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A taxonomic revision of the sponge-associated genus *Thoracactis* Gravier, 1918 (Anthozoa: Zoantharia) based on an integrated approach

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Abstract

The integrated approach of molecular phylogenetic and morphological analyses has revolutionized the systematics and our understanding of the evolutionary relationships of marine taxa. One such group is the hexacorallian order Zoantharia Rafinesque, 1815. The monotypic genus *Thoracactis* Gravier, 1918 has been little investigated since its placement within the order Zoantharia more than 100 years ago. Here, we examined museum specimens collected from the Cape Verde Islands (eastern Atlantic) and newly collected specimens from Brazil (southwestern Atlantic), using a combined molecular and morphological approach. Our results conclusively show *Thoracactis* to be referable to the family Parazoanthidae. Morphological data show that *Thoracactis topsenti* Gravier, 1918, the type species of this monotypic genus, has a cyclically transitional arrangement of its sphincter muscle, and this arrangement has previously been reported from the Parazoanthidae. *Thoracactis* can be distinguished from other hexasterophoran glass-sponge-associated genera (*Churabana* Kise, Montenegro & Reimer, 2022, *Parachurabana* Kise, 2023, and *Vitrumanthus* Kise, Montenegro & Reimer, 2022) by a combination of morphological, ecological and molecular phylogenetic data. In addition, molecular phylogenetic analyses clearly indicate that *Thoracactis topsenti* is placed within Parazoanthidae. These results are yet another demonstration of the utility of comprehensive combined approaches. From now, research attention should focus on the revision of remaining taxonomic questions within the family Epizoanthidae, with the goal of a comprehensively revised suborder Macrocnemina within reach.

Keywords

glass sponge – molecular phylogenetics – sphincter muscle – topotypes – zoantharian

Introduction

The combination of molecular phylogenetic and morphological datasets has revolutionized the systematics and our understanding of the evolutionary relationships of many marine taxa (e.g., Churchill et al., 2014; Huang et al., 2014; Hestetun et al., 2016; LaJeunesse et al., 2018; Hookabe et al., 2022). One such

group is the hexacorallian order Zoantharia Rafinesque, 1815 (e.g., Sinniger et al., 2005, 2010; Fujii & Reimer, 2011, 2013). In particular, this integrated approach has proved effective at implementing a comprehensive revision of the taxonomy and systematics of the suborder Macrocnemina Haddon & Shackleton, 1891 (e.g., Sinniger et al., 2005, 2010). As a result of recent research efforts, four macrocnemic

families and 16 genera have been erected in the last two decades (e.g., Reimer et al., 2007; Sinniger et al., 2010, 2013; Fujii and Reimer, 2011, 2013). However, taxonomic re-examinations of the family Epizoanthidae lag behind those of other Macrocnemina families, and the family Epizoanthidae still includes several taxonomic inconsistencies that have remained unresolved due to difficulties in specimen sampling (Kise et al., 2019; Reimer et al., 2019) and a lack of type materials and other museum specimens.

The family Epizoanthidae is among the largest of zoantharian macrocnemic families, and encompasses a diverse group of species found from the deep-sea (Ryland & Ward, 2016; Carreiro-Silva et al., 2017; Kise et al., 2019, 2022) to shallow water ecosystems (Phillipp & Fautin, 2009; Reimer & Sinniger, 2010; Di Camillo et al., 2014; Kise & Reimer, 2016).

Among Epizoanthidae, the genus *Thoracactis* Gravier, 1918 has remained a taxonomic enigma. This monotypic genus comprises only the type species *Thoracactis topsenti* Gravier, 1918, and is distinguished from other Epizoanthidae genera by having rudimentary sphincter muscles with no mesogleal channels or lacunae (Gravier, 1918; Pax & Müller, 1956), although the definition of this genus is not particularly well characterized due to relatively few diagnostic morphological characteristics. Indeed, Gravier (1918) originally described *T. topsenti* as an actinarian based on the absence of zooxanthellae and channels, gaps, or cell islets in mesoglea. However, a subsequent study by Pax and Muller (1956) redescribed *T. topsenti* and transferred the genus to Epizoanthidae based on encrustations, the shape and location of the sphincter muscle, and the presence of two rows of tentacles. More recent studies have suggested that *Thoracactis* may be referable to the family Parazoanthidae based on polyp color, as *T. topsenti* has bright yellow polyps, which

are also observed in some Parazoanthidae species (e.g., West, 1979) but are not known from Epizoanthidae (Reimer et al., 2010; but see Philipp & Fautin, 2009). Nevertheless, the taxonomic position of *Thoracactis* has remained unclear and has not been formally reconsidered since the work of Pax & Muller (1956).

Here, we revise the genus *Thoracactis* based on an integrated approach including molecular phylogenetic analyses combined with data from morphological observations of newly collected specimens. We provide detailed morphological characteristics as well as determine the phylogenetic position of this genus for the first time, and confirm the theory that *Thoracactis* is clearly located within the family Parazoanthidae.

Materials and methods

Specimen collection

We examined two *Thoracactis* specimens housed in the Porifera collection at Naturalis Biodiversity Center, Leiden, the Netherlands. These two specimens were collected during the CANCAP-VI expedition in the waters off Cape Verde in 1982 (van der Land, 1987). In addition, we examined 24 specimens from Rio Grande Rise, SW Atlantic collected during the Iata Piuna and Marine E-Tech expeditions and 22 specimens from Santos Basin, Brazil collected during the BIOIL expedition (fig. 1, table 1). The specimens collected from Brazil were registered in the SISGEN (Sistema Nacional de Gestão do Patrimônio Genético e do Conhecimento Tradicional Associado) under the accession number A6EB1FB.

