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Deciphering the Taxonomy, Phylogeny and Distribution of the Marine Polychaete *Eulalia viridis* (Linnaeus 1767) from Saurashtra Coast, Gujarat, India

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ABSTRACT

Phyllodocidae is one of the diverse families of class Polychaeta. The worms of this family generally live in intertidal habitats such as algal beds, rock crevices, mudflats also in the deep sea and some of the members are pelagic. *Eulalia viridis*, is a Marine Polychaeta and was observed along several coastal regions of Saurashtra. In this paper, a taxonomic and distributional description of *Eulalia viridis* (Linnaeus 1767) is undertaken along with its DNA Barcoding. This approach gave us broad spectra to understand its Morphology and Phylogeny. The study was conducted along different coastal stations such as Okha, Dwarka, Mangrol, Veraval, Sutrapada and Chhara. The samples were collected for Morphological study as well as DNA Barcoding. The COI region was amplified using specific primer for DNA Barcoding of *Eulalia viridis*. The phylogenetic tree was prepared for the confirmation of obtained species. The study revealed the distribution and abundance of *Eulalia viridis* along the Saurashtra coastline. The present study is the first to provide DNA Barcoding of *Eulalia viridis* from India.

Keywords Phyllodocidae, Taxonomy, Morphology, Distribution, DNA Barcoding.

INTRODUCTION

Polychaeta is one of the class of phylum Annelida often stumble across the marine environment, although some species are present in freshwater and rare are known from moist soil on land. Polychaetes are multi-segmented worms with huge morphological diversity, brightly colored, and have sizes ranging from less than 1 mm to several meters in length (Bartolomaeus *et al*. 2005, Garrison 2007, Eklöf 2010). Dales (1962), for instance, even differentiated several Polychaeta families based on structural variations of the proboscis (also called 'trunk' or 'eversible pharynx'), which shows radiated adaptation to multiple feeding

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and sensing strategies (Rodrigo *et al*. 2018). Among all the other families of Polychaeta worms, the family Phyllodocidae Örsted, 1843 is easily distinguishable by the enlarged tentacular cirri and flattened dorsal cirri on the body (Choi *et al*. 2015, Rouse and Pleijel 2001, Eklöf *et al*. 2007). It includes both benthic and pelagic forms, and its members show conspicuously bright color and unique pigment patterns on the body (Choi *et al*. 2015, Blake 1994, Tzetlin 1998). Phyllodocids are long and slender and may reach a length of more than half a meter. They have a pair of palps, which are similar in size and shape to the paired antennae, and there is often also a median antenna or nuchal papilla present (Eklöf 2010). To date, 18 genera of this family have been classified (Choi *et al*. 2015, Pleijel 1991, Eklöf *et al*. 2007).

Phyllodocida is among the most phylogenetically diverse groups of organisms (Nygren 2014, Ravara *et al*. 2017) while the key roles they play in marine ecosystems lead them to be a demanding component for morphology-based biomonitoring (Ravara *et al*. 2017). In recent taxonomic studies, DNA sequences of the mitochondrial cytochrome c oxidase subunit I (COI) and 16S ribosomal DNA (rDNA) regions have been used for DNA Barcoding to facilitate reliable species discrimination among closely related nereidids, in addition to morphological analyses (Park 2021, Park and Kim 2017, Tosuji *et al*. 2019, Sampieri *et al*. 2021, Villalobos-Guerrero *et al*. 2021). Together with the emergence of DNA metabarcoding and eDNA-based approaches for ecological and biological research (Deiner *et al*. 2018), the need to update molecular libraries becomes crucial (Weigand *et al*. 2019) not only for already known species but also for the remarkable hidden diversity that is being continuously revealed with the support of molecular data (Nygren and Pleijel 2011, Delic *et al*. 2017, Fiser *et al*. 2018).

