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Enenterum gomesae n. sp. (Enenteridae) in *Kyphosus incisor* (Kyphosidae) off the Rio de Janeiro coast, Brazil

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Research Paper

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Abstract

The genus *Enenterum* Linton, 1910 comprises species which parasitize herbivorous kyphosid fish. In the present study, a new species is described based on fresh specimens collected from *Kyphosus incisor* from Rio de Janeiro. The new species is characterized by having the oral sucker infundibuliform with 10 lobes, prepharynx two times longer than pharynx, presence of oesophagus, testes slightly lobed, round ovary and rectum with muscular sphincter connected to the anus. New genetic sequences include partial 18S and 28S rDNA and ITS1-5.8S-ITS2. The phylogenetic analyses place *Enenterum gomesae* n. sp. as sister of *Enenterum aureum*, corroborating the morphological analyses. *Enenterum aureum* (=*E. pimelopteri*) previously described from *Kyphosus* spp. from Rio de Janeiro is now considered *E. gomesae* n. sp. The new species represents the only South American species so far described for this genus.

Introduction

The family Enenteridae Yamaguti, 1958 comprises five genera, and most of its species have been reported from intestines of herbivorous marine teleost, mainly Kyphosidae (Bray & Cribb, 2001, Houston *et al.*, 2019, 2022). The genus *Enenterum* was established by Linton in 1910 with *Enenterum aureum* from *Kyphosus sectatrix* (Linnaeus) from Florida as type species. Currently, the genus *Enenterum* includes a total of 11 species with worldwide distribution (Huston *et al.*, 2022).

Gomes *et al.* (1974) collected specimens of *Enenterum* from the intestine of *Kyphosus* sp. off Rio de Janeiro, Brazil, and identified them as *Enenterum pimelopteri* Nagaty, 1942 a species originally described from *Kyphosus cinerascens* (Forsskål) (=*Pimelopterus tahmel*) from the Red Sea. A similar species, *E. pseudaureum* from *K. sectatrix* from Dakar, Africa, was described by Dollfus (1946). Manter (1947) redescribed *E. aureum* Linton, 1910 from *K. sectatrix* (type host) and *K. incisor* (Cuvier) from Florida suggesting that probably *E. pseudaureum* was a synonym of *E. pimelopteri*. Afterward Bray (1978) considered *E. pimelopteri* reported from South Atlantic by Fischthal & Thomas (1972) and Gomes *et al.* (1974), and *E. pseudaureum*, as synonyms of *E. aureum*.

Collection of new specimens of *Enenterum* from *Kyphosus incisor* (Cuvier) from off Rio de Janeiro, revealed it to be a new species now described with molecular data.

Material and methods

Sample collection and morphological analysis

Two kyphosid fish acquired from fishermen were examined: one from Jurujuba Beach, Niterói (22°55′35″S, 43°06′00″W) and the other from Copacabana Beach, Rio de Janeiro (22°59′08″S, 43°11′18″W). The fish were measured and weighed, and the intestine was removed and examined in a saline medium under a stereomicroscope. The trematodes were collected alive, washed in saline solution at room temperature, and fixed in alcohol 70% or hot 4% formalin under slight coverslip pressure. Specimens were stained in Mayer's paracarmine and Gomori's trichrome and mounted in Canada balsam. Measurements are presented in micrometres, with the range followed by the mean in parentheses. Drawings were made with the aid of a drawing tube. Representative specimens were deposited in the Helminthological Collection of Instituto Oswaldo Cruz (CHIOC), Rio de Janeiro, Brazil. For comparative purposes, specimens of *E. pimelopteri* previously reported by Gomes *et al.* (1974) and reassigned to *E. aureum* by Bray (1978), deposited at CHIOC 31012a-h, were reexamined. Comparative measurements are presented in Table 1.

Table 1. Comparative morphometric data of *Enenterum gomesi* n. sp., *Enenterum pimelopteri* and *Enenterum aureum*

