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Larvae trypanorhynch (Cestoda) infecting the dusky flounder, *Syacium papillosum* (Paralichthyidae: Pleuronectiformes) in the continental shelf of the Yucatan Peninsula, Mexico

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Summary

Trypanorhynch cestode larvae were found parasitizing specimens of dusky flounder *Syacium papillosum* (Linnaeus, 1758) in the Southeastern Gulf of Mexico (s-GoM). Plerocercoids were recovered from a total of 194 flatfish, embedded in the intestine and stomach wall. Trypanorhynch were identified using morphology and a molecular phylogeny using newly sequenced partial 28S (region D1-D3) ribosomal DNA in combination with data derived from other species of trypanorhynchs available from GenBank. Larvae representing three genera of trypanorhynch cestodes, *Nybelinia* Poche, 1926; *Kotorella* Euzet & Radujkovic, 1989 and *Oncomegas* Dollfus, 1929 were found in dusky flounder specimens in the s-GoM. These plerocercoids represent six species: *Nybelinia* sp. 1, *Nybelinia* sp. 2, *Nybelinia* sp. 3, *Nybelinia* sp. 4, *Kotorella pronosoma* (Stossich, 1901) and *Oncomegas wageneri* (Linton, 1890) Dollfus, 1929. All cestode specimens in this study represent new locality records for their respective species.

Keywords: Cestodes; Gulf of Mexico; Trypanorhynch; *Syacium papillosum*

Introduction

Cestodes of the order Trypanorhyncha Diesing, 1863 are the most frequent and abundant endoparasite helminth groups that infect elasmobranchs (Palm, 2004). In general, it is known that trypanorhynch infecting benthic invertebrates as their first intermediate hosts, vertebrates such as fishes (e.g., flatfishes) as their second intermediate hosts or paratenic hosts, and elasmobranchs (sharks and rays) as their definitive hosts (Beveridge *et al.,* 2017; Bennett *et al.,* 2019). This group has been well studied; approximately 303 species in 81 genera are known and 14 genera have been described at the larval stage (Caira & Jensen, 2014; 2017). Unlike cestode species in other orders, such as Tetraphyllidea and Rhinebothriidea, that can only be identified at the adult stage, trypanorhynch larvae possess well-defined scolex features that include eversible tentacles armed with the same hooks that remain in adults' stage, which allow accurate morphological identifications to the species level (Jensen & Bullard, 2010).

Notwithstanding, the identification of larvae stages may be complex due to the invagination of the tentacular apparatus inside the tentacular sheaths. Hence, when this happens, genetic/molecular tools may be useful in identifying larvae to the species level (Palm, 2004; Schaeffner, 2018; Vidal-Martínez *et al.,* 2019). The taxonomic description of adult and larval trypanorhynch cestodes

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has been made mainly in the Indo-Australian region (Palm, 2004; Beveridge & Campbell, 2005; Schaeffner & Beveridge, 2014; Beveridge *et al*., 2014; 2017;), on the Mediterranean Sea (Santoro *et al.,* 2021; Palomba *et al.,* 2021), the Southern Coast of Brazil (Knoff *et al.,* 2004; Pinto *et al.,* 2006; Felizardo *et al.,* 2010; Dias *et al.,* 2011), the North of the Gulf of Mexico and the Gulf of California (Palm & Overstreet, 2000; Jensen, 2009; Caira & Jensen, 2014; 2017). In the northern and western regions of the Gulf of Mexico, trypanorhynchs have been studied extensively, with approximately 42 species reported in the Actinopterygii and Elasmobranchii (Palm & Overstreet, 2000; Jensen, 2009). For the s-GoM (the Southeastern Gulf of Mexico), the description of marine cestodes is poor, with the exception of the infracommunities of flatfish (Vidal-Martínez *et al.,* 2019). The benthic habits of flatfish make them susceptible to parasitic infections, mainly of trypanorhynch larvae. In high intensity of infection, the plerocercoids cysts adhere to the serosal surfaces of the abdominal organs and invade the muscle and the submucosa of the stomach and intestine, which cause fish disorders such as inflammatory reaction, fibrosis and necrosis (Hassan *et al.,* 2002; Sales-Ribeiro *et al.,* 2021). *Epinephelus aerolatus* with heavy infection of *Floriceps* sp. has been associated with fibrosis of the skeletal muscle, abdominal cavity, mesentery and liver damage (Ibrahim, 2000). Many of the plerocercoids in flatfish have not yet been identified, and adult species that infect many elasmobranchs in the s-GoM have yet to be studied and taxonomically evaluated (Palm & Overstreet, 2000). In the s-GoM larval trypanorhynch have been reported from several flatfish species. For example, Trypanorhyncha gen. sp. was identified in *Symphurus plagiusa* (Rodríguez-González & Vidal-Martínez, 2008), *Nybelinia* sp., *Kotorella pronosoma* (Stossich, 1901), and *Oncomegas wageneri* (Linton, 1890) Dollfus, 1929 in *Syacium gunteri* (Yamaguti 1952), *O. wageneri* in *Cyclopsetta chittendeni* (Centeno-Chalé *et al.,* 2015). For the Yucatan shelf (YS), Vidal *et al*. (2019) provided molecular evidence of the presence of *Nybelinia* sp., *Lacisthorhynchus* sp., *Pterobothridae* gen. sp. in *S. papillosum*. Despite these records, there are still few trypanorhynch in larval stage identified at species level in the YS. By this reason, we consider relevant to increase the knowledge on the morphology and molecular identification of trypanorhynchs in this region. This knowledge would help studies regarding the infection dynamics of these cestodes in the different fish species in the YS, as well as life-stage matching between larvae and adults.

As part of a larger research project on the ecosystem health of the s-GoM (including the continental shelf of the Yucatan peninsula), we had the opportunity to collect parasitological material from flatfishes from three oceanographic cruises on the continental shelf of the Yucatan peninsula. These cruises gave us the opportunity to collected dusky flounders (*S. papillosum*) for parasitological studies at several sampling stations for each cruise. Therefore, the objective of this paper is to provide morphological descriptions and molecular identification of the larval stages recovered from new geographic locations in the Yucatan Shelf for *S. papillosum*.

