



A new species of the genus *Lucasioides* Kwon, 1993 (Isopoda, Oniscidea, Agnaridae) from Siberia, Russia

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Abstract

A new species of the family Agnaridae, *Lucasioides altaicus* **sp. nov.**, is described from the Altai Mountains, southwestern Siberia, based both on morphological characters and molecular data. This species is the first record of *Lucasioides* from Russia, whose location is the northernmost habitat of terrestrial isopods in indigenous habitats presently known to Eurasia. The diagnostic characters of the new species and a preliminary phylogenetic analysis within Agnaridae are provided.

Key words: woodlouse, *COI*, phylogenetic analysis, Altai, Siberia

Introduction

The northern range limit of isopod distribution in the Northern Palearctic is known to depend on the mean annual temperature (Kuznetsova & Gongalsky 2012), coinciding mainly with the border between the boreal and temperate forests. However, there are still unprospected areas close to the northern range limit of Oniscidea, many of them lying in southern Siberia. One of such spots is the Altai Mountains. The area attracts special attention due to its unique position in Asia, bordering on Mongolian and Kazakhstan steppes which are known to support woodlice. At present, only a few species have been described from the northern parts of these countries, the geographically closest species being *Desertoniscus mongolicus* Borutzky, 1978 from Bulgan, Mongolia, and *Desertoniscus zhe-lokhovtsevi* Borutzky, 1945 from northern Kazakhstan (Borutzky 1945, 1978; Gongalsky 2017). To date, there are at least seven synanthropic woodlouse species that are known to occur in southwestern Siberia (Khisametdinova *et al.* 2016; Nefediev *et al.* 2018), all recorded from the Altai Mountains within Russia. In this context, we report below the first indigenous woodlouse species from Siberia which belongs to the genus *Lucasioides* Kwon, 1993 as defined by Kwon (1993). *Lucasioides* has hitherto comprised 29 species, all confined only to China, Korea and Japan (Schmalfuss 2003; Nunomura 2008, 2010, 2013; Li 2017; Boyko *et al.* 2008 onwards).

Material and methods

The material treated here has been collected in 2015–2018 by the second co-author and students at the Altai State University in the lowlands of the Altai Mountains, environs of Charyshskoye Village, Altai Krai, southwestern Siberia, Russia. The specimens were taken by hand and fixed in 70% or 96% ethanol. Processing and dissections were executed using a Leica MZ8 dissection microscope. Micropreparations of diagnostic body appendages were

made in Euparal (Carl Roth GmbH). Line drawings were executed with the help of an Olympus BX41 compound microscope supplied with an Olympus U-DA camera lucida. Some individuals were attached to stubs, coated with gold in an S150A Sputter Coater, and studied under a Tescan Vega TS5130MM scanning electron microscope.

The material is deposited in the Zoological Museum of the Lomonosov Moscow State University, Russia (ZMMU), the Altai State University, Barnaul, Russia (ASU) and partly retained in the private collection of the first co-author (KG), as indicated below.

The terminology used in the species description mainly follows Vandel (1960).

DNA extraction and amplification

Total DNA was extracted from material fixed *in vivo* in 96% ethanol from one of the pereopods IV or V. Total cell DNA was isolated using a QIAamp DNA Investigator Kit (Qiagen, Germany). A fragment of the COI mtDNA locus was used to analyze genetic variation. The study was carried out using a full-length mtDNA fragment obtained by amplification with LCO1490 and HCO2198 primers (Folmer *et al.* 1994).

The polymerase chain reaction was carried out on a Bio-Rad T 100 thermocycler (Bio-Rad, USA) in a specially selected temperature regime: the initial denudation of 95°C was 5 min; annealing of 95°C for 35 sec, 48°C for 40 sec, 72°C for 40 sec (35 cycles), the final elongation of 72°C lasted for 7 min. For the PCR, a set of reagents for the amplification of “5x Mas Mix-2025” manufactured by Dialat Ltd (Moscow, Russia) was used. The 15 µl reaction mixture contained 1 µl total DNA, 3 µl mix and 1 µl of each primer. The amplification products were separated by electrophoresis in 1.5% agarose gel in 1x TBE and visualized with ethidium bromide. The DNA sequence was determined with a forward and reverse primer using the Big Dye 3.1 kits on an ABI 3500 genetic analyzer from Applied Biosystems, USA, in a POP7 polymer environment.

Phylogenetic analysis of molecular data

The sequences obtained were aligned with the help of BioEdit software version 5.0.9. The gene fragments of COI mtDNA, 652 bp in length, were used in the phylogenetic analysis. The best evolutionary substitution model was determined using MEGA version 7.0. The phylogenetic analysis was performed using MrBayes version 3.2.7a for Bayesian analysis (BA) with GTR + G + I evolutionary model for Maximum-Likelihood (ML), and Kimura-2-parameter (K2P) model. Bayesian analysis was carried out by sampling one tree every 1,000 replications over 1,000,000 generations. The divergence of pairwise genetic distances (*p*-distances) was calculated using the Kimura-2-parameter (K2P) model in MEGA version 7.0.

The COI mtDNA sequences of the new species were used for the phylogenetic analysis. We also used some sequences of congeners available in GenBank (NCBI) and confirmed in publications concerning the family Agnaridae Schmidt, 2003 (see Table 1). Genetic sequences for species of the genus *Orthometopon* Verhoeff, 1917 (Poulakakis & Sfenthourakis 2008) were not applied to the phylogenetic analysis due to the small number of base pair linear COI mtDNA, ranging from 381 to 450.

