

Article

# A New Species of *Paraturbanella* Remane, 1927 (Gastrotricha, Macrodasysida) from the Brazilian Coast, and the Molecular Phylogeny of Turbanellidae Remane, 1926

Ariane Campos <sup>1,\*</sup>, M. Antonio Todaro <sup>2</sup>  and André Rinaldo Senna Garraffoni <sup>1</sup> 

<sup>1</sup> Department of Animal Biology, Institute of Biology, University of Campinas, Campinas, SP 13083970, Brazil; arsg@unicamp.br

<sup>2</sup> Department of Life Sciences, University of Modena and Reggio Emilia, via G. Campi, 213/D, I-41125 Modena, Italy; antonio.todaro@unimore.it

\* Correspondence: arianeecampos@gmail.com

<http://zoobank.org/urn:lsid:zoobank.org:pub:EFA8F92E-7298-4982-B832-54F71E4BB995>



Received: 1 December 2019; Accepted: 15 January 2020; Published: 21 January 2020

**Abstract:** The family Turbanellidae includes *Paraturbanella* and five other genera. Despite the fact that the monophyly of these genera were not satisfactorily tested, species belonging to the genus *Paraturbanella* are distinguished from turbanellids by sharing a peculiar group of tubes on the ventrolateral side of the anterior pharyngeal region known as “*dohrni*” tubes. In this study, *Paraturbanella tricaudata* species nova (sp. nov.) from the intertidal zone of a sandy beach in Trindade (Rio de Janeiro State) and the sublittoral sand of Prumirim Island (São Paulo State), Brazil, is described. The new species can be distinguished from all other *Paraturbanella* species by the presence of three caudal cones (one medial and two laterals to it) and peculiar arrangement of the male system. This is the first description of a *Paraturbanella* species from Brazil and the third registered from the Southern Hemisphere (as opposed to 19 species in the Northern Hemisphere); thus, knowledge of marine gastrotrichs biodiversity in this region is far from satisfactory.

**Keywords:** gastrotricha; meiofauna; biodiversity; taxonomy; South America; South Hemisphere; nuclear genes

## 1. Introduction

Gastrotricha are meiofauna representatives (encompassing animals ranging in size from about 0.042 to 0.500 mm) living in freshwater and marine ecosystems around the world [1,2]. The *phylum* is divided into two orders—Chaetonotida Remane, 1925 [3] and Macrodasysida Remane, 1925 [3]. The latter group is composed in its majority of hermaphroditic and marine species, which live interstitially in sandy bottoms [2,4]. The order Macrodasysida includes 10 families, 36 genera and approximately 380 described species [2,5,6]. They have a strap-shaped body, a pharynx with pharyngeal pores, and, usually, numerous adhesive tubes in the anterior, lateral and posterior regions.

The family Turbanellidae Remane, 1927 [7] includes six genera: the monospecific *Prostobuccantia* Evans & Hummon, 1991 [8] and *Pseudoturbanella* d’Hondt, 1968 [9]; *Dinodasys* Remane, 1927 [7] (two species); *Desmodasys* Clausen, 1965 [10] (three species); *Paraturbanella* Remane, 1927 [7] (23 species); and *Turbanella* Schultze, 1853 [11] (32 species).

Although knowledge of phylogenetic relationships within Turbanellidae is still limited, *Turbanella* and *Paraturbanella* species have several common characteristics, but the presence of extraordinary adhesive tubes easily distinguishes the genus *Paraturbanella* from *Turbanella* [2,6,12–16].

The type species of the genus *Paraturbanella* was collected in the Gulf of Naples (Italy) and described as *Paraturbanella dohrni*, based on the presence of an extraordinary pair of accessory adhesive tubes (known also as “*dohrni*”, or “*Seitenfüßchen*” in German). Remane could distinguish these animals from the *Turbanella* species [7].

Nowadays, twenty-three species are currently known: *P. africana* Todaro, Dal Zotto, Bownes & Perissinotto, 2017 [17]; *P. aggregotubulata* Evans, 1992 [18]; *P. armoricana* (Swedmark, 1954) [19]; *P. boadeni* Rao & Ganapati, 1968 [20]; *P. brevicaudata* Rao, 1991 [21]; *P. cuanensis* Maguire, 1976 [22]; *P. dohrni* Remane, 1927 [7]; *P. dolichodema* Hummon, 2010 [23]; *P. eireanna* Maguire, 1976 [22]; *P. intermedia* Wieser, 1957 [24]; *P. levantia* Hummon, 2011 [25]; *P. manxensis* Hummon, 2008 [26]; *P. mesoptera* Rao, 1970 [27]; *P. pacifica* Schmidt, 1974 [28]; *P. pallida* Luporini, Magagnini & Tongiorgi, 1971 [29]; *P. palpibara* Rao & Ganapati, 1968 [20]; *P. pediballetor* Hummon, 2008 [26]; *P. sanjuanensis* Hummon, 2010 [23]; *P. scanica* Clausen, 1996 [30]; *P. solitaria* Todaro, 1995 [31]; *P. stradbrokei* Hochberg, 2002 [32]; *P. teissieri* Swedmark, 1954 [19]; and *P. xaymacana* Dal Zotto, Leasi & Todaro, 2018 [33].

Herein, we describe a new species of *Paraturbanella* from Brazil, previously reported as *Paraturbanella* sp. 2 [34,35].

## 2. Material and Methods

### 2.1. Sampling and Sample Processing

Information about the description of the new species is mainly taken from specimens found in samples collected by hand from the shallow intertidal area of Praia do Cachadaço, Trindade–Paraty (23°21′15.8″ S, 44°43′41.5″ W), Rio de Janeiro State, Brazil, in October 2017. The sandy sediment was filtered (0.40 µm mesh), and extraction of animals from the sediment was carried out by anaesthetization with MgCl<sub>2</sub>. The supernatant was poured into a Petri dish, and with a micro-pipette it was possible to separate the gastrotrichs into embryo dishes. Specimens were observed alive with a stereomicroscope ZEISS Stemi 2000. Additional information comes from specimens collected and documented by one of us (M. Antonio Todaro) in 2002 and 2003 [34,35]; the sampling sites and distribution of *Paraturbanella* species along the Brazilian coast were plotted on the map, elaborated using ArcGis [36] (Figure 1), and made available at <https://www.arcgis.com/home/webmap/viewer.html?webmap=fafb1d0942b4483a99ddf828fc24039a>.

