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GEOGRAPHICAL RANGE EXTENSION OF TWO PENAEID SHRIMP *PENAEUS PULCHRICAUDATUS* STEBBING, 1914 AND *KISHINOUYEPENAEOPSIS INCISA* (WANG & LIU *IN* LIU & WANG, 1987) IN BANGLADESH WATERS

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Abstract

Shrimps are among the most intriguing groups within the Decapoda order. In Bangladesh, penaeid shrimps hold significant economic value as a major export commodity. This study aimed to analyze the morphometric and molecular characteristics of penaeid shrimps in Bangladesh. Two species of penaeid shrimp, *Penaeus pulchricaudatus* and *Kishinouyepenaeopsis incisa* were identified as new records in Bangladesh waters. A distinguishing feature of *P. pulchricaudatus* is its dark brown transverse bands that do not extend to the lower half of the carapace. The identification of these species was confirmed using both morphometric characteristics and DNA barcoding techniques. Two sequences were generated for these species utilizing the 16S rRNA gene marker. The findings reveal two new records of shrimp species in Bangladesh, expanding the known diversity of shrimp in the region and highlighting the presence of previously undocumented species.

Introduction

Crustacean fisheries represent a crucial resource for Bangladesh, including commercially important species like shrimp, prawns, lobsters, and crabs. These species are vital to the food chain within tropical marine ecosystems. Worldwide, more than 30,000 marine crustacean species have been recorded(1). The Penaeidae family, commonly referred to as penaeid shrimp or prawn, encompasses 48 recognized genera, 23 of which are known solely from fossils. In Bangladesh, a total of 64 prawn and shrimp species from 8 families have been documented, with the Penaeidae family alone comprising 24 shrimp species, primarily originating from marine and coastal environment(2).

Shrimp is a major export commodity for Bangladesh. The total production of shrimp and prawn, including capture, has increased from 140,000 MT to 2,61,154 MT over the past 20 years, from 2000-01 to 2020-21(3). Among them 47,606 MT are marine production and the

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penaeid shrimp[3]. In 2020, Bangladesh exported 30,036.18 MT of frozen shrimp, generating approximately BDT 2,948.94 crore in revenue[4]. The prawn and shrimp sector accounts for 74.05% of the total export earnings from fisheries products[4]. The industry employs 1.2 million people directly in production, with an additional 4.8 million household members involved in the sector.

The Penaeidae family is distinguished by a range of sizes from small to large and features five pairs of well-developed legs, with the first three pairs forming pincers, none of which are notably large. The abdomen's posterior pleura overlap the anterior pleura of the next segment. Males have a prominent copulatory organ on the first pair of pleopods, known as the petasma, while females have a copulatory organ on the posterior thoracic sternites, called the thelycum. Eggs are released directly into the water and are not retained by the females. This paper presents the first documentation of two penaeid shrimp species, *Penaeus pulchricaudatus* Stebbing, 1914, and *Kishinouyepenaeopsis incisa* (Wang & Liu in Liu & Wang, 1987), from the marine waters of Bangladesh based on both morphological and molecular characteristics.

Materials and Methods

Sampling and Morphological Analysis

A specimen of *Penaeus pulchricaudatus* was collected on November 28, 2020 from Teknaf, (20.728 N 92.351 E) and another one specimen of *Kishinouyepenaeopsis incisa* on May 4, 2018 from Kuakata (21.847875 N 90.059220 E). Specimens were caught as a bycatch during pelagic fishing in the Bay of Bengal. After the collection, samples were immediately preserved in ice and transfer it to the Advanced Fisheries and DNA Barcoding lab, Department of Zoology, University of Dhaka. Fresh condition photographs were taken before the samples were stored in a refrigerator at -18°C for further analysis. Taxonomic identification of the specimen was conducted following Liu and Wang (1987)[5] and Tsoi et al., (2014)[6]. A portion of tissue (20mg) was transfer to a vial for genetic analysis, tagged the specimen as DUZM_CR_107B *(Kishinouyepenaeopsis incisa)* and DUZM_CR_094BS *(Penaeus pulchricaudatus)* and deposited at Kazi Zaker Hossain Zoological Museum.

Extraction and PCR amplification of genomic DNA

Using a Qiagen® Dneasy Blood & Tissue Kits (USA) and the manufacturer's instructions, DNA was extracted. A NanoDrop spectrophotometer was used to assess the extracted DNA's quality and quantity. The contigs were amplified using polymerase chain reaction (PCR) with the primers 16Sar (forward) 5′-CGCCTGTTTATCAAAAACAT-3′ and 16Sbr (reverse) 5′-CCGGTCTGAACTCAGATCATGT-3′[7]. The amplification protocol consisted of 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. The amplified gene bands were visualized on a 1% agarose gel. PCR purification and sequencing were carried out by Celemics Inc., Korea (outsourcing company).

