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MORPHOLOGY AND DNA BARCODING REVEAL FOUR NEW SPECIES OF HERMIT CRAB (CRUSTACEA: DECAPODA: ANOMURA) FOR THE FAUNA OF BANGLADESH

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Abstract. This study describes some morphometric and molecular aspects of four species of hermit crabs (*Clibanarius clibanarius, Clibanarius padavensis, Clibanarius infraspinatus,* and *Diogenes rectimanus*) reported for the first time from Bangladesh. A total of 15 COI sequences were generated for these four species. The average genetic distances found within species, genus, and family were 0.96 ± 0.02%, 22.80 ± 0.06%, and 25.13 ± 0.11%, respectively. For barcode gap analysis, the mean and maximum intra-specific values were compared to the nearest neighbour distance using the K2P model. The cluster sequence results revealed four OTUs. The phylogenetic tree based on the neighbor-joining (NJ) method showed a single cluster for each species. Thus, morphometric and molecular data confirmed the species identification. Based on the analysed material, we assume that there are many more species in the coastal areas of Bangladesh which could be discovered in future surveys.

INTRODUCTION

Hermit crabs are an intriguing decapod crustacean group that has adapted to fill empty scavenged mollusc shells in order to protect their fragile exoskeletons. Currently, there are approximately 1,100 recognized species of hermit crabs globally, with new species continually being documented (McLaughlin et al. 2010). Worldwide, the hermit crab communities are associated with a variety of habitats including terrestrial to marine (Fransozo and Mantelatto 1998; Williams and Mcdermott 2004). Additionally, a fascinating relationship between hermit crabs and gastropod shells, which plays a crucial role in their survival, adds complexity to the generalization of distributional models (Fransozo and Mantelatto 1998). Actually, despite the world-wide scientific interest on hermit crabs, their distributional aspects have been studied less frequently, possibly because of the difficulties in sampling designs on coastal zones, heterogeneity and intriguing aspects of relationship, and dependence of gastropod shell on hermit crab survivorship.

Although Bangladesh possesses a remarkable wealth of crustacean diversity (Ahmed et al. 2008), research on hermit crabs is very scanty in the country. Only five species of hermit crabs have so far been documented, *viz. Clibanarius longitarsus, Coenobita variabilis, C. violascens, Pagurus bernhardus* and *Parapagurus nudus* (IUCN 2015; Ahmed et al. 2008; Ahmed et al. 2021a). In contrast, a neighbouring country India has reported a total of 112 species (Trivedi and Vachrajani 2017). This clearly highlights the lack of attention given to hermit crab faunal diversity in Bangladesh.

Crabs are typically identified using morphometric and meristic characteristics. However, when the external appearance of closely related crab species is nearly identical, it becomes highly challenging to identify them accurately using traditional morpho-taxonomy. The limitations associated with morphology-based identification have been overcome by the development of DNA-based approaches (DNA barcoding) with a particular gene or genes (Hebert et al. 2003). The cytochrome c oxidase subunit I (COI) gene has been recognized as a remarkable molecular marker for species identification and phylogenetic analysis of animals (Ahmed et al. 2020, 2021b, 2022).

The present study reports the first records of four hermit

crabs: *Clibanarius padavensis, Clibanarius clibanarius, Clibanarius infraspinatus,* and *Diogenes rectimanus* from the coastal areas of Bangladesh. These species were identified for the first time in this region by using a combination of morphological key characteristics and DNA barcoding using COI gene sequences.

MATERIALS AND METHODS

Specimen collection and identification

The specimens were collected from several locations in the southeast and southwest part of Bangladesh including Cox's Bazar, St. Martin's Island, Sonadia Island, and Sundarbans (Table 1) from June 2021 to July 2022 by hand-picking. After collection during low tide from intertidal areas, the specimens were preserved in an icebox and transported to the DNA Barcoding Lab., Department of Zoology, University of Dhaka for morphological and molecular analysis. Photographs were taken after specimens had been carefully removed from the gastropod shells. The collected samples were morphologically identified initially following the taxonomic literature of Ahmed and Khan (1971), Tirmizi and Siddiqui (1981), Trivedi et al. (2015), and Fitrian et al. (2022). For molecular analysis, the tissue was extracted from the claws of each targeted specimen by using a sterile blade and preserved in 90% ethanol. The rest of the sample specimens were stored at -18 °C until further analysis. After completing all the morphological and molecular procedures, the specimens were finally deposited at Dhaka University Zoology Museum (DUZM) using an individual tag number.

DNA extraction, PCR amplification, and DNA sequencing

For each specimen, the protocol of Invitrogen™ Genomic DNA Mini Kit was followed to isolate DNA from 5 mg sample tissue. A NanoDrop™ spectrophotometer was used to measure the quality and quantity of extracted DNA. Primers LCO 5'TCAACAAATCATAAGGACATTGG 3' and HCO 5'TAAACTTCAGGGTGTCCAAAGAATCA 3' (Folmer et al. 1994) were used to amplify the COI gene. The PCR was carried out in 25 µl volumes which consisted of 23 µl of PCR Master Mix and 2 µl of DNA sample, mixed perfectly and spun for 30 s for homogenization of the mixture. A total of 12.5 µl of Taq Polymerase, 8.5 µl of Nano Pure water, 1 µl of forward primer, and 1 µl of reverse primer were used to prepare the PCR Master Mix. PCR amplifications were performed using an Applied Biosystems PCR Thermal Cycler (Thermo Fisher Scientific). The protocol included an initial denaturation temperature of 95 °C for 5 min, followed by 41 cycles of denaturation at 95 °C for 30 s, annealing at 54 °C for 30 s, and extension at 72 °C for 1 min, with a final extension step conducted at 72 °C for 5 min. After completing the PCR, the product was stored at -26 °C until further processing. The visualization of PCR products was conducted on a 1% agarose gel. The purification of PCR products was carried out using the PureLink™ PCR purification kit. If the DNA concentration was >10 ng/µl, those samples were sent to First BASE laboratories, Malaysia, for sequencing. Sanger dideoxy sequencing technology was applied for the sequencing using an ABI PRISM 3730xl Genetic Analyzer with the BigDye R Terminator v3.1 cycle sequencing kit chemistry.

