri sp. nov. is described from two caves in the town of Taga, in the mountainous flanks of ancient Lake Biwa, and a key to world species of *Morariopsis* is provided. Other known members of the genus live in two disjunct areas: three species are benthic dwellers in Lake Baikal, while three live in subterranean waters of the western Balkan Peninsula, in Slovenia and Croatia. In order to confirm the phylogenetic relationships of our new species, and to test whether the Baikal and Balkan groups represent one colonisation event each or more, a cladistic analysis was performed on all seven species and two outgroup taxa, based on 21 morphological characters. The resulting cladogram shows that the ingroup is well defined by at least two synapomorphies, and that the Baikal group is also a well-defined clade, probably representing a single colonisation event. The three Balkan species are only remotely related to each other, and all probably represent separate colonisations. The new species is not closely related to any of its presently known congeners, and also represents a separate colonisation event. We speculate that the ancestor of this species first colonised benthic habitats of ancient Lake Biwa, during its tectonic formation, and from there colonised subterranean waters in its surroundings during the major climatic changes of the Quaternary. We suggest, as a novel hypothesis, that ancient lakes also act as biodiversity pumps for subterranean habitats.

Key Words: Harpacticoida, ancient lakes, biodiversity pump, cladistics, phylogeny, stygofauna.

Introduction

The canthocamptid harpacticoid genus *Morariopsis* Borutzky, 1931 contains six species (Walter 2010) and has a disjunct distribution, being found so far only in the western Balkans (SE Europe) and the ancient Lake Baikal. The first record was that by Kiefer (1930), who reported on a small collection of subterranean copepods from the Cave of St. Canzian in Italy (now called Skocjanske Jame and in Slovenia), where he briefly described one unusual harpacticoid female as a new species of *Moraria* T. Scott and A. Scott, 1893, *M. scotenophila* Kiefer, 1930. Unlike any other *Moraria,* this species had one-segmented endopods of the second, third, and fourth swimming legs, but Kiefer was not sure about the significance of this, as the endopods' condition was not known in some representatives of the genus at the time. Almost at the same time, Borutzky (1931) was working on the very rich harpacticoid fauna of Lake Baikal, from which he described numerous representatives of the genus *Moraria*. In the same paper, he also described two benthic species with one-segmented endopods of the second, third, and fourth swimming legs; for them he erected a new genus, *Morariopsis* Borutzky, 1931. Both of Borutzky's species were described after females only, and *Morariopsis typica* Borutzky, 1931 was also nicely illustrated, while for *M. latifurcata* Bortuzky, 1931 only the drawings of the caudal rami and the fifth leg were provided. It seems that Bortuzky (1931) was not aware of Kiefer's (1930) paper, even though both were published in the same journal. It was Lang (1948) who first recognized that the Slovenian species belongs to the genus *Morariopsis*, and he also reproduced all of Borutzky's and all except one of Kiefer's drawings. However, a few years later, Borutzky (1952) placed the Slovenian species as *incertae sedis* in the family Canthocamptidae Sars, 1903, within his newly erected subfamily Morarinae Borutzky, 1952, although he noted its close relationship with *Morariopsis*. It is almost as though he was not aware of Lang's (1948) work, although he listed this monograph among the references. Borutzky (1952) also provided some additional illustrations and descriptions for *M. latifurcata*, but his drawing of the antenna (fig. 100-2) is clearly recycled from his 1930 description of *M. typica* (fig. 33), and he also misinterpreted some large spinules on the endopods of the swimming legs for armature elements and *vice versa*. Borutzky (1952) corrected the original drawing of the caudal rami ornamentation for *M. typica*, as the posterolateral seta was missing in Borutzky (1931, fig. 27). Petkovski (1959) described another new species of *Morariopsis* from a cave near Split in Yugoslavia (now Croatia), as *M. kieferi* Petkovski, 1959. He proposed that his new species is more closely related to the Slovenian one than to the two Baikal representatives. Although he found 35 females in that cave, not a single male was recorded. Borutzky and Okuneva (1975) described the third representative from Lake Baikal as *M. baicalensis* Borutzky and Okuneva, 1975, after both females and males, describing a male of *Morariopsis* for the first time. They still did not consider the Slovenian *M. scotenophila* to be a member of this genus and were apparently unaware of the Croatian species described by Petkovski (1959). Brancelj (1986, 2001) reported some new localities in Slovenia for *M. scotenophila* and redescribed the female, but again did not record any males. Finally, Brancelj (2000) described another new species of *Morariopsis* from Slovenia, both after males and females, as *M. dumonti* Brancelj, 2000. However, he was not aware of the paper by Borutzky and Okuneva (1975), and erroneously reported his record of the male as the first one in the genus. He also provided a revised generic diagnosis. Brancelj (2002) studied microdistributions of 12 different copepod species in the Velika Pasjica cave, which is the type locality of *M. dumonti*. He collected more than 250 specimens of this species and showed that its habitat preference is for locations with high amounts of organic material.

During our collecting of stygofauna in Japan, we found one new species of *Morariopsis* in two caves in the town of Taga, in the Suzuka Mountains that bound the eastern side of ancient Lake Biwa's watershed. Lake Biwa originated tectonically in the Pliocene, almost five million years ago and less than 100 km south of its present position, and shifted northwards in stages in the late Pliocene to early Quaternary (Takahashi 1989). It had become large and deep by 400,000 years ago, and is marked by the presence of more than 50 endemic animal species and subspecies (Uéno 1975, 1984; Kawanabe 1978, 1996; Nishino and Watanabe 2000). Some species reach their southern limit in Lake Biwa, where they persist in cooler temperatures of deep waters. The role of the lake as a refugium for cryophilic elements during interglacial periods and for thermophiles during glacial maximums of the Quaternary is well studied, especially for freshwater snails (Nishino and Watanabe 2000). Just like many other Asian lakes (including Lake Baikal), Lake Biwa was never glaciated, as most of the northern ice-cap was located over Europe and North America (Gualtieri *et al.* 2000). The role of Lake Biwa in the colonisation of surrounding subterranean waters has never been studied, but one can easily imagine how a benthic harpacticoid living at 50+ m (where there is hardly any light) would be well adapted for this kind of habitat shift. Our hypothesis is that ancient lakes act as a sort of a biodiversity pump for subterranean habitats, and wherever they are surrounded by suitable porous carbonate sediments a rich copepod stygofauna can be found (Petkovski and Karanovic 1997; Karanovic 2000). A broader aim of this study was to test this hypothesis on the relatively poorly studied copepod fauna of Lake Biwa, where we sampled a range of habitats from caves to lake interstitial and surface waters; this paper shows that we are on the right track and that continuing studies are likely to reveal many interesting endemic elements.

A summary of research on surface-freshwater copepods of Japan was given by Ishida and Kikuchi (2000) for harpacticoids and by Ishida (2002) for cyclopoids. The only endemic Lake Biwa element was a species of the cyclopoid genus *Diacyclops* Kiefer, 1927 that was left undescribed in Ishida (2002), as *Diacyclops* sp. B. Later on, Ishida (2005) described another endemic *Diacyclops* from Lake Biwa, *D. biwensis* Ishida, 2005, and commented on the affinities of the still undescribed *Diacyclops* sp. B, concluding that: "…the *Diacyclops* species of Lake Biwa and Lake Baikal show similar tendencies in segmentation of the swimming legs." Finally, Ishida (2006) described *Diacyclops* sp. B as *D. brevifurcus* Ishida, 2006, but found it also in a pond in Kyoto, proving that the species is not endemic to the lake. Two other cyclopoids, originally described from Lake Biwa (*Eucyclops biwensis* Ishida, 1998 and *Mesocyclops dissimilis* Defaye and Kawabata, 1993), also proved to be more widely distributed (see Ishida 1998, 2002; Defaye and Kawabata 1993; Kawabata and Defaye 1994), although the record of the former in the African Lake Victoria by Ishida (1998) will have to be verified, and the validity of this species thoroughly evaluated. The only other endemic Lake Biwa copepod (besides *Diacyclops biwensis*) is a subterranean harpacticoid, *Parastenocaris biwae* Miura, 1969, described from a sandy beach on the western shore at Shirahige (Miura 1969); the species was later synonymised with the Holarctic species, *P. brevipes* Kessler, 1913 by Reid (1995). The subterranean copepods have been studied in Japan only sporadically, but the fauna seems to be very rich (Kiefer 1938; Ito 1952, 1954, 1957; Chappuis 1955, 1958; Miura 1962a, b, 1964, 1969; Kikuchi 1970; Ueda *et al.* 1996; Tomikawa *et al.* 2005). Advances in this field in other parts of the world (see Galassi 2001; Karanovic 2004, 2005, 2006; Karanovic and Hancock 2009) show how systematic studies of subterranean copepods can help solving complex zoogeographic questions of freshwater colonisations, and they also represent a disproportionately large component of diversity in many regions (Humphreys 2008).

