



# A new species of freshwater amphipod (Crustacea, Amphipoda, Hyalellidae) from state of Rio Grande do Sul, Southern Brazil

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Abstract: The current study describes a new species of Hyalella found in a natural pond at the municipality of Palmeira das Missões, located in the northwestern region of state of Rio Grande do Sul, southern Brazil. The new species differs from other species found in state of Rio Grande do Sul (Hyalella bonariensis, H. castroi, H. kaigang, H. imbya, H. georginae, H. gauchensis, H. montenegrinae and H. pampeana) in the type and number of setae on uropods, on telson and on the inner face of gnathopod 1. Besides, Hyalella palmeirensis is morphologically similar to H. curvispina, especially in the shape and number of setae of uropods. However, H. palmeirensis differs from H. curvispina in having only two simple setae on telson, the inner face of gnathopod 1 with five serrate setae and only the posterior distal margin with denticles in comb-scales, and especially by the tubular sternal gills, present on segments 3 to 7. These new records increase from 11 to 12 the number of species known from state of Rio Grande do Sul.

Keywords: Freshwater crustaceans, Hyalella, Peracarida, taxonomy.

# Uma nova espécie de anfípodo dulcícola (Crustacea, Amphipoda, Hyalellidae) do estado do Rio Grande do Sul, sul do Brasil

Resumo: O presente trabalho descreve uma nova espécie de Hyalella encontrada numa nascente no município de Palmeira das Missões, localizado na região noroeste do estado do Rio Grande do Sul, sul do Brasil. A nova espécie difere das outras espécies encontradas no estado do Rio Grande do Sul (Hyalella bonariensis, H. castroi, H. kaigang, H. imbya, H. georginae, H. gauchensis, H. montenegrinae and H. pampeana) especialmente com relação ao tipo e número de setas dos urópodos, télson e superfície ventral do gnatópodo 1. Além disto, H. palmeirensis é similar a H. curvispina, especialmente na forma e número de setas dos urópodos. Entretanto, H. palmeirensis difere de H. curvispina por possuir somente duas setas simples no télson, cinco setas serradas na superfície ventral do gnatópodo 1 e dentículos (comb-scales) somente na margem posterior do gnatópodo 1 e brânquias esternais presentes do segmento 3 ao 7. Esse novo registro aumenta para 11 o número de espécies de Hyalella conhecidas para o estado do Rio Grande do Sul.

Palavras-chave: Crustáceo dulcícola, Hyalella, Peracarida, taxonomia.

#### Introduction

Amphipoda is a successful group of peracaridan crustaceans known in several aquatic environments, including freshwaters where it exerts dominance of the benthic community of lakes (Ishikawa and Urabe 2002; Rodrigues 2016). The latest classification of the Order is shown by Lowry and Myers (2013), and these authors presented a new Suborder called Senticaudata, comprising almost all freshwater Amphipoda species and many marine species that previously belonged to the defunct Suborder Gammaridea.

The Family Hyalellidae is represented only by *Hyalella* Smith, 1874, which occurs throughout the Nearctic and Neotropical biogeographic regions, and it is endemic of the Americas (Baldinger 2004), presenting restrict distribution of species (Bousfield 1996). There are 74 limnic crustacean species of *Hyalella* (Marrón-Becerra et al. 2014; Colla and César 2015; Streck et al. 2017; Bastos-Pereira et al. 2018), however the highest diversity of species occurs in South America, where 56 species are currently described (Rodrigues et al. 2014, Colla and César 2015; Streck et al. 2017; Bastos-Pereira et al. 2018).

Brazil has the greatest number of *Hyalella* species described in the world, 27 in total (Bueno et al. 2013; Bueno et al. 2014; Cardoso et al. 2014; Rodrigues et al. 2014; Streck et al. 2017; Rodrigues et al. 2017, Bastos-Pereira et al. 2018). According to Bueno et al. (2014) and Streck et al. (2017) the state of Rio Grande do Sul (Southern Brazil) is the region with the highest species diversity in the country, counting 11 described species. With the finding of this specie the state of Rio Grande do Sul account now with 12 *Hyalella* species, the same number of species observed in all Southeast region of Brazil.

