



Molecular phylogeny and morphological characteristics of the reef margin blenny, *Entomacrodus striatus* (Valenciennes, 1836) from the Gulf of Oman (Actinopteri: Blenniiformes Blenniidae)

ARTICLE INFO

Article Type
Original Research

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How to cite this article

Sharifiniya M., Mousavi-Sabet H., Alavi-Yeganeh M.S., Ghanbarifardi M. Molecular phylogeny and morphological characteristics of the reef margin blenny, *Entomacrodus striatus* (Valenciennes, 1836) from the Gulf of Oman (Actinopteri: Blenniiformes Blenniidae). *ECOPERSIA* 2022; 10(1): 37-46.

DOR:

20.1001.1.23222700.2022.10.1.4.7

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Article History

Received: June 24, 2021

Accepted: September 4, 2021

Published: December 23, 2021

ABSTRACT

Aim: Molecular phylogenetic relationship and morphological characteristics of the reef margin blenny, *Entomacrodus striatus* was undertaken to clarify its taxonomic status in the Iranian coasts of the Gulf of Oman. *Entomacrodus striatus* is distributed in the Indo-Pacific realm.

Materials and Methods: This study is based on four samples taken from Chabahar in the northern Gulf of Oman during February 2019. The mt-DNA marker (COI) of one *E. striatus* from the present study and 15 *Entomacrodus* species was used for verification over traditional morpho-taxonomy.

Findings: Bayesian Inference (BI) and Maximum Likelihood (ML) approaches result in five clades including, clade A (*Entomacrodus corneliae*, *E. chiostictus*, *E. nigricans*, *E. sealei*, *E. cymatobiotus*, *E. caudofasciatus*), clade B (*E. striatus*, *E. niuafoouensis*, *E. epalzeocheilos*, *E. randalli*), clade C (*E. macrospilus*, *E. thalassinus*), clade D (*E. vermiculatus*, *E. decussatus*), and clade E (*E. stellifer*). The morphological characters of the examined materials were in the range of its original description, except in having a pair of pores in one position of the preopercular series of sensory pores, which may be hypothesized a population variation associated with geographical distribution. The sexual dimorphism is distinguishable, especially in the color pattern of specimens in which the blotches and bands on the male bodies are more bolded than the female ones.

Conclusion: The inferred ML and BI trees resulted in a homological clustering of taxa grouped according to their morphological characteristics and agreed with molecular reconstructions based on nuclear markers.

Keywords: Chabahar, Indo-Pacific, Iranian coast, MtDNA marker (COI), Traditional morpho-taxonomy.

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Introduction

The genus *Entomacrodus* comprises 48 small and bottom-dwelling species in the Indo-Pacific and Atlantic Oceans [1]. The most comprehensive study was conducted by Springer [2], which identified seven species groups within the genus *Entomacrodus* based on the nature of the upper lip crenulae and other characters. The *striatus* group is one of these seven groups with five species consisting of *E. epalzeocheilus*, *E. jnarmoraius*, *E. niuafoouensis*, *E. randalli*, and *E. striatus*. *Entomacrodus striatus* (Valenciennes, 1836) is distributed in the Indo-Pacific biogeographic region with fewer predorsal commissural pores and simple preopercular pore positions than the other members of the *striatum* group. [2].

The genus *Entomacrodus* is mainly related to the genera *Blenniella* and *Istiblennius* based on molecular reconstructions [3, 4, 5] and body shape analysis [6]; moreover, the phylogenetic relationship of some species of *Entomacrodus* has been revealed in the mentioned studies.

Experimental surveys of the intertidal and subtidal zones of the Persian Gulf and the Gulf of Oman resulted in reporting 19 species to belong to 10 genera, including *Alticus*, *Antennablennius*, *Ecsenius*, *Entomacrodus*, *Istiblennius*, *Omobranchus*, *Parablennius*, *Petroscirtes*, *Salarias*, *Scartella* [7, 8, 9, 10, 11, 12, 13, 14, 15]. The previous studies briefly present some morphological characteristics of *Entomacrodus striatus* from the Gulf of Oman [13, 14].

