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Tubulovesicula lindbergi (Layman, 1930) (Digenea: Hemiuridae) in the Southwestern Atlantic Ocean: A Morphological and Phylogenetic Study Based on Specimens Found in *Nebris microps* (Actinopterygii: Sciaenidae) off the Brazilian Coast

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Abstract: This study presents the first record of *T. lindbergi* from the southwestern Atlantic Ocean, based on specimens collected from the smalleye croaker *Nebris microps* Cuvier (Sciaenidae), off the coast of Maranhão Island, State of Maranhão, Brazil. Our approach included a morphological analysis complemented by DNA sequencing (28S, ITS2 rDNA, and *cox*1 mtDNA). Our phylogenetic analysis revealed the affinity of *T. lindbergi* to its congener *T. laticaudi* Parukhin, 1969, a digenean parasite commonly found in hydrophiine snakes inhabiting the Pacific Ocean. The interspecific divergence between *T. lindbergi* and *T. laticaudi* measures 3.80% for 28S, 7.49–7.64% for ITS2, and 16.29–16.70% for *cox*1. Our findings expand the documented geographic range of *T. lindbergi* into the southwestern Atlantic Ocean, report a novel host record, and increase the number of hemiurids known from Brazil to 30 species. Additionally, this study represents the initial documentation of a marine digenean fish within the North Brazil Shelf.

Keywords: Dinurinae; marine fish; North Brazil Shelf; nuclear and mitochondrial DNA

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1. Introduction

Hemiurid trematodes of the genus *Tubulovesicula* Yamaguti, 1934, are found in a variety of fishes and rarely found in hydrophiine snakes, with records in the Atlantic, Indian, and Pacific Oceans [1–6]. The genus was established by Yamaguti [7] to accommodate *Tubulovesicula spari* Yamaguti, 1934, a parasite of the sparid *Acanthopagrus schlegelii* (Bleeker) (=*Sparus macrocephalus*) in Japan, Pacific Ocean. Yamaguti [7] also proposed transferring *Lecithaster lindbergi* Layman, 1930, described by Layman [8] from a variety of fishes in Peter the Great Bay, Russia, Pacific Ocean, to the genus *Tubulovesicula*. Later, Sogandares-Bernal [9] synonymised *T. spari* with *T. lindbergi* (Layman, 1930) due to the lack of conspicuous morphological differences between the two species. Currently, the World Register of Marine Species (WoRMS) [10] lists 25 species within the genus *Tubulovesicula*. Within the genus *Tubulovesicula*, genetic data are exclusively available for *T. laticaudi* Parukhin, 1969 (see Martin et al. [5]). The distinction among species of *Tubulovesicula* is frequently difficult because it relies solely on their morphological characteristics, often displaying subtle variations, thereby complicating species comparisons [2,11,12]. A representative instance is *T. lindbergi*, the most widely distributed species in the genus. Currently, there are more than 100 records of this species documented worldwide, including the data of synonymised species (*T. spari*; *T. californica* Park, 1936; *T. nanaimoensis* (McFarlane, 1936); *T. madurensis* Nigrelli, 1940) (Table 1).

The synonymity of some species has increased the complexity associated with *T. lindbergi*; therefore, the taxonomic history of this species needs to be reevaluated. *Tubulovesicula lindbergi* is reported mostly from the Pacific, but also in the Indian and Atlantic Oceans. The species purportedly has a broad spectrum of host species, including fish belonging to at least 29 families and 15 orders (Table 1). Given the complexity of the genus *Tubulovesicula*, molecular taxonomy can be a valuable tool for enhancing our understanding of its true diversity and distribution.

Numerous hemiurids have been documented in Brazil; nevertheless, presently, no *Tubulovesicula* species have been documented in the coastal waters of the southwestern Atlantic Ocean. However, the Hemiuridae family is well represented along the coast of the country, with 29 species in 10 genera, making it the second richest family of digeneans in marine fishes [13,14].

The demersal sciaenid fish studied, *Nebris microps* Cuvier, commonly known as the smalleye croaker, is found in the western Atlantic Ocean, ranging from Costa Rica to Brazil [15]. This fish is an important local fishery resource [16] and holds significant ecological value. However, the potential role of the smalleye croaker in parasite life cycles remains underexplored. To date, the only digenean reported in this host is the acanthocolpid *Pleorchis americanus* Lühe, 1906 (=*Pleorchis mollis*), collected off Macaé, Rio de Janeiro, Brazil, southwestern Atlantic Ocean, by Vicente and Santos [17]. Other studies have reported nematodes [*Anisakis* sp., *Procamallanus* (*S.*) *pereirai* (Annereaux, 1946), *Raphidascaris* (*I.*) *vicentei* Santos, 1970, and *Raphidascaris* sp.] and one monogenean (*Rhamnocercus micros* Chero, Cruces, Sáez & Luque, 2022) [18]. As part of our ongoing investigation of the Brazilian fish trematode fauna, we conducted a morphological and molecular analysis of hemiurid trematodes collected from the stomach of *N. microps* off Maranhão Island, North Brazil Shelf, Maranhão, Brazil. Our data represent the first record of *T. lindbergi* in the southwestern Atlantic Ocean, a novel host record, and the first assessment of its phylogenetic position.

Table 1. Host species and geographical distribution of *Tubulovesicula lindbergi* (Layman, 1930).

