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Monostephanostomum mesospinosum (Digenea: Acanthocolpidae) Infecting the Coastal Trevally *Carangoides caeruleopinnatus* (Carangiformes: Carangidae) from the Marine Water off Saudi Arabia

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ABSTRACT

Background: Carangids, like any other fish, can become infected with a variety of external and internal parasites. Little information on parasite infestations in these fish species is known. This study aimed to provide light on parasites that infect caragnids in Saudi Arabia.

Methods: A total of 20 *Carangoides caeruleopinnatus* specimens were collected from the Red Sea (Saudi Arabia) and then examined for the presence of digenetic trematodes. Parasitic species were isolated and studied morphologically by using light microscopic examination and molecularly via the partial sequencing of *18S rRNA* and *28S rRNA* genes.

Result: Only one parasitic species has been identified. This parasite is morphologically and morphometric compatible with previously *Monostephanostomum mesospinosum*, identified from *Carangoides malabaricus* in VSK, BOB. Phylogeny revealed that these putative acanthocolpid species nested well within a clade clustering *Monostephanostomum* species, which along with morphological data, suggests it is a member of the genus *Monostephanostomum*. Query sequences showed identities of 97.99% and 97.98% for *18S* (EF506762.1.1) and *28S* rRNA (EF506763.1) of *Monostephanostomum nolani*, respectively. This study reflects the third account of this genus as endoparasitic taxa of various carangid fish, as well as providing novel DNA data for this species.

Key words: Acanthocolpidae, Digenea, Morphology, Phylogeny.

INTRODUCTION

Fish serve as hosts to a range of parasites that are taxonomically diverse and exhibit a wide variety of life cycle strategies (Alshehri *et al*., 2022). Trematode parasites are considered one of the major invariably endo-parasitic taxa of helminths (Pichelin and Cribb, 2001). Family Acanthocolpidae Lühe (1906) is a family of digenetic trematodes mainly infecting marine teleost fish. Different studies have been carried out to highlight the complex relationships within the Acanthocolpidae and the place of this family in the Digenea system (Bray *et al*., 2009).

This family includes the following genera *Pleorchis* Railliet, 1896, *Stephanostomum* Looss, 1899, *Neophasis* Stafford, 1904, *Acanthocolpus* Lühe, 1906, *Lepidauchen* Nicoll, 1913, *Tormopsolus* Poche, 1926, *Pseudolepidapedon* Yamaguti, 1938, *Manteria* Caballero, 1950, *Spinoplagioporus* Skrjabin and Koval, 1958, *Acaenodera* Manter and Pritchard, 1960, *Ophiotreminoides* Coil and Kuntz, 1960, *Pseudacaenodera* Yamaguti, 1965, *Stephanostomoides* Mamaev and Oshmarine, 1966, *Monostephanostomum* Kruse, 1979, Gissutrema Machida and Kamegai, 1997, *Venusicola* Bray and Cribb, 2000 and *Ningalooia* Bray and Cribb, 2007.

Genus *Monostephanostomum* comprises nine valid species according to WoRMS (2021), which were: *Monostephanostomum manteri* Kruse, 1979, *Monostephanostomum krusei* Reimer, 1983, *Monostephanostomum roytmani* (Parukhin, 1974) Reimer, 1983, *Monostephanostomum yamagutii* Ramadan, 1984, *Monostephanostomum gazzae* (Shen, 1990) Bray and Cribb, 2002, *Monostephanostomum georgianum* Bray and Cribb, 2002, *Monostephanostomum loossi* (Pandey and Tewari, 1984) Saoud, Nahhas, Al Kuwari and Ramadan, 2002, *Monostephanostomum mesospinosum* (Madhavi, 1976)

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Bray and Cribb, 2002 and *Monostephanostomum nolani* Bray and Cribb, 2007.

The identification and taxonomic tools for the above-mentioned species depend mainly on their morphological, morphometric as well as ultrastructure characteristics which are considered as primary key features for their classification, except *Monostephanostomum nolani* that infecting *Plectropomus leopardus* and confirmed molecularly by Bray and Cribb (2007) *via* small subunit (SSU) and large subunit (LSU) of ribosomal rDNA gene sequences. Recently, DNA technology has been proven to be an alternative to addressing taxonomic parasites problems (Abdel-Gaber *et al*., 2021).

Carangidae forms one of the largest families of bony fish with worldwide distribution. Parasitic fauna of the Coastal trevally (*Carangoides caeruleopinnatus*), from Saudi Arabia are poorly known. Therefore, this study proved for the first time the digenetic parasitic infections from this fish species in Saudi Arabia's Red Sea Coasts.

