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

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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 c oxidase subunit ~~Cytochrome Oxidase~~ I gene region) together with

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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 are ~~reliably~~ consistently different 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*.

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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 COI data (from the successful realization of the Barcoding of Life initiative) is available for many taxa (*Hebert et*

Commented [AP3]: define abbreviation in the intro before use (despite mentioning the gene in the abstract) *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, we carried out 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 (*Taylor, Connelly & Kotov, 2020*). Lineage diversity was unexpectedly high in the Eastern Palearctic, while other regions, such as Africa, remained unexamined. Notably, the within-genus divergences for neustonic taxa were much greater than that found within other daphniid genera. We were unable to reconcile the novel diversity 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.

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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. 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 the 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 Subunit) based 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

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contigs were assembled in Geneious R7. The authenticity of newly obtained sequences was verified by BLAST comparisons. The alignment was carried out in the online version of MAFFT 7 using the default ~~form~~settings. Additional sequences were obtained from NCBI GenBank. 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. A neighbor joining tree was estimated with Kimura's 2 Parameter distance in Seaview. Violin plots were created in R for major taxa based on pairwise Kimura's 2-parameter distances (also calculated in Seaview). Support 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. 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.

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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 were lacking. Specimens of *Scapholeberis* from presorted samples were selected under a binocular stereoscopic microscope LOMO (Open Joint-Stock Company, Russia), and then studied *in toto* under optical microscopes Olympus BX41 or Olympus CX 41 (Olympus Corporation, Japan) 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. 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).

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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.

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; OLD, outer distal lobe; pep, preepipodite.

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 names proposed by *Taylor, Connelly & Kotov, 2020*. Lineages novel to the present study ~~included:~~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* (clade E) which groups with *S. armata* (clade N) (Figs. 1 and 2).

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S. mucronata (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 only in the vicinity of Churchill, Manitoba (*Jeffery, Elías-Gutiérrez & Adamowicz, 2011*).

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S. rammneri (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 (Direct submission to the GenBank).

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

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

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

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

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Morphological analysis

Order Anomopoda Sars, 1865

Family Daphniidae Straus, 1820

Subfamily Scapholeberinae Dumont & Pensaert, 1983

Genus *Scapholeberis* Schödler, 1858

Scapholeberis kingii species group

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

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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 middle (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 angle of valve broadly rounded, its ventral portion with a small protuberance (Fig. 7B). Valves with well-developed sculpture of polygonal reticulation.

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middle of body?

Head large for a daphniid (Figs. 4A and 7A). In lateral view head elongated, with a prominent rostrum, its distal portion roundish (Figs. 4A and 7A). In dorsal view head elongated, head shield with low lateral projections (fornices) covering ~~covered~~ bases of antennae II, a sclerotized ridge departs from the insertion of antenna II and extends to the side of head (Fig. 4B). In anterior view head slightly compressed from lateral sides (Figs. 4C and 7D). In ventral view postero-ventral portion of head forms a three-lobed rostrum, due to a shallow depression in points of antenna I insertion on each side~~,~~; its middle lobe rounded, with a minute frontal head pore (Figs. 4C and 7D–E). In anterior view, distance between the center of ocellus and eye slightly greater (almost twice) than distance from the center of ocellus to the tip of rostrum (Fig. 4C). Dorsal head pores absent. Labrum large, distal labral plate with bunches of long setules, in ventral view labrum triangular, with lateral projections (Fig. 4D, 7D).

Valve with straight ventral margin (Figs. 4A, 5D and 7A–B). Adhesive ventral rim of valves modified into "sucker-plate" (in terms of *Dumont & Pensaert, 1983*), no setae along most part of the sucker length except few rarely located setae at anteriormost portion and several sparsely located setae at posterior portion near mucro (Figs. 5A–C). 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. 5D–E, 7B–C).

Thorax relatively long, abdomen short (Fig. 4A).

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is this feature different in any other Scapholeberis?

Postabdomen almost rectangular, postabdomen length/height ratio about 3 (Figs. 5F–G). 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. 5G–I). Postanal portion of postabdomen armed with long, thin solitary teeth and bunches of fine setules (Figs. 5G–H). 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. 5H–I). Its external side armed by three rows of small denticles, decreasing in size distally. Denticles in middle portion of claw are stronger and located more sparsely as compared to other denticles. Basal spine absent (Figs. 5H–I).

