AMANDA DE SERPA NASCIMENTO

DESCRIÇÃO MORFOLÓGICA ROBUSTA E PRIMEIROS TRAÇOS ECOLÓGICOS PARA A POPULAÇÃO DE *HYALELLA* SMITH, 1874 (AMPHIPODA:HYALELLIDAE) DO GUAREÍ

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Profa. Dra. Alessandra Angélica de Pádua Bueno
Orientadora

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RESUMO

A presente dissertação apresenta uma nova espécie do gênero *Hyalella* Smith, 1874 e investiga traços ecológicos relacionados à sua estrutura populacional e parâmetros reprodutivos em um ambiente de água doce no sudeste do Brasil. Foram analisadas variáveis como razão sexual, tamanho corporal, fecundidade e volume de ovo das fêmeas. Resultados significativos foram observados para as variáveis analisadas, indicando que fatores ambientais influenciam diretamente nas estratégias de história de vida. Com isto, os dados morfológicos coletados subsidiaram a elaboração de um artigo taxonômico, que resultou na descrição formal da nova espécie, cujos traços que a diferenciam das outras 22 espécies registradas para o sudeste incluem, a ausência de seta curva no ramo interno do urópodo 1 dos machos; brânquias esternais presentes nos segmentos 3-7; a presença de comb-scales em ambos os gnatópodos; gnatópodo 2 com duas setas plumosas distais no dáctilo; urópodo 3 com pedúnculo apresentando três setas cuspidadas com seta acessória, e ramo com oito setas; maxilla 1 com sete setas serradas na placa externa; e telson com quatro setas cuspidadas distais e quatro setas plumosas lateral em cada lado. Sendo assim, os resultados obtidos reforçam a importância do ambiente na determinação de características populacionais e reprodutivas, contribuindo para a compreensão ecológica do gênero *Hyalella* e fornecendo subsídios para futuras ações de conservação.

Palavras chave: crustáceo; parâmetros populacionais; taxonomia; dulcícola.

ABSTRACT

The present dissertation describes a new species of the genus *Hyalella* Smith, 1874, and investigates its ecological traits related to population structure and reproductive parameters in a freshwater ecosystem in southeastern Brazil. Variables such as sex ratio, body size, fecundity, and egg volume of females were analyzed. Significant results were observed for the analyzed variables, indicating that environmental factors directly influence life-history strategies. The morphological data collected supported the preparation of a taxonomic article, which resulted in the formal description of the new species. Distinguishing traits from the other 22 species reported for southeastern Brazil include: absence of a curved seta on the inner ramus of uropod 1 in males; sternal gills present on segments 3–7; presence of comb-scales on both gnathopods; gnathopod 2 with two distal plumose setae on the dactylus; uropod 3 with peduncle bearing three cuspidate setae with an accessory seta, and ramus with eight setae; maxilla 1 with seven serrate setae on the outer plate; and telson with four distal cuspidate setae and four lateral plumose setae on each side. Therefore, the results obtained reinforce the importance of environmental conditions in determining population and reproductive characteristics, contributing to the ecological understanding of the genus *Hyalella* and providing a basis for future conservation efforts.

Keywords: crustacean; population parameters; taxonomy; freshwater.

INDICADORES DE IMPACTO

Esta pesquisa tem como finalidade ampliar o conhecimento sobre a biodiversidade de pequenos anfípodes dulcícolas pertencentes ao gênero *Hyalella* Smith, 1874, organismos essenciais para o equilíbrio ecológico dos ambientes aquáticos em que habitam. Esses crustáceos desempenham um papel fundamental na manutenção das cadeias tróficas e na decomposição da matéria orgânica, sendo considerados bioindicadores da qualidade ambiental. O estudo concentra-se em uma espécie do gênero *Hyalella*, descrita para o Brasil, contribuindo com uma nova descrição taxonômica detalhada, bem como com a avaliação de aspectos populacionais importantes para a compreensão de sua biologia e ecologia. A investigação de padrões ecológicos de espécies, permite obter informações e ferramentas valiosas que podem ser aplicadas em estudos futuros tanto de preservação como de conservação. Dessa forma, a pesquisa não apenas contribui para o avanço científico na área da taxonomia e ecologia de anfípodes, mas também propõe uma ferramenta eficaz para o monitoramento e preservação da biodiversidade aquática, promovendo um impacto positivo para o meio ambiente.

IMPACT INDICATORS

This research aims to expand knowledge about the biodiversity of small freshwater amphipods belonging to the genus *Hyalella* Smith, 1874, organisms that are essential for the ecological balance of the aquatic environments they inhabit. These crustaceans play a fundamental role in maintaining trophic chains and in the decomposition of organic matter, and are therefore considered bioindicators of environmental quality. The study focuses on a species of the genus *Hyalella* described for Brazil, contributing a new detailed taxonomic description as well as the assessment of important population aspects for understanding its biology and ecology. The investigation of ecological patterns in species provides valuable information and tools that can be applied in future studies related to both preservation and conservation. Thus, this research not only contributes to scientific advances in the taxonomy and ecology of amphipods, but also proposes an effective tool for monitoring and preserving aquatic biodiversity, promoting a positive environmental impact.

SUMÁRIO

PRIMEIRA PARTE

INTRODUÇÃO

Os anfípodes da família Hyalellidae, pertencentes ao gênero *Hyalella* Smith, 1874, são crustáceos amplamente distribuídos nas Américas, com destaque para sua diversidade e endemismo em ambientes de água doce (BAUER, 2004). Esses organismos desempenham um papel ecológico fundamental, atuando na decomposição de matéria orgânica, na ciclagem de nutrientes e como base alimentar para diversos vertebrados e invertebrados aquáticos (DERRAIK, 2002).

Apesar da ampla distribuição do gênero, muitas espécies ainda permanecem não descritas formalmente, devido à lacuna de conhecimento diante a sua distribuição, onde muitas vezes os indivíduos são encontrados acidentalmente em meio a outras coletas, como foi o caso deste trabalho. Além disso, a taxonomia de *Hyalella* é particularmente desafiadora devido à morfologia conservadora entre espécies e à presença de caracteres diagnósticos sutis, o que reforça a necessidade de descrições detalhadas e integradas com dados ecológicos e populacionais (PENONI *et al.*, 2021).

Além da importância taxonômica, estudar os traços ecológicos dessas espécies fornece subsídios cruciais para a compreensão de sua ecologia e estratégias de sobrevivência. O desconhecimento acerca da biodiversidade, como dados taxonômicos e populacionais, permite que se identifique lacunas de conhecimento. Tais lacunas possibilitam o desenvolvimento de novos estudos, que podem ser utilizados para mapear e quantificar as espécies, viabilizando a realização de projeções e, até mesmo, a conservação da espécie (HORTAL *et al.* 2015). Desta forma, facilitando medidas de proteção e preservação, às espécies e aos ambientes que habitam.

Neste contexto, a presente dissertação tem como objetivo principal, descrever morfologicamente uma nova espécie do gênero *Hyalella* encontrada em um trecho do rio Guareí, localizado na Estação Ecológica de Angatuba, situada na região sudoeste do estado de São Paulo, e adicionar alguns traços ecológicos da nova espécie. Os resultados visam contribuir tanto para o conhecimento taxonômico do grupo quanto para a redução da lacuna relacionada aos estudos ecológicos que envolvem as espécies do gênero. Apesar do aumento de pesquisas relacionadas ao gênero, ainda existe um desconhecimento acerca da ecologia populacional das espécies, parâmetros que são essenciais tanto para a conservação quanto para a proteção da espécie.

CONCLUSÃO

A descrição da nova espécie de *Hyalella* apresentada nesta dissertação amplia para 23 o número de espécies conhecidas para o sudeste brasileiro. A espécie mostrou um conjunto de caracteres morfológicos diagnósticos que a distinguem de espécies previamente descritas, reforçando a complexidade e a necessidade de revisões sistemáticas mais abrangentes dentro do grupo.

Além disso, este estudo apresenta características ecológicas da nova espécie, buscando uma nova tendência que incorpore dados taxonômicos e ecológicos, mesmo que limitados.

Dessa forma, os resultados obtidos contribuem não apenas para a sistemática do grupo, mas também para a compreensão de processos ecológicos em habitats de água doce, promovendo subsídios para ações de conservação e manejo da biodiversidade dulcícola da região.

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PENONI, L. R; LARES, M M. A. & BUENO, A. A. P.. **Description of two new species of the freshwater amphipod Hyalella Smith, 1874 (Amphipoda: Hyalellidae) from southeastern Brazil, with remarks on their population biology and reproduction.** Journal of Crustacean Biology, v. 41, n. 3, 2021.

