



RESEARCH ARTICLE

Seek and you shall find: new species of the rare genus Ornamentula (Gastrotricha: Chaetonotida) and first record outside of type-locality

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ABSTRACT. Ornamentula Kisielewski, 1991 is a monospecific genus in Order Chaetonotida. The sole species, O. paraensis Kisielewsk, 1991, is a semiplanktonic gastrotrich found in a single pond in the Amazon region of Brazil. Herein we describe a new species of the genus Ornamentula, collected in a small urban lagoon in the Atlantic Forest of southeast Brazil. Ornamentula miyazakii **sp. nov.** resembles O. paraensis, but it shows differences in the ornamented trunk scales and spinal spines distribution, sufficient to proposte it as it's a new species.

KEY WORDS. Atlantic rainforest, semiplanktonic gastrotrich, taxonomy, urban lagoon.

INTRODUCTION

Gastrotricha are free-living microinvertebrates found in benthic and epiphytic as well as periphytic biotopes in marine and freshwater habitats around the world (Balsamo et al. 2008, 2014, Kieneke and Schmidt-Rhaesa 2015, Todaro et al. 2019). The phylum is divided in two orders (Macrodasyida Remane, 1925, Chaetonotida Remane 1925), 18 families, 69 genera and more than 860 species (Balsamo et al. 2014, 2015, Garraffoni et al. 2017, Todaro et al. 2019).

Although genera such as *Chaetonotus* Ehrenberg, 1830, *Turbanella* Schultze, 1853, *Aspidiophorus* Voigt, 1903, *Tetranchyroderma* Remane, 1926, and *Macrodasys* Remane, 1924 are considered highly specious with dozens of species, other genera, e.g. *Dendrodasys* Wilke, 1954, *Crasiella* Clausen, 1968, *Diuronotus* Todaro et al., 2005, *Bifidochaetus* Kolicka & Kisielewski, 2016, have very few species and some even consist of a single species, e.g. *Ornamentula* Kisielewski, 1991, *Hummondasys* Todaro et al., 2014, *Thaidasys* Todaro et al., 2015 and *Cephalionotus* Garraffoni et al., 2017.

Ornamentula is a monotypic genus belonging to Dasydytidae Daday, 1905 (Chaetonotida). It was originally described by Kisielewski (1991) to accommodate the new species *Ornamentula paraensis* Kisielewsk, 1991, collected in a small pond from the Brazilian Amazon rainforest. Originally, Kisielewski (1991) spelled the species name as *Ornamentula paraënsis*, with a diacritic. However, the ICZN – Art. 32.5.2. states that: "A name published with a diacritic or other mark, ligature, apostrophe, or hyphen, or a species-group name published as separate words of which any is an abbreviation, is to be corrected.

Although most gastrotrich species are meiobenthic, *O. paraensis* has a semiplanktonic lifestyle, like few other gastrotrichs (Balsamo et al. 2014, Kieneke and Schmidt-Rhaesa 2015, Todaro et al. 2019, Minowa and Garraffoni 2017, 2020). These species show various morphological adaptations such as the complete loss of posterior adhesive tubes, a system of ciliary bands in the head region and short diagonal ciliary bands or tufts on the ventral trunk, and lateral motile spines to perform saltatory movements in the water column (Kisielewski 1991, Kieneke et al. 2008, Kieneke and Ostmann 2012).

Species of *Ornamentula* are characterized by a type of well-developed cuticular armature, unique among all Gastrotricha, that consists of a complex reticular ornamentation on the scales associated with thick and long spines (Kisielewski 1991). Kisielewski (1991) highlighted a possible proximity of *Ornamentula's* lineage with those of *Setopus* and *Haltidytes* based on the presence of scales and bifurcated spines. This hypothesis was also accepted by Kånneby et al. (2013) and Kånneby and Todaro (2015) based on a molecular analysis based on 3 genes, but it was not confirmed by studies based on morphological data (Kieneke et al. 2008, Kieneke and Ostmann 2012).

