Punjab University Journal of Zoology

OPEN ACCESS

37(2): 93-97 (2022)
https://dx.doi.org/10.17582/journal.pujz/2022.37.2.93.97

Check for updates

Research Article

Molecular Identification and Phylogenetic Analysis of *Turbinella pyrum*, (Linnaeus, 1767) (Mollusca: Gastropoda) using Cytochrome Oxidase Subunit 1

Amna Sulaman[1], Qurat-ul-Ain Ijaz[1], Muhammad Shafi[2] and Faiz Muhammad[1]*

[1]*Center of Excellence in Marine Biology, University of Karachi, Karachi-75270, Pakistan*
[2]*Lasbella University of Agriculture, Water and Marine Sciences, Lasbella, Uthal.*

Article History
Received: May 23, 2022
Revised: June 16, 2022
Accepted: July 24, 2022
Published: September 06, 2022

Authors' Contributions
AS collected samples, conducted experiments and data analysis. QAI participated in collection and typing. M.S. revised the manuscript. F.M. designed experiment, generated funds and wrote the article.

Keywords
COX1, DNA-barcode, Turbinellidae, Marine environment, Pakistan

Abstract | The marine environment is extremely important and has remarkable biodiversity. *Turbinella pyrum* is a marine species found only in the Indian Ocean. It is an economically important resource as well as a sacred animal. The meat of this animal is used in traditional medicine. *T. pyrum* is a less known species from Pakistani marine waters. This research validated its molecular identification as *T. pyrum*. The neighbor-joining results revealed that it clustered with the same species of family Turbinellidae except for the individual of the same species from India's west coast. Individuals of this species have demonstrated less genetic difference. The present findings will be beneficial for taxonomists and future researchers of the region.

Novelty Statement | An important species of gastropod known as Turbinella pyrum (Linneaus, 1967) has been identified for the first time using Cytochrome oxidase subunit 1 from Pakistani marine waters.

To cite this article: Sulaman, A., Ijaz, Q.A., Shafi M. and Muhammad, F., 2022. Molecular identification and phylogenetic analysis of *Turbinella pyrum, (*Linnaeus, 1767) (Mollusca: Gastropoda) using cytochrome oxidase subunit 1. *Punjab Univ. J. Zool.*, **37**(2): 93-97. https://dx.doi.org/10.17582/journal.pujz/2022.37.2.93.97

Introduction

Mollusca is the second largest phylum of invertebrates (Bhamrah and Juneja, 1991) and only the class Gastropoda consists of around 75,000 living species and 15,000 fossils. Of all the shells, *Turbinella* (=*Xancus*) *pyrum* is considered a sacred chank. This shell is limited to Indian waters but shares adjoining coasts of Sri Lanka and the Asian region (Hornell, 1949; Jones, 1966; Nayar and Mahadevan, 1973). *Turbinella pyrum,* also known as Chank shell or conch, and divine conch, belongs to the Turbinellidae family and is found in the Indian Ocean. It is not common along the western coast of the subcontinent, but it is found in large numbers in the Gulf

Corresponding Author: Faiz Muhammad
balouch_23@yahoo.com

of Kutch and along the coast of Sindh and Balochistan, West of Karachi (Kenoyer, 1984). Khan and Dastgir (1971) were the first to report it as *Xancus pyrum* from Pakistani marine waters. Its significance lies not only in its decorative aspects but also in its religious significance in Hinduism and Buddhism. According to Hindu beliefs, this species symbolizes success, harmony, and prosperity. (Panda *et al.*, 2011). It is exploited all year along the Indian Thoothukudi coast, but there is no record of it being fished or exploited in Pakistani marine waters. This species lives for 13 years and grows rapidly during its first three years (Panda *et al.*, 2011). This species has a wide range of morphological variants and has been gathered since the dawn of history (Arularasan *et al.*, 2016). Its shell is used for handicrafts and ethnic jewelry (Mukundan, 1968). *T. pyrum* meat is also used in traditional medicine as a remedy for many ailments (Gopal *et al.*, 2008). Hornell (1916) described five varieties based on their external form and color pattern, stating that each variety has its geographical distribution as well as physical and biological environmental characteristics (Hornel, 1915). However, Winckworth (1939) classified these varieties as synonyms of *T. pyrum* and *T. rapa* based on anatomical identification.

The species information is limited to physical and economic features (Shekar *et al.*, 2016), and yet only a few papers have detailed its genetic aspects. Investigations from Pakistani coastal waters are indeed limited for this species; therefore, it requires attention in all aspects including its molecular taxonomy.

