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The Diversity of Freshwater Stygobiotic Crustaceans in the Republic of North Ossetia–Alania Provides New Evidence for the Existence of an Ancient Glacial Refugium in the North Caucasus Region

Ivan N. Marin * and Dmitry M. Palatov

A.N. Severtsov Institute of Ecology and Evolution of RAS, 119071 Moscow, Russia; triops@yandex.ru
* Correspondence: coralliodecapoda@mail.ru

Abstract: A review and partial revision of the diversity of freshwater stygobiotic crustaceans in the territory of the Republic of North Ossetia–Alania, in the North Caucasus, is presented here. Previously, two species of the genus *Proasellus* Dudich, 1925 (Isopoda, Asellidae), *P. uallagirus* Palatov & Sokolova, 2020 and *P. irystonicus* Palatov & Sokolova, 2020, and one species of the genus *Niphargus* Schiödte, 1849 (Amphipoda, Niphargidae), *N. alanicus* Marin & Palatov, 2021, were described from the hyporhean/underground habitats (hyporhea) in the area. However, further research using an integrative approach has revealed that only a single species of the genus *Proasellus* (*P. uallagirus*) is actually widely distributed in the hyporhean riverbed habitats in the area, while the diversity of the genus *Niphargus* is higher than previously known. Six more new *Niphargus* species—namely, *N. ardonicus* **sp. nov.**, *N. sadonicus* **sp. nov.**, *N. fiagdonicus* **sp. nov.**, *N. tschertschesovae* **sp. nov.**, *N. osseticus* **sp. nov.** and *N. zeyensis* **sp. nov.**, were discovered from the various hypogean underground water sources (i.e., springs and seeps) and are described in this article. Their phylogenetic relationships with their congeners, as well as their ecology and known distribution, are discussed. Furthermore, molecular genetic analysis, with an interpretation of the estimated divergence time, suggests that the studied hyporheic/stygobiotic crustaceans started to diverge from related European and Balkan sister species during the Late Miocene, approximately 8–5.8 Mya, with the reduction in the Paratethys and the uplifting of the Caucasus Mountains. Local speciation was led by local geological processes and karst fragmentation during the Late Pliocene and Early Pleistocene periods, starting around 5.3 Mya. The obtained data suggest that the mountainous area of the North Ossetia–Alania could be considered as a post-Pliocene glacial refugium for subterranean and stygobiotic fauna—the first known for the North Caucasus region.

Keywords: Amphipoda; Isopoda; crustaceans; *Niphargus*; *Proasellus*; diversity; hyporheic; stygobiotic; new species; review; distribution; North Ossetia–Alania; Ciscaucasia; North Caucasus

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1. Introduction

The Caucasus region is currently hosting a unique and very diverse fauna of stygobiotic crustaceans and other subterranean animals [1–4]. This rich diversity has formed primarily due to the presence of an extensive karst landscape, a peculiar regional microclimate, and a complex history of climatic and geological changes, mostly associated with the Pleistocene glaciation period. During the Quaternary Ice Age (~2.59 Mya–present) and the last glacial maximum (LGM, about 23–18 Kya), which dramatically changed the biota of the Northern Hemisphere [5–11], many organisms were able to survive in local cryptic habitats, mainly underground. Today, they contribute to the unique biodiversity of the region, representing remnants (relicts) of the previous ancient fauna. This is relevant for the Western Palearctic glacial refugia [3,12–14], such as the Kolkhida coastal lowland plain of the eastern Black Sea (or Colchis), which formed during the Upper Pliocene [15–17].

Such refugia have also played an important role in the post-glacial recolonization during the post-Pleistocene times [3,9,13,18]. However, this is true for the southern slope of the Greater Caucasian Ridge, whereas the current knowledge of stygobiotic fauna is relatively poor on the northern slope, probably due to the lack of studies to date [19,20]. Nevertheless, there are exceptions, for example, the recently discovered ancient glacial refugium in the northern Black/Azov Sea Lowland near the mouth of the Don River [21].

The presence of ancient refugia can be determined by the existence of endemic relict organisms, which are typically not able to disperse over long distances and have been genetically isolated from their relatives for a long period of time. Stygobiotic crustaceans are ideal indicators of these refugia, as most of them are narrowly localized stenobiotic endemics [22,23], usually inhabiting only available underground habitats with stable environmental conditions that isolate them from climatic changes on the surface [24].

The members of the stygobiotic crustacean genera *Niphargus* Schiödte, 1849 (Amphipoda, Niphargidae) and *Proasellus* Dudich, 1925 (Isopoda, Asellidae) are among the most common and abundant animals in cave water reservoirs and hyporhean riverbed habitats in the Caucasus region [20,25–30]. Their relatively high diversity has now been described from the southern slope of the Greater Caucasus [19,25,27,29,31–36], while the stygobiotic fauna of the northern slope still remain mostly unknown. To date, only two species of the genus *Niphargus*—namely, *Niphargus ciscaucasicus* Marin & Palatov, 2019, from the foothills of the northern slope near Apsheronsk [19], and *Niphargus alanicus* Marin & Palatov, 2021 from an abandoned mine in the North Ossetia–Alania, have been discovered from the northern slope of the Great Caucasian Ridge [37]. Also, two species of the genus *Proasellus* Dudich, 1925 (Crustacea: Isopoda)—namely, *P. uallagirus* Palatov & Sokolova, 2021 and *P. irystonicus* Palatov & Sokolova, 2021, were recently described from hyporhean riverbed habitats of the Terek River Basin in the Alagir Gorge [20], and an undescribed species of *Proasellus* was also reported from the Digor Gorge (Iraf District) in the North Ossetia–Alania [38]. Such relatively low diversity can be explained by the impact of glaciation during the Quaternary period [5,14]. However, we believe that the lack of knowledge about stygobiotic species may be due to a lack of comprehensive research in the region.

In this article, we have attempted to fill this gap by providing a review of the stygobiotic crustacean fauna (*Niphargus* and *Proasellus*) that inhabit hyporhean/underground habitats in the territory of the Republic of North Ossetia–Alania, located in the middle part of the northern slope of the Greater Caucasian Ridge. This study uses an integrative approach to clarify the phylogenetic relationships and current distribution of the studied species, as well as to discuss the historical context and possible ways of entering this mountainous region.

2. Materials and Methods

Sample collection and processing: Stygobiotic crustaceans were collected from various hypogean and subterranean water resources (springs, wells, cave reservoirs, and river hyporhea) in the mountainous area of the Republic of North Ossetia–Alania in 2018–2022. In our study, we conducted random sampling from all available locations and water sources, as this was the first biogeographical survey in the area, which could not be conducted according to any scheme. A plastic hand-net with a pore size of about 1 mm was used to collect the crustaceans. The specific biotope where the stygobiotic crustaceans were found is described below for each species. All collected crustaceans were preserved in 90% ethanol for molecular genetic analysis. At least one individual of each collected species from each location was used for genetic analysis in order to understand their genetic diversity and distribution in the region. The type material (holotype and paratypes) was deposited at the collection of the Zoological Museum of Moscow State University, Moscow (ZMMU); additional material was deposited in the authors' personal collection at the A.N. Severtsov Institute of Ecology and Evolution of RAS, Moscow (LEMMI).

Morphological study: All collected specimens were preliminarily processed, sorted based on specific morphological features, and photographed under an Olympus SX10 light microscope at standard magnifications of ×5, ×7, and ×10. Scanning electron microscopy (SEM) images were taken using a Vega3 Tescan microscope at the Paleontological Museum of the Paleontological Institute of the Russian Academy of Sciences, Moscow. Specimens were cleaned in an ultrasonic cleaner, dehydrated with acetone, and then critical-point dried (CPD). After that, the specimens were fixed on stubs with double-sided Scotch tape and coated with gold by sputtering using a Polaron PS 100.

The body length (bl., mm) was measured as the dorsal length from the distal margin of the head to the posterior margin of the telson, with the exclusion of both antennae and uropod III.