	Enenterum aureum	*Enenterum pimelopteri*	*Enenterum pimelopteri**	*Enenterum gomesae* n. sp.
Host	*Kyphosus sectatrix* (type host), *Kyphosus incisor*	*Kyphosus cinerascens* (=*Pimelopterus tahmel*)	*Kyphosus* sp.	*Kyphosus incisor*
Reference	Manter, 1947	Nagaty, 1942	Gomes *et al*., 1974	Present study
Locality	Tortugas, Florida	Red Sea	Rio de Janeiro	Rio de Janeiro
Body (L)	3847–10.193	5053–10.105	9.88–12.70	9.04–13.9 (11.99)
Body (W)	0.870–1.323	0.71–1.505	0.84–1.50	0.76–1.88 (1.38)
Oral sucker (L)	0.337–0.405 (without lobes)	0.516–0.925	0.41–0.59	0.64–0.99 (0.81)
Oral sucker (W)		0.387–0.645	0.35–0.53	0.33–0.88 (0.74)
Lobes oral sucker	6: 2 dorsal, 2 ventral, 2 lateral. Dorsal and lateral processes notched looking like 10	10 petal-like divisions (the ventral-most are the largest)	10: 2 dorsal pairs, 2 ventral pairs and 1 e 1 ventral pair larger than others.	10 lobes: 3 groups of round bilobed tips (=6) and 2 groups deeply bilobed with pointed tips (4)
Ventral sucker	0.450–0.580	0.495–0.86	0.54–0.77 × 0.52–0.85	0.55–0.92 (0.72) × 0.56–0.95 (0.74)
Ratio oral: ventral suckers	1:1.25–1:1.66	1:1.04–1.07	1:1.30–1.50	1: 0.98–1.20 (1:1.12)
Prepharynx (L)	The same length as pharynx		0.55–0.63	0.45–0.80 (0.60)
Pharynx (L)	0.225–0.300	0.265–0.538	0.24–0.37	0.24–0.44 (0.32)
Pharynx (W)	0.195–0.255	0.258–0.43	0.24–0.37	0.26 × 0.36 (0.32)
Oesophagus (L)	Absent		0.1	0.46–0.62 (0.52)
Cirrus sac (L)			0.52–0.90	
Cirrus sac (W)			0.30–0.55	
Anterior testis (L)	0.70**	0.43–1.183	0.91–1.30	0.93–1.60 (1.06)
Anterior testis (W)	0.47** lobed	0.387–0.645	0.56–0.84	0.60–0.82 (0.68) slightly lobed
Posterior testis (L)	0.70**	0.43–1.183	0.97–1.40	0.87–1.38 (1.15)
Posterior testis (W)	0.47**	0.387–0.645	0.52–0.82	0.44–0.76 (0.63)
Ovary (L)	0.29**slightly lobed	0.265–0.538	0.34–0.55	0.36–0.52 (0.43)
Ovary (W)	0.35**		0.31–0.47	0.33–0.52 (0.41)
Eggs (L)	0.058–0.067	0.057–0.066	0.049–0.075	0.06–0.07 (0.06)
Eggs (W)	23–28 µm	0.035–0.044	0.029–0.046	0.03–0.04 (0.03)
Seminal receptacle		0.201	0.29–0.57 × 0.23–0.47	0.22–0.40 × 0.30–0.35
Posterior testis to body end	1.88**			1.90–2.74 (2.32)

*Considered *E. aureum* by Bray.
**Measured in the original drawing.

Genetic analysis

DNA extraction was performed using a QIAamp DNA Mini Kit (QIAGEN) according to the manufacturer's instructions, and a set of primers were used to amplify different regions of the DNA. The rDNA region 28S was amplified by polymerase chain reaction (PCR) using the primers LSU5 (5′- TAGGTCGACCCGCT-GAAYTTAAGCA- 3′) and 1500R (5′- GCTATCCTGAGG-GAAACTTCG- 3′) (Tkach *et al*., 2003). For partial 18S rDNA the primers SB3a (5'-GGAGGGCAAGTCTGGTGC-3') and A27a (5'-CCATACAAATGCCCCCGTCTG-3') (Hall *et al*., 1999) were used. For partial ITS1-5.8S-ITS2 region of the rDNA the BD1 (5′-GTCGTAACAAGGTTTCCGTA-3′) and BD2 (5′-TATGCT TAARTTCAGCGGGT-3′) primers were used (Luton *et al*., 1992). Forward and reverse primers were used for all regions. PCRs were carried out using cycling parameters as previously described by these authors.

PCR products were analyzed by electrophoresis in 1.5% agarose in Tris-borate EDTA gels, stained with SyberGreen (Invitrogen, Eugene, Oregon, USA) and photographed under ultraviolet trans-illumination. Amplified PCR products were purified using ExoSap-IT (USB Products Affymetrix Inc., Cleveland, Ohio, USA). DNA cycle sequencing reactions were performed using the BigDye Ter-minator v.3.1 (Applied Biosystems, Foster City, CA, USA) and automated Sanger sequencing was done using the Sequencing Platform at Fundação Oswaldo Cruz (PDTIS/Fiocruz) in Brazil. Sequences of both strands generated (.ab1 files) were oriented in the same direction, aligned (CLUSTAL W) and edited by using the MEGA11 software (Tamura *et al*., 2021). The low-quality trailing ends were removed. Sequences were compared to others available in the GenBank database using the Basic Local Alignment Search Tool (BLAST) program from the National Center for Biotechnol-ogy Information server (http://www.ncbi.nlm.nih.gov/BLAST)

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Table 2. List of the species of Digeneans used in the phylogenetic analyses of *Enenterum gomesae* n. sp. with respective GenBank accession numbers

