

NEW RECORDS OF TWO *LUTJANUS* SPECIES (TELEOSTEI: PERCIFORMES: LUTJANIDAE) WITH RE-DESCRIPTION OF SIX LUTJANIDS FROM SAINT MARTIN'S ISLAND OF THE BAY OF BENGAL, BANGLADESH

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Abstract: Investigations on Snapper fish of the genus *Lutjanus* were conducted from October 2015 to April 2016 in the coral ecosystem of St. Martin's Island, Bangladesh. Twenty one individuals of 8 species of *Lutjanus* were collected from local fishermen. Both morphological characters and DNA barcoding approach by mitochondrial cytochrome oxidase I subunit (COI) were used to confirm the identification of all species. Present study recorded the first national occurrence of two reef associated *Lutjanus* species (*L. xanthopinnis* and *L. indicus*). In addition six species (*L. rivulatus*, *L. lutjanus*, *L. vitta*, *L. lemniscatus*, *L. fulviflamma* and *L. johnii*) were re-described. Distinct morphological differentiation were found between more closely related species *L. xanthopinnis*, *L. vitta* and *L. lutjanus*. Three different appearances of *L. johnii*, two different appearances of *L. rivulatus* and *L. lemniscatus* were confirmed as same species. Phylogenetic analysis showed monophyletic clade for conspecific individuals and paraphyletic clade for congeneric individuals. Average genetic divergence for intraspecific level was 1.5% and 12.5% for interspecific level. Current study increased the number of *Lutjanus* species in Bangladesh from 15 to 17 and extended the distribution range of those new recorded species in northeastern part of the Bay of Bengal.

Key words: Coral ecosystem, morphomeristics, cytochrome oxidase I subunit (COI), *L. xanthopinnis*, *L. indicus*

INTRODUCTION

Lutjanus species were distributed along the Indo-West Pacific coast and recorded 43 species (Bloch 1790). In the past, total 15 *Lutjanus* species were recorded based on morphological characters only from the Bay of Bengal, Bangladesh. However, due to morphological similarities between species, taxonomy of *Lutjanus* is still highly complex. There are three species *L. stellatus*, *L. buccanella* and *L. campechanus* (Sarker *et al.* 2015), 8 species *L. argentimaculatus*, *L. bohar*, *L. gibbus*, *L. lemniscatus*, *L. lunulatus*, *L. lutjanus*, *L. rivulatus* and *L. sebae* (Rahman *et al.* 2009) and 4 species *L. sanguineus*, *L. fulviflamma*, *L. malabaricus* and *L. johnii* by (Tomascik 1997) in the bay of Bengal, Bangladesh were recorded.

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The variation of mitochondrial cytochrome oxidase I gene (COI) is moderately high between species and very little between the individuals of a given species (Gross 2012). As COI gene has proven to be highly effective at discovering new-recorded and new species (Gao *et al.* 2011, Qin *et al.* 2013). DNA barcoding can accurately link the larval stages of a species, which is usually difficult but using both tools the morphological and DNA barcoding approach apply on single species identification will lead more confirmation of the species. DNA barcoding can be a very effective tool in assessment of cryptic species. Iwatsuki *et al.* (2015) mentioned that *L. lutjanus*, *L. xanthopinnis* and *L. vitta* are cryptic species. Therefore they are morphologically similar but genetically distinct. Furthermore, *L. johnii*, *L. rivulatus* and *L. lemniscatus* show different marking patterns in different size also (Anderson *et al.* 2001). Therefore, misidentification could be done in systematics of *Lutjanus spp* using only one identification tool. The current paper will describe eight species of *Lutjanus* based on both morphometrics & DNA barcoding with two new records and differentiating them correctly from their related congeners and establishing link between conspecifics. This study will be helpful to fishery management, biodiversity conservation, and sustainable exploitation of these species.

MATERIAL AND METHODS

Specimen collection and deposition: Fish specimens were collected from local fishermen of St. Martin's Island (Fig. 1) (20°34'N - 20°38.8'N and 92°18'E - 92°20.8'E) during October, 2015 to April, 2016 visiting five times. Local fishermen catch these fishes using gear named small berjal, hooks and lines. Fresh fish deliveries from other areas to St. Martin's Island never occurred so, **fish found on fish shop and fish landing zone of this island were considered to originate from waters surrounding the Island.** All specimens were collected in full observance of local government regulation, and in obedience to appropriate animal care standards. Most specimens were photographed after collection. Then specimens were transported with ice box to fisheries lab, department of Zoology, Jagannath University, Dhaka and kept in -20°C within deepfreeze till further study. After study, all specimens were deposited in the museum of Zoology department, Jagannath University as voucher specimen.

