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Original Article

Morphological and molecular insights of a new species of trypanorhynchid cestode parasite, *Nybelinia exostigmi*, in the Narrowstripe cardinal fish *Apogon exostigma*

Abordagens morfológicas e moleculares de uma nova espécie de parasito cestóide tripanorrinquídeo, *Nybelinia exostigmi*, no peixe cardinal Narrowstripe *Apogon exostigma*

Saleh Al Quraishy[1]*; Mohamed Abdel Monem Dkhil[1,2]; Rewaida Abdel-Gaber[1,3]; Esam Al-Shaebi[1];
Ahmad Abu Jaffal[4]; Kareem Morsy[3,5]

[1] Zoology Department, College of Science, King Saud University, Riyadh, Saudi Arabia

[2] Department of Zoology and Entomology, Faculty of Science, Helwan University, Cairo, Egypt

[3] Zoology Department, Faculty of Science, Cairo University, Cairo, Egypt

[4] Clinical Laboratory Sciences Department, King Saud Bin Abdul Aziz University for Health Sciences, Riyadh, Saudi Arabia

[5] Biology Department, College of Science, King Khalid University, Abha, Saudi Arabia

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Abstract

Forty specimens of the Narrowstripe cardinal fish *Apogon exostigma* were examined for gastrointestinal helminthes, and 62.5% were infected with a new trypanorhynchid larval cestode parasite. The morphology of its larval stage was studied based on light and scanning electron microscopy. The data revealed plerocercoid larvae characterized by a pyriform body lined with prominent microtriches; the acraspedote scolex had four overlapping bothridia; four tentacles protruded through the pars bothridialis; the armature of the tentacles was homeocanthous, homeomorphous, and consisted of falcate compact rose-thorn-shaped tentacular hooks; four oval-shaped bulbs in pars bulbosa; and short appendix at terminal end of the body. Molecular analysis of the 18S rRNA sequences verified the taxonomy of this parasite and supported its morphology. We discovered that there was a close identity (up to 87%) with alternative species obtained for comparison from GenBank. The data also showed that there were high blast scores and low divergence values between this parasite and other Tentaculariidae species. The phyletic analysis showed that parasite sequences in conjunction with existing data places this trypanorhynchid species among the Tentaculariidae. This species is deeply embedded within genus *Nybelinia* with close relationships to *Nybelinia queenslandensis* as a putative sister taxon.

Keywords: Marine fish, trypanorhyncha, morphology, phylogeny.

Resumo

Quarenta espécimes do peixe cardinal *Apogon exostigma* da Narrowstripe foram examinados para identificar helmintos gastrointestinais, destes 62,5% foram infectados com um novo parasito larval cestóide tripanorrinquídeo. A morfologia de seu estágio larval foi estudada na microscopia de luz e eletrônica de varredura. Os dados revelaram larvas plerocercoides caracterizadas por uma forma piriforme com um corpo revestido por microtrícinos proeminentes; o escolex acraspedótico tinha quatro sobreposições; quatro tentáculos se projetavam através da pars botridialis; a armadura dos tentáculos era homeocante, homeomorfa e consistia de ganchos tentaculares em forma de espinhos, em forma de falcão; quatro bulbos ovais em pars bulbosa; e apêndice curto na extremidade terminal do corpo. A análise molecular das sequências de RNAr 18S verificou a taxonomia desse parasita e apoiou sua morfologia. Descobrimos que havia uma identidade próxima (até 87%) com espécies alternativas obtidas para comparação do GenBank. Os dados também mostraram que houve altos escores de brusone e baixos valores de divergência entre este parasita e outras espécies de Tentaculariidae. A análise filética mostrou que as sequências de parasitas em conjunto com os dados existentes colocam esta espécie de tripanorimidídeo entre os Tentaculariidae. Esta espécie está profundamente enraizada no gênero *Nybelinia*, tendo relações próximas com *Nybelinia queenslandensis* como um putativo táxon irmão.

Palavras-chave: Peixe marinho, trypanorhyncha, morfologia, filogenia.

***Corresponding author:** Saleh Al Quraishy. Zoology Department,
College of Science, King Saud University, Riyadh 11451, Saudi Arabia.
e-mail: squraishy@ksu.edu.sa

Introduction

The Red Sea is one of the major centers of global marine biodiversity and supports 1.248 species of fish representing 157 families (GALLI et al., 2007). Rohde (1993) stressed the commercial and economic importance of the marine fish resources and indicated that marine fish parasites are a potential threat to fish abundance. Trypanorhynch cestodes are one of the main metazoan parasites of marine fish species (PALM, 2010). While the adults are typically found in the stomach and intestine of marine elasmobranchs (CHERVY, 2002), larval forms infect a wide variety of marine teleosts and invertebrates (PALM et al., 2009). Infection with these cestodes on the flesh or musculature of commercially important fish species results in profound losses in fish-processing industries (PALM et al., 1997; BISEROVA et al., 2016).

The cestoda order Trypanorhyncha Diesing, 1863 is characterized by a scolex bearing 2 or 4 bothria (SOUTHWELL, 1929; JONES et al., 2004) and a tentacular apparatus, consisting of four retractile tentacles adorned with hooks as extensions of tentacle sheaths that are attached to four bulbs (PALM, 2004; LEE et al., 2016). Antagonistic bulbs and retractor muscles enable evagination and retraction of these structures that serve as holdfasts while the bothria are used for movement. This complex attachment apparatus is unique within the cestodes provides a strong synapomorphy that supports monophyly of this order. Palm (2004) recognized 254 different trypanorhynchid species, additionally, Beveridge & Campbell (2005), Friggens & Duszynski (2005), Beveridge & Justine (2006, 2007a,b,c), Campbell & Beveridge (2006a,b, 2007), and Beveridge (2008) have since added 23 more species to the order. Thus, a total of 277 trypanorhynchid species can be considered valid with additional species and genera being described every year.

There has been an extensive work on the genus *Nybelinia* within order Trypanorhyncha by Heinz & Dailey (1974), Shimazu (1975), Carvajal et al. (1976), Shah & Bilqees (1979), Kurshid & Bilquees (1988), São Clemente & Gomes (1992), Beveridge & Campbell (1994), Palm et al. (1994), Jones & Beveridge (1998), Palm (2000), Bray (2001), Hassan et al. (2002), Bannai (2008), Purivirojkul et al. (2009) and Haseli et al. (2011). The genus *Nybelinia* Poche, 1926 is considered to be the most species-rich genus with a wide distribution throughout the world (PALM et al., 1998) with 55 species described under the genus of which only 30 species are accepted as valid species of the genus; the other 21 species are placed in different genera such as *Heteronybelinia*, *Myxonybelinia*, *Kotorella*, *Tentacularia* and *Parabothrium* and the remaining 4 species have an uncertain status. Limited studies were done on trypanorhynchids in marine fish from the Red Sea. Egyptian studies include Abdou (2000, 2001, 2005), Abdou & Palm (2008), and Morsy et al. (2013). Papers from Saudi Arabia include those of Abu-Zinada (1998) and Toula (1999). The literature from Yemen includes Al-Zubaidy (2006) followed by Al-Zubaidy & Mhaisen (2011).

Therefore, this study aimed to report the occurrence of trypanorhynchids in the commercially exploited fish from the Egyptian coastal waters of the Red Sea. Morphological and molecular characterizations were performed to identify the most suitable characteristics for higher trypanorhynch classification.

Materials and Methods

Fish samples collection and parasitological examination

Forty specimens of the Narrowstripe cardinalfish *Apogon exostigma* (F: Apogonidae) were collected from fishermen of Hurghada City at the Gulf of Suez, Red Sea, Egypt; from January to December 2017. The fish were transported immediately to the laboratory and examined for trypanorhynch cestodes. All procedures contributing to this work comply with the ethical standards of the relevant national guides on the care and use of laboratory animals and have been approved and authorized by the Institutional Animal Care and Use Committee (IACUC) at Zoology Department, Faculty of Science, Cairo University, Egypt (No. CUFS/S/Para/38/2014). Parasite prevalence, intensity and mean abundance was calculated according to Bush et al. (1997). The isolated trypanorhynchid cestodes were washed in saline solution (0.9%), fixed in hot 4% neutral formaldehyde solution, stained with Semichon's acetocarmine, dehydrated in a graded ethanol series, cleared with clove oil and then mounted in Canada balsam. Terminology of the different body parts of these trypanorhynchids follows the guidelines proposed by Campbell & Beveridge (1994), and Palm (2004).