MATERIALS AND METHODS

Twenty specimens of the coastal trevally *Carangoides caeruleopinnatus* Rüppell, 1830 (F: Carangidae) were collected from commercial fishermen on the Red Sea coast in Jeddah, Saudi Arabia. Fish were brought to the Lab and identified using the rules of the website fishbase.org. Following that, fish specimens were necropsied and their organs were examined. Internal organs were transferred to Petri dishes with 0.65% saline and examined under a stereo-dissecting microscope (Nikon SMZ18, NIS ELEMENTS software) for detection of parasite infections. According to Bush *et al*. (1997), infection levels for the parasite species obtained were reported using prevalence and mean intensity.

Flukes were removed and preserved in 70% ethanol for morphological analysis or stored directly in absolute ethanol for DNA extraction. Semichon's acetocarmine was used to stain the whole mounts (Al Quraishy *et al*., 2019). After dehydrating the specimens in alcohol gradients, clearing them in clove oil, mounting them using Canada balsam and incubating at 60°C for 24 hrs to drive the air bubbles according to Schmidt (1992). Using a Leica DM 2500 microscope (NIS ELEMENTS software), specimens and relevant structural features were examined and photographed at various magnifications. Using a camera lucida, drawings were made to scale. Measurements were reported in millimeters.

Genomic DNA was extracted using Qiagen DNeasy tissue kit © (Hilden, Germany) according to the manufacturer's instructions. For parasite identification, partial *18S rRNA* and *28S rRNA* genes were amplified using PCR. Primers for *18S rRNA* were designed by Indaryanto *et al*. (2015) and those for *28S rRNA* were designed by Lee *et al*. (2007). BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, USA) was used to sequence PCR products via ABI PRISM 310 Genetic Analyzer (Applied Biosystems, CA). BLASTn compared sequences in NCBI database to find the most similar ones. BioEdit 7.0.5.3 was used to edit sequences. Sequences derived from *18S rRNA*, *28S rRNA* genes and those received from GenBank were aligned using ClustalW. Maximum Likelihood (ML) in MEGA 7.0 was used to construct phylogenetic trees with 1000 replicates.

RESULTS AND DISCUSSION

Fish are considered one of the important sources of animal protein (de Boer et al., 2020). Several studies have been conducted about the parasites of the Red Sea fish with digenea as the largest sector of endoparasitic species (Cribb *et al*., 2001). In total, 20 specimens of *Carangoides caeruleopinnatus* were examined, 13 (65%) of which were infected in the intestinal region with one acanthocolpid species having morphological features corresponding with the genus *Monostephanostomum*. The parasite's mean intensity per fish host does not exceed 5. The recovered acanthocolpid species identified as *Monostephanostomum mesospinosum* inhabited *C. caeruleopinnatus* for the first time.

Description (Fig 1)

Body elongated, parallel-sided over most of its length, and measured 6.102-7.923 (6.870) long × 0.201-0.243 (0.231) wide. Tegument covered with spines that increased gradually in size. Forebody contains eyespot pigment. A single uninterrupted row of enlarged 18 circum-oral spines surrounds oral sucker. The oral sucker measured 0.070-0.079 (0.075) long × 0.091-0.103 (0.098) wide. Prepharynx long, narrow, and measured 0.590-0.721 (0.611) long. Pharynx pyriform and measured 0.163-0.184 (0.172) long × 0.082-0.094 (0.086) wide. Just before ventral sucker, esophagus measured 0.078-0.098 (0.082) long and divided into two intestinal caeca. Ventral sucker has a spherical shape, pre-equatorial, and measured 0.118-0.147 (0.128) long × 0.124-0.168 (0.141) wide. The sucker ratio 1:1.2–1.4. Forebody is about 7% of body length. Caeca broad, long, opened into excretory bladder forming uroproct.

Testes two, oval, tandem and separated by vitelline follicles and found in posterior half of the body. The anterior testis measured 0.267-0.351 (0. 298) long × 0.173-0.185 (0.174) wide, while the posterior one was 0.291-0.371 (0.311) long × 0.189-0.184 (0.184) wide. Post-testicular region long. Cirrus-sac long, intercaecal that extends slightly more than halfway between ovary and ventral sucker, joining metraterm slightly posterior to ventral sucker. The cirrus sac measured 0.960-1.936 (1.721) long × 0.061-0.079 (0.065) wide. Seminal vesicle tubular. Pars prostatica short, sinuous, and bordered by prostate gland cells. Ejaculatory duct long, lined with knob-like protuberances. Genital atrium located anteriorly to ventral sucker and opened with a genital pore. Ovary rounded, pre-testicular, and measured 0.115-0.124 (0.119) long × 0.113-0.125 (0.119) wide. There is a uterine seminal receptacle. Uterus pre-ovarian, intercaecal, and runs dorsally to cirrus sac. Metraterm opened into genital atrium. Eggs large, operculated, and measured 0.049-0.054 (0.052) long × 0.020-0.025 (0.023) wide. Vitellaria of two lateral fields of numerous vitelline follicles that extend from base of cirrus-sac to posterior end of body, interrupted at level of ovary and testes. Excretory vesicle I-shaped and opened by a terminal excretory pore.