Material and Methods

The material studied here was collected in two caves in Kawachi, Taga town, Shiga Prefecture, Japan, within the drainage area of Lake Biwa (eastern side). Locality data and number of specimens are listed in the description of the new species below. All material is deposited in the Lake Biwa Museum (LBM), Kusatsu, Shiga Prefecture, Japan.

Samples were collected with hand-nets (mesh size 0.125 mm) and small rubber pumps of various sizes, often even from the smallest pools and cracks that contained no more than a few drops of water. All nets had an outer protective sleeve that prevented the inner mesh from being torn by sharp cave ornaments in the pools. Gongendo Cave was almost dry and contained no more than a couple of liters of water, while Kawachi-no-Kazaana Cave additionally had several larger pools. Small dripping waters and pools that form underneath were targeted in both caves. In larger pools, the bottom was disturbed before sampling and also the interstitial was sampled wherever possible along the edges. Samples were preserved in the field in 99.5% ethanol and sorted later in the laboratory. Specimens were dissected and mounted on microscope slides in Faure's medium, which was prepared following the old procedure, discussed most recently by Stock and von Vaupel Klein (1996), and dissected appendages were then covered by a coverslip. For the urosome or the entire animal, two human hairs were mounted between the slide and coverslip, so the parts would not be compressed. By manipulating the coverslip carefully by hand, the whole animal or a particular appendage could be positioned in different aspects, making possible the observation of all morphological details. During the examination water slowly evaporated and appendages eventually remained in completely dry Faure's medium, ready for long-term storage. All drawings were prepared using a drawing tube attached to a phase-interference compound microscope (Leica MB2500), with N-PLAN objectives. Specimens that were not drawn were examined in a mixture of equal parts of distilled water and glycerol and, after examination, were again preserved in 99.5% ethanol. Photographs of whole specimens were taken in glycerol with a micro-camera (Leica DFC420) attached to a dissecting microscope (Leica M205C). The software package Leica Application Suite (LAS), version 3.5.0, was used to create a multifocal montage image.

Morphological terminology follows Huys and Boxshall (1991), except for numbering of setae of the caudal ramus and small differences in the spelling of some appendages (antennula, mandibula, and maxillula instead of antennule, mandible, and maxillule), in an attempt to standardise the terminology for homologous appendages in different crustacean groups. Also, for the armature formula of the swimming legs a much simplified convension is used, because there are a number of transitional forms between spine and seta on these appendages in copepods. To avoid possible confusion we sometimes use the term "armature element" (or just "element") instead of spine or seta, especially for the fifth legs. Biospeleological terminology follows Humphreys (2000).

The cladistic analysis was performed on all seven species of *Morariopsis* and

Table 1. Character matrix for the phylogenetic analysis of seven species of the genus *Morariopsis* and two outgroup species of the genus *Moraria*.

Characters Species	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Morariopsis grygieri	1	0	0	0	1	0	0	0	0	0	1	0	1	1	1	0	1	1	0	1	0
M. dumonti	0	0	0	1	1	0	1	–	0	0	1	0	1	0	1	0	0	0	1	*	0
M. kieferi	1	0	0	0	0	1	–	–	0	0	1	0	0	0	0	0	0	0	1	1	0
M. scotenophilus	1	0	0	1	0	0	1	–	1	0	1	1	1	1	1	0	1	1	1	1	0
M. baicalensis	0	1	0	1	1	1	–	–	0	1	1	0	1	0	1	1	1	1	0	1	0
M. typica	0	1	1	2	1	1	–	–	0	1	1	1	1	1	1	1	1	1	0	1	1
M. latifurcata	0	1	1	2	1	1	0	0	0	1	1	0	0	1	1	1	1	1	0	1	0
Moraria tsukubaensis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
M. jana	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	1	0	0	0	0	0

Symbols used: 0, presumed plesiomorphic character state; 1, 2, presumed apomorphies; –, unknown value; *, polymorphic character. See text for more details.

two outgroup taxa: *Moraria tsukubaensis* Kikuchi, 1991 and *Moraria jana* Karanovic, 1997. The former species was described from forest litter in central Japan (Kikuchi 1991), while the latter was described from a cave in the Balkans (Karanovic 1997). A total of 21 morphological characters were used in the analysis (see below). Characters were coded, optimized, and weighted using the computer program WinClada, version 1.00.08 (Nixon 2002), and then analyzed using NONA, version 2 (Goloboff 1999). Standard coding was used in the analysis: "0" representing a presumably plesiomorphic character state and "1" and "2", presumed apomorphies. Unknown values were coded "–" and polymorphic characters are marked in the matrix with an asterisk (*). All characters were weighted 1 and coded as additive (representing ordered multistate character as a linked series of binary characters). A data matrix was created (Table 1) and the characters were analysed using Rachet Island Hopper searches with the following WinClada parameters: 1000 replications; 3 trees to hold; 4 characters to sample; 10 random constraint level and amb-poly=(amb- collapses a branch if the ancestor and descendant have different states under the same resolutions of multistate characters or of "–"; poly=treats trees as collapsed). Rachet Island Hopper is a method that searches tree space very effectively by reducing the search effort spent on generating new starting points and retaining more information from existing results of tree searches. Only characters 8 and 20 were uninformative (Table 1).

The 21 morphological characters used in the cladistic analysis are listed below, with character states in brackets (note: NONA requires character numbering to start with 0, rather than 1):

0. Anal operculum: smooth (0); serrated or ornamented externally (1).
1. Anal operculum: elongated (0); short (1).
2. Caudal rami: not inflated (0); inflated (1).
3. Caudal rami, outer terminal seta: strong and longer than ramus (0); as long as ramus (1); shorter than ramus (2).
4. Abdomen, ventral armature on preanal somite: present (0); absent (1).

5. Antenna, exopod, lateral seta: present (0); absent (1).
6. Mandibula, armature on basis: present (0); absent (1).
7. Maxilliped, seta on syncoxa: present (0); absent (1).
8. First leg, first endopodal segment, inner seta: present (0); absent (1).
9. First leg, second endopodal segment, inner subapical seta: present (0); absent (1).
10. Second to fourth legs, endopod: two-segmented (0); one-segmented (1).
11. Second leg, ultimate endopodal segment, proximal inner seta: present (0); absent (1).
12. Second leg, ultimate endopodal segment, outer (slender) apical seta: present (0); absent (1).
13. Third leg, ultimate endopodal segment, proximal inner seta: present (0); absent (1).
14. Third leg, ultimate endopodal segment, outer (slender) apical seta: present (0); absent (1).
15. Fourth leg, third exopodal segment, inner seta: present (0); absent (1).
16. Fourth leg, ultimate endopodal segment, proximal inner seta: present (0); absent (1).
17. Fourth leg, ultimate endopodal segment, outer (slender) apical seta: present (0); absent (1).
18. Female fifth leg endopod, first element from inner side: present (0); absent (1).
19. Female fifth leg exopod, proximal outer element: present (0); absent (1).
20. Female fifth leg exopod, distal outer element: present (0); absent (1).

Systematics

Genus ***Morariopsis*** Borutzky, 1931

Morariopsis grygieri sp. nov.

