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Research Article

Geographic range extension of zebra mantis shrimp *Lysiosquilla maculata* (Fabricius 1793) (Stomatopoda: Lysiosquillidae) through DNA barcoding from the Bay of Bengal, Bangladesh

Md. Sagir Ahmed*, Tonmoy Saha[1], Saikt Rahman[2], Durjoy Raha Antu[1] and Sujan Kumar Datta

Department of Zoology, Faculty of Biological Sciences, University of Dhaka, Bangladesh

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ABSTRACT

A new record of zebra mantis shrimp *Lysiosquilla maculata* (Fabricius 1793) was confirmed for the first time from the Bay of Bengal, Bangladesh. Morphological and molecular investigations were used to validate this new record. It possesses ten teeth on the dactylus of raptorial claw that can be differentiated easily from its congener species. A partial large subunit ribosomal RNA (16S rRNA) gene sequence was generated and revealed 100% similarity with pre-existing sequences that validated the morphological identification of the species. Including present species, the number of mantis shrimp has increased to nine in Bangladesh. Further extensive study on its biology, ecology, and conservation is highly recommended.

Introduction

Bangladesh is situated on the Ganges Delta and the Bay of Bengal stands in the southern portion of the country. The total marine area of Bangladesh is about 207,000 sq. km with a 711 km coastline rich with aquatic faunal diversity (Minar et al., 2013).

Stomatopods are marine crustaceans usually known as 'mantis shrimp' because of their large and powerful raptorial appendages. Worldwide, there are over 500 species of stomatopods that are reported to belong to over 120 genera, 18 families, and seven superfamilies (Ahyong, 2012). Since 2022, Lysiosquillidae has consisted of three genera *viz. Lysiosquilla* (Dana, 1852), *Lysiosquillina* (Manning, 1995), and *Lysiosquilloides* (Manning, 1977). Recently, Ahyong & Lin (2022) reassessed the status and composition of the lysiosquillid genera by cladistic analysis using all known species in the family. They found species transitional between *Lysiosquilla* and *Lysiosquillina*, which challenges the validity of the present generic system and recommends *Lysiosquillina* be synonymized with *Lysiosquilla*. *Lysiosquilla glabriuscula* (Lamarck, 1818), *L. lisa* (Ahyong and Randall, 2001), *L. maculata* (Fabricius, 1793), and *L. sulcata* (Manning, 1978) are the valid documented species from the family Lysiosquillidae. *L. maculata* is the largest mantis shrimp in the world with a length reaching up to 38.5 cm (Manning, 1978; Ahyong, 2001). It is distributed across the Indo-Pacific, ranging from East Africa to the Galápagos, Japan, Australia, and the Hawaiian Islands (Ahyong, 2001).

Stomatopods are economically utilized in various regions globally, with significant fisheries activity focused on *Squilla mantis* (Linnaeus, 1758) in the Mediterranean and *Oratosquilla oratoria* (de Haan, 1844) in Japan (Ahyong, 2001). Recently, these creatures have gained recognition as valuable bioindicators for assessing the impact of marine pollution stress on coral reefs (Erdmann and Caldwell, 1997). This newfound role impetus to understand the taxonomy and systematics of stomatopods.

*Corresponding author:<sagir@du.ac.bd>

[1]*Department of Zoology, Faculty of Life and Earth Science, Jagannath University, Bangladesh*

[2]*Department of Genetic Engineering and Biotechnology, Faculty of Biological Sciences,University of Dhaka, Bangladesh*

According to Ahmed et al. (2008) 185 crustacean species were documented from Bangladesh and the threat status of 141 species of crustaceans was assessed (IUCN Bangladesh, 2015). Eight species of mantis shrimps so far reported from Bangladesh water including *Acanthosquilla multifasciata* (Wood-Mason, 1895), *Bigelowina phalangium* (Fabricius, 1798), *Clorida decorata* (Wood-Mason, 1875), *C. latreillei* (Eydoux and Souleyet, 1842), *Harpiosquilla harpax* (de Haan, 1844), *H. raphidea* (Fabricius, 1798), *Oratosquillina interrupta* (Kemp, 1911) and *O. perpensa* Kemp, 1911) (Tabassum and Akash, 2022). *Oratosquillina perpensa* (Kemp, 1911) is the only species that is enlisted in these two recent vast documentations of crustaceans' diversity Bangladesh (Ahmed et al., 2008; IUCN Bangladesh, 2015).