DNA extraction, PCR amplification and sequencing

Specimens collected from Brazil were removed from the sponge using a delicate

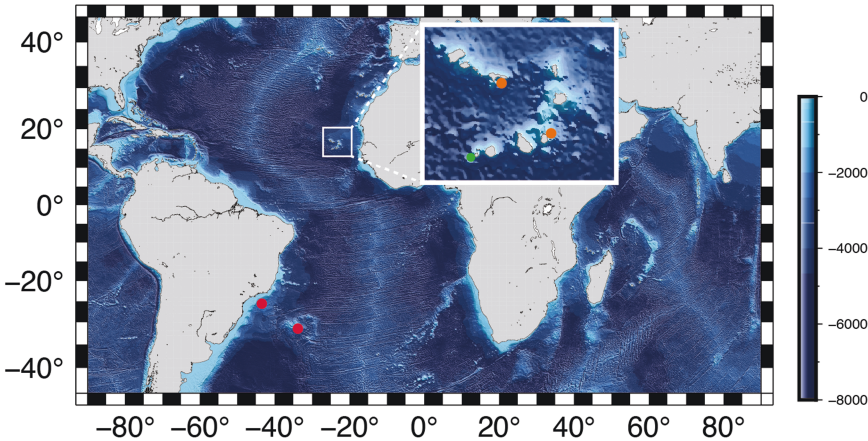


FIGURE 1 Distribution of *Thoracactis topsenti* examined in this study. Enclosed symbols indicate locations where *T. topsenti* was collected: type locality of *T. topsenti* (orange), location where topotypical specimens were collected (green), location where new specimens were collected (red).

pair of tweezers. We extracted DNA from the entire specimen using the Quick-DNA Zymo kit following the manufacturer's protocol. Polymerase chain reaction (PCR) amplification was performed using the GoTaq[®] Hot Start Polymerase (Promega) for the cytochrome oxidase subunit-I gene (COI), internal transcribed spacer region of ribosomal DNA (ITS) and mitochondrial 16S ribosomal DNA. We used the primers LCO and HCO (Folmer et al., 1994), ITSf and ITSr (Swain, 2009), and 16SarmL (Fujii & Reimer, 2011) and 16SBmoH (Sinniger et al., 2005), respectively. We used the following thermal cycles: 5 min at 95°C, 40 cycles of 30 s at 94°C, 1 min at 45°C, 2 min at 72°C and a final extension of 10 min at 72°C for COI; 5 min at 95°C, 40 cycles of 30 s at 94°C, 1 min at 49°C, 1 min 30 s at 72°C and a final extension of 10 min at 72°C for ITS; and 5 min at 95°C, 40 cycles of: 1 min at 95°C, 1 min at 52°C, 2 min at 72°C and a final extension of 7 min at 72°C for 16S. PCR products were purified using the QIAquick PCR Purification kit (QIAGEN) and sequenced either by Macrogen (Seoul, South Korea) or Myleus Biotecnologia (Belo Horizonte, Brazil).

Molecular and phylogenetic analyses

Sequences were initially assembled in Geneious Prime 2022.2.1. Thereafter, assembled sequences were manually trimmed and aligned with reference alignments consisting of previously published Parazoanthidae sequences (Kise et al., 2022, 2023) using MAFFT (Kato & Standley, 2013) with the auto algorithm under default parameters for all genetic markers. Phylogenetic analyses were performed on each genetic region as well as a concatenated dataset (COI+16S-rDNA+ITS-rDNA) using Maximum likelihood (ML) and Bayesian inference (BI). ModelTest-NG v0.1.6 (Darriba et al., 2020) under the Akaike information criterion was used to select the best fitting model for each molecular marker independently for both ML and BI analyses. The best selected models for ML and BI analyses were TPM2uf+I+G4 (HKY+I+G for BI) for COI, TIM3+G4 (GTR+G for BI) for mt 16S-rDNA, and TPM1uf+I+G4 (HKY+I+G for BI) for ITS-rDNA. Phylogenetic analyses were performed using model partitioning per each region in RAXML-NG v0.9.0 (Kozlov et al., 2019) for ML, and MrBayes v3.2.6 (Ronquist & Huelsenbeck,

2003) for BI based on Kise et al. (2022). For BI, MrBayes was configured to use the models and parameters above with the following settings: four MCMC heated chains were run for 5,000,000 generations with a temperature for the heated chain of 0.2. Chains were sampled every 200 generations. Burn-in was set to 1,250,000, at which point the average standard deviation of split frequency (ASDOSF) was steadily below 0.01.

In addition, ML phylogenetic analyses for each genetic marker were performed using PhyML v. 3.0 (Guindon et al., 2010) with the best selected model (TN93+G for COI, GTR+G for 16S-rDNA, GTR+R for ITS-rDNA) inferred by Smart Model Selection (SMS) implemented in PhyML, with 1000 bootstrap replicates.

Morphological observations

Preserved specimens (RMNH.COEL.45636, RMNH.COEL.45637) were examined whole, and then dissected and examined via serial sections. For internal morphological examinations, whole polyps of the specimens were embedded in paraplast after decalcification with Morse solution for 48 h (1:1 vol; 20% citric acid; 50% formic acid) and desilication with 20% hydrofluoric acid for 18–24h.

Ten to fifteen millimeter-thick serial sections were made with a microtome (LEICA RM2145; Leica, Germany) and stained with haematoxylin and eosin. Classification of marginal muscle shapes followed Swain et al. (2015). Cnidae analyses were conducted using undischarged nematocysts and spirocysts from the tentacles, column, actinopharynx, and mesenterial filaments of specimen RMNH.COEL.45636 using a Nikon Eclipse80i stereomicroscope (Nikon, Tokyo). Cnidae sizes were measured using ImageJ v1.45s (Rasband, 2012). The reported frequencies are the relative amounts based on numbers from all slides in the cnidae analyses. Cnidae classification generally followed England (1991)

and Ryland and Lancaster (2004) except for the treatment of basitrichs and microbasic b-mastigophores, which followed Kise et al. (2019).

Abbreviations

MOM	Musée Océanographique de Monaco
ColBIO	Coleção Biológica “Prof. Edmundo Nonato” do Instituto Oceanográfico, Universidade de São Paulo, Cidade Universitária
RMNH	Naturalis Biodiversity Center
MNRJ	National Museum of UFRJ in Rio de Janeiro

Results

Taxonomic account

Order Zoantharia Rafinesque, 1815
 Suborder Macrocnemina Haddon & Shackleton, 1891
 Family Epizoanthidae Delage & Hérouard, 1901

Genus *Thoracactis* Gravier, 1918

Synonymy. *Thoracactis* Gravier, 1918: 12, *Thoracactus* Walsh, 1967: 49, *Toracactis* Herberts, 1972: 80.

