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Research Article

Morphology and DNA barcode confirm three new records of gobies (Gobiiformes: Gobiidae) from Bangladesh

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Abstract: This paper deals with three new distributional records of gobies viz. *Amblyeleotris downingi* Randall, 1994, *Psammogobius biocellatus* (Valenciennes, 1837), and *Valenciennea muralis* (Valenciennes, 1837) from Bangladeshi waters in the northernmost part of the Bay of Bengal. The examined specimens are identified and described by morphomeristic characteristics in addition to DNA barcoding based on mitochondrial COI gene. The COI barcode sequence of *Amblyeleotris downingi* is submitted for the first time in the GenBank. In addition, an updated checklist of gobies of the country is also compiled in this paper.

Keywords: First record, Gobiid fish, Saint Martin's Island, Sonadia Island.

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Introduction

The family Gobiidae Cuvier, 1816 is one of the diverse groups of teleost fishes in the world comprising more than 258 genera and 1933 valid species under five subfamilies and one of the most species-rich families of vertebrates (Nelson et al. 2016, Frick et al. 2021). They are distributed worldwide in marine, estuarine and freshwater habitats of tropical and subtropical regions (Gill 1993; Nelson et al. 2016; Thacker and Roje 2011; Patzner et al. 2012; Esmaeili et al. 2017; Schliewen et al. 2018; Eagderi et al. 2019).

Generally, they are benthic, and many of them live on mud or sand and comprises a variety of mutualistic associations on coral reefs, including interactions between gobies and sessile invertebrates such as sponges, hard and soft corals, as well as free-living invertebrates including crustaceans (e.g. shrimps) and echinoderms (e.g. sea urchins) (Myers 1999; Allen et al. 2003; Jonna 2004; Thacker and Roje 2011; Nelson et al. 2016). In the Indo-pacific, coral reef gobies represent approximately 35% of the total fishes and about 25% of the fish diversity (Ackerman and Bellwood 2000; Winterbottom et al. 2011).

The variety of morphology, behavior, and habitat of this family is impressive; and due to their cryptic and secretive nature, they are poorly known and large number of new species are described each year, making them the greatest number of newly described species of the marine family (Thresher 1984; Hoese 1998; Jonna 2004; Nelson et al. 2016; Frick et al. 2021).

Forty-one goby species have been recorded from Bangladeshi waters (Hussain 1970; Ahmed 1991; Tomascik 1997; Rahman 2005; Rahman et al. 2009;

