

HELMINTHOLOGIA, 61, 4: 317 - 326, 2024

Morphomolecular identification of *Polylabris lingaoensis* Tingbao, Kritsky, and Jun, 2007 infecting the twobar Seabream (*Acanthopagrus bifasciatus*)

G. ALOJAYRI, S. AL-QURAI SHY, O. B. MOHAMMED, E. AL-SHAEBI, R. ABDEL-GABER*

Department of Zoology, College of Science, King Saud University, P.O. 2455, Riyadh 11451, Saudi Arabia,
*E-mail: rabelgaber@ksu.edu.sa

Article info

Received April 24, 2024
Accepted September 19, 2024

Summary

A total of 40 fish samples of the twobar seabream, *Acanthopagrus bifasciatus* (Sparidae), were collected from January to June 2023 in the Arabian Gulf (Saudi Arabia). Fish samples were surveyed for ectoparasitic taxa. This study revealed the presence of one monogenetic species in *A. bifasciatus* gills. Parasitic prevalence and the mean value of intensity were 32.5% and 10, respectively. This parasite species has all the morphological characteristics of *Polylabris*, a genus erected by Euzet and Cauwet, 1967 (Microcotylidae), and mostly resembles *Polylabris lingaoensis* Tingbao, Kritsky, and Jun, 2007 on the presence of follicular testes ranging between 6-8, two rows of parallel microcotylid clamps in the haptor structure with 35-45 per each, the conical form of the male copulatory organ, as well as the absence of genital armature. Molecular identification of this *Polylabris* species was conducted through sequencing of the partial nuclear large subunit (28S) of the ribosomal RNA (*rRNA*) and the mitochondrial gene of cytochrome c oxidase subunit I (*COI*). 28S *rRNA* genetic sequences were found to be conserved across several *Polylabris* spp., whereas, *COI* sequences were found to be more informative even with intraspecific variation. Therefore, the isolation of *P. lingaoensis*, provided herein, represents its first report on a marine sparid fish from Saudi Arabia. Furthermore, the reported sequences are also new genetic data for *P. lingaoensis*. Our findings will serve as the basis for any future study on monogeneans in Saudi waters.

Keywords: Marine fish; Monogenetic parasites; Microcotylidae; Morphology; 28S *rRNA*; *COI*

Introduction

Fish is considered an important nutritive niche for parasites in aquatic ecosystems. The twobar Seabream *Acanthopagrus bifasciatus* Forsskål, 1775 (Sparidae) is considered one of the main components for the fishery in different regions. Like all other fish species, they are affected by parasites. Monogeneans are mainly ectoparasitic platyhelminths that live on fish's fins, skin, gills and rarely in the urinary bladder, stomach, intestine, and blood system depending on their generic species (Whittington *et al.*, 2000).

These monogenoids cause serious damage, especially to the gill filaments, after the invasiveness of their haptor and accompanying structures at the attachment site, reducing the fish's marketability (Whittington & Chisholm, 2008). Within Mazocraeidea, the largest family is Microcotylidae Taschenberg, 1879, which accounts for 51 valid genera with parasitic species specific to marine fish (de Aguiar *et al.*, 2022). Members of the genus *Polylabris* Euzet and Cauwet, 1967 (Mazocraeidea: Polyospathocotylea: Microcotylidae), characterized by the conical shape of the sclerotized male copulatory organ, comprises 23 valid nominal species reported from a wide

* – corresponding author

range of fish inhabiting marine waters (WoRMS, 2024). Of these taxa, 18 host-specific species (termed as oioxenic), infect one or restrict to a few host species with a specific attachment site on the gills (Hayward, 1996).

Species of *Polylabris* show a high morphological plasticity, so, molecular techniques have been widely utilized to support the specific identification of monogeneoids (Hossen *et al.*, 2022). To discriminate between monogeneans, various gene regions, such as nuclear large subunit (28S) ribosomal RNA (*rRNA*) and mitochondrial gene of cytochrome c oxidase I (*COI*) have been used (Catalano *et al.*, 2010; Oliva *et al.*, 2014; Mendoza-Franco *et al.*, 2018; Villora-Montero *et al.*, 2020; Al-Nabati *et al.*, 2021; Alghamdi *et al.*, 2022; Abdel-Gaber *et al.*, 2023). *Polylabris* species has 25 genetic sequences deposited in GenBank, of which thirteen are recorded for 18S and 28S *rRNA* genes, and twelve for *COI*. However, none of the studies regarding the sequences of *Polylabris* available in GenBank provided morphological descriptions, except for: *P. sillaginae* (Woolcock, 1936) Dillon, Hargis & Hargises, 1983, *P. bengalensis* Sailaja & Madhavi, 2011 and *P. mamaevi* Ogawa & Egusa, 1980 (Dilon & Hargis, 1985; Tingbao *et al.*, 2007; Al-Daraji *et al.*, 2010; Sailaja & Madhavi, 2011).

This study aims to provide molecular data using 28S *rRNA* and *COI* gene sequences to a monogenean species that previously lacked genetic sequences, to facilitate further phylogenetic analyses and enhance our understanding of the evolutionary history of Microcotylidae and *Polylabris*.

Material and Methods

Fish samples and parasitological study

From January to June 2023, forty specimens of *Acanthopagrus bifasciatus* (Sparidae) were purchased from local fishermen in the coastal region along the Arabian Gulf (Dammam, Saudi Arabia). The gills were removed from the fish, washed with saline solution, and examined for ectoparasites using a dissecting microscope (Nikon SMZ18) supplied with NIS ELEMENTS software. The terms “prevalence” and “mean intensity” follow Bush *et al.* (1997). Monogeneans were fixed in AFA (70 % ethyl alcohol-formalin-acetic acid) for morphological analysis and 100 % ethyl alcohol for the molecular study. Some monogeneans were stained by Aceto carmine (Sigma-Aldrich, Missouri, USA), cleared in xylene, and mounted in Canada balsam (Du Preez *et al.*, 2017).

Table 1. Comparative metrical data for *Polylabris lingaoensis* and their congeneric species.

