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Two new subterranean species of *Hyalella* Smith, 1874 (Crustacea: Amphipoda: Hyalellidae) from Brazil

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Abstract

Two new species of *Hyalella* from Brazil are described. *Hyalella veredae* **sp. n.** shows the following characters: eyes reduced or absent in some specimens; antenna 1 and antenna 2 of similar size, and a curved seta on the inner ramus of male uropod 1. *Hyalella formosa* **sp. n.** is characterized by the absence of eyes, antenna 1 longer than antenna 2 and a curved seta on the inner ramus of male uropod 1. The species were found on caves located in two private properties, both under the impact of agricultural activities, which demonstrates a potential threat to these subterranean environments.

Key words: Neotropics, underground habitats, cave, amphipods, *Hyalella*, new species, taxonomy

Resumo

Duas novas espécies de *Hyalella* são descritas para o Brasil. *Hyalella veredae* **sp. n.** possui os olhos reduzidos ou ausentes em alguns espécimes; antena 1 e antena 2 com tamanhos semelhantes e uma seta curva no ramo interno do urópodo 1. *Hyalella formosa* **sp. n.** apresenta uma completa perda dos olhos; antena 1 mais longa que a antena 2 e uma seta curva no ramo interno do urópodo 1. Ambas as espécies foram encontradas em cavernas localizadas dentro de propriedades particulares e seus respectivos entornos encontram-se impactados pela ação de atividades agrícolas, demonstrando uma potencial ameaça sobre os ambientes.

Introduction

Although underground environments are usually considered "isolated" from external habitats, they can be easily influenced by external conditions, which can threaten this environment and consequently, the local fauna, through disturbance from the surface (Culver & Pipan, 2009). The vulnerability of these ecosystems highlights the importance of the knowledge on biological diversity, especially considering that most of the troglobitic species show a high degree of endemism (Sket, 1999). Furthermore, the occurrence of troglobitic species can safeguard underground environments through public policies for environmental conservation.

The amphipods belonging to the genus *Hyalella* Smith, 1874 occur in freshwater environments of the Americas (Grosso & Peralta, 1999), and include five hypogean species. Most of the subterranean species occur in Brazil: *H. caeca* Pereira, 1989 and *H. spelaea* Bueno & Cardoso, 2011 in São Paulo state (south-east) and *H. imbya* Rodrigues & Bueno, 2012 in Rio Grande do Sul state (south) (Pereira, 1989; Cardoso *et al*., 2011; Rodrigues *et al.,* 2012). The other two species, *H. anophthalma* Ruffo, 1957 and *H. muerta* Baldinger, Threloff & Shepard, 2000,

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are recorded in Venezuela and the U.S.A, respectively (Ruffo, 1957; Baldinger *et al.,* 2000). The aim of this paper is to describe two new troglobitic species of *Hyalella* from Brazil.

Material and methods

Samples were collected between the years 2010–2011 in different cave systems in Brazil, one from Vereda da Palha Cave, a limestone cave located in Presidente Olegário municipality (state of Minas Gerais) (Fig.1) and another from Andorinhas Cave, a sandstone cave located in Ponta Grossa municipality (state of Paraná) (Fig.2).

The material was preserved in 70% ethanol. In laboratory, specimens were measured (cephalothorax length and total body length), dissected and mounted on permanent slides. The appendages were studied under a microscopy and illustrated using a camera lucida (Gonzalez & Watling, 2003; Poore, 2005; Gonzalez *et al*., 2006). Images were generated using CorelDraw X15 software.

The appendages were prepared according to Zimmer *et al*., 2009, and analyzed through Scanning Electron Microscope (SEM) JSM 6060 in the Center for Electron Microscopy (CME) at Universidade Federal do Rio Grande do Sul (UFRGS), in order to examine the morphology, the arrangement of cuticular structures and to classify the setae.

The type material was deposited in the Museu de Zoologia da Universidade de São Paulo (MZUSP), Coleção de Crustáceos da Universidade Federal de Lavras (UFLA) and Coleção de Crustáceos da Universidade Federal do Rio Grande do Sul (UFRGS).

Results

Family Hyalellidae Bulycheva, 1957

Genus *Hyalella* Smith, 1874

Hyalella veredae Cardoso & Bueno, sp. n.

(Figs 3–6)

Type material. Holotype: male, cephalothorax length 0.4 mm, total length 4.7 mm, Brazil, Minas Gerais state, Presidente Olegário municipality, Vereda da Palha Cave (18°15'17"S 46°07'32"W), MZUSP 28420, 13.X.2010, Ferreira, R.L. & Cols. Paratypes: UFRGS 5542 (1 male on slide, 1 male, 1 female), MZUSP 28421 (1 male, 1 female), UFLA 0261 (1 male on slide, 2 males, 2 females), with the same data as holotype.

Diagnosis. Eyes reduced or absent in some specimens. Antenna 1 and antenna 2 of similar size. Gnathopod 1 and 2 carpus posterior lobe with polygonal pattern and two rows of denticles as comb scales. Gnathopod 1 propodus inner face with five serrate setae, posterior margin with denticles as comb scales; dactylus with denticles as comb scales. Gnathopod 2 propodus hammer-shaped, posterior margin with denticles as comb scales, irregular palm, longer than posterior margin. Uropod 1 inner ramus of male with one curved seta and five apical cuspidate setae with accessory seta.Uropod 3 peduncle with four cuspidate setae with accessory seta, ramus with three to four cuspidate setae.

Description of male. (Figs 1D and 3A). Mean body length: 4.3 ± 0.9 mm (n=15); mean cephalothorax length: 0.4 ± 0.09 mm. Body surface smooth, epimeral plates not acuminate. Round and pigmented eyes, with few ommatidia or eyeless (n=45; 4.4% of the sample).

Antenna 1 (Fig. 3B) total length reaches fifth pereonite; peduncle length exceeds first pereonite; flagellum with 9 to 11 articles, with two aesthetascs on each article after article 5.

Antenna 2 (Fig. 3C) total length reaches sixth pereonite; peduncle surpasses the first pereonite; flagellum with 9 to 11 articles.

Upper lip (Fig. 3D) margin rounded, distal border covered by setules on dorsal and ventral faces.

Mandible (Fig. 3E) basic amphipodan (in the sense of Watling 1993), but without palp; incisor toothed; left lacinia mobilis with five teeth and setal row with three pappose setae; right mandible with three pappose setae; molar process broad and cylindrical with accessory seta.

