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Morphometric and molecular studies on a diplectanid monogenean parasite infecting the twobar seabream (*Acanthopagrus bifasciatus*) in Saudi Arabia

[*Estudos morfométricos e moleculares sobre um parasita monogeneano diplectanídeo que infecta o pargo-de-duas-barras (Acanthopagrus bifasciatus) na Arábia Saudita*]

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ABSTRACT

Specimens of the genus *Lamellodiscus* Johnston and Tiegs, 1922 (Monogenea, Diplectanidae) were collected from the gills of the twobar seabream, *Acanthopagrus bifasciatus* (Sparidae), in the Arabian Gulf (Saudi Arabia). The overall prevalence and mean intensity were 12.5% (5 out of 40) and 14, respectively. All these parasite specimens belonged to the same species, which is morphologically very close to *Lamellodiscus spari* Zhukov, 1970 (Lamellodiscinae) belongs to the "elegans" group, characterized by rows of lamellodisc arranged as the 1st closed, 2nd – 9th paired elements, 10th of a single element, a "polymorphous" shaped male copulatory organ type, and the presence of the chitinous vaginal opening. A different host fish species, distant geographic locality, and small morphometric differences compared with the original description of *L. spari* acted as a stimulus for the taxonomic confirmation of this parasite species. Molecular identification of this *Lamellodiscus* species was conducted through sequencing of the nuclear large subunit of the ribosomal RNA (28S rRNA) gene. *Lamellodiscus* species reported in the present study formed a sister group with *L. spari*, and it was different from *L. spari* in 6 or 7 nucleotide bases. Findings obtained from the molecular analysis are concordant with data drawn from morphological identification, where the parasite recorded was morphologically similar to *L. spari* with a first record in Saudi marine fish. Further studies are recommended to include more genes for this monogenean parasite.

Keywords: fish, Monogenea, Lamellodiscinae, morphology, 28s rrna

RESUMO

Espécimes do gênero Lamellodiscus Johnston e Tiegs, 1922 (Monogenea, Diplectanidae) foram coletados das guelras do pargo-de-duas-barras, Acanthopagrus bifasciatus (Sparidae), no Golfo Árabe (Arábia Saudita). A prevalência geral e a intensidade média foram de 12,5% (5 em 40) e 14, respectivamente. Todos esses espécimes de parasitas pertenciam à mesma espécie, que é morfologicamente muito próxima do Lamellodiscus spari Zhukov, 1970 (Lamellodiscinae) pertence ao grupo "elegans", caracterizado por fileiras de lamellodiscos dispostos como o primeiro elemento fechado, o segundo ao nono emparelhados, o décimo como um único elemento, um órgão copulador masculino de forma "polimórfica" e a presença de uma abertura vaginal quitinosa. Uma espécie de peixe hospedeiro diferente, localização geográfica distante e pequenas diferenças morfométricas em comparação com a descrição original de L. spari serviram de estímulo para a confirmação taxonômica desta espécie de parasita. A identificação molecular desta espécie de Lamellodiscus foi realizada através do sequenciamento da subunidade nuclear grande do gene do RNA ribossômico (28S rRNA). As espécies Lamellodiscus relatadas no presente estudo formaram um grupo irmão com L. spari e eram diferentes de L. spari em 6 ou 7 bases nucleotídicas. As descobertas obtidas a partir da análise molecular são concordantes com os dados extraídos da identificação morfológica, em que o parasita registrado era morfologicamente semelhante a L. spari, com um primeiro registro em peixes marinhos da Arábia Saudita. Recomenda-se a realização de estudos adicionais para incluir mais genes para este parasita monogeneano.

Palavras-chave: peixes, Monogenea, Lamellodiscinae, morfologia, 28S rRNA

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INTRODUCTION

Monogeneans are the most numerous parasitic groups infecting fish, amphibians, and reptiles (Mladineo, 2004). They tend to select many specific microhabitats (i.e., skin, fins, gills, mouth cavity, and nostrils) on their hosts (Euzet and Combes, 1998). According to the morphology and accessory adhesive organs of the opisthohaptor, monogeneans are classified into two major groups, *Group I* monopisthocotylea and *Group II* polyopisthocotylea (Öztürk and Özer, 2014). The former group contains five accepted orders: Capsalidea, Dactylogyridea, Gyrodactylidea, Monocotylidea, and Montchadskyellidea. WoRMS (2023) recognized thirteen families based on morphological characters of haptor within Dactylogyridea (i.e., two pairs of main hooks joined by medial bars, 14 peripheral marginal hooks, and one or two groups of sclerotized rodlets or lamellae called 'squamodiscs' or 'lamellodiscs').