Materials and Methods

Host collection

The study area along the Yucatan platform in s-GoM included 67 sampling stations, but dusky flounders and their parasites were obtained only from 17 sampling stations from November 2015 to April 2016 (Fig. 1). Trawling was performed onboard the oceano-

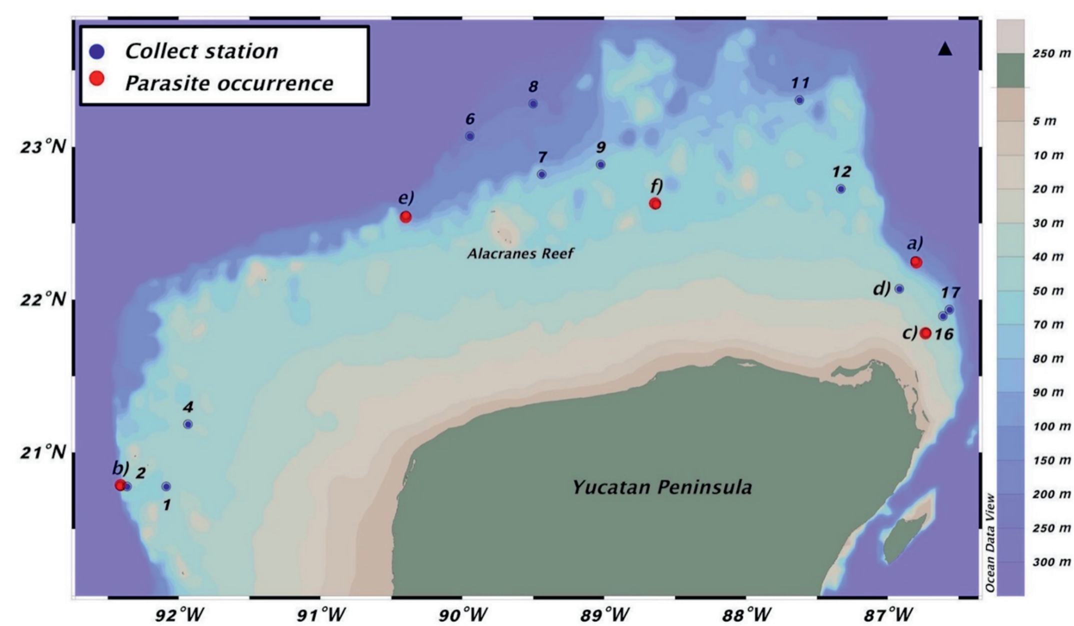

Fig. 1. Sampling sites from s-GoM. Blue dots show the sampling sites where *Syacium papillosum* were caught in trawls. The letters in each red dots show specimens collected for morphological description. **a**) *Nybelinia* sp. 1, **b**) *Nybelinia* sp. 2, **c**) *Nybelinia* sp. 3, **d**) *Nybelinia* sp. 4, **e**) *Kotorella pronosoma*, **f**) *Oncomegas wageneri*.

graphic vessel Riviera Maya. A total of 194 specimens of *S. papillosum* were collected in trawls of 0.5 h with two 20 m long shrimp nets at a speed of 2.0 – 2.3 knots for one nautical mile (whichever occurred first) and depths between 50 and 200 m from the oceanographic vessel Riviera Maya. The dead flatfish were kept in isolated plastic bags in coolers in the cold storage of the vessel and transported to CINVESTAV-IPN Unidad Mérida for parasitological examination. The collected fishes were identified in the Necton Laboratory and total length (TL, cm), standard length (SL, cm) and total weight (W, g) were recorded for each individual (Aguilar-Medrano *et al.,* 2020; Vega-Cendejas *et al*. 2023). Cavities and all internal organs were individually examined using a dissecting microscope. Once the cestode larvae were located, they were counted, fixed and preliminarily identified to each taxonomic group (Olson *et al.,* 2010). The cestode larvae were counted *in situ* and preserved in 4 % formalin or 96 % alcohol in labelled vials for subsequent morphological or molecular studies, respectively.

Morphological study

Cestode larvae were stained using Mayer's paracarmine technique for morphological identification (Palm & Overstreet, 2000; Palm, 2004; Jensen, 2009; Olson *et al.,* 2010). Voucher specimens were deposited in the Colección Nacional de Helmintos (CNHE) of the Universidad Nacional Autónoma de México (UNAM). Measurements were made using an OLYMPUS BX50 microscope (Waltham, USA) and ImageJ software (Wayne Rasband Scientific Software). Drawings were prepared using the Adobe Illustrator Software (2015 version). The morphometric measurements included in the description of the larvae (whenever possible) were: scolex length (SL), scolex width at level of pars bothridialis (SW), pars bothridialis (pbo), pars vaginalis (pv), pars bulbosa (pb), pars postbulbosa (ppb), velum (vel), pedunculus scolesis length (ps), appendix (app), bulb length (BL), bulb width (BW), bulb length to width ratio (BR), scolex proportions of pbo : pv : pb (SP), tentacle length (TL), tentacle width (TW) and tentacle sheath width (TSW). The tentacular armature was described as follows: homeomorphous or heteromorphous. Metabasal total hook length (L), hook height (H), total length base (B). Basal total hooks length (l), hook height (h), total length base (b). Hooks per half spiral row (hrs) (Palm & Overstreet, 2000; Palm, 2004; Jensen, 2009). All measurements are given in micrometers (µm) with the range followed by the mean in parentheses.

Scanning electron microscopy (SEM)

Specimens for SEM were fixed in 4 % glutaraldehyde in 0.1 M cacodylate buffer, postfixed in 1 % aqueous osmium tetroxide, dehydrated in ethanol (30, 40, 50, 60, 70) and dried overnight in hexamethyldisilasane. Dried samples were mounted on aluminum stubs, sputter coated with gold and examined in a HITACHI-SU1510 at an accelerating voltage of 0.3 kV to 30 kV.

DNA amplification and sequencing

For molecular data, total genomic DNA of several specimens from different specimens of trypanorhynch cestodes was extracted using DNeasy^{TM} Blood & Tissue Kit (QIAGEN, Hilden, Germany) following the standard manufacturer protocol. The 28S gene was amplified using the forward primer 391 5'-AGCGGAGGAAAAGAAACTAA-3' (Nadler & Hudspeth, 1998) plus the reverse primer 536: 5'- CAGCTATCCTGAGGGAAAC-3' (García-Varela & Nadler, 2005). PCR conditions were as follows: 94 °C for 5 min, followed by 35 cycles at 94 °C for 1 min, 50 °C for 1 min, 72 °C for 1 min, and a post-amplification extension for 10 min at 72 °C. Additionally, sequencing reactions were performed using the amplification primers plus two internal primers, the 504 5'- CGTCTTGAAACACGGACTAAGG-3' (García-Varela & Nadler, 2005) and the 503 5'-CCTTGGTCCGTGTTTCAAGACG-3' (Stock *et al.,* 2001). PCR products were sequenced at GENEWIZ (South Plainfield, NJ, USA). Sequences were assembled into contigs and then consensus sequences obtained by each primer were assembled with Geneious Pro 4.8.4® (Biomatters Ltd.). Subsequently the new sequences generated in this study were aligned with sequences of 28S belonging to other taxa of trypanorhynchs. The alignment was performed with the program ClustalW (Thompson *et al.,* 1994), which is implemented in the website http://www.genome.jp/tools/clustalw/, with the approach "SLOW/ACCURATE" and weight matrix "CLUSTALW (for DNA)". The nucleotide substitution model was estimated with the program jModelTest v.2 (Darriba *et al.,* 2012), and the selected model was GTR. Phylogenetic analysis was run under Maximum Likelihood (ML) with RAxML v. 7.0.4 (Stamatakis, 2006), and 10 replicates were implemented to obtain the best tree with 1,000 repetitions Bootstrap (bt). The ML tree was visualized in FigTree v.1.4.3. (Rambaut, 2016). The genetic distances in the dataset used in this study were calculated using the uncorrected *p*-value (p-distances) in MEGA v.6.0 (Tamura *et al.,* 2013).

