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Spelaeogammarus uai (Bogidielloidea: Artesiidae): a new troglobitic amphipod from Brazil

RAFAELA BASTOS-PEREIRA[1] & RODRIGO L. FERREIRA[2]
Universidade Federal de Lavras (UFLA), Centro de Estudos em Biologia Subterrânea, Departamento de Biologia. Lavras, MG, Brasil. E-mail: [1]rafaelabastospereira@gmail.com; [2]drops@dbi.ufla.br

Abstract

A new species was recently found in a cave from Southeastern Brazil, in the state of Minas Gerais, which is here described. Considering all the species of the genus, *Spelaeogammarus uai* **sp. nov.** is very similar to *S. santanensis* and *S. sanctus*, although the new species differs mainly in relation to the number of plumose setae in the apical margin of maxilliped inner plate, number of setae in the anterior margin of gnathopod 1 basis and number of setae in the posterior margin of gnathopod 2 basis. Furthermore, the ratio "length/width" of several articles of the new species is higher than in other species. An identification key of the *Spelaeogammarus* genus is provided, as well as a complemented multivariate statistical approach of the morphometry of the genus based on a previous work of the genus.

Key words: Subterranean biodiversity, Amphipoda, taxonomy, Brazil

Introduction

Currently there are 26 formally described stygobitic invertebrate species from Brazil. The sub-phyllum Crustacea represents the richest group in this scenario, containing 80% of these species (Bond-Buckup&Buckup, 1994; Messana et al, 2002; Prevorènik et al, 2012; Rodrigues *et al.*, 2014; Bastos-Pereira & Ferreira, 2015). The order Amphipoda stands out among the Brazilian subterranean crustaceans represented by 15 described species belonging to five families (Hyalellidae, Seborgidae, Mesogammaridae, Mesogammaridae and Bogidiellidae). Recent studies have revealed not only new species, but higher taxa like genera and even new occurrences of families (Koenemann & Holsinger 2000; Cardoso *et al.* 2014; Fišer *et al.* 2013; Senna *et al.* 2014). Such discoveries had shed light on biogeographical enigmas regarding the relatively poor biodiversity of amphipods in South America when compared to other continents, besides reinforcing how incipient is our knowledge regarding the stygobitic biodiversity in Brazil. Fišer *et al*. 2013 have found that the number of amphipod families in South America is not as low as thought. They also suppose that amphipods of the family Dogielinotidae with the species-rich genus *Hyalella* (now allocated in the family Hyalellidae) have outcompeted and depleted the ancestral epigean amphipod fauna, what have resulted on the subterranean habit of all the other amphipod families currently found in South America.

Spelaeogammarus belongs to the family Artesiidae and comprise six exclusively subterranean species: *S. bahiensis* Da Silva Brum, 1975 (type-species), *S. spinilacertus* Koenemann & Holsinger, 2000; *S. trajanoae* Koenemann & Holsinger, 2000; *S. santanensis* Koenemann & Holsinger, 2000, *S. titan* Senna *et al.*, 2014 and *S. sanctus* Bastos-Pereira & Ferreira, 2015. Until the present all records of these species were made for caves in the Brazilian state of Bahia. However, a new species was recently found in the state of Minas Gerais, thus expanding the occurrence of the genus. This new species is here described, an identification key for *Spelaeogammarus* is provided, as well as a morphometric analysis including all the described species of this genus.

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Material and methods

The collection was made in the "Lapa D'Água do Zezé" cave, municipality of Itacarambi, Northern Minas Gerais, Brazil (15°01'07.45" S 44°12'10.86" W) (Fig. 1). Specimens were collected with the aid of a hand net, individualized and preserved in ethanol 70%. Some of them were deposited in the Collection of Subterranean Invertebrates of Federal University of Lavras and other in the National Museum of Rio de Janeiro. The paratypes consisted of ten individuals, which were dissected and mounted on slides with glycerol gelatin. The morphology of appendages as well as number, type and arrangement of setae were observed in the slides through an optic microscope Primo Star ZEISS coupled with camera. Drawings were prepared based on the photographs of one paratype. The holotype, the allotype and other four paratypes remained entire. The nomenclature of seta followed Watling (1989).

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; Pl1–3, pleopods 1–3; U1–3, uropods 1–3; T, telson; R, right; L, left.

As well as Bastos-Pereira & Ferreira (2015) have done, a Principal Component Analysis was conducted, now including the new species. The same morphometric variables considered by the previously mentioned authors were used for this analysis.

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 uai sp. nov.

(Figs. 2–5)

Etymology. Until the present, all species known for the genus *Spelaeogammarus* were found in the Brazilian state of Bahia. The current work describes the first species recorded for the state of Minas Gerais where "uai" is a common interjection used by people who lives in the state meaning doubt, astonishment or surprise.

Material examined. Holotype male, 11.4 mm, July 2014, Lapa D'Água do Zezé cave (15°01'07.45" S 44°12'10.86" W) municipality of Itacarambi, state of Minas Gerais, Brazil, ISLA14962 (Fig. 2). Eight paratypes were dissected, four males and four females. Males mean body length: 10.25 mm (± 1.81), mean female body length: 8.64 mm (± 3.7). January 2015, Lapa D'Água do Zezé cave (15°01'07.45" S 44°12'10.86" W) municipality of Itacarambi, state of Minas Gerais, Brazil, ISLA14963, ISLA14964, ISLA14965, ISLA14966, ISLA14967, ISLA14968, ISLA14969. Other six paratypes were kept entire, ISLA14970, ISLA14971, ISLA14972, ISLA14973, MNRJ26136, MNRJ26137. These last were collected in the same locality on the same date.

