BF-1626
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RESEARCH ARTICLE Indian Journal of Animal Research

DNA Barcoding of *Argyrops filamentosus* (Perciformes: Sparidae) Based on Mitochondrial COI Gene and Histopathological Evaluation of Gills Infected by Monogeneans

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10.18805/IJAR.BF-1626

ABSTRACT

Background: Sparidae is a fish family that belongs to the order Perciformes and is commonly known as sea breams and porgies. Many species are included in this family and divided into 71 genera that could be barcoded using mitochondrial DNA genes. These fish are vulnerable to different parasitic taxa that affect fish status. The present study is aimed to confirm the molecular status of *Argyrops filamentosus* fish via the mitochondrial cytochrome c oxidase (COI) gene and to study the pathological changes of gills infested by monogenean parasites.

Methods: Thirty *Argyrops filamentosus* fish were collected from Jeddah (Saudi Arabia) and identified molecularly via the mtCOI gene. Also, gills were isolated and examined microscopically for presence of monogeneans. Histopathological impacts of monogeneans on fish gills were studied in comparison to the gills of non-infected fish.

Result: The DNA of fish species was barcoded and showed highly stringent criteria with the previously *Argyrops filamentosus* sequence data. The obtained host DNA sequences were deposited in NCBI database under accession number OP975758.1. Examination of the investigated fish gills revealed the presence of three monogenean species *Protolamellodiscus senilobatus* Kritsky, Jiménez-Ruiz and Sey, 2000, *Acleotrema maculatus* Morsy, El-Fayoumi and Fahmy (2014) and *Haliotrema susanae* Soo, 2019. Monogenean parasites penetrated deeply with their haptor to the gill lamella and caused damage and degeneration of epithelial cells leading to the formation of a cup-shaped depression. Therefore, the mtDNA gene has the ability for host identification and heavy monogeneans infections lead to severe damage to fish gills.

Key words: Barcoding, Fish, Histopathology, Monogeneans, Sparidae.

INTRODUCTION

The king soldier bream *Argyrops filamentosus* Valenciennes, 1830, belongs to the family Sparidae (Order: Perciformes) and this species is originally distributed in the western Indian Ocean from the Red Sea, Oman and Arabian Gulf to South Africa including Mauritius, Madagascar and La Réunion (Fricke *et al*., 2018). The soldier bream is an active carnivorous species feeding particularly on sardines and benthic invertebrates (Ghanem *et al*., 2021). 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 parasites, including monogeneans (Shawket *et al*., 2018).

Traditional morphology-based species identification is being replaced by DNA barcoding because it is becoming more generally available, reliable and is more widely adopted (Ali *et al*., 2014). According to Nerloviæ *et al*. (2015), the partial examination of the mitochondrial cytochrome c oxidase subunit I (COI) and 16S ribosomal RNA (16S rRNA) gene sequences validated the fish's identity. DNA-based techniques were used by Hendrick *et al*. (2019) and Cucalón *et al*. (2022) to guarantee successful host identification.

The knowledge of parasitic communities makes researchers recognize the role of host fish in the food web or ecosystem and provide a deeper assessment of the role of parasites in affecting fish health (Shawket *et al*., 2018). The gills in fish are important organs of examination in disease diagnosis, because of their direct contact with the environment, such as several irritants, parasites and pollutants present in the water. The host and parasite relationship in the case of monogeneans results in large-scale damage on the site of attachment (Shawket *et al*., 2018). To attach themselves to a host, monogeneans use a specialized organ called a haptor (Arya and Singh, 2020). By attachment and feeding, monogeneans induce pathological changes in the host epithelium, thus creating

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How to cite this article: Abdel-Gaber, R., Quraishy, S.A., Alghamdi, M., Aljawdah, H.M.A., Maodaa, S.N., Al-Shaebi, E.M. (2023). DNA Barcoding of *Argyrops filamentosus* (Perciformes: Sparidae) Based on Mitochondrial COI Gene and Histopathological Evaluation of Gills Infected by Monogeneans. Indian Journal of Animal Research. doi: 10.18805/IJAR.BF-1626.

