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Short Communication

Expanding the knowledge of *Hyalella* Smith, 1874 (Amphipoda: Hyalellidae) for southeast Brazil: two new troglophilic species from caves of the Atlantic Rainforest

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ABSTRACT

The present work describes two new species of *Hyalella* Smith, 1874 from two different state parks in Southeastern Brazil. Both species were collected in well-preserved and conserved areas of the Atlantic Rainforest, the first from the Parque Estadual Intervales (PEI) and the other from the Parque Estadual Turístico do Alto Ribeira (PETAR). The two species are troglophilic, i.e., facultative cave-dwellers. They are found inside the caves but do not present any noticeable adaptation to life underground. *Hyalella bocaina* sp. nov. most distinguishable features include the palp of maxilla 1 reaching almost half the distance between base of the palp and base of setae on the outer plate, gnathopod 1 propodus inner face with serrate setae, peduncle of the pleopod covered with several setules, uropod 1 of the male without curved seta, and the uropod 3 shorter than the telson. *Hyalella temimina* sp. nov., although found exclusively inside of the Temimina II cave, does not present troglomorphisms, and its main characteristics are the relatively long palp of maxilla 1, the rich ornamentation of the telson, gnathopod 1 propodus inner face with pappose setae, uropod 1 of the male without curved seta, and the excavation of the apex of the ramus of uropod 3. Here we increase the number of *Hyalella* species known for Brazil from 42 to 44.

KEYWORDS:

Cave fauna, diversity, freshwater amphipods, morphology, taxonomy

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INTRODUCTION

The genus *Hyalella* Smith, 1874 is restricted to the Nearctic and Neotropical biogeographic regions and is found from southern Canada to Patagonia (Bueno et al., 2014). The genus is exclusively freshwater (e.g., usually found associated with vegetation of wetlands or streams) adapted in both epigean (superficial) and hypogean (subterranean) environments (Grosso and Peralta, 1999). It is also the only known freshwater amphipod genus in Brazil to occur in superficial environments (Väinölä, 2008). There are 42 species described from Brazil (Penoni et al., 2021; Reis et al., 2020; 2023; Rangel et al., 2022; Limberger et al., 2022; 2024), eight of them are known from the state of São Paulo: *Hyalella caeca* Pereira, 1989; *Hyalella meinerti* Stebbing, 1899; *Hyalella warmingi* Stebbing, 1899; *Hyalella dielaii* Pereira, 2004; *Hyalella spelaea* Cardoso, Bueno and Ferreira, 2011; *Hyalella epikarstica* Rodrigues, Bueno and Ferreira, 2014; *Hyalella bala* Penoni, Lares and Bueno, 2021; and *Hyalella virgineae* Penoni, Lares and Bueno, 2021 (Serejo and Siqueira, 2018; Penoni et al., 2021). Here we expand the records of the genus for Brazil, with the description of two new species of *Hyalella* from the state of São Paulo.

MATERIAL AND METHODS

Study area

The Alto Ribeira (Upper Ribeira Valley) karst area is defined geologically by the presence of metasediments, deposited in a Pre-Cambrian tectonically active environment. The area is situated in the transition between the Rainforest and the Araucaria Forest domains, the climate is subtropical humid, without a typical dry season, and the mean annual temperature is around 18–19 °C (Nimer, 1989). The area is mostly protected by two contiguous conservation units, the Parque Estadual Intervales-PEI, and the Parque Estadual Turístico do Alto Ribeira-PETAR.

The aim of this study is to describe two new *Hyalella* species from two Southeastern Brazilian state parks (Fig. 1A). There are two populations from PEI (24°16'18.4" S 048°25'29.3" W and 24°16'34.7"S 048°27'22.7"W) for the first species. The first population was collected in 2000 in the rapids of the water course of Roda d'Água tourist trail, Bocaina River, municipality of Ribeirão Grande, state of São Paulo, along with other benthic invertebrates. The other population was sampled in 2009 in the Gruta do Minotauro (Fig. 1B) cave. The collections occurred during a speleological management plan project. This park is located in the Paranapanema Basin, an important Atlantic rainforest ecological corridor (De Paula Paciência, 2011).

The second new species was collected at Parque Estadual Turístico do Alto Ribeira – PETAR (24°22'36.8"S 048°33'56.2"W), at Temimina II cave (Fig. 1C), municipality of Apiaí, state of São Paulo. The collections occurred in 2009 during the speleological management plan project. Due to the small populations, only a few specimens were collected and the Fundação Florestal (FF) and ICMBIO granted the authorization for collections to MEB (COTEC and SISBIO 20165, respectively).

The specimens at both localities were collected using manual nets, fixed with 70% ethanol, and stored in labeled bottles until the time of preparation of slides.

Morphological description of the new species

For dissection and slide preparation, males and females of both species were colored with the "red congo" dye. Considering the small size of the population of Temimina II cave, only one male and one female were dissected for morphological description. All dissected specimens were measured (head and body length) under a Zeiss 2000-C stereomicroscope with an associated ocular micrometer. Body length was measured from the insertion of antenna I to the last segment base before the telson and the head was measured from the insertion of antenna I to before the beginning of the first thoracic segment.

Slides were photographed using a Zeiss AxioCam ERc5s camera (Carl Zeiss) coupled to a Carl Zeiss Primo Star microscope, and the resulting photographs were used as a guide for the illustrations in CorelDraw X7 software. The description of setae and cuticular structures followed Zimmer et al. (2009), and the appendage names follow Rogers et al. (2020).

Type materials for both new species are deposited in the Museu de Zoologia, Universidade de São Paulo (MZUSP), São Paulo, Brazil; Coleção de Crustáceos, Universidade Federal de Lavras (CCUFLA), state of Minas Gerais, Brazil; and at the Zoological Collection

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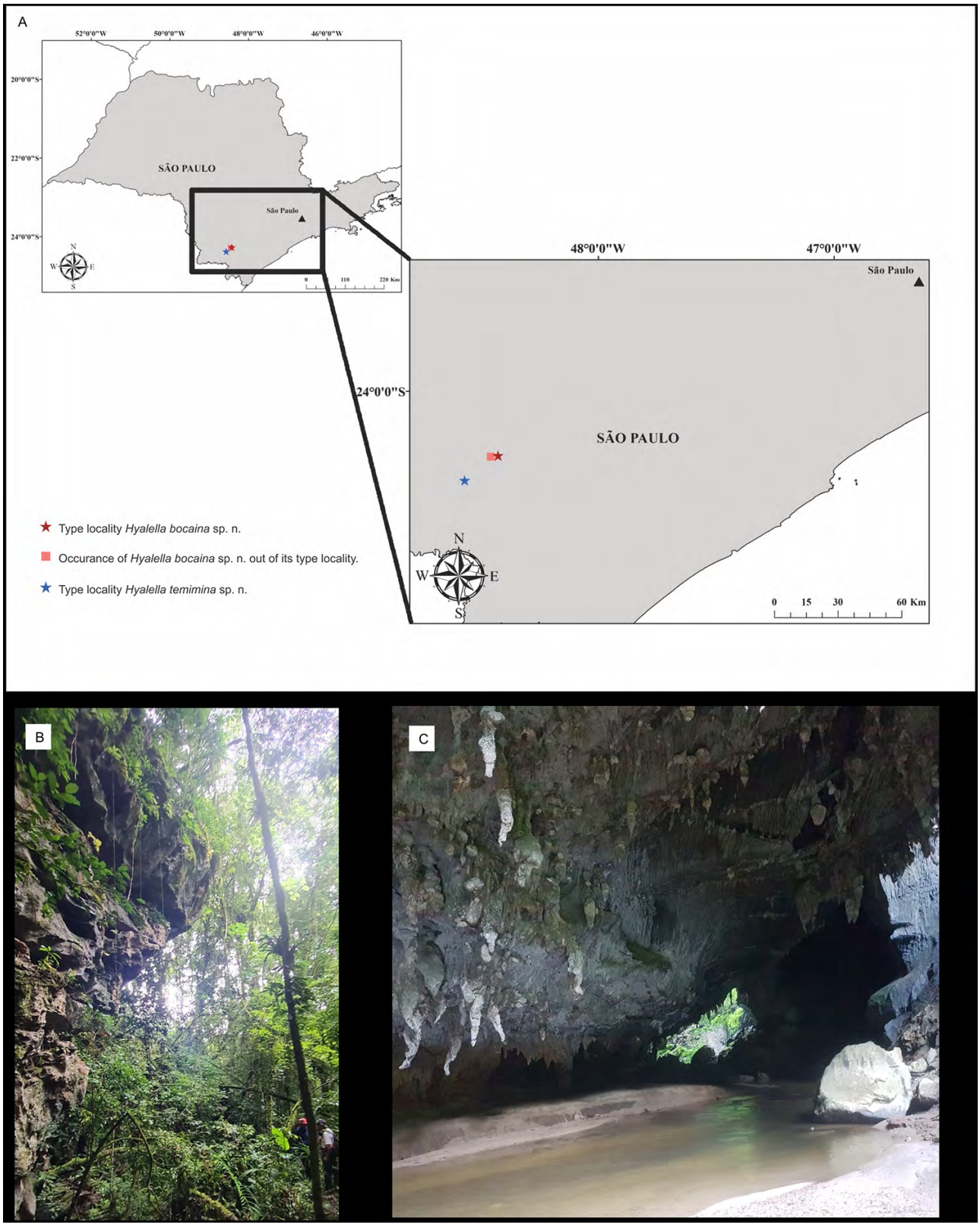

Figure 1. Collection sites of *Hyalella bocaina* sp. nov. and *Hyalella temimina* sp. nov. **A,** Map indicating collection sites of the new *Hyalella* species; **B**, Gruta do Minotauro cave, where one of the populations of *H. bocaina* sp. nov. was collected; **C**, Temimina II cave, type locality of *H. temimina* sp. nov. Photo authors: (**B**) J. Antunes; (**C**) J.S. Gallão.

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of Laboratório de Estudos Subterrâneos (LES), Universidade Federal de São Carlos, state of São Paulo, Brazil.

SYSTEMATICS

Order Amphipoda Latreille, 1816

Suborder Senticaudata Lowry and Myers, 2013

Family Hyalellidae Bulycheva, 1957

Genus *Hyalella* Smith, 1874

Hyalella bocaina sp. nov. Deotti, Penoni and Bueno

(Fig. 2–8)

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Type material. Holotype: adult male, (body length [L] 10.29 mm; head length [HL] 0.69 mm), MZUSP 45191, Brazil, state of São Paulo, municipality of Ribeirão Grande, Parque Estadual Intervales (24°16'18.42"S 048°25'29.3"W), March 2000, P.C. Bispo coll. — Paratypes: 1 adult female (L 9.8 mm; HL 0.50 mm), MZUSP 45192; 31 whole individuals and 3 males and females on slides CCUFLA 453 (same collection data as the holotype); 1 male on slides, CCUFLA 454; Brazil, state of São Paulo state, municipality of Iporanga, Parque Estadual Intervales, Gruta do Minotauro cave entrance (24°16'34.7"S 48°27'22.7"W), October 2009, M.E. Bichuette; T.L.C. Scatolini; A. Pereira and D. Monteiro-Neto colls.

Diagnosis. Body surface smooth. Eyes round, pigmented. Epimeron 1 and 3 postero-distal margin acute (acute angle). Antenna 2 not half the body length. Maxilla 1 palp longer than wide, reaching almost half of distance between base of palp and base of setae on outer plate. Gnathopod 1 propodus inner face with 10 serrate setae, anterior and posterior margins with comb-scales. Pleopods with 2 coupling spines and several setules present on margins. Uropod 3 shorter than telson, peduncle longer than ramus, with 6 cuspidate setae with accessory seta and ramus with distal cuspidate seta with accessory seta and some simple setae. Telson with 2 to 4 apical simple and symmetrical setae, with 3 simple setae on both sides, laterally. Coxal gills sac-like and present on segments 2 to 6. Sternal gills present on segments 3 to 7.

Description of male (Fig. 2A). Mean body length: 8.7 ± 1.3 mm ($N = 7$); mean head length: 0.68 mm ± 0.16 mm ($N = 7$). Body surface smooth. Epimeral plates 2–3 accuminate (Fig. 4B). Coxae 1–4 subequal in size and shape, slightly overlapping. Coxa 1 similar to 2 and 3. Coxa 4 longer than wide, excavated posteriorly. Coxa 5 posterior lobe narrower than anterior lobe. Coxa 6 and 7 longer than wide. Eyes round and pigmented.

Antenna 1 (Fig. 3A) about 3.8 × smaller than body length, 1.4 × smaller than antenna 2, 1.6 × longer than peduncle of antenna 2; peduncle 1.4 × longer than head; article 1 1.2 × longer than 2, article 3 1.1 × shorter than 1 and 1 × shorter than article 2; flagellum with 14 articles, 1.7 × longer than peduncle; aesthetascs (Fig. 3A, highlighted) occurring on flagellum from article 3 distally.

