Partial revision of the neustonic genus *Scapholeberis* Schoedler, 1858 (Crustacea: Cladocera): decoding of the barcoding results (#48120)

First revision

Guidance from your Editor

Please submit by **20 Oct 2020** for the benefit of the authors .

Structure and Criteria
Please read the 'Structure and Criteria' page for general guidance.

Custom checks
Make sure you include the custom checks shown below, in your review.

Author notes
Have you read the author notes on the guidance page?

Raw data check
Review the raw data.

Image check
Check that figures and images have not been inappropriately manipulated.

Privacy reminder: If uploading an annotated PDF, remove identifiable information to remain anonymous.

Files
Download and review all files from the materials page.

1 Tracked changes manuscript(s)
1 Rebuttal letter(s)
22 Figure file(s)
3 Table file(s)
1 Raw data file(s)

Custom checks

DNA data checks

- Have you checked the authors data deposition statement?
- Can you access the deposited data?
- Has the data been deposited correctly?
- Is the deposition information noted in the manuscript?

Field study

- Have you checked the authors field study permits?
- Are the field study permits appropriate?

New species checks

- Have you checked our new species policies?
- Do you agree that it is a new species?
- Is it correctly described e.g. meets ICZN standard?

For assistance email peer.review@peerj.com

Structure and Criteria

2

Structure your review

The review form is divided into 5 sections. Please consider these when composing your review:

1. **BASIC REPORTING**
2. **EXPERIMENTAL DESIGN**
3. **VALIDITY OF THE FINDINGS**
4. General comments
5. Confidential notes to the editor

You can also annotate this PDF and upload it as part of your review

When ready submit online.

Editorial Criteria

Use these criteria points to structure your review. The full detailed editorial criteria is on your guidance page.

BASIC REPORTING

- Clear, unambiguous, professional English language used throughout.
- Intro & background to show context. Literature well referenced & relevant.
- Structure conforms to PeerJ standards, discipline norm, or improved for clarity.
- Figures are relevant, high quality, well labelled & described.
- Raw data supplied (see PeerJ policy).

EXPERIMENTAL DESIGN

- Original primary research within Scope of the journal.
- Research question well defined, relevant & meaningful. It is stated how the research fills an identified knowledge gap.
- Rigorous investigation performed to a high technical & ethical standard.
- Methods described with sufficient detail & information to replicate.

VALIDITY OF THE FINDINGS

- Impact and novelty not assessed. Negative/inconclusive results accepted. *Meaningful* replication encouraged where rationale & benefit to literature is clearly stated.
- All underlying data have been provided; they are robust, statistically sound, & controlled.
- Speculation is welcome, but should be identified as such.
- Conclusions are well stated, linked to original research question & limited to supporting results.

Standout reviewing tips

The best reviewers use these techniques

Tip	*Example*
Support criticisms with evidence from the text or from other sources	*Smith et al (J of Methodology, 2005, V3, pp 123) have shown that the analysis you use in Lines 241-250 is not the most appropriate for this situation. Please explain why you used this method.*
Give specific suggestions on how to improve the manuscript	*Your introduction needs more detail. I suggest that you improve the description at lines 57- 86 to provide more justification for your study (specifically, you should expand upon the knowledge gap being filled).*
Comment on language and grammar issues	*The English language should be improved to ensure that an international audience can clearly understand your text. Some examples where the language could be improved include lines 23, 77, 121, 128 – the current phrasing makes comprehension difficult.*
Organize by importance of the issues, and number your points	*1. Your most important issue* *2. The next most important item* *3. …* *4. The least important points*
Please provide constructive criticism, and avoid personal opinions	*I thank you for providing the raw data, however your supplemental files need more descriptive metadata identifiers to be useful to future readers. Although your results are compelling, the data analysis should be improved in the following ways: AA, BB, CC*
Comment on strengths (as well as weaknesses) of the manuscript	*I commend the authors for their extensive data set, compiled over many years of detailed fieldwork. In addition, the manuscript is clearly written in professional, unambiguous language. If there is a weakness, it is in the statistical analysis (as I have noted above) which should be improved upon before Acceptance.*

Partial revision of the neustonic genus *Scapholeberis* Schoedler, 1858 (Crustacea: Cladocera): decoding of the barcoding results

Petr G Garibian [Equal first author, 1], **Anna N Neretina** [1], **Derek J Taylor** [2], **Alexey A Kotov** [Corresp. Equal first author, 1]

[1] A.N. Severtsov Institute of Ecology and Evolution, Russian Academy of Sciences, Moscow, Russia

[2] Department of Biological Sciences, State University of New York at Buffalo, Buffalo, New York, United States

Corresponding Author: Alexey A Kotov
Email address: alexey-a-kotov@yandex.ru

Water fleas (Crustacea: Cladocera) are among the most intensively studied freshwater invertebrates. But, ecologically important daphniids that live on the surface layer (neuston) remain taxonomically confused. Here we attempt to reconcile genetic and morphological information for the neustonic genus *Scapholeberis* Schoedler, 1858 (Cladocera: Daphniidae) and present the first revision of the *Scapholeberis kingii* species group. We analyzed new and existing mitochondrial DNA sequences (cytochrome oxidase I gene region) together with morphology for all but one of the known species of this neustonic daphniids genus. Morphological comparisons of available populations, belonging to the *Scapholeberis kingii* species group from several Australian, Asian and African localities, revealed, that they are almost identical according to parthenogenetic females. At the same time, Australian populations can be reliably distinguished from Asian ones based on the morphology of gamogenetic females. Mitochondrial DNA data analyses revealed divergent lineages (>17% for the DNA barcoding COI region) for the three different species (Australia, Asia and Africa). Based on this set of data, we redescribed *S. kingii* Sars, 1888 from Australia, its *terra typica*, and described a new species, *S. smirnovi* **sp.nov.** from the Russian Far East, Korea and Japan. The status of populations from Ethiopia and the Republic of South Africa remained unclear, because in the African material and the putative type material, we found only parthenogenetic females. Our results provide an integrative revision of the *S. kingii* species group and improve the taxonomic scaffold used for barcoding and genomics for the remaining species groups in the daphniid genus *Scapholeberis*.

Partial revision of the neustonic genus *Scapholeberis* Schoedler, 1858 (Crustacea: Cladocera): decoding of the barcoding results

Petr G. Garibian[1*], Anna N. Neretina[1], Derek J. Taylor[2], Alexey A. Kotov[1*]

[1] A.N. Severtsov Institute of Ecology and Evolution, Russian Academy of Sciences, Moscow, Russia.

[2] Department of Biological Sciences, the State University of New York at Buffalo, Buffalo, USA.

* Equal first authors

Corresponding author: Alexey A. Kotov[1]

A.N. Severtsov Institute of Ecology and Evolution, Moscow, Russia Leninsky prospect 33, Moscow, 119071, Russia

alexey-a-kotov@yandex.ru

Abstract

Water fleas (Crustacea: Cladocera) are among the most intensively studied freshwater invertebrates. But, ecologically important daphniids that live on the surface layer (neuston) remain taxonomically confused. Here we attempt to reconcile genetic and morphological

information for the neustonic genus *Scapholeberis* Schoedler, 1858 (Cladocera: Daphniidae) and present the first revision of the *Scapholeberis kingii* species group. We analyzed new and existing mitochondrial DNA sequences (cytochrome oxidase I gene region) together with morphology for all but one of the known species of this neustonic daphniids genus. Morphological comparisons of available populations, belonging to the *Scapholeberis kingii* species group from several Australian, Asian and African localities, revealed, that they are almost identical according to parthenogenetic females. At the same time, Australian populations can be reliably distinguished from Asian ones based on the morphology of gamogenetic females. Mitochondrial DNA data analyses revealed divergent lineages (>17% for the DNA barcoding COI region) for the three different species (Australia, Asia and Africa). Based on this set of data, we redescribed *S. kingii* Sars, 1888 from Australia, its *terra typica*, and described a new species, *S. smirnovi* **sp.nov.** from the Russian Far East, Korea and Japan. The status of populations from Ethiopia and the Republic of South Africa remained unclear, because in the African material and the putative type material, we found only parthenogenetic females. Our results provide an integrative revision of the *S. kingii* species group and improve the taxonomic scaffold used for barcoding and genomics for the remaining species groups in the daphniid genus *Scapholeberis*.

Subjects: Biodiversity, Taxonomy, Freshwater Biology

Key words: Biogeography, Genetics, Integrative Taxonomy, Morphology, New Species, *Scapholeberis*.

Running title

An integrative revision of the neustonic genus *Scapholeberis*

Introduction

Integrative taxonomy combines the evidence from disparate biological disciplines to better understand biodiversity. This approach has been particularly fruitful for taxonomically challenging, yet well-studied aquatic groups such as the water fleas (Crustacea: Branchiopoda: Cladocera). For some cladoceran taxa successful advances have been made by morphological (*Smirnov, 1992, 1996; Van Damme, Sinev & Dumont, 2011; Neretina & Sinev, 2016*) or genetic evidence alone (*Adamowicz et al., 2009; Bekker et al., 2016; Thielsch et al., 2017*). For some problematic cladoceran taxa, a combination of approaches has resulted in taxonomic progress (*Belyaeva & Taylor, 2009; Kotov, Ishida & Taylor, 2009; Quiroz-Vázquez & Elías-Gutiérrez, 2009*). The integrative approach has been particularly useful for taxa that lack distinguishing characters for parthogenetic females. For cladocerans, the sexual stages appear sporadically, but can be a rich source of diagnostic morphological characters (see review in *Kotov, 2013*). Genetic approaches, such as formal genetic barcoding (*Hebert et al., 2003*), have much value for the discovery of novel lineages and taxonomic diagnoses. However, taxonomic advances with genetic information alone are problematic because the existing taxonomic scaffold (i.e. from the 19th of 18th centuries) is based on morphology (*Kotov & Gololobova, 2016; Dupérré, 2020*). Moreover, as museum samples, including type materials, are generally not amenable to genetic study (but see *Umetsu et al., 2002; Turko et al., 2019*), taxonomic advances are often limited to morphological evidence.

At the same time, genetic data (sequences of different genes) for cladocerans (as well as other organisms) from different geographic regions are rapidly accumulating in specialized databases such as Genbank (*Benson et al., 2012*). A massive accumulation of cytochrome

oxidase I gene region sequences (COI data) is available for many taxa due to the successful realization of the Barcoding of Life initiative (*Hebert et al., 2003*). The coordination of this genetic information with formal taxonomic knowledge, even with the modest aim of accurate species identifications, is a considerable challenge.

The aim of the present paper is to apply the integrative approach to the considerable taxonomic problems of cladocerans associated with the surface layer of standing waters, with a focus on the genus *Scapholeberis* Schödler, 1858 (Anomopoda: Daphniidae: Scapholeberinae). Since the revision of *Dumont & Pensaert* (*1983*), most efforts to understand the diversity within this genus have been local (*Hudec, 1983; Elmoor-Loureiro, 2000; Elías-Gutiérrez et al., 2008; Quiroz-Vázquez & Elías-Gutiérrez, 2009; Hudec, 2000; Kotov, Jeong & Lee, 2012*). Recently, a global phylogenetic study of the subfamily based of 402 multigene sequences from the 12S rRNA, 16S rRNA, and tRNA (val) regions of the mitochondrial genomes was carried out (*Taylor, Connelly & Kotov, 2020*). Lineage diversity was unexpectedly high in the Eastern Palearctic. Other regions, such as Africa, remained unexamined according to current standards of cladoceran taxonomy. Notably, the within-genus divergences for neustonic taxa were much greater than that found within other daphniid genera (*Taylor, Connelly & Kotov, 2020*). We were unable to reconcile the newly uncovered taxa with existing databases, genome projects, and taxonomy or to assess if the marked divergences were limited to non-protein coding regions. Here we address some geographic sampling gaps (such as Africa), attempt to unify the genetic (including DNA barcoding and genome projects) and morphological knowledge, and revise the taxonomy of the genus *Scapholeberis*. We collect new COI sequences and revise the taxonomy of the widespread and historically confused *Scapholeberis kingii* Sars, 1888 species group using an integrated approach.

Material and methods

Collecting samples and their preliminary analysis

Numerous samples from different localities in different continents were collected by our team or by our colleagues via small-sized plankton nets (with mesh size 50 μm) and fixed via 4% formaldehyde or 96% ethanol in the fields, immediately after sampling. Sampling in non-protected water bodies of Russia do not require special permissions. Sampling in South Korea was conducted in frames of the program of the National Institute of Biological Resources (NIBR), of the Republic of Korea. Sampling in Ethiopia was conducted in frames of work of the Joint Ethiopian-Russian Biological Expedition (JERBE), with permission from the Ministry of Environment of Ethiopia to JERBE. Samples from Australia are obtained from colleagues having appropriate permissions.

All samples were preliminarily examined using a stereoscopic microscope. Individuals of *Scapholeberis* in them were initially identified via available references only according to morphological features (mainly, shape of head and rostrum from the ventral view) (*Dumont & Pensaert, 1983; Kotov et al., 2010*).

Genetics

Before genetic analysis, identification of each parthenogenetic female was re-checked under a binocular stereoscopic microscope in order to avoid mistakes, because some samples contained several *Scapholeberis* species simultaneously. Selected individuals were placed into 96 Well PCR plates and dried from ethanol on air. DNA of single individuals was extracted using DNA QuickExtract (Epicenter) as modified by *Ishida, Kotov & Taylor (2006*). PCR

reactions were carried out in 25 µL or 50 µL volumes using the Promega GoTaq Master mix protocol with 5 µL of DNA extraction, and the COI (cytochrome oxidase I subunit) via HCO/LCO primers of *Folmer et al.* (*1994*). PCR cycling conditions were 95 °C for 2 m, 95 °C for 30 s, 48 °C for 30 s, and 72 °C for 1 m for 39 cycles, followed by 72 °C for 5 m. The sizes of the PCR products were verified by agarose gel electrophoresis. PCR products were then purified and exposed to Sanger sequencing by TACGEN (California). Amplicons were sequenced in both directions and the contigs were assembled in Geneious R7. The authenticity of newly obtained sequences was verified by BLAST comparisons. Additional sequences were obtained from NCBI GenBank. The alignment was carried out in the online version of MAFFT 7 using the default settings. Phylogenetic trees were estimated using a Maximum Likelihood (ML) optimality criterion (with a GTR+I+gamma model) and the Subtree Pruning and Regrafting branch-swapping algorithm in Seaview 4.7. Violin plots (which show the full distribution of the data) were created in R for major taxa based on pairwise Kimura's 2-parameter distances (also calculated in Seaview). Branch support for the ML tree was estimated by the transfer bootstrap expectation method (using BOOSTER: https://booster.pasteur.fr/) which typically shows less "false" erosion of support compared to nonparametric bootstrap for deeper nodes (*Lemonie et al., 2018*). Bayesian analyses (BI) were performed in MrBayes v.3.2.6 (*Ronquist et al., 2012*). Four independent Markov chain Monte Carlo (MCMC) analyses were run simultaneously for 100000 generations and sampled every 500 generations. The site rate parameter (rates) was gamma plus invariable sites (invgamma) and the number of substitution types (nst) was six. The first 25% of the generations were discarded as the burn-in. Phylograms were visualized using the FigTree Version 1.4.4. The ML tree was rooted using specimens of the genus *Megafenestra* as outgroups.

Original sequences are deposited to the Genbank under Accession Numbers MT371605-MT371659.

Morphological analysis

The morphology of populations from Australia and Asia (southern part of the Russian Far East and South Korea), containing both parthenogenetic and ephippial females, was examined in detail with the aim of finding diagnostic characters. Parthenogenetic females from Ethiopia and the Republic of South Africa were examined because ephippial females and males were lacking. Specimens of *Scapholeberis* from presorted samples were selected under a binocular stereoscopic microscope LOMO, and then studied *in toto* under optical microscopes Olympus BX41 or Olympus CX 41 in a drop of glycerol formaldehyde or a glycerol-ethanol mixture. Then at least two parthenogenetic females and two ephippial females (if available) from each locality were dissected under a stereoscopic microscope for the study of appendages and postabdomen. Drawings were prepared via a *camera lucida* attached to optical microscopes. Several individuals from each population were dehydrated in a series of ethanol washes (30, 50, 70, 95%) and 100% acetone and then dried using hexametyldisilazane (*Laforsch & Tollrian, 2000*). Dried specimens were mounted on aluminum stubs, coated with gold in a S150A Sputter Coater (Edwards, United Kingdom), and examined under a scanning electron microscope (Vega 3 Tescan Scanning Electron Microscope, TESCAN, Czech Republic). We used a system of setae enumeration by *Kotov (2013)*, in cases of dubious homologies, the numbers are supplied by question marks.

Abbreviations

Abbreviations for collections: DAD, permanent slides from Collectio Dadayana, the Hungarian Natural History Museum, Budapest, Hungary; MGU ML, Invertebrate Collection of Moscow State University, Moscow, Russia; NIBR, collection of the National Institute of Biological Resources, Inchon, South Korea.

Abbreviations in illustrations and text: I–V, thoracic limbs I–V; acs, accessory seta; e1–e5, endites 1–5 of thoracic limbs; ejh, ejector hooks on limb I; epp, epipodite; ext, exopodite; IDL, inner distal lobe; ODL, outer distal lobe; pep, preepipodite.

Nomenclatural acts

'The electronic version of this article in Portable Document Format will represent a published work according to the International Commission on Zoological Nomenclature (ICZN), and hence the new names contained in the electronic version are effectively published under that Code from the electronic edition alone. This published work and the nomenclatural acts it contains have been registered in ZooBank, the online registration system for the ICZN. The ZooBank Life Science Identifiers (LSIDs) can be resolved and the associated information viewed through any standard web browser by appending the LSID to the prefix http://zoobank.org/. The LSID for this publication is: urn:lsid:zoobank.org:pub:A4A3415D-857E-42E5-9103-B8D48AC60832. The online version of this work is archived and available from the following digital repositories: PeerJ, PubMed Central and CLOCKSS'.

Results

COI Phylogeny

106 Scapholeberine sequences (58 from this study) were aligned and analyzed. We detected 21 main mitochondrial clades of Scapholeberinae (Figs. 1–2, and Supplementary Table 1). We used the clade labels proposed by *Taylor, Connelly & Kotov, 2020*. Lineages novel to the present study are labelled: X, Y, L2, J1–J4. Deep branches within *Scapholeberis* had low to moderate support in the ML tree. In contrast, the differentiation of terminal taxa (species) was well-supported, as was the separation of major morphologically-based species/species groups: *S. mucronata* (clades A–C and X, green in Fig. 2), *S. rammneri* (clades F–H and Y, red), *S. freyi* (clades J1–J4, black), *S. kingii* (clades K, L1, L2, grey), *S. spinifera* (clade M), and *S.* cf. *microcephala- armata* (clades E and N) (Figs. 1 and 2).

The *S. mucronata* species group (Figs. 1 and 2) had four main geographic clades (A+B+C+X). Clade A (*S. mucronata* s. str.) was detected only in Western and Central Europe; clade B was detected from European Russia to Yakutia and Alaska; clade C was found in Western Alaska only. Clade X was detected in the vicinity of Churchill, Manitoba (*Jeffery, Elías-Gutiérrez & Adamowicz, 2011*).

The *S. rammneri* species group (Figs. 1 and 2) had five main geographic clades (F+G+H+I +Y). Clade F (*S. rammneri* s.str.) was found in a single locality in Mongolia; clade G was present in two localities in Eastern Siberia; clade H was widely distributed in North America and in a single locality in Patagonia; clade I was detected only in a single locality in Patagonia. Clade Y was found in a single locality in Israel.

The *S. freyi* species group (Figs. 1 and 2) was represented by four main clades (J1–J4). Clade J1 (*S. freyi* s.str.) was detected in many localities in North America; clade J2 (*S. duranguensis*) was found in two localities in Mexico; clade J3 was found in three localities on the Yucatan Peninsula (sequences of *Elías-Gutiérrez et al., 2008* and *Prosser, Martínez-Arce &*

207 *Elías-Gutiérrez, 2013*); clade J4 was present in a single locality in Brazil (sequence directly
208 submitted to the GenBank) and a single locality in Belgium (also a direct submission to the
209 GenBank).

210 The *S. microcephala-armata* species group (Figs. 1 and 2) was represented by two main
211 clades, E from Alaska and Far east, and N from North America.

212 The *S. kingii* species group (Figs. 1 and 2) was represented by three clades (K, L1–L2).
213 Clade K (*Scapholeberis kingii* s. str.) was detected only in Australia; clade L1 was found in
214 Japan and China; clade L2 was found in a single locality in Ethiopia.

215 The genus *Megafenestra* (Figs. 1 and 2) was represented by three clades: clade O (*M.*
216 *aurita* s.str.) was found in Europe (Ukraine and Switzerland), clade P was present in Alaska
217 only; clade Q (*M. nasuta* s.str.) was present in New York State, USA.

218 Sequence pairs within each genus (*Megafenestra* and *Scapholeberis*) had maximum K2
219 parameter distances that exceeded 30% (Fig. 3). Indeed, the mean pairwise sequence divergence
220 within *Scapholeberis* exceeded 20%. Notably, within each major species group had some
221 pairwise sequence divergences exceeded 20% as well. The closest members of the *S. kingii*
222 complex were from Japan and Africa with a 17.4% distance estimate (Fig. 2).The large
223 divergences within genera for nucleotides were not accompanied by divergences in COI amino
224 acid sequences. The most common protein sequence for example was >99% similar to that found
225 in the genus *Daphnia* (e.g. AAL08864.1). Synapomorphic amino acid substitutions in
226 *Scapholeberis* included: a glycine to an alanine for *S. kingii* (Australia), and an alanine to a
227 serine for the *S. mucronata* group, and a serine to an alanine in *S. microcephala*.
228
229 ***Morphological analysis***

230

231 **Order Anomopoda Sars, 1865**

232 **Family Daphniidae Straus, 1820**

233 **Subfamily Scapholeberinae Dumont & Pensaert, 1983**

234 **Genus *Scapholeberis* Schödler, 1858**

235

236 ***Scapholeberis kingii* species group**

237 **Diagnosis.** Species of medium size for the genus (length of adult parthenogenetic female
238 up to 0.75 mm without mucro). Body with typical features of the genus (see *Dumont & Pensaert,*
239 *1983*), relatively elongated. In lateral view, head relatively large, without keel. Rostrum
240 relatively short and blunt. In ventral view posteroventral portion of head forms a three-lobed
241 rostrum, due to a shallow depression at the insertion point of antenna I on each side, its middle
242 lobe rounded, with minute frontal head pore. Dorsal head pores absent. Head and valves without
243 short denticles, spines or protuberances. Ventral margin of valve straight. Posteroventral angle
244 with short mucro. Adhesive ventral rim of valves modified into "sucker-plate" (in terms of
245 *Dumont & Pensaert, 1983*), no setae along most part of the sucker length except few rarely
246 located setae at anteriormost portion and several sparsely located setae at posterior portion near
247 mucro. Inner surface of posterior margin with broad "hyaline membrane" extending posterior rim
248 and "denticulated membrane" consisting of row of short setules along posterior rim. Five pairs of
249 thoracic limbs, proportions between seta 1’ and seta 2 of thoracic limb I are important for species
250 identification. Ephippium with single egg and two longitudinal keels.

251 Differentiation of species is based on characters listed in Table 1.

252

1. *Scapholeberis kingii* Sars, 1888

Figures 4–9

Daphnia mucronata (Müller) in *King, 1853*, p. 255–265, fig. 6E.

Scapholeberis kingii Sars, 1888, p. 68.

Scapholeberis kingi Sars in *Henry, 1919*, p. 465; *Henry, 1922*, p. 29, Pl. 4: Fig. 3; *Dumont, 1983*, 105–106, Pl. 3*; Dumont & Pensaert 1983*, p. 24–25, Fig. 2: 3; Fig. 4: 4; Fig. VI: 1–2; Pl. 1: 8; Pl. 2: 4; Pl. 3: 5, 7, 9; Pl. 4: 1–7; Pl. 5: 1–2, 4; Fig. 10: 3; Pl. 6: 6–8; Fig. 12 Fig. 21: 4 (partial); *Smirnov, 1995*, p. 5; *Shiel & Dickson, 1995*, p. 35.

