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A new species of *Schizopera* (Copepoda: Harpacticoida) from Japan, its phylogeny based on the mtCOI gene and comments on the genus *Schizoperopsis*

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The predominantly marine genus *Schizopera* Sars, 1905 has only two significant inland water species-flocks, one in the ancient African Lake Tanganyika and the other in subterranean waters of Western Australia. Discovery of *Schizopera abei* sp. nov. from several interstitial locations in the vicinity of the ancient Lake Biwa has wider implications for the study of morphological homoplasies in the genus, as well as for the study of freshwater invasions in harpacticoid copepods. The new *Schizopera* species belongs to a small group of congeners with a two-segmented endopod of the fourth leg, which used to be recognised as a separate genus, *Schizoperopsis* Apostolov, 1982. Our reconstructed phylogenies based on the mtCOI partial sequences suggest that this character probably evolved convergently in at least some *Schizopera*, thus rendering the genus *Schizoperopsis* polyphyletic. However, almost all basal nodes in our cladograms are weakly supported, which shows limitations of a single-gene approach for reconstructing phylogenetic relationships. The new species is the first member of its genus from Japanese inland waters, and it has no close relatives among extent congeners anywhere in the world. We speculate that its ancestor may have invaded Lake Biwa, and subsequently its surrounding subterranean waters, from brackish areas around central Japan, presumably during a period of high sea water level through its major outflow river. This discovery may provide further support for the hypothesis about the role of ancient lakes as biodiversity pumps for subterranean habitats.

http://zoobank.org/urn:lsid:zoobank.org:pub:1F71F7AD-B7C8-4AD3-BE44-5E1BEE4E2AA8

Keywords: ancient lakes; barcoding; phylogeny; stygofauna; taxonomy

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Introduction

The genus *Schizopera* was established by Sars (1905), with *S. longicauda* Sars, 1905 as the type species. Today, there are nearly 100 valid species and subspecies worldwide (Wells 2007; Karanovic and Cooper 2012; Karanovic and McRae 2013; Walter and Boxshall 2014). They are distributed in a variety of marine, brackish and freshwater habitats around the world, which makes them an ideal group for testing hypotheses of multiple invasions of freshwater (Karanovic and Cooper 2012), which was suggested for copepods generally (Boxshall and Jaume 2000; Karanovic 2008). The genus is,

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however, predominantly marine, with only two significant inland water species flocks, one in the ancient African Lake Tanganyika (Sars 1909; Gurney 1928; Lang 1948; Rouch and Chappuis 1960) and the other in subterranean waters of Western Australia (Karanovic 2004, 2006; Karanovic and Cooper 2012; Karanovic and McRae 2013). Unfortunately, a great number of species descriptions are incomplete and/or inadequate. Because of that, and because of the normal expansion of generic boundaries resulting from the inclusion of new species, systematics of the genus *Schizopera* has been very difficult at times. Lang (1948, 1965) maintained clarity in the generic diagnosis by suggesting the presence of a 'transformed spine' on the male third leg exopod as a synapomorphy. It was later proven that this structure is, in fact, an enormously enlarged tubular pore (Karanovic and Cooper 2012), similar in nature (although not in shape) to those observed in some other members of the same subfamily, Diossacinae Sars, 1906 (see Gee and Fleeger 1990). Attempts to split the genus based on the segmentation of endopods of first and fourth swimming legs (Wells and Rao 1976; Apostolov 1982; Bodin 1997; Boxshall and Halsey 2004) were subsequently questioned both based on morphological (Mielke 1992, 1995; Karanovic 2004; Wells 2007; Huys 2009) and molecular evidence (Karanovic and Cooper 2012; Karanovic and McRae 2013), and are now abandoned. The only exception is the genus *Eoschizopera* Wells and Rao, 1976, which was originally designed to accommodate all doubtful members of the genus *Schizopera*, but was later redefined by Karanovic (2004) to include only four species. Its validity has been supported by a set of morphological synapomorphies (see Wells 2007), but the subgeneric division proposed by Apostolov (1982) has also been abandoned (Boxshall and Halsey 2004; Wells 2007; Huys 2009). However, the monophyly of the genus *Schizoperopsis* as proposed by Apostolov (1982) has not yet been tested using molecular tools, and that was one of major aims of our study. Only four species were originally included in this genus: *Schizopera arenicola* Chappuis and Serban, 1953 from the Romanian coast of the Black Sea (type species); *S. gauldi* Chappuis and Rouch, 1961 from a sandy beach in Accra, Ghana; *S. nichollsi* Soyer from Kerguelen Island, Southern Ocean; and *S. varnensis* Apostolov, 1972 from the Bulgarian coast of the Black Sea (see Chappuis and Serban 1953; Chappuis and Rouch 1961; Apostolov 1972, 1982; Soyer). The only other *Schizopera* with a two-segmented endopod of the fourth swimming leg, a defining character of the genus *Schizoperopsis*, was described from subterranean waters of Western Australia by Karanovic and Cooper (2012): *S. akolos* Karanovic and Cooper, 2012.

During our collecting of stygofauna in Japan, we found one new species of *Schizopera* in several freshwater interstitial locations in streams and rivers on the eastern side of ancient Lake Biwa's watershed. This is the first endemic representative of its genus from Japan. Lake Biwa originated tectonically in the Pliocene, almost 4 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 about 400,000 years ago (Satoguchi 2012), 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; Satoguchi 2012). 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 inter-glacial periods and for thermophiles during glacial maximums of the Quaternary is well studied, especially for freshwater snails (Nishino and Watanabe 2000). Like many other

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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 in detail, but Karanovic and Abe (2010) suggested that ancient lakes may act as a sort of a biodiversity pump for subterranean habitats. This was further corroborated by the discovery of an endemic sister species pair from the cyclopoid genus *Diacyclops* Kiefer by Karanovic et al. (2013). *Diacyclops brevifurcus* Ishida, 2006, which lives in surface waters near Kyoto and in Lake Biwa, is very closely related to *D. ishidai* Karanovic, Grygier, and Lee, 2013, a subterranean species found in the vicinity of Lake Biwa. Our current study resulted from a research project aimed at further testing this hypothesis, with sampling carried out in a wide range of surface and subterranean habitats in and around Lake Biwa.

Summaries of research on surface-freshwater copepods of Japan were given by Ishida and Kikuchi (2000) for harpacticoids and by Ishida (2002) for cyclopoids. The only purportedly 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*. This species was later described as *D. brevifurcus* Ishida, 2006, but was also found in a pond in Kyoto, proving that the species is not endemic to the lake (Ishida 2006). 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. Ishida (2005) described another potentially endemic *Diacyclops* species from Lake Biwa, *D. biwensis* Ishida, 2005, and Karanovic et al. (2013) described a further two potentially endemic species of this genus from subterranean waters in the vicinity of Lake Biwa: *D. ishidai* Karanovic, Grygier and Lee, 2013 and *D. parahanguk* Karanovic, Grygier and Lee, 2013. Karanovic and Abe (2010) described a new and potentially endemic species of the harpacticoid genus *Morariopsis* Borutzky, 1931 from two caves in the town of Taga, in the mountainous flanks of Lake Biwa: *M. grygieri* Karanovic and Abe, 2010. Later, the presence of this copepod in a third cave in Taga, the Same-no-Komori-Ketsu, was confirmed (unpublished data). The only other purportedly endemic Lake Biwa copepod is a subterranean harpacticoid, *Parastenocaris biwae* Miura, 1969, described from a sandy beach on the western shore at Shirahige (now in Takashima city; Miura 1969). This species was later synonymised with the Holarctic *P. brevipes* Kessler by Reid (1995), but was reinstated as valid and redescribed from an interstitial locality in the outflow of Lake Biwa by Karanovic and Lee (2012). We have since confirmed it in phreatic waters of two inflowing rivers in this region as well, the Nyuu River and Hino River (unpublished data). 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, 1962b, 1964, 1969; Kikuchi 1970; Ueda et al. 1996; Tomikawa et al. 2005).

Employing molecular techniques in addition to traditional morphological ones is one of the most important recent developments in animal taxonomy and systematics. Recently, DNA-based species identification methods, referred to as 'DNA barcoding', have been widely employed to estimate levels of species diversity, with the 5' end of the mitochondrial cytochrome C oxidase subunit 1 gene (mtCOI) proposed as the 'barcode'

for all animal species (Hebert et al. 2003). The advantage of mtCOI is that it often shows low levels of genetic variation within species, but high levels of divergence (usually > 15% among crustacean species, Lefébure et al. 2006) between species. The availability of so-called 'universal' primers developed by Folmer et al. (1994) for the polymerase chain reaction (PCR)-amplification of mtCOI also greatly facilitates the use of this marker to investigate species boundaries in animals, and these primers have previously been employed successfully to PCR-amplify copepod DNA (Adamowicz et al. 2007; Bradford et al. 2010; Sakaguchi and Ueda 2010; Karanovic and Cooper 2011a, 2011b, 2012; Karanovic and Kim 2014; Karanovic et al. 2014). One of the aims of our study was to test the phylogenetic relationships of our new species, especially with other congeners that share some of its rare morphological characters, which were speculated to be homoplastic in the genus by Karanovic and Cooper (2012).

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Material and methods

All samples for this study were collected from freshwater interstitial habitats using special Bou-Rouch-style phreatic samplers similar to those used by Tanaka et al. (2014), but without the inner perforated sleeve and with a corked but distally perforated, hand-operated plastic kerosene pump instead of an electric peristaltic pump. The samples were packed in crushed ice for transport and later fixed in ca. 70% ethanol. Fixed samples were stored in a refrigerator in the lab, and sorted under a dissecting microscope. Locality data and number of specimens are given in the Type material examined section below. All material is deposited at the Lake Biwa Museum, Shiga, Japan (LBM).

Some specimens were dissected and mounted on microscope slides in Faure's medium (see Stock and von Vaupel Klein 1996), and dissected appendages were then covered with 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. All line drawings were prepared using a drawing tube attached to a Leica MB2500 phase-interference compound microscope. Specimens that were not drawn were examined in propylene glycol and, after examination, were stored in 99.9% ethanol in a refrigerator. Specimens for scanning electron micrography (SEM) were dehydrated in progressive ethanol concentrations, transferred into pure isoamyl-acetate, critical-point dried, mounted on stubs, coated in gold and observed under a Hitachi S-4700 scanning microscope on the in-lens detector, with an accelerating voltage of 10 kV and working distances between 12.3 and 13.4 mm; micrographs were taken with a digital camera.

The terminology for macro-morphological characters follows Huys and Boxshall (1991), except for the numbering of setae on the caudal rami and small differences in the spelling of some appendages (antennula, mandibula and maxillula instead of antennule, mandible and maxillule), as an attempt to standardise the terminology for homologous appendages in different crustacean groups. Sensilla and pores on each somite (body segment), the rostrum and the caudal rami were numbered consecutively from the anterior to posterior end of body and from the dorsal to ventral side, to aid in the recognition of serially homologous structures; sensilla were numbered using Arabic numerals, and pores using Roman numerals. The same numbers on different somites do not necessarily indicate serially homologous structures; serial homology was hypothesised in the description of cuticular organs (see below). As a tentative terminology for cuticular organs in the description,

we combined abbreviations for the rostrum (R), cephalothorax (C), free prosomites (FP1–FP3) and urosomites (U1–U6) with a given Arabic or Roman numeral, connected with a hyphen (for example, sensilla pair FP1-3).