The primary purpose of this study is to record the morphometric and meristic characters of the reef margin blenny, *E. striatus*, from the Iranian coast of the Gulf of Oman and clarify its molecular phylogenetic position within the congeners.

Material and Methods

Sampling and species identification.

Fish were collected from Chabahar, the Gulf of Oman, in February 2019. Samples

were caught from rocky intertidal pools using a hand net, and four specimens of *E. striatus* were regarded for morphological measurements and counts. Specimens were photographed and preserved in alcohol 96% for further examination. Specimens were considered with available identification keys [2, 16-18].

DNA extractions, amplification, and sequencing

Total genomic DNA of one specimen of *E. striatus* was extracted from the ethanol preserved fin clips using the standard phenol-chloroform method [19]. The COI marker was proliferated using primers Fish F1 and Fish R2 [20]. PCR was performed in 25 µl reactions containing 8.5 µl distilled water, one µl of each primer, two µl template DNA, and 12.5 µl PCR solution. PCR solution that contains suitable Taq polymerase was ordered from Pishgam Biotech Co. Amplification was performed under the following thermal program: initial 95°C for 5 min, 35 cycles at 94°C for 30 s, 54°C for 50 s and 72°C for 60 s, and a final extension step at 72°C for 10 min. One obtained COI sequence (Faza Pajoo Co) for the herein studied *E. striatus* is deposited in GenBank with the accession number MZ613311 (615 bp). An additional 41 sequences belonging to 15 species of the genus *Enomacrodus* and one sequence of *Istiblennius spilotus* as outgroup from GenBank (<http://www.ncbi.nlm.nih.gov/genbank>) (Table 1) were used to present molecular phylogenetic reconstruction of the genus *Enomacrodus* and the relationship of *E. striatus*. The assembled sequences were aligned using MAFFT v.7 [21]. Selecting the best model of nucleotide substitution and sequence evolution for this dataset was performed with MrModeltest v.2.3 [22]. According to the Akaike Information Criterion (AIC), the GTR + G + I model was used. Phylogenetic trees were concluded using maximum likelihood and Bayesian inference. Maximum likelihood

reconstruction was performed with RAxML v.8.24 [23] using the rapid hill-climbing algorithm. MrBayes v.3.2.6 [24] was applied for performing Bayesian inference analysis with two runs of four Markov Chain Monte Carlo (MCMC) for 10 million generations. The first 25% of the trees were discarded as 'burn in,' and most consensus trees were considered in the analysis. Support for internal branches was evaluated by nonparametric bootstrapping with 10000 replicates (ML) and posterior probabilities (BI).

Findings

Class: Actinopteri Cope, 1871

Order: Blenniiformes Rafinesque 1810

Family Blenniidae Rafinesque 1810 (blennies)

Subfamily Salariae Gill 1859 (Salariae blennies)

Genus: *Entomacrodus* Gill, 1859

Species: *Entomacrodus striatus* (Valenciennes, 1836)

Salarias striatus Valenciennes [A.] (ex Quoy & Gaimard) in Cuvier & Valenciennes 1836:309

Salarias fraenatus Valenciennes [A.] in Cuvier & Valenciennes 1836:342

Salarias arenatus Bleeker [P.] 1855:173 [Natuurkundig Tijdschrift voor Nederlandsch Indië v. 8 (no. 1),

Questionably a synonym of *Entomacrodus striatus* (Valenciennes 1836)

Entomacrodus wolffi Rofen [R. R.] 1958:202, Pl. 11 (Figure 31), Figure 1 [The Natural History of Rennell Island, British Solomon Islands, Copenhagen v. 1 (no. 10)

Entomacrodus plurifilis plurifilis Schultz [L. P.] & Chapman [W. M.] in Schultz et al.