Type species. *Thoracactis topsenti* Gravier, 1918 by original designation

“Actinies commensales” – Topsent, 1904: 5, fig. 1; *Thoracactis topsenti* – Gravier, 1922: 33–39, pl. II, figs. 17–18, pl. VII, fig. 81, pl. VIII, figs. 82–89, pl. IX, fig. 90; *Thoracactis topsenti* – Stephenson, 1922: 307; *Thoracactis topsenti* – Pax & Müller, 1954: 8; *Thoracactis topsenti* – Pax & Müller, 1956: 16–19, fig. 3; *Thoracactus topsenti* – Walsh, 1967: 49; *Thoracactis topsenti* – Reisinger, 2002: 1340, fig. 6; “zoanthids” – Dorhmann et al., 2011: 1009, fig. 3; *Thoractis topsenti* – Hajdu et al., 2017: 96–98, figs. 4–5; *Thoracactis topsenti* Corrêa et al., 2022: fig. 7.

TABLE 1 List of specimens examined in the study.

Species	Voucher number	Project	Ship	Station	Location	Sampling Gear	Date
<i>Thoracactis topsenti</i>	RMNH. COEL.45636	CANCAP-VI	HMS Tydema	6.035	sw of Brava, Cape Verde Islands, Santiago	Rectangular dredge	08-Jun-82
<i>Thoracactis topsenti</i>	RMNH. COEL.45637	CANCAP-VI	HMS Tydema	6.035	sw of Brava, Cape Verde Islands, Santiago	Rectangular dredge	08-Jun-82
<i>Thoracactis topsenti</i>	ColBIO- POR-00001	Marine E-Tech	RSS Discovery	DY094-22	Rio Grande Rise, sw Atlantic	Rock dredge	25-Oct-18
<i>Thoracactis topsenti</i>	ColBIO- POR-00001	Marine E-Tech	RSS Discovery	DY094-22	Rio Grande Rise, sw Atlantic	Rock dredge	25-Oct-18
<i>Thoracactis topsenti</i>	ColBIO- POR-00001	Marine E-Tech	RSS Discovery	DY094-22	Rio Grande Rise, sw Atlantic	Rock dredge	25-Oct-18
<i>Thoracactis topsenti</i>	ColBIO- POR-00001	Marine E-Tech	RSS Discovery	DY094-22	Rio Grande Rise, sw Atlantic	Rock dredge	25-Oct-18
<i>Thoracactis topsenti</i>	ColBIO- POR-00001	Marine E-Tech	RSS Discovery	DY094-22	Rio Grande Rise, sw Atlantic	Rock dredge	25-Oct-18
<i>Thoracactis topsenti</i>	ColBIO- POR-00001	Marine E-Tech	RSS Discovery	DY094-22	Rio Grande Rise, sw Atlantic	Rock dredge	25-Oct-18
<i>Thoracactis topsenti</i>	ColBIO- POR-00001	Marine E-Tech	RSS Discovery	DY094-22	Rio Grande Rise, sw Atlantic	Rock dredge	25-Oct-18
<i>Thoracactis topsenti</i>	ColBIO- POR-00001	Marine E-Tech	RSS Discovery	DY094-22	Rio Grande Rise, sw Atlantic	Rock dredge	25-Oct-18
<i>Thoracactis topsenti</i>	ColBIO- POR-00001	Marine E-Tech	RSS Discovery	DY094-22	Rio Grande Rise, sw Atlantic	Rock dredge	25-Oct-18
<i>Thoracactis topsenti</i>	ColBIO- POR-00002	Marine E-Tech	RSS Discovery	DY094-34	Rio Grande Rise, sw Atlantic	Rock dredge	31-Oct-18
<i>Thoracactis topsenti</i>	ColBIO- POR-00002	Marine E-Tech	RSS Discovery	DY094-34	Rio Grande Rise, sw Atlantic	Rock dredge	31-Oct-18

Latitude	Longitude	Depth (m)	Histo- logical section	Cnidae observa- tion	Molecular examina- tion	Accession numbers		
						ITS-rDNA	COI	16S-rDNA
14°49'00"N	24°45'00"W	600–675	◦	◦				
14°49'00"N	24°45'00"W	600–675	◦					
30°49'48"S	35°58'07"W	815			◦	PP338129		
30°49'48"S	35°58'07"W	815			◦	PP338130		
30°49'48"S	35°58'07"W	815			◦	PP338131	PP338157	
30°49'48"S	35°58'07"W	815			◦	PP338132	PP338158	
30°49'48"S	35°58'07"W	815			◦		PP338159	
30°49'48"S	35°58'07"W	815			◦		PP338160	PP344670
30°49'48"S	35°58'07"W	815			◦		PP338161	PP344671
30°49'48.9"S	35°58'07.5"W	815			◦		PP338173	PP344688
30°49'48.9"S	35°58'07.5"W	815			◦		PP338174	
30°49'48.9"S	35°58'07.5"W	815			◦			PP344689
30°49'30"S	35°58'00"W	927			◦		PP338175	
30°49'30"S	35°58'00"W	927			◦		PP338176	

TABLE 1 List of specimens examined in the study. (cont.)

