Gammarus liuruiyui sp. nov. (Amphipoda, Gammaridae), a new blind species from Tian Shan, China

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GAMMARUS LIURUIYUI SP. NOV. (AMPHIPODA, GAMMARIDAE), A NEW BLIND SPECIES FROM TIAN SHAN, CHINA

BY

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ABSTRACT

A new species of the genus *Gammarus* Fabricius, 1775 is described and illustrated from the Tian Shan, China. *Gammarus liuruiyui* sp. nov. is characterized by eyes being absent; pereopods V-VII bases not expanded; uropod III with scale-like inner ramus, second article of outer ramus vestigial; and posterior margins of epimeral plates nearly straight. Detailed morphological comparisons with related species are discussed. Genetic distances of the new species and related species are provided as proof of the species delimitation.

Key words. — Morphology, genetic distance, taxonomy

RÉSUMÉ

Une nouvelle espèce du genre *Gammarus* Fabricius, 1775 est décrite et illustrée de Tian Shan, Chine. *Gammarus liuruiyui* sp. nov. est caractérisée par l'absence d'yeux ; les bases des péréiopodes V-VII non élargies ; l'uropode III avec une rame interne en forme d'écaille ; le second article de la rame externe vestigial ; et les bords postérieurs des plaques épimérales presque droites. Des comparaisons morphologiques détaillées avec des espèces apparentées sont discutées. Les distances génétiques entre la nouvelle espèce et les espèces apparentées sont fournies comme preuves de la délimitation des espèces.

Mots clés. — Morphologie, distance génétique, taxonomie

INTRODUCTION

The Tian Shan is a large system of mountain ranges in Central Asia and stretches some 2900 kilometres westward from Xinjiang in China. The geological history of the Tian Shan was influenced by the uplift of the Tibetan Plateau and regression of

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the Tethys (Caves et al., 2017), which defined its biodiversity features, sustaining ancient Tethyan relicts (Hou et al., 2011). Thus, the discovery of species diversity will contribute to the understanding of the regional palaeogeographical history and the evolution of the region's fauna (Li & Quan, 2017).

The genus *Gammarus* Fabricius, 1775 contains more than 200 freshwater, brackish, and marine species in the northern hemisphere (Väinölä et al., 2008). It has been suggested that *Gammarus* originated from the ancient Tethys, then diversified in Eurasia (Hou et al., 2011). Up to now, four species have been recorded along the Tian Shan in China, including *Gammarus tianshan* Zhao, Meng & Hou, 2017, *G. takesensis* Hou, Li & Platvoet, 2004, *G. brevipodus* Hou, Li & Platvoet, and *G. decorosus* Meng, Hou & Li, 2003. In the current paper, a blind species attributable to the genus *Gammarus* was described and illustrated from a spring of the Tian Shan, China. A distribution map and a key to the species of the genus *Gammarus* known from Tian Shan in China are presented. Moreover, partial fragments of the mitochondrial cytochrome oxidase subunit I (COI) and nuclear 28S rRNA were obtained. The genetic distances between these species are calculated to confirm the species delimitation.

MATERIAL AND METHODS

Morphological observation

The specimens were collected in a spring with a fine-meshed hand net. Samples were preserved in 95% ethanol in the field, then deposited at −20 °C freezer for long-term preservation. The body length was recorded by holding the specimen straight and measuring the distance along the dorsal side of the body from the base of the first antenna to the base of the telson. All dissected appendages were mounted on slides according to the methods described by Holsinger (1967). Appendages were drawn using a Leica DM2500 compound microscope equipped with a drawing tube. Terminology and taxonomic descriptions follow the literature (Zheng et al., 2017), especially the terms "spines" and "setae" are used to distinguish between thin or fine and more robust setal structures. All types and other materials are maintained in the Institute of Zoology, Chinese Academy of Sciences (IZCAS), Beijing.

