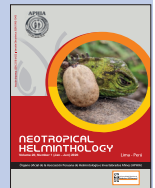




Neotropical Helminthology



ORIGINAL ARTICLE / ARTÍCULO ORIGINAL

NEW MORPHOLOGICAL AND MOLECULAR DATA ON *LECITHOCLADIUM EXCISUM* (RUDOLPHI, 1819) LÜHE, 1901 (DIGENEA: HEMIURIDAE) FROM BRAZIL: SEM ANALYSIS, NEW HOST RECORD, AND SYNONYMY WITH *LECITHOCLADIUM CHAETODIPTERI*

NUEVOS DATOS MORFOLÓGICOS Y MOLECULARES SOBRE *LECITHOCLADIUM EXCISUM* (RUDOLPHI, 1819) LÜHE, 1901 (DIGENEA: HEMIURIDAE) DE BRASIL: ANÁLISIS MEDIANTE MEB, NUEVO REGISTRO DE HOSPEDADOR Y SINONIMIA CON *LECITHOCLADIUM CHAETODIPTERI*

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ABSTRACT

This study aims to enhance the understanding of the parasitic digenean species *Lecithocladium excisum* (Rudolphi, 1819) Lühe, 1901 from *Chaetodipterus faber* (Broussonet, 1782) (Acanthuriformes: Ehippidae) in Brazil. The research uses scanning electron microscopy and molecular analyses. For electron microscopy, collected specimens were dehydrated through a graded ethanol series, critical point dried with carbon dioxide, and coated with gold. DNA was extracted and amplified using the partial 28S rDNA primer pair D1–D3 and the mitochondrial primer pair JB3 and CO1-R trema. Phylogenetic analyses were performed using maximum likelihood and Bayesian inference methods. Observations from electron microscopy corroborated those described in stained whole mounts in previous literature. Additionally, they revealed novel morphological features. Molecular and phylogenetic analyses demonstrated and supported that *Lecithocladium chaetodipteri* Amato, 1983 is a synonym of *L. excisum*. Analysis of morphometric data from the literature further corroborates this finding. This study also reports a new host record for *L. excisum*.

Keywords *Chaetodipterus faber* – digeneans – Integrative taxonomy – *Lecithocladium chaetodipteri* – *Lecithocladium excisum* – synonymy

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RESUMEN

Este estudio tiene como objetivo ampliar el conocimiento de la especie de digeneo parásito *Lecithocladium excisum* (Rudolphi, 1819) Lühe, 1901 en *Chaetodipterus faber* (Broussonet, 1782) (Acanthuriformes: Ephippidae) en Brasil, mediante microscopía electrónica de barrido y análisis moleculares. Para la microscopía electrónica de barrido, los especímenes recolectados fueron deshidratados en una serie creciente de etanol, secados al punto crítico con dióxido de carbono y recubiertos con oro. El ADN fue extraído y amplificado utilizando el par de cebadores parciales de 28S rDNA D1–D3 y el par mitocondrial JB3 y CO1-R. Los análisis filogenéticos se realizaron mediante métodos de máxima verosimilitud e inferencia Bayesiana. Las observaciones de la microscopía electrónica de barrido corroboraron las descritas en preparados teñidos de estudios previos, además de revelar características morfológicas novedosas. Los análisis moleculares y filogenéticos demostraron y respaldaron que *Lecithocladium chaetodipteri* Amato, 1983 es sinónimo de *L. excisum*. El análisis de datos morfométricos disponibles en la literatura corrobora este hallazgo. Además, este estudio registra un nuevo hospedador para *L. excisum*.

Palabras clave: *Chaetodipterus faber* – digeneos – *Lecithocladium chaetodipteri* – *Lecithocladium excisum* – Taxonomía integrativa – sinonimia

INTRODUCTION

Species of the genus *Lecithocladium* Lühe, 1901 (Digenea: Hemiuridae) are parasites of marine fishes, widely reported from the Atlantic Ocean and the Mediterranean Sea but poorly studied in the South Atlantic. Their taxonomy is challenging because of considerable morphological variability, overlapping morphometric ranges, and the scarcity of detailed redescrptions. Traditionally, species identification has relied on characters such as sucker ratio, development of the ecsoma, and the arrangement of reproductive organs. However, these traits often display high intraspecific variation, making species delimitation difficult (Lühe, 1901; Looss, 1907; Yamaguti, 1958; Gibson & Bray, 1986).

In recent decades, molecular phylogenetics has significantly improved the resolution of digenean taxonomy, helping to clarify relationships and confirm species identities (Pérez-Ponce de León & Hernández-Mena, 2019). Nevertheless, representatives of *Lecithocladium* remain underrepresented in genetic databases, and molecular data from South Atlantic populations are lacking. This gap restricts our understanding of their diversity, host associations, and biogeographic distribution.

In this study, we provide an integrative taxonomic approach to *Lecithocladium excisum* (Rudolphi, 1819) Lühe, 1901 recovered from the Atlantic spadefish *Chaetodipterus faber* (Broussonet, 1782) (Ephippidae) in Sepetiba Bay, southeastern Brazil. We combine detailed morphological observations, including scanning electron microscopy (SEM), with molecular analysis of 28S rDNA and CO1 mtDNA sequences to confirm species identity and assess

phylogenetic relationships within the Hemiuridae. This represents the first molecular evidence of *L. excisum* from the South Atlantic and contributes to a more comprehensive understanding of its taxonomy and distribution.

MATERIALS AND METHODS

Host collection and examination

Specimens of the Atlantic spadefish *C. faber* (Ephippidae) were obtained from artisanal fishermen operating in Sepetiba Bay, southeastern Brazil (22°57'18"S, 43°54'44"W). Fish were transported on ice to the laboratory and examined fresh whenever possible; some were stored frozen at –20 °C until analysis. Fish were identified following Menezes & Figueiredo (1985 and Carvalho-Filho 1999).

