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DNA BARCODING OF GASTROPOD *TELESCOPIUM TELESCOPIUM* (LINNAEUS, 1758) FOUND AT THE KARACHI COAST, PAKISTAN

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ABSTRACT

DNA barcoding is considered an effective tool for the identification of organisms on the molecular level. The present study aimed to focus on DNA barcoding of *Telescopium telescopium* (Gastropod) using mitochondrial cytochrome C oxidase subunit I (COI) gene and to correlate it with morphologically identified species found along the coast of Karachi, Pakistan. Our results of BLAST analyses show 95% similarity with the partial sequence of *T. telescopium* reported from Goa and Kerala, India whereas, the genomic distance of mt-COI gene among the *T. telescopium* population is 0% to 45%. *Telescopium telescopium* of Karachi, Pakistan showed 0% evolutionary divergence with the *T. telescopium* of Goa and Kerala, India and maximum distance 45% with *T. telescopium* of Bangladesh. This investigation confirmed the morphological and molecular identification of *T. telescopium* for the first time from Karachi, Pakistan.

Key words: DNA barcoding, mt-COI gene sequence, *Telescopium telescopium*, Molecular phylogeny.

INTRODUCTION

To understand the diversity of any region it is very important to have an accurate identification of taxon (Fontanilla *et al.,* 2014). According to taxonomists, traditional taxonomic characteristics are usually not enough for the accurate identification of species (Packer *et al.,* 2009), especially in the case of morphologically similar species and when samples are collected from juvenile life stages of organisms (Gossner and Hausmann, 2009). Traditional taxonomy in addition to molecular techniques is proved to be an authentic method especially DNA barcoding is the best-adopted method for the identification of species (Hebert *et al.,* 2003a; Hebert *et al.,* 2003b; Ferri *et al.,* 2009). Mitochondrial cytochrome C oxidase subunit, I (COI) serve as standard barcode for most of the animals (Hebert *et al.,* 2003b) in animal Kingdom and it is extensively used as a marker for phylogeographic studies and population genetics (Avise, 1994).

Gastropoda is the most diverse class of phylum Mollusca characterized by a complex variety of species exhibiting taxonomic problems in identification (Layton *et al.,* 2014). Taxonomic confirmation of marine organisms has been done effectively by DNA barcoding. Morphometric characteristics and DNA sequencing help to analyze the phylogeny of molluscs, especially gastropods were studied comprehensively (Barco *et al.,* 2016). DNA barcoding gives significant benefits to different fields i.e. biodiversity conservation (Kress *et al.,* 2015), ecological monitoring (Satheesh kumar *et al.,* 2010) and border biosecurity (Collins *et al.,* 2012). For this purpose, marine gastropods required a library of DNA barcodes for strong molecular identification where they match distinctly specimens to diagnostic barcode sequences (Borges *et al.,* 2016).

The single living species of genus *Telescopium* of subclass caenogastropoda is surface dweller found in mudflats (Lasiak and dye, 1986; Satheesh kumar *et al.,* 2012; Willian, 2013) and mangroves (Shamugan and Raja Gopal, 2006) and feeds on detritus (Alexander *et al.,* 1979; Lasiak and Dye, 1986; Das *et al.,*1988; Houbrick,1991; Rakshit *et al.,*1997 and Meziane and Tsuchiya, 2002). It is abundantly found in the Indian Ocean, West and Central Indo-Pacific regions. The advanced research will help to understand the evolutionary relationship of this species with the other members of their family Potamididae and ecological association with their mangrove's ecosystem (Reid *et al.,* 2008; Yap *et al.,* 2009). Molecular taxonomy is the well-organized method to find the phylogenetic relationships and identification of organisms at species-level through the DNA sequencing of mt-COI gene (Feng *et al.,* 2011).

The present study investigated the molecular phylogeny of *T. telescopium* using mitochondrial cytochrome C oxidase subunit I (COI) gene and a phylogenetic tree is constructed which helps to determine the evolutionary divergence and relationship among the same species. This is the first report on DNA barcoding of a gastropod *T. telescopium* from Pakistan using COI gene.