Molecular genetic study: A fragment of the mitochondrial gene that codes for cytochrome c oxidase subunit I (COI mtDNA gene marker) was amplified using a standard protocol and conditions, using Folmer's universal primers LCO1490 and HC02198 [39]. The obtained sequences were deposited in the GenBank (NCBI) public database, which is available for further genetic studies worldwide. The related congeneric species of the genera *Proasellus* and *Niphargus* were also separated from the general dataset through phylogenetic analysis of the available sequences from GenBank (NCBI) and the authors' personal databases.

The aligned sequences, including 658 bp, were used for the phylogenetic analysis. The best evolutionary substitution model was selected in MEGA 7.0. [40] and jModeltest2.1.141 (Diego Darriba, Universidade da Coruña, as part of the Computer Architecture Group (GAC), Coruña, Spain) from XSEDE via the CIPRES (Cyber Infrastructure for Phylogenetic Research) Science Gateway V. 3.3 (http://www.phylo.org/, accessed on 10 September 2023). Phylogenetic analysis was performed using the program PhyML 3.0 [41], with the selected GTR+G+I substitution model for the maximum-likelihood (ML) algorithm. The use of the COI mtDNA gene marker allowed us to address the challenges of the studied genetic diversity [42,43], whereas the analysis of more complex phylogenetic relationships using other gene markers, such as nuclear DNA, was not among the objectives of this study.

Pairwise uncorrected genetic distances (*p*-distances) were calculated in MEGA 7.0 using the Kimura 2-parameter (K2P) model [44]. Based on the obtained values of *p*-distances, the divergence times were estimated, with the minimum and maximum values being 5.16%/Mya^{-1} and 0.77%/Mya^{-1}, respectively [45]; the average time was calculated as 2.5%/Mya^{-1} [46,47].

3. Results

3.1. Phylogenetic Part

Genus Proasellus: A molecular genetic study revealed that all individuals of the genus *Proasellus* found in the North Ossetia–Alania belong to a single biological species. An intraspecific genetic divergence is 0.0105 ± 0.0025 substitutions per 100 nucleotides (approximately 1%). Therefore, the previously described *Proasellus uallagirus* Palatov & Sokolova, 2020 and *Proasellus irystonicus* Palatov & Sokolova, 2020 **syn. nov.** (junior synonym, see below) are conspecific. However, the studied individuals display a high degree of variation in numerous morphological features, and further research will allow us to determine the exact causes of such variability. Additionally, it is challenging to establish the phylogenetic relationships with other congeneric species from the Caucasus and nearby regions, due to the lack of molecular genetic data in well-established databases such as GenBank (NCBI).

Genus Niphargus: A molecular genetic study revealed that all collected specimens of the genus *Niphargus*, including the previously described *N. alanicus* Marin & Palatov, 2021, belong to the "*carpathicus*" species group also found on the Balkan Peninsula, the mountainous regions of Southern and Central Europe, western Ciscaucasia, and the southern Caucasus, including the Colchis Lowland. *Niphargus* species obtained from the North Ossetia–Alania belong to two phylogenetically distinct clades (see Figure 1): The first clade contains a single species, *Niphargus zeyensis* **sp. nov.** (from the Tseydon River Basin (Tsey

Gorge) (for the description, see below), which is phylogenetically closely related to several undescribed species from the Crimean Peninsula and the southern part of the Russian Caucasus (Sochi), as well as the members of the "*Niphargus borutzkyi*" species group from the Kutaisi area in western Georgia [30,36] (see Figure 1). The second clade includes six species, which are divided into three groups, namely, *N. alanicus* Marin & Palatov, 2021/*Niphargus ardonicus* **sp. nov.**/*Niphargus fiagdonicus* **sp. nov.** (group 1), *Niphargus osseticus* **sp. nov.** (group 2), and *Niphargus sadonicus* **sp. nov.**/*Niphargus tschertschesovae* **sp. nov.** (group 3) (for the descriptions, see below). This clade is closely related to the congeneric species from the southern slope of the Great Caucasian Ridge, such as the "*Niphargus alasonicus*" species group from eastern Georgia [25], the southern Georgian *Niphargus glontii* Behning, 1940, and an undescribed species from the Ablaskiri Cave in Abkhazia.

It should be noted that the specimens of the genus *Niphargus* from Lagodekhi, Kakhetia, Georgia, used in our phylogenetic study (see Figure 1) are currently considered to belong to the clade of the "*alasonicus*" species group [25]. At the same time, they differ significantly from the original description of *N. alasonicus* Derzhavin, 1945, which demonstrates the specific and different palm shape and smaller number of the outer setae on the dactyli of gnathopods I–II, the presence of an additional ventral spine on the dactyli of ambulatory pereiopods III–VII, the different armature of the urosome, and the presence of fewer groups of setae along the basal article of uropod III [48]. In this regard, further study is needed to confirm the taxonomic relationship between this group and the latter species.

The calculated *p*-distances between the studied *Niphargus* species from the North Ossetia–Alania exceeded 9–19% (see Table 1), suggesting their genetic divergence from one another for at least 7.6–3.6 Mya. Furthermore, based on the calculated *p*-distances and estimated divergence times (see Table 2), these North Ossetian *Niphargus* species became genetically isolated from their South Caucasian relatives much earlier than their local speciation occurred. Therefore, *Niphargus zeyensis* **sp. nov.** genetically diverged from the related South Caucasian congeneric species approximately 8–6 Mya. The species from the second clade (see above) also genetically separated from the related congeners about 8–6 Mya (see Table 2). These data are well correlated with the time of the reduction of the Paratethys and the active uplift of the central part of the Great Caucasian Ridge, which occurred approximately 10–5 Mya.

Table 1. Pairwise genetic distances (*p*-distances) (substitutions per 100 nucleotides ± SE (standard error)) of the studied species of the genus *Niphargus* from the Republic of North Ossetia–Alania. The lowest values of genetic divergence between the studied species are highlighted in bold.

Niphargus alanicus		*N. sadonicus* sp. nov.	*N. osseticus* sp. nov.	*N. ardonicus* sp. nov.	*N. tschertschesovae* sp. nov.	*N. zeyensis* sp. nov.
N. sadonicus **sp. nov.**	**0.130** ± 0.016					
N. osseticus **sp. nov.**	0.154 ± 0.019	0.158 ± 0.018				
N. ardonicus **sp. nov.**	0.158 ± 0.020	**0.127** ± 0.016	0.133 ± 0.017			
N. tschertschesovae **sp. nov.**	0.163 ± 0.018	**0.086** ± 0.013	0.162 ± 0.020	**0.122** ± 0.015		
N. zeyensis **sp. nov.**	0.160 ± 0.019	0.159 ± 0.019	0.178 ± 0.019	0.170 ± 0.019	0.176 ± 0.017	
N. fiagdonicus **sp. nov.**	0.191 ± 0.022	0.167 ± 0.021	0.180 ± 0.021	0.118 ± 0.015	0.150 ± 0.017	0.186 ± 0.019

