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

Ariane Campos [1,*], M. Antonio Todaro [2] and André Rinaldo Senna Garraffoni [1]

[1] Department of Animal Biology, Institute of Biology, University of Campinas, Campinas, SP 13083970, Brazil; arsg@unicamp.br

[2] 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

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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 Macrodasyida 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 Macrodasyida 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. stradbroki 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 $MgCl_2$. 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_2. 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].

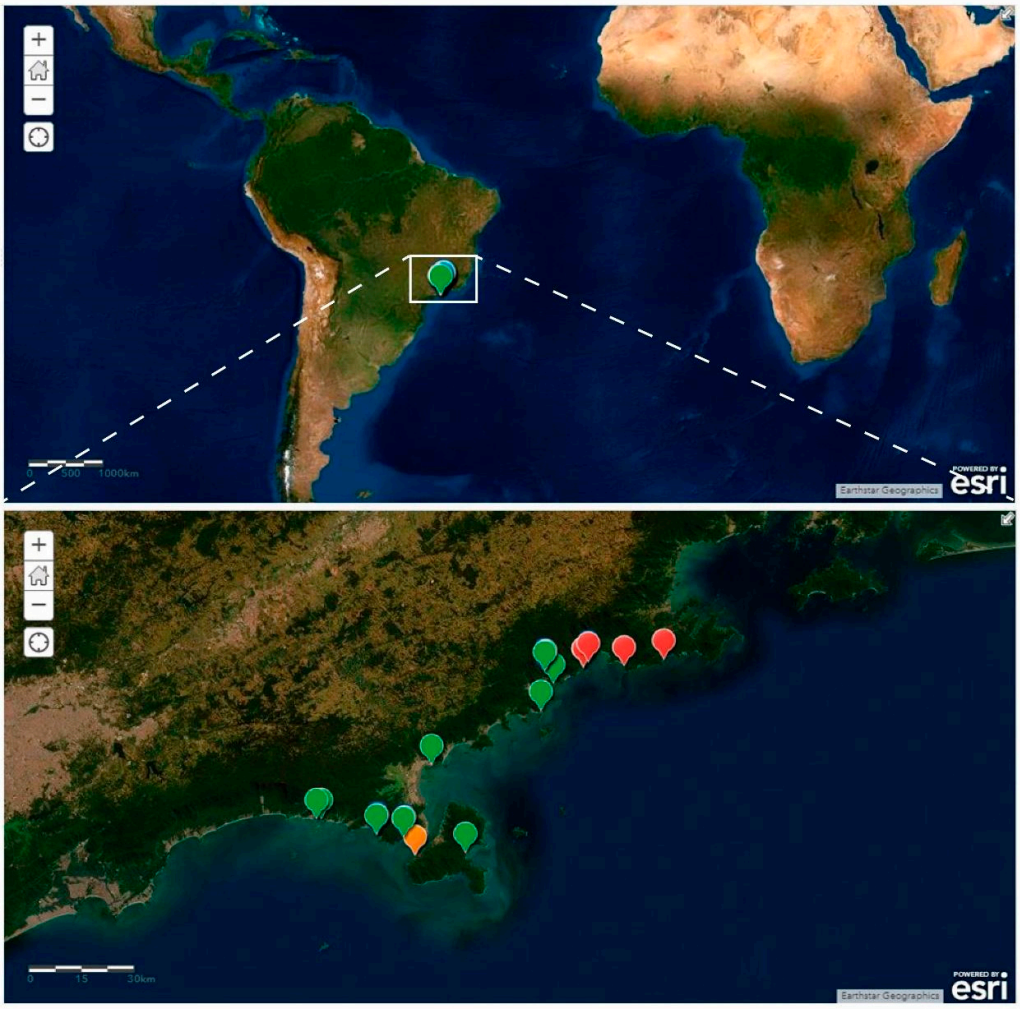

Figure 1. Map of Brazil showing the distribution of Paraturbanella species. Red: Paraturbanella tricaudata species nova (sp. nov.). Green: Paraturbanella sp. 1 [34,35]. Orange: Paraturbanella sp. 3 [34].

