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The white grunt, *Haemulon plumierii* (Lacepède, 1801) as paratenic and definitive host of two acanthocephalan species, with the description of a new species of *Dollfusentis* (Palaeacanthocephala: Leptohynchoididae) from the Yucatán Peninsula, Mexico

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Abstract

Acanthocephalans are a group of obligate endoparasites that alternate between vertebrates and invertebrates to complete their life cycles. Occasionally, the same individual host acts as a definitive or paratenic host for different acanthocephalan species. In this study, acanthocephalans were sampled in marine fish in three localities of the Yucatán Peninsula; adults and cystacanths were recovered from the intestine and body cavity, respectively, of *Haemulon plumierii* from off the coast of Sisal, Yucatán. Ribosomal DNA sequences (small and large subunits) were used to test the phylogenetic position of the species of the genus *Dollfusentis*, whereas the mtDNA gene *cox 1* was used for assessing species delimitation. The *cox 1* analysis revealed an independent genetic lineage, which is recognized herein as a new species, *Dollfusentis mayae* n. sp. The new species is morphologically distinguished from the other six congeners by having a cylindrical proboscis armed with 22–25 longitudinal rows bearing 12 hooks each. The cystacanths were morphologically identified as *Gorgorhynchus medius* by having a cylindrical trunk covered with tiny irregular spines on the anterior region, and a cylindrical proboscis armed with 17–18 longitudinal rows of 21 hooks each; small and large subunit phylogenetic analyses yielded *G. medius* within the family Isthomosacanthidae, suggesting that *Gorgorhynchus* should be transferred to this family from Rhadinorhynchidae where it is currently allocated.

Introduction

Acanthocephalans, also known as thorny-headed worms, belong to a relatively small group of obligate endoparasites occurring, as adults, in the alimentary tract of vertebrates (Kennedy, 2006). The phylum comprises four classes (Archiacanthocephala, Meyer 1931 Eoacanthocephala Van Cleave 1936, Palaeacanthocephala Meyer, 1931, and Polyacanthocephala Amin, 1987), with approximately 1300 species described worldwide (Amin, 2013). Currently, Palaeacanthocephala contains three orders, namely Echinorhynchida Southwell & Macfie, 1925, Heteromorphida Amin & Ha, 2008, and Polymorphida Petrochenko, 1956 (Amin, 2013). Palaeacanthocephalans are the most diverse, with approximately 470 species classified into 12 families (Pichelin & Cribb, 2001; Monks & Richardson, 2011; Smales, 2012; Amin, 2013; Huston *et al.* 2020; Huston & Smales 2020; García-Varela & Andrade-Gómez, 2021; Kita *et al.* 2023). The life cycle of acanthocephalans in this group is complex and diverse, including malacostracan crustaceans as intermediate hosts, and vertebrates as definitive hosts (Kennedy, 2006). Some species of palaeacanthocephalans modify the behaviour or coloration of their intermediate hosts increasing their susceptibility to predation (Kennedy, 2006); for this reason, these acanthocephalans have been the target of numerous studies related to their ecology, host-parasite relationships, pathogenicity, taxonomy and systematics (Kennedy, 2006), especially their phylogenetic position within Acanthocephala (Huston *et al.* 2020; Huston & Smales, 2021; García-Varela & Andrade-Gómez 2021; Kita *et al.* 2023; Li *et al.* 2023; Perrot-Minnot *et al.* 2023).

Sixty-five species of acanthocephalans have been described as parasites of vertebrates, which represent the 5% of the diversity of helminths in Mexico (Pérez-Ponce de León *et al.* 2011). Twenty-three of them occur in estuarine and marine fishes across the Pacific, Gulf of Mexico, and Caribbean Sea (García-Prieto *et al.* 2010). In the Yucatán Peninsula (in southeastern Mexico),

only 12 nominal species of acanthocephalans have been reported parasitizing 17 species of marine and estuarine fishes (García-Prieto *et al.* 2010). Two species of palaeacanthocephalans use a broad spectrum of definitive hosts. For example, *Dollfusentis chandleri* Golvan, 1969 is a leptorhynchoidid that has been reported from 10 fish species, i.e., *Mayaheros urophthalmus* (Günther), *Lutjanus griseus* (L.), *Eugerres plumieri* (Cuvier), *Oligoplites saurus* (Bloch & Schneider), *Haemulon melanurum* (L.), and *Scomberomorus maculatus* (Mitchill), *Centropomus parallelus* (Poey), *Micropogonias undulatus* (L), *Leiostomus xanthurus* (Lacepède), and *Diapterus auratus* (Ranzani), across the Gulf of Mexico and Caribbean Sea of Mexico (García-Prieto *et al.* 2010) and it has been also reported from haemulids in Brazil and the United States (see Paschoal *et al.* 2015). The isthomosacanthid *Gorgorhynchoides bullocki* Cable & Mafarachisi, 1970 has been reported from 14 fish species as cystacanth (*Atherinomorus stipes* (Müller & Troschel), *Caranx latus* (Günther), *Eucinostomus jonesii* (Günther), and *E. plumieri*, *L. griseus*, *L. analis* (Cuvier) and as adult (*Dasyatis guttata* (Bloch & Schneider), *D. sabina* (Lesueur), *Selene vomer* (L.), *Sphyraena barracuda* (Edwards), *Scomberomorus sierra* Jornan & Starks, *Gerres cinereus* (Walbaum), and *C. hippos* (L.), *Hexanematichthys assimilis* (Günther)) (García-Prieto *et al.* 2010).

During a survey of the parasite fauna of marine fishes of the Yucatán Peninsula, four species of acanthocephalans, three of them belonging to the genus *Dollfusentis* Golvan, 1969 (*Dollfusentis salgadoi* Monks, Alemán-García & Pulido-Flores, 2008; *Dollfusentis bravoae* Salgado-Maldonado 1976, and an undescribed species of *Dollfusentis*), and one species of the genus *Gorgorhynchus* Chandler, 1934[*Gorgorhynchus medius* (Linton 1908) Chandler, 1934], were collected and morphologically identified from the intestines of the white grunt, *Haemulon plumierii* (Lacepède) and the striped mojarra, *Eugerres plumieri* from three localities. The main objectives of the current study were *i*) to characterize molecularly and morphologically the species *Gorgorhynchus medius* and *Dollfusentis* sp; *ii*) to test the phylogenetic position of these two species; *iii*) to analyze in further detail the interrelationships among species of *Dollfusentis*; and *iv*) to describe *Dollfusentis* sp., sampled from *H. plumierii* from off the coast of Sisal, Yucatán, Mexico.

