

Arq. Bras. Med. Vet. Zootec., v.76, n.5, e13238, 2024

http://dx.doi.org/10.1590/1678-4162-13238 Original Article - Veterinary Medicine

Morphological identification tools of *Pseudodiscocotyla opakapaka* and *Kannaphallus raphidium* (Monogenea: Polyopisthocotylea) from *Acanthopagrus bifasciatus* (Spariformes: Sparidae)

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[Ferramentas de identificação morfológica de Pseudodiscocotyla opakapaka e Kannaphallus raphidium (Monogenea: Polyopisthocotylea) de Acanthopagrus bifasciatus (Spariformes: Sparidae)]

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ABSTRACT

Fish is considered one of the nutritive protein sources affected by various parameters including parasitism. Monogeneans are an ectoparasitic species infecting fish's skin, fins, and gills. During the present investigation, a total of 40 fish samples of the twobar seabream, *Acanthopagrus bifasciatus* (Perciformes: Sparidae) were collected from the coastal region along the Arabian Gulf (Saudi Arabia). The collected fish samples were dissected and examined for the presence of monogenetic parasites. The recovered parasite species were collected and identified by applying light microscopic examinations. Five out of 40 (12.5%) fish samples were found to be naturally infected with 2 monogenetic species belonging to two families within Polyopisthocotylea (order Mazocraeidea). They recorded two parasite species, namely, *Pseudodiscocotyla opakapaka* Yamaguti, 1965 and *Kannaphallus raphidium* Kritsky and Martin, 2023 belonging to families Discocotylidae and Heteraxinidae, respectively. Morphological and morphometric characterizations revealed some differences between the present species and others detected previously. The present findings of *P. opakapaka* and *K. raphidium* in *A. bifasciatus* (fish host) of the Arabian Gulf represent a new host and geographical record for these parasites. Furthermore, more studies are required to include the advanced molecular tools for these monogenetic species and their associated histopathological effects on the infection site.

Keywords: marine fish, discocotylidae, heteraxinidae, morphology, morphometry

RESUMO

O peixe é considerado uma das fontes de proteína nutritiva que é afetada por vários parâmetros, inclusive o parasitismo. Os monogenéticos são espécies ectoparasitas que infectam a pele, as barbatanas e as brânquias dos peixes. Durante a presente pesquisa, um total de 40 amostras de peixes da dourada Acanthopagrus bifasciatus (Perciformes: Sparidae) foi coletado na região costeira ao longo do Golfo Arábico (Arábia Saudita). As amostras de peixes coletadas foram dissecadas e examinadas quanto à presença de parasitas monogenéticos. As espécies de parasitas recuperadas foram coletadas e identificadas por meio de exames de microscopia óptica. Cinco das 40 (12,5%) amostras de peixes estavam naturalmente infectadas com duas espécies monogenéticas pertencentes a duas famílias de Polyopisthocotylea (ordem Mazocraeidea). Foram registradas duas espécies de parasitas, a saber, Pseudodiscocotyla opakapaka Yamaguti, 1965 e Kannaphallus raphidium Kritsky e Martin, 2023, pertencentes às famílias Discocotylidae e Heteraxinidae, respectivamente. As caracterizações morfológicas e morfométricas revelaram algumas diferenças entre a espécie atual e outras detectadas anteriormente. As descobertas atuais de P. opakapaka e K. raphidium em A. bifasciatus (peixe hospedeiro) do Golfo Arábico representam um novo hospedeiro e registro geográfico para esses parasitas. Além disso, são necessários mais estudos para incluir ferramentas moleculares avançadas para essas espécies monogenéticas e seus efeitos histopatológicos associados no local da infecção.

Palavras-chave: peixes marinhos, discocotylidae, heteraxinidae, morfologia, morfometria

INTRODUCTION

Sparid fish species have been identified as an important source of animal protein for trade and nutrition (Froese and Pauly, 2019). Seabreams,

members of the family Sparidae, can be found in both tropical and temperate coastal waters. They are an important species for both commercial and recreational fisheries because they make great eating fish (Prakash, 2020). The twobar sea

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Submitted: January 31, 2024. Accepted: March 11, 2024.

bream, *Acanthopagrus bifasciatus*, is found in the Western Indian Ocean, from South Africa's Natal to the Red Sea and Arabian Gulf.