Specimens for molecular analysis were examined without dissection under a compound microscope (objective 63× dry) in propylene glycol to confirm their identity, using a cavity well slide with a central depression. After examination, they were returned to 99.9% ethanol. Before amplification, whole specimens were transferred into distilled water for 2 hours for washing (to remove ethanol), and then minced with a small glass stick. DNA was extracted from whole specimens, except in one case when only one antennula was available, using the LaboPass™ extraction kit (COSMO Co. Ltd., Korea) and following the manufacturer's protocols for fresh tissue, except that samples were incubated in the Proteinase K solution overnight, step five was skipped, and 60 instead of 200 μL of Buffer AE was added in the final step, to increase the density of DNA. The mtCOI gene was amplified through PCR using PCR premix (BiONEER Co.) in TaKaRa PCR thermal cycler (Takara Bio Inc., Otsu, Shiga, Japan). The amplification primers used were the 'universal' primers LCO1490 and HCO2198 (Folmer et al. 1994). The amplification protocol was: initial denaturation 94°C for 300 s, 40 cycles of denaturation 94°C for 30 s, annealing at 42°C for 120 s, extension at 72°C for 60 s, and final extension at 72°C for 600 s. The final product was stored at 4°C. PCR results were checked by electrophoresis of the amplification products on 1% agarose gel with ethidium bromide. PCR products were purified with a LaboPass™ PCR purification kit and sequenced in both directions using a 3730xl DNA analyser (Macrogen, Korea). For this study, DNA was extracted and the mtCOI fragment successfully PCR-amplified from three specimens of the new species (see Table 1).

An additional 37 sequences were downloaded from GenBank and included in our analyses for the following 12 *Schizopera* species and subspecies: *S. akation* Karanovic and Cooper, 2012; *S. akolos* Karanovic and Cooper, 2012; *S. analspinulosa analspinulosa* Karanovic and Cooper, 2012; *S. analspinulosa linel* Karanovic and Cooper, 2012; *S. cf. uranusi* sp. 2 (an as-yet uncharacterised new species from the Yigarn region of Western Australia); *S. cooperi* Karanovic and McRae, 2013; *S. emphysema* Karanovic and Cooper, 2012; *S. knabei* Lang, 1965; *S. kronosi* Karanovic and Cooper, 2012; *S. leptafurca* Karanovic and McRae, 2013; *S.* sp. 2 (an undescribed new species from the Pilbara region of Western Austalia); and S. *uranusi* Karanovic and Cooper, 2012. As outgroups for our analyses, and for rooting the trees, an additional four sequences from GenBank were included, belonging to two species of the marine genus *Stenhelia* Boeck: *S. pubescens* Chislenko and *S. taiae* Mu and Huys (Table 1). The latter belong to the same family as *Schizopera*, the Miraciidae, but to a different subfamily, Stenheliinae Brady (see Karanovic and Kim 2014; Karanovic et al. 2014).

Obtained sequences were checked manually and aligned by the ClustalW algorithm (Thompson et al. 1994) in MEGA version 6 (Tamura et al. 2013). The alignment was checked again and all sites were unambiguously aligned. The best evolutionary model of nucleotide substitution for our dataset was established by the Akaike information criterion, performed with jModelTest (Posada and Crandall 1998; Guindon and Gascuel 2003). For the maximum likelihood (ML) analysis, the Hasegawa–Kishino–Yano model (Hasegawa et al. 1985) with gamma distributed rates with invariant sites (HKY + G + I) was selected. The neighbour joining (NJ) analysis used the Kimura two-parameter model (Kimura 1980; Nei and

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Table 1. List of copepod specimens for which the mtCOI fragment was successfully amplified and used in our molecular phylogenetic analysis. Generic abbreviations: S. = *Schizopera*; St. = *Stenhelia*. See text for full specific and subspecific names and more details.

Species	Country	Region	Locality	Date	Bases	GenBank
S. abei	Japan	Shiga	Ane River	28 May 2012	439	KP867870
S. abei	Japan	Shiga	Ane River	28 May 2012	407	KP867871
S. abei	Japan	Shiga	Ane River	28 May 2012	431	KP867872
S. akation	Australia	Yilgarn	YYAC284	12 November 2009	639	JQ390560
S. akation	Australia	Yilgarn	YYD26	15 March 2010	474	JQ390583
S. akation	Australia	Yilgarn	YYD22	15 March 2010	517	JQ390585
S. akation	Australia	Yilgarn	SB14-1	16 March 2010	475	JQ390587
S. akation	Australia	Yilgarn	LUNK1	16 March 2010	515	JQ390589
S. akolos	Australia	Yilgarn	YYD22	15 March 2010	538	JQ390584
S. analspinulosa linel	Australia	Yilgarn	LUNK1	12 January 2010	455	JQ390563
S. analspinulosa linel	Australia	Yilgarn	LUNK1	16 March 2010	538	JQ390588
S. analspinulosa s. str.	Australia	Yilgarn	SB14-1	16 March 2010	457	JQ390586
S. cf. uranusi sp. 2	Australia	Yilgarn	YYAC248	12 November 2009	515	JQ390571
S. cooperi	Australia	Pilbara	HAMB003	18 September 2010	515	JQ390555
S. emphysema	Australia	Yilgarn	YYAC1004C	27 August 2009	515	JQ390558
S. knabei	USA	unknown	unknown	culture	640	KF667527
S. kronosi	Australia	Yilgarn	YYAC1007A	27 August 2009	454	JQ390559
S. kronosi	Australia	Yilgarn	YYAC35	12 November 2009	639	JQ390567
S. kronosi	Australia	Yilgarn	YYAC328	17 March 2010	495	JQ390576
S. leptafurca	Australia	Yilgarn	YYHC085B	18 March 2010	538	JQ390557
S. leptafurca	Australia	Yilgarn	YYAC118	12 November 2009	518	JQ390565
S. leptafurca	Australia	Yilgarn	YYAC35	12 November 2009	451	JQ390566
S. leptafurca	Australia	Yilgarn	YYAC33	12 November 2009	513	JQ390568
S. leptafurca	Australia	Yilgarn	YYAC33	12 November 2009	487	JQ390569
S. leptafurca	Australia	Yilgarn	YYAC328	12 November 2009	515	JQ390570
S. leptafurca	Australia	Yilgarn	YYAC0014D	17 March 2010	520	JQ390574
S. leptafurca	Australia	Yilgarn	YYAC328	17 March 2010	517	JQ390575

(*Continued*)

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Table 1. (Continued).

S. leptafurca	Australia	Yilgarn	YYAC0016A	20 March 2010	517	JQ390578
S. leptafurca	Australia	Yilgarn	YYHC0049K	20 March 2010	522	JQ390580
S. leptafurca	Australia	Yilgarn	YYD26	15 March 2010	538	JQ390582
S. leptafurca	Australia	Yilgarn	YYAC118	21 March 2010	538	JQ390590
S. sp. 2	Australia	Pilbara	FMGSM1585	27 February 2010	487	JQ390556
S. sp. 2	Australia	Pilbara	FMGSM1585	20 January 2010	516	JQ390572
S. uranusi	Australia	Yilgarn	YYAC284	12 November 2009	639	JQ390561
S. uranusi	Australia	Yilgarn	YYAC284	12 November 2009	515	JQ390562
S. uranusi	Australia	Yilgarn	YYAC1007	12 November 2009	639	JQ390564
S. uranusi	Australia	Yilgarn	YYAC0019B	20 March 2010	516	JQ390573
S. uranusi	Australia	Yilgarn	YYAC0016A	20 March 2010	512	JQ390577
S. uranusi	Australia	Yilgarn	YYHC0139	17 March 2010	517	JQ390579
S. uranusi	Australia	Yilgarn	YYD26	15 March 2010	516	JQ390581
St. pubescens	Russia	Far East	Posyet Bay	6 May 2012	659	KF524870
St. pubescens	Russia	Far East	Posyet Bay	6 May 2012	667	KF524871
St. taiae	Korea	South Sea	Gwangyang Bay	18 November 2012	558	KF524884
St. taiae	Korea	South Sea	Gwangyang Bay	18 November 2012	662	KF524885

Kumar 2000) with uniform rates (K2P). Maximum parsimony (MP) analysis was conducted using an heuristic search option and the following default parameters: subtree pruning and regrafting branch swapping, 10 initial trees, maximum of 100 trees to retain. All phylogenetic and molecular evolutionary analyses were conducted using MEGA version 6 (Tamura et al. 2013). One thousand bootstrap replicates were performed to obtain a relative measure of node support for the resulting trees. Average pairwise NJ distances were also computed in MEGA version 6 using the K2P model.

Systematics

Subphylum **CRUSTACEA** Brünnich
Class **MAXILLOPODA** Dahl
Subclass **COPEPODA** H. Milne Edwards
Order **HARPACTICOIDA** G.O. Sars
Family **MIRACIIDAE** Dana
Subfamily **DIOSACCINAE** Sars
Genus ***Schizopera*** Sars, 1905

Schizopera abei sp. nov.
(Figures 1–7)

Type locality

Japan, Shiga prefecture, Nagahama city, Nomura-cho township, Ane River, a few meters upstream from the older of the two Nomurabashi bridges, cobble and large pebble shoal, interstitial water from Karaman–Chappuis hole, 35°24'56.3"N 136°19'26.8"E.

Specimens examined

Holotype female (LBM1430005760) dissected on one slide, allotype male (LBM1430005761) dissected on one slide, four paratype females (LBM1430005762-LBM1430005765) and three paratype males (LBM1430005766-LBM1430005768) dissected on one slide each, 36 paratypes (13 males, nine females, and 14 copepodids) (LBM1430005769) together in ethanol, and 21 paratypes (10 males and 11 females; LBM1430005773) together on one SEM stub; all collected from type locality, 28 May 2012, leg. M.J. Grygier, S. Kanao, and S. Nakaoka (sample no. 71–01).

Six paratypes (one male and five females) together in ethanol (LBM1430005770) from Japan, Shiga prefecture, Hikone city, Inukata township, Inukami River, fine sand in river bed, interstitial water pumped with Bou-Rouch style pump, 35°14'5.9"N 136°15'1.9"E, 26 May 2011, leg. K. Tanida party (sample no. 17–02).

One paratype male in ethanol (LBM1430005771) from Japan, Shiga prefecture, Hikone city, Obori township, Seri River, fine sand in river bed, interstitial water pumped with Bou-Rouch style pump, 35°14'48.0"N 136°16'5.4"E, 25 May 2011, leg. K. Tanida party (sample no. 2–08).