Molecular methods

Partial fragments of mitochondrial COI and nuclear 28S were amplified to confirm identifications and validate species delimitation (Costa et al., 2007; Hou et al., 2009). The primers used are LCO1490 (5′-GGTCAACAAATCATAAAGATAT TGG-3′) and HCO2198 (5′-TAAACTTCAGGGTGACCAAAAAATCA-3′) for

TABLE I
GenBank accession numbers and uncorrected pairwise distance of the COI and 28S partial sequences between species in this text

	Species	COI	28S				
			1	2	3	4	5
			MK455898	EF582971	JF965809	JF965681	EF582949
1	*Gammarus liuruiyui* sp. nov.	MK455899	–	0.056	0.070	0.049	0.056
2	*G. tianshan* Zhao, Meng & Hou, 2017	EF570327	0.216	–	0.045	0.042	0.016
3	*G. takesensis* Hou, Li & Platvoet, 2004	JF965979	0.271	0.216	–	0.056	0.043
4	*G. brevipodus* Hou, Li & Platvoet, 2004	–	–	–	–	–	0.039
5	*G. decorosus* Meng, Hou & Li, 2003	EF570303	0.229	0.210	0.232	–	–

COI (Folmer et al., 1994), and 28F (5′-TTAGTAGGGGCGACCGAACAGGGAT-3′) and 28R (5′-GTCTTTCGCCCCTATGCCCAACTGA-3′) for 28S (Hou et al., 2007). Genomic DNA extraction, amplification and sequencing procedures were performed as in Hou et al. (2007). Uncorrected pairwise distances between species were calculated using MEGA 7.0.16 (Kumar et al., 2016). The new sequences were deposited in GenBank and the accession numbers are provided in Table 1.

TAXONOMY

Family GAMMARIDAE Leach, 1814
Genus *Gammarus* Fabricius, 1775
Gammarus liuruiyui sp. nov.
(figs. 1-9)

Material examined.— Holotype male, 11.0 mm (IZCAS-I-A1634-1): Sumutash Town (78.19°E, 40.88°N), Aheqi County, Kizilsu Kirghiz Autonomous Prefecture, Xinjiang Uygur Autonomous Region, altitude 2170 m, 25 July, 2014, collected by Kaibaryer Meng. Paratype female, 9.0 mm (IZCAS-I-A1634-2): same data as holotype. Other material: six males, five females, and two juveniles, same data as holotype.

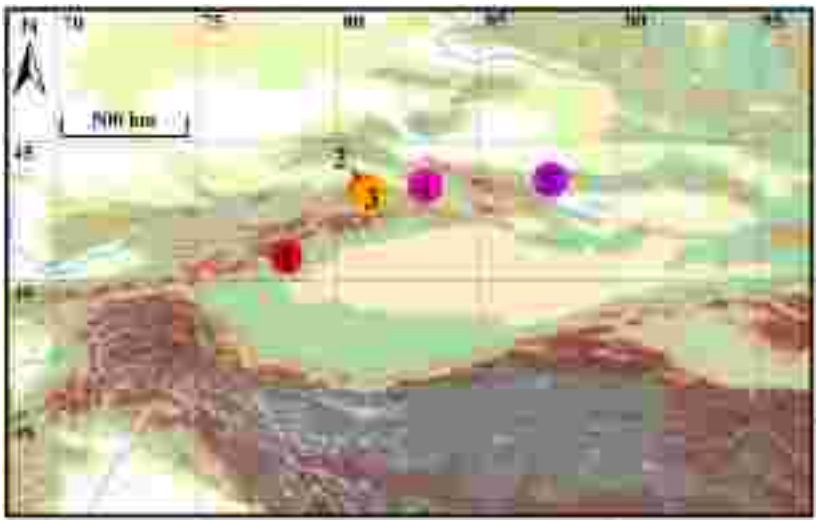

Fig. 1. Type localities of *Gammarus* species from Tian Shan, China. 1, *Gammarus liuruiyui* sp. nov.; 2, *Gammarus tianshan* Zhao, Meng & Hou, 2017; 3, *Gammarus takesensis* Hou, Li & Platvoet, 2004; 4, *Gammarus brevipodus* Hou, Li & Platvoet, 2004; 5, *Gammarus decorosus* Meng, Hou & Li, 2003.

