Bangladesh J. Zool. **48**(2): 253-262, 2020 ISSN: 0304-9027 (print)
2408-8455 (online)

TWELVE NEW RECORDS OF CORAL ASSOCIATED FISH FROM THE SAINT MARTIN'S ISLAND BASED ON MORPHOMETRIC AND MOLECULAR APPROACHES

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Abstract: The study was conducted to know the diversity of coral associated fishes of the Saint Martin Island from December 2017 to April 2019. A total of 46 coral associated marine fish species were recorded, among them 12 species; *guilcheri Lutjanus, Lutjanus russellii, Parupeneus indicus, Siganus sutor, Thalassoma lunare, Gazza achlamys, Pomadasys andamanensis, Pomadasys kaakan, Lethrinus crocineus, Argyrosomus regius, Istigobius ornatus and Cryptocentrus cyanotaenia* under six family were newly recorded from this Island. We assume that there are more species to be explored and need proper management strategy for conservation of coral reef and its associated biodiversity.

***Key words*:** New records, morphology, DNA barcode, COI gene.

INTRODUCTION

The St. Martin's Island is a small island in the northeastern part of the Bay of Bengal, about 9 km south of the tip of the main land of Teknaf peninsula and forming the southernmost part of Bangladesh. This is the only island in Bangladesh which has the coral reefs, are described as being situated along the north-west, west and south-west coast of the island. Enormous areas of sand ridge, some mangrove formations, pandanus vegetation and scattered boulder/dead corals are the major characteristics of this island. The main attraction for any coral island is their different, varieties of multicolor ornamental fishes. The first checklist of the fishes of St. Martine Island (Tomascik 1997) included 86 species belonging to 34 families from coastal and adjacent waters. Shafi and Quddus (2003) reported 14 species belonging to 11 families. Hossain and Islam (2006) mentioned 98 species were coral associated. Rahman *et al.* (2009) compiled 147 species belonging to 50 families. Sarker *et al.* (2015) found 26 species belonging to 11 families in short survey. It is evident

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 https://doi.org/ 10.3329/bjz.v48i2.52367

that most of the published reports on ichthyofauna of this island is inconsistent. Ahmed et al. (2020) described the molecular characterization of 185 species of marine and coastal fishes of Bangladesh based on mitochondrial Cytochrome C Oxidase subunit I gene (COI). They have reported 21 species of new records. But no in-depth survey was undertaken on fish diversity, especially associated with coral reef habitat. This study describes twelve coral associated fish species based on morphometric and molecular approaches.

MATERIAL AND METHODS

Sample collection and preservation: The study was conducted at the Saint Martin's Island (20°34′ N - 20°38.8′ N and 92° 18′ E - 92° 20.8′ E) from December, 2017 to April, 2019. Fish samples were collected from local fish market and landing station of Saint Martin's Island. Local fishermen caught coral associated fish using gill net, ber jal and hooks and lines. After collecting fish sample, taken photograph to get the original color pattern of fresh fish samples. Then the specimens were preserved in ice cool box till transfer at -20 ^{o}C in fisheries lab, Department of Zoology, Jagannath University till further morphometric and meristic analysis.

Taxonomic identification: Identification of all collected individual samples was performed following the published reference materials (Rahman *et al.* 2009, Allen 1985, Heemstra and Randall 1993, Humann 1994). All possible morphometric and meristic characteristics were carefully observed, measures and counted.

Molecular identification: Immediately after collecting the specimens, tissue samples were excised and stored in 90% ethanol. Voucher specimens were fixed with 10% formalin and then transferred to 70% ethanol solution for preservation. Voucher specimens were carried and deposited in the Dhaka University Zoology Museum (DUZM).

