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Morphological and molecular characterization of the thunder crab *Myomenippe hardwickii* Gray, 1831 (Brachyura: Menippidae) from the Malabar coast, India

by

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Abstract

The thunder crab, *Myomenippe hardwickii*, has been reported to be seen across Southeast Asia, including the Indian coast. However, previous research has lacked detailed taxonomic descriptions, color photographs, or the molecular characterization of Indian specimens. This study presents the first comprehensive documentation of *M. hardwickii* in India, combining both morphological and molecular taxonomy techniques. It also represents the first recorded occurrence of this species along the Malabar Coast. DNA barcoding was performed using mitochondrial cytochrome c oxidase subunit I (COI) and 16S ribosomal RNA (16S rRNA) gene markers to validate the species identity based on taxonomic classification. Previous taxonomic references indicate that the gonopods of *M. hardwickii* and its sister species, *Myomenippe fornasinii* Bianconi, 1851 are similar. However, this study marks the first instance of presenting a detailed description and illustration of the male gonopods (G1 & G2) and outlining the characteristics of the vulvae in female individuals of *M. hardwickii*. Consequently, we have provided species identification supported by appropriate illustrations and ecological information.

Key words: Cochin estuary, Brachyura; Menippidae, stone crabs, taxonomy

Abbreviations

CL, carapace length at midline; CW, carapace maximum width; G1, first male pleopod/first gonopod; G2, second male pleopod/second gonopod; mtDNA COI, mitochondrial DNA cytochrome c oxidase subunit I; mxp3, third maxillipeds; P5, fifth pereopods; 16S rRNA, 16S ribosomal RNA.

1. Introduction

Stone crabs, which belong to the family Menippidae, are a significant component of the benthic invertebrate fauna in many regions of the world. Species such as the Florida stone crab *Menippe mercenaria* (Say, 1818), the Maroon stone crab *Menippe rumphii* (Fabricius, 1798), and the thunder crab *Myomenippe hardwickii*, Gray, 1831 are of economic and commercial significance in the coastal communities (Tan & Ng, 1994). Currently, there are 3 genera and 11 species in the family Menippidae (WoRMS Editorial Board, 2025). One of these genera is *Myomenippe*, which consists of two species: *M. hardwickii* and *M. fornasinii,* both of which remain poorly studied. These two species can reach a relatively large size (5–10 cm carapace width [CW]), seem to be localized in the intertidal zone, and *M. fornasinii* has been reported from Australian waters (Tan et al., 2016) as well as from Madagascar (Serène, 1984) and Mozambique (Miguel et al., 2025), whereas *M. hardwickii* has been reported across Southeast Asia (Ng, 1998). Although *M. hardwickii* has been reported from the Indian coast (Chhapgar, 1957; Deb, 1995; Dev Roy, 2008, 2013; Dev Roy & Nandi, 2012; Dineshbabu et al., 2011; Shet et al., 2016; Trivedi & Vachhrajani, 2012), none of these studies has provided a detailed taxonomic description or color photographs. Additionally, it is noteworthy to highlight the absence of molecular characterization data for an Indian *M. hardwickii* (GenBank, National Center for Biotechnology Information (NCBI) accessed 6 December 2025). Species identification in brachyuran crabs is frequently hindered by morphological conservatism, intraspecific variation, and overlapping diagnostic characters, resulting in misidentifications and uncertain distributional records (Alam et al., 2020; Ampuero et al., 2010). These limitations are particularly evident within Menippidae, where closely related species of *Menippe* and *Myomenippe* exhibit strong external similarity despite clear evolutionary separation (Hanim et al., 2025; Ng et al., 2008). Under such conditions, morphology alone is often insufficient for reliable species delimitation. DNA barcoding using mitochondrial COI and 16S ribosomal RNA (16S rRNA) markers has proven effective in resolving closely related crustacean taxa (Pardo et al., 2009; Tang et al., 2010), with COI providing high discriminatory power (Barber & Boyce, 2006; Costa et al., 2007; Radulovici et al., 2009). Consequently, an integrative taxonomic approach combining morphological and molecular evidence can be very practical for ensuring accurate species identification and to validate regional records of morphologically conservative brachyuran crabs. In the present study, *M. hardwickii* is documented from the Malabar Coast using an integrative taxonomic approach in which detailed morphological examination is combined with mitochondrial COI and 16S rRNA markers. This research represents the first detailed morphological and molecular characterization of *M. hardwickii* in this region, adding information on male gonopods and female vulvae that was not included by the original author or found in the available literature.

2. Methods

2.1. Sample collection

In the present study, a total of five specimens (two males and three females) were collected from two intertidal to shallow subtidal sites (<6 m) of the Cochin estuary, Kerala, Malabar Coast, India: (1) Thevara (9°55′27.6″N 76°18′01.8″E); (2) Marine Science Boat Jetty, School of Marine Sciences, CUSAT (9°57′50.0″N 76°16′54.6″E) (Fig. 1). Specimens were collected during October and November 2022, either by handpicking at low tide or incidentally captured using a van Veen grab sampler during benthic sample collection.

