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Histopathological evaluation of the twobar seabream *Acanthopagrus bifasciatus* Forsskål, 1775 (Sparidae) infected by *Bivagina pagrosomi* (Microcotylidae)

[*Avaliação histopatológica do dourado* Acanthopagrus bifasciatus *Forsskål, 1775 (Sparidae) infectado por* Bivagina pagrosomi *(Microcotylidae*)]

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ABSTRACT

Fish, like all other species, can be impacted by a variety of environmental conditions, including parasitic infections. Although there are several parasitological researche on ectoparasitic monogeneans, few are published on those that infect sparid fish. Forty samples of Acanthopagrus bifasciatus were collected during this study from coastal regions along the Arabian Gulf (Saudi Arabia). Gills from all fish were isolated and examined to identify monogeneans. The parasites were studied morphologically using light microscopy. Overall prevalence and mean intensity were 20% and 9, respectively. Eight out of 40 (20%) fish samples were found to be naturally infected with a monogenetic species, namely, Bivagina pagrosomi (Murray, 1931) Dillon and Hargis, 1965 belonging to Microcotylidae (order Mazocraeidea). This parasite is characterized by the presence of haptor provided 43-47 clamps of microcotylid-type that deeply penetrated the gill lamellae and caused severe pathological impacts including hyperplasia, telangiectasis, and deformity of the respiratory epithelial cells Our finding indicates that this is the first report of A. bifasciatus being infected with B. pagrosomi from Saudi marine waters as well as the research of its deleterious effects on its host gills. More research is needed to confirm the parasite species' taxonomic status at the molecular level.

Keywords: marine fish, microcotylidae, morphology, morphometry, histopathology

RESUMO

Os peixes, como todas as outras espécies, podem ser afetados por uma variedade de condições ambientais, incluindo infecções parasitárias. Embora existam várias pesquisas parasitológicas sobre monogenéticos ectoparasitas, poucas foram publicadas sobre aqueles que infectam peixes esparsos. Quarenta amostras de Acanthopagrus bifasciatus foram coletadas durante este estudo em regiões costeiras ao longo do Golfo Arábico (Arábia Saudita). As brânquias de todos os peixes foram isoladas e examinadas para identificar monogênicos. Os parasitas foram estudados morfologicamente por meio de microscopia óptica. A prevalência geral e a intensidade média foram de 20% e 9, respectivamente. Verificou-se que oito das 40 (20%) amostras de peixes estavam naturalmente infectadas por uma espécie monogenética, a saber, Bivagina pagrosomi (Murray, 1931) Dillon & Hargis, 1965, pertencente à Microcotylidae (ordem Mazocraeidea). Esse parasita é caracterizado pela presença de um haptor com 43-47 grampos do tipo microcotylid que penetram profundamente nas lamelas das brânquias e causam impactos patológicos graves, incluindo hiperplasia, telangiectasia e deformidade das células epiteliais respiratórias. Nossa descoberta indica que este é o primeiro relato de A. bifasciatus infectado com B. pagrosomi de águas marinhas sauditas, bem como a pesquisa de seus efeitos deletérios nas brânquias de seus hospedeiros. São necessárias mais pesquisas para confirmar o status taxonômico das espécies de parasitas em nível molecular.

Palavras-chave: peixes marinhos, microcotylidae, morfologia, morfometria, histopatologia

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INTRODUCTION

Parasites are incredibly diverse and abundant in all environments, making this lifestyle one of the most successful on Earth (Lafferty *et al*., 2008). Fish are widely recognized to be parasitized by a variety of eukaryotic species, both unicellular and multicellular (M'Rabet *et al*., 2016). Fish gills and skin are continually in contact with the surrounding water, making them vulnerable to ectoparasitic species (Boualleg *et al*., 2010). Gill parasites are one of the most important problems in both wild and cultured fish, and they have a significant impact on their hosts' growth, reproduction, and survival (Reed *et al*., 2005).