Photomicrographs were made with the aid of Leica microscope DM 2500 (NIS ELEMENTS software, ver. 3.8). Measurements of the recovered parasites were made with an Olympus ocular micrometer and expressed in millimeters as a range followed by mean ± standard deviation (SD) in parentheses, unless otherwise stated. Illustrations were prepared using a drawing attachment for an Olympus BX51 microscope (Olympus Corporation, Tokyo, Japan) with the help of Nomarski differential contrast. For SEM studies, samples were fixed at 3% glutaraldehyde in 0.1 M sodium cacodylate buffer, washed in the same buffer, and dehydrated in a graded alcohol series (50%, 60%, 70%, 80%, 90% and 100%). Samples were then processed in a critical point drier "Bomer-900" with Freon 13, they were then sputter-coated with gold-palladium in a Technics Hummer V, and finally examined and photographed under an Etec Autoscan 10-kV JEOL scanning electron microscope (JSM-6060LV).

Molecular analysis

DNA extraction, PCR amplification, and sequencing

Total genomic DNA was extracted from ethanol-preserved samples using a QIAamp DNA mini Kit (Qiagen, Venlo, Netherlands) following the standard manufacturer-recommended protocol. Two microliters of gDNA (measured on a NanoDrop 1000; Thermo Scientific) were used as a template in 25 µl reactions using Ready-To-Go^{TM} PCR beads (Amersham Pharmacia Biotech). Partial 18S rRNA genes (domains D1-D3) were amplified using primers ZX-1F (5’-ACC CGC TGA ATT TAA GCA TAT-3’) and 1500R (5’-GCT ATC CTG AGG GAA ACT TCG-3’) reported by Palm et al. (2009) and the following cycling conditions: denaturation for 5 min at 95 °C, 40 cycles of 30 s at 95 °C, 30 s at 55 °C, 2 min at 72 °C, and 7 min extension at 72 °C. Each amplicon was examined by (1%) agarose gel electrophoresis in 1× Tris-acetate-EDTA (TAE) buffered gel stained with 1% ethidium bromide and then visualized with a UV transilluminator; the bands with predicted sizes were purified using QIAquick™ PCR Purification Kit (Qiagen, Venlo, Netherlands) following the standard manufacturer’s instructions. Amplicons were sequenced (in both directions) using an ABI Prism Dye Terminator Cycle Sequencing Core Kit (Applied Biosystems; Thermo Fisher Scientific, Waltham, MA, USA) with ABI 310 DNA Sequencer (Applied Biosystems, USA) and primers 300F (5’-CAA GTA CCG TGA GGG AAA GTT G-3’), ECD2R (5’-CTT GGT CCG TGT TTC AAG ACG GG-3’), 400R (5’-GCA GCT TGA CTA CAC CCG-3’) and 1090F (5’-TGA AAC ACG GAC CAA GG-3’) reported by Palm et al. (2009).

Sequence alignment and Phylogenetic analysis

Contiguous sequences were assembled and edited using Sequencher™ (GeneCodes Corp., version 4.6), and sequence identity was checked using the Basic Local Alignment Search Tool (BLAST) and the previously deposited sequences in GenBank database. The newly generated partial 18S rRNA sequences were aligned using CLUSTAL-X multiple sequence alignment (THOMPSON et al., 1997) and compared with some of previously recorded data from GenBank to analyze intra-specific differences. The alignment will be corrected manually using the alignment editor of software BioEdit 4.8.9 (HALL, 1999). Phylogeny reconstruction used neighbor-joining analysis with MEGA version 7 (KUMAR et al., 2016).

Results

Twenty-five (62.5%) of the forty specimens of the Narrowstripe cardinal fish *Apogon exostigma* (F: Apogonidae) were infected with plerocercoid larvae of *Nybelinia exostigmi* sp. nov. The infection was recorded in the intestine and stomach. Seasonally, the infection increased during winter to be 80% (16 out of 20) and fell to 45% (9 out of 20) during summer.

Morphological description (Figures 1-15, 16-17)

Plerocercoid larvae were pyriform in shape, rounded to slightly flattened anteriorly, tapering posteriorly, and covered with prominent microtriches. The scolex was acraspedote with the anterior half region overlapped by four bothridia. It measured 0.831-1.35 (1.20±0.1) mm in length and reached a maximum width at the level of the posterior portion of bothridia to be 0.323-0.642 (0.453±0.1) mm. The bothridia are broad, bean-shaped, and measured 0.491-0.760 (0.59±0.1) mm long and 0.08-0.13 (0.12±0.01) mm wide. The pars bothridialis was large, helmet shaped and measured 0.265-0.312 (0.291±0.1) mm long and 0.231-0.420 (0.354±0.1) mm wide; four tentacles protrude out through it. Tentacles sheathed and measured 0.58-0.65 (0.53±0.01) mm long. The width of the tentacles at the apical region measured 0.018-0.025 (0.020±0.001) mm, the metabasal region was 0.018-0.022 (0.021±0.001) mm, and the basal region was 0.010-0.017 (0.015±0.001) mm.

The armature was homeocanthous, homeomorphous, and consists of falcate compact rose–thorn–shaped tentacular hooks. However, the hooks diminish towards the basal end of tentacle with abruptly turned points and then increase in size towards the metabasal region and became longer near the apical portion with narrower implantation base. Four tentacle sheaths originated from the scolex as two anterior and two posterior these overlapped at the apices of the bulbs. The size of the bulbs reached 0.30-0.39 (0.32±0.01) mm long and 0.19-0.27 (0.22±0.01) mm wide. The bulb ratio was 1.45:1. The pars vaginalis started immediately behind tentacles and consists of four, thin tubes, reaching up to the parts of bulbosa. These measured 0.423-0.538 (0.491±0.1) mm long and 0.191-0.281 (0.201±0.1) mm wide. The pars bulbosa was characterized by four oval-shaped bulbs filled with glandular cells and measured 0.192-0.218 (0.201±0.1) mm long and 0.096-0.114 (0.101±0.01) mm wide. The body terminated by a short appendix measuring 0.071-0.142 (0.121±0.01) mm long and 0.043-0.063 (0.052±0.001) mm wide. Table 1 presents the maximum and minimum values, as well as the mean values found in the different structures studied in this species versus previously described *Nybelinia* species in fish.

Taxonomic summary

Parasite name: *Nybelinia exostigmi* sp. nov. (Family Tentaculariidae Poche, 1926)

Host: Narrowstripe cardinal fish *Apogon exostigma* (Family: Apogonidae)

Morbidity and mortality: Infected fish were generally symptomless externally

Site of infection: Intestine and stomach of infected host fish

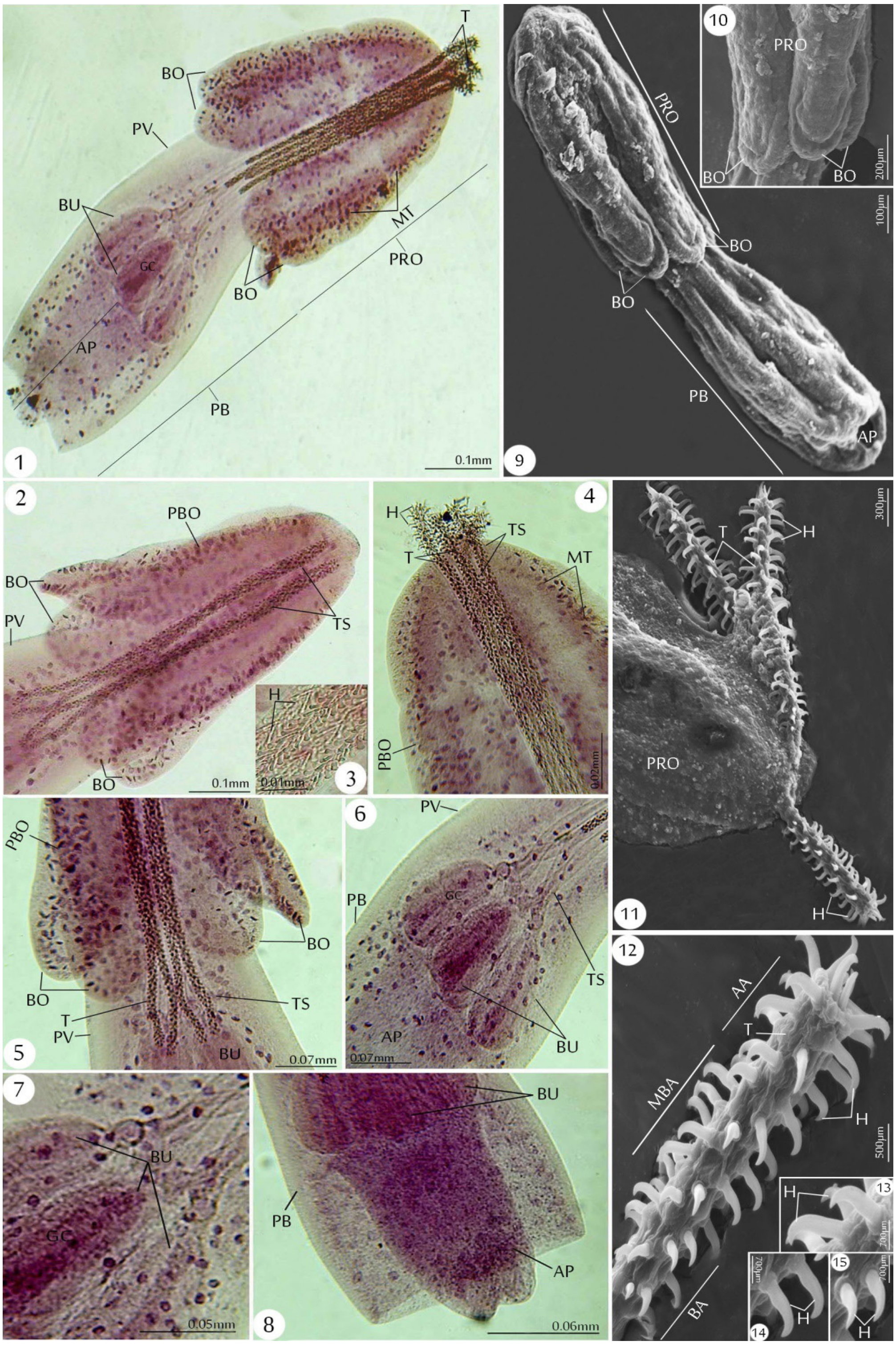
Locality: Hurghada City at the Gulf of Suez, Red Sea, Egypt

