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Research Article

Moving towards the west: Morphological and molecular documentation of the reef margin blenny *Entomacrodus striatus* (Teleostei: Blenniiformes) from the most western part of the Indo-Pacific Ocean

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Abstract

For most marine organisms, including fish, species diversity is very high in the Indo-Pacific region, and it reflects how past and present environmental conditions shape a link between biodiversity and ecosystem function. The distribution of a taxon in the region is due to the establishment of morphological, genetic, behavioral, and physiological aspects of species. Despite extensive surveys in the Indo-Pacific region still, there are new species and new records especially in the case of small cryptic species such as blennies. The family Blenniidae including members of the genus *Entomacrodus* are mostly small fishes of less than 15 cm having an elongate and slender body with a worldwide distribution. *Entomacrodus* comprises herbivorous combtooth blennies with about 27 species, 7 of which occur in the western Indian Ocean. In this study, the presence, general morphology, otolith shape variation, osteology, distribution, and molecular phylogenetic affinity of the reef margin blenny *Entomacrodus striatus* have been documented/presented from the westernmost part of the Indo-Pacific region (the Jask Port, Oman Sea). The morphological and meristic characteristics of specimens were well fit with *E. striatus*. The vertebral column includes 10 abdominal and 23 caudal vertebrae, for a total vertebral count of 33. Corroborating the morphological results, DNA barcoding based on mitochondrial COI confirmed that the specimens collected from the Jask Port area are conspecific with *E. striatus* from other Indo-Pacific localities. *Entomacrodus striatus* from the Oman Sea and other Indo-Pacific regions show a distinct clade sister to a clade of *E. epalzeocheilos*, *E. niuafoouensis*, and *E. randalli* with a closest phylogenetic relationship to *E. niuafoouensis* (0.108 K2P genetic distance). The presence of *E. striatus* in the coastal area of the Oman Sea in the Jask coastal area represents the first record of this species for the westmost of the northern Indian Ocean and markedly expands its known geographical distribution range.

Introduction

The family Blenniidae is distributed worldwide, with most species occurring in shallow coastal, tropical, and warm temperate marine waters, along with a few species known from fresh and/or brackish water (Nelson *et al.*, 2016). They are mostly small fish (<15 cm) with an elongate and slender body (slightly compressed posteriorly). The blennies are characterized by having a terminal or slightly ventral mouth; 1 row of comblike incisor-like teeth on each jaw; a well-developed lip; presence of cirri on head; absence of scales; 6–17 slender spines and 9–119 rays in dorsal fin; 2 spines in anal fin; 1 spine and 1–4 rays in pelvic fin, which is inserted anterior to pectoral-fin bases (Williams and Springer, 2022).

The family Blenniidae comprises 405 species and 59 genera worldwide (Fricke *et al.*, 2024) and 34 genera and at least 115 species in the western Indian Ocean including the genus *Entomacrodus* Gill, 1859 (see Williams and Springer, 2022). According to Esmaeili *et al.* (2022), the order Blenniiformes in most parts of the Northwest Indian Ocean (NIO) that encompasses the marine waters of the Persian Gulf, Sea of Oman/Oman Sea, Arabian Sea, Red Sea and the Gulf of Aden enumerates 81 species, representing 28 genera and two families. The family Blenniidae/combtooth blennies in the Northwest Indian Ocean present 25 genera and 64 species (Esmaeili *et al.*, 2022).

The genus *Entomacrodus* (Blenniiformes: Blenniidae: Salariinae) includes herbivorous combtooth blennies with about 27 species, 7 of which occur in the western Indian Ocean (Williams and Springer, 2022; Fricke *et al.*, 2024). The genus is distributed along rocky coasts and coral reefs throughout the world (Springer, 1967). Species of the genus are characterized by having teeth on the vomer; the presence of nasal, supraorbital and nape cirri (except no cirri on the nape in *E. thalassinus*); absence of fleshy blade-like crest on head; continuous gill openings across isthmus; tubular and continuous lateral line; free margin of upper lip either smooth or crenulate, the margin of lower lip smooth; dorsal fin with 13–14 (usually 13) spines and 13–18 rays, fin moderately notched between spines and rays; anal fin with 2 spines and 15–19 rays; pectoral fins with 12–15 (usually 14) rays; pelvic fins with 1 spine (embedded, readily visible only in skeletal preparations) and 4 rays; caudal fin usually with 13 rays, middle 9 rays branched. Maximum size ~16 cm TL (Williams and Springer, 2022).

In the investigations carried out on the tidal areas of the Persian Gulf and the Sea of Oman, 19 species belonging to 10 genera have been reported including *Alticus, Antennablennius, Ecsenius, Entomacrodus, Istiblennius*, *Omobranchus*, *Parablennius, Petroscirtes* and *Salaria* (Mehraban and Esmaeili, 2018; Eagderi *et al.*, 2019; Estekani *et al.*, 2020; Sharifiniya *et al.*, 2021; Sharifiniya *et al.*, 2024).

The first record of the reef margin blenny/pearly rockskipper, *Entomacrodus striatus* (Valenciennes, 1836) from the northern Oman Sea (Chabahar Bay) was made by Estekani *et al.* (2020). However, there is no record of this blenny from the western part of the Makran coast (Mehraban and Esmaeili, 2018; Eagderi *et al.*, 2019; Estekani *et al.*, 2020; Sharifiniya *et al.*, 2021). Some morphological characteristics and phylogenetic status of *E. striatus* from the Gulf of Oman in the Chabahar Bay have been provided by Estekani *et al.* (2020), and Sharifiniya *et al.* (2021), but additional characteristics remain to be described using newly collected materials.

This study aims i) to document further expansion of *E. striatus* to the western part of the Indian Ocean, ii) to provide a detailed description of the collected specimens, iii) to illustrate the otolith and caudal skeleton of *E. striatus*, iv) to reconstruct its phylogenetic tree, v) to compare the new findings with those available information, and vi) to provide a dichotomous key to all species in the western Indian Ocean.

Materials and methods

Study area and sampling

Thirteen specimens of *Entomacrodus striatus* were collected using hand nets from the Yekboni pier, Jask Port, Hormuzgan province, Iran (25.659592 N, 57.821020 E), from rocky intertidal pools in the most western part of the Indo pacific (Figs. 1, 2), in May 2023. After anesthesia (clove oil), specimens were fixed with 10% formaldehyde for morphological analysis or fixed with 96% alcohol for molecular analysis. The specimens are deposited in the Zoological Museum and Collection of Biology Department, Shiraz University, Shiraz (ZM-CBSU).

