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Molecular phylogenetic affinities of some subtidal gobies (Teleostei: Gobiidae) from Qeshm Island

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Abstract
Typical gobies are small in size and usually found as marine fishes. They inhabit on or whitin the substrates holes, and are mostly invisible. The present study identified two goby genera including *Valenciennea* and *Gobiodon* in Qeshm Island for the first time. Nuclear rhodopsin gene was sequenced in four goby species including *G. citrinus*, *V. puellaris*, *V. sexguttata*, as well as *V. strigata*. Furthermore, nuclear rhodopsin and mitochondrial cytochrome oxidase subunit I (COI) sequences of 38 Gobiidae species from GenBank were used to obtain a representative data-set in order to assess the phylogenetic position of the new samples and to compare the resultant tree with other related molecular reconstructions. Our phylogenetic tree was composed of two Clades. Clade 1 involved two genera including *Gobiodon* and *Paragobiodon* and Clade 2 involved three genera, including *Valenciennea*, *Signigobius* and *Amblygobius*. While, *Valenciennea* and *Signigobius* species were nested within Sub-Clade X, only *Amblygobius* species was nested within Sub-Clade Y. This study examined the phylogenetic relationships of four goby species using Rhodopsin and COI, as new markers. Our results on molecular phylogenetic analysis were in agreement with those of other studies using different molecular markers and morphological examinations.

Key words: *Valenciennea, Gobiodon, molecular phylogeny, Persian Gulf, Qeshm Island.*

INTRODUCTION

Gobiidae is the most species-rich family of teleost fishes, with approximately 1915 valid species (263 genera) (Fricke *et al.*, 2020). Typical gobies are small and usually marine fishes, with separate spinous dorsal fins, and pelvic fins often attached into a disc. Their color patterns range from colorful to camouflage (Thacker, 2011). Though 24 species from 19 genera of Gobiidae have been reported in waters of the Persian Gulf and Gulf of Oman (Blegvad & Loppenthin, 1944; Rahimian & Pehpuri, 2006; Ghanbarifardi & Malek, 2007, 2009; Bosaghzadeh *et al.*, 2015; Sadeghi *et al.*, 2017; Sadeghi & Esmaeili, 2019a; Sadeghi & Esmaeili, 2019b; Sadeghi *et al.*, 2019a; Sadeghi *et al.*, 2019b; Ghanbarifardi & Lagzian, 2019; Kovačić *et al.*, 2020), 61 species from 35 genera of this family have been identified in Oman coastal waters (Randall, 1995). Therefore, the biodiversity of Gobiidae in Iran southern marine waters has not been appreciated very well.

The genus *Valenciennea* Bleeker 1856 (bearing 16 valid species) was revised by Hoese and Larson (1994) in a study in which seven new species have been identified. Another species namely *Valenciennea yanoi* Suzuki, Senou and Randall, 2016 described from Japan. *Valenciennea parva* has been recently identified in the Red Sea for the first time, revising five species of this genus including *V. helsdingenii*, *V. parva*, *V. puellaris*, *V. wardii* as well as *V. sexguttata* (Kovačić *et al.*, 2018). Randall (1995) recorded four *Valenciennea* species, including *V. helsdingenii*, *V. persica*, *V. puellaris*, *V.*

sexguttata in Oman coastal areas (Persian Gulf, Gulf of Oman, Arabian Sea). *V. persica* and *V. sexguttata* have been identified in Kuwait, as well (Bishop, 2003). There is no record of *Valenciennea* in Iranian water bodies; though *V. persica* and *V. sexguttata* species have been reported in Arabian parts of the Persian Gulf (Eagderi *et al.*, 2019). Three species of this genus including *V. puellaris*, *V. sexguttata*, and *V. strigata* have been widely distributed throughout the Indian and Pacific Oceans (Hoese & Larson, 1994; Suzuki *et al.*, 2016).