So far, our knowledge on the biology, life cycle and distribution of many semiplanktonic gastrotrichs is very limited, probably due to a lack of specialists, but especially to the rareness

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of Dasydytidae specimens and their difficult preparation and handling (Kieneke et al. 2008, Kieneke and Ostmann 2012). In order to contribute to the poor knowledge on semiplanktonic gastrotrichs in the Neotropical region (Kisielewski 1991, Kånneby et al. 2013, Kånneby and Todaro 2015, Minowa and Garraffoni 2017, 2020), herein we describe the second species of the genus *Ornamentula* found in an urban lagoon surrounded by Atlantic rainforest, in Southeast Brazil.

MATERIAL AND METHODS

The material for the research was collected in a small urban lagoon, São Paulo, Brazil, with an area of 0.05 km² (22°79'S; 47°14'W), surrounded by fragments of Atlantic rainforest. The collection was carried out during an ongoing freshwater Gastrotricha sampling project, in which periodic biweekly collections have been carried out since 2017. The organisms were found in the samples from October 2017, from the upper 30 cm of the water surface, among floating vegetation roots, using 5 L buckets. The material was stored with constant aeration, with a temperature around 20 °C, and processed within seven days.

The sorting was carried out according to the protocol reported by Minowa and Garraffoni (2020) using 500 ml water samples sieved through a 30 μ m mesh, poured into Petri dishes, and observed under a Zeiss Stemi 2000 stereomicroscope, focusing on the water column searching for of semiplanktonic gastrotrichs.

Each individual was isolated, anesthetized with 2% MgCl₂, mounted individually and digitally documented under a Zeiss Axio Imager M2 light microscope equipped with DIC and AxioCam MRC5 digital video camera. The images were recorded and measurements were taken using the ZEN lite 2.5 2018 image software.

TAXONOMY

Gastrotricha Metschnikoff, 1865 Chaetonotida Remane, 1925 [Rao & Clausen, 1970] Paucitubulatina d'Hondt, 1971 Dasydytidae Daday, 1905

Ornamentula Kisielewski, 1991 (emended)

Dasydytidae of 106–132 µm in length. Body covered with very large and ornamented scales. Dorsal neck with one or two transversal rows of three spined scales. Dorsal trunk with two parallel columns of six large ornamented scales and a rearmost group made of three scales. Long cephalic and trunk dorsal spines present on all scales or just on anterior three. Each long spine provided with a single strong lateral denticle. Transverse band of cephalic cilia situated between large lateral plates. Four paired spines groups (ta-td) along the anterior trunk half, two pairs of rear spines (r1-r2) near the trunk end and in some cases a small pair of ventral rearmost spined scales. Posterior trunk half ventrally covered with fine ornamented and spined scales, oval or arrow-head in shape.

Two species: Ornamentula paraensis Kisielewski, 1991 (type species) and Ornamentula miyazakii sp. nov.

Ornamentula miyazakii sp. nov.

http://zoobank.org/33D78328-A59F-4468-8BD9-6999A90C9033 Figs 1-12, Tables 1, 2

Type material. Holotype. Photographs of one specimen (adult) collected from an urban lagoon in Paulínia, Brazil in October 2017 with floating vegetation. The specimen was examined alive with a compound microscope equipped with DIC, but due to the fragility of its body, it was destroyed and is no longer available (Garraffoni et al. 2019). The holotype is illustrated in Figs 1–3 and photomicrographs in Figs 5–7 (Garraffoni et al. 2019) and its digital image data are available at the Museum of Zoology, Universidade Estadual de Campinas, Brazil, under the accession number ZUEC GCH 52; Paratypes: Photographs of two additional specimens (adult and juvenile) collected from the same sampling site, with digital image data available under accession numbers ZUEC GCH 53 to ZUEC GCH 54.

Diagnosis. Ornamentula species 101–193 μ m in body length (126–227 μ m posterior spines included). Cone-shaped head, small cephalion. Two pairs of extremely long cephalic sensory bristles. Trunk dorsally covered by two columns each made of six large, ornamented scales each with a single spine. Ventral trunk side with small spined scales between ciliary tufts. Posterior end truncated; two pairs of dorsal terminal scales each with a thick spine and a similar terminal ventral scale with a thick spine.