Two paratype females together in ethanol (LBM1430005772) from Japan, Shiga prefecture, Taga town, Nakagawara, Seri River, fine sand in river bed, interstitial

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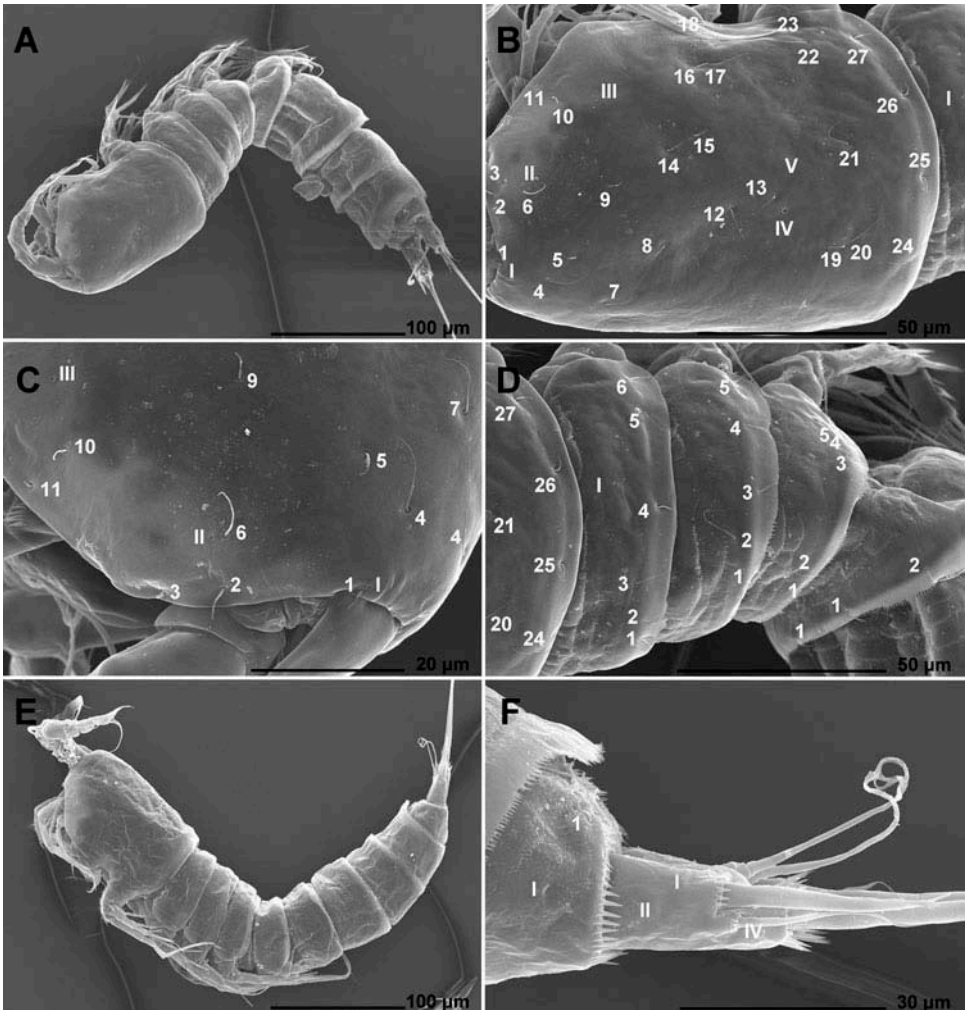

Figure 1. *Schizopera abei* sp. nov., scanning electron micrographs, (A–D) paratype female from LBM1430005773; (E–F) paratype male from the same lot. (A) Habitus, lateral; (B) cephalothoracic shield, lateral; (C) anterior part of cephalothoracic shield and rostrum, lateral; (D) tergites of free pedigerous somites, lateral; (E) habitus, lateral; (F) anal somites and caudal rami, lateral. Arabic numerals for sensilla and Roman numerals for pores consecutively from anterior to posterior end of each somite, rostrum, and caudal ramus, and from dorsal to ventral side.

water pumped with Bou-Rouch style pump, 35°14'9.9"N 136°16'56.9"E, 25 May 2011, leg. K. Tanida party (sample no. 19–01).

Etymology

The new species is named in honour of Dr. Yuji Abe, curator at the Taga Town Museum, Japan, in recognition of his continuing help in collecting subterranean copepods in and around Taga. The name is a noun in the genitive singular.

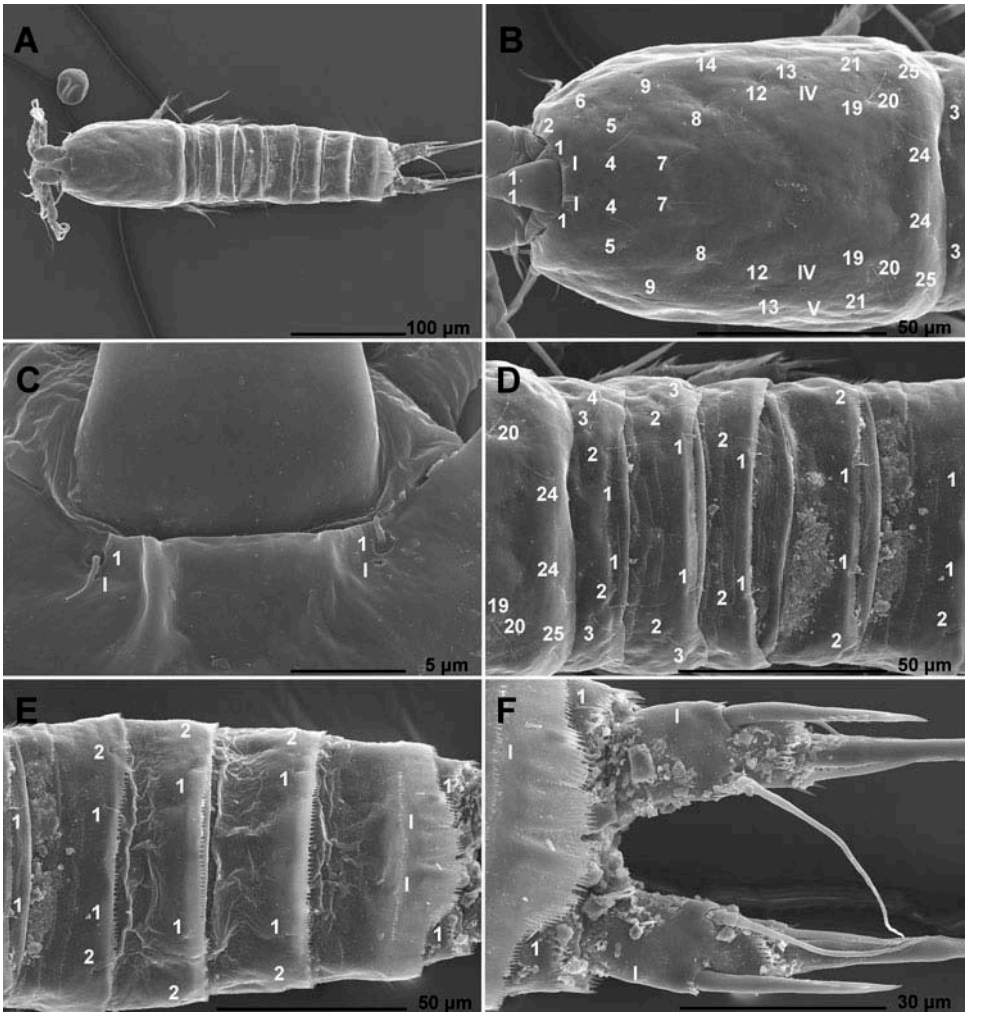

Figure 2. *Schizopera abei* sp. nov., scanning electron micrographs, a second paratype male from LBM1430005773. (A) Habitus, dorsal; (B) cephalothorax, dorsal; (C) anterior tip of cephalothorax and basal part of rostrum, dorsal; (D) free pedigerous somites, dorsal; (E) second to fifth urosomites, dorsal; (F) anal somite and caudal rami, dorsal. Arabic numerals for sensilla and Roman numeral for pores both assigned consecutively from anterior to posterior end of each somite, rostrum, and caudal ramus, and from dorsal to ventral side.

Description

Female (data from holotype and 16 paratypes). Total body length, measured from tip of rostrum to posterior margin of caudal rami (excluding caudal setae and appendages), from 330 to 355 µm. Colour of preserved specimens yellowish. Nauplius eye not visible. Prosome comprising cephalothorax with completely fused first pedigerous somite and three free pedigerous somites; urosome comprising fifth pedigerous somite, genital double-somite (fused genital and first abdominal somites) and three free abdominal somites. Habitus (Figure 1A) cylindrical but not particularly slender,

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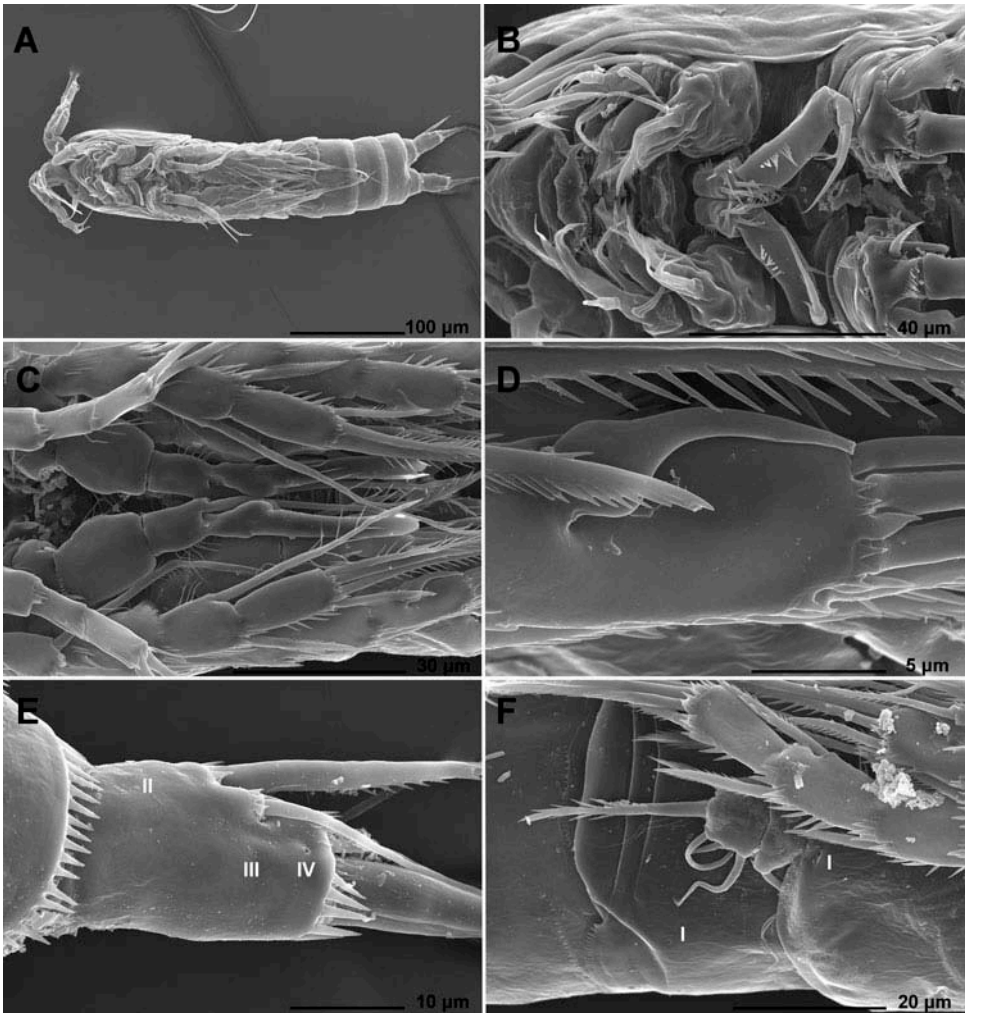

Figure 3. *Schizopera abei* sp. nov., scanning electron micrographs, (A–E) a third paratype male from LBM1430005773; (F) a fourth paratype male from the same lot. (A) Habitus, ventral; (B) mouth appendages, ventral; (C) swimming legs, anterior; (D) third exopodal segment of third swimming leg, anterior; (E) left caudal ramus, ventral; (F) left fifth and sixth legs, ventral. Roman numerals for pores assigned consecutively from anterior to posterior end of each somite and caudal ramus, from dorsal to ventral side.

without distinct demarcation between prosome and urosome; prosome/urosome ratio about 1.1 (in dorsal view); greatest width at posterior end of cephalothorax but difficult to establish, with cephalothorax only slightly wider than genital double-somite. Body length/width ratio about 4.5. Free pedigerous somites without pronounced lateral or dorsal expansions. Integument of all somites relatively well sclerotised, generally very smooth, without cuticular windows or pits. All somites (except cephalothorax) and caudal rami, besides other ornamentation, with two or more parallel rows of minute spinules. Hyaline fringe of all somites broad and at least

