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Note

First record of whitebarred goby *Amblygobius phalaena* (Valenciennes, 1837) from Indian waters

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ABSTRACT

Gobiid fishes are one of the dominant and cryptic taxa in tropical coral reefs. Andaman and Nicobar Islands of India consist of fringing and barrier reefs which harbour huge fish diversity. Due to morphological ambiguity and cryptic life styles, identification and delimitation of gobiids is difficult. In the present study, we report the occurrence of *Amblygobius phalaena* (Valenciennes, 1837) for the first time in the Indian waters using morphology and DNA barcoding approach. *A. phalaena* from Indian waters showed divergence values within the range of 0.00 to 0.047 with the reported conspecific individuals.

Keywords: *Amblygobius phalaena,* Coral reefs, DNA barcoding, Gobiids, India

Whitebarred goby *Amblygobius phalaena* (Valenciennes, 1837) (Order: Perciformes; Family: Gobiidae) is a marine, reef associated fish which inhabits sand burrows. It is a commercially important aquarium fish and has significant role in ecology (Myers, 1991). This species is distributed across Indo-Pacific region and is native of South Africa, Australia, Japan, Thailand, Malaysia and Indonesia (Myers, 1999). Valid synonymised names for this species are *Gobius annulatus* De Vis, 1884 and *G. phalaena* Valenciennes, 1837. Due to the cryptic morphological characteristics, *A. phalaena* has been erroneously synonymised as *Amblygobius albimaculatus* (Ruppell, 1830) (Bauchot *et al.,* 1991). The coral reefs of India are rich with diverse finfish and shellfish species. However, due to the morphological ambiguity among fish species, the ichthyofauna of coral reefs has not been completely documented. In the present paper, we report the occurrence of *A. phalaena* for the first time in Indian waters using morphology and DNA barcoding approach.

Two numbers of *A. phalaena* were caught from coral reefs (11^{0}41’4.69”N; 92^{0}47’2.77’E), Ross Island, South Andaman District, Andaman Islands, India on 2nd April 2017 using hand net from a depth of 2 m. The specimens were morphologically identified as per Smith *et al*. (1986). Later, fin clips were taken aseptically and preserved in absolute alcohol for molecular work. Total genomic DNA was extracted from these samples using standard phenol-chloroform method (Sambrook *et al.,* 2001). The mitochondrial cytochrome *c* oxidase subunit I partial gene (650 bp) was amplified as per Ward *et al.* (2005). The PCR amplified products were visualised on 2% agarose gel and purified using Qiaquick gel extraction kit (Qiagen, Germany) following manufacture’s protocol. The purified PCR products were sequenced bi-directionally at the sequencing facility of M/s SciGenom Pvt. Ltd., Kochi, India. Sequences were analysed using MEGA7 software for species delimitation (Kumar *et al.,* 2016). For estimating the genetic divergence value and species delimitation, 39 reported COI gene sequences of the genus *Amblygobius* were downloaded from NCBI GenBank (Table 1).

The collected fishes measured 10.0 and 10.50 cm in total length. The diagnostic features include seven dorsal spines with 15 branched rays; first dorsal spine longer than second; anal fin with one spine and 14 branched rays; rounded caudal fin; longitudinal scale series 55; ocellus on the first dorsal fin and a black spot at the upper caudal fin base (Fig. 1).

Body greenish brown in colour, with five dark brown to blackish bars; a pale dark stripe through the eye and another across cheek; head with white streaks; small white spots in 3-4 horizontal rows on the body.

Cytochrome *c* oxidase subunit I sequences were trimmed and the quality of the sequence (600 bp) was verified by Phred score of each nucleotide and lack of

Table 1. List of species along with COI gene sequence accession numbers

Species	Location details	GenBank Accession No.
Amblygobius albimaculatus	Mozambique: Pomene	JF492825
	Mozambique: Pomene	GU805111
	Saudi Arabia	KY676051
	Saudi Arabia	KY675893
	Saudi Arabia	KY675830
	Saudi Arabia	KY675639
	Saudi Arabia	KY675434
	Saudi Arabia	KX139519
Amblygobius decussates	Australia: Queensland, Lizard Island	KP194955
	Australia: Queensland, Lizard Island	KP194244
	Philippines: Manila	FJ582719-23
Amblygobius phalaena	Australia: Queensland, Lizard Island	KP194896
	Australia: Queensland, Lizard Island	KP194792
	Australia: Queensland, Lizard Island	KP194751
	Australia: Queensland, Lizard Island	KP194685
	Australia: Queensland, Lizard Island	KP194499
	Australia: Queensland, Lizard Island	KP194353
	Australia: Queensland, Lizard Island	KP194327
	Australia: Queensland, Lizard Island	KP194153
	Australia: Queensland, Lizard Island	KP194021
	French Polynesia, Society Islands	JQ431409
	Philippines: Manila	FJ582724-25
	Ross Island, Andaman*	MF537264-265*
Amblygobius sphnyx	Madagascar: Antananarivo, Ouest, Nosy Be, Ambanoro bay	JQ349688-95
Amblygobius hectori	Madagascar: Antananarivo, Ouest, Nosy Be, Ambanoro bay	JQ349683-87

