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MORPHOLOGICAL AND MOLECULAR IDENTIFICATION OF GASTROPODS FOUND IN MANGROVES OF SOMIANI BAY

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Abstract

Pakistan's coastline, extending 990 km along the northern Arabian Sea, lies entirely within the subtropical region. It comprises two distinct coastal segments the Sindh coast (about 320 km) and the Balochistan coast (about 670 km). In recent years, DNA barcoding has emerged as a strong and reliable scientific tool for species identification. Modern DNA barcoding, by contrast, provides an objective approach to taxonomic classification from kingdom to species level through the sequencing and analysis of a short, standardized region of an organism's genome. In this study, DNA barcoding proved to be a dependable molecular method for accurate taxonomic identification, with the cytochrome oxidase I (COI) mitochondrial gene serving as the most widely used marker due to its high specificity and authenticity. The analysis successfully confirmed the identification of specie belonging to the family Muricidae, demonstrating intraspecific genetic similarity ranging from 97% to 100%.. Phylogenetic analysis further revealed that the Muricidae species from Sonmiani Bay share close genetic affinities with populations from the United Kingdom (UK), Pakistan (PK), China (CN), New Zealand (NZ), India (IN), Australia (AU), Peru (PU) and Japan (JP), indicating a broad biogeographical connection within this family across diverse marine regions.

INTRODUCTION

At 1120 kilometers long, the coastline of Pakistan spans from the Iranian border in the northwest to the Indian border in the south. The length of Baluchistan's coast is 772 kilometres, whereas Sindh's is just 348. (UN commission march 2015). The United Nations Commission from 200 nm to 350 nm and from 250 nm to 290 nm, enabling the country to better explore and exploit its marine resources, expanded the borders of Pakistan's continental shelf exclusive economic zone (EEZ) in March 2015. Pakistan's Extended Economic Zone and Intertidal Zones are home to a wide variety of marine life, including both invertebrate and vertebrate species (FAO 2009).

The coastline is divided into three primary regions: Karachi, Lasbela, and Makran coasts. Coastal dynamics are influenced by tides, winds, waves, sea-level rise, geomorphic processes, and anthropogenic activities.Pakistan's Exclusive Economic Zone (EEZ) extends 200 nautical miles, encompassing an area of approximately 196,600 km², and was further extended by an additional 150 nautical miles in 2015 (Noman et al., 2022). Major fishing harbors include Korangi, Karachi, Pasni, and Gwadar, with Karachi accounting for 90% of the national catch and 95% of fish exports (Rafiq et al., 2017).In contrast, the aquaculture sector produces over 150,000 tons annually, driven by carp, tilapia, and trout farming (Shahzadi et al., 2020).

However, the sustainability of Pakistan's marine resources is under severe threat due to overfishing and habitat degradation (Khan et al., 2016). Miani Hor (Sonmiani Bay), a lagoon on the Balochistan coast approximately 145 km from Karachi, exemplifies a critical yet vulnerable habitat. The lagoon, about 60 km long and 7 km wide, receives freshwater inflows from the Winder and Porali rivers and supports a rich mangrove ecosystem (Gondal et al., 2012).

Gastropods, a class within the phylum Mollusca, comprise over 65,000 species, making them the largest mollusk group. This class includes snails, which typically retract into a protective shell, and slugs, which have evolved to have a significantly reduced or absent shell. Gastropods are remarkable for being one of the few animal groups to thrive in all three major habitats: ocean, fresh water, and land. (Ali et al., 2018).

Gastropods dominate mangrove ecosystems, where typically 44 to 65 species of mangrove-associated mollusks have been documented. The Potamididae is particularly associated with tropical and subtropical mangroves, comprising mudwhelks such as Terebralia, which feed on detritus, algae, and fallen leaves beneath mangrove canopies. Other genera, including Telescopium and Cerithidea, are smaller and climb mangrove vegetation for protection and thermoregulation.

