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Integrative taxonomy reveals a new species of *Dollfusentis* (Acanthocephala: Illiosentidae), in *Orthopristis ruber* (Osteichthyes: Haemulidae) from Rio de Janeiro, Brazil

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ABSTRACT

The taxonomy of species of *Dollfusentis* is rather confused due to the overlap of morphological traits. The aim of this study was to follow an integrative taxonomy approach over the acanthocephalans collected from *Orthopristis ruber* in Brazil. *Dollfusentis lenti* n. sp. is described and is characterised by having an elongate trunk with spines sparsely distributed (largest 60 − 85 μm long) extending from the neck to almost reach the end of proboscis receptacle; additionally, the new species possesses a long proboscis with 12–14 longitudinal rows of 16–17 hooks each; 3–4 posterior hooks reduced in size, well-spaced from the eight ventrolateral crescent hooks, and lemnisci longer than proboscis receptacle. New sequences of 18S rDNA, ITS1, 5.8S and ITS2, 28S rDNA and COI mtDNA are provided. *Dollfusentis bravoae* is morphologically similar because it possesses the same number of proboscis hooks, although it differs by the size of testes and uterus and by having a higher number of trunk spines; additionally, new scanning electron micrographs and genetic data for both species support its distinction. Phylogenetic analysis obtained either with two nuclear genes or mitochondrial COI gene showed that *Dollfusentis* spp. belong to Illiosentidae, and the new species is yielded as the sister species of *D. bravoae*, with *D. chandleri* as the sister species of the latter two.

1. Introduction

The marine fish, *Orthopristis ruber* (Cuvier, 1830) (Haemulidae), commonly known as "grunt", is widely distributed in the Western Atlantic and feeds mainly on invertebrate and small fishes in rocky and coralline seabeds [1–3]. During a parasitological survey on *O. ruber* off Rio de Janeiro coast, Brazil, specimens of acanthocephalans belonging to the genus *Dollfusentis* Golvan, 1969 were recovered from the intestine of their hosts. The genus *Dollfusentis* currently contains five valid species distributed in marine and brackish water fishes along the Atlantic coast of the Americas, most of them found in the Gulf of Mexico slope and the Caribbean Sea [4], although Amin [5] recognizes six species.

Previous reports of acanthocephalans parasitizing *O. ruber* in Brazil were listed in two checklists, indicating records of only two species, *Dollfusentis chandleri* Golvan, 1969 and *Serrasentis* sp. [6,7]. Considering the wide distribution of *D. chandleri* in marine fishes across the western Atlantic Ocean between North and South America, it is possible to expect morphological and molecular differences among specimens, raising the possibility, that *D. chandleri* consists of more than one species. Therefore, the aim of this study was to follow an integrative taxonomy approach with regard to the acanthocephalans collected from *O. ruber* in Brazil, including morphological, scanning electron microscopy analysis, distributional and genetic data. We uncovered a new species of *Dollfusentis*, which is described herein.

2. Materials and methods

2.1. Sample collection and taxonomic identification

From 2015 to 2018, 109 specimens of *O. ruber* were collected by local fisherman at Guanabara Bay near Urca beach, Rio de Janeiro, Brazil, (22°56′39.4″S, 43°09′46.0″W). The fish were transported on ice

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to the laboratory to be measured, weighed and identified according to Menezes and Figueiredo [8]. Acanthocephalans were collected from the intestine under a dissecting microscope. The specimens were washed and kept overnight at 4 °C in saline solution 0.7%, and then fixed in 70% ethanol for DNA study or 4% formalin for morphological study. For morphological identification, specimens were stained with Mayer's paracarmine, cleared in clove oil and mounted in Canada balsam. Measurements are presented in micrometers, with the mean values followed by range and sample size (n) of specimens measured in parentheses. Some individuals were cleared in Berlese's medium to inspect with more accuracy of the spines and hooks. Illustrations were made with the aid of a drawing tube. Specimens were deposited at the Helminthological Collection of Institute Oswaldo Cruz (CHIOC). Additionally, 20 specimens of *Dollfusentis bravoae* Salgado-Maldonado, 1976 were collected in February 2018 by MGV and GPPL after the study of 25 individuals of *Eugerres plumieri* (Cuvier, 1830) from the type-locality, Sontecomapan lagoon in Veracruz, Mexico, (18°31′45″N, 95°01′08″W), and processed for both molecular and morphological analyses, to be compared with the specimens sampled in Brazil.

For comparative purposes, specimens of *D. chandleri* previously deposited at CHIOC n° 31981, 31982 a-g, 32194, 32195, 32196, 32198, 32199 a-b, 32204 and 34022 were reexamined. Also, specimens of *D. chandleri* and other two species of *Dollfusentis*, *i.e. D. bravoae*, and *D. salgadoi* Monks, Alemán-García and Pulido-Flores, 2008 from the Colección Nacional de Helmintos, México City (CNHE) were examined: *D. chandleri* 673, 664, 665, 5719; *D. bravoae* 649 (Holotype), 651; *D. salgadoi* 6267 (Holotype), 6268, 6269.

2.2. Scanning electron microscopy (SEM)

The specimens of the new species fixed in 4% formalin were washed in 0.1 M sodium cacodylate buffer and post-fixed in 1% osmium tetroxide and potassium ferrocyanide 0.8% solution in the dark overnight, at room temperature. Specimens were dehydrated in alcohol series (30–100%), critical point dried with CO_2, coated with gold and observed in a JEOL JSM 6390 LV scanning electron microscope (JEOL USA Inc., Peabody, MA, USA) at Plataforma de Microscopia Electrônica Rudolf Barth of Institute Oswaldo Cruz at an accelerating voltage of 15 kV. Additionally, two specimens of *D. bravoae* were dehydrated through a graded series of ethyl alcohol, critical-point dried with CO_2, coated with gold, and examined in a Hitachi Stereoscan model SU1510 (Hitachi High-Technologies Mexico S.A. de C.V., Mexico) at 15 kV of the Biology Institute of Universidad Nacional Autónoma de México (UNAM).

2.3. Genetic analysis

The genomic DNA was extracted from mid-body of eight adult specimens from Brazil and seven specimens morphologically determined as *D. bravoae* from Mexico using QIAamp DNA Mini Kit (Qiagen, Hilden, Germany), according to the manufacturer's recommendations. The partial 28S rDNA region was amplified by polymerase chain reaction (PCR) using the primers C1 (5′ − ACCCGCTGAA TTTAAGCAT − 3′) and D2 (5′ − TGGTCCGTGTTTCAAGAC − 3′) [9,10]. The internal transcribed spacer (ITS) 1, 5.8S and ITS2 were amplified with the set of primers BD1 (5′ − GTCGTAACAAGGTTTCCGTA − 3′) and BD2 (5′ − TATGCTTAARTTCAGCGGGT − 3′) [11]. For the 18S rDNA fragment the primers (5′ − AGATTAAGCCATGCATGCGTAAG − 3′) and (5′ − TGATCCTTCTGCAGGTTCACCTAC − 3′) were used [12]. Primers used for the amplification of the mitochondrial cytochrome oxidase *c* subunit 1 gene (COI) were LCO1490 (5' − GGTCAACAAATCATAAAGA TATTGG − 3') and HC02198 (5' − TAAACTTCAGGGTGACCAAAAAA TCA − 3') [13].

