

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

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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.

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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. trspinosus* in mm and % to SL

Characteristics	Specimen					% to SL
	1	2	3	4	5	
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

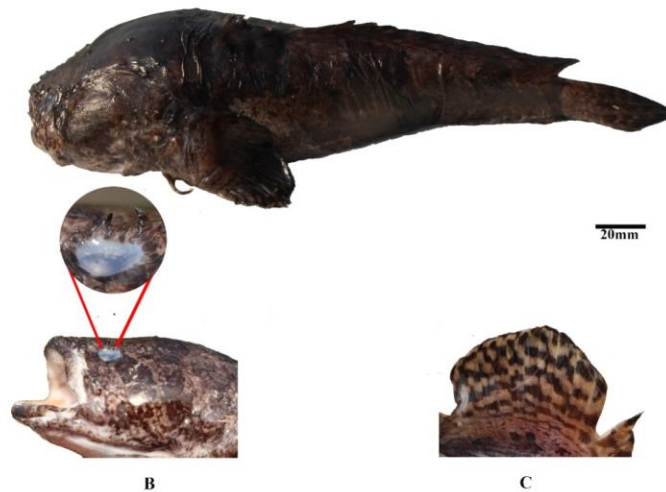


Fig. 1. *Batrachomoeus trispinosus*. (A) Whole specimen (B) Unequal tentacles and (C) Bars on pectoral fin.

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.

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