TABLE 1. List of specimens, sampling sites and accession numbers of the sequences for the COI mtDNA included in this study. References are given for sequences obtained from the GenBank (NCBI)

Species	Locality	GenBank No.	Sequence reference
<i>Desertoniscus zaitsevi</i>	Russia, Republic of Kalmykia	MH400725	Gongalsky <i>et al.</i> 2018
<i>Hemilepistus klugii</i>	Iran, Isfahan	MG887938	Dimitriou <i>et al.</i> 2018
<i>Hemilepistus schirasi</i>	Iran, Shahreza	MG887939	Dimitriou <i>et al.</i> 2018
<i>Lucasioides altaicus</i> sp. nov.	Russia, Altai Krai	MT499211, MT499212	this paper
<i>Protracheoniscus kryszanovskii</i>	Russia, Republic of Kalmykia	MH400727	Gongalsky <i>et al.</i> 2018
<i>Protracheoniscus major</i>	Russia, Republic of Kalmykia	MH400726	Gongalsky <i>et al.</i> 2018
<i>Protracheoniscus nogaicus</i>	Russia, Republic of Kalmykia	MH400724	Gongalsky <i>et al.</i> 2018
<i>Protracheoniscus pokarzhevskii</i>	Russia, Republic of Kalmykia	MG696253, MH400723	Gongalsky <i>et al.</i> 2018
<i>Protracheoniscus politus</i>	Hungary, Budapest	MG696252	Gongalsky <i>et al.</i> 2018

Taxonomy

Family Agnaridae Schmidt, 2003

Genus *Lucasioides* Kwon, 1993

Type species: *Lucasioides gigliotosi* (Arcangeli, 1927).

Diagnosis (based on Kwon 1993; Yamaki *et al.* 2009; Li 2017). Flat body granulated dorsally, gland pores absent. Cephalon with frontal line separated by a groove, bearing well-developed median and lateral lobes. Epimeron of pereonite 1 bent lateral. *Noduli laterales* on pereonites 2–4 farther from lateral margin than ones on pereonites 1 and 5–7. Merus and carpus of male pereopods 1–4 each with a brush of long setae at sternal margins. Pleopodal exopods 1–5 with *Protracheoniscus*-type pseudotracheae.

Remarks. *Lucasioides* is close to *Protracheoniscus* Verhoeff, 1917, *Mongoloniscus* Verhoeff, 1930 and *Desertoniscus* Verhoeff, 1930. It can be distinguished from *Protracheoniscus* by the granulated dorsum and the triangular median lobe of the cephalon; in *Protracheoniscus*, the dorsal surface of the body is smooth, and the median lobe of the cephalon is less strongly developed. *Lucasioides* differs from both *Mongoloniscus* and *Desertoniscus* by the *noduli laterales* on pereonites 2–4 located farther from the lateral margin than the ones on pereonites 1 and 5–7, and the epimeron of pereonite 1 bent lateral; in *Mongoloniscus*, the *noduli laterals* are generally placed at the same distance from the lateral margin, and the regularly convex pereonite 1 is not bent laterad. *Lucasioides* can be distinguished from *Desertoniscus* by the antennae reaching pereonite 2.

Lucasioides altaicus sp. nov.

Figs 1–7

Mongoloniscus sp. (pro parte)—Gongalsky *et al.* 2017: 26.

Material examined: Holotype, ♂ (ZMMU, Mc-1420), Russia, southwestern Siberia, Altai Krai, Charyshskoye District, near Komendantka, Altai State University (ASU) Field Station “Goluboi Utios”, 51°21'38.0"N, 83°38'02.7"E, 630 m a.s.l., N slope, plot 2, *Betula pendula*, *Populus tremula*, *Pinus sylvestris*, *Spiraea chamaedrifolia*, *Bergenia crassifolia*, hand sampling, 13 July 2016, leg. V.Yu. Slatina, Kh.Kh. Nedoev, S.T. Niyazov.

Paratypes: 1 ♂ (ZMMU, Mc-1421); 1 ♂, 1 ♀ (KG), Russia, southwestern Siberia, Altai Krai, Charyshskoye District, near Komendantka, ASU Field Station “Goluboi Utios”, 51°21'44.3"N, 83°37'42.6"E, 620 m a.s.l., N slope, plot 1, *B. pendula*, *P. tremula*, *P. sylvestris*, *S. chamaedrifolia*, *B. crassifolia*, soil sampling, sample 4 (0–10 cm), 12 July 2018, leg. V.Yu. Slatina, Kh.Kh. Nedoev; 3 ♂♂, 5 ♀♀ (ZMMU, Mc-1422); 1 ♀ (ZMMU, Mc-1424), 1 ♂ (KG), 4 ♂♂ (ASU), location, date, and collectors same as holotype; 1 ♂, 1 ♀ (ZMMU, Mc-1423), Russia, southwestern Siberia, Altai Krai, Charyshskoye District, near Komendantka, ASU Field Station “Goluboi Utios”, 51°21'20.3"N, 83°37'37.0"E, *S. chamaedrifolia*, *Caragana arborescens*, *Lonicera tatarica* on southern slope, 480 m a.s.l., soil sampling, sample 1 (0–10 cm), 6 September 2018, leg. Kh.Kh. Nedoev.

Diagnosis. Male exopod of pleopod 1 with concave tip and lower lobe is longer than the upper one; telson is elongated and shows sharp tip.