Ethical Approval and/or Informed Consent

All applicable institutional, national, and international guidelines for the care and use of animals were followed.

Results

A total of three trypanorhynch genera, *Nybelinia* Poche, 1926; *Kotorella* Euzet & Radujkovic, 1989 and *Oncomegas* Dollfus, 1929, were found in larval stage in *S. papillosum* in the s-GoM. Six trypanorhynch species were represented by these larvae: *Nybelinia* sp. 1, *Nybelinia* sp. 2, *Nybelinia* sp. 3, *Nybelinia* sp. 4, *Kotorella pronosoma* (Stossich, 1901) and *Oncomegas wageneri* (Linton, 1890) Dollfus, 1929 (Fig. 2). All species found represent new locality records. Most of the larval cestodes were isolated from the stomach wall and intestine. The morphological measurements, molecular identification, and comments on their distribution pattern and site of infection are given below:

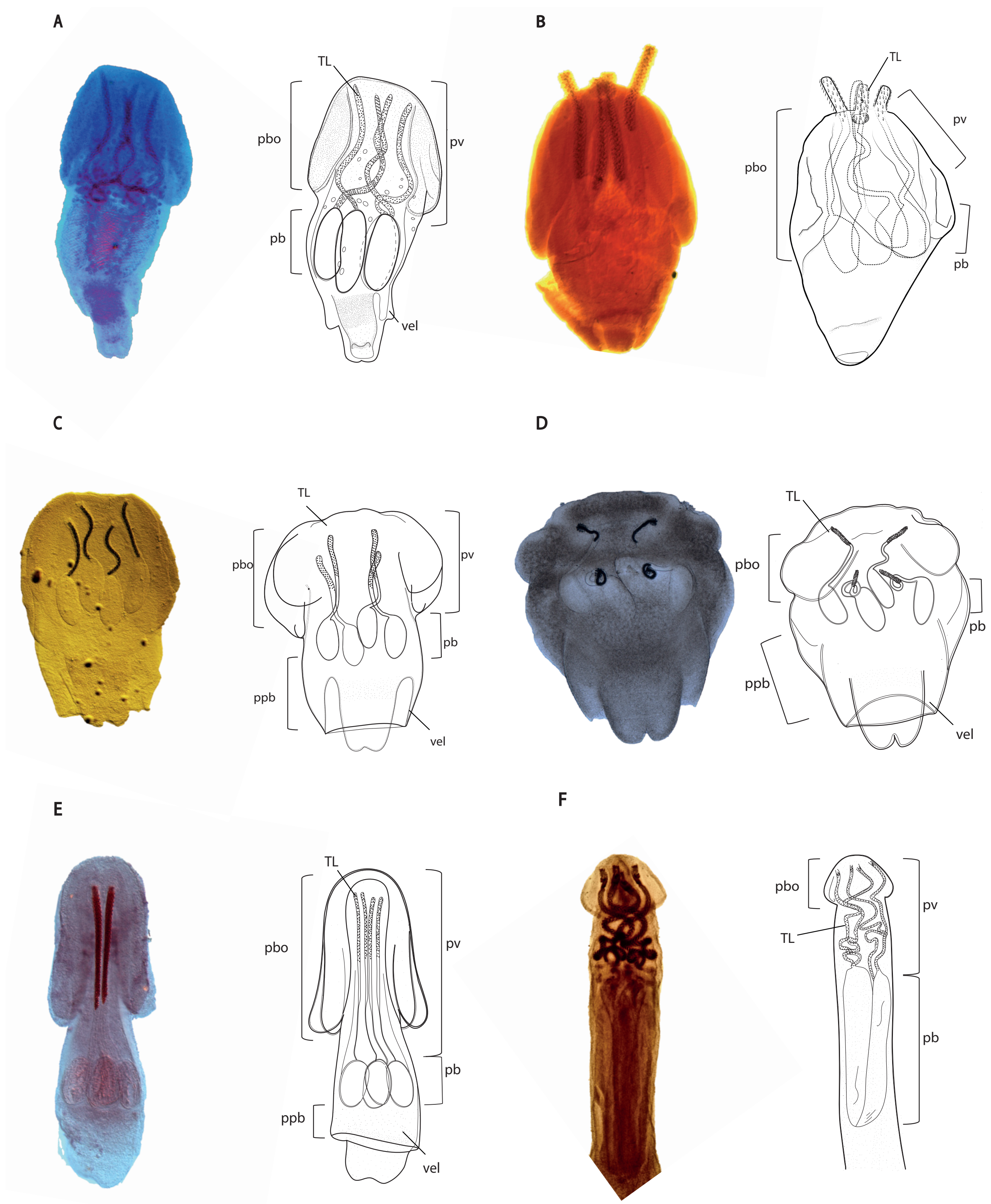

Fig. 2. Larval species of trypanorhynch cestodes. **A.** *Nybelinia* sp. 1, **B.** *Nybelinia* sp. 2, **C.** *Nybelinia* sp. 3, **D.** *Nybelinia* sp. 4, **E.** *Kotorella pronosoma*, **F.** *Oncomegas wageneri*. Some of the available measurements were made for morphological and morphometric description for larval cestodes: pars bothridialis (pbo), pars vaginalis (pv), pars bulbosa (pb), pars postbulbosa (ppb), velum (vel), tentacle length (TL).

