Zootaxa 5799 (2): 349–368
https://www.mapress.com/zt/
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Article

ISSN 1175-5326 (print edition)
ZOOTAXA
ISSN 1175-5334 (online edition)

https://doi.org/10.11646/zootaxa.5799.2.7
http://zoobank.org/urn:lsid:zoobank.org:pub:777681DA-C255-4543-A597-4F3EED1D1C74

A new *Tetranchyroderma* species (Gastrotricha, Macrodasyida) from Tunisia, with its phylogenetic position based on molecular markers

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Abstract

Data on marine gastrotrichs fauna from the southern coast of the Mediterranean Sea is scanty. To address the gap, a series of faunistic surveys along the northern Tunisian coastline is ongoing. In a shallow sublittoral sample collected at Chat Zwerâa, Béja, a new species of the genus *Tetranchyroderma* was found. Detailed morphological descriptions, along with molecular phylogenetic analysis based on ribosomal genes (18S and 28S) of the new species are provided. The new *Tetranchyroderma* has a pentancrous covering and prominent cephalic pestle organs; the caudal pedicles have three tubes each. The new species is also defined by a number and arrangement of adhesive tubes different from its congeners. Phylogenetic analyses using maximum likelihood (ML) and Bayesian inference (BI) methods, based on concatenated ribosomal gene sequences from 43 taxa, which included 31 thaumastodermatids, confirmed that the genus *Tetranchyroderma* is non-monophyletic. The analysis positioned the new species alongside other species that possess pestle organs, including *Tetranchyroderma antenniphorum, T. esarabdophorum, T. papii* and *Ptychostomella tyrrhenica*.

Key words: biodiversity, integrative taxonomy, Mediterranean Sea, meiofauna

Introduction

Marine gastrotrichs (Phylum Gastrotricha Mečnikow, 1865) recorded from the Mediterranean and the Black Sea represent approximately 38% of the global marine species. In this context, the fauna along the northern Mediterranean coastlines, particularly in Italy and France, are better studied compared to those in the southern regions (Todaro *et al*., 2003). Aside from Egypt, there have been no studies conducted in Morocco and Libya, and data from Algeria is limited (Todaro *et al*., 2003; Leasi & Todaro, 2004; Hummon, 2010). Research is currently ongoing along the

Accepted by T. Araújo: 17 Mar. 2026; published: 28 Apr. 2026

Tunisian coast, where a recent faunistic survey of the northern coastlines identified 24 marine gastrotrich species, including five of the family Thaumastodermatidae Remane, 1927 (Souid *et al*., 2025).

The marine family Thaumastodermatidae consists of two subfamilies, seven genera and 154 accepted species (Saponi & Todaro, 2024; WoRMS, 2025). This makes it the most diverse family within the order Macrodasyida (Remane, 1925a). Despite the considerable morphological diversity among the members of different genera, the family and the two subfamilies are strongly supported as monophyletic based on cuticular structures, reproductive anatomy, and molecular data (Rieger & Rieger, 1977; Ruppert, 1978; Hochberg & Litvaitis, 2001; Kieneke *et al*., 2008; Todaro *et al*., 2011). The genera are also mostly monophyletic, with the possible exception of the highly diverse genus *Tetranchyroderma* (Todaro *et al*., 2011).

The marine genus *Tetranchyroderma* is the second most species-rich group in the phylum Gastrotricha and the most diverse in the order Macrodasyida (Saponi & Todaro, 2024). It currently includes 86 species found all over the world; they thrive especially in shallow sublittoral medium-grained sediments, where often co-occur in clusters of five to six species, and can reach high densities in unpolluted sandy beach zones (e.g., Hummon, 1977, 2011; Todaro *et al*., 2003; Hummon & Todaro, 2009; Nicholas & Todaro, 2006; Araújo & Hochberg, 2017; Lee *et al*., 2017). Species of *Tetranchyroderma* are characterized by the dorsal and lateral cuticular covering made up of three-, four-, or five-pronged hooks called triancres, tetrancres, and pentancres. Although generic identification is straightforward (Todaro *et al*., 2019b), identifying species can be challenging (Todaro, 2002). Additionally, a phylogenetic analysis using molecular data indicated that the *Tetranchyroderma* species studied were separated into two clades, one clustered with *Pseudostomella* and the other with *Ptychostomella* (Todaro *et al.*, 2011).

During our survey of the gastrotrich fauna in Tunisia, we discovered several specimens of *Tetranchyroderma* whose morphometric traits do not match those of the previously described species. Therefore, using an integrative approach that combines morphological and molecular data, we provide the necessary information to formally classify them as a new species. Additionally, to contribute to the ongoing discussion regarding the status of *Tetranchyroderma*, we examined the phylogenetic relationships of this new species within the Thaumastodermatidae family using a dataset based on ribosomal gene sequences.

Material and methods

Sampling, sample processing and morphological analysis

Sandy samples were collected on September 21st, 2024 by skin diving, at 2 m depth from Chatt Zwerâa, Béja (37°01’42”N; 08°54’39”E), in the north of Tunisia (Fig. 1). The collected material was transported to Modena (Italy) the following day and analyzed within one week from sampling. In the laboratory, gastrotrichs were extracted from the sand using the narcotization decantation technique using a 7% solution of $MgCl_2$ (Todaro *et al*., 2019b; Souid *et al*., 2025). Specimens were sorted under a Wild M8 stereomicroscope, mounted alive to a glass slide, and observed under a Nikon Eclipse Ni-U microscope equipped with DIC (differential interference contrast). Micrographs and digital videos were captured with a Nikon F3i camera operated by NIS-Elements F software (v.5.21). After documentation, three specimens were retrieved from the slides, slightly rinsed with distillated water and transferred to absolute ethanol for molecular genetic analysis (see below). The measurements follow the standards given by Hummon *et al*., (1993) where the position of morphological traits along the body is reported in percentage of unit (U) relative to the total body length, from front to rear. Abbreviations used in the text are as follows: PhIJ—pharyngo-intestinal junction; TbA—anterior adhesive tube; TbDL—dorsolateral adhesive tube; TbP—posterior adhesive tube; TbVL—ventrolateral adhesive tube; TL—total body length.

Granulometric analysis

Granulometric analysis of the sandy substrate was carried out using dry sieving as described in Todaro *et al*., (2006; 2019a). The mean grain size, sorting coefficient, kurtosis, and skewness were calculated using a computerized program (Todaro, 1992) based on the equation developed by Seward-Thompson and Hails (1973).

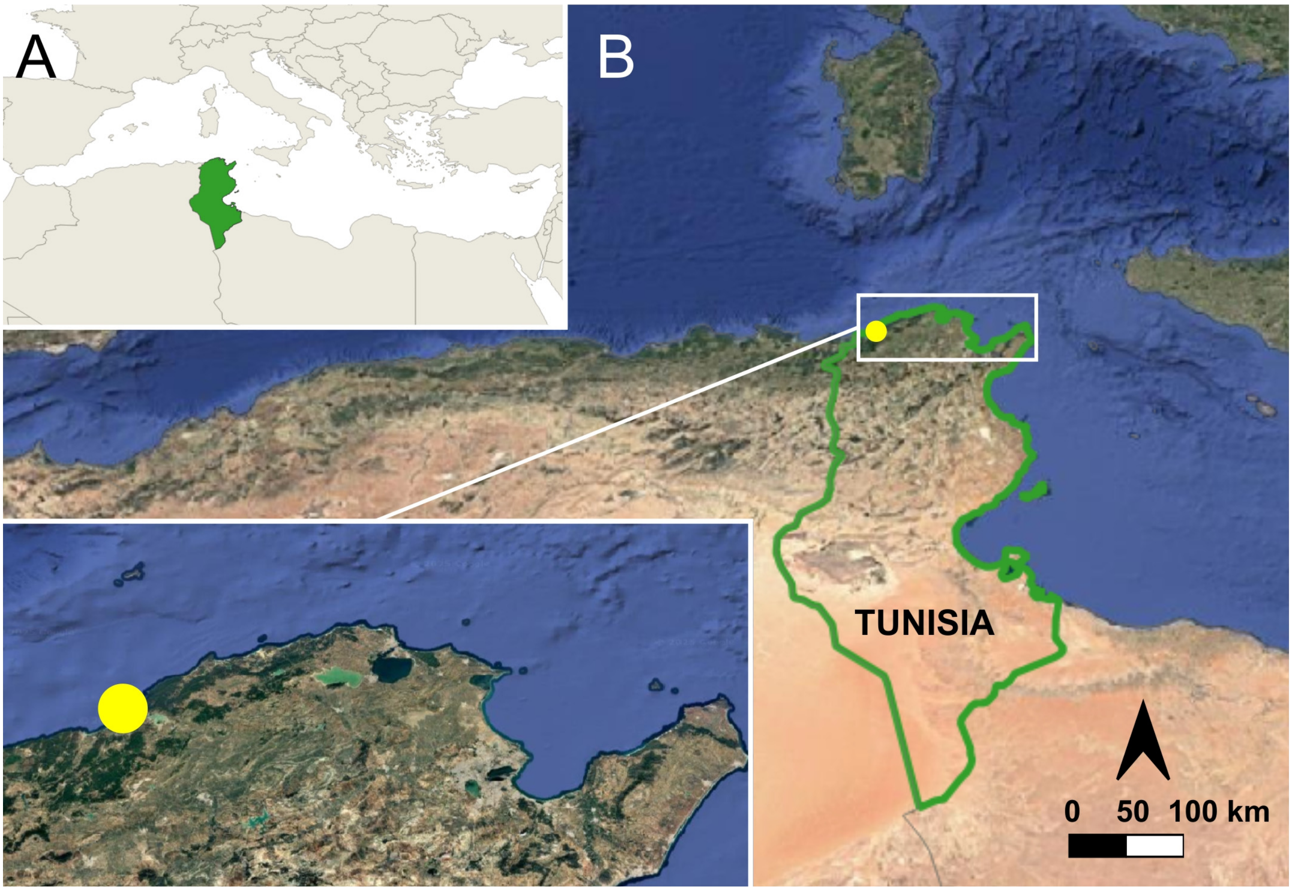

FIGURE 1. Maps of the Tunisian location where the new species was found. **A.** General map displaying the position of Tunisia in the Mediterranean Sea area. **B**. Satellite map of Tunisia with a close-up on the location (yellow dot) sampled in September 2024. See text and Table 1 for further details.