Using morphological characteristics for taxonomy does not always produce good results (Packer *et al.*, 2009). When both species and juvenile life stages are morphologically similar, traditional methods are limited (Galan *et al.*, 2018; Gossner and Hausmann, 2009). The DNA barcode identification method is widely used for the identification and understanding of phylogenetic relationships. It became a fashion to identify species using molecular markers, and the method has been used for many years (Ferri *et al.*, 2009). Cytochrome oxidase subunit 1 (CO1) is widely used as a barcode for most of the animals' taxa and as a marker for population genetics and phylogenetic studies (Hebert *et al.*, 2003). Some marine gastropod species from Hainan Island, China, have recently been the subject of research by Ran *et al.* (2020), additionally, a few chosen marine gastropods were examined by Galan *et al.* (2018) using a CO1 molecular marker.

The current study aimed to better understand the molecular taxonomy of *T. pyrum* found in Pakistani coastal waters, and to the best of our knowledge, it is the first time we have attempted to identify this rarely found species using a molecular marker (CO1) sequence. There are only a few molecular-based taxonomy studies on Mollusca species from Pakistani coastal waters (Zafar *et al.*, 2016; Humayun *et al.*, 2019; George *et al.*, 2021). The results of this study supported earlier taxonomic identifications made based on morphology.

Materials and Methods

Individuals of *T. pyrum* were picked at random and brought to the laboratory. The species was identified morphologically using Bosch *et al.* (1995). Once the shells were cracked open, the delicate tissues were kept at -20 °C for further investigation. Muscle tissue was used to isolate genomic deoxyribonucleic acid (gDNA) using a phenol-chloroform protocol (Sambrook *et al.*, 1989). The Cytochrome oxidase subunit 1 (COX1) gene was amplified by polymerase chain reaction (PCR) using universal primers (Table 1). The PCR was performed with 100mg DNA template, 2.5µl dNTP (2.5mM each), 2.5µl 10 X buffer, 2µl Mgcl2, (20mM), 1M primers (10M each), and 0.25µl Taq polymerase (5U Ml*1). Denaturation at 94°C for 5 minutes; 35 cycles at 94°C for 30 seconds, annealing at 50°C for 30 seconds, and extension at 72°C for 30 seconds; and a final extension at 72°C for 7 minutes. To validate effective amplification, 1.2 % gel electrophoresis was used. The PCR products were sequenced using the Sanger sequencing technique. The necessary insertion and deletion were performed using software (BIOEDIT and MEGA 6) (Tamura *et al.*, 2013). A neighbor-joining tree was generated using the Kimura 2 parameter (K2P) model and MEGA 6 to establish genetic relationships between the populations (Tamura *et al.*, 2013).

Results and Discussion

T. pyrum was identified morphologically based on the main traits reported by Tirmizi and Zehra (1982). Figure 1 depicts the photo morph of the species. For amplification, a set of Universal primers for Cytochrome oxidase subunit 1 (Table 1) was employed. This sequence was submitted to NCBI under accession number ON430599 and includes 655 base pairs (bp). In the present study, only 625 bp were used for analysis. The blast result revealed substantially comparable species sequences.

Table 1: The set of primer used in this study.

Gene	Primer name and sequence	Tm (°C)	Size (bp)	Reference
Cytochrome oxidase subunit1 (COX1)	LCO1490:5'GGTCAACAAATCATAAAGATATTGG-3'	50	710	Folmer *et al.*, 1994
	HCOR2198:5'-TAAACTTCAGGGTGACCAAAAAATCA-3'			

Table 2: The pairwise differences of the sequences of family Turbinellidae and distinctly related species. Accession numbers with red colors are representing the *T. pyrum*, the sequence with asterisk is from Pakistan. Green is representing *T. rapa*. The numbers in black color are representing *favartia alveata, Fasciolaria bullisi, Saxidomus ppurpuratus, Thais luteostoma, Reishia clavigera*, respectively.