Polymerase chain reactions were performed in 15 μl reactions, containing 7.5 μl of 2 × GoTaq® Colorless Master Mix (Promega), 1.5 μl of each primer with a final concentration of 10 pMol, 2.0 μl of DNA sample and ultrapure water to complete. For 28S rDNA and ITS1, 5.8S and ITS2 regions the thermocycling protocol followed [14]. The 18S rDNA and COI regions were amplified following [15]. PCR products were visualized after electrophoresis on a 1.5% agarose in Tris-borate EDTA gel, stained with SyberGreen (Invitrogen, Eugene, Oregon, USA) and photographed under UV transillumination. PCR amplicons were purified with ExoSAP-IT (Affymetrix, Cleveland, Ohio, USA). DNA cycle sequencing reactions were performed using BigDye v.3.1 chemistry (Applied Biosystems, Foster City, CA, USA) using the same primer set as those used for PCR amplification. Automated sequencing was performed in an ABI Prism 3730 sequence analyser at the Sequencing Platform of the Fundação Oswaldo Cruz-PDTIS/Fiocruz. Newly generated sequences of both strands were checked and edited using the MEGA 7 software [16]. The comparison for similarities with sequences from the GenBank database was performed using the BLAST program from the National Center for Biotechnology Information (NCBI) server (http://www.ncbi.nlm.nih.gov/BLAST) [17].

2.4. Phylogenetic analysis

In order to explore the phylogenetic relationships, the nucleotide sequences were aligned with the CLUSTAL W algorithm using the MEGA 7 software [18]. Sequences of COI region were translated into amino acid to check for the presence of premature stop codons, and the genetic divergence among isolates was estimated using the uncorrected (*p*-distance) method, as performed in the same software. Phylogenetic trees were constructed through Maximum likelihood (ML) with the program RAxML v7.0.4 [19]. Newly generated sequences of the new species were aligned with available sequences of members of five members of the Illiosentidae Golvan, 1960; additionally, sequences of representative species of six genera of Palaeacanthocephala were used as outgroups for rooting the trees. Nucleotide substitution model was selected for each molecular marker using jModelTest v0.1.1 [20] and applying the Akaike criterion. A GTRGAMMAI substitution model was used for the three data sets, and 10,000 bootstrap replicates were run to assess nodal support. Sequences from GenBank used for phylogenetic analysis are listed in Table 1.

3. Results

A total of 109 specimens of *Orthopristis ruber* measuring 9–24 (17) cm long were studied and we found 69 of them parasitized by specimens of *Dollfusentis.* After a detailed study of the morphological characteristics of the newly sampled specimens, we concluded that the species from Brazil corresponds with a new species, which is described below.

3.1. *Dollfusentis lenti* n. sp. (Figs. 1–2)

3.1.1. General description

Females larger than males. Trunk cylindrical and elongate with spines extending from the neck junction until almost the end of proboscis receptacle; armature similar in both sexes; anterior spines much longer than posterior ones. Proboscis long, cylindrical, with 12–14 longitudinal rows of 16–17 hooks each (Figs. 1A and B; 2A). Posterior 3–4 hooks of each row much reduced in size with a vertical pattern (Figs. 2A and D). Sensory papilla present laterally between the fifth and sixth or sixth and seventh hooks from the posterior end of hooks rows (Fig. 2A). Ventrolateral crescent of eight large hooks well-spaced from the posterior hooks of the proboscis. Small spines anteriorly pointed may be present at posterior trunk of males (Fig. 2F). Neck smooth. Proboscis receptacle double-walled. Two lemnisci, elongate, one slightly shorter than the other and both longer than proboscis receptacle.

Table 1
Acanthocephalans used in phylogenetic analyses with GenBank accesion numbers.

Species	Family	18S rDNA	28S rDNA	COI mtDNA
Acanthocephalus anguillae	Echinorhynchidae	AF469413		
Acanthocephalus dirus	Echinorhynchidae	AY830151	AY829106	DQ089718
Acanthocephalus lucii	Echinorhynchidae	AY830152	AY829101	
Acanthocephaloides propinquus	Echinorhynchidae	AY830149	AY829100	
Dentitruncus truttae	Illiosentidae	JX460863		
Dollfusentis lenti n. sp.	Illiosentidae	MK282744 MK282745	MK282746 MK282747 MK282748 MK282749 MK282750 MK282751 MK282752 MK282753	MK294053 MK294054 MK294055 MK294056 MK294057 MK294058 MK294059
Dollfusentis bravoae	Illiosentidae	MK282759	MK282754	MK294060 MK294061 MK294062 MK294063 MK294064
Dollfusentis chandleri	Illiosentidae			DQ320492 DQ320493 DQ320488 DQ320486 DQ320485 DQ320489 DQ320484 DQ320490 DQ320491 DQ320487
Echinorhynchus gadi	Echinorhynchidae	JX014222		
Echinorhynchus truttae	Echinorhynchidae		AY829097	DQ089710
Filisoma bucerium	Cavisomidae	AF064814	DQ089722	DQ089722
Illiosentis sp.	Illiosentidae	AY830158	AY829092	DQ089705
Koronacantha mexicana	Illiosentidae	AY830157	AY829095	DQ089708
Koronacantha pectinaria	Illiosentidae	AF092433	AY829094	DQ089707
Leptorhynchoides thecatus	Rhadinorhynchidae	AF001840	AY829093	DQ089706
Pomphorhynchus bulbocolli	Pomphorynchidae	JX014222	AY829096	DQ089709
Pomphorhynchus laevis	Pomphorynchidae	JX014223		
Pomphorhynchus zhoushanensis	Pomphorhynchidae	KY490051		
Pseudoleptorhynchoides lamothei	Rhadinorhynchidae	EU090950		EU090949
Rhadinorhynchus sp.	Rhadinorhynchidae		DQ089722	DQ089712
Rhadinorhynchus pristis	Rhadinorhynchidae	JX014226		
Serrasentis nadakali	Rhadinorhynchidae	KC291715		
Transvena annulospinosa	Transvenidae		AY829098	DQ089711

3.1.2. Male (based on 16 adult specimens)

Trunk 4783 (4075−5850, *n* = 15) long by 427 (375–500, n = 15) wide (Fig. 1A). Anterior trunk spines 66 (60–75, *n* = 12) long and posterior spines 16 (15–20, *n* = 12) long (Figs. 1B and C). Proboscis 798 (625–1000, *n* = 12) long by 92 (75–125, *n* = 16) wide, bearing 12–13 longitudinal rows with 16–17 hooks each (Figs. 1A and B). Apical hooks 48 (40–50, *n* = 11) long, middle hooks 31 (25–35, *n* = 13) long, basal vertical hooks 16 (15–20, *n* = 13) long (Fig. 2A). Ventrolateral crescent enlarged hooks 45 (40–50, *n* = 12) long with a distance of 25 (20–30, n = 11) from basal vertical hooks of proboscis. Neck 256 (200–300, *n* = 13) long by 104 (80–150, *n* = 14) wide (Fig. 1B). Proboscis receptacle 1241 (1000 − 1750, *n* = 14) long by 126 (100–200 *n* = 15) wide. Shorter lemniscus 1755 (1400 − 2030, *n* = 9) in length; longer lemniscus 2088 (2000 − 2200, *n* = 10) long. Male reproductive system occupies the third part of the trunk. Testes oval, anterior 314 (250–400, *n* = 13) long by 230 (180–290, *n* = 13) wide and posterior one 278 (200–340, *n* = 13) long by 225 (150–320, *n* = 13) wide (Fig. 1A). Vas efferens arise from the posterior end of each testis. Seminal vesicle, oval, elongated. Cement glands claviform, eight in number. Saefftigen's pouch pyriform, 716 (600–850, *n* = 9) long. Bursa well developed, 325 (200–420, *n* = 11) long by 285 (200–350, *n* = 11) wide when completely everted. Small spines anteriorly pointed are irregularly distributed at posterior trunk of males.