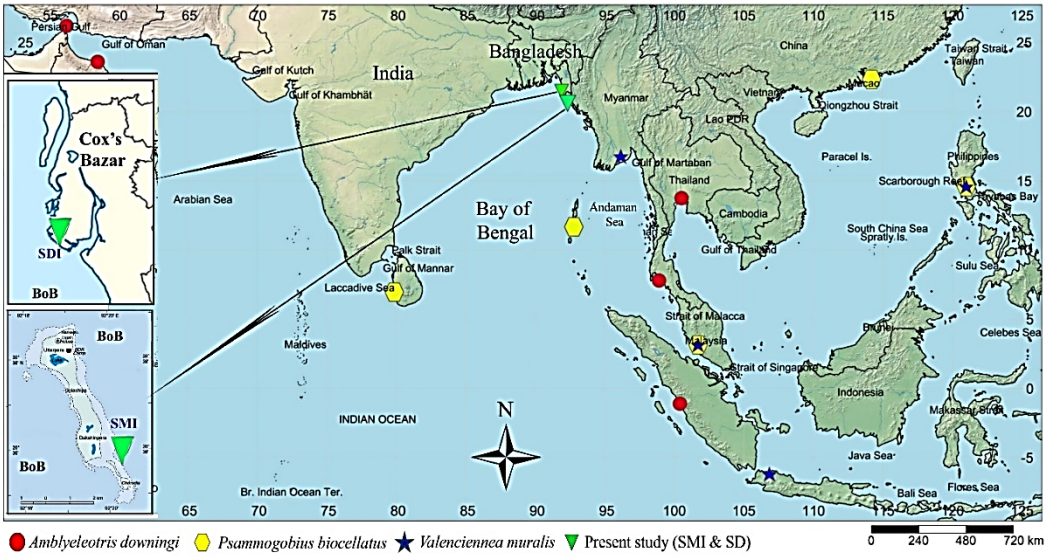

Fig.1. The sampling locations (▼): Saint Martin's Island (SMI) and Sonadia Island (SDI) of present study in Bangladesh; and previously reported locations of occurrence of *Amblyeleotris downingi* (●), *Valenciennea muralis* (★) and *Psammogobius biocellatus* (⬢).

Thompson & Islam 2010; IUCN Bangladesh 2015; Fanning et al. 2019; Ahmed et al. 2020; Habib et al. 2020; Naznin et al. 2020; Habib & Islam 2020; Sharifuzzaman et al. 2021). This study deals with the more three new records of goby species for Bangladesh.

Materials and Methods

Sample collection: A total of 7 specimens were collected from Saint Martin's (St. Martin's) Island and Sonadia Island, Bay of Bengal, Bangladesh (Fig 1). Three specimens were collected from the local fisherman of Saint Martin's Island. This dumbbell-shaped, small, continental island with an area of about 8 sq. km situated in the most northeastern part of the Bay of Bengal. It is located 9km south of the southern tip of the Cox's Bazar-Teknaf peninsula, forming the southeastern end of Bangladesh and it lies between 20°34'–20°38'N and 92°18'–92°21'E (Fig. 1). This is the only island in Bangladesh that supports a coral reef ecosystem. Another four specimens were collected from the local fishermen of Sonadia Island situated in Cox's Bazar district on the southeast coast of Bangladesh lies between 21°16' – 21°19'N and 91°30' – 91°33'E (Fig. 1). The island comprises a wide variety of wetland habitats including mudflats, sand dunes, mangroves, sand bars, lagoons, saltpans and beaches. The collected specimens were then transferred and preserved at Aquatic Bioresource Research Laboratory (ABR Lab.), Sher-e-Bangla Agricultural University (SAU) in Dhaka, Bangladesh.

Morphological study: Morphological study was conducted by following Talwar & Kacker (1984); Hoese (1986); Hoese & Larson (1994); Larson & Murdy (2001); Rahman et al. (2009). All measurements were taken with a Vernier caliper to 0.01mm. All of the measurements and counts are given in tables 1 and 2.

Genetic analysis: Genomic DNA was extracted from the collected muscle tissue using a Qiagen DNeasy Blood and Tissue Kit following the manufacturer's protocol. The partial fragment of the mitochondrial (mtDNA) cytochrome c oxidase subunit I (COI) gene was amplified with the primers FishF2 (5'-TCGACTAATCATAAAGATATCGGC AC-3') and FishR2 (5'ACTTCAGGGTGACCGAA GAATCAGAA-3') (Ward et al. 2005). Polymerase chain reaction (PCR) was performed in a 50μl reaction mixture in 0.2-ml small reaction tubes in a

Table 1. Meristic counts of the three new records of goby, *Amblyeleotris downingi*, *Psammogobius biocellatus* and *Valenciennea muralis* collected in the present study and comparison with reference data (n= number of individual).