Species-specific characters. A pair of extremely long, lateral cephalic sensory bristles inserted adjacent to the posterior cephalic ciliary tufts. Neck with one transversal row of three spined ornamented scales. Fish-shaped (oval scale with a posterior shallow constriction, and with convexe end) lateral ornamented scale (U44), immediately posterior to the tb spines group. An additional pair of dorsolateral rear spines, inserted on small ornamented scales. All dorsal scales with long, thick slightly curved spines with prominent denticles.

Description. Description based on characters and measurements of two adults (holotype and paratype) (Table 1).

Ornamentula of 101–193 µm length (spines excluded); 126–227 µm (spines included). Elongated-oval dorsoventrally flattened body, with distinct cone-shaped head, and well developed lobes (31 µm wide), with a short and narrow neck region. Sub-terminal mouth ring (8.4 µm diameter), with short tentacle-like projections around the mouth ring. Cephalion 18 µm wide (U04), pleura absent, and hypostomion not observed (Figs 1, 11). Narrow cylindrical pharynx (62 µm long) (Figs 6, 9). Intestine with maximum width at its middle (127 µm long, 44 µm wide, U62,) (Figs 4–9). Truncated posterior end with two thick caudal spines (r1-r2) inserted on dorsolateral scales (U85; U90).



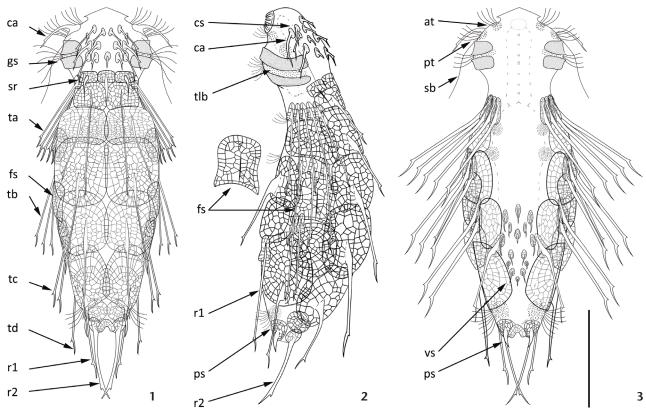
Table 1. Morphometric features of *Ornamentula miyazakii* sp. nov. and *O. paraensis* Kisielewski, 1991. All measures in μ m. x = average (without the juvenile measures in *O. miyazakii* sp. nov); n: number of specimens; ca: cephalic spine; ta, tb, tc, td: groups of trunk ventro-lateral long spine; r1-2: rear spines; d1-3: spines on dorsal scales; s1-7: dorsal scales.

Characters	_	O. miyazakii sp. nov.				O. paraensis		
characters		Ind1 (holotype)	Ind2 (paratype)	Ind3 (juvenile)	х	Range	х	r
Body length, spines excluded		193	175	101	184	106–132	122	1
Total body length		227	201	126	214	133–162	150	1
Maximum head width		23	-	40	23	28-44	35	e
Minimum neck width		18	-	22	18	17–26	22	4
Maximum trunk width		35	-	44	35	33–48	42	4
Pharynx length		62	68	28	65	35–43	38	4
Diameter of mouth ring		8	23	14	16	7	7	
Cephalion length		-	-	-	-	8	8	
Cephalion width		-	-	18	18	19	19	
	са	15	21	35	18	17.5–23	20	4
	ta1	25	19	50	22	31	31	
	ta2	23	21	-	22	29; 38	-	Ì
	ta3	19	23	-	21	41; 42	-	
	ta4	22	21	-	21	44	44	
	ta5	16	22	-	19	42	42	
	tb1	68	48	52	58	38-44	42	
	tb2	62	53	41	57	40-44	43	
	tb3	64	43	50	53	43; 44	-	
Spine length	tc1	63	48	_	55	36; 40	_	
	tc2	65	48	_	57	33–36	35	
	td	-	50	_	50	38–41	40	
	r1	33	24	-	29	26-34	29	1
	r2	27	21	57	24	26-31	28	1
	d1	-	73	84	73	31–33	32	
	d2	_	48	72	48	31–38	36	
	d3	_	50	50	50	30–34	32	
	d4	_	34	-	34	_	_	
	d5	_	30	-	30	_	-	
Scales length	s1	12	26	_	19	7	7	
	s2	16	-	-	16	15	15	
	s3	3	29	-	16	25	25	
	s4	30	50	-	40	21	21	
	s5	28	47	-	37	21	21	
	s6	11	51	-	31	8	8	
	s7	_	-	-	_	4	4	
	s1	14	47	_	31	12	12	
	s2	24	-	_	24	16	16	
Scales width	s3	26	30	_	28	19	19	
	s4	24	53	-	38	20	20	
	s5	18	48	-	33	15	15	
	s6	15	40	-	27	10	10	
	s7	_	_	_	_	5	5	