Antenna 2 (Fig. 3B) 2.6 × smaller than body length; peduncle slender, 2.2 × longer than head; article 4 with similar size than article 5; flagellum with 17 articles, 1.4 × longer than peduncle.

Mandible without palp; incisor toothed; left (Fig. 3C) mandible with 5 teeth and setal row with 5 pappose setae and some setules on apical margin, lacinia mobilis with 3 teeth, molar process with accessory seta; right mandible (Fig. 3D) with abundant setules on apical margin, setal row with 3 pappose setae; broad and cylindrical molar process with accessory seta.

Upper lip (Fig. 3E) margin rounded; distal border covered by setules on ventral and dorsal face. Lower lip (Fig. 3F) outer lobes rounded and distally notched, with several setules on dorsal and ventral face.

Maxilla 1 (Fig. 3G) inner plate slender, with 2 pappose setae and several setules distally. Outer plate with 9 papposerrate setae apically. Palp short, uniarticulate, longer than wide, reaching almost half of distance between base of palp and base of seta on

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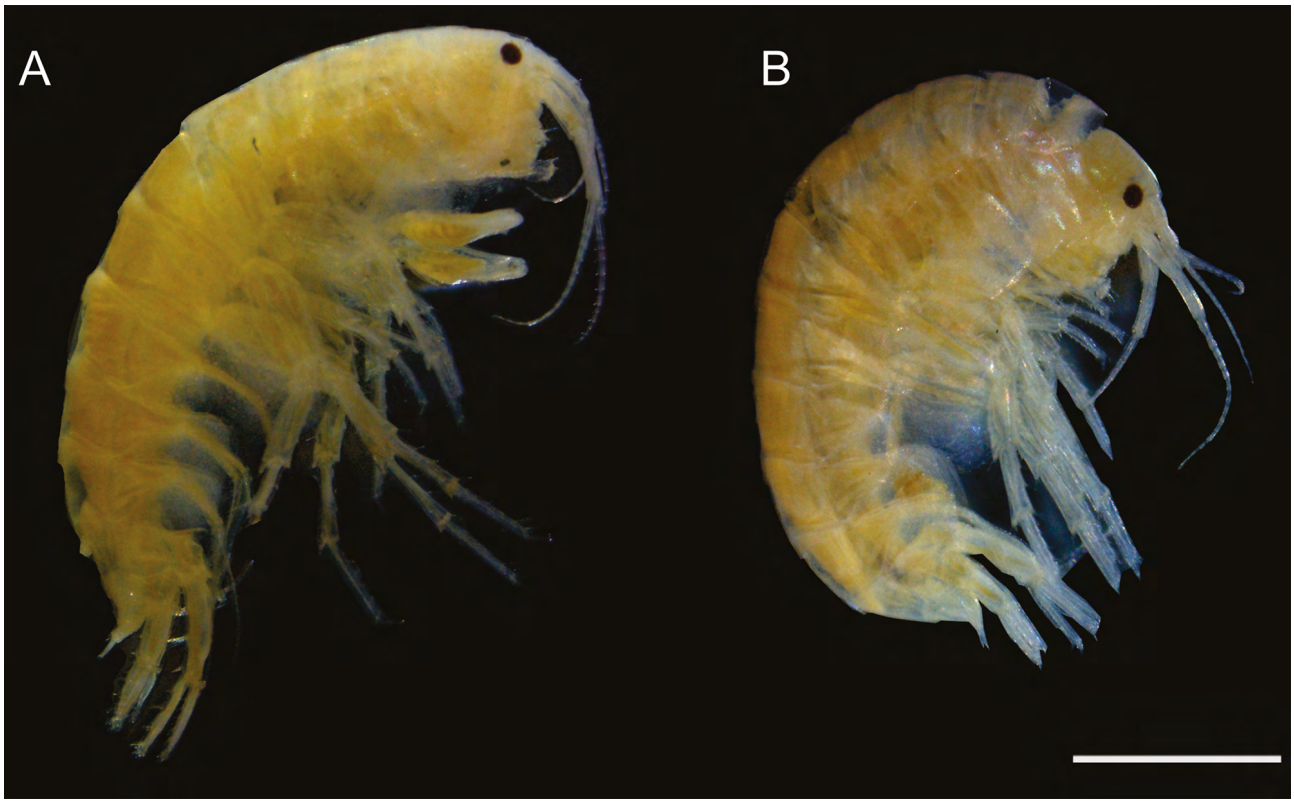

Figure 2. *Hyalella bocaina* sp. nov., Parque Estadual Intervales, state of São Paulo, Brazil. **A,** Holotype, male, 10.29 mm (MZUSP 45191); **B,** paratype, female, 9.8 mm (MZUSP 45192). Scale bars = 2 mm.

outer plate, with distal short seta and some setules on margin.

Maxilla 2 (Fig. 3H) inner plate subequal to outer plate, inner plate with 1 papposerrate seta, 6 pappose setae and several simple distal setae; outer plate with 3 pappose setae and several simple distal setae; inner and outer plates covered by several setules.

Maxilliped (Fig. 4A) inner plate with 3 cuspidate distal setae and several pappose setae, without comb-scales; outer plate 1.4 × smaller than inner plate, with several simple setae, without comb-scales; palp longer than outer plate and subequal to inner plate, with 4 articles, covered with several simple long setae on the inner margin; article 1 outer margin with few simple setae; article 2 outer margin with few long simple setae; article 3 outer margin with few long pappose setae, without comb-scales; article 4 (dactylus) unguiform, 2.2 × shorter than third article, with some pappose setae, shorter than nail, without comb-scales, and distal nail present. Variation: one specimen was also found with 7 cuspidate distal setae and several pappose setae on inner plate; outer plate with several simple and few pappose setae; article 2 of palp with some serrate setae apically on outer margin; article 4 (dactylus) with serrate setae instead of pappose.

Gnathopod 1 (Fig. 5A) subchelate; coxal plate 1.8 × wider than longer, with several short simple setae on margin; basis and ischium with dorsal and apical serrate setae and comb-scales on posterior margin of ischium; merus with some pappose setae on distal margin and polygonal pattern present; carpus 1.8 × longer than wide, 1.3 × longer than propodus, with lateral distal lobe produced, with 5 serrate setae, without comb-scales; posterior lobe, with polygonal pattern and pectinate border expressed, without comb-scales and with several serrate setae on margin and 4 pappose setae on inner face; propodus 1.4 × longer than wide, hammer-shaped, with several simple long setae on disto-anterior margin and polygonal pattern present, inner face with 10 serrate setae, with few simple setae and comb-scales absent; palm slope transverse, with row of many simple setae, posterior distal corner with 1 long and strong cuspidate seta with accessory seta, polygonal pattern present, and comb-scales absent; dactylus claw-like, polygonal pattern present on disto-anterior margin, terminal simple setae

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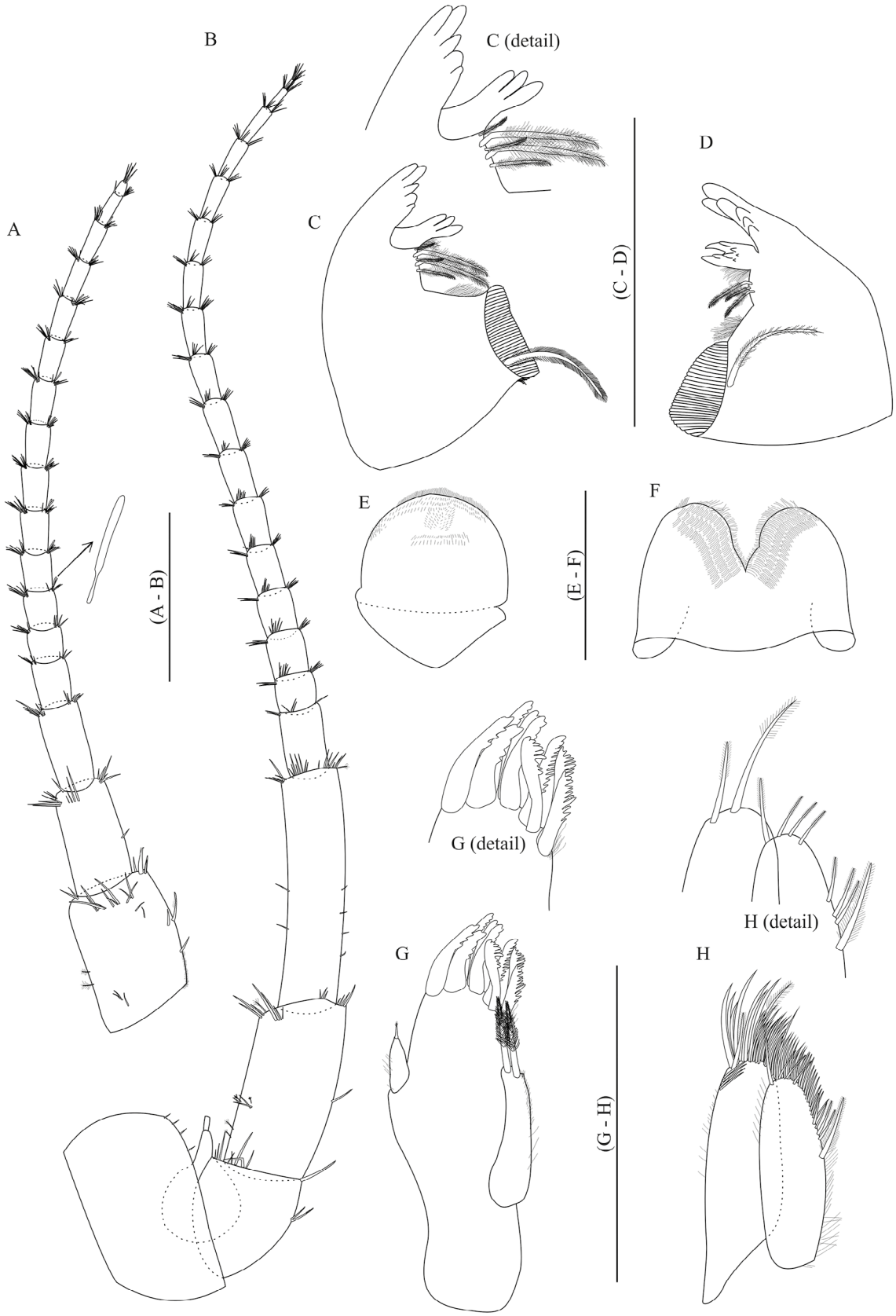

Figure 3. *Hyalella bocaina* sp. nov., holotype, male, CCUFLA 453. **A**, Antenna 1; **B**, antenna 2; **C**, left mandible; **D**, right mandible; **E**, upper lip; **F**, lower lip; **G**, maxilla 1; **H**, maxilla. Scale bars = 0.5 mm.

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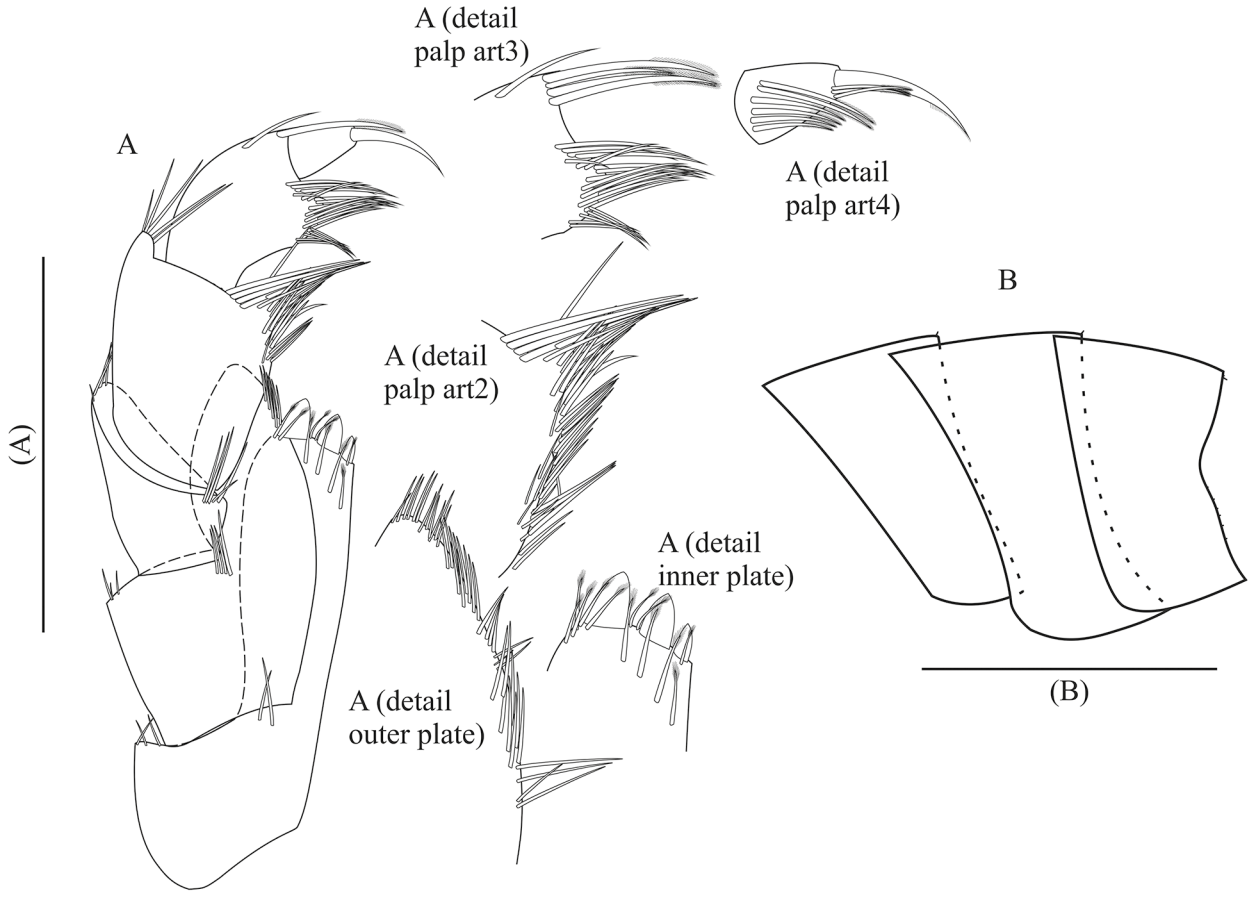

Figure 4. *Hyalella bocaina* sp. nov., holotype, male, CCUFLA 453. **A**, Maxilliped; **B**, epimeron. Scale bars = (**A**) 0.5 mm; (**B**) 2 mm.

and polygonal pattern present on proximal margin, with 1 plumose seta dorsally.