Table 1) Information of COI sequences referenced in this study.

| Species | number of used sequences in the present study | GenBank Accession numbers |
|------------------------------------|---|--|
| <i>Entomacrodus caudofasciatus</i> | 4 | MK658438, MK657407, KX301872, KX301871 |
| <i>Entomacrodus chiostictus</i> | 1 | HQ168561 |
| <i>Entomacrodus corneliae</i> | 2 | MK566898, KX301875 |
| <i>Entomacrodus cymatobiotus</i> | 2 | KJ968090, JQ431713 |
| <i>Entomacrodus decussatus</i> | 1 | KX301861 |
| <i>Entomacrodus epalzeocheilos</i> | 2 | KX301862, KX301863 |
| <i>Entomacrodus macrospilus</i> | 5 | MK566900, MK566899, MK657549, MK657013, KX301876 |
| <i>Entomacrodus nigricans</i> | 4 | JQ841165, JQ841164, JQ840835, JQ840836 |
| <i>Entomacrodus niuafoouensis</i> | 2 | KX301874, HQ168562 |
| <i>Entomacrodus randalli</i> | 2 | MK566901, KX301877 |
| <i>Entomacrodus sealei</i> | 1 | MK658447 |
| <i>Entomacrodus stellifer</i> | 1 | KX301860 |
| <i>Entomacrodus striatus</i> | 1 | This study MZ613311 |
| <i>Entomacrodus striatus</i> | 5 | KX301869, KX301866, KX301868, KX301865, KX301864 |
| <i>Entomacrodus thalassinus</i> | 8 | MK658549, MK658526, MK658114, MK658079, MK657149, MK657153, MK657097, MK657392 |
| <i>Entomacrodus vermiculatus</i> | 1 | KX301879 |
| <i>Istiblennius spilotos</i> | 1 | JF493690 |

1960:338, Figure 120

Entomacrodus plurifilis marshallensis Schultz [L. P.] & Chapman [W. M.] in Schultz et al. 1960:341, Figure 121

Common name: Black-spotted rock skipper
Remarks: Reported from the Iranian Coasts of Gulf of Oman (Chabahar) by Estekani et al. [13]. It is also reported from the Gulf of Oman (Daryabozorg, 25°16'N, 60°39'E) in the present study and Sharifiniya et al. [14]. This species is sympatric with *Antennablennius bifilum* in intertidal pools with rocky-sandy habitats.

Morphological description

The principal distinguishing morphological characteristics: Dorsal fin XIII (Thirteenth is very short, > 2.9% SL), 15-16; anal fin II, 16-17; segmented caudal fin rays 12-14; pectoral fin rays 14; pelvic fin rays I,4; total gill-rakers on first arch 14-16; pseudo-branchial filaments 7-8; supraorbital cirri branched with 4-5 lateral branches; a branched cirri on posterior margin of tube rim of each anterior nostril; a simple and short cirri on each side of nape; 3 predorsal commissural sensory pores; preopercular series of sensory pores occupying 7 spots in each side of head with simple pores in all spots, except in a position that has a pair of pores; circumorbital sensory pores 7-8 pairs; mandibular sensory pores 5 pairs; median supratemporal sensory pores 2; supratemporal sensory pores 4; One sensory pore before each anterior nostril in each side; lateral line pores ending on side in area below and between last dorsal fin spine and first dorsal ray; ventral margin of upper lip entirely serrate, with 26-29 crenulae; dorsal margin of lower lip entire, sometimes with weakly crenulate in corners; vomerine teeth and a relatively large recurved canine tooth inserted posteriorly on each articular bone were visible; over 110 freely movable teeth implanted in connective tissue in both jaws; a deep notch be-

tween spinous and soft portions of dorsal fin; posterior portion of dorsal segmented rays connected to anterior part of caudal peduncle by the membrane; longest dorsal spine shorter than longest dorsal ray; last dorsal spine is shortest dorsal element, greatly reduced and only visible on skeletal preparations or radiographs; posterior portion of segmented anal rays not bound to caudal peduncle; anal rays longer than anal spines; pelvic fin spine not visible externally; no fleshy crest on top of head in both sexes; males are often larger than females. Morphometric and meristic data are summarized in Table 2.

