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Insights on the Existence of Ancient Glacial Refugee in the Northern Black/Azov Sea Lowland, with the Description of the First Stygobiotic Microcrustacean Species of the Genus *Niphargus* Schiödte, 1849 from the Mouth of the Don River

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Abstract: A new species of the genus *Niphargus* Schiödte, 1849 (Crustacea: Amphipoda: Niphargidae), co-existing with other stygobiotic amphipods, *Diasynurella kiwi* Marin and Palatov, 2023 and *Pontonyx donensis* (Martynov, 1919) (Crangonyctidae), is described from a small spring on a shore of Kiziterinka River in Rostov-on-Don City in the mouth of the Don River. Two of the three species in the studied spring, *D. kiwi* and the discovered *Niphargus*, belong to microcrustaceans not exceeding the total body size of 3 mm. The new species, *Niphargus rostovi* **sp. nov.**, represents one of the smallest species within the genus and is mostly related to the Greek *Niphargus karkabounasi* Ntakis, Anastasiadou, Zakšek and Fišer, 2015, which is also not reaching the body size of 3 mm. One more related undescribed species is found on the Crete Island by the molecular genetic data. These species represent a separate phylogenetic lineage within the "*carpathicus*" species complex, which diverged from the congeners in the Late Miocene for more than 10 Mya. At the same time, the speciation within the ingroup started about 5–6 Mya, obviously correlating with the drainage of the Euxinian basin of the Eastern Paratethys, connecting the lower Don and southern Greece areas. *Niphargus potamophilus* Birštein, 1954 is also first recorded from the mouth of the Belbek River in the Crimean Peninsula, closing the known area from the Kuban River delta to Rostov-on-Don area and further along the western coast of the Black Sea to Bulgaria. Analysis of the recent records of long-time lineages of endemic/subterranean/stygobiotic animals unable to disperse for long distances assumed that glacial refugium existed at the mouth of the Don River, along with the South Caucasus (Colchis) and the southern Caspian (Hyrcania), where many species have survived several periods of glaciation since the late Miocene.

Keywords: diversity; phylogeny; refugee; barcoding; time-scale

1. Introduction

The genus *Niphargus* Schiödte, 1849 (Crustacea: Amphipoda: Niphargidae) is one of the most diverse freshwater amphipod genera, comprising over 450+ species living in a wide range of subterranean aquatic (stygobiotic) habitats, from deep cave lakes and small pores in the epikarst to helocrene spring, wells and hyporheic zones of rivers in the West Palaearctic [1–5]. Among the known diversity of the genus, only 49 species are currently found on the Crimean Peninsula and the Caucasus, mainly from the southern slope of the Greater Caucasian Ridge (41 species) [6–14], and only one species, *Niphargus alanicus* Marin and Palatov, 2021, has been recorded from the central mountainous parts of the northern slope of the Greater Caucasus in the Republic of Northern Ossetia-Alania [5,15]. However, the diversity of the genus *Niphargus* is still far from being fully studied in the Caucasus and Ciscaucasian Plain [5,9].

Similar to most of subterranean/stygobiotic animals, the representatives of the genus *Niphargus* are unable to live outside of their habitats as they are well adapted to ecologically narrow stygobiotic/subterranean conditions, very sensitive to environmental changes (stenobiotic) [16–18], and unable to travel or disperse for long distances [17,19–21]. These ecological features allowed using them for different evolutional and biogeographical studies [22]. It is also clear that the diverse fauna of stygobiotic crustaceans, and even the presence of the only long-time genetically isolated stygobiotic species of the genus *Niphargus*, is obviously associated with the presence of an ancient cryptic refugium in the area in the past [11,15].

Global climate changes that significantly changed the diversity and composition of fauna were observed during the Cenozoic Era (since 65.5 Mya until today) with glacial cycles during the Miocene, Pliocene and Pleistocene [23,24]. However, the most significant glacial periods were observed during the Pleistocene period (since 2.6 Mya) and the Last Glacial Maximum (LGM, 23–18 Tya) [25–28]. At this time, ice sheets and an unsuitable cold and dry climate made large areas of the Northern Hemisphere, almost reaching southern Europe, making the subterranean and water biota of Northern Europe virtually uninhabitable [28–30]. However, a number of species were able to survive unfavorable climatic conditions in the southern glacial refugium [31,32]. Southern glacial refugees in the Western Palearctic, such are the Iberian, Italian and Balkan peninsulas, Anatolia, the southern Caucasian Kolkhida coastal lowland (Colchis) and the southern coastal valley of the Caspian Sea (Hyrcania), as well as their diversity, conservation and post-glacial re-colonization paths for different taxa from there are currently quite well understood [28,31,33–40]. Most of these shelters are deeply indented protected mountain valleys, which have played a significant role in the survival of the species living there during the Ice Period [41]. In addition, in areas outside the aforementioned refugium protected by mountainous areas, for example, coastal plains, there is also a fairly high diversity of animals living in springs and groundwater [40,42–44], which are usually fragmented and poorly connected [45–47]. At the same time, it was believed that the areas adjacent to the Black/Azov Sea Lowland, not protected by mountains from the cold northern air masses, were seriously affected by periods of cooling/glaciation, especially during the Quaternary period, which is why their modern fauna is now severely impoverished. However, recent studies show that at least some endemic and sub-endemic stygobiotic/subterranean animals could have survived in their modern habitats along the northern lowland of the Black/Azov Seas during the past glacial periods [48–53], and the existence of an ancient glacial refugium has already been proposed in the Azov–Prikubanskaya Lowland [54].

In May–October 2022, studying the stygobiotic fauna of different water resources of the Black/Azov Sea Lowland, we discovered an unusual diversity of stygobiotic amphipods in a captured small spring on a shore of Kiziterinka River (Rostov-on-Don), 47°13′59.9″ N 39°47′00.1″ E, in the mouth of the Don River [48,55]. Two of the three crustacean species in the studied spring, namely *Diasynurella kiwi* Marin and Palatov, 2023 (Crangonyctidae) and undescribed species of the genus *Niphargus* (Niphargidae), belong to microcrustaceans not exceeding the total body size of 3 mm. It is obvious that all the discovered species, co-existing in the spring [55,56], are unable to spread over long distances [17,20,21] and survived here, isolated in the spring water system since Pliocene (see below). The discovery of these stygobiotic animals suggested the ancient glacial refugium in the mouth of the Don River and the Northern Black/Azov Sea Lowland since Late Miocene–Pliocene. The presented paper also discusses the current known diversity of the endemic and sub-endemic fauna of the non-mountainous habitats of the Northern Black/Azov Sea Lowland. At the same time, we did not consider or analyze the subterranean/stygobiotic diversity of the adjacent Caucasian, Crimean and Carpathian Mountains, since speciation there went on in different evolution ways, although we understand that these are certainly interrelated issues.

2. Materials and Methods

Study area. The study area includes the Northern Black/Azov Sea Lowland bordered with estuaries of the Kuban and Don (Sea of Azov), Dnieper and Dniester (Black Sea) rivers, representing the northern part of the Euxinian basin of the Eastern Paratethys during the Late Miocene (about 10–6 Mya) (see Figure 1) [57–59]. The lower Danube Lowland (former Dacian Basin) also connected with the studied region, but was rather isolated since Pliocene, and we consider the fauna only partially. It is also likely that in the past, similar to the modern times, the Danube riverbed connected the middle of the Black Sea with the Pannonian Lowland (Pannonian Basin), and was a channel for the distribution of many ancestral lines of modern groups of stygobiotic animals, such as the genus *Niphargus*. Currently, it is proven as a transit waterway for *Niphargus hrabei* S. Karaman, 1932 and *Niphargus valachicus* Dobreanu and Manolache, 1933 [14,60–63]. Moreover, the Danube Delta is the southernmost part of the studied area, and the influence of glaciation on it was significantly lesser, so that the evolutionary processes could occur there in a slightly different way than in the northern areas of the Northern Black/Azov Sea Lowland [64,65].

Morphological studies. Amphipods were collected using a hand net and then fixed in a 90% ethanol solution. Line drawings of collected amphipods were produced using *camera lucida* attached to Olympus SZX10 light stereomicroscope. The scanning electron microscopy (SEM) images were collected using the Vega 3 Tescan microscope in the Yu.A. Orlov Paleontological Museum of the Paleontological Institute of the Russian Academy of Sciences, Moscow. Amphipods were placed in 95% ethanol, cleaned in an ultrasonic cleaner, then dehydrated with acetone, critical-point dried (CPD), fixed on specimen stubs with double-sided tape and coated with gold by sputtering using Polaron PS 100. The body length (bl., mm), the dorsal straight body length from distal margin of head to the posterior margin of telson, without the length of uropod III and antennas, was used as a standard measurement. The type material was deposited at the collection of the Zoological Museum of Moscow State University, Moscow, Russia (ZMMU). Additional material was deposited in the author's personal collection at the A.N. Severtsov Institute of Ecology and Evolution of the Russian Academy of Sciences, Moscow, Russia (LEMMI).

Molecular and phylogenetic study. The mitochondrial cytochrome oxidase c subunit I (COI mtDNA) gene has been proven to be extremely informative in previous studies at both population and species level [66]. Total genomic DNA was extracted from muscle tissue using the innuPREP DNA Micro Kit (AnalitikJena, Germany). The COI mtDNA gene marker was amplified using universal primers LCO1490 (5′–GGTCAACAAATCATAAAGA TATTGG–3′) and HC02198 (5′–TAAACTTCAGGGTGACCAAAAAATCA–3′) under standard protocol conditions [67]. PCR products were then sequenced using Genetic Analyzer ABI 3500 (Applied Biosystems, Waltham, MA, USA) and BigDye 3.1 (Applied Biosystems, Waltham, MA, USA) with forward and reverse primers. The dataset of aligned sequences of COI mtDNA gene markers, about 617 base pairs in length used in the study, were obtained from the GenBank (NCBI) database and the author's personal dataset.

Pairwise genetic divergences (*p*-distances) and their standard errors (SE) were calculated based on the available COI sequences using MEGA 7.0 with the Kimura 2-Parameter (K2P) model of evolution [68].

Phylogenetic analysis is based on the dataset of consensus of the sequences (see Appendix) obtained with MEGA 7.0 [69]. The best evolutionary substitution model was determined using MEGA 7.0 and jModeltest2.1.141 (Diego Darriba, Universidade da Coruña as part of the Computer Architecture Group (GAC), Coruña, Spain) on XSEDE via the CIPRES (Cyber Infrastructure for Phylogenetic Research) Science Gateway V. 3.3 (http://www.phylo.org/, accessed on 10 November 2022). Phylogenetic analysis was conducted using PhyML 3.0 (http://www.atgc-montpellier.fr/phyml/; accessed on 12 January 2023) [70] with several models based on BIC (Bayesian Information Criterion) and AIC (Akaike Information Criterion). The general tree of all studied sequences/species (A) as well as the reconstruction between the studied species of the "*carpathicus*" complex (B) were visualized with FigTree v1.4.3 and presented in Figure 1.

