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ORIGINAL ARTICLE

A new species of the troglobitic genus *Spelaeogammarus* da Silva Brum, 1975 (Amphipoda: Artesiidae) from a cave in the Brazilian semi-arid region, with new records of its congener, *Spelaeogammarus spinilacertus* Koenemann and Holsinger, 2000

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ABSTRACT

We propose a new species of the troglobitic genus *Spelaeogammarus* da Silva Brum, 1975, with data on its distribution, ecology, aspects of behavior, and conservation status. *Spelaeogammarus ginae* sp. nov. occurs in a single cave in the Serra do Ramalho karst area, of the northeastern Brazilian semi-arid region. We also provide new data on the distribution and conservation status of its congener, *Spelaeogammarus spinilacertus* Koenemann and Holsinger, 2000 that occurs in the upper phreatic aquifer inside caves from Chapada Diamantina domain. Both species occur in caves in the state of Bahia, northeastern Brazil.

KEYWORDS

Amphipoda, conservation, state of Bahia, subterranean, taxonomy

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Introduction

Amphipods (Malacostraca) are macroscopic crustaceans that inhabit mainly marine water, but also occur in different freshwater habitats (Väinölä *et al.*, 2008). In Neotropical regions, 156 species, from 47 genera in 20 families of freshwater, or slightly brackish water, amphipods are recognized from inland waters (Väinölä *et al.*, 2008; Lowry and Myers, 2013; Rogers *et al.*, 2020). Moreover, the family diversity is highest in subterranean continental waters (Rogers *et al.*, 2020). In Brazil, to date, there are troglobitic amphipods species from five families: Mesogammaridae, Seborgiidae, Bogidiellidae, Hyalellidae, and Artesiidae (Gallão and Bichuette, 2018; Zepon *et al.*, 2021).

The family Artesiidae is composed of hypogean freshwater amphipods of the genus *Artesia* Holsinger, 1980, which has a distribution restricted to the southern USA, with two valid species (*Artesia subterranea* Holsinger, 1980 and *Artesia welbourni* Holsinger, 1992), and *Spelaeogammarus* da Silva Brum, 1975, which is restricted to Brazil (Lowry and Myers, 2013). *Spelaeogammarus* is considered a troglobitic genus, since all valid species are restricted to the subterranean environment, occurring only in phreatic aquifers. There are seven valid species in the genus: *Spelaeogammarus bahiensis* da Silva Brum, 1975, *Spelaeogammarus santanensis* Koenemann and Holsinger, 2000, *Spelaeogammarus trajanoae* Koenemann and Holsinger, 2000, *Spelaeogammarus spinilacertus* Koenemann and Holsinger, 2000, *Spelaeogammarus titan* Senna, Andrade, Castelo-Branco and Ferreira, 2014, *Spelaeogammarus uai* Bastos-Pereira and Ferreira, 2017, and *Spelaeogammarus sanctus* Bastos-Pereira and Ferreira, 2015 (see Horton *et al.*, 2021).

The state of Bahia in northeastern Brazil holds most of the species richness in the genus: *S. bahiensis* from Patamute cave (municipality of Curaçá) (da Silva Brum, 1975; Koenemann and Holsinger, 2000), *S. spinilacertus* from Baixa do Salitre and Jaburu caves (municipality of Iraquara) (Koenemann and Holsinger, 2000), *S. trajanoae* from Toca do Pitu, Buraco do Teodoro, Toca do Gonçalo, and Convento caves (municipality of Campo Formoso) (Koenemann and Holsinger, 2000), *S. titan* from PEA-445 cave (municipality of Santa Maria da Vitória) (Senna *et al.*, 2014), and *S. sanctus* from Gruta dos Milagres cave (municipality of Bom Jesus da Lapa) (Bastos-Pereira and Ferreira, 2015). *Spelaeogammarus uai* is the only species that occurs in the state of Minas Gerais, from a single cave, Lapa D'Água do Zezé (municipality of Itacarambi) (Bastos-Pereira and Ferreira, 2017). All these species show classic troglomorphism, the morphology typical of subterranean animals (*sensu* Christiansen, 1962), mainly the absence of eyes and absence of body pigmentation.

Recently, in an expedition to the Serra do Ramalho karst area, in the state of Bahia, specimens of *Spelaeogammarus*, proposed here as a new species, were found in a pool of the upper phreatic aquifer inside the Gruna da Serra Verde cave. Moreover, data about its taxonomy, aspects of ecology, behavior, and conservation are also considered. We also provide new data about the distribution, habitat characteristics and conservation status of its congener, *S. spinilacertus*, which is also a phreatic species. The conservation status of both species is evaluated following IUCN criteria (IUCN, 2012).

Materials and Methods

Specimens of *Spelaeogammarus ginae* sp. nov. were collected in a small pool inside the Gruna da Serra Verde cave, located at Serra do Ramalho region, in the state of Bahia, northeastern Brazil. Specimens were collected using fishing nets of 0.5 mm mesh, and later preserved in 70 % alcohol. In the laboratory, the head and body length of 10 randomly selected specimens were measured under a Stemi 508 stereoscope microscope with an ocular microscale attached.

For slide preparation, specimens were stained with "Red Congo" dye for 24h, dissected under the stereomicroscope, and mounted on slides. The appendages were then photographed using an AxioCam ERc5s camera coupled to a Carl Zeiss Primo Star microscope. The photographs were used as a basis for the drawings of the structures in CorelDraw X7 software. Images of preserved specimens of *Spelaeogammarus ginae* sp. nov. were produced using Celestron MicroCapture Pro. The morphological description follows Rogers *et al.* (2020). Diagnosis and description of structures are based on

three dissected males (paratypes) and any observed variation is indicated. Observations of female are based on one dissected specimen (paratype). Type material is deposited in the Crustacea Collection, Museu Nacional do Rio de Janeiro - MNRJcarcino (C.S. Serejo, curator) (one whole individual, not dissected), Zoological Collection of the Laboratório de Estudos Subterrâneos, Universidade Federal de São Carlos - LES (M.E. Bichuette, curator) (four whole individuals, not dissected), and Coleção de Crustáceos, Universidade Federal de Lavras – CCUFLA (A.A.P. Bueno, curator) (three whole individuals, not dissected, and four individuals mounted on slides).

For *S. spinilacertus*, distribution data from the literature (Koenemann and Holsinger, 2000) and new data collected during research conducted by the Laboratório de Estudos Subterrâneos of the Universidade Federal de São Carlos (LES/UFSCar) since 2004 were used. The identification of species followed the original description of *S. spinilacertus* (see Koenemann and Holsinger, 2000) and the key to *Spelaeogammarus* species (Rogers *et al.*, 2020). Images of preserved specimens of *S. spinilacertus* were taken with a Leica DFC 295 video camera attached to a Leica M205C microscope with a Planapo 1.0 × objective. Figures were produced from stacks of images using LAS (Leica Application Suite) v3.7.

We provide data about the physical and chemical variables (pH and temperature for *Spelaegammarus* sp. nov. and pH, temperature, conductivity, and salinity for *S. spinilacertus*), and the description of the microhabitat (area, depth, substrate type on the bottom, water current) for both species. For *Spelaegammarus ginae* sp. nov. individual counts, a visual census method (to calculate abundance and population density) was used, and observations of behavior through the *ad libitum* method (Altmann, 1974) were also considered.

We propose the categories of threat for both species using the IUCN criteria (IUCN, 2012), since *S. spinilacertus* is not yet included in the Brazilian Red List. To recognize the minimal extent of occurrence (IUCN, 2012) of *S. spinilacertus*, we drew a polygon encompassing the coordinates of the caves using Google Earth Pro version 7.3.4.

Maps of distribution of *Spelaeogammarus ginae* sp. nov. and of *S. spinilacertus* were produced using QGIS version 2.18 (QGIS, 2017) and information about land use and vegetation were based on MapBiomas products (MapBiomas Project, 2021).

