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Morphological and genetic identification of the gill monogenean parasite (*Diclidophora merlangi*) that infects Twobar Seabream Fish (*Acanthopagrus bifasciatus*) in the Arabian Gulf, Saudi Arabia

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Summary

Ectoparasites, particularly monogeneans, negatively affect fish health and growth. This study identified monogenean parasites in the twobar seabream, *Acanthopagrus bifasciatus* (Sparidae), inhabited the Arabian Gulf (Saudi Arabia). Following that, forty *A. bifasciatus* fish samples were visually examined for monogeneans. Parasite species were collected from the gills and then analyzed morphometrically, morphologically, and molecularly using the partial regions of the large subunit of ribosomal RNA (28S rRNA) and mitochondrial cytochrome C oxidase subunit I (COI) genes. Fish species were also identified using a DNA barcoding approach based on the COI gene. The monogenean species of *Diclidophora merlangi* (Diclidophoridae) were found in 45% of the fish species studied. The generic features of the *Diclidophora* genus distinguish this species. This species discriminated itself from congeners by having a muscular bulb with 17 grooved and recurved hooks, 218±10 (184-267) post-ovarian testes, and four pairs of pedunculated clamps of relative sizes. Partial 28S rRNA sequencing from monogeneans revealed that they grouped with members of the genus *Diclidophora*, forming a monophyletic group that supported the morphological descriptions. Molecular identification revealed that *D. merlangi* has a unique barcode made up of a COI sequence. The host identity was established as *A. bifasciatus* based on the COI gene sequences. Furthermore, a molecular phylogenetic study was performed to determine the phylogenetic affinity of parasite species and fish hosts. This study on *Diclidophora* species is considered the first record of this genus in the examined area.
Keywords: Monogenea; Polyopisthocotylea; Morphology; Host identity; 28S rRNA; COI

Introduction

Sparidae fish species have long been regarded as an important source of animal protein for trade and nutrition (Froese & Pauly, 2019). Like all other fish species, they are invaded by parasites. Monogenea are parasitic flatworms (Platyhelminthes) that typically infect the skin and gills of both marine and freshwater fish (Whittington *et al*., 2000). All monogeneans have a direct life cycle, with no intermediate hosts (Purivirojkul, 2008). Monopisthocotyleans and polyopisthocotyleans are the two primary categories based on their organ of attachment "opisthaptor" which is generally equipped with sclerotized structures such as hooks, clamps, and suckers (Öztürk & Özer, 2014).

The Diclidophoridae Cerfontaine, 1895 are polyopisthocotylean monogeneans of the Mazocraeidea order. According to the World Register of Marine Species (WoRMS) (2023), this family consists of 69 accepted genera, two taxon inquirendum, and one nomen nudum, characterized by the structure of the clamps and the mor-

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phology of the male copulatory organ. *Diclidophora* Krøyer, 1838 (Diclidophoridae) has a stenoxenic specificity for three families of Gadiformes (Gadidae, Macrouridae, and Moridae), with a particular attachment site on the host gills (Llewellyn *et al*., 1980). Taxa in this genus have complete or well-developed lamellate extensions in their haptoral clamps (Mamaev, 1976).

According to WoRMS (2023), there are presently twelve recognized species of the *Diclidophora* species: *D. merlangi* (Kuhn, 1829) Krøyer, 1838, *D. palmata* (Leuckart, 1830) Diesing, 1850, *D. luscae* (Van Beneden & Hesse, 1863) Price, 1943, *D. pollachii* (Van Beneden & Hesse, 1863) Price, 1943, *D. phycidis* (Parona & Perugia, 1889) Sproston, 1946, *D. pagelli* Gallien, 1937, *D. denticulate* (Olsson, 1876) Price, 1943, *D. esmarkii* (Scott, 1901) Sproston, 1946, *D. maccallumi* (Price, 1943) Sproston, 1946, *D. minor* (Olsson, 1876) Sproston, 1946, *D. micromesisti* Suriano & Martorelli, 1984, and *D. minuti* Tirard, Berrebi, Raibaut & Frenaud, 1992. However, recent studies have revealed the occurrence of *Diclidophora* species in different hosts: including *D. merlangi*, usually a parasite of the whiting *Merlangius merlangus* (L.), in cod, *Gadus morhua* (Perdiguero-Alonso *et al*., 2006) and brushtooth lizardfish, *Saurida undosquamis* (Morsy *et al*., 2018); *D. denticulata*, usually a parasite of *Pollachius virens*, in *Trisopterus minutus* (Strona *et al*., 2010); and *D. phycidis*, usually a parasite of *Phycis blennoides*, in *T. minutus* (Strona *et al*., 2010).

