Phyllodistomum vaili (Plagiorchiida: Gorgoderidae) infecting *Parupeneus rubescens* (Perciformes: Mullidae): morphology and phylogeny

Phyllodistomum vaili (Plagiorchiida: Gorgoderidae) infectando *Parupeneus rubescens* (Perciformes: Mullidae): morfologia e filogenia

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Abstract

Knowledge of the Arabian Gulf fish's parasite fauna is very poor. Until recently, only scattered reports from different locations are known for ecto- and endoparasites. Therefore, the present study aimed to investigate the digenean species that infects one of the most economically fish species in the Arabian Gulf, the rosy goatfish *Parupeneus rubescens*. One plagiorchiid species has been described, belonging to the Gorgoderidae family, and has been named as *Phyllodistomum vaili* Ho, Bray, Cutmore, Ward & Cribb, 2014 based on its morphological and morphometric characteristics. In order to accurately classify and characterize this plagiorchiid species, molecular analysis was carried out using both nuclear 18S and 28S rRNA gene regions and revealed that the present plagiorchiid species was associated with other species belonging to the Gorgoderidae family and deeply embedded in the *Phyllodistomum* genus, closely related to the previously described *P. vaili* (gb| KF013187.1, KF013173.1). The present study therefore revealed that the species *Phyllodistomum* is the first account as endoparasites from the rosy goatfish inhabiting the Arabian Gulf.

Keywords: Rosy goatfish, Digenean parasites, Gorgoderidae, Plagiorchiida, Arabian Gulf.

Resumo

O conhecimento da fauna de parasitas dos peixes do Golfo Árabe é escasso. Atualmente, apenas relatórios dispersos de diferentes locais são conhecidos para ecto e endoparasitas. Portanto, o presente estudo teve como objetivo investigar as especies digenéticas que infectam uma das espécies economicamente mais importantes do Golfo Arábico, o peixe-cabra rosado *Parupeneus rubescens*. Uma espécie de plagiorquídeo foi descrita, pertencente à família Gorgoderidae e foi denominada *Phyllodistomum vaili* Ho, Bray, Cutmore, Ward & Cribb, 2014, com base em suas propriedades morfológicas e morfométricas. A fim de classificar e caracterizar com precisão essa

espécie de plagiorquídeo, a análise molecular foi realizada usando as regiões nucleares do gene 18S e 28S rRNA, revelando que a atual espécie de plagiorchídeo estava associada a outras espécies pertencentes à família Gorgoderidae e, profundamente incorporada ao gênero *Phyllodistomum*, intimamente relacionado ao *P. vaili* descrito anteriormente (gb | KF013187.1, KF013173.1). O presente estudo revelou, portanto, que a espécie *Phyllodistomum vailli* é o primeiro relato como endoparasita do peixe-cabra rosado que habita o Golfo Arábico.

Palavras-chave: Peixe-cabra Rosado, Parasitas digenianos, Gorgoderidae, Plagiorchiida, Golfo Arábico.

Introduction

The Gorgoderidae was erected by Looss in 1899 for infecting the urinary bladder of actinopterygians and tetrapods, while those in chondrichthyans are generally in the body cavity; some species are recorded from other sites such as swim bladder, gall bladder and intestine (Campbell, 2008). It was a distinctive family of trematodes characterized by non-spinous tegument, simple male terminal genitalia, highly restrained vitellarium and extensive uterus (Campbell, 2008). The molecular phylogenetic analysis of Trematoda by Olson et al. (2003) included species of four Gorgoderidae genera that were recovered as monophyletic. Within the Gorgoderoidea, Olson et al. (2003) recognized the Gorgoderidae, one of four superfamilies within the Xiphidiata crown suborder. Gorgoderoidea consisted of Callodistomidae, Dicrocoeliidae, Encyclometridae, Gorgoderidae, Haploporidae (with the Atractotrematidae nested within it), Orchipedidae, Paragonimidae and Troglotrematidae. Relationships within the Gorgoderidae are unclear, except for the assumption that the peculiar deep-sea genus *Degeneria* Campbell, 1977 was indeed a gorgoderid, and that it was probably basal within the family.

Following Olson et al. (2003), Curran et al. (2006) extended the scope of related taxa studied and concluded that another family, the Allocreadiidae, should be integrated into the Gorgoderoidea, but that the Haploporidae and Atractotrematidae should be recognized in a separate Haploporoidea superfamily. In a study that paralleled some of that of Curran et al. (2006), Choudhury et al. (2007) also concluded that the Allocreadiidae are closely associated with the Callodistomidae and Gorgoderidae. Bray & Blair (2008) based on their Gorgoderoidea conception in the study of Olson et al. (2003), but added several families for "convenience" not included in that analysis.

Although understanding of the family's overall phylogenetic status seems to be relatively stable, the relationships within it are little understood. The family has two subfamilies Gorgoderinae Looss, 1899 and Anaporrhutinae Looss, 1901, as actually conceived (Campbell, 2008). The Gorgoderinae contains twelve genera all of which lack a pharynx except for enigmatic and monotypic Phyllodistomoides Brooks, 1977. The subfamily includes the genus *Phyllodistomum* Braun, 1899, one of the two largest genera of fish trematodes (Cribb et al., 2002). Species of this genus can be found in a wide range of marine and freshwater actinopterygians (Cribb et al., 2002). The Anaporrhutinae, the second subfamily, contains eight genera, all of which have a pharynx. Two of the eight genera as subgenera (*Nagmia* as *Petalodistomum* (Nagmia) and *Staphylorchis* as *Anaporrhutum* (Staphylorchis)) were recognized by Campbell (2008). Species of the two genera, *Bicornuata* Pearse, 1949 and *Plesiochorus* Looss, 1901, parasitize the urinary and gall bladders of marine turtles and the monotypic *Degeneria* infects a deep-sea teleost (Campbell, 1977). The remaining five genera infect elasmobranchs, and are found mainly in the body cavity. Brooks & Macdonald (1986) used 19 character series to create a morphology-based phylogenetic analysis of 14 gorgoderid genera. A basal division between the Gorgoderinae and the Anaporrhutinae is recognized in the study of Brooks & Macdonald (1986). A key finding was that autapomorphies were absent from *Phyllodistomum*.

The genus *Phyllodistomum* Braun, 1899 was erected for accommodating several trematode species from the urinary bladders of a variety of ectothermic vertebrates,

marine and freshwater fish and, more rarely, amphibians (Goodchild, 1943; Thomas, 1956; Rai, 1964; Schell, 1967; Ubelaker & Olsen, 1972; Bakke & Bailey, 1987). In the same year, Looss created the genus *Spathidium* unaware of Braun's publication for the same trematode. Lewis (1935) considered the genus *Catoptroides* to be a synonym of *Phyllodistomum* based on the full intergradation of generic characters. The genera *Phyllochorus* Dayal, 1938, *Plesiodistomum* Dayal, 1949 and *Vitellarinus* Zmeev, 1936 were considered synonyms of *Phyllodistomum*. The lack of a well-defined pattern of host specificity creates taxonomic ambiguity in the genus *Phyllodistomum*. In addition, considerable intra-specific variation was observed in most of these digeneans' morphological and morphometric characteristics (Gibson, 1996; Pérez-Ponce De León et al., 2015). The numerous published keys for gorgoderid subfamilies, genera, *Phyllodistomum* subgenera, and *Phyllodistomum* species (Holl, 1929; Lewis, 1935) were created on the basis of body shape differences, or the presence and absence of a groove separating the hind-body and fore-body, or the two suckers' size ratios.

Clearly, more extensive work is needed to get a better idea of the Arabian Gulf fish's parasitic infections in general and those off Saudi Arabia in particular. This study aimed to provide full data on parasitic trematodes and their indices in the rosy goatfish *Parupeneus rubescens* from the Arabian Gulf in Saudi Arabia.

Materials and Methods

Experimental animal's collection

A total of twenty specimens of the rosy goatfish *Parupeneus rubescens* (F: Mullidae) were collected from the boat landing sites at the coasts of the Arabian Gulf off Dammam City in Saudi Arabia during the time of the current study. Fish were immediately transported to the Laboratory of Parasitology Research, Zoology Department, College of Science, King Saud University, Riyadh, Saudi Arabia. All procedures that contribute to this work comply with the ethical standards authorized by Institutional Review Board (IRB) at King Saud University, Riyadh, Saudi Arabia. Within 48 h of sampling, all fish are dissected. Macro- and microscopic analysis of the collected fish specimens were performed externally and then necropsied and their internal organs under a stereo-dissecting microscope were examined for the presence of any parasite infection according to standard parasitological techniques of Vidal-Martínez et al. (2002). The site and number of parasite species was reported from each fish. Infection prevalence was estimated according to Bush et al. (1997).

Parasitological examination

According to Cribb & Bray (2010), we recommend killing and fixing the recovered trematodes with heat as an effective method. As, we advocate pipetting worms into the hot vertebrate saline, at this time, worms typically extend and assume a highly uniform and reproducible form. Some worms were fixed in a buffered formalin solution (10%) and others fixed for molecular analysis in a high grade 96% absolute alcohol. Worms fixed for whole mounts were stained with Semichon's acetocarmine, dehydrated through a graded ethanol series, cleared in clove oil and mounted with Canada balsam in permanent preparations. A Leica DM 2500 microscope (NIS ELEMENTS software, ver. 3.8) was used to examine, record and then photograph the stained specimens and the relevant structural details. All measurements in the descriptions and tables are in millimeters and are shown as the range followed by the mean ± standard deviation in parentheses.