Characters	*V. Muralis*		*A. downingi*		*P. biocellatus*	
	Present study (n=2)	Allen and Erdmann 2012	Present study (n=1)	Randaall 1995	Present study (n=4)	Hoese 1986
1st dorsal-fin spines	VI	VI	VI	VI	VI	VI
2nd dorsal-fin spines	I	I	I	I	I	I
2nd dorsal-fin soft rays	12	12	17	16	9	9
Pectoral-fin soft rays	19		20	18–20	17–18	17–19
Pelvic-fin spines	I	I	I	I	I	I
Pelvic-fin soft rays	5	5	5	5	5	5
Anal-fin spines	I	I	I	I	I	I
Anal-fin soft rays	12	12	17	17	8	8

thermal cycler (2720 Thermal Cycler, Applied Biosystems). The PCR condition profile consisted of a preheating at 95°C for 2 min followed by 35 cycles of denaturation at 94°C for 40s, annealing at 54°C for 40s, extension at 72°C for 1min, and a final extension at 72°C for 10 min. PCR samples with a single and clear visible band were purified with the QIAquick PCR Purification Kit (Qiagen). Sequencing was conducted with the same PCR primers by the Sanger standard method with automated sequencing (ABI 3730x1 DNA analyzer) at Macrogen Inc. (Korea). Nucleotide sequences were edited and aligned using the bioinformatics software MEGA-7 (Sudhir et al. 2016). The obtained COI Sequences were checked using BLAST search engine provided by National Center for Biotechnology Information (NCBI) and bold database. Finally, the consensus sequences obtained from collected specimens through DNA sequencing were submitted to GenBank.

Phylogenetic analysis was performed using Maximum likelihood (ML) methods through IQ Tree (Nguyen et al. 2015; Trifinopoulos et al. 2016) with bootstrap analysis of 10000 replications. ML tree was visualized using Figtree v1.4.3 and edited by Adobe Illustrator. We used the evolutionary model TPM2u+F+G4 in the phylogenetic analysis obtained as the best-fit model using the program Modelfinder (Kalyaanamoorthy et al. 2017). This model was selected by applying the Bayesian information criterion. Two sequences of gobies (Microdesmidae), *Gunnellichthys monostigma* Smith, 1958 and *G. curiosus* Dawson, 1968 retrieved from GenBank were used as outgroups. Kimura-2 parameter (K2P) distance model (Kimura 1980) was used for calculating the genetic distance among the sequences using MEGA-7. Nucleotide and haplotype diversity and polymorphic sites were analyzed by DNASP (Librado & Rozas 2009).

Results

We have identified three species of gobies i.e., *Amblyeleotris downingi* Randall, 1994, *Psammogobius biocellatus* (Valenciennes, 1837), and *Valenciennea muralis* (Valenciennes, 1837), and diagnosed characters are given hereunder.

Amblyeleotris downingi Randall, 1994

Downing's shrimpgoby (Fig. 2A)

Material examined: Specimen collected from Bangladesh: Bay of Bengal, Cox`s Bazar, Saint Martin's Island. Coordinate 20°36'39.6"N, 92°19'37.2"E (Fig. 1), collected by Md Jayedul Islam. One specimen; specimen voucher no. F1803SM-54 (SL 98 mm). Collection date: 27.III.2018 (GenBank accession no. MK340584).

Diagnostic characters: Body elongates, whitish in color; four pale broad dark brown bars at sides of the body; irregular dusky brown blotch at operculum; a dusky spot above the dorsal end of gill opening; a pale dusky spot on first dorsal fin. Lower jaw slightly

Table 2. Morphometric measurements of three new records of gobies, *Amblyeleotris downingi*, *Psammogobius biocellatus* and *Valenciennea muralis* collected in the present study.