? *Scapholeberis Kingi* n. sp. *in Sars, 1903*, p. 8–10, Pl. 1: Fig. 2a–c. – junior homonym of *S. kingi* Sars, 1888.

Type locality. "South Creek" and "Paramatta" (King, 1853), New South Wales, Australia.

Type material. Lost.

Material studied here. See Supplemental Table 2.

Redescription. Parthenogenetic female (Figs. 4A–E, 5, 6 and 7A–E). In lateral view body relatively elongated, dorsal margin regularly arched, ventral margin almost straight, maximum height at body midpoint (body height/length ratio about 0.6 for adults and 0.5 for juveniles) (Figs. 4A and 7A). In dorsal or ventral view body ovoid, moderately compressed from sides (Fig. 4B). In anterior view body moderately compressed, dorsal keel absent. Posterodorsal angle obtuse, posteroventral angle almost straight, with a long spine (mucro) (Figs. 4A, 5D–E and 7A–C). A row of numerous small setules on inner face of posterior margin of valve (Figs. 5D–E, 7B–C). Ventral margin covered by setae of different size (Figs. 5A–D). Anterovenral

276 angle of valve broadly rounded, its ventral portion with a small protuberance (Fig. 7B). Valves
277 with well-developed sculpture of polygonal reticulation.
278 Head large for a daphniid (Figs. 4A and 7A). In lateral view head elongated, with a
279 prominent rostrum, its distal portion roundish (Figs. 4A and 7A). In dorsal view head elongated,
280 head shield with low lateral projections (fornices) covering bases of antennae II, a sclerotized
281 ridge departs from the insertion of antenna II and extends to the side of head (Fig. 4B). In
282 anterior view head slightly compressed from lateral sides (Figs. 4C and 7D). In ventral view
283 postero-ventral portion of head forms a three-lobed rostrum, due to a shallow depression in
284 points of antenna I insertion on each side, its middle lobe rounded, with a minute frontal head
285 pore (Figs. 4C and 7D–E). In anterior view, distance between the center of ocellus and eye
286 slightly greater (almost twice) than distance from the center of ocellus to the tip of rostrum (Fig.
287 4C). Dorsal head pores absent. Labrum large, distal labral plate with bunches of long setules, in
288 ventral view labrum triangular, with lateral projections (Fig. 4D, 7D).
289 Valve with straight ventral margin (Figs. 4A, 5D and 7A–B). Adhesive ventral rim of
290 valves modified into "sucker-plate" (in terms of *Dumont & Pensaert, 1983*), no setae along most
291 part of the sucker length except few rarely located setae at anteriormost portion and several
292 sparsely located setae at posterior portion near mucro (Figs. 5A–C). Inner surface of posterior
293 margin with a broad "hyaline membrane" (in terms of *Dumont & Pensaert, 1983*) extending the
294 posterior rim and a "denticulated membrane" (in terms of *Dumont & Pensaert, 1983*) consisting
295 of row of short setules along the posterior rim (Figs. 5D–E, 7B–C).
296 Thorax relatively long for daphniids, abdomen short (Fig. 4A).
297 Postabdomen almost rectangular, postabdomen length/height ratio about 3 (Figs. 5F–G).
298 Ventral margin almost straight. Preanal margin two times longer than anal margin. Anal and

299 postanal margins almost equal in length. Basis of claws slightly inflated, bordered from distal
300 margin by a clear incision (Figs. 5G–I). Postanal portion of postabdomen armed with long, thin
301 solitary teeth and bunches of fine setules (Figs. 5G–H). Bunches of fine setules also on anal
302 margin and lateral surface of postabdomen. Postabdominal claw long (almost as long as anal
303 margin), slightly curved (Figs. 5H–I). Its external side armed by three rows of small denticles,
304 decreasing in size distally. Denticles in middle portion of claw are stronger and located more
305 sparsely as compared to other denticles. Basal spine absent (Figs. 5H–I).
306 Antenna I jointed to the head surface, relatively short, antennular body with aesthetascs
307 exceeds tip of rostrum in length (Figs. 5J–K, 7D–E). Antennular sensory seta slender, arising
308 subdistally, almost equal in length to antennular body. Nine aesthetascs unequal in size (Figs.
309 5J–K, 7E). All aesthetasc tips projecting beyond tip of rostrum.
310 Antenna II relatively long (Figs. 4A, 5L–M, 7A). Antennal formula for setae: 0-0-1-3/1-
311 1-3. Antennal formula for spines: 0-1-0-1/0-0-1. Coxal part folded, with two sensory setae. Basal
312 segment elongated, covered by concentric rows of fine setules with a very thin spine between
313 antenna II exopod and endopod branches on outer surface and a short bisegmented seta on outer
314 surface (Figs. 5L–M). Branches relatively elongated, all segments cylindrical, covered by
315 concentric rows of fine setules and tiny denticles around their distal margins. Apical setae typical
316 for daphniids (as long as antennal branches), setulated asymmetrically. Lateral setae arising from
317 basal and middle endopod segment long (reach tips of apical setae) (Fig. 5L). Lateral seta arising
318 from third exopod segment thin and relatively short (reaches the middle of apical setae). Spine
319 on the second exopod segment short and thin. Spines on apical segments of endopod and exopod
320 branches very small and short, subequal in size to concentric apical denticles, arising from distal
321 portions of apical segments.

Thoracic limbs: five pairs (Figs. 6A–E).

Limb I with ovoid epipodite (Fig. 6A). Accessory setae long, armed by long setules. Outer distal lobe with two setae unequal in size. Distal segment of the longest seta unilaterally armed by short setules; proximal portion of this seta bears especially long setules. Shorter seta of outer distal lobe bilaterally armed by short setules. Inner distal lobe (endite 5) with three setae unequal in size and shape (Fig. 5A: 1, 1', 1''). Two setae bisegmented, with elongated distal portions. A single seta 1 brush-shaped (in terms of *Dumont & Pensaert, 1983*), its distal end abrupt, bearing long thickened setules. Endite 4 with a short anterior seta 2 and two posterior setae (Fig. 6A: a–b). The ratio between seta 1' and seta 2 is almost 2.5 (i.e. seta 2 is relatively short as compared to *S.* cf. *intermedius* from Africa, see below). Endite 3 with a short and thin anterior seta 3 and two posterior setae (Fig. 6A: c–d). Endite 2 with a short anterior seta 4 and four posterior setae (Fig. 6A: e–h). Two ejector hooks subequal in size.

Limb II large (Fig. 6B). Limb distal portion (exopodite) as large ovoid setulated lobe with two soft setae unequal in size. Four endites fused (e5–e2), bearing in toto six setae. Distal segments of anterior setae a–d covered by short denticles. Two posterior setae (Fig. 6B: a, d) bear long setules. Gnathobase (endite 5) with two rows of setae: four anterior setae (Fig. 6B: 1–4, among them seta 1 as a small elongated sensillum) and six posterior setae of gnathobasic "filter plate".

Limb III with a large ovoid epipodite (Fig. 6C) and a flat round exopodite bearing four distal setae (Fig. 6C: 1–4), (among them seta 2 the longest) and two lateral setae (Fig. 6C: 5–6) unequal in length. Setae 3–5 covered by long setules. Setae 1–2 featured by long setules in their proximal portions and bearing shorter stiff setules on their distal segments. Inner distal portion of limb with four endites: endite 5 with a single, short anterior seta (1) and a posterior seta (a);

345 endite 4 with a single anterior seta (2) and a single posterior (b) seta; endite 3 with a short
346 anterior seta (3) and two posterior setae (c–d); endite 2 with two anterior seta (4–5?) and four
347 posterior (e–h) setae. The rest of limb inner-distal portion as a singular large lobe, modified
348 gnathobase, bearing numerous posterior soft setae, each with chitinous insertion within basal
349 portion of distal segment, and a single, relatively long anterior seta (1) in its distal corner (Fig.
350 6C).

351 Limb IV with a large ovoid epipodite (Fig. 6D) and wide, flat rounded exopodite with
352 two protruding setulated lobes, four distal (Fig. 6D: 1–4) and two lateral (Fig. 6D: 5–6) setae.
353 Among them seta 4 the longest. Inner-distal portion of this limb with completely fused endites,
354 distally with two setae (Fig. 6D: 1–2) of unclear homology, the most part of limb inner margin is
355 a gnathobase filter plate consisting of numerous posterior setae.

356 Limb V (Fig. 6E) with a setulated preepipodite, large, subovoid epipodite, triangular
357 exopodite supplied with two small, thin distal setae (Fig. 6E: 1–2) unequal in length and a large
358 lateral seta (Fig. 6E: 3). Inner limb portion as an ovoid flat lobe, with setulated inner margin and
359 a single, large seta.

360 **Ephippial female (Figs. 4F–H, 7F–L, 8A–G).** Body shape in general as in
361 parthenogenetic female. Dorsal portion of valves modified into ephippium. Ephippium dark
362 brown, ovoid, clearly bordered from ventral and lateral portions of valves separating during its
363 casting off (Figs. 4F, 7F–G, I–J). Egg chamber with a single egg, elongated, its sculpture
364 represented by shallow depressions (Figs. 4F–G, 7H, 7L, 8C). Sculpture of the rest of ephippium
365 is represented by small polygons. Lateral keels are well distinguishable from the lateral (Figs.
366 4F–G, 7F–G, I–J) and dorsal view (Figs. 8A–B). From the dorsal view, area between two keels
367 strongly elongated, keels not projected laterally out of body dorsal contour (Figs. 8A–B).

Preephippial female (Figs. 9A–F). Body shape in general similar to that in parthenogenetic female (Fig. 7A). Lateral keels already visible (Figs. 9A, D–E), but dorsal portion of valves almost weekly chitinized. Ventral and lateral borders between preephippium and the rest of valves not developed (Figs. 9A, D).

Male. Despite significant sampling effort, we failed to detect males in the investigated samples. Although males of *Scapholeberis* have been described by *Dumont & Pensaert (1983)*, it is difficult to detect them in nature or in laboratory cultures. In general view, males are similar to juvenile females and could not be distinguished without dissection. Also, it seems possible, that at least in some *Scapholeberis* species, ephippial females may appear in the natural populations and under laboratory conditions without males. The same situation is known for some *Daphnia* O.F. Mueller, 1785 *(Kotov, 2013)*.

Size. Medium-sized species, parthenogenetic female up to 0.55 mm in length without mucro (and 0.57 mm with mucro), ephippial female up to 0.57 mm in length without mucro (and 0.61 with mucro).

Variability. No significant variability was found among the investigated individuals.

Taxonomic notes. *King* (*1853, p. 255-256, plate V, fig. e*) found "*Daphnia mucronata* (Müller)" in "South Creek" and "Paramatta", New South Wales, Australia. In his diagnosis, he mainly reproduced the previous redescription of *Scapholeberis mucronata* by *Baird* (*1850, p. 99–100*) made for European populations, but pointed on two differences of the Australian specimens: (1) "European specimens have the upper part of the head sometimes terminated by a sharp-curved point, and directed upwards. I have not found any such variety here"; (2) "the head of each of Baird's figures is larger than that of the Australian species". Sars (1888: p. 68) took these differences into his consideration and established new taxon, *S. kingii* Sars, 1888, referring

391 to the description of *King* (*1853*) rather than based on his own original material. It is an
392 acceptable action according to the *ICZN* (*2000*). Specimens of this taxon from Australia are
393 absent from the collection of G.O. Sars in the Zoological Museum of the Oslo University,
394 Norway. King's specimens were eligible to be designated as types for *S. kingii ICZN* (*2000*), but
395 the specimens were apparently lost.
396 Then *Sars* (*1903, p. 8–10, plate 1, figs 2, 2a, 2b*) proposed the name "*Scapholeberis*
397 *Kingi*, G.O. Sars, n. sp. "for populations from Sumatra (unknown water bodies in "territories of
398 Deli and Langkat" collected by Mr. Iversen) with the following explanation: "The above-
399 described species is unquestionably identical with the Australian form recorded by King as
400 *Daphnia mucronata*. It is certainly very nearly allied to the European species, but apparently
401 specifically distinct, differing, as it does, not only in the much smaller size, but also in the shape
402 of the head and in the less sharply angulated anterior part of the valves. The sculpture of the shell
403 is, moreover, much coarser than in the European species". But, Sars' earlier species name "*S.*
404 *kingii"* of Australia has precedence over the Sumatran species. The Sumatran specimens are
405 present in the Collection of G.O. Sars (GOS F 9540, GOS F 12272, GOS F 12880). However,
406 these specimens are not regarded as types because they were not reported in the original taxon
407 description. According to the drawings of *Sars* (*plate 1, figs 2, 2a, 2b*), the specimens from
408 Sumatra belong to the *S. kingii* group. Presently it is unknown if the populations from Sumatra
409 belong to *S. kingii* s.str., *S. smirnovi* **sp.nov.**, or another taxon (tropical Asian populations are not
410 revised here).
411 *Dumont & Pensaert* (*1983*) correctly pointed out that *Dumont* (*1983*) erroneously stated
412 that *S. kingi* Sars, 1888 was a *nomen nudum* (and claimed that the species should have been
413 named *S. kingi* Sars, 1903).

414 **Distribution.** To date, we can confirm its presence in Australia only, where it is a
415 common taxon (*Dumont, 1983; Smirnov, 1995; Shiel & Dickson, 1995*), but we cannot fully
416 exclude the chance that there are several additional taxa within this group.
417 Records of *S. kingii* from Spain, Sicily and Central Europe have been declared dubious
418 (*Alonso 1996; Marrone, Barone & Naselli-Flores, 2005; Hudec, 2010*), but members of the *S.*
419 *kingii* species group (see below) were found to be common in Northern Africa (*Ghaouaci et al.,*
420 *2018; Neretina, 2018*). In the Eastern Palearctic, the range of *S.* cf. *kingii* extends northwards, up
421 to Japan (*Tanaka, 1998a; Tanaka, 1998b*), the Korean Peninsula (*Kotov, Jeong & Lee, 2012*) and
422 the Russian side of the Amur River (=Heilong Jiang in Chinese) basin (*Kotov et al., 2011*).
423 Therefore, the *S. kingii* species complex is regarded as a typical "tropicopolitan" taxon with a
424 very wide geographic range in the Eastern Hemisphere.
425
426 **2. *Scapholeberis intermedius* Daday, 1898**
427 Figure 10
428
429 *Scapholeberis mucronata var. intermedia* Daday, 1898, p. 59–60, Fig. 29a–b.
430 ? *Scapholeberis kingi* Sars in *Gurney, 1907*, p. 277–278; *Fernando, 1980*, p. 97; *Michael*
431 *& Sharma, 1988*, p. 73–74, Fig. 20a–c; *Chatterjee et al., 2013*, p. 20–21.
432
433 **Type locality.** "Sümpfe der Umgebung des Kalawewa-Sees", Sri Lanka (*Daday, 1898*).
434 **Type material (studied here).** See Supplemental Table 2.
435 **Brief redescription of museum material. Redescription. Parthenogenetic female.** In
436 lateral view body elongated and ovoid, dorsal margin regularly arched, ventral margin straight,

maximum height at middle of body (body height/length ratio about 0.61 for adults and 0.59 for juveniles) (Figs. 10A–B). Head large with well developed rostrum (Figs. 10A–B). Posterodorsal angle obtuse, posteroventral angle almost straight with long mucro (Figs. 10A–B). Posterior margin generally almost straight or slightly curved. Ventral margin almost straight. Anterovenral angle broadly rounded, its ventral side with small protuberance.

Head large (Figs. 10A–B). In lateral view head elongated with prominent rostrum. Distal portion of rostrum roundish. Compound eye large, ocellus is not recognizable (Figs. 10A–C).

Antenna II relatively long, endopod branch slightly longer than exopod (Fig. 10D). Antennal formula identical to previous species.

Ephippial female, male. Completely absent in the type material.

Size. Medium-sized species, parthenogenetic female up to 0.62 mm in length without mucro (and 0.63 mm with mucro).

Variability. No significant variability was found in the investigated individuals.

Taxonomic remarks. According to *Daday (1898)* this "variety" has intermediate morphological characters between *S. mucronata* O.F. Müller and *S. obtusa* Schödler. The latter is now regarded as a junior synonym of *Megafenestra aurita* Fischer. Unfortunately, type material of *S. intermedius* is represented by permanent slides with parthenogenetic females in the lateral or almost lateral position (Fig. 10). Gamogenetic females and males are completely absent in the type series. Thus, we have no opportunity to compare the morphological features (proportions of head and shape of ephippium from the dorsal position) of typical *S. intermedius*, *S. smirnovi* **sp.nov.** and African *S.* cf. *intermedius* (see below). Based on the genetic data, we demonstrated that populations from Ethiopia and the Russian Far East form unique lineages (Figs. 1 and 2). We propose here that *S. smirnovi* **sp.nov.** is a separate taxon, well delineated from other *S.*

460 *kingii*-like species (see below). Morphological and genetic investigations of *kingii*-like
461 populations from the type locality of *S. intermedius*, Sri Lanka (and South Asia as a whole) will
462 be carried out in future studies. To date we have no suitable material of *S. kingii* with ephippial
463 females from this area.
464
465 **3. *Scapholeberis* cf. *intermedius* Daday, 1898**
466 Figures 11–15
467
468 ? *Scapholeberis kingi* Sars in *Sars, 1916*, p. 314–315, Pl. XXXII: 3, 3a, 3f; *Brehm, 1937,*
469 p. 489; *Gauthier, 1951,* p. 48–50, text-figure in p. 49, C–D; *Harding, 1961,* p. 40; *Rey & Saint-*
470 *Jean, 1969*, p. 26, Fig. 5a–c; *Dumont & Van De Velde, 1977*, p. 80; *Dumont, Laureys &*
471 *Pensaert, 1979*, p. 265, 267; *Day et al., 1999*, p. 97, Fig. 4.6.B.
472
473 **Material studied here.** See Supplemental Table 2.
474 **Description. Parthenogenetic female (Figs. 11–15).** In lateral view, body regularly
475 elongated, dorsal margin broadly arched, ventral margin almost straight, maximum height at
476 middle of body (body height/length ratio about 0.59 for adults, juveniles not studied) (Figs. 11A,
477 15A). In dorsal and ventral view body ovoid, only moderately compressed from sides. In anterior
478 view body moderately compressed, dorsal keel absent. Head large with well developed rostrum
479 (Figs. 11A–B, 15A–C). Depression between head and rest of body absent, but dorsal contour
480 may be slightly concave under compound eye and antenna. Posterodorsal and posteroventral
481 angles expressed (Figs. 11A, E, 15A, D). Posterodorsal angle obtuse, posteroventral angle almost
482 straight with long mucro (Figs. 11A, E, 15A, D). Posterior margin generally almost straight or

slightly curved. A raw of numerous small setules on inner face of posterior margin of valve (Figs. 11F–G). Ventral margin almost straight, covered by setae of different size (Fig. 11E). Anteroventral angle broadly rounded, its ventral side with small protuberance (Figs. 11A, E, 15A, D). Valves with developed sculpture, consisting of polygons (Figs. 11E, 15D–E).

Head large for daphniids (Figs. 11A–B, 15A–C). In lateral view head elongated, with a prominent rostrum. Distal portion of rostrum roundish. In anterior view, head elongated and round, slightly compressed from lateral sides (Fig. 11C). Its ventral portion three-lobed with depression for antennulae. A central lobe is rostrum, its tip broadly rounded with small shallow incision. In anterior view, distance between the center of ocellus and eye significantly greater (almost in three times) than distance from the center of ocellus to the tip of rostrum (Fig. 11C). Dorsal head pores absent, frontal head pore was not studied. Labrum large (Fig. 11D). Distal labral plate with bunches of long setules.

Valve with straight ventral margin (Figs. 11E). Adhesive ventral rim of valves modified into "sucker-plate". Inner surface of posterior margin with a broad "hyaline membrane" (in terms of *Dumont & Pensaert, 1983*) extending the posterior rim and a "denticulated membrane" (in terms of *Dumont & Pensaert, 1983*) consisting of row of short setules along the posterior rim (Figs. 11F–G).

Postabdomen almost rectangular, slightly narrowing distally; postabdomen length/height ratio about 2.6 (Fig. 11I). Ventral margin straight. Preanal margin three times longer than anal margin. Anal and postanal margins almost equal in length. Basis of claws not inflated (Figs. 11I–J, 12A). Postanal portion of postabdomen armed with long and thin denticles and bunches of fine setules. Bunches of fine setules also on anal margin and lateral surface of postabdomen. Postabdominal claw long (almost as long as anal margin), slightly curved (Figs. 11I–J, 12A). Its

external side armed by three rows of small denticles, deceasing in size distally. Basal spine absent (Figs. 11I–J, 12A).

Antenna I relatively short, antennular body with aesthetascs exceeds tip of rostrum in length (Fig. 11L). Nine aesthetascs unequal in size.

Antenna II relatively long (Figs. 11A, 12B–J). Antennal formula for setae: 0-0-1-3/1-1-3. Antennal formula for spines: 0-1-0-1/0-0-1. General structure of antenna II identical to species described above.

Thoracic limbs: five pairs.

Limb I (Figs. 12K, 13A). Accessory setae very long, prominent. Outer distal lobe with two setae unequal in size. Distal segment of the longest seta unilaterally armed with short setules; proximal portion of this seta bears especially long setules. Shorter seta of outer distal lobe bilaterally covered by short setules. Inner distal lobe (endite 5) with three setae unequal in size and shape (Figs. 12K, 13A: 1, 1', 1"). Endite 4 with a short anterior seta 2 and two posterior setae (Figs. 12K, 13A: a–b). The ratio between seta 1' and seta 2 is almost 1.5 (i.e. seta 2 is relatively long in the comparison of other *Scapholeberis* species investigated here, see redescription of *S. kingii* above and description of *S. smirnovi* **sp.nov.** below). Endite 3 with a short and thin anterior seta 3 and two posterior setae (Figs. 12K, 13A: c–d). Endite 2 with a short anterior seta 4 and four posterior setae (Figs. 12K, 13A: e–h). Two ejector hooks almost similar in size.

Limb II large, basically similar to other *Scapholeberis* species investigated here (Figs. 13B–D).

Limb III (Fig. 13E–G) with a large ovoid epipodite and a flat round exopodite bearing four distal setae, (among them seta 2 the longest, Figs. 13E–F) and two lateral setae unequal in

length. Setae 3–5 covered by long setules. Setae 1–2 armed with long setules in their proximal portions and bear shorter stiff setules on their distal segments. Inner distal portion of limb (Fig. 13E, G) with four endites: endite 5 with a single, short anterior seta (1) and a posterior seta (a); endite 4 with a single anterior seta (2) and a single posterior (b) seta; endite 3 with a short anterior seta (3) and two posterior setae (c–d); endite 2 with two anterior seta (4–5) and four posterior (e–h) setae. The rest of limb inner-distal portion as a singular large lobe, modified gnathobase, bearing numerous posterior soft setae, each with chitinous insertion within basal portion of distal segment, and a single, relatively long anterior seta (1) in its distal corner. Also, two small sensillae recognizable in this portion.

Limb IV (Figs. 14A–C) with a large ovoid epipodite and wide, flat rounded exopodite with two protruding setulated lobes, four distal and two lateral setae. Among them seta 4 the longest (Figs. 14A–B). Inner-distal portion of this limb with completely fused endites, distally with two setae of unclear homology, the most part of limb inner margin is a gnathobase filter plate consisting of numerous posterior setae (Fig. 14C). Also, two small sensillae recognizable in this portion.

Limb V (Figs. 14D–E) with a setulated preepipodite, large, subovoid epipodite, triangular exopodite supplied with two small, thin distal setae (Figs. 14D–E: 1–2) unequal in length and a large lateral seta (Figs. 14D–E: 3). Inner limb portion as an ovoid flat lobe, with setulated inner margin and a single, large seta. A small sensillum recognizable near seta 2.

Ephippial female, male. Despite significant efforts, we did not find gamogenetic females and males in African localities. Other authors who dealt with the description of African populations also did not observe *Scapholeberis* ephippial females and males in their materials.

