

DNA BARCODING OF *MERETRIX CASTA* (GMELIN, 1791) COLLECTED FROM COAST OF PAKISTAN

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ABSTRACT

DNA barcode is authentic technique for the identification of organisms as compare to morphological characterization. The present study re-confirmed the bivalve species *Meretrix casta* (Bivalve) using a fragment of mitochondrial cytochrome C oxidase subunit I (COI) gene. The sequences were submitted to GenBank under accession numbers, MZ675502, and ON679527 respectively. The Neighbor-Joining tree showed three major clades of *M. casta* the Pakistani individuals clustered with individuals of Iran. The *M. casta* samples from Pakistan revealed lower genetic distance with individual of Iran.

Key-words: Bivalve, Veneridae, Bar-coding, COX1, Pakistan

INTRODUCTION

The systematic investigation of bivalve species was first initiated by Melvill (1893) in this region and until now it has been reported that 1100 species of Mollusca are found in Pakistan, among them 30 species are commercially important. Besides all Molluscan species only 68 species of family veneridae have been recorded in Pakistani territorial marine waters, two of them are commercially important (*Marcia marmorata* and *Meretrix casta* (Ranjha, 1960; Moazzam and Ahmed, 1994). The *M. casta* can be harvested from sandy, muddy bottoms, intertidal and shallow subtidal waters up to the depth of around 5 meters.

Moazzam and Ahmed (1994) were the first to report *M. casta* from the waters of Pakistan, basing their identification solely on morphological characters. However earlier, Hornel (1917) documented as variant of *M. casta*. Their work served as an initial reference point, although it lacked molecular validation. Years later, Jahangir *et al.* (2012) re-examined this classification and raised the possibility that the specimens found locally could be a variant of *M. casta*, hinting at either intraspecific diversity or a possible misidentification.

The morphological identifications are complex for certain groups like molluscan species, therefore, the well-known, easy and reliable method of molecular identification is being used in taxonomy of modern era.

The popular molecular marker like Cytochrome Oxidase sub-unit I is good to be used for identification of morphologically confused species. Therefore, several researchers of modern era have chosen molecular technique for identification of organism (Adamkewicz, 1997; Boore *et al.*, 1995; Sulaman *et al.*, 2022; Qurat-ul-Ain Ijaz *et al.*, 2023). Likewise the species of Veneridae have also been investigated based on the molecular techniques (Cheng *et al.*, 2006; Taylor *et al.*, 2007).

Meager information on molecular based identification for bivalve species are reported from Pakistan, therefore, present study has been designed to re-confirm the identification of *M. casta* using mitochondrial cytochrome Oxidase sub-unit I (COX1) marker. The outcomes of present efforts would be contribution of existing data for the biodiversity of this area.

MATERIALS AND METHODS

Sample collection:

The samples were collected from Miani Hor (Sonmianit Bay) during extreme low tide, samples were randomly handpicking and brought to the laboratory in 95% ethanol. Shells were opened and soft tissues were kept at -20C for further analysis. .

DNA isolation:

Genomic DNA (gDNA) was extracted from muscle tissue using the phenol-chloroform method, following the protocol described by Sambrook *et al.* (1989).

PCR Amplification and Gel electrophoresis:

The Cytochrome Oxidase Subunit 1 (COX1) gene was amplified through polymerase chain reaction (PCR) using universal primers LCO1490 5'-GGTCAACAATCATAAAGATATTGG-3' and HCOR2. 5'-TAAACTTCAGGGTGACCAAAAAATCA-3' (Folmer *et al.*, 1994). The PCR was carried out using 100mg DNA template, 2.5 µL dNTP (2.5mM each), 2.5 µL 10 X buffer, 2 µL MgCl₂ (20mM), 1 µM primers (10 µM each) and 0.25 µL of Taq polymerase (5 µU MI*1). PCR conditions were as: denaturation at 94 °C for 5 min; 35 cycles of 94 °C for 30 s, annealing at 50°C for 30 s, and a final extension for 7 min at 72 °C. The PCR product was confirmed using Gel electrophoresis (1.2 % agarose gel with ethidium bromide (stain)).