Material and methods

Host collection and morphological study

During several field expeditions in 2019 and 2020, two specimens of the white grunt, *H. plumierii* were collected off Champoton, Campeche (19° 21′ 40.3″ N, 90° 43′5.37″ W), and 24 from off Sisal, Yucatán (21° 08′ 1.5″ N, 90° 07′ 55.9″ W). Additionally,13 specimens of the striped mojarra, *E. plumieri* were sampled from off Chetumal, Quintana Roo (18° 29′ 29.8″ N, 88° 17′ 50.13″ W) (Fig. 1). Acanthocephalans were removed either from the intestines or from the body cavity of their hosts, where some cystacanths were found encysted. Later, the cystacanths were excysted and the adult specimens were washed in a 0.85% saline solution, placed in distilled water at 4°C overnight, and subsequently fixed in 70% or 100% ethanol. A few acanthocephalans were gently punctured in the body with a fine needle, stained with Mayer's paracarmine, destained in 70% acid ethanol, dehydrated in a graded ethanol series, cleared in methyl salicylate, and mounted as permanent slides with Canada balsam. Specimens were deposited in the Colección Nacional de Helmintos, Instituto de Biología, Universidad Nacional Autónoma de México, Mexico City (CNHE, 12844-12848). Acanthocephalans were initially identified by conventional morphological criteria following the key of Yamaguti (1963), and the original descriptions. Measurements of the specimens are presented in micrometers unless otherwise stated and with the mean

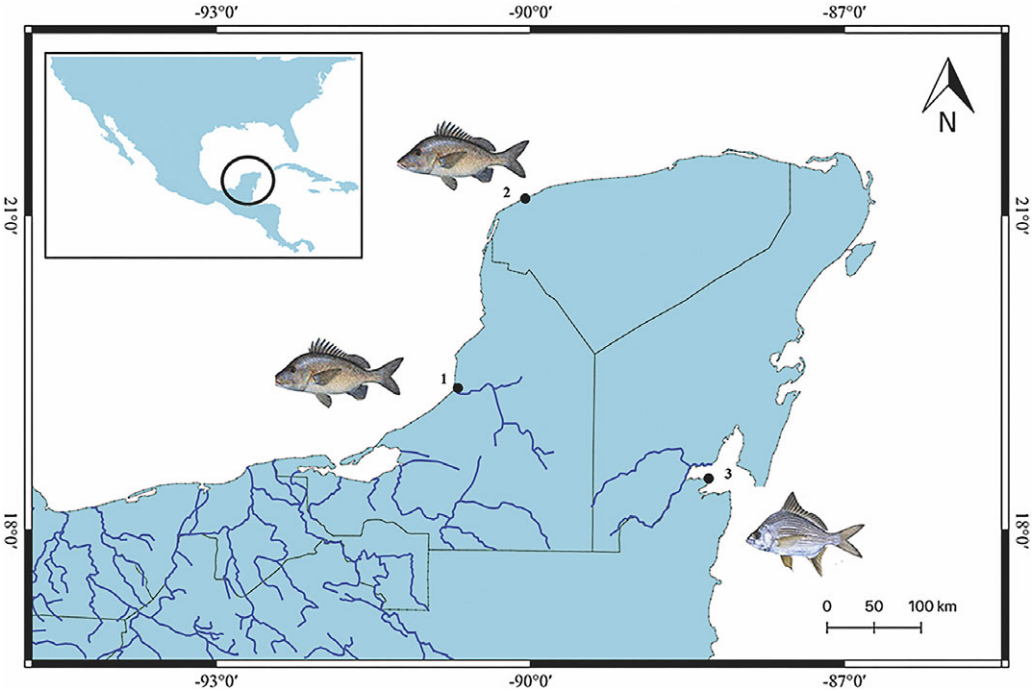

Figure 1. Map of Mexico showing the sampled sites. Locality, 1 Champoton, Campeche (19° 21′ 40.3′′ N, 90° 43′ 5.37′′ W); Locality 2, Sisal, Yucatán (21° 08′ 1.5′′ N, 90° 07′′ 55.9′′ W); Locality 3. Chetumal, Quintana Roo (18° 29′ 29.8′′ N, 88° 17′ 50.13′′ W).

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followed by range in parentheses. For scanning electron microscopy (SEM), specimens were dehydrated through an ethanol series, critical point dried with CO_2, sputter coated with gold, and examined with a Hitachi Stereoscan Model S-2469N scanning electron microscope operating at 10 kV at the Instituto de Biología, Universidad Nacional Autónoma de México (UNAM).

DNA sequencing and phylogenetic analyses

A total of 13 specimens, two of *Gorgorhynchus medius,* two of *Dollfusentis salgadoi*, five of *Dollfusentis bravoae*, and four of an undescribed species of *Dollfusentis* were individually digested overnight at 56°C in a solution containing 20 mM NaCl, 100 mM Na_2 EDTA (pH 8.0), 10 mM Tris–HCl (pH 7.6), 1% sarkosyl, and 0.1 mg/ml proteinase K. Following digestion, genomic DNA was extracted from the supernatant using DNAzol reagent (Molecular Research Center, Cincinnati, OH, USA) according to the manufacturer's instructions. Two regions of nuclear ribosomal DNA (rDNA) and one mitochondrial DNA region were amplified using polymerase chain reaction (PCR). A near-complete fragment from the small subunit from 18S rDNA (~1800 bp; SSU) was amplified using two overlapping PCR fragments of 1000 bp: the SSU amplicon 1 using the forward primer 5'-AGA TTA AGC CAT GCA TGC GT-3' and reverse primer 5'-AAC TTT TCG TTC TTG ATT AA TG-3' and the SSU amplicon 2 using the forward primer 5'-GCA GCG CGG TAA TTC CAG CTC-3' and reverse primer 5'-GCA GGT TCA CCT ACG GA AA-3' (García-Varela & Nadler, 2005). A near-complete fragment of the large subunit from 28S rDNA (~2900 bp; LSU) was amplified using three overlapping PCR fragments of 1200–1300 bp: the LSU amplicon 1 using the forward primer 5'-CAA GTA CCG TGA GGG AAA GTT GC-3' and reverse primer 5'-CAG CTA TCC TGA GGG AA AC-3', the LSU amplicon 2 using the forward primer 5'-ACC CGA AAG ATG GTG AAC TA TG-3' and the reverse primer 5'- CTT CTC CAA CGT CAG TCT TC AA-3', and the LSU amplicon 3 using the forward primer 5'- CTA AGG AGT GTG TAA CAA CTC ACC-3' and reverse primer 5'-CTT CGC AAT GAT AGG AAG AG CC-3' (García-Varela & Nadler, 2005). Finally, the cytochrome c oxidase subunit 1 (*cox 1*) from the mitochondrial DNA was amplified using the forward primer 5'-AGTTCTAATCATAA(R)GATAT(Y)GG-3′ and reverse primer 5' -TAAACTTCAGGGTGACCAAAAAAT CA-3′ (Folmer *et al.* 1994). PCR amplifications were performed in a total volume of 25 µL containing 2 µL of each primer, 10 pmol/µL, 2.5 µL of 10X buffer, 1.5 µL of 2 mM $MgCl_2$, 2 µL of the genomic DNA, and 1 U of Taq DNA polymerase (Platinum Taq, Invitrogen Corporation, California, United States). PCR cycling parameters for rDNA amplifications included denaturation at 94° C for 3 min, followed by 35 cycles of 94°C for 1 min, annealing at 50–58°C (optimized for each fragment amplified) for 1 min, and extension at 72°C for 1 min, followed by a post-amplification incubation at 72°C for 7 min. Sequencing reactions were performed with the primers mentioned above using ABI Big Dye (Applied Biosystems, Boston, Massachusetts) terminator sequencing chemistry. Reaction products were separated and detected using an ABI 3730 capillary DNA sequencer. Contigs were assembled and base-calling differences resolved using Codoncode Aligner version 9.0.1 (Codoncode Corporation, Dedham, Massachusetts).

Newly generated sequences were aligned with published sequences for other acanthocephalans retrieved from the GenBank dataset. Alignments for each molecular marker (SSU, LSU, and *cox 1*) were constructed using the software Clustal W (Thompson *et al.* 1994). A nucleotide substitution model was selected for the dataset using jModelTest version 2.1.7 (Posada, 2008). Phylogenetic analyses were inferred through maximum likelihood (ML) with the program RAxML version 7.0.4 (Stamatakis 2006). A GTRGAMMAI substitution model was used, and 10,000 bootstrap replicates were run to assess nodal support. In addition, a Bayesian analysis was carried out, using the program MrBayes 3.2.2 (Ronquist *et al.* 2012) with two Markov chain Monte Carlo runs for 10 million generations, sampling every 1000 generations, a heating parameter value of 0.2 and a burn-in of 25%. The resulting phylogenetics trees were visualized and edited using FigTree v.1.4.4 (Rambaut & Drumond 2007). The genetic divergence among taxa was estimated using uncorrected "p" distances with the program MEGA version 11 (Kumar et al. 2016).

