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NOTES ON GEOGRAPHIC DISTRIBUTION

First report of *Saccularina magnacetabula* Louvard et al., 2022 (Platyhelminthes, Trematoda, Didymozoidae) in the Gulf of Mexico based on morphological and molecular approaches

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Abstract. This study represents the first record of the parasitic trematode *Saccularina magnacetabula* Louvard et al., 2022 in the Atlantic basin. Using morphological and molecular data, we identified specimens isolated from *Elops saurus* Linnaeus, 1766 in Tampa Bay, Florida as *S. magnacetabula*, a species described from Australian waters. Although previous studies report putative undescribed species of *Saccularina* along the Atlantic coast of North America, we present the first record of *S. magnacetabula*, expanding the species' known range and host associations. We advise broader surveys exploring the distribution of this trematode.

Key words. 28S rDNA, Atlantic, *Elops saurus*, marine parasite, trematode

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INTRODUCTION

Saccularina magnacetabula is a parasitic trematode in the family Didymozoidae first described by Louvard et al. (2022) from adult worm specimens recovered from the fins of *Elops hawaiensis* Regan, 1909 (Hawaiian Ladyfish) and from larval sporocysts containing immature cercariae found in the gill tissues of *Anadara trapezia* (Deshayes, 1840) (Sydney Cockles) collected in Moreton Bay, Queensland, Australia. Adults of the species have a bright yellow (due to the presence of eggs) and filiform body that tapers at both ends. They measure 1.14–1.94 cm in length and 220–477 μm in width, and have an oval oral sucker and a prominent, subcircular ventral sucker. Adults are typically found in pairs in the fin membranes of host fish. While the full life cycle of *S. magnacetabula* has not been experimentally confirmed, it is thought that eggs are ingested by bivalves, the first intermediate host. Within the gill tissue of the bivalve host, eggs release miracidia that develop into sporocysts containing cystophorous cercariae. These sporocysts were described by Louvard et al. (2022: 420) as harboring "both next-generation sporocysts and cystophorous cercariae in *Anadara trapezia*". The sporocysts are thought to produce cystophorous cercariae with a body that is contractile and variable in shape with plicate teguments and no discernible or recognizable suckers, mouth, or digestive tracts. Cercariae are presumed to infect small crustaceans (second intermediate hosts) and later develop into metacercariae in planktivorous fish third intermediate hosts. The metacercariae are trophically transmitted to piscivorus fish definitive hosts where they mature into adults and sexually reproduce in fin tissue (Louvard et al. 2022).

To date, the sole records of *S. magnacetabula* are from the east coast of Australia (Louvard et al. 2022), with no works expanding on the broader geographic distribution of this species since its description. Nonetheless, reports from the Atlantic suggest the possibility of these parasites occurring in the region. A novel parasite was observed in the gill tissue of *Argopecten irradians* (Lamarck, 1819) (Bay Scallops) in North Carolina in 2012 and later found in *A. irradians* from the Gulf Coast of Florida (Boggess et al. 2024). Boggess et al. (2024) used molecular approaches to identify sporocysts present in the gill tissue of *A. irradians* from North Carolina and concluded that these parasites do not correspond to *S. magnacetabula* but potentially a new species of *Saccularina*.

These findings suggest the possible presence of *Saccularina* as well as *S. magnacetabula* in the Atlantic Basin. Additionally, two congeners of *E. hawaiiensis*, the definitive host reported by Louvard et al. (2022), are known to occur in western Atlantic: *Elops saurus* Linnaeus, 1766 and *Elops smithi* McBride et al., 2010 (McBride and Horodysky 2004; McBride et al. 2010).

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Herein, we use morphological characters and molecular data to identify trematode specimens infecting *E. saurus* collected from Tampa Bay, Florida as *S. magnacetabula*. Understanding the biogeography of *S. magnacetabula* and its unique host associations across its range of distribution may be instrumental for fisheries management. Total landings of *E. saurus* and *E. smithi* in 2024 exceeded $660,000 of estimated value in coastal waters of Florida, USA (Florida Fish and Wildlife Commission 2025). Also, the economic value of *A. irradians*, an intermediate host to reputedly *Saccularina* sp. (Scro et al. 2023; Boggess et al. 2024), is estimated at $1.6 million annually per county for select Florida gulf counties (Stevens et al. 2003).