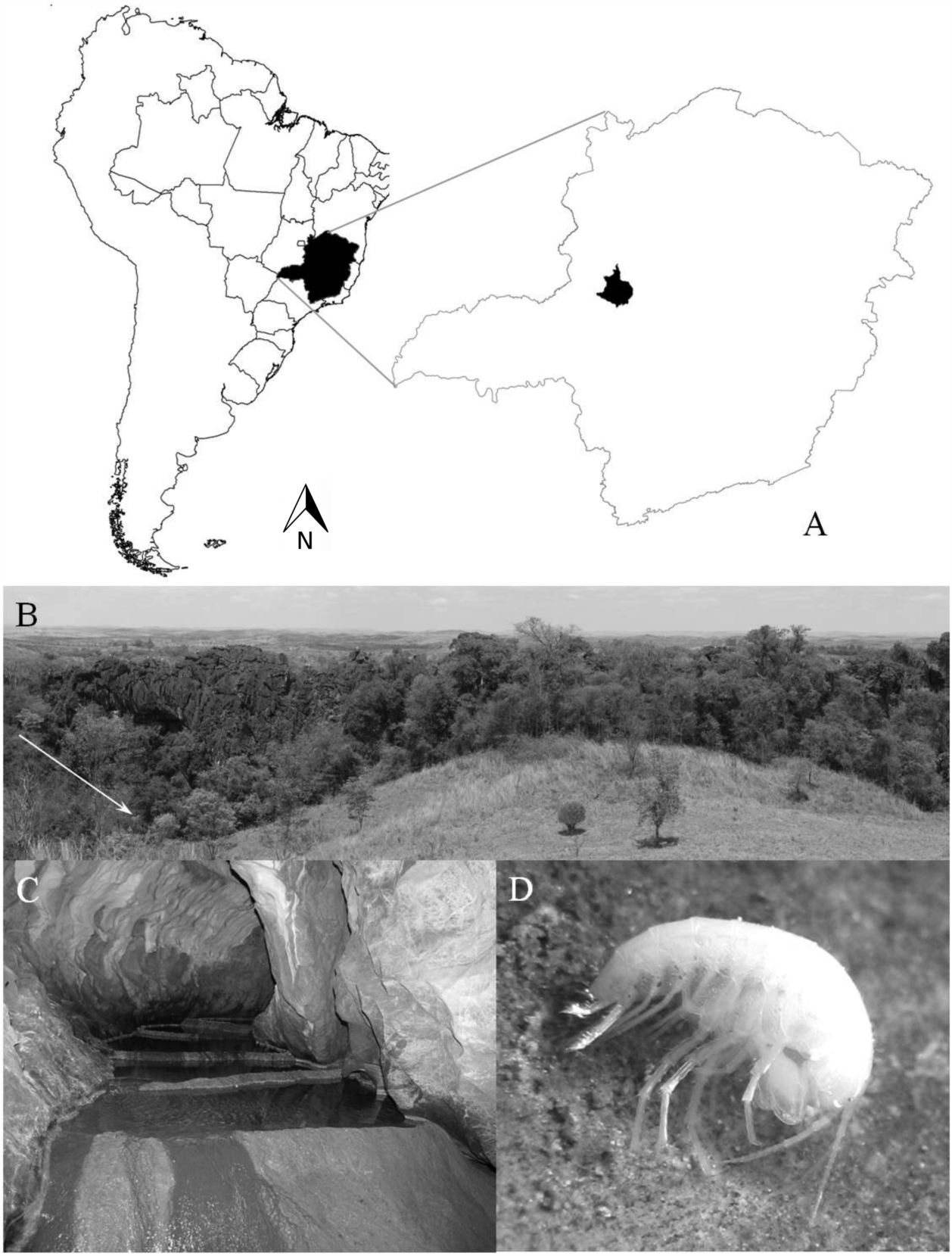

FIGURE 1. Habitat of *Hyalella veredae* **sp. n.** Cardoso & Bueno. A. Map of South America, showing the state of Minas Gerais and Presidente Olegario city; B. Location of Vereda da Palha Cave; C. Travertines in Vereda da Palha Cave; D. Habitus of male paratype.

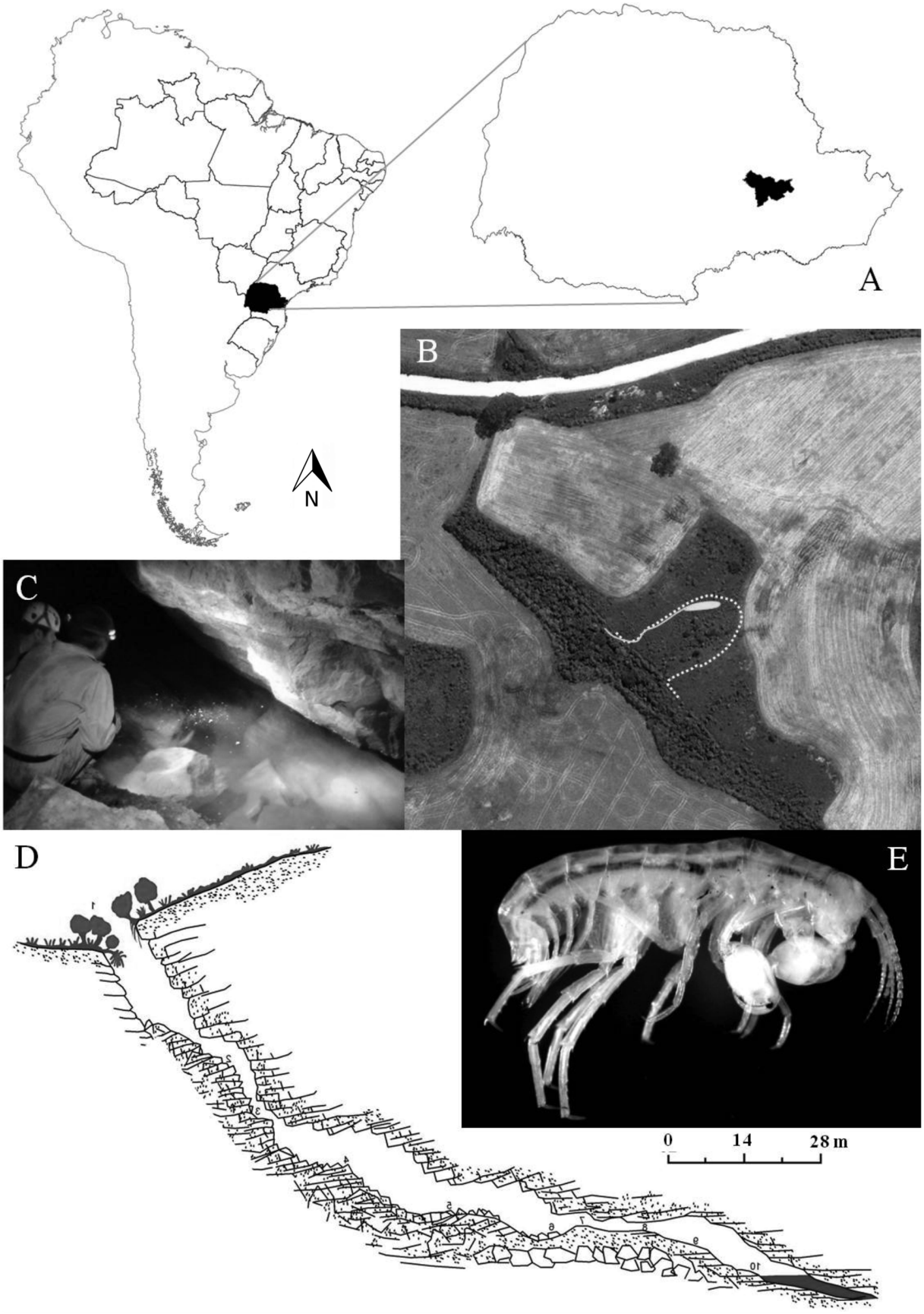

FIGURE 2. Habitat of *Hyalella formosa* **sp. n.** Cardoso & Araujo. A. Map of South America, showing the state of Paraná and Ponta Grossa city; B. Location of Andorinhas cave; C. Underground lake where the species were found; D. Map of Andorinhas cave; E. Habitus of male holotype.