Comparable parameters		Tingbao <i>et al.</i> (2007)	Bayoumy <i>et al.</i> (2015)	Present study (2024)
Host		<i>Ambassis gymnocephalus</i>	<i>Acanthopagrus bifasciatus</i>	<i>Acanthopagrus bifasciatus</i>
Location		China	Egypt	Saudi Arabia
Body	Length	1356 (1130-1597)	1231 (1120-1426)	1398 (1275-1723)
	Width	239 (159-298)	256 (179-286)	280 (230-310)
Buccal sucker	Length	41 (34-48)	43 (34-48)	49 (42-61)
	Width	43 (37-50)	46 (39-54)	54 (42-70)
Pharynx diameter		34 (27-42)	34 (27-42)	35 (30-52)
Oesophagus length		-	-	111 (102-120)
Number of genital spines		Unarmed	Unarmed	Unarmed
Male copulatory organ	Length	39 (34-45)	41 (37-48)	43 (39-50)
	Width	27 (22-33)	29 (25-36)	35 (30-45)
Germarium	Length	-	-	394 (387-411)
	Width	-	-	93 (81-102)
Vaginae diameter		-	-	49 (48-50)
Number of testes		5-7	6-8	6-8
Haptor	Length	583 (478-735)	623 (528-814)	1498 (1452-1511)
	Width	-	-	216 (207-232)
Number of clamp pairs		30-43	33-45	35-45
Anterior clamp	Length	-	-	58 (52-63)
	Width	43 (39-48)	-	33 (30-34)
Posterior clamp	Length	-	-	22 (21-25)
	Width	36 (33-40)	-	37 (36-38)

A few monogeneans were mounted in glycerin ammonium picrate (GAP; Sigma-Aldrich, Burlington, MA, USA) (Lim, 1991), to study haptor features and terminal genitalia organization. The mounted monogeneans were examined using a light microscope (Leica DM 2500, Leica Microsystems) and photomicrographs were taken for parasite specimens. Measurements (Table 1), all in micrometers, were taken with an ocular micrometer and presented as the mean followed by the range in parentheses.

Molecular phylogenetic analysis

Genomic DNA was extracted using the QIAamp® DNA Mini Kit (Qiagen, Germany) following the manufacturer's instructions. The partial *28S rRNA* and *COI* gene regions were amplified using standard PCR. The U178/L1642 primers were used for amplifying

28S rRNA as designed by Lockyer *et al.* (2003). The *COI* gene was amplified with primers COI-ASmit1/COI-ASmit2 designed by Littlewood *et al.* (1997) and Ward *et al.* (2005), respectively. To amplify the targeted genes, the cycling conditions were as follows: initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 30 sec, annealing at 56°C (*28S rRNA*) and 50°C (*COI*) for 30 sec, extension at 72°C for 1 min, and final extension 72°C for 10 min. Each amplicon (3 µl) was electrophoresed on a 1.5 % agarose gel, stained with SYBR green (Thermo Fischer Scientific, Ottawa, Canada) and 100 bp GeneRuler (Fermentas, Lithuania), and examined using a gel documentation system. Representative monogenean samples were sent to Macrogen's facilities unit in Seoul, South Korea, where they were subjected to Sanger sequencing using the same primer sets as PCR.

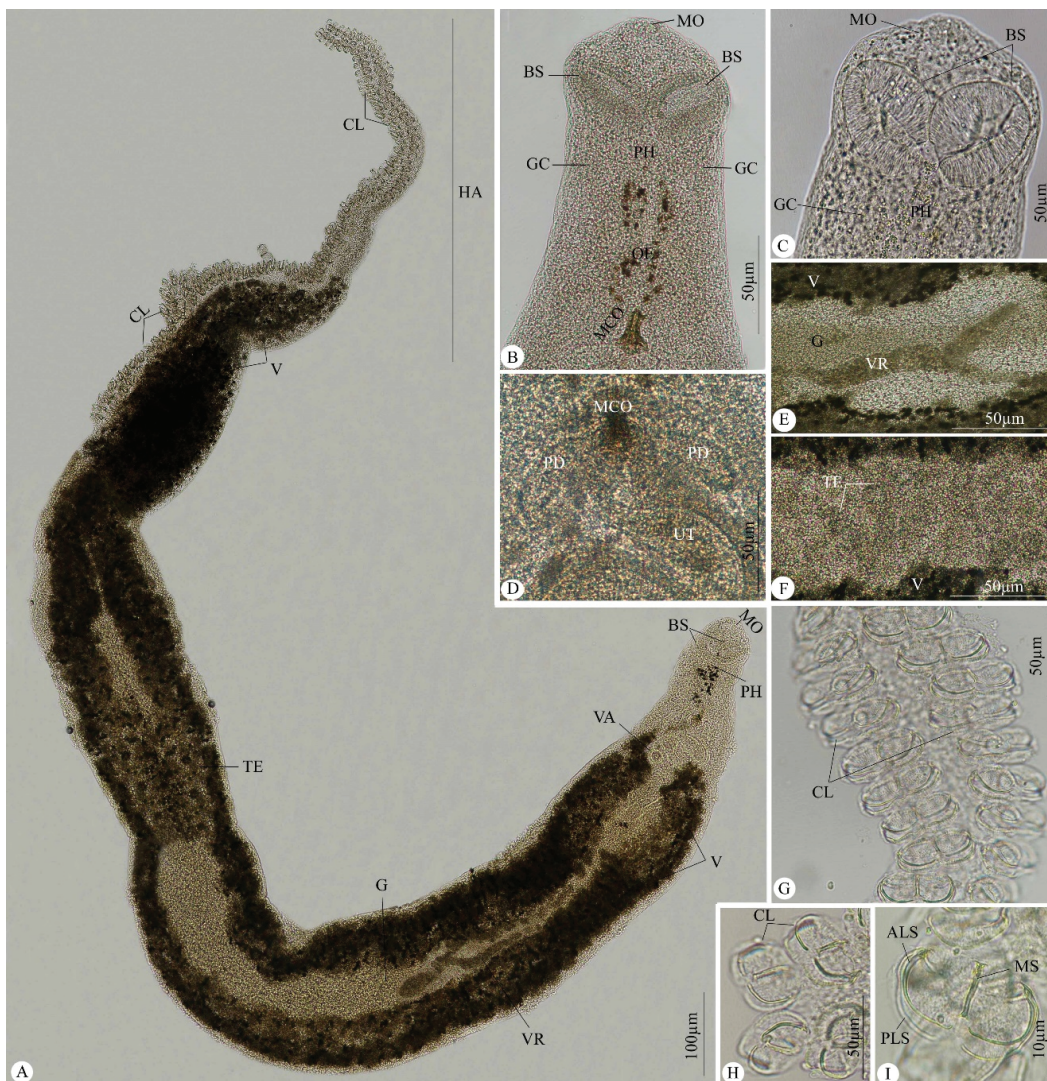


Fig. 1. Photomicrographs of *Polyabris lingaensis*. (A) Whole specimen. (B) Anterior part of prohaptor. (C) Detail of prohaptor. (D) Male copulatory organ and distal portion of seminal vesicle. (E) Germarium. (F) Testis. (G) Two clamp rows in the haptor. (H) Detail of haptoral clamps. (I) Structure of clamp. Note: ALS, antero-lateral sclerite; BS, buccal sucker; CL, clamps; G, Germarium; GC, gland cells; HA, haptor; MCO, male copulatory organ; Mo, mouth; MS, median sclerite; OE, oesophagus; PD, prostatic ducts; PH, pharynx; PLS, postero-lateral sclerite; TE, testes; UT, uterus; V, vitellaria; VA, vagina; VR, vitelline reservoir.