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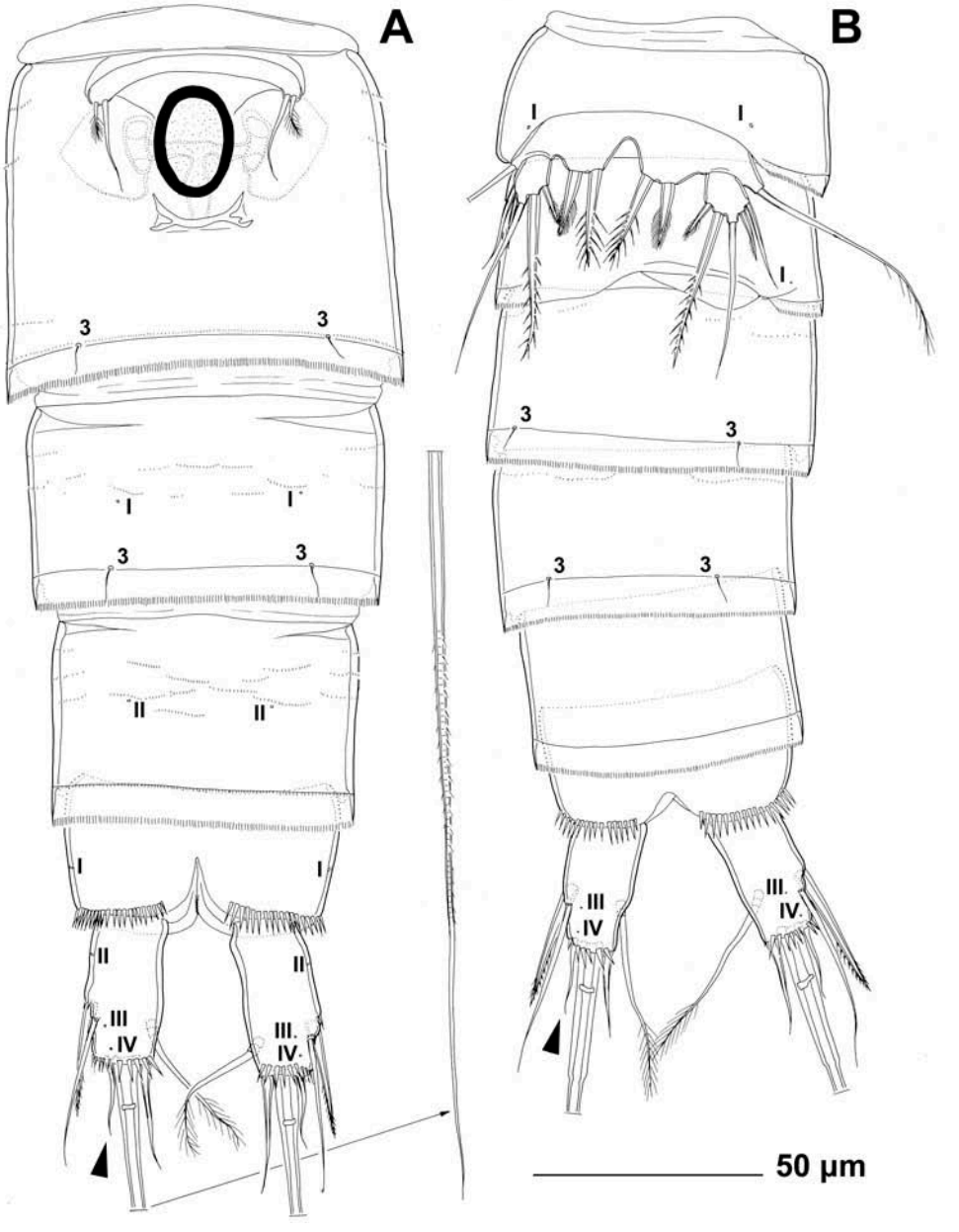

Figure 4. *Schizopera abei* sp. nov., line drawings. (A) Holotype female, urosome excluding fifth pedigerous somite, ventral; (B) allotype male, urosome, ventral. Arabic numerals for sensilla and Roman numerals for pores assigned consecutively from anterior to posterior end of each somite and caudal ramus, from dorsal to ventral side. Arrowheads point to the slender, minute outer principal seta on the caudal rami, the principal autapomorphy of the new species.

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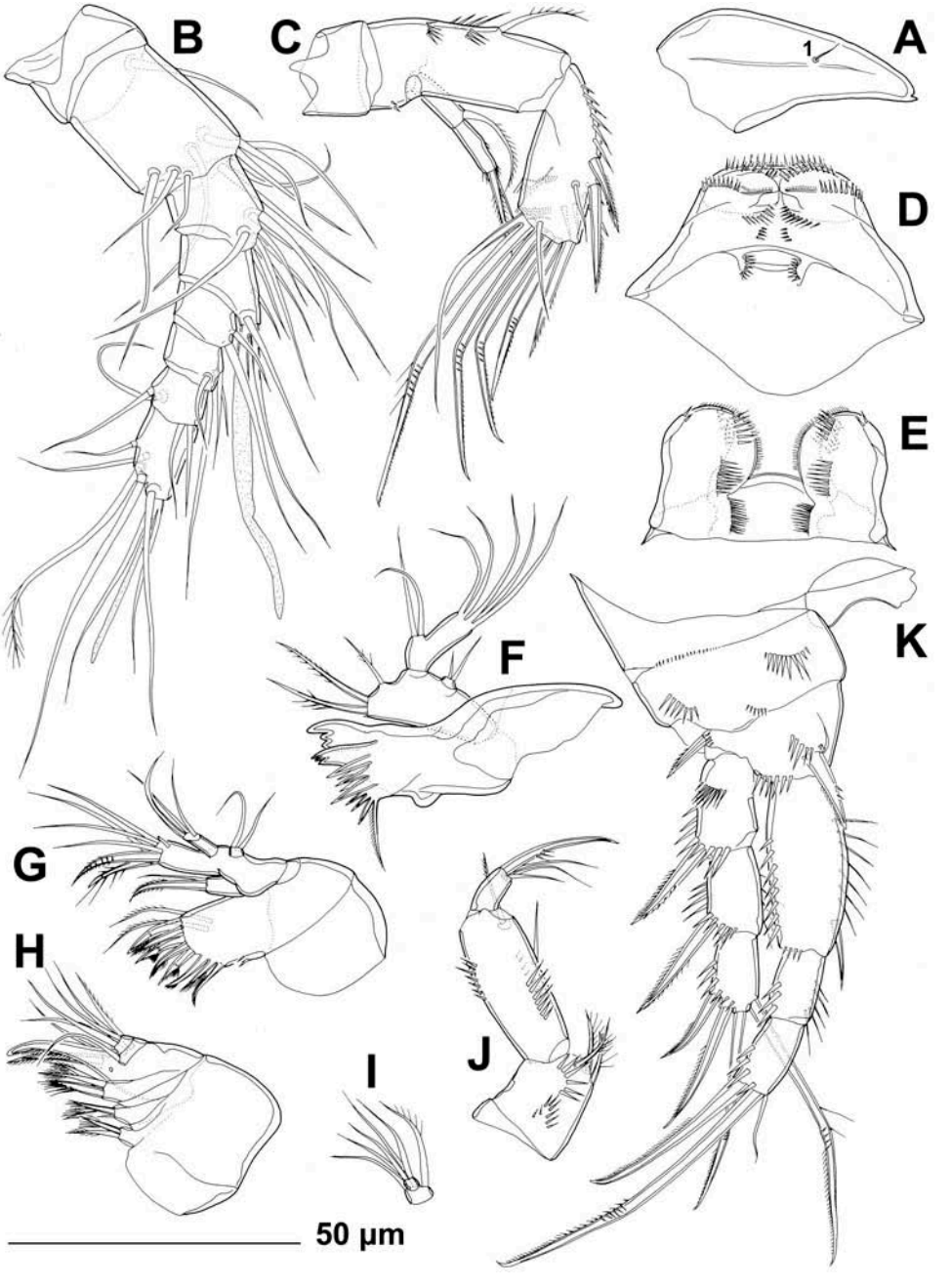

Figure 5. *Schizopera abei* sp. nov., line drawings, holotype female. (A) Rostrum dissected and flattened, lateral; (B) antennula, posterior; (C) antenna, posterior; (D) labrum, posterior; (E) paragnaths, anterior; (F) mandibula, posterior; (G) maxillula, posterior; (H) maxilla, posterior; (I) endopod of maxilla, antero-ventral; (J) maxilliped, anterior; (K) first leg, anterior. Arabic numeral 1 indicates the rostral sensillum.

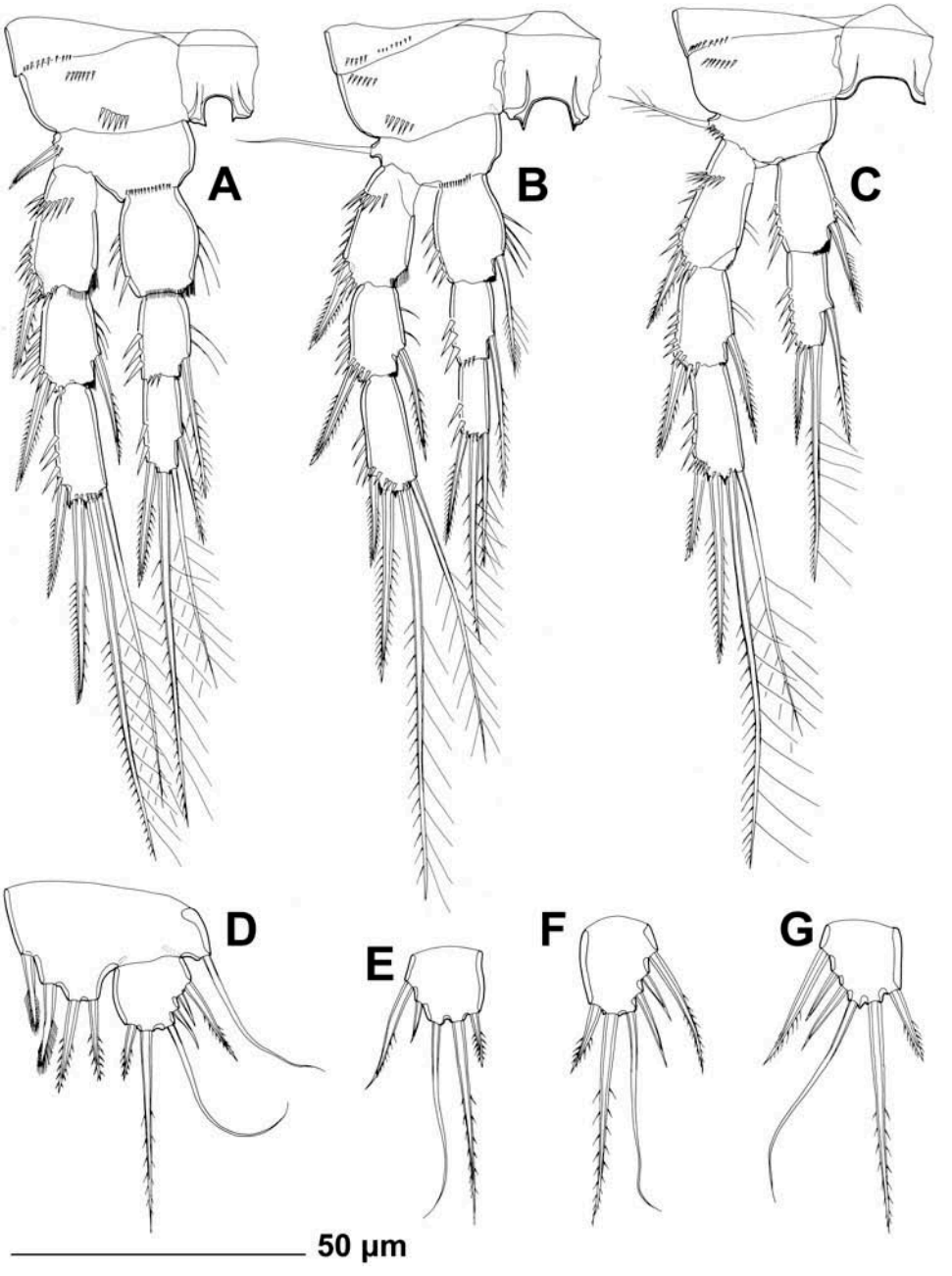

Figure 6. *Schizopera abei* sp. nov., line drawings, (A–E) holotype female; (F) paratype female (LBM1430005762); (G) another paratype female (LBM1430005763). (A) Second leg, anterior; (B) third leg, anterior; (C) fourth leg, anterior; (D) left fifth leg, anterior; (E) abnormal exopod of right fifth leg, anterior; (F) exopod of left fifth leg, anterior; (G) exopod of right leg, anterior.