Diplectanidae Monticelli, 1903 is a family of monopisthocotylean monogeneans that parasitize the gills of marine or freshwater fish. Diplectanids often show strict host-specificity (Oliver, 1992). As parasites, they can be extremely numerous, up to several thousand on an individual fish. Diplectanids from the genus *Lamellodiscus* Johnston and Tiegs, 1922 have one or two lamellodiscs that are formed by several overlapping sclerotized plates (lamellae). This genus includes 61 accepted species, 2 nomen nudum, 6 synonyms, 1 pre-occupied, 2 superseded combinations, and 1 misspelling species (WoRMS, 2023). *Lamellodiscus* from sparid fish has been used as a model for the study of co-evolution and speciation (Desdevises *et al*., 2002; Kaci-Chaouch *et al*., 2008). Members of *Lamellodiscus* are generally classified into three morphological groups, the 'ignoratus', the 'elegans', and the 'tubulicornis', according to the structure of the lamellodisc (Justine and Briand, 2010). Similarly, Diamanka *et al*. (2011a) distinguished among *Lamellodiscus* four different types based on the morphology of the male copulatory organ (MCO), the 'lyre', the 'forked', the 'polymorphous', and 'elongatus'.

Many attempts have been made to resolve phylogenetic relationships among *Lamellodiscus* species based on species-specific variability in the nuclear small subunit ribosomal RNA (ss rRNA) (Desdevises *et al*., 2000, 2002; Desdevises, 2001; Poisot *et al*., 2011), large subunit ribosomal RNA (lsrRNA) (Tingbao *et al*., 2006; Nitta, 2021), internal transcribed spacer (ITS) region (Desdevises *et al*., 2000; Kaci-Chaouch *et al*., 2008; Poisot *et al*., 2011), and mitochondrial cytochrome c oxidase subunit I (COI) gene (Poisot *et al*., 2011) which shown to be useful for precise monogeneans identification.

Previous studies were carried out on the monogeneans of fish in the Arabian Gulf (Al-Mathal, 2002; Kardousha, 2016; Kardousha *et al*., 2002; Mohamad and Razak, 2011; Bayoumy *et al*., 2012, 2015; Bayoumy and Baghdadi, 2013; Jawad, 2013; Bannai and Muhammad, 2015; Bayoumy *et al*., 2015; Hassan *et al*., 2015; Khosheghbal *et al*., 2017; Jassim and Al-Salim, 2020; Morsy *et al*., 2021; Baghdadi *et al*., 2022). Therefore, this study aims to study the natural occurrence of monogeneans infecting *Acanthopagrus bifasciatus* fish that inhabit the Arabian Gulf (Saudi Arabia), and the taxonomic status of these parasites was determined through morphometric features and confirmed molecularly.

MATERIALS AND METHODS

During the period of this study, forty specimens of *Acanthopagrus bifasciatus* (Sparidae) were collected from the fish market in Dammam province (along the coast of the Arabian Gulf), Saudi Arabia. Fish were identified using the external morphological standards set out by Abu Shusha *et al*. (2010). The gills of the host specimens were separated, submerged in 0.9% saline solution, and examined for monogeneans under a stereomicroscope (Mendoza-Franco *et al*., 2018). Definitions of prevalence and mean intensity were calculated according to Bush *et al*. (1997). Monogeneans were removed from the gills using a delicate dissection needle and then fixed in 4% formalin for species identification by the morphology of the body parts or in 96% ethanol for molecular genetic study. To remove excess fixatives, the fixed specimens were rinsed in distilled water. Some monogeneans were stained with Mayer-Schuberg's Aceto carmine, dehydrated in a series of ethanol (70, 80, 90, and 100% for 2 min/each), and then mounted in Canada balsam. For the study of the sclerotized structures, some unstained specimens were flattened with coverslips on slides with a mixture

of ammonium picrate glycerin, and then mounted in Canada balsam (Ergens, 1969). The parasites were examined using a light microscope (Leica DM 2500, Leica Microsystems). Using the ImageJ 1.53e software, measurements were taken in micrometers (µm) as a mean, followed by the range in parentheses.