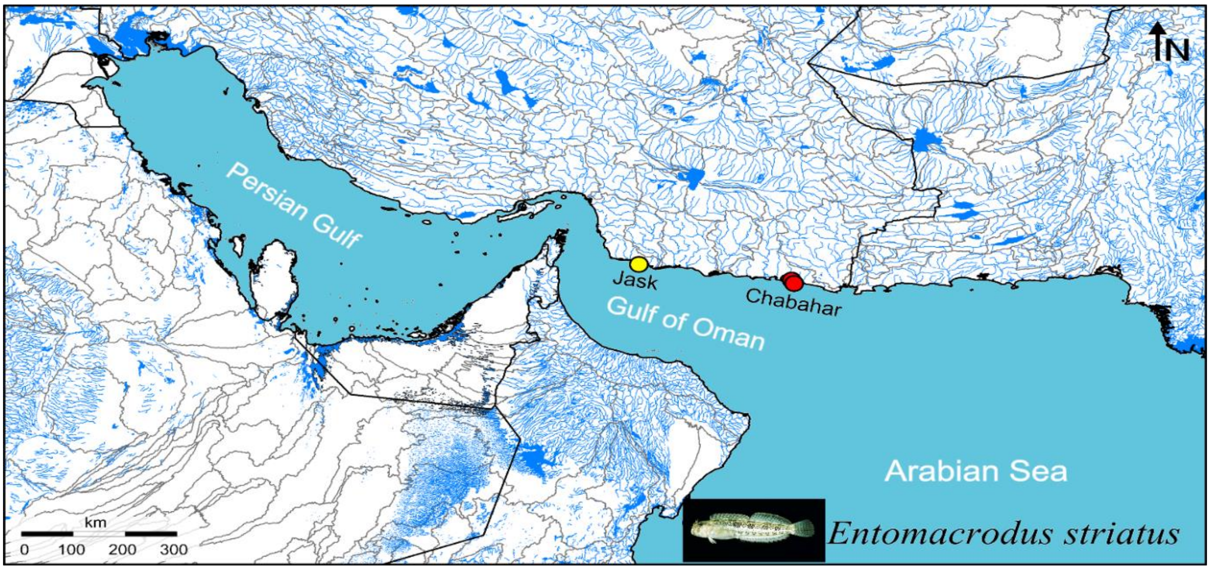

Figure 1: Collection site of *Entomacrodus striatus* from coastal area of the Makran Sea, Iran (25.659592 N 57.821020 E). Yellow circle, present study; red circle, previous studies.

Morphological studies

Morphometric measurements of *E. striatus* were taken to the nearest 0.1 mm using digital calipers. A stereomicroscope was used for the meristic characters. Morphometric characters were expressed as a percentage of standard length (SL) or a percentage of head length (HL) as appropriate. Morphometric and meristic measurements and counts followed Springer (1967). Identification was carried out using available identification keys (Randall, 1995; Williams and Springer, 2022).

Figure 2: Lateral view of *Entomacrodus striatus* collected from Jask Port, Hormozgan Province, Iran.

Otolith preparation and description

Left saccular otoliths were extracted from five specimens. The dorsal side of the skull was cut in the middle with a sharp scalpel, and the otoliths were gently removed with a pair of fine tweezers. Otoliths were incubated in 5% potassium hydroxide (KOH) solution for 5 min for stripping of adherent tissues, rinsed in distilled water, stored dry in a small plastic cell, and deposited in ZM-CBSU. Otolith morphology was examined with a stereomicroscope and analyzed using SEM images (TESCAN vega3). The general terminology of the otolith morphology follows Furlani *et al.* (2007), Tuset *et al.* (2008), and Mehraban *et al.* (2023) as it is illustrated in Figure 3 and Table 1.

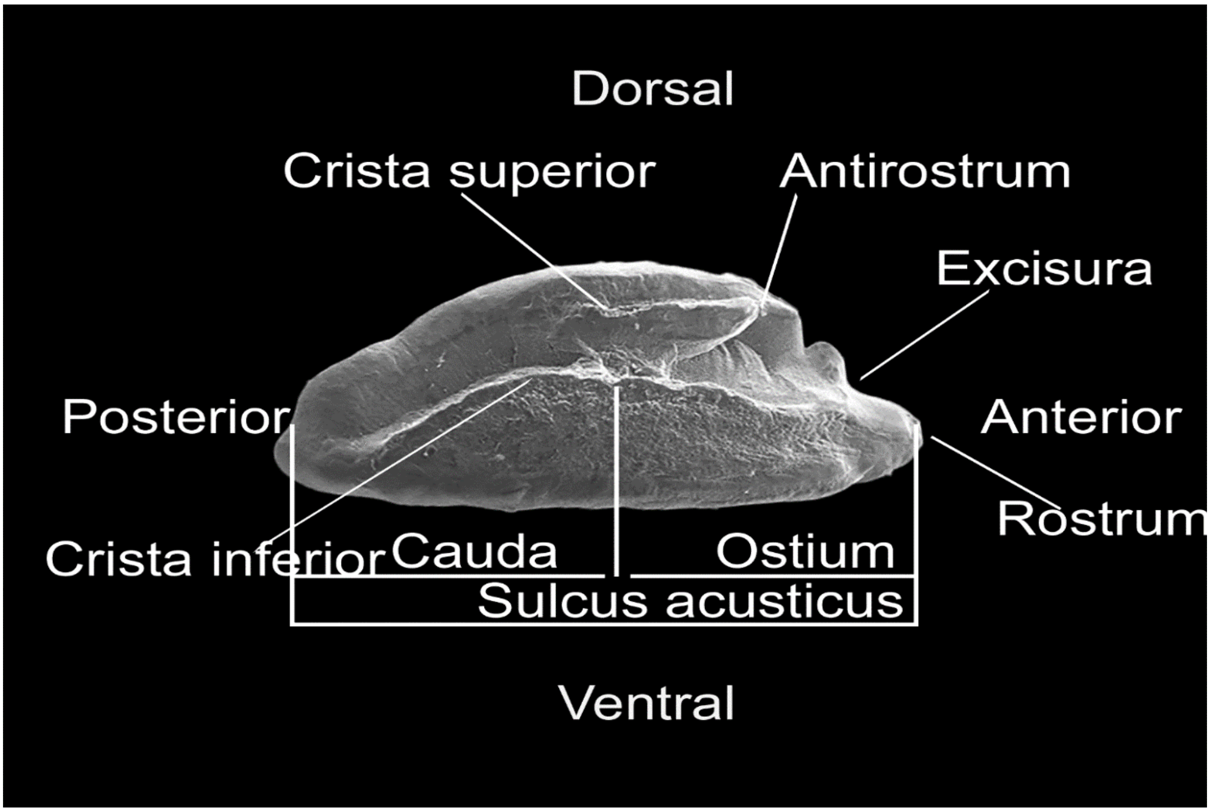

Figure 3: Schematic image of left sagittal otolith of *Entomacrodus striatus* showing terminology used in this study (after Furlani *et al.*, 2007).