Gastropods are also a valuable food resource, providing high-quality protein comparable to or exceeding that of beef and poultry. In developing countries, edible gastropods can play a key role in addressing malnutrition. Their biochemical composition rich in proteins, minerals, and

metabolites contributes significantly to human nutrition and has medicinal potential (Menabit et al., 2022).

Gastropods possess considerable economic and cultural significance. Species such as Tridacna spp. (giant clams) are valued for their meat and ornamental shells (Tabugo et al., 2013). Shell-based handicrafts, jewelry, and decorative items are commonly traded commodities in coastal communities.

Around the world, especially in areas where they are prevalent, gastropods are a nutrient-dense diet. Although their nutritional value varies depending on the species, climate, and season, they are typically an important source of vital nutrients. (Cumplido et al., 2024). Because of their high protein content, gastropods represent a significant source of protein for coastal communities and a viable substitute for aquaculture feed (Tan et al., 2021).

The most reliable and effective method for molecularly identifying species of mollusks is DNA barcoding. DNA barcodes are used to evaluate the molecular biodiversity of mollusks. DNA barcoding refers to the application of the conventional genomic sequencing approach with less genetic differentiation within the same species than between species. Having a thorough understanding of every known species of mollusks is useful for recognizing new species. From larvae to juvenile animals, these molecular-based identification methods resolve taxonomy at every stage of life (Hanner et al., 2011).

In DNA barcoding investigations, the identification of gastropod species frequently depends on molecular markers, such as nuclear markers like histone H3 and mitochondrial genes, especially when morphological information is scarce. For marine gastropods, where accurate taxonomic analysis of edible species is required for efficient fisheries management and food safety, this genetic method is crucial (Setiamarqa et al., 2025).

The identification of gastropod species and the evaluation of biodiversity have been transformed by developments in molecular taxonomy, especially DNA barcoding and COI sequencing. By combining morphological and genomic data, these methods improve taxonomic resolution and assist conservation efforts (Ke Ran et al., 2020).

MATERIALS AND METHODS

Specimen collection for the experiments was collected from the coastal regions of Dam, Balochistan (Fig. 1). Specimen was transferred to the Faculty of Marine Sciences Laboratory at Lasbela University of Agriculture, Water and Marine Sciences (LUAWMS), Uthal, for further analysis and identification. (Fig. 2) Primary morphological identification of gastropod specimens was carried out using standard taxonomic solutions and field guides (Grossu, 1956). Furthermore, diagnostic features like shell shape, size, coloration, space structure, and ornamentation were wisely examined under a stereomicroscope. Each specimen was catalogued, photographed, and labeled with a unique

identification code. Selected specimens represented separate morphotypes were subsequently subjected to molecular analyses for confirmation and species-level validation.

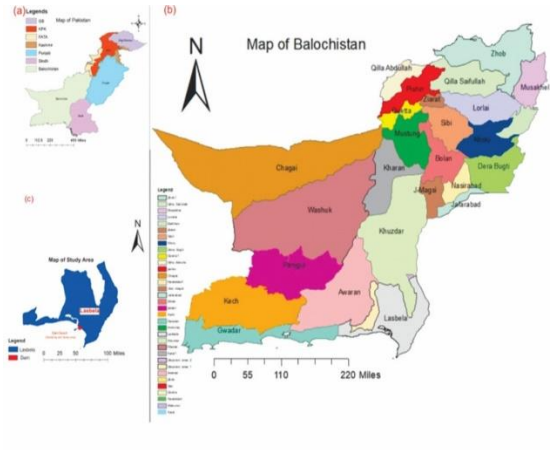


Figure 1: The geographical area of Somiani Bay of Balochishtan (a, b) and the c is indicating the sampling point.



