Population Ecology and Phylogenetic Relationship of Benthic Polychaete species along the West Coast of India

A thesis submitted to Goa University for the award of degree of

Doctor of Philosophy

In

MARINE SCIENCE

By

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Under the guidance of

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October 2018

Declaration

As required under the university ordinance, I hereby state that the present thesis for Ph.D. degree entitled "**Population Ecology and Phylogenetic Relationship of Benthic Polychaete species along the West Coast of India**" is my original contribution and that the thesis and any part of it has not been previously submitted for the award of any degree/diploma of any university or institute. To the best of my knowledge, the present study is the first comprehensive work of its kind from this area.

The literature related to the problem investigated has been cited. Due acknowledgement have been made whenever facilities and suggestions have been availed of.

PERIASAMY

Certificate

Certified that the research work embodied in this thesis entitled "**Population Ecology and Phylogenetic Relationship of Benthic Polychaete species along the West Coast of India**" submitted by Mr. R. Periasamy for the award of Doctor of Philosophy degree in Marine Science at Goa University, Goa, is the original work carried out by the candidate himself under my supervision and guidance.

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Acknowledgements

With heart-felt gratitude, I express my sincere thanks to Dr. Baban Ingole, Head, Biological Oceanography Division, CSIR-National Institute of Oceanography, Goa and my research supervisor for encouraging me to choose this topic. I am very grateful to him for valuable guidance and constant support throughout the study. His scientific experience in various fields of research along with effective suggestions has influenced my research throughout.

I wish to thank Director, CSIR-NIO for giving me an opportunity to work at CSIR-NIO and providing all the necessary facilities.

I owe my deepest gratitude to Dr. Eijiroh NISHI, College of Education and Human Sciences, Yokohama National University, Tokiwadai, Hodogaya, Yokohama, Japan, in taxonomy and extensive help and discussions during my research work.

I am grateful to Mr. Ram M. Meena for his constant support in DNA sequencing.

I am thankful to Dr. C. Mohandass, Principal Scientist, Biological Oceanography Division CSIR-National Institute of Oceanography, Goa for his valuable suggestions and encouragement during my research work.

I acknowledge to the MoES (Govt. of India; COMAPS project No.GAP-2741) for the fellowship during my study period.

I take this opportunity to thank Mr. Ilangovan and Dr. C.Rajasekaran, NITK, Surathkal, for their kind help and support.

My sincere thanks to BOD staffs Drs. J. Ravindran, C. Ravindran, Manikandan, Cathrine Sumathi, Ravail Singh and Dineshram for their help and support.

I am highly thankful to Mr. Areef Sardar, Dr. VK. Banakar and Mr. Sarath for scanning electron microscopy.

I thank for Dr M. Eashwar, CSIR-Central Electrochemical Research Institute, Karaikudi, Tamil Nadu, for discussion and valuable company to keep me cheerful and focused.

I express my gratitude to Dr. P. Laxmilatha, ICAR-CMFRI, Chennai for her support.

I sincerely appreciate the help and support rendered by my friends and well-wishers Viswanathan Karthik, Subha Anand, Muthukumar, Mangalaa, Sathish Kumar, Veerasingam, Kesava Priyan, Ranjith, Sam, Harish, Rashmi, Akhila, Sheba, Naveenan, Dr. Manimurali & Dr. Vidya, VijayRaj, Ravindra Pawar, Devika Joshi.

My sincere thanks to my wife P. Kalpna and all my Benthos lab colleagues, especially Dr. Sanita, Mr. Kalyan, Afreen, Sambhaji, Edward, Amit, Vishal, Jacky, Laxman for their help in field sampling and laboratory analysis.

I have no words to express my deep gratitude to my father, mother and families for moral support during my study.

Above all, I thank the almighty God for providing me this opportunity and granting me the capability to proceed successfully.

PERIASAMY

DEDICATED TO MY BELOVED PARENTS AND GUIDES

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Chapter 1

Introduction

1.1. General introduction of the west coast of India

India has a vast coastline of 8129 km. It is flanked by the Arabian Sea on the west coast and Bay of Bengal on the east coast. Although these two seas are situated at the same latitude, they have contrasting features, resulting in the heterogeneous environment along the Indian coast. The Arabian Sea has been considered as a 'hot spot' for marine speciation, and one of the most biologically productive regions of the world oceans (Ryther *et al*. 1966). The seasonal reversing monsoon results in two seasonal phytoplankton blooms followed by an organic enrichment in the benthic ecosystem (Madupratap *et al*. 1996; Prasanna kumar *et al*. 2010; Ingole *et al*. 2014). The unique feature of surface circulation is seasonally reversing in the North Indian Ocean, which is driven by the Asian monsoon winds. The seasonally reversing currents of Western India Coastal Current (WICC) and Eastern India Coastal Current (EICC) shows stronger and weaker along the Indian coast (Shankar *et al*. 2002). The flow of WICC is towards the equator during the Indian summer monsoon (May–September) and pole-wards during the winter monsoon (November–February). However, the coastal boundary currents showed a spatiotemporal variation (Durand *et al*. 2009, Amol *et al*. 2014, Mukherjee *et al*. 2014). The reversing monsoonal current is favorable for dispersing of planktonic larvae. Recently Sanitha and Ingole (2016) suggested that dispersal of planktonic larvae between the Arabian Sea and Bay of Bengal basins is due to seasonally reversing monsoon current.

The nutrient-rich surface waters are advantageous for major benthic recruitment that occurs during the monsoon season (Alongi 1990, Gaonkar *et al*. 2013; Ingole *et al*. 2014). The planktonic larval and adult benthic stages have a complex life cycle (Thiébaut *et al*.

1998). Consequently, larval dispersal of most benthic organisms happens primarily during the planktonic larval stage. The bottom living biological communities associated with sediment are collectively termed a "**BENTHOS**". They constitute largest faunal assemblage on the Earth (Snelgrove (1998). In addition to food for the bottom feeding higher organisms, benthic community performs a significant role in key ecosystem processes such as carbon and nutrient recycling within the sediment-water interface. Benthic macrofauna with their bioturbation and bio-irrigation activities are also considered as efficient ecosystem engineers inducing large changes in the sediment biogeochemistry. The macrobenthic communities are comprised of different benthic taxa such as polychaetes, crustaceans, molluscs, and echinoderms. Generally, the polychaetes are most dominant in organic-rich sediment areas (Hutchings 1998; Savidas *et al*. 2010). They play an important role in ecosystem functions such as mineralization, nutrient recycling, oxygenation of deeper sediment layer and benthopelagic coupling. Further, the alteration of sediment by these burrowing organisms would directly affect micro-niches as well as oxic-anoxic interphases (Kristensen and Kostka 2005).

1.2. General introduction of polychaete

The Polychaeta (having many chaetae) named as bristle worms, is a distinct group of the marine annelid. It is a very diverse group of animals living in marine environments (Stabili *et al*. 2013). The unique characters of polychaetes have parapodia-bearing chitinous chaetae which were first separated from clitellate annelids (Grube 1850). Polychaete diverses group from other annelids having a well-distinguished head with specialized sense organs, paired appendages, on most segments, and no clitellum. They

have many setae by their name. Polychaetes display an enormous morphological diversity, where different lifestyles have given rise to many disparate forms, with everything from free-living predators to filter-feeding, tube-builders and interstitial parasites. They occur in diverse marine habitats, including the intertidal, coral reefs, the deep-sea and hydrothermal vents, and are present in both soft and hard substrates.

1.3. Polychaetes

According to the American Heritage® Dictionary of the English language, pol•y•chete spelled as pol•y•chaete is distinct as "any form of the various annelid worms, including the lugworm, characterized by paired appendages tipped with bristles on each body segment". The word Polychaeta is obtained from the Greek word polukhaits, with much hair (polumeans poly and khait stands for long hair).

1.4. Morphology of Polychaetes

Polychaetes have a body with three fundamental regions. The presegmental region gives rise to the head. The bulk of the body is included of serially repeated segments. This form of segmentation is referred to as metamerism. The third region is the extreme posterior end of the body, the pygidium like the anterior end is non-segmental (Fig 1.1). Palps function and feeding structures with both sharing an identical pattern of innervation, are homologous structures (Orrhage 1993). The presence of dorsal and ventral cirri, ventral sensory palps, aciculae, compound chaetae and multiple prostomial antennae are define aciculates (Rouse and Pleijel 2001). The aciculates palps are derived from the prostomium and sensory organ (Rouse and Pleijel 2001). The close relationship between

Phyllodocida and Eunicida with jaws is suggested by evidence of morphological and molecular analysis (Rouse and Fauchald 1997; Zrzavy *et al*. 2009; Struck *et al*. 2011; Weigert *et al*. 2014). Canalipalpates are defined by the presence of grooved, ciliated palps and are located commonly between the peristomium and prostomium or are derived from the prostomium (Rouse and Pleijel 2001), although they have been secondarily lost in several taxa (Orrhage 2001).

1.5. Types

Polychaetes are often divided into two groups based on their activity: sedentary and errant (free-moving) polychaetes. Sedentary polychaetes spend much or all of their life span in tubes or permanent burrows (Fig 1.1). Many of them, especially those that live in tubes, have specialized structures for feeding and respiration. Errant polychaetes include free-swimming pelagic forms, active burrowers, crawlers, and tube worms that only leave their tubes for feeding or breeding (Glasby *et al*. 2000; Rouse and Pleijel 2001).

Fig 1.1. Errant polychaetes (A, Fire worm and B, Blood worm); sedentary polychaetes (C and D, Feather duster worms)

1.6. Reproduction

Most polychaetes show separate sexes. Many have broadcast spawning of gametes, external fertilization and planktotrophic (plankton feeding) larvae. Others have some form of sperm transfer to females from males and varying levels of parental care with yolky (lecithotrophic) eggs. Those polychaetes have been successful in occupying freshwater habitats. Hermaphroditism has possible brooding of larvae in some Namanereidins (Glasby 1999). Larval development in the freshwater serpulid *Marifugia cavatita* (Matjasic and Sket 1966) differs little from most marine serpulids in being a broadcast spawner, though the larvae are lecithotrophic (Fig 1.2). Benthic polychaetes constitute a discrete group of segmented worms, which play a significant role in marine ecology because of their high diversity as well as their high plasticity in reproductive modes and trophic strategies, and most diverse invertebrate groups. Polychaetes show many different reproductive modes (Wilson 1991, Giangrande 1997) and live in unique environments. They are belonging to twenty different trophic levels (Jumars and Fauchald 1979). Polychaetes worldwide make up a large proportion of the total macrofauna in soft bottoms (Hutchison 1998).The species richness of Polychaeta is placed fourth in the ranking of marine invertebrate (Blake 1995, Bouchet 2000).

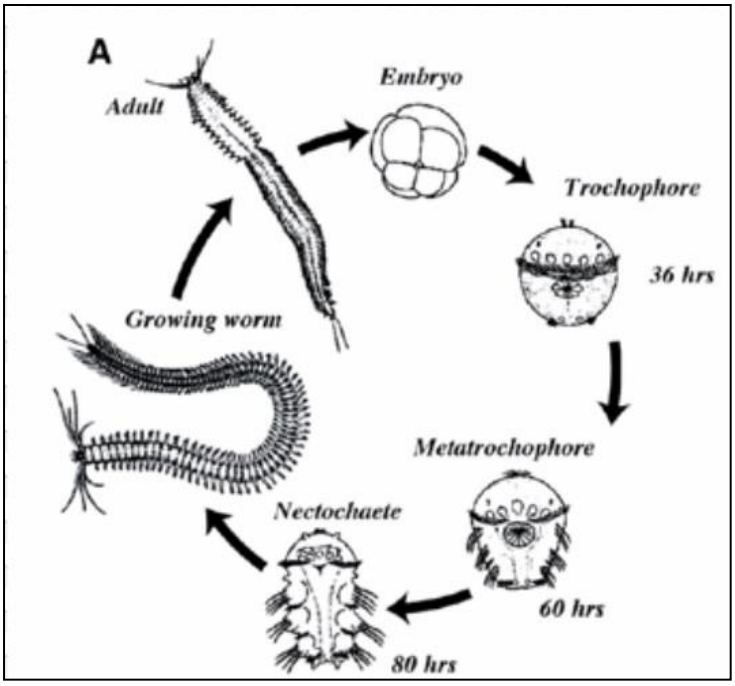

Fig 1.2. Life cycle of marine annelids (source from http://sunny.moorparkcollege.edu/~econnolly/wormsX3.htm).

1.7. Habit and habitat

Polychaetes worms are present in diverse habitats, including coastal, estuarine and rocky shore systems, continental shelf and deep-sea benthos, and some pelagic varieties (Glasby *et al*. 2000). The broad geographical distribution and variation in the environmental tolerance with dispersal ability suggest that there may be cryptic species associated with the name (Kelly *et al*. 2012). Those organisms can survive the harsh environmental conditions on top of rock usually live in colonies, protected inside tubes. Estuaries habitats are complex with varied physical and chemical conditions, causing the existence of many localized micro-environments or niches (Cognetti and Malatagliati 2000). The wide range of environments habitat promotes rapid speciation (Bilton *et al*. 2002) as seen in many estuarine species, including polychaetes, where they cover a range

of morphologically diverse types. Polychaetes have evolved a great diversity of feeding strategies including predation, parasitism, suspension feeding, and can be tube dwelling, epibenthic, burrowing or pelagic in the habit (Rouse and Pleijel 2001).

The polychaetes feeding are mostly raptorial (adapted for seizing prey) feeders. They include members of many families of surface dwelling, pelagic groups, and tubicolous groups. A scavenger or omnivorous habit has evolved in many polychaetes. Some polychaetes are categorized under non-selective deposit and selective feeders (Fig 1.3). Special head structures extend out over the substratum in selective feeders lack a proboscis. Deposit materials adhere to mucous secretions on the surface of feeding structure which is conveyed to the mouth (Srikrishnadhas *et al*. 1998).

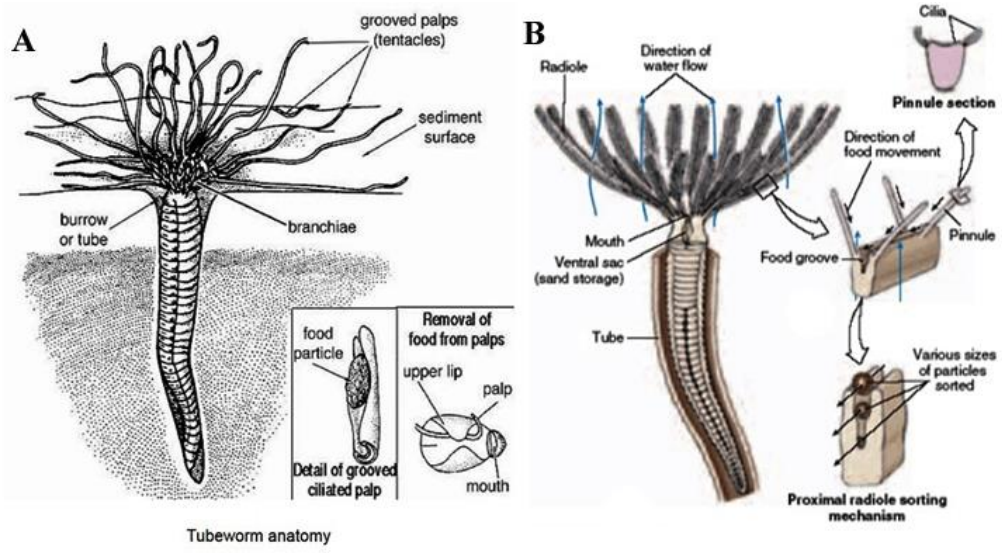

Fig 1.3.A. The Terebellidae on subtidal mudflat and long tentacles spread out over the bottom.**B,** Sabelladae: the extendedciliary feeder crown of feeding radioles from leathery secreted tube. (source from https://projects.ncsu.edu/project/bio402_315/Annelids/annelid.html).

1.8. Ecological role of polychaete species

Polychaetes are the ecologically vital inthe benthic region sincethey play a big role in nutrient cycling (digestion) with many species consuming organic particles through faecal deposition and when they die the nutrients are released back into the water column. Nutrient cycling is facilitated by burrowing polychaetes in soft sediment when they effectively aerates the mud to a depth (Waldbusser and Marinelli 2006). Aeration of the surface sediments through burrowing allows other sediment-dwelling speciesto live in the same area, when they may not otherwise, due to anoxic conditions. During their locomotion and feeding activities on the seafloor, polychaete plays an important role in mixing and reworking of sediment, a process known as bioturbation. The burrowing polychaetes incorporate organic matter and dissolved oxygen into the deeper sediment layer. Thereby enhance remineralization process. Many polychaetes also associated with other organisms such as coral, sponges, bivalves and also other invertebrates. Polychaetes are known in the ambulacral grooves of starfishes (Jones 1964).

Polychaetes are highly abundant and diverse in most marine benthic habitats, especially in soft sediments, algal turfs and fouling communities. Polychaete worms are represented about half of all the macrobenthic invertebrates (0.5) based on the sampling of marine environments. Polychaetes show a wide range of feeding strategies, including, carnivores, deposit feeders, suspension feeders, herbivores, and opportunistic species. They play an important role in marine food chains. Some groups such as the Capitellids and Arenicolids are deposit feeders and swallow mud feeders on the algae attached to the particles. Others, such as the Sabellids and Serpulids, are suspension feeders feed on

suspended particles. They also include active predators, scavengers, and grazers of microalgae. Many polychaetes are eaten by other polychaetes and other marine invertebrates including fish and wading birds. The polychates are connected in a variety of feeding behaviours ranging from ingestion of sediment, predation to filter feeding of plankton, hence they occupy several tropical levels of the food chain. Benthic polychaetes are important in the recycling of nutrients, irrigation, and oxygenation of sediment substratum. They sustain the demersal fishery resources of the region by offering trophic support. They are particularly important to environmental biomonitoring and considered to be an indicator of ecosystem health in coastal and marine ecosystems (Johnston and Roberts 2009; Yu *et al*. 2012; Sivadas *et al*. 2010; 2016).

1.9. Evolutionary position

The evolutionary history of polychaetes is very long and diverse and it is characterized as a sternly taxonomy. The first evolutionary relationship for the polychaetes group was suggested by Hatschek (1878). The evolution of polychaete species was considered based on the structure of the pharynx (Dales 1962). (Almeida *et al*. (2003) suggested the Annelida and Polychaeta are non-monophyletic, even when includeing Echiura, Clitellata, and Pogonophora. The phylogenetic revision of the errant polychaetes was led to argue for the paraphyly of the Polychaeta (Almeida and Christoffersen 2000).

1.10. Digitize inventory of Marine Biodiversity

The inventory on marine biodiversity over the wide geographic range is being carried out through international efforts such as Census of Marine Life (CoML), Ocean

Biogeographic Information System (OBIS), World Register of Marine Species (WoRMS) and Marine Barcode of Life (MarBOL). The CSIR-National Institute of Oceanography (NIO) which acted nodal agency in the Indian Ocean region. The bioSearch in NIO is a database purpose to develop the digitized Indian marine biodiversity (Kakodkar *et al*. 2013). These initiatives imply to evaluate the diversity, distribution and abundance of marine life on a global scale and make information available on their taxonomy and distribution to the public through searchable interfaces (Wafar *et al*. 2011). Further, these efforts also aim to enhance global capacity to identify marine organisms using DNA Barcodes. From the species distribution records in the aforementioned databases, preliminary assessments of the global distribution patterns of many taxonomic groups have become possible (Wafar *et al*. 2011).

1.11. Environment indicators

The polychaetes species are abundant with a wide range of size and short life cycle. They are very useful organisms for monitoring the marine environment. They are readily available, easy to sample and easy to maintain particularly responsive to changes in environmental conditions. The ability to monitor the recovery of the disturbed area is possible as different species of polychaetes appear after the end of the impact (Ansari *et al.* 1986; Sivadas *et al*. 2011). Opportunistic polychaetes species are dominat in the reduced oxygen level (hypoxia condition) areas and they can be useful for environmental indicators. Thus, polychaetes play a vital role in biomonitoring of the marine environmental quality and pollution indicators. The primary objective of biomonitoring is to assessthe impact of man-made changes for example, the introduction of toxic

chemicals on the biosphere. The polychaetes are useful in monitoring the marine environmental quality due to their direct contact with the water column. Thus, the sensitivity of polychaete species is expressed by anthropogenic compounds of sediments and the changes in their reproduction, growth, and mortality (Venturini and Tommasi 2004). The assessment of the presence and bioaccumulation of anthropogenic compounds such as polycyclic aromatic hydrocarbons (PAH) and polychlorinated biphenyls (PCB) and metal-organic complexes can be done from the polychaetes accumulated the deleterious materials intheir tissues. Nereidae and Dorvilleidae families are suitable for assessing the toxicity of sediments. Some countries are using polychaetes as pollution indicators include USA, UK, Canada, and Germany.

Nereidae has been reported as economically important polychaetespecies to be used as a food for many fishes (Ingole 2007). Since the polychaetes are used as a source of food for many commercial fishes they are also usedas an important factor in the estimation of fishing grounds, as well theirpresence and absence in sediments can be used as an indicator for thehealth of the benthic environment. Capitellidae and Spionidae families have been accepted widely as pollution indicators. The absence of sensitive species such as *Harmothoe imbricate*, *Maldane sarsi*, *Paramphinome*, *Ceratocephale*, *Harmothoe*, and *Lumbrineris* would be indicative of damaging environmental conditions while their low diversity indicates higher impacted areas (Rygg 1985). Species of Nereidae and Nephytidae are accepted as indicators of early succession phases of environmental recovery after pollution has been decreased (Pearson and Rosenberg 1978).

1.12. Polychaetes invasive

The random introductions of non-indigenous species into foreign habitats are generally the effect of human activity.Nevertheless, the translocation of organisms through ships' ballast water is considered to be important bio-invasion vector and threat to biodiversity.Consequences of such invasions are increasingly being realized in recent years (Ruiz *et al*. 1997, 2000; Carlton 1999; Anil *et al*. 2002; Tavares and DeMelo 2004; Subba Rao 2005; Gaonkar *et al*. 2010).

Some sabellids have been translocated from their native distribution range in ballast water. For example, *Sabella spallanzanii* (Gmelin, 1791) a common species in the Mediterranean Seawhich lives in shallow waters (< 30 m depth) inharbours over dock pilings, rocks, seagrass mats, or sand withhigh growth rate (10 cm/year)have been found in Australian waters competeing for food with native oysters and clams (Giangrande and Petraroli 1994; Giangrande *et al*. 2000).

1.13. Polychaete association

Polychaetes are commonly found close associated with marine sponges because the presence of holes, grooves, chambers, and channels provides a shelter and frequently supply of organic matters as a sources of food in their associates (Cinar and Ergen 1998; Martin and Britayev 1998; Sivadas *et al*. 2014). Polychaetes may inhabit in all sponge available space as they may live to attach to the sponge surface or within canals and choanosome (Peres 1982; Cinar and Ergen 1998). The relationships between polychaetes and sponges can be accidental where the involved organisms may inhabit a variety of

substrate. However, they may also involve a high degree of specificity which rises to either communalistic or parasitic relationships (Martin and Britayev 1998).

1.14. Polychaetes feeding types

The feeding guilds of polychaetes within sponges are defining the nature of the interrelationships between the organisms (Martin and Britayev 1998). The carnivorous species *H. spongicola* can point to its parasitic way of life in all sponge. The location and specific behavior of the species suggest that the sponge species probably provide habitat and enhance food sources to polychaetes. The position of filter feeders in the canals close to the sponge openings probably aids them in obtaining food from the waterflow created by the sponge (Ilan *et al*. 1994). Surface deposit-feeders of polychaete might obtain food from organic detritus accumulated on sponge canals. The importance of sponge has enhancing food supply for the associated fauna (Klitgaard 1998).

1.15. Molecular techniques for polychaete phylogeny

The first molecular studies including annelid taxa usually analyzed 18S rRNA or elongation factor 1α gene sequences (Winnepenninckx *et al*. 1995; Kim *et al*. 1996; McHugh 1997; Kojima 1998; Winnepenninckx *et al*. 1998; Eeckhaut *et al*. 2000; McHugh 2000; Struck *et al*. 2002a; Bleidorn *et al*. 2003a, b; Weigert and Bleidorn 2016). Genetic studies have great potential to explain one of the largest problems in polychaetes studies. Many polychaetes species are difficult to identify at the species level. The polychaetes have been classified into over 80 families in the traditional classification (Fauchald 1977). Typically phylogenetic studies based on molecular sequence data have not improved the higher taxa recognized by morphologists as a result it has been

attributed to short branches that are the consequence offast early diversification (Rousset *et al*. 2007). However, there is a lack of congruence between the earlier studies of molecular data which involved data from a diversity of sources including protein-coding genes and nuclear genes (Rousset *et al*. 2007), mitochondrial genes and gene order (Mwinyi *et al*. 2009; Shen *et al*. 2009); miRNAs (Sperling *et al*. 2009), ESTs (Struck *et al*. 2011) and combined morphological and molecular analyses (Zrzavy *et al*. 2009). There have beenmore molecular data which are available in GenBank and hence being used statistically in Bayesian and maximum likelihood methods for better fitting of models. The tree topology is emerging to the extent and fitting with a morphological scenario (Johnson and Omland 2004).

Recent analyses of molecular data have greatly improved our understanding of phylogeny. However, the phylogenetic relationships of polychaetes group are remaining unclear both among and within taxa. The lack of clearly homologous morphological characters across the evolutionary duration of protozoa to mammals requires, for instance in Indian coast polychaetes relationship among and within many of the major lineages remain unclear. Frequently ribosomal RNA sequences have been used to reconstruct deep branches of evolutionary history. The analyses of 18S rRNA gene sequences have been used to infer the early diversification of within eukaryotes (Embley, Hirt, and Williams 1994; Bhattacharya and Medlin 1995). These molecular techniques have revealed new phylogenetic lineages of benthic polychaetes where by several of which serve as the major component in a given polychaetes community. Although there is enough morphological information available for the polychaete but there has been a little effort to develop this information into comprehensive analyses of phylogenetic relationships of the

west coast of India. Recently, molecular data have made a huge impact on how we understand the evolutionary relationships of polychaetes and changed our view included in the Polychaeta taxon to make it monophyletic (Weigert and Bleidorn 2016).

1.16. Molecular studies of DNA sequencing methods

Investigation of various conserved genes is suitable for assessment of high-level relationships while other genes are evolving at higher rates such as 18S rRNA and 28S rDNA, revealing differences and similarities among related taxa and within species. The universal PCR primers (mitochondrial genes such as mtCOI, CytB and 16S, and Internal Transcriber Spacers (ITS) of the nuclear genome) are highly conserved regions which are used as a DNA barcoding in various polychaetes.

1.16.1. Nuclear gene (18S rRNA gene)

The exploration of diversity in organisms for traditional taxonomy by using molecular tools is very difficult (Tang *et al*. 2012). The 18S rRNA gene is one of the most important molecular markers to study the diverse group applications for molecular phylogenetic analyses and biodiversity screening. The 18S rRNA gene is commonly referred to as the nuclear small ribosomal subunit (SSU) and most frequently used in the phylogenetic studies. This gene is part of a tandem repeated element in the nuclear genome. There are hundreds of copies of the repeat in the genome that are typically homogenized by concerted evolution. The 18S rRNA gene data have been used to address intra-species relationships mainly for historical reasons. The conserved regions of genes have allowed for the development of universal primers for amplification through

polymerase chain reaction (PCR) and variation in nucleotide sequence in different gene regions makes easy to obtain the information at different phylogenetic study (Hillis and Dixon 1991; Abouheif *et al*. 1998; Halanych 2004). The 18S rDNA gene is part of the functional core in the ribosomal and it is exposed to similar selective forces in all living organisms (Moore and Steitz 2002). Therefore, the first large-scale phylogenetic studies of the animal kingdom based on 18S rRNA gene sequences were published and well-known as the prime candidate for reconstructing the metazoan tree of life (Field *et al*. 1988).

1.16.2. Internal transcribed spacers:

Ribosomal DNA (rDNA) is widely used as the phylogenetic marker for taxonomic studies and phylogenetic inferences. The rDNA is composed of three subunits (18S, 5.8S, and 28S) and two internal transcribed spacers (ITS1 and ITS2), each with a different evolution rate (Williams and Barclay 1988; Hillis and Dixon 1991; Eickbush and Eickbush 2007; Poczai and Hyvonen 2010) (Fig 1.4). The ITS regions are believed to be fast evolving and therefore may vary. Universal PCR primers designed from highly conserved regions of the ITS and its relatively small size (600-700 bp) enable easy amplification of ITS gene due to high copy number of rDNA repeats (up to-30000 per cell, Dubouzet and Shinoda 1999). The ITS region is an interesting subject for evolutionary and phylogenetic investigations (Baldwin *et al*. 1995; Hershkovitz *et al*. 1996; 1999) as well as biogeographic investigations (Suh *et al*. 1993). The ITS gene has been used for phylogenetic analyses at the species to generic level. The sequence data of the ITS gene has been evaluated as potential DNA barcodes for Fungi Plants and (Schoch

et al. 2012). However, limited information is available on its applicability to identify invertebrate species in the India coast.

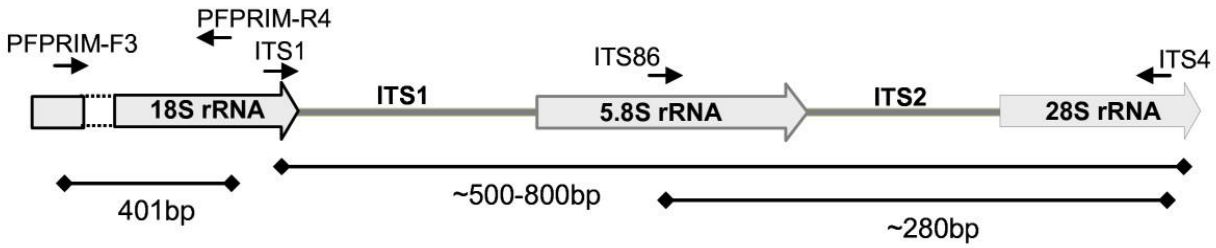

Fig 1.4. Schematic view of ribosomal DNA showing 18S rRNA, ITS genes region and the primers used for amplification. (source: Embong *et al*. 2008)

1.16.3. Mitochondrial gene

Mitochondrial genome in most animals is ~15000 bp length and embraces the phylogenetic information that can be usedfor gene rearrangement data and amino acid data or nucleotide data due to its dynamics in evolutionary rates of different genes and among different position (Brown 1985; Kondo *et al*. 1993) (Fig 1.4). The incomplete genomes are the result of difficulties with amplifying of mtDNA genomes (Boore and Brown 2000; Jennings and Halanych 2005). The mtDNA genomes which show a remarkable degree of conservation in gene order indicatingthat the analysis of concatenated coding and ribosomal genes may be more capable.

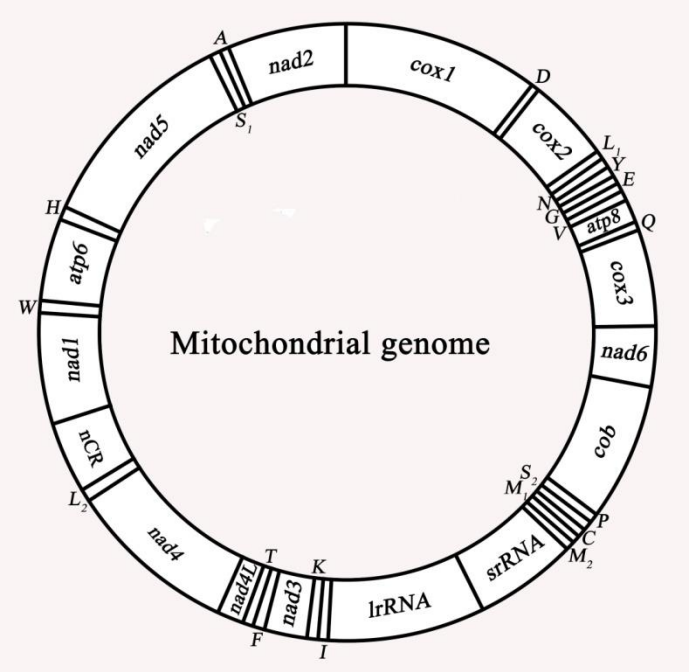

Fig 1.5. Genes encoded by Mitochondrial Genome (source: Lin *et al*. 2012)

1.16.4. DNA Barcoding

The barcoding of organisms is a DNA-based species identification method in which molecular biology and bioinformatics are combined. The PCR and sequencing techniques coupled with information technology have provided a new method of classification and termed DNA taxonomy. According to Hebert *et al*. (2003) had suggested a section of mitochondrial Cytochromec Oxidase Subunit I (mtCOI) gene could be used as a "barcode" to differentiate between species. The mtCOI gene is the best region for the taxonomic tool, as it has a high degree of conservation and insertions and deletions (Moritz and Cicero 2004). It has many rapidly evolving nucleotide sites, which will allow for the differentiation between recently evolved species (Nylander *et al*. 1999).

Compared to the nuclear genome and the mitochondrial genome lacks introns has had restricted exposure to recombination, and haploid mode of inheritance (Saccone *et al*. 1999).

However, there is evidence to suggest that cryptic species are common among all polychaete families making up a significant portion of their biodiversity (Nygren 2014). Whether or not cryptic speciesare more common within certain polychaete families, functional groups are unknown. For this reason, the use of molecular methods for accurate identification of morphologically different and cryptic polychaete species is essential if we are to understand their true diversity. The first major comprehensive DNA barcoding project of polychaetes was conducted by Carr *et al*. 2011 that sequenced 1876 specimens from waters surrounding Alaska and the Canadian Artic. In total, 25% of the species were collected of two or more separate genetic lineages and believed to contain cryptic species. Generally, the species identification based on morphological characters alone may significantly underestimate the species diversity.

1.17. Molecular techniques for genetic variation and population structure

The polychaete species is one of the most fundamental and important units of biology. It is impossible to recognize species ranges and boundaries, dispersal among populations and interactions between species without the ability to differentiate between species. These characteristics suggest that environmental factors directly modify the genetic patterns in the population (Gonza ´lez-Wanguemert *et al*. 2006). They could be contributing to the genetic diversity among and within the populations associated with physical and ecological factors (Bilton *et al*. 2002; Iannotta *et al*. 2008). The power of

genetics to study populations is irrefutable yet marine genetic research significantly pursues behind terrestrial work (Féral 2002). Investigating intra-specific genetic variation gives an estimate of gene flow and therefore an overall picture of the dispersal occurring among populations ('connectivity') (Bohonak 1999; Hellberg *et al*. 2002). This is particularly important in marine environments because direct observations of dispersal are rarely logistically possible (Palumbi 1995). Genetic connectivity has received considerable attention in tropical marine systems because of its role in informing spatial management. The population connectivity determines the degree of recruitment within coastal areas and interchange of populations outside of the coastal area (Botsford *et al*. 2001; Palumbi 2003; Sale and Kritzer 2003; Shanks *et al*. 2003). Generally the population genetic studies are showing that the duration of pelagic larval has been determined based on the genetic connectivity of benthic species and dispersal opportunity (Palumbi 1995).

Species boundaries in the ocean may be associated closely with chemical rather than morphological recognition; therefore genetic studies have increasingly revealed a wealth of 'cryptic species' (Knowlton 1993, 2000). Previously species determined genetically that have remained hidden to traditional morphological taxonomy. Hence, recently the molecular tools play an important role in marine biodiversity estimates (Bucklin *et al*. 2010), which resolves their evolutionary potential in the face of environmental change (Lande and Shannon 1996) and consequently the main concern of conservation in order to ensure species' long-term persistence (Bowen 1999).