Prevalence and intensity: 25 out of 40 (62.5%) specimens infected

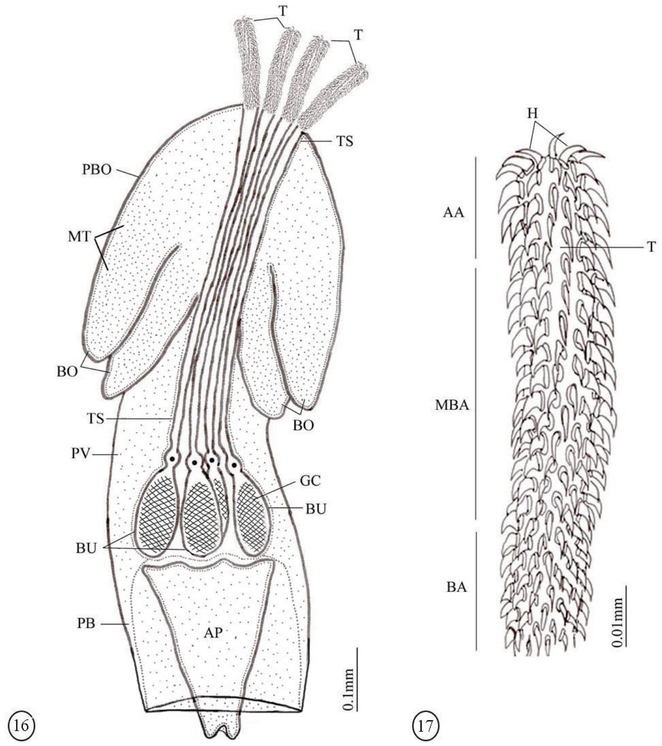
Etymology: New species name was given with respect to the specific host name, where the parasitized fish were discovered

Molecular analysis

An approximately 840 bp of 18S rRNA gene sequences for the recovered cestode parasite was deposited in GenBank (gb| MK084750.1) and GC content (50%) was obtained. Pairwise comparison of the isolated genomic sequence from this parasite species with a variety of alternative class species and genotypes disclosed a unique genetic sequence. Comparison of this novel genetic sequence with others retrieved from GenBank demonstrated a high degree of similarity up to 87% (Table 2). Comparison of the nucleotide sequences and divergence showed that the 18S rRNA sequences of our cestode species yielded



Figures 1-15. Plerocercoid larvae *Nybelinia exostigmi* sp. nov. infecting *Apogon exostigma.* **1-8** Photomicrographs showing: **1.** The body of plerocercoid larvae is divided into a scolex with three parts: pars bothridialis (PBO) with four bothridia (BO) and tentacles (T), pars vaginalis (PV), and pars bulbosa (PB) with four bulbs (BU); this ended with an appendix (AP). Note that the body is covered with prominent microtriches (MT); **2-4.** The pars bothridialis (PBO) with four bothridia (BO), and tentacles (T) is surrounded by the tentacle sheath (TS) and has hooks (H) at high magnification of the tentacle in (**3**); **5-7.** The pars vaginalis (PV), and pars bulbosa (PB) with four bulbs (BU); **8.** The body ends in an appendix (AP); **9-15** Scanning electron micrographs showing: **9.** The body of the plerocercoid larvae divided into a scolex with pars bothridialis (PBO) provided with four bothridia (BO) followed by pars bulbosa (PB) and ending with an appendix (AP); **10-15.** High magnifications of: **10, 12** Pars bothridialis (PBO) with four bothridia (BO) and four tentacles (T) protruding from it and provided with hooks (H); **12.** Tentacle (T) provided with hooks (H) and differentiated into apical (AA), metabasal (MBA), and basal (BA) armature; **13.** Hooks (H) with apical armature; **14.** Hooks (H) with metabasal armature; **15.** Hooks (H) with basal armature.



Figures 16-17. Line drawing with camera lucida of the plerocercoid larvae *N. exostigmi* sp. nov. showing different body parts. **16.** The whole plerocercoid larvae showing pars bothridialis (PBO), pars vaginalis (PV), pars bulbosa (PB), bothridia (BO), tentacles (T), tentacle sheath (TS), bulbs (BU), microtriches (MT), gubernaculum (GC), and appendix (AP); **17.** High magnification of the tentacle (T) provided with hooks (H) showing apical armature (AA), metabasal armature (MBA), and basal armature (BA).

the highest BLAST scores with lowest divergence values for *Nybelinia queenslandensis* (gb| AF287005.1), *Nybelinia sphyrnae* (gb| DQ642953.1), *Nybelinia africana* (gb| DQ642948.1), *Nybelinia surmenicola* (gb| AB626625.1), *Nybelinia indica* (gb| FJ572894.1), *Nybelinia aequidentata* (gb| DQ642952.1), *Kotorella pronosoma* (gb| DQ642950.1), *Heteronybelinia estigmena* (gb| DQ642951.1), *Mixonybelinia lepturi* (gb| FJ572898.1), and *Tentacularia coryphaenae* (gb| FJ572891.1) (Table 3, Figure 18).

A tree topology was automatically computed with 36 nucleotide sequences to estimate neighbor-joining (NJ) values (Figure 19). The constructed dendrogram showed two subclasses within class Cestoda: Eucestoda and Cestodaria. The first lineage includes Eucestoda species and consisted of subclades representing the most closely related orders with sequence similarities ranging between 96-91% with moderate bootstrap values: Trypanorhyncha, Diphyllobothriidea, Rhinobothriidea, Phyllobothriidea, Tetraphyllidea, and Diphyllidea. Additionally, there were two diagrammatic Trypanorhyncha suborders and each of them was represented by totally different families: Acystidea (Tentaculariidae, Hepatoxylidae) and Cystidea. The earliest divergent monophyletic sister cluster to the remaining taxa consists of families Gilquiniidae, Rhinoptericolidae, Otobothriidae, Eutetrarhynchidae, Tetrarhynchobothriidae, Lecistorhynchidae, Gymnorhychidae, Dasyrhynchidae, Aporhynchidae and Pseudotobothriidae, all representing taxon Gymnorhynchoidea at intervals of the taxonomic group Cystidea. At the family level, strong nodal support was seen for the monophyly of the Tentaculariidae as a sister taxon of *Rhinoptericola megacantha* (gb| DQ642954.1) and the type of species for the family Rhinoptericolidae.

The genus Tentacularia is a sister taxon to the four alternative existing genera of *Nybelinia*, *Mixonybelinia*, *Heteronybelinia*, and *Kotorella* within this family; these, in turn, form a polyphyletic assemblage. The second lineage contained the remaining order of Cestodaria represented by *Gyrocotyle rugosa* (gb| AF124455.2) and appeared as a sister taxon within the Eucestoda. Our phylogenetic analysis incorporated new and existing data and investigated the position of the examined trypanorhynchid species within the Tentaculariidae family. This species is deeply embedded within the genus *Nybelinia* with close relationships to the antecedently delineated *N. queenslandensis* as a putative sister taxon.