Accession Nos	1	2	3	4	5	6	7	8	9	10	11	12
KJ556552	-											
KJ556544	0.000	-										
KJ556549	0.000	0.000	-									
KJ556543	0.002	0.002	0.002	-								
KJ556539	0.003	0.003	0.003	0.005	-							
MG200031	0.003	0.003	0.003	0.004	0.006	-						
MK308478	0.187	0.187	0.187	0.18	0.187	0.185	-					
KT753988	0.201	0.201	0.201	0.201	0.201	0.199	0.185	-				
HM180839	0.219	0.219	0.219	0.219	0.224	0.224	0.198	0.219	-			
HM180831	0.219	0.219	0.219	0.219	0.224	0.224	0.198	0.219	0.000	-		
HM180820	0.217	0.217	0.217	0.217	0.2218	0.217	0.196	0.219	0.011	0.011	-	
*ON430599	0.008	0.008	0.008	0.009	0.011	0.011	0.197	0.2118	0.225	0.225	0.223	-

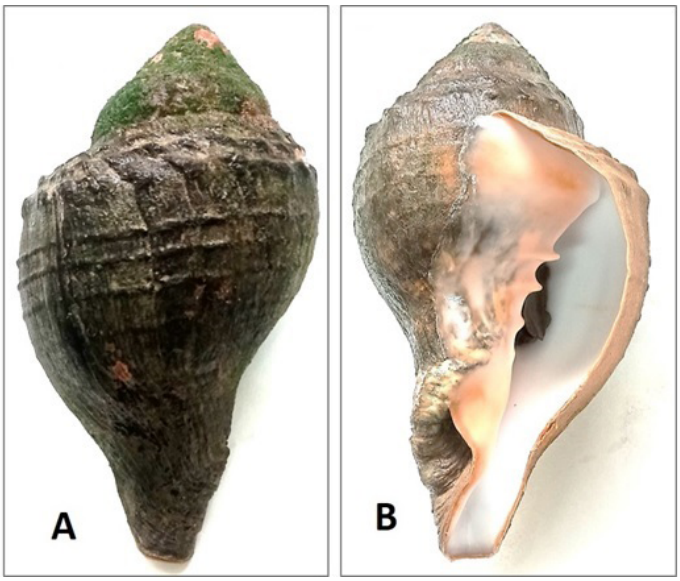

Figure 1: A and B show the photo morph of the *Turbinella pyrum*.

The blast result revealed substantially comparable species sequences. Figures 2A and B show the graphic blast visualization from NCBI (https://blast.ncbi.nlm.nih.gov/Blast.cgi) and the Kabalmoo (https://kablammo.wasmuthlab.org/#load). The barcode sequence of the current investigation is 98.93% similar to sequences under accession numbers KJ 556552, KJ556544, and KJ5565491. All these sequence submissions originated from India.

The average distance within the Turbinellidae family was 0.0046±00141, while the gap between Tubinellidae species and distinctly related species was 0.2107. The detailed pairwise differences are shown in Table 2. In the phylogenetic analysis, all Turbinellidae species were clustered together, except for sequence MG200031, which distinctly showed a split with 60 percent bootstrap support (Figure 3) and was submitted from Kerala, South West coast of India.

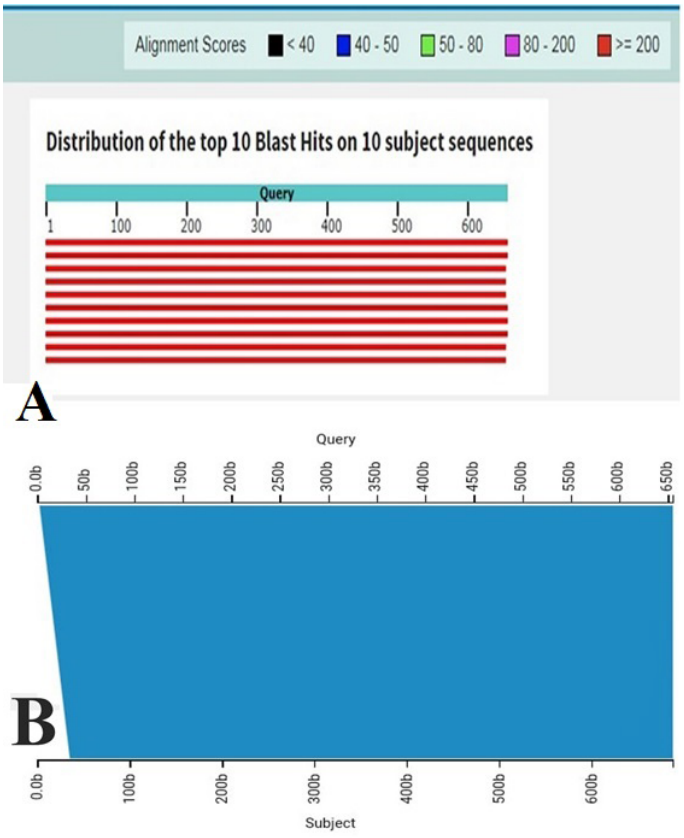

Figure 2: Graphic blast visualization of *Turbinella pyrum*. A: Taken from NCBI; B: Showing the results from Kabalommo.