Systematics

Order Amphipoda Latreille, 1816

Suborder Senticaudata Lowry and Myers, 2013

Superfamily Bogidielloidea Hertzog, 1936

Family Artesiidae Holsinger and Longley, 1980

Genus *Spelaeogammarus* da Silva Brum, 1975

***Spelaeogammarus ginae* sp. nov.**
Bueno and Penoni
(Figs. 1–4)
Zoobank: urn:lsid:zoobank.org:act:EE1300D7-465B-4E05-BD58-A06F26A407A1

Etymology. The epithet *ginae* is a tribute to Dr. Georgina Bond-Buckup, a devoted carcinologist that dedicated her life to the study of crustaceans.

Type material. Holotype: male, body length 12.24 mm, head length 0.84 mm, MNRJcarcino 030206, Brazil, state of Bahia, Gruna da Serra Verde cave, municipality of Coribe, (44°19'26.8"S 13°43'28.0"W), 05 Jul. 2021, M.E. Bichuette, J.S. Gallo, V.F. Sperandei, J.E. Gallão colls. Paratypes (same collection data as holotype): 4 whole individuals, LES 00027746; 3 whole individuals, CCUFLA 447; 3 males and 1 female on slides, CCUFLA 448.

Diagnosis. Antenna 1 less than half body length, flagellum with 18–20 articles, accessory flagellum 5-articulated. Antenna 2 subequal to antenna 1, flagellum with 8–10 articles. Maxilla 1 outer plate bearing 7 multicuspidate setae and article 2 of palp with 6–7 long, simple setae. Pleopods 1–3 inner rami with 9–10, 10–11, and 10 long plumose setae on distal margin, respectively; uropod 2 outer ramus outer

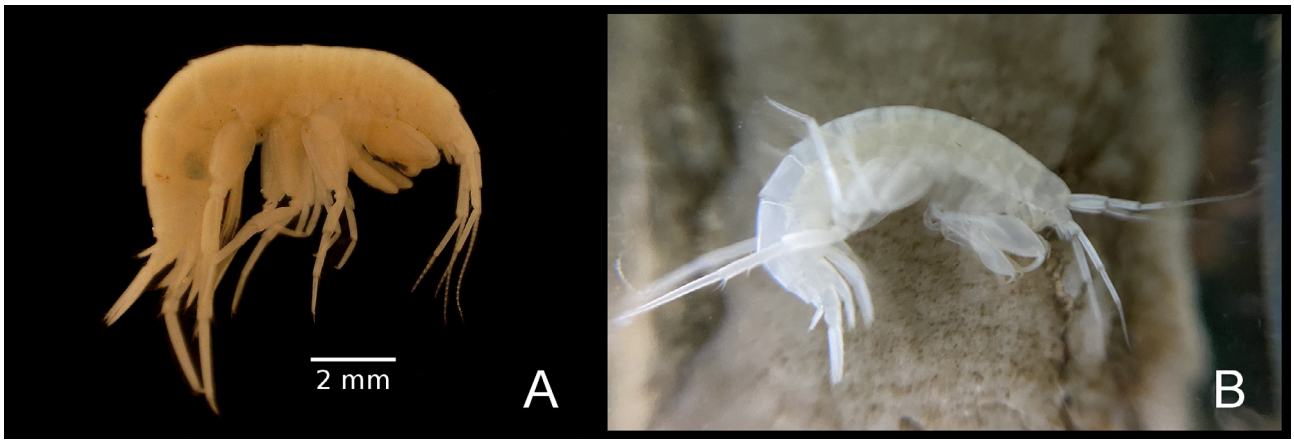

Figure 1. Preserved (**A**) and live specimen (**B**) of *Spelaeogammarus ginae* sp. nov., Gruna da Serra Verde cave, Serra do Ramalho karst area, state of Bahia, Brazil. Photographs: (**A**) T. Zepon, (**B**) M.E. Bichuette.

margin without seta; uropod 3 outer ramus internal margin with 0–1 simple seta, 8–19 long bifid setae and 3–7 cuspidate setae with accessory seta.

Description. Body slender (Fig. 1), completely depigmented, head without eyes (Fig. 1). Mean body length 10.39 ± 2.06 mm (N = 10) (7.44–12.24 mm); mean head length 0.71 ± 0.13 mm (N = 10) (0.50–0.84 mm). Epimeral plates 1–3 distoanterior margin rounded, distoposterior margin produced with a small spine.

Antenna 1 (Fig. 2A) less than half body length, peduncle subequal to flagellum, article lengths reducing gradually from 1 to 3, article 1 with 5 cuspidate setae with accessory setae on one side and 4–6 simple setae and 1–4 plumose setae on the other side, plumose and simple setae apically; flagellum with 18–20 articles, aesthetascs occurring distally on flagellum from article 2 to 18; accessory flagellum with 5 articles, reaching up to fifth article of flagellum.

Antenna 2 (Fig. 2B) subequal in length to antenna 1, peduncle shorter than flagellum, articles 4 and 5 elongated, subequal in size; flagellum with 8–10 articles.

Upper lip (Fig. 2C) apically rounded with setules. Lower lip (Fig. 2D) inner lobe with setules apically, rounded; outer lobe well developed, inner distal margin with some setules.

Maxilla 1 (Fig. 2E) inner plate apical margin with 3 strong plumose setae, subequal to outer plate; outer plate apical margin with 7–8 multicuspidate setae; palp 2-articulate, article 2 inner margin with 6–7 simple setae in a row.

Maxilla 2 (Fig. 2F) inner plate with about 11 plumose setae, 5–9 stout setae and 2 strong, longer plumose setae apically, setules laterally; outer plate slightly longer and slightly more slender than outer plate, apical margin with up to 14 slender setae and 7 plumose setae; slender setae and setules laterally.

Left mandible (Fig. 2G) molar cylindrical and semi-triturative; setal row with 5 plumose setae; *lacinia mobilis* present, oblong, apical margin with 5 crests; incisor multicuspidate; palp with 3 articles, article 2 the longest, article 3 subequal to 2, article 1 less than half the length of 2 and 3; article 1 almost as wide as long, article 2 slim, longer than wide, 3 slender setae with accessory seta on ventral margin either bifid or simple with accessory seta, article 3 with 1–2 stout long seta and 1–2 bifid long setae, apically.

Right mandible (Fig. 2H) slightly longer than left mandible, setal row with 4 plumose setae, 2 of them longer and stronger than the others; palp 3-articulate, article 2 ventral margin with 4–5 slender setae with accessory seta, 2 of them usually shorter, article 3 with 4 apical long setae with accessory setae.

Maxilliped (Fig. 2I) inner and outer plates subequal in size; inner plate with scattered long stout setae, apical margin rectangular bearing 3 slim plumose setae and 2 Y-shaped stout setae; outer plate with scattered long bi- or trifid stout setae, apical margin rounded with 3–6 stout or plumose setae on the inner distal margin; palp 4-articulate; article 1 conical; article 2 twice as long as wide, inner margin with several long,