Molecular analysis

DNA extraction and Polymerase Chain Reaction (PCR)

According to the manufacturer's instructions, genomic DNA was extracted from ethanol-preserved samples using a DNeasy tissue kit© (Qiagen, Hilden, Germany). A NanoDrop ND-1000 spectophotometer (Thermo Fischer Scientific, Inc., Wilmington, DE, USA) was used to quantify the concentration and purification of genomic DNA and 20 ng of genomic DNA was used for the polymerase chain reaction (PCR). Following the manufacturer's protocol, the nuclear 18S and 28S rRNA genes were amplified using the GeneJET™ PCR Purification kit [Thermo (Fermentas)]. The 18S rRNA gene was amplified with the forward primer 18SU467F (5'-ATC CAA GGA AGG CAG CAG GC-3') and the reverse primer 18SL1170R (5'-GTG CCC TTC CGT CAA TTC CT-3') designed by Indaryanto et al. (2015). While, the 28S rRNA gene was amplified using the forward primer JB10F (5'-GAT TAC CCG CTG AAC TTA AGC ATA-3') and the reverse primer JB9R (5'-GCT GCA TTC ACA AAC ACC CCG ACT C-3'), as designed by Lee et al. (2007). The amplification procedure was based on the schematic diagrams of the thermocycle profiles of the PCR and the primer combinations used to amplify the two genetic markers (Figure 1). All PCR products were verified on a 1% agarose gel in 1× Tris–acetate–EDTA (TAE) stained with 1% ethidium bromide and then visualized with UV trans-illuminator. PCR products of the intended size are excised, purified and cloned using a PureLink™ Quick Gel Extraction Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions.
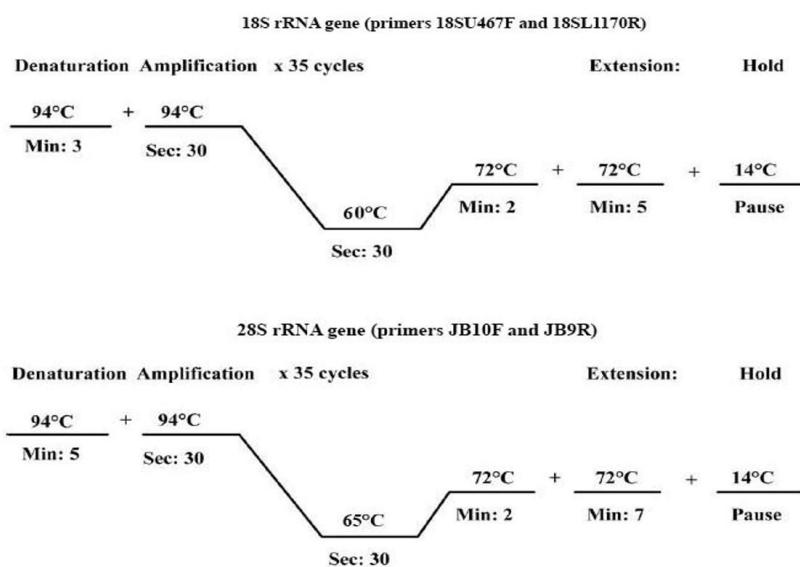

Figure 1. Schematic illustrations of the PCR thermocycle profiles and primer combinations used for amplification of the two genetic markers (18S and 28S rRNA), designed in this study.

Sequence alignment and Phylogenetic analysis

Sequencing was performed using BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA) on a 310 Automated DNA Sequencer (Applied Biosystems, Foster City, CA). Using ABI Editview (Perkin-Elmer) each sequence was manually edited for accuracy. A BLAST search was conducted on the NCBI database to classify associated sequences. These sequences are aligned directly with other gene region sequences available from GenBank™ using the multiple sequence alignment CLUSTAL-X (Thompson et al., 1997). Such sequences are selected to represent all available digenean lineages, with an emphasis on the taxa presumed to be linked to the groups being examined. The alignment was manually adjusted

using the BIOEDIT 4.8.9 alignment editor software (Hall, 1999). A phylogenetic tree was constructed using MEGA ver. 7.0 by using Maximum Likelihood method based on the Kimura 2-parameter model. Branch support under appropriate substitution models was calculated with 1000 bootstrap replicates. Tree has been drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer phylogenetic tree.

Results

Ten out of twenty (50%) specimens of the examined rosy goatfish *Parupeneus rubescens* were found to be naturally infected with the trematode parasite in the urinary bladder that identified as *Phyllodistomum vaili* Ho, Bray, Cutmore, Ward & Cribb, 2014.

Microscopic examination (Figure 2, Table 1).
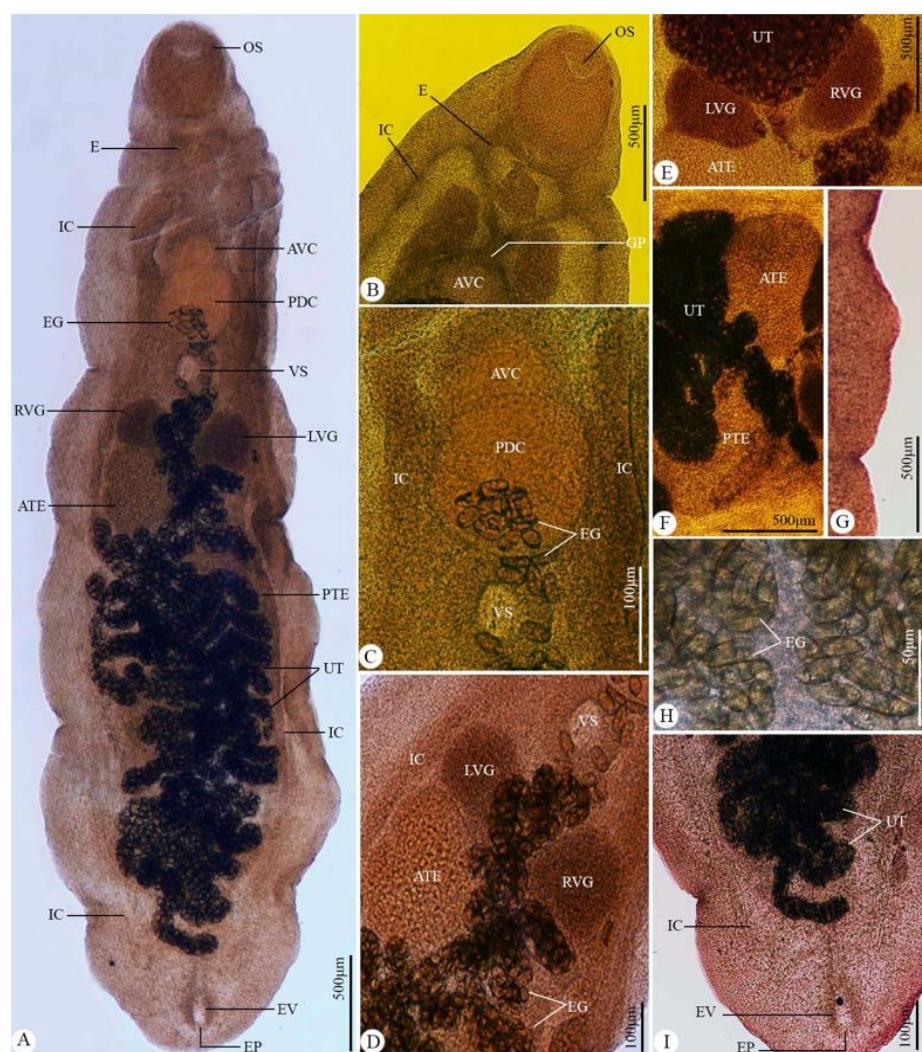
Figure 2. Photomicrographs of the adult *Phyllodistomum vaili* infecting *Parupeneus rubescens*. (A) Whole mount preparation. (B,C) Forebody. (D) Middle region of the body. (E) Vitelline glands. (F) Testes. (G) Marginal undulations. (H) Eggs. (I) Posterior extremity. Note: ATE, anterior testis; AVC, antero-ventral chamber; E, esophagus; EG, eggs; EP, excretory pore; EV, excretory vesicle; GP, genital pore; IC, intestinal ceca; LVG, left vitelline gland; OS, oral sucker; PDC, postero-dorsal chamber; PTE, posterior testis; RVG, right vitelline gland; UT, uterus; VS, ventral sucker.

Table 1. Comparative measurements of the adult *Phyllodistomum vaili* under study with those described previously.

