

Molecular evidence and morphological aspects of *Transversotrema licinum*, *Phyllodistomum hoggettae*, and re-description of *Gyuliauchen volubilis* (Digenea) from the Red Sea

Ali Al-Ghamdi¹, Kareem Morsy^{2,3*}, Saad Dajem¹, Ali Shati¹, Mohammed Al-Kahtani¹, Ahmed Baiomy³, Ahmed Ezzat^{1,4}

¹ Department of Biology, College of Science, Al Baha University, Al Baha, Saudi Arabia; ² Department of Biology, College of Science, King Khalid University, Abha, Saudi Arabia; ³ Department of Zoology, Faculty of Science, Cairo University, Cairo, Egypt; ⁴ Department of Theriogenology, Faculty of Veterinary Medicine, South Valley University, Qena, Egypt.

Article Info

Article history:

Received: 13 October 2018
Accepted: 26 December 2018
Available online: 15 March 2021

Keywords:

Digenea
Light microscopy
Molecular study
Morphology
Red Sea

Abstract

During a recent survey on digenean parasites infecting marine fish captured from water located along the Red Sea off Gizan Coasts, Saudi Arabia, thirty-two (29.09%) out of 110 fish were naturally infected with three different species of digenean parasites. The taxonomical status of the recovered worms was confirmed by light microscopy and molecular analysis. *Transversotrema licinum* infecting the Haffara seabream *Rhabdosargus haffara* possessed a transversely elongated body with eyespots and the anterior body margin with delineated velum along with its entire breadth. Oral sucker was absent, and eyespots were paired anterior to or at the level of ventral sucker. *Phyllodistomum hoggettae* from the urinary bladder of the Arabian Nagil *Plectropomus areolatus* was differentiated by its elongated body, narrow anterior end widened as going posteriorly with prominent marginal undulations in the hindbody. *Gyuliauchen volubilis* infecting the marbled spinefoot *Siganus rivulatus*, was with fleshy body, conical, tapered anteriorly, convex dorsally, and concave ventrally. Prepharynx was very long and convoluted and had occupied the greater part of the anterior half of the body. Multiple sequence alignment and phylogeny were based on maximum likelihood analysis for the ITS-2 and 28S rDNA for *Transversotrema licinum* and *Phyllodistomum hoggettae* were constructed, respectively. They were compared to species within their families and further deposited into the Genbank under accession numbers MG 722711 and MG722710, respectively.

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Introduction

The Red Sea is the habitat of over 1,000 invertebrate species and 200 soft and hard corals and is the most northern tropical sea.¹ Fishery in this sea area is of significant socio-economic importance to Red Sea countries in national food security and income generation for rural communities.¹ Of the Red Sea fish captured from Gizan coasts, Arabian Nagil *Plectropomus areolatus* (Serranidae), is used as ornamental fish and seafood in fish markets and restaurants. The haffara seabream *Rhabdosargus haffara* (Sparidae) inhabitants shallow waters, mainly around coral reefs, and over sandy or mud-sandy bottoms.² The marbled spinefoot *Siganus rivulatus* (Siganidae) is a herbivorous fish found in schools over

sandy substrates. These fish are of the most economically important marine species of the Red sea and considered as high value food fish species where most of the people in the Gizan region, Saudi Arabia, depends on them as food. Studies on helminth parasites of the Red Sea fish in Saudi Arabia tend to be limited and despite the importance of marine life and seafood resources in the Red Sea of Gizan coasts there are no reports regarding digenean parasites infecting fish inhabiting this region.² Many parasitological studies should be carried out in this area to explore the biogeographical range of marine digeneans, their richness and abundance through morphological and molecular methods. The second internal transcribed spacer (ITS2) rDNA and 28S have been demonstrated to be useful for species level differentiation in the study of the digenea

*Correspondence:

Kareem Morsy. PhD
Department of Biology, College of Science, King Khalid University, Abha, Saudi Arabia | Department of Zoology, Faculty of Science, Cairo University, Cairo, Egypt
E-mail: kareemmorsy@sci.cu.edu.eg



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which are highly conserved at species level.² *Transversotrema*.³ (Transversotrematidae) comprises digeneans that infect the epidermal surfaces beneath and between overlapping cycloid and ctenoid scales of freshwater and marine bony fish.⁴ The genus currently includes 25 accepted species. Members of Gorgoderidae, including *Phyllodistomum* sp., are parasitic flukes with a worldwide distribution that typically inhabit the urinary bladder of amphibians, marine and freshwater fishes.⁵ They are one of the most specious groups within class Digenea with more than 100 described species. *Gyliauchen* is the type genus of Gyliauchenidae which is a small family within the superfamily Lepocreadioidea.⁶ Members of this family constitute a group of intestinal trematodes, which are almost entirely found in certain herbivorous teleost fishes (e.g. siganids and acanthurids). In the present survey on helminth parasites infecting Red Sea fishes collected from Gizan coasts, Saudi Arabia, three different species of digenean parasites were isolated from three different fish species. The recovered parasites were described morphologically by light microscopy. Also, the taxonomy and phylogeny of two of the recovered parasites were confirmed by sequence analyses of their DNA.

Materials and Methods

Specimen collection and preparation. A total number of one hundred and ten fish specimens were collected from water locations along the Red Sea at Gizan coasts (16.8894° N, 42.5706° E) during the period from June 2017 to April 2018. These were the Haffara seabream *Rhabdosargus haffara* (Family: Sparidae, n = 30), the Arabian Nagil *Plectropomus areolatus* (Family: Serranidae, n = 43), and the marbled spinefoot *Siganus rivulatus* (Family: Siganidae, n = 37). Fish were transported alive or in an insulated icebox to the parasitology laboratory, College of Science, King Khalid University, Abha, Saudi Arabia. They were identified and classified.⁵ Fish were dissected, and the entire digestive system was removed and placed in a petri dish filled with a physiological saline 0.90% and examined for parasites under a compact

Greenough stereomicroscope (Stemi 305; ZEISS, Oberkochen, Germany). Parasitic worms were removed from the fish using fine forceps, immediately fixed, and preserved in 10.00% neutral buffered formalin for light microscopy and 70.00% ethanol for molecular analysis.

