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A new species of *Spelaeogammarus* (Amphipoda: Bogidielloidea: Artesiidae) with an identification key for the genus

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Abstract

There are five described species of the cave-dwelling amphipods of the genus *Spelaeogammarus*, all of them inhabiting caves on the Brazilian state of Bahia. A new species of this genus is here described, which is closely related to the already known species *S. santanensis* and *S. titan*. *Spelaeogammarus sanctus* **sp. nov.** differs from its congeneric species basically by the presence of more than 18 bifid setae on the dorsal margin of uropod 3 outer ramus and telson with one apical and two subapical stout setae, while the other species generally present less setae on the third uropod and more setae on telson. An identification key and an updated table of the *Spelaeogammarus* species diagnosis are provided, as well as a multivariate statistical approach of morphological variations among the species.

Key words: taxonomy, Subterranean amphipods, Cave biodiversity

Introduction

Amphipods represent a crustacean order of approximately 10,000 described species, and this number has been increasing in the last years (Fišer *et al.* 2013). These crustaceans are found on terrestrial, marine and freshwater environments, as well as in epigeal, hypogean and hypotelminorheic habitats (Fišer *et al.* 2010; Rodrigues *et al.* 2012). The stygobiotic fauna, which comprises the subterranean-restricted and usually specialized aquatic fauna is predominated by crustaceans (Sket 1999). Approximately 45% of the described freshwater amphipod species are stygobiotic, for which the highest diversity is found on karstic landscapes of Central and Southern Europe (Väinöla *et al.* 2008).

Currently five families of amphipods are known for subterranean ecosystems in Brazil: Hyaellidae, Mesogammaridae, Seborgidae, Bogidiellidae and Artesiidae (Koenemann & Holsinger 2000; Cardoso *et al.* 2012; Fišer *et al.* 2013; Senna *et al.* 2014). Although Botosaneanu & Stock (1989) had suggested that the name Artesiidae was leaved and its genera were included on Bogidiellidae, the most recent study regarding the phylogeny and classification of Amphipoda (Lowry & Myers, 2013) revalidates this family, which now groups the genera *Artesia* Holsinger, 1980 and *Spelaeogammarus* Da Silva Brum, 1975.

Spelaeogammarus is a genus found exclusively on Brazilian caves situated on the state of Bahia and comprises five species: *S. bahiensis* Da Silva Brum, 1975 (type-species), *S. spinilacertus* Koenemann & Holsinger, 2000; *S. trajanoae* Koenemann & Holsinger, 2000; *S. santanensis* Koenemann & Holsinger, 2000 and *S. titan* Senna *et al.*, 2014. All of them are anophthalmic and depigmented, presenting subtle morphological differences mainly on the flagellum of both antenna, number and type of seta on gnathopods, pleopods, uropods and telson, as well as body size. Koenemann & Holsinger (2000) stated that these differences seemed to be correlated with the spatial distribution, so that geographically nearest species show higher morphological resemblance.

A new stygobiotic Amphipoda of the genus *Spelaeogammarus* was found on the municipality of Bom Jesus da Lapa, state of Bahia, Brazil, which is being described in the present work. This new species presents subtle morphological differences in relation to its co-generics, for this reason in addition to the description of the new species we present a statistical approach of some morphological characteristics considering all the six species of the genus, besides an identification key for them and an updated table of the species diagnosis.

Material and methods

The new species was found in the Gruta dos Milagres cave (13°15'31.45" S 43°25'5.76" W) in the municipality of Bom Jesus da Lapa, Midwest of the Brazilian state of Bahia (Fig 1). The specimens were collected with the aid of a hand net and placed in vials with 70% ethanol. This material was deposited at the Collection of Subterranean Invertebrates of Federal University of Lavras and Museu Nacional of Universidade Federal do Rio de Janeiro. Ten paratypes were dissected and mounted on slides with glycerol gelatin. Each appendage was observed and photographed under an optic microscope Primo Star ZEISS coupled with camera, and drawings were made based on the holotype and one paratype. The nomenclature of seta followed Watling (1989).

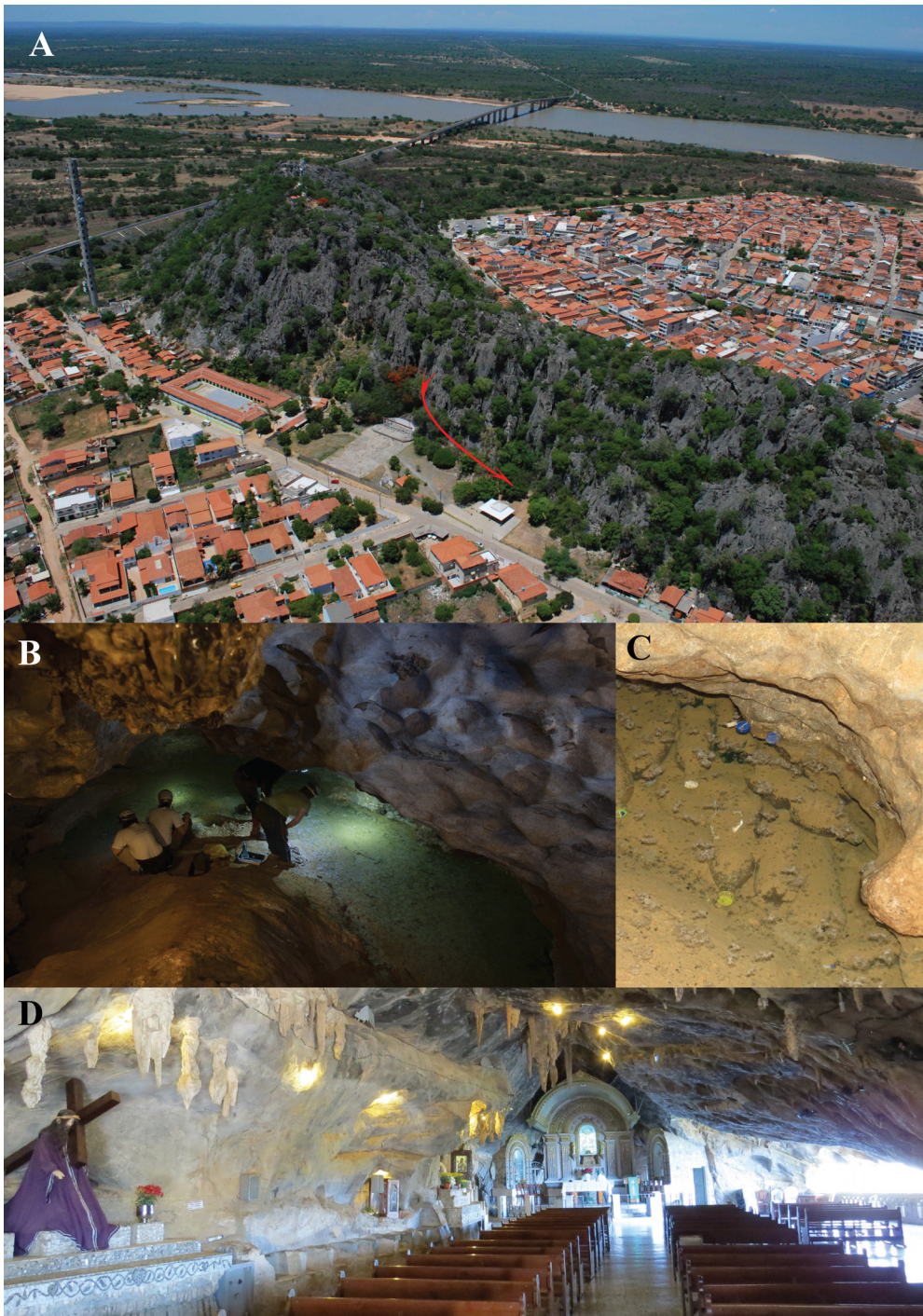


FIGURE 1. (A) Bom Jesus da Lapa municipality, state of Bahia, Brazil and the Gruta dos Milagres cave (the arrow indicates its entrance); (B) Visitor pilgrims inside the cave; (C) Water table in which amphipods were collected; (D) Gruta do Santuário de Bom Jesus, cave transformed in a church, Bom Jesus da Lapa municipality.

