http://dx.doi.org/10.1590/1678-4162-12993

Arq. Bras. Med. Vet. Zootec., v.75, n.4, p.673-681, 2023

Morphological and molecular characterization of the monogenean gill parasite, *Acleotrema maculatus*, infecting *Argyrops filamentosus* fish in the Red Sea, Saudi Arabia

[*Caracterização morfológica e molecular do parasita monogênico de brânquias,* Acleotrema maculatus, *que infecta o peixe* Argyrops *filamentosus no Mar Vermelho, Arábia Saudita*]

E. *Alshehri*, R. *Abdel-Gaber**, S. *Al Quraishy*, M. *Alghamdi*, E.M. *Al-Shaebi*

Department of Zoology, College of Sciences, King Saud University, Riyadh, Saudi Arabia

ABSTRACT

Fish are considered one of the important sources of protein which are invaded by different parasites. This study aimed to shed light on monogenean parasites that infect fish within the family Sparidae in Saudi Arabia. A total of 30 *Argyrops filamentosus* specimens were collected from the Red Sea, the city of Jeddah (Saudi Arabia), and then examined for the presence of monogenean parasites. Parasitic species were isolated and studied morphologically using light microscopic examination and molecularly via the partial sequencing of the *28S rRNA* gene. Only a monogenean parasitic species has been identified. This parasite is morphologically and morphometric compatible with previously *Acleotrema maculatus* Morsy, El-Fayoumi & Fahmy (2014), identified from *Plectropomus maculatus* in the Red Sea, Egypt. Phylogeny revealed that this putative diplectanid species nested well within a clade clustering Diplectanidae species, which along with morphological data, suggests it is a member of the genus *Acleotrema*. Query sequences showed identities of 98.92% for *28S rRNA* (AF026118.1) of *Acleotrema* sp. This study reflects the first account of this genus as endoparasite taxa of the examined sparid fish, as well as providing novel DNA data for this species.

Keywords: digenea, diplectanidae, morphology, phylogeny

RESUMO

Os peixes são considerados uma das fontes importantes de proteína e são invadidos por diferentes parasitas. O objetivo deste estudo foi esclarecer os parasitas monogênicos que infectam peixes da família Sparidae na Arábia Saudita. Um total de 30 espécimes de Argyrops filamentosus *foi coletado no Mar Vermelho, na cidade de Jeddah (Arábia Saudita), e depois examinado quanto à presença de parasitas monogênicos. As espécies parasitárias foram isoladas e estudadas morfologicamente por meio de exame microscópico leve e molecularmente por meio do sequenciamento parcial do gene 28S rRNA. Apenas uma espécie de parasita monogênico foi identificada. Esse parasita é morfologicamente e morfometricamente compatível com o* Acleotrema maculatus *Morsy, El-Fayoumi & Fahmy (2014), identificado anteriormente em* Plectropomus maculatus *no Mar Vermelho, Egito. A filogenia revelou que essa suposta espécie de diplectanídeo se aninhou bem em um clado que agrupa espécies de Diplectanidae, o que, juntamente com dados morfológicos, sugere que é um membro do gênero* Acleotrema. *As sequências de consulta mostraram identidades de 98,92% para 28S rRNA (AF026118.1) de* Acleotrema *sp. Este estudo reflete o primeiro relato desse gênero como táxon endoparasitário do peixe esparídeo examinado, além de fornecer novos dados de DNA para essa espécie.*

Palavras-chave: digenea, diplectanidae, morfologia, filogenia

*Corresponding author: rabdelgaber.c@ksu.edu.sa
Submitted: February 11, 2023. Accepted: March 27, 2023.

INTRODUCTION

Fish have great importance and significance in the life of mankind. Fish are well known to be parasitized by many eukaryotic organisms (M'Rabet *et al*., 2016), and the gills of fish represent one of the biotopes mostly exploited by different ectoparasites and invaded by many species of parasites, including monogeneans (Shawket *et al*., 2018). The genus *Acleotrema* was proposed for the first time by Johnston and Tiegs (1922). *Acleotrema* is a parasite of marine perciform fish (Carangidae, Kyphosidae, Sparidae, and Toxotidae) (Domingues and Boeger, 2008). This genus is currently composed of 16 described species according to the World Register of Marine Species (Acleoreema…, 2022). Rakotofiringa *et al.* (1987) proposed *Heteroplectanum* for diplectanids with squamodiscs consisting of divergent rows of dumbbell-shaped rodlets, with two more internal rows in a 'V'-shape. However, all species of *Acleotrema* share this feature and possess a relatively uniform male copulatory organ morphology that is fundamentally like that of *Heteroplectanum*. Species previously allocated to *Acleotrema* and *Heteroplectanum* are, thus, congeneric.