Morphometric, meristic and molecular analysis: All measurements were made to the nearest 0.01 cm. Methods of morphometrics and meristics are mostly same with those described in Allen and Talbot (1985). Most specimens were photographed when fresh. Counts included: dorsal fin spines and rays (D), anal fin spines and rays (A), pectoral fin rays (P), pelvic fin spines and rays (V), caudal fin rays (C), scales in lateral line (Li), scales above lateral line (aLi), and

scales below lateral line (bLi), gill raker on upper series (uGr), gill raker on lower series (lGr). Measurements included: standard length (SL), head length (HL), body width (BW), body depth (BD), caudal peduncle depth (CPD), caudal peduncle length (CPL), caudal concavity (CC), caudal fin base length (CBL), pre dorsal length (PDL), pre anal length (PAL), pre pectoral length (PPL), pre pelvic length (PVL), dorsal fin base length (DBL), longest dorsal spine (LDS), longest dorsal ray length (LDR), anal fin base length (ABL), longest anal spine (LAS), longest anal fin ray length (LArL), pectoral fin base length (PBL), longest pectoral fin ray length (LPrL), pelvic fin base length (VBL), longest pelvic fin ray length (LVrL), inter orbital length (IOL), pre orbital length (PrOL), post orbital length (PoOL), eye diameter (ED), snout length (SnL), upper jaw length (UJL), lower jaw length (LJL), jaw gape (JG).

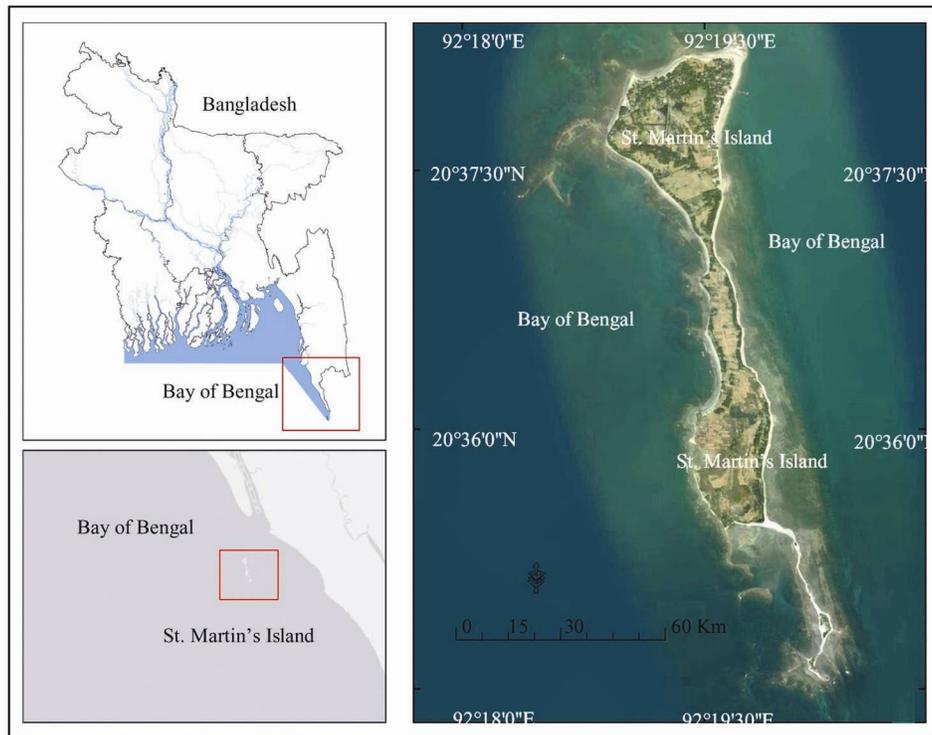


Fig. 1. The map of St. Martin's Island, Bay of Bengal, Bangladesh showing the location of sample sites of *Lutjanus* species.

Genomic DNA from 13 specimens representing 8 species was extracted by TIANamp Marine Animal DNA Kit. An ~655 bp fragment was amplified from the 5' region of the cytochrome oxidase subunit I (COI) gene of mitochondrial DNA using the C FishF1t1/ C FishR1t1 primer cocktails (Ivanova *et al.* 2007). The

PCR reactions were carried out in 20 μ l reaction mixture containing 6.0 μ l of distilled water, 10 μ l of master mix, 1 μ l of each primer (5 μ mol/l) and 2 μ l of DNA template. The thermal regime consisted of an initial step of 2 min at 94°C followed by 35 cycles of 30 sec at 94°C, 40 sec at 52°C, and 1 min at 72°C, followed in turn by 10 min at 72°C. Then soak at 4°C. PCR products were purified by using QIA quick PCR purification kit. After purification, the products were sequenced in both directions by using commercial sequencer, Macrogen, Korea. Sequences were manually edited using the software Chromas Lite. Alignment and neighbor-joining tree (Saitou and Nei 1987) was constructed in MEGA 6 (Tamura et al. 2013) with 1000 bootstrapping replications based on evolutionary distances calculated using the Kimura two parameter (K2P) model (Kimura 1980).