Diagnosis. Antenna 1 flagellum with 17–20 articles; accessory flagellum 5-articulate. Antenna 2 flagellum with 7–8-articles. Left mandible accessory setal row consisting of 6 curved plumose setae. Maxilla 1 outer lobe apical margin with 7 multi-cuspidate stout setae, palp article 2 with 4 slender apical setae. Maxilliped inner plate apical margin with 2 Y-shaped stout setae and 3 plumose setae, palp article 3, apical margin bearing a row of small, blunt and plumose nodular setae. Gnathopod 1 basis anterior margin with 6 small setae, posterior margin bearing 15 long setae, propodus palm acute, about 2.5 times longer than posterior margin. Gnathopod 2, basis subrectangular, about 3.8 times longer than wide, posterior margin with 18 long slender setae. Pereopod 5 propodus about 8.0 times longer than wide. Pleopods inner ramus with 9 setae. Pleopod 2 peduncle about 4.3 times longer than wide. Uropod 3 outer ramus bearing 16 dorsal bifid setae and 3 apical stout setae. Telson with 1 apical and 3 subapical stout setae.

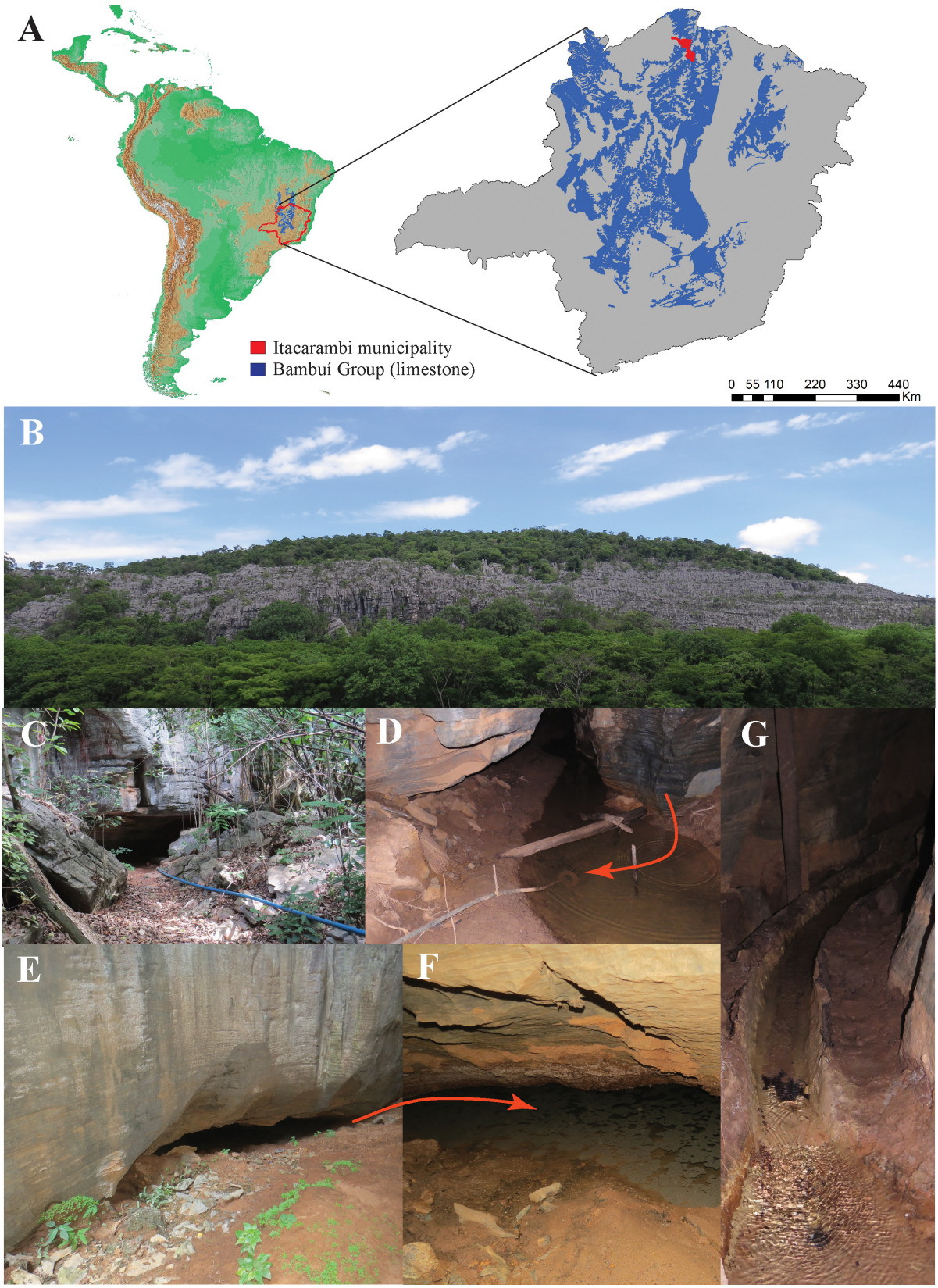

FIGURE 1. (A) Itacarambi municipality, state of Minas Gerais, Brazil and Lapa d'Água do Zezé cave; (B) External view of the outcrop in which the cave is inserted; (C) Cave entrance; (D) Ellectric pump installed inside the cave (indicated by the arrow); (E) Accessible part of the water table in a diaclasis close to a vertical entrance of the cave ; (F) The water covered by a thin layer of calcium carbonate is indicated by the red arrow; (G) Trunks installed inside the cave to prevent the water flow out of the water table.

FIGURE 2.*Spelaeogammarus uai* **sp. nov.** Holotype male, 10.25 mm, ISLA14962. Lapa D'Água do Zezé cave, municipality of Itacarambi, state of Minas Gerais, Brazil.

Description. Body slender. Head without eyes, slightly deeper than long, about as long as pereonites 1 and 2 combined; lateral cephalic lobe rounded, anteroventral corner subquadrate, without setae. Antenna 1, slightly less than half the body length, peduncle slightly longer than flagellum, article 1 0.7 times as long as articles 2 and 3 combined, ratio of peduncular articles 1–3 = 2 : 1.5 : 1; flagellum with 17–20 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 0.9 times the length of antenna 1, peduncle 3 times longer than flagellum, article 2 cone gland rising up to the distal margin of article 3, article 5 0.8 times the length of article 4; flagellum 7 or 8-articulate.