Molecular analysis

MUSCLE (Edgar 2004) was used to automatically align all the partial COI gene sequences with manual adjustments made subsequently. The NCBI BLAST searches were conducted to identify the species. The confirmed sequences were then deposited in both the Barcode of Life Data System (BOLD Systems) (Ratnasingham and Hebert 2007) creating a new dataset named DS-DUHC and NCBI GenBank. Kimura-2-parameter (K2P) distance (Kimura 1980) was calculated to assess the genetic divergence by using the BOLD system workbench. The neighbor-joining (NJ) method with the K2P model with gamma rate distribution was used for phylogenetic tree construction using MEGA 11 software (Tamura et al. 2021). To compare the sequences of the present study with the sequences of other regions (Japan, China, India, Pakistan, and Indonesia), several sequences (MK076143-44, LC474646-47, OM992273, MK747782, JX676123-24, MZ438271, and MW846617) were downloaded from GenBank. Operational taxonomic units (OTUs) were calculated using the BOLD system to classify the closely related species.

RESULTS AND DISCUSSION

Species identification

A total of 60 specimens of hermit crabs were collected from different habitats (Figure 1). Among them, four species belonging to two genera under one family were morphologically identified (Table 1).

The identified four species were *Clibanarius clibanarius, Clibanarius infraspinatus, Clibanarius padavensis* and *Diogenes rectimanus*. Identification keys for the species are as follows:

Identification key (modified from Ahmed and Khan (1971), Tirmizi and Siddiqui (1981), Trivedi et al. (2015), and Fitrian et al. (2022))

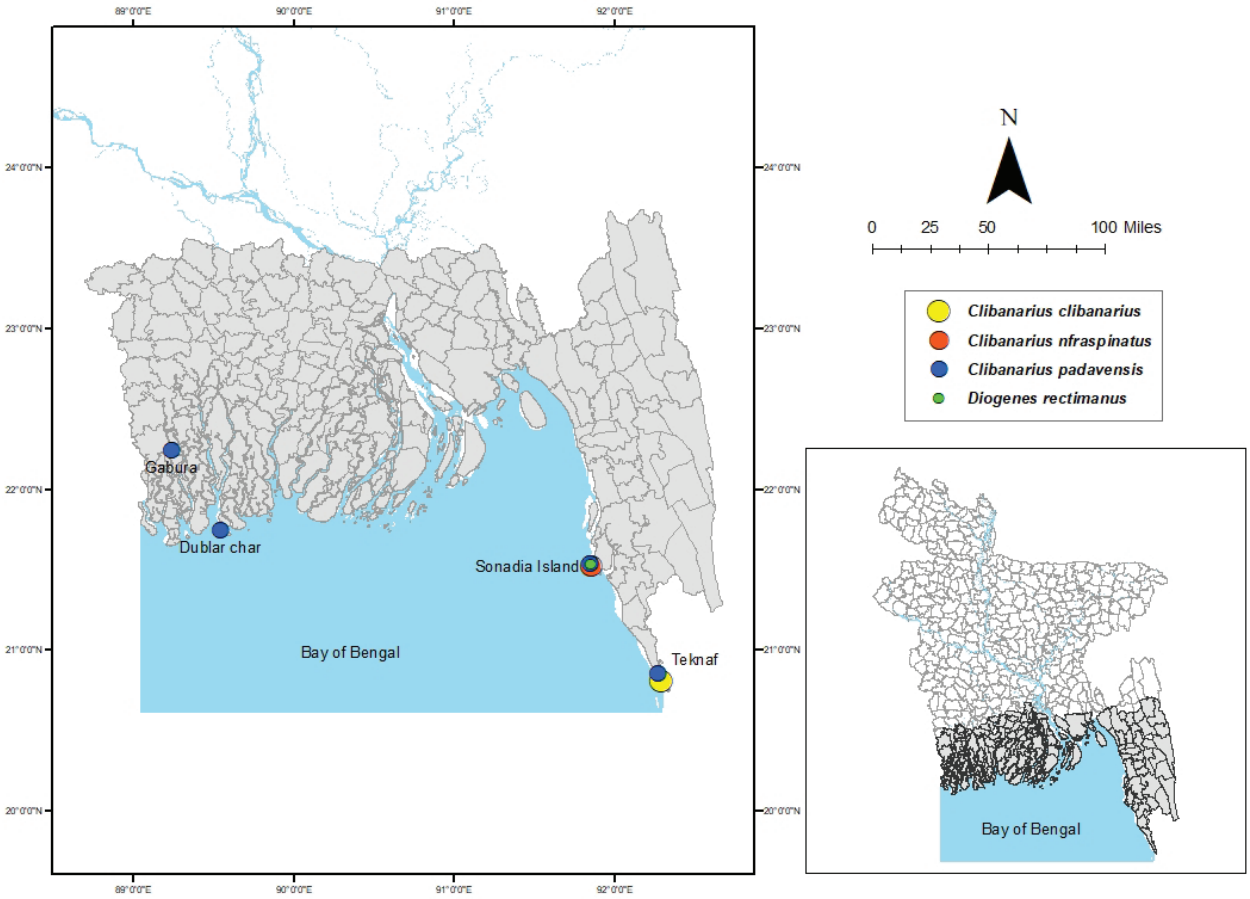

Figure 1. Map of the specimen collecting sites in Bangladesh. A symbol may cover more than one collecting site.