RESULTS AND DISCUSSION

Details morphometric measurements of 8 *Lutjanus* spp showed in Table 1 and 6 *Lutjanus* species (*L. vitta*, *L. rivulatus*, *L. johnii*, *L. fulviflamma*, *L. lutjanus* and *L. lemniscatus*) identifying morphological characters comparison with other authors are summarized in Table 2. Details morphological description are provided for only the two new recorded species (*L. xanthopinnis* and *L. indicus*) individually. *L. xanthopinnis* (4 individuals), *L. lutjanus* (2 individuals) and *L. vitta* (1 individual) showed morphology are very similar (Fig. 2a,b,c and Table 5) but differentiated as 3 distinct species using both tools. Three (3) individuals of *L. rivulatus* (Fig. 2f,g) and seven (7) individuals of *L. johnii* (Fig. 2h, i, j) morphologically showed 2 and 3 types of marking patterns but confirmed as same species by both tools. Also marking variation confirmation in *L. lemniscatus* (SL, 21.2-26 cm) (Fig. 2k, l).

Lutjanus xanthopinnis (Iwatsuki et al. 2015) represented by 4 individuals (7.5- 19.1 cm SL) were collected from fisherman at the fish landing zone of the study area. General body features are shown in Fig. 2a. Dorsal-fin with 10 spines and 13 rays, anal-fin with 3 spines and 8 rays, pectoral-fin with 16 rays, pelvic fin with 1 spine and 5 rays, caudal fin with 17 rays, lateral-line scales 50; scale rows transversely above lateral line 7; scale rows transversely below lateral line 14.

Description: Body elongated, laterally compressed. Dorsal profile convex, large in size. Head large and triangular, eye not at mid-level of head rather behind tip of snout. Posterior nostril is elliptical type (Fig. 3a). Small conical teeth on vomer, and both jaws have caniniform teeth with pair of dagger like canine in upper jaw anteriorly. Preopercle serrated. Large operculum with flap

Table 1. Comparisons of morphometric measurements (%SL) of 8 *Lutjanus* species

Body parameters	<i>L. vitta</i> (1)	<i>L. lutjanus</i> (2)	<i>L. xanthopinnis</i> (4)	<i>L. indicus</i> (1)	<i>L. lemniscatus</i> (2)	<i>L. johnii</i> (7)	<i>L. rivulatus</i> (3)	<i>L. fulviflamma</i> (1)
SL	15.1	10.5-14.5	7.5-19.1	28	21.2-26	20.4-32.5	21-36.9	11
HL	39.1	37.1-38.6	39.9-40	40	43.8-43.9	36.2-40.7	40.9-46.7	40
BD	31.1	33.3-34.5	36	37.9	38.7-38.8	38.8-40.7	43.9-46.7	37.3
BW	15.9	15.9-18.1	14.7-15	16.1	16.9-17	16.4-20.6	17.6-19	18.2
CPD	11.3	12.4-13.1	13.1-13.3	13.6	13.1-13.2	12.9-14.2	13-14.8	14.6
CPL	11.3	9.7-15.2	12-12.4	11.4	12.7	13.2-14.7	9.8-10.5	10.9
CC	5.3	6.9-8.6	6.5-6.7	5.4	5.2-5.4	2.2-2.9	6.8	5.5
CBL	13.3	12.4-13.1	14.4-14.7	15	14.2	13.8-15.7	14.1-23.8	14.6
PDL	44.4	38.1	42.5-42.7	41.1	40.6-40.8	48.5-49.6	35.2-47.6	34.6
PAL	75.5	70.5-78.6	74.7-75.2	72.9	73.1	66.4-69.1	68.3-76.2	64.6
PPL	35.8	33.3-34.5	37.3	32.5	36.2-36.3	35.8-37.1	33.9-41	36.4
PVL	41.1	39.1-39.3	41.3-41.8	37.1	41-41.2	38.8-39.7	36-43.3	39.1
DBL	55.6	53.3-55.2	56.9-57.3	53.2	52.7-52.8	52.6-54.9	51.8-53.8	50.9
LDS	19.2	15.2-16.6	18.3-18.7	14.6	14.6	17.2-18.8	14.4-16.7	15.5
LDR	13.9	10.3	13.1-13.3	-	-	16.4-17.2	15.5	-
ABL	15.9	14.3-15.9	16-16.3	15.4	16.9-17	16.8-17.2	16.8-18.1	18.2
LAS	13.9	10.3-14.3	16-16.3	8.6	13.1-13.2	15.2	11.1-13.8	12.7
LArL	13.9	11.7-14.3	14.4-14.7	14.6	14.6	16.7	20.6	18.2
PBL	5.3	5.5-5.7	5.2-5.3	5.4	6.9-7.1	6.9-7.3	7-7.1	7.3
LPtL	31.1	30.3-30.5	29.3-29.4	23.9	31.1-31.2	28.5-32.4	37.4-37.6	31.8
VBL	5.3	4.8	3.9-4	4.3	6.1-6.2	4.4-6	5.7-7.6	4.6
LVrL	23.8	20.7-23.8	21.3-21.6	20.4	24.5-24.6	21.6-24.5	19.8-26.2	23.6
IOL	9.3	9.5-9.7	7.8-8	7.5	6.5-6.6	6.9-7.3	9.5	7.3
PrOL	10.6	10.5-12.4	13.3-13.7	14.3	15.6-15.8	13.2-14.7	15.2	11.8
PoOL	17.2	17.9-18.1	18.3-18.7	19.3	21.2	20.7-21.1	20.1-21	18.2
ED	10.6	11-11.4	8-8.5	8.6	8-8.1	8.3-8.6	6-8.6	9.1
SnL	7.1	5.7-6.9	7.8-8	8.6	10.8-10.9	7.8-9.8	9.2	6.4
UJL	15.9	14.3-17.2	16-16.3	16.4	16.9-17	16-16.2	15.2-16.2	15.5
LJL	15.9	15.2-17.2	16-16.3	16.4	16.9-17	15.1-16.7	10-15.2	14.6
JG	18.5	16.2-17.2	17.3-17.7	12.9	16.5	11.6-14.7	10.6-13.8	18.2