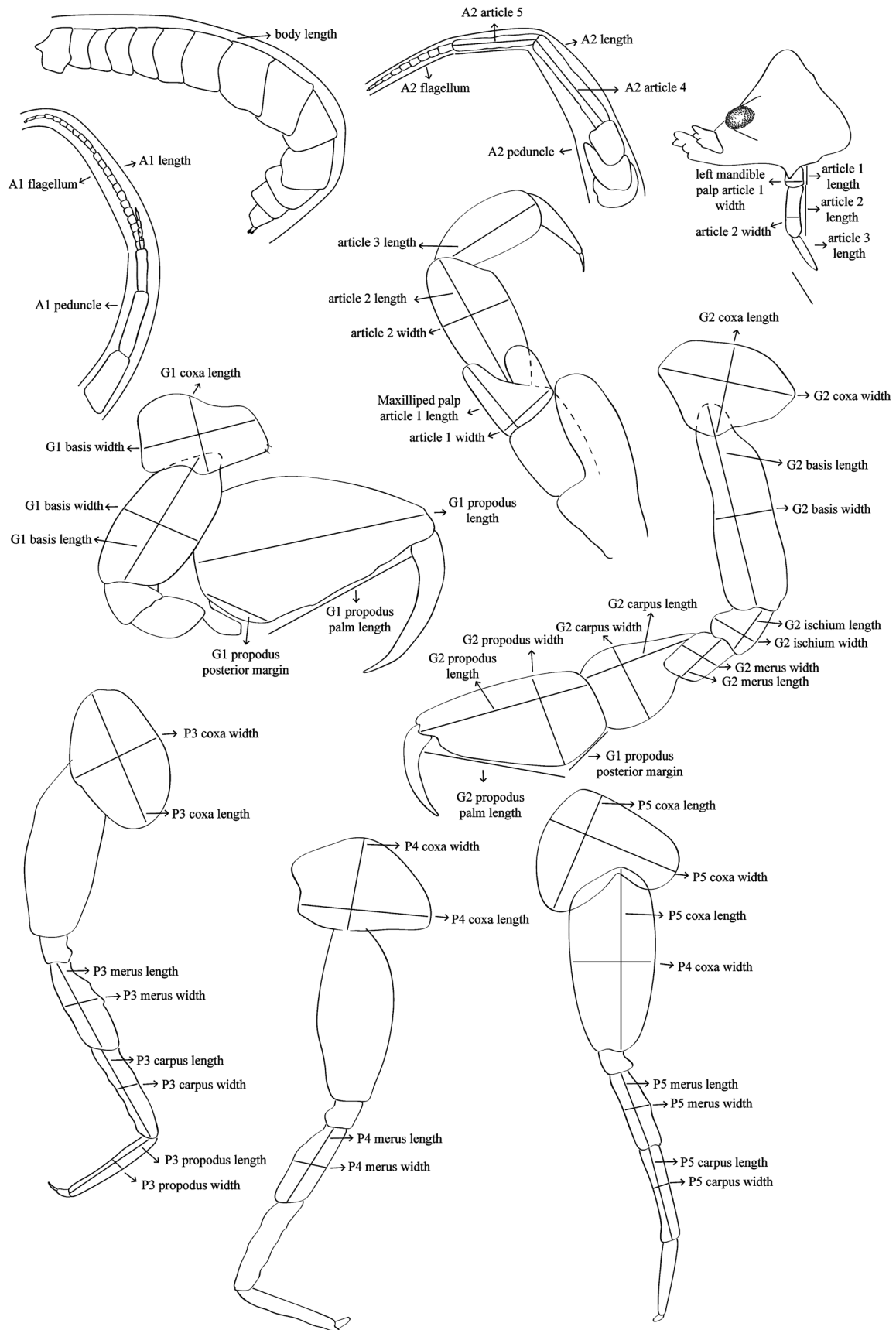


FIGURE 2. Morphometric characters measured to obtain the ratios used on Principal Component Analysis.