2.2. Morphological investigations

All of the specimens were transported to the lab for morphological analyses, and preserved in a 4% buffered formaldehyde solution. Tissue samples of leg muscle were preserved in 95% ethanol solution for molecular analysis. Photographs were taken using a Nikon D610 DSLR camera attached to a Nikkor 40 mm macro lens (Nikon Corporation, Japan) or to an Optika SLX-3 stereomicroscope (Optika S.R.L., Italy). Figures were edited using Adobe Photoshop 2021 (version 22.0). The morphometric measurements were taken with a digital Vernier caliper. CW was measured as the maximum distance across the carapace, including lateral teeth, while carapace length (CL) was taken from the frontal margin to the posterior margin along the midline. Abdominal width (AW) and abdominal length (AL) were measured on the ventral side of the crab. Propodus length (PL) and propodus height (PH) were measured from the major cheliped (crusher claw), from the carpus–propodus joint to the base of the fixed finger and at the maximum palm height, respectively. The identification process relied on key taxonomic characteristics outlined in the existing literature (De Man, 1887; Gray, 1831; Ng, 1998). The morphological terminology is in accordance with Davie et al. (2015). The following abbreviations are used: CW, carapace maximum width; CL, carapace length at midline; mxp3, third maxilliped; P5, fifth pereopod; G1, first male gonopod; and G2, second male gonopod.

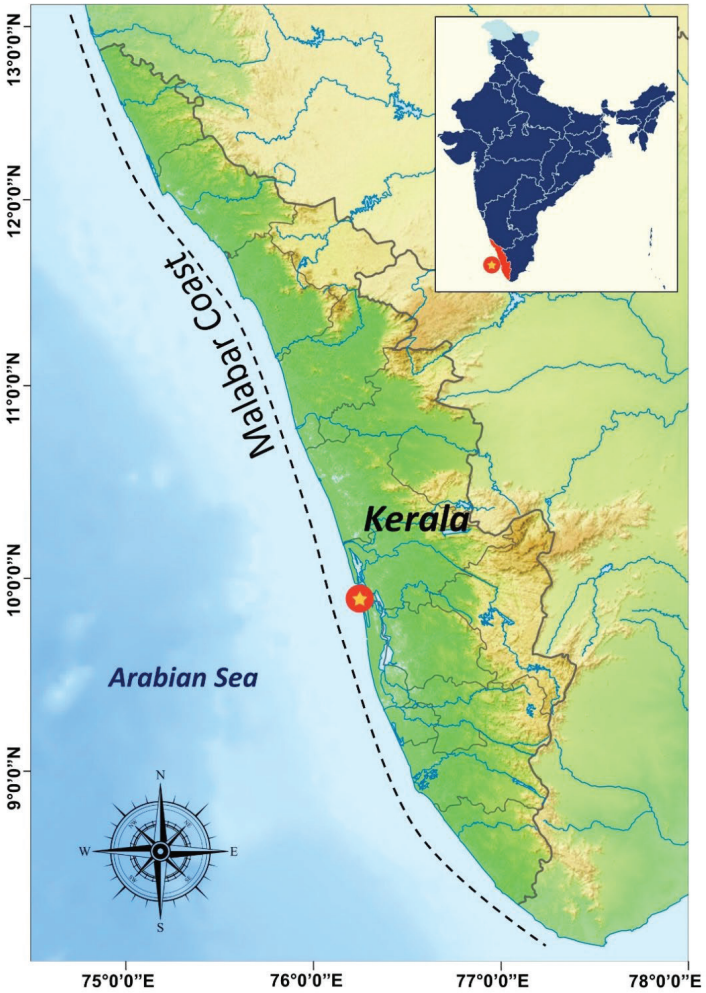

Figure 1

Map indicating the site of collection (red circle), in Cochin Estuary, Kerala, west coast of India.

2.3. Material examined

Three females (CW × CL: 42 mm × 31 mm, 56 mm × 42 mm, 64 mm × 47 mm), two males (CW × CL: 35 mm × 26 mm, 83 mm × 62 mm), Cochin estuary, coll. P. Hari Praved & K.V. Neethu, October & November 2022. The specimens examined were deposited in the collections of the Marine Biology Museum, Department of Marine Biology, Microbiology and Biochemistry, School of Marine Sciences, Cochin University of Science and Technology (CUSAT), Kochi, Kerala, India (MH/HPP1—HPP5/12-2022).

2.4. DNA barcoding

For the molecular study, total genomic DNA was extracted from the pereiopod muscle using the QIAamp Blood and Tissue Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Partial fragments of mitochondrial cytochrome c oxidase subunit I (COI) gene were amplified using universal primers LCO1490 5′ GTCAACAAATCATA AAGATATTGG-3′ and HCO2198 5′- TAAACTTCAGGGTG ACCAAAAAATCA -3′ (Folmer et al., 1994). The PCR mix included 12.5 μL of EmeraldAmp GT PCR Master Mix (Takara Bio), 0.4 μM primers, and 5 ng of template DNA in a 25 μL reaction volume. The amplification conditions for COI involved an initial denaturation step at 95°C for 180 s, followed by 35 cycles of 95°C for 30 s, 55°C for 60 s, and 72°C for 60 s, with a final extension of 72°C for 180 s. A mitochondrial DNA (mtDNA) region of 550 base pairs from the large subunit rRNA (16S rRNA) was amplified using the universal primers 16Sar (59- CGCCTGTTTATCAAAAACAT-39) and 16Sbr (59-CCGGTCTGAA CTCAGATCACGT-39) (Palumbi, 1996). The amplification conditions for 16S involved

an initial denaturation step at 94°C for 180 s, followed by 35 cycles of 94°C for 30 s, 50°C for 60 s, and 72°C for 60 s, followed by a final extension of 72°C for 180 s. The PCR products were purified with the GFX PCR DNA and Gel Band Purification Kit (Cytiva Life Technologies, Wilmington DE, United States). Bidirectional sequencing was performed with an ABI PRISM Big Dye Terminator v3.1 cycle sequencing kit in an AB 3730 DNA analyzer (Life Technologies).