Results

Taxonomy
Class: Palaeacanthocephala Meyer 1931
Order: Echinorhynchida Southwell & Macfine 1925
Family: Leptorhynchoididae Witenberg 1923
Genus: *Dollfusentis* Golvan 1969
Species: *Dollfusentis mayae* n. sp.
Type host: white grunt, *Haemulon plumierii* (Lacepède 1801).
Site of infection: Small intestine (prevalence 4% [1/25]).
Type locality: off the coast of Sisal, Yucatán (21° 08′ 1.5″ N, 90° 07′ 55.9″ W).
Type-material: CNHE: 12844 (holotype); 12845 (allotype); 12846 (paratype).
Representative DNA sequences: OR886452 (SSU); OR886455 (LSU); OR883550–OR883553 (*cox 1*).
Etymology: The specific epithet refers to the Maya civilization that inhabited the Yucatán Peninsula of Mexico for approximately 4000 years, between 2000 B.C and 1541 A.D.

Description

Dollfusentis mayae n. sp. (Figs. 2–3).

General

Sexual dimorphism evident, females larger than males. Trunk cylindrical, covered with spines on the anterior region of trunk, extending to level of 1/4 of proboscis receptacle in both sexes. Anterior spines larger than posterior spines. Proboscis long, cylindrical covered with 22–25 longitudinal rows with 12 hooks each, decreasing in size towards posterior end, with a transition to minute hooks and a ring of eight hooks in the posterior region of the proboscis. Neck smooth. Proboscis receptacle double-walled. Lemnisci elongate, one shorter than the other and both longer than proboscis receptacle. Gonopore terminal in both sexes.

Male (based on 1 mounted specimen and 1 for SEM). Trunk, 3.7 mm long × 330 wide. Anterior trunk spines large 53 (43–63), decreasing in size; posterior trunk spines 15 (11–18). Proboscis 429 × 54, with 22–25 longitudinal rows with 12 hooks each. Apical hooks (first 9–10, rows of hooks) 31 (21–36) long, middle hooks (11–16, rows of hooks) 17 (14–20) long, basal hooks (17–25, row of hooks) 11 (8–13). Neck 283 × 85. Proboscis receptacle 1151 × 141.

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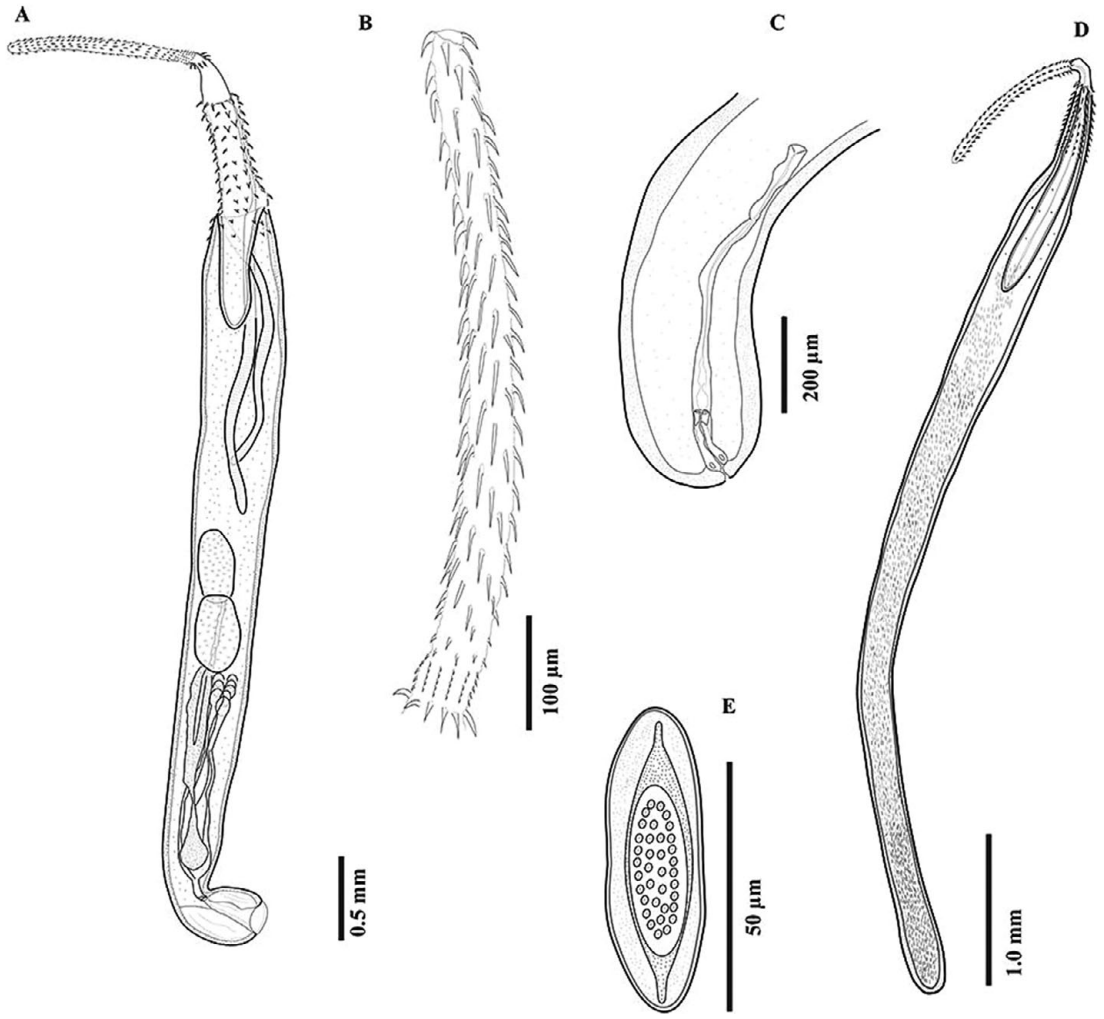

Figure 2. Line drawings of *Dollfusentis mayae* n. sp., from *Haemulon plumierii* from off Sisal, Yucatán, Mexico. Adult male, whole worm (**A**); proboscis (**B**); female reproductive system (**C**); adult female, whole worm (**D**); egg (**E**).

Lemnisci of different size, 1.9–2.0 mm long. Testes ovoid in tandem, anterior 365 × 145, posterior 354 × 185. Seminal vesicle oval, elongated. Cement glands claviform, eight in number. Saefftigen's pouch pyriform, 569 long. Copulatory bursa well-developed, 320 long × 220 wide.

Female (based on three gravid mounted specimens). Trunk 5.8 mm long (5.5–6.4 mm) × 392 wide (341–460). Anterior trunk spines longer 74 (59–89), decreasing in size, posterior spines 19 (13–25). Proboscis 959 (636–1265) × 99 (89–112), with 22–25 longitudinal rows with 12 hooks each. Apical hooks (first 9–10, rows of hooks) 38 (34–42) long, middle hooks (11–16, rows of hooks) 24 (19–29) long, basal hooks (17–25, row of hooks) 10 (7–13). Neck 169 (128–210) × 81 (70–92). Proboscis receptacle 1444 (1270–1591) × 177 (104–221). Neck 421 (399–443) × 379 (370–389). Female reproductive system from the apical part of the uterine bell to posterior end of the body, 814 long. Uterine bell 380 long. Uterus 307 long. Gonopore terminal. Mature eggs elongated (n = 90), with polar prolongations 56 (51–61) long × 16 (15–19) wide.