Molecular clock analysis was performed based on Bayesian Inference (BI) trees generated with the BEAST2 package [71]. Maximum Clad Credibility Tree was obtained using TreeAnnotator v2.5.1, with a 10% burn-in and selected mean node height [71,72]. The resulting trees were visualized with FigTree v1.4.3. Time calibration points were chosen based on the adapted time scale [17] and the analysis of possibly related historical events.

Abbreviations: Mx–maxilla; Gn–gnathopod; P–pereopod; Pp–pereopods; Pl–pleopod; Ep–epimeral plate; U–uropod.

3. Results

Phylogenetic Approach of Newly Discovered Species of the Genus Niphargus

According to the molecular genetic analysis (Figure 1), the newly discovered species is closely related to *Niphargus karkabounasi* Ntakis, Anastasiadou, Zakšek and Fišer, 2015, obtained from continental Greece [73], with which they form a distinct monophyletic lineage (Bayesian–PP = 1.00; ML–BS = 95%). Both species are related to the polyphyletic "*Niphargus carpathicus*" species complex (see Figure 1), including Romanian *Niphargus carpathicus* Dobreanu and Manolache, 1939, *N. decui* G. Karaman and Sarbu, 1995, N. *transsylvanicus* Schellenberg, 1934, as well as *N. grandii* Ruffo, 1936, *N. aberrans* Sket, 1972, *N. microcerberus* Sket, 1972, *N. barbatus* Karaman, 1985 and some others, which are found in stygobiotic mountainous habitats of Austria, Northern Italy and Slovenia (see Figure 1; [74,75]). The estimated time of the origin of the studied lineage and its separation from the related species of the "*carpathicus*" species complex is calculated as 0.215 ± 0.015015 substitutions per 100 nucleotides (about 21%) (about 10–9 Mya (95% HPD: 27.92–4.16) (min (0.0077/Mya) and max (0.0516/Mya) after [76], the average (about 0.025/Mya) for COI mtDNA gene marker (after [45,77]). It is probably related to the separation of the Eastern Basin from other basins of the former Paratethys (Euxinian, Alpine and Pannonian basins) (see Figure 1).

The interspecific uncorrected pairwise genetic distances (*p*-distances) between the studied individuals (n = 2) of *Niphargus rostovi* **sp. nov.** is very low, showing about 0.003 substitutions per 100 nucleotides (about 0.3%).

The intraspecific genetic difference (*p*-distances calculated using COI mtDNA gene marker) between *Niphargus rostovi* **sp. nov.** and *N. karkabounasi* is 0.135 ± 0.015 substitutions per 100 nucleotides (about 13%), showing the average divergence time for about 5.4 Mya (95% HPD: 17.53–2.61). Such divergence time is probably correlated with the disappearing (drying out) of the late Euxinian Basin of the Eastern Paratethys during the Messinian Crisis (5.96–5.33 Mya), which connected the regions of Greece and the modern mouth of the Don River [57–59,78,79]. There is also an undescribed species from a captured spring northeast of Arkadi Monastery, Arkadi, Crete, Greece, closely related to *N. karkabounasi*, which is known by molecular genetic data [73,74] (see Figure 1). Unfortunately, the sequences of the COI mtDNA gene marker for this species are not presented in GenBank (NCBI database), and we were not able to use it in our phylogenetic analysis.

The intraspecific genetic differences (*p*-distances of COI mtDNA gene marker) of *Niphargus rostovi* **sp. nov.** and other above-mentioned species of the "*carpathicus*" complex exceed 21% (Table 1), showing the absence of the gene flow and a long-time isolation for about 10–9 Mya (see above), which is well correlated with the time of the regression of the former Paratethys since the Late Miocene.
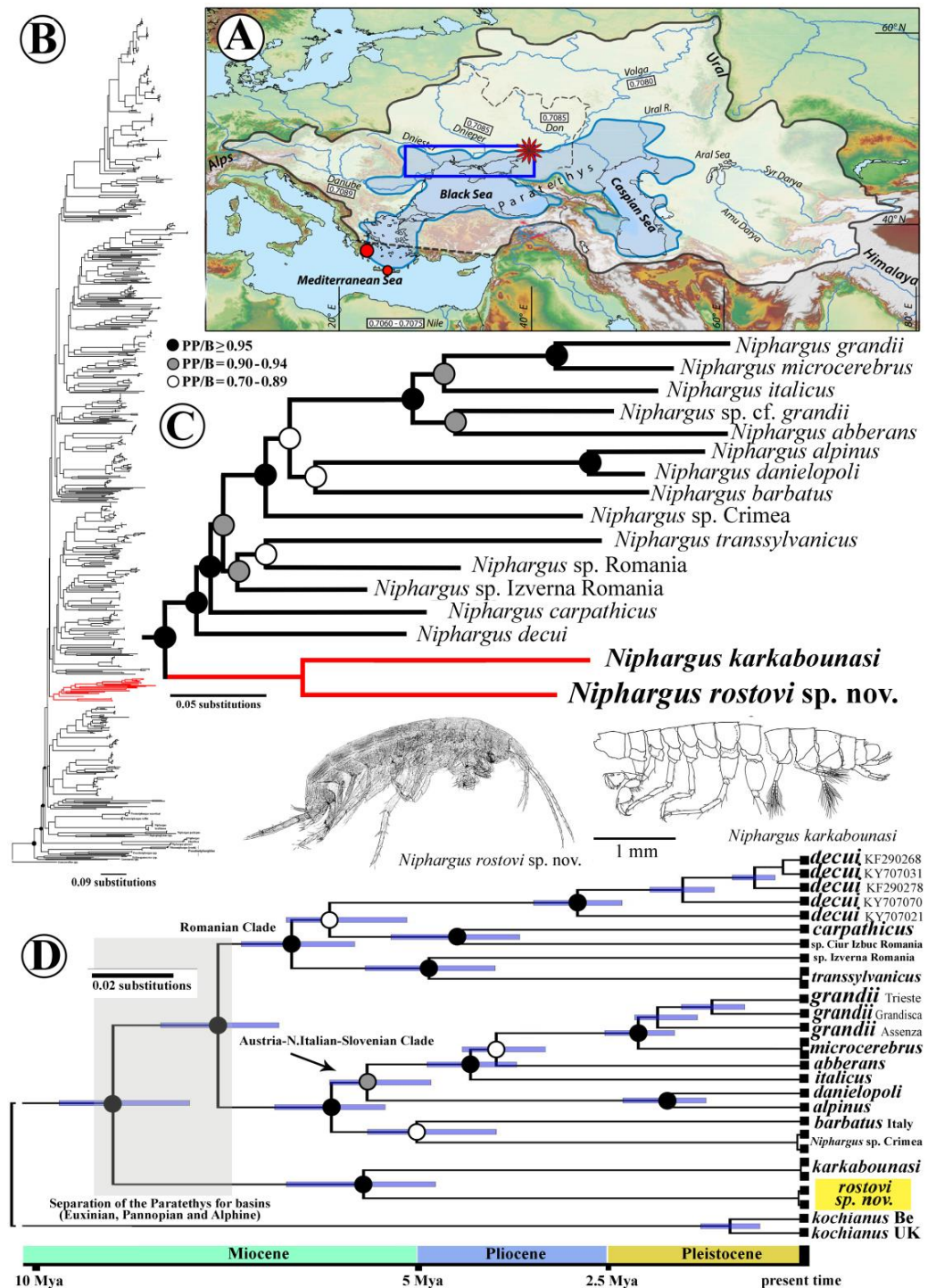
Figure 1. Phylogenetic tree (reconstruction) (based on COI mtDNA gene marker) of the relationships of the *Niphargus rostovi* **sp. nov.** from the mouth of the Don River. (**A**)—the study area with the records of the species within the outlined boundaries of the Paratethys and the later Eastern Paratethys (after [58]) taken from [59]; (**B**)—General phylogenetic tree of the used sequences of genus *Niphargus* with the indication of the studied Clade (red, arrow); (**C**)—the phylogenetic relationships (ML analysis) of the studied species of the "*carpathicus*" complex; (**D**)—time-calibrated tree (BI) with the estimated time scale. Map of the Paratethys during late Pontian regional stage (about 7–5 Mya) (modified) from [66]. Posterior probabilities of the nodes are reported. Blue horizontal bars show the 95% HPD (highest posterior density) of node ages on an arbitrary time scale. The drawing of *Niphargus karkabounasi* is taken from [74].

Table 1. Pairwise genetic (COI mtDNA) distances (substitutions per 100 nucleotides) and standard errors (SE) between *Niphargus rostovi* **sp. nov.** and related species of the "*carpathicus*" complex.

Species	*p*-Distance $\pm$ SE
Niphargus karkabounasi	0.135 $\pm$ 0.015
Niphargus italicus	0.211 $\pm$ 0.020
Niphargus microcerebrus	0.213 $\pm$ 0.018
Niphargus abberans	0.215 $\pm$ 0.019
Niphargus grandii	0.218 $\pm$ 0.018
Niphargus decui	0.222 $\pm$ 0.019
Niphargus alpinus	0.223 $\pm$ 0.019
Niphargus carpathicus	0.224 $\pm$ 0.019
Niphargus danielopoli	0.234 $\pm$ 0.020
Niphargus transsylvanicus	0.242 $\pm$ 0.021
Niphargus barbatus	0.270 $\pm$ 0.024

Taxonomic part

Order Amphipoda Latreille, 1816;
Family Niphargidae Bousfield, 1977;
Genus *Niphargus* Schiödte, 1849.
***Niphargus rostovi* sp. nov**.
Figures 2–7

Material examined: Holotype, ♂(bl. 3.0 mm), ZMMU Mb-1259, Russian Federation, the Northern Black/Azov Sea Lowland, Rostov Oblast', Rostov-on-Don, Proletarskiy district, 47°13′59.9″ N 39°47′00.1″ E, about 40 m asl, a small spring on a shore of Kiziterinka river, hand net sampling, coll. D. Palatov et I. Marin, 18 May 2022.

Paratypes, 1♂, 1♀(bl. 3.0 and 2.5 mm), ZMMU Mb-1260, same locality and data as for holotype.

Additional material: 4♀♀, LEMMI, same locality and data as for holotype.

Etymology: The new species is named after the locality, Rostov-on-Don, where this species was discovered.

Diagnosis: *Head* without eyes or pigmented spots on anterior lobe. Posteroventral corners of *epimeral plates I–II* rounded and bluntly, produced in *epimeral plate III*. *Urosomite I* unarmed, *urosomite II* with 1 strong spine in a posterodorsal angle, one on each side; *urosomite III* unarmed. Accessory flagellum of *antenna I* short, 2-articulated. Article III of *mandibular palp* equal to article II, with 1 A-seta; 1 C-seta; 8–10 D-setae and 4 E-setae. Outer plate of *maxilliped III* with strong spines. Dactyli of *pereopods III–VII* simple, with small simple seta at the inner margin near basis of nail, dactyli of *pereopods III–VII* elongated, about 4–5 times as long as it is wide. *Uropod I* rami of nearly equal length, equal in length to basal segment. *Pleopods* with 2 hooks in retinacules, without setae. *Telson* elongated, with 3 relatively long distal spines, accompanied by 1–2 simple setae on each side; dorsal surface with a tuft of 2–3 long simple setae in the medial part.

