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The shrimps from the bottom: a new species of stygobiotic atyid shrimps of the genus *Xiphocaridinella* from the world-deepest Verevkina Cave

Креветки с самого дна: новый вид стигобионтных креветок рода *Xiphocaridinella* из пещеры Веревкина, самой глубокой пещеры в мире

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КЛЮЧЕВЫЕ СЛОВА: баркодинг, стигобионтный, креветка, пещера Веревкина, Арабика, Кавказ.

ABSTRACT. A new species of the genus *Xiphocaridinella* Sadowsky, 1930 (Crustacea: Decapoda: Atyidae), *Xiphocaridinella demidovi* sp.n., and two specimens of *X. dbari* Marin, 2019 are described from the bottom section (–2212 m) of the world's deepest Verevkina Cave, located in the Arabika karst massif of the Gagra Ridge, southwestern Caucasus. Consequently, these species are the most deep living troglobionts/stygobionts in the world, whose taxonomic status is officially confirmed. Based on morphology and DNA analysis, both species are closely related representing sister clade separated for 6% by COI mtDNA gene marker. From the latter species and related congeners, the new species can be easily separated by a relatively long rostrum, pointed distally and reaching the distal margin of basal antennular segment, slender carpus of pereiopod II, the presence of 3 ischial spines and reduced exopods on pereiopods III–IV as well as stouter/wider telson. Due to the discovery of *Xiphocaridinella demidovi* sp.n., 14 species of the genus *Xiphocaridinella* are currently known from the Colchis Lowland of the southwestern Caucasus.

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РЕЗЮМЕ. Из донной секции пещеры Веревкина из карстового массива Арабика Гагрского хребта (юго-западный Кавказ), самой глубокой пещеры в мире (–2212 м), описан новый вид рода *Xiphocaridinella* Sadowsky, 1930 (Crustacea: Decapoda: Atyidae), *Xiphocaridinella demidovi* sp.n., а также два экземпляра *X. dbari* Marin, 2019. Следовательно, эти виды — наиболее глубоко живущие троглобионты/стигобионты в мире, таксономический статус которых официально подтвержден. Результаты исследования их морфологии и анализа ДНК позволяют утверждать, что оба вида близкородственны и образуют сестринские клады, отличающиеся примерно на 6% по генному маркеру COI мтДНК. Новый вид отличается от найденного месте с ним и от других родственных видов, относительно длинным заостренным рострумом, достигающим уровня дистального края базального антеннулярного сегмента, тонким карпусом переопод II (хелипед), наличием 3 шипов на исхиуме и редуцированными экзоподитами на переоподах III–IV, а также более широким тельсоном. Благодаря находке *Xiphocaridinella demidovi* sp.n., в роде *Xiphocaridinella* в настоящее время насчитывается 14 видов из Колхидской низменности юго-западного Кавказа.

Introduction

The stygobiotic atyid shrimp genus *Xiphocaridinella* Sadowsky, 1930 (Crustacea: Decapoda: Atyidae) currently includes 13 valid species described in the subterranean water habitats of the southwestern Caucasus, from the northern and northwestern part of Kolkhida coastal lowland plain (Colchis) of the southwestern Caucasus [Sadowsky, 1930; Birštein, 1939, 1948; Juzbaš'jan, 1940, 1941; Marin, Sokolova, 2014; Marin, Sinelnikov, 2017; Marin, 2017a, b, 2018a, b, 2019, 2020; Marin, Turbanov, 2021]. The knowledge about these stygobiotic shrimps living in underground (stygobiotic) habitats is still incomplete, and new species have been discovered to date.

The entrance to the Verevkina Cave is located in the eastern part of the Arabika plateau on the Gagra Ridge of the SW Caucasus. There are also entrances to the other world deepest karst cave — the Krubera-Voronja Cave (–2199 m) and the Sarma Cave (–1830 m). In March 2018, the speleoexpedition measured the depth of the siphon lake “Nemo”s Last Camp”, which added 8 m to the depth of the cave and increased it to –2212 m, making it the deepest cave in the World, 15 m deeper than the neighboring Krubera-Voronya Cave from the western part of the Arabika plateau on the Gagra Ridge. Based on one of sampled specimen, *Xiphocaridinella dbari* Marin, 2019 was recorded from the Verevkina Cave at a depth of –2100–2130 m (the depth in the original description is indicated incorrectly) [Marin, 2019].

Somewhat later in 2018 and 2019, the speleologists from the Perovo-Speleo team were able to go deeper into the cave, reaching in the siphon lake “Nemo”s Last Camp” (=Poslednaya Stoyanka Nemo), the present-day deepest part of the cave, where three more specimens of stygobiotic shrimps were caught. Detailed morpho-genetic research revealed that two of the specimens belong to *X. dbari*, while one of the sampled shrimps represent an undescribed species. Because sampling in such deep caves is extremely rare, the new species is described herewith based on the only available specimen. At the same time, the siphon “Nemo”s Last Camp” is located already above the basic non-karst rocks and, probably, this watercourse connects many caves starting on the Arabika plateau. Thus, the newly discovered species can be found in other caves of the Arabika karst massif as well.

Unfortunately, the head of numerous expeditions of the Perovo-Speleo team to the Verevkina Cave and one of the collectors of this material, Pavel Demidov, tragically died on August 23, 2020 in one of the caves of the Arabika karst massif. The species he found is named in his honour.

Material and methods

SAMPLING AND REPOSITORIES. Shrimps were collected by hand net in the subterranean siphon lake “Nemo”s Last Camp” located at –2204 m depth of the Verevkina Cave (43°24′56.0″N 40°21′23.0″E), Arabika plateau of Gagra Ridge, Western Abkhazia, SW Caucasus in September 2018 and August 2019. Research does not include endangered or protected species. All specimens were preserved and stored in 90% solution of ethanol for further DNA analysis. Postorbital carapace length (pcl., in mm), the length from the posterior orbit to the posterodorsal margin of carapace, and total body length (tbl., in mm), dorsal length from the tip of rostrum till the distal margin of telson, are used as standard measurements. The holotype is deposited in the: Zoological Museum of Moscow University (ZMMU), Moscow, Russia.

