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Research Article

CONVENTIONAL AND MOLECULAR TAXONOMY OF POLYCHAETE (GONIADA EMERITA) OF VELLAR ESTUARY, SOUTHEAST COAST OF INDIA

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ABSTRACT

In this present study, identification of polychaete species *Goniada emerita* both from morphological as well as molecular level, without taxonomist we cannot able to naming the species. Therefore, conventional and molecular method (DNA barcoding) are moving parallel to each other that's why we attain the targets like biodiversity conservation, palaeo-biology and evolution, census of life of earth, monitoring health and remediation of the environment and so on. With respect to genetic distance, with maximum it ranged between *Goniada emerita* and *Murex trapa* were 1.236 and minimum between *Goniada emerita* and *Goniada emerita* were 0.015.

Keywords: *Goniada emerita,* DNA barcoding, Taxonomist, Biodiversity, Evolution

INTRODUCTION

Taxonomy helps to know the diversity of living organisms and a better knowledge in taxonomy, leads to better understanding and utilization of bioresources globally. Biological diversity is at present receiving increasing attention. Proper identification of organisms is necessary to monitor biodiversity at any levels (Vecchione and Collette, 1996). Furthermore if decisions are to be made with regard to preserving species, then relationship among species must be known to determine the evolutionary uniqueness of the species. The ability to accurately identify species is fundamental to ecological research and environmental monitoring. Taxonomic identification of polychaete species may have been hampered by their morphological similarity to their fully marine counterparts (Bilton *et al*., 2002).

Geracitano *et al*. (2004) proved in histological studies showed that acute exposures to copper may cause changes in morphological and histological anomalies in *Laonereis acuta* (Polychaeta: Nereididae). This could cause problems in identification, even when the morphological identifications can be difficult already. Further, while collecting the benthic sample using Peterson grab or van Veen grab, some of the worms get broken into a number of species. During sieving and sorting of species, as well as the morphological characters lost or broken; since it will create some difficulties during identification. Besides, the possibility of cryptic taxa, which has been found in some species, may also be problematic. Studies using morphological structures to assign taxonomic identifications can be tedious and can lead to misidentify in cryptic species, and morphology of most species is described in detail only in the adult stages.

The morphological identification is heavily rely on morphological characters such as coloration patterns, the structure of wings (Yu *et al*., 1992), legs, head and mouth-parts arrangement, and genetalia (Jocque, 2002). Differentiation in these structures can be ambiguous and it can be hard to distinguish between species. Many cryptic species delineation depends on very specific complex structural components whose identification requires close and time-consuming viewing of structure under the microscope. Therefore, accurate species identification is very much essential as part of biological diversity and ecological health assessment (Hebert *et al*., 2003). In view of that, it is well understood that the conventional taxonomic identifications are orienting towards molecular techniques in the area of taxonomy.

DNA barcoding has the potential to be a practical method for identification of the estimated 10 million species of eukaryotic life on earth. As a uniform method for species identification DNA genetic barcode can be of significant help for taxonomical, ecological and biological studies mainly for specific identification in research on biological communities and biodiversity in general (Valentini *et al*., 2009). It can reliably assign unknown specimens to known species, also flagging potential cryptic species and genetically distant populations. Barcode will broaden scientific applications and greater utility in conservation biology, including biodiversity surveys.

DNA barcoding advocates the adoption of a "global standard", and a 650-base pair of the mitochondrial gene Cytochrome c Oxidase I (mtCOI) has gained designation as the barcode region for animals (Hebert *et al*., 2003). This fragment size has been selected so that a reliable sequence read can be obtained by a single sequence pass in conventional cycle-sequencing platforms. Shorter fragments of COI have also been shown to be effective for the identification of specimens with degraded DNA, however, where a 650-base sequence is not easily obtainable (Hajibabaei *et al*., 2006). In addition, the usability and robustness of COI in a standard high-throughput barcoding analysis have been extensively assessed (Hajibabaei *et al*., 2005). When compared to the nuclear genome, the mitochondrial genome lacks introns, has had restricted exposure to recombination, and has a haploid mode of inheritance (Saccone *et al*., 1999). Thus, the present study benthic polychaetes were collected from Vellar estuary and identified through both morphology and molecular point of view.