Description. Maximum body length: male 8.0 mm (holotype 7.2 mm), female 6.7 mm. *Body* dark grey-brown, with two rows of light grey-brown spots in medial part of coxal plates of pereon segments 2–7 (Fig. 1A). Body relatively elongated, pleon forming no continuous margin with pereon (Fig. 1A). Dorsal surface of tergites smooth. Posterior edges of coxal plates of pereonites 1–3 straight, those of pereonites 4–7 curved (Fig. 2A). *Head* covered with scattered, sharp, triangular, dorsal setae (Fig. 1B). *Noduli laterales* on pereonites 1–4 located at approximately same distance to edge of coxal plates (Figs 2A, 3). Cephalic lobes poorly developed. Distal edge of median lobe rounded (Figs 1B, 2B). Telson with an elongated distal part, distal corner forming a triangle (Figs 2C, 3A).

Antennula with three articles (Fig. 2D); first article wide and long; second article 0.5 times as long as first; third article narrow as almost as long as first article, bearing tuft of setae at apex. *Antenna* of medium length, reaching pereonite 3; flagellum with two articles, proximal article slightly shorter than distal one (Figs 1C, 2E). Aesthetascs absent from apical articles of antenna.

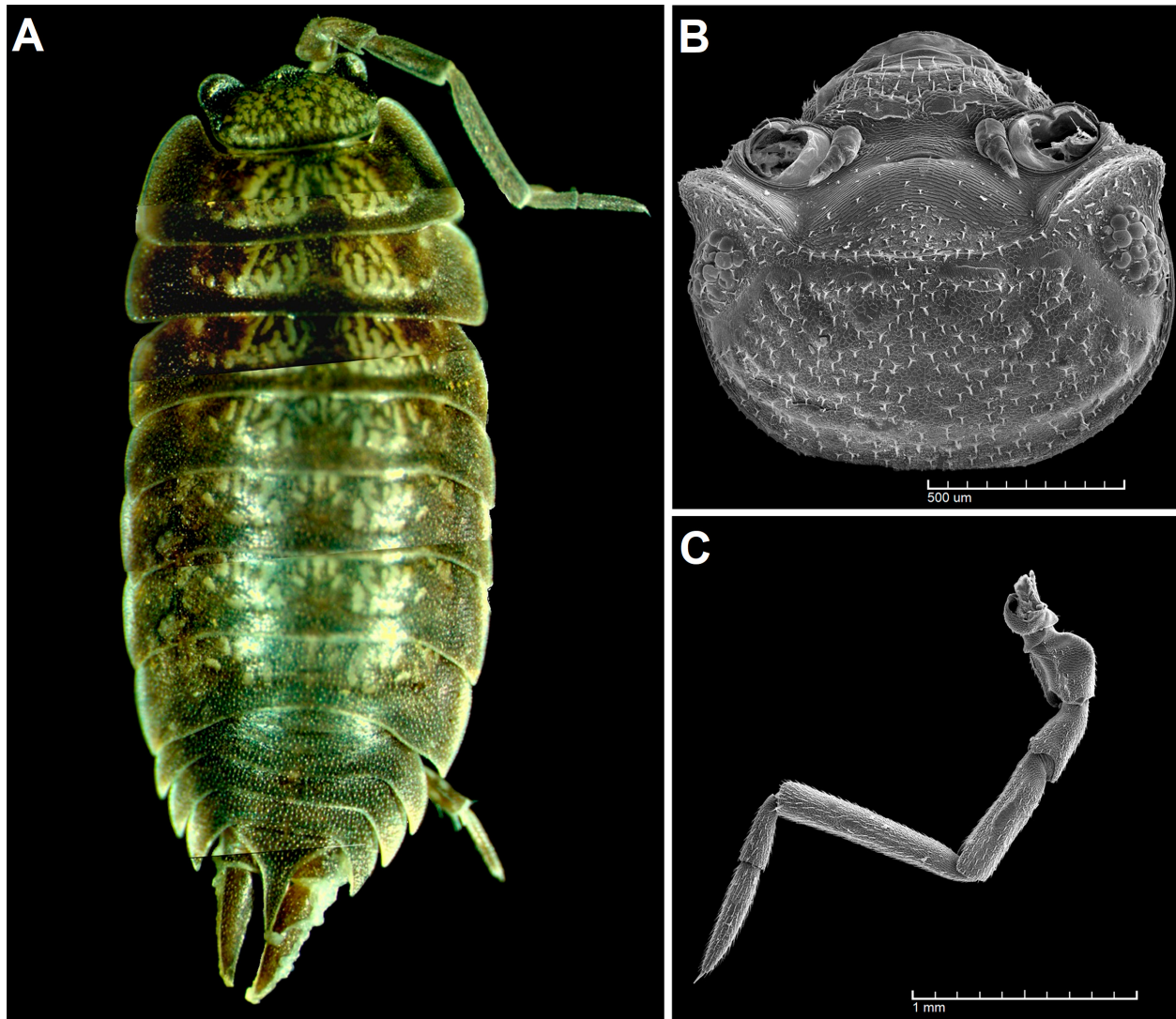


FIGURE 1. *Lucasioides altaicus* sp. nov., A—male paratype, dorsal view; female paratype: B—head; C—antenna.