A new species of *Hyalella* is described from the municipality of Palmeira das Missões, northwest region of state of Rio Grande do Sul, southern Brazil.

# **Material and Methods**

The municipality of Palmeira das Missões is located at the northwest region of state of Rio Grande do Sul, Southern Brazil. The specimens were collected in August/2012 and the sampling was made with the aid a hand net, with authorizations of the Instituto Chico Mendes de Conservação da Biodiversidade (MMA; ICMBio; SISBIO nº 32726-1).

Head length of the animals was measured through an optic microscope with a millimetric scale. Adult males and females were preserved in ethanol 70%, colored with Rose Bengal and dissected. A total of 15 individuals (10 males and 5 females) were dissected and the appendices were mounted on permanent slides. Illustrations were made using photos and the CorelDraw program.

The description was made based on main morphological characteristics such as the gnathopods, uropods and telson, according to González et al. (2003a, b), González et al. (2006), Cardoso et al. (2011), and Bastos-Pereira and Bueno (2012). The terminology used for the setae of the appendices follows Zimmer et al. (2009).

The description was based on male paratype and female allotype. The type material is deposited in the Museu Nacional do Rio de Janeiro (MNRJ).

#### Results

Taxonomy Order Amphipoda Latreille, 1816 Suborder Senticaudata Lowry & Myers, 2013 Family Hyalellidae Bulycheva, 1957 Genus *Hyalella* S. I. Smith, 1874

#### Hyalella palmeirensis n. sp. Streck-Marx & Castiglioni

**Type material:** Holotype male, total body length = 5.32 mm, head length = 0.50 mm (MNRJ 26584); Allotype female (MNRJ 26585). Brazil, state of Rio Grande do Sul, Palmeira das Missões municipality (27° 56.949' S – 53° 19.613' W), August, 24, 2012, Castiglioni, D.S. coll.

**Paratypes:** Mean total body length of male paratypes:  $5.50 \pm 0.78$  mm and female paratypes:  $5.00 \pm 0.50$  (20 males and 20 females) (MNRJ 26586), same date as holotype, Castiglioni, D.S. coll.

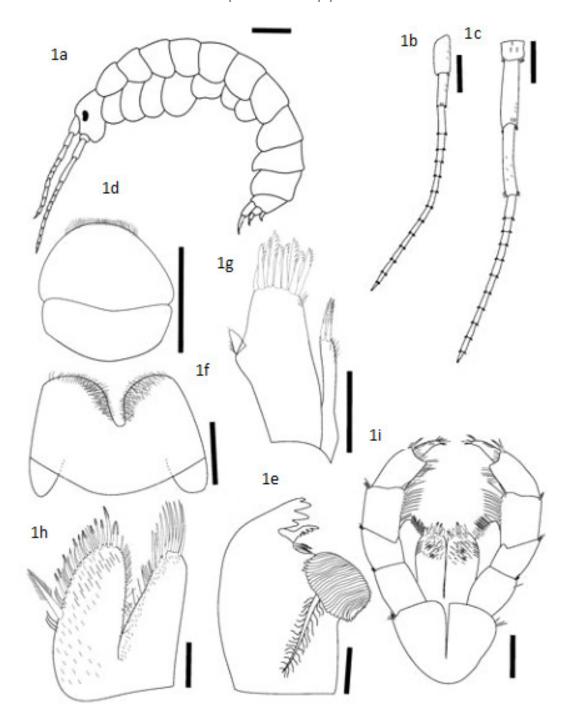
**Type-locality.** Brazil, state of Rio Grande do Sul (27°56.949'S 53°19.613'W), state of Rio Grande do Sul, Palmeira das Missões municipality, natural pond, 560 m, August, 24, 2012, Castiglioni, D.S. coll.