Species	Voucher number	Project	Ship	Station	Location	Sampling Gear	Date
<i>Thoracactis topsenti</i>	ColBIO-POR-00002	Marine E-Tech	RSS Discovery	DY094-34	Rio Grande Rise, sw Atlantic	Rock dredge	31-Oct-18
<i>Thoracactis topsenti</i>	ColBIO-POR-00002	Marine E-Tech	RSS Discovery	DY094-34	Rio Grande Rise, sw Atlantic	Rock dredge	31-Oct-18
<i>Thoracactis topsenti</i>	ColBIO-POR-00002	Marine E-Tech	RSS Discovery	DY094-34	Rio Grande Rise, sw Atlantic	Rock dredge	31-Oct-18
<i>Thoracactis topsenti</i>	ColBIO-POR-00002	Marine E-Tech	RSS Discovery	DY094-34	Rio Grande Rise, sw Atlantic	Rock dredge	31-Oct-18
<i>Thoracactis topsenti</i>	ColBIO-POR-00002	Marine E-Tech	RSS Discovery	DY094-34	Rio Grande Rise, sw Atlantic	Rock dredge	31-Oct-18
<i>Thoracactis topsenti</i>	ColBIO-POR-00002	Marine E-Tech	RSS Discovery	DY094-34	Rio Grande Rise, sw Atlantic	Rock dredge	31-Oct-18
<i>Thoracactis topsenti</i>	ColBIO-POR-00002	Marine E-Tech	RSS Discovery	DY094-34	Rio Grande Rise, sw Atlantic	Rock dredge	31-Oct-18
<i>Thoracactis topsenti</i>	ColBIO-POR-00002	Marine E-Tech	RSS Discovery	DY094-34	Rio Grande Rise, sw Atlantic	Rock dredge	31-Oct-18
<i>Thoracactis topsenti</i>	ColBIO-POR-00002	Marine E-Tech	RSS Discovery	DY094-34	Rio Grande Rise, sw Atlantic	Rock dredge	31-Oct-18
<i>Thoracactis topsenti</i>	ColBIO-POR-00003	Marine E-Tech	N/Oc. Alpha Crucis	491	Rio Grande Rise, sw Atlantic	Rock dredge	15-Feb-18
<i>Thoracactis topsenti</i>	MNRJ17626	Iata Piuna	RV Yokosuka	6K1338	Rio Grande Rise, sw Atlantic	Shinkai 6500	30-Apr-13
<i>Thoracactis topsenti</i>	MNRJ17626	Iata Piuna	RV Yokosuka	6K1338	Rio Grande Rise, sw Atlantic	Shinkai 6500	30-Apr-13
<i>Thoracactis topsenti</i>	ColBIO-POR-00004	BIOIL	N/Oc. Alpha Crucis	685	Santos Basin, Brazil	Box-corer	28-Jul-19
<i>Thoracactis topsenti</i>	ColBIO-POR-00004	BIOIL	N/Oc. Alpha Crucis	685	Santos Basin, Brazil	Box-corer	28-Jul-19
<i>Thoracactis topsenti</i>	ColBIO-POR-00004	BIOIL	N/Oc. Alpha Crucis	685	Santos Basin, Brazil	Box-corer	28-Jul-19

Latitude	Longitude	Depth (m)	Histo- logical section	Cnidae observa- tion	Molecular examina- tion	Accession numbers		
						ITS-rDNA	COI	16S-rDNA
30°49'30"S	35°58'00"W	927			°	PP338146	PP338177	PP344690
30°49'30"S	35°58'00"W	927			°		PP338178	PP344691
30°49'30"S	35°58'00"W	927			°		PP338179	
30°49'30"S	35°58'00"W	927			°		PP338180	PP344692
30°49'30"S	35°58'00"W	927			°			PP344693
30°49'30"S	35°58'00"W	927			°		PP338181	PP344694
30°49'30.3"S	35°58'00.6"W	927			°		PP338182	PP344695
30°49'30.3"S	35°58'00.6"W	927			°			PP344696
30°49'30.3"S	35°58'00.6"W	927			°		PP338183	PP344697
30°56'17"S	35°57'55"W	718			°		PP338184	PP344698
30°21'42"S	36°01'47"W	878			°	PP338133		PP344672
30°21'42"S	36°01'47"W	878			°	PP338134	PP338162	
24°55'56"S	44°28'25"W	550			°	PP338135	PP338163	PP344673
24°55'56"S	44°28'25"W	550			°		PP338164	
24°55'56"S	44°28'25"W	550			°		PP338165	

Latitude	Longitude	Depth (m)	Histo- logical section	Cnidae observa- tion	Molecular examina- tion	Accession numbers		
						ITS-rDNA	COI	16S-rDNA
24°55'56"S	44°28'25"W	550			o		PP338166	
24°55'56"S	44°28'25"W	550			o			PP344674
24°55'56"S	44°28'25"W	550			o	PP338136	PP338167	
24°55'56"S	44°28'25"W	550			o	PP338137		
24°55'56"S	44°28'25"W	550			o	PP338138		PP344675
24°55'56"S	44°28'25"W	550			o	PP338139		PP344676
24°55'56"S	44°28'25"W	550			o	PP338140		
24°55'56"S	44°28'25"W	550			o	PP338141		PP344677
24°55'56"S	44°28'25"W	550			o	PP338142		PP344678
24°55'56"S	44°28'25"W	550			o	PP338143		PP344679
24°55'56"S	44°28'25"W	550			o			PP344680
24°55'56"S	44°28'25"W	550			o			PP344681
24°55'56"S	44°28'25"W	550			o		PP338168	PP344681
24°55'56"S	44°28'25"W	550			o		PP338169	PP344683
24°55'56"S	44°28'25"W	550			o		PP338170	PP344684

TABLE 1 List of specimens examined in the study. (cont.)

Species	Voucher number	Project	Ship	Station	Location	Sampling Gear	Date
<i>Thoracactis topsenti</i>	ColBIO-POR-00004	BIOIL	N/Oc. Alpha Crucis	685	Santos Basin, Brazil	Box-corer	28-Jul-19
<i>Thoracactis topsenti</i>	ColBIO-POR-00004	BIOIL	N/Oc. Alpha Crucis	685	Santos Basin, Brazil	Box-corer	28-Jul-19
<i>Thoracactis topsenti</i>	ColBIO-POR-00004	BIOIL	N/Oc. Alpha Crucis	685	Santos Basin, Brazil	Box-corer	28-Jul-19
<i>Thoracactis topsenti</i>	ColBIO-POR-00004	BIOIL	N/Oc. Alpha Crucis	685	Santos Basin, Brazil	Box-corer	28-Jul-19

Diagnosis. Rudimentary sphincter muscles, azooxanthellate, with no mesogleal channels or lacunae. Obligate association with the Hexasterophora sponge *Sarostegia oculata* Topsent, 1904 (Gravier, 1918; Low et al., 2016).