Parasitological procedures

The digestive tract of each fish was removed and examined under a stereomicroscope. Trematodes were isolated, washed in saline, and fixed in AFA solution (2% acetic acid, 3% formaldehyde, 95% ethanol). Specimens were preserved in 70% ethanol for subsequent scanning electron microscopy (SEM) and molecular analyses. For SEM, specimens were dehydrated in graded ethanol, critical-point dried, sputter-coated with gold, and examined with a JEOL JSM-6390 microscope (Tokyo, Japan) at the Rudolf Barth Electron Microscopy Platform of Oswaldo Cruz Institute (IOC), Rio de Janeiro, Brazil. Voucher specimens of whole mounted slides were deposited in the Helminthological Collection of

the Oswaldo Cruz Institute (CHIOC), under accession numbers 40850 a-b.

Molecular procedures

Genomic DNA was extracted from individual worms using Qiagen QIAamp DNA Mini Kit, following the manufacturer's protocol. Amplification of the 28S rDNA was performed through three reactions: LSU5 and ECD2, LSU5 and 1200R, and LSU5 and 1500R (Pankov *et al.*, 2006). PCR reactions were performed in 25 μ L volumes containing 12.5 μ L of Promega PCR Master Mix (50 units/mL Taq DNA polymerase, 400 μ M of each dNTP, 3 mM MgCl₂), 0.5 μ L of each primer, 8.5 μ L ultrapure water, and 3 μ L DNA template. The thermal cycling profile followed Machado *et al.* (2024). CO1 mtDNA amplification was conducted using primers JB3 and CO1-R trema (Bowles & McManus, 1993; Miura *et al.*, 2005), in 25 volumes reactions containing 12.5 μ L Promega Master Mix, 1.2 μ L of each primer, 8.1 μ L ultrapure water, and 2 μ L DNA template. Mitochondrial PCR thermal cycling profile followed Miura *et al.* (2005).

PCR products were purified using Qiagen QIAquick PCR Purification Kit. Sequencing was performed using the Big Dye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) on an ABI 3730 DNA Analyzer, part of the RPT01A platform of IOC's Technological Platforms Network. Contigs were assembled and edited using Geneious R9.1 (Kearse *et al.*, 2012), producing consensus sequences. These were deposited in GenBank under accession numbers PX446276 (28S rDNA) and PX504246 (CO1 mtDNA) (*L. excisum*); and PX446277 (28S rDNA) PX503674 (CO1 mtDNA) (*L. excisum* with retracted ecsoma).

Phylogenetic analyses

Sequences were edited and aligned in MEGA 11 (Tamura *et al.*, 2021) using ClustalW with default parameters. Phylogenetic analyses were conducted with maximum likelihood (ML) and Bayesian inference (BI). ML trees were built using MEGA 11 with 1,000 bootstrap replicates, while BI analyses were run in MrBayes 3.2 (Ronquist *et al.*, 2012) through via CIPRES Science Gateway (Miller *et al.*, 2010). The best-fitting substitution model (GTR+G+I) was selected using MEGA 11. Sequences from Hemiuridae and Lecithasteridae were included, with Bunocotylidae species as outgroup, in 28S rDNA analyses (table 1). In CO1 mtDNA analyses, sequences from Hemiuridae (table 2) were similarly analyzed, with two Lecithasteridae species as outgroups.

Ethics declaration: All applicable institutional, national, and international guidelines for the care and

use of animals were followed. As no live animals were used and the fish specimens were obtained from a local market, approval from an ethics committee was not required, in accordance with Brazilian Law No. 11.794, Article 3, Item III.

RESULTS

SEM

Body elongated, with the greatest width occurring just before the ecsoma (Fig. 1a). Ecsoma elongated (Fig. 1a). Tegument plicated (Fig. 1a, 1d), with prominent folds observed in the dorsal region between the suckers (Fig. 1b). Tegument of ecsoma plicated, although the plications are smaller than those on the main body (Fig. 1d–f). Oral sucker is subterminal, funnel-shaped, and features two lateral notches delimiting a ventral lip (Fig. 1c). Dorsal tegument between the oral and ventral suckers displays a pronounced concavity (Fig. 1c). Ventral sucker is located anterior to midbody and presents a symmetrical form (Fig. 1c). Excretory pore terminal (Fig. 1f).

Phylogenetic analyses

The 28S rDNA gene matrix included 35 taxa and 1,291 characters, of which 712 were conserved and 487 were parsimony-informative. Bayesian analysis yielded a mean marginal likelihood of -9264.1401 and a median of -9263.786 . The effective sample size (ESS) for all parameters was 6,309. Maximum likelihood (ML) and Bayesian inference (BI) analyses produced similar topologies with minimal variation in node support values (fig. 2). Specimens of *L. excisum* and *L. excisum* with retracted ecsoma sequenced in this study clustered with a previously available *L. excisum* sequence with high ML bootstrap support (100%) and high Bayesian posterior probability (BBP = 1).

The CO1 mtDNA gene matrix included 14 taxa and 802 characters, of which 240 were conserved and 437 were parsimony-informative. Bayesian analysis yielded a mean marginal likelihood of -5138.5314 and a median of -5138.188 . ESS for all parameters was 14,460.4. ML and BI analyses produced similar topologies with minor differences in node support values and branch structure (fig. 3). *L. excisum* and *L. excisum* with retracted ecsoma clustered together with high ML bootstrap support (98%) and BBP = 1. Intraspecific genetic distances between both morphotypes were 0.000 for 28S rDNA and 0.010 for CO1 mtDNA sequences.

Table 1. Specimens included in the molecular analyses associated with their GenBank accession numbers for the 28S rDNA sequence, host and locality.

