

New records the genus *Schizopera* Sars, 1905 (Copepoda: Harpacticoida) species in Russia, with their molecular and morphological affinities

Новые находки видов *Schizopera* Sars, 1905 (Copepoda: Harpacticoida) на территории России, их генетические и морфологические особенности

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KEY WORDS: inland waters, brackish-water lake, aquarium fauna, DNA-barcoding, Russia, Copepoda, Harpacticoida, Miraciidae, Diosaccinae, *Schizopera*.

КЛЮЧЕВЫЕ СЛОВА: внутренние водоемы, солоноватоводное озеро, аквариумная фауна, ДНК-баркодинг, Россия, Copepoda, Harpacticoida, Miraciidae, Diosaccinae, *Schizopera*.

ABSTRACT. Two species of harpacticoids of the genus *Schizopera* Sars, 1905 were found in brackish lake in Chelyabinsk city and an aquarium in the exposition of the Museum-Aquarium of Papanin Institute for Biology of Inland Waters Russian Academy of Sciences (Borok Settlement, Yaroslavl region). Brief illustrated morphological descriptions of the females and males of both species: *S. neglecta* Akatova, 1935 and *S. samchunensis* Karaytuğ et Sak, 2005 are given, for the first time data on molecular-genetic barcodes (sequences of the mtDNA COI gene) of these species are provided. A key for the identification of Eurasian *Schizopera* species is presented.

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РЕЗЮМЕ. Два вида гарпактикоид рода *Schizopera* Sars, 1905 обнаружены в солоноватоводном озере в городе Челябинск и аквариуме из экспозиции Музея-аквариума Института биологии внутренних вод им. И.Д. Папанина РАН (пос. Борок Ярославской области). Приведены краткие иллюстрированные описания строения самки и самца обоих видов: *S. neglecta* Akatova, 1935 и *S. samchunensis* Karaytuğ et Sak, 2005. Впервые для этих видов предоставлены

данные по молекулярно-генетическим баркодам (последовательностям гена мтДНК COI). Также создан ключ для определения видов *Schizopera*, обитающих в Евразии.

Introduction

The genus *Schizopera* Sars, 1905 is distributed around the world. Totally 103 species of the genus are known (World of Copepods, <https://www.marinespecies.org/copepoda/aphia.php?p=taxdetails&id=115325>). Except seas they occur in a variety of brackish and freshwater inland water habitats [Lang, 1948; Borutsky, 1952]; lakes [Manuylova, 1966; Karaytuğ, Sak, 2005; Mirabdullayev, Ginatullina, 2007; Karanovic *et al.*, 2015; Connolly *et al.*, 2022], the subterranean [Pesce, 1988; Karanovic, Cooper, 2012; Karanovic, McRae, 2013; Turbanov *et al.*, 2016; Decu *et al.*, 2019; Watiroyram *et al.*, 2021], interstitial waters (i.e. Karanovic *et al.* [2015]). One of the species (*S. knabei* Lang, 1965) was noted from an artificial water-body (culture) somewhere in the USA [Karanovic *et al.*, 2015].

In Russia *Schizopera* were noted from marine and brackish-water habitats only: the White [Kornev, Chertoprud, 2008], Black, and Caspian [Borutsky, 1952; Alekseev, 2015; Chertoprud *et al.*, 2017] Seas, highly mineralized rivers in Volgograd region [Gusakov, 2019].

The monophyly of the genus *Schizopera* must be established by a complete revision [Karaytuğ, Sak, 2005]. It includes many morphological close species, validity of which is nevertheless confirmed by the molecular-genetics methods. To date, information on the structure of barcodes of 16 *Schizopera* species from the USA, Japan, Australia is presented in datasets GenBank and BOLDSYSTEMS and analyzed [Karanovic, Cooper, 2012; Karanovic, McRae, 2013; Karanovic *et al.*, 2015; Connolly *et al.*, 2022].

The main purpose of our work was to provide information on the morphological structure and structure of a DNA-marker of two *Schizopera* species recoded in Russia: lake of the South Urals and an aquarium.

Material and methods

Harpacticoid specimens were obtained from samples collected in 2021 from Lake Smolino (Chelyabinsk city) and in 2024 from an aquarium at Papanin Institute for Biology of Inland Waters Russian Academy of Sciences (Borok, Necouz Region, Yaroslavl Oblast).

Lake Smolino is located in the city, surrounded by residential and garden areas. At present its water regime and water chemistry is shaped by the influence of industrial wastewater and artificial water reduction: over the past 150 years, the lake has been transformed from a salty into a brackish water-body with a water mineralization of 1.2–1.8 g L⁻¹ [Isimetova, Shundeev, 2019]. The area of the lake is about 25.5 km², the average depth is 4.14 m.

Eight aquariums of the Museum-Aquarium of Papanin Institute for Biology of Inland Waters Russian Academy of Sciences were studied, but only one from them was inhabited by harpacticoid copepods. It was a 300 L glass aquarium with water temperature 24–25 °C, water conductivity 250 µS/cm. The aquarium is replenished with the tap water. This artificial water-body was inhabited by different plants and fishes *Kryptopterus bicirrhus* (Valenciennes, 1840) fed with dry food. In addition to harpacticoids, cyclopoids, ostracods, mollusks, flatworms, oligochaetes, and others invertebrates have been found in this aquarium.

In the laboratory living harpacticoids were sorted, fixed in 96% ethanol and stored at –20 °C. For morphological analysis we dissected harpacticoids and mounted in glycerin. Specimens were drawn using a Leica DM 4000B microscope compound microscope equipped at magnifications of 1000x. Congo Red staining and fluorescence microscopy were used to image the harpacticoids following Michels & Büntzow [2010]. Images of individuals were obtained using Nexcope NIB950FL-DIC v2.

Identification of harpacticoids from Lake Smolino and the aquarium at the species level was carried out using keys by K. Lang [1948] and J.B.J. Wells [2007]; publications on taxonomy of the genus *Schizopera* [Karaytuğ, Sak, 2005; Mirabdullayev, Ginatullina, 2007; Gomez, Vargas-Arriaga, 2008; Kornev, Chertoprud, 2008; Karanovic, Cooper, 2012; Karanovic, McRae, 2013; Karanovic *et al.*, 2015; Karanovic, Cho, 2016; Watirogram *et al.*, 2021] were also taken into account. The morphological species description is given for fixed individuals. Descriptive terminology (numbering of caudal setae, designation of swimming legs) follows [Huys, Boxshall, 1991]. The armature formula of swimming legs is given according to [Lang, 1934]. Abbreviations used in the text: P1–P6 — legs 1–6, Exp-1–Exp-3 — first–third segments of exopods, Enp-1–Enp-3 — first–third segments of endopods.

All morphological material is stored in the Department of Animals Ecology of the Institute of Biology of Komi Scientific Centre of the Ural Branch of the Russian Academy of Sciences (Syktyvkar).

For molecular genetic analysis, we used four *Schizopera* specimens from Lake Smolino and six specimens from the aquarium. A gene fragment of the first subunit of mitochondrial DNA cytochrome oxidase (COI) was analysed. The DNA extraction and sequencing were performed at the CCU “Molecular Biology” of the Institute of Biology of Komi Scientific Centre of the Ural Branch of the Russian Academy of Sciences using protocol described in Kochanova *et al.* [2018]. The COI gene was amplified using primers: H2198-COI (‘TAAACTTCAGGGTGACCAAAAAATCA’), L1490-COI (‘GGTCAACAAATCATAAAGATATTGG’) [Folmer *et al.*, 1994], and L1384-COI GGTCAATGTAATCATAAAGATATTGG [Machida *et al.*, 2004].