Monogenea samples were digested overnight in the DNA buffer (100µg/ml proteinase K) at 55ºC. DNA was extracted using QIAamp® DNA Mini Kit (Qiagen, Germany), according to the manufacturer's protocol. The partial 28S rRNA gene region was amplified by polymerase chain reaction (PCR) using U178 (Forward: 5′-GCA CCC GCT GAA YTT AAG -3′) and L1642 (Reverse: 5′-CCA GCG CCA TCC ATT TTC A-3′) primers designed by Lockyer *et al*. (2003). The PCR profile was set as follows: at 94°C was performed for 5min, followed by 35 cycles of denaturation at 94°C for 30sec, annealing at 56°C for 30sec, extension at 72°C for 1min, and final elongation at 72°C for 10min. Amplicons were electrophoresed in a 1.5% agarose gel in 1×TAE buffer (Tris 40 mM, Acetic Acid 20mM, EDTA 1mM), stained with SYBRsafe® (Thermo Fischer Scientific, Massachusetts, USA) alongside 100bp DNA Ladder, and then visualized under UV light. Representative monogenean samples were subjected to Sanger sequencing at the facility unit of Macrogen (Seoul, South Korea), using the same primer sets as for PCR. A BLAST search was conducted to verify the similarity of the obtained sequences with those of monogeneans available in the NCBI BioSystems database. Ambiguous sequence alignment was edited manually using BioEdit 4.8.9 software (Hall, 1999). Phylogenetic analysis was inferred with the help of appropriate models in MEGA X (Kumar *et al*., 2018), employing Maximum Likelihood and Neighbor-Joining methods using 1000 replicates.

RESULTS

Five (12.5%) out of 40 twobar seabream fish, *Acanthopagrus bifasciatus*, were found to have a monogenean parasite infecting the gill region with an infection intensity that did not exceed 20. This parasite was identified based on the morphological features of *Lamellodiscus spari* Zhukov, 1970.

(Figure 1 and Table 1). The body was elongated and measured 451 (412-504)×110 (98-129). The anterior region has three pairs of head organs, two pairs of eye spots, and two groups of glandular cells lateral to the pharynx. The mouth was situated between two pairs of eyespots. The pharynx was oval and measured 39 (35-46)×37 (35-47). The oesophagus was short and followed by an intestinal bifurcation, simple caeca. Vitellaria coextensive with caeca and filling the intercaecal region posterior to the testis.