Characters	***V. muralis*** **(n=2)**	***A. downingi*** **(n=1)**	***P. biocellatus*** **(n=4)**
Total length	115.00	133.00	69.00–74.00
Standard length	80.0—89.00	98.00	59.00–62.00
	Proportion of Standard length		
Body width	12.85–13.48	9.18	16.95–19.35
Body depth	15.71v16.85	13.27	16.95–19.67
Head length	28.57–29.21	25.51	1.8–1.9
Inter orbital wide	5.61–5.71	2.04	1.61–1.69
Pre orbital length	11.42–12.35	7.14	3.23–5.08
Post orbital length	14.28–15.73	14.29	12.90–15.25
Eye diameter	7.14–7.86	7.14	1.61–1.69
Caudal peduncle length	22.47–24.28	13.27	22.58–25.42
1st dorsal-fin base length	21.34–22.85	20.41	16.95–19.35
1st dorsal-fin length	24.71–2.71	21.43	22.58–24.59
2nd dorsal-fin base length	29.21–28.57	37.76	16.13–18.03
2nd dorsal-fin length	20.22–28.57	20.41	23.73–24.59
Pectoral-fin base length	10–11.23	7.14	8.06–9.84
Pectoral-fin length	22.47–23.71	21.43	27.42–28.81
Pelvic-fin base length	2.85–3.37	4.08	6.45–8.20
Pelvic-fin length	17.97–17.14	22.45	22.58–27.87
Anal-fin base length	24.71–25.71	32.65	20.97–22.95
Anal-fin length	17.97–17.14	16.33	19.35–21.31
Caudal-fin base length	12.35–11.42	8.16	9.68–11.48
Caudal-fin length	34.83–37	36.73	29.03–31.15

protruding beyond the upper jaw; gill opening extending anteriorly to the posterior margin of preopercle. Medially united pelvic fins with frenum; caudal fin moderately long. Median predorsal scales absent.

Distribution: *Amblyeleotris downingi* is known to occurs from Red Sea (e.g. Oman, Saudi Arabia, Bahrain etc.), Thailand, Malaysia, Indonesia (western Sumatra), and Andaman and Nicobar Islands, and Reef Mentawai Islands (Randall 1995; Allen & Erdmann 2012; Rajan et al. 2013; Froese & Pauly 2020; Frick et al. 2021). This study confirms the presence of this species from Andaman Island and Red sea to the northern Bay of Bengal.

Psammogobius biocellatus Valenciennes, 1837
Sleepy goby (Fig. 2B)

Material examined: Specimen collected from Bangladesh: Bay of Bengal, Cox`s Bazar, Sonadia Island. Coordinate 21°28'50.6"N, 91°53'15.3"E (Fig. 1), collected by Md Jayedul Islam. Four specimens; specimen voucher no. F1803ME-54 (SL 56mm), F1803ME-53 (SL-59mm), F1803ME-52 (SL 61mm), F1803ME-51 (SL 62mm). Collection date: 27.III.2018 (GenBank accession no. MN703106, MN703107, MN703108 and MN703109, respectively).

Diagnostic characters: Body moderately elongate, dark brown in color with indistinct black mottles and lines. Head depressed; lower jaw well protruding beyond upper jaw; small lappet over iris; gill opening extending well anteriorly to the preopercular margin. Paired fins dark gray in color; 3–4 white line on dorsal and anal fin. Pelvic fins united with frenum.