Remarks

Dollfusentis mayae n. sp. represents the seventh species described of the genus *Dollfusentis*, which contains *D. chandleri*, *D. bravoae*, *D. salgadoi*, *D. ctenorhynchus* (Cable & Linderoth, 1963) Golvan, 1969, *D. longispinus* (Cable & Linderoth, 1963) Golvan, 1969, and *D. lenti* Keidel, García-Varela, Brener, Pérez-Ponce de León & Santos, 2019 associated mainly with brackish and marine fishes in the Americas (see Keidel *et al.* 2019). The new species can be differentiated from the other six congenerics species by the number of hooks in the proboscis. *Dollfusentis mayae* n. sp. possesses the largest number longitudinal rows of hooks in proboscis, with 22–25, whereas *D. bravoae* possesses 16–17, *D. salgadoi* possesses 17–19 hooks, *D. chandleri* 19–21, *D. ctenorhynchus* 12–14, *D. longispinus* has 22 longitudinal rows of hooks, and *D. lenti* possesses 16–17 hooks.

Additionally, *Dollfusentis mayae* n. sp., can be differentiated by having smaller eggs of 56 (51–61) long × 16 (15–19) wide *vs* 63–75 × 15 in *D. chandleri*; 60 (41–72) × 15 (10–18) in *D. salgadoi*; 61–77 × 16 in *D. bravoae.*

Taxonomy

Class: Palaeacanthocephala Meyer 1931

Order: Polymorphida Petrochenko 1956

Family: Isthmosacanthidae Smales 2012

Genus: *Gorgorhynchus* Chandler 1934

Species: *Gorgorhynchus medius* (Linton 1908) Chandler 1934 (Figs. 4–5) (Cystacanth)

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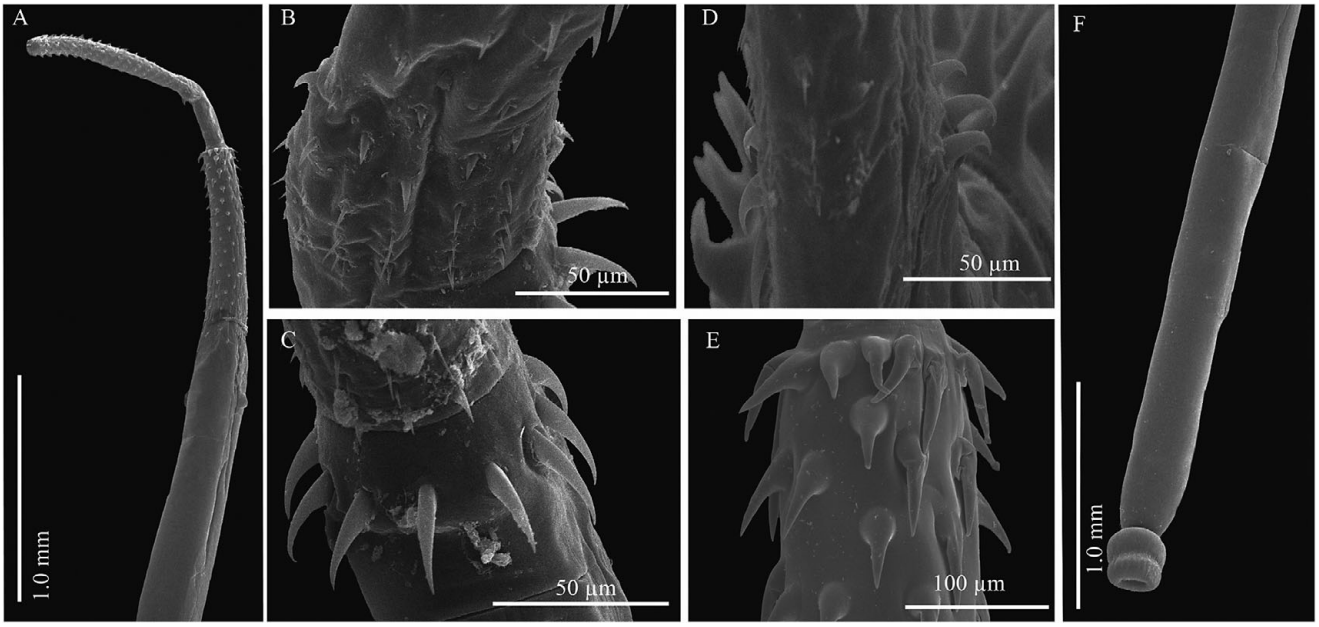

Figure 3. Scanning electron photomicrographs of *Dollfusentis mayae* n. sp., from *Haemulon plumierii* from off Sisal, Yucatán, Mexico. Adult male, anterior region (**A**); proboscis hooks, posterior region (**B-D**); anterior region of trunk (**E**); adult male, posterior end (**F**).

Host: white grunt, *Haemulon plumierii* Lacepède 1801
Site of infection: Mesentery (prevalence 12% [3/25]).
Locality: Off the coast of Sisal, Yucatán (21° 08′ 1.5″ N, 90° 07′ 55.9″ W).
Voucher: CNHE, 12847–12848.
Representative DNA sequences: OR886454 (SSU); OR886457 (LSU); OR883561–OR883562 (*cox 1*).

Redescription

General: Large acanthocephalans, sexual dimorphism evident, females larger than males.

Trunk cylindrical, swollen anteriorly, covered with tiny irregular spines on anterior region of trunk, reaching mid-level of proboscis receptacle in both sexes. Proboscis cylindrical armed with 17–18 longitudinal rows of 21 hooks each of different size; Apical hooks and basal hooks smaller. Neck short, conical, aspinose. Proboscis receptacle double-walled; cerebral ganglion sub-oval at posterior end; lemnisci tubular, longer than proboscis receptacle. Gonopore subterminal in both sexes.

Male (based on three immature mounted specimens and one for SEM). Trunk 8.9 mm (8.5–9.3 mm) × 1.09 (1.09–1.1); maximum width at anterior region. Trunk spines 50 (41–59). Proboscis 1080 × 377 (368–386). Neck 400 (365–436) × 402 (356–449). Proboscis receptacle 2.2 (2.1–2.2 mm) × 522 (505–540). Lemnisci 4.9 (4.1–5.4 mm) long. Testes ovoid, anterior testis 230 (215–245) × 173 (164–182). Posterior testis 224 (162–286) × 215 (181–250). Four tubular cement glands, 3.8 (3.5–4.0 mm) long.

Female (based on two immature mounted specimens). Trunk 10.3 mm (9.3–11.3 mm) × 988 (960–1016); maximum width anterior region. Trunk spines 40 (24–57) long. Proboscis 1.19 (1.18–1.21 mm) × 351 (330–372). Neck 421 (399–443) × 379 (370–389). Proboscis receptacle 2.06 mm (2.03–2.12 mm) × 512 (484–540). Lemnisci 4.5 mm (4.3–4.8 mm). Uterine bell short with thick body wall; uterus long; vagina complex with four bulbs connected to vagina; gonopore subterminal.

Remarks

The genus *Gorgorhynchus* includes 12 valid species as parasites of teleost marine fishes and elasmobranchs distributed in tropical and subtropical waters (Amin & Van Ha, 2011; Smales *et al.* 2019). In the Americas, five species of *Gorgorhynchus* have been described, three in South America, i.e., *G. clavatus* Van Cleave, 1940 (13–15 longitudinal rows of hooks); *G. lepidus* Van Cleave 1940 (14–16 longitudinal rows of hooks), and *G. trachinotus* Noronha, Vicente, Pinto & Fabio, 1986 (14 longitudinal rows with 11–12 hooks per row); one in North America (*G. gibber* Chandler, 1934) (24 longitudinal rows with 18 hooks per row); and one in the Caribbean Sea (*G. medius*) (22 longitudinal rows with 20 hooks per row); representing 41.6% of the species described in the genus. The acanthocephalans recovered from the white grunt, *H. plumierii* from off Sisal, Yucatán, show similar morphological characteristics compared to those assigned to *G. medius* including *i*) an elongated cylindrical trunk covered with tiny irregular spines on the anterior region of trunk, ii) neck short, conical, *iii*) a cylindrical proboscis, *iv*) proboscis hooks arranged in 17–18 longitudinal rows of 21 hooks per row, *v*) a double-walled proboscis receptacle, and *vi*) four long tubular cement glands very long in males (Figs. 4–5).

