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Molecular Insights into *Meretrix* Species Diversity from Tarakan Island, North Kalimantan, Indonesia: An Analysis of COI Sequences for Enhanced Marine Bivalve Identification

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ABSTRACT

Meretrix, a popular marine bivalve, features several species with significant ecological and commercial value, distributed in various marine habitats worldwide. Despite their importance, accurate global population estimates of these species are hindered by inadequate classification frameworks that often rely on external shell characteristics, which are susceptible to environmental influences. This limitation underscores the necessity for comprehensive research into the physiological and molecular attributes of these species for accurate identification. This study focused on identifying the local *Meretrix* species from Tarakan Island by analyzing their COI nucleotide sequences and comparing them with reference sequences in the BOLD and GenBank databases. The results demonstrate that the 14 specimens obtained with diverse morphological variations were found only within two major groups: *Meretrix meretrix* and *Meretrix lusoria*. These findings validate the efficacy of DNA barcoding as a tool for classifying marine bivalves, offering a robust framework for identifying unknown species, particularly in the Asian marine ecosystems. The study's approach and findings have broader implications for enhancing global marine biodiversity research and conservation strategies.

INTRODUCTION

Globally, the marine bivalve is rich and diverse, encompassing over 1000 species distributed across 82 families, among which the *Meretrix* spp. is notably prevalent in the West Pacific and various Asian regions, including species such as *Meretrix lyrata*, *Meretrix lusoria*, and *Meretrix petechialis* (**Torii *et al*., 2010**). Traditional classification of the *Meretrix* species has predominantly relied on morphological characteristics such as shell shape, size, and color, a method primarily documented in countries like Korea,

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Japan, Indonesia, and Malaysia (**Yamakawa & Imai, 2012**). However, this morphological approach has shown limitations in offering the precision required for accurate species identification and classification, highlighting a significant challenge within marine biodiversity research (**Hsiao & Chuang, 2023**).

In response to these limitations, DNA barcoding has been advanced as a robust and reliable methodology for the identification and discrimination of marine bivalves including *Meretrix* spp. This technique, which focuses on a 627bp segment of the conserved cytochrome oxidase subunit I (COI) gene, has proven effective for accurate species identification across a wide range of taxa, enhancing the resolution beyond that of traditional morphological analyses (**Lakra *et al*., 2011; Geller *et al*., 2013; Reunov *et al*., 2021**). The utility of DNA barcoding underscores a methodological shift toward genetic tools in marine taxonomy, offering a pathway to overcome the constraints of morphological identification (**Sarhan *et al*., 2021**).

This study sought to bridge the existing knowledge gap concerning the morphological and genetic diversity of marine bivalve species in the Celebes Sea, with a particular focus on the *Meretrix* spp. collected from Amal beach in Tarakan Island, Indonesia. By employing the COI DNA barcoding technique, the research aimed not only to document the morphological and genetic variations within these specimens but also to contribute to a more nuanced understanding of their classification and diversity. This endeavor is poised to enrich the global database of marine bivalves, facilitating more informed conservation strategies and offering insights into the ecological dynamics of the Celebes Sea. Through this investigation, we anticipate shedding light on the complex biodiversity of this region, underlining the critical role of genetic methodologies in modern marine biology.

MATERIALS AND METHODS

Sampling site

Specimens of *Meretrix*, a bivalve species colloquially known as "*kerang kapah*", were carefully collected from Amal Beach on Tarakan Island, situated between coordinates 3°17'9" N, 117°39'18" E and 3°20'52.89" N, 117°39'40.16" E, in North Kalimantan, Indonesia. These specimens were gathered using a hands-on, manual sampling technique, with the assistance of local fishers, resulting in over 100 clams being collected. Each collected *Meretrix* spp. specimen, noted for its unique morphological characteristics, was delicately placed in a designated sampling basket, displaying predominantly varied shell coloration. Every specimen was meticulously documented (Fig. 1). A portion of muscle tissue was excised from these specimens and placed in a sterile 1.5ml tube, filled with 96% ethanol to ensure preservation over an extended period (**Gaffar *et al*., 2021**). The preserved muscle samples were then transported to the Center for Life Science Laboratory at Universitas Borneo Tarakan in North Kalimantan,

Indonesia. For further study, the muscle tissue samples preserved in ethanol were stored at a temperature of -20°C.