Remarks

At the morphological and morphometric level, the current acanthocolpid species is compatible with other *Monostephanostomum* species by possessing all the species' characteristics exceptions. Kruse (1979) established the genus *Monostephanostomum* for the digenetic trematodes belonging to the family Acanthocolpidae. Parasites within this genus are characterized by relative sizes of suckers, presence of a single row of circumoral spines, small forebody, location of ventral sucker in middle or anterior third of body, forward and then backward loop of uterus, long metraterm, oval and contiguous gonads, anterior position of genital pore to ventral sucker, and distribution of vitelline follicles, caeca opened via uroproct, elongated cirrus sac, weekly developed pars prostatica, long ejaculatory duct, and excretory vesicle I-shaped reached ovary level. The present parasite species resembles the previously recorded species of Madhavi's (1976) and Madhavi and Bray (2018) for *M. mesospinosum* from *C. malabaricus* in VSK, BOB, especially in the long slender body and presence of 18 circumoral spines in uninterrupted rows. Our specimen falls within the range of measurements for different body parts suggested by Madhavi (1976), but with different localities and species for fish hosts. This species is distinguished from other species of genus by: smaller body size and number of circumoral spines of *M. yamaguttii* (12-16), *M. manteri* (16-18), *M. nolani* (17-20), *M. krusei* (17), *M. gazzae* (23), and *M. loossi* (32). These results have been obtained via comparison with other data from Ramadan (1984) and Bray and Cribb (2007).

There was a noticeable difference from our specimen that recorded by Ramadan (1984) by presence of two spines rows in *M. geogianum*, first ventral one with 18-20 (19), and second dorsal with 3-5 smaller spines, vitellarium is restricted to hind body and interruptions in vitelline fields at gonads level, and cirrus sac reaches only to about middle of ventral sucker to ovary distance. While, it differs from *M. manteri* which has a claviform body, continuous row of circum-oral spines, oval pharynx, longer post-testicular region, vitellarium reaches into forebody, and cirrus sac overlaps ovary; this agreed with Bray and Cribb (2007). Additionally, some differences were recorded with *M. yamaguttii* by Ramadan (1984) which has an elongate pharynx, smaller cirrus sac, and vitellaria that extends anteriorly only to posterior margin of ventral sucker. Extension of vitellarium into forebody was observed in *M. nolai* versus that in recovered species. Characteristic feature for ventral hiatus in circum-oral spine row was recorded in