1.18. Studies on diversity and distribution of macrobenthos along the west coast of India

The benthic studies have been conductedto understand the quantitative nature and community structure from different regions of the country. Ganapati and Rao (1959) made a preliminary work on benthos in the continental shelf of the north-east coast of India based on some grab and dredge in a widely separated stations. In the western continental shelf substantial amount of information is available on the benthic community structure (e.g. Neyman 1969; Harkantra *et al*. 1980, Parulekar *et al*. 1982; Sajan and Damodaran 2007; Jayaraj *et al*. 2007, 2008; Ingole *et al*. 2002; 2009, 2010, 2014; Joydas and Damodaran 2009; Sivadas *et al*; 2010, 2013; Musale and Desai 2010, Smitha 2011; Jaleel *et al*. 2014, 2015; Sivadas and Ingole 2016). Since these studies were carried out with different methodologies and objectives their utility under is limited in understanding the benthic community. The aspects of quantitative distribution, standing crop and annual production of benthos in the Indian coast and the effectiveness of the data for assessing the potential demersal resources were also studied by Parulekar *et al*. (1982). Bouillon *et al*. (2002) studied the relative importance of different primary carbon sources to invertebrates in the intertidal mangrove forest located along the Southeast coast of India. Ganesh and Raman (2007) studied the macrobenthic community structure of the northeast Indian shelf.

1.19. Research gap in the study of polychaete worms

Based on the review on various molecular markers are clear that the mtCOI, 18S rRNA and ITS fragments have successfully been used to study of polychaetes phylogenies

(Dahlgren *et al*. 2000), but most of the studies using a mtCOI gene divergence techniques have attempted to determine the phylogeny, rather than separate between species similarity. High levels of genetic variation have been observed between populations ofthe same Polychaete species (Von Soosten *et al*. 1998; Maltagliati *et al*. 2001) including the Nereid, *Hediste divericolor* (Hateley *et al*. 1992; Abbiati and Maltagliati 1996; Rohner *et al*. 1997). Moreover, most of the studies used morphological identification and in the case of molecular taxonomy the combined use of nuclear and mitochondrial genes are almost non-existentin Indian waters. Therefore, the present work mainly focuses on evaluating the discriminating efficiency of mtCOI, 18S rRNA and ITS genes in the polychaete species along the west coast of India. The grouping of sequence data from several conserved genes can give the possible approach to overcoming a problem and possibly moving towards the improved of understanding polychaete species diversity.

The main objectives of the present work are listed as follows:

- To examine the spatial variation of polychaetes species in relation to sediment texture along the west coast of India

- To understanding phylogeny of polychaetes

- To analyses of genetic variability on speciation along the west coast of India

Chapter 2

Polychaete assemblage driven by substrate composition along the coastal waters of the South-eastern Arabian Sea

2.1. Introduction

Free-living polychaetes are abundantly distributed in the soft sediment and play an ecologically important role in the benthic food web (Gray and Elliott 2009). The sediment burrowing polychaetes species are more or less integral part of coastal sediment and form the central role between the benthic and pelagic systems. They are often diverse in their feeding strategies and highly abundant, especially in areas of anthropogenic stress (Gray and Elliott 2009; Fauchald and Jumars 1979). Polychaetes are decisive in marine food chains, as important prey for many crustaceans, mollusks, fish, wading birds and other marine organisms. They play a major role in the break-down, subduction, and integration of organic matter into sediments and their bio-turbation. Being a major constituent of the soft bottom macrofauna, polychaete species composition, abundance, d iversity, and biomass have been successfully used to assess the health of the coastal ecosystem (Ingole *et al*. 2009; Sivadas *et al*. 2010, 2016).

The unique environmental condition along the west coast of India has influenced on the formation of the typical pelagic ecosystem, though, it does enhance the benthic ecosystems (Ingole *et al*. 2010; Singh and Ingole 2016). The benthic studies have been made to understand the quantitative nature and community structure from different regions of the country. In the western continental shelf, substantial amount of information is available on the benthic community structure (e.g., Sajan and Damodaran 2007; Jayaraj *et al*. 2007, 2008; Ingole *et al*. 2009, 2010; Joydas and Damodaran 2009; Sivadas *et al*. 2010, 2013, 2016; Jaleel *et al*. 2015). However, the above studies were carried out in different seasons following different methodologies and hence their utility for comparison is limited. Nevertheless, the aspects of quantitative distribution, standing

crop and annual production of benthos in Indian seas and the effectiveness of the data for assessing the potential demersal resources were studied by Parulekar *et al*. (1992). According to Joydas and Damodaran (2009), the contribution of polychaete was 57% in the totalmacrofaunal population with 122 species from 51-100m and 52 species in the shelf edge of the west coast of India. Musale and Desai (2011) reported 63 polychaete species along the Indian coast, of which 38 species were from the west and 25 were from the east coast of India. While reviewing the polychaete species of Indian coast, Sivadas and Ingole (2016) reported overall 564 species belonging to 262 genera and 54 families. The highest species richness is recorded in the eastern basin with 255 species, followed by 160 species western basins with and in the Andaman and Nicobar with 157 species. In general, the Neriididae was the most diverse family (71 species). Therefore, the aim of this study was to evaluate the variation in the polychaete community structures and its relation with the sediment texture from the shallow coastal areas.

2.2. Materials and Methods

2.2.1. Study area

The sampling was carried out on board *CVR Sagar Sukti* and *FORV Sagar Sampada* from May 2011 to March 2014, in order to generate local-scale benthic data. The sediment samples were collected at the following localities Mumbai 17°08'42" N 73°16'04" E, Ratnagiri 16°22'24" N 73°22'16" E, Goa 15°51'06" N 73°38'24" E, Karwar 15°41'18" N 73°42'14" E, Mangalore 12°59'19" N 74°47'58" E, Cochin 11°52'02" N 75°21'10" E, Trivandrum 08°04'38" N 77°31'50" E (Table 2.1 and Fig. 2.1).

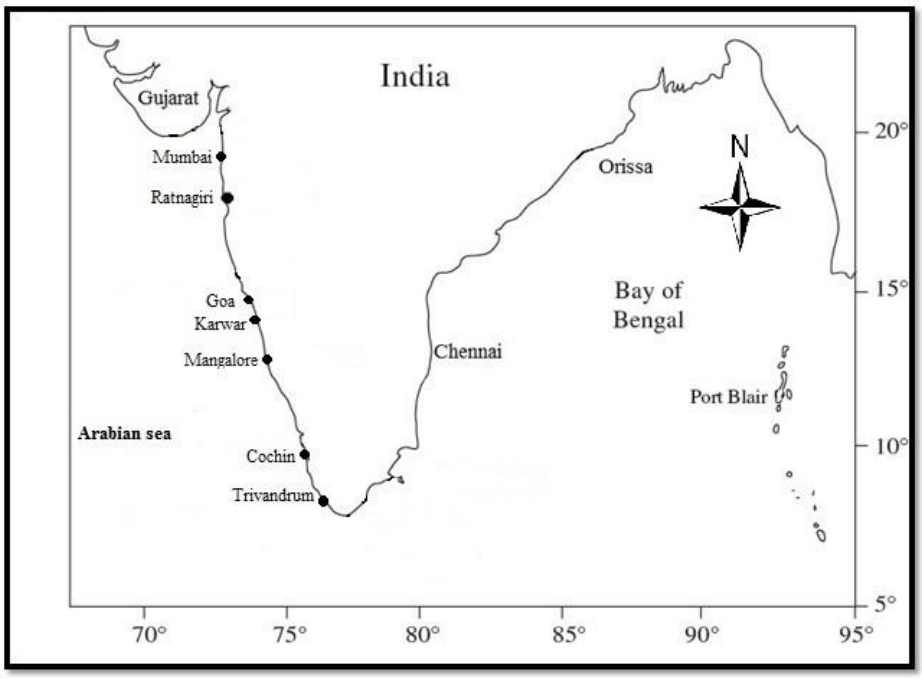

Fig 2.1. Sampling locations along the South-eastern Arabian Sea

2.2.2. Sample collection

Sediment samples were collected using 0.04 m² van Veen grabs. Three grabs were taken from each station for the analysis of benthic polychaetes and one for sedimentological study. The grabs were able to penetrate 15 cm into the sediments. The sediment samples after sieving through 500-µm mesh sieve were brought to the laboratory in polythene bags in the white-bottomed tray. The polychaetes were hand sorted by a forceps. After the preliminary examination, the whole sample was fixed in 5% formalin. Polychaete species were later identified and counted under a stereomicroscope to the lowest taxonomic level (Fauvel 1953; Day 1967).

2.2.3 Laboratory analysis

The sediment samples were washed again, sorted, and stored in 5% buffered formalin. Polychaete species were later identified to the lowest taxonomic level (Fauvel 1953; Day 1967) and counted under a stereomicroscope. Sediment texture was analyzed by Malvern Laser Analyzer (Model–Hydro 2000MU). Sediment samples of 50g were washed using de-ionized water and dried at 45˚C and treated over night with 30% H_2O_2 for removal of organic matter. The samples were sieved through a 62-m mesh to separate the sand from the siltyclay fraction. The sandy sediment samples were analysis with using sieve method (Carpenter and Deitz 1950) and silty clay samples were analyzed by Malvern laser particle size analyzer.

2.2.4. Data analysis

Polychaete data was processed using univariate and multivariate methods. Diversity indices were calculated using Margalef's index for species richness (d), Pielou's index (Pielou 1966) for species evenness (J'), Shannon-Wiener index (Shannon and Weaver 1963) for species diversity (H' log) and Simpson's dominance index (*C*') reflects the even occurrence of polychaete species within a community. The macrobenthic abundance data (after log transformation) based on bray–Curtis similarity analysis using PRIMER v.6. Following the division into groups from results of cluster analysis, the contribution of polychaete species was determined using similarity percentage program SIMPER using PRIMER 6 (Clarke and Warwick 2001). Feeding types of polychaetes species were assigned according to Fauchald and Jumars (1979). A Canonical Correspondence Analysis (CCA) was carried out to show in a single diagram the direct interpretation of

the relationship between sediment characteristics (Medium, fine sand, and silt clay) and polychaetes species using the Multivariate Statistical Package version (MSPV) 3.1 (Kovach 1998).

2.3. Results

2.3.1. Distribution of sediment texture

The distribution of grain-size sand–silt–clay of sediment varied from fine sand to mud along the study area. Two types of sediment textures were observed in the study area. The Northern Region (NR) was characterized by silty and clay, whereas the SR was characterized by fine sand. In general, the distribution of silt and clay was similar, while sand shows an opposite trend. In the SR low percentage of silt–clay content (0.34%) was observed, with coarse (20.47%), medium (47.6%), fine sand and silt-clay contents (31.61%). In the Central Region (CR) fine sand content varies from 24.49% to 30.1%, silt 17.7–95.99% and clay 46.46–52.2%. The poor sand content along CR and NR with near-equal proportions of fine sand 2.65–3.97%, silt 91.55–95.99% and clay 0.04–5.8% was present (Table 2.1).

Table 2.1. The distribution of sediment texture along the South-eastern Arabian Sea based on laser diffraction.

Stations	Date	Depth (m)	D_{50}	Coarse sand (%)	Medium sand (%)	Fine sand %	Silt (%)	Clay (%)
Trivandrum	March 2011	10 to 50	0.274	28.97	60.07	11.00	-	-
Cochin	March 2010	10 to 50	0.264	32.50	48.93	18.57	-	-
Mangalore	May 2010	10 to 50	0.234	-	34.70	65.26	0.04	-
Karwar	May 2012	10 to 30	0.003	-	-	30.10	17.70	52.20
Goa	May 2014	10 to 20	0.064	-	-	24.49	29.05	46.46
Ratnagiri	March 2012	10 to 30	0.003	-	-	3.97	95.99	0.04
Mumbai	May 2013	10 to 30	0.013	-	-	2.65	91.55	5.80

2.3.2. Diversity indices

Margalef‟s index (*d*) varied from 3.15 to 5.5 in the SR, whereas in CR it varied from 3.8 to 4.59, while a low *d* value was recorded from 2.5 to 3.3 in the NR. The species evenness (J) varied from 0.4 to 0.6 in SR whereas, in the NR *J* ranged from 0.5 to 0.7. However, the high value of species diversity (*H'*) of 3.5 to 5.71 was observed in the NR, whereas in SR (*H*') value ranged from 1.9 to 2.99. Applying Simpson's dominance index (*C*'), the Southwest coast had slightly lower values of dominance index (*C*') (0.45 to 0.68) than the Central west coast (0.68 to 0.69) and Northwest coast (0.80 to 0.86), indicating that there were clear dominance polychaete species (Table 2.2).

Table 2.2. Diversity indices along the South-eastern Arabian Sea

Location	N	*d*	*J'*	*H'*(log2)	(*C'*)
Trivandram	2987	5.50	0.55	2.99	0.68
Cochin	2648	3.15	0.40	1.87	0.45
Mangalore	3288	3.95	0.59	2.98	0.68
Karwar	2476	3.84	0.55	2.72	0.69
Goa	1336	4.59	0.63	3.22	0.80
Rathnagiri	1293	2.51	0.71	3.01	0.82
Mumbai	2444	3.33	0.74	3.48	0.86

Total abundance nos/m^2 (N); Margalef‟s index (*d*); species evenness (J'); species diversity (*H'(log2)*); dominance index (C')

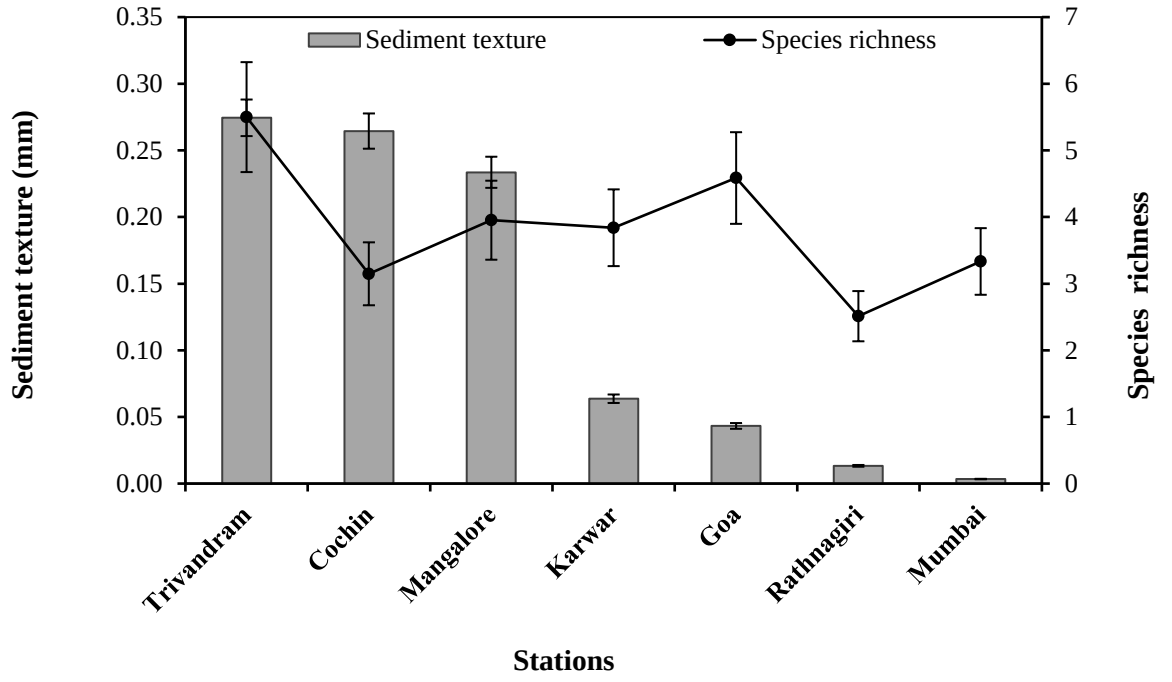

Fig. 2.2. Relationship between sediment texture and species richness along the South-eastern Arabian Sea

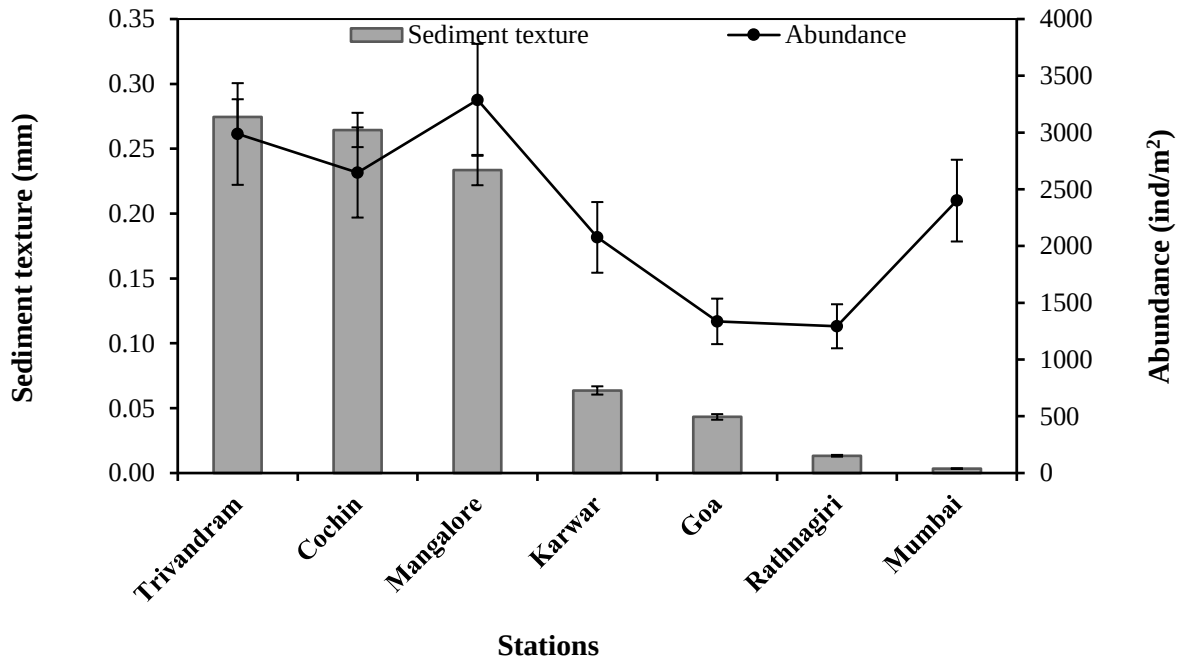

Fig. 2.2. Relationship between sediment texture and species abundances along the South-eastern Arabian Sea

2.3.3. Cluster analysis of polychaete assemblage

The cluster analysis showed that the clear difference in the polychaete species assemblage (Fig 2.3). We found that three groups were characterized with different ecosystem setting along the South-eastern Arabian Sea, with a barring of station taken close to shallow coastal water. Species richness, diversity, and density changed gradually from the high value in the SR to the low values in the NR. The data on the average abundance of polychaete species were placed into three different groups, which showed three structurally different communities in the study area. The SR group was differentiated by the dominance of *Aphelochaeta multifilis* and *Mediomastus capensis* and NR group were dominated by *C. delta* and *P. cordifolia* (Table 2.2). Bray-Curtis cluster analysis also showed three groups based on the species distribution. Group, I consisted of Trivandrum, Cochin and Mangalore stations with 66.3% similarity, whereas group II was clustered with 60.03% of Karwar and Goa and Group III at 57.68% similarity comprised of Mumbai and Ratnagiri. The polychaetes assemblage was subjected to the SIMPER analysis to find the species, which contributed to the similarity within each group. Consequently, Groups I (Trivandrum, Cochin and Mangalore) was dominated by *T. multifilis* (11%) and *M.capensis* (6.72%) with carnivore species of *Scoletoma funchalensis* (5.68%), Group II (Karwar and Goa) *Cossura delta* (10.59%), *P. cordifolia* (9.4%), and *Capitella capitata* (8.47%); Group III (Ratnagiri and Mumbai) by *C. delta* (13.48%) and *P. cordifolia* (12.31%) with carnivore species of *Glycera alba* (10.78%) (Table 2.2).

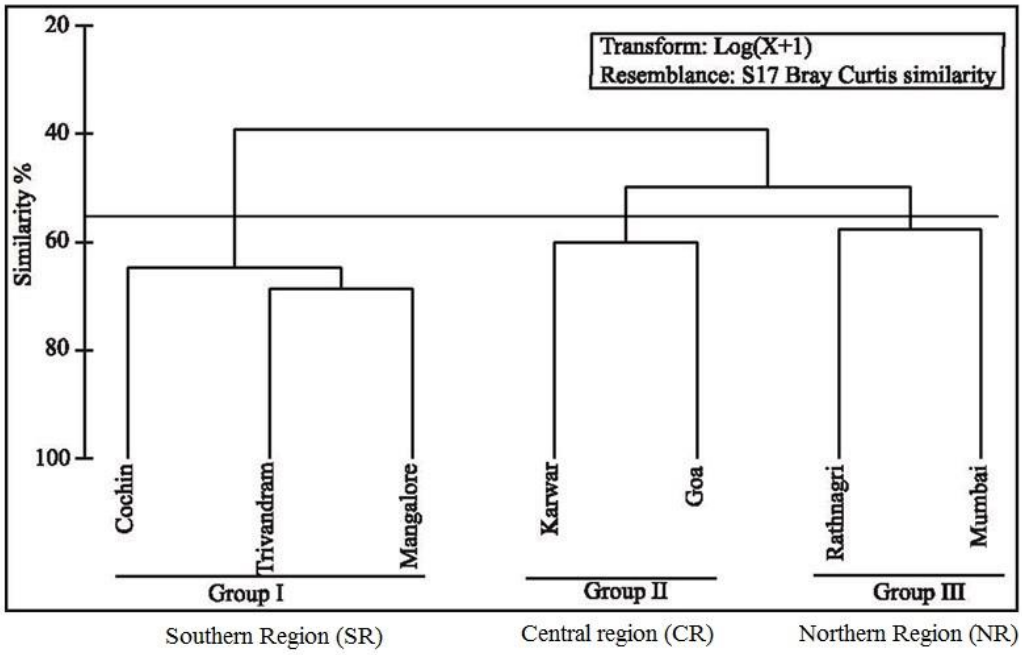

Fig. 2.3. Cluster analysis of similarity among sediment samples of NR, CR, and SR sites, based on log(X+1) transformed and the similarity level overlay on the figures 56% similarity

Table 2.2. SIMPER analysis show contribution of differences species assemblage and feeding types. Surface deposit feeder (SDF), sub-surface deposit feeder (SSDF), Sub-Surface Predator (SSP)

Species	Average dominance	Average Similarity	Contrib%	Cum.%	Feeding guilds
Group1: South west coast (Trivandrum, Cochin, and Mangalore)					
Average similarity: 66.03					
Aphelochaeta multifilis	7.41	6.96	10.55	10.55	SDF
Mediomastus capenis	5.14	4.43	6.72	17.26	SSDF
Scoletoma funchalensis	4.22	3.75	5.68	22.94	SSP
Inermonephtys inermis	3.85	3.12	4.73	27.67	SSP
Levinsenia sp.	3.94	3.05	4.63	32.3	SSDF
Group2: Central west coast (Karwar and Goa)					
Average similarity: 60.03					
Cossura delta	5.84	6.36	10.59	10.59	SDF
Paraprionospio cordifolia	4.85	5.64	9.4	19.99	SDF
Capitella capitata	4.33	5.08	8.47	28.46	SSDF
Inermonephtys inermis	3.78	4.20	6.99	35.45	SSP
Aphelochaeta multifilis	5.37	4.20	6.99	42.44	SDF
Group3: North west coast (Ratnagiri and Mumbai)					
Average similarity: 57.68					
Cossura delta	6.24	7.78	13.48	13.48	SDF
Paraprionospio cordifolia	5.60	7.10	12.31	25.8	SDF
Glycera alba	4.88	6.22	10.78	36.58	SSP
Scoletoma funchalensis	3.94	4.96	8.59	45.17	SSP
Aricidea sp.	4.07	4.25	7.36	52.53	SDF

2.3.4. Distribution of Polychaete feeding type

The SR was dominated by surface deposit feeders (dwelling polychaetes 54.4%), sub-surface deposit feeder (15.35%), carnivores (25.63%) and suspension feeders (4.63%), whereas, in CR surface and sub-surface feeder existed with 64.21% and carnivore (35.79%). *C. delta* and *P. cordifolia* were dominant in the surface and subsurface deposit feeder (63.59%) with carnivore (31.65%) as well as filter feeder in the NR. The suspension deposit feeder (4.76%) was common (Fig. 2.4).

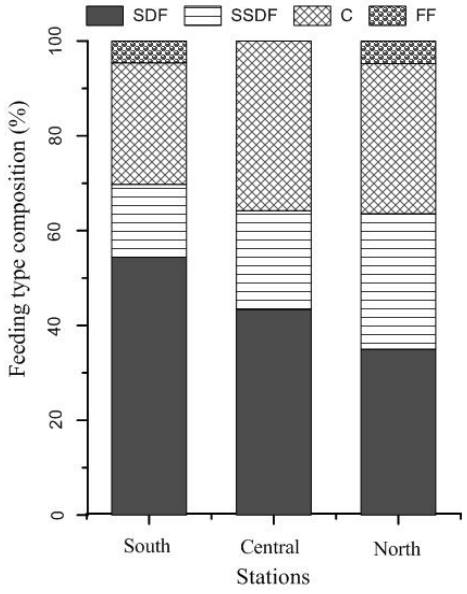

Fig. 2.4. The distribution of polychaetes feeding types, surface deposit feeder (SDF), sub-surface deposit feeder (SSDF), carnivores (C) and filter feeder (FF)

2.3.5. Canonical correspondence analysis

The two axes of CCA biplot explained 83% of the relationship between benthic polychaetes species and sediment variables. Medium, fine sand, and silt clay were the most important sediment variables influencing benthic species abundance. *C. delta* and *P. cordifolia* preferred silty clayey substratum whereas *A. multifilis*, *M. capensis S.*

funchalensis, and *I.inermis* preferred fine sand. The results show the group II polychaete species from the CR were favored by silt whereas the group III species favored by a higher percentage of clay in NR. The species indicated in group I preferred the low percentage of clay and those in group III preferred lower silt content (Fig. 2.5).

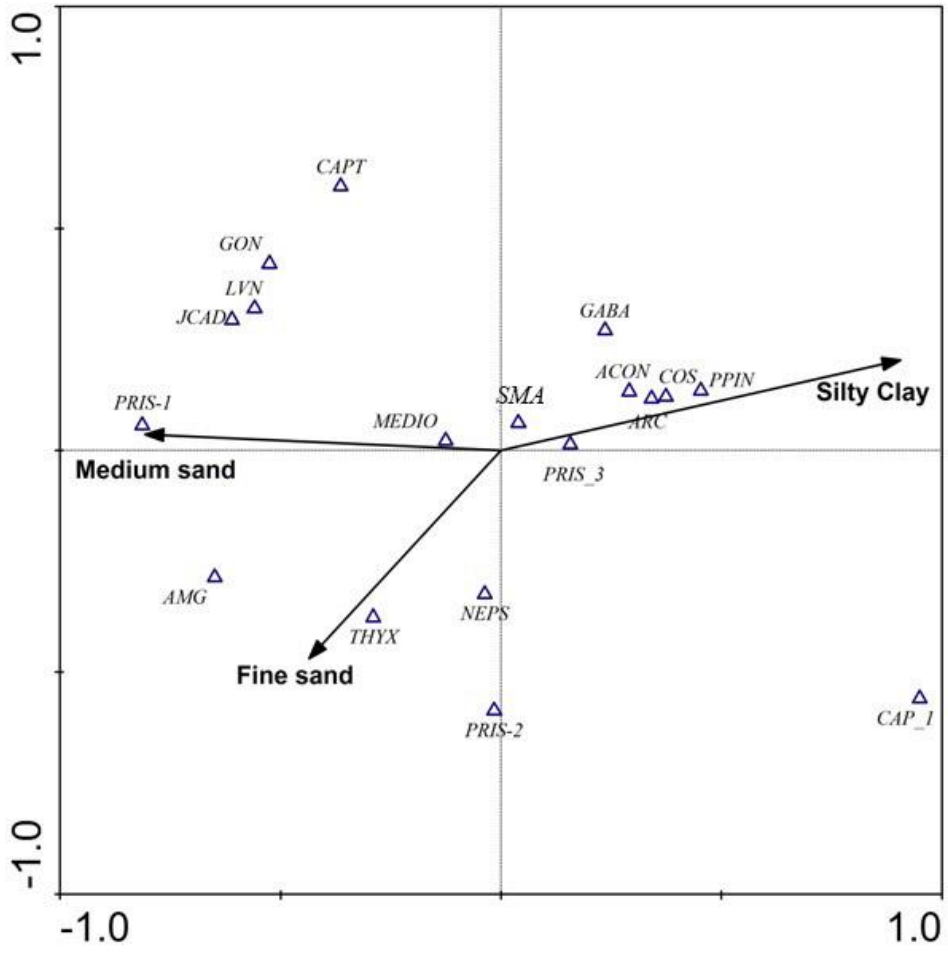

Fig. 2.5. Canonical correspondence analysis (CCA) showing polychaete species correlated with sediment type. CAPT: *Capitella capitata*, GON: *Goniadides* sp., LVN: *Levinsenia* sp., JCAD: *Jasmeneira caudate*, PRIS-1: *Prionospio aucklandica*, MEDIO: *Midiomastus capensis*, SMA: *Scoletoma funchalensis*, GABA: *Glycera alba*, ACON: *Sigambra constricta*, COS: *Cossura delta*, PPIN: *Paraprionospio cordifolia*, ARC: *Aricidea* sp., PRIS_3: *Prionospio cirrifera*, AMG: *Amage* sp., THYX: *Aphelochaeta multifilis*, NEPS: *Inermonephtys inermis*, PRIS_2: *Prionospiopygmaea*, CAP_1: *Capitellethus dispar*.

2.4. Discussion

The west coast of India provides a unique environmental condition to support the benthic polychaetes community response to sediment texture occurring at spatial scale. The sediment texture delineated three distinct areas along the west coast of India (Jayraj *et al*. 2008; Ingole *et al*. 2010; Sivadas and Ingole 2016). The fine sand is dominant in the SR, whereas CR has mixed sediments of fine sands. The silty clay was prevailing in the NR (Fig 2.3). The SR region showed a higher percentage of polychaetes species off Trivandrum, Cochin, and Mangalore and their contribution to the total polychaetes species was 36.6%. Similar findings were also made by earlier workers from the west coast of India (Joydas and Damodaran 2009; Musale and Desai 2011). Harkantra *et al*. (1985) also observed that fine sandy substrate harbor high macrobenthic diversity in the southern region, while the silty clay showed low diversity in the northern region. Thus, the specificity of polychaetes feeding types largely depends upon the type of substratum. Fine particles of sand might result in clogging of feeding apparatus of filter feeders hence they avoid fine sandy substratum although the adequate supply of food is available (Jayaraj 2008). Studies from tropical and temperate regions have shown that the grain size plays an important role in structuring the polychaetes community and high polychaete abundance is generally associated with fine sand (Defeo and McLachlan 2005; McLachlan and Dorvlo 2005).

The polychaetes species have more important role in the breakdown of the organic matter, and they provide recycling of nutrients into the pelagic system. Generally, the grain sizes increase from clayey to finer sand, with an increase in wave action towards the Southwest coast of India. *A. multifilis* dominate towards fine sand which can able to

hold the organic matter in southern region; while *C. delta* and second opportunistic species of *P. cordifolia* were dominant towards the higher silt clayey content of Northwest coast of India. Yokoyama and Sukumaran (2012) reported that the *Paraprionospio* specimens found from the northwest coast of India, and identified them as *P. cordifolia* Yokoyama 2007, *P. cristata* Zhou, Yokoyama and Li 2008, and *P. patiens* Yokoyama 2007. Some of the most dominance polychaete species identified during the present study are shown in figure 2.6.

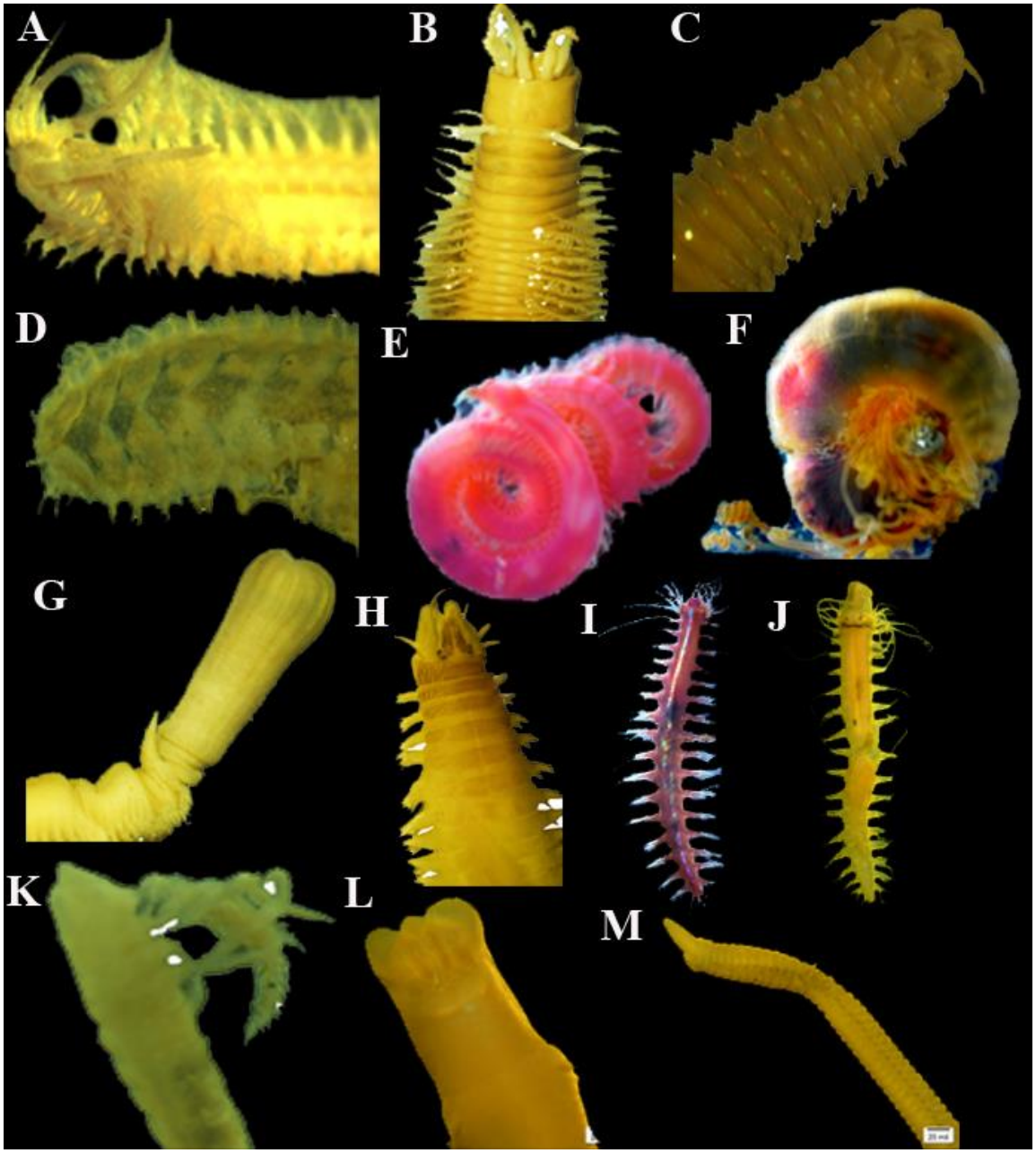

Fig. 2.6. Commonly occurring polychaete species- A: *Diopatra neapolitana*, B: *Eunice indica*, C: *Eunice* sp., D: *Thormora jukesii*, E and G: *Glycera capitata*, F: *Aphelochaeta multifilis*, H: *Nereis glandicincta*, I: *Hesione picta*, J: *Hesione* sp., K: *Paraprionospio cordifolia*, L: *Euclymene annandalei*, M: *Ninoe nigripes*

The species richness and polychaete abundance were high in the SR having fine sandy substratum. The proficiency of fine sand to detain more organic carbon is well documented. Gray (1974) observed the relationship between the macrofauna diversity on the continental shelf region and structural complexity in the sediment type. The faunal diversity in heterogeneous sediments tends to be higher than homogeneous sediments. However, Palacin *et al*. (1991) studied the benthic infauna of the Mediterranean Bay and reported the highest density in the sandy sediment. Robert (1979) also noticed increased species richness with increasing sand content, which was independent of bathymetric position. However, Bergen *et al*. (2000) and Ellingsen (2002) observed that water depth was the primary factor organizing benthic communities followed by sediment grain size in the Southern California and Norwegian.