Recently, morphological descriptions have been complemented with generic analysis, with both techniques allowing researchers to precise monogenean species identification (Mendoza-Franco *et al*., 2018; Víllora-Montero *et al*., 2020). Various target regions, including nuclear ribosomal DNA (rDNA), mitochondrial DNA (mtDNA), or repetitive DNA elements (microsatellite loci) which show considerable variation in the number of repeats within individuals have been employed to achieve the identification of parasite species or strains (Ahmed *et al*., 2011). The partial sequences of the ribosomal DNA coding regions (internal transcribed spacer (ITS)-1 and large subunit ribosomal (28S) RNA genes) were widely used to estimate the level of divergence at both intra- and interspecific levels among monogeneans (Domingues and Boeger, 2008; Kaci-Chaouch *et al*., 2008; Poisot *et al*., 2011; Mendoza-Franco *et al*., 2018).

There are, however, few studies on the monogeneans infecting fish in Saudi Arabia. This study is therefore designed to determine the study of the monogenean species infecting the king soldier bream (*Argyrops filamentosus*) from the Red Sea (Saudi Arabia) by using morphological and molecular analyses.

MATERIALS AND METHODS

Thirty specimens of the soldier bream fish, *Argyrops filamentosus* Valenciennes, 1830 (F: Sparidae) were collected from commercial fishermen on the Red Sea coast in Jeddah, Saudi Arabia. The fish's gills were separated, submerged in 0.9% saline solution to eliminate any extra gill mucus, and then inspected for monogeneans under a stereomicroscope (Nikon SMZ18, NIS ELEMENTS software). Using delicate dissection needles, monogeneans were extracted from the gills. Worms were preserved in 4% formalin for 2 h for microscopic studies or 96% ethanol for molecular analysis.

To remove excess fixatives, the fixed specimens (n=10) were rinsed in distilled water (Hassan *et al*., 2015). According to Malmberg (1973), worms were first mounted as semi-permanent preparations in a glycerin ammonium-picrate (GAP; Sigma-Aldrich, Missouri, USA) before being mounted in Canada balsam. Slides were incubated at 60ºC for 24 h to drive the air bubbles according to Schmidt (1992). The mounted specimens and the relevant structural details were examined, recorded, and photographed at different magnifications using a Leica DM 2500 microscope (NIS ELEMENTS software, version 3.8). Measurements were expressed in micrometers (µm). The site and the number of parasite species infecting fish were recorded. The parasitological terms of the prevalence and mean intensity were calculated according to Bush *et al*. (1997).

Genomic DNA was extracted using Qiagen DNeasy tissue kit© (Hilden, Germany) according to the manufacturer's instructions. For parasite identification, a partial *28S rRNA* gene was targeted and amplified using PCR. Primers for *28S rRNA* were LSU5 (TAGGTCGACCCGCTGAAYTTA) and LSU1200R (GCTATCCTGGAGGGAAACTTCG) designed by Littlewood *et al*. (2000) and Tkach *et al*. (2003), respectively. BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, USA) was used to sequence PCR products via ABI PRISM 310 Genetic Analyzer (Applied Biosystems,

CA). BLASTn compared sequences in the NCBI database to find the most similar ones and then aligned them using ClustalW. Maximum Likelihood (ML) in MEGA 7.0 was used to construct phylogenetic trees with 1000 replicates.

RESULTS

Fourteen out of thirty (46.66%) specimens of the examined soldier bream fish, *Argyrops filamentosus*, were naturally infected with a monogenetic parasite with a specific site for infection in the fish gills. This parasitic species was identified as *Acleotrema maculatus* Morsy *et al.* (2014) regarding the morphological identification criteria. The intensity of infection does not exceed eight in each of the parasitized fish with a mean of 7.14. This monogenetic species was examined in detail at both morphological and molecular phylogenetic levels to confirm its taxonomic position within the family Diplectanidae. Microscopic examinations **(**Figure 1**)**

