Integrative description of a new species of *Acanthodasys* Remane, 1927 (Gastrotricha, Macrodasyida, Thaumastodermatidae) based on four distinct morphological techniques and molecular data

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ABSTRACT

The number of undescribed animal species is still very high, in particular among the marine meiofaunal taxa. For these invertebrates, external and internal morphological information are often scarce and species delimitation and identification is a real problem. As a contribution to this shortfall, we describe a new gastrotrich species of the genus *Acanthodasys* (Macrodasyida, Thaumastodermatidae) from sublittoral sediments of the Brazilian southeastern coast based on four distinct morphological techniques (optical microscopy - DIC, CLSM, SEM, TEM) and molecular data. *Acanthodasys australis* sp. nov. is characterized by spined scales (uniancres) of different sizes and densities on the dorsal and ventral body sides, two types of spineless scales scattered among the spined scales, and 6 TbA per side forming two distinct groups (2+4) each arranged in two transverse rows. A molecular phylogeny of the family Thaumastodermatidae using a multigene approach found *Acanthodasys* to be monophyletic. This is the first species belonging to the genus *Acanthodasys* to be described from the Southern Hemisphere.

Keywords: Brazil, taxonomy, meiofauna, *Acanthodasys australis* sp. nov., Diplodasyinae, integrative morphology, molecular phylogeny

1. Introduction

Many meiofaunal taxa - an operational definition of motile, aquatic organisms, based on their retention in a mesh width of sieves between 500 and 44 μm (Giere 2009) - are considered "lesser known taxa" (Garey 2002). This poor knowledge is in part related to the small size and fragility of their bodies and the consequent difficulty in obtaining high-quality information about morphological diversity of external and internal structures (Artois et al. 2011) leading to a "Linnean" shortfall (*sensu* Fonseca et al. 2018). To make the study of meiofauna even more difficult, some meiofaunal organisms are frequently destroyed and/or lost during handling processes or deteriorate relatively quickly after preparation (Balsamo et al. 2014; Garraffoni & Freitas 2017; Garraffoni et al. 2019a). As a consequence, a large number of meiofauna species remains poorly described or even unknown (Appeltans et al. 2012), and our knowledge of homology among anatomical structures remains poor (Ramirez 2007; Vogt et al. 2010; Borkent 2018), due to the scarcity of proper anatomical data.

The phylum Gastrotricha contains soft-bodied animals commonly present in the meiofauna of marine and freshwater environments, where they glide by ciliary action (Balsamo et al. 2014; Todaro et al. 2019a). The taxon consists of more than 860 species (Todaro 2020) traditionally divided into two orders: Chaetonotida and Macrodasyida. Within the Macrodasyida, Thaumastodermatidae Remane, 1927 is the most speciose family, with more than 170 species (Todaro, 2020) and contains eight genera divided into two subfamilies: Thaumastodermatinae Remane, 1927 with the genera *Hemidasys* Claparède, 1867 (considered extinct by Hummon and Todaro 2010), *Oregodasys* Hummon, 2008, *Pseudostomella* Swedmark, 1956, *Ptychostomella* Remane, 1926, *Tetranchyroderma* Remane, 1926, *Thaumastoderma* Remane, 1926; and Diplodasyinae Ruppert, 1978, which includes *Acanthodasys* Remane, 1927 and *Diplodasys* Remane, 1927.

Currently, the genus *Acanthodasys* includes 12 valid species (Garraffoni & Balsamo 2017; Todaro 2020) and is characterized by a cuticular armature with uni-spined scales ("uniancres") (Todaro & Hummon 2008). To date, *Acanthodasys* species have been described from Europe (Germany, France, Norway, Italy and Portugal), United States,

Caribbean Sea and Korea; no nominal species are currently known from the Southern Hemisphere (Garraffoni & Balsamo 2017).

The marine gastrotrich fauna of Brazil was virtually unknown until about 15 years ago (Todaro & Rocha 2004, 2005); however, recent studies have revealed a hidden gastrotrich diversity along the Brazilian coast (Todaro 2012, 2013; Hochberg 2014; Araújo et al. 2014, 2016; Garraffoni et al. 2016, 2017; Campos & Garraffoni 2019; Todaro et al. 2019; Campos et al. 2020). Considering the length of the Brazilian coastline, approximately 7500 kilometers, we expect the biodiversity of the meiofauna to be equal to that of the Northern Hemisphere, and perhaps even to exceed it.

Here, we formally describe a new species of *Acanthodasys* from Brazil, previously reported by Garraffoni et al. (2017) as *Acanthodasys* sp. 1. We used four morphological methods to provide valuable taxonomic information on external and internal morphology: 1) differential interference contrast microscopy - DIC, optical microscopy - (e.g., cuticular structures, reproductive organ systems); 2) confocal laser scanning microscopy - CLSM - (muscle orientation and distribution); 3) scanning electron microscopy - SEM - (cuticle ultrastructure); and 4) transmission electron microscopy - TEM - (ultrastructure). We also applied nuclear ribosomal DNA (rDNA; 18S and 28S) sequencing for examining the affinity of the new *Acanthodasys* species with the other Thaumastodermatidae using a molecular approach.

2. Material and Methods

2.1 Taxonomic sampling

The sampled sites are located on the Northern coast of the State of São Paulo, at Ilhabela Island (23º44'27"S; 45º16'01"W) and coast of the State of Paraná, at Curral Island (25°44'6.13"S; 48°22'2.64"W). For Ilhabela Island, the top 15 cm of sediment at a depth of 8-10 m were placed in plastic buckets and gastrotrichs were extracted at Center of Marine Biology, University of São Paulo, in São Sebastião and at Laboratory of Evolutionary Meiofaunal Organisms, University of Campinas, in Campinas. The granulometric characteristics of the sediment at Fome Beach, Ilhabela Island are: size class coarse sand, sorting class well sorted, skewness 1.25, and kurtosis 5.40 (Garraffoni et al. 2017). For Curral Island, the top 10-20 of sediment at a depth of 5-10 m were placed in plastic buckets and gastrotrichs were extracted at Center for Marine Studies, Federal University of Paraná, in Pontal do Paraná. The granulometric characteristics of Curral

Island sediment are: size class medium sand, sorting class extremely poorly sorted, skewness 0.1847, and kurtosis 1.027.

2.2 Differential interference contrast (DIC) microscopy

Sediment screening for meiofauna was performed in the laboratory and gastrotrichs were sorted out following the protocol reported by Hochberg & Atherton (2010). Small amounts of sediment at a time were observed in a Petri dish under a stereomicroscope Zeiss Stemi 2000. Thirty-two narcotized specimens (30 from Ilhabela Island and 2 from Curral Island) and were isolated on glass slides and observed using a Zeiss Axio Imager M2 light microscope equipped with Differential Interference Contrast (DIC) light microscope and AxioCam MRC5 digital video camera. After, thirteen of the thirty-one live specimens were digitally documented, sixteen were recovered and subsequently fixed for further morphological analyses (eight for SEM, four for TEM and four for CLSM). The three remaining specimens were used for extraction of genomic DNA.