Cephalic ciliation consists of two pairs of lateral tufts (anterior one adjacent to the mouth, U04; posterior one medio-lateral, U06) and a pair of cephalic ciliary transversal bands (U10), bordering the cephalic lateral plates, interrupted in the dorsal and ventral medial region (Figs 1–3, 9–12). A pair of cephalic sensory bristles extremely long (4.3 µm; U03) inserted between the second lateral cephalic ciliary tuft and lateral cephalic transversal ciliary bands, anteriorly to granular lateral scales (U10) (Figs 1, 10, 11). Trunk ventral ciliation consists of three paired tufts (U43–U45, U59–U61, U86–U90) associated to lateral trunk spines tb1–3, tc1–2 and r2, respectively (Figs 3–4).

Body dorsum mostly covered with enormous scales with ornamentations described originally as scale reinforcements, probably due to their exaggerated size (Figs 1, 4–6). Cephalic cuticular armature: Two pairs of anterior lateral cephalic plates on the head (U10), slightly folded in its margin, with finely granulated surface, (Fig. 7), different from the scales of the trunk. These plates are positioned at both sides of lateral cephalic transverse bands (Figs





Figures 1–3. Schematic drawing of *Ornamentula miyazakii* sp. nov.: (1) dorsal view; (2) lateral view; (3) ventral view. at: anterior ciliary tuft, ca: cephalic long spine, cs: cephalic spines, fs: fish-shaped scale, gs: granular scale, ps: posterior spine, pt: posterior ciliary tuft, r1: rear spine 1, r2: rear spine 2, sb: sensory long bristle, sr: scales transverse row, ta-td: groups of trunk ventrolateral long spines, tlb: transversal lateral band, vs: ventral scales. Scale bar: 50 µm.

1–3). Several pairs of small rounded scales, each with a fine barbed spine, with fine barbed spines (U08–U12) on the cephalic dorsal space between granulated plates (Figs 1, 10, 11). Anterior cephalic spines short (3.7 μ m, 1.5x the length of inserted scale), lengthen gradually to the posterior longer ones (7 μ m, 2.4x the length of inserted scale) (Fig. 1). The lateral cephalic spines (ca) are inserted anteriorly adjacent to the granular plate (17.7 μ m long, U03), much thicker at the base and gradually getting thinner distally, until the denticle, where it gets thinnest (Figs 1–3, 7).

Trunk cuticular armature: Single dorsal anteriormost transversal row with three simple spined scales (U21). The dorsal trunk is covered with two parallel column of six scales each (U24, U30, U43, U58, U76, U90), with varying sizes (Table 2), each scale bears a long, straight, thick, and barbed spine at 4/5 of its length. Laterally, three scales positioned posterior to each spine group (U35, U50, U64), first rounded between ta and th spine groups; followed by fish-shaped scale, posterior to the spine group (i.e. rounded anterior half, with middle portion slightly constricted, and posterior half concave, with two pointed edges); posteriormost rounded scale lodged on the concavity of fish-shaped scale. Two pairs of rearmost ventrolateral scales

(U67, U96) with r1 and r2 spines inserted, followed by a ventral triangular scale (Figs 2, 3, 8) with short curved spine (U99).