SEGUNDA PARTE - ARTIGO

ARTIGO 1 - *Hyalella* sp. nov. (Crustacea, Amphipoda, Hyalellidae) - description of a new species of freshwater amphipod from Southeast, Brazil, with ecological traits

Artigo elaborado e formatado conforme as normas da publicação científica *Zootaxa*
Disponível em: <https://www.mapress.com/zt/about/submissions>

Abstract

The freshwater amphipod *Hyalella* Smith, 1874 currently includes 118 species distributed exclusively in the Americas. This study describes the twenty-third species for southeastern Brazil, characterized by the absence of the curved seta on the inner ramus of uropod 1 in males, sternal gills on segments 3–7, comb-scales on both gnathopods, two plumose distal setae on gnathopod 2 dactylus, three cuspidate setae with accessory seta on the peduncle of uropod 3, eight setae on the ramus, seven serrate setae on the outer plate of maxilla 1, and four distal cuspidate and four lateral plumose setae on each side of the telson. A detailed comparison with previously described southeastern Brazilian species is provided, and further studies are needed to better understand distribution patterns in the genus Hyalella.

Also, despite increasing research on *Hyalella* in Brazil, knowledge of population ecology remains limited, restraining the conservation of the genus. This article shows ecological traits for the new species. Whereas in *Hyalella* sp. nov., a female-biased sex ratio and sexual dimorphism, with larger males, reflect reproductive strategies. Female size correlates with fecundity, although environmental factors also play a role, and the mean number of eggs per female is lower than in other regional species. Incorporating taxonomic and ecological data, even limited, enhances the understanding of the genus.

Key words: Freshwater crustaceans; Amphipods; Taxonomy

Introduction

Currently, freshwater ecosystems are considered highly threatened due to human-induced degradation. As a result, aquatic biodiversity is increasingly at risk of extinction (Reid *et al*., 2019), and many species, along with their behaviors and ecological roles, remain unknown to the scientific community.

Among these ecosystems, there are the amphipods, which in Brazil, the genus *Hyalella* Smith, 1874 is an important representative. These crustaceans are exclusive to the Americas and are found only in freshwater ecosystems. Moreover, this genus exhibits high degree of species endemism, which makes it particularly vulnerable to any environmental disturbance (Väinölä *et al.*, 2008), also, these organisms could be a very important tool in toxicological analyses, being utilized as bioindicators (Graça *et al*., 2002).

Moreover, although this is an exclusively freshwater group, it can be found in a wide variety of habitats, whether in the water column, in bottom sediments, or attached to macrophytes (Wellborn, 1995; Bastos-Pereira & Bueno, 2016a and b; Talhaferro *et al.*, 2021).

Currently composed of a single genus within the family Hyalellidae, this group has undergone several taxonomic revisions over time. According to Bueno et al. (2014), it includes species that occur exclusively within the Nearctic and Neotropical biogeographic regions, ranging from southern Canada to Patagonia, in Brazil, *Hyalella* species are mostly found in environments characterized by low water flow.

Environmental conditions directly affect a species' population and its persistence over time. Some conditions that influence organisms include, for example, temperature, altitude, longitude, conductivity, and pH (Koffel *et al.*, 2021). At an optimal level, these conditions allow for the survival, growth, and reproduction of an organism (Thurman *et al.*, 2020).

Despite the number of studies related to the genus, there is still a huge gap around its ecology, an essential research to preserve and protect the species. Building on this assumption

and considering the growing impact on aquatic environments, research focused on the genus *Hyalella* has great potential to be explored (Martins & Bianchini, 2011). Therefore, ecological studies should be carried out alongside taxonomic studies, such as those conducted by Penoni e*t al.* (2021) when describing *H. bala* and *H. virgineae*.

To date, 48 species have been described for Brazil, with 12 recorded in the state of São Paulo (Pereira, 1989; González & Watling, 2003a; Pereira, 2004; Cardoso *et al.*, 2011; Rodrigues *et al*., 2014; Penoni *et al*. 2021; Penoni *et al.* 2025), 7 recorded in Minas Gerais (González & Watling, 2003b; Bueno *et al.,* 2011; Bastos-Pereira & Bueno, 2012; Bastos-Pereira & Bueno, 2013; Cardoso *et al*., 2014; Rodrigues et al. , 2017; Bastos-Pereira, Oliveira & Ferreira, 2018) and 3 recorded in Rio de Janeiro (Pereira, 1985; González and Watling, 2003b)

Here we (i) formally describe Hyalella sp. nov. from the Guareí River (SP) and (ii) quantify sex ratio, fecundity, egg size and size–fecundity relationships to contextualize reproductive strategies under local conditions.

Materials and Methods

Collection

Specimens were collected in 2012 from a section of the Guareí River, located within the Angatuba Ecological Station (Fig. 1), in the southwest region of São Paulo State, covering the municipalities of Angatuba and Guareí (23º28’6.66’’S 48º37’17.51’’W). This area consists of a preserved forest managed by the Forestry Institute of the State of São Paulo. It is part of the Upper Paranapanema River Basin, situated upstream from the Guareí River at the Jurumirim Reservoir, where the sampling site was a floodplain area along the margins of the Guareí River, within a dense forest region.

The collections took place between December 2012 and January 2013, at five distinct but closely spaced points along the river margins. Individuals were collected using a hand net and subsequently preserved in labeled vials containing 70% ethanol until the preparation of microscope slides, however this was not an amphipod focus collection, which lead to the ovigerous females and paired couples to not be isolated, so to have more complete information about reproduction aspects, new studies must be done.

Species description

The description was conducted in a laboratory, where ten males and five females, that had all the body structures complete were selected for dissection. Where each individual specimen was initially measured to obtain both head length and body length. Measurements were performed using a Carl Zeiss Stemi 2000-C stereomicroscope equipped with a millimetric ruler. Body length was measured from the insertion point of the antennae to the base of the telson. The telson itself was excluded from total length calculations due to the significant variation in its morphology among species. Head length was measured from the insertion point of the antennae to the beginning of the thoracic segments. Therefore, the total body length was calculated by summing the body length and head length.

Following measurement, the specimens were stained in Congo Red for a period of 12 to 24 hours. After staining, dissection was carried out under a Carl Zeiss Stemi 2000-C stereomicroscope, using a cavity slide containing glycerin. The appendages were then mounted on standard microscope slides with glycerin, covered with coverslips sealed with varnish, and subsequently photographed.

Photographs were taken using a Carl Zeiss Primo Star microscope equipped with an AxioCam ERc5s camera, operated via Carl Zeiss ZEN 2.3 (blue edition) software. The resulting images served as the basis for illustrations, which were produced using CorelDRAW 2019. This software was also used for measuring the appendages via the two-point line tool.

The description of cuticular structures in the new species followed the terminology established by Zimmer *et al*. (2009) and were compared to all 22 species known for São Paulo, Minas Gerais and Rio de Janeiro states (Pereira, 1989; González & Watling, 2003a; Pereira, 2004; Cardoso *et al.*, 2011; Rodrigues *et al*., 2014; Penoni *et al*. 2021; Penoni *et al.* 2025; González & Watling, 2003b; Bueno *et al.,* 2011; Bastos-Pereira & Bueno, 2012; Bastos-Pereira & Bueno, 2013; Cardoso et al., 2014; Rodrigues et al. , 2017; Bastos-Pereira, Oliveira and Ferreira, 2018; Pereira, 1985; González and Watling, 2003b).

Type materials for new species will be deposited in the Museu Nacional do Rio de Janeiro (MNRJcarcino), Rio de Janeiro, Brazil.

Observations on population and reproductive biology

Sex ratio was estimated as the number of males divided by the number of females that are non ovigerous.

Relation between head length (HL) and number of eggs in the marsupium were evaluated using a regression analysis.

The number of eggs in the marsupium of all ovigerous females were counted. Eggs were removed with the aid of insulin needles, and measured under the stereomicroscope with attached microscale. All ovigerous females found were used.

To obtain mean egg volume the formula was used:

$$V = \frac{4}{3\pi\, Rmax\, (Rmin)^2}$$

where Rmax and Rmin refer to the maximum and minimum radii of the egg, respectively. After that, a t- test was used to compare the different mean egg volume between stages 1 and 2.

Mean fecundity was calculated by dividing the total number of eggs (286) by the total number of ovigerous females (42).

All statistical analyses were performed using R software (version 4.0.5), using the base package for equations, estimates are given with 95 % confidence intervals.