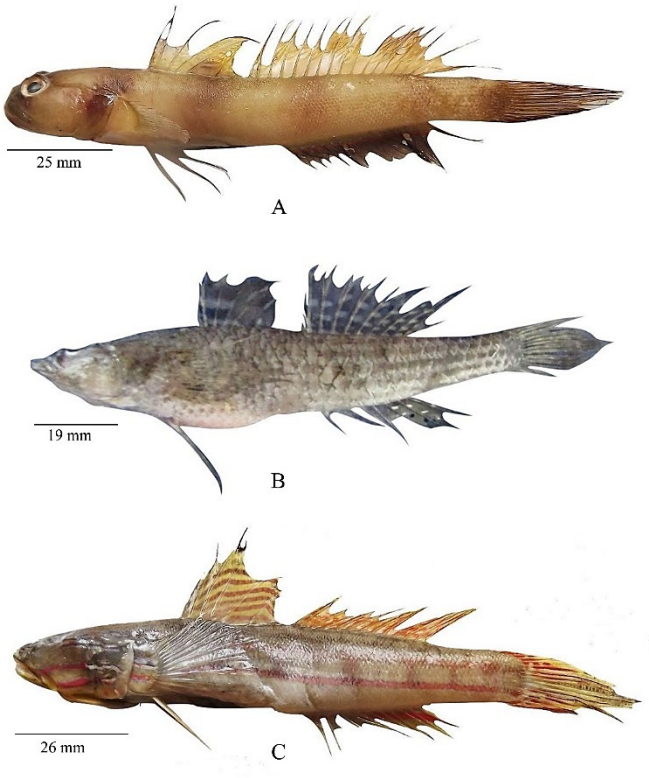

Fig.2. A. *Amblyeleotris downingi* (SL 98mm), B. *Psammogobius biocellatus* (SL 61mm), C. *Valenciennea muralis* (SL 89mm).

Distribution: *Psammogobius biocellatus* known to occur from Indo-Pacific: south to East London, South Africa, Western Central Pacific: Guam Madagascar Australia, Vietnam, Philippines, Japan, Taiwan, Fiji, Malaysia, Thailand, Srilanka, Andaman and Nicobar island (Kami 1975; Hoese 1986; Talwar & Jhingran 1991; Stiassny & Raminosoa 1994; Rajan et al. 2011; Froese & Pauly 2020; Frick et al. 2021). This study confirms the range expansion of this species from Andaman and Nicobar Island to the northern Bay of Bengal.

Valenciennea muralis Valenciennes, 1837
Mural Goby (Fig. 2C)

Material examined: Specimen collected from Bangladesh: Bay of Bengal, Cox`s Bazar, Saint Martin's Island. Coordinate 20°36'39.6"N, 92°19'37.2"E (Fig. 1), collected by Md Jayedul Islam. Two specimens; specimen voucher no. F1707SM-03 (SL 80mm), F1803SM-23 (89mm). Collection date: 15.VII.2017 and 12.III.2018 respectively (GenBank accession no. MK340745 and MK340746, respectively).

Diagnostic characters. Body moderately elongate, pale gray in color, with 4–5 longitudinal narrow pink stripes; lips yellowish; 3 blue-bordered red longitudinal stripes on the head slightly extends to the body, red basal stripes on the dorsal and anal fins, black spot at the distal part of the first dorsal fin just behind third spine. Gill opening relatively narrow restricted to the pectoral fin base. Third dorsal-fin spine slightly elongate, forming pointed fin; pelvic fins separated and frenum absent; caudal fin rounded, longer than head. Head and base of the pectoral fin without scales.

Distribution: *Valenciennea muralis* are known to occur from India, Myanmar (Yangon River), Thailand, Andaman Sea and Indonesia east to New Ireland (Papua New Guinea) and Solomon Islands, north to Hong Kong (China), south to northern Australia (Myers 1991; Hoese & Larson 1994; Ni & Kwok 1999; Werner & Allen 2000; Froese & Pauly 2020; Frick et al. 2021). This study confirms the range expansion of this species from Yangoon, Myanmer to the northern Bay of Bengal, Bangladesh.

Genetic description: We have successfully barcoded all of the three new records of goby species viz. *A. downingi, P. biocellatus, V. muralis* and submitted to GeneBank (NCBI) and BOLD system. Moreover, the COI sequence of *A. downingi* was submitted for the first time to GenBank as reference DNA barcode sequence. We got 7 COI barcode sequences of three species. Sequence alignment of COI gene yielded 551 nucleotide base pairs after removing the ambiguous sequences near primer ends. The COI sequences obtained from 3 species comprised 7 haplotypes with 129 polymorphic sites. The sequence analysis revealed that the average nucleotide compositions in seven COI sequences of three species were A=24±1%, T=28.2±0.7%, C=29.3 ±1.2%, G=18.5±0.5%. The overall GC content was 47.8%. The nucleotide diversity was calculated as