Raw Sanger chromatograms were inspected and trimmed for base-calling errors in SeqScanner, followed by the sequences being compiled, analyzed, and edited using BioEdit v7.2.5, Ibis Therapeutics (Hall, 1999). DNA sequences of 631 bp and 527 bp were derived from the COI fragments, and approximately 449 bp from the 16S fragments. The sequences were compared with the available entries in GenBank (National Center for Biotechnology Information, NCBI) using the standard nucleotide basic local alignment search tool (BLAST). To confirm species identification and assess the genetic divergence among closely related taxa using the COI gene, we retrieved sequences for other representatives of Menippidae from GenBank (accession numbers provided on the phylogenetic tree). Based on the phylogeny of Lai et al. (2014), *Carpilius convexus* was selected as an appropriate outgroup, representing a lineage closely related to but outside Menippidae, enabling reliable alignment and stable rooting of the phylogenetic tree. Multiple sequence alignments for COI were generated using MAFFT v7.505 (Katoh et al., 2019) under default parameters, following a conservative alignment strategy to preserve positional homology. Phylogenetic tree for the COI dataset were constructed using the maximum likelihood (ML) method in MEGA 11 using the Tamura Nei model (T93), the best-fit nucleotide substitution model identified by the Bayesian Information Criterion, and implemented with 1000 bootstrap replicates (Tamura & Nei, 1993; Tamura et al., 2021). The 16S rRNA sequences were used solely to support species identification and were not included in phylogenetic reconstruction due to the limited availability of comparable reference sequences. Pairwise genetic distances between species for COI were also calculated using the Tamura-Nei (T93) model. The obtained mtDNA COI and 16S rRNA sequences were deposited in GenBank (COI: OQ948136, OQ954126, and 16S: OQ955252).

3. Results

The crabs were identified as *M. hardwickii* based on the morphological features as elaborated below in Figs 2–4 and Table 1.

Figure 2

M. hardwickii, male (35 mm × 26 mm), MH/HPP4/12-2022. Dorsal habitus and live coloration.

3.1. Systematic part

Family Menippidae Ortmann, 1893
Genus *Myomenippe* Hilgendorf, 1879
Myomenippe hardwickii (Gray, 1831)
(Figs 2–4; Table 1)
Synonymized names
Cancer Hardwickii Gray, 1831
Menippe (Myomenippe) duplicidens Hilgendorf, 1879
Menippe granulosa A. Milne-Edwards, 1867

3.2. Morphological description of specimens

Carapace is dorsally granulated and is broader with a short length (CW/CL 1.33–1.36 times) (Table 1), appearing transversely ovate; the coloration is dirty brown to brownish yellow. The carapace regions are well defined, characterized by shallow depressions and adorned with numerous small granules. The cardiac region is distinctly separated from the gastric region; the gastric region is distinct and is subdivided into three portions—two anterolateral and one posterior (Fig. 3A). The surfaces of the two anterolateral gastric regions show a division into two slight, granule-covered convex lobes. The frontal margin is bilobed, separated from the orbit by a marked concavity, and the lobes are separated from each other by a median notch; each lobe possesses three teeth (Fig. 3D). The anterolateral margin is granular, thin, and sharp, and is rugose; it bears four broad lobiform