DNA amplification and sequencing

Two ethanol-preserved specimens of the new species were retrieved from the storing vial and washed three times with clean water, Milli-Q water, and then PBS, respectively, and then processed for DNA extraction and whole genome amplification using REPLI-g Single Cell Kit (QIAGEN®), following the manufacturer's instructions. The presence of gastrotrich genetic material in the amplified DNA product was ascertained through a validation step involving PCR (polymerase chain reaction) amplification and Sanger sequencing of the 18S rRNA gene of these animals (Cesaretti *et al*., 2025). The positively amplified DNA of one of the two specimens was then sent to Macrogen Europe (https://www.macrogen-europe.com/) and processed with a TrueSeq DNA PCR Free Library kit and whole genome de novo sequencing at NovaSeq 6000 Illumina Platform to generate a total of 40 million reads (paired-ends 2 × 150 bp).

Genes assembly and molecular characterization

Primary genome assembly of the processed specimens was obtained using our consolidate whole genome amplification pipeline (Cesaretti *et al*., 2024; Gammuto *et al*., 2024; Saponi *et al*., 2024; Kosakyan *et al*., 2026). In brief, the assembled contigs matching ribosomal genes were confirmed with Blastn analysis using available GenBank *Tetranchyroderma* sequences as queries. The reads mapped to these contigs were subsequently extracted and assembled with SPAdes v. 3.13.1 software (Bankevich *et al*., 2012) to obtain the whole ribosomal operon in a single separate contig each. The positions and boundaries of the 18S and 28S genes on the assembled contig were validated and delineated using the StructRNAFinder web tool (Arias-Carrasco *et al.*, 2018). The obtained rDNA, 18S and 28S sequences were used for phylogenetic analysis.

Selection of taxa

To compile the ingroup, we included the sequences of the new species along with all the marine Thaumastodermatidae described species available in GenBank that met the following criteria: i) taxa with 18S sequences shorter than 1500 base pairs and 28S sequences shorter than 1200 base pairs were excluded, and ii) low-quality sequences (those containing numerous N or ambiguous sites) were also discarded. The data retrieved from GenBank included a total of 46 sequences, 44 from 22 species used in the study by Todaro *et al.*, (2011) and 2 sequences from one species figuring in Golombek *et al.*, (2013). To enhance the study, we incorporated new sequences from seven additional thaumastodermatids species, obtained using the same protocol as for the new species. For the outgroup we included sequences from 12 species belonging to families likely to be the sister taxa of Thaumastodermatidae (see Cesaretti *et al.*, 2025). A comprehensive list of the taxa and the accession numbers of the sequences used in the present phylogenetic analysis is presented in Table 1.

TABLE 1. List of species used in this study, as ingroup and outgroup taxa along with sampling sites, coordinates, GenBank accession number and references. NA: not available.

Taxa	Sampling site	Coordinates	18S	28S	References
Ingroup taxa					
Acanthodasys aculeatus	Capraia, Italy	43°00'53"N; 09°49'24"E	JF357639	JF357687	Todaro *et al.*, 2011
Acanthodasys sp	Capraia, Italy	43°00'53"N; 09°49'24"E	JF357638	JF357686	Todaro *et al.*, 2011
Diplodasys meloriae 1	Meloria, Italy	43°33'11"N; 10°13'20"E	JF357632	JF357680	Todaro *et al.*, 2011
Diplodasys meloriae 2	Meloria, Italy	43°33'11"N; 10°13'20"E	JF357640	JF357688	Todaro *et al.*, 2011
Oregodasys ocellatus 1	Sardinia, Italy	41°03'9"N; 8°56'16" E	PX657506	PX657516	Present study
Oregodasys ocellatus 2	Meloria, Italy	43°33'11"N; 10°13'20"E	JF357642	JF357690	Todaro *et al.*, 2011
Oregodasys ruber	Meloria, Italy	43°33'11"N; 10°13'20"E	JF357625	JF357673	Todaro *et al.*, 2011
Oregodasys tentaculatus	Meloria, Italy	43°33'11"N; 10°13'20"E	JF357626	JF357674	Todaro *et al.*, 2011
Pseudostomella etrusca 2	Albinia, Italy	42°29'29"N; 11°11'28"E	JF357633	JF357681	Todaro *et al.*, 2011
Pseudostomella etrusca 1	Sardinia, Italy	39°07'14" N; 09°30'38" E	PX657507	PX657517	Present study
Pseudostomella mandela	Cape Vidal, RSA	28°07'18" S; 32°33'43" E	PX657508	PX657518	Present study
Ptychostomella tyrrhenica	Albinia, Italy	42°29'29"N; 11°11'28"E	JF357634	JF357682	Todaro *et al.*, 2011
Tetranchyroderma anomalopsum	Apulia, Punta Suina, Italy	40°00'09" N; 18°00'51" E	PX657509	PX657519	Present study

......*continued on the next page*

TABLE 1. (Continued)

Taxa	Sampling site	Coordinates	18S	28S	References
Tetranchyroderma antennatum	Kuwait	28°32’59”N; 48°25’18”E	JF357645	JF357693	Todaro *et al*., 2011
Tetranchyroderma cirrophorum	Capraia, Italy	43°00’53”N; 09°49’24”E	JF357635	JF357683	Todaro *et al*., 2011
Tetranchyroderma easrabdophorum	Mahdia, Tunisia	35°30’57”N; 11°03’00”E	JF357627	JF357675	Todaro *et al*., 2011
Tetranchyroderma hirtum	Capraia, Italy	43°00’53”N; 09°49’24”E	JF357628	JF357676	Todaro *et al*., 2011
Tetranchyroderma megastomum	Môvenberg, Danmark	55°01,506’N; 08°26,180’E	KC193100	KC193101	Golombek *et al*., 2013
Tetranchyroderma pachysomum	Meloria, Italy	43°33’11”N; 10°13’20”E	JF357636	JF357684	Todaro *et al*., 2011
Tetranchyroderma papii 1	Sardinia, Italy	40°35’02”N; 08°15’37”E	JF357637	JF357685	Todaro *et al*., 2011
Tetranchyroderma papii 2	Sicily, Italy	37°34’37” N; 12°53’43” E	PX657510	PX657520	Present study
Tetranchyroderma quadritentaculatum 1	Punta Ala, Italy	42°48’42”N; 10°44’46”E	JF357647	JF357695	Todaro *et al*., 2011
Tetranchyroderma quadritentaculatum 2	Albinia, Italy	42°29’22”N; 11°11’27”E	JF357629	JF357677	Todaro *et al*., 2011
Tetranchyroderma tunisiae **sp. nov**.	Béja, Tunisia	37°01’42”N; 08°54’39”E	PX657511	PX657521	Present study
Tetranchyroderma thysanophorum 1	Fautea, Corsica	41°42’40”N; 09°24’17”E	JF357646	JF357694	Todaro *et al*., 2011
Tetranchyroderma thysanophorum 2	Albinia, Italy	42°29’22”N; 11°11’27”E	JF357630	JF357678	Todaro *et al*., 2011
Thaumastoderma moebjergi	Bohuslàn, Sweden	NA	JF357671	JF357713	Todaro *et al*., 2011
Thaumastoderma ramuliferum 1	Torre Civette, Italy	42°00’00”N; 10°46’33”E	PX657512	PX657522	Present study
Thaumastoderma ramuliferum 2	Meloria, Italy	43°33’11”N; 10°13’20”E	JF357631	JF357679	Todaro *et al*., 2011
Thaumastoderma ramuliferum 3	Punta Ala, Italy	42°48’42”N; 10°44’46”E	JF357649	JF357697	Todaro *et al*., 2011
Thaumastoderma cf *mediterraneum*	Formiche Grosseto, Italy	42°34’32” N; 10°52?63” E	PX657513	PX657523	Present study
Outgroup taxa					
Crasiella sp1	Nicoya Peninsula, Costa Rica	09°59’38” N; 85°42’07” W	PX575827	PX575849	Cesaretti *et al*., 2025
Lepidodasys martini	Hallo Island, Sweden	58°20’27” N; 11°12’42” E	PX575825	PX575847	Cesaretti *et al*., 2025
Lepidodasys unicarenatus	Tuscany, Italy	42°34’32” N; 10°52’57” E	PX575826	PX575848	Cesaretti *et al*., 2025

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TABLE 1. (Continued)

Taxa	Sampling site	Coordinates	18S	28S	References
Megadasys sp	Grotta del Ciolo, Italy	39°50’38”N; 18°23’09”E	JF357655	JF357703	Todaro *et al*., 2011
Megadasys sp1	Porto Cesareo, Italy	40°15’33”N; 17°53’53”E	JF357656	JF357704	Todaro *et al*., 2011
Mesodasys laticaudatus	Albinia, Italy	42°29’29”N; 11°11’28”E	JF357657	JF357705	Todaro *et al*., 2011
Mesodasys littoralis	Bou Ficha, Tunisia	36°16’50”N; 10°29’41”E	JF357658	JF357706	Todaro *et al*., 2011
Paraturbanella dohrni	Punta Ala, Italy	42°48’42”N; 10°44’46”E	JF357659	JF357707	Todaro *et al*., 2011
Paraturbanella pallida	Capraia, Italy	43°00’53”N; 09°49’24”E	JF357660	JF357708	Todaro *et al*., 2011
Paraturbanella teissieri	Punta Ala, Italy	42°48’42”N; 10°44’46”E	JF357661	JF357709	Todaro *et al*., 2011
Turbanella bocqueti	Tramore, Ireland	52°09’24”N; 07°08’12”W	JF357662	JF357710	Todaro *et al*., 2011
Turbanella cornuta	Chioggia, Italy	45°12’57”N; 12°17’57”E	JF357663	JF357711	Todaro *et al*., 2011

Phylogenetic analysis

Alignments

Each gene was aligned separately. The 18S and 28S rDNA genes were aligned with MUSCLE algorithm embedded in the MEGA X software (Kumar *et al*., 2018). Individual alignments were trimmed at the length of the majority sequences, resulting in 1752 and 4080 nucleotides for 18S and 28S genes, respectively. Next, the two alignments were concatenated, using MEGA X software, into a single final matrix, resulting in 5832 sites (see supplementary material file S1).