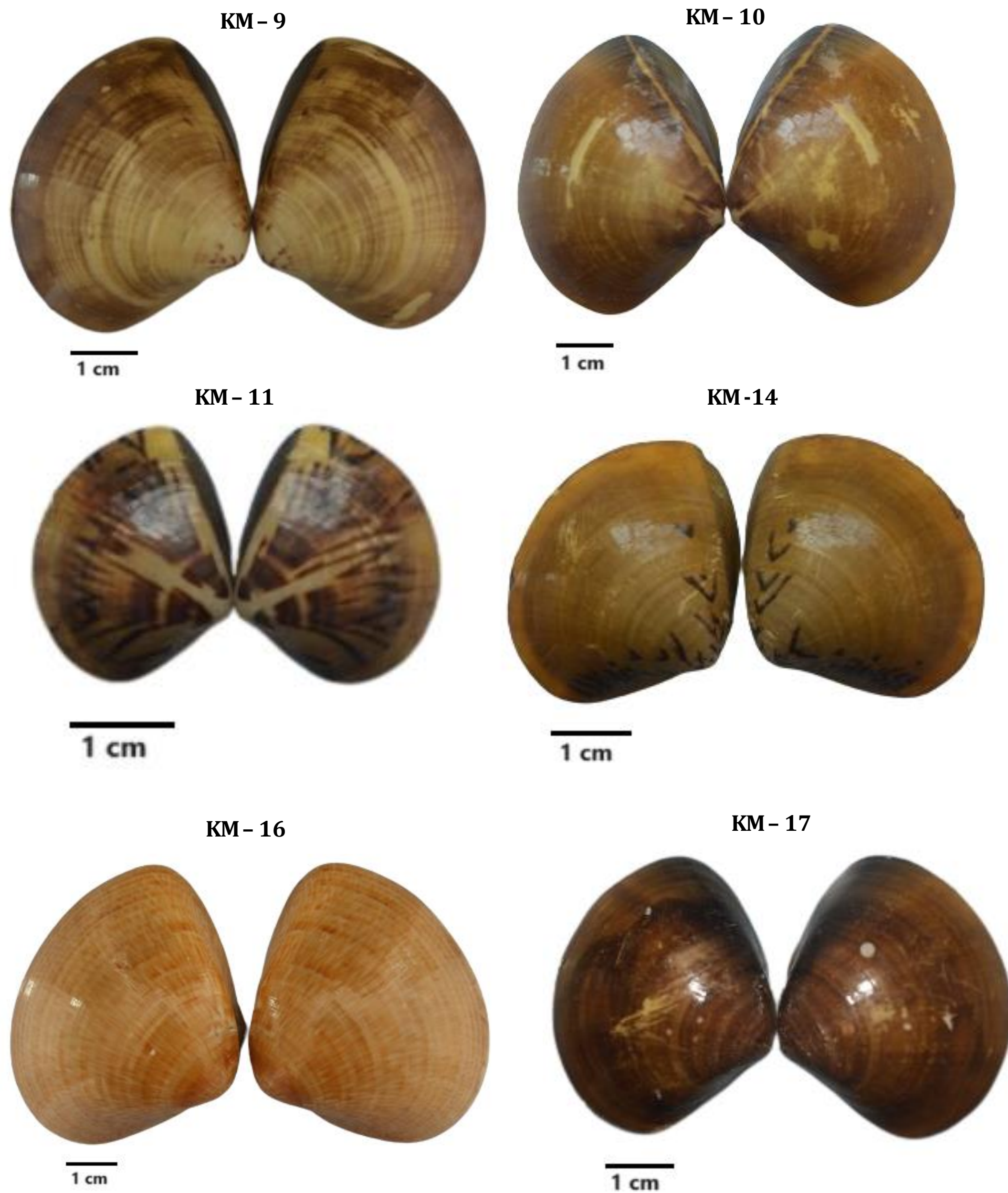

Fig. 1. *Meretrix* spp. specimens in this study

Extraction, amplification, and sequencing of DNA

Genomic DNA kit was used to extract genetic material including the mitochondrial COI from the specimen. The following invertebrate universal primers were obtained according to the model developed by **Folmer *et al.* (1994)**:

HCO2198 (R): 5'- TAAACTTCAGGGTGACCAAAAAATCA-3',
LCO1490 (F): 5'-GGTCAACAAATCATAAAGATATTGG-3'