### 2.2. Microscopical Study—Differential Interference Contrast (DIC)

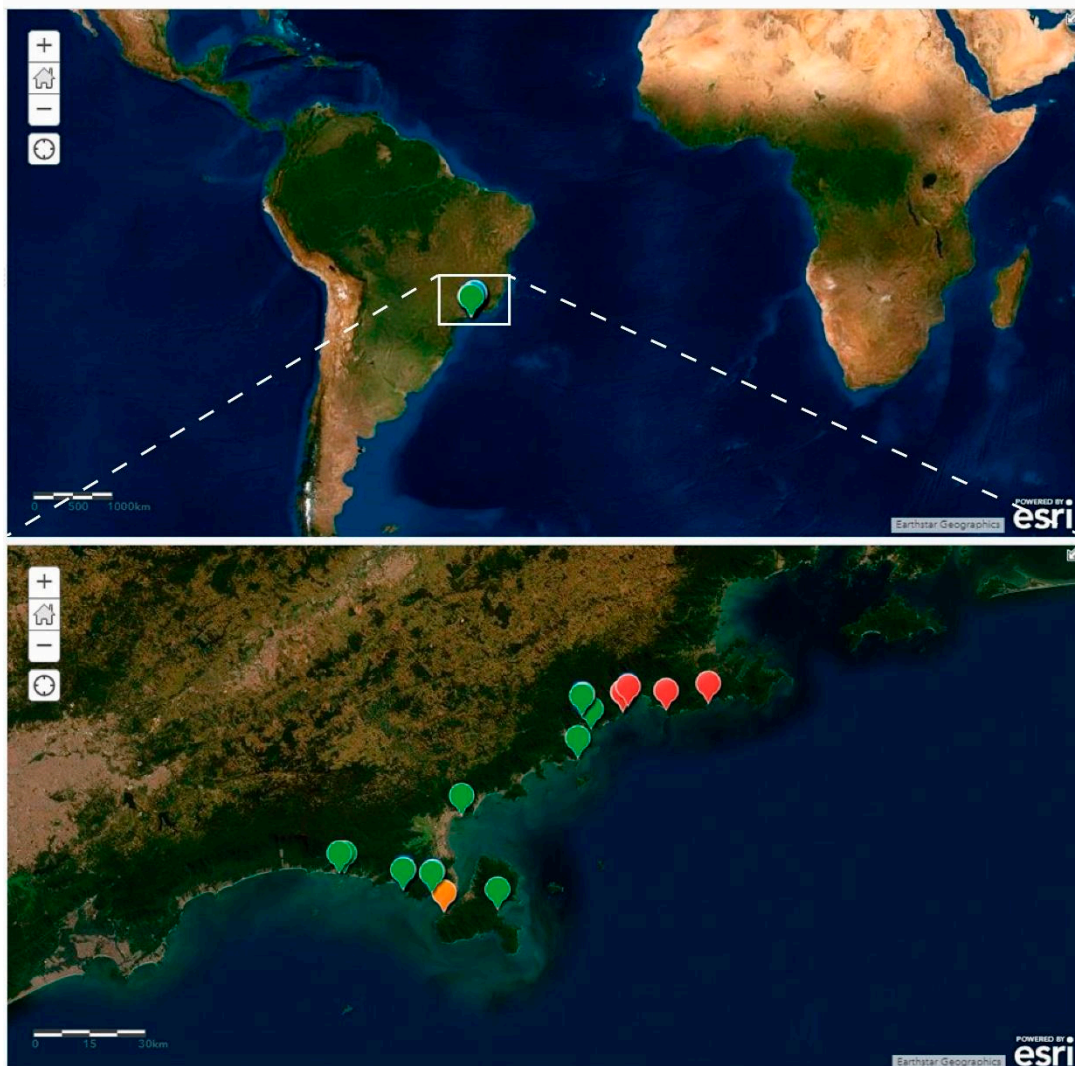
The gastrotrichs of interest were picked out with a micropipette, mounted on glass slides, and studied using Zeiss Axio Imager M2 plus Differential Interference Contrast optics, with Zen Lite Zeiss software (Zen Blue) and an Axiocam 105 color camera. The observation was taken using a slow-moving living specimen and the measures followed convention [37], according to the position of morphological characteristics along the body. The software used to measure the structures of specimens was Image J.

### 2.3. Scanning Electron Microscopy (SEM)

The specimens were fixed in glutaraldehyde 2.5%, rinsed with cacodylate buffer 0.1 M, dehydrated in a graded ethanol series, and critically point dried with CO<sub>2</sub>. Stubs were coated with gold and analyzed using a scanning electron microscope JSM 5800 LV, with 10 kV voltage.

### 2.4. Granulometric Analysis

The particle size analyses were made following the sediment screening method using different opening meshes [38]. A fraction of the sample was separated and oven dried at 60 °C for 24 h. The dried material was sieved in different Granutest sieves with progressively smaller openings, and the fraction retained in each sieve was weighed and noted. Through these values, sediment fractions were weighed and granulometric characters (median, standard deviation, skewness and kurtosis) were calculated using GRANPLOTS with line segments [39].



**Figure 1.** Map of Brazil showing the distribution of *Paraturbanella* species. Red: *Paraturbanella tricaudata* species nova (sp. nov.). Green: *Paraturbanella* sp. 1 [34,35]. Orange: *Paraturbanella* sp. 3 [34].

### 2.5. DNA Extraction, Amplification and Sequences

Genomic DNA was extracted from entire individuals of *Paraturbanella tricaudata* species nova (sp. nov.) using a QIAmp DNA Micro Kit (Quiagen, Hilden, Germany), following the manufacturer's instructions. PCR amplification was performed in a 20  $\mu$ L reaction mixture containing 3  $\mu$ L of genomic DNA, 13.5  $\mu$ L of water, 2  $\mu$ L of 10x buffer, 0.5  $\mu$ L of dNTP, 0.2  $\mu$ L of Taq Platinum (Quiagen) and 0.4  $\mu$ L (4 pmol) of specific primers [4]. The DNA fragments were sequenced using BigDye Terminator reactions in a 3500xL Genetic Analyzer (Life Technologies, Carlsbad, CA, USA) at the CBMEG laboratory (Campinas, Brazil). The 18S rDNA and 28S rDNA sequences of *Paraturbanella tricaudata* sp. nov. were deposited in GenBank (Table 1).

### 2.6. Phylogenetic Analysis

All the formal species of the family Turbanellidae from which sequences of 18S rDNA and/or 28S rDNA were available in GenBank were included as the ingroup in the present analysis (*Paraturbanella*: 5 species; *Turbanella*: 5 species). The species and respective GenBank accession numbers are listed in Table 1. The outgroup was composed of two *Megadasys* species and four *Mesodasys* species due to the

close phylogenetic position of these taxa with the taxon Turbanellidae [4,40]. Each gene dataset was aligned separately using BioEdit Sequence Alignment Editor and Mafft v.7.402 (L-INS-I approach) [41].

Parsimony analysis was performed using a heuristic search with equally weighted characters that was available using TNT software [42]. Most parsimonious trees were searched by the heuristic method, with 1000 replications, holding 1,000,000 trees per search (command line: mult = replications 1000 hold 100,000) and collapsing the tree after the search.

For maximum likelihood analysis, GTRCAT model was chosen and RAxML [43] was run with a with 1000 bootstrap replicates.

**Table 1.** Taxa of Gastrotricha included in this study, with respective GenBank accession numbers of 18S and 28S rDNA sequences.

Species	18S	28S	References
<i>Megadasys</i> sp. 1	JF357656	JF357704	Todaro et al. [44]
<i>Megadasys minor</i> Kisielewski, 1987	AY228131	-	Todaro et al. [45]
<i>Mesodasys littoralis</i> Remane, 1951	JF357658	JF357706	Todaro et al. [44]
<i>Mesodasys laticaudatus</i> Remane, 1951	JF357657	JF357705	Todaro et al. [44]
<i>Mesodasys</i> sp.	AY963690	KF921011	Petrov et al. [46]
<i>Mesodasys adenotubulatus</i> Hummon, Todaro & Tongiorgi, 1993	AM231780	-	Todaro et al. [45]
<i>Paraturbanella</i> sp.	KF921017	-	Petrov et al. [46]
<i>Paraturbanella teissieri</i> Swedmark, 1954	JF357661	JF357709	Todaro et al. [44,45]
<i>Paraturbanella pallida</i> Luporini, Magagnini & Tongiorgi, 1973	JF357660	JF357708	Todaro et al. [44]
<i>Paraturbanella dohrni</i> Remane, 1927	JF357659	JF357707	Todaro et al. [44]
<i>Paraturbanella tricaudata</i> sp. nov.	SUB6819988	SUB6819996	Present study
<i>Turbanella</i> sp.	JF970238	-	Paps & Riutort [47]
<i>Turbanella cornuta</i> Remane, 1925	AF157007	JF357711	Todaro et al. [44]
<i>Turbanella pilosum</i> Kolicka, 2018	MF325920	MF325905	Kolicka et al. [13]
<i>Turbanella lutheri</i> Remane, 1952	JF357669	-	Todaro et al. [44]
<i>Turbanella bocqueti</i> Kaplan, 1958	JF357662	JF357710	Todaro et al. [44]

### 3. Results

#### 3.1. Taxonomic Account

Phylum Gastrotricha Metschnikoff, 1865.

Order Macrodasysida Remane, 1925 [3] (Rao & Clausen, 1970) [48].