Diagnosis. Body surface smooth. Eyes round, pigmented. Antenna 1 shorter than antenna 2, flagellum with 11/12 articles and longer than peduncle, two aesthetascs occurring distally on flagellum after article 5. Antenna 2 flagellum with 10/11 articles and longer than peduncle. Maxilla 1 palp uniarticulate, short, longer than wide, inner plate shorter and slender than outer plate, with two long apical papposerrate setae, and some setules on the inner margin; outer plate with nine serrate setae. Maxilla 2 inner plate with only one long and strong papposerrate seta and several simple and serrate setae. Gnathopod 1 with inner face with five serrate setae, posterior distal margin with denticles in comb-scales, one cuspidate seta and some simple setae. Gnathopod 2 propodus longer than wide and with posterior margin of small lobe covered by denticles in comb-scales; palm smooth and sub-equal to posterior margin of propodus, slope oblique, palm margin convex; dactylus claw-like, short, not reaching the lobe of propodus; carpus wider than long, posterior lobe elongated, with pectinate border and one row of serrate setae, without polygonal pattern or comb-scales. Pleopods rami with long plumose setae. Uropod 1 inner ramus with a long-curved seta followed by a row of five small cuspidate setae with an accessory seta. Uropod 2 inner ramus apex with five cuspidate setae with an accessory seta and two cuspidate simple setae. Uropod 3 peduncle with three cuspidate setae with accessory setae apically, small cuspidate setae and simple setae distributed along the peduncle and ramus. Telson (male) longer than wide, with two apical simple setae, and three plumose setae close to each simple seta. Coxal gills sac-like present on segments 2 to 6 and sternal gills tubular, presents on segments 3 to 7.

**Description of male.** (Fig. 1a) Mean body length:  $5.50 \pm 0.78$ , mean head length:  $0.52 \pm 0.07$  mm (n=20). Head longer than the first thoracic segment, rostrum absent. Eyes round, pigmented.

Antenna 1 (Fig. 1b) shorter than antenna 2, peduncle not surpassing head length, flagellum with 11/12 articles, longer than peduncle; aesthetascs occurring distally on flagellum after article 5.

Antenna 2 (Fig. 1c) peduncle less than half body length, with very few setae; flagellum with 10/11 articles and longer than peduncle.



**Figure 1.** *Hyalella palmeirensis* n. sp. Paratype male (MNRJ 26586): (a) Habitus from holotype (MNRJ 26584), body length: 5.32 mm; (b) Antenna 1; (c) Antenna 2; (d) Upper lip; (e) Left mandible; (f) Lower lip; (g) Maxilla 1; (h) Maxilla 2; (i) Maxilliped; Scales: (1a-1c) represents 0.5 mm; (1d-1g) 0.2 mm; (1h) 0.05 mm; (1i) 0.2 mm.

Upper lip (Fig. 1d) margin rounded, distal border covered by several setules on dorsal and ventral faces.

Basic amphipodan mandible (Fig. 1e) (in sensu of Watling 1993), without palp; incisor toothed; left *lacinia mobilis* with five teeth and with three pappose setae; molar process large, cylindrical and with large accessory seta.

Lower lip (Fig. 1f) outer lobes rounded without notches or excavations, with several setules on dorsal and ventral faces.

Maxilla 1 (Fig. 1g) palp uniarticulate, short, longer than wide, reaching less than half length the distance between the base of the palp and tip of setae on outer plate; inner plate uniarticulate, shorter and slender than outer plate, with two long apical pappose setae. Outer plate uniarticulate, with 9 serrate setae.

Maxilla 2 (Fig. 1h) inner plate with one long and strong papposerrate seta, several simple and serrate setae; outer plate with one row of simple setae.

Maxilliped (Fig. 1i) inner plates apically truncated, with three connate setae and pappose and simple setae apically and medially; outer plates larger than inner plates, apically truncated, apical, medial, and facial setae simple. Palp of four articles with simple setae and few pappose setae; dactylus unguiform, shorter than propodus, with simple setae and comb-scales.