*Present study

Fig.1. *Amblygobius phalaena,* 90 mm SL from Ross Island, Andaman and Nicobar Islands

stop codons confirms absence of NUMTs. The sequences were submitted to NCBI with GenBank Accession nos. MF537264-265. Out of 600 nucleotides, the number of conserved and variable nucleotides was 523 and 77 respectively. Analysis using MEGA7 software revealed the average intra-specific genetic distance value for *A. phalaena* as 0.012±0.004 with a range of 0.000 to 0.051 (Table 2.). *A. phalaena* from Indian waters showed divergence values within the range of 0.00 to 0.047 and authenticated the species identification. The average interspecific divergence value between *A. phalaena* and *A. albimaculatus* is 0.102±0.009 which is 10x more than the average within species distance values of *A. phalaena* (Table 3). Further, Neighbor Joining tree constructed with

Table 2. Genetic divergence values (Kimura 2 Parameter) of COI gene within *Amblygobius phalaena* collected from different locations

Species	Ap1	Ap2	Ap3	Ap4	Ap5	Ap6	Ap7	Ap8	Ap9	Ap10	Ap11	Ap12	Ap13	Ap14
A. phalaena (Ap1)		0.002	0.004	0.004	0.005	0.003	0.004	0.004	0.004	0.004	0.004	0.004	0.002	0.009
A. phalaena (Ap2)	0.004		0.004	0.003	0.004	0.002	0.003	0.004	0.003	0.003	0.004	0.002	0.000	0.009
A. phalaena (Ap3)	0.012	0.009		0.003	0.003	0.004	0.002	0.000	0.002	0.002	0.000	0.004	0.004	0.009
A. phalaena (Ap4)	0.011	0.007	0.005		0.003	0.004	0.002	0.003	0.002	0.002	0.003	0.004	0.003	0.009
A. phalaena (Ap5)	0.014	0.011	0.005	0.007		0.005	0.003	0.003	0.003	0.003	0.003	0.004	0.004	0.009
A. phalaena (Ap6)	0.005	0.002	0.011	0.009	0.012		0.004	0.004	0.004	0.004	0.004	0.003	0.002	0.009
A. phalaena (Ap7)	0.011	0.007	0.002	0.004	0.004	0.009		0.002	0.000	0.000	0.002	0.004	0.003	0.009
A. phalaena (Ap8)	0.012	0.009	0.000	0.005	0.005	0.011	0.002		0.002	0.002	0.000	0.004	0.004	0.009
A. phalaena (Ap9)	0.011	0.007	0.002	0.004	0.004	0.009	0.000	0.002		0.000	0.002	0.004	0.003	0.009
A. phalaena (Ap10)	0.011	0.007	0.002	0.004	0.004	0.009	0.000	0.002	0.000		0.002	0.004	0.003	0.009
A. phalaena (Ap11)	0.012	0.009	0.000	0.005	0.005	0.011	0.002	0.000	0.002	0.002		0.004	0.004	0.009
A. phalaena (Ap12)	0.007	0.004	0.012	0.011	0.011	0.005	0.011	0.012	0.011	0.011	0.012		0.002	0.009
A. phalaena (Ap13)	0.004	0.000	0.009	0.007	0.011	0.002	0.007	0.009	0.007	0.007	0.009	0.004		0.009
A. phalaena (Ap14)	0.047	0.044	0.049	0.051	0.047	0.046	0.047	0.049	0.047	0.047	0.049	0.044	0.044	

Ap1-Ap2: Present study; Ap3-Ap11: Lizard Island, Australia; Ap12-13: Philippines, Manila; Ap14: French Polynesia, Society Islands, Morea.
Below diagonal - Divergence values; Above diagonal: Standard error values

Table 3. Genetic divergence values (Kimura 2 Parameter) of COI gene within the genus *Amblygobius* collected from different locations