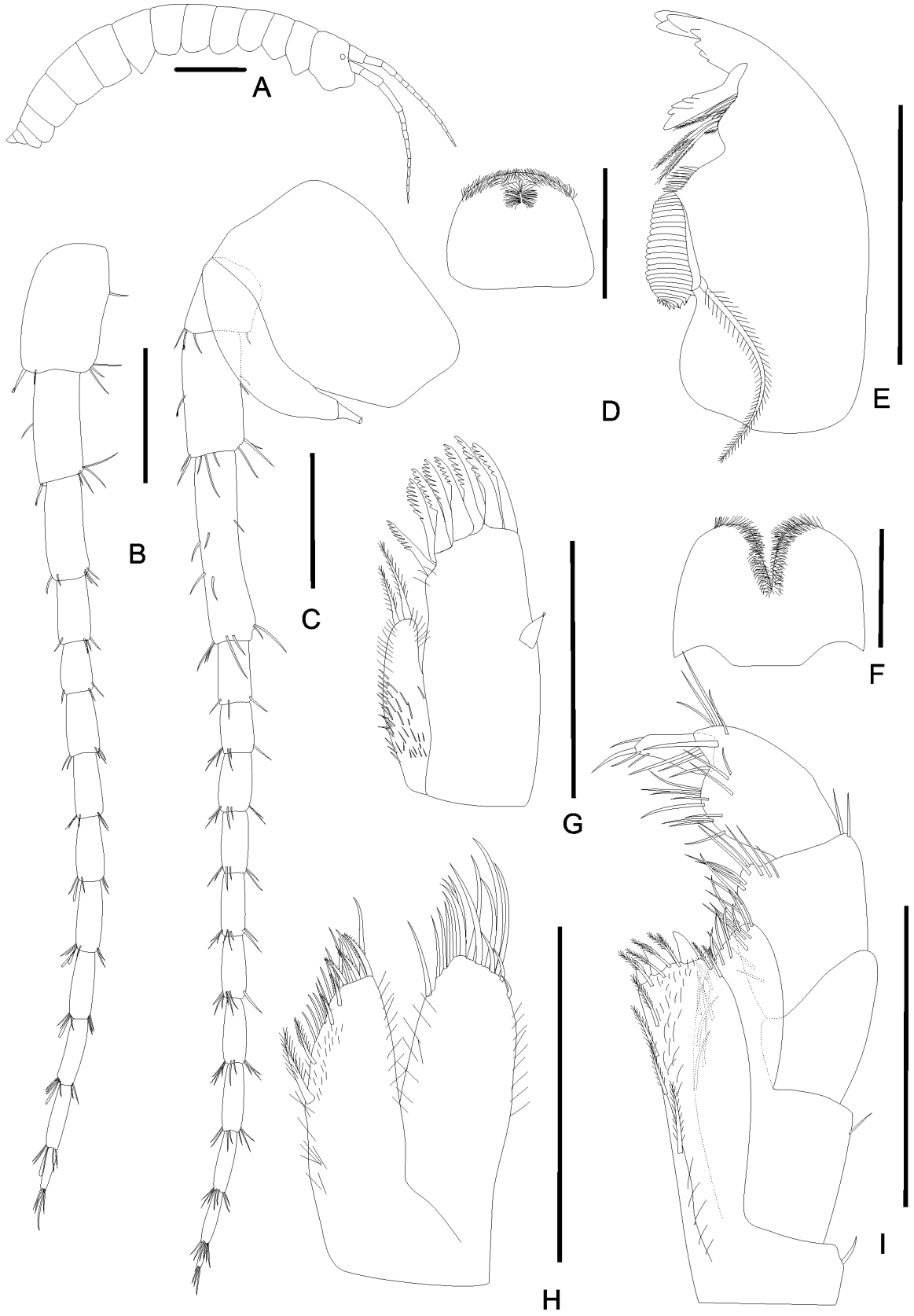

FIGURE 3. *Hyalella veredae* **sp. n.** Cardoso & Bueno (Male paratype, UFRGS 5542). A, habitus from holotype; B, antenna 1; C, antenna 2; D, upper lip; E, mandible; F, lower lip; G, maxilla 1; H, maxilla 2; I, maxilliped. Scale bars: (A) 1.0 mm, (B-I) 0.02 mm.

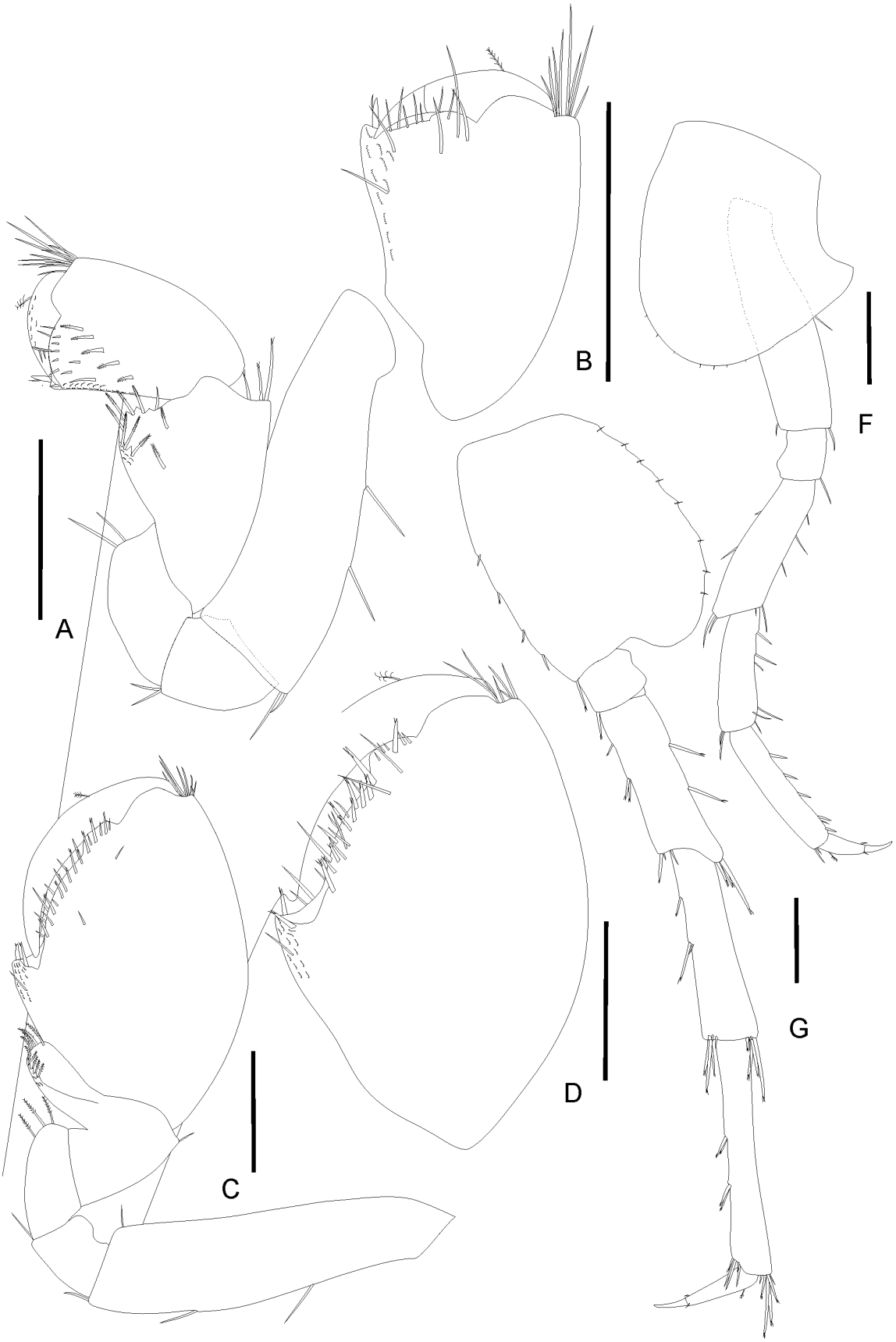

FIGURE 4. *Hyalella veredae* **sp. n.** Cardoso & Bueno (Male paratype, UFRGS 5542). A, gnathopod 1; B, detail of propodus and dactylus of gnathopod 1 dorsal view; C, gnathopod 2; D, detail of propodus and dactylus of gnathopod 2 ventral view; E, pereopod 3; F, pereopod 7.Scale bars: 0.02 mm.