Herler *et al.* (2013) described four new species of the coral-associated gobiid genus *Gobiodon* Bleeker 1856 (28 valid species) in the Red Sea and believed that there should be more than 30 species of this genus distributed in Indo-Pacific region; moreover, they have revised nine *Gobiodon* species (*G. ater*, *G. citrinus*, *G. histrio*, *G. rivulatus*, *G. fuscoruber*, *G. reticulatus*, *G. irregularis*, *G. prolixus*, *G. bilineatus*) in the Red Sea. *G. citrinus* and *G. reticulatus* have been recorded in Oman coastal waters (Randall, 1995). *G. reticulatus* has been recorded in Kuwait, as well (Bishop, 2003). Morphological and molecular phylogenetic relationship of some *Gobiodon* species have been devised using mitochondrial (12S, 16S, Cyt b) and nuclear (*S7* ribosomal protein gene Intron1) gene markers (Duchene *et al.*, 2013; Herler *et al.*, 2013; Harold *et al.*, 2008).

This study was aimed to present geographical data on *Valenciennea* and *Gobiodon* from subtidal zone of Qeshm Island and to use two new molecular markers in order to shed light on phylogenetic relationships of the mentioned genera and their relative taxa.

MATERIAL AND METHODS

Fish samples were collected from the Iranian coasts of the Persian Gulf (Qeshm Island, around Qeshm city) (Fig. 1). Two samples of each species were caught by diving in subtidal zones (26°55′38.8″ N 56°14′57.7″ E). The pelvic fin clips were preserved in absolute ethanol, and the samples were transferred and deposited in the Zoological Museum of the University of Sistan and Baluchestan (ZMUSB). Total genomic DNA extraction was carried out in ethanol-preserved tissue samples using a Yekta Tajhiz Azma kit. Rhodopsin gene (Rho) was amplified using Rho_Mi.F forward (5′ACGATCACAAGAATCTGCG3′) and, Rho_Mi.R (5′AGCTCCTGGCCATGAAGCC3′) reverse primers (Ghanbarifardi & Lagzian, 2019). All PCRs were conducted in 25 μl reaction tubes containing 9.5 μl distilled water, 0.5 μM of each primer, 2 μl of template DNA and 12.5 μl of Master mix solution (Taq DNA Polymerase, PCR buffer, dNTPs, $MgCl_2$). The amplifications were performed under the following temperature conditions: initial denaturation at 95°C for 15 min, followed by 35 cycles of 95°C for 45 sec, 61°C for 45 sec, 72°C for Rh 60 sec/Rag1 90 sec, and a final extension step at 72°C for 5 min. The partial Rhodopsin gene was sequenced using Big Dye Terminator v.3.1 Cycle Sequencing Kit (Applied Biosystems, Inc). Individual gene sequences were aligned with MAFFT v.7 software (Katoh & Standley, 2013) and adjusted using Geneious 11.1 (Biomatters Ltd, July 2018). The five resulting Rhodopsin sequences were deposited in GenBank under MT273939-MT273943 accession numbers.

FIGURE 1. Map of sampling area in the Persian Gulf (Qeshm city, red area, 26°55′38.8″ N 56°14′57.7″ E).

The additional 50 sequences of 38 Gobiidae species from the GenBank were used to obtain a representative data-set in order to assess the phylogenetic position of the new specimens and to compare the resultant tree with other molecular phylogenetic reconstructions (Table 1). *Callogobius bifasciatus* and *Callogobius sclateri* were selected as the outgroups (Agorreta *et al.*, 2013).

Phylogenetic trees were deduced using maximum likelihood (ML) (Felsenstein, 1981) and Bayesian inference (BI) (Huelsenbeck *et al.*, 2001). ML analysis was conducted with RAxML v.8.1.24 (Stamatakis, 2014) and BI analysis was performed with MrBayes v.3.2.6 (Ronquist *et al.*, 2012) by conducting two independent Markov Chain-Monte Carlo (MCMC) runs (four chains each) for 10 million generations. Internal nodes support was evaluated by nonparametric bootstrapping with 1,000 replicates (ML). For this dataset, the best fit combinations of partitioning schemes and nucleotide substitution models were determined by Partition Finder version 2 (Lanfear *et al.*, 2016). The GTRGAMMAI nucleotide substitution model was used for phylogenetic analyses (maximum likelihood and Bayesian inference).