DNA barcoding: Genomic DNA was extracted from the muscle tissue samples by the standard Proteinase-K/Phenol-Chloroform-isoamyl alcohol method (Green and Sambrook 2012, Sambrook and Russell 2001). The quality and quantity of the extracted DNA was measured using Nanodrop™ spectrophotometer. Approximately 658bp was amplified from the 5′ region of the MT-COI gene using the following primers: FishF2 5′TCGACTAATCATAAAGATATCGG CAC3′ and FishR2 5′ACTTCAGGGTGACCGAAG AATCAGAA3′ (Ward *et al.*, 2005). For this, 25 µl PCR reaction mixtures were prepared which included 17.25–18.75 µl of ultrapure water, 2.5 µl of 10× PCR buffer, 1.25 µl of $MgCl_2$ (50 mM), 0.25 µl of

each primer (0.01 mM), 0.125 µl of each dNTP (0.05 mM), 1 µl (0.625 U) of Taq polymerase, and 0.5–2.0 µl of DNA template. Amplifications were performed using ABI thermal cycler (ThermoFisher Scientific). The thermal regime consists of an initial step of 2 min at 95 °C followed by 35 cycles of 0.5 min at 94 °C, 0.5 min at 54 °C, and 1 min at 72 °C, followed in turn by 10 min at 72 °C and then held at 4 °C. PCR products were visualized on 1% agarose gel. The PCR products were purified using PureLink™ PCR purification kit and sequenced from First BASE Laboratories, Malaysia. All sequences were translated into amino acids to confirm the effectiveness of the sequences and to detect the presence of nuclear DNA pseudogenes, insertions, deletions, or stop codons. Sequences were checked and aligned using Sequencher v5.4.6 and were submitted to GenBank with referred accession numbers. All the data including taxonomic characteristics and accession numbers of GenBank were tagged with the voucher specimens preserved at the museum of Department of Zoology, University of Dhaka.

RESULTS AND DISCUSSION

In the present study, a total of 160 individuals were collected comprising of 46 species (41 upto species level and another 5 upto genus level) of coral associated fish belonging to 33 genera 26 families and 6 orders. Among 6 orders, Perciformes was dominant, which belongs to 20 families, 25 genera and 38 species. Based on morphometric and molecular characters 12 species under 10 genera were confirmed as new records from Bangladesh. Key identifying morphometric characteristics are briefly described below.

***Lutjanus guilcheri* Fourmanoir, 1959 (Plate A: 1)**

Identifying characteristics: Dorsal profile of head steeply sloped. Pectoral fin is yellowish. **Color:** Body Color is red or reddish-orange. Dorsal fin is reddish, and also tip of the dorsal fin is deeply reddish. Pectoral fin is yellowish. Pelvic and anal fins are also reddish. Fin formula is D X/14; P 17; V I/6; A III/9; C 20.

***Lutjanus russellii* (Bleeker, 1849) (Plate A: 2)**

Identifying characteristics: A black spot which is sometimes faint is on the lateral line below the anterior portions of the soft dorsal fin. Seven yellow horizontally curved stripes present on the body. **Color:** Generally, body whitish with silvery sheen. Head is silvery color. Seven yellow stripes present on the body. Pectoral and pelvic fin is whitish. Anal fin is yellowish in color. Caudal fin is blackish. Fin formula is D X/14; P 17; V I/5; A III/8; C 18.

***Parupeneus indicus* (Shaw, 1803) (Plate A: 3)**

Identifying characteristics: Horizontally elongate yellow spot (sometimes partly white) on lateral line below inter-dorsal space. Round black spot as large as or

larger than eye on side of caudal peduncle, two-thirds of which lies above the lateral line. Color: Body reddish browns dorsally, the scale edges narrowly dark, shading to whitish or pale pink ventrally. Dorsal fin is light greenish to pinkish. Pectoral, pelvic and anal fin is pinkish. Fin formula is D1 VIII; D2 9; P 16; V I/5; A I/7; C 21.

***Siganus sutor* (Valenciennes, 1835) (Plate A: 4)**

Identifying characteristics: Many rounded white spot present on the body. Forward-projecting spine is present. Color: After preservation, body deep blackish dorsally and light is whitish ventrally. Dorsal and anal fin is blackish. Pectoral and pelvic fin is light yellowish. Caudal fin is blackish with base light yellowish. Fin formula is D XIII/10; P 16; V II/3; A VII/9; C 18.