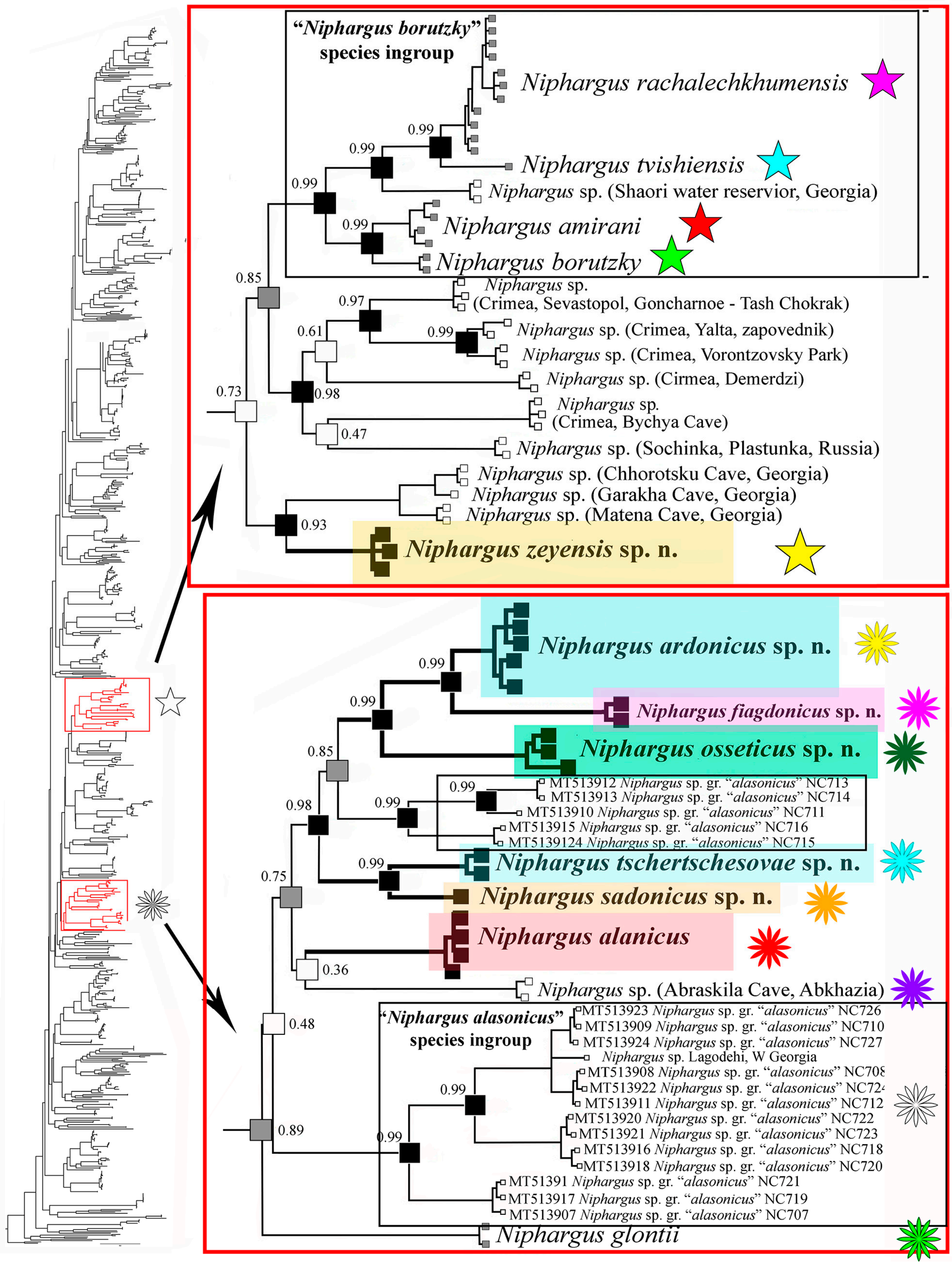

Figure 1. Phylogenetic reconstruction (tree) (COI mtDNA) showing the relationships of the studied species of the genus *Niphargus* (colored) from the Republic of North Ossetia–Alania with their congeners ((ML algorithm, GTR + G + I model). The presented COI sequences of related (sister) species were taken from the GenBank (NCBI) or/and authors personal databases.

Table 2. Pairwise genetic distances (*p*-distances) and estimated genetic divergence time between the studied *Niphargus* species from the Republic of the North Ossetia–Alania and their sister congeners from the southern part of the Great Caucasian Ridge and Crimean Peninsula.

Species	*p*-Distance	Estimated Divergence Time (Mya)		
		Min. (0.0516/Mya^{-1})	Average (0.025/Mya^{-1})	Max. (0.0077/Mya^{-1})
Niphargus zeyensis **sp. nov.**				
Chchortsku/Matena/Garakha	0.1495 ± 0.0156	2.89 ± 0.30	5.98 ± 0.624	19.41 ± 2.02
Crimea_Buchya_Cave	0.1839 ± 0.0178	3.56 ± 0.34	7.35 ± 0.71	23.88 ± 2.31
Niphargus clade (Crimea)	0.1946 ± 0.0144	3.77 ± 0.27	7.78 ± 0.57	25.27 ± 1.87
Sochi, Plastunka	0.1949 ± 0.0187	3.77 ± 0.36	7.79 ± 0.74	25.31 ± 2.42
"*borutzkyi*" species group	0.2029 ± 0.0167	3.93 ± 0.32	8.11 ± 0.66	26.35 ± 2.16
Niphargus alanicus				
Niphargus glontii	0.1856 ± 0.0176	3.59 ± 0.34	7.42 ± 0.70	24.10 ± 2.28
Abraskila Cave, Abkhazia	0.1971 ± 0.0184	3.81 ± 0.35	7.88 ± 0.73	25.59 ± 2.38
"*alasonicus*" species group	0.2112 ± 0.0161	4.09 ± 0.31	8.44 ± 0.64	27.42 ± 2.09
N. ardonicus **sp. nov.**, *N. fiagdonicus* **sp. nov.** and *N. osseticus* **sp. nov.** (Clade 2)	0.1790 ± 0.0144	3.46 ± 0.28	7.16 ± 0.57	23.24 ± 1.87
Niphargus sadonicus **sp. nov.** and *N. tschertschesovae* **sp. nov.** (Clade 3)	0.1828 ± 0.0190	3.54 ± 0.37	7.31 ± 0.76	23.74 ± 2.46
Niphargus ardonicus **sp. nov.**, *N. fiagdonicus* **sp. nov.** and *N. osseticus* **sp. nov.** (Clade 2)				
Niphargus glontii	0.1939 ± 0.0155	3.75 ± 0.30	7.75 ± 0.62	25.18 ± 2.01
Abraskila Cave, Abkhazia	0.2075 ± 0.0169	4.02 ± 0.32	8.30 ± 0.67	26.94 ± 2.19
"*alasonicus*" species group	0.2011 ± 0.0140	3.89 ± 0.27	8.04 ± 0.56	26.11 ± 1.81
Niphargus alanicus	0.1790 ± 0.0144	3.46 ± 0.28	7.16 ± 0.57	23.24 ± 1.87
N.sadonicus **sp. nov.** and *N. tschertschesovae* **sp. nov.** (Clade 3)	0.1703 ± 0.0134	3,.30 ± 0.26	6.81 ± 0.53	22.11 ± 1.74
Niphargus sadonicus **sp. nov.** and *N. tschertschesovae* **sp. nov.** (Clade 3)				
Niphargus glontii	0.1748 ± 0.0165	3.38 ± 0.32	6.99 ± 0.66	22.70 ± 2.14
Abraskila Cave, Abkhazia	0.1867 ± 0.0174	3.61 ± 0.33	7.46 ± 0.69	24.24 ± 2.26
"*alasonicus*" species group	0.1961 ± 0.0148	3.80 ± 0.28	7.84 ± 0.59	25.46 ± 1.92
N. ardonicus **sp. nov.**, *N. fiagdonicus* **sp. nov.** and *N. osseticus* **sp. nov.** (Clade 2)	0.1703 ± 0.0134	3.30 ± 0.26	6.81 ± 0.53	22.11 ± 1.74
Niphargus alanicus	0.1828 ± 0.0190	3.54 ± 0.37	7.31 ± 0.76	23.74 ± 2.46

3.2. Geographic Distribution

The obtained specimens of *Proasellus uallagirus* Palatov & Sokolova, 2020 from the territory of the Republic of North Ossetia–Alania (Figure 2, green squares) allowed us to conclude that it is a widely distributed permanent hyporheic inhabitant living in almost all river valleys (mountain gorges) in the studied area: Digorsky, Alagirsky, and Kabansky Gorges, over a wide range of altitudes, from 700 to 1800 m asl. However, the species has not been found in the Fiagdon River (Kurtatinsky Gorge) or lower Terek River Basins (Daryal Gorge) ([20] and present study), nor has it ever been observed in any hypogean water sources (e.g., springs).