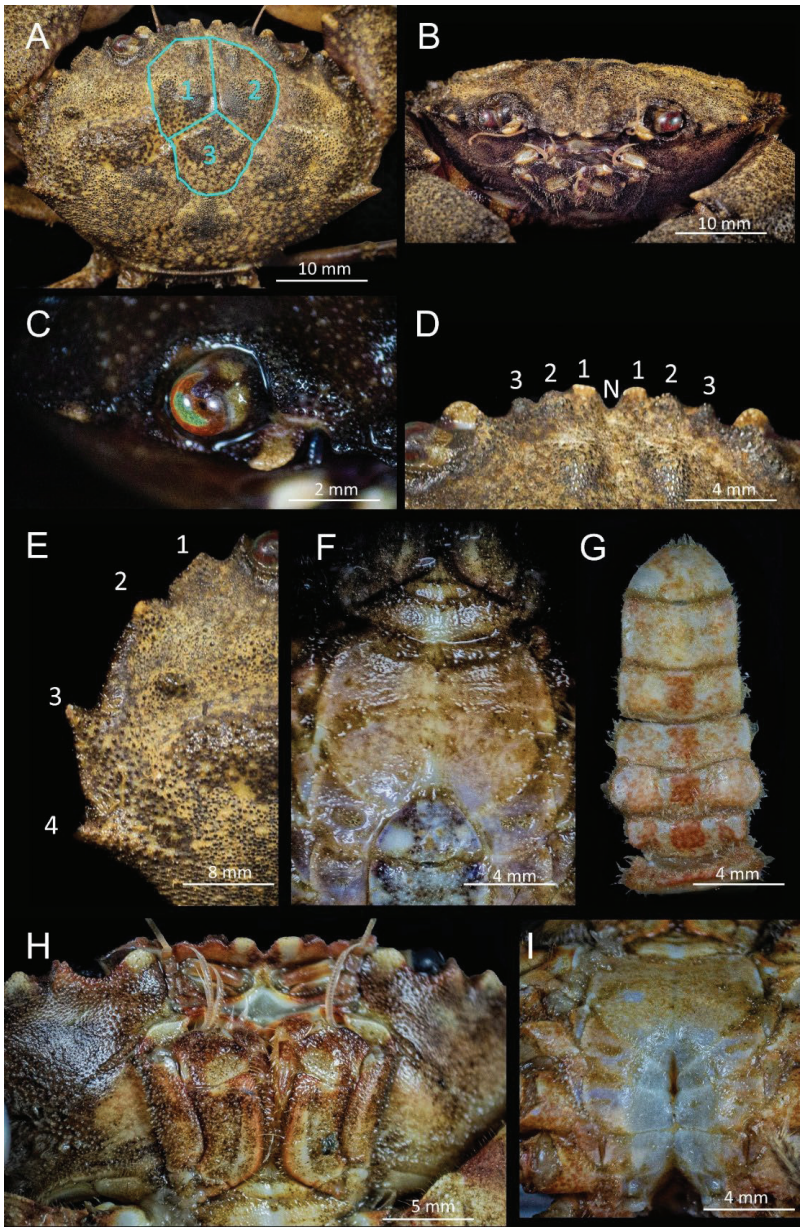

Figure 3

M. hardwickii, male, (35 mm × 26 mm), MH/HPP4/12-2022: **(A)** Dorsal view displaying three sections of the gastric region; **(B)** cephalothorax, frontal view; **(C)** green pigmented cornea ringed with red and extended infra orbital margin; **(D)** bilobed frontal margin with three teeth each; **(E)** anterolateral margin with four teeth; **(F)** anterior thoracic sternum and telson, ventral view; **(G)** detached pleon, ventral view; **(H)** mxp3, external view; **(I)** thoracic sternum and sternopleonal cavity. mxp3, third maxillipeds; N, notch.

crested teeth on each side. The first three teeth are broad and anteriorly acuminate, whereas the most posterior tooth is short and narrow (Fig. 3E). The orbits (Figs 3B and 3C) are wide, ovate, and closed internally; the eyes are large, filling the entire orbital cavity, and the corneas are pigmented green and ringed with red in life (Fig. 3C). The infraorbital margin is granular, and an exorbital tooth is present; the large internal lobe of the infraorbital margin extends slightly more forward than the third frontal tooth. The antennular fossae are subrectangular; the antennules are robust and fold transversely and obliquely; the antennary flagellum lies outside the orbital gap. The third maxilliped is large and fits tightly into the buccal cavity (Figs 3H and 4C);

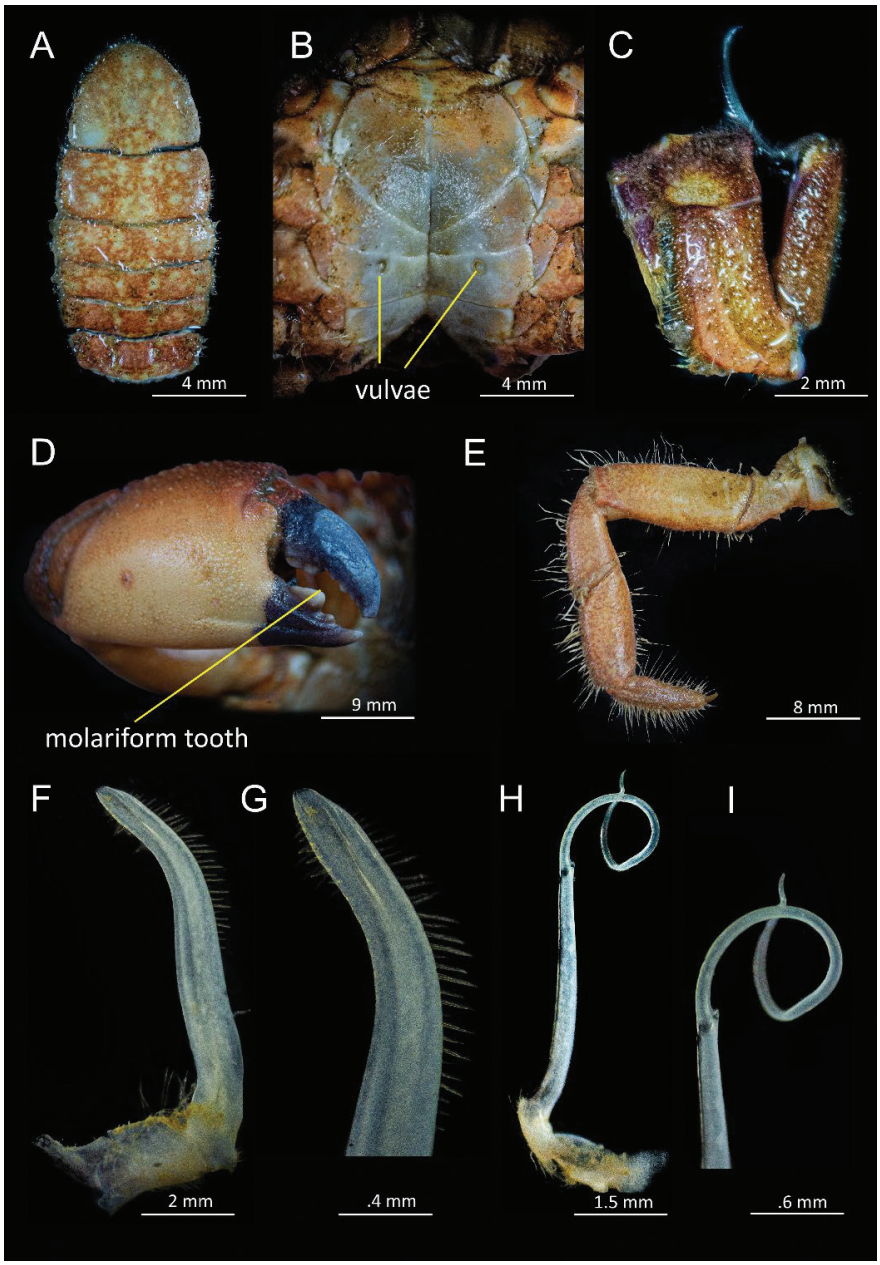

Figure 4

M. hardwickii, female (42 mm × 31 mm), MH/HPP1/12-2022: **(A)** detached female pleon, external view; **(B)** female thoracic sternum and vulvae, ventral view; **(C)** left third maxilliped, ventral view; **(D)** right cheliped with molariform tooth; **(E)** right P5, ventral view; **(F)** entire first male left G1, ventral view; **(G)** distal tip of left G1, ventral view; **(H)** entire second male right G2, ventral view; **(I)** distal tip of G2, ventral view.