Antenna I jointed to the head surface, relatively short, antennular body with aesthetascs exceeds tip of rostrum in length (Figs. 5J–K, 7D–E). Antennular sensory seta slender, arising subdistally, almost equal in length to antennular body. Nine aesthetascs unequal in size (Figs. 5J–K, 7E). All aesthetasc tips projecting beyond tip of rostrum.

Antenna II relatively long (Figs. 4A, 5L–M, 7A). Antennal formula for setae: 0-0-1-3/1-1-3. Antennal formula for spines: 0-1-0-1/0-0-1. Coxal part folded, with two sensory setae. Basal segment elongated, covered by concentric rows of fine setules with a very thin spine between antenna II exopod and endopod branches on outer surface and a short bisegmented seta on outer surface (Figs. 5L–M). Branches relatively elongated, all segments cylindrical, covered by concentric rows of fine setules and tiny denticles around their distal margins. Apical setae typical for daphniids (as long as antennal branches), setulated asymmetrically. Lateral setae arising from basal and middle endopod segment long (reach tips of apical setae) (Fig. 5L). Lateral seta arising from third exopod segment thin and relatively short (reaches the middle of apical setae). Spine on the second exopod segment short and thin. Spines on apical segments of endopod and exopod branches very small and short, subequal in size to concentric apical denticles, arising from distal 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); endite 4 with a single anterior seta (2) and a single posterior (b) seta; endite 3 with a short

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possible „feathered“?

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 (Fig. 6C).

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It is a bit inconvenient that multiple setae are indicated by the same number in the figure but I suppose the reader sho is actually interested in limb moprhology will manage.

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

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

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

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do you mean something like „separating“?

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Furthermore, I do not think „represented“ is a good word.

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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.

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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 between all investigated individuals.

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Differential diagnosis. On head, from anterior view, distance between the center of ocellus and eye slightly greater (almost twice) than distance from the center of ocellus to the tip of rostrum. On thoracic limb I, the ratio between seta 1’ and seta 2 is almost 2.5 (i.e. seta 2 is relatively short). In ephippial females, from the dorsal view, area between two keels of ephippium strongly elongated, keels not projected laterally out of body dorsal contour.

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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 to the description of *King* (*1853*) rather than based on his own original material. It is an acceptable action according to the *ICZN* (*2000*). Specimens of this taxon from Australia are absent from the collection of G.O. Sars in the Zoological Museum of the Oslo University, Norway. King's specimens had a chance to be regarded as types of *S. kingii ICZN* (*2000*), but they are apparently lost.

Then *Sars* (*1903, p. 8–10, plate 1, figs 2, 2a, 2b*) proposed the name "*Scapholeberis Kingi*, G.O. Sars, n. sp. "for populations from Sumatra (unknown water bodies in "territories of Deli and Langkat" collected by Mr. Iversen) with the following explanation: "The above-described species is unquestionably identical with the Australian form recorded by King as *Daphnia mucronata*. It is certainly very nearly allied to the European species, but apparently specifically distinct, differing, as it does, not only in the much smaller size, but also in the shape of the head and in the less sharply angulated anterior part of the valves. The sculpture of the shell is, moreover, much coarser than in the European species". But, Sars' earlier species name "*S. kingii"* of Australia has precedence over the Sumatran species. The Sumatran specimens are present in the Collection of G.O. Sars (GOS F 9540, GOS F 12272, GOS F 12880). However, these specimens are not regarded as types because they were not reported in the original taxon description. According to the drawings of *Sars* (*plate 1, figs 2, 2a, 2b*), the specimens from Sumatra belong to the *S. kingii* group. Presently it is unknown if the populations from Sumatra

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belong to *S. kingii* s.str., *S. smirnovi* **sp.nov.**, or another taxon (~~if~~ tropical Asian populations are not revised here).

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Dumont & Pensaert (*1983*) correctly pointed out that *Dumont* (*1983*) erroneously stated that *S. kingi* Sars, 1888 ~~is~~ was a *nomen nudum* (and claimed that the species ~~must~~ should have been ~~be~~ named *S. kingi* Sars, 1903).