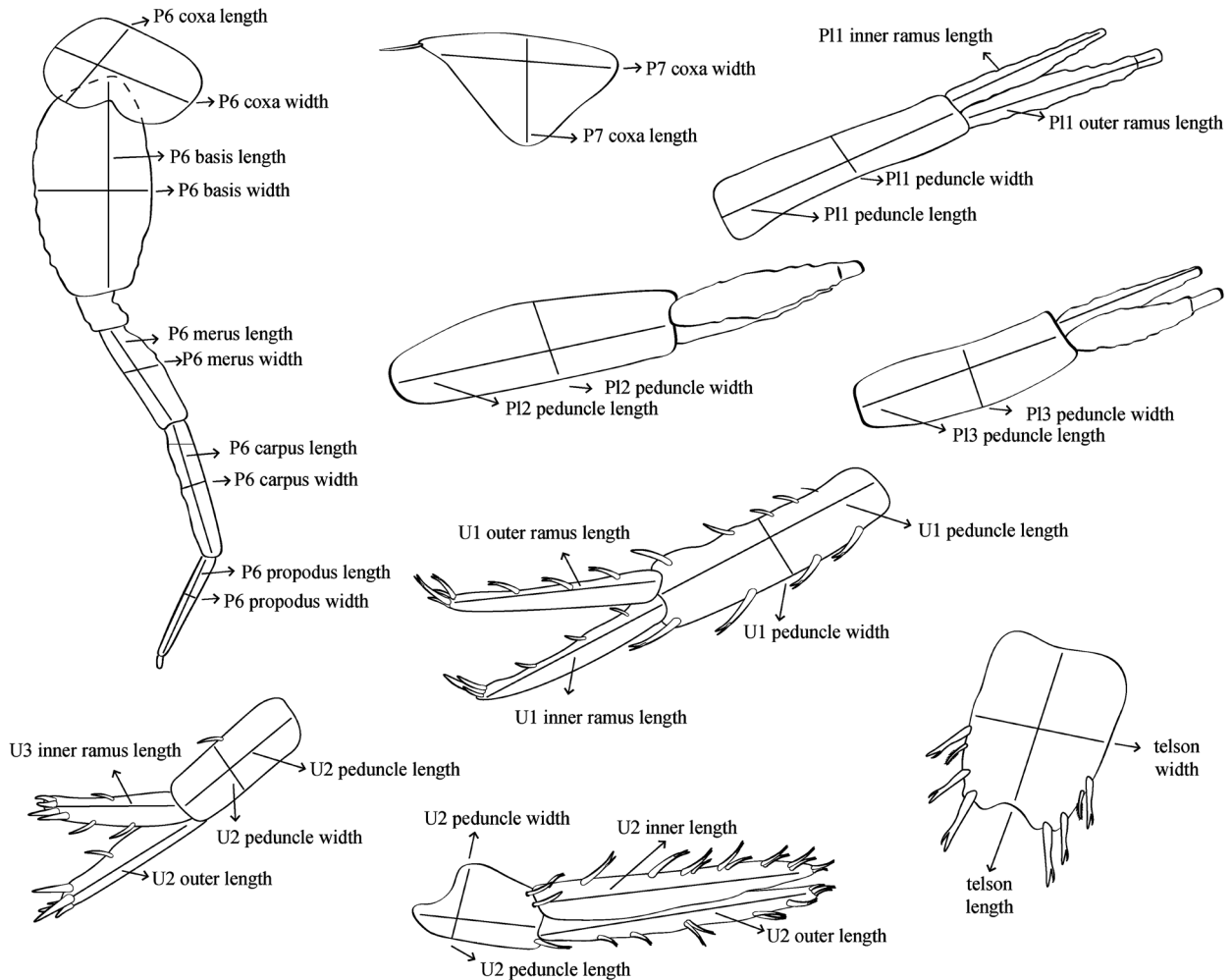


FIGURE 3. Morphometric characters measured to obtain the ratios used on Principal Component Analysis.

The following abbreviations were used on the figures: Hb, habitus; A1–2, antennae 1–2; Md, mandible; Mx1–2, maxillae 1–2; UL, upper lip; LL, lower lip; Mp, maxilliped; Gn1–2, gnathopods 1–2; P3–7, pereopods 3–7; P11–3, pleopods 1–3; U1–3, uropods 1–3; T, telson; R, right; L, left.

A Principal Component Analysis was conducted using the correlation matrix of morphometric variables of the six species of *Spelaeogammarus* to compare the interspecific morphological variations. This analysis takes several variables and combine them resulting on orthogonal (uncorrelated) components in which the variation is contained (Manly 1994). The measurements taken to calculate the morphometric ratios are illustrated on Figures 2 and 3.

Results

Order Amphipoda Latreille, 1816

Suborder Senticaudata Lowry & Myers, 2013

Superfamily Bogidielloidea Hertzog, 1936

Family Artesiidae Holsinger, 1980

Genus *Spelaeogammarus* Da Silva Brum, 1975

Spelaeogammarus sanctus sp. nov.

(Figs 4–8)

Etymology. The specific epithet *sanctus* refers to the place in which the new species was collected. The cave is inserted in a religious scenario situated in the municipality of Bom Jesus da Lapa in the Brazilian state of Bahia. Some people of the region say that the cave water is miraculous and thousands of people visit the cave every year looking for this water, which is collected from the species habitat, imposing some potential impacts on the species (see Habitat and Threats).

Material examined. Holotype male, 11.54 mm, July 2013, Gruta dos Milagres cave (13°15'31.45"S 43°25'5.76" W), municipality of Bom Jesus da Lapa, state of Bahia, Brazil, ISLA10106 (Fig. 3). Ten paratypes were dissected, from which 9 are males and 1 is female. Males mean body length: 12.01 mm (± 4.63), female body length: 12.8 mm. July 2013, Gruta dos Milagres cave (13°15'31.45" S 43°25'5.76" W), municipality of Bom Jesus da Lapa, state of Bahia, Brazil, ISLA10106, ISLA10107, ISLA10108, ISLA10109, ISLA10110, ISLA10111, ISLA10112, MNRJ25430, MNRJ25431, MNRJ25432. Other four paratypes were kept entire, which were collected in the same locality on the same date, ISLA10114, ISLA10210, ISLA10211, ISLA10212.



FIGURE 4. *Spelaeogammarus sanctus* sp. nov. Holotype, male 11.54 mm ISLA10106. Gruta dos Milagres Cave (13°15'31.45" S 43°25'5.76" W), municipality of Bom Jesus da Lapa, Bahia state, Brazil.

Diagnosis (Fig. 5–8). Antenna 1, accessory flagellum 5-articulate. Antenna 2, flagellum 7 to 10-articulate. Maxilla 1, outer lobe, apical margin with 7 multi-cuspidate stout setae. Maxilliped, inner plate, apical margin with 2 Y-shaped stout setae, 3 plumose setae, 2 of them slender setae, 1 submarginal small plumose setae; palp article 3, apical margin bearing a row of small, blunt and plumose nodular setae. Gnathopod 1, basis, anterior margin with 4 to 5 small setae, posterior margin with 15–17 long setae; propodus stout, about 1.3X longer than basis. Gnathopod 2, basis, posterior margin with 19–22 setae. Coxa 5 with 9 to 13 slender setae, posterior lobe round. Coxa 6 with 1 stout seta. Pleopods, inner ramus with 7 to 9 setae. Uropod 3, rami 2X longer than peduncle, outer ramus bearing more than 18 bifid setae. Telson bearing 1 apical and 2 subapical stout setae and 1 plumose seta in each lobe.

Description. Body slender. Head without eyes, slightly deeper than long, about as long as pereonites 1 and 2 combined; rostrum weakly produced, subacute, lateral cephalic lobe subacute, anteroventral corner subquadrate, without setae. Antenna 1, about 0.4X the body length, peduncle slightly shorter than flagellum, article 1 0.7 X as long as articles 2 and 3 combined, ratio of peduncular articles 1–3 = 1.8 : 1.4 : 1; flagellum with until 22 articles; accessory flagellum 5-articulate, article 5 reduced, total length of accessory flagellum as long as articles 1 to 5 of

flagellum combined. Antenna 2, about 0.9X the length of antenna 1, peduncle 2.6 X as long as flagellum, article 2 cone gland rising up to the distal margin of article 3, article 5 0.8X the length of article 4; flagellum 10-articulate.