Color in life: There is significant sexual dimorphism in the coloration of *E. striatus* (Figures 1a and 1b). The sides of the body have prominently numerous dark spots that are equal to or smaller than about half of the eye diameter. The spots are darker and denser along the midline of the body. The intensity and density of spots are decreased posteriorly to the caudal peduncle and dorsally and ventrally to the dorsal and ventral body areas. The central part of the body is pale. There are about five large blotches at the base of the dorsal fin. The dorsal fins are translucent, with oblique brown stripes and scattered spots. The caudal fin is transparent with a variable number of irregularly vertical brown strips on its radii. The anal fin is dusky with a brown band in the middle, and the tip of some of its radii is dark (especially in males). The pectoral fins are translucent, and the pectoral axils are covered with numerous light brown spots. The snout and the sides of the head are variably dusky that covered by numerous fine white and light brown stripes and spots, and the underside of the head has a pattern of dusky chevrons separated by pale stripes that are more obvious in females. A faint stripe stretches from the ventral margin of the eye to the lip. There is a large dark blotch on the cheek (darker



a



b

Figure 1) *Entomacrodus striatus*. a) male, 49 mm SL, b) female, 52 mm SL. Lateral view, Gulf of Oman.

and more evident in males) and a developed slender brown and blue band behind the eye. **Color in preserved specimens.** The upper half of the body is dark and covered with black and dark brown spots about half the diameter of the eye (Figure 2). The background of the lower half is light and has pale brown spots. The snout is gray, and the cheek is dark brown. Snout and under the head have several diffused fine white spots. Several white stripes and dashes under the head look more evident in female specimens.

Phylogenetic analysis

Topologies of the major clades were the same in Maximum Likelihood (ML), and Bayesian Inference (BI) approaches (Figure 3).

Bayesian Inference (BI) and Maximum Likelihood (ML) approaches support five clades including, clade A (*Entomacrodus corneliae*,

E. chiostictus, *E. nigricans*, *E. sealei*, *E. cymatobiotus*, *E. caudofasciatus*), clade B (*E. striatus*, *E. niuafoouensis*, *E. epalzeocheilos*, *E. randalli*), clade C (*E. macrospilus*, *E. thalassinus*), clade D (*E. vermiculatus*, *E. decussatus*), and clade E (*E. stellifer*).

Discussion

The counts and measurements of the examined materials are in the range of the original description by Springer [2], except in one feature. According to Springer [2], the preopercular series of sensory pores have simple pores in all positions, but one position has a pair of pores in all examined specimens.

The variation in the average number of segmented dorsal or anal fin rays, number of cirri branches on the main supraorbital cirrus, and relative length of the main supraorbital

Table 2) Morphometric and meristic data of *Entomacrodus striatus* from the Gulf of Oman (4 specimens).