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Distribution. To date, we can confirm its presence in Australia only, where it is a common taxon (*Dumont, 1983; Smirnov, 1995; Shiel & Dickson, 1995*), but we cannot fully exclude the chance that there are several additional taxa within this group.

Records of *S. kingii* from Spain, Sicily and Central Europe have been declared dubious (*Alonso 1996; Marrone, Barone & Naselli-Flores, 2005; Hudec, 2010*), but members of the *S. kingii* species group (see below) were found to be common in Northern Africa (*Ghaouaci et al., 2018; Neretina, 2018*). In the Eastern Palearctic, the range of *S.* cf. *kingii* extends northwards, up to Japan (*Tanaka, 1998a; Tanaka, 1998b*), the Korean Peninsula (*Kotov, Jeong & Lee, 2012*) and the Russian side of the Amur River (=Heilong Jiang in Chinese) basin (*Kotov et al., 2011*). Therefore, the *S. kingii* species complex is regarded as a typical "tropicopolitan" taxon with a very wide geographic range in the Eastern Hemisphere.

2. *Scapholeberis intermedius* Daday, 1898

Figure 10

Scapholeberis mucronata var. intermedia Daday, 1898, p. 59–60, Fig. 29a–b.

? *Scapholeberis kingi* Sars in *Gurney, 1907*, p. 277–278; *Fernando, 1980*, p. 97; *Michael & Sharma, 1988*, p. 73–74, Fig. 20a–c; *Chatterjee et al., 2013*, p. 20–21.

Type locality. "Sümpfe der Umgebung des Kalawewa-Sees", Sri Lanka (*Daday, 1898*).

Type material (studied here). See Supplemental Table 2.

Brief redescription of museum material. Redescription. Parthenogenetic female. In 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 with small, its ventral side with small protuberance.

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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. kingii*-like species (see below). Morphological and genetic investigations of *kingii*-like populations from the type locality of *S. intermedius*, Sri Lanka (and South Asia as a whole) will be carried out in future studies. To date we have no suitable ethanol-fixed material of *S. kingii* with ephippial females from this area.

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3. *Scapholeberis* cf. *intermedius* Daday, 1898

Figures 11–15

? *Scapholeberis kingi* Sars in *Sars, 1916*, p. 314–315, Pl. XXXII: 3, 3a, 3f; *Brehm, 1937*, p. 489; *Gauthier, 1951*, p. 48–50, text-figure in p. 49, C–D; *Harding, 1961*, p. 40; *Rey & Saint-Jean, 1969*, p. 26, Fig. 5a–c; *Dumont & Van De Velde, 1977*, p. 80; *Dumont, Laureys & Pensaert, 1979*, p. 265, 267; *Day et al., 1999*, p. 97, Fig. 4.6.B.

Material studied here. See Supplemental Table 2.

Description. Parthenogenetic female (Figs. 11–15). In lateral view, body regularly elongated, dorsal margin broadly arched, ventral margin almost straight, maximum height at middle of body (body height/length ratio about 0.59 for adults, juveniles not studied) (Figs. 11A, 15A). In dorsal and ventral view body ovoid, only moderately compressed from sides. In anterior view body moderately compressed, dorsal keel absent. Head large with well developed rostrum

(Figs. 11A–B, 15A–C). Depression between head and rest of body absent, but dorsal contour may be slightly concave under compound eye and antenna. Posterodorsal and posteroventral angles expressed (Figs. 11A, E, 15A, D). Posterodorsal angle obtuse, posteroventral angle almost 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 with small, its ventral side with small protuberance (Figs. 11A, E, 15A, D). Valves with developed sculpture, consisting of polygons (Figs. 11E, 15D–E).

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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 is not studied. Labrum large (Fig. 11D). Distal labral plate with bunches of long setules.

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Valve with straight ventral margin (Figs. 11E, D). 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 in three times longer than anal margin. Anal and postanal margins almost equal in length. Basis of claws not inflated (Figs. 12I–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. 12I–J, 12A). Its external side armed by three rows of small denticles, deceasing in size distally. Basal spine absent (Figs. 12I–J, 12A).

Antenna I relatively short, antennular body with aesthetascs exceeds tip of rostrum in length (Fig. 10L). 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 via 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 of different length. Two ejector hooks almost similar in size.

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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 featured by 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.

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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.