Table 1. Identified species used in this study (indicated by GPS coordinates) and downloaded from NCBI (indicated by country name) using COI gene with their GenBank accession No., BOLD process ID, and collection place.

Operational taxonomic unit ID	GB accession No	Bold process ID	BIN	Location (latitude–longitude)
Clibanarius infraspinatus	OM986469	GBMNF8315-22	BOLD: ACK3212	21.75 N 89.64 E
	OM986474	GBMNF8314-22	BOLD: AET0913	21.52 N 91.84 E
	OM986473	GBMNF8313-22	BOLD: AET0914	21.52 N 91.84 E
	OM986472	GBMNF8312-22	BOLD: ACK3212	21.52 N 91.84 E
	LC474647	GBMNB53058-20	BOLD: AED7095	Japan
	LC474646	GBMNB53059-20	BOLD: AED7095	Japan
	MK076143	GBMNB53060-20	BOLD: AEB3508	China
	MK076144	GBMNB53061-20	BOLD: AED7095	China
Clibanarius clibanarius	MW836020	GBMND85409-21	BOLD: ACC8938	22.23 N 89.23 E
	JX676124	GBCMD11934-13	BOLD: ACC8938	India
	JX676123	GBCMD11935-13	BOLD: ACC8938	India
Clibanarius padavensis	OM986467	GBMNF8318-22	BOLD: ADR7675	21.75 N 89.64 E
	OM986466	GBMNF8317-22	BOLD: ADR7675	21.75 N 89.64 E
	OM986465	GBMNF8316-22	BOLD: ADR7675	21.75 N 89.64 E
	MW847899	GBMND85411-21	BOLD: ADR7675	22.23 N 89.23 E
	MW836019	GBMND85410-21	BOLD: ADR7675	22.23 N 89.23 E
	OM986464	GBMNF8322-22	BOLD: ADR7675	21.75 N 89.64 E
	OM986463	GBMNF8321-22	BOLD: ADR7675	21.75 N 89.64 E
	OM986462	GBMNF8320-22	BOLD: ADR7675	21.75 N 89.64 E
	OM986468	GBMNF8319-22	BOLD: ADR7675	21.75 N 89.64 E
	MZ438271	GBMNF8323-22	BOLD: ADR7675	India
Diogenes rectimanus	ON997624	GBMNE75659-22		21.52 N 91.84 E
	MK747782	GBMNE20325-21	BOLD: AGC4217	China

Key to species

1. Finger tips of P.1 are spooned and comeous .... Genus *Clibanarius*

1(a). Eyestalks extend significantly beyond the front edge of the carapace.....*Clibanarius padavensis*

1(b). Serrulate P. I present, mesial lower margin of merus..... *C. clibanarius*

1(c). A single prominent tooth present, mesial lower margin of merus of P.I .....*C. infraspinatus*

2. Antennal flagellum setose. Tips of finger P.1 are pointed ..... Genus *Diogenes*

2(a). Mesial margin of merus of left P.I without spines .....*Diogenes rectimanus*

TAXONOMIC CHARACTERISTICS

Phylum: Arthropoda

Class: Malacostraca

Order: Decapoda Latreille, 1802

Family: Diogenidae Ortmann, 1892

Clibanarius infraspinatus (Hilgendorf 1869)

Synonyms. *Pagurus (Clibanarius) infraspinatus* Hilgendorf, 1869: 97. *Clibanarius infraspinatus* Yap Chiongco, 1938: 194, pl. 2, fig. 4; Fize and Serène 1955: 77, fig. 10; Tirmizi and Siddiqui 1982: 26, fig. 20.

Material examined. 2 males (SL: 11.8 mm, 12.5 mm) and 2 females (SL: 8.4 mm, 11.4 mm), DUZM_CR_148C.1–4 collected from Dublar Char, Sundarbans; Sonadia Island, Cox's Bazar; Bangladesh (Figure 3A).

Diagnosis. Carapace elongated and has clusters of lengthy setae along with a noticeable rostrum. Ocular peduncles slender and lengthy, roughly matching the length of the front edge of the shield (Figure 3A). The antennal peduncles shorter and do not extend to the cornea bases. Antennal acicles, on the other hand, reach past the base of the ultimate peduncular segment. Antennular peduncle equal or slightly longer than ocular peduncles. Propodus and carpus with three strong spines in a row on the upper inner surface and a few scattered spines on the rest of the upper surface. Toothed finger present contains bristles. Second and third walking legs are longer than chelipeds, with five to six spinules on the inner top border of the second leg's carpus. The telson nearly imperceptible central split and the rear lobes are notably uneven, with the left one being considerably larger. Each end with 5 or 6 distinct spines with corneous tips.