METHODS

Sample collection. Specimens of *Elops saurus* examined in this study were salvaged fish provided by a recreational fishing charter conducted on 7 February 2025. All fish used in this study were harvested by hook and line from a single location in Tampa Bay, Florida, USA (27°56′31″N, 082°32′34″W; Figure 1, hand drawn and assembled by LJA) and held in a live well before being transferred to ice prior to donation (~4 h). Upon receipt, fish were stored at –20 °C until further processing. Host identity was established based on vertebral counts of two randomly chosen fish from the donated material (84 vertebrae in both) and the reported exclusivity of *E. saurus* on the west coast of Florida (McBride et al. 2010). Vertebral isolation and counts were done using the fillet, steam, and scrape protocols described by McBride and Horodysky (2004).

For trematode examination and extraction, host fish were thawed and their fins removed before visually examining them with a dissecting microscope. Trematodes were carefully extracted from fin membranes of 22 fish using fine forceps and blunt probes. Once extracted, trematodes were either immediately stained and examined for morphological identification or preserved in 70% EtOH to be used for molecular identification.

Morphological identification. Freshly extracted worms were stained using Semichon's carmine stain for 8 min. Semichon's carmine stain was prepared using the protocol published by Cable (1958). Once stained, worms were rinsed and slide mounted in artificial seawater (32 ppt) for examination with a ZEISS Primostar 3 compound microscope and ZEISS Stemi 305 stereo-microscope equipped with Moticam X5 WiFi cameras. Captured images of specimens were measured using ImageJ (Schneider et al. 2012). The oral and ventral suckers, whole worm, body width, and eggs were examined and imaged on the compound scope under the weight of a glass coverslip. Total length measurements were generated from worms examined and imaged under a stereoscope without a coverslip.

A total of 21 worms were used for morphological measurements (3 worms per host specimen, 7 host specimens used). We measured and report the mean and range for worm body length from the anterior tip

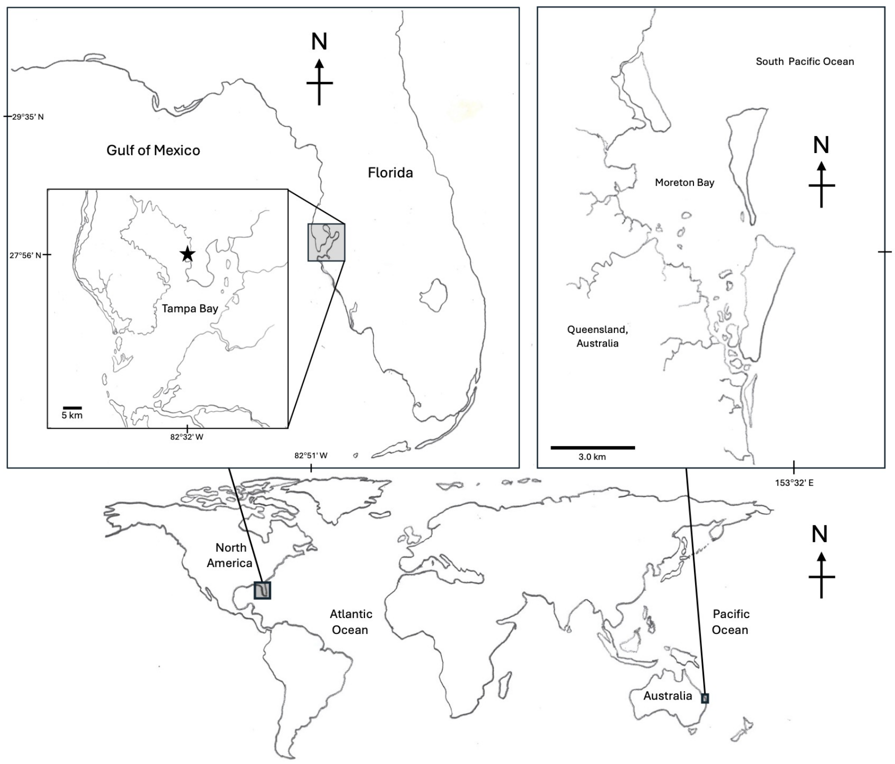

Figure 1. Map of Tampa Bay, Florida and collection site. Star indicates the location of our collection location.

of the oral sucker to the posterior end of the worm, body width as measured just posteriorly to the ventral sucker, ventral and oral sucker (length and width of each), and egg size (length and width of eggs near the genital pore). A subset of worms was removed prior to staining and preserved in 70% EtOH and deposited at the Florida Museum of Natural History in Gainesville, Florida, USA as voucher specimens.