The figure 2 showing the morphology of Muricidea species from Daam Lasbela Coast of Balochistan. *Semiricinula tissoti.*

DATA ANALYSIS

The sample has been analysis in the Hitech Bio-tech lab Lahore. Morphological data were analyzed to identify interspecies variations and to classified gastropods into morpho groups. Molecular data, included with COI sequences, were subjected to many sequences configuration and phylogenetic analysis using MEGA X software. Sequence parallel examinations were conducted using the NCBI BLAST database to confirm species identification and to assess genetic separation among populations.

DNA extraction

Primary morphological identification of gastropod specimens was carried out using standard taxonomic solutions and field guides (Grossu, 1956).

Total genomic DNA was extracted using the DNeasy Blood and Tissue Kit (Qiagen) with an enhanced protocol including a cell disruption stage. Tissue samples were homogenized in Tris-EDTA

(pH 8) buffer at 20°C using a SpeedMill PLUS Cell Homogenizer with a 0.2 mm ZR Bashing Bead lysis matrix. A partial region of the COI gene was amplified using universal metazoan primers (LCO1490 and HCO2198) in a 50 µL reaction mixture. The reaction mixture contained 1 unit of Taq DNA polymerase, 1 µL genomic DNA, 1 µL of each primer, 1× BSA, 0.1 mM dNTPs, and 1× Taq buffer with 2.5 mM $MgCl_2$. The thermal profile included an initial denaturation at 95°C for 2 min; 5 cycles of denaturation (94°C for 30 s), annealing (45°C for 90 s), and extension (72°C for 1 min); followed by 35 cycles with annealing at 50°C for 90 s, and a final extension at 72°C for 5 min. Amplified products were verified by electrophoresis on 1% agarose gel stained with ethidium bromide to confirm fragment size and integrity.

Molecular Taxonomy of Muricidea species from Pakistan

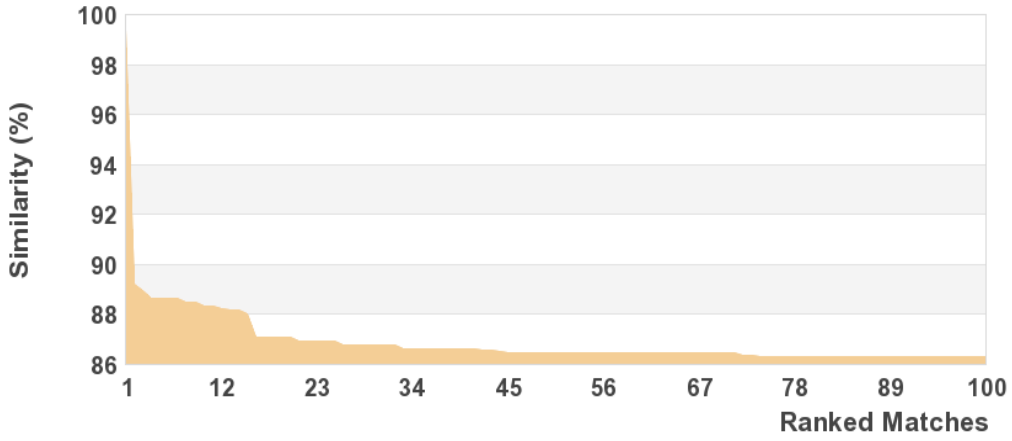


Figure 3: Higher similarity score of top 100 matches gained from BLAST and BOLDv4 system of Semiricinula tissoti.

Phylogenetic tree construction and sequencing

The Sanger sequencing method was used to sequence the PCR products. Sequences were edited and aligned using software (BIOEDIT and MEGA 6) (Tamura et al. 2013). The possibly correct species were known by the BOLDv4 systems (https://v4.boldsystems.org/) and Nucleotide Blast (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastnandBLAST SPEC=GeoBlastandPAGE_TYPE=BlastSearch). To analyze the evolutionary history among the inhabitants, a neighbor-joining tree was created using MEGA 6 and the Kimura 2 parameter (K2P) model (Tamura et al., 2013).

