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Research Article

Molecular phylogeny of combtooth blennies (Teleostei: Blenniidae) in the Persian Gulf and Oman Sea

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Abstract

This study collected 27 Blenniid specimens of 10 species from intertidal and subtidal habitats in the Persian Gulf and Oman Sea. The specimens were preserved and taxonomically identified before the genomic DNA extraction. The COI marker was then amplified and sequenced. Additional sequences from related subfamilies were obtained from GenBank. Phylogenetic reconstruction was carried out using Bayesian and phenetic methods. The study aimed to evaluate the phylogenetic position of the newly studied specimens and reconstruct the phylogenetic relationships among representatives of the Blenniidae. The amplification of the COI marker for *Istiblennius edentulus* and *Salarias fasciatus* in Iran is a significant advancement in understanding phylogeny, genetic diversity, and evolutionary connections in the area. A phylogenetic analysis was conducted using molecular data from this study and previous research from the southern waters of Iran. The study revealed that the subfamilies Blenniinae and Salariinae are paraphyletic and two tribes, Parablenniini and Omobranchini, form monophyletic groups. *Istiblennius* is considered a paraphyletic genus. *Omobranchus mekranensis* and *O. punctatus* are in a closer relationship than *O. fasciolatus*, which is grouped at a further distance. The phylogenetic tree places *Ecsenius* in a monophyletic clade at the furthest distance from other taxa, suggesting it may warrant a separate tribe rather than a genus.

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Introduction

The Blenniidae is considered a monophyletic family based on eight morphological characters, including the forms of coracoid and interopercle bones, the shape and position of teeth, two lateral projections on each side of urohyal, fleshy rugosities on the anal fins of males, lack of scales, and the nonprotractile premaxilla (Hastings and Springer, 2009).

The combtooth blennies, family Blenniidae Rafinesque, 1810, encompass 59 genera and 410 species (Fricke *et al.*, 2024) of small coastal and rocky reef benthic representatives with a worldwide distribution. These fishes reside in shallow coastal, tropical, and warm temperate marine waters, and a few species are reported from fresh and brackish waters (Lin and Hastings, 2013; Vecchioni *et al.*, 2019).

Major morphological studies have identified two subfamilies and six tribes within the Blenniidae. Blenniinae includes four tribes: Blenniini, Nemophini, Omobranchini, and Phenablenniini, while Salariinae includes two tribes: Parablenniini and Salariini (Hastings and Springer, 2009; Nelson *et al.*, 2016). The subfamily Blenniinae is established based on a single synapomorphy: the left and right dentaries are sutured to one another (Smith-Vaniz, 1976). The monophyly of Salariini is supported by three osteological characteristics (Springer and Spreitzer, 1978; Williams, 1990). The monophyly of the tribe Parablenniini is not fully supported (Hastings and Springer, 2009). However, the above classification is in doubt according to dentition traits (Bath, 2001). It seems the dentition of blenniids is not monotypic; rather, it is diverse, and small changes in tooth shape are accompanied by changes in size, number, attachment, and often diet; therefore, it could not be a reliable trait for phylogeny (Hundt and Simons, 2018).

Molecular phylogeny of the Blenniiformes, based on mitochondrial and nuclear loci, has confirmed the monophyly of Blenniidae; however, the relationships between genera are not consistently proposed (Lin and Hastings, 2013; Hundt *et al.*, 2014).