DNA EXTRACTION. The cytochrome c oxidase subunit I (COI) mtDNA gene marker is used as one of the most informative markers for genetic studies at population and species level for marking species within *Xiphocaridinella* species complex [Avise, 1993; Sket, Zakšek, 2009; Marin, 2020]. Total genomic DNA was extracted from muscle tissue using the innuPREP DNA Micro Kit (AnalitikJena, Germany). The COI mtDNA gene marker was amplified with the help of the universal primers LCO1490 (5'–GGTCAACAAATCATAAAGATATTGG–3') and HC02198 (5'–TAAACTTCAGGGTGACCAAAAAATCA–3') [Folmer *et al.*, 1994]. PCR products were performed on amplificator T100 (Bio-Rad, USA) under the following conditions: initial denaturation at 96 °C for 1.5 min followed by 42 cycles of 95 °C for 2 min, 49 °C for 35 sec, and 72 °C for 1.5 min, followed by chain extension at 72°C for 7 min. The volume of 10 μL of reaction mixture contained 1μL of total DNA, 2μL of 5xPCR mix (Dialat, Russia), 1μL of each primer and 5μL of H_2O. The amplification products were separated by using gel electrophoresis of nucleic acids on a 1.5% agarose gel in 1xTBE, and then stained and visualized with 0.003% EtBr using imaging UV software. DNA nucleotide sequences were determined using Genetic Analyzer ABI 3500 (Applied Biosystems, USA) and BigDye 3.1 (Applied Biosystems, USA) with direct and reverse primers. Dataset of aligned sequences of COI mtDNA gene markers, about 617 base pairs in length used in the study were taken from GenBank (NCBI) (see Table 1 in Marin & Turbanov [2021]).

PHYLOGENETIC ANALYSIS. Consensus of complementary sequences was obtained with MEGA 7.0. PartitionFinder v. 2 [Lanfear *et al.*, 2016] was used for finding best-fitting partitions for the concatenated dataset as COI mtDNA is usually characterized by a strong heterogeneity in nucleotide substitution rates among all three coding positions. The GTR+G+I evolutionary substitution models was suggested as the best by the Akaike Information Criterion (AIC) using jModeltest2.1.141. Different evolutionary substitution models and phylogenetic estimates were widely congruent in this study. Phylogenetic analysis was conducted using PhyML 3.0 (http://www.atgc-montpellier.fr/phyml/) [Guindon *et al.*, 2010] with several models based on BIC (Bayesian Information Criterion) and AIC (Akaike Information Criterion). Maximum likelihood bootstrap values are given for each node. Pairwise genetic divergences (p-distances) were calculated based on COI sequences using MEGA 7.0 with the Kimura 2-Parameter (K2P) model of evolution (Kimura, 1980).

Results

MOLECULAR-GENETIC ANALYSIS. The intraspecific *p*-distances between *Xiphocaridinella demidovi* sp.n. (n=1) and other Caucasian *Xiphocaridinella* species are more 0.06 substitutions per 100 nucleotide positions (more than 6%); the phylogenetically closest species is *X. dbari*, which is known also from Verevkina Cave (–2130 m) and the Gegskaya (Gega) Cave (43°23′43.7″N 40°27′28.4″E) of the same Gagra Ridge (see Marin [2020]).

TAXONOMY

Order Decapoda Latreille, 1802
Family Atyidae De Haan, 1849
Genus *Xiphocaridinella* Sadowsky, 1930
Xiphocaridinella demidovi **sp.n.**
Figs 1–3.

MATERIAL EXAMINED: HOLOTYPE, non-ovigerous ♀ (pcl. 7.5 mm, tbl. 24.0 mm), ZMMU Ma-6207, Abkhazia, Gagry region, Gagra Ridge, Arabika karst massif, Verevkina Cave, 43°24′56.0″N 40°21′23.0″E (the entrance), “Nemo”s Last Camp” siphon lake, about –2212 m from of the cave entrance, coll. Natalia & Andrey Sizikov, Sept. 2018.