Colouration. Cream-coloured carapace with greyish

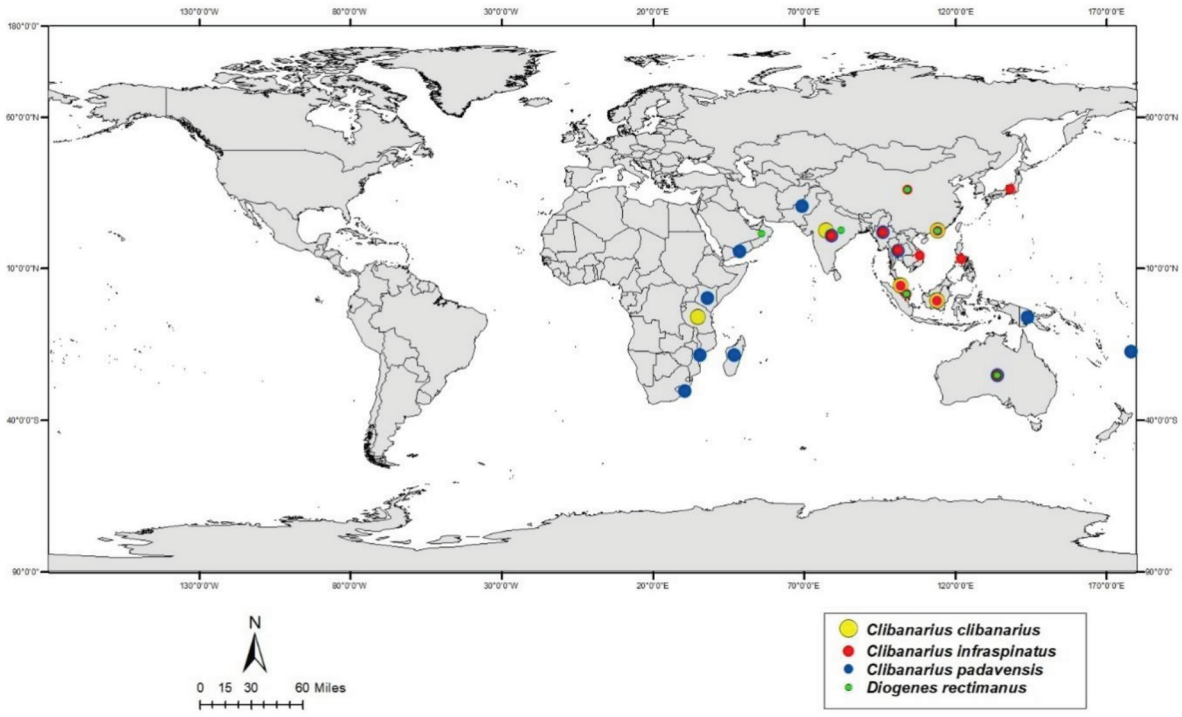

Figure 2. Global distribution map: *C. clibanarius* has been reported from India, Indonesia, Tanzania, Malaysia, and Hong Kong (GBIF 2024); *C. padavensis* from India, Thailand, Australia, Papua New Guinea, Fiji, Yemen, Madagascar, Kenya, South Africa, Myanmar, Mozambique, and Pakistan (GBIF 2024); *C. infraspinatus* from India, Singapore, Thailand, Japan, China, Malaysia, Hong Kong, Vietnam, Myanmar, Australia, the Philippines, and Indonesia (GBIF 2024); and *D. rectimanus* from Australia, China, Oman, Hong Kong, India, and Singapore (GBIF 2024).