551 **Size.** Medium-sized species, parthenogenetic female up to 0.70 mm in length without
552 mucro (and 0.73 mm with mucro).
553 **Variability.** No significant variability was found among the investigated individuals.
554 **Other records in Africa.** Distribution of *Scapholeberis* in Africa remains scarcely
555 studied. Reliable records of *S. kingii* populations are known from West Africa (*Dumont, 1981;*
556 *Egborge, Onwudinjo & Chigbu, 1994; Chiambeng & Dumont, 2005*), Central Africa (*Rey &*
557 *Saint-Jean, 1969*), and South Africa (*Sars, 1916; Day et al., 1999*).
558
559 **4. *Scapholeberis smirnovi* sp. nov.**
560 Figures 16–20
561
562 *Scapholeberis kingi* Sars in *Uéno, 1940*, p. 342; *Tanaka, 1998a*, p. 30–31, Fig. 2A–C;
563 *Tanaka, 1998b*: p. 15–16, Fig. 9–10; *Tanaka, Ohtaka & Nishino, 2004*, p. 173–174, Fig. 3; *Kotov*
564 *et al., 2011*, p. 403, Table 1; *Kotov, Jeong & Lee, 2012*, p. 58, Fig. 5; *Jeong, Kotov & Lee, 2014*,
565 p. 219.
566 ? (at least partially) *Scapholeberis kingi* Sars *in Chiang & Du, 1973*, p. 145–146, Fig.
567 97a-c; in *Du Nan-shan, 1973*, p. 44, Fig. 13; *Xiang et al., 2015*, p. 13–14.
568 *Scapholebeis mucronata* (O.F. Müller) in *Uéno, 1927*, p. 281, Fig. 9 (not 9a–9e!);
569 *Scapholeberis rammneri* Dumont & Pensaert in *Yoon, 2010*, p. 64–66, Fig. 34.
570
571 **Publication Zoobank ID.** See nomenclatural acta above.
572 **Zoobank taxon ID.** urn:lsid:zoobank.org:act:62ABBAFB-249D-453A-BB8D-
573 E59ECB1AB2B0.

574 **Etymology.** The taxon is named after Professor Nikolai N. Smirnov, a renowned Russian
575 zoologist and hydrobiologist, who established the Russian school of cladocerology and made
576 large advances in the study of freshwater zooplankton.
577 **Type locality.** A puddle near Lake Maloe Utinoe (N 43.4127°, E 131.8214°), Primorski
578 Territory, the Russian Far East.
579 **Type material.** Holotype: an ephippial female, fixed in 96% ethanol, deposited at the
580 collection of Zoological Museum of Moscow State University, MGU Ml-189. The label of
581 holotype is: "*Scapholeberis smirnovi* **sp. nov.**, 1 ephippial female from puddle near Lake Maloe
582 Utinoe, Holotype". Paratypes. See Supplemental Table 2.
583 **Description. Parthenogenetic female (Figs. 16A–F).** In lateral view body relatively
584 elongated, dorsal margin regularly arched, ventral margin almost straight, maximum height at
585 body middle (body height/length ratio about 0.6 for adults and 0.5 for juveniles) (Figs. 16A and
586 16E, correspondingly). In dorsal or ventral view body ovoid, moderately compressed from sides
587 (Fig. 16B). In anterior view body moderately compressed, dorsal keel absent. Posterodorsal
588 angle obtuse, posteroventral angle almost straight, with a long spine (mucro) (Figs. 16A, E and
589 17A–E). A row of numerous small setules on inner face of posterior margin of valve (Fig. 17E).
590 Ventral margin covered by setae of different size (Figs. 17A–D). Anteroventral angle of valve
591 broadly rounded, its ventral portion with a small protuberance (Fig. 16A, E). Valves with well-
592 developed sculpture of polygonal reticulation.
593 Head large for a daphniid (Fig. 16A). In lateral view head elongated, with a prominent
594 rostrum, its distal portion roundish (Fig. 16A). In dorsal view head elongated, head shield with
595 low lateral projections (fornices) covering bases of antennae II, a sclerotized ridge departs from
596 the insertion of antenna II and extends to the side of head. In anterior view head slightly

compressed from lateral sides. In ventral view postero-ventral portion of head forms a three-lobed rostrum, as there is a shallow depression at insertion points of antenna I on each side, its middle lobe rounded, with a minute frontal head pore (Figs. 16C). In anterior view, distance between the center of ocellus and eye significantly greater (almost in five times) than distance from the center of ocellus to the tip of rostrum (Figs. 16F). Dorsal head pores absent. Labrum large (Fig. 16D), similar to other *Scapholeberis* species.

Valve with straight ventral margin (Figs. 16A, 17A). Adhesive ventral rim of valves modified into "sucker-plate" (Figs. 16A–D), details of its structure identical to *S. kingii*.

Thorax relatively long, abdomen short (Fig. 16A).

Postabdomen almost rectangular, postabdomen length/height ratio about 2.8 (Figs. 17F–H). Ventral margin almost straight. Preanal margin two times longer than anal margin. Anal and postanal margins almost equal in length. Basis of claws slightly inflated, bordered from distal margin by a clear incision (Figs. 17G–I). Postanal portion of postabdomen armed with long, thin solitary teeth and bunches of fine setules. Bunches of fine setules also on anal margin and lateral surface of postabdomen. Postabdominal claw long (almost as long as anal margin), slightly curved (Figs. 17G–I). Its external side armed by three rows of small denticles, decreasing in size distally. Denticles in middle portion of claw are stronger and distributed more sparsely as compared to other denticles. Basal spine absent (Figs. 17G–I).

Antenna I relatively short, its proportions similar to other *Scapholeberis* species (Figs. 17J–K). Nine aesthetascs unequal in size.

Antenna II relatively long (Figs. 16A, 17L–M). Antennal formula for setae: 0-0-1-3/1-1-3. Antennal formula for spines: 0-1-0-1/0-0-1. Fine armature of antenna II similar to *S. kingii.*

Thoracic limbs: five pairs (Figs. 18A–H).

Limb I with ovoid epipodite (Figs. 18A–B). Accessory setae long, armed by long setules. Outer distal lobe with two setae unequal in size. Distal segment of the longest seta unilaterally armed by short setules; proximal portion of this seta bears especially long setules. Shorter seta of outer distal lobe bilaterally armed by short setules. Inner distal lobe (endite 5) with three setae unequal in size and shape (Fig. 18A: 1, 1', 1''). Two setae bisegmented, with elongated distal portions. A single seta 1 brush-shaped (in terms of Dumont & Pensaert, 1983), its distal end abrupt, bearing long thickened setules. Endite 4 with a short anterior seta 2 and two posterior setae (Fig. 18A: a–b). The ratio between seta 1' and seta 2 is almost 2.5 (i.e. seta 2 is relatively short as compared to *S.* cf. *intermedius* from Africa, and comparable to *S. kingii*, see above). Endite 3 with a short and thin anterior seta 3 and two posterior setae (Fig. 18A: c–d). Endite 2 with a short anterior seta 4 and four posterior setae (Fig. 18A: e–h). Two ejector hooks subequal in size.

Limb II large (Fig. 18C–D). Limb distal portion (exopodite) as large ovoid setulated lobe with two soft setae unequal in size. Four fused endites (e5–e2) bear six setae. Distal segments of anterior setae a–d covered by short denticles. Two posterior setae (a and d) bear long setules. Gnathobase (endite 5) with two rows of setae: four anterior setae (Fig. 18C: 1–4, among them seta 1 as a small elongated sensillum) and six posterior setae of gnathobasic "filter plate".

Limb III with a large ovoid epipodite (Fig. 18E) and a flat round exopodite bearing four distal setae (Fig. 18E: 1–4), (among them seta 2 the longest) and two lateral setae (Fig. 18E: 5–6) unequal in length. Proportions and armature of all setae similar to *S. kingii*.

Limb IV with a large ovoid epipodite (Fig. 18F–G) and wide, flat rounded exopodite with two protruding setulated lobes, four distal (Fig. 18F: 1–4) and two lateral (Fig. 18F: 5–6) setae. Proportions and armature of all setae similar to *S. kingii*.

643 Limb V (Fig. 18H) with a subovoid epipodite, triangular exopodite supplied with two
644 small, thin distal setae (Fig. 18H: 1–2) unequal in length and a large lateral seta (Fig. 18H: 3).
645 Inner limb portion as an ovoid flat lobe, with setulated inner margin and a single, large seta.
646 **Ephippial female (Figs. 16G–I, 19A–B, D–F, 20A–L).** Body shape in general as in
647 parthenogenetic female. Dorsal portion of valves modified into ephippium. Ephippium dark
648 brown, ovoid, clearly bordered from ventral and lateral portions of valves separating during its
649 casting off (Figs. 16G, 19A–B, 20A, D). Egg chamber with a single egg, elongated, its sculpture
650 represented by shallow depressions (Figs. 16G, 20F). Sculpture of the rest of ephippium is
651 represented by small polygons. Lateral keels are well distinguishable from the lateral (Figs. 16G,
652 19A–B, 20A–D) and dorsal view (Figs. 16H, 19–E, 20G, I–L). From the dorsal view, area
653 between two keels strongly rounded, keels strongly projected laterally out of body dorsal contour
654 (Figs. 16H, 19D, 20G).
655 **Preephippial female (Figs. 19C).** Body shape in general similar to that in
656 parthenogenetic female. Lateral keels already visible (Figs. 19C), but dorsal portion of valves
657 weakly chitinized. Ventral and lateral borders between preephippium and the rest of valves not
658 developed.
659 **Male.** Despite significant efforts, we did not find males in the investigated samples.
660 **Taxonomic notes.** Records of a "tropical" taxon, *S. kingii*, in northern regions such as
661 South Korea and the Russian Far East surprised cladoceran investigators (*Kotov, Jeong & Lee,*
662 *2012*). However, we now know that the Far Eastern populations belong to a separate taxon, the
663 real distribution of which needs to be accurately evaluated. To date, we had no DNA-available
664 samples of *S.* cf. *kingii* from SE Asia, South China and Indian subcontinent where that taxon is
665 common (*Michael & Sharma, 1988; Korovchinsky, 2013; Kotov et al., 2013; Sinev, Gu & Han,*

666 *2015*). Checking of the status of populations from different regions of the Palaeotropics is the
667 next step in the revision of this group..

668 Our revision confirms again that the Far East of Eurasia, in its temperate portion, is an
669 important source of new taxa, as it was already found previously (*Kotov, Ishida & Taylor, 2009;*
670 *Kotov et al., 2011*).

671 **Size.** Medium-sized species, parthenogenetic female up to 0.75 mm in length without
672 mucro (and 0.79 with mucro), ephippial female up to 0.70 mm in length (without mucro) (and
673 0.72 with mucro). Holotype 0.60 mm in length (without mucro), 0.37 mm in height.

674 **Distribution.** This taxon is known from the southern portion of the Far East of Russia,
675 the Korean Peninsula, Japan and an adjacent region of China (Dongbei = Manchuria). It has also
676 been recorded from a single locality in the southernmost portion of European Russia, but such a
677 disjunct population may be due to an anthropogenic introduction.

678

679 ## Discussion

680

681 ***Comparison of the COI and 12S+16S phylogenies***

682 The COI-based analyses reveal that the large genetic divergences within and among
683 species groups of neustonic daphniids exist for both rRNA (*Taylor, Connelly & Kotov, 2020*) and
684 protein-coding regions of the mitochondrial genome (the present study). However, the pattern
685 disparity between neustonic daphniids and *Daphnia* is greatest for within species/species group
686 variation. *Costa et al.* (*2007*) reported a 1.32 % average divergence within species of *Daphnia*
687 and a maximum divergence of 4.3%. In comparison, geographic clades within named species of
688 *Scapholeberis* are often beyond 20% in divergence. These unusually high maximum values for

689 *Scapholeberis* are unlikely to be reduced with further geographic sampling. These patterns are
690 consistent with greater rates of mitochondrial DNA evolution in Scapholeberinae than in
691 *Daphnia*. The COI data showed similar levels of within genus variation for *Daphnia (Costa et al.*
692 *2007*), *Scapholeberis* and *Megafenestra* at just over 30%, while the rRNA genes show greater
693 divergences within neustonic genera (*Scapholeberis* and *Megafenestra*) compared to those from
694 other cladoceran genera (*Taylor, Connelly & Kotov, 2020*). This outcome is expected for rate
695 increases in COI because the gene is prone to strong purifying selection resulting in
696 substitutional saturation (Pentinsaari, M., Salmela, H., Mutanen, M. *et al. 2016)*.
697 .
698 The COI based tree (Fig. 2) is similar to the tree estimated from 16S+12S rRNA
699 sequences (*Taylor, Connelly & Kotov, 2020*). The major groups in both trees are the same, while
700 the grouping of the deep branches is different. But, as the deep branches for COI have low
701 support, the discrepancies may be due to random error.
702 The *mucronata* group is well-supported in both trees, in each tree the group is represented
703 by four main clades. Our study confirms that the *mucronata*-group (clade X) is present in non-
704 Beringian North America. Clade X is known only from COI sequences from Manitoba, Canada
705 (Jeffery et al. 2011). Presently, the position of Clade X on the 12S-16S tree (*Taylor, Connelly &*
706 *Kotov, 2020*) is unknown .
707 All clades from the *rammneri* group represented in the rRNA tree (*Taylor, Connelly &*
708 *Kotov, 2020*) are also present in the COI tree (Fig. 2). New biogeographic information includes:
709 (1) Clade H penetrates further north in the Nearctic (though not beyond the boreal zone); (2)
710 there is a previously unknown clade Y in Israel; (3) the grouping of clade I (which is also basal
711 in the rRNA tree) with other clades is not well-supported in the COI tree.

712 The present study has much improved the geographic sampling of the S. freyi group
713 compared to our rRNA tree (this is largely due to the inclusion of sequences from previous DNA
714 barcoding projects). It is clear from the present results that *S. freyi* is indeed a diverse clade with
715 many closely related, but geographically differentiated phylogroups in the New World.
716 There is a new genetic clade within the *S. kingii* species group, *S.* cf. *intermedius* (clade
717 L2) (Figs 1–2) which was not sampled in the rRNA study. Therefore, the *S. kingii* group is more
718 diverse as it was expected before. In our COI tree, *S. armata* (clade N) grouped with *S.* cf.
719 *microcephala* (clade E) (Fig. 2), but they are distant branches on the rRNA tree. The source of
720 the incongruence is unknown but such discrepancies are common with long branches and short
721 internodes (see *Omilian & Taylor, 2001; Bergstren, 2005*).
722 Finally, the *Megafenestra* internal tree structure is different from that in rRNA tree, as the clade
723 P is sister group of Q in the COI tree and O – in the rRNA tree.
724
725 ***De-coding of the DNA barcoding results***
726 Before our study, 48 COI sequences were deposited to GenBank: *De Waard et al.* (*2006*)
727 (1 sequence); *Richter, Olesen & Wheeler* (*2007*) (1); *Elías-Gutiérrez et al.* (*2008*) (6); *Jeffrey,*
728 *Elías-Gutiérrez & Adamowicz* (2011) (2); *Elías-Gutiérrez & León-Regagnon* (*2013*) (3);
729 *Prosser, Martínez-Arce & Elías-Gutiérrez.* (*2013*) (2); *Yang et al.* (2017) (1); *Elias-Gutierrez et*
730 *al.* (*2018*) (14), and 20 sequences as direct submissions, including the iBOL releases. Because
731 the taxonomy of the Scapholeberinae is immature, identifications of the taxa by authors of these
732 data were tentative (Fig. 21), only 30% of taxa were identified to species group accurately, while
733 others were misidentified or identified to the genus level. In some publications, species were
734 assigned to numbers: e.g. "sp. 1, sp. 2 and sp. 3" of *Jeffrey, Elías-Gutiérrez & Adamowicz*

(*2011*). Subsequently, *S. duranguensis* was reasonably described from Mexico (*Quiroz-Vázquez & Elías-Gutiérrez, 2009*) based on specific COI sequences and morphological differences from other North American taxa, but no suggestions on the diversity within the genus were made.

Assessments of species diversity based on genetics can be confused by an immature taxonomic scaffold (as in *Scapholeberis* and *Megafenestra*). Indeed, before our study, GenBank was a source of misidentification, as 70% of sequences had incorrect labels. The barcoding data were an illegitimate alternative to real taxonomy based on the species typification and accurate descriptions/identifications (see *Kotov & Gololobova, 2016*). Moreover, when there are pervasive rate differences among taxa for mitochondrial DNA, as has been proposed for neustonic daphniids (*Taylor, Connelly & Kotov, 2020*) , mitochondrial DNA approaches may yield very different diversity results from morphological or nuclear genomic evidence.

Our recent decoding of the data from GenBank led to several interesting conclusions. The owners of sequences had no chance to make them because the barcoding data were not well-integrated with taxonomy. Note that the following our conclusions are mainly based on the analysis of the GenBank sequences rather than our original data: (1) *S. freyi* is not a subspecies of *S. armata*, and even not single monotypic species, but a monophyletic group of closely related genetic lineages (potential biological species) with a clear latitudinal differentiation in the Americas. Our previous hypothesis that *S. freyi* is a part of *S. rammneri* group (*Taylor, Connelly & Kotov, 2020*) was wrong. Note that to date only *S. freyi* s. lat. is genetically detected in tropical South and Central America. This conclusion agrees with opinions based on morphological data (*Elmoor-Loureiro, 2000*; *Elías-Gutiérrez, Kotov & Garfias-Espejo, 2006*). In contrast, *S. freyi* has not been detected in the western half of the Nearctic. Clade J4 was also found in Europe – this population is most probably the result of human-mediated introduction (see also *Taylor,*

758 *Connelly & Kotov, 2020*). The European population was used for a genomic study and identified
759 as "*S. mucronata* group*" (Cornetti et al., 2019)*.
760 (2) *S. duranguensis* is a member of a large group, namely the *S. freyi* species group. It is
761 not micro-endemic of a single locality in Durango State, but also present in the mountains of
762 Aguascalientes State.
763 (3) Members of the *S. mucronata* group (namely clade X) are present in non-Beringian
764 North America, but probably only in its northernmost (Arctic) portion.
765 (4) A new lineage (most probably, a separate biological species) of the *rammneri* group is
766 present in Israel.
767 (5) In contrast to our previous opinion (*Taylor, Connelly & Kotov, 2020*) representatives
768 of the American clade H of the *rammneri* group are found in the Beringian zone (although they
769 probably do not extend beyond the boreal zone in Alaska).
770 The information from "genetic barcoding" allows us to improve the biogeography of
771 neustonic daphniids, but only after integrating this information with morphological and other
772 genetic data (*Schlick-Steiner et al., 2010*).
773
774 ***Taxonomy***
775 There are two species within the genus *Megafenestra* (*Dumont & Pensaert, 1983*): *M.*
776 *aurita* (Fischer, 1849) and *M. nasuta* (Birge, 1879), and nine valid species within the genus
777 *Scapholeberis*: (1) *S. mucronata* (O.F. Müller, 1776); (2) *S. spinifera* (Nicolet, 1849); (3) *S.*
778 *armata* Herrick, 1882; (4) *S. kingi* Sars, 1888; (5) *S. microcephala* Sars, 1890; (6) *S. erinaceus*
779 Daday, 1903; (7) *S. rammneri* Dumont & Pensaert, 1983; (8) *S. freyi* Dumont & Pensaert, 1983;
780 (9) *S. duranguensis* Quiroz-Vázquez & Elías-Gutiérrez, 2009 (see *Dumont & Pensaert, 1983;*

781 *Quiroz-Vázquez & Elías-Gutiérrez, 2009*). But at least four "species" from this list (*S. kingi, S.*
782 *microcephala, S. mucronata, S. rammneri*) could be considered as taxa requiring special
783 attention to their taxonomy due to their very broad ranges both in the Eastern and Western
784 Hemispheres. Such taxa need careful taxonomic revisions according to the logic of "non-
785 cosmopolitanism" and "continental endemism" approaches (*Frey, 1982; Frey, 1987*) widely
786 accepted in the cladoceran taxonomy and biogeography.
787 After two subsequent revisions (*Taylor, Connelly & Kotov, 2020*; this study) we know
788 that the diversity of both genera has been strongly underestimated. The subfamily includes at
789 least 23–24 distinct lineages (note that rare *S. erinaceus* was not studied either here or by *Taylor,*
790 *Connelly & Kotov* (*2020*). In contrast to many other cladoceran groups, we can confidently say
791 that the phylogeny and taxonomy of *Scapholeberinae* is now relatively well-done. Main species
792 groups correspond well to those separated based on the morphological analysis. But it is very
793 obvious that further studies are necessary to find morphological differences between revealed
794 taxa and formulate diagnoses of the taxa which needs to be formally described (as *Megafenestra*
795 cf. *nasuta* clade P, *Scapholeberis* cf. *microcephala* clade E., *S.* cf. *rammneri* clades I, and
796 possibly other un-named clades). Therefore the revision of the taxonomy only starts with this
797 contribution. After all, from the lineages discovered up to now (and there are surely more to be
798 found), most remain unnamed and phenotypically not characterised.
799
800 **Conclusions**
801
802 To date we do not know if these taxa are morphologically different from congeneric taxa.
803 But, in this context, it is very premature to discuss a "lacking of resolution" of morphology and

804 the "limitations inherent in morphology-based identification system" (*Hebert et al., 2003*: *p.*
805 *313*), as nobody tried to find such differences. Such search is a task for future.
806 We can immediately recommend the main direction of such studies: gamogenetic
807 specimens must be analyzed for diagnostic characters first, as we did for the *S. kingii* species
808 group. We can assume, following ideas of *Goulden* (*1966*), that differences in the ephippial
809 morphology could provide a mechanism of reproductive isolation, as such differences could be
810 used by male during the copulation to recognize correct mate. Lateral keels on the ephippium,
811 characteristic of several, if not all, taxa of *Scapholeberis*, are analogous to the keels in
812 Bosminidae (*Kotov, 2013*). *Kerfoot & Peterson* (*1980*) proposed that the lateral keels and special
813 texture on the ephippium of *Bosmina* also contribute to pre-zygotic reproductive isolation. We
814 believe that differences between *Scapholeberis* ephippial females could also contribute to
815 reproductive isolation among congeneric species. Moreover, the situation with *Scapholeberis*
816 *kingii* and *S. smirnovi* **sp.nov.**, when parthenogenetic females are morphologically
817 indistinguishable, but gamogenetic specimens have morphological differences are usual among
818 the cladocerans (*Belyaeva & Taylor, 2009; Popova et al., 2016; Smirnov & Kotov, 2018*). Such
819 phenomena need further study to be accurately explained, but it is obvious that the
820 morphological evolution in parthenogenetic and gamogenetic specimens follow somewhat
821 different pathways. And the oft-reported morphological stasis in cladocerans (*Sacherová &*
822 *Hebert, 2003; Smirnov & Kotov, 2018*) is more characteristic of parthenogenetic females (the
823 sexual stages appear to evolve more rapidly in morphology).
824

825 ## Acknowledgements

826

827 Many thanks to E.I. Bekker, J.F. Cart, A. V. Chabovsky, S.E. Cherenkov, S.J. Connelly,
828 Y.R. Galimov, S. Ishida, P.D. Karabanov, Y. Kobayashi, T.P. Korobkova, L.E. Savinetskaya,
829 A.B. Savinetsky and D.V. Vasilenko for samples with the Scapholeberinae, M. Ballinger, K.S.
830 Costanzo, N.M., Korovchinsky and A. Medeiros for assistance in sampling trips.
831 We are deeply grateful to Sergei I. Metelev and Alexey N. Nekrasov for technical
832 assistance during SEM investigations. SEM works are carried out at the Joint Usage Center
833 “Instrumental Methods in Ecology” at A.N. Severtsov Institute of Ecol. Evol. of Russian
834 Academy of Sciences.

References

Adamowicz SJ, Petrusek A, Colbourne JK, Hebert PDN, Witt JDS. 2009. The scale of divergence: a phylogenetic appraisal of intercontinental allopatric speciation in a passively dispersed freshwater zooplankton genus. *Molecular Phylogenetics and Evolution* **50:** 423–436. DOI 10.1016/j.ympev.2008.11.026

Alexeev VR, Tsalolikhin SYM. 2010. *Identification guide of zooplankton and zoobenthos in freshwaters of European Russia. Vol. 1. Zooplankton*. Moscow: KMK.

Baird W. 1850. *The natural history of the British Entomostraca*. London: The Ray Society.