Molecular identification. We extracted total genomic DNA (gDNA) from six trematode specimens collected from three *E. saurus* individuals. Extractions were performed using the Quick g-DNA MiniPrep Kit (Zymo Research, Irvine, California, USA) following the manufacturer's protocol for animal tissues. For each specimen, we used previously published primers and reaction conditions to PCR-amplify a 522–bp fragment of the mitochondrial cytochrome c oxidase subunit I (COI) gene (Dig_cox1Fa, 5′–ATG ATW TTY TTY-TTY YTD ATG CC–3′ and Dig_cox1R, 5′–TCN GGR TGH CCR AAR AAY CAA AA–3′; Wee et al. 2017) and an ~1,200-bp fragment of the nuclear 28S rDNA gene (28S–A, 5′–TCG ATT CGA GCG TGA WTA CCC GC–3′; Matejusova and Cunningham 2004, and 1500R, 5′–GCA TAG TTC ACC ATC TTT CGG–3′; Tkach et al. 2003). We verified positive PCR amplification using electrophoresis on 1% agarose gels.

PCR amplicons were cycle-sequenced by Eurofins Scientific, with resulting chromatograms assembled, checked, and edited (e.g. primers were trimmed) in CodonCode Aligner v. 10.0.1. Assembled sequences were screened for indicators of pseudogenes (e.g. premature stop codons), heteroplasmy (e.g. multiple peaks in mitochondrial chromatograms), and/or heterozygosity (e.g. multiple peaks in nuclear 28S rDNA chromatograms), but no evidence suggestive of pseudogenes, heteroplasmy, or heterozygosity was observed. We determined the putative identity of each successfully sequenced gene fragment using nucleotide BLAST searches using the megaBLAST option in May 2025.

As no publicly available COI sequences are available for the *Saccularina* species reported from North Carolina by Boggess et al. (2024), we conducted phylogenetic reconstructions using only the 28S rDNA dataset. We combined 28S rDNA sequences produced herein with those included by Boggess et al. (2024) in their phylogenetic analyses as well as all those reported by Louvard et al. (2022) in their original description of *S. magnacetabula*. We aligned the 28S rDNA dataset in MAFFT v. 7.407.1 (Katoh and Standley 2013; Katoh et al. 2019) using default settings and the resulting alignment was curated using trimAI v. 1.4.1 (Capella-Gutiérrez et al. 2009), with automatic detection of trimming parameters. Finally, phylogenetic relationships were estimated using both maximum likelihood (ML) using IQ-TREE v. 1.6.12 (Nguyen et al. 2015) as implemented in the IQ-TREE webserver (Trifinopoulos et al. 2016) and Bayesian inference (BI) using MrBayes v. 3.2.7 (Ronquist et al. 2012). Searches in IQ-TREE consisted of searching for the best model of nucleotide evolution as indicated by Modeltest (Kalyaanamoorthy et al. 2017) followed by tree reconstruction under the best model of evolution identified by Modeltest using the Akaike Information Criterion (AIC). Support for relationships was estimated using 1,000 ultrafast bootstrap replicates (Hoang et al. 2018). Meanwhile, searches in MrBayes v. 3.2.7 were conducted under the GTR + Γ model of nucleotide evolution and consisted of two independent searches, each run for 10×10^6 generations with sampling every 1,000th generation and all other settings as default. Posterior probabilities (PP) for each node were estimated by calculating the majority-rule consensus tree of the stationary stage using the SumTrees command (Sukumaran and Holder 2010). The number of variable sites and Kimura 2-Parameter (K2P) pairwise genetic distances were estimated in MEGA v. 11.0.13 (Tamura et al. 2021) for the 28S rDNA dataset as well as the COI haplotypes reported herein and those previously reported for *S. magnacetabula* by Louvard et al. (2022).

RESULTS

Saccularina magnacetabula Louvard et al., 2022

New record (Figure 2). UNITED STATES OF AMERICA / GULF OF MEXICO — Florida • Tampa Bay; 27°56′31″N, 082°32′34″W; depth 2–3 m; 02.II.2025; from an estuarine embayment bordered by a residential shoreline and municipal interstate highway; 21 spec., UF 1511–1513; 8 spec., GenBank PV904786 (COI), PV911608–09 (28S rDNA gene).

