Dhaka Univ. J. Biol. Sci. **32**(1): 11-19, 2023 (January) DOI: https://doi.org/10.3329/dujbs.v32i1.64186

NEW RECORD OF SLIPPER LOBSTER *THENUS INDICUS* LEACH, 1816 (CRUSTACEA: DECAPODA: SCYLLARIDAE) FROM BANGLADESH WATERS

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Keywords: Thenus indicus, new record, DNA barcoding, COI, 16S rRNA

Abstract

Slipper lobster *Thenus indicus* Leach, 1816 (Crustacea: Decapoda: Scyllaridae) has been recorded for the first time from Cox's Bazar coast of the Bay of Bengal. The species was taxonomically identified by using the traditional morphometric method which was further validated by molecular approach based on partial cytochrome c oxidase subunit I (COI) and 16S rRNA gene sequences (DNA barcodes). The key characteristics of this species are spotless pereiopods, 1st pereiopod merus width less than 7% of carapace length, and 3rd pereiopod merus length more than 45% of carapace length.

Introduction

Lobsters fetch a high price in domestic and international markets. In Bangladesh, six species of lobsters are documented; scalloped spiny lobster *Panulirus homarus*, ornate spiny lobster *P. ornatus*, mud spiny lobster *P. polyphagus* and painted spiny lobster *P. versicolor* under the family Palinuridae and two slipper Lobster; flathead lobster *Thenus orientalis* and scaled slipper lobster *Scyllarus depressus* under the family Scyllaridae[1,2]. Palinurid lobsters are more popular than slipper lobster of the family Scyllaridae in Bangladesh.

The family Scyllaridae includes four subfamilies, 19 genera, 88 species, and two subspecies worldwide[3-7]. The four subfamilies are Arctidinae, Ibacinae, Scyllarinae, and Theninae. The subfamily Arctidinae contains two genera (*i.e.*, *Arctides* and *Scyllarides*), and the subfamily Theninae only one genus (*i.e.*, *Thenus)*. All slipper lobster species are bottom-dwelling, prefer sandy and muddy habitats and rest in extremely shallow water to a depth of more than 484 m[4]. *Thenus* considered a monotypic genus, with only species *i.e.*, *T. orientalis* Lund 1793[4,8,13]. Burton and Davie (2007) revised the genus throughout its geographic range by using the morphological, morphometric and molecular approaches. Basically, the genus *Thenus* is a complex of five species: *T. indicus*, *T. orientalis*, *T. unimaculatus*, *T. australiensis* and *T. parindicus*[9].

T. indicus occurred in Pakistan, India, the Gulf of Thailand, Singapore, Indonesia and Taiwan[9]. Previously, *T. orientalis* reported from the coastal waters of Bangladesh[1-2]. This

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study describes the presence of *Thenus indicus* based on morphometric and molecular (DNA barcoding) approaches.

Materials and Methods

Sampling and morphological analysis: A single specimen of *Thenus indicus* was collected on 10 December 2020 from BFDC fish landing center of Cox's Bazar, (21°27'6·15" N, 91°58'5.77" E) Bangladesh. It was caught by fishermen during fishing in the off coast of Cox's Bazar. Immediately after collection, the specimen was preserved in ice and transported to the DNA Barcoding lab, Department of Zoology, University of Dhaka. The specimen was kept frozen (−18 °C) until further use. Taxonomic identification of the specimens was performed based on morphometric and meristic characteristics following the guidelines of Burton and Davie (2007) and Chan (1998). The morphometric measurements were done in centimeter (cm) scale. The specimen was tagged with voucher ID (DUZM_CR_145B) and kept at Dhaka University Zoology Museum (DUZM).