Results

Taxonomy

Order Amphipoda Latreille, 1816

Suborder Senticaudata Lowry & Myers, 2013

Family Hyalellidae Bulycheva, 1957

Genus *Hyalella* Smith, 1874

***Hyalella XXXX* sp. nov.**

Type locality. Brazil, state of São Paulo, municipality of Angatuba, floodplain along margins of the Guareí river (23º28'6.66''S 48º37'17.51 " W) (Paranapanema River Basin) (Fig. 1 and Fig 2). **Type material. Holotype**, male, body length = 4.35 mm, head length = 0.48 mm, Brazil, municipality of Angatuba, state of São Paulo (23º28'6.66''S 48º37'17.51''W), December, 2012-January 2013 (Fig. 3A); **allotype**, female, body length = 3.60 mm, head length = 0.34 mm, Brazil, municipality of Angatuba, state of São Paulo (23º28'6.66''S 48º37'17.51''W), December 2012- January 2013 (Fig. 3B); paratypes (10 males in slides and 5 females in slide)

Diagnosis. Body surface smooth. Coxa 4 excavated posteriorly. Eyes round, pigmented. Antenna 1 is shorter than antenna 2. Antenna 2 is slightly more than half body length. Maxilla 1 palp longer than wide, reaching less than half the distance between base of palp and tip of setae on outer plate; inner plate slender, with two strong pappose apical setae; Outer plate with seven serrate setae. Maxilla 2 inner plate with two strong and six smaller pappose setae on inner margin. Gnathopod 1 propodus longer than wide, hammer-shaped, inner face with three simple setae, three small cuspidate setae and a row of seven pappose setae, anterior and posterior margins with comb-scales. Gnathopod 2 propodus ovate, palm longer than posterior margin of propodus, slope oblique. Uropod 1 of male without curved seta on inner ramus. Uropod 3 peduncle is wider and shorter than rami, with three cuspidate setae with accessory seta, rami with four distal cuspidate setae with accessory seta and four simple setae. Telson longer than wide, apically rounded with four apical cuspidate setae, and four lateral plumose setae. Coxal gills sac-like present on segments 2 to 6. Sternal gills present on segments 3 to 7.

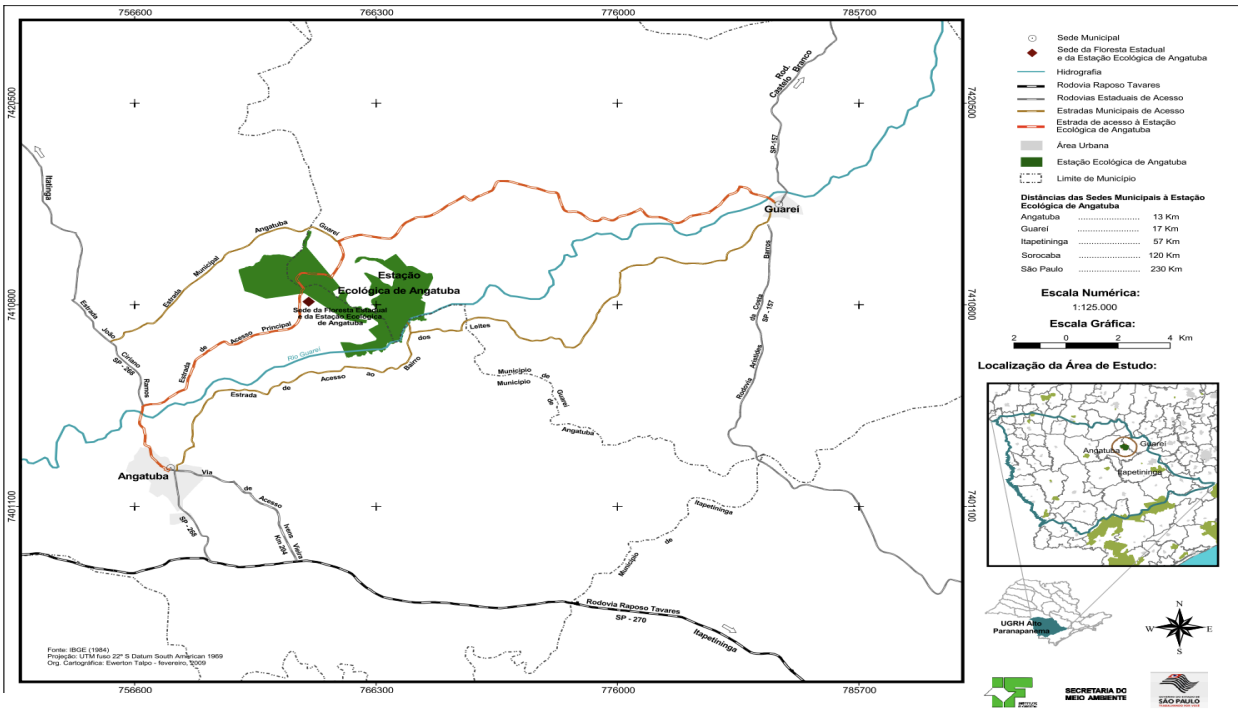

FIGURE 1. Map of Angatuba Ecological Station, state of São Paulo, Brazil. Extracted from the Angatuba Ecological Station Management Plan, prepared in February 2009.

FIGURE 2. Sampling area within the Angatuba Ecological Station. Image provided by Carolina Vieira da Silva

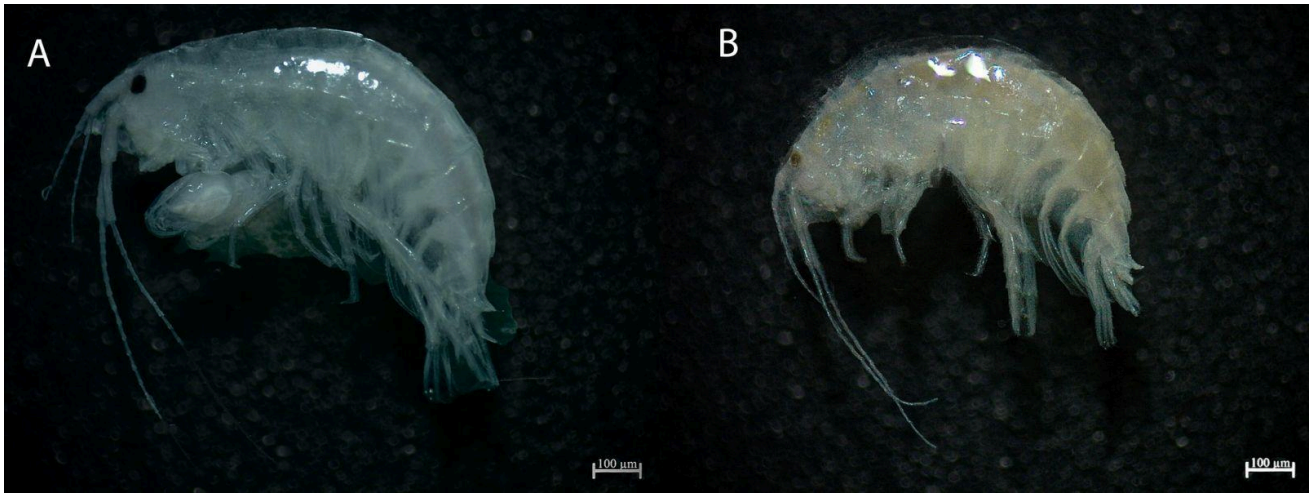

FIGURE 3. *Hyalella* **sp. nov.** (A) Holotype male and (B) Allotype female. Scale bars: 100 µm

Description of male (paratypes). Mean body length: 4.38 ± 0.6 mm, mean head length: 0.48 mm ± 0.1 mm (n=10). Body surface smooth. Epimeral plates with absent acumination. Coxae 1-4

subequal in size and shape, slightly overlapping. Acumination in coxae absent. Coxa 1 similar to 2 and 3. Coxa 3 narrower than 4. Coxa 4 longer than wide, excavated posteriorly. Coxa 5 posterior lobe deeper than anterior lobe. Coxa 6 anterior lobe small. Coxa 7 reduced. Head smaller than the first two thoracic segments. Eyes round and pigmented.

Antenna 1 (Fig. 4A) less than half body length, shorter than antenna 2, longer than peduncle of antenna 2; peduncle slightly shorter than head; article 1 longer than 2, article 3 shorter than 1, and longer than article 2; flagellum with 12 articles, longer than peduncle; 1-2 aesthetascs occurring on each flagellum from article 8 to article 10.

Antenna 2 (Fig. 4B) less than half body length; peduncle slender, longer than head, article 4 shorter than article 5; flagellum with 18 articles, longer than peduncle.

Basic amphipodan mandible (Fig. 4C-4D), with palp absent; left- *lacinia mobilis* with three teeth and setal row with three pappose setae; right mandible incisor with four teeth and two pappose setae; both mandibles with molar process broad and cylindrical with accessory seta.

Upper lip (Fig. 4F) margin rounded; distal border covered by setules on ventral and dorsal faces.

Lower lip (Fig. 4E) outer lobes rounded and distally notched, with setules on dorsal and ventral faces.

Maxilla 1 (Fig. 4G) inner plate slender, shorter than outer plate, with two long pappose apical setae and with many setules on the inner margin. Outer plate with seven serrate setae. Palp short, uniarticulate, longer than wide, reaching less than half length the distance between the base of palp and tip of setae on outer plate, presence of setules.

Maxilla 2 (Fig. 4H) inner and outer plates of similar sizes, inner plate with two strong and six smaller pappose setae and eleven simple setae; outer plate with twenty-one simple distal setae; inner and outer plates covered by several setules.