Description. BODY: Body length up to 2.5–3 mm, depigmented, moderately slender (Figure 2).

HEAD: length is approximately 9–10% of body length (Figure 7a); rostrum and eyes/pigmented spots on anterior lobe absent, with distally produced rounded lateral cephalic lobes and shallowly excavated anteroventral sinus.

PEREON: pereonites I–VII smooth, without setae (Figure 2).

PLEOSOMA: pleonites I–III with several short marginal setae on each postero-dorsal margin (Figure 7b).

EPIMERAL PLATES: Epimeral plates I–III with rounded posteroventral angles (Figures 6a–c and 7b). Epimeral plate I (Figure 6a): posterior and ventral margin convex; without spines along ventral margin; with 2 setae along the posterior margin; subrounded posteroventral angle with 1 strong seta. Epimeral plate II (Figure 6b): posterior margin bluntly rounded, ventral margin strongly convex, with 1 spine in the medial part; with

2 setae along the posterior margin; posteroventral angle bluntly rounded, with 1 strong seta. Epimeral plate III (Figure 6c): posteroventral margin bluntly produced posteriorly; posterior margin with 3 setae, ventral margin convex, with 1–2 spines in the anterior part.
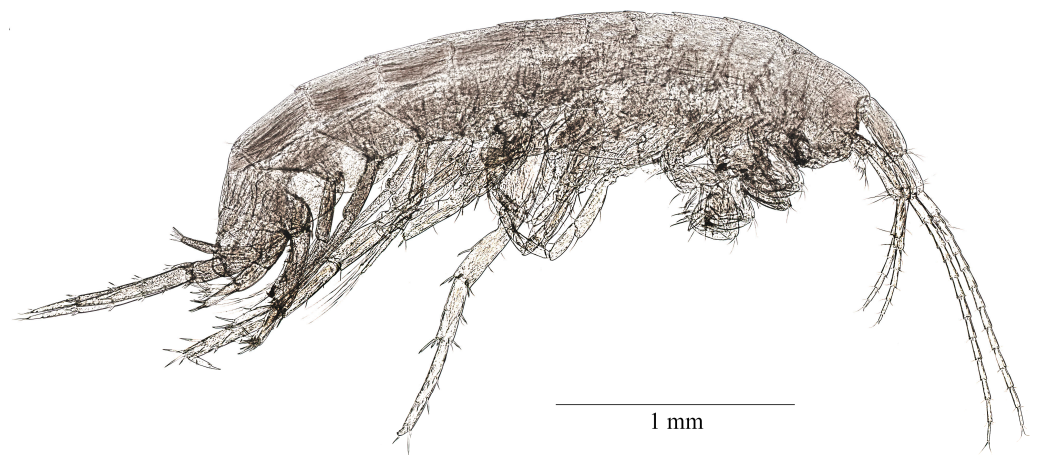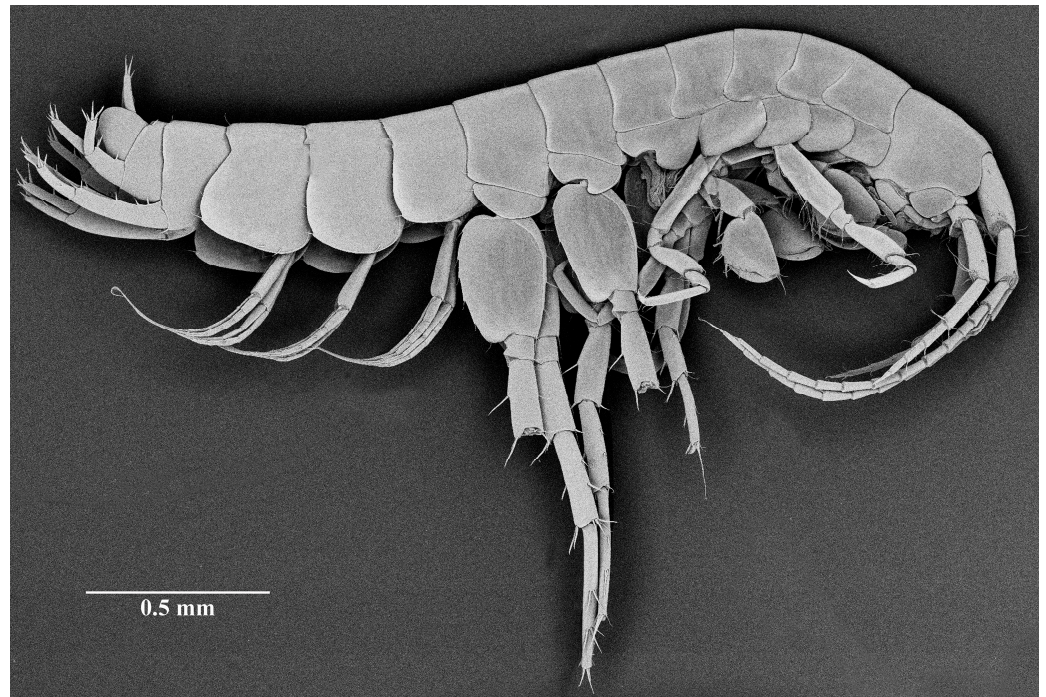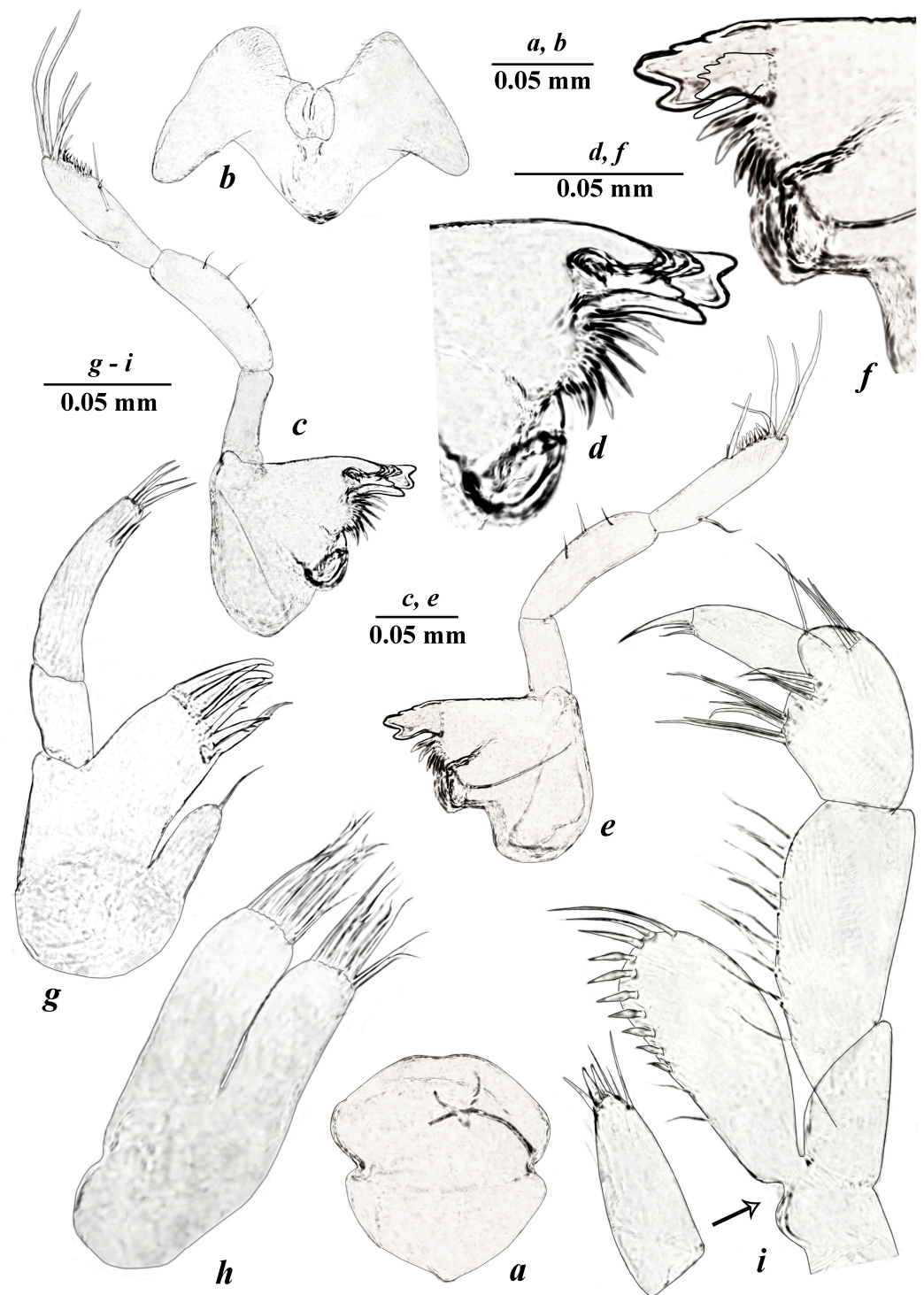

