



Populations of the stygobiotic amphipod *Palearcticarellus pusillus* (Martynov, 1930) maintain genetic connectivity between the mountain springs and the deep bottom habitats of Lake Teletskoye (Altai Mountains, Russia)

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Abstract: The diversity of underground fauna is often associated with the presence of karst areas, which have extensive underground cavities and connecting channels. Non-karst areas, however, make up a larger portion of the planet's surface, and our understanding of the distribution and dispersal abilities of underground fauna in these areas currently remains limited. During a recent study of the diversity of stygobiotic crustaceans in the mountain streams of the Kurai Mountain Range (Altai, Russia), a small-sized species of crangonyctid amphipod, *Palearcticarellus pusillus* (Crustacea, Amphipoda, Crangonyctidae) was found in high-altitude springs. This species has been previously known only from deep-water habitats in Lake Teletskoye, and molecular genetic analysis revealed that these amphipods maintain genetic connections between the springs and bottom lake habitats. We assume that individuals of this certainly stygobiotic species fall to the bottom of the lake as a result of leaching from tectonic faults connecting these habitats and are not permanent inhabitants of the lake bottom. This hypothesis also suggests that the very small body size of these animals may be related to the narrow crevices and pores of the tectonic faults, rather than neoteny, as it has been previously suggested. The estimated divergence time calculation has also revealed that *P. pusillus* obviously diverged from its sister species, *Palearcticarellus mikhaili* from valley springs in Kurai Steppe, ~5 Ma, at the beginning of the Pliocene, possibly due to the uplifting of the Kurai Mountain Range. Subsequent speciation occurred as a result of secondary tectonic activity and changes in groundwater flow, likely during the Pleistocene, approximately 2.7–0.7 Ma. Populations that were isolated for a long time continue to live near mountain springs in the study area.

Keywords: stygobiotic, Amphipoda, Crangonyctidae, diversity, phylogeny, Palaearctic

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INTRODUCTION

The understanding of the patterns of distribution of stygobiotic species, their ecological features and phylogenetic relationships can only be achieved through studying the morphological and genetic diversity, in combination with an analysis of local hydrogeological conditions, geological structures and historically documented tectonic processes. Additionally, the functional features of these species, such as their ability to disperse and local

geographic factors, e.g., environmental gradients and dispersal barriers, also play a significant role in their distribution (Kraft et al., 2015; Mittelbach & Schemske, 2015). The diversity of underground fauna is often associated with the presence of karst areas, which are characterized by the formation of significant underground cavities and channels due to the leaching of soluble ores. These channels and cavities allow stygobiotic animals, to disperse passively or actively over considerable distances, often crossing insurmountable surface barriers, such as

mountains and river valleys (e.g., Marin & Turbanov, 2021). However, the dispersal abilities of organisms in groundwater are greatly reduced (Holsinger, 1993; Strayer, 1994; Strayer & Reid, 1999) due to the limited passive dispersal caused by the high physical fragmentation of groundwater systems (Lefébure, 2005; Lefébure et al., 2007), as well as the small size of the hypogean pores, which act as one of a major physical filter for larger-bodied stygobiotic animals (Castellarini et al., 2007; Dole-Olivier et al., 2009; Hutchins et al., 2020). Despite the fact that non-karst regions make up a significantly larger portion on the planet, the distribution and dispersal abilities of underground fauna there are currently poorly understood, compared to karst areas.

The family Crangonyctidae Bousfield 1973 comprises predominantly freshwater amphipods that typically inhabit groundwater or epigean water reservoirs with a hypogean connection or origination (e.g., Holsinger, 1978; Zhang & Holsinger, 2003; Lowry & Myers, 2013, 2017; Copilaş-Ciocianu et al., 2019). Currently, 50 native species from 10 genera have been currently described from the Palearctic, showing a variety of morphological adaptations to a stygobiotic lifestyle (e.g., Marin & Palatov, 2023, 2024). Among them, several species of the genus *Palearcticarellus* Palatov & Marin, 2020 known

from Altai mountains, namely *Palearcticarellus pusillus* (Martynov, 1930), *Palearcticarellus mikhaili* (Sidorov, Holsinger & Takhteev, 2010) and recently described *Palearcticarellus ulagani* Marin, Yanygina, Ostroukhova & Palatov, 2023, demonstrates an extremely small body size, usually not exceeding 3–4 mm (see Palatov & Marin, 2020; Marin et al., 2023). Such a biological feature has been previously suggested to be due to the neotenic origin, which is thought to be related to living in rather extreme conditions in highland habitats (see Palatov & Marin, 2020). These crustaceans can be found in a variety of mountainous ecologically diverse environments, such as the deep bottom (about 100 m depth) of the mountain Lake Teletskoye and the high-altitude springs in the Kurai Steppe, respectively. Until now, the ecological variability of these species and its origin have not been fully understood.

The aim of this study was to explore the diversity of stygobiotic crustaceans in the mountain springs located on the Kurai Mountain Range, along the Bashkaus River which flows into Lake Teletskoye (Fig. 1). The collected material allowed us to redescribe partially *P. pusillus*, a tiny crangonyctid amphipod that was previously found only at the bottom of Lake Teletskoye, in order to clarify its phylogenetic relationships and some ecological features.