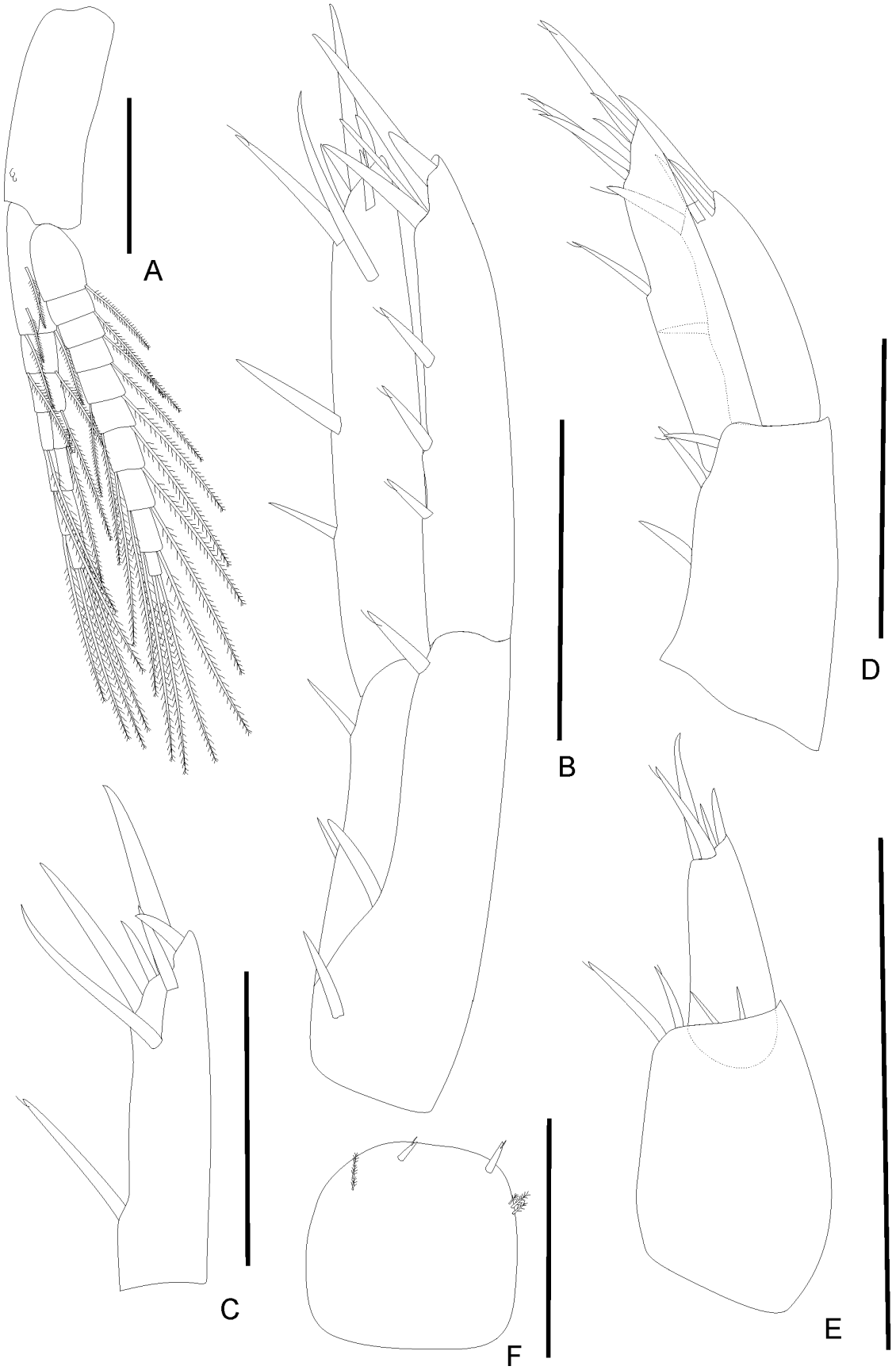

FIGURE 5. *Hyalella veredae* **sp. n.** Cardoso & Bueno (Male paratype, UFRGS 5542). A, pleopod; B, uropod 1; C, detail of curved seta of uropod 1; D, uropod 2; E, uropod 3; F, telson. Scale bars: 0.02 mm.

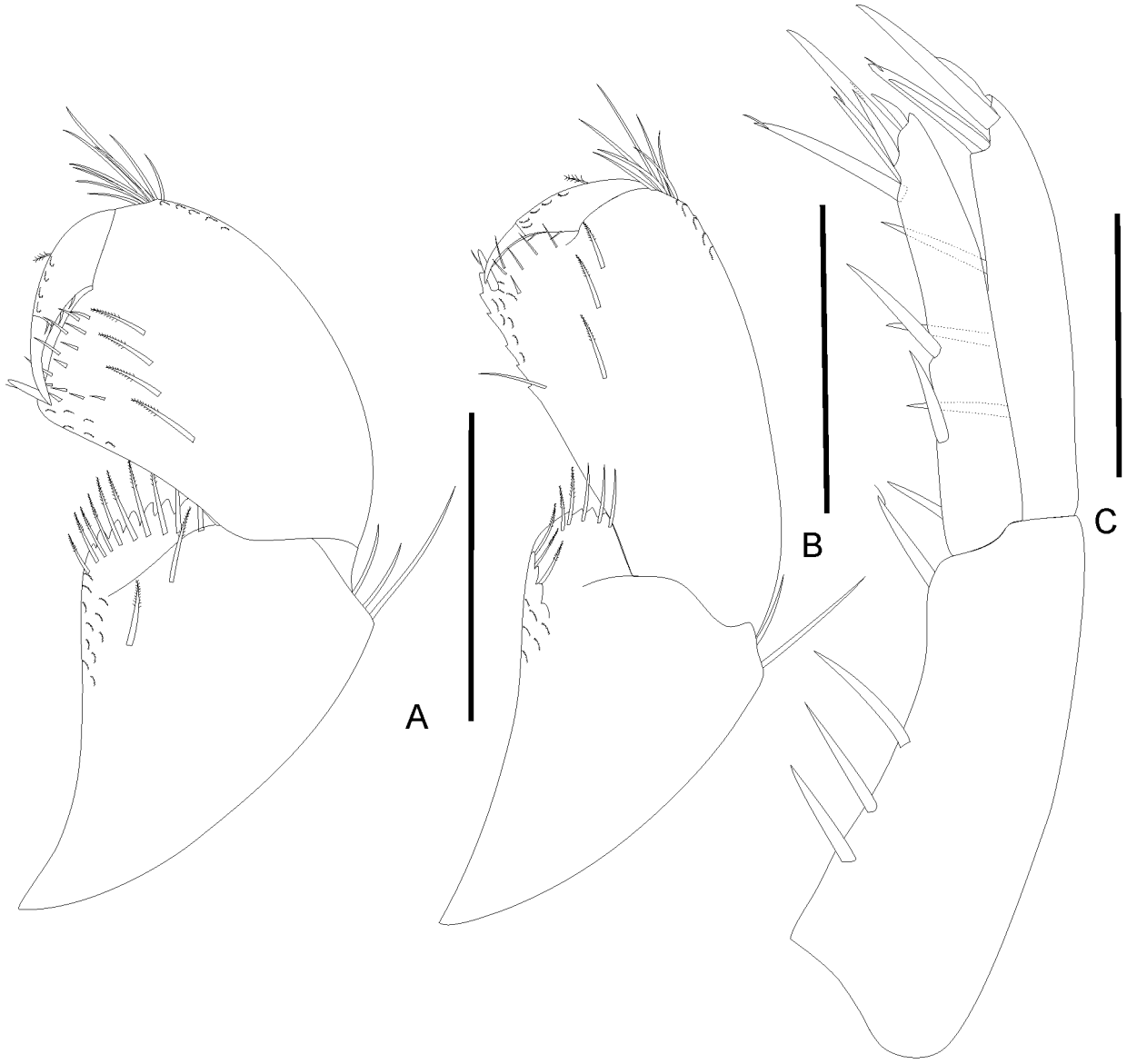

FIGURE 6. *Hyalella veredae* **sp. n.** Cardoso & Bueno (Female paratype, UFRGS 5542). A detail of carpus, propodus and dactylus of gnathopod 1 dorsal view; B, detail of carpus, propodus and dactylus of gnathopod 2; C, uropod 1. Scale bar: 0.02 mm.

Lower lip (Fig. 3F) lobes rounded, with setules on dorsal and ventral faces.

Maxilla 1 (Fig. 3G) inner plate shorter than outer plate, with two distal papposerrate setae, and several marginal setules. Outer plate with eight to nine serrate setae. Palp short, uniarticulated with few distal setules.

Maxilla 2 (Fig. 3H) inner and outer plates of similar sizes, inner plate with two papposerrate setae, five serrulate setae and several simple setae; outer plate with several simple distal setae.

Maxilliped (Fig. 3I) inner plate with three cuspidate distal setae and several pappose and simple setae; outer plate with simple setae on the margin; palp with four articles with several simple setae. Dactylus unguiform, smaller than third article, with simple seta and distal nail.

Gnathopod 1 (Fig. 4A) subchelate; basis, ischium and merus with simple setae; carpus longer than wider, longer than propodus, lobe posterior margin with polygonal pattern, two rows of denticles as comb scales and a row of serrate setae; propodus length 1.5 times the width (rectangular), hammer-shaped, inner face with five serrate setae, posterior margin with denticles as comb scales; dactylus claw-like with simple setae, one plumose seta and denticles as comb scales (Fig. 4B).