MATERIALS AND METHODS

Study site: The Sandspit is located in the southwest part of Karachi, at 24° 50′ N and 66° 56′ E between Hawks

Bay and Manora Channel. Manora channel connects Sandspit backwater to the Arabian Sea. During high and low tides, the seawater enters the Sandspit backwaters through Manora Channel frequently and drain back to Arabian Sea periodically (Sultana and Mustaquim, 2003). The area of Sandspit backwaters is covered with mangrove forest which is significantly important for the richness of biodiversity. The Lyari River is a source of the domestic and industrial waste but in rainy season (July to September) it brings freshwater into backwaters. *T. telescopium* is the dominating species of the mangrove area (Figure 1).

Figure 1. Map showing sampling site. 1.

Collection of samples: *T. telescopium* were randomly collected by hand, bought to the laboratory and kept in a glass aquarium filled with seawater brought from same habitat for further study. Morphometric measurements were taken after species identification described by (Bosch *et al*., 1995). Shell was cracked open and its soft tissues were kept at -20° C for further analyses.

DNA isolation: Total genomic DNA was extracted from muscle tissue of *T. telescopium* using Bio basic genomic DNA kit.

PCR Amplification and Gel electrophoresis: The polymerase chain reaction (PCR) amplification was performed in applied Biosystem 2720 thermal cycler, a pair of primers LCO1490: 5'-GGTCAACAAATCATAAAGATATTGG-3' and LCOR2198: 5'-TAAACTTCAGGGTGACCAAAAAATCA-3' (Folmer *et al.,* 1994) were used for the amplification of COI gene. The PCR conditions were as: denaturation at 94 °C for 5 min; 35 cycles of 94 °C for 30 s, annealing at 42 °C for 30 s, and extension at 72 °C for 30 s; and a final extension at 72 °C for 7 min. Gel electrophoresis was run using 1.2 % agarose gel with ethidium bromide (stain).

Sequencing and construction of phylogenetic tree: After successful amplification it was subsequently sequenced with the Sanger sequencing method. Softwares, including MEGA 6, and DNA Star were used for the editing and alignment of sequences. After necessary deletion and insertion, it was submitted to National Center for Biotechnology Information (NCBI) and accession number obtained (MT034052). Twelve partial sequences of *T. telescopium* from neighbor countries of the same continent (Asia) including India (JN190053.1, JN190058.1, JN190056.1, KT336817.1, AM932795.1, JX390723.1), Bangladesh (MF693397.1), Philippines (HE680642.1, HE680643.1), Thailand (HE680645.1) and from continent of Australia (HE680631.1, HE680633.1) were downloaded from gene bank in order to construct a neighbor joining phylogenetic tree using MEGA 6.

Evolutionary history of phylogenetic tree was computed and constructed by Neighbor-joining method on Kimura 2-parameter model (Kimura 1980) (Figure 3).

RESULTS AND DISCUSSION

Systematics

Class: Gastropoda
Subclass: Caenogastropoda
Super family: Cerithoidea (Fleming, 1822)
Family: Potamididae (Adams & Adams, 1854)

Genus: *Telescopium*
Species: *T. telescopium* (Linnaeus, 1758)
(WoRMS:
http://www.marinespecies.org/aphia.php?p=taxdetails&id=215140)

Morphological Characteristics: Shell 80 to 130 mm in length, black at first look but reddish-brown, thick, hard, moderately lustrous extended-conical, with 16 whorls, sutures poorly defined, even spiral ribs, few spiral ribs on flat base. Short siphonal canal and long snout (Figure 2).

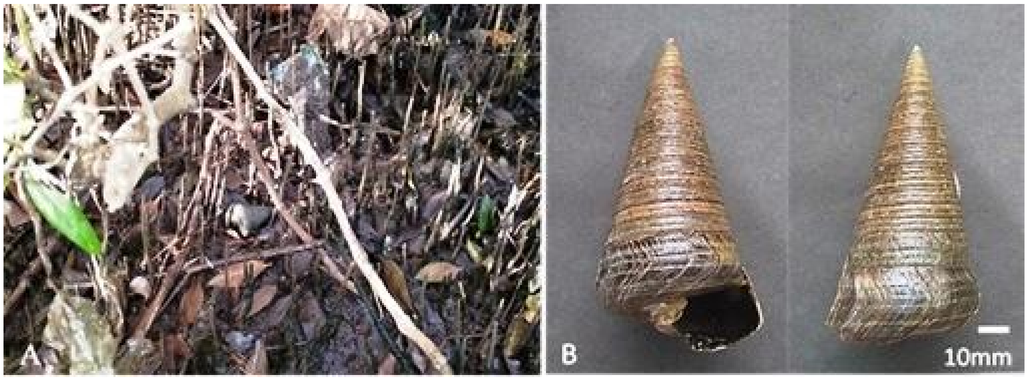

Figure 2. Photographs showing horn snail, *Telescopium telescopium*. A) mangrove area of Sandspit; B) Dorsal and ventral view of *T. telescopium* from Sandspit.