3.1.3. Female (based on 18 adult specimens)

Trunk 6800 (4125 − 9100, *n* = 9) long by 331 (250–400, *n* = 12) wide (Fig. 1D). Anterior trunk spines longer 74 (63–85, *n* = 10) than posterior ones 16 (15–20, *n* = 11). Proboscis 965 (775–1080, *n* = 7) long by 98 (75–125, *n* = 12) wide, bearing 12–14 longitudinal rows of 16–17 hooks each. Apical hooks 50 (45–55, *n* = 8) long; middle hooks 39 (30–45, *n* = 9) long; basal hooks 17 (15–20, *n* = 9) long. Eight ventrolateral crescent enlarged hooks 50 (45–60, *n* = 10) long with a distance of 26 (20–40, *n* = 9) from basal vertical hooks of proboscis. Neck 303 (260–350, *n* = 8) long, 108 (90–140, *n* = 9) wide at anterior end. Proboscis receptacle 1223 (1050–1390 *n* = 11) long, 150 (100–230 *n* = 8) wide. Shorter lemniscus 2207 (2000 − 2370, *n* = 3) long, longer lemniscus 2275 (2010 − 2600, *n* = 4) long (Fig. 1D). Female reproductive system from the apical part of uterine bell to posterior end of the body, 890 (690–1150, *n* = 4) long (Fig. 1E). Uterine bell 338 (225–550, *n* = 7) long by 45 (40–50, *n* = 6) wide. Uterus 279 (225–325, *n* = 6) long by 25 (20–30, *n* = 6) wide. Gonopore terminal. Mature eggs elongate with polar prolongations 68 (63–75, *n* = 10) long by 15 (15–20, *n* = 9) wide (Fig. 1F).

3.2. Scanning electron microscopy analysis

Proboscis elongate, cylindrical, armed with 16–17 hooks per row followed by smooth neck and trunk spined at anterior end

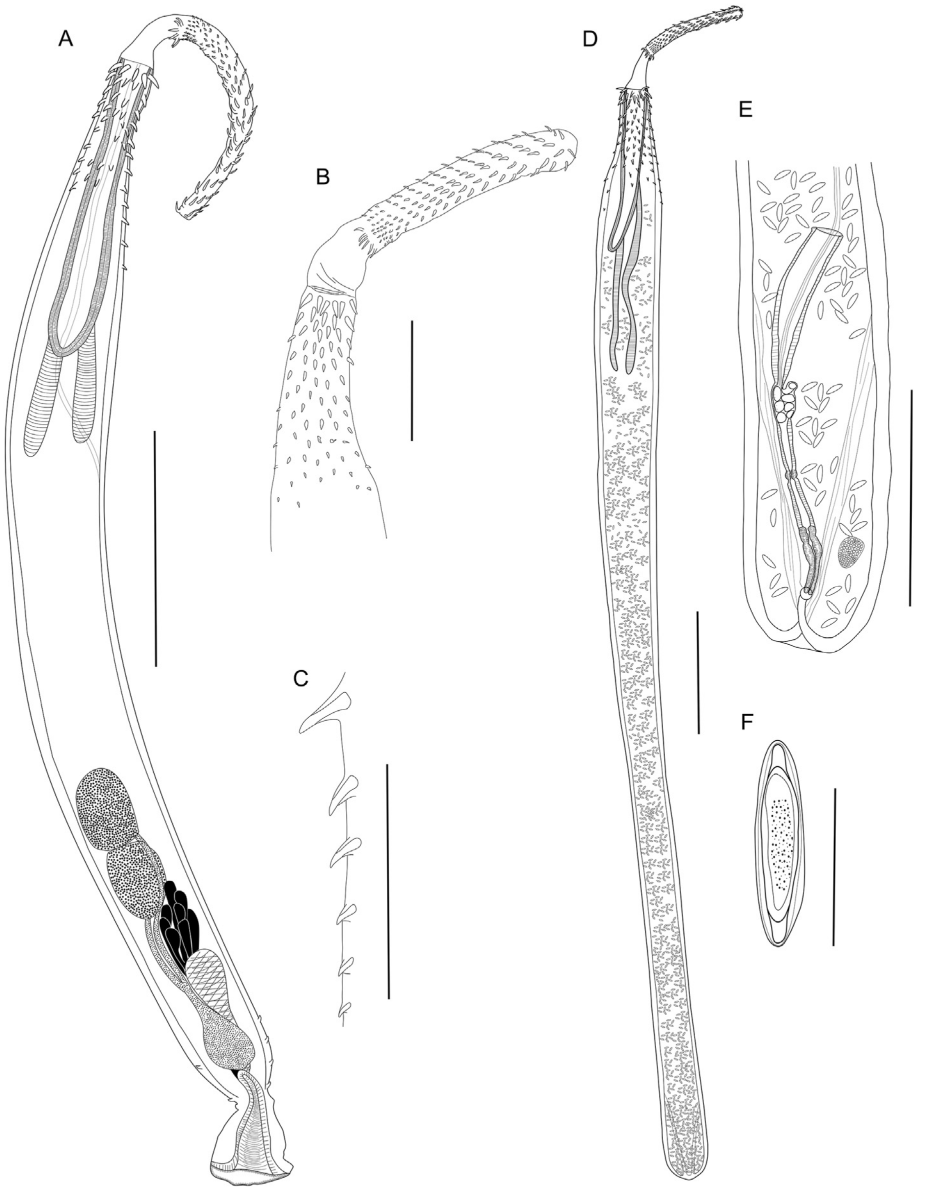

Fig. 1. *Dollfusentis lenti* n. sp. (A) Holotype, whole body of male showing trunk spined anteriorly, proboscis and receptacle, lemnisci, testes, cement glands, seminal vesicle, Saefftigen's pouch and copulatory bursa everted; (B) Detail of anterior end with arrangement of proboscis hooks and trunk spines; (C) Different sizes of trunk spines; (D) Allotype, female in total view; (E) Female reproductive system; (F) Egg. Scale bars: A = 1000 μm; B = 500 μm; C = 300 μm; D = 1000 μm; E = 500 μm; F = 50 μm.