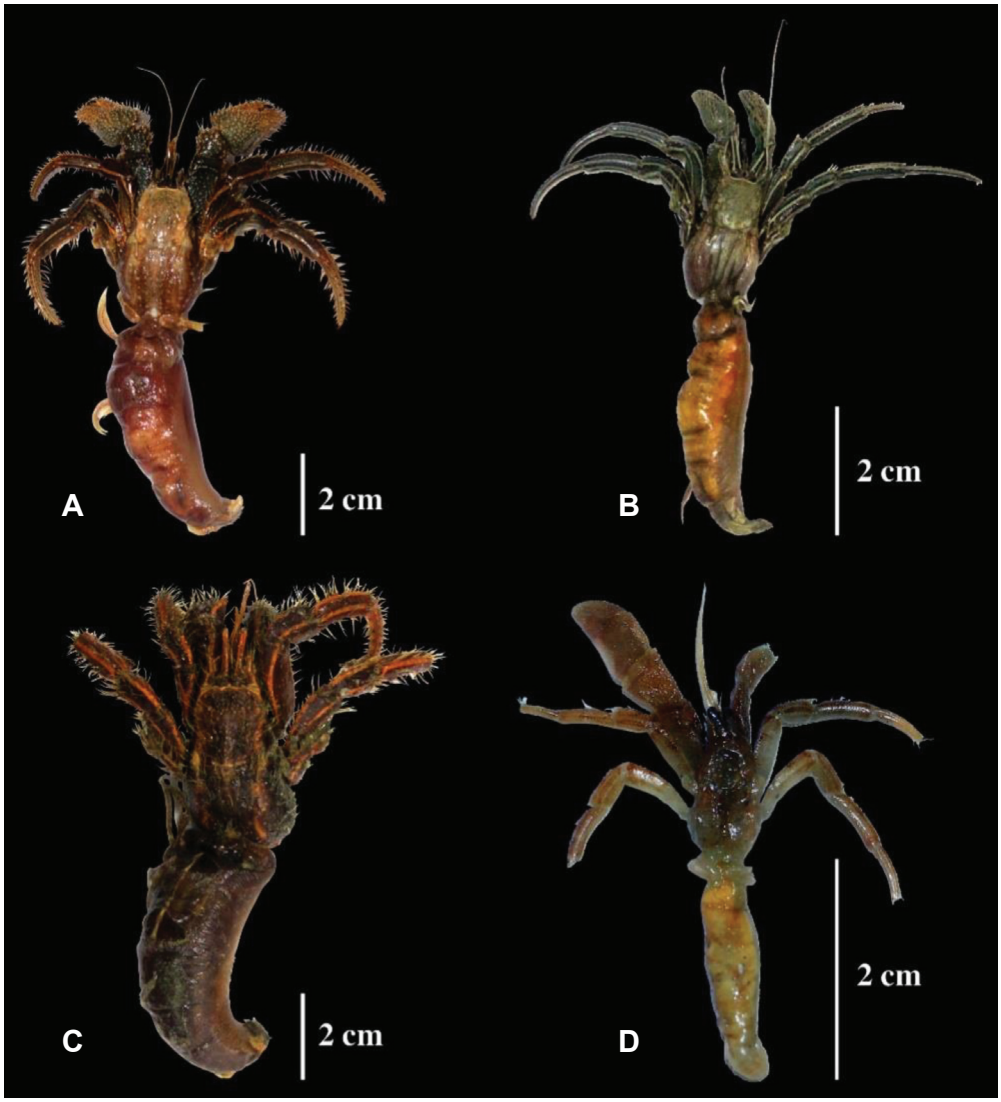

Figure 3. Images of hermit crabs: **(A)** *Clibanarius infraspinatus* (voucher ID: DUZM_CR_148C.4, collection date: 8 Feb 2021, place: Dublar Char); **(B)** *Clibanarius padavensis* (voucher ID: DUZM_CR_148B.12, collection date: 8 Feb 2021, place: Dublar Char); **(C)** *Clibanarius clibanarius* (voucher ID: DUZM_CR_148C, collection date: 28 Dec 2020, place: Satkhira); **(D)** *Diogenes rectimanus* (voucher ID: DUZM_CR_148E, collection date: 1 Sep 2021, place: Sonadia Island, Cox's Bazar).

orange shield (Figure 3A). Ocular peduncle is of dark blackish brown ground colour with longitudinal reddish white stripes. Chelipeds with light spines and dark brown to ash in colour. Fingertips dark and walking legs of dark ash ground colour with orange to yellow longitudinal l stripes bordered by red or chocolate lines.

Global distribution. India, Singapore, Thailand, Japan, China, Malaysia, Hong Kong, Vietnam, Myanmar, Australia, the Philippines, and Indonesia (Figure 2) (GBIF 2024).

Remarks. *Clibanarus infraspinatus* is closely similar to *Clibanarus clibanarius* but can be easily differentiated from *C. clibanarius* by the presence of a conspicuous tubercle or protuberance (Figure 4) on the ventral surface of the cheliped merus which is absent in *C. clibanarius*.

Clibanarius padavensis De Man 1888

Synonyms. *Clibanarius padavensis* de Man 1888, p. 242, pi. 16, fig. 1; Alcock 1905b, pp. 44–46, pi. 4, fig. 2; Southwell 1906, p. 215; Sundara Raj 1927, p. 130; Panikkar and Aiyar 1937, p. 296.

Material examined. 7 males (SL: 5–11.7 mm) and 5 females (SL: 5.2–6.4 mm), DUZM_CR_148B.3–13 collected from Dublar Char, Sundarbans, Bangladesh (Figure 3B).

Diagnosis. Carapace two thirds wider than length at the median line, fine smooth setae extant on the flanks of the carapace. Shield longer than broad with a well-developed rostrum that reaches the base of the ocular acicles (Figure 3B). Ocular acicles with 2–4 small spines. The ocular peduncle is almost as long as the antennular joint at the end. Antennular peduncles usually slightly longer

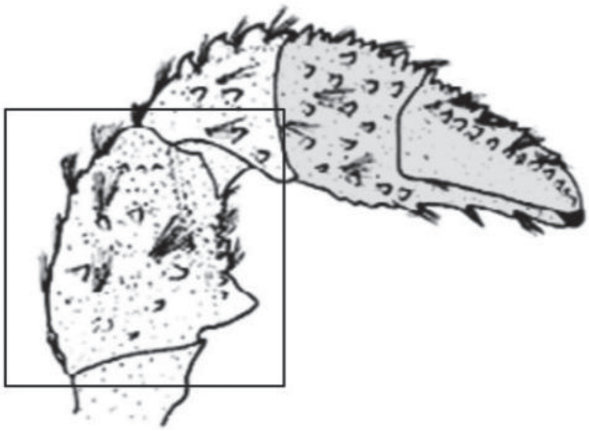

Figure 4. Tubercle or protuberance on the ventral surface of the cheliped merus of *Clibanarius infraspinatus* (Trivedi et al. 2015).

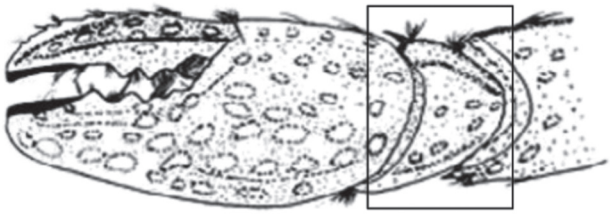

Figure 5. Carpus showing the presence of several strong spines in *Clibanarius padavensis* (Trivedi et al. 2015).

than ocular peduncles. The antennal acicles extend to or slightly surpass the proximal edges of the fifth peduncular segment. Carpus with a dorso-distal spine and several tiny spines on the outer and lower surface. Ambulatory legs longer than cheliped legs, with the right leg being longer than the left. Dactyls slightly longer than propodi and have ventral margins with 15–30 rows of very small corneous spines. Second pair of walking legs clearly longer than third. Second and third walking legs longer than chelipeds. The telson has a slight central split and rear lobe of nearly equal size. On each end, 5 or 6 spines with the strongest ones located on the sides. Telson round in shape, asymmetrical posterior lobe, long setae present, median clef quite detectable.