Microcotylidae Taschenberg, 1879 represents the largest family within Mazocraeidea and accounts for 74 taxonomic genera and almost all its species are parasites of marine fish (Aguiar *et al*., 2022). Members of the microcotylid genus *Bivagina* Yamaguti, 1963 (Polyopisthocotylea), characterized by the presence of a symmetrical haptor, the presence of few testes, a cirrus and/or genital atrium unarmed, and two vaginal pores armed or unarmed. This genus comprises 10 nominal species recorded from a wide range of marine fish (WoRMS, 2024). The accepted *Bivagina* species include *B. alcedinis*, *B. baumi*, *B. centrodonti*, *B. pagrosomi*, and *B. tai*. While the superseded combination included *B. australis*, *B. heterospina*, *B. kyphosi*, *B. punctipinnus*, and *B. sillaginae*, *most* of the previous studies about the *Bivagina* species were based on morphological identification criteria. Until now, little information was available about the genetic sequences of these species, that were published previously and deposited on the GenBank. These sequences are only available for *B. tai* and *B. pagrosomi* using the nuclear 18S and 28S rRNA (Littlewood *et al*., 1997, 1998, 1999) as well as the mt COI gene (Littlewood *et al*., 1997).

The twobar seabream *Acanthopagrus bifasciatus* is a sparid fish found in the Red Sea and the Arabian Gulf as well as South Africa and Mauritius. There is little information known about parasitic infections in this fish host. The current study aimed to provide more information about the *A. bifasciatus* parasites using light microscopic examination to determine their morphological features and validate their specificity in the Arabian Gulf fish (Saudi Arabia), as well as studying the pathological changes of the gills infected by monogenean parasites.

MATERIALS AND METHODS

Experimental animals and examination. A total of 40 twobar seabream fish, *Acanthopagrus bifasciatus*, were purchased from local fishermen in the coastal region along the Arabian Gulf (Dammam, Saudi Arabia). Samples were transferred to the Laboratory of Parasitology using an ice box at the Department of Zoology, College of Science, King Saud Arabia (Saudi Arabia). This research was approved by the Research Ethics Committee (REC) at King Saud University (approval number KSU-SU-23-76).

Gills were removed from fish and examined under a dissecting microscope (Nikon SMZ18, NIS ELEMENTS software) to find the parasites. Parasitological indexes (including prevalence and mean intensity) of parasites were calculated according to Bush *et al*. (1997). Monogeneans were detached from the gills, then fixed in 70% AFA (ethyl alcohol-formalin-acetic acid) and stained with Aceto carmine (Sigma-Aldrich, Missouri, USA). The stained parasites were dehydrated in ethyl alcohol series, then cleared in xylene, and mounted with Canada balsam (Palm, 2004).

Parasite specimens were examined using Leica DM 2500 microscope (NIS ELEMENTS software, ver. 3.8). Measurements were depicted in micrometers (µm) using the software ImageJ 1.53e (Wayne Rasband and contributors, National Institute of Health, USA) and indicated as the mean (range in parentheses).

Histopathological study. Pieces of gill arches (from both non-infected and infected fish) were fixed in 10% neutral-buffered formalin (NBF) for 24hr. The gills were decalcified with 10% ethylene-diamine-tetra-acetic acid (EDTA) solution for 7 days and were then neutralized with a saturated saline solution. Gills arches were then dehydrated in ethyl alcohol series (70%, 80%, 90%, and 100%), embedded in paraffin wax, and then serially sectioned at 5 µm using a rotary microtome. Histological sections were stained with hematoxylin-eosin (H&E). Sections were examined, and photomicrographs were taken by a Leica DM 2500 microscope (NIS ELEMENTS software, version 3.8).

RESULTS

Eight (20%) of 40 twobar seabream fish, *Acanthopagrus bifasciatus*, were discovered to have a monogenetic parasite infecting the gill region with an infection intensity of no more than nine. This parasite was identified using the morphological characteristics of *Bivagina pagrosomi* (Murray, 1931) Dillon and Hargis, 1965.

Microscopic examinations (Figure 1 and Table 1). The body was lanceolated and measured 3352 (2920-4967) × 466 (362-578). The mouth was ventrally sub-terminal. The anterior end was provided with a pair of septated buccal suckers, measured 105 (102-110) × 58 (53-62). The pharynx was circular and measured 38 (35-40) × 39 (37-42). The esophagus was relatively long, measured 550 (518-570 long, and bifurcated behind the male copulatory organ into two intestinal caeca that extended into the haptoral region. The genital atrium was unarmed and provided with a mid-ventral genital pore.