Lysiosquilla maculata as *Squilla maculate* was first described from the Indian Subcontinent (Fabricius, 1793) and the species was reported as *Lysiosquilla maculate* Kemp (1913) from several location of Indian water. Apart from this, Lyla et al. (1997), Roy and Gokul (2012), and Babu et al. (2023) reported the species from Indian waters. This paper represents the first documentation of *Lysiosquilla maculata* from the Bangladesh based on morphological and molecular characteristics.

Materials and Methods

Sampling and Morphological Analysis

Two specimens of *Lysiosquilla maculata were* collected on December 10, 2022 from Dublar Char, Sundarban area, (21.758693 N 89.616616 E) Bagerhat and one from Cox's Bazar (21.238105 N 91.756600 E) on September 4, 2022. Specimens were caught as a bycatch during pelagic fishing.Following collection, the Samples were quickly preserved in ice and transported to the Advanced Fisheries and DNA Barcoding Laboratory at the Department of Zoology, University of Dhaka. Photograph in fresh condition was taken, and kept in the refrigerator (-18℃) for further analysis. Taxonomic identification of the specimen was conducted following Manning (1978) and Ahyong (2001). A portion of tissue (20mg) was transfer to a vial for genetic analysis, tagged the specimen as DUZM_CR_084B, DUZM_CR_084B.2, DUZM_CR_084B.3, and deposited at Dhaka University Zoology Museum.

Extraction and PCR amplification of genomic DNA

DNA was extracted using a Monarch® Genomic DNA Purification Kit (USA) following the manufacturer's instructions. A NanoDrop spectrophotometer was utilized to evaluate the quality and quantity of the extracted DNA. The contigs were amplified using polymerase chain reaction (PCR) with the primers 16Sar 5′-CGCCTGTTTATCAAAAACAT-3′ and 16Sbr 5′-CCGGTCTGAACTCAGATCATGT-3′ (Palumbi et al., 1991). The amplification protocol included an initial denaturation at 95°C for 5 minutes, followed by 35 cycles of 94°C for 45 seconds, 48°C for 30 seconds, 72°C for 45 seconds, and a final extension at 72°C for 7 minutes.Amplified gene bands were visualized on a 1% agarose gel. PCR purification and sequencing were performed by an outsourcing company (Celemics Inc., Korea).

Bioinformatics analysis

The quality of the generated sequences was viewed using CHROMAS software. The sequence was confirmed by BLASTn search against the best-matching sequences in the nucleotide database and deposited in NCBI GenBank. Phylogenetic tree was constructed based on the neighbor-joining (NJ) statistical method with gamma distribution rates by bootstrap analysis with 1000 replicates in MEGA 11 (Tamura et al., 2021) and iTOL v5 (Letunic and Bork, 2021). Sequences of this species and some other species that were found in Bangladesh (not barcoded yet) were downloaded from GenBank to compare with the present species.

Results and Discussion

Systematic account

Order: Stomatopoda
Suborder: Unipeltata
Family: Lysiosquillidae
Genus: *Lysiosquilla*

***Lysiosquilla maculata* (Fabricius, 1793)**

Material examined: Bangladesh: Cox's Bazar, BFDC fish landing centre, 21.238105 N 91.756600 E, 4-September-2022, 1 female (TL: 92.0 mm); and Dublar char, Sundarban area, 21.758693 N 89.616616 E, 10-December-2022, 2 females (TL: 100.1 and 96.0 mm); DUZM_CR_084B & DUZM_CR_084B.2, DUZM_CR_084B.3

Diagnostic Character: The outer scale of *Lysiosquilla maculata* (Fabricius, 1793) is triangular, and erect and the anterior portion of the outer scale is inclined (Fig. 1). The rostrum shape is cordiform but sometimes sub-triangular in shape, and the length is smaller than the width.