MATERIALS AND METHODS

The specimens were observed in the month of October 2021, December 2021, January to April 2022 during regular field visits along several coastal zones of Saurashtra, Gujarat (Fig. 1) such as Okha (22°28’12.76”N,69°4’9.23”E), Dwarka (22°14’37.13”N, 68°58’6.61”E), Mangrol (21°06′21.92″N,70°06′21.92″E), Veraval (20°54′35.59″N,70°21′0.70″E), Sutrapa-

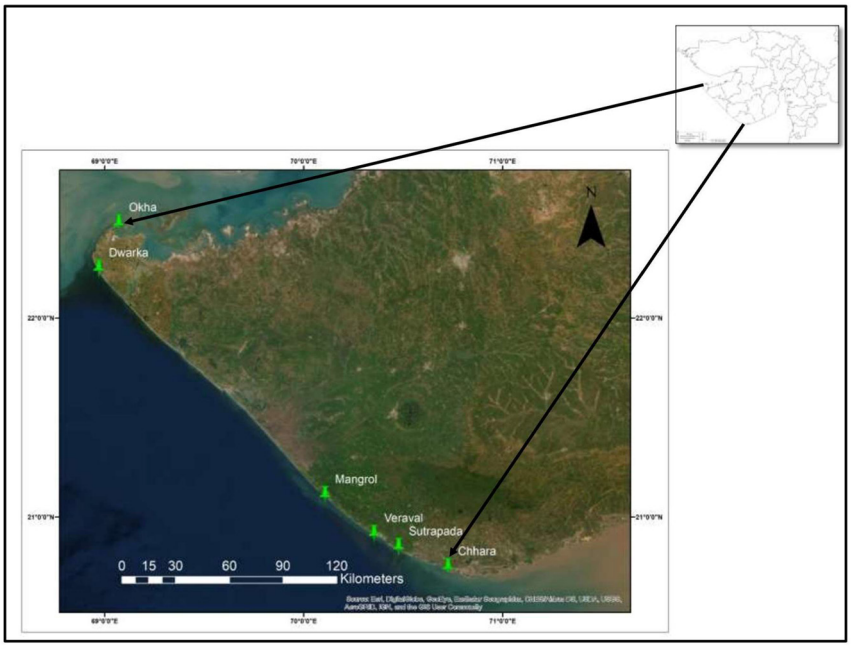

Fig. 1. Study sites along Saurashtra, Gujarat, India.

da (20°50'35.11"N,70°28'33.31"E), Chhara (20°44'34.55"N, 70°43'22.36"E). The specimen was examined in the natural habitat to note the characters like color, length. The voucher specimen was preserved in 70% alcohol and deposited in the Marine and Freshwater Biology Laboratory, Department of Zoology, The Maharaja Sayajirao University of Baroda, Vadodara. The specimen was observed under the stereomicroscope, a permanent slide was prepared to study parapodia, anterior part of the body, segmentation, posterior part of the body and photographs were captured. The morphological characters of the specimen were noted and identification was carried out. The identification was done based on morphological characters, using Marine Species Identification Portal, photographic evidence. The taxonomy was adopted from the World Register of Marine Species (WoRMS) as on 5-2-2022.

DNA isolation and sequencing

Genomic DNA was extracted using the tissue of the animal by the standard available QIAGEN DNeasy Blood and Tissue Kit. The first 658 bp of COI gene in the mitochondrial genome was amplified using Universal Primers in a Thermal cycler (Table 1).

Sequence analysis

Sequence analysis was done using sequencing analysis software version 5.4 (Applied Biosystems) and BioEdit, biological sequence alignment editor (Ibis Biosciences). Consensus sequences were generated after aligning gene sequences from forward and reverse primers. These sequences were subjected to Sequence match analysis using NCBI's Basic Local Alignment Search Tool (BLAST). Consensus sequences that showed a significant match with the earlier identified data on NCBI were submitted to BOLD SYSTEMS according to the guidelines provided on the BOLD website (http://www.boldsystems.org).

Table 1. List of universal primers used for sequencing.