Morphological identification. All 22 *E. saurus* examined were infected with at least one worm. The observed behavioral and morphological characteristics of the worms were generally consistent with the findings reported by Louvard et al. (2022) (Table 1). Worms were found unencapsulated in the dorsal, pectoral, pelvic, anal, and caudal fins with most extracted worms found embedded in pairs (71%) between fin rays, and less commonly alone (20%) or in trios (9%). The general body form of the worms was filiform with a total body length ranging from 4.49–21.14 mm and mean of 13.32 ± 3.79 mm and mean body width of 411 ± 50 μm. Worms possessed a pair of eye spots at the anterior end, a single testis intertwined with ovary, and a sac-shaped excretory vesicle located at the posterior end (Figure 2). The majority of worms extracted were bright yellow due to the presence of eggs (57 ± 4 μm x 26 ± 2 μm mean length × width respectively). Immature or not yet heavily gravid worms were white to translucent. The oral sucker was anteriorly terminal (101 ± 9 μm x 66 ± 4 μm mean length × width respectively) and posteriorly overlapped by the pharynx (Figure 2).

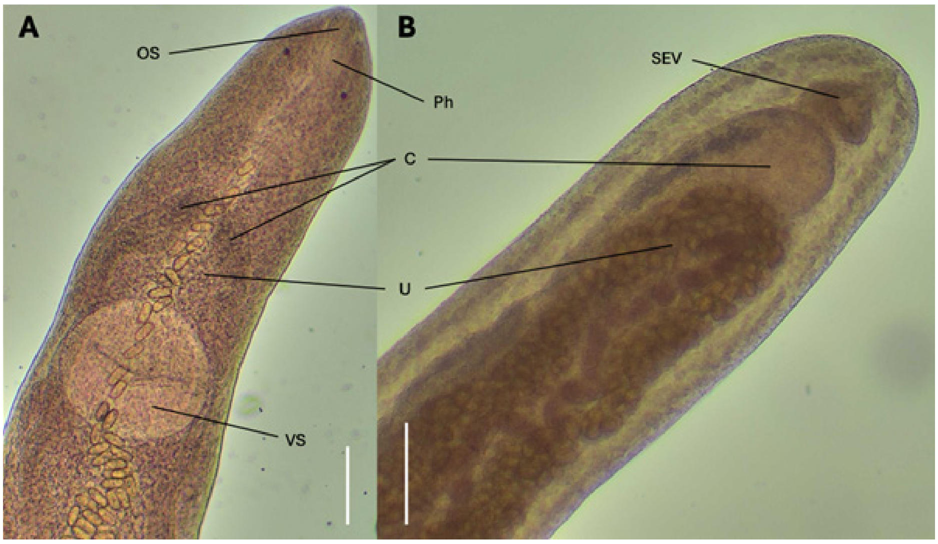

Figure 2. Identifying features of *Saccularina magnacetabula* collected in Tampa Bay, Florida, USA. **A.** Anterior end. **B.** Posterior end. C = Ceaca; OS = oral sucker; Ph = pharynx; U = uterus filled with eggs; VS = ventral sucker; SEV = saccular excretory vesicle. Scale bars: 200 µm.

Table 1. Comparison of morphometric measurements from *Saccularina magnacetabula* populations collected from Tampa Bay, Florida and reported from the east coast of Australia.

Source	Oral sucker (µm) range and (mean)		Ventral sucker (µm) range and (mean)		Egg dimensions (µm) range and (mean)		Body width (µm) range and (mean)	Body length (mm) range and (mean)
	Width	Length	Width	Length	Width	Length		
Present study	60–73 (66)	90–119 (101)	222–357 (296)	232–396 (305)	23–28 (26)	49–63 (57)	312–489 (411)	4.5–21.1 (13.32)
Louvard et al. 2022	29–41 (36)	40–62 (48)	117–188 (150)	136–169 (152)	10–18 (14)	33–39 (35)	220–477 (340)	11.4–19.5 (14.3)

The ventral sucker was subcircular to circular (305 ± 39 µm x 296 ± 37 µm mean length × width respectively). The esophagus was straight and opened to 2 blind caeca (Figure 2). The uterus was often heavily distended with eggs, looped once at the posterior end of the worm, and ascended to the genital pore (Figure 2).

Molecular identification. We successfully amplified both the COI and 28S rDNA genes for six trematode specimens extracted from three *E. saurus* individuals. All six trematode specimens shared a single COI haplotype (GenBank Acc. No: PV904786) that did not produce any matches of >98% similarity in BLAST searches. The best matches produced, as indicated by highest similarity scores, query cover, and E-values, were to the two COI haplotypes reported from *S. magnacetabula* by Louvard et al. (2022) [Query Coverage: 93–94%; E-values: 0.0–1×10–177, Percent Identity: 92.73–92.86%; up to 32-bp differences; COI K2P 7.6–7.8% K2P]. All other matches identified by BLAST produced low query cover scores, low identity scores, and/or high E-values.