The previously presented Iranian checklists of the Persian Gulf and the Oman Sea (Blegvad and Løppenthin, 1944; Ghanbarifardi and Malek, 2007; Ghanbarifardi and Malek, 2009; Mehraban and Esmaeili, 2018; Estekani *et al.*, 2020; Sharifiniya *et al.*, 2021a; Sharifiniya *et al.*, 2021b) confirm the existence of 18 species of blennies, including *Alticus kirkii*, *Antennablennius adenensis*, *Antennablennius bifilum*, *Antennablennius variopunctatus*, *Ecsenius pulcher*, *Entomacrodus striatus*, *Istiblennius edentulus*, *Istiblennius pox*, *Istiblennius spilotus*, *Parablennius opercularis*, *Parablennius pilicornis*, *Parablennius thysanius*, *Salarias emarginata*, *Salarias fasciatus* (subfamily Salariinae), *Omobranchus fasciolatus*, *Omobranchus mekranensis*, *Omobranchus punctatus*, *Petroscirtes mitratus* (subfamily Blenniinae). There is a scarcity of molecular phylogeny and phylogeographic studies on the blennies from the southern Iranian waters (The Persian Gulf and Oman Sea) (Attaran-Farimani *et al.*, 2016; Attaran-Farimani *et al.*, 2016; Mehraban *et al.*, 2020; Mehraban *et al.*, 2021;

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Sharifiniya *et al.*, 2022). This additional information will contribute to a better understanding of the genetic diversity and phylogenetic relationships within the Blenniidae family in Iran. It will also provide valuable information for conservation and management efforts for these species in the region.

The current study aims to introduce new COI sequences from the Blenniids of the Persian Gulf and the Oman Sea for the first time and reconstruct the phylogenetic relationship between representatives of the Blenniidae using available COI sequences in NCBI and the new ones from this study.

Materials and methods

A total of 27 fish specimens (Fig. 1) were collected from intertidal habitats along the northern coasts of the Persian Gulf and Oman Sea between 2018 and 2021 (Table 1). Specimens were obtained from tidal pools during low tide using scoop and hand nets, while those from the subtidal zone were captured by scuba diving up to a depth of 10 meters using hand nets. Sampling was conducted at five localities in the Persian Gulf (Ameri, Qeshm) and the Oman Sea (Abd, Gatan, Daryabozorg). The right pectoral fin of each individual was isolated, fixed in 96% ethanol, and stored at -20°C until DNA extraction.

The fish were preserved in 10% formaldehyde and then stored in 70% ethanol for long-term storage. Taxonomic identification of the samples was conducted using identification keys and primary taxonomic literature (Smith and Heemstra, 1986; Springer and Williams, 1994; Randall, 1995; Carpenter *et al.*, 1997). Morphological examinations and Molecular lab works have been conducted at Guilan University and Tarbiat Modares University. Genomic DNA was extracted from stored fin clips in alcohol using the salt method protocol (Bruford *et al.*, 1992). The entire COI genes were amplified via PCR using the primers (FishF1 and FishR2; Ward *et al.*, 2005). Amplification of COI was performed under the following temperature regime: initial 94°C for 3 min, 35 cycles at 94°C for 30 s, 58°C for 30 s, and 72°C for 90 s, and a final extension step at 72°C for 7 min. PCR products were purified with the PEG (Polyethylene glycol) method (Rosenthal *et al.*, 1993). Cleaned DNA was sequenced in a cycle sequencing reaction. Sequences were assembled, edited, and aligned with BioEdit 7.0.4 (Hall, 1999) and MAFFT v.7 (Katoh *et al.*, 2019). The 27 obtained COI sequences for this study are deposited in GenBank with accession numbers PP163345-PP163371 (Table 1).

An additional 60 sequences from subfamilies Salariinae and Blenniinae, belonging to 18 species, were included in Genbank (Table 2) to ensure a comprehensive dataset for evaluating the phylogenetic position of the new sequences in this study and for reconstructing the phylogenetic relationships among Blenniidae representatives. It is worth noting that the sequences retrieved from GenBank are predominantly from Iran.

The best-fitting nucleotide substitution model for the sequences, GTR+G+I, was selected using the Bayesian Information Criterion (BIC, Schwarz, 1978) in JModelTest 2.1.3 (Darriba *et al.*, 2012).