Genomic DNA extraction and amplification by PCR: DNA was extracted from a 5mg tissue sample of the specimen using Invitrogen™ PureLink™ Genomic DNA Mini Kit, following the manufacturer's protocol. The quality and quantity of the extracted DNA were measured using NanoDrop™ spectrophotometer. COI and 16S rRNA gene sequences were amplified by polymerase chain reaction with the primer LCO (forward) 5′-TCAACAAATCATAAGGACATTGG-3′ and HCO (reverse) 5′- TAAACTTCAGGGTGTCCAAAGAATCA-3′ for COI[10] and primer 16Sar (forward) 5′-CGCCTGTTTATCAAAAACAT-3′ and 16Sbr (reverse) 5′-CCGGTCTGAACTCAGATCATGT-3′ for 16S rRNA[11] genes. The PCR was conducted in 25 µl volumes containing 23 µl of PCR Master Mix and 2 µl of DNA sample, mixed and spun for 30s for homogenization of the mixture. PCR Master Mix consists of 12.5 µl Taq Polymerase, 8.5 µl Nano Pure water, 1 µl forward primer and 1 µl reverse primer. The PCR amplifications were performed following the conditions: initial denaturation at 95 ℃ for 5 min followed by 35 cycles of 94 ℃ for 45s, 50 ℃ (COI) and 42 ℃ (16S rRNA) for 30s, 72 ℃ for 45s, and a final extension at 72 ℃ for 10 min. The PCR products was kept at room temperature for 15 min, and then stored at -26 ℃ until further downstream application. PCR products were separated in 1% agarose gel and purified using PureLink™ PCR purification kit. The good quality purified PCR products of DNA concentration >10ng/µl were sent to First BASE laboratories, Malaysia for sequencing. Sequencing was done by Sanger dideoxy sequencing technology using ABI PRISM 3730xl Genetic Analyzer exploiting the BigDye R Terminator v3.1 cycle sequencing kit chemistry.

Sequencing analysis: The assembled contigs of each gene were prepared by the CAP3 DNA assembly program using bioinformatics software Unipro Ugene[12]. The sequences were confirmed via BLASTn against the best match sequences of the nucleotide database

(identity cut off ≥ 99%) and deposited in the NCBI GenBank. Our analysis includes DUZM sequences, along with sequences of three other *Thenus* species *T. parindicus, T. australiensis, T. orientalis* and *T. unimaculatus* retrieved from the NCBI GenBank database. *T. orientalis was* not included in the 16S rRNA sequences analysis due to the lack of similarity between existing sequences. All the COI and 16S rRNA sequences were aligned automatically using MUSCLE and then adjusted manually[13]. For the distance-based method, genetic pairwise divergence was determined by calculating Kimura-2-parameter (K2P)[14] distance using MEGA X[15]. Phylogenetic trees were constructed for COI and 16S rRNA sequences using Mega X based on the Maximum Likelihood (ML) statistical method. The phylogenetic construction uses the K2P substitution model with Gamma distributed rates. The robustness of clustering was determined by bootstrap analysis with 1000 replicates.

Results and Discussion

Diagnosis: Body markedly depressed and dark brown in color. Carapace trapezoid and narrowing posteriorly. Eyes small and subspherical; orbits situated at anterolateral angles of the carapace. Antennae broad flattened and plate-like. Five teeth present in second antennal segment. Antennules short and slender. Pereiopods and telson are yellowish in color without any spots. Rostral processes are sharply directed anteriorly and upward. Maxillipeds 1 and 3 lacks flagella (Fig. 1). A small spine present in the merus of the 3rd maxilliped. The inner margin of the ischium was prominently dentate along the total length. Abdominal segments expanded downward concealing the pleopods. The morphometric ratio of 1st pereiopod merus width and carapace length is 0.06 and 3rd pereiopod merus length and carapace length is 0.46 (Table 1).

Table 1. Morphological measurement ratios *Thenus indicus* following Burton and Davie (2007).

Morphometric characters	Present Study, n=1		Burton and Davie (2007)
	Measurement (cm)	Ratio with CL	
Carapace Length (CL)	6.3		
Carapace Width (CW)	8.1	1.27	
Length of the 3rd merus (ML3)	2.9	0.46	> 0.45
Width of the 1st merus (MW1)	0.4	0.06	< 0.07
Telson Length (TL)	0.85	0.13	
Telson Width (TW)	2.7	0.43	
Length of the 1st (and 2nd) propodus (PL1)	1.7	0.27	
Width of the 1st propodus (PW1)	0.35	0.05	

Remarks: T. indicus is closely related and often confused with the other species *T. orientalis.* The most obvious differences to distinguish this species is *T. indicus* tends to be brown whereas, *T. orientalis* is more reddish in body coloration. Spotless pereopods and telson; five teeth in the second antennal segment in *T. indicus* whereas four in *T. orientalis*[16]

and a spine on the merus of third maxilliped[17]. Abdominal segments, each with lateral margins expanded downward concealing the pleopods. The morphometric ratio of 1st pereiopod merus width and carapace length is less than 0.07 and 3rd pereiopod merus length and carapace length is more than 0.45 CL[9, 18-19].