Colouration. The colour of the shield and carapace yellowish brown (Figure 3B). Yellowish ocular peduncle with a reddish or brown stripe on the dorsal surface. Antennal and antennular peduncles yellowish with red or brown bands. Merus and carpus of chelipeds are dark green in colour with white stripes. The palm's outer surface dark green in colour, with a dark brown line on the upper part. Walking leg white with four or five dark brown longitudinal lines.

Global distribution. India, Thailand, Australia, Papua New Guinea, Fiji, Yemen, Madagascar, Kenya, South Africa, Myanmar, Mozambique, and Pakistan (Figure 2) (GBIF 2024).

Remarks. Tirmizi and Siddiqui (1982) described the left cheliped of what they believed to be *C. padavensis*, noting an unarmed carpus and the presence of several strong spines on the dorsomedial side of the segment (Figure 5). In contrast, the carpus of *C. laevimanus* is typically described as having spines, with no mention of an unarmed carpus, suggesting that this feature could help distinguish *C. padavensis* from *C. laevimanus.*

Clibanarius clibanarius (Herbst 1791)

Synonyms. *Cancer clibanarius* Herbst, 1891; *Pagurus clibanarius* Latreille 1803: 167 (no new locality); *Pagurus (Clibanarius) clibanarius* Hilgendorf 1878: 820 (no new locality). *Clibanarius vulgaris* Dana, 1851: 462 (invalid replacement name); *Clibanarius clibanarius* Henderson 1893.

Material examined. one male (SL: 11.8 mm), DUZM_CR_148C collected from Satkhira, Bangladesh (Figure 3C).

Diagnosis. Ocular acicles with two noticeable terminal spines and a smaller spine on the lateral portion (Figure 3C). Antennular peduncles approximately 0.50 times longer than the ocular peduncles. The antennal acicles reach the proximal edge of the fifth peduncular segment. Chelipeds identical in size and shape, with both carpi and palms having a series of spines on their upper inner edges, and all dorsal surfaces having either blunt or sharp spines. The dactyls and fixed fingers with a gap between them. Walking legs slightly cylindrical segments with clusters of stiff setae. The propodi are 1.35–1.50 times the length of the dactyls, and the carpi with only one spine on their upper outer edges. Telson with very faint central split, with the left posterior lobes being slightly longer. Each terminal margin has 6 or 7 spines with a corneous tip.

Colouration. The shield displays a maroon mottled pattern with cream-coloured edges, while the posterior carapace is a pale brown shade (Figure 3C). Ocular peduncles mostly brown, featuring a dark brown central longitudinal stripe each. The antennal and antennular peduncles typically exhibit a reddish-brown hue. Chelipeds are commonly maroon in colour. Ambulatory legs maroon with cream-coloured stripes running lengthwise. Dactyli with stripes on their sides, both on the dorsal and middle surfaces, while carpi feature a median stripe on their lateral surfaces.

Global distribution. India, Indonesia, Tanzania, Malaysia, and Hong Kong (Figure 2) (GBIF 2024).

Remarks. *Clibanarius clibanarius* closely resembles *C. infraspinatus* in terms of morphology, especially in features such as antennular peduncles extending beyond the distal corneal margins, the noticeably longer dactyli of pereopods 2 and 3 compared to the propodi,

and the presence of a dorsal row of spines on the carpi of pereopods 2. *Clibanarius clibanarius* can be easily differentiated from *C. infraspinatus* by the absence of a conspicuous tubercle or protuberance on the ventral surface of the cheliped merus, a feature present in *C. infraspinatus* (McLaughlin et al. 2007).

Diogenes rectimanus Miers 1884

Synonyms. *Diogenes rectimanus* Miers, 1884, p. 262, pl. 27, fig. c [type locality: Torres Strait]; Alcock 1905, p. 71, pl. 6, fig. 8, 8a, pl. 7, fig. 2, 2a; Wang 1991, p. 226, fig. 185; McLaughlin and Clark 1997, p. 37, fig. 10b; McLaughlin 2002b, p. 414, fig. 2A–C; McLaughlin et al. 2007, p. 151, unnumbered figure; McLaughlin et al. 2010, p. 21. Not *Diogenes rectimanus* – Lanchester 1902, p. 366. = *Diogenes goniochirus* Forest, 1956 and *D. avarus* Heller, 1865.

Material examined. one male (SL: 4.6 mm), DUZM_CR_148E collected from Sonadia Island, Cox's Bazar, Bangladesh (Figure 3D).

Diagnosis. Several small ridges with transverse spinulose running horizontally on the upper surface of the shield (Figure 3D). In each edge of the branchiostegal area, 5 or 6 reasonably well-developed spines present. Ocular peduncles stout and approximately 80% of the length of the shield. The ocular acicles possess three noticeable small spines along with several smaller spinules.

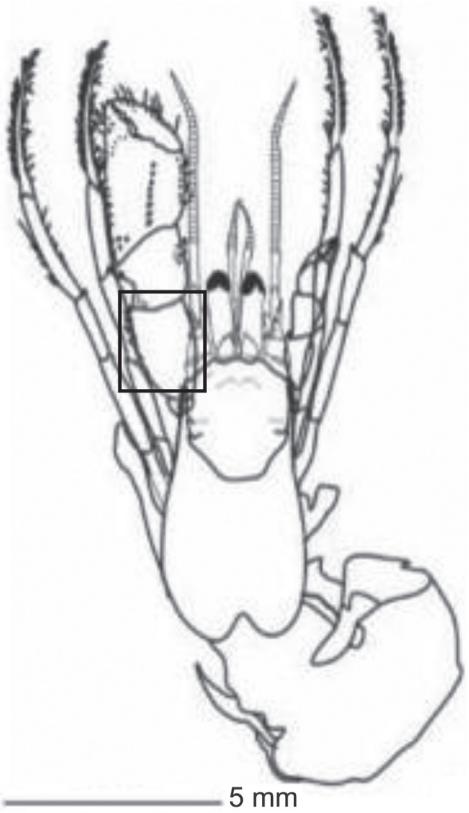

Figure 6. Mesial margin of merus in *D. rectimanus* (Fitrian et al. 2022).