The sequences were deposited in GenBank and compared to the database through Basic Local Alignment Search (BLAST). Phylogenetic relationships were inferred using MEGA X (Kumar *et al.*, 2018). Bootstrap values were determined for the 28S rRNA and COI molecular data sets using 1000 replicates each.

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

The gills of 40 twobar seabream fish, *Acanthopagrus bifasciatus*, were collected from Dammam (Eastern region Saudi Arabia) and then examined for monogenoids. Thirteen twobar seabreams were infected by a species of Microcotylidae, described below as *Polylabris lingoensis* Tingbao, Kritsky, and Jun, 2007. Intensity among infected fish did not exceed ten.

Description

Body lanceolate, 1398 (1275 – 1723) long, 280 (230 – 310) wide (Fig. 1 A). Anterior end equipped with pair of elliptical prohaptor suckers, septate, and open medially, with muscular anterior, posterior, and lateral walls; septum extended diagonally across the anterolateral half of sucker (Fig. 1 A – C). Each sucker 49 (42 – 61) long, 54 (42 – 70) wide. Pharynx spherical, 35 (30 – 52) in diameter (Fig. 1 A and B). Oesophagus relatively long, 111 (102 – 120)

long, bifurcated at the level of male copulatory organ, forming two intestinal caeca reaching haptor (Fig. 1 A – C). Genital atrium unarmed and equipped with a mid-ventral genital pore.

Testes 6 to 8 in number, and intercaecal in the posterior half of the body (Fig. 1 A and F). Male copulatory organ conical, 43 (39 – 50) long, 35 (30 – 45) wide, formed by inner tube and outer sheath (Fig. 1 D). Inner tube slightly expanded with parallel margins basally, narrowing before entering the distal portion of the outer sheath. Anterior portion of male copulatory organ flat and recurved dorsally. Pair of bilateral prostatic ducts join to produce a single common prostatic duct that enters a small circular pore on the dorsal side of the male copulatory organ's external sheath (Fig. 1 D). Germarium pre-testicular (Fig. 1 A and E), shaped as an interrogation mark, intercaecal, dorsal to vitelline ducts, 394 (387 – 411) long, 93 (81 – 102) wide. Uterus elongated, reaching from body's midline to genital atrium (Fig. 1 D). Vaginae unarmed, measuring 49 (48 – 50) in diameter, opening into a single medioventral aperture posterior to the common genital opening (Fig. 1 A). Vitelline reservoir vase-shaped and positioned ventrally to germarium (Fig. 1 A, E, and F). Vitelline follicles coextensive with the intestinal caeca and extended into haptor.

Haptor 1498 (1452 – 1511) long, 216 (207 – 232) wide with two parallel subequal rows of 35 – 45 pairs of microcotylid clamps each (Fig. 1 A and G). Each clamp bilaterally symmetrical with paired antero- and postero-lateral sclerites, as well as a median sclerite with bifid ends (Fig. 1 H and I). Anterior and posterior clamps were 58 (52 – 63) long × 33 (30 – 34) wide and 22 (21 – 25) long × 37 (36 – 38) wide.

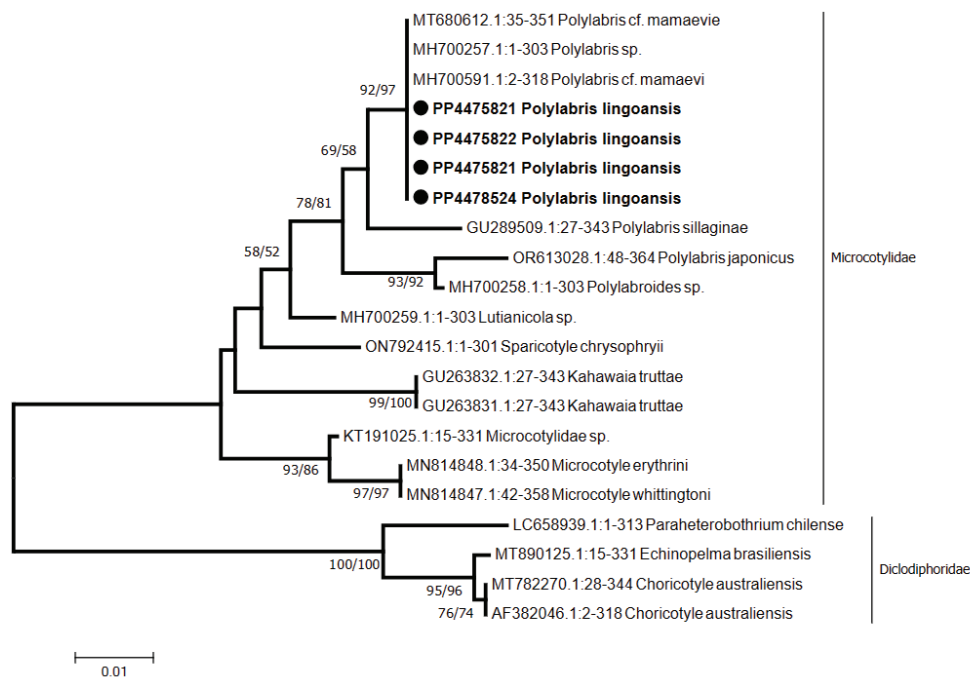


Fig. 2. A consensus phylogenetic tree constructed with maximum likelihood (ML) and Neighbor-Joining (NJ) methods, inferred from the partial 28S rRNA. Numbers indicated at branch nodes are bootstrap values. Only bootstraps > 50% are shown.

Taxonomic summary

Type host: Twobar seabream *Acanthopagrus bifasciatus* Forsskål, 1775 (Sparidae)

Type locality: Gills of the infected fish.

Prevalence and mean intensity: 32.5 % (13 fish infested of a total of 40); mean of 10 monogeneans per infested fish (range 8 – 13).