Species	28S rDNA	18S rDNA	5.8S-ITS2	Reference
Enenteridae				
Enenterum aureum Linton, 1910	AY222232	AY222124	–	Olson *et al.* (2003)
Enenterum kyphosi Yamaguti, 1970	ON228454 ON228455	–	ON228451	Huston *et al.* (2022)
Enenterum petrae Huston, Cutmore & Cribb, 2022	ON228456	–	ON228453	Huston *et al.* (2022)
Enenterageitus huxleyi (Bray & Cribb, 2001)	MN080864	–	MN080855	Huston *et al.* (2019)
Koseiria sp.	–	–	MN080853	Huston *et al.* (2019)
Koseiria argalea Huston, Cutmore & Cribb, 2019	MN080858	–	MN080850	Huston *et al.* (2019)
Koseiria laiphopharophora Huston, Cutmore & Cribb, 2019	MN080860	–	MN080851	Huston *et al.* (2019)
Koseiria pyknophora Huston, Cutmore & Cribb, 2019	MN080861	–	MN080852	Huston *et al.* (2019)
Koseiria xishaense Gu & Shen, 1983	–	AY222125	–	Olson *et al.* (2003)
Proenenterum allanwilliamsi (Bray & Cribb, 2002)	MN080863	–	MN080854	Huston *et al.* (2019)
Proenenterum ericotylum Manter, 1954	FJ788499	–	–	Bray *et al.* (2009)
Proenenterum isocotylum Manter, 1954	FJ788500	–	–	Bray *et al.* (2009)
Leprocreadiidae				
Preptetos caballeroi Pritchard, 1960	–	AJ287563	–	Cribb *et al.* (2001)
Preptetos trulla (Linton, 1907)	–	AY222128	–	Olson *et al.* (2003)
Lepidapedidae				
Labrifer secundus Manter, 1940	–	MF414434	–	Ñacari *et al.* (2018)
Outgroup				
Affecauda annulata Hall & Chambers, 1999	FJ788501	–	–	Bray *et al.* (2009)
Endochortophagus protoporus Huston, Miller, Cutmore & Cribb, 2019	MK396257	–	–	Huston *et al.* (2019b)
Haplosplanchnus pachysoma (Eysenhardt, 1829)	–	LK932143	KY852459	Besprozvannykh *et al.* (2016); Huston *et al.* (2017)
Pygidiopsis macrostomum Travassos, 1928	–	KT877408	–	Borges *et al.* (2017)
Schikhobalotrema acutum (= *Schikhobalotrema huffmani*) (Linton, 1910)	–	–	KY852465	Huston *et al.* (2017)

(Altschul *et al.*, 1990). Evolutionary divergence estimates between sequences were conducted in MEGA11 using the Kimura 2-parameter (K2p) model (Kimura, 1980).

To examine phylogenetic relationships, nucleotide sequences were aligned using MEGA11. Bayesian inference phylogenetic trees were conducted using Monte Carlo Markov Chain analysis available in the BEAST v2.6.3 software (Bouckaert *et al.*, 2019). Likelihood parameters set for the BI analysis were based on the Akaike Information Criteria test in jModelTest2 (Nylander, 2004). The selected model was the General Time-Reversible for 28S, and the Hasegawa-Kishino-Yano for the 18S and ITS1-5.8S-ITS2, employing the birth-death model. Posterior probabilities were calculated via 10,000,000 generations, sampling every 1000th tree. Tracer v1.7.2 (Rambaut *et al.*, 2018) was used to validate the convergence and mixing to ensure all effective sample size values greater than 200. Trees were presented as Maximum-Clade Credibility trees using the TreeAnnotator v2.6.3 software after discarding the first 10% as burn-in and visualized using the FigTree v1.4.4 (Rambaut *et al.*, 2018). For tree rooting, the best sequences used as outgroups were *Pygidiopsis macrostomum* Travassos, 1928 (KT877408) and *Haplosplanchnus pachysoma* (Eysenhardt, 1829) (LK932143) for 18S, *Affecauda annulata* Hall & Chambers, 1999 (FJ788501) and *Endochortophagus protoporus* Huston, Miller, Cutmore & Cribb, 2019 (MK396257) for 28S, and *H. pachysomus* (KY852459) and *Schikhobalotrema acutum* (Linton, 1910) (KY852465) for 5.8S-ITS2. The ITS1 region was not included in the phylogenetic analysis because the GenBank sequences for comparison only had 5.8S-ITS2. Sequences from GenBank that were used for the phylogenetic analysCis are listed in Table 2.

Results

Lepocreadioidea Odhner, 1905
Enenteridae Yamaguti, 1958
***Enenterum* Linton, 1910**
***Enenterum gomesae* n. sp.**
Syns: *Enenterum pimelopteri* of Gomes, Fabio & Rolas (1974), re-identified as *E. aureum* by Bray (1978)
http://zoobank.org/urn:lsid:zoobank.org:pub:21E575C6-F226-4CD8-94EC-94EF015CB233
(Figs 1-3; 2 Tables)

Description based on eight specimens: Body elongate, tapering at each end, 9.04–13.90 (11.99) long by 0.76–1.88 (1.38) wide (Fig. 1a). Tegument spinous. Eyespot pigment sparce in forebody. Oral

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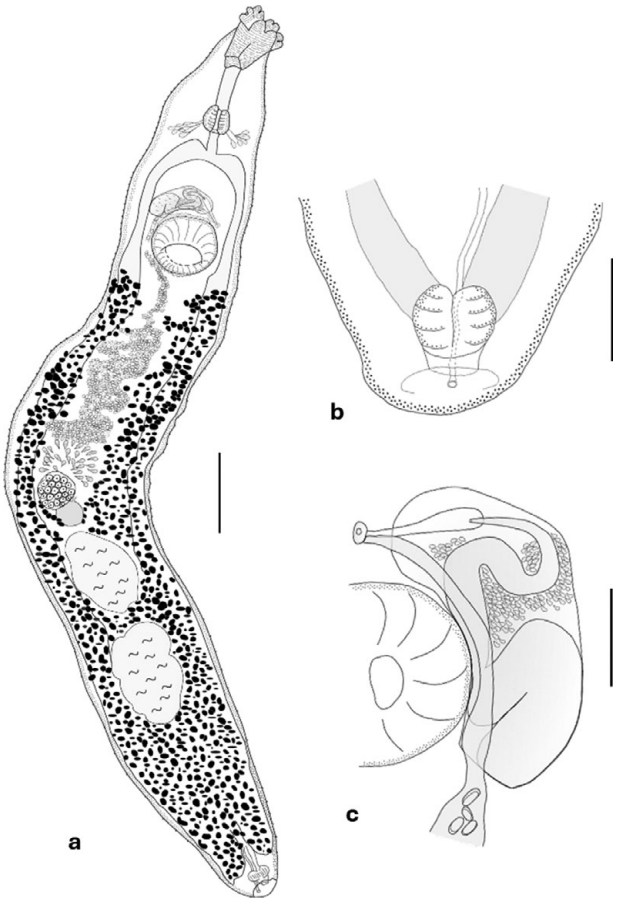