We identified two 1,122-bp 28S rDNA haplotypes that differed at a single nucleotide position (GenBank accession nos.: PV911608–09). These two haplotypes produced matches of >99.5% similarity to *S. magnacetabula* sequences reported by Louvard et al. (2022), including the 28S rDNA haplotype reported for the *S. magancetabula* holotype (GenBank acc. no: OL336034). Matches also exhibited high query cover and low E-values (GenBank acc. no: OL336032–OL336035, Query cover: 89–100%, E-values = 0). No other sequences returned a similarity score >93%, with the putative *Saccularina* haplotype reported by Boggess et al. (2024) from North Carolina (GenBank acc. no: PP666254) producing a similarity score of only 92.89% (Query cover = 99%; E-value = 0).

The combined alignment of 28S rDNA sequences consisted of 3,019 nucleotides, of which 1,955 positions were flagged as poorly aligned by trimAl and excluded from further analysis. The final alignment thus consisted of 1,064 nucleotide positions. The topology of our inferred phylogenetic trees under ML and BI were consistent and match that of Boggess et al. (2024), apart from the inclusion of the 28S rDNA haplotypes from specimens collected in Tampa Bay and reported herein (Figure 3). These haplotypes are placed in a well-supported clade (BS = 100; PP = 100) with those reported for *S. magnacetabula* by Louvard et al. (2022). This clade includes the 28S rDNA haplotype reported for the *S. magnacetabula* holotype (GenBank acc. no: OL336033). The sister taxon to this clade was the putative *Saccularina* reported from North Carolina (GenBank acc. no: PP666254) by Boggess et al. (2024), a relationship that was strongly supported by Bayesian analyses (PP = 97) but only moderately so by maximum-likelihood analyses (BS = 81).

DISCUSSION

Herein, we used morphological characters and molecular data to identify the trematode specimens infecting *Elops saurus* collected in Tampa Bay, Florida as *Saccularina magnacetabula*, a species first described by Louvard et al. (2022) from specimens collected in Australian waters. This study thus represents the first record of this species in the Gulf of Mexico, and by extension the Atlantic Ocean.

We identified the specimens as *S. magnacetabula* based on their morphological and molecular similarities to those reported by Louvard et al. (2022). The adult worm morphologies and behavioral tendencies we observed were generally similar to those of the *S. magnacetabula* recovered from *E. hawaiiensis* hosts in Australia (Louvard et al. 2022). For instance, both body length and body width of the Tampa Bay collected *S. magnacetabula* closely resemble those reported from Australia (Table 1). Similarly, the 28S rDNA haplotypes recovered from our specimens differ solely by 1–2 nucleotides to those reported by Louvard et al. (2022) and are within the levels of intra-specific variation reported for this gene in parasitic flatworms (Blair 2006; Linh et al. 2022; Bray et al. 2022). Furthermore, phylogenetic reconstructions place the haplotypes recovered from Tampa Bay specimens in a well-supported clade (BS = 100; PP = 100) with the *S. magnacetabula* sequences produced by Louvard et al. (2022) including that of the species' holotype (Figure 3). Lastly, COI distances between haplotypes reported herein and those reported by Louvard et al. (2022), though relatively high (up to 32 differences of 522-bp; 7.6–7.8% K2P), fall within the range of intraspecific variation reported for some trematode species (Ghatani et al. 2014; McNamara et al. 2014; Bray et al. 2022).

Comparable levels of intraspecific variation in the COI gene have been documented across Indo-Pacific fish trematodes. Bray et al. (2022) reported maximum intraspecific differences of 28–54 nucleotides when comparing a 474-bp fragment of the COI gene for *Preptetos laguncula* Bray & Cribb, 1996, *P. paracaballeroi* Bray et al., 2022, and *P. zebravaranus* Bray et al., 2022 individuals collected across the Indo-Pacific. Similarly, McNamara et al. (2014) analyzed a 601-bp region of the COI gene in *Hurleytrematoides coronatum* Manter & Pritchard, 1961, *H. morandi* McNamara & Cribb, 2011, *H. loi* McNamara & Cribb, 2011, *H. deblocki* McNamara et al., 2012, and *H. sasali* McNamara & Cribb, 2011, and reported maximum intraspecific differences of 40–82 nucleotides. Highest intraspecific divergences were often observed between geographically distant localities, such as between Heron Island (Australia) and the Gambier Archipelago (French Polynesia) for *P. laguncula*, Moorea (French Polynesia) and Ningaloo (Australia) for *H. coronatum*, and Palau and Moorea for *H. deblocki* (McNamara et al. 2014). The divergence between the *S. magnacetabula* COI haplotypes reported here and