Tree construction

The Maximum likelihood (ML) and Bayesian inference (BI) algorithms were used to build the phylogenetic trees. The ML analysis was performed in IQ-TREE v.3.0.1 (Nguyen *et al*., 2015), with the following settings and considerations: i) the best substitution model obtained with the MEGA X dedicated tool for two partitions as such: GTR+G+I for both 18S and 28S; ii) edge-unlinked partition option, iii) 1000 standard bootstrap replicates.

Bayesian analyses were performed in the program MrBayes v.3.2.7 (Ronquist *et al*., 2012) with the following settings and considerations: i) setting up all the parameters of evolutionary models as estimated with MEGA (see above), ii) edge-unlinked partition option was considered, iii) 6.000.000 Markov chain Monte Carlo (MCMC) simulations, with a sampling frequency of trees and parameters at 100, and with a relative burn-in fraction of 25%. Convergence of the MCMC analyses was confirmed with the in-built diagnostics of the program with the average standard deviation of split frequencies was 0.002320, the potential scale reduction factor converged to 1.00 for all parameters, the effective sample sizes (ESS) of all parameters were >200 (i.e., min. ESS = 6544.15, av. ESS = 6568.75). ML and BI trees were computed as unrooted and then were rooted in FigTree v.1.4.3 (http://tree.bio.ed.ac.uk/software/figtree/), using the clade of non-Thaumastodermatidae species (*Crasiella* sp1; *Mesodasys littoralis* Remane, 1951; *Mesodasys laticaudatus* Remane, 1951; *Paraturbanella dohrni* Remane, 1927; *Paraturbanella teissieri* Swedmark, 1954; *Paraturbanella pallida* Luporini, Magagnini & Tongiorgi, 1973; *Turbanella bocqueti*

Kaplan, 1958 sensu Boaden, 1974; *Turbanella cornuta* Remane, 1925b; *Megadasys* sp; *Megadasys* sp1; *Lepidodasys martini* Remane, 1926 and *Lepidodasys unicarenatus* Balsamo, Fregni & Tongiorgi, 1994) as outgroup. The final tree was edited using CorelDraw X7 (Corel Corporation, Ottawa, Canada).

Results

Taxonomic account

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

Family Thaumastodermatidae Remane, 1927

Subfamily Thaumastodermatinae Remane, 1927

Genus *Tetranchyroderma* Remane, 1926

Tetranchyroderma tunisiae sp. nov.

urn:lsid:zoobank.org:act:3F96282B-6D87-4064-A457-11623009C149
(Figs. 2–5)

Holotype. An adult specimen with sperm and a mature egg inside (Figs. 3,4), collected by AS on September 21st, 2024. The holotype is no longer extant (International Code of Zoological Nomenclature 19, Articles 73.1.1 and 73.1.4; also see the recommendation 73 G–J of Declaration 45 Addition of Recommendations to Article 73 (ICZN, 1999, 2017).

Additional examined specimens. Six specimens, five of which showing a maturing egg and /or spermatozoa inside. One specimen lacked sperm and egg altogether and therefore was considered subadult. Three specimens were destroyed during observation; three were retrieved from the slides and stored in alcohol for DNA analysis (see above).

Type locality. Chatt Zwerâa, Béja (Tunisia; Latitude 37°01’42”N; Longitude 08°54’39”E); sandy samples collected at a depth of 2 m. Granulometric analysis classified the substrate as medium to coarse sand, with mean grain size of 1.106 (phi), moderately well sorted, exhibiting a 2.41 Kurtosis and a negative skewness (–0.37).

Etymology. The name *tunisiae* refers to Tunisia, the country where the species was first found.

Diagnosis

A *Tetranchyroderma* with a TL of 372 µm. Pharynx length 80.7 µm with pharyngeal pores at U35.3; PhIJ at U39.7. A unilobed head with a pair of pestle organs at U10, a wide oral opening covered by an oral hood projected forward. A symmetric body margins and a caudal region occupied by a pair of pedicles measuring 20 µm in length. Sensory cilia inserted around the oral opening and scattered along body sides. Epidermal glands are irregular in shape and size, about 7–8 glands per side. Cuticular covering of the dorsal and lateral margins composed of pentancres with equal tines. Ancres are smaller in size at both ends. Adhesive apparatus composed of TbA, 5 per side, inserted around the mouth ventral border at about U15, with one median and four lateral; TbVL, up to 11 per side, one in the anterior pharyngeal region at U18, one inserted slightly after the PhIJ at U50, six evenly spaced from U60 to U81, and up to three tubes forming a row at U88; TbP, 6 per side, two emerging from each pedicle, one tube located medially at the base of each pedicle; TbDL, one per side, in the rear region at U96. Locomotor cilia form an uninterrupted field of transverse rows covering the entire ventral area. Reproductive system, hermaphroditic; testis on right side; vas deferens appears to open into the rear of the caudal organ, in front of the anus; eggs develop back to front, large egg above the mid-gut; caudal organ pear-shaped, thin-walled; frontal organ vesicular, filled with a round mass of spermatozoa.

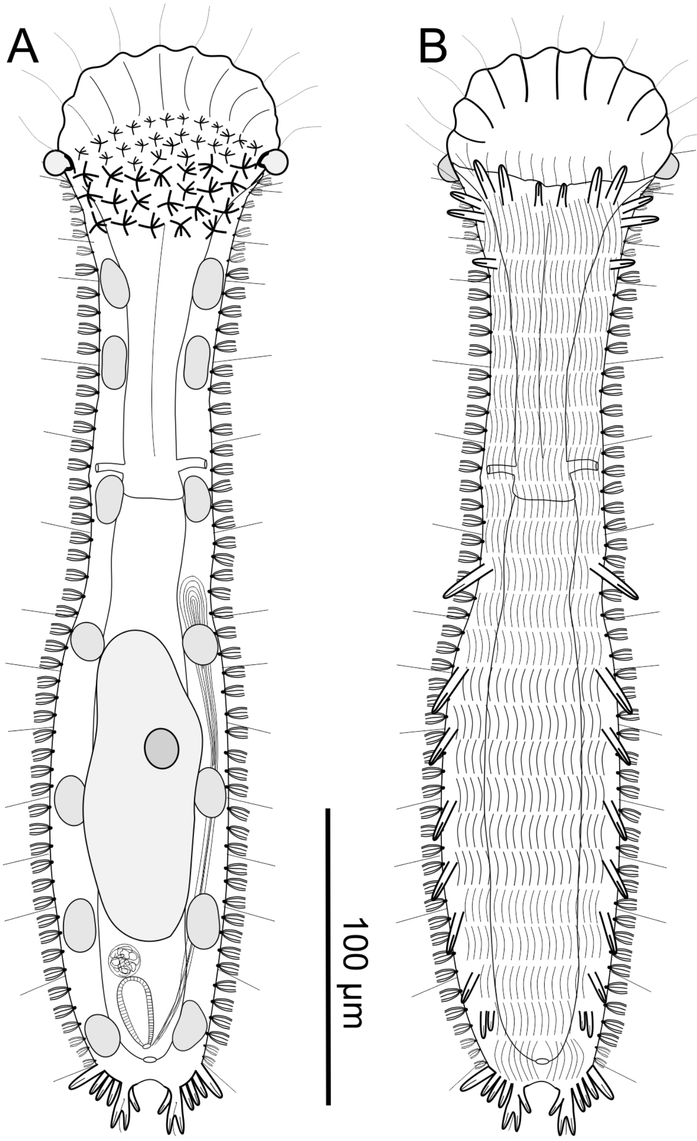

FIGURE 2. Schematic drawings of *Tetranchyroderma tunisiae* **sp. nov. A.** Habitus, dorsal view, showing the head covered with pentancres, the pestle organs and the internal organs as seen from above. The dorsal sides and the lateral margins are covered with pentancres, although not all are represented here for clarity. **B.** Habitus, ventral view, showing the adhesive apparatus and the ventral ciliation.

Description

The description is mainly based on the adult holotypic specimen, measuring 378 µm in TL (Figs. 3–5). The body is rather oblong, ventrally flattened, dorsally vaulted, with slightly inflated trunk but narrowing toward the bilobed caudum. The head end is slightly undulating, with perceptible, round sensorial knobs at U10; the caudal pedicles are naked, measuring 20 µm, indenting medially to U98. Widths pestle organs/ behind the mouth rim /PhIJ/ mid-trunk and caudal base with locations along the length of the body are as follows: 71 µm / 54 µm / 48 µm / 58 µm and 27 µm at U10/ U17/ U42 /U80 and U96, respectively. The epidermal glands, distributed in two columns along the sides, with 7 to 8 located on each side, exhibit a distinctive round or nearly elliptic shape, measuring 3 to 5 µm in diameter or length.

Cuticular armature. the dorsal surface and the lateral margin of the body are covered with pentancres, extending from U06 at the oral hood, where forming an arc, onto the caudal pedicles. Smaller in the head and posterior region than in the rest of the body. Each anchor is equipped with five tines of similar length (about 6.5 µm in the trunk region and 2 µm on head and posterior region) (Figs. 2A, 3B, D, 5B).

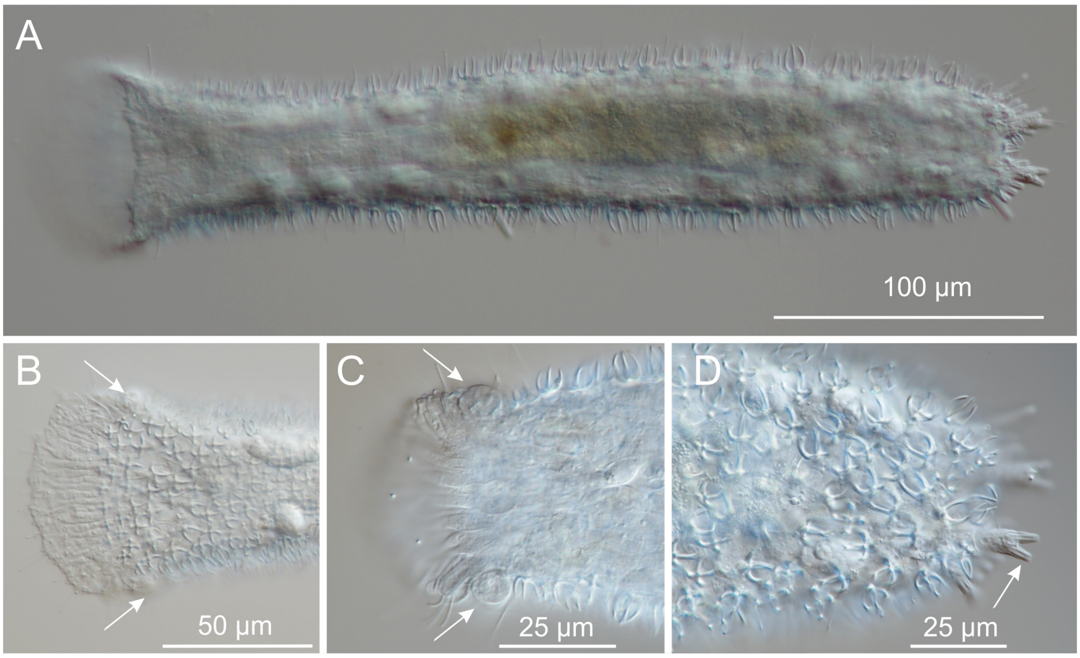

FIGURE 3. Photomicrographs of *Tetranchyroderma tunisiae* **sp. nov**. holotype. **A.** Habitus. **B.** Close-up of the anterior region, dorsal view**,** showing the cuticular covering and position of the pestle organs (arrows). **C.** Close-up of the anterior region at a different focal plane showing the round pestle organ (arrows). **D**. Close-up of the posterior region, dorsal view showing the cuticular covering and the naked pedicles (arrow). Differential interference contrast microscopy (Nomarski).