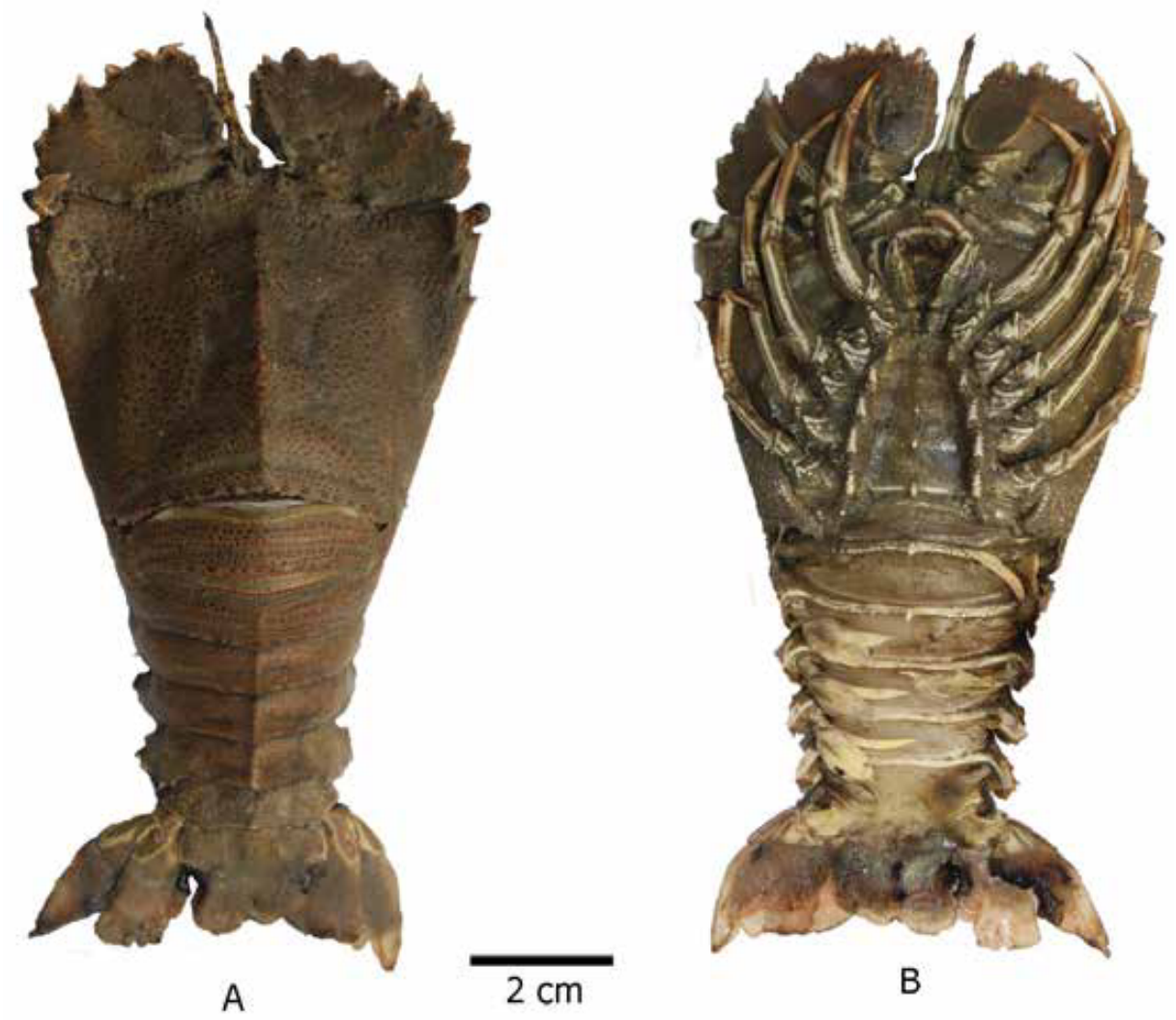

Fig. 1. *Thenus indicus* A. Dorsal view; B. Ventral view.