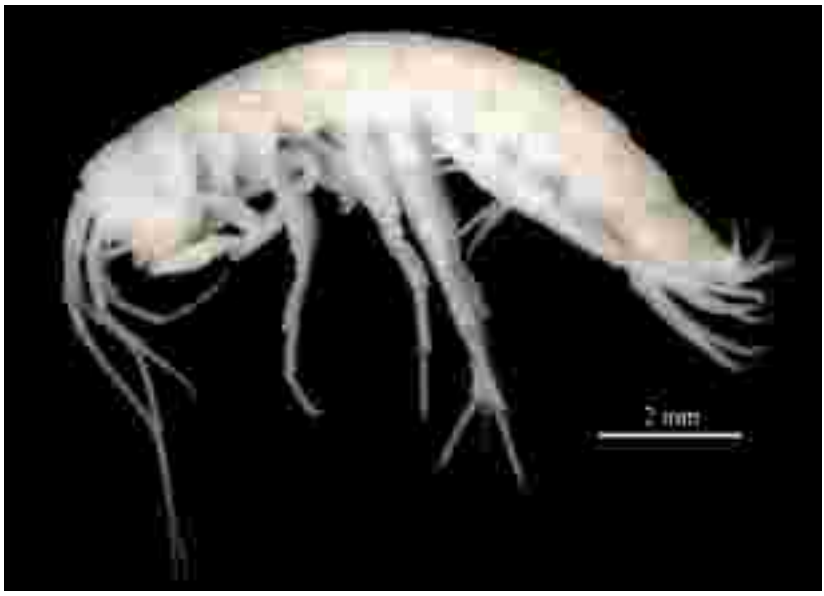

Fig. 2. *Gammarus liuruiyui* sp. nov. male holotype.

Etymology.— The species name honours Prof. Liu Ruiyu for his contributions to crustacean research; the name is a noun in the genitive singular.

Diagnosis.— Eyes absent; pereopod IV coxal plate weakly lobate; pereopods V–VII bases similar, not expanded; epimeral plates nearly straight on posterior margins; urosomites I-III with spines on dorsal margins; uropod III with scale-like inner ramus, second article of outer ramus vestigial; telson deeply cleft, with three distal spines.

Description of holotype male (IZCAS-I-A1634-1).— Body length 11.0 mm.

Head (fig. 3A): eyes absent, inferior antennal sinus shallow.

Antenna I (fig. 3B): longer than antenna II, peduncle articles 1-3 in length ratio 1.0 : 0.6 : 0.4, with distal setae; flagellum with 23 articles; accessory flagellum with two articles; both primary and accessory flagella with short distal setae.

Antenna II (fig. 3C): peduncle articles 3-5 in length ratio 1.0 : 2.5 : 2.2, peduncle article 3 with a seta on posterior margin, three setae on anterior margin, articles 4-5 nearly same length, each with stiff setae along anterior and posterior margins; flagellum with eight articles, each article with distal setae; calceoli absent.

Upper lip (fig. 3D): ventral margin convex, bearing short minute setae.

Mandible (fig. 3E): asymmetrical, right mandible incisor with four teeth; lacinia mobilis with three teeth, setal row with eight plumose setae; palp composed of three articles, third article with one long seta and four short setae on apical margin.

Lower lip and maxilla I: broken, similar to those of female (fig. 6G-I).

Maxilla II (fig. 3F): inner plate with 11 lateral setae; outer plate with 12 apical setae.

Maxilliped (fig. 3G): inner plate with ten apical setae; outer plate with eight peg-like spines and three setae; palp with four articles, second article stout with a row of long simple setae on inner margin and four setae on outer margin, third article with setae on both margins, terminal article hooked, nail small.