Discussion

Parasitic infection of the Narrowstripe cardinal fish *A. exostigma* seen here with *N. exostigmi* sp. nov. was 62.5%; this concurs with previous studies by Eyo et al. (2013) who stated that the total infection rate for the larval cestodes in *Synodontis batensoda* ranged between 48-61.8%. The current prevalence is higher than that recorded by Obiekezie et al. (1992) in Nigeria (0.44%), or El Naffar et al. (1992) who reported on *Nybelinia* sp. from *Upeneus tragula* at a prevalence of 11.4% and *Saurida tumbil* at a prevalence of 2.7% along the coast of the United Arab Emirates. Other reports include Palm et al. (1993) in the Philippines (2.25%), São Clemente et al. (1997) in Brazil (55.75%), and Abu-Zinada (1998) on the coast of the Red Sea in Saudi Arabia (29.4%). Kardousha (1999) reported *Nybelinia indica* from *Alepes djedaba* and *Tentacularia coryphaenae* from *Euthynnus affinis* at a prevalence of 6.7% along the coasts of the United Arab Emirates. Hassan et al. (2002) described the prevalence of trypanorhynchid cestodes in the Arabian Gulf (7.73%), Costa et al. (2003) studied trypanorhynchids infecting marine fish from Madeira (9.6%), and Abo-Esa (2007) stated that the prevalence of trypanorhyncha *Nybelinia* metacestode in the wall and lumen of the stomach of the Barbony fish was 11%. Al-Zubaidy & Mhaisen (2011) found that the infection rate of trypanorhynchids in the body cavity and mesenteries of fish in the Yemeni waters of the Red Sea was 24.3%. Such differences can be explained by the fact that the combination of selected fish species and sizes is the major factor underlying the prevalence of musculature infection during a survey for trypanorhynchids.

A possible explanation for the differences and similarities of the above results might be attributed to many factors as stated by Sabas & Luque (2003): the positive correlation of host-parasite interaction, the influence of regional ecological disturbances and the ontogenetical changes in the feeding behavior of fish. The plerocercoid larvae *Nybelinia* was found encapsulated in the body cavity on sides of the stomach and intestine of *A. exostigma*. This data agreed with Bates (1990) and Al-Zubaidy & Mhaisen (2011) who reported that most *Nybelinia* specimens were found

Table 1. Comparative measurements (in millimeters) of the present *Nybelinia exostigmi* sp. nov. and those described previously.

Species/ Parameters	Host	Scolex		Pars bothridialis Length	Pars vaginalis Length	Bulb			Pars bulbosa Length	Tentacle Width			Hooks Shape	Armature	Appendix Length
		Length	Width			Length	Width	Ratio		Apical	Metabasal	Basal			
N. rougetcampanae São Clemente and Gomes, 1992	*Sphyrna lewini*	0.51-0.69 (0.60)	---	0.28-0.38 (0.33)	0.31-0.41 (0.36)	---	0.03-0.05 (0.04)	---	0.18-0.22 (0.20)	---	0.013-0.026	0.005-0.008	Falcate	Heteromorphous	—
N. lingualis Pascual et al., 1996	*Todaropsis eblanae*	0.8-1.2	0.9-1.1	0.60-0.62	0.24-0.28	0.75	---	---	0.24-0.28	---	---	---	Uncinate	Homeomorphous	—
N. beveridgei Palm et al., 1997	*Chascomopsetta lugubris*	4.00-5.28 (4.640)	2.176-3.104 (2.604)	2.784-3.040 (2.912)	2.368-3.040 (2.704)	0.928-0.992 (0.960)	0.256-0.320 (0.288)	3.3:1	---	---	0.081-0.098	---	Uncinate	Heteromorphous	0.608-1.120 (0.864)
N. anthicosum Palm et al., 1997	*Todarodes angolensis*	1.762-2.112 (1.936)	0.864-1.376 (1.120)	0.800-1.186 (0.992)	0.640-0.992 (0.816)	---	---	3.4:1	0.416-0.576 (0.496)	---	0.048-0.055	---	Uncinate	Homeomorphous	0.352-0.507 (0.430)
N. gopalai Palm et al., 1997	*Coelorhinchus flabellispinus*	1.144-2.240 (1.692)	0.416-1.105 (0.761)	0.520-1.014 (0.767)	0.611-1.300 (0.956)	---	---	7.1:1	0.286-0.715 (0.501)	---	0.016-0.035	---	Uncinate	Heteromorphous	0.156-0.325 (0.241)
N. robusta Palm et al., 1997	*Bassango albescens*	1.600-2.528 (2.064)	0.416-1.472 (0.944)	0.832-1.352 (1.092)	0.767-1.472 (0.982)	---	---	4.4:1	0.384-0.637 (0.511)	---	0.029-0.033	---	---	Homeomorphous	0.273-1.408 (0.841)
N. yamagutii Palm et al., 1997	*Todarodes angolensis*	1.183-3.904 (2.544)	0.546-1.440 (0.993)	0.529-1.888 (1.240)	0.689-2.016 (1.353)	---	---	6.7:1	0.286-1.568 (0.927)	---	0.042-0.080	---	Falcate	Heteromorphous	0.169-0.512 (0.341)
N. queenslandensis Jones and Beveridge, 1998	*Carcharhinus melanopterus*	0.97-1.20 (1.10)	0.90	---	0.38-0.61 (0.54)	0.34-0.58 (0.45)	0.09-0.13 (0.11)	---	---	---	0.07-0.08 (0.08)	---	Falcate	Homeomorphous	—
N. sakanariae Palm, 1999	*Xiphiurus capensis*	1.507-1.512	0.747-0.775	0.700	0.647-0.680	0.335-0.387	0.113-0.116	3.3:1	0.386-0.397	---	0.051-0.056	---	---	Homeomorphous	0.335-0.360
N. aequidentata Palm, 1999	*Lepturacanthus savala*	3.400	1.700	1.510	1.890	0.756-0.813 (0.780)	0.227-0.265 (0.237)	3.3:1	0.813	0.046-0.051	0.054-0.058	---	Falcate	Homeomorphous	0.585
N. africana Palm, 1999	*Carcharhinus obscurus*	0.536	0.420	0.327	0.205	0.168-0.178 (0.174)	0.070-0.075 (0.073)	2.4:1	0.178	---	0.023-0.024	0.027-0.028	Uncinate and falcate	Heteromorphous	—
N. schmidti Palm, 1999	*Isurus glaucus*	1.172	0.832	0.794	0.473	0.289	0.104	2.8:1	0.289	---	0.018-0.023	---	---	Homeomorphous	—
N. jayapaulazariahi Palm, 1999	*Harpadon nehereus*	0.530	0.326	0.298	0.285	0.150-0.165 (0.157)	0.054	2.9:1	0.165	---	0.016-0.0185	---	Uncinate	—	0.114
N. southwelli Palm and Walter, 1999	*Rhina ancylostoma*	1.701	---	1.078	0.982	0.474	0.166	2.9:1	0.485	---	0.033-0.038	0.046-0.051	---	Homeomorphous	—
N. perideraeus Palm and Walter, 1999	*Glyphis gangeticus*	1.222	0.700-0.767 (0.715)	0.533-0.546 (0.637)	0.390-0.520 (0.540)	0.340-0.390 (0.445)	0.103-0.114 (0.115)	3.8:1	0.408-0.461 (0.429)	0.020	0.030-0.033 (0.035)	0.033-0.039 (0.042)	---	Heteromorphous	—
N. riseri Palm, 1999	*Trachyurus felicipes*	1.380-1.587 (1.455)	0.510-0.680 (0.580)	0.585-0.662 (0.630)	0.567-0.700 (0.636)	0.270-0.303 (0.284)	0.084-0.117 (0.100)	2.8:1	0.280-0.303 (0.294)	---	0.051-0.056	---	Uncinate	Homeomorphous	0.312-0.360 (0.331)
Nybelinia sp. Palm, 1999	*Coryphaena hippurus*	1.172-1.228	0.775-0.907	0.850-0.888	0.548-0.624	0.234-0.247 (0.246)	0.084-0.112 (0.099)	2.5:1	0.252-0.257	---	0.032-0.035	---	---	Homeomorphous	0.294-0.364
N. scoliodoni Palm, 1999	*Carcharhinus limbatus*	0.667	0.320	0.267	0.227	0.125-0.144 (0.133)	0.056-0.064 (0.059)	2.2:1	0.144	0.012-0.013	0.014-0.017	0.023-0.025	---	Homeomorphous	—

Table 1. Continued...