Figure 1. *Enenterum gomesae* n. sp. a. Whole specimens, ventral view. Bar 1 mm. b. Posterior region showing the presence of a muscular sphincter and the anus. Bar 0.2 mm. c. Cirrus-sac, lateral view. Bar 1 mm.

sucker terminal, infundibuliform, bordered by 10 lobes disposed as: two pairs of dorsal lobes with anterior notches separated by a sagittal cleft; two lateral pairs, one at each side of the sucker, with anterior notches; one pair of strong ventral lobes, with pointed tip, separated by a deep central cleft (Figs. 1a, 2a). At the level of this central cleft a longitudinal groove, as an inverted "Y", runs down to the base of the oral sucker (Figs. 1a, 2a). Oral sucker longer than wide, 0.64–0.99 (0.81) long by 0.33–0.88 (0.56) wide. Ventral sucker round 0.55–0.92 (0.72) long by 0.56–0.95 (0.74) wide, in anterior third of body. Prepharynx 0.45–0.80 (0.60) long. Pharynx well-developed 0.24–0.44 (0.32) long by 0.26–0.36 (0.32) wide. Sucker width ratio 1: 0.98-1.20 (1:1.12). Oesophagus short 0.46–0.62 (0.52) long. Intestine bifurcates anterior to cirrus-sac forming broad caeca with irregular contour that unite posterior to testes forming a single cecum, which opens through a rectum with funnel-shaped muscular sphincter into the anus (Figs. 1b, 3b). Excretory pore opens posterior to anus. The anus and excretory pore open in a terminal common cavity. Testes slightly lobed, posterior to midbody, intercaecal. Anterior testis 0.93–1.60 (1.06) long by 0.60–0.82 (0.68) wide; posterior one 0.87–1.38 (1.15) by 0.44–0.76 (0.63). Distance from posterior testis to posterior end of body 1.60–2.74 (2.23). Cirrus sac preacetabular, between the ventral sucker and caecal bifurcation contains tubular and coiled seminal vesicle, large pars prostatica and muscular ejaculatory duct (Figs. 1c, 2d). Ovary round, pretesticular, postequatorial, 0.36–0.52 (0.43) long by 0.33–0.52 (0.41) wide. Seminal receptacle round, large 0.22–0.40 (0.33) long by 0.30–0.35 (0.32) wide, posterior to ovary. Laurer's

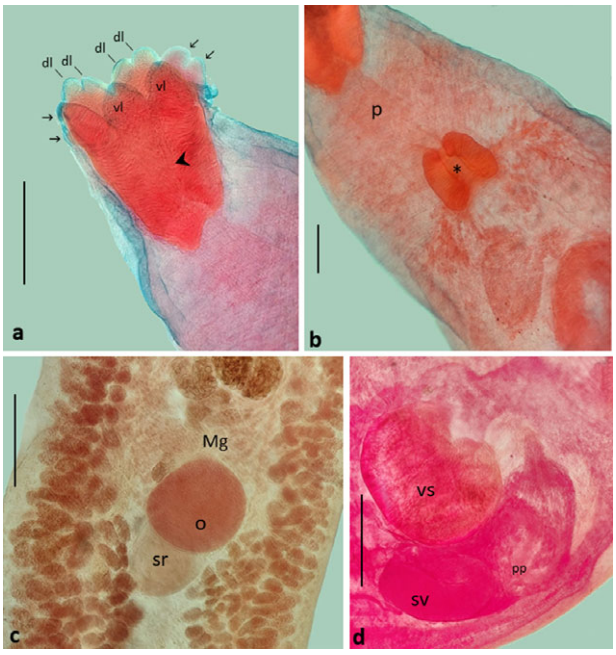

Figure 2. *Enenterum gomesae* n. sp. a. Detail of oral sucker terminal bordered by 10 lobes, being two pairs of dorsal lobes (dl), two lateral pairs (thin arrow) and one pair of strong ventral lobes (vl). A longitudinal groove, as an inverted "Y", runs down to the base of the oral sucker (arrow head). Bar 0.5 mm. b. Detail of prepharynx (p) and pharynx (asterisk). Bar 0.3 mm. c. Detail of ovary (o) and seminal receptacule (sr). Mg, Mehlis' gland. Bar 0.5 mm. d. Cirrus-sac with a coiled seminal vesicle (sv) and pars prostatica (pp). vs, ventral sucker. Bar 0.17 mm.

canal present. Mehlis' gland anterior to ovary (Figs. 2c, 3a). Uterus pre-ovarian; metraterm long, muscular, passes dorsal to acetabulum to open in the genital pore at the genital atrium. Vitellarium follicular extends from posterior end of ventral sucker to almost the body end (ventral and lateral to caeca in uterine region; ventral, lateral and dorsal to caeca posteriorly) (Figs. 3b-c). Eggs numerous 0.06-0.07 × 0.03-0.04 (0.06 × 0.03). In total, six new sequences were generated for this study: two partial 18S rDNA, two partial 28S