Sequencing was carried out in both directions, using the BigDye Terminator v3.1 (Life Technology) reagent kit in an ABI PRISM 310 Genetic Analyzer (Applied Biosystems, Waltham, Massachusetts, USA) in the Syntol Company (Moscow), and using reagents set Ready Reaction Mix (Life Technology) on the genetic analyzer NANOFOR 05 (Russia) in the CCU “Molecular Biology”. Obtained nucleotide sequences were deposited at the NCBI GenBank database.

Phylogenetic analyses of the COI sequence data were conducted using the MEGA 10 model: GTR+G+I [Kumar *et al.*, 2018]. The analysis involved 58 nucleotide sequences (original and from NCBI GenBank and BOLDSYSTEMS databases). Codon positions included were 1st+2nd+3rd+Noncoding. There were a total of 509 positions in the final dataset. A maximum likelihood (ML) phylogenetic tree was created using the GTR+G+I model using 500 bootstrap pseudoreplicates. Next, data were handled in the iTOL v7 program (<https://itol.embl.de/>). For visualization of results, some sequences from NCBI GenBank and BOLDSYSTEMS databases were excluded from the final phylogenetic tree. Estimates of Evolutionary Divergence between Sequences were conducted using the Kimura 2-parameter model [Kimura, 1980]. Codon positions included were 1st+2nd+3rd+Noncoding. All ambiguous positions were removed for each sequence pair (pairwise deletion option).

Results

Subclass Copepoda Milne Edwards, 1840
 Superorder Podoplea Giesbrecht, 1882
 Order Harpacticoida Sars, 1903
 Family Miraciidae Dana, 1846
 Subfamily Diosaccinae Sars G.O., 1906
 Genus *Schizopera* Sars G.O., 1905
Schizopera neglecta Akatova, 1935
 Figs 1–3.

Schizopera neglecta: Akatova, 1935: 324, figs 13–18; Lang, 1948: 751, fig. 305; Apostolov, 1973: 33, fig. 2; Chertoprud, Kornev, 2005: 281; Mirabdullayev, Ginatullina, 2007: 313; Wells, 2007: 558; Alekseev, 2015: 179, fig. 110; Karanovic, Cho, 2016: 2; Chertoprud *et al.*, 2017: 3.

Schizopera (s.str.) *neglecta*: Apostolov, Marinov, 1988: 194, fig. 74.

MATERIAL. RUSSIA • 9 ♀♀ and 1 ♂ dissected on seven slides; Chelyabinsk city, Lake Smolino; approx. 55.088 N, 61.438 E; 31 August 2021; M.A. Baturina leg; ZMIB: HRP-102 – HRP-109.

DESCRIPTION. **Female.** Body more or less cylindrical, gradually tapering posteriorly (Fig. 1A–C). Length from tip of rostrum to distal margin of caudal rami about 500 µm.

Cephalothorax width about 130 µm. Nuchal organs absent (Fig. 1A, C). Rostrum (Figs 1A; 2C, D) separated from cepha-



Fig. 1. Fluorescence micrographs of female of *Schizopera neglecta* from Smolino Lake (A–C) and *Schizopera samchunensis* from the aquarium (D–F). A, D — dorsal view; B, E — ventral view; C, F — lateral view.

Рис. 1. Сделанные с помощью флуоресцентного микроскопа фотографии *Schizopera neglecta* из оз. Смолино (A–C) и *Schizopera samchunensis* (D–F) из аквариума. А, D — дорсально; В, Е — вентрально; С, F — латерально.

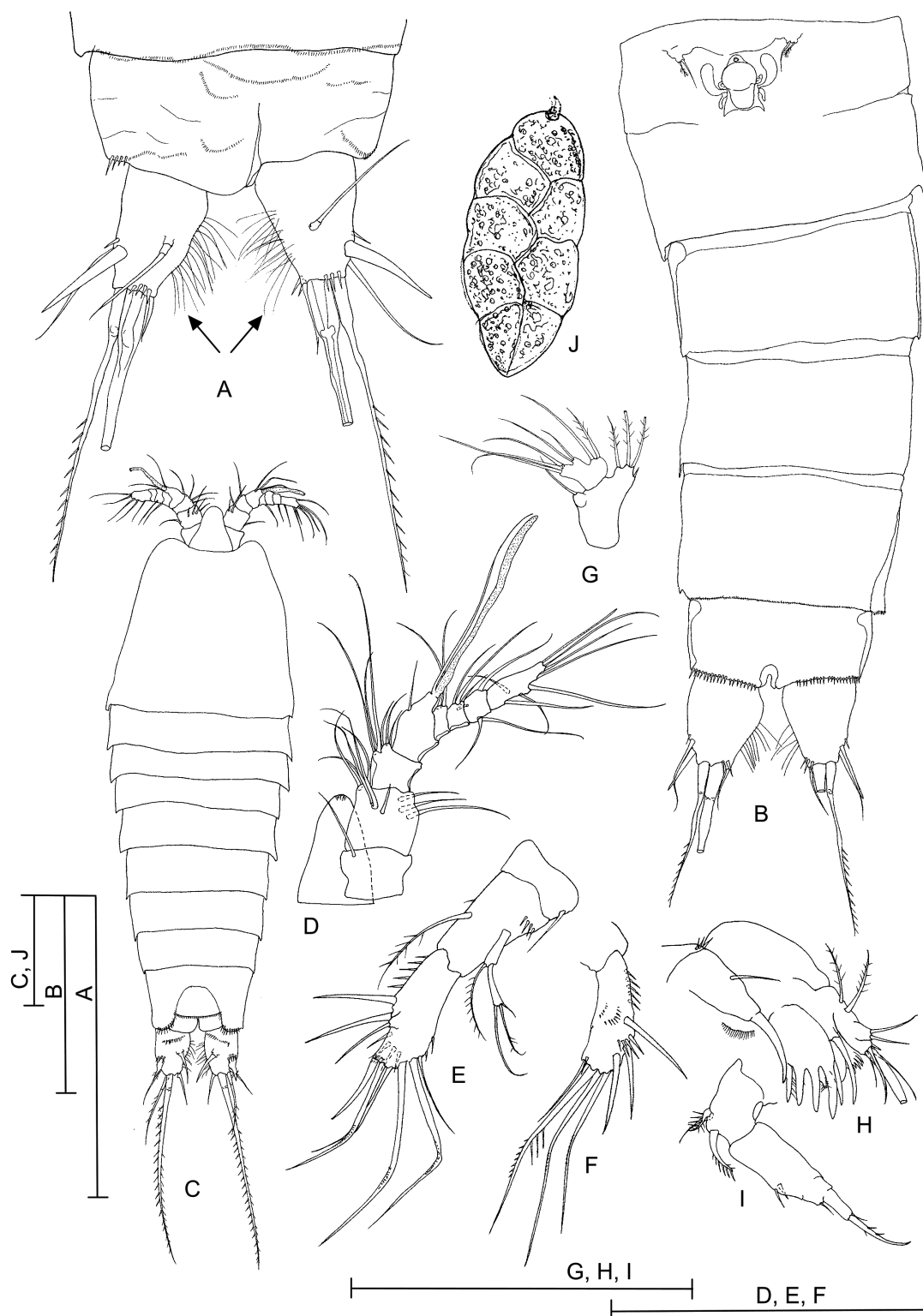


Fig. 2. *Schizopera neglecta*. ♀: A — caudal rami, dorsal view; B — abdomen, ventral view; C — habitus of copepodite V, dorsal view; D — rostrum and A1; E — A2; F — distal segment (endopod) of A2; G — mandible palp (mandible exopod); H — maxillule; I — maxilliped. Hereinafter, in Figs 3, 4, some characteristics are indicated by arrows (see text). Scale bar: 100 μ m.