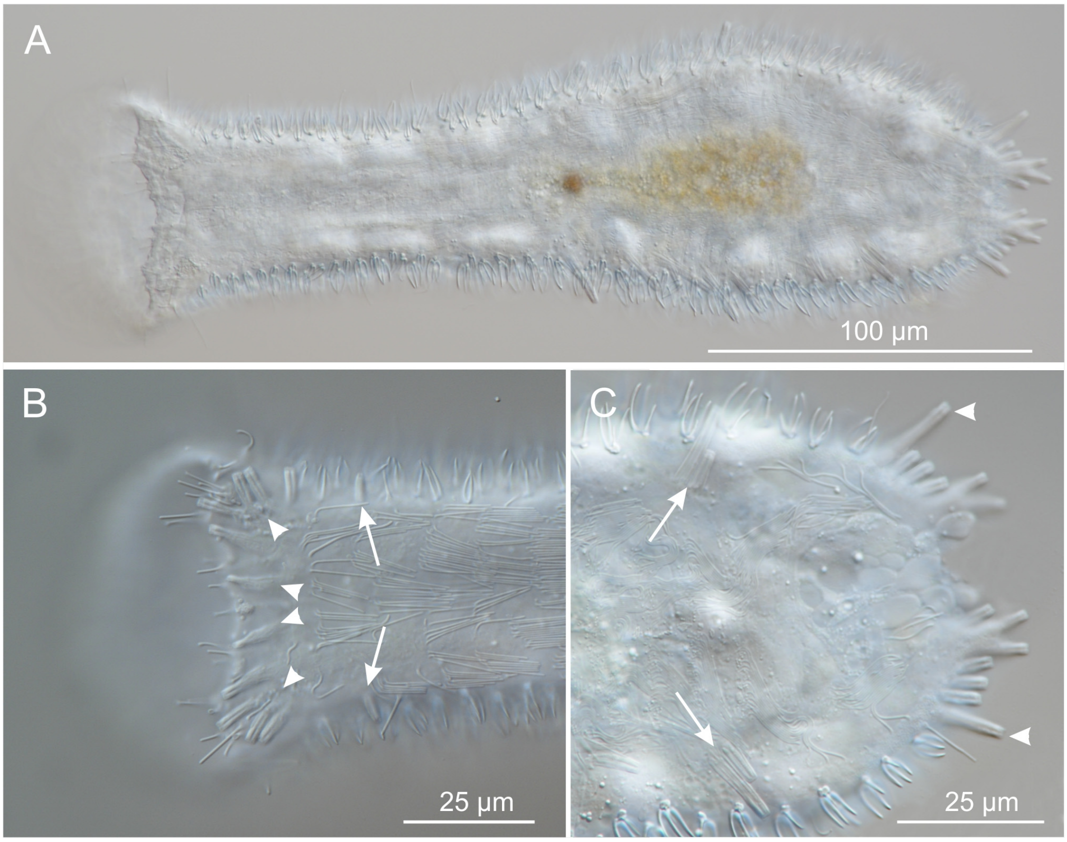

FIGURE 4. Photomicrographs of *Tetranchyroderma tunisiae* **sp. nov.** holotype. **A.** Habitus, ventral view. **B.** Close-up of the anterior region, ventral view, showing the anterior adhesive tubes (arrowheads) and the anteriormost ventrolateral adhesive tubes (arrows). **C.** Close-up of the posterior region, ventral view, showing the caudal pedicles and the arrangement of the posterior adhesive tubes; moreover, the posteriormost ventrolateral adhesive tubes arranged in rows (arrows) and the dorsolateral adhesive tubes (arrowheads) are visible. Differential interference contrast microscopy (Nomarski).

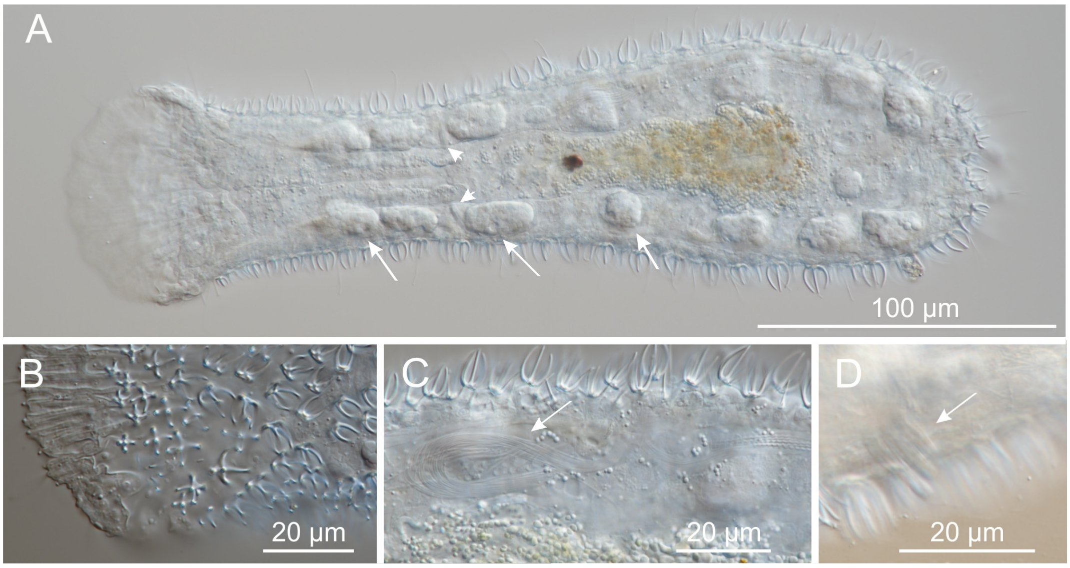

FIGURE 5. Photomicrographs of *Tetranchyroderma tunisiae* **sp. nov. A.** Habitus of the holotype, dorsal view, showing the distribution of the epidermal glands (arrows) and the position of the pharyngeal pores (arrowhead). **B-D.** Another adult specimen. **B.** Close up of the anterior region, dorsal view**,** showing the cuticular covering made up of pentancres. **C.** Close-up of the trunk region, internal view, showing anterior portion of the testis (arrows). **D**. Close-up of the posterior region, ventral view, showing the posteriormost ventrolateral adhesive tubes, in this specimen arranged in a row of three tubes (arrow). Differential interference contrast microscopy (Nomarski).

Adhesive apparatus. TbA, 5 per side, inserted around the ventral mouth rim, at U15, with one median shorter (about 6 µm in length) and four positioned more laterally, longer (8 µm to 10 µm) (Figs. 2A, 4B). TbVL, 10 tubes per side, arranged in a longitudinal column. One tube, measuring 6 µm in length, is located in the anterior pharyngeal region at U18 (Figs. 2B, 4B); a second tube, 17 µm long, is inserted just after the PhIJ at U50 (Figs. 2B, 6B); six tubes (15 µm to 18 µm in length) are evenly spaced from U60 to U81 (Figs. 2B, 6B), and two tubes forming a row are at U88; these tubes are of different length; the lateral tube is longer, measuring 13 µm, while the inner tube is shorter measuring 9 µm in length (Figs. 2B, 4C, 6B). TbP, 6 per side, two emerging distally from each pedicle (4–6 µm in length), one tube (5 µm in length) located medially at the base of each pedicle, forming the typical arrangement of the family "two fingers and a thumb" and three tubes (7–10 µm in length) located outside each pedicle (Figs. 2, 3A, D, 4A, C). TbDL, 1 tube per side (16 µm in length), in the rear body region, emerging just anterior to the posterior tubes at U96 (Figs. 2, 4C).

Ciliation. Short sensory cilia are found around the ventral oral opening, measuring approximately 4–6 µm in length. There are additional cilia on the oral hood, ranging from 10 to 12 µm long, and numerous cilia are present laterally, measuring between 12 and 16 µm. Additionally, other cilia, similar in length to the lateral ones, are regularly distributed on the dorsolateral surfaces (Figs 2, 4B). The ventral locomotor cilia are arranged in transverse rows that span the whole ventral surface from behind the TbA, at U15, to the base of the caudal pedicles, at U96 (Figs 2B, 4B, 6B). Individual cilia length ranges from 10 µm to 12 µm.

Digestive tract. A wide oral opening (width 63 µm × length 45 µm), covered anteriorly by an oral hood extending from U00 to U12 (Figs, 2A, 3B). An 87.7 µm long pharynx featuring a pair of pharyngeal pores at U35 (Figs. 2A, 5A, 6A). The PhIJ is at U40 (Figs. 2A, 6A). The intestine is 174 µm in length. The anus opens at U83 (Figs. 2A, 6B).

Reproductive system. Simultaneous hermaphrodite; a single, elongate testis is positioned on the anatomical right side, starting well behind the PhIJ at U48 (Figs. 2A, 5C); vas deferens joining the caudal organ, in front of the anus (Figs. 2A, 6C, E); eggs develop back to front, large egg above the mid-gut measuring 90 µm × 26 µm (Figs. 3A, 6C); caudal organ at U82, pear-shaped, 24 µm in length, showing a slightly muscular wall; frontal organ at U79, vesicular, 12 µm in diameter, filled with a round mass of spermatozoa (Figs. 2A, 6C, D).