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 µL reaction mixture containing 3 µL of genomic DNA, 13.5 µL of water, 2 µL of 10x buffer, 0.5 µL of dNTP, 0.2 µL of Taq Platinum (Quiagen) and 0.4 µL (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
Megadasys sp. 1	JF357656	JF357704	Todaro et al. [44]
Megadasys minor Kisielewski, 1987	AY228131	-	Todaro et al. [45]
Mesodasys littoralis Remane, 1951	JF357658	JF357706	Todaro et al. [44]
Mesodasys laticaudatus Remane, 1951	JF357657	JF357705	Todaro et al. [44]
Mesodasys sp.	AY963690	KF921011	Petrov et al. [46]
Mesodasys adenotubulatus Hummon, Todaro & Tongiorgi, 1993	AM231780	-	Todaro et al. [45]
Paraturbanella sp.	KF921017	-	Petrov et al. [46]
Paraturbanella teissieri Swedmark, 1954	JF357661	JF357709	Todaro et al. [44,45]
Paraturbanella pallida Luporini, Magagnini & Tongiorgi, 1973	JF357660	JF357708	Todaro et al. [44]
Paraturbanella dohrni Remane, 1927	JF357659	JF357707	Todaro et al. [44]
Paraturbanella tricaudata sp. nov.	SUB6819988	SUB6819996	Present study
Turbanella sp.	JF970238	-	Paps & Riutort [47]
Turbanella cornuta Remane, 1925	AF157007	JF357711	Todaro et al. [44]
Turbanella pilosum Kolicka, 2018	MF325920	MF325905	Kolicka et al. [13]
Turbanella lutheri Remane, 1952	JF357669	-	Todaro et al. [44]
Turbanella bocqueti Kaplan, 1958	JF357662	JF357710	Todaro et al. [44]

3. Results

Taxonomic Account

Phylum Gastrotricha Metschnikoff, 1865.
Order Macrodasyida 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