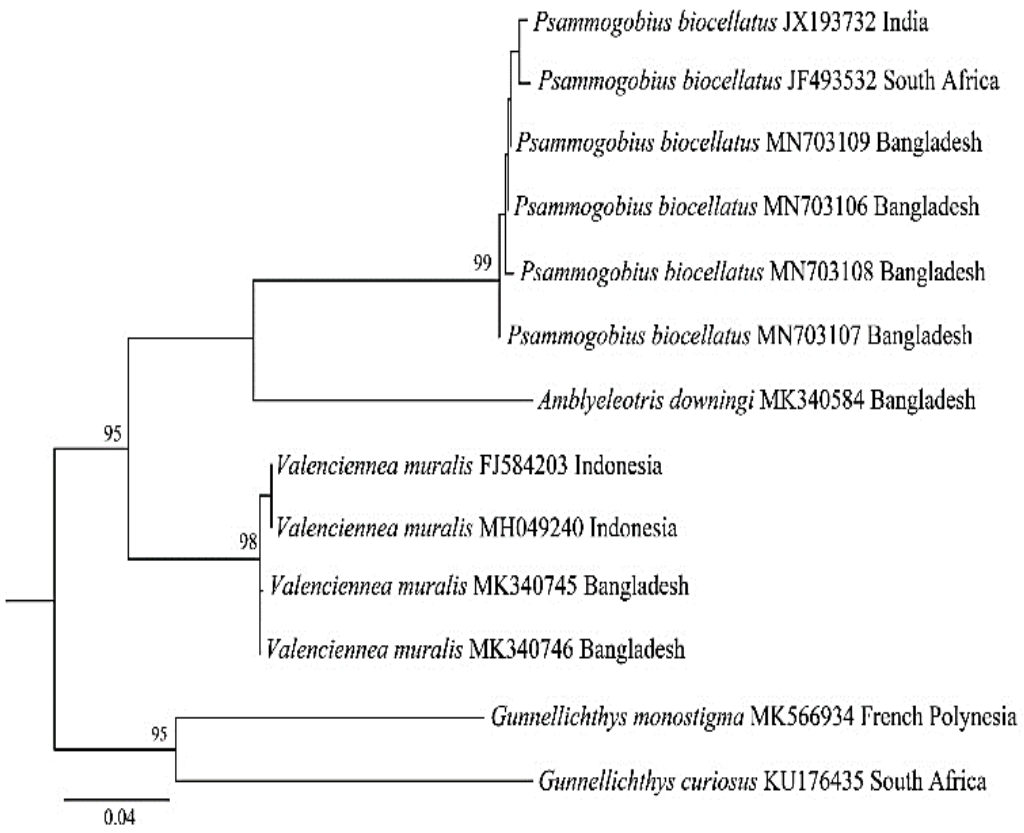

Fig.2. Maximum likelihood tree constructed based on mitochondrial COI sequences. Bootstrap support of >90% are shown above branches. Scale bar indicates nucleotide substitutions per site.

0.11 and the haplotype diversity was 1±0.01 for the seven sequences obtained in the present study. Among the three goby species of the present study, the highest pairwise genetic distance was found as 20.5% between *V. muralis* and *P. biocellatus*. The overall genetic distance was 13.6% among the studied sequences of COI gene. In the phylogeny, we used all of the seven COI sequences of three species obtained in the present study and four other sequences of *V. muralis* and *P. biocellatus* retrieved from GenBank. The phylogenetic tree showed three clades, each belonging to the separate species (Fig. 3). Four COI sequences of *P. biocellatus* of Bangladesh obtained in this study formed a single clade with the sequences from India and South Africa with 99 bootstrap value. Genetic distances between the sequences of Bangladesh and each of the sequence of India and South Africa are 1.2-2.2% and 1.4-2.2%**,** respectively. Likewise, two sequences of *V. muralis* of this study made a single clade with the sequences of Indonesia, and the genetic distance between the sequences of the two countries were found 0.8 to 1.0%. No conspecific sequence of *A. downingi* was found in GenBank for comparison. However, the COI sequence of this species clearly formed separate clades from other species of goby in the ML tree.