Parasite species	Host (Locality)	Body size	Dimensions of different body parts						
			Oral sucker	Ventral sucker	Vitelline glands	Anterior testis	Posterior testis	Ovary	Eggs
P. magnificum Cribb, 1987	*Anguilla reinhardtii* (New Zealand)	1.92-4.54 (2.93) × 0.66-2.18 (1.33)	0.20-0.36 (0.27) × 0.17-0.31 (0.24)	0.20-0.45 (0.34) × 0.19-0.47 (0.34)	--	0.16-0.39 (0.27) × 0.11-0.33 (0.21)	0.22-0.48 (0.31) × 0.11-0.33 (0.23)	0.08-0.22 (0.16) × 0.07-0.18 (0.13)	0.036-0.043 (0.038) × 0.023-0.027 (0.024)
P. funduli Helt et al., 2003	*Fundulus sciadicus* (Nebraska)	1.10-5.49 (2.06) × 0.46-2.73 (1.09)	0.20-0.83 (0.33) × 0.20-0.78 (0.32)	0.15-0.73 (0.29) × 0.12-0.71 (0.29)	L: 0.07-0.36 (0.18) × 0.05-0.27 (0.09) R: 0.07-0.49 (0.18) × 0.05-0.24 (0.08)	0.07-0.32 (0.21) × 0.05-0.54 (0.19)	0.10-0.85 (0.24) × 0.07-0.73 (0.23)	0.10-0.51 (0.20)	0.01-0.04 (0.03) × 0.01-0.03 (0.02)
P. simile Sey et al., 2004	*Cirrhinus marginipinnis* (Thailand)	1.6-1.9 ×1.0	0.20-0.22	0.31-0.35 × 0.30-0.35	L: 0.75 × 0.10 R: 0.06-0.07 × 1.0-1.25	0.25-0.40 × 0.20-0.25	0.40-0.42 ×0.20-0.25	0.15-0.17 × 0.10	0.026-0.030 × 0.018-0.020
P. inecoli Razo-Mendivil et al., 2013	*Heterandria bimaculata* (Mexico)	1.15-2.08 (1.60) × 0.49-1.13 (0.80)	0.16-0.30 (0.23) × 0.14-0.25 (0.21)	0.14-0.27 (0.20) × 0.14-0.27 (0.19)	L: 0.06-0.12 (0.09) × 0.04-0.10 (0.07) R: 0.06-0.16 (0.09) × 0.04-0.10 (0.07)	0.10-0.39 (0.23) × 0.06-0.21 (0.13)	0.08-0.39 (0.23) × 0.06-0.21 (0.14)	0.05-0.20 (0.13) × 0.06-0.17 (0.11)	0.023-0.028 (0.026) × 0.014-0.018 (0.016)
P. biringo Shimazu, 2005	*Gymnogobius breunigii* (Japan)	0.70-1.44 ×0.37-0.71	0.08-0.12 × 0.09-0.11	0.12-0.14 × 0.11-0.16	0.05-0.06 × 0.09-0.11	0.09-0.17 × 0.09-0.14	0.09-0.17 × 0.09-0.14	0.06-0.15 × 0.09-0.14	0.033-0.048 × 0.021-0.024
P. carassii Shimazu, 2005	*Carassius auratus* (Japan)	3.92-5.37 × 2.96-3.42	0.56 × 0.45-0.48	0.61-0.62 × 0.53-0.64	0.48-0.75 × 0.05-0.12	0.35 × 0.27	0.19-0.29 × 0.10-0.24	--	0.017-0.019 ×0.013
P. mogurndae Shimazu, 2005	*Odontobutis obscura* (Japan)	1.74-4.01 × 1.03-1.98	0.25-0.43 × 0.20-0.41	0.19-0.33 × 0.21-0.34	0.09-0.25 × 0.11-0.25	0.18-0.41 × 0.17-0.28	0.20-0.34 × 0.16-0.41	0.13-0.43 × 0.19-0.35	0.016-0.017 × 0.011-0.013
P. hoggettae Ho et al., 2014	*Plectropomus leopardus* (Great Barrier Reef)	3.48-4.76 (4.14) × 0.96-1.85 (1.50)	0.28-0.44 (0.33) × 0.28-0.41 (0.30)	0.36-0.55 (0.43) × 0.36-0.51 (0.44)	L: 0.12-0.27 (0.17) × 0.07-0.21 (0.12) R: 0.10-0.28 (0.19) × 0.05-0.22 (0.12)	0.15-0.34 (0.27) × 0.09-0.39 (0.25)	0.17-0.45 (0.30) × 0.15-0.54 (0.28)	0.07-0.33 (0.20) × 0.06-0.23 (0.16)	0.036-0.057 (0.049) × 0.021-0.033 (0.029)
P. vaili Ho et al., 2014	*Mulloidichthys vanicolensis*	1.49-2.30 (1.84) × 0.40-0.68 (0.54)	0.13-0.20 (0.16) × 0.12-0.20 (0.15)	0.11-0.18 (0.14) × 0.09-0.17 (0.14)	L: 0.052-0.093 (0.074) × 0.051-0.083 (0.054) R: 0.049-0.125 (0.078) × 0.033-0.081 (0.053)	0.08-0.19 (0.14) × 0.06-0.18 (0.12)	0.06-0.21 (0.15) × 0.06-0.17 (0.12)	0.05-0.15 (0.10) × 0.04-0.13 (0.08)	0.027-0.050 (0.041) × 0.014-0.029 (0.022)
P. parasiluri Shimazu, 2005	*Silurus asotus* (Japan)	1.13-3.49 × 0.41-1.04	0.09-0.25 × 0.11-0.24	0.16-0.35 × 0.16-0.33	0.04-0.16 × 0.09-0.22	0.22-0.63 × 0.12-0.41	0.25-0.82 × 0.14-0.51	0.09-0.32 × 0.09-0.25	0.011-0.022 × 0.008-0.016
P. cribbi Pérez-Ponce de León et al., 2015	*Zoogoneticus quiteoensis* (Mexico)	0.92-1.84 (1.46) × 0.47-1.22 (0.90)	0.17-0.29 (0.23) × 0.14-0.28 (0.23)	0.14-0.25 (0.20) × 0.14-0.27 (0.22)	L: 0.05-0.10 (0.07) × 0.06-0.14 (0.10) R: 0.04-0.09 (0.06) × 0.06-0.14 (0.11)	0.12-0.27 (0.19) × 0.09-0.26 (0.18)	0.08-0.30 (0.17) × 0.10-0.28 (0.18)	0.06-0.17 (0.11) × 0.12-0.15 (0.13)	0.018-0.037 (0.028) × 0.013-0.026 (0.020)
P. kanae Nakao, 2015	*Hynobius retardatus* (Japan)	4.0-4.9 × 2.0-2.1	0.48-0.49 × 0.51-0.55	0.83-0.85 × 0.60-0.70	L: 0.22-0.29 × 0.16-0.17 R: 0.23-0.27 ×	0.69-0.83 × 0.52-0.54	0.88-0.92 × 0.41-0.52	0.39-0.40	0.049-0.054 × 0.034-0.037

Table 1. Continued...

Parasite species	Host (Locality)	Body size	Dimensions of different body parts						
			Oral sucker	Ventral sucker	Vitelline glands	Anterior testis	Posterior testis	Ovary	Eggs
					0.14-0.18				
P. wallacei Pérez-Ponce de León et al., 2015	*Xenotaenia resolanae* (Mexico)	0.71-1.61 (1.24) × 0.44-1.22 (0.79)	0.14-0.28 (0.19) × 0.11-0.32 (0.19)	0.11-0.18 (0.16) × 0.09-0.21 (0.16)	L: 0.03-0.11 (0.06) × 0.03-0.13 (0.08) R: 0.03-0.11 (0.07) × 0.03-0.13 (0.08)	0.06-0.25 (0.12) × 0.07-0.13 (0.10)	0.09-0.19 (0.13) × 0.08-0.16 (0.11)	0.05-0.20 (0.13) × 0.12-0.15 (0.13)	0.019-0.032 (0.025) × 0.013-0.23 (0.018)
P. hyporhamphi Cutmore and Cribb, 2018	*Hyporhamphus regularis* (Australia)	1.83-5.08 (3.40) × 0.87-2.92 (1.71)	0.25-0.52 (0.41) × 0.19-0.44 (0.34)	0.13-0.28 (0.22) × 0.14-0.31 (0.23)	L: 0.10-0.28 (0.19) × 0.12-0.29 (0.18) R: 0.11-0.30 (0.19) × 0.12-0.32 (0.19)	0.13-0.42 (0.22) × 0.14-0.43 (0.26)	0.16-0.53 (0.26) × 0.14-0.43 (0.26)	0.14-0.32 (0.23) × 0.133-0.44 (0.23)	--
P. pacificum Cutmore and Cribb, 2018	*Carangoides equula* (Japan)	4.57-5.33 (4.95) × 1.11-1.32 (1.22)	0.31-0.41 (0.35) × 0.31-0.38 (0.33)	0.26-0.34 (0.29) × 0.26-0.32 (0.28)	L: 0.09-0.17 (0.12) × 0.11-0.18 (0.14) R: 0.08-0.16 (0.11) × 0.12-0.19 (0.14)	0.32-0.48 (0.39) × 0.24-0.42 (0.30)	0.41-0.59 (0.50) × 0.27-0.48 (0.35)	0.16-0.30 (0.20) × 0.12-0.24 (0.16)	0.032-0.051 (0.042) × 0.021-0.032 (0.26)
P. punctati Jithila and Prasadan, 2018	*Channa punctata* (India)	1.51-6.13 (2.92) × 1.03-4.32 (2.92)	0.33-0.82 (0.47) × 0.35-0.76 (0.46)	0.28-0.29 (0.44) × 0.29-0.73 (0.45)	L: 0.09-0.32 (0.20) × 0.05-0.30 (0.17) R: 0.09-0.29 (0.17) × 0.06-0.27 (0.16)	0.12-0.51 (0.29) ×0.07-0.32 (0.18)	0.09-0.68 (0.28) × 0.11-0.33 (0.17)	0.11-0.43 (0.26) × 0.10-0.37 (0.23)	0.024-0.029 (0.025) × 0.012-0.020 (0.016)
P. vaili (Present study)	*Parupeneus rubescens* (Saudi Arabia)	1.38-2.10 (1.75±0.1) × 0.41-0.63 (0.52±0.01)	0.11-0.18 (0.14±0.01) × 0.10-0.18 (0.12±0.01)	0.10-0.16 (0.13±0.01) × 0.08-0.14 (0.12±0.01)	L: 0.054-0.098 (0.081±0.001) × 0.051-0.083 (0.054±0.001) R: 0.049-0.118 (0.086±0.001) × 0.035-0.087 (0.064±0.001)	0.09-0.17 (0.15±0.01) × 0.07-0.15 (0.13±0.01)	0.06-0.19 (0.16±0.01) × 0.06-0.16 (0.13±0.01)	0.06-0.17 (0.11±0.01) × 0.04-0.15 (0.09±0.001)	0.032-0.059 (0.050±0.001) × 0.015-0.027 (0.020±0.001)

Body lanceolate, 1.38-2.10 (1.75±0.1) mm in length and 0.41-0.63 (0.52±0.01) mm as a maximum width at the anterior testis level. Forebody narrow and tapering, 0.50-0.87 (0.63±0.1) mm long. Hindbody with 4-5 weak marginal undulations on each side produced by distinct lateral muscular loops. Oral sucker opening subterminally, 0.11-0.18 (0.14±0.01) mm long and 0.10-0.18 (0.12±0.01) mm wide. Intestinal bifurcation midway between oral and ventral suckers. Ventral sucker of 0.10-0.16 (0.13±0.01) mm long and 0.08-0.14 (0.12±0.01) mm wide, usually slightly smaller than oral sucker, pre-equatorial, and rounded in shape. Absence of prepharynx and pharynx. Esophagus 0.13-0.31 (0.27±0.01) mm long. There are penetration glands at the end of the esophagus. Caeca simple, blind tubes ending from the posterior extremity at 0.11-0.49 (0.26±0.01) mm.

Testes slightly lobed, oblique, in mid-hindbody; anterior testis 0.09-0.17 (0.15±0.01) × 0.07-0.15 (0.13±0.01) mm in size; posterior one 0.06-0.19 (0.16±0.01) × 0.06-0.16 (0.13±0.01) mm in size. Cirrus sac absent. Seminal vesicle saccular, bipartite; postero-

dorsal chamber large, dorsal to genital pore, anteriorly constricting to small antero-ventral chamber situated anterior to the genital pore. Prostatic chamber and surrounding cells ventrally to the posterodorsal chamber of seminal vesicle. Seminal ducts arising from two testes unite at slightly posterior to seminal vesicle into a common seminal duct and opens to seminal vesicle. Genital pore median, 0.18-0.28 (0.23±0.01) mm anterior to the ventral sucker.