Superfamily Tentacularioidea Poche, 1926
Family Tentaculariidae Poche, 1926
Genus *Nybelinia* Poche, 1926

***Nybelinia* sp. 1**
Host: *Syacium papillosum* (Linnaeus, 1758) (Paralichthyidae).
Locality: Southeast of the Gulf of México (latitude 22.15144, longitude -86.48403).
Site of infection: Stomach wall.
Material deposited: Voucher (1 slide, 1 specimen) was deposited in Helminthological Collection of the Universidad Nacional Autónoma de Mexico (access number CNHE No. 11114).
GenBank number: MK558803.
Supplementary observations: (Based on 4 larvae, Fig. 2 A): Scolex craspedote, SL = 494–517 (525), SW = 230–243 (215), 4 small bothridia, pbo = 289–382 (327), pv = 291–397 (325), pb = 194–230 (207), ppb = 72–105 (92), vel = 64–84 (76) (based on 3 specimens), ps = 566–681 (612), app = 96–160 (133), 4 bulbs fluted and elongated, BL = 171–227 (196), BW = 52–58 (55), BR = 1.31:1, SP = 1.4:1.1:1, tentacle with small hooks invagination, TL = 344–391 (371), TW = 16–19 (17) provided with invaginated hooks. Hooks solid invaginated, uncinate in shape.
Remarks: The specimens examined in the present study allowed us identify larvae within the genus *Nybelinia* recognized by Palm (2004). They presented morphological and morphometric similarity with members of this genus such as the presence of small scolex, craspedote, four triangular sessile bothria, a short pars vaginalis, four short to elongated tentacles, and short to elongated bulbs. Since the hooks were invaginated in our specimen, it was only possible to identify them at genus level. However, their morphology was different with respect to the rest of available descriptions. By this reason we designated them as *Nybelinia* sp. 1. For logistical reasons during the cruise in the oceanographic vessel Riviera Maya, the majority of the plerocercoids were preserved in 4 % formalin or 96 % alcohol, after arrival into the laboratory which made impossible to obtain evertion of the hooks in the samples. In terms of some morphological characteristics, this specimen were similar to *Nybelinia africana* Dollfus 1960 (e.g., pb, BL, TL) (Palm, 1999), *N. jayapaulazariahi* Reimer, 1980 (app, BW, SP, TW) (Palm & Beveridge, 2002) and *N. indica* (TL) (Palm, 2004) (Annex 1).

***Nybelinia* sp. 2**
Host: *Syacium papillosum* (Linnaeus, 1758) (Paralichthyidae).
Locality: Yucatan shelf, Gulf of México (latitude 20.46603, longitude -92.0524).
Site of infection: Stomach wall.
Material deposited: Voucher (1 slide, 1 specimen) was deposited in Helminthological Collection of the Universidad Nacional Autónoma de Mexico (access number CNHE No. 11117).
GenBank number: OR750422 and OR750423.
Supplementary observations: (Based on 10 larvae, Fig. 2 B; Fig. 3 A–D): Scolex craspedote, SL = 662–835 (745), SW = 219–389 (310), 4 small bothridia, pbo = 322–350 (310), pv = 274–320 (298), pb = 115–165 (110), ppb = 158 (1 specimen), vel = 65–90 (81), ps = 489 (1 specimen), app = 55–59 (57), 4 bulbs elongated, BL = 120–140 (130), BW = 49–86 (69), BR = 1.61:1, SP = 1.8:1.4:1 , tentacles with small hooks invagination, TL = 180–220 (206) , TW = 20–25 (23), TWS = 15–25 (19) (Annex 1). Metabasal tentacular armature homeoacanthous homeomorphus; hooks solid. 11–12 rows of uncinate hooks. Metabasal hooks: L = 6.4, H = 4.2, B = 4.15, Basal hooks: l = 3.6, h = 2.6, b =2.9, 7–8 hsr metabasal, 5–6 hsr basal. Number total of rows = 12.
Remarks: Ten specimens have been identified as belonging to *Nybelinia* sp. 2. The genus *Nybelinia*, is characterized by having a compact scolex, craspedote, four sessile bothria arranged oppositely, and four short tentacles (Palm, 2004). However, their morphology was different with respect to the rest of available descriptions. By this reason we designated them as *Nybelinia* sp. 2. Scolex measurements of the present specimens are similar to those given for *N. indica* Chandra, 1986 by Palm (1997; 1999), e.g., SL, SW, pb, BL, BW, BR, SP, TL, TW, TSW, l/h/b (basal). While, pbo, pv, L/H/B (Metabasal), hsr metabasal and number total of rows were similar to *N. scoliodoni* (Vijayalakshmi, Vijayalakshmi & Gangadharam, 1996) that described by Palm & Overstreet (2000) (see Annex 1).

***Nybelinia* sp. 3**
Host: *Syacium papillosum* (Linnaeus, 1758) (Paralichthyidae).
Locality: Yucatan shelf, Gulf of México (latitude 21.56311, longitude -86.34387).
Site of infection: Stomach wall.
Material deposited: Voucher (1 slide, 1 specimen) was deposited in Helminthological Collection of the Universidad Nacional Autónoma de Mexico (access number CNHE No. 11115).
GenBank number: MK558804.
Supplementary observations: (Based on 7 larvae, Fig. 2 C; Fig. 3 E-H): Scolex craspedote, SL = 501–803 (608), SW = 240–496 (308), 4 small bothridia, pbo = 213–367 (268), pv = 137–306 (193), pb = 88–157 (150), ppb = 108–343 (210), vel = 318 (1 specimen), ps = 411–755 (550), app = 38–119 (61), 4 bulbs elongated, BL = 77–180 (116), BW = 36–92 (57), BR = 1.54:1, SP = 1.65:1.1:1, tentacle with small hooks invagination, TL = 108–249 (162), TW = 8–20 (13), TSW = 10–17 (13) (based on 5 specimens). Metabasal tentacular armature homeoacanthous homeomorphus; hooks solids. 6–7 rows of uncinate hooks. Metabasal hooks: L = 11.42 , H = 9.7, B = 6. Basal hooks: l = 6.42 , h = 4.34, b = 4.5, 8 hsr basal, 7-8 hsr metabasal. The hooks form continuous spirals around the tentacles.
Remarks: The specimens examined conform to several morphological and morphometric characteristics of *Nybelinia africana* collected by (Palm, 1997; 1999), obtained from *Todarodes angolensis* and *Carcharhinus obscurus* stomach in South Africa with the exception that the velum (vel) was slightly larger (up to 318) (Annex 1). Some elements of their morphology resemble that of *Nybelinia mehlhorni* Palm & Beveridge, 2002 (SL, SW, pbo, pv, BL BW,

TSW) in *Hemigaleus microstoma* (Palm & Beveridge, 2002), and *N. jayapaulazariahi* Reimer, 1980 (SL, SW, pbo, pv, vel, app, BL, BW) in *Synaptura nigra* (Palm & Beveridge, 2002). The majority of the plerocercoids were found with the tentacles invaginated.