Morphological analysis. Following fixation, worms were stained overnight in acetocarmine, dehydrated in ethanol series, cleared in clove oil, and permanently mounted on glass slides using Canada balsam. Photomicrographs of stained whole mounted specimens were made with a research photomicroscope supplied with a built-in camera (BX53F; Olympus, Tokyo, Japan). Drawings were made with the aid of a drawing tube. Measurements were in micrometers, otherwise stated. Identification of the recovered parasites was according to the keys of trematode parasites of vertebrates.⁶

Molecular analysis. In general, the total genomic DNA (gDNA) was extracted from 70.00% ethanol preserved samples using the DNeasy tissue kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. Amplification was carried out on a MJ Research PTC-150 thermocycler. The PCR steps and profiles were shown in Table (1). Amplicons were either gel-excised using a QIAquick gel extraction kit (Qiagen) or purified directly using QIAquick™ PCR purification kit (Qiagen) following the standard manufacturer-recommended protocol. Cycle-sequencing from both strands was carried out on an ABI 3730 DNA Analyser, Big Dye using ABI BigDye™ chemistry (version 1.1; Applied Biosystems, Inc., Carlsbad, USA).

Phylogenetic analysis. Sequence identity for the recovered data was checked using the Basic Local Alignment Search Tool (BLAST). The sequence trimming for the congeneric species recovered was carried out by Bioedit (version 7.2.5; Ibis Therapeutics, Carlsbad, USA). Sequence alignment was done by the CLUSTAL W Multiple Sequence Alignment Program (version 2.0; UCD, Dublin, Ireland). The phylogenetic trees were constructed using the MEGA 6 program based on Maximum likelihood analysis. *Crusziella Formosa* and *Nagmia* sp. were employed as outgroups during tree construction for *Transversotrema* sp and *Phyllodistomum* sp. respectively.

Table 1. PCR cycle settings and primers used in the molecular analysis of *T. licinum* (MG 722711) and *P. hoggettae* (MG722710).

Parasite	Region	Cycles	Primers ⁷⁻¹⁰	PCR profile and thermal settings ¹¹
<i>T. licinum</i>	ITS-2 rDNA	1		95.00 °C for 3 min* 45.00 °C for min** 72.00 °C for 90 sec†
		4	F: 3S (5'-GGT ACC GGT GGA TCA CGT GGC TAG TG-3') GA1 (5'-AGA ACA TCG ACA TCT TGA AC-3') (sequencing) R: ITS2.2 (5'-CCT GGT TAG TTT CTT TTC CTC CGC-3')	95.00 °C for 45 sec* 50.00 °C for 45 sec** 72.00 °C for 90 sec† 95.00 °C for 20 sec* 52.00 °C for 20 sec**
		30		72.00 °C for 90 sec, 72.00 °C for 5 min†
<i>P. hoggettae</i>	28S rDNA	1	F: DigI2 (5'-AAG CAT ATC ACT AAG CGG-3') R: L0 (5'-ATC CTG AG (AG) GAA ACT TCG-3')	95.00 °C for 3 min* 94.00 °C for 30 sec**
		36		72.00 °C for 2 min; 72.00 °C 10 min†

* denaturation; ** annealing; † extension.

Results

Thirty-two (29.09%) out of 110 fish were found to be naturally infected with three different species of digenean parasites. The infection site included the urinary bladder of *P. areolatus*, beneath the host scales of *R. haffara* and the intestine of *S. rivulatus*.

Morphology

Family Transversotrematidae.

Transversotrema licinum.¹²

Diagnosis (n = 7): The body was transversely elongated with two eyespots. Anterior body margin was relatively straight with delineated velum (flap), (Figs. 1A and 2A, Table 2)

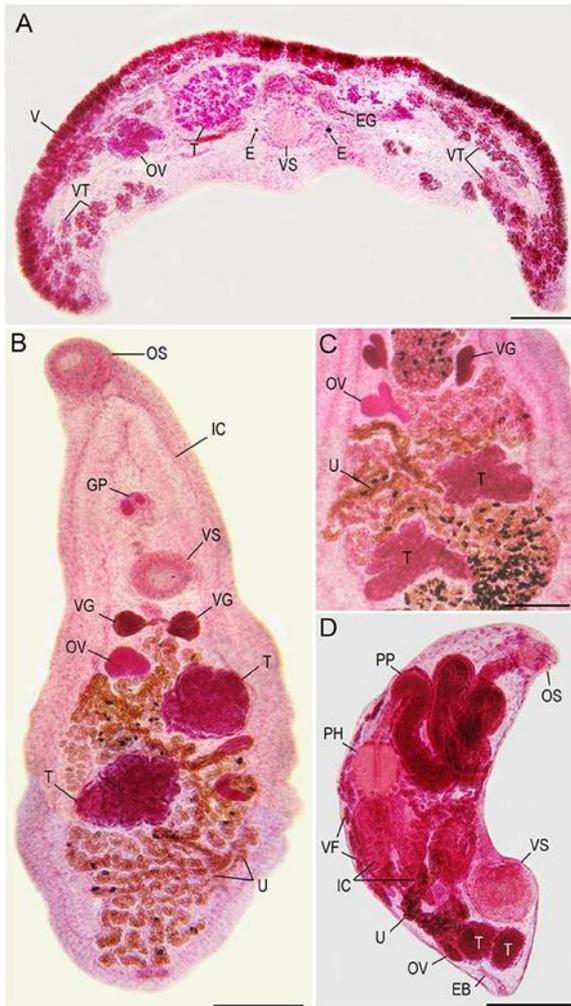


Fig. 1. Photomicrographs of whole-mount preparations of **A)** *Transversotrema licinum* (ventral view), Scale bar = 110 µm. **B)** *Phyllodistomum hoggettae*, Scale bar = 60.00; **C)** High magnification, Scale bar = 80.00 µm, and **D)** *Gyliochaen volubilis* (ventrolateral view), Scale bar = 45.00 µm. E: Eyespots, EB: Excretory bladder, EG: Eggs, IC: Intestinal caeca, OS: Oral sucker, OV: Ovary, PH: Pharynx, PP: Prepharynx, T: Testes, U: Uterus, V: Velum, VF: Vitelline follicles, VG: Vitelline glands, VS: Ventral sucker, VT: Vitellaria.