Size. Medium-sized species, parthenogenetic female up to 0.70 mm in length without mucro (and 0.73 mm with mucro).

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

Differential diagnosis. On head, from 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. On thoracic limb I, the ratio between seta 1' and seta 2 is almost 1.5 (i.e. seta 2 is relatively long).

Other records in Africa. Distribution of *Scapholeberis* in Africa remains scarcely studied. Reliable records of *S. kingii* populations are known from West Africa (*Dumont, 1981; Egborge, Onwudinjo & Chigbu, 1994; Chiambeng & Dumont, 2005*), Central Africa (*Rey & Saint-Jean, 1969*), and South Africa (*Sars, 1916; Day et al., 1999*).

4. *Scapholeberis smirnovi* sp. nov.

Figures 16–20

Scapholeberis kingi Sars in *Uéno, 1940*, p. 342; *Tanaka, 1998a*, p. 30–31, Fig. 2A–C; *Tanaka, 1998b*: p. 15–16, Fig. 9–10; *Tanaka, Ohtaka & Nishino, 2004*, p. 173–174, Fig. 3; *Kotov et al., 2011*, p. 403, Table 1; *Kotov, Jeong & Lee, 2012*, p. 58, Fig. 5; *Jeong, Kotov & Lee, 2014*, p. 219.

? (at least partially) *Scapholeberis kingi* Sars *in Chiang & Du, 1973*, p. 145–146, Fig. 97a-c; in *Du Nan-shan, 1973*, p. 44, Fig. 13; *Xiang et al., 2015*, p. 13–14.

Scapholebeis mucronata (O.F. Müller) in *Uéno, 1927*, p. 281, Fig. 9 (not 9a–9e!);

Scapholeberis rammneri Dumont & Pensaert in *Yoon, 2010*, p. 64–66, Fig. 34.

Publication Zoobank ID. urn:lsid:zoobank.org:pub:A4A3415D-857E-42E5-9103-B8D48AC60832

Zoobank taxon ID. urn:lsid:zoobank.org:act:62ABBAFB-249D-453A-BB8D-E59ECB1AB2B0.

Etymology. The taxon is named after Professor Nikolai N. Smirnov, a renowned Russian zoologist and hydrobiologist, who established the Russian cladoceran school and made large advances in the study of freshwater zooplankton.

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Type locality. A puddle near Lake Maloe Utinoe (N 43.4127°, E 131.8214°), Primorski Territory, the Russian Far East.

Type material. Holotype: an ephippial female, fixed in 96% ethanol, deposited at the collection of Zoological Museum of Moscow State University, MGU Ml-189. The label of holotype is: "*Scapholeberis smirnovi* **sp. nov.**, 1 ephippial female from puddle near Lake Maloe Utinoe, Holotype". Paratypes. See Supplemental Table 2.

Description. Parthenogenetic female (Figs. 16A–F). In lateral view body relatively elongated, dorsal margin regularly arched, ventral margin almost straight, maximum height at body middle (body height/length ratio about 0.6 for adults and 0.5 for juveniles) (Figs. 16A and 16E, correspondingly). In dorsal or ventral view body ovoid, moderately compressed from sides (Fig. 16B). In anterior view body moderately compressed, dorsal keel absent. Posterodorsal angle obtuse, posteroventral angle almost straight, with a long spine (mucro) (Figs. 16A, E and 17A–E). A row of numerous small setules on inner face of posterior margin of valve (Fig. 16E). Ventral margin covered by setae of different size (Figs. 17A–D). Anteroventral angle of valve broadly rounded, its ventral portion with a small protuberance (Fig. 16A, E and 16B). Valves with well-developed sculpture of polygonal reticulation.

Head large for a daphniid (Fig. 16A). In lateral view head elongated, with a prominent rostrum, its distal portion roundish (Fig. 16A). In dorsal view head elongated, head shield with low lateral projections (fornices) ~~covered~~ covering bases of antennae II, a sclerotized ridge departs from 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. 16F). 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. 17A).

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 located more sparsely as compared to other denticles. Basal spine absent (Figs. 17G–I).

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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.

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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*.

Limb V (Fig. 18H) with a subovoid epipodite, triangular exopodite supplied with two small, thin distal setae (Fig. 18H: 1–2) unequal in length and a large lateral seta (Fig. 18H: 3). Inner limb portion as an ovoid flat lobe, with setulated inner margin and a single, large seta.