Ovary subspherical, entire, postero-sinistral to the ventral sucker and anterior-sinistral to testes, 0.06-0.17 (0.11±0.01) mm long and 0.04-0.15 (0.09±0.001) mm wide. Oviduct extends into a distinct chamber prior to entering mehlis' gland. Paired vitelline glands posterolateral to the ventral sucker; left lobe immediately posterior to and occasionally overlapped by ovary, 0.054-0.098 (0.081±0.001) × 0.051-0.083 (0.054±0.001) mm in size; right lobe 0.049-0.118 (0.086±0.001) × 0.035-0.087 (0.064±0.001) mm in size. Laurer's canal short, running transversely, opening on the antiovarian side of the body dorsally close to the vitellarium. Median ootype, between vitellaria, anterior to vitelline ducts. Mehlis' gland large. There was no seminal receptacle. Uterus almost entirely intracaecal in hindbody with extensive coils reaching just posterior to ends of intestinal caeca; uterine chamber distinct and prominent dorsal to ventral sucker and genital pore, packed with numerous eggs. Metraterm well developed, surrounded by small gland cells and opened at gonopore. Eggs elongate elliptical, not operculated, 0.032-0.059 (0.050±0.001) mm long and 0.015-0.027 (0.020±0.001) mm wide. Excretory vesicle tubular and extending anteriorly to the ovarian level. Excretory pore dorsally subterminal, close to the posterior end of the body. Table 1 shows the maximum and minimum values of the different body parts of this species in comparison to the previously described *Phyllodistomum* species, as well as the mean values.

Molecular analysis

For 28S rRNA gene region

A total of 237 bp with 56.5% GC content for 28S rRNA gene region of the present digenea species was analysed and deposited in GenBank under the accession number MK881623.1. Phylogenetic analysis was performed based on the alignment of partial and complete 28S rRNA sequences for 29 taxa using maximum likelihood method based on the Kimura 2-parameter model representing two plagiorchiid suborders (Xiphidiata and Lepocreadiata) (Table 2, Figure 3).
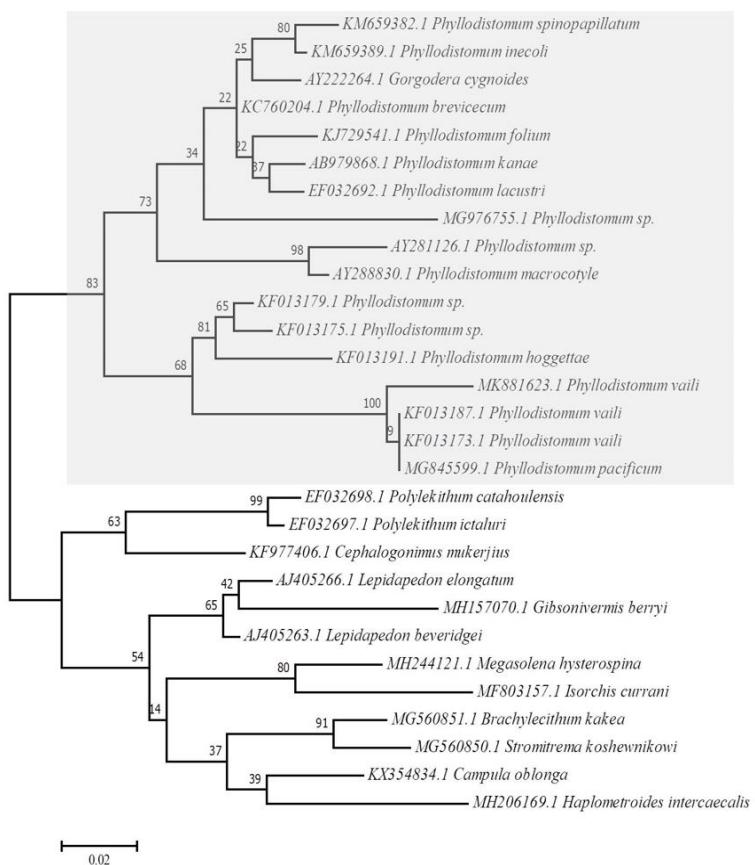
Figure 3. Molecular Phylogenetic analysis of 28S rRNA of *Phyllodistomum vaili* by Maximum Likelihood method based on the Kimura 2-parameter model. The tree with the highest log likelihood (-1497.51) is shown. The percentage of trees shown above the branches in which the related taxa clustered together. By applying the Maximum Parsimony method, initial tree(s) for the heuristic search are obtained automatically. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. Evolutionary analyses were conducted in MEGA7.

Table 2. Digenea species used in the 28S rRNA nuclear gene analysis of *Phyllodistomum vaili* specimens obtained in this study.

Parasite species	Order/Family	Host species	Accession no.	Percent identity (%)	GC content
Phyllodistomum vaili	Plagiorchiida/Gorgoderidae	*Mulloidichthys vanicolensis*	KF013187.1	99.50%	53.25%
Phyllodistomum vaili	Plagiorchiida/Gorgoderidae	*Mulloidichthys flavolineatus*	KF013173.1	99.50%	53.34%
Phyllodistomum pacificum	Plagiorchiida/Gorgoderidae	*Pantolabus radiatus*	MG845599.1	98.73%	52.93%
Phyllodistomum sp.	Plagiorchiida/Gorgoderidae	*Epibulus insidiator*	KF013179.1	91.58%	54.30%
Phyllodistomum sp.	Plagiorchiida/Gorgoderidae	*Cephalopholis boenak*	KF013175.1	91.09%	54.24%
Phyllodistomum hoggettae	Plagiorchiida/Gorgoderidae	*Plectropomus leopardus*	KF013191.1	90.10%	55.14%
Gorgodera cygnoides	Plagiorchiida/Gorgoderidae	*Pelophylax ridibundus*	AY222264.1	89.08%	53.88%
Polylekithum ictaluri	Plagiorchiida/Allocreadiidae	*Ictalurus furcatus*	EF032697.1	88.66%	52.06%

Table 2. Continued...

Parasite species	Order/Family	Host species	Accession no.	Percent identity (%)	GC content
Phyllodistomum kanae	Plagiorchiida/Gorgoderidae	*Hynobius retardatus*	AB979868.1	88.53%	54.02%
Phyllodistomum brevicecum	Plagiorchiida/Gorgoderidae	*Umbra limi*	KC760204.1	88.39%	53.84%
Phyllodistomum spinopapillatum	Plagiorchiida/Gorgoderidae	*Profundulus balsanus*	KM659382.1	88.24%	52.64%
Phyllodistomum folium	Plagiorchiida/Gorgoderidae	*Pisidium amnicum*	KJ729541.1	88.24%	53.22%
Phyllodistomum inecoli	Plagiorchiida/Gorgoderidae	*Profundulus sp.*	KM659389.1	87.82%	52.38%
Polylekithum catahoulensis	Plagiorchiida/Allocreadiidae	*Ictalurus furcatus*	EF032698.1	87.82%	52.35%
Cephalogonimus mukerjius	Plagiorchiida/Cephalogonimidae	*Pila globosa*	KF977406.1	87.76%	49.27%
Phyllodistomum lacustri	Plagiorchiida/Gorgoderidae	*Ameiurus melas*	EF032692.1	87.56%	53.76%
Phyllodistomum sp.	Plagiorchiida/Gorgoderidae	*Pisidium amnicum*	AY281126.1	87.39%	53.74%
Phyllodistomum macrocotyle	Plagiorchiida/Gorgoderidae	*Dreissena polymorpha*	AY288830.1	87.39%	50.95%
Brachylecithum kakea	Plagiorchiida/Dicrocoelidae	*Acrocephalus arundinaceus*	MG560851.1	86.55%	52.94%
Stromitrema koshewnikowi	Plagiorchiida/Dicrocoelidae	*Hirundo rustica*	MG560850.1	86.55%	54.53%
Campula oblonga	Plagiorchiida/Brachycladiidae	*East Asian finless porpoise*	KX354834.1	86.55%	55.60%
Megasolena hysterospina	Plagiorchiida/Haploporidae	*Archosargus rhomboidalis*	MH244121.1	86.55%	52.02%
Phyllodistomum sp.	Plagiorchiida/Gorgoderidae	*Channa striata*	MG976755.1	86.46%	53.75%
Lepidapedon elongatum	Plagiorchiida/Lepocreadiidae	*Gadus morhua*	AJ405266.1	86.19%	52.94%
Lepidapedon beveridgei	Plagiorchiida/Lepocreadiidae	*Coryphaenoides armatus*	AJ405263.1	86.19%	53.60%
Gibsonivermis berryi	Plagiorchiida/Gibsonivermidae	*Sillago ciliata*	MH157070.1	86.19%	53.41%
Isorchis currani	Plagiorchiida/Atractotrematidae	*Selenotoca multifasciata*	MF803157.1	86.13%	54.09%
Haplometroides intercaecalis	Plagiorchiida/Plagiorchiidae	*Phalotris matogrossensis*	MH206169.1	85.77%	52.88%

Comparison of nucleotide sequences and divergence showed that the 28S rRNA of this species revealed taxa sequence identities belonged to Xiphidiata as 99.50-85.77% with Plagiorchioidea, 88.66-86.55% with Allocreadioidea, and 86.13% with Haploporoidea. While, the sequence identities for nucleotide sequences within Plagiorchioidea were 99.50-86.46% with taxa under the Gorgoderidae family, 87.76% with Cephalogonimidae, 86.55% with Dicrocoeliidae, and 85.77% with Plagiorchiidae (Table 2). Among Gorgoderidae, the maximum identity (99.50%) with lowest divergent value was recorded between the present digenea species and the previously described *P. vaili* (gb| KF013187.1, KF013173.1) followed by *Phyllodistomum pacificum* (98.73%, gb| MG845599.1), *Phyllodistomum sp.* (91.58%, gb| KF013179.1),

Phyllodistomum sp. (91.09%, gb| KF013175.1), and *Phyllodistomum hoggettae* (90.10%, gb| KF013191.1) (Table 2).

