A new semi-terrestrial *Cryptorchestia* Lowry et Fanini, 2013 (Amphipoda: Talitridae) from the southwestern Caucasus and the Ciscaucasian Plain

Новая амфибиотическая *Cryptorchestia* Lowry et Fanini, 2013 (Amphipoda: Talitridae) с юго-западного Кавказа и Предкавказской равнины

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ABSTRACT. A new species of the semi-terrestrial talitrid genus *Cryptorchestia* Lowry et Fanini, 2013, *Cryptorchestia ciscaucasica* sp.n., is described from western Abkhazia and the western part of the Ciscaucasian Plain, reaching Rostov-on-Don and the Tsimlyansk Reservoir, Russia. The new species is obviously related to the Western European *C. garbinii* Ruffo, Turaco et Latella, 2014. Both species are genetically separated for 2.5% by COI mtDNA gene marker, geographically isolated and can be easily distinguished by the shape of antennas I–II and the spinulation of propodus (palm) of both gnathopods I–II in males and females. It is very likely that these species were separated by episodes of cooling/glaciation, the most powerful of which occur during the Cromerian Stage of the Middle Pleistocene, about 0.5–0.8 Mya, and survived during some unfavorable periods in the southern refugium of the Caucasus and southwestern Europe, respectively.

РЕЗЮМЕ. Описан новый вид амфибиотических амфипод-талитрид рода *Cryptorchestia* Lowry et Fanini, 2013, *Cryptorchestia ciscaucasica* sp.n., распространенный от центральной Абхазии и западной части Предкавказской равнины до Ростова-на-Дону и Цимлянского водохранилища, Россия. Новый вид является близкородственным для западноевропейской талитриды *C. garbini* Ruffo, Tarocco et Latella, 2014. В тоже время эти виды генетически разделены на 2.5% по генному маркеру COI мтДНК, географически изолированы и могут быть легко различимы по форме антенн I–II и вооружению проподуса (ладони) гнатопод I–II у самцов и самок. Весьма вероятно, что виды были разделены эпизодами похолодания/оледенения, наиболее мощные из которых пришлись на Кромерианский этап Среднего Плейстоцена, около 0,5–0,8 млн. лет назад, и пережили неблагоприятные периоды в рефугиумах южной части Кавказа и юго-западной Европы, соответственно.

Introduction

The family Talitridae (beach- and landhoopers) (Crustacea: Amphipoda) includes semi-terrestrial amphipods widely distributed across warm temperate and subtropical regions, where they live in a wide variety of coastal to inland terrestrial habitats. Several species are known as synanthropic, while humans have transported others accidentally [Greenslade *et al*., 2008; Simpson, 2011; Wildish *et al*., 2016].

Talitird amphipods from the coastal habitats of the Black and Azov Seas include ten species, with six species known from its northwestern coastline [Grintzov, 2022]. Among them, *Cryptorchestia garbinii* Ruffo, Tarocco et Latella, 2014, originally described from Lake Garda, Italy, is one of the large-size semi-terrestrial talitrid species (body size reaches 18 mm) living along the shores of freshwater reservoirs, such as lakes and rivers, reaching areas covered with snow and

ice in winter. Previously, this species was confused with *Orchestia cavimana* Heller, 1865 (= *Cryptorchestia cavimana* (Heller, 1865)) from Cyprus (see Lowry and Fanini [2013] and Ruffo *et al*. [2014]). In Europe, *C. garbinii* is currently spreading in the inland freshwater ecosystems [Ruffo *et al*., 2014; Davolos *et al*., 2018; Rewicz *et al*., 2020], and it is believed that its current known distribution extends from the Ponto-Caspian region to the Balkans and Italy France and the UK [Ruffo *et al*., 2014; Rewicz *et al*., 2020]. The genetic diversity of European populations is extremely low, and all of them are almost identical in COI mtD-NA gene marker [Rewicz *et al*., 2020]. However, eastern populations, for example, from the territory of Russian Federation and southwestern Caucasus have never been studied in terms of genetic diversity.

We examined landhoppers from the southwestern part of the Caucasus (Lake Abrau, Russia and New Athos, Abkhazia), as well as the nearby Ciscaucasian Plain in the Rostov region (Rostov-on-Don and Tsimlyansk Reservoirs), Russia, which were previously identified as *C. garbinii* or *C. cavimana* (e.g., Takhteev *et al*. [2015], Grintsov [2022]). The studied specimens can be distinguished from the type population of *C. garbinii* from Lake Garda (after Ruffo *et al*. [2014]) by several morphological features, as well as extensive genetic data using the gene marker of COI mtDNA (barcoding), and described herewith as a new species.

Material and methods

SAMPLE COLLECTION AND PROCESSING. Amphipods were collected in various coastal habitats in the southwestern Caucasus in 2020–2022. All samples were fixed in 90% ethanol. Photographs of alive coloration in situ were made using a digital camera CanonG16. Photographs of morphological features were made with a digital camera attached to a light microscope Olympus ZX10 or Olympus CX21. Scanning electron microscopy (SEM) images were made using a Vega3 Tescan microscope in the Yu.A. Orlov Paleontological Museum of the Paleontological Institute of the Russian Academy of Sciences, Moscow. The body length (bl., mm), the dorsal length from the distal margin of head to the posterior margin of telson, without uropod III and both antennas, was used as a standard measurement. The type material is deposited at the collection of Zoological Museum of Moscow State University, Moscow, Russia (ZMMU). Additional material is deposited in the author's personal collection (IM) at the A.N. Severtsov Institute of Ecology and Evolution of the Russian Academy of Sciences, Moscow, Russia (LEMMI).

DNA AMPLIFICATION AND SEQUENCING. Total genomic DNA was extracted from muscle body tissue using the innuPREP DNA Micro Kit (AnalitikJena, Germany). The COI mtDNA gene fragment was amplified using the universal primers LCO1490 (5'–GGTCAACAAATCAT-AAAGATATTGG–3') and HC02198 (5'–TAAACT-TCAGGGTGACCAAAAAATCA–3') under the standard protocol [Folmer *et al*., 1994]. PCR products were then sequenced using Genetic Analyzer ABI 3500 (Applied Biosystems, USA) and BigDye 3.1 (Applied Biosystems, USA)

with forward and reverse primers. Dataset of aligned sequences, 617 base pairs in length was used in the study.