(Figs 1–6)

Type material. Holotype: adult female dissected on 1 slide (LBM 1430004928), Japan, Shiga Prefecture, Taga town, Kawachi, Kawachi-no-Kazaana Cave, 35°15.136′N 136°20.979′E (note: GPS coordinates taken at the cave entrance, not the precise sampling site), 13 October 2009, leg. T. Karanovic. Allotype: adult male dissected on 1 slide (LBM 1430004933), same data as holotype. Paratypes: 1 adult female on 1 slide (LBM 1430004929) *in toto*, 1 copepodid on the same slide (LBM 1430004930) *in toto*, 1 adult female in 1 vial in 99.5% ethanol (LBM 1430004931), and 1 adult female in 1 vial in 99.5% ethanol (LBM 1430004932), same data as holotype; 1 male and 1 female in 1 vial in 70% ethanol (LBM 1430004934), Japan, Shiga Prefecture, Taga town, Kawachi, Gongendo Cave, 35°15.418′N 136°21.985′E (note: GPS coordinates taken at the cave entrance, not the precise sampling site), 30 September 2009, leg. T. Karanovic.

Description. *Female* (holotype). Total body length, measured from tip of rostrum to posterior margin of caudal rami (excluding appendages and caudal setae), 0.445 mm. Preserved specimen colourless. Nauplius eye absent. Prosome comprising cephalothorax with completely fused first pedigerous somite and three free pedigerous somites; urosome five-segmented, comprising fifth pedigerous somite, genital double somite, and three abdominal somites. No sclerotized joint between

Fig. 1. *Morariopsis grygieri* sp. nov., holotype female (A, B) and allotype male (C). A, C, Habitus, dorsal view; B, habitus, lateral view. Arrows indicate continuations of caudal rami setae. Scale bar: 0.1 mm.

prosome and urosome. Habitus (Fig. 1A, B) cylindrical, very slender, without distinct demarcation between prosome and urosome; prosome/urosome length ratio 0.81 and greatest width at posterior end of cephalothorax. Body length/width ratio about 5.1; cephalothorax 1.2 times as wide as genital double somite. Free pedigerous somites without pronounced lateral or dorsal expansions. Integument smooth, not very strongly chitinized.

Rostrum (Figs 1A, 3A) relatively large, membranous, linguiform, reaching just beyond distal margin of first antennular segment, about as long as wide and only internally demarcated at base, ornamented with two dorsal sensilla.

Cephalothorax (Fig. 1A, B) almost conical in dorsal view, about 1.2 times as long as wide (including rostrum), representing 23% of total body length. Surface of cephalic shield ornamented with 24 pairs of large sensilla and with small dorsal cuticular window. Tergites of three free pedigerous somites ornamented with ten, ten, and eight large sensilla respectively; first two free prosomites also with oval lateral cuticular windows, and third one additionally ornamented with two dorsal pores. Hyaline fringes of all prosomites narrow and smooth. Fifth pedigerous (first urosomal) somite also ornamented with eight sensilla (two dorsal and six lateral (three on each side)), as well as with three dorsal pores; hyaline fringe smooth.

Genital double somite (Figs 1A, B, 2A) with traces of original separation visible only as internal lateral cuticular ridges; hyaline fringe smooth and integument smooth on surface. Ornamentation consisting of 12 large sensilla almost evenly distributed around double somite, six posterior and six at midlength belonging to ancestral genital somite; no spinules or cuticular pores. Genital complex (Fig. 2A) with single relatively large, ovoid copulatory pore at middlength ventrally, well sclerotized, wide copulatory duct, and two small, ovoid seminal receptacles. Single large genital aperture covered by fused reduced sixth legs, representing 51% of somite width. Third urosomite (Figs 1A, B, 2A) ornamented with six posterior sensilla (two dorsal, two ventral, and one lateral on each side) and single dorsal anterior pore; hyaline fringe smooth. Fourth urosomite (preanal) without any surface ornamentation, completely smooth, and also with smooth hyaline fringe. Anal somite (Figs 1A, B, 2A) ornamented with pair of large dorsal sensilla at base of anal operculum, two large cuticular pores next to them (dorsolaterally), two lateral pores on each side (one posterior and one anterior), two lateral anterior rows of minute spinules on each side, and transverse row of large spinules along posterior margin, dorsal ones not demarcated at base, lateral ones smallest and ventral ones largest. Anal operculum (Figs 1A, 3J) convex, reaching beyond posterior end of anal somite, representing 50% of somite's width, ornamented with 11 strong spinules on posterior margin; these spinules being fused basally to create serrated edge. Anal sinus ornamented with two diagonal rows of minute spinules converging medially at posterior margin.

Caudal rami (Figs 1A, B, 2A) about as long as anal somite, almost cylindrical, with pronounced dorsal cuticular ridge, 1.7 times as long as wide (ventral view), somewhat divergent, with space of about one ramus width between them, armed with seven elements (three lateral, one dorsal, and three terminal (=apical)). Only ornamentation consisting of five large spinules posteroventrally, at base of terminal setae (Fig. 2A). Dorsal seta slightly shorter than ramus, slender, inserted at 3/4 of ramus length, triarticulate at base (i.e. inserted on two pseudojoints). Anterolateral seta half as long as ramus length, strong, arising dorsolaterally at 1/3 of ramus length, and accompanied at base ventrally by minute anterolateral accessory seta, latter smaller than dorsal sensillum on anal somite. Posterolateral seta somewhat shorter than anterolateral one, also strong, arising slightly dorsolaterally at 2/3 of ramus length. Dorsal and all three lateral setae smooth. Terminal accessory seta

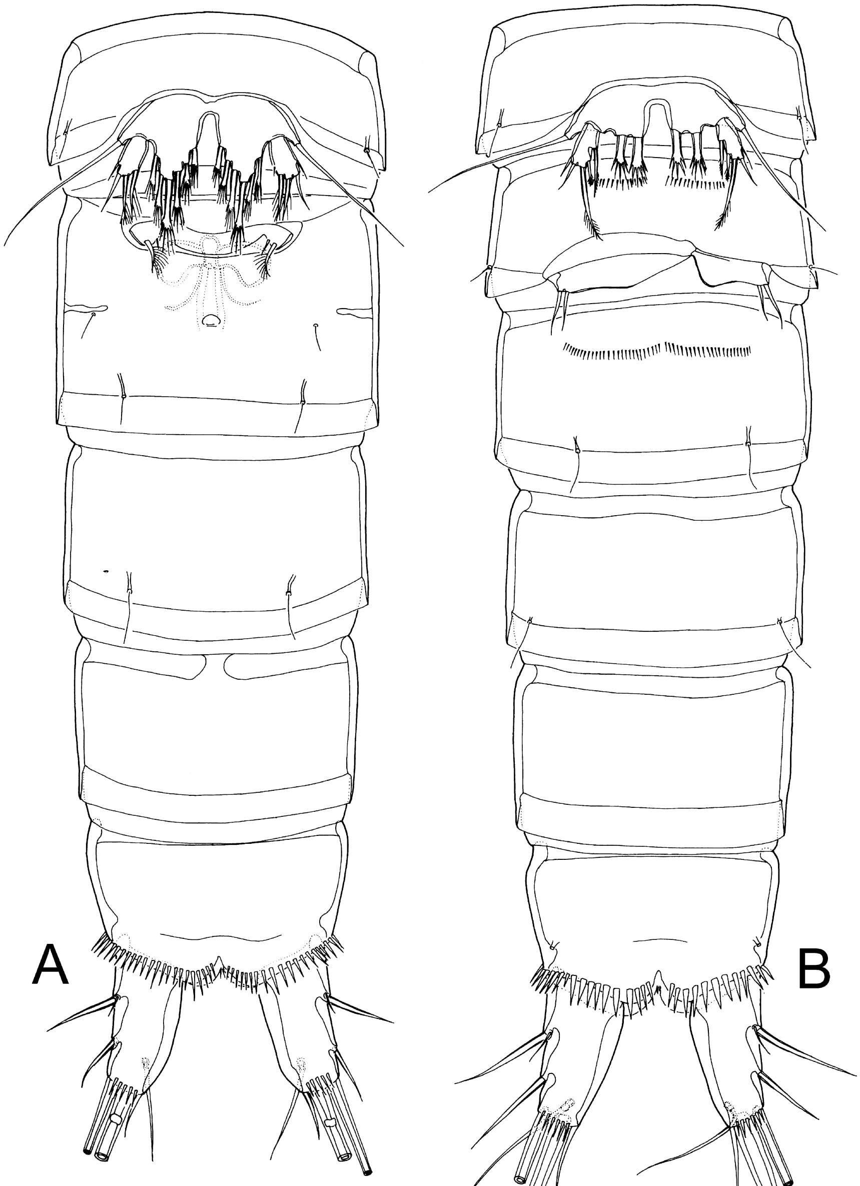

Fig. 2. *Morariopsis grygieri* sp. nov., holotype female (A) and allotype male (B). A, B, Urosome, ventral view. Scale bar: 0.1 mm.