Usually, 10 teeth are present in the dactylus of raptorial claw, but this number may be varied from 10 to 11 in larger females. TS8 sternal keel round shape. Mandibular palp present and three segment presents on mandibular palp. 7–9 movable spines present on the outer margin of the uropodal exopod proximal segment and triangular lobes with ventro laterally anterior to each uropod's articulation make up the uropodal protopod. Distal ¾ of the uropodal endopod is black in color.

Fig. 1. Dorsal view of *Lysiosquilla maculata* (TL: 92 mm) voucher ID DUZM_CR_084B, collection date: 4-September-2022, place: Cox's Bazar, Bangladesh.

Description

Large eye with bilobed cornea, set slightly obliquely on stalk. The carapace is convex and broad in size. Antennular peduncle short, about ½ of the carapace. The antennal scale is broad and curved with a large dark spot on the surface. The rostrum shape is cordiform but sometimes sub-triangular in shape, and the length is smaller than the width. The representation of blunt longitudinal carina on the anterior third plate. Dactylus of raptorial claw with 8-11 teeth (usually 10-11). The number and size of teeth reduced in large females. Propodus and carpus are comparatively more inflated in large females and males. The ventral keel of the eighth thoracic somite rounded. Abdomen smooth, unarmed. Sixth somite smooth medially, with low, broad lateral boss flanked mesially by a shallow longitudinal groove. Telson is much broader than long, smooth or slightly wrinkled. About 3-4 pairs of fixed projections in the posterior margin of the telson. Basal segment of uropod with slender dorsal spine. The proximal segment of the exopod is shorter than the distal, with 8 movable spines, distalmost not exceeding midlength of distal segment. Endopod broad, length about or less than twice greatest width.

Coloration

During collection, Pale yellow dorsum base with black transverse bands was noted. A carapace with three dark transverse bands intervened by pale bands of about the same width. Distal ½ of the proximal segment and proximal 2/3 of the distal segments are black in uropodal exopod. About ¾ of the distal uropodal endopod is black in color.

Measurement

Three female specimens (TL 92-100.1 mm) were collected and morphometric measurements were taken. Morphometric data of the specimens are given in Table 1.

Table 1. Morphometric data of the specimens of *Lysiosquilla maculata* (DUZM_CR_084B, DUZM_CR_084B.2 and DUZM_CR_084B.2) and comparisons with other species.

Characteristic	Mean Length (mm) n=3	% to TL	*L. lisa* (Ahyong and Randall, 2001)	*L. maculata* (Ahyong, 2001)	*L. sulcata* (Manning, 1978)
Total Length	96.03		192-308	81-335	41-150
Carapace Length	18.95	19.73			
Eye Diameter	05.33	05.55			
Dactylus length	21.87	22.78			
Telson Length	12.90	13.44			
Telson Wide	19.29	20.09			
Abdomen Length	51.51	53.64			
Thorax Length	18.37	19.13			
Teeth on Raptorial claw dactylus	10		9-10	7-11 (usually 10-11)	7-8
Color of uropodal endopod	Dark		Dark Brown	Dark	Light/Pale
Color ratio of endopod	¾ dark			¾ dark	

Remarks

This species is widespread in the Indo-Pacific, ranging from East Africa to the Hawaiian Islands and the Galápagos (Ahyong, 2001). Recently Babu et. al. (2023) reported this species from the Chennai coast, Tamil Nadu, India. *L. maculata* is very close to *L. sulcirostris* and *L. sulcata.* The two species can be differentiated easily through the teeth on raptorial claw dactylus and colouration of the uropodal endopod. *L. maculata* has 10 teeth on the raptorial claw dactylus whereas *L. sulcirostris* has 7-8 teeth. On the other hand, the distal half of the uropodal endopod is dark in *L. maculata* but not in *L. sulcata*.