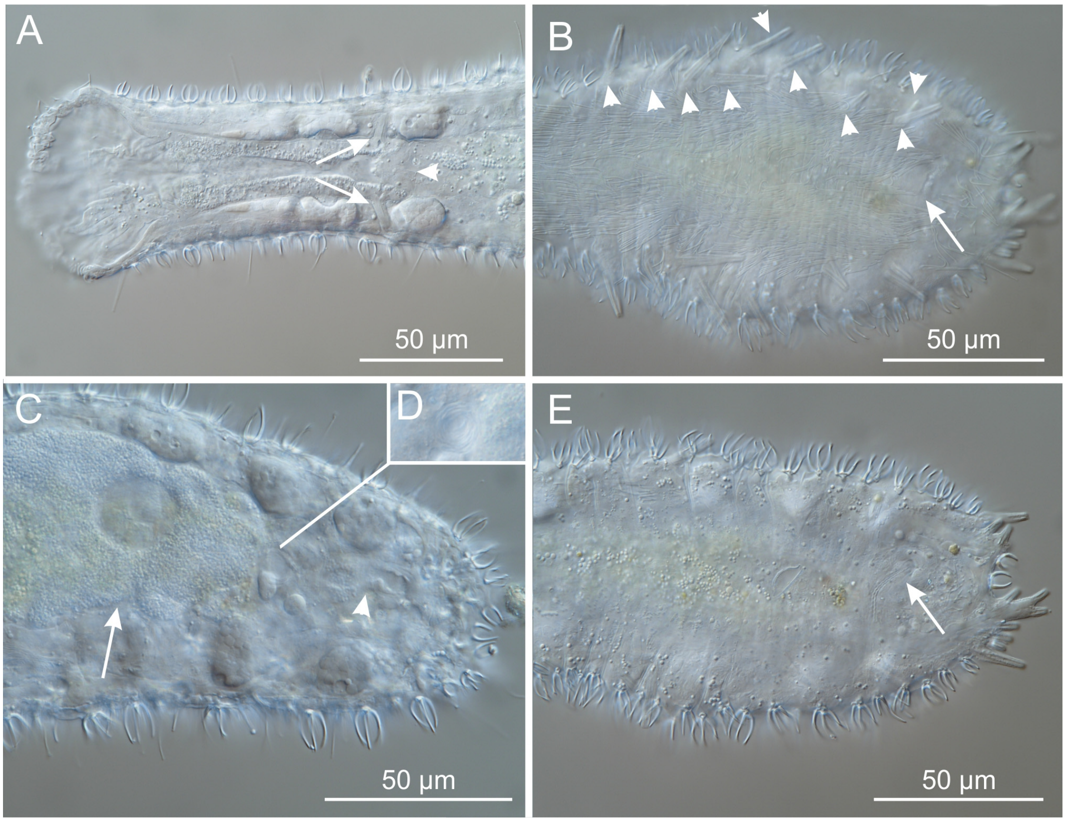

FIGURE 6. Photomicrographs of *Tetranchyroderma tunisiae* **sp. nov.** another full adult specimen, partially contracted. **A**. Anterior, and pharyngeal region, internal view, showing the position of the pharyngo-intestinal junction (arrowhead) and the pharyngeal pores (arrows) **B**. Trunk region, ventral view, showing the anal opening (arrow), and the adhesive apparatus; curiously, the ventrolateral tubes under the left side number 9 (arrowheads) while under the right-side number just 8. **C**. Posterior trunk region, internal view, showing the mature egg (arrow), the caudal organ (arrowhead) and the position of the frontal organ. **D**. The frontal organ at a slightly different focal plane. **E**. Trunk region, internal view, showing the confluence of the vas deferens to the caudal organ (arrow). Differential interference contrast microscopy (Nomarski).

Variability and remarks

The general appearance and the morphological characteristics of the additional studied specimens are very similar to the holotype (Table 2). The differences in key traits among the adult specimens examined are negligible, specifically regarding: i) total body length, which varied from 366.0 µm to 378.0 µm; ii) pharynx length, which ranged from 80 µm to 88.7 µm; and iii) pedicle length, which spanned from 17.5 µm to 21 µm. The number and arrangement of the adhesive tubes also show similar patterns, with only two specimens exhibiting 11 TbVL instead of 10, as they possess three posterior-most tubes arranged in a row rather than two. During our observations, we noted instances of asymmetry in the number of adhesive tubes on the left and right sides. Specifically, in one specimen, the asymmetry was recorded as TbA, while in another, it was noted as TbVL (Table 2).

In the 346 µm-long subadult, the adhesive apparatus was very similar to that of the adults, except for fewer TbP (5 vs 6 per side); specifically, the subadult has two tubes instead of three outside the pedicles (Table 2). The measured subadult specimen indicates that in this species, the reproductive apparatus begins to form in individuals larger than 346 µm in total length.

TABLE 2. *Tetranchyroderma tunisiae* **sp. nov.** Main taxonomic characters and measurements (in µm) of the examined specimens. Specimen 1 is the holotype. Some data for specimens 3–6 were not taken (NA) because the animals became contracted during the observation.

Trait	Adult							subadult
	1	2	3	4	5	6	Mean ± SD	6
Total body length	378	372	366	NA	NA	NA	372	346
Pharynx length	87.7	80	nv	88.7	82	80	80.7	NA
Pedicles length	20	19	nv	21	17.3	20	20	16.5
Oral hood	U12	U11	U11	NA	U11	U14	U11.8	U11
Pharyngo-intestinal junction	U40	U39	U40	NA	NA	NA	U39.7	U42
Pharyngeal pores	U35	U36	U35	NA	NA	NA	U35.3	U37
Width at pestle organs	71	78	79	76	63	76	73.8	76
Width behind mouth rim	54	56	NA	61	51	60	56.4	58
Width at PhIJ	48	48	NA	59	47	52	50.8	47
Width at mid-trunk	58	68	NA	NA	NA	NA	63	46
Width at caudal base	27	30	NA	27	26	28	27.6	25
TbA per side	5	5	5	5	5–6*	5	5	5
TbVL per side	10	10	10–11*	11**	11**	10	10.3	10
TbDL per side	1	1	1	1	1	1	1	1
TbP per side	6	6	6	6	6	6	6	5
Testis starts at	U48	U47	U44	U48	-	-	U46.3	-
Egg	90×26	-	102×60	75×50	-	-	89×45	-

**In this specimen the number of tubes on the right and left side was different.*

*** this specimen displayed the terminal most TbVL arranged in rows of three tubes each.*

Taxonomic affinities

The specimens from Chatt Zwerâa (Tunisia) bear among others, pentancres and a pair of pestle organs. Among the 46 pentancrous *Tetranchyroderma* species described so far, six are known to have also pestle organs, these are: *T. megabitubulatum* Lee & Chang, 2012; *T. monokerosum* Lee & Chang, 2007 and *T. multicirratum* Lee & Chang, 2007, from Korea, and *T. antenniphorum* Hummon & Todaro, 2010; *T. esarabdophorum* Tongiorgi & Balsamo, 1984; and *T. sardum* Todaro, Balsamo & Tongiorgi, 1988, known to occur in the Mediterranean Sea.

The Korean species can be easily distinguished from our species due to several distinctive features. For instance, *T. megabitubulatum* has an additional pair of elongated TbD in the mid-trunk region (Lee & Chang, 2012); *T. multicirratum* possesses even more TbD on the trunk, about ten pairs, that are also notably long; *T. monokerosum* has an extra pair of TbD in the mid-trunk region, along with a unique, long rod-like cephalic tentacle located in a mid-dorsal position on the head (Lee & Chang, 2007).

The specimens from Tunisia generally resemble the three species identified in the Mediterranean Sea. However, *T. antenniphorum***,** *T. esarabdophorum*, and *T. sardum* can be easily distinguished from our species by several key characteristics: i) they possess a pair of rod-like cephalic tentacles, which are absent in our species; ii) they show a lower number of TbA, four versus five tubes per side; iii) they exhibit a higher number of TbVL, up to 22 compared to 10–11 tubes; and iv) they bear fewer TbP, with four instead of six tubes (Luporini *et al*., 1973; Tongiorgi & Balsamo, 1984; Todaro *et al*., 1988). Therefore, it is appropriate to classify the specimens from Tunisia as a new species, for which we propose the name *Tetranchyroderma tunisiae* **sp. nov**.

Molecular phylogeny

The overall branching patterns of the ML and BI trees resulting from the phylogenetic analyses of concatenated ribosomal gene sequences are highly consistent with each other (Figs. 7, 8). In both trees, the terminals of the

Thaumastodermatidae family are organized into two fully supported clades that correspond to the currently recognized subfamilies: Diplodasyinae Ruppert, 1978 and Thaumastodermatinae Remane, 1927. The Diplodasyinae subfamily includes the genera *Diplodasys* and *Acanthodasys*, while the Thaumastodermatinae includes the remaining genera.