Most of the dominant and widespread polychaete species belonged to the surface and sub-surface deposit feeding families such as Spionidae, Cirratulidae, Cossuridae, and Capitellidae in deep depth where other families have low representation or even absent showed their ability to survive in adverse conditions, especially with a reduced amount of sediment grain size. Carnivorous species belonging to the Eunicidae and Glyceridae were also represented in terms of abundance and dominance. Mirza and Gray (1981) attributed low diversity of benthos and dominance of polychaetes (viz., *Capitella* and *Polydora*) to the oxygen-poor mud in Norway. The present study showed an increased density, richness and polychaete diversity in the southern region with the dominance of sandy substrate which could hold high organic carbon matter. Stable benthic conditions allow many specialized species such as *C. delta* and *A. multifilis* to be present in the study area, but competition for food is probably higher, leading to low densities (Duineveld *et al*.

1991). The biological factors include competition, predator-prey relations, and a larval settlement which could be the important factors in polychaetes assemblage.

The significant spatial variation in polychaetes assemblage was directly related to change in the occurrence of the dominant species and their present/absent at each station. Overall, abundance was higher for sub-surface deposit feeder *C. delta* species in the northern stations. *C. delta* is considered as a shallow water muddy species. Polychaete assemblages along the South-eastern Arabian Sea were characterized by high species richness, density, and environment related characteristics. Recently, Sivadas and Ingole (2016) reported that the macrofaunal assemblages in diverse communities were dependent on the substratum type. Therefore, knowledge on the substratum type, species diversity, and species assemblages are necessary to understand the ecosystem function.

Resulds suggest that the polychaete species response to substrate composition in the study area. Differences in the distribution of polychaete species between SR and NR habitats may be supported statistically in the present study, however, the variability of polychaete species distribution between central parts with fine sandy sites reflects the increased heterogeneity and complexity of substrate when compared with clay silty substrate. The sediment composition has mainly influenced the distribution and diversity of polychaetes species, with diversity increasing in the sandy sediment (Herna´ndez Arana *et al*. 2003). The data generated through this study provides valuable baseline information of polychaete assemblage from an ecologically important area with differentiate polychaete community structure and use to focus on future benthic research. It is necessary to monitor the nature of polychaete species–relation with environmental

changes and estuary-related coastal environment. As described by Gambi and Giangrande (1986) the macrobenthic distribution to test the efficacy of polychaetes as 'markers' of different ecological conditions. The distribution of polychaetes species between habitats has also been observed earlier by Ingole *et al*. (2010), Joydas and Damodaran, (2009) and Sivadas *et al*. (2010). The present study shows the substratum composition determine the type of polychaetes assemblage.

This study provides strong effects of substratum composition on benthic polychaete communities. Changes in assemblage patterns were due to the physical forcing (coastal current) together with changes in sediment texture and its potential role towards ecosystem understanding. Seventy-one polychaete species belonging to 16 families were identified. Increased richness and diversity patterns in the SR were in contrast with northern region. The west coast of India is an ecologically productive region which promotes higher biodiversity in the Arabian Sea. The regional species assemblage dominated by *C. delta* in the Northwest coast of India, whereas *A. multifilis* dominated in the Southwest coast of India. Therefore, long-term studies could be directed towards the demographic, behavioral, population genetics and ecological responses of species assemblages. Further considering the seasonal monsoonal variability and reversing surface current in the region, studies on oceanic connectivity and gene flow could be very helpful in establishing the possible east-west larval dispersal of benthic species.

Table 2.3. The abundance of polychaete (nos/m-^{2}) in the sub-tidal area along the South-eastern Arabian Sea

	Assemblage	Southwest coast			Central west coast		Northwest coast	
S. No	Species	Trivandrum	Cochin	Mangalore	Karwar	Goa	Ratnagiri	Mumbai
1	*Aglaophamus uruguayi* Hartman, 1953	44	44	0	0	0	0	0
2	*Alitta succinea* (Leuckart, 1847)	0	0	0	44	88	44	44
3	*Amphicteis posterobranchiata* Fauvel, 1932	0	0	0	0	0	0	44
4	*Amage* sp.	44	44	0	0	0	0	0
5	*Aphelochaeta multifilis* (Moore, 1909)	1643	1511	1820	1332	132	44	44
6	*Sigambra constricta* (Southern, 1921)	44	44	44	44	0	0	0
7	Aphroditidae Malmgren, 1867	0	0	44	88	44	88	44
8	*Arabella (Arabella) iricolor* (Montagu, 1804)	0	0	0	0	0	44	0
9	*Aricidea* sp.	44	0	0	0	0	0	44
10	*Armandia sampadae* Gopal, Jaleel, Parameswaran & Vijayan, 2016	44	44	88	44	0	132	0
11	*Axiothellao bockensis* (Gravier, 1905)	44	0	37	0	0	0	0
12	*Aonides* sp. Claparède, 1864	44	44	0	0	0	0	0
13	*Bylgides sarsi* (Kinberg in Malmgren, 1866)	0	0	0	88	88	44	0
14	*Capitella capitata* (Fabricius, 1780)	44	44	44	0	0	0	44
15	*Timarete dasylophius* (Marenzeller, 1879)	0	0	44	0	0	0	44
16	*Cirratulus spectabilis* (Kinberg, 1866)	44	88	44	0	0	0	0
17	*Cirriformia grandis* Verrill, 1873	0	0	0	0	0	0	44
18	*Cossura delta* Reish, 1958	44	88	132	266	710	844	577
19	*Capitellethus dispar* (Ehlers, 1907)	44	44	88	0	0	0	132
20	*Diopatra neapolitana* Delle Chiaje, 1841	0	0	44	0	0	0	0
21	*Trochochaeta* Levinsen, 1884	44	0	0	0	0	0	0
22	*Eunice indica* Kinberg, 1865	0	44	0	44	44	0	0
23	*Hypereteone heterepoda* (Hartman, 1951)	44	0	0	0	0	0	0
24	*Euclymene annandalei* Southern, 1921	0	88	0	0	0	0	0
25	*Goniadides* sp. Hartmann-Schröder, 1960	44	0	44	0	0	0	0
26	*Glycera capitata* Setosa Örsted, 1843	44	0	0	44	44	0	44
27	*Glycera alba* (O.F. Müller, 1776)	88	44	88	0	0	0	178
28	*Hesione* sp. Savigny in Lamarck, 1818	0	0	0	0	0	0	0
29	*Hesione picta* Müller in Grube, 1858	0	0	44	44	132	132	176
30	*Idanthyrsus* sp. Kinberg, 1876	44	0	0	0	44	0	0
31	*Jasmineira caudata* Langerhans, 1880	0	0	0	0	44	0	0
32	*Levinsenia* sp. Mesnil, 1897	44	88	88	0	0	0	0
33	*Scoletoma funchalensis* Kinberg, 1865	132	44	132	0	0	0	44

34	*Magelona* sp. F. Müller, 1858	0	0	132	44	44	88	44
35	*Magelona cincta* Ehlers, 1908	44	44	44	0	0	0	44
36	*Maldanella harai* (Izuka, 1902)	311	84	222	0	0	0	0
37	*Mediomastuscapensis* Day, 1961	7	0	0	0	44	0	0
38	*Micronereides capensis* Day, 1963	0	0	0	44	0	0	0
39	*Micronephthys sphaerocirrata* (Wesenberg-Lund, 1949)	0	0	0	0	0	44	0
40	*Inermonephtys inermis* (Ehlers, 1887)	44	0	0	44	44	0	0
41	*Ninoe nigripes* Verrill, 1873	0	44	132	0	0	0	44
42	*Neanthes glandicincta* (Southern, 1921)	0	0	0	44	0	0	0
43	*Nereis sandersi* Blake, 1985	44	132	88	178	88	44	355
44	*Notomastus aberans* Day, 1957	44	0	0	0	0	0	0
45	*Onuphis eremita* Audouin & Milne Edwards, 1833	0	44	0	0	44	0	44
46	*Phyllodoce* sp. Lamarck, 1818	0	0	0	44	0	44	0
47	*Lagis koreni* Malmgren, 1866	44	0	44	0	44	0	44
48	*Paraprionospio patiens* (Yokoyama 2007)	0	44	88	132	44	132	222
49	*Prionospio aucklandica* Augener, 1923	44	44	0	0	0	0	0
50	*Prionospio pygmaeus* Hartman, 1961	0	0	0	88	0	0	0
51	*Prionospio (Minuspio) cirrifera* Wirén, 1883	88	44	0	44	44	132	132
52	*Paraprionospio cordifolia* Yokoyama, 2007	44	44	44	132	222	178	266
53	*Polydora ciliate* (Johnston, 1839)	0	44	0	44	0	0	0
54	*Paraonides lyra capensis* (Day, 1955)	0	88	0	0	0	0	0
55	*Potamilla leptochaeta* Southern, 1921	0	44	0	44	44	0	44
56	*Polyphysiacrassa* (Örsted, 1843)	0	44	44	0	0	0	0
57	*Protodorvillea egena* (Ehlers, 1913)	44	0	0	88	44	0	0
58	*Poecilochaetus serpens* Allen, 1904	0	44	0	44	0	44	44
59	*Paralacydonia paradoxa* Fauvel, 1913	0	0	0	0	44	0	0
60	*Linopherus abyssalis* (Fauchald, 1972)	44	88	0	0	0	0	0
61	*Schistomeringos neglecta* (Fauvel, 1923)	0	88	44	44	0	0	0
62	*Sternaspis scutata* (Ranzani, 1817)	0	0	44	44	0	0	44
63	*Spiochaetopterus costarum* (Claparède, 1869)	44	0	0	0	0	0	0
64	Sabellidae Latreille, 1825	0	0	0	44	0	0	0
65	*Scoloplos (Scoloplos) marsupialis* (Southern, 1921)	44	44	44	0	0	0	0
66	*Scoloplos armiger* (Müller, 1776)	44	0	0	88	0	0	0
67	*Schistocomus* sp. hiltoni Chamberlin, 1919	0	44	0	0	0	0	0
68	*Synelmis* sp. Chamberlin, 1919	44	0	0	44	0	0	0
69	*Sylliscornuta* Rathke, 1843	44	0	88	0	0	0	0
70	*Terebellides stroemi* Sars, 1835	0	44	0	0	0	44	88
71	*Thormora jukesii* Baird, 1865	0	0	0	88	44	0	0

Chapter 3

Phylogeny and Molecular Identification of Polychaete Species along the West Coast of India

3.1. Introduction

The molecular taxonomy is refreshing the traditional taxonomy and helps to increase the taxonomic crisis, alternative and complementary approaches particularly successful in the identification and delimitation of new species from various groups (Valentini *et al*. 2009). The challenging in the traditional taxonomy of species is based on the body arrangement, the ground pattern, which would determine the direction of species evolution. The molecular studies are increasingly being used in addition to the classical taxonomy for species identification and delimitation, for estimating the levels of biodiversity, and distributional ranges (Bucklin *et al*. 2011). Recently, the increased identification of the abundance and importance of cryptic species, those are morphologically identical but genetically different (Brasier *et al*. 2016). Moreover, the molecular studies have been reformed the exploration of biodiversity for which traditional taxonomy is difficult (Tang *et al*. 2012). The use of genetic identification can clearly help to improve the biodiversity and important assessment of morphological traits used in taxonomy (Valentini *et al*. 2009). The primary step in understanding the patterns and controls of diversity levels is important for accurate documentation of species diversity, biogeography and functional ecology and important to the management of marine ecosystems.

The first single gene molecular studies of annelid taxa based on the analysis of 18S rRNA and elongation factor 1α gene sequences were done by Winnepenninckx *et al*. (1995); McHugh (1997); Winnepenninckx *et al*. (1998); Eeckhaut *et al*. (2000); McHugh (2000); Struck *et al*. (2002); Bleidorn *et al*. (2003). However, the available molecular data was slowly and covering only a fraction of the annelid diversity. As suggest by Rouse and

Fauchald (1997) the species diversity of polychaete taxa were much higher. Moreover, the resolutions of relationships among families were poor and highly inconsistent between analyses. Whereas, the monophyly of many annelid families could be confirmed, however, the relationships among them remained difficult to understand (Bleidorn 2009). The incorporation of molecular data provided important evidence for the addition of Echiura and Siboglinida within Annelida and further support the Clitellata should also be a sister group within Polychaeta (McHugh 2000; Bleidorn *et al*. 2003). As demonstrated by Bleidorn (2009) the relationships of many annelid families could be resolved with molecular data.

In the western continental shelf rich in benthic diversity based on the following substantial amount of information is available on the benthic community structure (e.g., Neyman 1969; Harkantra *et al*. 1980, Parulekar *et al*. 1982; Jayaraj *et al*. 2007, 2008; Ingole *et al*. 2009; Joydas and Damodaran 2009; Sivadas *et al*. 2010, 2013, 2016). According to Joydas and Damodaran (2009), the polychaete species was a 57 % in the total population with 122 species from 51-100m and 52 species in the shelf edge belonging to 32 polychaete families along the shelf waters of the west coast of India, Arabian Sea. Musale and Desai (2011) reported 63 polychaetes and Jaleel *et al*. (2014) noted 195 polychaete species belonging to 107 genera and 37 families from the South Eastern Arabian Sea continental margin. Jaleel *et al*. (2015) reported 189 polychaete species belonging to 43 families from the southeastern Arabian Sea shelf. Sivadas and Ingole (2016) report 564 species belonging to 54 families and 262 genera. In general, the Neriididae was the most diverse family (71 species). These studies have continuing to acquire knowledge on the benthic community from a traditional morphological point of

view. The beginning of the molecular systematic approach to identify the species which are important in understanding the interspecific and intraspecific variation in the genetic level, and their population expansion keeps genetic diversity. Since these studies were carried out with different methodologies and objectives. The health of the ecosystem is predicted based on the diversity of polychaete species which is frequently used asindicator taxa in the environmental pollution (Dean 2008; Sivadas *et al*. 2010, 2016).

The limited DNA barcodes, short sequences of DNA from a single organism are availablefor Indian marine species. Considering the seasonal monsoon variability and reversing surface current along the west coast of India, is important to understand the phylogeny and benthic diversity which could be very helpful in establishing the possibility of east-west pelagic larval dispersal. The study is to identify the benthic polychaetes species based on 18S rRNA gene.

3.2. Materials and Methods

3.2.1. Study area

The sediment samples were collected at the following localities (Fig 2.1). Sediment samples were collected using 0.04 m^2 van Veen grabs. The grabs were able to penetrate 15 cm into the sediments. Samples were sieved on a 500-µm mesh using filtered seawater. The samples were washed again, sorted, and fauan was stored in 95% ethanol. The specimens were temporarily placed in plastic bottles and then kept in vials containing absolute ethanol. Some of the middle segments of polychaete species were removed from these specimens and kept in vials containing absolute ethanol until further

use for DNA isolation. The most commonly occurring polychaete species are shown in figure 3.2. Identification of polychaete species was done by observing diagnostic characters parapodia-bearing chitinous chaetae under stereo zoom microscope using keys (Fauvel 1953; Day 1967).

3.2.2. DNA extraction, PCR amplification, purification, and sequencing

Genomic DNA was extracted from the specimen using Qiagen DNeasy Tissue Kit according to manufacturer's instructions. The 18S rDNA gene was used for PCR amplification in overlapping fragments of 1800 bp length each with modified primer pairs (Table 3.2) with standard cycle sequencing protocols. Amplifications had been carried out using an Eppendorf Mastercycler gradient. The following PCR temperature file was used: 95C for 3 min; 35 cycles at 95°C for 45 s, 60°C for 1 min, and 72C for 2 min; final extension at 72C for 5 min. After detection by gel electrophoresis, the products had been purified using the Qiaquick PCR Purification Kit (Qiagen). Sequences were produced using the same primers and determined on an Applied Biosystems (ABI) 3730xl automated DNA sequencer using the ABI Big-dye Ready-Reaction mix kit, following the standard cycle sequencing protocol. All sequences were submitted to NCBI GenBank (Table 3.1).

Table 3.1. The 18S DNA sequences submitted to NCBI GenBank and Accession Number.

Sequence ID	Specimen voucher	Accession number	Organism	Sequence ID	Specimen voucher	Accession number	Organism
Seq1	GP0155	KT900259	*Listriolobus sorbillans*	Seq45	GP0199	KT900303	*Eunice miurai*
Seq2	GP0156	KT900260	*Notoplana australis*	Seq46	GP0200	KT900304	*Paraprionospio cordifolia*
Seq3	GP0157	KT900261	*Phascolosoma scolops*	Seq47	GP0201	KT900305	*Paraprionospio cordifolia*
Seq4	GP0158	KT900262	*Phascolosoma sp.*	Seq48	GP0202	KT900306	*Paraprionospio cordifolia*
Seq5	GP0159	KT900263	*Paraplanocera oligoglena*	Seq49	GP0203	KT900307	*Paraprionospio patians*
Seq6	GP0160	KT900264	*Micrura verrilli*	Seq50	GP0204	KT900308	*Paraprionospio patians*
Seq7	GP0161	KT900265	*Eurythoe complanata*	Seq51	GP0205	KT900309	*Paraprionospio cordifolia*
Seq8	GP0162	KT900266	*Eurythoe complanata*	Seq52	GP0206	KT900310	*Scolelepis* sp.
Seq9	GP0163	KT900267	*Eurythoe complanata*	Seq53	GP0207	KT900311	*Scolelepis* sp.
Seq10	GP0164	KT900268	*Notopygos caribea*	Seq54	GP0208	KT900312	*Magelona cincta*
Seq11	GP0165	KT900269	*Eurythoe complanata*	Seq55	GP0209	KT900313	*Neosabellaria indica*
Seq12	GP0166	KT900270	*Pareurythoe borealis*	Seq56	GP0210	KT900314	*Neosabellaria indica*
Seq13	GP0167	KT900271	*Thormora* sp.	Seq57	GP0211	KT900315	*Neosabellaria indica*
Seq14	GP0168	KT900272	*Thormora* sp.	Seq58	GP0212	KT900316	*Neosabellaria indica*
Seq15	GP0169	KT900273	*Chloeia viridis*	Seq59	GP0213	KT900317	*Sabellaria chandraae*
Seq16	GP0170	KT900274	*Chloeia viridis*	Seq60	GP0214	KT900318	*Sabellaria chandraae*
Seq17	GP0171	KT900275	*Eurythoe complanata*	Seq61	GP0215	KT900319	*Sabellaria intoshi*
Seq18	GP0172	KT900276	*Eurythoe complanata*	Seq62	GP0216	KT900320	*Terebella* sp.
Seq19	GP0173	KT900277	*Eurythoe complanata*	Seq63	GP0217	KT900321	*Terebella* sp.
Seq20	GP0174	KT900278	*Hermenia verruculosa*	Seq64	GP0218	KT900322	*Paraeupolymnia uspiana*
Seq21	GP0175	KT900279	*Chloeia viridis*	Seq65	GP0219	KT900323	*Parasabella saxicola*
Seq22	GP0176	KT900280	*Notopygos ornate*	Seq66	GP0220	KT900324	*Parasabella saxicola*
Seq23	GP0177	KT900281	*Notopygos ornate*	Seq67	GP0221	KT900325	*Hydroides sanctaecrucis*
Seq24	GP0178	KT900282	*Haplosyllis* sp.	Seq68	GP0222	KT900326	*Chitinopoma serrula*
Seq25	GP0179	KT900283	*Pseudonereis* sp.	Seq69	GP0223	KT900327	*Pomatoceros triqueter*
Seq26	GP0180	KT900284	*Perinereis cultrifera*	Seq70	GP0224	KT900328	*Spirobranchus latiscapus*
Seq27	GP0181	KT900285	*Platynereis dumerlii*	Seq71	GP0225	KX290696	*Thormora* sp.
Seq28	GP0182	KT900286	*Platynereis dumerilii*	Seq72	GP0226	KX290697	*Bhawania cryptocephala*
Seq29	GP0183	KT900287	*Namalycastis abiuma*	Seq73	GP0227	KX290698	*Bhawania cryptocephala*
Seq30	GP0184	KT900288	*Dendronereis aestuarina*	Seq74	GP0228	KX290699	*Perinereis* sp.
Seq31	GP0185	KT900289	*Namalycastis abiuma*	Seq75	GP0229	KX290700	*Perinereis* sp.
Seq32	GP0186	KT900290	*Platynereis australis*	Seq76	GP0230	KX290701	*Nectoneanthe soxypoda*
Seq33	GP0187	KT900291	*Nereis sandersi*	Seq77	GP0231	KX290702	*Hermenia verruculosa*
Seq34	GP0188	KT900292	*Glycera capitata*	Seq78	GP0232	KX290703	*Hermenia verruculosa*
Seq35	GP0189	KT900293	*Glycera alba*	Seq79	GP0233	KX290704	*Hediste atoka*
Seq36	GP0190	KT900294	*Eunice miurai*	Seq80	GP0234	KX290705	*Terebellides* sp.
Seq37	GP0191	KT900295	*Lysidice* sp.	Seq81	GP0235	KX290706	*Terebellides* sp.
Seq38	GP0192	KT900296	*Lysidice* sp.	Seq82	GP0236	KX290707	*Paralacydonia paradoxa*
Seq39	GP0193	KT900297	*Lumbrineris funchalensis*	Seq83	GP0237	KX290708	*Paralacydonia paradoxa*
Seq40	GP0194	KT900298	*Marphysa viridis*	Seq84	GP0238	KX290709	*Hesione* sp.
Seq41	GP0195	KT900299	*Ninoe nigripes*	Seq85	GP0239	KX290710	*Spiochaetopterus* sp.
Seq42	GP0196	KT900300	*Marphysa* sp.	Seq86	GP0240	KX290711	*Spiochaetopterus* sp.
Seq43	GP0197	KT900301	*Marphysa* sp.	Seq87	GP0241	KX290712	*Euclymene* sp.
Seq44	GP0198	KT900302	*Diopatra* sp.	Seq88	GP0242	KX290713	*Clymenura* sp.

3.2.3. Sequence alignment

Sequences alignment were used by CLUSTAL W (Thompson *et al*. 1994) using the default parameters for gap opening and gap penalty. The sequences were manually edited by eye using BioEdit (Hall 1999). For estimating the appropriate model of sequence evolution, a hierarchical likelihood ratio test (hLRT) was carried out as implemented in the program modeltest version 3.06 (Posada and Crandall 1998, 2001). The modeltest criteria show the Tamura-Nei substitution model (Tamura and Nei 1993) with equal base frequencies, invariant sites, and gamma distribution (TrNef + I + C) represents the optimal model with respect to the data. The maximum likelihood analysis was carry out by Phylogenetic Analysis using Parsimony (PAUP), version 4.0b8 (Swofford 2001) under the likelihood settings suggested by the result of the modeltest using the heuristic search option with TBR branch swapping (Fig 3.1). The phylogentic tree was viewed by the FigTree v1.3.1 (Rambaut and Drummond 2010).

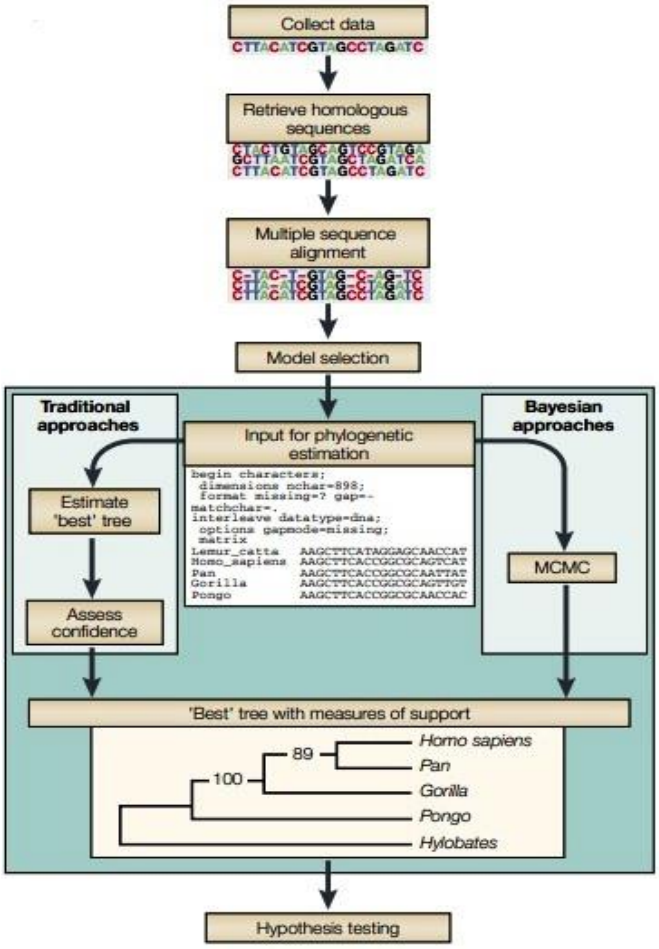

Fig 3.1. Flow chart showinging the phylogenetic analysis (source Holder and Lewis 2003)

Table 3.2. List of 18S rRNA gene primers used for PCR amplification and sequencing.

Name	Sequence (5'-3')	Position	Direction	Reference
18F	CTG GTT GAT CCT GCC AGT	3/21	Forward	Hillis and Dixon 1991
18F509	CCC CGT AAT TGG AAT GAG TAC A	548/569	Forward	Struck et al. 2002
18L	GAA TTA CCG CGG CTG CTG GCA CC	609/632	Reverse	Halanych et al. 1995
18R925D	GAT CYA AGA ATT TCA CCT CT	955/974	Reverse	Burnette et al. 2005
18F997	TTC GAA GAC GAT CAG ATA CCG	1044/1065	Forward	Struck et al. 2002
18R	GTC CCC TTC CGT CAA TTY CTT TAA G	1191/1215	reverse	Passamaneck et al. 2004
18F1435	AGG TCT GTG ATG CCC TTA GAT	1489/1509	forward	Burnette et al. 2005
18R1779	TGT TAC GAC TTT TAC TTC CTC TA	1811/1834	reverse	Struck et al. 2002
18R1843	GGA TCC AAG CTT GAT CCT TCT GCA GGT TCA CCT AC	1843/1877	reverse	Cohen et al. 1998

Table 3.3. Results of BLAST and polychaetes species identification tool at NCBI. Species identification cut-off of 99 to 100% was used. Samples showing similarity of less than 99% were not identified.

S. No	Specimen voucher	Morphological ID	NCBI Accession number	BLAST identification Species name (Aceession number)	Similarity (%)
1	GP0161-GP0163	*Eurythoe complanata*	KT900265-KT900267	*Eurythoe complanata* (JN086539)	99
2	GP0164	*Notopygos caribea*	KT900268	*Notopygos caribea* (KM055065)	99
3	GP0165	*Eurythoe complanata*	KT900269	*Eurythoe complanata* (JN086539)	99
4	GP0166	*Pareurythoe borealis*	KT900270	*Pareurythoe borealis* (JN086541)	100
5	GP0167-GP0168	*Thormora* sp.	KT900271-KT900272	*Thormora jukesii* (JN852840)	99
6	GP0169-GP0170	*Chloeia viridis*	KT900273-KT900274	*Chloeia viridis* (JN086537)	99
7	GP0171-GP0173	*Eurythoe complanata*	KT900275-KT900277	*Eurythoe complanata* (JN086539)	99
8	GP0174	*Hermenia verruculosa*	KT900278	*Hermenia verruculosa* (JN852830)	99
9	GP0175	*Chloeia viridis*	KT900279	*Chloeia viridis* (JN086537)	99
10	GP0176-GP0177	*Notopygos ornate*	KT900280-KT900281	*Notopygos* sp. (KM055065)	100
11	GP0178	*Haplosyllis* sp.	KT900282	*Haplosyllis* sp. (JF903907)	99
12	GP0179	*Pseudonereis* sp.	KT900283	*Platynereis dumerlii* (KT900285)	99
13	GP0180	*Perinereis cultrifera*	KT900284	*Platynereis dumerlii* (KT900285)	99
14	GP0181-GP0182	*Platynereis dumerlii*	KT900285-KT900286	*Platynereis dumerlii* (AY894303)	98
15	GP0183	*Namalycastis abiuma*	KT900287	*Namalycastis abiuma* (HQ157237)	100
16	GP0184	*Dendronereis aestuarina*	KT900288	*Namalycastis abiuma* (KT900289)	90
17	GP0185	*Namalycastis abiuma*	KT900289	*Namalycastis abiuma* (HQ157237)	100
18	GP0186	*Platynereis australis*	KT900290	*Platynereis dumerilii* (AY894303)	99
19	GP0187	*Nereis sandersi*	KT900291	*Nereis sandersi* (AM159579)	97
20	GP0188	*Glycera capitata*	KT900292	*Glycera capitata* (GQ426559)	99
21	GP0189	*Glycera alba*	KT900293	*Glycera unicornis* (KT989348)	99
22	GP0190	*Eunice miurai*	KT900294	*Eunice miurai* (GQ497480)	99
23	GP0191-GP0192	*Lysidice* sp.	KT900295-KT900296	*Lysidice* sp. (GQ497517)	99
24	GP0193	*Lumbrineris funchalensis*	KT900297	*Lumbrineris funchalensis* (AF412797)	99
25	GP0194	*Marphysa viridis*	KT900298	*Marphysa viridis* (GQ497508)	99
26	GP0195	*Ninoe nigripes*	KT900299	*Ninoe nigripes* (AY838852)	100
27	GP0196-GP0197	*Marphysa* sp.	KT900300-KT900301	*Marphysa cf. bellii* (GQ497511)	99
28	GP0198	*Diopatra* sp.	KT900302	*Diopatra aciculata* (AY838845)	99
29	GP0199	*Eunice miurai*	KT900303	*Eunice miurai* (GQ497480)	99
30	GP0200-GP0202	*Paraprionospio cordifolia*	KT900304-KT900306	*Aurospio foodbancsia* (EU340097)	96
31	GP0203-GP0204	*Paraprionospio patians*	KT900307-KT900308	*Aurospio foodbancsia* (EU340097)	96
32	GP0205	*Paraprionospio cordifolia*	KT900309	*Aurospio foodbancsia* (EU340097)	97
33	GP0206-GP0207	*Scolelepis* sp.	KT900310-KT900311	*Scolelepis squamata* (AF448164)	98
34	GP0208	*Magelona cincta*	KT900312	*Magelona* sp. (AY611454)	99
35	GP0209-GP0212	*Neosabellaria indica*	KT900313-KT900316	*Gunnarea capensis* (DQ317111)	99
36	GP0213-GP0214	*Sabellaria chandraae*	KT900317-KT900318	*Gunnarea capensis* (DQ317111)	100

37	GP0215	*Sabellaria intoshi*	KT900319	*Gunnarea capensis* (DQ317111)	100
38	GP0216-GP0217	*Terebella* sp.	KT900320-KT900321	*Terebella lapidaria* (JX423653)	98
39	GP0218	*Paraeupolymnia uspiana*	KT900322	*Lanice conchilega* (X79873)	98
40	GP0219-GP0220	*Parasabella saxicola*	KT900323-KT900324	*Sabella crassicornis* (AY527059)	93
41	GP0221	*Hydroides sanctaecrucis*	KT900325	*Hydroides sanctaecrucis* (EU184061)	99
42	GP0222	*Chitinopoma serrula*	KT900326	*Chitinopoma serrula* (DQ779643)	93
43	GP0223	*Pomatoceros triqueter*	KT900327	*Pomatoceros triqueter* (DQ317121)	99
45	GP0224	*Spirobranchus latiscapus*	KT900328	*Pomatoceros lamarckii* (DQ140404)	99
46	GP0225	*Thormora* sp.	KX290696	*Thormora Jukesii* (JN852840)	97
47	GP0226-GP0227	*Bhawania cryptocephala*	KX290697-KX290698	*Bhawania reyssi* (EU555035)	99
48	GP0228-GP0229	*Perinereis* sp.	KX290699-KX290700	*Platynereis dumerlii* (AY894303)	99
49	GP0230	*Nectoneanthe soxypoda*	KX290701	*Nereis succinea* (AY210447)	96
50	GP0231-GP0232	*Hermenia verruculosa*	KX290702-KX290703	*Hermenia verruculosa* (JN852830)	99
51	GP0233	*Hediste atoka*	KX290704	*Hediste* sp. (DQ442617)	100
52	GP0234-GP0235	*Terebellides* sp.	KX290705-KX290706	*Terebellides californica* (JN936462)	94
53	GP0236-GP0237	*Paralacydonia paradoxa*	KX290707-KX290708	*Paralacydonia paradoxa (DQ790088)*	98
54	GP0238	*Hesione* sp.	KX290709	*Hesione* sp. (DQ442617)	99
56	GP0239-GP0240	*Spiochaetopterus* sp.	KX290710-KX290711	*Phyllochaetopterus socialis* (DQ209212)	99
57	GP0241	*Euclymene* sp.	KX290712	*Euclymene oerstedi* (FJ612475)	99

3.3. Results

The phylogenetic relationship of polychaete taxa was reconstructed based on 18S rRNA gene sequence data. The most of the families form monophyletic clade were strongly supported by the ML method. We have performed 18S rRNA gene as a DNA barcoding of 54 polychaete species for the first molecular data set from the west coast of India. Identification of 34 polychaetes species were confirmed based on the DNA barcoding. It demonstreated that 18S rRNA gene as a reliable for DNA barcoding differentiated the species. In fact 54 polychaete species were newly sequenced based on the 18S rDNA and all together 88 sequences were submitted to NCBI GenBank (Table 3.1).

The result shows the two major clades; one clade (Errantia) comprising 30 species belonging to six families (Nereididae, Gyceridae, Lumbrineridae, and Eunicidae) and the next clade residing only Capitellidae. The entailing of the clade (Nereididae, Eunicidae, Lumbrineridae and Glyceridae families) has perfect distinct between families by strong bootstrap value support of 99 and 100 (Table 3.3). The classification of Polychaeta was based on the observed lifestyle and feeding strategies: Errantia, connecting the more or less free moving and predatory forms.

Aciculata is characterized by the presence of aciculae including mobile lifestyle, actively foraging and predating (Eunicida, Phyllodocida). The Errantia clade comprises the following taxa of Phyllodocidae, Glyceridae, Syllidae, Nereididae, Nephtyidae, Amphinomidae, Eunicidae families are strongly supported in ML analysis (Fig 3.3). The topology of Canalipalpata obtained by ML differs slightly in the position, which forms sister group and a close relationship to a clade consisting of Spionidae, Sabellaridae,

Serpulidae, Sabellidae families (Fig 3.3). These clades show adaptations of polychaete lifestyle (errant and sedentary), with the modification of morphological traits such as peristaltic movement, parapodia, elongated gills, and sensory organs (Struck *et al*. 2011).