(innermost apical seta) smooth, slightly shorter than distolateral seta and about 0.4 times as long as ramus. Inner terminal seta (middle apical seta) strongest, with breaking plane, unipinnate at distal end, about twice as long as outer apical seta and 0.45 times as long as total body length. Outer terminal seta also pinnate at

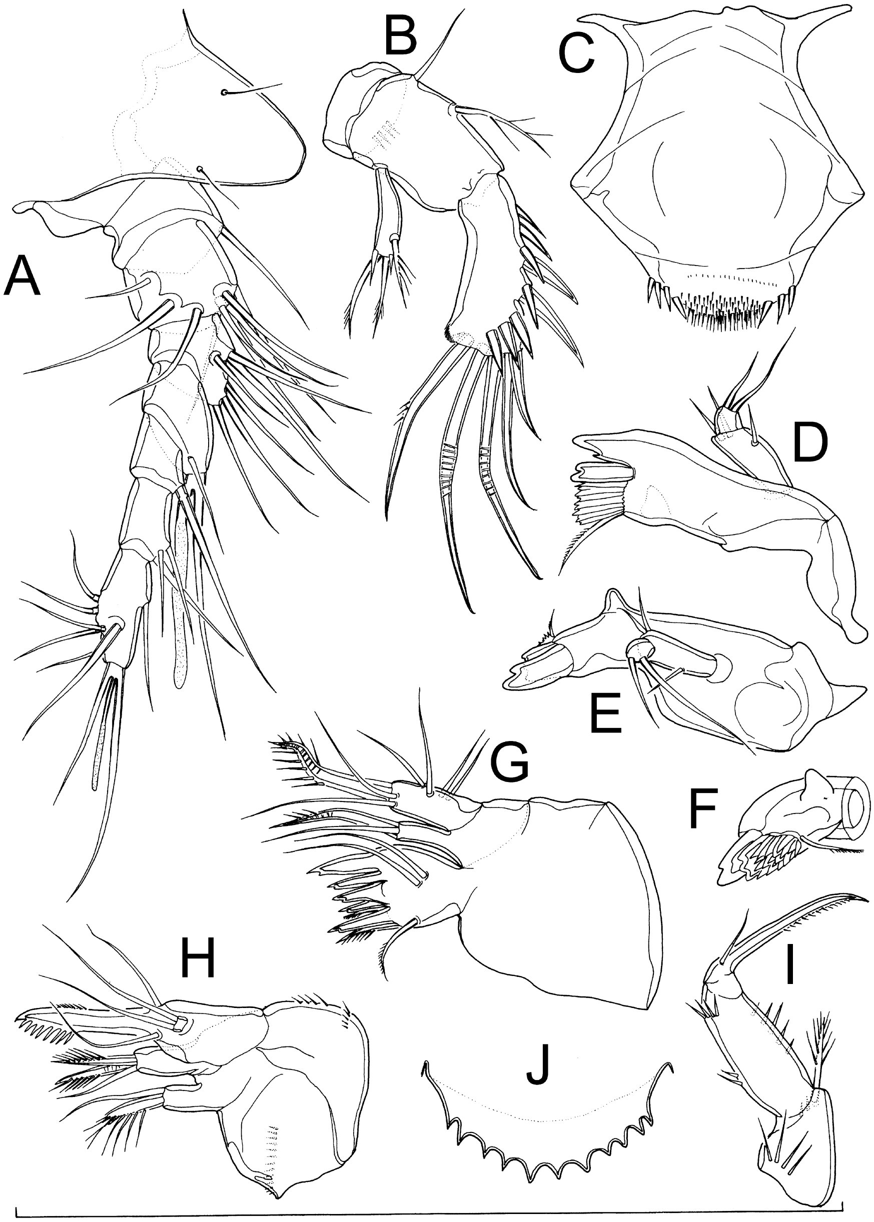

Fig. 3. *Morariopsis grygieri* sp. nov., holotype female. A, Antennula and rostrum, dorsal view; B, antenna, anterior view; C, labrum, ventral view; D, mandibula, anterior view; E, mandibula, ventral view; F, mandibular cutting edge, inner view; G, maxilla, anterior view; H, maxilla, anterior view; I, maxilliped, posterior view; J, anal operculum, dorsal view. Scale bar: 0.1 mm.

distal end, but without breaking plane.

Antennula (Fig. 3A) seven-segmented, joined to cephalotholax with small, triangular pseudosegment laterally, approximately half as long as cephalothorax with rostrum. Long aesthetasc on fourth segment fused basally with adjacent large seta and reaching slightly beyond tip of appendage; slender apical aesthetasc on seventh segment fused basally with two apical setae, forming apical acrothek. Setal formula: 1.9.6.2.1.3.9. All setae smooth; four lateral setae on seventh segment biarticulate at base (i.e. inserted on one pseudojoint); only large seta on fifth segment with breaking plane; all others uniarticulate without breaking planes. No surface ornamentation visible. Length ratio of antennular segments, from proximal end along caudal margin 1 : 1.5 : 0.8 : 1.1 : 1.4 : 1.6 : 2.5.

Antenna (Fig. 3B) short and strong, composed of coxa, allobasis (formed by fused basis and first endopodal segment), one-segmented endopod, and one-segmented exopod. Coxa very short, unarmed but ornamented with four large posterior spinules. Allobasis 1.4 times as long as wide, more than four times as long as coxa, unornamented, armed with two inner setae; proximal inner seta smooth, inserted at 1/5 of segment length, 0.6 times as long as segment; distal inner seta plumose, inserted at midlength, as long as proximal inner seta. Endopod the largest segment, about 2.8 times as long as wide and widest in its distal part, 1.3 times as long as allobasis, with one surface frill subdistally on outer (dorsal) side, armed laterally with two strong, smooth spines; apical armature consisting of five geniculate elements, outermost one with several pinnules on convex margin; two largest apical elements with flexible joints at midlength, others simple; ornamentation consisting of seven large spinules along inner (anterior) surface. Exopod slender, narrowest at base, 0.7 times as long as allobasis and 3.6 times as long as wide, ornamented with two large spinules at distal corners, armed with one lateral and two apical pinnate setae; outer apical seta as long as segment and somewhat longer than inner apical seta, latter somewhat longer than lateral seta; lateral seta inserted at 4/5 of segment length.

Labrum (Fig. 3C) large compared to cephalothorax, with trapezoidal free part and very long and strong base, rigidly sclerotized, with relatively short and somewhat concave cutting edge, ornamented subapically at lateral corners with two rows of three or four strong spinules and apically with numerous slender spinules. No fields of gustatory papillae visible on dorsal (posterior) surface.

Paragnaths small, ellipsoidal, not dissected in satisfactory position to allow detailed observation.

Mandibula (Fig. 3D–F) with wide cutting edge on elongated coxa, armed with two complex teeth in ventral half, numerous finer teeth in dorsal half, and one dorsal unipinnate seta. Palp uniramous, comprising basis and one-segmented endopod. Basis cylindrical, somewhat narrower at base, about 2.3 times as long as wide, unornamented, armed with three short, smooth setae: two subapically on dorsal surface, one laterally on ventral surface. Endopod very small, about as long as wide and 0.3 times as long as basis, unornamented, armed with two slender apical setae: ventral one as long as basis and endopod combined, and 1.6 times as long as dorsal one, latter 1.2 times as long as longest basal seta.

Maxillula (Fig. 3G) with large praecoxa, its arthrite rectangular, unornamented, and armed with two anterior surface setae, three lateral unipinnate setae, and five apical smooth and bilobate spines. Coxal endite much shorter than praecoxal arthrite, armed with one curved, stout, unipinnate seta and one smooth, slender seta. Basis slightly longer than coxal endite, armed with four smooth, slender setae laterally on ventral surface, two slender setae subapically, and one curved, bipinnate strong spine apically. Endopod and exopod completely fused to basis.

