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Research note

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New record, with a summary of the distribution and an appraisal of the conservation status of the stone crab *Myomenippe fornasinii* (Bianconi, 1851) (Crustacea: Decapoda: Menippidae) in Mozambique, Western Indian Ocean

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ABSTRACT

Myomenippe fornasinii (Bianconi, 1851) is a poorly known species of brachyuran crab previously recorded from northern Australia. We provides the first genetically validated record of *M. fornasinii* from Mozambique, southwestern Indian Ocean. Morphological identification and DNA barcoding using the COI and 16S rRNA genes confirmed 100% genetic similarity with low intraspecific divergence (< 1%) with Australian populations. Phylogenetic analyses confirmed species boundaries and extended the known range of the species. These findings suggest a broader or underestimated range, possibly influenced by anthropogenic or natural dispersal, and highlight the ecological importance of estuarine habitats. The study reinforces the value of integrative taxonomy for conservation planning and biodiversity assessment in coastal ecosystems in the western Indian Ocean.

KEYWORDS: Crustacea, DNA barcoding, estuaries, genetic fingerprinting

Myomenippe Hilgendorf, 1879 (Menippidae) comprises two currently recognized species: the mangrove stone crab M. hardwickii (Gray, 1831) found across the Indo-West Pacific (Prusty *et al.*, 2023; Akhmadi *et al.*, 2024) and the smooth stone crab M. fornasinii (Bianconi, 1851), which remains relatively unknown, with limited records primarily from northern Australia (Tan *et al.*, 2016). The first documented record of *M. fornasinii* in Mozambique, southwestern Indian Ocean, was based on specimens described as *Galene fornasinii forlanini* by Bianconi (1851). No specimens were collected ever since.

Myomenippe fornasinii plays a significant role in shallow-water coastal ecosystems influenced by tidal dynamics such as estuaries, rocky reefs, coral reefs, and mangrove forests (Dahdouh-Guebas *et al.*, 1999). The robust chelipeds of M. fornasinii are adapted for breaking hard-shelled prey such as mollusks, reflects its impact on local trophic dynamics (Carpenter & Niem, 1998; Rahayu & Setyadi 2009).

Specimens of M. fornasinii were collected from the Bons Sinais River estuary, Inhassunge District, Zambezia province, Mozambique (18°04'01.82"S, 36°54'34.39"E) (Fig. 1A). The estuary is characterized by predominantly clay-rich sediments with high organic matter content and exhibits considerable variability in carbon and nitrogen levels (Groeneveld *et al.*, 2021). Water temperatures ranged 30°C–35°C, creating favorable environmental conditions for a diverse array of aquatic species (Hoguane *et al.*, 2021).

Sampling was conducted during the rainy season in February 2023. Five individuals with a carapace width of 3–6 cm were

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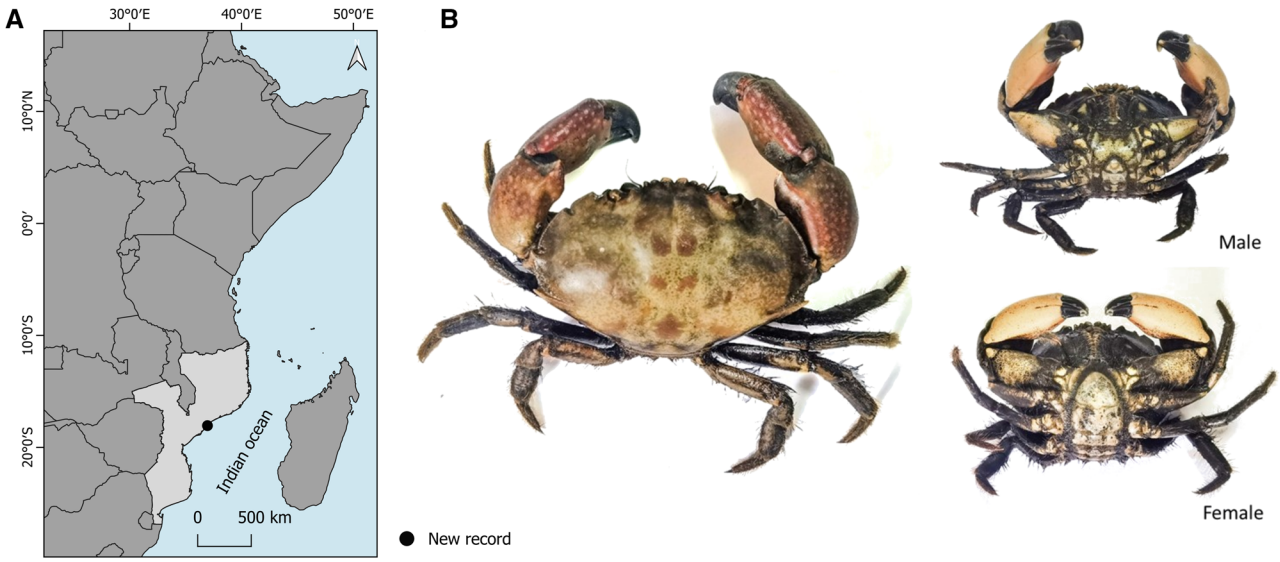