Maxilliped (Fig. 5C) inner plate longer than wide, with three cuspidate distal setae, thirteen pappose setae and five serrate setae; outer plate smaller than inner plate, with several simple setae on the margin; palp slightly shorter than inner plate and longer than outer plate, five articles; article 1 longer than wide, margins without setae; article 2 longer than wide, outer margin with one simple seta; article 3 slightly longer than wide, outer margin with two simple setae and inner margin with several long simple setae; article 4 slightly longer than wide, outer margin with five simple setae, outer margin with several simple setae and seven serrate distal setae; article 5 (dactylus) unguiform, shorter than fourth article, distal setae simple and shorter than nail, distal nail present.

Gnathopod 1 (Fig. 5A) subchelate; coxal plate wider than long, with simple setae on the margins; basis with one serrate seta on posterior margin and three serrate setae on distal posterior margin; ischium with simple seta; merus with simple seta, one strong and two smaller serrate setae and presence of comb-scales; carpus longer than wide, longer than propodus, lateral distal lobe with twelve paposerrate setae, anterior margin with three serrate setae, two simple setae with accessory seta, absence of polygonal pattern and presence of comb-scales; propodus longer than wide, hammer-shaped, with eight simple setae on disto-anterior margin, comb-scales present, inner margin with two simple setae, and comb-scales on the disto-posterior margin, inner face with five simple setae, three small cuspidate setae and a row of seven pappose setae; palm slope transverse, margin convex, palm with many simple and cuspidate setae, posterior distal corner

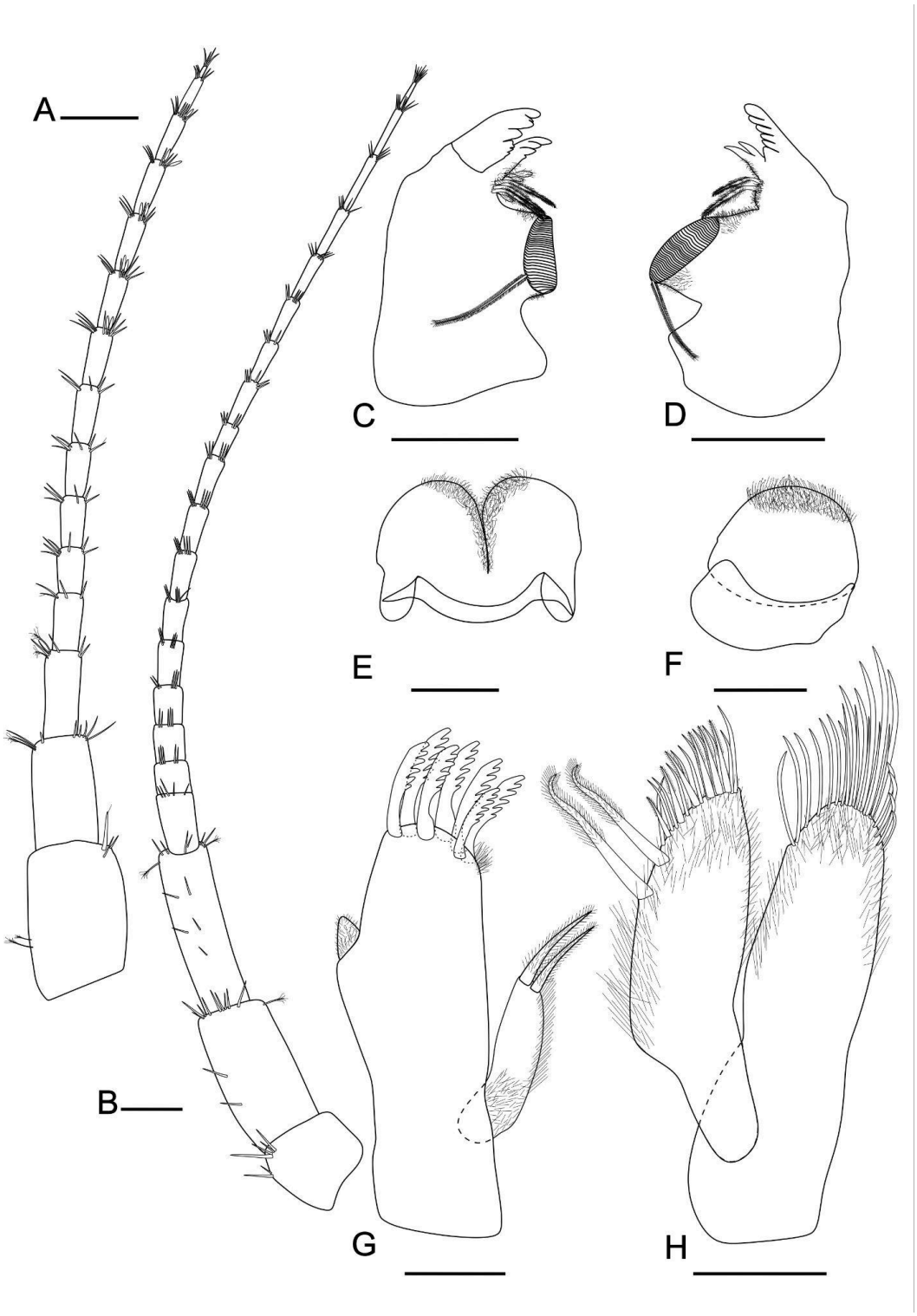

FIGURE 4. *Hyalella* sp. nov. Male paratype, municipality of Angatuba, state of São Paulo, southeast Brazil. (A) Antenna 1; (B) Antenna 2; (C) Left mandible; (D) Right mandible; (E) Lower-lip; (F) Upper-lip; (G) Maxilla 1; (H) Maxilla 2. Scale bars: A-F: 50 µm, G-H: 25 µm.

with two short cuspidate setae with accessory seta and comb-scales; dactylus claw-like, comb-scales present, with one plumose seta dorsally.

Gnathopod 2 (Fig.5B) subchelate; coxal plate wider than long, with simple setae on the margin; basis with one serrate seta on posterior margin and two long simple setae with accessory seta on posterior distal corner; ischium with one simple seta, comb-scales presence on the posterior distal corner and absence of polygonal pattern; merus with one simple seta and comb-scales presence on the posterior margin and three serrate setae and one simple seta on the posterior distal corner; carpus wider than longer, posterior lobe slim produced between merus and propodus with eleven paposerrate setae, anterior distal corner with one serrate seta, one cuspidate seta with accessory seta and one paposerrate seta, comb-scales and polygonal pattern absent; propodus ovate, lobe almost straight, longer than wide, posterior margin with three simple setae and comb-scales present; palm longer than posterior margin of propodus, slope oblique, with one row of several cuspidate setae, one with an accessory seta and simple setae, posterior distal corner with two strong cuspidate setae with accessory seta and one simple seta; dactylus claw-like, congruent with palm, two plumose setae dorsally, comb-scales absent.

Pereiopods 3 to 7 (Fig. 6A-E) simple, coxal plates with margins covered by small simple setae. pereiopods 3 and 4 merus and carpus posterior margin with several cuspidate setae; propodus posterior margin of pereiopod 3 with several cuspidate setae and some simple setae pereiopod 4 with some cuspidate setae with accessory setae and one simple seta; dactylus less than half-length of propodus. Pereiopods 5-7 merus, carpus and propodus posterior margin with marginal clusters of 1-3 cuspidate setae with accessory seta, dactylus less than half-length of propodus. Pereiopods 3-7 presents one plumose seta on the dactylus. Pereiopod 3 and pereiopod 4 with similar sizes; pereiopod 5 smaller than the others; pereiopod 6 smaller than pereiopod 7.

Pleopods (Fig. 7E) peduncle shorter than rami, with coupling spines; both rami with several plumose setae.

Uropod 1 (Fig. 7A) longer than uropod 2; peduncle longer than rami, with five cuspidate setae without accessory seta; rami subequal; inner ramus with two dorsal cuspidate setae with an accessory seta on the margin and three distal cuspidate setae, one with accessory seta, male without curved seta; outer ramus with three dorsal cuspidate setae with accessory seta on the margin and two distal cuspidate setae with accessory seta.

Uropod 2 (Fig. 7B) shorter than uropod 1, peduncle shorter and wider than rami, with four cuspidate setae, three with accessory seta; inner ramus with two dorsal cuspidate setae with accessory seta and three distal cuspidate setae, one with accessory seta; outer ramus with three dorsal cuspidate setae with accessory seta and three distal cuspidate setae, one with accessory seta.

Uropod 3 (Fig. 7C) shorter than peduncle of uropod 1 and peduncle of uropod 2; peduncle longer than wide, with three cuspidate setae with accessory seta; inner ramus absent; outer ramus uniarticulate; ramus longer than peduncle; basal width less than twice apex of ramus, with four distal cuspidate setae with accessory seta and four distal simple setae.

Telson (Fig. 7D) entire, longer than wide, apically triangular, with four apical cuspidate setae and four plumose setae laterally.