Bioinformatics analysis

The quality of the generated sequences was assessed using CHROMAS software. Sequence confirmation was performed by conducting a BLASTn search against the best-matching sequences in the nucleotide database, and the sequences were subsequently deposited in the NCBI GenBank. A phylogenetic tree was constructed using the neighbor-joining (NJ) statistical method with gamma distribution rates, employing bootstrap analysis with 1000 replicates in MEGA 11[8] and iTOL v5[9].

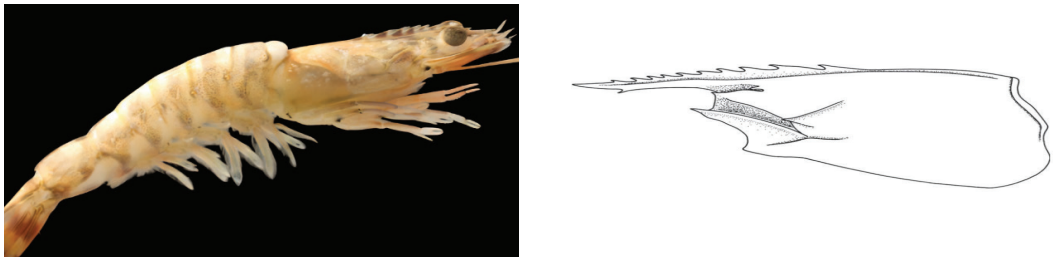
Results and Discussion

Taxonomy

Penaeus pulchricaudatus Stebbing, 1914

Material examined

Teknaf, Bangladesh, 20.728 N 92.351 E, 28 November, 2020, carapace length 37.2 mm, DUZM_CR_094BS (deposited at the Kazi Zaker Hossain Zoological Museum), 16S GenBank accession number MW483130



A B

Fig. 1. *Penaeus pulchricaudatus* Stebbing, 1914, A. A specimen from Teknaf, Bangladesh B. Carapace[10]

Description

The carapace features well-developed ridges and grooves, devoid of longitudinal and transverse sutures. The rostrum has 9 dorsal teeth, with 3 on the carapace and 1 ventral tooth (Fig. 1 A,B). The integument is smooth. The postrostral carina nearly extends to the posterior carapace, featuring a deep median groove along its length. The adrostral groove is as wide as the postrostral carina and extends close to the posterior carapace. There is no postocular sulcus. The gastrofrontal groove is distinct, with the posterior end divided into two. The cervical carina is sharp, accompanied by a well-marked groove. The hepatic spine is very pronounced, and the hepatic carina is well-marked, curved, and ventrally inclined at the anterior part. The ischial spine of the first pereiopod is either absent or barely visible. The sixth abdominal somite lacks a dorsolateral groove and has three cicatrices. The thelycum is double-tubed and pouch-like, opening anteriorly. The spermatophore is deposited on the thelycum as a large subtriangular wing-like process. The telson has three sets of movable lateral spines. The morphometric measurement was shown in Table 1.

Table 1. Biometry of *Penaeus pulchricaudatus* and *Kishinouyepenaeopsis incisa* (in mm and % to TL)

Characteristics	*Penaeus pulchricaudatus*		*Kishinouyepenaeopsis incisa*	
	Measurement (mm)	**% to total length**	**Measurement (mm)**	**% to total length**
Total length	137.7		77.5	
Ocular/Body length	122.2	88.74	65.4	84.39
Abdomen length	85.0	61.73	47.9	61.81
Carapace length	37.2	27.02	17.5	22.58
Rostrum length	15.5	11.26	12.1	15.61
Telson length	15.2	11.04	8.1	10.45
Rostral formula				

Color

The body is pale yellowish, marked with dark brown transverse bands. These bands extend from the top to about the middle of the carapace, with the rearmost band on abdominal somite VI being interrupted. The eyes are black-brown. The scaphocerite has a somewhat greenish hue with white tips, while the antennal flagella range from reddish-brown to yellowish-brown. The pereiopods are whitish to yellowish, and the pleopods are yellowish to reddish, featuring brown and white spots at the bases.

P. pulchricaudatus is almost identical to *P. japonicus*, but can be differentiated by the coloration of the ventrolateral carapace. In *P. pulchricaudatus*, the dark brown transverse bands do not extend to the lower half of the carapace, whereas they do in the latter species.