Various target regions, such as large subunit ribosomal RNA (28S rRNA) and mitochondrial cytochrome c oxidase I (COI), have been used to differentiate monogenean species (Catalanoet *et al*., 2010; Oliva *et al*., 2014; Mendoza-Franco *et al*., 2018; Víllora-Montero *et al*., 2020; Al-Nabati *et al*., 2021; Alghamdi *et al*., 2023; Abdel-Gaber *et al*., 2023). *Acanthopagrus* (Perciformes, Sparidae) is now a distinct genus with 20 accepted species (Pombo-Ayora *et al*., 2022). The twobar seabream, *Acanthopagrus bifasciatus* (Forsskål, 1775), is found in the Western Indian Ocean from the Red Sea and Arabian Gulf to Natal in South Africa. However, due to their morphological resemblance, several *Acanthopagrus* species are classified as cryptic taxa (Zhou *et al*., 2011; Wu *et al*., 2018). The COI gene is a standard molecular marker for fish DNA barcoding (Prioli Sônia *et al*., 2002; Ward *et al*., 2005; Saitoh *et al*., 2006; Zhang, 2011; Tan *et al*., 2012; Abbas *et al*., 2017; Wang *et al*., 2018) and has been successfully used for Sparidae barcoding (Ahmed *et al*., 2021; Abdel-Gaber *et al*., 2023; Nuryanto *et al*., 2023).

Some studies have been conducted on monogenean parasites infecting marine fish species in Saudi Arabia to date (Bayoumy *et al*., 2012; Mohamed *et al*., 2015; Bakhraibah, 2018; Dajem *et al*., 2019; Al-Nabati *et al*., 2021; Morsy *et al*., 2021; Abdel-Gaber *et al*., 2023, Alshehri *et al*., 2023; Alghamdi *et al*., 2022). The purpose of this study to investigate the natural occurrence of monogenean parasites in twobar seabream (*Acanthopagrus bifasciatus*) from the Arabian Gulf (Saudi Arabia), as well as to describe their morphology and phylogenetic relationships. It also sought to conduct host record investigations and genetically characterize fish species using the partial COI gene to validate their taxonomic and geographic status.

Materials and Methods

Fish samples collection

During the study period (January – June 2023), 40 samples of the twobar seabream, *Acanthopagrus bifasciatus* (Family Sparidae), were bought from local fishermen in the coastal region of the Arabian Gulf (Dammam, Saudi Arabia). Samples were transported to the laboratory in an ice box and classified using the standard taxonomic guidelines of Abu Shusha *et al*. (2010). Animals were used in the experiment following the institution's guidelines on the care and use of animals in research (approval no. KSU-SU-23-76).

Parasitological examination

To prepare for parasite investigation, the fish's gills were removed and cleaned in a standard saline solution (0.9 %) to eliminate any excess gill mucus. Furthermore, a small portion of the fish's hepatopancreas was excised, washed with saline, and kept in 96 % ethanol for molecular analysis. Monogeneans were removed from the gills using fine forceps under a stereomicroscope (Nikon SMZ18, NIS ELEMENTS software), fixed in AFA (70 % ethyl alcohol-formalin-acetic acid), and kept in 70 % ethyl alcohol till inspection. Following Georgiev *et al*. (1986), specimens were stained with Aceto carmine (Sigma-Aldrich, Missouri, USA). This was followed by washing in an ascending ethyl alcohol series, clearing in clove oil, and finally mounting in Canada balsam (Palm, 2004). Some specimens were mounted in glycerin ammonium picrate (GAP; Sigma-Aldrich, Burlington, MA, USA) to clarify the sclerotized features and examined under a Leica DM 2500 microscope (NIS ELEMENTS software, version 3.8). According to Bush *et al*. (1997), the prevalence (%) and mean intensity of monogeneans were evaluated. Photomicrographs of several body parts of monogenean parasites were taken with a Leica DM 2500 microscope (NIS ELEMENTS software, ver. 3.8). According to Al-Zubaidy (2013), measurements were taken using the ImageJ 1.53e program (Wayne Rasband and contributors, National Institute of Health, USA) and expressed in millimeters (mm).

Molecular analysis

Total genomic DNA was extracted from 70 % ethanol-preserved parasite and fish's hepatopancreas using the methods outlined in QIAamp® DNA Mini Kit (Qiagen, Germany). For monogenean parasite, PCR targeting the partial coding region of the 28S rRNA gene was then amplified using the forward primer (U178: 5'-GCA CCC GCT GAA YTT AAG-3') and the reverse primer (L1642: 5'-CCA GCG CCA TCC ATT TTC A-3') designed by Lockyer *et al*. (2003), as well as, the COI gene was amplified using the forward primer (COI-ASmit1: 5'-TTT TTT GGG CAT CCT GAG GTT TAT-3') and the reverse primer (COI-ASmit2: 5'-TAA AGA AAG AAC ATA ATG AAA ATG-3') designed by Littlewood *et al*. (1997). The COI