Bekker EI, Karabanov DP, Galimov YR, Kotov AA. 2016. DNA barcoding reveals high cryptic diversity in the North Eurasian *Moina* Species (Crustacea: Cladocera). PLoS ONE **11(8):** e0161737. DOI 10.1371/journal.pone.0161737

Belyaeva M, Taylor DJ. 2009. Cryptic species within the *Chydorus sphaericus* species complex (Crustacea: Cladocera) revealed by molecular markers and sexual stage morphology. *Molecular Phylogenetics and Evolution* **50:** 534–546. DOI 10.1016/j.ympev.2008.11.007

Benson DA, Cavanaugh M, Clark K, Karsch-Mizrachi I, Lipman DJ, Ostell J, Sayers EW. 2012. GenBank. *Nucleic acids research* **41(D1):** D36–D42. DOI 10.1093/nar/gks1195

Brehm V. 1938. Cladoceren and Ostracoden (d'Angola). *Archiv für Hydrobiologie* **32:** 488–502.

Chatterjee T, Kotov AA, Van Damme K, Chandrasekhar SVA, Padhye S. 2013. An annotated checklist of the Cladocera (Crustacea: Branchiopoda) from India. Zootaxa **3667:** 1–89. DOI 10.11646/zootaxa.3667.1.1

Chiambeng GY, Dumont HJ. 2005. The Branchiopoda (Crustacea: Anomopoda, Ctenopoda and Cyclestherida) of the rain forests of Cameroon, West Africa: low abundances, few endemics and a boreal-tropical disjunction. *Journal of Biogeography* **32:** 1611–1620. DOI 10.1111/j.1365-2699.2005.01280.x

Cornetti L, Fields PD, Van Damme K, Ebert D. 2019. A fossil-calibrated phylogenomic analysis of Daphnia and the Daphniidae. *Molecular Phylogenetics and Evolution* **137**:250-262. DOI 10.1016/j.ympev.2019.05.018

Day JA, Stewart BA, de Moor IJ, Louw A.E. 1999. Guides to the Freshwater Invertebrates of Southern Africa: Crustacea I: Notostraca, Anostraca, Conchostraca and Cladocera. *Water Research Commission Report No. TT 121/00, Pretoria*: 1–126.

DeWaard JR, Sacherova V, Cristescu MEA, Remigio EA, Crease TJ, Hebert PDN. 2006. Probing the relationships of the branchiopod crustaceans. *Molecular Phylogenetics and Evolution* **39 (2):**491–502. DOI 10.1016/j.ympev.2005.11.003

Dumont HJ. 1981. Cladocera and free-living Copepoda from the Fouta Djalon and adjacent mountain ares in West Africa. *Hydrobiologia* **85(2):** 97–116. DOI 10.1007/BF00006620

Dumont HJ. 1983. Genus *Simocephalus* Schoedler, 1858. Genus *Scapholeberis* Schoedler, 1858. In: Smirnov NN, Timms BV, eds. *A revision of the Australian Cladocera (Crustacea). Records of the Australian Museum* **Suppl. 1**: 97–106.

Dumont HJ, Laureys P, Pensaert J. 1979. Anostraca, Conchostraca, Cladocera and Copepoda from Tunisia. *Hydrobiologia* **66(3):** 259–274.

Dumont HJ, Pensaert J. 1983. A revision of the Scapholeberinae (Crustacea: Cladocera). *Hydrobiologia* **100:** 3–45.

DOI 10.1007/BF00027420

Dumont HJ, & Van de Velde I. 1977. Cladocères et Conchostracéc récoltes par le professeur Th. Monod dans la moyenne vallée du Niger en décembre 1972 et janvier 1973. *Bull. Inst. fondam. Afr. Noire (sér. A)* **39 (1):** 75–93.

Du Nan-shan. 1973. *Cladocera of China*. Peking: Science Press, Academia Sinica.

Dupérré N. 2020. Old and new challenges in taxonomy: what are taxonomists up against? *Megataxa* **1(1):** 59–62.

DOI 10.11646/megataxa.1.1.12

Egborge ABM, Onwudinjo CC, Chigbu PC. 1994. Cladocera of coastal rivers of western Nigeria. *Hydrobiologia* **272(1/2):** 39–46.

DOI 10.1007/BF00006511

Elías-Gutiérrez M, Kotov AA, Garfias-Espejo T. 2006. Cladocera (Crustacea: Ctenopoda, Anomopoda) from southern Mexico, Belize and northern Guatemala, with some biogeographical notes. *Zootaxa* **1119:** 1–27.

DOI 10.11646/zootaxa.1119.1.1

902 **Elías-Gutiérrez M, Martínez Jerónimo F, Ivanova NV, Valdez Moreno M, Hebert**
903 **PDN. 2008.** DNA barcodes for Cladocera and Copepoda from Mexico and Guatemala,
904 highlights and new discoveries. *Zootaxa* **1839:** 1–42.
905 DOI 10.11646/zootaxa.1839.1.1
906 **Elías-Gutiérrez M, León-Regagnon V. 2013.** DNA barcoding in Mexico: an
907 introduction. Molecular Ecology Resources **13(6):** 1093–1096.
908 DOI 10.1111/1755-0998.12149
909 **Elías-Gutiérrez M, Valdez-Moreno M, Topan J, Young MR, Cohuo-Colli JA. 2018.**
910 Improved protocols to accelerate the assembly of DNA barcode reference libraries for freshwater
911 zooplankton. *Ecology and evolution* **8(5):** 3002–3018.
912 DOI 10.1002/ece3.3742
913 **Elmoor-Loureiro LMA. 2000.** Ocorrência de *Scapholeberis armata* freyi Dumont &
914 Pensaert (Crustacea, Anomopoda, Daphniidae) no estado de São Paulo, Brasil. *Revista Brasileira*
915 *de Zoologia* **17(1):** 301–302.
916 DOI 10.1590/S0101-81752000000100026
917 **Fernando CH. 1980.** The freshwater zooplankton of Sri Lanka, with a discussion of
918 tropical freshwater zooplankton composition. *Internationale Revue der gesamten Hydrobiologie*
919 *und Hydrographie* **65(1):** 85–125.
920 DOI 10.1002/iroh.19800650105
921 **Frey DG. 1982.** Questions concerning cosmopolitanism in Cladocera. *Archiv für*
922 *Hydrobiologie* **93:** 484–502.
923 **Frey DG. 1987.** The taxonomy and biogeography of the Cladocera. *Hydrobiologia* **145:**
924 5–17.

925 DOI 10.1007/978-94-009-4039-0_2

926 **Ghaouaci S, Amarouayache M, Sinev AY, Korovchinsky NM, Kotov AA. 2018.** An
927 annotated checklist of the Algerian Cladocera (Crustacea: Branchiopoda). *Zootaxa* **4377 (3):**
928 412–430.

929 DOI 10.11646/zootaxa.4377.3.5

930 **Goulden CE. 1966.** Co-occurence of moinid Cladocera and possible isolating
931 mechanisms. *Verhandlungen der Internationalen Vereinigung für Theoretische und Angewandte*
932 *Limnologie* **16(3):** 1699–1672.

933 DOI 10.1080/03680770.1965.11899491

934 **Gurney R. 1907.** On some freshwater Entomostraca in the collection of the Indian
935 Museum, Calcutta. *Journal and Proceedings of the Asiatic Society of Bengal, New Series* **2(7):**
936 273–281.

937 **Hebert PDN, Cywinska A, Ball SL, De Waard JR. 2003.** Biological identifications
938 through DNA barcodes. *Proceedings of the Royal Society, Series B* **270:** 313–321.

939 DOI 10.1098/rspb.2002.2218

940 **Henry M. 1919.** On some Australian Cladocera. *Journal and Proceedings of the Royal*
941 *Society of New South Wales* **52:** 463–485.

942 **Henry M. 1922.** A monograph of the freshwater Entomostraca of New South Wales. Part
943 I. Cladocera. *Proceedings of the Linnean Society of New South Wales* **47(1):** 186: 26–52.

944 **Hudec I. 1983.** Redescription of *Scapholeberis echinulata* Sars 1903 (Crustacea:
945 Cladocera). *Hydrobiologia* **107:** 63–69.

946 DOI 10.1007/BF00126705

International Commission on Zoological Nomenclature (ICZN). 2000. *International code of zoological nomenclature. Fourth edition*. London: The Natural History Museum.

Jeffery NW, Elías-Gutiérrez M, Adamowicz SJ. 2011. Species Diversity and Phylogeographical Affinities of the Branchiopoda (Crustacea) of Churchill, Manitoba, Canada. *PLoS ONE* **6(5):** e18364.

DOI 10.1371/journal.pone.0018364

Jeong HG, Kotov AA, Lee W. 2014. Checklist of the freshwater Cladocera (Crustacea: Branchiopoda) of South Korea. *Proceedings of the Biological Society of Washington* **127(1):** 216–228.

DOI 10.2988/0006-324X-127.1.216

Kerfoot WC, Peterson C. 1980. Predatory copepods and *Bosmina*: replacement cycles and further influences of predation upon prey reproduction. *Ecology* **61:** 417–431.

DOI 10.2307/1935198

King RL. 1853. On Australian Entomostraca – in continuation. *Papers and proceedings of the Royal Society of Tasmania* **2:** 253–263.

Korovchinsky NM. 2013. Cladocera (Crustacea: Branchiopoda) of South East Asia: history of exploration, taxon richness and notes on zoogeography. *Journal of Limnology* **72(s2):** 109–124.

DOI 10.4081/jlimnol.2013.s2.e7

Kotov AA. 2013. *Morphology and phylogeny of the Anomopoda (Crustacea: Cladocera)*. Moscow: KMK Press.

Kotov AA, Gololobova MA. 2016. Traditional taxonomy – quo vadis? *Integrative Zoology* **11**: 500–505.

970 DOI 10.1111/1749-4877.12215

971 **Kotov AA, Ishida S, Taylor DJ. 2009.** Revision of the genus *Bosmina* Baird, 1845
972 (Cladocera: Bosminidae), based on evidence from male morphological characters and molecular
973 phylogenies. *Zoological Journal of the Linnean Society* **156 (1):** 1–56.

974 DOI 10.1111/j.1096-3642.2008.00475.x

975 **Kotov AA, Jeong HJ, Lee W. 2012.** Cladocera (Crustacea: Branchiopoda) of the south-
976 east of the Korean Peninsula, with twenty new records for Korea. *Zootaxa* **3368:** 50–90.

977 DOI 10.11646/zootaxa.3368.1.4

978 **Kotov, AA, Sinev AY, Glagolev SM, Smirnov NN. 2010.** Water fleas (Cladocera). In:
979 Alexeev VR & Tsalolokhin SY, eds. *Key book for zooplankton and zoobenthos of fresh waters of*
980 *European Russia*. Moscow: KMK, 151–276.

981 **Kotov AA, Korovchinsky NM, Sinev AY, Smirnov NN. 2011.** Cladocera (Crustacea,
982 Branchiopoda) of the Zeya basin (Amurskaya Area, Russian Federation). 3. Systematic-faunistic
983 and zoogeographic analysis. *Zoologichesky Zhurnal* **90:** 402–411.

984 **Kotov AA, Van Damme K, Bekker EI, Siboualipha S, Silva-Briano M, Adabache**
985 **Ortiz A, Galván de la Rosa R, Sanoamuang L. 2013.** Cladocera (Crustacea: Branchiopoda) of
986 Vientiane province and municipality, Laos. *Journal of Limnology* **72(s2):** 81–108.

987 DOI 10.4081/jlimnol.2013.s2.e6

988 **Laforsch C, Tollrian R. 2000.** A new preparation technique of daphnids for Scanning
989 Electron Microscopy using hexamethyldisilazane. *Archiv fuer Hydrobiologie* **149:** 587–596.

990 **Lemoine F, Entfellner JBD, Wilkinson E, Correia D, Felipe MD, De Oliveira T,**
991 **Gascuel O. 2018.** Renewing Felsenstein's phylogenetic bootstrap in the era of big data. *Nature*
992 **556:** 452–456.

993 DOI 10.1038/s41586-018-0043-0

994 **Marrone F, Barone R, Naselli-Flores L. 2005.** Cladocera (Branchiopoda: Anomopoda,
995 Ctenopoda, and Onychopoda) from Sicilian inland waters: An updated inventory. *Crustaceana*
996 **78:** 1025–1039.

997 **Michael RG, Sharma BK. 1988.** *Fauna of India and ajancent countries. Indian*
998 *Cladocera (Crustacea: Branchiopoda: Cladocera)*. Calcutta: Zool. Surv. India.

999 **Neretina AN. 2018.** Fauna of the Cladocera (Crustacea: Cladocera) of Ethiopia.
1000 Unpublished D. Phil. Thesis, A.N. Severtsov Institute of Ecology and Evolution.

1001 **Neretina AN, Sinev AY. 2016.** A revision of the genus *Leberis* Smirnov, 1989
1002 (Cladocera: Chydoridae) in the Old World and Australia. *Zootaxa* **4079(5):** 501–533.
1003 DOI 10.11646/zootaxa.4079.5.1

1004 **Popova EV, Petrusek A, Kořínek V, Mergeay J, Bekker EI, Karabanov DP,**
1005 **Galimov YR, Neretina TV, Taylor DJ, Kotov AA. 2016.** Revision of the Old World *Daphnia*
1006 *(Ctenodaphnia) similis* group (Cladocera: Daphniidae). *Zootaxa* **4161(1):** 1–40.
1007 DOI 10.11646/zootaxa.4161.1.1.

1008 **Prosser S, Martínez-Arce A, Elías-Gutiérrez M. 2013.** A new set of primers for COI
1009 amplification from freshwater microcrustaceans. *Molecular ecology resources* **13(6):** 1151–
1010 1155.
1011 DOI 10.1111/1755-0998.12132.

1012 **Omilian AR, Taylor DJ. 2001.** Rate acceleration and long-branch attraction in a
1013 conserved gene of cryptic daphniid (Crustacea) species. *Molecular Biology and Evolution*
1014 **18(12):** 2201–2212.
1015 DOI 10.1093/oxfordjournals.molbev.a003767

Pentinsaari M, Salmela H, Mutanen M, Roslin T. 2016. Molecular evolution of a widely-adopted taxonomic marker (COI) across the animal tree of life. *Scientific Reports* **6:** 35275.

DOI 10.1038/srep35275

Quiroz-Vázquez P, Elías-Gutiérrez M. 2009. A new cryptic species of the freshwater cladoceran genus *Scapholeberis* Schoedler, 1858 (Cladocera: Anomopoda) from the Semidesert Northern Mexico, highlighted by DNA barcoding. *Zootaxa* **2236:** 50–64.

DOI 10.11646/zootaxa.2236.1.4

Rey J, Saint-Jean L. 1969. Les Cladocères (Crustacés, Branchiopodes) du Tchad (Deuxième note). *Cahiers ORSTOM, série Serie Hydrobiologie* **3:** 21–42.

Richter S, Olesen J, Wheeler WC. 2007. Phylogeny of Branchiopoda (Crustacea) based on a combined analysis of morphological data and six molecular loci. *Cladistics* **23(4):** 301–336.

DOI 10.1111/j.1096-0031.2007.00148.x

Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP. 2012. MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic biology* **61(3):** 539–542.

DOI 10.1093/sysbio/sys029

Sacherová V, Hebert PDN. 2003. The evolutionary history of the Chydoridae (Crustacea: Cladocera). *Biological Journal of the Linnean Society* **79:** 629–643.

DOI 10.1046/j.1095-8312.2003.00216.x

Sars GO. 1903. Fresh-water Entomostraca from China and Sumatra. *Archiv for Mathematik og Naturvidenskab* **25(8):** 1–44.

Sars GO. 1916. The fresh-water Entomostraca of the Cape Province (Union of South Africa). Part 1: Cladocera. *Annals of the South African Museum* **15:** 303–351.

Schlick-Steiner BC, Steiner FM, Seifert B, Stauffer C, Christian E, Crozier RH. 2010. Integrative taxonomy: a multisource approach to exploring biodiversity. *Annual Review of Entomology* **55:** 421–438.

DOI 10.1146/annurev-ento-112408-085432

Shiel RJ, Dickson JA. 1995. Cladocera recorded from Australia. *Transactions of the Royal Society of South Australia* **119(1):** 29–40.

Sinev AY, Gu Y, Han BP. 2015. Cladocera of Hainan Island, China. *Zootaxa* **4006 (3):** 569–585.

DOI 10.11646/zootaxa.4006.3.9

Smirnov NN. 1992. *The Macrothricidae of the world. Guides to the identification of the microivertebrates of the Continental Waters of the world*. The Hague: SPB Academic Publishing.

Smirnov NN. 1995. Check-list of the Australian Cladocera (Crustacea). *Arthropoda Selecta* **4(1):** 3–6.

Smirnov NN. 1996. *Cladocera: the Chydorinae and Sayciinae (Chydoridae) of the world. Guides to the identification of the microivertebrates of the Continental Waters of the world*. Amsterdam: SPB Academic Publishing.

Smirnov NN, Kotov AA. 2018. On morphological radiation of Cladocera (Crustacea). *Invertebrate Zoology* **15(3):** 231–248.

Tanaka S. 1998a. Microcrustaceans (Cladocera and Copepoda) of Ryushou-ike Pond located in the central part of the Hida Mountain Range. *Bull. Toyama Biol. Soc.* **37:** 29–34.

Tanaka S. 1998b. Notes on the Cladocera of Japan - 6. Family Daphniidae Straus, 1820 - 3. Genus *Ceriodaphnia* and genus *Scapholeberis*. *Memoirs of the Faculty of Education Toyama University, Series B. Natural Sciences* **52:** 9–18.

Tanaka S, Ohtaka A, Nishino M. 2004. Cladoceran fauna in littoral zones and Naikos (attached lakes or lagoons) of Lake Biwa, central Japan. *Japanese Journal of Limnology* **65:** 167–179.

Taylor DJ, Connelly SJ, Kotov AA. 2020. The Intercontinental phylogeography of neustonic daphniids. *Scientific Reports* **10(1):** 1–12.

DOI 10.1038/s41598-020-58743-8

Thielsch A, Knell A, Mohammadyari A, Petrusek A, Schwenk K. 2017. Divergent clades or cryptic species? Mito-nuclear discordance in a *Daphnia* species complex. *BMC Evolutionary Biology* **17(1):** 227.

DOI 10.1186/s12862-017-1070-4

Turko P, Wolinska J, Tellenbach C, Dziuba MK, Monchamp ME, Spaak P. 2019. Using DNA from formaldehyde-preserved *Daphnia* to reconstruct past populations. *Hydrobiologia* **841(1):** 153–161.

DOI 10.1007/s10750-019-04015-0

Uéno M. 1927. The freshwater Branchiopoda of Japan I. *Memoirs of the College of Science, Kyoto Imperial University, Series B* **2(5):** 259–311.

Uéno M. 1940. Cladocera of Manchoukuo. Phyllopoda of Manchoukuo. *Report of the Limnological Survey of Kwantung and Manchoukuo, Dairen*: 323–367.

Umetsu K, Iwabuchi N, Yuasa I, Saitou N, Clark PF, Boxshall G, Osawa M, Igarashi K. 2002. Complete mitochondrial DNA sequence of a tadpole shrimp (*Triops cancriformis*) and analysis of museum samples. *Electrophoresis* **23:** 4080–4084.
DOI 10.1002/elps.200290024

Van Damme K, Sinev AY, Dumont HJ. 2011. Separation of *Anthalona* gen.n. from Alona Baird, 1843 (Branchiopoda: Cladocera: Anomopoda): morphology and evolution of scraping stenothermic alonines. *Zootaxa* **2875:** 1–64.
DOI 10.11646/zootaxa.2875.1.1

Xiang XF, Ji GH, Chen SZ, Yu GL, Xu L, Han BP, Kotov AA, Dumont HJ. 2015. Check-List of Chinese Cladocera (Crustacea: Branchiopoda). Part 1. Haplopoda, Ctenopoda, Onychopoda and Anomopoda (families Daphniidae, Moinidae, Bosminidae, Ilyocryptidae). *Zootaxa* **3904:** 1–27.

Yang J, Zhang X, Zhang W, Sun J, Xie Y, Zhang Y, Burton G.A, Yu H. (2017). Indigenous species barcode database improves the identification of zooplankton. *PloS one **12(10).***
DOI 10.1371/journal.pone.0185697

Yoon SM. 2010. Arthropoda: Branchiopoda: Anostraca, Notostraca, Spinicaudata, Laevicaudata, Ctenopoda, Anomopoda, Haplopoda Branchiopods. *Invertebrate fauna of Korea* **21(2):** 1–156.

1102 **Captions**

1103

1104 **Figure 1. Map of populations of *Scapholeberis* and *Megafenestra* studied here**
1105 **genetically.** Symbols correspond to mitochondrial clades (see Figure 2): (A) populations of the
1106 *S. mucronata* species group (northern hemisphere); (B) populations of the *S. rammneri* species
1107 group in the northern hemisphere; (C) populations of the *S. freyi* species group (western
1108 hemisphere); (D) populations of *Megafenestra* (clear symbols), *S. microcephala*, *S. smirnovi*
1109 **sp.nov.**, *S. armata*, *S.* cf. *microcephala* in northern hemisphere; (E) all populations revealed in
1110 southern hemisphere. The base maps are from the public domain atlas in the desktop app, Marble
1111 2.2.20 (http://edu.kde.org/marble). Symbols were placed manually in Microsoft PowerPoint
1112 using the output from DIVA-GIS 7.5 (https://www.diva-gis.org/) as a guide. Note that the base
1113 maps and symbols are basically same as in Taylor et al. (2020), but just the only localities are
1114 represented from where the COI sequences were obtained here in addition to Taylor et al. (2020).
1115

1116 **Figure 2. Maximum likelihood mitochondrial phylogeny of neustonic daphniids**
1117 **(*Scapholeberis* and *Megafenestra*).** Bold letters (A–Q, X–Y) indicate geographic clades.
1118 Numbers at the nodes indicate Bayesian posterior probabilities and Transfer Bootstrap
1119 Expectations (TBE). Colours represent major species groups in the Scapholeberinae:
1120 *Scapholeberis mucronata* group (green), *S. rammneri* group (red), *S. freyi* group (black), *S. kingii*
1121 group (grey), *S. armata-microcephala* clade (blue), genus *Megafenestra* (white). The tree is
1122 outgroup-rooted using sequences from the genus *Megafenestra*. See Appendix S1 for individual
1123 sequences.

Figure 3. Violin plots of pairwise Kimura's 2 Parameter Distances from the COI region of mitochondrial DNA in clades of neustonic daphniids (Scapholebeberinae). Horizontal bars indicate means. Gray rectangles show the ranges. Taxa are genera or species groups in the Scapholeberinae. See Appendix S1 for individual sequences.

Figure 4. *Scapholeberis kingii* Sars, 1888, parthenogenetic and ephippial females from Farm Dam, New South Wales, Australia. A–D, Adult parthenogenetic females, E, Juvenile parthenogenetic female, F–H, Ephippial females. A, Parthenogenetic female, lateral view. B, Adult parthenogenetic female, dorsal view. C, Head, ventral view. D, Labrum. E, Juvenile parthenogenetic female, lateral view. F, Ephippial female, lateral view. G, Ephippial female, dorsal view. H, Ornamentation of ephippium. Scale bars = 0.1 mm.

Figure 5. *Scapholeberis kingii* Sars, 1888, parthenogenetic females from Farm Dam, New South Wales, Australia. A, Valve, ventral view. B–C, Armature of valve. D–E, Posteroventral portion of valve, inner view. F–G, Postabdomen. H–I, Postabdominal claw. J–K, Antenna I. L–M, Antenna II. Scale bars = 0.1 mm.

Figure 6. *Scapholeberis kingii* Sars, 1888, parthenogenetic females from Farm Dam, New South Wales, Australia. A, Thoracic limb I. B, Thoracic limb II. C, Thoracic limb III. D, Thoracic limb IV. E, Thoracic limb V. Scale bar = 0.1 mm.

Figure 7. *Scapholeberis kingii* Sars, 1888, parthenogenetic and ephippial females from Farm Dam, New South Wales, Australia. A–E, Parthenogenetic females, F–L, Ephippial

females. A, Ephippial female, lateral view. B, Valve, inner view. C, Posteroventral portion of valve, inner view. D, Head, ventral view. E, Antenna I. F, I, Ephippial females, lateral view. G, J, Ephippia, lateral view. K, Head, lateral view. H, L, Ornamentation of central portion of ephippia. Scale bars = 0.2 mm for A–B, F–G, I–J, 0.1 mm for D, K, 0.05 mm for H, 0.02 mm for C, E, L.