Sl. No.	Primer	Sequence
1	Forward LCO 1490	GGTCAACAAATCATAAAGATATTGG
2	Reverse HCO 2198	TAAACTTCAGGGTGACCAAAAAATCA

RESULTS AND DISCUSSION

A) Systematics

Phylum: Annelida
Class: Polychaeta
Subclass: Errantia
Order: Phyllodocida
Suborder: Phyllodociformia
Family: Phyllodocidae Örsted 1843
Subfamily: Eteoninae Bergström 1914
Genus: *Eulalia Savigny* 1822
Species: *Eulalia viridis* (Linnaeus 1767).

B) Synonyms of *Eulalia viridis* (Linnaeus 1767)

Eracia virens (Ehlers 1864)
Eulalia microceros (Claparede 1868)
Eulalia annulata (Verrill 1873)
Eulalia brevisetis (Saint-Joseph 1899)
Eulalia virens (Ehlers 1864)
Eumidia vivida (Verrill 1873)
Nereis viridis (Linnaeus 1767)
Phyllodoce gervillei (Audouin and Milne Edwards 1833)
*Adopted from WoRMS (as of 5-02-2022).

C) Material examined: In the mentioned study sites along the Saurashtra coast, a total of 46 individuals of *Eulalia viridis* were observed (Fig. 1).

D) Habitat: The specimen was found in the different habitats along all the sites; It was observed on *Palythoa tuberculosa* (Zoanthid), on the algal bed covering of rocky shore, rock crevices, inside the tidepools and into the holes (Fig. 2).

E) Morphological features: Dark greenish. Body stout, anteriorly and posteriorly tapered, about 75 mm long (Fig. 2). Prostomium rounded triangular, about as long as wide. Proboscis widest distally, covered with diffusely distributed rounded papillae. Eversible pharynx present (Fig. 3). Two pairs of palps, four pairs of long tentacular cirri (Fig. 3). Ventral tentacular cirri reaching about segments 3-4, often thick and slightly flattened. Parapodia unimarous (Fig. 3) with neuropodia, notopodia and a varying number

Fig. 2. *Eulalia viridis.*

of chaetae (Fig. 4). Ventral cirri oval, slightly longer than parapodial lobes. Pygidial cirri are three or four times as long as wide.

F) Phylogenetic analysis: The evolutionary history was inferred using the Neighbor-Joining method (Saitou and Nei 1987). The bootstrap consensus tree inferred from 1000 replicates (Felsenstein 1985) is taken to represent the evolutionary history of the taxa analyzed (Felsenstein 1985). Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches (Felsenstein 1985). The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura *et al*. 2004) and are in the units of the number of base substitutions per site. This analysis involved 9 nucleotide sequences. All ambiguous positions were removed for each se-

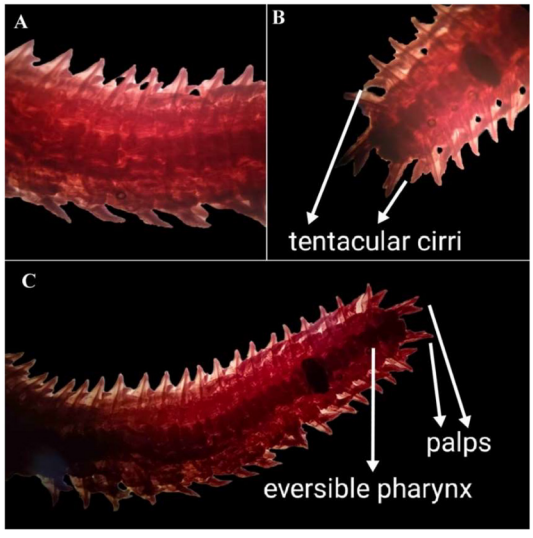

Fig. 3. Morphological features of *Eulalia viridis*, A) Series of parapodia B) Anterior end, dorsal view C) Anterior end, permanent slide.