The percentage body unit (total body length = 100 units [U]) was used to indicate lengths and positions of structures and organ systems.

2.3 Scanning electron microscopy (SEM)

Specimens were fixed in 2.5% glutaraldehyde in 0.2 M cacodylate buffer (pH 7.4) at room temperature and storage within fixative for few months at 4ºC. They were dehydrated with a graded ethanol series with increasing concentration (20%, 30%, 40%, 50%, 60%, 70%, 95%, 100%), and then dried through a passage in a Hexamethyldisilazane (HMDS) solution (Hochberg & Litvaitis, 2000), mounted on aluminium stubs, sputter coated (Baltec SCD 050) with gold-palladium and finally observed with a JSM 5800LV scanning electron microscope at an accelerating voltage of 10 kV; images recorded with software Semafore (v.5.2).

2.4 Transmission electron microscopy (TEM)

Specimens were fixed in 2.5% glutaraldehyde in a 0.2 M sodium cacodylate buffer (pH 7.4), at room temperature and storage within fixative for several months at 4ºC. After they were post-fixed in 1% osmium tetroxide in the same buffer for 2 hours at room temperature, washed in a clean 0.1 M sodium cacodylate buffer, dehydrated in a graded ethanol series and embedded in Araldite. Ultra-thin sections were cut with a LKB

Ultrotome 2088 V microtome and were contrasted with a saturated solution of 3% uranyl acetate in ethanol 50% and lead citrate. The ultra-thin sections (70-80 nm) were observed under a Philips CM10 Transmission Electron Microscope at the University of Urbino (Italy).

2.5 Confocal laser scanning microscopy (CLSM)

Subadults specimens were fixed in 2.5% paraformaldehyde in 0.1 M buffered PBS saline (pH 7.2) at room temperature and storage within fixative for 2 weeks at 4ºC. After they were placed in buffered PBS saline with 1 % Triton X (PBT) for 1 h, and stained overnight at 4ºC with Alexa Fluor 488 phalloidin. Stained specimens were briefly rinsed in PBS before mounting in Fluoromount G on glass slides. Specimens were visualized under Olympus FV 300 confocal laser-scanning microscope excited with Argon laser (488 nm). Confocal z-stacks were collected and processed in as TIF files and MOV video files. Files were further processed with the software Volocity to generate z-projections.

2.6 DNA extraction and amplification

DNA extraction, amplification of nuclear 18S and 28S rDNA genes fragments and sequencing followed the protocols presented in Garraffoni et al. (2019b). Sequences of *Acanthodasys australis* sp. nov. were deposited in GenBank (GenBank accession numbers, 18S rDNA: MT028550; 28S rDNA: MN943644).

2.7 Phylogenetic analysis

The ingroup and outgroup dataset for this study was basically the same combined dataset of 18S and 28S rDNA used by Todaro et al. (2011) with 22 species belonging to Thaumastodermatidae (supplementary material) plus the 18S and 28S rDNA sequences of *Acanthodasys australis* sp. nov. As outgroups we also followed Todaro et al. (2011) and chose the final combined sequences of the chaetonotid *X. intermedia* (that rooted the tree) and other macrodasyids (*Dactylopodola mesotyphle*, *Mesodasys littoralis* and *Macrodasys* sp. 1).

Each gene dataset was aligned separately using Mafft v.7.402 (L-INS-I approach) (Katoh & Standley 2013) and concatenated using Sequence Matrix (Vaidya et al., 2011). The concatenated database was analyzed using maximum likelihood using RAxML (Stamatakis et al., 2008) with with GTRGAMMA model and 1000 bootstrap replicates.

The alignments and maximum likelihood analysis were performed using the CIPRES Science Gateway, San Diego Supercomputer Center (Miller et al., 2010).

3. Results

3.1 Taxonomy

Order Macrodasyida Remane, 1925 [Rao & Clausen, 1970]

Family Thaumastodermatidae Remane, 1927

Subfamily Diplodasyinae Ruppert, 1978

Genus *Acanthodasys* Remane, 1927

Acanthodasys australis sp. nov.

(Figs. 1-34, Table 1)

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3.1.1. Material examined

Holotype. Photographs of an adult specimen, collected from Ilhabela Island Island (municipality of Ilhabela), Fome Beach, State of São Paulo, Brazil (23º44’27’’S; 45º16’01’’W) at 8-10 m depth, on 16/07/2015. The narcotized specimen was examined with a compound microscope, but due to the fragility of the body, it was destroyed during microscopic examination and is no longer available (Garraffoni et al. 2019a). The holotype is illustrated in Fig. 8, 11 (International Code of Zoological Nomenclature, 2017: Articles 73, Recommendation 73G, in Declaration 45), and photos are available at the Museum of Zoology, University of Campinas, Brazil, under accession number ZUEC GCH 36.

Paratypes. Photographs of 7 (subadults and adults) specimens collected from São Sebastião Island (municipality of Ilhabela), Fome Beach, State of São Paulo, Brazil (23º44’27’’S; 45º16’01’’W) at 8-10 m depth, on 16/07/2015; photographs of 4 (sub adults and adult) specimens collected at the same locality on 21/02/2018. All of them were examined narcotized with a compound microscope and as the holotype, they were destroyed due to microscopic examination and are no longer available; 2 (adults) specimens collected from Curral Island (municipality of Pontal do Paraná), State of Paraná, Brazil (25°44'6.13"S; 48°22'2.64"O) at 5-10 m depth, on 29/01/2019, mounted on a glass slide. Photos and slides of paratypes (International Code of Zoological Nomenclature, 2017: Articles 73, Recommendation 73G, in Declaration 45) are available

at the Museum of Zoology, University of Campinas, Brazil, under accession numbers ZUEC GCH 37 to 50.

3.1.2. Additional material

Eight specimens were mounted for SEM (kept at University Of Campinas) and four specimens were mounted on microscope slides for confocal analysis (kept at University Massachusetts-Lowell). Three specimens were prepared for DNA sequencing and are no longer extant. All of them were adult specimens, collected on 07/07/2015 in São Sebastião Island (municipality of Ilhabela), Fome Beach, State of São Paulo, Brazil.

3.1.3 Etymology

The specific epithet '*australis*' (Latin australis = southern) was chosen because these *Acanthodasys* specimens were the first that have been collected in the Southern Hemisphere.