Рис. 2. *Schizopera neglecta*. ♀: A — каудальные ветви, дорсально; B — abdomen, вентрально; C — общий вид копепода V, дорсально; D — роstrum и A1; E — A2; F — дистальный сегмент эндоподита A2; G — пальпа мандибулы (экзоподит мандибулы); H — максиллula; I — максиллярная ножка. Здесь и далее (рис. 3, 4) стрелками показаны некоторые признаки (см. текст). Масштаб: 100 μ m.

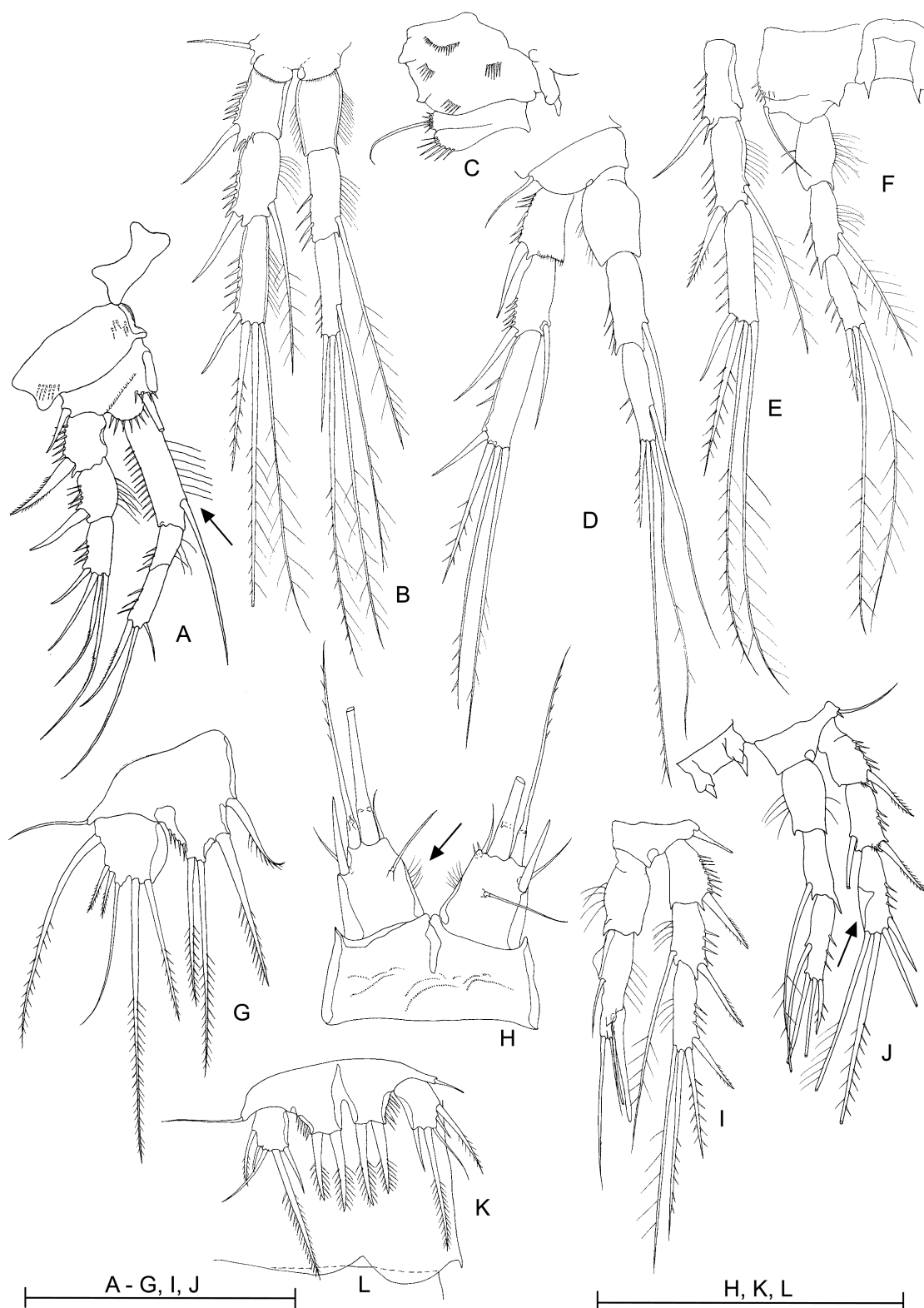


Fig. 3. *Schizopera neglecta*. ♀ (A-G), ♂ (H-L): A — P1; B, I — P2; C — P3 coxa and basis; D, J — P3; E — P4 exopod; F — P4 endopod; H — caudal rami, dorsal view; G, K — P5; L — P6. Scale bar: 100 μ m.

Рис. 3. *Schizopera neglecta*. ♀ (A-G), ♂ (H-L): A — P1; B, I — P2; C — P3 кокса и базис; D, J — P3; E — экзоподит P4; F — эндоподит P4; H — каудальные ветви, дорсально; G, K — P5; L — P6. Масштаб: 100 μ m.

lothorax, about twice as long as wide. Genital field as figured (Fig. 2B). Sixth leg represented by two setae. Egg sacs two (Fig. 1B) with 6–10 large eggs in each sac (Fig. 2J). Anal operculum is missing in mature individuals (Fig. 2A) and rounded in copepodites (Fig. 2C).

Caudal rami (Fig. 2A–B) conical, 1.2–1.6 times as long as width in medium part of ramus, with a group of sensillae row on the inner margins (shown by arrows in Fig. 2A); furnished with six setae. Seta I absent, seta III spiniform, setae IV and V well developed.

Antennule (Fig. 2D) short, 8-segmented. Acrothek on the fourth segment extends the tip of last segment. Armature formula: 1–[1], 2–[9], 3–[6], 4–[1 + 1 + ae], 5–[2], 6–[4], 7–[3], 8–[7 + acrothek].

Antenna endopod (Fig. 2E–F) one-segmented with 9 setae and rows of spinules on integument. Antenna exopod (Fig. 2E) two-segmented with one pinnate seta on the first segment and three setae on the second segment, from which external seta very small.

Mandible palp (Fig. 2G) biramous; basis with three pinnate setae; exopod small; endopod with 7 setae.

Maxillule (Fig. 2H), maxilla (not drawn), and maxilliped (Fig. 2I) common for the genus in structure.

P1 (Fig. 3A) with 3-segmented exopod and endopod. Exopod shorter than endopod, reaching the end of second endopodal segment, Exp-1 – Exp3 of about same length. Endopod with first segment slightly longer (in 1.2 times) than two first exopodal segments in sum (shown by arrow in Fig. 3A). The length of the endopodal segments is 55% (Enp-1), 17% (Enp-2), and 28% (Enp-3) of the length of P1 endopod.

P2–P4 (Fig. 3B–F) with 3-segmented exopod and endopod. Intercoxal sclerites with spinous protuberances at distal corners. Spine and seta formula as follows:

	Exopod	Endopod
P1	0; 0; 0, 2, 2	1; 0; 1, 1, 1
P2	0; 1; 0, 2, 2	0; 1; 1, 2, 1
P3	0; 1; 0, 2, 2	0; 1; 1, 2, 1
P4	0; 1; 0, 2, 2	0; 1; 0, 2, 1

P5 (Fig. 3G) biramous. Baseoendopod with outer basal seta and four pinnate setae on baseoendopodal lobe. Baseoendopodal lobe with spinules on inner margin. Exopod wide, square, with six setae, from which one unipinnate and five pinnate.

Male. Body length from tip of the rostrum to the caudal rami posterior margin 492 μ m. Cephalothorax width 128 μ m.

Caudal rami (Fig. 3H) about 1.3 times the width with a group of sensillae row on the inner margin (shown by arrow in Fig. 3H).