Pereon. Gnathopod I (fig. 4A, B): coxal plate sub-rectangular, with three setae on anterior margin, one seta on inner face, three setae on posterior margin; basis sub-linear, bearing long setae on posterior margin; merus with five long setae on posterior margin; carpus with setae on anterior and posterior margins, with tapered distolateral lobe; propodus twice as long as wide, oval, palm with six spines on posterodistal corner, medial spine absent; dactylus reaching approx. 60% length of propodus.

Gnathopod II (fig. 4C, D): coxal plate with three setae on anterior margin and two setae on posterior margin; basis with three long setae on anterior margin and one seta on distal margin, posterior margin with a row of long setae; merus with five setae on posterior margin; carpus with long setae on posterior margin; propodus 1.7 times as long as wide, sub-rectangular, palm with one medial spine and three spines on posterior margin; dactylus reaching approx. 67% length of propodus.

Fig. 3. *Gammarus liuruiyui* sp. nov. male holotype. A, Head; B, antenna I; C, antenna II; D, upper lip; E, right mandible; F, maxilla II; G, maxilliped; H, epimeral plate I; I, epimeral plate II; J, epimeral plate III.

Fig. 4. *Gammarus liuruiyui* sp. nov. male holotype. A, Gnathopod I; B, propodus of gnathopod I; C, gnathopod II; D, propodus of gnathopod II.

Fig. 5. *Gammarus liuruiyui* sp. nov. male holotype. A, Pereopod III; B, pereopod IV; C, pereopod VI; D, pereopod VII; E, pleopod I; F, uropod I; G, uropod II; H, uropod III; I, telson; J, dactylus of pereopod III; K, dactylus of pereopod IV; L, dactylus of pereopod VI; M, dactylus of pereopod VII; N, urosomites (dorsal view).