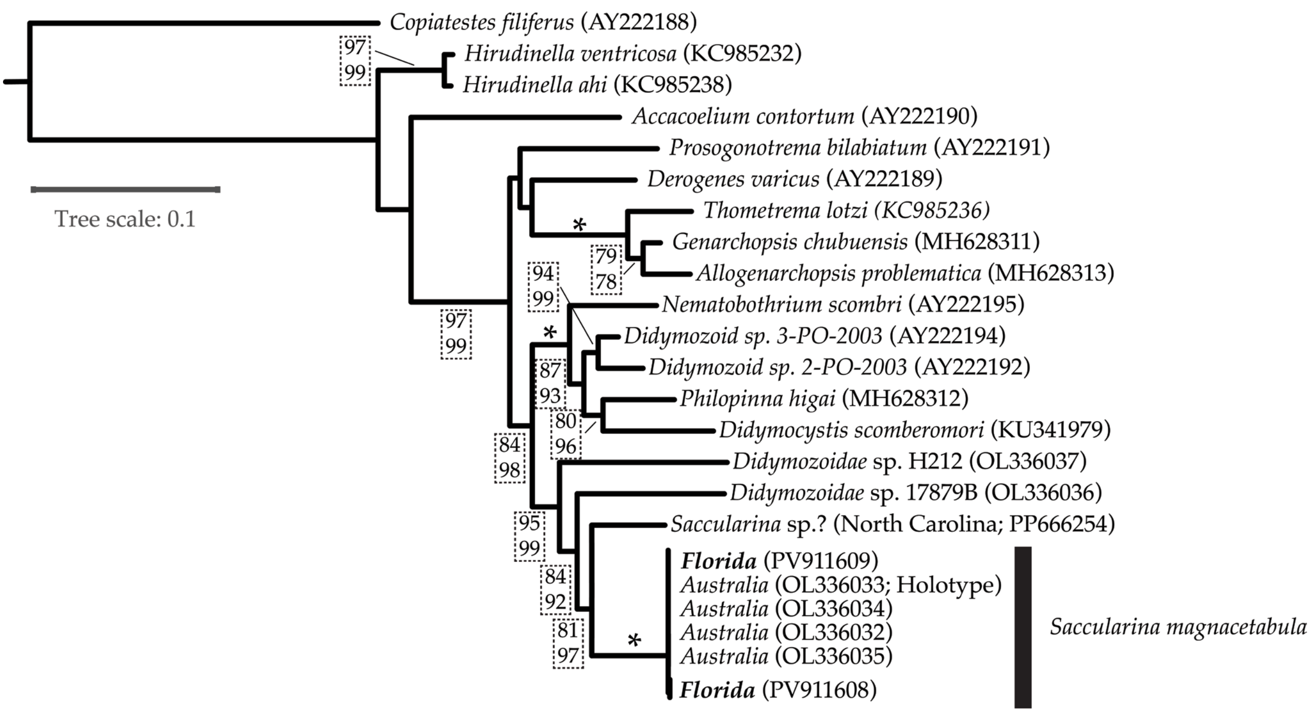

Figure 3. Bootstrap consensus tree of 28S rDNA haplotypes produced by IQ-TREE with support values as produced in both maximum-likelihood (ML) and Bayesian-inference (BI) analyses. Phylogenetic relationships were inferred under the best model of evolution indicated according to the AIC criterion and are rooted using *Copiastes filiferus*. Numbers by clades indicate bootstrap support in ML analyses (top) and posterior probabilities from BI analyses (bottom), with asterisks indicating 100% support in both analyses. Support values under 75 are not shown. 28S rDNA haplotypes recovered from Florida specimens are indicated in bold. Accession numbers are provided for all sequences used.

those reported by Louvard et al. (2022) may similarly reflect intraspecific variation among localities; however, additional surveys and molecular characterizations of *S. magnacetabula* across the Indo-Pacific remain needed.