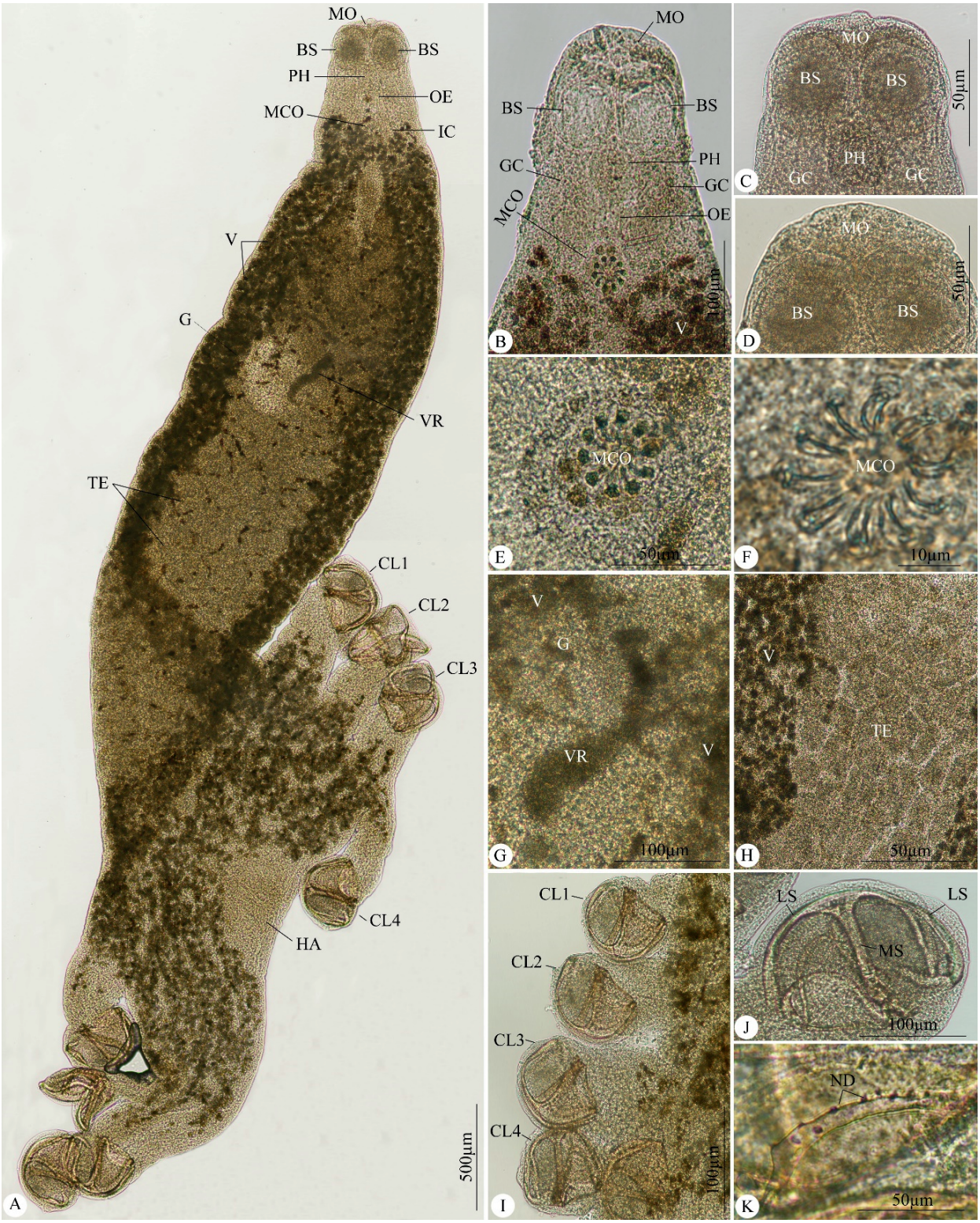

Fig. 1 Photomicrographs of *Diclidophora merlangi* infecting *Acanthopagrus bifasciatus*. (A) Whole-mount preparation. (B-K) High magnifications for different body parts, as follows: (B-F) Anterior portion of the prohaptor. (G) Germarium. (H) Testes. (I-K) Clamps in haptor. Note: MO, mouth; BS, buccal sucker; PH, pharynx; GC, gland cells; OE, oesophagus; MCO, male copulatory organ; IC, intestinal crura; V, vitellaria; VR, vitelline reservoir; G, germarium; TE, testes; CL, clamps; HA, haptor; LS, lateral sclerite; MS, median sclerite; ND, nodular openings.