Molecular characterization: The accession numbers of the partial sequences of COI and 16S rRNA genes obtained are MW514207, MW504993, respectively. The acquired 630 bp COI sequence showed 99.21% species identity with 100% query cover with the *Thenus indicus* from India (JQ229892). The sequence was also in monophyly with the other species sequences from different Asian countries. The 463 bp long 16S rRNA sequence showed 100% identity and query coverage with the species from India (JQ229878) and Singapore (MT704568).

The average K2P distance between *T. indicus* and three other congeneric species sequences was calculated to understand their genetic relationships (Tables 2-3). Our species showed the lowest genetic divergence with the *T. parindicus,* with a genetic distance of 11.54% for COI and 4.234% for 16S rRNA genes. For both the genes, the distance was greater than the standard threshold for species identification of 3%[20]. The wide genetic divergence between COI sequences confirms the organism collected to be different from the compared *Thenus* species. On the other hand, the fewer genetic divergence between 16S rRNA sequences and the distinct morphological characteristics confirms the accuracy in delimiting the genus *Thenus*. The other species sequences compared showed the highest mean divergence with *T. unimaculatus* of 22.09% for COI and *T. australiensis* of 11.80% for 16S rRNA sequences.

Table 2. The genetic divergence (K2P distance %) between T. *indicus* (DUZM) and *T. parindicus, T. australiensis, T. orientalis* and *T. unimaculatus* based of COI gene (Burton and Davie 2007).

Species (COI)	*T. parindicus*	*T. australiensis*	*T. orientalis*	*T. unimaculatus*
Mean	11.54±0.286	15.01±0.582	14.13±0.687	15.68±1.071
Range	11.04-12.06	13.74-16.06	13.06-15.24	14.01-18.30
No. of comparison	25	20	25	35

Table 3. The genetic divergence (K2P Distance %) between *T. indicus* (DUZM) and *T. parindicus, T. australiensis* and *T. unimaculatus* based on 16S rRNA gene (Burton and Davie 2007).

Species (16S rRNA)	*T. parindicus*	*T. australiensis*	*T. unimaculatus*
Mean	4.234±0.649	5.686±0.806	5.221±1.034
Range	3.490-5.271	4.465-6.835	3.437-6.326
No. of comparison	15	12	12

Maximum likelihood (ML) tree was constructed for phylogenetic analysis where the lineage support was interpreted based on the bootstrap percentage (BP). The sequences of each species formed monophyletic clade, and our species clustered with the pre-existing *T. indicus* of the GenBank database. *Ibacus peronii* was taken as an out group as the species has morphological similarity with the *Thenus* species[21]. The monophyly within *T. indicus* was supported with a strong bootstrap value of 94% in the COI evolutionary tree (Fig. 2). Among the four *Thenus* species compared, *T. indicus* shared the common ancestor with *T. parindicus* with strong clade support of 96% BP. The 16S rRNA sequence of our species was grouped with the *T. indicus* from India with 95% BP support (Fig. 3). Thus, proving the effectiveness of the COI and 16S rRNA in species delimitation.