Left mandible (Fig. 4A) with molar process carrying two teeth and *lacinia mobilis* with straight edge. Lobe covered by setae with three plumose penicils followed by row of 18–20 penicils located basal to *lacinia*. Right mandible smaller than left mandible, molar process with three teeth and *lacinia mobilis* with two teeth and hairy bilobate lobe with row of 18–20 penicils (Fig. 4B). *Maxillula* (Fig. 4C) medial corner of inner endite with two strong hirsute setae. Apical edge of outer endite bearing nine spines divided into two groups; five medial spines slender and with split tips, and four lateral spines stouter and with simple tips. Tip of endite covered with brush of dense setae. *Maxilla* with bilobate edge, medial half of apical edge of inner lobe with brush of short dense setae (Fig. 4D). Inner margin with a subapical tubercle. Maxilliped with outer corner of endite with two acute tips and large spine near inner corner (Fig. 4E). Basal article of endopod with large spine.

Pereopods (Figs 2F–H, 5). Ventral edge of male pereopod 7 carpus slightly curved.

Uropods (Fig. 1A, 2C) colored as dorsal surface of body. Exopods elongated, with widest part at 1/3 proximal distance (Figs 2C, 3A).

Male. Shape of genital papilla typical of the genus. All exopods at lateral margin with lungs. Exopod of *pleopod 1* (Figs 6A, 7B) with long hind lobe bearing deep hollow and 9–10 setae at apex; inner margin slightly concave and devoid of setae. Endopod of pleopod 1 with a dorsal furrow and row of spines (Figs 6B, 7C, D). *Pleopod 2* exopod triangular, with concave outer margin bearing 12 setae (Figs 6C, 7A); endopod much longer than exopod, narrow and parallel-sided (Figs 6D, 7A). *Pleopods 3–4* exopods (Fig. 6E–G) trapezoidal, slightly decreasing in size from 3 to 4. *Pleopod 5* exopod triangular.

Etymology. The specific epithet refers to the *locus typicus* (the Altai Mountains).

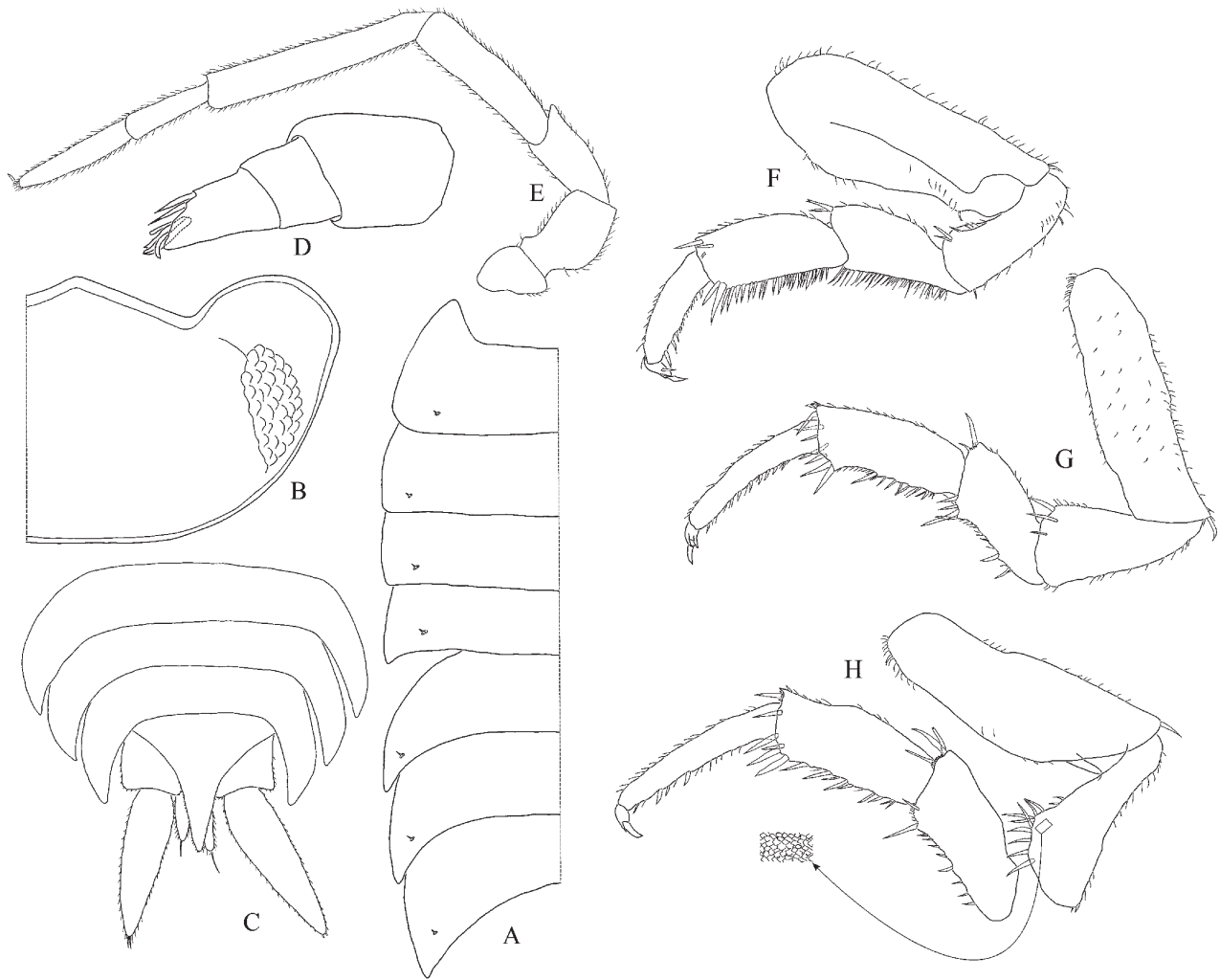


FIGURE 2. *Lucasioides altaicus* sp. nov., female paratype: A—pereion edge; B—head; C—telson; D—antennula; E—antenna; F—pereopod 1; G—pereopod 6; H—pereopod 7.