Kishinouyepenaeopsis incisa (Wang & Liu in Liu & Wang, 1987)

Material examined

Kuakata, Bangladesh, 21.847875 N 90.059220 E, 4 May, 2018, carapace length 17.5 mm, DUZM_CR_107B (deposited at the Kazi Zaker Hossain Zoological Museum), 16S GenBank accession number ON264685.

Fig. 2. K*ishinouyepenaeopsis incisa* (Wang & Liu in Liu & Wang, 1987), A specimen from Kuakata, Bangladesh

Description

The body is slender and setose. The rostrum is mostly straight, with a slight upward curve at the tip, bearing 7-8 dorsal teeth except for a short toothless distal portion (Fig. 2). It does not extend beyond the third antennular segment of the peduncle. The antennular flagella are equal in length and shorter than the carapace, while the antennal flagellum is larger and exceeds the total body length. The distolateral projections of the petasma are longer than the distomedian projections, slender, horn-like, diverging at the base and curving inward at the tips. The anterior plate of the thelycum is rectangular with rounded corners, fused to the posterior plate by a broad posteromedian process. The posterior plate features a pair of lateral depressed regions, and a tuft of long hairs is located behind the thelycum. All five pairs of legs are well developed, with the first three pairs forming pincers. The morphometric measurement was shown in Table 1.

Color

The body is pale greenish, marked with numerous minute dark spot. Eyes are black-brown. The scaphocerite has a somewhat greenish hue with white tips, while the antennal flagella range from reddish-brown to black-brown. The pereiopods are whitish to yellowish, and the pleopods are yellowish to reddish, featuring brown and greenish spots at the bases.

Molecular Analysis

Two partial ribosomal RNA sequences were generated, with lengths of 475 and 478 base pairs for the two identified species. The aligned partial sequences were deposited in GenBank with accession numbers ON264685 for *K. incisa* and MW483130 for *P. pulchricaudatus.* In the

BLAST search results, *P. pulchricaudatus* and *K. incisa* showed 100% query coverage, with similarity percentages of 100% and 97.56%, respectively, compared to existing sequences from India and China. The nucleotide base frequencies for *P. pulchricaudatus* were A: 32.43%, T: 32.85%, C: 13.39%, and G: 21.34%. For *K. incisa*, the frequencies were A: 30.74%, T: 33.68%, C: 13.47%, and G: 22.11%. Both sequences exhibited a strong AT bias, with percentages of 64.42% for K. incisa and 65.28% for *P. pulchricaudatus*. A Neighbor Joining (NJ) phylogenetic tree was constructed using a total of 7 sequences from 7 penaeid shrimp species (Fig. 3).

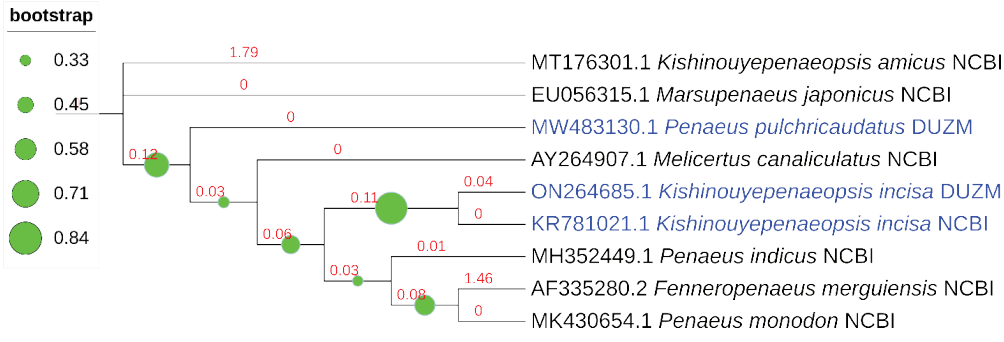


Fig. 3. Phylogenetic analysis of penaeid shrimp species by the Neighbor-joining method in MEGA 11 and iTOL v5. The values in red color denote the branch length and round circle bootstrap values (33-83%). DUZM represents the generated sequence of the present study.

The sequences from the present study are denoted as DUZM, while the remaining sequences were retrieved from the NCBI database. In the phylogenetic tree, each species forms a distinct clade (Fig. 3). *P. pulchricaudatus* is almost identical to *P. japonicus* which was referred to as I and II. They were distinguished by diagnostic color banding patterns on the carapace, previously considered merely a color variant in taxonomic studies[11-12]. Despite the lack of differences in other morphological traits or morphometric parameters[12], phylogenetic analyses using mitochondrial (mt) DNA markers consistently show that while these two forms are closely related to each other , they are genetically distinct[10].

The results reveal two new records of shrimp species from Bangladesh. These findings expand the known diversity of shrimp in the region and highlight the presence of previously undocumented species.

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