In aquatic environments, fish play a significant role as parasite hosts (Whittington *et al.*, 2000). The largest group of fish parasites are the monogeneans (Platyhelminthes) (Al-Azizz *et al.*, 2017), a group of ectoparasitic flatworms that live on fish's skin, fins, and gills, which have a negative economic impact (Benovics *et al.*, 2021). Purivirojkul (2008) stated that all monogeneans have a direct life cycle. According to their attachment organs, monogeneans are provided with hooks and hooklets and hence they are known as Monopistocotylea or with clamps and hence they are known as Polyopisthocotylea (Pečínková *et al.*, 2007; Öztürk and Özer, 2014; Konstanzová *et al.*, 2017).

The Discocotylidae Price, 1936 is a family of polyopisthocotylean monogeneans within the order Mazocraeidea. According to WoRMS (2023a), this family included 6 accepted genera. The genus *Pseudodiscocotyla* Yamaguti, 1965 (Discocotylidae) is distinguished from other genera by the following features: the elliptical and bulbous shape of the male copulatory organ and the armature of the genital atrium. There are 2 recognized species of the *Pseudodiscocotyla* species: *P. opakapaka* Yamaguti, 1965 infecting *Pristipomoides microlepis* (Hawaii) and *Pristipomoides filamentosus* (Japan) as well as *P. mikiae* Kamio *et al.*, 2023 infecting *P. filamentosus* (Japan).

The Heteraxinidae Unnithan, 1957 is also a family of polyopisthocotylean monogeneans (order Mazocraeidea). This family included 19 accepted genera, 1 preoccupied, 1 unjustified emendation, and 1 unaccepted (WoRMS, 2023b). The genus Kannaphallus Unnithan, 1957 (Heteraxinidae) is distinguished from other genera by the presence of asymmetric clamps, paired vaginas, and the male copulatory complex shape. The previously described Kannaphallus species were K. virilis Unnithan, 1957 infecting Atropus atropos (India), K. lateriporis Mamaev, 1988 infecting Caranx sexfasciatus (India), K. mochimae Zambrano, 1998 infecting Acanthurus coeruleus (Venezuela), K. leptosomus Nitta et al., 2022 infecting Scyris indica (Malaysia), and K. raphidium Kritsky and Martin, 2023 infecting Gnathanodon speciosus (Australia).

Several investigations on monogenean parasites infecting marine fish species in Saudi Arabia have been undertaken to date (Bayoumy *et al.*, 2012; Hassan *et al.*, 2015; Bakhraibah, 2018; Dajem *et al.*, 2019; Al-Nabati *et al.*, 2021; Morsy *et al.*, 2021; Alghamdi *et al.*, 2022; Abdel-Gaber *et al.*, 2023; Alghehri *et al.*, 2023). Therefore, this investigation aimed to study the natural occurrence and describe the morphological traits of monogenean parasite species to validate their specificity within the twobar seabream fish (*Acanthopagrus bifasciatus*) for the first record in the Arabian Gulf fish (Saudi Arabia).

MATERIALS AND METHODS

Fish samples collection. Throughout January-June 2023, the twobar seabream Acanthopagrus bifasciatus Forsskål, 1775 (Family Sparidae), were purchased from the local fishermen in the coastal region along the Arabian Gulf, Dammam (Saudi Arabia). Samples were transferred using icebox to Parasitology Laboratory at Department of Zoology, College of Science, King Saud University (Saudi Arabia). They were identified using keys provided by Abu Shusha *et al.* (2010).

Parasite collection and preparation. Fish's gills were removed, placed in a Petri dish containing normal saline solution (0.9%), and observed under a dissecting microscope (Nikon SMZ18, NIS ELEMENTS software) to find the parasites. The monogenean parasites were detached from the gills using fine dissection needles and fixed in AFA (70% ethyl alcohol-formalin-acetic acid). Parasites were stained with Aceto carmine (Sigma-Aldrich, Missouri, USA), dehydrated in an ascending ethyl alcohol series (70%, 90%, and 100%), then cleared in xylene, and finally mounted on glass slides in Canada balsam (Palm, 2004). Some parasite specimens were mounted in glycerin ammonium picrate (GAP; Sigma-Aldrich, Burlington, MA, USA) to observe the sclerotized structures.