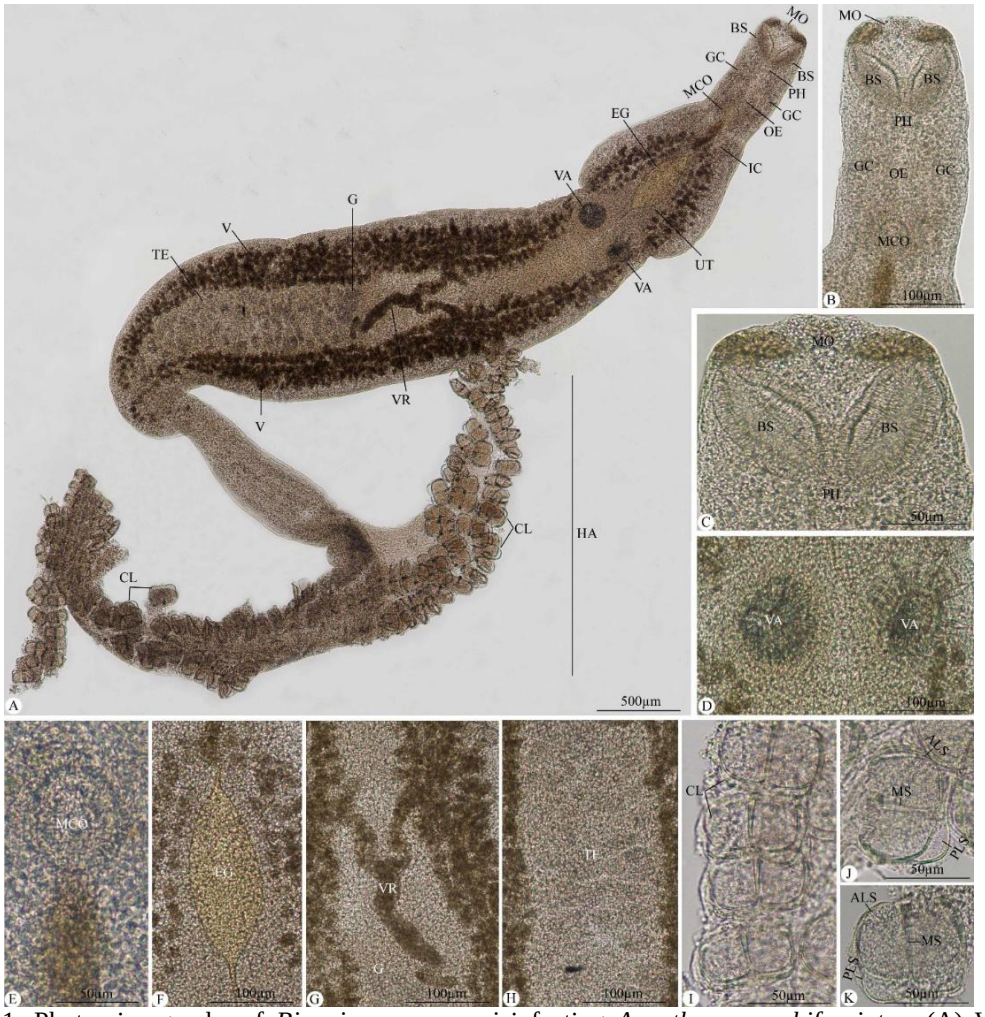

Figure 1. Photomicrographs of *Bivagina pagrosomi* infecting *Acanthopagrus bifasciatus*. (A) Whole-mount preparation. (B-K) High magnifications for different body parts, as follows: (B and C) Anterior portion of the prohaptor. (D) Vagina. (E) Male copulatory organ. (F) Egg. (G) Germarium. (H) Testis. (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; UT, uterus; G, Germarium; TE, testes; VA, vagina; VR, vitelline reservoir; EG, egg; CL, clamps; HA, haptor; ALS, antero-lateral sclerite; PLS, postero-lateral sclerite; MS, median sclerite.