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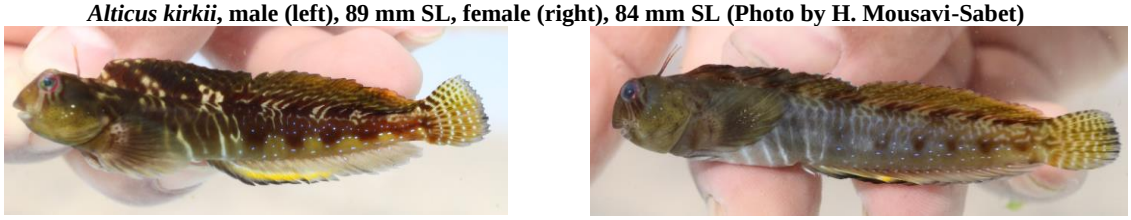
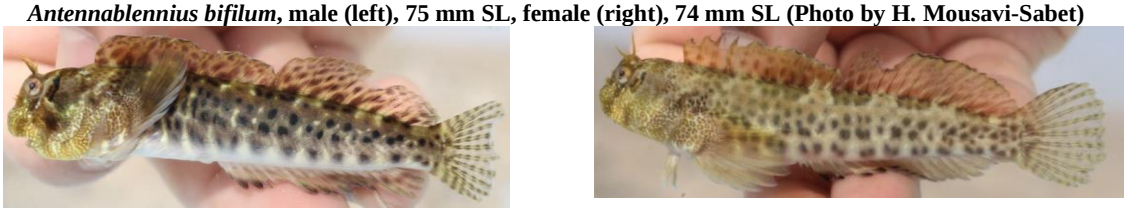
***Alticus kirkii*, male (left), 89 mm SL, female (right), 84 mm SL (Photo by H. Mousavi-Sabet)**



***Antennablennius bifilum*, male (left), 75 mm SL, female (right), 74 mm SL (Photo by H. Mousavi-Sabet)**



***Entomacrodus striatus*, male (left), 68 mm SL, female (right), 63 mm SL (Photo by H. Mousavi-Sabet)**

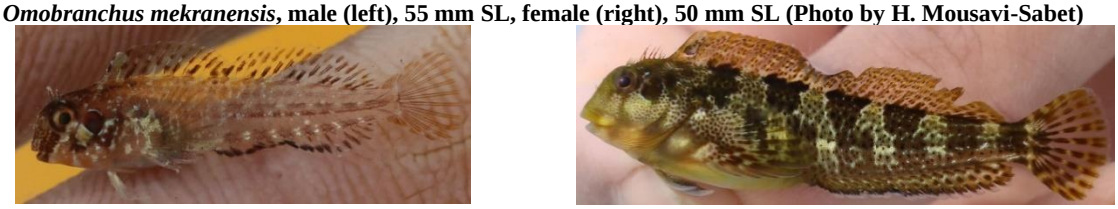
***Istiblennius pox*, male (left), 111 mm SL, female (right), 98 mm SL (Photo by H. Mousavi-Sabet)**

***Istiblennius spilotus*, 108 mm SL (Photo by H. Mousavi-Sabet)**

***Omobranchus punctatus*, 58 mm SL (Photo by H. Mousavi-Sabet)**

***Omobranchus fasciolatus*, female (left), 52 mm SL, male (right), 59 mm SL (Photo by H. Mousavi-Sabet)**

***Omobranchus mekranensis*, male (left), 55 mm SL, female (right), 50 mm SL (Photo by H. Mousavi-Sabet)**



***Parablennius opercularis*, 20 mm SL (Photo by H. Mousavi-Sabet)**

***Scartella emarginata*, 50 mm SL (Photo by H. Mousavi-Sabet)**

Figure 1: Pictures of some species studied in the present molecular phylogeny.

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Table 1: Blenniid Species collected from the Iranian coasts of the Persian Gulf and the Oman Sea.