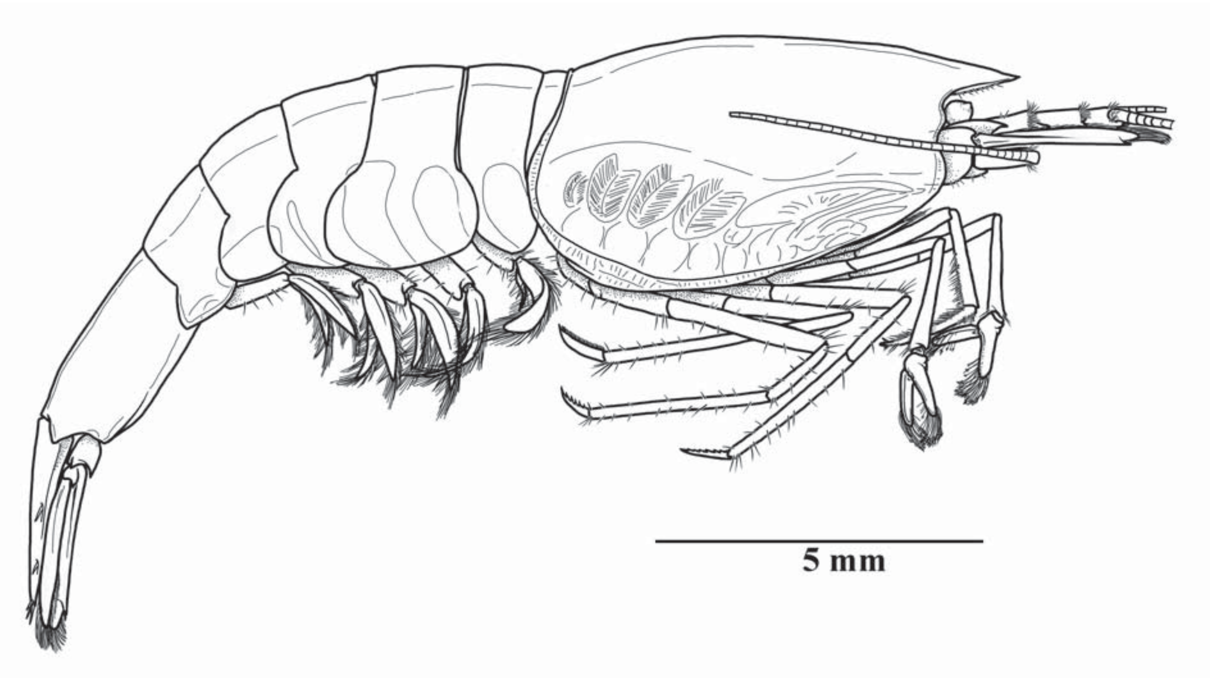


Fig. 1. *Xiphocaridinella demidovi* sp.n., holotype ♀, pcl. 7.5 mm, tbl. 24.0 mm, ZMMU Ma-6207, Verevkina Cave, Western Abkhazia, southwestern Caucasus: general lateral view.

Рис. 1. *Xiphocaridinella demidovi* sp.n., ♀, голотип, pcl. 7,5 мм, tbl. 24,0 мм, ZMMU Ma-6207, пещера Веревкина, Западная Абхазия, юго-западный Кавказ: общий вид сбоку.

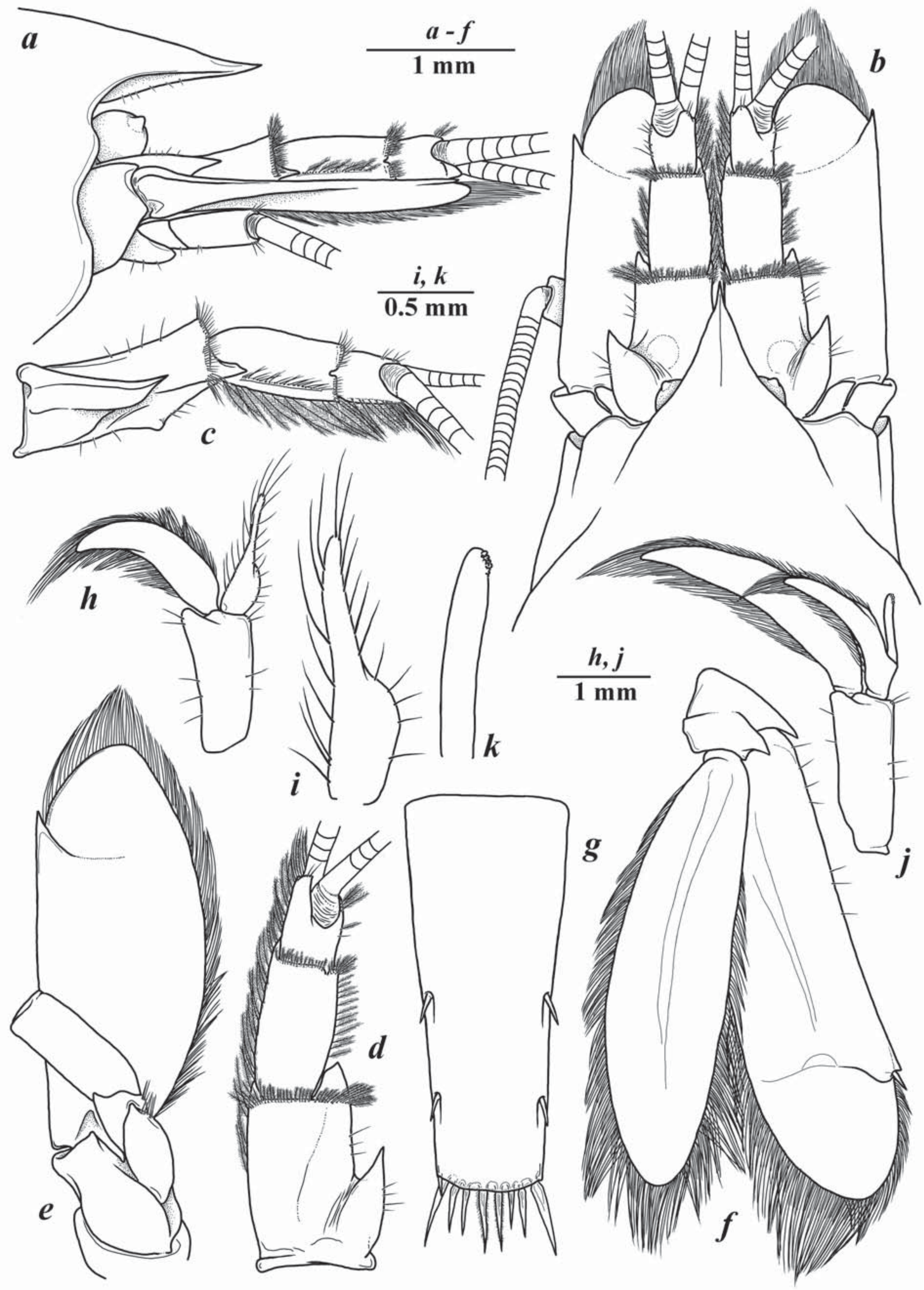
Table1. Pairwise genetic (COI mtDNA) distances (*p*-distances±SE) and estimated divergence time (Mya) (Min. and Max. after Guy-Haim *et al.* [2018]) between *Xiphocaridinella demidovi* sp.n. (n=1) and other species of the genus *Xiphocaridinella* (data from GenBank (NCBI) database).

Таблица 1. Попарные генетические дистанции (COI mtDNA) (p-distances±SE) и предположительное время дивергенции (Mya) (min. и max. по Guy-Haim et al. [2018]) между *Xiphocaridinella demidovi* sp.n. (n=1) и другими видами рода *Xiphocaridinella* (данные базы данных GenBank (NCBI)).