(Figs. 2A – C). Proboscis hooks decrease in size towards the basal vertical ones. Clear separation between basal vertical hooks and the ventral crescent hooks (Figs. 2C and D). Trunk slightly thinner at anterior end armed with strong spines, which decrease in size and become sparse (Figs. 2B and C). Copulatory bursa of males bell-shaped; small spines anteriorly pointed may appear at end of trunk (Fig. 2F). Trunk of female end bluntly; gonopore terminal (Fig. 2E). SEM micrographs of specimens of *D. bravoae* show trunk armed with spines which gradually decrease in size (Fig. 3B). Some similarity with specimens from Brazil include a proboscis armed with 16–17 hooks per row, a smooth neck (Fig. 3A and B) and clear separation between basal vertical hooks and the ventral crescent hooks (Fig. 3C); also, males possess anteriorly pointed spines in the posterior end of body (Fig. 3D).

3.3. Taxonomic summary

Type host: *Orthopristis ruber* (Cuvier, 1830).

Type locality: Guanabara Bay, Urca beach, Rio de Janeiro, Brazil (22°56′39.4”S, 43°09′46.0”W).

Site of infection: intestine.

Type material: Holotype 40.019a; Allotype 40.019b; Paratypes 40.019c-g.

Voucher material: 40.018.

Material collected: 343 specimens.

Prevalence: 63% (109 fish examined/ 69 parasitized).

Intensity: 1–27 worms per fish.

Representative DNA sequences: Sequences of *Dollfusentis lenti* n. sp. were deposited in GenBank under the accession numbers: 18S rDNA (MK282744, MK282745); 28S rDNA (MK282746– MK282753); COI mtDNA (MK294053– MK294059) and ITS1, 5.8S and ITS2 rDNA (MK282742, MK282743). New comparative sequences of *D. bravoae* were also deposited under accession numbers for 18S rDNA (MK282759); 28S rDNA (MK282754) and COI mtDNA (MK294060–MK294064).

Etymology: The specific name is in honor of Prof. Herman Lent from Instituto Oswaldo Cruz in recognition of his valuable contributions to

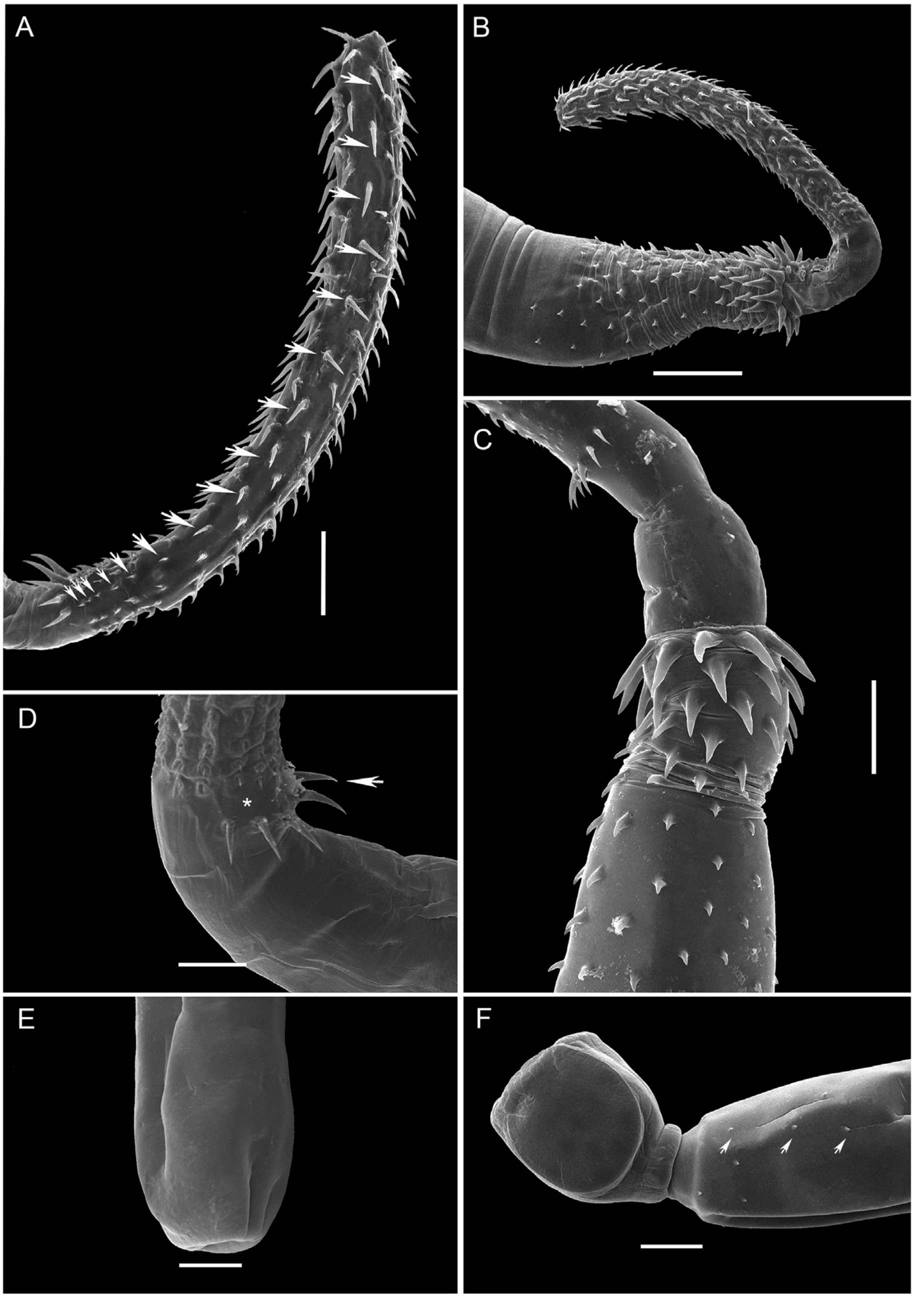

Fig. 2. Scanning electron microscopy of *Dollfusentis lenti* n. sp. (A) Proboscis with arrangement of 16 hooks (apical, middle and basal vertical ones) (arrows) and ventral crescent hooks; (B) Proboscis, neck and trunk armed anteriorly with large anterior spines decreasing in size; (C) Ventral crescent hooks, neck and anterior trunk spines; (D) Ventral crescent hooks (arrow) spaced (asterisk) from basal proboscis hooks; (E) Body end of female and (F) Copulatory bursa everted with anteriorly pointed spines in the posterior end of body. Scale bars: A = 100 μm; B = 200 μm; C = 100 μm; D = 500 μm; E–F = 100 μm.

our knowledge in the field of Helminthology.