Upper lip rounded and smooth, with sparse setules apically. Lower lip, inner lobe apically rounded with few setules; outer lobe well developed, broadly rounded. Left mandible, molar broad, semi-triturative, subcircular; accessory setal row consisting of 6 curved plumose setae, 3 of them longer and stronger than the others; *lacinia mobilis* present, well developed, broad and apically 5-cuspidate; incisor multi-cuspidate; palp 3-articulate, article 1 about 1.5 longer than wide, article 2 slightly robust, about 2.8 times longer than wide, longer than article 3, ventral margin bearing 4 slender setae, article 3 tapering distally, with 5 slender 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; *lacinia mobilis* present, multi-cuspidate. Maxilla 1, inner plate apically round, bearing 3 slender setae on apical margin, about 0.7 times the length of outer plate; outer plate subrectangular, bearing 7 multi-cuspidate setae on apical margin; palp 2-articulate, article 2 tapering distally, with 4 slender apical setae. Maxilla 2, short, inner plate slightly wider than outer plate, apical margin bearing a fringe of long setae and 2 plumose setae; outer plate slightly longer than inner plate, apical margin bearing a fringe of slender setae, lateral margin with 2 shorter setae. Maxilliped, inner and outer plates short; inner plate suboval, apical margin with 2 Y-shaped stout setae, 3 plumose setae; outer plate longer than inner, suboval, bearing 9 stout setae apically; palp 4-articulate, article 1 about 1.8 times longer than wide, article 2, the largest, about 2.4 times longer than wide and 1.4 times longer than article 3, apical margin bearing a row of small, blunt and plumose nodular setae, article 4 tapering distally, inner margin setose, claw present distally.

Gnathopod 1, coxa subrectangular; basis stout, about 1.9 times longer than wide, anterior margin with 6 small

setae, posterior margin bearing 15 long setae; ischium 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 1.5 times longer than basis, palm acute, about 2.5 times longer than posterior margin, covered by a dense fringe of small setae, bearing 8 stout setae; dactylus long, curved, with 5 sets of 1-2-1-3-3 small simple setae dorsally, not reaching the palmar corner.

Gnathopod 2, basis subrectangular, about 3.8 times longer than wide, posterior margin with 18 long slender setae; ischium slightly wider than long, posterior margin with 1 slender setae; merus subrectangular, about 2.9 times longer than wide; carpus subtrapezoidal, about 1.3 times longer than wide, posterior margin setose; propodus suboval, slightly elongate, about 2.0 times longer than wide, anterior and posterior margins setose, palm acute, about 0.7 times shorter than posterior margin, covered by a dense fringe of small setae, bearing 6 stout setae, dactylus long, curved, naked, almost reaching the palmar corner.

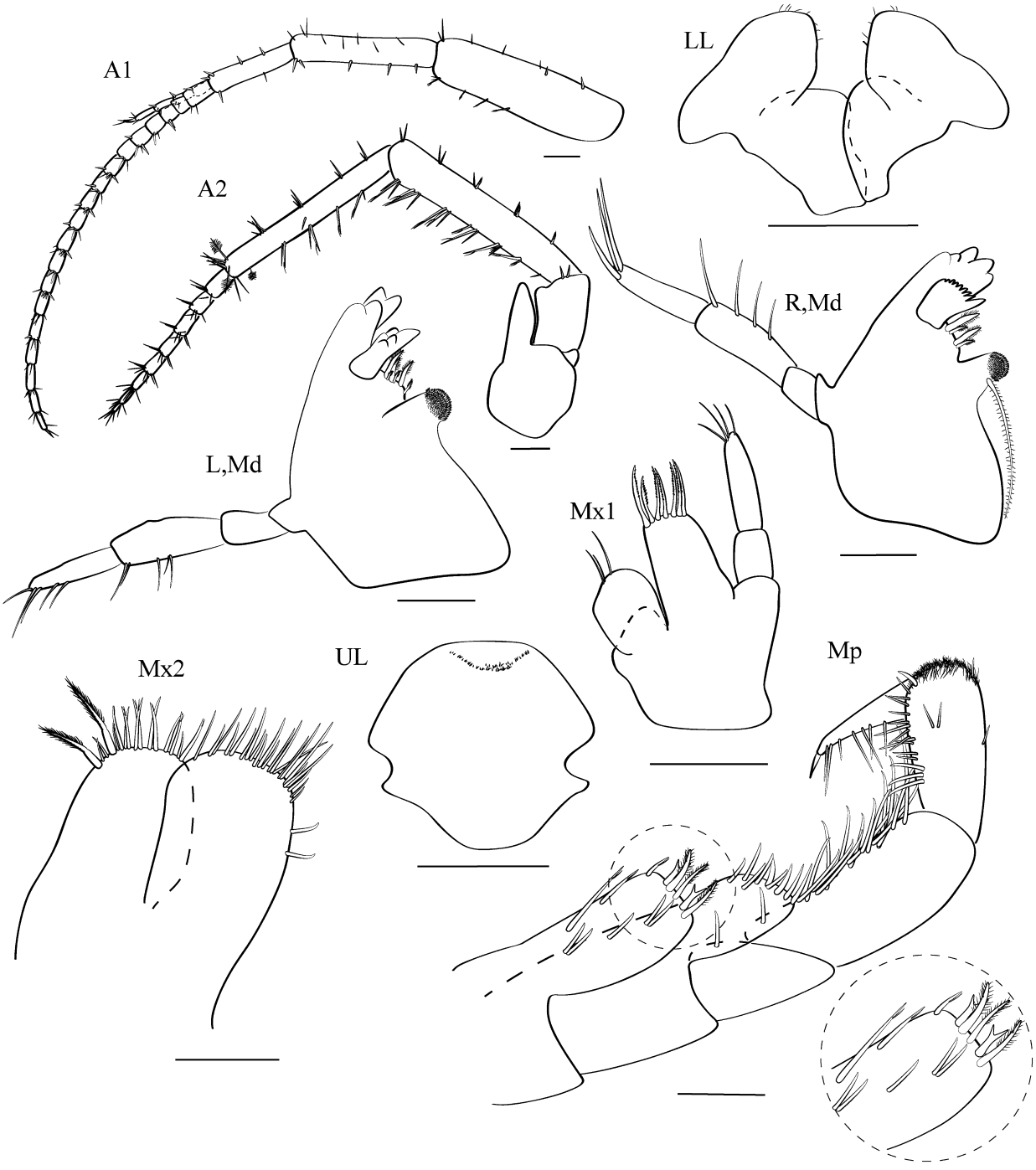

FIGURE 3. *Spelaeogammarus uai* **sp. nov.** Paratype male, 9.98 mm, ISLA14963. Scale bars: 200 µm.