Host Family	**Host Species**	**Locality**	**Reference**
Acipenseridae	*Acipenser transmontanus*	Columbia River, Washington, DC, USA	Becker [19]
	Huso dauricus	Amur River basin, Russia	Akhmerov [20]
Congridae	*Conger conger* (=*Leptocephalus conger*)	Puerto Real, Porto Rico, Atlantic Ocean	Siddiqi and Cable [21]
Muraenesocidae	*Cynoponticus ferox* (=*Phyllogramma regani*)	Tema, Ghana, Atlantic Ocean; Gulf of Guinea, Nigeria, Atlantic Ocean	Fischthal and Thomas, [22]; Siddiqi and Hafeezullah [23]
Anguillidae	unidentified eel	Pelado Island, Panama, Pacific Ocean	Sogandares-Bernal [9]
Synodontidae	*Saurida tumbil*	South China Sea, Pacific Ocean; Gulf of Mannar, Indian Ocean	Shen [24]; Gupta and Sehgal [25]
	Synodus sp.	Panama Bay, Panama, Pacific Ocean	Sogandares-Bernal [9]
Batrachoididae	*Porichthys notatus*	Burke Channel, Canada, Pacific Ocean	Arai [26] *
Hemiramphidae	*Hyporhamphus sajori*	Japan, Pacific Ocean	Zhukov [27]
Echeneidae	*Echeneis naucrates*	South China Sea, Pacific Ocean	Parukhin [28]
Alestidae	*Hydrocyon brevis*	Volta River, Ghana	Fischthal and Thomas [22]
Sparidae	*Pagrus* sp. (=*Pagrosomus unicolar*)	Inland Sea, Japan, Pacific Ocean	Yamaguti [29] *
	Sparus macrocephalus	Inland Sea, Japan, Pacific Ocean	Yamaguti [7] *
Gadidae	*Gadus chalcogrammus* (=*Theragra chalcogramma*)	Friday Harbor, Washington, USA, Pacific Ocean	Ching [30]
	Gadus macrocephalus (Gadus morhua macrocephalus)	Japan, Pacific Ocean	Zhukov [27]
Lophiidae	*Lophiomus setigerus*	Japan, Pacific Ocean	Zhukov [27]
	Lophius litulon	Japan, Pacific Ocean	Machida et al. [31]
	Lophius piscatorius	Indian Ocean	Parukhin [28]
Embiotocidae	*Cymatogaster aggregata*	Burke Channel, Canada, Pacific Ocean	Arai [26] *
	Hyperprosopon ellipticum	Tomales and Bodega Bays, USA, Pacific Ocean	Rodella and Nahhas [32]
Agonidae	*Hemitripterus villosus*	Japan, Pacific Ocean	Zhukov [27]
Anarhichadidae	*Anarrhichthys ocellatus*	Bering Sea, Russia, Pacific Ocean	Gordeev and Sokolov [33]
Cottidae	*Enophrys bison*	Dillon's Beach, USA, Pacific Ocean; Newport, USA, Pacific Ocean	Park [34] **; McCauley, [12]
	Hemilepidotus hemilepidotus	Friday Harbor, USA, Pacific Ocean	Ching [30]
	Leptocottus armatus	San Quintín Bay, Baja California, Mexico, Pacific Ocean; Friday Harbor, Washington, USA, Pacific Ocean; Newport, Oregon, USA, Pacific Ocean; Burke Channel, Canada, Pacific Ocean	King [35]; Ching [30]; McCauley [12]; Arai [26]
	Myoxocephalus brandtii (=Myoxocephalus brandti)	Japan, Pacific Ocean	Zhukov [27]
	Myoxocephalus polyacanthocephalus	Burke Channel, Canada, Pacific Ocean	Arai [26,36]
	Oligocoitus maculosus	Burke Channel, Canada, Pacific Ocean	Arai [26]
	Synchirus gilli	Friday Harbor, USA, Pacific Ocean	Ching [30]
Gasterosteidae	*Gasterosteus aculeatus*	Friday Harbor, USA, Pacific Ocean	Ching [30]
Hexagrammidae	*Ophiodon elongatus*	Friday Harbor, USA, Pacific Ocean; Newport, USA, Pacific Ocean; Burke Channel, Canada, Pacific Ocean	Ching [30]; McCauley [12]; Arai [26,36]
	Pleurogrammus azonus	Japan, Pacific Ocean	Zhukov [27]
Jordaniidae	*Scorpaenichthys marmoratus*	Departure Bay, Canada, Pacific Ocean	McFarlane [37] ***
Platycephalidae	*Platycephalus indicus*	Yellow Sea and Bo-hai Sea, China, Pacific Ocean	Li et al. [38]; Shen and Qiu [39]
Scorpaenidae	*Scorpaena madurensis*	Ilha da Madeira (Origin) Collected in an aquarium in NY	Nigrelli [6] ****

Table 1. *Cont.*

Host Family	Host Species	Locality	Reference
Sebastidae	*Sebastes alutus*	Northeastern Pacific Ocean	Sekerak and Arai [40,41]
	Sebastes brevispinis	Northeastern Pacific Ocean	Sekerak and Arai, [41]
	Sebastes borealis	Northeastern Pacific Ocean	Sekerak and Arai, [41]
	Sebastes caurinus (=Sebastodes caurinus)	Friday Harbor, USA, Pacific Ocean; Northeastern Pacific Ocean	Ching [30]; Sekerak and Arai [41]
	Sebastes crameri	Northeastern Pacific Ocean	Sekerak and Arai [41]
	Sebastes maliger	Northeastern Pacific Ocean	Sekerak and Arai [41]
	Sebastes melanops (=Sebastodes melanops)	Friday Harbor, USA, Pacific Ocean	Ching [30]
	Sebastes nigrocinctus (=Sebastodes nigrocinctus)	Friday Harbor, USA, Pacific Ocean	Ching [30]
	Sebastes paucispinis	Northeastern Pacific Ocean	Sekerak and Arai [41]
	Sebastes pinniger	Northeastern Pacific Ocean	Sekerak and Arai [41]
	Sebastes serranoides	Off Central California, Pacific Ocean	Love et al. [42]
	Sebastes trivittatus	Japan, Pacific Ocean	Zhukov [27]
Stichaeidae	*Anoplarchus purpurescens*	Newport, USA, Pacific Ocean	McCauley [12]
	Stichaeus grigorjewi	Japan, Pacific Ocean	Zhukov [27]
Cyclopsettidae	*Citharichthys sordidus*	Newport, USA, Pacific Ocean	McCauley [12]
	Citharichthys stigmaeus	Newport, USA, Pacific Ocean; Burke Channel, Canada, Pacific Ocean	McCauley [12]; Arai [26,36]
Paralichthyidae	*Platichthys bicoloratus* (=*Kareius bicoloratus*)	Japan, Pacific Ocean	Zhukov [27]
	Paralichchthys californicus	San Quintín Bay, Mexico, Pacific Ocean; San Quintín Bay, Todos Santos Bay and Estero de Punta Banda, Mexico, Pacific Ocean	King [35]; Castillo-Sánchez et al. [43]
	Paralichthys olivaceus	Sagami Sea, Japan, Pacific Ocean	Kuramochi [44]
	Paralichthys stellatus (=*Pleuronectes stellatus*)	Japan, Pacific Ocean	Zhukov [27]
Pleuronectidae	*Atheresthes stomias*	Bering Sea, Russia, Pacific Ocean	Mamaev [45]
	Cleisthenes pinetorum (=*Cleisthenes herzensteini*)	Japan, Pacific Ocean	Zhukov [27]
	Eopsetta grigorjewi	Sagami Sea, Japan, Pacific Ocean	Kuramochi [44]
	Hippoglossus hippoglossus	Bering Sea, Russia, Pacific Ocean	Mamaev [45]
	Hippoglossus stenolepis	Japan, Pacific Ocean; Canada, Pacific Ocean	Zhukov [27]; Machida et al. [30]; Blaylock et al. [46]
	Limanda aspera	Peter the Great Bay, Russia, Pacific Ocean	Tsimbalyuk [47]
	Pleuronichthys guttulatus (=Hypsopsetta guttalata)	San Quintín Bay, Mexico, Pacific Ocean	King [35]
	Isopsetta isolepis	Friday Harbor, USA, Pacific Ocean	Ching [30]
	Lepidopsetta bilineata	Netarts Bay, USA, Pacific Ocean	McCauley [12]
	Parophrys vetulus	Departure Bay, Canada, Pacific Ocean; Friday Harbor, USA, Pacific Ocean	McFarlane [37] ***; Ching [29]
	Platichthys stellatus	Newport, USA, Pacific Ocean; Far Eastern Seas, Pacific Ocean	McCauley [12]; Mamaev et al. [48]
	Psettichthys melanostictus	Newport, USA, Pacific Ocean; Puget Sound, USA, Pacific Ocean	McCauley [12]
	Pseudopleuronectes herzensteini	Japan, Pacific Ocean	Zhukov [27]
	Pseudopleuronectes obscurus (=*Liopsetta obscura*)	Japan, Pacific Ocean	Zhukov [27]
	Pseudopleuronectes yokohamae	Japan, Pacific Ocean	Zhukov [27]
	Unidentified	Peter the Great Bay, Russia, Pacific Ocean	Layman [8] *****
	Verasper moseri	Hokkaido, Japan, Pacific Ocean	Machida et al. [30]
Psettodidae	*Psettodes eruei*	Gulf of Tonkin, Vietnam, Pacific Ocean	Parukhin [49]