The Palpata were characterized by the possession of palps and were further subdivided into two distinct groups: Canalipalpata, which feature the presence of peristomial grooved palps and include mainly burrowing deposit feeders or sessile tube-dwelling filter feeders (Spionidae, Sabellaridae, Serpulidae, Sabellidae). The monophyletic groups of Maldanidae, Trichobranchidae, Pectinariidae, Terebellidae families are highly dependent on the choice of method. The taxon is supported by bootstrap analysis of the unequally weighted ML analysis (<50%). Chaetopteridae, Ophellidae, Cirratulidae families form a monophyletic group when 18S rRNA molecular data sets were analyzed.

3.4. Discussion

3.4.1. Errantia

Yokoyama and Sukumaran (2012) reported the *Paraprionospio* specimens found from the west and northwest coast of India and identified them as *Paraprionospio cordifolia* Yokoyama, 2007, *Paraprionospio cristata* Zhou, Yokoyama and Li, 2008, and *Paraprionospio patiens* Yokoyama, 2007. The 18S rRNA sequence from *P. patiens* and *P. cordifolia* were submitted in NCBI webside. The *Eurythoe complanata* (Pallas, 1766) was observed in the study area which is commonly known as a fireworm and considered a circumtropical species, occurring in the Atlantic, Pacific and Indian oceans and the Mediterranean and Red seas. However, The Barroso *et al.* (2010) studied the morphological features, allozyme analyses, DNA analysis and molecular divergence of *E.*

complanata suggests that the true range was limited to the Atlantic Ocean, specifically from the Caribbean Sea to southern Brazil. A recent study focusing on two Syllidae (Errantia) species recovered two highly rearranged gene orders for *Ramisyllis multicaudata* and *Trypanobia cryptica*, suggesting a higher variability in some taxa (Aguado *et al.* 2015).

The 18S rRNA gene is evidently appropriate to solve relationships at family level. The clades may have emerged too fast in time to enable the accumulation of mutations. The phylogenetic tree gives evidence to confirm the polychaetes relationships. The fact that some closely related polychaetes do not cluster together in phylogenetic trees is all the more puzzling, since they belong to either the same genus or same superfamily (Fauchald 1977). The l8S rRNA gene of these taxa are essential to establish whether these anomalies result from biases (bad sequence, wrong identification, contamination) for true biological meaning.

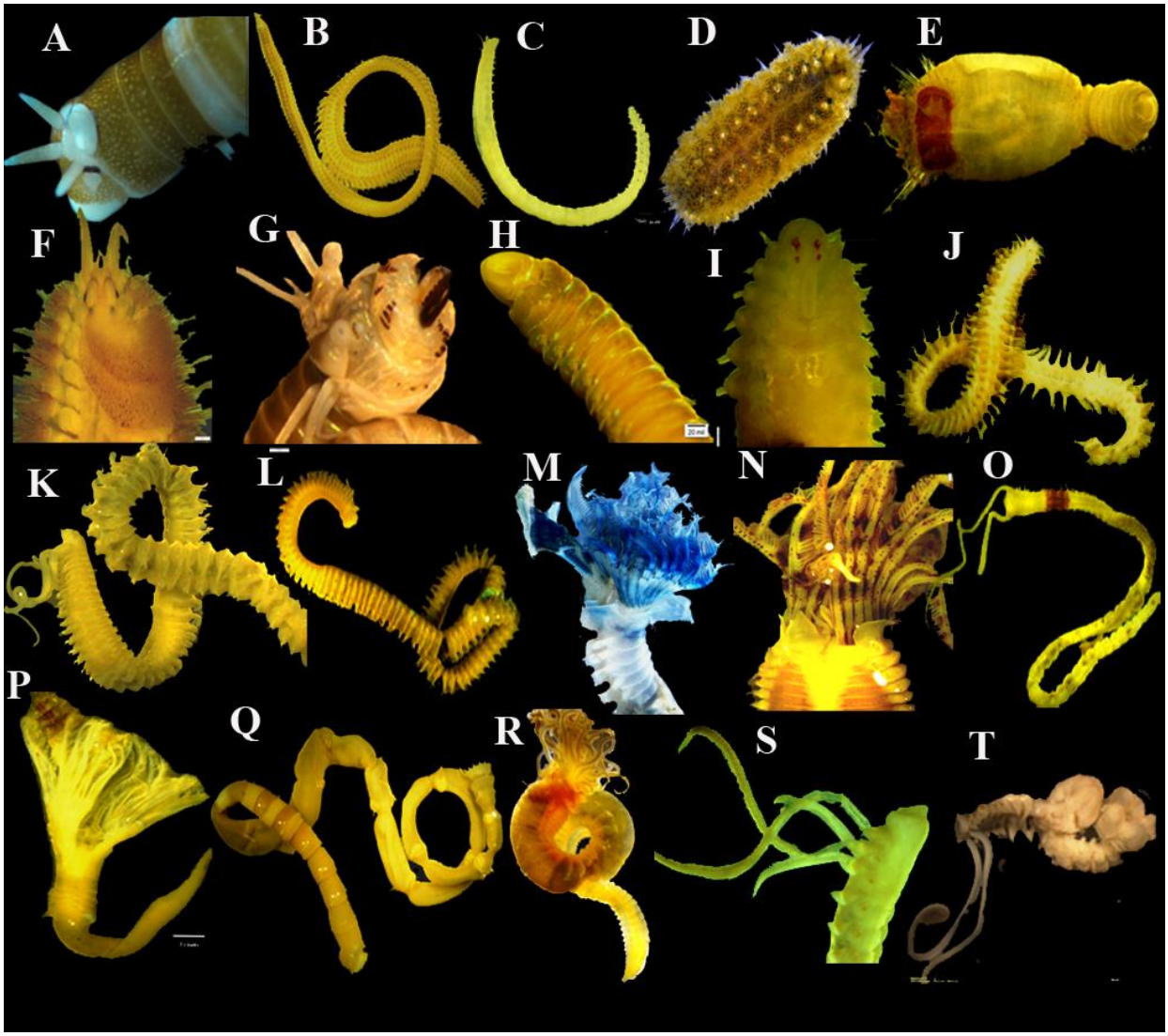

Fig. 3.2.Commonly occurring polychaete species-A: *Lysidice* sp., B: *Eteone heteropoda,* C: *Haplosyllis* sp., D: *Thormora* sp., E: *Sternapsis suctata*, F: G: *Perinereis cultrifera*, H: *Scoletoma funchalensis* I: *Pareurythoe borealis*J: *Ceratonereis japonica* K-L: *Scolelepis* sp., M: *Pomatoceros triqueter*, N: *Parasabella saxicola*, O: *Magelona cincta*, P: *Pomatostegus actinoceros* Q: *Euclymene* sp., R: *Terebella* sp., S: *Paraprionospio cordifolia*, T: *Spiochaetopterus* sp.

3.4.2. Sedentaria

The second clade (Sedentaria) comprised the sessile and tube-dwelling forms. As shown in figure 3.3 Polychaeta subdivided into two separate taxonomic clades: Palpata and Scolecida (Rouse and Fauchald 1997). The phylogenetic relationship within Chaetopteridae family was assessed by Moore *et al*. (2017), and placed as a monophyletic group based on the COI, nuclear 28S, and 18S rDNA genes as well as the morphological features. *Chaetopterus* and *Mesochaetopterus* taxa formed well-supported sister clades based on 18S rDNA and mtCOI genes sequence analysis (Osborn *et al*. 2007; Martin *et al*. 2008; Moore *et al*. 2017). However, the *Spiochaetopterus* and *Phyllochaetopterus* were the paraphyletic/polyphyletic groups (Osborn *et al*. 2007; Zhang *et al*. 2015).

The most investigated polychaetes belonging to Errantia and Sedentaria allow an identical order of ribosomal RNA gene, the phylogenbetic reconstruction allows the common ground pattern for polychaetes taxa. The Sedentaria comprise most of the polychaete families classified as Canalipalpata or Scolecida. Oweniidae, Magelonidae, and Chaetopteridae form a separate branch as a basal evaluation of major radiation (Weigert and Bleidorn 2016). Whereas the Trichobranchidae, Pectinariidae and Terebellidae families to delineate the sister group is strongly supported by ML analysis. Similar results were reported by various authors using 18S gene sequence (Rouse and Fauchald 1997; Bleidorn *et al*. 2003; Rousset *et al*. 2007; Lehrke *et al*. 2007).The highlights of DNA barcoding should be considered a complementary method of species identification for diversity investigations (DeSalle *et al*. 2005). The lacking of reference

sequences on public databases is most important in species or families and as DNA barcodes would not be able to connect individuals to a known species. There have been increased numbers of unidentified specimens which limits the use of their sequences in future studies of biogeography or for management tools.

The ML analyses in the present study recovered many relationships and consistent with results obtained from analyses of 18S rRNA gene sequences. The results conclude that the 18S rRNA gene may contain the historical signal, perhaps as a consequence of the evolutionary constraints imposed on the sequences. The slow evolving regions are informative regarding recent divergences and relationships within major groups of benthic polychaetes. Consequently, the contribution of new data on molecular sequence of 18S rRNA gene will support the execution of further studies to help discover new patterns that trigger the diversity. Most of the polychaete families have monophyletic groups. However, the biogeography, and functional traits are remaining to be investigated, which could be a primary focus of future DNA barcoding projects. This study contributes to the ongoing research effort to document, describe and underst the diversity, biogeography and functionality of polychaetes along the west coast of India. The present study is important for effective research-driven ecosystem based management of the rapidly changing benthic ecosystem. These results suggest that the assumption on the basis of a broader polychaetes sampling and the phylogenetic position of controversial discussed taxa could be inferred by using 18S rRNA sequence data.

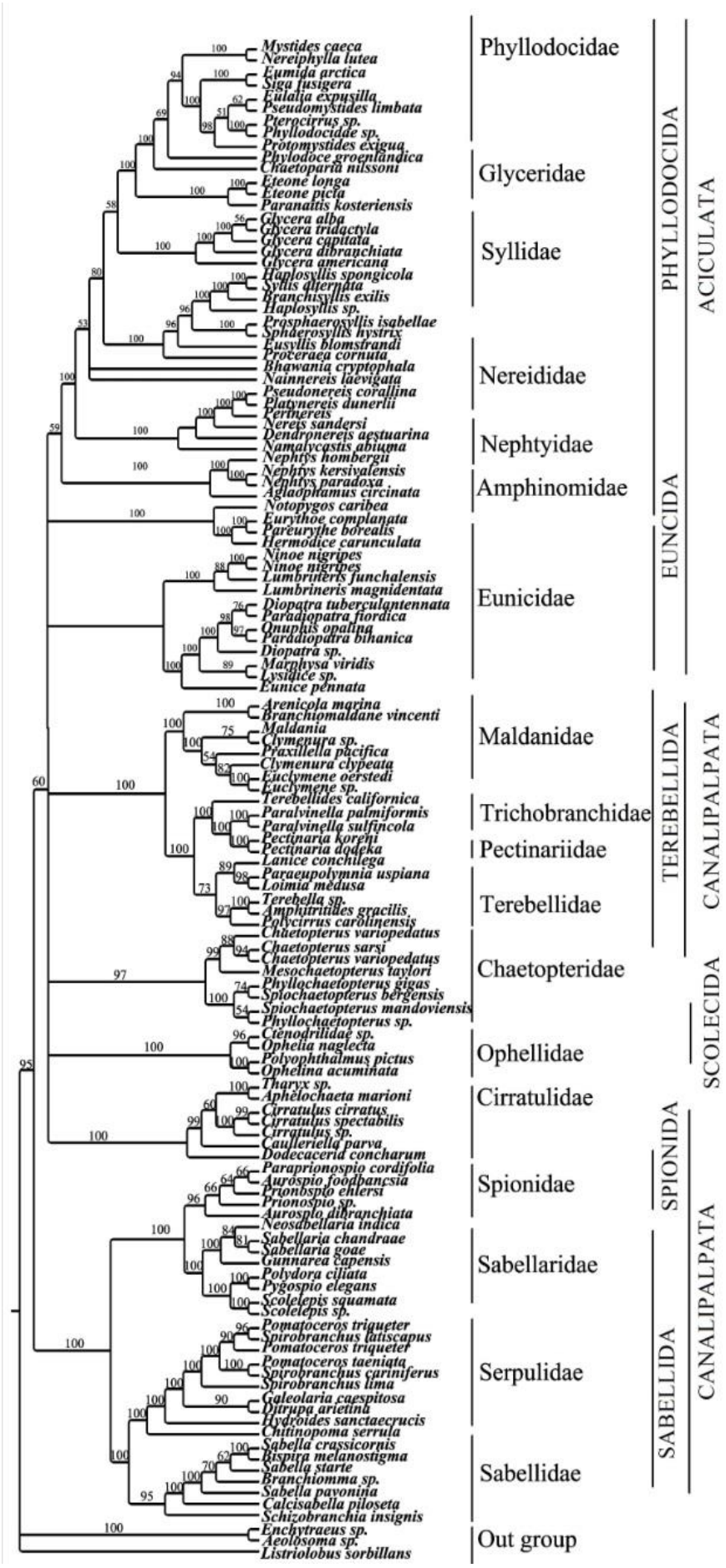

Fig 3.3 Maximum Likelihood tree of studied polychete species based on 18S rRNA gene sequence data.

Chapter 4

Low genetic diversity in the reef-building polychaete *Sabellaria chandraae* de Silva, 1961 along the west coast of India

4.1. Introduction

Distribution and genetic diversity of organisms are influenced by water circulation pattern, availability of suitable habitat, population, fecundity and recruitment success (Siegel *et al*. 2008; Weersing and Toonen 2009; Villamor *et al*. 2014; Dawson 2014). The larval dispersal plays an important role in both the genetic structure and connectivity of populations in marine benthic species. Traditionally, planktotrophic larvae have been considered to have higher dispersal capability than lecithotrophic larvae (Cowen and Sponaugle 2009). Generally, the very low geographic genetic variations are expected to show in the marine organisms due to the lack of clear barriers to dispersal and even for species with pelagic larvae in many species (Palumbi 1992). Increasing marine based genetic studies have revealed a wealth of 'cryptic species' (Knowlton 1993; Knowlton 2000).

Molecular tools are playing an important role in marine biodiversity within populations, which determine their evolutionary beginning in the face of environmental change (Lande and Shannon 1996; Bucklin *et al*. 2010). Hence, the population connectivity studies receiving considerable attention in marine ecosystem management (Stephenson and Clark 2002). Marine benthic invertebrates serve as an excellent model to underst and how the interaction among dispersal dynamics, environmental conditions, biotic interactions or historical isolation influences the species distribution pattern (Avise 1992; Palumbi 1994). Flavia *et al*. (2017) found that reef-building polychaetes *Phragmatopoma caudata* was able to maintain genetic connectivity across the geographical range and long-distance connectivity due to long-lived larvae that tolerant to a wide range of environmental conditions. The distribution patterns of benthic fauna along the Indian

coast are affected by ‘soft’ biogeographic barriers: differences in temperature, habitat heterogeneity, habitat availability and spatio-temporal variation in the coastal boundary currents, upwelling, Oxygen Minimum Zone and freshwater discharge (Sivadas and Ingole 2016).

Marine polychaetes from the family Sabellariidae Johnston, 1865 are gregarious and are important reef-building organisms in coastal environments world-wide (Goldberg 2013). The Sabellariidae reef provides various spatial and trophic niches for various organisms to colonize and develop (Dubois *et al*. 2006). The reef of many polychaetes is the refuge for the juveniles of some commercially important species (Rabaut *et al*. 2010). Their distribution is inhibited by the availability of stable substrates for the settlement of immature larval stages and sedentary adult stages (Kirtley 1992). They are filter feeders and need a constant food supply, high levels of dissolved oxygen, and suspended sediments for tube building (Volvelle 1965) and naturally appear in large colonies along the west coast of India. Reefs of *Sabellaridae* have been reported along the South and West coast of India (Badve 1996). These sandy reefs were observed to be inhabited in the rocky surfaces as well as boulders extending upto a depth of 12 to 15m. The polychaete species found on this coast were *Sabellaria chandrae* and *S. simplex* which form sandy coral structures. While *Sabellaria spinulosa* is distributed on the rocky areas of the Southwest coast of India thatis characterized by high wave action. The other reef forming sabelaridea species include *Phragmatopoma* and *Neosabellaria clandestina* (Menon 1966; Badve 1996).The *S. chandraae* de Silva, 1961 are broadly distributed along the intertidal zone of the Indian and Sri Lanka coast (Nishi *et al*. 2010).

Eckelbarger (1977) and Pawlik (1988) reported that the larvae growth rate reared from fertilization to metamorphos is stage was observed from 6 to 32 weeks in the laboratory adult and a long planktonic larval stage. Once they have settled and metamorphosed, the worms quickly become reproductively mature and could live for 1–2 years (Kirtley 1966; McCarthy *et al*. 2003; McCarthy 2001; Simmon *et al*. 2005). As a result, the larval dispersal pattern and thus the distribution range of the adults would be expected to reflect their interaction with oceanographic conditions especially the coastal currents (Sherman, 2008). The distribution of wide-range species should have relatively high dispersal capabilities with low genetic structuring (Jones *et al*. 2004). The habitat selection by larvae of reef-building polychaete species and massive construction reefs in the intertidal rocky shore area, require the strong flow of water for the transport of the tube-building material and reasons its presents in the west coast of India (Achari 1974).

Indian subcontinent with its unique geological history and natural gradient in environmental features and complex oceanographic processes provides the useful model to understand the marine biogeography patterns and the underlying factors. However, few studies have focused the effect of environmental heterogeneity on the distribution of coastal species assemblage (Sivadas and Ingole 2016). Despite the ecological value and wide distribution of *S. chandraae* along the west coast of India, no molecular study has been carried out to examine the genetic variation and population structure. This study is to assess the genetic connectivity among the population of *S. chandraae,* the dominant reef-building species in the west coast of India.

4.2. Materials and Methods

4.2.1 Study area and Sample collection

The survey was conducted along the 1260 km coastline of the west coast of India to study the distribution pattern of reef-forming Saberallridae. For molecular study, the specimens of *S. chandraae* collected during September and October 2015 from eight localities: the Northern Konkan coast (Mumbai-Goa) include localities of Ganapati Pule (GP), Devgad (DG), Vengrula (VG), Anjuna (AJ), and Palolem (PL); whereas the Southern region Mangalore (MG), Kannur (KN) and Kanyakumari (KK) (Fig 4.1, Table 4.1) were used for molecular work. A total of 143 individuals of *S. chandraae* specimens were collected during low-tide, from breaking small blocks of reefs and extracting 2-4. Specimens were also collected using a hand net from the reefs and preserved in 95% ethanol until DNA extraction. The species were identified based on the key characteristic described in Nishi *et al*. 2010.

Table 4.1. Details of sampling locations and GPS coordinate with numbers of individuals NCBI Genbank accession numbers.

Samplinglocations	Latitude	Longitude	COI	ITS
Ganapatipule (GP)	17°08'42" N	73°16'04" E	KX525516-KX525585	KX470627-KX470706
Devgad (DG)	16°22'24" N	73°22'16" E		
Vengurla (VG)	15°51'06" N	73°38'24" E		
Arampol (AP)	15°41'18" N	73°42'14" E		
Palolem (AK)	15°00'32" N	74°1'13" E		
Mangalure (MG)	12°59'19" N	74°47'58" E		
Kannur (KN)	11°52'02" N	75°21'10" E		
Kanyakumari (KK)	08°04'38" N	77°31'50" E		

Fig 4.1. Sampling sites along the west coast of India

4.2.2. DNA extraction and PCR amplification

Total genomic DNA was extracted using the commercial QIAamp Tissue Kit (QIAGEN). Fragments of one mitochondrial cytochrome oxidase I gene (mtCOI) and the internal transcribed spacer (ITS2) region were used as molecular markers (Table 4.2). The partial sequences of mitochondrial COI fragments were amplified using the universal primers PolyCO1_R and PolyCO1_F, under the polymerase chain reaction (PCR) (Carr et al. 2011). Internal transcribed spacer one (ITS) regions were amplified using the primer pair ITS1, 5.8S, and ITS2 (Nygren 2009). The PCR reactions were carriedout in a total

volume of 50 µl that consisted of 50–100 ng genomic DNA, 1 × PCR buffer, 2.5 mM $MgCl_2$, 0.2 mMd NTPs, 10 µM primers, and 5U Taq DNA polymerase (Sigma). The temperature profile for the mtCOI was as follows: 94°C for 3 min; 30 cycles of denaturation at 94°C for30 s, annealing at 54.5°C for the 30s and extension at 72°C for 60s; followed by a final extension at 72°C for 5 min. Polymerase chain reactions for the ITS1 region were carried out using the following PCR protocol: 95°C for 3 min; 35 cycles of denaturation at 95°C for 60s, annealing at 48°C for 60 s and extension at 72°C for 90 s; followed by a final extension at 72°C for 1 min. The PCR products were examined on 1% agarose gels, stainedwith Genecolour TM (Biotium, USA), and photographed with transmitted illumination. PCR-amplified DNA fragments were purified using QIAquick gel purification kit according to manufacturer's instructions (QIAGEN). Sequences were produced using the same primers and determined on an Applied Biosystems (ABI) 3730xl automated DNA sequencer, following the standard cycle sequencing protocol. The sequences were submitted to NCBI web site.

Table 4.2. List of primers used for amplifying COI and ITS markers in *S. chandraae*

Primer	**Orientation**	**Sequence (5´- 3´)**	**Reference**
ITS Gene			
ITS18SFPOLY	Forward	GAGGAAGTAAAAGTCGTAACA	Nygren *et al*. 2009
ITS5.8SFPOLY	Forward	GAATTGCAGGACACATTGAAC	Nygren *et al*. 2009
ITS5.8SRPOLY	Reverse	GTTCAATGTGTCCTGCAATTC	Nygren *et al*. 2009
ITS28SRPOLY	Reverse	ATGCTTAAATTCAGCGGGT	Nygren *et al*. 2009
COI Gene			
PolyCOI_R	Reverse	GAYTATWTTCAACAAATCATAAAGATATTGG	Carr *et al*. 2011
PolyCOI_F	Forward	TAMACTTCWGGGTGACCAAARAATCA	Carr *et al*. 2011

4.2.3. Molecular data analyses

DNA sequence chromatograms were checked for errors and edited with Sequencher 4.5 (Gene Codes Corp, Ann Arbor, MI, USA). Published sequences of *S. chandraae* are given the Genbank accession numbers in Table 1. Sequences of mtCOI and ITS genes were aligned using CLUSTAL W (Thompson 1994) implemented in BIOEDIT (Hall 1999) with default gap weighting parameters and adjusted by eye as necessary. Hierarchical Analysis of MOlecular VAriance (AMOVA) was used to separating of genetic variance within and among the localities and between the two regions (Northern and Southern). Genetic differentiation was examined by means of pairwise F_{st} values, using 10, 000 permutations and to determine significance in Arlequin 3.5 (Excoffier and Lischer 2010). The Haplotype (h) and nucleotide (π) diversities were estimated using DnaSP v5 (Librado and Rozas 2009). The demographic history of *S. chandraae* was inferred using mismatch distribution analyses applied in DnaSP v5 (Librado and Rozas 2009). The population distribution is multimodal drawn from populations at demographic equilibrium in samples; however, it is usually unimodal following a recent demographic expansion (Librado and Rozas 2009). Mismatch distribution analyses were used to estimate possible historical events of population growth and the decline in the assumption of selective neutrality (Rogers and Harpending 1992). Gene flow (*Nm*) was calculated to assess the degree of genetic differentiation between populations using DnaSP v5. The clustering method was carried out using the Spatial Analysis of MOlecular Variance, SAMOVA 1.0 (Dupanloup *et al*. 2002) was implemented to identify the groups of populations, based on the proportion of the total molecular variance explained by differences between groups (F_{CT}). The number of groups was denoted as k. The statistical

variance was evaluated with 1000 random permutations repeatedly running. The k value that maximized F_{CT} was understood as the most likely geographic subdivision. The package Network 4.6.0.0 was used for a median-joining network based on maximum parsimony to reconstruct the relationships among haplotypes. The oretical distributions in the assumption of constant population size and the sudden expansion model were evaluated to the observed data.

4.3. Results

4.3.1. Genetic diversity

A total of 63 sequences of *S.chandraae* had the length of 610 base pair (bp) from the mtCOI gene (KX525516-KX525585) and 80 nuclear ITS gene sequences were 850 bp length (KX470627-KX470706). Total haplotype diversity values from the mtCOI, ITS1, and ITS2 gene sequences being 0.21, 0.352 and 0.423, respectively (Table 4.3). From the COI fragment, two different haplotypes were obtained among the sampled locations, one haplotype restricted to Northern population and one to the Southern population. Total nucleotide diversity of mtCOI, ITS1 and ITS2 genes sequence were 0.0005, 0.001 and 0.0018%, respectively (Table 4.3). In total, seven unique haplotypes were identified among the ITS2 gene sequences that contained polymorphic sites at the end of the region were parsimony informative, whereas, the ITS1 gene sequences revealed asingle haplotype.

Table 4.3. Details of nucleotide and haplotyde diversity showed from mt COI and ITS genes sequences in *Sabellaria chandraae* along the west coastal India waters

	COI				ITS1				ITS2		
Geographic region	**No. of sequences**	**No. of haplotypes**	**Haplotype diversity h**	**Nucleotide diversity π**	**No. of sequences**	**No. of haplotypes**	**Haplotype diversity h**	**Nucleotide diversity π**	**No. of haplotypes**	**Haplotype diversity h**	**Nucleotide diversity π**
Northern region											
GP	8	2	0.250	0.0005	10	2	0.355	0.0009	3	0.511	0.0024
DG	9	4	0.694	0.001	10	2	0.555	0.0015	3	0.377	0.0022
VG	9	2	0.200	0.0007	10	2	0.466	0.0015	3	0.511	0.0028
AJ	6	1	0.333	0.0005	10	2	0.355	0.0009	3	0.666	0.0039
PL	10	1	0	0	10	2	0.355	0.0009	3	0.644	0.0028
Southern region											
KN	0	0	0	0	10	2	0.200	0.0005	2	0.533	0.0028
MG	10	1	0	0	10	2	0.200	0.0005	3	0.533	0.0019
KK	10	1	0	0	10	2	0.333	0.0009	3	0.533	0.0019
Total	63	7	0.21	0.0005	80	2	0.352	0.001	7	0.423	0.0018

4.3.2. Population genetic structure

The Analysis of MOlecular VAriance (AMOVA) test showed a lock genetic structure based on the distance method (Tamura and Nei 1993). The analysis revealed that most of the genetic variation retained both among populations and within populations for mtCOI and ITS genes (Table 4.4). The AMOVA of the (North and South) two genetic clusters of structure analysis showed that 7.61 and 3.61% variation among regions and 94.19 and 99.32% within populations from the ITS1 and ITS2 genes, respectively. These results suggested that populations were still no structured within populations. The AMOVA revealed no genetic differentiation among the *S. chandraae* population within the west coast population (between North and South) for mtCOI, ITS1 and ITS2 (F_{CT} = 0.036, 0.076 and 0.036; p>0.795, 0.097 and 0.099, respectively) and among populations (F_{ST} = 0.086, 0.058 and 0.007; p>0.1, 0.5 and 0.7, respectively). The mismatch frequency spectra for the eight populations are shown in 4. 2. The observed mismatch distributions of all the populations sampled along the west coast of India showed a deficiency of mutations shared by many individuals in the population, a scenario in conformity with the recent expansion model (Fig 4.2). The network analyse for *S. chandraae* was generated with all the ITS2 haplotype sequences because very few haplotypes were identified. The median-joining network analysis revealed one direction of haplotype network (Fig 4.3). The most frequent haplotype (Hap 1), shared by all geographic regions, was inferred to be the ancestral haplotype, whereas the most derived ones are linked to this haplotype with a maximum branch length of one mutational steps. The SAMOVA results showed genetic structure with no significant (Table 4.4) and, to characterize the spatial genetic structure. The optimal k=2, that defines the probable number of geographic subdivisions.

Table 4.4. Analysis of molecular variance (AMOVA) of *Sabellaria chandraae* between: two-groups (North and South groups) identified by phylogenetic analysis. Northern regions group I Ganapati Pule (GP), Devgad (DG), Vengrula (VG), Anjuna (AJ), and Palolem (PL); Southern regions group II Mangalore (MG), Kannur (KN) and Kanyakumari (KK). Significant values ($P< 0.05$).

Source of variation	df	Sum of squares	Percentage of variation	Fixation index	*P*
COI					
Among groups	1	16.58	100	F_{SC}=0	0.000
Among populations within groups	5	0.00	0.00	F_{ST}=1	1
Within populations	63	0.00	0.00	F_{CT}=1	0.029
ITS1					
Among groups	1	4.166	7.61	F_{SC}=-0.0194	0.3030
Among populations within groups	4	12.42	-1.80	F_{ST}=0.0581	0.518
Within populations	64	0	94.19	F_{CT}=-0.0760	0.097
ITS2					
Among groups	1	0.470	3.61	F_{SC}=-0.030	0.543
Among populations within groups	7	1.204	-2.93	F_{ST}=0.007	0.690
Within populations	66	15.006	99.32	F_{CT}=-0.036	0.099

*Significant values ($P< 0.01$).

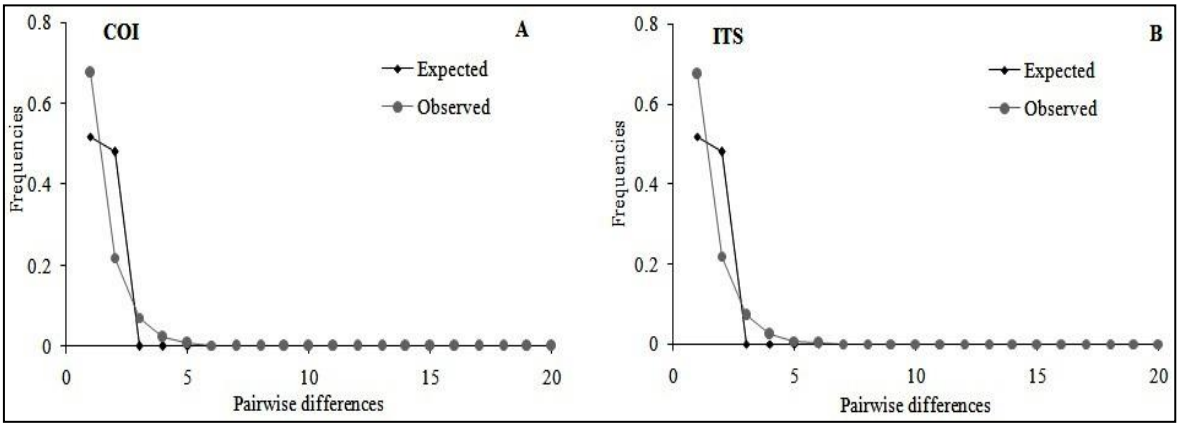

Fig 4.2. Mismatch distribution of COI and ITS haplotypes in *Sabellaria chandraae* along the west coast of India. The expected frequency is based on the population growth-decline model (initial theta = 0.96, final theta =1000, tau = 1.427), determined using the DNAsp v5 program (Librado and Rozas 2009).

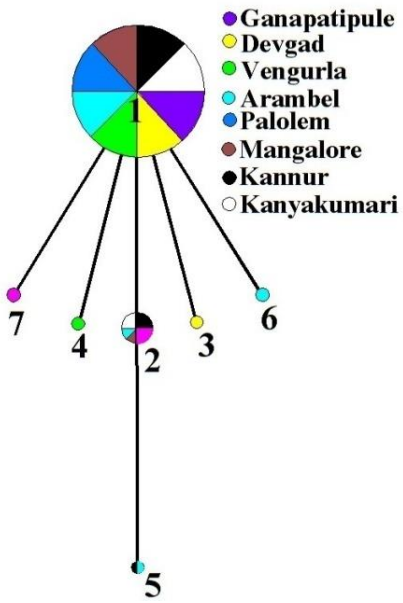

Fig 4.3. Median-joining network for all *Sabellaria chandraae* ITS2 haplotypes and size of the circle represent the haplotype frequency. The size of each circle is proportional to the number of individuals sharing this haplotype. Lines between circlers present mutational steps. Colours indicate the geographical origin of the populations.

4.4. Discussion

The reef-building polychaetes of S. *chandraae* along the west coast of India have low genetic variation based on the partial mitochondrial COI and ITS gene sequences. The AMOVA tests showed no significant genetic differentiation between the northern and the southern coast of India (Table 4). The results indicate that *S.chandraae* may have a similar origin or come from single population along the west coast of India. The different level of genetic variation in many benthic species is determined by coastal water circulation, availability of substrates, population size, richness and stochasticity of recruitment success (Siegel *et al*. 2008; Weersing and Toonen 2009; Villamor *et al*. 2014; Dawson *et al*. 2014). A low genetic variation of *S. chandraae* population of west coast suggests that the genetic exchangeability is not constrained along the ~ 1260 km heterogeneous west coast of India. Further, the estimation of genetic diversity suggested the *S. Chandraae* is genetically comprised of a single population indicates high interbreeding among the population. The phylogeography of *Phragmatopoma* population have studies using cox-1 and ITS-1 genes in the western Atlantic Ocean (Nunes *et al*. 2016). The high levels of connectivity across the species range, possibly due to high gamete density upon spawning, long pelagic larval stage. The planktonic larvae were tolerant to a wide range of temperatures, and salinity. However, the larval dispersal was not efficient barriers by Amazon plume, other major rivers along the coast of Brazil or the upwelling in Cabo Frio. The population connectivity maintained along the entire coast of Brazil and between Brazil and the eastern Caribbean (Flavia *et al*. 2017). Generally, the *Sabellaria* spp. has long pelagic larvae stage more than

two weeks, (Eckelbarger 1977; Pawlik 1988), which could have allowed displacement of planktonic larvae along the ~ 1260 km coast line of the study area with high current speed (as high as 3.7 m/sec). Therefore, the lack of genetic structure with low genetic diversity among the *S. chandraae* populations could be due to the high level of gene flow along the west coast.

The boundary currents play an important role in the species distribution patternin the coastal system. The different biogeographic regions of the North Indian Ocean have the potential to connect by the reversing of monsoonal currents (Obura 2012). The Western India Coastal Current (WICC) and Eastern India Coastal Current (EICC) are seasonally reversing coastal boundary currents, along with the west and east coast of India, respectively. The WICC flows toward the equator during the Indian summer monsoon. The benthic polychaetes showed 34% of the species being common between the western and eastern basins which was also confirmed from the genetic (Menezes *et al*. 2002; Divya *et al*. 2012; Kumar *et al*. 2012; Kunal *et al*. 2013) and larval dispersal studies (George *et al*. 2011). Sivadas and Ingole (2016) reported the high similarity of benthic species from Mumbai to North of Kerala coast. Hence, the similar habitats and reversing currents would have attributed for the similarity within the west coast region of India. Further, the major recruitment period for most benthic organisms along the Indian coast is during the monsoon season. This period is characterized by the upwelling induced high primary productivity to provide food for the benthic planktonic larvae. Therefore, food availability, current pattern and similar habitats along the west coast of India favoured the dispersal and successful recruitment of *S. chandraae*. There is no barrier for

larval movement among study sites is further confirmed by the low values of F_{ST}. In addition, in substantial genetic differentiation of mtCOI and ITS genes may result from the high dispersal of larvae that can travel long distances. Moreover, the dispersal ability of *S. chandraae* has positive implications for their elasticity, as evidenced by long planktonic larval phase, constant with the potential of wide spread dispersal. A wide-ranging species with high dispersal capabilities should have the relatively low genetic structure (Jones *et al*. 2004).