***Thalassoma lunare* (**Linnaeus**, 1758) (Plate A: 5)**

Identifying characteristics*:* Head green to blue with irregular pink to violet bands. Blue pectoral fins with a large elongate pink area distally. Large yellow crescent posteriorly is present in caudal fin. Color: Body dark green to blue with vertical red to purplish red lines. Head green to blue with irregular pink to violet band. Dorsal fin is bluish. Pectoral fin is blue with a large elongate pink area. Pelvic and anal fins were greenish to bluish. Yellow crescentic posteriorly present in caudal fin. Fin formula is D VIII/13; P 17; V I/5; A III/11; C 19.

***Gazza achlamys* Jordan & Starks, 1917 (Plate A : 6)**

Identifying characteristics: With distinct canine teeth in both jaws. Mouth is pointing forward when protracted. Scales present on the body, absent anterior to a line from origin of soft dorsal fin to behind pectoral fin bases. Color: After preservation, body surface in general silvery color, blackish dorsally and ventral silvery. Head color including nostril and above the upper lips light yellowish. Two lips color light is yellowish. Silvery is operculum. Dorsal fin is whitish, tip of spine blackish irregular marks. Membrane of spinous dorsal fin is black at its distal portion. Black dots are present all over ventral half of the body and head. Pectoral and pelvic fin is colorless. Anal fin yellow anteriorly. Caudal fin base is yellowish. Fin formula is D VIII/16; P 16; V I/5; A III/14; C 27.

***Pomadasys andamanensis* McKay and Satapoomin, 1994 (Plate A: 7)**

Identifying characteristics: Four blackish horizontal stripes are present on the back and flanks. Soft anal fin is blackish. Color: Generally, body light silver dorsally and whitish ventrally. Head is blackish in color. Among four blackish body stripes, 1st stripe starts from slightly above the origin of spinous dorsal and join with the starting base of soft dorsal; 2nd stripe starts just below the first one and reaches upto the anterior portion of soft dorsal; 3rd stripe starts from the nape and reaches upto the last basal portion of soft dorsal fin; 4th

stripe starts from just behind the eye, run along the eye and reaches upto the base of caudal fin. All the fins are blackish without pectoral fin, which is brownish in color. Fin formula is D XII/16; P 17; V I/5; A III/9; C 20.

***Pomadasys kaakan* (Cuvier, 1830) (Plate A: 8)**

Identifying characteristics: Spots and blotches on the dorsal fin and the bands of double spots or blotches on the body. Large individuals may lose the body spots. Color: The body is silver dorsally and whitish ventrally with a golden tint on the sides. It has seven pairs of dark spots arranged into vertical interrupted bars on the upper sides The head is olive yellowish and the snout is blackish. The spinous part of the dorsal fin is white with a black membrane tip. Two rows of black spots are found in the inter-membranous portion of the 3rd spine to the 10th spine, and one black spot between the 10th and 11th spine. The soft part is white and bordered with black. The pectoral fin is brownish, and the basal 1/3 portion is reddish. The pelvic and anal fins are whitish. The caudal fin is whitish at the basal half and blackish at the rest half toward the tip. Fin formula is D1 XII/14; P 16; V I/5; A III/8; C 17.

***Lethrinus crocineus* Smith, 1959 (Plate A: 9)**

Identifying characteristics: Pectoral fin is yellowish. Dorsal and caudal fin's tip reddish. **Color:** Body light blackish dorsally, whitish ventrally. Head is greenish. Dorsal fin, anal fin and caudal fin are reddish. Pectoral fin is yellowish. Fin formula is D X/; P 13; V I/5; A III/9; C 19.

***Argyrosomus regius* (Asso, 1801) (Plate A: 10)**

Identifying characteristics: Rough scales present. Mouth is protractile with caniniform teeth. Color: In general, body and all the fins blackish in color. Fin formula is D X/29; P 19; V I/5; A II/8; C 17.