Phylogenetic analyses

Nuclear ribosomal markers

The alignment of the SSU consisted of 93 taxa and 2219 sites, whereas the LSU alignment included 48 taxa with 3077 sites. The phylogenetic trees inferred with the SSU dataset recovered Echinorhynchida as paraphyletic with the highest bootstrap (100%) and Bayesian posterior probability support values (1.0) (Fig. 6). On the one hand, *Dollfusentis* spp. were recovered as members of Leptorhynchoididae, which included species from eight genera (*Leptorhynchoides* Kostylew 1924; *Pseudoleptorhynchoides* Salgado-Maldonado 1976; *Metacanthocephalus* Yamaguti 1959; *Illiosentis* Van Cleave 1921; *Dentitruncus* Sinzar 1955; *Neotegorhynchus* Lisitsyna, Xi, Orosová, Barčák & Oros 2022; *Koronacantha*

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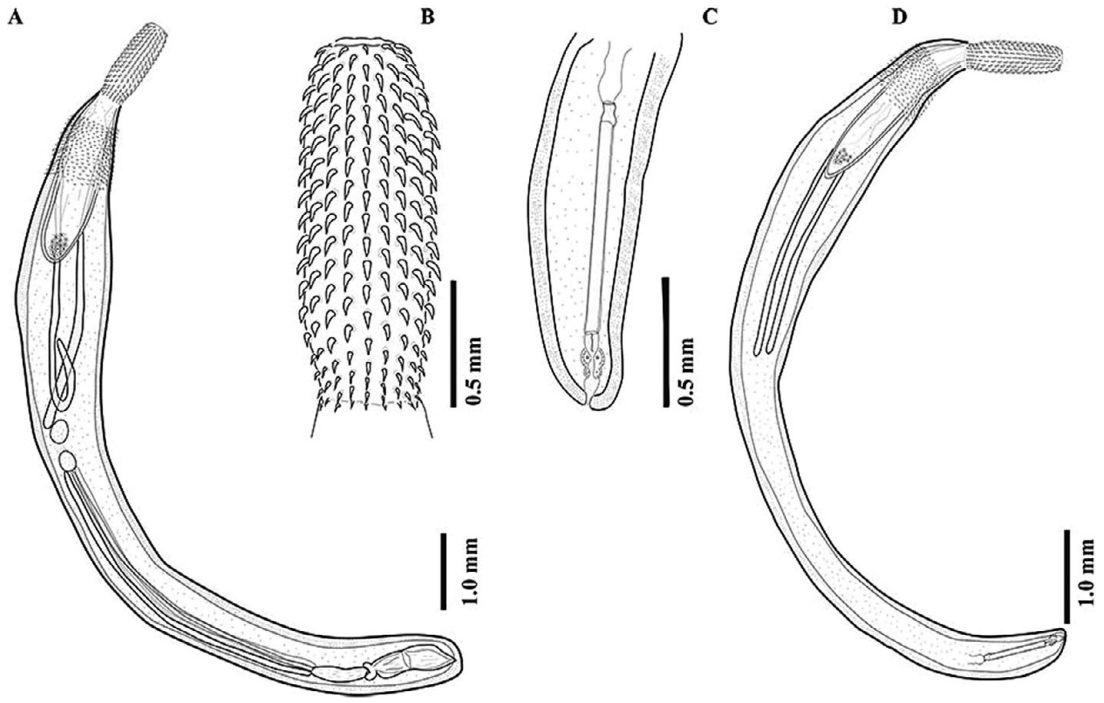

Figure 4. Drawing of *Gorgorhynchus medius* from *Haemulon plumierii* from off Sisal, Yucatán, Mexico. Adult male, whole worm (**A**); proboscis (**B**); female reproductive system (**C**); adult female, whole worm (**D**).

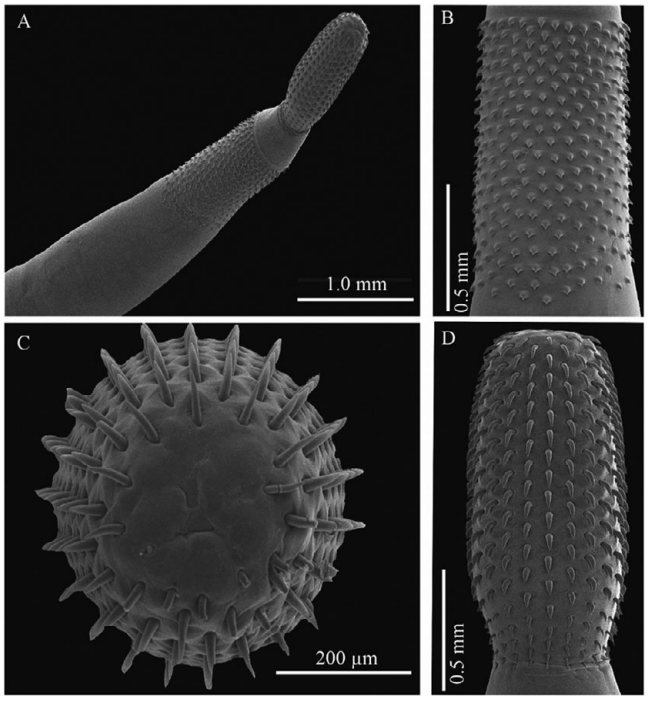

Figure 5. Scanning electron photomicrographs of *Gorgorhynchus medius* from *Haemulon plumierii* from off Sisal, Yucatán, Mexico. Adult male, anterior region (**A**); anterior region of trunk (**B**); proboscis (**C-D**).

Monks and Pérez-Ponce de León 1996; and *Dollfusentis* Golvan 1969). The three species of *Dollfusentis* from the Gulf of Mexico and Caribbean Sea included *D. bravoae*, as sister species of the newly sampled specimen of *D. salgadoi* and *D. mayae* n. sp., which formed a monophyletic clade with moderate nodal support (Fig. 6). They appeared in an unresolved clade along with *D. lenti* described from the grunt, *Orthoprisris ruber*, from off Rio de Janeiro, Brazil, and *N. cyprini* + *D. truttae*. On the other hand, the phylogenetic trees showed the sequence of *G. medius* as the sister clade of isolates of two genera, *Serrasentis* Van Cleave 1923 and *Gorgorhynchoides* Cable & Linderoth 1963, both members of the Isthomosacanthidae, with strong bootstrap (100%) and Bayesian posterior probability (1.0) support values (Fig. 6).