Figure 2. *Niphargus rostovi* **sp. nov.**, Rostov-on-Don, ♂, general lateral view, SEM and light microscope photos, non-type specimens, LEMMI. Uropod III are detached on the above SEM figure.
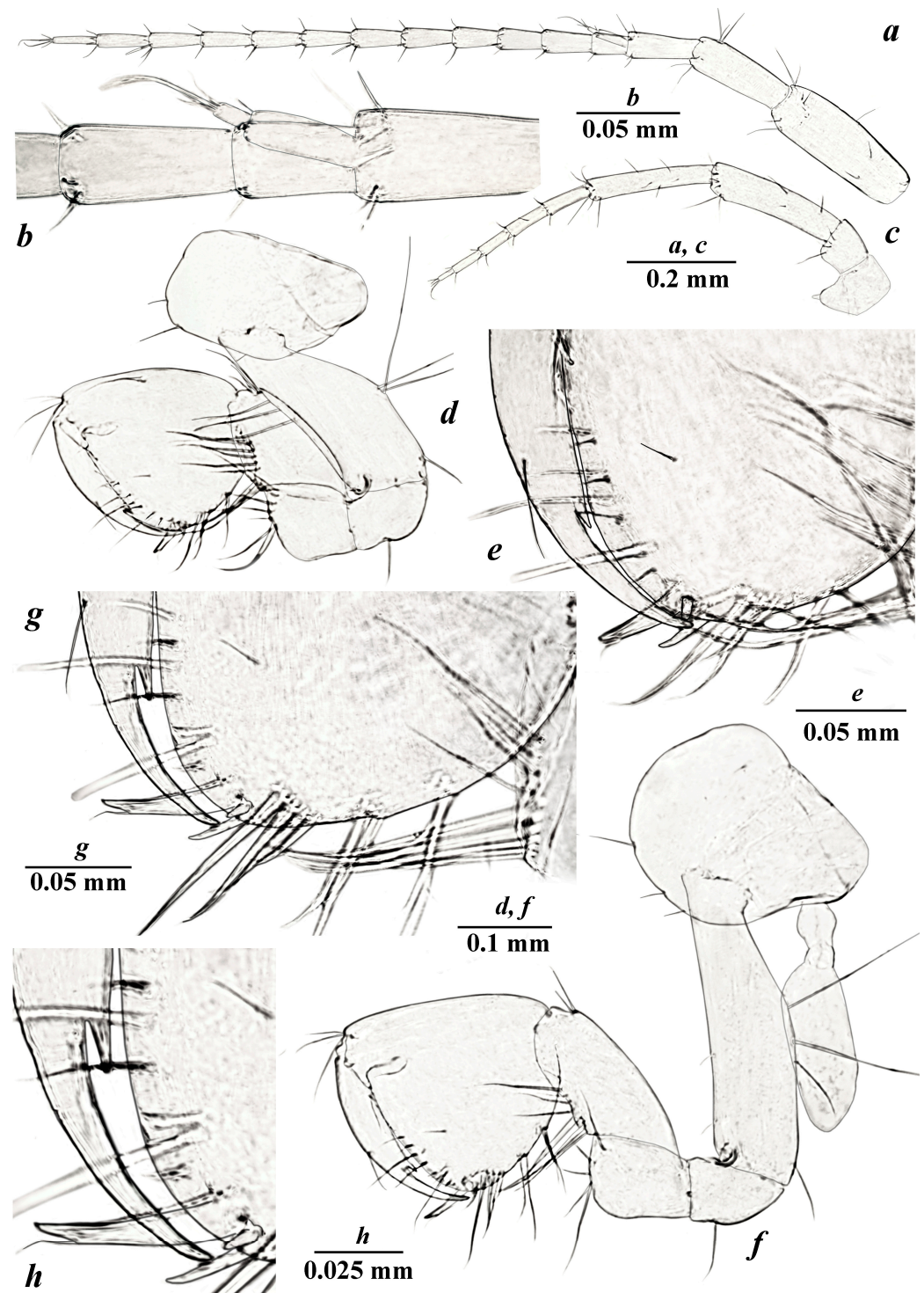
Figure 3. *Niphargus rostovi* **sp. nov.**, Rostov-on-Don, holotype ♂, ZMMU Mb-1259: (*a*)—antenna I; (*b*)—accessory flagellum of antenna I; (*c*)—antenna II; (*d*)—gnathopod I; (*e*)—distoventral palmar margin of chela of GnI; (*f*)—gnathopod II; (*g*,*h*)—distoventral palmar margin of chela of GnII.

Figure 4. *Niphargus rostovi* **sp. nov.**, Rostov-on-Don, holotype ♂, ZMMU Mb-1259: (*a*)—labrum (upper lip); (*b*)—labium (lower lip); (*c*)—left mandible; (*d*)—same, incisor process and pars incisiva; (*e*)–right mandible; (*f*)—same, incisor process and pars incisiva; (*g*)—maxilla I; (*h*)—maxilla II; (*i*)—maxilliped.

UROSOMITES (Figure 7d,e): Urosomite I unarmed; urosomite II with 1 small strong spine posterolaterally; urosomite III unarmed.

ANTENNA I (Figure 3a): slender, relatively short, 0.50–0.51 of body length; peduncular articles moderately slender, ratio is 1/0.68/0.45; flagellum consists of 12 articles, most

of them with 2 short aesthetascs each; accessory flagellum short, 2-articulated (Figure 3b). Length ratio of antennas I/II is 1/0.57.

ANTENNA II (Figure 3c): peduncular articles moderately stout, with several long setae along ventral margin, dorsal setae shorter than inner ones; flagellum relatively short, consisting of 5 articles with relatively short setae; lengths of peduncle articles 4/5 is 1/0.9; flagellum about 0.67 times of the length of peduncular articles 4 + 5.

LABRUM (Figure 4a) typical, dorsally rounded.

LABIUM (Figure 4b): with entire, subrounded outer lobes and well-developed smaller inner lobes.

MANDIBLES (Figure 4c–f). Left mandible (Figure 3c,d): incisor with 5 teeth, lacinia mobilis with 4 teeth; with a row of 6 serrated setae between lacinia and molar, few spatulate setae and one long seta at the base of molar (Figure 4d). Right mandible (Figure 3e,f): incisor process with 4 teeth, lacinia mobilis 6-dentate, with a row of 5 serrated setae between lacinia and molar (Figure 4f); ratio of mandibular palp article 2/3 (distal) is 1/1–1.05; proximal article of palp without setae; article 2 with 3 setae; distal article with 1 A-seta; 1 C-seta; 8–10 D-setae and 4 E-setae (Figure 3c,e).

MAXILLA I (Figure 4g): inner lobe with 1 distal seta, outer lobe with 7 robust spines (4 spines without lateral tooth, and 2 with 1 and 1 with 2 strong lateral teeth, respectively (0–0–0–0–1–2–1); palp 2-articulated, distal article with 4–6 simple setae distally and 2 subdistal setae.

MAXILLA II (Figure 4h): both plates with numerous long distal simple setae, outer lobe without setae along the outer margin.

MAXILLIPED (Figure 4i): inner plate relatively slender with 2 distal robust setae intermixed with 5 distal simple setae, subdistally with 1–2 simple thin lateral setae; outer plate reaching half of palpal article 2, with a row of 7 strong lateral and 3 longer distolateral spines and several distal simple setae; palpal article 3 about 2 times longer than article 4, with long simple setae along the outer margin; palpal article 4 about as long as it is wide, with produced rounded distodorsal lobe; nail shorter than pedestal, with 2 setae near basis.

GNATHOPOD I (Figure 3d): smaller than GnII; coxal plate nearly rectangular, with rounded corners, with 2 apical setae, width/depth ratio 0.64/1; basis width/length ratio is 0.40/1, 3 long setae on anterior and posterior surfaces; ischium rectangular, about as long as it is wide, with 1 subdistal setae; merus rectangular, about as long as it is wide, similar in size to ischium, with 6–7 distal and subdistal setae; carpus about 1.6 times as long as it is wide, 1.7 times of merus, with 6–8 simple setae in the inner margin, 2–3 distodorsal setae and unarmed in the outer margin; propodus subtrapezoidal, feebly setose, with 3–4 rows of setae at anterodorsal angle, palmar corner bluntly rounded, armed with 1 long spiniform palmar seta, 2 serrated stout spiniform setae, supported by 1 stout spiniform seta on the inner surface (Figure 3e); dactylus with 1 seta along the anterior margin, and strong well-marked subdistal inner tooth under the nail, with the nail 0.4 times the length of dactylus.