gene was amplified in a fish host with the primers F1 (5'-TCA ACC AAC CAC AAA GAC ATT GGC AC-3') and R1 (5'-TAG ACT TCT GGG TGG CCA AAG AAT CA-3') (Ward *et al*., 2005). The thermal cycling conditions were: initial denaturation at 94°C for 5 min, 35 cycles of amplification {94°C for 30 sec of DNA denaturation, annealing step at 56°C (28S rRNA parasite), 50 °C (COI parasite), 52°C (COI host [fish]) for 30 sec, and 72°C for 1 min of extension}, and final extension at 72°C for 10 min. PCR products were analysed on a 1.5 % w/v agarose gel (Sigma-Aldrich, Missouri, USA) in 1× Tris-acetate-EDTA (TAE) and stained with SYBR Safe DNA gel dye (Thermo Fischer Scientific, Ottawa, Canada) against the GeneRuler 100bp Plus ready-to-use DNA ladder (Fermentas, Lithuania). The Sanger method was used for sequencing at Macrogen's facilities in Seoul, South Korea. The COI sequences were deposited in GenBank™ and compared to those accessible in the NCBI database. The phylogenetic analysis was done using MEGA X (Kumar *et al*., 2018). Maximum parsimony was used to generate the phylogenetic tree, with 1000 bootstrapping replicates.

Ethical Approval and/or Informed Consent

This research was approved by the Research Ethics Committee (REC) at King Saud University (approval number KSU-SU-23-76).

Results

Eighteen (45 %) of 40 twobar seabream fish, *Acanthopagrus bifasciatus*, had a monogenetic parasite in their gills. The parasite species *Diclidophora merlangi* (Kuhn, 1829) Krøyer, 1838 was identified based on morphological features. Each parasitized fish had an infection intensity of no more than 4, with a mean of 2.13.

Microscopic description (Fig. 1)

The body was flask-shaped, with a pronounced bottle-necked-shaped anterior end and broad posterior end, measuring 3.67±0.12 (2.97–4.26) × 0.72±0.01 (0.61–0.95). The anterior end has two spherical buccal suckers measuring 0.096±0.006 (0.089–