***Nybelinia* sp. 4**

Host: *Syacium papillosum* (Linnaeus, 1758) (Paralichthyidae).
Locality: Yucatan shelf, Gulf of México (latitude 22.04241, longitude -86.5512).
Site of infection: Stomach wall.
Material deposited: Voucher (1 slide, 1 specimen) was deposited in Helminthological Collection of the Universidad Nacional Autónoma de Mexico (access number CNHE No. 11116).
GenBank number: MK558805, OR750424 and OR750425.
Supplementary observations: (Based on 6 larvae, Fig. 2 D; Fig. 3 I-L): Scolex craspedote, SL = 645–814 (736), SW = 348–458 (394), 4 small bothridia, pbo = 312–424 (362), pv = 151–229 (185), pb = 186–282 (238), ppb = 255–294 (279), vel = 111–291 (183), ps = 660–795 (733), app = 68 (1specimen), 4 bulbs elongated, BL = 180–241 (225), BW = 71–106 (83), BR = 1.56:1, SP = 1.76:1.34:1, long tentacle with small hooks invagination, TL = 138–234 (200), TW = 19–21 (18), TSW = 9–16 (14) (based 2 organism). Metabasal tentacular armature homeoacanthous homeomorphus; hooks solids of uncinate hooks. L = 7.77, H = 6.18, B = 3.05. 6–7 hrs metabasal. Basal armature absent**.**
Remarks: This specimen agrees with the characteristics of the genus *Nybelinia* described by Palm (Palm, 2004). However, it was impossible to observe all of the everted tentacles in the measured specimens. Some measurements were similar to those of *N. indica* (SL, pv, vel, BL, TL, TW, but differ in tentacular armature) obtained from several hosts e.g., in the stomach of *Carcharhinus limbatus* from South Africa (Palm, 1999), in *Balistes carolinensis* from France (SL, BL, BW and metabasal length) (Palm & Walter, 2000), and in *Coryphaena hippurus* from Mississippi, Gulf of Mexico (pbo, metabasal length) (Palm & Overstreet, 2000) (Annex 1). These specimens also resemble *N. lingualis* (Cuvier, 1817) Dollfus, 1929 (TSW, metabasal length), *N. mehlhorni* (SW, pbo, metabasal length) (Palm, 2004), *N. jayapaulazariahi* (SL, pb, BL, BW, SP, TW, hsr metabasal) (Palm & Beveridge, 2002) and *N. africana* (SL, SW, pbo, pv, pb, vel, BL, BW, TL) (Palm, 1999) (Annex 1).

***Kotorella* Euzet & Radujkovic, 1989**
***Kotorella pronosoma* (Stossich, 1900)**

Host: *Syacium papillosum* (Linnaeus, 1758) (Paralichthyidae).
Locality: Yucatan shelf, Gulf of México (latitude 22.33033, longitude -90.23738).
Other hosts and localities: Reported in *Dasyatis pastinaca* from Boka Kotorska, Montenegro (Euzet & Radujkovic, 1989), *Dasyatis pastinaca* from the Mediterranean (Palm & Walter, 2000), *Cynoscion nebulosus* from Mississippi, Gulf of Mexico (Palm & Overstreet, 2000), adults from *Hemitrygon fluviorum* from East Australia (Palm & Beveridge, 2002), *Pagrus pagrus* and *Mullus barbatus* from Red Sea (Morsy *et al.,* 2013), *Maculabatis gerrardi* from Borneo (Schaeffner & Beveridge, 2014), *Syacium gunteri* and *S. papillosum* from southern Gulf of México (Vidal-Martínez *et al.,* 2014; 2019).
Site of infection: Stomach wall.
Material deposited: Voucher (1 slide, 1 specimen) was deposited in Helminthological Collection of the Universidad Nacional Autónoma de Mexico (access number CNHE No. 11118).
GenBank number: MK558802.
Supplementary observations: (Based on 10 larvae Fig. 2 E; Fig. 3 M): Scolex craspedote, SL = 607–781 (695), SW = 240–270 (255), 4 small bothridia, pbo = 370–430 (400), pv = 420–430 (440), pb = 80–125 (115), ppb = 35–154 (166), vel = 42–96 (73) (based in 7 specimens), ps = 527–668 (643), app = 160–186 (173), 4 small bulbs, BL = 101–140 (114), BW = 56–59 (57), BR = 1.7:1, SP = 2.8:4:1, long tentacles with small invaginated hooks, TL = 251–276 (261), TW = 14–18 (17), TSW = 13–17. Tentacular armature homeoacanthous, heteromorphous, hooks solid, arranged in quincunxes, of similar size along tentacle. Metabasal hooks: L = 8–10, H = –, B = 1.8–3. Basal hooks: l = 3–5, h = not measured, b = 1.5–3.5, 6–7 hsr metabasal, 8 hsr basal. Almost, all specimens were found with the hooks invaginated.
Remarks: These specimens match the description of the genus *Kotorella* described by Palm (2004), with an elongated scolex and craspedote, four elongated bothria, a par vaginalis longer than the pars bothrialis, and four short invaginated tentacles with hooks that appear to be arranged in a homeoacanthous heteromorphous pattern. Our specimens conform with most of the measurements of *K. pronosoma* reported for the Gulf of Mexico by Palm & Overstreet (2000) and Palm & Walter (2000), but differ slightly in vel, BR and SP (see Annex 1). This species has a worldwide distribution. Vidal-Martínez *et al.* (2014; 2019) reported *K. pronosoma* in *Syacium gunteri* from the southern Gulf of Mexico.

Superfamily Eutetrarhynchoidea Guiart, 1927
Family Eutetrarhynchidae Guiart, 1927
***Oncomegas* Dollfus, 1929**

***Oncomegas wageneri* (Linton, 1890) Dollfus, 1929**

Host: *Syacium papillosum* (Linnaeus, 1758) (Paralichthyidae).
Locality: Yucatan shelf, Gulf of México (latitude 22.49475, longitude -89.26.324).
Other hosts and localities: *Conger myriaster*, *Ophisurus macrorhynchus* from the Pacific coastal waters of Japan (Yamaguti, 1934), *Conger myriaster*, *Cepola schlegeli* from Japan (Yamaguti, 1952), *Lutjanus aya* from the Gulf of Mexico (Thatcher, 1961), *Acanthocepola limbata*, *Cepola schlegelii*, *Cepola* sp. from the Gulf of Tonkin (Mamaev, 1970), *Dasyatis centroura* from Massachusetts (Toth *et al.,* 1992), *Lutjanus aya*, *Ophidion* sp. from the Gulf of Mexico (Jensen 2009), *Carcharhinus limbatus* from Veracruz (Méndez & Dorantes-González, 2017), *Syacium gunteri* from Southern the Gulf of Mexico (Vidal-Martínez *et al.,* 2014; 2015;