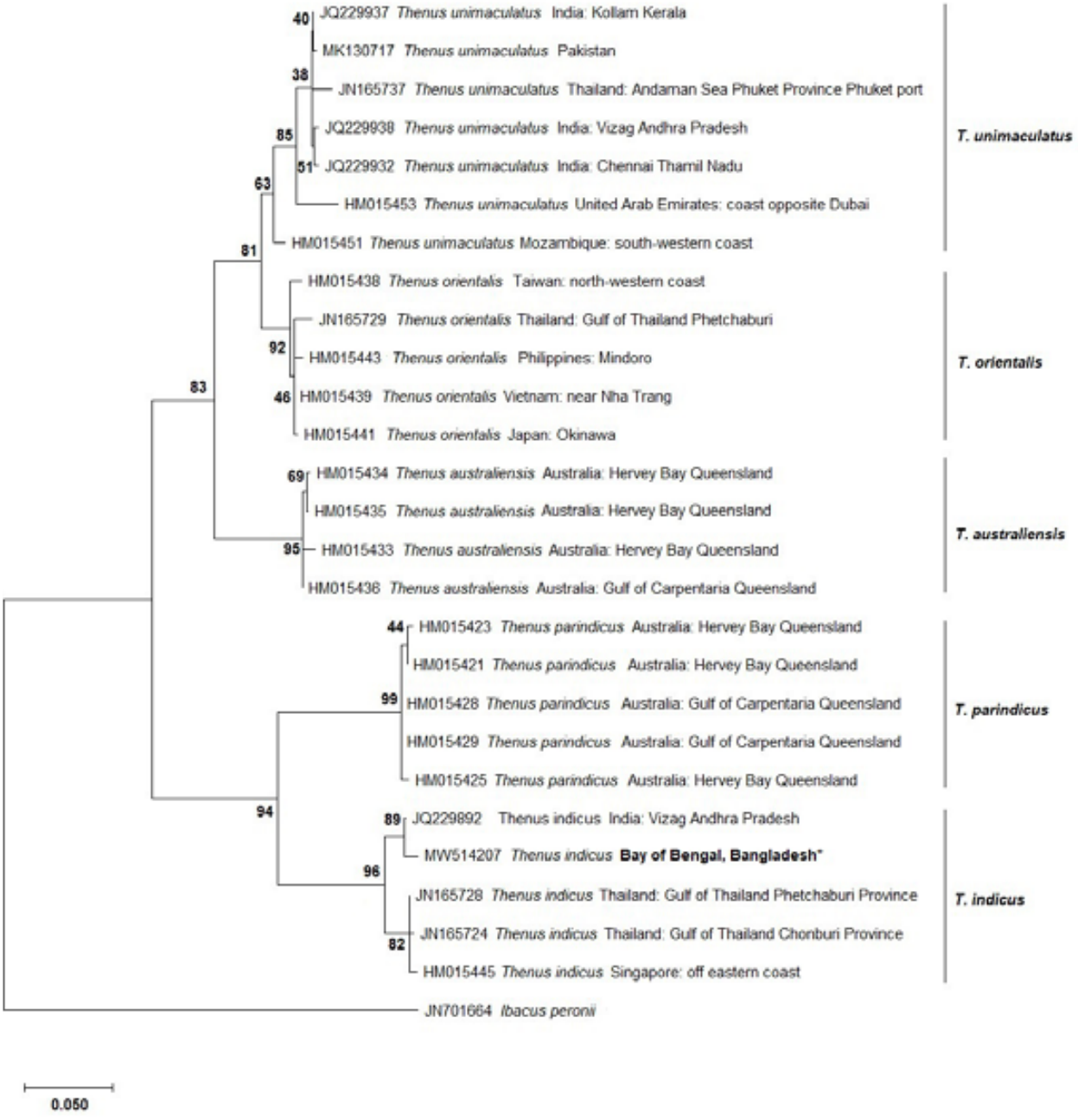

Fig. 2. Maximum Likelihood tree of the COI sequences showing the relationships among *Thenus indicus* with the pre-existing sequences of *Thenus* species of the NCBI GenBank. *Ibacus peronii* was taken as an outgroup.

Fig. 3. Maximum Likelihood tree of the 16S rRNA sequences showing the relationships among *Thenus indicus* with the pre-existing sequences of *Thenus* species of the NCBI GenBank. *Ibacus peronii* was taken as an outgroup.

Conclusion

The morphological characterization and the overall sequence analysis based on two marker genes supports the identification of the species as *T. indicus*. It confirms the species occurrence for the first time reported in Cox's Bazar, Bay of Bengal, Bangladesh. Thus, extending the geographical distribution range of the species.

Acknowledgements

We acknowledge the financial support from Bangladesh Academy of Science under the BAS-USDA Endowment Program for Applied Research in Natural Sciences Focused on Food Security, (Grants No. FI 18).

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(Manuscript received on 06 April, 2022; accepted on 8 June, 2022)