The phylogenetic trees inferred with the LSU dataset yielded different topology than the SSU trees. Taxa representation for both molecular markers is somewhat different, although both analyses agreed on the systematic position of the taxa under study. For instance, *D. bravoae*, *D. salgadoi*, and *D. mayae* n. sp. were placed together within a clade formed by species of the genera *Pseudoleptorhynchoides, Leptorhynchoides*, *Metacanthocephalus*, *Illiosentis*, and *Koronacantha*, with strong bootstrap (100%) and Bayesian posterior probability support values (1.0) (Fig. 7). LSU sequences of *D. lenti* are available in GenBank but they are very short; hence, they were not included in the present analysis. The new species was not clearly distinguished from the other two congeners using this molecular marker. Additionally, the LSU sequence of *G. medius* nested as the sister taxa of two isolates of *Serrasentis sagitiffer* (Linton 1889) Linton 1932 and these two as the sister group of three species of *Gorgorhynchoides,* i.e., *G. gnathanodontos* Smales 2014 and *G. bullocki* + *G. pseudocarangis* Huston & Smales 2021, all of them members of Isthomosacanthidae. These relationships were highly supported by bootstrap and Bayesian posterior probability support values (Fig. 7). In summary, the phylogenetic trees inferred with each nuclear ribosomal marker consistently placed *D. bravoae*, *D. salgadoi*, and *D. mayae* n. sp. inside Leptorhynchoididae (order Echinorhynchida), and the species *G. medius* inside Isthomosacanthidae (order Polymorphida), both clades highly supported by bootstrap and Bayesian posterior probability support values (Figs. 6–7).

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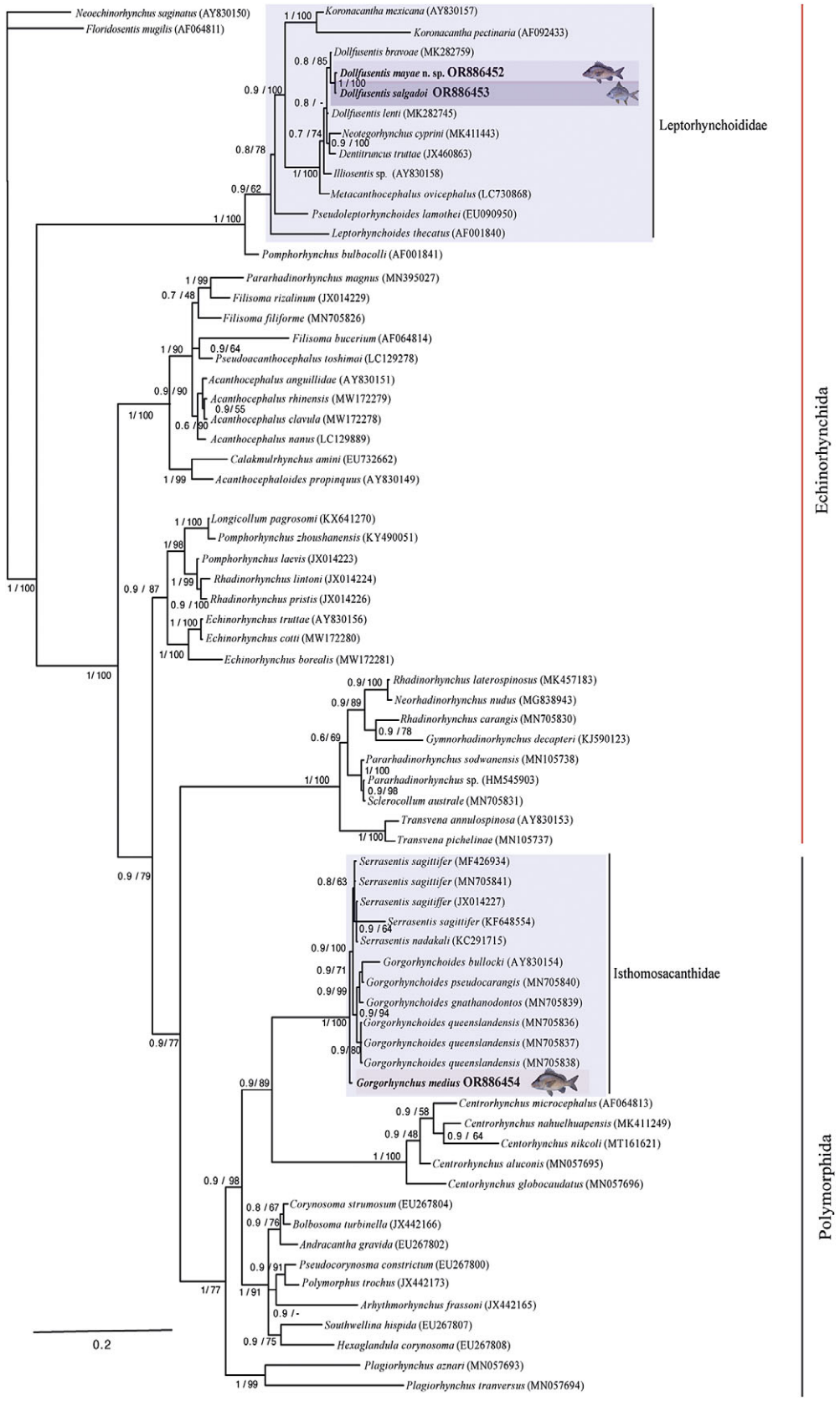

Figure 6. Maximum likelihood tree and consensus Bayesian Inference trees inferred with small subunit from 18S rDNA; numbers near internal nodes show posterior probabilities (BI) and ML bootstrap values. Sequences in bold were generated in this study.

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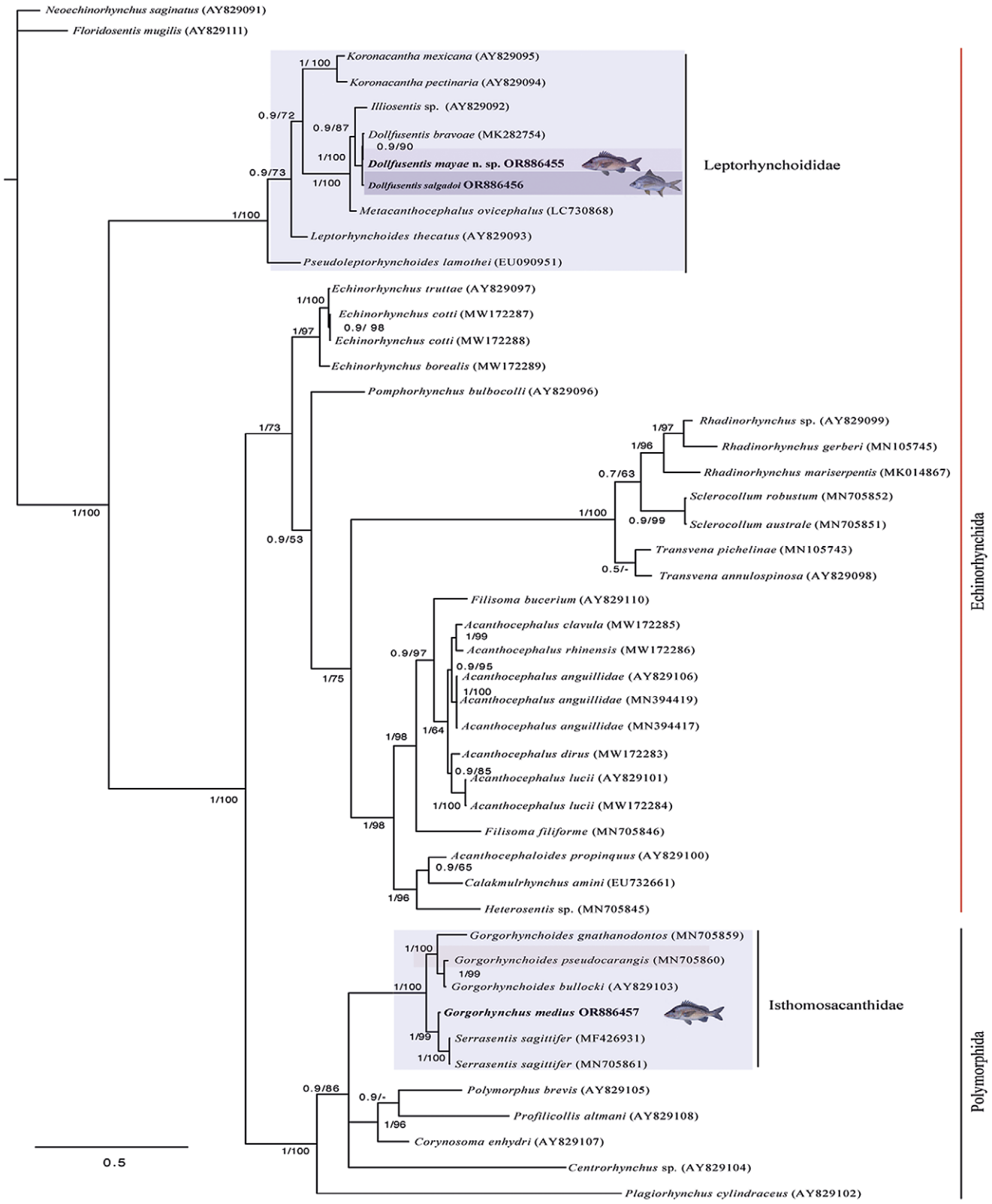