Table 2. Identifying morphological characters of additional 6 *Lutjanus* species ((●), (■), (♦), (+), (*), (◆), (⬠), (⬢), (⬣), (⬤)) marks represent : This study, Matsumura et al. (2011), Anderson et al. (2001), Karna et al. (2018), Jawad et al. (2018), Allen (1985), Vella et al. (2015) Chyung (1977) Iwatsuki et al. (1993) where n is the number of individuals examined in the present study and reference values are shown in parentheses

Character	<i>L. lutjanus</i> ●, ■, ■ (n = 2)	<i>L. rivulatus</i> ●, ◆, + (n = 3)	<i>L. johnii</i> ●, ■ (n = 7)	<i>L. fulviflamma</i> ●, ◆, * (n = 1)	<i>L. vittata</i> ●, ■, ◆, * (n = 1)	<i>L. lemniscatus</i> ●, ■ (n = 2)
Dorsal fin rays	XI,12 (X-XII,12)	X,15-16	X,14-15 (X,13-14)	X,13 (X,12-14)	XI,12 (X-XII,12-14)	X,15 (X,12-16)
Anal fin rays	III,8 (III,8)	III,8 (III,8)	III,9 (III,9)	III,8 (III,8)	III,8 (III,8-9)	III,8 (III,8)
Pectoral fin rays	16 (16-17)	17 (15-17)	17	15 (15)	16 (16-17)	17
Lateral line scales	51 (48-50)	53	55-57	50 (49)	53 (49-53)	53
Standard length						
Body depth	2.9-3 (2.9-3.3)	2.1-2.3 (2.1-2.4)	2.5-2.6	2.7 (2.7)	3.2 (2.6-3.2)	2.6 (2.5-2.8)
Head length	2.6-2.7	2.1-2.4 (2.7)	2.5-2.8	2.5 (2.8)	2.6 (2.5-3.1)	2.3
Head length						
Interorbital width	3.9-4.1	4.3-4.9	5.0-5.9 (5.6-7.3)	5.5	4.2 (4.3-6.8)	6.6 (5.5-7.6)
Eye diameter	3.4-3.5	5.4-6.8 (6.7)	4.2-4.9	4.4 (4.4)	3.7 (3-4.9)	5.5
Stripe/spot	Fine yellow lines, 1 per scale row with broad midlateral yellow stripe (Thin stripes at 1 per scale row with broad midlateral yellow stripe on body)	Numerous wavy bluish lines on head; white spot on lateral line below junction of spinous and soft parts of dorsal fin in 21 & 26.7 cm SL but absent 36.9 cm SL (lost with age)	Each scale with a brown spot forming horizontal lines on side; large black spot above lateral line present at the level of last dorsal spine and 1 st six dorsal soft ray (disappears with age and replaced by pale ground)	Black spot on lateral line at the position of last spine and 1 st 3 soft ray of dorsal fin and 6 orange yellow stripes on side (mostly below lateral line or bisected by it and 4-7 yellow stripes on side)	Fine yellow lines, 1 per scale row below the broad yellow stripe horizontally (same)	Broad, black, midlateral stripe in 21.2 cm SL, slightly appears in 26 cm SL (Large juveniles with black mid-lateral stripe)
Temporal scale						