3.4. Remarks

Dollfusentis lenti n. sp. is the sixth described species of the genus, which includes *D. chandleri*, *D. bravoae*, *D. salgadoi*, *D. ctenorhynchus* (Cable and Linderoth, 1963) and *D. longispinus* (Cable and Linderoth, 1963). All these species have 12–14 longitudinal rows of hooks, most of them differing in the number of hooks per row. A comparison of the data between *D. lenti* n. sp. and *D. chandleri*, a species previously reported from the same geographic area, shows respectively 16–17 hooks *vs* 22–24 after Golvan [21] and 19–21 after Salgado-Maldonado [22]. Additionally, *D. chandleri* can also be differentiated by the smaller size of basal vertical hooks (10–15 *vs* 15–20) and size of eggs (80 × 12–18 *vs* 63–75 × 15). *Dollfusentis bravoae* is morphologically more similar to the new species with respect to the number of hooks, since it possesses 16–17 hooks, while *D. salgadoi* possess 17–19 hooks. The testes of *D. lenti* n. sp. are larger (280–350; 200–340) than in *D. chandleri* (187–300; 187–283) and *D. bravoae* (192–236, 164–222). The new species also possesses longer uterus 225–325 in comparison to *D. bravoae* (102–114), *D. salgadoi* (100–175) and is slightly longer than *D. chandleri* (150–281). The trunk spines of *D. lenti* n. sp. are sparsely distributed with longer spines measuring 60–85; in *D. bravoae* a larger number of spines are found with the longest ones measuring 41–57. *Dollfusentis salgadoi* can also be distinguished from the new species by the presence of anteriorly pointed spines in the posterior end of body of females. *Dollfusentis ctenorhynchus* and *D. longispinus* can be readily differentiated from the new species by the number of proboscis hooks (19–21

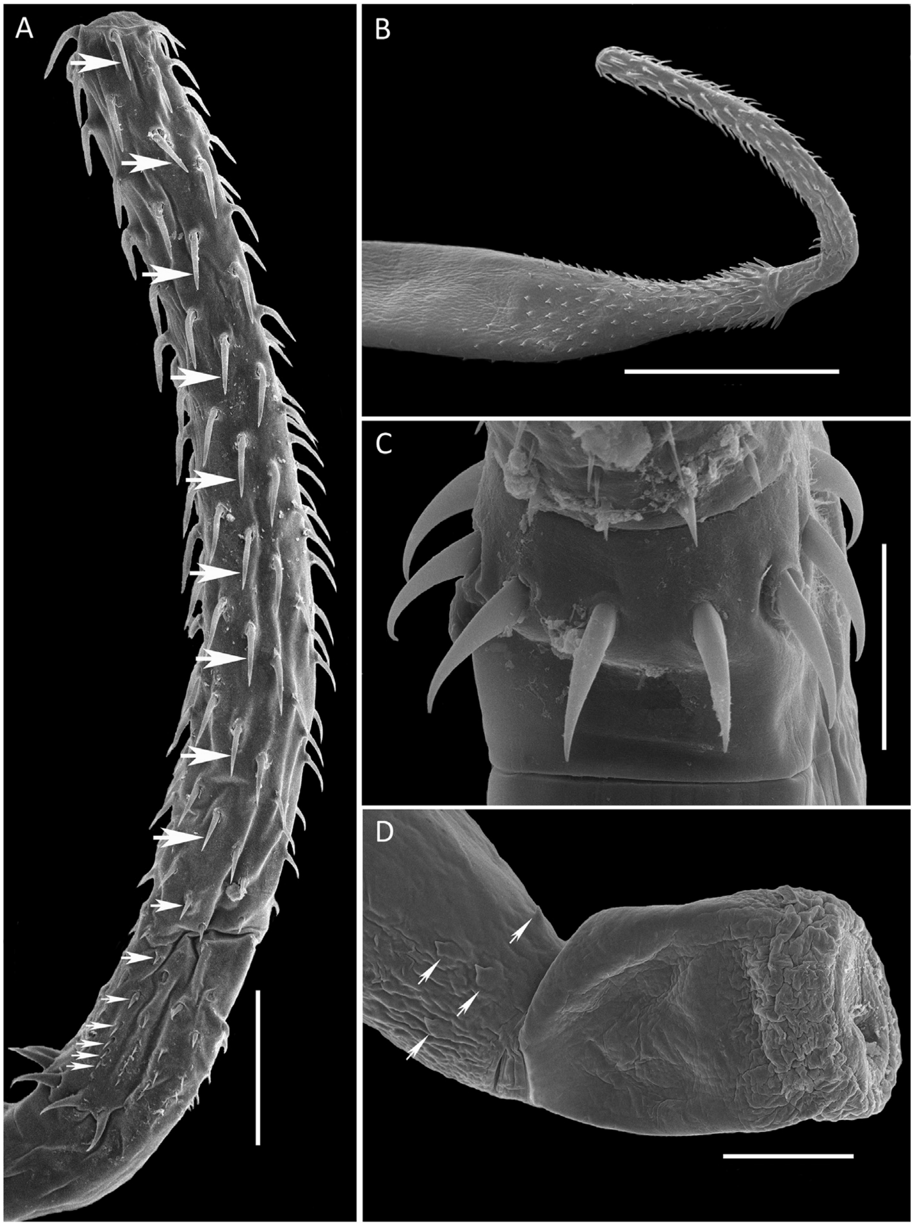

Fig. 3. Scanning electron microscopy of *Dollfusentis bravoae*. (A) Proboscis with arrangement of 16 hooks (arrow), ventral crescent hooks and neck; (B) Proboscis, neck and trunk armed anteriorly; (C) Ventral crescent hooks well spaced from basal proboscis hooks; (D) Anteriorly pointed spines in the posterior end of body and copulatory bursa everted. Scale bars: A = 100 μm; B = 500 μm; C = 40 μm; D = 100 μm.

and 22, respectively), and smaller size of female reproductive system (540 and 600, respectively × 690–1150). The host association, the geographical distribution and a genetic distinctiveness from the closest related species *D. chandleri* and *D. bravoae* support the recognition of the new species.

3.5. Phylogenetic analysis

Although ITS sequences were added to GenBank, they were not used for phylogenetic analysis due to the lack of comparable sequences for other taxa. Therefore, phylogenetic analyses were conducted using three independent datasets. The first two alignments were built with nuclear rDNA sequences (18S and 28S, Figs. 4 and 5) of the new species and *D. bravoae,* along with sequences of other illiosentids and other palaeoacanthocephalans used as outgroups. These analyses recovered members of the Illiosentidae as monophyletic groups, with high bootstrap support values; the 18S rDNA gene tree showed no difference between two isolates of the new species, and *D. bravoae*; conversely the 28S rDNA gene tree showed these two species of *Dollfusentis* as sister taxa. We also built an alignment for COI sequences that included seven sequences of the new species, along with five sequences of *D. bravoae* and 10 sequences available in the GenBank (unpublished) from *D. chandleri*. The alignment also included five other species of illiosentids and six palaeoacanthocephalans as outgroups. In the COI tree, the family Illiosentidae is recovered as monophyletic, and the three species of *Dollfusentis* also form a monophyletic group, in both cases with very high levels of bootstrap support (Fig. 6). In addition, Fig. 6 showed that *D. chandleri* and *D. bravoae* are sister species (relationships supported by low bootstrap value), and the new species from Brazil is the sister species from the latter two. Genetic divergence values estimated using *p*-distance values for COI between the *D. lenti* n. sp. and *D. bravoae* from Mexico ranged from 6.5 to 7.1% and between *D. lenti* n. sp. and unpublished sequences of *D. chandleri* was from 6.6 to 8.2%. The new species differs from the other two species from 6.5 to 8.2%.