Sequencing and construction of phylogenetic tree:

PCR products were sequenced using the Sanger sequencing method. Sequence editing, including necessary insertions and deletions, was performed using Molecular Evolutionary Genetic Analysis (MEGA) and BioEdit (Tamura *et al.*, 2013; Hall, 1999). The neighbor joining tree was constructed using constructed on Kimura 2-parameter model (Kimura, 1980) using MEGA 6 (Tamura *et al.*, 2013). The obtained sequences were submitted to National Center for Biotechnology Information (NCBI) under accession number MZ675502 and ON679527.

RESULTS***M. casta* Morphological characteristics:**

Shell ovate to oblong with or without two obscure and often imperfect radial bands. Hinge elongate and strong. It has various colors, outside of shell variable under the light to dark brown or dirty grey. It is feeding mainly dependent on phytoplankton and detritus. They are widely distributed such as Pakistan, India, Bangladesh, Philippines, Thailand, Australia, Malaysia, Indonesia, Japan, Singapore, and Madagascar (Liang *et al.*, 2004; Silva *et al.*, 2006; Alfonso *et al.*, 2008) (Fig.1 A & B)



Fig. 1. Showing the morphology of *Meretrix casta*. A. Dorsal B. ventral view, respectively.

Molecular Identification:

In present investigation the cytochrome oxidase sub unit 1 (CO1) a mitochondrial gene was utilized to identify *Meretrix casta*, a bivalve species earlier reported based on morphological features. The amplified sequences were submitted to GenBank under accession numbers MZ675503 and ON 679527. The obtained sequences were aligned with existing CO1 sequences of *M. casta*, confirming the species identification. The phylogenetic relationship was analyzed using Neighbor-joining method (Fig. 2). The tree denoted three distinct clades within *M. casta*. Pakistani specimens clustered closely with individuals from Iran, showing a low genetic distance between these populations. Some of the Indian specimen also showed a moderate genetic distance (Table 1). Present results validate the initial morphological identification of *M. casta*.

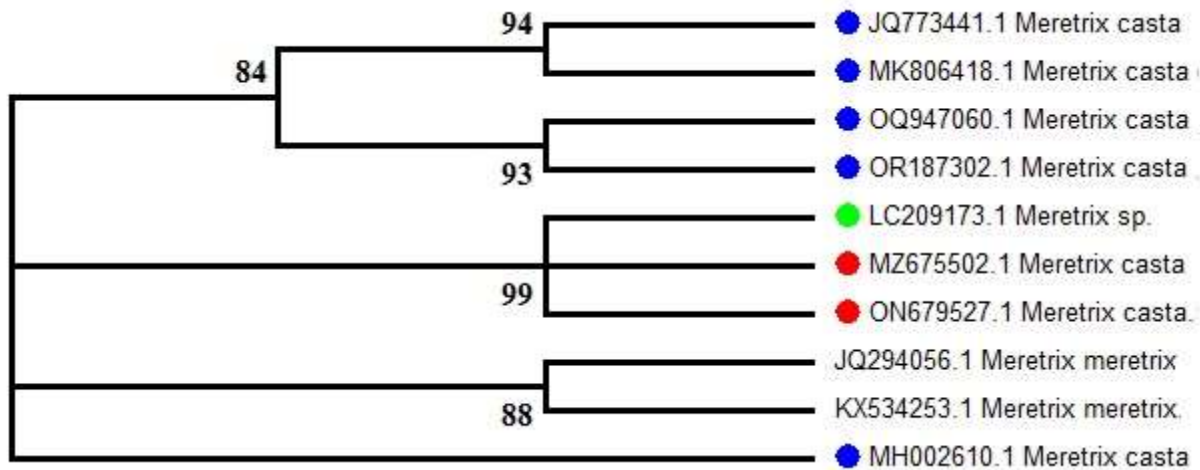


Fig. 2. Showing the neighbor joining phylogenetic tree. Individuals with red, blue and green circles are denoting the samples of Pakistan, India and Iran, respectively.