Table 1. Comparative metrical data for *Bivagina pagrosomi* and their congeneric species

Comparable parameters		Dajem *et al*. (2019)	Present study
Host		*Sparus aurata*	*Acanthopagrus bifasciatus*
Location		Saudi Arabia	Saudi Arabia
Body	Length	3266 (2895-5347)	3352 (2920-4967)
	Width	435 (395-544)	466 (362-578)
Buccal sucker	Length	104 (90-125)	105 (102-110)
	Width	60 (54-83)	58 (53-62)
Pharynx	Length	35 (30-45)	38 (35-40)
	Width	37 (32-53)	39 (37-42)
Esophagus length		130 (100-145)	550 (518-570)
Number of genital spines		Unarmed	Unarmed
Male copulatory organ	Length	-	50 (48-52)
	Width	-	39 (36-43)
Egg	Length	200 (185-230)	220 (216-223)
	Width	85 (80-98)	85 (81-90)
Vaginae diameter		-	73 (68-77)
Number of testes		Numerous	Numerous
Haptor diameter		-	2630 (2437-2977)
Number of clamp pairs		43-47	43-47
Anterior clamp	Length	34 (30-40)	42 (38-45)
	Width	62 (58-70)	72 (70-77)
Middle clamp	Length	85 (80-88)	76 (73-80)
	Width	40 (36-45)	47 (45-49)
Posterior clamp	Length	35 (30-38)	37 (34-40)
	Width	58 (48-64)	58 (56-60)

Testes were 50 to 70 in number, post-ovarian intercaecal in the posterior half of the body and did not extend into the haptoral region. The male copulatory organ consisted of a muscular bulb measuring 50 (48-52) × 39 (36-43). The germarium is pre-testicular, shaped as an interrogation mark, intercaecal, and dorsal to vitelline ducts. The uterus is relatively straight, extending anteriorly along the body midline and opening into the genital atrium. Eggs were ovoid, provided with anterior and posterior filaments, and measured 220 (216-223) × 85 (81-90). Vaginae were paired, measured 73 (68-77) in diameter, and armed with a crown of spines. The vitelline reservoir was Y-shaped and located ventrally to the germarium. The vitelline follicles coextensive with the intestinal ceca and extend into the haptor.

The haptor was symmetrical and delineated from the body, measured 2630 (2437-2977) in diameter, and provided with two equal rows of 43-47 pairs of dissimilarly sized clamps of microcotylid-type. The clamp was bilaterally symmetrical, each one consisted of paired antero-lateral sclerites, postero-lateral sclerites, and a median sclerite with bifid ends. The anterior clamp measured 42 (38-45) × 72 (70-77), the middle one measured 76 (73-80) × 47 (45-49), and the posterior one measured 37 (34-40) × 58 (56-60).

Histopathological changes (Figure 2). The findings of this study indicated the histological alterations in the gills of *A. bifasciatus* (fish host) collected from the Arabian Gulf (Figure 2). Figure (2A) depicts the gill morphology of the control fish, which consisted of horizontal flat filaments supported by bony gill arches. These filaments contained secondary lamellae. The secondary lamellae were bordered with a thin layer of epithelial cells that protected the pillar cells, which in turn surrounded the blood sinusoids. Furthermore, histological studies on parasite-infected gills revealed that parasites use their haptor to penetrate deeper into gill lamella, causing secondary lamellar degeneration. The fish host response included significant mucus secretion, hyperplasia, the occasional appearance of neutrophils, telangiectasis, and deformation of the respiratory epithelial cells lining the secondary lamella (Figure 2B-F).