Its entire breadth and the demonstrable oral sucker was absent. Eyespots were paired and equal in size. The ventral sucker was spherical. There were two symmetrical and deeply lobed testes. The ovary was smooth to slightly irregular, sinistral, and anterior to the left testis. The uterus was extended between the testes and anterior limbs of the caeca. Vitellarium occupied space between cyclocoel and body margin and was extended medially to a relative level of the eyespots, comprised large follicles with spheroid, ova-like structures, and putative vitelline material. The seminal receptacle was absent. Eggs were large. The excretory vesicle was short, tubular with a terminal excretory pore.

Taxonomic summary

Host: *Rhabdosargus haffara* (Family: Sparidae)

Locality: Coasts of Gizan along the Red Sea, Saudi Arabia.

Infection site: Beneath scales.

Prevalence: Seven fish out of 30 (23.30%) were naturally infected.

Voucher material: Three slides of acetocarmine stained whole mounts (acc. no. KKU. BIO18.1-3) were deposited in the parasite collection of the parasites section, Biology Department Museum, College of Science, King Khaled University, Saudi Arabia.

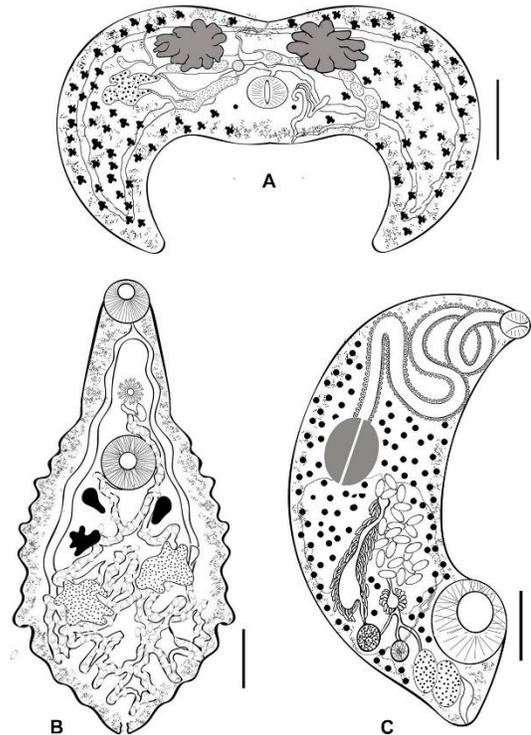


Fig. 2. **A)** *Transversotrema licinum*, a line diagram, ventral view. Scale bar = 160 µm. **B)** *Phyllodistomum hoggettae*, a line diagram, Holotype ventral view. Scale bar = 60.00 µm. **C)** *Gyliochaen volubilis*, a line diagram (fully gravid specimen, ventrolateral view). Scale bar = 30.00 µm.

Table 2. Collected data for some transversotrematid taxa used in the phylogenetic analysis of *T. licinum* recorded in the present study*.

Species	Body length	Body width	Eyespots apart	Ventral sucker	Testes width (µm)	Ovary width (µm)	Eggs size	Eggs in uterus	Vitelline follicles	Host	Family
<i>T. manteri</i>	0.33-0.46	1.29- 1.91	0.09-0.21	0.01-0.02	L:0.11-0.36 R:0.10-0.37	0.02-0.60	0.09-0.14× 0.03-0.07	0-6	21-47	<i>Caesio cunning</i>	Caesionidae
<i>T. borboleta</i>	0.27-0.91	0.94-2.67	0.07-0.26	0.02-0.04	L:0.45-0.95 R:0.09-0.44	0.02-0.56	0.06-0.12× 0.03-0.05	-	15-35	<i>Chaetodon lunula</i>	Chaetodontidae
<i>T. witenbergi</i>	0.34-0.49	1.08-1.91	0.10-0.18	0.01-0.04	L:0.08-0.25 R:0.07-0.24	0.20-0.90	0.07-0.08× 0.03-0.06	1-8	26-45	<i>Pterocaesio marri</i>	Caesionidae
<i>T. atkinsoni</i>	0.43-0.88	1.76-2.78	0.15-0.29	0.04-0.07	L:0.12-0.50 R:0.11-0.50	0.20-0.90	0.08-0.13× 0.03-0.09	0-4	48-72	<i>Scolopsis monogramma</i>	Nemipteridae
<i>T. carmenae</i>	0.32-0.92	0.88-2.17	0.13-0.22	0.02-0.04	L:0.12-0.38 R:0.11-0.43	0.40-0.78	0.06-0.11× 0.03-0.05	1-4	18-46	<i>Scolopsis margaritifera</i>	Nemipteridae
<i>T. damsella</i>	0.69-0.96	1.50-2.41	0.19-0.24	0.03-0.06	L:0.10-0.14 R:0.10-0.13	0.09-0.18	0.06-0.11× 0.03-0.07	15	55	<i>Abudefduf bengalensis</i>	Pomacentridae
<i>T. espanola</i>	0.54-0.74	1.60-2.51	0.12-0.19	0.03-0.06	L:0.17-0.48 R:0.16-0.45	0.14-0.70	0.09-0.12× 0.03-0.05	1-11	31-47	<i>Lutjanus carponotatus</i>	Lutjanidae
<i>T. cardinalis</i>	0.49-1.60	1.47-4.08	0.09-0.35	0.03-0.07	L:0.11-0.86 R:0.12-0.88	0.24-0.76	0.08-0.13× 0.03-0.04	1-6	10-46	<i>Lutjanus bohar</i>	Lutjanidae
<i>T. fusilieri</i>	0.41-0.64	1.29-2.22	0.07-0.21	0.02-0.02	L:0.12-0.34 R:0.13-0.17	0.30-0.80	0.07-0.13× 0.05-0.07	2-6	21-46	<i>Caesio cunning</i>	Lutjanidae
<i>T. elegans</i>	1.11-1.57	2.43-3.31	0.20-0.29	0.00-0.01	L:0.27-0.46 R:0.29-0.48	0.17-0.28	0.07-0.11× 0.04-0.06	8-56	37-59	<i>Choerodon cephalotes</i>	Labridae
<i>T. cutmorei</i>	0.39-0.67	0.93-1.92	0.11-0.18	0.04-0.06	L:0.09-0.25 R:0.06-0.25	0.60-0.80	0.05-0.09× 0.03-0.08	-	26	<i>Upeneus tragula</i>	Mullidae
<i>T. tragorum</i>	0.43-1.08	1.13-2.77	0.13-0.22	0.01-0.07	L:0.10-0.53 R:0.09-0.52	0.17-0.30	-	3-12	33-62	<i>Parupeneus indicus</i>	Mullidae
<i>T. licinum</i>	0.45-0.70	0.74-2.20	0.06-0.24	0.06-0.09	L:0.30-0.47 R:0.20-0.49	0.08-0.16	0.08-0.12× 0.03-0.05	2-28	0-42	<i>Acanthopagrus australis</i>	Scorpaenidae
<i>T. licinum*</i>	0.13-0.45	0.82-0.89	0.013-0.019	0.04-0.08	L:0.10-0.16 R:0.12-0.18	0.09-0.16	0.03-0.07× 0.02-0.04	3-8	33-62	<i>Rhabdosargus haffara</i>	Sparidae