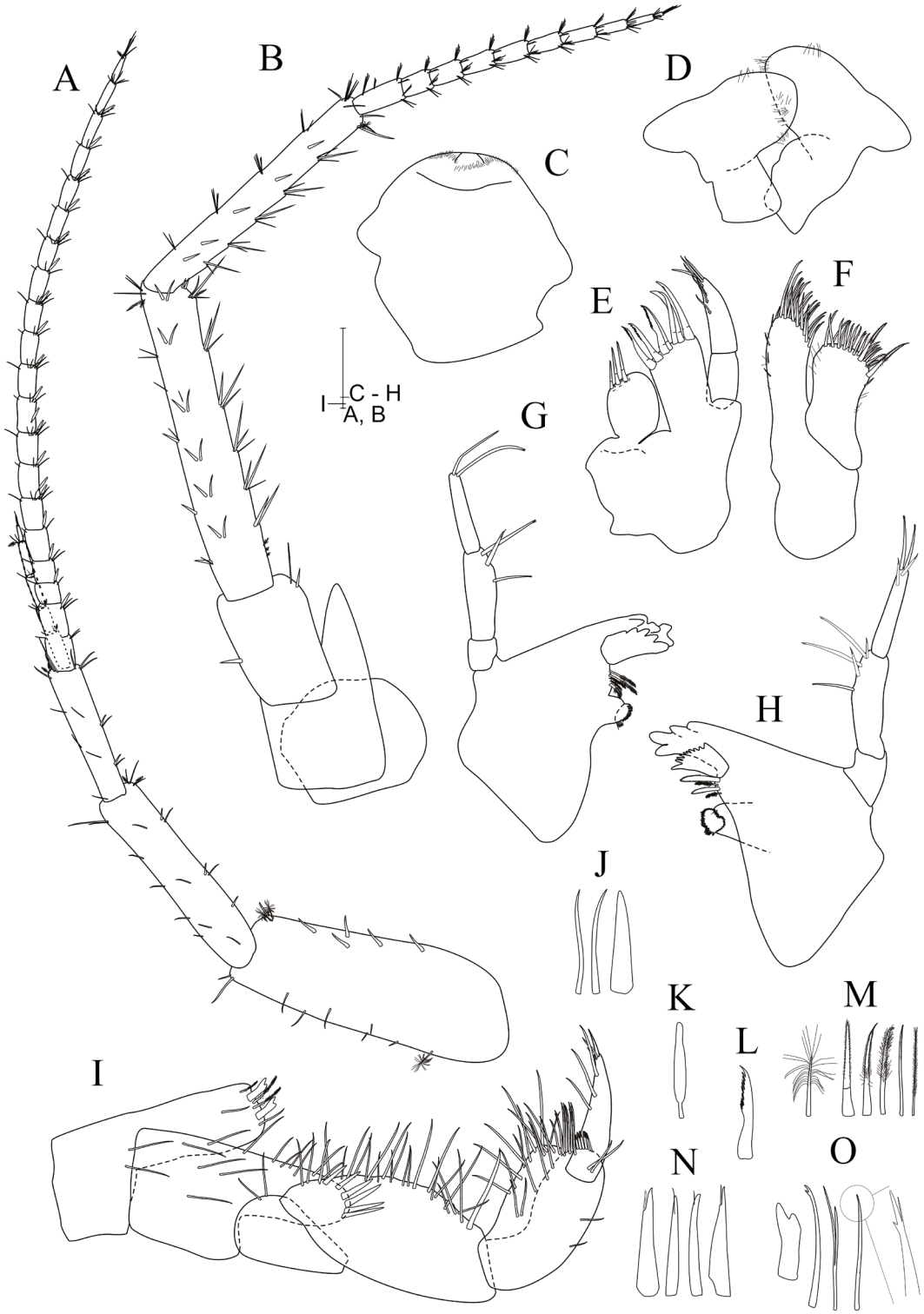

Figure 2. *Spelaeogammarus ginae* sp. nov., Gruna da Serra Verde cave, Serra do Ramalho, state of Bahia, Brazil. Paratype male, body length 11.31 mm; antenna 1 (**A**); antenna 2 (**B**); upper lip (**C**); lower lip (**D**); maxilla 1 (**E**); maxilla 2 (**F**); left mandible (**G**); right mandible (**H**); maxilliped (**I**); simple setae, present in various structures, the last one is more common on the uropods (**J**); aesthetasc, from antenna 1 (**K**); multicuspidate seta, from maxilla 1 outer plate (**L**); plumose setae, from antenna 1, maxilla 1 inner plate, maxilla 2 inner plate, mandibles and maxilliped, maxilliped and gnathopods, pleopods (**M**); (stout) cuspidate setae with accessory seta, present in various structures, first one more common on gnathopods and last one from telson (**N**); bifid and trifid setae, from maxilliped inner ramus, mandibles palp, gnathopods and uropod 3, pleopod 7, maxilliped palp (**O**). Scale bars: A, B = 600 µm; C–I = 200 µm; J–O = enlarged setae to see details.

trifid setae; article 3 inner margin with several long, trifid setae, inner face with row of plumose setae and some strong and short plumose setae apically; article 4 triangular, with long stout setae at inner margin, distal claw present.

Gnathopod 1 (Fig. 3A) coxa rectangular; basis twice as long as wide, 4 small simple setae on anterior margin, up to 14–16 long, bifid setae on posterior margin, 1 simple small seta on posterodistal margin; ischium small, 1 bifid slender seta on posterodistal margin; merus quadrate, with long bifid setae and several setules along posterior margin; carpus subtriangular, posterodistal margin with setules, up to 7 plumose setae and 2 simple stout setae; propodus suboval, slightly longer than basis, palm oblique, about 2.5 × longer than posterior margin, 19–20 simple short setae, 10–11 short and strong cuspidate setae with accessory setae, 3–7 long slim simple setae and 3 long bifid setae; some scattered, long and thin trifid seta on inner margin; dactylus curved, congruent in length with palm, not reaching the palmar corner, some short simple setae dorsally.

Gnathopod 2 (Fig. 3B) coxa subtriangular, wider than long, basis 3 × longer than wide, posterior margin with 14–18 stout long simple setae, anterior margin with 1 small simple seta, 0–1 simple seta on posterodistal margin; ischium and merus subrectangular, posterior margins with 1 and 2–4 small simple setae, respectively; carpus longer than wide, posterior margin with several short and long simple setae and some setules, anterior margin with 4–5 simple short setae; propodus suboval, almost twice as long as wide, anterior margin with 9 groups of 1–5 simple short and long simple setae, posterior margin with 8 groups of 2–8 short and long simple setae and setules, palm oblique, subequal to posterior margin, with 14 short setae, 2 long, thin simple setae and 4–6 strong cuspidate setae with accessory seta; dactylus curved, claw-like, not reaching the palmar corner.

Pereopods 3–4 (Fig. 3C–G) pereopods 3 and 4 alike, coxae 3 and 4 longer than wide; basis with 1 single small plumose seta on anterior margin, basis and ischium posterodistal margins with 1–2 simple short setae; merus with 2 simple or cuspidate short setae with accessory seta on anteroproximal margin, and up to 5 groups of 1–2 cuspidate short setae with accessory seta on posterior margin; propodus distal anterior margin with 3 simple setae, posterodistal margin with up to 7 groups of 1–3 cuspidate setae with accessory seta; dactylus short, with a plumose seta distally and 2 short setae coupled with the nail.

Pereopod 5 (Fig. 3E) longer than pereopods 3 and 4, coxa slightly wider than long, anterior lobe with 7–8 simple setae, more developed than posterior, posterior lobe with 1 long seta with accessory seta; basis almost twice as long as wide, anterior and posterior margins each with 7–10 groups of 1–2 simple or stout cuspidate setae with accessory seta; ischium with 2–3 simple long setae on anterodistal margin; merus 3 × longer than wide, anterior margin with 3–6 simple setae, posterior margin with 2–4 cuspidate setae with accessory seta, and 1 cuspidate seta with accessory seta on anteriodistal margin; carpus 5–6 × longer than wide, anterior margin with 4–6 groups of 1–2 cuspidate setae with accessory seta, posterodistal margin with 1 strong seta, long and slender setae occurring as fringe; propodus 7–8 × longer than wide, with 14–15 cuspidate setae with accessory seta on anterior margin, 1 simple thin seta on posterior margin, distally, 1 cuspidate seta with accessory seta on posterodistal margin, long and slender setae occurring as fringe; merus, carpus and propodus subequal in length; dactylus short, with a plumose seta distally and 2 short setae coupled with nail.