Gnathopod 1 (Fig. 2a) subchelate; dactylus claw-like, with denticles in comb-scales and one plumose seta dorsally; propodus longer than wide, width about 3/4 of maximum length (rectangular), hammer-shaped, palm slope slightly oblique and margin sinuose with several simple setae, posterior distal margins with comb scales, one cuspidate setae and some simple setae, anterodistal margin without comb scales, but with six or seven simple setae, propodus inner face (ventral) with five serrate setae; carpus longer than wide, slightly longer than propodus, with strong lateral distal lobe produced and forming a scoop-like structure, posterior lobe of carpus with one row of serrate setae and without comb-scales; basis, ischium and merus with simple setae dorsally, without comb-scales posterodistally; coxal plates longer than wide.

Gnathopod 2 (Fig. 2b) subchelate; dactylus claw-like, short, not reaching the lobe of propodus, with one plumose seta dorsally, without comb-scales; propodus longer than wide (ovate), propodus length 1.5 times the maximum width, posterior margin of lobe almost straight and covered with denticles in comb-scales; palm margin convex, with several cuspidate setae with accessory setae and simple setae, posterior distal corner with few simple setae; carpus wider than long, posterior lobe elongated, with pectinate border with one row of serrate setae and without polygonal pattern or comb-scales; ischium and merus with few simple setae on posterior margin; basis, ischium and merus without comb-scales on posterior margin; coxal plate wider than long.

Peraeopods 3 to 7 (Fig. 3a to 3e) dactylus, propodus, carpus, merus, ischium and basis with simple and cuspidate setae with accessory seta. All coxal plates with simple setae on the border.

Pleopods (Fig. 4a) not modified, peduncle slender, smaller than flagellum rami, and two distal coupling spines; both rami with long plumose setae.

Uropod 1 (Fig. 4b) peduncle slightly (1.2 times) longer than rami, with four cuspidate setae with an accessory seta dorsally; inner ramus longer than outer ramus; inner ramus with two dorsal cuspidate setae

with an accessory seta, male with a curved seta followed by a row of five small cuspidate setae with an accessory seta, two cuspidate setae apically; outer ramus with three dorsal cuspidate setae with an accessory seta and four cuspidate setae apically (two of them with an accessory seta).

Uropod 2 (Fig. 4c) shorter than uropod 1; peduncle rectangular with similar size as rami, with four cuspidate setae with an accessory seta dorsally; inner ramus slightly longer than outer ramus, with two dorsal cuspidate setae with an accessory seta and seven cuspidate setae apically (five with an accessory seta); outer ramus with two dorsal cuspidate setae with an accessory seta and apex with four cuspidate setae (two with an accessory seta).

Uropod 3 (Fig. 4d) peduncle slightly longer than wide, wider than ramus, with three cuspidate setae with an accessory seta and one simple seta apically, two simple setae distributed along the peduncle; ramus with five simple setae and a short, strong cuspidate setae.

Telson (Fig. 4e) is longer than wide, with two long simple setae and three plumose setae close to each simple seta.

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

**Female.** Mean total body length:  $5.00 \pm 0.50$ , mean head length:  $0.47 \pm 0.05$  (n= 20). Antenna 1 similar shape to male but with flagellum of 7–8 articles. Antenna 2 similar in shape to male, flagellum of 8–9 articles.