Ephippial female (Figs. 16G–I, 19A–B, D–F, 20A–L). Body shape in general as in parthenogenetic female. Dorsal portion of valves modified into ephippium. Ephippium dark brown, ovoid, clearly bordered from ventral and lateral portions of valves refusing during its casting off (Figs. 16G, 19A–B, 20A, D). Egg chamber with a single egg, elongated, its sculpture represented by small holes (Figs. 16G, 20F). Sculpture of the rest of ephippium is represented by small polygons. Lateral keels are well distinguishable from the lateral (Figs. 16G, 19A–B, 20A–D) and dorsal view (Figs. 16H, 19–E, 20G, I–L). From the dorsal view, area between two keels strongly rounded, keels strongly projected laterally out of body dorsal contour (Figs. 16H, 19D, 20G).

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Preephippial female (Figs. 19C). Body shape in general similar to that in parthenogenetic female. Lateral keels already visible (Figs. 19C), but dorsal portion of valves almost weakly chitinized. Ventral and lateral borders between preephippium and the rest of valves not developed.

Male. Despite significant efforts, we did not find males in the investigated samples.

Differential diagnosis. On head, from anterior view, distance between the center of ocellus and eye significantly greater (almost five times) than distance from the center of ocellus to the tip of rostrum. On thoracic limb I, the ratio between seta 1' and seta 2 is almost 2.5 (i.e. seta 2 is relatively short). In ephippial females, from the dorsal view, area between two keels of ephippium strongly rounded, keels strongly projected laterally out of body dorsal contour.

Taxonomic notes. Records of this "tropical" taxon in so northern territories as South Korea and Russian Far East cased a surprise for the cladoceran investigators (*Kotov, Jeong & Lee, 2012*), but now we know that the Far Eastern populations belong to a separate taxon, real distribution of which needs to be accurately evaluated. To date, we had no DNA-available samples of *S.* cf. *kingii* from SE Asia, South China and Indian subcontinent where ~~this~~ that taxon is ~~usual~~ common (*Michael & Sharma, 1988; Korovchinsky, 2013; Kotov et al., 2013; Sinev, Gu & Han, 2015*). Checking of the status of populations from different regions of the Palaeotropics is the next step in the revision of this group. To date, distribution of *S. smirnovi* **sp.nov.** could be regarded as similar to that in *Daphnia sinensis* (see *Popova et al., 2016*), but further studies are needed to confirm this hypothesis.

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

Commented [AP55]: what does this mean?
I think the word "alomost" is unnecessary.

Commented [AP56]: I am not sure these are "taxonomic notes", in fact – this is an assemblage of various discussion-like statements

Commented [AP57]: surely you do not refer to S. smirnovi – do you mean S. kingi? State it.

Commented [AP58]: this seems very unusual claim. D. sinensis extends from Far East to Subsaharan Africa, which is probably not the case of S. smirnovi. Below, you do not provide any evidence for such broad distribution.

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

Distribution. To date, this taxon is found in southern portion of Far East of Russia, Korean Peninsula, Japan and closest region of China (Dongbei = Manchuria). It has also been recorded from a single locality in southernmost portion of European Russia, but such population could appear due to an anthropogenic-mediated invasion. Exact distribution ranges of this taxon are unknown, see Discussion.

Commented [AP60]: you mention distribution also in the "taxonomic notes above" but some statements in the two sections are contradictory...

Discussion

Comparison of the COI and 12S+16S phylogenies

The COI-based analyses reveal that the large genetic divergences within and among species groups of neustonic daphniids exist for both rRNA and protein coding regions of the mitochondrial genome. However, the pattern disparity between neustonic daphniids and *Daphnia* is greatest for within species/species group variation. *Costa et al.* (*2007*) reported a 1.32 % average divergence within species of *Daphnia* and a maximum divergence of 4.3% (30.65% was found within the genus *Daphnia*). Geographic clades within named species of *Scapholeberis* are often beyond 20% in divergence. The strong divergence extends beyond the existence of cryptic species on separate continents (as in the *S. kingii* complex). The pattern is consistent with evolutionary rate differences among genera of daphniids. Note that the COI data showed similar levels of within genus variation regardless of genus at just over 30%, while the rRNA genes show greater divergences within neustonic genera compared to those from other cladoceran

Commented [AP61]: refer here to the Taylor et al. paper?