Number of individual are shown in parentheses

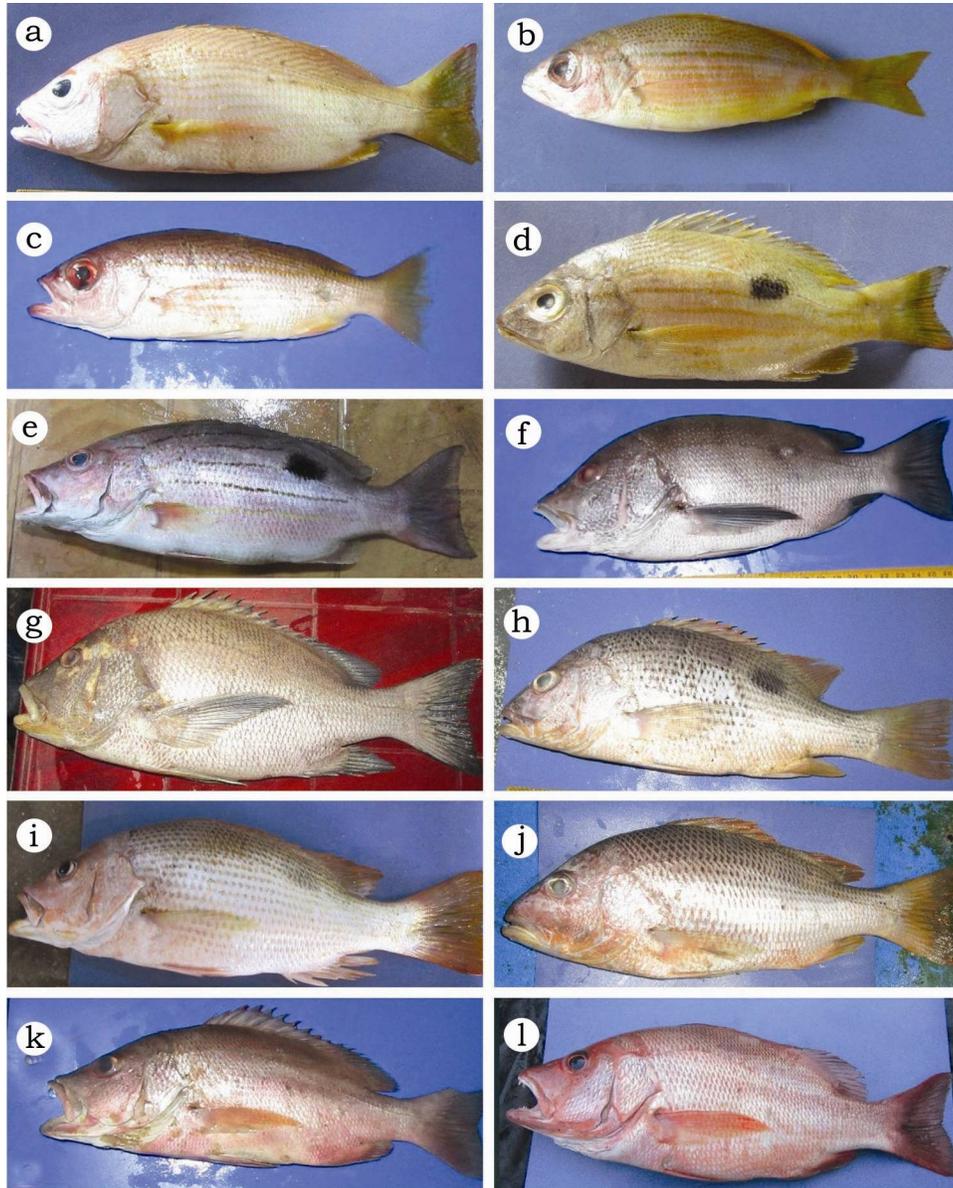


Fig. 2. Morphological representation of twelve species of *Lutjanus* used in the present study (a) *Lutjanus xanthopinnis* (15.3 cm SL); (b) *L. lutjanus* (10.5 cm SL); (c) *L. vitta* (15.1 cm SL); (d) *L. fulviflamma* (11 cm SL); (e) *L. indicus* (28 cm SL); (f) *L. rivulatus* (21 cm SL, white spot on lateral line); (g) *L. rivulatus* (36.9 cm SL, no spot on lateral line); (h) *L. johnii* (20.4 cm SL, black spot on lateral line distinctly appear); (i) *L. johnii* (25.9 cm SL, black spot being disappear) and (j) *L. johnii* (32.5 cm SL, black spot completely disappear); (k) *L. lemniscatus* (21.2 cm SL) and (l) *L. lemniscatus* (26 cm SL).

and large opening included 2 spines at the end of operculum. One rounded serrated bone above operculum. Scales are ctenoid type on body. Scale rows on back rising obliquely above lateral line. Five scale rows has on cheek (Fig. 3b). Pre-dorsal scales extending forward to about the middle of inter-orbital space (Fig. 3c). Moderately incised dorsal fin with 4th spine longest 2.8 cm and longest dorsal ray length 2 cm. Three rows of small scale on the soft dorsal fin base. Pectoral fin pointed. Caudal fin emarginated.

Color: Thin yellow stripes on the body. Body yellowish dorsally with 18 yellow oblique lines, light yellowish ventrally. Head light pinkish. Twelve fine yellow stripes horizontally, 1 per scale row below the lateral line. Dorsal, pectoral, pelvic, anal and caudal fin yellow.

Distribution: Indo-western Pacific: Japan, Taiwan, Indonesia and Sri Lanka. First described as a new species by Iwatsuki *et al.* (2015) from Indo-western Pacific region on the basis of the 11 specimen from Japan, Taiwan, Indonesia and Sri Lanka. All of the identifying characters in the present study show similarity with Iwatsuki *et al.* (2015) which is summarized in Table 3.