Species	Locality	Nª	Coordinates	GenBank No.
Alticus kirkii	Oman Sea, Daryabozorg	6	25°58′N 57°15′E	PP163361-PP163366
Antennablennius bifilum	Oman Sea, Gatan	3	25°16′N 60°39′E	PP163367-PP163369
Istiblennius edentulus	Oman Sea, Daryabozorg	1	25°58′N 57°15′E	PP163357
Istiblennius pox	Oman Sea, Abd	3	25°38′N 58°51′E	PP163358-PP163360
Istiblennius spilotus	Oman Sea, Daryabozorg	2	25°58′N 57°15′E	PP163355-PP163356
Omobranchus fasciolatus	Persian Gulf, Qeshm, Bostanou	1	26°55′N 56°15′E	PP163354
Omobranchus mekranensis	Oman Sea, Daryabozorg	5	25°58′N 57°15′E	PP163349-PP163353
Omobranchus punctatus	Persian Gulf, Ameri	2	28°30′N 51°06′E	PP163347-PP163348
Parablennius opercularis	Oman Sea, Daryabozorg	2	25°58′N 57°15′E	PP163345-PP163346
Salarias fasciatus	Persian Gulf, Qeshm, Bostanou	2	26°55′N 56°15′E	PP163370-PP163371

For phylogenetic reconstruction, the Bayesian method was run based on four simultaneous runs of four Markov chains for 10000000 generations and a burn-in of 15% of the initial trees in MrBayes 3.2.6 (Ronquist *et al.*, 2012). The tree was rooted with two COI sequences of *Labrisomus nuchipinnis* (Blenniiformes: Labrisomidae) as outgroups. Additionally, a phenetic method, the Neighbour-Joining (NJ) analysis using the K2P distance (as suggested by Hebert *et al.* (2003)), implemented in MEGA X, was employed to examine relationships among the COI sequences.

Results

In total, 27 COI sequences from 10 blenniid species have been added to the previously deposited data of this marker from Iran. The COI marker has been amplified and reported for the first time for two species from Iran, including *Istiblennius edentulus* (one sequence) and *Salarias fasciatus* (two sequences). Additionally, two COI sequences of *Omobranchus punctatus* have been reported from the Persian Gulf for the first time.

All COI sequences from various blenniid species found in the Southern waters of Iran, as well as the 27 new sequences obtained in this study, were utilized to construct a phylogenetic tree (Fig. 2). Neighbour-Joining and Bayesian Inference analyses produced trees with identical topologies. The Phylogenetic tree consists of six major clades. Clade 1 consists of the *Parablennius* and *Scartella* genera, corresponding to the Parablenniini group of Salariinae. Clade 2 includes *Petroscirtes mitratus*, a member of the Nemophini group of Blenniinae. Clades 3 (*Istiblennius*, *Entomacrodus*, *Antennablennius*), 5 (*Alticus*, *Salarias*), and

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6 (*Ecsenius*) belong to the Salariini group of Salariinae; however, they do not form a monophyletic lineage. Clade 4 consists of *Omobranchus*, a member of the Omobranchini group of Blenniinae.

Table 2: List of COI sequences archived in GenBank and used in the phylogenetic analyses. The number in the parenthesis indicates the quantity of sequences used in this study. Subfamilies and tribes are based on Hastings and Springer, 2009.