Caudal skeleton preparation and description

The lateral view of the samples was imaged using an X-ray imaging system with a mammography machine in the mammography clinic (Dr. Hamida Raisi), and the images were studied for skeletal morphology. Naming skeletal elements and counting caudal fin rays were based on Springer's method (1967).

Table 1: Terminology of otolith.

Antirostrum	where present, the portion of the otolith extending dorsally and anteriorly from the excisura notch.
Colliculum	a raised part of the sulcus floor, occurring in the ostium (anterior colliculum) or in the cauda (posterior colliculum).
Collum	a wall-like structure separating ostium and cauda in some otoliths.
Crista	the rim of the sulcus dorsally (crista superior) and ventrally (crista inferior), ranging in development from absent or broken, through to a continuous, extended feature, which in turn may be poorly developed, or well developed, through to a raised, ridge-like margin to the sulcus.
Dorsal area and ventral areas	the area on the proximal surface of the otolith lying dorsal/ventral to the sulcus.
Excisura	where present, the opening of the sulcus onto the otolith anterior margin; often with an associated notch.
Margin	shape and sculpturing of otolith edges.
Otoliths	three pairs of ear stones in the inner ear, including asterisci (otoliths of the lagena), lapilli (otoliths of the utriculus) and sagittae (otoliths of the sacculus).
Rostrum	where present, the portion of the otolith extending ventrally and anteriorly from the excisura notch.
Sulcus	(=sulcus groove, sulcus acusticus), a longitudinal depression on the proximal surface of the otolith. Divided into ostium (that portion of the sulcus anterior to the neck) and cauda (that portion of the sulcus posterior to the neck). The sulcus acusticus houses the sensory epithelium of the sacculus (=macula sacculi).

Molecular analysis

Mitochondrial DNA was extracted from fin clips using the standard salt method described by Bruford *et al.* (1998). The standard DNA barcode region *E. striatus* of the COI was amplified using primer pairs named FishF1 5'TCAACCAACCACAAAGACATTGGCAC3’ and FishR1 5'TAGACTTCTGGGTGGCCAAAGAATCA3’ (Ward *et al.*, 2005) with the basis amplification protocol: 94°C one minute for primary denaturing, 35 cycles of 94°C for 30 seconds, 52°C for 45 seconds, and 72°C for 45 seconds, followed by 72°C for 5 minutes as ending extension on a Bioer thermal cycler. Purification and sequencing of the PCR products were conducted in the Niagenenoor Laboratory (Tehran). The phylogenetic tree was reconstructed using maximum likelihood and Bayesian.

One obtained COI sequence for the herein studied *E. striatus* is deposited in GenBank with the accession number PQ203340 (576 bp). An additional 65 sequences belonging to 15 species of the genus *Entomacrodus* and two sequences of *Istiblennius spilotus* as outgroup from

GenBank (http://www.ncbi.nlm.nih.gov/genbank) (Table 2) were used to present molecular phylogenetic reconstruction of the genus *Entomacrodus* and the relationship of *E. striatus*. The assembled sequences were aligned using MAFFT v.7 (Katoh and Standley, 2013). Estimates of mean evolutionary divergence between species were calculated using the Kimura-2-Parameter model (K2P; Kimura, 1980) implemented in MEGA X.

Table 2: Number of obtained COI sequences from the GenBank and related accession numbers, and also COI sequence of *E. striatus* in the present study*.

Species	Sequences No. in the present study	GenBank Accession numbers
Entomacrodus caudofasciatus	7	MK658438, MK657407, KX301872, KX301871, MK657833, MK657353, MK657152
Entomacrodus chiostictus	1	HQ168561
Entomacrodus corneliae	2	MK566898, KX301875
Entomacrodus cymatobiotus	9	KJ968090, KJ968089, KJ968088, KJ968087, KJ968086, KJ968085, KJ968084, JQ431714, JQ431713
Entomacrodus decussatus	1	KX301861
Entomacrodus epalzeocheilos	2	KX301862, KX301863
Entomacrodus macrospilus	5	MK566900, MK566899, MK657549, MK657013, KX301876
Entomacrodus nigricans	14	JQ841165, JQ841164, JQ840835, JQ840836, JQ841163, JQ841162, JQ841160, JQ840498, JQ842111, JQ842110, JQ842109, JQ842108, JQ842107, JQ841566
Entomacrodus niuafoouensis	2	KX301874, HQ168562
Entomacrodus randalli	2	MK566901, KX301877
Entomacrodus sealei	2	MK658447, MK657699
Entomacrodus stellifer	1	KX301860
Entomacrodus striatus *	1	This study (PQ203340)
Entomacrodus striatus	7	KX301869, KX301866, KX301867, KX301868, KX301865, KX301864, MZ613311
Entomacrodus thalassinus	9	MK658549, MK658526, MK658114, MK658079, MK657149, MK657153, MK657097, MK657392, MK658127
Entomacrodus vermiculatus	1	KX301879
Istiblennius spilotus	2	JF493690, JF493691

Selecting the best model of nucleotide substitution and sequence evolution for this dataset was performed with MrModeltest v.2.3 (Darriba *et al.*, 2013). According to the Akaike Information Criterion (AIC), the TIM2+G model was used for Bayesian inference. Phylogenetic trees were concluded using maximum likelihood and Bayesian inference. Maximum likelihood reconstruction was performed with RAxMLv.8.24 (Stamatakis *et al.*, 2007) using the rapid hill-climbing algorithm. MrBayes v.3.2.6 (Ronquist *et al.*, 2012) 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 the *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).

Results

Systematics

Class: Actinopterygii Klein, 1885
Order: Blenniiformes Bleeker, 1860
Family: Blenniidae Rafinesque, 1810 (blennies)
Subfamily: Salariinae Gill, 1859 (salariid blennies)
Genus: *Entomacrodus* Gill, 1859
Species: *Entomacrodus striatus* (Valenciennes, 1836)
Synonyms:
Salarias fraenatus Valenciennes [A.] in Cuvier & Valenciennes, 1836:342.
Entomacrodus plurifilis marshallensis Schultz [L. P.] & Chapman [W. M.] in Schultz *et al.* 1960:341.
Entomacrodus plurifilis plurifilis Schultz [L. P.] & Chapman [W. M.] in Schultz *et al.* 1960:338.
Entomacrodus wolffi Rofen [R. R.] 1958:202, Pl. 11.