Sexual dimorphism in antennule, P1–P3, P5, P6.

Antennule (not drawn) 9-segmented with geniculations between fourth and fifth and seventh and eighth segments.

P1 (not drawn) as in female except for basis with a thick spiniform chitinous formation on inner edge.

P2 (Fig. 3I) exopod as in female, endopod two-segmented; second endopodal segment with inner unipinnate seta, three setae on the posterior margin, and strong fused naked spine on the outer side.

P3 (Fig. 3J) as in female except for Exp-3 with a hyaline broad spine instead of inner seta (shown by arrow in Fig. 3J).

P5 (Fig. 3K) medially fused; baseoendopodal lobe with two spines, exopod with five setae.

P6 (Fig. 3L) medially fused, without armatures.

DIAGNOSIS. From other species of the genus *S. neglecta* is distinguished in follow marks. Caudal rami conical, 1.2–1.6 times as long as width (longer in female than in male), with a group of sensillae on the inner margins in female and male.

Female P5 with six setae on exopod and four setae on baseoendopodal lobe. First endopodal segments of P2–P4 without setae. The length of first endopodal segment of P1 slightly longer than the common length of the first and second exopodal segments. The third endopodal segment of P1 in 1.5 times longer than the second endopodal segment. Egg sacs with 6–10 eggs each.

VARIABILITY. Morphological variability of *S. neglecta* of studied population was detected in the body size and eggs number in egg sacs of females.

ECOLOGY. The species was found by us in brackish-water lake of the South Trans-Urals together with other copepods: calanoid *Eurytemora velox* (Lilljeborg, 1853), harpacticoid *Onychocamptus mohammed* (Blanchard et Richard, 1891), and not-identified cyclopoids. In the population of *S. neglecta* investigated females dominated. About half of these females were with egg sacs.

Schizopera samchunensis Karaytuğ et Sak, 2005

Figs 1, 4, 5.

Schizopera samchunensis: Karaytuğ, Sak, 2005: 34, figs 1–4; Wells, 2007: 556.

MATERIAL. RUSSIA • 8 ♀♀ and 2 ♂♂ dissected on seven slides, 6 ♀♀ stored in a vessel; Borok, Necouz Region, Yaroslavl Oblast, aquarium; approx. 58.066° N, 38.238° E; water temperature: 24–25 °C, water conductivity: 250 μ S cm⁻¹; 25 October 2024; M.A. Golubev, A.S. Bakashkina leg; ZMIB: HRP-110 – HRP-117, HRP-129.

DESCRIPTION. Female. Body more or less cylindrical (Fig. 1D–F). Average length from tip of rostrum to distal margin of caudal rami 450 μ m (n = 11; 362–509 μ m). Cephalothorax width 100–105 μ m (n = 2). Nuchal organs absent (Fig. 1D, E).

Egg sacs two (Fig. 1E) with 4–5 large eggs in each.

Rostrum (Fig. 4D) large and elongate (length 40–60 μ m) with two delicate sensillae.

Genital field as figured (Fig. 4C). Sixth leg represented by two setae.

Anal operculum is missing in mature individuals (Fig. 4A).

Caudal rami (Fig. 4A, B) conical, about 2 times the width with a group of sensillae row on the inner margin (shown by arrow in Fig. 4A); furnished with six setae. Seta I absent, seta III spiniform, setae IV and V well developed.

Antennule (Fig. 4D) short, 8-segmented. Acrothek on the fourth segment extends the tip of last segment. Armature formula: 1–[1], 2–[9], 3–[6], 4–[1 + 1 + ae], 5–[2], 6–[4], 7–[3], 8–[5 + acrothek].

Antenna exopod (not drawn) two-segmented with one seta on the first segment and three setae on the second segment, from which external very small.

Mandible palp (Fig. 4E) biramous; basis with three setae; exopod small with one seta; endopod with 7 setae.

Maxillule, maxilla (not drawn), and maxilliped (Fig. 4F) common for the genus in structure.

P1 (Fig. 4G) with 3-segmented exopod and endopod. Exopod shorter than endopod, reaching the end of first endopodal segment, Exp-1 – Exp3 of about same length (shown by arrow in Fig. 4G). Endopod with first segment a bit longer than exopod. The length of the endopodal segments is 68% (Enp-1), 12% (Enp-2), and 20% (Enp-3) of the length of P1 endopod.

P2–P4 (Fig. 4H–J) with 3-segmented exopod and endopod. Intercoxal sclerites with spinous protuberances at distal corners. Spine and seta formula as follows:

	Exopod	Endopod
P1	0; 0; 0, 2, 2	1; 0; 1, 1, 1
P2	0; 1; 0, 2, 2	0; 1; 1, 2, 1
P3	0; 1; 0, 2, 2	1; 1; 1, 2, 1
P4	0; 1; 0, 2, 2	1; 1; 0, 2, 1