This analysis (Figure 3) demonstrated the basal position of Plagiorchiidae in which forming sister group to Brachycladiidae+ Dicrocoeliidae + Haploporidea + Atractotrematidae with weak nodal support. Also, Allocreadiidae forming sister group to Cephalogonimidae with high nodal support. In addition, Lepocreadiidae forming sister group to Gibsonivermidae within Lepocreadiata, while, Lepocreadiidae forming more related sister group with Haploporidea + Atractotrematidae with moderate nodal support. The ME tree showed the cluster containing all *Phyllodistomum* spp. was clearly divided into two distinct clades which was strongly supported by a high bootstrap value. For the present plagiorchiid species, the ME tree revealed a well-resolved distinct clade with other members of the digenea species belonging to the Gorgoderidae family and deeply embedded in the *Phyllodistomum* with close relationship to the previously described *Phyllodistomum vaili* (gb| KF013187.1, KF013173.1) and *Phyllodistomum pacificum* (98.73%, gb| MG845599.1) as a more related sister taxons.

For 18S rRNA gene region

A total of 666 bp with 47.14% GC content was analysed and the resulting sequences of the present digenea species were deposited in GenBank under the accession number MK883474.1. No identical sequences found in the DNA databases through the basic local alignment search tool (BLAST). Phylogenetic analysis was performed based on the comparison with 25 related species using a maximum likelihood method based on the Kimura 2-parameter model representing three orders of Plagiorchiida, Opisthorchida, and Echinostomida (Table 3, Figure 4).

Table 3. Digenea species used in the 18S rRNA nuclear gene analysis of *Phyllodistomum vaili* specimens obtained in this study.

Parasite species	Order/Family	Host species	Accession no.	Percent identity (%)	GC content
Gorgoderina lufengensis	Plagiorchiida/Gorgoderidae	*Nanorana yunnanensis*	MH285258.1	93.11%	50.50%
Gorgodera sp.	Plagiorchiida/Gorgoderidae	*Rana ridibunda*	AJ287518.1	92.81%	49.87%
Ragaia lizae	Plagiorchiida/Haploporidae	*Liza aurata*	FJ211231.1	91.19%	51.77%
Haploporus benedeni	Plagiorchiida/Haploporidae	*Liza ramado*	FJ211228.1	90.92%	51.81%
Dicrogaster perpusilla	Plagiorchiida/Haploporidae	*Liza ramado*	FJ211230.1	90.88%	51.17%
Saccocoelium cephali	Plagiorchiida/Haploporidae	*Mugil cephalus*	FJ211232.1	90.75%	51.52%
Orchipedum tracheicola	Echinostomida/Orchipedidae	*Cygnus olor*	AJ287551.1	90.45%	51.05%
Prosthenhystera obesa	Plagiorchiida/Callodistomidae	*Hoplias sp.*	AY222108.1	90.27%	50.56%
Rubenstrema exasperatum	Plagiorchiida/Plagiorchiidae	*Crocidura leucodon*	AJ287572.1	90.16%	50.43%
Stephanostomum gaidropsori	Opisthorchiida/Acanthocolpidae	*Gaidropsarus mediterraneus*	DQ248208.1	90.16%	50.97%
Campula oblonga	Plagiorchiida/Brachycladiidae	*Phocoena phocoena*	KM258665.1	90.02%	52.03%
Opisthioglyphe ranae	Plagiorchiida/Plagiorchiidae	*Rana arvalis*	AY222157.1	90.01%	49.97%
Haematoloechus longiplexus	Plagiorchiida/Haematoloechidae	*Rana catesbeiana*	AJ287520.1	89.88%	50.23%
Oschmarinella	Plagiorchiida/Brachycladiidae	*Stenella*	KM258667.1	89.87%	51.05%

Table 3. Continued...

| Parasite species	Order/Family	Host species	Accession no.	Percent identity (%)	GC content
rochebruni		*coeruleoalba*			
Paragonimus iloktsuenensis	Plagiorchiida/Troglotrematidae	*Rattus norvegicus*	AY222141.1	89.85%	51.77%
Nasitrema globicephalae	Opisthorchiida/Nasitrematidae	--	AJ004968.1	89.85%	51.05%
Synthesium pontoporiae	Plagiorchiida/Brachycladiidae	--	FJ357162.1	89.85%	51.23%
Nephrotrema truncatum	Plagiorchiida/Troglotrematidae	*Neomys anomalus*	AY222139.1	89.85%	51.07%
Brachycladium goliath	Plagiorchiida/Brachycladiidae	*Balaenoptera acutorostrata*	KR703279.1	89.85%	51.68%
Stephanostomum cestillium	Opisthorchiida/Acanthocolpidae	*Lophius piscatorius*	DQ248213.1	89.85%	51.05%
Skrjabinoeces similis	Plagiorchiida/Plagiorchiidae	*Rana ridibunda*	AJ287575.1	89.51%	50.17%
Auridistomum chelydrae	Plagiorchiida/Auridistomidae	*Chelydra serpentina*	AY222159.1	89.40%	50.88%
Pseudomegasolena ishigakiense	Plagiorchiida/Haploporidae	*Scarus rivulatus*	AJ287569.1	88.97%	49.92%
Lissorchis kritskyi	Plagiorchiida/Lissorchiidae	*Carpiodes cyprinus*	AY222136.1	88.79%	50.25%
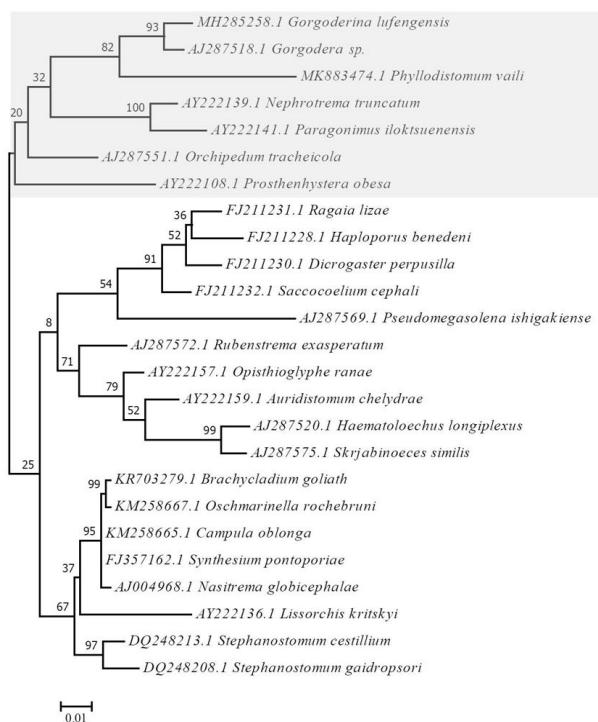
Figure 4. Molecular Phylogenetic analysis of 18S rRNA of *Phyllodistomum vaili* by Maximum Likelihood method based on the Kimura 2-parameter model. This displayed the tree with the highest log likelihood (-3184.75). The percentage of trees shown above the branches in which the associated taxa clustered together. Initial tree(s) for the heuristic search are obtained automatically by applying the Maximum Parsimony method. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. In MEGA7, evolutionary analyses are performed.

Comparison of the nucleotide sequences and divergence revealed that the 18S rRNA of this species revealed sequence identities with taxa belonged to Plagiorchiida as 93.11-89.40% with Plagiorchioidea, 91.19-88.97% with Haploporoidea, 90.02-89.85% with Allocreadioidea, 89.85% with Troglotrematidea, and 88.79% with Monorchioidea (Table 3). The maximum identity with lowest divergent values were recorded with other gorgoderid species, *Gorgoderina lufengensis* (93.11%, gb| MH285258.1) and *Gorgodera* sp. (92.81%, gb| AJ287518.1) (Table 3).

The constructed dendrogram (Figure 4) is divided into two clades, the major one clustering all Gorgoderidae species to forming sister group with Troglotrematidae + Callodistomidae + Orchipedidae. While, the minor clade was subdivided into lineages; the former clustered all species belonging to Haploporidae, Omphalometridae, Telorchiidae, Auridistomidae, Haematoloechidae, and Plagiorchiidae; the latter lineage demonstrated the basal position of Acanthocolpidae within Opisthorchida forming sister group to Lissorchiidae + Brachycladiidae with moderate nodal support. The ME tree showed a well-resolved distinct clade for the present plagiorchiid species with other members of the digenea species belonging to the Gorgoderidae family and deeply embedded in the *Phyllodistomum* genus with close relationship to *Gorgoderina lufengensis* (gb| MH285258.1) and *Gorgodera* sp. (gb| AJ287518.1) as a sister taxon associated with it.

Discussion

Phyllodistomum Braun, 1899 is the most specious gorgoderid genus, with a common infection site of urinary bladder and/or ureters of marine and freshwater fish, more rarely amphibians (Goodchild, 1943; Thomas, 1956; Rai, 1964; Schell, 1967; Ubelaker & Olsen, 1972; Bakke & Bailey, 1987; Cribb, 1987; Hoffman, 1999; Mendoza-Garfias & Pérez-Ponce de León, 2005; Campbell, 2008). This genus has a worldwide distribution and is one of the most specious groups in the Digenea, describing about 120 species (Cribb et al., 2002; Helt et al., 2003).