Pereopod 6 (Fig. 3F) coxa slightly wider than long, anterior lobe more developed than posterior, without setae, posterior lobe with 1 cuspidate seta with accessory seta; basis almost twice as long as wide, 9–10 cuspidate setae with accessory seta on both anterior and posterior margins, posterodistal margin with 1–2 small plumose setae or simple seta with accessory seta; ischium with 2 simple long setae on anterodistal margin; merus about 3 × longer than wide, anterior margin with 3 simple setae, 1 simple seta and 1 cuspidate seta with accessory seta on anterodistal margin, posterior margin with 3 cuspidate setae with accessory seta, and 1 cuspidate seta with accessory seta on anteriodistal margin; carpus 5–6 × longer than wide, anterior margin with 4–5 groups of 1–3 cuspidate setae with accessory seta, long and slender setae occurring as fringe, posterior margin with 2 simple slender setae, posterodistal margin with 2–3 cuspidate setae with accessory seta; propodus 8–9 × longer than wide, with 15 cuspidate setae with accessory seta

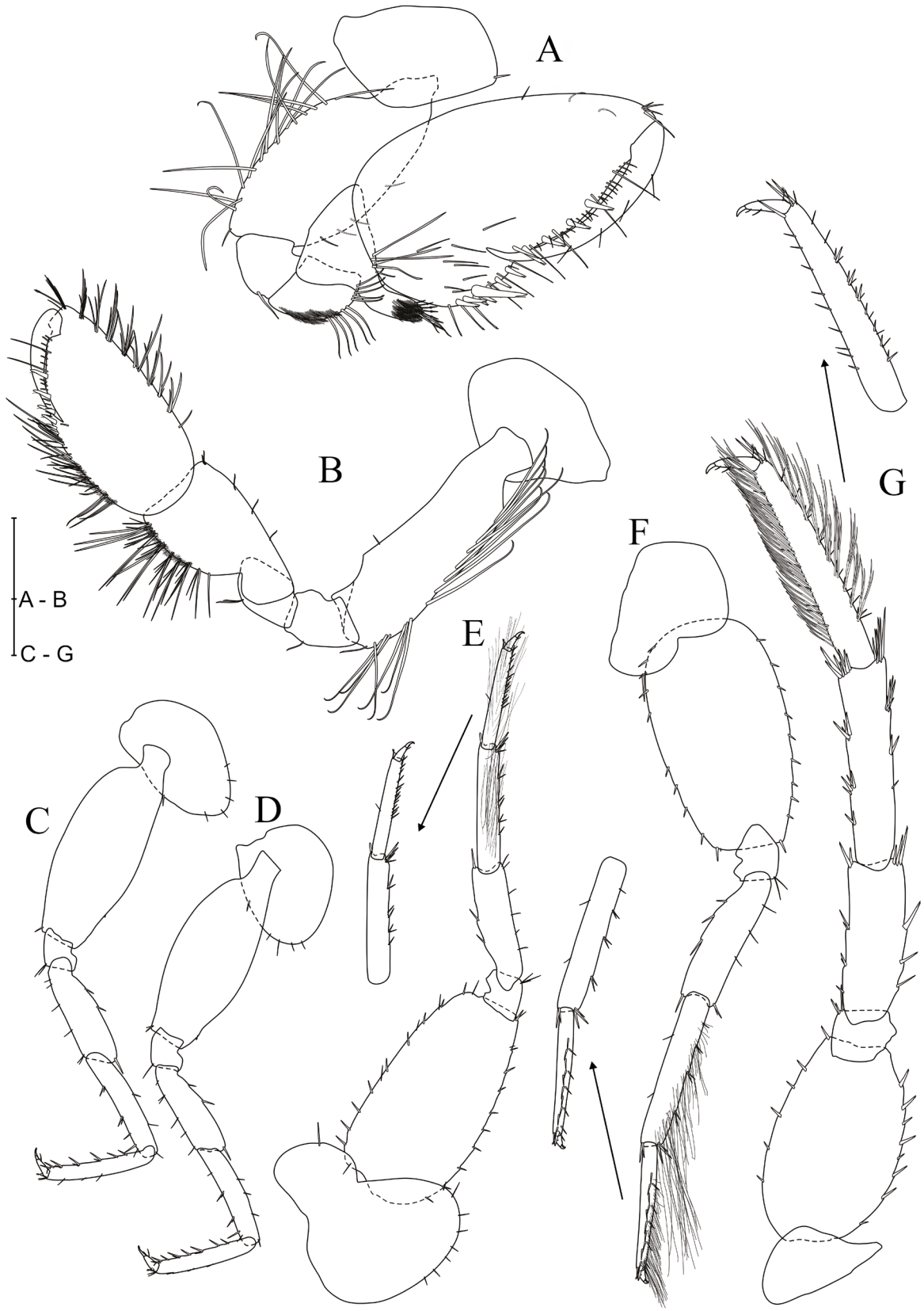

Figure 3. *Spelaeogammarus ginae* sp. nov., Gruna da Serra Verde cave, Serra do Ramalho, state of Bahia, Brazil. Paratype male, body length 11.31 mm; gnathopod 1 (**A**); gnathopod 2 (**B**); pereopod 3 (**C**); pereopod 4 (**D**); pereopod 5 (**E**); pereopod 6 (**F**); pereopod 7 (**G**). Scale bars: A, B = 400 µm; C–G = 1 mm.

on anterior margin, long and slender setae occurring as fringe, 4 cuspidate setae with accessory seta on distal margin; merus, carpus and propodus subequal in length; dactylus short, with a plumose seta distally and 2 short setae coupled with nail.

Pereopod 7 (Fig. 3G) coxa almost as wide as long, 0–1 simple seta with accessory seta on anterior margin; basis longer than wide, anterior and posterior margins with 6–10 and 6–8 cuspidate setae with accessory seta, respectively; ischium with 2 cuspidate setae with an accessory seta and 1 simple, long seta on the anterodistal margin; merus 2–3 × longer than wide, anterior and posterior margins with 3 groups of 1–2 cuspidate setae with accessory seta, anterodistal margin with 2 cuspidate setae with an accessory seta, posterodistal margin with 2–3 cuspidate setae with an accessory seta; carpus 4–5 × longer than wide, anterior margin with 3 groups of 2–4 cuspidate setae with accessory seta, 8 cuspidate setae with accessory seta on the anterodistal margin, posterior margin with 2–3 groups of 1–5 cuspidate setae with accessory seta, 7–8 cuspidate setae with accessory seta on the posterodistal margin; propodus about 7 × longer than wide, anterior margin with a variable number of cuspidate setae with accessory seta and several long, slim bifid setae, posterior margin with 9 groups of variable numbers of bifid, simple and cuspidate setae with accessory seta, posterodistal margin with 1 simple and 4 cuspidate setae with accessory seta, 2 of them longer than the others; merus smaller than carpus and propodus; dactylus short, with a plumose seta distally and 2 short setae coupled with the nail.

Pleopods 1, 2 and 3 (Fig. 4A–C) similar; peduncle elongated, respectively 3–5 × longer than wide, with 2 coupling spines each; inner rami 1-articulate, with 9–10, 10–11 and 10 plumose long setae on distal margin, respectively; outer ramus 3-articulate, third article the smallest; pleopod 1 outer ramus article 1 inner margin with 8–9 plumose setae, outer margin with 13–18 plumose setae, article 2 with 2 plumose setae, article 3 with 2 apical plumose setae; pleopod 2 outer ramus article 1 inner margin with 7–9 plumose setae, outer margin with 9–16 plumose setae, article 2 with 2 long plumose setae, article 3 with 2 long plumose setae; pleopod 3 outer ramus article 1 inner margin with 7–8 plumose setae, outer margin with 9–13 plumose setae, article 2 with 2 plumose setae, article 3 with 2 plumose setae.