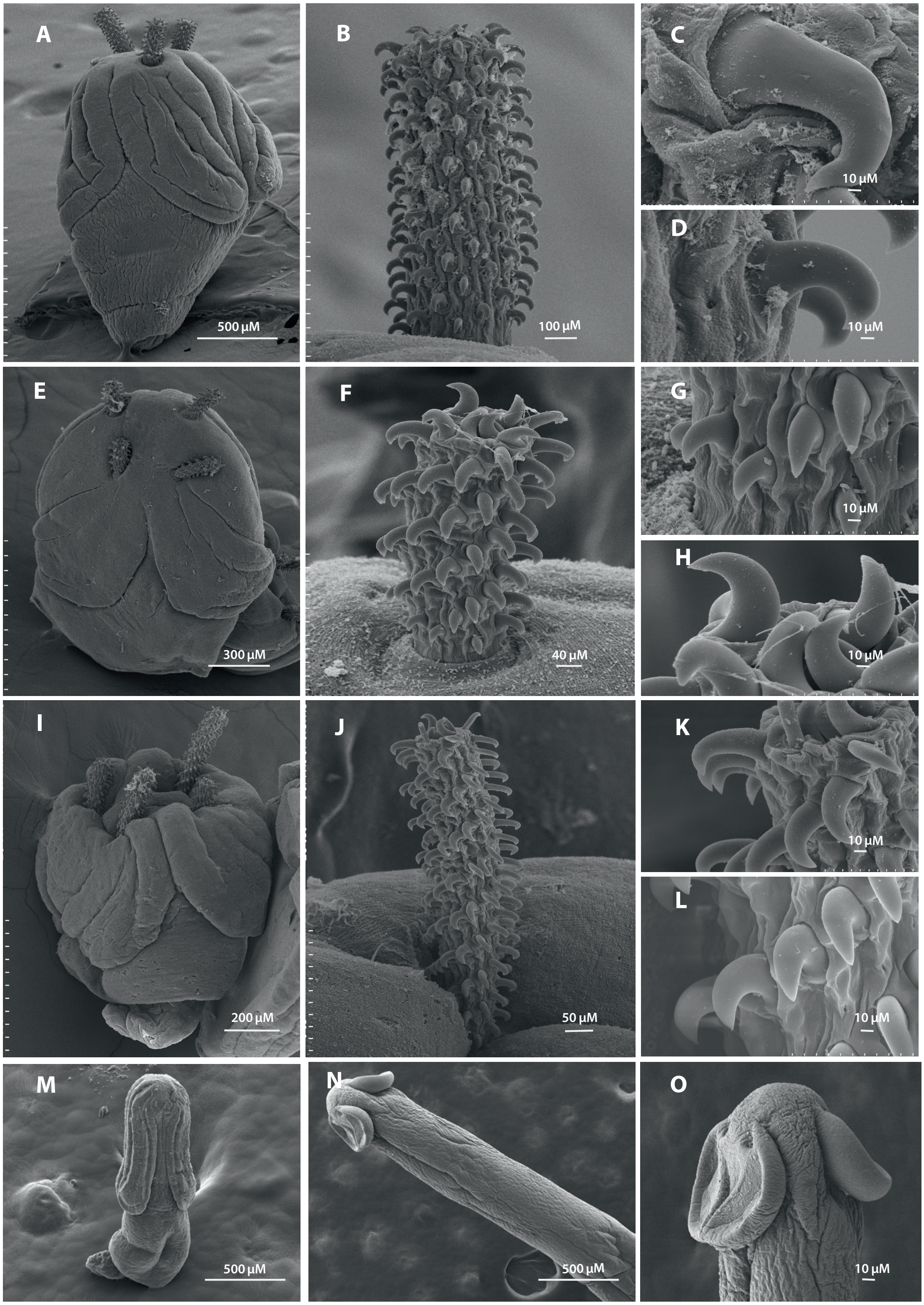

Fig. 3. Scanning electron micrographs of larvae species of trypanorhynch cestodes in *Syacium papillosum* from s-GoM. **A-D.** *Nybelinia* sp. 2, **B.** External armature, **C.** Metabasal hook, **D.** Basal hook. **E-H.** *Nybelinia* sp. 3, **F.** External armature, **G.** Metabasal armature, **H.** Basal armature. **I-L.** *Nybelinia* sp. 4, **J.** External armature, **K.** Metabasal armature, **L.** Basal armature. **M.** *Kotorella pronosoma*. **N-O.** *Oncomegas wageneri*.

2019), *Hypanus guttatus* from the southwestern Atlantic Ocean off Maceio, Brazil (Schaeffner, 2018), *Rhizoprionodon terraenovae*, *Hypanus americanus* from the Gulf of Mexico (Martínez-Aquino *et al.,* 2020), *Cyclopsetta chittendeni* from Southern the Gulf of Mexico (Soler-Jiménez *et al.,* 2020).
Site of infection: Intestine.
Material deposited: Voucher (1 slide, 1 specimen) was deposited in Helminthological Collection of the Universidad Nacional Autónoma de Mexico (access number CNHE No. 11119).
GenBank number: MK908867.
Supplementary observations: (Based on 8 larvae Fig. 2 F; Fig. 3 N-O): Scolex length acraspedote, SL = 2020–2440 (2105), SW = 346–380 (350), 2 bothridia, pbo = 285–304 (295), pv = 215–320 (230), pb = 267–464 (571), pars postbulbosa absent, 2 bulbs fluted and elongated muscular, BL = 430–1011 (650), BW = 114–152 (133), BR = 8.6–10.1:1, SP = –, long tentacle with hooks invagination, TL = 1480–1565 (1320), TW = 44–55 (49), TSW = 202–212 (208). Basal armature with single macrohook on external surface. Macrohook uncinate, L = 35–40 (38), H = 18–22 (21), B = 26–29 (27).
Remarks: The presence of an armature asymmetrical basal swelling on the internal face of the tentacle opposite to a single macrohook in the basal armature of the external face is a distinguishing feature of *Oncomegas* (Toth *et al.,* 1992). The measurements of our larvae conform with the description of *O. wageneri* (Toth *et al.,* 1992; Palm, 2004), i.e., basal armature consisting of a single macrohook on the external surface (Annex 1). However, the bulb length (BL) was shorter in s-GoM than that reported by Toth *et al.* (1992). The majority of the plerocercoids were found with the tentacles invaginated which made the description of the hook pattern difficult.