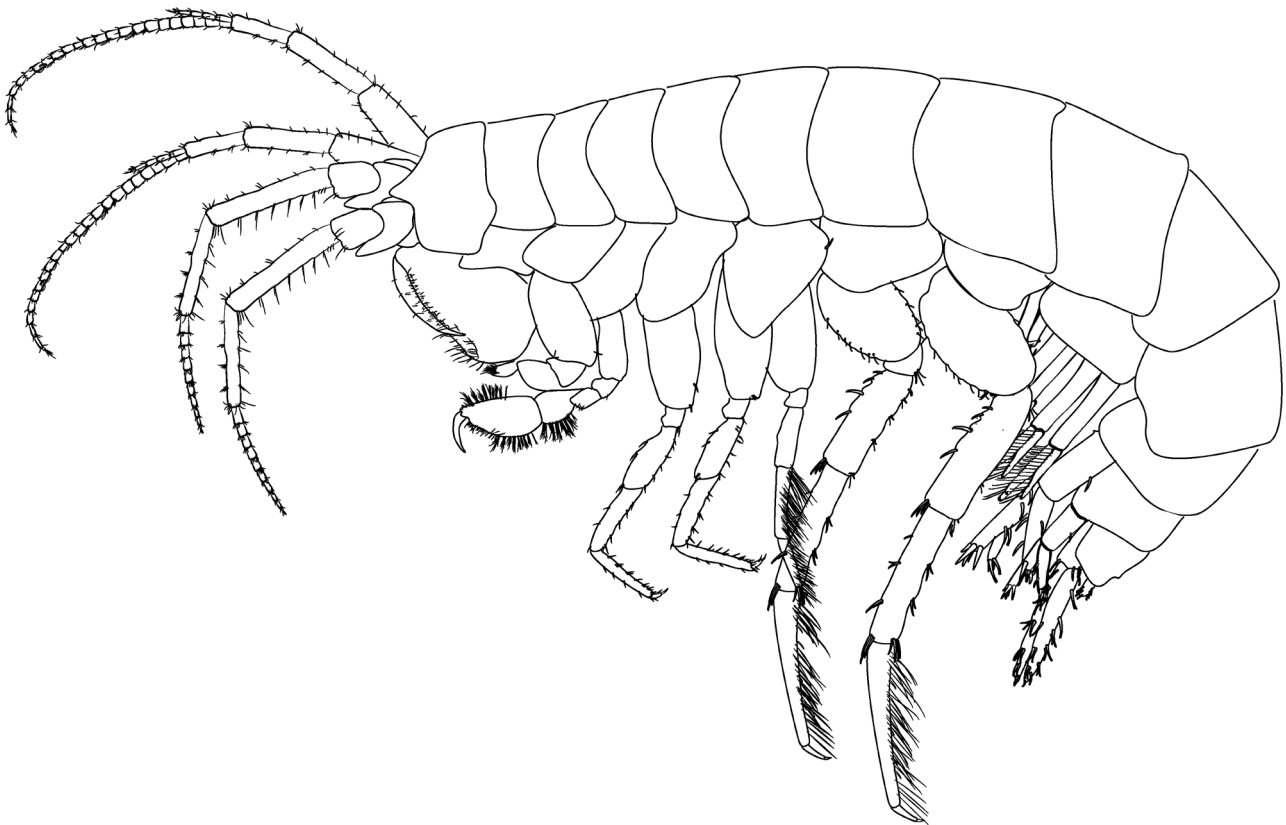


FIGURE 5. *Spelaeogammarus sanctus* sp. nov. habitus (body length 11.92 mm). Scale bar: 1 mm.

Upper lip rounded and smooth, with sparse setules apically. Lower lip, inner lobe apically rounded and setulose; outer lobe well developed, broadly rounded, inner distal margin setulose. Left mandible, molar broad, semi-triturative, subcircular; accessory setal row consisting of 4 curved plumose setae, one of them longer and stronger than the others; *lacinia mobilis* present, well developed, broad and apically multi-cuspidate; incisor multi-cuspidate; palp 3-articulate, article 1 about twice longer than wide, article 2 slightly robust, about 2.4 X longer than wide, subequal in size in relation to article 3, ventral margin bearing 5 slender setae, article 3 tapering distally, with 2 apical long setae. Right mandible subequal to left mandible, accessory setal row consisting of 4 curved plumose setae, 2 of them longer and stronger than the others. Maxilla 1, inner plate apically round, bearing 3 stout plumose setae on apical margin, about 0.8X the length of outer plate; outer plate subrectangular, bearing 7 multi-cuspidate setae on apical margin; palp 2-articulate, article 2 tapering distally, with a row of setae downwards from the apex to the inner margin. Maxilla 2, short, inner plate about 1.4 X wider than outer plate, apical margin bearing a fringe of plumose setae; outer plate slightly longer than inner plate, apical margin bearing a fringe of slender setae. Maxilliped, inner and outer plates short; inner plate suboval, apical margin with 2 Y-shaped stout setae, 3 plumose setae and 1 slender subapical plumose setae; outer plate slightly elongate, suboval, bearing 5 stout setae apically and 7 slender setae on the inner margin; palp 4-articulate, article 1 about 2.2X longer than wide, article 2, the largest, about 2.1X longer than wide and 1.1X longer than article 3; article 3 apical margin bearing a row of small, blunt and plumose nodular setae, article 4 tapering distally, inner margin setose, a small claw present.

Gnathopod 1, coxa subquadrate; basis stout, about 2.2X longer than wide, anterior margin with 4 to 5 small setae, posterior margin bearing 15 to 17 long setae; isquium subtriangular, distoventral corner with 1 slender seta; merus, ventral margin setose; carpus subtriangular, posterodistal corner produced, without comb-scales, posterodistal margin with a fringe of plumose setae; propodus stout, suboval, about 1.3X longer than basis, palm acute, about 2X longer than posterior margin, covered by a dense fringe of small setae, bearing 10 stout setae; dactylus long, curved, with three small simple setae dorsally, not reaching the palmar corner.

Gnathopod 2, basis subrectangular, about 3.0X longer than wide, posterior margin with 20 long slender setae; isquium slightly wider than long, posterior margin with 1 slender setae; merus subrectangular, about 2.9X longer than wide; carpus subtrapezoidal, about 1.5X longer than wide, posterior margin setose; propodus suboval, slightly elongate, about 1.9X longer than wide, anterior and posterior margins setose, palm acute, about 1.2X longer than posterior margin, covered by a dense fringe of small setae, bearing 7 stout setae, dactylus long, curved, naked, almost reaching the palmar corner.