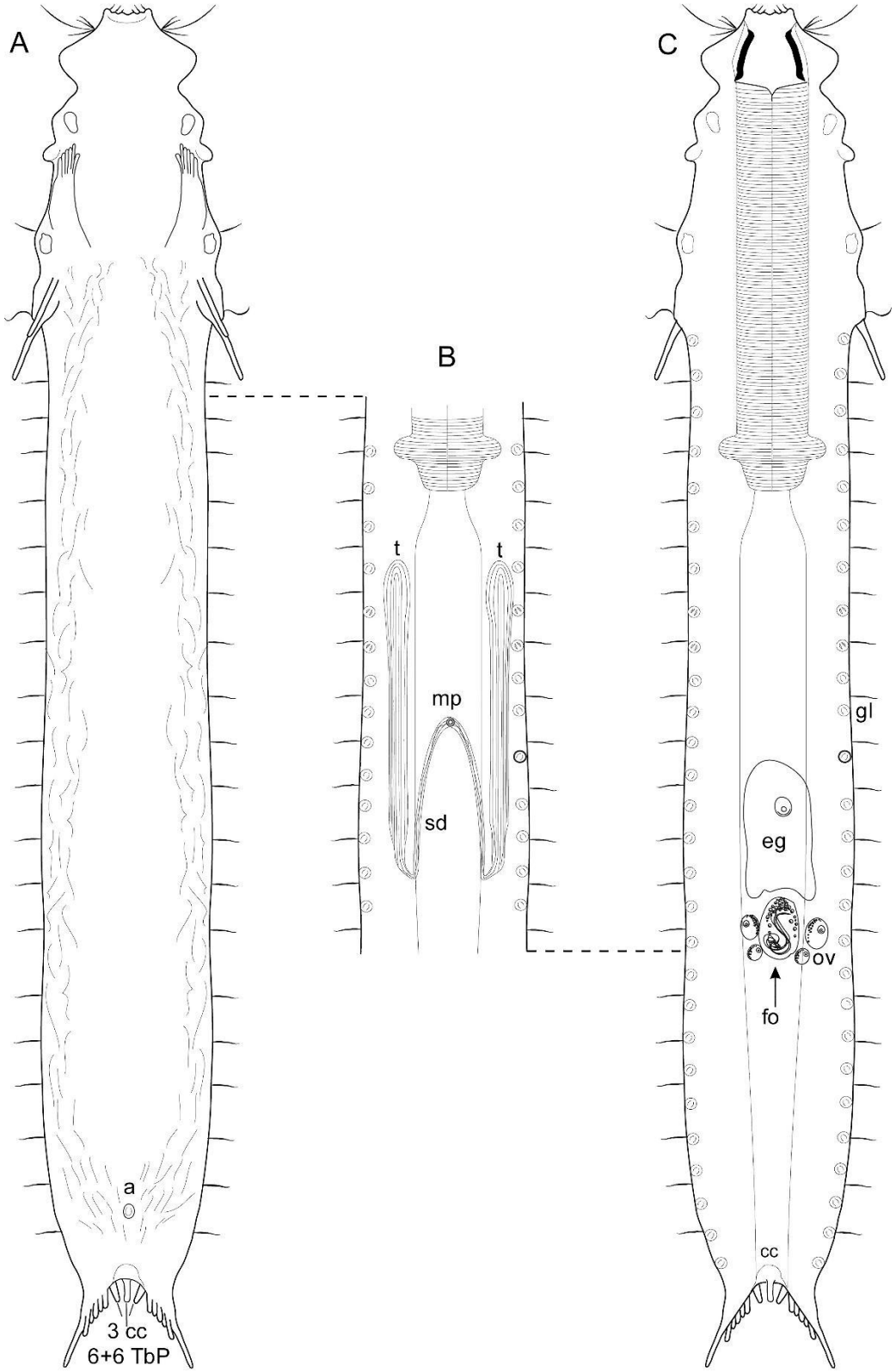

Figure 2. Paraturbanella tricaudata sp. nov. schematic drawing. (**A**) Ventral view of the Habitus. (**B**) Detail of the mid-trunk region, showing a ventral view of the male system. (**C**) Dorsal view of the Habitus, showing a mature egg, the frontal organ and the ovaries. Abbreviations: a = anus,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