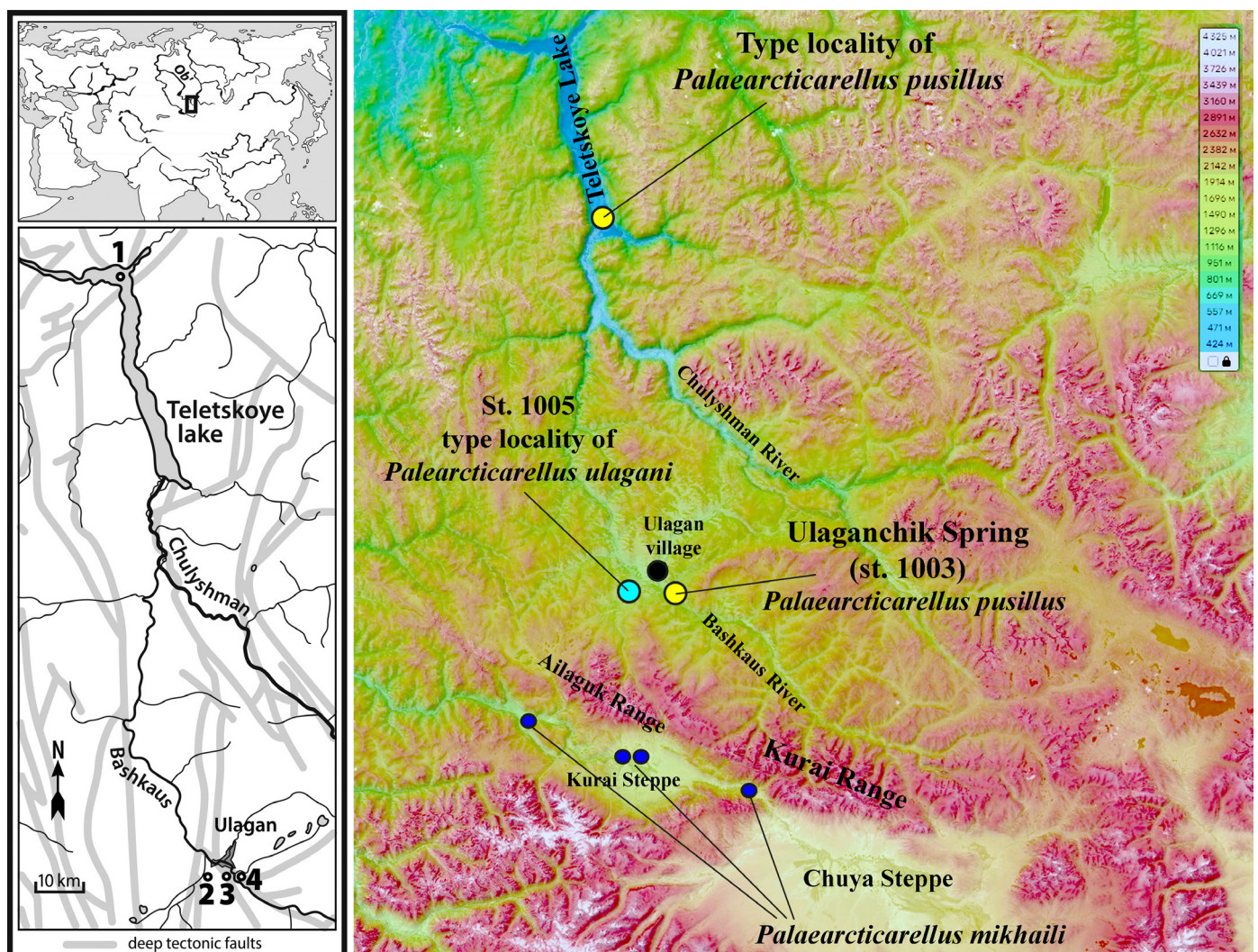


Fig. 1. The map of the studied area indicating the locality where amphipods were collected (see topographic map on the right panel), and underlying deep tectonic faults (grey lines, lower left panel). The spring numbering is according to Table 1.

MATERIAL AND METHODS

Sampling and processing

Stygobiotic amphipods were collected by hand net in several springs on the upper reaches of the Bashkaus River in the northern slope of the Kurai Mountain Range in the Altai Mountains in 2022–2023 (see Table 1). Additionally, the specimens of *P. pusillus*, representing the topotypic population of the species (see Martynov, 1930), were dredged from the bottom of Lake Teletskoye from a depth of 100 m in 2019–2023. After sampling, all collected amphipods were

preserved in 96% ethanol for further phylogenetic analysis. The body length (bl., mm), measured from as the dorsal length from distal margin of head to the posterior margin of the telson, without the length of uropod III and antennae, was used as the standard measurement. The specimens were deposited in the Zoological Museum of Moscow University (ZMMU), Moscow, Russia, and the author's personal zoological collection in the A.N. Severtsov Institute of Ecology and Evolution of Russian Academy of Sciences (LEMMI), Moscow, Russia. This research does not involve any endangered or protected species.

Table 1. The mountain springs of the Kurai Mountain Range in Altai Mountains, Russia, which were studied for their diversity of stygobiotic fauna. The bold indicates the springs, where stygobiotic amphipods, identified as *P. pusillus* and *P. cf. pusillus*, were collected.

Nr.	Coordinates	Altitude (m)
1	50°38'8.35"N 87°57'33.67"E	1228
2 (Ulaganchik, st. 1003)	50°35'44.65"N 87°58'56.00"E	1237
3	50°32'37.24"N 88° 5'41.75"E	1342
4	50°43'58.00"N 88° 2'24.76"E	1315
5	50°34'10.66"N 87°50'39.79"E	1365
6	50°34'20.50"N 87°50'47.50"E	1361
7 (Mandilu, st. 1004)	50°35'58.51"N 87°58'8.13"E	1240
8 (st. 1005)	50°36'2.57"N 87°52'34.49"E	1278
9	50°36'4.12"N 87°52'38.02"E	1275

Phylogenetic analysis

For the phylogenetic analysis, a fragment of the mitochondrial cytochrome oxidase C subunit I (COI mtDNA) gene was used. Total genomic DNA was extracted from abdominal and pereopod muscle tissue using the innuPREP DNA Micro Kit (AnalytikJena, Germany) following the standard protocol. The gene marker was amplified using the universal primers LCO1490 and HC02198 (Folmer et al., 1994) with the T100 amplification (Bio-Rad, USA), under the standard protocol conditions. A consensus of complementary sequences of COI mtDNA gene marker of representatives of the genus *Palearcticarellus* from author's personal database and one sequence of *P. anastasiae* (HE794980), taken from the GenBank (NCBI) database, was obtained using MEGA 7.0 (Kumar et al., 2016). The final dataset included 22 sequences, with 586 base pairs in length, displaying 198 variable (polymorphic) sites, of which 212 were parsimony informative. The best evolutionary substitution model was determined using MEGA 7.0. and jModeltest2.1.141 on XSEDE via the CIPRES (Cyber Infrastructure for Phylogenetic Research) Science Gateway V. 3.3 (<http://www.phylo.org/>). Phylogenetic analysis based on GTR+G+I model for Maximum-Likelihood (ML) algorithm was performed using PhyML 3.0 (Guindon et al., 2010).