Uropod 1 (Fig. 4D) peduncle 3× longer than wide, with 4–5 stout, cuspidate setae with accessory seta on each side; inner ramus subequal in length to peduncle, with 3 dorsal stout simple setae with accessory seta and 5 apical setae, 2 out of 5 apical setae with accessory seta; outer ramus subequal in length to peduncle and to inner ramus, with 1–3 stout, cuspidate setae with accessory seta on inner margin and 4 apical setae, 1 longer than others.

Uropod 2 (Fig. 4E) peduncle twice as long as wide, with 2 dorsal and 2–3 distolateral stout, cuspidate setae with accessory seta; inner ramus subequal in length to peduncle, with 3 dorsal strong and 3 short cuspidate setae with accessory seta on internal margin, and 4 apical setae, 2 of them longer than others; outer ramus subequal in length to peduncle and to inner ramus, with 2 ventral cuspidate setae with accessory seta and 3–4 apical stout setae, 2 of them with accessory seta.

Uropod 3 (Fig. 4F) peduncle short, slightly longer than wide and less than half length of rami, with 1–3 small cuspidate setae with accessory seta dorsally and 0–1 longer cuspidate seta with accessory seta dorsally on the distolateral margin; inner ramus with 7 groups of 1–2 cuspidate setae with accessory seta dorsally, 4 groups of 1–2 cuspidate setae with accessory seta distally on internal margin, and 2 short cuspidate setae with accessory seta apically; outer ramus with row of 0–1 simple long seta plus 8–19 bifid long setae plus 3–7 stout, cuspidate setae with accessory seta distally on internal margin, 4–5 groups of 1–4 stout, cuspidate setae with accessory seta dorsally, and 3 strong and 1–2 thin setae apically.

Telson (Fig. 4G) almost as long as wide, apical margin with concave excavation, with 2 apical, 2 subapical stout, cuspidate setae with accessory seta and 3 plumose setae on one side, and 1 apical stout, cuspidate seta with accessory seta and 2 subapical stout, cuspidate setae with accessory seta and 1 plumose seta on the other side. Variations: symmetrical distribution of setae, with 1 apical and 2 subapical stout, cuspidate setae with accessory seta plus 3 subapical plumose setae on each side.

Sternal gills occurring on segments 2–7. Coxal gills sac-like on segments 3–6.

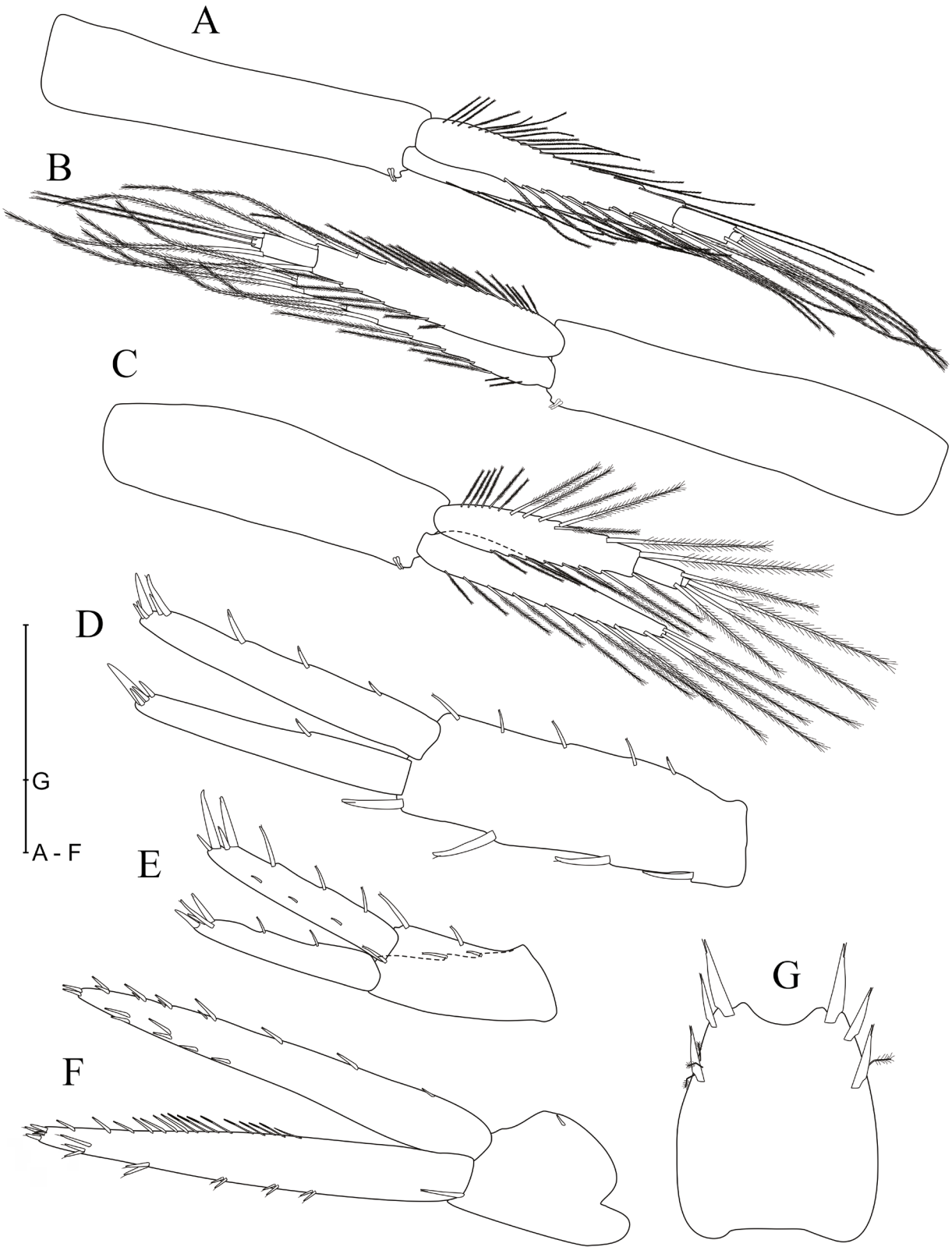

Figure 4. *Spelaeogammarus ginae* sp. nov., Gruna da Serra Verde cave, Serra do Ramalho, state of Bahia, Brazil. Paratype male, body length 11.31 mm; pleopod 1 (**A**); pleopod 2 (**B**); pleopod 3 (**C**); uropod 1 (**D**); uropod 2 (**E**); uropod 3 (**F**); telson (**G**). Scale bars: A–F = 1 mm; G = 200 µm. Specimens of *Spelaeogammarus ginae* sp. nov. were found (**C**); map indicating the state of Bahia, the Gruna da Serra Verde cave, and the land use and land cover of the area (**D**). Photographs: M.E. Bichuette.

Female: no apparent sexual dimorphism. Oostegites slim, observed on pereonites 2–6, with curly pointed setae.

Taxonomic remarks. A thorough morphological comparison between the known *Spelaeogammarus* species is provided (Tab. 1), including the new one here described. We highlight the following differences: *Spelaeogammarus ginae* sp. nov. differs from *S. bahiensis*, *S. spinilacertus,* and *S. titan* in the number of articles on the accessory flagellum of antenna 1; the basis of pereopod 5 of *S. ginae* sp. nov. is quite setose (12–14 cuspidate setae with accessory seta on the posterior margin), while *S. sanctus* has no observed seta in this structure; the gnathopod 2 posterior margin of the basis of *S. ginae* sp. nov. (with 14–18 setae) differs from those of *S. santanensis* (21–23 setae) and of *S. trajanoae* (8–9 setae); and the palp of the maxilla 1 of *S. ginae* bears 6–7 setae, while *S. uai* palp has only 4.