In this study, *Phyllodistomum vaili* Ho, Bray, Cutmore, Ward & Cribb, 2014 found to be naturally infected the urinary bladder of the rosy goatfish *P. rubescens* inhabited the Arabian Gulf and this species represented by a moderate parasite prevalence (50.0%). Firstly, the type of host species and the geographical location for the recovered gorgoderid species is differed from all other species within this genus due to the lack of a well-defined pattern of host specificity. In addition, considerable intraspecific variation was observed in most of these digeneans' morphological and morphometric characteristics, which were agreed with Bakke (1988) and Gibson (1996). Secondly, the rate of parasitic infection is higher than that observed for *P. funduli* infecting *Fundulus scaidicus* from Cedar Creek in Western Nebraska (21-42.4%; Helt et al., 2003); *P. simile* Nybelin, 1926 infecting *Cirrhinus marginipinnis* from Bhumipol Dam, Tak Province, Thailand (16.6%, Sey et al., 2004); *P. pseudofolium* Nybelin, 1926 and *P. folium* Olfers, 1816 infecting *Gymnocephalus cernuus* from Lithuania (19, 12%, respectively; Stunžėnas et al., 2017); *P. punctati* infecting *Channa punctata* from Ozhakkodi Wayanad, Kerala, India (7%, Jithila & Prasadan, 2018); and *P. thunni* Baudin-Laurencin & Richard, 1973 infecting *Thunnus albacares* from Gulf of Guinea (14.8%, Guidelli et al., 2018). However, it is lower than *P. inecoli* infecting *Heterandria bimaculata* from Creek at Aqua Bendita, Xico, Veracruz, Mexico (96%, Razo-Mendivil et al., 2013); *P. hohhettae* infecting *Plectropomus leopardus* from Lizard Island, Northern Great Barrier Reef (80%, Ho et al., 2014); *P. vaili* infecting *Mulloidichthys vanicolensis* from Lizard Island, Northern Great Barrier Reef (100%, Ho et al., 2014); and *P. angulatum* infecting *S. lucioperca* from Lithuania (100%; Stunžėnas et al., 2017).

According to Campbell (2008), smooth tegument, thin body muscles, well-developed suckers, a wide hindbody where the gonads are located, pretesticular ovary, seminal vesicle, pars prostatica and undeveloped ejaculatory duct, genital pore close to the

intestinal bifurcation, uterus extending to the hindbody, and small and non-operculated eggs are the characteristic features described members within the Gorgoderidae family. The specimen recovered here from *P. rubescens* was described as belonging to the genus *Phyllodistomum* according to Campbell (2008) due to the site of infection, more-or-less foliate hindbody, blind caeca, a slender excretory bladder, arrangement and appearance of vitellarium and gonads, adjusting to the morphological variability recognized for the genus by Ho et al. (2014). At morphological and morphometric levels, the present gorgoderid species resembles species of *Phyllodistomum* Braun, 1899 with reference to the previously described *P. vaili* Ho, Bray, Cutmore, Ward & Cribb, 2014 that are closely resemble to each other by having all the characteristic features with similar body proportions and infection-specific site. The current study of *P. vaili* represents the first account of an endoparasite from the rosy goatfish. Also, it compared with adult stages of other *Phyllodistomum* species that infect different types of host species with the same sites of infection.

In addition, the present *P. vaili* is somewhat similar to: *P. mamaevi* Parukhin, 1970, *P. hoggettae* Ho, Bray, Cutmore, Ward & Cribb, 2014, and *P. marinae* Bravo-Hollis & Manter, 1957 by having strong marginal undulations; *P. crenilabri* Dolgikh & Naidenova, 1968, *P. hoggettae*, *P. lancea* Mamaev, 1968, *P. leilae* Nagaty, 1956, *P. lewisi* Srivastava, 1938, *P. mamaevi*, *P. marinae*, *P. pacificum* Yamaguti, 1951, *P. parukhini* Yamaguti, 1971, *P. pomacanthi* Nahhas & Cable, 1964, *P. sobolevi* Parukhin, 1979, *P. thalassomum* Soheir & Ahmed, 2000, *P. unicum* Odhner, 1902, *P. caudatum* Steelman, 1938, *P. carangis* MacCallum, 1913, *P. kanae* Nakao, 2015 by having the uterus is strongly developed intracaecally within hindbody; *P. borisbychowskyi* Caballero & Caballero, 1969, *P. centropomi* Mendoza-Garfias & Pérez-Ponce de Leon, 2005, *P. hoggettae*, *P. parukhini*, *P. unicum*, *P. cribbi* Pérez-Ponce de León et al., 2015, *P. magnificum* Cribb, 1987 by having entire lobed vitelline follicles; *P. cribbi* having ovary is smaller than the testes; *P. hyporhamphi* Cutmore & Cribb (2018) having a ventral sucker that is much smaller than an oral sucker; bipartite and saccular seminal vesicle; *P. pacificum* using the same body shape, 4–5 weak marginal undulations, slightly smaller ventral sucker than oral sucker; *P. pacificum* and *P. magnificum* by a bipartite and saccular seminal vesicle with posterodorsal chamber larger than anteroventral one; *P. simile* Nybelin, 1926 by having aspinous tegument.

However, it differs from other species of *Phyllodistomum*, as; *P. psettodi* Parukhin, 1966 by having the forebody occupy over half the body length; *P. borisbychowskyi*, *P. acceptum* Looss, 1901, *P. centropomi*, *P. lancea*, *P. mirandai* Lamothe-Argumedo, 1969, *P. tongaatense* Bray, 1985, *P. trinectes* Corkum, 1961, *P. scrippsi* Brooks & Mayes, 1975, *P. funduli*, *P. simile* by having the uterus is highly developed extracaecally or in the forebody; *P. acceptum*, *P. crenilabri*, *P. lancea*, *P. leilae*, *P. lewisi*, *P. mirandai*, *P. pomacanthi*, *P. scrippsi*, *P. sobolevi* and *P. tongaatense* by having firmly to deeply indented vitelline lobes; *P. sobolevi* by having deeply lobed vitelline follicles; *P. acceptum*, *P. borisbychowskyi*, *P. lancea*, *P. leilae*, *P. mamaevi*, *P. marinae*, *P. parukhini*, *P. scrippsi*, *P. sobolevi*, *P. thalassomum* and *P. unicum* which all of them have reported minimum lengths of at least 5.4 mm; *P. acceptum*, *P. borisbychowskyi*, *P. crenilabri*, *P. lancea*, *P. leilae*, *P. mirandai*, *P. pacificum*, *P. parukhini*, *P. pomacanthi*, *P. scrippsi*, *P. sobolevi*, *P. thalassomum*, *P. tongaatense* and *P. trinectes* by lacking of marginal undulations in the hindbody; *P. hoggettae*, *P. hyporhamphi*, *P. simile* by having a spatulated body, number of marginal undulations (7-9), and ventral sucker that is clearly larger than an oral sucker for the former; both seminal vesicle chambers of similar size for the latter species.

It also has some distinctions with *P. cribbi* by having spatulated body, tegument with numerous tiny papillae, lack of cephalic glands, oral sucker with 8 papillae, ventral sucker with 6 papillae, seminal vesicle saccular and relatively short, ovary lobed (3-5 lobes); *P. wallacei* Pérez-Ponce de León et al., 2015 by having oral sucker bears 14 well-developed papillae, ventral sucker has six well-developed papillae;

P. wallacei, *P. inecoli* Razo-Mendivil, Perez-Ponce de Leon, & Rubio-Godoy, 2013 and *P. spinopapillatum* Pérez-Ponce de León et al. (2015) by having spatuled to elongate body, presence of irregular number of dome-like papillae on the ventral surface; *P. cribbi*, *P. wallacei*, *P. hyporhamphi*, *P. magnificum*, *P. biringo* Shimazu (2005), *P. punctati* by having uterus extensive in the hindbody and developed inter- and extracaecal; *P. carangis* by possession of broadly rounded posterior and anterior ends, tri-lobed vitelline masses.

Some differences have also been reported with *P. magnificum* Cribb (1987), *P. punctati* having a spatulated body without prominent undulations, a ventral sucker with six prominent papillae for the former, and terminal oral sucker for the latter; *P. biringo*, *P. carassii* Long and Wai, 1958, *P. mogurndae* Yamaguti, 1934 by having banji-shaped body, pyriform seminal vesicle; *P. parasiluri* Yamaguti, 1934 by having lanceolate-oblong shape of the body; *P. carassii*, *P. mogurndae*, *P. parasiluri* with uterus much folded in post-vitelline field of hindbody; *P. folium* Olfers, 1816 by having oral sucker is smaller than the ventral one; *P. caudatum* by having suckers of equal size, 6-lobed ovary; *P. americanum* Osborn, 1903, *P. pseudofolium* Nybelin, 1926 by having three undulations on each lateral side of the hindbody, both suckers have the same size with six papillae on the oral sucker and four around the ventral sucker, *P. angulatum* Linstow, 1907 by the presence of 14 sensory papillae are found around the oral sucker and 6 around the ventral one; *P. funduli* by having terminal oral sucker, sac-like seminal vesicle, no marginal undulations in the hindbody; *P. etheostomae* Fischthal, 1943 by having 2 marginal demarcation in the hindbody; *P. kanae* by having body pyriform in shape, hindbody foliates without crenulate margin, ventral sucker larger than oral sucker.

The combination of morphological and molecular data has become a common practice in the parasite classification and characterization (Curran et al., 2013; Tkach et al., 2013). Even though *Phyllodistomum* is one of the most diverse genera in the Digenea, from a molecular perspective, few species have been studied. As a result, few sequences of cox1, 28S rRNA genes and the ITS regions are available to classify different *Phyllodistomum* species molecularly (Razo-Mendivil et al., 2013). In this analysis, both nuclear 28S and 18S rRNA genes were used to determine the exact taxonomic position of the recovered gorgoderid species and genetic variations were found in the two molecular markers studied, this is consistent with Parker et al. (2010), Razo-Mendivil and Pérez-Ponce De León (2011), Snyder & Tkach (2011), and Pulis et al. (2013) who reported that these genes are considered as the important genes used to differentiate and classify closely related digenean species. As following the molecular information obtained, the establishment of the recovered species described herein is clearly supported and improved.

The phylogeny presented herein demonstrated the relationship between the three plagiorchiid suborders Xiphidiata, Lepocreadiata, and Monorchiata, this was agreed with Brooks et al. (2000), Cribb et al. (2001), and Olson et al. (2003) who reported that the relationship between these suborders, due to the presence of a uniformity, the cercarial penetration gland dorsally located to the oral sucker, which exhibited among members of Apocreadiata, Lepocreadiata, Monorchiata, Opisthorchioidea and Xiphidiata, which form a nested clade within the higher Plagiorchiida. Furthermore, Olson et al. (2003) stated that the crown clade of digenea is the Xiphidiata consisting of four superfamilies Microphalloidea Ward, 1901, Gorgoderoidea Looss, 1901, Allocreadioidea Looss, 1902, and Plagiorchioidea Lühe, 1901, and the union of these superfamilies is confirmed by the presence of a penetrating stylet in the cercariae, reflected in the name 'Xiphidiata', and this unique character absent in Haploporidae and some Acanthocolpidae. This consistent with our data that the last three superfamilies have shared.