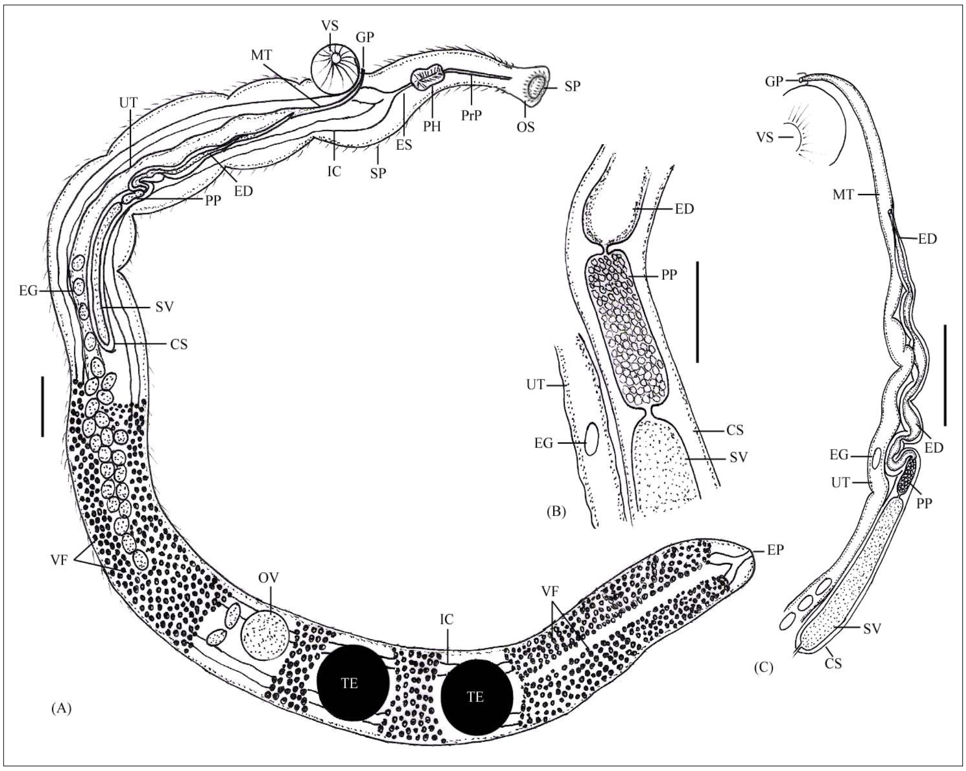

Fig 1: Line drawing for *Monostephanostomum mesospinosum*. Scale bar= 200 µm. (A) Whole-mount preparation. (B and C) Terminal genitalia and ventral sucker. Note: CS: Cirrus sac; ED: Ejaculatory duct; EG, egg(s); EP: Excretory pore; ES: Esophagus; GP: Genital pore; IC: Intestinal ceca; MT: Metraterm; OS: Oral sucker; OV: Ovary; PH: Pharynx; PP: Pars prostatica; PrP: Pre-pharynx; SP: Spine(s); SV: Seminal vesicle; TE: Testes; UT: Uterus; VS: Ventral sucker.

Table 1: Taxa used for the *18S rRNA* sequence analysis for *Monostephanostomum mesospinosum.*

Parasite species	Order, family	Host species	% Identity	% GC content
EF506762.1 Monostephanostomum nolani	Opisthorchiida, Acanthocolpidae	*Carangoides plagiotaenia*	97.99	51.1
DQ248209.1 Stephanostomum pristis	Opisthorchiida, Acanthocolpidae	*Phycis phycis*	93.98	50.9
DQ248214.1 Stephanostomum cf. cestillium	Opisthorchiida, Acanthocolpidae	*Zeus faber*	93.55	51
DQ248213.1 Stephanostomum cestillium	Opisthorchiida, Acanthocolpidae	*Lophius piscatorius*	93.55	51.1
EF506760.1 Stephanostomum sp.	Opisthorchiida, Acanthocolpidae	*Plectropomus leopardus*	93.54	50.8
DQ248212.1 Stephanostomum bicoronatum	Opisthorchiida, Acanthocolpidae	*Sciaena umbra*	93.41	51.1
DQ248208.1 Stephanostomum gaidropsori	Opisthorchiida, Acanthocolpidae	*Gaidroopsarus mediterraneus*	93.41	51
DQ248207.1 Stephanostomum tantabiddii	Opisthorchiida, Acanthocolpidae	*Carangoides fulvoguttatus*	93.12	50.6
DQ248211.1 Stephanostomum minutum	Opisthorchiida, Acanthocolpidae	*Uranoscopus scaber*	92.69	51.1
DQ248210.1 Stephanostomum interruptum	Opisthorchiida, Acanthocolpidae	*Menticirrhus americanus*	92.69	50.9
AJ224885.1 Stephanostomum baccatum	Opisthorchiida, Acanthocolpidae	*Eutrigla gurnardus*	92.69	50.9
DQ248206.1 Stephanostomum cf. uku	Opisthorchiida, Acanthocolpidae	*Aprion virescens*	91.98	51.3
DQ248203.1 Pleorchis uku	Opisthorchiida, Acanthocolpidae	*Aprion virescens*	92.84	50.7
MW730771.1 Neophasis oculata	Opisthorchiida, Acanthocolpidae	*Myoxocephalus scorpius*	92.98	51.1
KY512768.1 Stephanostomoides tenuis	Opisthorchiida, Acanthocolpidae	-	91.98	49.3
DQ248204.1 Tormopsolus orientalis	Opisthorchiida, Acanthocolpidae	*Seriola dumerli*	91.54	50.7
AB521800.1 Euryhelmis costaricensis	Opisthorchiida, Heterophyidae	*Martes melampus*	91.14	51.8
HQ832624.1 Metagonimus miyatai	Opisthorchiida, Heterophyidae	*Plecoglossus altivelis*	90.86	51.6
KR703279.1 Brachycladium goliath	Plagiorchiida, Brachycladiidae	*Balaenoptera acutorostrata*	93.91	51.7
AY245702.1 Dexiogonimus ciureanus	Opisthorchiida, Heterophyidae	-	90.86	51.4
MF077366.1 Stellantchasmus falcatus	Opisthorchiida, Heterophyidae	*Homo sapiens*	90.40	50.9
MF980222.1 Ascocotyle longa	Opisthorchiida, Heterophyidae	*Mesocricetus auratus*	90.71	51.9
MW361240.1 Cryptocotyle lingua	Opisthorchiida, Heterophyidae	*Littorina littorea*	90.70	51.6
KX815126.1 Haplorchis taichui	Opisthorchiida, Heterophyidae	*Homo sapiens*	90.57	51.8
JF823987.1 Opisthorchis viverrini	Opisthorchiida, Opisthorchiidae	*Homo sapiens*	91.84	51.4
AY222121.1 Amphimerus ovalis	Opisthorchiida, Opisthorchiidae	*Apalone mutica*	91.12	51.8
AJ287542.1 Mitotrema anthostomatum	Opisthorchiida, Cryptogonimidae	*Cromileptes altivelis*	91.54	50.6
AJ287572.1 Rubenstrema exasperatum	Plagiorchiida, Omphalometridae	*Crocidura leucodon*	90.97	50.4
EU196355.1 Choanocotyle platti	Plagiorchiida, Choanocotylidae	*Chelodina rugosa*	90.24	51.1
OL413039.1 Glypthelmins Africana	Plagiorchiida, Plagiorchiidae	*Hyperolius viridiflavus*	90.83	50.3
AY222157.1 Opisthioglyphe ranae	Plagiorchiida, Telorchiidae	*Rana arvalis*	90.69	50
MT218366.1 Tremiorchis ranarum	Plagiorchiida, Brachycoeliidae	*Euphlyctis cyanophlyctis*	90.41	50
FJ211255.1 Dicrogaster contracta	Plagiorchiida, Haploporidae	*Liza ramado*	90.26	51.9
MN700961.1 Skrjabinopsolus nudidorsalis	Plagiorchiida, Deropristidae	-	91.70	50.4
MT303882.1 Liliatrema skrjabini	Plagiorchiida, Liliatrematidae	-	91.01	51.5
KM258668.1 Pholeter gastrophilus	Plagiorchiida, Troglotrematidae	*Tursiops truncatus*	91.57	51.9