Figure 8. *Scapholeberis kingii* Sars, 1888, ephippial females from Farm Dam, New South Wales, Australia. A, Ephippial female, dorsal view. B, Ephippium, dorsal view. C, Ephippium, dorsal view on higher magnification. D, Head, dorsal view. E, Ephippial female, ventral view. F, Head, ventral view. G, Head on higher magnification, ventral view. Scale bars = 0.2 mm for A–B, E, 0.1 mm for C–D, 0.05 mm for F–G.

Figure 9. *Scapholeberis kingii* Sars, 1888, preephippial female from the roadside pool near Lake Bantic, West Coast, Tasmania, Australia. A, Preephippial female, lateral view. B, Head, lateral view. C, Postabdominal claw, lateral view. D, Posterior portion of body. E–F, Posterior portion of body on higher magnifications. Scale bars = 0.2 mm for A, D, 0.1 mm for B, E, 0.05 mm for F, 0.02 mm for C.

Figure 10. *Scapholeberis intermedius* Daday, 1898, parthenogenetic females from Collectio Dadayana. A, Adult parthenogenetic female, lateral view (DAD 10-70-159). B, Juvenile parthenogenetic female, lateral view (DAD 10-70-156). C, Head, dorsal (?) view (DAD 10-70-156). D, Antenna II (DAD 10-70-156). Scale bars = 0.1 mm.

Figure 11. *Scapholeberis* cf. *intermedius* Daday, 1898, a parthenogenetic female from Bahir Dar Bay of Lake Tana, Amhara, Ethiopia. A, Parthenogenetic female, lateral view. B, Head, lateral view. C, Head, ventral view. D, Labrum. E, Valve. F–H, Armature of posteroventral angle of valve. I, Postabdomen. J, Distal portion of postabdomen. K, Postabdominal seta. L, Antenna I. Scale bars = 0.1 mm.

Figure 12. *Scapholeberis* cf. *intermedius* Daday, 1898, a parthenogenetic female from Bahir Dar Bay of Lake Tana, Amhara, Ethiopia. A, Distal portion of postabdomen. B, Antenna II. D–J, Fragments of antenna II. K, Thoracic limb I. Scale bars = 0.1 mm.

Figure 13. *Scapholeberis* cf. *intermedius* Daday, 1898, a parthenogenetic female from Bahir Dar Bay of Lake Tana, Amhara, Ethiopia. A, Thoracic limb I. B, Thoracic limb II. C–D, Fragments of thoracic limb II. E, Thoracic limb III. F–G, Fragments of thoracic limb III. Scale bars = 0.1 mm.

Figure 14. *Scapholeberis* cf. *intermedius* Daday, 1898, a parthenogenetic female from Bahir Dar Bay of Lake Tana, Amhara, Ethiopia. A, Thoracic limb IV. B–C, Fragments of thoracic limb IV. D, Thoracic limb V. E, Fragment of thoracic limb V. Scale bars = 0.1 mm.

Figure 15. *Scapholeberis* cf. *intermedius* Daday, 1898, a parthenogenetic female from Bahir Dar Bay of Lake Tana, Amhara, Ethiopia. A, Parthenogenetic female, lateral view. B, Anterior portion of body. C, Head, lateral view. D, Posterior portion of body. E, Ornamentation of valve. Scale bars 0.2 mm for A, D, 0.1 mm for B, 0.05 mm for C, E.

Figure 16. *Scapholeberis smirnovi* sp.nov. from the puddle near Lake Maloe Utinoe, Primorski Territory, Far East, Russia. A–D, Adult parthenogenetic females, E–F, Juvenile parthenogenetic female, G–I, Ephippial females. A, Adult parthenogenetic female, lateral view. B, Parthenogenetic female, dorsal view. C, Head, ventral view. D, Labrum. E, Juvenile parthenogenetic female. F, Head, ventral view. G, Ephippial female, lateral view. H, Ephippial female, dorsal view. I, Ornamentation of ephippium. Scale bars = 0.1 mm.

Figure 17. *Scapholeberis smirnovi* sp.nov. from the puddle near Lake Maloe Utinoe, Primorski Territory, Far East, Russia. A, Valve, outer view. B, Valve, ventral view. C–D, Armature of valve. E, Posteroventral portion of valve, inner view. F–H, Postabdomen. I, Postabdominal claw. J–K, Antenna I. L–M, Antenna II. Scale bars 0.1 = mm.

Figure 18. *Scapholeberis smirnovi* sp.nov. from the puddle near Lake Maloe Utinoe, Primorski Territory, Far East, Russia. A–B, Thoracic limb I. C–D, Thoracic limb II. E, Thoracic limb III. F–G, Thoracic limb IV. H, Thoracic limb V. Scale bar = 0.1 mm.

Figure 19. *Scapholeberis smirnovi* sp.nov., ephippial and preephippial females from the puddle near Lake Maloe Utinoe, Primorski Territory, Far East, Russia. A–B, D–F, Ephippial females, C, Preephippial female. A, Ephippial female, lateral view. B, Ephippium, lateral view. C, Preephippial female, lateral view. D, Ephippial female, anterodorsal view. E, Ephippium, anterodorsal view. F, Ephippial female, ventral view. Scale bars = 0.2 mm for A–D, F, 0.1 mm for E.

Figure 20. *Scapholeberis smirnovi* sp.nov., ephippial females from a pond in Choenggye Mountains, Seoul, the Republic of South Korea. A, Ephippial female, lateral view. B–C, Head, lateral view. D, Ephippium, lateral view. E–F, Ornamentation of ephippium. G, Ephippial female, dorsal view. H, Head, dorsal view. I–J, Ephippium, dorsal view. K–L, Armature of ephippium on higher magnifications. Scale bars = 0.2 mm for A, D, G, I, 0.1 mm for B–C, H, J–L, 0.5 mm for F, 0.2 mm for E.

Figure 21. Analysis of identification for four species groups of *Scapholeberis* based on GenBank data.

Table 1. Differentiation of the species of the *Scapholeberis kingii* group based on morphological characters.

Supplemental Figure 1. Neighbor joining tree of neustonic daphniids (*Scapholeberis* and *Megafenestra*) using Kimura's 2 parameter distances. Bold letters (A–Q, X–Y) indicate geographic clades. Colours represent major species groups in the Scapholeberinae: *Scapholeberis mucronata* group (green), *S. rammneri* group (red), *S. freyi* group (black), *S. kingii* group (grey), genus *Megafenestra* (white). The tree is midpoint rooted supporting a basal position of the genus *Megafenestra*. See Appendix S1 for individual sequences.

Supplemental Table 1. Complete list of original sequences obtained in the frame of this study and GenBank sequences with information on specimen ID and locality provided for each individual.

Supplemental Table 2. List of material studied morphologically.

Figure 1

Map of populations of *Scapholeberis* and *Megafenestra* studied here genetically.

Symbols correspond to mitochondrial clades (see Figure 2): (A) populations of the *S. mucronata* species group (northern hemisphere); (B) populations of the *S. rammneri* species group in the northern hemisphere; (C) populations of the *S. freyi* species group (western hemisphere); (D) populations of *Megafenestra* (clear symbols), *S. microcephala*, *S. smirnovi* **sp.nov.**, *S. armata*, *S*. cf. *microcephala* in northern hemisphere; (E) all populations revealed in southern hemisphere. The base maps are from the public domain atlas in the desktop app, Marble 2.2.20 (http://edu.kde.org/marble). Symbols were placed manually in Microsoft PowerPoint using the output from DIVA-GIS 7.5 (https://www.diva-gis.org/) as a guide. Note that the base maps and symbols are basically same as in Taylor et al. (2020), but just the only localities are represented from where the COI sequences were obtained here in addition to Taylor et al. (2020).

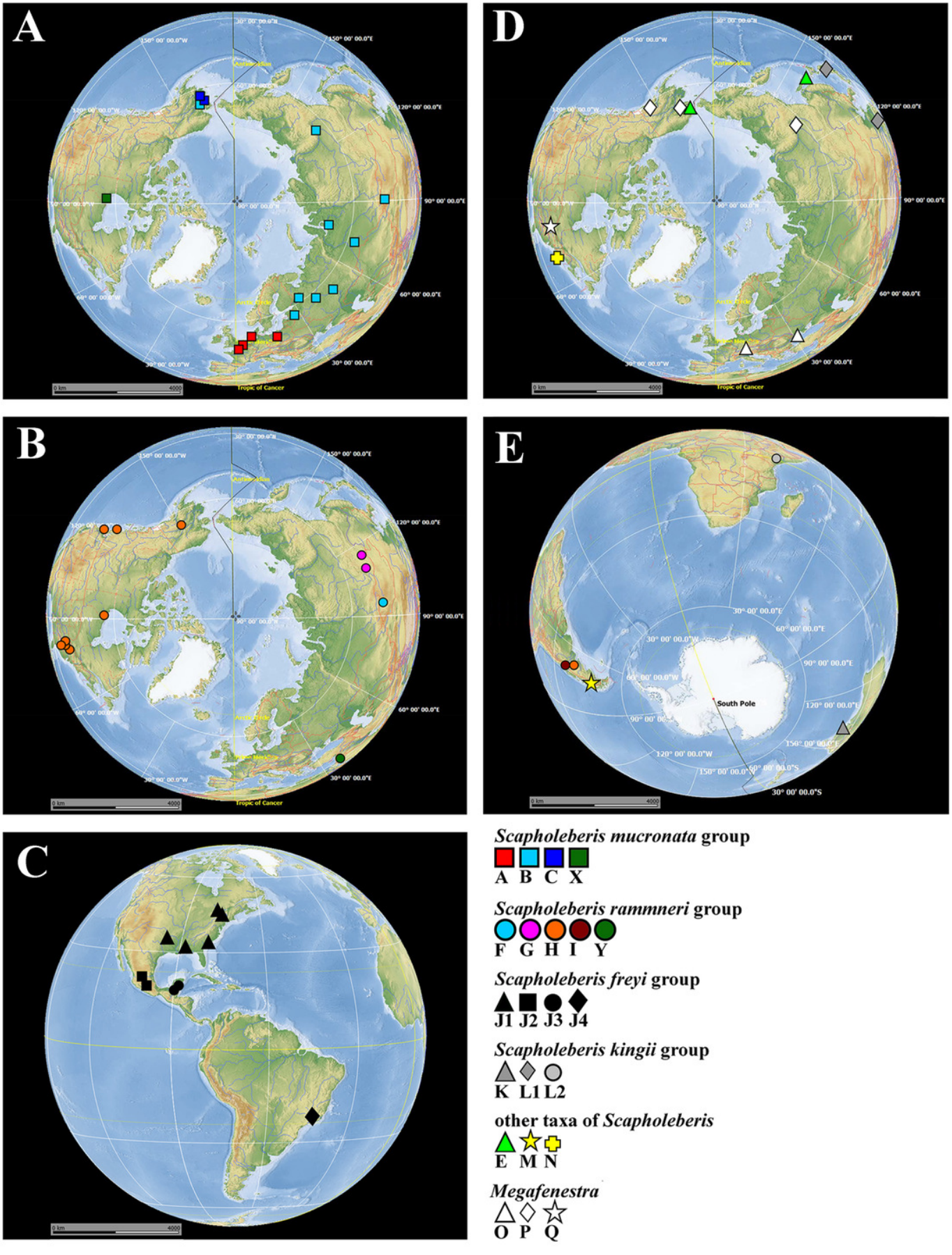
A
D
B
E
C
Tropic of Cancer
South Pole
Scapholeberis mucronata group
A B C X
Scapholeberis rammneri group
F G H I Y
Scapholeberis freyi group
J1 J2 J3 J4
Scapholeberis kingii group
K L1 L2
other taxa of Scapholeberis
E M N
Megafenestra
O P Q

Figure 2

Maximum likelihood mitochondrial phylogeny of neustonic daphniids (*Scapholeberis* and *Megafenestra*).

Bold letters (A–Q, X–Y) indicate geographic clades. Numbers at the nodes indicate Bayesian posterior probabilities and Transfer Bootstrap Expectations (TBE). Colours represent major species groups in the Scapholeberinae: *Scapholeberis mucronata* group (green), *S. rammneri* group (red), *S. freyi* group (black), *S. kingii* group (grey), *S. armata-microcephala* clade (blue), genus *Megafenestra* (white). The tree is outgroup-rooted using sequences from the genus *Megafenestra*. See Appendix S1 for individual sequences.

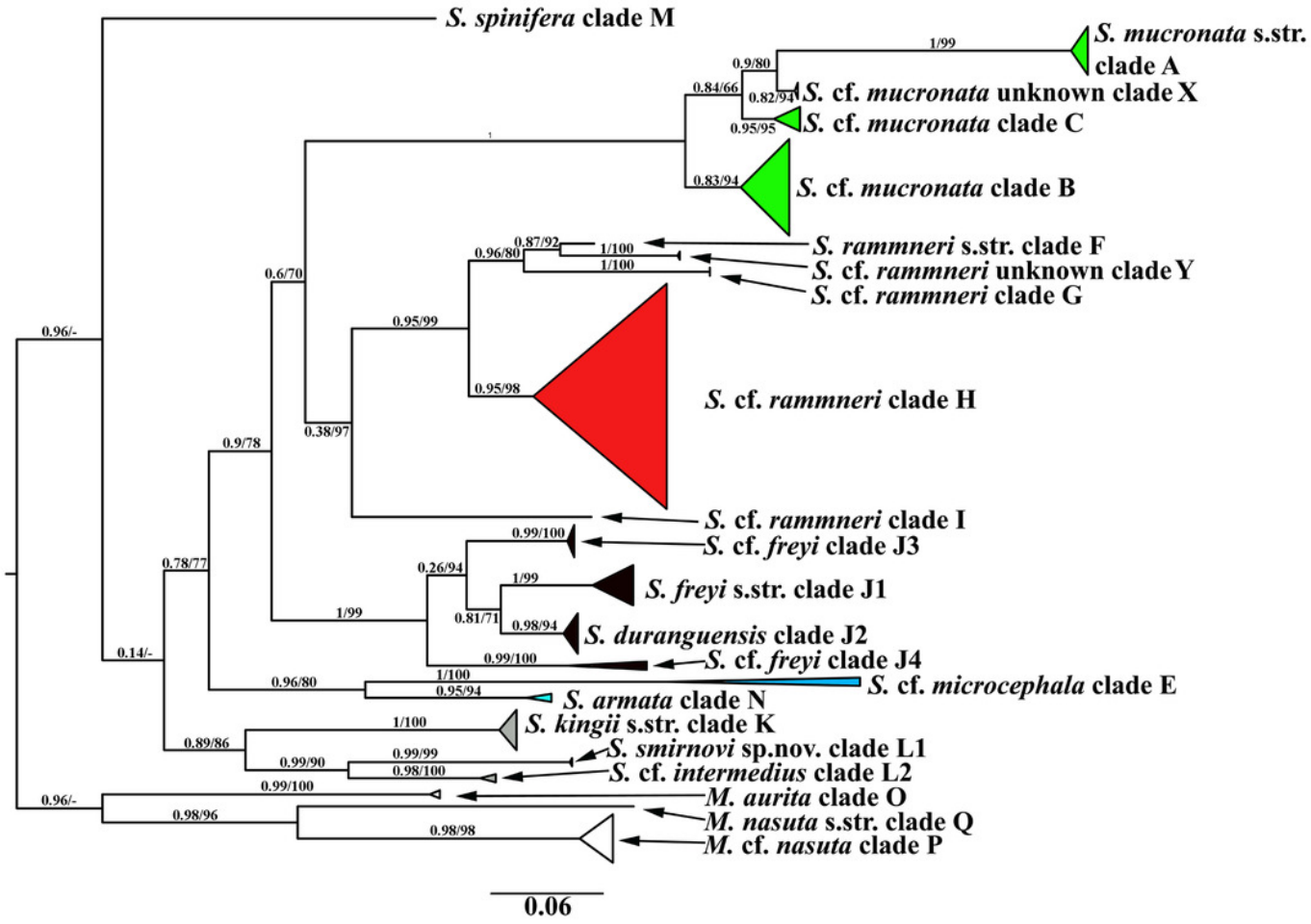

Figure 3

Violin plots of pairwise Kimura's 2 Parameter Distances from the COI region of mitochondrial DNA in clades of neustonic daphniids (Scapholebeberinae).

Horizontal bars indicate means. Gray rectangles show the ranges. Taxa are genera or species groups in the Scapholeberinae. See Appendix S1 for individual sequences.

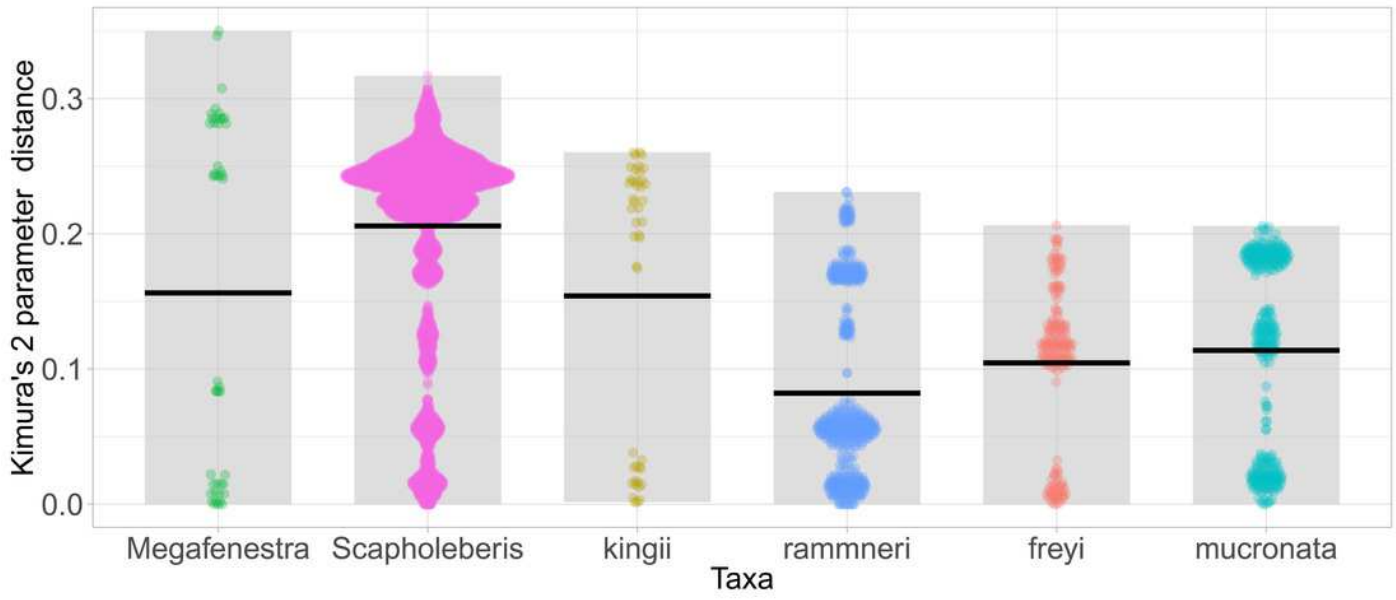

Figure 4

Scapholeberis kingii Sars, 1888, parthenogenetic and ephippial females from Farm Dam, New South Wales, Australia.

A–D, Adult parthenogenetic females, E, Juvenile parthenogenetic female, F–H, Ephippial females. A, Parthenogenetic female, lateral view. B, Adult parthenogenetic female, dorsal view. C, Head, ventral view. D, Labrum. E, Juvenile parthenogenetic female, lateral view. F, Ephippial female, lateral view. G, Ephippial female, dorsal view. H, Ornamentation of ephippium. Scale bars = 0.1 mm.

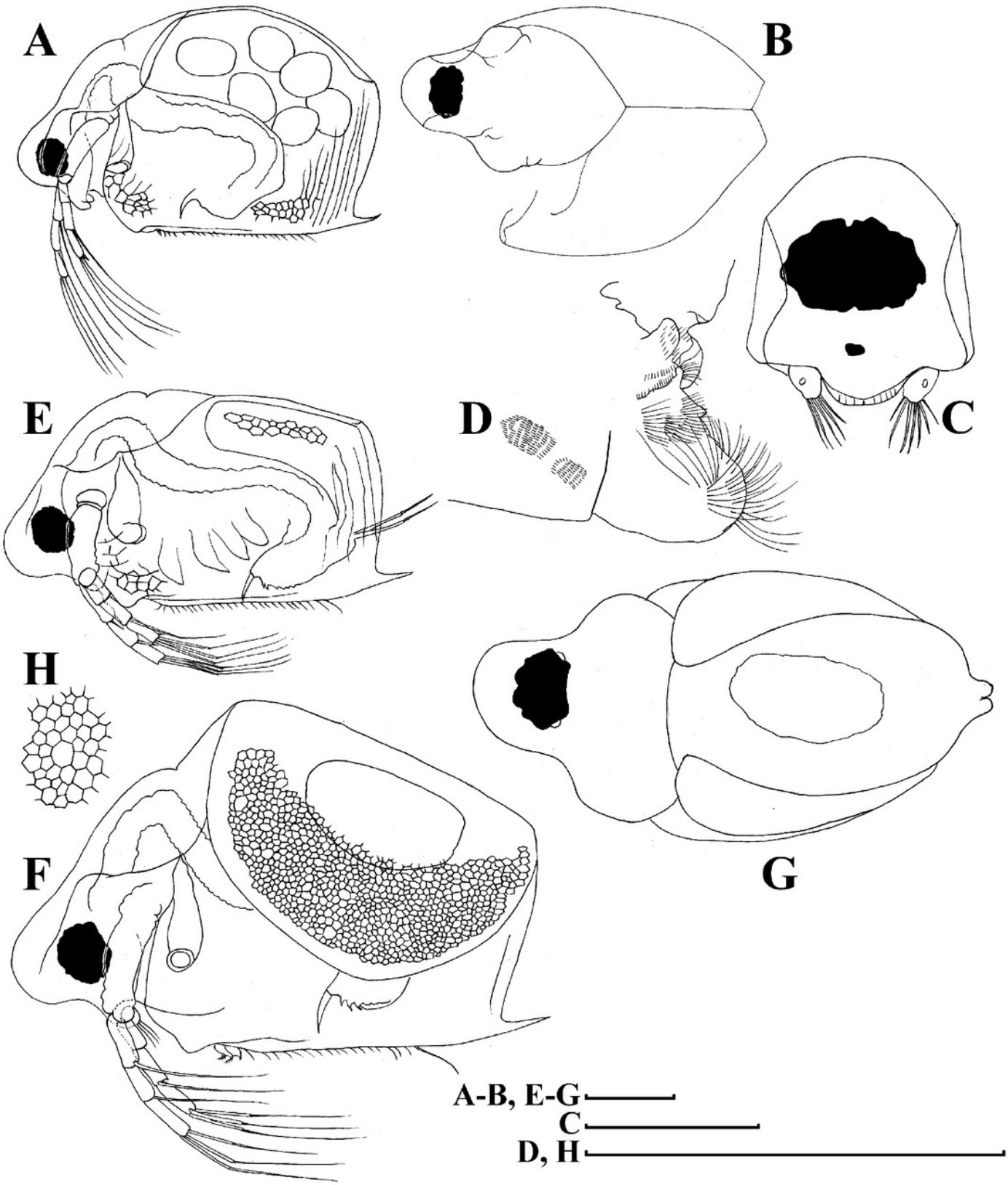
A
B
C
D
E
F
G
H
A-B, E-G
C
D, H

Figure 5

Scapholeberis kingii Sars, 1888, parthenogenetic females from Farm Dam, New South Wales, Australia.

A, Valve, ventral view. B–C, Armature of valve. D–E, Posteroventral portion of valve, inner view. F–G, Postabdomen. H–I, Postabdominal claw. J–K, Antenna I. L–M, Antenna II. Scale bars = 0.1 mm.