Pairwise uncorrected genetic distances (*p*-distances) were calculated based on the obtained sequences using MEGA 7.0 with the Kimura 2-Parameter (K2P) model of evolution (Kimura, 1980). The data on the genetic diversity of *P. mikhaili* from the Kurai Steppe (after Palatov & Marin, 2020) were also used for the analysis.

Poisson Tree Process (PTP) and the Bayesian variant of the method (bPTP) (Zhang et al., 2013)

([https:// species.h-its.org/](https://species.h-its.org/)) was run on the RAxML gene trees for 1×10^6 MCMC (Markov chain Monte Carlo) generations thinning every 1000 events and removing the distant outgroup that can improve the delimitation results.

Median joint network (Bandelt et al., 1999) was reconstructed with PopArt (Population Analysis with Reticulate Trees) software (Leigh & Bryant, 2015).

Time calibration

The estimated minimum (5.16%/Ma) and maximum (as 0.77%/Ma) divergence times were calculated after Guy-Haim et al. (2018), with an average divergence time of 2.5%/Ma for COI mtDNA gene marker (Lefébure et al., 2006; Copilaş-Ciocianu & Petrusek, 2015; Guy-Haim et al., 2018). Additionally, a divergence time estimates as 1.773%/Ma was calculated according to Copilaş-Ciocianu et al. (2019).

RESULTS

Molecular genetic study

Tiny crangonyctid amphipods morphologically identified as *P. pusillus*, were collected from two mountain springs, located in the intake of the Bashkaus River, which flows down into Lake Teletskoye from the northern slope of the Kurai Mountain Range (see Fig. 1; Table 1).

Molecular genetic analysis of the collected specimens revealed that amphipod individuals collected from the Ulaganchik Spring (st. 1003) are closely related to the topotypic *P. pusillus* from Lake Teletskoye. In contrast, amphipods collected from the neighboring Mandilu spring (st. 1004) appear to be phylogenetically more distant (see Figs 2, 3). Species delimitation analyses conducted using the coalescent tree-based approach

(PTP and bPTP) yielded almost identical results, demonstrating 7 OTUs (Fig. 2). This supports the hypothesis that populations from Ulaganchik Spring and Lake Teletskoye belong to the same biological species, while Ulaganchik and Mandilu springs are inhabited by genetically distinct species.

The calculated intraspecific pairwise uncorrected genetic distances (p -distances) within the populations of *P. pusillus* (Ulaganchik spring + Lake Teletskoye) ($n = 6$) is 0.0102 ± 0.0030 (about 1.0%), *P. cf. pusillus* (st. 1004) ($n = 4$) is 0.007 ± 0.0028 (about 0.7%), and *P. mikhaili* ($n = 6$) is 0.014 ± 0.003 substitutions per 100 nucleotides (about 1.4%). These values also indicate that the genetic divergence between the

samples of *P. pusillus* collected from the mountain spring and Lake Teletskoye could be considered as intraspecific.

The interspecific genetic differences (p -distances) within the genus *Paeleartcarellus* (Table 2) is lowest between *P. pusillus* (Ulaganchik Spring + Lake Teletskoye) and *P. cf. pusillus* (Mandilu Spring), representing 0.058 ± 0.008 (about 0.6%), and *P. cf. pusillus* (Mandilu Spring) and *P. mikhaili*, being 0.067 ± 0.008 substitutions (about 0.7%), showing their close phylogenetic relationship. At the same time, the genetic divergence between *P. pusillus* (Ulaganchik Spring + Lake Teletskoye) and *P. mikhaili* is much higher, being 0.102 ± 0.012 substitutions (about 10%).