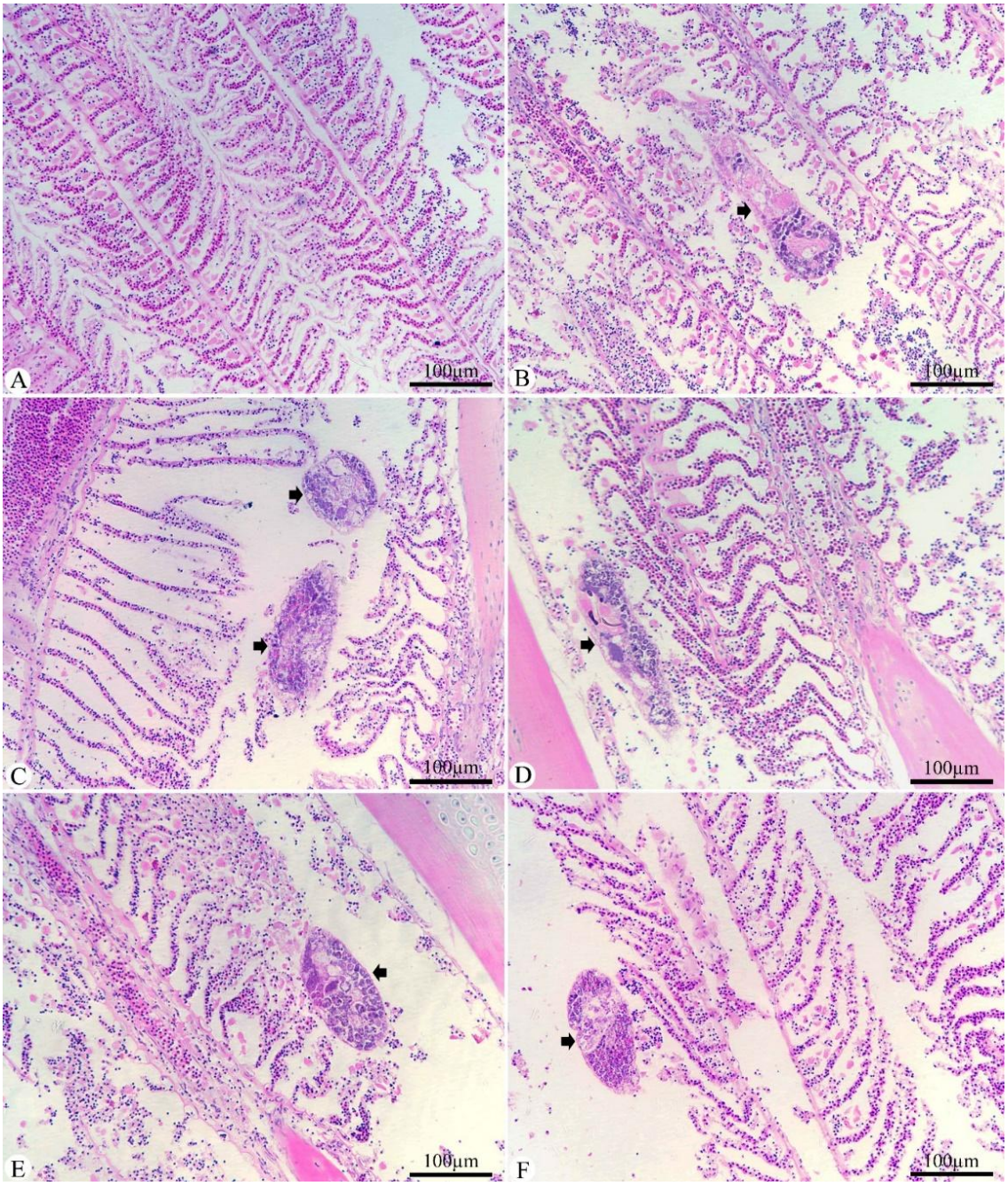

Figure 2. Photomicrographs of histological sections stained with hematoxylin and eosin for the gills isolated from *Acanthopagrus bifasciatus*. (A) non-infected fish gills. (B-F) infected fish gills with monogenean parasites. Note the presence of monogeneans attached to the secondary lamellae (black arrow).

DISCUSSION

Bivagina Yamaguti, 1963 is a genus within the family Microcotylidae Taschenberg, 1879 with 5 valid species, 4 superseded combinations, and 1 Iapsus (WoRMS, 2024). The recovered specimens from *A. bifasciatus* in Saudi waters have the diagnostic morphological features of *Bivagina* provided by Yamaguti (1963) from the gills of marine fish. Members of the genus

Bivagina could be differentiated from other Microcotylidae species based on the presence of a symmetrical haptor, the presence of few testes, a cirrus and/or genital atrium unarmed, and two vaginal pores armed or unarmed. Few taxonomic studies have been performed on the *Bivagina* species.

The present species is compared morphologically and morphometrically with other *Bivagina* species. The appearance of the present species is closely related to that of *B. pagrosomi* (Murray, 1931) Dillon & Hargis, 1965 from *Pagrus aurata* and Dajem *et al*. (2019) from *Sparus aurata* (Saudi Arabia) in having all the characteristic generic features of that species such as the presence of a pair of armed vaginae with a full corona of spines opposing each other and occupying almost the entire width of the worm. However, there are slight differences in the measurements of the different body parts of the recovered species from those described previously of *B. pagrosomi* might be due to the degree of maturity and the infection to the other host type.