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

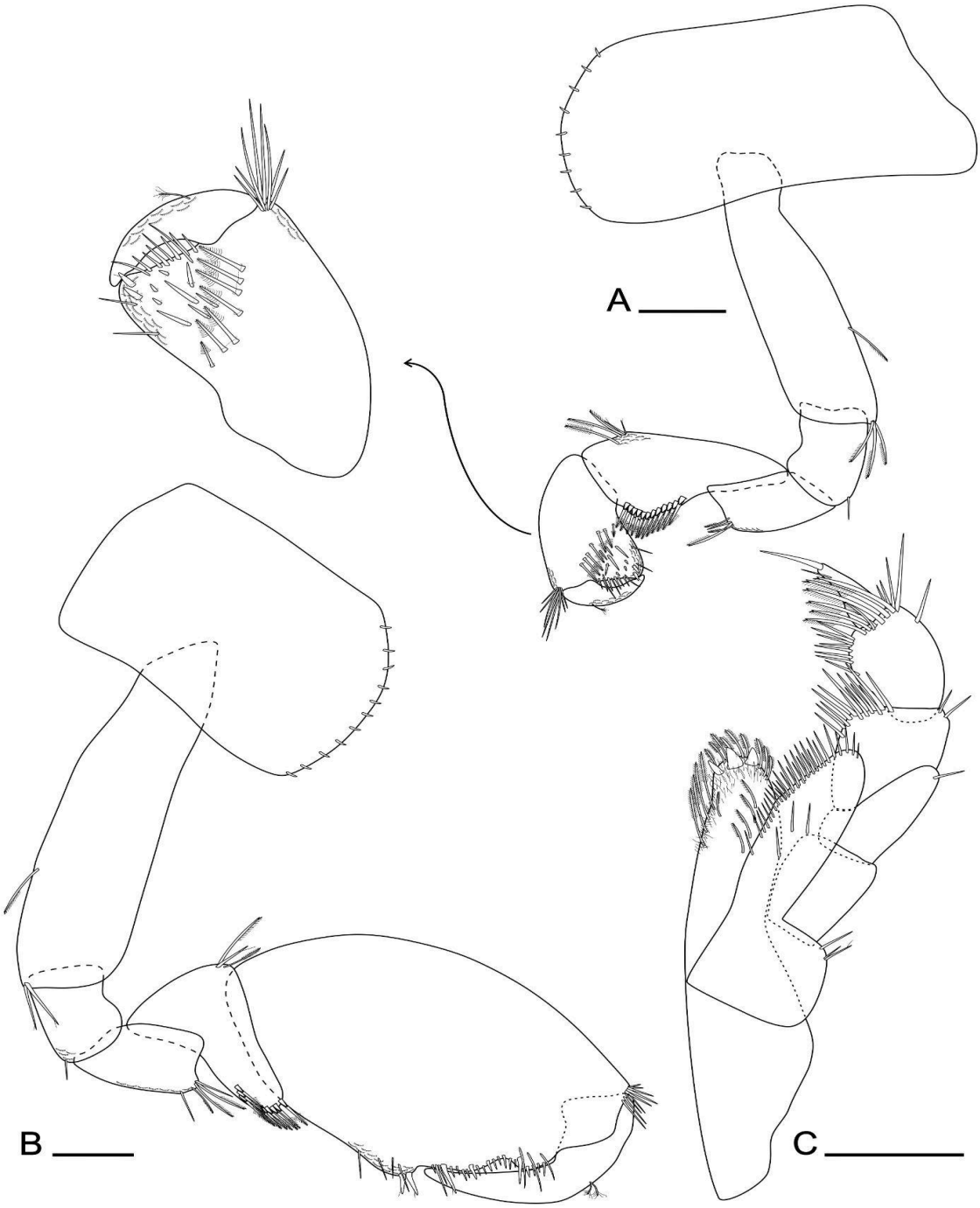

FIGURE 5. *Hyalella* sp. nov.*,* municipality of Angatuba, state of São Paulo, southeast Brazil. Male paratype. (A) Gnathopod 1; (B) Gnathopod 2; (C) Maxilliped. Scale bars: A-C: 50 µm.

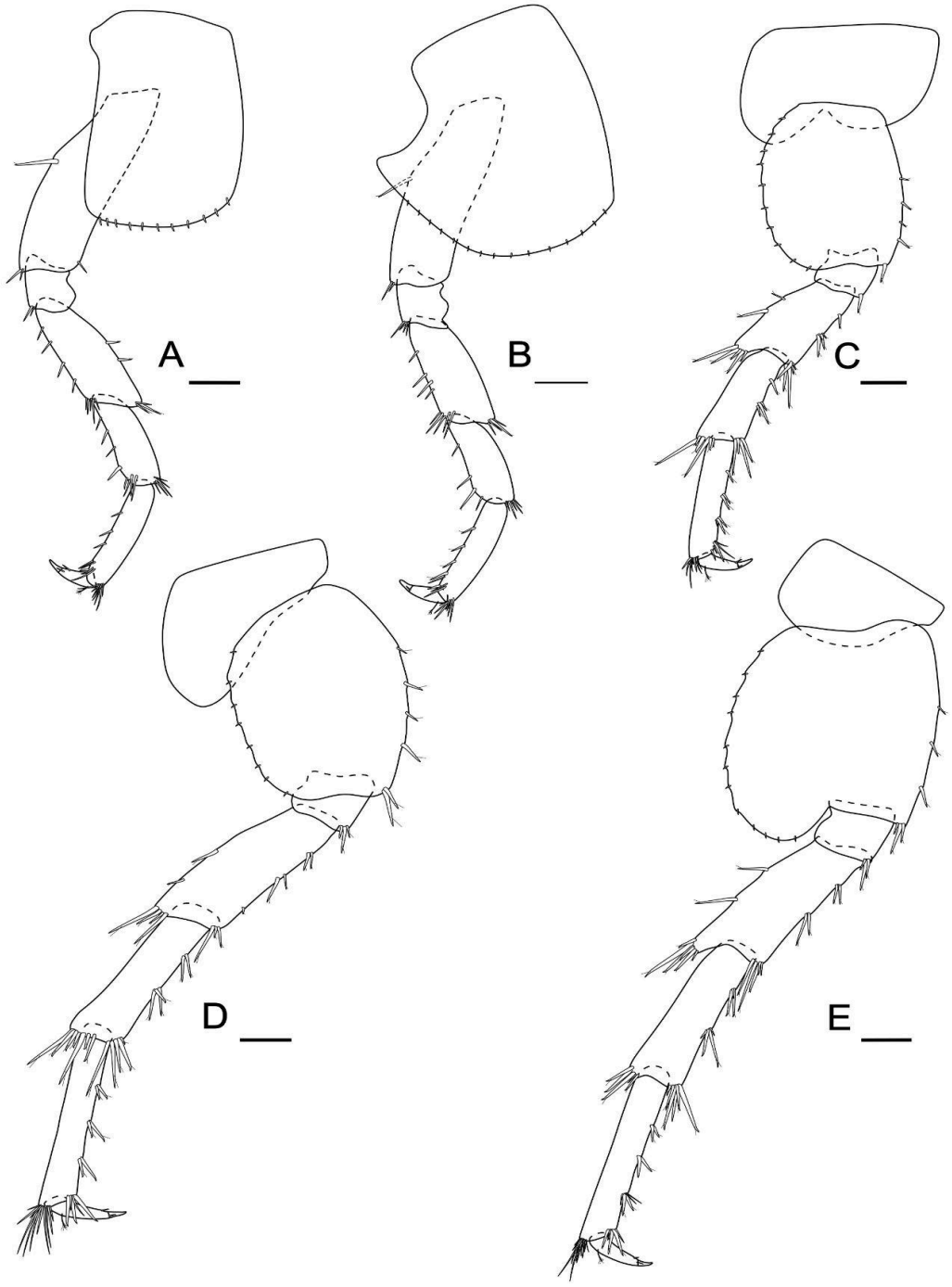

FIGURE 6. *Hyalella* sp. nov., municipality of Angatuba, state of São Paulo, southeast Brazil Male paratype. (A) Pereiopod 3; (B) Pereiopod 4; (C) Pereiopod 5; (D) Pereiopod 6; (E) Pereiopod 7. Scale bars: A-E: 50 μm.