Commented [AP62]: state more clearly where the large divergence is observed? The reader may not remember it.

Commented [AP63]: this is a very vague statement – be more specific, and/or provide references

Commented [AP64]: what do you mean? that the maximum divergence within Daphnia and within Scapholeberis is comparable? Or within various genera of cladocerans? again, no reference is provided, and the statement is too vague genera (*Taylor, Connelly & Kotov, 2020*). This outcome is expected for rate increases in COI because the gene is prone to strong purifying selection resulting in substitutional saturation.

Commented [AP65]: reference?

The COI based tree (Fig. 2) is similar to tree estimated from 16S+12S rRNA sequences (*Taylor, Connelly & Kotov, 2020*). The major groups in both trees are the same, while the grouping of the deep branches is different. But, as the deep branches for COI have low support, the discrepancies may be due to random error.

Commented [AP66]: I would not call this "random" – there is simply insufficient information, and the topology is not supported.

The *mucronata* group is well-supported in both trees, in each tree the group is represented by four main clades. Presently, we cannot ascertain if clade D from *Taylor, Connelly & Kotov, 2020* is identical to the COI clade X. The present study does confirm that the *mucronata*-group (clade X) is present in non-Beringian North America.

Commented [AP67]: it would be good to state why – I suppose you do not have data for both genes (would it be so difficult to get them, by the way?). Do you have any opinion whether this match is likely or not?

All clades from the *rammneri* group represented in the rRNA tree (*Taylor, Connelly & Kotov, 2020*) are also present in the COI tree (Fig. 2). New biogeographic information includes: (1) Clade H penetrates further north in the Nearctic (though not beyond the boreal zone); (2) there is a previously unknown clade Y in Israel; (3) the grouping of clade I (which is also basal in the rRNA tree) with other clades is not well-supported in the COI tree.

Commented [AP68]: this is not a biogeographic information

The present study has much improved the geographic sampling of the S. freyi group compared to our rRNA tree (this is largely due to the inclusion of sequences from previous DNA barcoding projects). It is clear from the present results that *S. freyi* is indeed a diverse clade with many closely related, but geographically differentiated phylogroups in the New World.

There is a new genetic clade within the *S. kingii* species group, *S.* cf. *intermedius* (clade L2) (Figs 1–2) which was not sampled in the rRNA study. Therefore, the *S. kingii* group is more complicated as it was expected before. In our COI tree, *S. armata* (clade N) grouped with *S.* cf. *microcephala* (clade E) (Fig. 2), but they are distant branches on the rRNA tree. The source of

Commented [AP69]: diverse?

Commented [AP70]: this is not surprising, though – an in-depth genetic study of any as widespread cladoceran taxon as S. kingi s.l. is likely to reveal some additional diversity.

the incongruence is unknown but such discrepancies are common with long branches and short internodes (see *Omilian & Taylor, 2001; Bergstren, 2005*).

Finally, the *Megafenestra* internal tree structure is different from that in rRNA tree, as the clade P is sister group of Q in the COI tree and O – in the rRNA tree.

The COI tree fully confirms existence of several clades with trans-Beringian distribution ranges among the Scapholeberinae as it was suggested by *Taylor, Connelly & Kotov, 2020*.

Commented [AP71]: this is a king of "orphan" statement, with no connection to other sentences.
To avoid this, you may consider first to describe the key consistent patterns between the two studies, and then go to details. (In principle, both trees being based on mtDNA markers, they should share lots of features.)

De-coding of the DNA barcoding results

Before our study, 48 COI sequences were deposited to GenBank: *De Waard et al.* (*2006*) (1 sequence); *Richter, Olesen & Wheeler* (*2007*) (1); *Elías-Gutiérrez et al.* (*2008*) (6); *Jeffrey, Elías-Gutiérrez & Adamowicz* (2011) (2); *Elías-Gutiérrez & León-Regagnon* (*2013*) (3); *Prosser, Martínez-Arce & Elías-Gutiérrez.* (*2013*) (2); *Yang et al.* (2017) (1); *Elias-Gutierrez et al.* (*2018*) (14), and 20 sequences as direct submissions, including the iBOL releases. Because the taxonomy of the Scapholeberinae is immature, identifications of the taxa by authors of these data were tentative (Fig. 21), only 30% of taxa were identified to species group accurately, while others were misidentified or identified to the genus level. In some publications, species were 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.