Family	Species	Access Number in GenBank	Host	Locality	Reference
Hemiuridae	<i>Aphanurus mugilus</i> Tang, 1981	LT607809	<i>Osteomugil ergeli</i> (Bleeker, 1858)	Cat Ba Island, Vietnam	Atopkin <i>et al.</i> (2017)
	<i>Brachyballus crenatus</i> (Rudolphi, 1802) Odhner, 1905	MH628299	<i>Sabellinus leucomacris</i> (Pallas, 1814)	Sea of Okhotsk, Western Pacific Ocean	Sokolov <i>et al.</i> (2019)
	<i>Dinosoma synphobranchi</i> Yamaguti, 1938	MH628304	<i>Animora microlepis</i> Bean, 1890	Sea of Okhotsk, Western Pacific Ocean	Sokolov <i>et al.</i> (2019)
	<i>Dimurus longisimus</i> Looss, 1907	AY222202	<i>Coryphaena hippurus</i> Linnaeus, 1758	Port Royal, Jamaica	Olson <i>et al.</i> (2003)
	<i>Ectenurus virgula</i> Linton, 1910	OP918121	<i>Decapterus punctatus</i> (Cuvier, 1829)	Rio de Janeiro coastal zone, Brazil	Pantoja & Kudlai (2022)
	<i>Hemimurus appendiculatus</i> (Rudolphi, 1802) Looss, 1899	KR349121	<i>Alosa fallax</i> (Lacepède, 1803)	Western Iberian	Bao <i>et al.</i> (2015)
	<i>Hemimurus luebei</i> Odhner, 1905	MH628316	<i>Ophidion rochei</i> Müller, 1845	Peninsula Rivers	Sokolov <i>et al.</i> (2019)
	<i>Lectiochirium caesionis</i> Yamaguti, 1942	AY222200	<i>Caesio cunning</i> (Bloch, 1791)	Black Sea, Russia	Olson <i>et al.</i> (2003)
	<i>Lectiochirium floridense</i> (Manter, 1934) Crowcroft, 1946	MK558793	<i>Syacium papillosum</i> (Linnaeus, 1758)	Heron Island, Australia	Vidal-Martinez <i>et al.</i> (2019)
	<i>Lectiochirium microstromum</i> Chandler, 1935	KC985235	<i>Trichurus lepturus</i> Linnaeus, 1758	Yucatan Peninsula, Mexico	Calhoun <i>et al.</i> (2013)
	<i>Lectiochirium muraenae</i> Manter, 1940	OP918128	<i>Gymnothorax vicinus</i> (Castelnau, 1855)	Gulf of Mexico, USA	Pantoja & Kudlai (2022)
	<i>Lectiochirium synodi</i> Manter, 1931	OP918132	<i>Pseudoperis numida</i> Miranda Ribeiro, 1903	Rio de Janeiro coastal zone, Brazil	Pantoja & Kudlai (2022)
	<i>Lectiochirium excisum</i> (Rudolphi, 1819) Lühe, 1901	PX446276	<i>Chaetodipterus faber</i> (Broussonet, 1782)	Rio de Janeiro coastal zone, Brazil	Present study
	<i>Lectiochirium excisum</i> (Rudolphi, 1819) Lühe, 1901 with retracted ecsoma	PX446277	<i>Chaetodipterus faber</i> (Broussonet, 1782)	Rio de Janeiro coastal zone, Brazil	Present study
	<i>Lectiochirium excisum</i> (Rudolphi, 1819) Lühe, 1901	AY222203	<i>Scomber scombrus</i> Linnaeus, 1758	North Sea, United Kingdom	Olson <i>et al.</i> (2003)
<i>Myosaccium ecaude</i> Montgomery, 1957	OP918123	<i>Sardinella brasiliensis</i> (Steindachner, 1879)	Rio de Janeiro coastal zone, Brazil	Pantoja & Kudlai (2022)	
<i>Parahemimurus merus</i> (Linton, 1910) Manter, 1940	OP918125	<i>Harengula clupeiola</i> (Cuvier, 1829)	Rio de Janeiro coastal zone, Brazil	Pantoja & Kudlai (2022)	
<i>Plerurus digitatus</i> (Looss, 1899) Looss, 1907	AY222201	<i>Scomberomorus commerson</i> (Lacepède, 1800)	Heron Island, Australia	Olson <i>et al.</i> (2003)	
<i>Pulmonernis cyanovirellus</i> Coil & Kuntz, 1960	MH628314	<i>Laticauda semifasciata</i> (Reinwardt, 1837)	Ishigaki Island, Japan	Sokolov <i>et al.</i> (2019)	
<i>Tibulovesicula laticauda</i> Parukhin, 1969	OR209733	<i>Hydrophis cyanocinctus</i> Daudin, 1803	Bay of Bengal, Sri Lanka	Martin <i>et al.</i> (2023)	

(continued Table 1)

Table 1. Specimens included in the molecular analyses associated with their GenBank accession numbers for the 28S rDNA sequence, host and locality (continuation).