Thoracactis topsenti Gravier, 1918

Figs. 2, 3

Type locality. Cape Verde Islands.

Type material. Syntypes. MOM INV-0020239 and MOM INV-0006591, collected from Cape Verde Islands, station 1193 (15°17'00"N, 23°2'00"W) at 1311 m depth, coll. RV *Princess Alice* II, 15 August 1901, fixed in ethanol, Musée Océanographique de Monaco. Syntypes. MOM INV-0006589 and MOM INV-0021095, Cape Verde Islands, station 1144 (16° 44'00"N, 24°49'00"W) at depth of 828 m, coll. RV *Princess Alice* II, 22 July 1901, in ethanol, Musée Océanographique de Monaco.

Other material examined. RMNH.COEL. 45636, RMNH.COEL.45637; collected from SW of Brava, Cape Verde Islands, Santiago (14°49'00"N, 24°45'00"W) at depths of 600–675 m, coll. RV HMS *Tydemia*, 08 June 1982, fixed in 99.5% ethanol, Naturalis Biodiversity Center. MNRJ17626, collected from Rio Grande

Rise (30°21'42"S, 36°01'47") at 878 m depth, coll. HOV *Shinkai 6500*, 30 Apr 2013, fixed in 70% ethanol, Museu Nacional do Rio de Janeiro. ColBIO-POR-00001, collected from Rio Grande Rise (30°49'48"S, 35°58'07"W) at 815 m average depth, coll. RSS *Discovery*, 25 Oct 2018, fixed in 96% ethanol, Coleção Biológica "Prof. Edmundo Nonato". ColBIO-POR-00002, collected from Rio Grande Rise (30°49'30"S, 35°58'00"W) at 972 m average depth, coll. RSS *Discovery*, 31 Oct 2018, fixed in 96% ethanol. RSS *Discovery*, 25 Oct 2018, fixed in 96% ethanol, Coleção Biológica "Prof. Edmundo Nonato". ColBIO-POR-00003, collected from Rio Grande Rise (30°56'17"S, 35°57'55"W) at 718 m average depth, coll. N/Oc. Alpha Crucis, 15 Feb 2018, fixed in 96% ethanol, Coleção Biológica "Prof. Edmundo Nonato". ColBIO-POR-00004, collected from Santos Basin, Brazil (24°55'56"S, 44°28'25"W) at 550 m depth, coll. N/Oc. Alpha Crucis, 28 Jul 2019, fixed in 96% ethanol, Coleção Biológica "Prof. Edmundo Nonato".

Description. External morphology. Preserved specimens consist of flat-cylindrical polyps (fig. 2A,B). Solitary polyps completely

Latitude	Longitude	Depth (m)	Histological section	Cnidae observation	Molecular examination	Accession numbers		
						ITS-rDNA	COI	16S-rDNA
24°55'56"S	44°28'25"W	550			o		PP338171	PP344685
24°55'56"S	44°28'25"W	550			o		PP338172	PP344686
24°55'56"S	44°28'25"W	550			o	PP338144		PP344687
24°55'56"S	44°28'25"W	550			o	PP338145		

embedded in the body of *Sarostegia oculata* and surrounded by sponge ectosomal formations (fig. 2B). Preserved polyps dark gray in coloration (fig. 2A–C). Living polyps light beige in coloration (fig. 2D). Polyp located all over the branch shape structured sponge body (fig. 2A, C). Surface of column rough, and ectoderm and mesoglea of scapus and coenenchyme encrusted with numerous sand and silica particles (fig. 2B). Contracted preserved polyps 0.5–1.0 mm in height, 3.0–5.0 mm in diameter. Tentacles approximately 26–30 with 13–15 inner endocoelic and 13–15 outer exocoelic tentacles. Capitulary ridges discernible, 13–15 in number when contracted.

Internal morphology. Zooxanthellae absent. Cyclically transitional marginal musculature (fig. 3B). Encircling sinus or mesogleal canal present. Mesenteries approximately 26–30 in number. Heavy sand, foraminiferan shells and silica incrustation in ectoderm to mesoglea (fig. 3C). Single siphonoglyph. Topotypic specimens examined fertile (Fig. 3A). Mesenterial filaments present (fig. 3D).

Cnidae. Basitrichs and microbasic b-mastigophores, microbasic p-mastigophores,

holotrichs, and spirocysts (for sizes, see fig. 4, table 2).

Distribution. The type locality of *Thoracactis topsenti* is Cape Verde (15°17'00"N–16°44'00"N, 23°02'00"W–24°49'00"W) (fig. 1). This species has been reported from the North and South Atlantic Ocean: Cape Verde, Rio Grande Rise, and Santos Basin, Brazil, at depths of 550–1311 m (Topsent, 1904; Gravier, 1918; Hajdu et al., 2017; Carrerette et al., 2022). Dendy (1916) reported *Sarostegia oculata* from Saya de Malha in the Indian Ocean, and a specimen of this sponge species examined in his study was also associated with zoantharian that has similar external morphology with *T. topsenti*. Therefore, *Thoracactis topsenti* may be distributed in the Indian Ocean as well.

Associated host. *Sarostegia oculata* Topsent, 1904

Molecular phylogeny. ML and BI phylogenetic analyses using a concatenated dataset yielded similar topologies (fig. 5, supplementary fig. S1); the BI phylogenetic reconstruction indicated that the Hexasterophora sponge-associated species Parazoanthidae sp. MISE-JMG50J was sister to *Vitrumanthus*, although

TABLE 2 Cnidae types and sizes observed in this study. Frequency: relative abundance of cnidae type in decreasing order; numerous, common, occasional, rare. n = number of cnidae measured.