Maxilla (Fig. 3H) with large syncoxa, ornamented with two short rows of spinules on outer ventral surface and one long row basally on posterior surface, with two well developed and highly mobile cylindrical endites; proximal endite shorter and armed apically with one smooth, slender seta and one strong and unipinnate spine; distal endite longer and larger, armed apically with one smooth, slender seta and two strong, unipinnate spines. Basis drawn out into long and robust claw, with slender, smooth seta at base, ornamented with minute spinules along convex margin and large, basally fused spinules along concave margin distally. Endopod represented by minute segment, armed with two long, smooth, subequal apical setae. Exopod completely fused to basis, represented by single smooth, slender ventral seta.

Maxilliped (Fig. 3I) with short syncoxa, armed with one bipinnate seta subapically, ornamented with four large spinules basally and two smaller ones at base of subapical seta. Basis about 2.5 times as long as wide and 1.3 times as long as syncoxa, unarmed, ornamented with short, transverse row of spinules subapically, one longitudinal row of five spinules along inner margin, and two spinules on outer margin proximally. Endopod represented by long, curved claw, about as long as basis, ornamented with row of spinules along concave side distally, accompanied at base by thin and smooth, short seta.

Swimming legs (Fig. 4A–E) very short compared to body size (Fig. 1B), with three-segmented exopods; endopod of first leg (Fig. 4A) somewhat shorter than exopod and two-segmented; endopods of other legs one-segmented and about as long as first exopodal segment. Armature formula of swimming legs in Table 2.

Intercoxal sclerites of all swimming legs with concave distal margin and unornamented. Praecoxa with posterior row of minute spinules on anterior surface. Coxa of all swimming legs unarmed, ornamented with several large spinules on distal outer corner. Basis also ornamented with very long spinules on outer margin and large cuticular pore on anterior surface, armed with outer spine on first and second swimming legs and with outer smooth seta on third and fourth legs; basis of first leg with stout spine near inner distal corner and additional row of spinules posteriorly at base of endopod. Exopodal and endopodal segments of all swimming legs ornamented with strong spinules along outer margin and some segments also with spinules along inner and distal margins. All exopodal segments of about same length. First endopodal segment of first swimming leg (Fig. 4A) stout, about 1.7 times as long as wide, somewhat shorter than first two exopodal segments, and 1.4 times as long as second endopodal segment. Most setae on rami strong and spiniform, except for long, smooth, prehensile apical setae on first leg; inner apical seta on third exopodal segment of second, third, and fourth legs much shorter than outer apical one.

Fifth leg (Figs 2A, 4F) small compared to somite, biramous, baseoendopods not fused. Baseoendopod with outer basal seta long and smooth, arising from long setophore. Endopodal lobe wide and long, almost triangular, extending beyond posterior margin of exopod, unornamented, armed with six subequal stout and short elements, each with apical tuft of spinules; spinules on all but two innermost ele-

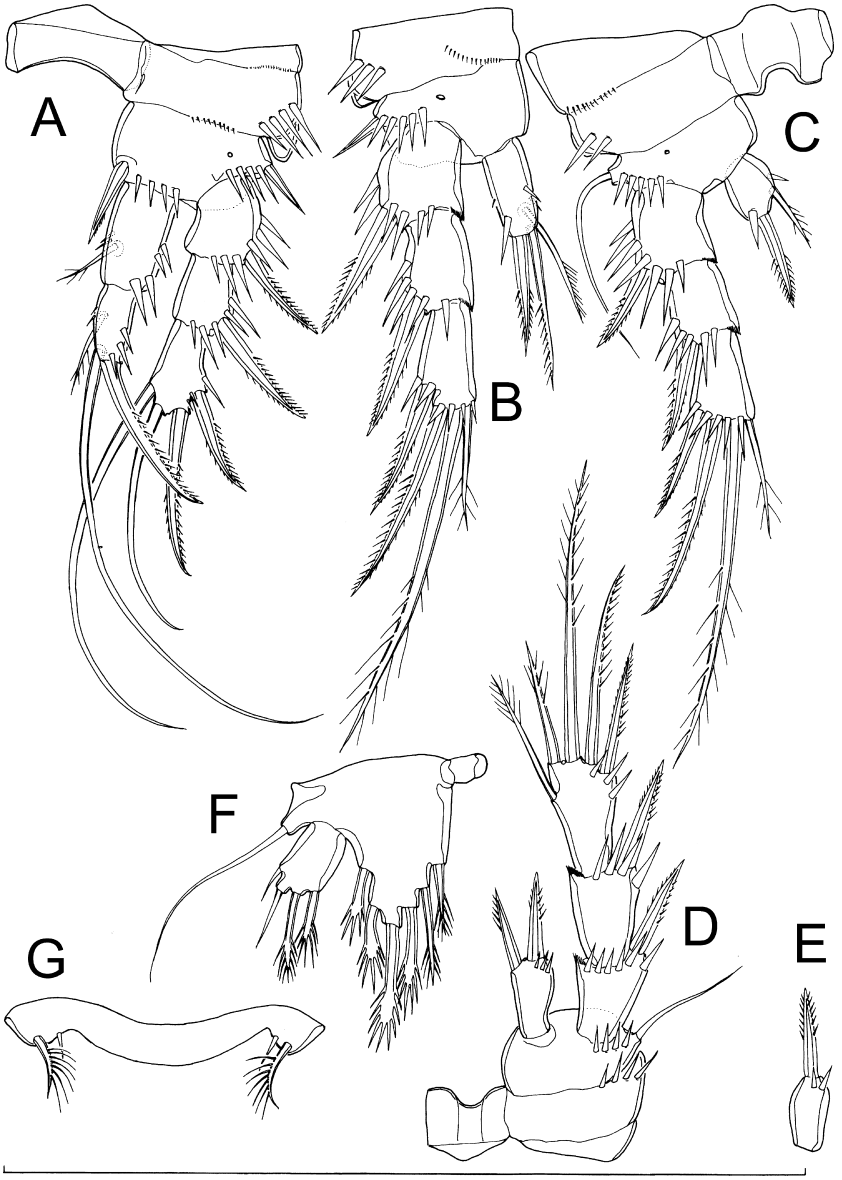

Fig. 4. *Morariopsis grygieri* sp. nov., holotype female. A, First swimming leg, anterior view; B, second swimming leg, anterior view; C, third swimming leg, anterior view; D, fourth swimming leg, anterior view; E, endopod of left fourth swimming leg, anterior view; F, fifth leg, anterior (ventral) view; G, sixth legs, ventral view. Scale bar: 0.1 mm.

ments characteristically fused basally. Exopod quadrate, about 1.7 times as long as maximum width, also unornamented, armed with four elements; two inner elements strong and each with apical tuft of strong spinules, latter being fused

Table 2. Armature formula of the swimming legs of *Moraripsis grygieri* sp. nov.

Leg	Ramus	Exopod			Endopod	
	Segment	1	2	3	1	2
First leg		0/1	0/1	0/2/2	1/0	1/1/1
Second leg		0/1	0/1	0/2/2	1/1/1	–
Third leg		0/1	0/1	0/2/2	0/1/1	–
Fourth leg		0/1	0/1	1/2/2	0/1(0)/1	–

Elements are arranged in inner/outer for penultimate segments, and inner/terminal/outer for ultimate segments.

basally; and two shorter and smooth outer elements; outermost seta about half as long as other three.

Sixth legs (Fig. 4G) completely fused together, indistinct, forming short, simple operculum covering single gonopore, without any ornamentation, each armed with short, curved, unipinnate seta and much shorter, smooth inner seta.

Male (allotype). Body length, excluding caudal setae, 0.466 mm. Habitus (Fig. 1C), ornamentation of prosomites, rostrum, colour, and nauplius eye similar to those of female. Hyaline fringe of all prosomites smooth and cuticular windows present on second (Fig. 5A) and third pedigerous somites laterally and on cephalothorax dorsally. Small, longitudinally positioned spermatophore (Figs 1C, 5I) visible inside third pedigerous somite, with its neck in caudal half of second pedigerous somite; neck thin, long and coiled, spermatophore body ellipsoidal, 2.7 times as long as its greatest width. Third pedigerous somite with two small sensilla dorsally in place of two cuticular pores in female, while dorsal pair of pores missing from fourth pedigerous somite (present in female). Prosome/urosome length ratio 0.84, greatest width at posterior end of cephalothorax, body length/width ratio about 5.4; cephalothorax 1.1 times as wide as genital somite.