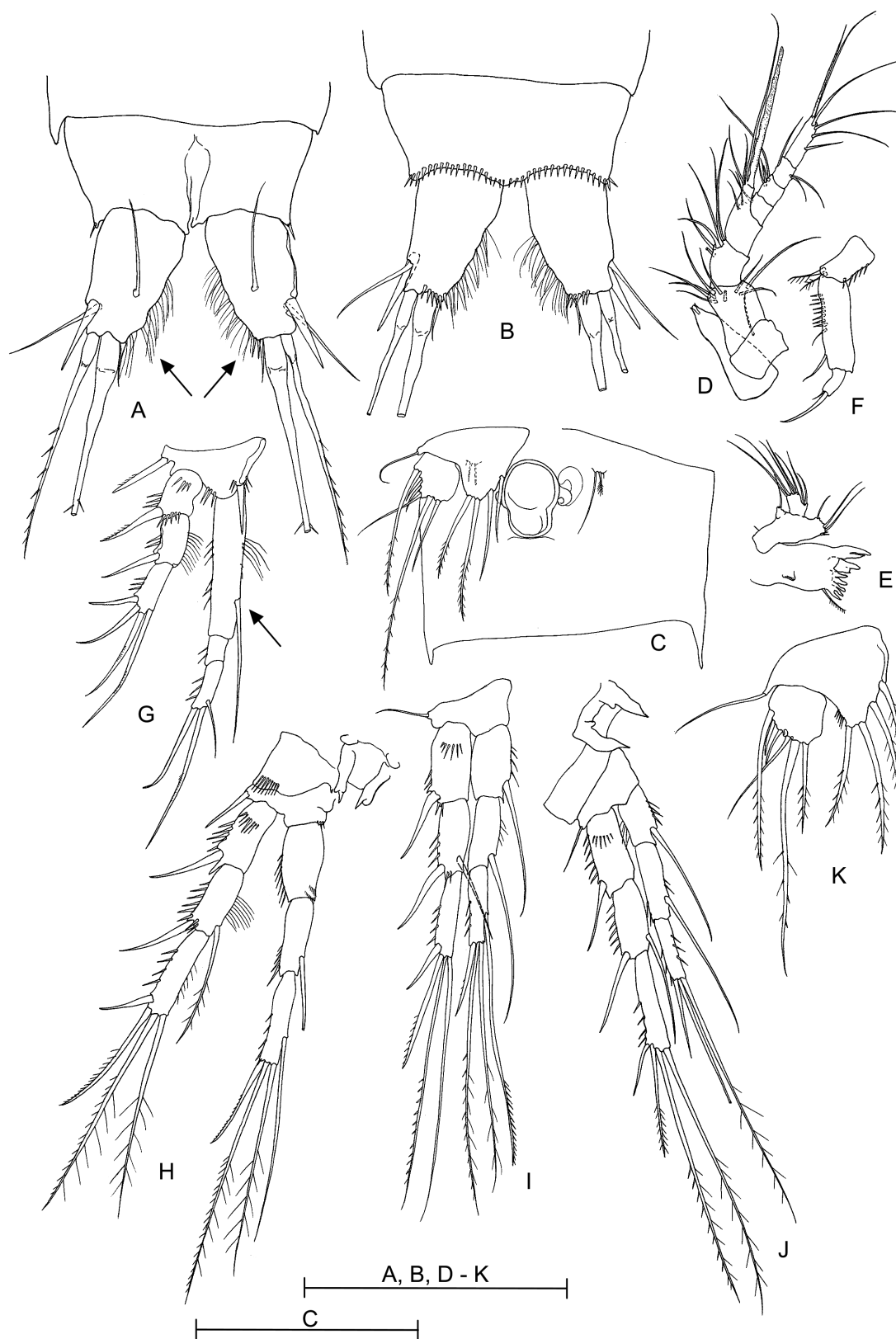


Fig. 4. *Schizopera samchunensis*, ♀: A — caudal rami, dorsal view; B — caudal rami, ventral view; C — genital somite, ventral view; D — rostrum and A1, dorsal view; E — mandible; F — maxilliped; G-K — P1-P5. Scale bar: 100 μm.

Рис. 4. *Schizopera samchunensis*, ♀: A — каудальные ветви, дорсально; B — каудальные ветви, вентрально; C — генитальный сегмент, вентрально; D — рострум и A1, дорсально; E — мандибула; F — максиллярная ножка; G-K — P1-P5. Масштаб: 100 μm.

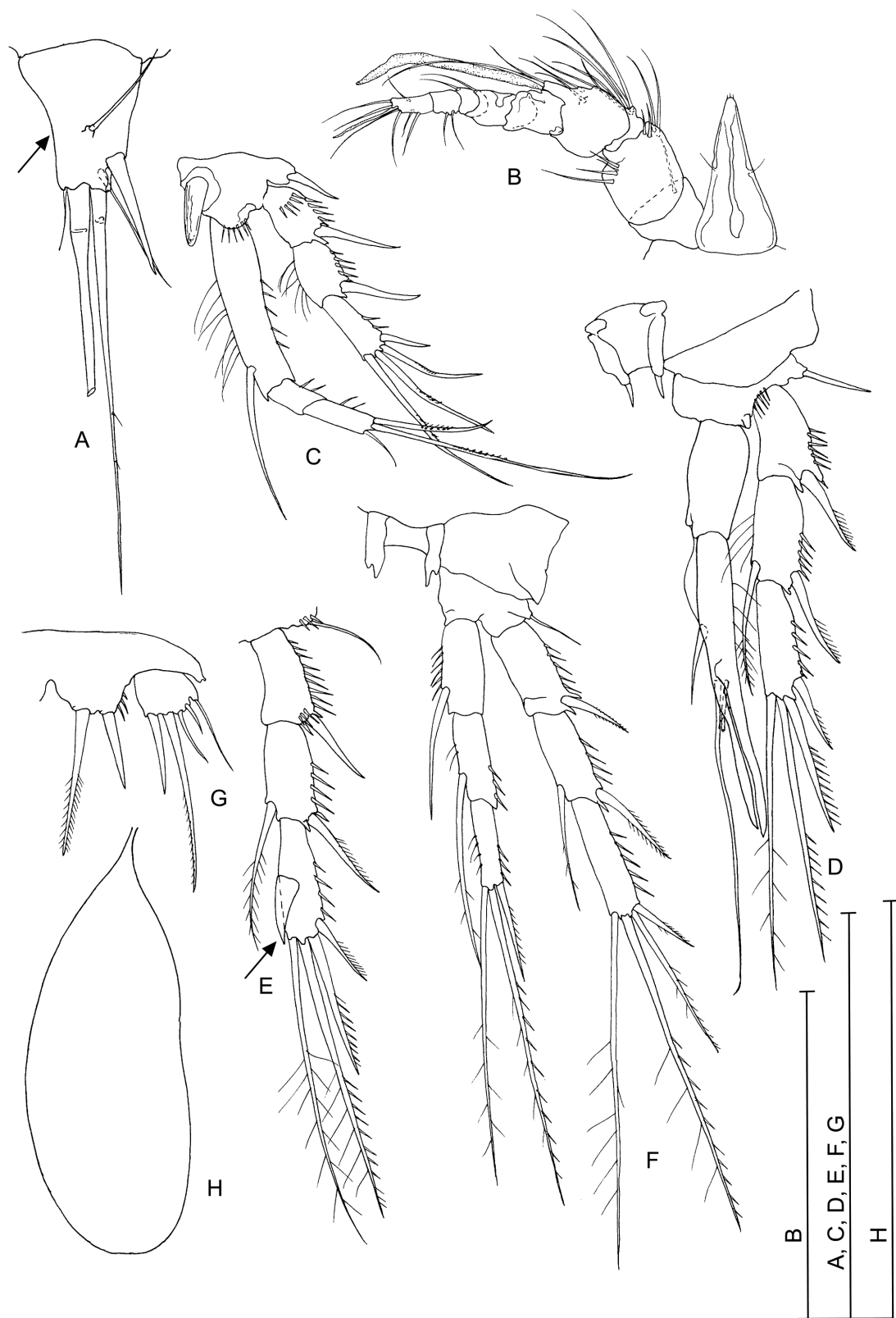


Fig. 5. *Schizopera samchunensis*, ♂: A — caudal ramus, dorsal view; B — rostrum and A1, ventral view; C, D — P1, P2; E — P3 exopod; F, G — P4, P5; H — spermatophore. Scale bar: 100 μm.

Fig. 5. *Schizopera samchunensis*, ♂: A — каудальные ветви, дорсально; B — рostrum и A1, вентрально; C, D — P1, P2; E — экзоподит P3; F, G — P4, P5; H — сперматофор. Масштаб: 100 μm.

P5 (Fig. 4K) biramous. Baseoendopod with outer basal seta and four pinnate setae on baseoendopodal lobe. Baseoendopodal lobe with spinules on inner margin. Exopod wide square, with six setae.

Male. Body length from tip of the rostrum to the caudal rami posterior margin 485 μ m. Cephalothorax width 100 μ m. Sexual dimorphism in caudal rami, antennule, P1–P3, P5, P6.

Spermatophore (Fig. 5H) large, about three times as long as wide.

Caudal rami (Fig. 5A) about 1.5 times the width without sensillae row on the inner margin (shown by arrow in Fig. 5A).

Rostrum (Fig. 5B) as in female; antennule 9-segmented with geniculations between fourth and fifth and seventh and eighth segments.

P1 (Fig. 5C) as in female except for basis with a thick spiniform chitinous formation on inner edge.

P2 (Fig. 5D) exopod as in female, endopod two-segmented; second endopodal segment with inner unipinnate seta, three setae on the posterior margin, and strong fused naked spine on the outer side.

P3 as in female except for Exp-3 (Fig. 5E) with a hyaline broad spine instead of inner seta (shown by arrow in Fig. 5E)).

P4 (Fig. 5F) as in female.

P5 (Fig. 5G) medially fused; baseoendopodal lobe with two spines of which inner spine in two times shorter than outer sine; exopod wide and short with five setae.

DIAGNOSIS. From other species of the genus *S. samchunensis* is distinguished in follow marks. Caudal rami conical, elongated, 1.5–2.1 times as long as width (longer in female than in male). Female caudal rami with a group of sensillae row on the inner margins, male caudal rami can be without sensillae on the inner margins. Female P5 with six setae on exopod and four setae on baseoendopodal lobe. The first endopodal segment of P2 without inner seta, the first endopodal segments of P3, P4 with seta on the inner side. The first endopodal segment of P1 length same or slightly longer than exopod. The third endopodal segment of P1 in about 2 times longer than the second endopodal segment. Egg sacs with 4–5 eggs each.