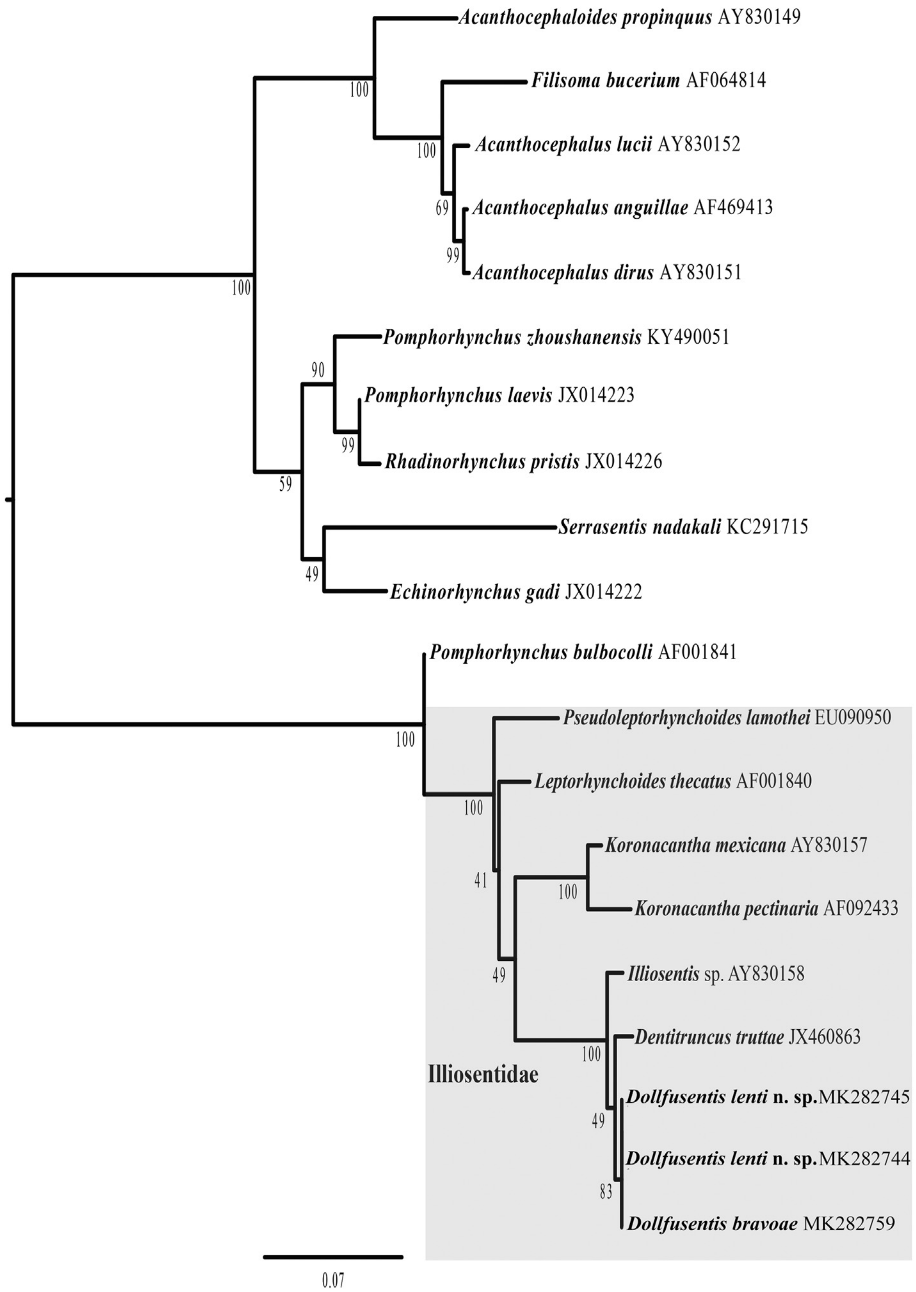

Fig. 4. Maximum likelihood tree inferred with 18S rDNA data set. Numbers near internal nodes show ML bootstrap values inferred with 10,000 replicates. Grey shadow refers to species included in Illiosentidae in comparison with other members of the class Palaeacanthocephala.

4. Discussion

The taxonomic history and species composition of *Dollfusentis* is rather confused mostly due to the overlap of morphological traits. Currently, five species of *Dollfusentis* are considered valid, *i.e.*, *D. chandleri*, *D. bravoae*, *D. salgadoi*, *D. ctenorhynchus* and *D. longispinus* [4], even though Amin [5] still considers *Illiosentis heteracanthus* as a member of *Dollfusentis*. Their phylogenetic relationships are also understudied, as until the present study, almost no molecular data was available.

Golvan [21] erected the genus *Dollfusentis* with the type species *Dollfusentis chandleri* (= *Telosentis tenuicornis* (Linton, 1905) Van Cleave, 1947, *Echinorhynchus pristis tenuicornis* Linton, 1905, *Rhadinorhynchus tenuicornis* Van Cleave, 1918 and *Echinorhynchus incrassatus* Molin *sensu* Linton, 1888). He also included in the genus *Illiosentis longispinus* Cable and Linderoth, 1963, *Illiosentis heteracanthus* Cable and Linderoth, 1963 and *Illiosentis ctenorhynchus* Cable and Linderoth, 1963. Monks and Pulido-Flores [23] returned *D. heteracanthus* to *Illiosentis heteracanthus.*

Bullock and Mateo [24] have suggested that specimens of *R. tenuicornis* redescribed by Chandler [25] corresponded with *D. longispinus* but Buckner et al. [26] highlighted that Bullock, in personal communication, was uncertain about the status of the species and considered all specimens from the Northern Gulf of Mexico to be *D. chandleri.* Additionally, the length of lemnisci originally reported for the single specimen of *D. longispinus* as twice the length of proboscis receptacle was revealed to be variable among species of the genus. Although Amin [5] considers *D. longispinus* as the type species, in his previous paper [27], he discussed that the suggested "synonymy with *D. chandleri* may be validated upon careful study of a long series of specimens and data analysis".

Dollfusentis chandleri was originally described as a parasite of *Micropogonias undulatus* (Linnaeus, 1766) and *Leiostomus xanthurus*