PHYLOGENETIC ANALYSIS. Consensus of complementary sequences was obtained with MEGA 7.0 [Kumar *et al*., 2016]. PartitionFinder v.2. [Lanfear *et al*., 2016] was used for finding best-fitting partitions for the concatenated dataset as COI mtDNA gene marker is usually characterized by a strong heterogeneity in nucleotide substitution rates among all three coding positions. The GTR+G+I evolutionary substitution model was suggested as the best using jModeltest2.1.141 and MEGA 7.0. Different evolutionary substitution models and phylogenetic estimates were widely congruent in this study. Further, RAxML [Kozlov *et al*., 2019] with 1000 bootstrap replicates was used to create Maximum Likelihood (ML) phylogeny. Pairwise genetic divergences (p-distances) were calculated based on COI sequences using MEGA 7.0 with the Kimura 2-Parameter (K2P) model of evolution [Kimura, 1980]. Median-joining network [Bandelt *et al*., 1999] was reconstructed with PopArt (Population Analysis with Reticulate Trees) software [Leigh, Bryant, 2015].

Results

Order Amphipoda Latreille, 1816
Infraorder Gammarida Latreille, 1802
Family Talitridae Rafinesque, 1815
Genus *Cryptorchestia* Lowry et Fanini, 2013

Cryptorchestia ciscaucasica **sp.n.**
Figs 1–4.

MATERIAL EXAMINED. Holotype, ♂ (bl. 12.0 mm), ZMMU Mb-1261, Russian Federation, northwestern Ciscaucasian Plain, Rostov Oblast', Rostov-on-Don, Proletarskiy district, 47°13′59.9″N 39°47′00.1″E, about 40 m a.s.l., a small spring on a shore of Kiziterinka river, hand net sampling, 18.05.2022, coll. D. Palatov et I. Marin.

Paratypes, 1♂, 2♀♀, ZMMU Mb-1262 and ZMMU Mb-1263, same locality and date as for the holotype.

Additional material: 5♂♂, 9♀♀ (LEMMI), Russian Federation, Krasnodar Krai, Novorossiysk area, Lake Abrau, 44°42′41.2″N 37°35′31.9″E, on the shore, under large stones, hand net sampling, 12.06.2022, coll. I. Marin et D. Palatov; 2♂♂, 7♀♀ (LEMMI), Rostov Oblast, Rostov-on-Don, Proletarskiy district, the bank of the Kiziterinka River, 47°13′59.9″N 39°47′00.2″E, under large stones, hand net sampling, 10.05.2022, coll. I. Marin et D. Palatov; 6♂♂, 10♀♀ (LEMMI), Rostov Oblast, Tsimlyansky district, Tsimlyansk Reservoir, 47°41′47.34″N, 42°11′16.04″E, on the shore, under large stones and boulders, hand net sampling, 26.08.2022, coll. D. Palatov; 1♂, 3♀♀ (LEMMI), SW Caucasus, Abkhazia, New Athos, 43°05′28.9″N 40°49′01.0″E, under large stones, hand net sampling, 7.01.2023, coll. D. Palatov.

DESCRIPTION (based on the original description of *C. garbinii* from Garda Lake, Italy [Ruffo *et al*., 2014], and an additional re-description from the Republic of Türkiye (Turkey) [Davalos *et al*., 2018]).

Head. Eyes large, subcircular, black. *Antenna I* (Fig. 2*a*) short, frequently reaching distal margin of article III of antenna II, article II about 1.9 times longer than wide, subequal to article III, flagellum with 5 articles. *Antenna II* (Fig. 2*b*) about 1/2 of total body length, peduncle not swollen, and with sparse small robust setae, flagellum relatively short with 19–20 articles.

Mouthparts. Labrum (Fig. 3*a*) and *labium* (Fig. 3*b*) with very fine setules on anterior margin. *Mandible* (Fig. 3*c*) left
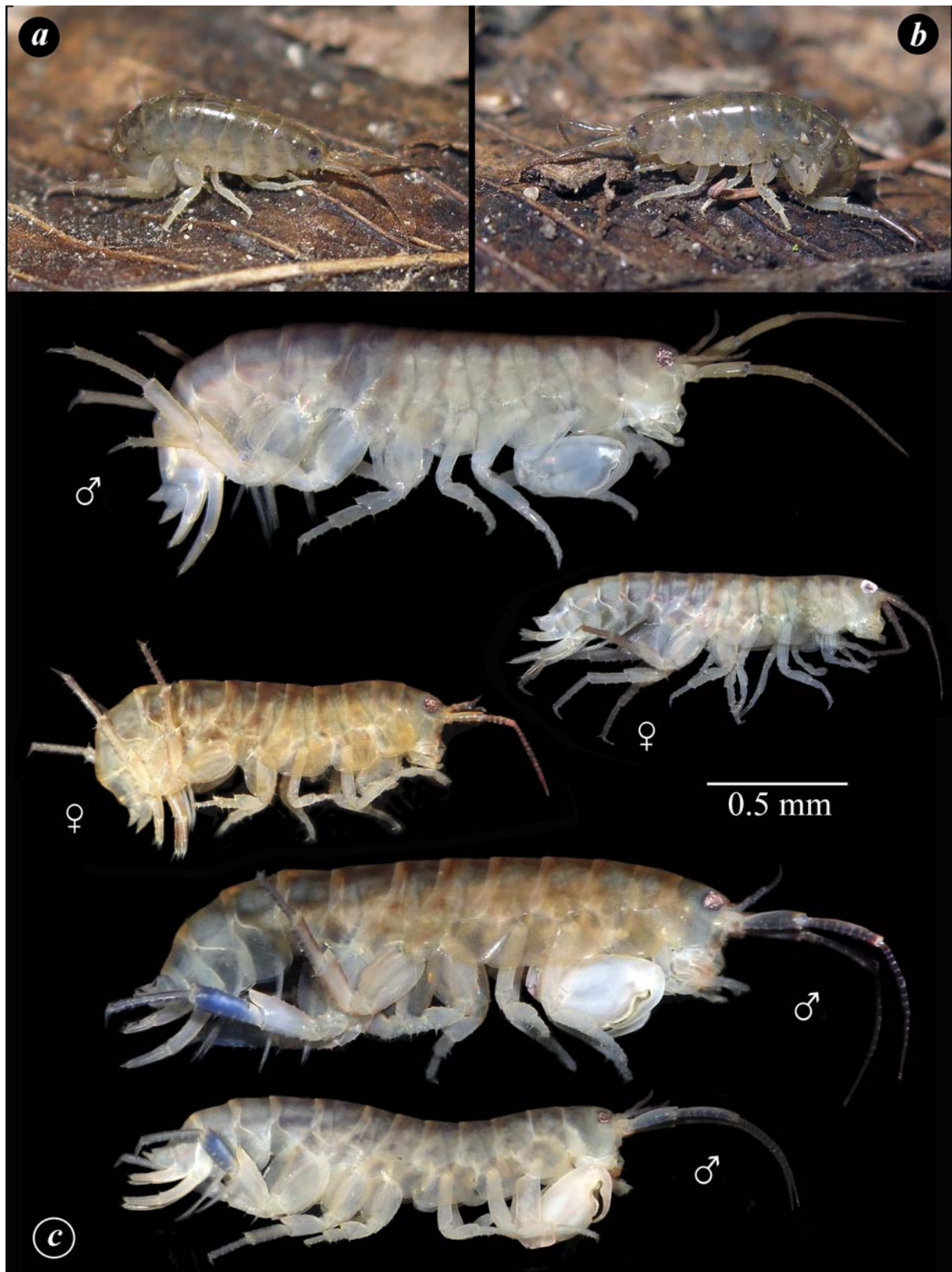
Fig. 1. *Cryptorchestia ciscaucasica* sp.n., Rostov-on-Don, Russia: *a*, *b* — general view *in situ*; *c* — relaxed individuals (with clove oil), alive coloration, general lateral view.