Distribution: *Telescopium telescopium* is found in muddy areas of the Mangrove of Indo-pacific regions. Literature shows their distribution in India, Bangladesh, Philippines, Thailand, Australia, Malaysia, Indonesia , Japan, Singapore, and Madagascar (Satheesh kumar *et al*., 2020).

The present study is aimed to identify horn snail, *T. telescopium* morphologically as well as phylogenetically using COI gene sequence.

Phylogenetic analysis: Phylogenetic tree of COI gene was constructed with the twelve similar sequences obtained from the NCBI and it confirms the morphological identification of *T. telescopium.* Previous study identified *T. telescopium* at molecular level using COI gene from Australia, Thailand, Philippines, Bangladesh and different areas of India (Table 1). *T. telescopium* reported from Goa and Kerala, India having accession no: AM932795.1 and JX390723.1 showed 95% similarity, while the sequence from the Bay of Bengal, India showed 85% similarity. However, the sequence from Philippines (HE680642.1, HE680643.1), and Thailand (HE680645.1) showed 84 % similarity while the sequence from Bay of Bengal, India having accession # JN190056.1 showed 83% similarity (Figure 3).

The neighbor joining phylogenetic tree revealed that *T. telescopium* from Karachi (MT034052.1) clustered with the individuals of Goa and Kerala (AM932795.1, JX390723.1), which is reasonable understanding, only individual of Bangladesh (MF693397.1) denoted a separated clade, it may because of several complex reasons, such as pattern of current or any other physical force handicap the gene flow (Figure 3). Furthermore, it has been confirmed that among *T. telescopium,* there is intra-species diversification.

The geographical locations play an important role in evolutionary divergence, it might increase with increase in their geographic distance (Deza and Deza, 2013). The divergence between *T. telescopium* from Karachi, Sindh and Bangladesh is 0.45 (45 %) which lies at the peak of Bay of Bengal. Australia showed 0.16 (16%) divergence and the samples from Philippines, Thailand and India indicated evolutionary divergence of 13% to 14 %. Various features, i.e. geographic barrier, geographic distance and larval behavior were reported to be responsible to bound gene flow among the different populations (Schmidt and Rand, 1999; Riginos and Nachman, 2001). Physical, climatic landscapes and biotic geography could be one of the reasons of limited gene flow even in the same continent, particularly those physical barriers produced by spatial, temporal patterns and bathymetry of oceanographic currents (Galarza *et al*., 2009). For example, consistent counter flow patterns and limitations among divergent water masses (Werner *et al*., 2007) could be a factor of great importance. Numerous other aspects may be significant, including partial dispersal ability (Waples, 1987; Duffy, 1993; Hunt, 1993; Doherty *et al*., 1995), limited adaptations (Koehn *et al*., 1980; Powers *et al.,* 1991; Schmidt and Rand, 1999), ocean currents (Shulman and Bermingham, 1995; Benzie and Williams, 1997; Palumbi *et al*., 1997; Rocha-Olivares & Vetter, 1999; Stepien, 1999; Snidvong and Sojisuporn, 1999), and isolation through distance (Hellberg, 1996; Lavery *et al.,* 1996; Palumbi *et al*., 1997). Moreover, some Indo-Pacific taxa show increased divergence among Indian and Pacific ocean populations (Lavery *et al*., 1995, 1996; McMillan and Palumbi, 1995; Chenoweth *et al*., 1998b; Duda and Palumbi, 1999).

Further sampling from Indian Ocean has the potential to reveal diversity of species and biogeographic influence on them globally and to reconfirm our results.