Distribution and habitat. The species has been collected in the lowlands of Altai Mountains in the environs of Charyshskoye Village. The area supports a steppe habitat with *S. chamaedrifolia*, *C. arborescens* and *L. tatarica* bushes and scattered *P. sylvestris*, *B. pendula* and *P. tremula* stands with *S. chamaedrifolia* and *B. crassifolia*, growing on the southern and northern rocky slopes of a mountain, respectively. The above is the first record of the genus *Lucasioides* Kwon, 1993 from Russia, showing its presently northwestern range limit.

Remarks. The new species is assigned to *Lucasioides* since it has five pairs of pleopodal lungs; the flagellum of antenna consisting of two articles, and the position of the *noduli laterales* distinctive for the genus. This species seems to be particularly close to *Desertoniscus mongolicus* due to the similar shape of most body parts. Yet the taxonomic position of *D. mongolicus* within *Desertoniscus* needs to be revised. In the original description, Borutzky (1978) noted that *D. mongolicus* occupies a position intermediate between the genera *Desertoniscus* and *Mongoloniscus*, but in fact both *D. mongolicus* and *L. altaicus* sp. nov. also bear characteristics of both *Protracheoniscus* and *Lucasioides* (e.g., the position of the *noduli laterales*). However, these two species differ in the shape of the male exopod of pleopod 1: *D. mongolicus* has a longer upper lobe at the tip, while in *L. altaicus* sp. nov. the lower lobe is longer; *D. mongolicus* has antenna reaching only the posterior edge of tergite I, while they are longer in *L. altaicus* sp. nov. and reaching the posterior edge of tergite II. Within the genus *Lucasioides*, there are no species which could be mixed up with the new species. The morphologically closest species are *L. isseli* (Arcangeli, 1927) from China, *L. sinuosus* (Nunomura, 1987) from Korea and Japan, *L. toyamaensis* Nunomura 2008, *L. sagarai* Nunomura 2008, and *L. yokahatai* Nunomura 2010 from Japan (Nunomura 2008, 2010). The new species readily differs from all by

the sharp and elongated telson, and in the shape of the exopod of pleopod 1. A molecular analysis is needed for the complex of those small species of Agnaridae, and a complete taxonomic revision of these sister genera is needed.