Cutmore et al. (2013) stated that Gorgoderoidea consisted of Gorgoderidae, Dicrocoeliidae, Callodistomidae, Encyclometridae, Haploporidae, Paragonimidae, Orchipedidae, and Troglotrematidae. The present phylogenetic analysis found that Opisthorchioidea to be Haploporoidea's sister group. In addition, it was found that the

clade clustered Haploporoidea + Opisthorchioidea is closely associated with Lepocreadioidea. Bakhoum et al. (2011, 2015) agreed on this data. In addition, as described by Olson et al. (2003), Haploporidae nested in the same clade with Atractotrematidae. The current study showed that Gorgoderidae species formed sister group with Troglotrematidae + Callodistomidae + Orchipedidae, consistent with Bray & Blair (2008) and Olson et al. (2003) which reported that Callodistomidae + Gorgoderidae forming sister lineage with Orchipedidae and Dicrocoelidae + Encyclometridae. Choudhury et al. (2007) and Olson et al. (2003) reported that Acanthocolpidae, as defined herein, is probably reflected by the position of *Stephanostomum* as a sister-group to the Brachycladiidae within the Allocreadioidea. The Cephalogonimidae closely related in the present study to Plagiorchiidae, which is consistent with Mehra (1937), Prudhoe & Bray (1982), and Brooks et al. (1985). Furthermore, as described by Fernández et al. (1998a, b), and Cribb et al. (2001), there is a sister taxon relationship between Brachycladiidae and Acanthocolpidae.

Molecular phylogenetic analyses of Gorgoderidae members have recently shown that *Phyllodistomum* is paraphyletic (Razo-Mendivil et al., 2013; Cutmore et al., 2013; Petkevičiūtė et al., 2015), as described herein. The phylogenetic trees from 18S rRNA and 28S rRNA data sets were analysed separately in the present study. Both trees clearly showed that the recovered species represent independent lineage, and is part of a paraphyletic group, with 28S rRNA presenting the current species as the sister taxon to a clade containing at least eleven species of *Phyllodistomum*, Gorgodera, and Gorgoderina infecting fish. This data is consistent with that obtained by Cutmore et al. (2013), Nakao (2015), and Pérez-Ponce de León et al. (2015) who reported that this clade appeared to be related to species in which cystocercous cercariae develop in bivalves with family Sphaeriidae. While the present species nested as the sister taxon of *Gorgoderina* and *Gorgodera* species, which are parasites of the amphibian urinary bladder, in the tree of the current species with 18S rRNA phylogeny, which agreed with Mata-López & León-Règagnon (2006) and Campbell (2008) who stated that the broad and foliate hindbody of *Phyllodistomum* is a key morphological character that distinguishes this genus from *Gorgoderina* and *Gorgodera*, and furthermore *Gorgodera* has more testes than *Phyllodistomum* and *Gorgoderina*. The above-mentioned molecular phylogenies, however, indicate a probability that the hindbody shape and number of testes are homoplasious traits.

Hence, this study represents the consistency of *Phyllodistomum* classification. The current study reflects a combination of morphometric data and morphological analysis in conjunction with the molecular data used to accurately identify and determine the exact taxonomic status of the recovered gorgoderid species. In addition, for this parasite species in Saudi Arabia, *P. rubescens* was considered a new host with new locality records.

Acknowledgements

References

Bakke TA, Bailey RE. *Phyllodistomum umblae* (Fabricius) (Digenea, Gorgoderidae) from the British Columbia salmonids: A description based on light and scanning electron microscopy. *Can J Zool* 1987; 65(7): 1703-1712. http://dx.doi.org/10.1139/z87-263.

Bakke TA. Morphology of adult *Phyllodistomum umblae* (Fabricius) (Platyhelminthes, Gorgoderidae): The effect of preparation, killing and fixation procedures. *Zool Scr* 1988; 17(1): 1-13. http://dx.doi.org/10.1111/j.1463-6409.1988.tb00082.x.

Bakhoum AJS, Bâ CT, Shimalov VV, Torres J, Miquel J. Spermatological characters of the digenean *Rubenstrema exasperatum* (Rudolphi, 1819) (Plagiorchioidea, Omphalometridae). *Parasitol Res* 2011; 108(5): 1283-1293. http://dx.doi.org/10.1007/s00436-010-2178-2. PMid:21127907.

Bakhoum AJS, Quilichini Y, Justine JL, Bray RA, Miquel J, Feliu C, et al. First spermatological study in the Atractotrematidae (Digenea, Haploporoidea): the case of *Atractotrema sigani*, intestinal parasite of *Siganus lineatus. Parasite* 2015; 22: 26. http://dx.doi.org/10.1051/parasite/2015026. PMid:26475645.

Bray RA, Blair D. Superfamily Gorgoderoidea Looss, 1899. In: Bray RA, Gibson DI, Jones A. *Keys to the Trematoda, volume 3*. Wallingford: CAB International, Natural History Museum; 2008. p. 187-190.

Brooks DR, MacDonald CA. A new species of *Phyllodistomum* Braun, 1899 (Digenea: Gorgoderidae) in a Neotropical catfish, with discussion of the generic relationships of the Gorgoderidae. *Can J Zool* 1986; 64(6): 1326-1330. http://dx.doi.org/10.1139/z86-197.

Brooks DR, O'Grady RT, Glen DR. Phylogenetic analysis of the Digenea (Platyhelminthes: Cercomeria) with comments on their adaptive radiation. *Can J Zool* 1985; 63(2): 411-443. http://dx.doi.org/10.1139/z85-062.

Brooks DR, Pérez-Ponce de León G, León-Régágnon V. Phylogenetic analysis of the Enenteridae (Digenea, Lepocreadiidae) and discussion of the evolution of the digenean fauna of kyphosid fishes. *Zool Scr* 2000; 29(3): 237-246. http://dx.doi.org/10.1046/j.1463-6409.2000.00042.x.

Bush AO, Lafferty KD, Lotz JM, Shostak AW. Parasitology meets ecology on its own terms: Margolis et al. revisited. *J Parasitol* 1997; 83(4): 575-583. http://dx.doi.org/10.2307/3284227. PMid:9267395.

Campbell RA. *Degeneria halosauri* (Bell 1887) gen. et comb. n. (Digenea: Gorgoderidae) from the deep-sea teleost *Halosauropsis macrochir. J Parasitol* 1977; 63(1): 76-79. http://dx.doi.org/10.2307/3280106. PMid:845742.

Campbell RA. Family Gorgoderidae Looss, 1899. In: Bray RA, Gibson DI, Jones A. *Keys to the Trematoda, volume 3*. Wallingford: CAB International, Natural History Museum; 2008. p. 191-213.

Choudhury A, Rosas Valdez R, Johnson RC, Hoffmann B, Pérez-Ponce de León G. The phylogenetic position of Allocreadiidae (Trematoda: Digenea) from partial sequences of the 18S and 28S ribosomal RNA genes. *J Parasitol* 2007; 93(1): 192-196. http://dx.doi.org/10.1645/GE-966R.1. PMid:17436963.

Cribb TH, Bray RA, Littlewood DTJ, Pichelin S, Herniou EA. The Digenea. In: Littlewood DTJ, Bray RA. *Interrelationships of the Platyhelminthes*. London: Taylor and Francis; 2001. p. 168-185.

Cribb TH, Bray RA. Gut wash, body soak, blender, and heat-fixation: approaches to the effective collection, fixation and preservation of trematodes of fishes. *Syst Parasitol* 2010; 76(1): 1-7. http://dx.doi.org/10.1007/s11230-010-9229-z. PMid:20401574.

Cribb TH, Chisholm LA, Bray RA. Diversity in the Monogenea and Digenea: Does lifestyle matter? *Int J Parasitol* 2002; 32(3): 321-328. http://dx.doi.org/10.1016/S0020-7519(01)00333-2. PMid:11835972.

Cribb TH. A new species of *Phyllodistomum* (Digenea: Gorgoderidae) from Australian and New Zealand freshwater fishes with notes on the taxonomy of *Phyllodistomum* Braun, 1899. *J Nat Hist* 1987; 21(6): 1525-1538. http://dx.doi.org/10.1080/00222938700770951.

Curran SS, Tkach VV, Overstreet RM. A review of *Polylekithum* Arnold, 1934 and its familial affinities using morphological and molecular data, with description of *Polylekithum catahoulensis* sp. nov. *Acta Parasitol* 2006; 51(4): 238-248. http://dx.doi.org/10.2478/s11686-006-0037-1.

Curran SS, Tkach VV, Overstreet RM. A new species of *Homalometron* (Digenea: Apocreadiidae) from fishes in the northern Gulf of Mexico. *J Parasitol* 2013; 99(1): 93-101. http://dx.doi.org/10.1645/GE-3169.1. PMid:22924927.

Cutmore SC, Cribb TH. Two species of *Phyllodistomum* Braun, 1899 (Trematoda: Gorgoderidae) from Moreton Bay, Australia. *Syst Parasitol* 2018; 95(4): 325-336. http://dx.doi.org/10.1007/s11230-018-9784-2. PMid:29417344.

Cutmore SC, Miller TL, Curran SS, Bennett MB, Cribb TH. Phylogenetic relationships of the Gorgoderidae (Platyhelminthes: Trematoda), including the proposal of a new subfamily (Degeneriinae n. subfam.). *Parasitol Res* 2013; 112(8): 3063-3074. http://dx.doi.org/10.1007/s00436-013-3481-5. PMid:23760874.

Fernández M, Aznar FJ, Latorre A, Raga JA. Molecular phylogeny of the families Campulidae and Nasitrematidae (Trematoda) based on mtDNA sequence comparison. *Int J Parasitol* 1998a; 28(5): 767-775. http://dx.doi.org/10.1016/s0020-7519(98)00027-7. PMid:9650057.

Fernández M, Littlewood DTJ, Latorre A, Raga JA, Rollinson D. Phylogenetic relationships of the family Campulidae (Trematoda) based on 18S rRNA sequences. *Parasitology* 1998b; 117(4): 383-391. http://dx.doi.org/10.1017/S0031182098003126. PMid:9820860.

