**Three New Records of Cardinalfish (Apogonidiae) from the Northern Bay of Bengal, Bangladesh**

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**Abstract**—Three species of cardinalfishes viz. *Ostorhinchus cookii* (Macleay, 1881), *Lepidamia kalosoma* (Bleeker, 1852) and *Apogonichthyoides sialis* (Jordan et Thompson, 1914)recorded for the first time from the Northern Bay of Bengal. Furthermore, for *L. kalosoma*, this is the first report in the entire Bay of Bengal. Specimens were collected from the Saint Martin’s Island, Bay of Bengal, Bangladesh, during a survey on coral associated fishes of Bangladesh from April 2017 to June 2018. The examined specimens are identified by morphomeristic characteristics in addition with DNA Barcoding.

**Keywords:** Saint Martin’s Island, morphology, underwater, DNA barcoding

INTRODUCTION

Cardinalfishes (family Apogonidae) are one of the numerically dominant reef fish families inhabitant of warm temperate to tropical areas in the Pacific, Indian and Atlantic Oceans. The family carried 381 valid species, in which most of them are marine and some thrive in brackish water (Allen, 1993; Fraser and Allen, 2010; Nelson et al., 2016; Froese and Pauly, 2019; Fricke et al., 2020) They are cryptic during daylight and active at night, and known as oral egg brooding by males (Mabuchi et al., 2014; Vagelli, 2019). Most of the apogonids are small in size (usually less than 120 mm), but some species grow to larger sizes (maximum 200 mm) (Fraser, 1973; Allen, 1999; Froese and Pauly, 2019).

Most cardinal fishes occupy in coral or rocky reefs habitat, while some species in seagrass and soft-bottom communities, coralline algal meadows, estuaries, and lowland freshwater. In terms of species diversity and abundance, cardinalfishes are a significant part of reef fish assemblages (Wainwright and Bellwood, 2002; Brandl et al., 2019). Most apogonids are carnivorous feeding on benthic organisms, small fish, and plankton as their feeding (Vivien, 1975; Chave, 1978; Marnane and Bellwood, 2002; Barnett et al., 2006).

Cardinal fishes in Bangladesh have been poorly studied. Only seven species of this family have been reported from Bangladesh waters which is comparatively much lower than Indian side where 69 species have been reported so far (Hussain, 1970; Fraser, 2005; Suresh and Thomas, 2007; Rahman et al., 2009; Thompson and Islam, 2010; Saravanan et al., 2018) The present paper reports the addition of three new species from the marine reef habitat of the country.

MATERIALS AND METHODS

*Sample Collection and Preparation*

During field survey between April 2017 to June 2018, specimens were collected from Saint Martin’s Island. The island is located at the southern-most tip of Bangladesh in the Bay of Bengal (Fig. 1). Underwater photographs of the species were also taken during scuba diving in the island. The fishes were caught in March 2018 by the local fishermen while they set gill net on the coral reef. After tagging, the collected samples were photographed following the technique described by Randall (1961). Then samples were transferred to research laboratory for further analysis. After morphological analysis in lab, a small piece of muscle tissue was cut from each specimen and stored in a sterile 1.5 mL tube containing 98% alcohol for subsequent molecular work. All examined specimens deposited in the Aquatic Bioresource Research Lab. (ABR Lab), Department of Fisheries Biology and Genetics, Sher-e-Bangla Agricultural University, Dhaka, Bangladesh.

*Morphometric Analyses*

Morphometric study was performed following Weber and de Beaufort (1916), Allen (1999), Mabuchi et al. (2014), and Psomadakis et al. (2019).