Table 2: Taxa used for the *28S rRNA* sequence analysis for *Monostephanostomum mesospinosum.*

Parasite species	Order, family	Host species	% Identity	% GC content
EF506763.1 Monostephanostomum nolani	Opisthorchiida, Acanthocolpidae	*Carangoides plagiotaenia*	97.98	53.6
DQ248227.1 Stephanostomum cf. cestillium	Opisthorchiida, Acanthocolpidae	*Zeus faber*	92.21	54
DQ248226.1 Stephanostomum cestillium	Opisthorchiida, Acanthocolpidae	*Lophius piscatorius*	92.21	54
DQ248225.1 Stephanostomum bicoronatum	Opisthorchiida, Acanthocolpidae	*Sciaena umbra*	91.92	54.4
DQ248222.1 Stephanostomum pristis	Opisthorchiida, Acanthocolpidae	*Phycis phycis*	91.22	53.1
DQ248221.1 Stephanostomum gaidropsori	Opisthorchiida, Acanthocolpidae	*Gaidropsarus mediterraneus*	91.20	53.7
DQ248220.1 Stephanostomum tantabiddii	Opisthorchiida, Acanthocolpidae	*Carangoides fulviguttatus*	89.64	55
MW115577.1 Stephanostomum cf. uku	Opisthorchiida, Acanthocolpidae	-	88.94	55.1
DQ248223.1 Stephanostomum interruptum	Opisthorchiida, Acanthocolpidae	*Menticirrhus americanus*	88.90	53.9
DQ248224.1 Stephanostomum minutum	Opisthorchiida, Acanthocolpidae	*Uranoscopus scaber*	88.76	53.9
AY222256.1 Stephanostomum baccatum	Opisthorchiida, Acanthocolpidae	*Eutrigla gurnardus*	88.52	53.6
LC269095.1 Oschmarinella macrorchis	Plagiorchiida, Brachycladiidae	*Mesoplodon carlhubbsi*	87.37	55.6
EF506761.1 Stephanostomum sp.	Opisthorchiida, Acanthocolpidae	*Plectropomus leopardus*	90.63	54.4
KR703279.1 Brachycladium goliath	Plagiorchiida, Brachycladiidae	*Balaenoptera acutorostrata*	87.37	51.7
MW730791.1 Neophasis anarrhichae	Opisthorchiida, Acanthocolpidae	*Buccinum undatum*	87.39	55.2
MW730783.1 Neophasis oculata	Opisthorchiida, Acanthocolpidae	*Neptunea despecta*	87.39	55.2
KM258671.1 Campula oblonga	Plagiorchiida, Brachycladiidae	*Phocoena phocoena*	87.23	55.5
DQ248217.1 Tormopsolus orientalis	Opisthorchiida, Acanthocolpidae	*Seriola dumerli*	87.23	53.7
KJ820760.1 Pseudolepidapedon balistis	Opisthorchiida, Acanthocolpidae	*Balistes capriscus*	86.96	53
MN295500.1 Synthesium tursionis	Plagiorchiida, Brachycladiidae	*Sotalia guianensis*	86.96	55.2
MZ265266.1 Nasitrema sunameri	Plagiorchiida, Brachycladiidae	*Neophocaena asiaeorientalis*	86.96	55.8
MT153593.1 Orthosplanchnus arcticus	Plagiorchiida, Brachycladiidae	*Serripes groenlandicus*	86.84	55.1
AY222255.1 Zalophotrema hepaticum	Plagiorchiida, Brachycladiidae	*Zalophus californianus*	86.80	55.2
MT103410.1 Palaeorchis incognitus	Plagiorchiida, Monorchiidae	*Lithoglyphus naticoides*	85.88	53.2
MT928353.1 Lissorchis cf. gullaris	Plagiorchiida, Monorchiidae	*Ictiobus niger*	85.59	51.6
DQ248216.1 Pleorchis uku	Opisthorchiida, Acanthocolpidae	*Aprion virescens*	85.53	54.5
MN700998.1 Skrjabinopsolus nudidorsalis	Plagiorchiida, Deropristidae	*Acipenser ruthenus*	84.98	53.6
KJ658287.1 Helicometroides longicollis	Plagiorchiida, Monorchiidae	*Diagramma labiosum*	84.59	53.2
EF411200.1 Sigmapera cincta	Plagiorchiida, Plagiorchiidae	*Emydura kreffti*	84.59	51.8
EU196358.1 Choanocotyle nematoides	Plagiorchiida, Choanocotylidae	*Emydura kreftii*	84.57	53.4
JF820592.1 Telorchis bonnerensis	Plagiorchiida, Telorchiidae	*Chelydra serpentina*	84.45	52.1
HQ900670.1 Paragonimus kellicotti	Plagiorchiida, Troglotrematidae	-	84.43	53.2
AF184253.1 Astiotrema monticellii	Plagiorchiida, Plagiorchiidae	-	84.43	54.2