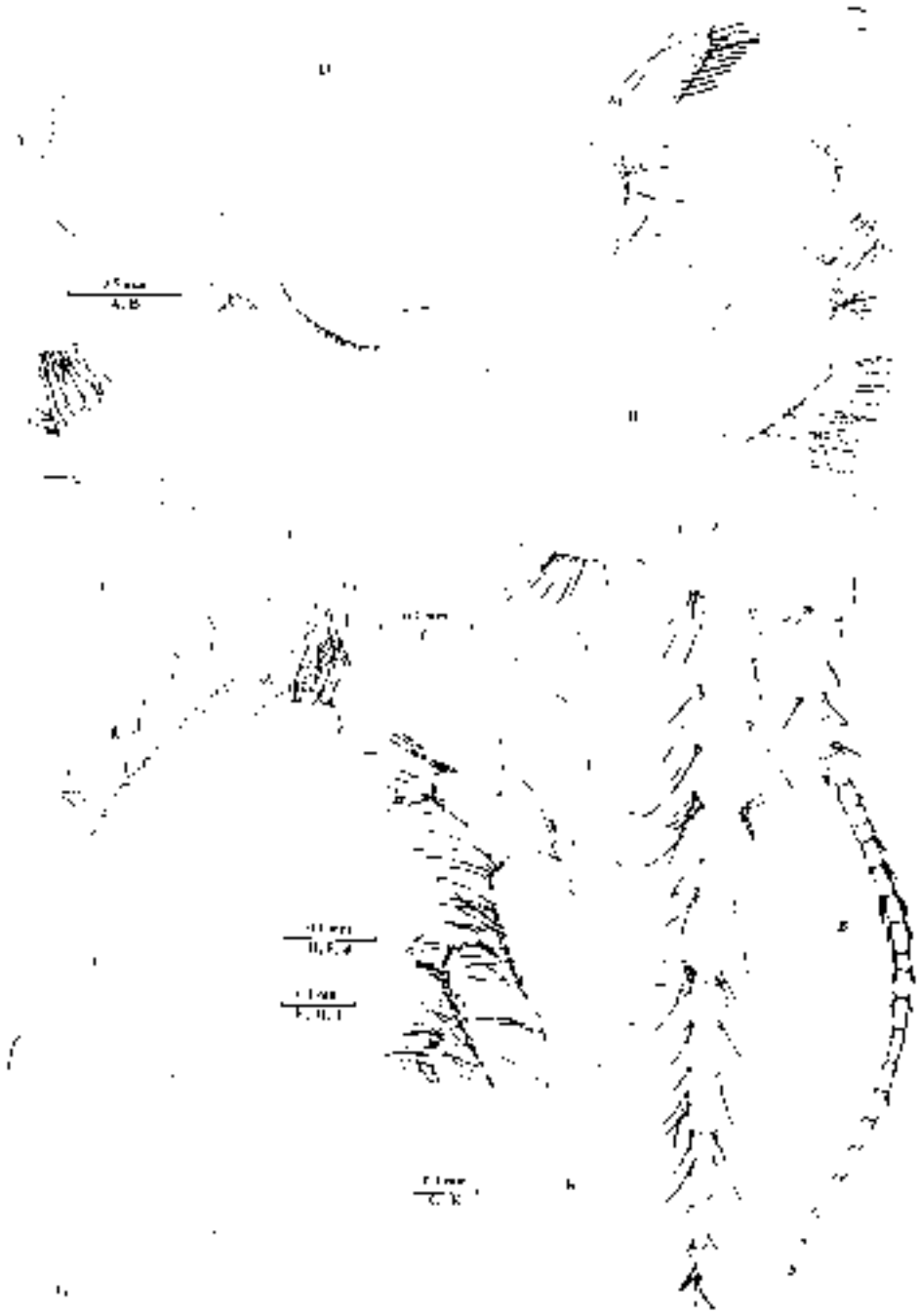

Fig. 6. *Gammarus liuruiyui* sp. nov. female paratype. A, Head; B, antenna I; C, antenna II; D, upper lip; E, right mandible; F, left mandible; G, lower lip; H, maxilla I (right); I, maxilla I (left); J, maxilla II; K, maxilliped.

Fig. 7. *Gammarus liuruiyui* sp. nov. female paratype. A, Gnathopod I; B, propodus of gnathopod I; C, gnathopod II; D, propodus of gnathopod II.

Pereopods III-IV (fig. 5A-B, J-K): similar to each other, coxal plate III with six setae on anterior margin; coxal plate IV weakly concave, with two setae on anterior margin; bases sub-linear, bearing one to four long setae and short spines on both margins; merus to propodus with spines accompanied by short setae along anterior and posterior margins; dactylus with one seta at hinge of unguis.

Pereopod V: missing.

Pereopod VI (fig. 5C, L): coxal plate weakly bilobate, with four short setae on posterior margin; basis with six spines on anterior margin and nine spines on posterior margin; merus to propodus with spines and setae along anterior and posterior margins; dactylus with one seta at hinge of unguis.

Pereopod VII (fig. 5D, M): coxal plate small, irregular, with four setae on posterior margin; basis linear, with three short spines on anterior margin and seven spines on posterior margin; merus to propodus with spines and setae along anterior and posterior margins; dactylus with one seta at hinge of unguis.

Coxal gills (fig. 5B-D): present on gnathopod II and pereopods III-VII, sac-like.

Pleon. Epimeral plates (fig. 3H-J): plate I ventrally rounded, with one seta on ventral margin and four setae on posterior margin; plates II-III nearly straight on posterodistal corners, with long setae along ventral and posterior margins.

Pleopods I–III (fig. 5E): similar to each other, peduncle with some marginal setae; inner and outer rami nearly the same length, both rami fringed with plumose setae.

Urosome. Urosomites I-III (fig. 5N): urosomite I with one spine on dorsal margin; urosomite II with three spines on dorsal margin; urosomite III with four spines on dorsal margin.

Uropod I (fig. 5F): peduncle longer than rami, with one basofacial spine, one and six spines on inner and outer margins, respectively; inner ramus with two spines on inner margin; outer ramus with three spines on outer margin; both rami with five terminal spines.

Uropod II (fig. 5G): peduncle with three spines on inner margin; inner ramus with three spines on inner margin, one spine on outer margin; outer ramus with two spines on inner and outer margins, respectively; both rami with four terminal spines.