Рис. 1. *Cryptorchestia ciscaucasica* sp.n., Ростов-на-Дону, Россия: *a*, *b* — общий вид *in situ*; *c* — особи, расслабленные гвоздичным маслом, прижизненная окраска, общий вид сбоку.
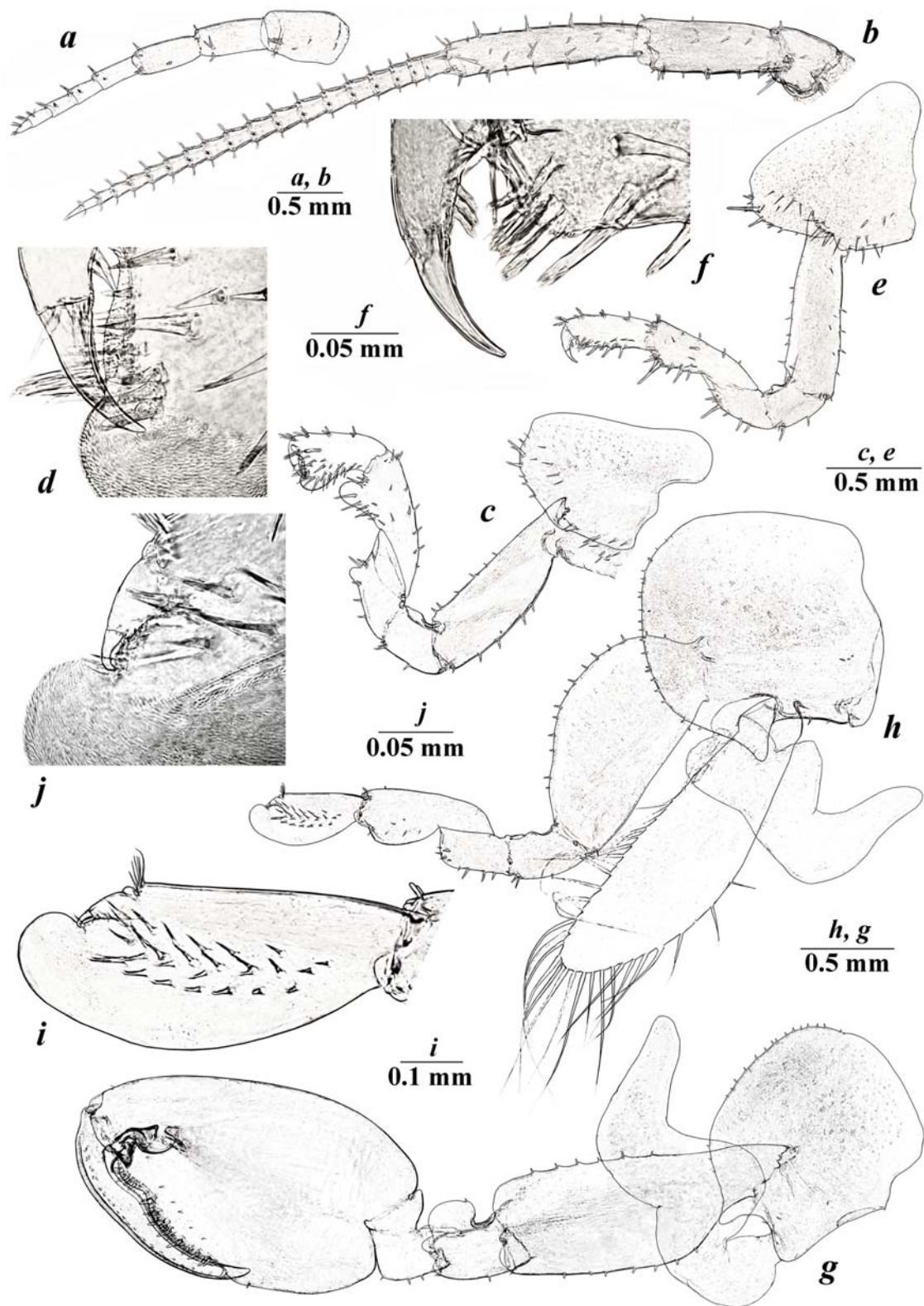
Fig. 2. *Cryptorchestia ciscaucasica* sp.n., Rostov-on-Don, Russia, ♂ (*a–d, g*) and ♀ (*e, f, h–j*): *a* — antenna I; *b* — antenna II; *c, e* — gnathopod I; *e, f* — distoventral corner of chela of GnI; *g, h* — gnathopod II; *i* — propodus (chela) of GnII; *j* — distoventral corner of chela of GnII.