Family Turbanellidae Remane, 1926 [49]

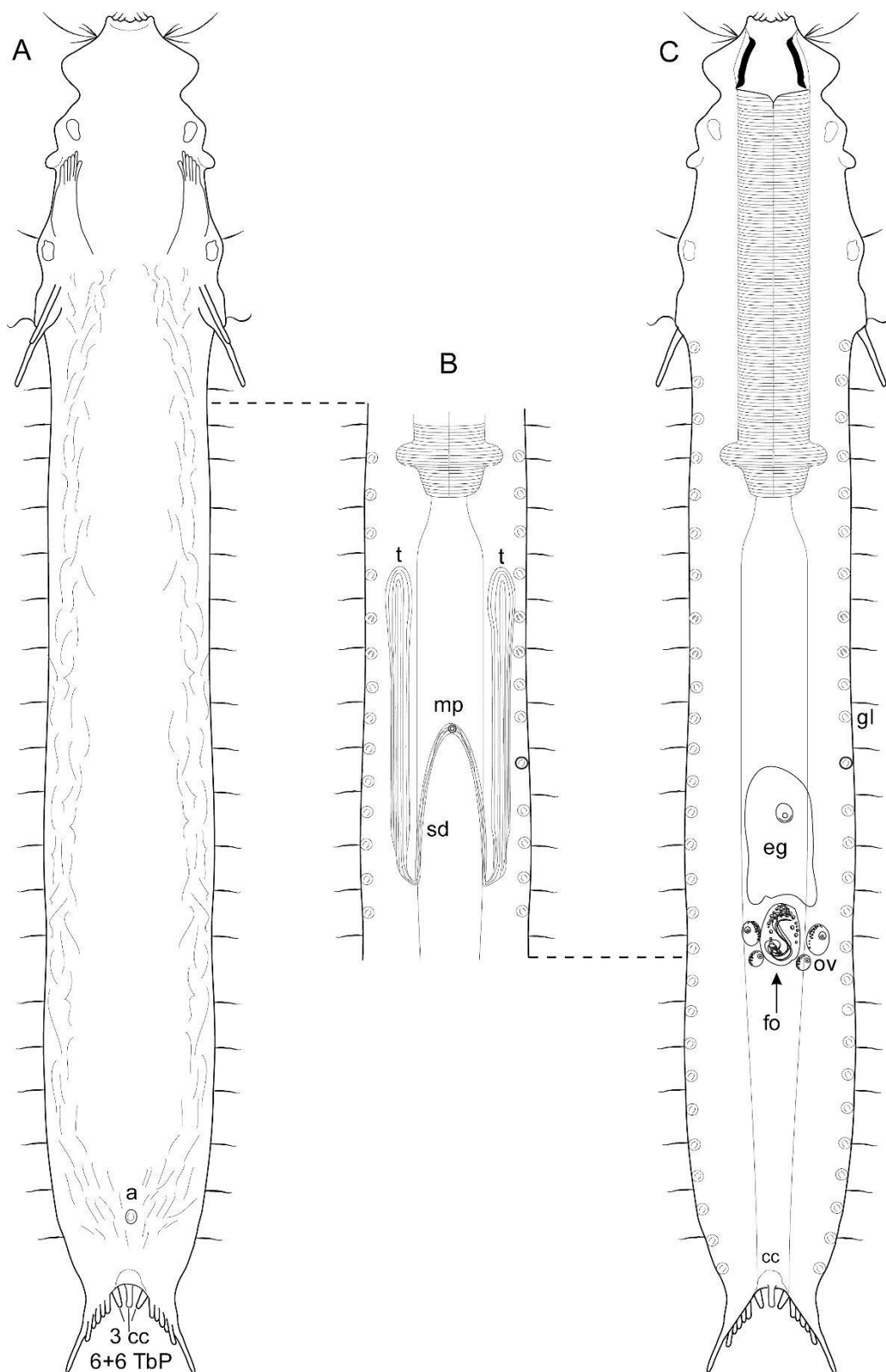
Genus *Paraturbanella* Remane, 1927 [7]

*Paraturbanella tricaudata* sp. nov. (Table 2 and Figures 2–6).

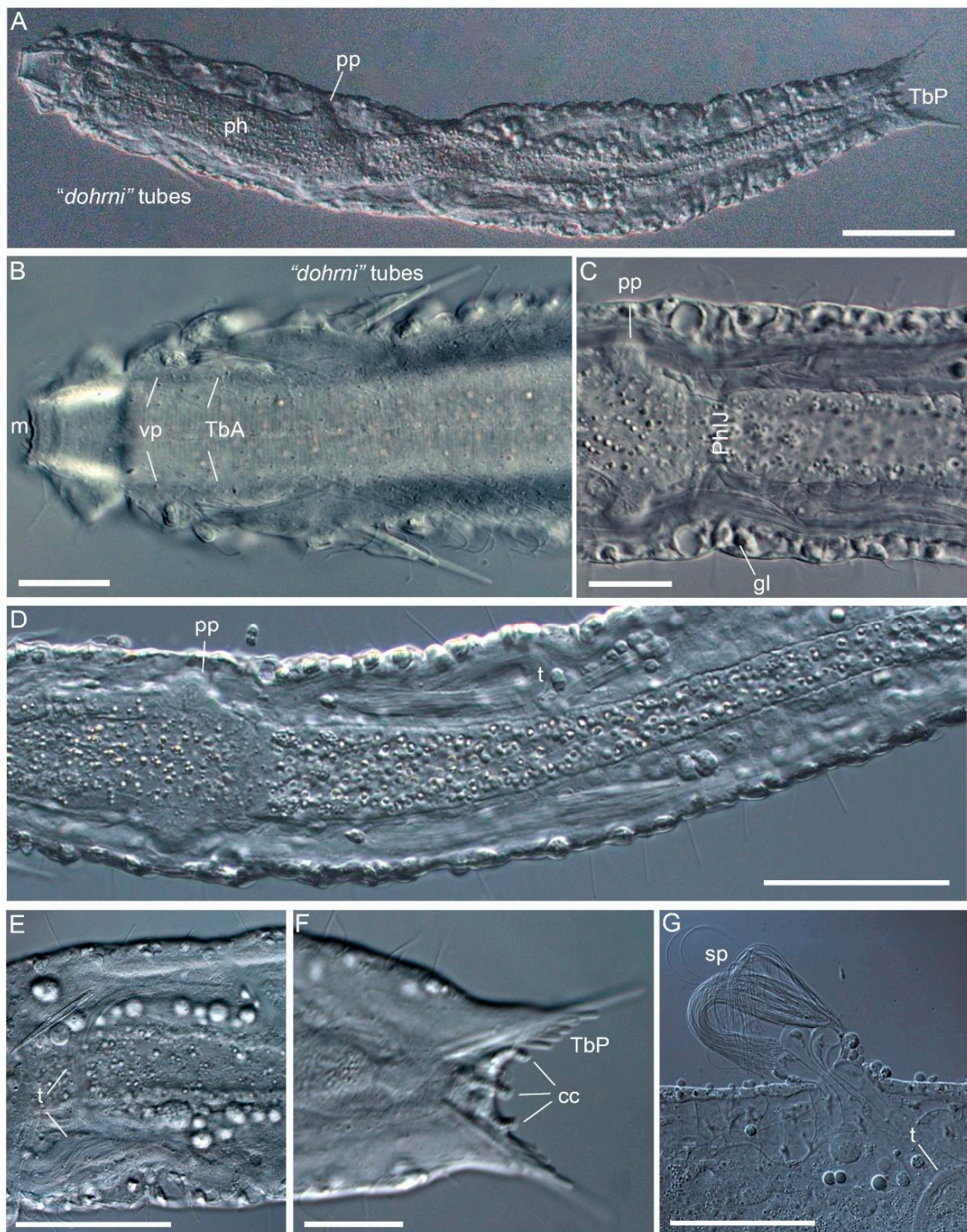
Synonym *Paraturbanella* sp. 2—Todaro & Rocha [34,35].

**Table 2.** Morphometric features of *Paraturbanella tricaudata* sp. nov. Min: minimum value. Max: maximum value. N: total number of measured adult specimens. SD: standard deviation.