Figure 1 New confirmed record (●) of Myomenippe fornasinii in the Rio dos Bons Sinais estuary, Zambezia province, Mozambique (**A**). Dorsal and ventral views of *M. fornasinii* specimens: adult male (center and top right) and female (bottom right) (**B**).

collected from decomposing mangrove wood. Preliminary identification used taxonomic keys (De Man, 1899; Carpenter & Niem, 1998) based on morphological features.

Muscle tissue samples were extracted from each specimen and preserved in 96% ethanol for molecular analyses. Genomic DNA was isolated from tissue using the Wizard Genomic DNA Purification Kit (Promega, Madison, WI, USA) following the manufacturer's protocol. The quality of the extracted DNA was assessed by electrophoresis on a 1% agarose gel stained with GelRed and visualized under ultraviolet light using a transilluminator.

The mitochondrial cytochrome c oxidase subunit I (COI) and 16S rRNA genes were targeted for amplification using the polymerase chain reaction (PCR) with universal primers LCO1490 and HCO2198 (Folmer *et al.*, 1994) for the COI gene, and 16Sar and 16Sbr for the 16S rRNA gene (Palumbi & Benzie 1991). PCR reactions were performed in a final volume of 15 µl, consisting of 2.4 µl of dNTPs (1.25 mM), 1.5 µl of buffer solution (10×), 1.2 µl of $MgCl_2$ (25 mM), 0.8 µl of each primer (50 ng µl–1), 1–3 µl of genomic DNA (100 ng µl–1), 0.2 µl of Taq DNApPolymerase (5 U µl–1), and nuclease-free water to complete the volume. The thermal cycling protocol consisted of an initial denaturation step at 95°C for 5 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 40 s, and extension at 72°C for 40 s, with a final extension at 72°C for 7 min.

Positive PCR products were purified using a polyethylene glycol (PEG) 8000 M protocol, as described by Dunn &Blattner (1987). Purified amplicons were sequenced using the Sanger dideoxy termination method (Sanger *et al.*, 1977) with the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) following the manufacturer's instructions. Electrophoresis of the DNA fragments was conducted on an ABI 3500 XL genetic analyzer (Applied Biosystems). Negative controls were included in all PCR reactions to monitor potential contamination. All sequences generated were deposited in the GenBank database (http://ncbi.nlm.nih.gov) and are available under the accession numbers listed in Supplementary material Table S1.

The sequences were automatically aligned using ClustalW (Thompson *et al.*, 1994) implemented in GENEIOUS 9.0.5 (www.geneious.com). Phylogenetic relationships were assessed through maximum likelihood (ML) analysis using RAxML 7.2.7 (Stamatakis, 2006). The analysis was performed under the GTR-GAMMA model, and branch support for the best-scoring tree was evaluated based on 1,000 bootstrap pseudoreplicates. The resulting trees were edited using FigTree 1.4.3 (http://tree.bio.ed.ac.uk/software/figtree). The genetic distance between groups was estimated using two methods: the net number of nucleotide substitutions per site (Da), as described by Nei (1987: equation 10.21), and the uncorrected genetic distance (p-distance). All analyses were conducted using the DNAsp software (Librado & Rozas, 2009).