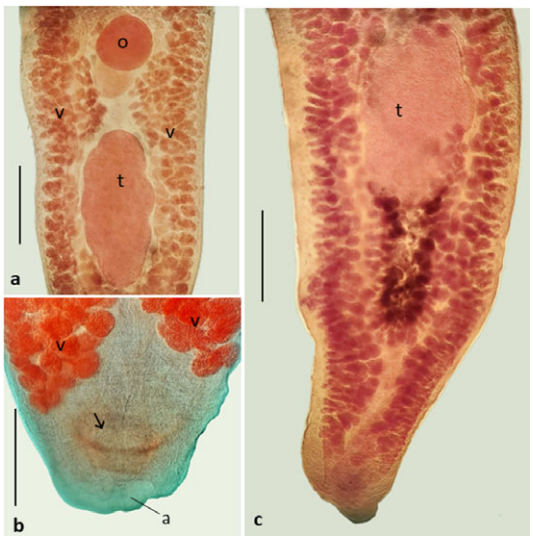

Figure 3. *Enenterum gomesae* n. sp. a. Detail of anterior testis (t). o., ovary; v, vitelline follicles. Bar 0.55 mm. b. Detail of posterior region showing the presence of a muscular sphincter and the anus v, vitelline follicles. Bar 0.55 mm. c. Posterior region showing the distance from the posterior testis to the end of the body. Bar 0.55 mm.

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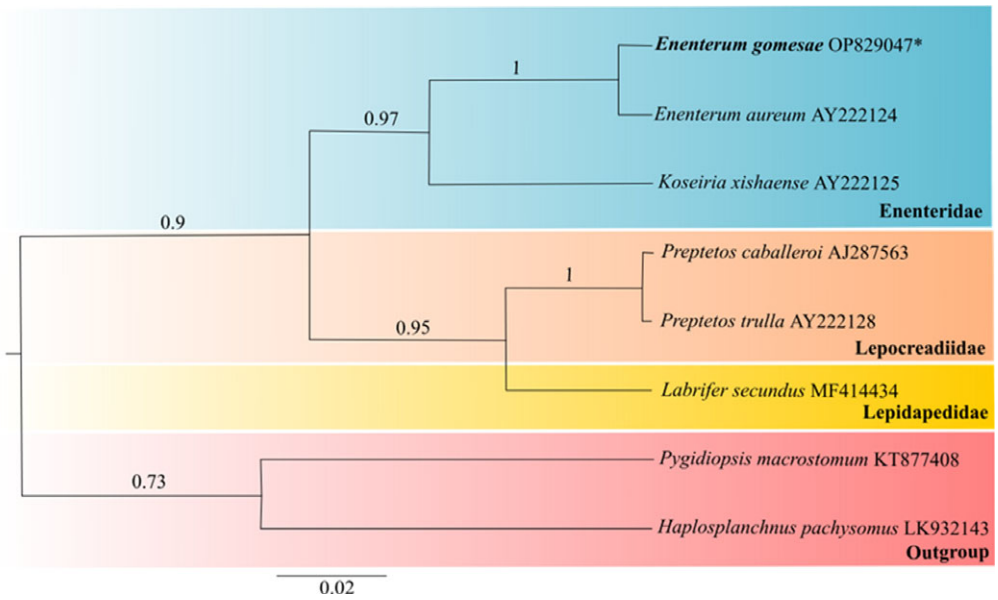

Figure 4. Bayesian phylogenetic topology with posterior probabilities indicating node support based on the 18S rDNA to show the relationships of *Enenterum gomesae* n. sp. with other Enenteridae, Lepocreadiidae and Lepidapedidae species. The GenBank accession numbers are shown, and the scale bar indicates the nucleotide mutations per site. *New sequence data.