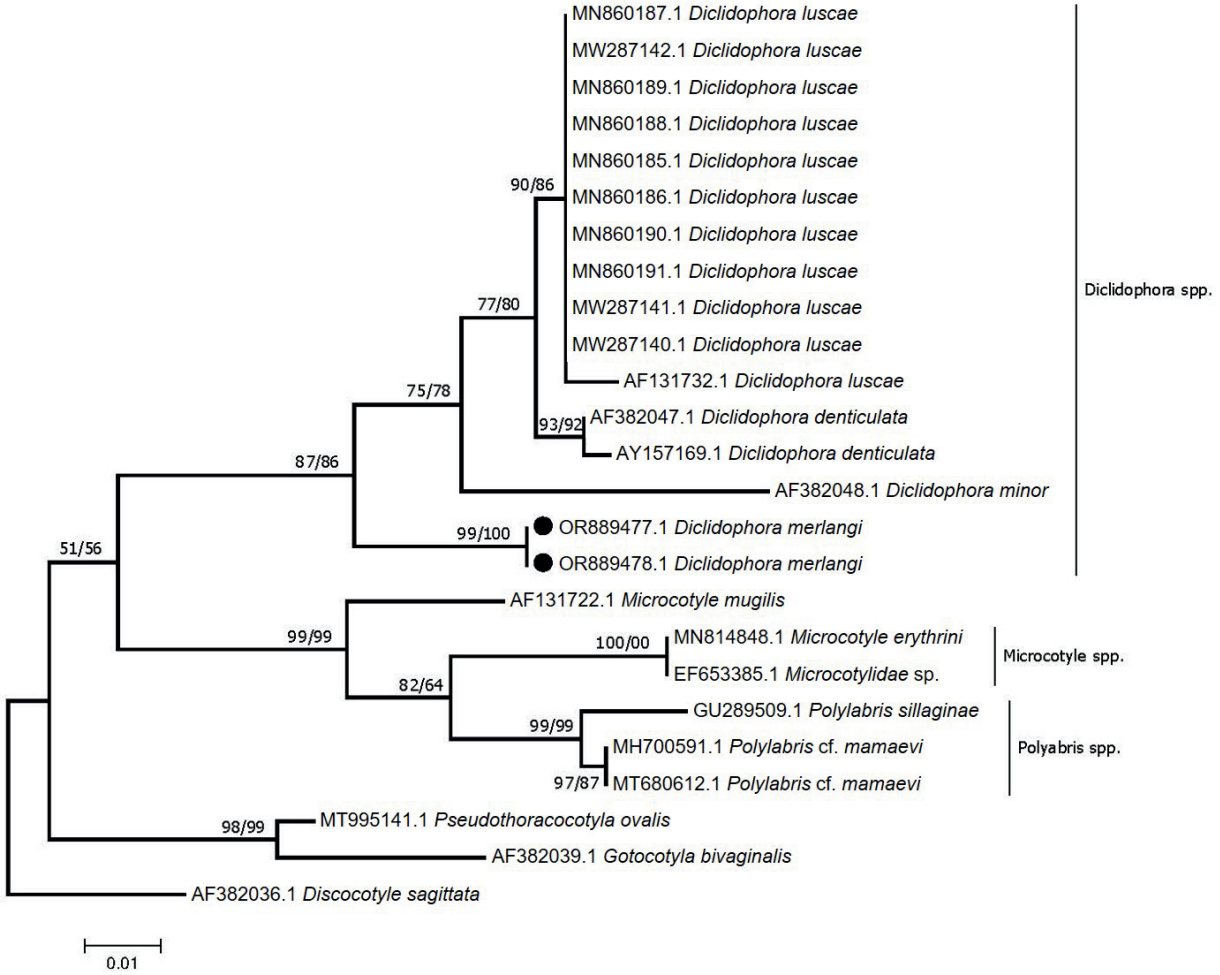

Fig. 2. A consensus phylogenetic tree constructed with maximum likelihood (ML) and Neighbor Joining (NJ) methods from the partial *28S rRNA* sequences, showing phylogenetic relationships between *Diclidophora merlangi* infecting *Acanthopagrus bifasciatus* (2 sequences (OR889477-OR889478)) and 22 related taxa in NCBI GenBank with *Discocotyle sagittata* as an outgroup. Numbers indicated at branch nodes are bootstrap values (ML/NJ).

0.105) × 0.076±0.004 (0.071–0.089), with a rounded mouth opening between them. The pharynx was oval, measuring 0.059±0.013 (0.041–0.075) × 0.048±0.008 (0.036–0.056). The oesophagus was short. The intestinal bifurcation occurred just before to the muscular bulb. The intestinal cecal branches varied in their extension into the haptoral region. The testes were sub-spherical or irregular, numbered 218±10 (184–267) and scattered in a pre-testicular region of 0.981±0.023 (0.801–1.26). They were positioned in the post-ovarian and extended to lateral fields among intestinal branches. The copulatory organ is funnel-shaped, with a muscular bulb (0.049±0.008 (0.039–0.055) in diameter) and a crown of 17 grooved and recurved hooks. The germarium had a median pre-testicular location and measured 0.232±0.034 (0.191–0.270). The vitelline follicles were small and abundant, covering the whole caeca. The haptor was 0.665±0.128 (0.459–0.752) long and armed with four pairs of pedunculated clamps. The first three pairs of clamps were nearly equal in size and measured 0.160±0.008 (0.150–0.166) × 0.142±0.008 (0.133–0.149), while the fourth pair was slightly smaller and measured 0.134±0.001 (0.130–0.140) × 0.112±0.003 (0.108–0.113). Each clamp is closed and consists of three pairs of lateral sclerites and one pair of median sclerites with small nodular openings that protrude as spiny processes on the ventromedial surface.

Molecular analysis (Figs. 2 – 3)

DNA amplification of the partial 28S rRNA region from *D. merlangi* yielded a fragment of ~350 bp. Two identical sequences were obtained from the organism detected in the current investigation, which was morphologically recognized as *D. merlangi* and deposited in GenBank™ under the accession numbers OR889477 and OR889478. GenBank™ had 14 sequences from members of the *Diclidophora* genus, which includes three species. *D. luscae* was