The body was fusiform with parallel lateral margins. Tegument smooth. Cephalic lobes were well developed with 3 pairs of head organs and two groups of cephalic glandular cells at the level of the pharynx. Two pairs of eye spots anterior to the pharynx. Mouth subterminal, opening ventrally. The pharynx was sub-globular in shape. Esophagus was very short. The intestinal caeca are not confluent and end posteriorly close to the peduncle. Haptor with 2 similar lamellodiscs with radial rows of contiguous dumbbell-shaped rodlets; internal rows of rodlets form a 'V'-shape. The two types of anchors are different; ventral anchor with an outer root that was very long, stout, and slightly notched at the broad proximal end; the inner root was conical in shape, with a short shaft; as well as the dorsal anchor with a base large, stout, with only lateral rudiment of roots; the blade and point were long and curved with a long shaft. The two types of bars are different. Lateral (dorsal) bars are two stout, dumbbell shaped. The ventral bar is slender, with a transverse groove. Seven pairs of hooks were observed; hook pair 1 was located close to the ventral anchor, followed by hook pairs 2, 3, and 4 surrounding squamodiscs; hook pair 5 was behind the ventral bar; hook pair 6 close to the dorsal anchor's point, and hook pair 7 close to the dorsal anchor's base. The male copulatory organ (MCO) was a sclerotized part of a curved tube with a broad distal loop and a subterminal recurved spine as well as an accessory piece that bifurcates at the midpoint.

Total length 0.912-0.963 (0.930), maximum width 0.101-0.163 (0.131); pharynx width 0.033-0.038 (0.035); haptor width 0.051-0.093 (0.076); lamellodisc 0.041-0.084 (0.063) × 0.022-0.071 (0.040); ventral anchor 0.032-0.071 (0.053); dorsal anchor 0.038-0.042 (0.040); ventral bar 0.080-0.132 (0.113); dorsal bar 0.055-0.060 (0.058); and male copulatory organ 0.060-0.065 (0.063).

The examined monogenetic species' partial *28S rRNA* sequence was 462 bp with a GC content of 48.3% [A(27.06% 125) | C(19.7% 91) | G(28.57% 132) | T(24.68% 114)] and deposited in GenBank under the accession number OP870550.1 (Figure 2). The ML approach was used to align nucleotide sequence data from 30 taxa over 460 positions to produce a phylogenetic dendrogram that represented different monogenetic species (Table 1). The overall mean distance among all specimen sequences was 0.128. A pairwise comparison with the GenBank *28S rRNA* gene data set confirmed the identification of the genus *Acleotrema* (Table 1). The phylogenetic analysis included taxa of the order monopisthocotylean represented by two families Diplectanidae and Ancylodiscoididae (Figure 2). Comparable species have identity ranges, 98.92-86.15% for Diplectanidae and 85.82-83.93% for Ancylodiscoididae (Table 1). The current dendrogram is split into two major clades (Figure 2), the first of which was strongly supported by species belonging to the Diplectanidae, and the latter of which was represented by taxa belonging to Ancylodiscoididae. The phylogenetic dendrogram showed a well-resolved distinct clade with Diplectanidae species and the recovered monogenetic species (Figure 2). High sequence identity (98.92%) is shown by the examined species for *Acleotrema* species, and this is highly confirmed by a value of 98. With a high bootstrap value of 100, the current species was robustly grouped in the same clade as *Acleotrema* sp. (AF026118.1).