Material collected

ZM-CBSU ES001-13 (13 specimens, TL=59.2-96.9 mm; SL=44.9-79.4 mm), Iran: Hormuzgan Prov., Jask Port, Yekboni pier, 25.659592 N., 57.821020 E., F. Pourhosseini, M. Pasalari, J. Pourhosseini, M. Karami, 5 May 2023.

Key to species of the genus Entomacrodus from the western Indian Ocean

1a Free margin of upper lip entirely smooth……………………*E. thalassinus*
1b Free margin of upper lip somewhat crenulate (portions of the lip may be smooth)..................................………… 2
2a Free margin of the upper lip with central third smooth, sides crenulate…...……….. 3
2b Free margin of the upper lip with at least central third crenulate…………………. 6
3a Preopercular pore positions with single pore; 1 pore anterior to nostril on each side……………………………..*E. striatus*
3b Most preopercular pore positions with pairs or clusters of pores; 2–4 pores anterior to nostril on each side…………...………. 4
4a Supraorbital cirri heavily branched, usually 10+ branches in fish >4 cm SL; upper lip irregularly dusky or with 2 or 3 broad dusky and pale bars.. *E. vermiculatus*
4b Supraorbital cirri weakly branched (<10 branches); upper lip with ~20 narrow dusky and pale bars....………………………... 5
5a Nape with ≥2 cirri on each side……………………...*E. epalzeocheilos*
5b Nape with 1 cirrus on each side…………………….. *E. niuafoouensis*
6a Preopercular pore positions each with single pore; upper lip, head and body with small white spots………………………………*E. solus*
6b Usually 3–5 (rarely 1 or 2) preopercle pore positions with ≥2 pores; upper lip with alternating dark and pale bars, and no small white spots……………………..*E. lemuria*

General morphology

Diagnosis

Entomacrodus striatus is distinguished from its congeneric species in the western

Indian Ocean by free margin of the upper lip with central third smooth, sides crenulate, preopercular pore positions with single pore; and 1 pore anterior to nostril on each side.

Morphology

See Figures 2 and 4 for general appearance and Table 3 for morphometric and meristic data. Dorsal fin with 13 spines and 12-16 rays; anal fin with 2 spines and 15-17 rays; pectoral fin with 14 rays; pelvic fin I,3 rays; caudal fin with 14-15 rays; supraorbital cirri on each side 4-9; nape with 1 cirrus on each side; free margin of upper lip entirely crenulate (crenulae sometimes weakly developed); edge of lips 23-30; preopercular pore positions each with 1 pore; 1 pore before each anterior nostril; lateral line pores terminating on the side in the area below and between dorsal-fin spine 11 and dorsal ray 10 (usually between dorsal spine 13 and dorsal ray 6).

Figure 4: Dorsal, lateral and ventral views of *Entomacrodus striatus* (ZM-CBSU-ES7; SL: 57 mm) collected from Jask Port, Hormozgan Province, Iran.

Coloration

Head and body color variable: usually pale with small dark spots along sides, and sometimes mottled with faint dark bars; upper lip uniformly tan or with alternating dusky and pale bars; irregular blue-green mark behind the eye and just above pectoral-fin base. It reaches 96.9 mm TL (Table 3).

Sexual dimorphism

Males bear fleshy rugose modifications of the skin of the anal-fin spines and the anterior first to fourth anal-fin rays (Fig. 5).

Table 3: Morphometric and meristic data of *Entomacrodus striatus* from the westernmost part of the Indo-Pacific.

Proportion	Min-Max	Mean±SD
Total weight(g)	1.6-6.1	3.7±1.3
Morphometric characters		
Total length (TL)	59.2-87.3	73.7±8.3
Standard length (SL)	48.3-70.1	59.3±7.1
In % of SL		
Head length (HL)	24.5-29.8	26.4±1.3
Length of dorsal fin (LD)	90.8-73.1	77.1±5.2
Length of anal-fin (LA)	37.8-52.2	44.3±4.05
Pre anal distance (PaD)	49.2-61.1	52.7±2.8
Post anal distance (PoAD)	4.1-7.8	6.5±1.2
Length of pectoral fin (LP)	9.6-12.1	10.7±0.6
Length of longest pectoral-fin ray (LPF)	24.1-27.5	26.1±0.9
Length of longest caudal-fin ray (LCF)	24.2-28.6	26.5±1.4
Minimum body depth (MinBD) (Caudal peduncle depth)	9.1-11.7	10.6±0.7
Maximum body depth (MaxBD)	19.4-23.4	20.7±1.1
In % of HL		
Head depth (HD)	70.3-88.1	78.2±5.2
Pre pelvic distance (PpD)	63.5-97.3	85.3±9.0
Post orbital distance (PoD)	57.4-75.4	65.9±6.2
Inter orbital distance (InD)	12.09-17.4	15.1±1.6
Eye diameters (ED)	13.7-21.09	17.6±2.4
Pre dorsal distance (PD)	70.3-92.2	83.6±6.6
Supraorbital cirrus length (OCL)	16.3-32.0	22.5± 5.2
Nuchal cirrus length (NCL)	5.1-10.0	7.6± 1.5
Third dorsal spine length (DS3)	33.3- 44.8	36.5± 3.5
Meristic characters		
Dorsal-fin spine	12	
Dorsal-fin ray	14-16	
Anal-fin spine	2	
Anal-fin ray	15-17	
Pectoral-fin ray	14	
Caudal-fin ray	14-15	
Preopercular pore	6	
Supraorbital cirri: (supraorbital cirri on each side)	5-9	
Nape cirri:	1	
Nostril cirri	present	
Edge of lips	23-30	

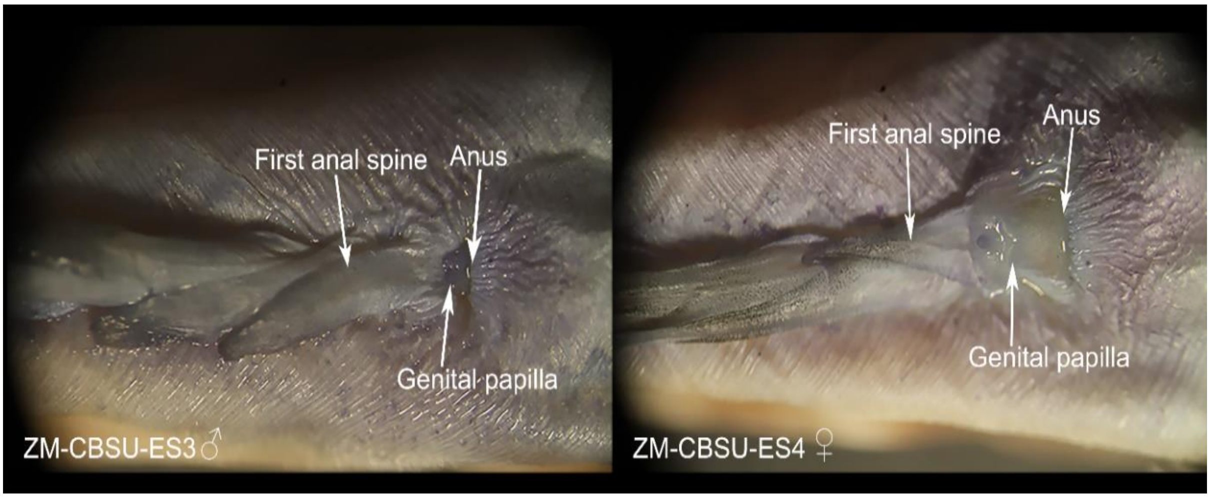

Figure 5: Sexual dimorphism in *Entomacrodus striatus* collected from Jask Port, Hormozgan Province, Iran.