Such work may also help explain the presence of *S. magnacetabula* in both Australian and Gulf of Mexico waters. Distribution data for marine trematodes remain limited (Cribb et al. 2016), and cosmopolitan species appear to be rare. For instance, a review of 9,880 marine fish trematode records from the Atlantic and Eastern Pacific found only 53 of 1,274 species to be cosmopolitan (Bray et al. 2016). Cosmopolitan species are typically associated with pelagic fishes such as tuna (Aiken et al. 2007) or with parasites that have a wide host breadth (Bray et al. 2016). While *Elops* are not pelagic, the genus inhabits tropical and temperate waters around the world with species exhibiting broad geographic distributions (Adams et al. 2014). In the same vein, multiple congeners of the first intermediate host described from Australian waters (*Anadara trapezia*) also occur in the Gulf of Mexico (*A. brasiliana* (Lamark, 1819), *A. secticostata* Reeve, 1844, and *A. transversa* (Say, 1822)). Of those, *A. brasiliana* has been documented to host hemiurid cercariae (Wardle 1975). Flexible host use may provide a path for *S. magnacetabula* to exhibit a broad geographic distribution. Dispersal may also occur via intermediate hosts, which remain only partially identified. Alternatively, the presence of *S. magnacetabula* in the Gulf of Mexico may reflect human-mediated dispersal, as hypothesized for other trematodes (Gérard and Le Lannic 2003; Saito et al. 2025). Given the limited knowledge of this recently described species, these explanations remain speculative and underscore the need for additional study.

Further work on *S. magnacetabula* may also help determine the extent of morphological variation within this species. Oral and ventral suckers, and the eggs from our worms were consistently about twice the size of those reported by Louvard et al. (2022) (Table 1). Trematode morphologies can vary significantly within a species as a result of infection intensity (Fischthal et al. 1982) and host species identity (Kinsella 1971; Toledo et al. 2004). Given our molecular results, morphometric differences observed herein could be the consequence of phenotypic variability resulting from different environmental conditions (i.e. different host species). Alternatively, differences between worm morphometrics in the two studies may also be an artifact of methodology. Measurements reported by Louvard et al. (2022) were taken from prepared slides of preserved worms mounted in Canada balsam. Worms in that study were reported to be “slightly” or “strongly” flattened prior to measurement, while our measurements were taken from unpreserved specimens that experienced a freeze/thaw cycle and mounted under the weight of a coverslip.

Our findings represent the first record of *S. magnacetabula* in the Gulf of Mexico and the Atlantic Ocean more broadly. It also may represent the first report of the *Saccularina* genus in these waters. Both the genus *Saccularina* and species *S. magnacetabula* were first described by Louvard et al. (2022) from specimens collected in Australia. Boggess et al. (2024) reported the presence of an unknown *Saccularina* species in North Carolina based on molecular identifications conducted on sporocysts found in the gill tissue of *A. irradians*. Boggess et al. (2024) conducted phylogenetic reconstructions based on 28S rDNA haplotypes and identified their specimens as an undescribed *Saccularina* species based on their well-supported sister-taxon relationship with *S. magnacetabula* (BS = 94%). Our findings, however, cast doubt on this identification. While our phylogenetic reconstructions recover this relationship between *S. magnacetabula* and the haplotype reported by Boggess et al. (2024) from North Carolina, support for the relationship is inconsistent across methods (BS = 81%, PP = 97%). Furthermore, the K2P distance between the putative *Saccularina* haplotype from North Carolina and those reported for *S. magnacetabula* herein and by Louvard et al. (2022) exceed intra-genus levels reported for trematode taxa (e.g. Linh et al. 2022). The North Carolina 28S rDNA haplotype is about 6.9% divergent from those reported for *S. magnacetabula*, a value that exceeds inter-genic distances seen in our 28S rDNA dataset (e.g. 3.2–6.0% K2P distances in comparisons between *Allogenarchopsis problematica* (Faust, 1924), *Genarchopsis chubuensis* Shimazu, 2015, and *Thometrema lotzi* Curran et al. 2002; Table 2). The divergence observed between the haplotype recovered in NC and those for *S. magnacetabula* also exceed inter-genic distances reported for other trematode taxa including gastrothylacid flukes (Ghatani et al. 2014) and digeneans from the Mediterranean (Blasco-Costa et al. 2009).

To conclude, although putative undescribed species of *Saccularina* have been suggested to occur along the Atlantic coast of North America (Scro et al. 2023; Boggess et al. 2024), this study appears to present the first record of *S. magnacetabula* in the Gulf of Mexico, and by extension the Atlantic Ocean. This finding expands the known range of the species and highlights the need for broader surveys to assess the distribution and host specificity of this trematode. Additional sampling of *Elops* species and additional molecular characterizations of *S. magnacetabula* may help elucidate the levels of intra-specific variation in this species, its natural geographic distribution, and the dispersal pathways that may explain their presence in both Australian and Gulf of Mexico waters.

Table 2. Estimates of evolutionary divergence, as measured by Kimura 2-parameter distances, for 28S rDNA haplotypes included in this study.