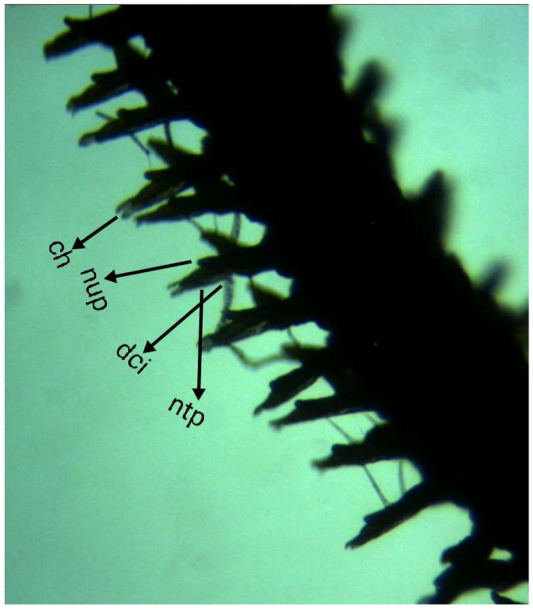

Fig. 4. Parapodia (dorsal view), Abbreviations: ch – chaetae, nup – nuropodium, dci – dorsal cirri, ntp - notopodium.

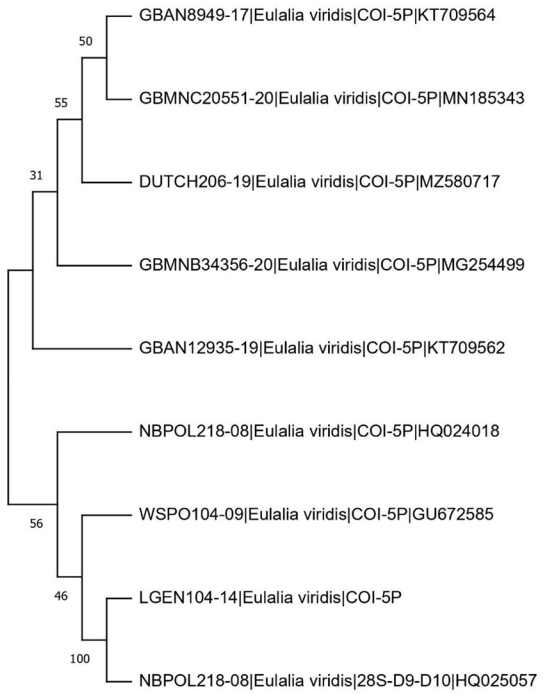

Fig. 5. Neighbor-joining tree constructed using partial sequences of COI. Numbers on each clade indicate the bootstrap values with 1,000 replications.

quence pair (pairwise deletion option). Evolutionary analyses were conducted in MEGA11 (Tamura *et al*. 2021) The summarized form of the Neighbor-joining tree of cytochrome oxidase I gene sequences of *Eulalia viridis* is shown (Fig. 5).

CONCLUSION

The Morphological study carried out in the present study was preliminary for identification purposes. The species was observed in different habitats along all the selected study sites, and was found relatively more abundant in all the selected areas. It suggests that *Eulalia viridis* is distributed in different habitats based on several convenient factors along the entire Saurashtra coastline. The Phylogenetic tree suggests interspecific variations based on the COI gene sequences of *Eulalia viridis* collected from different countries. The tree was constructed using the sequence of the present study and others available on BOLD. The Phylogenetic tree suggested that LGEN104-14 *Eulalia viridis* COI-5P, collected from Dwarka, Gujarat (present study), is 100% similar to NBPOL218-08 *Eulalia viridis* 28S-D9-D10|HQ025057 which is collected in Canada, New Brunswick, St. Andrews and showed no interspecific variation. The present study is the first to provide DNA Barcoding of *Eulalia viridis* from India. Considering the abundance of this species, the future aspects would be to study its population structure and dynamics.

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