Species	Ap1	Ap2	Ap3	Aa1	Aa2	Aa3	Ad1	Ad2	Ad3	As1	As2	As3	Ah1	Ah2	Ah3
A. phalaena (Ap1)		0.002	0.005	0.015	0.015	0.015	0.021	0.021	0.021	0.022	0.022	0.023	0.021	0.021	0.021
A.phalaena (Ap2)	0.004		0.004	0.014	0.014	0.015	0.020	0.020	0.020	0.022	0.022	0.023	0.021	0.021	0.021
A. phalaena (Ap3)	0.012	0.009		0.014	0.014	0.014	0.020	0.020	0.020	0.023	0.023	0.023	0.021	0.021	0.021
A.albimaculatus (Aa1)	0.103	0.099	0.103		0.000	0.002	0.017	0.017	0.018	0.021	0.021	0.022	0.019	0.019	0.019
A. albimaculatus (Aa2)	0.103	0.099	0.103	0.000		0.002	0.017	0.017	0.018	0.021	0.021	0.022	0.019	0.019	0.019
A. albimaculatus (Aa3)	0.105	0.101	0.105	0.002	0.002		0.017	0.017	0.017	0.021	0.021	0.021	0.019	0.019	0.019
A. decussates (Ad1)	0.205	0.201	0.205	0.163	0.163	0.160		0.000	0.002	0.021	0.021	0.022	0.021	0.021	0.021
A. decussates (Ad2)	0.205	0.201	0.205	0.163	0.163	0.160	0.000		0.002	0.021	0.021	0.022	0.021	0.021	0.021
A. decussates (Ad3)	0.208	0.203	0.208	0.165	0.165	0.163	0.002	0.002		0.021	0.022	0.022	0.021	0.021	0.021
A. sphinx (As1)	0.232	0.227	0.237	0.205	0.205	0.203	0.210	0.210	0.212		0.002	0.004	0.023	0.023	0.023
A. sphinx (As2)	0.234	0.229	0.239	0.207	0.207	0.205	0.212	0.212	0.215	0.002		0.003	0.023	0.023	0.023
A. sphinx(As3)	0.244	0.239	0.249	0.217	0.217	0.214	0.222	0.222	0.224	0.009	0.007		0.024	0.024	0.024
A. hectori (Ah1)	0.213	0.208	0.203	0.177	0.177	0.175	0.217	0.217	0.220	0.235	0.237	0.247		0.002	0.000
A. hectori (Ah2)	0.210	0.206	0.201	0.180	0.180	0.177	0.220	0.220	0.222	0.232	0.234	0.244	0.002		0.002
A. hectori (Ah3)	0.213	0.208	0.203	0.177	0.177	0.175	0.217	0.217	0.220	0.235	0.237	0.247	0.000	0.002	

Below diagonal - Divergence values; Above diagonal - Standard error values

COI divergence values showed distinct clusters shared by conspecific individuals with significant bootstrap value (Fig. 2). Within *A. phalaena* cluster, specimen from French Polynesia and Australia formed different branches, while specimen from India clustered with samples of Philippines.

Gobiidae is one of the most speciose families with more than 1700 described fish species (Eschmeyer and Fong, 2013). A total of 174 species of gobiid fishes have been reported from Indian waters (Chatterjee and Mishra, 2012). Globally, these fishes form one of the dominant taxa in tropical coral reef and coastal fish diversity. Andaman and Nicobar Islands located in Bay of Bengal consist of 352 islands and the shelf topography of these islands is characterised by fringing and barrier reefs. These ecosystems are habitat to a total of 1434 fish species including gobies (Rajan *et al.,* 2013). Due to their small size and cryptic life styles, identification and delimitation of gobiids using morphological characters is difficult. Because of this reason, gobiids have been under-represented in ichthyofaunal survey of different ecosystems (Close and Gouws, 2007; Thacker, 2011). DNA barcoding approach along with morphological characters has been successfully used to delineate fishes including gobiids (Ward *et al.,* 2005; Viswambaran *et al.,* 2013; Knebelsberger and Thiel, 2014)