3.1.4 Diagnosis

Acanthodasys with body length from 373 to 795 μm. Body width at mouth from 34 to 63 μm, at the pharyngeointestinal junction (PhJIn) from 58 to 61 μm, posterior body from 25 to 33 μm. Pharynx from 133 to 205 μm long. Cuticle with spined scales (uniancres) and small spineless scales of two morphological types: eye-shaped scales with a central depression, and often a small central oval bump, and very elongate lanceolate scales with only a central depression. Uniancres cover both the dorsal and ventral body sides, being much larger dorsally, 4.6 - 6.2 μm in length, and 1.7 - 2.8 μm in length ventrally. At least 50-55 epidermal glands per side, distributed along the whole body. On the head six ventrally inserting TbA per side, forming two distinct groups (2+4), each one arranged into two transversal rows. Six elongate TbL per side, present only in the trunk region. Twenty TbVL per side from the PhJIn up to the outer edge of the caudal lobes. Three TbP per side, 2 terminal and 1 medial on each caudal lobe. Hermaphroditic, with paired testes and a single ovary anterior to the caudal organ and dorsal to the posterior intestine. Caudal organ located ventrally to the terminal gut. Rosette organ dorsolateral on the left body side. Frontal organ located dorsally and anterior to the mature oocyte, in continuity with a compact band of large Y-shaped cells containing myofilaments and hemoglobin.

3.1.5 Species-specific characters

Two types of spineless scales: 1) eye-shaped scales with a central depression, a slightly thickened rim and often a small central oval bump (Figs. 5-7, 16) ; 2) lanceolate scale present on ventral side with a honeycomb arrangement (Figs. 2, 11, 12); 6 TbA per side arranged into two series (2+4) (Figs. 2, 4, 14-15).

3.1.6 Description

The description is based on the holotype. Body is mostly strap-shaped, 650 µm long (Figs. 3-4, 8) and entirely covered with spined scales and spineless scales except for the hood-like region around the mouth (approximately 8 µm long). Body inflates at U17, narrows at U30, and slightly narrows again until the caudal base. Body widths are: at the level of mouth (U03) 44 µm, PhJIn (U30) 55 µm, maximum trunk width (U42) 66 µm, at the caudal base (U89) 36 µm. Pharynx 197 µm long and 36 µm wide, with pharyngeal pores at U33 (observed only in the confocal micrographs, Fig. 34). Mouth fringed with numerous sensory cilia (7 µm long) and on either side of the head with longer cilia (26 µm long) (Figs. 9, 11). Numerous epidermal glands of various size (5-11 µm diameter) (Figs. 3, 8), arranged into two longitudinal columns of about 50-55 per side. Ventral locomotory cilia arranged into numerous small patches limited by lanceolate scales (see below), from U06 to the base of the caudal appendages (Fig. 2).

Cuticular armature. Both uniancres and eye-shaped spineless scales cover the whole surface of the body. Conspicuous uniancres are arranged around the head edge, along the dorsal and ventrolateral sides of the trunk and on the caudal lobes (Figs. 1, 9-10, 16-18, 20). Other smaller spines uniformly cover the dorsal and ventral body sides (Figs. 14-18). Approximately 25 ancres, each about 6 µm long, extend transversely across the dorsal side of the head just behind the cilia of the spineless region in terminal mouth, whereas they are absent along the ventral outline (Figs. 9). The number of ancres in a longitudinal row is much higher on dorsal and ventrolateral sides of the body (approximately 90) than on the ventral side, 10 ancres at U14. Spines increase in size from the dorsal side (5 - 6.2 µm long) to the lateral ones (6.2 - 7.6 µm long), and decrease in size from ventrolateral sides (2.8 - 4 µm long) to the ventral side (1.6 - 2.3 µm long) (Figs. 14, 18). Each uniancre arises from a quadrangular-shaped base, is cross-shaped in transverse section and shows a tapering apex: four small opening are visible at the base of each spine longitudinal groove (Figs. 7, 17).

Two types of small spineless scales are interposed between the uniancres: eye-shaped scales, with a central depression and often a small central oval bump, and very elongate, lanceolate scales with only a central depression (Figs. 5-6, 12, 16-18). Eye-shaped scales are distributed on the dorsal and ventrolateral body sides, whereas lanceolate scales are present only on the ventral side with a honeycomb-like arrangement (Fig. 12).

Eye-shaped scales are ca. 1.4 - 2.6 μm long and 0.6 - 1.0 μm wide, with a central depression 1.2 - 1.9 μm long and 0.3 - 0.6 μm wide (measures from paratype 4 - Fig. 5). Lanceolate scales ca. 2.0 - 3.5 μm long and 0.8 - 1.3 μm wide, central depression in scales is 1.3 - 2.8 μm long and 0.4 - 0.7 μm wide (measures from paratype 4 - Fig. 6). All the eye-shaped spineless scales are arranged in several different orientations (longitudinal, transverse and oblique).

Adhesive tubes. TbA: a group of 6 per side, 4 - 5.5 μm long, arranged into two transverse series, the anterior series consists of 2 and the posterior series consists of 4 tubes (Figs. 4, 14, 15). TbL: 6 per side, 13 μm long, only present in the trunk region (U55, U62, U70, U77, U80 and U90). TbVL: 20 per side, 10-11 μm long, arranged in a column from the PhJIn to the lateral edge of the caudal lobe, U45 - U95 (Figs. 4, 19). TbP: 3 per side, grouped on each caudal lobe as 2 terminal tubes, 15 μm long, and one medial tube 9 μm long (Figs. 4, 13, 19). Tubes in TEM sections show duo-gland adhesive organs, *sensu* Tyler and Rieger (1980): two glands presumably produces the adhesive secretion (‘viscid gland cells’) and the other produces a releaser material (‘releasing gland cell’) (Figs. 21-22).

Digestive tract. Mouth terminal and circular, 44 μm wide, surrounded by an area of naked cuticle that forms a rim 8 μm thick (Fig. 9, 14). Pharynx Pharynx 197 μm long and 36 μm wide, cylindrical, with pharyngeal pores at the base (U33) (only observed in the confocal micrographs, Fig. 34). Intestine narrow and tapering toward the posterior body end (49 μm wide at U35, 28 μm wide at U90. Anus ventral at U93.

Reproductive system. The genital system is composed of two testes lateral to the intestine (Fig. 27), starting at the level of PhJIn (U33) and extending posteriorly into two sperm ducts that flow into the glando-muscular caudal organ. The latter is ovoid, 60 μm long, and lies at U80-U90, opening into a ventral pore anterior to the anus (Figs. 3, 28). The internal lumen of the caudal organ was often filled with numerous mature spermatozoa (Figs. 27-29).

An unpaired ovary lies dorsally to the middle intestine, from U70 to U80. Oogenesis proceeds in a caudo-cephalic direction: numerous oocytes at various stages of maturation were observed (Figs. 3, 25). The 'rosette' opening (Figs. 3, 23-24) is well developed (6.5 µm in diameter) and is dorsolateral on the left body side (U40). A compact band of large cells runs from the rosette to a cellular sac, the frontal organ (from U62 to U70), that is in contact with the full-grown oocyte (Figs. 3, 23, 25). Mature spermatozoa are filiform cells up to 150 µm long, composed of a cork-screw-shaped acrosome (30 µm long), a nuclear-mitochondrial region (40 µm long) with a large mitochondrion surrounded by a helical nucleus and a perinuclear helix, and a posterior long flagellum (80 µm long) (Fig. 28). The latter has a 9x2+2 axoneme surrounded by an obliquely striated cylinder. A detailed and complete study of the spermatozoon, spermatogenesis with additional notes on the reproductive system of *A. australis* is in preparation (Guidi et al. in preparation).