Molecular analysis (Figs. 2,3)

PCR amplification of the *28S rRNA* and *COI* regions yielded 350 bp and 420 bp, respectively. The *28S rRNA* gene provided four sequences, while the *COI* gene amplification produced two sequences. The sequences derived from the *28S rRNA* region were all identical and were deposited in GenBank with the accession numbers PP375821 to PP375824. *COI* sequences were also deposited in GenBank, with accession numbers PP372692 and PP372693. The *28S rRNA* sequences were grouped with those from the Family Microcotylidae, with two sequences from *Polylabris cf. mamaevi* (MT680612 and MH700591) and *Polylabris sp.* (MH700257) demonstrating a significant bootstrap value (Table 2). The sequences obtained in this study had a 100 % similarity

to those of *Polylabris cf. mamaevi* and *Polylabris sp.* The present study's sequences indicated 98 % similarity to *Polylabris sillaginae* (GU289509), *Polylabroides sp.* (MH700258), and *Lutianicola sp.* (MH700259), as well as 97 % similarity to *Polylabris japonicus* (OR613028). Both Maximum Likelihood (ML) and Neighbor-Joining (NJ) phylogenetic trees based on *28S rRNA* sequences revealed the same topology, with sequences related to *P. lingaoensis* obtained in the current study clustering with *Polylabris cf. mamaevi* and *Polylabris sp.* The present sequences were distinct from those of *P. sillaginae* and *P. japonicus* (Fig. 2).

Sequences for the recovered monogenoid species from the *COI* region (PP372692 and PP372693) were not similar, and the alignment revealed one mutation (transversion) at position 201, which was an A on PP372692 and a T on PP372693. However, the amino acids produced during translation remained unchanged. Phylogenetic trees (NJ and ML) derived from the analysis of *P. lingaoensis COI* sequences and sequences from other *Polylabris* species available in GenBank (Table 3) revealed two separate clades of *Polylabris* spp. One clade included *P. sillaginae* and *P. australi-*

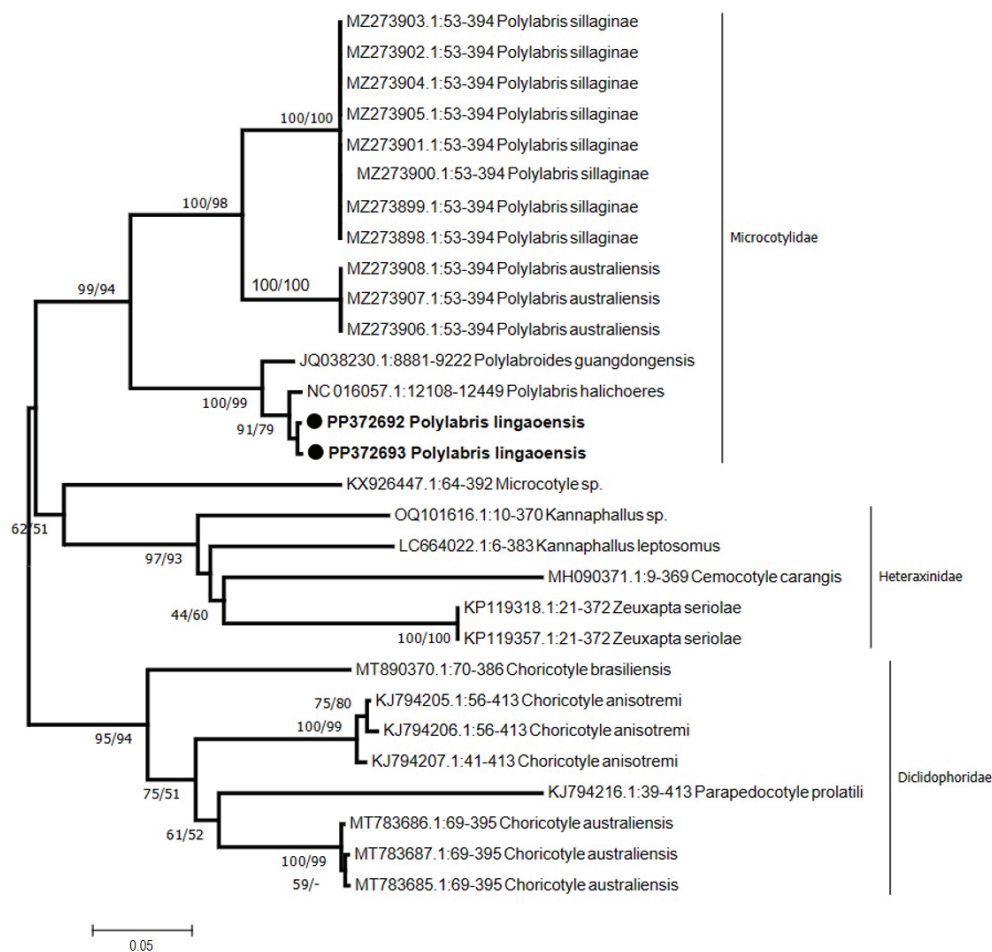


Fig. 3. A consensus phylogenetic tree constructed with maximum likelihood (ML) and Neighbor-Joining (NJ) methods, inferred from the partial *COI*. Numbers indicated at branch nodes are bootstrap values. Only bootstraps > 50% are shown.

aensis, while another included *P. lingaoensis*, *P. halichoeres*, and *Polylabroides guangdongensis* (Fig. 3). The closest relation of *P. lingaoensis* was seen with *P. guangdongensis*, with variations in 4 to 5 nucleotides. The difference with *P. guangdongensis* was seen on 10 to 11 nucleotides.

Discussion

Monogenea are ectoparasitic platyhelminths that live on the body surfaces, fins, head, gills, eyes, and oral and branchial cavities of several fish species (Whittington & Chisholm, 2008). These worms cause significant damage due to the invasiveness of their suckers, clamps, and hooks at the attachment site (Hutson *et al.*, 2007). Many monogenean taxa have been described globally from marine fish species. Little information is available on species of the family Microcotylidae. *Polylabris* currently contains twenty-three species found in various fish hosts and locations (WoRMS, 2024). The monogenean specimens found herein were identified following the dichotomous keys of Hussey (1986), Hayward (1996), and Tingbao *et al.* (2007) for the genus *Polylabris* for the first time in the sparid fish from Saudi Arabia.