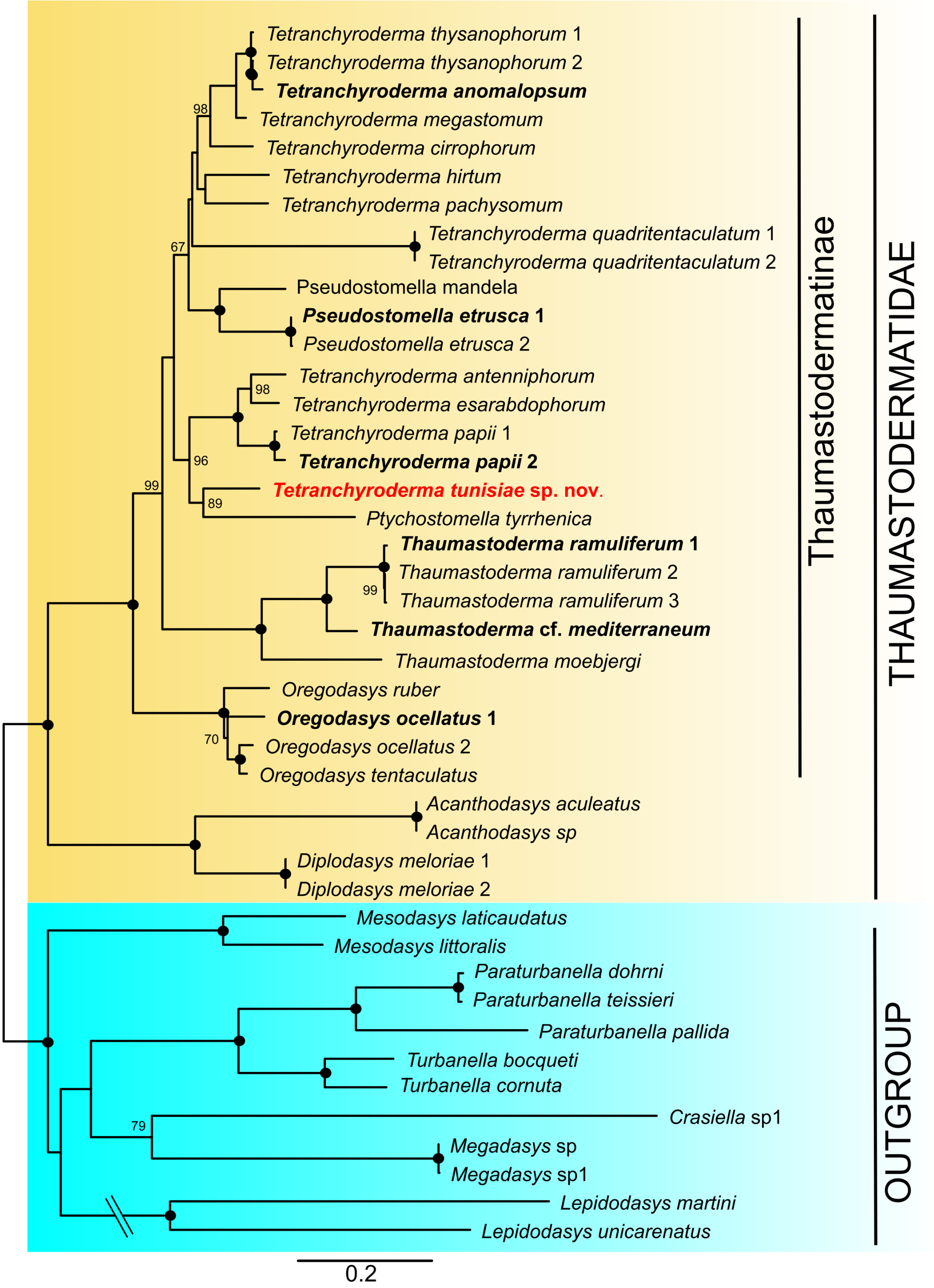

FIGURE 7. Phylogenetic relationships of 43 macrodasyidans inferred from maximum likelihood (ML) based on 18S rDNA and 28S rDNA concatenated alignment. From the 43 terminals, 31 belongs to the family Thaumastodermatidae and 12 are used as the outgroup. In bold, taxa sequenced in the current study, with the new species in red color. Numbers at nodes represent bootstrap support (1000 replicates). A black dot at the node indicates full bootstrap support for the branch. Bootstrap values <65 are not reported. The scale bar indicates the number of nucleotide substitutions per site.

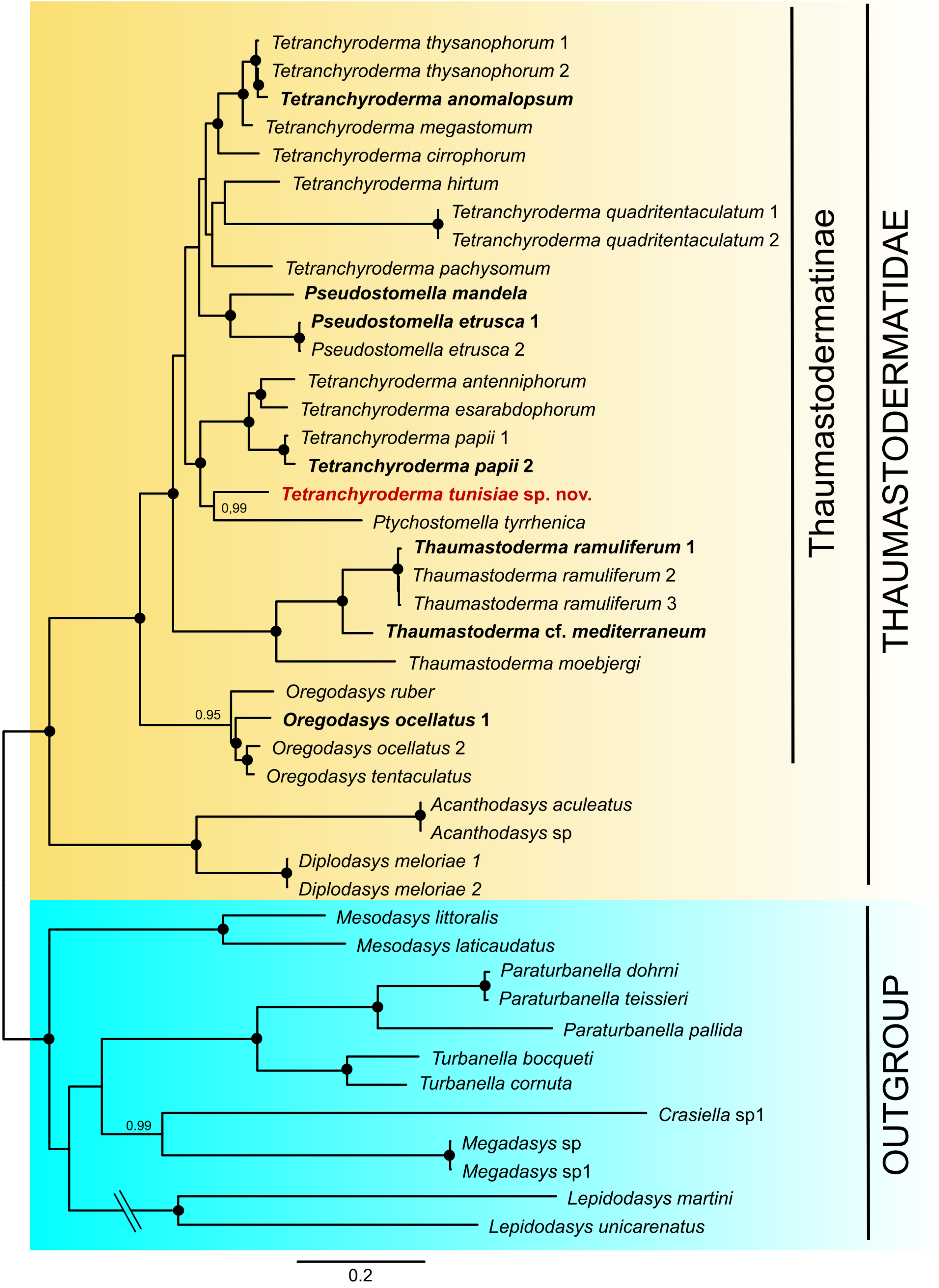

FIGURE 8. Phylogenetic relationships of 43 macrodasyidans inferred from Bayesian inference (BI) based on 18S rDNA and 28S rDNA concatenated alignment. From the 43 terminals, 31 belongs to the family Thaumastodermatidae and 12 are used as the outgroup. In bold, taxa sequenced in the current study, with the new species in red color. Numbers at nodes represent posterior probability support. A black dot at the node indicates full posterior probability support for the branch. Posterior probability values <0.95 are not reported. The scale bar indicates the number of nucleotide substitutions per site.

Within the Thaumastodermatinae, both trees recover *Oregodasys* species as a fully supported clade, which is inferred as the sister group to the remaining Thaumastodermatinae. Among these, *Thaumastoderma* species form a monophyletic, early-branching, cluster. In both clades, the species sequenced in this study, namely *O. ocellatus* 1, *Th. cf mediterraneum*, and *Th. ramuliferum* 1 are covered (Figs. 7, 8), indicating the reliability of the sequences obtained.

The evolutionary scenario for the Thaumastodermatinae at deep nodes revealed in this study compellingly supports the conclusions drawn by Todaro *et al.* (2011), despite their use of different outgroups. This alignment highlights the robustness of the results and increases the likelihood of inferences about the possible evolution of the morpho-functional traits of these organisms (e.g., a primary state for the tetrancres vs pentancres).

None of the analyses identified *Tetranchyroderma* as a monophyletic group. In both topologies, the *Tetranchyroderma* species with pentancres and recognizable sensory organ on the lateral margin of the head (e.g., pestle organs and sensory palps) form a well-supported monophyletic group, which also includes *Ptychostomella tyrrhenica* Hummon, Todaro & Tongiorgi, 1993, interestingly possessing pestle organs (Hummon *et al*., 1993).

The other *Tetranchyroderma* species form a distinct cluster, serving as a sister group to a fully supported clade comprising the three species of *Pseudostomella* Swedmark, 1956 included in the analyses. However, the support for the *Tetranchyroderma*-*Pseudostomella* relationship revealed by the analyses is not that strong (67% in the ML tree).

Once again, species sequenced de novo for this study were found clustering with taxa sharing general morphology and current classification, except for the new species.

In more details, the species with pestle organs or sensorial palps are categorized into two subclades of different sizes. The larger subclade includes *T. antenniphorum*, *T. esarabdophorum*, and the two specimens of *T. papii* Gerlach, 1953. The smaller subclade consists of *P. tyrrhenica* and the newly identified species *T. tunisiae* (Figs. 7, 8).

In a morpho-functional framework, the close phylogenetic relationships between the palp-bearing species, *T. papii*, and the pestle-bearing species suggest that these two organs have a homologous origin. They likely serve the same function as chemosensory organs, a topic that has been explored in detail only for the palp of *T. papii* (Gagné, 1980).

In a taxonomic context, it is challenging to draw general conclusions about the relationship between the new species and the genus *Ptychostomella* due to the limited sampling of the latter (here represented solely by *P. tyrrhenica*). However, the placement of the species from Tunisia along a distinct evolutionary lineage, separate from the species that appears most similar (see taxonomic affinities above), supports and reinforces the decision to classify it as a new species.

With regard *Tetranchyroderma* species clustering with *Pseudostomella*, we notice that *T. quadritentaculatum* Todaro, Balsamo & Tongiorgi, 1992, is the only species bearing cephalic structures that may serve as sensory organs (Todaro *et al*., 1992). Our phylogenetic analyses indicate these as autoapomorphic for the species, i.e., not related to palps or pestle organs, nor to the rod-like cephalic tentacles present in *T. antenniphorum*, *T. esarabdophorum*, and *T. papii* (Gerlach, 1953; Hummon & Todaro, 2010; Tongiorgi & Balsamo, 1984).