Species of Menippe De Haan, 1833 (family Menippidae) and species from other families of Decapoda were used as outgroups. Sequences were retrieved from GenBank (https://www.ncbi.nlm.nih.gov) and BOLD Systems (https://v3.boldsystems.org) databases (Supplementary material Table S1).

The specimens of *M. fornasinii* were identified based on diagnostic morphological characters, including the quadrate carapace with four well-defined anterolateral teeth, the granular surface of the dorsal carapace, robust chelae with dark tips, and walking legs covered with rigid bristles. The robust chelae tips were strongly calcified and dark brown (Fig. 1B).

The mitochondrial DNA dataset comprised 631 bp from the COI gene and 543 bp from the 16S rRNA gene. Phylogenetic analyses revealed a well-defined cluster, grouping other *M. fornasinii* sequences available in GenBank and BOLD systems into a single monophyletic group characterized by a highly supported clade (Fig. 2). These analyses demonstrated a supported separation between the clusters corresponding to all specimens identified as *M. fornasinii* and those identified as *M. hardwickii* (Fig. 2A, B). The genetic divergence, assessed by Da (0.0061) and

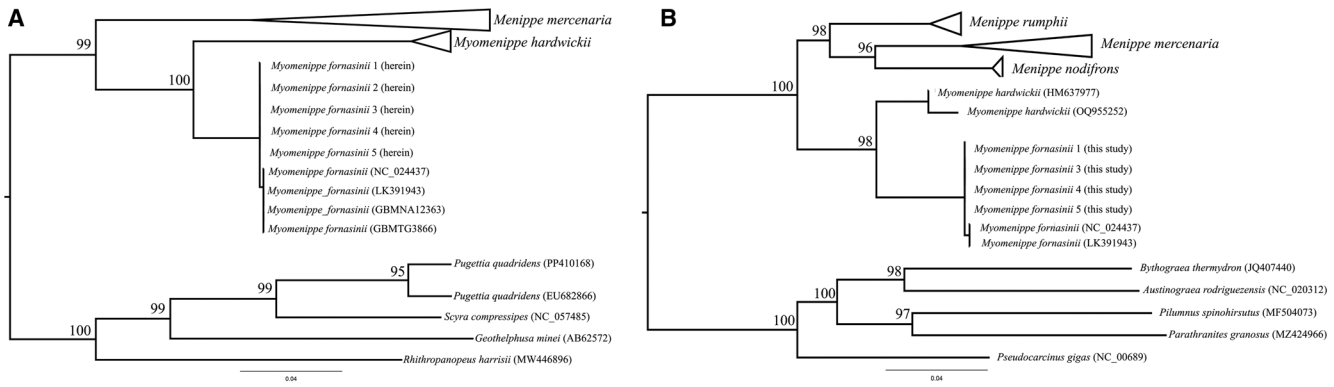

Figure 2. Phylogenetic tree inferred using the maximum likelihood (ML) method based on mitochondrial cytochrome c oxidase subunit I (COI) (**A**) and (**B**) 16S rRNA gene sequences from Myomenippe fornasinii and related species of Menippidae. Bootstrap values are shown at the nodes, indicating the statistical support for each clade.

Table 1. Genetic divergence between the main mtDNA defined group measured by p-uncorrected distance and Da (average pairwise net nucleotide divergence following equation 10.21 of Nei (1987)).

	p-distance	Da
M. fornasinii (herein) × *M. fornasinii* (Genbank)	0.006805	0.006090
M. fornasinii (Genbank and herein) × M. hardwickii	0.5338	0.4669

uncorrected p-distance (0.0068; ~0.7%) between the specimens analyzed and *M. fornasinii* sequences retrieved from GenBank, was very low (Table 1), corroborating the genetic homogeneity and validating the accurate taxonomic identification of the sequenced specimens.