*Genomic DNA Extraction, PCR Amplification and DNA Sequencing*

Genomic DNA was extracted from the collected muscle tissue using a DNeasy® Blood and Tissue Kit from QIAGEN following the protocol provided inside the kit box. The concentration of genomic DNA were measured by Qubit 3.0 fluorometer. Polymerase chain reaction (PCR) was performed in a 50 μL reaction mixture in small reaction tubes (0.2 mL) using Thermal cycler (2720 Thermal Cycler, Applied Biosystems). The partial sequences of mitochondrial DNA (mtDNA) *COI* gene of collected specimens were amplified using the following primers FishF2-5′TCGACTAATCATAAAGATATCGGCAC3′ (Forward primer) and FishR2-5′ACTTCAGGGTGACCGAAGAATCAGAA3′ (Reverse primer) (Ward et al., 2005). The thermal regime consisted 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 until gel electrophoresis. PCR products were visualized on 1% agarose gel (Invitrogen, USA) stained with ethidium bromide in gel documentation chamber (Syngene InGenius³). The flow of UV ray was kept on to watch the band in the connected computer by using GeneSys software. PCR samples with a single and clear visible band were purified with the QIAquick PCR Purification Kit (QIAGEN) for sequencing. The concentration of the purified DNA was estimated with the help of Qubit 3.0 fluorometer. Sequencing was conducted with the PCR primers by Sanger standard method in a normal automatic sequencing 3730xI DNA analyzer (Macrogen Inc., Korea). The Software Geneious 9.0.5 was used for editing nucleic acid sequences. The obtained consensus sequences were edited based on the chromatogram peak clarities with the help of Chromas Lit. Sequences were matched using BLAST search engine provided by NCBI and Bold database. Finally, the nucleotide sequences of partial *COI* gene obtained in the present study were deposited in the NCBI GenBank under the accession numbers MK340670 for *Ostorhinchus cookii*, and MK340634 and MK560520 for *Lepidamia kalosoma*, respectively. Unfortunately, we failed to get good DNA sequences for *Apogonichthyoides sialis* using different primers and applying different thermal conditions in PCR.

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RESULTS

In the present study, we identified total five species of cardinalfish where 3 species i.e., *Ostorhinchus cookie,* *Lepidamia kalosoma*, and *Apogonichthyoides sialis* recorded for the first time from Bangladesh.

*Ostorhinchus cookii (Macleay, 1881) or Cook’s cardinalfish*

**Material.** Specimen collected from Bangladesh: Cox’s Bazar, Teknaf, St. Martins Island, Bay of Bengal; coordinate: 20°36'47" N, 92°19'36" E. Specimen voucher F1802SM-13, standard length (*SL*) 62 mm, coll. K.A. Habib and M.J. Islam; 16. 02. 2018 (Fig 2a). Moreover an underwater photograph was captured during the foraging of *O. cookii* around 3 m of depth in the Chera Dip, northeast side of the St. Martin’s island (Fig 2b). NCBI GenBank accession no MK340670.

**Remarks.** Body with 6 dark brown stripes on lateral surface of the body; 3rd stripe beginning from upper eye, ending at middle of body (below second dorsal-fin origin); fourth stripe (middle stripe) posteriorly reaching to a dusky spot on caudal-fin base which is sub-equal in size to pupil diameter. Fins sub translucent or a little tinged with red (Fig. 1). Morphometric measurements of *O. cookii* are in Tables 1, 2.

*Lepidamia kalosoma (Bleeker, 1852) or Pinstripe Cardinalfish*

**Material.** Specimen collected from Bangladesh: Cox’s Bazar, Teknaf, St. Martins Island, Bay of Bengal; coordinate 20°36'47" N, 92°19'36" E (Fig. 1). Specimen voucher no F1803SM-33, F1803SM-34; *SL* 83−85 mm, coll. K.A. Habib and M.J. Islam; 21. 03. 2018 (Fig. 3a). Moreover an underwater photograph was captured during the foraging of *L. kalosoma*around 4 m of depth in jetty of the St. Martin’s island (Fig. 3b). GenBank accession number of *COI* sequences of two *L. kalosoma* specimen are MK340634 and MK560520, respectively.

**Remarks.** Colour of live specimen with a dark lateral band from head to base of caudal, where it ends in a large caudal spot. A second band from snout, along sides at some distance below profile of back. Upper part of first dorsal fin blackish. All the fin slightly reddish in color in live. In preserved condition, body color reddish with slightly dark band over the body and caudal spot may be absent or disappear (Fig. 3). Morphometric count and measurements of *Lepidamia kalosoma* given into Tables 1, 2.

*Apogonichthyoides sialis (Jordan et Thompson, 1914) or Twinbar Cardinalfish*

**Material.** Specimen collected from Bangladesh: Cox’s Bazar, Teknaf, St. Martins Island, Bay of Bengal; coordinate 20°36'47" N, 92°19'36" E (Fig. 1). Specimen voucher no F1803SM-16, *SL* 64 mm, coll. K.A. Habib and M.J. Islam; 21. 03. 2018 (Fig. 4a). Moreover an underwater photograph was captured during the foraging of *A. sialis* around 4 m of depth in the jetty of the St. Martin’s island (Fig. 4b).