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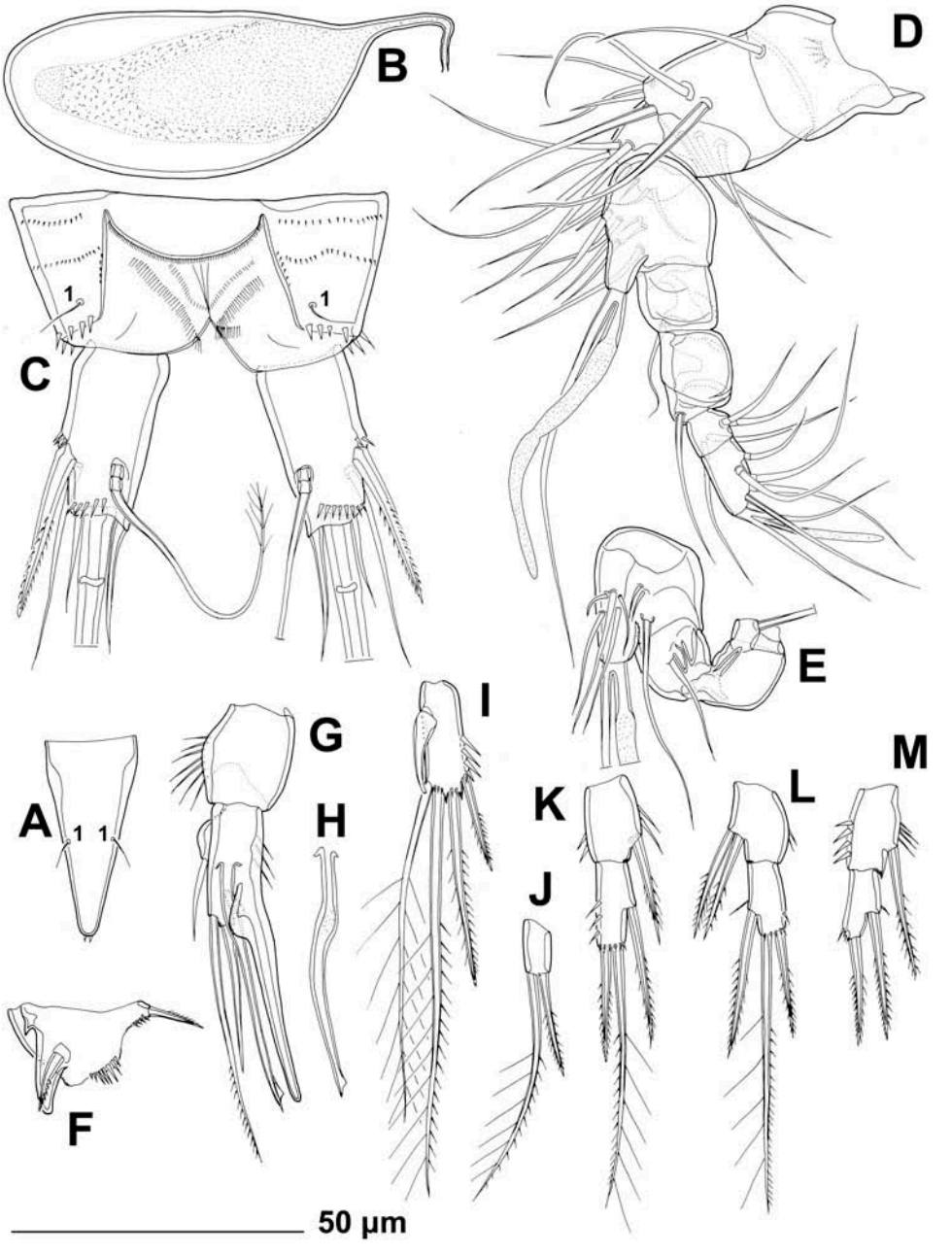

Figure 7. *Schizopera abei* sp. nov., line drawings, (A, B, J) paratype male (LBM1430005766); (C) another paratype male (LBM1430005767); (D–G, I, K, L) allotype male; (H, M) a third paratype male (LBM1430005768). (A) Rostrum dissected and flattened, dorsal; (B) spermatophore; (C) anal somite and caudal rami slightly compressed, dorsal; (D) antennula, anterior; (E) fourth to seventh segments of antennula, posterior; (F) basis of first leg, anterior; (G) endopod of second leg, anterior; (H) transformed lanceolate element on endopod of second leg, anterior; (I) third exopodal segment of third leg, anterior; (J) abnormal third endopodal segment of third leg, anterior; (K) endopod of fourth leg, anterior; (L) abnormal endopod of fourth leg, anterior; (M) abnormal endopod of fourth leg, anterior. Arabic numerals indicating dorsal sensilla on rostrum and anal somite.

partly serrated, except for smooth posterior end of cephalothoracic shield. Surface of somites, rostrum and caudal rami with total of 70 pairs of cuticular organs (15 pairs of cuticular pores and 55 pairs of sensilla; see Figures 1–4).

Rostrum (Figures 1C, 5A) long and clearly demarcated at base, reaching two thirds of second antennular segment, linguiform, about twice as long as wide, with two short spiniform processes on tip and single dorsal pair of sensilla (R-1) at about midlength.

Cephalothorax (Figure 1A–C) about 1.3 times as long as wide in dorsal view (without rostrum); representing 30% of total body length, tapering towards anterior end in dorsal view only in anterior third. Hyaline fringe of cephalothoracic shield wide and smooth. Cephalothoracic shield (Figure 1B, C) with five pairs of pores (C-I to C-V) and 27 pairs of sensilla (C-1 to C-27); sensilla C-16 and C-17 very close to each other, as well as sensilla C-19 and C-20; pores C-I very close to sensilla C-1 (both at base of rostrum) and pores C-II relatively close to sensilla C-6; pores C-IV more than twice as large as any other prosomal pores; sensilla C-19 to C-27 and pores C-XI and C-XII probably belonging to the first pedigerous somite, incorporated into cephalothorax; lateral marginal zone includes sensilla C-2, C-3, C-11, C-18, and C-23 (see Figure 1B); posterior marginal zone including sensilla C-24 to C-27 (see Figure 1D).

Pleuron of first free prosomite (second pedigerous somite; Figure 1D) with two rows of minute spinules in anterior half, one pair of anterior dorsal-lateral pores (FP1-I) and six pairs of long sensilla (FP1-1 to FP1-6); posterior marginal zone including sensilla FP1-1, FP1-2 and FP1-4 to FP1-6, while sensilla pair FP1-3 situated slightly more anteriorly and longer than any other pair; sensilla FP1-1 and FP1-6 probably serially homologous to sensilla C-24 and C-27, respectively, on first pedigerous somite; other serial homologies difficult to determine; hyaline fringe wide, serrated only dorsally, smooth dorso-laterally and laterally.

Pleuron of second free prosomite (third pedigerous somite; Figure 1D) slightly longer and with several more short rows of minute spinules than pleuron of first free prosomite, without pores, with only five pairs of long sensilla (FP2-1 to FP2-5); posterior marginal zone including sensilla FP2-1 and FP2-3 to FP2-5, while sensilla pair FP2-2 situated slightly more anteriorly and longer than any other pair; dorsal-most pair of sensilla more widely spaced than on pleuron of first free prosomite but recognition of serially homologous pairs relatively easy (FP2-1 = FP1-1, FP2-2 = FP1-3, FP2-3 = FP1-4, FP2-4 = FP1-5 and FP2-5 = FP1-5); hyaline fringe wide, serrated dorsally and dorso-laterally, smooth laterally.

Pleuron of third free prosomite (fourth pedigerous somite; Figure 1D) slightly narrower and significantly shorter (especially dorsally) than pleuron of second free prosomite, but with similar number of minute spinules and also without pores, with only five pairs of long sensilla (FP3-1 to FP3-5); all sensilla probably serially homologous to their counterparts with same Arabic numerals on pleuron of second free prosomite; hyaline fringe narrow dorsally, wide laterally, serrated dorsally and dorso-laterally, smooth laterally.

First urosomite (fifth pedigerous somite; Figure 1D) about as long as pleuron of third free prosomite but with fewer minute spinules, with one pair of ventro-lateral pores near base of fifth legs (U1-I) and two pairs of dorsal sensilla (U1-1 and U1-2); sensilla pair U1-1 probably serially homologous to sensilla pair FP3-1, but serial homology of sensilla pair U1-2 not obvious (perhaps FP3-3?); hyaline fringe wide and finely serrated dorsally and dorso-laterally, smooth only near base of fifth legs.

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Second urosomite (Figures 1A and 4A) in female almost completely fused with third urosomite into genital double-somite, with three dorsal and dorso-lateral parallel rows of minute spinules and only two pairs of dorsal posterior sensilla (U2-1 and U2-2), both probably serially homologous to their counterparts with same Arabic numerals on first urosomite. No remnants of hyaline fringe. Female genital complex (Figure 4A) with single copulatory pore posterior to epicopulatory bulb (latter also serving as copulatory duct), two small seminal receptacles placed inside large, paired, genital apertures; apertures with two ventral gonopores, each covered by reduced sixth leg. Epicopulatory bulb large, ovoid, strongly sclerotised, about 1.4 times as long as wide. Seminal receptacles very small, kidney-shaped, not reaching anterior margin of epicopulatory bulb, about 0.6 times as long as epicopulatory bulb.

Third urosomite (Figures 1A and 4A) with posterior row of minute spinules, wide and finely serrated hyaline fringe, and three pairs of posterior sensilla: one dorsal (U3-1), one lateral (U3-2) and one ventral (U3-3); establishing serially homologous sensilla of third and second urosomites not easy (possibly, U3-1 = U2-2). Genital double somite as a whole about 0.8 times as long as wide (ventral view), with only small internal ridge dorso-laterally (but no external suture) indicating original segmentation.

Fourth urosomite (Figures 1A and 4A) slightly narrower and significantly shorter than genital double-somite, with several anterior rows of minute spinules, wide and finely serrated hyaline fringe, one pair of anterior ventral pores (U4-I), and three pairs of posterior sensilla (U4-1 to U4-3); all sensilla with homologous pairs on third urosomite (i.e., U4-1 = U3-1, U4-2 = U3-2, and U4-3 = U3-3) but ventral pair (U4-3) slightly closer together.

Fifth urosomite (preanal; Figures 1A and 4A) narrower than third urosomite but not shorter, without sensilla, with one pair of posterior dorsal pores (U5-I), one pair of anterior ventral pores (U5-II), several short and slightly arched rows of minute spinules in anterior half, and posterior continuous row of minutes spinules; ventral pores serially homologous with those on fourth urosomite (i.e., U5-II = U4-I); hyaline fringe sharply serrated, ventrally as wide as that in third urosomite but dorsally extended into wide and long pesudoperculum, this nearly reaching posterior margin of anal somite, with about 35 sharp teeth.