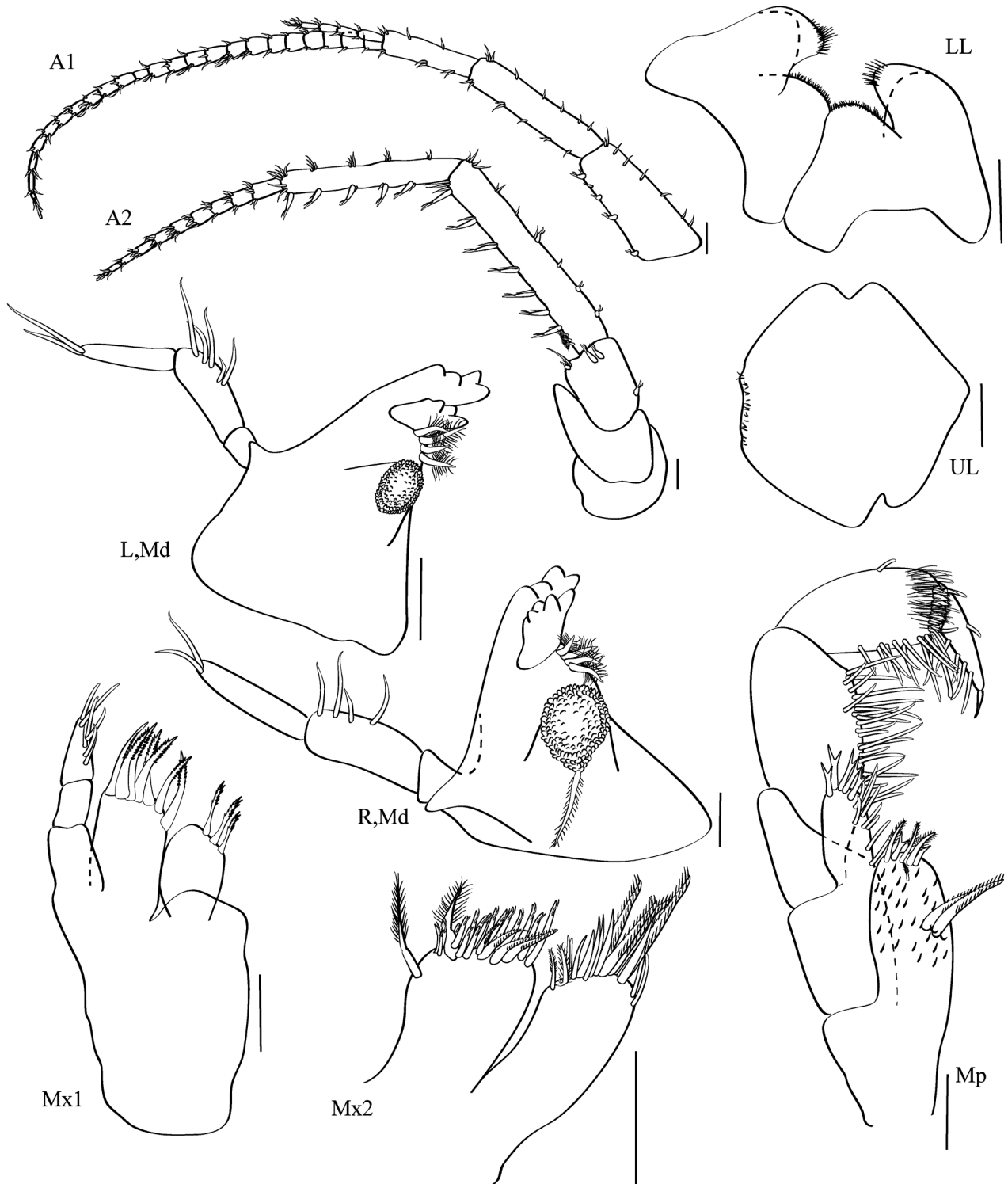


FIGURE 6. *Spelaeogammarus sanctus* sp. nov. (body length 11.92 mm). Scale bars: 200 μ m.

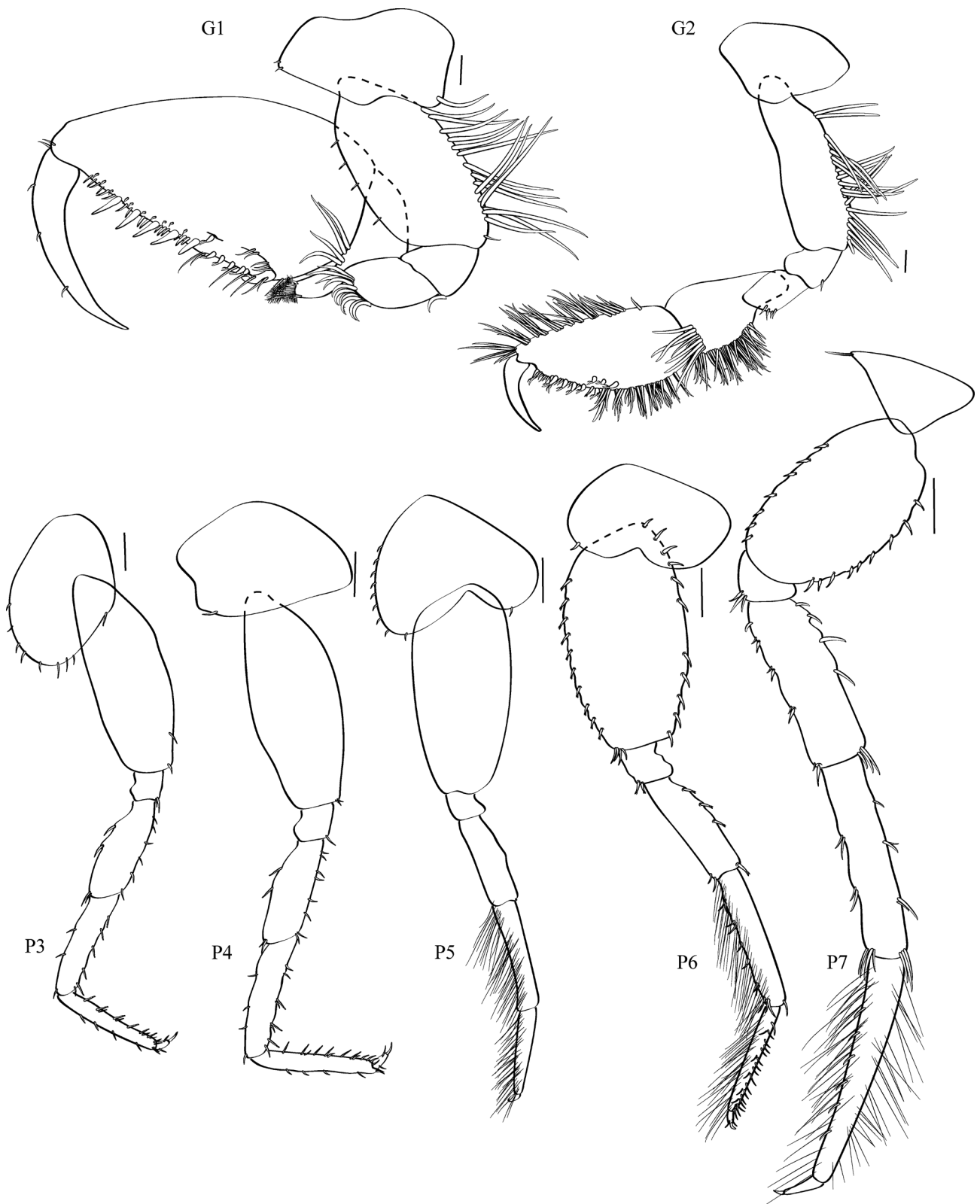


FIGURE 7. *Spelaeogammarus sanctus* sp. nov. (body length 11.92 mm). Scale bars: G1–G2 200 μ m; P3–P7 500 μ m.

Pereopod 3, coxa suboval, about 1.4X longer than wide, ventral margin with 8 setae; basis suboval, slightly elongate, anterior and posterior margins without setae, posterodistal corner with 2 small seta; merus, about 2.9X longer than wide, slightly shorter than carpus, anterior margin with 2 stout setae, anterodistal corner with 2 stout setae, posterior margin with 6 setae; carpus elongate, about 4.1X longer than wide, slightly longer than merus,

anterior margin with 2 stout setae, posterior margin with 5 stout setae; propodus elongate, about 7.1X longer than wide, slightly longer than carpus, anterior margin with 4 slender setae, posterior margin with bearing 7 lateral sets of stout setae (1-2-2-1-2-1-2); dactylus slightly curved, apical nail present. Pereopod 4 subequal in length to pereopod 3. Pereopod 5, coxa bilobate, anterior lobe well developed, about 1.1X wider than long, with 9–13 slender setae, posterior lobe small, posterior margin slightly concave, bearing 2 stout seta; basis suboval, about 2.1X longer than wide, anterior and posterior margins bearing 3 marginal stout setae and 2–3 distal stout setae; merus about 3.6X longer than wide, anterior margin with 5 slender setae, posterior margin with 2 stout setae; carpus slightly elongate, 7.3X longer than wide, about 1.4X longer than merus, anterior margin with a dense fringe of slender setae, anterodistal corner with two stout setae, posterior margin with 3 sparse slender setae; propodus slightly elongate, about 6.4X longer than wide, about 0.6X the carpus length, bearing 10 lateral sets of stout setae (2-2-2-2-1-1-1-1-1-2), anterior margin with a dense fringe of small slender setae, posterior margin naked; dactylus slightly curved, apical nail present. Pereopod 6 about 1.3X longer than pereopod 5, coxa bilobate, anterior lobe slightly more developed than posterior, anterior margin with 1 stout seta; basis suboval, about 1.8X longer than wide, anterior and posterior margins bearing several stout setae, merus slightly elongate, about 3.3X longer than wide, anterior margin bearing 3 stout setae, posterior margin naked; carpus slightly elongate, 5.5X longer than wide, about 1.2X longer than merus, anterior margin with a dense fringe of slender setae, distal setae longer than the proximal, anterodistal corner with 3 stout setae, posterior margin naked, posterodistal corner with 1 stout seta; propodus slightly elongate, about 9.5X longer than wide, about 0.8X the length of carpus, bearing 15 lateral sets of stout setae (1-2-1-1-1-2-1-1-2-1-2-2-1), anterior margin with a dense fringe of small slender setae, distal setae longer than the proximal, anterodistal corner with 1 stout seta, posterior margin naked; dactylus slightly curved, apical nail present. Pereopod 7 about 1.2X the length of pereopod 6, coxa subtriangular, about 1.4X wider than long, with 1 posterior stout seta; basis suboval, about 1.7X longer than wide, anterior and posterior margins with several stout setae; ischium slightly stout; merus about 2.8X longer than wide, anterior margin with 4 sets of stout setae (1-2-2-2), posterior margin with 4 stout setae, anterodistal corner with 2 stout setae, posterodistal corner with 3 stout setae; carpus about 4.7X longer than wide, about 1.1X longer than merus, anterior margin with 2 sets of 2 stout setae each, posterior margin with 3 sets of stout setae (3-2-1), anterodistal corner with 4 stout setae, posterodistal corner with 3 stout setae; propodus elongate, about 8.1X longer than wide, about 1.3X longer than carpus, anterior and posterior margins densely setose; dactylus slightly curved, apical nail present.