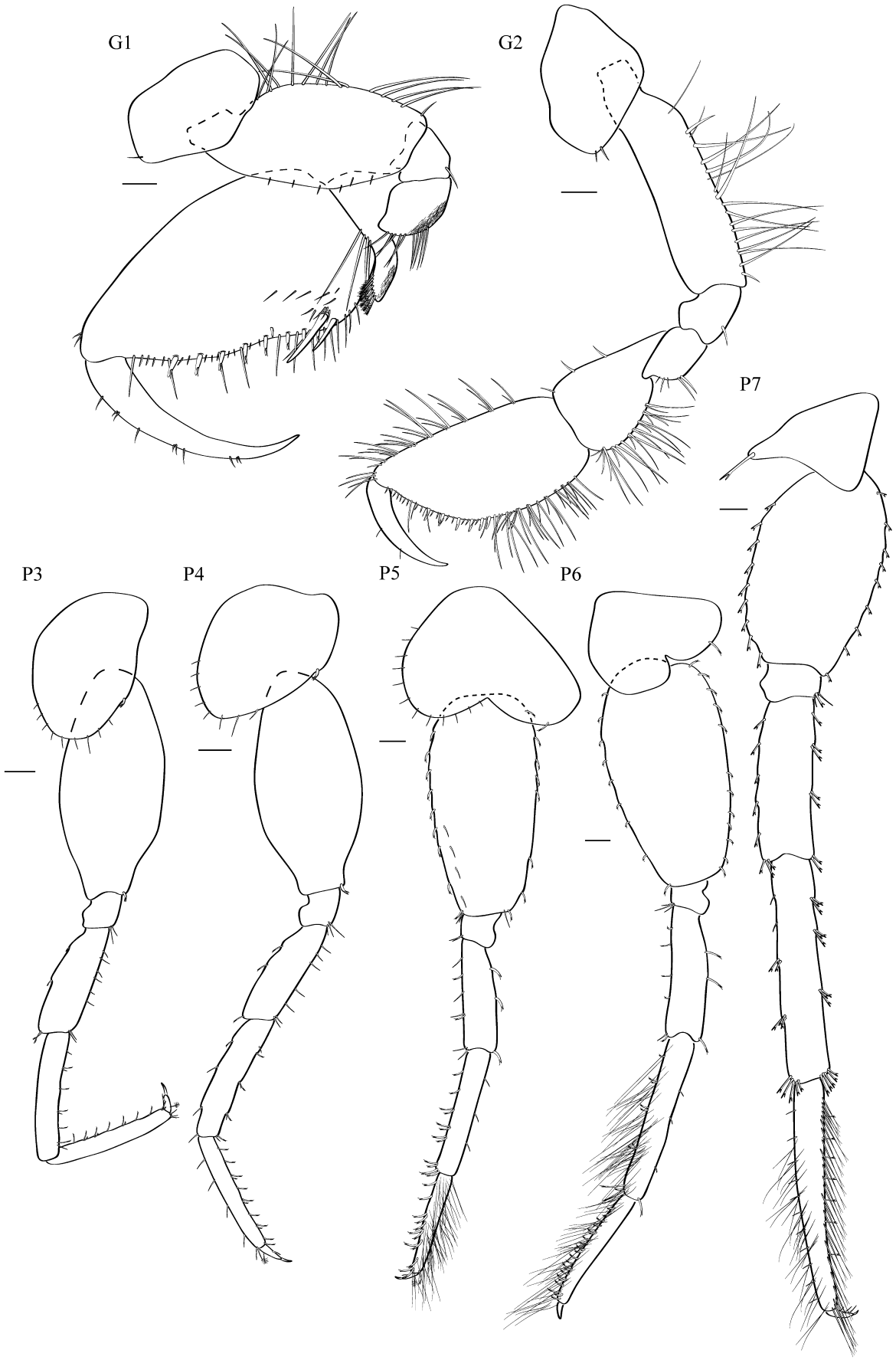

FIGURE 4. *Spelaeogammarus uai* **sp. nov.** Paratype male, 9.98 mm, ISLA14963. Scale bars: 200 µm.