Рис. 2. *Cryptorchestia ciscaucasica* sp.n., Ростов-на-Дону, Россия, ♂ (*a–d, g*) и ♀ (*e, f, h–j*): *a* — антенна I; *b* — антенна II; *c, e* — гнатопода I; *e, f* — дистовентральный край ладони клешни GnI; *g, h* — гнатопода II; *i* — проподус (клешня) GnII; *j* — дистовентральный край ладони клешни GnII.
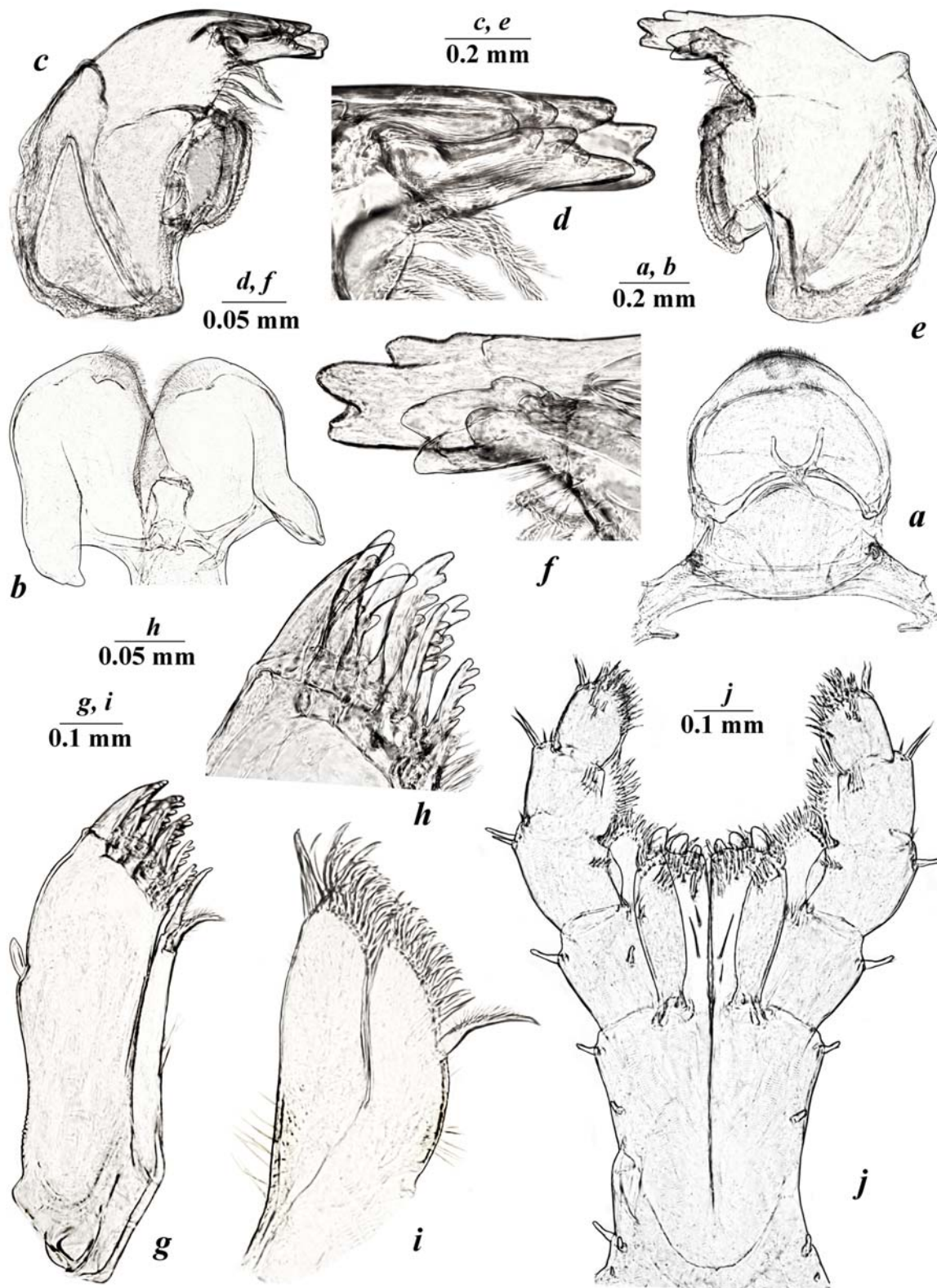
Fig. 3. *Cryptorchestia ciscaucasica* sp.n., ♂, Rostov-on-Don, Russia: *a* — labrum (upper lip); *b* — labium (lower lip); *c, e* — mandibles; *d, f* — incisor process and *pars incisiva* of mandibles; *g* — maxilla I; *h* — same, distal margin of outer lobe; *i* — maxilla II; *j* — maxilliped.