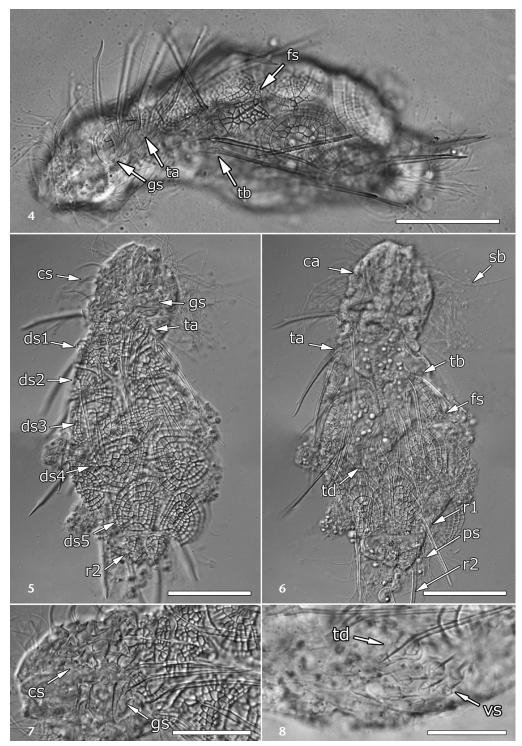
Ventrolateral trunk with thick and straight spines organized in four groups (ta, tb, tc, td) arranged in 5–3–2–1 spines each (U27, U43, U61, U68), with a conspicuous denticle at 4/5 of its length, and inserted on triangular scales (Figs 2–3, 8). The ta group is the most laterally inserted, with ta1–2 spines dorsolateral (Fig. 2).

Ventral trunk anteriorly smooth in interciliar space, and posterior region with 15 small rounded scales (U55–70) between tb, tc, and td spine groups. Anterior scales with longer spines (3.4 μ m, 1.5x the length of inserted scale) followed by posterior ones shorter spines (1.7 μ m, 1.5x the length of inserted scale) (Fig. 8).

Sexuality unknown, as we could not observe any specimen with eggs, nor the sexual organs.

Etymology. Species dedicated to animation director Hayao Miyazaki, a highly notorious animator and filmmaker. He animated the fantasy film "Princess Mononoke", and illustrated the character Forest Spirit (1h01'20" in the original film), that at nighttime turns to Daidarabocchi, resembling the specimen drawn in Fig. 1.





Figures 4–8. Ornamentula miyazakii sp. nov. photomicrographs of mature specimens: (4) paratype, lateral view; (5) holotype, dorsal view; (6) holotype, ventral view; (7) holotype, dorsal cephalic view; (8) paratype, ventral trunk view. ca: cephalic long spine, cs: cephalic spines, ds1-5: dorsal scales, fs: fish-shaped scale, gs: granular scale ps: posterior spine, r1: rear spine 1, r2: rear spine 2, sb: sensory long bristle, sr: ta-td: groups of trunk ventrolateral long spines, vs: ventral scales. Scale bars: $4-6 = 50 \mu m$.



Species	Ornamentula miyazakii sp. nov.	Ornamentula paraensis				
Cephalic ciliation	Two anterior tufts followed by a ventral transverse band	Two anterior tuft followed by transversal discontinuous band, two pairs of ventral medial tufts				
Cephalic dorsal scales	15 spined scales between cephalic plates	16 spined scales between cephalic ciliary tufts				
Cephalic long spine (ca)	Extremely thick, barbed, inserted on small scale	Thin, barbed, inserted directly to the cuticle				
Cephalic sensory bristle	A pair of dorsolateral long bristle	No bristle				
Granular lateral plates	Two pairs of lateral scales adjacent to transversal ciliary band	Two pairs of lateral scales adjacent to transversal ciliary band				
Trunk ciliation	Three pairs of ventral tufts	Three ventral tufts				
Trunk dorsal scales parallel rows	Six pairs of dorsal scales, all spined	Six pairs of dorsal scales, anterior three spined, posterior spineless				
Trunk lateral scales	Three lateral oval to fish-shaped scales posterior to each spine group	Three lateral rounded scales posterior to each spine group				
Trunk ventral scales	12 small oval to round spined scales	25 small round to triangular spined scales				
Ventral long spines (t)	Groups of 5 – 3 – 2 – 1 thick, straight barbed spines, inserted on triangular to round ventrolateral scales	Groups of 5–3–2–1 thick, straight barbed spines, inserted on triangular to round ventrolateral scales				
Rear spines (r)	Two barbed spines inserted on lateral scales, one simple short spine inserted on ventral scale	Two barbed long spines inserted on lateral scales				

Table 2. Comparison of several morphological structures between Ornamentula miyazakii sp. nov. and O. paraensis.