Measurements in (mm), otherwise stated.

Family Gorgoderidae.

Phyllodistomum hoggettae.¹³

Diagnosis (n = 10): Body was elongated, with narrow anterior end widened as going posteriorly with 1:3 length to width ratio. The oral sucker was opened subterminally. The oral sucker was opened subterminally. The ventral sucker was almost equal in size to the oral sucker. Intestinal bifurcation was midway between oral and ventral suckers. Testes were slightly lobed and oblique in the mid-body. The ovary was entire, sinistrally posterior to ventral sucker and anterior to testes. Vitelline lobes were complete and almost round. The left lobe was immediately anterior to the ovary and posterior to the ventral sucker. The uterus was intracaecal in the hindbody as extensive coils were extended just posterior to intestinal caeca ends. The excretory vesicle was tubular, and the excretory pore was terminal, (Figs. 1B, C and 2B, Table 3).

Taxonomic summary

Host: *Plectropomus areolatus* (Family: Serranidae)

Locality: Coasts of Gizan along the Red Sea, Saudi Arabia.

Infection site: Urinary bladder.

Prevalence: Fifteen fish out of forty-three (34.90%) were naturally infected.

Voucher material: Five slides of acetocarmine stained whole mounts (acc. no. KKU. BIO18.4-8) were deposited in the parasite collection of the parasites section, Biology

Department Museum, College of Science, King Khaled University, Saudi Arabia.

Family: Gyliauchenidae

Gyliauchen volubilis.¹⁴

Diagnosis (n = 10): Body was fleshy, robust, conical, tapered anteriorly, convex dorsally, and concave ventrally with small tail-like protuberance posterodorsal to the acetabulum. The oral sucker was pyriform. Prepharynx was very long, convoluted, and occupied the more significant part of the anterior half of the body. The pharynx was elongated, close to an intestinal bifurcation. Ventral sucker was well developed. Oesophagus was highly coiled with an esophageal bulb round. There were two oval, symmetrical, dorsal to ventral sucker testes. The ovary was spherical, submedian between right testis and cirrus sac. The uterus was relatively short. Eggs were thin-shelled, and yellowish. Vitelline follicles were small, irregular, numerous, and extended in lateral fields. The excretory vesicle was claviform and excretory pore postero-terminal, (Figs. 1D and 2C, Table 4).

Taxonomic summary

Host: *Siganus rivulatus* (Family: Siganidae)

Locality: Coasts of Gizan along the Red Sea, Saudi Arabia.

Infection site: Intestine

Prevalence: Ten fish out of 37 (27.00%) were naturally infected.

Table 3. Collection data for some gorgoderid specimens compared to *P. hoggettae* recovered in the current study*.