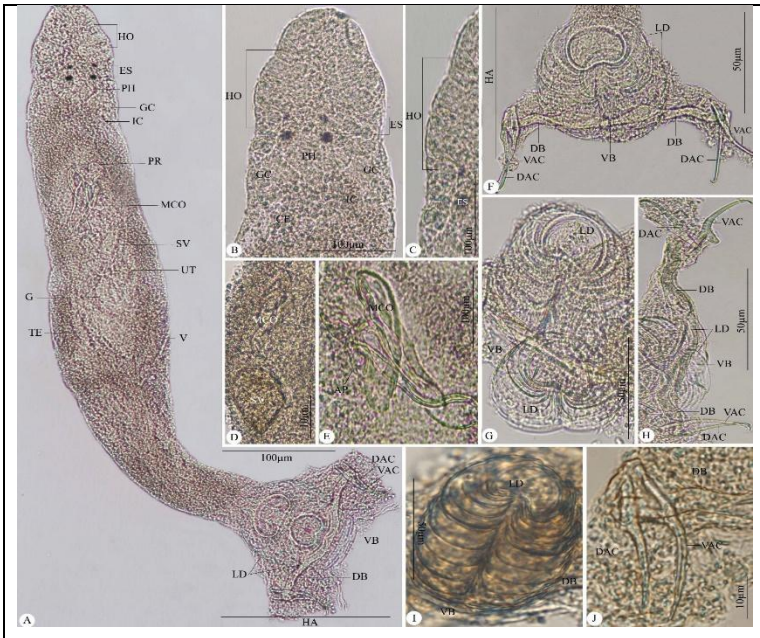

Figure 1. Photomicrographs of *Lamellodiscus spari* infecting *Acanthopagrus bifasciatus*. (A) Whole-mount preparation. (B-J) High magnifications for different body parts, as follows: (B-E) Anterior portion of the prohaptor. (F-H) Haptor and related structures. (I) Lamellodisc. (J) Ventral and dorsal anchors. Note: Ho, head organs; ES, eye spots; PH, pharynx; IC, intestinal ceca; GC, glandular cells; MCO, male copulatory organ; SV, seminal vesicle; UT, uterus; G, germarium; TE, testis; DA, dorsal anchor; VA, ventral anchor; VB, ventral bar; DB, dorsal bar; LD, lamellodiscs; HA, haptor; AP, accessory piece.

Table 1. Comparative metrical data for *Lamellodiscus spari* and those described previously

Comparable parameters	Ogawa and Egusa, 1978	Present study
Host	*Acanthopagrus schlegeli*	*Acanthopagrus bifasciatus*
Location	Japan	Saudi Arabia
Body length	425-580	451 (412-504)
Body width	108-136	110 (98-129)
Pharynx length	36-48	39 (35-46)
Pharynx width	38-53	7 (35-47)
Testis length	88-122	95 (84-115)
Testis width	62-93	76 (69-86)
Copulatory organ morphology	Polymorphous type	Polymorphous type
Copulatory sclerites length	-	25 (21-33)
Germarium length	80-125	88 (79-113)
Germarium width	22-32	25 (20-29)
Haptor width	152-189	164 (156-178)
Haptor morphology	Elegans group	Elegans group
Lamellodisc length	-	81 (79-89)
Lamellodisc width	70-111	73 (69-98)
Ventral bar length	79 (71-84)	70 (68-81)
Dorsal bar length	28-34	30 (27-31)
Ventral anchor length	46 (42-51)	44 (040-47)
Dorsal anchor length	40 (6-45)	39 (34-42)
Marginal hook length	9-10	9 (8-11)

A single post-ovarian testis was observed intercaecally and measured 95 (84-115) × 76 (69-86). The vas deferens arises from the antero-sinistral portion of the testis, enlarges into a broad seminal vesicle, and then simply dilates to form a saccate prostatic reservoir that is located anterior to the copulatory complex. The male copulatory organ is of a polymorphous type, with two articulated sclerites and a simple one of 25 (21-33) in length. Germarium was pyriform, intercaecal, pre-testicular, and measured 88 (79-113) × 25 (20-29). The oviduct extends anteriorly from the receptaculum seminis. The vagina consists of a chitinous opening on the right lateral margin of the body and a very lightly chitinous duct that leads into the posterior part of the receptaculum seminis. The uterus extends anteriorly to the gonopore located ventrally to the copulatory sclerites.

Haptor measured 164 (156-178) in width and was associated with a truncated posterior margin. Dorsal and ventral lamellodiscs large of the "elegans" group measured 81 (79-89)×73 (69-98), each covering the entire median section of the haptor and extending slightly onto the body proper. Ten concentric lamellae make up lamellodiscs, with the anterior one forming a complete ring 201 (196-208) in width, and others crescentic. Seven pairs of hooks are present in the lateral margins of the haptor, and each measured 9 (8-11) long.

Two pairs of anchors situated posterolaterally of the haptor, connected by three crossbars. The ventral pair of anchors measured 44 (40-47) long, with internal and external processes of 13 (10-14) and 5 (4-7) long, respectively. Dorsal anchors measured 39 (34-42) long. The ventral

bar was plate-like with an anterior median groove and tapered ends and measured 70 (68-81) long. Paired dorsal bars were slightly curved, with the inner end distinctly wider than the outer, and prominent anterior process slightly curved towards the lateral extremity of the bar, and measured 30 (27-31).