Submitted: 03-01-2022 **Accepted:** 28-03-2023 **Online:** 10-04-2023

a gateway for secondary bacterial and viral infections (Feist and Longshaw, 2008).

The present study is aimed to confirm the molecular status of *A. filamentosus* fish targeting the COI gene and to study the pathological changes of gills infected by monogenean parasites in comparison with non-infected gills.

MATERIALS AND METHODS

Fish and samples collection

Thirty fish specimens of the solider bream, *A. filamentosus* were collected from commercial fishermen in Jeddah (Saudi Arabia), during the period January-December 2022. The fish's gills were separated, submerged in 0.9% saline solution, then examined under Nikon SMZ18 stereomi croscope. Monogeneans were isolated from the gills and identified according to the diagnostic criteria. A piece of fish muscle was removed from the dorsal side and preserved in 96% ethanol for molecular analysis.

Molecular barcoding of fish

DNA was extracted using a DNeasy tissue kit© (Qiagen, Hilden, Germany) following the recommended steps. From the mitochondrial genome, the cytochrome c oxidase I (COI) gene was amplified by PCR using primers of Baldwin *et al*. (2009). PCR amplification under the following conditions: 95°C for 3 min, followed by 35 cycles at 95°C for 20 s, 55°C for 20 s and 72°C for 40 s, with a final extension at 72°C for 10 min. PCR products were sequenced using ABI PRISM 310 Genetic Analyzer (Applied Biosystems, Foster City, CA) at Prince Naif Health Research Center, King Saud University, Riyadh, Saudi Arabia. Sequences were deposited at a public sequence database, GenBank of the National Center for Biotechnology Information (NCBI, https://www.ncbi.nlm.nih.gov/). A search on BLASTn was performed to determine the most related sequences available in GenBank for the COI dataset. Analysis of the aligned DNA sequences of the partial COI gene region was done by the maximum likelihood (ML) using MEGA7 with the best-fit substitution models. Statistical support for each node was evaluated using a non-parametric bootstrap test with 1000 replicates.

Histopathological studies

Small portions of gills from non-infected fish and those naturally infected with monogeneans were collected and fixed in 10% neutral buffered formalin. The tissues were processed by routine paraffin embedding method and the sections were stained with Hematoxylin and Eosin (H & E). The stained sections were examined and photographed using a Leica DM 2500 microscope (NIS ELEMENTS software, version 3.8).

RESULTS AND DISCUSSION

Prevalence of parasitic infections

Fish are considered one of the important sources of animal protein (de Boer *et al*., 2020). Sea breams are fish of considerable economic importance and are commonly affected by several pathological problems, which can lead to mortalities and loss of production (Bannai, 2018). The

Table 1: GenBank accession numbers for COI sequences used in ML analysis for fish host.

Family	Species	Accession No.	Site location	Source	% identity
Sparidae	*Argyrops filamentosus*	JF492876.1	South Africa	GenBank	99.26
Sparidae	*Argyrops filamentosus*	KJ012288.1	Indian Ocean	GenBank	99.07
Sparidae	*Argyrops filamentosus*	KJ012287.1	Indian Ocean	GenBank	99.07
Sparidae	*Argyrops filamentosus*	HQ945946.1	South Africa	GenBank	99.07
Sparidae	*Argyrops filamentosus*	HQ945852.1	South Africa	GenBank	99.44
Sparidae	*Argyrops spinifer*	LC543877.1	Egypt	GenBank	89.98
Sparidae	*Argyrops spinifer*	MT325507.1	Qatar	GenBank	89.98
Sparidae	*Argyrops spinifer*	MN380038.1	Egypt	GenBank	89.98
Sparidae	*Argyrops spinifer*	MT076869.1	United Arab Emirates	GenBank	89.98
Sparidae	*Argyrops spinifer*	KU499786.1	Saudi Arabia	GenBank	89.98
Sparidae	*Argyrops spinifer*	KU499566.1	Saudi Arabia	GenBank	89.98
Sparidae	*Argyrops spinifer*	LC151617.1	Egypt	GenBank	89.98
Sparidae	*Argyrops spinifer*	KJ012289.1	Indian Ocean	GenBank	89.98
Sparidae	*Argyrops spinifer*	HQ149792.1	Iran	GenBank	89.98
Sparidae	*Argyrops spinifer*	HQ149793.1	Iran	GenBank	89.98
Sparidae	*Argyrops spinifer*	DQ884969.1	South Africa	GenBank	89.98
Sparidae	*Argyrops spinifer*	MT325506.1	Qatar	GenBank	89.90
Sparidae	*Argyrops spinifer*	MT076870.1	United Arab Emirates	GenBank	89.90
Sparidae	*Argyrops spinifer*	KU499788.1	Saudi Arabia	GenBank	89.90
Sparidae	*Pagellus erythrinus*	MW575730.1	Tunisia	GenBank	89.98
Sparidae	*Porcostoma dentata*	KT883630.1	USA	GenBank	89.90
Sparidae	*Porcostoma dentata*	JX282351.1	South Africa	GenBank	89.90
Sparidae	*Oblada melanura*	HM590726.1	Greece	GenBank	89.42