Pleopod 1, peduncle rectangular, about 6X longer than wide, a pair of hook setae present (probably a cuticular structure of unknown nature); inner ramus 1-articulate, bearing 11 plumose slender setae; outer ramus 3-articulate, subequal in length to inner ramus, article 1, inner margin with 9 plumose setae, outer margin with 18 plumose setae, article 2 with 2 plumose setae, article 3, smallest, with 2 apical plumose setae. Pleopod 2, peduncle rectangular, about 3.5 X longer than wide, a pair of hook setae present; inner ramus 1-articulate, bearing 12 plumose slender setae; outer ramus 3-articulate, slightly longer than inner ramus, article 1, inner margin with 9 plumose setae, outer margin with 16 plumose setae, article 2 with 2 plumose seta, article 3, smallest, with 2 apical plumose setae. Pleopod 3 peduncle rectangular, about 3.2X longer than wide, a pair of hook setae present; inner ramus 1-articulate, bearing 10 plumose slender setae; outer ramus 3-articulate, slightly longer than inner ramus, article 1, inner margin with 9 plumose setae, outer margin with 18 plumose setae, article 2 with 2 plumose seta, article 3, smallest, with 2 apical plumose setae.

Uropod 1, peduncle elongate, about 3.7X longer than wide, bearing 3 ventral stout setae, 4 dorsal stout setae and 2 distolateral stout seta; inner ramus slightly longer than outer ramus, both lanceolate; inner ramus about 0.8X the peduncle length, bearing 3 dorsal, and 4 apical setae; outer ramus bearing 3 dorsal and 3 apical setae. Uropod 2, peduncle about 2.4X longer than wide, bearing 1 dorsal and 2 distolateral stout setae; rami subequal in length, 1.2X longer than peduncle; inner ramus bearing 2 dorsal, 2 ventrodiscal, and 3 apical stout setae; outer ramus bearing 2 dorsal, 2 ventral, and 3 apical stout setae. Uropod 3 peduncle short, about 1.7X longer than wide, bearing 1 distolateral stout setae; rami elongate, lanceolate; inner ramus bearing 6 ventral sets stout setae (1-3-3-3-3-2), more than 18 dorsal stout setae and 3 apical stout setae; outer ramus subequal in length in relation to inner ramus, about 2.3X longer than peduncle, bearing 7 dorsal sets of stout setae (1-1-1-2-2-2-2), 4 ventrodiscal sets of stout setae (1-1-2-2), and 2 apical stout setae.

Telson 1.4X longer than wide, apical margin with shallow U-shaped excavation, bearing in each lobe bearing 1 apical and 2 subapical stout setae and 1 plumose seta in each lobe.

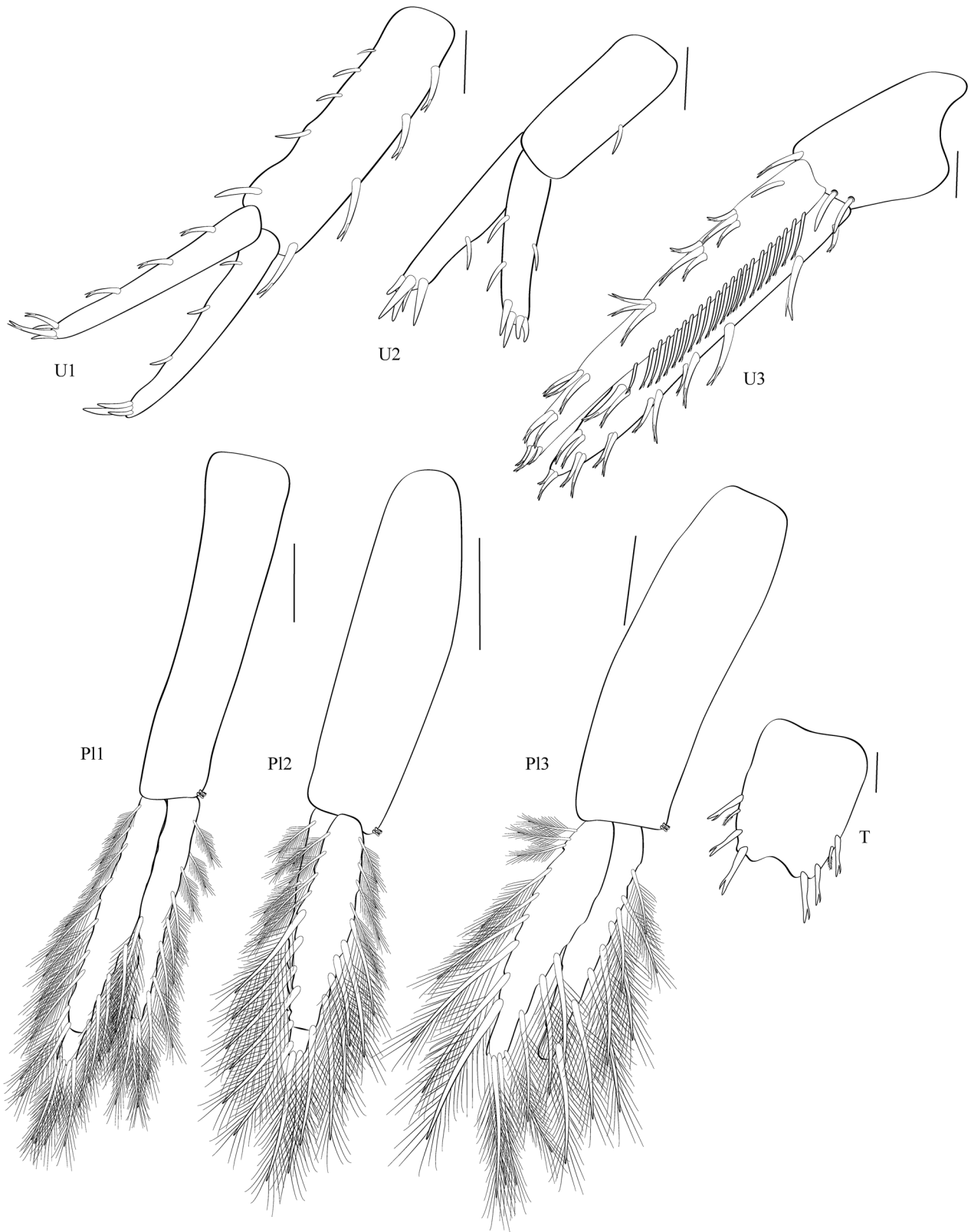


FIGURE 8. *Spelaeogammarus sanctus* sp. nov. Scale bars: U1, U2, U3, telson 200 μm ; P11–3 400 μm .