Species	***p*-distance**	**Approximate divergence time (Mya)**		
		Min (0.0516/Mya)	Average (0.029/Mya)	Max (0.0077/Mya)
Xiphocaridinella dbari (n=7)	0.057±0.008	1.10±0.15	1.96±0.27	7.40±1.03
Xiphocaridinella fagei (n=17)	0.065±0.009	1.26±0.17	2.24±0.31	8.44±1.16
Xiphocaridinella jusbaschjani (n=14)	0.068±0.009	1.32±0.17	2.34±0.31	8.83±1.16
Xiphocaridinella ablaskiri (n=15)	0.101±0.012	1.96±0.23	3.48±0.41	13.11±1.55
Xiphocaridinella otapi (n=18)	0.103±0.012	2.0±0.23	3.55±0.41	13.37±1.55
Xiphocaridinella osterloffi (n=35)	0.104±0.012	2.02±0.23	3.58±0.41	13.50±1.56
Xiphocaridinella falcirostris (n=11)	0.109±0.013	2.11±0.25	3.75±0.44	14.15±1.68
Xiphocaridinella kumistavi (n=18)	0.112±0.014	2.17±0.27	3.86±0.48	14.54±1.81
Xiphocaridinella shurubumu (n=15)	0.115±0.015	2.23±0.29	3.96±0.51	14.93±1.94
Xiphocaridinella kutaissiana (n=16)	0.116±0.015	2.25±0.29	4.0±0.51	15.06±1.95
Xiphocaridinella smirnovi (n=1)	0.118±0.016	2.29±0.31	4.07±0.55	15.32±2.07
Xiphocaridinella motena (n=10)	0.125±0.017	2.42±0.32	4.31±0.58	16.23±2.20

DESCRIPTION. Small-sized shrimp with swollen subcylindrical body and fragile integument. Carapace (Fig. 1) smooth dorsally, almost transparent, without any tubercles/carina, without supraorbital teeth (Fig. 2*a*, *b*). Rostrum (Fig. 2*a*, *b*) unarmed, slender in distal part and sharply pointed distally, turned forward, reaching the distal margin of basal antennular segment, with well-developed lateral lamina, and feeble developed dorsal carina.

Abdominal somites smooth, unarmed (Fig. 1); tergites non-carinate, without dorsal lobes, not posteriorly produced; pleura of pleomeres I–IV posteroventrally and ventrally rounded, distoventral margins of pleomere VI sharply produced posterodorsally. Telson (Fig. 2*g*) about 2.5 times as long as wide proximally, slightly expanded distally, usually with 2 pairs of slender dorsal spines, inserted at about 0.5 and 0.8 of telson length, respectively; distal margin convex, with 4 pairs of distal spines.


a
a - f
1 mm
b
i, k
0.5 mm
c
h
i
k
h, j
1 mm
g
j
d
e
f

Eyes (Fig. 2*a*, *b*) reduced, swollen, partly covered by carapace; cornea rounded, with small blunt projection, without pigment; eyestalk stout, swollen, cylindrical, about as long as wide.

Antennula (Fig. 2*a*–*d*) with robust basal peduncular segment, about 1.5 times as long as wide, with mostly reduced ventromedial tooth (Fig. 2*c*), distolateral angle with broadly produced sharp triangular projection (Fig. 2*d*); stylocerite short, sharpening distally, not reaching the distal margin of basal segment; second segment about 2 times as long as wide, subcylindrical, smooth. about 1.5 times as long as distal segment; distal segment about as long as wide, unarmed.

Antenna (Fig. 2*e*) with basicerite stout, about 1.3 times as wide as long, with distolateral margin unarmed; carpocerite robust, about 2 times as long as wide, almost reaching the midlength of scaphocerite; scaphocerite well developed, broad, with small but well-marked distolateral tooth, lamella bluntly rounded distally.

Mouthparts characteristic for the genus, without specific features. Mandible well developed, without palp; molar process well developed, relatively slender, with several small and large distodorsal teeth; incisor process slender, tapering distoventrally, with slender serrated sharp lamina distally. Maxillula with well-developed simple palp armed with 2 setae distally, well developed, produced, blunt distally, dorsal lobe with completely fused lacinia, ventral lobe small, covered with curved setae distally. Maxilla normal, with simple tapering small palp; endites stout, well developed; basal endite feebly bilobed, furnished with elongated stiff setae; coxal endite obsolete; scaphognathite broad, furnished with short plumose setae. Maxilliped I normal; epipod small, reduced; exopodite small and slender, with expanded caridean lobe bearing plumose marginal setae; basal and coxal endites completely fused, excavate, furnished with long simple setae distally. Maxilliped II with relatively robust segments; without epipod; exopodite slender overreaching propodal segment, with long plumose setae distally; distolateral margin of propodus straight, dorsal margin convex, furnished with slender setae; dactylus about twice as long as broad, with straight distal margin, with numerous medium stout spines and numerous simple spines along distal margin. Maxilliped III (Fig. 3*a*, *b*) slender, with small epipodite and arthrobranch; exopodite short, partly reduced, slender, almost reaching the midlength of antepenultimate segment; antepenultimate segment slender, about 7 times as long as wide; penultimate segment about 6 times as long as wide, with straight lateral margins; ultimate (distal) segment (Fig. 3*b*) slender, about 6.5–7 times as long as wide, tapering distally, with tufts of short simple stick-like setae along ventral and lateral margins.

Pereiopods I (Fig. 3*c*, *d*) with basis about as long as wide; coxa with well-developed slender epipodite and tuft of setobranchs; basis about as long as wide, with slightly reduced slender exopodite almost reaching carpo-meral articulation; ischium about 2 times longer than wide; merus slender, about 5 times as long as wide, about 2 times longer than ischium and about 1.2 times longer than carpus; carpus relatively stout, widening distally, about as 4 times as long as maximal width; palm relatively stout, about as long as wide, subcylindrical in cross-section; fingers subcylindrical, about 3–3.5 times as long as proximal width, armed with distal tufts of strong curved distally plumose setae (Fig. 3*d*).