**Remarks.** Body brownish to greenish grey anteriorly, shading to dark grey posteriorly in live condition; two black bars below dorsal fins extending ventrally beyond pectoral fin; iris dark: a white ring around pupil; pelvic fins dark brown with white leading edge; distinct dark, small basicaudal spot (Fig. 4). Morphometric measurements of the specimen of *A. sialis* given in Tables 1, 2.

*Genetic Description*

For genetic confirmation, we constructed phylogenetic tree using the *COI* sequence of *Ostorhinchus cookii*, *Lepidamia kalosoma* from the present study, 10 conspecific, and 1 congeneric sequences reported in GenBank from different regions of the world. The phylogeny shows that one sequence of *O. cookii* collected from the Bay of Bengal formed a single clade with the sequence of India and Madagascar with 100 bootstarp value (Fig. 5). Two distinct clades were formed containing the sequence of Australia in one clade, and China, Taiwan sequences in another clade. Genetic distance between Bangladesh, and Madagacar and Indian sequences is 1.55–2.13% (SE ± 0.005–0.006). Genetic distance between Bangladesh and Australia sequences is 6.88% (SE ± 0.012). China and Tawan sequences showed the highest genetic distances from Bangladesh sample calculating 9.30–10.23% (SE ± 0.015). These apparent populations need further study.

One the other hand, two specimen of *L. kalosoma* of the present study have got identical nucleotide sequence (i.e., single haplotype). In the phylogeny, two specimen collected from Bangladesh (Bay of Bengal) formed a separate clade with Taiwan (KU943672) and Indoinesia (HM422431) over 95% bootstrap value (Fig. 5). Genetic distance between the species of Bangladesh, and Taiwan and Indonesia was calculated as 1.2 (SE ±0.005) and 2.8% (SE ±0.007), respectively.

DISCUSSION

Cardinal fish are one of the least studied group in Bngladesh. Among the 38 genera found worldwide, only seven species of three genera have been reported from Bangladesh waters. From the analysis of the present study, we added three more cardinal fishes belonging to the genera *Apogonichthyoides*, *Lepidamia*, *Ostorhinchus* (Table 3).

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*Apogonichthyoides sialis* is identified by the combination of its morphometry and color pattern.This species is confused with *A. pseudotaeniatus* with its colour pattern and morphometry such as: third dorsal spine 2 in head length(2.00–2.45 in *A. pseudotaeniatus*) and smaller dark basicaudal spot, spot diameter 4 in caudal peduncle depth length (4.6–6.1 in *A. pseudotaeniatus*). However, *A. pseudotaeniatus* is probably restricted to the Red Sea, Arabian coast and the Persian Gulf and prefers reefs (Gon and Randall, 2003). *Apogonichthyoides sialis* specimens were collected from the Saint Martin’s Island located at the northern-most part of the Bay of Bengal. Suresh and Thomas (2007) assumed that *A. pseudotaeniatus* reported from the west coast of India could be *A. sialis* (Manjebrayakath et al., 2012)*.* *Apogonichthyoides sialis* also occurs in Brunei, China, the Philippines and Suruga Bay, Japan, west coast of India and Srilanka and Andaman sea of Myanmar (Manjebrayakath et al., 2012; Russel, 2016; Howard, 2018; Psomadakis et al., 2019; GBIF.org., 2020; Froese and Pauly, 2019). So, the present study confirms the first report of *A. Sialis* from the northern Bay of Bengal including Bangladesh.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

AUTHOR CONTRIBUTION

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Kazi Ahsan Habib, Md Jayedul Islam, Najmun Nahar and Amit Kumer Neogi. The first draft of the manuscript was written by Md Jayedul Islam and all authors commented on previous versions of the manuscript. All authors read and approved the final version of the manuscript.