Family	Species	Access Number in GenBank	Host	Locality	Reference	
Lecithasteridae	<i>Aponurus laguncula</i> Looss, 1907	OQ676198	<i>Trachinotus rhodopus</i> Gill, 1863	Pacific coast, Mexico	Martínez-Flores <i>et al.</i> (2023)	
	<i>Aponurus laguncula</i> Looss, 1907	KU527430	<i>Rhomboplites aurorubens</i> (Cuvier, 1829)	Alabama, USA	Claxton <i>et al.</i> (2017)	
	<i>Aponurus nulli</i> Carreras-Aubets, Repulles-Albelda, Kostadinova & Carrasson, 2011	HQ713441	<i>Mullus barbatus</i> Linnaeus, 1758	Besos, Spain	Carreras-Aubets <i>et al.</i> (2011)	
	<i>Aponurus</i> Looss, 1907 sp.	DQ354368	<i>Mullus surmuletus</i> Linnaeus, 1758	Spain	Pankov <i>et al.</i> (2006)	
	<i>Lecithaster confusus</i> Odhner, 1905	MH625976	<i>Acanthogobius flavimanus</i> (Temminck & Schlegel, 1845)	Southern Russian Far East	Atopkin <i>et al.</i> (2020)	
	<i>Lecithaster gibbosus</i> (Rudolphi, 1802) Lühe, 1901	AY222199	<i>Merlangius merlangus</i> (Linnaeus, 1758)	North Sea, United Kingdom	Olson <i>et al.</i> (2003)	
	<i>Lecithaster mugilis</i> Yamaguti, 1970	LN865021	<i>Moolgarda seheli</i> (Fabricius, 1775)	Cat Ba Island, Vietnam	Besprozvannykh <i>et al.</i> (2017)	
	<i>Lecithaster salmonis</i> Yamaguti 1934	MH625981	<i>Hypomesus japonicus</i> (Brevoort, 1856)	Hokkaido, Japan	Atopkin <i>et al.</i> (2020)	
	<i>Lecithaster sayori</i> Yamaguti, 1938	MH625977	<i>Hemirhamphus marginatus</i> (Forsskål, 1775)	Cat Ba Island, Vietnam	Atopkin <i>et al.</i> (2020)	
	<i>Lecithaster</i> Lühe, 1901 sp.	MH625978	<i>Siganus fuscescens</i> (Houttuyn, 1782)	Cat Ba Island, Vietnam	Atopkin <i>et al.</i> (2020)	
	<i>Lecithaster sudzubensis</i> Besprozvannykh, Atopkin, Ngo, Ermolenko, Van Ha, Van Tang & Beloded, 2016	LN865024	<i>Mugil cephalus</i> Linnaeus, 1758	Kievka River, Russia	Besprozvannykh <i>et al.</i> (2017)	
	<i>Lecithophyllum botryophorum</i> (Olsson, 1868)	AY222205	<i>Alepocephalus bairdii</i> Goode & Bean, 1879	Goban Spur, Northeast Atlantic	Olson <i>et al.</i> (2003)	
	<i>Opisoblecithum micropsi</i> (Zdzitowiecki, 1992) Vainutis & Voronova, 2025	MH628305	<i>Dissostichus mausoni</i> Norman, 1937	Amundsen Sea, Antarctic Ocean	Sokolov <i>et al.</i> (2019)	
	Bunocotylidae (Outgroup)	<i>Saturnius gibsoni</i> Marzoug, Rima, Boutiba, Georgieva, Kostadinova & Pérez-del-Olmo, 2014	KJ010542	<i>Mugil cephalus</i> Linnaeus, 1758	Oran, Algeria	Marzoug <i>et al.</i> (2014)
		<i>Saturnius</i> Manter, 1969 sp.	DQ354366	<i>Mugil cephalus</i> Linnaeus, 1758	Santa Pola, Spain	Pankov <i>et al.</i> (2006)

Table 2. Specimens included in the molecular analyses associated with their GenBank accession numbers for the CO1 mtDNA sequence, host and locality. NA: not available.

Family	Species	Access Number in GenBank	Host	Locality	Reference
Hemiuridae	<i>Ectenurus virgula</i> Linton, 1910	OP948304	<i>Prionotus punctatus</i> (Bloch, 1793)	Rio de Janeiro coastal zone, Brazil	Pantoja & Kudlai (2022)
	<i>Eriplepturus</i> Woolcock, 1935 sp.	OP451903	<i>Luijanus argenteimaculatus</i> (Forskål, 1775)	Kerala, India	NA
	<i>Hemiurus luehei</i> Odhner, 1905	DQ489709	NA	NA	NA
	<i>Lecithochirium</i> cf. <i>muraenae</i> Manter, 1940	OP918023	<i>Gymnothorax vicinus</i> (Castelnau, 1855)	Rio de Janeiro coastal zone, Brazil	Pantoja & Kudlai (2022)
	<i>Lecithochirium floridense</i> (Manter, 1934) Crowcroft, 1946	OP918025	<i>Perophis brasiliensis</i> Quoy & Gaimard, 1825	Rio de Janeiro coastal zone, Brazil	Pantoja & Kudlai (2022)
	<i>Lecithochirium microstomum</i> Chandler, 1935	OP918021	<i>Trichurus lepturus</i> Linnaeus, 1758	Rio de Janeiro coastal zone, Brazil	Pantoja & Kudlai (2022)
	<i>Lecithochirium synodi</i> Manter, 1931	OP918024	<i>Anisotremus virginicus</i> (Linnaeus, 1758)	Rio de Janeiro coastal zone, Brazil	Pantoja & Kudlai (2022)
	<i>Lecithocladium excisum</i> (Rudolphi, 1819) Lühe, 1901	PX504246	<i>Chaetodipterus faber</i> (Broussonet, 1782)	Rio de Janeiro coastal zone, Brazil	Present study
	<i>Lecithocladium excisum</i> (Rudolphi, 1819) Lühe, 1901 with retracted ecsoma	PX503674	<i>Chaetodipterus faber</i> (Broussonet, 1782)	Rio de Janeiro coastal zone, Brazil	Present study
	<i>Stomachicola muraenesocis</i> Yamaguti, 1934	MT682639	<i>Muraenox cinereus</i> (Forskål, 1775)	Bushehr, Iran	Ghanei-Motlagh <i>et al.</i> (2024)
Lecithasteridae (Outgroup)	<i>Tubulovesicula laticauda</i> Parukhin, 1969	OR221149	<i>Hydrophis spinalis</i> (Shaw, 1802)	Sri Lanka	Martin <i>et al.</i> (2023)
	<i>Tubulovesicula lindberg</i> (Layman, 1930) Yamaguti, 1934	PP891444	<i>Nebria microps</i> Cuvier, 1830	Maranhão, Brazil	Pantoja <i>et al.</i> (2024)
	<i>Lecithaster</i> cf. <i>salmonis</i> Yamaguti, 1934	PQ165659	<i>Coregonus lavaretus</i> (Linnaeus, 1758)	White Sea, Russia	Krupenko <i>et al.</i> (2022)
	<i>Lecithasteridae</i> Odhner, 1905 sp.	ON506029	<i>Oncorhynchus keta</i> (Walbaum, 1792)	Primorsky region, Russia	NA