Pereiopods II (Fig. 3*e*) with relatively slender segments, longer than PI; coxa with well-developed slender epipodite and a tuft of small setobranchs; basis about as long as wide, with relatively short exopodite reaching carpo-meral articulation; ischium about 3–3.5 times as long as wide; merus slender, about 4 times as long as wide, equal to ischium and about 1.4 times shorter than carpus; carpus relatively slender, about 6.5–7 times as long as wide, slightly widening distally; palm relatively stout, similar to palm of pereiopod I, about as long as wide, subcylindrical in cross-section; fingers relatively stout, subcylindrical, blunt distally, about 4 times as long as proximal width, with simple and straight cutting edge, with distal tufts of strong curved distally plumose setae.

Pereiopod III (Fig. 3*f*) relatively slender; coxa with tuft of setobranchs and well-developed epipodite; basis about as long as wide, with reduced exopodite slightly overreaching carpo-meral articulation; ischium about 2.5 times as long as wide, with well-marked distoventral spine; merus about 6.5–7 times as long as wide, with 3 small spines along ventral margin; carpus relatively slender, about 5 times as long as wide, slightly widening distally, about twice shorter than merus and propodus, without subdistal spine; propodus about 11 times as long as wide, with straight margins, armed with small spine-like setae a row of small simple setae; dactylus (Fig. 3*g*) about 3 times longer than maximal wide, main unguis smooth, curved and sharp, accessory unguis small, triangular, sharp, signifacantly shorter than main unguis and subequal to ventral spines, ventral margin armed with a row of spines accompanying with small simple setae.

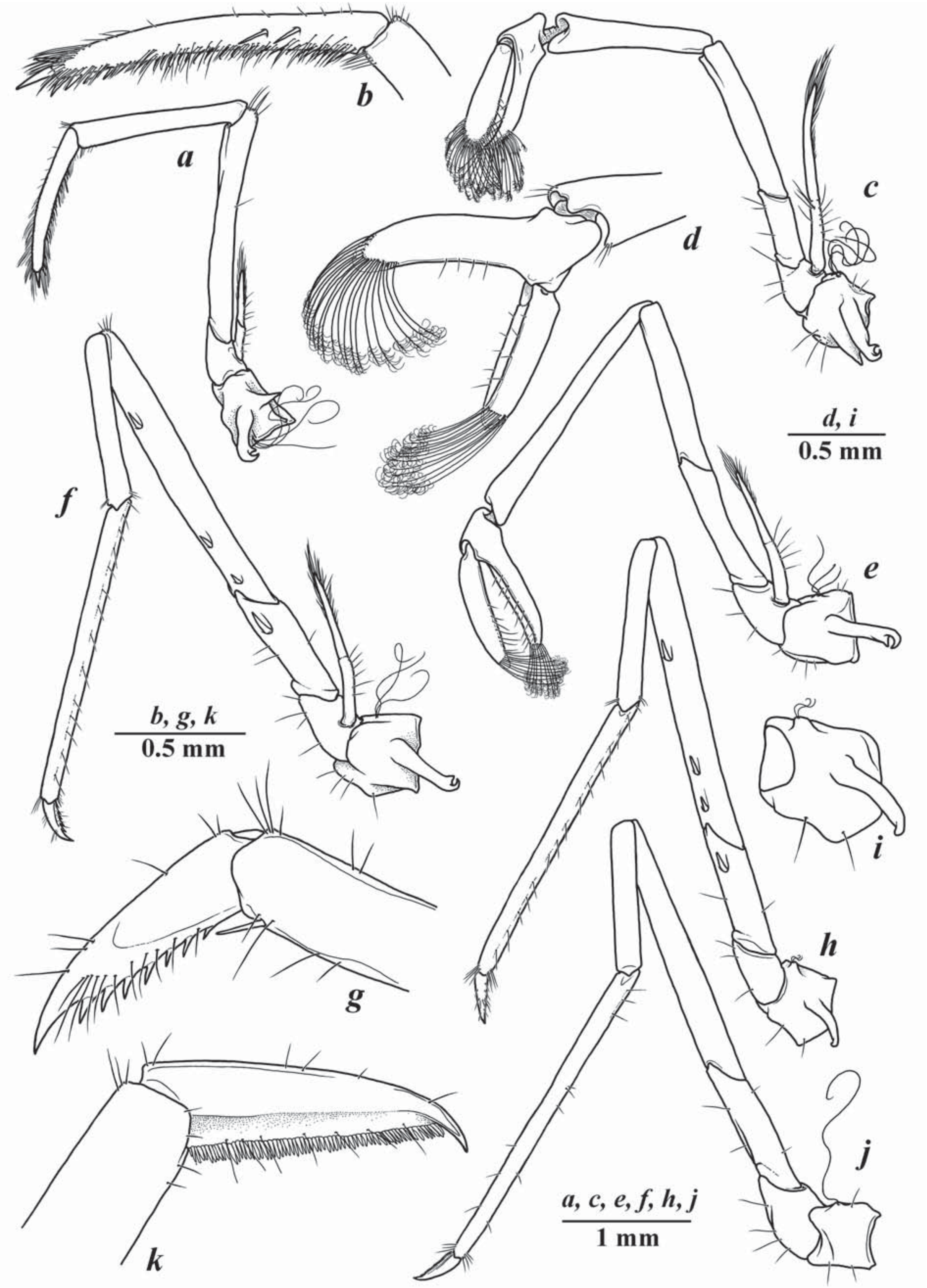
Pereiopod IV (Fig. 3*h*) generally similar to pereiopod III; coxa without tuft of setobranchs and slightly reduced epipodite (Fig. 2*i*); basis about as long as wide, without exopodite; ischium about 2.5 times as long as wide, with small distoventral spine; merus about 5.5 times as long as wide, armed with 3 spines; carpus about 5 times as long as wide, without subdistal spine; propodus about 12 times as long as wide, with straight margins, armed with 10–11 spine-like setae along ventral margin; dactylus about 3 times longer than wide, biunguiculate, with small accessory unguis; ventral margin armed with 4–5 small spines.