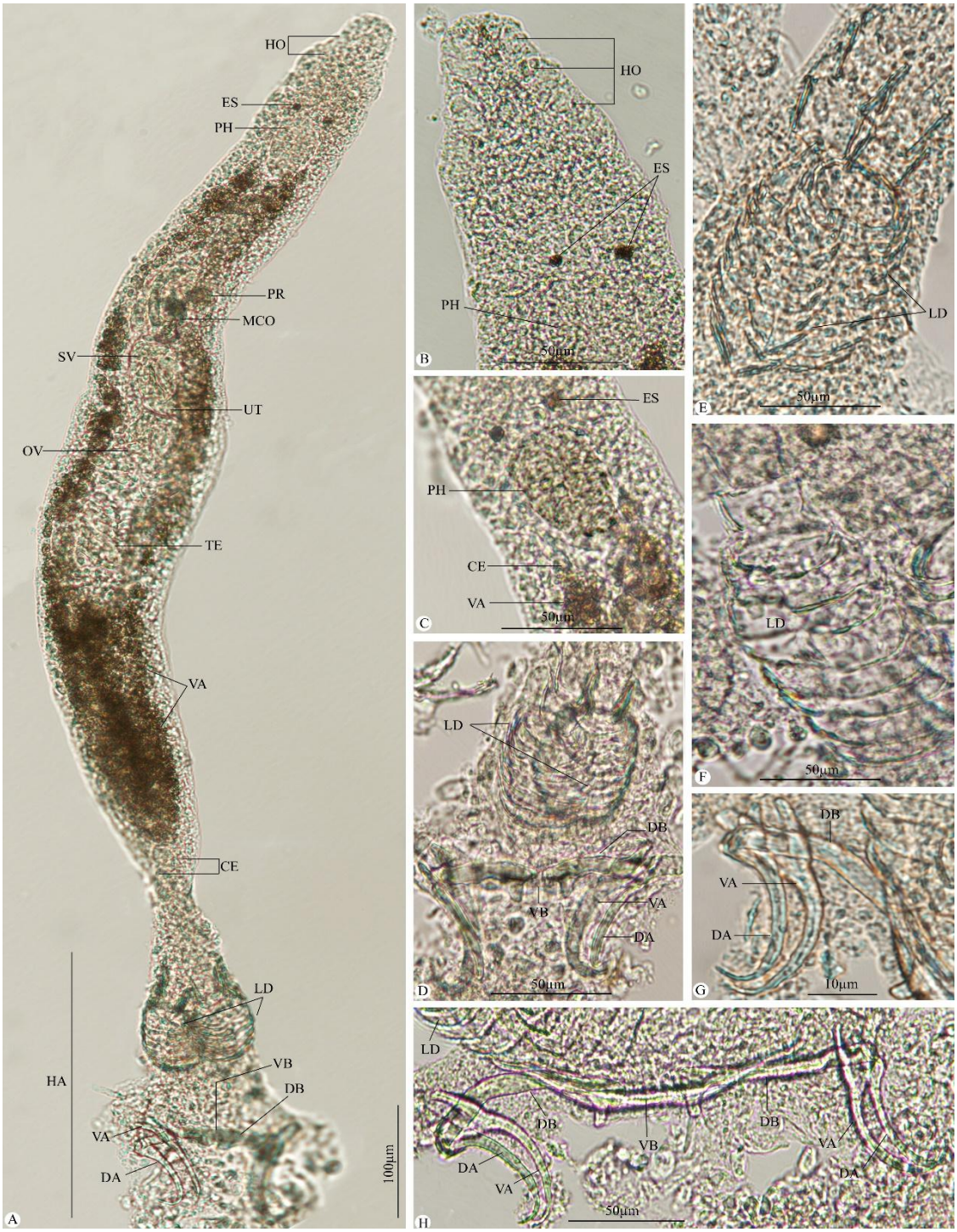

Figure 1. Photomicrographs of *Acleotrema maculatus* infecting *Argyrops filamentosus*. (A) whole mount preparation. (B, C) anterior region of the body. (D) haptor with related structures. (E-G) Lamellodiscs. (H) anchors and bars of haptor. Note: HO, head organs; ES, eye spots; PH, pharynx; PR, prostatic reservoir; MCO, male copulatory organ; SV, seminal vesicle; OV, ovary; TE, testis; VA, vitellaria; CE, Ceca; HA, haptor; LD, lamellodiscs; DA, dorsal anchor; VA, ventral anchor; VB, ventral bar, DB, dorsal bar.