Species	Body length	Length to width ratio	Marginal undulations	Width oral/ventral sucker	Uterus	Vitellarium	Host	Family
<i>P. acceptum</i>	7.20	2.06	Absent	0.95	Moderately extra-caecal	Deeply lobed	<i>Symphodus cinereus</i>	Labridae
<i>P. unicum</i>	5.40	1.97	Absent or indistinct	0.95	Mainly intra-caecal	Entire	<i>Epinephelus tauvina</i>	Serranidae
<i>P. lewisi</i>	2.40	2.28	Strong	1.26	Mainly intra-caecal	Strongly lobed	<i>Strongylura strongylura</i>	Belonidae
<i>P. pacificum</i>	2.20-3.10	3.33	Absent or indistinct	0.95	Mainly intra-caecal	Slightly lobed	<i>Carangoides equula</i>	Carangidae
<i>P. leilae</i>	6.30	3.44	Absent or indistinct	0.88	Mainly intra-caecal	Strongly lobed	<i>Hipposcarus harid</i>	Scaridae
<i>P. trinectes</i>	0.82-2.10	1.72	Absent or indistinct	101.20	Moderately extra-caecal	Slightly lobed	<i>Trinectes maculatus</i>	Achiridae
<i>P. parukhini</i>	7.50-13.00	2.11	Absent	1.46	Mainly intra-caecal	Entire	<i>Rachycentron canadum</i>	Rachycentridae
<i>P. pomacanthi</i>	3.28	2.20	Absent or indistinct	0.71	Mainly intra-caecal	Strongly lobed	<i>Pomacanthus arcuatus</i>	Pomacanthidae
<i>P. crenilabri</i>	2.70-3.04	2.83	Absent	1.03	Mainly intra-caecal	Deeply lobed	<i>Symphodus tinca</i>	Labridae
<i>P. lancea</i>	12.00	2.90	Absent	0.87	Mainly intra-caecal	Strongly lobed	<i>Auxis thazard</i>	Scombridae
<i>P. borisbychowskyi</i>	4.56	1.84	Absent	0.82	Strongly extra-caecal	Entire	<i>Gibbonsia metzi</i>	Clinidae
<i>P. mirandai</i>	2.27-3.23	1.99	Absent	2.60-2.80	Moderately extra-caecal	Strongly lobed	<i>Sphoeroides annulatus</i>	Tetraodontidae
<i>P. mamaevi</i>	4.40	1.67	Strong	0.91	Mainly intra-caecal	Slightly lobed	<i>Mastacembelus armatus</i>	Labridae
<i>P. scrippsi</i>	3.93-4.58	1.45	Absent	0.80	Strongly extra-caecal	Strongly lobed	<i>Semicossyphus pulcher</i>	Labridae
<i>P. sobolevi</i>	6.50	1.81	Absent or indistinct	0.89	Mainly intra-caecal	Deeply lobed	<i>Mullus barbatus</i>	Mullidae
<i>P. tongaatense</i>	2.90-3.22	1.73	Absent	1.10-1.25	Moderately extra-caecal	Strongly lobed	<i>Dichistius multifasciatus</i>	Dichistiidae
<i>P. thalassomu</i>	6.40-10.80	3.08	Absent	1.00	Mainly intra-caecal	Slightly lobed	<i>Cheilinus abudjubbe</i>	Labridae
<i>P. vaili</i>	1.49-2.30	2.85-3.85	Strong	0.74-1.12	Mainly intra-caecal	Entire	<i>Mulloidichthys vanicolensis</i>	Mullidae
<i>P. hoggettae</i>	3.48-4.76	2.39-3.60	Strong	1.12-1.71	Mainly intra-caecal	Entire	<i>Plectropomus leopardus</i>	Serranidae
<i>P. hoggettae*</i>	0.32-0.48	2.96	Strong	1.08	Mainly intra-caecal	Entire	<i>Plectropomus areolatus</i>	Serranidae

Measurements in (mm).

Voucher material: Three slides of acetocarmine stained whole mounts (acc. no. KKU. BIO18.9-11) in addition to 70.00% preserved samples in vials were deposited in the parasite collection of the parasites section, Biology Department Museum, College of Science, King Khaled University, Saudi Arabia.

Molecular study

Transversotrema licinum.¹²

The ITS-2 rDNA sequences recovered in the present study were aligned with sequences from other transversotrematid species available from GenBank. It was observed that the present transversotrema were monophyletic relative to the transversotrematid genera. Three strongly supported clades of *Transversotrema* species were demonstrated, and clade A included transversotrematid species infecting mullids. Clade B included

labrids, lethrinids, scarids, and clade C, which included species infecting a wide range of families. The latter clade included two subclades, clades Ci and Cii. Clade Ci included those species infecting Lutjanidae, Nemipteridae while Clade Cii was almost called the *T. licinum* clade and included three genotypes of *T. licinum* to which the present *T. licinum* was the sister taxon, in addition to *T. witenbergi* and *T. borboleta* infecting some caesionines and chaetodontids. *Transversotrema licinum* was differed from Clade A species by 41.00 - 66.00 bp and from clade B species by 25.00 - 50.00 bp. Within *T. licinum* clade (Cii), the present sequences were differed by eight bp from the same species recorded previously from different hosts, and from *T. borboleta*, *T. witenbergi* by 30, 36 bp, respectively. The sequencing data were deposited in the Genebank under accession number MG 722711, (Fig. 3).

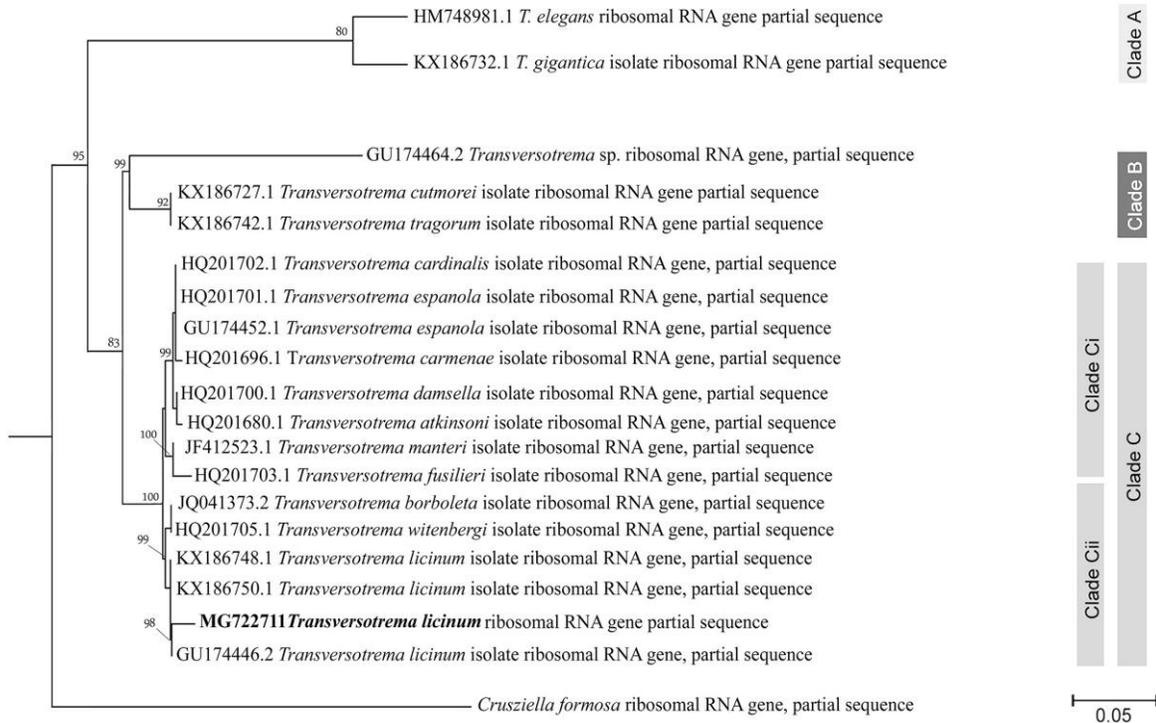


Fig. 3. A dendrogram shows the relationship between *Transversotrema licinum* (Acc. no. MG722711) and other transversotrematids taxa. The tree was constructed using the MEGA 6 program. Maximum likelihood bootstrap support values above the nodes.