These primers were utilized in the process of gene amplification. Executing the polymerase chain reaction (PCR) involved composing a reaction mixture with a total volume of 25µL. This mixture was constituted of 12.5µL from the My Taq® HS Red Mix PCR Kit, provided by Bioline, alongside 2µL of genomic DNA previously extracted. Additionally, each primer was present at a final concentration of 0.6µM (0.75µL; 20µM). To complete the mixture, 9µL of nuclease-free water was added, ensuring the total volume reached 25µL. The PCR reactions were conducted in an Eppendorf X50s Thermal Cycler. The PCR protocol involved an initial denaturation phase at 95°C for 4 minutes, followed by a series of 30 cycles. Each cycle involved denaturation at 95°C for 15 seconds, annealing at 45°C for 15 seconds, and elongation at 72°C for 10 seconds, resulting in a final extension at 72°C for two minutes. Post-amplification, the PCR products derived from the samples were subjected to electrophoresis on 1% agarose gels and visualized with FloroSafe DNA Stain supplied by 1st BASE. The COI sequencing reactions were subsequently conducted in both forward and reverse directions, in accordance with the established protocols utilizing the ABI Big Dye Terminator v3.1 cycle sequencing kit from Applied Biosystems. This process entailed the addition of 5 to 7µL of the purified PCR product and 0.8µL of either primer to each reaction. Finally, the prepared sequence-reaction products were analyzed using an ABI 3500 Genetic Analyzer, also from Applied Biosystems, allowing for the sequencing of amplicons in both the forward and reverse directions **(Gaffar *et al*., 2023)**.

Molecular identification and phylogenetic analysis

The validation of data processed by the sequencing analysis was conducted using the sequence scanner software, compatible with applied biosystems genetic analyzer instruments. For the examination of DNA segments within the COI region, the DNA baser was employed, a tool essential for generating consensus fragments as outlined in DNA Sequence Assembler v4 **(BioSoft, 2013) (Gaffar *et al*., 2021; Gaffar & Sumarlin, 2021)**. Subsequent to this phase, the consensus sequences derived from each *Meretrix* were translated to detect the presence of stop codons, employing the invertebrate mitochondrial code as a reference (available at: Expasy - Translate tool). These COI sequences were subsequently formatted into fasta files, a crucial step in preparation for species identification. To ascertain sequence similarity, we conducted searches on two renowned public databases: BLASTn (accessible via https://blast.ncbi.nlm.nih.gov/Blast.cgi) and BOLDsystems (details at https://www.boldsystems.org/index.php/IDS_OpenIdEngine). The use of both databases allowed for cross-verification of the results, enhancing the reliability of species identification. Genetic distances among sequences were determined using MEGA 11 software **(Tamura *et al*., 2021)**, aiding in the reconstruction of the phylogenetic tree based on the method of **Kimura (1980)**. The TN93+G model was selected as the most suitable nucleotide substitution model using the Maximum Likelihood (ML) method,

incorporating Akaike and Bayesian information criterion scores for assessment, with 1000 bootstrap replications for robustness **(Gaffar *et al*., 2023)**.

RESULTS AND DISCUSSION

Genetic analysis and species identification

In this study, we successfully sequenced 14 hard clam specimens collected from the east coast of Tarakan Island. Following validation and translation to identify stop codons, we observed that the base lengths of these sequences varied between 630 and 663 base pairs. The nucleotide sequences from the sampled *Meretrix* species yielded intriguing results. Utilizing the Expasy online tool, we translated the nucleotide bases from all 14 sequenced samples into proteins, confirming the absence of stop codons within these sequences **(Duvaud *et al*., 2021)**. The resulting proteins consisted of 210 to 221 amino acids each, with no insertions or deletions observed, indicative of a high degree of genetic stability.

For species identification, we employed two public databases, BLASTn and BOLD. The sequence analysis revealed that the specimens could be categorized into two distinct species groups: *Meretrix meretrix* and *Meretrix lusoria*, as detailed in Table (1). This table presents the results of species identification based on consensus barcoded sequences analyzed via BLASTN searches from the GenBank database and the BOLD Identification System (BOLD-IDs). We adopted similarity ratings for this study as follows: 97– 100% to indicate significant similarity and 92– 96% for moderate similarity **(Sarhan *et al*., 2021)**.

The application of DNA sequence analysis techniques to two *Meretrix* species yielded pivotal insights into their genetic variations. Seven specimens identified as *Meretrix lusoria* exhibited a remarkable genetic homogeneity, with similarity levels exceeding 98%. In contrast, the eight *Meretrix meretrix* specimens showed a more genetic diversity, with similarity percentages ranging from 96.17 to 96.72%. The critical question arising from these findings pertains to the species delimitation threshold, particularly in relation to the cytochrome c oxidase subunit I (COI) gene. The established threshold for genetic distance in COI gene sequences, widely accepted in molecular taxonomy, is 2%. Organisms with a genetic distance less than this threshold are typically considered the same species **(Yang *et al*., 2014; Ge *et al*., 2021; Gaffar *et al*., 2023)**. Given the observed genetic similarities within the *Meretrix lusoria* group, these specimens clearly fall within the same species under the 2% threshold. However, the variation observed in the *Meretrix meretrix* group, although below the interspecies threshold, suggests a potential substructuring within the species. This raises the possibility that what is currently classified as *Meretrix meretrix* could encompass more than one genetically distinct group **(Chen *et al*., 2009)**.