Distribution, habitat, ecology and behavior. Freshwater, hypogean. Specimens of *S. ginae* sp. nov. were found in a single pool formed by the upper phreatic aquifer at Gruna da Serra Verde cave (Fig. 5A–D). The cave is located at Bambuí Group, a geomorphological unit, in a region called Serra do Ramalho, with typical dry-forest vegetation. The region has dozens of caves, all of carbonate rock (limestone caves), some with several kilometers of passages (Rubbioli *et al.*, 2019). The pool has a soft bottom of silt, with little organic matter (some vegetation and drops of guano) and limestone riffles (Fig. 5B). In the wet season, the extension of the pool reaches 150 m in length and approximately 1.5–2.0 m in width (Fig. 5D); in the dry season (May–October) the pool showed a smaller dimension (Fig. 5B) of 1.38 m in length, 0.52 m in width, and approximately 0.18 m in depth (0.72 m^2 of area and 0.13 m^3 of volume), with a high abundance (48) of individuals trapped in the pool. The temperature was relatively high (24.1 °C) and the pH followed the values for karstic waters (pH = 7.2). The individuals were found only in the dry season, when the population densities per area and per volume were particularly high for troglobitic species (66.6 inds.m^{-2} and 369 inds.m^{-3}, respectively). All individuals (approximately 48) observed in the pool showed a fast swimming-behavior (observation from 12:15 PM until 13:40 PM), mainly on the soft-bottom (Video S1), with a few stops to forage on particulate vegetal debris and drops of guano. The individuals did not show photophobic behavior, *i.e.*, did not react to the light of the flash-lamps, with an intensity of about 600 lumens. Despite the large concentration of individuals in a small pool, the species did not appear to have a gregarious habit.

Conservation. We propose that *S. ginae* sp. nov. should be classified as Critically Endangered (CR) according to the IUCN criteria B1ab(iii), *i.e.*, the extent of occurrence (EOO) estimated less than 100 km^2 (criteria B1); one location (criteria a); continuing decline in the quality of habitat (criteria b). The species is possibly endemic to its type locality and the surroundings of the cave have several anthropogenic impacts including cattle grazing and deforestation. These impacts contribute to decline in the quality of habitat. Another impact could be related to climate change or due to cyclical events of drought and flood. The single pool where the species occurs has been suffering a decrease in the water level during the last three yearly dry-seasons (MEB., pers. obs.).

Spelaeogammarus spinilacertus Koenemann and Holsinger, 2000

(Fig. 6).

Material examined (all from Brazil, state of Bahia). 1 adult, LES 0016806, municipality of Iraquara, Buraco do Cão cave, 12°23'37.5"S 41°36'08.2"W, 20 Jun. 2009, M.E. Bichuette, L. Senna-Horta colls.; 3 adults, LES 0000230, municipality of Palmeiras, Canoa Quebrada cave, 12°25'29.6"S 41°33'28.1"W, 12 Jul. 2004, M.E. Bichuette coll.; 1 adult, LES 0000231, municipality of Palmeiras, Canoa Quebrada cave, 12°25'29.6"S 41°33'28.1"W, 13 Nov. 2004, M.E. Bichuette coll.; 3 adults, LES 0004906, municipality of Iraquara, Gruta da Lagoa Seca cave, 12°18'42.7"S 41°34'03.6"W, 23 Jan. 2007, M.E. Bichuette coll.; 2 adults, LES 0022569, municipality of Iraquara, Gruta da Lagoa Seca cave, 12°18'42.7"S 41°34'3.6"W, 23 Jan. 2007, M.E. Bichuette coll.; 1 adult, LES 0016823, municipality of Iraquara, Gruta Umburana cave, 12°20'25.4"S

Table 1. Morphological characteristics of the species of the genus *Spelaeogammarus* from Brazil. Legend: "—" not specified or unidentified.

Morphological characteristic	*S. ginae* sp. nov.	*S. bahiensis*	*S. sanctus*	*S. santanensis*	*S. spinilacertus*	*S. titan*	*S. trajanoae*	*S. uai*
A1 length in relation to body length	<0.5	—	0,4	0.4-0.5	0.5	~0.5	—	~0.5
A1 flagellum number of articles	18–20	16	22	20-21	17-20	26	—	17-20
A1 accessory flagellum number of articles	5	3-4	5	5	4	6	—	5
A2 flagellum number of articles	8–10	7	10	8-10	7	10	—	7-8
M1 outer plate number of multi-cuspidate setae	7–8	7	7	7	6-7	6	—	7
M1 palp article 2 number of apical setae	6–7	0	~7	—	5-6	~7	—	4
G1 basis posterior margin number of setae	14–18	7-9	15-17	20	6-8	20	9-10	15
G2 basis posterior margin number of setae	14–18	0	20	21-23	9-10	23	8-9	18
P5 basis posterior margin number of setae	12-14	—	0	12	10-11	~20	10-12	8
P7 basis posterior margin number of setae	6–8	—	~9	—	7-9	~11	10	~9
Pleopod 1 inner ramus distal margin number of plumose setae	9–10	—	11	—	—	10	—	9
Pleopod 2 inner ramus distal margin number of plumose setae	10–11	—	12	—	—	11	—	9
Pleopod 3 inner ramus distal margin number of plumose setae	10	—	10	—	—	13	—	9
U1 outer ramus number of setae on outer margin	0	0	0	—	0	7	—	0
U1 outer ramus number of setae on inner margin	1–3	2	3	—	3	21	—	5
U2 outer ramus number of setae on outer margin	0	—	1	—	2-3	3	—	1
U2 outer ramus number of setae on inner margin	2	—	2	—	0	2	—	2
U3 outer ramus number of setae on outer margin	11–13 cuspidate with accessory seta	—	15	—	12-18	12	4-18	18 cuspidate with accessory seta
U3 outer ramus number of setae on inner margin	0–1 simple + 8–19 bifid long + 3–7 cuspidate with accessory seta	—	21 bifid long + 4 cuspidate with accessory seta	—	~20 bifid	21 simple	—	16 bifid + 5 cuspidate with accessory seta