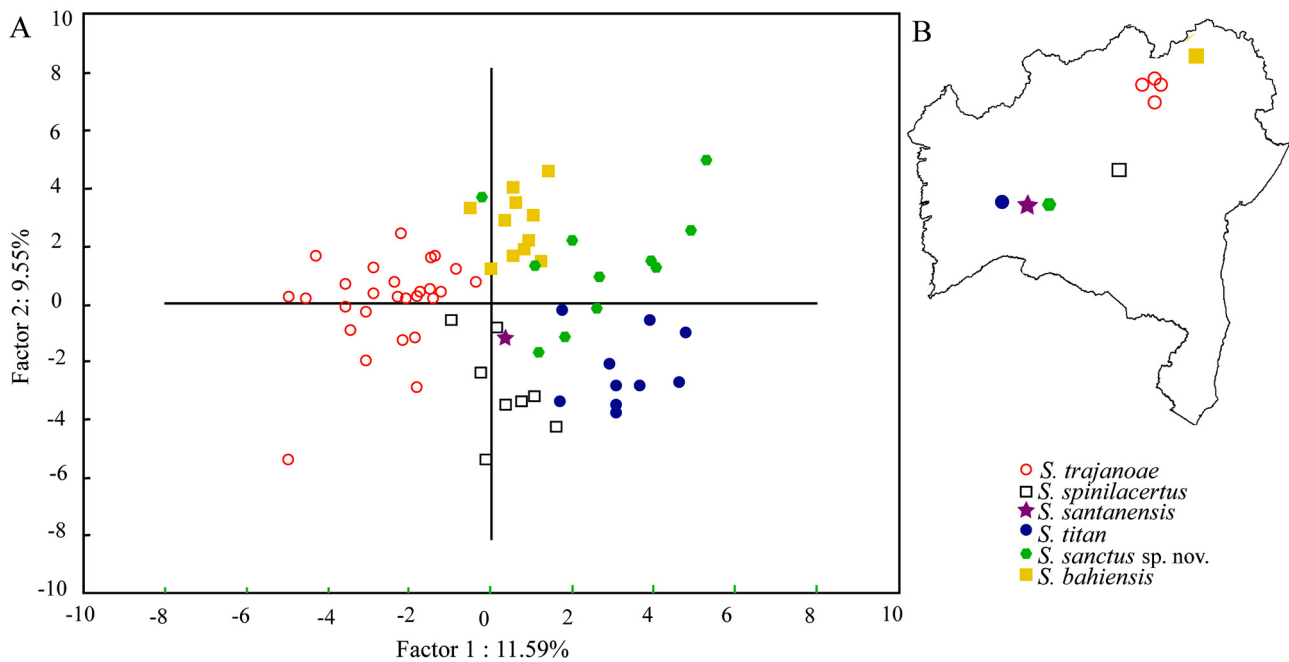


FIGURE 9. (A) Principal Component Analysis of morphometric ratios obtained from the six known *Spelaeogammarus* species; (B) geographical distribution of the genus. BA: Brazilian state of Bahia.

Identification key

1. Accessory flagellum of antenna 1 with 4 articles; flagellum of antenna 2 with 7 articles; propodus of gnathopod 1 slightly larger than basis. 2
- Accessory flagellum of antenna 1 with 5 or more articles, flagellum of antenna 2 with 8 or more articles, propodus of gnathopod 1 much larger than basis. 4
2. Coxal plate 6 bearing 1 spine, setae absent; outer plate of maxilla 1 with 6 multi-cuspidate setae and 1 plumose seta; inner plate of maxilliped with 4 plumose setae. 3
- Coxal plate 6 bearing 1 spine and about 20 setae; outer plate of maxilla 1 with 7 multi-cuspidate setae; inner plate of maxilliped without plumose setae *Spelaeogammarus bahiensis*
3. Anterior margin of gnathopod 1 basis with 5-9 short setae; coxal plate 5 bearing 1 stout setae and 17-18 short setae *Spelaeogammarus trajanoae*
- Anterior margin of gnathopod 1 basis with 2-4 long setae and 1 long seta; coxal plate 5 bearing 1 stout setae and about 9 short setae. *Spelaeogammarus spinilacertus*
4. Accessory flagellum of antenna 1 with 5 articles; coxa 5 with posterior lobe round. 5
- Accessory flagellum of antenna 1 with 6 articles; coxa 5 with posterior lobe slightly concave *Spelaeogammarus titan*
5. Posterior margin of gnathopod 1 basis with 15-17 simple setae; inner ramus of uropod 3 with more than 18 dorsal bifid setae *Spelaeogammarus sanctus sp. nov.*
- Posterior margin of gnathopod 1 basis with 20 simple setae; inner ramus of uropod 3 with 8 dorsal bifid setae *Spelaeogammarus santanensis*

Affinities. The new species is morphologically very similar to *S. santanensis* and *S. titan*. In relation to the number of articles of antenna 1 accessory flagellum, antenna 2 flagellum, number of setae on apical margin of maxilla 1 outer plate, as well as on apical margin of maxilliped inner plate and anterior margin of gnathopod 1 basis, the three species are identical. However, on the posterior margin of gnathopod 1 basis *S. sanctus* differs from them by presenting less setae (15 to 17), while 20 setae are observed on this structure of *S. santanensis* and *S. titan*. *Spelaeogammarus sanctus* and *S. santanensis* present a round posterior lobe on coxa 5 as their congeneric species, except *S. titan*, which presents a slightly concave posterior lobe on such coxa. Another difference observed in *S. sanctus sp. nov.* is the presence of more than 18 bifid setae on the dorsal margin of uropod 3 outer ramus, and its telson presents one apical and two subapical stout setae, while the other species generally present more setae both apically and subapically.

TABLE 1. Diagnostic characters of the species in *Spelaeogammarus* (modified from Senna *et al.* 2013)