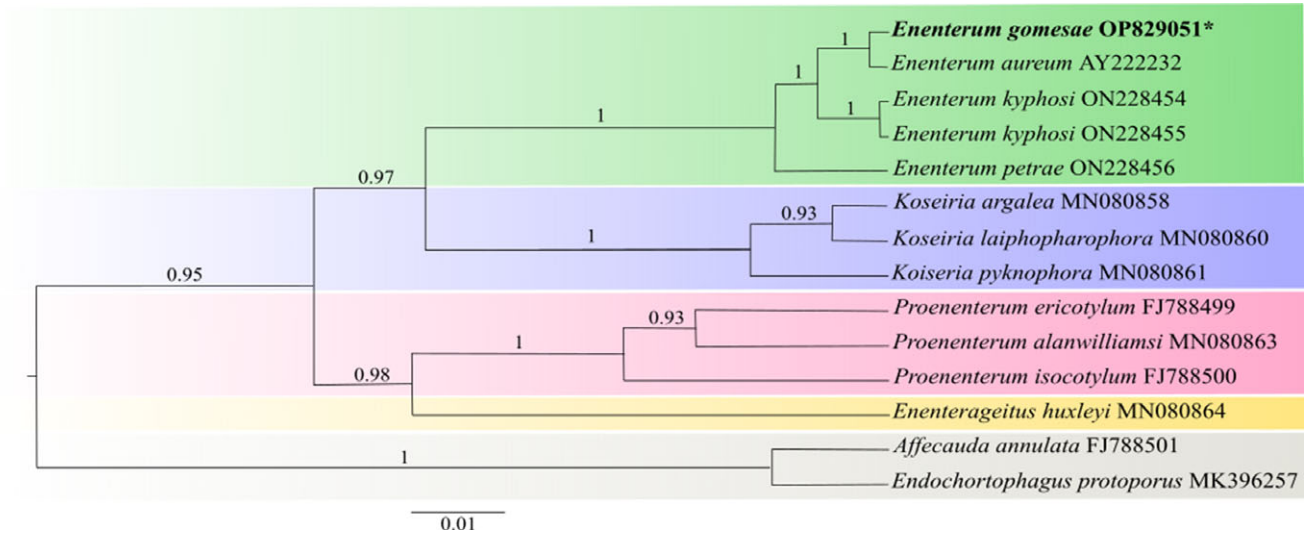

Figure 5. Bayesian phylogenetic topology with posterior probabilities indicating node support based on the 28S rDNA gene to show the relationship of *Enenterum gomesae* n. sp with other species of Enenteridae. The GenBank accession numbers are shown, and the scale bar indicates the nucleotide mutations per site. *New sequence data.

rDNA and two ITS1-5.8S-ITS2 sequences. The 18S rDNA sequences of *Enenterum gomesae* n. sp. were 406 bp long in both sequences (GenBank OP829047 and OP829048), the 28S sequences were 1076 and 1075 bp long (GenBank OP829051 and OP829052) and the ITS1-5.8S-ITS2 sequences were 985 and 1002 bp long (GenBank OP829053 and OP829054). There was no genetic variation between the new sequences generated in all regions.

The partial 18S rDNA sequence of *E. gomesae* n. sp. indicated similarity of 99.02% with *E. aureum* (AY222124). The K2p distance between these species was 0.74%, with four divergent nucleotides in a 406 bp. The partial 28S rDNA sequence of *E. gomesae* n. sp. indicated 99.72% similarity with *E. aureum* (AY222232); the K2p distance was 0.28% with three divergent nucleotides in a 1075 bp. The 5.8S-ITS2 sequence of *E. gomesae* n.sp. indicated 95.87% similarity with *Enenterum kyphosi* Yamaguti, 1970 (ON228452) with K2p distance of 4.27%, with 17 divergent nucleotides in a 388 bp.

The Bayesian phylogenetic 18S rDNA tree showed that *E. aureum* was the closest species to the new sequence, with a node support of one, and in the same clade as *Koseiria xishaensis* Gu Shen, 1983. Both genera, belonging to Enenteridae, were separated from species of Lepocreadiidae Odhner, 1905 and Lepidapedidae Yamaguti, 1958 (Fig. 4). The topologies of the 28S rDNA and 5.8S-ITS2 trees were similar and the new sequences formed a clade with *E. aureum*, *E. kyphosi* and *Enenterum petrae* Huston, Cutmore & Cribb, 2022, with the exception of *E. aureum* for 5.8S-ITS2, for which there is no sequence available to date. The *Enenterum* clade was separated from other genera of the Enenteridae, including

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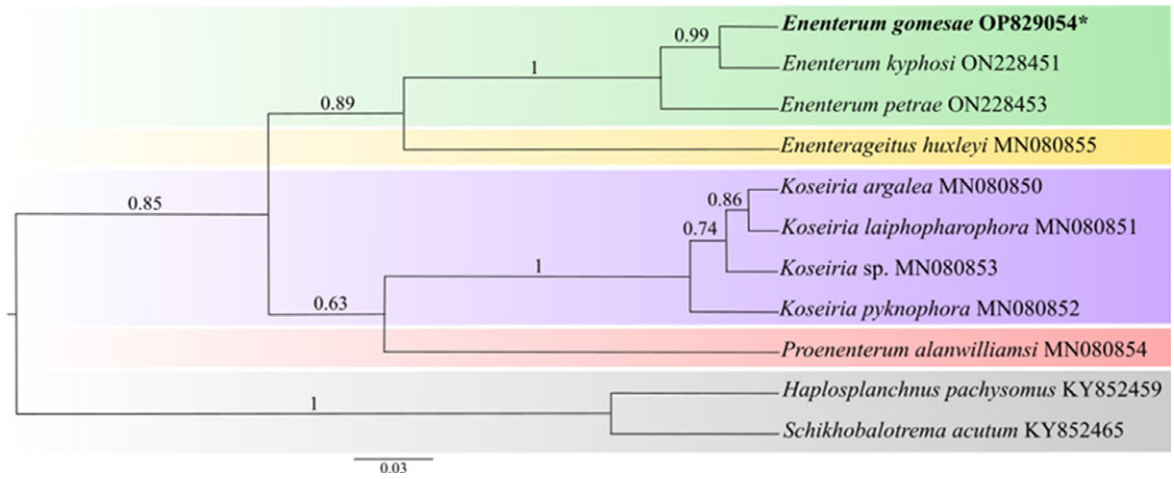

Figure 6. Bayesian phylogenetic topology with posterior probabilities indicating node support based on the 5.8S-ITS2 region to show the relationship of *Enenterum gomesae* n. sp. with other species of Enenteridae and Lepocreadiidae. The GenBank accession numbers are shown, and the scale bar indicates the nucleotide mutations per site. *New sequence data.

