NEW GEOGRAPHICAL RECORD OF THREE-SPINE FROGFISH, 
BATRACHOMOEUS TRISPINOSUS (GÜNther 1861) FROM THE RIVER 
RUPSA, BANGLADESH

Md. Sagir Ahmed1*, Sujan Kumar Datta2, Tonmoy Saha2, Ayesha Akhter Zhilik1, 
Nishat Zahan Chowdhury1 and Sumaiya Ahmed2

* Author for Corresponding: <sagir@du.ac.bd>  
2Department of Zoology, Jagannath University, Dhaka 1100, Bangladesh

Department of Zoology, University of Dhaka, Dhaka 1000, Bangladesh

Marine and coastal waters of Bangladesh are rich in ichthyofaunal diversity. There is a record of over 450 species of fin fish including sharks and rays (Rahman et al. 2009). No extensive survey has been done on the ichthyofaunal diversity in marine and coastal waters of Bangladesh. The works so far done indicates the existence much more fauna than described so far (Hussain 1969, 1971, Shafi and Quddus 1983).

The family Batrachoididae consists of 25 genera and 78 species (Greenfield 2008). This is the only family of the order Batrachoidiformes which belongs small to medium-sized fishes (max 57 cm). They are easily recognized by their peculiar head shape and a tapering body. The head is large, broad and flattened often with barbels and/or fleshy flaps around their large mouths. The genus Batrachomoeus was given by Ogilby in 1908 which was the revision of Pseudobatrachus by Castelnau (1875) and Pelophiletor by Ogilby (1906). Most of the members of the family, Batrachoididae, can make a croaking sound when they are pestered or the males are courting females, hence they are named frogfish. The species of this genus distributed in Indo-West Pacific: northwestern Australia, the Arafura Sea (Russell and Houston 1989), Thailand, Indonesia and Papua New Guinea (Rainboth 1996). These toadfish inhabit in a variety of habitats including intertidal flats near mangroves, estuaries, and reefs, preferably the muddy bottoms of estuaries.

This paper deals with the occurrence of the three-spine frogfish, B. trispinosus, from the coastal area of Bangladesh. The identity of B. trispinosus was confirmed based on morphometric and molecular (DNA barcoding) approaches.
Five specimens of *B. trispinosus* were collected from the fishermen on April 20, from Rupsa river, Dacop, Khulna (22°34’18.9” N 89°33’16.2” E) that is connected with the mangroves of Khulna region. The specimens were preserved in ice immediate after collection and carried to the DNA Barcoding lab, Department of Zoology, University of Dhaka and kept frozen (-18°C) until further use. Taxonomic identification of the specimens was performed based on morphometric and meristic characteristics following the guideline of Hutchins (1976). The measurements were taken in cm scale. For genomic DNA extraction about 20 mg of tissue was taken from each specimen. The specimens were examined in fresh to the nearest 0.1mm, fixed in 10% formalin for two weeks and then transferred to 70% ethanol solution for long term preservation. The specimens were stored in the Professor Kazi Zaker Husain Museum of the Department of Zoology, University of Dhaka (Voucher ID No. DUZM_MF_121B, DUZM_MF_121B.2, DUZM_MF_121B.3, DUZM_MF_121B.4, DUZM_MF_121B.5).

Genomic DNA was extracted from the muscle tissue samples by the standard Proteinase-K/Phenol-Chloroform-isoamyl alcohol method (Green & Sambrook 2012; Ahmed et al. 2019). Quantification and visualization of extracted DNA, PCR amplification of target MT-COI gene, purification of amplified PCR products was performed following the procedure described by Ahmed et al. (2019). Approximately 658 bp was amplified from the 5′ region of the MT-COI gene using the following primers: FishF1 5′ TCAACCAACCACAAAGACATTGGCAC 3′ and FishR1 5′ TAGACTTCTGGGTGGCCAAAGAATCA 3′ (Ward et al. 2005). The purified products were sequenced bidirectionally using an ABI 3730 automated sequencer following the manufacturer’s instructions. The raw sequences were edited manually using MEGA X. The sequence was verified by BLAST and only the best matches (>98%) were submitted to the NCBI GenBank.

*Morphological description:* The body of *B. trispinosus* is cylindrical, compressed posteriorly and head considerably depressed with moderate snout (Fig. 1). Mouth wide, lower jaw protruding and gape generally horizontal. Maxilla extends to below posterior half of eye or even further. The eyes are large with concave or flat interorbital space and the grooves in frontal bone wide and deep. Sub-operculum with one spine, either equal to or somewhat shorter than the upper opercular one. A flexible spine always presents on upper base of sub-opercular spine, extend into near tip of upper-opercular spine (Hutchins 1976). The moderate size of gill opening encircles the base of pectoral fin. Body scaleless, smooth and slimy skin loosely attached to head, body and fins. Tentacles on the head is considerably reduced and unequal.
Table 1. Biometry of B. trispinosus in mm and % to SL