Morphology and morphometry. The mounted specimens were examined and photographed using a Leica DM 2500 microscope (NIS ELEMENTS software, ver. 3.8). Morphometric measurements (in micrometers) of 10 specimens were considered for statistical analysis and indicated as the mean followed by the range in parentheses using ImageJ 1.53e program (Wayne Rasband and contributors, National Institute of Health, USA).

Parasitological indexes. Sites and number of each parasite species from each fish were recorded. Parasitological terms of the prevalence ((number of infected fish/number of examined fish) \times 100) and mean intensity (number of parasite species/number of infected fish) were calculated according to Bush *et al.* (1997).

RESULTS

Five out of 40 samples (12.5%) of the twobar seabream fish, *Acanthopagrus bifasciatus*, were found to have two monogenetic parasites. The recovered parasites were collected from the gill region of the infected fish samples, and all of them belonged to subclass Polyopisthocotylea. Two species found were *Pseudodiscocotyla opakapaka* Yamaguti, 1965 and *Kannaphallus raphidium* Kritsky and Martin, 2023 which were described morphologically as mentioned below.

Pseudodiscocotyla opakapaka Yamaguti, 1965 (Figure 1 and Table 1). The body was elongated, tapered at the anterior end, and measured 1580 (1400-1970) \times 260 (170-340). The head was truncated and provided with a pair of two elliptical buccal suckers, each measuring 56 (50-59) \times 76 (60-91). Gland cells were present at the anterior end of the body. The pharynx was globular and measured 54 (42-60) \times 43 (39-49). The oesophagus was short and bifurcating just behind the muscular bulb. Branches of intestinal cecal with short inconspicuous diverticula, extending into haptor, terminating very close to each other near the wide posterior sinus between two lobes of the haptoral region.

The testes were irregular in shape, 65 (54-96) in number, occupying nearly the whole postovarian inter-cecal field and extending into lateral fields among intestinal branches. The copulatory organ was ellipsoidal with a muscular bulb [76 (71-82) \times 63 (60-67)] provided with numerous spines. Genital atrium with a crown of two alternating rows of minute spines and opening mid-ventrally at a distance of 27 (19-34) from the anterior end. The germarium was situated on the right of the median line just in front of the middle of the body and its size was 154 (140-165) \times 85 (53-105). The uterus was mid-ventral with no eggs observed. A pair of elliptical vaginae lined with corrugated cuticle was observed on the ventrolateral surface of the body, measured 56 (47-79) \times 28 (25-37) and located at 277 (265-287) from the head end. Vitelline reservoir was Y-shaped, slender, median, ventral to germarium. The vitelline follicles commenced behind the vaginae and extended along the whole length of the intestinal ceca.

The haptor was not set off from the body proper, measured 243 (239-278) \times 469 (421-489), and armed with a median pair of larval hooklets and two symmetrical lobes, each bearing an oblique row of symmetrical four clamps measured 53 (42-66) \times 109 (94-114). Each clamp is made up of a pair of anterolateral sclerites and posterolateral sclerites, small sclerites, and ventro midsclerite with bifid ends and dorsal mid-sclerite with bifid ends.

Taxonomic summary.

Type species: *Pseudodiscocotyla opakapaka* Yamaguti, 1965 (Discocotylidae Price, 1936)

Type of host species: Twobar seabream fish *Acanthopagrus bifasciatus* Forsskål, 1775 (Family Sparidae)

Type of habitat and infection site: Gills of the infected fish

Prevalence of parasitic infection: 2 (5%) out of 40 specimens were naturally infected

Mean intensity of infection: 5 (4-7)

Etymology: The specific name of the parasite is derived from the local name "*opakapaka*" for the host fish from which the parasite was isolated for the first time.

Remarks: Pseudodiscocotyla Yamaguti, 1965 is a genus within the family Discocotylidae Price, 1936 with two species that infect the gill lamellae of marine fish (Yamaguti, 1965; Kamio et al., 2023). This agreed with Ilgová et al. (2021) stated that gills are considered a preferred feeding and attachment site for monogeneans. The recovered specimens from A. bifasciatus in Saudi waters have the diagnostic morphological features of *Pseudodiscocotyla* genus provided by Yamaguti (1965), especially for the unjointed intestinal branches posteriorly and the armature of cirri and genital atrium. Few studies have been conducted on Pseudodiscocotyla species. The present species is compared morphologically and morphometrically with other Pseudodiscocotyla species such as P. opakapaka and P. mikiae. The appearance of the present species is closely related to that of P. opakapaka Yamaguti, 1965 from P. microlepis (Hawaii) and Kamio et al. (2023) from P. filamentosus (Japan) in having all