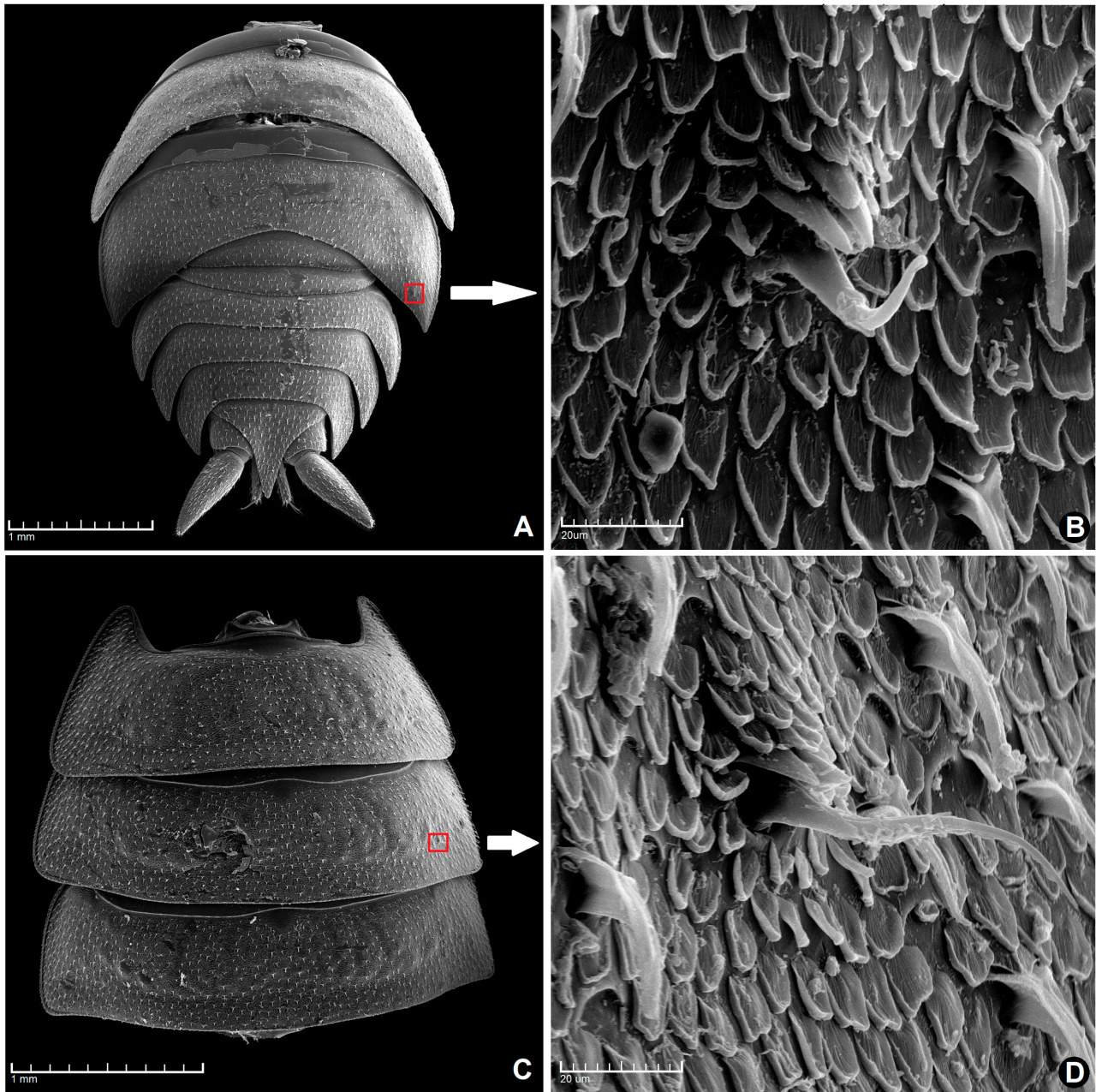


FIGURE 3. *Lucasioides altaicus* sp. nov., female paratype: A—pereionites 6, 7, pleon and telson; B—nodulus lateralis on pereonite 7; C—pereionites 1–3; D—nodulus lateralis on pereonite 2.

Phylogenetic analysis. A total of 11 nucleotide sequences were used to assess both species and intraspecific variation (Fig. 8): two of them were obtained from *L. altaicus* sp. nov., and further nine revealed earlier from *Protracheoniscus politus* (C. Koch, 1841) from Budapest, Hungary, *P. pokarzhevskii* Gongalsky et Turbanov, 2018, *P. kryszanovskii* Borutzky, 1957, *P. nogaicus* Demianowicz, 1932, *P. major* (Dollfus, 1903) and *Desertoniscus zaitsevi* Gongalsky, 2017 from the Republic of Kalmykia, Russia (Gongalsky *et al.* 2018). The loci of the *COI* mtDNA of *Hemilepistus klugii* (Brandt, 1833) and *H. schirasi* Lincoln, 1970 from Iran (Dimitriou *et al.* 2018) were used as outgroup taxa.

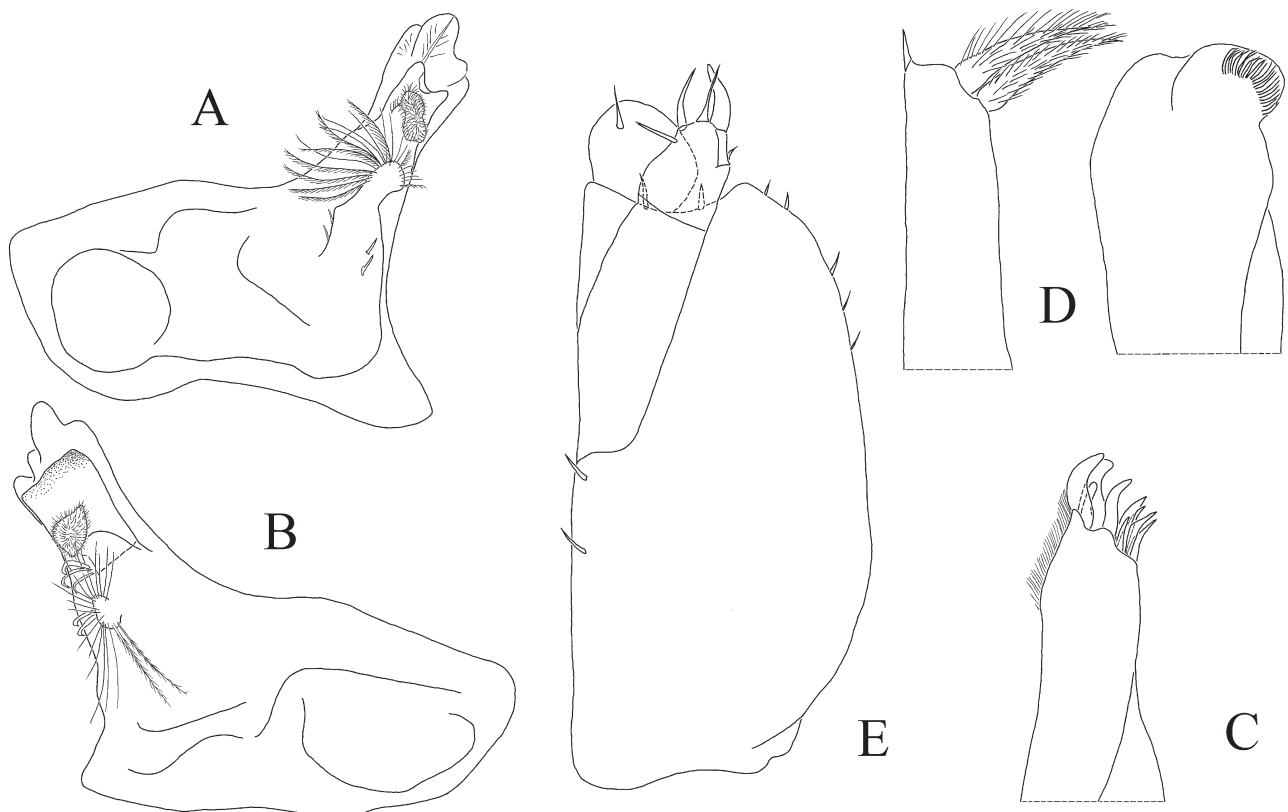


FIGURE 4. *Lucasioides altaicus* **sp. nov.**, female paratype: A—left mandible; B—right mandible; C—maxillula; D—maxilla; E—maxilliped.