MATERIALS AND METHODS

Description of study area:

The river Vellar originates from Servaryana hills of Salem district and it flows for a total distance of 480 km; it forms the estuarine system at Parangipettai (formerly known as Portonovo) before its confluences with the Bay of Bengal (Fig.1). Its confluences into the Bay of Bengal near Parangipettai on the east coast of India. Vellar estuary considers as a "True estuary" it receives a large quantity of fresh water during monsoon season (October-December). The tides in the Vellar estuary are semi-diurnal in nature with maximum tidal amplitude of about 1 meter. It fall on the GPS coordination (Lat: $11^0$29'998"N and Long: 79^0 46'588"E).

Figure 1: Map showing the study area

Benthic sample collection:

The benthic samples were collected using Peterson grab which covering an area of 0.1m^2. The sample was washed over sieves with meshes of 0.5mm, and the polychaetes species with 90% ethanol.

For morphological identification:

The morphological identification is heavily rely on morphological characters such as coloration patterns, the structure of wings (Yu *et al*., 1992), legs, head and mouth-parts arrangement, and genetalia (Jocque, 2002). Differentiation in these structures can be ambiguous and it can be hard to distinguish between species. Many cryptic species delineation depends on very specific complex structural components whose identification requires close and time-consuming viewing of structure under the microscope. For example, Neredidae one of the most cryptic family and their structures such as parapodial and chaetal organization (Bakken, 2002; Sato and Nakashima, 2003), and the number and arrangement of paragnaths on the eversible pharynx (Fiege and Damme, 2002; Breton *et al*., 2003; Bakken and Wilson, 2005) is used for conformation. Some of the structures used in identification are very small and fragile, such as the parapodia in many species of polychaetes. Therefore, accurate species identification is very much essential as part of biological diversity and ecological health assessment (Hebert *et al*., 2003). The sorted specimens were

counted under stereomicroscope and identified to the lowest possible taxonomic level with the consultation of available literature (Fauvel, 1953; Day, 1967; http://www.marinespecies.org/polychaeta/).

For molecular Identification:

For molecular identification DNA barcoding were adopted and the methodology are given below:

DNA extraction:

A small piece of tissue from individual was placed in 1.5mL eppendorf tube and 500µL of solution I (0.1M NaCl, 10mM Tris-HCl, pH 8.0 and 1mM EDTA pH 8.0 and 2% SDS) was added. The tissue was homogenised using a sterile homogenizer following which 5 µL of proteinase K (1mg/mL) was added and vortexed for 5 minutes. The sample was incubated at 55°C in a water bath for 2 hours. After incubation, the samples were chilled over ice for 10 min and solution II (6m NaCl) was added and mixed by inverting the tubes several times. The tube was chilled on ice for 5 min and centrifuged at 8000rpm for 15 min. About 500µL of the supernatant was carefully collected into a new tube and two volume of (i.e., 1mL) 100% ethanol was added in order to precipitate the DNA. After precipitation, the tube was centrifuged at 8000rpm for 5 min and the supernatant was removed without touching the pellet. The DNA pellet was washed with 500µL of ice cold ethanol and centrifuged at 11,000 rpm for 5 min. The supernatant was carefully removed and the excess liquid was decanted. The pellet was air dried and re-suspended with 50µL of MilliQ water.

PCR amplification:

Primer pairs of Folmer *et al*. (1994) were used for amplification. It is been found that not a single pair of primer was effective in amplifying all the specimens collected and specific primers used in amplifying specific species is listed in Table.1.