Ecology. Freshwater, periphytic and semiplanktonic among roots of floating vegetation mainly composed by *Eichhornia* sp.

Remarks. Although the body plan of the taxon *Ornamentula* seems to be different from other members of Dasydytidae, it shows some common characteristics with other semiplanktonic species. Like its counterparts, *Ornamentula* species are characterized by a tenpin-shaped body, with evident head and neck constriction, cephalic ciliature arranged by discontinuous tufts and/or bands and body with paired groups of ventrolateral single-barbed spines.

Both Atlantic and Amazonian *Ornamentula* species share several unique morphological features characteristic of the taxon bauplan, such as: i) two parallel columns of dorsal ornamented scales forming a rigid armature, ii) two pairs of lateral cephalic plates (granular scales) on both sides of cephalic transverse ciliary bands, iii) a pair of lateral cephalic spines, iv) two pairs of ventrolateral cephalic ciliary tufts followed by the transverse band, and three ventral ciliary tufts on the trunk, v) four groups of movable ventrolateral spines with a conspicuous denticle inserted on ornamented scales and organized in 5–3–2–1 (ta–tb–tc–td) and vi) two pairs of terminal ventral spines with denticles (r1 and r2).

However, the new species can be distinguished from *O*. *paraensis* by some remarkable differences:

- 1. The dorsolateral cephalic spines (ca) thicker and curved, followed by a pair of long cephalic bristles adjacent to the second cephalic tuft, absent in former species.
- 2. The group of dorsal cephalic scales provided with spines with prominent denticles, in contrast to simple and shorter spines in former species.
- 3. The absence of second dorsal transverse rows of scales on the neck, present in *O. paraensis*.
- 4. The lateral trunk scale, posterior to tb spine group, fish-like shaped, in contrast to the rounded scale of *O. paraensis*.
- Ventral spined scales with rounded uniform shape through the ventrum, unlike *O. paraensis*, with rounded in anterior portion and posteriorly triangular shaped.
- 6. A third terminal ventral scale with a short, simple and curved spine, much shorter than r1 and r2 spines, absent in *O. paraensis*.

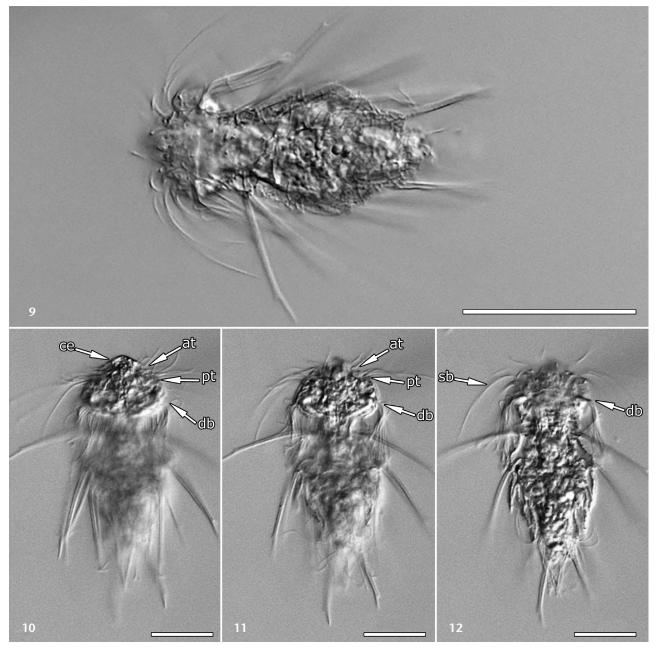
DISCUSSION

It is well known in the literature how difficult the identification process is for meiofauna species (Fonseca et al. 2018, Garraffoni et al. 2019) and how remarkably poor the biodiversity knowledge is of these microscopic organisms compared to other groups of animals (Appeltans et al. 2012, Fonseca et al. 2018). Consequently, accurate diagnosis and meiofaunal species delimitation are difficult to access, leading to shortfalls (sensu Hortal et al. 2015) regarding identity, distribution and evolution of meiofaunal taxa.