VARIABILITY. One female of the aquarium *Schizopera* population has abnormal P1: endopod of one from leg branches was two-segmented instead of three-segmented. Another P1 endopod of this specimen was three-segmented and extends approximately to the end of exopod. Morphological variability of *S. samchunensis* was detected also in the body size and eggs number in egg sacs.

ECOLOGY. We found *S. samchunensis* in fresh-water aquarium. The females and egg sacs bearing females dominated in the aquarium population.

DNA-analysis

DNA was extracted and the COI fragment successfully PCR-amplified from 10 *Schizopera* specimens using a nested combination of primers given in Material and methods section. All the sequences were translated into protein shown to have no evidence of stop codons, ambiguities or insertions–deletions. BLAST analyses of GenBank revealed that the obtained sequences are copepod in origin. The sequences were deposited in the GenBank database under the numbers: OP970225, PQ76352–PQ763522 (*S. neglecta*) and PQ763516–PQ763519, PV176547, PV176548 (*S. samchunensis*).

Each studied species was separated from each other *Schizopera* species on the phylogenetic tree (Fig. 6). We noted relatively large molecular-genetic gaps (according

the Kimura 2-parameter) between them and other species. The minimum distance (21.7 %) between *S. samchunensis* and *Schizopera* sp. (WWBRL007-23) were observed, maximum distance (31.2%) between *S. neglecta* and *S. knabeni* (KF667527.1).

Discussion

An integrative approach to species description has more value for taxonomic, phylogenetic and phylogeographic studies [Padial *et al.*, 2010]. And it is apply to more evidential harpacticoid copepods delimitation including *Schizopera*, which is difficult identified in connection with using for species identification morphometric (relative) features [Karanovic, Cooper, 2012; Karanovic, McRae, 2013; Karanovic *et al.*, 2015].

So, the relative length of the first segment of the P1 endopod is an important character for identification of the genus members [Wells, 2007]. Mainly on this feature for example found by us *S. samchunensis* (Enp-1 extends approximately to the end of exopod) and *S. compacta* Lint, 1922 (Enp-1 extends beyond exopod, and exopod never extends beyond approximately 85% of the length of Enp-1) are differed. The authors of the first description of *S. samchunensis* also discuss about the great morphological similarity of these two species [Karaytuğ, Sak, 2005]. Meanwhile, *S. samchunensis* and *S. compacta* might vary in ecology: the first species was found by us and by S. Karaytuğ & S. Sak [2005] in freshwater habitats, whereas *S. compacta* is known for the brackish-water habitats only [Lang, 1948; Por, 1960; Apostolov, 1973; Apostolov, Marinov, 1988]. We took into account that when determining the species from the fresh-water aquarium. But it had slight differences from Israel *S. samchunensis*: males from the population we examined had no setules on the inner side of the caudal rami. Further studies of *S. samchunensis* and *S. compacta*, comparing them primarily in molecular structures, will determine whether these morphological species are the same taxon or different. At present, information on the molecular sequences of COI (or other genetic markers) of *S. compacta* is absent in the databases, and we have obtained the barcodes of *S. samchunensis* for the first time for the species.

Most sequences of *Schizopera* in the databases published were obtained for endemics of Australia [Karanovic, Cooper, 2012; Karanovic, McRae, 2013] or Japan [Karanovic *et al.*, 2015]. Then it was not a surprise to us that the molecular genetic sequences of COI found by us individuals were well different from these members of the genus.

Data on only two wide-spread *Schizopera* species were presented in GenBank: *S. borutzky* from Lake Ontario [Conolly *et al.*, 2022] and *S. knabeni* Lang, 1965 from culture [Karanovic *et al.*, 2015]. *Schizopera borutzky* occurs in estuaries of the Black Sea also [Monchenko, 1967], *S. knabeni* is common in the natural brackish waters at Mexico and the USA [Gomez, Vargas-Arriaga, 2008]. According a revision by V.A. Gusakov [2019] *S. paradoxa* (Daday, 1903) was noted for the salt and fresh water-bodies. But this author writes finds the species in

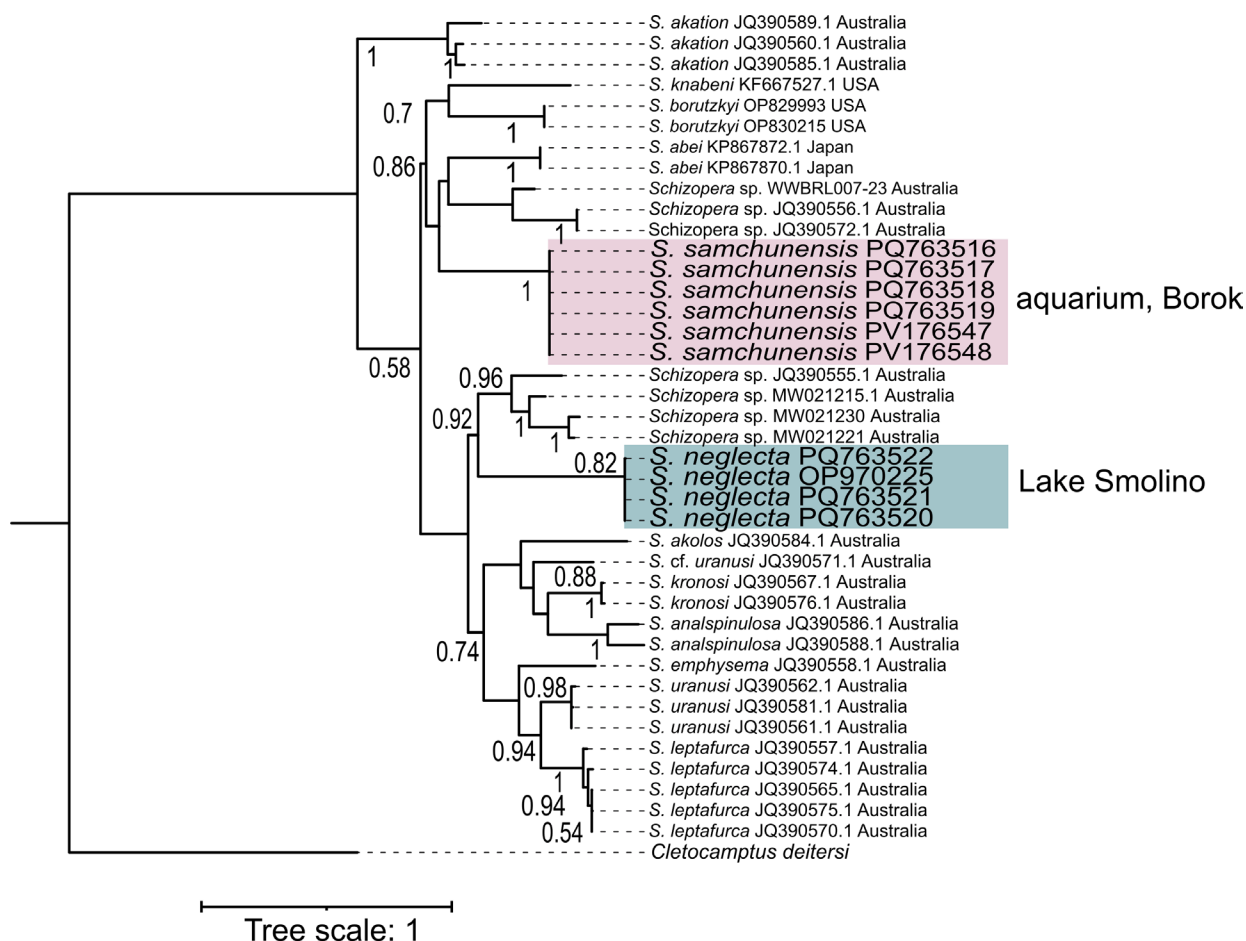


Fig. 6. Maximum likelihood (ML) tree based on COI sequences of *Schizopera* specimens. Numbers on the branches represent bootstrap values. The tree is rooted with *Cletocamptus deitersi* (Richard, 1897) with number in GenBank: AF315010.1. The sequences numbers represented in GenBank and BOLD SYSTEMS are shown.