T. pyrum is only found in the Indian Ocean, according to reports. The studies from Pakistani coastal waters are meager and were first time recorded by Khan and Dastgir (1971). The findings confirmed its earlier morphological-based identification. The neighbor-joining tree resulted that *T. pyrum* clustered closely with other identical species,

with exception of one which distinctly clustered in the NJ tree with 60% bootstrap support (Figure 2). The mean genetic distance between the individuals of *T. pyrum* was lower, indicating a significant pattern of gene flow.

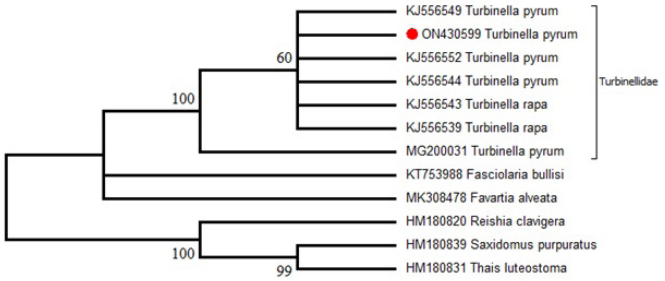

Figure 3: Neighbor-joining tree showing the phylogenetic relationship among species of family Turbinellidae and other distinctly relative gastropod species. The red circle is showing the species collected from the Pakistani marine environment.

Conclusions and Recommendations

The cytochrome oxidase I (COI) gene has proven to be an extremely useful tool for species identification. *T. pyrum* has been genetically verified in the present investigation. It has been difficult to find evidence that organisms from other places have diverged over time. The results obtained in the present study support previous morphological based taxonomy done in the regional investigations and future research in population genetics, reproduction, and stock assessment is advised.

Acknowledgements

The Petroleum Marine Development Committee of Malir District, Karachi, has generously supported this research. We extend our sincere gratitude to the anonymous reviewers for their insightful criticism and enhancement of this paper.

Ethics statement

All the methods were carried out in line with international norms for an invertebrate.

Conflict of interest

The authors have declared no conflict of interest

References

Arularasan, S., Viswanathan, M.S., Venmathi-Maran, B.A., Zaman, G.U. and Chandra, S.M., 2016. Variation in shell morphology and adult specimen weight in three varieties of a commercially important gastropod *Turbinella pyrum* (Linnaeus, 1767) from Southeast Coast of India. *J. Mar. Sci. Res. Dev.*, **6**(184). https://doi.org/10.4172/2155-9910.1000184

Bhamrah, H.S. and Juneja, K., 1991. An introduction to Mollusca. Anmol. Publ. New Delhi, pp. 203.

Bosch, D.T., Dance, S.P., Moolenbeck, R.G. and Oliver, P.G., 1995. Sea shells of the eastern Arabia (ed. P. dance). Motivate Publishing, Emirates printing press, Dubai, UAE. pp. 1-296.

Shekar, C.M., Arularasan, S., Nathani, N.M., Zaman, G.U. and Joshi, C.G., 2016. Genetic architecture of three *Turbinella pyrum* varieties (Linnaeus, 1758) from the southeast coast of India. *Mar. Ecol.*, **37**: 588-598. https://doi.org/10.1111/maec.12312

Ferri, E., Barbuto, M., Bain, O., Galimberti, A., Uni, S., Guerrero, R. and Casiraghi, M., 2009. Integrated taxonomy: traditional approach and DNA barcoding for the identification of filarioid worms and related parasites (Nematoda). *Front. Zool.*, **6**: 1-12. https://doi.org/10.1186/1742-9994-6-1

Folmer, O., Black, M., Hoeh, W., Lutz, R. and Vrijenhoek, R., 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Mol. Mar. Biol. Biotechnol.,* **3**(5): 294-299.

Galan, G.L., Mendez, N.P. and dela Cruz, R.Y., 2018. DNA barcoding of three selected gastropod species using cytochrome oxidase (COI) gene. *Annls West Univ. Timisoara Ser. Biol.*, **21**: 93-102.