Gnathopod 2 (Fig. 4C) subchelate; basis, ischium and merus with simple setae on the posterior margin; carpus

wider than longer, lobe posterior margin elongated with polygonal pattern, two rows of denticles as comb scales and a row of serrate setae; propodus longer than wider (rectangular), posterior margin with denticles as comb scales, irregular palm, slope oblique strongly inclined, palm 1.1 times longer than posterior margin, margin convex with several simple setae and cuspidate setae with accessory seta (15-18); dactylus claw-like with a plumose seta (Fig. 4D).

Pereopods 3 to 7 (Figs. 4E and 4F) merus, carpus, propodus posterior margin with cluster of cuspidate setae with accessory seta; dactylus length 1/3 of propodus; propodus dorsal and distal margins with simple setae; pereopod 3 and 4 with similar sizes; pereopod 5 smaller than others; pereopod 6 smaller than pereopod 7, which is about 1.2 times longer than pereopod 3.

Pleopods (Fig. 5A) peduncle shorter than rami, with distal coupling spines; both rami with several plumose setae.

Uropod 1 (Fig. 5B) peduncle shorter than rami, with five cuspidate setae with accessory seta; inner ramus longer than outer ramus with two cuspidate setae on inner margin, one curved seta and five apical cuspidate setae (Fig. 5C); outer ramus with three cuspidate setae with accessory seta on inner margin, and apex with three cuspidate setae and a simple seta (U1 about 5 times the length of U3).

Uropod 2 (Fig. 5D) shorter than uropod 1; peduncle shorter than rami, with three cuspidate setae on the margin; inner ramus longer than outer ramus, margin with cuspidate seta with accessory seta, apex with three cuspidate setae and two cuspidate setae with accessory seta; outer ramus with a cuspidate seta with accessory seta on the margin, apex with four cuspidate setae, one with accessory seta.

Uropod 3 (Fig. 5E) shorter than uropod 2; peduncle longer than wider with four distal cuspidate setae with accessory seta; ramus uni-articulated, as long as peduncle, with three to four distal cuspidate setae.

Telson (Fig. 5F) as wide as long, with two apical cuspidate setae and three plumose setae laterally.

Coxal gills sac-like present on pereonites 2 to 6. Sternal gills tubular present on pereonites 2 to 7.

Female. Mean cephalothorax length: 0.4 ± 0.05 mm, mean body length: 4.5 ± 0.9 mm (n=3). Gnathopod 1 (Fig. 6A) shorter than gnathopod 2, but wider; carpus longer than wider, lobe posterior margin with polygonal pattern and denticles as comb scales on the margin, propodus longer than wider, inner face with four serrate setae. Gnathopod 2 (Fig. 6B) carpus longer than wider, lobe posterior margin with polygonal pattern and denticles as comb scales; propodus rectangular, longer than wider (2 times), inner face with three serrate setae. Uropod 1 (Fig. 6C) peduncle longer than rami, with four cuspidate setae; inner ramus with two cuspidate setae on the margin, apex with five cuspidate setae, three of them with accessory seta; outer ramus with three cuspidate setae on the margin and four distal setae, one of them with accessory seta.

Etymology. The specific epithet *veredae* refers to the location where the species was found.

Habitat and ecological considerations. The species was found in a freshwater underground stream, in Vereda da Palha Cave, located in the municipal district of Presidente Olegário, near Varjão de Minas city (Fig. 1A). The cave is situated in the karst area belonging to the Bambui group, which is extended through the states of Minas Gerais, Goiás, Tocantins and Bahia. The known cave extension corresponds to 1,300 meters, with at least three levels. The lower level is characterized by the presence of a drainage formed by a stream that descends in one of the cave entrances (Fig. 1B). No individual was observed in this drainage. Specimens were only found at the second level, which has a succession of travertines (Fig. 1C) filled by percolating water probably from epikarst compartments, especially in dry seasons. The main resource observed in these pools consisted of organic matter. In the first sampling (13.X.2010), dozens of individuals were observed swimming on small branches, in a very deep puddle. The water was crystal clear, allowing to observe the bottom and of the specimens. On the same visit, several couples were found in pre-copulatory behavior. On a subsequent visit to the cave (31.I.2011), only one specimen was found. On the occasion, the water from the travertines was quite turbid, indicating that the habitat changes over the year, not only receiving waters from the epikarst compartments. The absence of individuals in this occasion might indicate that the organisms are capable of migrating to other underground compartments, thus avoiding turbid waters.

Remarks. *Hyalella veredae* represents the first troglobitic species described for the state of Minas Gerais, south-eastern Brazil. *H. veredae* resembles *H. spelaea* due to the presence of reduced eyes, while in *H. caeca*, *H. imbya*, *H. anophthalma* and *H. muerta* the eyes are absent. However, a small part of the population (4.4%) did not present eyes. Culver *et al*. (1995) described a similar process in cave populations of *Gammarus minus* Say, 1818 that had different degrees of eyes reduction and different sizes of the antennae. These authors suggest that intraspecific variation may be consequence of time and degree of isolation of each population in underground

environments. In addition to the eyes, *H. veredae* shows different antenna sizes, in which antenna 1 and 2 are subequal in length, The shape of male gnathopod 2 of *H. veredae* is similar to *H. spelaea*, *H. anophthalma* and *H. muerta*, showing irregular palm, differing from *H. caeca* and *H. imbya,* both having a smooth palm. The palm inclination of male gnathopod 2 of *H. veredae* is similar to *H. imbya*, both showing palm strongly inclined. The presence of one curved seta in uropod 1 inner ramus is similar to *H. imbya*. Moreover, it was observed that the number of serrate setae on propodus gnathopod and on uropod 3 is different in *H. veredae* in comparison to other troglobitic species.

Hyalella formosa Cardoso & Araujo, sp. n.

(Figs. 7–10)

Type material. Holotype: male, cephalothorax length 0.4 mm, total length 5.6 mm, Brazil, Paraná state, Ponta Grossa municipality, Andorinhas Cave (25°08'39"S 49°55'58"W), MZUSP 28419; 24.VII.2011, Ferreira R.L. & cols. Paratypes: UFRGS 5543 (2 males on slides, 1 female on slide), UFLA 0260 (1 male, 1 female). All samples have the same data as holotype.

Diagnosis. Eyes absent. Antenna 1 flagellum with 11 to 13 articles, longer than antenna 2. Antenna 2 with eight to ten articles. Gnathopod 1 and 2 carpus posterior lobe with polygonal pattern and one row of serrate setae. Gnathopod 1 propodus oval shape, without projections on the posterior margin, inner face with five serrate setae. Gnathopod 2 propodus elongated, oval shape, without projections on the posterior margin; palm smooth, slope oblique, strongly inclined, longer than posterior margin; dactylus long, exceeding half length of propodus. Uropod 1 inner ramus of male with one curved seta and four apical cuspidate setae. Uropod 3 peduncle with one cuspidate seta distally; ramus with one cuspidate seta and two to three distal simple setae.

Description of male. (Figs 2E and 7A). Mean body length: 5.4 ± 0.9 mm (n=3); mean cephalothorax length: 0.4 ± 0.08 mm. Body surface smooth, epimeral plates not acuminate. Eyes absent.

Antenna 1 (Fig. 7B) longer than antenna 2; total length reaches fourth pereonite; peduncle not surpassing first pereonite; flagellum with 11 to 13 articles, each with one or two aesthetascs after article 2.

Antenna 2 (Fig. 7C) total length reaches fourth segment; peduncle not surpassing first pereonite; flagellum with eight to ten articles.

Upper lip (Fig. 7D) margin rounded, distal border covered by setules on dorsal and ventral faces.

Mandible (Fig. 7E) basic amphipodan (in the sense of Watling 1993), but without palp; incisor toothed; left lacinia mobilis with five teeth and setal row with two pappose setae, right mandible with three pappose setae; molar process broad and cylindrical with accessory seta.

Lower lip (Fig. 7F) lobes rounded, with setules on dorsal and ventral faces.

Maxilla 1 (Fig. 7G) inner plate shorter than outer plate, with two distal papposerrate setae and several setules on the margin. Outer plate with nine serrate setae; palp short, uniarticulated with one distal setae.

Maxilla 2 (Fig. 7H) inner and outer plate of similar sizes, inner ramus with two papposerrate setae, nine serrulate and several simple setae; outer plate with several simple setae and one long plumose seta distally.