In this investigation, the gills of thirteen two-bar seabreams (32.5 %) were found to be infected with a polyopisthocotylean parasite from the *Polylabris* genus, with an average intensity of

10. The current prevalence is lower than the previous data of *Polylabris bengalensis* reported by Sailaja and Madhavi (2011) in *Siganus javus* and *S. oramin* from Visakhapatnam coast (Bay of Bengal, India) (Prevalence =50 %) and mean intensity of 17.8, *Polylabris lingaoensis* reported by Bayoumy *et al.* (2015) in *Acanthopagrus bifasciatus* from Red Sea (Hurghada, Egypt) (Prevalence =53.3 %), and *Polylabris sillaginae* reported by Hossen *et al.* (2022) in *Sillago flindersi* from NSW coast and Victorian coast (Australia) (prevalence =55 % and 3 %) with a mean intensity of 1.93 and 1 parasites/host, respectively. Furthermore, this prevalence is higher than the previous data of *Polylabris tubicirrus* reported by Santos *et al.* (1996) in *Diplodus argenteus* Copacabana beach (Rio de Janeiro, Brazil) (prevalence =12 %), and *Polylabris mamaevi* reported by Al-Daraji *et al.* (2010) in *Acanthopagrus latus* from Khor Abdullah (Northwest Arabian Gulf) (prevalence =17 %) with a mean intensity of 1.3 parasites/host.

Members of the *Polylabris* genus differ from other microcotylids in that they have a single sclerotized male copulatory organ generally conical (Hayward 1996). These *Polylabris* species might be distinguished by the morphology of the male copulatory organ, the number of vaginal pores (uni- or bi-vaginate), the number and form of testes, the number of clamp pairs, and the extent of caeca. The current microcotylid specimens are identical to *P. lingaoensis* identified previously by Tingbao *et al.* (2007) from the gills of *Ambassis*

Table 2. Monogenean species used in the phylogenetic analysis of 28S rRNA gene of *Polylabris lingaoensis*.

Parasite species	Host species	Infection site	Locality	Reference
PP375821 <i>Polylabris lingaoensis</i>	<i>Acanthopagrus bifasciatus</i>	Gills	Saudi Arabia	Present study
PP375822 <i>Polylabris lingaoensis</i>	<i>Acanthopagrus bifasciatus</i>	Gills	Saudi Arabia	Present study
PP375823 <i>Polylabris lingaoensis</i>	<i>Acanthopagrus bifasciatus</i>	Gills	Saudi Arabia	Present study
PP375824 <i>Polylabris lingaoensis</i>	<i>Acanthopagrus bifasciatus</i>	Gills	Saudi Arabia	Present study
MT680612 <i>Polylabris</i> cf. <i>mamaevii</i>	<i>Siganus fuscescens</i>	Gills	China	GenBank
MH700257 <i>Polylabris</i> sp.	-	-	China	GenBank
MH700591 <i>Polylabris</i> cf. <i>mamaevii</i>	-	-	China	GenBank
GU289509 <i>Polylabris sillaginae</i>	<i>Sillaginodes punctatus</i>	Gills	Australia	GenBank
OR613028 <i>Polylabris japonicus</i>	<i>Acanthopagrus schlegelii</i>	-	South Korea	GenBank
MH700258 <i>Polylabroides</i> sp.	-	-	China	GenBank
MH700259 <i>Lutianicola</i> sp.	-	-	China	GenBank
ON792415 <i>Sparicotyle chrysophryii</i>	<i>Sparus aurata</i>	Gills	Tunisia	GenBank
GU263832 <i>Kahawaia truttae</i>	<i>Arripis trutta</i>	Gills	Australia	GenBank
GU263831 <i>Kahawaia truttae</i>	<i>Arripis trutta</i>	Gills	Australia	GenBank
KT191025 <i>Microcotylidae</i> sp.	<i>Dentex dentex</i>	Gills	Greece	GenBank
MN814848 <i>Microcotyle erythrinii</i>	<i>Pagellus erythrinus</i>	Gills	Spain	GenBank
MN814847 <i>Microcotyle whittingtoni</i>	<i>Dentex dentex</i>	Gills	Spain	GenBank
LC658939 <i>Paraheterobothrium chilense</i>	<i>Hippoglossina macrops</i>	-	Japan	GenBank
MT890125 <i>Echinopelma brasiliensis</i>	<i>Pagrus pagrus</i>	-	Brazil	GenBank
MT782270 <i>Choricotyle australiensis</i>	<i>Chrysophrys auratus</i>	Gills	Australia	GenBank
AF382046 <i>Choricotyle australiensis</i>	<i>Choricotyle australiensis</i>	-	Australia	GenBank

gymnocephalus (India) and Bayoumy *et al.* (2015) from the gills of *Acanthopagrus bifasciatus* (Egypt). The only variation between *P. lingaoensis* from *A. bifasciatus* and two specimens from *A. gymnocephalus* and *A. bifasciatus*, is the size of the haptor concerning the body length, the number of clamps, and the copulatory organ size. In contrast, body size is not considered a reliable feature to be used for discriminating *Polylabris* species, especially in the presence of various fish species (type host), and this is supported by Bayoumy *et al.* (2015), who reported that *Polylabris* species can be significantly smaller than the average even when isolated from their usual host.

Some *Polylabris* species exhibit a high level of host specificity by being restricted to fish of the family Sparidae including *P. diplodi*, *P. acanthopagri*, *P. tubicirrus*, *P. japonicus*, *P. angifer*, *P. rhabdosargi*, and *P. lingaoensis*, whilst those appearing on several host species

within different families (i.e. Kuhliidae, Gobiidae, Gerreidae, Pomacentridae, Siganidae, Sillaginidae, Leiognathidae, Mugilidae, Kyphosidae, and Labridae) with diversity in geographical distribution are limited to fishes with relatively tight phylogenetic relationships, such as Tingbao *et al.* (2007) stated that this pattern of host ranges shows a low possibility of cospeciation among *Polylabris* species, with adaptive forms of speciation dominating the genus *Polylabris*' evolutionary history and development.

Moreover, *P. lingaoensis* could be differentiated from other *Polylabris* species based on the morphology of testes (oval and arranged linearly in *P. mamaevi*), as well as the number of testes (5 in *P. sigani*; 5 – 6 in *P. carnarvonensis*; 18 – 20 in *P. virgatarum*; 18 – 24 (by Mamaev & Parukhin, 1976) and 9 – 14 (by Tingbao *et al.*, 2007) in *P. mamaevi*; numerous follicular in *P. bengalensis*; 9 – 10 in *P. indica*; 9 – 13 in *P. halichoeres*; 9 – 17 in *P. angifer*,

Table 3. Monogenean species used in the phylogenetic analysis of *COI* gene of *Polylabris lingaoensis*.