<i>Thoracactis topsenti</i>						
Tissue	Type of cnidae	Length (min-max, mean)	Width (min-max, mean)	Frequency	n	
Tentacle	Spirocysts	11.0-23.0, 15.7	2.0-4.0, 2.9	Numerous	156	
	Basitrichs and microbasic b-mastigophores	11.0-19.0, 15.8	2.0-4.0, 3.3	Numerous	125	
Column	Holotrich (L)	33.0-47.0, 41.0	19.0-24.0, 21.2	Common	39	
	Microbasic p-mastigophores	14.0-18.0, 15.6	3.0-4.0, 3.9	Common	18	
Actinopharynx	Basitrichs and microbasic b-mastigophores	11.0-18.0, 14.0	2.0-4.0, 3.0	Common	33	
	Holotrichs (S)	7.0-9.0, 8.0	2.0-4.0, 2.7	Occasional	6	
	Holotrichs (M)	18.0	8.0-9.0	Rare	2	
Mesenterial filament	Microbasic p-mastigophores	7.0-13.0, 12.0	2.0-4.0, 3.2	Common	16	
	Holotrichs (M)	10.0-12.0, 11.1	4.0-6.0, 5.0	Occasional	8	
	Holotrichs (L)	39.0-43.0, 40.3	16.0-22.0, 19.1	Common	16	

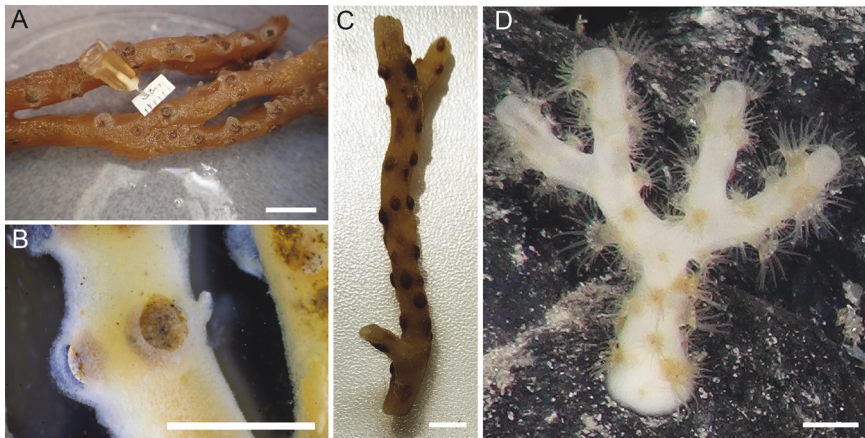


FIGURE 2 Images of external morphology of topotype of *Thoracactis topsenti*. (a) preserved topotypic specimen (RMNH.COEL.45636). (b) close-up image of a single preserved polyp (RMNH.COEL.45636). (c) image of museum specimen taken by Michèle Brunmi (MOM INV-0017721). (d) living polyps on a hexactinellid sponge *Sarostegia oculata*. Scale bars: 10 mm

support was low ($BI < 0.95$, supplementary fig. S1). The results based on the concatenated dataset demonstrated that *Thoracactis topsenti* was placed within Parazoanthidae, sister to *Churabana* ($ML = 77\%$, $BI = 0.97$). Sequences of examined specimens formed a monophyletic clade ($ML = 100\%$, $BI = 1.0$). Furthermore, phylogenetic analyses based on each genetic marker yielded similar topologies to the concatenated dataset (supplementary figs. S2–S4).

Discussion

Taxonomic position of the genus Thoracactis

Thoracactis topsenti was described in detail by Gravier (1918) from two specimens collected from Cape Verde (fig. 2C). Details of the external and internal morphology are provided in the description by Gravier (1918), who described *T. topsenti* as an actiniarian based on the absence of zooxanthellae and channels, gaps, or cell islets in mesoglea. However, *Paleozoanthus* Carlgren, 1924, within Epizoanthidae (although now known

to very likely be within Hydrozoanthidae based on results in Fourreau et al. (2023); see below), also has no zooxanthellae and channels, gaps, or cell islets in mesoglea. Therefore, a subsequent study by Pax and Muller (1956) redescribed *T. topsenti* and transferred it to the family Epizoanthidae in the order Zoantharia based on the presence of encrustations, the shape and location of the sphincter muscle, and the presence of two rows of tentacles. Pax and Muller (1956) also indicated that *Thoracactis* could be distinguished from *Epizoanthus* Gray, 1867, and *Paleozoanthus* by the location of gonads that do not originate from mesenteries but from the endodermal of the body wall. Although we observed the gonads in the topotypic specimens (fig. 3A), the location of these gonads was not confirmed. Further study is required to confirm whether the gonads are derived from the endoderm of the body wall.

The ectoderm and mesoglea of *T. topsenti* are encrusted by empty foraminiferan shells, sand, and silica particles, and the sphincter muscle of *T. topsenti* consists of 15 meshes of an oval or slit shape located in the mesoglea.