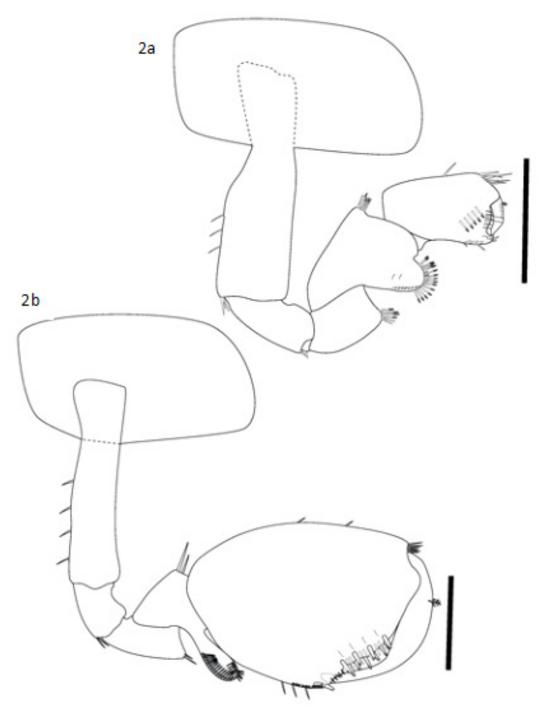
Gnathopod 1 (Fig. 5a) similar to male gnathopod 1, similar size to gnathopod 2; dactylus with comb-scales and plumose seta; propodus longer than wide, hammer-shaped, inner face with five serrate setae and two long simple setae and several short simple setae, anterior distal margin without comb-scales but two simple setae and distally with six or seven short simple setae, posterior distal margin with comb-scales and two simple setae and posterior distal corner with one strong cuspidate seta; carpus longer than wide, with lateral distal lobe produced and forming a scoop-like structure, posterior lobe margin with polygonal pattern and with serrate setae and without comb-scales; base, merus and ischium without comb-scales on posterior margin.

Gnathopod 2 (Fig. 5b) similar in size to gnathopod 1, but the propodus of gnathopod 2 is slightly longer than gnathopod 1; different to male gnathopod 2 in shape and smaller; dactylus with comb-scales and one plumose seta; propodus rectangular, longer than wide, inner face with four serrate setae and two long simple setae and several short simple setae, palm transverse, posterior distal corner with one strong cuspidate seta, posterior distal margins with comb-scales and two simple setae and anterior margin without comb-scales; base, merus and ischium without comb-scales on posterior margin. Telson similar in shape to male, with two long simple setae and three plumose setae close to each simple seta.

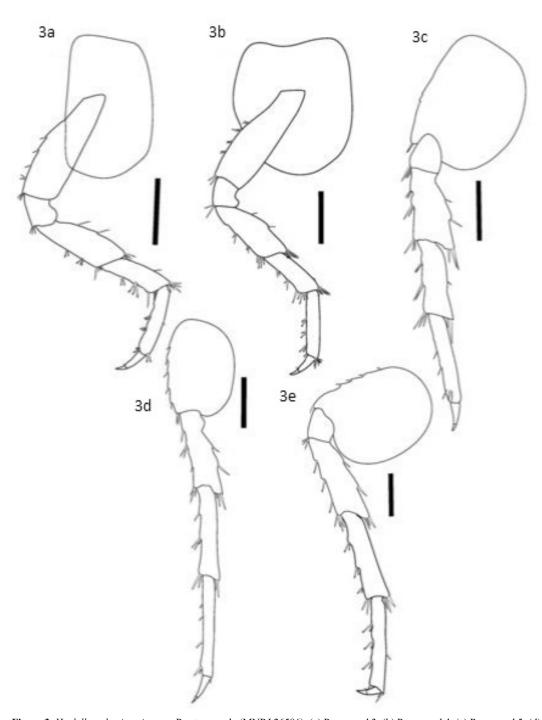
**Habitat.** Freshwater, epigean. *Hyalella palmeirensis* was found in a natural pond of shallow depth (around 40 cm) (Fig. 6a and 6b). The pond contains a large amount of macrophytes of the genus *Egeria* which were used as shelter for the specimens of *H. palmeirensis*.

**Etymology.** The specific name, *palmeirensis*, refers to native residents of Palmeira das Missões municipality, southern Brazil.