it is finely granular externally, and the inner and outer margins of the ischium and merus are sparsely setose. The merus of the third maxilliped is subquadrate, with the distolateral angle projecting and rounded; the ischium is subrectangular and about twice as long as the merus. The palps are thick and subcylindrical, and hairy tufts occur on the inner apical region of the carpus, propodus, and dactylus. The exopod is elongate and not tapering; its distal tip reaches three-quarters of the lateral margin of the merus, and the flagellum is long and well developed. The male thoracic sternum (Fig. 3F) is broad, with a smooth, lightly setose surface; a distinct suture separates the sternites. The abdomen is seven-segmented (Fig. 3G);

Table 1

Morphological measurements of *M. hardwickii* (Gray, 1831)

Measurements (mm)								
No.	CW	CL	AW	AL	PL	PH	Sex	CW/CL
1	42	31	14	22	27	16	Female	1.355
2	56	42	19	28	38	23	Female	1.333
3	64	47	11	32	53	29	Female	1.362
4	35	26	12	18	23	11	Male	1.346
5	83	62	26	43	68	36	Male	1.339

AL, abdominal length; AW, abdominal width; CL, carapace length; CW, carapace width; PH, propodus height; PL, propodus length.

the pleon is sparsely granulose and setose, with all somites and the telson freely articulating. The lateral margin is convex, and the telson is subtriangular, bluntly rounded distally, with the base as broad as it is wide. The female pleon (Fig. 4A) is much wider than that of the male; somite 3 is the widest in males, whereas somite 5 is the widest in females. The male first gonopod (G1) is stout, with the apical process gently curved; a fine line of setae is present distally along the margins (Figs 4F and 4G). The male second gonopod (G2) is elongate, subequal in length to the G1, and its distal segment is coiled inward (Fig. 4H). The vulvae in females are located 2–3 mm laterally on the sixth thoracic sternite and are ovate in outline (Fig. 4B). The chelipeds are massive and robust, slightly unequal, with the right chela larger in all specimens; males have proportionally larger and more robust chelipeds than females; the carpus is curved, and a small inner spine extends anteriorly from it. The digits are stout and black; the base of the dactylus of the larger chela bears a prominent molariform tooth (Fig. 4D). The dorsal surfaces of the carpus and palm of the chelipeds are granulose. The walking legs are slender; the margins of all legs are fringed with setae, with the thicker setae occurring on the propodus and dactylus.

3.3. Molecular characterization of *M. hardwickii* from India

We successfully amplified and sequenced two mtDNA COI fragments of 631 bp and 527 bp from *Myomenippe* specimens collected from the Cochin Estuary. The nucleotide BLAST analysis showed that both fragments exhibited 100% sequence identity with *M. hardwickii* reference sequences in GenBank (accession numbers: PX457434 and PQ536003), confirming a perfect match across the full sequence length. In addition, a 449 bp fragment of the 16S rRNA gene was generated, which displayed >98.89% sequence identity to the only available *M. hardwickii* 16S reference sequence in GenBank (accession number: HM637977) from Singapore. The sequences obtained in this study are deposited in GenBank under accession numbers OQ948136, OQ954126 (COI), and OQ955252 (16S).

The evolutionary relationships of *M. hardwickii* to other menippid crabs were examined using an ML phylogenetic tree constructed from mtDNA COI sequences (Fig. 5). The ML phylogeny divides the taxa into two major clades within Menippidae. The two sequences generated in this study (OQ954126 and OQ948136) cluster tightly with previously published *M. hardwickii* sequences from Thailand (PX457434), Malaysia (PQ536003), and Singapore (HM638052), forming a strongly supported monophyletic group with a bootstrap value of 100%. *M. fornasinii* forms the immediate sister clade, represented by sequences PV053328, LK391943, and NC024437, confirming its close evolutionary proximity to *M. hardwickii*. Other menippid species, including *M. rumphii* (OL960453), *Menippe nodifrons* (MW264437), *Menippe adina* (MW094166), and *M. mercenaria* (MW094171), form distinct and well-separated lineages outside the *Myomenippe* clade. The tree is appropriately rooted with *C. convexus* (HM638025). Pairwise TN93 distances illustrate the same phylogenetic patterns (Table 2). Genetic divergence between *M. hardwickii* from India and its GenBank representatives was found to be low (0.01). The mean TN93 distance between *M. hardwickii* and *M. fornasinii* within *Myomenippe* was 0.118, whereas distances between *Myomenippe* and *Menippe* species were notably higher (mean = 0.176). Net TN93 distances also revealed clear species boundaries: the net divergence between *M. hardwickii* and *M. fornasinii* was 0.114, compared with 0.116 between *Myomenippe* and the other *Menippe* species, indicating deeper intergeneric separation. Together, the phylogenetic topology and genetic distance metrics robustly confirm the identity of the present specimens as *M. hardwickii* and demonstrate their strong genetic affinity with the Southeast Asian populations.