Parasite species	Host species	Infection site	Locality	Reference
PP372692 <i>Polylabris lingaoensis</i>	<i>Acanthopagrus bifasciatus</i>	Gills	Saudi Arabia	Present study
PP372693 <i>Polylabris lingaoensis</i>	<i>Acanthopagrus bifasciatus</i>	Gills	Saudi Arabia	Present study
MZ273903 <i>Polylabris sillaginae</i>	<i>Sillago flindersi</i>	-	Australia	GenBank
MZ273902 <i>Polylabris sillaginae</i>	<i>Sillago flindersi</i>	-	Australia	GenBank
MZ273904 <i>Polylabris sillaginae</i>	<i>Sillago flindersi</i>	-	Australia	GenBank
MZ273905 <i>Polylabris sillaginae</i>	<i>Sillago flindersi</i>	-	Australia	GenBank
MZ273901 <i>Polylabris sillaginae</i>	<i>Sillago flindersi</i>	-	Australia	GenBank
MZ273900 <i>Polylabris sillaginae</i>	<i>Sillago flindersi</i>	-	Australia	GenBank
MZ273899 <i>Polylabris sillaginae</i>	<i>Sillago flindersi</i>	-	Australia	GenBank
MZ273898 <i>Polylabris sillaginae</i>	<i>Sillago flindersi</i>	-	Australia	GenBank
MZ273908 <i>Polylabris australiensis</i>	<i>Engraulis australis</i>	-	Australia	GenBank
MZ273907 <i>Polylabris australiensis</i>	<i>Engraulis australis</i>	-	Australia	GenBank
MZ273906 <i>Polylabris australiensis</i>	<i>Engraulis australis</i>	-	Australia	GenBank
JQ038230 <i>Polylabroides guangdongensis</i>	-	-	China	GenBank
NC016057 <i>Polylabris halichoeres</i>	-	-	-	GenBank
KX926447 <i>Microcotyle</i> sp.	<i>Scorpaena notata</i>	Gills	France	GenBank
OQ101616 <i>Kannaphallus</i> sp.	<i>Gnathanodon speciosus</i>	Gills	Australia	GenBank
LC664022 <i>Kannaphallus leptosomus</i>	<i>Scyris indica</i>	Gills	Malaysia	GenBank
MH090371 <i>Cemocotyle carangis</i>	<i>Caranx latus</i>	Gills	Brazil	GenBank
KP119318 <i>Zeuxapta seriola</i>	<i>Seriola lalandi</i>	Gills	Chile	GenBank
KP119357 <i>Zeuxapta seriola</i>	<i>Seriola lalandi</i>	Gills	Chile	GenBank
MT890370 <i>Choricotyle brasiliensis</i>	<i>Orthopristis ruber</i>	Gills	Brazil	GenBank
KJ794205 <i>Choricotyle anisotremi</i>	<i>Anisotremus scapularis</i>	Gills	Chile	GenBank
KJ794206 <i>Choricotyle anisotremi</i>	<i>Anisotremus scapularis</i>	Gills	Chile	GenBank
KJ794207 <i>Choricotyle anisotremi</i>	<i>Anisotremus scapularis</i>	Gills	Chile	GenBank
KJ794216 <i>Parapedocotyle prolatili</i>	<i>Prolatilus jugularis</i>	Gills	Chile	GenBank
MT783686 <i>Choricotyle australiensis</i>	<i>Chrysophrys auratus</i>	Gills	Australia	GenBank
MT783687 <i>Choricotyle australiensis</i>	<i>Chrysophrys auratus</i>	Gills	Australia	GenBank
MT783685 <i>Choricotyle australiensis</i>	<i>Chrysophrys auratus</i>	Gills	Australia	GenBank

12 – 16 in *P. diplodi*; 13 – 15 in *P. tubicirrus*).

Regarding to the number of clamps per row, the present species could be distinguished from other related ones (19 – 25 in *P. acanthogobii*; 20 – 35 (Hossen *et al.*, 2022), 19 – 36 (Hayward, 1996) in *P. australiensis*; 23 – 32 in *P. halichoeres*; 25 – 44 (Mamaev & Parukhin, 1976) and 27 – 47 (Tingbao *et al.*, 2007) in *P. mamaevi*; 30 in *P. sigani*; 27 – 40 (Hossen *et al.*, 2022), 22 – 34 (Dillon *et al.*, 1985), 21 – 25 (Williams, 1991), and 27 – 39 (Hayward, 1996) in *P. sillaginae*; 32 – 39 in *P. bengalensis*; 41 – 47 in *P. carnarvonensis*; 48 – 50 in *P. virgatarum*; 58 in *P. tubicirrus*; 55 – 60 in *P. diplodi*; 53 – 63 in *P. japonicus*; 50 – 70 in *P. angifer*).

According to the reproductive organs features, there are some points differentiate the current species from those described previously, as following the number of vaginae (bi-vaginate in *P. sigani*, *P. sillaginae*, *P. australiensis*, *P. williamsi*, *P. carnarvonensis*), the genital atrium armature (armed in *P. acanthopagri*), and the anterior portion of the male copulatory organ (straight with outer sheath strongly sclerotized with broad base and parallel proximal margins in *P. kuhliiae*).

The morphological identification of *Polylabris* species requires molecular analysis to establish its generic position utilizing the partial 28S rRNA and COI gene sequences obtained during this study. Sequences from the 28S rRNA related to *P. lingaoensis* were identical to those from *Polylabris* cf. *mamaevi* and *Polylabris* sp., indicating that the 28S rRNA region is unsuitable for differentiation between congeners of *Polylabris*, as stated by previous works (Mendoza-Franco *et al.*, 2018). Mendoza-Franco *et al.* (2018) found that all microcotylids exhibited little variation at the molecular level with their relevant organisms from distant geographic locations. Even when they showed remarkable morphologic differences, as seen herein. That was attributed to the region of the 28S rRNA being highly conserved in microcotylids, which demonstrated that using sequences from the mitochondrial DNA such as COI should be prioritized when distinguishing between species of monogeneans, which is reinforced by the results of the present work (Mendoza-Franco *et al.*, 2018). Even within the two sequences we described herein there was a mutation at one point which has shown intraspecific variation. Therefore, COI is a useful marker to differentiate between different members of the family Microcotylidae. Mitochondrial DNA sequences, represented by COI, from specimens of the present study clearly showed distinction from *P. australiensis*, *P. sillaginae*, *Polylabroides guangdongensis*, and *Polylabris halichoeres*. Based on the morphological characteristics the organism under study is related to *P. lingaoensis*.