GNATHOPOD II (Figure 3f–h): coxal plate subrogate, with 3 apical setae, width/depth ratio is 0.88/1; basis width/length ratio is 0.27/1, 3 long setae on the posterior margin; ischium rectangular, about 1.3 times as long as it is wide, with 1 subdistal setae; merus rectangular, about 1.3 times as long as it is wide, similar in size to ischium, with 6–7 distal and subdistal setae; carpus about 2.0 times as long as it is wide, 1.8 times of merus, with 6–8 simple setae in the inner margin, 2–3 distodorsal setae and unarmed in the outer margin; propodus close to triangular shape, feebly setose, with 3–4 rows of setae at an anterodorsal angle, palmar corner bluntly rounded, armed with 1 long spiniform palmar seta, 2 serrated stout spiniform setae, supported by 1 stout spiniform seta on the inner surface (Figure 4g); dactylus with 1 seta along the anterior margin, and strong well-marked subdistal inner tooth under the nail (Figure 3h), with the nail 0.4 times the length of dactylus.
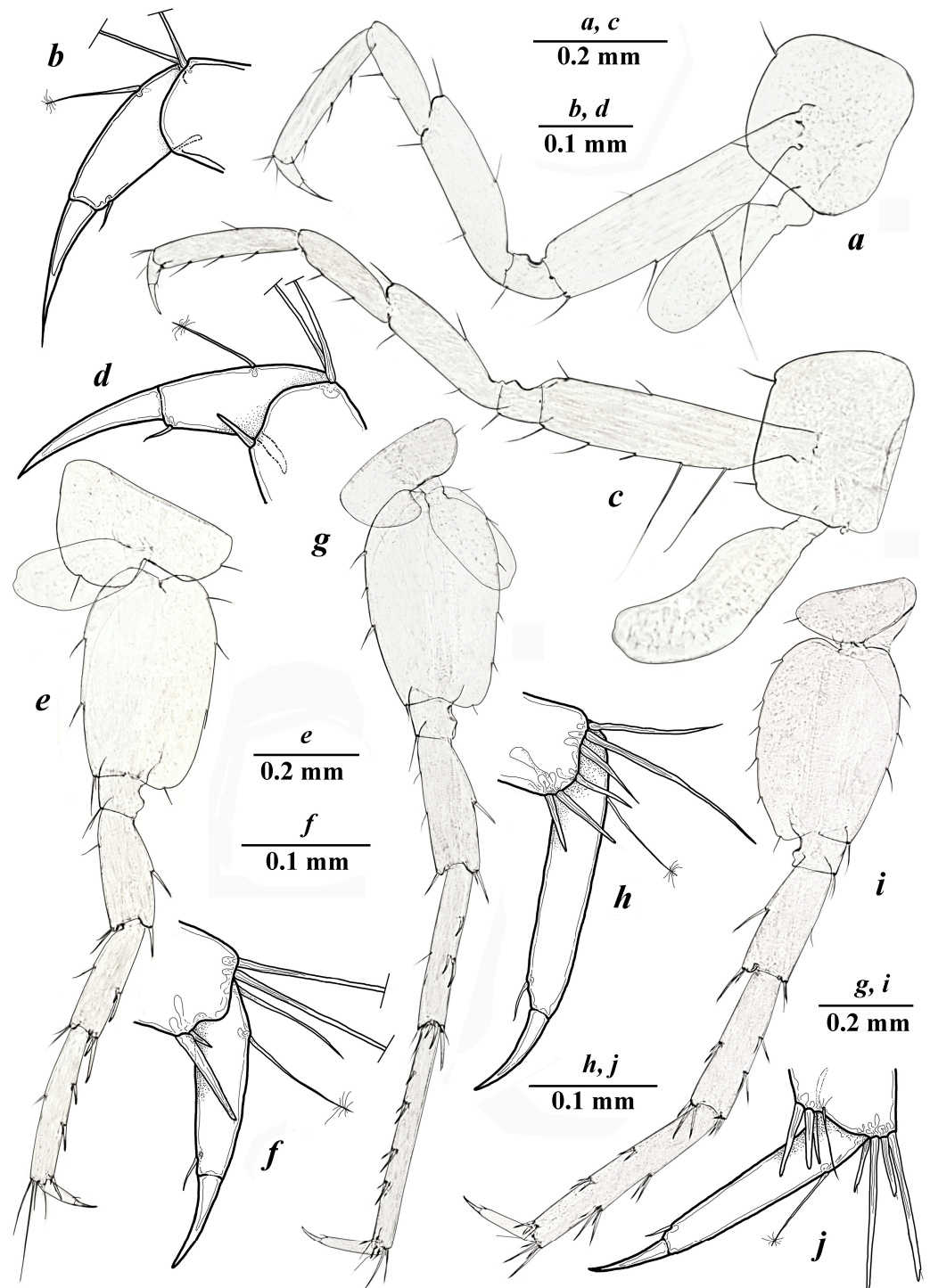
Figure 5. *Niphargus rostovi* **sp. nov.**, Rostov-on-Don, holotype ♂, ZMMU Mb-1259: (*a*)—pereopod III; (*b*)—dactylus of PIII; (*c*)—pereopod IV; (*d*)—dactylus of PIV; (*e*)—pereopod V; (*f*)—dactylus of PV; (*g*)—pereopod VI; (*h*)—dactylus of PVI; (*i*)—pereopod VII; (*j*)—dactylus of PVII.
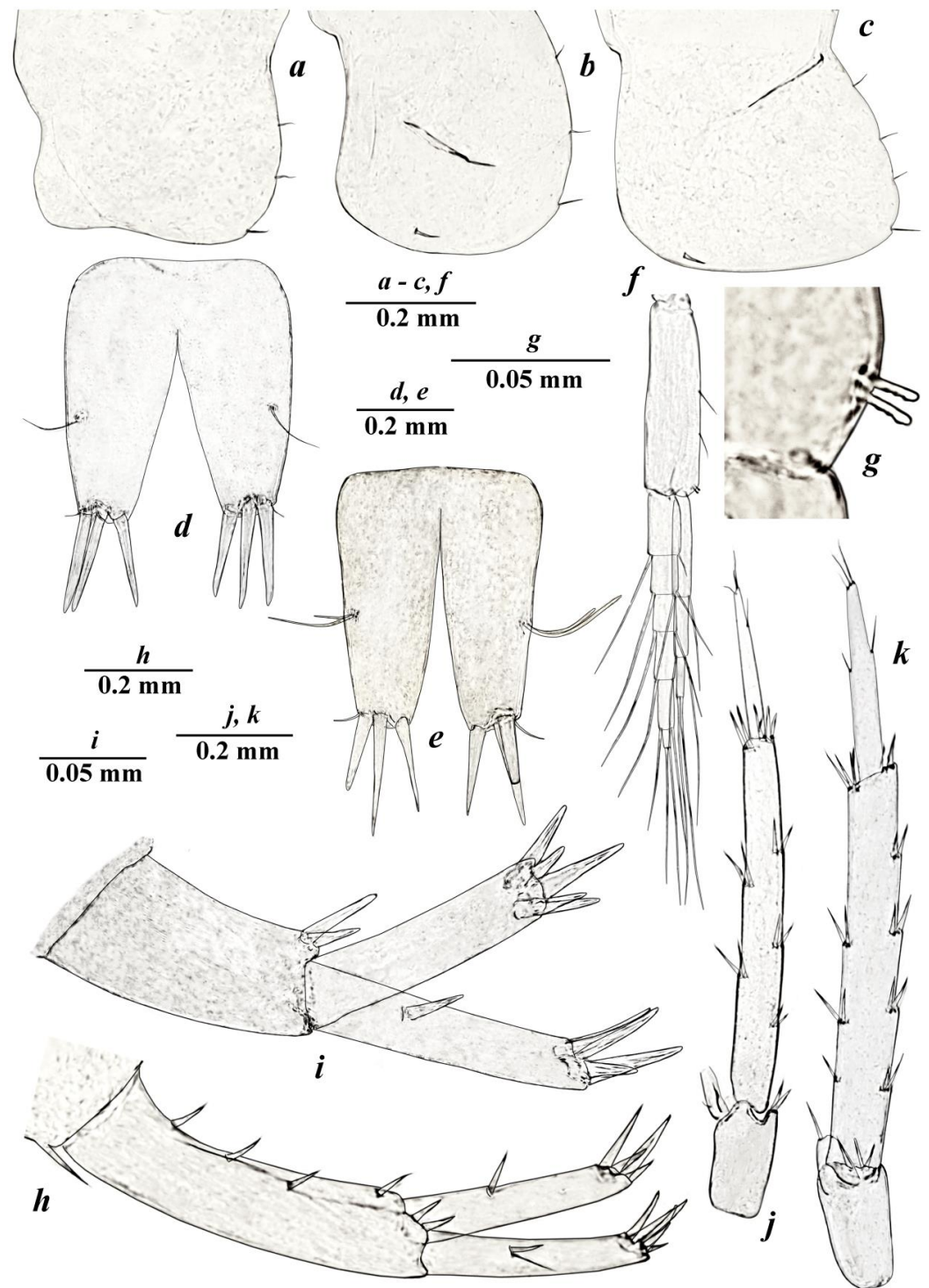
Figure 6. *Niphargus rostovi* **sp. nov.**, Rostov-on-Don, holotype ♂, ZMMU Mb-1259 (*a–i,k*), paratype ♀, ZMMU Mb-1260 (*j*): (*a*)—epimeral plate I; (*b*)—epimeral plate II; (*c*)—epimeral plate III; (*d*)—telson, female; (*e*)—telson, male; (*f*)—pleopod III; (*g*)—hooks of retinacula of pleopod II; (*h*)—uropod II; (*i*)—uropod I; (*j,k*)—uropod III.

PEREOPODS III–IV (Figure 4a–d) almost similar in size and shape; coxa mostly quadrate, straight ventrally, about as long as it is wide, with 2 setae along the anterior margin; basis about 4.5 times as long as it is wide, with the posterior margin bearing long marginal setae, with distoventral simple seta; ischium short, length/width ratio is 1.1–1/1, with ventrodistal simple seta; merus with slender simple setae, about 3.8 times as long as it is wide, along dorsal and ventral margins; ratio carpus/propodus is 0.7–0.8/1; propodus with several simple setae along posterior margin; dactylus (Figure 5b) relatively stout, curved, sharp distally, with 1 tiny ventral seta at the base of the nail and 1 long seta at the outer margin; the ratio of dactyli of propodus of PIII/IV is 0.9/1, the nail length is about 0.50–0.52 of the total length of dactylus.

PEREOPOD V (Figure 5e) coxal plate anteriorly with large lobe, with 1 anterior and 1 posterior setae; basis almost rectangular, length/width ratio is 1/0.64, with feebly developed posteroventral lobe, with almost straight posterior margin armed with several simple setae, anterior margin slightly convex armed with a row 2–3 marginal and a group of setae in the distal part; ischium short, about as long as it is wide; merus about 2.4 times as long as it is wide, with 2 slender spine-like setae along the posterior margin; carpus about 3 times as long as it is wide, equal to merus, with 2 bunches of spine-like setae along the posterior margin; propodus slender, 5–6 times as long as it is wide, with several short setae and a tuft of long simple setae anterodorsally; dactylus (Figure 5f) with basal part about 3 times as long as it is wide, with 1 tiny ventral seta at the base of the nail and 1 short seta at the outer margin.

PEREOPOD VI (Figure 5g) coxal plate anteriorly with a large lobe, without anterior and with 1 posterior seta; basis almost rectangular, length/width ratio is 1/0.58, with feebly developed posteroventral lobe, with almost straight posterior margin armed with several simple setae, anterior margin slightly convex armed with a row 3 marginal and a group of setae in the distal part; ischium short, about as long as it is wide; merus about 3.1 times as long as it is wide, with 2 slender spine-like setae along the posterior margin; carpus about 5 times as long as it is wide, subequal to merus, with 2 bunches of spine-like setae along the posterior margin; propodus slender, 9–10 times as long as it is wide, with several short setae and a tuft of long simple setae anterodorsally; dactylus (Figure 5h) with basal part about 5 times as long as it is wide, with 1 tiny ventral seta at the base of the nail and 1 short seta at the outer margin.

PEREOPOD VII (Figure 5i): coxa half-rounded shaped with 1 posterior tiny seta; basis length/width ratio is 1/0.69, without distinct posteroventral lobe and convex posterior margin bearing a row of 5–6 short marginal setae, anterior margin slightly convex, with a row of 3 longer marginal and a group of setae in distal part; ischium short, about as long as it is wide; merus about 2.6 times as long as it is wide, with several slender spine-like setae along the anterior and posterior margins; carpus about 3.8 times as long as it is wide, equal to merus, with 2 bunches of spine-like setae along the posterior margin; propodus slender, about 5–6 times as long as it is wide, with several groups of short spine-like setae and a tuft of long simple setae anterodorsally; dactylus (Figure 5j) with basal part about 4–5 times as long as it is wide, with 1 small ventral seta at the base of the nail a and 1 long seta at the outer margin.

PEREOPODS V–VII (Figure 5e–j); length ration of PpV/VI/VII is 1/1.43/1.48; length of PVII is about half of total body length.

GILLS II–VI ovoid. Coxal gills II–VI ovoid, length ratios of gills/bases of pereopods are 0.58/1, 0.57/1, 0.79/1, 0.62/1 and 0.51/1, respectively.