examined solider bream fish, *A. filamentosus*, was found to be naturally infected with three Monogeneans, ectoparasitic flatworms. Identification of parasitic taxa of sea breams associated with their morphological and biological peculiarities. Molecular techniques provide useful alternatives to morphology for the identification of parasites (Chaudhary *et al*., 2015; Abdel-Gaber *et al*., 2023). Monogeneans isolated from the gills of the solider bream fish were identified as *Protolamellodiscus senilobatus* Kritsky, Jiménez-Ruiz and Sey, 2000 (F: Diplectanidae), *Acleotrema maculatus* Morsy, El-Fayoumi and Fahmy (2014) (F: Diplectanidae) and *Haliotrema susanae* Soo, 2019 (F: Ancyrocephalidae) with a prevalence rate of 70 (21/30) for *P. senilobatus*, 46.66% (14/30) for *A. maculatus* and 50.00% (15/30) for *H. susanae*. Mixed infection for the recovered parasites was recorded to be 33.33% (10/30). Little information is available on parasitic infections of the soldier bream fish and the specificity with its host. Therefore, the present study provided more data on parasite fauna in this sparid fish species and confirm its taxonomic position.

Molecular barcoding of *Argyrops filamentosus* fish

DNA barcoding is considered to be an efficient tool to identify fish species within an ecosystem and to evaluate the genetic variability within species (Rajkumar *et al*., 2015; Panprommin *et al*., 2019; Ghouri *et al*., 2020; Alshehri *et al*., 2022). In the present study, the partial sequences of the mt COI gene were amplified by PCR that generated 539 bp fragment. The average base-pair proportions for the COI gene were A(24.49% 132) | C(28.01% 151) | G(16.14% 87) | T(31.35% 169), which is consistent with Ward *et al*. (2005), Ivanova *et al*. (2007), Armani *et al*. (2012), Consuegra *et al*. (2015) and Cutarelli *et al*. (2018). PCR products were deposited in GenBank under the accession number OP975758.1. The ML approach was used to align nucleotide sequence data from 24 taxa over 530 positions to produce a phylogenetic dendrogram that represented different marine species (Table 1). There is a low intra-species variation (0.076) for COI among all specimen sequences, agreeing with Xia *et al*. (2008), Renxie *et al*. (2018) and Ceruso *et al*. (2020) who also