Muscular system.

Splanchnic Circular Muscles: Circular muscles are present as individual rings that wrap around the pharynx and intestine (Figs. 30-31). They are evenly spaced along the entire digestive tract, at the anterior end where a set with large number of wide (in diameter) circular muscles forming the oral mouth ring and a compact set o few number of small (in diameter) circular muscles they forming the PhJIn sphincter (Fig. 30). The circular muscles appear to enclose all longitudinal muscles except the thick ventrolateral longitudinal muscles. The helicoidal muscles (see below) are external to the circular muscles.

Splanchnic Longitudinal Muscles: The dorsal longitudinal muscles extend from the oral ring to the caudal end (Fig. 33), splitting in two insertions at U07. There is at least one pair of dorsal medial longitudinal muscles. Two pairs of ventral longitudinal muscles extend along the ventral side of the pharynx and intestine which are wrapped by circular muscles and helical muscles (Figs. 30-32).

Splanchnic Helicoidal Muscles: These are the thinnest muscles of the body. They are located in the pharyngeal region (up to the PhJIn junction) and consist of at least two distinct muscle bands intersecting with each other in a double-helix fashion, appearing to cover circular muscle bands (Fig. 30).

Somatic Longitudinal Muscles: The ventrolateral longitudinal muscles (*musculi principales* - Remane 1936; Kieneke & Nikoukar 2017) are the thickest muscles (8.5 µm

in wide) in the body, and appear to consist of several closely aligned muscle fibers. They extend over the length of the body, from the lateral margin of the mouth rim where they form anterior, bifurcating insertions, to the posterior body end. A set formed by ventrolateral longitudinal (somatic) and ventral longitudinal (splanchnic) muscles appear to supply the caudal lobes.

3.1.7 Taxonomic remarks

The defining morphological features of species of the subfamily Diplodasyinae are the presence of paired testes and a set of a frontal and a caudal organ that are anatomically and functionally disjointed (Ruppert 1978). Species of the genus *Acanthodasys* are characterized by cuticular spines called uniancres, that are hooks with a single point, with a cruciform cross-section, arising from a quadrangular base (Todaro & Hummon 2008; Todaro et al. 2011).

Currently there are twelve valid species described and recognized in the genus *Acanthodasys*. The new species appears to be especially closest to three species of the genus that possess both spined and spineless scales and also have dorsal uniancres longer than ventral ones: *A. arcassonensis*, *A. caribbeanensis* and *A. paurocactus*. However, there are several differences worth noting.

1) *A. arcassonensis* has only one type of spineless scales that can be compared with eye-shaped of *A. australis* sp. nov., but they cover also the ventral body side.

2) *A. caribbeanensis* has not only just one type of spineless scales (eye-shaped scales) but they are also much larger, 15 µm vs 2-3 µm, and show a quite various shape. Also the uniancres of *A. caribbeanensis* are much longer of those of *A. australis* sp. nov., 50 µm vs 8 µm.

3) *A. paurocactus* is the closest species to *A. australis* sp. nov. for qualitative parameters, in particular the presence of both eye-shaped scales and lanceolate scales. However, both types of scales consistently show a central ornament (oval bump or bar-shaped ridge) unlike the lanceolate scales of the new species, always lacking this feature. *A. paurocactus* has uniancres up to 15 µm long *vs* 8 µm in *A. australis*, and its ventral ciliation is arranged into an anterior field and two narrow columns on the trunk whereas *A. australis* sp. nov. shows small ciliary patches limited by lanceolate scales along the whole ventral body surface. In addition, the new species has much larger total length compared to *A. paurocactus* (350-400 µm vs. almost 650 µm).

3.1.8 Phylogenetic analysis

The final alignment of the 18S rDNA yielded 2,997 positions, 28S rDNA 4,359 and combined dataset 7,357. The phylogenetic reconstruction based on a multigene analysis revealed the taxon Thaumastodermatidae as monophyletic and within this taxon, the Diplodasyinae clade appears as a sister-group of the Thaumastodermatinae clade (all three clades emerge as highly supported groups). Within the Diplodasyinae clade, the three included *Acanthodasys* sequences analyzed in the present study nested together in a monophyletic group (with high bootstrap value) as the sister clade of *Diplodasys* (Fig. 35).

4. Discussion

It is well known that achievement of good morphological data for meiofauna taxa can be a challenging task due to their microscopic size and body fragility (Giere 2009; Balsamo et al. 2014; Fonseca et al. 2018; Garraffoni et al. 2019a). Consequently, species description, morphological traits, and understanding the species evolutionary history can be inaccurate in these organisms (Fonseca et al. 2018).

For this reason, taxonomists often need to access to several imaging techniques (e.g., DIC, SEM, TEM and confocal microscopy) to obtain a sufficient number of morphological characters of high reliability that can resolve the relationships of closely-related species. Here, we chose to use a number of microscopical techniques to explore the morphology of the new species. Although all of them provide potentially useful data, the best morphological information still comes from two "traditional" techniques: DIC microscopy and SEM. Gastrotrich taxonomy was founded on bright field microscopy (e.g., Remane 1927), and even if recent studies have shown the utility of other optical techniques (CLSM e.g., Leasi and Todaro 2009; TEM e.g. Guidi et al. 2014), these are mostly useful when comparable data are also available from closely-related species. The same is also true for molecular data, which are still very limited for the species of *Acanthodasys*. The good news is that more integrative investigations are becoming commonplace (e.g., Todaro et al. 2015; Kieneke & Nikoukar 2017; Yamaucki & Kajihara 2018; Garraffoni et al. 2019b), and so the expectation is that our knowledge of gastrotrich taxonomy and evolution will be more precise and begin to move at a faster pace.