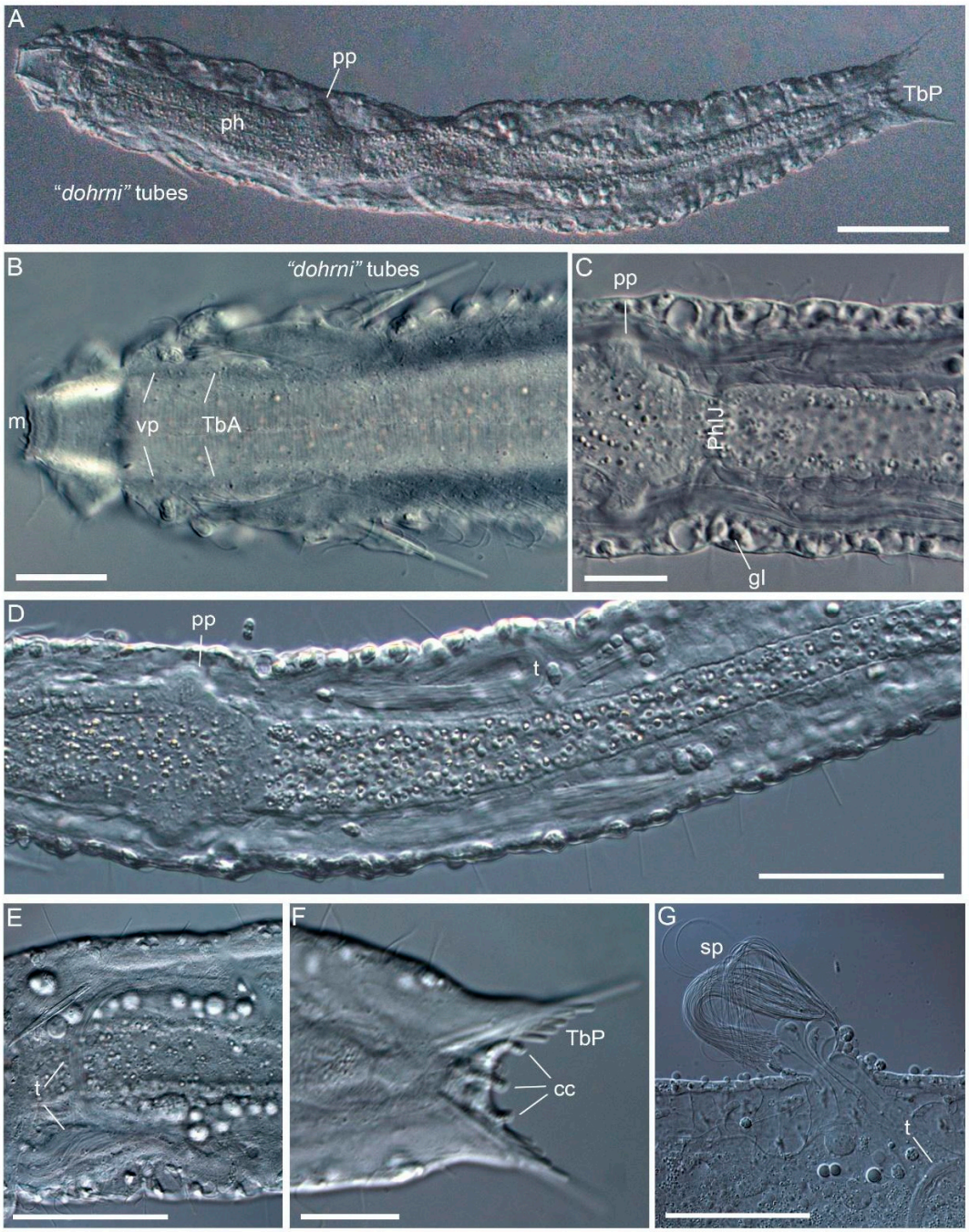

Figure 3. Paraturbanella tricaudata sp. nov. DIC photomicrographs. (**A**) Dorsal view of the Habitus. (**B**) Anterior region showing the mouth (m), peribuccal swellings, anterior adhesive tubes (TbA), "dohrni" tubes and ventral papillae (vp). (**C**) Pharyngeal pores (pp), pharyngo-intestinal junction (PhIJ), and epidermal glands (eg). (**D**) Paired testes (t)—they are lateral and extend posteriorly, with sperm ducts. (**E**) Sperm duct recurving 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 μm.

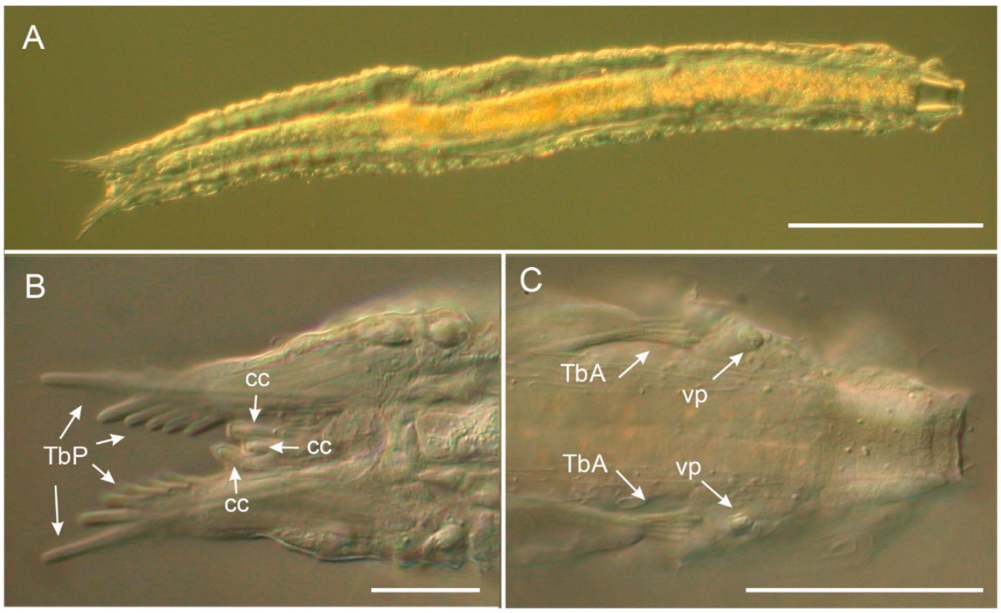

Figure 4. Adult specimen of Paraturbanella tricaudata sp. nov. (2002–2003 sampling). (**A**) Habitus; (**B**) ventral view of the posterior body end, showing posterior adhesive tubes (TbP) and tree caudal cones (cc); (**C**) ventral view of the anterior region, showing the anterior adhesive tubes (TbA)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