From our analyses, a close phylogenetic relationship emerges between *T. thysanophorum* Hummon, Todaro & Tongiorgi, 1993 and *T. anomalopsum* Hummon, Todaro, Balsamo & Tongiorgi, 1996. Pentancres characterize both species; however, the latter is one of the so-called bikini-trix, a complex of species, in which the ancrous covering is reduced, with some species showing only epaulets (Hummon *et al*., 1996).

The inclusion in future analyses of other species with reduced covering, could clarify if the reduction is a trend in close related species or an unrelated widespread phenomenon. In the last case it may have affected also species currently classified in the genus *Ptychostomella*, making the key traits of this taxon (cuticle smooth, lacking a cuticular covering made up of ancres) less reliable for grouping. The potential evolution of *Ptychostomella* from a lineage of *Tetranchyroderma* with a reduced covering has been proposed by Todaro *et al*., (2011); however, our study does not support the possibility that *T. anomalopsum* is part of such a lineage.

Our analysis reveals that in this cluster, species with ancres exhibiting different numbers of tines tend to cluster together. For instance, *T. cirrophorum*, which has tetrancres, clusters alongside *T. thysanophorum* and *T. anomalopsum*, both of which possess pentancres. This observation suggests caution when using this morphological characteristic to determine relationships within the genus *Tetranchyroderma* (see also Todaro *et al*., 2011), even though it is instrumental in species determination (Todaro *et al*., 2002).

Conclusions

Our research shows that even in areas renowned for their gastrotrich fauna, like the Mediterranean Sea, there are still undiscovered species. An integrative taxonomic approach that combines both morphological and molecular data is especially effective in uncovering relationships that are often hard to identify. This method facilitates a deeper understanding of the evolution of morphological traits, and beyond (Saponi *et al*., 2026).

Acknowledgements

Staying of AS at The University of Modena and Reggio Emilia has been made possible thanks to a grant from ACHIEVE-IT—Academic Collaboration through Higher International Education for a Viable and Equitable Africa with Italy". Codice TNE23-00071. Finanziamenti destinati a iniziative educative transazionali finanziate nell'ambito del PNRR—Missione 4 "Istruzione e ricerca"—Componente 1 "Potenziamento dell'offerta dei servizi all'istruzione: dagli asili nido all'Università", Investimento 3.4 "Didattica universitaria e competenze avanzate", sotto-investimento T4 "Iniziative Transnazionali in materia di istruzione", finanziato dall'Unione Europea—NextGenerationEU. Codice tipo progetto: PNRR_TNE_2023. CUP D91I23001030006.

This research benefitted of grant from the National Recovery and Resilience Plan (NRRP), Mission 4 Component 2 Investment 1.4—Call for tender No. 3138 of 16 December 2021, rectified by Decree n. 3175 of 18 December 2021 of the Italian Ministry of University and Research funded by the European Union—Next- GenerationEU. Project Code CN_00000033, Concession Decree No. 1034 of 17 June 2022 adopted by the Italian Ministry of University and Research, CUP E93C22001090001, Project title "National Biodiversity Future Center-NBFC".

References

Araújo, T.Q. & Hochberg, R. (2017) First description of a gastrotrich with four scale types, including feathered biancres, a novel find for Gastrotricha. *Journal of the Marine Biological Association of the United Kingdom*, 97 (2), 269–275. https://doi.org/10.1017/S0025315416000333

Arias-Carrasco, R., Vásquez-Morán, Y., Nakaya, H.I. & Maracaja-Coutinho, V. (2018) StructRNAfinder: an automated pipeline and web server for RNA families prediction. *BMC Bioinformatics*, 19 (1), 55. https://doi.org/10.1186/s12859-018-2052-2

Balsamo, M., Fregni, E. & Tongiorgi, P. (1994) Marine and freshwater Gastrotricha from the Island of Montecristo (Tuscan Archipelago, Italy), with the description of new species. *Bollettino di Zoologia*, 61 (3), 217–227. https://doi.org/10.1080/11250009409355889

Bankevich, A., Nurk, S., Antipov, D., Gurevich, A.A., Dvorkin, M., Kulikov, A.S., Lesin, V.M., Nikolenko, S.I., Pham, S., Prjibelski, A.D., Pyshkin, A.V., Sirotkin, A.V., Vyahhi, N., Tesler, G., Alekseyev, M.A. & Pevzner, P.A. (2012) SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. *Journal of Computational Biology*, 19 (5), 455–477. https://doi.org/10.1089/cmb.2012.0021

Boaden, P.J.S. (1974) Three new thiobiotic Gastrotricha. *Cahiers de Biologie Marine*, 15 (3), 267–378. https://doi.org/10.7202/041847ar

Cesaretti, A., Kosakyan, A., Saponi, F. & Todaro, M.A. (2024) Gaining and losing on the way: the evolutionary scenario of reproductive diversification in genus *Urodasys* (Macrodasyida, Gastrotricha) inferred by multi-gene phylogeny. *Zoological Journal of the Linnean Society*, 202 (4), zlae148. https://doi.org/10.1093/zoolinnean/zlae148

Cesaretti, A., Kosakyan, A., Saponi, F. & Todaro, M.A. (2025) Improved taxonomic and gene sampling advance the knowledge of deep relationships within Macrodasyida (Gastrotricha). *Cladistics*, 42 (1), 25–45. https://doi.org/10.1111/cla.70013

Gagné, G.D. (1980) Ultrastructure of the sensory palps of *Tetranchyroderma papii* (Gastrotricha, Macrodasyida). *Zoomorphologie*, 95 (2), 115–125. https://doi.org/10.1007/BF00997905

Gammuto, L., Serra, V., Petroni, G. & Todaro, M.A. (2024) Molecular phylogenetic position and description of a new genus and species of freshwater Chaetonotidae (Gastrotricha: Chaetonotida: Paucitubulatina), and the annotation of its mitochondrial genome. *Invertebrate Systematics*, 38 (7), IS23059. https://doi.org/10.1071/IS23059

Gerlach, S.A. (1953) Gastrotrichen aus dem Küstengrundwasser des Mittelmeeres. *Zoologischer Anzeiger*, 150, 203–211.

Golombek, A., Tobergte, S., Nesnidal, M.P., Purschke, G. & Struck, T.H. (2013) Mitochondrial genomes to the rescue–Diurodrilidae in the myzostomid trap. *Molecular Phylogenetics and Evolution*, 68 (2), 312–326.
https://doi.org/10.1016/j.ympev.2013.03.026

Hochberg, R. & Litvaitis, M.K. (2001) Macrodasyida (Gastrotricha): A cladistic analysis of morphology. *Invertebrate Biology*, 120 (2), 124–135.
https://doi.org/10.1111/j.1744-7410.2001.tb00116.x

Hummon, W.D. (1977) Introgressive hybridization between two intertidal species of *Tetranchyroderma* (Gastrotricha, Thaumastodermatidae) with the description of a new species. *Mikrofauna Meeresboden*, 61, 113–136.

Hummon, W.D. (2010) Global distribution of marine Gastrotricha (ed. 2001–2010). Available from: http://www.gastrotricha.unimore.it/checklist.htm (accessed 5 December 2025)

Hummon, W.D. (2011) Marine Gastrotricha of the Near East: 1. Fourteen new species of Macrodasyida and a redescription of *Dactylopodola agadasys* Hochberg, 2003. *ZooKeys*, 94, 1–59.
https://doi.org/10.3897/zookeys.94.794

Hummon, W.D. & Todaro, M.A. (2009) Italian marine Gastrotricha: VI. Seven new species of Macrodasyida. *Zootaxa*, 2278 (1), 47–68.
https://doi.org/10.11646/zootaxa.2278.1.3

Hummon, W.D. & Todaro, M.A. (2010) Analytic taxonomy and notes on marine, brackish-water and estuarine Gastrotricha. *Zootaxa*, 2392 (1), 1–32.
https://doi.org/10.11646/zootaxa.2392.1.1

Hummon, W.D., Todaro, M.A., Balsamo, M. & Tongiorgi, P. (1996) Italian marine Gastrotricha: III. Four new pentancrous species of the genus *Tetranchyroderma* (Macrodasyida, Thaumastodermatidae). *Italian Journal of Zoology*, 63 (1), 73–79.
https://doi.org/10.1080/11250009609356110

Hummon, W.D., Todaro, M.A. & Tongiorgi, P. (1993) Italian marine Gastrotricha: II. One new genus and ten new species of Macrodasyida. *Bollettino di Zoologia*, 60 (1), 109–127.
https://doi.org/10.1080/11250009309355798

ICZN International Commission of Zoological Nomenclature, I. (1999) *International Code of Zoological Nomenclature. 4th Edition*. International Trust for Zoological Nomenclature, London, 306 pp. Available from: https://code.iczn.org/?frame=1 (accessed 10 December 2025)

ICZN International Commission of Zoological Nomenclature (2017) Declaration 45—Addition of Recommendations to Article 73 and of the term “specimen, preserved” to the Glossary. *The Bulletin of Zoological Nomenclature*, 73 (2–4), 96–97.
https://doi.org/10.21805/bzn.v73i2.a2

Kaplan, G. (1958) Premières observations sur les gastrotriches psammophiles des côtes du Calvados. *Archives de Zoologie Expérimentale et Générale*, 1, 27–37.

Kieneke, A., Riemann, O. & Ahlrichs, W.H. (2008) Novel implications for the basal internal relationships of Gastrotricha revealed by an analysis of morphological characters. *Zoologica Scripta*, 37 (4), 429–460.
https://doi.org/10.1111/j.1463-6409.2008.00334.x

Kosakyan, A., Gammuto, L., Cesaretti, A., Saponi, F., Serra, V., Petroni, G., Macher, J.N. Wallnoefer, O., Plazzi, F., Todaro, M.A. (2025) From rigid order to radical variation: mitogenome evolution in the main lineages of a lesser-known animal phylum (Gastrotricha). *Genome Biology and Evolution*, 18, evag001. [https://academic.oup.com/gbe/article/18/2/evag001/8419271]
https://doi.org/10.1093/gbe/evag001

Kumar, S., Stecher, G., Li, M., Knyaz, C. & Tamura, K. (2018) MEGA X: Molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution*, 35 (6), 1547–1549.
https://doi.org/10.1093/molbev/msy096

Leasi, F. & Todaro, M.A. (2004) Egyptian marine Gastrotricha. Available from: http://www.gastrotricha.unimore.it/Egypt-marine.pdf (accessed 5 December 2025)

Lee, J. & Chang, C.Y. (2007) Two new marine gastrotrichs of the genus *Tetranchyroderma* (Macrodasyida: Thaumastodermatidae) from South Korea. *Zoological Studies*, 46 (4), 474.