Species/ Parameters	Host	Scolex		Pars bothridialis Length	Pars vaginalis Length	Bulb			Pars bulbosa Length	Tentacle Width			Hooks Shape	Armature	Appendix Length
		Length	Width			Length	Width	Ratio		Apical	Metabasal	Basal			
N. riseri Palm and Walter, 2000	*Raja binoculata*	1.465-1.890 (1.716)	0.630-0.980 (0.928)	0.770-1.005 (0.938)	0.798-1.150 (0.1000)	0.350-0.371 (0.362)	0.116-0.131 (0.121)	3.0:1	0.252-0.350 (0.294)	0.024-0.025	0.031-0.034	0.052-0.055	Uncinate	Homeomorphous	0.623
N. strongyla Palm and Walter, 2000	*Sphoeroides pachygaster*	1.920	1.100	0.845	0.503	0.533-0.572 (0.553)	0.182-0.208 (0.198)	2.8:1	0.585	---	0.059	0.062	Uncinate	Homeomorphous	—
N. lingualis Palm and Walter, 2000	*Thunnus thynnus*	2.700	1.620	1.755	1.350	0.378-0.420 (0.392)	0.121-0.138 (0.131)	3.0:1	0.462	---	0.038-0.041	---	Uncinate	Homeomorphous	0.924
N. lingualis Palm and Walter, 2000	*Chelidonichthys lucerna*	1.100-1.620	0.672-0.765	0.616-0.966	0.714	0.350-0.378 (0.364)	0.294-0.308 (0.299)	3.1:1	0.392	---	0.035-0.040	---	Uncinate	Homeomorphous	0.182-0.470
N. thyrsites Palm and Walter, 2000	*Squalus* sp.	1.148-1.162	0.840	0.518	0.434-0.364	0.294-0.322 (0.310)	0.120-0.140 (0.129)	---	0.350	---	0.045-0.048	0.048-0.052	Falcate	Homeomorphous	0.294-0.322
N. scoliodoni Palm and Walter, 2000	*Balistes carolinensis*	0.770-0.980	0.685-0.686	0.434-0.560	0.245-0.294	0.224-0.228 (0.226)	0.069-0.097 (0.083)	2.7:1	0.224-0.228	0.029-0.031	0.032-0.034	---	---	Homeomorphous	0.350-0.462
N. surmenicola Palm and Walter, 2000	*Gurnardus fabric*	0.450-0.693	1.57-2.43	1.98-2.56	1.98-2.56	0.630-0.728 (0.672)	0.266-0.294 (0.280)	2.4:1	0.700-1.035	0.080-0.120	0.070-0.107	0.057	Uncinate	Homeomorphous	1.50-2.42
N. africana Palm and Beveridge, 2002	Unidentified shark	1.780	1.134	0.930	0.840	0.423-0.459 (0.439)	0.157-0.171 (0.163)	2.7:1	0.523	---	0.047-0.050	---	Uncinate and falcate	Heteromorphous	—
N. schmidti Palm and Beveridge, 2002	*Isurus oxyrinchus*	1.600	0.850	1.040	0.680	0.270-0.330 (0.298)	0.110-0.150 (0.131)	2.3:1	0.340	---	0.030-0.032	0.045-0.048	Falcate	Homeomorphous	—
N. jayapaulazariahi Palm and Beveridge, 2002	*Aseraggodes macieayanus*	1.060	0.700	0.470	0.450	0.320-0.325 (0.323)	0.085-0.135 (0.119)	2.7:1	0.365	---	0.032-0.035	---	Uncinate	—	—
N. thyrsites Palm and Beveridge, 2002	*Carcharhinus brachyurus*	1.550-2.032	0.700-1.520	0.610-1.024	0.500-0.640	0.355-0.365 (0.358)	0.114-0.120 (0.117)	3.1:1	0.368-0.520	0.027-0.032	0.057-0.062	0.038-0.044	Falcate	Homeomorphous	0.496
N. hemipristis Palm and Beveridge, 2002	*Hemipristis elongata*	2.030	1.230	1.000	0.860	0.550-0.560 (0.553)	0.210-0.220 (0.218)	2.5:1	0.560	0.037-0.062	0.075-0.085	0.075-0.085	Falcate	Homeomorphous	—
N. mehlhorni Palm and Beveridge, 2002	*Hemigaleus microstoma*	0.560	0.400	0.330	0.125	0.150-0.200 (0.178)	0.050-0.070 (0.058)	3.1:1	0.250	---	0.022-0.027	---	---	Homeomorphous	—
N. strongyla Palm and Beveridge, 2002	*Argyrosomus hololepidotus*	1.303-1.566	0.648-0.796	0.700-0.781	0.647-0.764	0.244-0.292 (0.286)	0.082-0.090 (0.086)	3.3:1	0.292-0.355	---	0.050-0.058	---	Uncinate	Homeomorphous	0.374-0.429
N. scoliodoni Palm and Beveridge, 2002	*Makaira indica*	1.115-1.350	0.800-0.860	0.520-0.670	0.210-0.230	0.280-0.300 (0.288)	0.110-0.120 (0.115)	2.5:1	0.320-0.300	0.025-0.030	0.045-0.050	0.030-0.035	---	Homeomorphous	0.200-0.310
N. victoria Palm and Beveridge, 2002	*Lepidotrigla modesta*	1.030-1.050	0.650-0.700	0.570-0.450	0.350-0.320	0.300-0.315 (0.310)	0.115-0.135 (0.124)	2.5:1	0.315-0.320	---	0.040-0.050	---	---	Homeomorphous	—
Nybelinia sp. Haseli et al., 2010	*Rhizoprionodon acutus*	0.456	0.279	0.270	0.147	0.159	---	1.9	0.189	---	---	0.037	Uncinate and falcate	—	—
N. bisulcata Al-Zubaidy and Mhaisen, 2011	*Lethrinus lentjan*	1.44-2.3 (2.1)	---	0.46-0.49 (0.478)	---	---	---	---	---	---	0.019-0.022 (0.020)	---	---	—	0.31-0.34 (0.324)
N. pintneri Khamkar, 2012	*Trygon sephen*	1.668	0.568-1.053	0.379	0.666-0.667	0.417-0.462	0.076-0.114	---	0.417-0.462	---	0.030-0.053	---	---	Homeomorphous	—
N. narinari Morsy et al., 2013	*Pagrus pagrus*	1.19-1.26 (1.22±0.02)	1.00–1.32 (1.04±0.02)	---	---	0.21–0.27 (0.25±0.02)	0.09–0.13 (0.10±0.02)	---	---	---	---	---	---	—	0.28–0.36 (0.32±0.02)

Table 1. Continued...

Species/ Parameters	Host	Scolex		Pars bothridialis Length	Pars vaginalis Length	Bulb			Pars bulbosa Length	Tentacle Width			Hooks Shape	Armature	Appendix Length
		Length	Width			Length	Width	Ratio		Apical	Metabasal	Basal			
N. bisulcata Morsy et al., 2013	*Mullus barbatus*	0.84-0.93 (0.87±0.02)	0.48–0.58 (0.55±0.02)	---	---	0.35–0.44 (0.39±0.02)	0.26-0.33 (0.29±0.02)	---	---	---	---	0.03–0.07 (0.04±0.002)	Falcate	Heteromorphous	0.07-0.14 (0.12±0.02)
N. indica kalyan and Prasanna, 2016	*Rhizoprionodon acutus*	1.20-1.24	0.44-0.47	0.55-0.61	0.65-0.68	0.44-0.47	---	---	0.42-0.47	---	0.03	---	Uncinate	Homeomorphous	---
***N. exostigmi* sp. nov. (Present study)**	***Apogon exostigma***	**0.831-1.35 (1.20±0.1)**	**0.323-0.642 (0.453±0.1)**	**0.265-0.312 (0.291±0.1)**	**0.423-0.538 (0.491±0.1)**	**0.30–0.39 (0.32±0.01)**	**0.19–0.27 (0.22±0.01)**	1.45:1	**0.192-0.218 (0.201±0.1)**	**0.018-0.025 (0.020±0.001)**	**0.018-0.022 (0.021±0.001)**	**0.010-0.017 (0.015±0.001)**	Falcate	**Homeomorphous**	**0.071-0.142 (0.121±0.01)**

Table 2. Comparative measurements (in millimeters) of the present *Nybelinia exostigmi* sp. nov. and those described previously.