Gnathopod 2 (Fig. 5B) subchelate; coxal plate 1.8 × wider than long, with simple setae on margin; basis and ischium with several serrate setae and polygonal pattern on posterior margin of ischium; merus with few serrate setae on posterior margin and polygonal pattern; carpus 3.6 × wider than long, posterior lobe slim produced between merus and propodus, forming scoop-like structure, margin with several pappose setae, polygonal pattern, pectinate border and serrate setae on lateral distal lobe, without comb-scales; propodus ovate, 1.4 × longer than wide, comb-scales absent; palm shorter than posterior margin of propodus, slope transverse, margin with 1 row of several cuspidate setae with accessory seta and simple setae, posterior distal corner with 2 long and strong cuspidate setae and with cup for dactylus, polygonal pattern present; dactylus claw-like, congruent with palm, plumose seta dorsally, and terminal simple setae and polygonal pattern present on the proximal and disto-anterior margin.

Pereopods 3 to 7 (Fig. 6A–E) simple. Pereopods 3 and 4 merus and carpus posterior margin with several serrate and simple setae and some simple with accessory seta in carpus; propodus posterior margin of pereopod 3 and 4 with some simple setae and several cuspidate with accessory seta; dactylus 3.6 × and 4.1 × shorter than propodus, respectively, with plumose seta dorsally. Pereopods 5 to 7 merus, carpus and propodus posterior margin with several cuspidate setae some of them with accessory seta and few simple setae, dactylus 4.0 ×, 3.9 ×, and 4.3× shorter than propodus, respectively, with plumose seta dorsally. Pereopod 3 and pereopod 5 similar in size; pereopod 4 smaller than others; pereopod 6 longer than pereopod 7.

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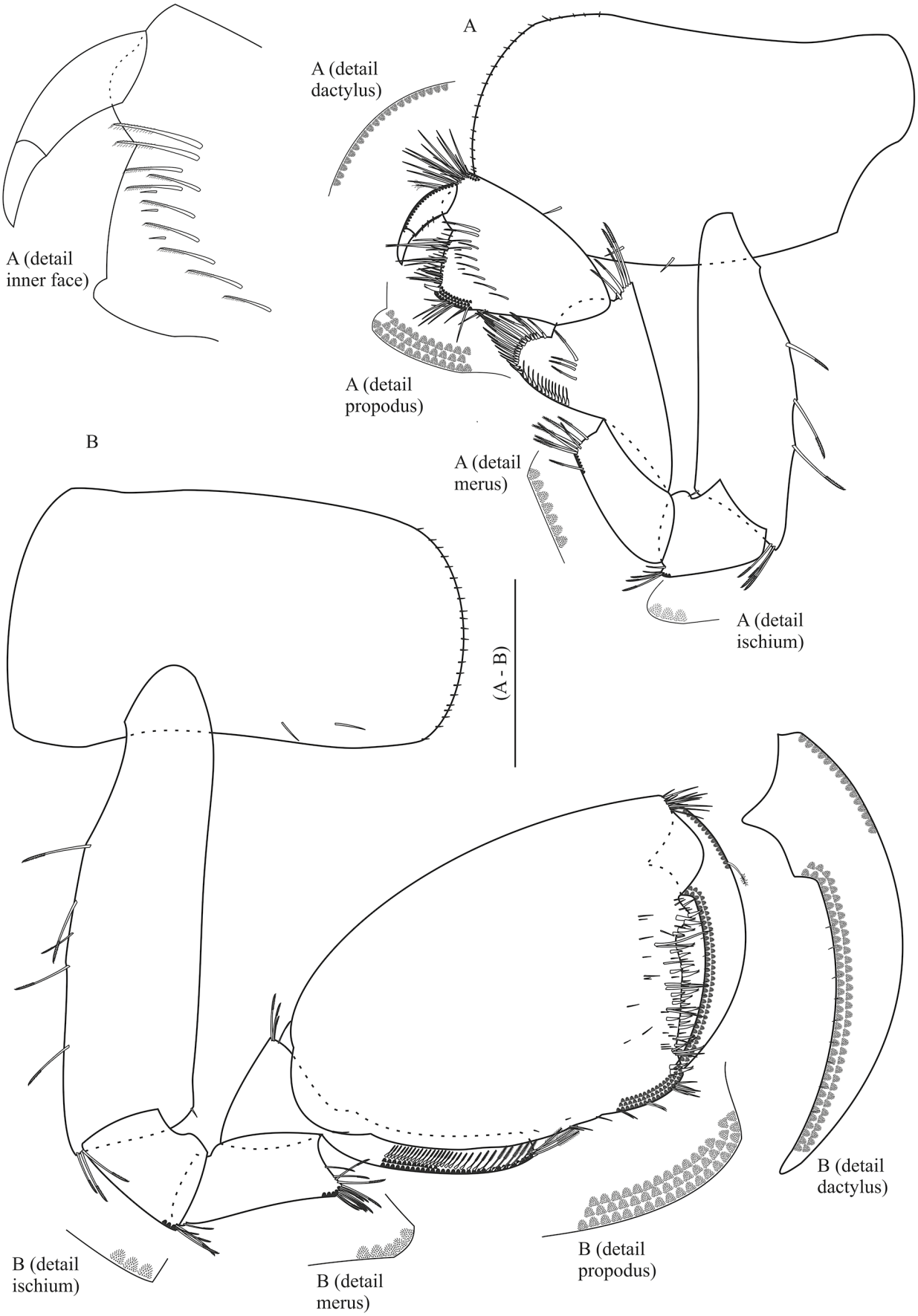

Figure 5. *Hyalella bocaina* sp. nov., holotype, male, CCUFLA 453. **A**, Gnathopod 1; **B**, gnathopod 2. Scale bars = 0.5 mm.

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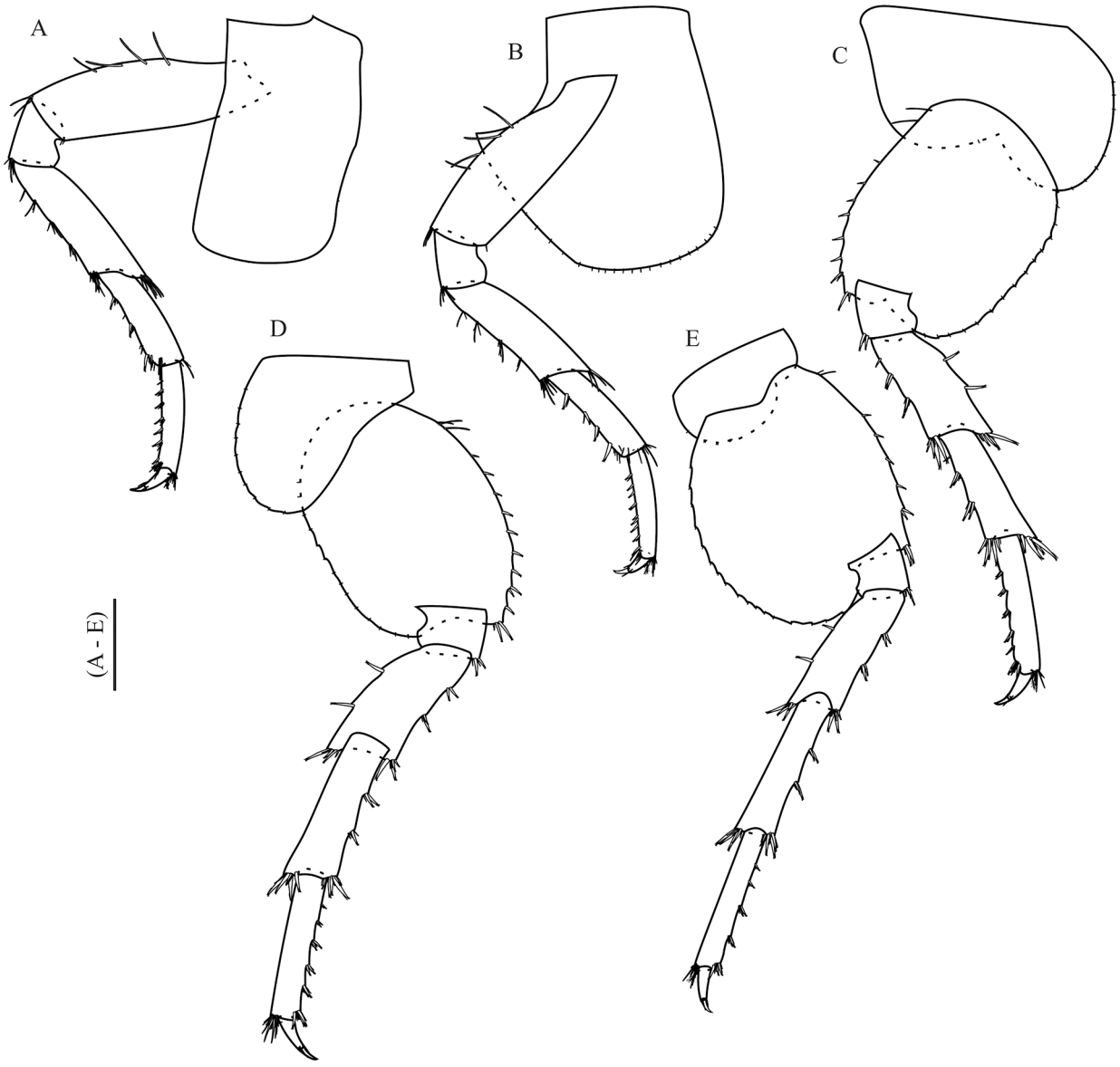

Figure 6. *Hyalella bocaina* sp. nov., holotype, male. CCUFLA 453. **A**, Pereopod 3; **B**, pereopod 4; **C**, pereopod 5; **D**, pereopod 6; **E**, pereopod 7. Scale bars = 0.5 mm.

Pleopods (Fig. 7A) peduncle 3.3 × longer than wide, 1.7 × mean size of rami, with 2 coupling spines and several setules present on margins; both rami with several plumose setae.

Uropod 1 (Fig. 7B) peduncle 3.1 × longer than outer ramus and subequal to inner ramus, with 4 cuspidate setae with accessory seta and 1 simple seta; inner ramus slightly longer than outer ramus, with 3 dorsal cuspidate setae with accessory seta on margin and 7 cuspidate setae with accessory seta apically, without curved seta; outer ramus with 5 dorsal cuspidate setae with accessory seta on the margin and 3 cuspidate setae with accessory seta apically.

Uropod 2 (Fig. 7C) 1.4 × shorter than uropod 1, peduncle longer than inner ramus, with 4 cuspidate setae with accessory seta; inner ramus with 4 dorsal cuspidate setae and 6 cuspidate setae apically, all with accessory seta; outer ramus with 4 dorsal cuspidate setae and 4 apical cuspidate setae, all with accessory seta.