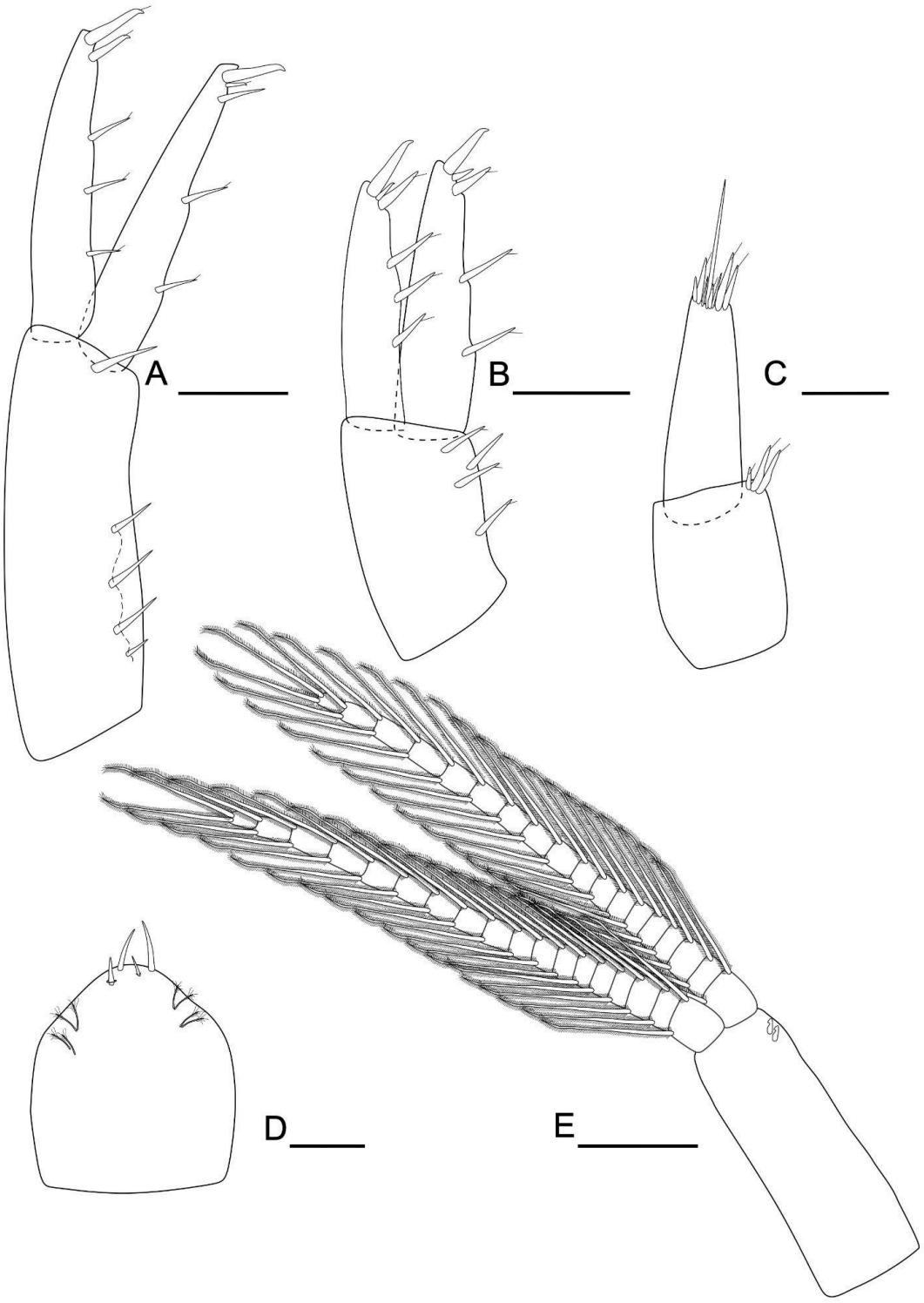

FIGURE 7. *Hyalella* sp. nov.*,* municipality of Angatuba, state of São Paulo, southeast Brazil Male paratype. (A) Uropod 1; (B) Uropod 2; (C) Uropod 3; (D) Telson; (E) Pleopod. Scale bars: A, B, E,: 50 µm, C-D: 25 µm.

Description of female (paratypes). Mean body length: 3.60 ± 1.2 mm, mean head length: 0.34 ± 0.1 mm (n=5).

Gnathopod 1 (Fig. 8B) similar to male gnathopod 1; carpus longer than wide, without comb-scales, with posterior lobe produced and forming a scoop-like structure, with several paposerrate setae, one paposerrate seta on inner face and two serrate setae on anterior distal margin; propodus longer than wide, hammer-shaped, palm shorter than posterior margin of propodus, posterior and anterior margins with comb-scales present, inner margin with few simple setae, inner face with couple clusters of simple setae, palm slope transverse, dactylus claw-like. Gnathopod 2 (Fig. 8C) similar in size and shape to gnathopod 1; different in shape to male gnathopod 2 and smaller; propodus longer than wide, subchelate, inner margin with two simple setae, inner face with a row of seven paposerrate setae, palm transverse with several long simple setae and a couple cuspidate setae, with comb-scales.Telson (Fig. 8A) longer than wide, apically rounded, with two apical simple setae.

Habitat and ecological conservation: Freshwater, epigean. The specimens of *Hyalella* **sp. nov.** were found along a stretch of the Guareí River located within the Angatuba Ecological Station, situated in the southwestern region of the state of São Paulo , in the municipalities of Angatuba and Guareí. This is a preserved forest area managed by the São Paulo State Forestry Institute, and it is part of the Upper Paranapanema River Basin, in a more remote area upstream from the confluence of the Guareí River with the Jurumirim Reservoir. The site selected for this collection is a floodplain area along the Guareí River, located within a dense forest region.

Taxonomic Remarks: The analysis was based on the morphological characteristics and data shown in articles describing all 22 species for the genus *Hyalella* published for southeast Brazil.

Where there are 12 species for the state of São Paulo (*H. meinerti* (Stebbing, 1899); *H. caeca* Pereira, 1989; *H. dielaii* Pereira, 2004; *H. spelaea* Bueno & Cardoso, 2011; *H. epikarstica* Rodrigues, Bueno & Ferreira, 2014; *H. bala* Penoni & Bueno, 2021; *H. virgineae* Lares, Penoni & Bueno, 2021; *H. sumida* Penoni & Bueno, 2025; *H. ceciliae* Penoni & Bueno, 2025; *H. roncador* Penoni & Bueno, 2025; *H. bocaina* Deotti, Penoni & Bueno, 2025 and *H. temimina* Penoni, Deotti & Bueno, 2025), 7 species for the state of Minas Gerais (*H. warmingi* (Stebbing, 1899); *H. xakriaba* Bueno & Araujo, 2011; *H. carstica* Bastos-Pereira & Bueno, 2012; *H. minensis* Bastos-Pereira & Bueno, 2013; *H. veredae* Cardoso & Bueno 2014; *H. montana* Rodrigues, Senna, Quadra & Bueno, 2017 and *H. troglofugia* Bastos-Pereira, Oliveira & Ferreira, 2018) and 3 species for the state of Rio de Janeiro (*H. longistila* (Faxon, 1876); *H. gracilicornis* (Faxon, 1876) and *H. pernix* (Moreira, 1903)).

Comparative analysis shows that *Hyalella* sp. nov. does not correspond to any of the species above, presenting unique set of characteristics: absence of a curved seta on the inner ramus of uropod 1 in males; presence of comb-scales on both gnathopods; gnathopod 2 with two distal plumose setae on the dactylus; uropod 3 with peduncle bearing three cuspidate setae with an accessory seta, and ramus with eight setae; maxilla 1 with seven serrate setae on the outer plate; and telson with four distal cuspidate setae and four lateral plumose setae on each side. Where gnathopod 2 presents two plumose setae on the dactylus, maxilla 1 with seven serrate setae on the outer plate and telson with four lateral plumose setae, differentiates *Hyalella* sp. nov. from all other 22 species.

The species that shows closest affinities with epigean taxa from state of São Paulo, is *H. meinerti*, which can be differentiated by having only one plumose seta present on the dactylus, and absence of comb-scales, both on gnathopod 1 and 2, less articles in both antennas 1 and 2, higher count of setation on uropod 1, a lower count on uropod 3 and the lack of lateral setae on