| Proportion | Min-Max | Mean±SD |
|--|-------------|------------|
| Weight (g) | 0.98-1.81 | 1.37±0.44 |
| Morphometric characters (mm) | | |
| Standard length | 38-52 | 44.96±7.19 |
| % In standard length | | |
| Head length | 21.93-24.47 | 22.82±1.28 |
| Head height at the nape | 18.24-18.68 | 18.46±0.22 |
| Snout length | 11.40-13.34 | 11.89±1 |
| Postorbital distance | 12.71-13.74 | 12.76±0.58 |
| Interorbital distance | 1.18-3.94 | 3.06±1.41 |
| Preorbital distance | 5.45-5.57 | 5.56±0.06 |
| Length of longest dorsal-fin spine | 4.88-5.78 | 5.22±0.45 |
| Length of longest dorsal-fin soft ray | 6.83-8.15 | 7.31±0.66 |
| First dorsal fin base length | 36.21-36.26 | 36.25±0.02 |
| Second dorsal fin base length | 38.68-44.44 | 40.03±3.01 |
| Pectoral fin length | 24.06-25.78 | 24.08±0.98 |
| Pectoral fin base length | 10.73-10.94 | 10.94±0.11 |
| Pelvic fin length | 13.26-16.29 | 14.05±1.56 |
| Pelvic fin base length | 3.44-3.57 | 3.46±0.07 |
| Longest anal soft-ray length | 4.05-8.21 | 6±2.08 |
| Anal fin base length | 44.73-47.85 | 45.95±1.57 |
| Caudal fin length | 19.90-23.39 | 21.28±1.75 |
| Caudal fin height | 10.84-18.75 | 15.50±3.97 |
| Caudal peduncle height | 8.21-9.83 | 9.07±0.81 |
| Caudal peduncle length | 4.68-4.93 | 4.73±0.13 |
| Meristic characters | | |
| Dorsal fin spines | | 13 |
| Dorsal fin soft rays | | 15-16 |
| Pectoral fin rays | | 14 |
| Pelvic fin spines | | 1 |
| Pelvic fin soft rays | | 4 |
| Anal fin spine | | 2 |
| Anal fin soft rays | | 16-17 |
| Caudal fin ray | | 12-14 |
| Gill rakers | | 14-16 |
| Predorsal commissural sensory pores | | 3 |
| Preopercular series of sensory pores (on each side of the head) | | 7 |
| Circumorbital sensory pores 7-8 pairs (on each side of the head) | | 7-8 |
| Mandibular sensory pores five pairs (on each side of the head) | | 5 |
| Median supratemporal sensory pores | | 2 |
| Supratemporal sensory pores (on each side of the head) | | 4 |
| Anterior nostril pores (on each side of the head) | | 1 |