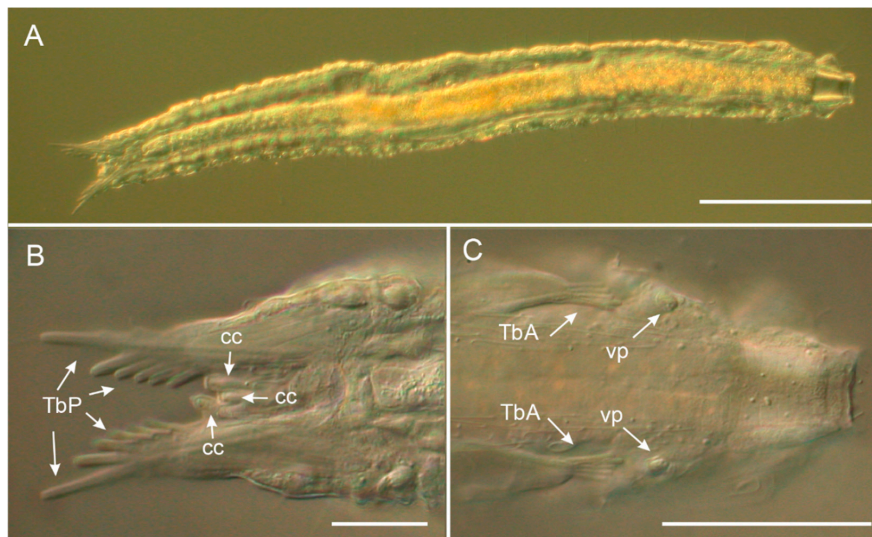
Character	Min–Max (µm)	Average (µm)	SD (µm)	N
Lt: total body length	417–480	449.00	31.51	5
Lbc: length of buccal cavity	18.6–22.3	19.9	1.2	10
Diameter of mouth opening	13.3–17.4	15.0	1.3	10
Head width at cephalic swellings	35.8–51.5	40.4	5.1	8
Neck constriction width	30.1–34.1	32.3	1.7	8
Maximum trunk width	40.9–49.1	43.2	2.8	8
Lph: length of pharynx	127.1–141.9	136.9	4.9	6
Distance of the pharyngeal pores from PhJIn	20.2–27.1	23.5	2.8	6
“Dohrni” longer tube length	22.6–33.8	27.8	4.9	6
“Dohrni” shorter tube length	15.6–19.4	18.5	2.5	5
Maximum length of TbA	8.7–9.6	9.2	0.4	4
Length of TbP	6.2–7.9	7.0	0.9	5



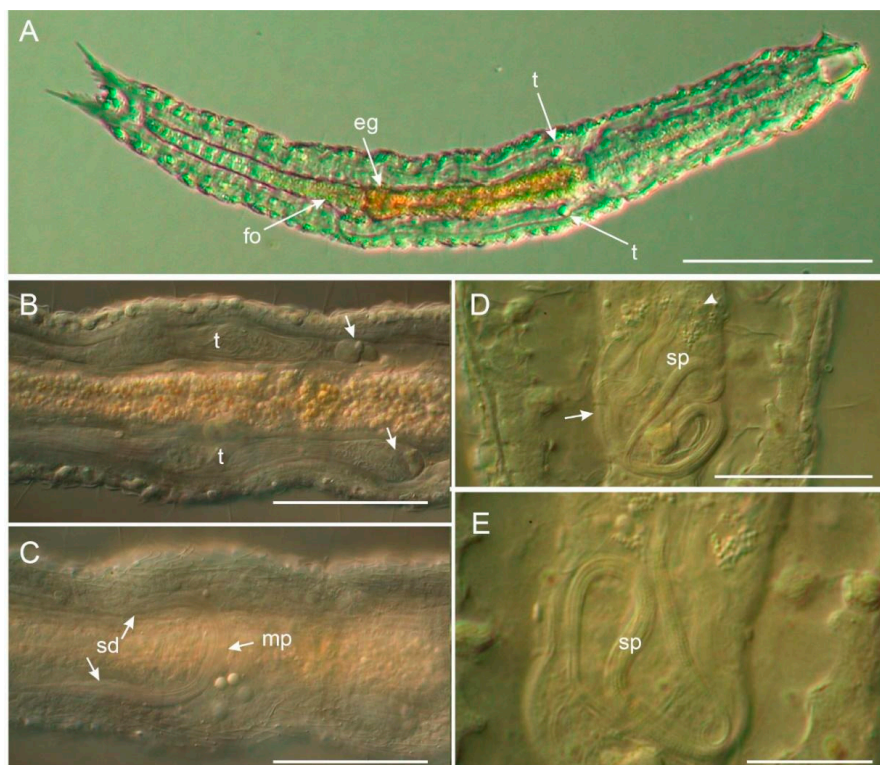
**Figure 2.** *Paraturbanella tricaudata* sp. nov. schematic drawing. (A) Ventral view of the *Habitus*. (B) Detail of the mid-trunk region, showing a ventral view of the male system. (C) Dorsal view of the *Habitus*, showing a mature egg, the frontal organ and the ovaries. Abbreviations: a = anus, fo = frontal organ, gl = gland, mp = male pore, ov = ovary, sd = sperm duct, and t = testes.



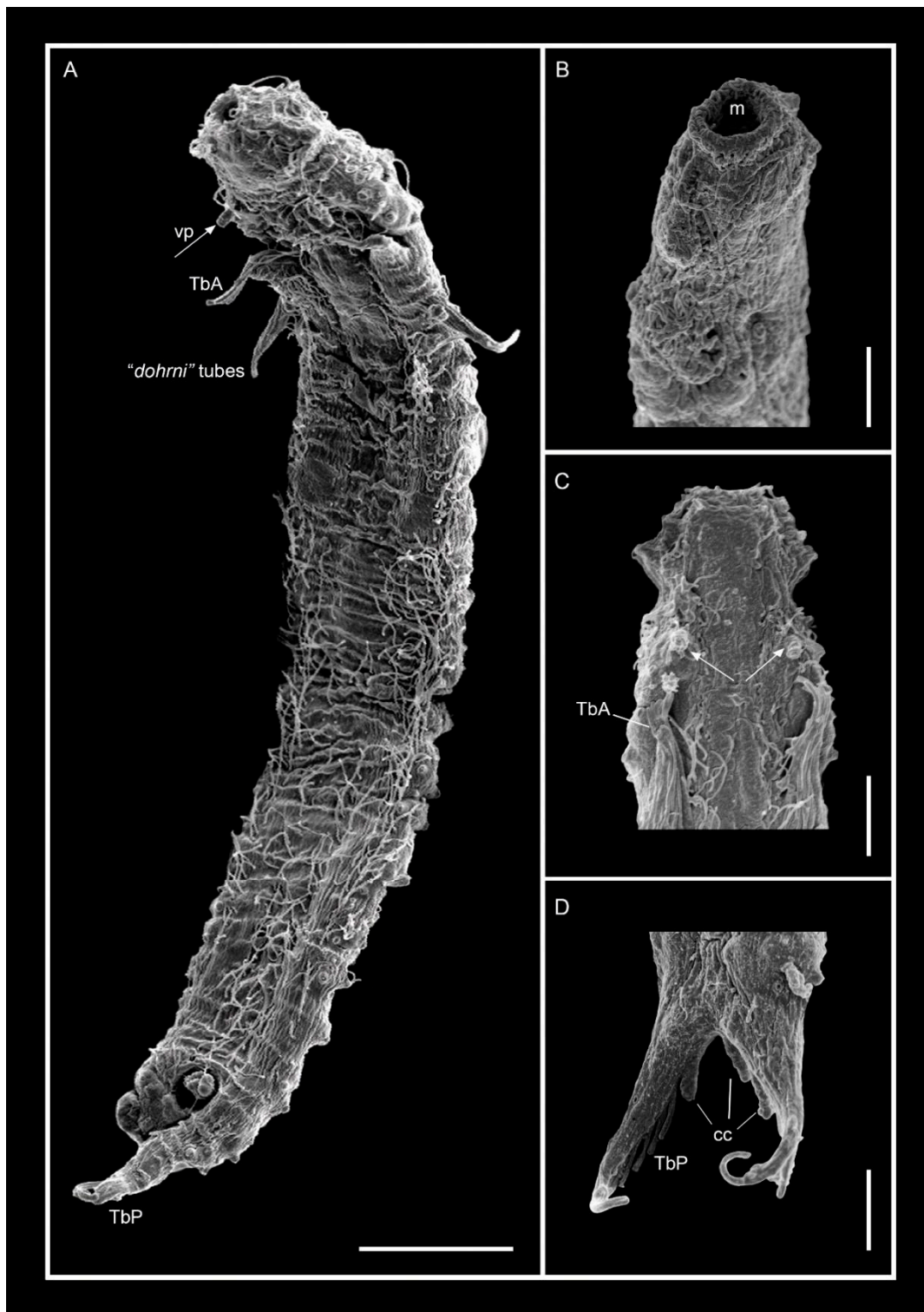
**Figure 3.** *Paraturbanella tricaudata* sp. nov. DIC photomicrographs. (A) Dorsal view of the *Habitus*. (B) Anterior region showing the mouth (m), peribuccal swellings, anterior adhesive tubes (TbA), “dohrni” tubes and ventral papillae (vp). (C) Pharyngeal pores (pp), pharyngo-intestinal junction (PhIJ), and epidermal glands (eg). (D) Paired testes (t)—they are lateral and extend posteriorly, with sperm ducts. (E) Sperm duct recurring to the front of the mid-intestine. (F) Caudal lobes, posterior adhesive tubes (TbP), and caudal cones (cc). (G) Sperm (sp) released from the side of the body due to excessive compression. Scale bar: A = 50; B, C, E, F = 20; D = 40; G = 10  $\mu$ m.