Commented [AP72]: I do not think this figure is needed. One or two sentences of the text will deliver the same message.

An exclusively genetic approach to understand diversity and taxonomy is limited 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

Commented [AP73]: these are two very different aspects – in fact, genetic analyses may provide a reasonable insight into diversity of a given group but the patterns may lack proper taxonomic interpretation.

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, genetic approaches may yield very different diversity results from morphological or nuclear genomic evidence.

Commented [AP74]: provide reference

Commented [AP75]: how does "nuclear genomic evidence" differ from "genetic approaches"? Are there any data about the rate of evolution of nuclear genomes in neustonic daphniids, in comparison with mtDNA and with other cladoceran taxa?

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 is the result of human-mediated introduction (see also *Taylor, Connelly & Kotov, 2020*). The European population was used for a genomic study and identified as "*S. mucronata*".

Commented [AP76]: do you mean that only genetic data of this group are available for those regions, or that no other Scapholeberis group was reported from there?

Commented [AP77]: provide reference for the genomic study in question

(2) *S. duranguensis* is a member of a large group, namely the *S. freyi* species group. It is not micro-endemic of a single locality in Durango State, but also present in the mountains of Aguascalientes State.

(3) Members of the *S. mucronata* group (namely clade X) are present in non-Beringian North America, but only in its northernmost (Arctic) portion.

Commented [AP78]: can you be sure of this? The fact that you do not have genetic data from elsewhere may not mean that the group is as restricted.

(4) A new lineage (most probably, a separate biological species) of the *rammneri* group is present in Israel.

(5) In contrast to our previous opinion (*Taylor, Connelly & Kotov, 2020*) representatives of the American clade H of the *rammneri* group are found in the Beringian zone (although they probably do not extend beyond the boreal zone in Alaska).

The information from "genetic barcoding" allows us to improve the biogeography of neustonic daphniids, but only after integrating this information with morphological and other genetic data (*Schlick-Steiner et al., 2010*).

Taxonomy

Presently, there are two species within the genus *Megafenestra* (*Dumont & Pensaert, 1983*): *M. aurita* (Fischer, 1849) and *M. nasuta* (Birge, 1879), and nine valid species within the genus *Scapholeberis*: (1) *S. mucronata* (O.F. Müller, 1776); (2) *S. spinifera* (Nicolet, 1849); (3) *S. armata* Herrick, 1882; (4) *S. kingi* Sars, 1888; (5) *S. microcephala* Sars, 1890; (6) *S. erinaceus* Daday, 1903; (7) *S. rammneri* Dumont & Pensaert, 1983; (8) *S. freyi* Dumont & Pensaert, 1983; (9) *S. duranguensis* Quiroz-Vázquez & Elías-Gutiérrez, 2009 (see *Dumont & Pensaert, 1983; Quiroz-Vázquez & Elías-Gutiérrez, 2009*). But at least four "species" from this list (*S. kingi, S. microcephala, S. mucronata, S. rammneri*) could be considered as species groups rather than separate species due to their very broad ranges both in the Eastern and Western Hemispheres. Such taxa need careful taxonomic revisions according to the logic of recent "non-

Commented [AP79]: With this paper, S. smirnovi becomes valid as well. The word "presently" at the beginning the sentence is thus misleading.

Commented [AP80]: this argument is partly flawed. The wide range itself cannot be a reason for claiming something is a species group. It is an indication that the taxon requires attention, especially if spanning multiple biogeographic regions, and exhibiting some phenotypic variation. However, we do have cladocerans that have conspecific populations across unusually broad ranges (for example, Daphnia sinensis cited above, or D. magna).

Commented [AP81]: I do not think we can call this approach recent any more.

cosmopolitanism" and "continental endemism" approach (*Frey, 1982; Frey, 1987*) widely accepted in the cladoceran taxonomy and biogeography.