Distribution

Red Sea; Indo-West Pacific: KwaZulu-Natal (South Africa), East Africa (Mozambique, Tanzania, Kenya), Seychelles, Comoros, Madagascar and Mascarenes (La Réunion, Mauritius, Rodrigues) east to Wake Atoll and Pitcairn Group, north to southern Japan, south to Rottnest Island (Western Australia), Coral Sea (Queensland, Australia), Lord Howe Island (Australia) and Rapa (French Polynesia), see Fricke *et al.* (2024). Recorded from subtidal stations in the Chabahar Bay and Makran Coast by Estekani *et al.* (2020), and from Daryabozorg (Gulf of Oman) by Sharifiniya *et al.* (2021), both in Sistan and Baluchestan Province of Iran. Here, it is reported for the first time from Jask Port (Hormuzgan Province), west to the previously recorded sites.

Otolith morphology

Fusiform shape; anterior region peaked to blunt; rostrum short, wide and relatively pointed; antirostrum short, broad, round and peaked; posterior region blunt, double peaked and oblique; excisura relatively wide with a shallow notch; dorsal margin entire; ventral margin entire to slightly sinuate; sulcus acusticus heterosulcoid, ostial, median; crista superior relatively distinct and ridge-like; crista Inferior distinct and ridge-like; ostium funnel-like to elliptic, shorter than or as long as the cauda; cauda curved, descending, approaches ventral margin or conclusion closed to the posterior margin (Fig. 6).

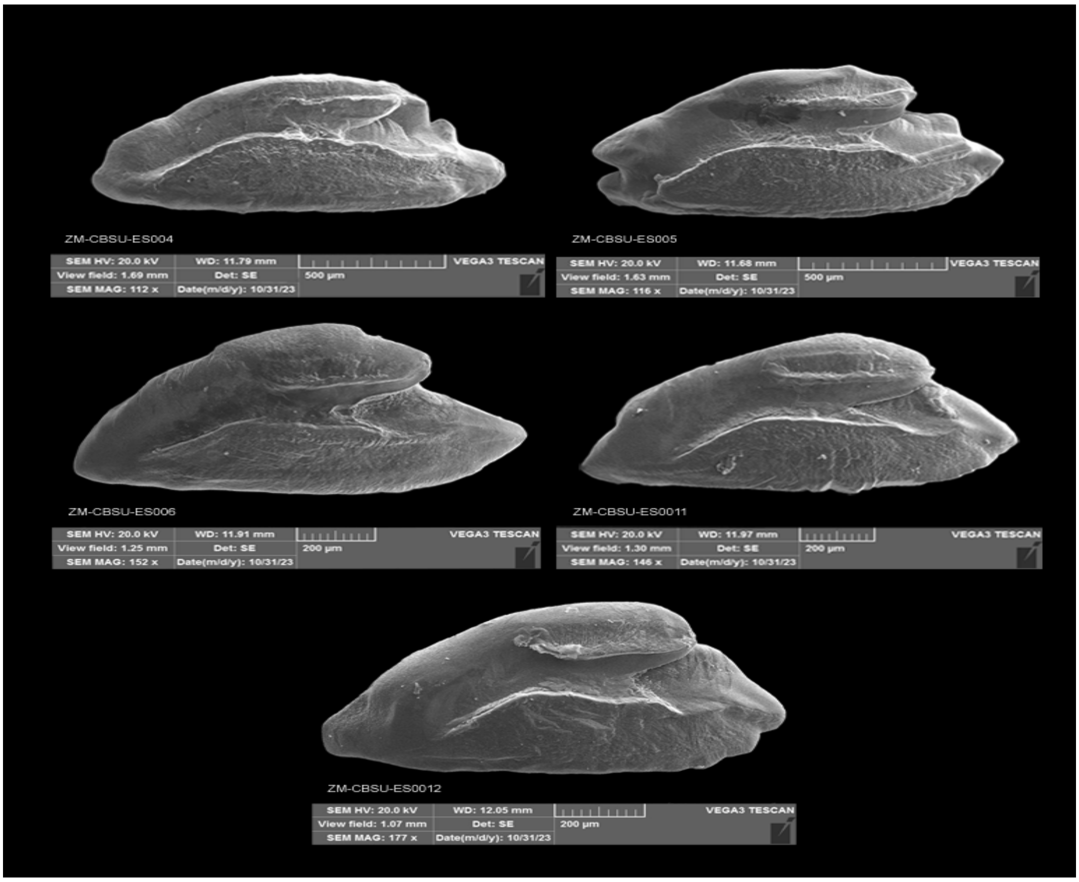

Figure 6: Otolith variation of *Entomacrodus striatus* collected from Jask Port, Hormozgan Province, Iran.

Osteology

Total vertebral count 33 vertebrae: 10 precaudal and 23 caudal vertebrae including terminal centrum (TC), all with neural spine, except TC. The neural spines are well-developed. The eleventh vertebra is the first caudal vertebra and is specified by bearing the first haemal spine. The segmented rays in the caudal fin range 14, the middle nine of which are branched.

Only one epural is present that is delicate. The hypurals 1 to 4 are triangular-like-shaped bones, while the hypural 5 is narrow. Hypurals 1+2 and Hypurals 3+4 are fused.

The minimal hypural (hypural 5) is not attached posterodorsally to the fused dorsal hypural and appears to support only the procurrent caudal ray (Fig. 7). The same has been reported for two other species of the genus *Entomacrodus*: *E. stellifer* (Jordan and Snyder, 1902) and *E. rofeni* Springer, 1967 by Springer (1967). Five branched caudal-fin rays also attach to the fused hypural plate (hypurals 3+4). There is one ventral hypural plate (fused hypurals 1+2) bearing four branched and 2 unbranched caudal rays.