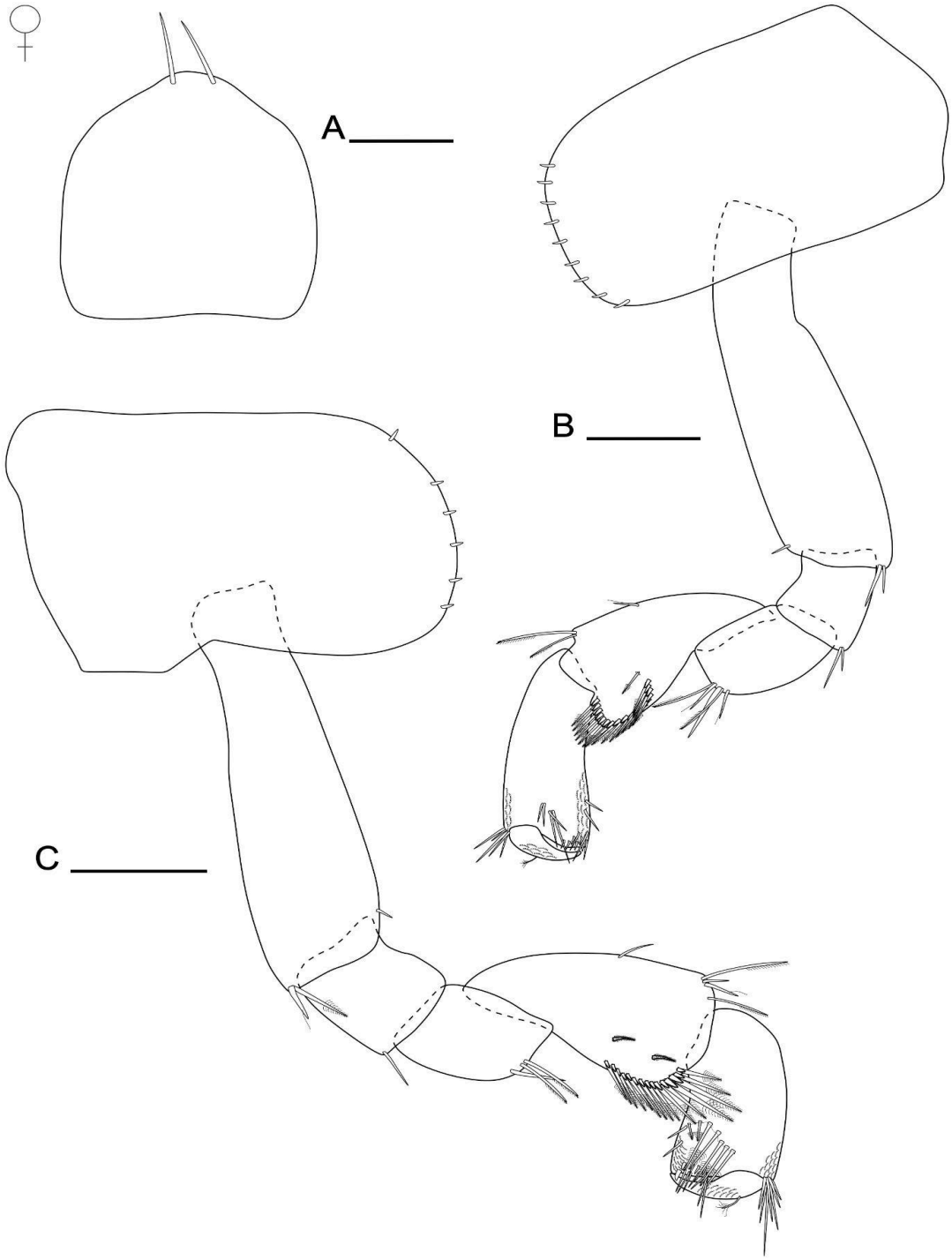

FIGURE 8. *Hyalella* sp. nov.*,* municipality of Angatuba, state of São Paulo, southeast Brazil Female paratype. (A) Telson; (B) Gnathopod 1; (C) Gnathopod 2. Scale bars: C: 50 µm, A-B: 25 µm.

telson.

Table 1 sums up all the morphological differences between *Hyalella* sp. nov. and all other species that occur in southeast Brazil.

Ecological traits

2,367 individuals of *Hyalella* sp. nov. were analyzed: 703 males, 992 females (950 non ovigerous + 42 ovigerous females), and 672 juveniles, which showed a sex ratio of 0.74.

The mean head length was 0.41 ± 0.04 mm for males, 0.35 ± 0.07 mm for females, 0.40 ± 0.05 mm for ovigerous females and 0.22 ± 0.04 mm for juveniles.

Regression analysis of fecundity against size indicated a positive relation between head length and number of eggs, meaning that larger females have a larger brood (Fig. 9).

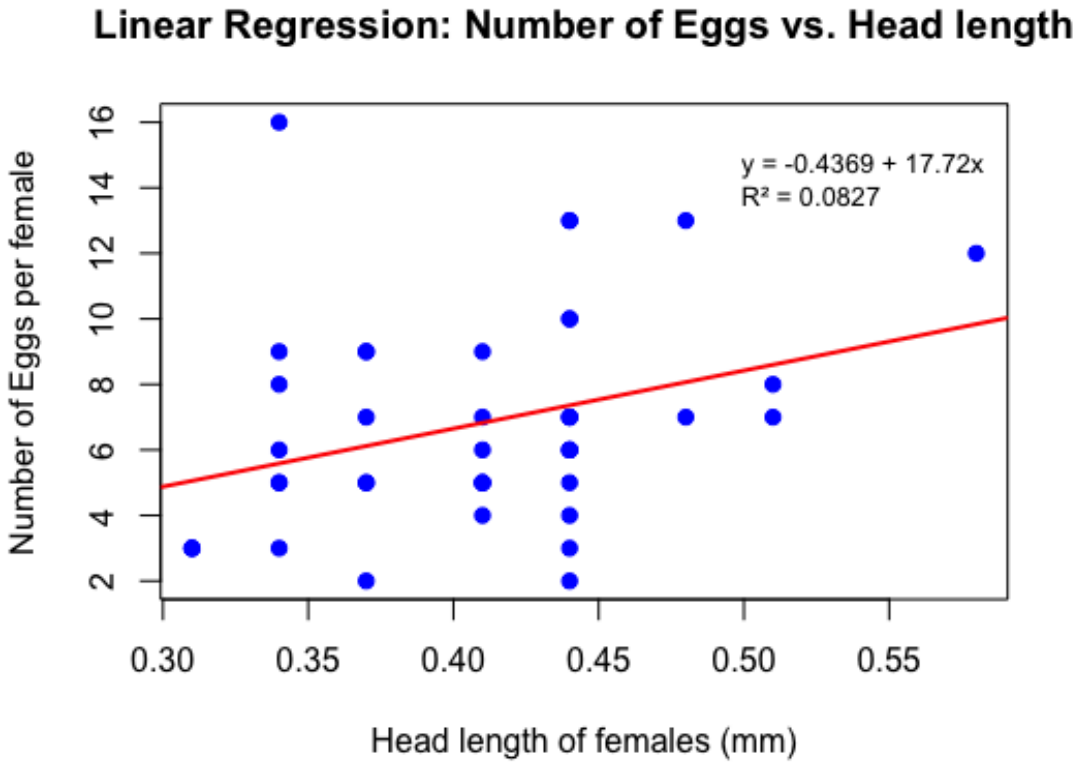

FIGURE 9. Linear regression showing the relation between head length (mm) and the number of eggs per female of *Hyalella* sp. nov.

Mean fecundity found was 6.8. Mean egg volume (Fig. 10) is 0.016 ± 0.02 mm^3 for stage 1 and 0.020 ± 0.003 mm^3 for stage 2 where there is an expected growth

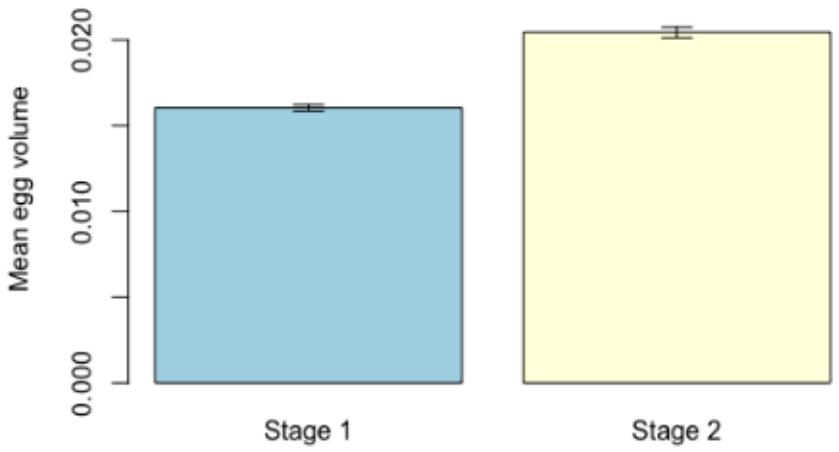

FIGURE 10. t-test showing different mean egg volume between stages 1 and 2.

Discussion

This article describes a new species, along with its ecological data, following a trend that can be seen in Penoni *et al.* (2021), of merging a taxonomic study accompanied by its ecological traits.While taxonomy is essential, incorporating even a few ecological points, represents a significant advancement in expanding the knowledge base for the genus, especially for its habitat and conservation.

Description of new species

Freshwater ecosystems are among the most threatened by anthropogenic degradation, placing aquatic biodiversity under constant pressure (Reid *et al*., 2019). As a result, species are often driven to extinction before their identity or ecology is even understood.

Populations of the genus *Hyalella,* are generally isolated, where most species are endemic (Bueno *et al.*, 2014). The accurate identification of new species are crucial for establishing conservation strategies aimed at preserving the diversity of these amphipods (Wellborn & Capps, 2013).

Hyalella sp. nov. represents a morphologically distinct species that can be clearly differentiated from all other species of southeastern Brazil through a combination of key characteristics: absence of a curved seta on the inner ramus of uropod 1 in males; sternal gills present on segments 3–7; presence of comb-scales on both gnathopods; gnathopod 2 with two

distal plumose setae on the dactylus; uropod 3 with peduncle bearing three cuspidate setae with an accessory seta, and ramus with eight setae; maxilla 1 with seven serrate setae on the outer plate; and telson with four distal cuspidate setae and four lateral plumose setae on each side.

The description of the new species provides solid information for future studies with the genus, and its habitat, meaning that the comprehension of biodiversity is essential, so effective measures can be taken for conservation practices, ensuring environmental balance.

Ecological Traits

Despite an increase in research on the genus *Hyalella* in Brazil, there remains a significant knowledge gap regarding the population ecology of its species. Given the escalating impact on aquatic environments, investigating these ecological parameters is crucial for the conservation and protection of these species, making this a promising area for future research (Martins & Bianchini 2011).