M. nolai, *M. krusi*, and *M. roytmani*, this is consistent with Bray and Cribb (2007). In addition, it distinguished by smaller egg size from *M. yamaguttii* (0.068-0.074 × 0.036-0.052), *M. geogianum* (0.075-0.086 × 0.039-0.050), *M. manteri* (0.080-0.094 × 0.035-0.048), and *M. krusei* (0.062-0.064 × 0.039-0.044), these measurements regarding studies of Ramadan (1984), Bray and Cribb (2002, 2007). Regarding sucker ratio by Bray and Cribb (2007), the current species has a smaller ratio than *M. krusi* (1:1.8-2.7), *M. gazzae* (1:2.0-2.5), and *M. nolani* (1:1.6-3.1). *Monostephanostomum* species occupied many hosts belonging to different families (Lethrinidae, Arripidae, and Carangidae) in different geographical locations (Egypt, Australia and Tasmania) (Madhavi, 1976, Kruse, 1979, Reimer, 1983, Ramadan, 1984, Bray and Cribb, 2002, Bray and Cribb, 2007, Madhavi and Bray, 2018). Our findings represent the third record for this acanthocolpid species in carangid fish.

Molecular analysis

Identification and taxonomic characterization of different parasitic taxa are primarily based on their morphological and ultrastructural features, which are considered mainly criteria for their classification (Indaryanto *et al.,* 2015). Molecular biological tools are recently considered sensitive and advanced tests for parasite taxonomy (Chaudhary *et al.*, 2016). In our molecular analyses, one *Monostephanostomum* species was confirmed to belong to other Opisthorchiida taxa, especially Acanthocolpidae, based on *18S* and *28S rRNA* sequencing.

18S rRNA gene

PCR amplification was successfully carried out to amplify a 697 bp with 51.5% GC content and submitted to NCBI database with accession number OL770106.1. The query sequence was compared with *18S rRNA* sequences of other digenean taxa available in the GenBank database (Table 1). Phylogenetic tree was represented by two orders Opisthorchiida and Plagiorchiida within Digenea (Fig 2). First major clade of the dendrogram was subdivided into two lineages, the first one containing taxa of Opisthorchiida and represented by Acanthocolpidae with strong support value (82), the second lineage supported taxa of two suborders of Plagiorchiida: Xiphidiata, Lepocreadiata, and remaining taxa of Acanthocolpidae. Second clade contains remaining taxa of Opisthorchiata (represented by Cryptogonimidae, Heterophyidae, Opisthordiidae), and other plagiorchiids