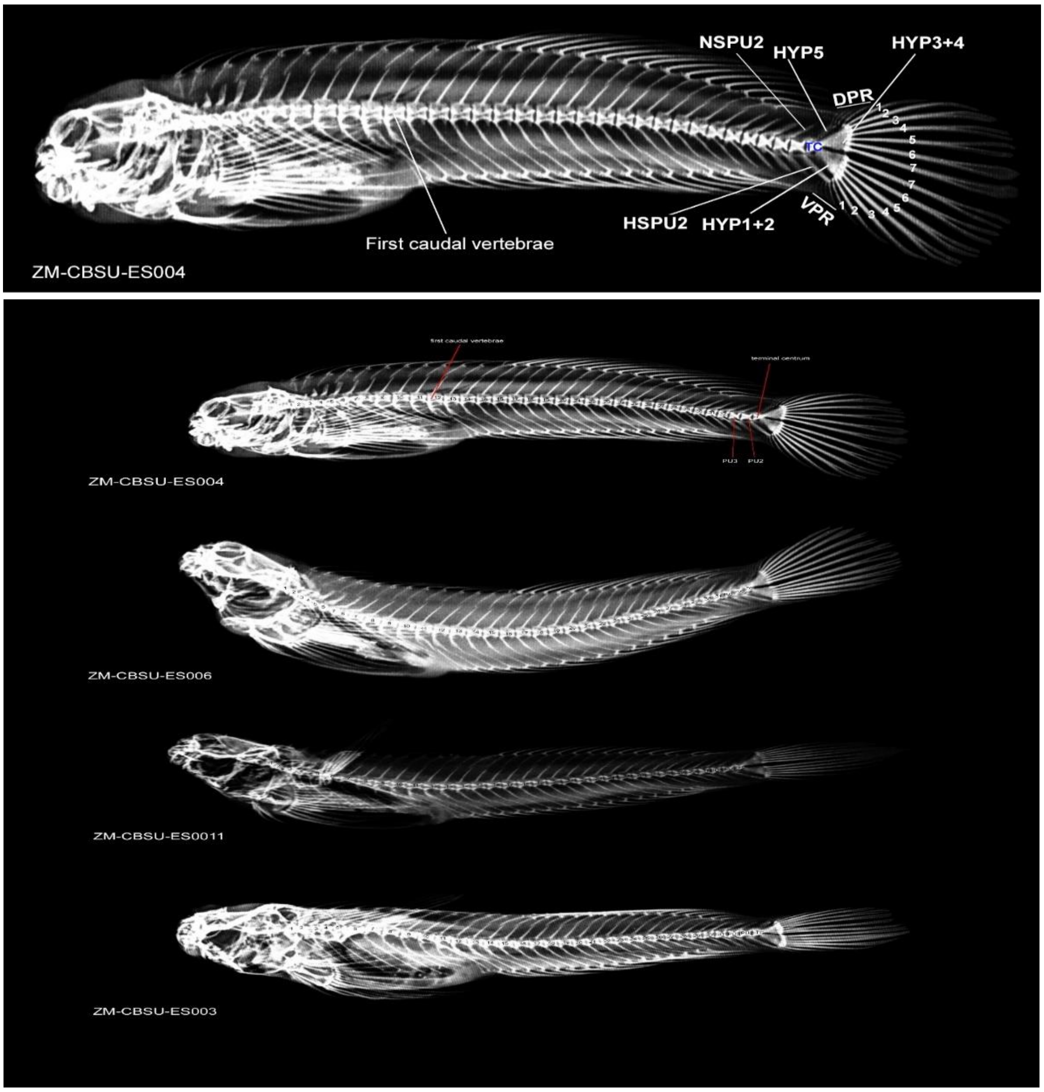

Figure 7: Osteology of *Entomacrodus striatus* collected from Jask Port, Hormozgan Province, Iran.

Phylogenetic relationship

The reconstructed phylogenetic tree shown in Figure 8 is based on COI sequences of 68 specimens belonging to 16 blenniid species including the genus *Entomacrodus* from the Iranian Makran coast of Jask (present record). As shown in Figure 8, the COI sequences from the Jask area (present study) were nested within the other COI sequences of *Entomacrodus striatus* including one from Chabahar Bay with accession number MZ613311. The closest sister group to the species *E. striatus* were *E. epalzeocheilos*, *E. niuafoouensis*, and *E. randalli*, which is not supported much in Maximum likelihood, but MrBayes v.3.2.6 supports it. The closest sister group is clade *E. epalzeocheilos*, *E. niuafoouensis*, and *E. randalli* and the furthest sister group is clade *E. vermiculatus, E. decussatus,* and *E. striatus* has the closest genetic distances with *E. niuafoouensis*, *E. epalzeocheilos* and *E. randalli*, respectively (Table 4).