	Copiatestes filiferus AY222188	*Hirudinella ventricosa* KC985232	*Hirudinella ahi* KC985238	*Accacoelium contortum* AY222190	*Prosogonotrema bilabiatum* AY222191	*Derogenes varicus* AY222189	*Thometrema lotzi* KC985236	*Genarchopsis chubuensis* MH628311	*Allogenarchopsis problematica* MH628313	*Nematobothrium scombri* AY222195	Didymozoidae sp. AY222194	Didymozoidae sp. AY222192	*Philopinna higai* MH628312	*Didymocystis scomberomori* KU341979	Didymozoidae sp. OL336037	Didymozoidae sp. OL336036	*Saccularina* sp. NC? PP666254	*Saccularina magnacetabula* FL, USA PV911608–09	*Saccularina magnacetabula* AUS OL336032–35
Copiatestes filiferus AY222188	N/A																		
Hirudinella ventricosa KC985232	22.4	N/A																	
Hirudinella ahi KC985238	22.4	0.8	N/A																
Accacoelium contortum AY222190	23.9	11.1	10.5	N/A															
Prosogonotrema bilabiatum AY222191	27.0	12.5	12.5	14.3	N/A														
Derogenes varicus AY222189	24.7	11.7	12.0	14.3	10.6	N/A													
Thometrema lotzi KC985236	25.0	12.7	13.1	14.6	11.7	11.6	N/A												
Genarchopsis chubuensis MH628311	25.7	12.0	12.5	15.0	12.1	10.3	4.7	N/A											
Allogenarchopsis problematica MH628313	25.2	12.1	12.4	15.4	12.4	11.8	6.0	3.2	N/A										
Nematobothrium scombri AY222195	24.6	13.0	12.9	14.3	12.4	10.0	12.3	10.2	10.8	N/A									
Didymozoidae sp. AY222194	24.0	11.3	11.2	13.1	10.9	9.2	11.4	11.0	11.6	6.4	N/A								
Didymozoidae sp. AY222192	24.0	12.4	12.3	13.9	11.2	10.6	12.4	12.3	12.5	7.3	3.2	N/A							
Philopinna higai MH628312	25.8	13.2	13.1	13.3	12.6	11.2	13.3	12.4	13.2	6.7	6.1	7.2	N/A						
Didymocystis scomberomori KU341979	22.9	14.1	13.8	13.7	13.5	13.1	13.9	13.8	14.3	9.6	7.6	8.3	8.4	N/A					
Didymozoidae sp. OL336037	27.6	16.9	17.3	16.6	15.7	14.0	15.2	15.1	15.1	13.8	11.1	12.5	15.0	15.4	N/A				
Didymozoidae sp. OL336036	24.4	15.7	16.0	16.1	12.2	12.3	13.5	13.3	14.2	12.7	11.1	12.2	12.5	13.9	13.6	N/A			
Saccularina sp.? NC, USA PP666254	23.6	13.6	13.7	14.6	11.6	11.1	12.4	11.6	11.8	10.7	8.4	9.9	10.9	11.4	12.0	9.8	N/A		
Saccularina magnacetabula FL,USA PV911608–09	24.7	14.2	14.3	15.1	11.6	11.0	13.3	12.9	13.6	10.2	8.9	9.9	10.6	11.7	12.0	9.9	6.9	0.1	
Saccularina magnacetabula AUS OL336032–35	24.8	14.3	14.4	15.2	11.6	11.0	13.3	12.9	13.6	10.2	8.9	9.9	10.6	11.8	12.0	9.9	6.9	0.1	0.0

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ADDITIONAL INFORMATION

Conflict of interest

The authors declare that no competing interests exist.

Ethical statement

No ethical statement is reported.

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Author contributions

Conceptualization: CAS, ESD, KEG, LJA . Data curation: CAS, ESD, KEG, LJA, LW.. Formal Analysis: CAS. Funding acquisition: ESD. Investigation: CAS, ESD, KEG, LJA, LW. Methodology: CAS, ESD, KEG, LJA. Project administration: CAS, ESD, LJA. Resources: ESD, LJA. Software: CAS. Supervision: CAS, ESD, LJA. Validation: CAS, ESD, LJA. Visualization: CAS, ESD, LJA. Writing-original draft: CAS, ESD, LJA. Writing-review and editing: CAS, ESD, KEG, LJA, LW.

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Data availability

All data that support the findings of this study are available in the main text.
Voucher specimens have been deposited at the Florida Museum of Natural History in Gainesville, FL, USA (Catalog Number: UF73965). Unique haplotypes uncovered in this study have been deposited in GenBank under accession numbers PV904786 (COI) and PV911608–09 (28S rDNA).

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