Antennal peduncles extend 0.20 length of the ultimate segment beyond the corneal distal margins. Antennal acicles with four strong spines on the mesial margin and strong bifid terminal spines. Left cheliped of dactyl with a double row of spines on the upper margin. The upper surface of the palm covered with an irregular triple row of spines. Carpus with a row of blunt spines along the upper border, with the distal two or three being stronger. In ambulatory legs, dactyls are 0.25 times longer than propodi. Dactyls nearly double rows of long, stiff, dense setae on their dorsal margins. Left propodi with rows of setae clusters, each paired with a row of small spines on the upper surfaces, with the third row being the most prominent. On each carpus, a line of sharp spines, with the third one being slightly shorter. Telson has a narrow median cleft. Both lobes of terminal margins have long spines interspersed with smaller spines.

Colouration. The ocular, antennal, and antennular peduncles display a pale orange-yellow hue. The cheliped, dactyls, and propodi are either cream-coloured or pale orange, with green-grey tubercles that are darker in colour. The propodi, carpi, and meri feature patches of darker green or brown (Figure 3D).

Global distribution. Australia, China, Oman, Hong Kong, India, and Singapore (Figure 2) (GBIF 2024).

Remarks. *Diogenes rectimanus* can be differentiated from *Diogenes lophochir* by the absence of spine in mesial margin of merus in *D. rectimanus* (Figure 6) where scattered spines are present in dorsal, ventral, outer and inner margin of merus in the later species.

MOLECULAR ANALYSES

More than three specimens for the representative species were taken for molecular identification to validate the morphologically identified species. An attempt was initiated to identify them by the COI gene marker. A total of 15 COI sequences were generated (Table 1). Another 11 sequences were downloaded from Genbank and used in this study. Sequences were submitted to GenBank and BOLD with GB Accession numbers and BOLD process ID, respectively (Table 1). Analysis was done in the BOLD system creating a dataset named "DS-DUHC".

The lengths of all the COI barcode sequences ranged from 319 to 635 bp, with an average of 566 bp. No stop codons, insertions, or deletions were observed in any sequences. The average nucleotide composition frequency was G: 21.86 ± 0.59%; C: 21.09 ± 1.10%; A: 24.58 ± 0.52%, and T: 32.47 ± 1.29%. The GC content was found as 42.95 ± 1.17%. The average percentages of GC contents were 49.76 ± 0.79% in the first, 43.09 ± 0.20% in the second, and 35.98 ± 2.84% in the third codon

positions. The average genetic distances were calculated from 15 COI sequences and found within species, genus, and family as 0.96 ± 0.02%, 22.80 ± 0.06%, and 25.13 ± 0.11%, respectively (Table 2). For the barcode gap analysis, the mean and maximum intra-specific values are compared to the nearest neighbour distance using the K2P model (Table 3). Where the species is a singleton, N/A is displayed for intra-specific values.

In K2P models, the species comparison advocated four species with max-intra and two with mean-intra distances higher than the nearest neighbouring species. A scatter plot of high genetic divergence species based on K2P distances is provided in Figure 7A, B. The summary of barcode gap comparisons is presented in Table 3 and indicates a clear gap among the species and the intra-specific distances also minimal for the identified species except *C. infraspinatus*.

The distribution of sequence divergence at each taxonomic level was calculated using 23 COI sequences, and the cluster sequence results revealed four OTUs (Table 1). The COI tree based on the neighbour-joining (NJ) method was constructed for COI sequences. The tree showed a single major clade for each species without any anomaly (Figure 8). *Clibanarius infraspinatus* showed a distance from the species of Japan and China. On the other hand, our sequence of *Diogenes rectimanus* formed a clade with the species of China with a bootstrap value of 99%, and another sequence of this species from Indonesia showed some divergence with these two sequences (95%).

Thus, the morphometric and molecular analysis confirms the geographic extension of these four species of hermit crabs. Further extensive surveys may explore new species or new records from the coastal and diverse mangrove habitats of Bangladesh.

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Table 2. Genetic divergence (K2P distance %) within species, genus and family for COI gene.

Label	n	Taxa	Comparisons	Min dist (%)	Mean dist (%)	Max dist (%)	SE dist (%)
Within species	14	3	40	0.00	0.96	3.45	0.02
Within genus	12	1	29	17.99	22.80	26.11	0.06
Within family	13	1	12	22.20	25.13	26.71	0.11

Table 3. Summary of mean and max intra-specific variation and the distance to the nearest-neighbour species (NN).