the characteristic generic features of that species but with a slight difference in the clamp structure from those in the original description. Clamps are considered the characteristic feature of polyopisthocotyleans and are the main organ used for attachment to the host, which is often armed with sclerotize elements (Hayward, 2006). Yamaguti (1965) indicated that the distal ends of the mid-sclerites were bifurcated in the *Pseudodiscocotyla* species. In the recovered *Pseudodiscocotyla* species, an accessory skeletal piece represented by a projecting V-shaped process was observed on the distal end of the dorsal midsclerite that was not mentioned in the original description, this coincided with Kamio *et al.* (2023). Also, it differs from *P. mikiae* Kamio *et al.*, 2023 infecting *P. filamentosus* (Japan) which is characterized by the absence of spines around the male genital pore (*vs.* two alternating rows of spines in *P. opakapaka*), circular vaginal pore (*vs.* elliptical with corrugated cuticle in *P. opakapaka*), and the spanner-shaped dorsal mid-sclerites (*vs.* V-shaped in *P. opakapaka*). Such differences between *Pseudodiscocotyla* species justify the need to extend the generic diagnosis.



Figure 1. Photomicrographs of *Pseudodiscocotyla opakapaka* infecting *Acanthopagrus bifasciatus*. (A) Whole-mount preparation. (B-H) High magnifications for different body parts, as follows: (B-E) Anterior portion of the prohaptor. (F-H) Clamps in haptor. Note: BS, buccal sucker; PH, pharynx; GC, gland cells; OE, oesophagus; MCO, male copulatory organ; IC, intestinal crura; V, vitellaria; UT, uterus; VA, vagina; G, germium; TE, testes; CL, clamps; HA, haptor; ALS, antero-lateral sclerite; PLS, postero-lateral sclerite; SS, small sclerite; VMS, ventral mid-sclerite; DMS, dorsal mid-sclerite; LH, larval hooklets.

Comparable parameters		Pseudodiscocotyla opakapaka		Pseudodiscocotyla mikiae	Present study
Reference		Yamaguti, 1965	Kamio et al., 2023	Kamio et al., 2023	Present study
Host		Pristipomoides microlepis	Pristipomoides filamentosus	Pristipomoides filamentosus	Acanthopagrus bifasciatus
Location		Hawaii	Japan	Japan	Saudi Arabia
Body	Length	1600-2700	3875 (3200-4375)	5807 (4575-6600)	1580 (1400-1970)
-	Width	220-600	425 (375-500)	1115 (750-1300)	260 (170-340)
Buccal sucker	Length	40-70	58 (50-70)	100 (70-120)	56 (50-59)
	Width	60-110	97 (40-100)	133 (50-150)	76 (60-91)
Pharynx	Length	30-60	53 (50-60)	79 (70-90)	54 (42-60)
	Width	30-45	43 (40-50)	59 (50-70)	43 (39-49)
Germarium	Length	100-170	190 (180-200)	303 (150-350)	154 (140-165)
	Width	60-112	150 (240-260)	467 (230-570)	85 (53-105)
Number of testes		50-100	108 (99-127)	127 (93-146)	65 (54-96)
Muscular bulb	Length	75	108 (99-127)	54 (50-62)	76 (71-82)
	Width	60		25 (17-32)	63 (60-67)
Distance of genital atrium from the anterior end		17-35			27 (19-34)
Vaginae	Length	42-82	62 (55-67)	30 (25-37)	56 (47-79)
	Width	26-42	29 (20-35)	25 (22-32)	28 (25-37)
Distance of vaginae from the anterior end		270-450			277 (265-287)
Haptor length					243 (239-278)
Clamp	Length		118 (100-145)	157 (65-260)	53 (42-66)
-	Width	100-180	159 (100-205)	228 (147-300)	109 (94-114)

Table 1. Comparative metrical data for Pseudodiscocotyla opakapaka and their congeneric species

Genus Kannaphallus Unnithan. 1957. Kannaphallus raphidium Kritsky and Martin, 2023 (Figure 2 and Table 2). The body was elongated, measured at 3375 (3174-3577) \times 501 (411-553). The mouth was subterminal with a tapered end. Prohaptoral suckers were two in number, oval, widely spaced, and measured 48 $(46-50) \times 53$ (49-56). Gland cells were present in the anterior end of the body. The pharynx was oval and measured 59 $(57-61) \times 54$ (52-56). The oesophagus was slender, bifurcated above the muscular bulb forming two intestinal ceca. Branches of ceca terminated blindly in haptor, with lateral and usually medial diverticula. The genital atrium was oval, measured 90 (87-93) \times 71 (69-72), provided with a midventral common pore at the level of oesophageal bifurcation.