Lee, J. & Chang, C.Y. (2012) Three new gastrotrich species of the genus *Tetranchyroderma* (Macrodasyida: Thaumastodermatidae) from Korea. *Zootaxa*, 3368 (1), 245–261.
https://doi.org/10.11646/zootaxa.3368.1.12

Lee, J., Chang, C.Y. & Kim, I.H. (2017) A new species of the genus *Tetranchyroderma* (Gastrotricha: Macrodasyida: Thaumastodermatidae) from Korea, with a peculiar pentancrous armature. *Proceedings of the Biological Society of Washington*, 130 (1), 156–165.
https://doi.org/10.2988/17-00016

Luporini, P., Magagnini, G. & Tongiorgi, P. (1973) Gastrotrichi macrodasioidei delle coste della Toscana. *Pubblicazioni Della Stazione Zoologica Di Napoli*, 38, 267–288.

Mečnikow, E., (1865) Ueber einige wenig bekannte niedere Thierformen. *Zeitschrift für wissenschaftliche Zoologie*, 15, 450–463.

Nguyen, L.T., Schmidt, H.A., Von Haeseler, A. & Minh, B.Q. (2015) IQ-TREE: A fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Molecular Biology and Evolution*, 32 (1), 268–274.

https://doi.org/10.1093/molbev/msu300

Nicholas, W. & Todaro, M.A. (2006) Two new species of *Tetranchyroderma* (Gastrotricha, Macrodasyida) from a sandy beach in southeastern Australia. *New Zealand Journal of Marine and Freshwater Research*, 40 (2), 249–258.
https://doi.org/10.1080/00288330.2006.9517418

Rao, G.C. & Clausen, C. (1970) *Planodasys marginalis* gen. et sp. nov. and Planodasyidae fam. nov. (Gastotricha Magrodasyoidea). *Sarsia*, 42 (1), 73–82.
https://doi.org/10.1080/00364827.1970.10411164

Remane, A. (1925a) Organisation und systematische Stellung der aberranten Gastrotrichen. *Verhandlungen Der Deutschen Zoologischen Gesellschaft*, 30, 121–128.

Remane, A. (1925b) Neue aberrante Gastrotrichen II: *Turbanella cornuta* nov. spec. und *T. hyalina* M. Schultze, 1853. *Zoologischer Anzeiger*, 64, 309–314.

Remane, A. (1926) Morphologie und Verwandtschaftsbeziehungen der aberranten Gastrotrichen I. *Zeitschrift Für Morphologie Und Ökologie Der Tiere*, 5 (4), 625–754.
https://doi.org/10.1007/BF00408293

Remane, A. (1927) Neue Gastrotricha Macrodasyoidea. *Zoologische Jahrbüecher Abteilung Fuer Systematik Oekologie Und Geographie Der Tiere*, 54, 203–242.

Remane, A. (1951) *Mesodasys*, ein neues Genus der Gastrotricha Macrodasyoidea aus der Kieler Bucht. *Kieler Meeresforschungen*, 8 (1), 102–105.

Rieger, G.E. & Rieger, R.M. (1977) Comparative fine structure study of the Gastrotrich cuticle and aspects of cuticle evolution within the Aschelminthes 1. *Journal of Zoological Systematics and Evolutionary Research*, 15 (2), 81–124.
https://doi.org/10.1111/j.1439-0469.1977.tb00533.x

Ronquist, F., Teslenko, M., Van Der Mark, P., Ayres, D.L., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M.A. & Huelsenbeck, J.P. (2012) MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology*, 61 (3), 539–542.
https://doi.org/10.1093/sysbio/sys029

Ruppert, E.E. (1978) The Reproductive System of Gastrotrichs. III. Genital organs of Thaumastodermatinae subfam. n. and Diplodasyinae subfam. n. with discussion of reproduction in Macrodasyida 1. *Zoologica Scripta*, 7 (1–4), 93–114.
https://doi.org/10.1111/j.1463-6409.1978.tb00592.x

Saponi, F., Cesaretti, A., Kosakyan, A., Serra, V. & Todaro, M.A. (2026) Phylogenetic position of *Setopus* (Gastrotricha, Paucitubulatina) among planktonic Gastrotricha, with the description of a new species. *Zoologischer Anzeiger*, 321, 248–261.
https://doi.org/10.1016/j.jcz.2026.01.012

Saponi, F., Kosakyan, A., Cesaretti, A. & Todaro, M. A. (2024) A contribution to the taxonomy and phylogeny of the genus *Chaetonotus* (Gastrotricha, Paucitubulatina, Chaetonotidae), with the description of a new species from Italian inland waters. *The European Zoological Journal*, 91 (2), 1078–1092.
https://doi.org/10.1080/24750263.2024.2397473

Saponi, F. & Todaro, M.A. (2024) Status of the Italian freshwater Gastrotricha biodiversity, with the creation of an interactive GIS-based web map. *Diversity*, 16 (1), 17.
https://doi.org/10.3390/d16010017

Seward-Thompson, B.L. & Hails, J.R. (1973) An appraisal of the computation of statistical parameters in grain size analysis. *Sedimentology*, 20 (1), 161–169.
https://doi.org/10.1111/j.1365-3091.1973.tb01612.x

Souid, A., Gammoudi, M., Saponi, F., El Cafsi, M. & Todaro, M.A. (2025) First investigation of the marine gastrotrich fauna from the waters of North Tunisia, with the description of a new species of *Halichaetonotus* (Gastrotricha, Chaetonotida). *Diversity*, 17 (1), 17.
https://doi.org/10.3390/d17010017

Swedmark, B. (1954) Description de *Paraturbanella teissieri*, n. sp. (Gastrotriche Macrodasyoide). *Bulletin de la Société zoologique de France*, 79 (1), 46–49.

Swedmark, B. (1956) Nouveaux gastrotriches macrodasyoides de la région de Roscoff. *Archives de Zoologie Expérimentale et Générale*, 94, 43–57.

Todaro, M.A. (1992) Contribution to the study of the Mediterranean meiofauna: Gastrotricha from the Island of Ponza, Italy. *Bollettino di Zoologia*, 59 (3), 321–333.
https://doi.org/10.1080/11250009209386689

Todaro, M.A. (2002) An interesting new gastrotrich from littoral meiobenthos (Long Beach Island, USA), with a key to species of *Tetranchyroderma* (Gastrotricha: Macrodasyida). *Journal of the Marine Biological association of the United Kingdom*, 82 (4), 555–563.
https://doi.org/10.1017/S0025315402005878

Todaro, M.A. (2025) Gastrotricha. Available from: http://www.gastrotricha.unimore.it/ (accessed 5 December 2025)

Todaro, M.A., Balsamo, M. & Tongiorgi, P. (1988) *Tetranchyroderma sardum*, a new species of the family Thaumastodermatidae (Gastrotricha, Macrodasyida). *Bollettino di Zoologia*, 55 (1–4), 69–72.
https://doi.org/10.1080/11250008809386602

Todaro, M.A., Balsamo, M. & Tongiorgi, P. (1992) Marine gastrotrichs from the Tuscan archipelago (Tyrrhenian Sea): I. Macrodasyida, with description of three new species. *Bollettino di Zoologia*, 59 (4), 471–485. https://doi.org/10.1080/11250009209386709

Todaro, M.A., Balsamo, M. & Tongiorgi, P. (2002) Marine gastrotrich fauna in Corsica (France), with a description of a new species of the genus *Tetranchyroderma* (Macrodasyida, Thaumastodermatidae). *Sarsia*, 87 (3), 248–257. https://doi.org/10.1080/00364820260294879

Todaro, M.A., Cesaretti, A. & Dal Zotto, M. (2019a) Marine gastrotrichs from Lanzarote, with a description of a phylogenetically relevant species of *Urodasys* (Gastrotricha, Macrodasyida). *Marine Biodiversity*, 49 (5), 2109–2123. https://doi.org/10.1007/s12526-017-0747-7

Todaro, M.A., Kånneby, T., Dal Zotto, M. & Jondelius, U. (2011) Phylogeny of Thaumastodermatidae (Gastrotricha: Macrodasyida) inferred from nuclear and mitochondrial sequence data. *PLoS ONE*, 6 (3), e17892. https://doi.org/10.1371/journal.pone.0017892

Todaro, M.A., Leasi, F., Bizzarri, N. & Tongiorgi, P. (2006) Meiofauna densities and gastrotrich community composition in a Mediterranean Sea cave. *Marine Biology*, 149 (5), 1079–1091. https://doi.org/10.1007/s00227-006-0299-z

Todaro, M.A., Matinato, L., Balsamo, M. & Tongiorgi, P. (2003) Faunistic and zoogeographical overview of the Mediterranean and Black Sea marine Gastrotricha. *Biogeographia—The Journal of Integrative Biogeography*, 24 (1), 131–160. https://doi.org/10.21426/B6110169

Todaro, M.A., Sibaja-Cordero, J.A., Segura-Bermúdez, O.A., Coto-Delgado, G., Goebel-Otárola, N., Barquero, J.D., Cullell-Delgado, M. & Dal Zotto, M. (2019b) An introduction to the study of Gastrotricha, with a taxonomic key to families and genera of the group. *Diversity*, 11 (7), 117. https://doi.org/10.3390/d11070117

Tongiorgi, P. & Balsamo, M. (1984) A new *Tetranchyroderma* species (Gastrotricha, Macrodasyoidea) from the Adriatic coast. *Bollettino di Zoologia*, 51 (3–4), 335–338. https://doi.org/10.1080/11250008409439471

WoRMS (2025) World Register of Marine Species. Available from: https://www.marinespecies.org/aphia.php?p=taxdetails&id=2078 (accessed 5 December 2025)

The legend for supplementary material S1: Final matrix of the concatenated 18S and 28S rDNA alignments from 43 species used for phylogenetic analyses.