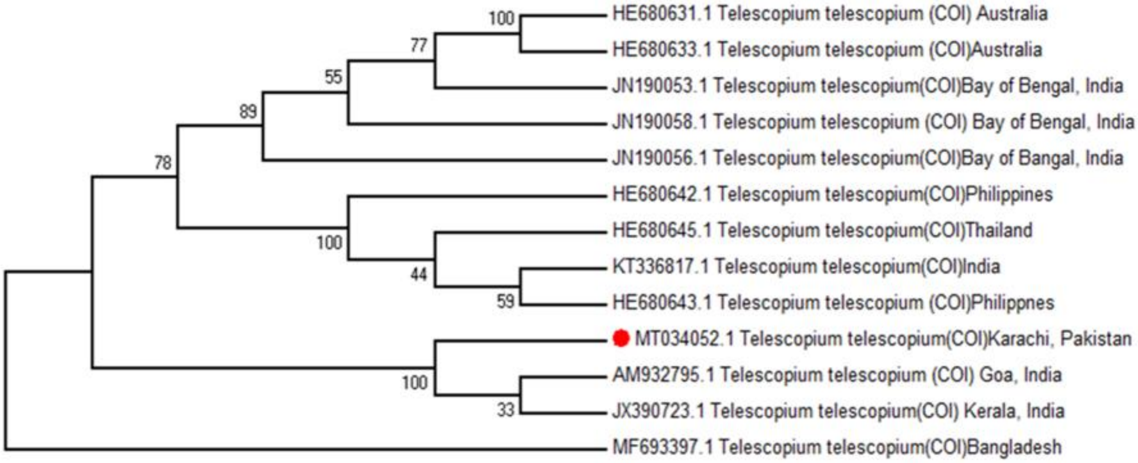

Figure 3. Neighbour- joining tree denoting the *T. telescopium* species from Karachi clustered with Goa and Kerala individuals.

Table 1. List of accession numbers used for phylogenetic analysis in study.

S. no	Taxa	Country	Accession number
1	*T. Telescopium*	Australia	HE680631.1
2		Australia	HE680633.1
3		Bay of Bengal, India	JN190053.1
4		Bay of Bengal, India	JN190058.1
5		Bay of Bengal, India	JN190056.1
6		India	KT336817.1
7		Philippines	HE680642.1
8		Philippines	HE680643.1
9		Thailand	HE680645.1
10		Karachi, Pakistan	MT034052.1
11		Bangladesh	MF693397.1
12		Goa, India	AM932795.1
13		Kerala, India	JX390723.1

Table 2. Evolutionary divergence of different countries between sequences of *T. telescopium*.

S.no		1	2	3	4	5	6	7	8	9	10	11	12	13
1	India	-												
2	Karachi	0.13												
3	Philippines	0.01	0.13											
4	Philippines	0.01	0.14	0.01										
5	Goa, India	0.13	0.00	0.13	0.14									
6	Thailand	0.01	0.13	0.00	0.01	0.13								
7	Australia	0.12	0.16	0.11	0.12	0.16	0.11							
8	Australia	0.12	0.16	0.11	0.12	0.16	0.11	0.00						
9	Bangladesh	0.43	0.45	0.44	0.44	0.45	0.43	0.42	0.42					
10	India	0.09	0.14	0.08	0.09	0.14	0.08	0.06	0.06	0.46				
11	India	0.09	0.13	0.08	0.09	0.13	0.08	0.05	0.05	0.45	0.06			
12	India	0.09	0.14	0.09	0.10	0.14	0.09	0.03	0.03	0.43	0.03	0.02		
13	Kerala, India	0.13	0.00	0.13	0.14	0.00	0.13	0.16	0.16	0.45	0.14	0.13	0.14	-

Conclusion: DNA barcoding, cytochrome oxidase I (COI) gene is proved to be a beneficial tool for the authentic identification of organisms at species level. In present study member of Family Potamididae, *T. telescopium* is analyzed morphologically as well as taxonomically and the barcoding results are assisting morphological results. These results indicate the relation between geographical locations and evolutionary divergence found between sequences of same species. The genetic distance among sequences increases with regards to increase in their geographic distance. Gene flow limitation occurs even at the same continent due to physical barriers (Ngeve *et al.,* 2016) and geographical barriers (Riginos and Nachman 2001). These factors can differentiate individuals of same species into clades. Biogeographical partitions between lineages on the Indian Ocean waters are have been reported (Satheeshkumar *et al.*, 2020). In future, worldwide genetic diversity studies are needed to be performed.

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