Uropod III (fig. 5H): shorter than uropods I-II, peduncle shorter than outer ramus, with three spines on distal margin; inner ramus with one distal seta, scale-like; outer ramus with three spines on inner margin, one spine on outer margin, and five terminal spines, second article very small.

Telson (fig. 5I): deeply cleft, each lobe with three distal spines.

Description of paratype female (IZCAS-I-A1634-2).— Body length 9.0 mm.

Head. Antenna I (fig. 6B): peduncle articles 1-3 in length ratio 1.0 : 0.7 : 0.4, with distal setae; flagellum with 20 articles; accessory flagellum with two articles.

Antenna II (fig. 6C): peduncle articles 3-5 with setae along anterior and posterior margins; flagellum with seven articles, both with distal setae.

Upper lip (fig. 6D). convex, with minute setae.

Mandible (fig. 6E-F): asymmetrical, right incisor with four teeth; lacinia mobilis with three teeth; spine row with six setae; second article of palp with eight setae on inner margin, third article with two setae on outer face, a row of eight setae on inner margin, three long setae on apical margin. Incisor of left mandible with four teeth; lacinia mobilis with three teeth.

Lower lip (fig. 6G): inner lobes lacking.

Maxilla I (fig. 6H-I): inner plate of right maxilla I with ten long plumose setae; outer plate with seven pectinate robust spines; article 2 of palp bearing five spines apically; left palp with five stiff setae.

Maxilla II (fig. 6J): outer plate broader than inner plate, both with several setae.

Maxilliped (fig. 6K): inner plate and outer plate with spines and setae on both margins; second article of palp expanded, with a row of setae on inner margin, four setae on outer margin, third article with marginal setae, fourth article claw-shaped.

Pereon. Gnathopod I (fig. 7A-B): basis sub-linear bearing several long setae on both margins; propodus oval, palm with two spines on posterodistal corner.

Gnathopod II (fig. 7C-D): merus and carpus with setae; propodus twice as long as wide, palm with two spines on posterodistal corner.

Pereopods III-VII (fig. 8A-J): similar to those of male. Bases of pereopods V-VII slender.

Oostegites present on gnathopod II and pereopods III-V.

Pleon. Epimeral plates I-III (fig. 9A-C): plates I-III with three, four and four setae on posterior margins, respectively; plates II-III with one and two setae on ventral margins, respectively.

Pleopods I-III (fig. 9D): both rami fringed with plumose setae.

Urosome. Urosomites I-III (fig. 9I): with three spines on dorsal margins, respectively.

Uropod I (fig. 9E): peduncle with two and five spines on outer and inner margins, respectively; inner ramus with three spines on inner margin; outer ramus with one and two spines on inner and outer margins, respectively; both rami with five terminal spines.

Uropod II (fig. 9F): peduncle with three spines on outer margin and one spine on inner margin; outer ramus slightly shorter than inner ramus, with three spines

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Fig. 8. *Gammarus liuruiyui* sp. nov. female paratype. A, Pereopod III; B, pereopod IV; C, pereopod V; D, pereopod VI; E, pereopod VII; F, dactylus of pereopod III; G, dactylus of pereopod IV; H, dactylus of pereopod V; I, dactylus of pereopod VI; J, dactylus of pereopod VII.

Fig. 9. *Gammarus liuruiyui* sp. nov. female paratype. A, Epimeral plate I; B, epimeral plate II; C, epimeral plate III; D, pleopod I; E, uropod I; F, uropod II; G, uropod III; H, telson; I, urosomites (dorsal view).

on inner margin; inner ramus with two and three spines on inner and outer margins, respectively; both rami with five terminal spines.

Uropod III (fig. 9G): peduncle shorter than outer ramus, with one and three spines on anterior and posterior margins, respectively; inner ramus scale-like; outer

ramus with three spines on outer margin and several terminal spines, second article vestigial.

Telson (fig. 9H): deeply cleft, each lobe with two to three distal spines.