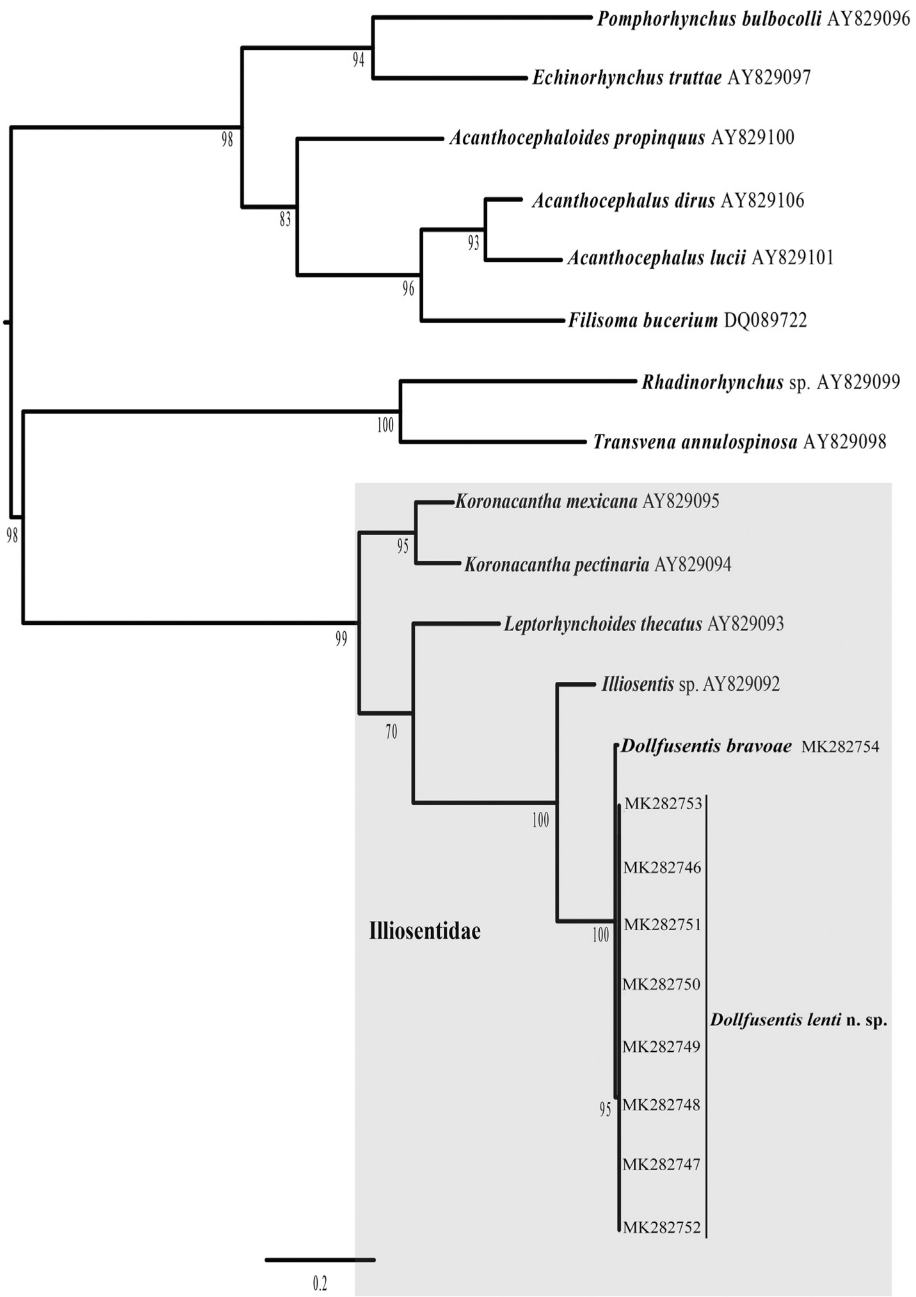

Fig. 5. Maximum likelihood tree inferred with 28S rDNA data set. Numbers near internal nodes show ML bootstrap values inferred with 10,000 replicates. Grey shadow refers to species included in Illiosentidae in comparison with other members of the class Palaeacanthocephala.

Lacepède, 1802 in Galveston Bay, Texas, also including *Cynoscion arenarius* Ginsburg, 1930 and *Orthopristis chrysopterus* Linnaeus, 1766 [21]. Additional records of this species include a wide array of marine and brackish water fishes. For instance, in Mexico, the species has been recorded in 10 fish species, apparently showing very low host-specificity [28]. The other two species reported from the Atlantic and Caribbean Sea coast of Mexico are apparently host-specific, with *D. bravoae* parasitizing *Pomadasys croco* (Cuvier, 1830) from the Sontecomapan lagoon, Veracruz [22], and *D. salgadoi* a species described as a parasite of *Eugerres plumieri* (Cuvier, 1830) from Chetumal Bay [4]. However, specimens of *D. bravoae* are now reported from *Eugerres plumieri* from Sontecomapan lagoon in Veracruz, Mexico. Finally, another two species were described from marine fishes in the Caribbean, *D. ctenorhynchus* reported from the mullid *Upeneus martinicus* (= *Mullodichthys martinicus* Cuvier, 1829), and *D. longispinus* from the haemulid *Anisotremus virginicus* (Linnaeus, 1758), both in Jamaica [21]. Even though the distributional range of some fish species extends from North to South America, there seems to be a gap in the distribution of species of *Dollfusentis*. Still more data are required from Central American marine fishes to test this hypothesis.

Dollfusentis chandleri is widely distributed along the Atlantic coast of the Americas, from the Gulf of Mexico southwards to Brazil [6,21,22,27,28], in addition, the species exhibits very low host-specificity because even though it parasitizes mostly grunts belonging to the family Haemulidae Gill, 1885, it is also found in fishes from unrelated families such as sparids, gerreids, sciaenids, carangids.

In Brazil, Kohn and Macedo [29] were the first authors to record *D. chandleri* off the coast of Rio de Janeiro, parasitizing *Haemulon sciurus* (Shaw, 1803). Later, Noronha et al. [30] reported this acanthocephalan from *Archosargus rhomboidalis* (Linnaeus, 1758), *Eucinostomus argenteus* Baird & Girard, 1855, *Umbrina coroides* Cuvier, 1830, *Haemulon sciurus* (Shaw, 1803), Luque et al. [31] from *Orthopristis ruber* (Cuvier, 1830)