Conclusion

The present findings update the available data about *P. lingaoensis* (Microcotylidae) from the gills of *A. bifasciatus*. This is the first report of this monogenean parasite in marine fish in Saudi Arabia. Moreover, we have also shown both 28S rRNA and COI sequences from *P. lingaoensis* for the first time. Sequences of COI were

found to be more informative in elucidating the phylogenetic position of *P. lingaoensis* with related taxa.

Conflict of Interest

No conflict of interest was declared by the authors regarding the content of this article.

Consent to Participate

All authors in this study agreed to participate.

Consent to Publish

All authors in this study agreed to publish the data.

Availability of Data and Materials

All the datasets generated or analyzed during this study are included in this published article.

Acknowledgement

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

References

- ABDEL-GABER, R., ALGHAMDI, M., AL QURAI SHY, S., AL-SHAEBI, E.M., ELKHADRAGY, M.F., EL-ASHRAM, S., DKHIL, M.A. (2023): A monogenean gill parasite within the genus *Haliotrema* (Ancyrocephalidae) infecting *Argyrops filamentosus* fish: Morphology and molecular studies. *Animals*, 13 (6): 1010. DOI: 10.3390/ani13061010
- AL-DARAJI, S., BANNAI, M.A.A., ABBAS, A.A.K. (2010): Some parasites of the yellow-finned sea bream *Acanthopagrus latus* (Houttuyn, 1782) in the Iraqi marine waters. *Iraqi J Aquacult*, 7(2): 115 – 122. DOI: 10.58629/ijaq.v7i2.249
- ALGHAMDI, M., AL-QURAI SHY, S., AL-SHAEBI, E.M., ABDEL-GABER, R. (2022): Morphological and molecular analyses *Protolamellodiscus senilobatus* (Monogenea: Diplectanidae), a gill parasite infecting the soldier bream *Argyrops filamentosus* (Sparidae). *J King Saud Uni Sci*, 35: 102417. DOI: 10.1016/j.jksus.2022.102417
- AL-NABATI, E., ALI, S., AL-QURAI SHY, S., ALAJMI, R., AL-SHAEBI, E.M., ALJAWDAH, H.M.A., DKHIL, M.A., ABDEL-GABER, R. (2021): *Heteromicrocotyla polyorchis* Unnithan, 1961 (Monogenea: Heteromicrocotylidae), a gill parasite of the yellow-spotted trevally, *Carangoides fulvoguttatus* (Carangidae) from Saudi Arabia: Morphology and phylogeny. *Microb Pathog*, 160: 105165. DOI: 10.1016/j.micpath.2021.105165
- BAYOUMY, E.M., EL-LAMIE, M.M.M., DERWA, H.I.M. (2015): First report of *Polylabris lingaoensis* (Monogeneoidea: Polyopisthocotylea) infesting the gills of *Acanthopagrus bifasciatus* from the Red Sea,