Uropod 3 (Fig. 7D) 0.7 × shorter than peduncle of uropod 1 and 0.6 × than peduncle of uropod 2;

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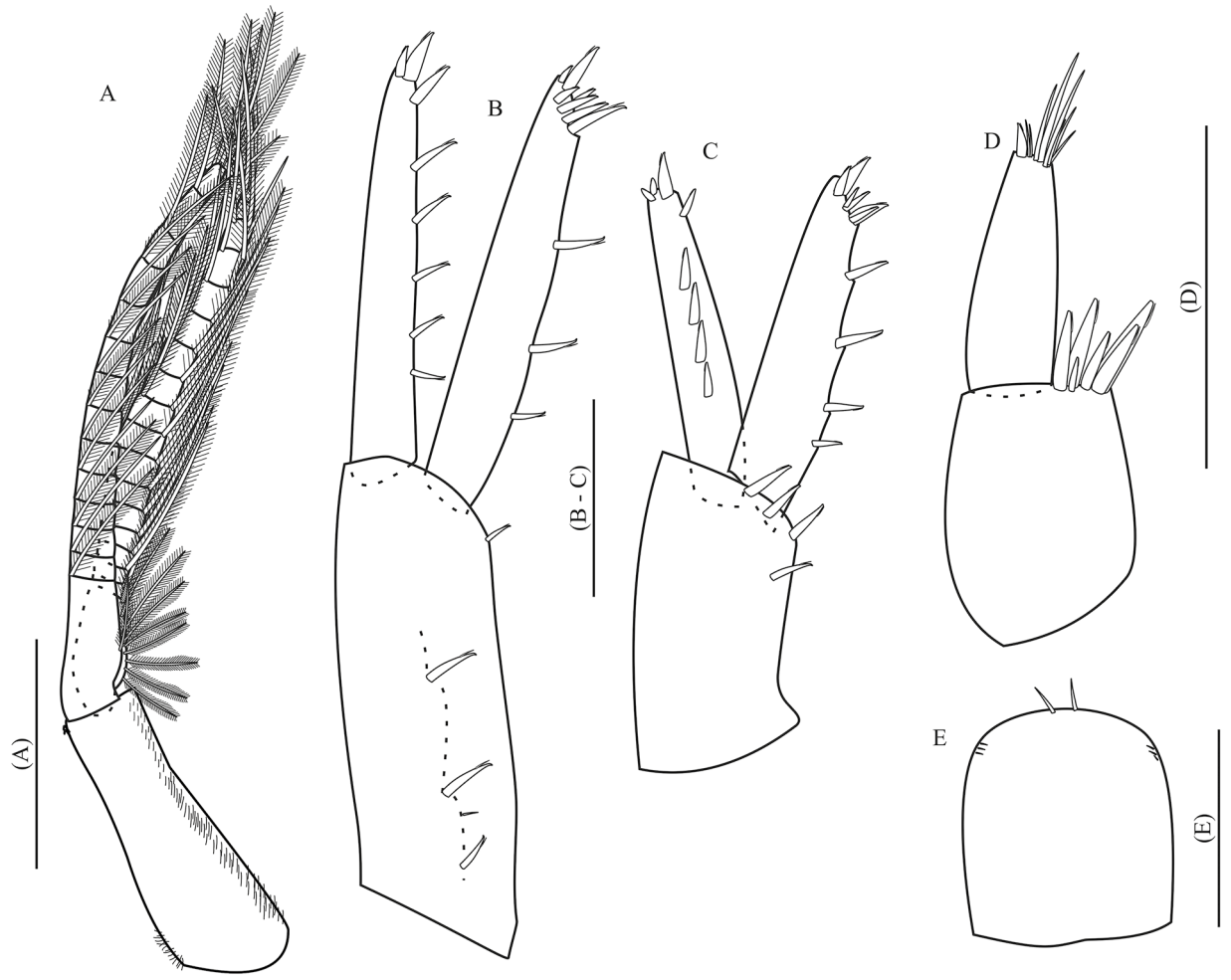

Figure 7. *Hyalella bocaina* sp. nov., holotype, male, CCUFLA 453. **A**, Pleopod; **B**, uropod 1; **C**, uropod 2; **D**, uropod 3; **E**, telson. Scale bars = 0.5 mm.

1.1 × shorter than telson; peduncle with 6 cuspidate setae with accessory seta; inner ramus absent; outer ramus uniarticulate, subequal to peduncle, with one cuspidate with accessory seta and some simple setae.

Telson (Fig. 7E) entire, 1.1 × longer than wide, apically rounded, with 2 to 4 apical simple setae, symmetrically, with 3 simple setae laterally on both sides. Variations: 3 plumose setae laterally on each side.

Epimeron (Fig. 4B) 2 and 3 postero-distal margin acute. Coxal gills sac-like and present on pereonites 2 to 7. Sternal gills tubular and present on pereonites 3 to 7.

Description of female. (Fig. 2A) Mean body length: 8.3 ± 1.1 mm ($N = 7$); mean head length: 0.68 ± 0.07 mm ($N = 7$).

Gnathopod 1 (Fig. 8A) similar to male gnathopod 1 in size and shape; ischium with polygonal pattern; carpus 2.2 longer than wide, without polygonal pattern and comb-scales, with pectinate border and some serrate and simple setae; propodus 1.7 × longer than wide, disto-anterior margin with few simple setae and polygonal pattern present, inner face with 10 serrate setae; palm transverse with several long simple setae, posterior distal corner with 2 long and strong cuspidate setae with accessory seta, without comb-scales and polygonal pattern; dactylus claw-like, with polygonal pattern on disto-anterior margin, with 1 plumose seta dorsally and terminal simple setae on proximal margin.

Gnathopod 2 (Fig. 8B) similar in size and shape to gnathopod 1; ischium and merus with comb scales; carpus 2.3 × longer than wide, without comb-scales, posterior lobe produced and forming scoop-like structure, without pectinate margin and polygonal

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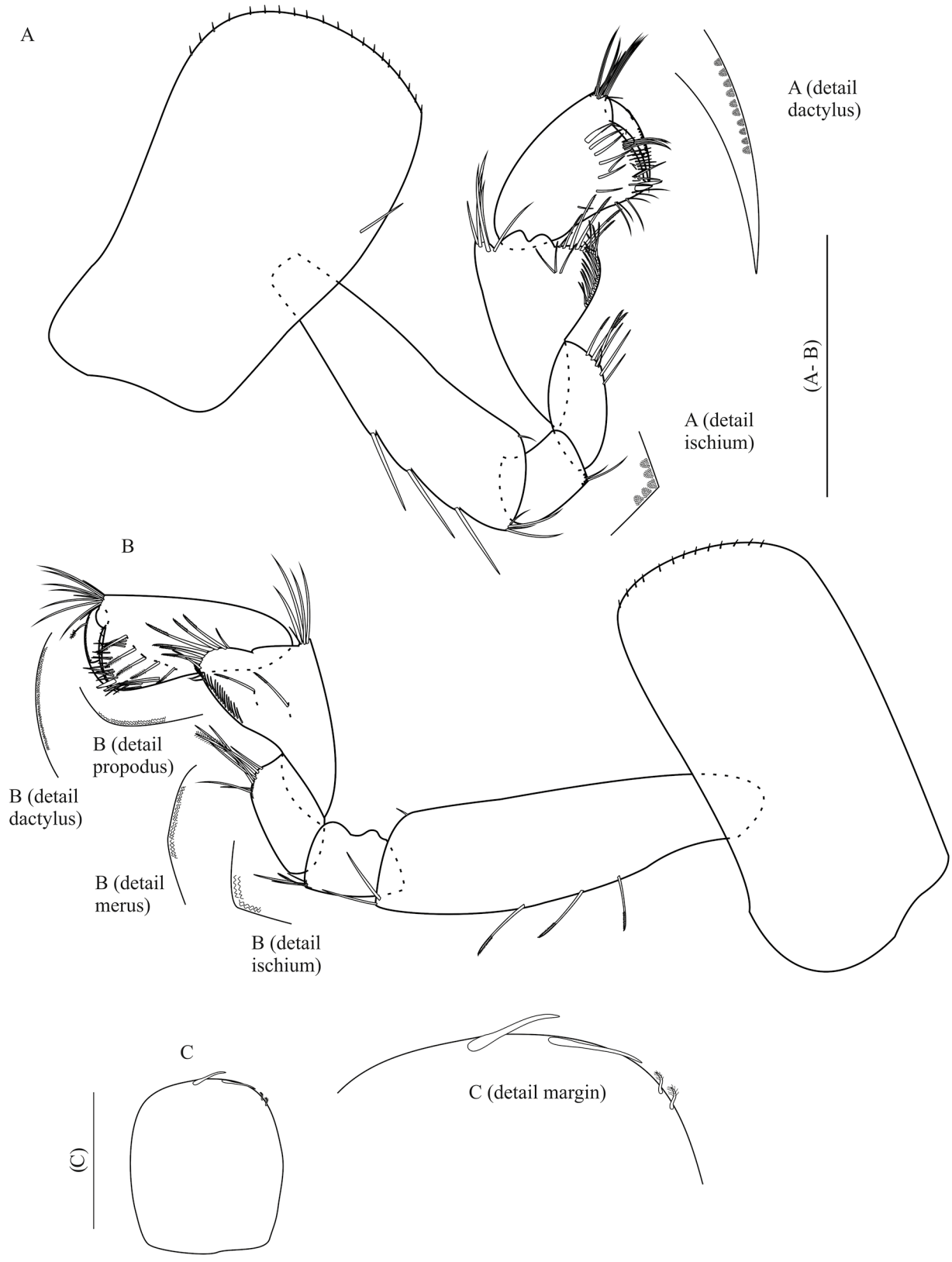

Figure 8. *Hyalella bocaina* sp. nov., paratype, female, CCUFLA 453. **A**, Gnathopod 1; **B**, gnathopod 2; **C**, telson. Scale bar = 0.5 mm.

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pattern, with several serrate setae and 2 long serrate setae on inner face; propodus 2.3 × longer than wide, hammer-shaped, inner face with some pappose setae; palm 1.1 × shorter than posterior margin of propodus, without polygonal pattern, inner margin with few simple setae, palm slope transverse, posterior distal corner with 2 long and strong cuspidate seta with accessory seta and comb-scales present; dactylus claw-like, with comb-scales on disto-anterior margin, with 1 plumose seta dorsally and terminal simple setae on proximal margin.

Telson (Fig. 8C) 1.1 × longer than wide, similar in shape to male, with 2 to 4 simple apical setae and 1 to 3 plumose setae on each side of margin.

Etymology. The epithet *bocaina* is a recognition of the river where the two populations of this new species are found.

Habitat. Freshwater, troglophilic. The species is categorized as troglophilic because it has well-established subterranean and epigean (surface) populations. They are sometimes also called facultative cave-dwellers. Specimens of *H. bocaina* sp. nov. were collected at two different sites, including the rapids of the Roda d'Água tourist route and at the cave entrance zone of the Gruta do Minotauro. The surrounding forest is well preserved. The rapids of PEI present an irregular flow, allowing their interaction with the substrate and, thus, the formation of micro-currents with different speeds. In addition, from this interaction, microhabitats are formed between the rocks, allowing the accumulation of sand and leaves (de Paula Paciência, 2011).

Remarks. Among all *Hyalella* species that occur in Brazil, *H. bocaina* sp. nov. is distinguished from the other 26 species by the absence of a curved seta on the inner ramus of uropod 1 of the male. Table 1 presents the main morphological differences between *H. bocaina* sp. nov. and the other *Hyalella* species from Brazil. We highlight that *H. bocaina* sp. nov., differs from *H. caeca*, *H. spelaea* and *Hyalella troglofugia* Bastos-Pereira, Oliveira and Ferreira, 2018, for being epigean, with no evident troglomorphic features; also, *H. bocaina* sp. nov. presents a pectinate border expressed on the carpus of the female and male gnathopod 1, which is different from *Hyalella montana* Rodrigues, Senna, Quadra and Bueno, 2017; there are no flanges — or dorsal carina — in the new species, different from *Hyalella pseudoazteca* González and Watling, 2003b; and *H. bocaina* sp. nov. has antenna 2 shorter than half the body length, which is different from *Hyalella longipropodus* Limberger, Graichen and Castiglioni, 2021.

***Hyalella temimina* sp. nov.**
Penoni, Deotti and Bueno

(Fig. 9–15)

Zoobank: urn:lsid:zoobank.org:act:B4E06617-3FC0-4A94-B3C5-63DB735B2AB3

Type material. Holotype: adult male (Fig. 9A) (L 10.62 mm, HL 1.01 mm), MZUSP 45193, Brazil, state of São Paulo, municipality of Apiaí, Parque Estadual Turístico do Alto Ribeira, Temimina II cave (24°22'36.8"S 48°33'56.2"W), November 2017, M.E. Bichuette, T.L.C. Scatolini, A. Pereira, D. Monteiro-Neto colls. — Paratypes: 1 adult female (Fig. 9B) (L 6.44 mm; HL 0.49 mm), MZUSP 45190, same locality as the holotype, April 2018, M.E. Bichuette, T.L.C. Scatolini, A. Pereira, D. Monteiro-Neto colls.; 1 male on slide CCUFLA 451, and 1 female on slide CCUFLA 452, same locality as the holotype, March 2009, M.E. Bichuette, T.L.C. Scatolini, A. Pereira, D. Monteiro-Neto colls.