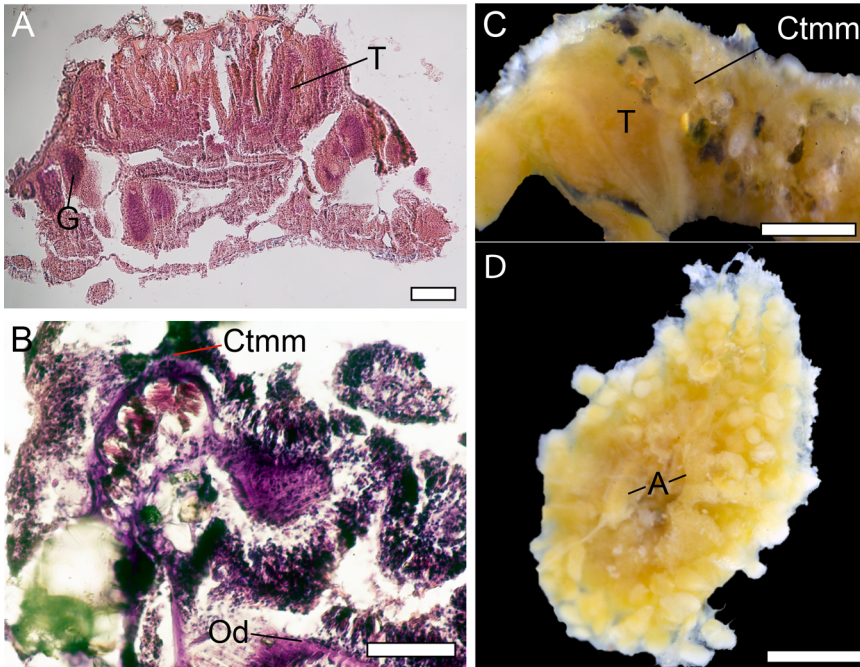


FIGURE 3 Images of internal morphology of topotype of *Thoracactis topsenti* (RMNH.COEL.45636). (a) longitudinal section of a polyp. (b) closed-up image of cyclically transitional marginal musculature. (c) longitudinal section of a polyp obtained by hand-cutting. (d) cross-section of a polyp by hand-cutting. Abbreviations: A: actinopharynx, Ctmm: cyclically transitional marginal musculature, G: gonads, Od: oral disk, T: tentacles. Scale bars: 100 μm (a), 30 μm (b), 500 μm (c, d).

Recent studies have shown that there are at least 12 different types of the sphincter muscles in zoantharians (Swain & Swain, 2014; Swain et al., 2015). The sphincter muscle of *Epizoanthus* is of reticulate mesogleal arrangement with irregularly-shaped lacunae that appear as a reticulate mesh arranged along the length of the sphincter muscle. The sphincter muscle of *Paleozoanthus* is also considered as reticulate mesogleal in arrangement based on illustrations of the original description by Carlgren (1924). On the other hand, upon close examination using topotypic specimens in this study, the sphincter muscle of *T. topsenti* has muscle attachment sites that transition between mesogleal pleats and lacunae, an arrangement known as cyclically transitional, and this arrangement is found in some

genera within family Parazoanthidae such as *Corallizoanthus* Reimer in Reimer Nonaka, Sinniger & Iwase, 2008, *Savalia* Nardo, 1844, and *Vitrumanthus* Kise, Montenegro & Reimer, 2022. The mesogleal sphincter muscle as illustrated by Pax and Muller (1956) is also more similar to that of Parazoanthidae than to that of Epizoanthidae (fig. 3b).

Hexasterophoran glass sponge-associated species have been reported in three Parazoanthidae genera (*Churabana*, *Parachurabana*, *Vitrumanthus*). Our molecular phylogenetic results clearly indicated that *Thoracactis* is sister to the genus *Churabana* (fig. 4), but these two genera can be distinguished by sphincter muscle (cyclically transitional vs cteniform endodermal arrangement) and host sponges (*Pararete*

Thoracactis topsenti

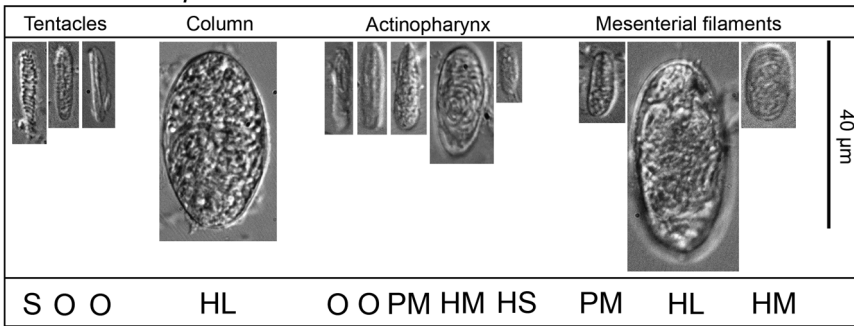


FIGURE 4 Cnidae in the tentacles, column, actinopharynx and mesenterial filaments of topotype of *Thoracactis topsenti* (RMNH.COEL.45636). Abbreviations: HL, holotrich large; HM, holotrich medium; HS, holotrich small; O, basitrichs and microbasic b-mastigophores; PM, microbasic p-mastigophores; S, spriocysts.

Ijima, 1927 vs *Sarostegia* Topsent, 1904). In addition, *Thoracactis* can be separated from *Parachurabana* based on sphincter muscle (cyclically transitional vs cteniform endodermal arrangement) and host sponges (*Farrea* Bowerbank, 1862 vs *Sarostegia*). Although *Thoracactis* and *Vitrumanthus* share a similar shape of the sphincter muscle, these two genera are phylogenetically distinct. *Vitrumanthus* associated with both hexasterophoran glass sponge and Demospongiae sponge, while *Thoracactis* is associated with *Sarostegia*. The association with *Sarostegia* has not been observed in *Vitrumanthus*. Regarding external morphology, the four parazoanthid genera associated with hexasterophoran glass sponge have similar features. However, *Thoracactis* can be distinguished from the other genera by relatively flatter polyps apart from *Parachurabana*. Although *Thoracactis* and *Parachurabana* have similar polyp sizes, *Thoracactis* can be distinguished from *Parachurbana*, as polyps of *Thoracactis* are completely embedded in the body of the host sponge, whereas those of *Parachurabana* are attached to the surface of the host sponge.

Although the new specimens used for sequencing were collected from Brazil, which is not the type locality of *T. topsenti*, their

morphological characteristics are identical to those of the topotypic museum specimens collected from the Cape Verde Islands. Therefore, based on the sum of evidence, we formally transfer the genus *Thoracactis* with its species *T. topsenti* to the family Parazoanthidae.