Gibson DI. Trematoda. In: Margolis L, Kabata Z. *Guide to the parasites of fishes of Canada. Part IV*. Ottawa: NRC CNRC; 1996. pp. 1-373. (Canadian Special Publication of Fisheries and Aquatic Sciences; vol. 124).

Goodchild CG. The life-history of *Phyllodistomum solidum* Rankin, 1937, with observations on the morphology, development and taxonomy of the Gorgoderinae (Trematoda). *Biol Bull* 1943; 84(1): 59-86. http://dx.doi.org/10.2307/1538050.

Guidelli G, Tavechio WLG, Ferreira BP. New host and geographic distribution of *Phyllodistomum thunni* (Trematoda, Gorgoderidae). *Mar Biodivers Rec* 2018; 11(9): 1-5. http://dx.doi.org/10.1186/s41200-018-0144-3.

Hall TA. BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp Ser* 1999; 41: 95-98.

Helt J, Janovy J Jr, Ubelaker J. *Phyllodistomum funduli* n. sp. (Trematoda: Gorgoderidae) from *Fundulus sciadicus* Cope from Cedar Creek in western Nebraska. *J Parasitol* 2003; 89(2): 346-350. http://dx.doi.org/10.1645/0022-3395(2003)089[0346:PFNSTG]2.0.CO;2. PMid:12760652.

Ho HW, Bray RA, Cutmore SC, Ward S, Cribb TH. Two new species of *Phyllodistomum* Braun, 1899 (Trematoda: Gorgoderidae Looss, 1899) from Great Barrier Reef fishes. *Zootaxa* 2014; 3779(5): 551-562. http://dx.doi.org/10.11646/zootaxa.3779.5.5. PMid:24871750.

Hoffman GL. *Parasites of north american freshwater fish*. 2nd ed. Ithaca, New York: Comstock Publishing Associates, Cornell University Press; 1999.

Holl EJ. The phyllodistomes of North America. *Trans Am Microsc Soc* 1929; 48(1): 48-53. http://dx.doi.org/10.2307/3222458.

Indaryanto FR, Abdullah MF, Wardiatno Y, Tiuria R, Imai H. A description of *Lecithocladium angustiovum* (Digenea: Hemiuridae) in Short Mackerel, *Rastrelliger brachysoma* (Scombridae), of Indonesia. *Trop Life Sci Res* 2015; 26(1): 31-40. PMid:26868591.

Jithila PJ, Prasadan PK. Prevalence, intensity, mean abundance and description of *Phyllodistomum punctati* n. sp. (Digenea: Gorgoderidae) from the urinary bladder of *Channa punctata* (Bloch) from the Western Ghats, India. *Inter J Fish Aquat Stud* 2018; 6(5): 1-5.

Lee SU, Chun HC, Huh S. Molecular phylogeny of parasitic Platyhelminthes based on sequences of partial 28S rDNA D1 and mitochondrial cytochrome c oxidase subunit I. *Korean J Parasitol* 2007; 45(3): 181-189. http://dx.doi.org/10.3347/kjp.2007.45.3.181. PMid:17876163.

Lewis FG. The trematode genus *Phyllodistomum* Braun. *Trans Am Microsc Soc* 1935; 54(2): 103-117. http://dx.doi.org/10.2307/3222118.

Mata-López R, León-Règagnon V. Comparative study of the tegumental surface of several species of Gorgoderina Looss, 1902 (Digenea: Gorgoderidae), as revealed by scanning electron microscopy. *Comp Parasitol* 2006; 73(1): 24-34. http://dx.doi.org/10.1654/4186.1.

Mehra HR. Certain new and already known distomes of the family Lepodermatidae Odhner (Trematoda), with a discussion on the classification of the family. *Z Parasitenkd* 1937; 9(4): 429-469. http://dx.doi.org/10.1007/BF02120292.

Mendoza-Garfias B, Pérez-Ponce De Léon G. *Phyllodistomum centropomi* sp. n. (Digenea: Gorgoderidae), a parasite of the fat snook, *Centropomus parallelus* (Osteichthyes: Centropomidae), in the Papaloapan River at Tlacotalpan, Veracruz State, Mexico. *Zootaxa* 2005; 1056(1): 43-51. http://dx.doi.org/10.11646/zootaxa.1056.1.3.

Nakao M. *Phyllodistomum kanae* sp. nov. (Trematoda: Gorgoderidae), a bladder fluke from the Ezo salamander *Hynobius retardatus. Parasitol Int* 2015; 64(5): 314-318. http://dx.doi.org/10.1016/j.parint.2015.04.003. PMid:25892565.

Olson PD, Cribb TH, Tkach VV, Bray RA, Littlewood DTJ. Phylogeny and classification of the Digenea (Platyhelminthes: trematoda). *Int J Parasitol* 2003; 33(7): 733-755. http://dx.doi.org/10.1016/S0020-7519(03)00049-3. PMid:12814653.

Parker JH, Curran SS, Overstreet RM, Tkach VV. Examination of *Homalometron elongatum* Manter, 1947 and description of a new congener from *Eucinostomus currani* Zahuranec, 1980 in the Pacific Ocean off Costa Rica. *Comp Parasitol* 2010; 77(2): 154-163. http://dx.doi.org/10.1654/4451.1.

Pérez-Ponce de León G, Aquinoa AM, Mendoza-Garfias B. Two new species of *Phyllodistomum* Braun, 1899 (Digenea: Gorgoderidae), from freshwater fishes (Cyprinodontiformes: Goodeidae: Goodeinae) in central Mexico: An integrative taxonomy approach using morphology, ultrastructure and molecular phylogenetics. *Zootaxa* 2015; 4013(1): 87-99. http://dx.doi.org/10.11646/zootaxa.4013.1.6.

Petkevičiūtė R, Stunžėnas V, Stanevičiūtė G, Zhokhov AE. European *Phyllodistomum* (Digenea, Gorgoderidae) and phylogenetic affinities of Cercaria duplicata based on rDNA and karyotypes. *Zool Scr* 2015; 44(2): 191-202. http://dx.doi.org/10.1111/zsc.12080.

Prudhoe S, Bray RA. *Platyhelminth parasites of the Amphibia*. London: British Museum (Natural History); Oxford University Press; 1982.

Pulis EE, Fayton TJ, Curran SS, Overstreet RM. A new species of *Intromugil* (Digenea: Haploporidae) and redescription of *Intromugil mugilicolus*. *J Parasitol* 2013; 99(3): 501-508. http://dx.doi.org/10.1645/12-106.1. PMid:23327447.

Rai SL. Observations on the life-history of *Phyllodistomum srivastavai* sp. nov. (Trematoda: gorgoderidae). *Parasitology* 1964; 54(1): 43-51. http://dx.doi.org/10.1017/S0031182000074308.

Razo-Mendivil U, Pérez-Ponce de León G, Rubio-Godoy M. Integrative taxonomy identifies a new species of *Phyllodistomum* (Digenea: Gorgoderidae) from the two spot livebearer, *Heterandria bimaculata* (Teleostei: Poeciliidae), in Central Veracruz, Mexico. *Parasitol Res* 2013; 112(12): 4137-4150. http://dx.doi.org/10.1007/s00436-013-3605-y. PMid:24022129.

Razo-Mendivil U, Pérez-Ponce de León G. Testing the evolutionary and biogeographical history of Glypthelmins (Digenea: Plagiorchiida), a parasite of anurans, through a simultaneous analysis of molecular and morphological data. *Mol Phylogenet Evol* 2011; 59(2): 341-351. http://dx.doi.org/10.1016/j.ympev.2011.02.018. PMid:21356320.

Schell SC. The life history of *Phyllodistomum staffordi* Pearse, 1924 (Trematoda: gorgoderidae Looss, 1901). *J Parasitol* 1967; 53(3): 569-576. http://dx.doi.org/10.2307/3276717. PMid:6026845.

Sey O, Wongsawad C, Wongsawad P. Trematodes from fish in the Bhumipol Dam, Tak Province, Thailand. *Southeast Asian J Trop Med Public Health* 2004; 35(Suppl. 1): 284-287.

Shimazu T. Digeneans found in fresh- and brackish-water fishes of Lake Ogawara in Aomori Prefecture, Japan. *Bull Natn Sci Mus* 2005; 31(4): 137-150.

Snyder SD, Tkach VV. *Aptorchis kuchlingi* n. sp. (Digenea: Plagiorchioidea) from the Oblong Turtle, *Chelodina oblonga* (Pleurodira: Chelidae), in Western Australia. *Comp Parasitol* 2011; 78(2): 280-285. http://dx.doi.org/10.1654/4401.1.

Stunžėnas V, Petkevičiūtė R, Poddubnaya LG, Stanevičiūtė G, Zhokhov AE. Host specificity, molecular phylogeny and morphological differences of *Phyllodistomum pseudofolium* Nybelin, 1926 and *Phyllodistomum angulatum* Linstow, 1907 (Trematoda: Gorgoderidae) with notes on Eurasian ruffe as final host for *Phyllodistomum* spp. *Parasit Vectors* 2017; 10(1): 286. http://dx.doi.org/10.1186/s13071-017-2210-9. PMid:28587614.

Thomas JD. Life-history of *Phyllodistomum simile* Nybelin. *Nature* 1956; 178(4540): 1004. http://dx.doi.org/10.1038/1781004a0.

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG. The CLUSTAL-X windows interface: Flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* 1997; 25(24): 4876-4882. http://dx.doi.org/10.1093/nar/25.24.4876. PMid:9396791.

Tkach VV, Curran SS, Bell JA, Overstreet RM. A new species of *Crepidostomum* (Digenea: Allocreadiidae) from *Hiodon tergisus* in Mississippi, and molecular comparison with three congeners. *J Parasitol* 2013; 99(6): 114-1121. http://dx.doi.org/10.1645/13-279.1. PMid:23915018.

Ubelaker JE, Olsen AW. Life cycle of *Phyllodistomum bufonis* (Digenea: Gorgoderidae) from the boreal toad, *Bufo boreas. Proc Helminthol Soc Wash* 1972; 39: 94-100.

Vidal-Martínez V, Aguirre-Macedo ML, Scholz T, González-Solís D, Mendoza EF. *Atlas de los helmintos parásitos de cíclidos en México.* México: Instituto Politécnico Nacional; 2002. 182 p.