Diagnosis. Body surface smooth. Eyes round, pigmented. Epimeron 1 and 3 postero-distal margin subacute (angular and slightly rounded), epimeron 2 postero-distal margin acute (acute angle). Antenna 2 smaller than body length. Maxilla 1 palp longer than wide, longer than half distance between base of palp and base of seta on outer plate. Gnathopod 1 propodus posterior margins without comb-scales. Uropod 3 ramus longer than peduncle, with excavation close to apex, bearing cuspidate seta with accessory seta, simple seta, and short triangular cuspidate seta apically. Telson with 8 apical cuspidate setae with accessory seta and 3 small plumose setae on each side,

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Table 1. Differences and similarities between the two new species (*Hyalella bocaina* sp. nov. and *H. temimina* sp. nov.) and the other species of *Hyalella* recorded previously from Brazil. Legend: U, uropod; G, gnathopod; M, maxilla; P, peduncle; R, ramus; *, relative length of palp of maxilla 1 in relation to base of setae on the outer ramus of maxilla 1.

Species authorship	Characters										
	Evidence of troglomorphisms	Curved seta present on male U1	Propodus of male G1 with comb scales	Setae type on inner face of G1 propodus	Pectinate border on carpus of G1	Pectinate border on carpus of G2	Length ratio between U1 peduncle and U2 total size	Length ratio between U2 peduncle and U3 total size	Length ratio between peduncle and ramus of U3	M1 palp relative size*	Epimeral plates
H. bocaina sp. nov.	No	No	No	Serrate seta	Yes	Yes	U1 > U2	U2 P > U3	R < P	~1/2	2 -3 acuminate
H. temimina sp. nov.	No	No	No	Pappose seta	No	No	U1 > U2	U2 P > U3	R > P	>1/2	1 and 3 subacute, 2 acute
H. bala Penoni and Bueno in Penoni, Lares and Bueno, 2021	No	No	No	No	No	No	U1 < U2	U2 P > U3	R ≈ P	=1/2	2 acute, 3 subacute
H. bonariensis Bond-Buckup, Araujo and Santos in Santos, Araujo and Bond-Buckup, 2008	No	Yes	Yes	No	No	No	U1 > U2	Unavailable information	Unavailable information	Unavailable information	Not acuminate
H. brasiliensis Bousfield, 1996	No	Yes	No	No	Yes	No	Unavailable information	Unavailable information	R > P	Unavailable information	1 – 3 acuminate
H. caeca Pereira, 1989	Yes	No	No	No	Yes	No	U1 > U2	Unavailable information	R < P	=1/2	Unavailable information
H. carsone Reis, Bueno and Araujo, 2023	No	No	Yes	Serrate seta	No	No	U1 > U2	U2 P > U3	R > P	<1/2	1 – 3 acuminate
H. carstica Bastos-Pereira and Bueno, 2012	No	Yes	Yes	Pappose seta	Yes	Yes	U1 > U2	U2 P < U3	R > P	<1/2	Not acuminate
H. castroi González, Bond-Buckup and Araujo, 2006	No	Yes	Yes	Pappose seta	Yes	Yes	U1 > U2	U2 P > U3	R > P	<1/2	1 – 3 acuminate
H. catarinensis Reis and Bueno in Reis, Penoni and Bueno, 2020	No	Yes	Yes	No	No	No	U1 > U2	U2 P > U3	R ≈ P	<1/2	Not acuminate
H. curvispina Shoemaker, 1942	No	Yes	Yes	Serrate seta	No	No	U1 > U2	U2 P > U3	R < P	<1/2	Unavailable information
H. dielaii Pereira, 2004	No	No	No	No	Yes	No	Unavailable information	Unavailable information	R > P	=1/2	Unavailable information
H. epikarstica Rodrigues, Bueno and Ferreira, 2014	Yes	Yes	No	Serrate seta	No	No	U1 > U2	U2 P > U3	R < P	<1/2	Not acuminate
H. formosa Cardoso and Araujo in Cardoso, Araujo, Bueno and Ferreira, 2017	Yes	Yes	No	Serrate seta	No	No	U1 > U2	Unavailable information	R < P	Unavailable information	Not acuminate

Table 1. Cont.

Species authorship	Characters										
	Evidence of troglomorphisms	Curved seta present on male U1	Propodus of male G1 with comb scales	Setae type on inner face of G1 propodus	Pectinate border on carpus of G1	Pectinate border on carpus of G2	Length ratio between U1 peduncle and U2 total size	Length ratio between U2 peduncle and U3 total size	Length ratio between peduncle and ramus of U3	M1 palp relative size*	Epimeral plates
H. gauchensis Streck and Castiglioni in Streck, Cardoso, Rodrigues, Graichen and Castiglioni, 2017	No	Yes	Yes	Serrate seta	Yes	Yes	U1 > U2	Unavailable information	R ≈ P	<1/2	Not acuminate
H. georginae Streck and Castiglioni in Streck, Cardoso, Rodrigues, Graichen and Castiglioni, 2017	No	Yes	Yes	Serrate seta	No	Yes	U1 > U2	Unavailable information	R ≈ P	<1/2	Not acuminate
H. gracilicornis and (Faxon, 1876)	No	No	Yes	Pappose seta	Yes	Yes	U1 > U2	U2 P < U3	R > P	=1/2	1 – 3 acuminate
H. imbya Rodrigues and Bueno in Rodrigues, Bueno and Ferreira, 2012	Yes	Yes	No	Serrate seta	Yes	Yes	U1 > U2	U2 P > U3	R < P	<1/2	Not acuminate
H. insulae Rangel, Limberger and Castiglioni in Rangel, Silva, Siegloch, Limberger and Castiglioni, 2022	No	Yes	Yes	Serrate seta	Yes	Yes	U1 ≈ U2	Unavailable information	R < P	<1/2	Not acuminate
H. kaigang Araujo and Cardoso in Bueno, Araujo, Cardoso, Gomes and Bond-Buckup´, 2011	No	Yes	Yes	Serrate seta	No	No	U1 > U2	Unavailable information	R > P	Unavailable information	1 rounded, 2-3 acuminate
H. jaboticabensis Castiglioni, Limberger and Santos, in Limberger, Castiglioni and Santos, 2024	No	Yes	Yes	Serrate seta	Yes	Yes	U1 > U2	Unavailable information	R ≈ P	<1/2	Not acuminate
H. lagoana Talhaferro and Bueno in Talhaferro, Bueno, Pires, Stenert, Maltchik and Kotzian, 2021	No	Yes	Yes	Serrate seta	No	No	Unavailable information	Unavailable information	R < P	<1/2	Not acuminate
H. longipropodus Limberger, Graichen and Castiglioni, in Limberger, Castiglioni and Graichen, 2021	No	No	Yes	Serrate seta	Yes	Yes	U1 > U2	Unavailable information	R ≈ P	<1/2	Not acuminate
H. longistila (Faxon, 1876)	No	No	Yes	Pappose seta	Yes	Yes	U1 > U2	U2 P < U3	R > P	<1/2	1 – 3 acuminate
H. luciae Limberger, Santos and Castiglioni, 2022	No	Yes	Yes	Serrate seta	Yes	Yes	Unavailable information	Unavailable information	R ≈ P	<1/2	Not acuminate

Table 1. Cont.

Species authorship	Characters										
	Evidence of troglomorphisms	Curved seta present on male U1	Propodus of male G1 with comb scales	Setae type on inner face of G1 propodus	Pectinate border on carpus of G1	Pectinate border on carpus of G2	Length ratio between U1 peduncle and U2 total size	Length ratio between U2 peduncle and U3 total size	Length ratio between peduncle and ramus of U3	M1 palp relative size*	Epimeral plates
H. meinerti Stebbing, 1899 (González and Watling, 2003b)	No	No	Yes	Pappose seta	Yes	Yes	U1 > U2	U2 P < U3	R > P	<1/2	1 – 3 acuminate
H. minensis Bastos-Pereira and Bueno, 2013	No	No	Yes	Pappose seta	Yes	Yes	U1 > U2	U2 P < U3	R ≈ P	<1/2	Not acuminate
H. minuana Talhaferro and Bueno in Talhaferro, Bueno, Pires, Stenert, Maltchik and Kotzian, 2021	No	Yes	Yes	Serrate seta	No	No	Unavailable information	Unavailable information	R ≈ P	=1/2	Not acuminate
H. montana Rodrigues, Senna, Quadra and Bueno, 2017	No	No	No	Serrate seta	No	No	U1 < U2	U2 P > U3	R > P	<1/2	1-3 slightly acuminate
H. montenegrinae Bond-Buckup and Araujo, 1998	No	Yes	No	No	No	No	Unavailable information	Unavailable information	R ≈ P	Unavailable information	Unavailable information
H. palmeirensis Streck-Marx and Castiglioni, 2020	No	Yes	Yes	Serrate seta	No	Yes	U1 > U2	Unavailable information	Unavailable information	<1/2	Unavailable information
H. pampeana Cavalieri, 1968	No	Yes	No	No	Yes	Yes	U1 < U2	Unavailable information	Unavailable information	Unavailable information	Unavailable information
H. pernix (Moreira, 1903)	No	Yes	Yes	No	Yes	No	Unavailable information	Unavailable information	R ≈ P	<1/2	Unavailable information
H. pleoacuta González, Bond-Buckup and Araujo, 2006	No	Yes	Yes	Pappose seta	Yes	Yes	U1 > U2	U2 P > U3	R ≈ P	<1/2	1-3 acuminate
H. pseudoazteca González and Watling, 2003a	No	No	Yes	Pappose seta	Yes	Yes	U1 > U2	U2 P > U3	R ≈ P	<1/2	1-3 acuminate
H. rioantensis Penoni and Bueno in Reis, Penoni and Bueno, 2020	No	Yes	Yes	No	No	No	U1 > U2	U2 P < U3	R > P	>1/2	1-3 acuminate
H. sambaqui Talhaferro and Bueno in Talhaferro, Bueno, Pires, Stenert, Maltchik and Kotzian, 2021	No	Yes	Yes	Serrate seta	No	No	U1 > U2	U2 P ≈ U3	R > P	<1/2	1 - 3 slihtly acuminate
H. spelaea Bueno and Cardoso, in Cardoso, Bueno and Ferreira, 2011	Yes	No	Yes	Serrate seta	Yes	Yes	U1 > U2	U2 P ≈ U3	R ≈ P	=1/2	Not acuminate
H. troglofugia Bastos-Pereira, Oliveira and Ferreira, 2018	Yes	No	Yes	Serrate seta	No	No	U1 > U2	Unavailable information	R > P	Unavailable information	Not acuminate

Table 1. Cont.

Species authorship	Characters										
	Evidence of troglomor-phisms	**Curved seta present on male U1**	**Propodus of male G1 with comb scales**	**Setae type on inner face of G1 propodus**	**Pectinate border on carpus of G1**	**Pectinate border on carpus of G2**	**Length ratio between U1 peduncle and U2 total size**	**Length ratio between U2 peduncle and U3 total size**	**Length ratio between peduncle and ramus of U3**	**M1 palp relative size***	**Epimeral plates**
H. veredae Cardoso and Bueno, in Cardoso, Bueno, Araujo and Ferreira, 2014	Yes	Yes	Yes	Serrate seta	No	No	U1 > U2	Unavailable information	R ≈ P	Unavailable information	Not acuminate
H. virgineae Lares, Penoni and Bueno, in Penoni, Lares and Bueno, 2021	No	No	No	Pappose seta	Yes	No	U1 > U2	U2 P > U3	R > P	=1/4	2 acute, 3 subacute
H. wangarie Reis, Bueno and Araujo, 2023	No	Yes	Yes	Serrate seta	No	No	U1 > U2	U2 P > U3	R ≈ P	<1/2	1-3 acuminate
H. warmingi Stebbing, 1899	No	No	No	Pappose seta	Yes	Yes	U1 > U2	U2 P ≈ U3	R > P	<1/2	1 -3 acuminate
H. xakriaba Bueno and Araujo in Bueno, Araujo,Cardoso, Gomes and Bond-Buckup, 2011	No	Yes	Yes	Serrate seta	No	No	U1 > U2	Unavailable information	R ≈ P	Unavailable information	Not acuminate