As pointed out by Fonseca et al. (2018), to overcome these shortfalls it is highly recommended that meiofaunologists use the available microscopical techniques (optical, scanning, transmission, and confocal microscopy) as much as possible to better describe the external morphology and internal anatomy of new species. Thus, we are aware of the risk of conducting a nomenclatural act based on morphological information obtained only with the use of light microscopy with DIC gathered from two adult specimens. However, our decision to designate a new species is grounded in practical and philosophical standpoints that supported us to consider *O. paraensis* and *O. miyazakii* sp. nov. as two distinct evolutionary entities, or two distinct explanatory hypotheses for the intrinsic or relational properties of organisms that can be accounted for reproductive events (Fitzhugh 2005, 2009).

In some cases due to environmental remoteness, sampling depth (e.g. deep sea), or rarity of some taxa, only one or very few meiofaunal specimens are collected and can be used to observe a sufficient number of morphological features to identify and delimit the species. In the last five years, we have been continuously sampling the lagoon where *O. miyazakii* sp. nov. was found and we were able to find many specimens of three unknown species of semiplanktonic gastrotrich (two of them were described by Minowa and Garraffoni 2017, 2020) and at least five unknown benthic gastrotrich (one of them described by Garraffoni and Melchior 2015). However, until





Figures 9–12. Ornamentula miyazakii sp. nov., photomicrographs of a juvenile in dorsal view. at: anterior ciliary tuft, ce: cephalion, pt: posterior ciliary tuft sb: sensory long bristle, sr: scales of the transverse row, tlb: transversal lateral band. Scale bars: $9 = 100 \mu m$, $10-12 = 50 \mu m$.

October 2019, this was the only sampling moment we found specimens belonging to *O. miyazakii* sp. nov. Thus, taking into account these specificities and the ontological status of species taxa as a species-as-individuals hypothesis (e.g. Coleman and Wiley 2001, Fitzhugh 2009), we agree with Kieneke and Nikoukar (2017) and interpret the description of new species based on very few specimens as "primary species hypothesis". In this

case, the use of "primary" is only an indication of the initial essay for species delimitation, because *O. miyazakii* sp. nov. or *Thaumastoderma antarctica* Kieneke, 2010 (among many other examples in gastrotrichs) were hypothesized as entities that have unique common evolutionary origins and are spatiotemporally located (Rieppel 2009), in the same way that any taxonomist would do if they had many specimens to analyze.



Besides, hypotheses of homology between characteristics of species of distinct lineages are as important as the hypotheses of species themselves. Highlighting the existence of the pairs of granular transverse scales on both species of Ornamentula, inserted on both sides of the head, arranged in such a way that the transverse ciliary band lies between them. However, these granular scales, despite the similar relative positions on the head, are not homologous to the cephalic pleura present in other Paucitubulatina (Kieneke and Schmidt-Rhaesa 2015). This statement is supported by the position relative to the ciliary arrangement, as such structures are arranged adjacent to the cephalion, and each ciliary cephalic tuft is inserted ventrally (Minowa and Garraffoni 2020) on benthic representatives. Even compared with the semiplanktonic species, the cephalic reinforcement (pleura) is inserted anteriorly to the ciliary band, in contrast with the posterior insertion of the second scale in the Ornamentula species.

Garraffoni and Araújo (2020) provided a taxonomic key for Neotropical Gastrotricha species, which we updated here with the *Ornamentula* identification and included the new species.

Gastrotricha: Chaetonotida: Dasydytidae: Ornamentula

- 1a. Cephalic spined scaled simple; dorsal scales arranged in two parallel columns of six scales each, three anterior with long barbed spines, three posterior spineless, dorsal neck with two transversal rows of three spined scales
- Description of three spined scales
 Ornamentula paraensis
 Cephalic spined scaled barbed; dorsal scales arranged in two parallel columns of six scales each, all of them with long barbed spines, dorsal neck with single transversal row of three spined scales
 Ornamentula miyazakii sp. nov.

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