Phylogenetic relationships
A total of nine specimens from six species of trypanorhynchs were sequenced for 28SrDNA gene. The nucleotide frequencies of the alignment were as follows: A= 0.214, C=0.218, G=0.323 and T=0.245. The ML value of our phylogenetic tree was –15620.259117. The phylogenetic hypothesis was congruent with the trees previously obtained by Palm *et al.* (2009), Olson *et al.* (2010) and Haseli *et al.* (2017) (Fig. 4). The species of *Nybelinia* and *Kotorella* sequenced in this study were grouped into a clade, strongly supported bootstrap support values (bt=100) that included species of the genus *Nybelinia*, *Heteronybelinia* Palm, 1999, *Mixonybelinia* Palm, 1999, *Kotorella* Euzet & Ra 1989 and *Tentacularia* Bosc 1797, all belonging to Tentaculariidae (Fig. 4). Within this clade *Nybelinia*, *Heteronybelinia* and *Kotorella* were paraphyletic. *Nybelinia* sp. 1 grouped with *N. indica* Chandra 1986 (bt=54); larvae identified as *Nybelinia* sp. 2 was grouped as the sister species of the clade *Heteronybelinia yamagutii* (Dollfus, 1960) + *N. sphyrnae* Yamaguti, 1952 (bt=97); *Nybelinia* sp. 3 was the sister species of *N. aequidentata* (Shipley & Hornell, 1906) (bt=12); and the larvae of *Nybelinia* sp. 4 was the sister group of *N. africana* Dollfus, 1960 (bt=94). Our sequence of *K. pronosoma* was grouped with the sequence DQ642788 of the same species (bt=100), although other sequences also identified as *Kotorella* (DQ642787 and FJ572935) were phylogenetically positioned in another unrelated clade (Fig. 4). In particular, within the clade *Oncomegas*, our sequenced specimen was grouped with the *O. wageneri* specimens found by Martínez-Aquino *et al.* (2020) from the s-GoM (bt=100). They found high genetic variation, in the population genetic analyses for *O. wageneri* and revealed a lack of genetic structure among the sampled sites from the s-GoM. Thus, apparently there are several cryptic species within *O. wageneri*.

Discussion

The present study provides the first morphological and molecular data on larvae infections of trypanorhynchs in *S. papillosum* from the waters of the Yucatan shelf. With the exception of the study of Vidal-Martinez *et al*. (2019), new taxonomic and molecular data of plerocercoid trypanorhynchs have not been reported in flatfish in the Gulf of Mexico since the work of Palm and Overstreet (2000). In this study, a total of three genera of trypanorhynch cestode larvae were found in the stomach wall and intestine of *S. papillosum,* represented by plerocercoids of six species of cestodes. The family Tentaculariidae was the most represented, with five species registered. The taxonomic and molecular sequences identified the larvae of four cestode species in the genus *Nybelinia*. This genus is characterized morphologically by homeoacanthous homeomorphous metabasal armature and solid hooks form continuous spirals around the tentacles, which were evident in the plerocercoids found in this study and allowed us to assign them to this genus (Palm, 2004). In its adult form, *Nybelinia* infects approximately 53 species of 12 batoid and 23 shark genera (Palm, 2004; Palm & Bray, 2014; Schaeffner & Beveridge, 2014; Haseli, 2017). The low host specificity within this genus, especially in the second intermediate host, allows them to be widely distributed worldwide in many teleosts fish and cephalopods (Palm & Caira, 2008). In the northern of Gulf of Mexico, Palm and Overstreet (Palm & Overstreet, 2000) reported *Nybelinia* cf. *bisulcata*, *N. scoliodoni*, *K. pronosoma* and *Dasyrhynchus pacificus* Robinson, 1959 in teleosts and elasmobranchs. Likewise, Jensen (2009) reported adults of *Nybelinia* cf. *bisulcata*, *N. lingualis* and, in larval stage, *N. indica*, *N. mehlhorni* and *Nybelinia* sp. in several host species also in the northern Gulf of Mexico. It appears that the genus comprises many species with a cosmopolitan distribution pattern (Palm, 2004). Currently, nine trypanorhynchs families (Tentaculariidae, Pterobothriidae, Lacistorhynchidae, Otobothriidae, Eutetrarhynchidae, Sphyriocephalidae, Gymnorhynchidae, Otobothriidae, Pseudotobothriidae) have been collected from elasmobranchs and marine mammals in the Gulf of Mexico (Jensen, 2009).
Others species of trypanorhynch cestodes such as *Nybelinia surmenicola* Okada in Dollfus, 1929, *N. bisulcata* (Linton, 1889) Dollfus, 1929, *N. lingualis*, *Lacistorhynchus dollfussi* Beveridge & Sakanari, 1987, *Grillotia* sp., are found in other flatfish species