Primers were synthesised in a commercial company Bioserve biotechnologies, Pvt. Ltd. (India). Polymerase chain reaction (PCR) was conducted with final concentrations of 1µM primers, 3mM MgCl2, 0.4mM each dNTP and 2.5 units of Taq polymerase (Merck, India). The thermocycle profile for amplifying barcode region consisted of warm up at 94˚C for 1 minute, 5 cycles at 94°C for 30 sec, annealing at 45 – 50°C for 40 sec, and extension at 72°C for 1 minute, followed by 30-35 cycles of 94°C for 30 sec, 51 – 54°C for 40 sec, and final extension at 72˚C for 10 minutes.

Specimen ID	**Species name and Accession number**	**Best primer pairs**	**Primer sequences**
SIBER	*Goniada emerita* and KM094190	LCO1490/ HCO2198	5'-GGTCAACAAATCATAAAGATATTGG / 5'-TAAACTTCAGGGTGACCAAAAAATCA-3'

Table 1: Folmer's primers used for amplifying COI sequences of polychaetes

PCR amplicons of COI gene was gel checked using 1.2% China agarose prepared in 1X TAE buffer. About 3µl Ethidium Bromide was used as staining dye and 5µl of Bromothymol blue was used as tracking dye. About 100V DC was maintained between the electrodes of gel apparatus for 20 minutes. Following electrophoresis the gel was analyzed and pictured in gel doc system (Bioserve Biotechnologies, Pvt.Ltd.).

DNA sequence analysis and BLAST:

COI gene sequences produced in the study were subjected to BLAST analysis through BLASTN 2.2.26 (Zhang *et al*., 2000). The sequence chromatograms are read manually and double checked for miscall and base spacing using Chromas Pro (Ver.1.5) (www.technelysium.com.au/ChromasPro.html).

The DNA sequences were aligned using ClustalX (ver.2.0), (Larkin *et al*., 2007). The phylogenetic trees are constructed using MEGA 5.2 (Molecular Evolutionary Genetic Analysis) software. MEGA is an integrated tool for conducting automatic and manual sequence alignment, inferring phylogenetic trees, mining the web base data bases, estimating the rates of molecular evolution, and testing evolutionary hypothesis.

RESULTS

Morphological identification:

Systematic position

Kingdom	–	Animalia
Phylum	–	Animalia
Class	–	Polychaeta
Subclass	–	Errantia
Order	–	Phyllodocida
Family	–	Goniadidae
Genus	–	Goniada
Species	–	Emerita

The specimens *Gonaida emerita* grow upto the maximum length 360mm (Fig. 2). Body divided in to two regions; the anterior one with uniramous feet and the posterior one with biramous feet. Prostomium has long cone with about 8-10 annulations and four short antennae but no eyes. Proboscis covered with small rounded papillae with a curved flange around a central pore and groove; chevrons on either side of the base. The anterior region consists of 60-70 uniramous segments and the feet each with tapered dorsal cirrus rather flattened at the base, a setigerous lobe with two digitiform presetal lips and a triangular postsetal lobe. The last few feet before the posterior region are transitional but lack notosetae.