The testicular mass was intercaecal, occupying the whole post-ovarian space and extended into the anterior part of the haptor. The copulatory organ consisted of a muscular bulb measuring 39 $(36-42) \times 20$ (18-24) connecting at the base with a sclerotized straight duct measured 122 (115-129) × 13 (10-15). The germarium was elongated in shape, intercaecal, and situated on the middle third of the body. The vitelline reservoir was Yshaped and located ventrally to the germarium. The vitelline follicles coextensive with intestinal ceca and extend into the haptor. The haptor was asymmetrical in two unequal rows of clamps; the long row was 1204 (1195-1243) in length with 28-31 clamps; the short row was 430 (412-453) in length with 12-15 clamps. Clamps of a microcotyle-type were measured 44 (41-48) \times 51 (47-55) and consisted of a pair of anterolateral and postero-lateral sclerites, mid-sclerite, accessory sclerite, and thin muscular base. The eggs were spindle in shape, measured 177 (171-187) \times 53 (49-57) with anterior and posterior filaments.

Taxonomic summary

Type species: *Kannaphallus raphidium* Kritsky and Martin, 2023 (Heteraxinidae Unnithan, 1957)

Type of host species: Twobar seabream fish Acanthopagrus bifasciatus Forsskål, 1775 (Family Sparidae)

Type of habitat and infection site: Gills of the infected fish

Prevalence of infection: Three (7.5%) out of 40 specimens were naturally infected.

Mean intensity of infection: 7 (5-9)

Etymology: The specific name of the parasite is derived from the Greek term "raphidium" which refers to the needlelike tip of the male copulatory organ.

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Figure 2. Photomicrographs of *Kannaphallus raphidium* infecting *Acanthopagrus bifasciatus*. (A) Wholemount preparation. (B-J) High magnifications for different body parts, as follows: (B and C) Anterior portion of the prohaptor. (D) Germarium. (E) Egg. (F) Testes. (G-I) Clamps. Note: Mo, mouth; BS, buccal sucker; PH, pharynx; GC, gland cells; OE, oesophagus; MCO, male copulatory organ; V, vitellaria; VR, vitelline reservoir; G, germarium; EG, egg; TE, testes; CL, clamps; HA, haptor; ALS, antero-lateral sclerite; PLS, postero-lateral sclerite; MB, muscular base; MS, median sclerite.

Morphological identification...

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Comparable	K. virilis	К.	К.	К.	K. raphidium	Present study
parameters		lateriporis	mochimae	leptosomus		
Reference	Unnithan (1957)	Mamaev (1988)	Zambrano (1998)	Nitta <i>et al.</i> (2022)	Kritsky and Martin (2023)	Present study
Host	Atropus	Caranx	Acanthurus	Scvris	Gnathanodon	Acanthopagrus
	atropos	sexfasciatus	coeruleus	indica	speciosus	bifasciatus
Location	India	India	Venezuela	Malaysia	Australia	Saudi Arabia
Body length	3500-3600	3980-4370	1511-1990	5441-8162	3210	3375
Body length	5500 5000	3700 1370	1511 1770	5111 0102	(2770-3630)	(3174-3577)
Body width	825-840	1000-1060	556-733	442-946	677	501
Duccel qualter length	15 60		40.51	50.60	(363-733)	(411-333)
Buccal sucker lengui	43-00	-	40-51	50-09	(38-53)	(46-50)
Buccal sucker width	60-75	50	32-46	49-95	48	53
					(39-56)	(49-56)
Pharynx length	45	60	40-41	50-76	-	59 (57-61)
Pharvnx width	-	-	26-32	39-53	52	54
					(47-58)	(52-56)
Genital atrium length	135-150	40-41	62-64	104-189	75	90
-					(58-94)	(87-93)
Genital atrium width	90-105	37-40	-	75-134	48	71
					(40-59)	(69-72)
Number of genital spines	Absent	Absent	28	Absent	-	Absent
Number of testes	>100	110-130	15-20	100-151	Testicular mass	Testicular mass
Testicular zone length	1100	-	-	1864-2823	772	1423
C					(574-943)	(1356-1459)
Testicular zone width	255	-	-	201-420	231	289
					(176-275)	(280-305)
Muscular bulb length	40	-	-	46-74	209	39
e					(175-265)	(36-42)
Muscular bulb width	20	-	-	24-32	-	20
						(18-24)
Muscular duct length	150	74-82	-	199-228	196	122
					(167-227)	(115-129)
Muscular duct width	15	-	-	20-26	-	13
						(10-15)
Haptor length	1050-1100	-	-	625-1185	-	1123
						(1105-1145)
Haptor width	-	-	-	346-1562	-	496
						(469-518)
Length of the long	-	1460-1580	1156-1378	1325-1874	1230	1204
row					(963-1430)	(1195-1243)
Number of clamps in	28-31	29-38	47-51	24-32	29-39	29-39
a long row						
Length of short row	-	400-500	489-578	632-1061	376	430
					(291-444)	(412-453)
Number of clamps in	12-15	7-12	22-25	10-12	7-12	7-12
short row						
Clamp length	32-52	65	58-70	76-92	-	44
						(41-48)
Clamp width	40-60	62	40-58	82-97	51	51
					(41-57)	(47-55)