(Figures 2 and Table 2). Partial 28S rRNA region amplification from *L. spari* revealed a PCR product of ~350 bp. Two sequences were generated from the organism under study, which showed morphological and morphometric data related to *L. spari* described from Japan (Ogawa and Egusa, 1978; Nitta, 2021). The two sequences were homologous with two mutations at positions 193 and 212 on the alignment. The two sequences were deposited in GenBank and were given the accession numbers PP504853 and PP505854. There were 11 sequences related to members of the genus *Lamelodiscus*, two of which are from *L. spari* (DQ054823 and LC565450). The sequences from the present study showed differences in 6-7 bases. They also showed the same difference in 2 sequences from *L. chin* (LC565448 and LC565449). Phylogenetic analysis using both Maximum Likelihood (ML) and Neighbor Joining (NJ) revealed the same topology, placing the sequences obtained in the present study in a sister group for the clade that grouped *L. spari* and *L. chin* with significant bootstrap values of 94 and 96, respectively (Figure 2). Sequences from *L. spari* detected in the present study were different in 23 bases from *L. pagrosomi* (KY640620 and EF100562). Sequences from *L. spari* (DQ054823 and LC565450) and *L. chin* (LC565448 and LC565449) showed 99.7% similarity (Table 2).

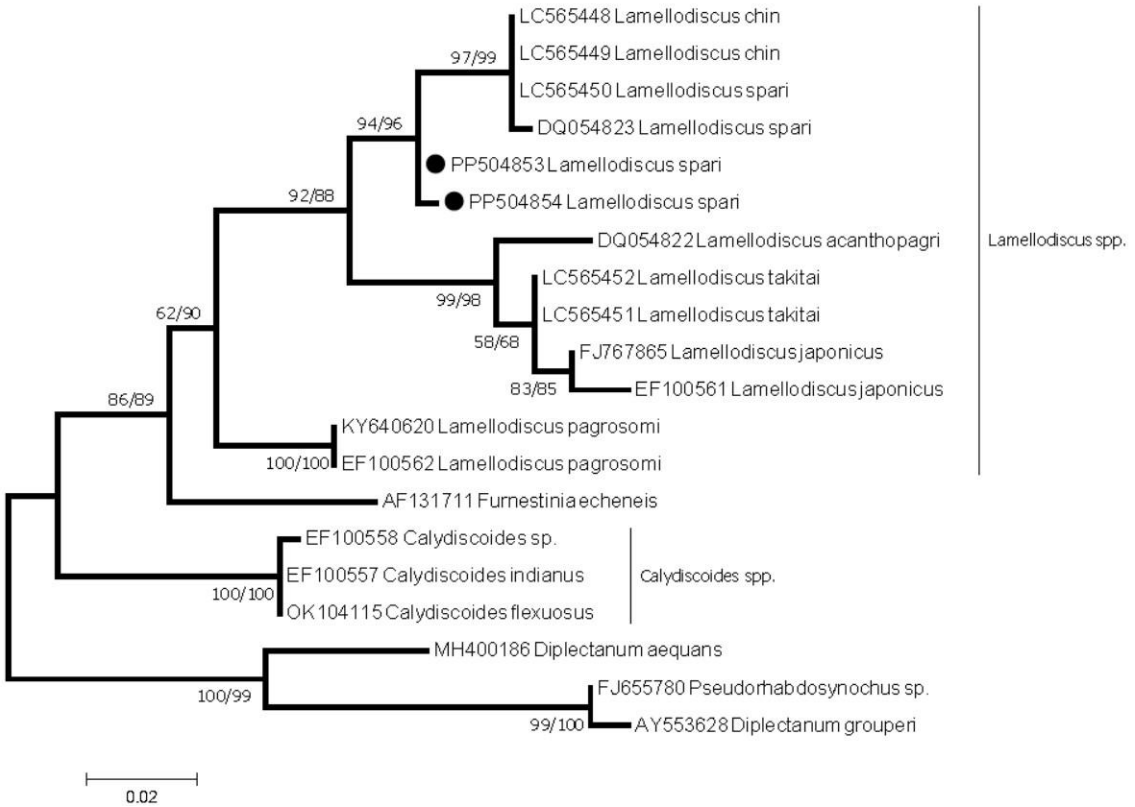

Figure 2. A consensus phylogenetic tree generated with maximum likelihood (ML) and Neighbor Joining (NJ) methods, showing phylogenetic relationships between *Labellodiscus spari* (2 sequences) and 18 related taxa in the family Diplectanidae at NCBI GenBank. The ML and NJ trees are inferred from the partial 28S rDNA sequence data (338 bp) generated from the *L. spari* detected from the gills of *Acanthopagrus bifasciatus* (PP504853 and PP505854 shown with solid circles) and 18 related taxa from GenBank. Numbers indicated at branch nodes are bootstrap values (ML/NJ).