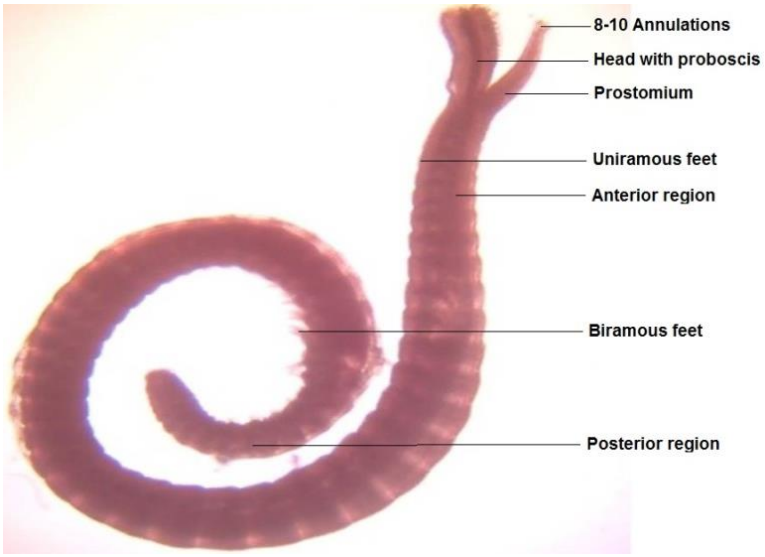

Figure 2: Light microscopic view of polychaete species *Gonaida emerita*

Molecular identification:

The reference sequences showed a similarity between 99% and 82%. In the present study, SIBER was showed 99% similarities with the reference specimen EU835659 *Goniada emerita* in the geographical location of Australia. Similarly, 82% similarities showed with the reference stain HM892188 *Pleuroptya* sp. along the Canadian coastal waters (Table 2).

Specimen ID	Species name with accession no.	Reference species name with accession no.	Similarities %	Geographical location
SIBER	*Goniada emerita*	*Goniada emerita* EU835659	99	Australia
		Pleuroptya sp. HM892188	82	Canada
		Murex trapa HM583721	Out groups	China

Table 2: Similarity between the COI sequences produced in the present study and the reference sequences from Genbank database with its geographical identity.

Mitochondrial DNA sequencing analysis:

The phylogenitic analysis of CO1 dataset is presented as a NJ dendogram with bootstrap analysis (Fig. 2). The phylogenetic tree showed two distinct clades, the first clade showed the similarities with reference strain EU835659 *Goniada emerita* in Australian waters followed by the strain *Pleuropty* sp. with Canadian waters. The strain *Murex trapa* was included in the tree in order to show the out groups.

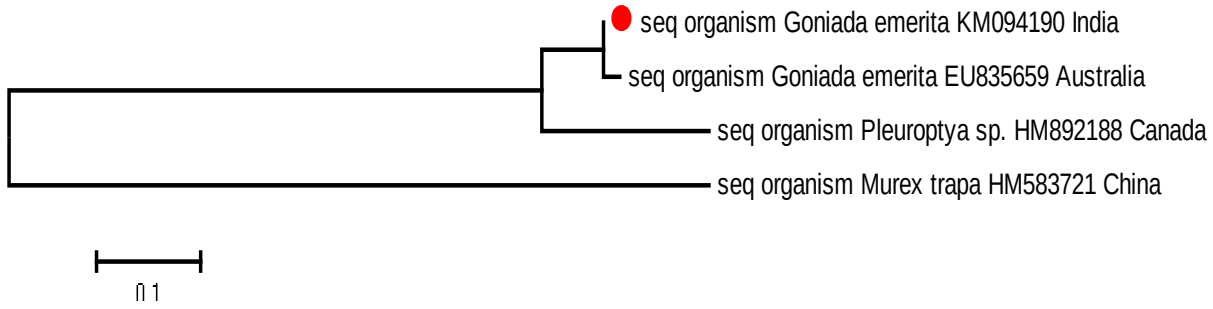

Figure 2: Phylogenetic tree analysis of the COI sequence of *Goniada emerita*

Genetic distance:

Inter-generic pair-wise distance between polychaete genera showed high levels of intraspecific gene flow among the polychaete species. The genetic distance between the genera of polychaets was calculated using pair wise distance analysis via pairwise distance method. The overall genetic distance among the species is 0.720. The maximum genetic distance between *Goniada emerita* and *Murex trapa* were 1.236 and minimum genetic distance between *Goniada emerita* and *Goniada emerita* were 0.015 (Table 3).