Table 1. *Cont.*

Host Family	Host Species	Locality	Reference
Salmonidae	*Oncorhynchus keta*	Amur River, Russia; British Columbia coast, Canada, Pacific Ocean; Indian Ocean	Akhmerov [20]; Strelkov [50]; Margolis and Boyce [51]; Parukhin [31]
	Oncorhynchus gorbuscha	British Columbia coast, Canada, Pacific Ocean	Margolis and Boyce [51]
	Oncorhynchus kisutch	Friday Harbor, USA, Pacific Ocean	Ching [30]
	Oncorhynchus tschawytscha	Alsea Bay, USA, Pacific Ocean; Mad River, California, USA	McCauley [12]; Jennings and Hendrickson [52]
	Salvelinus leucomaenis	Japan, Pacific Ocean	Zhukov [27]
	Salvelinus malma	Burke Channel, Canada, Pacific Ocean	Arai [26,36]
Syngnathidae	*Syngnathus califoniensis* (=*Syngnathus griseolineatus*)	Burke Channel, Canada, Pacific Ocean	Arai [26,36]

* Referred as *T. spari*; ** Referred as *T. californica*; *** Referred as *Dinurus nanaimoensis*; **** Referred as *T. madurensis*. ***** Referred as *Lecithaster lindbergi*.

2. Materials and Methods

Twelve specimens of *Nebris microps* Cuvier, 1830 (Sciaenidae), were obtained in October 2022 from artisanal fishermen off the Maranhão Island (2°24′29″ S, 44°05′52″ W), near the municipality of Raposa, State of Maranhão, Brazil. The specimens were recently deceased. The fish were examined for the presence of infection with helminth parasites. Trematode individuals collected from the examined fish were washed in 0.9% saline and fixed in 80% ethanol. A small piece of the ecsoma of each specimen selected for molecular analyses was excised and used for DNA extraction, and the remaining piece was used for morphological analysis (hologenophore, see Pleijel et al. [53]). Hologenophores and remaining specimens (paragenophores) were stained in Mayer's hydrochloric carmine solution, dehydrated in ethanol, cleared in clove oil, and mounted in Canada balsam, and thereafter used for morphological evaluation. The fish identification was determined according to Marceniuk et al. [16]. Drawings were made using a drawing tube attached to a light microscope Olympus CH-2 and then digitised. Measurements were taken using Leica Application Suite software (LAZ EZ), v.2.0. software adapted to the Leica DM 750 optical microscope (Leica Microsystems, Wetzlar, Germany) and were given in micrometres (µm). Voucher material was deposited in the Helminthological Collection of the Oswaldo Cruz Institute, CHIOC (CHIOC–40430a,b; CHIOC–40431a–e), Rio de Janeiro, and in the Collection of Trematodes of the Federal University of Minas Gerais, UFMG (UFMG-TRE137), Belo Horizonte, Brazil.

Total genomic DNA was extracted from trematodes following Georgieva et al. [54]. The D1–D3 region of the large ribosomal subunit (28S rDNA) was amplified using the primers digl2 (forward; 5′–AAG CAT ATC ACT AAG CGG–3′) and 1500R (reverse; 5′–GCTA TCC TGA GGG AAA CTT CG–3′) (Snyder and Tkach, [55]), following the protocol described by Tkach et al. [56]. The second internal transcribed spacer region (ITS2) was amplified using the primers 3S (forward; 5′–GGT ACC GGT GGA TCA CGT GGC TAG TG–3′) (Bowles et al. [57]) and ITS2.2 (reverse; 5′–CCT GGT TAG TTT CTT TTC CTC CGC–3′) (Cribb et al. [58]), following the protocol described by Cutmore et al. [59]. The partial fragment of the *cox*1 gene was amplified using the primers Digcox1Fa (forward; 5′–ATG ATW TTY TTY TTY YTD ATG CC–3′) and Dig_cox1R (reverse; 5′–TCN GGR TGH CCR AAR AAY CAA AA–3′) (Wee et al. [60]), following the PCR protocol as described by Wee et al. [60]. PCR amplicons were purified with the Exo-SAP-IT KitTM Express Reagent (Thermo Fisher Scientific, Waltham, MA, USA) and subjected to Sanger for sequencing. The original PCR primers were used for sequencing and two additional internal primers: 300F (forward; 5′–CAA GTA CCG TGA GGG AAA GTT G–3′) (Littlewood et al. [61]) and ECD2 (reverse; 5′–CCT TGG TCC GTG TTT CAA GAC GGG–3′) (Littlewood et al. [62]) were used for the sequencing of the 28S rDNA amplicons. The sequences were assembled and edited using Geneious Prime® 2023.0.1. The newly generated sequences were deposited in GenBank with accession numbers PP889615 (28S, 1275 bp), PP889614 (ITS2, 574 bp), and PP891444 (*cox*1, 490 bp). Three alignments (28S, ITS2 and *cox*1), including new and previously published sequences were built using ClustalW implemented in Geneious Prime® 2023.0.1. The 28S alignment included 24 sequences for species of the family Hemiuridae. Phylogenetic relationships of hemiurids (28S) were assessed using maximum likelihood (ML) and Bayesian inference (BI) analyses. The lecithasterid *Lecithaster gibbosus* (Rudolphi, 1802) (AY222199) was used as an outgroup based on the topology in the phylogenetic tree of the family Hemiuridae provided by Martin et al. [5]. The analyses were performed using the GTR+ I + G model, which was predicted as the best model by the Akaike Information Criterion in jModelTest 2.1.2 [63]. ML analysis was performed using PhyML ver. 3.0 [64] and run on Geneious Prime® 2023.0.1 with a nonparametric bootstrap value of 100 pseudoreplicates. BI was performed using MrBayes software (ver. 3.2.3) [65] through the CIPRES Science Gateway ver. 3.3 [66] accessed on 5 December 2023. Markov Chain Monte Carlo chains were run for 10,000,000 generations, log-likelihood scores were plotted, and only the final 75% of trees were used to build the consensus tree. The ITS2 and *cox*1 alignments were built with sequences generated in the present study (PP889614, ITS; PP891444, *cox*1) and sequences of the only *Tubulovesicula* species, *T. laticaudi* (OR209735 and OR209736, ITS2; OR221151, OR221153 and OR221154, *cox*1), available in GenBank. Pairwise genetic distances (uncorrected p-distance) for the three datasets were calculated in MEGA ver. 11 [67].