RESULTS

Our results identified *Gobiodon citrinus* (Rüppell, 1838), *Valenciennea puellaris* (Tomiyama, 1956), *V. sexguttata* (Valenciennes, 1837) as well as *Valenciennea strigata* (Broussonet, 1782) in Iranian waters of the Persian Gulf. The reports were based on two sample collections from a subtidal zone of Qeshm City. Morphological identifications were conducted using main references (Hoese & Larson, 1994; Herler *et al.*, 2013; Kovačić *et al.*, 2018).

A nuclear marker gene (Rhodopsin) was amplified in all four species (*Gobiodon citrinus*, *V. puellaris*, *V. sexguttata*, *V. strigata*). This marker gene was sequenced in *Gobiodon citrinus* for the first time.

TABLE 1. Taxa, molecular markers, and GenBank accession numbers of the sequences used in the phylogenetic analyses.

rows	Species	GenBank acc. Numbers, COI	GenBank acc. Numbers, Rhodopsin Rho
1	*Amblygobius albimaculatus*	JF492825	-
2	*Amblygobius buanensis*	MH514923	-
3	*Amblygobius decussatus*	KP194955	-
4	*Amblyogobius esakiae*	-	KF235546
5	*Amblygobius nocturnus*	MK658014	HQ536939
6	*Amblygobius phalaena*	MK658090	HQ536897
7	*Amblygobius stethophthalmus*	MH049211	-
8	*Gobiodon acicularis*	MK496383	-
9	*Gobiodon aoyagii*	MK496374	-
10	*Gobiodon axillaris*	MK496341	-
11	*Gobiodon brochus*	MK496344	-
12	*Gobiodon ceramensis*	MK496349	-
13	*Gobiodon citrinus*	MF123902	-
14	*Gobiodon citrinus*	MT273939	This study for the first time
15	*Gobiodon erythrospilus*	MK496354	-
16	*Gobiodon fuscoruber*	MK496377	-
17	*Gobiodon histrio*	FJ583434	HQ536895
18	*Gobiodon micropus*	KP194730	-
19	*Gobiodon oculolineatus*	MK658647	KF235538
20	*Gobiodon okinawae*	KP194150	-
21	*Gobiodon prolixus*	JQ350006	-
22	*Gobiodon quinquestrigatus*	KP194949	JF261550
23	*Gobiodon reticulatus*	MF123909	-
24	*Gobiodon rivulatus*	MK658681	-
25	*Gobiodon unicolor*	MK658708	-
26	*Paragobiodon echinocephalus*	KP194948	-
27	*Paragobiodon lacunicolus*	MK658131	-
28	*Paragobiodon modestus*	JQ431971	JF261551
29	*Paragobiodon xanthosoma*	MK496380	-
30	*Signigobius biocellatus*	KP194237	HQ536947
31	*Valenciennea helsdingenii*	MK567145	-
32	*Valenciennea longipinnis*	MH049201	HQ536923
33	*Valenciennea muralis*	MK340746	-
34	*Valenciennea puellaris*	KU176381	HQ536910
35	*Valenciennea puellaris*	MT273942	This study
33	*Valenciennea puellaris*	MT273943	This study
36	*Valenciennea sexguttata*	FJ584224	KF235532
37	*Valenciennea sexguttata*	MT273941	This study
38	*Valenciennea strigata*	HQ945877	HQ536900
39	*Valenciennea strigata*	MT273940	This study
40	*Valenciennea wardii*	FJ584238	-
41	*Callogobius bifasciatus* (outgroup)	MH160734	HQ536904
42	*Callogobius sclateri* (outgroup)	MK657563	KF235554