3.4. Habitat and ecology

M. hardwickii was found on submerged structures, decaying aquatic weeds, and mangroves in the Cochin Estuary. The species is usually abundant during the pre-monsoon period

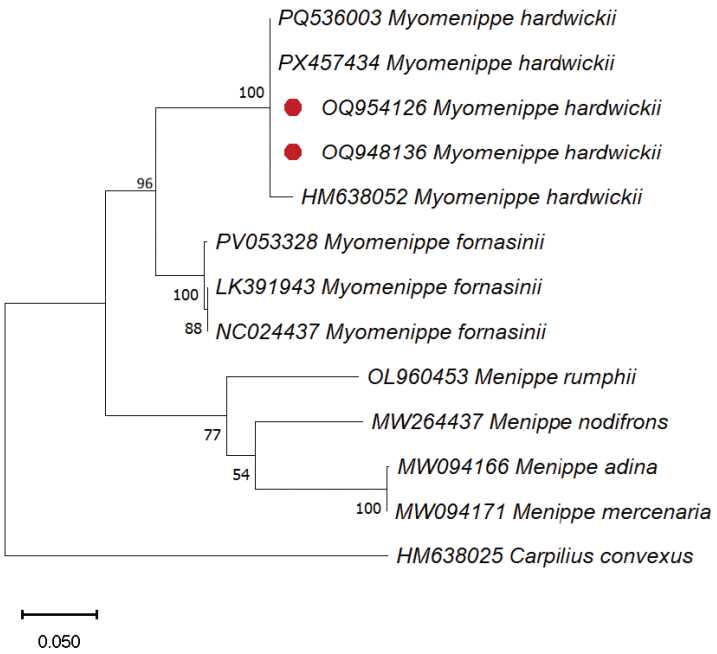

Figure 5

Molecular phylogenetic tree inferred using the ML method based on the Tamura–Nei model (T93) showing the relationships among *M. hardwickii*, related *Menippe* species, and the outgroup *Dromia personata*. The analysis is based on a COI gene alignment generated after sequence trimming and multiple sequence alignment using MAFFT v7.505. Bootstrap support values (>50%) are indicated at the nodes and branch lengths drawn to scale as substitutions per site. Sequences generated in this study are represented by a red circle. ML, maximum likelihood.

(January–May) at sites with a salinity of 10–33 ppt and depth of 2–6 m. During the sampling period (October & November 2022), the organic content in the sediment was 1.7%–2.8%. The habitat is muddy to clayey–silty, the pH of the sediment ranged from 7.5 to 8.3, and the average redox potential was from –135 to –275 mV. The total carbon of the sediment ranged from 29 to 37 g/kg. In the present observation, they were found to occur together with encrusting communities such as clumps of mussels (*Mytella strigata* and *Perna virdis*), oyster bed of *Magallana bilineata,* gastropods (*Nassodonta insignis*), amphipods (*Grandidierella gilesi*), and other brachyuran crabs such as *Thranita crenata*, *Neorhynchoplax alcocki*, *Neorhynchoplax demeloi*, and *Aniptumnus bijoyi*.

3.5. Remarks

M. hardwickii is morphologically most similar to its sister species *M. fornasinii* (Fig. 6A), in the general form of the carapace, male pleon, and pereopods. Nevertheless, *M. hardwickii* is readily distinguished from *M. fornasinii* by a combination of carapace sculpture, anterolateral tooth morphology, cheliped ornamentation, and gonopod structure. The dorsal surface of the carapace in *M. hardwickii* is distinctly granulose, whereas it is smooth in *M. fornasinii* (Figs 6A–6C). In *M. hardwickii*, the first two anterolateral teeth are strongly produced and closely spaced, the second tooth is relatively robust, and the supraorbital margin is continuous, lacking visible fissures (Fig. 6B). By contrast, *M. fornasinii* possesses weakly produced first and second anterolateral teeth, with the second tooth more elongate and more widely separated from the third (Fig. 6C) (De Man, 1887, 1899), and a supraorbital margin divided by two distinct fissures (Fig. 6C) (Haswell, 1881; Milne-Edwards, 1867). Chelipeds of *M. hardwickii* are dorsally granulose, with coarse granules on the propodus and carpus and faint punctation on the mesial surfaces (Fig. 2) (De Man, 1887), whereas those of *M. fornasinii* are smooth and distinctly punctate, with the punctae being larger and more

Pairwise comparisons based on TN93 distances between species included in the study

No.	Species name	1	2	3	4	5	6	7	8	9	10	11	12	13
1	*Myomenippe hardwickii* OQ948136 (India)													
2	*Myomenippe hardwickii* PQ536003 (Malaysia)	0.0000												
3	*Myomenipp hardwickii* PX457434 (Thailand)	0.0000	0.0000											
4	*Myomenippe hardwickii* OQ954126 (India)	0.0000	0.0000	0.0000										
5	*Myomenippe hardwickii* HM638052 (Singapore)	0.0137	0.0160	0.0160	0.0160									
6	*Myomenippe fornasinii* LK391943 (Australia)	0.0979	0.1170	0.1157	0.1223	0.1299								
7	*Myomenippe fornasinii* NC024437 (Australia)	0.0979	0.1170	0.1157	0.1223	0.1299	0.0000							
8	*Myomenippe fornasinii* PV053328 (Australia)	0.1027	0.1213	0.1213	0.1258	0.1352	0.0046	0.0046						
9	*Menippe adina* MW094166	0.1813	0.1726	0.1697	0.1805	0.1757	0.1717	0.1717	0.1756					
10	*Menippe mercenaria* MW094171	0.1817	0.1761	0.1731	0.1843	0.1757	0.1684	0.1684	0.1720	0.0015				
11	*Menippe nodifrons* MW264437	0.1690	0.1734	0.1731	0.1816	0.1611	0.1849	0.1849	0.1862	0.1355	0.1325			
12	*Menippe rumphii* OL960453	0.1699	0.1740	0.1740	0.1759	0.1682	0.1812	0.1812	0.1827	0.1511	0.1480	0.1506		
13	*Carpilius convexus* HM638025	0.2414	0.2361	0.2360	0.2361	0.2446	0.1957	0.1957	0.2011	0.2127	0.2127	0.2588	0.2607	