3. Results

3.1. Morphological Description

Hemiuridae Looss, 1899
Dinurinae Looss, 1907
***Tubulovesicula lindbergi* (Layman, 1930)**

Synonyms: Lecithaster lindbergi Layman, 1930, *Tubulovesicula spari* Yamaguti, 1934; *T. californica* Park, 1938; *T. madurensis* Nigrelli, 1940; *T. nanaimoensis* (McFarlane, 1936).

Site of infection: stomach.

Infection rates: two out of twelve (16.7%).

Intensity of infection: 11 and 12 specimens.

Description (based on seven whole mounts and two hologenophores; measurements of hologenophores in description and paragenophores in Table 2; Figure 1A,B): Soma elongate, dorso-ventrally flattened, maximum width at level of ventral sucker or close to posterior soma extremity, 4554–4975 long, 1551–1716 wide. Tegument smooth. Forebody short, 1575–1743. Ecsoma well developed, protruded. Pre-oral lobe distinct, 25–43 long. Oral sucker muscular, well developed, subspherical, ventro-terminal, 432–586 long, 531–657 wide. Prepharynx indistinct. Pharynx muscular, well developed, subspherical or elongate-oval, 196–327 long, 184–282 wide. Oesophagus absent. “Drüsenmagen” present. Intestinal bifurcation immediately posterior to pharynx. Caeca blind, with thin walls and wide lumen, terminates in ecsoma, near posterior extremity. Ventral sucker muscular, well developed, subspherical or elongate-oval, 904–1219 long, 1008–1328 wide, larger than oral sucker (1:1.90–2.02), pre-equatorial. Testes two, symmetrical or obliquely symmetrical, separated, entire, pre-ovarian, in anterior hindbody, contiguous or separated from ventral sucker; right testis subspherical 304–334 long, 325–395 wide; left testis subspherical or elongate-oval, 255–317 long, 310–385 wide; distance between ventral sucker and right testis 240 (*n* = 1). Seminal vesicle thin-walled, tubular, sinuous, 679–1104 long, 169–196 wide (Figure 1B). Seminal vesicle entirely in anterior hindbody or extends to posterior margin of ventral sucker, anterior to testes. Pars prostatica long, sinuous, bent or straight, densely invested by prostatic cells, connected to seminal vesicle, between sinus sac and posterior margin of ventral sucker or reaches into the hindbody, 679–1104 long, 169–196 wide (Figure 1B). Sinus sac subspherical, elongate-oval or transversely oval, posterior to pharynx, with muscular wall, 221–380 long, 233–358 wide. Aglandular ejaculatory duct short, within sinus sac, immediately joined by metraterm to form hermaphroditic duct. Hermaphroditic duct straight within sinus sac. Temporary sinus organ retracted or extruded through genital pore. Genital atrium distinct. Genital pore median, posterior to pharynx. Ovary median, submedian or sinistral, entire, subspherical, elongate-oval or transversely oval, 272–293 long, 270–373 wide, in middle of hindbody, separated or contiguous with sinistral testis. Vitellarium seven elongate tubular lobes (three dextral and four sinistral), between testes level and posterior soma extremity, 981–1180 long, 1155–1209 wide. Juel’s organ and Mehlis’ gland not observed. Uterus coiled, extensive in hindbody, extends approximately up to middle length of ecsoma. Metraterm passes into sinus-sac ventrally, joins male duct forming hermaphrodict duct. Eggs numerous, 35–28 × 21–29 (*n* = 10).

Table 2. Comparative metrical data (µm) of *Tubulovesicula lindbergi* (Layman, 1930).