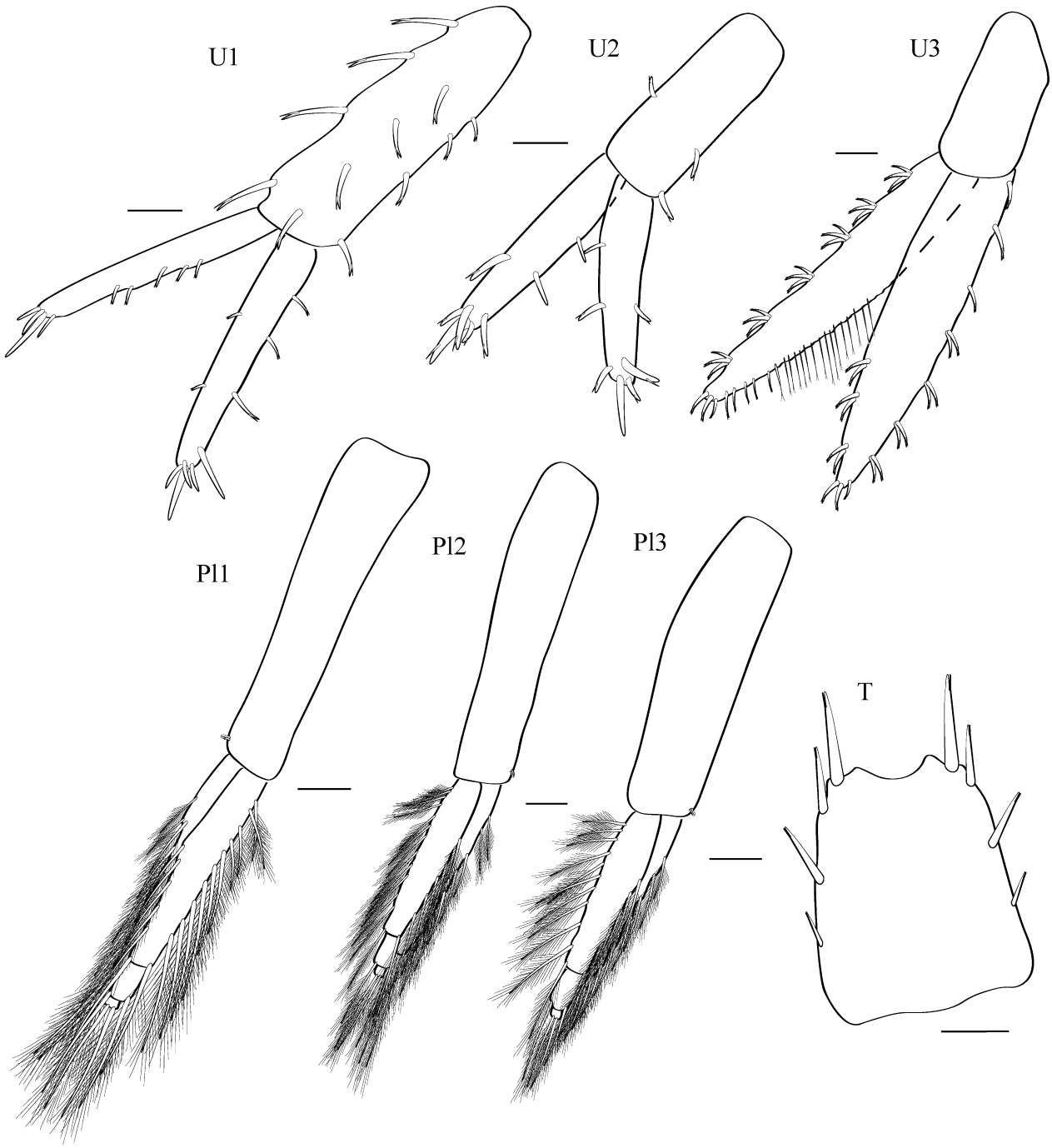

FIGURE 5. *Spelaeogammarus uai* **sp. nov.** Paratype male, 9.98 mm, ISLA14963. Scale bars: 200 μm.

Pereopod 3, coxa suboval, about 1.4 times longer than wide, ventral margin with 9 setae; basis suboval, elongate, anterior and posterior margins without setae, posterodistal corner with 1 small seta; merus, about 3.1 times 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 5 times longer than wide, slightly longer than merus, anterior margin without setae, posterior margin with 6 sets of 1-2-1-2-1-2 setae; propodus elongate, about 7.2 times longer than wide, slightly shorter than carpus, anterior margin without setae, posterior margin with bearing 8 lateral sets of (1-2-2-1-2-1-1-2) setae; dactylus slightly curved with 1 small plumose setae on dorsal margin, apical nail present. Pereopod 4 subequal in length to pereopod 3. Pereopod 5, coxa bilobate, anterior lobe well developed, about 1.3 times wider than long, with 12 slender setae, posterior lobe small, posterior margin slightly concave, bearing 1 stout seta; basis suboval, about 2.0 times longer than wide, anterior margin bearing 8

stout setae and 3 distal stout setae, anterior margin with 7 marginal stout setae 2 distal stout setae; merus about 3.1 times longer than wide, anterior margin with 4 sets of 1-1-1-2 slender setae, posterior margin with 2 stout setae; carpus slightly elongate, 6.0 times longer than wide, about 1.2 times longer than merus, anterior margin with 6 sets of 1-1-2-2-2-2 stout setae, anterodistal corner with 2 stout setae, posterior margin with 3 sparse slender setae; propodus slightly elongate, about 8.0 times longer than wide, about 1.5 times the carpus length, bearing 8 lateral sets of stout setae (1-1-2-1-2-2-1-2), anterior margin with a dense fringe of small slender setae; dactylus slightly curved, apical nail present. Pereopod 6 slightly shorter than pereopod 5, coxa bilobate, anterior lobe slightly more developed than posterior, posterior margin with 1 stout seta; basis suboval, about 1.9 times longer than wide, anterior margin bearing 8 stout setae, posterior margin with 12 stout setae, merus slightly elongate, about 3.4 times longer than wide, anterior margin bearing 4 stout setae and 2 on the anterodistal corner, posterior margin bearing 2 stout setae and 1 stout setae on posterodistal corner; carpus slightly elongate, 5.6 times longer than wide, about 1.2 times longer than merus, anterior margin with a dense fringe of slender setae and 4 sets of 1-2-2-2 stout setae, anterodistal corner with 3 stout setae, posterior margin with 3 setae, posterodistal corner with 1 stout seta; propodus slightly elongate, about 8.5 times longer than wide, about 0.8X the length of carpus, bearing 15 lateral sets of stout setae (1-1-1-1-2-1-1-2-1-1-2-1-2-1-2), anterior margin with a dense fringe of small slender setae, anterodistal corner with 1 stout seta, posterior margin naked; dactylus slightly curved, apical nail present. Pereopod 7 about 1.3 times the length of pereopod 6, coxa subtriangular, about 0.6 times wider than long, with 1 posterior stout seta; basis suboval, about 1.6 times longer than wide, anterior and posterior margins with several stout setae; ischium slightly stout; merus about 2. 8 times longer than wide, anterior margin with 5 stout setae, anterodistal corner with 3 stout setar, posterior margin 3 sets of 1-2-2 stout setae, posterodistal corner with 2 stout setae; carpus about 5.8 times longer than wide, about 1.4 times longer than merus, anterior margin with 4 sets of 1-1-3-3 setae, anterodistal corner with 5 stout setae, posterior margin with 3 sets of 3-3-3 stout setae, posterodistal corner with 5 stout setae; propodus elongate, about 7.9 times longer than wide, about 1.3 times longer than carpus, anterior and posterior margins densely setose; dactylus slightly curved, apical nail present.