All studied species of the genus *Niphargus* from the territory of the North Ossetia–Alania (Figure 2) have been found to be associated with various hypogean/underground water sources and hyporhean riverbed habitats in various gorges/river valleys: *Niphargus alanicus* Marin & Palatov, 2021 is known from the Sardidon and Dargonkom River Valleys ([37] and present study); *Niphargus ardonicus* **sp. nov.** from the Ardon, Urukh, Mayramadag, and Gizeldon River Valleys; *Niphargus sadonicus* **sp. nov.** from the Sadon River Valley, a tributary of the Ardon River; *Niphargus tschertschesovae* **sp. nov.** from the

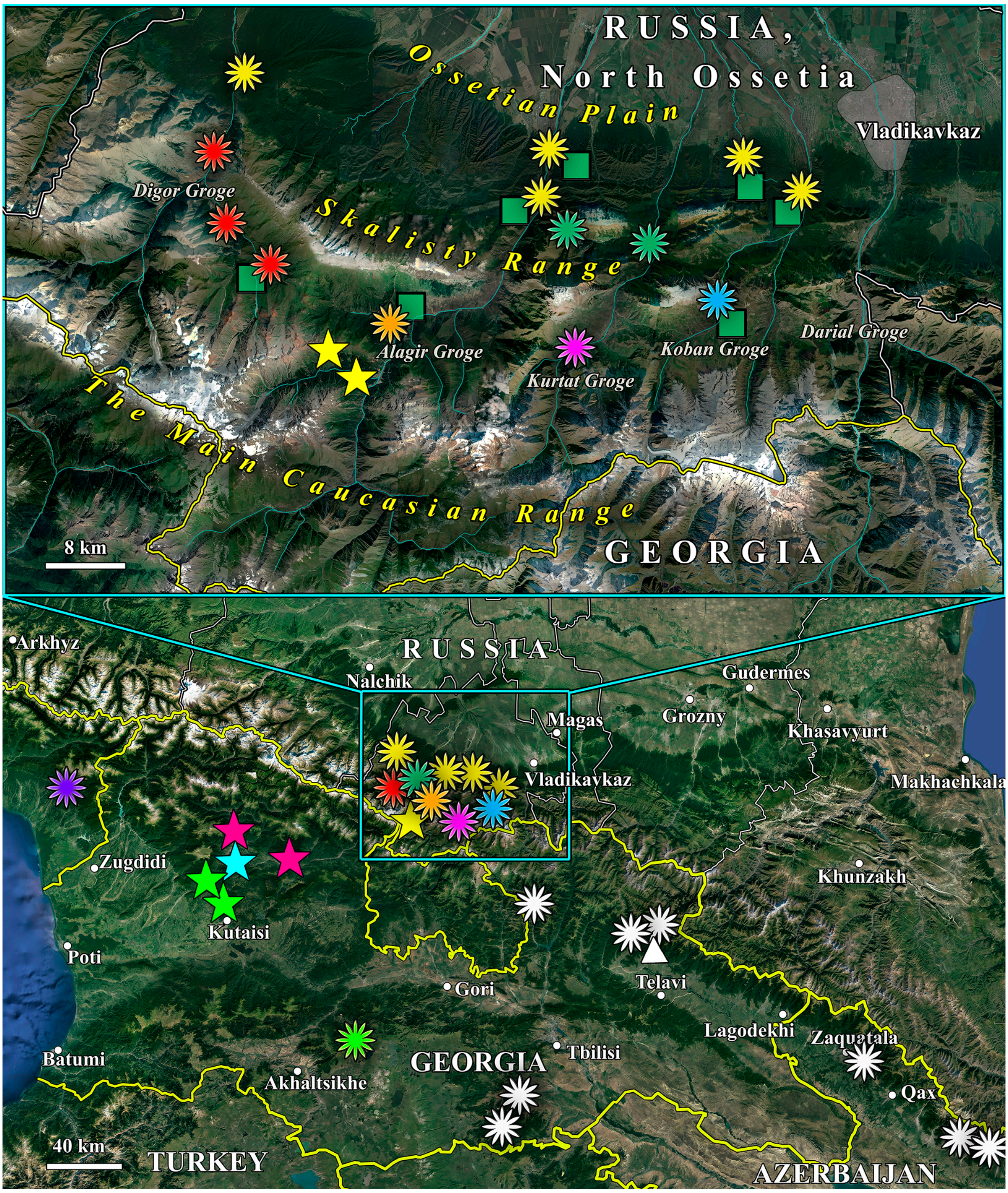

Figure 2. The distribution of *Niphargus* species in the territory of the Republic of North Ossetia–Alania, with the known distribution area of the related (sister) taxa along the southern slope of the Great Caucasian Ridge. Green squares (upper map only)—*Proasellus uallagirus* Palatov & Sokolova, 2020. White triangle—the type locality for *N. alasonicus* Derzhavin, 1945. Twelve-pointed stars: white—undescribed *Niphargus* spp. gr. "*alasonicus*" (after [25]); dark violet—*N. ablaskiri* Birštein, 1940; light green—*N. glontii* Behning, 1940; red—*N. alanicus* Marin & Palatov, 2021; yellow—*N. ardonicus* **sp. nov.**; orange—*N. sadonicus* **sp. nov.**; pink—*N. fiagdonicus* **sp. nov.**; light blue—*N. tschertschesovae* **sp. nov.**; green—*N. osseticus* **sp. nov.** Five-pointed stars represent "*Niphargus borutzkyi*" species ingroup [30,36]: light green—*N. borutzkyi* Birštein, 1933; red—*N. amirani* Marin, 2020; blue—*N. tvishiensis* Marin, Barjadze, Maghradze & Palatov, 2023; pink—*N. rachalechkhumensis* Marin, Barjadze, Maghradze & Palatov, 2023; yellow—*N. zeyensis* **sp. nov.**

Gizeldon River Valley; and *Niphargus zeyensis* **sp. nov.** from the Tseydon River Basin (Tsey (Zey) Gorge). The closely related *Niphargus fiagdonicus* **sp. nov.** and *Niphargus osseticus* **sp. nov.** inhabit various areas (upper and lower, branched) of the Fiagdon River Valley (gorge), while *Niphargus tschertschesovae* **sp. nov.** and *Niphargus ardonicus* **sp. nov.** were also found in different areas (upper (mountainous) and lower parts, respectively) of the Gizeldon River. Only a single *Niphargus* species was found in any of the studied locations.

3.3. Taxonomic Part

Order Isopoda Latreille, 1817
Family Asellidae Rafinesque-Schmaltz, 1815
Genus *Proasellus* Dudich, 1925
***Proasellus uallagirus* Palatov & Sokolova, 2020**
Proasellus irystonicus Palatov & Sokolova, 2020 **syn. n.**

Material: 2 ♂♂, 4 ♀♀ (LEMMI)—Russian Federation, Republic of North Ossetia–Alania, Alagirsky District, a spring in the valley of the Ardon River, about 8 km south of the town of Alagir, 42°55′31.79″ N 44°11′26.14″ E, 823 m asl, coll. D. Palatov, 3 October 2020; 1 ♂, 3 ♀♀ (LEMMI)—a stream in an abandoned mine near the village of Sadon, 42°50′40.28″ N 44°01′16.77″ E, 1155 m asl, coll. D. Palatov, 8 October 2020; 6 ♂♂, 10 ♀♀ (LEMMI)—a spring in the valley of the Mayramadag River, 2 km south of the village, 42°59′47.96″ N 44°29′37.87″ E, 670 m asl, coll. D. Palatov, 9 May 2022; 3 ♂♂, 7 ♀♀ (LEMMI)—Irafsky District, a spring seeping on the banks of the Dargonkom River Valley, 42°53′26.8″ N 43°51′58.3″ E, 1860 m asl, coll. D. Palatov, 3 November 2021; 5 ♂♂, 12 ♀♀ (LEMMI)—Prigorodny District, groundwater of the Gizeldon River near the village of Dargavz, 42°51′08.76″ N 44°26′55.55″ E, 1395 m asl, coll. D. Palatov, M. Antipova, 22 May 2023; 1 ♂, 2 ♀♀ (LEMMI)—a spring stream in the floodplain of the Gizeldon River, opposite the "Alpina Park" complex, 42°58′39.84″ N 44°34′11.35″ E, 763 m asl, coll. D. Palatov, M. Antipova, 24 May 2023.