Moreover, it is compared with other accepted *Bivagina* species such as *B. alcedinis*, *B. baumi*, 1963, *B. centrodonti*, *B. pagrosomi*, *B. tai*. The clamps of the haptor help the *Bivagina* species to attach to the gills of their fish host. *B. pagrosomi is* characterized by the presence of 43-47 clamps (*vs.* 120-160 in *B. centrodonti* from *Pagellus bogaraveo,* and 80-130 in *B. tai* from *Pagrus major*). It has also 50-70 testes (*vs.* 14-23 in *B. centrodonti*) and an unarmed genital atrium (*vs.* numerous spines surrounding the genital atrium of *B. centrodonti*). In addition, monogeneans are viewed as highly host-specific but variation in specificity occurs between species of monogeneans (Strona *et al*., 2010). In this study, the type of host of *B. pagrosomi* was *A. bifasciatus* which varied from other *Bivagina* species (*vs. B. baumi* from *Spondyliosoma cantharus* and *B. alcedinis* from *Spicara smaris*). Such differences between the *Bivagina* species justify the need to extend the generic diagnosis.

This study also describes the histopathological effects of parasitic monogeneans on infected *A. bifasciatus* gills, revealing functional impairment of the gill lamellae, parasite attachment at the tips of the gill lamellae, and swelling of the host tissue at the attachment site. The number of parasites on the gills determined the severity of respiratory damage to the fish host. This is consistent with Pahor *et al*. (2017), who stated that when the intensity of monogenean parasites increases, gill damage can be severe, with a marked impact on histology, leading to fish mortality. Similar response was recorded previously in three species of monogeneans *Anacanthorus spathulatus*, *Notozothecium janauachensis*, and *Mymarothecium boegeri* infecting *Colossoma macropomum* (Tavares-Dias *et al*., 2021), as well as in three species of monogeneans *Protolamellodiscus senilobatus*, *Acleotrema maculatus*, and *Haliotrema susanae* infecting *Argyrops filamentosus* (Abdel-Gaber *et al*., 2023).

The present study found that the host response to monogenetic parasitic infections includes the accumulation of cutaneous mucus secreted by mucous cells present in the epidermis at the site of infection, which is consistent with Zhao *et al*. (2008), who reported that mucus secretion is considered the first line of defense against infection. Spotte (1970) found that it forms a protective sheath against ectoparasitic invasion. Yolanda *et al*. (2017) revealed that the mucus produced has a negative impact on the fish respiration system, as the mucus can cover the surface of the gill lamellae, inhibiting the exchange of O_2 and CO_2. Neutrophils were also found at the site of parasite attachment, which is consistent with El-Naggar *et al*. (2019), who indicated that a higher neutrophils count is a useful indicator of host response to monogenetic infections.

Furthermore, this study found hyperplasia at the site of monogenean attachment in the gill lamellae, together with necrosis and deformities. According to Haqqawiy *et al*. (2013), the hyperplasia can occur in both secondary and primary lamellae. Similar response was recorded previously in *Seriola lalandi* infected with *Zeuxapta seriola* (Mansell *et al*., 2005), as well as *Pagellus erythrinus* fish infected with *Diplectanum aequans* (Adawy *et al*., 2016), *Gyrodactylus cichlidarum* infecting *Oreochromis niloticus* (Grano-Maldonado *et al*., 2018), and *Cichlidogyrus philander* infecting *Pseudocrenilabrus philander* (Igeh and Avenant-Oldewage, 2020). Mora *et al*. (2022) proposed that the hyperplasia may entangle attached monogenean parasites, protect underlying tissues

from pathogenic organisms, and/or replace deteriorated tissues with new healthy ones. According to Yolanda *et al*. (2017), the increase in mucus during hyperplasia represents a self-protection strategy against infections, as observed in this study.

In addition, the high intensity of parasitic infections causes the formation of an aneurysm because of pillar cell rupture, which agrees with Brown *et al*. (2004), who stated that this issue results from a disturbance in blood flow in the lamellae caused by pillar cell damage, followed by the dilation of marginal canal after a specific reaction of the gill to the toxic substances secreted by monogeneans. According to Lestari *et al*. (2018), telangiectasia develops because of additional tissue damage such as hyperplasia and necrosis.

CONCLUSION

This study, which is part of the collection of data for the genus *Bivagina* that will aid future studies and species circumscription, should be regarded as a first report on the morphological description of *B. pagrosomi* isolated from the *A. bifasciatus* (Sparidae) from the Arabian Gulf coasts (Saudi Arabia), as well as its histopathological impacts in the gill region. To better comprehend the taxonomic classification of this parasite species, substantial phylogenetic research should be conducted.

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