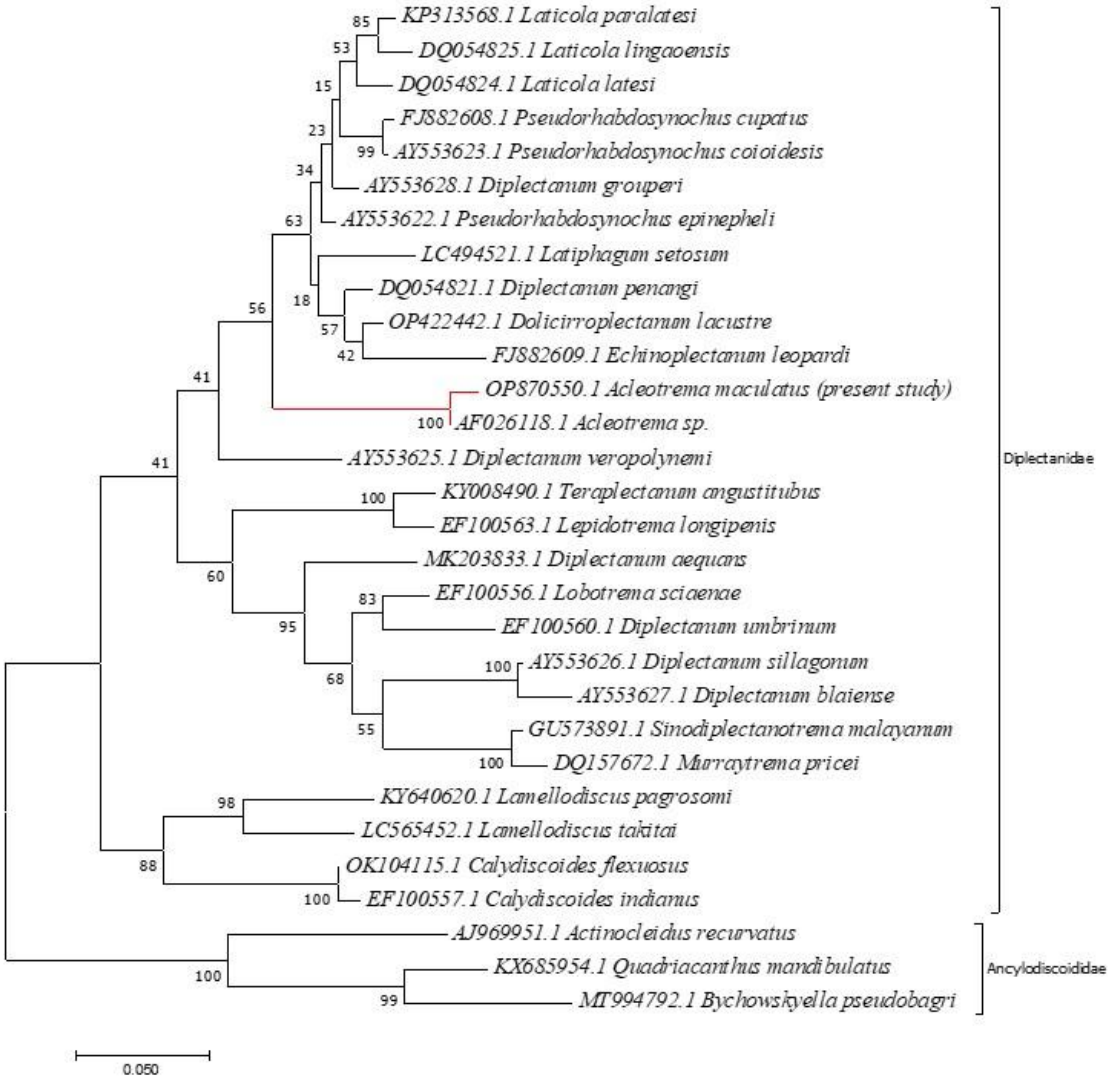

Figure 2. Molecular Phylogenetic analysis by Maximum Likelihood method based on the Tamura 3-parameter model. The tree with the highest log likelihood (-3780.14) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site.

Table 1. Monopisthocotylean taxa used for phylogenetic analysis of the *28S rRNA* sequence

Parasite species	Family	Source	% Identity	GC content
AF026118.1 *Acleotrema sp.*	Diplectanidae	GenBank	98.92	50.2
AY553622.1 *Pseudorhabdosynochus epinepheli*	Diplectanidae	GenBank	94.55	51.4
AY553628.1 *Diplectanum grouperi*	Diplectanidae	GenBank	93.90	51.9
DQ054825.1 *Laticola lingaoensis*	Diplectanidae	GenBank	93.81	50.8
KP313568.1 *Laticola paralatesi*	Diplectanidae	GenBank	93.68	50.7
FJ882608.1 *Pseudorhabdosynochus cupatus*	Diplectanidae	GenBank	93.32	52.6
AY553623.1 *Pseudorhabdosynochus coioidesis*	Diplectanidae	GenBank	93.25	53.1
DQ054821.1 *Diplectanum penangi*	Diplectanidae	GenBank	92.84	51.9
OP422442.1 *Dolicirroplectanum lacustre*	Diplectanidae	GenBank	92.47	52.6
LC494521.1 *Latiphagum setosum*	Diplectanidae	GenBank	92.03	49.7
FJ882609.1 *Echinoplectanum leopardi*	Diplectanidae	GenBank	91.90	53.7
AY553625.1 *Diplectanum veropolynemi*	Diplectanidae	GenBank	91.82	52.5
DQ054824.1 *Laticola latesi*	Diplectanidae	GenBank	91.01	50.6
MK203833.1 *Diplectanum aequans*	Diplectanidae	GenBank	90.07	48.1
EF100560.1 *Diplectanum umbrinum*	Diplectanidae	GenBank	89.14	50.2
KY640620.1 *Lamellodiscus pagrosomi*	Diplectanidae	GenBank	88.81	47.7
KY008490.1 *Teraplectanum angustitubus*	Diplectanidae	GenBank	88.28	54.5
GU573891.1 *Sinodiplectanotrema malayanum*	Diplectanidae	GenBank	88.14	49.3
EF100556.1 *Lobotrema sciaenae*	Diplectanidae	GenBank	88.07	48.3
DQ157672.1 *Murraytrema pricei*	Diplectanidae	GenBank	87.95	49.6
AY553626.1 *Diplectanum sillagonum*	Diplectanidae	GenBank	87.50	49.3
AY553627.1 *Diplectanum blaiense*	Diplectanidae	GenBank	86.97	48.1
EF100563.1 *Lepidotrema longipenis*	Diplectanidae	GenBank	86.93	55.2
EF100557.1 *Calydiscoides indianus*	Diplectanidae	GenBank	86.26	49.2
OK104115.1 *Calydiscoides flexuosus*	Diplectanidae	GenBank	86.18	47.8
LC565452.1 *Lamellodiscus takitai*	Diplectanidae	GenBank	86.15	47
KX685954.1 *Quadriacanthus mandibulatus*	Ancylodiscoididae	GenBank	85.82	47.4
MT994792.1 *Bychowskyella pseudobagri*	Ancylodiscoididae	GenBank	85.19	47.5
AJ969951.1 *Actinocleidus recurvatus*	Ancylodiscoididae	GenBank	83.93	48.7