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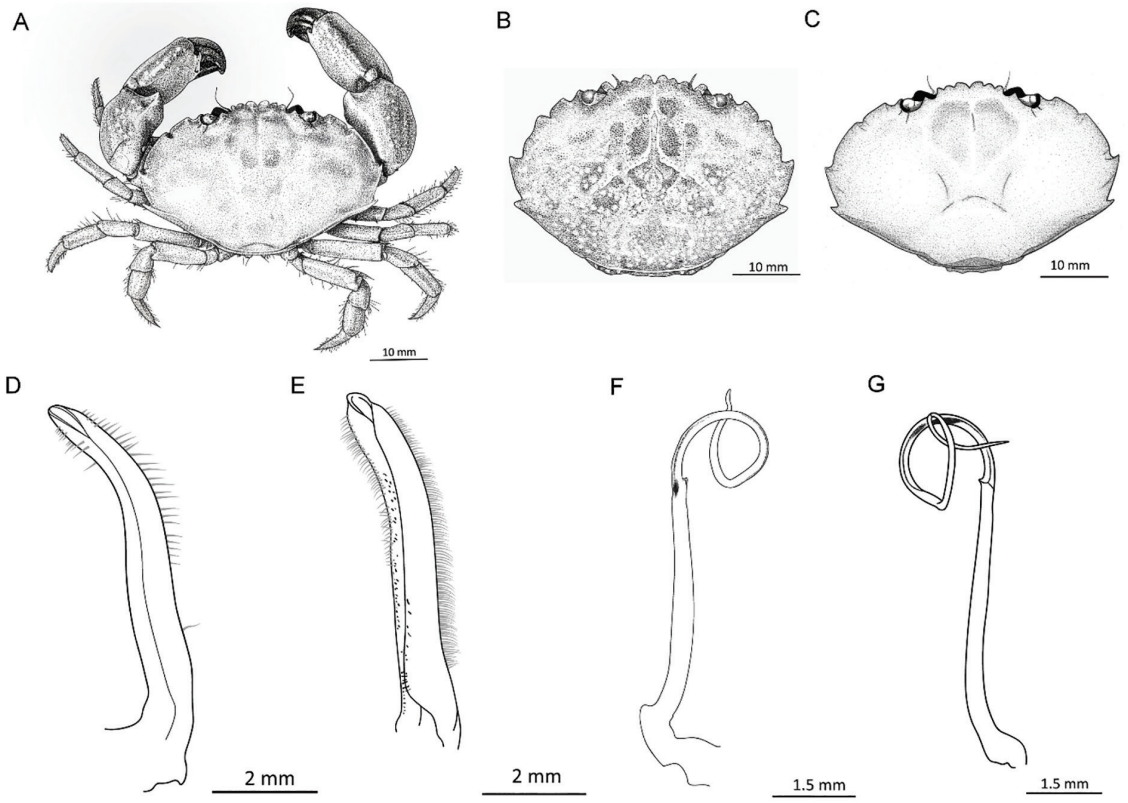

Figure 6

M. hardwickii, male (35 mm × 26 mm), MH/HPP4/12-2022: **(B)** carapace, dorsal view; **(D)** left first pleopod (G1), ventral view; **(F)** right second pleopod (G2), ventral view. *M. fornasinii*, male: **(A)** dorsal habitus; **(C)** carapace, dorsal view; **(E)** left first pleopod (G1), ventral view; **(G)** left second pleopod (G2), ventral view. Panels **(A)** and **(C)** are adapted and redrawn from Miguel et al. (2025); panels **(E)** and **(G)** are redrawn from Serène (1984).

densely arranged along the dorsal margin of the palm (Fig. 6A) (De Man, 1899; Miguel et al., 2025). Earlier accounts of *M. hardwickii* are limited. Gray (1831) provided only a brief description of the type material, lacking detailed information on diagnostic characters, such as the male gonopods (G1, G2) or the position and shape of the female vulvae. Subsequent treatments by Milne-Edwards (1867) and Hilgendorf (1879) added little beyond Gray's original account. Serène (1984) presented a key to the genus *Myomenippe* and illustrated the gonopods of *M. fornasinii*, noting the apparent similarity of gonopods in both species, while Ng (1998) largely reiterated earlier observations without additional morphological detail. The present study documents and illustrates, for the first time, the male gonopods (G1, G2) and the position and shape of the female vulvae in *M. hardwickii*. The first gonopod (G1) of *M. hardwickii* is stout, with the apical process more gently curved than in *M. fornasinii* (Fig. 6D); in *M. fornasinii*, the G1 bears denser marginal setation (Fig. 6E). The distal segment of the second gonopod (G2) in *M. hardwickii* is singly coiled (Fig. 6F), whereas it is doubly coiled in *M. fornasinii* (Fig. 6G). In females of *M. hardwickii*, the vulvae are ovate in outline and located laterally on thoracic sternite 6 (Fig. 4B).