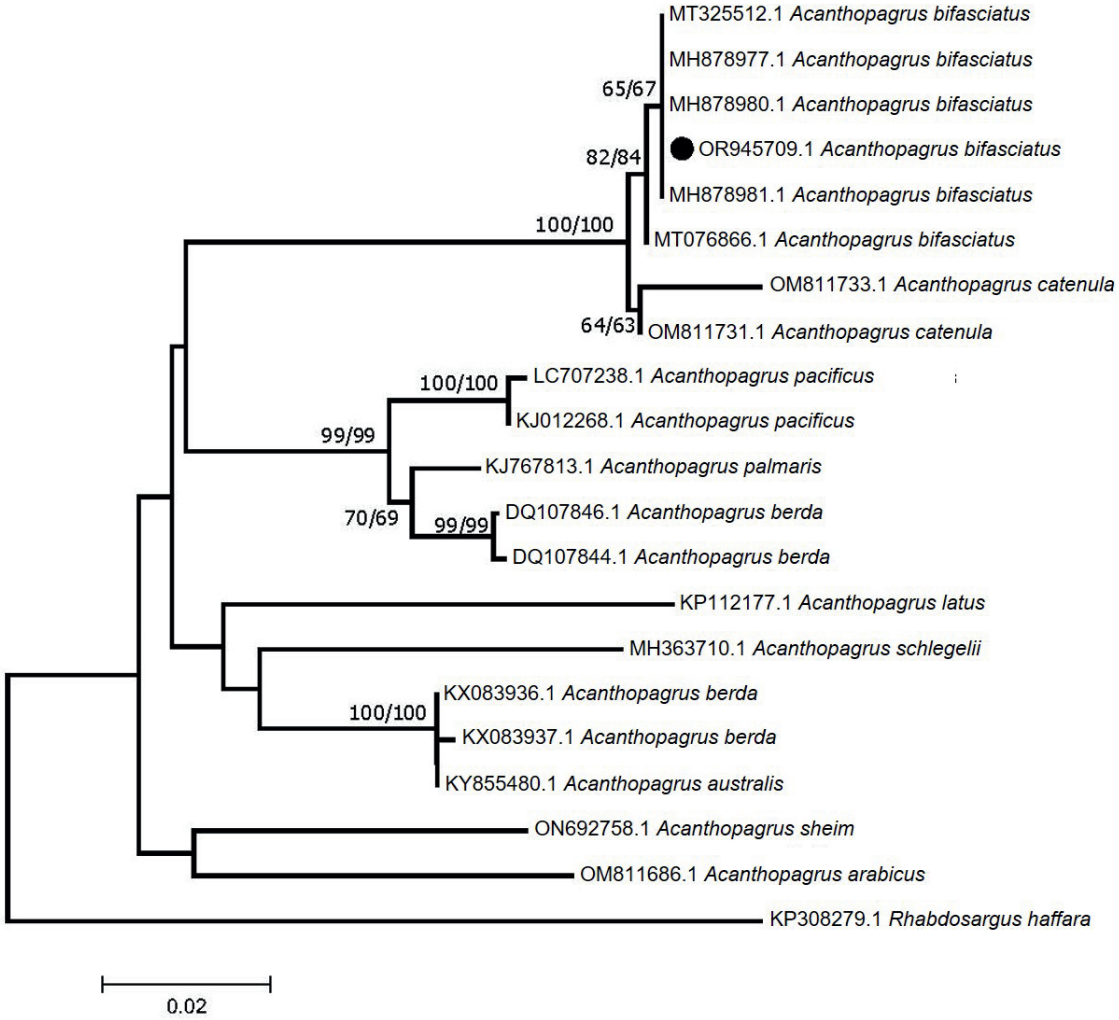

Fig. 3. A consensus phylogenetic tree constructed with maximum likelihood (ML) and Neighbor Joining (NJ) methods from the partial *COI* sequences, showing phylogenetic relationships between *Acanthopagrus bifasciatus* (one sequence (OR945709)) and 19 related taxa in NCBI GenBank with *Rhabdosargus haffara* as an outgroup. Numbers indicated at branch nodes are bootstrap values (ML/NJ).

represented by 11 sequences, *D. denticulata* was represented by 2, and *D. minor* by a single sequence. The current sequence identity ranged from 92.95 % in *D. minor* to 95.57 % in *D. luscae*, respectively. Phylogenetic analysis using Maximum Likelihood (ML) and Neighbour Joining (NJ) revealed that the sequences obtained in the current study clustered with the clade that included members of the genus *Diclidophora*, including *D. luscae, D. denticulata* and *D. minor* with strong bootstrap support (Fig. 2). *Diclidophora* members differed from *Microcotyle* spp. and *Polyabris* spp. (Fig. 2). *Discocotyle sagittata* (AF382036.1) was used as an outgroup.

Amplification of the partial mitochondrial cytochrome oxidase c subunit 1 (COI) gene yielded 400 bp. GenBank™ had COI sequences relating to members of the *Diclidophora* genus. As a result, it was not possible to compare the sequence we obtained in this study with comparable sequences from members of the genus *Diclidophora*. A sequence was obtained from the organism in this study and deposited in GenBank™ under the accession number OR936714. *C. brasiliensis* (MT890370.1) and *Choricotyle australiensis* (MT783687.1) were the closest matches to the *D. merlangi* sequence obtained in the current investigation, with nucleotide matches of 84.39 % and 83.75 %, respectively.

The host's identification was validated molecularly by amplifying the partial COI, yielding ~ 660 bp. Phylogenetic analysis revealed that both the ML and NJ trees gave the same topology. The host's DNA sequence was found to be 100 % identical to that *Acanthopagrus bifasciatus* from Kuwait (MH878977.1). The clade that included individuals from *A. bifasciatus* and *A. catenula* was distinct from the clades that included other *Acanthopagrus* members (Fig. 3). According to the results of the phylogenetic analysis of the sequence obtained in the current study (OR945709), the host for *D. merlangi* is *A. bifasciatus*. *Rhabdosargus haffara* (KP308279.1) from the Red Sea was used as an outgroup.

Discussion

Fish parasites have an essential role in aquatic biodiversity (Quiazon, 2015). Monogeneans are regarded one of the pathogens that cause the fish mortality, either directly or indirect (Mohamed *et al*., 2015). There are few researches on parasite taxa belonging to the Diclidophoridae family among various fish species. In this study, the gills of eighteen twobar seabream (45 %) were found to be naturally infected with a monogenean parasite within the polyopisthocotylean group, based on Öztürk and Özer (2014). The haptor of this group is equipped with clamps. The current prevalence rate of parasite infection is similar with prior data for *Diclidophora* species reported by Morsy *et al*. (2018, 40 %) in *Saurida undosquamis* in Hurghada coastlines along the Red Sea (Egypt). This prevalence rate, however, is higher than that reported for *Diclidophora* by Perdiguero-Alonso *et al*. (2006, 0.67 % and 0.72 %) in *Gadus morhua* (Table 1).