Table 4. Comparative measurements between *G. volubilis* (present study)* and some species of family Gyliuacheniidae.

Species	Body length	Body width	Oral sucker	Pharynx	Ventral sucker	Testes size	Ovary size	Eggs size	Host	Family
<i>G. pomacentri</i>	2.50	1.13	0.36×0.37	0.21×0.15	0.69×0.61	L: 0.10×0.23 R: 0.14×0.17	0.17× 0.20	0.06-0.08× 0.03-0.04	<i>Pomacentrus philippinus</i>	Pomacentridae
<i>G. parapapillatus</i>	1.43- 0.21	0.40-0.83	0.20-0.25× 0.14-0.21	0.23-0.34 × 0.17-0.32	0.28-0.36× 0.22-0.31	0.14-0.30	0.04- 0.19	0.06-0.07× 0.030-0.035	<i>Siganus rivulatus</i>	Siganidae
<i>G. zancli</i>	1.70	0.65	0.20×0.16	0.26×0.15	0.33×0.30	L: 0.26×0.18 R: 0.21×0.18	0.14× 0.11	0.05-0.08× 0.03-0.05	<i>Zanclus cornutus</i>	Zanclidae
<i>Gyliuachen</i> sp.	1.33	0.40-0.45	0.12-0.14	0.16-0.19× 0.12-0.13	0.30×0.25	L: 0.13×0.11 R: 0.12×0.10	0.06	0.07×0.03	<i>Siganus spinus</i>	Siganidae
<i>Apharyngogyliuachen</i> sp.	2.03	1.05	0.31×0.24	-	0.46	L: 0.20×0.10 R: 0.13×0.10	0.16× 0.13	-	<i>Scarus ghobban</i>	Scaridae
<i>P. magnacetabulum</i>	1.98- 3.20	610-950	-	0.16-0.25× 0.12-0.19	0.49-0.75	0.241-0.383× 0.177-0.281	-	0.06-0.08× 0.04-0.05	<i>Siganus luridus</i>	Siganidae
<i>G. volubilis</i>	1.81	0.80	0.13×0.15	-	0.29×0.31	L: 0.24×0.13 R: 0.25×0.13	0.13× 0.10	0.091-0.098× 0.055-0.059	<i>Siganus rivulatus</i>	Siganidae
<i>G. volubilis</i> *	0.11- 0.35	0.04-0.08	0.008-0.019× 0.009-0.025	0.01-0.02× 0.011-0.016	0.020-0.030	0.01-0.04× 0.01-0.02	0.01- 0.02	0.02-0.04	<i>Siganus rivulatus</i>	Siganidae

Measurements in (mm).

Phyllodistomum hoggettae.¹³

Sequence alignment for the data obtained from 28S rDNA analysis of the digenean parasite isolated from the host examined yielded 584bp aligned with 17 species of gorgoderid taxa. Maximum likelihood analysis of all datasets resulted in the gorgoderids forming a monophyletic clade. Two strongly supported clades of *Phyllodistomum* species were demonstrated, clade A (subclades Ai and Aii) included species of

Phyllodistomum infecting several families of fishes and mussels, while clade B included the parasite species infecting Serranidae, Caragnidae, and mullidae hosts. Within this clade, *P. hoggettae* (KF013191.1) isolated from *Plectropomus leopardus* (Serranidae) showed a percentage of sequence identity 98.00% to the sequence data obtained for the parasite recovered herein, which was a sister taxon to *P. pacificum* (MG845599.1)