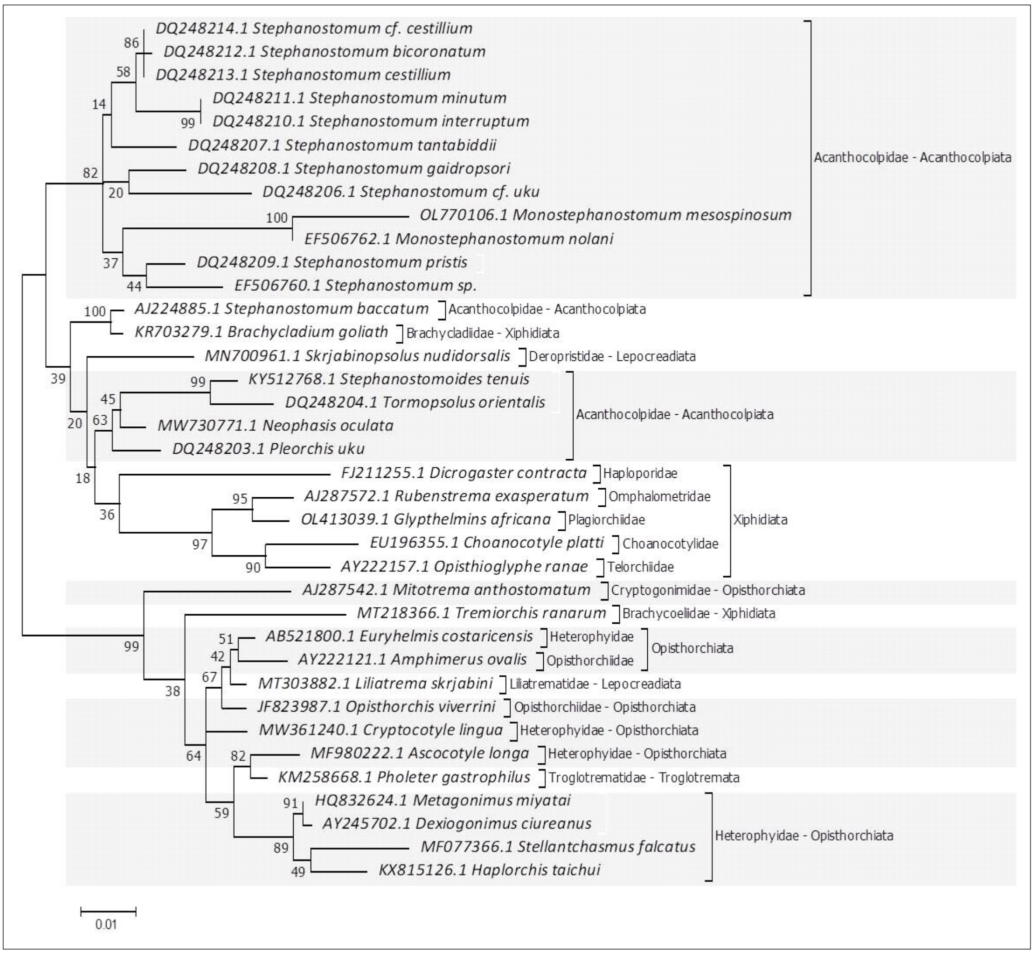

Fig 2: Molecular analysis using ML method based on Tamura-Nei model for 18S rRNA gene.

belonging to Xiphidiata, Lepocreadiata, and Troglotremata. Among Acanthocolpidae, the maximum identity (97.99%) with the lowest divergent value was recorded between the present species and *M. nolani*. Monophyly of *Monostephanostomum* species was supported sister group generally to *Stephanostomum*. Query sequence is well aligned and placed within Opisthorchiida with special reference to Acanthocolpidae. Sequence representing *M. mesospinosum* grouped with strong bootstrap value (100) with sequence represented by EF506762.1 for the previously deposited sequence for *M. nolani* parasitizes the barcheek trevally *Carangoides plagiotaenia* collected from Lizard Island, Great Barrier Reef (Australia), as expected based on sequence comparisons.

28S rRNA gene

The sequence analysis revealed a band size of 693 bp with 53.5% GC content that submitted to NCBI database with accession number OL773371.1. The query sequence was compared with *28S rRNA* sequences of other related digenean available in the GenBank database (Table 2). Phylogenetic tree was constructed with two major clades (Fig 3), the first one clustered taxa of the family Acanthocolpidae within the subclass Digenea. Whereas, the second clade contains taxa of four orders Xiphidiata (represented by Brachycladiidae, Plagiorchiidae, Choanocotylidae, and Telorchiidae), Monorchiata (represented by Monorchiidae and Lissorchiidae), Lepocreadiata (represented by Deropristidae), and Troglotremata (represented by Troglotrematidae). Among Acanthocolpidae, the maximum identity (97.98%) with the lowest divergent value was recorded between the present digenean species and *Monostephanostomum nolani*. Monophyly of *Monostephanostomum* species was supported sister group generally to *Stephanostomum*. Query sequence *M. mesospinosum* is well aligned and placed within Opisthorchiida with special reference to Acanthocolpidae with a close relationship in a well-supported taxon (100) with the previously recorded *M. nolani* (EF506763.1) parasitizes the barcheek trevally *Carangoides plagiotaenia* collected from Lizard Island, Great Barrier Reef (Australia), as expected based on sequence comparisons.