The morphological features of the monogenean species recovered from the gills of the twobar seabream fish were consistent with Rubec and Drobeb (1994) key to the *Diclidophora* genus. Members of this genus have a posterior haptor that is not separate from the body proper, no vagina, a triangular body tapering to its maximum width at the level of the first pair of clamps, and clamp morphology. The morphological features of the diclidophorid species described herein similar to those of *D. merlangi*, which was previously isolated from *Gadus morhua* and *Merlangius merlangus* (Perdiguero-Alonso *et al*., 2006) and *Saurida undosquamis* (Morsy *et al*., 2018), particularly the number of testes in the testicular area and hooks supporting the muscular bulb, which were within the range of those *Diclidophora* species, as well as the asymmetrical clamp length with small nodules in sclerites. The key distinction between *D. merlangi* from *A. bifasciatus* and two specimens from *M. merlangus* and *S. undosquamis* is the latter's significantly larger in the body size, which is consistent with Perdiguero-Alonso *et al*. (2006). However, the body size is not considered a reliable trait to differentiate *Diclidophora* species, especially when they are recorded from alternative hosts, and this is supported by Sproston (1946), Lewellyn and Tully (1969), and Llewellyn *et al*. (1980) who reported that some parasite species can be significantly smaller than the average even when isolated from their usual host.

Furthermore, the basic morphology and clamp structure of *D. paracoelorhynchi* is similar to *D. merlangi*, with distinction that the former *Diclidophora* species has a much larger and more powerful muscular sucker in each clamp than *D. merlangi*. However, *D. merlangi* might be separated from other *Diclidophora* species in the following ways: (I) *D. macruri* has clamps that are clearly longer than wide, (II) *D. coelorhynchi* has 18 cirrus hooks and pedunculated clamps in which the diagram is not entirely joined with the lateral sclerites or the base of the central sclerite, such that no ring is formed to hold the sucker, (III) *D. paracoelorhynchi* is up to twice as large as *D. merlangi*, with a lobed seminal receptacle and 40 – 60 testes, (IV) *D. srivastavai* isolated from *Setipinna phasa* has sessile clamps and a plumped body, (V) *D. indica* has varied forms of testes, (VI) *D. embiotocae* isolated from three embiotocid fish (*Amphisticus* rhodoterus, *Hyperprosopon ellipticum*, and *Hyperprosopon argenteum*), had 11 – 13 recurved hooks and 91 (78 – 96) testes, (VII) *D. nezumiae* isolated from *Nezumia bairdii* has short peduncles for clamps, cirrus with 10 – 13 (11) recurved hooks and 18 – 30 testes, (VIII) *D. luscae* (from *Trisopterus luscus*), *D. esmarkii* (from *Trisopterus esmarki*), *D. phycidis* (from *Phycis blennoides*) have 8 – 10 cirrus hooks, (IX) *D. palmata* isolated from *Molva molva* has palmate shaped haptoral peduncles and 19 genital corona spines, and (X) *D. denticulata* isolated from *Pollachius virens* has extremely large haptoral clamps and peduncles as well as muscular bulb with 13 – 15 spines, (XI) *D. minor* isolated from *Micromesistius poutassou* has distinctly demarcated haptor and the number of testes was 150, and (XII) *D. micromesisti* isolated from *Micromesistius australis* has buccal suckers distinctly smaller than pharynx.

Molecular confirmation of the parasite was achieved by analyzing sequence variation in both the 28S rRNA and COI gene regions.

Table 1. Comparative metrical data for *Diclidophora merlangi* and their congeneric species.