***Istigobius ornatus* (Rüppell, 1830) (Plate A: 11)**

Identifying characteristics: Five vertical rows of white spots present on pectoral fins. Blue spot present on the body and a series of black bar mid of the body. Color: Body color pale gray; operculum with 5 small blue spots interspersed with brownish red spots; 5 vertical rows of white spots on pectoral fins; anterior tip of first dorsal fin bright yellow. Fin formula is D VII/11; P 19; V 6; A I/9; C 12.

***Cryptocentrus cyanotaenia* (Bleeker, 1853) (Plate A: 12)**

Identifying characteristics: Eleven blue band on the body and small numerous blue line on head. 4 brownish stripes present on the anal fin. **Color:** Body brownish grey color with 11 vertical narrow whitish blue lines on the sides; largely greenish yellow on head and mandible; head and base of pectoral fin with numerous short blue oblique broken lines and spots with markings on the head

Table 1: Morphometric characteristics of newly recorded coral associated fish collected from Saint Martin's Island area, Bangladesh (in cm).

Scientific Name	TL	SL	FL	BW	BD	HL	PDL	PPL	PVL	PAL	IOL	PrOL	PoPL	ED	CPL	CPD	CC	UJL	LJL	JG	DBL	D_1BL	D_2BL	LDS	PBL	LPrL	VBL	LVrL	ABL	LAS	CBL
L. guilcheri	35.5	28.3		5.6	12	11.4					2.2	3.7	5.8	1.9	3.8	4				3.8	14.7			A	1.9		1.9		4.9		3.9
L. russellii	18.1	14.5		3.6	8.2	9.3	8.6	7.7	8.7	15.5	1.4	3.3	4.5	1.6	2.7	2.8	1.1	3.6	3.6	3.5	11.2				1.5		1.3		3.6		3
P. indicus	23.2	18.5		1.3	2.1	2.5								0.8								1.7	1.4		0.5		0.4		1		1.1
S. sutor	15.7	12.1	14.5	1.5	5	3.4	3.4	3.1	4.1	6.8	1	1.2	1.1	1.1	0.8	0.7	1.2	0.9	0.8	1.3	9.2			2	1	2.6	1.4	1.8	5.5	1.8	0.9
T. lunare	9.8	8				2.8								0.5							5				0.6		0.3		3.1		1
G. achlamys	13.1	9.9	11.6	1.6	5.5	3.6	5.1	3.5	4.3	6	1	1.1	1.3	1.2	0.5	0.7	1.1	1.1	0.9	1.4	5.9			2.2	0.7	2.1	0.3	1.2	4.9	2.2	0.8
P. andamanensis	13	11.5		3	8.3	6.4	8.7	6.4	7.3	14	1.9	2.1	3	1.7	2.3	2.5	1.2	1.9	1.9	1.8	11				1.2		1.3		3.2		2.8
P. kaakan	18.3	14.5		86.2	45.5	44.1	49.7	42.8	47.6	80.7	17.2	25		21.9	16.6	11.7	3.44	25	26.6	29.7	55.2			22.8	6.2	32.4	4.83	24.1	15.2		13.1
L. crocineus	25.5	20		3.2	9.6	7.7	9.8	7.5	8.2	13.3	2	3.6	3.1	1.8	3.4	3.1	1.7	3.1	3.1	2.2	11.1			3	1.5	7.4	1.4	5.4	4.2	2.5	2.8
A. regius	27.1	21.5		3	7.6	7.8	8.6	7.3	7.6	15.1	1.6	2	4.5	1.3	1.8	2.1		3	2.8	2.6	13.8			3.3	1.9	5.1	1.3	6.3	2.3	3.7	2.3
I. ornatus	9	7.5				1.4								0.3							0.7				1		0.3		0.6		0.6
C. cyanotaenia																															