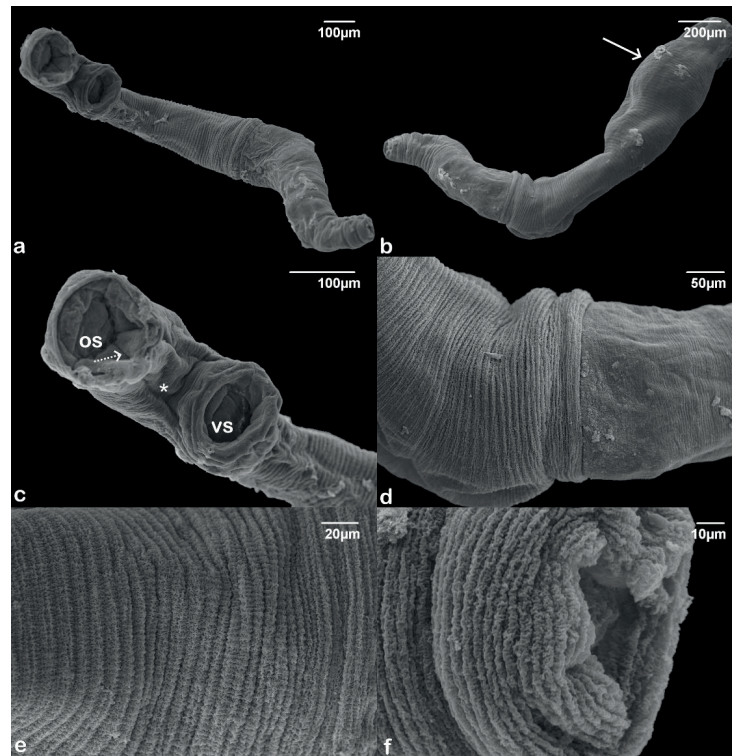


Figure 1. Micrographs of *Lecithocladium excisum* under SEM. **a:** ventral view; **b:** dorsal view indicating the wider plications of the dorsal region of tegument between the suckers (solid arrow); **c:** anterior end in ventral view indicating the lateral incisions of the ventral sucker (dotted arrow) and the tegument concavity between suckers (*), abbreviations os: oral sucker; vs: ventral sucker; **d:** detail of the transition between the soma and ecsoma tegument; **e:** ecsoma tegument; **f:** posterior end, showing detail of the excretory pore.

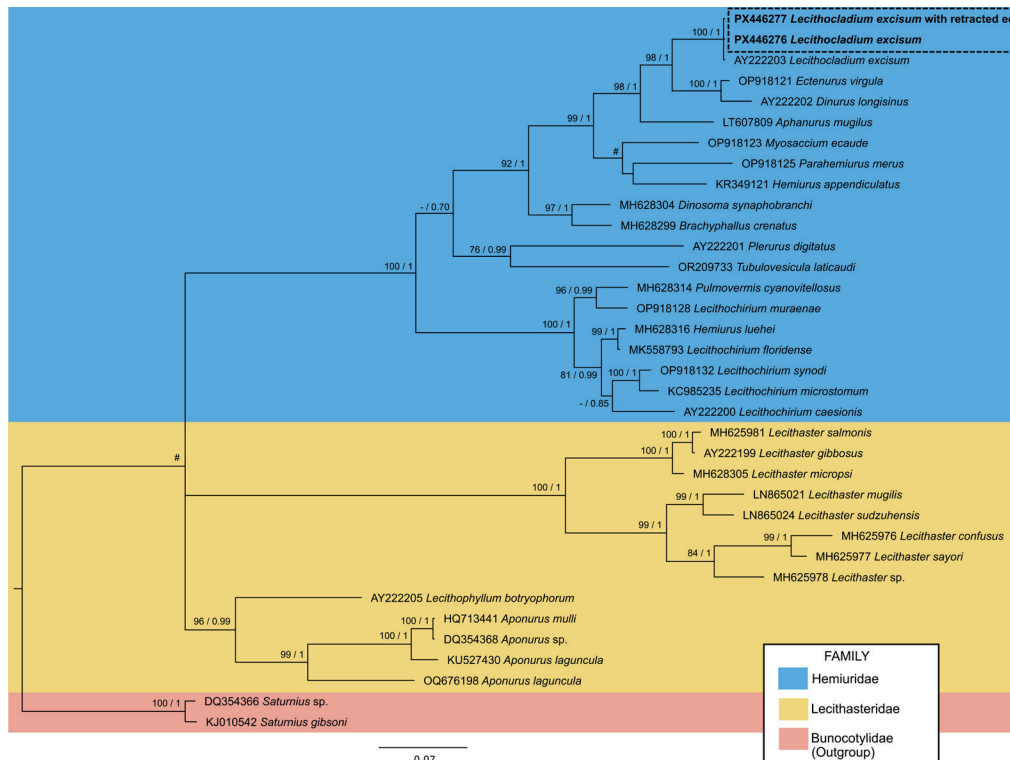


Figure 2. Phylogenetic relationships among selected species sequences from families Hemiuridae, Lecithasteridae and Bunocotylidae based on analyses of partial 28S rDNA. The tree was inferred by using the ML and BI. The nodal support is described at the left by bootstrap replicates and at the right by posterior probability. * Indicates that this node value was not evaluated. - indicates nodal support values below 70 or 0.7. # indicates a distinct clade topology between ML and BI analyses.