Comparable parameters	Perdiguero-Alonso *et al*. 2006			Morsy *et al*. 2018	Present study
Host	*Gadus morhua*	*Gadus morhua*	*Merlangius merlangus*	*Saurida undosquamis*	*Acanthopagrus bifasciatus*
Location	North Sea	Celtic Sea	Celtic Deep	Red Sea, Egypt	Arabian Gulf, Saudi Arabia
Body Length	2.24	3.30	9.07±1.98 (4.25-13.10)	6±2 (4-10)	3.67±0.12 (2.97-5.26)
Width	1.18	1.06	3.69±0.77 (2.12-5.43)	1.25±0.23 (0.45-1.39)	0.72±0.01 (0.61-0.95)
Buccal sucker diameter	-	-	-	0.12±0.02 (0.08-0.15)	0.076±0.004 (0.071-0.089)
Pharynx length	0.15	0.08	0.27±0.04 (0.15-0.39)	0.12±0.006 (0.08-0.15)	0.059±0.013 (0.041-0.075)
Muscular bulb diameter	0.077	0.069	0.12±0.02 (0.08-0.15)	-	0.049±0.008 (0.039-0.055)
Number of copulatory organ hooks	17	17	17±2 (13-20)	15-19	17
Germarium length	0.53	0.78	1.96±0.43 (1.00-2.83)	0.60±0.01 (0.04-0.80)	0.232±0.034 (0.191-0.270)
Number of testes	223	256	201±31 (167-290)	220±40 (190-250)	218±10 (184-267)
Testicular area length	0.826	0.908	0.670±0.031 (0.177-1.413)	-	0.981±0.023 (0.801-1.26)
Haptor length	0.85	1.15	2.68±0.63 (1.35-4.10)	-	0.665±0.128 (0.459-0.752)
First clamp length	0.17	0.22	0.35±0.05 (0.22-0.43)	-	0.160±0.008 (0.150-0.166)
First clamp width	0.14	0.17	0.28±0.40 (0.18-0.36)	0.39±0.01 (0.28-0.47)	0.142±0.008 (0.133-0.149)

Sequences from the partial 28S rRNA gene revealed that the two sequences acquired from the organism in the present investigation clustered with members of the *Diclidophora* genus. The branch that contained members of the genus *Diclidophora* differed from the branch grouped related organisms from genera *Microcotyle* and *Polylabris*. Both genera constituted a monophyletic group. Molecular findings validated the distinction of the organism's identity as *D. merlangi*, which differs from *D. luscae*, *D. minor* and *D. denticulata*. Ramos *et al*. (2022) studied *D. luscae* from *Trisopterus luscus* and validated its molecular identification, grouping it alongside *D. minor* and *D. denticulata* into a single clade. Ramos *et al*. (2022) sequences were determined to be more than 99 % identical to those described before (Mollaret *et al*., 2000; Jovelin & Justine, 2001), who classified it as *D. luscae capelanii*. The organism (*D. luscae capelanii*) was recovered from *T. capelanus* in the Mediterranean Sea and after the host harboring it (*T. luscus*) from the Atlantic Ocean (Jovelin & Justine, 2001). Ramos *et al*. (2022) recovered the same organism from *T. lusca*, hence, the International Code of Zoological Nomenclature (ICZN) used the name *D. luscae*. Furthermore, there are no COI sequences related to the genus *Diclidophora* in GenBank, so the sequence reported from the organism (*D. merlangi*) is considered a unique sequence. The closest match for the sequence was that from *Choricotyle australiensis* and *C. brasiliensis*, with nucleotide matches of 84.39 % and 83.75 %, respectively. Thus, the sequence is novel and can be employed as a barcoding sequence for *D. merlangi* in future research.

Most monogenean species that parasitize fish are very unique to their hosts. However, this study is the first to show that *A. bifasciatus* harboured *Diclidophora* species in Saudi Arabia, which is consistent with the findings of Perdiguero-Alonso *et al*. (2006), Strona *et al*. (2010), and Morsy *et al*. (2018), who all stated that *D. merlangi* was found on an unusual host. The novel fish host observation could be explained by interaction between hosts, which aids in parasite transmission. DNA barcoding is thought to be a method for species discrimination, which helps in comprehending the diversity within an ecosystem and assessing genetic variability between them (Rajkumar *et al*., 2015; Abbas *et al*., 2017; Ghouri *et al*., 2020). Previous research has used the COI gene to confirm the taxonomic status of many *Acanthopagrus* species (Hsu *et al*., 2011; Wu *et al*., 2018; Alam *et al*., 2020; Liu *et al*., 2021; Pan *et al*., 2021; Islam *et al*., 2022; Pombo-Ayora *et al*., 2022). The host's morphological identification was established by comparing COI sequences to comparable organisms. The COI sequence recovered from *A. bifasciatus* in the current investigation was determined to be 100 % identical to *A. bifasciatus*, confirming morphological identification. Sequences from *A. bifasciatus* and *Acanthopagrus catenula* shared a common ancestor, as previously observed by Pombo-Ayora *et al*. (2022).

Conclusion

This is the first study to present the morphological description and molecular characterization (28S rRNA and COI genes), resulting in the identification of the monogenean *D. merlangi* and DNA barcoding of the fish host (*A. bifasciatus*) in the Arabian Gulf (Saudi Arabia), utilizing the COI gene as a genetic marker. The COI sequence acquired for *D. merlangi* is unique and will serve as the foundation for all future research by members of the *Diclidophora* genus on this gene.

Conflict of Interests

The author(s) declare that they have no conflict of interest regarding the content of this article.

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Consent to participate All authors agreed to participate in this study.
Consent to publish All authors agreed to publish the data in this study.
Availability of data and materials All the datasets generated or analyzed during this study are included in this published article.

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