RESULTS

Cytochrome oxidase subunit I (COI) gene fragments were used for molecular identification. In China, the Sanger sequencing technique was used to magnify the COI gene. The NCBI acknowledged the confirmed sequences with Accession Number PV826588. The NCBI Nucleotide BLAST and BOLD

v4 systems were used for class identification; the maximum resemblance calculation was selected (Fig. 3). Inside the similar species, the noticed species presented 97% to 100% resemblance. COI gene sequences were used to generate a neighbor-joining phylogenetic tree that assembled the exposed species with mention sequences from diverse geographical sites. The phylogenetic relations of *Semiricinula tissoti* from Somiani Bay showed close heritable similarities with inhabitants from United Kingdom (UK), Pakistan (PK), China (CN), New Zealand (NZ), India (IN), Australia (AU), Peru (PU) and Japan (JP), (Fig. 4).

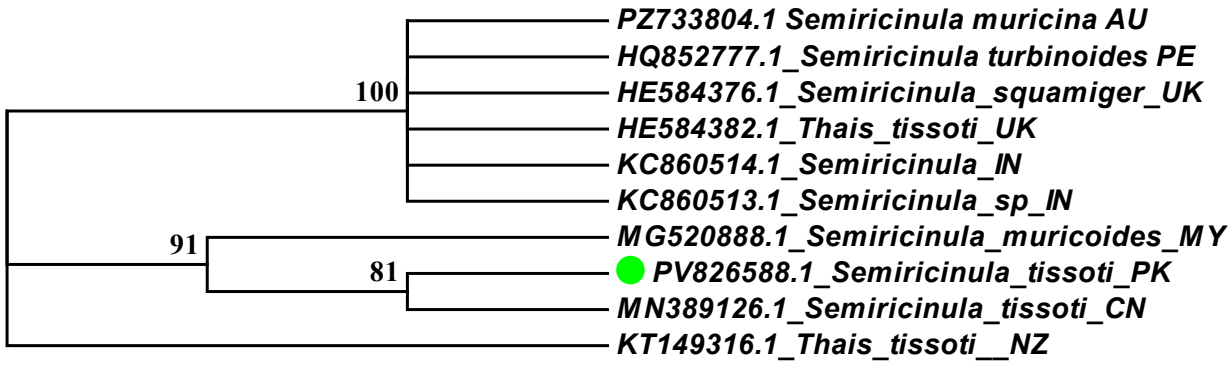


Figure 4: Neighbor Joining tree of CO1 barcode for Semiricinula tissoti species greater than 70 % are shown with samples collected from the Somiani Bay, Balochistan.

DISCUSSION

Pakistan's entire coastline, approximately 990 km long, is located in the subtropics of the northern Arabian Sea. It consists of the Sindh province coastline (320 km) and the Balochistan province coastline (670 km). The Balochistan coast features many characteristic bays, locally known as *Hors*. Sonmiani Bay, or Miani Hor, is a lagoon situated about 90 km from Karachi on the easternmost part of the Balochistan coast. This tortuous and contorted bay is 60 km long and 7 km wide, connecting to the sea at its southeastern end through a 4 km wide mouth (Saifullah and Rasool, 1995). The primary sources of freshwater for Miani Hor are the seasonal run-off from the Porali and Windor rivers (Rasool et al., 2002; Saifullah et al., 2004).

The mangrove forests of Miani Hor represent 42% (3,000 hectares) of the total mangrove cover in Balochistan. This area is extremely important as it supports the natural growth of three mangrove species: *Avicennia m*arina, Rhizophora mucronata, and Ceriops tagal. These mangroves play a vital role in the lagoon's productivity (Gondal et al., 2012).

The phylum Mollusca is considered one of the most diverse groups, with an estimated 50,000 to 55,000 species that have been evaluated and characterized . This number increases annually by approximately 443 new species, over half of which (51.5%) are discovered in the tropical Indo-Pacific region(Alongi et al., 2025). Molluscs are identified by key morphological characteristics such as their shell, mantle, the presence of a radula, and their nervous system configuration (Herbert et al., 2018).