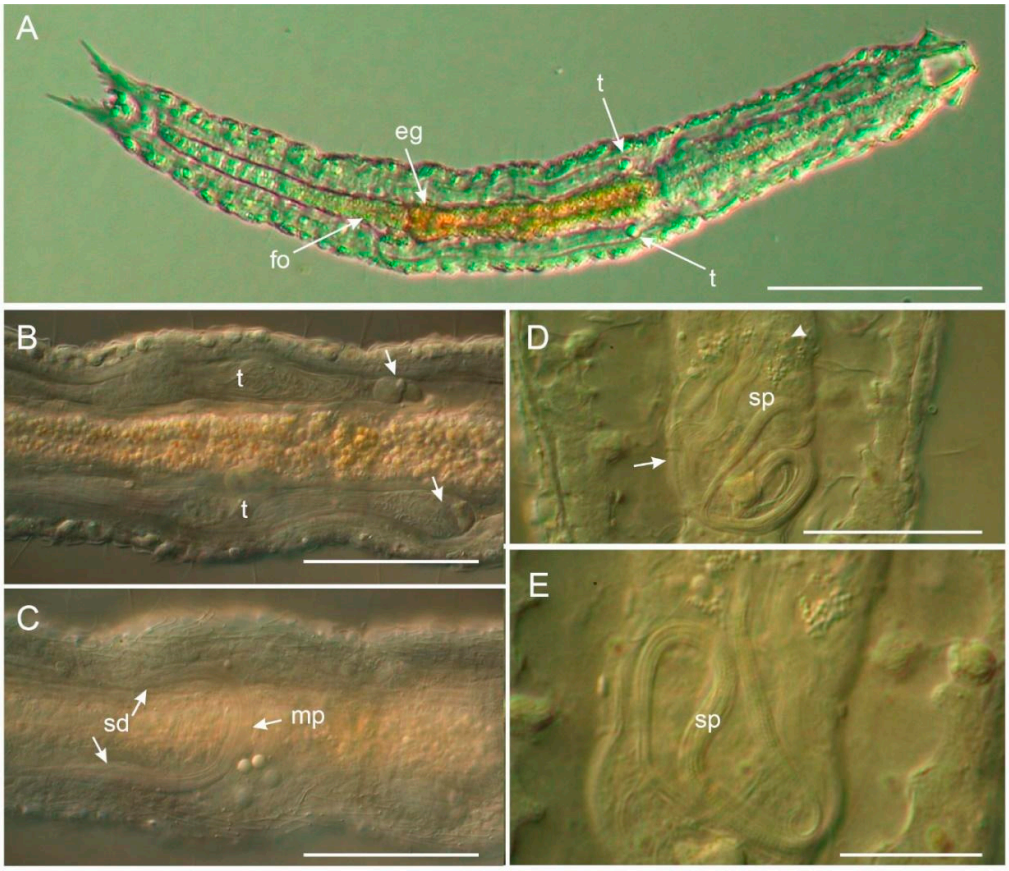

Figure 5. Adult specimen of Paraturbanella tricaudata sp. nov. (2002–2003 sampling). (**A**) Habitus, indicating the anterior most portion of the testes (t), frontal organ (fo) and the largest egg (egg). (**B**) Trunk region showing the testes (t), including the anterior most portion (arrows). (**C**) 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 μm.