**Figure 4.** Adult specimen of *Paraturbanella tricaudata* sp. nov. (2002–2003 sampling). (A) *Habitus*; (B) ventral view of the posterior body end, showing posterior adhesive tubes (TbP) and tree caudal cones (cc); (C) ventral view of the anterior region, showing the anterior adhesive tubes (TbA) and ventral papillae (vp). Scale bars: A = 100; B, C = 50  $\mu$ m.



**Figure 5.** Adult specimen of *Paraturbanella tricaudata* sp. nov. (2002–2003 sampling). (A) *Habitus*, indicating the anterior most portion of the testes (t), frontal organ (fo) and the largest egg (egg). (B) Trunk region showing the testes (t), including the anterior most portion (arrows). (C) Trunk region from a slightly different focal plane showing the anastomosis of the sperm ducts (sd) and the ventral, ephemeral male pore (mp). (D) Dorsal view of the trunk region showing the frontal organ with a bundle of allosperm inside; the arrowhead indicates the location of the dorsal external pore through which sperm (sp) have been injected. (E) Close-up of the spermatozoa bundle inside the frontal organ. Scale bars: A = 100; B, C = 50; D, E = 20  $\mu$ m.



**Figure 6.** *Paraturbanella tricaudata* sp. nov. (SEM). (A) Ventrolateral view of the *Habitus*, showing ventral papillae (vp), anterior adhesive tubes (TbA), “dohrni” tubes and ventral ciliation. (B) Anterior region showing the terminal mouth (m). (C) Ventral view of the anterior region, showing the peribuccal swellings, anterior adhesive tubes (TbA) and ventral papillae (arrows). (D) Posterior body region showing the caudal lobe carrying adhesive tubes (TbP) and caudal cones (arrows). Scale bar: A = 20; B, C and D = 10  $\mu$ m.



**Examined material:** Holotype. Photographs of an adult specimen, collected on 9th November 2017 from Praia do Cachadaço, in the Trindade district in the municipality of Paraty, Rio de Janeiro State, Brazil (23°21'15.8" S 44°43'41.5" W). Bare sand of 30 cm depth had the following sediment characteristics: mean = 1.4111 phi (medium sand), SD = 0.8137, skewness = −0.8573, kurtosis = 4.4435, and median = 1.3215.

The specimen was observed alive with a compound microscope, but due to the fragility of the organisms, it was inadvertently destroyed during the study and is no longer available [50]. The holotype is illustrated in Figure 2 (according to the International Code of Zoological Nomenclature, 2017: Article 73, Recommendation 73G, in Declaration 45), and photos are available at the Museum of Zoology, University of Campinas, Brazil, under the accession number ZUEC GCH 61.

Paratypes. Photographs of five adult specimens (adults), collected on 9th November 2017 from Praia do Cachadaço, in Trindade district in the municipality of Paraty, Rio de Janeiro State, Brazil. The specimens were observed alive with a compound microscope, but due to the fragility of the organisms, physical specimens were inadvertently destroyed during the study and are no longer available [50]. Photographs of each specimen are available at the Museum of Zoology, University of Campinas, Brazil, under accession numbers ZUEC GCH 62–66.

**Additional material:** From the type locality, ten specimens were prepared for SEM and from locations sampled between 2002–2003 [34,35], two specimens are shown in Figures 4 and 5.

**Etymology:** The specific name refers to the triple caudal cone.

**Repository:** <http://zoobank.org/urn:lsid:zoobank.org:pub:EFA8F92E-7298-4982-B832-54F71E4BB995>

**Diagnosis:** The body is strap-shaped, and its length ranges from 417 to 480 µm. It has a large terminal mouth with a diameter ranging from 13.3 to 17.4 µm. The buccal cavity is heavily cuticularized, and it has a head with noticeable peribuccal swelling and ventral papillae. The pestle organ is absent. The pharyngo-intestinal junction (PhJIn) is between U34 and U38, and the distance of the pharyngeal pores from PhJIn varies from 20.2 to 27.1 µm. Epidermal glands are arranged in a single column on each side. There are five to six anterior adhesive tubes (TbA) on each side, all occurring on fleshy hands. There are two accessory adhesive tubes (“*dohrni*” tubes) of unequal length per side (the longer tube maximum length is 33.8 µm and the shorter is 19.4 µm). There are six posterior adhesive tubes (TbP) per side, the outermost being the longest. Dorsal adhesive tubes (TbD), ventral adhesive tubes (TbV), ventrolateral adhesive tubes (TbVL), and lateral adhesive tubes (TbL) are absent. In the posterior region, body tapering occurs gradually to the caudal base, and the caudum has three caudal cones, one medial and two laterals. Paired testes extend into sperm ducts, which turn anteriorly at U65 and fuse in a mid-ventral pore at U53. The frontal organ is at U71. About 20–30 epidermal glands are distributed along both lateral body margins.

**Description:** The description is based on both the holotype and ten paratypes (see Table 2). The body is strap-shaped and 417 µm in total length. The head bears noticeable lateral peribuccal swelling (U03) and a pair of papillae ventrally but no pestle organ; the cephalic region is delimited by a neck constriction 34.1 µm wide (U05). Body tapering occurs gradually to the caudal base, and the caudum bilobed has three caudal cones (U96). Widths at the outer oral opening, neck constriction, mid-pharynx, PhJIn, mid-intestine, furcal base, and their locations along the body length are: 16.4, 37.1, 25.2, 22.2, 19.7, and 33.0 µm at U0, U05, U20, U37, U64, and U93, respectively. Epidermal glands are arranged along the body in one column per side of 30 glands, which vary in size (1.6–4.7 µm in diameter).

**Adhesive tubes:** There are five or six anterior adhesive tubes (TbA) per side, all of which occur on fleshy hands that insert at U15. The innermost, mimicking a thumb, is the shortest, while the second from the inner side is the longest. Accessory adhesive tubes (“*dohrni*” tubes) are posterolaterally directed, and there are two per side of unequal length (the longer tube is 14 µm and the shorter is 9 µm) at U17. Dorsal adhesive tubes (TbD), ventral adhesive tubes (TbV), ventrolateral adhesive tubes (TbVL), and lateral adhesive tubes (TbL) are absent. There are six posterior adhesive tubes (TbP) per

side, the outermost being the longest. The distance between the external TbP is greater than the width of the body's caudal base.