| Characteristics                      | Specimen 1 | Specimen 2 | Specimen 3 | Specimen 4 | Specimen 5 | % to SL |
|--------------------------------------|------------|------------|------------|------------|------------|---------|
| Total length                         | 197        | 155        | 154        | 168        | 151        |         |
| Standard length                      | 162        | 127        | 128        | 137        | 125        |         |
| Head length                          | 62         | 41         | 46         | 46         | 41         | 32.3-38.3 |
| Head width                           | 52         | 38         | 38         | 32         | 29         | 23.2-32.1 |
| Head depth                           | 29         | 25         | 25         | 27         | 22         | 17.6-19.7 |
| Bony inter orbital length            | 10         | 8          | 9          | 9          | 8          | 06.2-07.0 |
| Fleshy inter orbital length          | 21         | 15         | 15         | 16         | 15         | 11.7-13.0 |
| Orbital diameter                     | 8          | 6          | 5          | 6          | 5          | 03.9-04.9 |
| Snout length                         | 12         | 8          | 6          | 9          | 6          | 04.7-07.4 |
| Upper jaw length                     | 45         | 38         | 41         | 42         | 36         | 27.8-32.0 |
| Mouth width at rictus                | 37         | 27         | 26         | 35         | 26         | 20.8-25.5 |
| Predorsal fin distance               | 67         | 51         | 45         | 58         | 45         | 35.2-42.3 |
| Dorsal fin base length               | 91         | 72         | 71         | 80         | 71         | 55.2-58.4 |
| Preanal fin distance                 | 107        | 81         | 80         | 91         | 82         | 62.5-66.0 |
| Greatest body depth                  | 40         | 26         | 28         | 31         | 29         | 20.5-24.7 |
| Caudal peduncle depth                | 16         | 13         | 14         | 15         | 14         | 09.9-11.2 |
| Caudal peduncle length               | 6          | 5          | 5          | 5          | 4          | 03.2-03.9 |
| Anal fin base length                 | 65         | 43         | 46         | 51         | 50         | 33.9-40.1 |
| Caudal fin length                    | 35         | 28         | 26         | 31         | 26         | 20.3-22.6 |
| Pectoral fin length                  | 43         | 33         | 31         | 31         | 31         | 22.6-26.5 |
| Pelvic fin length                    | 35         | 25         | 26         | 26         | 27         | 19.0-21.6 |
| Pelvic fin distance                  | 23         | 15         | 20         | 22         | 17         | 11.8-16.1 |
| 1st dorsal spine length              | 5          | 5          | 5          | 4.5        | 4          | 03.0-03.9 |
| 2nd dorsal spine length              | 10.5       | 8          | 8          | 8.1        | 8          | 05.9-06.4 |
| 3rd dorsal spine length              | 8          | 7          | 7.5        | 7          | 7          | 04.8-05.9 |

Dorsal fin spinous covered by thick skin and a low fold of skin joined it to soft dorsal. All soft dorsal rays and anal rays are almost equal in length except the last 2-3. Rounded pectoral and caudal are almost equal in length. Ventral fin consists of one hidden spine, one fleshier ray and one branched ray having 3-4 branches.

The irregular dark brown transverse bars and blotches all over the pale brown body and head make distinct appearance of this fish (Fig. 1A). The bars are well
contrasted with the base color and sharply separated usually on posterior part of the body. The abdomen and lower surface of head with uniform creamy to pale brown. Fins pale brown with series of dark brown irregular transverse bars which is prominent on pectoral (Fig. 1C) and caudal fins. Body bars are continuous on soft dorsal and anal fins. Counts and measurements of specimens are provided in tables 1 & 2.

Table 2. Morphometrics of *B. trispinosus* in comparison with previous record (Hutchins, 1976)

| Characteristics                        | Hutchins, 1976 | Present Study |
|----------------------------------------|----------------|--------------|
| Head Length in SL                      | 2.4-2.7        | 2.61-3.09    |
| head depth in SL                       | 3.9-5.4        | 5.07-5.68    |
| Pectoral in SL                         | 4.2-4.7        | 3.77-4.42    |
| Eye in head length                     | 4.0-7.2        | 6.83-7.75    |
| Snout in head length                   | 5.9-8.4        | 5.11-7.66    |
| head depth in head length              | 1.6-2.0        | 1.6-2.14     |
| head width in head length              | 1.1-1.4        | 1.07-1.44    |
| head length in anal base               | 0.9-1.0        | 0.9-1.0      |
| Interorbital in eye                    | 0.9-2.6        | 1.25-1.80    |
| first dorsal spine in eye              | 1.3-2.1        | 1.2-1.6      |
| Dorsal rays                            | III+21-23      | III+21       |
| Pectoral rays                          | 22-23          | 22-23        |
| Anal rays                              | 17-20          | 17           |
| Caudal rays                            | 14-16          | 14-15        |

Fig. 1. *Batrachomoeus trispinosus*. (A) Whole specimen (B) Unequal tentacles and (C) Bars on pectoral fin.