According to Borges, 2024 until 2023 only 17% of all new descriptions for South America has its populational aspects known. This knowledge gap causes severe damage, such as the difficulty in listing the species on a threatened species list like the IUCN, which requires these parameters (Cazalis *et al.* 2022).

For *Hyalella* sp nov. there was a sex ratio leaning towards females, which is the most seen in amphipods (Kevrekidis, 2005), this female predominance may be a strategy to maximize reproductive output.

The study revealed a clear size difference between males and females, with males having a larger mean head length (0.41 ± 0.04 mm) than females (0.35 ± 0.07 mm). This larger size in males is a common pattern in the genus and is associated with their reproductive role of seeking and defending mates, a behavior that favors larger body size in male competitors (Borges, 2024).

The positive relationship between female size and fecundity indicates that larger females produce a larger brood. Female size is often linked to their capacity to carry eggs, which reflects their fertility (Castiglioni & Bond-Buckup 2008a). This reproductive effort, which is dedicated to egg production, is directly related to how females allocate energy for reproduction, which, in turn, influences their body size (Castiglioni & Bond-Buckup 2009; Goos *et al*. 2016). However, the weak correlation ($p=0.03$) suggests that fecundity is also influenced by a combination of complex biotic and abiotic factors, such as the physiological condition of the female and local environmental parameters.

Egg volume differences between developmental stages 1 and 2 confirmed the expected growth during embryogenesis, as previously described for amphipods (Castiglioni & Bond-Buckup, 2009). The mean fecundity of *Hyalella* sp. nov. is 6.8, is considerably lower than other species from the southeastern region of Brazil. For instance, a study on *Hyalella carstica* from Minas Gerais found a mean fecundity of 12.6 ± 7.2 eggs per female (Torres *et al.*, 2015), while research on *Hyalella longistila* from Rio de Janeiro reported a mean of 12.88 ± 2.00 eggs per female (Bastos-Pereira & Bueno, 2016). This comparison highlights that the fecundity of *Hyalella* sp. nov. is low relative to these other regional species.

Acknowledgments

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Table 1. Morphological characteristics of species of *Hyalella* of southeast Brazil.

	Plumose setae G2	Comb-scales G2	Polygonal pattern G2	Coxa 4	Polygonal pattern G1	Comb-scales propodus G1	Comb-scales dactylus G1	Articles A1
H. sp. nov.	2	present	absent	excavated	absent	present	present	12
H. bocaina Deotti, Penoni & Bueno, 2025	1	absent	present	excavated	present	absent	absent	14
H. temimina Penoni, Deotti & Bueno, 2025	1	absent	present	excavated	present	absent	absent	20
H. bala Penoni & Bueno, 2021	none	absent	absent	excavated	absent	absent	absent	10
H. virgineae Lares, Penoni & Bueno, 2021	1	absent	absent	excavated	present	absent	absent	14
H. caeca Pereira, 1989	1	absent	absent	excavated	present	absent	absent	10-11
H. dielaii Pereira, 2004	1	absent	absent	excavated	absent	absent	absent	15-17
H. epikarstica Rodrigues, Bueno & Ferreira, 2014	1	absent	absent	excavated	absent	absent	absent	9
H. meinerti (Stebbing, 1899)	none	absent	absent	excavated	absent	present	present	10
H.spelaea Bueno & Cardoso, 2011	1	present	absent	non exca.	absent	absent	absent	9
H. sumida Penoni & Bueno, 2025	1	absent	present	excavated	present	absent	absent	17-18
H. ceciliae Penoni & Bueno, 2025	1	absent	absent	excavated	absent	absent	absent	13
H. roncador Penoni & Bueno, 2025	3	absent	absent	excavated	present	absent	absent	19-21
H. warmingi (Stebbing, 1899)	1	absent	absent	excavated	absent	absent	absent	13
H. veredae Cardoso & Bueno 2014	1	present	present	excavated	present	absent	absent	9-11
H. carstica Bastos-Pereira & Bueno, 2012	1	present	absent	excavated	absent	present	present	12-13
H. montana Rodrigues, Senna, Quadra & Bueno 2017	1	absent	present	excavated	present	absent	absent	9
H. troglofugia Bastos-Pereira, Oliveira & Ferreira, 2018	1	present	absent	excavated	absent	absent	absent	9-11
H. xakriaba Bueno & Araujo, 2011	1	present	absent	excavated	absent	absent	present	9-12
H. minensis Bastos-Pereira & Bueno, 2013	none	present	absent	excavated	absent	absent	present	11-12
H. longistila (Faxon, 1876)	none	present	absent	excavated	absent	absent	absent	13
H. gracilicornis (Faxon, 1876)	none	present	absent	excavated	absent	present	present	12
H. pernix (Moreira, 1903)	1	present	absent	non exc.	absent	absent	present	12

Table caption: G1- gnathopod 1; G2 gnathopod 2; A1- antenna 1.

Table 1. (continued).

	Articles A2	Main setae LM	Palp setae Mx1	outer plate setae Mx1	Inner plate setae Mx2 (type)	Inner ramus setae U1	Curved seta U1	Ramus setation pattern U3	Lateral plumose setae telson
H. sp. nov.	18	3	absent	7	pappose	2 dorsal; 3 distal	absent	8	4
H. bocaina Deotti, Penoni & Bueno, 2025	17	5	1	9	papposerrate; pappose	3 dorsal; 7 apical	absent	9	3
H. temimina Penoni, Deotti & Bueno, 2025	23	3	1	9	papposerrate	3 dorsal; 3 apical	absent	8	3
H. bala Penoni & Bueno, 2021	14	5	1	8	papposerrate	4 dosal; 4 apical	absent	5	absent
H. virgineae Lares, Penoni & Bueno, 2021	16	7	2	9	papposerrate; simple; serrulate	3 dorsal; 7 apical	absent	5	1-2
H. caeca Pereira, 1989	13-14	3	1	9	pappose; simple ; serrate	3 dorsal; 6 apical	absent	4	3
H. dielaii Pereira, 2004	18-22	6	1	9	simple; serrate; pappose	3 dorsal; 5 apical	absent	10	3
H. epikarstica Rodrigues, Bueno & Ferreira, 2014	7	2	1	9	papposerrate; serrulate; simple	1 dorsal; 4 apical	present	2	absent
H. meinerti (Stebbing, 1899)	12	3	absent	9	pappose	2 dorsal; 4 apical	absent	4	absent
H.spelaea Bueno & Cardoso, 2011	16	4	1	9	papposerrate; simple; serrate	2 dorsal; 8 apical	absent	6	absent
H. sumida Penoni & Bueno, 2025	15	4	1	9	pappose; simple	3 dorsal; 5 apical	absent	7	2
H. ceciliae Penoni & Bueno, 2025	17	4	absent	8	pappose; simple	3 dorsal; 5 apical	absent	3	2
H. roncador Penoni & Bueno, 2025	27	3	1	9	pappose; simple; papposerrate	2 dorsal; 6 apical	absent	up to 9	3
H. warmingi (Stebbing, 1899)	18	3	absent	9	pappose	3 dorsal; 6 apical	absent	6	absent

Table 1. (continued)

	Articles A2	Main setae LM	Palp setae Mx1	outer plate setae Mx1	Inner plate setae Mx2 (type)	Inner ramus setae U1	Curved seta U1	Ramus setation pattern U3	Lateral plumose setae telson
H. veredae Cardoso & Bueno 2014	9-11	3	absent	8-9	papposerrate;serrate;simple	2 dorsal; 5 apical	present	3-4	3
H. carstica Bastos-Pereira & Bueno, 2012	14-16	3	1	8	pappose	2 dorsal; 4 apical	present	5	1
H. montana Rodrigues, Senna, Quadra & Bueno 2017	15	4	7	8	papposerrate;simple	3 dorsal; 3 apical	absent	4	absent
H. troglofugia Bastos-Pereira, Oliveira & Ferreira, 2018	9-12	3	1	8	papposerrate; serrate;simple	3-5 dorsal; 4 apical	absent	4	3
H. xakriaba Bueno & Araujo, 2011	12-15	4	absent	8-9	papposerrate; serrulate;simple	3 dorsal; 2 apical	present	5-6	3
H. minensis Bastos-Pereira & Bueno, 2013	14-17	4	1	7 or less	pappose	4 dorsal; 4 apical	absent	6	absent
H. longistila (Faxon, 1876)	18	3	1	9	pappose	2 dorsal; 5 apical	absent	6	3
H. gracilicornis (Faxon, 1876)	19-25	3	absent	9	pappose	2 dorsal; 5 apical	absent	3-4	absent
H. pernix (Moreira, 1903)	14	3	1	8	pappose; simple	2 dorsal; 6 apical	present	6	3

Table caption: A2- antenna 2; LM- left mandible; Mx1- maxilla 1; Mx2- maxilla 2; U1 - uropod 1; U3 - uropod 3

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