- off Hurghada; Egypt. *World J Fish Mar Sci*, 7 (3): 209 – 213. DOI: 10.5829/idosi.wjfm.2015.7.3.95269
- BUSH, A.O., LAFFERTY, K.D., LOTZ, J.M., SHOSTAK, A.W. (1997): Parasitology meets ecology on its own terms: Margolis *et al.* revisited. *J Parasitol*, 83: 575 – 583. DOI: 10.2307/3284227
- CATALANO, S.R., HUTSON, K.S., RATCLIFF, R.M., WHITTINGTON, I.D. (2010): Redescriptions of two species of microcotylid monogeneans from three arripid hosts in southern Australian waters. *Sys Parasitol*, 76: 211 – 222. DOI: 10.1007/s11230-010-9247-x
- DE AGUIAR, J.C.C., DOMINGUES, M.V., SILVA, W., CECCARELLI, P.S., ADRIANO, E.A., SOARES, G.B. (2022): Morphology and molecular phylogeny of *Pauciconfibuloides amazonica* gen. n. sp. n. (Platyhelminthes, Monogeneoidea) parasitizing the Amazonian croaker *Plagioscion squamosissimus*. *Parasitol Inter*, 87: 102489. DOI: 10.1016/j.parint.2021.102489
- DILLON, W.A., HARGIS, W.J., HARRISES, A.E. (1983/1985): Monogeneans from the southern Pacific Ocean, Polyopisthocothleids from Australian fishes, The subfamilies Polylabrinae and Microcotylinae. *Zool J*, 62 (6): 821 – 828
- DU PREEZ, L.H., BADETS, M., HÉRITIER, L., VERNEAU, O. (2017): Tracking platyhelminth parasite diversity from freshwater turtles in French Guiana: First report of *Neopolystoma* Price, 1939 (Monogenea: Polystomatidae) with the description of three new species. *Parasit Vectors*, 10: 53. DOI: 10.1186/s13071-017-1986-y
- EUZET, L., CAUWET, A. (1967): *Polylabris diplodi* n. g., n. sp. (Monogenea, Microcotylidae) parasite de teleosteens du genre *Diplodus* (Sparidae). *Bull Mus Natl Hist Nat*, 39 (1): 213 – 220
- FORSSKÅL, P. (1775): *Descriptiones animalium avium, amphibiorum, piscium, insectorum, vermium; quae in itinere orientali observavit. Post mortem auctoris edidit Carsten Niebuhr. Hauniae. Descr. Animalium Descriptiones animalium avium, amphibiorum, piscium, insectorum, vermium; quae in itinere orientali observavit... Post mortem auctoris edidit Carsten Niebuhr.* Mölleri, Copenhagen, 20 pp., xxxiv+164 pp. (Pisces on pp. x–xix + 22 – 76)
- HAYWARD, C.J. (1996): Revision of the monogenean genus *Polylabris* Microcotylidae). *Invertebr Syst*, 10 (5): 995 – 1039. DOI: 10.1071/IT9960995
- HOSSEN, M.S., BARTON, D.P., WASSENS, S., SHAMSI, S. (2022): Molecular (cox1), geographical, and host record investigation of monogeneans *Mazocraes australis* (Mazocraeidae), *Polylabris sillaginae*, and *P. australiensis* (Microcotylidae). *Parasitol Res*, 121 (12): 3427 – 3442. DOI: 10.1007/s00436-022-07664-8
- HUSSEY, C.G. (1986): Some monogenean parasites of marine perciform fishes of Kuwait. *J Nat Hist*, 20: 415 – 430. DOI: 10.1080/00222938600770311
- HUTSON, K.S., ERNST, I., WHITTINGTON, I.D. (2007): Risk assessment for metazoan parasites of yellowtail kingfish *Seriola lalandi* (Perciformes: Carangidae) in South Australian sea-cage aquaculture. *Aquaculture*, 271: 85 – 99. DOI: 10.1016/j.aquaculture.2007.03.020
- KUMAR, S., STECHER, G., LI, M., KNYAZ, C., TAMURA, K. (2018): MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. *Mol Biol Evol*, 35(6): 1547 – 1549. DOI: 10.1093/molbev/msy096
- LIM, L.H.S. (1991): Preparation of museum specimens – Monogenea. *Fish Health Section Newsletter*, 2: 10 – 11
- LITTLEWOOD, D.T.J., ROHDE, K., CLOUGH, K.A. (1997): Parasite speciation within or between host species? – Phylogenetic evidence from site-specific polystome monogeneans. *Inter J Parasitol*, 27: 1289 – 1297. DOI: 10.1016/S0020-7519(97)00086-6
- LOCKYER, A.E., OLSON, P.D., LITTLEWOOD, D.T.J. (2003): Utility of complete large and small subunit rRNA genes resolving the phylogeny of the Neodermata (Platyhelminthes): implications and a review of the cercomer theory. *Biol J Linn Soc*, 78: 155 – 171. DOI: 10.1046/j.1095-8312.2003.00141.x
- MAMAIEV, Y.L., PARUKHIN, A.M. (1976): On the genus *Polybris* Euzet et Cauwet, 1967 and some closely allied species of microcotylids (Monogeneoidea: Microcotylidae). *Parazitologiya*, 10(3): 245 – 254
- MENDOZA-FRANCO, E.F., TUN, M.D.C.R., ANCHEVIDA, A.J.D., RODRÍGUEZ, R.E.D.R. (2018): Morphological and molecular (28S rRNA) data of monogeneans (Platyhelminthes) infecting the gill lamellae of marine fishes in the Campeche Bank, southwest Gulf of Mexico. *ZooKeys*, 783: 125 – 161. DOI: 10.3897/zookeys.783.26218
- OGAWA, K., EGUSA, S. (1980): Two species of microcotylid monogeneans collected from black seabream *Acanthopagrus schlegali* (Bleeker) (Teleostei: Sparidae). *Jpn J Parasitol*, 29(6): 455 – 462
- OLIVA, M.E., SEPULVEDA, F.A., GONZÁLEZ, M.T. (2014): *Parapedocotyle prolatili* gen. n. et sp. n., a representative of a new subfamily of the Dicliphoridae (Monogenea), a gill parasite of *Prolatilus jugularis* (Teleostei: Pinguipedidae) from Chile. *Folia Parasitol*, 61(6): 543. DOI: 10.14411/fp.2014.067
- SAILAJA, B., MADHAVI, R. (2011): *Polylabris bengalensis* sp. nov. (Monogenea, Microcotylidae) from siganid fishes of the Visakhapatnam coast, Bay of Bengal, India. *Acta Parasitol*, 56 (3): 290 – 295. DOI: 10.2478/s11686-011-0058-2
- SANTOS, C.P., SOUTO-PADRÓN, T., LANFEREDI, R.M. (1996): *Atrистер heterodus* (Lebedev and Paruchin, 1969) and *Polylabris tubicirrus* (Paperna and Kohn, 1964) (Monogenea) from *Diplodus argenteus* (Vail., 1830) (Teleostei: Sparidae) from Brazil. *J Helminthol Soc Wash*, 63 (2): 181 – 187
- TASCHENBERG, E.O. (1879): Zur Systematik der monogenetischen Trematoden. *Z. Naturw. Berl.*, 52: 232 – 265
- TINGBAO, Y., KRITSKY, D.C., JUN, P. (2007): *Polylabris lingaoensis* sp. n. and *Polylabris* cf. *mamaevi* Ogawa et Egusa, 1980 (Monogeneoidea: Microcotylidae) from perciform fishes in the Gulf of Tonkin, South China Sea. *Folia Parasitol*, 54: 27 – 33. DOI: 10.14411/fp.2007.004
- TUBANGUI, M.A. (1931): Trematode parasites of Philippine vertebrates, IV. Ectoparasitic flukes from marine fishes. *Philipp J Sci*, 45: 109 – 117
- VILLORA-MONTERO, M., PÉREZ-DEL-OLMO, A., GEORGIEVA, S., RAGA, J., MONTERO, F. (2020): Considerations on the taxonomy and morphology of *Microcotyle* spp.: redescription of *M. erythrini* van Beneden & Hesse, 1863 (*sensu stricto*) (Monogenea: Microcotylidae) and

- the description of a new species from *Dentex dentex* (L.) (Teleostei: Sparidae). *Parasit Vectors*, 13. DOI: 10.1186/s13071-020-3878-9
- WARD, R.D., ZEMLAK, T.S., INNES, B.H., LAST, P.R., HEBERT, P.D. (2005): DNA barcoding Australia's fish species. *Philos Trans R Soc Lond B Biol Sci*, 360: 1847 – 1857. DOI: 10.1098/rstb.2005.1716
- WHITTINGTON, I.D., CHISHOLM, L. (2008): Diseases caused by Monogenea. In: EIRAS, J.C., SEGNER, H., WAHLII, T., KAPOOR, B.G. (Eds) *Fish Diseases. Science Publishers Ltd, Manchester, NH*, pp 683 – 816
- WHITTINGTON, I.D., CRIBB, B.W., HAMWOOD, T.E., HALLIDAY, J.A. (2000): Host-specificity of monogenean (platyhelminth) parasites: a role for anterior adhesive areas? *Inter J Parasitol*, 30(3): 305 – 320. DOI: 10.1016/S0020-7519(00)00006-0
- WILLIAMS, A. (1991): Monogeneans of the families Microcotylidae Taschenberg, 1879 and Heteraxinidae Price, 1962 from Western Australia, including the description of *Polylabris sandarsae* n. sp. (Microcotylidae). *Syst Parasitol*, 18: 17 – 43. DOI: 10.1007/BF00012221
- WOOLCOCK, V. (1936): Monogenetic trematodes from some Australian fishes. *Parasitology*, 28(1): 79 – 91. DOI: 10.1017/S0031182000022277
- WoRMS (2024): *Polylabris Euzet & Cauwet, 1967*. Retrieved April 19, 2024 from: <https://www.marinespecies.org/aphia.php?p=tax-details&id=119382>