	Goniada emerita	***Goniada emerita***	***Pleuroptya* sp.**	***Murex trapa***
Goniada emerita				
Goniada emerita	0.015			
***Pleuroptya* sp.**	0.227	0.227		
Murex trapa	1.236	1.269	1.346	

Table 3: Variations among the COI pair-wise distances within the polychaetes

DISCUSSION

In the marine realm, annelid are one of the most dominant animal phyla exhibiting an amazing variety of morphological forms with each species having specific characters, yet they are less studied compared to other taxa of similar ecological importance and complicated morphological evidence (Fauchald, 1977). Morphological identifications of polychaete species is time consuming and somewhat inaccurate leading to possible misidentifications and morphological traits are complex due to the high levels of homoplasy (Eklof, 2010). Although morphological identification has been performed following the early description in monographs of Day (1967); Fauvel (1930, 1932) and (Fauchald, 1977).

DNA barcoding is a well-accepted taxonomic method which uses a short genetic marker to facilitate identification of a particular species even by non-specialist. DNA barcoding can reliably assign unknown specimens to known species, also flagging potential cryptic species and genetically distant populations (Radulovici *et al*., 2010). The popularity of CO1 DNA 'barcoding' is increasing rapidly, with mass amounts of invertebrates and vertebrates collected in the field inevitably becoming a mass of data to be added. With so much data needing to be processed, 'taxonomic impediment' exists just as much for molecular data as it does for traditional collections (Brower, 2006). Hebert *et al*., (2004) proposed DNA barcoding works under the principle that interspecies variations are greater than the intra-species variations allowing one to distinguish the species using nucleotide sequences. CO1 has been accepted as universal barcode to delineate animal life. Sequence variation in a segment of the mitochondrial cytochrome c oxidase 1 gene was employed to compare morphological versus molecular diversity estimates. The phylogenetic relationships of these polychaete taxa are matter of ongoing debates in recent papers on annelid morphology (Westheide *et al*., 1999). Based on multiple studies Hebert *et al*. (2003a) suggested an approximately 650bp of the CO1 gene which is relatively easy to amplify with standard primers and is sufficient enough to obtain resolution on all levels between species and phylum for majority of the groups albeit with some exceptions (Ward, 2009). CO1 has been proposed as the principle gene for barcoding organisms. Sequence variation in a segment of the mitochondrial cytochrome c oxidase1 gene was employed to compare morphological versus molecular diversity.

Morphological identification of the polychaetes in this study was supported by the molecular data as shown by the congruence and high similarity between the sequences produced in the present study and those available in GenBank. Our strain showed the 2 genera closest matches in the molecular database are most likely the result of the limited taxonomic coverage of CO1 sequences available in GenBank relative to the vast diversity of marine polychaetes. The likely applicability of a CO1 identification system to new animal groups and geographical settings suggests the feasibility of creating an identification system for animals-at-large scale.

Gonaida emerita barcoded in the present study, members of the same species clustered in the same clade with 99% similarity proving the reliability of CO1 gene sequences in identifying polychaetes. *Pleuroptya* sp. HM892188 was found to be another genus with 82% similarity was observed during the blast due to the lack of close sequences in the database it was transparently placed outside of the cluster. This indicates the necessity to expand the polychaete barcodes data in the Genbank. Maturana *et al*. (2011) assessed intraspecific and interspecific genetic divergence among marine polychaetes of 13 polychaetes species and identified high levels of interspecific variation among 31 analyzed sequences. Mean pairwise sequence distance comparisons ranged from 0.2 to 0.4% in previous study, but the in the present study interspecific comparisons between 0.015 and 1.236.

CONCLUSION

Morphological identification of polychaetes is fully hampered on morphological key character of polychaetes whereas molecular taxonomy, the identification relay on nucleotide base pairs as well as conventional taxonomist. Hence, the present study concluded that the morphology and molecular taxonomy needs to be holds-up each other. That's why we identified the exact name of polychaete species, and in order to extract the immediate application of monitoring health and protecting endangered and vulnerable species.

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