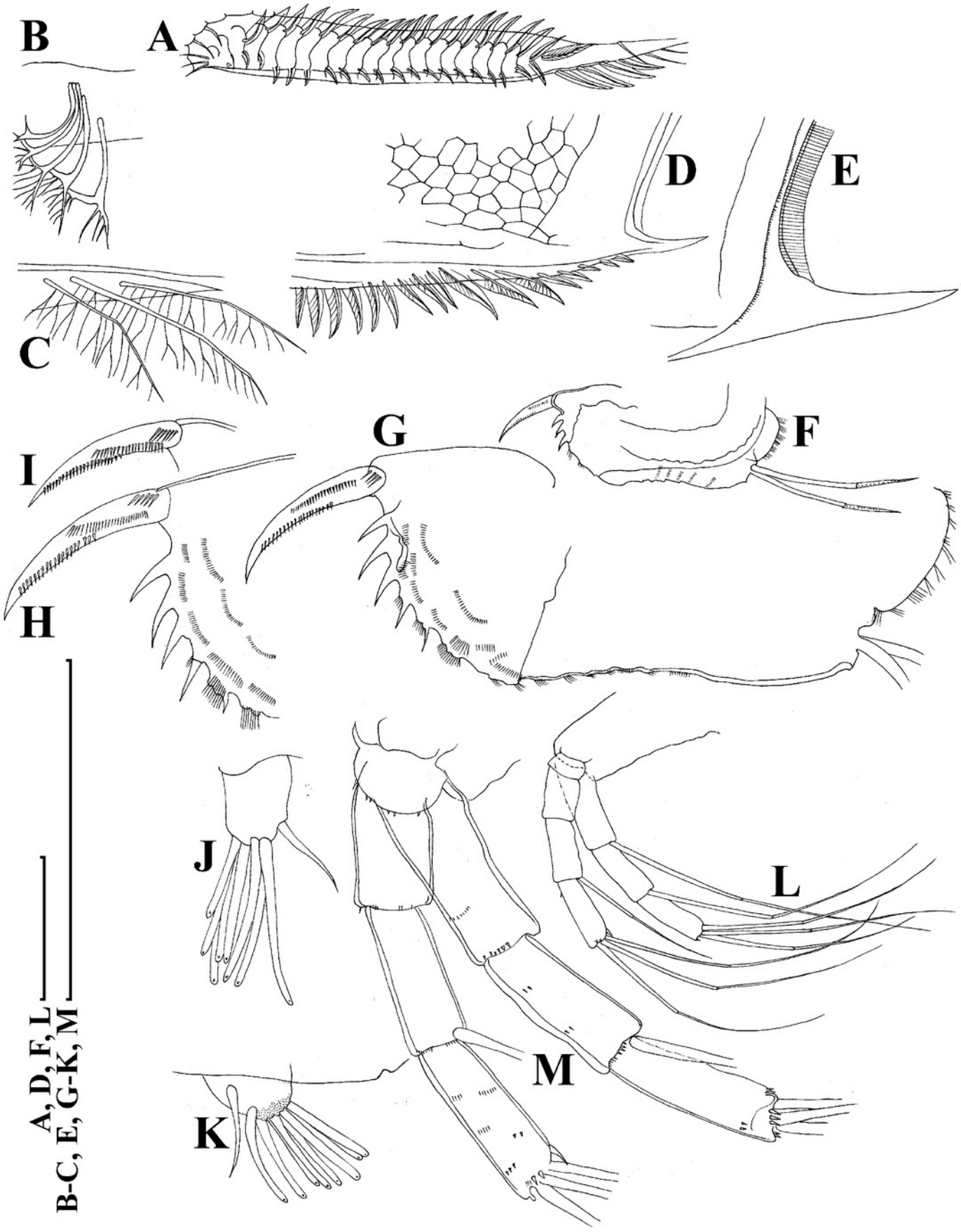
B
A
D
E
C
G
F
I
H
J
L
M
K
A, D, F, L
B-C, E, G-K, M

Figure 6

Scapholeberis kingii Sars, 1888, parthenogenetic females from Farm Dam, New South Wales, Australia.

A, Thoracic limb I. B, Thoracic limb II. C, Thoracic limb III. D, Thoracic limb IV. E, Thoracic limb V. Scale bar = 0.1 mm.

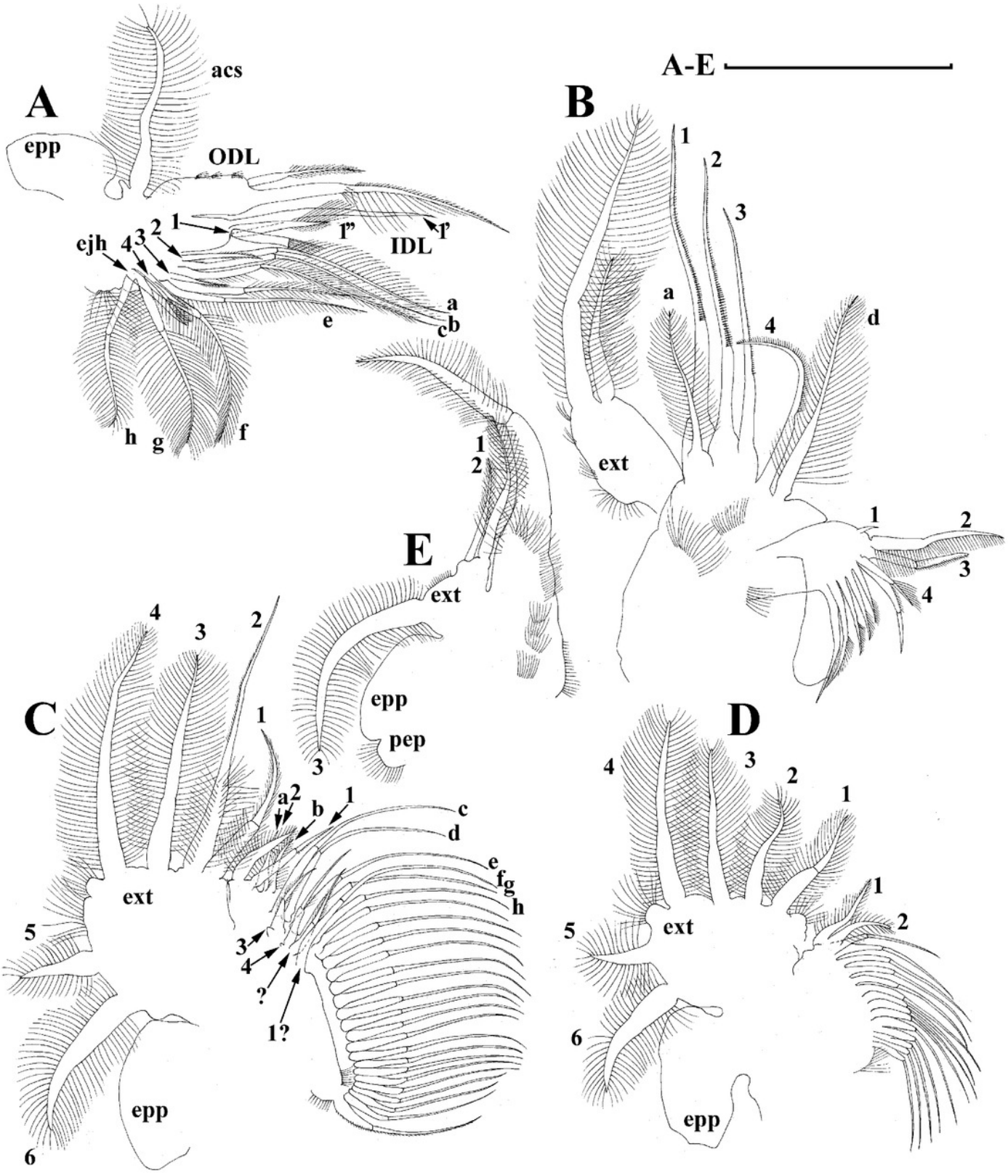
A-E
A
acs
epp
ODL
IDL
ejh
1
2
3
4
a
b
c
d
e
f
g
h
B
ext
C
ext
epp
pep
5
6
?
1?
D
ext
epp
E
ext
epp

Figure 7

Scapholeberis kingii Sars, 1888, parthenogenetic and ephippial females from Farm Dam, New South Wales, Australia.

A–E, Parthenogenetic females, F–L, Ephippial females. A, Ephippial female, lateral view. B, Valve, inner view. C, Posteroventral portion of valve, inner view. D, Head, ventral view. E, Antenna I. F, I, Ephippial females, lateral view. G, J, Ephippia, lateral view. K, Head, lateral view. H, L, Ornamentation of central portion of ephippia. Scale bars = 0.2 mm for A–B, F–G, I–J, 0.1 mm for D, K, 0.05 mm for H, 0.02 mm for C, E, L.

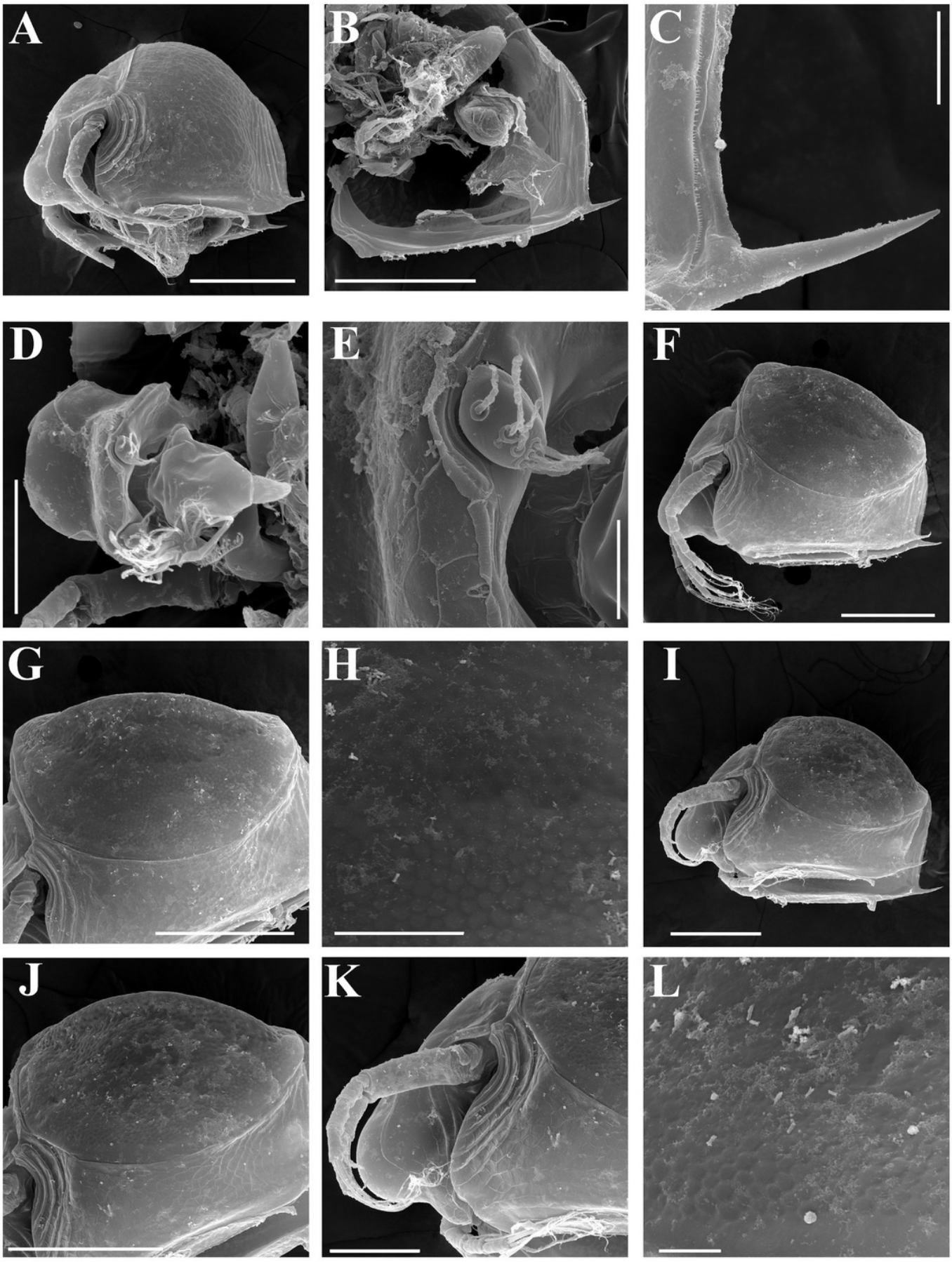
A
B
C
D
E
F
G
H
I
J
K
L

Figure 8

Scapholeberis kingii Sars, 1888, ephippial females from Farm Dam, New South Wales, Australia.

A, Ephippial female, dorsal view. B, Ephippium, dorsal view. C, Ephippium, dorsal view on higher magnification. D, Head, dorsal view. E, Ephippial female, ventral view. F, Head, ventral view. G, Head on higher magnification, ventral view. Scale bars = 0.2 mm for A–B, E, 0.1 mm for C–D, 0.05 mm for F–G.

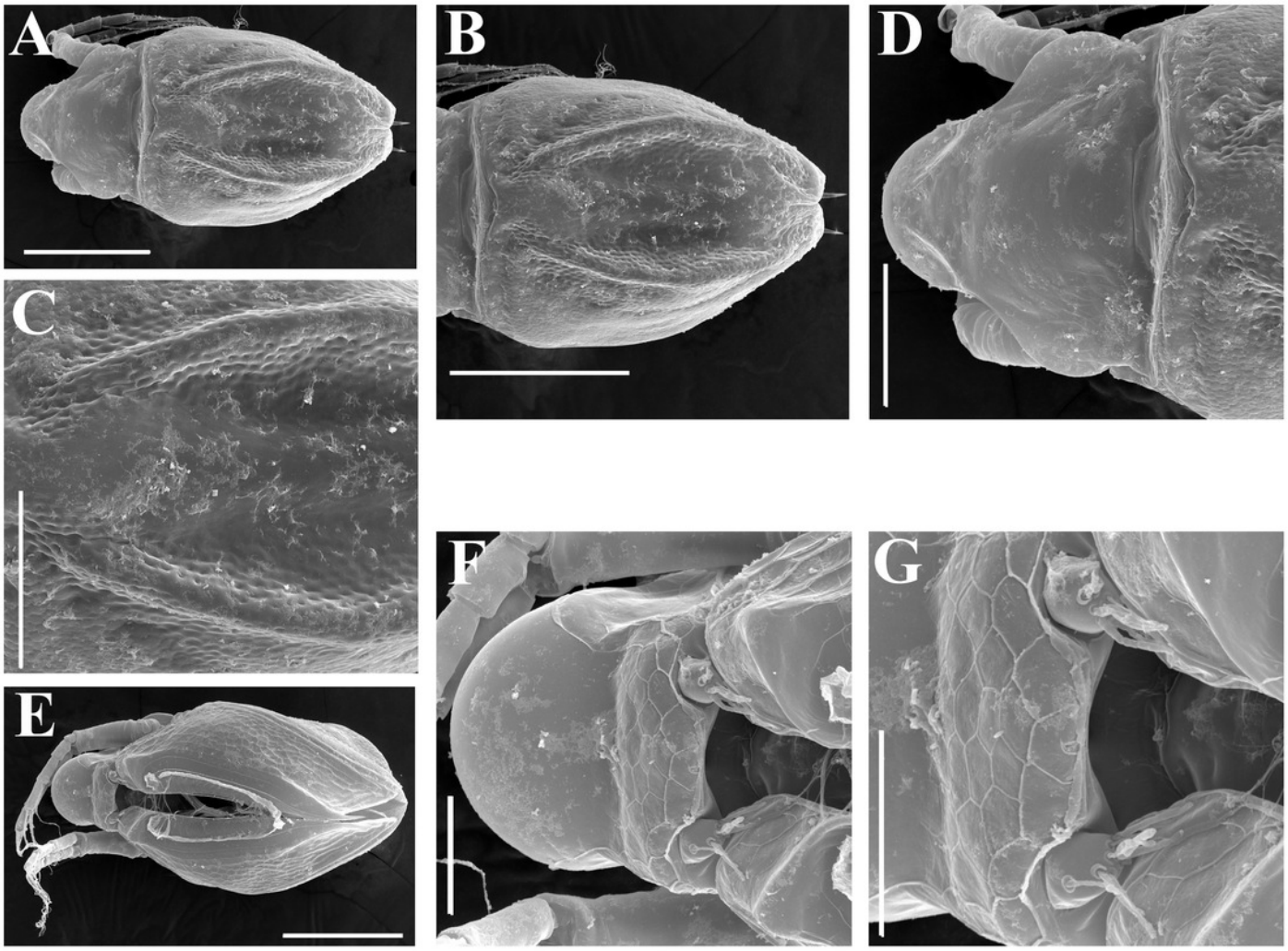

Figure 9

Scapholeberis kingii Sars, 1888, preephippial female from the roadside pool near Lake Bantic, West Coast, Tasmania, Australia.

A, Preephippial female, lateral view. B, Head, lateral view. C, Postabdominal claw, lateral view. D, Posterior portion of body. E–F, Posterior portion of body on higher magnifications. Scale bars = 0.2 mm for A, D, 0.1 mm for B, E, 0.05 mm for F, 0.02 mm for C.

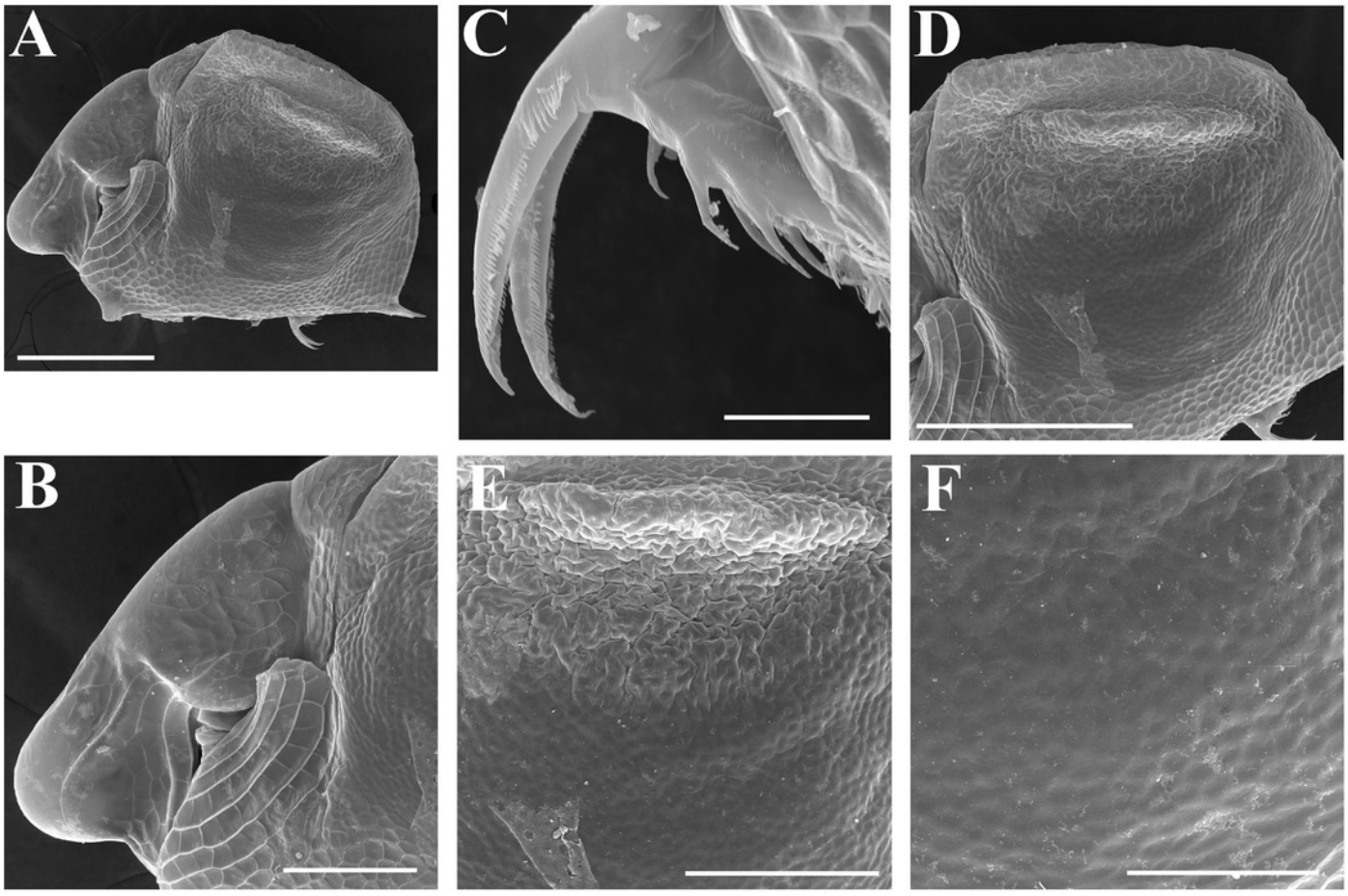

Figure 10

Scapholeberis intermedius Daday, 1898, parthenogenetic females from Collectio Dadayana.

A, Adult parthenogenetic female, lateral view (DAD 10-70-159). B, Juvenile parthenogenetic female, lateral view (DAD 10-70-156). C, Head, dorsal (?) view (DAD 10-70-156). D, Antenna II (DAD 10-70-156). Scale bars = 0.1 mm.

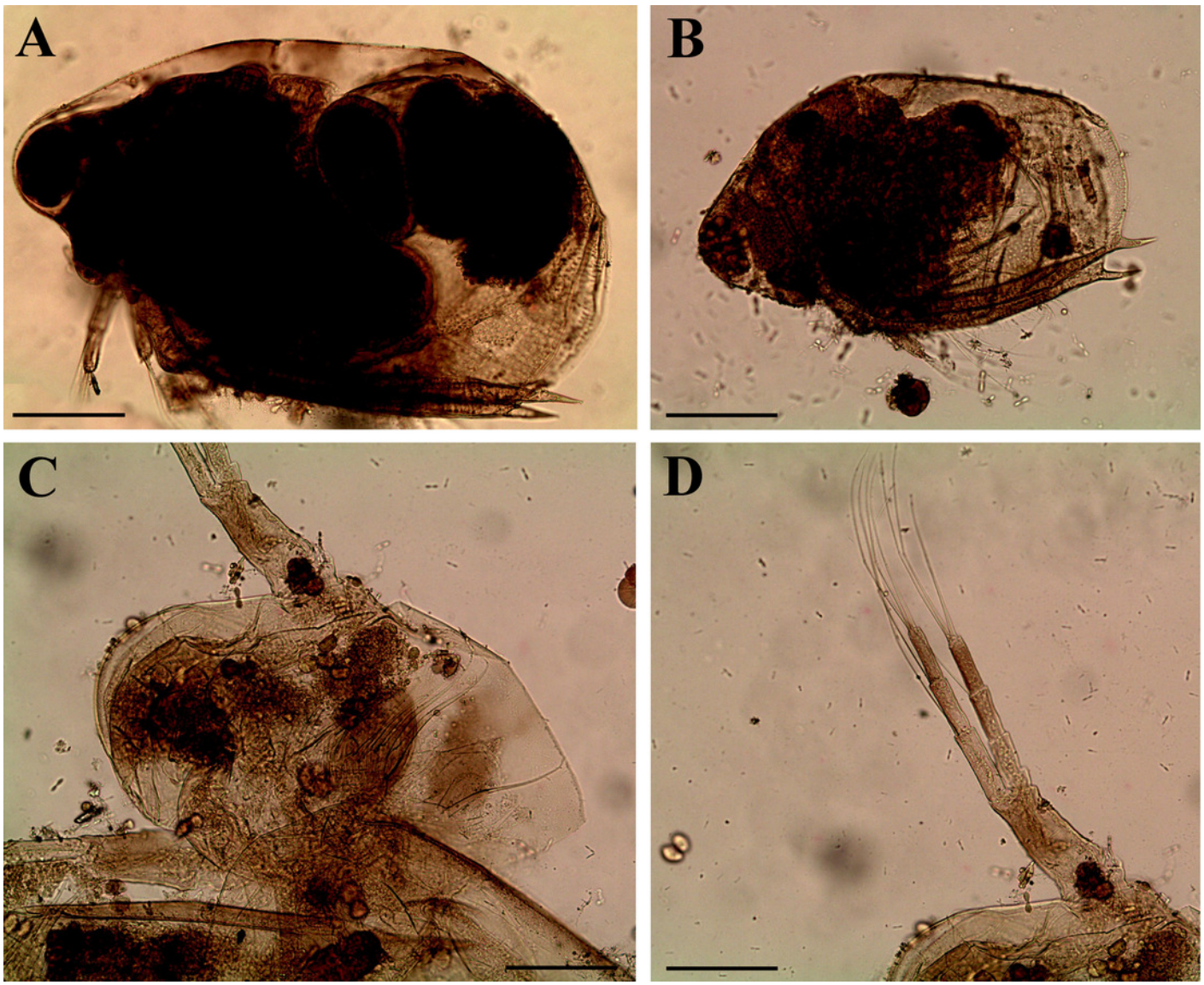

Figure 11

Scapholeberis cf. *intermedius* Daday, 1898, a parthenogenetic female from Bahir Dar Bay of Lake Tana, Amhara, Ethiopia.