The combined dataset (Table 1) included 1419 bp (COI: 654, Rhodopsin: 765), of which 374 bp (COI: 246, Rhodopsin: 128) were variable and 346 bp (COI: 239, Rhodopsin: 107) were parsimony-informative. Both ML and BI analyses yielded highly congruent trees with differences only in the branch lengths and levels of support (Fig. 2).
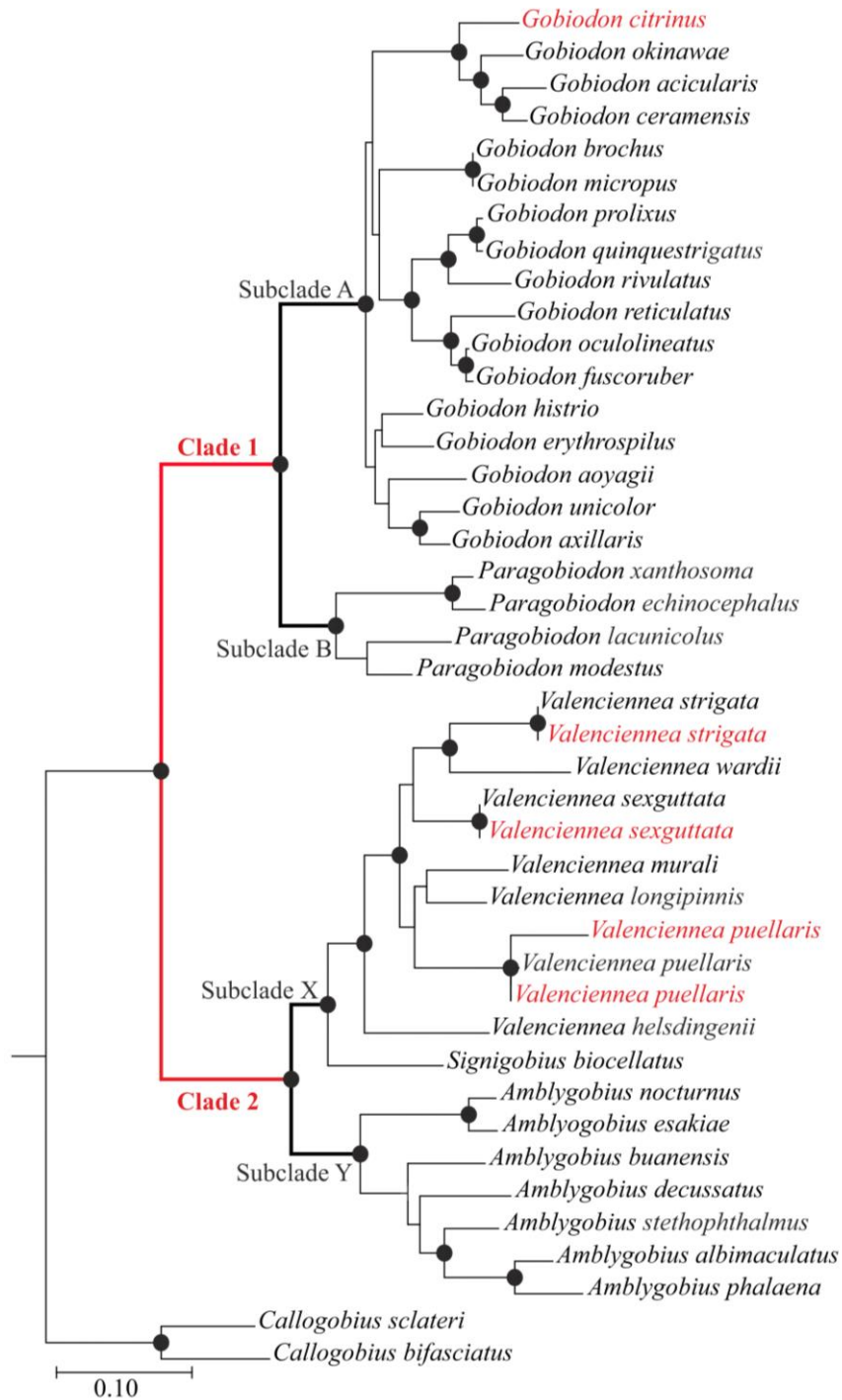
FIGURE 2. Phylogenetic relationships (ML phylogram) based on the main dataset comprising 1419 bp of two mitochondrial and nuclear (COI and Rhodopsin) markers. Filled bullets on nodes denote support for both ML (BP P≥70%) and BI (PP P≥0.95). Absence of bullet on a node denotes no support for either ML (BP<70%) or BI (PP<0.95). Four species sequenced for this study are in red.

