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

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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 espécies digenéticas que infectam uma das espécies economicamente mais importantes do Golfo Arábico, o peixe-cabra rosado Parupeneus rubescens. Uma espécie de plagiorquide 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
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espécie de plagiorquideo, 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 Phyl lodistomum, intimamente relacionado ao P. vaili descrito anteriormente (gb | KF013187.1, KF013173.1). O presente estudo revelou, portanto, que a espécie Phyl lodistomum vaili 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 Gorgodoidea, Olson et al. (2003) recognized the Gorgoderidae, one of four superfamilies within the Xiphidiata crown suborder. Gorgodoidea 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 Gorgodoidea, 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 Gorgodoidea 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 Phyl lodistomoides Brooks, 1977. The subfamily includes the genus Phyl lodistomum 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 Phyl lodistomum. The genus Phyl lodistomum Braun, 1899 was erected for accommodating several trematode species from the urinary bladders of a variety of ectothermic vertebrates,
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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 (NIKON 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 spectrophotometer (Thermo Fisher 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 vailii* 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** | *Anguilla* rehardtii (New Zealand) | 1.92-4.54 (2.93) | 0.20-0.36 (0.27) | 0.20-0.45 (0.34) | 0.16-0.39 (0.27) | 0.22-0.48 (0.31) | 0.08-0.22 (0.16) | 0.036-0.043 (0.038) |
| Cribb, 1987     |                 | (1.33)     | (0.24)         | (0.34)          | (0.21)         | (0.23)         | (0.13) |      |
| **P. funduli**  | *Fundulus* sciadicus (Nebraska) | 1.10-5.49 (2.06) | 0.20-0.83 (0.33) | 0.15-0.73 (0.29) | 0.07-0.36 (0.18) | 0.05-0.27 (0.09) | 0.10-0.85 (0.21) | 0.10-0.51 (0.20) |
| Helt et al., 2003 |                 | (1.09)     | (0.32)         | (0.29)          | (0.05-0.24) (0.08) | (0.05-0.24) (0.19) | (0.23) |      |
| **P. simile**   | *Cirrhinus* marginipinnis (Thailand) | 1.6-1.9 ×1.0 | 0.20-0.22 (0.21) | 0.31-0.35 ×0.30-0.35 | 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 |
| Sey et al., 2004 |                 |            |              |                | (1.0)          | (0.10)         | (0.011) |      |
| **P. inceolii** | *Heterandria* bimaculata (Mexico) | 1.15-2.08 (1.60) | 0.16-0.30 (0.23) | 0.14-0.27 (0.20) | 0.06-0.16 (0.09) | 0.04-0.06 ×0.07-1.0 | 0.06-0.20 (0.13) | 0.014-0.018 (0.016) |
| Razo-Mendivil et al., 2013 |                 | (0.80)     | (0.21)         | (0.19)          | (0.01-0.04) (0.07) | (0.01-0.04) (0.14) | (0.11) |      |
| **P. biringo**  | *Gymnogobius* breunigii (Japan) | 0.70-1.44 ×0.37-0.71 | 0.08-0.12 ×0.12-0.14 | 0.05-0.06 ×0.09-0.11 | 0.09-0.17 ×0.09-0.19 | 0.09-0.17 ×0.09-0.19 | 0.06-0.15 ×0.09-0.14 | 0.033-0.048 ×0.021-0.024 |
| Shimazu, 2005   |                 |            |              |                | (0.11-0.16) | (0.09-0.11) | (0.09-0.14) |      |
| **P. carassii** | *Carassius auratus* (Japan) | 3.92-5.37 ×2.96-3.42 | 0.56-0.61 ×0.62 | 0.48-0.75 ×0.50-0.12 | 0.35 ×0.27 | 0.19-0.29 ×0.10-0.24 | -- | 0.017-0.019 ×0.013 |
| Shimazu, 2005   |                 | (0.45-0.48) | (0.53-0.64) | (0.25-0.30) | (0.19) | (0.14) | (0.16) |      |
| **P. mogurnda** | *Odontobatis* obscura (Japan) | 1.74-4.01 ×1.03-1.98 | 0.25-0.43 ×0.19-0.33 | 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 |
| Shimazu, 2005   |                 | (0.20-0.41) | (0.21-0.34) | (0.21-0.34) | (0.19-0.22) (0.12) | (0.11-0.25) (0.078) | (0.033-0.081) (0.053) |      |