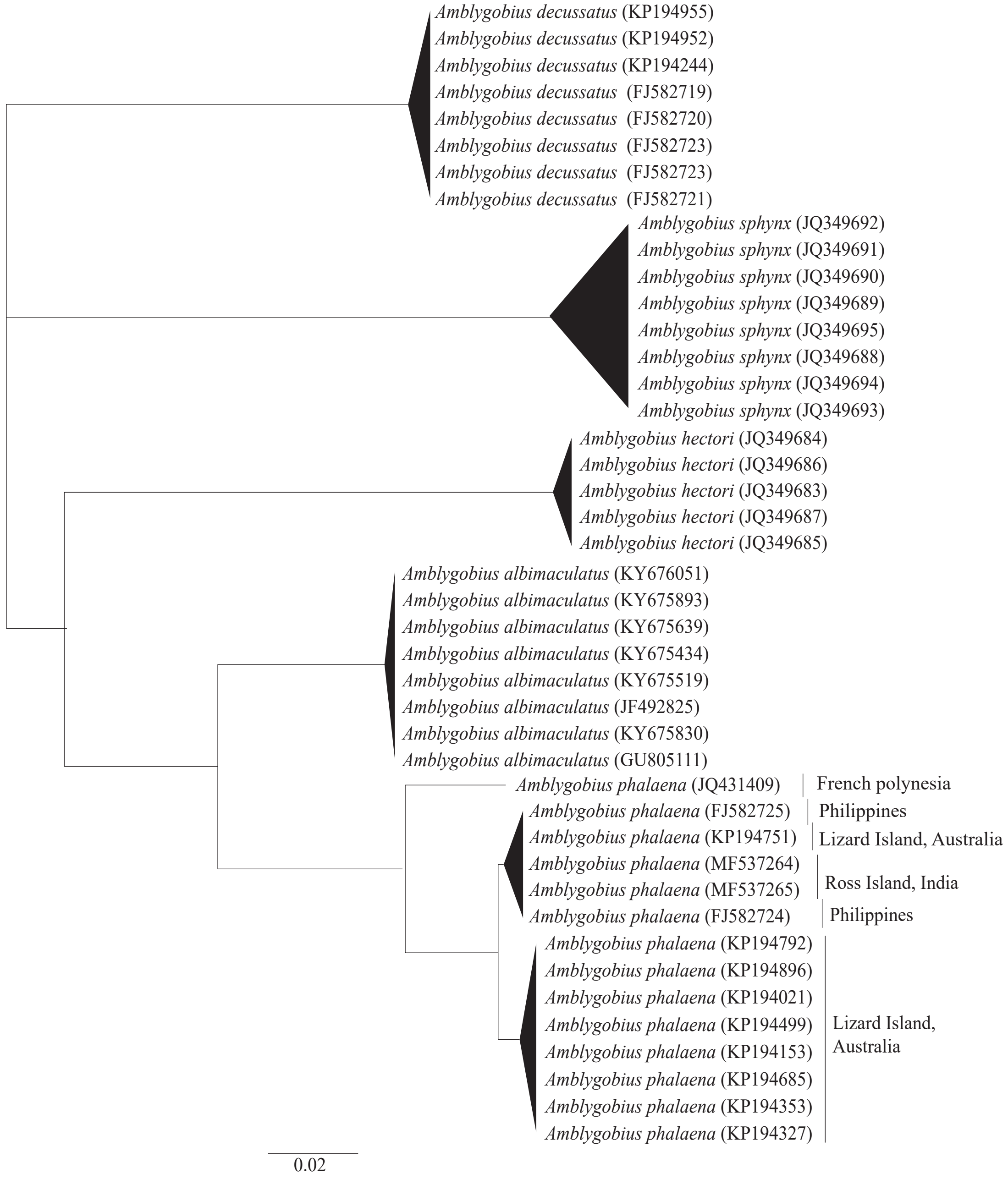

Fig.2. Neighbour joining tree of COI gene sequences of *Amblygobius* species

In the present study, two adult specimens of *A. phalaena* were collected from Ross Islands, Andaman and Nicobar Islands. Due to their cryptic life style, it was difficult to collect adequate number of samples in a single exploration. Initially, Valenciennes described *Gobius phalaena* from Vanikoro Island, south-western Pacific (Cuvier and Valenciennes, 1837). Later valid synonymisation of this species as *Amblygobius albimaculatus* resulted in taxonomic instability (Bauchot *et al.,* 1991). However, several studies have disproved this synonymisation and reported *Amblygobius phalaena* as a valid species (Allen and Swainston, 1988).

In the present study, the species status of *A. phalaena* was confirmed using DNA barcodes and morphological characters. Comparison of COI sequences generated in the present study with 15 reported sequences from Philippines, Australia and French Polynesia showed high similarity (99%) and less genetic divergence value against *A. phalaena.* Whereas higher genetic divergence values *i.e.* 10 times more than the average within species divergence value of *A. phalaena* were observed between congeners of *Amblygobius*. This high divergence value confirms that *A. phalaena* and *A. albimaculatus* are two distinct species. This observation is in congruence with the previous studies that disproved synonymisation of *A. phalaena* and *A. albimaculatus* (Randall *et al.,* 1990). Several previous studies have successfully used DNA barcodes for accurately identifying gobiids (Viswambaran *et al*., 2013; Knebelsberger and Thiel, 2014).

Larvae/juvenile dispersal through sea currents could be responsible for extended distribution of this species in Indian waters. The distribution and abundance of *A. phalaena* has been reported to be positively correlated with topography and grain size and negatively correlated with depth and disturbance (Syms and Jones, 2004). Several previous studies have also reported new distributional records of gobiid fishes such as two stripe goby, *Valenciennea helsdingenii* and *Cryptocentrus cyanotaenia* from Gulf of Mannar, India (Kannan *et al.,* 2013; Kumar *et al.,* 2015). Further, extensive ichthyological surveys employing morphological and molecular tools would be required to study its population status. The present study would enrich the present ichthyofaunal diversity database of India and help in fish resource management.

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