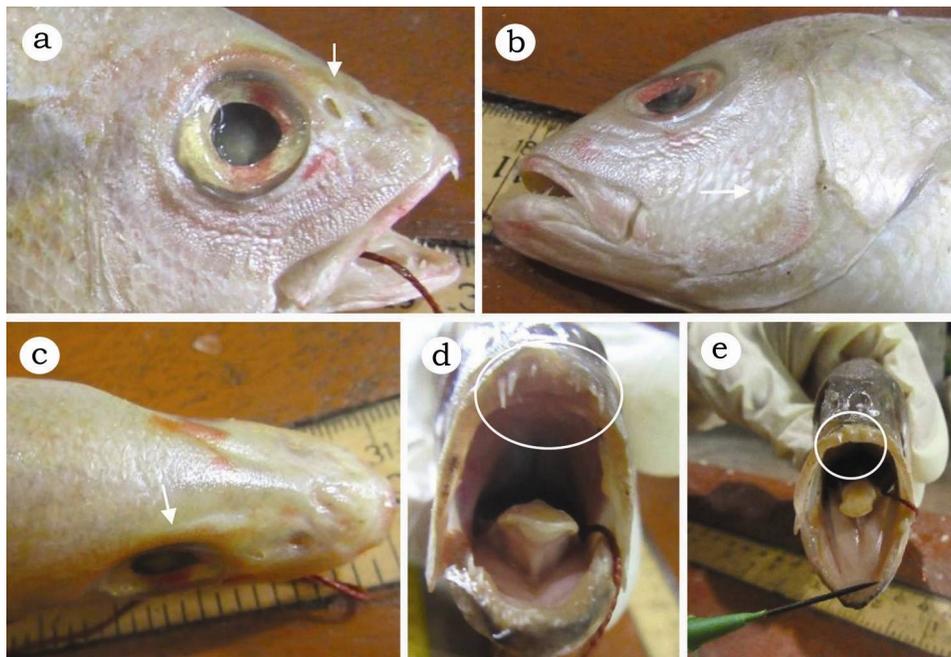


Fig. 3(a). Shape of posterior nostril, (b) scales on cheek and (c) extension of anterior predorsal scales in *L. xanthopinnis*; (d) 3 pairs of large canines in upper jaw of *L. lutjanus*; (e) pair of large canines in upper jaw of *L. vitta*.

Table 3. Identifying morphological characters of four individuals of *L. xanthopinnis*

Character	<i>L. xanthopinnis</i> (Present study)	<i>L. xanthopinnis</i> (Iwatsuki <i>et al.</i> 2015)	Character	<i>L. xanthopinnis</i> (Present study)	<i>L. xanthopinnis</i> (Iwatsuki <i>et al.</i> 2015)
Dorsal fin rays	X,13	X,13	Scale rows above lateral line	7	6-7
Anal fin rays	III,8	III,8	Scale rows on cheek	5	4-5
Pectoral fin rays	16	16-17	Size of midlateral yellow stripe	Very thin	Very thin
Body depth	2.7-2.8 in SL	2.7-2.9 in SL	Shape of posterior nostril	Elliptical	Elliptical
Lateral line scales	50	48-50	Extension of anterior predorsal scales	middle of interorbital space	middle of interorbital space

Lutjanus indicus (Allen *et al.* 2013) represented by single individual (28.0 cm SL) and was collected from fish shop of the study area on 10 January 2016. General body features are shown in Fig. 2e.

Dorsal fin with 10 spines and 14 rays, anal fin with 3 spines and 8 rays, pectoral fin rays 15; pelvic fin with 1 spines and 5 rays, caudal fin rays 17, lateral-line scales 49; scale rows transversely above lateral line 8; scale rows transversely below lateral line 17.

Description: Body elongated, laterally compressed. Dorsal profile convex, large in size. Head large and triangular. Anterior and posterior nostrils elliptical shaped. Five scale rows on cheek. Small conical teeth on vomer, tongue and palatine. Upper jaw with pair of large dagger like canines anteriorly with smaller one middle of it and 12 relatively smaller canines on remaining lateral portion; lower jaw with 12 small canines on each side which become largest posteriorly and at back of jaw single row of 8 minute conical teeth. Preopercle posteriorly serrated. Large operculum with flap and large opercular opening. One rounded serrated bone just above the opercular opening. Dorsal fin incised, fifth spines longest 4.1 cm. Pectoral fin elongated, 1.7 in HL. Pelvic fin relatively short, 2.0 in HL. Caudal fin double emarginated.