A close relatedness to *M. nolani* (EF506762.1 and EF506763.1) was determined based on molecular similarities of those described by Bray and Cribb (2007) infecting the carangid, the barcheek trevally, *Carangoides*

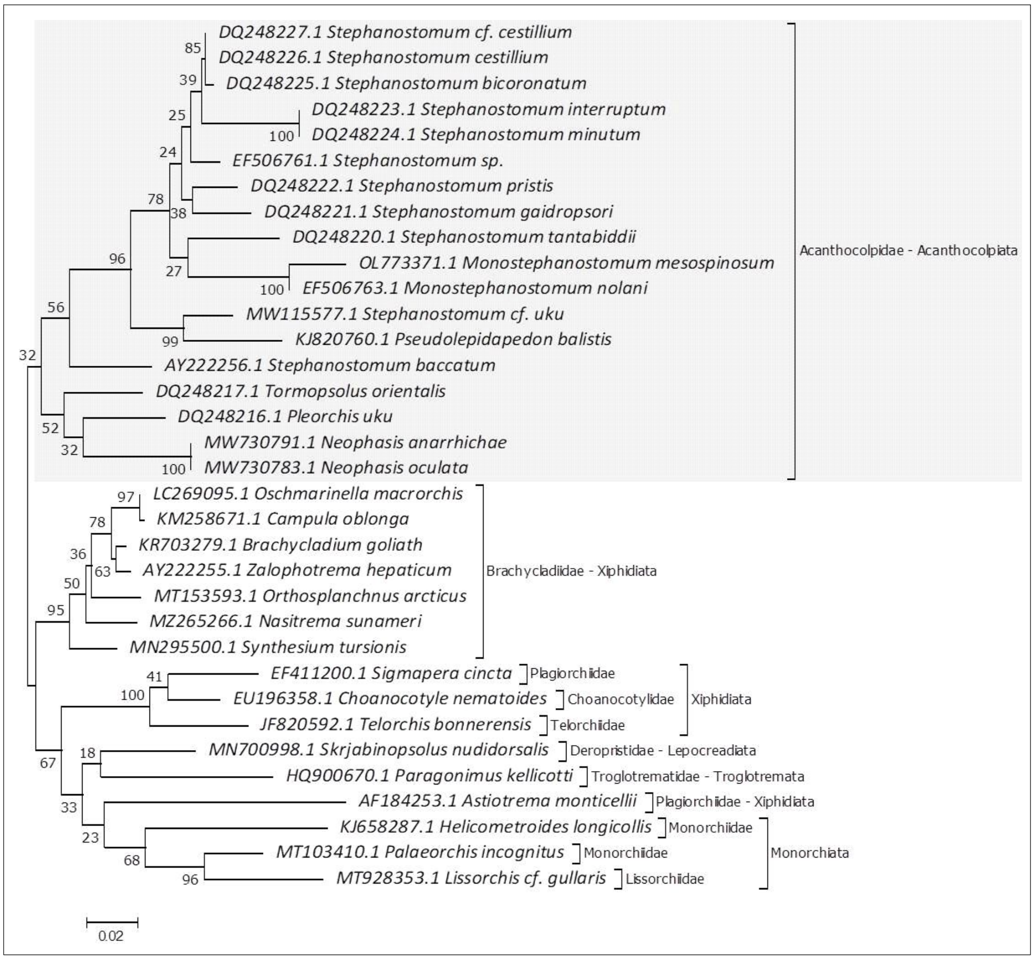

Fig 3: Molecular analysis using ML method based on Tamura-Nei model for 28S rRNA gene.

plagiotaenia from Lizard Island, Great Barrier Reef. This is consistent with Bray *et al.* (2009) who reported that complete SSU and partial LSU rDNA sequence data enhance significantly the chance for an accurate differentiation between plagiorchiids. Thus, the nuclear RNA analyses herein showed that the *Monostephanostomum* was consistent with the morphological taxa of trematodes within the same genus. Our phylogeny assured morphological evidence for a close relation between *Stephanostomum* and *Monostephanostomum* to be clustered together in the well-supported clade with consideration for the number and arrangement of circum-oral spines for both genera, this agreed with previous studies by Bray and Cribb (2002, 2007) and Bray *et al.* (2007). The current dendrograms demonstrated Acanthocolpidae polyphyly, which is in agreement with Abdel-Gaber et al. (2020) who reported the same issue due to presence of relation with taxa included within Xiphidiata and Lepocreadiata. Furthermore, the current study confirms the position of the Acanthocolpidae in the superfamily Brachycladioidea along with Brachycladiidae, as consistent with Bray *et al.* (2009) and Bakhoum *et al.* (2015).

CONCLUSION

It could be concluded that valuable information on the natural occurrence of acanthocolpid species identified as *M. geogianum* was given in the present study. In addition, to our knowledge, this indicates the third report of the genus *Monostephanostomum* in the carangid fish, as well as, providing information about the new host and locality records of *C. caeruleopinnatus* off the Red Sea, Saudi Arabia. It is proposed that future studies include more parasite samples and different genes to provide more knowledge of this parasite.

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Conflict of interest

The authors declare that they have no conflict of interest regarding the content of this article.

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