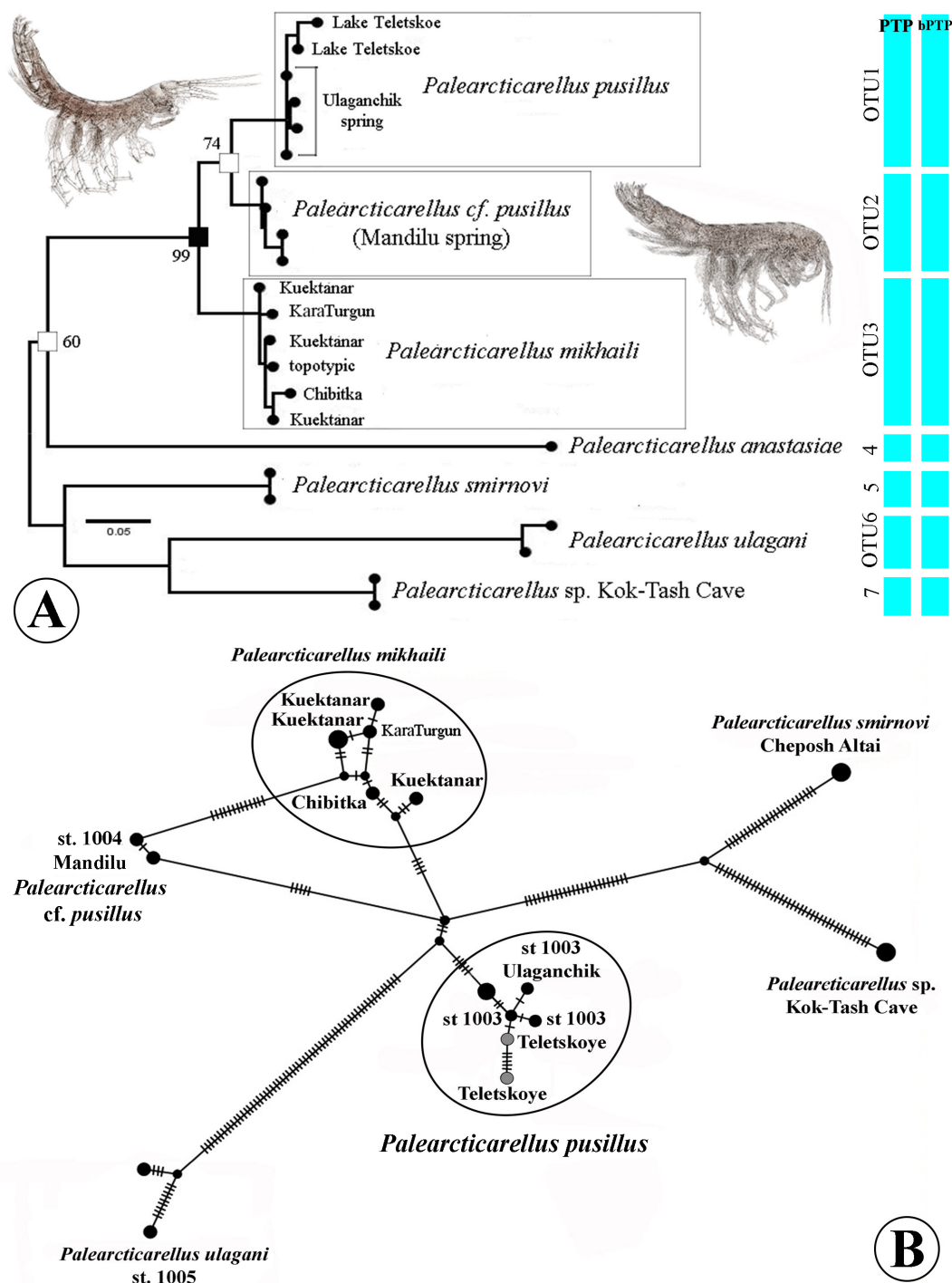


Fig. 2. Phylogenetic reconstruction (tree) (ML) of relationships and median-joint network (TCS) between known species of the genus *Paeleartcarellus* from Altai Mountains. The sequence of *P. anastasiae* (HE794980) is taken from the GenBank (NCBI) database. The support of nodes is marked. Results (Consensus) of Poisson Tree Processes (PTP and bPTP) delimitation approaches are presented by yellow lines.

The divergent rates between other congeneric species are usually greater, exceeding 0.20 substitutions (more than 20%) (see Table 2), indicating a long period of genetic isolation (see below).

The estimated genetic divergence between *P. pusillus* (Ulaganchik Spring + Lake Teletskoye) and *P. mikhaili* from the springs of the Kurai Steppe is about 10.2%. This divergence is corresponding to a time period

ranging from approximately 1.95(min)–13.2(max) Ma, with an average estimated as 5.76–4.08 Ma. At the same time, the genetic divergence between closely related *P. pusillus*/*P. cf. pusillus* (st. 1004) and *P. mikhaili*/*P. cf. pusillus* (st. 1004) are generally close to 6% (see Table 2), corresponding to about 1.16(min)–7.8(max) Ma, with an average divergence time of approximately 3.38–2.4 Ma.

Table 2. Pairwise genetic (COI mtDNA) distances (p-distances) between the studied species of the genus *Palearticarellus*. The sequence of *P. anastassiae* (HE794980) is taken from the GenBank (NCBI) database.

<i>P. pusillus</i> (st. 1003 -Teletskoye)		1004	<i>P. mikhaili</i>	<i>P. smirnovi</i>	Kök-Tash Cave	<i>P. ulagani</i>
St. 1004	0.058±0.008					
<i>P. mikhaili</i>	0.102±0.012	0.067±0.008				
<i>P. smirnovi</i>	0.23±0.024	0.18±0.019	0.21±0.022			
Kök-Tash Cave	0.25±0.024	0.21±0.019	0.23±0.022	0.22±0.022		
<i>P. ulagani</i>	0.28±0.031	0.24±0.028	0.26±0.028	0.27±0.028	0.26±0.029	
<i>P. anastassiae</i>	0.29±0.027	0.23±0.022	0.26±0.023	0.29±0.026	0.28±0.026	0.34±0.031

Taxonomic part

Order Amphipoda Latreille 1816

Infraorder Gammarida Latreille 1802

Family Crangonyctidae Bousfield 1973

Genus *Palearticarellus* Palatov & Marin, 2020

***Palearticarellus pusillus* (Martynov, 1930)**

(Figs 3–5)

Material examined

5♀♀, LEMMI, Russian Federation, Altai Republic, Ulagan District, about 5 km southeast of the village Ulagan, mountain spring (Ulaganchik), st. 1003, 50°35'44.65"N, 87°58'56.00"E, 1237 m asl, hand net sampling, 13.06.2022; 2♀♀, Russian Federation, Altai Republic, Turochak District, the Lake Teletskoye near Kokshi River, st. 5.2, depth 297 m, bottom dredging in Lake Teletskoye, 51°34'18.24"N, 87°40'06.27"E, 434 m asl., 05.06.2020.

Comparative material

Palearticarellus cf. pusillus

5♀♀, LEMMI, Russian Federation, Altai Republic, Ulagan District, about 5 km southeast of the village Ulagan, mountain spring (Mandilu), st. 1004, 50°35'58.51"N 87°58'8.13"E, 1240 m asl, hand net sampling, 22.06.2022.