Here, TL=Total length, SL=Standard length, FL= Fork length, BW= Body width, BD= Body depth, HL= Head length, Pre-dorsal length (PDL), Pre-pectoral length (PPL), Pre-pelvic length (PVL), Pre-anal length (PAL), Inter-orbital length (IOL), Pre-orbital length (PrOL), Post- orbital length (PoOL), Eye diameter (ED), Caudal peduncle length (CPL), Caudal peduncle depth (CPD), Caudal concavity (CC), Upper jaw length (UJL), Lower jaw length (LJL), Jaw gape (JG), Dorsal fin base length (DBL), Longest dorsal spine (LDS), Pectoral fin base length (PBL), Longest pectoral fin ray length (LPrL), Pelvic fin base length (VBL), Longest pelvic fin ray length (LVrL), Anal fin base length (ABL), Longest anal fin (LAS), Caudal fin base length (CBL).

Plate A. 1. *Lutjanus guilcheri;* 2. *Lutjanus russellii;* 3. *Parupeneus indicus;* 4. *Siganus sutor;* 5. *Thalassoma lunare;* 6. *Gazza achlamys;* 7. *Pomadasys andamanensis;* 8. *Pomadasys kaakan;* 9. *Lethrinus crocineus;* 10. *Argyrosomus regius;* 11. *Istigobius ornatus;* 12. *Cryptocentrus cyanotaenia*

and snout; 4-5 diffuse dark bars on side of head; yellow margin on 1st and 2nd dorsal fin; several longitudinal dark, orange and yellow stripes on anal fin; yellow edges on anal and caudal fin; pelvic slightly darkish violet and finely dotted with dark, pectorals hyaline and dotted with dark.

Molecular identification: As a promising alternative to the traditional species identification based on morphological characters, partial cytochrome c oxidase

Table 2. Species of new records barcoded base of COI gene along with their GB Accessions number

Order	Family	Species	No. of individual	GB Accession Number
Perciformes	Mullidae	*Parupeneus indicus*	1	MT012679
	Siganidae	*Siganus sutor*	3	MH429334 MH882461 MT012651
	Haemulidae	*Pomadasys kaakan*	1	MH311286, NW509666
	Lethrinidae	*Lethrinus crocineus*	1	MH429360
	Sciaenidae	*Argyrosomus regius*	1	MH429361
	Gobiidae	*Istigobius ornatus*	1	MT012661
	Gobiidae	*Cryptocentrus cyanotaenia*	1	MT012659

subunit I (COI) sequences (DNA barcodes) have been suggested for standardized and routine species identification (Hebert et al. 2003). Partial sequences of COI gene of seven species (*Parupeneus indicus, Siganus sutor, Pomadasys kaakan, Lethrinus crocineus, Argyrosomus regius, Istigobius ornatus and Cryptocentrus cyanotaenia* were generated. BLAST results showed 98-100% identity with pre-existing sequences in the NCBI GenBank. Sequences were submitted to the Gen Bank and accordingly assigned GB Accession numbers which validated their taxonomic identification (Table 2).

Teleost fishes are an important component of coral reef ecosystems, fulfilling many critical ecological roles (McClanahan 2000; Bellwood *et al.* 2004; Dulvy *et al.* 2004), and are a major source of food and livelihood for people in tropical coastal areas (Pauly *et al.* 2002). The extent of habitat damage and initial reef condition will also influence fish responses to disturbance. It is often assumed that the relationship between coral cover and fish diversity is linear (Bell and Galzin 1984). In the coral reef ecosystem, reef fish diversity is an important indicator to assessment reef ecology. In the studies, we have recorded 46 coral reefs associated marine fish that is helpful for further extension research on ecology and fish diversity. All reef fish species have significant value in fishery, tourism, local economy, nutrition, socio-economic activities and biodiversity.

Acknowledgments: We acknowledge the financial support from the Ministry of Science and Technology, Government of the People's Republic of Bangladesh as a grant for Special Allocation in Research (2016-2017).

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(Manuscript received on 20 September, 2020 revised on 25 December, 2020)