Sixth urosomite (anal; Figures 1A and 4A) slightly narrower and only about 0.7 times as long as fifth urosomite, cleft medially in posterior half, with one pair of large dorsal sensilla (U6-1), one pair of large lateral pores (U6-I), posterior row of large spinules at base of each caudal ramus, and several short curved rows of minute spinules (mostly on dorsal and lateral surfaces); anal operculum short, narrow, convex, situated anterior to dorsal sensilla, completely covered by pseudoperculum, with posterior row of numerous hair-like minute spinules, representing 55% of somite's width; anal sinus widely opened, without any chitinous projections, with weakly sclerotised walls and two diagonal rows of long, hair-like spinules.

Caudal rami (Figures 1A and 4A) strongly sclerotised, about 1.9 times as long as greatest width in ventral view, almost cylindrical (somewhat tapering towards caudal end in posterior third but with almost straight inner margin), with space between them slightly less than one ramus width; ornamented with posterior row of large spinules, several smaller spinules at base of lateral setae, and four pairs of pores: anterior dorso-lateral pores (CR-I), anterior ventro-lateral pores (CR-II) and two pairs of posterior ventral pores (CR-III and CR-IV); armed with six elements (two

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lateral, one dorsal and three apical). Dorsal seta slender and apically pinnate, about 1.3 times as long as ramus, inserted at about 2/3 of ramus length in deep recess, triarticulate at base (i.e. inserted on two pseudojoints). Lateral proximal spine stout, bipinnate, inserted at 2/3 of ramus length and 0.8 times as long as ramus. Lateral distal seta slender, smooth, inserted slightly ventrolaterally at 3/4 of ramus length, and about as long as ramus. Inner apical seta with wide and strong base, smooth, about 0.7 times as long as ramus. Inner principal apical seta with breaking plane, very strong, distally pinnate, about six times as long as caudal ramus. Outer principal apical seta smooth, slender and very short, only about half as long as caudal ramus (arrowed in Figure 4A).

Antennula (Figure 5B) eight-segmented, approximately half as long as cephalothorax, with slender aesthetasc on eighth segment fused basally to two apical setae, large aesthetasc on fourth segment reaching significantly beyond tip of appendage and fused basally to slightly shorter seta, and setal formula 1.9.8.3.2.4.4.7. Two lateral setae on seventh segment and four on eighth segment biarticulate (i.e. inserted on short pseudojoint). All setae slender, all except for one on eighth segment smooth, and most ending apically in pore (except apical and subapical setae); apical pores only observable under scanning electron microscope. Length ratio of antennular segments, from proximal end and along caudal margin, 1:2.2:0.8:0.9:0.8:0.8:0.8:1.6.

Antenna (Figure 5C) comprising coxa, basis, two-segmented endopod and much smaller but also two-segmented exopod. Coxa short, 0.7 times as long as wide, without ornamentation or armature. Basis and first endopodal segment partly fused along posterior surface. Basis also short and unarmed, about 0.6 times as long as wide, ornamented with small spinules along inner margin. First endopodal segment twice as long as wide and 2.8 times as long as basis, with two arched rows of large spinules in proximal half on outer margin, and one short, unipinnate lateral seta at middle. Second endopodal segment 1.2 times as long as first, more slender proximally, with two surface frills distally; lateral armature consisting of two strong spines flanking small, slender seta; apical armature consisting of seven elements: one smooth, slender, short seta, one unipinnate short spine and four geniculate setae, longest fused basally to another smooth and slender but long seta; all geniculate setae with minute spinules along outer (concave) margin distally, longest one with several long spinules along inner (convex) margin as well. Ornamentation of second endopodal segment consisting of longitudinal row of large spinules along anterior margin and diagonal row of large spinules between lateral and apical armature elements. Both exopodal segments of about same width and length; first segment armed with 1 unipinnate subapical seta, unornamented; second segment ornamented with transverse apical row of slender spinules and several small lateral spinules, armed apically with one smooth, slender seta and one strong, bipinnate spine of about same length as former, both about 1.5 times as long as segment.

Labrum (Figure 5D) large, trapezoidal, rigidly sclerotised, with slightly concave cutting edge, ornamented with numerous slender apical and subapical spinules, as well as several rows of spinules of various length and orientation along posterior surface.

Paragnaths (Figure 5E) slightly smaller than labrum, also rigidly sclerotised, almost linguiform, connected by medial trapezoidal lobe resembling labrum in shape, with numerous spinules along inner and apical margins (apical ones much more robust), as well as two longitudinal rows of spinules on anterior surface.

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Mandibula (Figure 5F) with cutting edge of coxa narrow, armed with two complex teeth in ventral part (both tricuspidate), eight simple (unicuspidate) teeth in dorsal part, and one unipinnate dorsalmost seta; coxa unornamented. Basis smaller and shorter than coxa, about twice as long as wide, armed with three bipinnate slender setae along inner margin; ornamented with several minute spinules at base of ventralmost seta. Endopod one-segmented, twice as long as wide, armed with two lateral and five apical smooth setae. Exopod very small but distinct segment, armed with two smooth apical setae.

Maxillula (Figure 5G) with large praecoxa, arthrite highly mobile, armed apically with six strong, unipinnate spines, and two dorsalmost unipinnate setae; armed laterally with two smooth, slender setae and ornamented with short row of spinules at base of arthrite. Coxa small, armed with two setae on inner margin; distal seta slender and smooth, proximal seta very strong, spiniform and bipinnate. Inner margin of basis furnished with two strong, curved, bipinnate spines and five smooth, slender setae; distalmost seta minute, others as long as spine or longer. Endopod one-segmented, small, about twice as long as wide, armed with three apical smooth setae, innermost seta longest and strongest. Exopod also distinct but very small segment, half as long as wide, armed with two slender and smooth apical setae.

Maxilla (Figure 5H, I) composed of syncoxa, basis and two-segmented endopod. Syncoxa unornamented, large, ovoid, with three endites, proximal and central ones each armed with two subequal setae, distal one armed with two setae and one spine, all pinnate near distal tip. Basis much smaller than coxa, elongate, armed with one apical claw-like spine (partly fused to basis), one unipinnate and strong apical seta and one smooth, slender lateral seta on anterior surface; ornamented with one pore on posterior surface. Endopod very small, short and wide, armed with three slender setae on each segment.

Maxilliped (Figure 5J) prehensile, three-segmented, composed of coxobasis and two-segmented endopod. Coxobasis 1.2 times as long as wide, cylindrical, ornamented with two arched rows of large spinules on anterior margin, armed with three strong, unipinnate setae on inner (median) margin, all about half as long as coxobasis. First endopodal segment about 2.5 times as long as wide and 1.6 times as long as coxobasis, ornamented with two longitudinal rows of large spinules on anterior surface and one row of smaller spinules on posterior surface; armed with two short, slender setae, one centrally on inner margin and other subapically on posterior surface. Second endopodal segment smallest, only 0.3 times as long as first and twice as long as wide, armed apically with one claw-like unipinnate spine and three smooth, slender setae; spine more than twice as long as second endopodal segment and 1.3 times as long as longest seta.

All swimming legs (Figures 1A, 5K and 6A–C) slender, short in comparison to body length and width, composed of small unarmed triangular praecoxa, large unarmed quadrate coxa, smaller armed basis, three-segmented armed exopod, and three-segmented armed endopod. Coxae in all pairs of legs connected by unornamented intercoxal sclerite. All exopodal and endopodal segments of about same length, except for much longer first endopodal segment of first leg.

First swimming leg (Figure 5K) with small, short and wide intercoxal sclerite, concave at distal end and unornamented. Praecoxa ornamented with posterior row of minute spinules on anterior surface. Coxa also ornamented with several short arched rows of spinules of various sizes on anterior surface. Basis with one inner and one outer

strong, pinnate spine, inner one stronger and about 1.5 times as long as outer; ornamentation consisting of several spinules at base of each spine and one additional row of large spinules along distal margin between endopod and exopod, all on anterior surface. Exopod armed with single outer-distal spine on first and second segments, and with two outer spines and two apical geniculate setae on third segment; all exopodal segments ornamented with strong spinules along outer margin and subdistally, and additionally along inner margin of second segment; first exopodal segment with additional arched row of strong spinules on anterior surface proximally; inner geniculate seta on third segment slightly longer than entire exopod and about 1.3 times as long as outer geniculate seta. Endopod geniculate, with first segment 0.65 times as long as entire exopod, 2.4 times as long as second endopodal segment, about twice as long as wide; third endopodal segment about 1.1 times as long as second endopodal segment; endopodal armature consisting of one strong but short inner seta on first segment (inserted at about 4/5), and three setae on third segment [innermost slender and smooth, middle longest and geniculate, outermost spiniform seta (or spine?) 0.6 times as long as middle one]; endopodal ornamentation consisting of strong spinules along outer margin of all segments, and also along inner margins of first two segments.

Second swimming leg (Figure 6A) with even smaller praecoxa than in first leg, also ornamented with posterior row of spinules on anterior surface. Coxa ornamented with two short horizontal rows of large spinules on anterior surface. Intercoxal sclerite with paired, pointed, distal protrusions. Basis armed only with outer bipinnate spine, ornamented with small spinules at base of outer spine and with minute spinules along distal margin at base of endopod. Distal inner corners of first and second exopodal and endopodal segments with serrated hyaline frills. All exopodal and endopodal segments ornamented with strong spinules on outer margins; first and second segments also with weaker spinules along inner margins. Exopod armed with outer-distal spine on first and second segments, inner spiniform seta on second segment, two outer spines and two apical setae on third segment; all spines and setae strong and bipinnate; outer apical seta on third segment appearing transitional in form between spine and seta, with outer margin furnished with short spinules and inner margin with long, slender spinules. Endopod slightly shorter than exopod, armed with single inner seta on second segment, and four elements on third segment: outer-distal short spine, two apical long setae and one inner strong seta (inserted at 2/3).

Third swimming leg (Figure 6B) very similar to second, except basis armed with outer slender seta instead of spine, first endopodal segment with one inner seta, and third endopodal segment with only three elements (inner seta missing).

Fourth swimming leg (Figure 6C) similar to third leg, except endopod two-segmented and only about 0.6 times as long as exopod, innermost armature element on ultimate endopodal segment representing inner seta (inner distal seta absent), and intercoxal sclerite without pointed processes.

Fifth leg (Figure 6D–G) unornamented, biramous, composed of large, broad baseoendopod and small, ovoid exopod, with division line visible on anterior surface but not complete on posterior surface. Baseoendopod with outer basal smooth seta arising from relatively short setophore. Endopodal lobe almost triangular, extending to middle of exopod, armed with four very stout, spiniform elements (two inner ones probably spines, two outer ones probably spiniform setae); length ratio of endopodal armature elements, from inner side, 1:1.1:1.2:1. Exopod from 0.9 to 1.25 times as long as maximum width, normally armed with six elements: two innermost apical ones

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strong and bipinnate, outer apical one smooth and slender, distal and central outer ones short, smooth and spiniform, and proximal outer one long, strong and pinnate; length ratio of exopodal armature elements, from inner side, 1:3:3:1:0.8:1.8. One exopod with only five elements (Figure 6E).

Sixth leg (Figure 4A) indistinct, very small cuticular plate covering gonopore, armed with two slender setae; inner seta smooth, about 2.4 times as long as outer bipinnate seta.

Male (data from allotype and 14 other paratypes). Body length ranging from 324 to 347 μm. Habitus (Figures 1E, 2A and 3A) slightly more slender than in female, but also cylindrical, and with similar proportions of prosome/urosome, and cephalothorax/genital somite. Body length/width ratio about 4.2. Ornamentation of rostrum (Figure 7A), prosomites (Figure 2B–D) and first urosomite (Figures 2D, 3F and 4B), as well as colour and nauplius eye, as in female.

Genital somite (Figures 2E, 3F and 4B) more than twice as wide as long. Single, completely formed, longitudinally placed spermatophore (Figure 7B) inside first two urosomites in most specimens. Ornamentation consists of two pairs of large dorsal sensilla as in female (U2-1 and U2-2) and additional pair of ventral pores at base of sixth legs (U2-I).