| **P. hoggettae**| *Plectropomus* leopardus (Great Barrier Reef) | 3.48-4.76 ×0.96-1.85 | 0.28-0.44 (0.33) | 0.36-0.55 (0.43) | 0.12-0.27 (0.17) | 0.07-0.36 (0.27) | 0.17-0.45 (0.30) | 0.07-0.33 (0.20) |
| Ho et al., 2014 |                 | (1.50)     | (0.30)         | (0.44)          | (0.12)         | (0.12)         | (0.12) | 0.036-0.057 (0.049) |
| **P. vailii**   | *Mulliodichthys vanicolensis* | 1.49-2.30 (1.84) | 0.13-0.20 (0.16) | 0.11-0.18 ×0.09-0.17 | 0.051-0.083 (0.054) | 0.08-0.19 | 0.06-0.21 (0.10) | 0.05-0.15 (0.041) | 0.027-0.050 (0.041) |
| Ho et al., 2014 |                 | (0.40-0.68) | (0.15)         | (0.14)          | (0.12)         | (0.12)         | (0.12) | 0.014-0.029 (0.022) |
| **P. parasiluri**| *Silurus* asotus (Japan) | 1.13-3.49 ×0.41-1.04 | 0.09-0.25 ×0.16-0.35 | 0.04-0.16 ×0.11-0.24 | 0.22-0.63 ×0.22-0.54 | 0.25-0.82 ×0.14-0.21 | 0.09-0.25 ×0.04-0.13 | 0.011-0.022 ×0.008-0.016 |
| Shimazu, 2005   |                 | (0.54)     | (0.15)         | (0.14)          | (0.12)         | (0.14)         | (0.12) |      |
| **P. cribbi**   | *Zoogoneticus* quiteensis (Mexico) | 0.92-1.84 (1.46) | 0.17-0.29 ×0.23 | 0.14-0.25 ×0.20 | 0.12-0.27 (0.07) | 0.06-0.14 (0.10) | 0.08-0.30 (0.17) | 0.06-0.17 (0.11) |
| Pérez-Ponce de León et al., 2015 |                 | (0.47-1.22) | (0.23)         | (0.20)          | (0.19)         | (0.17)         | (0.17) | 0.018-0.037 (0.028) |
| **P. kanae**    | *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 | 0.22-0.29 (0.16-0.17) | 0.69-0.83 ×0.52-0.54 | 0.88-0.92 ×0.41-0.52 | 0.39-0.40 (0.049-0.054) |
| Nakao, 2015     |                 | (0.14-0.011) | (0.22)         | (0.22)          | (0.27)         | (0.24)         | (0.27) | 0.034-0.037 |
| Parasite species | Host (Locality) | Body size | Oral sucker | Ventral sucker | Vitelline glands | Anterior testis | Posterior testis | Ovary | Eggs |
|-----------------|----------------|-----------|-------------|---------------|-----------------|----------------|----------------|-------|------|
| P. wallacei     | 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) × 0.06-0.25 (0.12) × 0.06-0.19 (0.13) × 0.05-0.20 (0.13) × 0.019-0.032 (0.025) × 0.013-0.23 (0.018) | 0.14-0.18 | |
| P. hyporhamphi  | 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) | R: 0.03-0.11 (0.07) × 0.07-0.13 (0.10) × 0.08-0.16 (0.11) × 0.12-0.15 (0.13) × 0.013-0.23 (0.018) | |
| P. pacificum    | 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) | R: 0.08-0.16 (0.11) × 0.12-0.19 (0.14) × 0.16-0.30 (0.20) × 0.032-0.051 (0.042) × 0.021-0.032 (0.016) | 0.12-0.16 (0.16) × 0.054-0.098 (0.081±0.001) | 0.051-0.083 (0.081±0.001) × 0.09-0.17 (0.11±0.01) × 0.06-0.19 (0.11±0.01) × 0.06-0.17 (0.11±0.01) × 0.032-0.059 (0.050±0.001) | |
| P. puntat i     | 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) | R: 0.09-0.29 (0.17) × 0.06-0.27 (0.16) × 0.10-0.37 (0.23) × 0.11-0.43 (0.24) × 0.024-0.029 (0.025) × 0.012-0.020 (0.016) | 0.11-0.43 (0.16) × 0.054-0.098 (0.081±0.001) | 0.035-0.087 (0.064±0.001) | |
| P. vaili        | 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) × 0.10-0.16 (0.13±0.01) | 0.10-0.16 (0.13±0.01) × 0.051-0.083 (0.15±0.01) × 0.09-0.17 (0.16±0.01) × 0.06-0.19 (0.11±0.01) × 0.06-0.17 (0.11±0.01) × 0.032-0.059 (0.050±0.001) | R: 0.049-0.118 (0.13±0.01) × 0.07-0.15 (0.13±0.01) × 0.06-0.16 (0.13±0.01) × 0.04-0.15 (0.13±0.01) × 0.015-0.027 (0.09±0.001) × 0.020-0.001 (0.086±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 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 antiovian side of the body dorsally close to the vitellaria. 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% |
| *Polykleithum ictaluri* | Plagiorchiida/Allocrastiidae | *Ictalus furcatus* | EF032697.1 | 88.66% | 52.06% |
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), *Phylodistomum sp.* (91.58%, gb| KF013179.1).
Plagiorchiida of Parupeneus rubescens