Koseiria Nagaty, 1942, *Proenenterum* Manter, 1954 and *Enenterageitus* Huston, Cutmore & Cribb, 2019 (Figs. 5-6).

Type host. Kyphosus incisor

Type locality. Jurujuba Beach, Niterói, RJ.

Site of infection. Intestine.

Intensity. Two fish with eight and 18 specimens each.

Additional material studied. CHIOC 31012a-h

Deposition of types. CHIOC 40451 a (holotype) and 40451b-h.… (paratypes) and 40452 a-j (voucher).

Etymology. The specific name of this species is in honour of Dr Delir Correa Gomes Maués da Serra Freire for her contribution to the study of Helminthology in Brazil.

Remarks

The main diagnostic characters of the new species include 10 lobes on the oral sucker, prepharynx two times longer than pharynx, testes slightly lobed, ovary round, and presence of a rectum with muscular funnel-shaped sphincter. *E. pimelopteri* previously reported by Gomes *et al.* (1974) from Rio de Janeiro and reassigned to *E. aureum* by Bray (1978) is now considered *E. gomesae* n. sp. The species of *Enenterum* which have 10 lobes on the oral sucker include *E. aureum*, *E. pimelopteri*, *Enenterum elongatum* Yamaguti, 1970, *E. kyphosi* and *Enenterum ghardaguensis* Saoud & Ramadan, 1985. The new species is closer to *E. aureum* from *K. sectatrix* from Florida, which can be distinguished by having a longer prepharynx, while in *E. aureum* the prepharynx is about the same length of pharynx when extended. *Enenterum gomesae* n. sp. presents an oesophagus (absent in *E. aureum*), the testes are slightly lobed and ovary is round, while in *E. aureum* the testes are lobed and the ovary is slightly lobed. Besides this, *E. gomesae* n. sp. has a rectum with muscular funnel-shaped sphincter connected to the anus and the ventral sucker is longer than in *E. aureum. Enenterum gomesae* n. sp. can be distinguished from *E. pimelopteri* from *K. cinerascens*, from Red Sea by the prepharynx longer than oesophagus (in *E. pimelopteri* oesophagus is longer than prepharynx) and the testes of *E. pimelopteri* are entire (not lobed). The new species differs from *E. elongatum*, a parasite from *K. cinerascens* off Hawaii and *Kyphosus sydneyanus* (Günther) from Australia, by the size of oesophagus (300-507 *vs.* 460-620) and position of ovary near midbody, while in *E. gomesae* n. sp. it is postequatorial. *Enenterum gomesae* n. sp. differs from *E. kyphosi* originally found in *K. cinerascens* off Hawaii, by presenting a larger length of body (10.290–14.800 *vs.* 9.04–13.90), a larger prepharynx (0.50–0.70 *vs.* 0.12–0.45) and larger oesophagus (0.30–0.51 *vs.* 0.10–0.20), and by presenting testes slightly lobed (*vs.* deeply lobed in *E. kyphosi*). *Enenterum ghardaguensis* from *K. cinerascens* (=*Pimelopterus tahmel*) off the Red Sea differs from *E. gomesae* n. sp. by the smaller length of body, by presenting a small prepharynx (0.03–0.04 *vs.* 0.45–0.80 in *E. gomesae* n. sp.) and oesophagus (0.05 *vs.* 0.30–0.51 in *E. gomesae* n. sp.). The testes are deeply lobed, while in *E. gomesae* n. sp. testes are slightly lobed.

Discussion

The genus *Enenterum* was erected by Linton in 1910 with *E. aureum* from *Kyphosus sectatrix* from Florida as type species. Now, this genus comprises 12 valid species: *E. pimelopteri*, *E. elongatum*, *E. kyphosi*, *Enenterum elsti* Bray, 1978, *Enenterum prudhoei* Bray, 1978, *Enenterum mannarense* Hafeezullah, 1980, *E. ghardaguensis*, *Enenterum stinkvis* Bray, 1986, *Enenterum tongaatensis* Bray, 1986, *E. petrae* and *E. gomesae* n. sp. (Nagaty, 1942; Yamaguti, 1970; Zaidi & Khan, 1977; Bray, 1978; Hafeezullah, 1980; Saoud & Ramadan, 1985; Bray, 1986; Huston *et al.*, 2022). Two other species, *Enenterum minutum* Yadav, 1977 and *E. theraponii* have been considered with a doubtful status (Bray, 1986).

The Bayesian-inference analysis of the 28S rDNA dataset resulted in a phylogram in which the four Enenterid genera (*Enenterum*, *Koseiria*, *Proenenterum* and *Enenterageitus*) are in different clades, similar to that observed by Huston *et al.* (2019). In our 5.8S-ITS2 Bayesian-inference analysis, this pattern was also observed. However, the genera closest to *Enenterum* was *Koseiria* in the 28S phylogenetic tree, while for the 5.8S-ITS2 analysis, it was *Enenterageitus. Enenterogeitus huxleyi* was transferred from *Koseiria* by Huston *et al.* (2019). These four genera can be easily differentiated by the morphology of oral sucker, presence/absence of anus and presence/absence of muscular post-oral ring. In our phylogenetic analyses of 28S rDNA, *E. huxleyi* was found sister to, but distinct from, *Proenenterum*, similar to that demonstrated by Huston *et al.* (2019, 2022). However, our ITS analysis showed *E. huxleyi* as sister to the *Enenterum* clade. Future studies are suggested to better understand the relationship between these genera.