Table 2. The number of base differences per sequence between sequences is shown. This analysis involved 20 nucleotide sequences, including sequences from the present study (given in bold). All ambiguous positions were removed for each sequence pair (pairwise deletion option)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
PP504853_ *Lamellodiscus_spari*																				
PP504854_ *Lamellodiscus_spari*	2																			
LC565450_ *Lamellodiscus_spari*	6	7																		
DQ054823_ *Lamellodiscus_spari*	6	7	1																	
LC565448_ *Lamellodiscus_chin*	6	7	0	1																
LC565449_ *Lamellodiscus_chin*	6	7	0	1	0															
LC565452_ *Lamellodiscus_takitai*	14	13	17	16	17	17														
LC565451_ *Lamellodiscus_takitai*	14	13	17	16	17	17	0													
FJ767865_ *Lamellodiscus_japonicus*	16	15	19	18	19	19	2	2												
EF100561_ *Lamellodiscus_japonicus*	19	18	22	20	22	22	6	6	4											
KY640620_ *Lamellodiscus_pagrosomi*	20	20	18	16	18	18	22	22	25	26										
EF100562_ *Lamellodiscus_pagrosomi*	23	23	22	16	22	22	26	26	28	30	4									
DQ054822_*Lamellodiscus_acanthopagri*	16	17	19	20	19	19	8	8	8	11	23	23								
AF131711_ *Furnestinia_echeneis*	23	23	28	26	28	28	24	24	26	29	23	26	26							
EF100557_*Calydiscoides_indianus*	29	29	33	31	33	33	31	31	33	36	27	31	31	27						
EF100558_ *Calydiscoides*_sp.	30	30	34	32	34	34	32	32	34	37	28	32	32	28	1					
OK104115_ *Calydiscoides_flexuosus*	29	29	33	31	33	33	31	31	33	36	26	28	31	27	0	1				
MH400186_ *Diplectanum_aequans*	40	40	40	40	40	40	37	37	39	42	36	39	38	39	33	32	33			
FJ655780_ *Pseudorhabdosynochus*_sp.	45	43	45	46	45	45	42	42	44	47	40	43	43	41	41	42	41	25		
AY553628_ *Diplectanum_grouperi*	44	42	44	45	44	44	42	42	44	47	35	39	42	39	40	41	40	27	2	

DISCUSSION

Although many studies are available about the parasitofauna of marine fish, little is known about Lamellodiscinae (Alghamdi *et al*., 2023). In the present study, the gills of five twobar seabreams (12.5%) with a mean intensity of 14 were found to be infected with the monopisthocotylean parasite within the *Lamellodiscus* genus. Based on previous studies on the specificity of *Lamellodiscus* species to the host type, these species parasitized mainly sparids and lethrinids, with few cases reported from centracanthids, pomacanthids, and pomacentrids (Amine and Euzet, 2005; Machkewskyi *et al*., 2014; Kritsky and Bakenhaster, 2019; Nitta, 2021). The present prevalence is similar to the previous data of *L. dentexi* reported by Diamanka *et al*. (2011b) in *Dentex macrophthalmus* (Northwest coast of Africa) (prevalence = 12%). Also, this prevalence is lower than the previous data of *L. elegans* reported by Mladineo (2004) in sharp-snout bream (*Diplodus puntazzo*) (Adriatic Sea) (prevalence = 64.95%); *L. crampus* reported by Neifar (2008) in *D. maroccanus* (Tunisia) (prevalence = 65%); Justine and Briand (2010) reported that *Gymnocranius grandoculis* (New Caledonis) infected with 100% of *L. magnicornis* and *L. parvicornis*; Diamanka *et al*. (2011a,b) reported that *D. macrophthalmus* (Senegal) infected with 94% of *L. toguebayei*, 27% of *L. vicinus*, 56% of *L. triacies*, and *D. gibbosus* (Senegal) infected with 70% of *L. euzeti*; *L. elegans* and *L. fraternus* reported by Özer *et al*. (2015) in *Diplodus annularis* (Turkish Black Sea coast) (prevalence = 83.3%); *L. iraqensis* reported by Jassim and Al-Salim (2020) in *Acanthopagrus arabicus* (Iraq) (prevalence =

25%); *L. indicus* reported by Al-Darwesh *et al*. (2022) in *Rhabdosargus haffara* (Iraq) (prevalence = 97%) and *R. sarba* (Iraq) (prevalence = 95%).