Palearticarellus ulagani: 2♀♀, LEMMI, Russian Federation, Altai Republic, Ulagan District, about 7 km southwest of the village Ulagan, 50°36'2.57"N 87°52'34.49"E, 1278 m asl, in a small mountain spring in a drainage basin of a small mountainous river, a tributary of Bashkaus River flowing into Lake Teletskoye, coll. S.A. Ostroukhova, 24.06.2022.

***Palearticarellus mikhaili* (Sidorov, Holsinger et Takhteev 2010)** (GenBank MT231256–MT231259): Russian Federation, Altai Republic, Kurai Steppe, Ulagan area: 10♀♀, near Chibit village, in a small spring in the valley of the Chibitka River,

50°19'12.7"N 87°31'26.0"E, about 1200 m asl, hand net sampling, 11.09.2019; Kosh-Agach area: 3♀♀ LEMMI, valley of the Chuya River near Karaturgun River, 50°13.762'N 87°51.051' E, 1470 masl, hand net sampling, 12.09.2019; 5♀♀, LEMMI, valley of the Karaturgun River, 50°13.691'N 87°51.284' E, 1466 m asl, hand net sampling, 12.09.2019; 7♀♀, LEMMI, a small spring near the beginning of the Karaturgun River, 50°15.228'N 87°51.643' E, 1596 m asl, hand net sampling, 13.09.2019; 8♀♀, a small spring near the beginning of the Kuektanar River, 50°09.592' N 88°18.785' E, 1775 m asl, hand net sampling, 13.09.2019.

Diagnosis

Only females are known. Stygomorphic, eyeless, completely unpigmented, with total body length reaching only 4 mm (Fig. 3). Urosomites free, unarmed. Telson (Fig. 4h) subquadrate, distal margin with tiny median notch, armed with 6–7 strong spine-like setae on each lobe. Pleonal plates 1–3 (Fig. 5a–c) with bluntly rounded posteroventral corners, each armed with small simple setae; ventral margin of plate 3 with 1 spine (Fig. 5c). Antenna 1 (Figs 3, 4a) about 30% of body length, twice longer than antenna 2, primary flagellum with 8–9 segments bearing aesthetascs accompanied by setae; accessory flagellum 2-segmented, distal segment about 3 times smaller than basal one (Fig. 4a). Antenna 2 with 4-segmented flagellum (Fig. 5b). Inner plate of maxilla 1 with only 1 (Fig. 3c), rarely 2 plumose apical setae. Maxilliped (Fig. 4) with inner plate armed with 4 serrated spines and 2 naked setae apically; outer plate with row of 7 simple setae distally; nail of dactyl (palp segment 4) long and sharply pointed, accompanied with long simple setae on basal part of segment 4. Gnathopods 1 and 2 mostly similar in size and shape (Fig. 4e, f);

propodus (palm) of both gnathopods armed with 4 spines at defining angle, with palmar region smooth, unarmed. Paired median sternal gills on pereonites 2–4 (Fig. 5d–f), 1 pair simple lateral sternal gills on pereonite 7. Uropod 1 (Fig. 5) with subequal inner and outer rami, armed with strong spine-like setae; rami subequal to peduncle in length. Uropod 2 (Fig. 5j) with subequal inner and outer rami, which are about 1.4 times longer than peduncle. Uropod 3 (Fig. 6k) with ramus about half length of peduncle, armed with 4 prominent apical spines.

Distribution

The species is known from a small mountain Ulaganchik Spring, 50°35'44.65"N, 87°58'56.00"E, located in the Bashkaus River basin at an altitude of 1200 m asl and bottom habitats (deeper than 100 m) in Lake Teletskoye, Altai Republic, Russia.

Taxonomic remarks

All studied individuals of *P. pusillus* from both Lake Teletskoye and a mountain spring are mostly identical to the previous descriptions of the species (see Martynov, 1930; Holsinger, 1987; Sidorov et al., 2010). Compared to phylogenetically close *P. mikhaili*, *P. pusillus* can be distinguished by mostly smooth body (vs. densely covered with sensillae) and an inner plate of maxilla 2 that is rounded, not tapered, and armed with fewer plumose setae (vs. narrowing

apically inner plate, with a row of 4 plumose setae) (see Sidorov et al., 2010).

Ecological remarks

One of the important results of this study is also the fact that *P. pusillus* could now be considered as a stygobiotic species that hypothetically occurs at the bottom of Lake Teletskoye, possibly, due to leaching from groundwater. Previously, this species was described as a deep-lake dweller (Martynov, 1930; Holsinger, 1987; Sidorov et al., 2010), the only known within the family. All other known members of the genus *Palearticarellus* (see Marin & Palatov, 2020), as well as Palaearctic members of the family Crangonyctidae, are mostly stygobiotic, inhabiting springs, caves, and other hypogean water sources (see review in Marin & Palatov, 2023). Among them, only some Palaearctic species from *Synurella* Wrześniowski, 1877 and *Pontonyx* Palatov & Marin, 2021 show an epigeal lifestyle, living in shallow forest lakes and peat bogs (Palatov & Marin, 2021; Marin & Palatov, 2022). These species are actually have adapted to these conditions, for example, having sufficiently developed eyes and body pigmentation, as well as certain behavioral features, such as folding into a ball, to avoid predators like fish (Palatov & Marin, 2021). However, *P. pusillus* has a completely different morphology (stygomorphic) that is characteristic of stygobionts (e.g., Sidorov et al., 2010; present study).