Molecular Analysis

One partial large subunit ribosomal RNA gene sequence of 473 bp was generated and submitted to the NCBI GenBank database with an accession number PP065678. The BLAST search results have shown 100% query coverage with the pre-existing sequences of *L. maculata* (MT490885) that was submitted from Japan which validated the morpho-taxonomic identification of the species. The nucleotide base frequencies were found as T (31.71%), C (12.68%), A (34.88%) and G (20.72%). The percentage of GC was found lower than AT and was 33.41% and 66.59%, respectively. To compare with other species of squilla found in Bangladesh (not barcoded yet, collected from NCBI) were used in this study from GenBank. *Lysiosquilla sulcirostris* was found as the nearest species of *L. maculata* with a distance of 8.01% and the divergence with the other species was more than 13% (Table 2).

A Neighbor-Joining (NJ) phylogenetic tree was constructed using a total of 10 sequences belonging to 9 species of mantis shrimp. One sequence obtained from this study was designated as DUZM, and the remaining nine sequences were retrieved from the NCBI database (Fig. 2). In the phylogenetic tree, *L. maculata* forms a distinct clade with the same species from Japan with a 100% bootstrap value (Fig. 2).

Table 2. Genetic divergence (% K2P distance) of *L. maculate* with other species.

Species		Interspecies Distance %
Lysiosquilla maculata	*Lysiosquilla sulcirostris*	8.01
	Oratosquilla perpensa	13.42
	Oratosquilla oratoria	13.82
	Clorida decorata	13.84
	Harpiosquilla harpax	15.33
	Acanthosquilla multifasciata	18.09
	Bigelowina phalangium	18.81

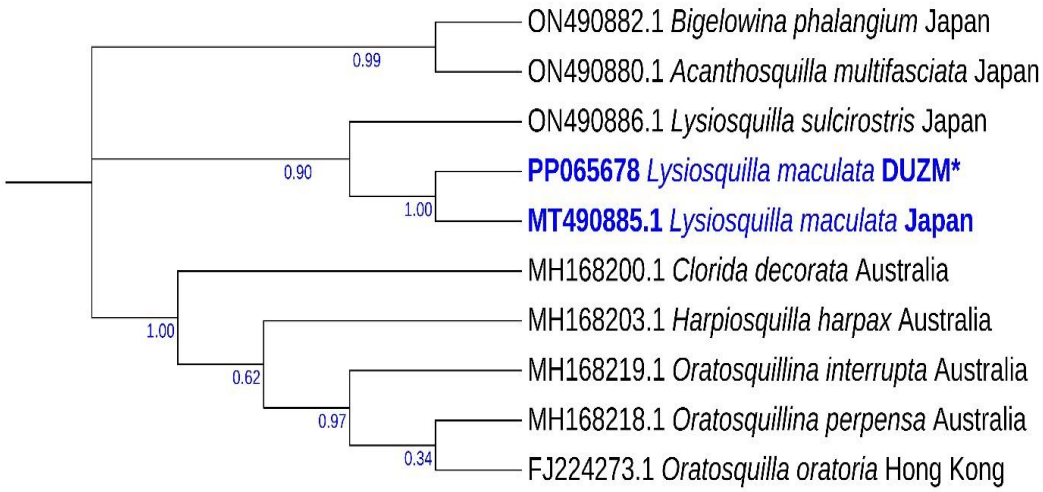

Fig. 2. Phylogenetic analysis of *Lysiosquilla maculata* with closely related species by the Neighbor-joining method in MEGA 11 and iTOL v5. DUZM* represents the generated sequence of the present study.

Conclusions

Classical taxonomy based on morphometric and meristic characters, along with DNA barcoding based on 16S rRNA marker confirms the taxonomic identity and the first occurrence of *L. maculata* from the Bangladesh coast. It is suspected that this species could be distributed throughout the entire coastal belt of the country. More extensive research is needed to know about its biology, local distribution and population status.

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Author contributions

Md. Sagir Ahmed: Conceptualization, Supervision, Methodology, Data curation, Writing - original draft, Writing - review & editing. **Tonmoy Saha, Saikt Rahman and Durjoy Raha Antu:** Field collection, Data curation, Formal analysis, Writing - original draft, Writing - review & editing. **Sujan Kumar Datta:** Conceptualization, Methodology, Field collection, Laboratory analysis, Data curation & analysis, Writing - original draft, Writing - review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Ethical Approval

No ethical approval was required as the studied animals were not listed in CITES appendices I or II or in the threatened categories of the IUCN Red List Species.

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