Diagnosis: Completely depigmented, small species. *AI* with 5–8 flagellar articles, flagellum of *AII* with 28–40 articles. Inner plate of *Mx* with five apical pappose setae. Propodus of *PpI* elongated and oval, its inferior margin without proximal apophyses, with 1–2 robust spiniform setae. Dactylus of *PpI* with 3–4 short robust setae on the inferior margin and five or six simple setae along the superior margin. Dactylus of *PpII–VII* with a single robust stiff-like seta on the inferior margin. Retinacula of *PlI* with a single hook. Endopodite of *PlII* in ♂♂ with weakly expressed basal apophysis, short distal apophysis, and small goulot with developed lips. Endopodite of *PlII* in ♀♀, subtriangular, with three short, marginal, simple setae. Lateral and terminal margins of *PlIII* with 10–11 short simple setae, with endopodites about 1.6 times shorter than exopodites. Lateral margin of exopodite of *PlIV* without setae. Endopodite of *PlIV* suboval, about 1.3 times shorter than the exopodite. Exopodite of *PlV* ovoid, elongated, 2.2 times as long as wide, and with lateral margins without setae; endopodite suboval and about 90% of exopodite. *Uropods* similar in ♂♂ and ♀♀; proto-, endo-, and exopodite length ratio of 1/1.2/1.3, respectively.

GenBank accession number: PP715899–PP715902, PP715904.

Distribution and ecology: Currently, the species is known from the hypogean underground water habitats, located in the in the upper (mountainous) basin of the Terek River: Urukh River (Digorskoe Gorge), Ardon River (Alagirskoe), and Gizeldon River (Kobanskoe), in a wide range of altitudes, from 700 to 1800 m asl. However, it has not been discovered in the Fiagdon River (Kurtatinsky Gorge) or upper Terek River (Daryal Gorge) Basins.

Taxonomic remarks: The species is characterized by a high degree of morphological variation, which is expressed in the average body size of individuals, the number of segments in the antennae, the pubescence and shape of the pleural shield, the structure of the uropods, and the structure of pleopods II in ♂♂ [20]. However, molecular genetic study indicates that individuals with various morphologies belong to the same biological species (see above).

For the difference from congeners from the Caucasus, see the original description [20].

Order Amphipoda Latreille, 1816
Family Niphargidae Bousfield, 1977
Genus *Niphargus* Schiödte, 1849
***Niphargus alanicus* Marin & Palatov, 2021**

Material: 5 ♀♀ (bl. 10 mm) (ZMMU Mb-1163)—Russian Federation, Republic of North Ossetia–Alania, Irafsky District, Sardidon River Valley, Fansal village, 42°56.0980′ N 43°49.0340′ E, about 1430 m asl, in an abandoned mine, coll. D. Palatov, 31 December 2017; 35 ♀♀ (LEMMI)—same locality, coll. D. Palatov, 3 August 2019; 3 ♀♀ (LEMMI)—Dargonkom River Valley, 42°53′26.0″ N 43°52′53.0″ E, about 1960 m asl, in helocrene springs, coll. D. Palatov, 30 December 2017.

Newly discovered material: ♀ (bl. 10 mm) (LEMMI)—Urukh Cave, coll. S.A. Kapralov, 19 September 2020.

Diagnosis: *Head* without pigmented spots on anterior lobe. Posteroventral corners of *EpI–III* rounded. *UrI–II* with 1 strong spine at a posterodorsal angle on each side. Flagellum of *AI* with 2 short aesthetascs on each article. Third article of the *mandibular palp* slightly shorter than the previous one. Dactyli of *PpIII–IV* without spines near the base of nails. Dactyli of *PpV–VII* with a small additional spine. Rami of *UI* of almost equal length. Retinacula of *PlI–III* with 4–5 hooks. *Telson* with 5–6 long distal spines, 2–3 long or medium lateral spines, and 2 plumose setae.

Coloration and body size: Body coloration is completely white. The largest collected ♀ has bl. 10.5 mm.

GenBank accession number: MW771599, MW771600, PP715910.

Ecology and distribution: The species inhabits hypogean underground waters underlying karst areas of the Sardidon and Dargonkom River Basins (Digoria historical region) in the subalpine zone of the northern slope of the Great Caucasian Ridge. Sometimes washed out to the surface through numerous cracks, springs, and artificial mines.

Taxonomic remarks: Taxonomic remarks are given in the original description [37]. For the comparison with the other newly discovered species, see below.

***Niphargus ardonicus* sp. nov.**

Material: Holotype: ♀ (bl. 10 mm) (ZMMU Mb-1269)—Russian Federation, North Caucasus, the Republic of North Ossetia–Alania, Alagirsky District, a spring in the Ardon River Valley, about 8 km south of Alagir, 42°55′31.79″ N 44°11′26.14″ E, 823 m asl, coll. D. Palatov, 3 October 2020.

Paratypes: 2 ♀♀ (ZMMU Mb-1270)—same data as for holotype.

Additional material: 6 ♀♀ (LEMMI)—same data as for holotype; 5 ♀♀ (LEMMI)—Russian Federation, Republic of North Ossetia–Alania, Alagirsky District, a spring in the Ardon River Valley, about 2 km south of Alagir, 42°58′31.8″ N 44°13′11.2″ E, about 670 m asl, coll. D. Palatov, 23 September 2020; 12 ♀♀ (LEMMI)—Alagirsky District, a spring in the Mayramadag River Valley, 2 km south of Mayramadag, 42°59′47.96″ N 44°29′37.87″ E, about 655 m asl, coll. D. Palatov, 9 May 2022; 7 ♀♀ (LEMMI)—Irafsky District, a spring in a damp beech forest, located 2 km south of Kalukh, 43°4′37″ N 43°49′22.7″ E, about 970 m asl, coll. D. Palatov, 5 November 2021; 2 ♀♀ (LEMMI)—Prigorodny District, a spring stream in the floodplain of the Gizeldon River, opposite the "Alpina Park" complex, 42°58′39.84″ N 44°34′11.35″ E, 763 m asl, coll. D. Palatov, M. Antipova, 24 May 2023.

Etymology: The new species was named after the Ardon River.

Diagnosis: *Head* without pigmented spots on anterior lobe. Posteroventral corners of *EpI–III* rounded. *UrII* with 2 strong spines at posterodorsal angles on each side. Distal article of *mandibular palp* with a group of 4–6 A-setae, 2–3 B-setae, 14–15 D-setae, and 4–5 E-setae. Outer lobe of *MxI* with 7 robust comb-like spines, carrying 5–6 thin teeth each. Dactyli of *PpIII–IV* at the inner margin without strong spines near the bases of nails. Dactyli of *PpV–VII* with a small additional spine. Rami of *UI* of equal length. Retinacula of *PlI–III* with 5 hooks. *Telson* with 3–4 relatively long distal spines, 1–2 long or medium lateral spines, and 2 plumose setae on each side.

The complete morphological description is presented in the Supplementary Materials (Figures S1–S4 and S11a,b).

Coloration and body size: Body coloration is transparent whitish. The largest collected ♀ had bl. 10.5 mm.

GenBank accession number: PP715911–PP715916.

Distribution and ecology: This species is found in the forest zone of the foothills of Ossetia, at the base of all river valleys (gorges) facing the plain, in the foothills of the Urukh, Ardon, Gizeldon, and Mayramadag River Valleys. Of all the species described in North Ossetia–Alania, it has the widest known range, living in the hypogean underground (springs) and hyporhean riverbed habitats (hyporhea) of the abovementioned rivers.

Taxonomic remarks: The species can be easily separated from the already described *N. alanicus* [37] by (1) the presence of numerous smaller spine-like setae along the outer margins of robust spines of maxilla I (vs. absent), (2) the presence of simple setae on the dorsolateral margin of urosomal somite I (vs. strong spines), (3) a strong additional spine-like seta on dactyli of ambulatory pereiopods (vs. simple seta), (4) retinacula with 5 hooks (vs. 4 hooks), (5) smaller coxal gills on gnathopod II, and (6) telson lobes with 1 lateral and 3 distal strong spines (vs. 2–3 lateral and 6 distal spines).

For the differences from other newly discovered species, see below.