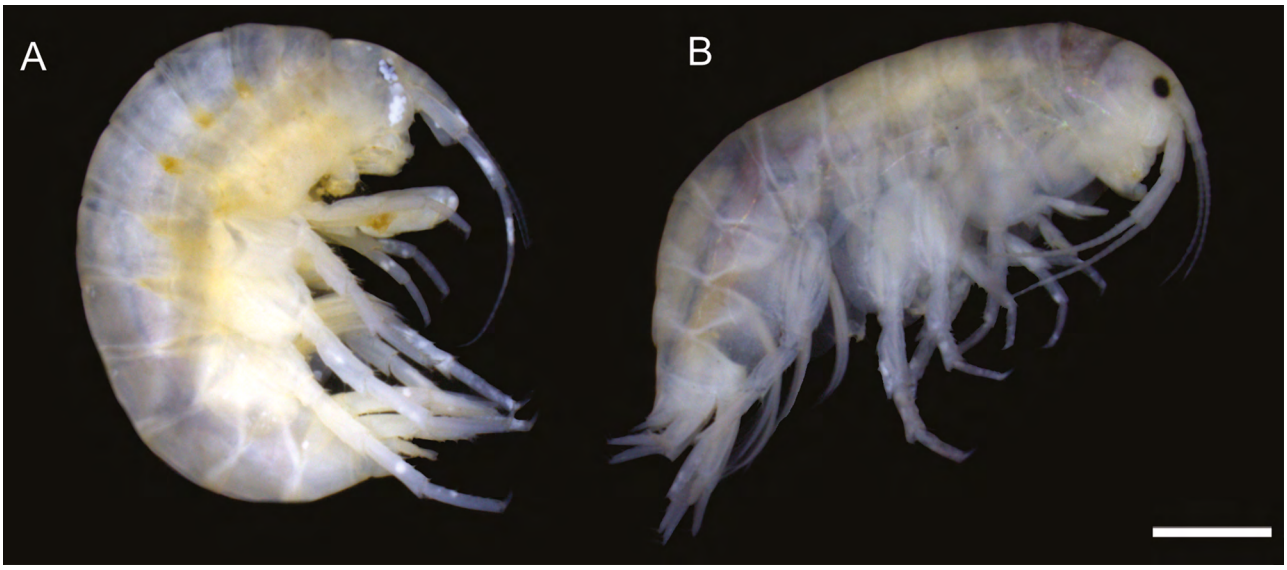

Figure 9. *Hyalella temimina* sp. nov., Parque Estadual Turístico Alto do Ribeira, state of São Paulo, Brazil. **A,** Holotype, male, 10.62 mm (MZUSP 45193); **B,** paratype, female, 6.44 mm (MZUSP 45190). Scale bars = 2 mm.

laterally. Coxal gills sac-like and present on segments 2 to 6. Sternal gills present on segments 3 to 7.

Description of male (Fig. 9A). Mean body length: 10.77 mm ($N = 2$); mean head length: 0.96 mm ($N = 2$). There were smaller animals observed at the collection site. Body surface smooth. Epimeral plates 2 acute and 1 and 3 subacute (Fig. 7J). Coxae 1–4 subequal in size and shape, slightly overlapping. Coxa 1 similar to 2 and 3. Coxa 3 wider than 4. Coxa 4 longer than wide, excavated posteriorly. Coxa 5 posterior lobe narrower than anterior lobe. Coxa 6 as long as wide. Coxa 7 reduced. Eyes round and pigmented.

Antenna 1 (Fig. 10A) about 3.2 × smaller than body length, 1.5 × smaller than antenna 2, 1.7 × longer than peduncle of antenna 2; peduncle 1.2 × longer than head; article 1 1.3 × longer than 2, article 3 1.3 × shorter than 1 and 1x shorter than article 2; flagellum with 20 articles, 2.5 × longer than peduncle; aesthetascs occurring on flagellum from article 7 to 19 distally.

Antenna 2 (Fig. 10B) 2.2 × smaller than body length; peduncle slender, 2.1 × longer than head; article 4 1.2 × shorter than article 5; flagellum with 23 articles, 1.5 × longer than peduncle.

Mandible without palp; incisor toothed; left (Fig. 10C) lacinia mobilis with 5 teeth and setal row with 3 long and strong pappose setae and 1 shorter pappose seta, molar process without accessory seta; right mandible (Figure 10D) with 2 longer central and 2 shorter pappose setae; molar process broad and cylindrical without accessory seta.

Upper lip (Fig. 10E) margin rounded; distal border covered by setules. Lower lip (Figure 4F) outer lobes rounded and distally notched, with setules on dorsal and ventral faces.

Maxilla 1 (Fig. 10G) inner plate slender, shorter than outer plate, with 2 apical papposerrate setae and setules distally. Outer plate with 9 serrate setae, increasing in size from inner to outer margin. Palp relatively long, uniarticulate, longer than wide, reaching more than half of distance between base of palp and base of seta on outer plate, with distal long seta.

Maxilla 2 (Fig. 10H) inner plate subequal to outer plate, inner plate with 1 proximal and 1 distal strong papposerrate seta; inner and outer plates with several serrulate and simple setae, and setules distally.

Maxilliped (Fig. 11A) inner plate longer than wide, with 3 cuspidate distal setae and up to 8 small pappose setae apically, without comb-scales; outer plate smaller than inner plate, with several long simple setae, without comb-scales; palp longer than inner, longer than outer plate, with 4 articles; article 1 triangular, outer margin with up to 3 long simple setae; article 2 longer than wide, inner margin with

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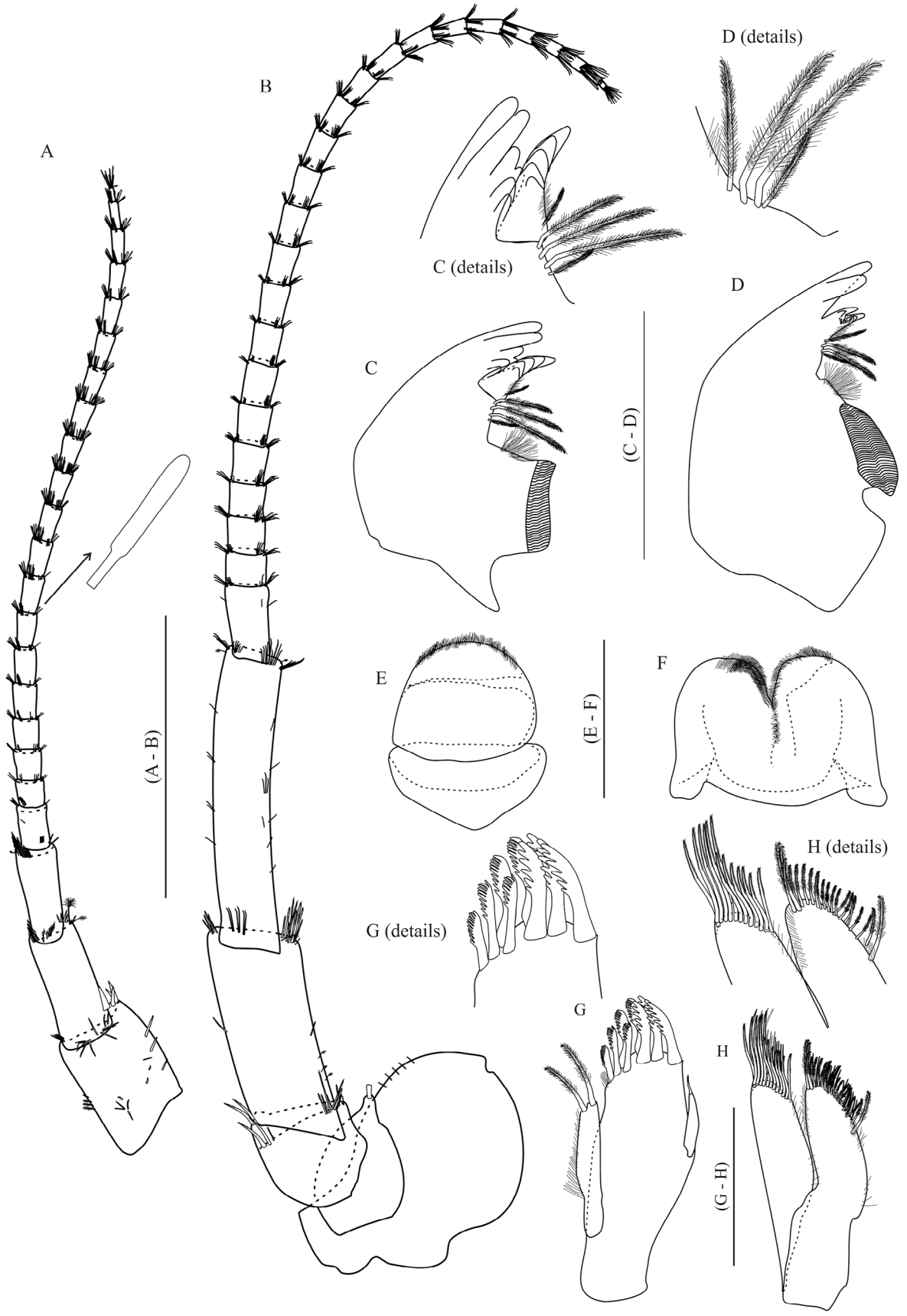

Figure 10. *Hyalella temimina* sp. nov., holotype, male, CCUFLA 451. **A**, Antenna 1; **B**, antenna 2; **C**, left mandible; **D**, right mandible; **E**, upper lip; **F**, lower lip; **G**, maxilla 1; **H**, maxilla 2. Scale bars = **A**, **B**: 1 mm; **C**–**H**: 0.5 mm.

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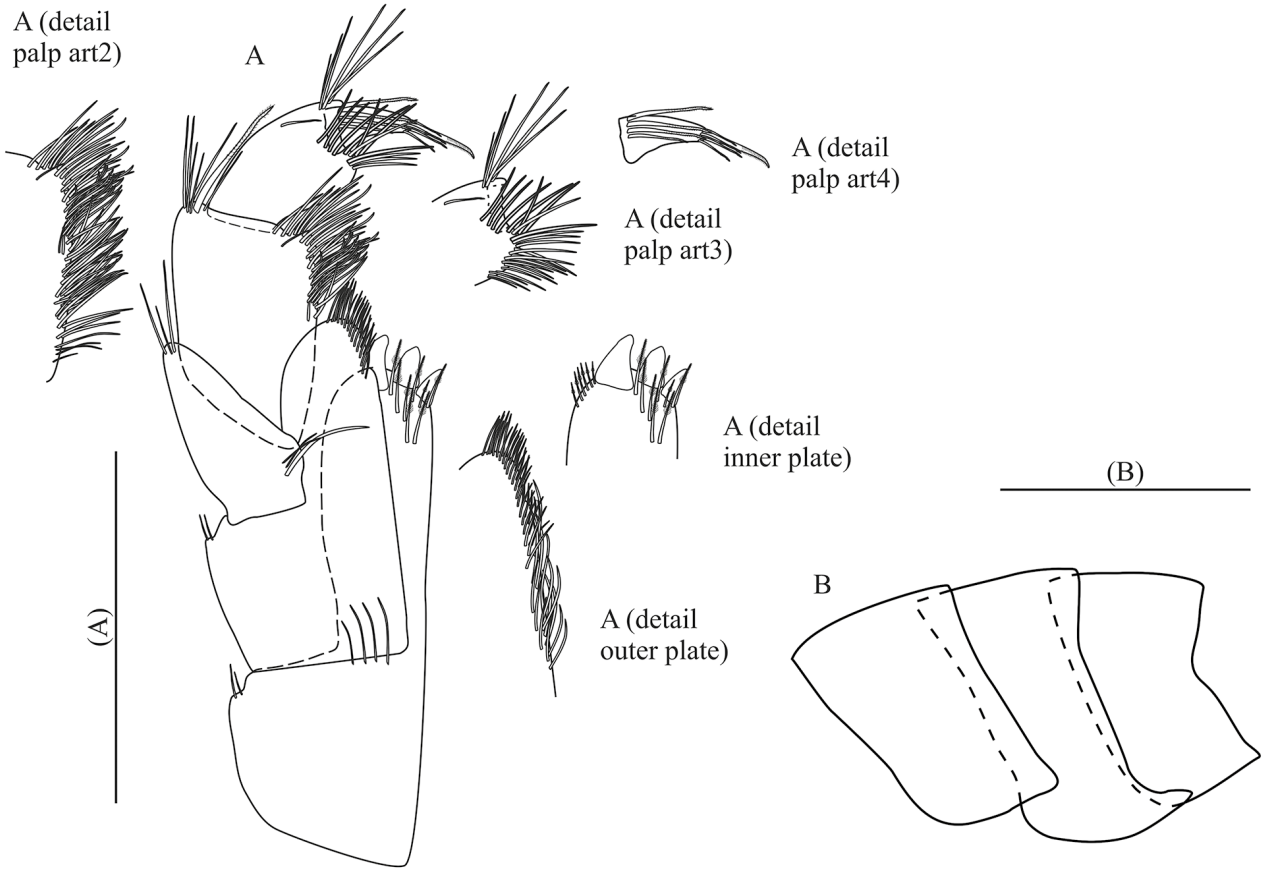

Figure 11. *Hyalella temimina* sp. nov., holotype, male, CCUFLA 451. **A**, Maxilliped; **B**, epimeron. Scale bars = **A**: 0.5 mm; **B**: 2 mm.

several long simple setae, outer margin with up to 6 long simple setae and 1 long and strong pappose seta; article 3 longer than wide, inner margin with several long simple setae, outer margin with pappose and long simple setae, without comb-scales; dactylus unguiform, relatively small, longer than wide, with distal setae simple and shorter than nail, without comb-scales, and distal nail present.