Sl. No.	Species	Mean intra-sp. variation	Max intra-sp. variation	Nearest species	Nearest neighbour	Distance to NN
1	*C. clibanarius*	N/A	0.00	*C. infraspinatus*	GBMNF8312-22	17.99
2	*C. infraspinatus*	3.12	3.87	*C. clibanarius*	GBMND85409-21	17.99
3	*C. padavensis*	0.94	3.45	*C. clibanarius*	GBMND85409-21	21.32
4	*D. rectimanus*	N/A	0.00	*C. infraspinatus*	GBMNF8312-22	22.20

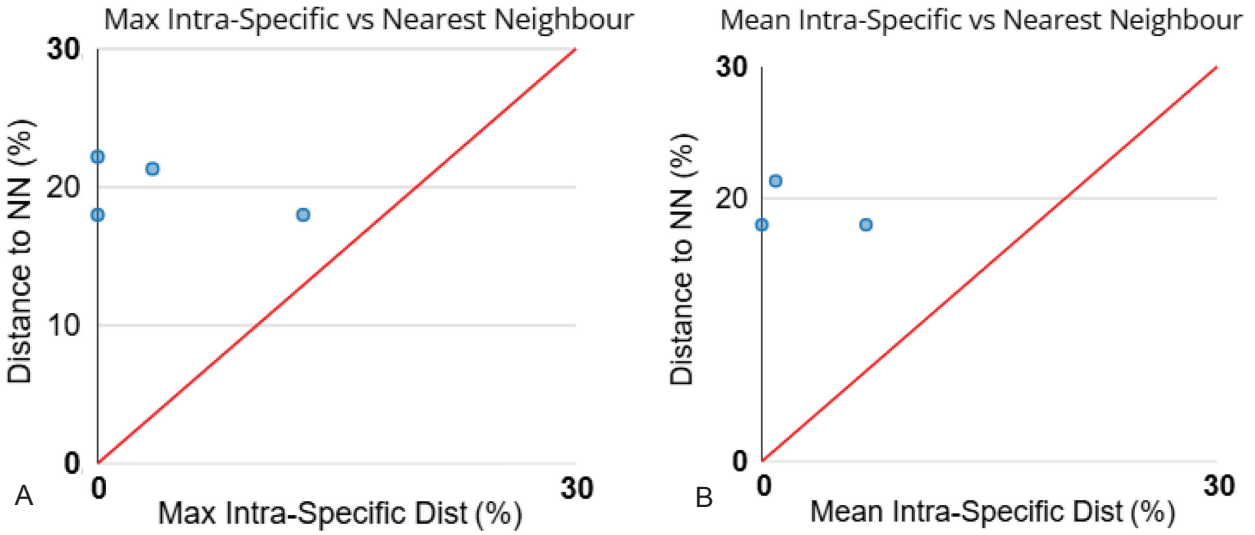

Figure 7. Comparison of **(A)** maximum intra-specific variation and distance to nearest-neighbour species and **(B)** mean intra-specific variation and distance to nearest-neighbour species.

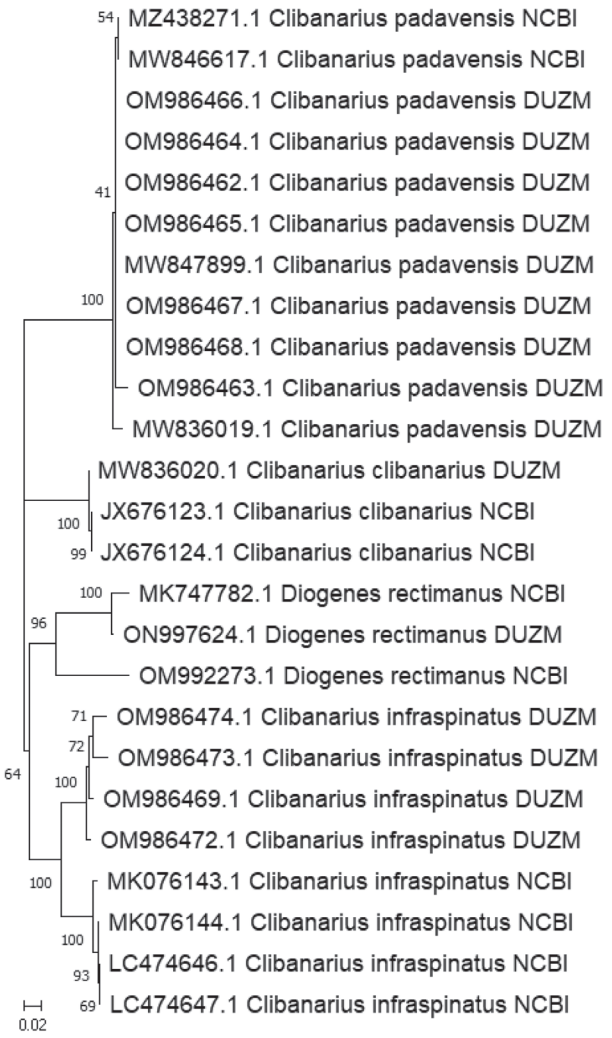

Figure 8. Neighbour-joining (NJ) tree of hermit crab species, using K2P distances. DUZM refers to the sequences from the present study, while NCBI to those downloaded from GenBank.

CRediT authorship contribution statement

Md. Sagir Ahmed: Conceptualization, Supervision, Methodology, Data curation, Writing – original draft, Writing – review & editing. Sumaiya Ahmed: Logistic, Sample collection, Writing – review & editing; Sujan Kumar Datta: Conceptualization, Methodology, Field collection, Laboratory analysis, Data curation & analysis, Writing – original draft, Writing – review & editing. Durjoy Raha Antu: Field collection, Data curation, Formal analysis, Writing – original draft, Writing – review & editing. Israt Jahan: Laboratory analysis, Data curation & analysis, Writing – review & editing. Mysha Mahjabin and Tasfia Tanjim Islam: Laboratory analysis, 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.

Availability of data and material

These sequence data have been submitted to the NCBI GenBank databases under accession number and BOLD system. Data can be retrieved from https://www.ncbi.nlm.nih.gov/nuccore and https://www.boldsystems.org/

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Declarations

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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