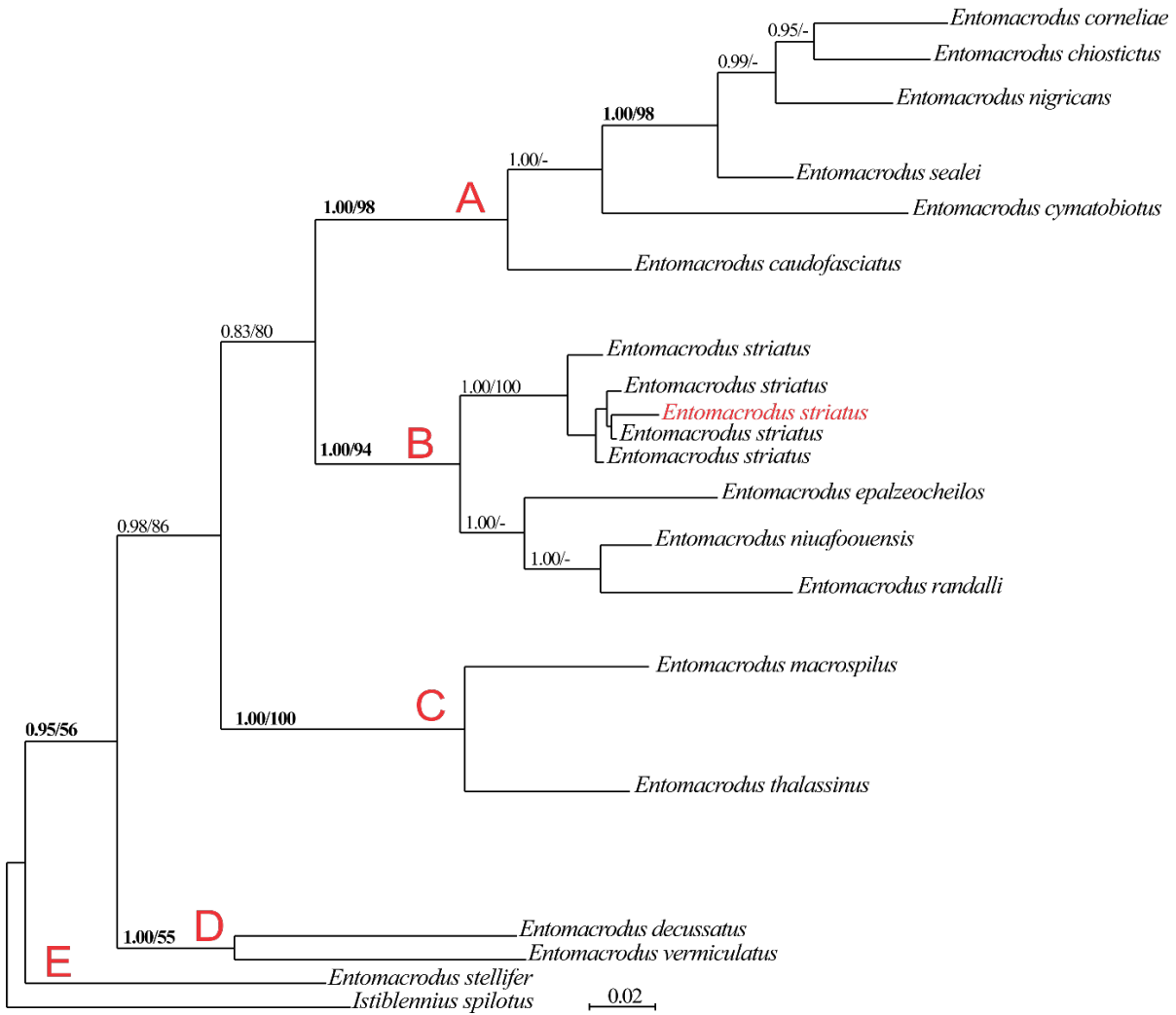


Figure 3) Phylogenetic relationships of the species of *Entomacrodus* based on COI gene sequences. The above branches define support for BI and ML, respectively; however, some branches are supported only by BI. Our new obtained sequence is marked with a star. The scale bar indicates the number of substitutions per site.



Figure 2) *Entomacrodus striatus* in preservative. 48 mm SL. Ventral side of the head, a) female, b) male.

cirrus could be correlated to geographical, sexual, and ontogenetical factors [2]. Therefore, the differences in the sensory pores of the examined specimens in this study with the type specimens described by Springer [2] taken from the Indian Ocean, Western Australia, South Africa, South China Sea, and the Pacific Ocean may be hypothesized geographical variation between different populations.

DNA barcoding based on COI markers has been abundantly applied in species identification and biodiversity examinations [25, 26]. In this study, we examined the morphological results and the DNA sequencing of the COI gene to confirm the species identity of *E. striatus*. We generated a COI barcode for an *E. striatus* collected from the Iranian coast of the Gulf of Oman. Phylogenetic analyzes provided well-resolved hypotheses of relationships at species level among the genus *Entomacrodus*, and sequence analysis of the COI gene strongly supported the identity of this species as *E. striatus*. The inferred phylogenetic tree in the present study resulted in a mostly congruent clustering with groups based on morphological characteristics [2]. Springer [2] divided all species of the genus *Entomacrodus* into seven groups consist of *thalassinus* group (*E. thalassinus*, *E. macrospilus*), *nigricans* group (*E. cadenati*, *E. caudofasciatus*, *E. chiostictus*, *E. corneliae*, *E. nigricans*, *E. sealei*, *E. textilis*, *E. vomerinus*), *stellifer* group (*E. stellifer*), *rofeni* group (*E. rofeni*), *cymaiohiotus* group (*E. cymaiobioius*, *E. sirasburgi*, *E. chapmani*), *striatus* group (*E. epalzeocheilus*, *E. marmoratus*, *E. niuafoouensis*, *E. randalli*, *E. striatus*) and *decussatus* group (*E. decussatus*, *E. vermiculatus*) with regarding the morphological characteristics, especially the nature of the upper lip crenulae. There are some similarities between the morphologically based grouping [3, 6] and our molecular phylogenetic reconstruction (Figure 3). Species of *thalassinus*, *decussa-*