A, Parthenogenetic female, lateral view. B, Head, lateral view. C, Head, ventral view. D, Labrum. E, Valve. F–H, Armature of posteroventral angle of valve. I, Postabdomen. J, Distal portion of postabdomen. K, Postabdominal seta. L, Antenna I. Scale bars = 0.1 mm.

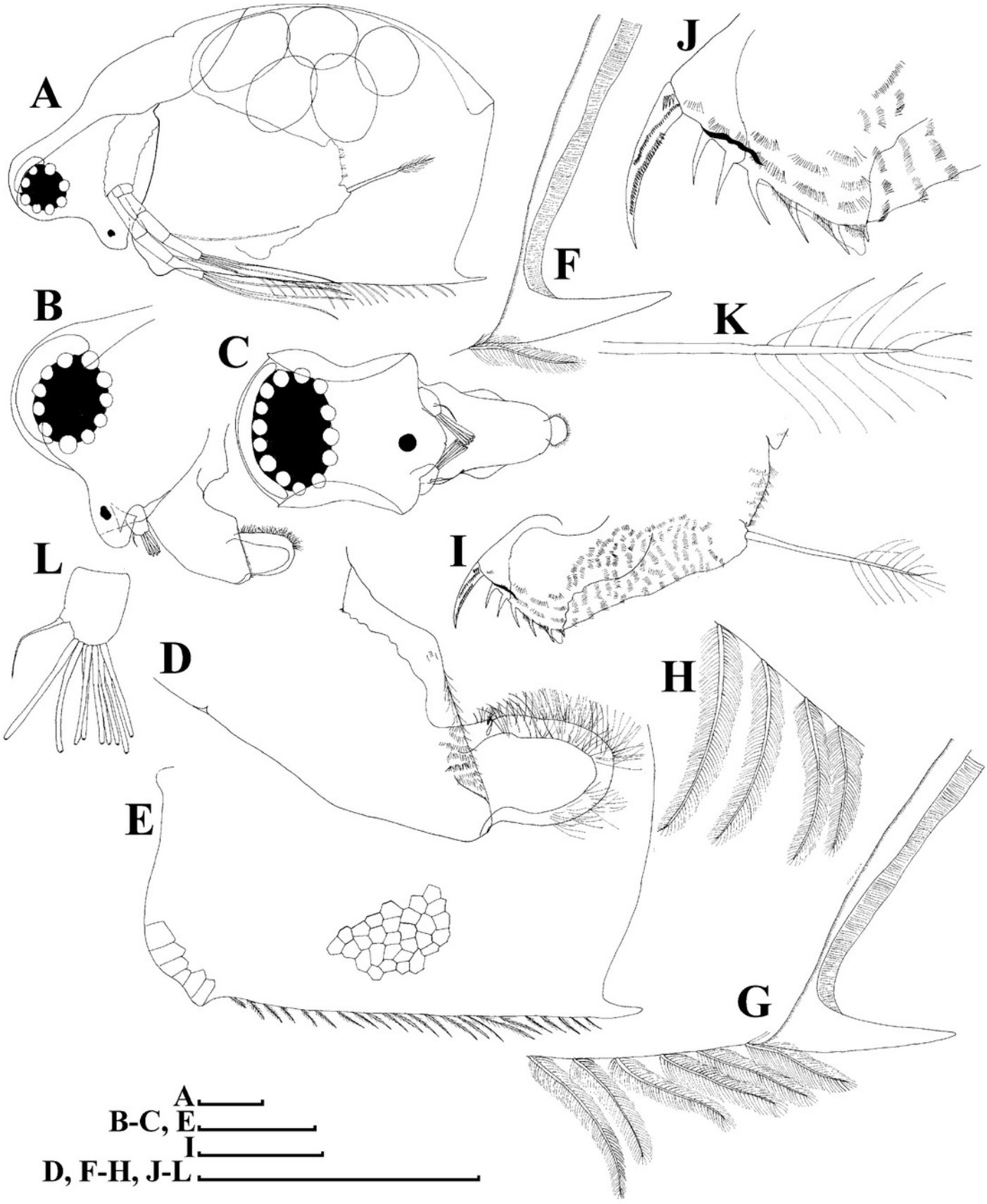
A
B
C
D
E
F
G
H
I
J
K
L
A
B-C, E
I
D, F-H, J-L

Figure 12

Scapholeberis cf. *intermedius* Daday, 1898, a parthenogenetic female from Bahir Dar Bay of Lake Tana, Amhara, Ethiopia.

A, Distal portion of postabdomen. B, Antenna II. D–J, Fragments of antenna II. K, Thoracic limb I. Scale bars = 0.1 mm.

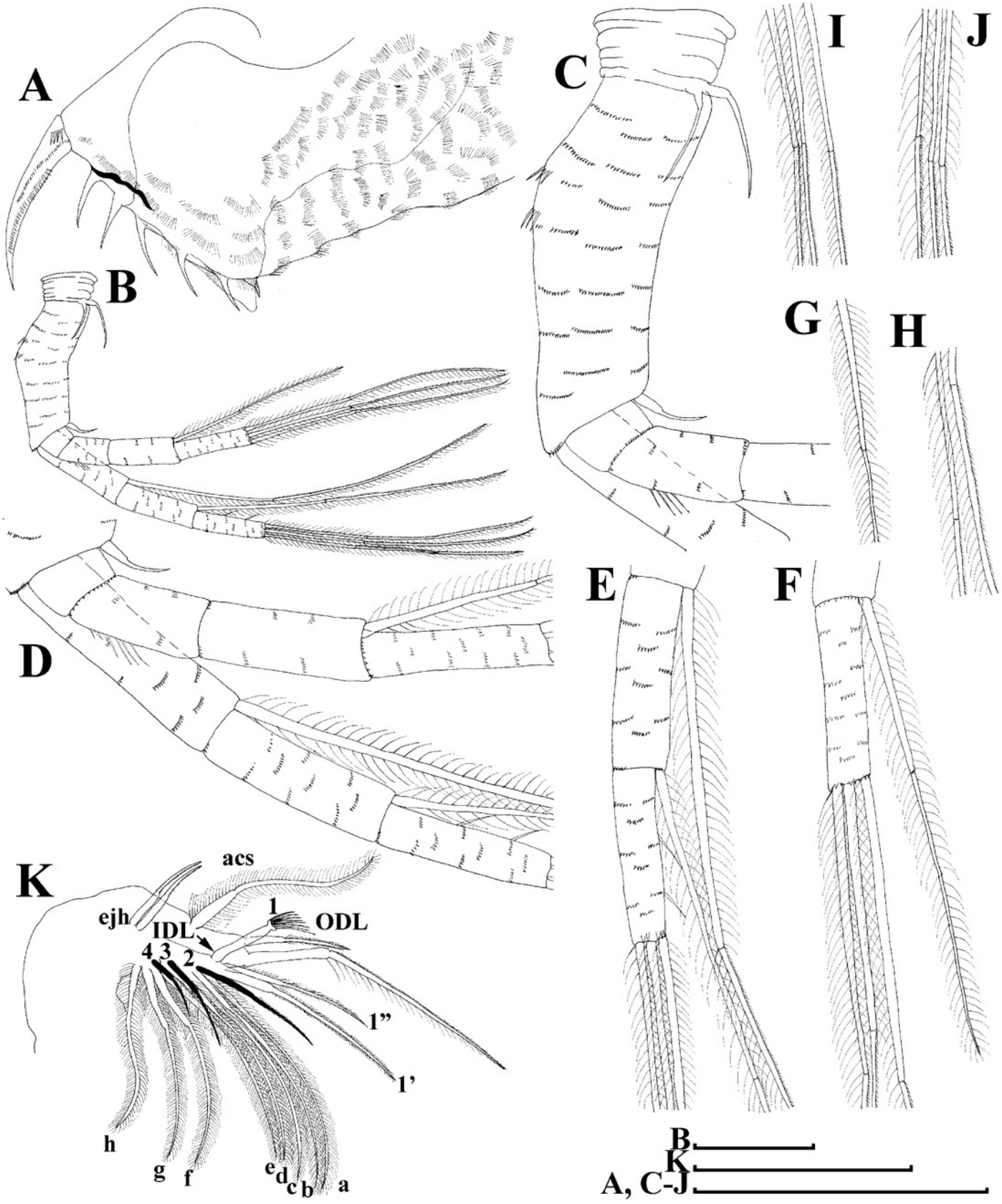
A
B
C
D
E
F
G
H
I
J
K
acs
ejh
IDL
1
ODL
4 3 2
1"
1'
h
g
f
e
d
c
b
a
B
K
A, C-J

Figure 13

Scapholeberis cf. *intermedius* Daday, 1898, a parthenogenetic female from Bahir Dar Bay of Lake Tana, Amhara, Ethiopia.

A, Thoracic limb I. B, Thoracic limb II. C–D, Fragments of thoracic limb II. E, Thoracic limb III. F–G, Fragments of thoracic limb III. Scale bars = 0.1 mm.

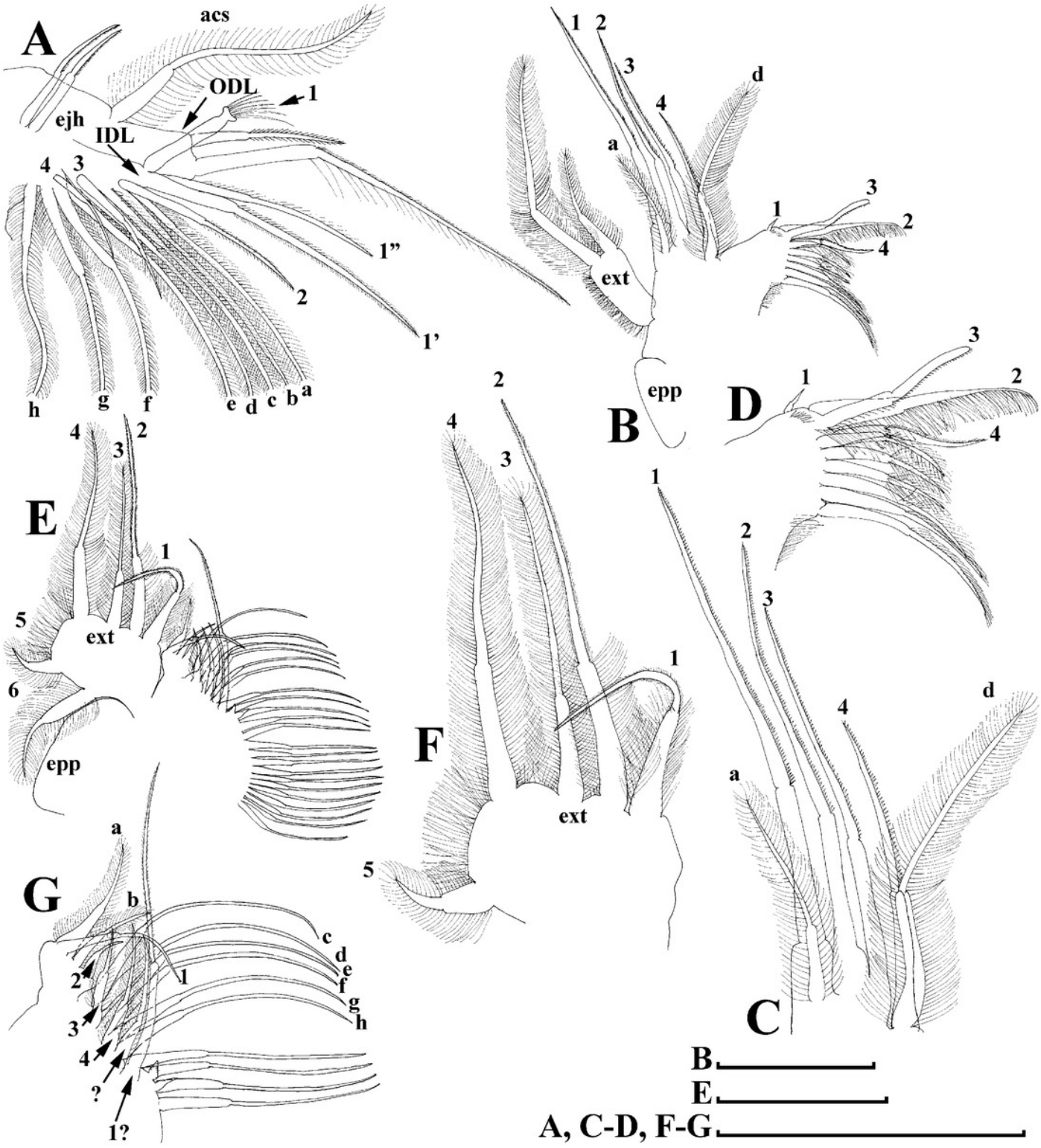
A
acs
ODL
1
ejh
IDL
4
3
1"
2
1'
h
g
f
e
d
c
b
a
B
1
2
3
4
d
a
ext
epp
C
D
E
5
6
F
G
b
c
d
e
f
g
h
?
1?
B
E
A, C-D, F-G

Figure 14

Figure 14. *Scapholeberis* cf. *intermedius* Daday, 1898, a parthenogenetic female from Bahir Dar Bay of Lake Tana, Amhara, Ethiopia.

A, Thoracic limb IV. B–C, Fragments of thoracic limb IV. D, Thoracic limb V. E, Fragment of thoracic limb V. Scale bars = 0.1 mm.

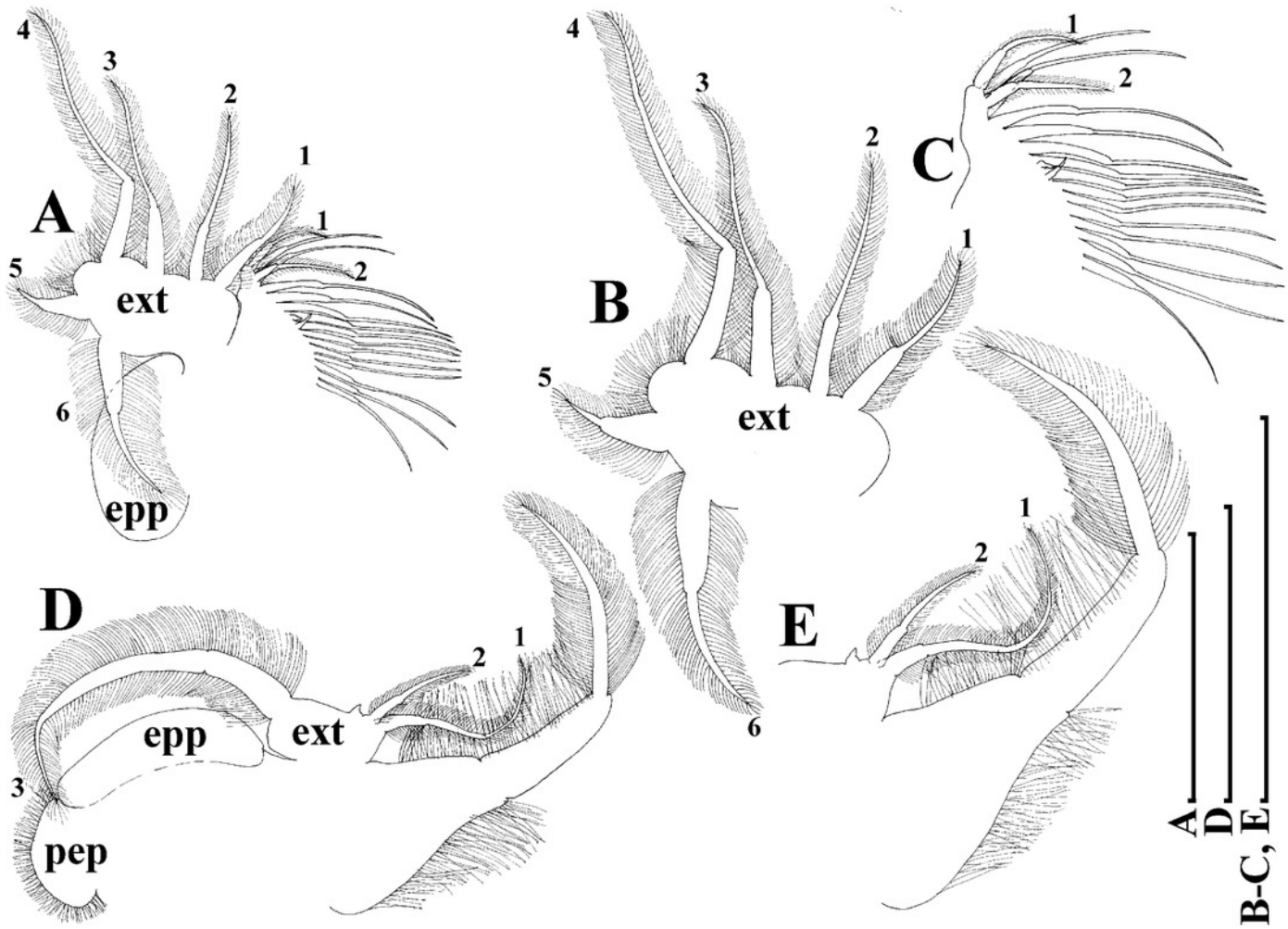

Figure 15

Scapholeberis cf. *intermedius* Daday, 1898, a parthenogenetic female from Bahir Dar Bay of Lake Tana, Amhara, Ethiopia.

A, Parthenogenetic female, lateral view. B, Anterior portion of body. C, Head, lateral view. D, Posterior portion of body. E, Ornamentation of valve. Scale bars 0.2 mm for A, D, 0.1 mm for B, 0.05 mm for C, E.

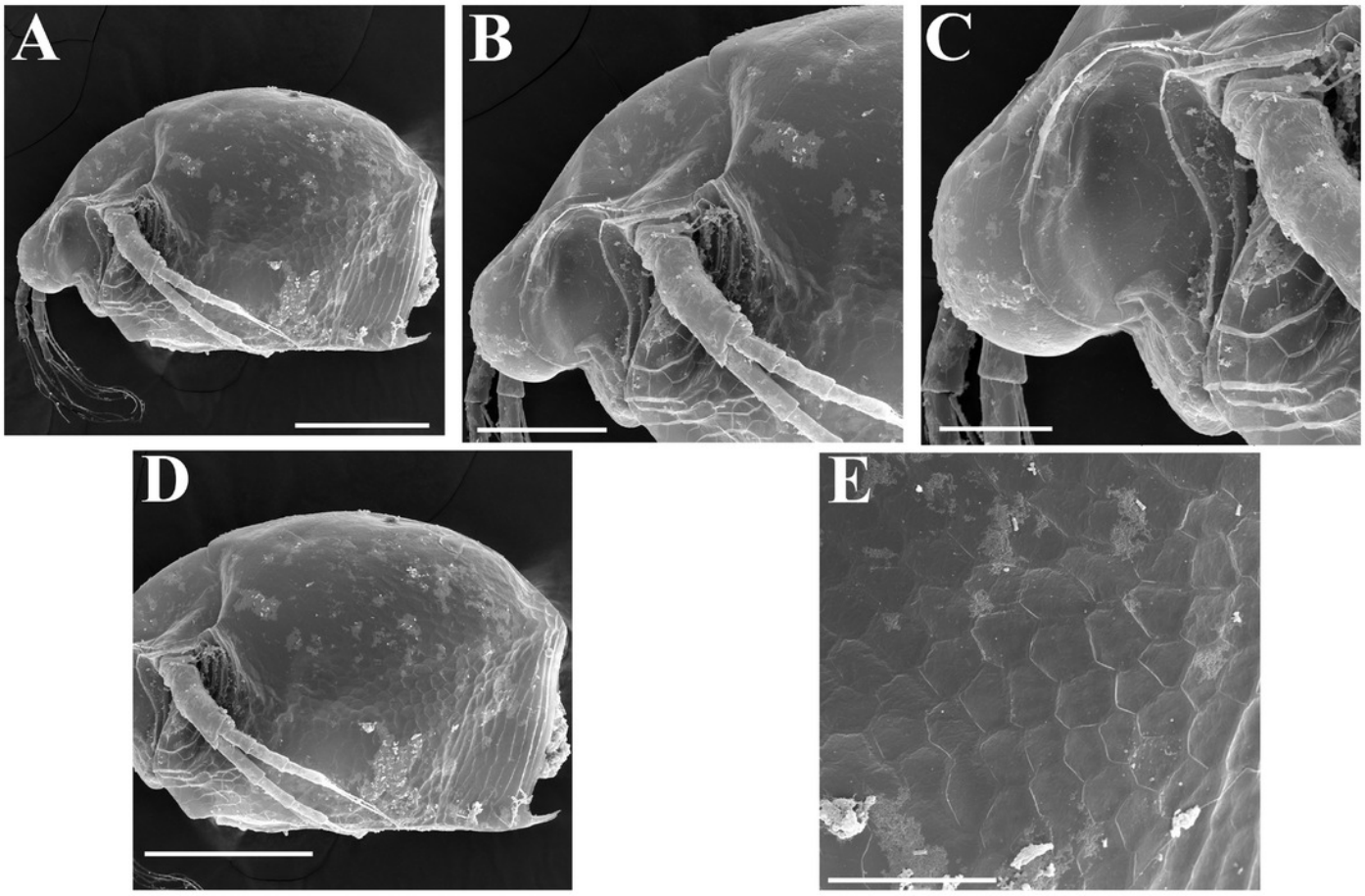

Figure 16

Scapholeberis smirnovi sp.nov. from the puddle near Lake Maloe Utinoe, Primorski Territory, Far East, Russia.

A–D, Adult parthenogenetic females, E–F, Juvenile parthenogenetic female, G–I, Ephippial females. A, Adult parthenogenetic female, lateral view. B, Parthenogenetic female, dorsal view. C, Head, ventral view. D, Labrum. E, Juvenile parthenogenetic female. F, Head, ventral view. G, Ephippial female, lateral view. H, Ephippial female, dorsal view. I, Ornamentation of ephippium. Scale bars = 0.1 mm.