Genital somite (Figs 1C, 2B) 2.8 times as wide as long, with only one dorsal cuticular pore (three in female). Urosomite ornamentation similar to that of female, although genital and first abdominal somites ornamented with additional transverse row of minute spinules ventrally (Fig. 2B). Anal somite lacking second short lateral row of minute spinules proximally (Fig. 5B). Anal operculum (Fig. 5C) similar in size to that of female, but with only eight teeth.

Caudal rami (Figs 1C, 2B, 5B) with the same armature and ornamentation as in female, although slightly more conical (i.e. with wider base).

Antennula (Fig. 5D) of similar size and proportions to that of female, but nine-segmented and strongly geniculate between sixth and seventh segment; antennula also unornamented as in female. Armature of first, second, and ultimate segments similar to that of female. Aesthetasc on apical acrothek of fourth segment much shorter and more slender than in female, reaching only midlength of remaining distal segments. Slender aesthetasc on tenth segment fused basally to two apical setae as in female, but additional aesthetasc present on sixth segment and fused basally to slender seta. Seventh segment lacking armature, but strongly sclerotized and with three thick-walled teeth on inner (anterior) margin. Setal formula: 1.9.5.8.1.3.0.1.9. All setae smooth and slender; only five lateral setae on ultimate segment biarticulate basally. No setae with breaking plane.

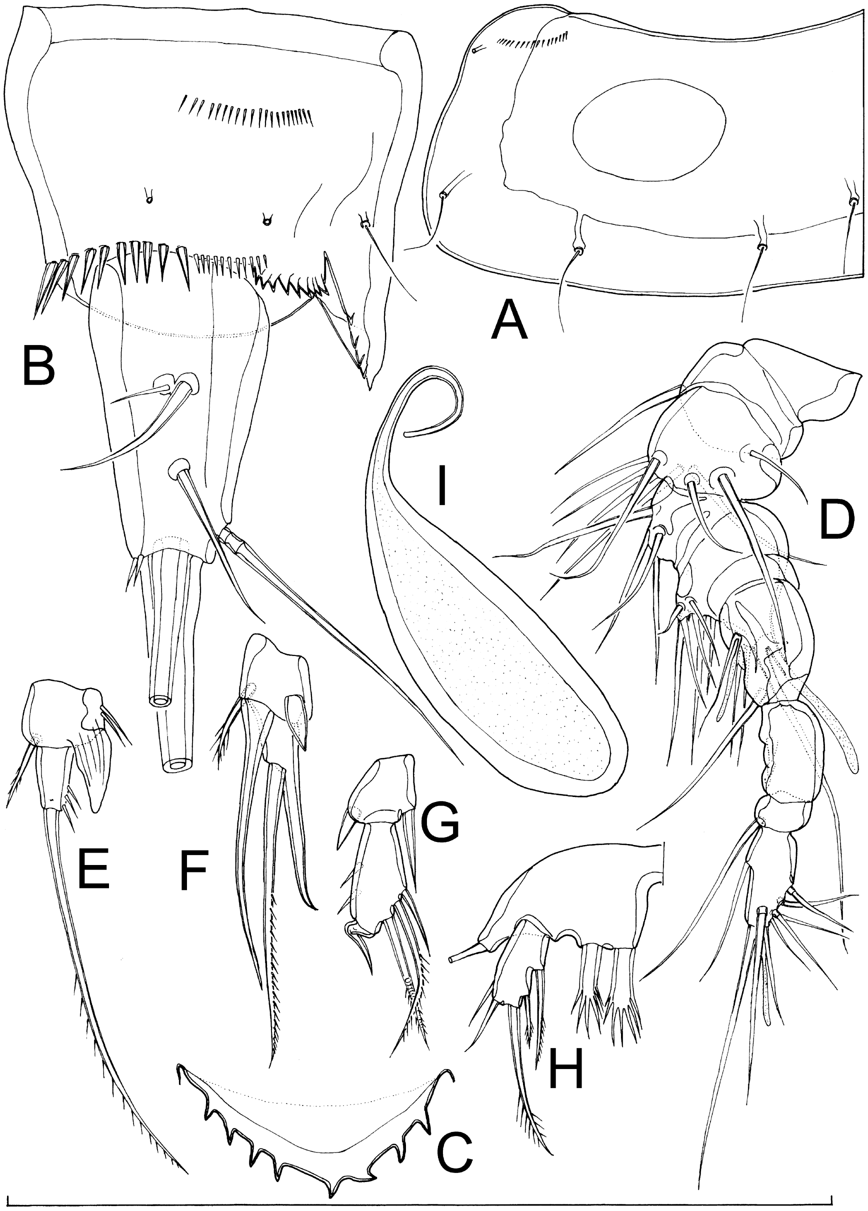

Fig. 5. *Morariopsis grygieri* sp. nov., allotype male. A, Left pleura of second pedigerous somite, flattened; B, anal somite and left caudal ramus, lateral view; C, anal operculum, dorsal view; D, antennula, dorsal view; E, endopod of left second swimming leg, anterior view; F, endopod of left third swimming leg, anterior view; G, endopod of fourth swimming leg, posterior view; H, fifth leg, anterior (ventral) view; I, spermatophore. Scale bar: 0.1 mm.

Antenna, labrum, paragnaths, mandibula, maxillula, maxilla, maxilliped, first swimming leg, and exopods of second, third, and fourth swimming legs similar to those of female.

Endopod of second swimming leg (Fig. 5E) two-segmented, but very short. First segment rectangular, armed with inner bipinnate seta and ornamented with two spinules on outer margin proximally and with enormous foliaceous spinule distally, this being fused basally to this segment and reaching distal margin of second segment. Second segment slender, armed apically with single long, unipinnate element, ornamented with three spinules along outer margin distally.

Endopod of third swimming leg (Fig. 5F) also two-segmented but short. First segment rectangular, armed with short and bipinnate proximal inner seta and long, lanceolate subapical distal element, latter 4.2 times as long as segment and smooth; this segment ornamented only with large foliaceous spinule on outer distal corner, this spinule not being fused basally and smaller than that on endopod of second leg. Second segment small and almost conical, unornamented, armed with smooth subapical outer spine, this being fused basally to segment, and inner apical element, this being pinnate along outer margin and longer than any other element; outer subapical spine geniculate.

Endopod of fourth swimming leg (Fig. 5G) two-segmented, second segment much better developed, 1.7 times as long as first segment and almost as wide. First segment armed with short, smooth inner element and ornamented with large spinule on outer distal corner. Second segment ornamented with two smaller spinules on outer margin, armed with three inner setae (proximal smooth and shortest, middle unipinnate and longest, distal bipinnate), and transformed outer subapical spine, this being smooth and characteristically coiled like pig's tail.

Fifth legs (Figs 2B, 5H) with baseoendopods fused only basally, forming narrow bridge, even smaller than in female. Baseoendopod with very long and smooth outer basal seta arising from long setophore but not reaching posterior margin of genital somite. Endopodal lobe broad and short, almost straight, not extending to 1/3 length of exopod, unornamented, but armed with two short elements bearing apical tufts of spinules. Exopod similar to that of female but without ornamentation along inner margin, and with additional spiniform inner seta; also, apical spiniform element much longer than in female.

Sixth legs (Fig. 2B) not fused basally to each other and demarcated at base (i.e. not fused basally to somite), asymmetrical in shape, formed as simple cuticular plates, without ornamentation, but each armed with two short and smooth setae; outer seta slightly longer than inner, but still shorter than some sensilla on urosomal somites. Right leg wider, with narrower inner part, and with more obvious, wider, demarcation at base.