Рис. 3. *Cryptorchestia ciscaucasica* sp.n., ♂, Ростов-на-Дону, Россия: *a* — лабрум (верхняя губа); *b* — лабиум (нижняя губа); *c, e* — мандибулы; *d, f* — резцовый отросток и *pars incisiva* мандибул; *g* — максилла I; *h* — то же, дистальный край наружной доли; *i* — максилла II; *j* — максиллипед.
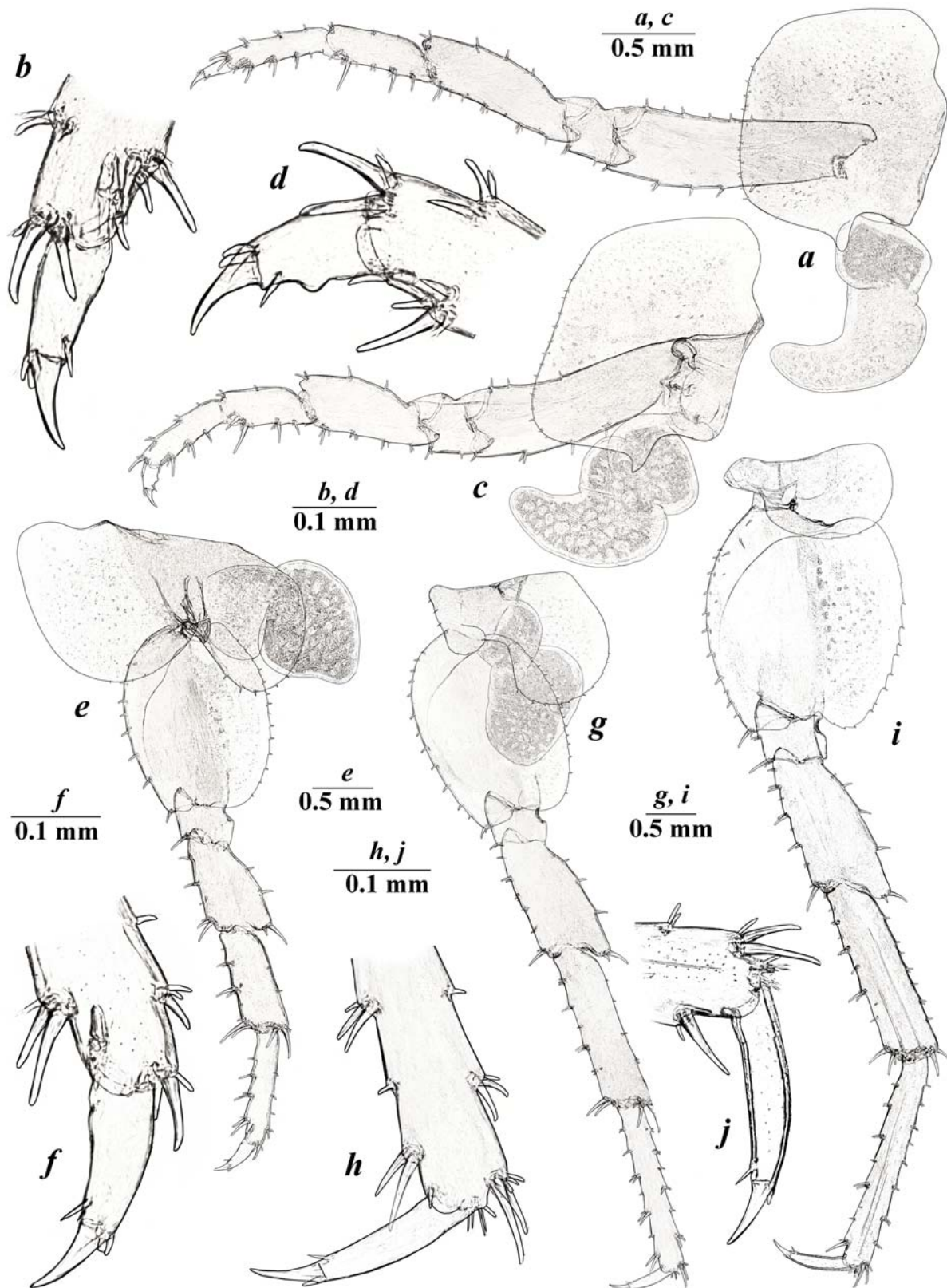
Fig. 4. *Cryptorchestia ciscaucasica* sp.n., ♂, Rostov-on-Don, Russia: *a* — pereopod III; *b* — dactylus of PIII; *c* — pereopod IV; *d* — dactylus of PIV; *e* — pereopod V; *f* — dactylus of PV; *g* — pereopod VI; *h* — dactylus of PVI; *i* — pereopod VII; *j* — dactylus of PVII.

Рис. 4. *Cryptorchestia ciscaucasica* sp.n., ♂, Ростов-на-Дону, Россия: *a* — переопода III; *b* — дактилус PIII; *c* — переопода IV; *d* — дактилус PIV; *e* — переопода V; *f* — дактилус PV; *g* — переопода VI; *h* — дактилус PVI; *i* — переопода VII; *j* — дактилус PVII.
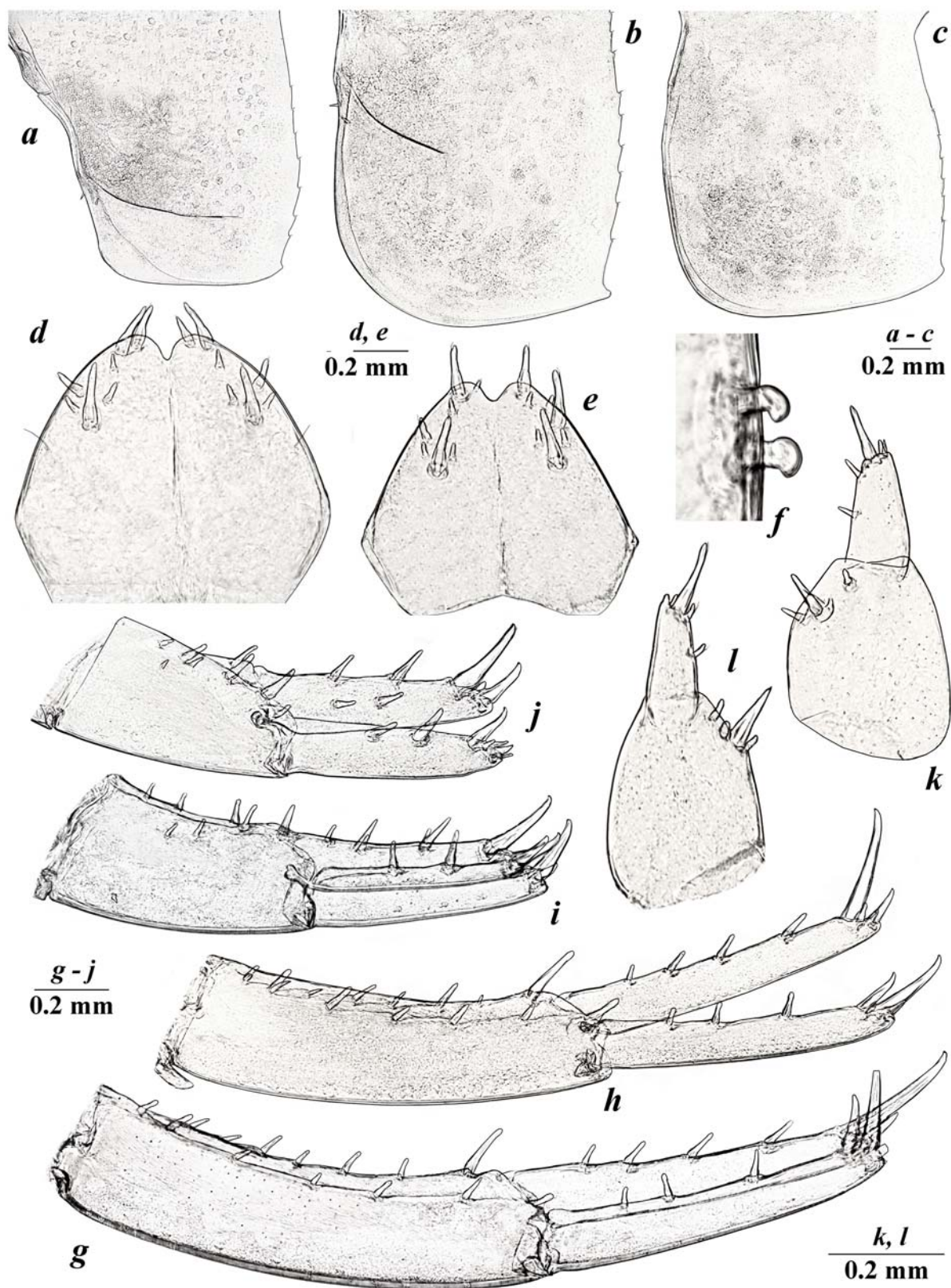
Fig. 5. *Cryptorchestia ciscaucasica* sp.n., Rostov-on-Don, Russia: ♂ (*a–d, i, g, k*) and ♀ (*e, j, h, l*): *a–c* — epimeral plates I–III; *d, e* — telson; *f* — retinacula of pleopod I; *i, j* — uropod II; *g, h* — uropod I; *k, l* — uropod III.