Source	Present study		Layman [8]	Yamaguti [7]	Park [34]	McFarlane [37]	Nigrelli [6]	McCauley [12]	Shen [24]
Locality	Maranhão Island, Maranhão, Brazil, Atlantic Ocean		Peter Great Bay, Russia, Pacific Ocean	Inland Sea, Japan, Pacific Ocean	Dillon Beach, California, USA, Pacific Ocean	Departure Bay, Vancouver Island, British Columbia, Canada, Pacific Ocean	New York Aquarium, New York, USA	Newport, Alsea Bay, Netarts Bay, Oregon; Pujet Sound, Washington, USA, Pacific Ocean	Hainan Island, China, Pacific Ocean,
Host	*Nebris microps* (Sciaenidae)		Variety of fishes (most Pleuronectiformes)	*Acanthopagrus schlegelii* (= *Sparus macrocephalus*) (Sparidae)	*Enophrys bison* (Cottidae)	*Parophrys vetulus* (Pleuronectidae) and *Scorpaenichthys marmoratus* (Jordaniidae)	*Scorpaena madurensis* (Scorpaenidae)	Variety of fishes (most Pleuronectidae)	*Saurida tumbil* (Synodontidae)
	Range (*n* = 7)	Mean	Range (*n* = NP)	Range (*n* = 1)	Range (*n* = 1)	Range (*n* = NP)	Range (*n* = 4)	Range (*n* = NP)	Range (*n* = 6)
Body (soma) length	3356–4645	4019	2400−3800	5570	2560	1510–2680	–	1110–3800	2688–5367
Body (soma) width	1211–1677	1370	852−1310	–	–	–	1630	300–1137	935–1403
Ecsoma length	2102–2813	2376	1147 (maximum)	–	1530	–	2690	1600	1313–2272
Total length	5867−6879	6395	3600−5240	–	4090	–	7350	–	3557–7047
Forebody length	1232−1725	1498	−	–	–	–	–	–	–
Hindbody length	1359−2279	1758	−	–	–	–	–	–	–
Preoral lob length	24−78	61	−	74	–	–	44	–	33–50
Oral sucker length	403−494	452	229−327 (diameter)	300	210	168–212	251	100–350	184–301
Oral sucker width	410−516	466	−	320	280	336–420	287	100–380	251–334
Pharynx length	194−227	215	98−147	140	110	89 (diameter)	161	80–120 (diameter)	134–167 (diameter)
Pharynx width	182−236	205	81−147	150	130	–	194	–	
Ventral sucker length	792−1076	934	409−606 (diameter)	640 (diameter)	460	–	643	230–380 (diameter)	434–585 (diameter)
Ventral sucker width	788−1015	903	−	–	470	–	659	–	–
DIBAE *	501−647	584	−	–	260	–	–	–	–
Anterior testis (or right) length	253−370	328	295−376 (diameter)	260	210 (diameter)	224 (diameter)	444	120–200	248–351
Anterior testis (or right) width	287−402	330	−	290	–	–	498	100–300	198–367
Posterior testis (or left) length	208–380	376	−	320	240 (diameter)	–	413	–	198–367
Posterior testis (or left) width	290–378	334	−	360	–	–	532	–	228–334
DTVS *	95–378 (*n* = 6)	249	–	–	–	–	–	–	–
Post-testicular region	785–1617	1164	–	–	–	–	–	–	–
Seminal vesicle length	437−1336	842	590–655	1000	670	–	465	–	835–1336
Seminal vesicle width	65−208	128	–	63	120	–	165	–	50–117
Pars prostatica length	1591−2171	1822	737−835	1000	920	–	1280	–	585–969
Pars prostatica width	179−270	222	−	–	–	–	–	–	–
Sinus-sac length	197–289	236	376−458	–	270	224	150	–	217–418
Sinus-sac width	204–262	229	−	–	180	112	270	–	150–284
Ovary length	216−310	251	114−229	200	310 (diameter)	240	348	140–350	167–267
Ovary width	201−345	290	229−360	390	–	130	442	–	267–384
Vitellarium length	777–1332	1062	653−885 (ray)	–	–	–	–	–	–
Vitellarium width	821–1160	1000	−	–	–	–	–	–	–
Egg length	29–34 (*n* = 10)	32	27−29	32	28–32	32–36	12–15	18–23	24–27
Egg width	25–31 (*n* = 10)	28	18−20	21	16–24	19–20	18–25	12–22	18–20
Body length/body width	1:2.28–3.56	1:2.96	−	–	1:1.70	–	–	–	–
Oral/ventral sucker width	1:1.79−2.06	1:1.94	−	–	–	–	–	–	1:2.2
Ecsoma/body length, %	48−84	60	−	–	–	–	–	–	–
Forebody/body length, %	31−51	38	−	–	–	–	–	–	–
Post-testicular region/body length, %	21–35	29	−	–	–	–	–	–	–

* Abbreviation: DIBAE, Distance of intestinal bifurcation from anterior extremity; DTVS, Distance of testes from ventral sucker; NP, Not provided.

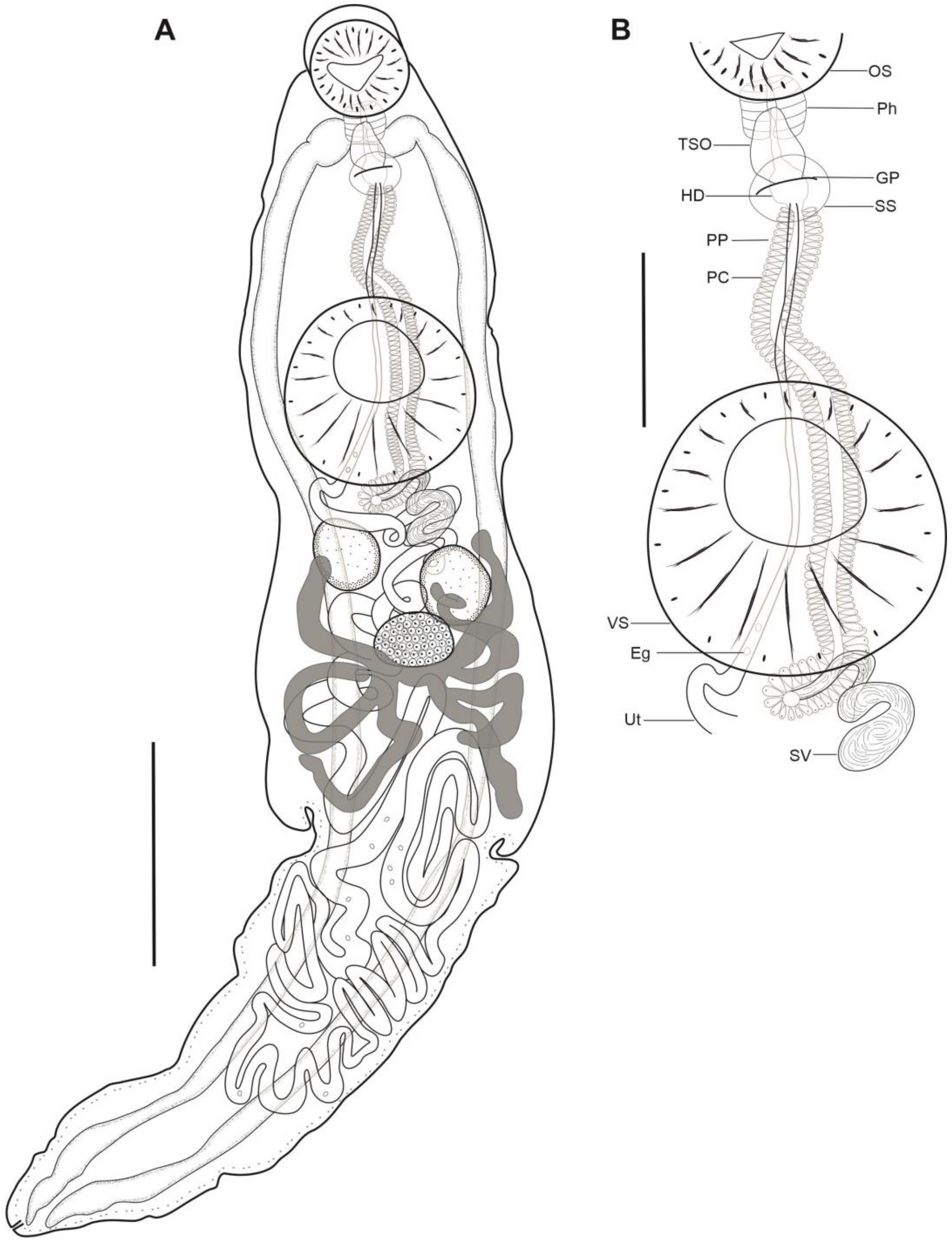

Figure 1. Adult of *Tubulovesicula lindbergi* (Layman, 1930) ex *Nebris microps*, Maranhão Island, State of Maranhão, Brazil. (**A**) Complete specimen, ventral view, (**B**) Detail of the terminal genitalia. Scale bars: (**A**), 1 mm; (**B**), 500 µm. Abbreviations: Eg, egg; GP, genital pore; HD, hermaphroditic duct; OS, oral sucker; PC, prostatic cells; Ph, pharynx; PP, pars prostatica; SS, sinus sac; SV, seminal vesicle; TSO, temporary sinus organ; Ut, uterus; VS, ventral sucker.