They inhabit a wide range of marine environments, from intertidal and splash zones to the depths of marine trenches. For example, they have been found in the Puerto Rico Trench at a depth of 8,595 meters a depth comparable to the height of Mount Everest (Leal and Harasewych, 1999). The molluscan body is typically divided into three parts: a head, a visceral mass that contains the internal organs, and a muscular foot. The body is often enclosed within a hard shell composed of calcium carbonate (Dennis et al., 2021).

In current study of family Muricidea was examined using CO1 gene. The specie *Semiricinula tissoti,* . The class signifies an obvious taxonomic group limited with Muricidae family, which is familiar for its predatory behaviour and important biological role in aquatic systems. The arrangement of class significantly diverse in catches from areas of Damb Lasbela DNA barcoding examines the close boundary of identical species, which is obliging in the deviation of close approaching neighbors within the same species.

Universally, DNA barcoding has revealed to be an crucial molecular method for freshwater aquatic fish identification and biodiversity. (Wang et al., 2010)DNA barcoding overwhelmed the problems related with morphological identification to allow fish identification. DNA barcoding has exposed itself to be an effective molecular procedure for the identification and genetic variety of aquatic species from Damb Lasbela, with outstanding Molecular Taxonomy of Muricidea from Pakistan. In thoroughly connected genetically similar species, hybridization can principal to species misidentification; DNA barcoding can benefit with this. Gastropod taxonomy and genetic identification can benefit from DNA barcoding. In terms of gastropode taxonomy and biodiversity, DNA barcoding reduces doubt and difficulty. (Lamendin et al.,2015)

CONCLUSION

The species of the Muricidea family from the coastal region of Damb Baluchistan (*Semiricinula tissoti)* was effectively recognized molecularly using the mt DNA gene CO1. The study determined that the CO1 gene is a dependable and valid genetic marker for recognizing Muricidea found along the coast of Lasbela. We expect that upcoming researchers and conservation fisheries managers will find the variety of Muricidea species to be a valuable resource for precise data on the makeup of Baluchistan's Muricidea species. Ultimately, it will help conservation and protection organisations with their species management strategies. Precise species identification will be vital for increasing the economics of marine resources due to the coast's variety and abundance of marine animals. Large-scale diversity survey of Muricidea is feasible through wide sampling, and several genetic markers are mandatory to close the gaps in the present research.

REFERENCES

FAO, J. (2009). Fishery and aquaculture country profiles. Food and Agriculture Organization of the United Nations, 8.

Rafiq, N., Ahmad, S. Z., Yasmeen, G., Baset, A., Iqbal, M. A., Khan, A., ... & Ali, A. (2017). Identification of terrestrial gastropods families found in district Swat, Pakistan. Brazilian Journal of Biology, 83, e248420.

Gondal, M. A., Saher, N. U., & Qureshi, N. A. (2012). Diversity and biomass distribution of intertidal fauna in sonmiani bay (MianiHor), balochistan (pakistan). *Egyptian Academic Journal of Biological Sciences*, B. Zoology, 4(1), 219-234.

Noman, M., Mu, Y. T., Nisar, U., Mohsin, M., & Memon, A. M. (2022). Constraint analysis of major problems facing the marine fisheries sector in accordance with the national fisheries policy of Pakistan. *Indian Journal of Geo-Marine Sciences (IJMS)*, *51*(01), 94-103.

Shahzadi, A., Kainat, S., & Ammara, S. (2020). Climate-smart fisheries production in Pakistan: A policy brief and way forward for decision-makers. *Science Letters*, *14*, 15.

Khan, M. W., & Abbas, G. (2024). Review on fisheries resources and the effect of marine pollution in coastal waters of Pakistan. *Journal of Zoology and Systematic*, *2*(1), 23-43.