**Diagnosis:** No scale and maxillary flap; foramen at top of pectoral fin axil a distinct round hole, not funnel shaped and lacking glandular tissue on ventral
margin; supraorbital tentacles present and is unequal (Fig. 1B); anterior nasal tentacle not elongate; lower gill opening at lower pectoral-fin base; subopercle with one strong spine and one filament.

**Molecular characterization:** We have generated partial sequence of COI gene from the three different individual specimens and submitted to the GenBank (NCBI) through individual submission system. The partial sequence of mitochondria COI gene produced a mean value of 617 nucleotide base pairs. BLAST tool was used to match the sequences with the pre-deposited sequences in the GenBank. Our obtained sequence of *B. trispinosus* (accession no MN234104, MN234105 and MN234107) showed 99.68% identity with pre-existing sequence of accession number AP006738 (University of Tokyo, Japan). The COI sequences having 98-100% similarity can confidently be considered as the same species in DNA barcoding concept. Thus, our molecular characterization supplements the morpho taxonomic identification of this species. The taxonomic identity and the occurrence of *B. trispinosus* from Rupsa river, Khulna also suggested further research on its biology, abundance and conservation status in region.

**Remarks:** Identification of *B. trispinosus* can easily be confused with *B. dahli* and *B. dubius* based on their body color and appearance. In case of *B. trispinosus*, the last three body bars usually well separated rather than they are joined medially in *B. dahli*. Moreover, *B. trispinosus* recorded from Bangladesh can be distinguished from *B. dahli* having head length 0.9-1.0 in length of anal fin base where in later 0.7-0.8 (Hutchins, 1976) and relatively wider interorbital. *B. trispinosus* can be distinguished from *B. dubius* having 21-23 soft dorsal fin rays in former and 19-20 in later.

This species is locally known as ‘Gongonia’ and usually consumed by the lactating mothers. It is locally believed that consuming this fish can increase the breast milk secretion of a lactating mother.

**Acknowledgements:** We acknowledge the financial support from the Ministry of Education, Government of the People’s Republic of Bangladesh as a grant for advanced research in Education (Grants No. MRS2017448). We are also thankful to Mr. Abdus Salam, Manager Administration, HEED Bangladesh for his kind cooperation during sample collection.
LITERATURE CITED

AHMED, M.S., CHOWDHURY, M.M.K. and NAHAR, L. 2019. Molecular characterization of small indigenous fish species (SIS) of Bangladesh through DNA barcodes. *Gene*, **684**: 53-57.

CASTELNAU, F.L. de, 1875. Researches on the fishes of Australia. Official Record, Philadelphia Centennial Exhibition of 1876 (Melbourne 1875): Intercolonial Exhibition Essays 1875-6. **2**:1-52.

GREENFIELD, D.W., WINTERBOTTOM, R. and COLLETTE, B.B. 2008. Review of the Toadfish Genera (Teleostei: Batrachoididae). *Proceedings of the California Academy of Sciences*. **59**(15):665–710.

GUNTER, A. 1861. Catalogue of the acanthopterygian fishes in the collection of the British Museum. 3. London: The Trustees of the British Museum (Natural History).

HUSSAIN, M.M. 1969. Marine and estuarine fishes of the north-east part of Bay of Bengal. *Sci. Res.* **7**(1): 26-55.

HUSSAIN, M.M. 1971. The commercial fishes of the Bay of Bengal, survey for the Development of Fisheries in East Pakistan. UNDP Project PAK 22, Project Publication No. **1**:60.

OGILBY, J.D. 1906. Honorary Curator’s report. *Amat. Fisherm. Assoc. Qd A. Rep*. 1905-6:3-13.

OGILBY, J.D. 1908. Revision of the Batrachoididae of Queensland. *Ann. Qd. Mus.* **9**: 43-57.

QUDDUS, M.M.A. and SHAHI, M. 1983. *Bangopasagarer Matsya Sampad* (Fisheries Resources of the Bay of Bengal) in Bangla. Bangla Academy, Dhaka, Bangladesh. 426 p.

RAHMAN, A.K.A., KABIR, S.M.H., AHMAD, M., AHMED, A.T.A., AHMED, Z.U., BEGUM, Z.N.T., HASSAN, M.A. and KHONDKER, M.(eds) 2009. *Encyclopedia of Flora and Fauna*. Vol. 24. Marine Fishes. Asiatic Society of Bangladesh. 485pp.

RAINBOTH, W.J. 1996. *Fishes of the Cambodian Mekong*. FAO species identification field guide for fishery purposes. FAO, Rome, 265 pp.

RUSSELL, B.C. and HOUSTON, W. 1989. *Offshore fishes of the Arafura Sea*. Beagle **6**(1):69-84.

SAMBROOK, J. and RUSSELL, D.W. 2001. *Molecular cloning: A laboratory manual*. 3rd ed., Cold Spring Harbor Lab. Press, NY; 2344 pp.

(Manuscript received on 12 June, 2020 revised on 25 June, 2020)