Species	Subfamily> tribe	GenBank acc. Numbers, Locality
Alticus kirkii (3)	Salariinae> Salariini	MW323472, MW323474, MW323475 (Iran)
Antennablennius bifilum (3)	Salariinae> Salariini	MW323476, MW323477, MW323478 (Iran)
Ecsenius anomalus (1)	Salariinae> Salariini	KJ411425 (Iran)
Ecsenius pulcher (4)	Salariinae> Salariini	KJ411424, KU140947, KU140948, KU140949 (Iran)
Entomacrodus striatus (3)	Salariinae> Salariini	JQ349959, JQ349960 (Madagascar), MZ613311 (Iran)
Istiblennius edentulus (2)	Salariinae> Salariini	KX301847 (Cook Islands), KX301850 (Australia)
Istiblennius lineatus (6)	Salariinae> Salariini	HQ168572, KU944798, KX301854 (Taiwan), KX301853 (Guam), KJ411429, MW323479 (Iran)
Istiblennius pox (8)	Salariinae> Salariini	MW323486, MW323489, MW323490, MW323492, MW323493, MW323494, MW323495, MW323496 (Iran)
Istiblennius spilotus (3)	Salariinae> Salariini	JF493690, JF493691 (South Africa), KJ411431 (Iran)
Omobranchus fasciolatus (3)	Blenniinae> Omobranchini	MW323499, MW323502, MW323509 (Iran)
Omobranchus mekranensis (4)	Blenniinae> Omobranchini	MW323510, MW323511, MW323512, MW323513 (Iran)
Omobranchus punctatus (2)	Blenniinae> Omobranchini	MW323514, MW323515 (Iran)
Parablennius cornutus (1)	Salariinae> Parablenniini	KJ411426 (Iran)
Parablennius opercularis (3)	Salariinae> Parablenniini	MW323516, MW323517, MW323518 (Iran)
Parablennius pilicornis (3)	Salariinae> Parablenniini	HQ561509, HQ945848 (South Africa), KJ411427 (Iran)
Petroscirtes mitratus (4)	Blenniinae> Nemophini	HQ168580 (Seychelles), KJ411430 (Iran), KY675936 (Saudi Arabia), MT076514 (the United Arab Emirates)
Salarias fasciatus (4)	Salariinae> Salariini	FJ584072 (Indonesia), MH049261 (Indonesia), MW630756, OQ386337 (Philippines)
Scartella emarginata (3)	Salariinae> Parablenniini	JF494436, JF494437 (South Africa), KJ411428 (Iran)

According to the phylogenetic tree, *Parablennius* is considered a paraphyletic genus in relation to *Scartella*. Similarly, *Istiblennius* is also a paraphyletic genus because *I. spilotus* is grouped with *Entomacrodus striatus* and *Antennablennius bifilum* instead of other species of *Istiblennius*.