Taxonomic revision of the family Epizoanthidae

Taxonomic re-examination of the family Epizoanthidae lags behind research on other Macrocnemina families, although it is known that the family Epizoanthidae includes several taxonomic inconsistencies that have remained unstudied due to difficulties in sampling (e.g., Reimer et al., 2010; Kise et al., 2019). Based on this study, the family Epizoanthidae now consists of only two genera, *Epizoanthus* and *Paleozoanthus*. However, there still a need for comprehensive taxonomic revision of Epizoanthidae. *Paleozoanthus* is currently a taxon inquirendum (Reimer, 2023). Kise et al. (2022) reported the presence of *Paleozoanthus* for the first time since its original description, although the genus' phylogenetic position was unclear. However, a recent subsequent study found that *Paleozoanthus* was placed in the family Hydrozoanthidae Sinniger, Reimer & Pawlowski, 2010 (Fourreau

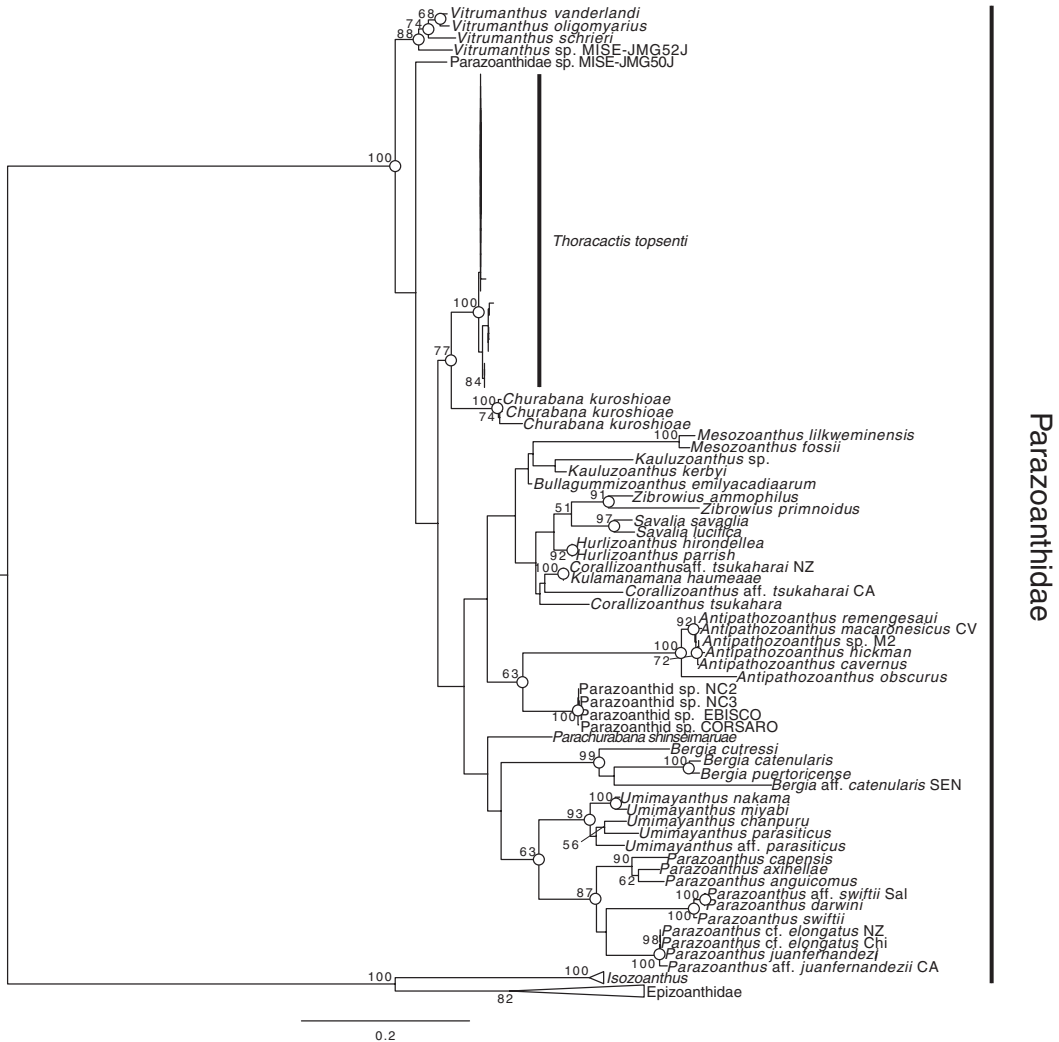


FIGURE 5 Maximum likelihood tree based on combined dataset of COI, 16S-rDNA and ITS-rDNA. Number at nodes represent ML bootstrap values (> 50% are shown). White circles on nodes indicate high support of Bayesian posterior probabilities (>0.95).

et al., 2023). Interestingly, Low et al. (2015) suggested that *Paleozoanthus* may correspond to *Terrazoanthus* Reimer & Fujii, 2011 within Hydrozoanthidae based on morphological features such as the sphincter muscle (Swain et al., 2015).

Thus, *Paleozoanthus* may belong to Hydrozoanthidae rather than Epizoanthidae. Unfortunately, Kise et al. (2022) did not examine the internal morphology of the

Paleozoanthus specimen due to its poor condition. To confirm the taxonomic position of *Paleozoanthus*, examination of topotypic specimens is needed, although based on molecular evidence it is almost certain the genus is referable to Hydrozoanthidae (Fourreau et al., 2023).

Epizoanthus consists of various species, some associated with marine invertebrates, some living on hard substrates (e.g., rocks

and dead empty shells), as well as soft substrate-living species within sediment, and the host range of *Epizoanthus* is comparatively broad (see Kise et al., 2023). Host specificity has been indicated as a key for the modern taxonomic framework of Parazoanthidae, with monophyletic clades formed of species with specific associated host marine organisms used as substrates (Sinniger et al., 2005, 2010, 2013; Reimer et al., 2008; Montenegro et al., 2015; Kise et al., 2022). On the other hand, several studies suggested that host specificity in *Epizoanthus* may not strictly follow molecular phylogenetic results (Reimer et al., 2010; Kise et al., 2018). However, more recently, Kise et al. (2023) reported that the compositions of host organisms were different within the genus *Epizoanthus* based on comprehensive molecular phylogenetic analyses of specimens collected across a broad range of host organisms. Thus, host specificity still has at least some utility in the taxonomy of this genus when combined with molecular phylogenetic analyses. Taxonomic re-examinations of the genus *Epizoanthus* based on an integrated approach including molecular phylogenetic analyses, host specificity, and morphology provide a framework for further revision of the family Epizoanthidae, and can improve our knowledge of the evolutionary history of zoantharians.

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Supplementary material

Supplementary material is available online at: <https://doi.org/10.6084/m9.figshare.25224728>

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