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One of the main characteristics of the genus *Enenterum* is the shape of the oral sucker with variable number of lobes in the anterior margin. Some species have 10 lobes (*E. aureum*, *E. pimelopteri*, *E. elongatum*, *E. kyphosi*, *E. ghardaguensis* and *E. gomesae* n. sp.), others have eight (*E. stinkvis* and *E. prudhoei*), seven (*E. elsti*), six (*E. petrae* and *E. mannarense*), and two lobes (*E. tongaatensis*).

Species with eight oral lobes like *E. prudhoei* were reported from southwestern Indian Ocean and *E. stinkvis* from *Neoscorpis lithophilus* (Gilchrist & Thompson) from South Africa. *Enenterum elsti* with about seven irregularly conical projections and *E. tongaatensis* with four lobes were also described from *N. lithophilus* from South Africa. *Enenterum petrae* which appears to have three-lobed when protracted and six-lobed oral sucker when retracted was described from *K. vaigiensis* from Australia. *Enenterum mannarense* also described with six pointed oral lobes was found in Kyphosidae from Australia and India. *Enenterum theraponii* described by Zaidi & Khan (1977) from the intestine of *Terapon jarbua* (Forsskål, 1775) from the Arabian was considered *incertae sedis* by Gibson & Bray, 1982.

Considering the closest species with 10 oral lobes, *E. aureum* was considered a cosmopolitan species based on their attributed wild geographical distribution ranging from Gulf of Mexico, Caribbean Sea to French Polynesia, Great Barrier Reef, Indian Ocean and Tropical Eastern Pacific (Bray & Cribb 2001, 2002). The specimens from Australia and French Polynesia presented prepharynx smaller than pharynx, smaller bodies length, ventral suckers, testes and ovary, apart from their geographical distribution. The full picture of the entire concept of *E. aureum* would include some populations from the Western Indo-Pacific but also from the Eastern Indo-Pacific realms that we believe need to be revised.

The concept of species delineation over geographic range was discussed by Huston *et al.* (2021) considering *Gorgocephalus yaaji* Bray & Cribb, 2005, which parasitises kyphosid fish in an expansive marine ecoregion stretching from the east coast of Africa to Australia and French Polynesia (see figure 1 in Huston *et al.*, 2021). They reported molecular variation suggesting the possibility of multiple species with specimens morphologically indistinguishable from *G. yaaji*. They concluded that additional specimens collected between Australia and South Africa would be necessary to split *G. yaaji* into multiple morphologically cryptic species. The marine ecoregion reported for *G. yaaji* shares not only the Western, Central Indo-Pacific and Eastern Indo-Pacific realms, after the Marine Ecoregions of the world (Spalding *et al.*, 2007), giving additional evidence for future new species to be described.

Enenterum gomesae n. sp., for instance, is described from the lowest level of the Tropical Southwestern Atlantic realm, far distant from the type locality of *E. aureum* from Florida, which is in the Tropical Northwestern Atlantic realm, both well-separated by the North Brazil Shelf (see maps in Spalding *et al.*, 2007). We believe that the *E. aureum* population described from Western Indo-Pacific and the Eastern Indo-Pacific must contain several cryptic species, as they do not present monophyly and have a variety of hosts. That's why we focused on the North Atlantic original description of *E. aureum* which is probably distributed only in the Atlantic and the other *E. aureum*–type worms can constitute a group of cryptic species, with few morphological differences.

Bray (1978) considered *E. pimelopteri*, with 10 oral lobes, reported by Fischthal & Thomas (1972) and Gomes *et al.* (1974) from Senegal and Brazil, respectively, a synonym from *E. aureum*. In the Pacific and Atlantic Oceans, additional references included Winter (1957), Sogandares-Bernal (1959), Overstreet (1969) and Pérez-Ponce de León *et al.* (2007).

A review of the specimens reported by Gomes *et al.* from Rio de Janeiro showed that although the oesophagus was contracted in their specimens, in the fresh material now collected from *K. incisor* the oesophagus ranged from 0.46 to 0.62 (0.52). The testes of *E. gomesae* n. sp. are much larger than in the type of *E. aureum* from Florida based on its original figure (0.93–1.6 vs. 0.70). The ratio between the length of the ventral and oral suckers also differed, being 1:1.25–1.66 in *E. aureum* and 1:0.98–1.20 (1.12) in *E. gomesae* n. sp. Additionally, the results of our molecular phylogenetic analyses of 18S and 28S rDNA place *Enenterum gomesae* n. sp. as sister of *E. aureum* from *K. vaigiensis* from French Polynesia (Table 2). Therefore, we describe here that *Enenterum gomesae* n. sp. representing the only species described so far in South America. Other species of *Enenterum* observed in 28S rDNA and the 5.8S-ITS2 trees were in the same clade as *Enenterum gomesae* n. sp. but on different and well-supported branches.

Enenterum gomesae n. sp. is described based on morphological differences, genetic data and distribution on well separated marine ecoregion realms. New sequences of *E. aureum* from type–host and locality are necessary for future comparison with *E. gomesae* n. sp. and entire concept of *E. aureum.* To date, there is scarce molecular data available for *Enenterum* species, limiting the understanding of the phylogenetic relationship of this family. The new sequences generated, the partial 28S and 18S rDNA genes and the ITS1-5.8S-ITS2 of *E. gomesae* n. sp., contribute to further comprehension of this group.

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