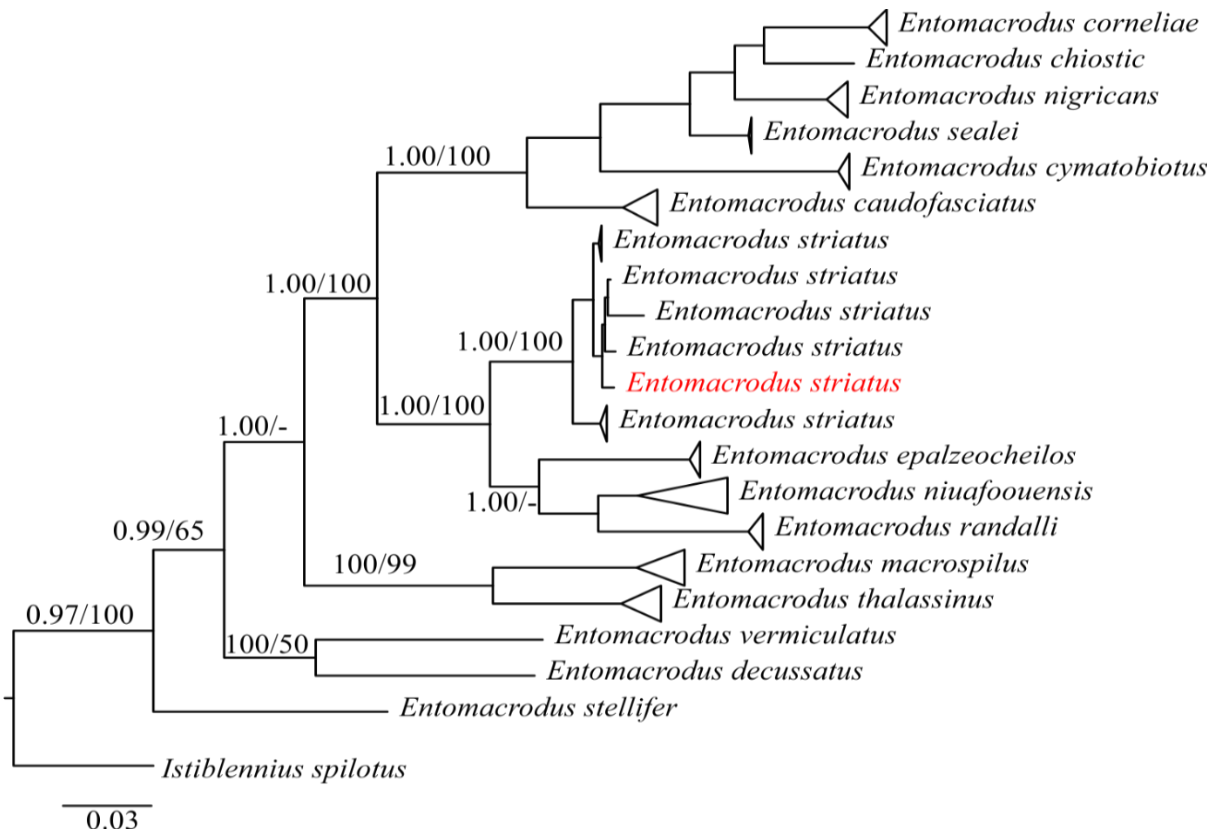

Figure 8: Phylogenetic relationships of the species of *Entomacrodus* based on COI gene sequences. The numbers define support for BI and ML, respectively; however, some branches are supported only by BI. Our newly obtained sequence is red. The scale bar indicates the number of substitutions per site.

Table 4: Estimates of mean K2P sequence divergence between the *Entomacrodus* species.

	E. striatus	***E. randalli***	***E. niuafoouensis***	***E. epalzeocheilos***	***E. corneliae***	***E. sealei***	***E. nigricans***	***E. chiostictus***	***E. caudofasciatus***	***E. cymatobiotus***	***E. macrospilus***	***E. thalassinus***	***E. vermiculatus***	***E. decussatus***
E. randalli	0.125	0.000												
E. niuafoouensis	0.108	0.095	0.000											
E. epalzeocheilos	0.116	0.132	0.111	0.000										
E. corneliae	0.196	0.216	0.185	0.171	0.000									
E. sealei	0.166	0.185	0.161	0.173	0.092	0.000								
E. nigricans	0.168	0.192	0.177	0.177	0.082	0.073	0.000							
E. chiostictus	0.179	0.183	0.157	0.189	0.081	0.075	0.079	0.000						
E. caudofasciatus	0.158	0.212	0.186	0.181	0.128	0.116	0.122	0.116	0.000					
E. cymatobiotus	0.209	0.224	0.189	0.192	0.164	0.146	0.161	0.155	0.158	0.000				
E. macrospilus	0.196	0.184	0.179	0.196	0.220	0.220	0.197	0.221	0.218	0.232	0.000			
E. thalassinus	0.185	0.175	0.177	0.194	0.216	0.220	0.194	0.201	0.207	0.255	0.115	0.000		
E. vermiculatus	0.209	0.210	0.216	0.231	0.249	0.233	0.224	0.236	0.230	0.224	0.231	0.212	0.000	
E. decussatus	0.199	0.210	0.198	0.207	0.194	0.192	0.193	0.199	0.188	0.217	0.235	0.221	0.180	0.000
E. stellifer	0.208	0.188	0.197	0.197	0.223	0.239	0.219	0.226	0.189	0.227	0.227	0.229	0.193	0.195

Discussion

The decline and extinction of vertebrate populations including fishes have been a defining feature of the Anthropocene (a proposed geological epoch dating from the commencement of significant human impact on Earth), and thus monitoring, distribution range record, and thus the study of different aspects of their biology and ecology are necessary. During the ichthyological survey of the coastal area of the Oman Sea, we collected blenny specimens which were later confirmed as *Entomacrodus striatus* using both morphological (counts and measurements), and molecular (COI sequences). The fish systematics emphasizes holomorphology (total form). Holomorphology describes the concept of using many different characteristics from different types (levels) of organismic sources to complete the description of a species. The process combines information from gross morphology, structural morphology (*e.g*., otolith, skeleton) anatomy, and molecular characteristics (*e.g*., mitochondrial gene sequences) which have been implemented for many fish species (*e.g.*, Zarei *et al.*, 2022; Esmaeili *et al.*, 2023). Herein, we followed the same concept and used general morphology, counts and measurements, otolith morphology, osteological characteristics, and mtDNA sequences to document and describe *E. striatus* from the Iranian coastal area of the Makran Sea.

The specimens collected from the coastal area of Makran (Oman Sea) were identified as a member of the genus *Entomacrodus* in having distinctive salariid morphology including presence of teeth on vomer; presence of nasal, supraorbital and nape cirri; absence of fleshy blade-like crest on head; continuous gill openings across isthmus; tubular and continuous lateral line; and meristic counts of fin rays (Williams and Springer, 2022). The collected specimens showed good accordance with *E. striatus* in general morphology, morphometrics, and meristic characteristics (Springer, 1967; Williams and Springer, 2022). The counts and measurements of the examined materials are almost in the range of those given by Sharifiniya *et al.* (2022) except in one feature. Conforming to Springer (1967), the preopercular series of sensory pores have simple pores in all positions, and it does not match Sharifiniya *et al.* (2022). Variation in the number of lateral cirri branches on the main supraorbital cirrus has been reported to occur in some populations of *E. striatus* (Springer, 1967). Variability and plasticity among conspecific populations are a common, widespread, natural phenomenon that occurs in a vast array of different taxa. (reviewed by Foster, 1999; Foster and Endler, 1999).

Similar to the other members of the genus *Entomacrodus*, the reef margin blenny *E. striatus* shows sexual dimorphism. Sexual dimorphism in the genus *Entomacrodus* has been well documented by Springer (1967). According to Springer (1967), males show the following characteristics: the anal papilla is small, just posterior to the anus and anterior to the first anal spine; both anal spines distinct, the anterior slightly shorter than the posterior; the skin of the anal spines and as many as four anteriormost rays becoming swollen, fleshy, rugose, or plicate in presumably mature specimens.

The flesh of the dorsal portion of the head becomes thickened, and swollen in appearance, sometimes forming a low distinct fold medianly, never very prominent. Color patterns are frequently darker, and frequently more uniform than those of females in the same collection. Supraorbital cirri frequently relatively longer than cirri of females from the same collection.