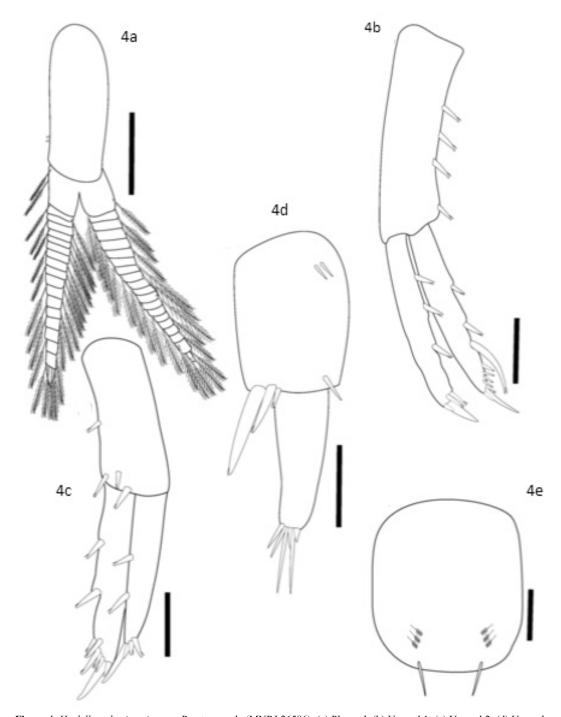
**Remarks.** We noted using the taxonomic key of Rodrigues et al. (2014) that *H. palmeirensis* differs from the other species of the genus in Brazil. *Hyalella palmeirensis* resembles *H. bonariensis*, *H. montenegrinae*, *H. curvispina*, *H. castroi*, *H. imbya*, *H. pseudoazteca*,



**Figure 2.** *Hyalella palmeirensis* n. sp. Paratype male (MNRJ 26586); (a) Gnathopod 1; (b) Gnathopod 2; Scales: (2a-2b) represents 0.5 mm.



**Figure 3.** *Hyalella palmeirensis* n. sp. Paratype male (MNRJ 26586); (a) Peraeopod 3; (b) Peraeopod 4; (c) Peraeopod 5; (d) Peraeopod 6; (e) Peraeopod 7; Scales: (3a-3e) represents 0.5 mm.



**Figure 4.** *Hyalella palmeirensis* n. sp. Paratype male (MNRJ 26586); (a) Pleopod; (b) Uropod 1; (c) Uropod 2; (d) Uropod 3; (e) Telson; Scales: (4a) represents 0.5 mm; (4b-4d) 0.2 mm; (4e) 0.05 mm.

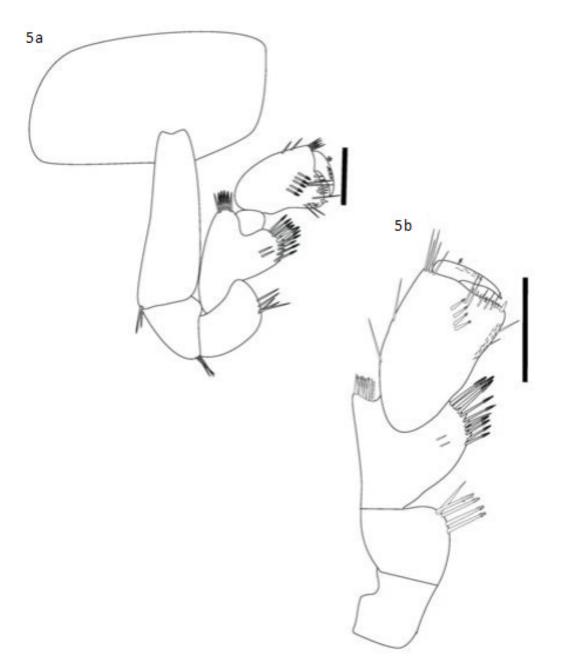


Figure 5. Hyalella palmeirensis n. sp. Allotype female (MNRJ 26585); (a) Gnathopod 1; (b) Gnathopod (2) Scales: (5a-5b) represents 0.2 mm.

H. kaingang, H. pampeana, H. pleoacuta, H. carstica, H. xakriaba, H. georginae and H. gauchensis, by having a curved seta on the inner ramus of uropod 1 of the males.