Remarks: The characters of the specimens found in the present study are consistent, within the Hemiuridae Looss, 1899, with membership of the subfamily Dinurinae Looss, 1907, in having tegument smooth, well-developed sinus sac, temporary sinus organ, well-developed genital atrium, and vitellarium composed of tubular arms and in the absence of an ejaculatory vesicle. Specimens collected in the present study agree well with the generic diagnosis of *Tubulovesicula* Yamaguti, 1934 provided by Gibson et al. [3] and Martin et al. [5] in having tubular, sinuous and not partitioned seminal vesicle, temporary sinus-organ, pars prostatica undivided not separated from seminal vesicle via aglandular duct and body without plications.

Morphologically, our specimens differ from *T. diacopae* Nagaty & Abdel-Aal, 1962 and *T. hebrae* Nagaty & Abdel-Aal, 1962 in position of ventral sucker (pre-equatorial vs. post-equatorial); from T. magnacetabulum Yamaguti, 1939, *T. alviga* Aleshkina, 1983 and *T. microcaudum* Shaukat, Bilqees & Haseed, 2008 in having smaller sucker ratio (1:1.91–2.06 vs. 1:3.02 vs. 1:3.5 vs. 1:3.5–3.7); from *T. magnacirrosa* Shaukat & Bilqees, 2011 in having larger sucker width ratio (1:1.91–2.06 vs. 1:0.76–0.77); from *T. angusticauda* (Nicoll, 1915), *T. marsupialia* Oshmarin, 1965, *T. spasskii* Lebedev, 1968, *T. laticaudi* Parukhin, 1969, *T. olivaceus* Shaukat & Bilqees, 2011, *T. microrchis* Bilqees, Khalil, Khatoon, Rehman & Perveen, 2010, *T. macrovesicula* Bilqees, Khalil, Khan, Haseeb & Perveen, 2010, *T. dorabi* Bilqees, Khalil, Khatoon, Rehman & Perveen, 2010, *T. zonichthydis* Shen, 1990 and *T. longicorporis* Shen, 1990 in length of pars prostatic relative to the ventral sucker (from the posterior margin of ventral sucker or anterior hindbody vs. from middle of ventral sucker or anteriorly to it); from *T. pinguis* Linton, 1940 in size of testes and ovary in comparison to ventral sucker (testes and ovary smaller than ventral sucker vs. testes and ovary equal or larger than ventral sucker); from *T. karachiensis* Shaukat, 2008 in intestinal caeca length (extending into ecsoma vs. not extending into ecsoma); from *T. trichiuri* (Gu & Shen, 1978) in ecsoma/body length (ecsoma smaller than body vs. ecsoma larger than body); from *T. lycodontis* Toman, 1992 in position of testes (hindbody vs. at level of ventral sucker or in forebody); from *T. sexaginta* Li & Sun, 1994 in having longer forebody (31−51% vs. approximately 24%).

Our specimens agree with the original description of *T. lindbergi* (=*Lecithaster lindbergi*) provided by Layman [8] and collected from a variety of fishes (most Pleuronectiformes) in the Peter Great Bay, Russia, Pacific Ocean, particularly in body shape (maximum width at level of ventral sucker or at posterior body extremity), in having soma longer than ecsoma, in having testes and ovary smaller than ventral sucker, in position of pars prostatica (between sinus-sac and posterior margin of ventral sucker or anterior hindbody), in sucker ratio (ventral sucker approximately two times larger than oral sucker) and in extension of intestinal caeca (extending into ecsoma). However, our specimens differ from the material of Layman [8] by having larger dimensions, except for the soma length, soma width, testes and ovary, where the dimensions overlap, and in having smaller sinus-sac (Table 2). Later, Yamaguti [7] described *Tubulovesicula spari* Yamaguti, 1934 from the Inland Sea, Japan, Pacific Ocean, which was considered identical, except for the egg size (slightly smaller in material of Layman [8]), with *T. lindbergi* by Sogandares-Bengal [9] who put *T. spari* as its synonym. In comparison with the material of Yamaguti [7], our specimens possess larger dimensions, except for body (soma) length, for ovary width and egg length, where our specimens are smaller, as well as for the preoral lobe length and for testes, where the dimensions overlap (Table 2).

McFarlane [37] described *Dinurus nanaimoensis* MacFarlane, 1936, which was transferred to the genus *Tubulovesicula* by Manter [11] and later synonymised with *T. lindbergi* by McCauley [12]. In comparison with material of McFarlane [37], our specimens differ in having larger dimensions, except in sinus sac length and ovary length, for which the dimensions overlap, and in having smaller eggs (Table 2). Park [34] described *T. californica*, (1936) which was later considered synonymous of *T. lindbergi* by McCauley [12]. In comparison with material of Park [34], the maxima for most internal organs of our specimens are higher, except for the body length, posterior testis length, sinus sac length, ovary length, and egg width, for which the dimensions overlap (Table 2). Nigrelli [1] described *T. madurensis* Nigrelli (1940), and then the species was synonymised with *T. lindbergi* by Manter [11]. In comparison with material of Nigrelli [6], our specimens differ mainly in having smaller total length, smaller testes, larger oral sucker, larger pharynx length, larger ventral sucker, and longer eggs (Table 2). In comparison with material of McCauley [12], our possess larger dimensions except for body length and eggs where the dimensions overlap. The dimensions of our material overlap within those of Shen [24], except for in oral sucker, pharynx length, ventral sucker, and in pars prostatica length, which our dimensions are higher (Table 2).

Tubulovesicula muraenesocis Yamaguti, 1934, and *T. pseudorhombi* Yamaguti, 1938, were considered synonyms of *T. lindbergi* by Manter [11]. Bray [2], in a detailed taxonomic review, synonymised both species with *T. angusticauda*. Later, Madhavi and Bray [4] listed these two species as synonymous of *T. lindbergi*. Checking the original descriptions, we agree with Bray [2] because the lengths of pars prostatica of *T. muraenesocis* and *T. pseudorhombi* are more related to the description of *T. angusticauda*. Sogandares-Bernal [9] considered *T. anguillae* Yamaguti, 1934, a synonym of *T. lindbergi*. Later, Bray [2] considered *T. anguillae* a synonym of *T. angusticauda*. However, we express doubt about the interpretation of Bray [2] regarding the length of the pars prostatica in *T. anguillae*. Although Yamaguti [7] did not provide a detail description of the terminal genitalia, the figure provided by him, in our interpretation, seems to show that the pars prostatica is starting from the posterior margin of the ventral sucker.