As our practices for describing gastrotrichs improves, so does our geographic sampling. As an example, the first observations of marine gastrotrichs in Brazil were done in the last half of the 20th century (du Bois-Raymond Marcus 1952; Forneris 1985), but

since then, new surveys have revealed more diversity along this extensive coastline (e.g., Todaro & Rocha 2004, 2005; Todaro 2012, 2013; Araújo et al. 2014, 2016; Hochberg 2014; Garraffoni et al. 2016, 2017; Campos & Garraffoni 2019; Todaro et al. 2019; Campos et al. 2020). Today, the marine Gastrotricha fauna of Brazil is known to comprise 243 species and 40 that are awaiting to be described (Campos & Garraffoni 2019). Most of them, 16, have broad geographic ranges and can also be found along the coastlines of North America, Europe, and Asia, while the remaining 96 species so far are known only from the Brazilian coast appear to be endemic to the Brazilian coast (*Macrodasys fornerise* Todaro & Rocha, 2004; *Pseudostomella dolichopoda* Todaro, 2012; *Ptychostomella lamelliphora* Todaro, 2013; *Pseudostomella squamalongispinosa* Araújo, Balsamo & Garraffoni, 2014; *Crasiella fonseci* Hochberg, 2014; *Dactylopodola todaroi* Garraffoni, Di Domenico & Hochberg, 2017; *Kryptodasys carlosrochai* Todaro, Dal Zotto, Kånneby & Hochberg, 2019; *Paraturbanella tricaudata* Campos, Todaro & Garraffoni, 2020; the new species of *Acanthodasys* described herein).

Despite our growing efforts to characterize marine gastrotrichs from across the globe, we are still far away from truly approaching the full extent of this phylum diversity and biogeography. Brazil has approximately 7.500 km of coastline, and only a small fraction of this has been explored in detail, at least regarding this phylum. It is clear that new techniques will improve our understanding of this diversity, as will do wider sampling regimes and a new and larger generation of taxonomists.

Finally, the inclusion of the new sequences data of *Acanthodasys australis* sp. nov. in the molecular database provided by Todaro et al. (2011) did not change the overall scenario unraveled by them. The two major lineages within Thaumastodermatidae, as well as most of taxa assigned to Linnean rank of genus were recovered as monophyletic groups., and the genus *Tetranchyroderma* as was again decomposed as a polyphyletic group which confirms the need for its systematics revision.

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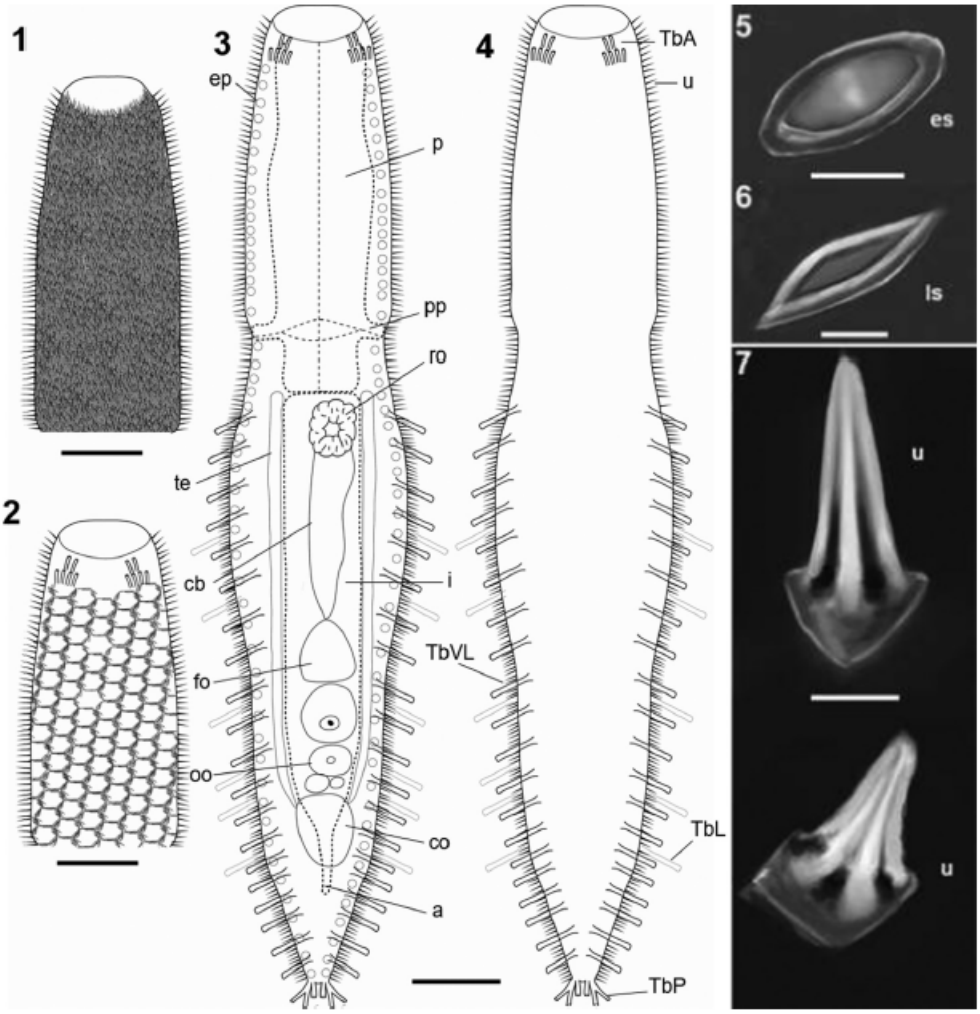

Fig. 1. 1-7. Schematic drawing of *Acanthodasys australis* sp. nov. 1. Dorsal view of the anterior body region showing the covering of uniancres; 2. Ventral view of the anterior body region, showing the honeycomb-like arrangement of lanceolate scales and locomotory ciliation; 3. Optical section showing the digestive, epidermal gland and reproductive systems; 4. Composite sketch of the ventral side showing the adhesive tubes; 5e7. Graphic reconstruction of the spineless and spined scales based on SEM photographs: 5. Eye-shaped scale; 6. Lanceolate scale; 7. Spined scales (uniancre). a - anus, c - cilia, cb - cellular band, co - caudal organ, ep - epidermal glands, es - eye-shaped scales, fo - frontal organ, i - intestine, ls -lanceolate scales, o - opening visible at the base of each ancre, oo - oocytes, p - pharynx, pp - pharyngeal pores, ro - Rosette organ, TbA - anterior adhesive tubes, TbVL – ventrolateral adhesive tubes, TbL - lateral adhesive tubes, TbP - posterior adhesive tubes, te- testes u - uniancres. Scale bar: 1, 2,3, 4: 50 mm; 5, 6, 7: 2 mm.