Our results provide robust evidence for the phylogenetic delimitation of M. fornasinii, unequivocally distinguishing it from M. hardwickii. The combined analysis of the mitochondrial markers revealed a highly supported monophyletic cluster that includes sequences from both the specimens analyzed and those available in public databases (GenBank and BOLD) (Fig. 2).

Integrating morphological and molecular approaches substantially improved the accuracy of species identification, effectively resolving previous taxonomic ambiguities, which is further supported by the 100% genetic similarity between specimens collected in Mozambique and a lineage from northern Australia (Supplementary material Table S1). The observed intraspecific divergence values were consistently below 1%, a level lower than the threshold commonly employed to delineate lineages or species based on DNA barcoding (e.g. Raupach *et al.*, 2015; Landschoff & Gouws, 2018).

The family Menippidae was not listed among the brachyuran families previously recorded from Mozambique, where the known crab fauna comprises approximately 200 species across 25 families (Muñoz *et al.*, 2021). The absence of subsequent taxonomic revisions highlights the significant gap in current biodiversity assessments for the region, underscoring the need for integrative taxonomic efforts combining morphological and molecular approaches to reassess species inventories and inform conservation strategies to preserve the region's potentially underestimated crab diversity.

The original description of *M. fornasinii* warrants a re-evaluation. Bianconi (1851) did not conclusively identify the specimens he examined as Galene fornasinii, instead deferring this determination to future studies. The original record was based on limited and poorly documented observations, which complicated efforts to confirm the actual presence and distribution of the species in Mozambique.

The new Mozambique record suggests that *M. fornasinii* may possess a much wider and possibly continuous distribution along the western Indian Ocean and East Africa than previously recognized. Between northern Australia and Mozambique lies a coastline spanning over 20,000 km with no published records of the species. Comprehensive distributional records are warranted to accurately delineate the species distribution, assess potential cryptic diversity, and better understand its ecological roles.

Despite this apparent distributional gap, coastal regions have been the subject of ongoing surveys, which have frequently resulted in records, as in the case of recent new records of shallow- and deep-water marine species from the Indian Ocean (e.g. Padate *et al.*, 2018, 2021; Akash *et al.*, 2020; Trivedi *et al.*, 2021).

Understanding the geographic distribution of *M. fornasinii* is a key factor for any effective conservation strategies, particularly given the current scarcity of data on its status. The evidence of a broad distribution, potentially including connected populations between Mozambique and northern Australia, suggests that the species' range may be considerably more extensive than previously documented, as in the case of M. hardwickii, the sister species of *M. fornasinii* (Nurqadri *et al.*, 2023).

It remains plausible that the occurrence of *M. fornasinii* in Mozambique is the result of anthropogenic introduction, whether through deliberate or inadvertent translocation. The pronounced genetic congruence with Australian populations and the absence of documented intermediate occurrences strongly suggest that maritime transport is a potential vector. Ships facilitate transport to new environments (Hewitt *et al.*, 2009; review by McLay, 2015). Ballast water and hull fouling alone are considered responsible for 60–90% of marine bioinvasions (Costa-Areglado *et al.*, 2025). Should the population of *M. fornasinii* in Mozambique be confirmed as non-native, its apparent establishment in local mangrove ecosystems needs a rigorous evaluation as a potential invasive species. This exemplifies the complexity of marine biogeographic patterns, such

as in the red lionfish (*Pterois volitans* Linnaeus, 1758), a well-documented invasive species in Mozambique that has spread through ocean transport mechanisms (Rojas-Sandoval, 2024). The dynamics of the Indian Ocean current connect Australia and East Africa via the South Equatorial Current, which supplies waters to the Mozambique Channel (Schott *et al.*, 2009). The new record from the Bons Sinais River estuary underscores the ecological importance of this habitat as a reservoir of previously undocumented biodiversity, with species occurrences not previously anticipated for this region (Muhala *et al.*, 2023, 2024).

Our findings highlight the estuarine system's considerable potential to support non-endemic or range-expanding taxa, reinforcing the urgent need for continued and systematic biodiversity assessments to refine species inventories and guide effective conservation and management strategies in the region.

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SUPPLEMENTARY MATERIAL

Supplementary material is available at *Journal of Crustacean Biology* online.

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