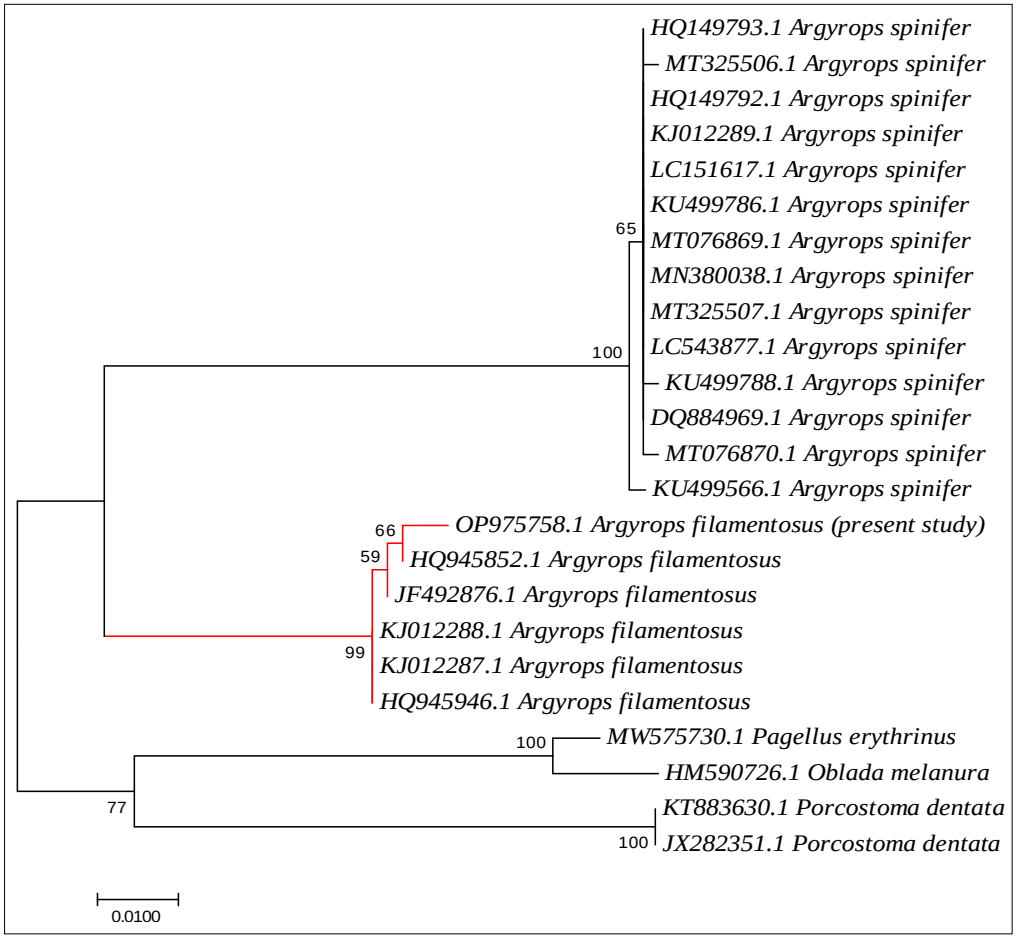

Fig 1: Phylogenetic analysis by Maximum Likelihood method based on the Tamura-Nei model. The tree with the highest log likelihood (-1426.29) is shown. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances 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.

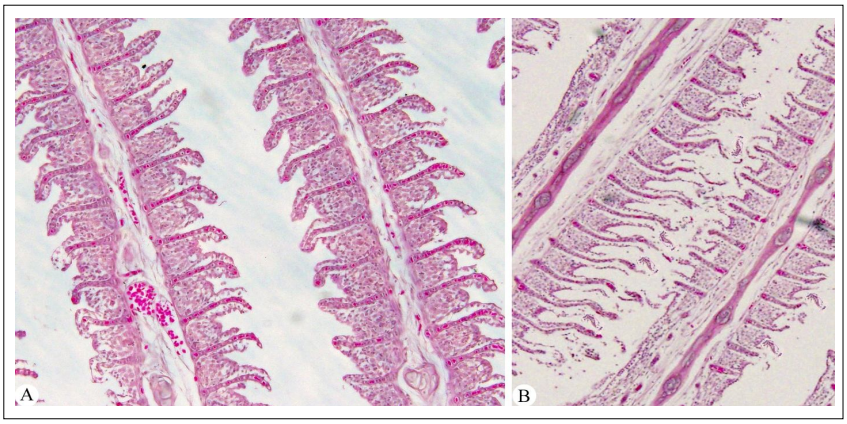

Fig 2: Gills isolated from *Argyrops filamentosus*. (A) non-infected fish gills. (B) fish gills infected with monogenean parasites. 400 ×.