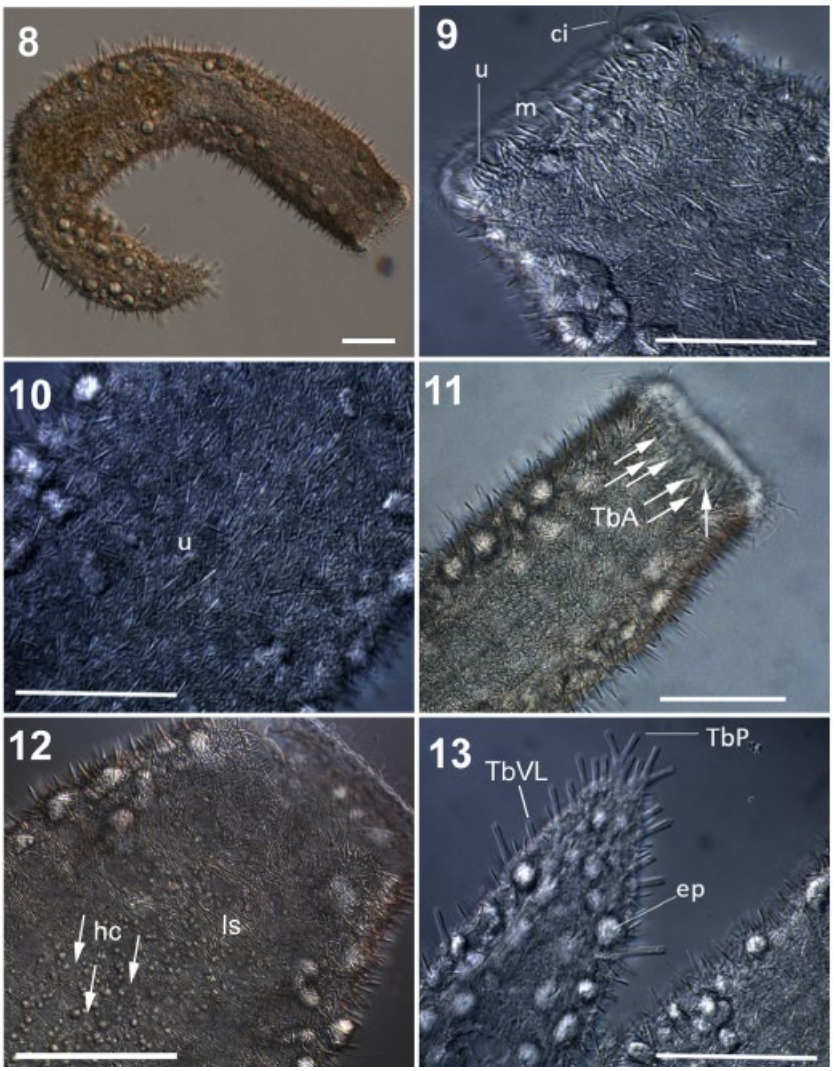

Fig. 2. 8-13. *Acanthodasys australis* sp. nov. DIC-micrographs of the Holotype. 8. Ventral view; 9. Close-up of the dorsal anterior head showing the mouth edge with uniancres and sensory cilia; 10. Detail of the dorsal trunk, showing the uniancres; 11. Close-up of the ventral head with the TbA; 12. Detail of the ventral trunk, showing the honeycomb-like arrangement of the lanceolate scales; 13. Ventral posterior trunk and body end, with TbVL and TbP; epidermal glands are visible. ci - cilia, ep - epidermal gland, ls – lanceolate scales, m - mouth, TbA - anterior adhesive tubes, TbL - lateral adhesive tubes, TbVL - ventrolateral adhesive tubes, u - uniancre. Scale bars: 50 mm.

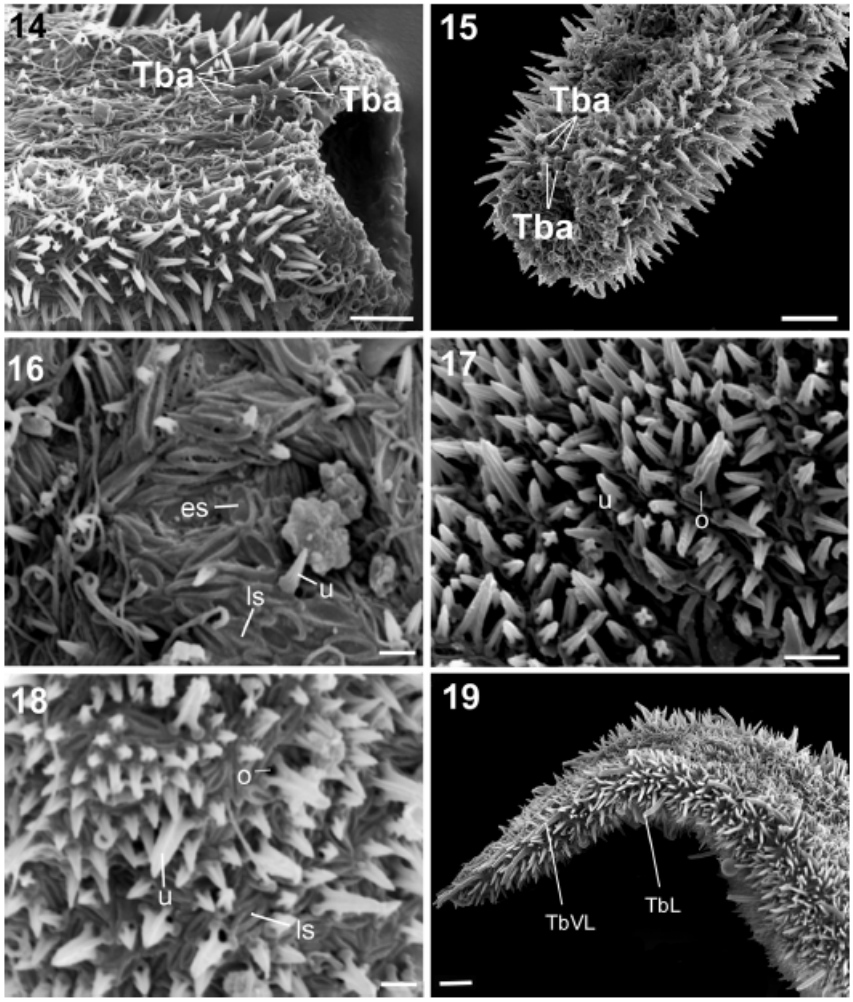

Fig. 3. 14-19. *Acanthodasys australis* sp. nov. SEM photos. 14e15. Closeup of the ventral head: the mouth opening, TbA anterior adhesive tubes, ventrolateral and ventral uniancres and locomotory cilia are visible; 16. Detail of the ventrolateral trunk, showing small uniancres, eye-shaped scales and lanceolate scales; 17e18. Detail of the dorsal trunk with the uniancre covering; 19. Ventrolateral view of the trunk showing the TbL, TbVL, lateral uniancres and the ventral locomotory ciliation. es - eye-shaped scales, ls- lanceolate scales, o -opening visible at the base of each ancre, TbA - anterior adhesive tubes, TbL - lateral adhesive tubes, TbP - posterior adhesive tubes, TbVL - ventrolateral adhesive tubes, u - uniancres. Scale bars: a, b, e: 10 mm; c: 2 mm; d, f: 5 mm.