Figure 7. Maximum likelihood tree and consensus Bayesian Inference trees inferred with large subunit from 28S rDNA; numbers near internal nodes show posterior probabilities (BI) and ML bootstrap values. Sequences in bold were generated in this study.

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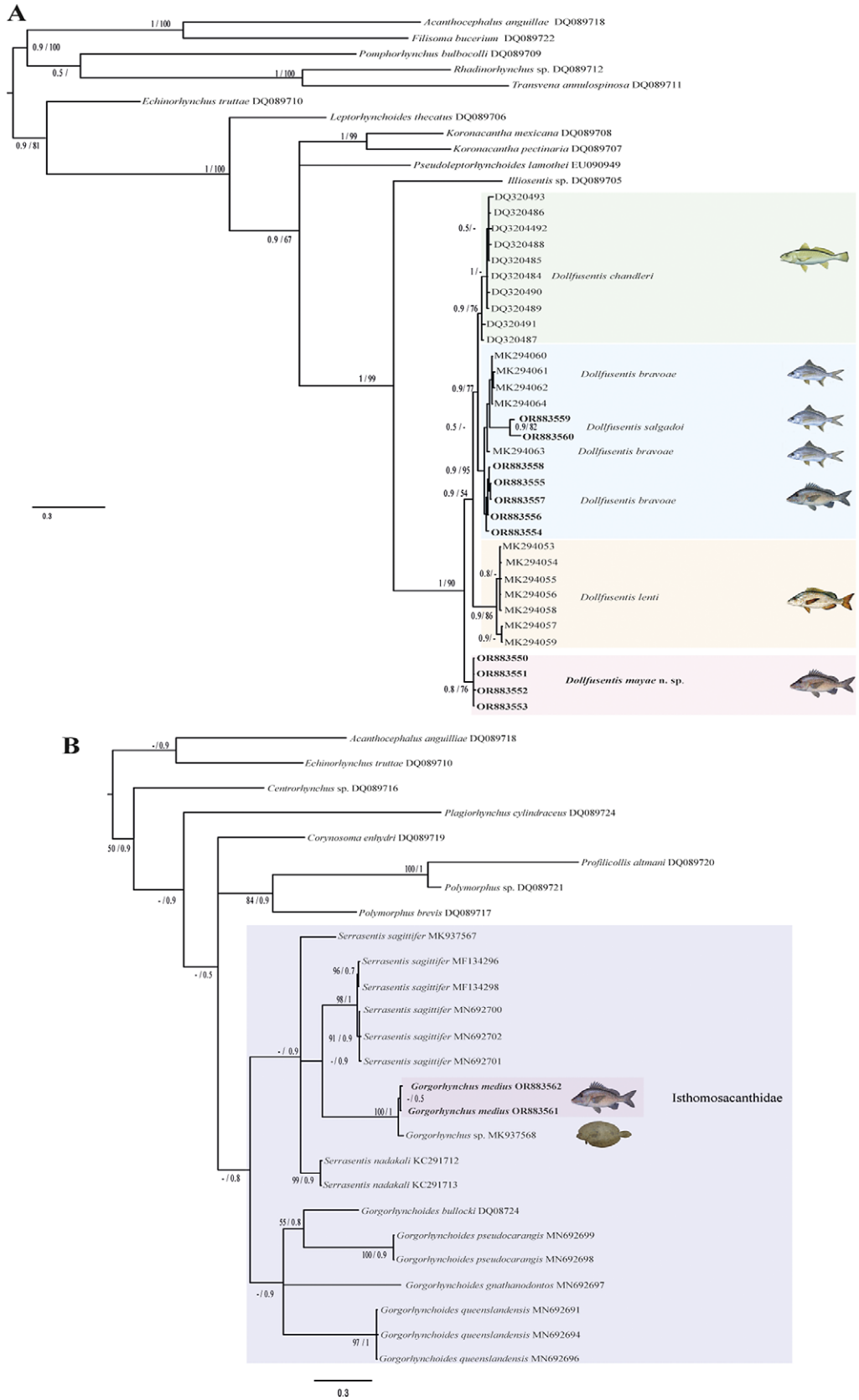

Figure 8. Maximum likelihood tree and consensus Bayesian Inference trees inferred with cytochrome c oxidase subunit 1 from the mitochondrial DNA of *Dollfusentis* spp., (**A**); members of the family Isthomosacanthidae (**B**). Numbers near internal nodes show posterior probabilities (BI) and ML bootstrap values. Sequences in bold were generated in this study.

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Cox 1

The *cox 1* alignment for the *Dollfusentis* analysis included 655 sites and 44 sequences. The phylogenies inferred with the ML and posterior probabilities (BI) methods yielded *Dollfusentis* as a monophyletic group, with strong bootstrap and Bayesian posterior probabilities support. The phylogenetic trees showed that *Dollfusentis* consisted of four main subclades. The first subclade was formed by four sequences of *D. mayae* n. sp. (Fig. 8A). The second subclade was formed by seven sequences of *D. lenti.* The third subclade was formed by five sequences of *D. bravoae* collected from the type host and locality, the striped mojarra, *E. plumieri* in Sontecomapan, Veracruz, in the Gulf of Mexico, and five newly generated sequences of *D. bravoae* from *E. plumieri* from Champoton, Campeche. Surprisingly, within this clade, two newly generated sequences of the species *D. salgadoi* (collected from the type host and locality, also the striped mojarra, *E. plumieri* from Isla Tamalcab, Chetumal, Quintana Roo, in the Caribbean Sea), were nested inside of a clade formed by sequences of *D. bravoae.* Finally, the fourth subclade included 10 sequences identified as *D. chandleri*, all from the Atlantic Croaker, *Micropogonias undulatus* from the Atlantic coast of the United States (Fig. 8A). The genetic divergence estimated among the isolates of *Dollfusentis mayae* n. sp., ranged from 0.0% to 0.04%; among isolates of *D. chandleri* and among those of *D. lenti*, divergence ranged from 0.02% to 2.0, and among the 12 isolates of *D. bravoae*, it ranged from 0.0% to 7.0%. Finally, the genetic divergence between *D. mayae* n. sp. and *D. chandleri* ranged from 3. 5% to 5.7%; between *D. mayae* n. sp., and *D. bravoae* ranged from 4.5% to 9.2%; and the genetic divergence between *D. mayae* n. and *D. lenti,* was 7%.