Рис. 5. *Cryptorchestia ciscaucasica* sp.n., ♂, Ростов-на-Дону, Россия: ♂ (*a–d, i, g, k*) и ♀ (*e, j, h, l*): *a–c* — эпимеральные пластинки I–III; *d, e* — тельсон; *f* — ретинакула плеоподы I; *i, j* — уропода II; *g, h* — уропода I; *k, l* — уропода III.

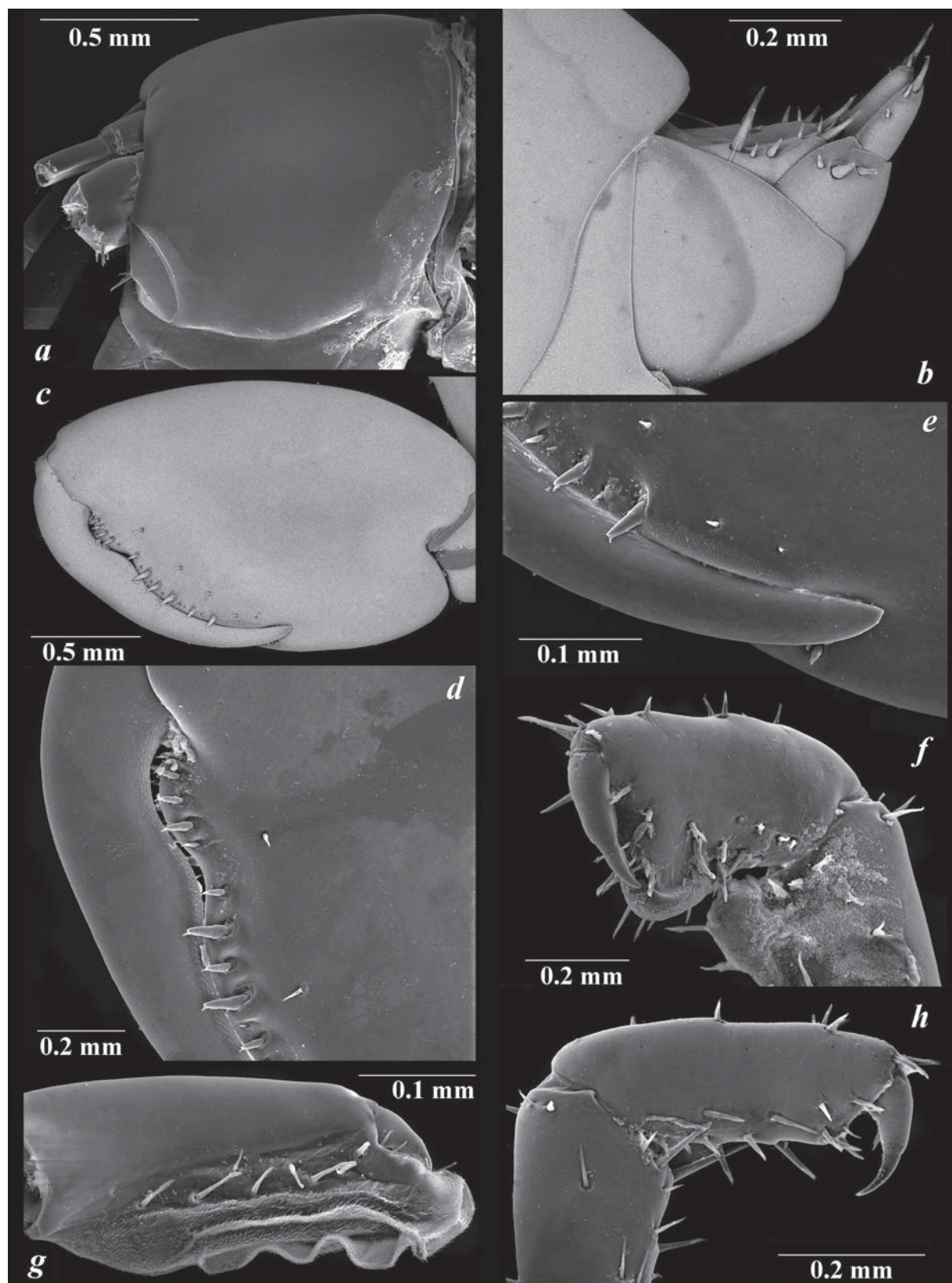Fig. 6. *Cryptorchestia ciscaucasica* sp.n., Rostov-on-Don, Russia: ♂ (*a–f*) and ♀ (*g, h*): *a* — head; *b* — urosoma, uropod III and telson; *c, d, e, g* — chela of gnathopod II; *f, h* — chela of gnathopod I.