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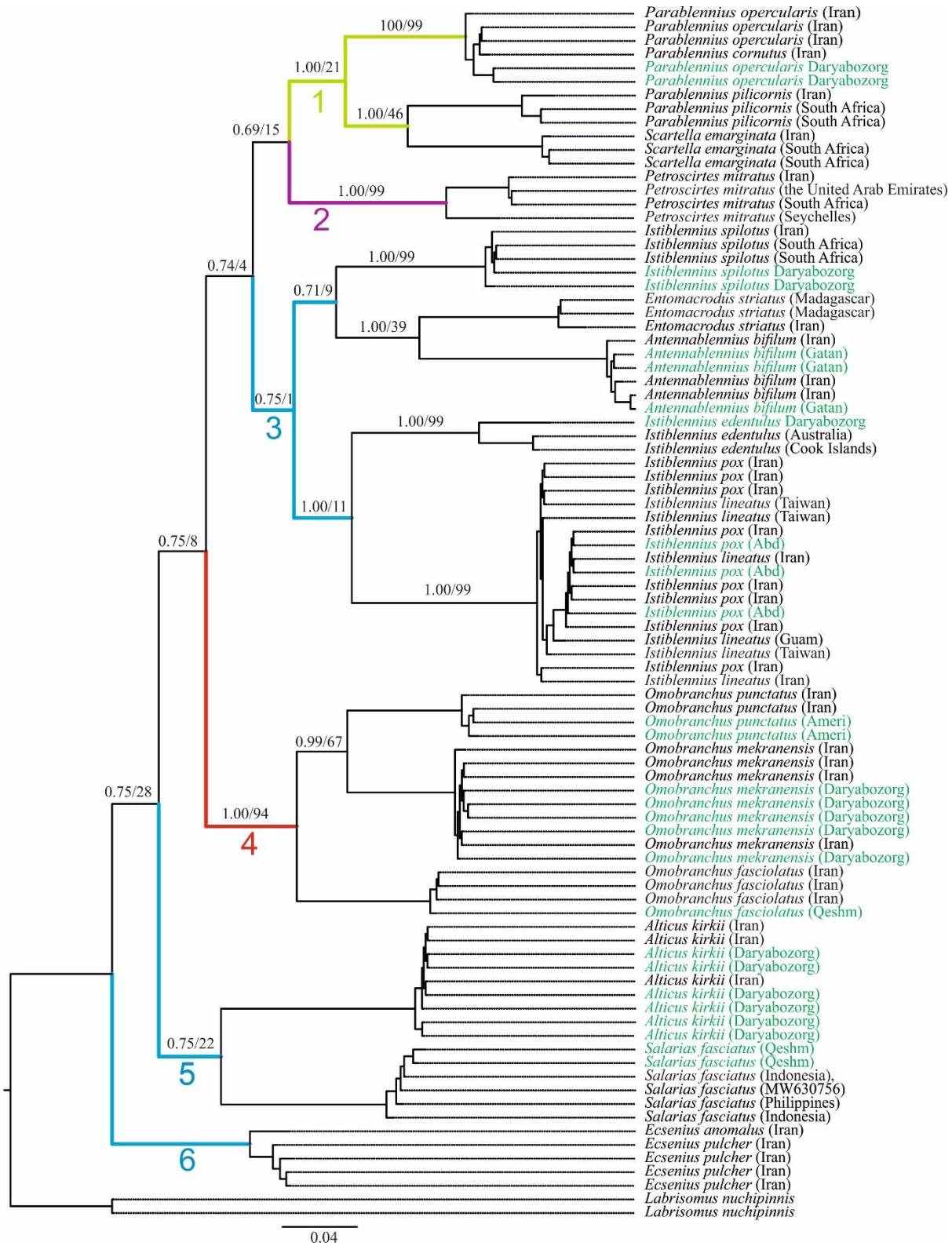

Figure 2: BI and NJ phylogeny of the studied blenniid species reconstructed using COI sequences. Sequences produced in this study are indicated in green fonts. The values next to the branches before and after a slash represent Bayesian posterior probability (PP) and NJ bootstrap (BP) values, respectively. The locality of each sequence is in parentheses. There are six major clades explained in the results.

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Discussion

The identification and reporting of the COI marker for *Istiblennius edentulus* (Daryabozorg, Table 1) and *Salarias fasciatus* (Qeshm, Table 1) in Iran represent a significant advancement in comprehending the genetic diversity and evolutionary connections of these species in the area. This information will be beneficial for future research on the population genetics, phylogeography, and conservation of these species. It also contributes to the expanding pool of genetic data for marine species in the Persian Gulf and the wider Indian Ocean region (Mehraban *et al.*, 2020; Mehraban *et al.*, 2021). Utilizing the molecular data from this study, 27 COI sequences from 10 species, and previous research from the southern waters of Iran (Attaran-Farimani *et al.*, 2016, in Persian; Attaran-Farimani *et al.*, 2016; Mehraban *et al.*, 2021; Sharifiniya *et al.*, 2022), phylogenetic analysis has been carried out.

Based on the phylogenetic tree (Fig. 2), the subfamilies Blenniinae (2: purple and 4: red clades) and Salariinae (3, 5, 6: blue and 1: green clades) are found to be paraphyletic, which is in line with previous molecular surveys (Lin and Hastings, 2013; Hundt *et al.*, 2014; Mehraban *et al.*, 2021) and contradicts major morphological studies (Smith-Vaniz, 1976; Williams, 1990; Hastings and Springer, 2009; Nelson *et al.*, 2016). We concur with other molecular studies that Parablenniini and Omobranchini form monophyletic groups (Lin and Hastings, 2013; Hundt *et al.*, 2014; Mehraban *et al.*, 2021). It is worth noting that *Parablennius* is not a monophyletic taxon concerning *Scartella* (Fig. 2, green clade), which is consistent with previous molecular surveys that recognize *Parablennius* as a paraphyletic genus with *Hypleurochilus*, *Salaria*, and *Scartella* (Almada *et al.*, 2005; Levy *et al.*, 2013).