Table 1. Summary of species identification based on consensus barcoded sequences

No.	Sample ID	Species' match by name		Similarity (%)		Query cover (%)	Acc. Number	DNA length (bp)
		BLASTN	BOLD-IDS	BLASTN	BOLD-IDS			
1	KM-1	*M. lusoria*	*M. lusoria*	98.25	98.39	100	JN898935	686
2	KM-2	*M. meretrix*	*M. meretrix*	96.52	96.62	100	JN043623	633
3	KM-3	*M. meretrix*	*M. meretrix*	96.72	96.71	99	JN043623	646
4	KM-4	*M. meretrix*	*M. meretrix*	96.58	96.56	98	JN043623	652
5	KM-5	*M. meretrix*	*M. meretrix*	96.36	96.52	100	JN043623	687
6	KM-6	*M. meretrix*	*M. meretrix*	96.50	96.49	100	JN043623	627
7	KM-7	*M. lusoria*	*M. lusoria*	98.25	98.39	100	JN898935	685
8	KM-8	*M. meretrix*	*M. meretrix*	96.33	96.33	100	JN043623	682
9	KM-9	*M. lusoria*	*M. lusoria*	98.24	98.24	100	JN898935	682
10	KM-10	*M. lusoria*	*M. lusoria*	98.08	98.23	100	JN898935	676
11	KM-11	*M. lusoria*	*M. lusoria*	98.14	98.41	100	JN898935	698
12	KM-14	*M. lusoria*	*M. lusoria*	98.21	98.39	100	JN898935	669
13	KM-16	*M. meretrix*	*M. meretrix*	96.39	96.38	100	N043623	693
14	KM-17	*M. lusoria*	*M. lusoria*	98.12	98.39	100	JN898935	691

Haplotype diversity and mutational insights

In our detailed genetic analysis of the *Meretrix lusoria* group, 14 distinct haplotypes were identified, reflecting a substantial genetic diversity within this species. Notably, a key genetic feature in this group was a transversion mutation at the ninth base position, accompanied by a predominance of transitional substitutions, as delineated in Table (2). This combination of transversion and transition mutations contributes to our understanding of the genetic variation mechanisms in *Meretrix lusoria* and could be indicative of specific evolutionary pressures or historical adaptation processes. In contrast, our examination of *Meretrix meretrix* revealed a different genetic landscape, with the identification of 21 haplotypes. These haplotypes were primarily characterized by two distinct types of transversion substitutions at base positions of 132 and 288, as shown in Table (3). The presence of these specific transversion mutations suggests potential hotspots for genetic variation that may be critical in understanding the species' evolutionary biology and could have implications for its ecological adaptability.

In the context of DNA barcoding, the occurrence of transition or transversion mutations among species can affect the interpretation of genetic distance and species identification. A study found that transversions have a greater impact on the regulatory element activity than transitions, suggesting that transversions may have larger regulatory effects than transitions **(Guo *et al.*, 2017)**. However, another study found that the conservativeness of transitions is a rather weak effect, and the chance of a transition mutation being more fit than a transversion is not large enough to explain the several-fold bias toward transition replacements observed in evolution **(Stoltzfus & Norris, 2016)**.

Table 2. Polymorphic sites in the partial mtDNA nucleotide sequence encoding COI of *Meretrix lusoria* group

Sampel \ Position	9	45	90	180	204	271	277	357	375	450	504	510	534	660
KM-1	A	G	A	G	A	T	A	A	C	C	G	A	T	C
KM-7	●	●	●	●	●	●	●	●	●	●	●	●	●	●
KM-9	●	●	●	●	●	●	●	●	●	●	●	●	●	●
KM-10	●	●	●	●	●	●	G	●	●	●	●	●	●	●
KM-11	●	●	●	●	●	●	●	●	●	●	●	●	●	●
KM-14	●	●	●	●	●	●	●	●	●	●	●	●	●	●
KM-17	●	●	G	●	●	●	●	●	●	●	●	G	●	●
JN898935.1_*M. lusoria*	T	A	●	A	G	C	●	G	T	T	A	G	C	T
Substitution type	Tv	Ts	Ts	Ts	Ts	Ts	Ts	Ts	Ts	Ts	Ts	Ts	Ts	Ts

Note: (●) is a conserved nucleotide base; Tv is Transversion; and Ts is Transition.