Pleopod 1, peduncle rectangular, about 5.0 times longer than wide, a pair of hook setae present (probably a cuticular structure of unknown nature); inner ramus 1-articulate, bearing 9 plumose slender setae; outer ramus 3-articulate, subequal in length to inner ramus, article 1, inner margin with 6 plumose setae, outer margin with 11 plumose setae, article 2 with 2 plumose setae, article 3, smallest, with 2 apical plumose setae. Pleopod 2, peduncle rectangular, about 4.3 times longer than wide, a pair of hook setae present; inner ramus 1-articulate, bearing 9 plumose slender setae; outer ramus 3-articulate, slightly longer than inner ramus, article 1, inner margin with 7 plumose setae, outer margin with 13 plumose setae, article 2 with 2 plumose seta, article 3, smallest, with 2 apical plumose setae. Pleopod 3 peduncle rectangular, about 3.4 times longer than wide, a pair of hook setae present; inner ramus 1-articulate, bearing 9 plumose slender setae; outer ramus 3-articulate, slightly longer than inner ramus, article 1, inner margin with 6 plumose setae, outer margin with 11 plumose setae, article 2 with 2 plumose seta, article 3, smallest, with 2 apical plumose setae.

Uropod 1, peduncle elongate, about 3.4 times longer than wide, bearing 4 ventral stout setae, 4 dorsal stout setae and 4 lateral stout seta; inner ramus slightly shorter than outer ramus, both lanceolate; inner ramus about 0.8 times the peduncle length, bearing 3 dorsal, and 5 apical setae; outer ramus bearing 5 dorsal and 4 apical setae. Uropod 2, peduncle about 2.5 times longer than wide, bearing 1 dorsal and 2 distolateral stout setae; rami subequal in length, 1.2times longer than peduncle; inner ramus bearing 2 dorsal, 1 ventral and 4 apical stout setae; outer ramus bearing 1 dorsal, 2 ventral, and 5 apical stout setae. Uropod 3 peduncle short, about 1.9 times longer than wide, without setae; rami elongate, lanceolate; outer ramus bearing 6 ventral sets stout setae (3-3-3-3-3-3), 21 dorsal bifid setae and 3 apical stout setae, 2.3 times longer than peduncle; inner ramus subequal in length to outer ramus, bearing 7 dorsal sets of stout setae (1-1-1-2-2-2-2), 3 ventrodistal sets of 2-2-2 stout setae and 3 apical stout setae.

Telson slightly longer than wide, apical margin with shallow U-shaped excavation, bearing in each lobe 1 apical stout seta and 3 subapical stout setae.

Identification key for the genus *Spelaeogammarus*

1. Accessory flagellum of antenna 1 with 4 articles 2
- Accessory flagellum of antenna 1 with 5–6 articles 4

2. Maxilla 1 outer plate apical margin with 6 multi-cuspidate stout setae and 1 plumose setae 3
- Maxilla 1 outer plate apical margin with 7 multi-cuspidate stout setae ..... *Spelaeogammarus bahiensis*
3. Gnathopod 1 basis anterior margin with 2–4 stout setae and 1 small seta ..... *Spelaeogammarus spinilacertus*
- Gnathopod 1 basis anterior margin with 5–9 small setae ..... *Spelaeogammarus trajanoae*
4. Accessory flagellum of antenna 1 with 5articles 5
- Accessory flagellum of antenna 1 with 6articles ..... *Spelaeogammarus titan*
5. Uropod 3 outer ramus dorsal margin with more than 15 bifid setae 6
- Uropod 3 outer ramus dorsal margin with less than 10 bifid setae ..... *Spelaeogammarus santanensis*
6. Telson with 1 apical and 2 subapical stout setae per lobe ..... *Spelaeogammarus sanctus*
- Telson with 1 apical and 3 subapical stout setae per lobe ..... *Spelaeogammarus uai* **sp. nov.**

Affinities. Regarding the taxonomy of *Spelaeogammarus,* there is not a single characteristic proper of each species, but a set of morphological aspects that combined allows identifying the species of this genus (Table 1). As well as *S. santanensis* and *S. sanctus*, the here described *S. uai* **sp. nov.** presents 5-articulate accessory flagellum of antenna 1 and other characteristics in common with these two previously mentioned species (e. g. 7 to 10 articles in the flagellum of antenna 2 and 7 multi-cuspidate stout setae in the apical margin of maxilla 1 outer plate). The main differences of *S. uai* **sp. nov.** in relation to *S. santanensis* and *S. sanctus* is the presence of 3 plumose setae in the apical margin of maxilliped inner plate, the number of setae in the anterior margin of gnathopod 1 (*S. santanensis*: 4; *S. sanctus:* 4–5; *S. uai* **sp. nov.**: 6) and the number of setae in the posterior margin of gnathopod 2 basis (*S. santanensis*: 20–23; *S. sanctus:* 19–22; *S. uai* **sp. nov.**: 18). While *S. santanensis* has 20 simple setae in the posterior margin of gnathopod 1 basis, *S. sanctus* has 15 to 17 and *S. uai* **sp. nov.** has 14 to 16. And in relation to the stout setae per lobe of telson, while *S. santanensis* and *S. uai* **sp. nov.** have 1 apical and 3 sub-apical, *S. sanctus* has 1 apical and 2 sub-apical setae.

Morphometric Analysis. Considering the same metrics (ratios) than Bastos-Pereira and Ferreira (2015), the principal component analysis (Factors 1 and 2 jointly) explained 21.15% of the total variation among species, the first explaining 11.6% and the second 9.55% (Fig. 6). The variables most correlated to Factor 1 were "P4 coxa length/width", "P3 propodus length/carpus length" and "P3 coxa length/width", while "P3 merus length/width", "P4 merus length/width" and "P6 carpus length/width" were the most correlated metrics with Factor 2.