In the female specimens, the anal papilla present included posteromedially in a fleshy, posteriorly directed swelling behind the anus; swelling incorporating much reduced first anal spine (frequently visible only in skeletal preparations or on radiographs); first anal spine frequently visible in young females. The flesh of the head is not thickened or swollen and the head is without a median fold (see Springer, 1967). In the collected specimens of *E. striatus* from the Jask Port of Oman Sea, males develop fleshy rugose modifications of the skin of the anal spines and the anterior first to fourth anal rays, and the anal papilla is small, just posterior to the anus and anterior to the first anal spine. Sexual dimorphism in the coloration of *E. striatus* was observed in *E. striatus* population from the Chabahar Bay (Oman Sea) by Sharifiniya *et al.* (2022). Sexual color dimorphism/dichromatism (SCD) refers to the differences in coloration between male and female individuals, while sexual structural dimorphism (SStD) is characterized by the presence or absence of specific macrostructures. For instance, males of sharks and rays possess claspers, male mosquitofish have gonopodia, and male cyprinid fishes of the genus Garra exhibit large and numerous breeding tubercles. Additionally, variations in the shape, size, and relative position of urogenital papillae are observed across many fish taxa. These differences arise from sexual selection, primarily driven by either female mate choice or male–male competition (Esmaeili *et al.*, 2023).

Since the studies of Koken (1884), morphological differences in otoliths have been applied to identify teleost species. These calcified structures are now considered a source of informative taxonomic characters at both the species and population levels, and ontogenetic growth for different fish species (Tuset *et al.*, 2008; Mehraban *et al.*, 2023). Tuset *et al.* (2008) described the sagitta morphology of nine species of blennies from five genera (*Blennius*, *Coryphoblennius*, *Lipophrys*, *Parablennius*, and *Scartella*), and suggested that the overall shape of the sagitta in the studied species of *Lipophrys* and the posterior region of the sagitta in the species of *Parablennius* could be suitable diagnostic characters. Based on the obtained results by Mehraban *et al.* (2023) on the otolith morphology of 10 blennies, sagitta morphology could be considered as diagnostic characters distinguishing the genera *Alticus*, *Antennablennius*, *Istiblennius*, *Omobranchus* and *Parablennius* showing differences related to the general shape, that is, elliptic/oval to triangular; shape of antirostrum and its degree of development; the degrees of convexity of ventral and dorsal margins, and shape of ostium. The morphology of the sulcus acusticus/sulcus, which is functionally, as well as taxonomically the most important element/part of an otolith (especially the ostium part) could be a proper character to recognize some genera and species of the blennies. The common/general otolith characters shared in all studied combtooth blennies, and *E. striatus* form the Jask Port is the heterosulcoid condition, which is a shared character with Perciformes and Gobiiformes. Similar to several other blennies, the type of sulcus in *E. striatus* is ostial, and it can be considered a plesiomorph character shared with a basal group of actinopterygian fishes. In the heterosulcoid condition, ostium and cauda are distinct and do not have the same shape, so that the sulcus is more or less asymmetrical (Mehraban *et al.,* 2023). The description of otolith morphology in *E. striatus* could be useful in taxonomical, paleontological, ecological and food and feeding habits studies.

The vertebral column and caudal skeleton of fishes is a complex structure made up of bones derived from either cartilage or dermal tissues (Rojo, 1991), and has been used as a significant source of information in the study of systematic and phylogenetic relationships of actinopterygian fishes (Purrafee Dizaj *et al.,* 2022; Echreshavi *et al.*, 2021). Springer (1967) for the first time, performed a comprehensive study on the osteology of the family and distinguished two subfamilies based on osteological characters. Later, Springer (1993) hypothesized the monophyly of suborder Blennioidei on the basis of the osteology of five specialized character complexes involving: dorsal gill arches, pectoral fin and girdle, pelvic fin and girdle, caudal fin, and anal fin. However, morphology of the caudal peduncle skeleton in blennies has not been well considered. Recent work of Mehraban (2018), focuses on the caudal peduncle skeleton. According to Mehraban (2018), in the study of osteology across 10 species of blennies, the caudal peduncle skeleton exhibits a stable morphological character in comb-tooth blennies, and its primary structure appears to be conserved in eight of the examined species. There are minor changes or variations in some features such as the shape of hypural plates the dorsal part is mostly triangular and the ventral part is trapezoid and the presence of hypural 5 (Mehraban, 2018). At the genus level, the most noticeable trait that distinguishes Omobranchus from the other three is the presence of hypural 5, which is absent in Omobranchus but present in the other genera. Springer (1967) compared the morphology of the caudal peduncle of five species of blennies (*Aspidontus taeniatus, Hypleurochilus geminatus*, *Lipophrys trigloides*, and *Salarias guttatus*). The variations were mainly based on the presence of hypural 5 and the number of epurals. There are 33 vertebrae in *E. striatus* which is in the lower range of vertebrae recorded for other species of the genus *Entomacrodus* being 33-36 for other species according to Springer (1967).

DNA barcoding using the COI marker has been widely used for species identification and biodiversity surveys (Ghanbarifardi *et al.,* 2016; Damadi *et al.*, 2020; 2023; Mehraban *et al.*, 2021; Alavi-Yeganeh *et al.*, 2022; Khandan-Barani *et al.,* 2023). COI marker has been utilized to identify *E. striatus* and investigate its phylogenetic relationship with other conspecifics (Fig. 8). The topology of our phylogenetic tree is consistent with

Sharifiniya *et al.* (2022), and both place *E. striatus* in a clade including *E. randalli*, *E. epalzeocheilus*, and *E. niuafoouensis*. The molecular phylogenetic relationship above is in line with the morphological classification of the *striatus* group (Springer, 1967).

The otolith characteristics of 10 blenniids from the Persian Gulf and Gulf of Oman are described and analyzed; however, the otolith shape characteristics of *E. striatus* are examined in the present study for the first time. A dendrogram based on otolith traits (Mehraban *et al.*, 2023) and two molecular phylogenetic trees of blennies (Mehraban *et al.*, 2021; Sharifiniya *et al.*, 2024) show some similarities; therefore, otoliths could be useful tools for taxonomic examinations.

Conclusions

Morphological characteristics and mtDNA sequences reveal the presence of *Entomacrodus striatus* in the western part of the Indian Ocean. It shows a distinct clade sister to a clade of *E. epalzeocheilos*, *E. niuafoouensis*, and *E. randalli* with the closest phylogenetic relationship to *E. niuafoouensis.* The slender body, caudal skeleton, and otolith morphology are well-adapted for living in intertidal pools. The distribution of *E. striatus* in the most western part of the Indian Ocean can be due to the establishment of morphological, genetic, behavioral, and physiological aspects of species.

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Conflicts of interest

Authors hereby declare that there is no conflict of interest.

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