Variability. The body length of females ranged from 0.387 to 0.445 mm (mean 0.408 mm; n=5), while only two males were collected, 0.466 and 0.391 mm long (mean 0.429 mm). The holotype female exhibits asymmetry in the armature of the fourth leg endopod, that on the left leg being armed with only one apical element (Fig. 4E), but the right leg with two (Fig. 4D). All other females have two armature elements on both fourth legs. Differing lengths of specimens are more a consequence of the body somites being fully extended (Fig. 1A–C) or telescopically contracted (Fig. 6A, B) than a reflection of real differences in size.

Etymology. The new species is named in honour of Dr Mark J. Grygier, of the

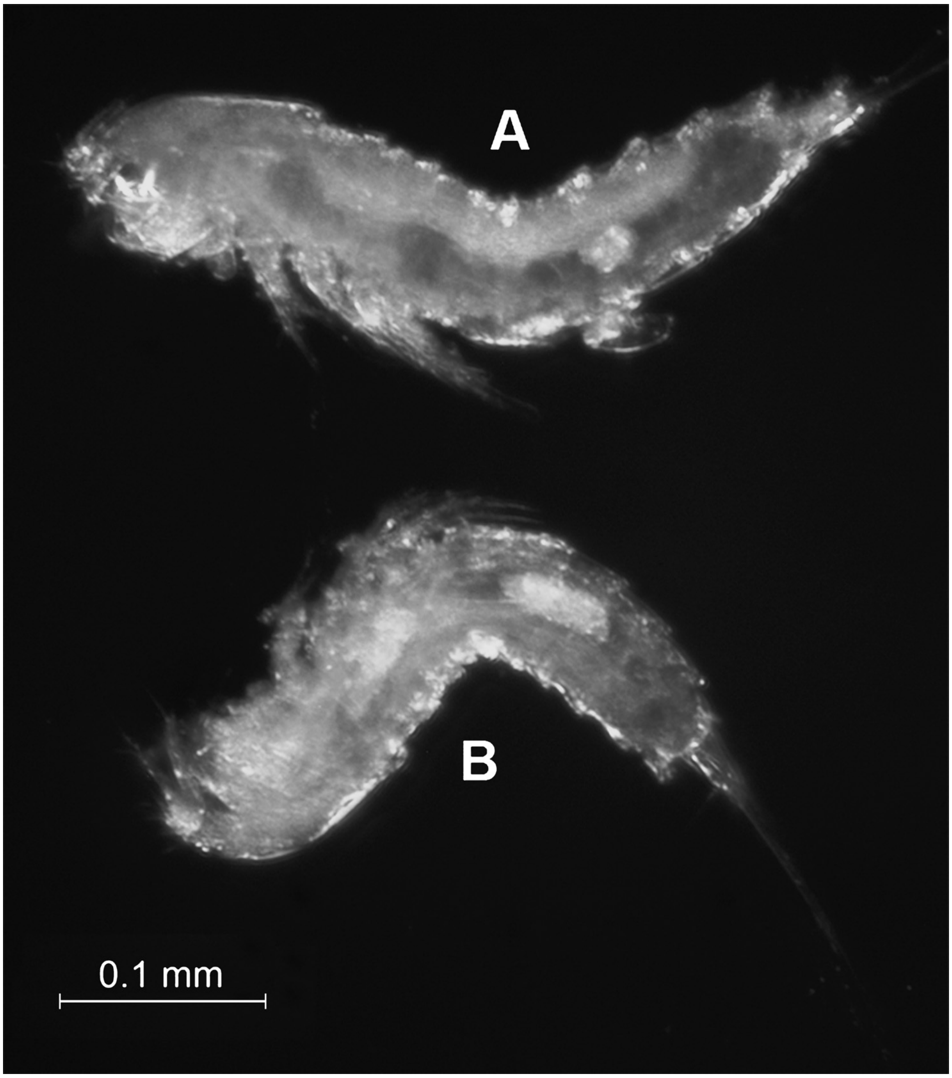

Fig. 6. *Morariopsis grygieri* sp. nov., montage micrograph from a dissecting microscope. A, Paratype female from Gongendo Cave with attached spermatophore; B, paratype male from Gongendo Cave.

Lake Biwa Museum, who kindly invited the senior author to Japan and made this collaboration possible. The specific name is a noun in the genitive singular.

Discussion

As was mentioned in the Introduction, the presently known members of the genus *Morariopsis* live in two disjunct areas: three species are benthic dwellers in

Lake Baikal (*M. typica, M. latifurcata,* and *M. baicalensis*), while three live in the subterranean waters of the western Balkan Peninsula, in Slovenia and Croatia (*M. scotenophilus, M. kieferi,* and *M. dumonti*). These two groups are supported by some morphological characters, and a superficial examination of published descriptions shows that all three Baikal species have six elements on the baseoendopod of the female fifth leg (five or less in the Balkan species), while all three Balkan species have an inner seta on the third exopodal segment of the fourth leg (absent in the Baikal species). Our new Japanese species, *M. grygieri*, displays the plesiomorphic state of both of these characters (six elements on the female fifth leg and the inner seta present on the third exopodal segment of the fourth leg), a situation which does not tell us anything about its closer relationships with these two groups. Also, we wanted to know whether the Baikal and Balkan species represent one colonisation event each or more. One can speculate in the latter case that one ancestral species invaded the subterranean waters of the Balkans and then diversified into three daughter species, or that each of these colonised this habitat independently from surface-water ancestors, or that two of them are sister species, while the third is a result of a separate colonisation event. The same applies to Baikal. In order to test these zoogeographic hypotheses, as well as to learn the phylogenetic relationships of the new Japanese species, we performed a cladistic analysis of all seven *Morariopsis* species and two outgroup taxa, as outlined above. The two outgroup taxa were chosen from the closely related genus *Moraria*. Males of both genera have a characteristically curled apical spine on the fourth leg's endopod (Fig. 5G), indicating that together they may comprise a monophyletic group, as it would be hard to imagine a convergent evolution of such an unusual feature. *Moraria tsukubaensis* is a surface-water species from Japan, with all chosen characters in their plesiomorphic state, while *Moraria jana* lives in the subterranean waters in the Balkans and has some reductions that can be interpreted either as synapomorphic character states or (more probably) homoplasies with those of *Morariopsis* species. Obviously, the two outgroups were also symbolically chosen from the same zoogeographic regions as some ingroup taxa.

Character selection for this study was influenced mostly by the data available in published descriptions, as well as the choice of outgroup taxa. We were able to score 19 informative characters, and two uninformative ones were also added in the cladistic analysis (Table 1), but most autapomorphic (uninformative) characters were left out. The list of characters and their states are given in the Material and Methods section above. The majority of them (ten characters) are related to the armature of the swimming legs, but we found other informative characters in the shape and armature of the caudal rami, as well as in the cephalic appendages.

Unfortunately, some species descriptions, and even redescriptions, were based on a limited set of characters. Although they are adequate to confirm separate specific status and generic affinities, limited descriptions are a serious impediment to phylogenetic analyses and the testing of monophyly of many harpacticoid genera and species groups (Karanovic and Hancock 2009; Karanovic *et al.* in press). Here we should discuss a couple of issues related to the problems in recognizing homologous structures in different taxa of *Morariopsis*. The original drawings of *M. baicalensis* are very unreliable, as it is obvious that Borutzky and Okuneva (1975) did not use a camera lucida, and the proportions of some armature and ornamentation elements on the swimming legs appear to be quite exaggerated. We used their written interpretation of the armature of the swimming legs in our matrix (Table 1), although it is possible that they mistook some of the larger spinules for armature elements.

Recognizing homologous structures in the different species was the hardest task, and in some cases almost impossible. We used indirect evidence from the two chosen outgroups to determine homologous armature elements in more reduced species. For example, it is impossible to say with any certainty whether the inner apical element on the endopod of the fourth leg in *M. grygieri* and *M. scotenophilus* represents the ancestral distal inner element or inner apical element, as both are present in our outgroups and some ingroup taxa as well. However, when both are present the condition of the latter element is always that of a very thin seta, while the former is a very robust, spiniform seta, so we presume the evolutionary trend was towards the reduction of the weaker one (see Character 17; Table 1), even though the alternative, reduction of the ancestral distal inner seta, cannot be excluded completely.