George, N., Siddiqui, G., Muhammad, F. and George, Z., 2021. Dna barcoding of gastropod *Telescopium telescopium* (linnaeus, 1758) found at the Karachi coast, Pakistan. *J. Anim. Pl. Sci.*, **31**(5): 1530-1536. https://doi.org/10.36899/JAPS.2021.5.0355

Gopal, R., Vijayakumaran, M., Venkatesan, R. and Kathiroli, S., 2008. Marine organisms in Indian medicine and their future prospects. *Nat. Prod. Radiance*, **7**(2): 139-145.

Gossner, M.M. and Hausmann, A., 2009. DNA barcoding enables the identification of caterpillars feeding on native and alien oak (Lepidoptera: Geometridae). *Mitteilungen der Münchner Entomologischen Gesellschaft*, **99**: 135-140.

Hebert, P.D., Ratnasingham, S. and De Waard, J.R., 2003. Barcoding animal life: cytochrome c oxidase subunit 1 divergences among closely related species. *Proc. R. Soc. Lon. Ser. B Biol. Sci.*, **270**(**suppl_1**): S96-S99. https://doi.org/10.1098/rsbl.2003.0025

Hornell, J., 1915. *The Indian varieties and races of the genus Turbinella. Mem. Ind. Mus.,* 109-122.

Hornell, J., 1916. An explanation of the cyclic character of the pearl fisheries of the Gulf of Mannar. *Madras Fish. Bull.*, **8**: 11-22.

Hornell, J., 1949. The study of Indian molluscs, Part I. *J. Bombay Nat. Hist. Soc.*, **48**: 303-334.

Humayun, F., Ayub, Z., Haider, M.S. and Ali, S.A., 2019. Morphological identification, molecular barcoding and nutritional value of Siphonaria species from

Sindh Coast, Pakistan. *Biologia (Pakistan)*, **65**: 1-10.

Jones, S., 1966. The molluscan fishery resources of India. *Proc. Symp. Mollusca Mar. Biol. Ass. India*, **III**: 907-917.

Kenoyer, J.M., 1984. Shell industries at Moenjo Daro, Pakistan. *Interim Rep.*, **1**: 99-115.

Khan, M.D. and Dastagir, S.G., 1971. On the mollusca: Gastropod fauna of Pakistan. *Rec. Zool. Surv., Pakistan*, **1**: 17-130.

Mukundan, C., 1968. Molluscs in Indian tradition and economy. In: *Proceedings of the Symposium on Mollusca*: MBAI, 12-16 January 1968, Cochin

Nayar, K.N. and Mahadevan, S., 1973. *Chank resources of India*. Proc. Symp Living resources of the seas around India. CMFRI Spl. Pub., Cochin, pp. 672-686.

Packer, L., Gibbs, J., Sheffield, C. and Hanner, R., 2009. DNA barcoding and the mediocrity of morphology. *Mol. Ecol. Resour.*, **9**: 42-50. https://doi.org/10.1111/j.1755-0998.2009.02631.x

Panda, D., Jawahar, P. and Venkataramani, V.K., 2011. Growth and mortality parameters of *Turbinella pyrum* (Linnaeus, 1758) exploited off Thoothukudi, south-east coast of India. *Indian J. Fish.*, **58**: 29-33.

Sambrook, J., Fritsch, E.F. and Maniatis, T., 1989. *Molecular cloning: A laboratory manual*. Cold Spring Harbor Laboratory.

Ran, K., Li, Q., Qi, L., Li, W. and Kong, L., 2020. DNA barcoding for identification of marine gastropod species from Hainan Island, China. *Fish. Res.*, **225**: 105504. https://doi.org/10.1016/j.fishres.2020.105504

Tamura, K., Stecher, G., Peterson, D., Filipski, A. and Kumar, S., 2013. MEGA6: Molecular evolutionary genetics analysis version 6.0. *Mol. Biol. Evol.*, **30**: 2725-2729. https://doi.org/10.1093/molbev/mst197

Tirmizi, N.M. and Zehra, I., 1982. *Illustrated key to families of Pakistani marine molluscs*. Published by Pakistan Science Foundation. pp. 1-46.

Winckworth, R., 1939. On the species of *Xancus* or *Turbinella*. *J. Molluscan Stud.*, **23**: 345-347. https://doi.org/10.1093/oxfordjournals.mollus.a064362

Zafar, F.H.S., Ayub, Z., Begum, S., Siddiqui, G. and Roberts, D., 2016. Limpets of the genus Cellana (Patellogastropoda) from Pakistan, North Arabian Sea: Species identification based on DNA sequencing. *Mitochondrial DNA A*, **27**: 2868-2872. https://doi.org/10.3109/19401736.2015.1053133