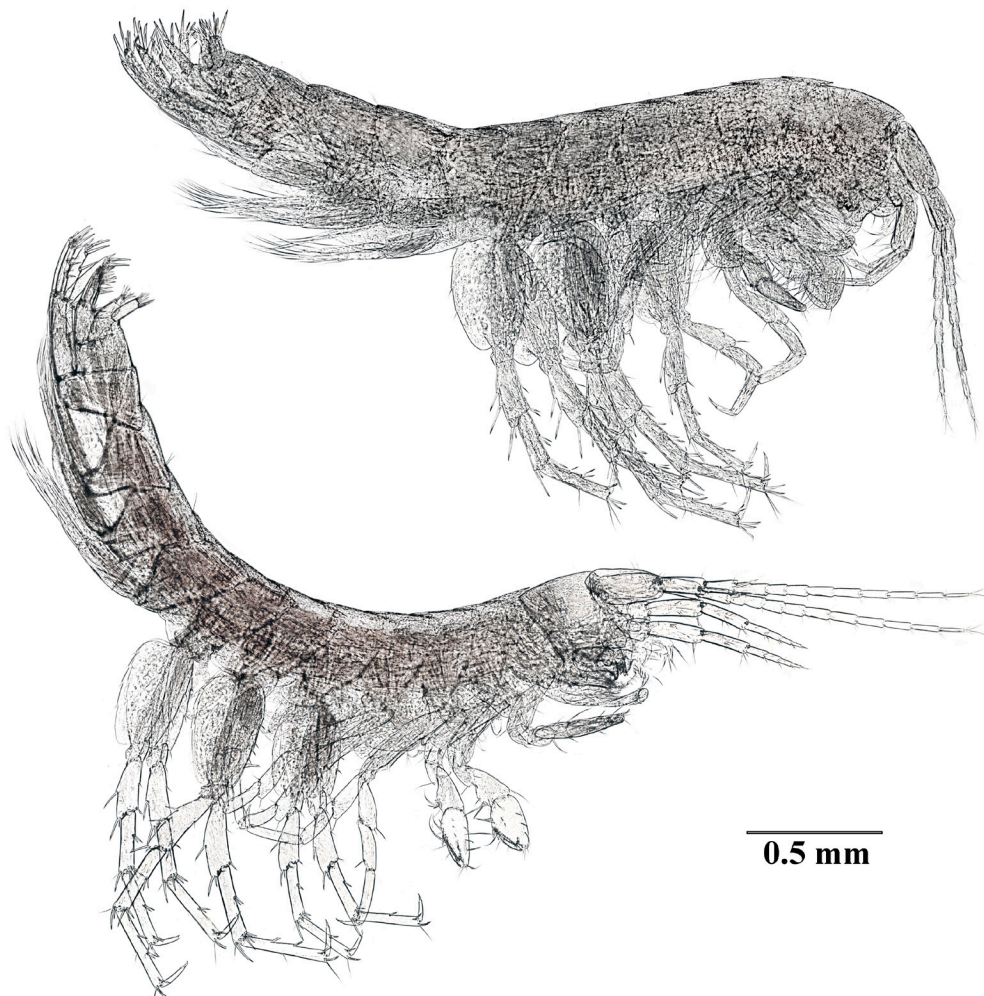


Fig. 3. General view of *Palearticarellus pusillus* (Martynov 1930) from Lake Teletskoye (upper) and the Ulaganchik Mountain Spring (st. 1003) in the intake of the Bashkaus River (lower).