Discussion

Most of the species of gobiids of Bangladesh were found in marine and brackish waters environment. Among 41 species found in Bangladesh waters, only seven species were found in brackish and freshwater (Table 3) (Hussain 1970; Ahmed 1991; Tomascik 1997; Rahman 2005; Rahman et al. 2009; Thompson & Islam 2010; IUCN Bangladesh 2015; Fanning et al. 2019; Ahmed et al. 2020; Habib et al. 2020; Naznin et al. 2020; Sharifuzzaman et al. 2021). In the present study, we have identified three species of goby viz., *V. muralis, A. downingi,* and *P. biocellatus*

Table 3. List of goby fish species found in Bangladesh.

	Species	Literatures consulted	Habitat
1	*Acentrogobius caninus* (Valenciennes, 1837)	Rahman 2005, Ahmed 1991	M, B, F
2	*Acentrogobius cyanomos* (Bleeker, 1849)	Ahmed 1991, Rahman 2005, Habib and Islam 2020	M, B
3	*Acentrogobius nebulosus* (Forsskål, 1775)	Sharifuzzaman et al. 2021	M, B, R
4	*Acentrogobius viridipunctatus* (Valenciennes, 1837)	Rahman 2005, Habib and Islam 2020	M, B, F
5	*Amblyeleotris downingi* Randall, 1994	Present study	M
6	*Amblyeleotris steinitzi* (Klausewitz, 1974)	Thompson and Islam 2010, Habib and Islam 2020	M
7	*Apocryptes bato* (Hamilton, 1822)	Ahmed 1991, Hussain 1970, Rahman 2005, Habib and Islam 2020	M, B, F
8	*Awaous grammepomus* (Bleeker, 1849)	Rahman 2005	B, F
9	*Awaous guamensis* (Valenciennes, 1837)	Rahman 2005, Habib and Islam 2020	M, B, F
10	*Bathygobius coalitus* (Bennett, 1832)	Sharifuzzaman et al. 2021	M
11	*Bathygobius curacao* (Metzelaar, 1919)	Sharifuzzaman et al. 2021	M
12	*Boleophthalmus boddarti* (Pallas, 1770)	Hussain 1970, Ahmed 1991, Habib et al. 2018, Habib and Islam 2020	M, B, F
13	*Boleophthalmus dussumieri* Valenciennes, 1837	IUCN Bangladesh 2015, Fanning et al. 2019, Habib and Islam 2020	M, B, F
14	*Brachygobius nunus* (Hamilton, 1822)	Ahmed 1991, Rahman 2005	B, F
15	*Callogobius hasseltii* (Bleeker, 1851)	Sharifuzzaman et al. 2021	M, B, F
16	*Cryptocentrus cinctus* (Herre, 1936)	Thompson and Islam 2010, Habib and Islam 2020	M
17	*Cryptocentrus cyanotaenia* (Bleeker, 1853)	Naznin et al. 2020	M
18	*Exyrias puntang* (Bleeker, 1851)	Ahmed 1991, Ahmed et al. 2020	M, B
19	*Favonigobius gymnauchen* (Bleeker, 1860)	Ahmed et al. 2020	M, B, F
20	*Glossogobius giuris* (Hamilton, 1822)	Hussain 1970, Rahman 2005, Habib et al. 2018, Habib and Islam 2020	M, B, F
21	*Gobiopsis macrostoma* Steindachner, 1861	Ahmed 1991, Rahman 2005, Habib and Islam 2020	M, B, F
22	*Gobiopterus chuno* (Hamilton, 1822)	Ahmed 1991, Rahman 2005	B, F
23	*Gobiopterus chuno* (Hamilton 1822).	Ahmed 1991	B, F
24	*Istigobius decoratus* (Herre, 1927)	Sharifuzzaman et al. 2021	M
25	*Istigobius diadema* (Steindachner, 1876)	Present study	M, B, F
26	*Istigobius ornatus* (Rüppell, 1830)	Sharifuzzaman et al. 2021	M, B
27	*Odontamblyopus rubicundus* (Hamilton, 1822)	Hussain 1970, Habib and Islam 2020	M, B
28	*Oligolepis acutipennis (*Valenciennes, 1837)	Ahmed et al. 2020	M, B, F
29	*Oxyurichthys microlepis* (Bleeker, 1849)	Ahmed 1991, Rahman 2005	M, B
30	*Oxyurichthys papuensis* (Valenciennes, 1837)	Fanning et al. 2019	M, B
31	*Oxyurichthys petersii* (Klunzinger, 1871)	Fanning et al. 2019	M
32	*Periophthalmodon schlosseri* (Pallas, 1770)	Ahmed 1991, Rahman 2005, Habib and Islam 2020	M, B, F
33	*Periophthalmus barbarus* (Linnaeus, 1766)	Rahman et al. 2009, Habib and Islam 2020	M, B, F
34	*Pseudapocryptes elongatus* (Cuvier, 1816)	Rahman 2005	B, F
35	*Pseudogobiopsis oligactis* (Bleeker, 1875)	Ahmed 1991, Rahman 2005	B, F
36	*Scartelaos histophorus* (Valenciennes, 1837)	Ahmed 1991, Rahman 2005, Habib and Islam 2020	M, B
37	*Stigmatogobius sadanundio* (Hamilton, 1822)	Ahmed 1991, Rahman 2005	B, F
38	*Taenioides anguillaris* (Linnaeus, 1758)	Rahman et al. 2009, Habib and Islam 2020	M, B, F
39	*Taenioides buchanani* (Day, 1873)	Hussain 1970, Habib and Islam 2020	M, B
40	*Taenioides cirratus* (Blyth, 1860)	IUCN Bangladesh 2015, Habib and Islam 2020	M, B, F
41	*Tridentiger barbatus (Günther, 1861)*	Ahmed et al. 2020	B
42	*Trypauchen vagina* (Bloch & Schneider, 1801)	Rahman et al. 2009, Habib and Islam 2020	M, B
43	*Valenciennea muralis* (Valenciennes, 1837)	Present study	M
44	*Zappa confluentus* (Roberts, 1978)	Bernacsek 2001, Habib and Islam 2020	M, B, F