For the *Gorgorhynchus* analysis the *cox 1* alignment included 26 sequences with 655 nucleotides. The phylogenies inferred with the ML and posterior probabilities (BI) methods showed that the family Isthomosacanthidae is monophyletic, albeit with weak nodal support values (Fig. 8B). Two main unresolved subclades were formed, one containing four species of *Gorgorhynchoides*, including *G. bullocki* from Mexico, two sequences of *G. pseudocarangis*, one of *G. gnathanodontos*, and three sequences of *G. queenslandensis* Smales 2014 all from Australia. The second subclade, also unresolved, consisted of two sequences of *Serrasentis nadakali* George & Nadakal 1978 from India, single sequence of *Serrassentis sagittifer* from the dusky flounder (*Syacium papillosum*) from Yucatán Peninsula, Mexico, and a group consisting of five sequences of *S. sagittifer* from the cobia, *Rachycentron canadum* and the emperor red snapper, *Lutjanus sebae* from Australia as the sister group of the two newly sequenced specimens of *G. medius* from the white grunt, *H. plumierii* from off Sisal, Yucatán, Mexico, plus a sequence of an specimen identified as *Gorgorhynchus* sp., also from the dusky flounder, *Syacium papillosum* from Yucatán Peninsula, Mexico (Fig. 8B). The genetic divergence estimated among the two isolates *G. medius* was 0.07%, and between both isolates and the unidentified sequence of *Gorgorhynchus* was 2.0%. However, the divergence between *G. medius* and the other congeners varied from 18% to 25%.

Discussion

The transmission strategy of the acanthocephalans is complex and contains an ecological framework; sometimes the same individual host may act as definitive and paratenic host during the transmission (see Perrot-Minnot *et al.* 2019). In the current study, we observed that the white grunt, *H. plumierii* can harbor adult acanthocephalans in the intestine, and cystacanths in the body cavity. The adult specimens were described as *Dollfusentis mayae* n. sp., representing the fourth species recorded in coasts of the Gulf of Mexico and Caribbean Sea, and the seventh in the genus *Dollfusentis*, which is solely distributed across the Atlantic coast of the Americas in association to brackish water and marine fishes (see Keidel *et al.* 2019). The new species can be morphologically differentiated from the other congeneric species described by the number of longitudinal rows of hooks in the proboscis. Our phylogenetic analyses based on sequences of two nuclear ribosomal markers consistently placed the new species nested within a well-supported clade formed by *D. bravoae* + *D. salgadoi* (Figs. 6–7). In addition, the phylogenetic relationship inferred with *cox 1,* which has a faster substitution rate also supported the monophyly of *Dollfusentis* (Fig. 8A). The *cox 1* sequences placed all the isolates of *Dollfusentis mayae* n. sp. (Fig. 8A) in a reciprocally monophyletic subclade. The five sequences of *D. bravoae* from the type host and locality plus five sequences of *D. bravoae* from Champoton, Campeche, plus two sequences of *D. salgadoi* from the type host and locality, conformed a subclade (see Fig. 8A), suggesting that all these isolates belong to the same species. This is suggested because *D. salgadoi* was described from the same host species than *D. bravoae*, and mitochondrial DNA sequences were nested within the latter. Therefore, *D. salgadoi* must be synonymized with *D. bravoae*. The type host of *D. bravoae* is the striped mojarra (*E. plumieri*), a demersal fish widely distributed along the western Atlantic, from North Carolina south along the U.S. coast, in the Gulf of Mexico, in the Caribbean Sea from Cuba to Puerto Rico, and along the Central and South American coast from Mexico to Colombia (see Miller *et al.* 2005). It is plausible then to postulate that *D. bravoae* is specific of *E. plumieri* and its present distribution extends from Veracruz, where it was originally described as Campeche in the Gulf of Mexico and as Chetumal in the Caribbean Sea.

The second acanthocephalan species found as cystacanth in the body cavity of the white grunt was identified as *G. medius*, a parasite that uses *H. plumierii* as a paratenic host. The phylogenetic analyses based on sequences of two nuclear ribosomal markers consistently placed *G. medius* within a clade formed by species of the genera *Gorgorhynchoides* and *Serrasentis*, both members of the Isthomosacanthidae (Figs. 6–7). However, both trees differed in the position of *G. medius* with respect to the other two, rendering the interrelationships among the genera controversial. The SSU recovered *Gorgorhynchus* as the sister taxa of the other two (Fig. 6), whereas the LSU tree recovered the genus *Gorgorhynchus* as the sister taxa of *Serrasentis*, and these two as sister taxa to *Gorgorhynchoides* (Fig. 7). However, regarldess of their sister group relationships, the genus *Gorgorhynchus* has been traditionally placed in the family Rhadinorhynchidae (Amin, 2013; Pichelin *et al.* 2016), even though the family has been found to be non-monophyletic (see García-Varela & Nadler, 2005; Verweyen *et al.* 2011). Smales (2012) reviewed the systematic and classification of *Gorgorhynchoides* and *Golvanorhynchus* Noronha, Fabio & Pinto, 1978 using morphological traits and transferred both genera to the family Isthomosacanthidae, and erected the type-genus *Isthmosacanthus* Smales, 2012. With the inclusion of *Gorgorhynchus* to Isthomosacanthidae, the family was proposed to contain five genera: *Isthmosacanthus, Gorgorhynchoides*, *Golvanorhynchus*, *Gorgorhynchus*, and *Serrasentis* Van Cleave 1923. More recently, Huston *et al.* (2020), performed phylogenetic analyses inferred with the small and large subunit from the ribosomal DNA and found support for the monophyly of Isthomosacanthidae, placing

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it inside Polymorphida and not in Echinorhynchida as previously classified. Our phylogenetic analyses inferred with the same molecular markers support the conclusion of Huston et al. (2020).

In the current study, we also generated *cox 1* sequences for two samples of *G. medius*. The alignment included sequences from the genera *Gorgorhynchoides*, *Serrasentis*, and *Gorgorhynchus* (Fig. 8B). The two isolates of *G. medius* were nested with an unidentified species of *Gorgorhynchus* from the dusky flounder (*S. papillosum*) from the Yucatán Peninsula forming a clade (Fig. 8B). Interestingly, these sequences nested within *Serrasentis*, a genus that seems to need a deep revision. The intraspecific genetic divergence among the three samples of *Gorgorhynchus* ranged from 0.07% to 2.0%, which is similar to that found in other genera of Isthomosacanthidae. For instance, the divergence among three isolates of *Gorgorhynchoides queenslandensis* from Australia ranged from 0.03% to 0.06%; between two isolates of *G. pseudocarangis*, divergence was 0.01%, and between two sequences of *S. nadakali*, it was 0.05%. It seems that the sequences from the dusky flounder (*S. papillosum*) reported by Vídal-Martínez et al. (2019) are conspecific with *G. medius*.

Finally, the life cycle of acanthocephalans is very complex and the inclusion of paratenic hosts is considered an ecological trait that has been conserved across the phylum (Kennedy, 2006). Since the same individual host can act alternatively as definitive and paratenic host, such as the white grunt, *H. plumierii*.

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Author contribution. M.G.V. and G.P.P.L.: conceptualization, phylogenetic analysis, writing – original draft, review, editing. A.L.S.U. and B.S.G.: sampling, investigation, genetic data, morphological study. G.P.P.L. and M.G.V.: funding acquisition, review, and editing.

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Competing interest. The authors declare that they have no competing financial interests or personal relationships that could have appeared to influence the work reported in this manuscript.

Ethical standard. All applicable institutional, national, and international guidelines for the care and use of animals were followed. Fish analysed in this study were obtained from commercial capture; specimens from Yucatán were sampled under the collecting permit granted to the Laboratorio de Biología de la Conservación (BioCon) by the Comisión Nacional de Acuacultura y Pesca (CONAPESCA), No. PPF/DGOPA-001/20. Fish were humanely euthanized following the protocols described by the 2020 edition of the American Veterinary Medical Association (AVMA) Guidelines for the Euthanasia of Animals.

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