Istiblennius is considered a paraphyletic genus because *I. spilotus* is classified with *Entomacrodus* and *Antennablennius* rather than other representatives of *Istiblennius* (Fig. 2, blue clade 3). Mehraban *et al.* (2021) also introduce *Istiblennius* as a paraphyletic taxon, and *I. spilotus* is grouped with *Antennablennius bifilum* (Fig. 2); however, they do not use *Entomacrodus* in their phylogenetic analysis. *Entomacrodus* and *Istiblennius* are closely related according to molecular surveys (this study, Lin and Hastings, 2013; Hundt *et al.*, 2014) and morphological examination, mainly the upper-lip crenulae and sixth branchiostegal ray (Springer and Williams, 1994). Bath (2001) also grouped *Istiblennius* and *Entomacrodus* based on open premaxillary and dentary capsules and the inferior excavation of the premaxillary and superior excavation of the dentary.

Omobranchus mekranensis and *O. punctatus* are in a closer relationship with *O. fasciolatus* (Fig. 2, red clade), which is consistent with previous molecular phylogenetic analysis (Mehraban *et al.*, 2021). The dendrogram based on the otolith phenotypic relations classifies *Omobranchus mekranensis* and *O. fasciolatus* together (Mehraban *et al.*, 2023), contradicting the mentioned molecular studies. Molecular phylogenetic analyses provide evidence for the monophyly of *Omobranchus* and support for the *elongatus* and *banditus* species groups (Gibbs *et al.*, 2018). However, *O.*

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mekranensis and *O. fasciolatus* were not considered in the mentioned study, so we could not compare their results with ours.

Our phylogenetic tree places *Ecsenius* in a monophyletic clade at the furthest distance from other taxa (Figure 2, blue clade 6), which is consistent with the major molecular phylogeny of Blenniidae (Hundt *et al.*, 2014). *Ecsenius* is the most distinct genus in the Salariini based on morphological characteristics. It occupies a unique position in the Blenniidae and may even warrant a separate tribe. However, the general nature of its dentition at least places *Ecsenius* as an offshoot of the Salariini. *Ecsenius* differs from all other blenniids in having dorsal processes extending from the proximal portions of the epipleural ribs on at least the third through fifth vertebrae; in having both anterior and posterior canines on the dentary (the anterior canines are difficult to distinguish from the incisoriform teeth in most species, but their nature is apparent in osteological preparations); and in lacking an ossified median ethmoid. In addition to the above characters, *Ecsenius* also differs from the other Salariini genera in having the lateral extrascapular fused with the pterotic (Smith-Vaniz and Springer, 1971).

Conclusions

The discovery and documentation of the COI marker for *Istiblennius edentulus* and *Salarias fasciatus* in Iran represent a significant step forward in understanding the genetic diversity and evolutionary relationships of these species in the region. This information will be valuable for future research on conservation efforts for these species. It also contributes to the growing body of genetic data for marine species in the Persian Gulf and the wider Indian Ocean region. Utilizing molecular data from this study and previous research, revealing that the subfamilies Blenniinae and Salariinae are paraphyletic, consistent with previous molecular surveys but contradicting major morphological studies. The study also found that Parablenniini and Omobranchini form monophyletic groups. Additionally, the phylogenetic tree places *Ecsenius* in a distinct clade, consistent with previous molecular phylogenies, indicating its unique position within the Blenniidae family.

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Conflicts of interest

The authors declare no conflicts of interest.

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