Pereiopod V (Fig. 3*j*) generally similar to pereiopods III–IV, but without armature on basal segments and exopodite on basis; basis about as long as wide, with a single setobranch; other segments subequal to segment of PIV; propodus about 12 times as long as wide, with straight margins, unarmed ventrally; dactylus (Fig. 3*k*) with ventral margin armed with a dense "brush" consisting of small simple sharp setae; without accessory unguis, main unguis curved, triangular, sharp distally.

Pleopod I (Fig. 2*h)* normal, without specific features, with endopod bearing well marked appendix interna (Fig.

Fig. 2. *Xiphocaridinella demidovi* sp.n., holotype ♀, pcl. 7.5 mm, tbl. 24.0 mm, ZMMU Ma-6207, Verevkina Cave, Western Abkhazia, southwestern Caucasus: *a* — front of carapace, lateral view; *b* — front of carapace, dorsal view; *c* — antennula, lateral view; *d* — antennula, dorsal view; *e* — antenna; *f* — uropods; *g* — telson; *h* — pleopod I; *i* — same, appendix interna; *j* — pleopod II; *k* — distal part of *appendix interna*.

Рис. 2. *Xiphocaridinella demidovi* sp.n., ♀, голотип, pcl. 7,5 мм, tbl. 24,0 мм, ZMMU Ma-6207, пещера Веревкина, Западная Абхазия, юго-западный Кавказ: *a* — передняя часть карапакса, вид сбоку; *b* — передняя часть карапакса, вид сверху; *c* — антеннула, вид сбоку; *d* — антеннула, вид сверху; *e* — антенна; *f* — уроподы; *g* — тельсон; *h* — плеопод I; *i* — то же, appendix interna; *j* — плеопод II; *k* — дистальная часть *appendix interna*.


a
b
c
d
e
f
g
h
i
j
k
d, i
0.5 mm
b, g, k
0.5 mm
a, c, e, f, h, j
1 mm

2*i*). Pleopod II (Fig. 4*j*) in males with well-developed appendix interna (Fig. 2*k*).

Uropods (Fig. 2*f*) relatively stout, slightly exceeding telson; lateral margin of uropodal exopodite straight, with blunt triangular distolateral angle and 1 large distolateral spine; dieresis simple, without spines.

COLORATION. Body and appendages of shrimps are translucent white or yellowish in color; cornea of eyes is albescent; internal organs (gonads and hepatopancreas) are whitish or yellowish.

ETYMOLOGY. The species is named after the famous cave explorer Pavel Evgenievich Demidov, the head of the expeditions of the "Perovo-Speleo" team (Moscow), which explored the Verevkina Cave for a long time. Unfortunately, Pavel died tragically on August 23, 2020 in the Integral Cave, located to the north of the Verevkina Cave. He led speleoexpeditions that collected all known specimens of cave shrimps from the Verevkina Cave.

GENBANK ACCESSION NUMBERS. OK165439.

DISTRIBUTION. The species is known from the deepest part of the Verevkina Cave (43°24′56.0″N 40°21′23.0″E), Gagra Ridge, Abkhazia, SW Caucasus, in subterranean siphon lake "Nemo"s Last Camp" located about –2204 m from the entrance.

TAXONOMIC REMARKS. The new species is mostly phylogenetically close and represent a sister clade to *X. dbari* Marin, 2019 (see Fig. 4), also described from the caves of Gagra Ridge, including one specimen collected inside Verevkina Cave at the depth -2130 m, where there seems to be a different water flow [Marin, 2019]. From the latter, the new species can be easily separated by 1) longer rostrum, sharpening distally and reaching the distal margin of basal antennular segment (Fig. 2*a*, *b*) (vs. stout triangular rostrum reaching only the midlength of basal antennular segment in *X. dbari* [Marin, 2019: Fig. 3*a–d*]; slender pereiopod II with carpus about 6.5–7 times as long as wide (Fig. 3e) (vs. carpus of pereiopod II about 4 times as long as wide in females of *X. dbari* [Marin, 2019: Fig. 4*h*]); 3) the presence of 3 ischial spines and more reduced exopod on pereiopod III (Fig. 3f) (vs. 2 ischial spines on pereiopod III in *X. dbari* [Marin, 2019: Fig. 4*d*]); 4) the presence of 3 ischial spines and completely reduced exopod on pereiopod IV (Fig. 3*h*) (vs. 2 ischial spines and vestigial expopod on pereiopod IV in *X. dbari* [Marin, 2019: Fig. 4e]); 5) stouter and wider telson (Fig. 2*f*). Molecular genetic divergence between the species by COI mtDNA gene marker is about 6% (Table 1).

From the related *Xiphocaridinella jusbaschjani* Birštein, 1948 known from Agura river, Sochi, Russia and *Xiphocaridinella fagei* Birštein, 1939 from the New Athos (=Novo-Afonskaya) and the Habu caves as well as the neighboring Mchishta karst system of the eastern Abkhazia [Marin, Sokolova, 2014], the new species can be also separated by longer and distally sharpening rostrum reaching the distal margin of basal antennular segment, slenderer pereiopods I and, especially, III, the less number of smaller ischial spines and reduced exopods on pereiopods III–V. Molecular genetic divergence from these species by COI mtDNA gene marker is more than 7% (Table 1).