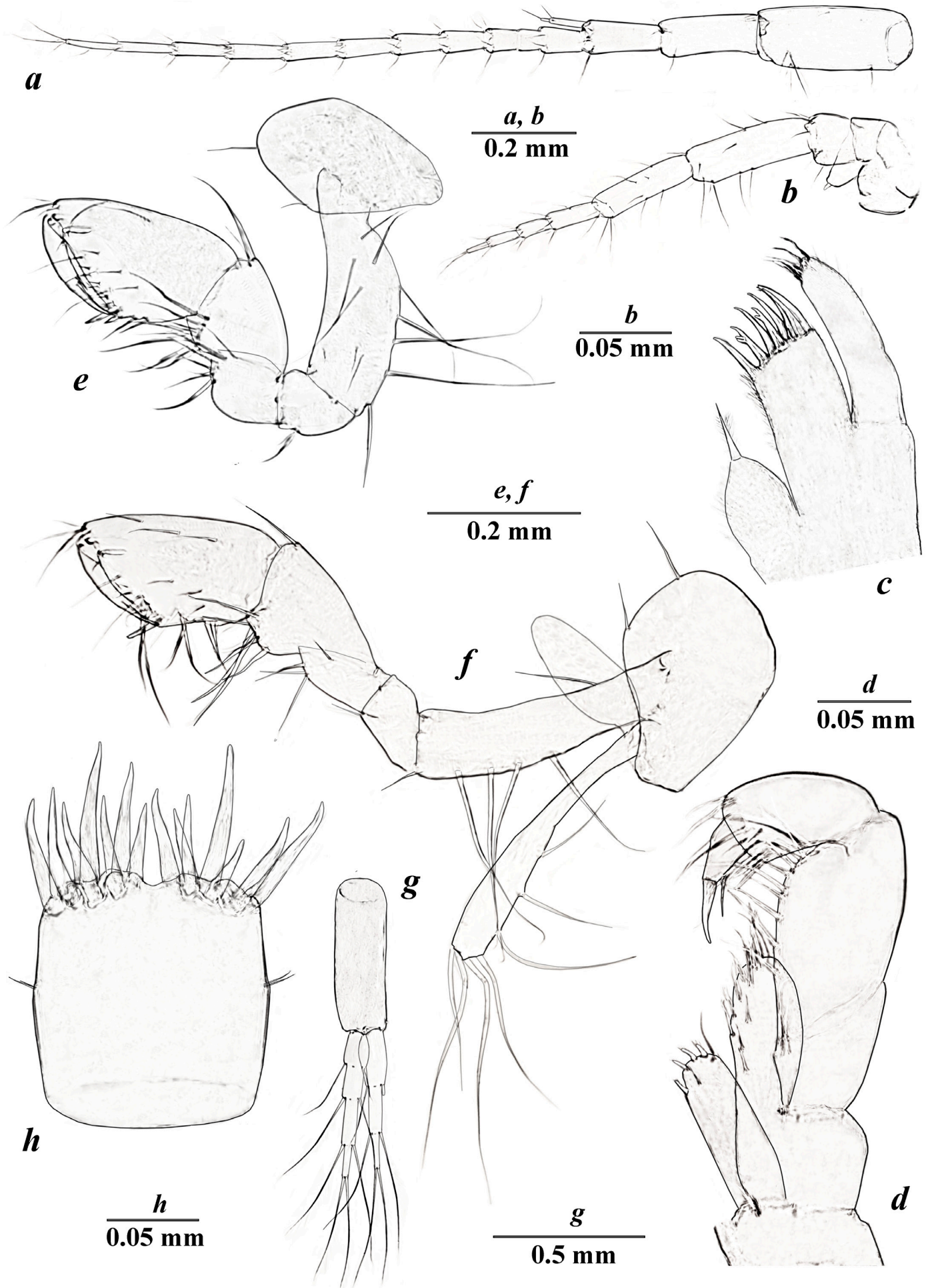


Fig. 4. *Paelearticarellus pusillus* (Martynov 1930) from the Ulaganchik Mountains Spring (st. 1003) in the intake of the Bashkaus River, ♀: a – antenna I; b – antenna II; c – maxilla II; d – maxilliped; e – gnathopod I; f – gnathopod II; g – pleopod II; h – telson.

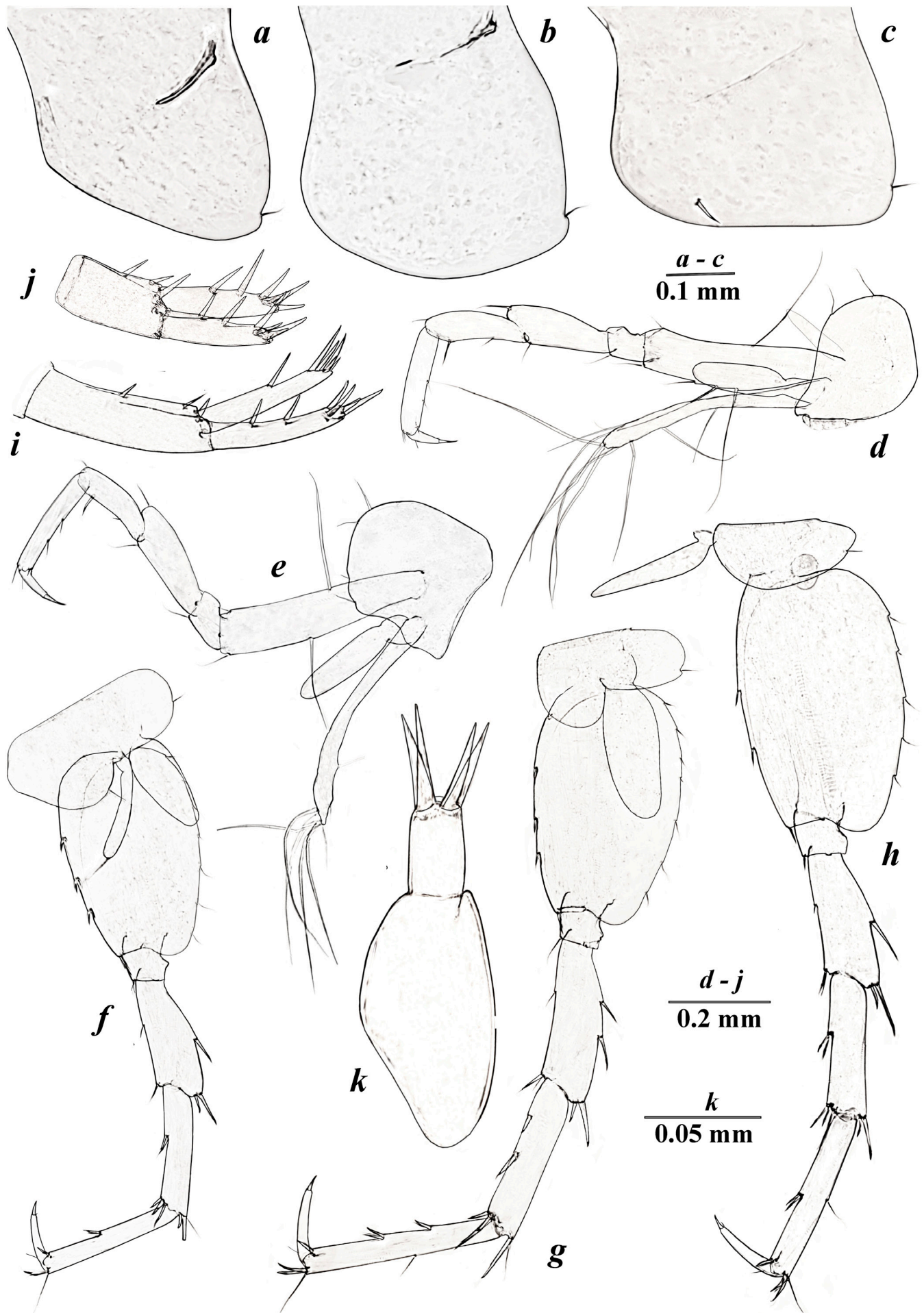


Fig. 5. *Palearcticarellus pusillus* (Martynov 1930) from the Ulaganchik Mountain Spring (st. 1003) in the intake of the Bashkaus River, ♀: a–c – epimeral plates I–III; d – pereopod III; e – pereopod IV; f – pereopod V; g – pereopod VI; h – pereopod VII; i – uropod I; j – uropod II; k – uropod III.

DISCUSSION

The Altai Mountain country, and especially the southeastern part of Altai, is located in an area of high tectonic activity. The slow arched uplift of this area began during the Paleogene period (66–56 Ma), while the most intense tectonic movements started during the Middle Oligocene (28.4 Ma) and continued until the Late Miocene (5.3 Ma) (Bogachkin, 1981). During the Pliocene period, the intensity of tectonic activity decreased, but the height of the mountains continued to increase for 300–400 m over the entire period (5.3–1.8 Ma). The modern outlines of the terrain acquired at the end of the Pliocene ~1.8 Ma (Bogachkin, 1981).