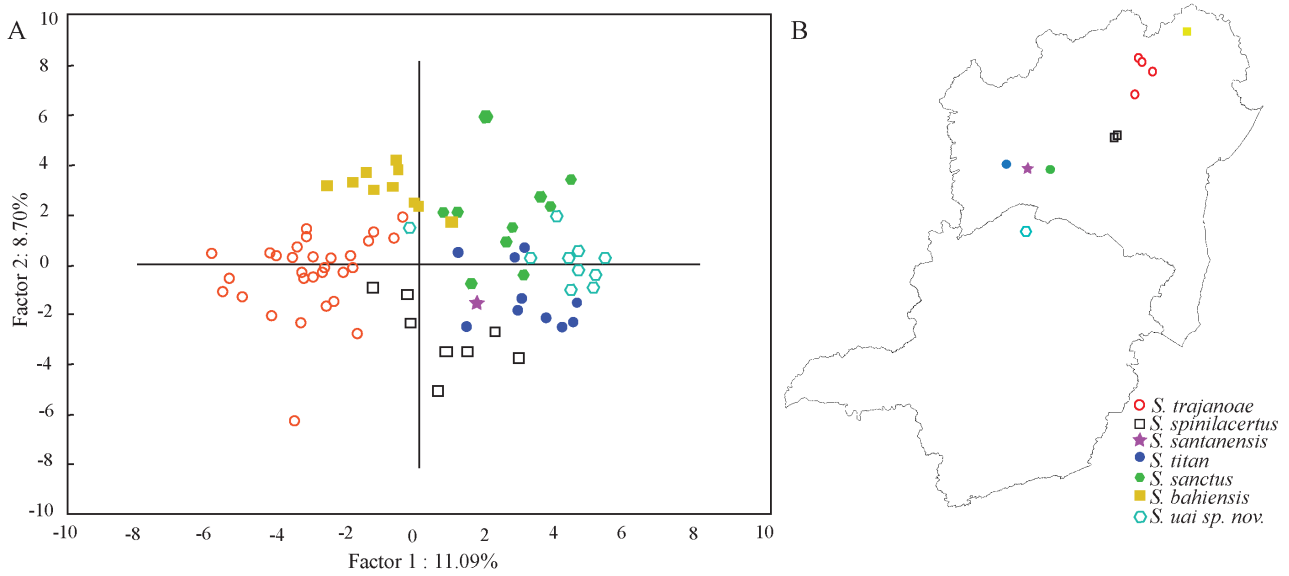

FIGURE 6. (A) Principal Component Analysis of morphometric ratios obtained from the seven known *Spelaeogammarus* species; (B) geographical distribution of the genus in the state of Bahia (BA) and Minas Gerais (MG).

Habitat and threats. Lapa D'Água do Zezé cave comprises the only known habitat of *S. uai* **sp. nov.** Although other caves with water bodies have been inventoried in the surroundings, no amphipods were found. However, it is important to highlight that most of the caves with perennial water in the area possess streams, instead of being connected to the water table, as the case of Lapa D'Água do Zezé cave. At least one other cave connected to the water table (Lapa do Saco Velho cave) was visited, and no specimens were observed.

TABLE 1. Morphological characters distinguishing the*Spelaeogammarus* species (modified from Bastos-Pereira & Ferreira, 2015).

Characters	*S. spinilacertus*	*S. trajanoae*	*S. santanensis*	*S. bahiensis*	*S. titan*	*S. sanctus*	*S. uai* **sp. nov.**
Accessory flagellum	4-articulate	4-articulate	5-articulate	4-articulate	6-articulate	5-articulate	5-articulate
Antenna 2, flagellum	7-articulate	7-articulate	8 to 10-articulate	7-articulate	10-articulate	7 to 10 articles	7 to 10 articles
Maxilla 1, outer plate, apical margin	6 multi-cuspidate stout setae + 1 plumose seta	6 multi-cuspidate stout setae + 1 plumose seta	7 multi-cuspidate stouts etae	7 multi-cuspidate stout setae	6 multi-cuspidate stout setae + 1 plumose seta	7 multi-cuspidate stout setae	7 multi-cuspidate stout setae
Maxilliped, inner plate, apical margin	4 plumose setae	4 plumose setae	2 plumose setae	Without plumose setae	2 plumose setae	2 plumose setae	3 plumose setae
Gnathopod 1, basis, anterior margin	2–4 stout setae + 1 small seta	5–9 small setae	4 small setae	3–5 small setae	7 small setae	4–5 small setae	6 small setae
Gnathopod 1, basis, posterior margin	6–8 setae (some bifid)	9–10 simplesetae	20 simplesetae	7–9 simplesetae	20 setae	15–17 simple setae	14–16 simple setae
Gnathopod 1, propodus length	Slightly longer than basis	Slightly longer than basis	About 1.5 times longer than basis	Slightly longer than basis	About 1.8 times longer than basis	About 1.3 times longer than basis	About 1.5 times longer than basis
Gnathopod 2, basis, posterior margin	9–10 setae	8–9 setae	20–23 setae	9 setae	23 setae	19–22 setae	18setae
Coxa 5	1 stout seta + 9 slender setae	1 stout seta + 17– 18 slender 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 slender setae	1 stout setae + 12 slender setae
Coxa 5, posterior lobe	Round	Round	Round	Round	Slightly concave	Round	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	1 stout seta
Pleopods, inner ramus	4–5 setae	5–7 setae	7–8 setae	7 setae	10–13 setae	7–9 setae	9 setae
Uropod 3, outer ramus, dorsal margin	20 bifid setae	20 bifid setae	8 bifid setae	19 bifid setae	22 simple setae	18-25 bifid setae	21 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	1 apical + 3 subapical