Рис. 6. *Cryptorchestia ciscaucasica* sp.n., ♂, Ростов-на-Дону, Россия: ♂ (*a–f*) и ♀ (*g, h*): *a* — голова; *b* — уросома, уроподы III и тельсон; *c, d, e, g* — клешня гнатоподы II; *f, h* — клешня гнатоподы I.

with 5-dentate *lacinia mobilis* and 5-dentate *pars incisiva* (Fig. 3*d*), right mandible with multi-teethed lacinia mobilis (Fig. 3*e, f*). *Maxilla I* (Fig. 3*g*) with 9 robust and crenelated setae on inner lobe (Fig. 3*h*). *Maxilla II* (Fig. 3*i*) with numerous apical setae, long and finely pinnate seta on inner margin of inner lobe. *Maxilliped* (Fig. 3*j*) basal lobe with 3 blunt teeth on anterior margin, axial margin lined with robust setae armed with setules; palp article 4 reduced, but clearly visible.

Epimeral plates I–III (Fig. 5*a–c*) wider than deep, with bluntly produced convex anterior margins. Epimeral plate I with 2 short robust setae only on distal rounded margin. Epimeral plates II with 2 short robust setae only on distal rounded margin. Epimeral plate III unarmed.

Gnathopod I (Fig. 2*c*) larger in males, posterior margins of merus, carpus and propodus with lobes covered with numerous palmate setae; propodus sub-triangular, with bluntly produced distoventral lobe in males (Fig. 2*d*), distally excavated; dactylus shorter than palm. Gnathopod I subchelate in females (Fig. 2*e*), carpus and propodus without rugose lobe, dactylus longer than palm.

Gnathopod II subchelate in females (Fig. 2*h*) and with strong chela in males (Fig. 2*g*). Propodus (chela) oviform in males (Fig. 2*g*), stout with rounded protuberance near dactylus insertion; palmar margin with a large sinus in anterodistal part, dactylus somewhat longer than palm. Propodus (chela) subchelate, with distinct bluntly produced lobe in females (Fig. 2*h*), lateral margin armed with numerous stout spines; dactylus shorter than palm (Fig. 2*j*). Oostegites longer than wide; setae with simple straight tips.

Pereopods III–VII (Fig. 4) cuspidactylate. *Pereopods III–IV* (Fig. 4*a, c*) similar, without special feature; merus of pereopod III longer than that of pereopod IV; dactylus simple (Fig. 4*b, d*). *Pereopod V* (Fig. 4*e, f*) basis oval, elongate, posterodistal lobe wide, convex. *Pereopod VI* (Fig. 4*g*) slightly shorter than pereopod VII (Fig. 4*i*); basis oval, elongate, posterior margin convex; propodus as long as carpus, dactylus simple (Fig. 4*h*). *Pereopod VII* (Fig. 4*i*) basis wide with distinct, rounded posterodistal lobe; merus and carpus not enlarged; propodus longer than carpus, dactylus simple (Fig. 4*j*).

Pleopods I–III well-developed, biramous, peduncle without marginal setae, slightly longer than ramus; rami with slender setae; inner ramus slightly longer than outer; with 2 hooks in retinacules (Fig. 5*f*).

Uropods I–II (Fig. 5*g–j*) with peduncle and both rami spinose, distal robust setae longer than others; uropods I rami slightly shorter than peduncle both in males (Fig. 5*g*) and females (Fig. 5*h*), armed with large strong spines. Uropod II rami longer than peduncle both in males (Fig. 5*i*) and females (Fig. 5*j*), armed with large strong spines. Uropod III peduncle with 3–4 robust setae anterodorsally; ramus shorter than peduncle, with 1–2 lateral stout spine-like setae, and 1 long and 2–3 smaller spine-like setae.

Telson (Fig. 5*d, e*) about as long as broad, dorsal midline entire, with 7–8 marginal and distal robust spine-like setae per lobe.

GENBANK ACCESSION NUMBERS. OR211848 (Rostov-on-Don), OR211846 (Lake Abrau), OR211847 (New Athos), OR211849 (Tsimlyansk Reservoir).

TAXONOMIC REMARKS. New cryptic species is most closely related to *Cryptorchestia garbinii* Ruffo, Tarocco et Latella, 2014 described from Lake Garda (Italy) [Ruffo *et al*., 2014; Davalos *et al*., 2017] and belongs to the genus *Cryptorchestia* sensu stricto. The latter also includes *C. cavimana* from Cyprus, and *C. ruffoi* Latella et Vonk, 2017

from Rhodes in south-eastern Greece. All these species have a small lobe on the merus of gnathopod I [Davalos *et al*., 2018a]. Davalos *et al*. [2017] also added *C. kosswigi* (Ruffo, 1949) from the Turkish coast to this group of species, which also has a small lobe on merus of gnathopod I in males.

Nevertheless, some morphological features are clearly separating these species. The new species can be distinguished from *G. gabrinii* from Lake Garda [Ruffo *et al*., 2014] by: (1) peduncular articles I–III of antenna I are 1.6, 1.9 and 2.3 times longer than wide, respectively (Fig. 2*a*) vs. 0.85, 1.6 and 2.1 times (after Ruffo *et al*. [2014]: Fig. 1B); (2) peduncular articles II–III of antenna II are 2.6 and 4.5–4.7 times longer than wide, respectively (Fig. 2*b*), vs. 3.5 and 8.0 times (after Ruffo *et al*. [2014]: fig. 1A, C)); (3) anterodistal lobe of propodus (palm) of gnathopod I in males armed with a much larger amount of spikes (Fig. 2*c*); (4) less convex anterodistal lobe of propodus (palm) of gnathopod II in males (Fig. 2*g*); (5) medialateral margin of propodus (palm) and carpus of gnathopod II in females armed with a much larger amount of spikes (Fig. 2*h, i*) and (6) smaller body size up to 12–13 mm (vs. 17–18 mm).

At the same time, the new species distinguishes from *G.* cf. *gabrinii* from the northwestern Turkey [Davalos *et al*., 2018] by: (1) peduncular articles I–III of antenna I are 1.6, 1.9 and 2.3 times longer than wide, respectively (Fig. 2*a*), vs. articles are about as long as wide (after Davalos *et al*. [2018]: fig. 3A); (2) peduncular articles II–III of antenna II are 2.6 and 4.5–4.7 times longer than wide, respectively (Fig. 2*b*), vs. 2.2 and 3.5–4 times (after Davalos *et al*. [2018]: fig. 3B)); (3) anterodistal lobe of propodus (palm) of gnathopod I in males armed with a much larger amount of spikes (Fig. 2*c*); (4) less convex anterodistal lobe of propodus (palm) of gnathopod II in males (Fig. 2*g*); (5) lateral medial margin of propodus (palm) and carpus of gnathopod II in females armed with a much larger amount of spikes (Fig. 2*h, i*); (6) elongated basal (basis) articles of pereiopods V–VI, which are about 1.3–1.4 times as long as wide (Fig. 4*e, g*) vs. almost round, about as long as wide (after Davalos *et al*. [2018]: fig. 5C, D); and (7) ventral margin of epimeral plates I–III more convex, especially in distal part (Fig. 5*a–c*).