According to the WoRMS Editorial Board [10], *Tubulovesicula* has been reported to comprise a total of 25 species. To investigate the taxonomic history of *T. lindbergi,* a review of the literature was conducted. Through our examination, we were able to assess the validity and synonymies associated with this species. Previously, McCauley [12] synonymised *T. californica* and *T. madurensis* with *T. lindbergi,* while Sogandares-Bernal [9] concluded that the type species *T. spari* is a synonym of *T. lindbergi*.

3.2. Molecular Results

In the present study, four novel sequences were generated for two isolates of *T. lindbergi* (2 = 28S; 1 = ITS2; 1 = *cox*1). Phylogenetic analyses were performed using the 28S rDNA alignment (1150 pb), and the resulting tree provided insights into the phylogenetic relationships of *T. lindbergi* within the Hemiuridae (Figure 2). The newly obtained 28S rDNA sequences of *T. lindbergi* from *N. microps* were closely related to *T. laticaudi* (OR209733), collected from *Hydrophis cyanocinctus* Daudin (Elapidae), in Sri Lanka. This relationship was supported by high nodal values (Figure 2). The intraspecific divergence between the two generated sequences (28S) was null; therefore, we deposited only one sequence (PP889615) and the interspecific divergence between *T. lindbergi* and *T. laticaudi* was 3.80% (42 bp), which is of the same order of magnitude as other congeneric species of the family included in the analysis. The other sequences utilised for the phylogenetic analysis displayed a difference of more than 12% compared to those generated in the present study. Additionally, pairwise genetic distances were calculated between *T. lindbergi* and *T. laticaudi* for ITS2 and *cox*1. The interspecific divergence between *T. lindbergi* (PP889614) and *T. laticaudi* (OR209735 and OR209736) based on an ITS2 comparison was 7.49−7.64% (42−43 bp). The interspecific divergence between both species (PP891444; *T. lindbergi*) and (OR221151, OR221153 and OR221154, *T. laticaudi*) based on a *cox*1 comparison was 16.29−16.70% (79−81 bp).

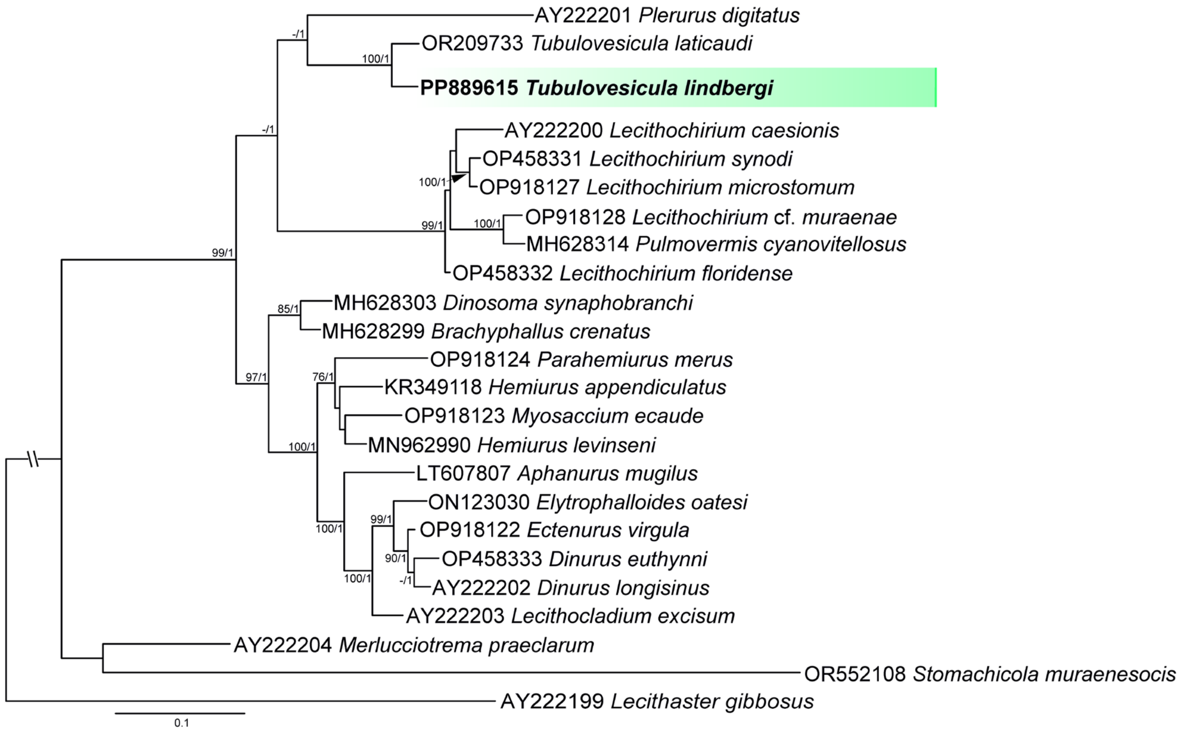

Figure 2. Phylogram from maximum likelihood (ML) analysis based on the 28S rDNA sequences of the Hemiuridae. Nodal support values is given as ML/BI (Bayesian inference). Support values lower than 70 (ML) and 0.70 (BI) are not shown. The scale bar indicates the expected number of substitutions per site. The newly generated sequence is highlighted in bold.

4. Discussion

The morphological analysis of digeneans collected from *Nebris microps* off the Maranhão Island, Maranhão State, Brazil, concluded that they represent *T. lindbergi*, particularly in accordance with the original description provided by Layman [8], thus reporting the first record of this species in the southwestern Atlantic Ocean. Our study represents the first documentation of a marine fish trematodes from the North Brazil Shelf, an overlooked region for diversity of fish and their parasites (see Bray et al. [68]). Moreover, we provide, for the first time, DNA sequences for *T. lindbergi*.

Although our study represents the first record of *T. lindbergi* in the southwestern Atlantic Ocean, this species has been previously reported in other parts of the Atlantic Ocean. Siddiqi and Cable [21] reported the species off Puerto Rico from the congrid *Conger conger* (Linnaeus) (=*Leptocephalus conger*), and Fischthal and Thomas [22] reported the species from specimens collected from the alestid *Hydrocynus brevis* Günther (= *Hydrocyon brevis*) and from the muraenesocid *Cynoponticus ferox* Costa (=*Phyllogramma regani*) in the Volta River and Tema, Ghana. The most recent documentation of *T. lindbergi* in the Atlantic Ocean was provided by Siddiqi and Hafeezullah [23], who reported the presence of this species in *C. ferox* along the Nigerian coast.