Members of the *Lamellodiscus* genus are distinguished from other diplectanids by the morphology of the vagina opening, the sclerites of the copulatory complex, and the type and shape of haptorial lamellodiscs, as mentioned in Machkewskyi *et al*. (2014). The present specimen has all the morphological features of *L. spari*, which was isolated previously from *Sparus macrocephalus czerskii* (Japan) by Zhukov (1970) and *Acanthopagrus schlegeli* (Japan) by Ogawa and Egusa (1978). Although the shapes of the chitinous parts are much the same between the present specimen and those of Zhukov (1970) and Ogawa and Egusa (1978), the former is considerably smaller in their measurements. Taking this into consideration, the difference in the body size and its associated parts is not a reliable feature to be used for discriminating this *Lamellodiscus* species, especially in the presence of various fish species (type host) in different habitats, and this agreed with Ogawa and Egusa (1978) reported that *Lamellodiscus* measurements lie within the limits of specific variations.

Moreover, there is a high similarity with *Lamellodiscus vaginalis* isolated from Australian fish of *Acanthopagrus australis* and *Acanthopagrus butcheri* by Byrnes (1968) due to the presence of large lamellodiscs in both samples, which are considered the most important morphological features for identification. As well as it is sufficient to note that the vagina opens on the right side of the body, which seems to be an exceptional case in the *Lamellodiscus* genus as well as in *L. corallinus* and *L. minousi*. According to the morphology of the haptoral structures within the "elegans" group, the present specimen is similar to some *Lamellodiscus* species (i.e. *L. dentexi*, *L. coronatus*, *L. drummondi*, *L. furcosus*, *L. gracilis*, *L. mirandus*, *L. obeliae*, *L. parisi*, *L. virgula*, *L. echeneis*, *L. mormyrid*, *L. verberis*, *L. elegans*, *L. flagellates*, *L. impervious*, *L. bidens*, *L. hilii*, *L. acanthopagri*, *L. caballeroi*, *L. indicus*, *L. butcheri*, *L. cirrusspiralis*, *L. major*, *L. squamosus*, *L. vaginalis*, *L. typicus*, *L. niedashui*, *L. japonicus*, and *L. takitai*) in which rows of lamellodisc arranged as the row 1 closed, rows 2-9 paired elements, row 10 of a single element, this agreed with Justine and Briand (2010) and Machkewskyi *et al*. (2014). The current specimen is similar to other *Lamellodiscus* species in the polymorphous type of male copulatory organ (i.e., *L. dentexi*, *L. drummondi*, *L. impervious*, *L. hilii*, *L. caballeroi*, *L. indicus*, *L. butcheri*, *L. cirrusspiralis*, *L. major*, *L. squamosus*, *L. vaginalis*, *L. typicus*, *L. takitai*, and *L. epsilon*), which agrees with Machkewskyi *et al*. (2014).