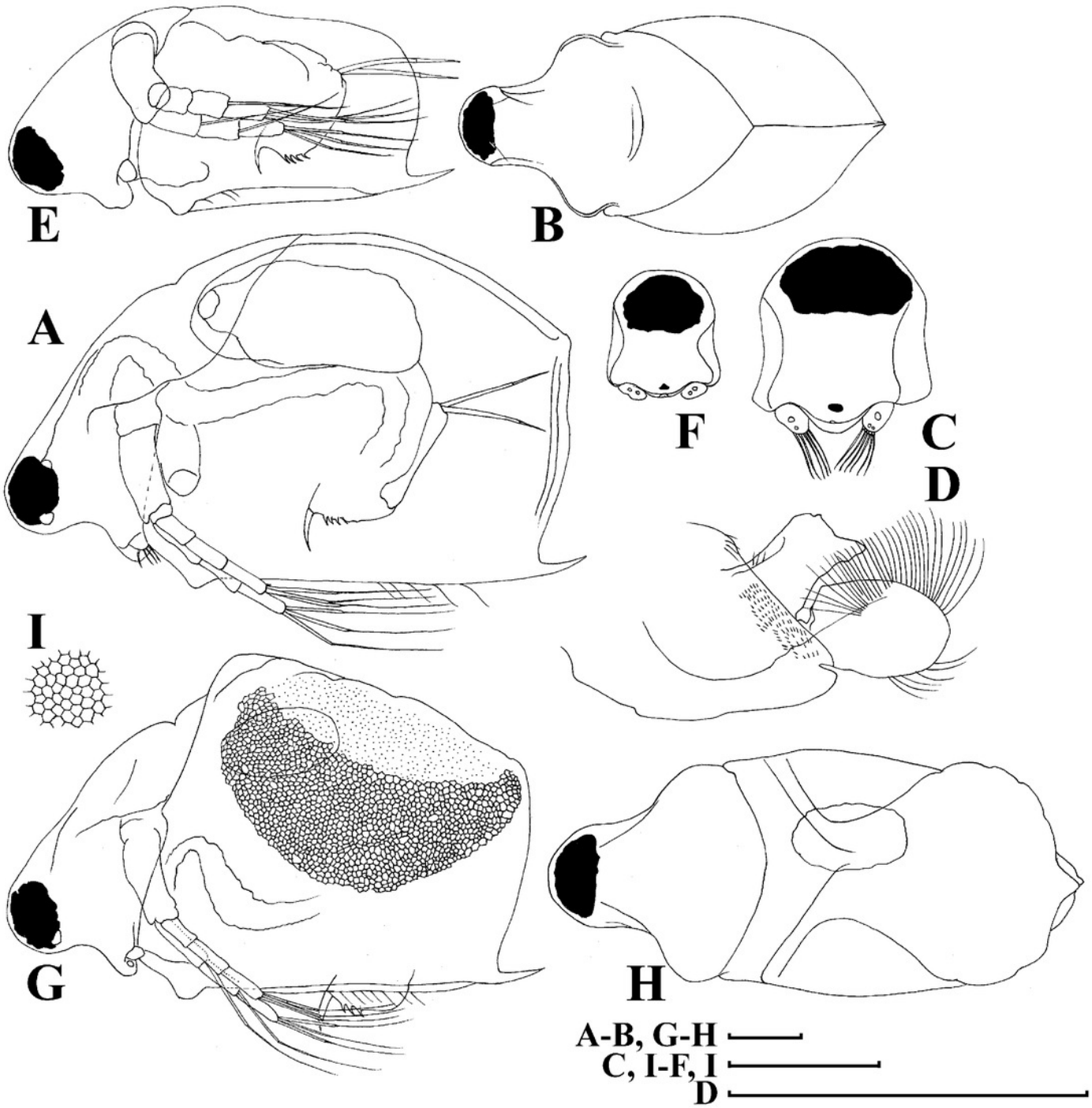
E
B
A
F
C
D
I
G
H
A-B, G-H
C, I-F, I
D

Figure 17

Scapholeberis smirnovi sp.nov. from the puddle near Lake Maloe Utinoe, Primorski Territory, Far East, Russia.

A, Valve, outer view. B, Valve, ventral view. C–D, Armature of valve. E, Posteroventral portion of valve, inner view. F–H, Postabdomen. I, Postabdominal claw. J–K, Antenna I. L–M, Antenna II. Scale bars 0.1 = mm.

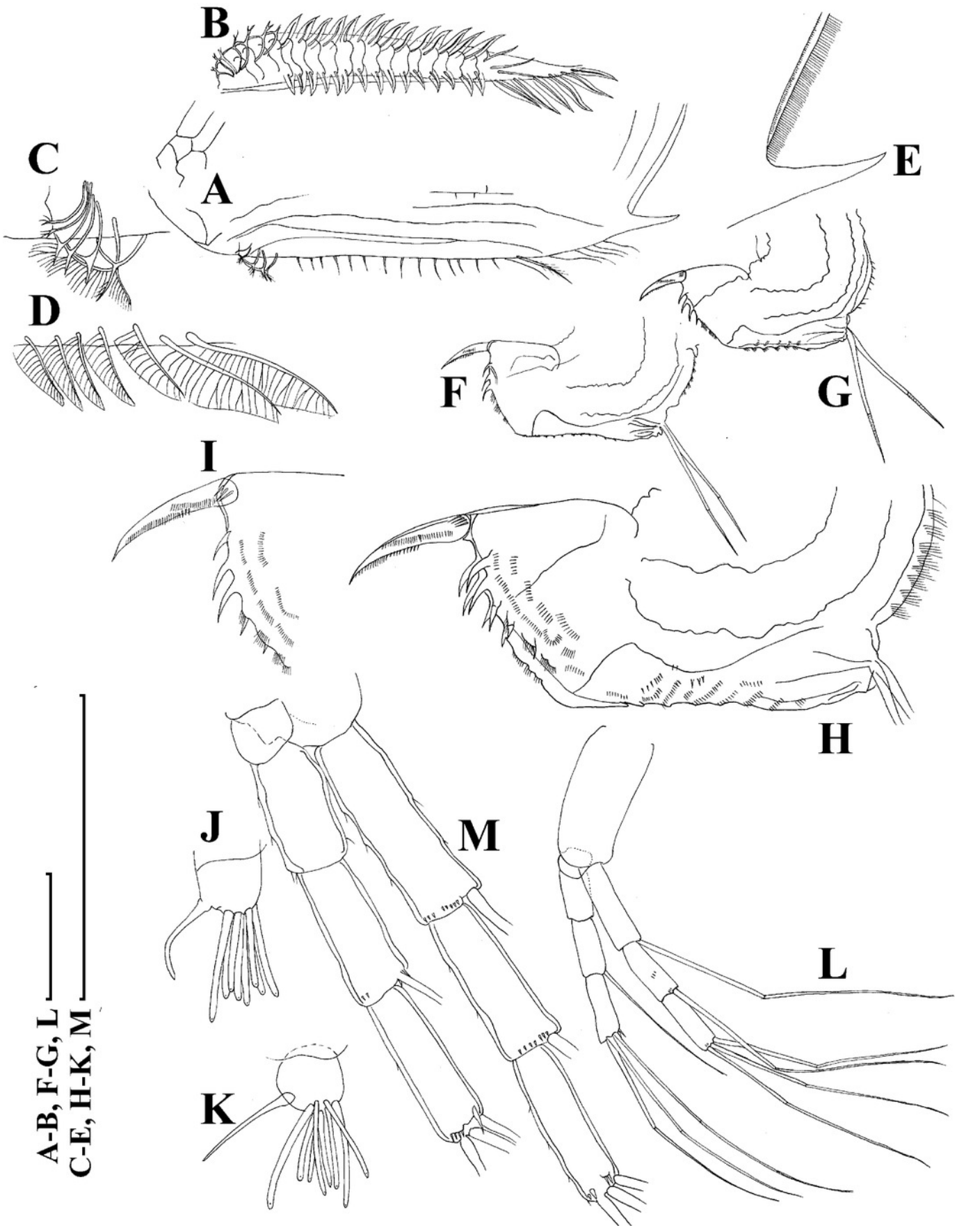
B
C
A
E
D
F
G
I
H
J
M
L
K
A-B, F-G, L
C-E, H-K, M

Figure 18

Scapholeberis smirnovi sp.nov. from the puddle near Lake Maloe Utinoe, Primorski Territory, Far East, Russia.

A–B, Thoracic limb I. C–D, Thoracic limb II. E, Thoracic limb III. F–G, Thoracic limb IV. H, Thoracic limb V. Scale bar = 0.1 mm.

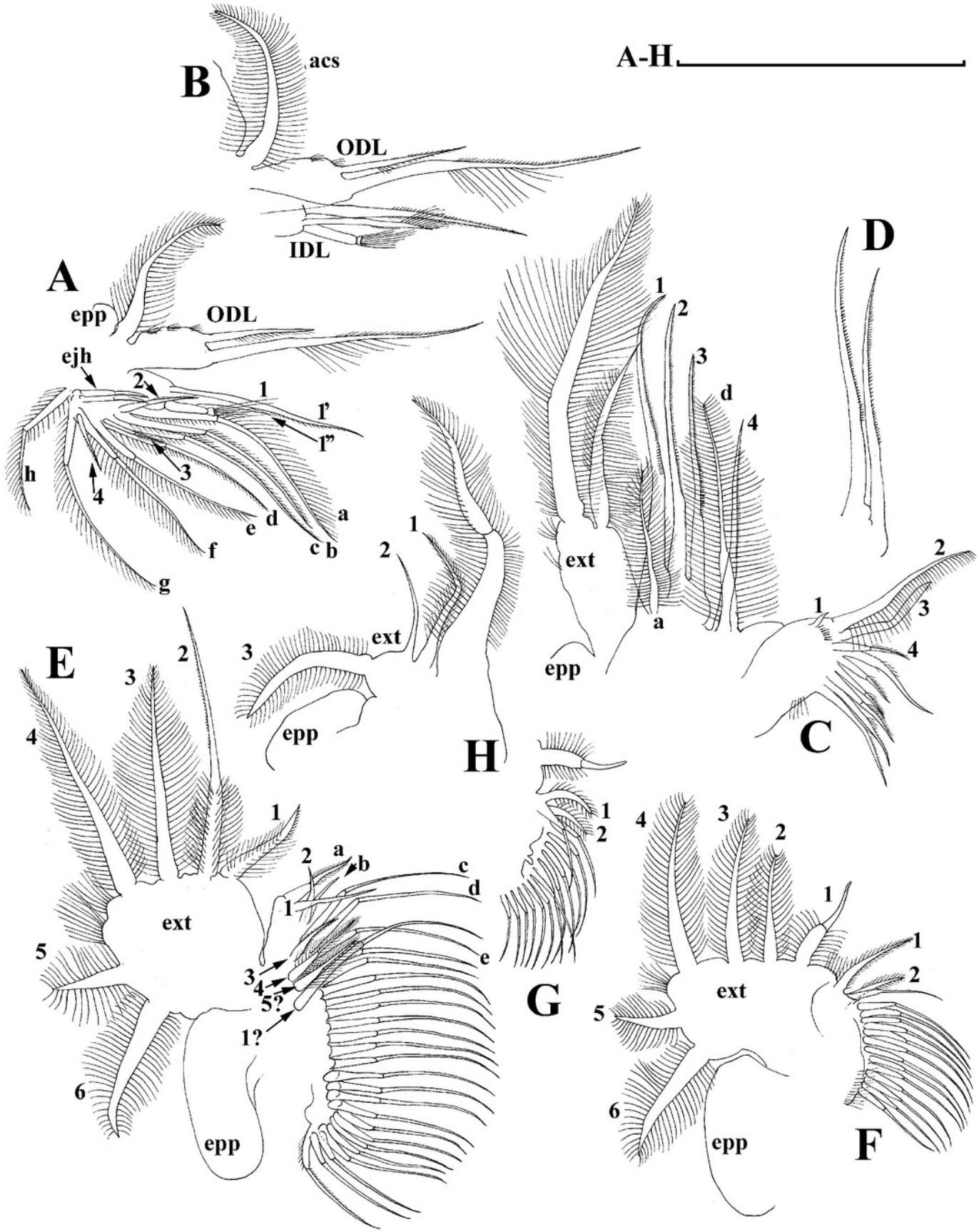
A-H
B
acs
ODL
IDL
A
epp
ODL
ejh
2
1
1'
1"
h
3
4
e
d
a
f
c
b
g
H
1
2
3
ext
epp
D
1
2
3
d
4
ext
a
epp
C
2
1
3
4
E
4
3
2
1
2
a
b
c
d
1
ext
5
e
3
4
5?
1?
6
epp
G
1
2
F
4
3
2
1
1
2
ext
5
6
epp

Figure 19

Scapholeberis smirnovi sp.nov., ephippial and preephippial females from the puddle near Lake Maloe Utinoe, Primorski Territory, Far East, Russia.

A–B, D–F, Ephippial females, C, Preephippial female. A, Ephippial female, lateral view. B, Ephippium, lateral view. C, Preephippial female, lateral view. D, Ephippial female, anterodorsal view. E, Ephippium, anterodorsal view. F, Ephippial female, ventral view. Scale bars = 0.2 mm for A–D, F, 0.1 mm for E.

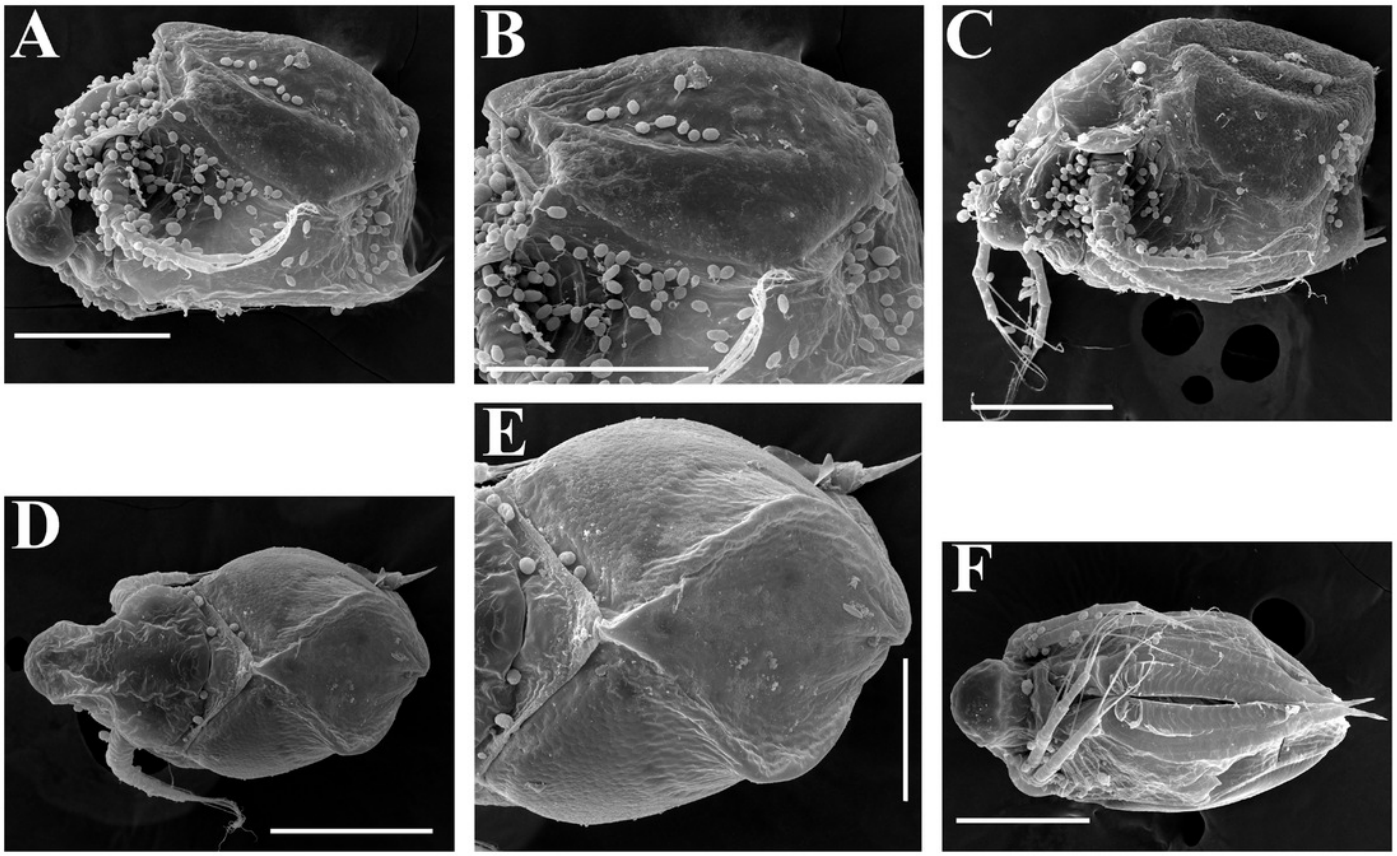

Figure 20

Scapholeberis smirnovi sp.nov., ephippial females from a pond in Choenggye Mountains, Seoul, the Republic of South Korea.

A, Ephippial female, lateral view. B–C, Head, lateral view. D, Ephippium, lateral view. E–F, Ornamentation of ephippium. G, Ephippial female, dorsal view. H, Head, dorsal view. I–J, Ephippium, dorsal view. K–L, Armature of ephippium on higher magnifications. Scale bars = 0.2 mm for A, D, G, I, 0.1 mm for B–C, H, J–L, 0.5 mm for F, 0.2 mm for E.

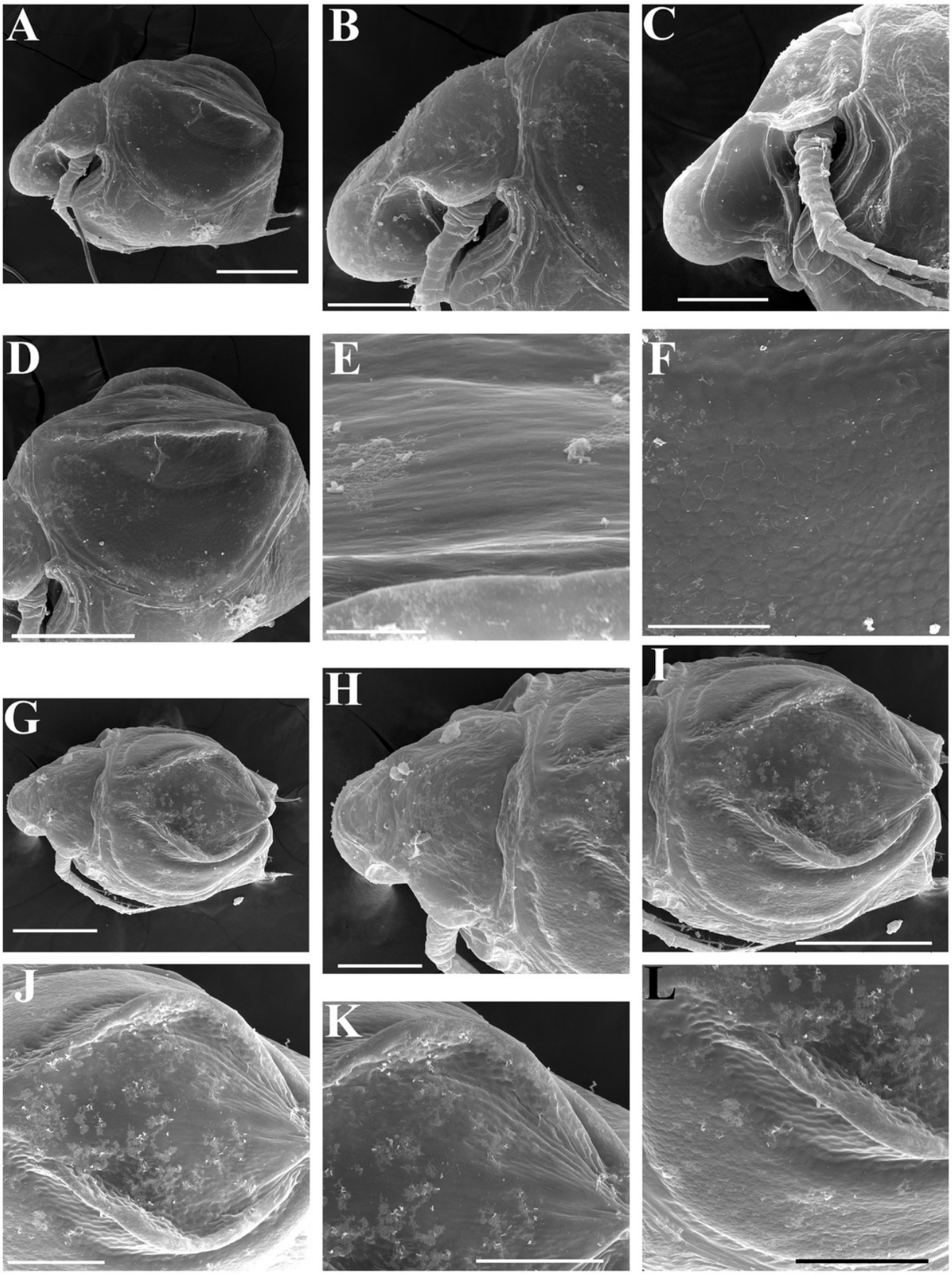
A
B
C
D
E
F
G
H
I
J
K
L

Figure 21

Analysis of identification for four species groups of *Scapholeberis* based on GenBank data.

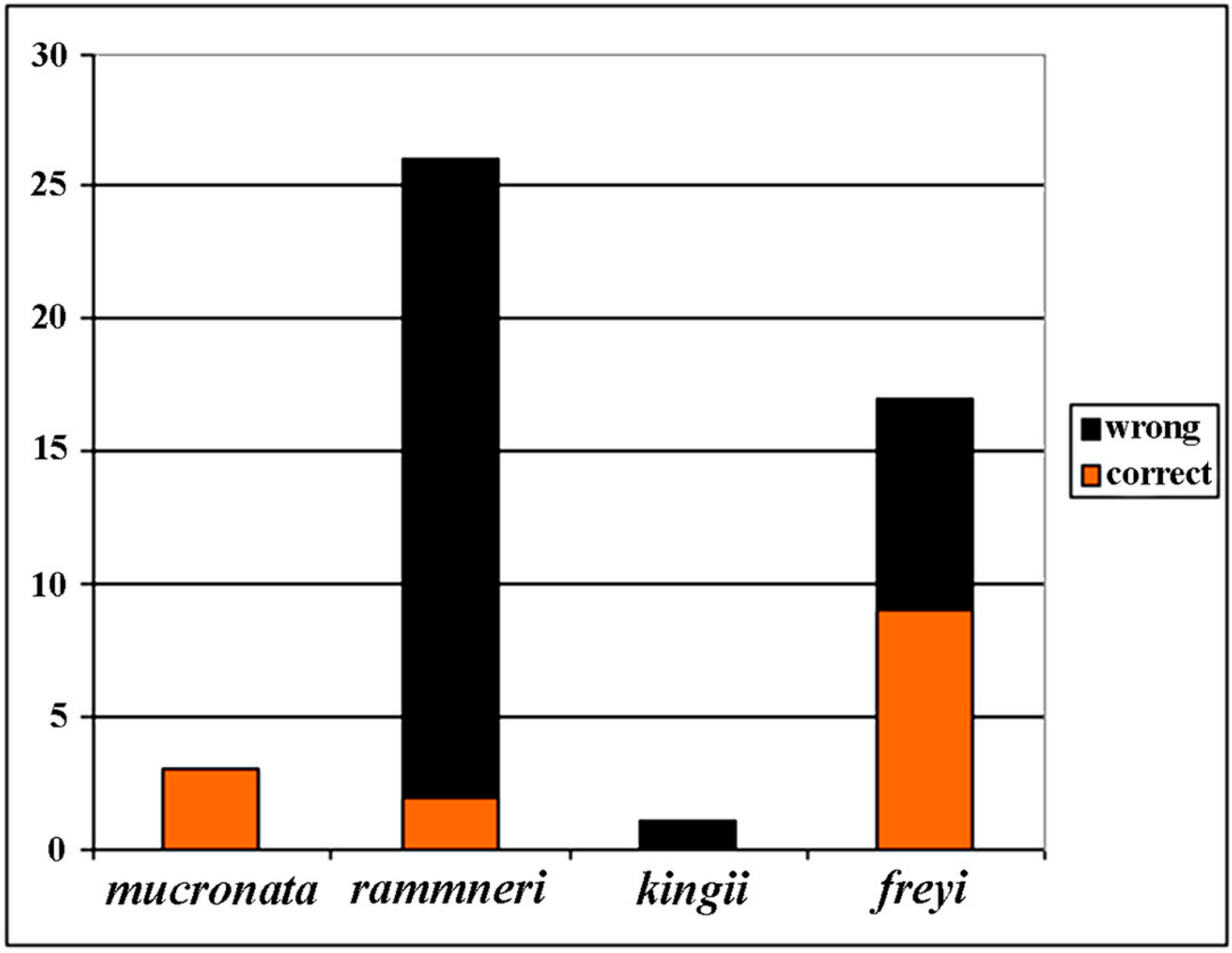

Table 1(on next page)

Table 1. Differentiation of the species of the *Scapholeberis kingii* group based on morphological characters

Taxon	***S. kingii***	***S. intermedius***	***S. smirnovi* sp.nov.**
Distance between the center of ocellus and eye/ distance from the center of ocellus to the tip of rostrum	almost 2	almost 3	about 5
On thoracic limb I, the ratio between seta 1' and seta 2	almost 2.5 (i.e. seta 2 is relatively short)	almost 1.5 (i.e. seta 2 is relatively long)	almost 2.5 (i.e. seta 2 is relatively short)
In ephippial females, area between two keels of ephippium	strongly elongated, keels not projected laterally out of body dorsal contour	Unknown	strongly rounded, keels strongly projected laterally out of body dorsal contour

1

2