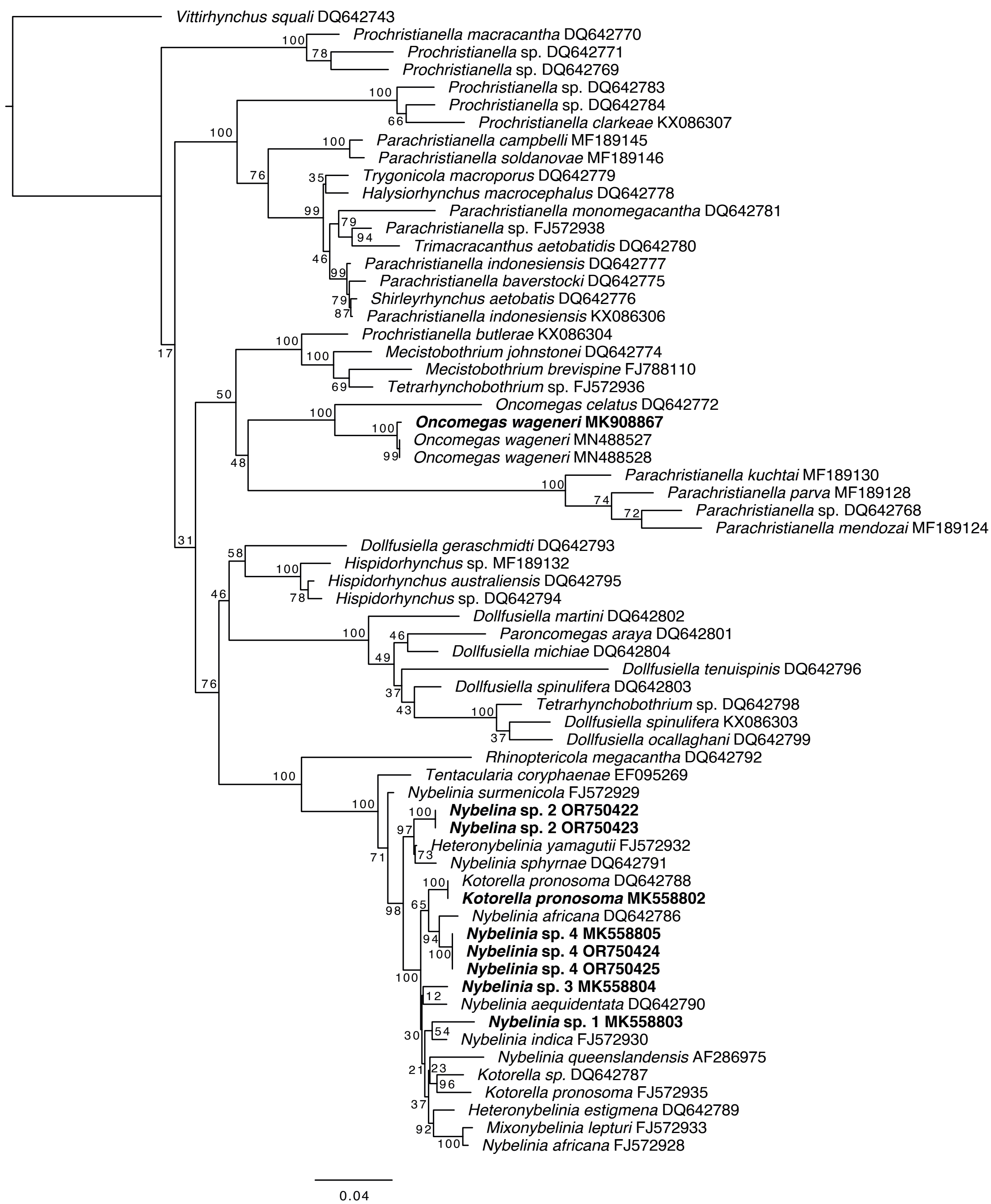

Fig. 4 Phylogenetic relationships of the trypanorhynch cestodes found in *Syacium papillosum* resulting from the Maximum Likelihood analysis with the 28S gene. The newly generated sequences are highlighted in bold. Numbers near the tree nodes represent the Bootstrap support values and the scale-bar indicates the number of substitutions per site.

(*Hippoglossina macrops*, *Hippoglossus stenolepis*, *Hippaglossoides platessoides*, *Oncopterus darwinii*, *Paralichthys adspersus*, *P. isosceles*, *P. microps*, *P. patagonicus*) in different regions of the world (Jansen & Burreson, 1990; Oliva *et al.,* 1996; Blaylock *et al.,* 1998; Palm & Overstreet, 2000; Álvarez *et al.,* 2002; Felizardo *et al.,* 2010; Fonseca *et al.,* 2012; Alves *et al.,* 2018; Vidal-Martínez *et al.,* 2014; 2019). Thus, possibly some of these trypanorhynch cestodes will apper in the s-GoM in the near future.

Additionally, this study represents the first taxonomic record of *K. pronosoma* in the s-GoM waters. This species has a cosmopolitan distribution, as it has been reported in the Mediterranean, the Gulf of Mexico, Sri Lanka and Indonesia (Euzet & Radujkovic, 1989; Palm *et al.,* 1997; Palm, 1997; Palm & Overstreet, 2000). It appears that *K. pronosoma* parasitizes many host species with a worldwide distribution. This is supported by the fact that adults and larvae of this plerocercoid have been recorded in *Dasyatis pastinaca* (Euzet & Radujkovic, 1989), *Dasyatis pastinaca* (Palm & Walter, 2000), *Cynoscion nebulosus* (Palm & Overstreet, 2000), *Dasyatis fluviorum* (Palm & Beveridge, 2002), *Pagrus pagrus* and *Mullus barbatus* (Morsy *et al.,* 2013), *Himantura gerrardi* (Schaeffner & Beveridge, 2014), *Syacium gunteri* and *S. papillosum* (Vidal-Martínez *et al.,* 2014; 2019). The adult form is found in the stomach of rays and plerocercoids in the stomach wall of marine teleosts and cephalopods (Jensen, 2009). An elongated scolex and four elongated bothria are the main morphological characteristics that characterise this genus (Palm, 2004). Trypanorhynch species (*Callitetrarhynchus gracilis* (Rudolphi, 1819) Pintner, 1931, *Floriceps* sp., *Pintneriella musculicola* Yamaguti, 1934) can generate injuries in fish, however, information regarding the pathological changes caused by these parasites is still limited. In visceral organs and musculature of marine teleosts histopathological changes are observed including necrosis, inflammatory, and fibrosis (Ibrahim, 2000; Hassan *et al.,* 2002; Abdelsalam *et al.,* 2016; Sales-Ribeiro *et al.,* 2021). Molecular analysis supports the presence of *Oncomegas wageneri* belonging to the Eutetrarhynchid genus. Palm (2004) described the distinctive features of this genus e.g., a slender scolex, an acraspedote, and two bothria in an opposite arrangement, four long tentacles with asymmetrical basal swelling, metabasal tentacular armature heteroacanthous typical, homeomorphous or heteromorphous and basal swelling armature with single macrohook on external surface. This genus has a wide geographical distribution. For example, *O. paulinae* Toth, Campbell & Schmidt, 1992 from *Urolophus halleri* has been reported in the Pacific Ocean, Mexico; *O. australiensis* Toth, Campbell & Schmidt, 1992 from *Aetobatus narinari* in Australia, *O. javensis* sp. nov. Palm, 2004 from *Dasyatis thetidis* in Indonesia, *O. wageneri* from *D. centroura* in Northwestern Atlantic Ocean (Gulf of Mexico), *Hypanus guttatus* in the southwestern Atlantic in Brazil; and from *S. papillosum* in s-GoM (Toth *et al.,* 1992; Palm, 2004; Schaeffner, 2018; Vidal-Martínez *et al.,* 2019). Recently, Martinez-Aquino *et al.* (2020) reported adults of *O. wageneri* from *Rhizoprionodon terraenovae* and *Hypanus americanus* in the s-GoM, representing the first record of the genus *Oncomegas* in sharks. This genus infects several Actinopterygii intermediate hosts (5 orders, 7 families, and 12 species), with a wide worldwide distribution e.g., in the Atlantic Ocean and Pacific Ocean. In conclusion, this study confirms, for the first time, the identity of larvae stages of cestodes found in *S. papillosum* based on their morphological and molecular information. Additional sequences linked to a detailed morphological characterization need to be added to public databases to clarify the identity of those larvae unidentified to date. These preliminary reports of the three genera of trypanorhynch cestodes increase our knowledge of the parasite species composition in the southern Gulf of Mexico. Finally, it is highly recommended to proceed with the identification of the adult stages of these cestodes in sharks and rays to complete the information on their life cycles on the region.

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Conflict of Interests

The authors declare that they have no competing interests.

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