The low genetic variability observed in *S. chandraae* population is consistent to findings of other species from Sabellaridae family. The populations of *Idanthyrsus cretus* were found to have high rates of gene flow in the Las Perlas Archipelago, Peru influenced by the Peru Current and the South Equatorial Current (Barrios *et al*. 2009). The populations of *Eurythoe complanata* was well connected along 2500 km coast of the Caribbean and Brazil as the teleplanic larvae were elated by the westward South Equatorial Current and the eastward South Equatorial Counter current (Barroso *et al*. 2010). The influence of ocean currents in the dispersal of larvae is further confirmed from the wide spread population connectivity of *Pectinaria koreni* along the north coast of France (Jolly *et al*. 2004).

The findings support that the *S. chandraae* population sampled along the ~1260 km west coast of India represents a single population. A combination of historical factors, reversing WICC, habitat availability and local adaptation may, therefore, explain the low genetic diversity the west coast populations.

Therefore, the environmental condition favors benthic population with low levels of genetic diversity making them particularly unsuitable for the rapidly changing marine environment.

Chapter 5_A

New species of *Neosabellaria indica* sp. nov. (Annelida: Polychaeta: Sabellariidae) from the Central West Coast of India

5A.1. Introduction

Sabellariidae Johnston, 1865 is a well-defined and highly specialized group of marine annelids commonly known as honeycomb or sandcastle worms (Capa and Hutchings 2014). Sabellariids are well-known as reef builders in shallow waters throughout temperate to tropical oceans (Kirtley 1992, 1994; Pandolfi *et al*. 1998; Bailey-Brock *et al*. 2004). Currently, there are 130 nominal species in the family (Hutchings et al. 2012; Capa and Hutchings 2014). Also, two species *Lygdamis indicus* Kinberg, 1866 and *L. porrectus* Ehlers, 1908 (currently belonging to the genus *Tetreres* Caullery 1913; WoRMS 2015) were also known from the area adjacent to the Indian waters (Achari 1974). *Phragmatopoma* sp. recorded by Achari (1974) might be *N. clandestina* (Kirtley 1994). *Sabellaria simplex* was synonymized with *Neosabellaria clandestina* (Kirtley 1994). Currently, 16 species of five genera-*Idanthyrsus*, *Sabellaria*, *Neosabellaria*, *Lygdamis*, and *Tetreres*, were recorded in India and adjacent region.

Recent taxonomical information is summarized in Capa and Hutchings (2014) and other taxonomic papers (e.g. Nishi *et al*. 2009, 2015). *Neosabellaria* includes seven species restricted to the Indo-Pacific region (Bailey-Brock *et al*. 2007; Capa and Hutchings 2014). The sabellariid species, one is *Neosabellaria* was recently collected around Kunakeshwar from the west coast of India.

5A.2. Materials and methods

5A.2.1. Study area

The polychaete samples were collected during October of 2014 from the rocky intertidal zone of the Kunakeshwar (16°59′40″N, 73°18′00″E) beaches located along the central west coast of India, (Fig. 5A.1). The distance between the two stations is ~150 km.

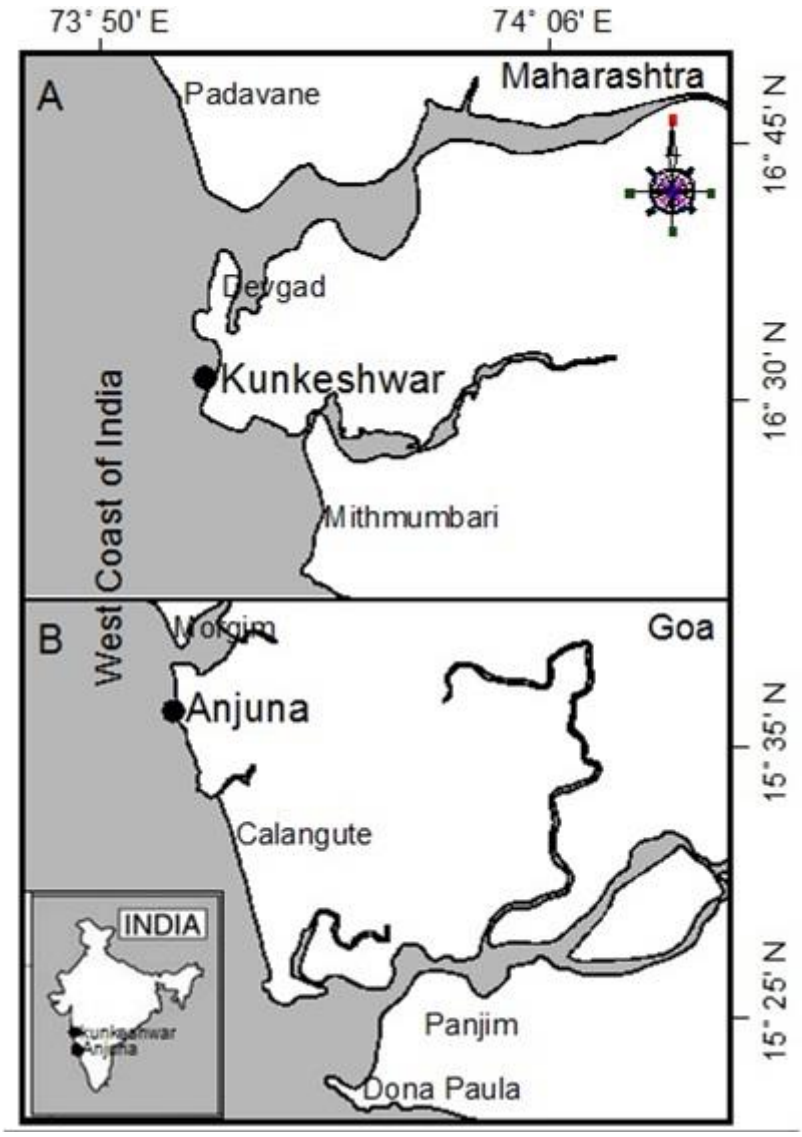

Fig 5A.1. Map showing sample collection sites: Anjuna and Kunkeshwar from the west coast of India.

Fig 5A.2. Sample collection area in the Kunkeshwar along the west coast of India

Neosabellaria indica sp. nov. is distributed from the west coast of India and their distribution is related to the sediment type. The builds their sand tubes on rocky shores (Fig. 5A.2). *Neosabellaria indica* sp. nov. prefer very fine sand (particle size is 65–125 µm) (Fig 5B.2B) and were observed mostly in riverine mouth area (Fig 5B.2B). The availability of food and sand particle on the water column were enhanced during monsoon season and as the river carry the fine sand into the coastal area.

5A.2.2 Laboratory analysis

Specimens were collected by hand, fixed with neutralized formalin, and preserved in 70% ethanol. Specimens were studied under a dissecting and a

compound microscope. As in Capa *et al*. (2015), some specimens were stained with methyl green to improve the contrast of structures for photography. Data referring to the holotype are given in the species description, with the variation observed among paratypes provided in parentheses. Two worms were dissected, mounted on stabs, coated with palladium, and viewed under scanning electron microscope (SEM) JEOL 5600LV. Type specimens and comparative material were deposited in National Museum of India, belonging to the Zoological Survey of India (ZSI), India.

Family Sabellariidae Johnston, 1865

Genus *Neosabellaria* Kirtley, 1994

Neosabellaria indica sp. nov.

Figures 3, 4, 5

5A.2.4. Material examined:

5A.2.4.1. Holotype: CMNH ZW 01900

Intertidal rocky zone at Kunkaleswar (16°59′40″N, 73°18′00″E), Maharashtra, India, October, 2014, coll. by Periasamy, R. and Baban Ingole.

5A.2.4.2. Paratypes, ZSI/ANRC-14816-14820, 5 specimens, same collection data as for holotype

5A.2.4.3. Comparative material:

Neosabellaria vitiensis Bailey-Brock et al. 2007, non-type, YNU M-Iv.-Pol. – 0005, Suva, Fiji; mangrove area, intertidal, 15 June 2004, coll. S. Pohler. *Neosabellaria cementarium* (Moore, 1906), Nakanose, Akkeshi, Hokkaido, by dredge, collected by T. Kato, CMNH-ZW00873, 00874, 1 July 1995; (same site

and by same collector):-ZW01202, 5 July 1994;-ZW01204, 15 April 1998;-ZW01205, 21 June 2001 (cited by Nishi and Kato 2002).

5A.2.5. Description: Holotype 15 mm in body length excluding cauda, cauda 2.0 mm in length, width at thorax 2.0 mm. Three parathorax chaetigers has 28 abdominal chaetigers. CMNH ZW 01900 with the short compact body, colourless except for scattered pigment around the lateral side of opercular lobes (Fig. 5A.5). Operculum short, width similar to length, with lobes, completely separated along their length. Paleae arranged in three semicircular concentric rows (Fig 5A.4A, B, D), inner and middle row paleae overlapping. Outer row with 30 pairs [30–34] of geniculate golden paleae with flattened blades, approximately 0.3 mm long, 0.1 mm wide (Fig 5A.5B), blade with thecal bands, smooth lateral margins and small tip without midline plume (Figs 5A.4B, C); distal part of some outer paleae partly broken, cleaved into 2–5 smaller lateral lacina (laciniate tip) (Fig 5A.5F). The middle row of the single form, with 16–18 pairs of paleae, approximately 0.2 mm long, shoe-shaped, geniculate, with broad and concave blades directed outwards and bluntly tapering tips. Inner row with 12 [10–14] pairs, approximately 0.1–0.2 mm long, broadly flattened, geniculate, smooth in lateral side, with pointed tips (Figs 5A.4G, 4D). Outer paleae extended from inner and middle rows, middle paleae not covering inner opercular paleae (Fig 5A.4F). Twelve pairs [12–16 pairs] of short opercular have conical papillae (Figs 5A.3C, D) peripheral to outer paleae. Three pairs [two to three] of nuchal spines are slightly curved inwards with blunt tips. Tentacular filaments compound (branching) arranged in eight horizontal or diagonal rows

(Fig 5A.3B, C). Palps are slightly longer than the length of the operculum. Median organ present at the dorsal junction of lobes of operculum, median ridge present, with eye spots on lateral margins. Segment 1 (chaetiger 1) is with one pair of neuropodial cirri bearing a small tuft of fine capillary chaetae on either side of the U-shaped buccal organ (Fig 5A.4B). Chaetiger 2 is with one pair of elongated lateral lobes, connecting branchiae to neuropodia (Fig 5A.4C, D). A pair of branchiae present in thorax, parathorax and anterior abdominal segments. The branchiae narrow-based, strongly ciliated, long and tapering to fine tip. Segment 3–5 (parathoracic) with two types of notochaetae arranged transversely (Fig 5A.4B, C); seven to twelve lanceolate chaetae, tapering to an elongate frayed tip (Fig 5A.5H) and fine capillaries with frayed margins inserted between lanceolate ones. The abdominal region is with 28 [20–35] chaetigers. Branchiae present only in anterior chaetigers; anterior 6 chaetigers in holotype having branchia, at most anterior 10 chaetigers having branchia in paratype. Notopodia as transverse tori, with long-handled uncini, numbers per torus decreasing posteriorly, each uncinus with two vertical rows, each row with 7–8 major teeth. Neuropodia with the ventral bundle of fine notochaetal capillaries with extended the fine tips, shafts composed of stacks of thecae with margins extending to fine filamentous tip. Cauda smooth and long bent backward on ventrum (Fig. 5A.4E).

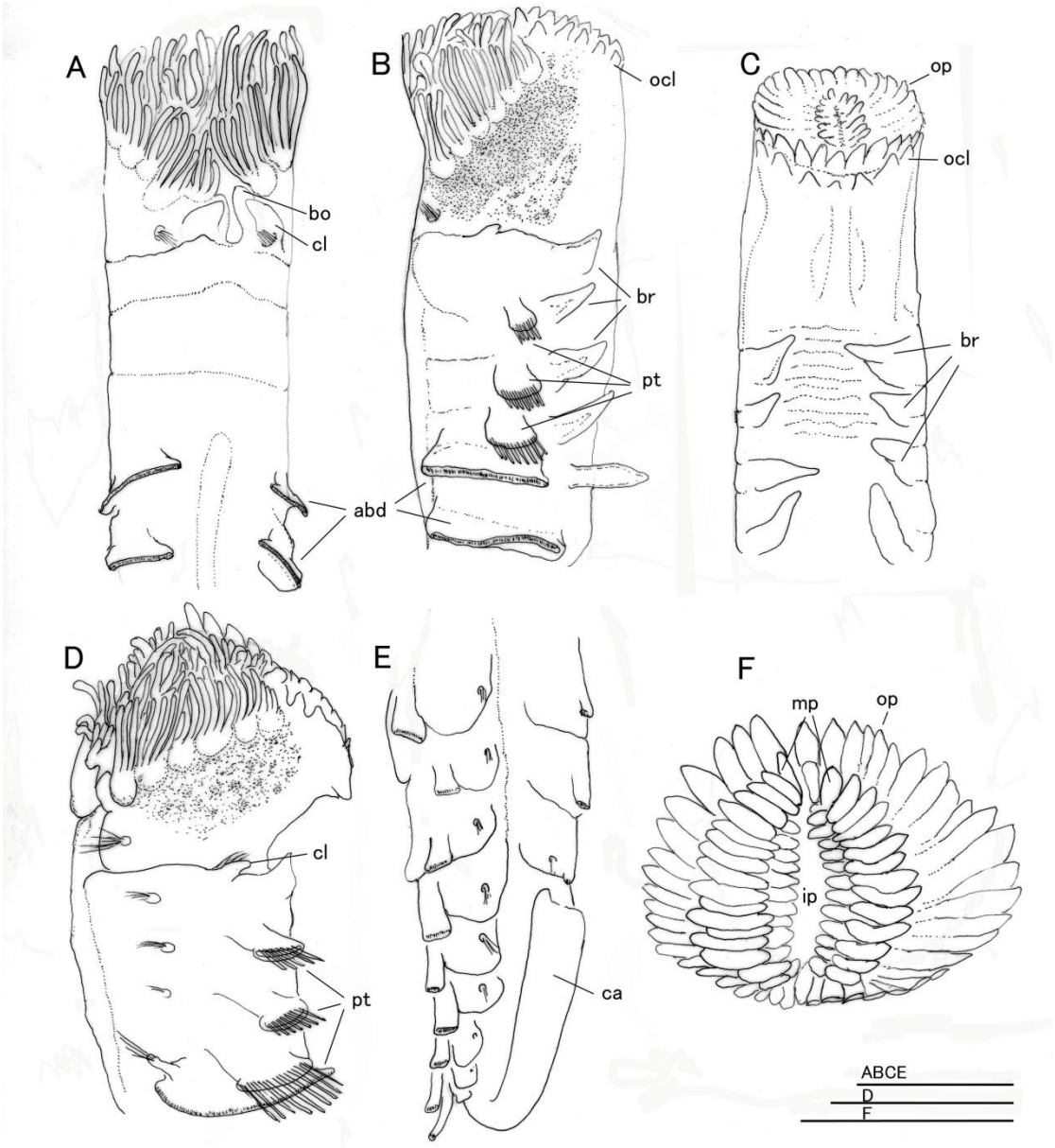

Fig 5A.3. *Neosabellaria indica* new species. A–C, E, F – holotype, D – paratype. A, ventral view. B, lateral view. C, dorsal view. D, leteral view. E, posterior part. F, opercular crown, upper view. abd, abdominal notopodium. bo, building organ. br, branchia. ca, cauda. cl, conical lobe. ip, inner palea. mp, middle palea. pcl, opercular conical lobe. op, outer palea. pt, parathoracic chaetiger. Scales are 1mm.

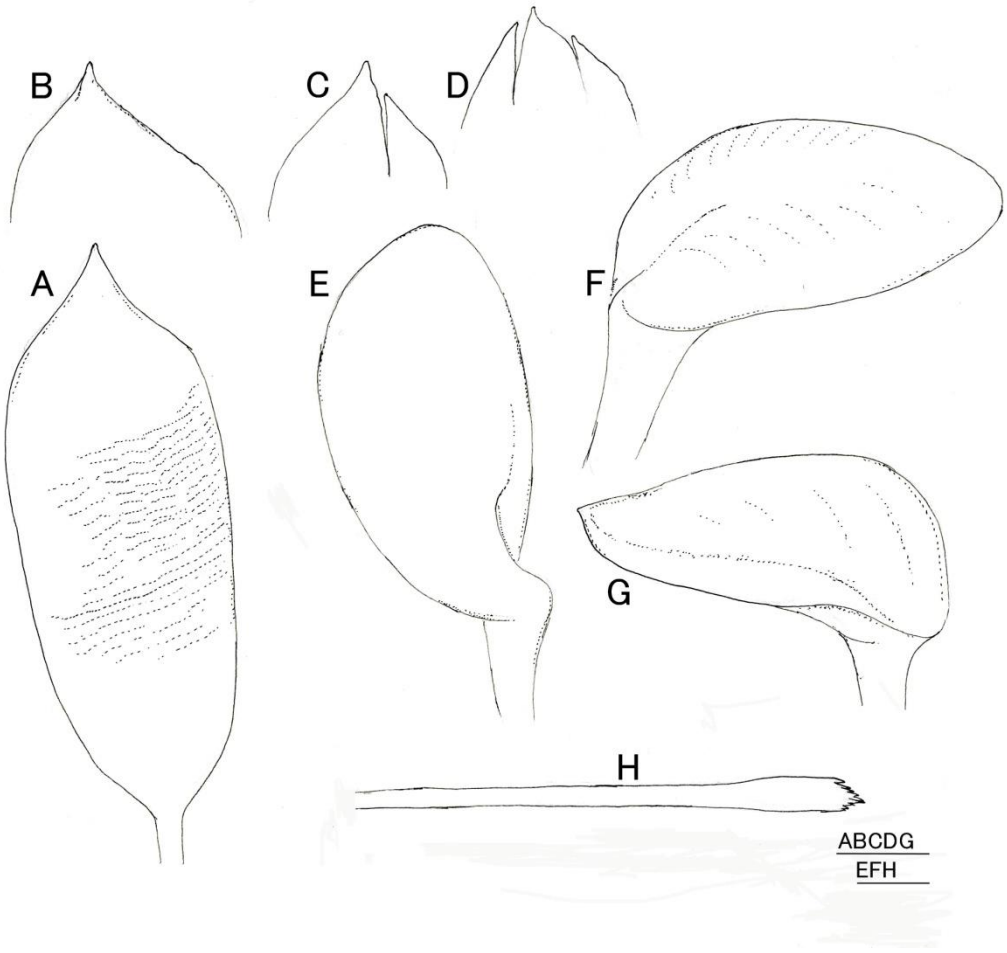

Fig 5A.4. Paleae and chaeta of *Neosabellaria indica* new species. A–D, outer paleae. B–D, close-up of outer paleae distal part. E, F, middle paleae. G, inner palea. H, parathoracic nochaeta. Scales are 0.1mm in A, B, C, D, 0.05mm in E, F, G, H.

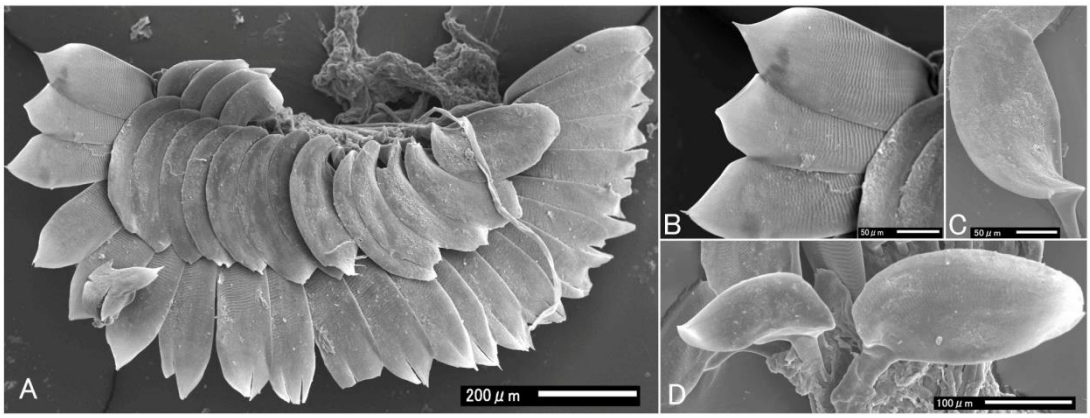

Fig 5A.5. Scanning electron microscope images of paleae of *Neosabellaria indica* new species. A, right rows of paratype: ZSI/ANRC-14816-14820. B, outer paleae. C, middle paleae. D, inner (left) and middle (right) paleae.

5A.2.6. Taxonomic remarks

Neosabellaria indica nov. sp. is characterized by the presence of a single form of middle paleae and spinouts tip without dentition of outer paleae. In the genus, only *Neosabellaria uschakovi* Kirtley, 1994 has two forms (long and short blades) of middle paleae (Kirtley 1994; Nishi *et al*. 2009), while other 6 species described have a single form of middle paleae. Among these six species (*N. antipoda* (Augener 1926), *N. cementerium* (Moore 1906), *N. kaiparaensis* (Augener, 1926), and *N. rupicaproides* (Augener 1926)), only *N. vitiensis* Bailey-Brock *et al*. (2007) has distal lateral teeth and median plume on outer paleae. *Neosabellaria indica* sp. nov. has outer paleae with spinous central tooth lacking distal lateral teeth (Fig 5A.3, 4); although distal lateral teeth sometimes were found, the paleae had broken and distal part split, lacinate tip (Figs 5A.4B, C, D, 5A). *Neosabellaria indica* nov. sp. is most similar to *N. clandestina* Menon and Sareen (1966), as both species have shoe-shaped middle paleae,

while other congeners, such as *N. antipoda, N. cementerium, N. kaiparaensis,* and *N. rupicaproides,* have long, tapering middle paleae. *Neosabellaria clandestina* has spines on the medial plume of outer paleae (Fig 5A.5 Menon and Sareen 1966a) but our new species lacks spines on the central tooth of outer paleae.

5A.2.7. Distribution

The new species were distributed in the intertidal to the subtidal region of Kunakeshwar, Maharashtra along, the west coast of India.

5A.3. Discussion

Kirtley (1994) was the first to suggest a key to all species of the genus *Neosabellaria*, and later Bailey-Brock et al. (2007) revised the key based on paleal morphology. The key to all *Neosabellaria*, including *Neosabellaria indica* sp. nov.

Key to the species of *Neosabellaria*

1a. Middle opercular paleae of two kinds ……...*N. uschakovi*

1b. Middle opercular paleae of a single kind ……. 2

2a. Middle opercular paleae shoe-shaped …….. 3

2b. Middle opercular paleae longer than outer paleae ……... 4

2c. Middle opercular paleae length similar to that of outer paleae …..… 5

3a. Outer paleae with distal lateral teeth and denticulate median plume …

N. vitiensis

3b. Outer paleae with frayed, small spinous distal tip ……..… *N. clandestina*

3c. Outer paleae with small pointed tip ……. ***N. indica* sp. nov.**

4a. Middle opercular paleae recurved, forming a hook-like tip …

N. rupicaproides

4b. Middle opercular paleae blade gradually tapered to tip ……*N. antipoda*

5a. Middle opercular paleae taper gradually to a what kind of point …….. *N. cementerium*

5b. Middle opercular paleae tapering abruptly to acute tip … …..*N. kaiparaensis*

Chapter 5_B

New species of *Sabellaria goae* sp. nov. (Annelida: Polychaeta: Sabellariidae) from the Central West Coast of India

5B.1. Introduction

The honeycomb or sandcastle worm (Sabellariidae, Polychaeta) is a well-defined and highly specialized group of marine annelids commonly known as honeycomb or sandcastle worms (Capa and Hutchings 2014). Sabellariids are well-known as reef builders in shallow waters throughout temperate to tropical oceans (Kirtley 1992, 1994; Pandolfi *et al*. 1998; Bailey-Brock *et al*. 2004). Sabellariids reef-building species have been the focus of several taxonomic, biological and ecological studies. The three dimensional structures, provided that refuge and food for many invertebrate species (e.g. Dubois, Retiere and Olivier 2002; Sepulveda, Moreno and Carrasco 2003; McCarthy *et al*. 2008; Fournier, Etienne and Le Cam 2010).Currently, there are 130 nominal species in the family (Hutchings *et al*. 2012; Capa and Hutchings 2014). The following 15 species from four genera have been recorded around India (Fauvel 1953; Achari 1974; Rajasekaran and Fernando 2012):

Sabellaria alveolata Linnaeus, 1767,
S. cementarium Moore, 1906,
S. chandraae De Silva, 1961,
S. clandestina Menon & Sareen, 1966,
S. floridensis Hartman, 1944,
S. myriaensis Parab & Gaikwad, 1990,
S. pictinata Fauvel, 1928,
S. rupicaproides Augener, 1926,
S. simplex Day, 1973
S. spinulosa Leuckart, 1849,
S. spinulosa var. *alcocki* Gravier, 1906,

S. spinulosaranjhi Hasan, 1960,
Idanthyrsus pennatus (Peters, 1854),
I. bihamatus (Caullery, 1944),
Phragmatopoma sp.

Also, two species *Lygdamis indicus* Kinberg, 1866 and *L. porrectus* Ehlers, 1908 (currently belonging to the genus *Tetreres* Caullery 1913; WoRMS 2015) were also known from the area adjacent to the Indian waters (Achari 1974). Among the above species, *Sabellaria pictinata* is possibly a misspelling of *Sabellaria pectinata*. *Sabellaria cementarium, S. clandestina* and *S. rupicaproides* had been moved to the genus *Neosabellaria* and *S. spinulosa ranjhi* and *S. spinulosa* var. *alcocki* had been raised to the species rank from subspecies or variant by Kirtley (1994).

Recent taxonomical information is summarized in Capa and Hutchings (2014) and other taxonomic papers (e.g., Nishi *et al*. 2009, 2015). *Sabellaria* genus is found mainly in the Atlantic and Indo-Pacific Oceans (Capa *et al*. 2012; Capa and Hutchings 2014) with 41 valid species including two recently described (Dos Santos *et al*. 2014; Nishi *et al*. 2015). Nishi et al. (2010) examined the type material of *S. chandraae* de Silva, 1961 in detail but no other species from Indian waters and adjacent waters were examined. The sabellariid species of *Sabellaria* was recently collected around Anjuna from west coast of India, and were compared to other members of *Sabellaria*. Then, we describe species here as the 43th species in the genus *Sabellaria* Lamarck, 1812.

5B.2. Materials and methods

5B.2.1. Study area

The polychaete samples were collected during October of 2014 from the rocky intertidal zone of the Anjuna (15°34′59″N, 73°43′59″E) beaches located along the central west coast of India, (Fig. 5A.1). The distance between the two stations is around ~150 km.

5B.2.2. Sample collection

The Western Ghats are a mountain range that runs almost parallel to the western coast of the Indian Peninsula. *Sabellaria goae* sp. nov. are distributed from the west coast of India and their distribution is related to the sediment type. *Sabellaria goae* sp. nov. prefers sand with medium particle size (125–250 µm) (Fig 5B.2A) and builds their sand tubes on rocky shores (Fig. 5A.1). The availability of food and sand particle on the water column were enhanced during monsoon season and as the river carry the fine sand into the coastal area.

Fig 5B.1. Sample collection area of Anjuna in the west coast of India

5B.2.3. Laboratory analysis

Specimens were collected by hand, fixed with neutralized formalin, and preserved in 70% ethanol. Specimens were studied under a dissecting and a compound microscope. As in Capa *et al*. (2015), some specimens were stained with methyl green to improve the contrast of structures for photography. Data referring to the holotype are given in the species description, with the variation observed among paratypes provided in parentheses. Two worms were dissected, mounted on stabs, coated with palladium, and viewed under scanning electron microscope (SEM) JEOL 5600LV. National Museum of India, belonging to the Zoological Survey of India (ZSI), India.

Sabellaria **Lamarck, 1812**

Sabellaria goae new species

Figures 6, 7, 8

5B.2.4. Material examined:

Holotype:

ZSI/ANRC-14821, fixed in 10% seawater formalin, intertidal rocky zone at Anjuna Goa (15°34′59″N, 73°43′59″E) west coast of India, October, 2014, coll. By Periasamy R and Baban Ingole

Paratypes:

ZSI/ANRC-14822-14825, 4 specimens, same collection data as for holotype

Comparative material:

Sabellaria isumiensis Nishi *et al.* 2010, *Sabellaria jeramae* Nishi *et al.* 2015

5B.2.5. Description.

Holotype 18 [12–20] mm long excluding cauda, cauda 2 mm long, width at parathorax 4.0 [3.0–4.2] mm, 25 [20–30] chaetigers, with speckled faint brown pigment on ridged ciliated dorsum (Fig. 5B.2C), rectangular pigment patches across ventrum of chaetiger 5 (3rd parathoracic). Operculum width similar to its length, with lobes completely separated, and three rows of golden paleae arranged in concentric semicircles (Figs 5B.2A, B, D). Outer row with 28 [24–30] pairs of geniculate paleae with flattened blades, smooth lateral margins and serrated distal margins with a middle tapering denticle occupying half of distal end which has marked surface ornamentation (Figs 5B.3A, 4B, C). Middle paleae of two forms, long and short ones arranged alternately; seven [5–8] pairs of geniculate long paleae, with flat and tapering blades, tip slightly recurved, dull pointing (Figs 5B.3D, E, 4D); seven [5–8] short form shoe-shaped, circular tip (Fig 5B.3F, 4C). Twelve pairs of inner paleae, with strongly geniculated, concave blades, similar to middle paleae, but wider, shorter and pointing inwards center of operculum (Fig 5B.3B). Outer paleae are longer than those of inner rows. Opercular is conical papillae, 12 [10–15] pairs, short, peripheral to outer paleae on each lobe (Fig 5B.2A, B). Three [2–3] pairs of nuchal spines present, stout, flattened, slightly curved, with smooth blunt tips. Tentacular filaments compound is arranged in 9 horizontal rows (Fig 5B.2B, C). Median ridge and edian organ present. Eyespots were not visible. Palps are slightly longer than opercular lobes. Segment 1 (chaetiger 1) is with lobe-shaped neuropodia bearing capillary neurochaetae on either side of U-shaped buccal organ (Fig 5B.2C). Segment 2 (chaetiger 2) is with one elongate triangular

shaped lateral lobe, connecting branchiae to neuropodia, with fine capillary neurochaetae (Fig 5B.2C). Seventeen [15–20] pairs of dorsal branchiae present from segment 2 (Fig 5B.6A, B), continuing along middle abdomen, with broad base and strongly ciliated ridges and slightly pigmented, all detached except one, which tapers distally with filiform tip (Fig 5B.2A, B). Segments 3–5 (parathoracic) with two types of notochaetae arranged transversely, 7–10 (many represented by stumps only) lanceolate with frayed tips and short, flattened smooth shafts with frayed tips, and fine short capillaries with expanded theca inserted between lanceolate (Fig. 5B.2B). Segments 3–5 with two types of neurochaetae are arranged in compact fascicle, with lanceolate and fine capillaries. Parathoracic notopodia is larger than neuropodia (Fig 5B.2B, C). Abdominal region is with 21 [16–26] chaetigers. Notopodia as elongate, erect tori, with long-handled uncini, numbers per torus decreasing posteriorly, each uncinus with two vertical rows, each with 7–8 teeth. Neuropodia has becoming considerably elongated posteriorly, with ventral bundle of capillaries. Cauda smooth is more than half length of abdomen.

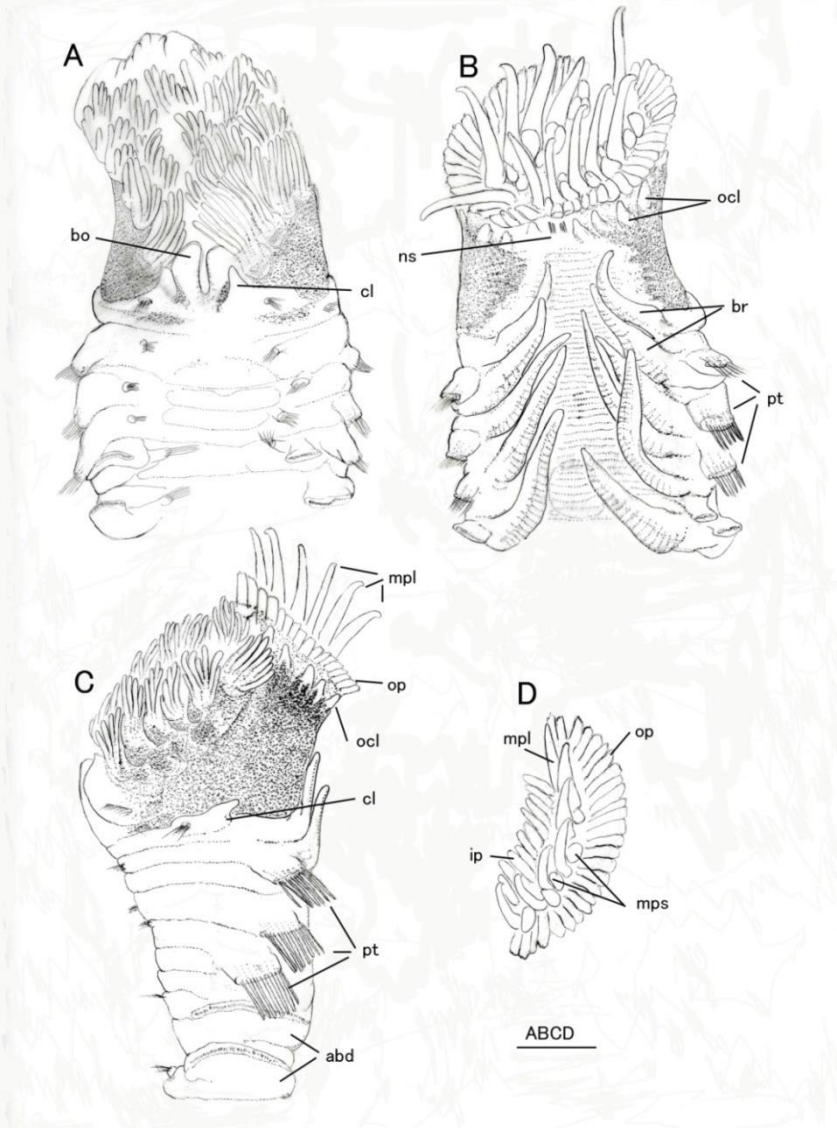

Fig 5B.2. *Sabellaria goae* new species holotype. A, ventral view. B, dorsal view. C, lateral view. D, right rows of opercular paleae. abd, abdominal notopodium. bo, building organ. br, branchia. cl, conical lobe. ip, inner palea. mpl, middle palea long form. mps, middle palea short form. ns, nucal spine. pcl, opercular conical lobe. op, outer palea. pt, parathoracic chaetiger. Scales are 1mm.