Parasite species	Order/Family	Host/Host group	Source	Accession no.	Sequence length (bp)	Percent identity (%)
Aporhynchus norvegicus	Trypanorhyncha / Aporhynchidae	*Etmopterus spinax*	GenBank	FJ572911.1	2028	92
Sagittirhynchus aculeatus	Trypanorhyncha / Gilquiniidae	*Centrophorus* sp.	GenBank	DQ642907.1	2015	92
Chimaerarhynchus rougetae	Trypanorhyncha / Gymnorhynchidae	*Squalus megalops*	GenBank	DQ642906.1	1842	92
Molicola sp.	Trypanorhyncha / Gymnorhynchidae	*Xiphias gladius*	GenBank	KX712332.1	1998	92
Gymnorhynchus isuri	Trypanorhyncha / Gymnorhynchidae	*Isurus oxyrinchus*	GenBank	DQ642909.1	1967	92
Hepatoxylon trichiuri	Trypanorhyncha / Hepatoxylidae	*Taractes rubescens*	GenBank	FJ572907.1	2026	92
Diphyllobothrium pacificum	Diphyllobothriidea / Diphyllobothriidae	*Homo sapiens*	GenBank	DQ925310.1	2182	92
Diplogonoporus balaenopterae	Diphyllobothriidea / Diphyllobothriidae	*Homo sapiens*	GenBank	KY552792.1	2022	92
Ligula intestinalis	Diphyllobothriidea / Diphyllobothriidae	*Podiceps cristatus*	GenBank	KY552785.1	2023	92
Digramma interrupta	Diphyllobothriidea / Diphyllobothriidae	*Hemiculter lucidus*	GenBank	DQ925308.1	2177	92
Dasyrhynchus giganteus	Trypanorhyncha / Dasyrhynchidae	*Caranx hippos*	GenBank	FJ788112.1	2000	92
Callitetrarhynchus gracilis	Trypanorhyncha / Lacistorhynchidae	*Sardinella brasiliensis*	GenBank	MG693781.1	1931	92
Proemotobothrium linstowi	Trypanorhyncha / Otobothriidae	*Rhynchobatus djiddensis*	GenBank	DQ642917.1	1947	92
Fossobothrium perplexum	Trypanorhyncha / Otobothriidae	*Anoxypristis cuspidata*	GenBank	DQ642914.1	1952	92
Otobothrium cysticum	Trypanorhyncha / Otobothriidae	*Peprilus triacanthus*	GenBank	FJ572926.1	2020	92
Calyptrobothrium sp.	Phyllobothriidea / Phyllobothriidae	*Torpedo nobiliana*	GenBank	KF685848.1	1978	92
Pedibothrium mounseyi	Tetraphyllidea / Onchobothriidae	*Nebrius ferrugineus*	GenBank	KF685803.1	1986	91
Calliobothrium cf. *verticillatum*	Tetraphyllidea / Onchobothriidae	*Mustelus canis*	GenBank	KF685812.1	1989	92
Phoreiobothrium lewinense	Tetraphyllidea / Onchobothriidae	*Sphyrna lewini*	GenBank	KF685830.1	1983	92
Anthocephalum ruhnkei	Rhinebothriidea	*Urogymnus granulatus*	GenBank	MH577332.1	1865	92
Dollfusiella geraschmidti	Trypanorhyncha / Eutetrarhynchidae	*Urolophus paucimaculatus*	GenBank	DQ642955.1	1922	93
Paroncomegas araya	Trypanorhyncha / Eutetrarhynchidae	*Potamotrygon motoro*	GenBank	DQ642963.1	1916	92
Ditrachybothridium macrocephalum	Diphyllidea / Echinobothriidae	*Apristurus laurussonii*	GenBank	DQ642903.1	1961	92
Tetrarhynchobothrium sp.	Trypanorhyncha / Tetrarhynchobothriidae	*Himantura gerrardi*	GenBank	DQ642960.1	1866	92
Rhinoptericola megacantha	Trypanorhyncha / Rhinoptericolidae	*Rhinoptera bonasus*	GenBank	DQ642954.1	1927	91
Nybelinia sphyrnae	Trypanorhyncha / Tentaculariidae	*Sphyrna lewini*	GenBank	DQ642953.1	1902	94
Nybelinia surmenicola	Trypanorhyncha / Tentaculariidae	---	GenBank	AB626625.1	837	96

Table 2. Continued...

Parasite species	Order/Family	Host/Host group	Source	Accession no.	Sequence length (bp)	Percent identity (%)
Tentacularia coryphaenae	Trypanorhyncha / Tentaculariida	*Selar crumenophthalmus*	GenBank	FJ572891.1	1975	93
Mixonybelinia lepturi	Trypanorhyncha / Tentaculariida	*Heteropriacanthus cruentatus*	GenBank	FJ572898.1	1935	94
Heteronybelinia estigmena	Trypanorhyncha / Tentaculariidae	*Carcharhinus limbatus*	GenBank	DQ642951.1	1939	95
Kotorella pronosoma	Trypanorhyncha / Tentaculariidae	*Dasyatis say*	GenBank	DQ642950.1	1909	93
Nybelinia africana	Trypanorhyncha / Tentaculariidae	*Lamiopsis temminckii*	GenBank	DQ642948.1	1950	94
Nybelinia queenslandensis	Trypanorhyncha / Tentaculariidae	*Carcharhinus melanopterus*	GenBank	AF287005.1	1940	96
Nybelinia indica	Trypanorhyncha / Tentaculariidae	*Heteropriacanthus cruentatus*	GenBank	FJ572894.1	1975	95
Nybelinia aequidentata	Trypanorhyncha / Tentaculariidae	*Rhinoptera neglecta*	GenBank	DQ642952.1	1914	95
Gyrocotyle rugosa	Gyrocotylidea / Gyrocotylidae	*Hydrolagus colliei*	GenBank	AF124455.2	1982	87

in the stomach, stomach wall, or the body cavity, these appear to be the preferred sites of the post-larvae. In addition, Palm (1995) proved that this corresponds to the site preference of the adults, which often infests the stomach of their elasmobranch final hosts.

The present trypanorhynch cestode was included in the genus *Nybelinia* Poche, 1926 on the basis of the scolex shape, tentacular apparatus, presence of the characteristic basal armature, and bulb ratio. A morphometric comparison between the described species seen here and other previously described *Nybelinia* species showed some similarities in the measurements of the different body parts. This *Nybelinia* species was similar to *N. queenslandensis* in having all characteristic features of the body, but all measurements including the hooks are substantially smaller; these, can be distinguished from *N. queenslandensis* in having an acraspedote scolex. The pars bothridialis extends to the posterior ends of the bulbs, and the hooks on one side of the tentacle are more sharply recurved than the hooks on the opposite surface of the tentacle. In *N. queenslandensis*, all hooks in the metabasal region are similarly shape. These differ from other species (*Nybelinia rougetcampanae*, *Nybelinia africana*, *Nybelinia jayapaulazariahi*, *Nybelinia scoliodoni*, *Nybelinia surmenicola*, *Nybelinia* sp., *Nybelinia bisulcata*, *Nybelinia mehlhorni* and *Nybelinia strongyla*) because of the smaller scolex.

Completely evaginated tentacles were reported in the present plerocercoid larvae and seen in the acraspedote scolex as compact thorn-shaped basal hooks lacking an anterior extension of the basal plate. Similar findings were seen in *N. scoliodoni*, *Nybelinia* sp., *Nybelinia schmidti*, *N. strongyla*, *N. surmenicola*, *Nybelinia victoria* and *N. bisulcata*. These features differ from *Nybelinia riseri* and *N. scoliodoni* in having incompletely evaginated tentacles. There is a close relationship between the present *Nybelinia* species and *Nybelinia lingualis*, *Nybelinia anthicosum*, *Nybelinia robusta*, *Nybelinia sakanariae*, *Nybelinia aequidentata*, *N. schmidti*, *Nybelinia southwelli*, *N. riseri*, *Nybelinia* sp., *N. scoliodoni*, *N. strongyla*, *N. lingualis*, *Nybelinia thyrsites*, *N. surmenicola*, *Nybelinia hemipristis*, *N. mehlhorni*, *Nybelinia pintneri*, and *Nybelinia indica* in having similar tentacular homeomorphous armature. In addition, it differs from them in having a smaller bulb ratio. However, it differs from *Nybelinia beveridgei* in having a homeoacanthous armature of heteromorphous metabasal hooks (on opposite tentacle surfaces) and a characteristic basal armature of homeomorphous hooks. It differs from *N. rougetcampanae* and *Nybelinia yamagutii* in both having heteromorphous hooks together with a characteristic basal armature. The hook shape is similar to *N. rougetcampanae*, *N. yamagutii*, *Nybelinia queenslandensis*, *Nybelinia aequidentata*, *N. africana*, *N. thyrsites*, *N. schmidti*, *N. hemipristis*, and *N. bisulcata* in having the apical hook form remaining similar to that seen on the metabasal part of the tentacle, and the hooks increase slightly in size. This study is the third report of *Nybelinia* species from fish from the Gulf of Suez, Red Sea, Egypt. The first report was by Morsy et al. (2013) as it reported the presence of *Nybelinia narinari* and *N. bisulcata* from *Pagrus pagrus*. The second report for Palm & Walter (1999) recognized adults of *N. africana* from *Carcharhinus melanopterus*.

This study identified a combination between the morphological features, and a molecular estimate was used to resolve the interrelationships within the order. This also, assessed conflicting hypotheses on the phylogeny and classification of different elasmobranch tapeworms from Trypanorhyncha. Strong nodal support was seen for some superfamilies, families, and genera as recognized within the recent classification by Palm (2004). The supported morphology and cladistics can offer larger systematic stability inside the order. This represents a major advance considering this cluster has been the foremost chaotic and confusing tapeworm groups until now (WARDLE & MCLEOD, 1952; CAMPBELL

Table 3. Estimates of Evolutionary Divergence between Sequences. The number of base substitutions per site from between sequences is shown. Analyses were conducted using the Tamura-Nei model. The analysis involved 37 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were a total of 321 positions in the final dataset. Evolutionary analyses were conducted in MEGA version 7.