Xiphocaridinella dbari Marin, 2019

MATERIAL EXAMINED: 1♀ (pcl. 5.5 mm, tbl. 18.0 mm), ZMMU Ma-6208, Abkhazia, Gagra Ridge, Arabika karst massif, Verevkina Cave, 43°24′56.0″N 40°21′23.0″E (the entrance), "Nemo"s Last Camp" siphon lake, about –2212 m from of the cave entrance, coll. Pavel Demidov, 4.03.2018; 1♀ (pcl. 4.7 mm, tbl. 16.0 mm), ZMMU Ma-6209 (damaged), Verevkina Cave, 43°24′ 56.0″N 40°21′23.0″E, "Nemo"s Last Camp" siphon lake, about – 2212 m from of the cave entrance, coll. Pavel Demidov, 22.08.2019.

MATERIAL FOR COMPARISON (described in the original description by Marin [2019]): 4♀♀, 5♂♂ (LEMMI), Abkhazia, Gagry region, Bzyb River Canyon, Gegskaya (Gega) Cave, 43°23′ 43.7″N 40°27′28.4″E (the entrance), about 273 m above sea level, in cave lake, coll. I. Marin, 17 Sept. 2017; 1♀ (LEMMI), Abkhazia, Gagry region, Arabika karst massif, Verevkina Cave, 43°24′56.0″N 40°21′23.0″E (the entrance), about –2100–2130 m from of the cave entrance, in cave lake, coll. Pavel Demidov, Sept. 2018.

TAXONOMIC REMARKS. The collected specimens are mostly similar to the original description (see Marin [2019]). The species can be easily separated from *Xiphocaridinella demidovi* sp.n. by stout triangular rostrum reaching only the midlength of basal antennular segment, the armature of ischium of ambulatory pereiopods (2 ischial spines on PIII and 2 ischial spines on PIV) and the presence of vestigial exopod on pereiopod IV (see above). The molecular divergence from the new specie is about 6% (0.057±0.008 substitutions per 100 nucleotides) by COI mtDNA gene marker (see Tab. 1; Fig. 4).

GENBANK ACCESSION NUMBERS. OK165440, OK165441.

DISTRIBUTION. The species is known from an underground lake inside the Gegskaya Cave, 43°23′43.7″N 40°27′28.4″E, Abkhazia, Western Caucasus (the type locality) and from the Verevkina Cave (43°24′56.0″N 40°21′23.0″E) at a depth –2100–2130 m and –2212 m (the siphon "Nemo"s Last Camp"). Three known specimens from Verevkina Cave differ from the population from Gegskaya Cave for about 1% by COI mtDNA gene marker (0.01 substitutions per 100 nucleotides).

Discussion

Currently, the world deepest troglobiont is the springtail *Plutomurus ortobalaganensis* Jordana et Baquero, 2012 (Collembola: Tomoceridae), which lives in the Krubera-Voronja Cave (Arabika karst massif, Gagra Ridge, Abkhazia) from –60 m to –1980 m from the entrance [Sendra, Reboleira, 2012], and previously the deepest dwelling stygobiotic animal is *Kruberia abchasica* Sidorov et Samokhin, 2016 (Crustacea: Amphipoda: Gammaridae), also collected in the Krubera-Voronja Cave from the siphon "Two Captains" (=Dva Kapitana) at a depth of –2175 m from the entrance [Sidorov, Samokhin, 2016]. At the same time, the current record of *Xiphocaridinella demidovi* sp.n. and *X. dbari* from a depth of –2212 m in bottom

Fig. 3. *Xiphocaridinella demidovi* sp.n., holotype ♀, pcl. 7.5 mm, tbl. 24.0 mm, ZMMU Ma-6207, Verevkina Cave, Western Abkhazia, southwestern Caucasus: *a* — maxilliped III; *b* — same, ultimate (distal) segment; *c* — pereiopod I; *d* — same, chela; *e* — pereiopod II; *f* — pereiopod III; *g* — same, dactylus; *h* — pereiopod IV; *i* — same, coxal segment; *j* — pereiopod V; *k* — same, dactylus.

Рис. 3. *Xiphocaridinella demidovi* sp.n., ♀, голотип, pcl. 7,5 мм, tbl. 24,0 мм, ZMMU Ma-6207, пещера Веревкина, Западная Абхазия, юго-западный Кавказ: *a* — максиллипед III; *b* — то же, дистальный сегмент; *c* — переопод I; *d* — то же, клешня; *e* — переопод II; *f* — переопод III; *g* — то же, дактилус; *h* — переопод IV; *i* — то же, коксальный сегмент; *j* — переопод V; *k* — то же, дактилус.