PLEOPODS (Figure 6f): pleopods I–III with basal segments with 2 long simple setae, with 2 hooks in retinacules (Figure 6g).
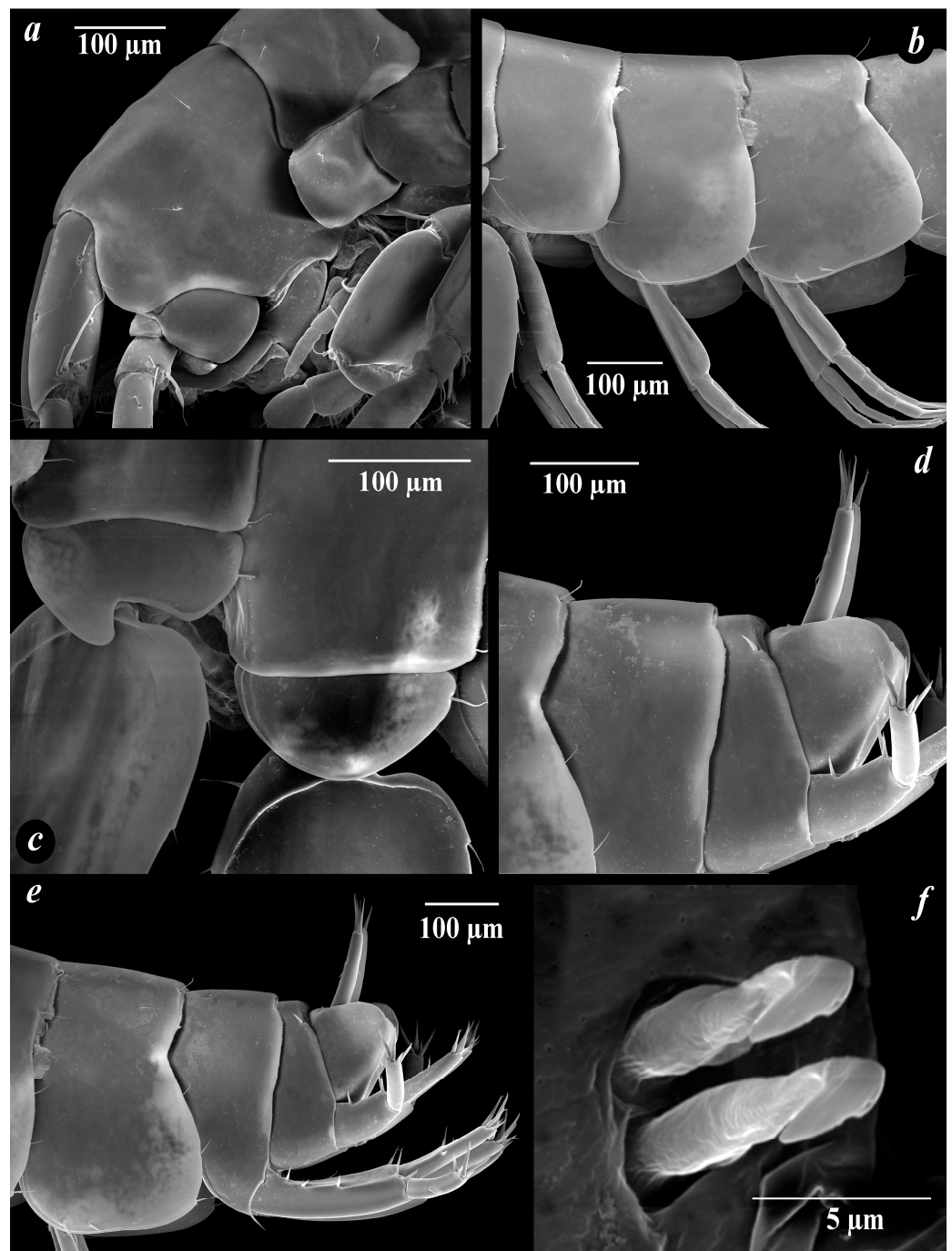
Figure 7. *Niphargus rostovi* **sp. nov.**, Rostov-on-Don, ♂, non-type specimen, LEMMI: (*a*)—head; (*b*)—epimeral plates I–III; (*c*)—coxal segments of pereiopod VI–VII; (*d*,*e*)—urosomal segments, lateral view; (*f*)—hooks of retinacula of pleopod II.

UROPOD I (Figure 6h): protopodite about 4.5 times as long as it is wide, with 3 dorsointernal and 2 subdistal spines; rami straight, equal in length and equal to protopodite; exopodite not paddle-like, straight, with 1 medial spiniform setae laterally and 4 strong spines apically; endopodite without setae laterally, with 4–5 spines apically.

UROPOD II (Figure 6i): protopodite about 2.0 times as long as it is wide, about 1.2 times shorter than rami in length, with 2 subdistal spines only; rami with 1 lateral and 4–5 distal slender spines; ratio of exopodite/endopodite is 1.1/1.

UROPOD III (Figure 6k,j): 0.18–0.20 of body length in females and males; protopodite about 2.0 times as long as it is wide, without lateral setae and 2–3 apical spines; rami unequal, endopodite short, about 9 and 10–11 times shorter than the exopodite in females and males, respectively, with 1 small setae laterally and 1 long setae apically; exopodite with proximal article with 5–7 groups of thin-flexible, spiniform setae along inner and outer margins and 2 distolateral groups of simple setae distally; distal article is 0.4 and 0.5 of the length of proximal article in females and males, respectively, with and 1–2 lateral setae and 2–3 long simple setae apically.

TELSON (Figure 6d,e): ratio of length/width is 1/1.2–1.4; cleft about 0.9 of telson length; margins straight and narrowing apically; with 1–2 medial long simple setae on each side, and 3 strong distal spines accompanied with 1–2 small simple setae; apical setae are about 0.5 of length of telson.

Coloration and Body size: Body coloration is completely white. The largest collected male has bl. 3 mm.

GenBank accession numbers: OQ918541, OQ918542.

Distribution and ecology: Currently, the species is known only from a single spring on the slope of the Kiziterinka River in the Proletarskiy district (Nakhichevan) within the borders of the city of Rostov-on-Don. It is possible that the species may live in other nearby springs, but the careful study in 2021–2022 did not allow for collecting more specimens in other localities. However, the collecting of this species is rather difficult due to its very small size (about 2.5–3 mm of body length for adults), as well as habitat in the thickness of the pebbles covering the bottom of springs and wells. The other subterranean crustaceans inhabiting the same spring are crangonyctid *Diasynurella kiwi* Marin and Palatov, 2023 and *Pontonyx donensis* (Martynov, 1919) (Amphipoda: Crangonyctidae) [56,57].

Taxonomic remarks: According to morpho-molecular analysis (see above, Figure 1), the new species clearly belongs to the obviously polyphyletic "carpathicus" species complex, mostly related to *Niphargus karkabounasi* Ntakis, Anastasiadou, Zakšek and Fišer, 2015 found in continental Greece [73] (Figure 1). Both species form a distinct phylogenetic lineage (see above) and possess only 2 hooks in the retinacules of pleopods in contrast to other related species from the "*carpathicus*" complex, for example, Romanian *Niphargus carpathicus*, *N. decui*, *N. transsylvanicus*, as well as *N. grandii*, *N. aberrans*, *N. microcerberus*, *N. barbatus*, *N. italicus* G. Karaman, 1976, *N. danielopoli* G. Karaman, 1994 and *N. alpinus* G. Karaman and Ruffo, 1989, found in Austria, Northern Italy and Slovenia and one undescribed species from the Crimean Peninsula, all of which have 4–5 hooks in the retinacules [73]. This feature most easily and reliably allows separating these species from the congeners.

The new species can be easily separated from *N. karkabounasi* by the presence of unarmed (smooth) spines on the outer lobe of maxilla I (Figure 4g) (vs. usually with 1–2 lateral teeth ([73]: Figure 8D); trapezoidal form of palm (propodus) of both gnathopods I–II (Figure 3d,f) (vs. distinctly triangular [73]: Figure 9); unarmed protopodite of uropod II (Figure 6i) (vs. 1–2 dorsointernal spines [73]: Figure 7e); rounded and non-produced posteroventral angle of epimeral plate I (vs. sharply posteriorly produced [73]: Figure 7a), and stouter and shorter distal spines of each telson lobe (Figure 6d,e).

From *N. decui* (after [80]), the new species can be also separated by significantly shorter accessory flagellum of antenna I, unarmed (smooth) spines on the outer lobe of maxilla I, rounded posteroventral angles of epimeral plates I–III, slender basal segments of pereiopod III-VII without well-developed posterior lobes, 2 hooks in the retinacules of pleopods, different armature (mostly reduced) of the urosomal segments, uropods I–II and distal margins of lobes of telson.

From *N. italicus* (after [81]), the new species can be separated by the slender basal segment of antenna I, significantly shorter accessory flagellum of antenna I, unarmed (smooth) spines on outer lobe of maxilla I, rounded posteroventral angles of epimeral plates I–III, non-produced palmar corner of gnathopods I–II, more slender basal segments of pereiopods III–VII without well-developed posterior lobes, 2 hooks in retinacules of

pleopods, different armature (mostly reduced) of the urosomal segments, uropods I–II and distal margins of lobes of telson.

Niphargus potamophilus **Birštein**, 1954
Figure 8
Niphargus potamophilus Birštein, 1954: 1025–1028, Figure 1 (type locality–mouth of the Don River)

Material examined: 5♂♂, 7♀♀, LEMMI, Crimean Peninsula, Sevastopol area, the mouth of the Belbek River, Lubimovka, in a small canal flowing from a small artificial pond, 44°39′41.6′′ N 33°32′47.7′′ E, about 3–5 m asl, hand net sampling, 15 December 2022, coll. I. Marin.

**GenBank accession numbers:** OQ913572, OQ913573.

Ecology: *Niphargus potamophilus* lives in channels and small, shallow, densely overgrown, well-warmed ponds and parts of the rivers where large predators (e.g., fish) and other epigean species of the genus Niphargus are absent.

Distribution: The species was originally described from small water reservoirs in the delta of the Don River near Rostov-on-Don [50]. Sub-endemic of the Northern Black/Azov Sea Lowland, currently found in the Azov–Prikubanskaya Lowland (from Rostov-on-Don to the lower delta of Kuban River) [14] and the coastal area of the Northern Black/Azov Sea Lowland from southern Ukraine to Bulgaria [63] (Figure 9). A new discovery in the Crimean Peninsula connected the known eastern area of its distribution in the delta of the Kuban and Don rivers with the western one in the deltas of the Dnieper, Dniester and Danube.