Niphargus fiagdonicus **sp. nov.**

Material: Holotype: ♀ (bl. 12 mm) (ZMMU Mb-1271)—Russian Federation, Republic of North Ossetia–Alania, Alagirsky District, a spring in the Fiagdon River Valley, near the Alan Holy Dormition Monastery in Hidikus, 42°49′18.5′′ N 44°16′17.8′′ E, 1295 m asl, coll. D. Palatov, 16 May 2023.

Etymology: The new species was named after the Fiagdon River.

Diagnosis: *Head* without pigmented spots on anterior lobe. Posteroventral corners of *EpI–III* rounded. *UrII* with 2 strong spines at posterodorsal angles on each side. Distal article of *mandibular palp* with a group of 4–5 A-setae, 2 B-setae, 17–19 D-setae, and 4–5 E-setae. Outer lobe of *MxI* with 7 robust comb-like spines, carrying 3–10 teeth each. Dactyli of *PpIII–IV* at the inner margin without strong spines near the bases of nails. Dactyli of *PpV–VII* with a small additional spine. Rami of *UI* of nearly equal length. Retinacula of *PlI–III* with 5 hooks. *Telson* with 4 with long distal spines, 2–3 long or medium lateral spines, and 2 plumose setae on each side; dorsal surface without spines.

The complete morphological description is presented in the Supplementary Materials (Figures S5, S6, and S11c,d).

Coloration and body size: Body coloration is completely white. The largest collected ♀ had bl. 12.0 mm.

GenBank accession number: PP715920.

Distribution and ecology: This species is found only in the type habitat, small hyporhean springs in the middle course of the Fiagdon River (Kurtatinsky Gorge).

Taxonomic remarks: The species is morphologically similar to *N. ardonicus* **sp. nov.**, described above, and can be easily separated from the already-described *N. alanicus* [37] by (1) the presence of numerous small spine-like setae along the outer margin of robust spines of maxilla I (vs. absent), (2) the presence of a simple seta on the dorsolateral margin of urosomal somite I (vs. strong spine), (3) a strong additional spine-like seta on the dactyli of ambulatory pereiopods (vs. a simple seta), (4) retinacula with 5 hooks (vs. 4 hooks), (5) significantly smaller coxal gills on gnathopod II, and (6) telson lobes with 4 apical strong spines on each (vs. 6 apical spines). For the differences from other newly discovered species, see below.

From the closely related *Niphargus ardonicus* **sp. nov.**, this species can be separated by (1) smaller coxal gills, which are smaller than the basis of pereiopod IV (vs. larger or similar in size to the basis of pereiopod IV), (2) less beveled posterior margin of the epimeral plate III, and (3) the presence of 2–3 lateral spines on each lobe of the telson (vs. 1 lateral spine). For the differences from other newly discovered species, see below.

Niphargus osseticus **sp. nov.**

Material: Holotype: ♂ (bl. 11.5 mm) (ZMMU Mb-1272)—Russian Federation, Republic of North Ossetia–Alania, Alagirsky District, a spring inside the Agomskaya Cave, Ardon River Basin, bank of Akhshkadon Gorge, 2.9 km northwest from Oldukhankhokh mt., about 1209 m asl, coll. S. Kapralov, 17 September 2020.

Paratypes: 2 ♀♀ (ZMMU Mb-1273)—same data as for holotype.

Other material: 3 ♀♀ (LEMMI)—same data as for holotype; 3 ♀♀ (LEMMI)—Russian Federation, the Republic of North Ossetia–Alania, Prigorodny District, a spring on the right bank of the Fiagdon River opposite the "cascading waterfall", 42°55′3.7″ N 44°22′10.1″ E, about 1000 m asl, coll. D. Palatov, 18 July 2021.

Etymology: The new species is named after the Republic of North Ossetia–Alania.

Diagnosis: *Head* without pigmented spots on anterior lobe. Posteroventral corners of *EpI–III* rounded. *UrII* with 2 strong spines at posterodorsal angles on each side. Distal article of *mandibular palp* with a group of 6–7 A-setae, 3–4 B-setae, 16–18 D-setae, and 4–5 E-setae. Outer lobe of *maxilla I* with 7 robust spines, carrying 2–4 teeth each. Dactyli of *PpIII–IV* at the inner margin without strong spines near the bases of nails. Dactyli of *PpV–VII* with a small additional spine. *Uropod I* rami of nearly equal length. Retinacula of *PlI–III* with five hooks. *Telson* with 3 relatively long distal spines, 2–3 long or medium lateral spines, and 2 plumose setae on each side; dorsal surface without spines.

The complete morphological description is presented in the Supplementary Materials (Figures S7–S10 and S11e,f).

Coloration and body size: Body coloration is completely whitish. The largest collected ♂ had bl. 11.5 mm.

GenBank accession number: PP715917–PP715919.

Distribution and ecology: Currently, this species is in two nearby locations—the Agomskaya Cave in the basin of the Ardon River (Akshakadon Gorge) and a spring in the valley of the Fiagdon River (Kurtatinsky Gorge). It is very likely that these locations are connected through underground water systems.

Taxonomic remarks: This species belongs to the same group of species as *N. alanicus*, and it is very morphologically similar to the latter species. It can be separated from *N. alanicus* [37] by (1) retinacula of pleopods with 5 hooks (vs. 4 hooks), (2) the presence of a simple seta on the dorsolateral margin of urosomal somite I (vs. strong spine), (3) large coxal gills on gnathopod II and pereiopod IV exceeding the length of the basis (vs. significantly smaller), (4) a strong additional spine-like seta on the dactyli of ambulatory pereiopods (vs. simple seta), and (5) the presence of 3 lateral and 3 apical strong spines on each lobe of the telson (vs. 2–3 lateral and 5–6 apical spines).

From the closely related *Niphargus ardonicus* **sp. nov.** and *N. fiagdonicus* **sp. nov.**, this species can be separated by (1) the presence of only several spine-like setae along the outer margin of robust spines of maxilla I (vs. numerous smaller spines along the outer margin), (2) a larger coxal gill similar in length to the basis of pereiopod IV (vs. smaller in size than the basis of pereiopod IV), (3) the presence of two tufts of 5–6 setae on the dorsal margin of the palm of gnathopod I (vs. only one simple seta), and (4) the presence of 3 lateral spines on each lobe of the telson (vs. 1 lateral spine in *N. ardonicus* **sp. nov.** and 2 lateral spines in *N. fiagdonicus* **sp. nov.**). For the differences from other newly discovered species, see below.

Niphargus sadonicus **sp. nov.**

Material: Holotype: ♀ (bl. 10 mm) (ZMMU Mb-1274)—Russian Federation, Republic of North Ossetia–Alania, Alagirsky District, a stream in an abandoned mine near the village of Sadon, 42°50′40.28″ N 44°01′16.77″ E, about 1155 m asl, coll. D. Palatov, 8 October 2020.

Paratypes: 2 ♀♀ (ZMMU Mb-1275)—same data as for holotype.

Other material: 3 ♀♀ (LEMMI)—same data as for holotype.

Etymology: The new species is named after the type locality, the village of Sadon.

Diagnosis: *Head* without pigmented spots on anterior lobe. Posteroventral corners of *EpI–III* rounded. *UrII* with 1 strong spine at a posterodorsal angle on each side. Distal article of *mandibular palp* with a group of 4–5 A-setae, 1–2 B-setae, 12–14 D-setae, and 4–5 E-setae. Outer lobe of *MxI* with seven robust spines, carrying 1–4 teeth each. Dactyli of *PpIII–IV* at inner margins without strong spines near the bases of nails. Dactyli of *PpV–VII* with small additional spiniform setae. *UI* rami different in length: exopodite shorter than endopodite. *PlI–III* with 3–5 hooks in retinacula. *Telson* with three relatively long distal spines, one long lateral spine, and two plumose setae on each side; dorsal surface without spines.

The complete morphological description is presented in the Supplementary Materials (Figures S12–S15 and S22a,b).

Coloration and body size: Body coloration is completely white. The largest collected ♀ had bl. 10 mm.

GenBank accession number: PP715903.

Distribution and ecology: The species is known only from the type locality, from an abandoned mine near the village of Sadon.