Table 1. Pairwise genetic distance between the individuals of different countries.

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|-------------|-------------|------|------|------|------|-------------|------|------|----|
| JQ294056 <i>Meretrix meretrix</i> | - | | | | | | | | | |
| LC209173 <i>Meretrix sp.</i> (Iran) | 0.26 | - | | | | | | | | |
| MH002610 <i>Meretrix casta</i> (India) | 1.33 | 1.15 | - | | | | | | | |
| KX534253 <i>Meretrix meretrix</i> | 0.00 | 0.25 | 1.34 | - | | | | | | |
| JQ773441 <i>Meretrix casta</i> (India). | 0.16 | 0.25 | 1.38 | 0.16 | - | | | | | |
| MK806418 <i>Meretrix casta</i> (India). | 0.16 | 0.25 | 1.39 | 0.16 | 0.01 | - | | | | |
| MZ675502 <i>Meretrix casta</i> (Pak) | 0.26 | 0.01 | 1.47 | 0.25 | 0.25 | 0.25 | - | | | |
| ON679527 <i>Meretrix casta</i> (Pak) | 0.26 | 0.01 | 1.47 | 0.25 | 0.25 | 0.25 | 0.00 | - | | |
| OQ947060 <i>Meretrix casta</i> (India) | 0.16 | 0.25 | 1.36 | 0.16 | 0.05 | 0.04 | 0.25 | 0.25 | - | |
| OR187302 <i>Meretrix casta</i> (India) | 0.15 | 0.24 | 1.38 | 0.16 | 0.05 | 0.04 | 0.24 | 0.24 | 0.01 | - |

DISCUSSION

The accurate identification of marine invertebrates remains a cornerstone in understanding marine biodiversity and ecosystem health. Our study focused on the molecular identification of *Meterix casta* from the coastal waters of Pakistan, an area that, while biologically diverse, remains relatively under-explored in terms of molecular taxonomy. *M. casta* was first reported from Pakistani waters by Moazzam and Ahmed (1994), based on morphological characteristics. Their identification set a foundational reference, without molecular support. Later, Jahangir *et al.* (2012) revisited this classification and remarked that the local specimens might represent a variety of *M. casta*, suggesting potential intraspecific variation or even misidentification.

Adding to this taxonomic complexity, Yoosukh and Matsukuma (2001) identified similar specimens as *Meterix ovum* from other regional waters, indicating the potential for morphological convergence among closely related

species or cryptic speciation. This highlights the limitations of morphology-based identification, especially in taxa where phenotypic plasticity or developmental variation can obscure species boundaries.

In the absence of historical molecular data or preserved reference samples from earlier studies, our approach combined detailed morphological analysis with DNA barcoding, specifically using the cytochrome c oxidase subunit I (COI) gene, which has proven effective in resolving taxonomic ambiguities in marine invertebrates (Hebert *et al.*, 2003). The COI sequences obtained from our specimens showed strong similarity with published sequences of *M. casta*, supporting the identification despite the noted morphological overlap with *M. ovum*.

Our findings not only affirm the presence of *Meterix casta* in Pakistani marine waters but also underscore the value of integrating molecular techniques in regional taxonomic studies. This is especially critical in regions like the northern Arabian Sea, where faunal inventories remain incomplete and are often based on outdated or morphologically ambiguous identifications.

Conclusion

To improve our understanding of biodiversity and ensure the accuracy of taxonomic identifications, it is imperative to develop a comprehensive and well-curated reference collection of marine invertebrate specimens from Pakistani waters. Coupling morphological data with molecular barcoding will allow for more precise species identification and will be instrumental in future biodiversity assessments, conservation planning, and ecological research.

Declaration

We declare no conflict of interest.

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