Habitat.— Fifteen individuals were collected from a spring (area 0.2 m^2), which is connected to a stream flowing from Tian Shan two kilometers away. The spring is influenced by the groundwater in the deep strata. When the snow melts, the river flows through the desert and forms subterranean water. A spring will erupt from the shallow layer. All specimens were found under rubble in the bottom of the spring, with eyes totally absent without subcutaneous ocelli. The blind species were considering as isolated populations from a species widespread in surface water that have adapted to subterranean life (Simon, 2012). However, epigean gammarids have not been seen around the type locality.

Remarks.— Freshwater *Gammarus* are characterized by direct development of fertilized eggs in a marsupium and no free-swimming larvae to be transported passively by currents (Hughes & Ahyong, 2016). Their weak dispersal potential means that they are easily influenced by geological barriers, such as mountain ranges (Hou et al., 2014). Previous studies indicated that most are narrowly endemic species (Copilaş-Ciocianu et al., 2018). We have made comparisons between *Gammarus liuruiyui* sp. nov. and the species of *Gammarus* from Central Asia, finding that the new species can be easily distinguished by the scale-like inner ramus of uropod III (Sidorov et al., 2018). Therefore, we focused our discussion on the differences between the new species and the species of *Gammarus* known from Tian Shan, China.

Gammarus liuruiyui sp. nov. is most similar to *Gammarus brevipodus* in that the calceoli are absent in antenna II and that pereopods III and IV have a few short setae. The new species differs from *G. brevipodus* in that the eyes are absent in *Gammarus liuruiyui*, but eyes of *G. brevipodus* are present; bases of pereopods V-VII are slender, but bases of *G. brevipodus* are slightly expanded on medial margin; uropod III has the scale-like inner ramus, but inner ramus of *G. brevipodus* is about one third the length of the outer ramus.

Gammarus liuruiyui sp. nov. resembles *Gammarus takesensis* in that pereopods III-VII have a few short setae, the peduncle of uropod I has a basofacial spine, and the telson is deeply cleft. It can be distinguished from *G. takesensis* by the eyes being absent in *Gammarus liuruiyui* while present in *G. takesensis*; the coxal plate of pereopod IV is weakly concave, while it is concave in *G. takesensis*; epimeral plates II and III are nearly straight on posterodistal corners, while subacute in *G. takesensis*; uropod III has a scale-like inner ramus, while the inner ramus of *G. takesensis* is about three-fourth the length of the outer ramus.

We downloaded COI and 28S sequences of the genus *Gammarus* from Tian Shan, China. Molecular analyses showed high interspecific divergences. The uncorrected pairwise distances between *Gammarus liuruiyui* sp. nov. and *G. tianshan*, *G. takesensis*, *G. decorosus* range from 21.6-27.1% for COI, and 4.9-7.0% for 28S. These COI distance values are larger than the 16% divergence recommended by Lefebure et al. (2006) as a threshold for crustacean species delimitation. The 28S differentiation is comparable to interspecific distances calculated for several species of *Gammarus* well delineated in morphological terms (Hou & Li, 2010; Copilaş-Ciocianu & Petrusek, 2016). Therefore, morphological and molecular data support *G. liuruiyui* sp. nov. being a new species.

The comparison between *Gammarus* species from Tian Shan, China is presented in the following key.

KEY TO *GAMMARUS* SPECIES FROM THE TIAN SHAN, CHINA

1. Eyes present, inner ramus of uropod III longer than one-third of outer ramus 2
– Eyes absent, inner ramus of uropod III scale-like ..... *Gammarus liuruiyui* sp. nov.
2. Pereopods III-IV and uropod III bearing long setae 3
– Pereopods III-IV and uropod III with a few short setae 4
3. Uropod III inner ramus about half the length of outer ramus, both rami with simple setae ..... *Gammarus tianshan*
– Uropod III inner ramus about three-fourth of outer ramus, both rami with simple and plumose setae ..... *Gammarus decorosus*
4. Calceoli of antenna II present ..... *Gammarus takesensis*
– Calceoli of antenna II absent ..... *Gammarus brevipodus*

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