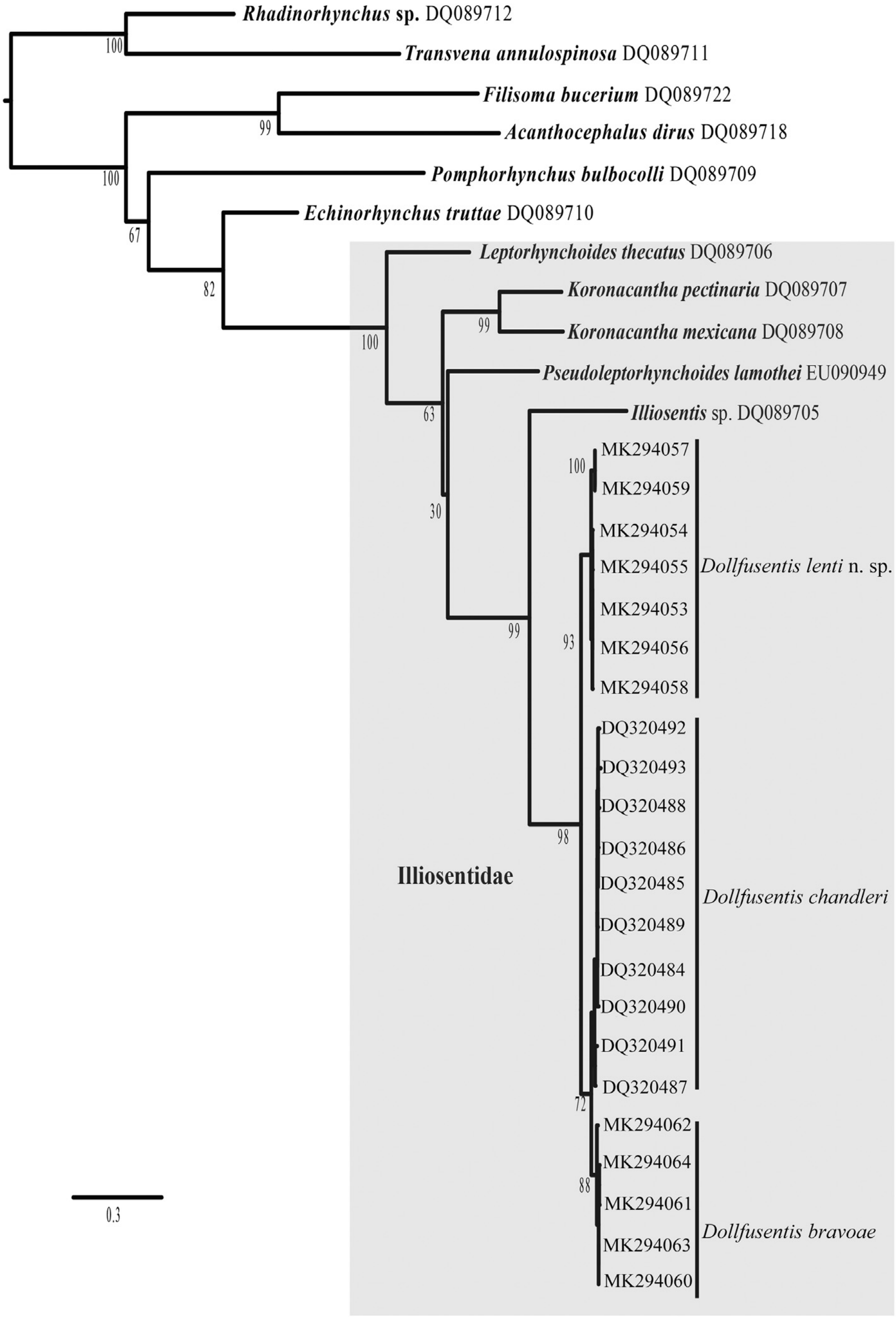

Fig. 6. Maximum likelihood tree inferred with COI data set. Numbers near internal nodes show ML bootstrap values inferred with 10,000 replicates. Grey shadow refers to species included in Illiosentidae in comparison with other members of the class Palaeacanthocephala.

and more recently, Alves and Luque [32] from *Micropogonias furnieri* (Desmarest, 1823). The examination of specimens of *D. chandleri* from *H. sciurus* off Rio de Janeiro, deposited at CHIOC n° 31,981, 31,982 a – g, and 32,199 a – b, showed that they all possess 12–14 longitudinal rows of 16–17 hooks, corresponding with the range and morphology of the new species, and should therefore be renamed as *D. lenti* n. sp. The slides CHIOC n° 32,195 and 32,196 from *E. argenteus*, 32,204 from *A. rhomboidalis*, 34,022 from *M. furnieri* and 32,194 and 32,198 from *U. coroides* from nearby areas were not in good condition and new material is necessary to confirm their identification.

Species of *Dollfusentis* possess a high intraspecific variability, very similar interspecific morphology and reduced morphological parameters for identification as reported by Salgado Maldonado [22]. All measurements given for morphological traits used to describe species in the genus *Dollfusentis* show some level of overlap, making it difficult to distinguish reliably differences among them. The main distinction criteria seemed to be the difference in the number of hooks per row of the proboscis, although there is no consensus in the literature about how many hooks may represent a gap to distinguish among species. Golvan [21] reported *D. chandleri* with 12–14 rows of 22–24 hooks each from specimens obtained in Galveston, Texas, USA. Salgado-Maldonado [22] redescribed this species as having 13–14 rows of 19–21 hooks with specimens sampled from a haemulid off the Cozumel Island in the Mexican Caribbean. Salgado-Maldonado [22] argued that *D. chandleri* was very similar to *D. bravoae* by the general shape of the worm, but the later species could be differentiated by possessing 13–14 rows of 16–17 hooks each, larger separation between crescent basal and basal hooks, larger anterior spines on the trunk and absence of female genital vestibule. Furthermore, Monks et al. [4] noticed characters such as the shape of musculature of the terminal end of female and the presence/absence of genital vestibule that could be used to distinguish among *D. chandleri, D. bravoae* and the species these authors described from Chetumal Bay, *D. salgadoi*. However, the reliability of these characters for taxonomical differentiation is uncertain. The absence of female genital vestibule may be due to the muscular contraction and/or the fixation process [33] and the width difference of the attachment of the muscle bands is not clear yet. Additionally, Monks et al. [4] used the so-called genital spines to separate species of *Dollfusentis*. We caution about the use of the term `genital spines´ because even though such spines are located in the posterior end of the trunk, they are not associate with the genital pore. In any case, these authors averred that *D. longispinus, D. bravoae* and *D. salgadoi* possessed these spines (in *D. salgadoi* occurs in males and females), and that character separated these species from *D. chandleri* and *D. ctenorhynchus.* This is still controversial because although Monks et al. [4] mentioned that *D. chandleri* lacks such spines, the redescription of the species in Salgado-Maldonado [22] mentioned the presence of irregularly distributed spines in the posterior end of trunk in males.

Considering the geographical distribution of fish hosts of *Dollfusentis* spp. from the Gulf of Mexico and the Caribbean Sea, most of them occur from the Gulf of Mexico up to Brazil [3]. However, it is possible that an *inhibiting area* between the latitudes 10°S to 10°N [34], may affect the distribution of parasites. This environmental barrier includes: 1) low salinity levels in the delta of the Amazon River, which extends the estuarine condition into the open sea with excess of sediment in suspension and 2) North-Brazil and Guyana currents flow directed to the northwest of the continent.

In this study, we used morphological information along with distributional and host association data to describe a new species of *Dollfusentis* from marine fishes off the coast of Brazil. The recognition of the new species was further supported by molecular data. The two nuclear molecular rDNA genes supported the contention that *Dollfusentis* belongs in the Illiosentidae, although they are not variable enough to clearly distinguish among the new species and *D. bravoae*. However, the evidence gathered from the COI analysis showed that the individuals of *Dollfusentis* sequenced from Brazil represent a reciprocally monophyletic lineage, albeith with moderate bootstrap support value, and all isolates were separated from the other two species of the genus. In addition, COI genetic divergence values support the contention that they represent separate species. The new species differs from the other two species by 6.5 to 8.2%. The COI divergence values among species (or lineages) of acanthocephalans belonging to other families is variable, since it ranges between 6 and 21%. For instance, Steinauer et al. [35] reported divergence values from 6.3 to 11.6% among three lineages of *Leptorhynchoides thecatus*, a parasite of freshwater fishes. García-Varela and Pérez-Ponce de León [36] found divergence values for the same molecular marker of 11–21% among species allocated in different genera of polymorphids that infect aquatic birds and marine mammals; Pinacho-Pinacho et al. [37] reported a large divergence (20.3–21.03%) between two species of *Neoechinorhynchus*, parasites of brackish water fishes.

5. Conclusion

We believe, with the results of the present study, that current taxonomic work in acanthocephalans should address an integrative taxonomy approach where several sources of information may be used to establish more robust species delimitation criteria. In this case, the use of morphology, DNA sequences and geographical distribution was very important to recognize that the specimens of *Dollfusentis* from Brazil represented a new species.

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Ethical standards

This study was authorized by the Brazilian Institute of Environment and Renewable Natural Resources (IBAMA, license no. 15898-1) in accordance with the guidelines of the Brazilian College for Animal Experiments (COBEA). In Mexico, specimens were collected under the Cartilla Nacional de Colector Científico (FAUT 0202 and 0057) issued by the Secretaría del Medio Ambiente y Recursos Naturales (SEMARNAT), to MGV and GPPL, respectively, and all applicable national and international guidelines for the care and use of animals were followed.

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