4. Discussion

Recognizing species accurately remains challenging in the absence of comprehensive morphological documentation, particularly in morphologically conservative brachyuran crabs (Paransa et al., 2024; Spiridonov et al., 2014). Such taxonomic uncertainty has broader implications beyond systematics: misidentification can pose risks to biodiversity assessment, and insufficient taxonomic or geographic

resolution is a persistent concern in the food industry. This issue is especially relevant for crabs, which constitute some of the most heavily exploited benthic marine resources worldwide and in India (Josileen et al., 2021; Penn et al., 2018; Stevens & Miller, 2020). Stone crabs (Menippidae) are harvested in commercial and recreational claw-based fisheries, and represent one of the most valuable crab fisheries in the United States (Hogan & Griffen, 2014). In India, they remain underutilized within the broader marine crab fishery; however, they are considered a local delicacy along the Maharashtra and Konkan coasts (Vartak et al., 2015b). The practice of discarding the body and retaining only the claws complicates morphology-based species identification, thereby increasing the importance of DNA-based genetic authentication for processed crab meat products (Haye et al., 2012; Vartak et al., 2015a). Consequently, robust species identification and inventorying require rigorous and systematic approaches, for which DNA barcoding has become a powerful and widely accepted tool for verifying species identity and geographic origin in exploited marine taxa (Lockley & Barsley, 2000). Within this framework, the integrative morphological and molecular approach applied in the present study provides a reliable and independently validated record of *M. hardwickii* from Indian waters. Although *M. hardwickii* has been reported previously from several regions along the Indian coastline (Chhapgar, 1957; Deb, 1995, 1999; Dev Roy, 2008, 2013; Dev Roy & Nandi, 2012; Dineshbabu et al., 2011; Shet et al., 2016; Trivedi & Vachhrajani, 2012), most of these records lack detailed morphological documentation or molecular validation, and misidentification with *M. fornasinii* or *M. rumphii* has been frequent. As a result, the reliability of earlier distributional records has remained uncertain. The present study therefore provides the first unequivocally validated record of *M. hardwickii* from the Malabar Coast, supported by comprehensive morphological descriptions, reproductive anatomy, and DNA sequence data, filling a significant gap in the verified distribution of the species along the southwest coast of India, and complementing recent confirmed records from the eastern Indian coast (Prusty et al., 2022).

Molecular evidence generated in this study robustly supports the morphological identification of the specimens. DNA barcoding has been widely applied to crustacean taxonomy and has proven particularly effective for resolving closely related species (Bezeng & van der Bank, 2019; Costa et al., 2007; Radulovici et al., 2009). The mitochondrial COI and 16S rRNA sequences obtained from Indian *M. hardwickii* exhibit extremely low divergence from Southeast Asian reference sequences and form a strongly supported monophyletic clade in ML analyses. Clear separation between *M. hardwickii* and *M. fornasinii* is consistently supported by both phylogenetic topology and pairwise genetic distance estimates, corroborating the morphological distinctions observed between the two taxa. The low level of mitochondrial divergence detected among geographically distant populations of *M. hardwickii* indicates genetic cohesion for the markers examined, suggesting either effective larval dispersal or historical connectivity across the Indo–West Pacific (Fig. 5A). These results reinforce the value of integrating classical morphology with mtDNA barcoding in resolving species boundaries within morphologically conservative brachyuran groups (Alves et al., 2025; Chu et al., 2015; Geller et al., 1997; Mendoza et al., 2022). Although broader population-level sampling will be required to assess fine-scale phylogeographic structure, the present data demonstrate that even limited mitochondrial markers can be sufficient to resolve species-level relationships in *Myomenippe*, a genus currently comprising only two recognized species.

The availability of mitochondrial barcode data for menippid crabs remains limited, particularly from Indian waters. The COI and 16S sequences generated here represent the first publicly available mitochondrial data for *M. hardwickii* from the region and substantially improve the geographic representation of the genus in global sequence databases. These data provide an essential baseline for future studies on population structure, connectivity, and evolutionary history of menippid crabs in the Indian Ocean. Although detailed morphological comparisons are addressed in the 'Remarks' section, the inclusion of previously undocumented reproductive characters strengthens the overall taxonomic framework for *M. hardwickii* and enhances confidence in species-level diagnoses. Together, the integration of classical morphology and molecular evidence in this study contributes to a more reliable understanding of brachyuran diversity along the southwest coast of India.

5. Conclusion

This study confirms the presence of *M. hardwickii* on the Malabar Coast using an integrative taxonomic framework that combines diagnostic morphology with mitochondrial DNA markers. The first detailed documentation of male gonopods and female vulvae provides reliable diagnostic characters for distinguishing this species from its sister taxon *M. fornasinii* and establishes a solid morphological

foundation for future taxonomic and comparative studies of the genus *Myomenippe*. The validated COI and 16S rRNA sequences generated here represent the first genetic reference data for *M. hardwickii* from India and link the Indian population with conspecific populations across Southeast Asia. Collectively, these findings provide an important baseline for future taxonomic, biogeographic, and population-level studies of Menippidae in the Indo–West Pacific and contribute to improved accuracy in regional biodiversity assessments.

Declarations

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Authors contributions

P. Hari Praved: Field collection, Conceptualization, Methodology, Morphological investigation, Molecular investigation, Writing original draft & editing. K.V. Neethu: Field collection, Formal analysis, Writing original draft & editing. E.H. Aravind: Formal analysis, Writing - review & editing. S. Bijoy Nandan: Resources, Supervision, Writing - review & editing, Validation. P.C. Shamily Catherine: Molecular analysis, Writing review & editing.

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Competing interests

The authors declare that they have no competing interests.

Availability of data and material

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

Not applicable.

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