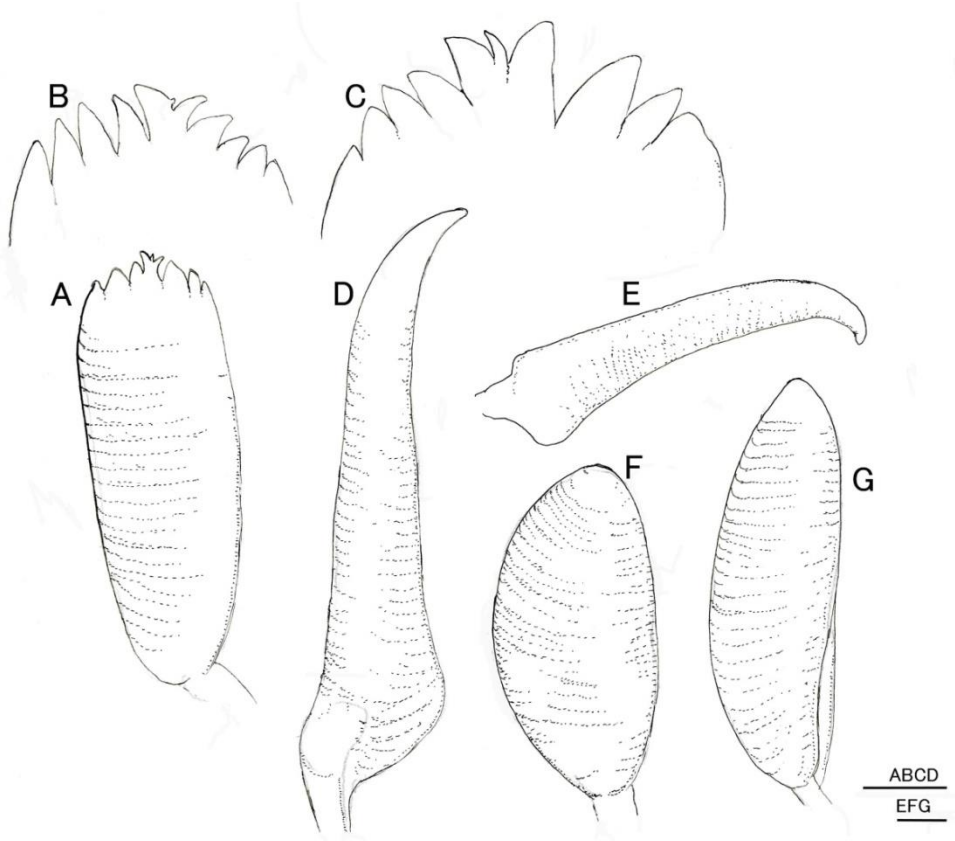

Fig 5B.3. *Sabellaria goae* new species. A–C, outer paleae. D, E, middle paleae, long form. F, middle paleae short form. G, inner paleae. Scales are 0.1mm in A, 0.2 mm in B, C, D, E, 0.05mm in F, G.

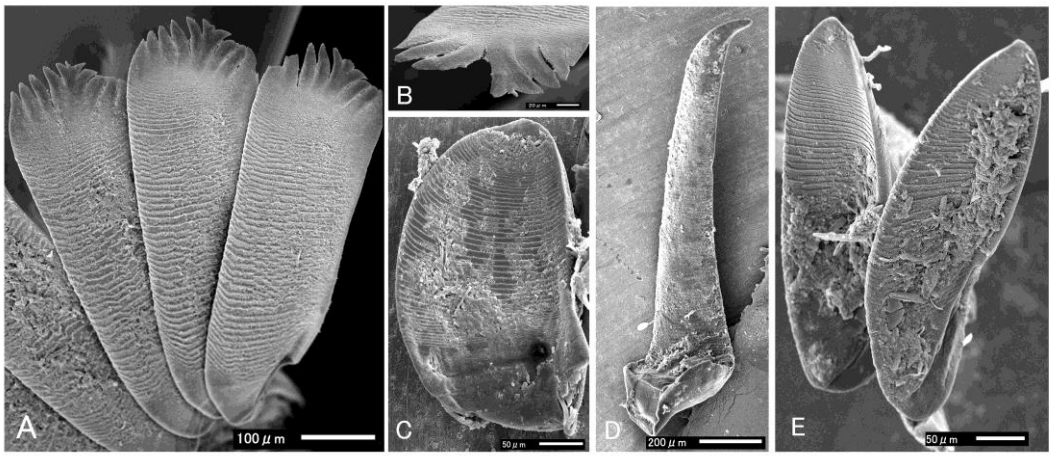

Fig 5B.4. *Sabellaria goae* new species. Scanning electron microscope images of paleae. A, outer paleae. B, close-up view of outer palea. C, middle palea short form. D, middle palea, long form. E, inner paleae

5B.2.6. Taxonomic Remarks

Sabellaria could be separated into two groups-having middle paleae with a single form and having middle paleae with two (long and short) forms. *Sabellaria goae* n. sp. has long and short forms of middle paleae, and thus belongs to the latter group. Among the members of the latter group, *S. goae* n. sp. is similar to *S. ranjhi* (Hasan, 1960), *S. chandraae* (De Silva, 1961), and *S. isumiensis* Nishi *et al.* (2010) in the morphology of outer paleae distal parts. Those four species have distal lateral teeth on both sides of central tooth, distal lateral teeth 2–4 in each side (some times asymmetrical), and a central tooth with a plume or a small spine. *Sabellaria ranjhi* and *S. chadraae* have outer paleae with a plume bearing small lateral teeth (Nishi *et al*. 2010). *Sabellaria isumiensis* has outer paleae with a central tooth and extended plume on distal part of central tooth (Nishi *et al*. 2010). *Sabellaria goae* sp. nov. has a central

tooth and minute spine on the tooth (Figs 5B.3A, B, C, 4A, B).

Hutchings et al. (2012) had suggested a presence or absence of branchiae on posterior abdominal segments as a diagnostic character. According to their suggestion, we have compared the absence or presence of branchiae on posterior abdominal segments in the recently described species, *S. jeramae* Nishi et al. (2015) and *S. isumiensis* Nishi *et al*. (2010). *Sabellaria jeramae* has well-developed branchiae in parathoracic and abdominal chaetigers and only two or three posterior segments lack branchiae in this species (Fig. 5B.9). But in *S. goae* n. sp. branchiae are poorly developed, no branchia crossed to the dorsal side and to the anterior abdomen, branchia are small, and are absent in posterior from 6 to 12 segments (Fig 5B.9). *Sabellaria isumiensis* showed a pattern similar to that in *S. goae* n. sp. where branchaie are poorly developed, and are absent on posterior abdominal segments (Fig 5B. 9). Then, *S. jeramae* and other two species could be distinguished by the absence or presence of branchiae in posterior abdominal segments. Although the diagnostic characters of Sabellariidae are mainly the morphology of paleae as noted in Kirtley (1994), absence or presence of branchiae and median organ should be a good character for a classification of the taxa as suggested by Hutchings *et al.* (2012).

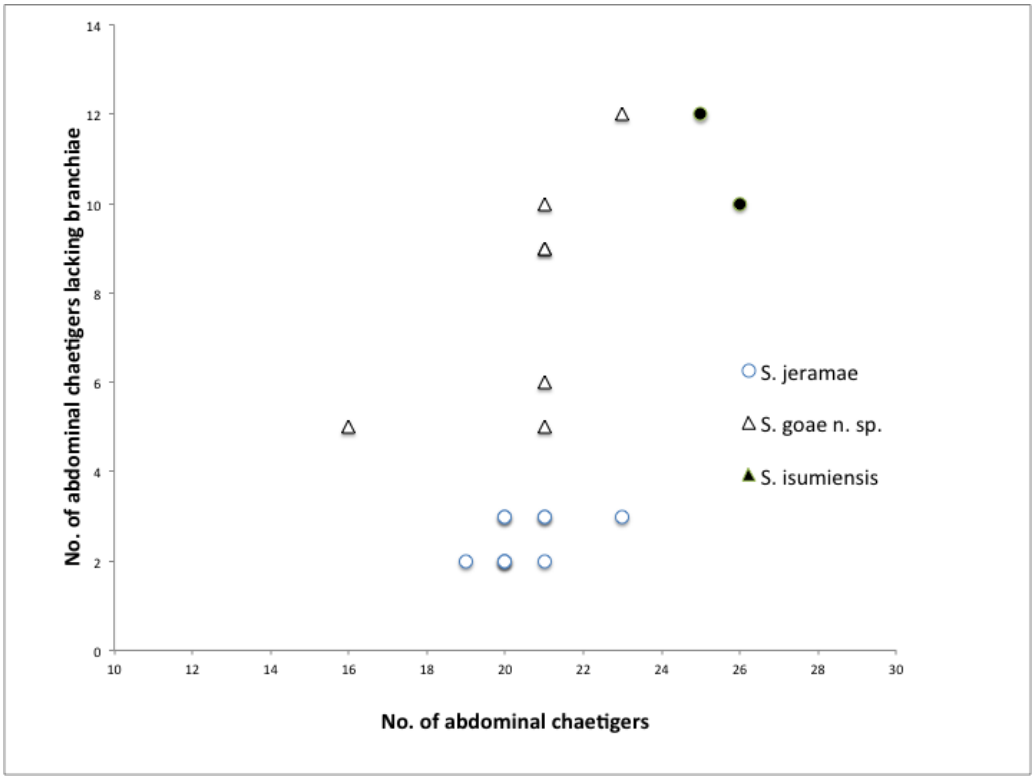

Fig 5B.5. Comparison of absence or presence of branchiae in posterior abdominal segments in three species of *Sabellaria*. All the materials used here are types (holo- and paratypes).

5B.2.7. Distribution

Along the west coast of India

5B.3. Discussion

Recently, new diagnostic characters such as, absence or presence of median organ (aboral cirrus), form of oral feeding tentacles, presence or absence of dorsal branchiae on posterior segments, have been suggested in Sabellariidae taxonomy (Hutchings *et al*. 2012, Dos Santos *et al*. 2014). In genus *Sabellaria*, all valid species have been summarized in Hutchings *et al*. (2012). We here, provide three recently described species in 2014, 2015 and in this study, as in their Table1 format.

Table 5B.1. Description on key features of *Sabellaria* species

Table 1.

Species	Type locality	Length exc. cauda (mm)	Max width (mm)	Outer paleae (pairs)	Medial plume on outer paleae	Teeth on distal margin of outer paleae (pairs)	Middle paleae (pairs)	Type of middle paleae	Middle paleae, 1. long form	Middle paleae, 2. short form	Inner paleae (shape)
S. guamare Dos Santos et al. 2014	Guamare Bay, Brasil	55	2	28–30	present, minute denticulate	3	12	1	–	with dominant and distal teeth	geniculate, bluntly rounded point in tip
S. jeramae Nishi et al. 2015	Jeram, Malaysia	35	2	24–36	present, denticulate	3–4	20–25	1	–	slender, shoe–shaped	geniculate, shoe–shaped
S. goae n. sp.	Goa, India							2	flat, tip curved	shoe–shaped	geniculate, shoe–shaped

Table 1. (continued)

Species	Eyespots on median organ	Opercular papillae (pairs)	Nuchal spines (pairs)	Neuroch seg. 1	Median organ	Lateral lobes seg. 2 (pairs)	Branchiae, posterior segments	Reference
S. guamare Dos Santos et al. 2014	present (small black eyespots)	NA	3	Present	Present	NA	absent	Dos Santos et al. 2014
S. jeramae Nishi et al. 2015	absent	16	2 – 3	Present	Present	1	present	Nishi et al. 2015
S. goae n. sp.	absent	12 – 15	3	Present	Present	1	absent	this study

The key to species of Indian Sabellariidae

Key to Indian Sabellariidae

1a. Opercular disc (distal end) clearly oblique to longitudinal margin, with paleae arranged in a dorsal slope from lateral view..... 2

1b. Opercular disc (distal end) flat, with paleae arranged perpendicular to longitudinal axis ... (*Sabellaria*) …..... 8

2a. Outer paleae with smooth margins. Four parathoracic segments ……... (*Lygdamis*) *L. indica*

2b. Outer paleae with with large and pointed denticles on margins. Three parathoracic segments …….. (*Idanthyrsus*) 3

3a. Outer paleae with denticles not all straight ……..*I. pennatus*

3b. Outer paleae with denticles all nearly straight ……*I. bihamatus*

4a. Outer paleae with denticulate distal plume ……..… 5

4b. Outer paleae without denticulate distal plume ……..……..7

5a. Long distal plume with dense denticulation …….……..… *S. alcocki*

5b. Distal plume without dense denticulation ……..…….6

6a. Blades of middle paleae laterally compressed ……..*S. spinulosa*

6b. Blades of middle paleae not compressed …… *S. floridensis*

7a. Outer paleae with pilose distal plume …….8

7b. Outer paleae without any distal plume ……...9

8a. Outer paleae with distal teeth on both sides of medial plume …..*S. ranjhi*

8b. Outer paleae with distal teeth, either side of medial plume … *S.chandraae*

8c. Outer paleae with distal teeth, central tooth with minute spine … ***S.goae* sp. nov.**

9a. Outer paleae with many distal teeth ……*S. pectinata*

9b. Outer paleae with large distal teeth ……..……..*S. myriaensis*

9c. Outer paleae with distal teeth all curved toward lateral margins of outer paleae ……..*S. alveolata*

Chapter 5_C

New species *Pseudonereis corallinsis* sp. nov. (Nereidae: polychaeta) from Goa, the west coast of India

5C.1. Introduction

The family Nereididae, Blainville (1818) is one of the most common and diverse polychaete group, which is probably well known and it includes 44 genera and 677 species (Read and Fauchald 2012). Although the number of species could be much more than anticipated. The importance of this family is clear by its high diversity and abundance. The genera *Pseudonereis* has one of the diverse group in the family described by Kinberg (1865) including two species *P. gallapagensis* and *P. formosa* from Galapagos and Hawaii islands, respectively. It is characterized by closely spaced conical paragnaths in 'pectinate-like' rows on the pharynx, and with elongated dorsal notopodial ligules with terminally attached cirri. The distribution of the taxa belonging to *Pseudonereis* is mostly tropical and subtropical, even though specimens have been reported from the Pacific coast of South America and Puerto Montt (Hartmann-Schröder, 1962b). The most of the species are described from Central and South America and the Indo-Pacific region and nevertheless are habitat from intertidal and shallow waters (Bakken 2007).

The corallinales is a unique and diverse group of macrophytes distributed in marine wave-exposed littoral and sub-littoral habitats (Johansen 1976). Ecologically, they are considered to be inhabited as distinct zones in the littoral and sub-littoral and supply niches for many other species (Chamberlain and Cooke, 1984; Kinzie and Buddemeier, 1996). They are known to be the third most species-rich within the red algae (Brodie and Zuccarello 2007). Calcareous algae are important constituents of the carbonate deposits of tropical Indian coastal areas. They play an important role in ecosystem management

including a diverse range of niches (Kamenos *et al.* 2004). They are considered to be high biodiversity shelters (Nelson 2009) by providing food, substratum, habitat, and refuge for a certain number of important marine organisms (Kelaher 2003).

A new species of *P. corallinsis* sp. nov. from rocky shore area at Anjuna, Goa, the west coast of India is described here, compared with all the existing congeners and provided with a phylogenetic relationship for the new species based on the analysis of 18S rDNA sequence data.

5C.2. Material and methods

5C.2.1. Study area

The polychaete samples were collected from the rocky shore area at Anjuna, Goa, West coast of India (Lat 15°24″50′ N, long 073°54″40′ E) in January 2015. Specimens were obtained from association with the corallinsis algae *Corallina crossmanni*. Polychaetes separated from algae were fixed with 10% seawater–formalin and transferred to 70% ethanol for subsequent dissection followed by examination using a stereomicroscope. Parapodia and chaetae were examined using a compound microscope. Identifications were facilitated by previous contributions and morphological keys (Bakken 2007). Approximately 10–20 segments of the posterior portion were used for DNA extraction. Samples used for DNA work had been fixed in 95% ethanol, while the ones for morphological investigation were relaxed in isotonic 7% $MgCl_2$ and rapidly submerged in 95% ethanol. From the dissected individuals, parapodia were preserved in polyvinyl lactophenol on microscope slides. Pictures have been captured using an Olympus SZ61 stereomicroscope.

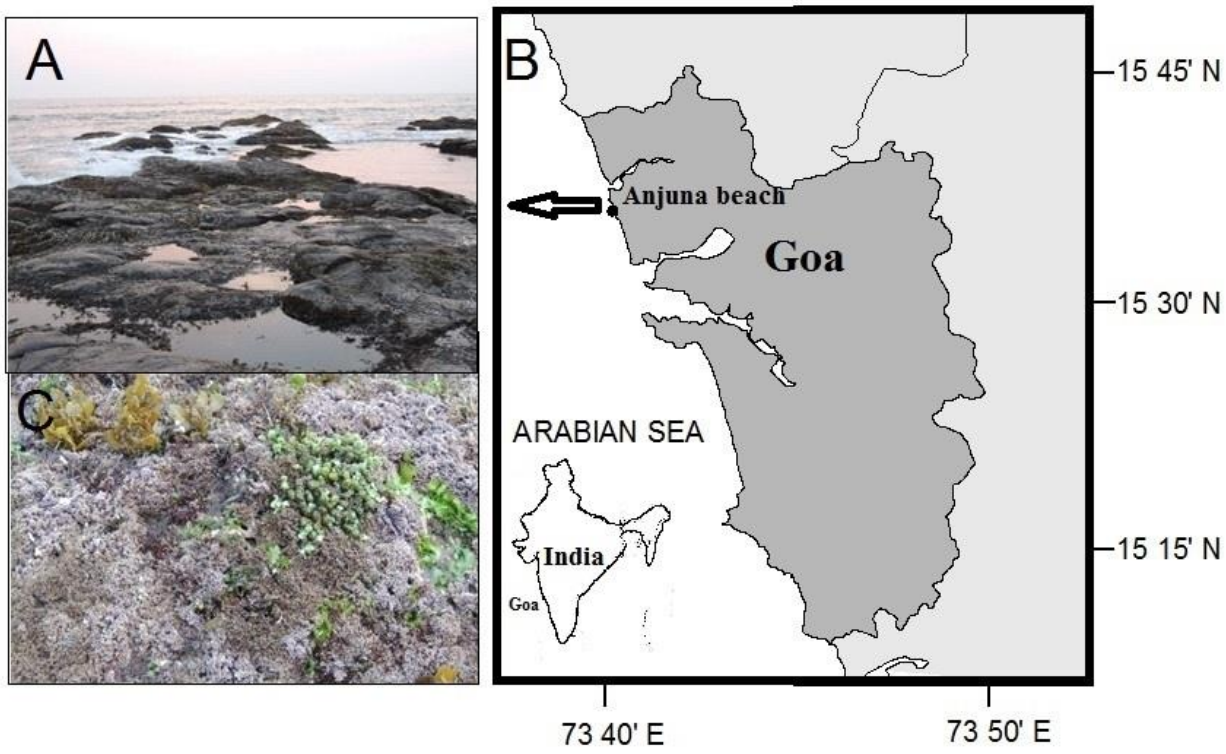

Fig 5C.1. Map showing the positions of the collection localities of *Pseudonereis corallinsis* sp. nov. Goa, India. Anjuna beach (Lat 15°24′50′ N, Long 073°54′40′ E)

5C.2.2. DNA Extraction and PCR amplification

Total genomic DNA was extracted from the tissue samples following extraction protocol of Miller *et al.* (1988). Partial sequences of 18S rDNA were PCR-amplified using subsequent primers for 18S (Medlin *et. al.*, 1988). The PCR used the following protocol: *(i)* an initial 3 minute denaturation step at 95°C for all samples, (*ii*) followed by 35 cycles of 45 seconds denaturation step at 94°C, 1 second annealing step at 55°C, 2 minutes extension step at 72°C and, *(iii)* a final 5 minute extension step at 72°C for all samples. The quality of all PCR products was checked using gel electrophoresis (1% Agarose gel); successfully amplified products were purified using Qiaquick PCR Purification Kit (Qiagen). Thereafter, cycle sequencing and ethanol precipitation were carried out, and

nucleotide sequencing was performed on an ABI 3037 XL Genetic Analyzer (Applied Biosystems, Foster City, CA). Nucleotide sequences were deposited at NCBI (accession numbers: KT900283) and type specimens were deposited in the collections of the CSIR-NIO.

5C.2.3. Phylogenetic analyses

Sequence alignment of forward and reverse sequences was carried out using BioEdit ver. 7.0.5.2 (Hall 1999). For estimating the appropriate model of sequence evolution, a hierarchical likelihood ratio test (hLRT) was carried out as implemented in the program Modeltest ver. 3.06 (Posada and Crandall 1998, 2001). The test criteria indicate that the Tamura Nei substitution model (Tamura and Nei 1993) with equal base frequencies, invariant sites, and gamma distribution (TrNef + I + C) represents the optimal model with respect to the data. The maximum likelihood analysis was carried out by Phylogenetic Analysis using Parsimony (PAUP) ver. 4.0b8 (Swofford 2001) under the likelihood settings suggested by Modeltest using the heuristic search option with TBR branch swapping and simple sequence addition.

5C.3. Results

5C.3.1. Systematic

Phylum: Annelida

Class: Polychaeta

Order: Phyllodocida Dales, 1962

Family: Nereididae Blainville, 1818

Subfamily: Nereidinae Hartman, 1959

Genus: *Pseudonereis*

Species: *Pseudonereis corallinsis* sp. nov.

Figure 5C. 2,3 Table 1

5C.3.2. Type locality- The central west coast of India, Goa, Anjuna, Lat 15°24′50″ N, Long 073°54′40″ E (Fig 5C.1, site 2).

5C.3.3. Type material- Holotype - NIO-SP 1150-56, 112.0 mm SL. from the west coast of India: 04°33'09.7"S, 56°17'59.6"W, 11 June 2014, R. Periasamy.

5C.3.4. Diagnosis: 72-79 chaetigers, length 25-45 mm, wide 3-4 mm at chaetiger 8, biarticulate palps globose, with four pairs of thread-like tentacular cirri. Posterio-dorsal pair extending back to posterior margin of 6-7 chaetiger. Prostomium is slightly wider than long with entire anterior margin. Two pairs of eyes of similar size in the quadrilateral with two sides parallel arrangement. One apodous anterior segment is greater than the length of chaetiger. The smooth edge of jaws is with black/brown like the plate. Maxillary ring of pharynx is with paragnaths arranged in discrete areas. Areas II–IV comb-like arranged in regular rows. Area I = 2 conical paragnaths; Area II = 13–34 p-bar paragnaths in three rows; Area III = 59–76 p-bar paragnaths in four rows; Area IV with 63–87 p-bar, paragnaths in 4–5 rows, and 2–4 p-bars towards jaws. Different groups present in area V-VI. Area V = 1 conical paragnath. One large shield-shaped bar present in area VI (Figs 5C.2A, B); Area VII–VIII = 18–21, conical paragnaths and large p-bars in two alternating rows, p-bars being more posteriorly placed (Figs 5C.2A).

Notopodium with dorsal notopodial ligule stout rounded as small as ventral notopodial ligule in anterior chaetigers (Figs 5C.3A, B), markedly elongate and markedly broader on posterior chaetigers, expanded in breadth from about chaetiger 30 (Fig 5C.3C), in length from about chaetiger 40 (Figs 5C.3D, E). Prechaetal notopodial lobe is absent, acicular

process is also absent. Dorsal cirrus simple, lacking basal cirrophore, two times length of ventral notopodial ligule at chaetiger 10–20, basally attached in anterior chaetigers (Fig 5C.3 B), sub-terminally attached from about chaetiger 30 (Figs 5C.3D, E), terminally attached to dorsal notopodial ligule on posteriormost chaetigers. Ventral notopodial ligule stout rounded, in posterior chaetigers digitiform. Neuropodial inferior lobe is prominent in anterior chaetigers; small superior lobe present throughout (Figs 5C.3E). Neuropodial postchaetal lobe present throughout low rounded not projecting beyond the end of acicular ligule, in posterior chaetigers with a small triangular tip (Figs 5C.3D). Ventral neuropodial ligule of anterior chaetigers presents well developed, similar in length to acicular neuropodial ligule throughout. Ventral cirrus 0.5–1 times as long as neuropodial acicular ligule. Notochaetae: homogomph spinigers. Neurochaetae, dorsal fascicle: homogomph spinigers (Fig 5C.2C) and heterogomph falcigers present throughout, blades serrated. Neurochaetae, ventral fascicle: heterogomph spinigers present from about chaetiger 40, with blades finely serrated; heterogomph falcigers with short blades (Fig 5C.2C) present throughout. The multiple ventral incisionsin the pygidium, and anal cirri reach back five chaetigers.

5C.3.5. Etymology This specific name is derived from its association with coralline algal genera *Corallina*, which is widely distributed along the west coast of India.

Table 5C.1. Comparison table for taxa in *Pseudonereis* Kinberg, 1865 showing important characters used for identification. *P. corallinsis* n. sp. is easily distinguished by presence of homogomph falcigers in neuropodial fascicle

Taxon	Paragnaths							Dorsal cirrus, attached in posterior chaetigers	Neuropodia				Type locality
	Area I	Area II	Area III	Area IV	Area V	Area VI	Area VII-VIII		Superior lope	Postchaetal lope	Ventral ligue: anterior, ×long as acicular ligule	Ventral ligue: Posterior, ×long as acicular ligule	
P. anomala	1–3	11–31	30–72	20–52	0	3–15 cones	10–24	sub terminal	present	present	1	1	Djibouti, Gulf of Aden
P. multisetosa	2	17-18	20	29-33	0	4, cones	8	sub terminal	present	present	1	0.5	Rangiroa, French Polynesia
P. palpata	2	39-40	109	108-120	1	1-2, bar+cones	19	terminal	present	present	1	0.5	Rio de Janeiro, Brazil
P. cortezi	4-6	17-31	33-54	31-55	8-15	1 shield-shaped	62-87	terminal, from c. 50-55	present	absent	0.5	<0.5 diminishing	Punta La Cholla, Mexico
P. pseudonoodti	4	15-27	28-40	20-61	3	1 shield-shaped	69-100	terminal, from c. 40	absent	present, poorly developed	0.5	<0.5, reduced	Paitilla, Panama
P. trimaculata	1-2	23-38	51-69	50-78	1-3	1 shield-shaped	20-24	terminal, posterior 1/4	present	present	0.5	0.5	Geser, Indonesia
P. atopodan	1	37-49	56	?, 4-5	0-1	1 shield-shaped	14-24	terminal, from c. 60-65	present	present	1	0.5	Tonga Islands, Oceania
P. gallapagensis	1	17-20	51-68	38-57	1	1 shield-shaped	17-20	terminal, from c. 15	present	present	1	0.5	Galapagos Islands
P. noodti	1	25-27	64	70-75	1	1 shield-shaped	16-17	terminal, from c. 25	present	present	1	0.5	Chimbote, Peru
P. variegata	1-2	13-34	59-76	63-87	1	1 shield-shaped	18-21	terminal, last few only	present	present	1	1	Valparaiso, Chile
***P.corallinsis* n.sp.**	2	15-34	42-64	54-91	1	1 shield-shaped	24-26		present	present	0.5	0.5	Anjuna Goa, India

** (source from Bakken, 2007)*

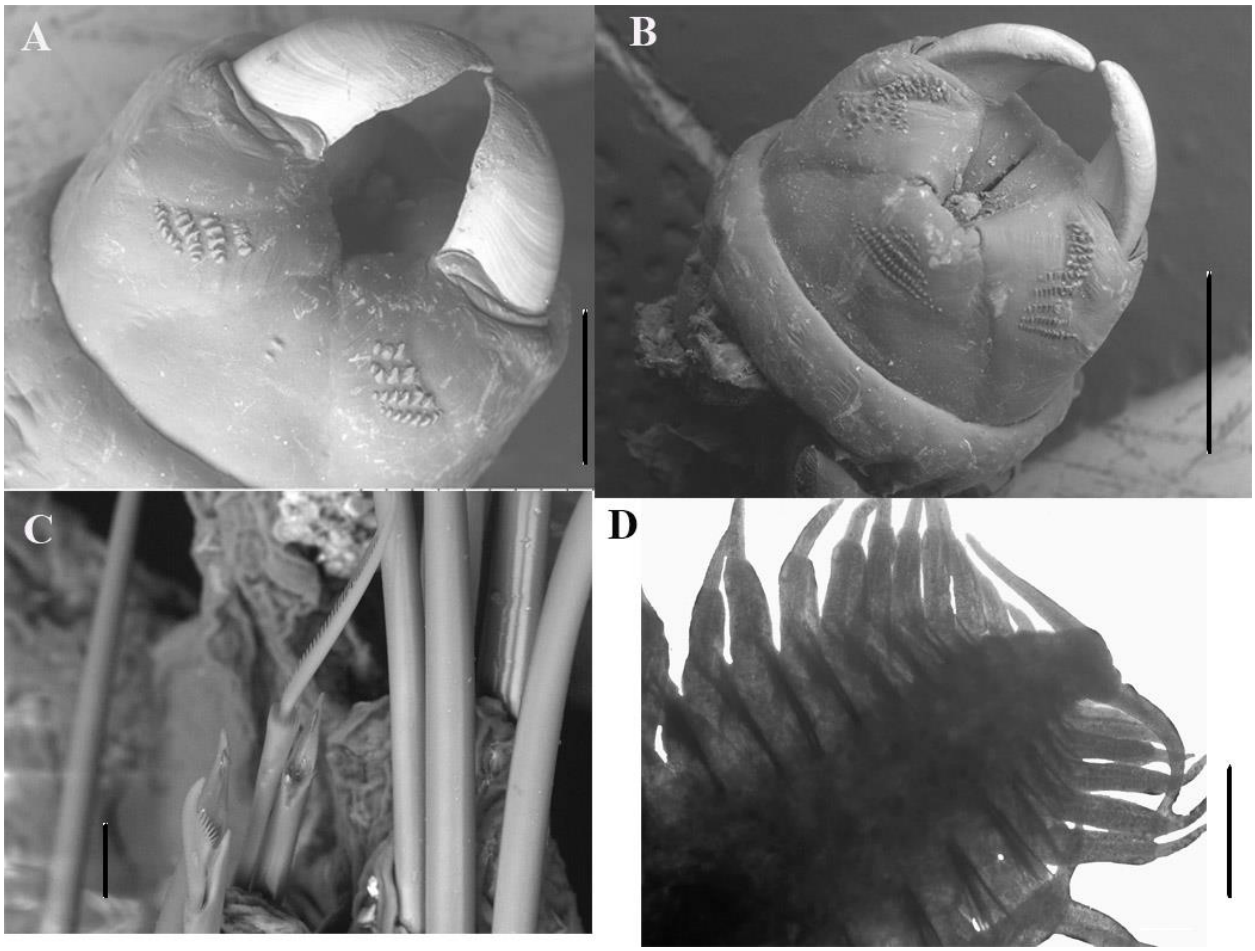

Fig 5C.2. *Pseudonereis corallinsis* sp. nov. **A.** paragnath dorsal view with smooth edge of jaws like plate; **B.** paragnath ventral view; **C.** Notopodium homogomph spinoger and Heterogomph falcigers from neuropodium in chaetiger 40; **D.** Pygidium. A, B–D is scale bar=0.5 mm scale and C is scale bar=50 µm.

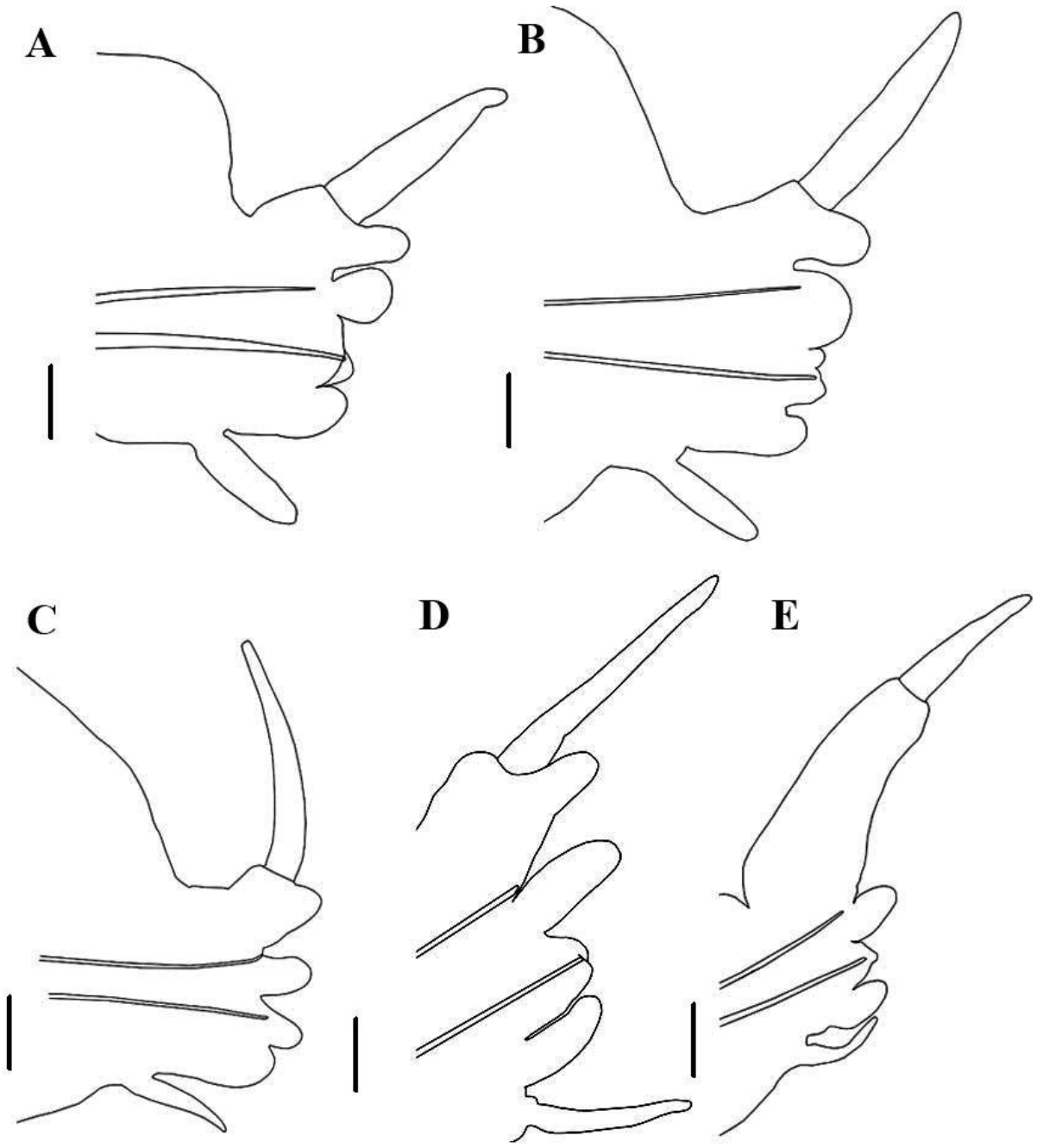

Fig 5C.3. *Pseudonereis corallinsis* sp. nov. A, parapodium 3th chaetiger posterior view. B, 11th chaetiger posterior view. C, 30th chaetiger posterior view. D, parapodium 56th chaetiger anterior view. E, parapodium 72th chaetiger posterior view. Scale bar in 0.2 mm.

5C.3.6. Distribution: This species is known only from the west coast of India and was collected in association with coralline algae in intertidal rocky environments.

5C.3.7. Ecological note: *P. corallinsis* sp. nov. was collected solely from coralline algae (*Corallina crossmanni*) association within rocky shore. The coralline algae are found along the west coast of India on the intertidal rocky shores located in water depths of 0-1.5 m, with ~80% of the rocky shore exposed during low tide. Salinity at the collection sites ranged from 34 to 38 PSU.

5C.3.8. Phylogeny

The systematic placement of new species has been placed within the Nereididae family as well as the only two members of Nereididae for which data were available for all of the 18S (*Nereis pelagica* Linnaeus, 1758 and *Nereis vexillosa* Grube, 1849). The final molecular matrix contained 3789 aligned sites. The TNT analysis recovered 5 equally parsimonious trees, 8426 steps long and the strict consensus and corroborates the morphological analysis in confirming the novelty of the species. The specimens of *P. corallinsis* form a monophyletic group among the sampled nereidid taxa. This position is supported by a bootstrap value of 100% and the monophyly of the specimens received maximum support. The details of the remainder of the tree are presented in Fig. 5C.4.