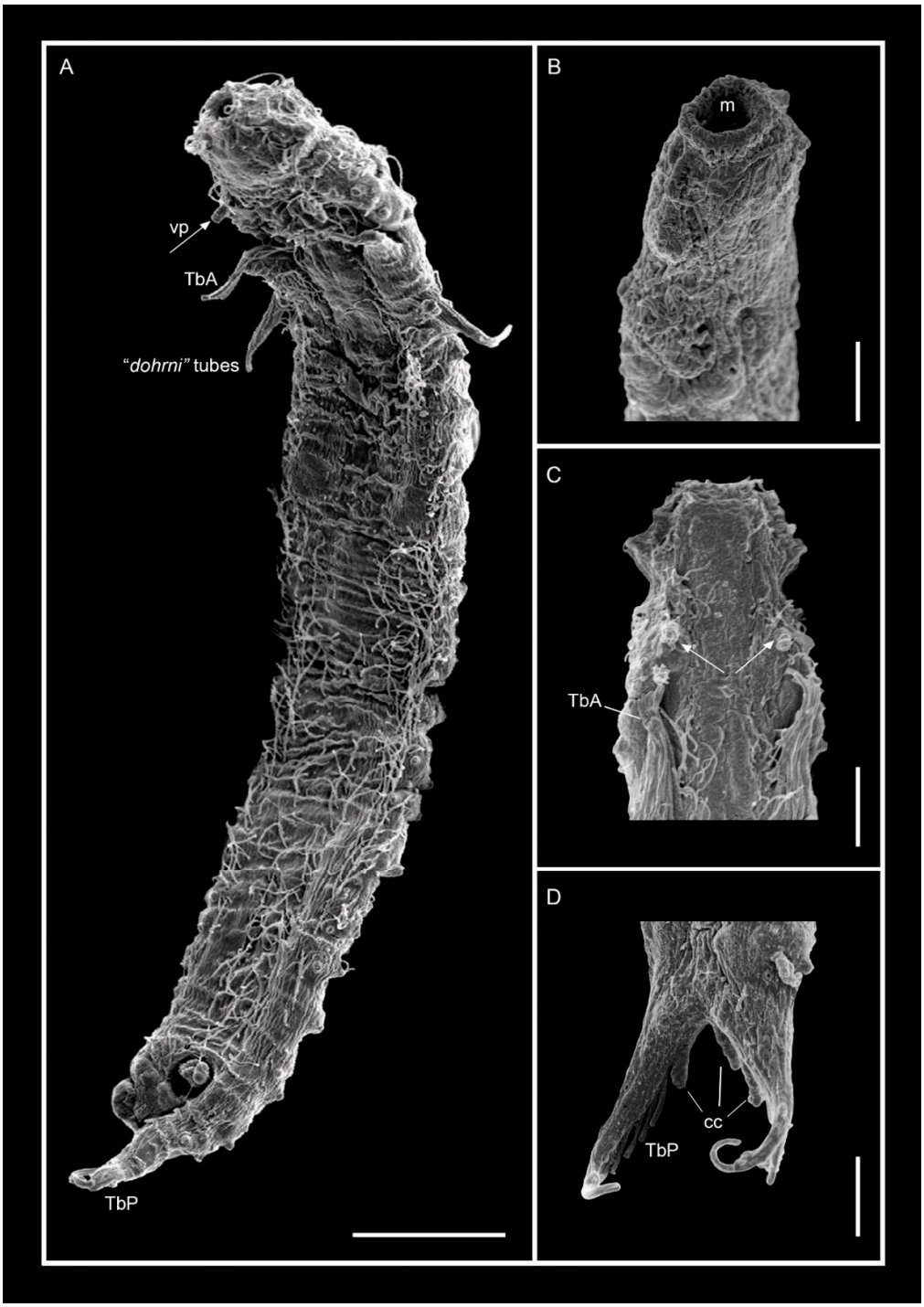

Figure 6. Paraturbanella tricaudata sp. nov. (SEM). (**A**) Ventrolateral view of the Habitus, showing ventral papillae (vp), anterior adhesive tubes (TbA), "dohrni" tubes and ventral ciliation. (**B**) Anterior region showing the terminal mouth (m). (**C**) Ventral view of the anterior region, showing 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 μm.

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 μm wide and 19.1 μm long) mug shaped with walls heavily cuticularized. The pharynx is 142 μm in length and 22.6 μm 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.

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 μm (Figure 5) possessed only five TbA per side while another attaining 332 μm in total length had six TbA.

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).

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).