tus, and *stellifer* groups are congruent with clades C, D, and E, respectively. Clades A and B are also similar to *nigricans* and *striatus* groups, correspondingly. Molecular phylogenetic relationships of Blenniiformes have been examined [3], and four species of *Entomacrodus* (*E. niuafoouensis*, *E. chiostictus*, *E. striatus*, *E. nigricans*) are regarded; however, the topology of *Entomacrodus* representatives is not resembling morphological study [2], and our constructed tree probably because of involving only four species. Molecular analyses of Blenniidae using four nuclear loci resolved the relationship among *Entomacrodus*, *Istiblennius*, and *Blenniella* [4]. Six species of *Entomacrodus* (*E. niuafoouensis*, *E. striatus*, *E. nigricans*, *E. sealei*, *E. stellifer*, *E. decussatus*) were classified similar to clades A, B, D, and E of the phylogenetic tree (Figure 3) in the present study. Another molecular examination of Blenniidae using five nuclear markers regarded ten representatives of *Entomacrodus* (*E. thalassinus*, *E. niuafoouensis*, *E. striatus*, *E. cadenati*, *E. nigricans*, *E. sealei*, *E. caudofasciatus*, *E. stellifer*, *E. vermiculatus*, *E. decussatus*) [5] and put these species in lineages similar to clades A, B, C, D, E of our examination (Figure 3) with matching topology. Survey of the body shape of Blenniidae via 2D landmarks [6] clustered six species of *Entomacrodus* (*E. niuafoouensis*, *E. striatus*, *E. nigricans*, *E. sealei*, *E. stellifer*, *E. decussatus*) similar to clades A, B, D, and E of the phylogenetic tree (Figure 3) in the present study.

Springer [2] reviewed the genus *Entomacrodus* and identified 22 valid species. Two new blenniid species, including *Entomacrodus lemuria* from the western Indian Ocean and *Entomacrodus williamsi* from the western Pacific Ocean [27]. Manilo and Bogorodsky [28] provided a list of the littoral fishes of the Arabian Sea and reported three species, including *E. epalzeocheilus*, *E. striatus*, and *E. vermiculatus*. Williams and Bogorodsky [29]

described *Entomacrodus solus* from the shallow rocky shore of the Ras Mohammed, Red Sea.

Conclusion

The present study reports the morphometric and meristic characteristics and COI gene sequence of *E. striatus* from the Iranian coasts of the Gulf of Oman. The inferred ML and BI trees were concordant with traditional morphological taxonomy and recent molecular phylogenies.

Acknowledgments

We are greatly thankful to the University of Guilan and the University of Sistan, and Baluchestan for their support during this study period. We honor Tarbiat Modarres University for providing laboratory services and facilities.

Ethical Permissions: Data and information in this research did not require any ethical permission.

Authors' Contribution: Sampling, morphological measurements, and molecular lab work were performed by Maryam Sharifiniya (40%), Hamed Mousavi-Sabet (20%), and Mohammad Sadegh Alavi-Yeganeh (20%). Data analysis was conducted by Maryam Sharifiniya and Mehdi Ghanbarifardi (20%).

Conflicts of Interests: None declared by Authors.

Funding

This study was financially supported by the University of Guilan, and the University of Sistan, and Baluchestan.

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