Taxonomic remarks: This species belongs to the same species group as *N. alanicus* [37], but it can be separated from the latter species by (1) 3–5 hooks in retinacula (vs. 4 hooks), (2) the presence of a simple seta on the dorsolateral margin of urosomal somite I (vs. strong spine), and (3) the presence of 1 lateral and 3 apical strong spines on each lobe of the telson (vs. 2–3 lateral and 5–6 apical spines).

From the closely related *Niphargus ardonicus* **sp. nov.** and *N. fiagdonicus* **sp. nov.**, it can be separated by (1) the presence of only several spiny setae along the outer margin of robust spines of the maxilla I (vs. numerous smaller spines along the outer margin), (2) a small additional seta on the dactyli of ambulatory pereiopods (vs. a strong spine), (3) the presence of three tufts of 5–6 setae on the dorsal margin of the palm of gnathopod I (vs. only a simple seta), and (4) the presence of 1 lateral spine on each lobe of the telson (vs. 2 lateral spines in *N. fiagdonicus* **sp. nov.**).

From the closely related *N. osseticus* **sp. nov.**, the new species can be distinguished by (1) smaller gills on gnathopod II and pereiopod IV, which are smaller than the basis (vs. equal to the basis), and (2) the presence of one lateral spine on each lobe of the telson (vs. three lateral spines in *N. osseticus* **sp. nov.**). Some smaller distinguishing features are present in the armature of gnathopod I (4 additional distoventral spines in *N. sadonicus* **sp. nov.** vs. 2 spines in *N. osseticus* **sp. nov.**; outer plate of maxilla I with 1 simple seta in *S. sadonicus* **sp. nov.** vs. 3 setae in *N. osseticus* **sp. nov.**).

For the differences from other newly discovered species, see below.

Niphargus tschertschesovae **sp. nov.**

Material: Holotype: ♀ (bl. 10 mm) (ZMMU Mb-1276)—Russian Federation, Republic of North Ossetia–Alania, Prigorodny District, groundwater of the Gizeldon River near the village of Dargavz, 42°51′08.76″ N 44°26′55.55″ E, 1395 m asl, coll. D. Palatov, M. Antipova, 22 May 2023.

Paratypes: 2 ♀♀ (ZMMU Mb-1277)—same data as for holotype.

Other material: 3 ♀♀ (LEMMI)—same data as for holotype.

Etymology: The species is named in honor of the renowned hydrobiologist and entomologist Susanna Konstantinovna Tchertchesova (Cherchesova) (North Ossetian State

University after K.L. Khetagurov, Vladikavkaz), who made a significant contribution to the study of aquatic invertebrates in the North Caucasus.

Diagnosis: *Head* without pigmented spots on anterior lobe. Posteroventral corners of *EpI–III* rounded. *UrII* with 1 strong spine at a posterodorsal angle on each side. Distal article of *mandibular palps* with a group of 4–5 A-setae, 1–2 B-setae, 12–14 D-setae, and 4–5 E-setae. Outer lobe of *MxI* with seven robust spines, carrying 2–10 teeth each. Dactyli of *pereiopods III–IV* at inner margins without strong spines near the bases of nails. Dactyli of *PpV–VII* with small additional spiniform setae. *UI* rami different in length: exopodite shorter than endopodite. Retinacula of *PlI–III* with 3–4 hooks. *Telson* with 3 relatively long distal spines, 2–3 long lateral spines, and 2 plumose setae on each side.

The complete description is presented in the Supplementary Materials (Figures S16, S17, and S22c,d).

Coloration and body size: Body coloration is completely white. The largest collected ♀ has bl. 10.5 mm.

GenBank accession number: PP715908, PP715909.

Distribution and ecology: This species is known only from hypogean habitats of the Gizeldon River Valley (Kobanskoe Gorge) near the village of Dargavz.

Taxonomic remarks: This species is closely related to *Niphargus sadonicus* **sp. nov.** and belongs to the same species group as *N. alanicus*. It can be separated from *N. alanicus* [37] by (1) the presence of a strong spine-like seta accompanied by a simple seta on the dorsolateral margin of urosomal somite I (vs. a strong spine only), (2) a larger coxal gill on gnathopod II, which is equal to the basis (vs. significantly smaller than the basis), and (3) the presence of 3 apical strong spines on each lobe of the telson (vs. 5–6 apical spines).

From the closely related *Niphargus ardonicus* **sp. nov.** and *N. fiagdonicus* **sp. nov.**, it can be separated by (1) the presence of only several spine-like setae along the outer margin of robust spines of maxilla I (vs. numerous smaller spines along the outer margin), (2) the presence of two tufts of 5–6 setae on the dorsal margin of the palm of gnathopod I (vs. only 1 simple seta), (3) the presence of a strong spine-like seta accompanied by a simple seta on the dorsolateral margin of urosomal somite I (vs. a simple seta only), and (4) the presence of 2 lateral spines on each lobe of the telson (vs. 1 lateral spine in *N. ardonicus* **sp. nov.**).

From the closely related *N. osseticus* **sp. nov.** and *N. sadonicus* **sp. nov.**, the new species can be separated by (1) smaller gills on gnathopod II and pereiopod IV, which are smaller than the basis (vs. equal to the basis in *N. osseticus* **sp. nov.**); (2) the presence of 2 lateral spines on each lobe of the telson (vs. 1 lateral spines in *N. sadonicus* **sp. nov.** and 3 lateral spines in *N. osseticus* **sp. nov.**); and (3) a high and narrow coxal plate on pereiopod IV (vs. almost subquadrate). For the differences from *N. zeyensis* **sp. nov.**, see below.

***Niphargus zeyensis* sp. nov.**

Material: Holotype: ♂ (bl. 12.5 mm) (ZMMU Mb-1278)—Russian Federation, Republic of North Ossetia–Alania, Alagirsky District, Tsey (Zey) Gorge, a stream on the western outskirts of the village of Abaytikau, 42°48′31.7″ N 43°57′18.2″ E, about 1985 m asl, coll. D. Palatov, 28 July 2021.

Paratypes: 2 ♀♀ (ZMMU Mb-1279)—same data as holotype.

Other material: 4 ♀♀ (ZMMU Mb-1279)—same data as holotype; 10 ♀♀ —Russian Federation, North Caucasus, the Republic of North Ossetia–Alania, Alagirsky District, a small spring near the road to the Tsey mountain camp (Tsey Gorge), 42°48′11.51″ N 43°57′21.56″ E, about 1722 m asl, coll. D. Palatov, 7 October 2020.

Etymology: The new species is named in honor of the Tsey (Zey) Gorge.

Diagnosis: *Head* without pigmented spots on anterior lobe. Posteroventral corners of *EpI–III* rounded. *UrII* with one strong spine accompanied by a simple seta at posterodorsal angles on each side. Distal article of *mandibular palp* with a group of 5–6 A-setae, 2–3 B-setae, 14–16 D-setae, and 4–5 E-setae. Outer lobe of *MxI* with seven robust spines, carrying 1–4 teeth each. Dactyli of *PpI–II* with 5–6 long setae on the outer margins, 3 of which are grouped together. Dactyli of *PpIII–IV* at inner margins without strong spines near the bases of nails. Dactyli of *PpV–VII* with small additional spine-like setae. *UI* rami of nearly equal length. *PlI–III* with 5–6 hooks in retinacula. *Telson* with 4–5 relatively long distal spines, 2–3 long lateral spines accompanied by 2 thin plumose setae on each outer side, 0–1 short spines on inner margins of lobes and 0–1 short setae on the dorsal surface of each lobe.

The complete description is presented in the Supplementary Materials (Figures S18–S21 and S22e,f).

Coloration and body size: Completely whitish. The largest collected ♂ had bl. 12.5 mm.

GenBank accession number: PP715905–PP715907.

Distribution and ecology: Currently, this species is known only from two neighboring springs on the slope of the valley of the Tseydon River (Tsey (Zey) Gorge).