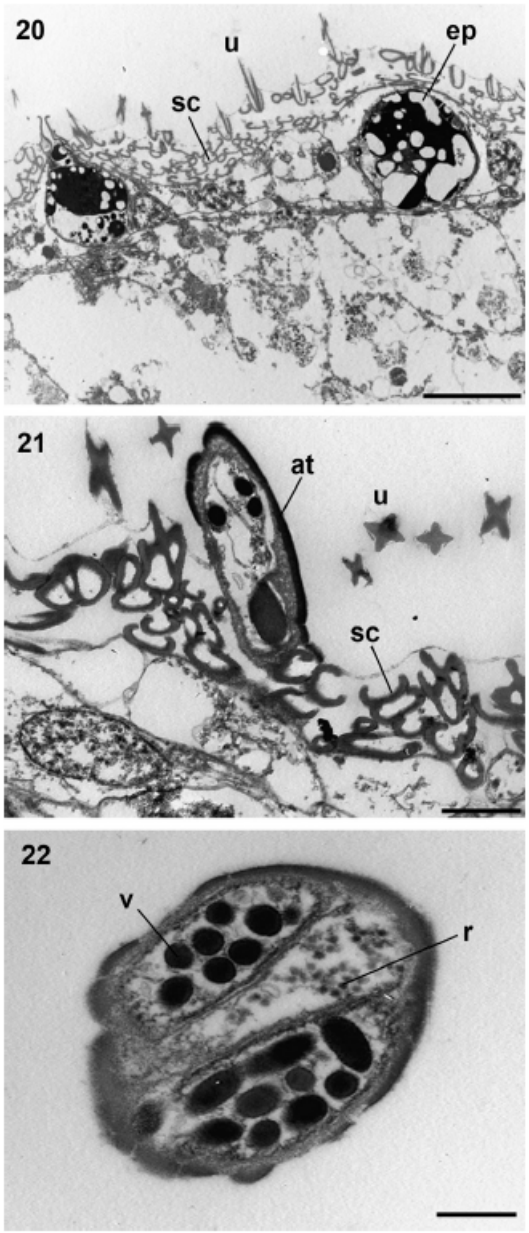

Fig. 4. 20-22. *Acanthodasys australis* sp. nov. TEM photos. 20e21. Longitudinal sections of the body wall, showing the uniancres, the spineless scales, the epidermal glands and an adhesive tube; 22. Transverse section of an adhesive tube showing detail viscid gland cells and detail realizing gland cell. at - adhesive tubes, ep – epidermal gland, r - releasing gland, sc- scales u - uniancres, v - viscid gland,. Scale bars: a: 5 mm, b: 1 mm, c: 0,5 mm.

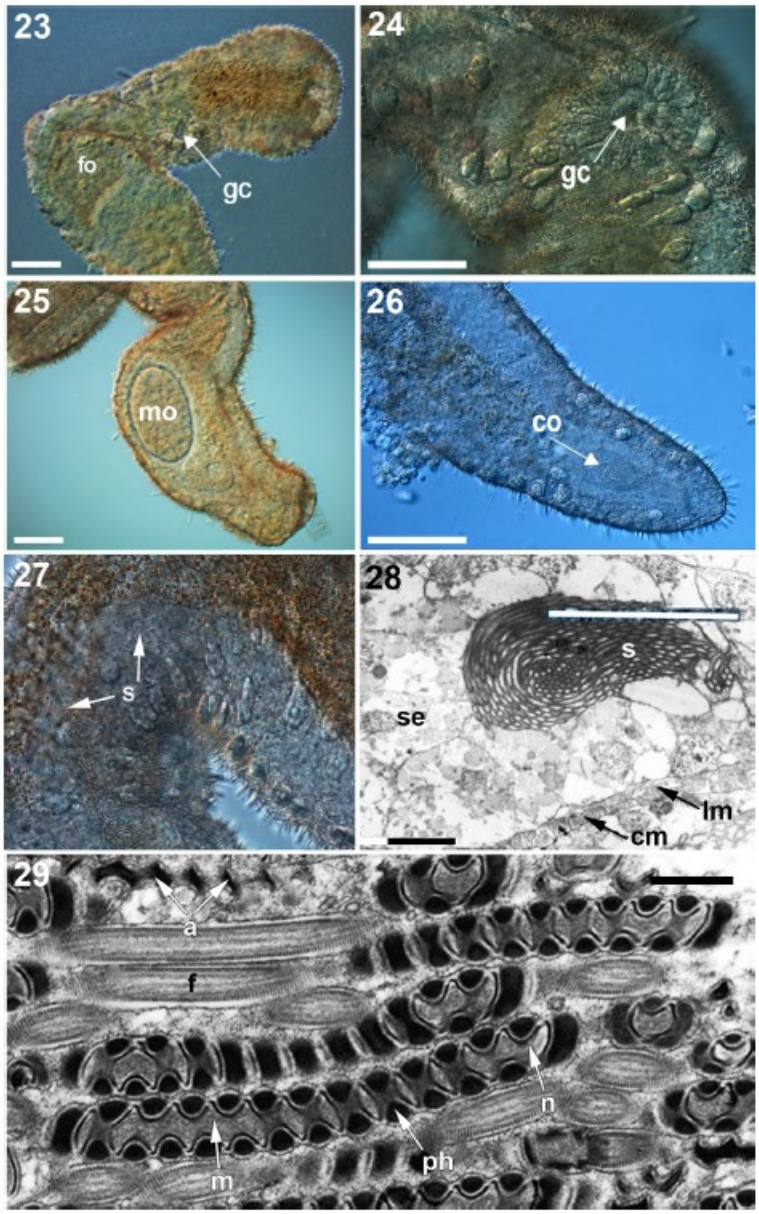

Fig. 5. 23-29. Reproductive system of *Acanthodasys australis* sp. nov. 23e24. Anterior trunk with the ’rosette organ’: the central pore is surrounded by gland cells full of secretory vesicles arranged in a radial pattern; the frontal organ is also visible (DIC); 25. Posterior trunk showing oocytes in different stages of maturation (DIC); 26. Posterior trunk showing the caudal organ (DIC), animal strongly twisted; 27. Dorso-lateral view showing sperms; 28. Section of the caudal organ with secretory cells, circular and longitudinal musculature, and several spermatozoa inside its lumen (TEM); 29. Sections of mature spermatozoa, with a cork-screw-shaped acrosome, a nuclear-mitochondrial region, a perinuclear helix and a flagellum. a - acrosome, cm - circular musculature, co - caudal organ, f - flagellum, fo - frontal organ, gc - gland cells, lm - longitudinal musculature, m - mitochondrion, mo – mature oocyte, n - nucleus, ph - perinuclear helix, s - spermatozoa, se - secretory epithelium. Scale bars: 23, 25: 25 mm, 24, 27: 35 mm, 26: 50 mm, 28: 2 mm, 29: 0.5 mm.