Maxilliped (Fig. 7I) inner ramus with three cuspidate distal setae and several pappose and simple setae on the margin; outer ramus with simple setae and two serrate setae on the margin; palp with four articles with several simple setae; dactylus unguiform, shorter (1/2) than the article 3, with simple seta and distal nail.

Gnathopod 1 (Fig. 8A) subchelate; basis, ischium and merus with simple setae; carpus longer than wider, shorter than propodus, lobe posterior margin with polygonal pattern and one row of serrate seta; propodus length 1.5 times the width (rectangular), oval shape, without projections on the posterior margin, inner face with five serrate setae; dactylus claw-like with simple setae and one plumose seta (Fig. 8B).

Gnathopod 2 (Fig. 8C) subchelate; basis, ischium and merus with simple setae on the posterior margin; carpus wider than longer, lobe posterior margin with polygonal pattern and one row of serrate setae; propodus elongated, oval shape, without projections on the posterior margin, length 1.4 times the width (rectangular); smooth palm, slope oblique, strongly inclined, palm 1.5 times longer than posterior margin, with 12 to 20 cuspidate setae with accessory seta; dactylus claw-like, long, exceeding half the length of the propodus (Fig. 8D).

Pereopods 3 to 7 (Figs. 8E and 8F) merus, carpus, propodus posterior margin with cluster of cuspidate setae with accessory seta; dactylus length 1/3 of propodus; propodus dorsal and distal margins with simple setae; pereopod 3 and 4 with similar sizes; pereopod 5 smaller than others; pereopod 6 smaller than pereopod 7; which is about 1.5 times greater than pereopod 3.

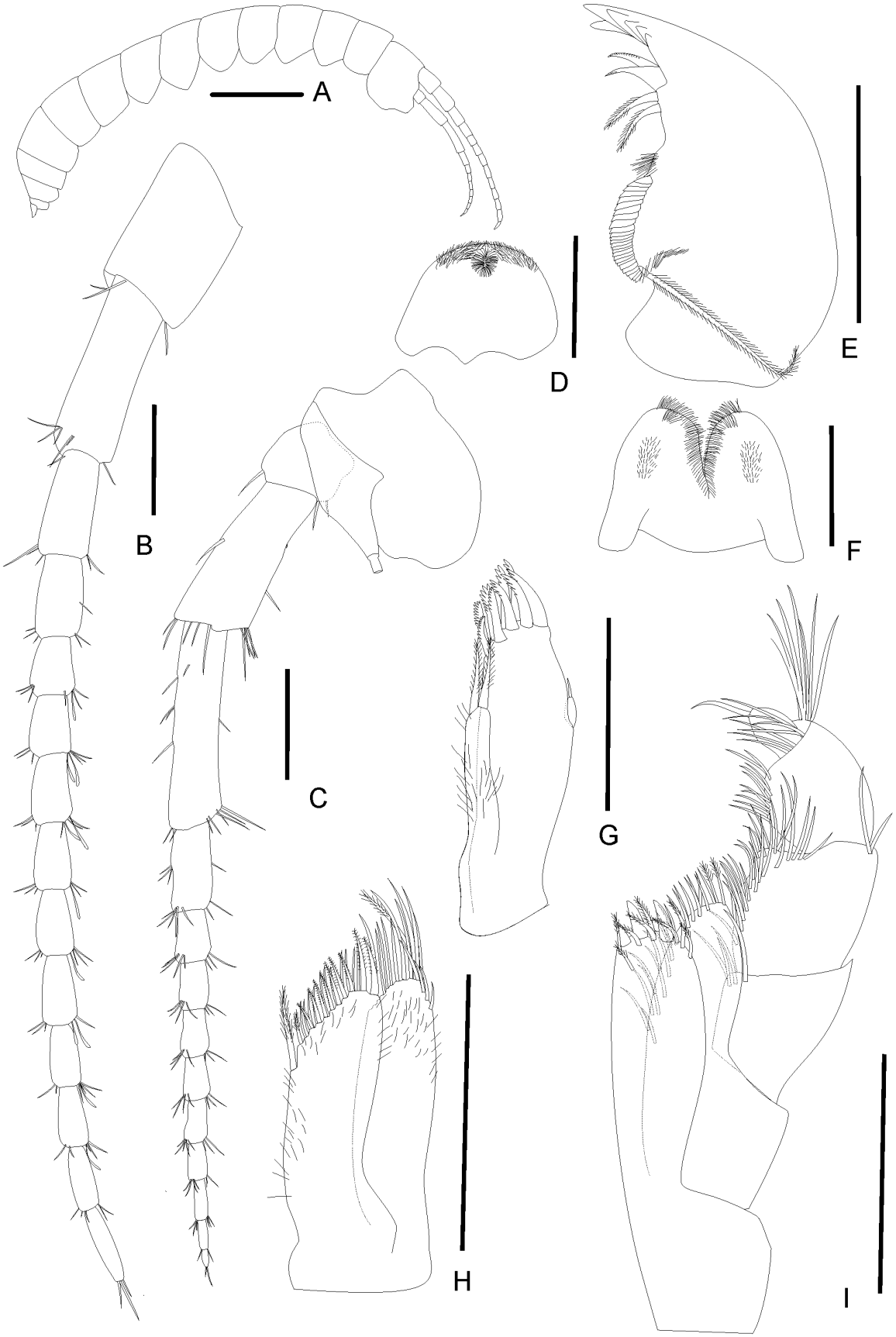

FIGURE 7. *Hyalella formosa* **sp. n.** Cardoso & Araujo (Male paratype, UFRGS 5543). A, habitus from holotype; B, antenna 1; C, antenna 2; D, upper lip; E, mandible; F, lower lip; G, maxilla 1; H, maxilla 2; I, maxilliped. Scale bars: (A) 1.0 mm, (B-I) 0.02 mm.