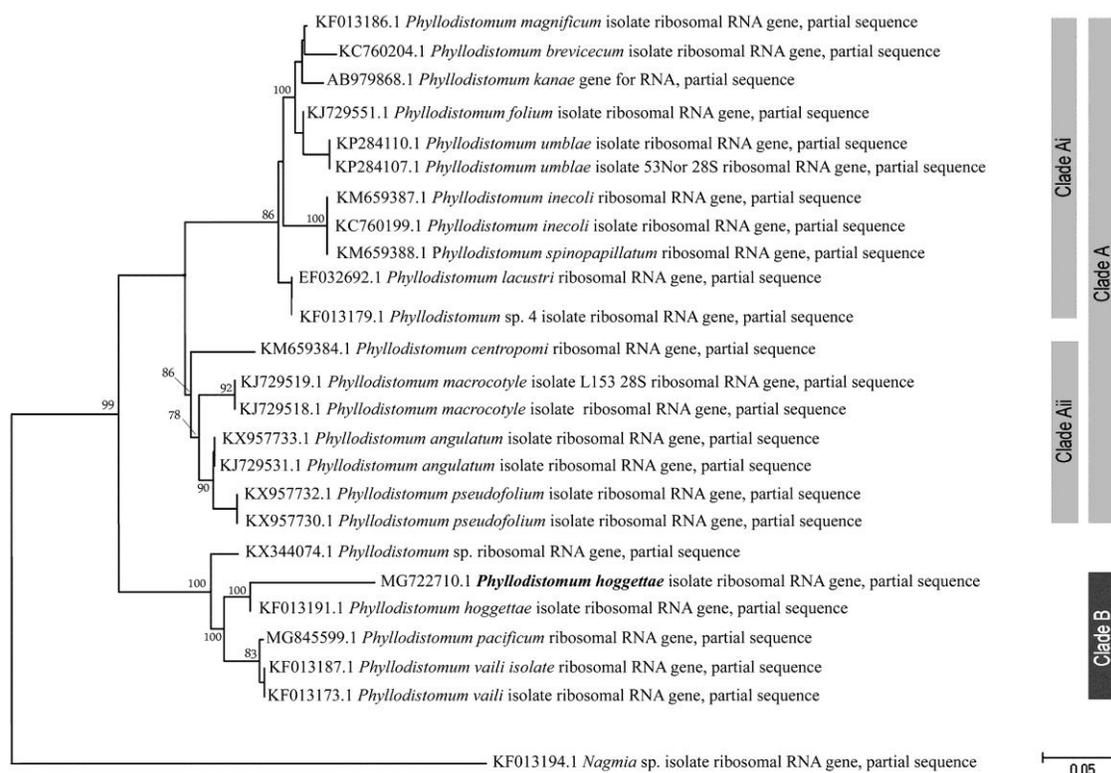


Fig. 4. A dendrogram shows the relationship between *Phyllostomum hoggettae* (Acc. no. MG722710) and some gorgoderid species. The tree was constructed using the MEGA 6 program. Maximum likelihood bootstrap support values above the nodes.

with an identity of 84.00% and *P. vaili* (KF013187.1 and KF013173.1) both with identities of 85.00%. It was observed that the parasite isolated in the present study from the Arabian Nagil *Plectropomus areolatus* from the Red Sea coasts at Gizan, Saudi Arabia, was the same as the host family of *P. hoggettae* (KF013191.1) described previously from Off Lizard Island, northern Great Barrier Reef. The sequencing data were deposited in the Genbank under accession number MG722710, (Fig. 4).

Discussion

Transversotrematids are morphologically unique in having adults with an extremely dorsoventrally flattened body that is markedly broader than long lacking oral sucker and possessing a diminutive ventral sucker. This species was first described from *Scorpius* sp. and *Microcanthus strigatus* (Cuvier), (Kyphosidae) from Moreton Bay, Australia.¹³ Family Transversotrematidae was reviewed, redescribing the species in detail, based on material obtained from several fish species belonging to 7 host families. They were previously recovered from *Diplodus sargus* (Sparidae) from the Kuwaiti coast, and their surface topography and ultrastructure were described. *T. licinum* was described from two species of fishes from Moreton Bay,

Queensland, and subsequently reported from 13 further species from six families in the Indo-West Pacific region. Ten species of this genus were described; *T. atkinsoni*¹⁵ from nemipterids from Heron Island (southern GBR) and Ningaloo Reef (Western Australia), *T. borboleta*¹⁵ from chaetodontids and lutjanines (Lutjanidae) from Lizard Island and Heron Island, *T. cardinalis*¹⁵ from lutjanines and a haemulid from Lizard Island, *T. carmenae*¹⁵ from nemipterids from Lizard Island, *T. damsella*¹⁵ from pomacentrids, a labrid and a mugilid from Lizard Island, *T. espanola*¹⁵ from lutjanines from Heron and Lizard Islands, *T. fusilieri*¹⁵ from caesionines (Lutjanidae) from Lizard Island, *T. manteri*¹¹ from caesionines from Lizard Island and Ningaloo Reef, *T. nova*¹⁵ from a nemipterid from New Caledonia, and *T. witenbergi*¹⁵ from caesionines from Heron Island. The present parasite is the most similar to *T. atkinsoni*, *T. manteri*, and *T. witenbergi* in the presence of a highly transversely elongate body. It can be distinguished from *T. witenbergi* in possessing vitelline follicles that do not extend anterior to the cyclocoel and from *T. atkinsoni* in possessing fewer vitelline follicles enclosed within the cyclocoel. It is distinctly smaller than *T. atkinsoni*, *T. manteri*, and *T. witenbergi*. Also, it has a comparatively larger ventral sucker than *T. manteri* and *T. witenbergi* and a comparatively larger pharynx than *T. manteri*. Also, it is distinct from *T. witenbergi* in being crescent-shaped rather

than spindle-shaped. The current specimen is consistent with *T. licinum* in possessing a crescent-shaped body, few vitelline follicles anterior to the cyclocoel, and many eggs with a non-significant difference in the size range.

Phylogeny and analysis of the 28S dataset resulted in a phylogram consistent with the previous findings¹⁵ demonstrated the presence of three marine clades of *Transversotrema* species. *T. licinum* formed a well-supported clade infecting a range of families with species from caesionids, chaetodontids, lutjanids and sillaginids, sister to a clade infecting lutjanids and pomacentrids, and this clade was that to which the present specimen belongs. The sequence data of *T. licinum* (present study) were different from that of the comparable species of *Transversotrema* by a minimum of 8 bases, a difference strongly consistent with species-level distinction. Numerous other species are known from different hosts including lutjanids (*T. espanola*, *T. fusilieri*, *T. licinum*, *T. manteri* and *T. witenbergi*, *T. borboleta* complex). The host examined in the present study included within family Sparidae which is a new host and supported by a previous study.¹⁵ isolated the same parasite species from *Acanthopagrus pacificus* (Sparidae) off Moreton Bay, Queensland, Australia. A collective data for some species of transversotrematid taxa used in the sequence analysis and comparing their morphometric criteria with the parasite isolated herein were shown in Table (2). Following the combined morphological and molecular analysis of the present parasite, it was concluded that this species belonged to *Transversotrema* as *T. licinum* with new locality/host.