Table 2. Comparative metrical data for Kannaphallus raphidium and their congeneric species

Remarks: *Kannaphallus* Unnithan, 1957 is a genus within the family Heteraxinidae Unnithan, 1957 with 5 species that infect the gill lamellae of marine fish (Unnithan, 1957; Mamaev, 1988; Zambrano, 1998; Nitta *et al.*, 2022; Kritsky and Martin, 2023). The recovered specimens from *A. bifasciatus* in Saudi waters have the diagnostic morphological features of genus *Kannaphallus*

provided by Unnithan (1957), especially for the vaginal opening position, the morphology of the clamp, and the genital atrium. Bouguerche *et al.* (2020) reported that the presence/absence and number of vaginae is a major feature of the systematics of monogeneans. The present species is compared morphologically and morphometrically with other *Kannaphallus* species such

as K. virilis, K. lateriporis, K. mochimae, K. leptosomus, K. raphidium. There are few studies have been conducted on Kannaphallus species. Appearance of present species is closely related to that of K. raphidium Kritsky & Martin, 2023 infecting Gnathanodon speciosus (Australia) in having all characteristic generic features of that Kannaphallus species. It is differentiated from other Kannaphallus species by the following: (1) the absence of genital spines (vs. 28 in K. mochimae), (2) the dorsal vaginal pores lateral to the intestinal ceca (vs. dorsal vaginal pores intercecal in K. virilis and K. leptosomus), (3) the male copulatory organ with a short sclerotized straight duct (vs. long sclerotized straight duct in K. virilis and K. leptosomus and apparently present in K. lateriporis), (4) copulatory canal surrounded by a large glandular bulb (vs. absence of glandular bulb in K. virilis and K. leptosomus and apparently present in K. lateriporis), (5) presence of testicular mass (vs. >100 in K. virilis, K. lateriporis, K. leptosomus and 15-20 in K. *mochimae*). (6) uterus is ventral to that portion of germarium and the entirety of male reproductive system (vs. uterus extends anteriorly from the öotype dorsal to transverse arm of germarium, the vas deferens, and the distal male genitalia), (7) bilaterally symmetrical haptoral clamps (vs. asymmetrical clamps in K. lateriporis), and (8) 3-pronged projection arising from the dorsal termination of the median clamp sclerite (vs. 1pronged projection on median sclerite in K. virilis). Such differences between Kannaphallus species justify the need to extend the generic diagnosis.

CONCLUSION

Collectivelyy, the present findings enrich and update monogenean parasite list for the marine fish family Sparidae (*A. bifasciatus*) in the Arabian Gulf (Saudi Arabia). In addition, to our knowledge, this indicates the first report of the genera *Pseudodiscocotyla* and *Kannaphallus* in Saudi waters. This study was supported by morphological and morphometric criteria for the present monogeneans. Further studies are required to include the molecular phylogenetic analysis for these monogenetic taxa.

FUNDING

This study was supported by the Researchers Supporting Project (RSP2024R25), King Saud University, Riyadh, Saudi Arabia.

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