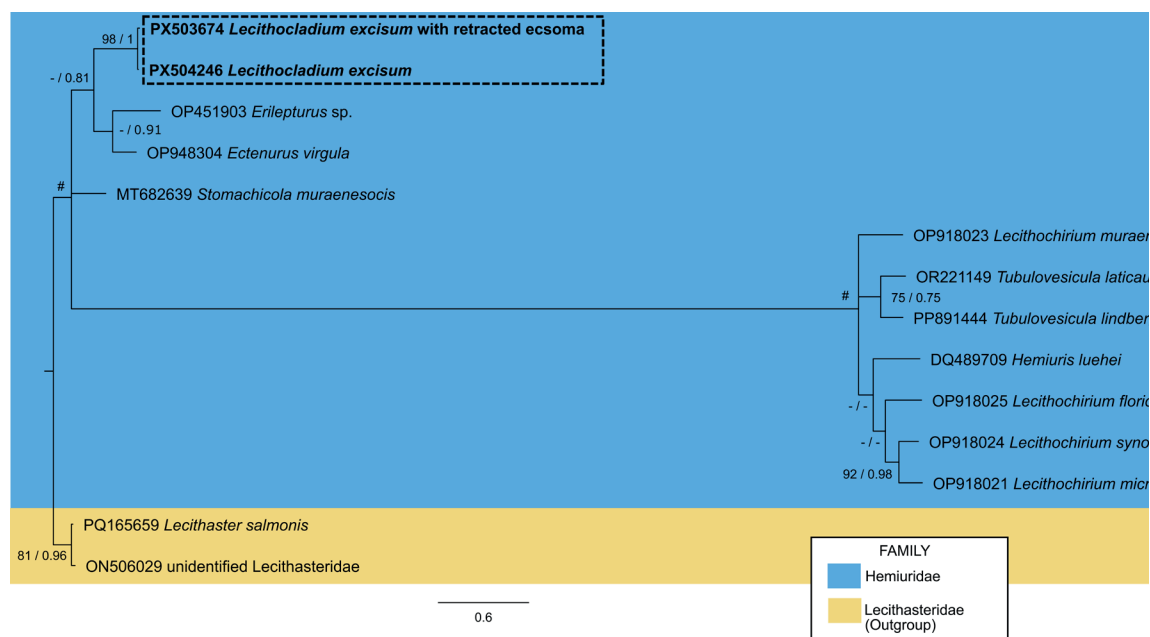


Figure 3. Phylogenetic relationships among selected species sequences from families Hemiuridae and Lecithasteridae based on analyses of COI mtDNA. The tree was inferred by using the ML and BI. The nodal support is described at the left by bootstrap replicates and at the right by posterior probability. * Indicates that this node value was not evaluated. - indicates nodal support values below 70 or 0.7. # indicates a distinct clade topology between ML and BI analyses.

DISCUSSION

The subfamily Elytrophallinae Skrjabin & Guschanskaja, 1954 comprises species characterized by a well-developed ecsoma, a seminal vesicle with a thick muscular wall, and a long tubular sinus-sac (Gibson & Bray, 1979; Gibson *et al.*, 2022). This subfamily was originally proposed as the family *Elytrophallidae* by Skrjabin & Guschanskaja (1954) and was later reclassified as a subfamily within Hemiuridae by Gibson & Bray (1979). The genus *Lecithocladium* was established by Lühe (1901) to accommodate *Lecithocladium excisum*, its type species, which had previously been described as *Distoma excisum* Rudolphi, 1819. The following genera are considered synonymous with *Lecithocladium*: *Bengalotrema* Malhotra *et al.*, 1989; *Cleftocolleta* Sahai & Srivastava, 1978; *Colletostomum* Sahai & Srivastava, 1978; and *Magnapharyngium* Bilquees, 1971 (WoRMS, 2024).

Lecithocladium excisum was originally described from *Scomber scombrus* [as *Scombrus scombrus*] Linnaeus, 1758 and *Scomber colias* [as *Scombrus colias*] Gmelin, 1789, collected in Rimini and Naples, respectively, along the Italian coast (Rudolphi, 1819). Looss (1907) reported additional hosts for juvenile stages, including *Spicara maena* (Linnaeus, 1758) [as *Maena vulgaris*], *Gaidropsarus vulgaris* (Cloquet, 1824) [as *Motella vulgaris*], *Trachurus trachurus* (Linnaeus, 1758) [as *Caranx trachurus*], *Boops boops* (Linnaeus, 1758) [as *Box boops*], *Lophius piscatorius* Linnaeus, 1758,

Spondyliosoma cantharus (Linnaeus, 1758) [as *Cantharus orbicularis*], and *Cepola macrophthalmia* (Linnaeus, 1758) [as *Cepola rubescens*], all from the Mediterranean region.

Further records of *L. excisum* include occurrences in *S. scombrus* from the Baltic Sea (Poland) (Markowski, 1933), Marseille (France) (Mazza, 1963), the Faeroe Isles (NE Atlantic) (Gibson *et al.*, 2002), Categate and Öresund (Denmark) (Koie, 1991), and the Dardanelles (Turkey) (Keser *et al.*, 2007); in *Scomber japonicus* Houuttuyn, 1782 from Toyama Bay, Kyushu, and Sagami Bay (Japan) (Ichihara *et al.*, 1968; Yamaguti, 1934); in *S. japonicus* from Dakar (Senegal) (Ndiaye *et al.*, 2012) and the Aegean Sea (Turkey) (Akmirza, 2013); in *Caesioperca lepidoptera* (Forster, 1801) and *Paraperca colias* (Forster, 1801) from Wellington (New Zealand) (Manter, 1954); in *Peprius triacanthus* (Peck, 1804) from Pensacola Bay (Mexico) (Nahhas & Powell, 1971); and in *Boops boops* (Linnaeus, 1758), *Dentex fillosus* (Rafinesque, 1810), *Scyris alexandrina* (Geoffroy Saint-Hilaire, 1817), *Caranx rhonchus* Geoffroy Saint-Hilaire, 1817, and *Alepes djedaba* (Fabricius, 1775) from Haifa, Tel Baruch, and Jaffa (Israel) (Fischthal, 1980). Additional records include *Mullus surmuletus* Linnaeus, 1758 from Israel (Fischthal, 1980) and the Algerian coast (Brahim *et al.*, 2009), *Trachurus trachurus* (Linnaeus, 1758) from Denmark (Koie, 1991), *Rachycentron canadum* (Linnaeus, 1766) [as *Apolectus niger*] and *Pampus argenteus* (Euphrasen, 1788) from the Hugli Estuary (India)