Characters	<i>S. spinilacertus</i>	<i>S. trajancae</i>	<i>S. santanensis</i>	<i>S. bahiensis</i>	<i>S. titan</i>	<i>S. sanctus sp. nov.</i>
Accessory flagellum	4-articulate	4-articulate	5-articulate	4-articulate	6-articulate	5-articulate
Antenna 2, flagellum	7-articulate	7-articulate	8 to 10-articulate	7-articulate	10-articulate	7 to 10 articles
Maxilla 1, outer plate, apical margin	6 multi-cuspidate stout setae + 1 plumose seta 4 plumose setae	6 multi-cuspidate stout setae + 1 plumose seta 4 plumose setae	7 multi-cuspidate stout setae	7 multi-cuspidate stout setae	6 multi-cuspidate stout setae + 1 plumose seta 2 plumose setae	7 multi-cuspidate stout setae
Maxilliped, inner plate, apical margin	2-4 stout setae + 1 small seta	5-9 small setae	2 plumose setae	Without plumose setae	7 small setae	2 plumose setae
Gnathopod 1, basis, anterior margin	6-8 setae (some bifid)	9-10 simple setae	4 small setae	3-5 small setae	7 small setae	4-5 small setae
Gnathopod 1, basis, posterior margin	Slightly longer than basis	Slightly longer than basis	20 simple setae	7-9 simple setae	20 setae	15-17 simple setae
Gnathopod 1, propodus length	Slightly longer than basis	Slightly longer than basis	About 1.5X longer than basis	Slightly longer than basis	About 1.8X longer than basis	About 1.3X longer than basis
Gnathopod 2, basis, posterior margin 9	9-10 setae	8-9 setae	20-23 setae	9 setae	23 setae	19-22 setae
Coxa 5	1 stout seta + 9 setae	1 stout seta + 17-18 setae	1 stout seta + 12 slender setae	1 stout seta + 20-21 slender setae	1 stout seta + 14 slender setae	1 stout setae + 9-13 setae
Coxa 5, posterior lobe	Round	Round	Round	Round	Slightly concave	Round
Coxa 6	1 stout seta	1 stout seta	1 stout seta + 1 slender seta	1 stout seta + 20-21 slender setae	1 stout seta	1 stout seta
Pleopods, inner ramus	4-5 setae	5-7 setae	7-8 setae	7 setae	10-13 setae	7-9 setae
Uropod 3, outer ramus, dorsal margin	20 bifid setae	20 bifid setae	8 bifid setae	19 bifid setae	22 simple setae	More than 18 bifid setae
Telson, stout setae per lobe	2 apical + 3-4 subapical	3 apical + 2-3 subapical	1 apical + 3 subapical	2 apical + 3-4 subapical	1 apical + 3 subapical	1 apical + 2 subapical

Morphometric Analysis. The Principal Component Analysis revealed morphological variations among the *Spelaeogammarus* species, considering the whole of metrics (ratios) in the analysis (Fig. 9). The first two principal components (Factors 1 and 2) jointly explained 21.14% of the total variation among species, the first explaining 11.59% and the second 9.55% of the variation. Among the fifty eight morphological ratios used we highlight “telson length/width”, “pleopod 1 peduncle length/width” and “pereopod 3 carpus length/merus length”, which were the most correlated variables with Factor 1, and “pereopod 3 merus length/width”, “pereopod 4 merus length/width” and “pereopod 6 carpus length/width”, the most correlated with Factor 2. Furthermore, these variables consist of important measurements to be considered when intending to differentiate species. The correlation values of the remainder variables are presented on Appendix 1.

Habitat and Threats. The Gruta dos Milagres cave is located in a karst massif inserted in the middle of the urban center of Bom Jesus da Lapa Municipality (Figure 1A). This massif embraces a dozen of caves, from which some are used for religious purposes. In some cases, the cave was deeply altered, being transformed in a church, as the Gruta do Santuário de Bom Jesus cave (Figure 1D). This cave was discovered in 1691 and there are records of visitor pilgrims since the beginning of the 20th century. The Gruta dos Milagres cave has been periodically visited in the last centuries and pilgrims always remove water from the water table where specimens of *S. sanctus* live. Such removal is due to a belief that this water have miraculous properties. During our collections, visitors were collecting water from the single accessible pond (Figure 1B). Unfortunately during such practice several bottles and other materials (such as batteries, wires and plastic) are frequently left behind and then settle on the bottom of the pond (Figure 1C). Such materials, especially batteries and iron components, can release toxic elements and compounds on the water table, thus seriously threatening the amphipod population. Considering the lack of a management plan to control the use of this cave, the population of this species is critically endangered, since this cave is the only known habitat of this species. All other caves in the massif (and in surroundings) are not connected to the water table.

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APPENDIX 1. Correlation of all variables with Factor 1 and 2.

N. of variable	Morphometric ratios	Factor 1	Factor 2
1	A1/ body length	-0.215874	0.006774
2	A1 peduncle/A1 flagellum	0.258385	0.213822
3	A2 length/A1 length	0.312602	0.167703
4	A2 peduncle/A2 flagellum	-0.199164	-0.076155
5	A2 article 5/article 4	0.157392	0.181516
6	Left mandible article 1 of palp length/width	-0.201670	0.162861
7	Left mandible article 2 length/width	-0.125984	-0.035770
8	Left mandible article 2 length/article 3	-0.455357	0.151426
9	Maxilliped palp article 1 length/width	-0.267252	0.009940
10	Maxilliped article 2 length/width	-0.414566	-0.216864
11	Maxilliped article 2 length/article 3	-0.068873	-0.070033
12	G1 coxa length/width	-0.039494	-0.082386
13	G1 basis length/width	-0.459397	-0.115035
14	G1 basis length/propodus length	-0.378260	0.040781
15	G1 propodus palm length/posterior margin length	-0.140189	0.107185
16	G2 coxa length/width	-0.117940	-0.048098
17	G2 basis length/width	-0.459397	-0.115035
18	G2 ischium length/width	-0.356732	-0.024915
19	G2 merus length/width	-0.014296	-0.279512
20	G2 carpus length/width	-0.603472	-0.243839
21	G2 propodus length/width	-0.379658	-0.326849
22	G2 propodus palm length/posterior margin	0.066877	-0.066210
23	P3 coxa length/width	-0.525960	0.060424
24	P3 merus length/width	-0.255238	-0.794178
25	P3 carpus length/width	0.313938	-0.676215
26	P3 carpus length/merus length	0.628216	0.150726
27	P3 propodus length/width	-0.397444	-0.013130
28	P3 propodus length/carpus length	-0.611676	-0.044190
29	P4 coxa length/width	-0.628188	-0.104579
30	P4 merus length/width	-0.262338	-0.793165

.....continued on the next page

APPENDIX 1. (Continued)

N. of variable	Morphometric ratios	Factor 1	Factor 2
31	P5 coxa length/width	0.340769	0.230625
32	P5 basis length/width	0.400074	-0.573543
33	P5 merus length/width	0.043499	-0.674365
34	P5 carpus length/merus length	0.190482	-0.334928
35	P5 propodus length/width	-0.334584	-0.121814
36	P5 propodus length/carpus length	-0.004515	0.620667
37	P6 length/P5 length	0.468076	-0.064261
38	P6 coxa length/width	0.405275	-0.035481
39	P6 basis length/width	0.487885	-0.435836
40	P6 merus length/width	0.058634	-0.476051
41	P6 carpus length/width	0.153154	-0.750052
42	P6 carpus length/merus length	0.123376	-0.333333
43	P6 propodus length/width	-0.119422	-0.315524
44	P6 propodus length/carpus length	0.226526	0.246610
45	P7 coxa length/width	0.230501	-0.253827
46	Pleopod 1 peduncle length/width	0.630800	-0.446191
47	Pleopod 1 outer ramus length/inner ramus length	0.203995	-0.053465
48	Pleopod 2 peduncle length/width	0.271599	-0.203989
49	Pleopod 3 peduncle length/width	0.012081	0.110375
50	U1 peduncle length/width	0.152761	-0.181603
51	U1 inner ramus length/outer ramus length	-0.116476	0.073637
52	U1 inner ramus length/peduncle length	-0.010479	-0.071708
53	U2 peduncle length/width	0.340268	-0.172430
54	U2 inner ramus length/outer ramus length	-0.144443	-0.043734
55	U3 peduncle length/width	-0.270051	-0.302131
56	U3 inner ramus length/peduncle length	-0.438242	-0.262042
57	U3 inner ramus length/outer ramus length	-0.270051	-0.302131
58	Telson length/width	0.796003	-0.125100