**Ciliation:** The cephalic region has ciliary patches and circumcephalic rows in the mouth. Ventral locomotor cilia are arranged in two longitudinal bands that trace the lateral body sides and join posteriorly near the anal opening. Additional sensory bristles are organized in lateral, dorsolateral and dorsal columns.

**Digestive tract:** The mouth is terminal and surrounded by the mouth ring, composed of a strengthened cuticle; buccal cavity (6.4  $\mu\text{m}$  wide and 19.1  $\mu\text{m}$  long) mug shaped with walls heavily cuticularized. The pharynx is 142  $\mu\text{m}$  in length and 22.6  $\mu\text{m}$  in wide (U30), with pharyngeal pores near the base at about U33 and PhJIn at U37; the intestine is straight and the anus ventral is at U85.

**Reproductive tract:** It is hermaphroditic, with paired testes from U52 to U67, which extend into two sperm ducts at U60, turning anteriorly and fusing in a mid-ventral pore at U90. The frontal organ at U70 is vesicular and filled with bundled spermatozoa; the caudal organ is absent; the paired ovaries are at U68, and mature egg dorsal (to the intestine) occurs at U60.

### 3.1.1. Variability and Remarks on General Morphology

Seven additional adult measured specimens showed six TbP per side and three caudal cones, meaning that these traits are rather constant within and among the investigated populations. On the other hand, the number of TbA is slightly variable but not related to the size of the animal; in fact, one of the specimens attaining a maximum length of 480  $\mu\text{m}$  (Figure 5) possessed only five TbA per side while another attaining 332  $\mu\text{m}$  in total length had six TbA.

### 3.1.2. Taxonomic Remarks

Currently, there are 23 described species belonging to the genus *Paraturbanella* [6,17,33]. The new species bares closest resemblance to five species: *P. africana* Todaro, Dal Zotto, Bownes & Perissinotto, 2017 [17]; *P. teissieri* Swedmark, 1954 [19]; *P. sanjuanensis* Hummon, 2010 [23]; *P. solitaria* Todaro, 1995 [31]; and *P. xaymacana* Dal Zotto, Leasi & Todaro, 2018 [33], showing similar body and head shape, as well as peribuccal swelling [33].

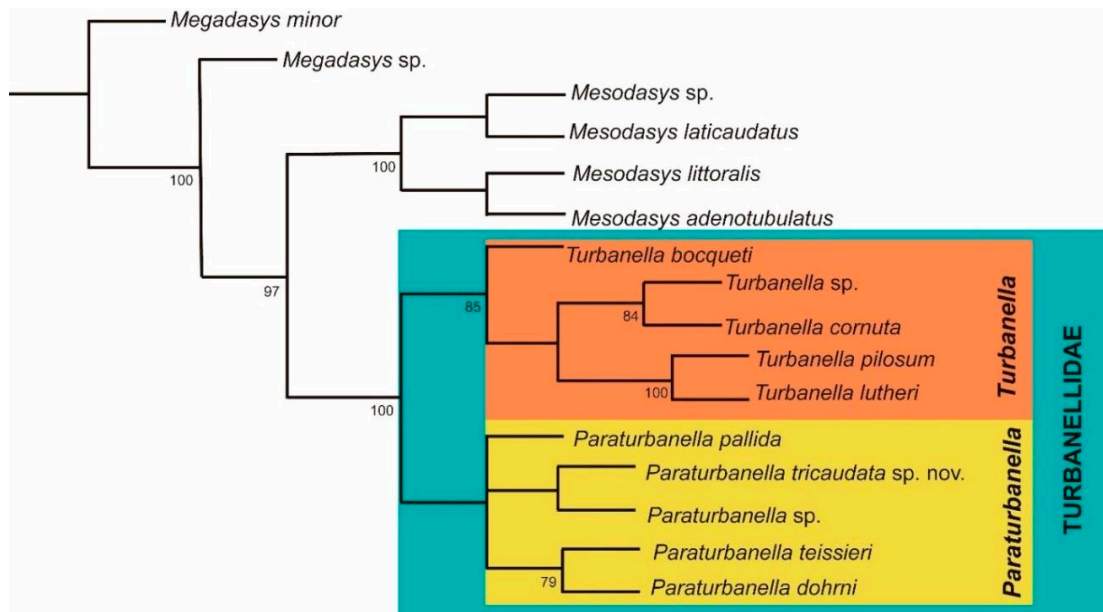
The species *Paraturbanella tricaudata* sp. nov. is considered new because it has three caudal cones, while the others have just one cone; moreover, the arrangement of the reproductive system, and in particular the location of the male pore, in combination with the extension and anatomical positioning of the testes is unique among congeneric species (two sperm ducts at U60 turning anteriorly and fusing in a mid-ventral pore).

### 3.1.3. Phylogenetic Analyses

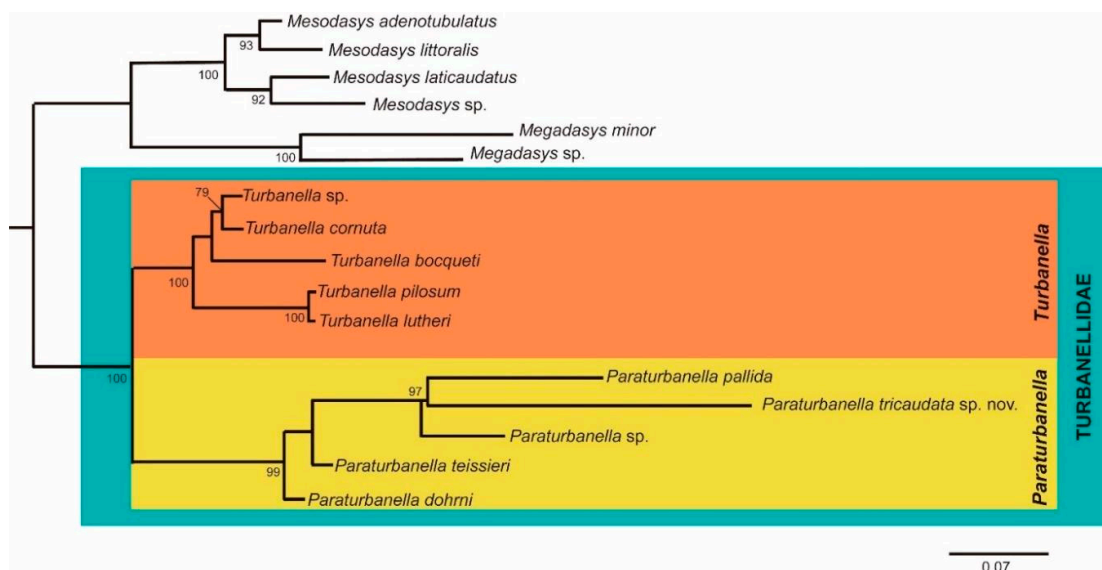
The final alignment of the combined dataset yielded 3525 positions (1693 in 18S rDNA and 1832 in 28S rDNA). The parsimony analysis yields only one most-parsimonious tree with 4626 steps (Figure 7).

Parsimony and Maximum Likelihood analyses yielded congruent topologies. In both analyses the phylogenies showed that the family Turbanellidae (both supported by very high bootstrap value - 100) and the genera *Turbanella* and *Paraturbanella* were monophyletic (Parsimony: *Turbanella* with good bootstrap values - 85; Maximum Likelihood: both genera supported by very high bootstrap value, respectively 100 and 99) and sister groups.

The phylogenetic position of *Paraturbanella tricaudata* sp. nov. was not stable in both analyses. The new species appeared as the sister-group of *Paraturbanella* sp. and both species were closely related with *P. pallida* in Maximum Parsimony analysis (Figure 7). However, *Paraturbanella tricaudata* sp. nov. was sister-group of *P. pallida* and both species were closely related with *Paraturbanella* sp. in Maximum Likelihood analysis (Figure 8).



**Figure 7.** Maximum parsimony reconstruction based on molecular data (18S and 28S rDNA). Molecular dataset aligned in MAFFT. Numbers at nodes indicate bootstrap support (> 75).



**Figure 8.** Phylogenetic relationships of Turbanellidae species inferred from Maximum Likelihood analysis of 18S and 28S rRNA. Numbers at nodes represent bootstrap support (1000 bootstrap replicates).