Third urosomite (Figures 2F, 4B) ornamented as in female, but not fused to second (genital) urosomite.

Fourth and fifth urosomites (Figures 2E, F and 4B) as in female, except ventral pair of pores absent and fewer minute spinules present.

Sixth urosomite (Figures 1F, 2F, 3E, 4B and 7C) as in female.

Caudal rami (Figures 1F, 2F, 3E, 4B and 7C) slightly shorter in comparison with anal somite and more slender than in female but without any difference in armature or ornamentation. Outer principal seta also very short and slender (arrowed in Figure 4B).

Antennula (Figure 7D, E) half as long as cephalothorax, strongly prehensile and nine-segmented (basically, female's sixth segment subdivided in male), with geniculations between fourth and fifth and seventh and eighth segments. Segments participating in geniculations strengthened with cuticular plates along anterior surface, with largest such plates on fifth segment. Aesthetascs as in female, on fourth and last segments; that on fourth segment somewhat wider than in female. First two and last two segments similar to female. Setal formula: 1.9.8.9.1.0.1.4.7. Most setae smooth and with pore on tip; same setae biarticulate as in female.

Antenna, labrum (Figure 3B), mandibula (Figure 3B), maxillula (Figure 3B), maxilla (Figure 3B), maxilliped (Figure 3B), exopod and endopod of first swimming leg (Figure 3C), exopod of second swimming leg (Figure 3C), endopod of third swimming leg and fourth swimming leg (Figures 3F and 7K) as in female.

First swimming leg (Figures 3B and 7F) with modified basis, inner margin very rigidly sclerotised, with spiniform smooth distal process and smaller sharp process at its base. Inner spine on basis smaller than in female, without spinules at its base, inserted more proximally and about as long as larger spiniform process.

Second swimming leg (Figures 3C and 7G, H) with transformed endopodal second and third segments. Second segment with part of inner margin protruded as rounded indistinct lobe, without ornamentation on its surface; inner seta shorter than in female, smooth and slender. Third segment completely modified; inner apical seta unipinnate and longer than in female, outer apical seta smooth and strong, outer

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apical spine transformed into smooth, lanceolate implement with slightly swollen part at about first third of its length and abruptly tapering tip; outer distal corner produced into long, blunt spiniform process, about as long as lanceolate outer spine. As result of these transformations, third segment medially cleft.

Third swimming leg (Figures 3D and 7I) with very characteristic element on anterior surface of third exopodal segment probably representing hugely enlarged tubular pore: swollen in basal part, with pore on tip, inserted at 2/5 and close to inner margin, reaching distal margin of third segment.

Fifth legs (Figures 3F and 4B) with medially fused baseoendopods. Endopodal lobe much smaller and shorter, trapezoidal, extending to first third of exopod in length, armed with two very strong apical spines; inner spine about 1.5 times as long as outer one and with fewer but stronger spinules. Exopod about as long as wide, demarcated basally on both anterior and posterior surfaces, armed with only five elements (one short lateral element missing); length ratio of exopodal armature elements, from inner side, 1:3.8:4:1.1:2.1.

Sixth legs (Figures 3F and 4B) expressed as pair of small, short cuticular plates, without armature or ornamentation; left one better demarcated at base and probably functioning as genital flap.

Variability

In addition to the slight variability in body length (see above), several other features were observed as variable. The exopod of the female fifth leg was about as long as wide in most specimens (see Figure 6G), but the length/width index could vary from 0.9 to 1.25 (Figure 6D, F); one paratype female was observed with only five elements on one fifth leg exopod (Figure 6E), while the opposite leg has a normal condition of six elements (Figure 6D). One paratype male had only two elements on the third endopodal segment of the third leg (Figure 7J), while the opposite leg and all other specimens examined had the normal condition of three elements. Another paratype male had an abnormal endopod of the fourth leg, with two inner setae on the first segment (Figure 7L), while its opposite leg is normal (i.e. same as in the female; Figure 7K). One paratype male had only two elements on the second endopodal segment of the fourth leg (Figure 7M), while its opposite leg was normal.

Molecular results

DNA was extracted, and the mtCOI fragment successfully PCR-amplified from three specimens of our new species (Table 1). All sequences were translated into protein using MEGA and were shown to have no evidence of stop codons, ambiguities or insertions/deletions indicative of non-functional copies of mtCOI. BLAST analyses of GenBank revealed that the obtained sequences are copepod in origin and not contaminants. All analyses were run with all additional 37 *Schizopera* mtCOI sequences downloaded from GenBank, and with four sequences belonging to two outgroup species (Table 1). The complete mitochondrial genome of *S. knabei* (see Easton et al. 2014) was trimmed for the largest overlapping range with any other congener after alignment (640 bases), while all other sequences were used with their original lengths, ranging from 407 to 639 bases. Our alignment showed no gaps. Average pairwise distances between species were found to be very high (Table 2), with the

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Table 2. Average pairwise neighbour joining (NJ) distances [Kimura two-parameter (K2P) model] among mtCOI sequences between each morphospecies (lower diagonal) and within morphospecies (diagonal). Generic abbreviations: S. = *Schizopera*; St. = *Stenhelia*.

Species	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.	13.	14.	15.
1. *S. abei*	0.000														
2. *S. akation*	0.255	0.122													
3. *S. akolos*	0.235	0.274	–												
4. *S. analspinulosa s.str.*	0.219	0.256	0.236	–											
5. *S. analspinulosa linel*	0.242	0.268	0.224	0.150	0.000										
6. *S. cf. uranusi* sp. 2	0.190	0.259	0.208	0.210	0.191	–									
7. *S. emphysema*	0.232	0.278	0.277	0.245	0.250	0.221	0.000								
8. *S. kronosi*	0.241	0.219	0.197	0.202	0.185	0.188	0.227	0.011							
9. *S. leptafurca*	0.238	0.272	0.236	0.225	0.234	0.214	0.215	0.210	0.020						
10. *S. cooperi*	0.255	0.288	0.262	0.274	0.271	0.238	0.256	0.245	0.266	–					
11. *S.* sp. 2	0.228	0.256	0.223	0.339	0.262	0.222	0.263	0.246	0.314	0.270	0.000				
12. *S. uranusi*	0.230	0.277	0.226	0.241	0.253	0.203	0.193	0.229	0.169	0.216	0.235	0.007			
13. *S. knabei*	0.242	0.270	0.292	0.296	0.304	0.287	0.285	0.238	0.287	0.289	0.287	0.293	–		
14. *St. taiae*	0.299	0.295	0.293	0.309	0.317	0.315	0.338	0.288	0.341	0.347	0.269	0.299	0.318	0.000	
15. *St. pubescens*	0.291	0.295	0.295	0.317	0.361	0.285	0.339	0.273	0.329	0.336	0.281	0.299	0.315	0.229	0.002

lowest divergence (16.9%) between *S. leptafurca* and *S. uranusi*. Divergences between all other taxa were in excess of 18%. These high divergence values are generally indicative of distinct species by comparison with other crustaceans (Lefébure et al. 2006) and other harpacticoid copepods (Karanovic and Cooper 2011a, 2012; Karanovic and Kim 2014; Karanovic et al. 2014). There was evidence for multiple divergent lineages (12.2% average sequence divergence; 4.9–17% divergence between haplotypes) within the species *S. akation*, but the question remains as to whether these lineages represent the presence of cryptic species or are just divergent mtDNA sequences within a species. All other divergences within morphospecies were below 2%, and these are indicative of intraspecific variability (Lefébure et al. 2006). Our three sequences of *S. abei* did not show any differences, which is not surprising considering that all three specimens were collected in the same locality and are probably kin.

The ingroup was recovered in all three cladistics analyses (Figure 8), and was supported with relatively high bootstrap values: 90% for ML, 83% for MP and 99% for NJ. All our analyses also supported the presence of at least 13 genetically divergent ingroup lineages, corresponding to 13 morphospecies, and all seven of the multi-sample lineages were supported with high bootstrap values (between 90 and 100%). A sister group relationship between *S. uranusi* and *S. leptafurca* was only relatively weakly supported, despite the overwhelming morphological evidence suggesting this (see Karanovic and Cooper 2012), but the monophyly of a group comprising *S. uranusi*, *S. leptafurca* and *S. emphysema* was moderately well supported, in accordance with morphological evidence. A relatively well-supported sister group relationship between *S. analspinulosa s. str*. and *S. analspinulosa linel* (95% in our NJ analysis) was also shown. Although morphological evidence suggests a relatively close relationship among *S. kronosi*, *S. analspinulosa s. str.* and *S. analspinulosa linel*, this relationship was only recovered in our NJ analysis, and with extremely low bootstrap support (24%). Most basal ingroup clades had very low bootstrap support, which is likely to be the result of the low phylogenetic resolution of the mtCOI gene in basal nodes of the tree, possibly due to saturation at third codon positions (Karanovic and Cooper 2012) and also to various lengths of the fragments amplified (see Table 2). For example, our MP analysis resulted in a single most parsimonious tree of 494 steps, but the consistency and retention indices were low (0.41 and 0.77 respectively), as was the number of parsimony-informative sites (270), while the number of variable sites was 298 and there were 28 singletons. Not surprisingly, the tree topology differed between different methods employed. For example, our NJ analysis suggested *S.* sp. 2 as a sister clade to all other *Schizopera*, while our MP analysis grouped this species together with *S. abei* and *S. knabei* (albeit

Figure 8. Maximum likelihood (ML) tree based on mtCOI sequences of 40 *Schizopera* specimens from Australia, Japan, and the USA (see Table 1), constructed using MEGA V6.0 and an HKY+G + I model of evolution. Numbers on the branches represent bootstrap values above 50% for three different methods (NJ/MP/ML) from 1000 pseudoreplicates. The tree is rooted with *Stenhelia taiae* Mu and Huys, 2002 from Korea and *Stenhelia pubescens* Chislenko, 1978 from Russia. The cladogram is drawn to scale and the specimen codes represent their GenBank accession numbers. Arrowheads point to two *Schizopera* species with a two-segmented endopod of the fourth leg.