observed the lower intraspecific distance between comparable sparid fish. The results validate that COI is a useful marker for species diagnosis in the family Sparidae. One family (Sparidae) for the fish host (*Argyrops filamentosus*) was identified for the recovered monogenean parasites. The phylogenetic relationship of the family Sparidae is supported by previous data from Day (2002) and Chiba *et al*. (2009). All the GenBank entries that matched our COI sequence under the highly stringent criteria (89.90-99.44% identity, 98-100% query coverage and E-value 0.0) were assigned to two monophyletic groups within family Sparidae for *A. spinifer* and *A. filamentosus*. In ML analyses (Fig 1), the taxa of *Argyrops* were grouped in a distinct clade with high bootstrap values. Dendrograms confirmed the association of our specimen with the *Argyrops* group, with special reference to *Argyrops filamentosus* (JF492876.1, KJ012288.1, KJ012287.1, HQ945946.1 and HQ945852.1) that found in South Africa and the Indian Ocean. This study confirms the high efficacy of DNA barcodes for the identification of sparid fish.

Histopathological studies

The histopathological alterations of the infected gills were observed and compared to the control sections of *A. filamentosus* (Fig 2). The damages are consistent with Purivirojkul (2012) reported that infection with monogeneans is one of the most prevalent parasitic agents affecting gills and destroying gills which leads to impairment of fish respiration as well as a general deterioration of fish health. Microscopic examinations revealed that the gills from control fish consisted of horizontal flat filaments and were supported by bony gill arches. On these filaments, secondary lamellae were found. These lamellae are composed of a thin epithelial cell layer covering pillar cells, which in turn surround the blood sinusoids. Monogenean parasites were attached to the secondary gill lamella of infected fish. Parasites penetrated deeply with their haptor to the gill lamella and caused damage and degeneration of epithelial cells leading to the formation of a cup-shaped depression. Nevertheless, the major host response to monogenean infection was represented by hyperplasia of the gill filament. The severity of respiratory damage for the fish host was directly proportional to the number of parasites on the gills. This agreed with Campos *et al*. (2011) and Pahor *et al*. (2017) stated that when the intensity of monogenean parasites increases, the gill damage may be serious with the pronounced impact of the histology and lead to fish mortality. Because of these damages, hyperplasia of gill epithelium associated with monogenean anchors was observed, which agreed with Shamsi *et al*. (2009), Raissy *et al*. (2011), Pahor *et al*. (2017) and Hoai (2020) also noticed hyperplasia of the epithelial cells, subsequent lamellar fusion and extensive secretion of mucus. Moreover, these results agreed with those observed on the gill filament of *Plectropomus maculatus* infecting *Acleotrema maculatus* (Morsy *et al*., 2014), *Pagellus erythrinus* infecting *Diplectanum aequans* (Adawy *et al*., 2016), *Gyrodactylus cichlidarum* infecting *Oreochromis niloticus* (Grano-Maldonado *et al*., 2018), *Cichlidogyrus philander* infecting *Pseudocrenilabrus philander* (Igeh and Avenant-Oldewage, 2020), *Dactylogyrus* spp. infecting *Epinephelus coioides* (Ramudu *et al*., 2020); and three species of monogeneans *Anacanthorus spathulatus*, *Notozothecium janauachensis* and *Mymarothecium boegeri* infecting *Colossoma macropomum* (Tavares-Dias *et al*., 2021).

CONCLUSION

Therefore, it could be concluded that the ability of mtDNA barcoding for host identification determines the specificity of monogenean parasites to their host species. There is an effect of heavy parasitic infections on fish gills and epithelial destruction of lamellae. Further studies should include some tools for controlling the parasite distribution.

ACKNOWLEDEGEMENT

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

Data availability statement

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

Conflict of interest

The author(s) declare that they have no conflict of interest regarding the content of this article.

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