An analysis of the average values of pairwise sequence divergences (p -distances) of partial *COI* mtDNA (Table 2) shows that the intraspecific difference of two *loci* of *L. altaicus* **sp. nov.** is 0%, which indicates their complete identity. Within the considered species, p -distances range from 15.7% between *L. altaicus* **sp. nov.** and *P. kryszanovskii* up to 24.9% between *P. politus* and *P. major*. Similar genetic distances correspond to possible variations within the *COI* mtDNA gene for woodlice (Dimitriou *et al.* 2018).

It seems noteworthy that *D. zaitsevi* falls within the p -distances range for the genus *Protracheoniscus*, whose average values of pairwise sequence divergences reach 18.0% with *P. major* and 23.4% with *P. nogaicus*. *Protracheoniscus* appears to be paraphyletic (Fig. 8). However, this aspect and the question of the validity of the genera *Desertoniscus* Verhoeff, 1930 and *Lucasioides* Kwon, 1993 is to be left outside present paper, requiring a special study based on a more representative material.

TABLE 2. Estimates of pairwise sequence divergence (uncorrected p -distances) of the gene *COI* mtDNA haplotypes between *Lucasioides altaicus* **sp. nov.** and eight species of Agnaridae.

<i>Lucasioides altaicus</i> sp. nov.								
<i>Desertoniscus zaitsevi</i>	0.223							
<i>Protracheoniscus kryszanovskii</i>	0.157	0.183						
<i>Protracheoniscus major</i>	0.203	0.180	0.175					
<i>Protracheoniscus nogaicus</i>	0.247	0.234	0.247	0.214				
<i>Protracheoniscus pokarzhevskii</i>	0.218	0.223	0.243	0.245	0.259			
<i>Protracheoniscus politus</i>	0.202	0.224	0.216	0.249	0.239	0.225		
<i>Hemilepistus klugii</i>	0.216	0.211	0.213	0.212	0.262	0.235	0.245	
<i>Hemilepistus schirasi</i>	0.237	0.241	0.197	0.221	0.249	0.216	0.227	0.080

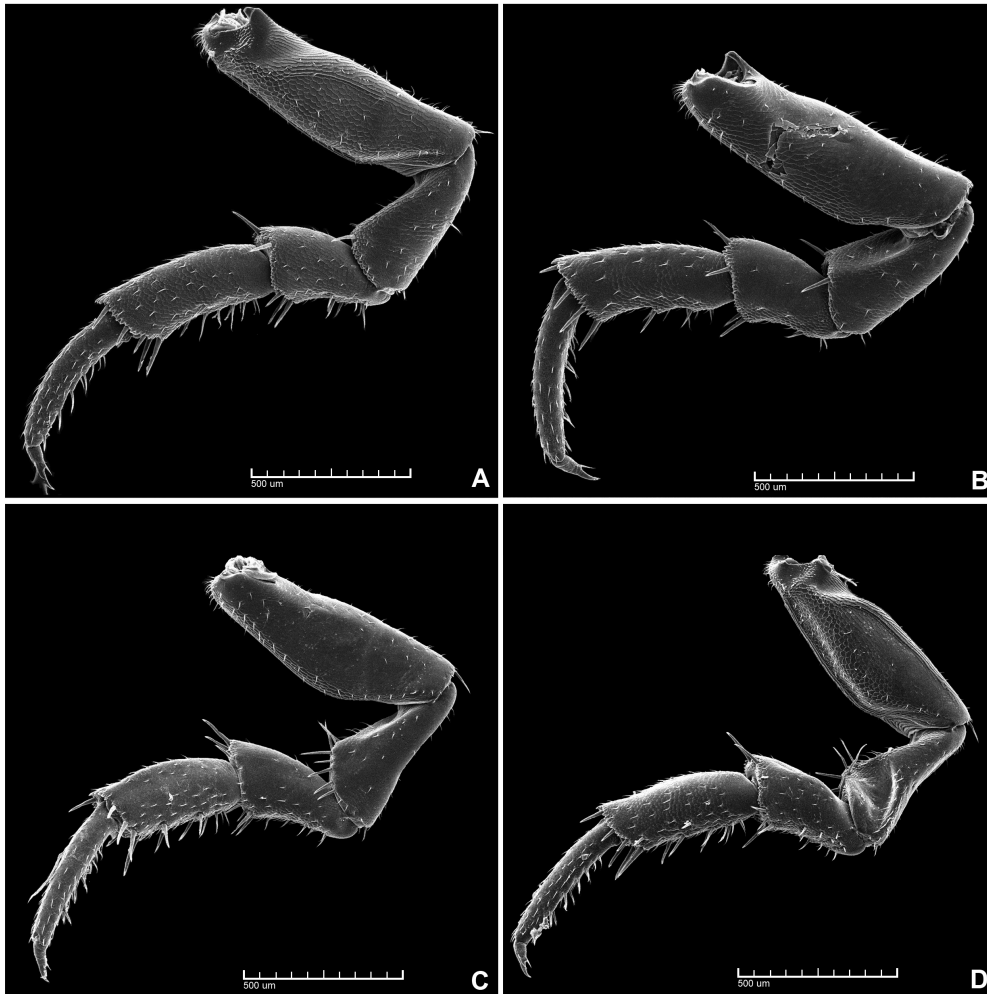


FIGURE 5. *Lucasioides altaicus* sp. nov., male paratype: A, B—pereopod 6; C, D—pereopod 7.