Color, stripe and spot: Blackish dorsally, side of the body light reddish and whitish ventrally. Head gray with preopercle and lip reddish. Lower side of body with series of 6 dark brown and 2 yellow (total 8) stripes on posterior head and side, first dark brown stripe starts from nape and reaches up to the fourth dorsal fin spine base; second dark brown stripe starts from just above the eye

reaches up to the eighth dorsal fin spine base; third dark brown stripe starts from eye and reaches up to the fourth soft dorsal fin base; fourth dark brown stripe starts from lower margin of eye and reaches upto just above of broad black spot; fifth dark brown stripe starts from eye and reaches up to the lowermost part of broad black spot; sixth dark brown stripe starts from below eye, run along middle of opercle and reaches upto the caudal peduncle; seventh yellow stripe starts from the in front of preopercle, run along the above of pectoral fin and reaches up to the caudal peduncle; eighth yellow stripe starts from opercular opening and reaches up to the end of anal fin base. Prominent black spot, about 1.3 size of eye, on posterior back below base of first 8 soft dorsal rays, its lowermost part occupying about one scale row below lateral line.

Distribution: Distributed in northern continental margin of the Indian Ocean including western Thailand, Myanmar, Andaman Islands, Sri Lanka, India, Gulf of Oman and Arabian Gulf (FAO, 1985). Most of the identifying characters in the present study show similarity with Allen *et al.* (2013) and are shown in Table 4 except interorbital width in head length which is 5.3 in the present study and 5.9–7.0 as described in Allen *et al.* (2013); number of stripes on posterior head and side that are six dark brown and two yellow in the present study, where five dark brown and two yellow as described in Allen *et al.* (2013) and position of prominent black spot below base of first 8 soft dorsal rays (first 6–7 soft dorsal rays, Allen *et al.* 2013). Minor differences in above mentioned characters may be due to sampling from different geographical area.

Table 4. Identifying morphological characters of *L. indicus* of the present study with that of reported data

Character	<i>L. indicus</i> (Present study)	<i>L. indicus</i> (Allen <i>et al.</i> 2013)	Character	<i>L. indicus</i> (Present study)	<i>L. indicus</i> (Allen <i>et al.</i> 2013)
Dorsal fin rays	X,14	X,13-14	Head length		
Anal fin rays	III,8	III,8	Interorbital width	5.3	5.9-7.0
Lateral line scales	49	47-49	Eye diameter	4.6	4.2-4.6
Standard length			Stripes on posterior head and side	6 dark brown and 2 yellow	7 dark brown to yellow stripes
Body depth	2.6	2.5-2.9	Size of prominent black spot	1.3 size of eye	about 1.2-1.5 size of eye
Head length	2.5	2.5-2.7	Position of prominent black spot	below base of first 8 soft dorsal rays	below base of first 6-7 soft dorsal rays

According to the yellow lined snapper complex of Allen and Talbot (1985) and Iwatsuki *et al.* (2015) four more confusing *Lutjanus* species are *L. lutjanus*, *L. vitta*, *L. xanthopinnis* and *L. madras*. As present study described *L. lutjanus*, *L. vitta* and *L. xanthopinnis*, and *L. madras* (Iwatsuki *et al.* 2015) so, identifying meristic and morphological characters between them are summarized in Table 5.

Table 5. Comparison between 4 more closely related *Lutjanus* species

Character	<i>L. lutjanus</i>	<i>L. vitta</i>	<i>L. xanthopinnis</i>	<i>L. madras</i>
Dorsal fin rays	XI,12-13	XI,12	X,13	X,13
Anal fin rays	III,8-9	III,8	III,8	III,9
Lateral line scales	51	53	50	49-51
Horizontal scale rows above lateral line	5-6	8	7	7-8
Scale rows on cheek	5	6	5	7-8
Number of dagger-like canines in upper jaw	3 pair*	1 pair#	1 pair	1 pair
Size of midlateral yellow stripe	Broad	Broad	Very thin	Broad
Shape of posterior nostril	Rounded	Rounded	Elliptical	Rounded
Extension of anterior predorsal scales	1/3 anterior of eye	1/3 anterior of eye	middle of interorbital space	posterior edge of the orbit

*Fig. 3d & #Fig. 3e.

Sequence analysis of the COI gene: We amplified the COI gene fragments of 12 individuals representing 8 species of *Lutjanus*. All the COI sequences were submitted to GenBank. Including the downloaded COI sequences of congeners and conspecifics, totally 32 sequences were used in the analysis. GenBank accession numbers of all COI sequences are provided in the associated figure. The mean intraspecies evolutionary divergence (K2P distance) was 1.5%. The minimum and maximum intraspecies evolutionary divergence were 0% (*L. lutjanus*) and 3.5% (*L. lemniscatus*) respectively. The mean, minimum and maximum interspecies evolutionary divergence were 12.5%, 0.8% (between *L. lutjanus* and *L. vitta*) and 16.9% (between *L. xanthopinnis* and *L. rivulatus*) respectively far exceeding the threshold of species delimitation (3.5%) which was proposed and applied by Ward *et al.* (2005, 2009) based on the metric of 10× the average intra-species genetic variation (Herbert *et al.* 2004) except the minimum divergence, 0.8% between *L. lutjanus* and *L. vitta*. But these two species were distinctly separated based on morphometric measurements and meristics (details in Tables 1,2,5).