Taxonomic remarks: This species is mostly phylogenetically distant from all other species of the genus *Niphargus* known from the North Ossetia–Alania. It can be separated from other species in the area by (1) similar length of distal and proximal articles of uropod III in ♂♂ (vs. distal article about 1/3 of the proximal one), (2) epimeral plates II–III armed with paired spines along the ventral margins (vs. single spines in a row), (3) dactyli of gnathopods I–II with several tufts of setae along the outer margins (vs. only one seta), (4) telson with small and thin setae on the dorsal surface and inner margins of lobes of the telson (vs. absent), (5) 6 apical spines on each lobe of the telson (vs. 3–5 spines, except *N. alanicus*, which also has 6 spines), and (6) 5–6 (usually 6) hooks in the retinacula of pleopods (vs. maximum 5 hooks, usually less).

From the species of the related "*Niphargus borutzkyi*" species ingroup [30,35], the new species can be separated by (1) the armature of the urosome, with only 1 strong spine present on somite II posterodorsally (vs. 2–3 spines); (2) the longer distal article of uropod III in ♂♂ , which is almost equal to the basal article; (3) the almost subrectangular basis of pereiopod VII; (4) the presence of small additional setae on the dactyli of ambulatory pereiopods ventrally (vs. well-marked strong spines); and (5) the presence of paired ventral spines on epimeral plate II (vs. single ventral spines).

Zoobank taxon ID. The electronic version of this article in Portable Document Format will represent a published work according to the International Commission on Zoological Nomenclature (ICZN), and hence the new names contained in the electronic version are effectively published under that Code from the electronic edition alone. The LSID for this publication is: urn:lsid:zoobank.org:pub:CB1BB16F-2225-4A04-8438-5E37F2B2EDE8. The online version of this work is archived and available from the following digital repository: MDPI Water.

Niphargus ardonicus **sp. nov.:** urn:lsid:zoobank.org:pub:CB1BB16F-2225-4A04-8438-5E37F2B2EDE8
Niphargus fiagdonicus **sp. nov.:** urn:lsid:zoobank.org:pub:CB1BB16F-2225-4A04-8438-5E37F2B2EDE8
Niphargus osseticus **sp. nov.:** urn:lsid:zoobank.org:pub:CB1BB16F-2225-4A04-8438-5E37F2B2EDE8
Niphargus sadonicus **sp. nov.:** urn:lsid:zoobank.org:act:CC4DD058-25E6-4C11-92BB-27CC3FBEE368
Niphargus zeyensis **sp. nov.:** urn:lsid:zoobank.org:pub:CB1BB16F-2225-4A04-8438-5E37F2B2EDE8

4. Discussion

Currently, hypogean groundwater habitats are considered to be among the most underexplored and undervalued, but nevertheless a global key ecosystem [49]. The biospeleological research conducted in the Caucasus, and in particular the application of this knowledge, for both fundamental and applied research purposes—for example, the search for pure drinking water sources—represents important fields of scientific activity in the region [50,51]. These tasks are also relevant for all areas of the Caucasus.

The current study contributes to the knowledge of the hyporheic/stygobiotic crustacean diversity in the Republic of North Ossetia–Alania [20,37]. This study has allowed us to explore the diversity at a larger number of locations. As a result, seven hyporheic/stygobiotic species of the genus *Niphargus* have been discovered and identified in the area. Moreover, molecular genetic data allowed us to clarify the taxonomy of the hyporheic genus *Proasellus*. However, we believe that there may still be undiscovered, narrowly distributed hyporheic/stygobiotic/subterranean species from both these crustacean genera and other taxa.

Based on the data obtained, the diversity of stygobiotic crustaceans in the North Ossetia–Alania stands out noticeably against the background of other areas along the central and western parts of the northern slope of the Great Caucasian Ridge (North Caucasus). Until now, hyporheic/stygobiotic crustaceans have only been found on the most eastern part of the northern slope, from the territory of Krasnodar Krai (e.g., *Niphargus ciscaucasicus* Marin & Palatov, 2019) [19] and the Republic of Adygea (pers. data, unpubl.). No representatives of these taxa have been discovered in nearby mountainous areas, such as the Stavropol Region, Republic of Ingushetia, Kabardino-Balkaria, Karachay-Cherkessia, or the Chechen Republic (Chechnya). It is likely that a more thorough and extensive search for these hyporheic/stygobiotic animals in these areas is necessary.

The majority of hyporheic and stygobiotic animals are narrowly localized stenobiotic endemics that are well adapted to the stable conditions of their habitats, e.g., [22,23]. Based on the geographical references of the obtained data, different hyporheic/stygobiotic crustaceans exhibit various distribution patterns. Thus, the representatives of the genus *Proasellus* most likely permanently inhabit the hyporhean riverbed habitats. For example, *P. uallagirus* in particular is found in the mountainous part of the Terek River Basin and may even be able to move from one tributary basin to another. These isopods likely move actively along riverbeds, as studied individuals of *P. uallagirus* from fairly distant locations are genetically similar (see above). In contrast, our study revealed that different representatives of the genus *Niphargus* are found only in separate basins of mountainous rivers (gorges) and, likely, in the underlying karst aquifers. These amphipods are likely unable to disperse over long distances and cross significant geographic barriers [22–24], even through riverbed hyporhea, and their distribution and speciation are most likely related to karst fragmentation and isolation of underground water basins [27], which limit their distribution.

According to the well-known historical records of glacial cycles, most of the area along the northern slope of the Caucasian Ridge experienced significant temperature fluctuations, mainly during the Pleistocene [3,52]. These fluctuations were caused by the expansion and contraction of glaciers, which affected both the valleys and higher parts of the neighboring mountains. Such events presumably led to total extinction in this area, especially in parts covered with ice sheets. Later, in warmer times during the Late Pleistocene/Holocene, when glaciers had decreased, terrestrial fauna colonized this region again, coming from areas along the southern slopes or nearby regions [12,53–56]. Therefore, the surface fauna of this region exhibit low levels of endemism and are dominated by widespread post-glacial colonizers [3,7,8,57–59]. However, our research suggests that older, more ancient endemic species still inhabit hypogean/underground habitats. The stable conditions of these environments have allowed hyporheic/stygobiotic invertebrates to survive significant climatic changes that have occurred in the past. The previously reconstructed phylogenetic relationships of *N. alanicus* revealed that the time of its divergence from the only identified sister taxa (the European *Niphargus ambulator* G. Karaman, 1975) occurred approximately 7–5 Mya [37]. Since then, it has been possible to gather genetic data on a greater number of Caucasian and Crimean congeneric species and identify a whole series of related sister species. As a result, the approximate time of divergence for all North Ossetian *Niphargus* species from their sister taxa falls within the range of 8–5.8 Mya, suggesting that they separated from related South Caucasian and European species during the Late Miocene, due to the shallowing and drying of Paratethys basins [60] and the uplift of the central part of the Great Caucasus [61–64]. These historic geological processes disrupted the genetic connections between populations, leading to allopatric speciation. This hypothesis is also supported by the discovery of the related (sister) species on both sides of the Caucasian Ridge, but these species have never been able to cross this significant barrier.

The discovery of these new species in the North Ossetia–Alania allows us to hypothesize much more widespread distribution of the representatives of the genus *Niphargus* in the Ciscaucasian Plain and the plain areas north of the Great Caucasian Ridge during the Miocene. These ancient species are obviously genetically connected with their European and Balkan congeners through the freshwater periods of the Paratethys [60,65]. The uplifting of the Caucasus Mountains and the reduction in the Paratethys interrupted these genetic connections about 10–5 Mya. Cryptic hypogean/subterranean habitats in mountain gorges with safe and stable conditions provided a stable shelter for hyporheic/stygobiotic fauna during the climatic (temperature) fluctuations of the Pleistocene (Quaternary) glacial episodes (2.6 Mya–present), and especially during the last glacial maximum (LGM) (23–18 Kya), allowing them to survive there until the present day. The related fauna of the northern foothill plains underwent significant temperature cooling and became extinct. This hypothesis suggests that the mountainous region of the Republic of North Ossetia–Alania may be considered to be a post-Pliocene glacial refugium for stygobiotic fauna—the first known in the North Caucasus region.

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.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/w16091212/s1.

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