4. Discussion

The diversity and endemism of the stygobiotic fauna of the mountainous regions of the Caucasus and the Crimean Peninsula in the south, especially their southern slopes, as well as the Carpathian Mountains in the west of the studied region, are very well known, and many endemic species have been described from there (see above; Table 2) [82–86]. In the past, the abovementioned mountainous regions were interconnected with the Middle East, representing a West Asian transition region for subterranean/stygobiotic animals [86–89] within the boundaries of Paratethys [57,58]. The diversity of these regions largely exists due to maintaining of a stable warm climate during periods of cooling and glaciation, determined by the presence of glacial refugees during the Pliocene/Pleistocene time, such as well-known Colchis and Hyrcania [90,91]. A small refugium, or several scattered ones, probably also existed on the northern slope of the Greater Caucasian Ridge [15,92,93]. However, the adjacent foothill and coastal plains, for example, the Black Sea Lowland, Azov–Prikubanskaya Lowland and the northern Crimean Lowland, and especially their parts significantly removed from the modern sea line and mountains, have been studied much worse in terms of diversity and endemism. At the same time, only a few endemics have been found in these areas (see Table 2). Low diversity in these areas can also be explained by the influence of ancient periods of cooling and glaciation, especially the significant influence of the cold climate in Pleistocene [94,95].
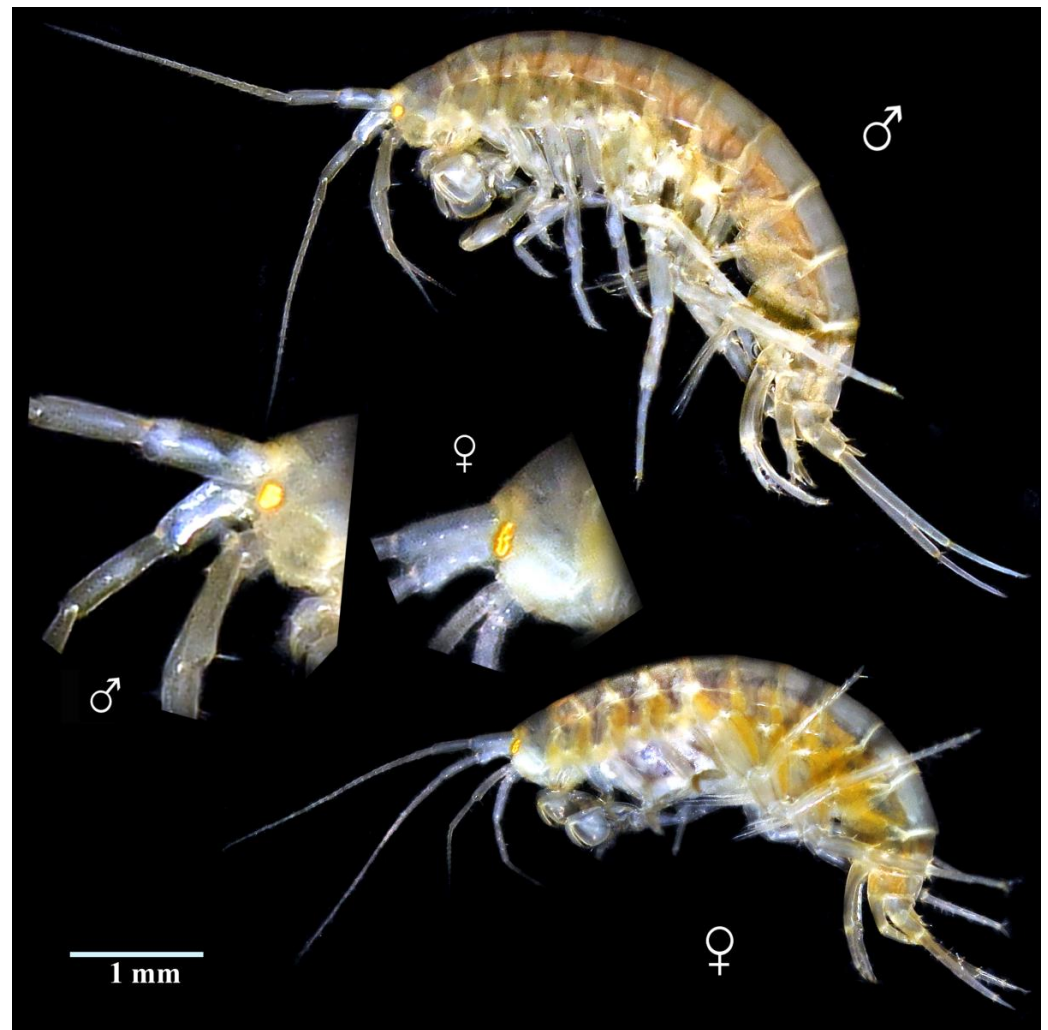
Figure 8. *Niphargus potamophilus* Birštein, 1954 from the mouth of the Belbek River, Crimean Peninsula, general view, alive coloration.

According to the latest molecular genetic data, most of the endemic and sub-endemic species of the genus *Niphargus* for the Northern Black Sea and the Azov–Prikubanskaya lowlands, Caucasus and Crimean Peninsula are usually related to southwestern and western European relatives [6,9–11,13,15,96–99], which indicates the presence of a common freshwater or brackish water ancestor during the existence of the Eastern Paratethys [57–59]. The formation of the Carpathian arc–shaped mountain belt led to the final division of Paratethys into two parts in the Late Miocene (about 11.63–5.333 Mya)—the Central Paratethys (Pannon Lake, or Pannonian Basin) and the Eastern Paratethys (Dacian, Euxine and Caspian basins) [64,65,98,99]. Later, the Messinian crisis (5.96–5.33 Mya), fluctuations in the level/salinity of the separated abovementioned basins of the Eastern Paratethys during the Pliocene (5.333–2.58 Mya), divided ancestral genetic lineages into various refuges, where they survived during the Pleistocene periods of cooling/glaciation (2.58 Mya–11.7 Tya) [100,101], also representing the recent speciation [54]. Formation of the northwestern foothills of the Great Caucasian Ridge and salinization of the Azov Sea basin during the last 1.0–1.5 Mya probably led to the formation of a rather isolated Azov-Prikubanskaya Lowland and its specific fauna.
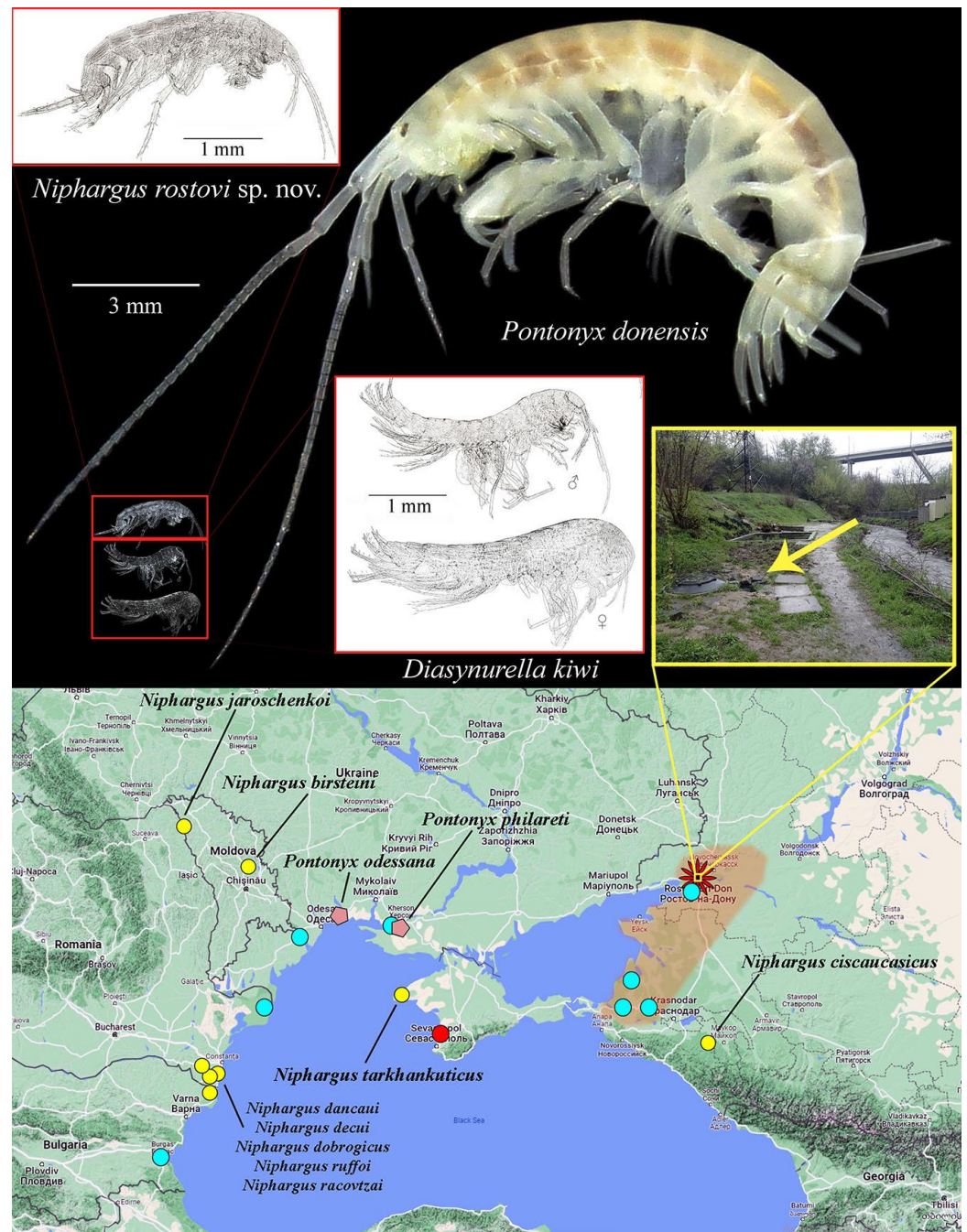
Figure 9. Stygobiotic crustaceans from the studied spring on a shore of the Kiziterinka River (Rostov-on-Don) in the mouth of the Don River (above) and (below) currently known diversity of different endemic and sub-endemic amphipods (Crustacea: Amphipoda), showing different types of the distribution (local endemic and sub-endemic) in the Northern Black/Azov Sea Lowland bordered with estuaries of the rivers Kuban and Don (Sea of Azov), Dnieper, Dniester and Danube (Black Sea). Star indicates the well-studied region in the Rostov-on-Don, inhabited by endemic and stygobiotic *D. kiwi*, *P. donensis* and *N. rostovi* **sp. nov**. Yellow circles indicate different species of the genera *Niphargus* (signed); red (new record in the Crimean Peninsula) and blue circles indicate the distribution of *N. potamophilus*. Rhombs indicate the known records of the genus *Pontonyx*; red-colored area indicates *Cryptorchestia* cf. *garbinii* (see Table 2). Fauna of the mountainous (Caucasian, Crimean and Carpathian) areas are not presented.

Recent studies indicate the existence of a number of narrowly distributed endemics on the Northern Black/Azov Sea Lowland, among the species that are not capable of moving and expanding their range, for example, slave-making ants of the genus *Strongylognathus* Mayr, 1853 (Hymenoptera, Formicidae) [102,103], stygobiotic amphipods of the genera *Niphargus* Schiödte, 1849 (Amphipoda, Niphargidae) [6,12,52,64,81,104–106], *Diasynurella* Behning, 1940 and *Pontonyx* Palatov and Marin, 2021 (Amphipoda, Crangonyctidae) [52,55,56] (see Figure 9), as well as, for example, sandy blind mole-rat *Spalax arenarius* Reshetnik, 1939, strictly distributed in steppe habitats of the most southern left the left bank of the Dnieper river [53,107]. The unique subterranean sulfide cave ecosystems of eastern Romania (for example, the Movile Cave), isolated for about 5.0–2.5 Mya, are inhabited by a whole complex of endemic animals of different taxonomic groups [108–110]; in addition, a kind of endemism is observed for the fish of the Kuban River [111]. However, such studies are limited, and present-day records are largely random, whereas the number of endemic and narrowly distributed species discovered at the moment may be underestimated. There are also a number of sub-endemic species characteristic of the Azov–Prikubanskaya Lowland (e.g., form separate lineages of landhooper of *Cryptorchestia* cf. *garbinii* Ruffo, Tarocco and Latella, 2014 (pers. observ, in prep.) and freshwater mussel *Anodonta anatina* (Linnaeus, 1758) [54], as well as the whole Black/Azov Sea Lowland (e.g., *N. potamophilus* [14,63]) (see Figure 9) or different freshwater mollusks and snails [112] (see Table 2).