Moreover, *L. spari* could be differentiated from other *Lamellodiscus* species, as follows: (I) The sclerites in the male copulatory organ (two articulated sclerites and a simple one in *L. spari vs.* hooked in *L. squamosus*, long spiral sclerite in *L. cirrusspiralis* and *L. drummondi*), which agreed with the finding of Byrnes (1968). (II) The morphology of the male copulatory organ (polymorphous type in *L. spari vs.* "lyre" type within *L. toguebayei*, *L. triacies*, *L. vicinus*, *L. knoffleri*, *L. rastellus*, *L. sarculus*, *L. sigilatus*, *L. ergensi*, *L. erythrini*, *L. ignoratus*, *L. euzeti*, *L. aff. euzeti*, *L. baeri*, *L. confusus*, *L. crampus*, *L. falcus*, *L. kechemirae*, *L. neifari*, *L. sanfilippoi*, *L. theroni*, *L. tomentosus*, *L. fraternus*, *L. donatellae*, *L. furcillatus*, and *L. pagrosomi*, "furca" type within *L. coronatus*, *L. furcosus*, *L. gracilis*, *L. mirandus*, *L. obeliae*, *L. parisi*, *L. virgula*, *L. echeneis*, *L. mormyrid*, *L. verberis*, *L. elegans*, *L. flagellates*, *L. bidens*, *L. acanthopagri*, *L. japonicus*; and without accessory piece in *L. corallinus*), which consistent with Machkewskyi *et al*. (2014). (III) The shape of the vaginal opening (chitinous opening in *L. spari vs.* flower-like in *L. vaginalis*, cup-like in *L. bucheri*, funnel-shaped in *L. japonicus*), which agrees finding of Byrnes (1968). (IV) The size of haptorial lamellodiscs (larger size in *L. spari vs.* smaller one in other *Lamellodiscus* species, which is consistent with data of Ogawa and Egusa (1978) and Machkewskyi *et al*. (2014). (V) The morphology of haptoral structures ("elegans" groups in *L. spari vs.* ignoratus group within *L. toguebayei*, *L. triacies*, *L. vicinus*, *L. knoeffleri*, *L. rastellus*, *L. sarculus*, *L. sigilatus*, *L. ergensi*, *L. erythrini*, *L. ignoratus*, *L. euzeti*, *L. aff. euzeti*, *L. baeri*, *L. confusus*, *L. crampus*, *L. falcus*, *L. Kechemirae*, *L. neifari*, *L. sanfilippoi*, *L. theroni*, *L. tomentosus*, *L. fraternus*, *L. corallinus*, *L. donatellae*, *L. furcillatus*, and *L. pagrosomi*) in which rows of lamellodisc arranged as the row 1 closed, rows 2-10 single elements, this agreed

with Justine and Briand (2010), as well as "tubulicornis" group within *L. parvicornis*, *L. magnicornis*, and *L. tubulicornis*) in which rows of lamellodisc arranged as the row 1 closed, row 2 almost closed, rows 3-9 paired elements, row 10 a single element, which agreed with data of Justine and Briand (2010) and Machkewskyi *et al*. (2014).

For the classification of the *Lamellodiscus* species, molecular studies should support the degree of morphological variation between the current parasite and other taxa within Lamellodiscinae. The phylogenetic position of *Lamellodiscus* species identified from Saudi Arabia was validated in this study using the partial genetic sequences of the 28S rRNA gene. Sequences obtained from the partial 28S rRNA region from *L. spari* collected in the present study from *Acanthogarus bifaciatus* showed a strong relationship with sequences obtained from *L. spari* and *L. chin* from Japan (Nitta, 2021). The phylogenetic position of the samples investigated in the present study fits well with the morphological description of *L. spari*. Intraspecific variation between *L. spari* sequences was noticed previously, as two sequences described by Nitta (2021) were different from each other in a single base. In the present study, it was found that the two sequences obtained were different from each other in two sites. Furthermore, variation at the interspecific level was noticed between *L. spari* and *L. chin*, where in four sequences, two from the first and two from the latter have shown 99.7% to 100% similarity. This suggests that they are highly related despite significant morphological differences. Therefore, it is suggested to study different genes, including the 28S rRNA gene, when studying the phylogenetic relationships of *Lamellodiscus* spp. Although *Furnestinia echeneis* was grouped with *Lamellodiscus* spp. in the phylogenetic tree generated from the partial 28S rDNA data, morphological data suggested that it is a unique genus. According to the World Register of Marine Species (https://www.marinespecies.org/aphia.php?p=taxdetails&id=119523), the identity of *F. echeneis* is considered unaccepted and suspended. In a study by Desdevises (2001), it was suggested that the hypertrophy of its unique lamellodisc is probably a morphological adaptation for attachment to the host. Therefore, the feature of having one lamellodisc rather than 2, as in the case of members of *Lamellodiscus* spp., is an adaptation to attach to its host. The difference in nucleotides between *F. echeneis* and the sequences from *L. spari* reported in the present study was similar to that between *L. pargrosomi* and *L. spari*. Additional molecular studies may resolve the situation of the monospecific genus *Furnestinia*.

CONCLUSION

A parasitological investigation of Lamellodiscinae parasites of *Acanthopagrus bifasciatus* (Sparidae) in Saudi marine waters, herein, was carried out for the first time. Moreover, the exploration of the 28S rRNA genetic sequences of the recovered *Lamellodiscus* species revealed that it belongs to *L. spari*. There was intraspecific variation with the 28S rRNA studied. Additional molecular studies investigating more genes are required to clarify the classification of this group of parasites.

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