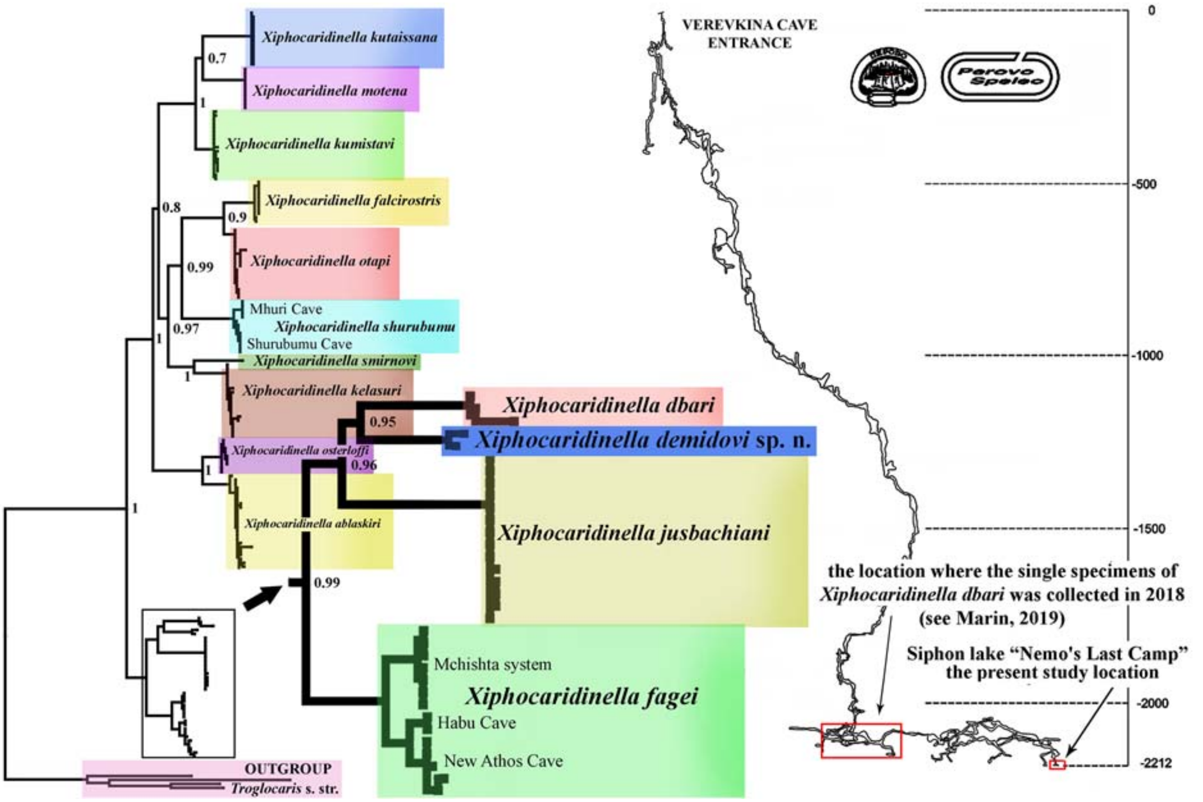


Fig. 4. Phylogenetic (COI mtDNA) reconstruction (tree) of the Caucasian *Xiphocaridinella* (ML algorithm, GTR+G+I model, (AIC)–aLRT SH-like) with the Maximum likelihood bootstrap values presented for each node; and the map of the Verevkina Cave indicating the localities, where stygobiotic shrimps where collected.

Рис. 4. Филогенетическая (COI мтДНК) реконструкция (дерево) Кавказских *Xiphocaridinella* (ML алгоритм, GTR+G+I, (AIC)–aLRT SH-like) с максимальной вероятностью значения для каждого узла; и карта пещеры Веревкина с указанием локаций, где были собраны стигобионтные креветки.

section of the Verevkina Cave makes them the world's deepest living troglobiotic/stygobiotic animals officially recorded. Although, of course, other stygobiotic species live in this watercourse of the lower part of the Verevkina Cave. It is very likely that the co-occurring of the species in the bottom section of the cave is caused by the convergence of underground streams from flowing from the different parts of the Arabika plateau, as well as extensive high floods characteristic of the caves of this karst massif.

According to research of Klimchuk [2018], the caves of the western part of the Arabika plateau (Krubera-Voronja, Kuibyshevskaya, Ilyukhina, etc.) are unloaded in submarine and coastal resources, including coastal Reprua River (43°19′52.4″N 40°12′17.1″E), while the unloading of the caves from the eastern part of the Arabika plateau (Verevkina, Sarma, Moskovskaya, Dzou, etc.) occurs in the canyon of the Bzyb River. Specifically, according to his assumptions [Klimchuk, 2018], unloading from the Verevkina and Sarma caves takes place in the area of the Blue Lake (43°21′02.8″N 40°24′40.0″E), and the Moskovskaya and Dzou caves are unloaded upstream in the area of the Gegsky waterfall (43°26′08.4″N 40°26′36.5″E). At the same time, according to our data, the water flows of the Verevkina and Gegskaya caves (43°23′43.7″N 40°27′28.4″E) are interconnected, since they contain the same species of stygobiotic shrimp *Xiphocaridinella dbari* (see above; Marin [2019]). The biospeleological method, which studies the DNA of subterranean stygobiotic animals, allowing establishing biogeographic connections between underground watercourses, has already been used along with traditional hydrogeological methods to establish the connection of remote caves in Abkhazia [Marin, 2020; Marin, Turbanov, 2021]. This method can also be used on the example of other stygobiotic animals, namely the widespread amphipods of the genus *Niphargus* Schiødte, 1847 (Crustacea: Amphipoda: Niphargidae), which are more widespread and common in subterranean watercourses.

The new described species belongs to the clade of the genus *Xiphocaridinella*, which lives in the north-western part of the Colchis lowland, on the territory of Russia (*X. jusbaschjani*), as well as on the territory of western Abkhazia (*X. dbari* and *X. fagei*). This clade separated from the other species of the genus quite a long time ago, from 2–13 Mya (see Table 1), very likely about 4–5Mya. It is still unknown to which species belongs the specimen, identified as *Troglocaris* sp. from the lower part of the Krubera-Voronya Cave

[Sendra, Reboleira, 2012], but it certainly also belongs to this clade and is related/conspecific to either the new species or *X. dbari*. According to the data obtained (Table 1), the approximate divergence time of 5Mya for separation of the new species from other species of the genus, as well as all other species within the genus, seems unlikely to exceed such a global catastrophe as Messinian Crisis, as a result of which the entire aquatic fauna of the Caucasus was likely to become extinct. At the same time, data less than 5 Mya on the main division of species within the genus, as well as the separation of the new species from the most phylogenetically related *X. dbari*, which inhabit the same Gagra Ridge within the late 1–2 Mya, seems quite plausible. The active growth of the Caucasian mountain massifs, and, accordingly, the occurrence of karst caves, begins approximately in the Pliocene–Pleistocene, and the age of many Caucasian caves, including the caves of the Arabika massif, is just defined as approximately 1–2 Mya [Klimchuk, 2018]. The pools of the siphon lake "Nemo"s Last Camp" siphon lake and the water source in the Verevkina Cave at a depth of –2100–2130 m, where stygobiotic shrimps were found, are located almost above the basic non-karst rocks, which indicates a long process of formation of these cavities. Thus, the rate of evolutionary changes in shrimps of the genus *Xiphocaridinella* is quite high and is close to the values of 5–3% Mya^{-1}.

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