Gnathopod 1 (Fig. 12A) subchelate; coxal plate 1.6 × wider than longer, with simple setae on margin; basis and ischium with dorsal and apical serrate setae, without comb-scales; merus with pappose setae on distal margin, without comb-scales; carpus 2.6 × longer than wide, 1.1 × longer than propodus, with lateral distal lobe produced, with 8 serrate seta on disto-anterior margin, without comb-scales; posterior lobe without pectinate border or polygonal pattern, several pappose setae on margin and in inner face; propodus 1.4 × longer than wide, hammer-shaped, with several simple long setae on disto-anterior margin, comb-scales absent, inner face with many pappose setae, disto-posterior margin with simple setae and polygonal pattern; palm slope transverse, with many pappose setae with accessory seta, posterior distal corner with 2 long and strong cuspidate setae with accessory seta and polygonal pattern; dactylus claw-like, polygonal pattern present on disto-anterior margin, with 1 plumose seta dorsally and terminal simple setae on proximal margin.

Gnathopod 2 (Fig. 12B) subchelate; coxal plate 1.7 × wider than long, with simple setae on margin; basis and ischium with several distal and apical serrate setae, without comb-scales; merus with up to 12 papposerrate setae on posterior margin, without comb-scales; carpus 2.6 × wider than long, posterior lobe slim produced between merus and propodus, forming scoop-like structure, margin with many papposerrate setae, polygonal pattern forming pectinate border; propodus ovate, 1.2 × longer than wide, polygonal pattern present and few small pappose setae on inner face; palm subequal to posterior margin of propodus, slope oblique, margin with row of several cuspidate

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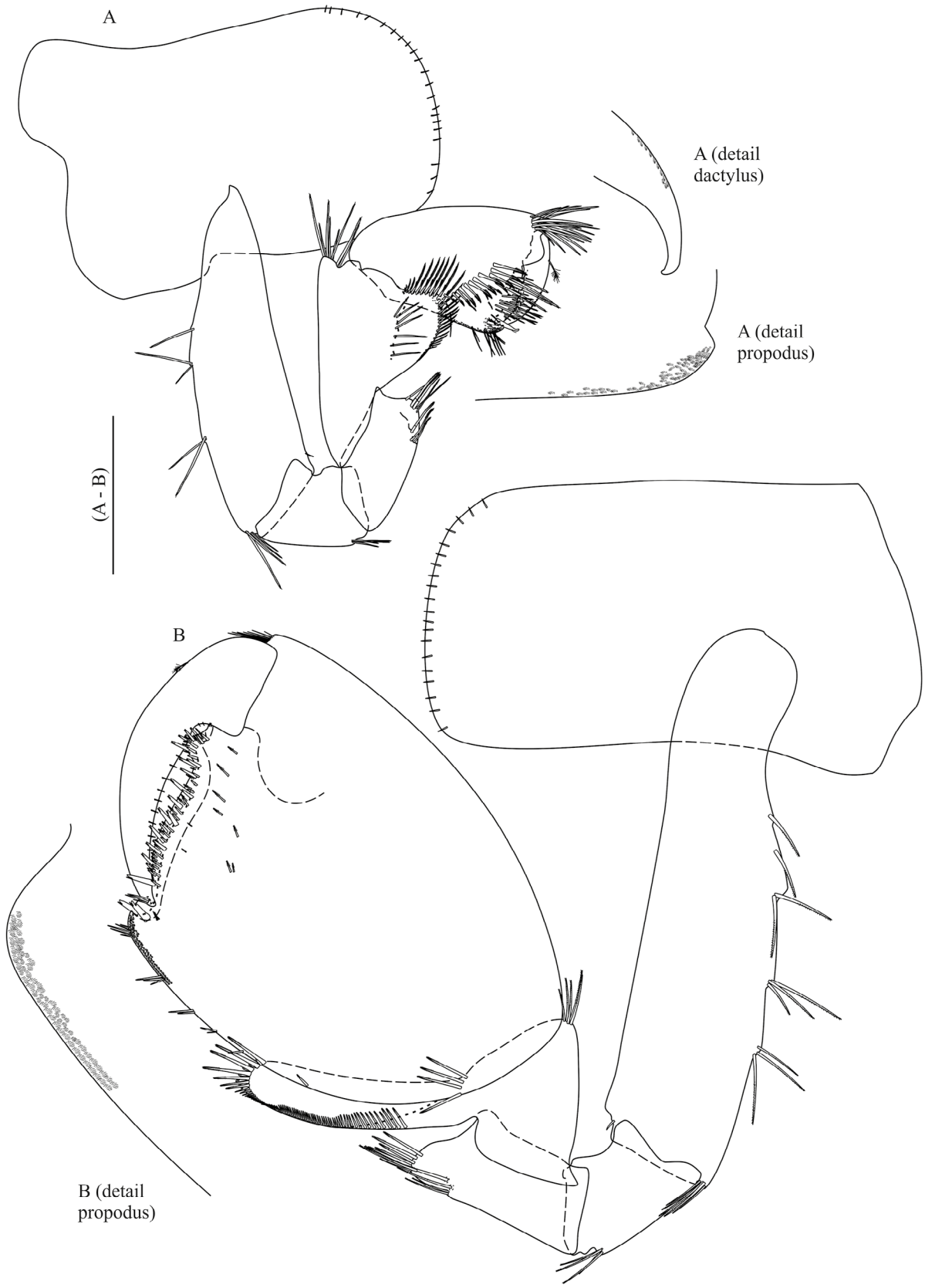

Figure 12. *Hyalella temimina* sp. nov., holotype, male, CCUFLA 451. **A**, Gnathopod 1; **B**, gnathopod 2. Scale bar = 0.5 mm.

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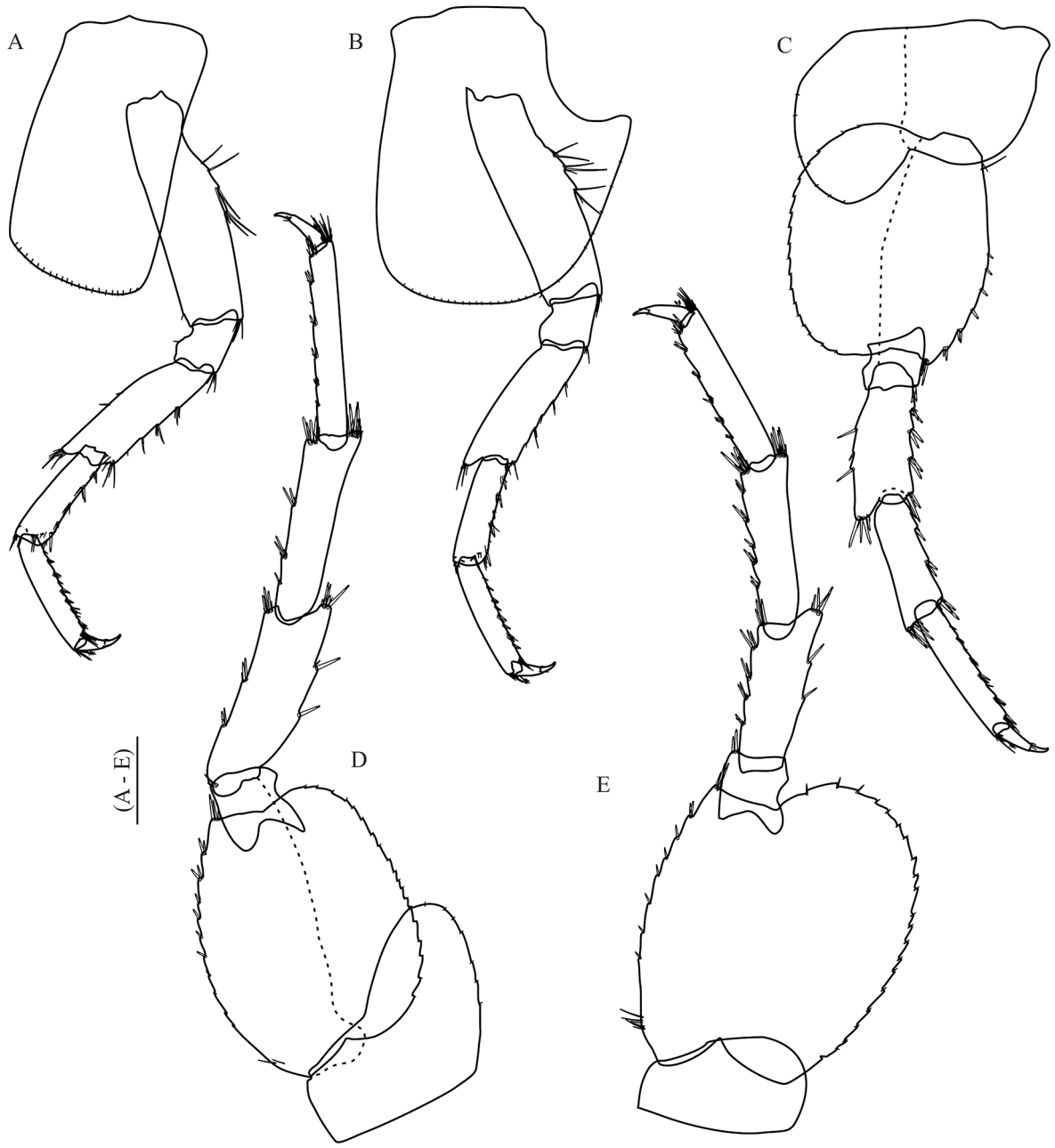

Figure 13. *Hyalella temimina* sp. nov., holotype, male, CCUFLA 451. **A**, Pereopod 3; **B**, pereopod 4; **C**, pereopod 5; **D**, pereopod 6; **E**, pereopod 7. Scale bar = 0.5 mm.

setae with accessory seta and few simple and pappose setae, posterior distal corner with 2 short cuspidate setae and with cup for dactylus; dactylus claw-like, congruent with palm, plumose seta dorsally, terminal simple setae on proximal margin and comb-scales absent.

Pereopods 3 to 7 (Fig. 13A–E) simple. Pereopods 3 and 4 merus and carpus posterior margin with several simple setae, some cuspidate with accessory seta and some serrate setae; propodus posterior margin of pereopod 3 and 4 with several cuspidate setae with accessory seta, some simple setae and few serrate setae; dactylus 2.7 × and 2.8 × shorter than propodus, respectively, with plumose seta dorsally. Pereopods 5 to 7 merus, carpus and propodus posterior margin with several cuspidate setae most of them with accessory seta and some simple setae, dactylus 2.5 ×, 3.1 ×, and 2.8 × shorter than propodus, respectively, with

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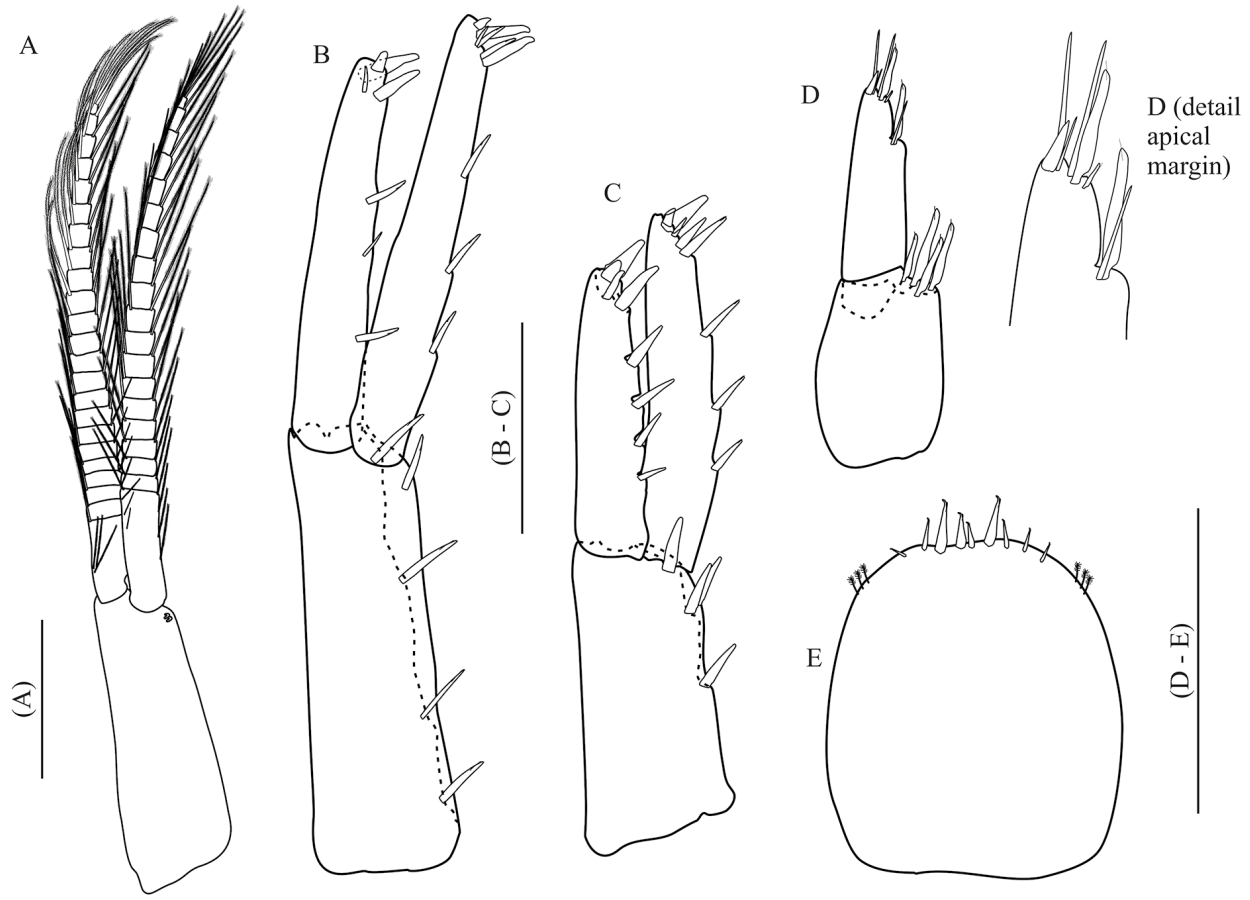