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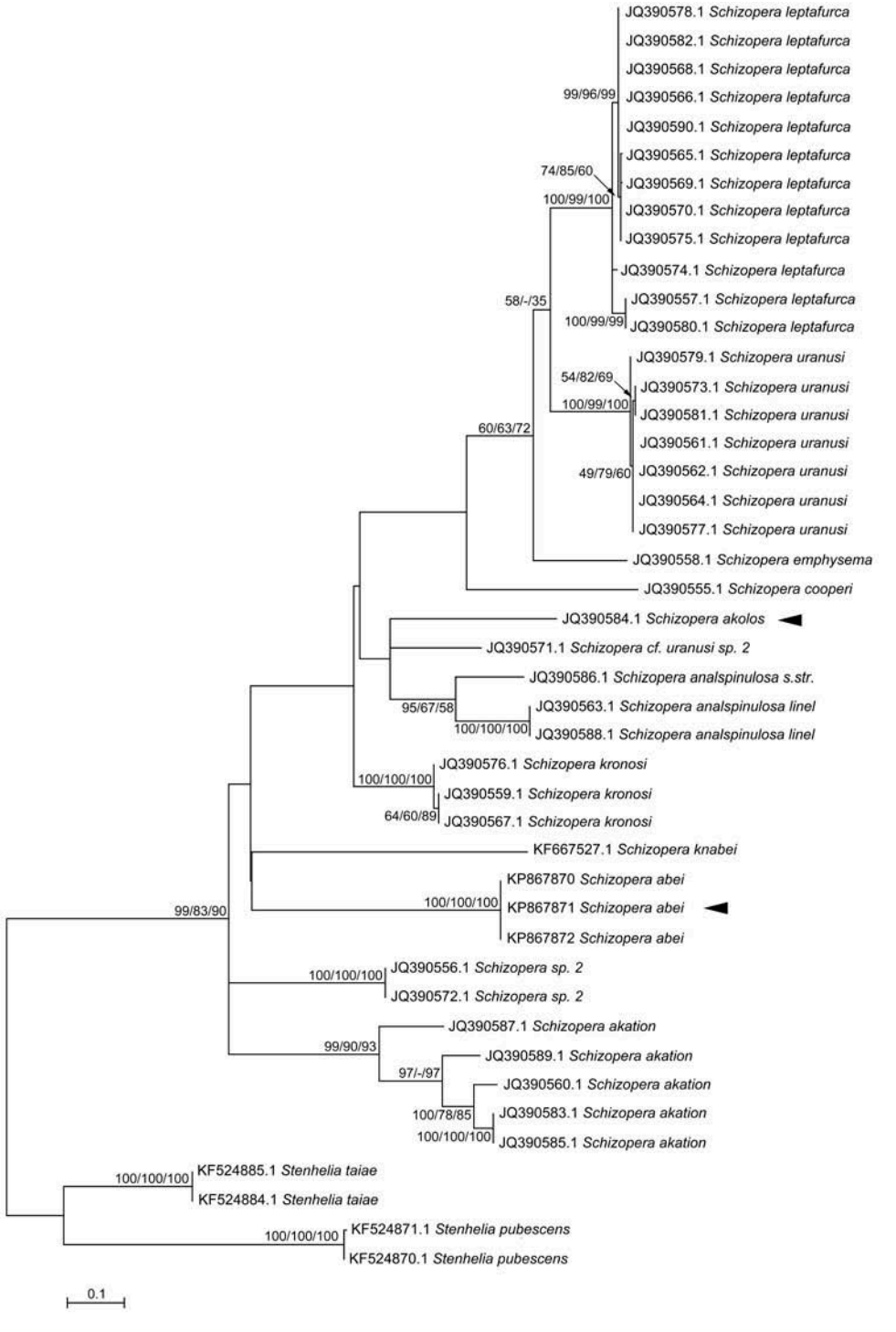

with a bootstrap support of only 27%), and our ML analysis suggested an unresolved basal position (Figure 8). The one specimen that did not match our morphospecies (JQ390571.1; preliminary identification as *S.* cf. *uranusi*) formed a separate lineage and is likely to represent an uncharacterised species of *Schizopera*.

The phylogenetic position of *S. abei* among its other congeners could not be established based on the cladistic analysis of mtCOI with any confidence. Our ML analysis suggested a sister relationship with the American marine *S. knabei*, but this clade was very weakly supported (30%) and was not recovered in our NJ and MP analyses. Instead, our MP analysis suggested a sister relationship with *S.* sp. 2 (13% bootstrap support) with them as a sister clade to *S. knabei*, while our NJ analysis suggested *S. abei* as a sister clade to a large group of nine species (25% bootstrap support). This all suggests that our new Japanese species has no close relatives among the congeners for which we had mtCOI sequences. This is not surprising as most of them come from inland waters of Australia. None of our analyses suggested a particularly close relationship of *S. abei* and *S. akolos*, the only two species in this group with a two-segmented endopod of the fourth leg, that would have been considered members of the genus *Schizoperopsis* based on morphological characters proposed by Apostolov (1982).

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Discussion

The presence of a large, transformed tubular pore on the male third leg exopod (Figures 3D and 7I) shows that the new species unquestionably belongs to the genus *Schizopera*, and this conclusion is further supported by a number of morphological characters that it shares with nearly all 100 congeners: the shape of the female genital field with a large and characteristically shaped epicopulatory bulb (Figure 4A), the segmentation and armature of the antennula (Figure 5B), antenna (Figure 5C) and mouth appendages (Figure 5D–J), the segmentation and armature of the first three swimming legs (Figures 3C, 5K and 6A, B), the shape and armature of the fifth leg (Figures 3F, 4B and 6D) and the armature of the caudal rami (Figures 1F, 2F, 3E, 4A, B and 7C).

Schizopera abei sp. nov. differs from all congeners in the armature of the caudal rami in both sexes, with a minute and slender outer principal seta (arrowed in Figure 4A, B) and a long and strong inner principal seta. A number of *Schizopera* species have a relatively short outer principal seta on the caudal ramus, including, for example, *S. oldcuei* Karanovic, 2004 from Western Australia (see Karanovic 2004), but in no species is this seta so reduced in thickness and size as to be shorter and more slender than the innermost apical seta. This character state is a clear autapomorphy of the new species. Several species of *Schizopera* have females with both principal setae dramatically reduced in length (for example, *S. bradyi* Soyer, 1975 from Kerguelen Island; *S. elatensis* Kahan and Bar-El, 1982 from Israel; and *S. soyeri* Kunz, 1983 from the Azores), but they are always very bulbous; that is, they look like the basal parts of normally developed elements (see Soyer 1975; Kahan and Bar-El 1982; Kunz 1983).

The new species has a two-segmented endopod of the fourth leg (see Figures 6C and 7K–M), which is a character found so far only in five other congeners: *S. akolos* Karanovic and Cooper, 2012 from subterranean waters in arid Western Australia; *S. arenicola* Chappuis and Serban, 1953 from the Romanian coast of the Black Sea;

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S. gauldi Chappuis and Rouch, 1961 from a sandy beach in Accra, Ghana; *S. nichollsi* Soyer, 1975 from Kerguelen Island, Southern Ocean; and *S. varnensis* Apostolov, 1972 from the Bulgarian coast of the Black Sea (see Apostolov 1972; Chappuis and Serban 1953; Chappuis and Rouch 1961; Soyer 1975; Karanovic and Cooper 2012). This character was used by Apostolov (1982) to erect the genus *Schizoperopsis*, which was criticised by Mielke (1992, 1995), Willen (2000), Karanovic (2004) and Karanovic and Cooper (2012). In fact, the only morphological character that unites these species is the segmentation of the fourth leg endopod, while most other characters that show interspecific variability in *Schizopera* differ among them (for a summary of major differences see Table 3). Other differences include the length proportions of different armature elements, as well as the ornamentation of some somites and appendages. Several of these species are as yet known only from a limited set of morphological characters and cannot be properly compared with all members. Even so, several other morphological differences could be observed between the new species and some of these five congeners. For example, even though *S. abei* and *S. gauldi* have the same armature formula of the fourth leg endopod (1.3), the nature of the innermost seta on the second segment is clearly different; this seta is inserted on the inner margin in *S. abei*, while in *S. gauldi* it has a terminal position. Clearly, this armature formula originated convergently by a reduction of different armature elements. Similarly, the same armature formula of the third leg endopod in *S. abei* and *S. nichollsi* (1.1.3; see Table 3) is probably a result of convergent evolution. Morphological data suggest that our new species has no close relatives among these five species, and they all probably originated independently from more primitive *Schizopera* species, with a three-segmented endopod of the fourth leg, each one developing its own set of reductions. Note that the majority of *Schizopera* species from around the world have exactly the same armature formula of the swimming legs.

Our molecular phylogenies (Figure 8) substantiate this hypothesis, as no sister relationship was suggested between *S. abei* and *S. akolos* in any of our analyses. In fact, the two are only remotely related, with their average pairwise distances exceeding 23% (see Table 2). We interpret this as a further evidence for the polyphyletic nature of the genus *Schizoperopsis*, as already argued by Mielke (1992) and Karanovic and Cooper (2012). Our ML analysis suggested a sister relationship between *S. abei* and *S. knabei*, but this clade was very weakly supported (30%), and was not recovered in our NJ and MP analyses. Their average pairwise distances are too large to support a close relationship (in excess of 24%), and the two species differ by a number of morphological characters, including the armature and length of the caudal rami, the length of the first endopodal segment of the first leg, the armature of the third leg's endopod, the segmentation of the fourth leg's endopod and the relative lengths of the fifth leg's armature (see Lang 1965). It should be noted, however, that almost all basal clades in our trees were very weakly supported, which shows limitations of a single-gene approach for reconstructing phylogenetic relationships. Further studies should aim to include more molecular markers, in addtition to a wider taxon sampling. Our study shows the mtCOI gene to be quite adequate for barcoding in this group of copepods, with individuals of the same species grouped nicely together and separately from other species.

Schizopera abei is the second harpacticoid of marine origin described from subterranean waters in the drainage area of ancient Lake Biwa. The first one was *Morariopsis grygieri* Karanovic and Abe, 2010, described from two caves in the

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Table 3. Major morphological differences among six *Schizopera* species with a two-segmented endopod of the fourth leg.

Characters/species	*S. abei*	*S. akolos*	*S. arenicola*	*S. gauldi*	*S. nichollsi*	*S. varnensis*
Caudal rami, number of dorsal rows of spinules	1	2	1	2	4	1
Caudal rami, proximal lateral seta enlarged	no	yes	yes	no	no	no
Caudal rami, outer principal seta minute	yes	no	no	no	no	no
First leg, endopod, number of segments	3	3	3	2	3	3
First leg, first endopodal segment, inner seta present	yes	yes	yes	no	yes	yes
Second leg exopod, second segment, inner seta	yes	no	no	no	no	yes
Second leg endopod in female, armature formula	0.1.4	0.1.4	0.0.3	0.0.2	0.1.4	0.1.3
Third and fourth leg exopods, second segment, inner seta present	yes	no	no	no	no	no
Third leg endopod, armature formula	1.1.3	0.1.2	0.0.3	0.0.2	1.1.3	1.1.2
Fourth leg endopod, armature formula	1.3	0.3	0.2	1.3	1.2	0.2
Fifth leg exopod in female, number of elements	6	4	5	5	6	6
Fifth leg endopodal lobe in female, number of elements	4	4	4	3	4	4

town of Taga, in the mountainous flanks of Lake Biwa (Karanovic and Abe 2010), and also now known from another cave in the same region (unpublished data). Other known members of *Morariopsis* 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 (see Kiefer 1930; Borutzky 1931, 1952; Lang 1948, Petkovski 1959; Borutzky and Okuneva 1975; Brancelj 1986, 2000, 2001). Karanovic and Abe (2010) performed a cladistic analysis of this genus based on 21 morphological characters and concluded that *M. grygieri* is not closely related to any of its presently known congeners, and probably represents a separate colonisation event. They speculated 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.

This scenario of independent colonisation from marine or (more probably) brackish benthic or interstitial habitats in the first phases of the formation of Lake Biwa would be a plausible explanation for the presence of an endemic *Schizopera* there today. However, the known history of Lake Biwa and its predecessors does not include a marine or estuarine stage (Kawanabe 1996). The closest salt water got to any of the lakes would have been when Osaka Bay reached up nearly to Kyoto, between 1 million years ago and 100,000 years ago (Satoguchi 2012), but no study has yet explicitly addressed the question of the lake's proximity to such estuaries. On the other hand, there are other endemic animals in Lake Biwa with marine or estuarine relatives, such as one amphipod crustacean (Ariyama 2007), one clam (Nishino and Watanabe 2000) and perhaps one or two goby fishes (Takahashi 1989). We interpret the discovery of the endemic *Schizopera* there as further evidence that ancient lakes have a significant role as biodiversity pumps for subterranean habitats (see Karanovic and Abe 2010), in addition to their role as refugia (Matzinger et al. 2006; Albrecht and Wilke 2008), even though it is entirely plausible that the ancestor of *S. abei* may have colonised subterranean waters in the vicinity of Lake Biwa directly through marine/brackish/freshwater interstitial (i.e. without ever colonising the lake itself). We agree with Ariyama (2007) that colonisation routes of recent Lake Biwa endemics could be very different, but that colonisation from estuarine environments along its outflow is certainly a significant component.

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No potential conflict of interest was reported by the author(s).

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