The genus *Phyllodistomum* is included within the family Gorgoderidae, and its species composition remains controversial.¹³ Three distinct groups of marine species of *Phyllodistomum* were recognized as follows: Group A comprises just *P. psettodi*, which have the forebody occupy over half the body length. Group B comprises six species in which the length to width ratio reaches 4.00 or more: *P. marinum*, *P. mugilis*, *P. notosinicum*, *P. sampaioi*, *P. strictum*, and *P. thunni*. Group C comprises 19 species in which the forebody is relatively short, and adults' length width ratio does not exceed 4.00. Morphologically, it was observed that the present species belong to members of group C. As recently reviewed⁵ species of the genus are characterized by having a more-or-less foliate hindbody, blind caeca, two testes, and the uterus was strongly developed in the hindbody but not in the forebody with a slender excretory vesicle. The present described species were morphometrically different from some species within group C; it is smaller in body dimensions than *P. acceptum*, *P. lancea*, *P. leilae*, *P. parukhin*, *P. sobolevi*, and *P. thalassomum*, and *P. unicum*. It has a more considerable length than the remaining species except for *P. hoggettae* which has the most similar dimensions to the current species. The present parasite is distinguished by the presence of prominent marginal undulations in the hindbody, which

was absent in most of the comparable species as *P. borisbychowskyi*, *P. leilae*, *P. lancea*, *P. pacificum*, *P. pomacanthi*, *P. sobolevi*, *P. tongaatense*, *P. trinectes*, *P. scrippsi*, *P. mirandai*, *P. parukhini*, *P. crenilabri*, *P. thalassomum*, *P. acceptum*, *P. mamaevi*, with strong marginal undulations than that of the present study, while *P. unicum* possessed invisible undulations. Also, the entire vitelline shape of the present parasite distinguished from most of the species compared, where some of them have a deeply lobed vitellaria: *P. acceptum*, *P. crenilabri*, *P. lancea*, *P. leilae*, *P. lewisi*, *P. mirandai*, *P. pomacanthi*, *P. scrippsi*, *P. sobolevi*, and *P. tongaatense*. It is concluded that most of the morphological and morphometric characteristics are close to *P. hoggettae*.

The recent molecular phylogenetic analysis of the Gorgoderidae,⁵ recognized three subfamilies. The Anaporrhutinae, infects marine elasmobranchs and turtles, the Degeneriinae,¹⁵ infecting deep sea teleosts, and the Gorgoderinae, which infect amphibians and marine and freshwater teleost fishes. Within the Gorgoderinae, *Phyllodistomum* by far there is the largest genus containing over 100 species and is one of the largest genera of trematodes. In the recent years, considerable advances in the understanding of the systematics and phylogeny of gorgoderid digeneans⁹ took place, where, the difficulties of proper identification among *Phyllodistomum* spp. are related to the great intraspecific morphological variation in many species and numerous inadequate morphological descriptions were previously reported. According to the analysis of 28S gene sequences, the present data supported the validity of the present parasite to the family Gorgoderidae as *P. hoggettae*. In the phylogenetic analysis, this *Phyllodistomum* sp. Was clustered in one clade with *P. hoggettae* (KF013191.1), *P. pacificum* and *P. valli*,¹³ with a close sequence identity to *P. hoggettae*. This was in agreement with the previous molecular phylogenetic studies involving members of the Gorgoderidae which showed that the genus *Phyllodistomum*, was the most species-rich genus in the family Gorgoderidae and one of the largest genera in the Digenea.⁹ Table (3) showed a collective data for some species of family Gorgoderidae used in the phylogenetic analysis and compared their morphometric criteria with the parasite isolated herein.

The studied parasite was isolated from the intestine of the marbled spinefoot *Siganus rivulatus* belonging to the family Siganidae. This fluke was closely related to *Gyiliauchen volubilis*, which was collected previously from *Siganus rivulatus*¹⁶ starting from Hurghada City off the shore of the Red Sea in Egypt.¹⁴ Mangrove swamps on the Egyptian coast of the Gulf of Aqaba and mangrove swamps near Rabigh on the western Red Sea coast of Saudi Arabia. It ended at Sharm El-Naga coast southern of Hurghada City, Red Sea, Egypt. The peculiar body shape of gyiliauchenids may be due to the failure of the hind body to

grow during the post-cercarial development with a compensating elongation of the forebody so-called excretory papilla and would correspond to the hind body of other trematodes.^{17,18} Based on the ultrastructure, the peculiar gut morphology of gyliachenids may be interpreted to be an adaptation to the predominantly herbivorous diets of the definitive fish host.¹⁹ The method of collection of fishes may affect the normal distribution of helminths along their gastrointestinal tracts. Certain methods of capture (traps or nets) resulted in significant stress to fish. According Mackenzie and Gibson, such a stress causes regurgitation and contributes towards the expelling of some intestinal helminthes.²⁰ The migration of parasitic helminths along the gastrointestinal tract of fish during periods of starvation or after death may also affect their normal distribution. The present data of the recorded parasite were compared to the results obtained from the same species previously recorded by many authors. Morphologically, the present parasite shared the most characteristic features of genus *Gyliachen*; the body was tapered anteriorly, convex dorsally, and concave ventrally, and the oral sucker was absent and functionally replaced by the pharynx or was embedded in parenchyma. The ventral sucker was either at or close to the posterior end of the body and the esophagus was very long formed many coils within the fore body and erminated in an oesophageal bulb. In the genus *Gyliachen*, the ovary was described as anterior to the oblique testes and the testes were dorsal to the acetabulum. The topography of the gonads was on the whole more like that of *Gyliachen* and for that reason the present species was assigned to that genus. *G. volubilis* resembled most *G. papillatus*,²¹ However, different from that species: (a) in the more posterior position of the ovary; (b) in the greater development and extent of the vitellaria; (c) in the constant disposition of the pre-pharyngeal convolutions which were also longer and more voluminous; (d) in the better developed excretory protuberance. Table (4) presents a comparative data between the parasite isolated in the present study and some gyliachenids.

Acknowledgments

The authors extend their appreciation to AlBaha University for funding this work through Research group Project under grant number (1439/36). Also, the authors extend their gratitude to Deanship of Scientific Research at King Khalid University through Research group Project under grant number (R.G.P.1-112-40).

Conflict of interest

The authors declare that there is no conflict of interest.

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