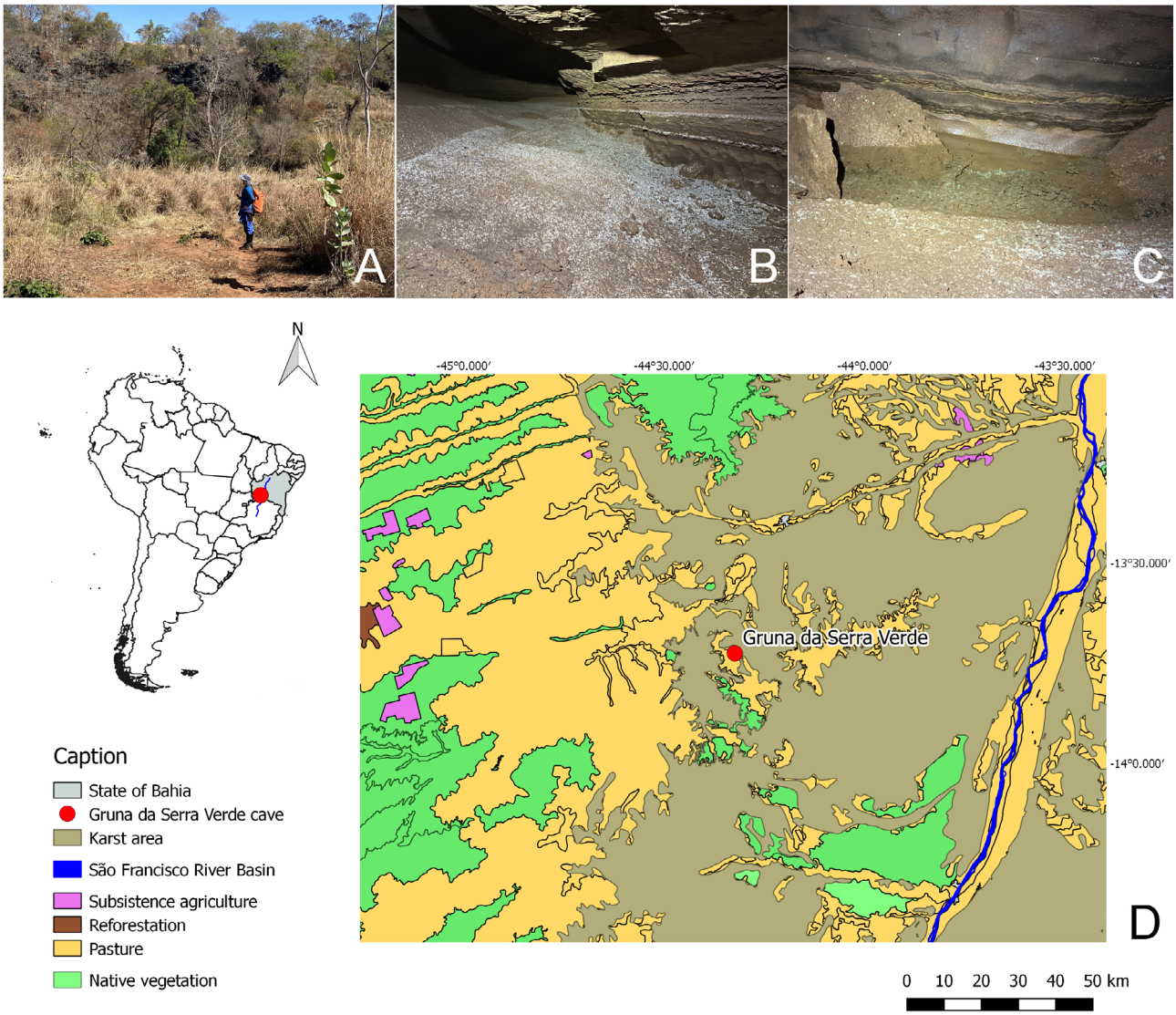

Figure 5. Outcrops and pastures close to the Gruna da Serra Verde cave (**A**); cave gallery in the dry season (**B**) and detail of the small pool formed by the upper phreatic aquifer, where specimens of *Spelaeogammarus ginae* sp. nov. were found (**C**); map indicating the state of Bahia, the Gruna da Serra Verde cave, and the land use and land cover of the area (**D**). Photographs: M.E. Bichuette.

41°36'32.0"W, 19 Jun. 2009, M.E. Bichuette coll.; 2 adults, LES 0000225, municipality of Iraquara, Lapa Doce System, 12°20'01.7"S 41°36'16.5"W, 30 Mar. 2005, M.E. Bichuette, D.R. Pedroso colls.; 3 adults, LES 0022568, municipality of Iraquara, Lapa Doce System; 12°20'01.7"S 41°36'16.5"W, 30 Oct. 2016, M.E. Bichuette, M. Rosendo, J.E. Gallão colls.; 3 adults, LES 0001804, municipality of Iraquara, Lapa Doce System, 12°20'01.7"S 41°36'16.5"W, 25 Mar. 2000, D. Allegrini coll.; 2 adults, LES 0027745, municipality of Iraquara, Lapa Doce System, 12°20'01.7"S 41°36'16.5"W, M.E. Bichuette, J.P.M. Araujo, G.H.N. Basílio, M.A. Rasteiro colls.

Identification. Taxonomic treatment followed the original description of *S. spinilacertus* (see Koenemann and Holsinger, 2000) and the key to *Spelaeogammarus* species (Rogers *et al.*, 2020). *Spelaeogammarus spinilacertus* is characterized by 4 articles on antenna 1 accessory flagellum; antenna 2 is 25 % shorter than antenna 1, flagellum with 7 segments; 6 multicuspidate setae on maxilla 1 outer plate; and 4 plumose setae on maxilliped inner plate (Koenemann and Holsinger, 2000; Rogers *et al.*, 2020). The original description of the species was based on one holotype and one allotype (Koenemann and Holsinger, 2000), hampering the observation of

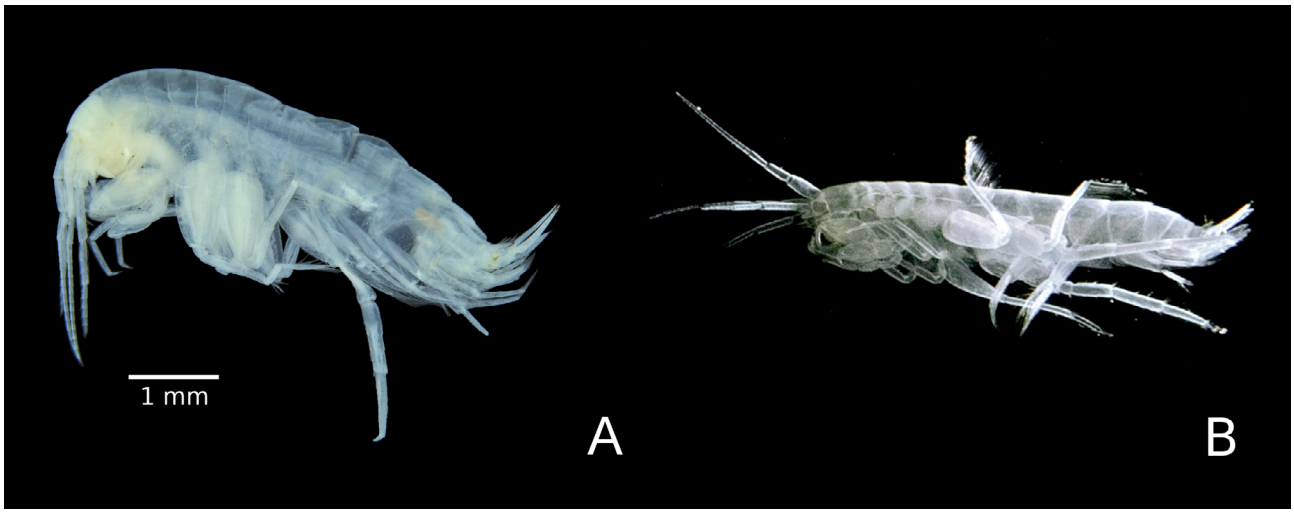

Figure 6. Preserved specimen of *Spelaeogammarus spinilacertus* Koenemann and Holsinger, 2000 from Buraco do Cão cave, Iraquara municipality, state of Bahia, Brazil (**A**) and live specimen of *S. spinilacertus* from Canoa Quebrada cave, municipality of Iraquara, state of Bahia, Brazil (**B**). Photograph: (**A**) Luciana B.R. Fernandes, (**B**) Adriano Gambarini.

variation. All but one diagnostic characteristic were observed on the analyzed material, *i.e.*, instead of 6 multicuspidate setae on maxilla 1 outer plate, we found 7 setae, which could represent variability in this character.

Geographic distribution and ecological data. The distributional range of *Spelaeogammarus spinilacertus* increased from two (Baixa do Salitre and Jaburu caves) to seven caves (new records: Lapa Doce System, Gruta da Umburana, Gruta da Lagoa Seca, Buraco do Cão, and Canoa Quebrada caves) that define a polygon of approximately 101 km^2 (Fig. 7C). All caves are located in the Chapada Diamantina region, central portion of the state of Bahia, northeastern Brazil, upper Paraguaçu River basin. The limestone rocks from Chapada Diamantina region are dated to the Neoproterozoic age, and belong to the Una Geological Group, Salitre Formation (Schobbenhaus *et al.*, 1984). These caves present lentic water bodies of the upper phreatic zone. *Spelaeogammarus spinilacertus* occurs in shallow, soft-bottom pools (observations of individuals were to 2 m depth), with slow water current (Fig. 7A, B). Populations show a preference for swimming close to the substrate, formed mainly of silt, limestone riffles, and guano piles and, less frequently, in the mid-water and surface (MEB., pers. obs.). The abundance is relatively high in two caves, Canoa Quebrada (observed in June/2005) and Lapa Doce System (observed in October/2016), with 40 and 20 individuals respectively. Physical and chemical variables showed little variation between the cave waters and three of them are typical of karst waters (alkaline pH and high conductivity): pH from 7.7 to 7.9; conductivity from 0.474 to 0.639 µS.cm^{-1}; temperature from 22.5 to 26.3 °C; and salinity from 0.1 up to 0.2 %.