M= Marine water, B= Brackish water, F= Freshwater

confirmed by morphology and DNA barcoding those were not previously recorded from Bangladesh waters. Sourcing from different valid reports including present study, we have updated the checklist of gobies of Bangladesh (Table 3) which indicates that 44 species of gobies from 29 genera

have been reported in the country until now. Reporting of the three new records viz. *A. downingi*, *P. biocellatus* and *V. muralisfrom* Bangladesh in the present study confirms range extension of distribution of these species in the northern Bay of Bengal. The study also indicates that more fish species of the family Gobiidae might be found in Bangladesh if more extensive study is conducted.

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مقاله پژوهشی

ریخت‌سنجی و DNA بارکدینگ تایید کننده سه گونه گاو ماهی (گاوماهی شکلان: گاوماهیان) گزارش شده از بنگلادش

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چکیده: در این مقاله سه گزارش جدید از پراکنش گونه‌های *Amblyeleotris downingi* Randall, 1994، *Psammogobius biocellatus* (Valenciennes, 1837) و *Valenciennea muralis* (Valenciennes, 1837) از آب‌های بنگلادش در شمالی‌ترین قسمت خلیج بنگال ارائه می‌دهد. نمونه‌های بررسی شده علاوه بر DNA بارکدینگ بر اساس ژن COI، با ویژگی‌های مورفومریستیک شناسایی و توصیف می‌شوند. توالی بارکد COI، گونه *A. downingi* برای اولین بار در بانک ژن ارائه می‌شود. علاوه بر این، یک چک لیست به روز شده از گاوماهیان کشور نیز در این مقاله گردآوری شده است.

کلمات‌کلیدی: اولین گزارش، گاوماهیان، جزیره سنت مارتین، جزیره سونادیا.