Comparable samples	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36
1. MK084750.1 *Nybelinia exostigmi*																																				
2. DQ642953.1 *Nybelinia sphyrnae*	0.04																																			
3. DQ642948.1 *Nybelinia africana*	0.04	0.00																																		
4. AB626625 *Nybelinia surmenicola*	0.04	0.00	0.00																																	
5. AF287005.1 *Nybelinia queenslandensis*	0.04	0.00	0.00	0.00																																
6. FJ572894.1 *Nybelinia indica*	0.04	0.00	0.00	0.00	0.00																															
7. DQ642952.1 *Nybelinia aequidentata*	0.04	0.01	0.00	0.01	0.00	0.00																														
8. DQ642950.1 *Kotorella pronosoma*	0.04	0.00	0.00	0.00	0.00	0.00	0.00																													
9. DQ642951.1 *Heteronybelinia estigmena*	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.00																												
10. FJ572898.1 *Mixonybelinia lepturi*	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00																											
11. FJ572891.1 *Tentacularia coryphaenae*	0.04	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00																										
12. DQ642955.1 *Dollfusiella geraschmidti*	0.06	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02																									
13. DQ642954.1 *Rhinoptericola megacantha*	0.06	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02																								
14. DQ642963.1 *Paroncomegas araya*	0.05	0.01	0.02	0.01	0.02	0.02	0.02	0.02	0.02	0.02	0.01	0.02	0.02																							
15. DQ642960.1 *Tetrarhymchobothrium* **sp.**	0.06	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.01	0.02	0.01																						
16. FJ572907.1 *Hepatoxylon trichiuri*	0.06	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.02	0.03	0.02	0.02																					
17. KF685812.1 *Caliobothrium* **cf.** *verticillatum*	0.07	0.03	0.03	0.03	0.03	0.03	0.04	0.03	0.03	0.03	0.03	0.03	0.04	0.02	0.03	0.03																				
18. KX712332.1 *Molicola* **sp.**	0.07	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.02	0.04	0.03	0.02	0.01	0.02																			
19. DQ642909.1 *Gymnorhynchus isuri*	0.07	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.02	0.04	0.03	0.02	0.01	0.02	0.00																		
20. FJ788112.1 *Dasyrhynchus giganteus*	0.06	0.02	0.02	0.02	0.02	0.02	0.03	0.02	0.02	0.02	0.02	0.02	0.03	0.01	0.02	0.02	0.01	0.01	0.01																	
21. DQ642907.1 *Sagittirhynchus aculeatus*	0.07	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.04	0.03	0.03	0.02	0.02	0.01	0.01	0.01																
22. DQ642906.1 *Chimaerarhyynchus rougetae*	0.07	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.04	0.03	0.03	0.02	0.02	0.01	0.01	0.01	0.00															

Table 3. Continued...

Comparable samples	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36
23. DQ925310.2 ***Diphyllobothrium pacificum***	0.06	0.02	0.03	0.02	0.03	0.03	0.03	0.03	0.03	0.03	0.02	0.02	0.03	0.02	0.02	0.02	0.02	0.02	0.02	0.01	0.02	0.02														
24. DQ642917.1 ***Proemotobothrium linstowi***	0.06	0.02	0.02	0.02	0.02	0.02	0.03	0.02	0.02	0.02	0.02	0.02	0.03	0.01	0.02	0.02	0.01	0.01	0.01	0.00	0.01	0.01	0.01													
25. DQ642914.1 ***Fossobothrium perplexum***	0.07	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.02	0.03	0.02	0.02	0.02	0.02	0.01	0.01	0.01	0.02	0.02	0.02	0.01												
26. KF685830.1 ***Phoreiobothrium lewinense***	0.07	0.03	0.03	0.03	0.03	0.03	0.04	0.03	0.03	0.03	0.03	0.03	0.04	0.02	0.03	0.03	0.00	0.02	0.02	0.01	0.02	0.02	0.02	0.01	0.02											
27. DQ642903.1 ***Ditrachybothridium macrocephalum***	0.06	0.02	0.03	0.02	0.03	0.03	0.03	0.03	0.03	0.03	0.02	0.02	0.03	0.01	0.01	0.02	0.02	0.03	0.03	0.01	0.03	0.03	0.02	0.01	0.02	0.02										
28. KF685803.1 ***Pedibothrium mounseyi***	0.07	0.03	0.04	0.03	0.04	0.04	0.04	0.04	0.04	0.04	0.03	0.03	0.04	0.03	0.03	0.03	0.01	0.02	0.02	0.01	0.03	0.03	0.02	0.01	0.02	0.01	0.03									
29. FJ572911.1 ***Aporhynchus norvegicus***	0.06	0.03	0.03	0.03	0.03	0.03	0.04	0.03	0.03	0.03	0.03	0.03	0.04	0.03	0.03	0.02	0.02	0.01	0.01	0.02	0.00	0.00	0.02	0.02	0.02	0.02	0.01	0.02								
30. MG693781.1 ***Callitetrahynchus gracilis***	0.06	0.02	0.02	0.02	0.02	0.02	0.03	0.02	0.02	0.02	0.02	0.02	0.03	0.01	0.02	0.02	0.01	0.01	0.01	0.00	0.01	0.01	0.01	0.00	0.01	0.01	0.02	0.02	0.01							
31. KY552792.1 ***Diplogonoporus balaenopterae***	0.06	0.02	0.03	0.02	0.03	0.03	0.03	0.03	0.03	0.03	0.02	0.02	0.03	0.02	0.02	0.02	0.02	0.02	0.02	0.01	0.02	0.02	0.00	0.01	0.02	0.02	0.02	0.02	0.01	0.00						
32. KY552785.1 ***Ligula intestinalis***	0.06	0.02	0.03	0.02	0.03	0.03	0.03	0.03	0.03	0.03	0.02	0.02	0.03	0.02	0.02	0.02	0.02	0.02	0.02	0.01	0.02	0.02	0.00	0.01	0.02	0.02	0.02	0.02	0.01	0.02	0.02					
33. KF685848.1 ***Calyptrobothrium* sp.**	0.07	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.05	0.03	0.03	0.03	0.00	0.02	0.02	0.01	0.02	0.02	0.02	0.01	0.02	0.00	0.01	0.02	0.00	0.01	0.01	0.02				
34. FJ572926.1 ***Otobothrium cysticum***	0.06	0.02	0.03	0.02	0.03	0.03	0.03	0.03	0.03	0.03	0.02	0.02	0.03	0.02	0.01	0.02	0.01	0.01	0.01	0.00	0.0	0.02	0.01	0.00	0.00	0.01	0.02	0.02	0.01	0.00	0.00	0.02	0.01			
35. DQ925308.1 ***Digramma interrupta***	0.06	0.02	0.03	0.02	0.03	0.03	0.03	0.03	0.03	0.03	0.02	0.02	0.03	0.02	0.02	0.02	0.02	0.02	0.02	0.01	0.02	0.02	0.00	0.01	0.02	0.02	0.02	0.04	0.02	0.03	0.03	0.04	0.02	0.03		
36. AF124455.2 ***Gyrocotyle rugosa***	0.09	0.04	0.05	0.04	0.05	0.05	0.05	0.05	0.05	0.05	0.04	0.05	0.05	0.05	0.05	0.05	0.04	0.04	0.04	0.04	0.05	0.05	0.04	0.04	0.04	0.04	0.04	0.05	0.04	0.04	0.04	0.05	0.04	0.04	0.05	