As a result of the long-term process of mountain formation, a complex system of tectonic faults and fractures has developed on the territory of Altai, which differ significantly from each other both in terms of both their formation time and the environmental groundwater conditions filling them (Dutova et al., 2020; https://www.vsegei.ru/ru/info/gisatlas/sfo/altay/10-geol_karta.jpg). Recent studies have revealed that some spring waters in the Ulagan area have an increased helium content, which indicates a deep occurrence of the groundwater (Dutova et al., 2020). Lake Teletskoye is also fed by groundwater from deep tectonic faults, and the presence of a significant proportion of groundwater from tectonic faults was previously proposed for this basin (e.g., Maloletko, 2009). According to a proposed map of tectonic faults in the area (Fig. 1), adapted from Dutova et al. (2020), Mandilu Spring is located near a system of tectonic faults that originate from the Kurai Mountain Range. On the other hand, Ulaganchik Spring, is connected to deeper-lying faults that run along the riverbed of the Bashkaus River towards Lake Teletskoye (Fig. 1). At the same time, the nearby Ulaganchik and Mandilu springs, belong to different tectonic fault systems according to the presented map (Fig. 1). In addition to the geological data, molecular genetic analysis indicates that populations/species of stygobiotic amphipods from the studied nearby springs (Ulaganchik and Mandilu) have lost genetic contact for about 2 Ma, while individuals from Ulaganchik and Lake Teletskoye are still connecting genetically. Based on the resulting data, as well as an assumption about the possible dispersal of these crustaceans through a system of deep tectonic faults, it may help to explain why genetically isolated species of stygobiotic amphipods have been found in nearby springs, despite some more distant populations still being genetically related. We can also assume that some stygobiotic crustaceans might even live permanently in the groundwater of the tectonic faults, with some individuals occasionally being washed away.

Hypothesizing about the habitat of these crustaceans in tectonic faults may shed light on some aspects of their biology. Their minimum total body size, not exceeding 2–3 mm, could be a direct adaptation to the narrow spaces (pores) in the faults. This idea could contradict the hypothesis that their small body size is due to neoteny (Marin & Palatov, 2020), although both hypotheses could be true in certain cases.

A possible way for the dispersal of these crustaceans, namely the movement in the riverbed of Bashkaus River and possible habitation within hyporhean habitats, seem unlikely to us. This mountain river has a hard rocky bottom, with a very steep slope and strong current, especially during spring time, which makes it difficult for sediment accumulation in continuous layers over a significant distance (personal observation). Furthermore, the Bashkaus and Chulyshman river systems extensively studied, and no stygobiotic crustaceans have been found there (e.g., Koveshnikov, 2014; personal data). It seems that these river systems are not suitable for fragile stygomorphic animals, such as representatives of the genus *Palearticarellus*. No any other related taxa have been described from such powerful mountain river habitats so far.

It is also worth noting here that the formation of the landscape in the area is also influenced by climate changes. Glaciation has been identified as one of the main factors that limit the distribution of stygobiotic species. For example, analysis in the UK has shown that only 1% of recent habitats of stygobiotic animals are located in the historically glaciated zone, while the remaining 99% are in areas that were not subjected to glaciers in the past (Robertson et al., 2009; Mcinerney et al., 2014). The Ulagan Plateau experienced glaciation of the Altai during the Middle Pleistocene period, when an ice sheet may have even formed in this area (Okishev & Narozhny, 2006), as well as during the several smaller-scale glaciation events occurred later (e.g., Blyakharchuk et al., 2004; Dirksen et al., 2007). Thus, a large glacier tongue descended from the northern slopes of the Kurai Mountain Range along the valley of the Kubadru River and flowed into the Ulagan Ice Sheet about 70,000 years ago. Despite this, a third of the surveyed springs on the Ulagan Plateau once contained stygobiotic animals (Marin et al., 2023; present paper). The estimated divergence time between the studied species of the genus *Palearticarellus* living in different parts of the Ailaguk and Kurai mountain ranges indicates that they genetically diverged around 5–4 Ma, much earlier than the glacial times in Pleistocene period. However, these data correlate well with the active uplift phase of the Kurai Range and its spurs at the beginning of the Pliocene, about 5 Ma (Bogachkin, 1981) that possible started the fragmentation of underground habitats and the separation of these species with a higher probability. Therefore, we do not consider glaciation to be of the main factors limiting the distribution of the subterranean/stygobiotic fauna of Altai.

The wide distribution of *P. mikhailli* in the Kurai Steppe, which has also been greatly influenced by glaciation in the past (Blyakharchuk et al., 2004; Dirksen et al., 2007), could also be explained by hydrogeological factors rather than any climatic influence. The water sources found in the intermountain depressions of the Chuya and Kurai steppes are represented by a widespread groundwater artesian basin, which can sometimes be multi-level (Nevedrova et al., 2019; Deev et al., 2023), and through which these crustaceans can obviously be passively transported or can move freely.

The current ideas about the location of tectonic and geological faults and groundwater deposits in the vast area between Lake Teletskoye and the Ulagan Plateau, allows us to only speculate about the dispersal routes of stygobiotic animals. However, the discovery of common species in such distant locations represent new challenges for researchers studying the biospeleological and hydrogeological conditions in the region. One such challenge is the active use of molecular genetic studies of stygobiotic fauna as an indicator of hydrogeological connections and historical changes in geological conditions. We would like to emphasize that molecular identification can be used in combination with traditional biospeleological and hydrogeological methods, using specific chemical markers (Goldscheider et al., 2008). This approach is simpler and more convenient in some cases, as samples (animals) can be collected at different times and do not require research in multiple geographical/geological areas simultaneously.

We also suggest that subterranean/stygobiotic species are narrowly localized and require strict protection and attention to their habitats, since the destruction of populations leads to the forever loss of unique ancient genetic lineages, which are impossible to restore.

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