Homologous armature elements were named according to their position in the outgroup with the most plesiomorphic character states, in this case *M. tsukubaensis*. One should consult Kikuchi (1991) for more details about the morphology of this species and to understand more easily the present cladistic analysis. For example, Characters 19 and 20 (Table 1) are called the “proximal outer element” and “distal outer element” respectively, because there is one more seta (proximal) on the fifth leg’s exopod in *M. tsukubaensis,* even though none of the ingroup taxa has that element, aside from an aberrant specimen of *M. dumonti* in which it is present on one side of the body. Also, when the number of elements on baseoendopod of the female fifth leg is reduced from six to five, it is very hard to know which one of these is lost, but the positions and proportions of the remaining ones suggest that the innermost one is lost (Character 18). For the current analysis this is not so important, assuming it is the same element in all species.

Our analysis resulted in only one most parsimonious tree (Fig. 7), with a length of 33 steps, a consistency index (Ci) of 63, and a retention index (Ri) of 71. The values of the Ci and Ri indices indicate a relatively high proportion of convergences (homoplastic changes). This is obviously a result of the choice of characters, but it also reflects a general pattern: copepod groups with a high proportion of subterranean species display an unusually high proportion of convergences (Karanovic and Hancock 2009; Karanovic *et al.* in press). Nevertheless, the ingroup is well defined by at least two synapomorphies: reduced endopodal segmentation of the second to fourth swimming legs (Character 10), and reduced armature of the exopod of the female fifth leg (Character 19), which are precisely the two features that Borutzky (1931, 1952) used to define the genus *Morariopsis*.

The three Baikal species also represent a well-defined clade, which is a good indicator of a single colonisation event. The Baikal clade is defined by at least two synapomorphies: a short anal operculum (Character 1), and a reduced inner subapical seta on the second endopodal segment of the first leg (Character 9). They also all lack the inner seta of the third exopodal segment of the fourth leg (Character 15), which almost certainly originated independently (as a homoplasy) in the second outgroup as a consequence of a subterranean lifestyle. We also believe that the reduced armature of the antennal exopod (Character 5), which they share with *M. kieferi*, originated convergently in the latter species, while it is a synapomorphy

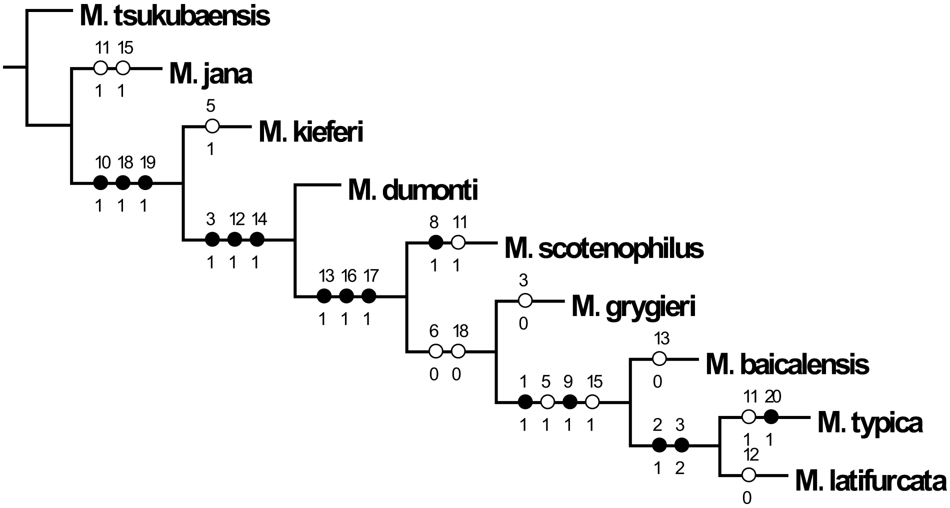

Fig. 7. The only cladogram resulting from the analysis of 21 morphological characters (Table 1), scored for seven species of *Morariopsis* Borutzky, 1931 and the two outgroups: *Moraria tsukubaensis* Kikuchi, 1991 and *Moraria jana* Karanovic, 1997. Full circles, presumed apomorphies; empty circles, presumed plesiomorphies; numbers above branches, characters; numbers below branches, character states. See text for more details.

for *M. baicalensis, M. typica,* and *M. latifurcata.* Of the three Baikal species, the latter two are more closely related. Our new species is a sister clade to the Baikal clade, but this branch is based just on plesiomorphic character states and does not suggest a closer phylogenetic relationship between *M. grygieri* and any of the three Baikal represenatives. We think that *M. grygieri* is a result of a separate colonisation event, and that it originated from a different surface-water ancestor than the three Baikal representatives.

The most surprising discovery of this cladistic analysis is that the three Balkan species are not closely related to each other at all, and probably represent three separate colonisation events of subterranean habitats in a relatively small area of the Croatian and Slovenian Dinaric karst. Both Petkovski (1959) and Brancelj (2000) discussed the affinities of *M. scotenophilus, M. kieferi,* and *M. dumonti* and suggested their close relationship, but their assumption was clearly based on symplesiomorphies and cannot be supported here.

We can also quite confidently reject the hypothesis that all seven species of *Morariopsis* originated from a single surface-water ancestor with a Palaearctic distribution. The genus probably originated in marginal environments along the northern shore of the ancient Tethys Sea from a *Moraria*-like ancestor, but by the time of the closure of Tethys it was probably differentiated into at least five different species. Reduced armature of the swimming legs probably resulted from a life in estuarine muds and the anchialine interstitial, where there is very little need for swimming. This made them perfectly suited for the colonisation of subterranean habitats in Europe and Japan. The ancestor of the Baikal clade may have colonised the lake from estuarine environments during one of the interglacial phases (or even during the Tertiary), or it was trapped there during the origin of the lake itself, as a marine/brackish element. The ancestor of *M. grygieri* probably first colonised benthic habitats of Lake Biwa during the lake's tectonic formation and from there colonised subterranean waters in its surroundings, during major climatic changes. We hope that continuing research of the deepest habitats in this ancient lake will reveal more species of *Morariopsis*, despite the very prolonged anthropogenic influence here (Kawanabe 1996).

If the ecological results obtained for *M. dumonti* by Brancelj (2002) can be applied across the genus, this would suggest their preference for locations with high amounts of organic material. However, tolerances of these animals to chemical pollution may be very different between species, and that is one of the reasons we recommend an urgent survey of the benthic habitats in ancient Lake Biwa, in addition to all other measures of minimising pollution and other anthropogenic influences. If ancient lakes indeed act as biodiversity pumps for the surrounding subterranean and surface-water habitats, their importance is even greater than currently perceived (mostly as refugia; see Matzinger *et al.* 2006; Albrecht and Wilke 2008), especially when we face a major biodiversity crisis. One of the species, *M. baicalensis,* was collected from a depth of 74 m in Lake Baikal (Borutzky and Okuneva 1975), which is an environment with very little light, and ideal for the evolution of subterranean adaptations (stygomorphies) that would enable it to easily colonize subterranean habitats.

A key to species of the genus *Morariopsis* has not been provided since Lang (1948) for three species and Borutzky (1952) for his two species. With the inclusion of the new species described in this paper, the number has risen to seven, and we think it would be beneficial to provide an updated key here. The characters employed in this key are not only chosen for their convenience but also for their phylogenetic importance, whenever possible (see also Fig. 7).

Key to species of the genus *Morariopsis* Borutzky, 1931

1\. Endopod of third leg with less than four elements 2
– Endopod of third leg with four elements .... *M. kieferi* Petkovski, 1959
2\. Endopod of fourth leg with two elements 3
– Endopod of fourth leg with four elements .... *M. dumonti* Brancelj, 2000
3\. First endopodal segment of first leg with inner seta 4
– First endopodal segment of first leg without inner seta .... *M. scotenophilus* (Kiefer, 1930)
4\. Third exopodal segment of fourth leg without inner seta 5
– Third exopodal segment of fourth leg with inner seta .... *M. grygieri* sp. nov.
5\. Caudal rami inflated 6
– Caudal rami slender .... *M. baicalensis* Borutzky and Okuneva, 1975
6\. Exopod of female fifth leg with three elements .... *M. typica* Borutzky, 1931
– Exopod of female fifth leg with four elements .... *M. latifurcata* Borutzky, 1931

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