Ali, S. A., Humayun, F., Munir, I., Ahmad, S., Ayub, Z., Fatima, H., ... & Haider, M. S. (2018). Morphological, molecular, biochemical and nutritional characterization of three major Thais species from the Sindh coast of Pakistan. *The Open Food Science Journal*, 10(1), 33-45.

Menabit, S., Begun, T., Teac ˘a, A., Mure¸san, M.,Lavin, P., Purcarea, C. (2022). DNA Barcoding and Distribution of Gastropods and Malacostracans in the Lower Danube Region. Diversity, 14, 533. https://doi.org/10.3390/d14070533.

El-Gayar, H. A., Abd El-Maoula, L. M., Mohamed, E. S. N., El-Naggar, H. A., & Ageba, M. F. ARTICLE INFO ABSTRACT.

Grossu, A.V. (1956). Mollusca Gastropoda Prosobranchia si Opistobranchia; Fauna Republicii Populare Române: Bucharest, Romana, Volume 3.

Cumplido, M., Marinho, C., & Bigatti, G. (2020). Nutritional composition of Patagonian marine gastropods during reproductive seasonality. *Journal of the Marine Biological Association of the United Kingdom*, 100(4), 567–576. https://doi.org/10.1017/s0025315420000454

Tan, K., Zhang, H., Ma, H., Li, S., & Zheng, H. (2021). Effects of tidal zones and seasons on nutritional properties of commercially importance gastropods. Estuarine, *Coastal and Shelf Science*, 254, 107289. https://doi.org/10.1016/j.ecss.2021.107289

Hanner, R., Floyd, R., Bernard, A., Collette, B. B., &Shivji, M. (2011). DNA barcoding of billfishes. Mitochondrial dna, 22(sup1), 27-36.

Ke Ran, Qi Li, Lu Qi, Weidong Li, Lingfeng Kong, (2020). DNA barcoding for identification of marine gastropod species from Hainan Island, China. *Fisheries Research*, 225:105504. https://doi.org/10.1016/j.fishres.2020.105504.

Setiamarga, D. H. E., Shimizu, M., Nakashima, S., Osaki, C., Hirota, K., Anggraeni, L., Van Tu, D., & Sasaki, T. (2025). DNA barcoding of museum-vouchered samples collected from fish markets reveals an unexpected diversity of consumed gastropods in Vietnam. *Future Foods*, 12, 100689. https://doi.org/10.1016/j.fufo.2025.100689

Lamendin, R., Miller, K. and Ward, R.D., 2015. Labelling accuracy in Tasmanian seafood: An investigation using DNA barcoding. Fd. Contr., 47: 436–443.https://doi.org/10.1016/j.foodcont.2014.07.039

Rasool, F., Tunio, S., Hasnain, S., & Ahmad, E. (2002). Mangrove conservation along the coast of Sonmiani, Balochistan, Pakistan. *Trees*, *16*(2), 213-217.

Gondal, M. A., Saher, N. U., & Qureshi, N. A. (2012). Diversity and biomass distribution of intertidal fauna in sonmiani bay (MianiHor), balochistan (pakistan). *Egyptian Academic Journal of Biological Sciences*, B. Zoology, 4(1), 219-234.

Selvavinayagam, K. (2008). Shoreline change monitoring in Tuticorin coast using remote sensing and GIS tools.

Wang, Z., Guo, Y., Tan, W., Li, L., Tang, E., Liu, C. and Liu, Y., 2010. DNA barcoding, phylogenetic relationships and speciation of snappers (genus Lutjanus). Sci. China Life Sci., 53: 1025-1030. https://doi.org/10.1007/s11427-010-4034-0

Dennis, M. M., Molnár, K., Kriska, G., & Lőw, P. (2021). Mollusca: gastropoda. *Invertebrate histology*, 87-132.

Alongi, D. M. (2025). Global meta-analysis of mangrove primary production: implications for carbon cycling in mangrove and other coastal ecosystems. *Forests*, *16*(5), 747.s