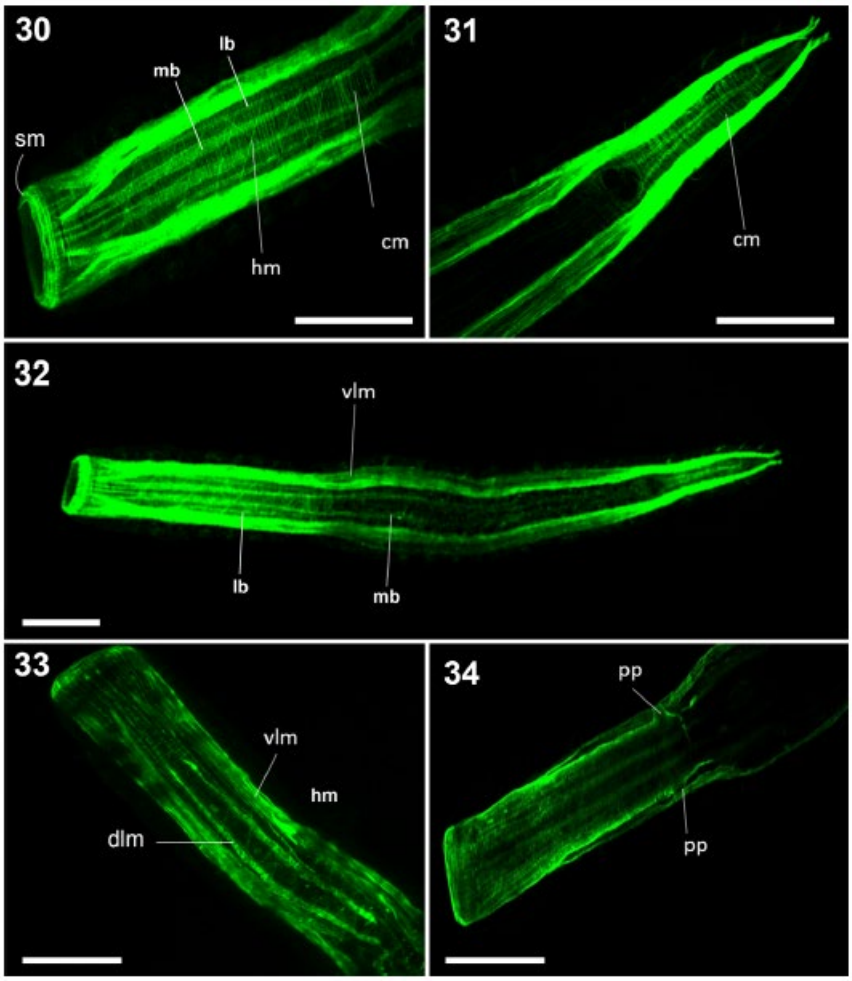

Fig. 6. 30-34. *Acanthodasys australis* sp. nov. CLSM photos. 30. Ventral anterior body region: circular muscles of the mouth, circular muscles and helicoidal muscles along the pharyngeal region; 31. Ventral view of the posterior body region, with circular muscles 32. Ventral view of the whole specimen: ventrolateral longitudinal muscles and medial longitudinal muscles extend over the entire length of the body; 33. Dorsal anterior body region: ventrolateral longitudinal muscles, medial longitudinal muscles and helicoidal muscles; 34. Dorsal anterior body region: pharyngeal pores. cm - circular muscles, dlm - dorsal longitudinal muscles, hm - helicoidal muscles, lb - ventral longitudinal muscle, mb -ventral median muscle, pp - pharyngeal pore, sm - sphincter muscles, vlm - ventrolateral longitudinal muscles. Scale bars: 60 mm.

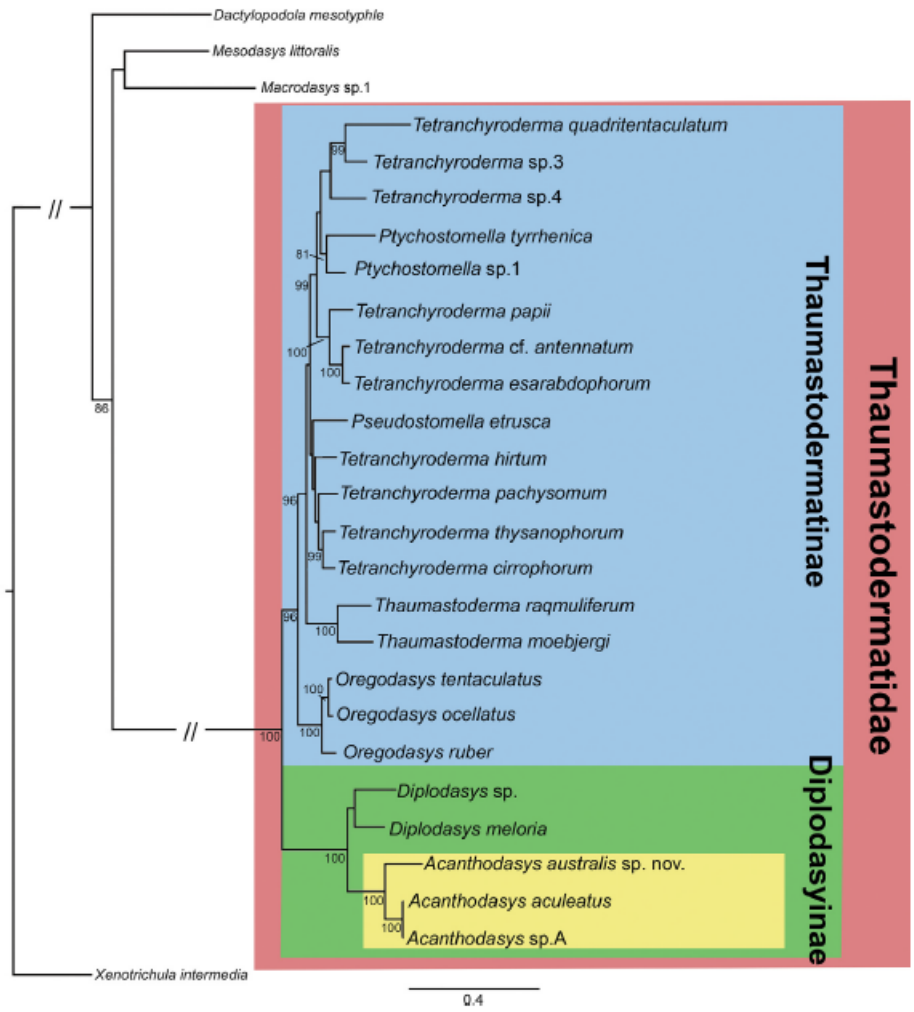

Fig. 7. 35. Phylogenetic relationships of Thaumastodermatidae inferred from maximum likelihood of 18S and 28S rDNA using the GTRGAMMA model for nucleotide substitution. Sequences of *Xenotrichula intermedia* (Chaetonotida, Xenotrichulidae) was chosen in order to root the tree. Family, subfamilies and the genus *Acanthodasys* are indicated. Numbers at nodes represent bootstrap support values. For bootstrap support (1000 replicates) only nodes with ≥70 values are shown.