After two subsequent revisions (*Taylor, Connelly & Kotov, 2020*; this study) we know that the diversity of both genera has been strongly underestimated. The subfamily includes at least 23–24 distinct lineages (note that rare *S. erinaceus* was not studied either here or by *Taylor, Connelly & Kotov* (*2020*). In contrast to many other cladoceran groups, we can confidently say that the phylogeny and taxonomy of *Scapholeberinae* is now relatively well-done. Main species groups correspond well to those separated based on the morphological analysis. But it is very obvious that further studies are necessary to find morphological differences between revealed taxa and formulate diagnoses of the taxa which needs to be formally described (as *Megafenestra* cf. *nasuta* clade P, *Scapholeberis* cf. *microcephala* clade E., *S.* cf. *rammneri* clades I, and possibly other un-named clades).

Commented [AP82]: I would say the phylogeny is relatively well resolved but the revision of the taxonomy only starts with this contribution. After all, from all the lineages discovered up to now (and there are surely more to be found), most remain unnamed and phenotypically not characterised.

~~Concludions~~Conclusions

To date we do not know if these taxa are morphologically different from congeneric taxa. But it is very premature to discuss "lacking of resolution" of morphology and the "limitations inherent in morphology-based identification system" (*Hebert et al., 2003*: *p. 313*), as nobody tried to find such differences. Such a search is time-consuming and requires expertise – but such this work is necessary to move the taxonomy of this group forward.

Commented [AP83]: You are whipping a dead horse here – the concept of integrative taxonomy is well established at the moment, and we all know that the claims in the early papers introducing the molecular barcoding were not appropriate.
This whole paragraph in fact brings very little information. Criticizing 17 years old paper that had indeed premature claims is no longer relevant.

We can immediately recommend the main direction of such studies: gamogenetic specimens must be analyzed for diagnostic characters first, as we did for the *S. kingii* species group. We can assume, following ideas of *Goulden* (*1966*), that differences in the ephippial

Commented [AP84]: What about laboratory induction of males (which are apparently rare in natural samples) by methyl farnesoate? Would you recommend trying this approach?

morphology could provide a mechanism of reproductive isolation, as such differences could be used by male during the copulation to recognize correct mate. Lateral keels on the ephippium, characteristic of several, if not all, taxa of *Scapholeberis*, are analogous to the keels in Bosminidae (*Kotov, 2013*). *Kerfoot & Peterson* (*1980*) proposed that the lateral keels and special texture on the ephippium of *Bosmina* also contribute to pre-zygotic reproductive isolation. We believe that differences between *Scapholeberis* ephippial females could also contribute to reproductive isolation among congeneric species. Moreover, the situation with *Scapholeberis kingii* and *S. smirnovi* **sp.nov.**, when parthenogenetic females are morphologically indistinguishable, but gamogenetic specimens have morphological differences, are usual among the cladocerans (*Belyaeva & Taylor, 2009; Popova et al., 2016; Smirnov & Kotov, 2018*). Such phenomena need further study to be accurately explained, but it is obvious that the morphological evolution in parthenogenetic and gamogenetic specimens follow somewhat different pathways. And the oft-reported morphological stasis in cladocerans (*Sacherová & Hebert, 2003; Smirnov & Kotov, 2018*) is more characteristic of parthenogenetic females (the sexual stages appear to evolve more rapidly in morphology).

Commented [AP85]: That is obvious indeed – if there is any selection on specific features related to sexual reproduction, these may not have to be expressed in parthenogenetic specimens.

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Captions

Figure 1. Map of populations of *Scapholeberis* and *Megafenestra* studied here. 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 in contrast to Taylor et al. (2020).

Commented [AP86]: populations studied genetically?

Possibly specifically state here that these are populations from which new sequence data were obtained. In such case, the last sentence may be modified, just noting that the symbols are the same as in Taylor et al. to facilitate comparison.
(In fact, the figures are only superficially similar to that paper.)

Commented [AP87]: better "in addition to those in Taylor et al."

but see comment above

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), genus *Megafenestra* (white). The tree is outgroup rooted using sequences from the genus *Megafenestra*. See Appendix S1 for individual sequences.

Commented [AP88]: You mention most colours but not the blue ones. They may not be the "major species group" but the caption might be modified.

I think vertical lines indicating all branches belonging to that particular species group (including those that are not in colour) would be helpful.

Commented [AP89]: outgroup-rooted?

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.

Commented [AP90]: see my general comment to violin plots.

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.

Commented [AP91]: I find this figure unnecessary

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.