Рис. 6. ML-филогенетическое дерево основанное на анализе COI-сиквенсов особей *Schizopera*. Числа на ветвях — бутстрэп-поддержки. В качестве внешней группы — *Cletocamptus deitersi* (Richard, 1897) с номером из GenBank: AF315010.1. Показаны номера сиквенсов в GenBank и BOLD SYSTEMS.

fresh-water habitats are questionable: incorrect identification cannot be excluded.

Salt tolerance range of *Schizopera* species poorly understood [Gusakov, 1919]. A few information we have on inter-species morphological variability of some other copepods in the salinity gradients [Borutsky *et al.*, 1991; Dvoretzkii, Dvoretzkii, 2009; Shadrin, Anufrieva, 2018]. It was shown morphometry of euryhaline species can vary depending on the water salinity. So, it may be likely that *S. samchunensis* from aquarium is fresh-water morph of *S. compacta*.

Other studied *Schizopera* species — *S. neglecta* — was found in the brackish-water condition, and it is consistent with the known ecological characterization of this species. In the Caspian Sea *S. neglecta* occurs at salinity 9–40 g L⁻¹ [Apostolov, Marinov, 1988; Chertoprud *et al.*, 2017], in the Black Sea at salinity 2–18 g L⁻¹ [Apostolov, Marinov, 1988].

Finding of *Schizopera* in the aquarium was interesting also in connection with follow reasons. It is known [Reid, 2001; Duggan, 2010; Watson *et al.*, 2015; Duggan *et al.*,

2018], that non-indigenous harpacticoid populations are able to spread around the world, including through aquarium husbandry. Until now, from fresh-water harpacticoids two such “species-hitchhikers” of genera *Elaphoidella* and *Nitokra* have been known [Duggan, 2010; Watson *et al.*, 2015; Duggan *et al.*, 2018]. Special studies [Duggan *et al.*, 2018] revealed they have spread widely around the world through the trade of at least aquarium plants, which are transported in water and substrata over long distances. Meanwhile, such copepods can be cultured and used as highly nutritive and inexpensive live food in aquaculture industry [Souza-Santos *et al.*, 2006; Zaleha, Bursa, 2012]. And definitely data on COI sequences can also be helpful to investigate the source of non-indigenous species and have been previously used to highlight potential invasion vectors by identifying the probable source populations [Watson *et al.*, 2015; Fefilova *et al.*, 2021].

As a conclusion result we provide a key for identification of *Schizopera* species occurring around Eurasia. Morphological characteristics of only females of 34 species were taken into account in the key. Unfortunately, to

include in the key several species was problematic and impossible, because their descriptions are incomplete and/or inadequate. There are *S. longirostris* (Daday, 1901) from Thailand, *S. stephanidesi* Pesta, 1937 from island Corfu (Greece), *S. grimalchii* Jakubisiak, 1938 from the Black Sea, *S. parvula* Noodt, 1955 from the Bay of Biscay, and *S. issykkulica* Manulova, 1966 inhabiting Lake Issyk Kul.

So, the male but not female of *S. parvula* was described [Noodt, 1955]. About *S. grimalchii* we know that the species is close to *S. neglecta*, but data on several features of *S. grimalchii* important for species diagnostic haven't been provided [Jakubisiak, 1938]. The same situation is with *S. longirostris* and *S. stephanidesi*. About i.e. *S. longirostris* they know that it belongs to a species group with three-segmented swimming legs, five setae on the fifth leg exopod, and with rather long first segment of the first leg endopod [Daday, 1901; Watiroyram *et al.*, 2021]. The description of *S. issykkulica* [Manulova, 1966] consists of literally two sentences of the text and is not accompanied by illustrations. It should be mentioned that the description of included in the key *S. nana* [Noodt, 1955] is also a bit inconsistent. In the figure (43, page 97 by W. Noodt [1955]) three setae on the last segment of the second leg endopod are shown, at the same time according the setae formula in page 95 [Noodt, 1955] these setae are four. We followed the figure in our key for the Eurasian members of the genus *Schizopera*.

KEY TO FEMALES OF EURASIAN SPECIES OF THE GENUS *SCHIZOPERA*

- 1 (58). P1 endopod 3-segmented.
- 2 (55). P5 baseoendopod with 4 setae.
- 3 (17). P5 exopod with 5 setae.
- 4 (14). Enp-3 of P2 with 4 setae.
- 5 (8). Enp-3 of P3 with 4 setae.
- 6 (7). Caudal rami conical, about 1.5-1.7 times as long as width *S. brusinai* Petkovski, 1954.
Bulgaria: Black Sea [Apostolov, Marinov, 1988], Greece: Aegean Sea [Apostolov, 2008].
- 7 (6). Caudal rami subcylindrical, about 2 times as long as width
S. pactaii Watiroyram, Sanoamuang et Brancelj, 2021.
Thailand: Khao Chang Hai Cave [Watiroyram *et al.*, 2021].
- 8 (5). Enp-3 of P3 with 3 or 2 setae.
- 9 (13). Enp-3 of P3 with 3 setae.
- 10 (11). P5 baseoendopod and exopod fused
..... *S. rybnikovi* Chertoprud et Kornev, 2005.
Caspian Sea [Chertoprud, Kornev, 2005; Chertoprud *et al.*, 2017].
- 11 (12). P5 baseoendopod and exopod separated, caudal rami almost cylindrical, more than 2 times as long as width, lateral caudal seta (seta III) deform to very short thick spike
..... *S. pontica* Chappuis et Serban, 1953.
Bulgaria, Romania: Black Sea [Apostolov, Marinov, 1988].
- 12 (11). P5 baseoendopod and exopod separated, caudal rami almost cylindrical, about 1.5 times as long as width with triangular appendix on outer side
..... *S. langi* Petkovski, 1954.
Adriatic, Black Seas (Apostolov, Marinov, 1988).
- 13 (9). Enp-3 of P3 with 2 setae
..... *S. gangneungensis* Karanovic et Cho, 2016.
Korea: Sea of Japan [Karanovic, Cho, 2016].
- 14 (4). Enp-3 of P2 with 3 setae.
- 15 (16). Caudal rami almost square: the same length as width
..... *S. kunzi* (Apostolov, 1967).
Bulgaria: Black Sea [Apostolov, Marinov, 1988].
- 16 (15). Caudal rami conical, about twice as long as wide, lateral caudal seta (seta III) swollen at the basis
..... *S. nana* Noodt, 1955.
France: coastal ground waters of Bay of Biscay [Noodt, 1955].
- 17 (3). P5 exopod with 6 setae.
- 18 (52). Enp-3 of P2 with 4 setae.
- 19 (30). Enp-1 of P2–P4 without seta.
- 20 (27). Enp-1 of P1 visibly shorter than exopod.
- 21 (26). Enp-1 of P1 longer than common length of Exp-1 and Exp-2.
- 22 (23). Enp-3 of P4 with 3 setae
..... *S. neglecta* (Akatova, 1935).
Caspian, Black Seas [Chertoprud *et al.*, 2017], Korea: Sea of Japan, marshes and estuaries [Karanovic, Cho, 2016].
- 23 (22). Enp-3 of P4 with 2 setae.
- 24 (25). Inner apical seta on caudal rami absent.....
..... *S. cicolanii* (Galassi et Pesce, 1988).
Italy: brackish waters [Galassi, Pesce, 1988].
- 25 (24). Inner apical seta on caudal rami presented.....
..... *S. setulosa* Mirabdullayev et Ginatullina, 2007.
Uzbekistan: eastern slope of the Ustyurt Plateau, streams, Lake Sudochie [Mirabdullayev, Ginatullina, 2007].
- 26 (21). Enp-1 of P1 shorter than common length of Exp-1 and Exp-2, caudal rami with a notch in the middle of the inner side, segments of P5 fused.....
..... *S. aralensis* Borutsky, 1971.
Aral Sea [Borutsky, 1971].
- 27 (20). Enp-1 of P1 approximately the same length than exopod.
- 28 (29). Caudal rami conical, about 1.3–1.5 times as long as width *S. jugurtha* (Blanchard et Richard, 1891).
Issyk-Kul Lake, Aral, Azov, Black seas; Africa: Algeria, Nyasa Lake [Borutsky, 1952; Apostolov, Marinov, 1988].
- 29 (28). Caudal rami narrowed in the basal and distal parts, maximum width in the proximal part, about 2.5 times as long as width..... *S. pseudojugurtha* Borutsky, 1972.
Kyrgyzstan: ground waters near Issyk-Kul Lake [Borutsky, 1972].
- 30 (19). Enp-1 at least of P3–P4 with seta.
- 31 (34). Enp-1 of P2 with seta.
- 32 (33). Enp-1 of P1 longer than first two segments of exopod, exopod and baseoendopod of P5 separated
..... *S. paradoxa* (Daday, 1903).
Russia: Elton Lake; Kyrgyzstan: Karakol city [Borutsky, 1952], a well near Issyk-Kul Lake [Turbanov *et al.*, 2016]; Turkmenistan: lake in the Kaptarhana cave.
- 33 (32). Enp-1 of P1 a little shorter than first two segments of exopod, exopod and baseoendopod of P5 fused
..... *S. lindae* Apostolov et Pesce, 1987.
Italy: ground waters [Apostolov, Pesce, 1987].
- 34 (31). Enp-1 of P2 without seta.
- 35 (42). Enp-1 of P1 equal by length to first two segments of exopod.
- 36 (37). Third outer seta of female P5 exopod in three times longer than second seta *S. clandestina* (Klie, 1924).
Baltic, Black seas [Apostolov, Marinov, 1988], Korea: Sea of Japan, marshes and estuaries [Karanovic, Cho, 2016].
- 37 (36). Second and third outer setae of female P5 exopod equal by length.
- 38 (41). Enp-3 of P3 with 4 setae, Enp-3 of P4 with 3 setae.