Neighbor-joining tree was constructed based on K2P model with 1000 replications of bootstrapping test. While DNA barcoding provides taxonomic

identification for a specimen, the accuracy of such an assignment depends on whether species are monophyletic with respect to sequence variations of the COI gene. That is, individuals of a given species are more closely related to all other conspecifics than to any member of other species (Junbin 2011). In the phylogenetic analysis 5 individuals of the *L. johnii* formed a group in a monophyletic clade. Similarly, all other individuals of a species (conspecific) are monophyletic and formed a single clade. Individuals of the different species of the genus *Lutjanus* (congeneric species) are paraphyletic. Totally 10 species formed a polyphyletic tree (Fig. 4).

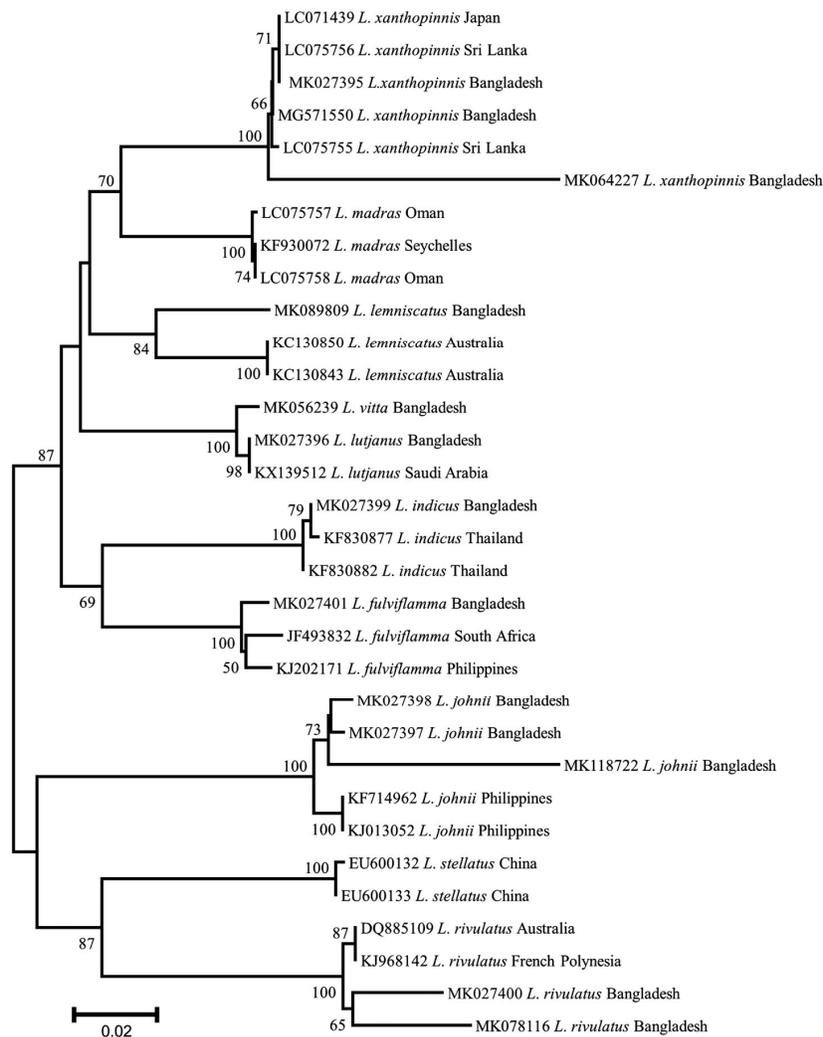


Fig. 4. Neighbor- joining tree constructed using the K2P model for COI gene sequences of ten species of *Lutjanus*.

L. vitta has always been confused with its sibling species, *L. lutjanus*, *L. xanthopinnis* from Saint Martin's Island and *L. madras* from India. Genetic divergence of about 0.8, 10.4 and 7.7% was found between *L. vitta* and its closest congener, *L. lutjanus*, *L. xanthopinnis* and *L. madras*, respectively from comparison of the mitochondrial cytochrome c oxidase subunit 1 (CO1) genetic marker utilized in DNA barcoding. Also morphologically distinctly different from them. Close relation of *L. xanthopinnis* with *L. madras* from India, can be particularly differentiated by its elliptical posterior nostril. Genetic divergence of about 8.1% and 9.9% was found between *L. xanthopinnis* and its closest congeners, *L. madras* and *L. lutjanus*, respectively from comparison of the mitochondrial CO1 gene utilized in DNA barcoding.

CONCLUSION

The two newly recorded fish that are presented in this study provide new knowledge about their specific distributions and give an indication about their original distributions or possible range extensions. In either case, this information is important when it comes to conservation efforts for the species in question or, in a broader sense, for the entire Western Indo-Pacific or Northeastern part of the Bay of Bengal biodiversity. With every new record, the marine biodiversity of the St. Martin's Island increases and may be much higher than previously assumed. This study also determines differentiation between cryptic *L. lutjanus*, *L. xanthopinnis* and *L. vitta*, and confirmation of standard length wise phenotypic variation in *L. johnii* and *L. rivulatus*.

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