With our results, the total count of digenean hemiurids in marine fishes in Brazil has now risen to 30. These hemiurids have been associated with 28 fish families, with carangids having the highest number of recorded instances. Notably, most of these records originate from the state of Rio de Janeiro (Tropical Southwestern Atlantic) [13,14]. No records of species of the genus *Tubulovesicula* have previously been made from the sciaenid *N. microps*. However, previous records of *Tubulovesicula* species in related sciaenid fishes include *T. karachiensis* and *T. magnacirrosa*, which were described from *Protonibea diacanthus* (Lacepède) (=*Pseudosciaena diacanthus*), and *T. microcaudum* was reported from *Otolithes ruber* (Bloch & Schneider (=*Otolithes argenteus*) off the Karachi coast, Pakistan, Indian Ocean [69–72], *T. pinguis* was reported from *Cynoscion regalis* (Bloch & Schneider), and *Menticirrhus saxatilis*

(Bloch & Schneider) was reported in the Woods Hole Region, Massachusetts, USA, Atlantic Ocean [73].

The host specificity of the *Tubulovesicula* species varies. Six species are apparently euryxenous: *Tubulovesicula lindbergi* and *T. angusticauda* were recorded several times, being found in at least fishes from 29 families in 15 orders and from 26 families and 13 orders, respectively [10]. *Tubulovesicula pinguis*, although less frequently reported, was found parasitising fishes from 13 families in 11 orders [73], and *Tubulovesicula trichiuri* was reported in two sympatric fishes, *Trichiurus lepturus* (Forsskål) (=*Trichiurus haumela*) (Trichiuridae) and *Synodus* sp. (Synodontidae) from China, Pacific Ocean [74,75]. *Tubulovesicula laticaudi* has been recorded from sympatric sea snakes from six species in three families [5], and *T. magnacetabulum* was reported in *Epinephalus akaara* (Temminck & Schlegel), *E. fasciatus* (Forsskål) (Epinephelidae), and *Sebastes marmoratus* (Sebastidae) [10].

From our perspective, the accuracy of some of these reports may be revaluated. Our opinion is primarily based on poorly written descriptions provided in many studies. Additionally, it is worth noting that molecular data have been provided for only one species until the present study (see Martin et al. [5]). However, the euryoxenous nature of these species might be true. Low host specificity has been recorded from hemiuroids species (see Miller et al. [76]), and some studies have been demonstrated a euryoxenous nature within hemiurids through molecular data [14,77], including, recently, in the genus *Tubulovesicula* [5]. However, a critical analysis of the host specificity of these species of *Tubulovesicula* will be possible when further studies including integrative taxonomy approaches will be available.

Tubulovesicula marsupialia is the only stenoxenous species of the genus. It has been found only in *Saurida tumbil* and *S. undosquamis* (Synodontidae) [31,78]. The majority of the *Tubulovesicula* species—*T. alviga*, *T. diacopae*, *T. dorabi*, *T. hebrae*, *T. karachiensis*, *T. longicorporis*, *T. lycodontis*, *T. macrovesicula*, *T. magnacirrosa*, *T. microrchis*, *T. microcaudum*, *T. olivaceus*, *T. spasskii*, *T. sexaginta* and *T. zonichthydis*—to the best of our knowledge, were never reported again after their original descriptions. This lack of further reports has led to a poor understanding of their host specificity.

The genus *Tubulovesicula* comprises 22 species, many of which have been insufficiently documented in terms of their descriptions. Inadequate detail, i.e., incomplete information regarding the terminal genitalia, coupled with the lack of distinctive morphological characteristics, makes it difficult to distinguish species. Certain species within the genus, like *T. diacopae* and *T. hebrae*, do not exhibit typical *Tubulovesicula* traits as observed by Bray [2], such as the post-equatorial ventral sucker and the presence of plications in the body. This raises doubts about whether these species should be classified within the genus. Previous studies have suggested synonyms to simplify the taxonomy of poorly understood forms within the genus, particularly for *T. angusticauda* and *T. lindbergi*. However, a comprehensive revision remains necessary. DNA sequencing can provide valuable additional information for accurate identification, especially for species like *T. lindbergi*, which exhibits widespread occurrence and slight morphometric differences across diverse hosts and geographic regions. A comprehensive taxonomic revision of *T. lindbergi* should be conducted when more data are accumulated, including DNA sequences and life cycle information. Our findings, with careful consideration due to the absence of molecular data from outside Brazilian waters, suggest that the geographical distribution of *T. lindbergi* is even broader. Therefore, it is important to revisit the Indo-West Pacific and examine the hosts from which *T. lindbergi* (primarily Pleuronectiformes) was described to conduct an integrative taxonomy study to add confidence to the identification of the new material.

Our findings also show, molecularly, that the species of the genus *Tubulovesicula* distributed in fishes and sea snakes are closely related. Recently, Martin et al. [5] provided the first molecular information for the only species of the genus known from non-fish hosts, *T. laticaudi*, collected from elapid sea snakes. Their findings demonstrated the polyphyly of the subfamily Dinurinae Looss, 1907, proposing an alternative classification primarily based on the nature of the sinus organ and on the molecular information of the family available. The resurrection of the subfamily Mecoderinae aimed to relocate species possessing a temporary sinus organ, including members of *Tubulovesicula*, *Mecoderus* Manter, 1940, *Allostomachicola* Yamaguti, 1958, and *Stomachicola* Yamaguti, 1934. The authors propose restricting the Dinurinae to accommodate species with a permanent sinus organ, such as members of *Dinurus* Looss, 1907, *Ectenurus* Looss, 1907, *Erilepturus* Woolcock, 1935, *Paradinurus* Vigueras, 1958, and *Qadriana* Bilqees, 1971. However, Ghanei-Motlagh et al. [79] provided the phylogenetic position of *Stomachicola muraenesocis*, revealing that this species is not closely related to *T. laticaudi*. The recent findings and previous studies indicate that further investigation into the molecular data of the Hemiuridae is necessary to propose a new subfamilial classification and determine which morphological characters hold taxonomic value for this classification.

5. Conclusions

Using morphological and genetic analyses, we were able to identify *T. lindbergi* and report this species in the Southeastern Atlantic Ocean for the first time. This discovery also represents the first observation of a marine fish digenean within the "North Brazil Shelf". Our study highlights the importance of investigating this rich and poorly known region in terms of marine diversity. Consequently, our findings contribute to expanding the number of hemiurid species identified off the Brazilian coast to 30 species.

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Data Availability Statement: The data generated in this study are available from the corresponding author upon request.

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Conflicts of Interest: The authors declare no conflicts of interest.

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