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 Gibsoniervinidae 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 taxa.

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, Opisthorchiida, 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 ramada        | FJ211228.1   | 90.92%               | 51.81%     |
| Dicrogaster perpusilla    | Plagiorchiida/Haploporidae | Liza ramada        | 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%     |
| Rubenstremat 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       | coeruleoaalba                 |                       |               |                      |                |
| Paragonimus     | Plagiorchiida/Troglotrematidae| Rattus norvegicus     | AY222141.1    | 89.85%               | 51.77%         |
| iloktsuenensis   | --                            |                       |               |                      |                |
| Nasitrema       | Opisthorchiida/Nasitrematidae | --                    | AJ004968.1    | 89.85%               | 51.05%         |
| globicephalae    | Plagiorchiida/Brachycladiidae | --                    | FJ357162.1    | 89.85%               | 51.23%         |
| Synthesium       | Plagiorchiida/Troglotrematidae| Neomys anomalus       | AY222139.1    | 89.85%               | 51.07%         |
| pontoporariae    | Plagiorchiida/Brachycladiidae | Balanopeneta          | KR703279.1    | 89.85%               | 51.68%         |
| Nephrotrema      | Plagiorchiida/Troglotrematidae| --                    |               |                      |                |
| truncatum        | Plagiorchiida/Brachycladiidae | --                    |               |                      |                |
| Brachycladium    | Plagiorchiida/Brachycladiidae | --                    |               |                      |                |
| goliath          | --                            |                       |               |                      |                |
| Stephanostomum   | Opisthorchiida/Acanthocolpidae| Lophius piscatorius   | DQ248213.1    | 89.85%               | 51.05%         |
| cestillium       | --                            |                       |               |                      |                |
| Skrjabinoeces    | Plagiorchiida/Plagiorchiidae  | Rana ridibunda        | AJ287575.1    | 89.51%               | 50.17%         |
| similis          | --                            |                       |               |                      |                |
| Auridistomum     | Plagiorchiida/Auridistomidae  | Chelydra serpentina   | AY222159.1    | 89.40%               | 50.88%         |
| chelydrae        | --                            |                       |               |                      |                |
| Pseudomegasolena | Plagiorchiida/Haploporidae    | Scars rivulatus       | AJ287569.1    | 88.97%               | 49.92%         |
| ishigakiense     | --                            |                       |               |                      |                |
| Lissorchis       | Plagiorchiida/Lissorchidae    | Carpiodes cyprinus    | AY222136.1    | 88.79%               | 50.25%         |
| kritskyi         | --                            |                       |               |                      |                |

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 Plagiophorida, 91.19-88.97% with Haploporida, 89.85% with Troglotrematidae, 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 Opisthorthidae 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-Garfías & 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 Parupeneus 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 sciaicus from Cedar Creek in Western Nebraska (21-42.4%; Helt et al., 2003); P. similis Nybelin, 1926 infecting Cirrhinus marginipinnis from Bhumipol Dam, Tak Province, Thailand (16.6%, Sey et al., 2004); P. pseudofolium Nybelin, 1926 and P. folium Offers, 1816 infecting Gymnocephalus cernuus from Lithuania (19, 12%, respectively; Stunženas 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 Mullolidichthys vanicolensis from Lizard Island, Northern Great Barrier Reef (100%, Ho et al., 2014); and P. angulatum infecting S. lucioperca from Lithuania (100%; Stunženas 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 *Phylloidostomum* 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 *Phylloidostomum* 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 *Phylloidostomum* 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. pomacenthi* 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 highly developed extra caecally or in the forebody; *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 posteroventral chamber larger than anteroventral one; *P. simile* Nybelin, 1926 by having aspinous tegument.

However, it differs from other species of *Phylloidostomum*, 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* Lamotho-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 extra caecally or in the forebody; *P. acceptum*, *P. crenilabri*, *P. lancea*, *P. leilae*, *P. lewisi*, *P. mirandai*, *P. pomacenthi*, *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. pomacenthi*, *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;
Plagiorchiida of *Parupeneus rubescens*

*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 spatulated 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. parasiliuri* Yamaguti, 1934 by having lanceolate-oblong shape of the body; *P. carassii, P. mogurndae, P. parasiliuri* 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 Monochiata, 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, Monochiata, 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, Gorgoderoidae Looss, 1901, Allocrea rioidea 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 Haploporoidea and some Acanthocolepidae. This consistent with our data that the last three superfamilies have shared.

Cutmore et al. (2013) stated that Gorgoderoidae consisted of Gorgoderidae, Dicrocoeliidae, Cal lodistomidae, Encyclometridae, Haploporidae, Paragonimidae, Or chipedidae, 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. Bakhoun 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 Troglocreatidae + Calloidistomiidae + Orchipediae, consistent with Bray & Blair (2008) and Olson et al. (2003) which reported that Calloidistomiidae + Gorgoderidae forming sister lineage with Orchipediae and Dicroceliidae + 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 Brachyclostomidae within the Allocreadioidea. The Cephalogonimidae closely related in the present study to Plagiorchiidae, which is consistent with Mehr (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 Brachyclostomidae 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-Régagnon (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.

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