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TABLES

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**Table 2.** Morphometric measurements of the specimen of *Ostorhinchus cookii*, *Lepidamia kalosoma* and *Apogonichthyoides sialis* collected in the present study (*n* = number of individual)

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| --- | --- | --- | --- |
| Morphometric measurements, mm | *L. kalosoma**n* = 2 | *O. cokii**n* = 1 | *A. sialis**n* = 1 |
| Standard length (*SL*) | 83–85 | 62.00 | 64 |
| Proportion of *SL* |
| Body width | 35.29–37.35 | 9.68 | 28.13 |
| Body depth | 15.66–16.47 | 32.26 | 9.38 |
| Body depth at dorsal-fin origin  | 33.73–35.29 | 30.65 | – |
| Body depth at pelvic-fin origin  | 32.53–34.12 | 33.06 | – |
| Body depth at anal-fin origin  | 27.71–31.76 | 32.26 | – |
| Head length | 38.55–41.18 | 37.10 | 39.06 |
| 1st dorsal fin base length | 14.46–15.88 | 16.13 | 14.06 |
| Dorsal-fin base length | 15.66–15.29 | 16.13 | – |
| Pectoral-fin base length | 9.64–8.24 | 6.45 | 3.13 |
| Pectoral-fin length | 25.30–28.24 | 16.13 | 21.88 |
| Pelvic-fin base length | 6.02–4.71 | 4.84 | 3.13 |
| Pelvic-fin length | 21.69–25.88 | 31.77 | 18.75 |
| Anal-fin base length | 10.84–12.94 | 13.71 | 10.94 |
| Caudal-fin base length | 16.47–16.87 | 16.13 | 15.63 |
| Caudal peduncle length | 27.06–30.12 | 25.32 | 17.19 |
| Caudal peduncle depth length | 15.66–16.47 | 15.32 | – |
| Pre-dorsal length | 44.58–56.47 | 41.94 | 42.19 |
| Pre-pectoral length  | 37.35–38.82 | 39.52 | 37.50 |
| Pre-pelvic length | 40.96–42.35 | 38.71 | 34.38 |
| Pre-anal length | 67.05–72.29 | 62.90 | 60.94 |
| Pelvic spine length | 13.25–14.12 | 16.13 | – |
| 1st dorsal-fin spine length  | 2.35–2.41 | 4.84 | – |
| 2st dorsal-fin spine length  | 8.43–10.59 | 9.68 | – |
| 2nd dorsal-fin spine length | 15.66–17.65 | 20.97 | 21.88 |
| 3rd dorsal-fin spine length | 14.12–14.46 | 17.74 | 18.75 |
| 4th dorsal-fin spine length  | 12.05–12.94 | 14.52 | – |
| 5th dorsal-fin spine length | 9.64–10.00 | 10.97 | – |
| 6th dorsal-fin spine length  | 5.88–6.02 | 64.52 | – |
| 1st spine of 2nd dorsal-fin length | 14.46–16.35 | 14.52 | – |
| 1st ray of 2nd dorsal-fin length | 24.10–24.71 | 24.19 | – |
| 1st anal fin spine length | 2.35–2.41 | 3.23 | – |
| 2nd anal fin spine length | 12.05–13.35 | 12.90 | – |
| Longest anal ray length | 19.28–20.00 | 19.35 | – |
| Proportion of head length |
| Inter orbital width | 17.14–21.88 | 21.74 | 20.00 |
| Post orbital length | 45.71–46.88 | 10.87 | 40.00 |
| Eye diameter | 34.29–34.38 | 39.13 | 36.00 |
| Snout length | 17.14–18.75 | 21.74 | 16.00 |
| Upper jaw length | 68.75–77.14 | 47.83 | – |
| Jaw gap | 18.75–20.00 | 26.09 | – |

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FIGURE CAPTIONS

**Fig. 1.** Location of the Saint Martin’s Island of Bangladesh in the northern Bay of Bengal from where fish samples were collected (▼) in the present study.

**Fig. 2.** *Ostorhinchus cookii*,(a) F1802SM-13, *SL* 62 mm; K.A. Habib and M.J. Islam, 16.02. 2018; (b) phographed from Chera Dip southern-most side of Saitn Martin’s Island, Sharif Sarwar.

**Fig. 3.** *Lepidamia kalosoma*, (a)F1803SM-33, *SL* 83 mm, K.A. Habib and M.J. Islam, 21.03.2018; (b) phographed from the Jetty of Saitn Martin’s Island, SM. Atiqur Rahman.

**Fig. 4.** *Apogonichthyoides sialisi*, (a) F1803SM-16, *SL* 64 mm, K.A. Habib and M.J. Islam, 21.03.2018; (b) phographed from the Jetty of Saitn Martin’s Island, SM. Atiqur Rahman.

**Fig. 5.** Neighbor-joining tree for *COI* gene sequences of *Ostorhinchus cookie* and *Lepidamia kalosoma.* Bootstrap supports of >95%are shown above branches. Scale represents genetic distance.

FIGURES

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(a) (b)

Fig. 2

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