The Phylogenetic tree (Fig. 2) was composed of two Clades. Clade 1 (Sub-Clades A and B) included *Gobiodon* and *Paragobiodon* genera, with *Gobiodon* being placed in Sub-Clade A and *Paragobiodon* in Sub-Clade B. Clade 2 (Sub-Clades X and Y) involved three genera, including

Valenciennea, *Signigobius* and *Amblygobius*, with *Valenciennea* and *Signigobius* being nested within Sub-Clade X and *Amblygobius* within Sub-Clade Y.

DISSCUSSION

Valenciennea sexguttata and *Valenciennea puellaris* can be regarded as two frequent taxa in the Middle East water bodies, as they have been reported in Red Sea (Kovačić *et al.*, 2018), Oman coasts (Randall, 1995), Kuwait (Bishop, 2003), and Qeshm Island in the present study. Though *Valenciennea strigata* is identified in our studied area and adjacent regions; it is expected to be widely distributed throughout the Indian and Pacific oceans (Hoese & Larson, 1994). *Gobiodon citrinus* was also another approximately abundant species (Herler *et al.*, 2013; Randall, 1995; Bishop, 2003) identified in the Qeshm Island. *Gobiodon citrinus* rhodopsin gene was sequenced for the first time in the present study.

Clade 1 (Fig. 2) was composed of *Gobiodon* (Sub-Clade B) and *Paragobiodon* (Sub-Clade B) genera. Various studies have proposed *Gobiodon* and *Paragobiodon* as the sister taxa, based on morphological characteristics and molecular markers (Harold *et al.*, 2008; Duchene *et al.*, 2013; Herler *et al.*, 2013). Though Phylogenetic analysis of some *Gobiodon* species has been investigated using osteological and molecular characteristics (12S, 16S, Cyt b, S7I1) (Harold *et al.*, 2008; Duchene *et al.*, 2013; Herler *et al.*, 2013), this study for the first time examined the phylogenetic relationship of *Gobiodon* species using the combination of rhodopsin and COI as new gene markers.

Our results are in accordance with those of previous studies (Harold *et al.*, 2008; Duchene *et al.*, 2013; Herler *et al.*, 2013), placing all four *Gobiodon* species (*Gobiodon citrinus*, *G. acicularis*, *G. okinawae*, and *G. ceramensis*) within one cluster. Two clades have been similarly presented by the present study and a previous one by Duchene *et al.* (2013) in which *Gobiodon oculolineatus*, *G. reticulatus*, *G. rivulatus*, *G. quinquestriagatus* were placed in one clade, while *G. histrio*, *G. erythrospilus*, *G. unicolor* and *G. axillaris* (Harold *et al.*, 2008; Duchene *et al.*, 2013) were placed together within another clade. It should be noticed that clustering using different markers (morphological, mitochondrial and nuclear) has more credibility than clustering using only one marker.

The Clade 2 (Fig. 2) was composed of three genera including *Valenciennea*, *Signigobius* and *Amblygobius*. Sub-Clade X grouped *Valenciennea* and *Signigobius* as sister taxa, a result which was further confirmed based on the presence of similar morphological characters, including dorsal placement of fleshy pads on the gill arches (Hoese & Larson, 1994). Sub-Clade Y was only composed of *Amblygobius* genus. Though *Amblygobius* was expected to be a close relative of *Signigobius* and *Valenciennea* genera, it shows difference in a number of characteristics including the structure of the first gill arch and the presence of more than one row of teeth in the upper jaw of some *Amblygobius* species (Hoese & Allen, 1977). Therefore, clustering Clade 1 in the present study is in agreement with the results of morphological (Hoese & Larson, 1994; Hoese & Allen, 1977) and molecular studies (Agorreta *et al.*, 2013; Thacker & Roje, 2011). Future studies including more *Gobiodon*, *Paragobiodon*, *Valenciennea* and *Amblygobius* species is required to assess phylogenetic relationships among the species of these genera with more details.

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