DISCUSSION

Many studies are available about parasites infecting marine fish inhabiting the Red Sea. Little information is available about the monogenean parasites infecting *A. filamentosus* (family Sparidae) (Yoon *et al*., 2013, 2015; Hassan *et al*., 2015; Sánchez-García *et al*., 2015; Alghamdi *et al*., 2023). Therefore, this study is designed to investigate the presence of monogenean parasites in the gill region of this sparid host collected from the Red Sea (Jeddah, Saudi Arabia). In this study, only 14 specimens of the total 30 *A. filamentosus* had an infection rate of 46.66% for the recovered parasite. The recovered parasite species was isolated from the gills of the infected soldier bream fish. This rate is quite similar to Morsy *et al*. (2014) stated that 53.3% of *Plectropomus maculatus* (Serranidae) (from the Coasts of Hurghada City, Red Sea, Egypt) were infected with *Acleotrema maculatus*.

The current species is compatible with other diplectanids, *Acleotrema* species, that have inhabited perciform fish, by sharing all the species' morphological distinguishing features. *Acleotrema* was originally proposed by Johnston and Tiegs (1922) for *Acleotrema girellae* from *Girella tricuspidata* (Quoy and Gaimard) in Australian waters. *Acleotrema* species were reported in perciform fish within families of Carangidae, Kyphosidae, Sparidae, and Toxotidae. Our results corroborated with Domingues and Boeger (2007) and Morsy *et al*. (2014) that the primary key feature for *Acleotrema* species is the presence of accessory adhesive organ (squamodiscs) with articulated rodlets, tubular MCO comprised of two nested tubes surrounded by proximally by a slightly sclerotized sac and accessory piece absent, and

the genital atrium was heavily sclerotized. The recovered species has all morphological features with the previously recorded *A. maculatus*, with special reference to the squamodiscs with close rings of rodlets. Intraspecific variations between the measurements of this *Acleotrema* species and those of *A. maculatus* were reported complete. Therefore, the host species was considered as a new host and locality records for the recovered parasite in Saudi Arabia. It differs from all previous *Acleotrema* species in measurements of different body parts especially haptor and its related structures. It has some observable discriminating features from *A. lamothei* which has a wider haptor (twice the width of the body) and the open rings of squamodiscs with more rows of rodlets and *A. serrulopenis* with a spined male copulatory organ.

The molecular analysis combined with morphological data has helped to resolve phylogenetic relationships and species identity in diplectanid monogeneans (Domingues and Boeger, 2006; Yoon *et al*., 2013). The reported parasite has most similarities with the previously described *A. maculatus* which was not phylogenetically analyzed before. Wu *et al*. (2005) reported that *28S rRNA* is known to allow excellent phylogenetic resolution among monogenean groups. The phylogenetic position of *Acleotrema* species identified from Saudi Arabia was validated in this study using the partial genetic sequences of the *28S rRNA* gene. The family Diplectanidae is unambiguous and located in a distinct clade, as demonstrated by the sequence alignment and phylogenetic trees in the current study. Similar findings have been reported by Chotnipat *et al*. (2015), Tambireddy *et al*. (2016), Villar-Torres *et al*. (2019), and Nitta (2021). The recorded parasite cannot be assigned to any of the aligned sequences since the percentage of sequence identities between the present parasite and the aligned sequences exhibited a maximum identity of 98.92% with *Acleotrema* sp. (AF026118.1) that isolated from the gill region of *Xyphosus vaigienis* from Heron Island, Australia. This agreed with previous studies by Mollaret *et al*. (1997), Domingues and Boeger (2007), Santos *et al*. (2008), and Morsy *et al*. (2014) reported the key identification criteria for *Acleotrema* species. This study is regarded as the first report combining the morphological description and molecular analysis of the partial *28S rRNA* sequences of *A. maculatus* isolated from the *A. filamentosus* (Sparidae) from the coasts of the Red Sea at Jeddah, Saudi Arabia.