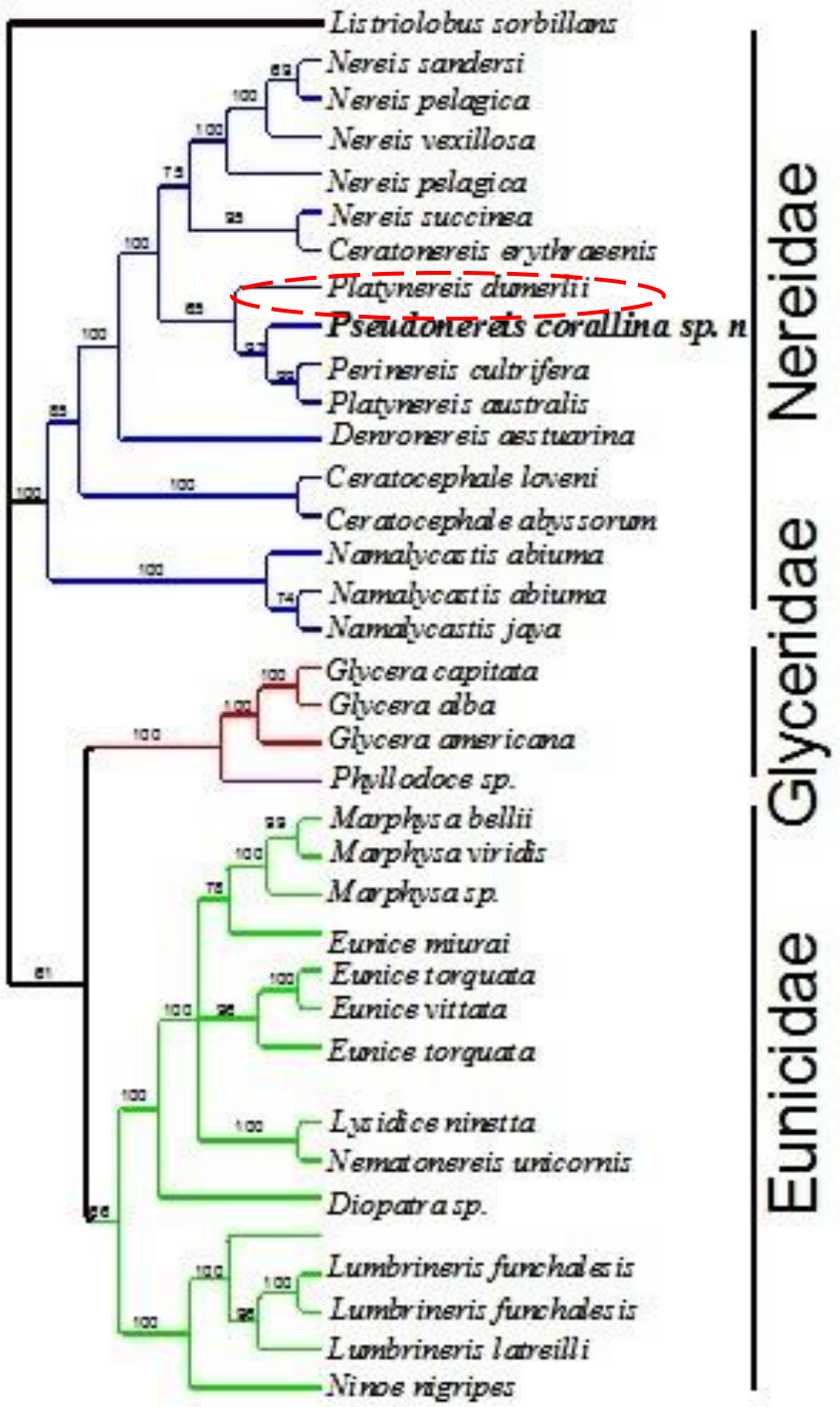

Fig 5C.4. Strict consensus of five equally parsimonious trees from the TNT analysis (Length: 8426; CI: 0.357; RI: 0.543). Bootstrap support values are shown above the nodes and representatives of the new species are shown in bold font. See text for further discussion.

5C.3.9. Remarks

The possible groupings within *Pseudonereis* based on the maxillary ring of pharynx with paragnaths arranged. The taxa belonging to *Pseudonereis* are predominantly distributed in tropical and subtropical regions, although specimens are found rather far south on the Pacific side of South America (Ehlers 1901; Hartmann-Schröder 1962). However, a majority are described from Central and South America and the Indo-Pacific region. The *P. gallapagensis* (Kinberg, 1865) and *P. variegata* (Grube, 1857) showed geographical distribution and well-known striking morphological similarity to less well-known taxa with resemblance distribution (Bakken 2007). A cladistic analysis using parsimony is included to test for monophyly of *Pseudonereis*. A monophyletic clade including all *Pseudonereis* taxa is given bootstrap support (Bakken 2007). The monophyletic status of the subfamily Nereidinae has been confirmed by phylogenetic analyses (Fitzhugh 1987; Glasby 1991, 1999); the status of the genera within the subfamily has been discussed. The species shared distinctive elongated dorsal cirri of posterior chaetigers.

Species of the genus have dominance from shallow waters ranging significantly from rocky shore area. This tolerance for varying environments may also indicate that the abundance of the genus is richer and its distribution is wider than currently recognized. *P. corallinsis* sp. nov. related species occur in a clade with 88% bootstrap support, the sister group to a clade including *Platynereis* to *Perinereis*. The *Pseudonereis* clade is equivocally supported by the synapomorphies presence of paragnaths in Areas II-IV arranged in regular comb-like rows and dorsal cirrus terminally attached to dorsal

notopodial ligule in posterior chaetigers. New species of *P. corallinsis* sp. nov. has been associated with coralline algae, rich calcareous coralline algae are found in most intertidal coastal areas, particularly, along the west coast of India (Singh *et al*. 2010). It seems that *P. corallinsis* sp. nov. specimens, if earlier collected, have seemingly been misidentified or left at a higher taxonomic level in earlier research from the west coast of India. This is only one species of *Pseudonereis* found within the west coast of India. The new ecological groupings that later will be tested on the reproductive cycle of this species. The future study, therefore, is necessary to document the seasonality, distribution and reproductive biology of *P. corallinsis* sp. nov. in the west coast of India. However, further collection and study of the morphology of this population are required, including association with coralline algae from the west coast of India.

Chapter 5_D

New record of *Spiochaetopterus costarum* (Chaetopteridae: Polychaeta) from the central west coast of India

5D.1. Introduction

Chaetopterids are a curious polychaete group in the benthic community and tubiculous asadults. They have three distinct regions of the body divided into anterior, middle, and posterior referred to as A, B, and C (Crossland 1904; Bhaud *et al*. 1994).The widespread descriptions of *Spiochaetopterus* genus showed the potential of larval dispersal and adult distribution ofspecies are far more limited (Bhaud 2003). However, the *S. costarum*is now conserved a species complex (Bhaud and Fernandez-Alamo 2000, Bhaud and Petti 2001, Bhaud 2003, Bhaud *et al*. 2003), and usually considered to be a putative cosmopolitan species (Okuda 1935; Day 1967; Blake 1996). The biogeographic distribution of *S. costarum* has been in habited extensively in Pacific: West Canada to South California, Japan, Indian Ocean, Madagascar, Atlantic-Mediterranean area: East to West North Atlantic, Mediterranean (Blake 1996; Day 1967). Bhaud (1998) studied from Arcachon beach in the Atlantic Ocean, Galician Rias Bajas (Spain, Atlantic coast), Banyuls and Naples (Mediterranean Sea).

The wider geographical distribution of *S. costarum*is showed that ability of planktonic larvae is distributed throughout ocean (Bhaud 2003). Therefore, the morphological differences have been identified, supporting the existence of distinct species of *Spiochaetopterus* in geographical areas as diverse as Japan and the Persian Gulf (Nishi *et al*. 1999, Nishi and Bhaud 2000), Brazil (Bhaud and Petti 2001), the Yellow Sea (Bhaud *et al*. 2002). Recently, Bhaud (1998a) reported that existence of similar but different species on both western and eastern coasts of the North Atlantic Ocean, where earlier studies reported of diverse subspecies of *S. costarum* (Gitay 1969).

The new record of *S. costarum* species from the west coast of India, identified in the benthic samples collected at near the Marmagoa port, Goa, where the benthic community was investigated for the purpose of creating a database for baseline surveys of the Coastal Ocean Monitoring and Prediction System (COMAPS) Program under the Government of India, during March 2016. The phylogenetic analyses showed that the two genera *Phyllochaetopterus* and *Spiochaetopterus* are the paraphyletic groups based on mtCOI, 18S and 28S rRNA genes (Osborn *et al*. 2007, Martin *et al*. 2008, Morineaux *et al*. 2010; Zhang *et al*. 2015). However, the records of *S. costarum* from the Indian coast are rare. The aim of the study focuses on morphology and phylogenetic relationship within the chaetopterids family based on partial sequences of mtCOI and 18S rRNA genes of *S. costarum.*

5D.2 Materials and Methods

5D.2.1. Study area and sample collection

The sediment sample was collected on board *CRV Sagar Paschimi*, on the Mandovi estuaries in the state of Goa, central west coast of India. The Mandovi river is a major west coast river with a length of 87 km and the basin area of 2032 km^2 (Rao 1975). Benthic samples were collected in replicates using a Van Veen grab (0.11 m^{-2}) during May 2016. Sub-sampling was done with a metallic quadrant (225cm^2). All samples were preserved in 10% buffered formalin Rose-Bengal with seawater solution.

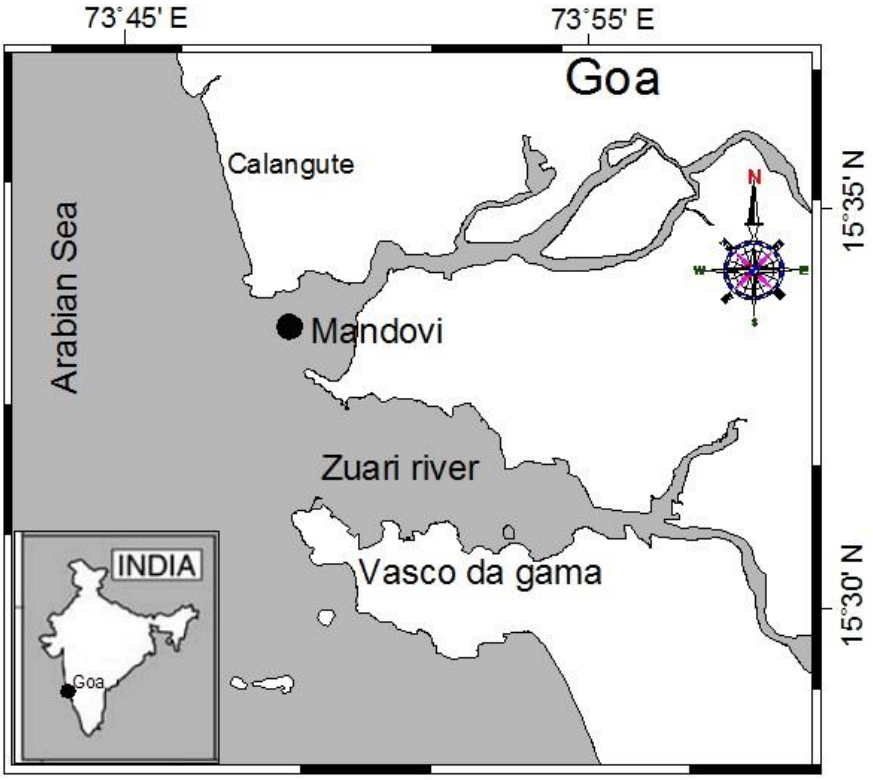

Fig 5D.1. Map of collection sites at Mandovi estuary, Goa, India.

5D.2.1. Laboratory analysis

All samples were sieved through a 0.5 mm mesh sieve and the fauna retained on sieve mesh were sorted and preserved in 5% buffered formalin. An initial sorting was done under a stereomicroscope (Zeiss Stemi 2000-C) to separate the tubes containing *Spiochaetopterus* worms. For the morphological observations, more than 20 worms were carefully extracted by cutting their tubes under the stereomicroscope.

5D.2.2. Morphological analysis

For Scanning Electron Microscope (SEM) observations of the specialized setae with enlarged cutting an A4 parapodium was dissected from the body and isolated in a glass

dish containing a nearly saturated solution of potassium peroxide. The parapodia were rinsed with distilled water, andthe specialized seta was removed with fine forceps under a compound microscope. Immediately prior to viewing in a Hitachi S.520 SEM (University of Perpignan, Centre of Electron Microscopy), they were transferred to 100% alcohol, critical-point dried, attached to a stub, and coated.The morphological description of the genus *Spiochaetopterus* prepared in three different sections based on the three body regions; Anterior (region-A), mid-body (region-B) and posterior region (region-C) (Bhaud et al. 1994).

5D.2.3. DNA extraction and PCR amplification

Some specimens of *S. costarum* were preserved in 99.5% ethanol directly for molecular analysis. Total genomic DNA was extracted using the CTAB method (Winnepenninckx *et al*. 1993). The Polymerase chain reaction (PCR) amplification was completed from mtCOI genes (Folmer *et al*. 1994) and 18S rRNA geneswith using master mixture reagent (Sigma-Aldrich) with the manufacturer's instructions. The final products of PCR were sequenced by ABI 3130xl sequencer. The obtained DNA sequence chromatogram was edited by ABI sequence scanner software 1.0v. Then, BLAST was used to find similar sequences in the NCBI database and finally representative all sequences were deposited in NCBI GenBank (Table 5D.1).

Table 5D.1. Sequences of chaetopterids with GenBank accession numbers used for phylogenetic analysis. The outgroup *Owenia* sp. is labeled as *Owenia fusiformis* in NCBI database.

Outgroup	**COI**	**18S**	**References**
Owenia sp.	GU67220 5.1	AY17629 8.1	Carr et al. 2011
Ingroup			
Chaetopterus cf. luteus	DQ209253.1	DQ209220.1	Osborn et al. 2007
Chaetopterus pugaporcinus PB1	DQ209257.1	DQ209224.1	Osborn et al. 2007
Chaetopterus pugaporcinus PB2	DQ209256.1	DQ209223.1	Osborn et al. 2007
Chaetopterus sarsi	DQ209254.1	DQ209221.1	Osborn et al. 2007
Chaetopterus sarsi 1	N/A	DQ779642.1	Carr et al. 2011
Chaetopterus sp. 1	DQ209252.1	DQ209219.1	Osborn et al. 2007
Chaetopterus sp. 2	DQ209255.1	DQ209222.1	Osborn et al. 2007
Chaetopterus sp. KP-2005	DQ087501.1	N/A	Peterson & Butterfield 2005
Chaetopterus sp. NKP-2014	N/A	KM206141.1	Carr et al. 2011
Chaetopterus sp. SEG_CHARREA	N/A	AH001603.1	Field et al. 1988
Chaetopterus variopedatus CvB-Fr	AM503095.1	AJ966759.1	Martin et al. 2008
Chaetopterus variopedatus CvN-It	AM503094.1	AJ966758.1	Martin et al. 2008
Chaetopterus variopedatus CvNo-Uk	AM503096.1	N/A	Martin et al. 2008
Chaetopterus variopedatus CVU67324	N/A	U67324.1	Direct submission
Mesochaetopterus japonicus	N/A	DQ209218.1	Osborn et al. 2007
Mesochaetopterus rogeri	AM503098.1	AJ966762.1	Martin et al. 2008
Mesochaetopterus taylori	DQ209251.1	DQ209217.1	Osborn et al. 2007
Mesochaetopterus tingkokensis	KP222296	KP222297	Zhang et al. 2015
Mesochaetopterus xerecus	AM503097.1	AJ966763.1	Martin et al. 2008
Phyllochaetopterus polus	GQ891958.1	N/A	Morineaux et al. 2010
Phyllochaetopterus prolifica BAMPOL0306	HM473565.1	N/A	Carr et al. 2011
Phyllochaetopterus prolifica BAMPOL0308	HM473567.1	N/A	Carr et al. 2011
Phyllochaetopterus socialis	DQ209247.1	DQ209212.1	Osborn et al. 2007
Phyllochaetopterus socialis PsB-Fr	N/A	AJ966761.1	Martin et al. 2008
Phyllochaetopterus gigas	DQ209248.1	DQ209213.1	Osborn et al. 2007
Phyllochaetopterus sp. 2	DQ209250.1	DQ209216.1	Osborn et al. 2007
Phyllochaetopterus sp. 1	DQ209249.1	DQ209215.1	Osborn et al. 2007
Phyllochaetopterus sp. SAM E3512	N/A	DQ779665.1	Rousset et al. 2007
Phyllochaetopterus sp. SAM E3513	N/A	DQ779666.1	Rousset et al. 2007
Spiochaetopterus bergensis	N/A	DQ209214.1	Osborn et al. 2007
Spiochaetopterus solitarius SsPV-Fr	N/A	AJ966760.1	Martin et al. 2008
Spiochaetopterus sp.	N/A	AF448165.1	Carr et al. 2011
Spiochaetopterus costarum	KT307698	N/A	Aylagas et al. 2016
Spiochaetopterus costarum	**KX525513**	**KX290710**	This study
Spiochaetopterus costarum	**KX525514**	**KX290711**	This study
Spiochaetopterus costarum	**KX525515**	-	This study

5D.2.4. Phylogenetic analysis

All available chaetopterid COI and 18S rRNA genes sequences in NCBI GenBank were used in phylogenetic analysis (Table 1). *Owenia* sp. was chosen as outgroup. These sequences were alignedby Mesquite using the MUSCLE algorithm, respectively. Poorly aligned positions and divergent regions were removed with the Gblocks Server. The most suitable models of molecular evolution for each gene and the concatenated data were determined using the bestfit substitution model GTR + Γ + I as indicated by the Modeltest2 based on the Akaike Information Criterion (AIC) (Darriba *et al*. 2012). Node support was assessed by 20000 bootstrap replicates. The Maximum Likelihood (ML) analysis was conducted with Phylogenetic Analysis Using Parsimony (PAUP)* V4.0 using settings previouslyreported by Osborn *et al*. (2007).

5D.3.1. Results

SYSTEMATICS

Phylum: Annelida

Class: Polychaeta

Subclass: Sedentaria

Family: Chaetopteridae

Genus: *Spiochaetopterus*

Species: *Spiochaetopterus costarum*

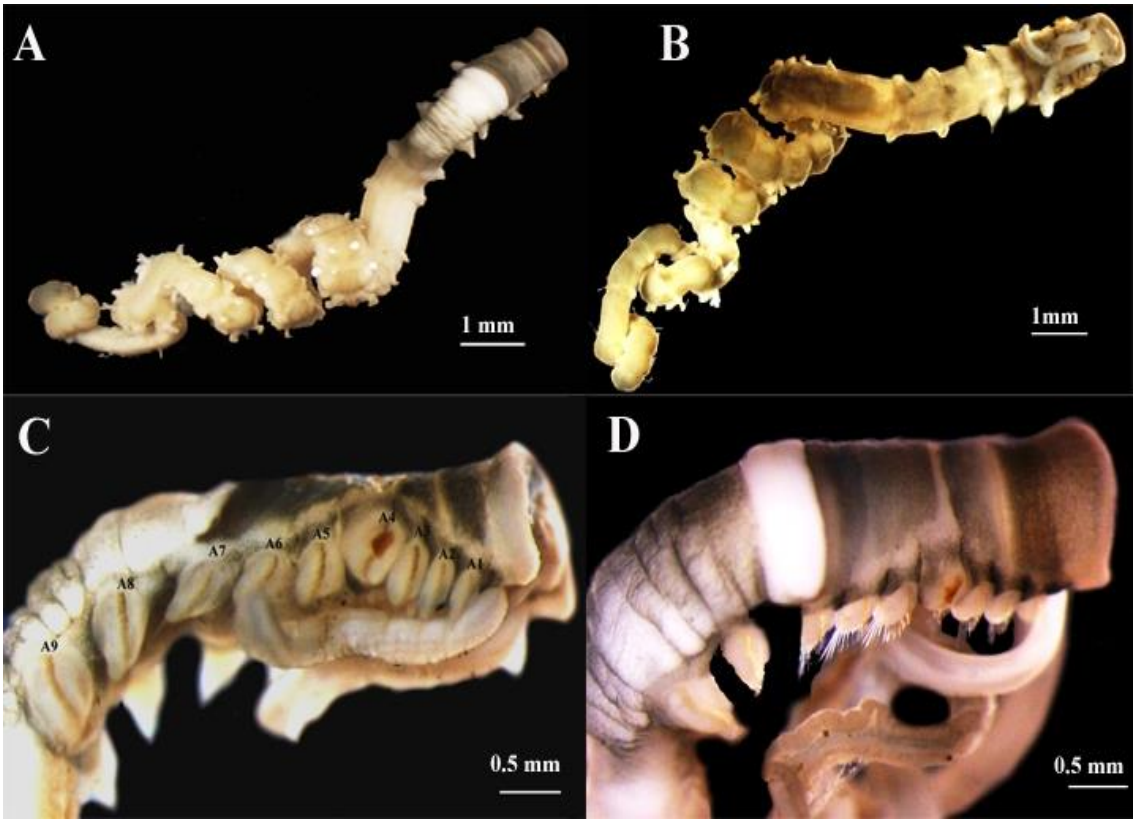

Fig 5D.2. *Spiochaetopterus costarum* from paratype ZSI/ANRC-14827. **A** and **B** whole specimens **C** and **D**: lateral view of anterior region giving coloration pattern with brown pigmentation on lateral side

5D.3.2. Morphological Identification

The *Spiochaetopterus* specimens were characterized by of smallsize, eyes present, with two pairs of longpalps from the west coast of India. The modified (cutting) chaetae of chaetiger A4, each with inflated head, pear-shaped in upper-ventral view, with teeth, and light yellow in color. Segments A6 to A7 with whitish ventral gland observed in alcohol-preserved specimens. Region B is with more than 2 chaetigers. Region C with neuropodia

unilobed, notopodia with small 2 or 3 pointed chaetae. Tube is transparent or translucent, partly amberto light white in color, branched, weakly annulated.

Region A narrow, 2.2 mm long for nine chaetigers in paratypes. Prostomium distinct, peristomium extended plate like (Fig 5D.3A). Eyespots present. Long paired palpi grooved, arising from near posterolateral border of prostomium (Fig 5D.2). Dorsal groove extending from base of palps through regions A, B, and C with ciliated. A single row of 10 to 20 lanceolate chaetae,chaetiger A1 to A3 short; A4 elongate, with two to four large (cutting) chaetae, and more than 10 lanceolate chaetae; A5 to A9 longer and wider than anterior three chaetigers, with single row of 30 to 40 lanceolate chaetae. The A4 chaetiger is obliquely semi-circular with large modified (cutting) chaetae. The total length of body 350–420 mm, head 70 mm in width, and 100 mmlength (Figs. 5D.2A, B). Head of modified chaetae slightly inflated, head wider than shaft, pear-shaped in upper ventral view, tip slightly pointed (Figs 5D.2B, C). Lateral or ventral grooves absent and shaft nearly semi-circular in horizontal section. These characters are based on the relative size of several parts and also on the asymmetry of the head (Fig 5D.2).

Middle region (region B) chaetigers similar in length to anterior chaetigers, this portion poorly preserved with mucous contamination, notopodia present but only one lobe present in types; paddle and cupule sometimes absent. B1 and B2 were of nearly same length. Neuropodia is unilobed with one row of uncini (partly discernible; Fig 5D.2B). Uncini is very small, bluntly triangular, with the single row of ca. 30 min teeth (Fig 5D.2F).

Posterior region (region C) is with 25 chaetigers. Knob-like tip is with 2 or 3 chaetae, notopodia of region-C with unilobed. Neuropodia is unilobed. The larger size of uncini is with similar morphology in the region-B; the 25 to 30 numbers of teeth, length ca. 10 mm and width ca. 8 mm (Fig 5D.2G). Tube is 1.0 to 1.5 mm in diameter, fragile, slender, nearly straight, weakly annulated (Fig 5D.2I). Tube wall is thin, transparent to translucent and light amber.

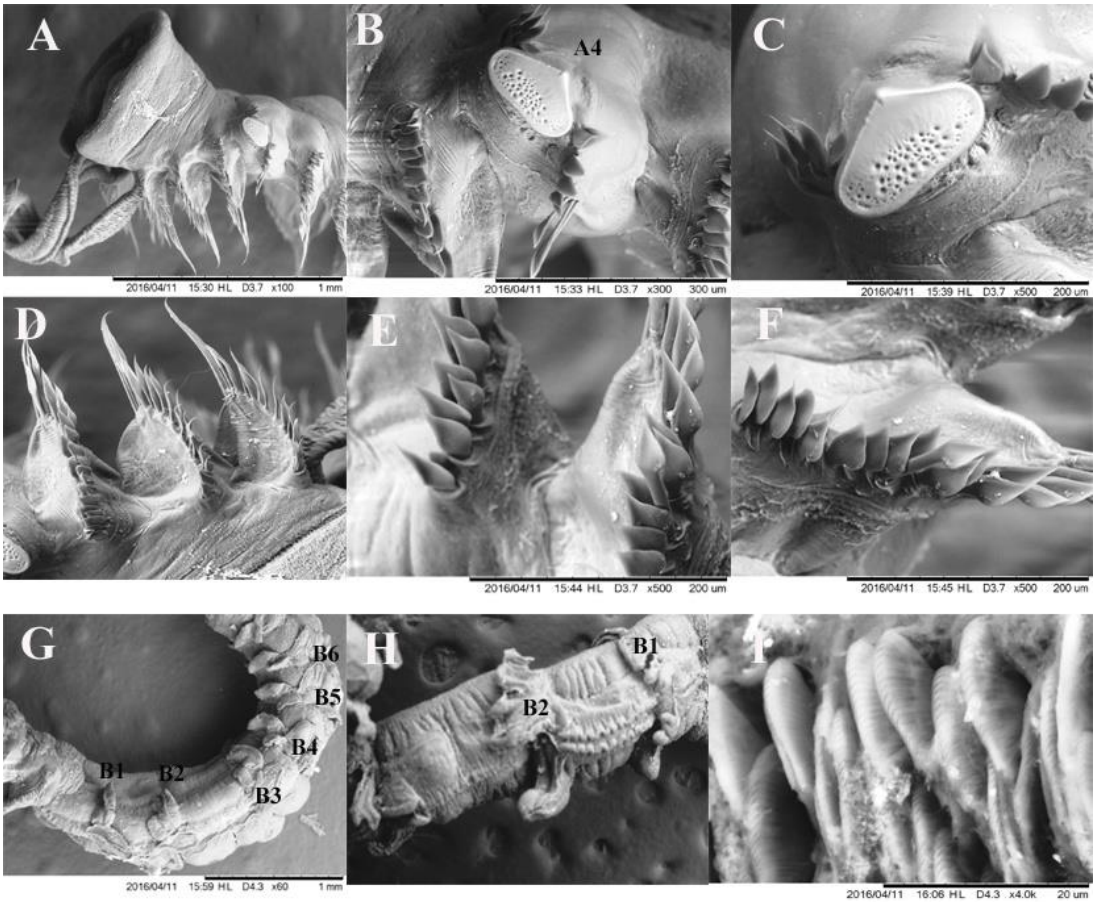

Fig. 5D.3 (A), lateral view of head, (B-C) chaetal arrangement of the modified A4 chaetiger. (E-F) chaetal arrangement of the knife-like chaeta. I, uncini from neuropodial. Scale bar is in µm.

5D.3.3. Geographic Distribution

Pacific: West Canada to South California, Japan, Indian Ocean, Madagascar, Atlantic-Mediterranean area: East to West North Atlantic, Mediterranean (Blake 1996, Day 1967). Bhaud (1998) studied from Arcachon beach in the Atlantic Ocean, Galician Rias Bajas (Spain, Atlantic coast), Banyuls and Naples in the Mediterranean Sea.

5D.3.4. Molecular phylogeny

The mtCOI and rDNA 18S sequences from specimens collected from the west coast of India were 645 bp and1800 bp long, respectively. These DNA gene sequences provide support that the specimens from belonging to *S. costarum*. The DNA gene sequences obtained from the west coast of India show that the specimens fall within the chaetopteridae family. TheCOI and 18S rDNA genes sequence have been submitted to NCBI (National center for Bioinformatics Institute) GenBank website and accession numbers obtained. The Maximum Likelihood trees of both COI and 18S rDNA sequences of *S. Costarum* were clustered with specimens from Spain, Italy and Basque Coast (Aylagas *et al*. 2016). In the Maximum Likelihood, this clade was sister to a poorly supported clade of the *Phyllochaetopterus* and *Spiochaetopterus*. They were polyphyletic and recovered in a well-supported clade with nested well inside the assemblage (Fig 5D.4).

Table 5D.2. Summary of the main features observed on four species in the Genus *Spiochaetopterus*

Characters Species	*S. solitarius*	*S. oculatus*	*S. costarum*	
		Gulf of Mexico	India	Italy
Worm length (mm)	20 mm	30-60 [8]	15-20 mm	50-60 C: 80[1]
Size ratio pro/peri	pro < peri	pro = peri	pro = peri	pro = peri
Eyespots	+	+	+	+
color	black	black very marked	black very marked	black
No. set. A	9 (10) [11]	9 [3]	9[10]	9 [1 11]
No. set. B	>2 <30:7-28 (N-55) [3] >2 <30:6-31 (N-335)[11]	21-74 (18-37) [8] 20-73 [7] 14-23 [3]	20-23 [5]	>30:35-54(N=35) [3] >30:34-54(N=72) [11]
No. set. C.	9-26 [3]	large number, variable [8] >38	24-27[3]	variable [1] >60 [5]>18 [3]
Dorsal cupules	B2 [9]	B2-B14, varies [8]	B2[8]	B2 [7]
Modified setae A4 (Fig 5)	clearly cordate 3: ventral curve very marked;	clearly cordate3: ventral curve very marked;	Clearly cordate 3(Fig 4) ventral curve very marked;	boadly cordate 3: sinus of the ventral curve not deep;
Shape of upper oblique plan	upper plane oblique	upper plane vertical	upper plane vertical	upper plane oblique
Number	1(2) 3	1 (2) [3 8 9]		1 [1] 1 [3]
Color	yellow to amber	yellow to amber	yellow to amber	yellow to amber
No. rami:	3	3	3	3
Notopodia B	1	1	1	1
Neuropodia B1	B2 to last: 2	B2 to last: 2	B2 to last: 2	B2 to last : 2
B2	1	1	1	1
Notopodia C	2	2	2	2
Neuropodia C				
Color in life	pale pinkish?	?	brown violet	brown violet
peristomium	violet stripes	white/br. Specks	white/br.	violet spots
dorsum A	pinkish: lt. grey	brownish purple	Specks	rose/white
VS-6	?	yellow-white: lt br	brownish	greenish[5]
B – C	pale (immature)		purple	?
eggs				
Tube width (mm)	0.63 0.6-0.8[10]	0.5-0.6[7] 0.7-1.2[8]	0.8-1.2[7]	1-1.5[1] 1.1.7[2]

		0.9[3]		1.6-1.7[3]
Tube length	75-110[10]	80-120 (500)[8]	65-70 [9]	1.2-1.4[9]
Tube septa	+[10]	+[83]	+[76]	=or > 250[9]
Type locality	Santander, N Spain Atlantic	Norhampton Co, Virginia, USA	West coast of India	+[9] Naples, Italy
Depth range	2-7 m[10]	Low water [7] Shallow Sub-tidal [8]	3-9 m [5]	4-5 m[9]
Substratum	Fine sand slightly muddy And dead Posidonia[3]	Sand [7] and mud [8]	Fine sand and silt	fine sand and silt
Distribution	temperate: NW Mediterranean (France+Spain) and Atl (NW Spain)	temperate: USA East Coast, from Massachusetts to the Gulf of Mexico (DAY 1973)	tropical: India	temperate + sub-tropical Ati+Med.

(*Source from: Bhaud 1998*)

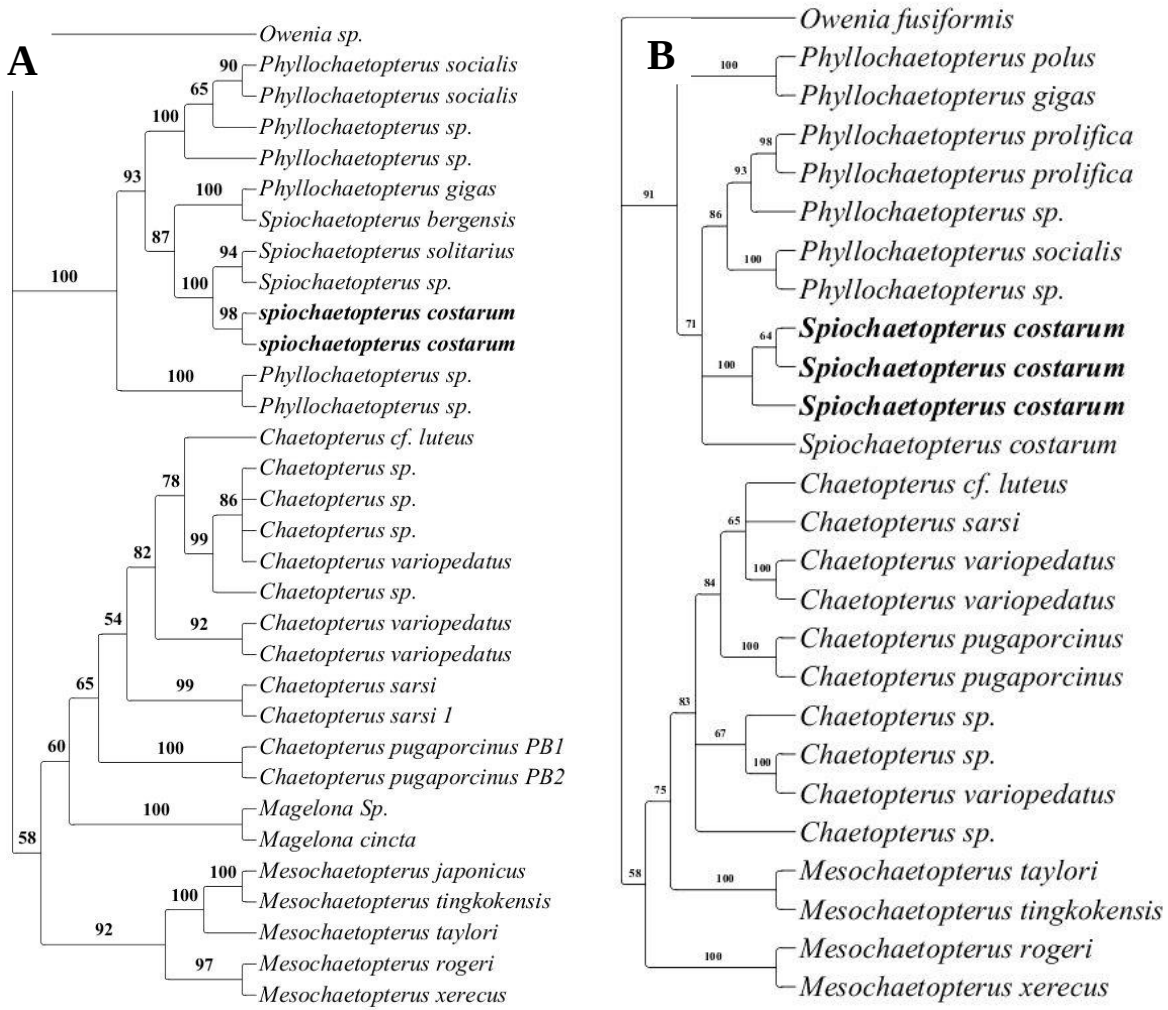

Fig 5D.4. Phylogenetic tree generated by analysis (ML). A, based on 18S gene sequences; B, based on COI genes. Numbers above the branches represent ML bootstrap values based on 20, 000 replicates. Highest possible support is 100. Values below 70 are considered weak, and values below 50 are not shown.