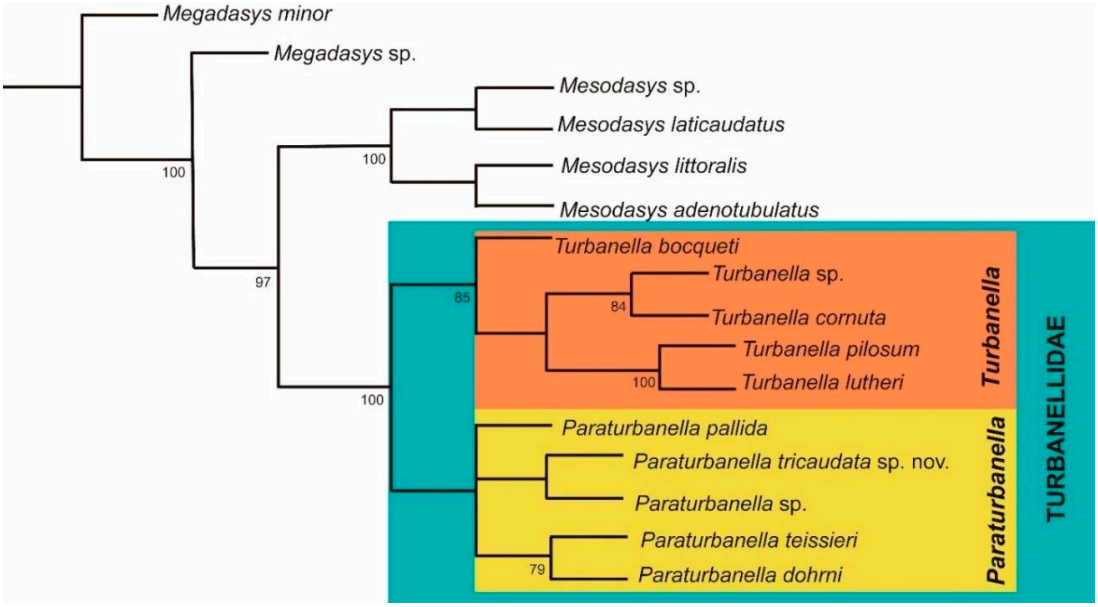

Figure 7. Maximum parsimony reconstruction based on molecular data (18S and 28S rDNA). Molecular dataset aligned in MAFFT. Numbers at nodes indicate bootstrap support (> 75).

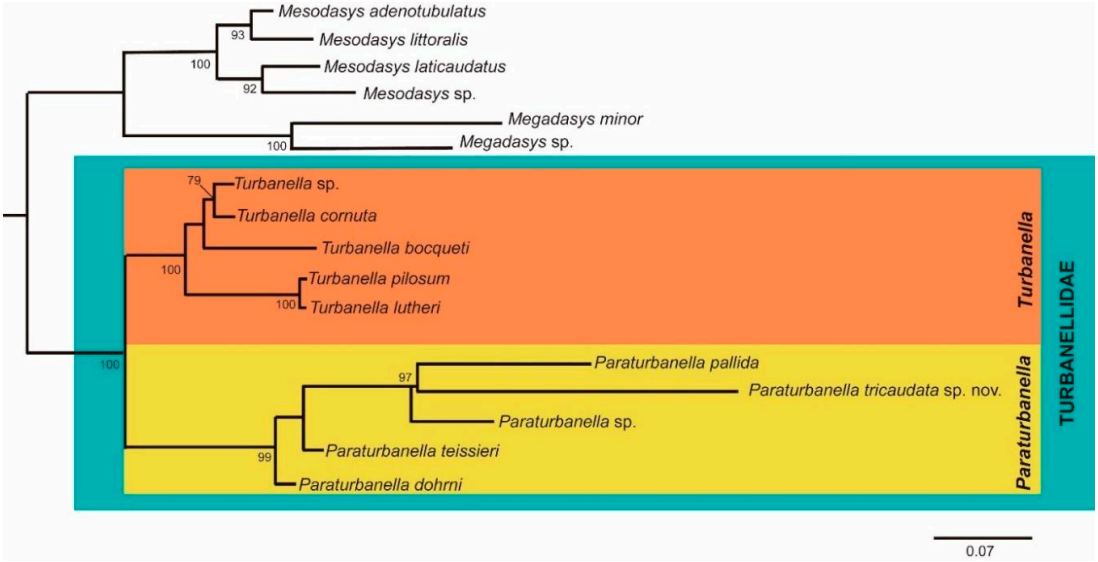

Figure 8. Phylogenetic relationships of Turbanellidae species inferred from Maximum Likelihood analysis of 18S and 28S rRNA. Numbers at nodes represent bootstrap support (1000 bootstrap replicates).

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: For research articles with several authors, a short paragraph specifying their individual contributions must be provided. The following statements should be used "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.

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