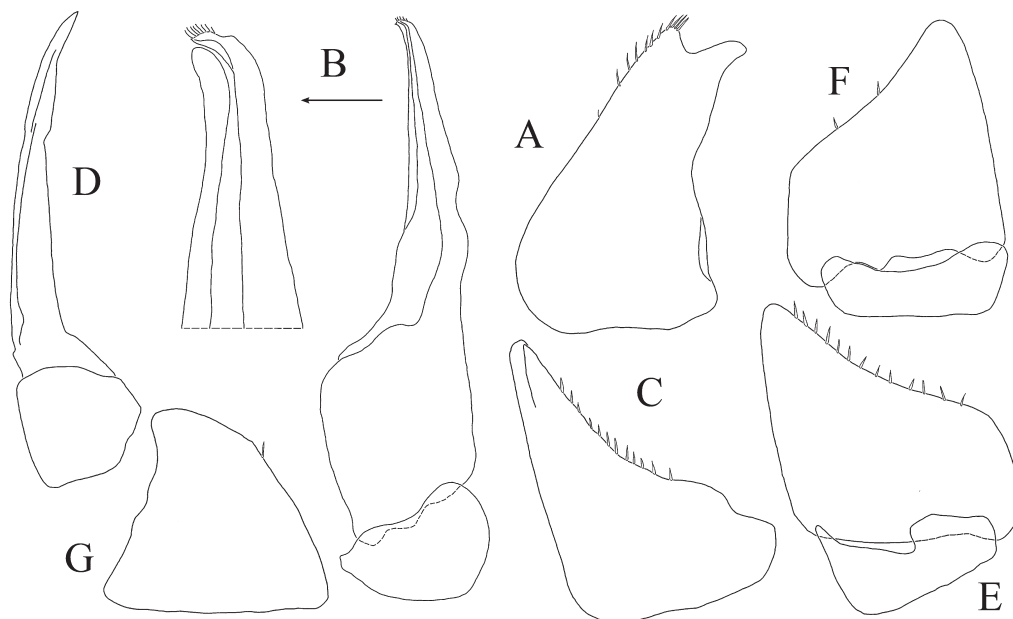


FIGURE 6. *Lucasioides altaicus* sp. nov., male paratype: A—exopod of pleopod 1; B—endopod of pleopod 2; C—exopod of pleopod 2; D—endopod of pleopod 2; E—exopod of pleopod 3; F—exopod of pleopod 4; G—exopod of pleopod 5.



FIGURE 7. *Lucasioides altaicus* sp. nov., male paratype: A—exopod and endopod of pleopod 2; B—exopod of pleopod 1; C, D—endopod of pleopod 2.

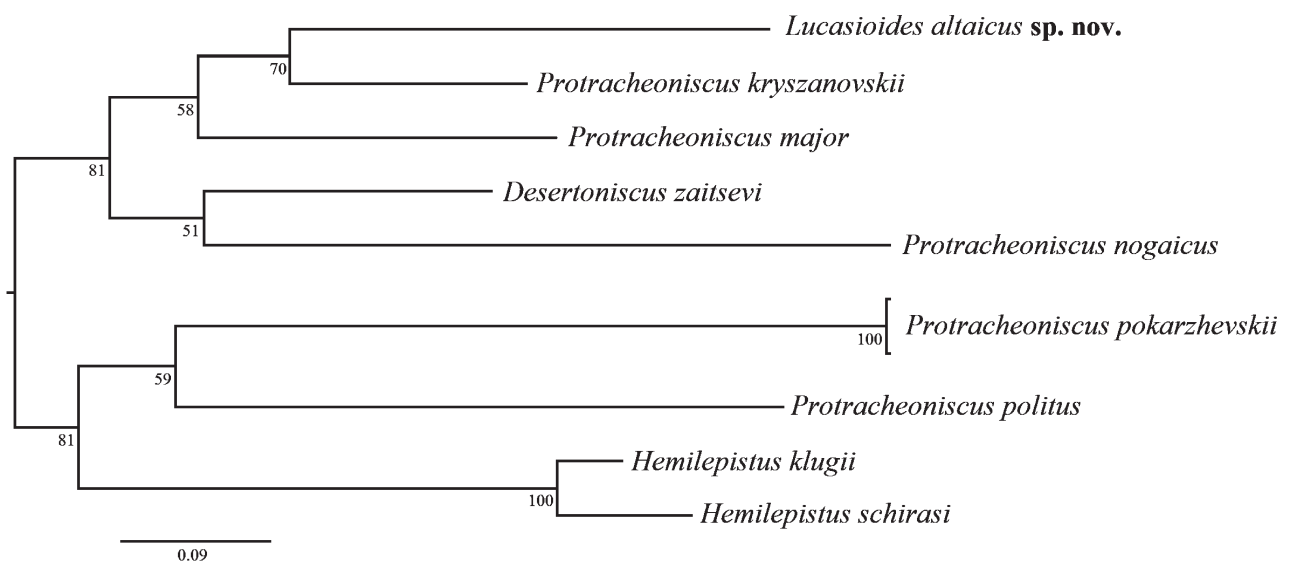


FIGURE 8. Phylogenetic reconstruction of selected species of family Agnaridae, based on the analysis of COI mtDNA gene sequences using the Bayesian analysis (BA) with the GTR + G + I evolutionary model for Maximum-Likelihood (ML), using Kimura2-parameter (K2P) model; bootstrap support for branch nodes shown for 1,000 replications.

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