### 3.1.4. Conclusive Remarks

The majority of marine gastrotrich sampling sites are in the Northern Hemisphere [51], while in tropical countries the investigated areas are very scattered [14,16,17,30,52]. The Brazilian coast is poorly sampled, although it shows a high diversity of marine gastrotrichs, according to current literature [5,51]. In terms of knowledge of Turbanellidae diversity from Brazil, there are at least four candidate species to be formally described (one *Turbanella* species and three *Paraturbanella* species) from the north coast of São Paulo State [34,35].

The Brazilian coast represents a potentially important area for the discovery of new species, and it is worth noting that this region is of particular interest in the study of marine meiofauna and the diversity of Gastrotricha, as recorded in previous studies [2,34,35,51–54]. We emphasize the importance

of investigating new geographic areas in order to improve our understanding of morphological diversity and the species richness of gastrotrich species.

Finally, the inclusion of new sequences of data concerning *Paraturbanella tricaudata* sp. nov. and the use of distinct phylogenetic approaches did not change the scenario found by Todaro et al. [44], Kolicka et al. [13], and Garraffoni et al. [4]. However, it is important to highlight that the low number of Turbanellidae species sequenced (from two of the six genera) may result in misleading the phylogenetic relationships within this clade.

**Author Contributions:** Conceptualization, A.C. and A.R.S.G.; methodology, A.C., M.A.T., A.R.S.G.; validation, A.C., M.A.T., A.R.S.G.; formal analysis, A.C., M.A.T., A.R.S.G.; investigation, A.C., M.A.T., A.R.S.G.; resources, A.R.S.G.; data curation, A.C., M.A.T., A.R.S.G.; writing—original draft preparation, A.C., M.A.T., A.R.S.G.; writing—review and editing, A.C., M.A.T., A.R.S.G.; visualization, A.C., M.A.T., A.R.S.G.; supervision, A.R.S.G.; project administration, A.R.S.G.; funding acquisition, A.R.S.G. All authors have read and agreed to the published version of the manuscript.

**Funding:** This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior—Brasil (CAPES) Finance Code 001 and Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP-2014/23856-0).

**Acknowledgments:** The authors would like to thank K.R. Seger for help with molecular analysis. A.C.Z Amaral for providing the nested column of sieves and T.A. Quintão for grain size data analysis. We are also grateful to three anonymous reviewers for their constructive criticism that greatly improving the first version of the manuscript.

**Conflicts of Interest:** The authors declare no conflict of interest.

## References

- Balsamo, M.; d’Hondt, J.L.; Kisielewski, J.; Todaro, M.A.; Tongiorgi, P.; Guidi, L.; Grilli, P.; de Jong, Y. Fauna Europaea: Gastrotricha. *Biodivers. Data J.* **2015**, *3*, e5800. [CrossRef]
- Todaro, M.A.; Sibaja-Cordero, J.A.; Segura-Bermúdez, O.A.; Coto-Delgado, G.; Goebel-Otárola, N.; Barquero, J.D.; Cullerell-Delgado, M.; Dal Zotto, M. An introduction to the study of Gastrotricha, with a taxonomic key to families and genera of the group. *Diversity* **2019**, *11*, 117. [CrossRef]
- Remane, A. Neue aberrante Gastrotrichen II: *Turbanella cornuta* nov.spec. und *T. hyalina* M. Schultze, 1853. *Zool. Anz.* **1925**, *64*, 309–314.
- Garraffoni, A.R.S.; Araújo, T.Q.; Lourenço, A.P.; Guidi, L.; Balsamo, M. Integrative taxonomy of a new *Redudasys* species (Gastrotricha: Macrodasysida) sheds light on the invasion of freshwater habitats by macrodasysids. *Sci. Rep.* **2019**, *9*, 2067. [CrossRef] [PubMed]
- Campos, A.; Garraffoni, A.R.S. A synopsis of knowledge, zoogeography and an online interactive map of Brazilian marine gastrotrichs. *PeerJ* **2019**, *7*, e7898. [CrossRef]
- Todaro, M.A. (Ed.) Marine. In *Gastrotricha World Portal*; 2019; Available online: <http://www.gastrotricha.unimore.it/marine.htm> (accessed on 30 November 2019).
- Remane, A. Neue Gastrotricha Macrodasysoidea. *Zool. Jahrb. Abt. Anat. Ontog. Tiere* **1927**, *54*, 203–242.
- Evans, W.A.; Hummon, W.D. A new genus and species of Gastrotricha from the Atlantic coast of Florida, U.S.A. *Trans. Am. Microsc. Soc.* **1991**, *110*, 321–327. [CrossRef]
- d’Hondt, J.L. Contribution a la connaissance des Gastrotriches intercotidaux du Golfe de Gascogne. *Cah. Biol. Mar.* **1968**, *9*, 387–404.
- Clausen, C. *Desmodasys phocoides* gen. et. sp. n., family Turbanellidae (Gastrotricha Macrodasysoidea). *Sarsia* **1965**, *21*, 17–21. [CrossRef]
- Schultze, M. Über *Chaetonotus* und *Ichthydium* (Ehrb.) und eine neue verwandte Gattung *Turbanella*. Müller’s Arch. *Anat. Physiol.* **1853**, *6*, 241–254.
- Kieneke, A.; Riemann, O.; Ahlrichs, W.H. Novel implications for the basal internal relationships of Gastrotricha revealed by an analysis of morphological characters. *Zool. Scr.* **2008**, *37*, 429–460. [CrossRef]
- Kolicka, M.; Kotwicki, L.; Dabert, M. Diversity of Gastrotricha on Spitsbergen (Svalbard Archipelago, Arctic) with a description of seven new species. *Ann. Zool.* **2018**, *68*, 609–740. [CrossRef]
- Todaro, M.A.; Dal Zotto, M.; Perissinotto, R.; Bownes, S.J. First records of Gastrotricha from South Africa, with description of a new species of *Halichaetonotus* (Chaetonotida, Chaetonotidae). *Zookeys* **2011**, *142*, 1–13. [CrossRef] [PubMed]