Table 3. Polymorphic sites in the partial mtDNA nucleotide sequence encoding COI of *Meretrix meretrix* group

Sampel \ Position	21	39	120	132	165	171	205	231	258	273	288	294	315	456	474	480	525	528	540	570	613
KM-2	A	G	T	G	A	A	T	T	G	A	T	C	G	T	A	A	T	G	T	A	A
KM-3	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
KM-4	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
KM-5	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
KM-6	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
KM-8	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
KM-16	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
JN043623.1_*M. meretrix*	G	A	C	T	G	G	C	C	A	G	G	T	T	C	G	G	C	A	C	G	G
Substitution type	Ts	Ts	Ts	Tv	Ts	Ts	Ts	Ts	Ts	Ts	Tv	Ts	Tv	Ts	Ts	Ts	Ts	Ts	Ts	Ts	Ts

Note: (●) is a conserved nucleotide base; Tv is Transversion, and Ts is Transition.

Table 4. Nucleotide composition and sequence lengths of *Meretrix* species specimens

Specimen	Nucleotide composition (%)						Length (bp)
	T(U)	C	A	G	A+T	G+C	
KM-1	44.01	14.33	21.08	20.57	65.09	34.9	593
KM-2	43.68	14.50	20.40	21.42	64.08	35.92	593
KM-3	43.68	14.50	20.40	21.42	64.08	35.92	593
KM-4	43.68	14.50	20.40	21.42	64.08	35.92	593
KM-5	43.68	14.50	20.40	21.42	64.08	35.92	593
KM-6	43.68	14.50	20.40	21.42	64.08	35.92	593
KM-7	44.01	14.33	21.08	20.57	65.09	34.9	593
KM-8	43.68	14.50	20.40	21.42	64.08	35.92	593
KM-9	44.01	14.33	21.08	20.57	65.09	34.9	593
KM-10	44.01	14.33	20.91	20.74	64.92	35.07	593
KM-11	44.01	14.33	21.08	20.57	65.09	34.9	593
KM-14	44.01	14.33	21.08	20.57	65.09	34.9	593
KM-16	43.68	14.50	20.40	21.42	64.08	35.92	593
KM-17	44.01	14.33	20.74	20.91	64.75	35.24	593
JN898935.1 *M. lusoria*	44.01	14.33	20.91	20.74	64.92	35.07	593
JN043623.1 *M. meretrix*	43.00	15.35	19.73	21.92	62.73	37.27	593
OM292826.1 *M. planisulcata*	44.18	15.35	19.90	20.57	64.08	35.92	593
OQ567566.1 *M. lyrata*	43.68	14.33	20.91	21.08	64.59	35.41	593

Nucleotide composition and ecological implications

The comparative genetic analysis of the *Meretrix* clams—*Meretrix lusoria* and *Meretrix meretrix*—has yielded insights that deepen our understanding of these species' evolutionary trajectories and environmental adaptabilities. The nucleotide composition, particularly the purine-pyrimidine ratio and the %GC content, underscores distinct genetic divergences between the two. *Meretrix lusoria* was found to have an average %GC content of 34.9 and an %AT of 65.09, while *Meretrix meretrix* demonstrated a slightly higher %GC content of 35.92 and an %AT of 64.08. Such disparities, notably in the GC content, suggest variations in environmental adaptations and evolutionary histories of these groups **(Wang *et al*., 2017)**.

High GC content, which is typically correlated with increased DNA stability and gene richness, posits that *Meretrix meretrix* may be genetically primed for survival in more variable or extreme environmental conditions. This adaptability is corroborated by the resilience of *M. meretrix* in the face of heavy metal pollution, with tissue concentrations of heavy metals observed to be within acceptable standards, indicating a potential robustness against such contaminants **(Alyahya *et al*., 2011)**. The species' presence also appears to influence erosion and accretion processes in intertidal flats, highlighting its ecological importance **(Shi *et al*., 2020)**.

These findings not only offer a window into the adaptive mechanisms and resilience of these species within their ecological niches but also inform conservation and

management strategies, especially pertinent in an era of changing environmental conditions and anthropogenic impacts. The genetic nuances discerned through this analysis reflect the intricate dance of life these species navigate, shaped by the forces of nature and evolution, and underscore the importance of understanding these dynamics for their preservation and sustainable utilization.