Figure 14. *Hyalella temimina* sp. nov., holotype, male, CCUFLA 451. **A**, Pleopod; **B**, uropod 1; **C**, uropod 2; **D**, uropod 3; **E**, telson. Scale bars = 0.5 mm.

plumose seta dorsally. Pereopod 3 and pereopod 4 similar sizes; pereopod 5 smaller than others; pereopod 6 longer than pereopod 7.

Pleopods (Fig. 14A) peduncle longer than wide, shorter than both rami, with 2 coupling spines; both rami with several plumose setae.

Uropod 1 (Fig. 14B) 1.4 × longer than uropod 2; peduncle 1.1 × longer than outer ramus and subequal to inner ramus, with 5 cuspidate setae with accessory seta; inner ramus 1.2 × longer than outer ramus, with 3 dorsal cuspidate setae with accessory seta on margin and 3 small cuspidate setae with accessory seta, 2 longer cuspidate and 1 short triangular cuspidate seta apically, without curved seta; outer ramus with 3 dorsal cuspidate setae with accessory seta on margin and 2 longer, 1 shorter and 1 triangular cuspidate setae apically.

Uropod 2 (Fig. 14C) peduncle subequal to outer ramus and 0.83 × size of inner ramus, with 4 cuspidate setae; inner ramus with 3 dorsal cuspidate setae with accessory seta and 5 cuspidate setae apically, only 1 with accessory seta; outer ramus with 4 dorsal cuspidate setae, sequentially increasing in size, and 2 short and 2 longer apical cuspidate setae.

Uropod 3 (Fig. 14D) 0.8–0.9 × shorter than peduncle of uropod 2; peduncle longer than wide, with 4 cuspidate setae with accessory seta; inner ramus absent; outer ramus uniarticulate, 1.2 × longer than peduncle, with excavation close to apex, bearing 1 cuspidate seta with accessory seta and 1 simple seta, and 2 long, 2 short simple setae, 1 long cuspidate seta with accessory seta and 1 short triangular cuspidate seta apically.

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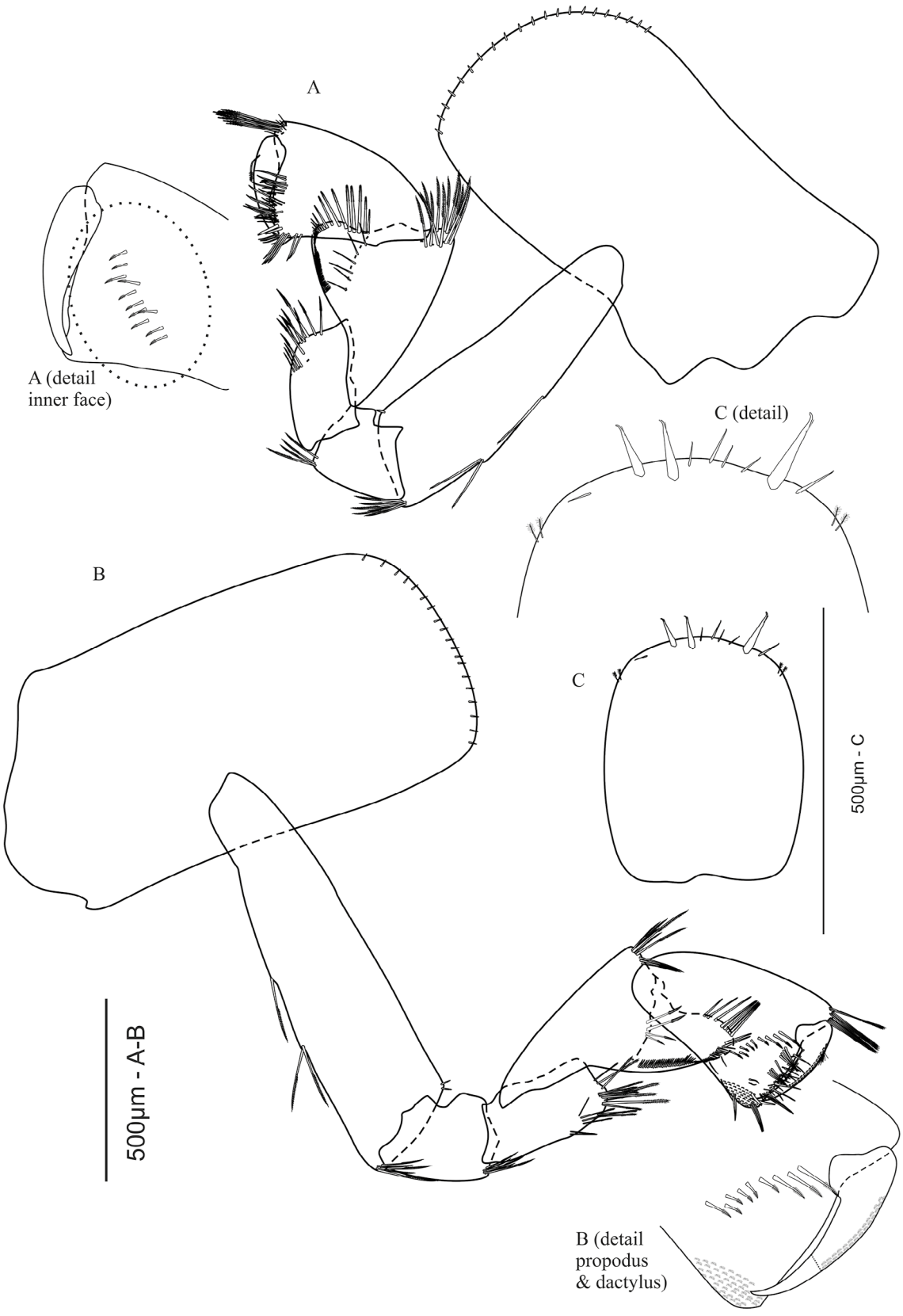

Figure 15. *Hyalella temimina* sp. nov., paratype, female, CCUFLA 452. **A**, Gnathopod 1; **B**, gnathopod 2; **C**, telson. Scale bars = 0.5 mm.

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Telson (Fig. 14E) entire, 1.2 × longer than wide, apically rounded, with up to 8, almost symmetrical, small cuspidate setae with accessory seta apically, with 3 small plumose setae on each side, laterally.

Epimeron (Fig. 11B) 1 and 3 subacute; epimeron 2 acute.

Coxal gills sac-like and present on pereonites 2 to 6. Sternal gills tubular, present on pereonites 3 to 7.

Description of female (Fig. 9B). Mean body length: 10.24 mm (N = 3); mean head length: 0.73 mm (N = 3).

Gnathopod 1 (Fig. 15A) similar to male gnathopod 1; carpus 1.4 × longer than wide, without comb-scales, posterior lobe with pectinate margin, with several serrate setae; propodus 1.5 × longer than wide, hammer-shaped, with comb-scales, palm 0.7 × size of posterior margin of propodus, without comb-scales, inner margin with 11 papposerrate setae, palm slope transverse, dactylus claw-like, with comb-scales.

Gnathopod 2 (Fig. 15B) similar in size and shape to gnathopod 1; carpus similar to gnathopod 1; propodus 1.4 × longer than wide, with polygonal pattern on disto-posterior margin, inner margin with up to 9 papposerrate setae, palm transverse with several long simple setae, without comb-scales; dactylus claw-like, congruent to palm, with 1 plumose seta apically and polygonal pattern on posterior margin.

Telson (Fig. 15C) 1.2 × longer than wide, similar in shape to male's, with up to 9 cuspidate setae with accessory seta asymmetrically distributed apically, and 2 small plumose setae on each side, laterally.

Etymology. The epithet *temimina* is a recognition of the cave, Temimina II, where the specimens of this new species were collected.

Habitat. Freshwater, hypogean, typical lentic waters, with bottom formed by silt and pebbles. Specimens of *Hyalella temimina* sp. nov. were found only at cave Teminina II. Despite the absence of specimens in the surface streams, *Hyalella temimina* sp. nov. does not show troglomorphic traits, and we hypothesize that the new species is troglophilic, similar to *Hyalella bocaina* sp. nov.

Remarks. Table 1 presents the main morphological features that *H. temimina* sp. nov. differs from the other 42 species that occur in Brazil. It is a hypogean species with no signs of troglomorphism, having large and pigmented eyes. In this way, it differs from classically troglomorphic species, such as *H. caeca*, *H. spelaea*, and *H. troglofugia*. *Hyalella temimina* sp. nov. body is smooth, which is different from *H. pseudoazteca*; *H. temimina* sp. nov. has pappose setae and does not have comb-scales on the propodus of male gnathopod 1, while *H. longipropodus* has serrate setae on the inner face of the propodus and comb scales on propodus of male ganthopod 1. Moreover, *H. temimina* sp. nov. is characterized by the presence of pappose setae on the inner surface of the propodus of gnathopod 1, which differentiates the new species from *H. bala*, *H. dielaii*, and *H. montana*. Finally, *H. temimina* sp. nov. is different from *Hyalella bocaina* sp. nov. because the former has only two teeth on the right mandible incisor, palp of maxilla 1 relatively longer, male gnathopod 1 with polygonal pattern only on the propodus and dactylus, gnathopod 2 with polygonal pattern only on the propodus, and uropod 3 with an apical excavation on the ramus, whereas *H. bocaina* sp. nov. has six teeth on the right mandible incisor, relatively shorter palp of maxilla 1, and polygonal pattern on the merus and ischium of both male gnathopods, with no excavations on the uropod 3 ramus.

Conservation. We propose for both species, *H. bocaina* sp. nov. and *H. temimina* sp. nov., the categorization as vulnerable. Vulnerable (VU) - (D2) = typically, area of occupancy (AOO < 20 km^2) or number of locations (≤ 5) (ICMBio, 2013). Their populations are restricted to small areas and, similar to other *Hyalella* species, they are possibly endemic and restricted to the sites, or even micro-basins, where they were collected.

CONCLUSION

With this paper we increase from eight to ten the number of *Hyalella* species that occur in the Brazilian state of São Paulo and from 42 to 44 the number of known species in Brazil. In the past decade the knowledge of the diversity and richness of *Hyalella* species has grown substantially, mostly with the description of new species in the southern region

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of Brazil (Penoni et al., 2021, Rangel et al., 2022; Limberger et al., 2024). *H. bocaina* sp. nov. and *H. temimina* sp. nov. were collected, respectively, in Parque Estadual Intervales (PEI) and Parque Estadual Turístico Alto do Ribeira (PETAR). They are both of special ecological importance since the Parks are intended for biological conservation (SNUC, 2000). Thus, as they are Atlantic Rainforest conservation areas, the description of new species for these locations provides evidence for an effective contribution to the conservation of biological diversity, and facilitates cooperation with the creation of planning and management strategies for Conservation Units such as State Parks.

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ADDITIONAL INFORMATION AND DECLARATIONS

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Conceptualization and design: CMD., AAPB. Data collection: MEB. Performed research; analysis and interpretation of data, preparation of table and drawings: CMD, LRP. Writing – original draft: CMD, LRP. Critical review and editing: AAPB, LRP, MEB.

Consent for publication

All authors declare that they have reviewed the content of the manuscript and gave their consent to submit the document.

Competing interests

The authors declare no competing interest.

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