We propose that *Spelaeogammarus spinilacertus* should be classified as Endangered (EN) according to the IUCN criteria B1ab(iii). The species has an extent of occurrence (EOO) estimated less than 5,000 km^2 (criteria B1); one location (defined as a geographically or ecologically distinct area in which a single threatening event can rapidly affect all individuals of the taxon present) (criteria a); and continuing decline in the quality of habitat (criteria b). The species is associated with phreatic water and, therefore, a single threatening event can rapidly affect the individuals of different caves. The region is influenced by several anthropogenic impacts including cattle grazing, agriculture, but the main impact could be pollution of the phreatic waters by pesticides, as the caves are located outside protected areas such as the Chapada Diamantina National Park (Gallão and Bichuette, 2018). These impacts contribute to decline in the quality of habitat.

Spelaogammarus spinilacertus co-occurs with the troglobitic catfish *Rhamdiopsis krugi* Bockmann and Castro, 2010, also endemic to limestone caves of the Chapada Diamantina (Bichuette *et al.*, 2015) and considered one of the predators of the amphipod species.

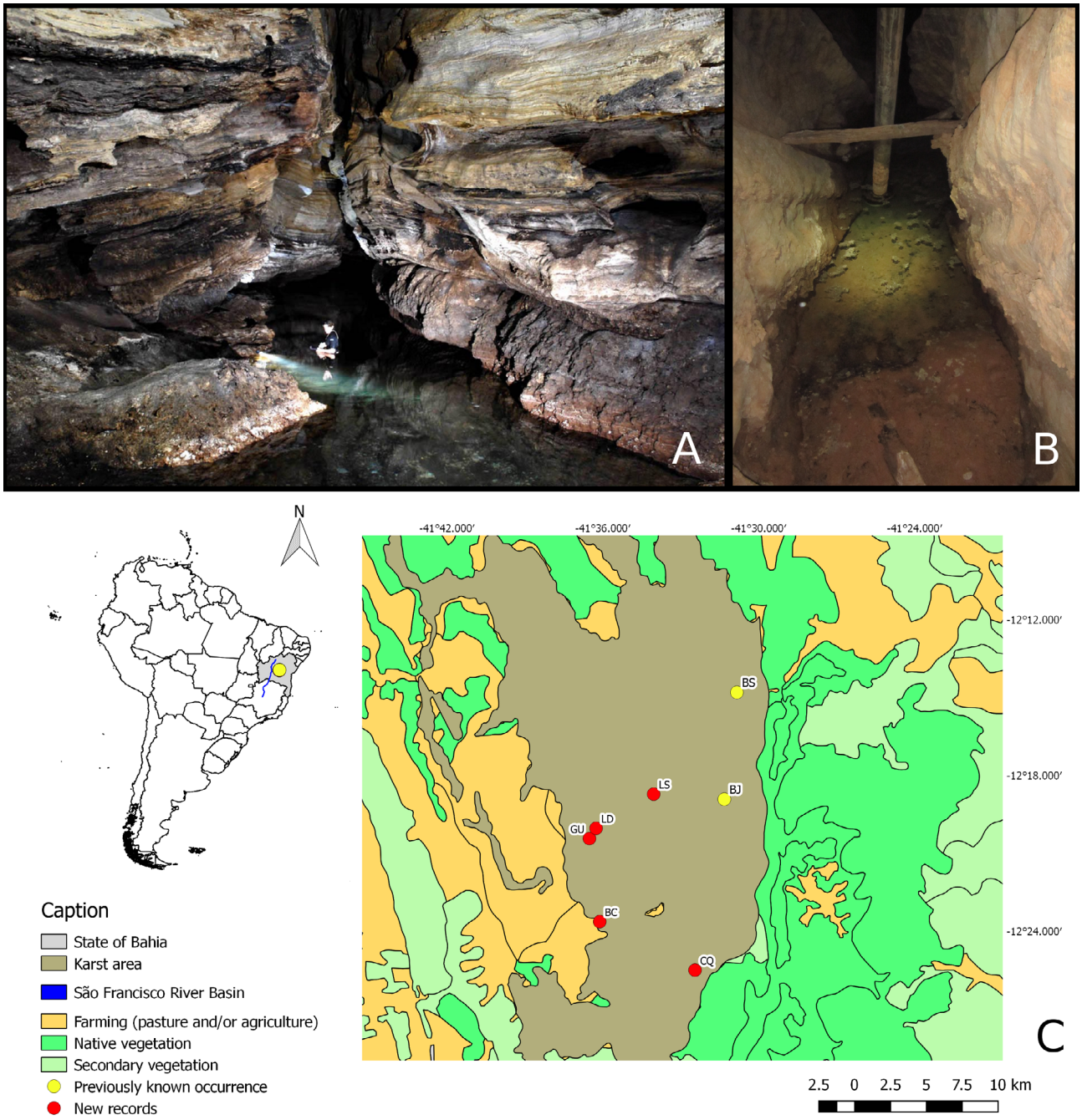

Figure 7. Lake formed by upper phreatic aquifer at Canoa Quebrada cave (**A**); small pool of upper phreatic aquifer at Lapa Doce System (**B**); distribution of *Spelaeogammarus spinilacertus* Koenemann and Holsinger, 2000, indicating caves with records of the species showing the land use and land cover of the area (**C**). Caption: LD = Lapa Doce System, GU = Gruta da Umburana cave, LS = Gruta da Lagoa Seca cave, BC = Buraco do Cão cave, CQ = Canoa Quebrada cave, BS = Baixa do Salitre cave, and BJ = Jaburu cave. Photographs: (**A**) Adriano Gambarini, (**B**) Jonas E. Gallão.

Discussion

Spelaeogammarus ginae sp. nov. shows the troglomorphisms observed in the other *Spelaeogammarus* species and many other cave animals, in the absence of eyes and body pigmentation, which corroborates the hypothesis that these character-states are typical autapomorphies, with no taxonomic correlation. This idea has been discussed exhaustively in the subterranean literature, from Barr (1968) to recent works (*e.g.*, Bichuette *et al.*, 2015), and reinforces the need for caution in discussing homoplastic characters.

The new species described herein is already threatened, as it is possibly endemic to a single cave, with the surroundings being highly impacted by agricultural pastures and other anthropogenic activities. The scenario becomes even worse with the lack of legal protection for the region of Serra do Ramalho as a whole (Gallão and Bichuette, 2018), since there is no conservation unit in this region, and habitat alteration due to climate change, with severe decline of the upper phreatic aquifer that supplies the habitat of *S. ginae* sp. nov. (MEB., pers. obs. from 2017 to 2021). Official and legal protection, in addition to long-term monitoring projects and more investigation for more populations, are urgently needed to understand these threats and to effectively protect this unique and fragile species.

The new distributional range of *S. spinilacertus* (Fig. 7C), expanded from two to seven caves and reaching 101 km^2, shows that these different populations deserve more study focusing on biogeography. Koenemann and Holsinger (2000) suggested that the apparent isolation of some *Spelaeogammarus* species (*S. trajanoae*, *S. spinilacertus*, *S. santanensis*, and *S. bahiensis*) in different areas may reflect a sequence of allopatric speciation events over a relatively short time period. Species of *Spelaeogammarus* currently have no studies focusing on population parameters, genetic data, or physiology, to know the degree of their resilience, which makes the threats to them more drastic. To date, *S. spinilacertus* is not included in the Brazilian Red List, as it was only evaluated in 2017, and has not been officially published yet.

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Supplementary Material

Video S1. Swimming-behavior of *Spelaeogammarus ginae* sp. nov. Available at: https://youtu.be/q_LvxpxqAzg

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