15. Todaro, M.A.; Leasi, F.; Hochberg, R. A new species, genus and family of marine Gastrotricha from Jamaica, with a phylogenetic analysis of Macrodasysida based on molecular data. *Syst. Biodivers.* **2014**, *12*, 473–488. [[CrossRef](#)]
16. Todaro, M.A.; Perissinotto, R.; Bownes, S.J. Two new marine Gastrotricha from the Indian Ocean coast of South Africa. *Zootaxa* **2015**, *3905*, 193–208. [[CrossRef](#)]
17. Todaro, M.A.; Dal Zotto, M.; Bownes, S.J.; Perissinotto, R. Two new interesting species of Macrodasysida (Gastrotricha) from KwaZulu-Natal (South Africa). *Proc. Biol. Soc. Wash.* **2017**, *130*, 140–155. [[CrossRef](#)]
18. Evans, W.A. Five new species of marine Gastrotricha from the Atlantic coast of Florida. *Bull. Mar. Sci.* **1992**, *51*, 315–328.
19. Swedmark, B. *Turbanella armoricana* n. sp., nouveau gastrotriche macrodasyoïde de la côte nord de Bretagne. *Bull. Soc. Zool. Fr.* **1954**, *79*, 469–473.
20. Rao, G.C.; Ganapati, P.N. Some new interstitial gastrotrichs from the beach sands of Waltair coast. *Proc. Sci. Acad. India* **1968**, *67*, 35–53.
21. Rao, G.C. Meiofauna, In: Fauna of Lakshadweep, State Fauna Series. *Zool. Surv. India* **1991**, *2*, 73–75.
22. Maguire, C. Two new species of *Paraturbanella cuanensis* and *P. eireanna*. *Cah. Biol. Mar.* **1976**, *17*, 405–410.
23. Hummon, W.D. Marine Gastrotricha of San Juan Island, Washington, USA, with notes on some species from Oregon and California. *Meiofauna Mar.* **2010**, *18*, 11–40.
24. Wieser, W. Gastrotricha Macrodasyoidea from the intertidal of Puget Sound. *Trans. Am. Microsc. Soc.* **1957**, *76*, 372–381. [[CrossRef](#)]
25. Hummon, W.D. Marine Gastrotricha of the Near East: 1. Fourteen new species of Macrodasysida and a redescription of *Dactylopodola agadasys* Hochberg, 2003. *ZooKeys* **2011**, *94*, 1–59. [[CrossRef](#)] [[PubMed](#)]
26. Hummon, W.D. Gastrotricha of the North Atlantic Ocean: 1. Twenty-four new and two redescribed species of Macrodasysida. *Meiofauna Mar.* **2008**, *16*, 117–174.
27. Rao, G.C. 3 New interstitial Gastrotrichs from Andhra Coast, India. *Cah. Biol. Mar.* **1970**, *11*, 109–120.
28. Schmidt, P. Interstitielle Fauna von Galapagos. IV. Gastrotricha. *Mikrofauna Meeresbodens.* **1974**, *26*, 1–76.
29. Luporini, P.; Magagnini, G.; Tongiorgi, P. Contribution a la connaissance des gastrotriches des côtes de Toscane. *Cah. Biol. Mar.* **1971**, *12*, 433–455.
30. Clausen, C. Three new species of Gastrotricha Macrodasysida from the Bergen area, western Norway. *Sarsia* **1996**, *81*, 119–129. [[CrossRef](#)]
31. Todaro, M.A. *Paraturbanella solitaria*, a new psammic species (Gastrotricha: Macrodasysida: Turbanellidae), from the coast of California. *Proc. Biol. Soc. Wash.* **1995**, *108*, 553–559.
32. Hochberg, R. Two new species of Turbanellidae (Gastrotricha: Macrodasysida) from a high-energy beach on North Stradbroke Island, Australia. *N. Zeal. J. Mar. Fresh.* **2002**, *36*, 311–319. [[CrossRef](#)]
33. Dal Zotto, M.; Leasi, F.; Todaro, M.A. A new species of Turbanellidae (Gastrotricha, Macrodasysida) from Jamaica, with a key to species of *Paraturbanella*. *ZooKeys* **2018**, *734*, 105–119. [[CrossRef](#)] [[PubMed](#)]
34. Todaro, M.A.; Rocha, C.E. Diversity and distribution of marine Gastrotricha along the northern beaches of the State of São Paulo (Brazil), with description of a new species of Macrodasys (Macrodasysida, Macrodasysidae). *J. Nat. Hist.* **2004**, *38*, 1605–1634. [[CrossRef](#)]
35. Todaro, M.A.; Rocha, C.E. Further data on marine gastrotrichs from the State of São Paulo and the first records from the State of Rio de Janeiro (Brazil). *Meiofauna Mar.* **2005**, *14*, 27–31.
36. ArcGis. *Paraturbanella* in Brazil [basemap], Scale Not Given. 2019. Available online: <https://www.arcgis.com/home/webmap/viewer.html?webmap=fafb1d0942b4483a99ddf828fc24039a> (accessed on 17 November 2019).
37. Hummon, W.D.; Todaro, M.A.; Tongiorgi, P. Italian Marine Gastrotricha: II. One new genus and ten new species of Macrodasysida. *Boll. Zool.* **1993**, *60*, 109–127. [[CrossRef](#)]
38. Suguio, K. *Introdução à Sedimentologia*; Edgard Blucher: São Paulo, Brazil, 1973; p. 307.
39. Balsillie, J.H.; Donoghue, J.F.; Butler, K.M.; Koch, J.L. Plotting equation for Gaussian percentiles and a spreadsheet program for generating probability plots. *J. Sediment. Res.* **2002**, *72*, 929–933. [[CrossRef](#)]
40. Todaro, M.A.; Dal Zotto, M.; Kånneby, T.; Hochberg, R. Integrated data analysis allows the establishment of a new, cosmopolitan genus of marine Macrodasysida (Gastrotricha). *Sci. Rep.* **2019**, *9*, 7989. [[CrossRef](#)]
41. Katoh, K.; Standley, D.M. MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. *Mol. Biol. Evol.* **2013**, *30*, 772–780. [[CrossRef](#)]
42. Goloboff, P.A.; Farris, J.S.; Nixon, K.C. TNT, a free program for phylogenetic analysis. *Cladistics* **2008**, *24*, 774–786. [[CrossRef](#)]

43. Stamatakis, A.; Hoover, P.; Rougemont, J. A rapid bootstrap algorithm for the RAxML web servers. *Syst. Biol.* **2008**, *57*, 758–771. [[CrossRef](#)]
44. Todaro, M.A.; Kånneby, T.; Dal Zotto, M.; Jondelius, U. Phylogeny of Thaumastodermatidae (Gastrotricha: Macrodasysida) inferred from nuclear and mitochondrial sequence data. *PLoS ONE* **2011**, *6*, e17892. [[CrossRef](#)] [[PubMed](#)]
45. Todaro, M.A.; Littlewood, D.T.J.; Balsamo, M.; Herniou, E.A.; Cassanelli, S.; Manicardi, G.; Tongiorgi, P. The interrelationships of the Gastrotricha using nuclear small rRNA subunit sequence data, with an interpretation based on morphology. *Zool. Anz.* **2003**, *242*, 145–156. [[CrossRef](#)]
46. Petrov, N.B.; Pegova, A.N.; Manylov, O.G.; Vladychenskaya, N.S.; Mogue, N.S.; Aleshin, V.V. Molecular phylogeny of Gastrotricha on the basis of a comparison of the 18S rRNA genes: Rejection of the hypothesis of a relationship between Gastrotricha and Nematoda. *Mol. Biol.* **2007**, *41*, 445–452. [[CrossRef](#)]
47. Paps, J.; Riutort, M. Molecular phylogeny of the phylum Gastrotricha: New data brings together molecules and morphology. *Mol. Phylogenet. Evol.* **2012**, *63*, 208–212. [[CrossRef](#)]
48. Rao, G.C.; Clausen, C. *Planodasys marginalis* gen. et sp. nov. and Planodasyidae fam. nov. (Gastrotricha Macrodasyoidea). *Sarsia* **1970**, *42*, 73–82. [[CrossRef](#)]
49. Remane, A. Morphologie und Verwandtschaftbeziehungen der aberranten Gastrotrichen I. *Z. Morph. Oekol. Tiere* **1926**, *5*, 625–754. [[CrossRef](#)]
50. Garraffoni, A.R.; Kieneke, A.; Kolicka, M.; Corgosinho, P.H.; Prado, J.; Nihei, S.S.; Freitas, A.V. ICZN Declaration 45: A remedy for the nomenclatural and typification dilemma regarding soft-bodied meiofaunal organisms? *Mar. Biodivers.* **2019**, *49*, 2199–2207. [[CrossRef](#)]
51. Garraffoni, A.R.S.; Balsamo, M. Is the ubiquitous distribution real for marine gastrotrichs? Detection of areas of endemism using Parsimony Analysis of Endemicity (PAE). *Proc. Biol. Soc. Wash.* **2017**, *130*, 197–210. [[CrossRef](#)]
52. Araújo, T.Q.; Balsamo, M.; Garraffoni, A.R.S. A new species of *Pseudostomella* (Gastrotricha, Thaumastodermatidae) from Brazil. *Mar. Biodivers.* **2014**, *44*, 243–248. [[CrossRef](#)]
53. Garraffoni, A.R.S.; Di Domenico, M.; Amaral, A.C.Z. Patterns of diversity in marine Gastrotricha from Southeastern Brazilian Coast is predicted by sediment textures. *Hydrobiologia* **2016**, *773*, 105–116. [[CrossRef](#)]
54. Hochberg, R.; Atherton, S.; Kieneke, A. Marine Gastrotricha of Little Cayman Island with the description of one new species and an initial assessment of meiofaunal diversity. *Mar. Biodivers.* **2014**, *44*, 89–113. [[CrossRef](#)]



© 2020 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<http://creativecommons.org/licenses/by/4.0/>).