Besides, *H. palmeirensis* resembles *H. montenegrinae*, *H. carstica* and *H. montana* Rodrigues et al., 2017 in having sternal gills on segments 3–7. The new species resembles *H. curvispina*, *H. castroi*, *H. pseudoazteca*, *H. kaingang* and *H. pleoacuta* in having the dactylus of gnathopod 2 shorter than the propodus palm. Differs from *H. bonariensis*, *H. castroi*, *H. kaigang H. imbya*, *H. georginae*, *H. gauchensis*, *H. montenegrinae* and *H. pampeana* in the type and number of setae on uropods, telson and inner face of gnathopod 1. *Hyalella palmeirensis* is similar to *H. curvispina*, especially in the shape

and number of setae of uropods. However, *H. palmeirensis* differs of *H. curvispina* in having only two simple setae on telson, inner face of gnathopod 1 with five serrate setae and only the posterior distal margin with denticles in comb-scales, and especially by tubular sternal gills present on segments 3 to 7.

The new species *Hyalella palmeirensis* resembles *H. misionensis*, from the Province of Misiones in Argentina, in the telson (male) with two long apical simple setae. However, it differs in the curved seta on the inner ramus of uropod 1, inner face of propodus of gnathopod 1 and sternal gills. The area where *H. misionensis* was found is geographically close to Brazil. Comparisons between morphological characters of *Hyalella* species of Rio Grande do Sul are presented in Table 1.





**Figure 6.** Type-locality of *Hyalella palmeirensis* n. sp. Streck-Marx & Castiglioni; (a) Showing the natural pond of shallow depth (around 40 cm). Note the large amount of macrophytes of the genus *Egeria*, which are used as shelter for the specimens of the new species; (b) Showing the natural pond of shallow and surrounding cultivation of any type of crops, as soy and rice which could be considered a threat to this species as the pesticides used in these cultures can be harmful to the amphipods.

## Discussion

The new species described here, improves the diversity of *Hyalella* in the Southern region of Brazil, increasing the number of species found in the region, to 14, whereas 12 of them are observed in the state of Rio Grande do Sul, the same number of all southeastern region of Brazil (Bueno et al. 2014; Cardoso et al. 2014; Rodrigues et al. 2014).

In addition to morphological differences, other evidence that *H. palmeirensis* is a distinct species from others of the genus comes from a matrix of pairwise nucleotide sequence divergences using concatenated

genes 16 S (~650bps), 12 S (~550bps) and H3 (~375bps) (Rodrigues 2016). This analysis showed that *H. palmeirensis* (called *Hyalella* sp. 20 by Rodrigues 2016) diverge at least 15.3% to 25.4% from *H. curvispina*, (the closest species), and 16.2% from *H. gauchensis* (called *Hyalella* sp. 18 by Rodrigues 2016) and 17.6% from *H. georginae* (called *Hyalella* sp. 19 by Rodrigues 2016) (Rodrigues 2016). It should be noted that *H. gauchensis* and *H. georginae* are two species that occur in nearby areas where *H. palmeirensis* was sampled (Streck et al. 2017). Moreover, Rodrigues (2016) estimated the phylogeny of *Hyalella* reconstructed by Bayesian analysis and according to it, *H. palmeirensis* is statistically

**Table 1.** Main morphological differences between *Hyalella palmeirensis* n. sp. Streck-Marx & Castiglioni and 11 *Hyalella* species from nearby areas in the state of Rio Grande do Sul, Southern, Brazil.