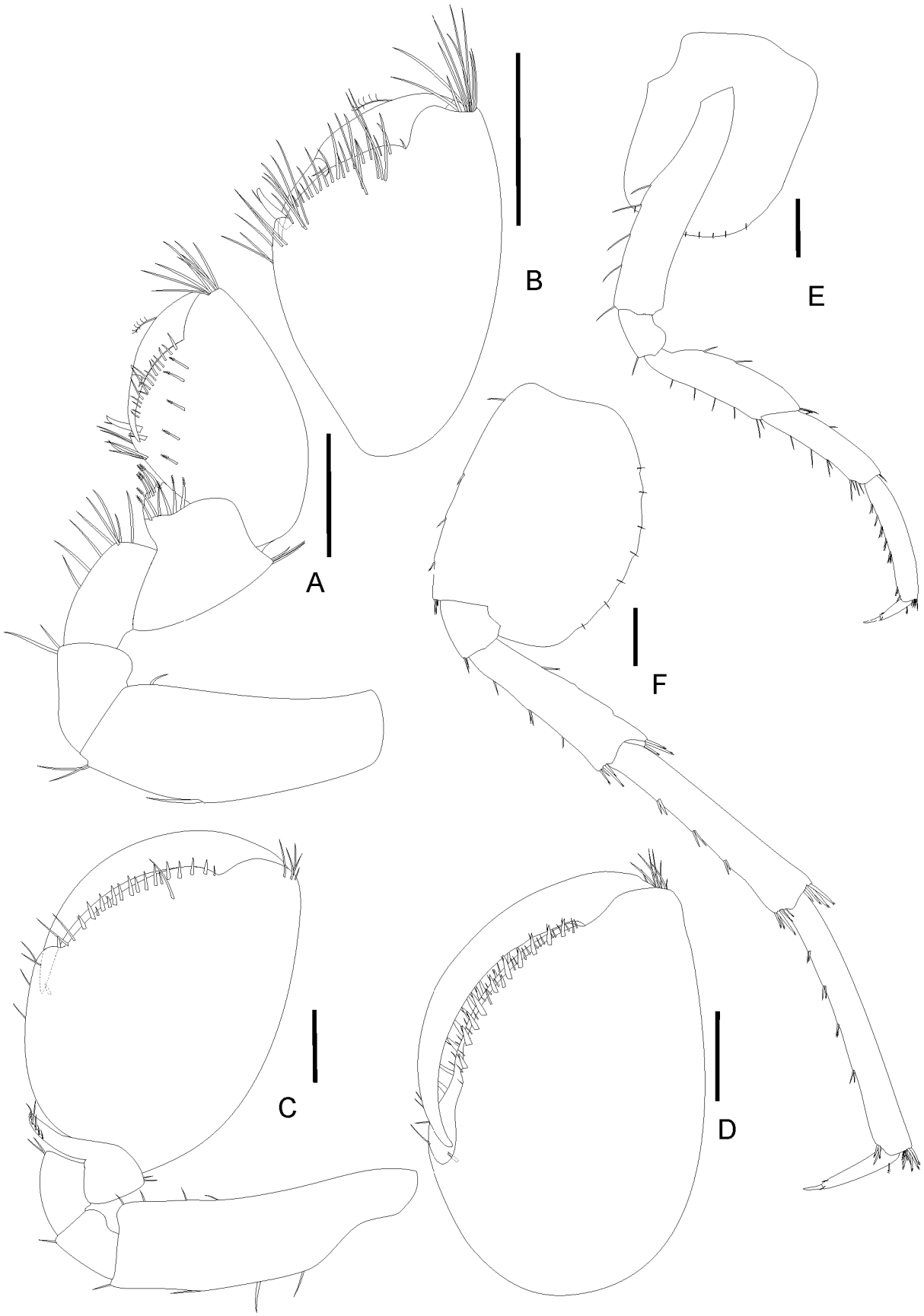

FIGURE 8. *Hyalella formosa* **sp. n.** Cardoso & Bueno (Male paratype, UFRGS 5543). A, gnathopod 1; B, detail of propodus and dactylus of gnathopod 1 dorsal view; C, gnathopod 2; D, detail of propodus and dactylus of gnathopod 2 ventral view; E, pereopod 3; F, pereopod 7.Scale bars:0.02 mm.

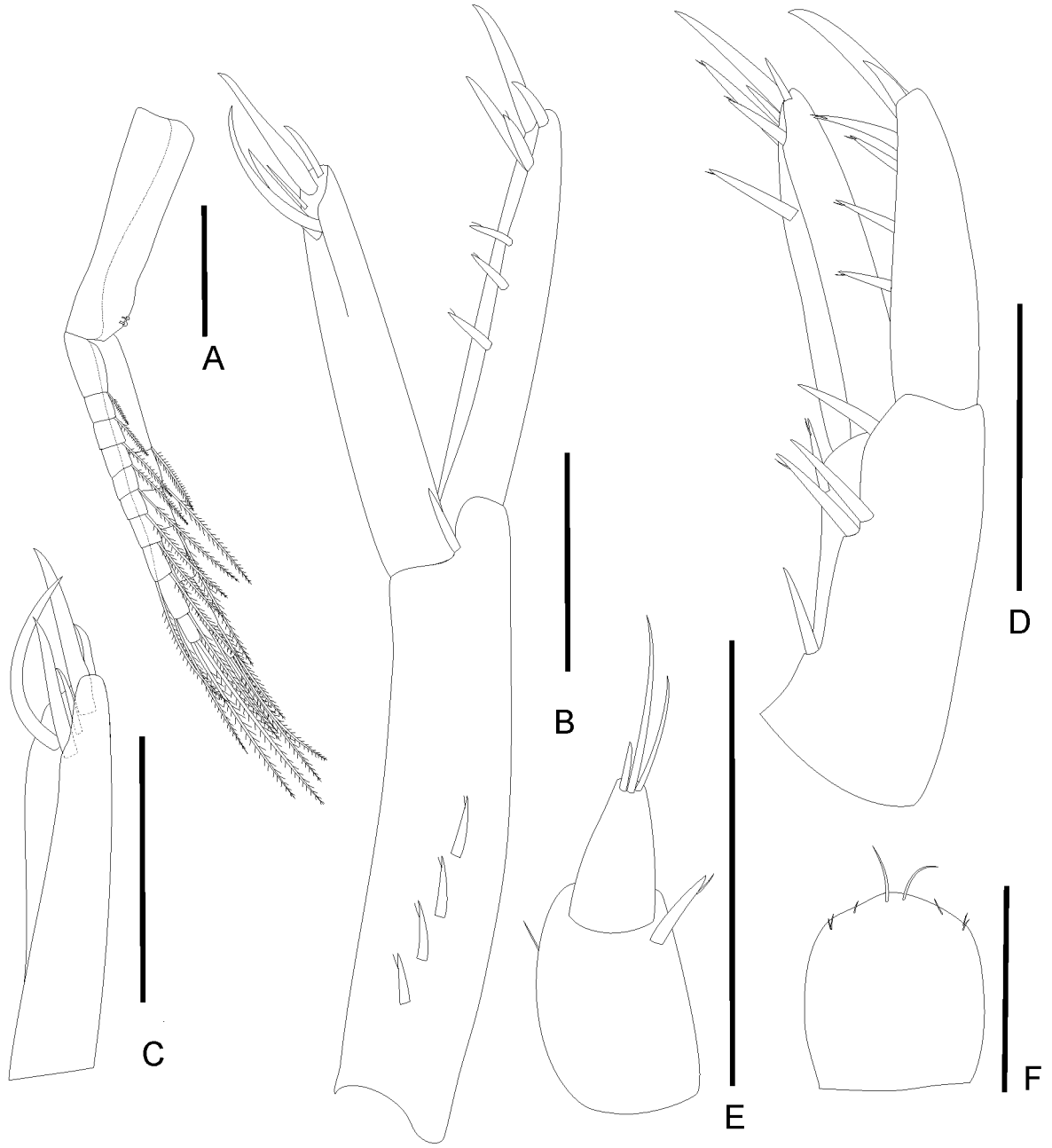

FIGURE 9. *Hyalella formosa* **sp. n.** Cardoso & Bueno (Male paratype, UFRGS 5543). A, pleopod; B, uropod 1; C, detail of curved seta of uropod 1; D, uropod 2; E, uropod 3; F, telson. Scale bars: 0.02 mm.

Pleopods (Fig. 9A) peduncle shorter than rami, with distal coupling spines; both rami with several plumose setae.

Uropod 1 (Fig. 9B) peduncle longer than rami, with four cuspidate setae with accessory seta; inner ramus with one curved seta and four apical cuspidate setae (Fig. 9C); outer ramus with three cuspidate setae with accessory seta on inner margin, and apex with four cuspidate setae, one of them with accessory seta (U1 about 5 times the length of U3).

Uropod 2 (Fig. 9D) shorter than uropod 1; peduncle with four cuspidate setae with accessory seta on the margin; inner ramus longer than outer ramus with one or two cuspidate setae with accessory seta on the margin, apex with five cuspidate setae with accessory seta; outer ramus with three cuspidate setae with accessory seta, apex with three to four cuspidate setae, two of them with accessory seta.