Lapa D'Água do Zezé cave is inserted in a huge limestone outcrop belonging to the Bambuí formation (Neoproterozoic limestone) with a well-preserved deciduous forest on the top and surroundings (Figure 1B). The area is located in the transition of two phytogeographic types: Cerrado (Brazilian savannah) and Caatinga (Velloso, 2002). In this transition several sub-types of vegetation in different degrees of conservation can be distinguished. The cave is labyrinthic with one horizontal entrance (main entrance—Figure 1C) and at least two vertical entrances. The farmers from the area installed an electric pump inside the cave (near the main entrance—Figure 1D) to drag water for consumption in the houses and irrigation of crops. The cave has two distinct areas (although both are interconnected) regarding the presence of water: the first one is the only accessible part of the water table, corresponding to a narrow passage in the base of a diaclasis, close to a vertical entrance of the cave, then it is located in a photic/disphotic area (Figure 1E). A thin layer of calcium carbonate often covers the accessible part of the water table, preventing the direct observation of the water column (Figure 1F) and for the collection, this layer had to be displaced. Most of the specimens observed in the cave were collected in this area, although two specimens were also found in another pond, without any visible contact with other watercourses inside the cave. This pond may have originated from a flooding event, and such specimens were probably trapped on it. The second area with water in the cave comprises a very small drainage, which is formed by the overflow of the water table. This drainage trespasses part of the cave, which is altered by human activities. The farmers frequently remove the deposited sediment that could prevent the water to flow out of the water table, and some trunks were installed to favor the flow (Figure 1G). In this area, dozens of troglobitic isopods (Styloniscidae) from two distinct species were found (both species are under description), but no amphipods were observed. Probably they avoid such area due to the water flow. Although this small stream is frequently altered by human activities, it seems that this does not represent a huge impact to the cave communities, since most part of the populations is certainly associated to inaccessible areas in the water table.

The physico-chemical parameter of the water where the specimens were collected was measured during one of the visits to the cave (January 2015): dissolved oxygen 3.46 mg/L, temperature 25.35 ºC, pH 8.45, electrical conductivity 0.565 µS/cm, total dissolved solids 0.359 g/L.

Regarding the species *Spelaeogammarus sanctus*, during one of our visits to the type locality, we also measured the hydrochemistry of the lake where specimens were collected (Gruta dos Milagres cave, municipality of Bom Jesus da Lapa, Bahia state), and those data were not presented in the original description. Accordingly, we also add such data of *S. sanctus* habitat here, which were collected in March 2014: dissolved oxygen 2.03 mg/L, temperature 26.46 ºC, pH 6.82, electrical conductivity 0.904 µS/cm, total dissolved solids 0.578 g/L.

Discussion

Morphometric analysis. Despite adding the metrics related to *S. uai* **sp nov.** the explanation provided by each factor was very similar to those found by the previously mentioned authors (Bastos-Pereira & Ferreira, 2015 found 21.14% of explanation for Factors 1 and 2, while in the present work 21.15% of explanation was found). However, differences were observed when comparing the most correlated variables with Factors 1 and 2 of Bastos-Pereira and Ferreira (2015) and the current work. Bastos-Pereira and Ferreira (2015) found that the variables most correlated to Factor 1 were ratios taken from telson, pleopod 1 and P3, while ratios from P3-6 were the most correlated variables to Factor 2. On the other hand, the addition of *S. uai* **sp nov.** to the analysis provided slightly different results, in which for both Factors 1 and 2 the most correlated variables were ratios mainly taken from P3 and P4 (Factor 1: P4 coxa length/width, P3 propodus length/carpus length and P3 coxa length/width; Factor 2: P3 merus length/width, P4 merus length/width and P6 carpus length/width)

Since in general only subtle morphological differences are observed among the *Spelaeogammarus* species, these differences on the results of PCA reinforce that different sets of characters are peculiar to each species, what leads to changes on the most correlated variables to each factor as new species are added to the analysis. However it is important to highlight that ratios taken from pereopods (mainly 3 and 4) were recurrent among the most correlated variables distinguishing species in the genus.

Considering Factor 1, greater ratios (length x width) were found especially for *S. sanctus, S. santanensis, S. titan* and *S. uai* **sp. nov.**, what means that in these species the articles of P3 and P4 are longer than wide. The elongation of appendages is one of the most commonly observed troglomorphisms (Culver & Pipan, 2009), and

although all *Spelaeogammarus* species known until the present are clearly troglobitic, the group formed by these four previously mentioned species seems to present more elongated pereopods.

It is also important to highlight that the morphological proximity of species in the PCA graph coincides with their geographical proximity, what may suggest and eventually support the existence of at least two divergent lineages (one group formed by for *S. sanctus, S. santanensis, S. titan* and *S. uai* **sp nov**., with occurrence on Southwestern Bahia and Northern Minas Gerais, and other formed by *S. trajanoae*, *S. spinilacertus* and *S. bahiensis* inhabiting caves on Northeastern Bahia), which may have been separated by the main channel of São Francisco River. This reinforces the need to analyze the phylogenetic relationships of this group for more accurate conclusions.

Habitat and threats. Although the cave fauna in Brazil has been accessed for decades, most studies were more focused in specific groups (e.g. fishes), what have led to a scenario of huge ignorance regarding the Brazilian cave fauna as a whole. Only in the last few years, more robust and systematic inventories started to be made, including different areas in the country, what have revealed hundreds of new troglobitic species (e. g. Rodrigues et al, 2014; Iniesta & Ferreira, 2015; Souza et al, 2015; Hoch & Ferreira, 2016; Shear et al, 2016; Souza & Ferreira, 2016; Vasconcelos & Ferreira, 2016a, 2016b). However, this fauna is endangered especially due to anthropogenic activities, which are threatening their habitats. After 2008 Brazilian caves, which used to be totally protected, started to be target of irreversible impacts, including activities leading to the total destruction of the caves.This occurred due to some changes in the Brazilian speleological legislation in 2008. After those changes, to ensure the complete conservation of a cave in Brazil, it is necessary, from a biological point of view, to attest the occurrence of at least one endemic troglobitic species. Therefore, the description of *S. uai*, besides contributing to the knowledge of this genus in Brazil, ensures the protection of the cave in which the species inhabits and the surrounding landscape.

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