The discovery of subterranean/stygobiotic species in this area, especially from the genera *Niphargus*, *Pontonyx* and *Diasynurella* (see Figure 9; Table 2), in our opinion, clearly indicates the existence of an ancient refugium in this area, since they cannot move into new territories [17]. The new species, *Niphargus rostovi* **sp. nov.**, diverged from the most related *Niphargus karkabounasi* also for about 5 Mya (see above), as well as co-existing crangonyctid *D. kiwi* and *P. donensis* for more than 20 Mya or an even longer time [55,56], indicating that they have existed here at least since the Plio-Pleistocene times, and probably successfully survived these events. A number of strictly endemic and a whole group of sub-endemic species for the Northern Black Sea region (see Table 2) indicates the existence of a glacial refugia in this area, similar to the Colchis and Hyrcania in the southeast, as well as the Carpathian and Italian in the southwest.

A significant morpho-geological difference of the glacial refugium in the Northern Black/Azov Sea Lowland is the absence of a restraining (protecting) northern ridge of mountains or a separate mountain range, in contrast to the Great Caucasian Ridge, for example, mostly protecting the Colchis from the cold air masses. In this case, the Black and Azov Seas basins probably maintained the microclimate stability, restraining a large intake of cold air, as it is happening at present. The deepest known ancient episodes of glaciation to the south reached the northern borders of Ukraine and the modern Rostov and Samara regions (for example, Don Glaciation (Donian Glaciation or the Donian Stage)), the major glaciation of the Eastern European Plain, 0.5–0.8 Mya, during the Cromerian Stage of the Middle Pleistocene [113]. The northernmost of the currently known representatives, for example, of the genus *Niphargus*, are distributed up to the northern slopes of the Carpathian Mountains and further north have been not found, indicating that their northward distribution boundary was probably limited by the Pleistocene glaciation [51,114–117]. Moreover, *Volgonyx dershavini* (Behning, 1928) is one of the northernmost stygobiotic crangonyctid amphipods (Amphipoda: Crangonyctidae), survived in the Saratov region of the Volga Upland since at least Miocene [118], also pointing to the southeastern border of the past events of glaciations. The post-glacial re-colonization also largely explained the presence in the fauna of some elements of the Western European and Caucasian fauna in the Black/Azov Sea Lowlands [119–123]. Two epigean species of the genus *Niphargus*, *N. hrabei* S. Karaman, 1932 are *N. valachicus* Dobreanu and Manolache, 1933, currently found in the coastal swamp area of SW Caucasus [14,63] and also the Pannonian River Basin [60–62]. Both species are of the Ponto-Caspian origin, dispersed in the Black/Azov Sea Lowland from the Pannonian region throughout the Danube riverbed possibly during or just after the Last Glacial Maximum (30–25 Tya). At that time, the area of the Black and Azov Sea including

the estuary of the Dnieper, Dniester, Danube and Don Rivers represented a freshwater lake, isolated from the World Ocean [62,63,124–126], and lately (about 7–9 Tya) separated by flooding of the saline waters from the Mediterranean Sea [127–129]. At the same time, another epigean species, *Niphargus potamophilus* Birštein, 1954, is most likely native to the Black/Azov Sea Lowland, while currently its populations are somewhat scattered [14,63] or still undiscovered.

We believe that the current distribution of subterranean/stygobiotic species in the Northern Black/Azov Sea Lowland is determined in the past by both the periods of glaciation and the boundary of the Eastern Parathethys. The findings of a new stygobiotic species of *Niphargus* in the mouth of the Don River and related species in continental Greece confirm this fact, since they are all located along the boundaries of the Parathethys during the Miocene, about 10–7 Mya [57,59]. Ancient historical events significantly modernized the original fauna both by advancing the seawater boundary with the modern Black Sea coast, which flooded the available habitats of stygobiotic animals while water masses of the Black and Azov seas helped to maintain a warm and stable climate, and by shifting the northern distribution boundary to the south under the influence of deeply penetrating episodes of glaciation. At present, the primordial relict habitats of the time of the Eastern Parathethys are probably represented only by a narrow landline (lowland) along the northern coastline of the Black and Azov seas, which we consider as a refugium.

Table 2. The endemic/sub-endemic species of non-mountainous part of the northern Black and Azov Sea area (the diversity of the Movile Cave, eastern Romania, is excluded [110]).

Species	Distribution	Lifestyle and Habitats	Reference
	CRUSTACEA: AMPHIPODA		
Synurella philareti Birštein, 1948 (=*Pontonyx philareti*)	Endemic, a spring in a mouth of Dnieper	stygobiotic, in springs	[130]
Pontonyx odessana (Sidorov and Kovtun, 2015)	Endemic, catacombs of Odessa	subterranean water reservoirs in the catacombs	[52]
Pontonyx donensis (Martynov, 1919)	Endemic, several springs in the Rostov-on-Don	stygobiotic, in springs	[48,56]
Diasynurella kiwi Marin and Palatov, 2023	Endemic, a spring in the Rostov-on-Don	stygobiotic, in spring	[55]
Niphargus ciscaucasicus Marin and Palatov, 2019	Endemic, Apsheronsk area	stygobiotic, inside well	[11]
Niphargus birsteini Dedyu, 1963	Endemic, a spring in Pyatra village, Moldova	stygobiotic, in spring	[51]
Niphargus jaroschenkoi Dedyu, 1963	Endemic, a spring in Novye Badrazi village, Moldova	stygobiotic, in spring	[51]
Niphargus tarkhankuticus Marin, Turbanov, Prokopov and Palatov, 2022	Endemic, Tarkhankut Plain, Crimean Peninsula	stygobiotic, in wells	[9]
Niphargus dancaui Brad, Fišer, Flot and Sarbu, 2015	Endemic, Movile Cave and surrounding area in Mangalia	sulphidic groundwater, caves	[97]
Niphargus dobrogicus Dancau, 1964	Endemic, Doi Mai, Schitu and Vama Veche (Dobrogea) in Eastern Romania	sulphidic groundwater	[104,108]
Niphargus ruffoi (Karaman and Sarbu, 1993)	Endemic, Hagieni Spring near Mangalia	sulphidic groundwater	[106,108]
Niphargus racovitzai (Dancau, 1968)	Endemic, Movile Cave, Mangalia	sulphidic groundwater	[105,108]
Niphargus decui G. Karaman and Sarbu, 1995	Endemic, in southern Dobrogea in Mangalia	stygobiotic, in well	[80,108]
Antrobathynella stammeri stammeri (Jakobi, 1954)	Endemic, Danilo-Ivanovka village, Zaporozhye region	stygobiotic, in spring	[131]
Bathynella natans ukrainica Monchenko, 1968	Endemic, from the Chumshe spring in the vicinity of the village of Vladimirovka, Odessa region	stygobiotic, in spring	[131]
Niphargus potamophilus Birštein, 1954	Sub-endemic, the Northern Black/Azov Sea Lowland	epigean, in ponds and other water reservoirs	[14,53,63]
Cryptorchestia cf. *garbinii* Ruffo, Tarocco and Latella, 2014 (a separate mitochondrial DNA (COI) lineage)	The Azov–Prikubanskaya Lowland	terrestrial, cryptic	pers. observ, in prep.
Pontogammarus cf. *maeoticus* (Sovinskij, 1894) (a separate mitochondrial DNA (COI) lineage)	Eastern Crimean Peninsula	free living, fresh water	[132]
	INSECTA		
Ecdyonurus dispar gratificus Martynov and Godunko, 2013	Endemic to the Donetsk Ridge	fresh water, stream	[49]
Dorcadion spp. (*ciscaucasicum*-group, *cinerarium*-group)	Endemic or sub-endemic to Northern Black and Azov Sea Lowland	terrestrial	[133]
Colletes tardus Noskiewicz, 1936	Kherson Province	terrestrial	[134]
Melitta budashkini Radchenko and Ivanov, 2012	Cape Chauda, the Kerch Peninsula	terrestrial, xerophytic steppe	[135]
Andrena stepposa Osytshnjuk, 1977	Donetsk region, Khomutovskaya steppe, valley of the Gruzsky Elanchik river, Kharkiv and Voronez regions	terrestrial	[136]
Strongylognathus arnoldii Radchenko, 1985	Endemic, Tarkhankut Plain, Crimean Peninsula	terrestrial	[137,138]
Strongylognathus chelifer Radchenko, 1985	Endemic, Kherson oblast, Askania-Nova	terrestrial	[102,103,138]

Table 2. *Cont.*

Species	Distribution	Lifestyle and Habitats	Reference
	GASTROPODA		
Elia novorossica nagolnica Balashov, 2013	Endemic of Donetsk Upland	terrestrial	[139]
Clathrocaspia knipowitchi (Makarov, 1938)	Sub-endemic. In the Dnieper River near Kherson City, Don River and Caspian Sea	in rivers and freshwater parts of limans	[140,141]
Clathrocaspia stanislavi (Alexenko and Starobogatov, 1987)	Endemic, in the mouth of the Don river	in the stream of the river; probably crenobiotic [see 142]	[140–142]
Laevicaspia lincta (Milaschewitsch, 1908)	Sub-endemic, along the northern Black and Azov Sea coastal area	in fresh and brackish waterbodies	[141–143]
Laevicaspia ismailensis (Golikov and Starobogatov, 1966)	Endemic, along the northern Black and Azov Sea coastal area	in fresh and brackish waterbodies	[141–143]
Clessiniola variabilis (Eichwald, 1838)	Sub-endemic, northern Black and Azov Sea coastal area	in fresh and brackish waterbodies	[141–143]
Turricaspia chersonica Alexenko and Starobogatov, 1987	Endemic, northern Black and Azov Sea coastal area	in fresh and brackish waterbodies	[141–143]
	BIVALVIA		
Anodonta anatina (a separate mitochondrial DNA (COI) lineage)	The Azov–Prikubanskaya Lowland	fresh water	[54]
Adacna fragilis Milaschewitsch, 1908	Endemic, the northern Black Sea maritime area	fresh and brackish waterbodies	[141,144]
Adacna colorata (Eichwald, 1829)	Sub-endemic, the northern Black Sea maritime area	fresh and brackish waterbodies	[141,144]
Hypanis plicata relicta (Milaschewitch, 1916)	Endemic, the northern Black Sea maritime area	fresh and brackish waterbodies	[141,144]
	FISHES		
Barbus kubanicus Berg, 1912	Endemic of the Kuban River	fresh water	[11]
Sabanejewia maeotica Vasil'eva and Vasil'ev, 2023	Endemic of the lower Don River	fresh water	[145]
Sabanejewia spp., *Romanogobius* spp., *Alburnus* spp., *Leuciscus* spp. (some species)	Local endemics of Ponto-Caspian Basin	fresh and brackish waters	[145–147]
	MAMMALIA		
Spalax arenarius Reshetnik, 1939	The southern part of the left bank of the Dnieper River, opposite the city of Kherson	terrestrial, burrowing	[54,107,148]

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