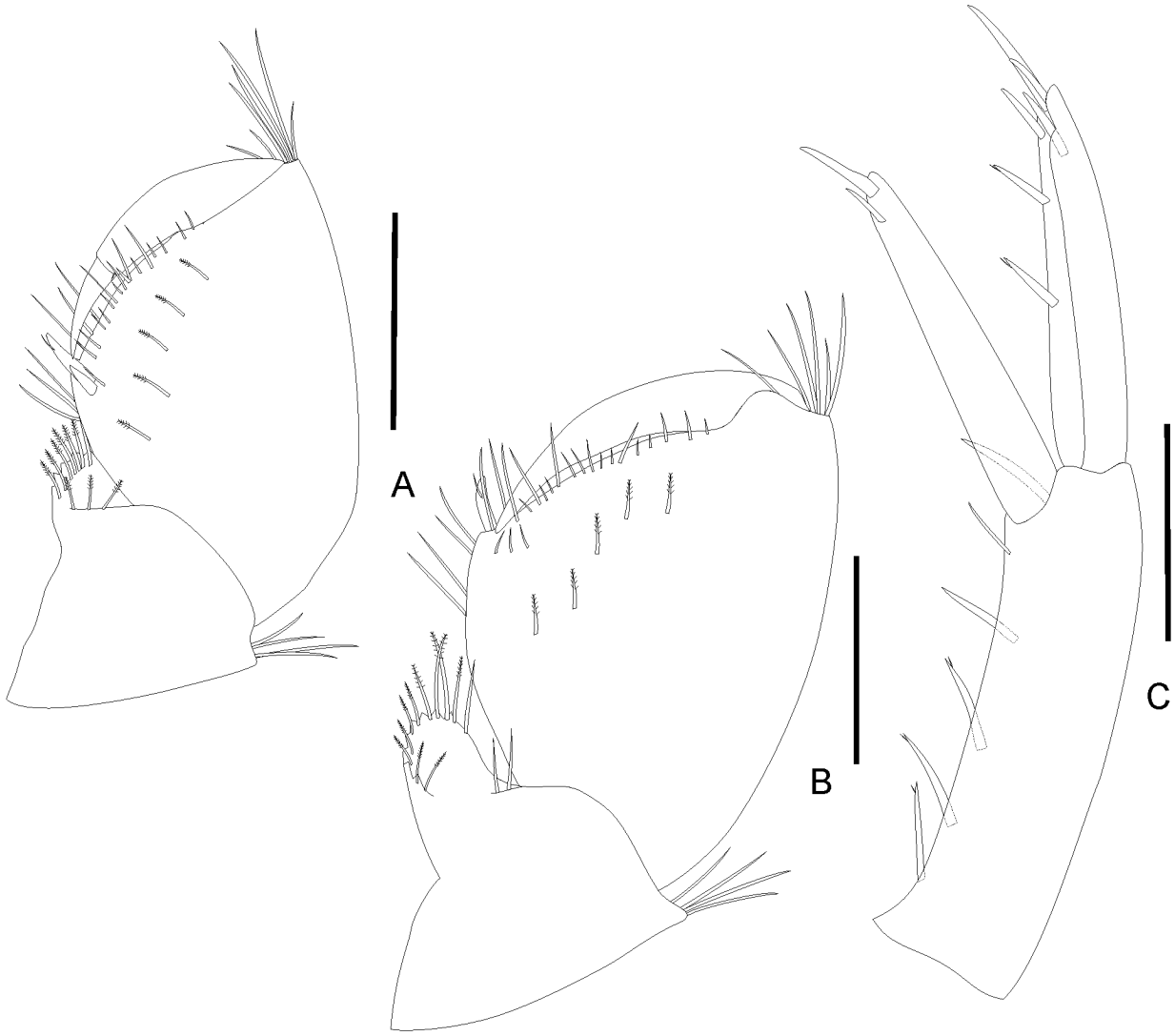

FIGURE 10. *Hyalella formosa* **sp. n.** Cardoso & Bueno (Female paratype, UFRGS 5543). A, detail of carpus, propodus and dactylus of gnathopod 1 dorsal view; B, detail of carpus, propodus and dactylus of gnathopod 2; C, uropod 1. Scale bar: 0.02 mm.

Uropod 3 (Fig. 9E) shorter than uropod 2, peduncle longer than ramus, longer than wide with one distal cuspidate setae; ramus uniarticulated with one cuspidate setae and two to three distal simple setae.

Telson (Fig. 9F) longer than wider, with two long simple setae and three short simple setae laterally.

Coxal gills sac-like present on pereonites 2 to 6. Sternal gills tubular present on pereonites 2 to 7.

Female. Cephalothorax length: 0.4 mm, body length: 5.0 mm (n = 1). Gnathopod 1 (Fig. 10A) smaller than gnathopod 2; carpus wider than longer, lobe posterior margin with polygonal pattern; propodus longer than wider, inner face with five serrate setae. Gnathopod 2 (Fig. 10B) similar to gnathopod 1, slightly larger, with carpus wider than longer, lobe posterior margin with polygonal pattern; propodus rectangular, longer than wider, inner face with five serrate setae. Uropod 1 peduncle longer than rami, inner margin with five cuspidate setae with accessory seta; inner ramus with two apical cuspidate setae; outer ramus with two cuspidate setae with accessory seta on the margin and apex with four cuspidate setae (Fig. 10C).

Habitat and ecological considerations. *Hyalella. formosa* was found in an underground lake in Andorinhas Cave, located near Ponta Grossa city (Fig. 2A). The cave is mainly composed of sandstone; its entrance is within a fracture covered by dense vegetation, while the surrounding is quite modified, especially due to agricultural activities (Fig. 2B). The cave comprises an elliptical hall approximately 140 meters long, its floor is strongly declined (Fig. 1D) and, in the deepest portion, there is the lake (Fig. 1C) where the species was found (Spinardi & Lopes, 1990). Dozens of individuals were observed swimming in the water column. There is organic material

composed by plant fragments that are flushed in from the outside by water during rainy periods. The water table appears to vary over the year so the configuration of the pond level may be variable depending on the season. Other fractures were observed in the region, although apparently not connected with the water table. Although the only possible habitat for this species appears to be Andorinhas Cave, it is quite likely that the distribution of the species is more extensive, comprising part of the water table in the area.

Remarks. The troglomorphic features found in *H. formosa*, such as the absence of eyes resembles *H. caeca*, *H. imbya*, *H. anophthalma* and *H. muerta*; and the antenna 1 being longer than antenna 2, resemble *H. imbya* and *H. muerta*. The propodus of gnathopod 2 with a smooth palm in *H. formosa* is similar to *H. caeca* and *H. imbya*, while in *H. spelaea*, *H. anophthalma* and *H. muerta* the palm is irregular. In addition *H. formosa* shares with *H. imbya* the strongly inclinated palm of gnathopod 2, the absence of denticles on the posterior margin of gnathopod 2 carpus (Fig. 23), the presence of one curved seta in uropod 1 and long pereopods 6 and 7. The new species has unique characteristics such as the shape of gnathopod 1 distinct from the hammer-shape type, which is present in all other species (González & Watling, 2003), as well as different numbers of setae on the gnathopod propodus, uropod 1 and 3.

Key to subterranean species of *Hyalella* from Brazil

1. Uropod 1 of male with curved setae on inner ramus. 2
- Uropod 1 of male without curved setae on inner ramus 4
2. Sternal gills present on pereonites 2–7 3
- Sternal gills present on pereonites 3–7 . . . . . *H. imbya*
3. Propodus of gnathopod 2 with irregular edge and posterior margin with comb scales . . . . . *H. veredae*
- Propodus of gnathopod 2 with regular edge and posterior margin without comb scales . . . . . *H. formosa*
4. Eyes present, but reduced . . . . . *H. spelaea*
- Eyes absent . . . . . *H. caeca*

Discussion

Researches carried out in underground habitats have certainly contributed to the increased knowledge on the diversity of amphipods in Brazil, revealing new species and also allowing inferences about the species richness and evolution of this group in South America (Fišer *et al*., 2013).

The apparent low richness of freshwater amphipods in South America has intrigued researchers for decades. However, new families recently recorded in Brazil showed that the diversity in South America is far from being low (Fiser *et al*., 2013).

In the last decade, taxonomic studies about *Hyalella* have grown substantially in Brazil, adding nine new species to the group, which currently has 17 species (Bueno *et al.*, 2013). The description of two new species of hypogean *Hyalella* brings the number of troglomorphic species known for Brazil to five species (25%), highlighting that the diversity of species in the genus is certainly underestimated.

Both species are restricted to their respective underground habitats, which are vulnerable to external threats, since the regions have a high degree of degradation due to agricultural activities. Such activities not only remove the original vegetation for cultivation but also often bring out chemicals for soil remediation, pest control or fertilization. These practices often alter the natural supply of nutrients to the caves, and may also cause pollution of underground drainages. Consequently, the dynamics of underground environments can be altered, affecting the survival of their fauna. A situation that exacerbates these threats is the lack of information on the extent of the distribution and ecology of the new species described here, as it occurs for most troglobitic species present of this group.

Up to the year 2008, all Brazilian caves were fully protected by law. With legislation changes that occurred in 2008, the caves are no longer out of reach and are now highly vulnerable to various human activities. In order to determine which caves may be obliterated and which should be preserved, categories of relevance were created based on geological and biological parameters. For a cave to reach the maximum level of relevance and be entirely preserved, the law requires the presence of an endemic or rare troglobitic species. In this context, the description of *H. veredae* and *H. formosa*, not only represents a contribution to the knowledge of the genus, but also ensures the

permanent preservation of the caves in which they occur, ensuring the continuity of these so endangered and unique species.

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