(Hafeezullah & Dutta, 1998), *Mugil cephalus* Linnaeus, 1758 from Libya (Al-Bassel & Hussein, 2012), *Caranx rhonchus* Geoffroy Saint-Hilaire, 1817 from Senegal (Olson *et al.*, 2003), and *Tenualosa ilisha* (Hamilton, 1822) from West Bengal (Ghosh *et al.*, 2024). In Brazil, it has been reported in *Peprilus paru* from the southeastern coast in Rio de Janeiro (Fabio, 1988). The present study reports *Chaetodipterus faber* as a new host species for this parasite.

SEM analysis confirmed previously described features and revealed a new one: a plicated ecsoma. Lühe (1901), Ichihara *et al.* (1968), Gibson & Bray (1986), and Hafeezullah & Dutta (1998) described a ventral lip delimited by two lateral notches, which was corroborated in our study. A ventral neck concavity was also observable under SEM, consistent with Rudolphi's (1819) original description.

Previous studies have documented a plicated body tegument in *L. excisum* (Rudolphi, 1819; Looss, 1907; Markowski, 1933; Ichihara *et al.*, 1968; Gibson & Bray, 1986; Hafeezullah & Dutta, 1998; Akmirza, 2013), and our SEM analysis confirmed this feature, in agreement with Koie (1991). Our observations also revealed annular plications in the ecsoma tegument, although smaller than those in the soma, contradicting Gibson & Bray's (1986) report of a smooth ecsoma surface. This suggests variability in ecsoma morphology within Hemiuridae. Notably, Koie (1991) did not describe the ecsoma tegument in her SEM study.

Markowski (1933) and Gibson & Bray (1986) reported more pronounced, crenated folds in the dorsal region, particularly at the level of the pharynx, referred to by Looss (1907) as "Krauselung". Our SEM images revealed wider plications in this region, consistent with their observations.

Amato (1983) described *L. chaetodipteri* from *C. faber* in Santa Catarina, and Cezar & Luque (1999) also reported it in *C. faber* from Rio de Janeiro. These are the only two records of this species, with *C. faber* as its sole known host. Amato (1983) distinguished *L. chaetodipteri* from *L. excisum* using sucker size ratios. However, a comparison of morphometric data from the literature (table 3) shows overlapping measurements. Some descriptions of *L. excisum* even report shorter ecsoma lengths (Mazza, 1963; Ichihara *et al.*, 1968; Gibson & Bray, 1986) than those of *L. chaetodipteri* (Amato, 1983).

Moreover, the unflattened *L. excisum* illustrated by Gibson & Bray (1986) closely resembles Amato's original depiction of *L. chaetodipteri*. The observed morphological variations, particularly in ecsoma size and insertion, likely

represent intraspecific variability rather than valid species-level differences, especially since both morphotypes were collected from the same host in this study. Phylogenetic analyses strongly support this interpretation.

Ribosomal and mitochondrial markers were employed to corroborate the synonymy of *L. chaetodipteri* with *L. excisum*. Ribosomal genes are widely used in molecular studies of digenetic trematodes (Perkins *et al.*, 2011; Toledo *et al.*, 2014; Graça *et al.*, 2016; Chan *et al.*, 2021), whereas mitochondrial markers provide greater resolution due to their higher variability (Chan *et al.*, 2021). This study provides the first 28S rDNA sequences of *L. excisum* from *C. faber* and from Brazilian waters, as well as the first CO1 mtDNA sequence for the species. In the 28S rDNA analysis, both morphotypes (*L. excisum* with normal and retracted ecsoma) clustered with a GenBank sequence of *L. excisum* from *Scomber scombrus* (North Sea, United Kingdom) (Olson *et al.*, 2003). No base differences were observed among the three sequences. These specimens, representing Elytrophallinae, formed a strongly supported monophyletic clade with Dinurinae members *Ectenurus virgula* and *Dinurus longisinus*, consistent with previous Hemiuroidea phylogenies based on partial 28S rDNA (Sokolov *et al.*, 2019).

In the CO1 mtDNA analysis, the two specimens also clustered together. Unfortunately, no other mitochondrial sequences are available for comparison within *Lecithocladium*. The eight base differences observed between the two sequences are within the expected range of intraspecific variation. As in the ribosomal phylogenies, Elytrophallinae representatives grouped with Dinurinae, including *Eriolepterus* sp. and *Ectenurus virgula* Linton, 1910.

The present study provides a comprehensive taxonomic update of *L. excisum*, integrating SEM, molecular, and phylogenetic data. It reports a new host species and establishes a new junior synonym. These findings, supported by both ribosomal and mitochondrial evidence, reinforce the interpretation that ecsoma protrusion represents morphological variability within the species. The compilation and comparison of morphometric data from previous studies further support this synonymy. Future molecular studies, particularly those using mitochondrial markers and specimens from different hosts and geographic regions, are essential to clarify intraspecific variation and evolutionary relationships within *Lecithocladium*.

Table 3. Morphometric characteristics (range and average values) from *Lecithocladium chaetodipteri* (first column) and *Lecithocladium excisum* specimens of previous studies. NA: not available.