Genetic distance and species delimitation

Table (5) offers a genetic distance within the *Meretrix* genus, employing mitochondrial cytochrome c oxidase I (COI) gene sequences to reveal subtle differences and suggest potential speciation. This research, incorporating 19 samples from Tarakan Island alongside data from the GeneBank. The findings demonstrate a significant genetic consistency within certain groups, with distances ranging from 0.00 to 0.02 among the selected *M. lusoria* and *M. meretrix* samples, affirming their categorization as a single species. In contrast, genetic distances between 0.13 to 0.17 in other samples, including the novel *Meretrix* sp. n. STH-2020 (*M. taiwanica*), indicate considerable genetic diversity within the genus, pointing to a potential speciation. These observations are instrumental in refining taxonomic classifications and illustrate the complex genetic fabric of the *Meretrix* genus. Moreover, this analysis paves the way for further research into the evolutionary paths and adaptive mechanisms of *Meretrix* species, thereby deepening our understanding of their ecological roles, conservation requirements, and management needs. This study significantly advances our knowledge of marine biodiversity, with a particular focus on Southeast Asian marine ecosystems, and underscores the essential role of molecular techniques in biodiversity research for a thorough exploration of genetic diversity and the formulation of effective conservation strategies.

The phylogenetic tree presented in Fig. (2) illustrates the genetic relationships among various *Meretrix* spp. samples from Tarakan Island, alongside reference sequences from the GenBank. The tree, constructed based on cytochrome c oxidase I (COI) gene sequences, employed the bootstrap method with 1000 replicates to ensure accuracy. Genetic distance analysis substantiated the formation of two distinct clades within the phylogenetic tree. The *M. meretrix* group, encompassing samples KM-2, KM-3, KM-4, KM-5, KM-6, KM-8, and KM-16, formed a coherent clade with a strong bootstrap support of 99%. This indicates a close genetic relationship among these samples. Another clade, comprising samples KM-1, KM-7, KM-9, KM-10, KM-11, KM-14, and KM-17, displayed varied bootstrap values (100, 96, 85), suggesting a wider genetic variance within this subset of the *Meretrix* population. Specifically, the positioning of KM-10 and KM-14, with a lower bootstrap value of 45, signifies further genetic differentiation. Notably, the samples labeled as *Meretrix* sp. n. S.TH-2020 (GenBank accessions MZ453103.1 and MN275934.1) exhibited a closer genetic resemblance to *Meretrix lusoria* (JN989935.1), suggesting a more recent common ancestor with this species. In contrast, other *Meretrix* species such as *M. meretrix* (JN043623.1), *M. lyrata* (OQ567566.1), and *M. planisulcata* (OM292826.1) were positioned on separate branches of the tree, highlighting their distinct genetic identities.

Table 5. Genetic distances among *Meretrix* spp. in the study

Code	1	2	3	4	5	6	7	8	9	10	11	14	16	17	18	19	20	21	22
1		0.02	0.02	0.02	0.02	0.02	0.02	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.01	0.02	0.02	0.02	0.02
2	0.17		0.00	0.00	0.00	0.00	0.00	0.02	0.02	0.02	0.02	0.02	0.00	0.02	0.02	0.01	0.02	0.02	0.02
3	0.17	0.00		0.00	0.00	0.00	0.00	0.02	0.02	0.02	0.02	0.02	0.00	0.02	0.02	0.01	0.02	0.02	0.02
4	0.17	0.00	0.00		0.00	0.00	0.00	0.02	0.02	0.02	0.02	0.02	0.00	0.02	0.02	0.01	0.02	0.02	0.02
5	0.17	0.00	0.00	0.00		0.00	0.00	0.02	0.02	0.02	0.02	0.02	0.00	0.02	0.02	0.01	0.02	0.02	0.02
6	0.17	0.00	0.00	0.00	0.00		0.00	0.02	0.02	0.02	0.02	0.02	0.00	0.02	0.02	0.01	0.02	0.02	0.02
7	0.17	0.00	0.00	0.00	0.00	0.00		0.02	0.02	0.02	0.02	0.02	0.00	0.02	0.02	0.01	0.02	0.02	0.02
8	0.00	0.17	0.17	0.17	0.17	0.17	0.17		0.00	0.00	0.00	0.00	0.02	0.00	0.01	0.02	0.02	0.02	0.02
9	0.00	0.17	0.17	0.17	0.17	0.17	0.17	0.00		0.00	0.00	0.00	0.02	0.00	0.01	0.02	0.02	0.02	0.02
10	0.00	0.18	0.18	0.18	0.18	0.18	0.18	0.00	0.00		0.00	0.00	0.02	0.00	0.01	0.02	0.02	0.02	0.02
11	0.00	0.17	0.17	0.17	0.17	0.17	0.17	0.00	0.00	0.00		0.00	0.02	0.00	0.01	0.02	0.02	0.02	0.02
14	0.00	0.17	0.17	0.17	0.17	0.17	0.17	0.00	0.00	0.00	0.00		0.02	0.00	0.01	0.02	0.02	0.02	0.02
16	0.17	0.00	0.00	0.00	0.00	0.00	0.00	0.17	0.17	0.18	0.17	0.17		0.02	0.02	0.01	0.02	0.02	0.02
17	0.00	0.17	0.17	0.17	0.17	0.17	0.17	0.00	0.00	0.01	0.00	0.00	0.17		0.01	0.02	0.02	0.02	0.02
18	0.02	0.17	0.17	0.17	0.17	0.17	0.17	0.02	0.02	0.02	0.02	0.02	0.17	0.02		0.02	0.02	0.02	0.02
19	0.18	0.03	0.03	0.03	0.03	0.03	0.03	0.18	0.18	0.19	0.18	0.18	0.03	0.18	0.18		0.02	0.02	0.02
20	0.17	0.13	0.13	0.13	0.13	0.13	0.13	0.17	0.17	0.17	0.17	0.17	0.13	0.17	0.17	0.14		0.02	0.02
21	0.17	0.14	0.14	0.14	0.14	0.14	0.14	0.17	0.17	0.17	0.17	0.17	0.14	0.17	0.17	0.15	0.17		0.02
22	0.02	0.17	0.17	0.17	0.17	0.17	0.17	0.02	0.02	0.03	0.02	0.02	0.17	0.02	0.18	0.17	0.16	0.18	