CONCLUSION

This study is regarded as the first report combining the morphological description and molecular analysis of the partial *28S rRNA* sequences of *A. maculatus* isolated from the *A. filamentosus* (Sparidae) from the coasts of the Red Sea at Jeddah, Saudi Arabia.

ACKNOWLEDGMENTS

This study was supported by the Researchers Supporting Project (RSP2023R25), King Saud University, Riyadh, Saudi Arabia.

REFERENCES

ACLEOTREMA Johnston & Tiegs, 1922. [s.l.]: WoRMS, 2022. Available in: https://www.marinespecies.org/aphia.php?p=taxdetails&id=517904. Accessed in: 26 Dec. 2022.

AHMED, M.; SINGH, M.N.; BERA, A.K. *et al.* Molecular basis for identification of species/isolates of gastrointestinal nematode parasites. *Asian Pac. J. Trop. Med*., v.4, p.589-593, 2011.

ALGHAMDI, M.; AL-QURAISHY, S.; AL-SHAEBI, E.M.; ABDEL-GABER, R. Morphological and molecular analyses *Protolamellodiscus senilobatus* (Monogenea: Diplectanidae), a gill parasite infecting the soldier bream *Argyrops filamentosus* (Sparidae). *J. King Saud Univ. Sci*., v.35, p.102417, 2023.

BUSH, A.O.; LAFFERTY, K.D.; LOTZ, J.M.; SHOSTAK, A.W. Parasitology meets ecology on its own terms: Margolis *et al*. revisited. *J. Parasitol*., v.83, p.575-583, 1997.

CHOTNIPAT, S.; MILLER, T.L.; KNUCKEY, R.M.; HUTSON, K.S. Molecular and morphological evidence for the widespread distribution of *Laticola paralatesi* infecting wild and farmed *Lates calcarifer* in Australia. *Dis. Aquat. Organ*., v.113, p.195-205, 2015.

DOMINGUES, M.V.; BOEGER, W.A. Phylogeny and revision of Diplectanidae Monticelli, 1903 (Platyhelminthes: Monogenoidea). *Zootaxa*, v.1698, p.1-40, 2008.

DOMINGUES, M.V.; BOEGER, W.A. Revision and phylogeny of Rhamnocerciane Monaco, Wood et Mizelle, 1954 (Monogenoidea: Diplectanidae). *Folia Parasitol*., v.53: p.107-116, 2006.

DOMINGUES, M.V.; BOEGER, W.A. The status of *Acleotrema* Johnston & Tiegs, 1922 and *Heteroplectanum* Rakotofiringa, Oliver & Lambert, 1987 (Monogenoidea: Diplectanidae), with the redescription of *Acleotrema girellae* Johnston & Tiegs, 1922. *Syst. Parasitol*., v.66, p.35-41, 2007.

HASSAN, M.A.; OSMAN, H.A.M.; ASWATHAN, M. Infestation of cage-cultured fish with *Benedenia acanthopagri* (Monogenea: Capsalidae) in eastern province of Saudi Arabia. *Global Vet*., v.14, p.219-227, 2015.

JOHNSTON, T.H.; TIEGS, O.W. New gyrodactyloid trematodes from Australian fishes together with a reclassification of the super-family Gyrodactyloidea. Proc. Linnean Soc. N. S. Wales, v.47, p.83-131, 1922.

KACI-CHAOUCH, T.; VERNEAU, O.; DESDEVISES, Y. Host specificity is linked to intraspecific variability in the genus *Lamellodiscus* (Monogenea). *Parasitology*, v.135, p.607-616, 2008.

LITTLEWOOD, D.T.J.; CURINI-GALLETTI, M.; HERNIOU, E.A. The interrelationships of Proseriata (Platyhelminthes: Seriata) tested with molecules and morphology. *Mol. Phylogenet. Evol*., v.16, p.449-466, 2000.

M'RABET, C.; ENSIBI, C.; DHAOUADI, R.; YEHIA, O.K. A preliminary study on gill parasites of gilthead sea bream *Sparus aurata* (Linnaeus 1758) (Pisces: Teleostei) from the eastern Tunisian sea-cage aquaculture. *Biol. Environ. Sci*., v.7, p.1-5, 2016.

MALMBERG, G. On a *Gyrodactylus* species from Northern Sweden and the subgeneric position of *G. hrabei* Ergens, Trematoda, Monogenea. *Zool. Scripta*, v.2, p.39-42, 1973.

MENDOZA-FRANCO, E.F.; TUN, M.D.C.R.; ANCHEVIDA, A.J.D. 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*, v.783, p.125-161, 2018.