Species	Body surface	Inner margin of maxila 2	Articles of flagellum of antenna 1	Articles of flagellum of antenna 2	Inner face of propodus of gnathopod 1	Setae on peduncle of uropod 3	Telson	Sternal gills	Tipe locality
H. bonariensis	Smooth	Two pappose setae	9–12	12–15	5 setae	6 setae	Quadrangular, with 2–3 cuspidate setae apically	2–7	Province of Buenos Aires, Salto, Argentina
H. castroi	Smooth	One pappose setae	10–17	14–18	More than 10 serrate setae	7 setae	Wider than long, with 8 setae	2–7	Vale of trout, Rio Grande do Sul, Brazil
H. curvispina	Smooth	Two plumose setae	11	13	5–7 setae	3 setae	Wider than long, with 3 simple spines	2–7	Lake Tramandai, Rio Grande do Sul, Brazil
H. georginae	Smooth	Two robust papposerrate apical setae	15	16	9 serrate setae	9 setae	Wider than long, with 7 apical cuspidate setae with an accessory seta, and 4 plumose setae laterally	2–7	Palmeira das Missões, Rio Grande do Sul, Brazil
H. gauchensis	Smooth	Two papposerrate apical setae	11	15	9–20 serrate setae	6 setae	Wider than long, with 6 apical cuspidate setae, and 6 plumose setae laterally	2–7	Palmeira das Missões, Rio Grande do Sul, Brazil
H. imbya	Smooth	Two papposerrate setae	18–23	14–16	7 serrate setae	1 seta	Two long simple apical setae	3–7	Roque Gonzales, Rio Grande do Sul, Brazil
H. kaigang	With dorsal flanges on pleonites 1-2	Two papposerrate setae	17–18	18–24	2 rows of serrate setae	6 setae	Wider than long, with 6–7 cuspidate seta with acessory setae	2–7	Garapiá stream, municipality of São Francisco de Paula, Rio Grande do Sul, Brazil
H. montenegrinae	Smooth	Two plumose setae	14–16	14–19	9–10 plumose setae	4 or 5 setae	Wider than long, 7 to 9 setae	3–7	São José dos Ausentes, Rio Grande do Sul, Brazil
H. pampeana	Smooth	Two bipectinada setae	11–12	Up to 18	5–6 setae	5 to 7 setae	As wide as long, apically rounded, with 2–5 spines	2–7	Province of Buenos Aires, Argentina
H. pleoacuta	With dorsal flanges on some segments	Two pappose setae	14	15	9 serrate setae	4 setae	As long as wide, with 2 simple setae	2–7	Vale of Trout, Rio Grande do Sul, Brazil
H. pseoudoazteca	With dorsal flanges on some segments	One pappose seta	10	8–9	1–3 pappose setae	1 seta	Wide as long, 2 closely spaced, long simple setae	3–7	Taim Ecological Reserve, Rio Grande do Sul, Brazil
H. palmeirensis	Smooth	One pappose setae	11–12	10–11	5 serrate setae	3 setae	Longer than wide, with 2 long simple setae and 6 plumose setae laterally	3–7	Palmeira das Missões, Rio Grande do Sul, Brazil

well supported in a different clade from *H. curvispina*, *H gauchensis* and *H. georginae*, forming distinct groups which have divergence at 28.55 million of years ago.

The species *Hyalella palmeirensis* lives in association with macrophytes in a natural pond of shallow depth. The pond contains a large amount of macrophytes of the genus *Egeria*, which are used as shelter by *H. palmeirensis*. However, *H. palmeirensis* occurs in a natural pond surrounded by the cultivation of many types of crops, which could be considered a threat to this species as the pesticides used during cultivation can be harmful to the amphipods. Agricultural expansion, especially rice and soybean, is one of the main factors that affect and hinders conservation, because this activity not only remove the original vegetation for cultivation but also often bring out chemicals for soil remediation, pest control or fertilization, and cause fragmentation and deterioration of these ecosystems (Maltchik and Rolon 2010; Rodrigues et al. 2012).

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## **Authors Contributions**

Morgana Taís Streck Marx: contribution to data collection, manuscript preparation, data analysis and interpretation.

Daniela da Silva Castiglioni: contribution to data collection and concept and design of the study.

### **Conflicts of interest**

The authors declare that they have no conflict of interest related to the publication of this manuscript.

## **Ethics**

The sampling was made with authorizations of the Instituto Chico Mendes de Conservação da Biodiversidade (MMA; ICMBio; SISBIO nº 32726-1).

# **Data availability**

The type material is deposited in the Museu Nacional do Rio de Janeiro (MNRJ).

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