Notes: 1 = KM-1; 2 = KM-2; 3 = KM-3; 4 = KM-4; 5 = KM-5; 6 = KM-6; 7 = KM-8; 8 = KM-7; 9 = KM-9; 10 = KM-10; 11 = KM-11; 14 = KM-14; 16 = KM=16; 17 = KM-17; 18 = JN898935.1 *M. lusoria*; 19 = JN043623.1 *M. meretrix*; 20 = OQ567566.1 *M. lyrata*; 21 = OM292826.1 *M. planisulcata*; 22 = MN275934.1 *Meretrix* sp. n. STH-2020 *(M. taiwanica)*

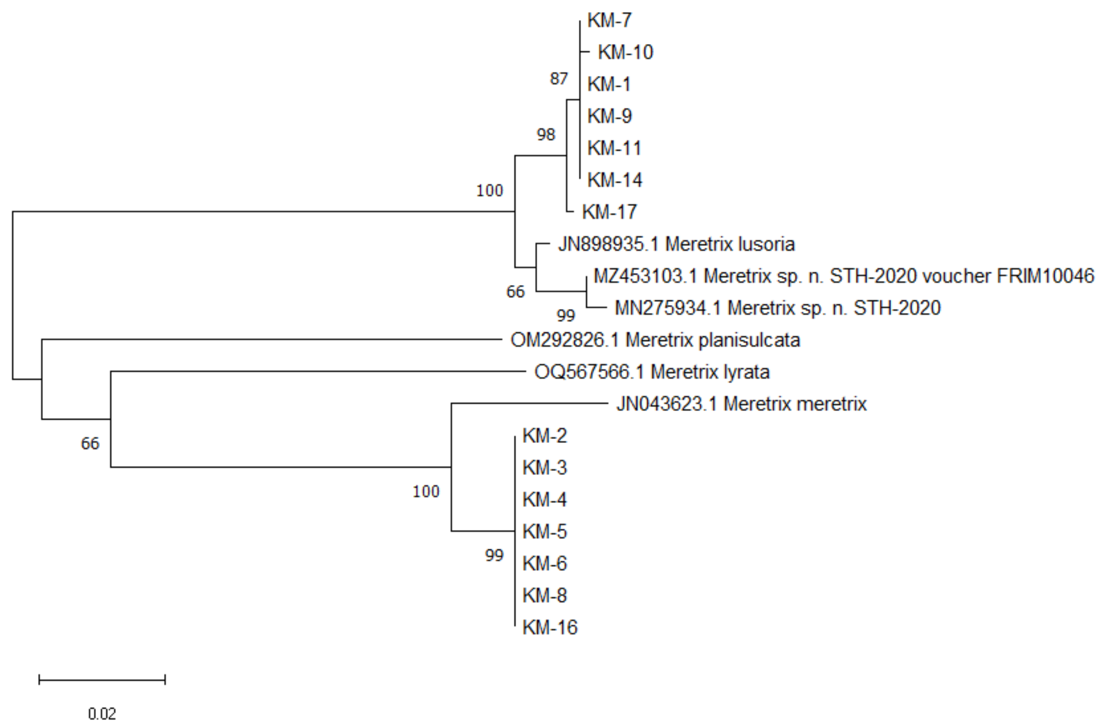

Fig. 2. Depiction of the phylogenetic relationships between *Meretrix* spp. from Tarakan Island and *Meretrix* sequences acquired from GenBank, with the tree generated through 1000 bootstrap replications