- 39 (40). Caudal rami conical, without notches
.....*S. subterranea* Lang, 1948.
Italy: Adriatic Sea [Lang, 1948]; Thailand: coastal region
of inner gulf [Watiroyram *et al.*, 2021].
- 39 (40). Caudal rami conical, without notches
.....*S. subterranea* Lang, 1948.
Italy: Adriatic Sea [Lang, 1948]; Thailand: coastal region
of inner gulf [Watiroyram *et al.*, 2021].
- 40 (39). Caudal rami angular, with notch in the distal margin of
inner side.....*S. baltica* Lang, 1965.
Sweden: Baltic Sea, ground waters [Lang, 1965].
- 41 (38). Enp-3 of P3 with 3 setae, Enp-3 of P4 with 2 setae
..... *S. lagrecai* Pesce, 1988.
Italy: Sicily, ground waters [Pesce, 1988].
- 42 (35). Enp-1 of P1 longer than first two segments of exopod.
43 (46). Enp-1 of P1 extends to the middle of Exp-3 of P1.
- 44 (45). Caudal rami conical, about twice as long as wide ..
.....*S. daejinensis* (Karanovic et Cho, 2016).
Korea: Sea of Japan [Karanovic, Cho, 2016].
- 45 (44). Caudal rami almost cylindrical, about three times longer
as wide, lateral caudal seta (seta III) deform to very short
thick spike.....*S. minuta* Noodt, 1955.
France: coastal ground waters of Bay of Biscay [Noodt,
1955].
- 46 (43). Enp-1 slightly extends approximately to the end of
exopod or extends beyond exopod.
- 47 (48). Enp-1 slightly extends beyond exopod
.....*S. compacta* (Lint, 1922).
Bulgaria, Romania: Black Sea; Holland: North Sea [Apos-
tolov, Marinov, 1988]; Korea [Karanovic, Cho, 2016].
- 48 (51). Enp-1 slightly extends approximately to the end of
exopod, caudal rami conical.
- 49 (50). Caudal rami about twice as long as wide, inner seta on
Enp-1 of P3 about twice longer than this segment
.....*S. samchunensis* Karaytuğ et Sak, 2005.
Israel: Hula Lake [Karaytuğ, Sak, 2005]; Russia: Yaroslavl
region, aquarium.
- 50 (49). Caudal rami as long as wide, inner seta on Enp-1 of P3
as long as this segment
.....*S. sindoensis* (Karanovic et Cho, 2016).
East Coast of Korea [Karanovic, Cho, 2016].
- 51 (48). Caudal rami almost cylindrical, about twice as long as
wide *S. yeonghaensis* Karanovic et Cho, 2016.
Korea: Sea of Japan [Karanovic, Cho, 2016].
- 52 (18). Enp-3 of P2 with 3 setae.
- 53 (54). Enp-3 of P3 with 3 setae
.....*S. borutzky* Montschenko, 1967.
Black Sea, Danube river delta [Apostolov, Marinov, 1988];
Ontario Lake [Connolly *et al.*, 2022].
- 54 (53). Enp-3 of P3 with 2 setae
.....*S. reducta* Borutsky, 1971.
Aral Sea [Borutsky, 1971].
- 55 (2). Female P5 baseopodopod with 5 setae.
- 56 (57). Enp-3 of P3 with 5 setae
.....*S. akatovae* Borutsky, 1953.
Caspian Sea [Alekseev, 2015].
- 57 (56). Enp-3 of P3 with 4 setae
.....*S. petkovskii* (Apostolov, 1971).
Bulgaria: Black Sea [Apostolov, 1973; Apostolov, Marinov,
1988].
- 58 (1). P1 endopod 2-segmented.
- 59 (64). Exp-2 of P3 with seta on inner side.
- 60 (63). Caudal rami with spinular rows on dorsal side.
- 61 (62). Caudal rami with two spinular rows on dorsal side, P5
exopod rounded with 6 unipinnate setae
.....*S. chaetosa* Petkovski, 1954.
Adriatic, Black seas [Apostolov, Marinov, 1988].
- 62 (61). Dorsal side of caudal rami covered rows of tiny spi-
nules, P5 exopod prolongate (about 2 times as long as width)
with 6 pinnate setae
.....*S. ornata* Nood et Purasjoki, 1953.
Russia: White Sea, coastal waters of Karelia; Germany:
Baltic Sea, coastal waters [Kornev, Chertoprud, 2008].
- 63 (60). Caudal rami without spinular rows on dorsal side ..
.....*S. pratensis* Noodt, 1958.
Germany: salt marshes [Noodt, 1958].
- 64 (59). Exp-2 of P3 without seta on inner side
.....*S. meridionalis* (Petkovski, 1954).
Adriatic, Black Sea; Sweden: coastal waters [Apostolov,
Marinov, 1988].

Compliance with ethical standards

CONFLICT OF INTEREST: The authors declare that they have no conflict of interest.

Ethical approval: No ethical issues were raised during our research.

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