GENETIC DATA. The genetic divergence of *Cryptorchestia ciscaucasica* sp.n. from *G. garbinii* is about 2.5% (0.025±0.007 substitutions) and about 19% (0.192±0.019 substitutions) from *G. cavimana* from the Crete Island by COI mtDNA gene marker (see Fig. 7).

ECOLOGY. Like other representatives of the genus, this species lives in moist places along the coast of rivers or fresh-water lakes, usually avoiding brackish water reservoirs. Different-sized and heterosexual individuals form aggregations, especially in moist areas under large stones, branches or in the forest floor.

DISTRIBUTION. The currently known distribution of *Cryptorchestia ciscaucasica* sp.n. is limited to the coastal areas of the southwestern Caucasus from Abkhazia (New Athos) to Lake Abrau and further north to deltas of Kuban and Don rivers (Rostov-on-Don) and the Tsimlyansk reservoir in the Rostov region (see Fig. 1). At the same time, previously published records of the talitrid species identified as *C. cavimana* are wider in the region, reaching westward to the Crimean Peninsula [Rewicz *et al*., 2020] and southeastern Black Sea coast of the Republic of Türkiye (Turkey) [Davolos *et al*., 2018]. Russo *et al*. [2014] also mentioned that the studied individuals from the Republic of Türkiye (Turkey) (Lake Sapanka, Lake Iznik, Kiyikoy) are not belong either to *C. garbinii* nor to *C. cavimana*, although clearly belonging to the genus *Cryptorchestia*.

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Fig. 7. Distribution of *Cryptorchestia ciscaucasica* sp.n. (stars and yellow square) and *Cryptorchestia garbinii* Ruffo, Tarocco et Latella, 2014 (A), reconstruction of COI mtDNA phylogeny by the highest likelihood method (B) and median-joining network of the studied haplotypes, strokes on the branches denote substitutions (C). Yellow icons indicate type localities.

Рис. 7. Распространение *Cryptorchestia ciscaucasica* sp.n. (звездочки и желтый квадрат) и *Cryptorchestia garbinii* Ruffo, Tarocco et Latella, 2014 (А), реконструкция филогении COI мтДНК методом наибольшего правдоподобия (Б) и медианная объединенная сеть изученных гаплотипов (C). Желтыми значками отмечены типовые области обитания.

Discussion

The periods of cooling/glaciation during the Pleistocene greatly changed the habitats and faunas of the European part of Eurasia, where many species survived in refugia only [Hewitt, 2000, 2003, 2004; Zachos *et al.*, 2001; Provan, Bennett, 2008]. Currently, the southern glacial refugia in the Western Palearctic, such as the Iberian, Italian and Balkan peninsulas, Anatolia, the southern Caucasian Kolkhida coastal lowland (Colchis) and the southern coastal valley of the Caspian Sea (Hyrcania), are well recognized [Taberlet *et al.*, 1988; Schmitt, 2007; Bennett, Provan, 2008; Keppel *et al.*, 2012]. Also recently, a glacial refugia have been found on the northern slope of the Greater Caucasus Ridge [Marin, Palatov, 2021; Palatov, Sokolova, 2021; Anistratenko *et al.*, 2022] and the Northern Black/Azov Sea coastal plain [Marin, Palatov, 2023]. They were localities of conservation and subsequent settlement of species, and contributed to the speciation of divided populations in the Pleistocene [Marin, 2020; Marin *et al.*, 2021].

The studied genetic structure of the type species *G. garbinii* in the Western Europe indicates the Holocene dispersion of one of the populations, which was preserved, presumably, in the Italian refugium [Davalos *et al.*, 2018a]. At the same time, this species currently cannot penetrate into the Northern Black/Azov Seas region, as it is currently "cut off" by zones of passage of cold winter air, for example, through the territory of eastern Ukraine and the Crimean Peninsula, where periodically the air temperature can drop to –30 ºC in winter. At the same time, we assume some ancient populations of landhoopers survived in the southern refugium of the Caucasus during the Pleistocene. It is very likely that *Cryptorchestia ciscaucasica* sp.n. also distributed from western Abkhazia and Lake Abrau to the north, to the Kuban Delta and the Rostov region during the Holocene.

To determine the species boundaries, a threshold barcoding interval based on *p*-distances calculated for the variability of COI mtDNA gene marker was proposed [Kimura, 2005; Hebert *et al.*, 2003b, 2004]. It ranges from 2% [Hebert *et al.*, 2003a; Matzen da Silva *et al.*, 2011] to 16% of the threshold value for crustacean and amphipod species [Lefébure *et al.*, 2006]. Nevertheless, the threshold of about 3% is most commonly used to determine the species boundaries [Hebert *et al.*, 2003a], the threshold of 3.75% is adopted for amphipods of the genus *Hyalella* S.I. Smith, 1874 (Amphipoda: Hyalellidae) [Witt *et al.*, 2006] and the threshold of 4% proposed for other amphipods [Costa *et al.*, 2009; Haversmans *et al.*, 2011]. In the case of *Cryptorchestia ciscaucasica* sp.n., the genetic divergence is somewhat lower (2.5%) than the generally accepted values about (3.75–4%), however, the presence of well-marked morphological differences and geographic isolation with strict geographical distribution (local endemism) of both species suggests allopatric speciation since the middle of the Pleistocene (Qua-ternary period), during the last 1.8–1.0 Mya [Knowlton, Weight, 1998; Schubart *et al.*, 1998] or 0.5 Mya [Loeza-Quintana *et al.*, 2019]. It is very likely that these species were separated by episodes of cooling/glaciation since 1.65 Mya, the most powerful of which occurred during the Cromerian Stage in the Middle Pleistocene, about 0.8–0.5 Mya [Turner, 1996; Marin, Palatov, 2023].

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