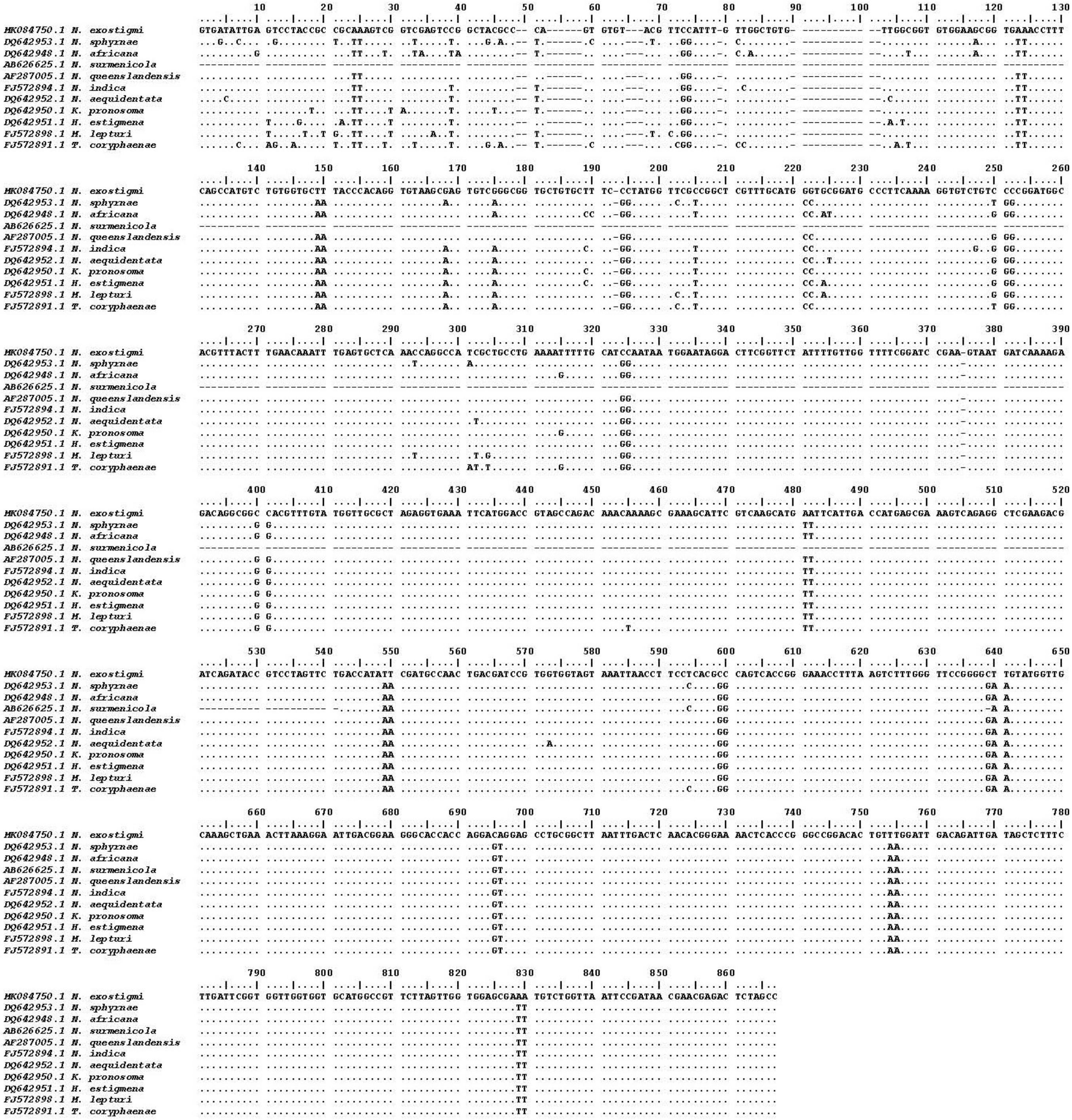


Figure 18. Sequence alignment of 18S rRNA of *N. exostigmi* sp. nov. with the most closely related tentaculariid species. (Only variable sites are shown. Dots represent the bases identical to those of the first sequences, and dashes indicate gaps).

& BEVERIDGE, 1994; PALM, 1995, 1997). This sentence with our analysis represented strong to moderate nodal support values. We demonstrated that Tentacularioidea, Gymnorhynchoidea, and Otobothrioidea were monophyletic, but that Eutetrarhynchoidea was paraphyletic. This may be due to insufficient taxon sampling, species descriptions, or a lack of resolution offered by SSU rRNA genes that require additional gene and taxon sampling to provide greater resolution. This hypothesis is consistent with Beveridge & Campbell (1994), Palm (1995), Beveridge et al. (1999), and Palm et al. (2009).

The trypanorhynchs are monophyletic according to the ordinal level relationships described for tapeworms Waeschenbach et al. (2007). This data agreed with our results. This unequivocal morphological evidence including the presence of a highly complex unique

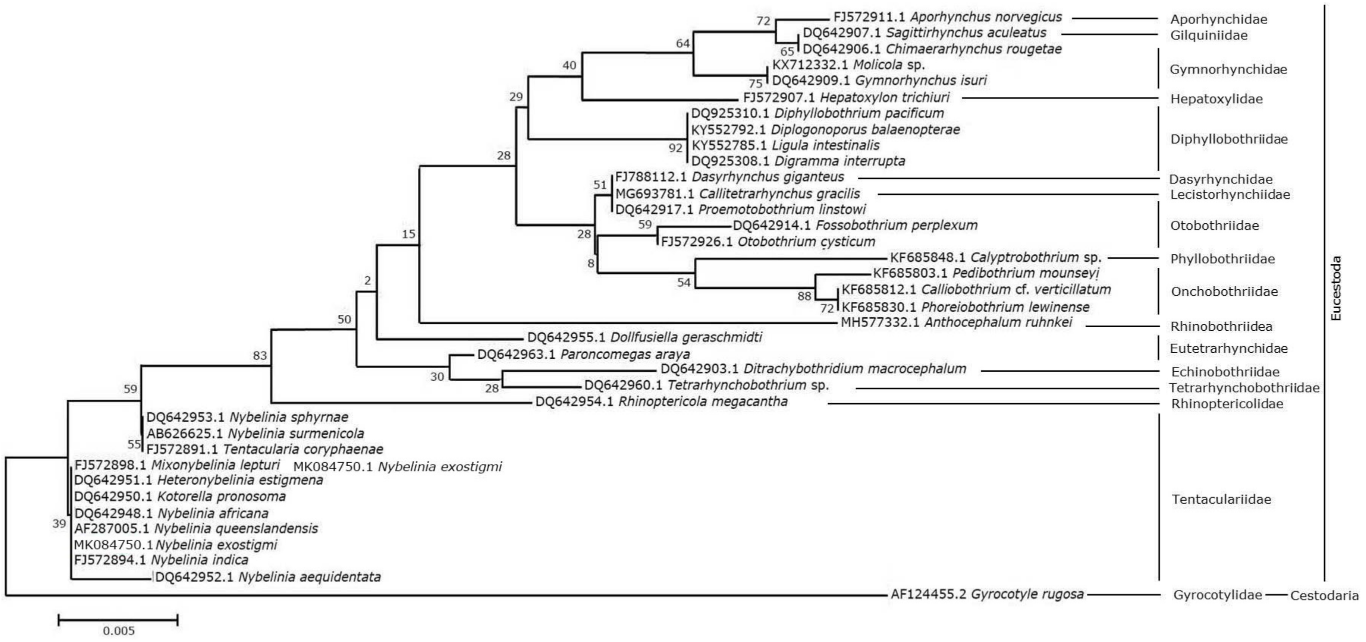


Figure 19. Evolutionary relationships of taxa. The evolutionary history was inferred using the Neighbor-Joining method. The optimal tree with a sum of branch length = 0.20056368 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) are shown next to the branches. The tree is drawn to scale with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Tamura-Nei method and are in the units of the number of base substitutions per site. The analysis involved 37 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were a total of 321 positions in the final dataset; evolutionary analyses were conducted in MEGA version 7.

tentacular armature system suggests that Trypanorhyncha is a monophyletic group. Our analysis supported a sister group relationship between the two orders of Trypanorhyncha and Diphyllidea because both groups are bothriate and hosted by elasmobranchs. This observation concurs with others including Hoberg et al. (1997) who demonstrated that the exact placement of Diphyllidea has long been problematic although most researchers have usually allied them with the Trypanorhyncha because both groups are bothriate and hosted by elasmobranchs. Ivanov & Hoberg (1999) and Olson et al. (2001) strongly supported the monophyly of Diphyllidea, whereas genus *Echinobothrium* was found to be paraphyletic. Caira et al. (2001) supported a sister group relationship between the two orders based on the morphological analyses. The current analysis showed that *Anthocephalum ruhnkei* within Rhinebothriinae is basal to the remaining tetraphyllidean taxa, the other members of Onchobothriidae are generally basal to those of Phyllobothriidae. This agrees with Mariaux (1998), Olson & Caira (1999), Caira et al. (2001), and Hoberg et al. (2001) who demonstrated the paraphyly of the order based on morphological and molecular levels.

Our analysis also showed that the genera of the Tentaculariidae (*Tentacularia* and *Nybelinia*) are united via uteri developing from an anlage considered here to be an apomorphic character. The arrangement of this family agrees with Palm (1995, 1997). In addition, this current study supports the taxonomic position of the sampled trypanorhynchid species as deeply embedded in a genus including the described species of *Nybelinia aequidentata* as a putative sister taxon. We concluded that the Narrowstripe cardinal fish *Nybelinia exostigma* of the current study are now considered to be a new host record for the recovered *Nybelinia exostigmi* within the Egyptian coastal waters of the Red Sea.

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