MOLLARET, I.; JAMIESON, B.G.M.; ADLARD, R.D. *et al*. Phylogenetic analysis of the Monongenea and their relationships with Digenea and Eucestoda inferred from 28S rDNA sequences. *Mol. Biochem. Parasitol*., v.90, p.433-438, 1997.

MORSY, K.; EL FAYOUMI, H.; FAHMY, M. Description of *Acleotrema maculatus* sp. nov. (Monogenea: Diplectanidae) infecting the spotted coral grouper *Plectropomus maculatus* (F: Serranidea) from the Red Sea and its histopathological impact. Beni-Suef University *J. Basic Appl. Sci*., v.3, p.222-228, 2014.

NITTA, M. *Lamellodiscus* (Monogenea: Diplectanidae) species parasitic on Japanese *Acanthopagrus* Peters, with proposals of *L. chin* n. sp. infecting *A. sivicolus* Akazaki and *L. egusai* nom. nov. for *L. japonicus* Ogawa & Egusa, 1978, a junior homonym of *L. japonicus* Pillai & Pillai, 1974. *Syst. Parasitol.*, v.98, p.177-188, 2021.

POISOT, T.; VERNEAU, O.; DESDEVISES, Y. Morphological and molecular evolution are not linked in *Lamellodiscus* (Platyhelminthes, Monogenea). *PLoS One*, v.6 p.e26252, 2011.

RAKOTOFIRINGA, S.; OLIVER, G.; LAMBERT, A. *Heteroplectanum* n. gen., un nouveau genre de Diplectanidae Bychowsky, 1957 (Monogenea, Monopisthocotylea) parasite de Téléostéens marins de Madagascar. *Bull. Muséum Nat. d'Hist. Nat., Paris*, 4éme Série, 9, Sect A (1), p.145-157, 1987.

SÁNCHEZ-GARCÍA, N.; RAGA, J.A.; MONTERO, F.E. (2015) Morphological and attachment changes of *Lamellodiscus theroni* (Monogenea: Diplectanidae) during its post-larval development on fish. *Folia Parasitol*., v.62, p.26, 2015.

SANTOS, C.P.; BIANCHI, L.G.; GIBSON, D.I. *Acleotrema lamothei* n. sp. (Monogenea Diplectanidae) from the gills of *Kyphosus incisor* in Brazilian waters. *Rev. Mex. Biodiversidad*, v.79, p.69S-73S, 2008.

SCHMIDT, G.D. *Essentials of parasitology*.5.ed. Brown: C. Publishers, 1992.

SHAWKET, N.; ELMADHI, Y.; M'BARECK, I. *et al*. Distribution of two monogenean (Gastrocotylidae) from the North Atlantic coast of Morocco. *Beni Suef Univ. J. Basic Appl. Sci*., v.7, p.270-275, 2018.

TAMBIREDDY, J.D.; GIBSON, T.J.; PLEWNIAK, F. *et al.* The CLUSTAL-X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res*., v.24, p.4876-4882, 2016.

TKACH, V.V.; LITTLEWOOD, D.T.J.; OLSON, P.D. *et al.* Molecular phylogenetic analysis of the Microphalloidea Ward, 1901 (Trematoda: Digenea). *Syst. Parasitol*., v.56, p.1-15, 2003.

VILLAR-TORRES, M.; REPULLÉS-ALBELDA, A., MONTERO, F.E. *et al.* Neither *Diplectanum* nor specific: a dramatic twist to the taxonomic framework of *Diplectanum* (Monogenea: Diplectanidae). *Int. J. Parasitol.*, v.49, p.365-374, 2019.

VÍLLORA-MONTERO, M.; PÉREZ-DEL-OLMO, A.; GEORGIEVA, S. *et al.* 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*, v.13, p.45, 2020.

WU, X.Y.; CHILTON, N.B.; ZHU, X.Q. *et al*. Molecular and morphological evidence indicates that *Pseudorhabdosynochus lantauensis* (Monogenea: Diplectanidae) represents two species. *Parasitology*, v.130, p.660-677, 2005.

YOON, G.; AL-ADAWI, H.; SHINN, A. Gill monogenean communities on three commercially important sparid fish in Omani waters. *J. Agric. Mar. Sci*., v.20, p.54-61, 2015.

YOON, G.H., AL-JUFAILI, S., FREEMAN, M.A. *et al. Omanicotyle heterospina* n. gen. et n. comb. (Monogenea: Microcotylidae) from the gills of *Argyrops spinifer* (Forsskål) (Teleostei: Sparidae) from the Sea of Oman. *Parasit. Vectors*, v.6, p.170, 2013.