As of 2024, the World Register of Marine Species (WoRMS) has cataloged a total of 17 species within the genus *Meretrix* **(WoRMS, 2024)**. This represents an addition of four new species compared to the 13 species previously listed by **Huber (2010)**. The newly recognized species are *Meretrix lagiensis* **(MolluscaBase, 2024a)**, *Meretrix marisarabicum* **(MolluscaBase, 2024b)**, *Meretrix taiwanica* **(MolluscaBase, 2024c)**, and *Meretrix tigris* **(MolluscaBase, 2024d)**. This biodiversity necessitates the need for precise species identification. However, DNA barcoding, a vital tool for species identification in *Meretrix* spp., remains underutilized, as indicated by the limited number of relevant scholar articles. Traditionally, research on *Meretrix* has predominantly concentrated on morphological features, leading to inconsistencies and confusion in species nomenclature.

A notable case in point is a recent study on samples from Pantai Amal, Tarakan island. Here, 14 morphologically distinct specimens were initially classified. Yet, molecular analysis revealed a surprising finding: these specimens were actually members of only two species, *Meretrix lusoria* and *Meretrix meretrix*. This stark contrast between morphological and molecular identifications underscores the critical importance of molecular techniques in ensuring accurate species identification.

In a realm where precision in species identification is paramount, our research presents a compelling contrast to earlier studies by **Herlintos *et al.* (2012)** and

Wiharyanto (2013), particularly in the context of the *Meretrix* specimens KM-6 and KM-10. Initially classified as *Meretrix lyrata* and *Meretrix meretrix*, respectively, our study elucidates a significant genetic divergence, reclassifying KM-6 as *M. meretrix* and KM-10 as *M. lusoria*, with genetic similarities of approximately 96.50 and 98.08%. This revelation not only hints at the potential existence of a hitherto unidentified species but also underscores the imperative for comprehensive phylogenetic analyses employing additional genetic markers to reinforce this hypothesis. The quintessence of molecular research in this scenario is to mitigate misidentification, a critical factor in preserving the integrity and accuracy of scientific research, particularly in publications devoid of seasoned taxonomic consultation.

Further intriguing is the case of specimen KM-5, morphologically akin to *M. taiwanica* as per **Hsiao and Chuang (2023)**, yet exhibiting 17% genetic distance based on COI gene sequence analysis, thereby affirming distinct species status. This finding accentuates the taxonomic intricacies and the prevalence of 'cryptic species' within the *Meretrix* genus, categorically identifying KM-5 as *M. meretrix*. Additionally, this investigation diverges from **Jabarsyah and Arizono (2016)**, who through Gel SDS-PAGE electrophoresis postulated the presence of three distinct species within the Amal Beach Tarakan area. They identified specimens D and G as *M. lyrata*, correlating to KM-16 and KM-3 in our study. However, our COI gene analysis designates both KM-16 and KM-3 as *M. meretrix*, highlighting the disparate outcomes derived from protein-based versus DNA-based methodologies, where codon variations may not translate into amino acid changes or molecular weight alterations in proteins.

Complementing these findings, **Akhmadi and Trijoko (2016)** differentiated species based on palial sinus patterns, categorizing KM-7, KM-9, KM-10, and KM-14 as *M. meretrix*, and KM-3, KM-4, KM-6, and KM-8 as *M. lusoria*. This classification starkly contrasts with our results, further illustrating how methodological variations can lead to divergent species identifications. Conclusively, our study advocates for a multidisciplinary approach, amalgamating morphological data, molecular analysis, and potentially other techniques like behavioral ecology, to attain a more comprehensive and accurate depiction of the diversity and taxonomy of the *Meretrix* species.

CONCLUSION

This study successfully identified various *Meretrix* spp. specimens inhabiting the Tarakan Island. A total of 14 specimens were collected, exhibiting a range of morphological diversity. However, genetic analysis using the COI gene through DNA barcoding revealed that these specimens predominantly clustered into two major groups: *Meretrix meretrix* and *Meretrix lusoria*. This discovery not only contributes to the taxonomic knowledge of *Meretrix* spp. but also underscores the importance of molecular techniques in the accurate identification of bivalve species.

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