Parasitic species	<i>Lecithocladium chaetodipteri</i> Amato, 1983		<i>Lecithocladium excisum</i> (Rudolphi, 1819) Lühe, 1901	
Host	<i>Chaetodipterus faber</i> (Broussonet, 1782)	State of Santa Catarina, Southern Brazil	<i>Spicara maena</i> [as <i>Maena vulgaris</i>] (Linnaeus, 1758), <i>Gaidropsarus vulgaris</i> [as <i>Motella vulgaris</i>] (Cloquet, 1824), <i>Trachurus trachurus</i> [as <i>Caranx trachurus</i>] (Linnaeus, 1758), <i>Boops boops</i> [as <i>Box boops</i>] (Linnaeus, 1758), <i>Lophius piscatorius</i> Linnaeus, 1758, <i>Spondylisoma cantharus</i> [as <i>Cantharus orbicularis</i>] (Linnaeus, 1758) and <i>Cepola macrophthalma</i> [as <i>Cepola rubescens</i>] (Linnaeus, 1758)	<i>Caesioperca lepidoptera</i> (Forster, 1801) and <i>Paraperca colias</i> [as <i>Paraperca colias</i>] (Forster, 1801)
Features / Location		NA	Scomber scombrus Linnaeus, 1758	Scomber scombrus Linnaeus, 1758
		NA	Baltic Sea, Poland	Wellington, New Zealand
		NA		Toyama Bay and Kyushu, Japan
Total length	1.590 - 2090	6000 - 8000	6000	4700 - 7500
Total width	540 - 675	500 - 1000	NA	630 - 740
Ecsoma length	910 - 1490	3000 - 4000	NA	NA
Ecsoma width	NA	NA	NA	NA
Oral sucker length	220 - 350	650	225	400 - 450
Oral sucker width	285 - 326	500	585	420 - 460
Pharynx length	204 - 269	500	495	300 - 380
Pharynx width	122 - 163	250	225	200
Ventral sucker length	244 - 261	350 - 450	420	270 - 390
Ventral sucker width	301 - 326		450	NA
Sucker width ratio	1:1	NA	NA	NA
Seminal vesicle length	285 - 457	NA	NA	NA
Seminal vesicle width	NA	NA	NA	470 - 680
Anterior testis length	106 - 122	NA	165	190 - 260
Posterior testis length	89 - 114	NA	360	190 - 280
Anterior testis width	122 - 138	NA	450	NA
Posterior testis width	97 - 122	NA	480	NA
Ovary length	106 - 122	NA	345	210 - 240
Ovary width	163 - 195	NA		290 - 340
Eggs length	18 - 20	NA	19 - 27	18 - 23
Eggs width	10	NA	11	11 - 12
Reference	Amato (1983)	Looss (1907)	Markowski (1933)	Manter (1954)
				Yamaguti (1934)
				Mazza (1963)

Table 3. Morphometric characteristics (range and average values) from *Lecithocladium chaetodipteri* (first column) and *Lecithocladium excisum* specimens of previous studies (continuation).
NA: not available.

Parasitic species		<i>Lecithocladium excisum</i> (Rudolphi, 1819) Lühe, 1901				
Host	<i>Scomber japonicus</i> [as <i>Pneumatophorus japonicus</i>] Houttuyn, 1782	<i>Scomber scombrus</i> Linnaeus, 1758	<i>Peprilus paru</i> (Linnaeus, 1758)	<i>Rachycentron canadum</i> [as <i>Apolectus niger</i>] (Linnaeus, 1766) and <i>Pampus argenteus</i> (Euphrasen, 1788)	<i>Mugil cephalus</i> Linnaeus, 1758	<i>Scomber japonicus</i> Houttuyn, 1782
Features / Location	Sagami Bay, Japan	Faeroe Isles, Northeast Atlantic	Rio de Janeiro, Brazilian Southeast coast	Hugli estuary, West Bengal	Missurata, Libya	Aegean Sea, Turkey
Total length	2000 – 3700	3500 – 7200	3320 – 4810	4880 – 8410	1870 – 1970	1400 – 2300
Total width	340 – 750	600 – 1200	640 – 660	560 – 900	340 – 370	300 – 400
Ecsoma length	750 – 4900	150 – 400	NA	NA	NA	NA
Ecsoma width	NA	NA	NA	NA	NA	NA
Oral sucker length	310 - 540	350 – 650	280 – 370	NA	63 - 64	NA
Oral sucker width	410 - 690	380 – 600	320 – 400	NA	98 - 99	NA
Pharynx length	NA	NA	270 – 370	NA	120 - 160	NA
Pharynx width	NA	NA	130 – 180	NA	180 - 190	NA
Ventral sucker length	230 - 400	300 – 470	290 – 400	NA	88 - 85	NA
Ventral sucker width	220 - 490	350 – 500	280 – 410	NA	62 - 84	NA
Sucker width ratio	NA	1 – 0.8-0.9	1 – 1.05	NA	NA	NA
Seminal vesicle length	270 - 700	NA	280 – 400	NA	NA	NA
Seminal vesicle width	160 - 270	NA	150 – 160	NA	NA	NA
Anterior testis length	120 - 340	230 – 420	200	NA	33 - 34	NA
Posterior testis length	200 - 440	230 – 430	210 – 260	NA	NA	NA
Anterior testis width	90 - 340	150 – 300	240	NA	30 - 32	NA
Posterior testis width	100 - 400	280 – 390	230 – 250	NA	55 - 58	NA
Ovary length	20 - 23	21 – 24	180 – 210	NA	57 - 59	NA
Ovary width	10 - 12.5	11 – 14	240	NA	24 - 25	NA
Eggs length			17 – 19	18 - 20	12 - 13	NA
Eggs width			9 – 12	11 - 12		NA
Reference	Ichihara <i>et al.</i> (1968)	Gibson & Bray (1986)	Fabio (1988)	Hafeezullah & Durta (1998)	Al-Bassel & Hussein (2012)	Akmirza (2013)

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