5D.4.1. Discussion

We found that new record of the *Spiochaetopterus* from the west coast of India was confirmed to be *S. costarum* based on morphological features and molecular analyses. The molecular study of mtCOI and 18S rDNA genes were identical to previously published DNA sequences of *S. costarum* (Aylagas *et al*. 2016). Particularly, the species were divided into two groups of different sizesin each geographic unit of the Atlantic-Mediterranean area. The *S. typicus* and *S. bergensis* are consisting with boreal biogeographic similarity and the temperate biogeographic species affinity consisting of *S. costarum, S. solitarius* and *S. oculatus* (Bhaud 1998). However, the implying of long planktonic larval and gene flowconsist that individuals from widely separate areas all belong to one species and justified that planktonic larvae are transported over long distances (Scheltema 1974, 1986). Nevertheless, the extensive larval dispersals were considered to be promoting geographical homogeneity and most important to the existence of potentially cosmopolitan species (Bhaud 2003). However, Jablonski (1986) suggested the species with high dispersal of larval phase have wider geographic ranges, longer species duration on a geological scale, and lower rates of speciation than similar species with low dispersal. These futures suggested that *S. costarum* is a pseudo-sibling complex species.

Phylogenetic analyses placed *S. costarum* within clade of *Spiochaetopterus*, suggesting that the species found here belongs to genus *Spiochaetopterus*. The molecular analysis of *S. costarum* used for the phylogenetic relationship based on slowly evolving 18S rRNA genes and a faster evolving mtCOI gene. These genes have been used in the phylogentic study of chaetopteridae family by Osborn *et al*. (2007), Martin *et al*. (2008), Morineaux

et al. (2010). The ML analyses showed the two gene fragments were concatenated to generate a phylogenetic tree and *S. costarum* in the same clade, with species being most closely relate to *S. costarum* in Basque Coast, Spain. In addition, *Mesochaetopterus* and *Chaetopterus* are sister groups, whereas *Phyllochaetopterus* and *Spiochaetopterus* are paraphyletic.

SUMMARY

- The west coast of India is an ecologically productive region which promotes higher biological diversity in the region.
- In a recent review Sivadas and Ingole (2016) reported overall 564 species belonging to 262 genera and 54 families from the Indian waters. The highest species richness is recorded in the eastern basin with 255 species, followed by 160 species from the west coast of India.
- In the present study, 71 polychaete species belonging to 16 families were collected and identified based on themorphological characterstics and molecular analaysis.
- The regional polychaete species assemblage was dominated by *Cossura delta* (13.48% in the Northern Region), whereas *Aphelochaeta multifilis* (10.55%) dominated in the Southern Region.
- The increasing trends in polychaete diversity were observed from southern to northern region.
- Changes in assemblage patterns were due to the physical forcing together with changes in substratum and its potential role towards ecosystem functioning.
- The Maximum Likelihood analyses recovered many relationships and consistent with results obtained from analyses of 18S rDNA gene sequences.
- The contribution of new data on the molecular sequence of 18S rDNA gene will support the execution of further studies to help discover new patterin that trigger the high diversity.
- Most of the polychaete families have monophyletic groups.

- The analysis of genetic diversity data revealed that there is a single population of reef building *S. chandraae* habited along the ~1260 km west coast of India. A combination of historical factors, reversing WICC, habitat availability and local adaptation could have been responsible for wider distribution range with the low genetic diversity.
- The detailed taxanomic and molecular analysis resulted in describing three new polychaete species (*Neosabellaria indica* nov. sp., *Sabellaria goae* nov. sp., and *Pseudonereis corallinsis* sp. nov.) from the west coast of India. Whereas *Spiochaetopterus costarum*is identified from the estuarine region of Goa (Mandovi River) is a new record from the region.
- The phylogenetic analyses supported the monophyly of the family and revealed two well-supported clades: *Chaetopterus*/ *Mesochaetopterus* and *Spiochaetopterus*/ *Phyllochaetopterus*. However, *S. costarum* could be an introduced cosmopolitan species.
- The biogeography and functional traits remains to be investigated and could be a primary focus of future DNA barcoding studies.
- The present study contributes to the ongoing research effort to document the biodiversity of the region. It has successfully added few new species, new records and described diversity patterns.
- The results presented here are important for effective research-driven ecosystem-based management of the rapidly changing benthic ecosystem.

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Publications

1. **Periasamy R**, Ingole BS. (2018). 18S rDNA sequencing data of Benthic Polychaetes from the Eastern Arabian Sea. Data in Brief. 20, 1749-1752

2. **Periasamy R**, Kalyan D, Ingole BS (2017) Polychaete assemblage driven by substrate composition along the coastal waters of the South-eastern Arabian Sea. Reg Stud Mar Sci. 16, 208-215.

3. Ingole BS, **Periasamy R**, Kalyan D (2016) Macrobenthic Community Structure Response to Coastal Hypoxia off Southeastern Arabian Sea. J Coast Zone Manag. 19(4), 1-10.

4. Manikandan B, Ravindran J, Mohan H, **Periasamy R**, ManiMurali R, Ingole BS (2016) Community structure and coral health status across the depth gradients of Grande Island, Central west coast of India. Reg Stud Mar Sci. 7, 150-158.

5. **Periasamy R**, BS Ingole and Ram Murthi Meena (2017). Phylogeny and Genetic variation within Population of the *Tachypleus gigas* (Müller, 1785). Curr Sci. 112(10), 2029-2033.

6. **Periasamy R,** Chen X, Ingole BS, Liu W (2016) Complete mitochondrial genome of the Spadenose shark *Scoliodon laticaudus* (Carcharhiniformes: Carcharhinidae). Mitochondrial DNA Part A. 27(5), 3248-3249.

Papers presented at National Conference

1. Kalyan De, Sambhaji Mote, Lobsang Tsering, **Periasamy R**, Vishal Patil, Sautya, S., Ingole, B.S. Insight of scleractinian coral diversity of Malvan Marine Sanctuary. World Ocean Science Congress, 2015, Kochi, India

Data in Brief 20 (2018) 1749–1752

Contents lists available at ScienceDirect

Data in Brief

journal homepage: www.elsevier.com/locate/dib

Data Article

18S rDNA sequencing data of benthic polychaetes from the Eastern Arabian Sea

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ARTICLE INFO

Article history:
Received 21 August 2018
Received in revised form
30 August 2018
Accepted 7 September 2018

ABSTRACT

The limited DNA sequence data of the polychaetes species are available from the Eastern Arabian Sea. We have sequenced 18S rDNA gene from 54 polychaetes species and 37 species identified up to the species level. The DNA bar-coding data provides for molecular identification of benthic polychaetes that will provide imminent into drivers of species diversity in the Eastern Arabian Sea. The 18S rDNA sequence data set is made publicly available to enable critical or extended analyzes of DNA bar-coding.

Specifications table

Subject area	Marine biology
More specific subject area	Molecular biology, Benthic polychaetes
Type of data	Figures, Table
How data was acquired	Applied biosystems (ABI) 3730xl DNA sequencer
Data format analysed	Raw data (Fasta)
Experimental factor	Benthic polychaetes species
Experimental features	Datasets for body of tissues
Data source location	West coast of India
Data accessibility	Data is with this article and available online at https://www.ncbi.nlm.nih.gov/nuccore/KX525515

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https://doi.org/10.1016/j.dib.2018.09.015

Value of the data

- These data are the first generated using 18S rRNA genes of polychaetes in west coast of India.
- This project presents the diversity of benthic polychaetes communities by using 18S rRNA gene sequencing.
- This data provides other researchers to extend the molecular identification (DNA barcoding).

1. Data

The molecular taxonomy is refreshing traditional taxonomy and helps to increase the taxonomic crisis, alternative and complementary approaches, particularly successful in the identification and delimitation of new species from various groups [1]. Recently, the increased identification of abundance and importance of cryptic species, those are morphologically identical but genetically different [2]. Moreover, the molecular identification has been reformed the exploration of biodiversity for which traditional taxonomy is difficult [3]. There has been increased numbers of unidentified specimens in our collection which limits their use in future studies involving the biogeography. The most commonly occurring polychaete species are shown in the Fig. 1. A total 54 polychaete species were newly sequenced based on the 18S rDNA gene together with 88 sequences submitted to NCBI GenBank (Table 1) including *Paraprionospio cristata* Zhou, Yokoyama and Li, 2008, and *Paraprionospio patiens* Yokoyama, 2007. They are most dominant and opportunistic species along the study area.

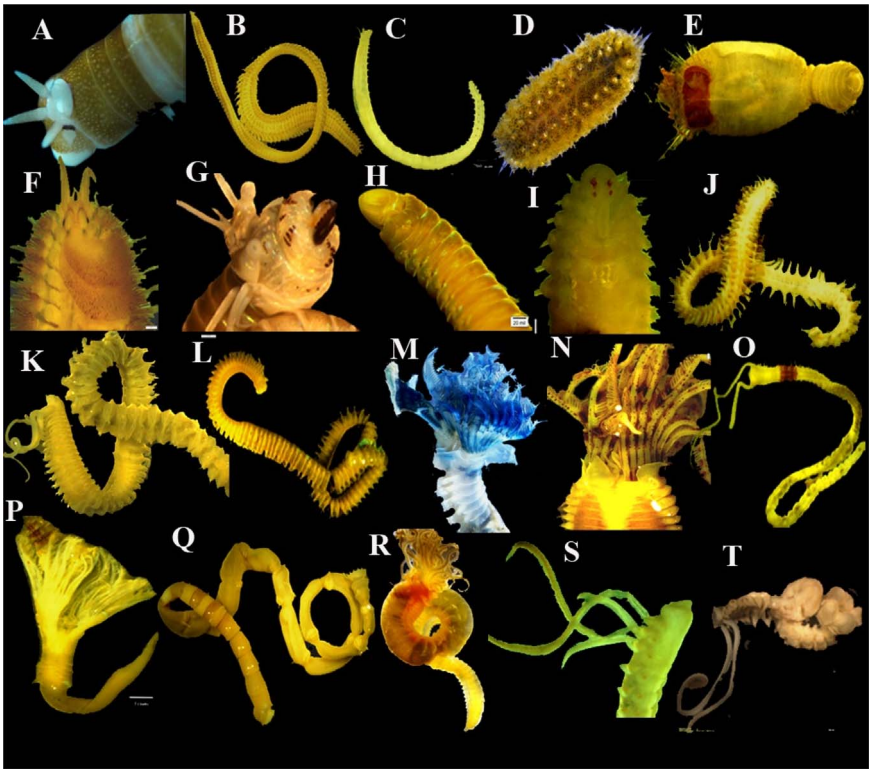

Fig. 1. Commonly occurring polychaete species-A: Lysidice sp., B: Eteone heteropoda, C: Haplosyllis sp., D: Thormora sp., E: Sternapsis suctata, F: G: Perinereis cultrifera, H: Lumbrineris funchalensis, I: Pareurythoe borealis, J: Ceratonereis japonica, K-L: Scolelepis sp., M: Pomatoceros triqueter, N: Parasabella saxicola, O: Magelona cincta, P: Pomatostegus actinoceros, Q: Euclymene sp., R: Terebella sp., S: Paraprionospio cordifolia, T: Spiochaetopterus sp.

Table 1
NCBI Accession number for benthic polychaetes species along the west coast of India.

Specimen voucher	Morphological ID	NCBI Accession number
GP0161–GP0163	*Eurythoe complanata*	KT900265–KT900267
GP0164	*Notopygoscaribea*	KT900268
GP0165	*Eurythoe complanata*	KT900269
GP0166	*Pareurythoe borealis*	KT900270
GP0167–GP0168	*Thormora* sp.	KT900271–KT900272
GP0169–GP0170	*Chloeiaviridis*	KT900273–KT900274
GP0171–GP0173	*Eurytho ecomplanata*	KT900275–KT900277
GP0174	*Hermenia verruculosa*	KT900278
GP0175	*Chloeia viridis*	KT900279
GP0176–GP0177	*Notopygos ornate*	KT900280–KT900281
GP0178	*Haplosyllis* sp.	KT900282
GP0179	*Pseudonereis* sp.	KT900283
GP0180	*Perinereis cultrifera*	KT900284
GP0181–GP0182	*Platynereis dumerlii*	KT900285–KT900286
GP0183	*Namalycastis abiuma*	KT900287
GP0184	*Dendronereis aestuarina*	KT900288
GP0185	*Namalycastis abiuma*	KT900289
GP0186	*Platynereis australis*	KT900290
GP0187	*Nereis sandersi*	KT900291
GP0188	*Glycera capitata*	KT900292
GP0189	*Glycera alba*	KT900293
GP0190	*Eunice miurai*	KT900294
GP0191–GP0192	*Lysidice* sp.	KT900295–KT900296
GP0193	*Lumbrineris funchalensis*	KT900297
GP0194	*Marphysa viridis*	KT900298
GP0195	*Ninoe nigripes*	KT900299
GP0196–GP0197	*Marphysa* sp.	KT900300–KT900301
GP0198	*Diopatra* sp.	KT900302
GP0199	*Eunice miurai*	KT900303
GP0200–GP0202	*Paraprionospio cordifolia*	KT900304–KT900306
GP0203–GP0204	*Paraprionospio patians*	KT900307–KT900308
GP0205	*Paraprionospio cordifolia*	KT900309
GP0206–GP0207	*Scolelepis* sp.	KT900310–KT900311
GP0208	*Magelona cincta*	KT900312
GP0209–GP0212	*Neosabellaria indica*	KT900313–KT900316
GP0213–GP0214	*Sabellaria chandraae*	KT900317–KT900318
GP0215	*Sabellaria intoshi*	KT900319
GP0216–GP0217	*Terebella* sp.	KT900320–KT900321
GP0218	*Paraeupolymniauspiana*	KT900322
GP0219–GP0220	*Parasabella saxicola*	KT900323–KT900324
GP0221	*Hydroides sanctaecrucis*	KT900325
GP0222	*Chitinopomaserrula*	KT900326
GP0223	*Pomatoceros triqueter*	KT900327
GP0224	*Spirobranchuslatiscapus*	KT900328
GP0225	*Thormora* sp.	KX290696
GP0226–GP0227	*Bhawaniacryptocephala*	KX290697–KX290698
GP0228–GP0229	*Perinereis* sp.	KX290699–KX290700
GP0230	*Nectoneanthes oxypoda*	KX290701
GP0231–GP0232	*Hermeniave rruculosa*	KX290702–KX290703
GP0233	*Hedisteatoka*	KX290704
GP0234–GP0235	*Terebellides* sp.	KX290705–KX290706
GP0236–GP0237	*Paralacydonia paradoxa*	KX290707–KX290708
GP0238	*Hesione* sp.	KX290709
GP0239–GP0240	*Spiochaetopterus* sp.	KX290710–KX290711
GP0241	*Euclymene* sp.	KX290712

2. Experimental design, materials and methods

The sediment samples were collected at the following localities. Sediment samples were collected using 0.04 m² van Veen grabs. Samples were sieved on a 500 μm mesh. In the laboratory, the sediment samples were washed again, sorted, and stored in 95% ethanol. Some of middle segments of polychaete species were removed from these specimens and kept in vials containing absolute ethanol until further use for DNA isolation. Identification of polychaete species was done by observing diagnostic characters parapodia-bearing chitinous chaetae under stereo zoom microscope using keys [4,5].

2.1. DNA extraction, PCR amplification, purification, and sequencing

Genomic DNA was extracted from the specimen using the Qiagen DNeasy Tissue Kit according to manufacturer's instructions. The 18S rRNA gene amplifications were carried out using primer pair 18F/18R1843 [6]. PCR amplification of the 18S rDNA gene changed into done in overlapping fragments of ~1800 bp length each with modified primer pairs with standard cycle sequencing protocols. Amplifications had been carried out using an Eppendorf Master Cycler Gradient. The following PCR temperature file was used: 95 C for 3 min; 35 cycles at 95 °C for 45 s, 60 °C for 1 min, and 72 C for 2 min; final extension at 72 C for 5 min. After detection by gel electrophoresis, the products had been purified using the Qiaquick PCR Purification Kit (Qiagen). Sequences were produced using the same primers and determined on an Applied Biosystems (ABI) 3730xl. All sequences were submitted to NCBI GenBank (Table 1).

Acknowledgements

The authors are grateful to the Director, CSIR-National Institute of Oceanography Goa, for encouragement and facilities. The authors also wish to thank Mr. Ram Murti Meena, Biology Division for providing sequencing facilities. We also acknowledge the financial support from Maharashtra Gene bank project GAP 2871.

Funding sources

This work is part of a Ph.D. thesis of Periasamy Rengaiyan and was funded by MoES (Govt. of India) under COMAPS project (No. GAP-2741).

Transparency document. Supporting information

Transparency data associated with this article can be found in the online version at https://doi.org/10.1016/j.dib.2018.09.015.

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Regional Studies in Marine Science 16 (2017) 208–215

Contents lists available at ScienceDirect

Regional Studies in Marine Science

journal homepage: www.elsevier.com/locate/rsma

Polychaete assemblage driven by substrate composition along the coastal waters of the South-eastern Arabian Sea

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HIGHLIGHTS

- The regional species assemblage dominated by *Cossura delta* (13.48% in the NR), whereas *Aphelochaeta multifilis* (10.55%) dominated in the SR.
- The increasing trends in polychaete diversity with its maximum value were observed in the SR.
- Increased richness and diversity patterns in southern region were in contrast with northern region.

ARTICLE INFO

Article history:
Received 10 July 2017
Received in revised form 26 August 2017
Accepted 7 September 2017
Available online 21 September 2017

Keywords:
Polychaetes
Functional groups
Diversity
Abundance
Sediment texture

ABSTRACT

The spatial variation in polychaete community structure and sediment composition was studied along the coastal waters of the South-eastern Arabian Sea. Sampling was conducted during the pre-monsoon season (2011–2014) in <100 m water depth with the help of van Veen grab. We identified a total of 71 polychaetes species belonging to 16 families. Cluster analysis showed clear separation based on the species distribution. Canonical Corresponding Analysis (CCA) revealed the close dependence of polychaete species to the sediment texture (fine sand & silty clay). The first assemblage was in the South Region (SR) as the sediment texture between Trivandrum, Cochin and Mangalore were characterized by fine sand. The second species assemblage was observed in the Central Region (CR) at Karwar and Goa where the substrate in this area was characterized by a moderate content of silt. The third assemblage was in the North Region (NR) between Ratnagiri and Mumbai and the sediment in this region was characterized by high content of silt and clay. The SIMPER analysis showed regional species assemblage dominated by *Cossura delta* (13.48% in the NR), whereas *Aphelochaeta multifilis* (10.55%) dominated in the SR. Increased richness and diversity patterns in the SR were in contrast with NR. The study suggests that the distinct polychaete assemblages in the region were may be influenced by sediment texture pattern. Therefore, long-term studies could be directed towards the demographic, behavioural, and ecological responses of species assemblages.

1. Introduction

Free-living polychaetes are abundantly distributed in the soft sediment and play an ecologically important role in the benthic food web (Gray and Elliott, 2009). The sediment burrowing polychaetes species are more or less integral part of coastal sediment and form the central role between the benthic and pelagic systems. They are often diverse in their feeding strategies and highly abundant, especially in areas of anthropogenic stress (Gray and Elliott, 2009; Fauchald and Jumars, 1979). Polychaetes are decisive in marine food chains, as important prey for many crustaceans, mollusks, fish, wading birds and other marine organisms. They play a major role in the breakdown, subduction, and integration of organic matter into sediments and their bio-turbation. Being a major constituent of the soft bottom macrofauna, polychaete species composition, abundance, diversity, and biomass have been successfully used to assess the health of the coastal ecosystem (Ingole et al., 2009; Sivadas et al., 2010, 2016).

The unique environmental condition along the west coast of India has influenced on the formation of the typical pelagic ecosystem, though, it does enhance the benthic ecosystems (Ingole et al., 2010; Singh and Ingole, 2015). The benthic studies have been made to understand the quantitative nature and community structure from different regions of the country. In the western continental shelf, substantial amount of information is available on the benthic community structure (e.g., Sajan and Damodaran, 2007; Jayaraj et al., 2007, 2008; Ingole et al., 2009, 2010; Joydas and Damodaran, 2009; Sivadas et al., 2010, 2016; Jaleel et al., 2015). However, the above studies were carried out in different seasons following

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http://dx.doi.org/10.1016/j.rsma.2017.09.004
2352-4855/

Table 1
The distribution of sediment texture along the South-eastern Arabian Sea based on laser diffraction.

Stations	Date	Depth (m)	D_{50}	Coarse sand (%)	Medium sand (%)	Fine sand %	Silt (%)	Clay (%)
Trivandrum	March 2011	10 to 50	0.274	28.97	60.07	11.00	–	–
Cochin	March 2010	10 to 50	0.264	32.50	48.93	18.57	–	–
Mangalore	May 2010	10 to 50	0.234	–	34.70	65.26	0.04	–
Karwar	May 2012	10 to 30	0.003	–	–	30.10	17.70	52.20
Goa	May 2014	10 to 20	0.064	–	–	24.49	29.05	46.46
Ratnagiri	March 2012	10 to 30	0.003	–	–	3.97	95.99	0.04
Mumbai	May 2013	10 to 30	0.013	–	–	2.65	91.55	5.80

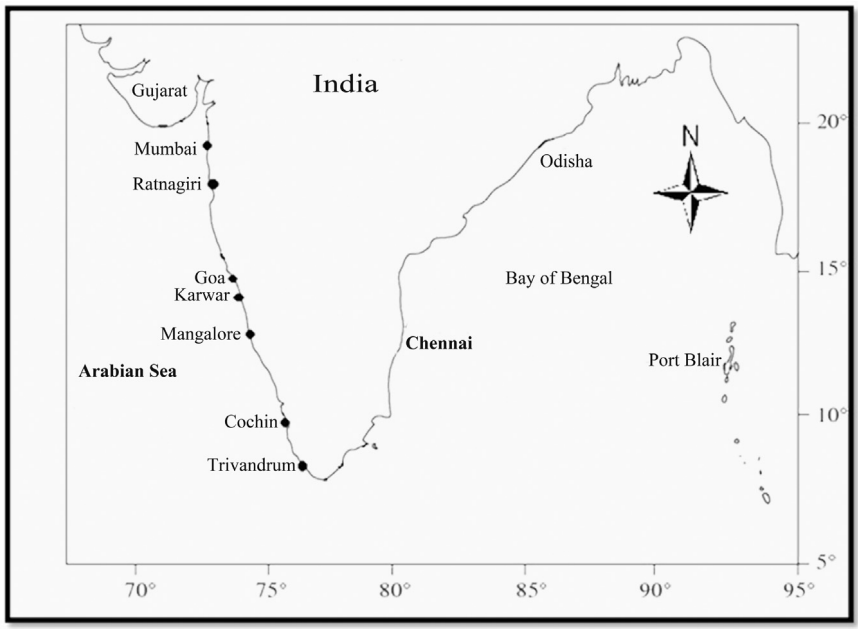

Fig. 1. Sampling locations along the South-eastern Arabian Sea.

different methodologies and hence their utility for comparison is limited. Nevertheless, the aspects of quantitative distribution, standing crop and annual production of benthos in Indian seas and the effectiveness of the data for assessing the potential demersal resources were studied by Parulekar et al. (1992). According to Joydas and Damodaran (2009), the contribution of polychaete was 57% in total macrofaunal population with 122 species from 51–100 m and 52 species in the shelf edge of the west coast of India. Musale and Desai (2011) reported 63 polychaete species along the Indian coast, of which 38 species were from the west and 25 were from the east coast of India. While reviewing the polychaete species of Indian coast, Sivadas and Ingole (2016) reported overall 564 species belonging to 262 genera and 54 families. The highest species richness is recorded in the eastern basin with 255 species, followed by 160 species western basins with and in the Andaman and Nicobar with 157 species. In general, the Neredidae was the most diverse family (71 species). Therefore, the aim of this study was to evaluate the variation in the polychaete community structures and its relation with the sediment texture from the shallow coastal areas.

2. Materials and methods

2.1. Study area and sample collection

The sampling was carried out on board *CVR Sagar Sukti* and *FORV Sagar Sampada* from May 2011 to March 2014, in order to generate local-scale benthic data. The sediment samples were collected at the following localities Mumbai 17°08'42'' N 73°16'04'' E, Ratnagiri 16°22'24'' N 73°22'16'' E, Goa 15°51'06'' N 73°38'24'' E, Karwar 15°41'18'' N 73°42'14'' E, Mangalore 12°59'19'' N 74°47'58'' E, Cochin 11°52'02'' N 75°21'10'' E, Trivandrum 08°04'38'' N 77°31'50'' E (Table 1 and Fig. 1). Sediment samples were collected using 0.04 m^2 van Veen grabs. Three grabs were taken from each station for the analysis of benthic polychaetes and one for sedimentological study. The grabs were able to penetrate 15 cm into the sediments. The sediment samples after sieving through 500 μm mesh sieve were brought to the laboratory in polythene bags in the white-bottomed tray. The polychaetes were hand sorted by a forceps. After the preliminary examination, the whole sample was fixed in 5% formalin. Polychaete species were later identified and counted under a stereomicroscope to the lowest taxonomic level (Fauvel, 1953; Day, 1967).

2.2. Laboratory analysis

The sediment samples were washed again, sorted, and stored in 5% buffered formalin. Polychaete species were later identified to the lowest taxonomic level (Fauvel, 1953; Day, 1967) and counted under a stereomicroscope. Sediment texture was analysed by Malvern Laser Analyzer (Model—Hydro 2000MU). Sediment samples of 50 g were washed using de-ionized water and dried at 45 °C and treated overnight with 30% H_2O_2 for removal of organic matter. The samples were sieved through a 62 m mesh to separate the sand from the mud fraction. Sand samples were analysis used of sieve analysis (Carpenter and Deitz, 1950) and mud samples were analysed by Malvern laser particle size analyzer.

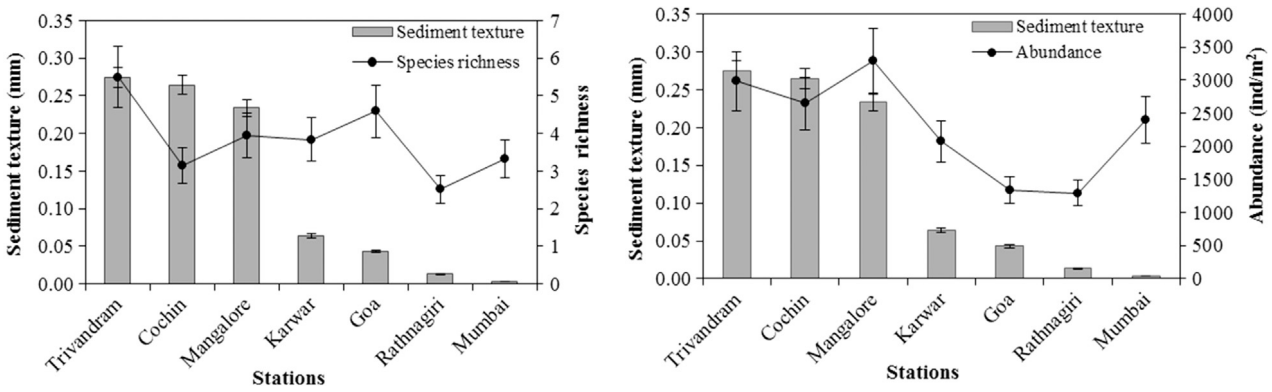

Fig. 2. Sediment texture, species abundances, and richness along the South-eastern Arabian Sea.

2.3. Data analysis

Polychaete data was processed using univariate and multivariate methods. Diversity indices were calculated using Margalef's index for species richness (d), Pielou's index (Pielou 1966) for species evenness (J'), Shannon–Wiener index (Shannon and Weaver 1963) for species diversity (H' log) and Simpson's dominance index (*C*') reflects the even occurrence of polychaete species within a community. The Bray–Curtis similarity cluster analysis shows that among the regions measure for abundance data after log transformation. Following the division into groups from results of cluster analysis, the contribution of polychaete species was determined using similarity percentage program SIMPER using PRIMER 6 (Clarke and Warwick, 2001). Feeding types of polychaetes species were assigned according to Fauchald and Jumars (1979). A Canonical Correspondence Analysis (CCA) was carried out to show in a single diagram the direct interpretation of the relationship between sediment characteristics (Medium, fine sand, and silt–clay) and polychaetes species using the Multivariate Statistical Package version (MSPV) 3.1 (Kovach, 1998).

3. Results

3.1. Distribution of sediment texture

The distribution of grain-size sand–silt–clay of sediment varied from fine sand to mud along the study area. Two types of sediment textures were observed in the study area. The Northern Region (NR) was characterized by silty and clay, whereas the SR was characterized by fine sand. In general, the distribution of silt and clay was similar, while sand shows an opposite trend. In the SR low percentage of silt–clay content (0.34%) was observed, with coarse (20.47%), medium (47.6%), fine sand and silt–clay contents (31.61%). In the Central Region (CR) fine sand content varies from 24.49% to 30.1%, silt 17.7%–95.99% and clay 46.46%–52.2%. The poor sand content along CR and NR with near-equal proportions of fine sand 2.65%–3.97%, silt 91.55%–95.99% and clay 0.04%–5.8% was present (Table 1).

3.2. Diversity indices

Margalef's index (d) varied from 3.15 to 5.5 in the SR, whereas in CR it varied from 3.8 to 4.59, while a low d value was recorded from 2.5 to 3.3 in the NR. The species evenness (J) varied from 0.4 to 0.6 in SR whereas, in the NR J ranged from 0.5 to 0.7. However, the high value of species diversity (H') of 3.5 to 5.71 was observed in the NR, whereas in SR (H') value ranged from 1.9 to 2.99. Applying Simpson's dominance index (*C*'), the Southwest coast had slightly lower values of dominance index (*C*') (0.76 to 0.79) than the Central west coast (0.82 to 0.86) and Northwest coast (0.81 to 0.91), indicating that there were clear dominance polychaete species (Fig. 2).

3.3. Cluster analysis of polychaete assemblage

The cluster analysis showed that the clear difference in the polychaete species assemblage (Fig. 3). We found that three groups were characterized with different ecosystem setting along the South-eastern Arabian Sea, with a barring of station taken close to shallow coastal water. Species richness, diversity, and density changed gradually from the high value in the SR to the low values in the NR. The data on the average abundance of polychaete species were placed into three different groups, which showed three structurally different communities in the study area. The SR group was differentiated by the dominance of *A. multifilis* and *Mediomastus capensis* and NR group were dominated by *C. delta* and *P. cordifolia* (Table 2). The Bray–Curtis cluster analysis also showed three groups based on the species distribution. Group, I consisted of Trivandrum, Cochin and Mangalore stations with 66.3% similarity, whereas group II was clustered with 60.03% of Karwar and Goa and Group III at 57.68% similarity comprised of Mumbai and Ratnagiri. The polychaetes assemblage was subjected to the SIMPER analysis to find the species, which contributed to the similarity within each group. Consequently, Groups I (Trivandrum, Cochin and Mangalore) was dominated by *T. multifilis* (11%) and *M. capensis* (6.72%) with carnivore species of *Scoletoma funchalensis* (5.68%), Group II (Karwar and Goa) *Cossura delta* (10.59%), *P. cordifolia* (9.4%), and *Capitella capitata* (8.47%); Group III (Ratnagiri and Mumbai) by *C. delta* (13.48%) and *P. cordifolia* (12.31%) with carnivore species of *Glycera alba* (10.78%) (Table 2).

3.4. Distribution of polychaete feeding type

The SR was dominated by surface deposit feeders (dwelling polychaetes 54.4%), sub-surface deposit feeder (15.35%), carnivores (25.63%) and suspension feeders (4.63%), whereas, in CR surface and sub-surface feeder existed with 64.21% and carnivore (35.79%). *C. delta* and *P. cordifolia* were dominant in the surface and subsurface deposit feeder (63.59%) with carnivore (31.65%) as well as filter feeder in the NR. The suspension deposit feeder (4.76%) was common (Fig. 4, Table 3).

The two axes of CCA biplot explained 83% of the relationship between benthic polychaetes species and sediment variables. Medium, fine sand, and silt–clay were the most important sediment variables influencing benthic species abundance. *C. delta* and *P. cordifolia* preferred silty clayey substratum whereas *A. multifilis, M. capensis L. funchalensis* and *I. inermis* preferred fine sand. The results show the group II polychaete species from the CR were

Table 2
SIMPER analysis show contribution of differences species assemblage and feeding types. Surface deposit feeder (SDF), sub-surface deposit feeder (SSDF), sub-surface predator (SSP).

Species	Average dominance	Average similarity	Contrib%	Cum.%	Feeding guilds
Group1: South west coast (Trivandrum, Cochin, and Mangalore)					
Average similarity: 66.03					
Aphelochaeta multifilis	7.41	6.96	10.55	10.55	SDF
Mediomastuscapenis	5.14	4.43	6.72	17.26	SSDF
Scoletoma funchalensis	4.22	3.75	5.68	22.94	SSP
Inermonephtys inermis	3.85	3.12	4.73	27.67	SSP
Levinsenia sp.	3.94	3.05	4.63	32.3	SSDF
Group2: Central west coast (Karwar and Goa)					
Average similarity: 60.03					
Cossura delta	5.84	6.36	10.59	10.59	SDF
Paraprionospio cordifolia	4.85	5.64	9.4	19.99	SDF
Capitella capitata	4.33	5.08	8.47	28.46	SSDF
Inermonephtys inermis	3.78	4.2	6.99	35.45	SSP
Aphelochaeta multifilis	5.37	4.2	6.99	42.44	SDF
Group3: North west coast (Ratnagiri and Mumbai)					
Average similarity: 57.68					
Cossura delta	6.24	7.78	13.48	13.48	SDF
Paraprionospio cordifolia	5.6	7.1	12.31	25.8	SDF
Glycera alba	4.88	6.22	10.78	36.58	SSP
Scoletoma funchalensis	3.94	4.96	8.59	45.17	SSP
Aricidea sp.	4.07	4.25	7.36	52.53	SDF

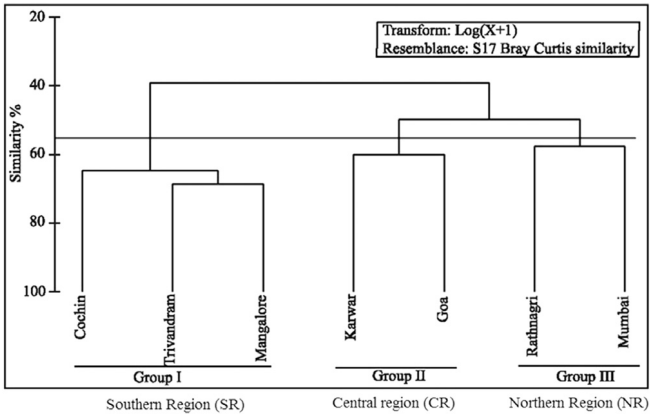

Fig. 3. Cluster analysis of similarity among sediment samples of NR, CR, and SR sites, based on log(X + 1) transformed and the similarity level overlay on 56% similarity.

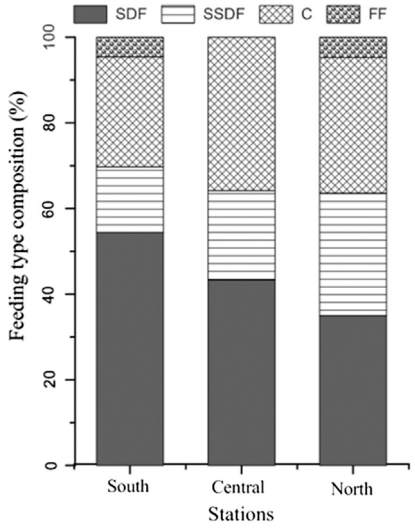

Fig. 4. The distribution of polychaete species feeding types, surface deposit feeder (SDF), sub-surface deposit feeder (SSDF), carnivores (C) and filter feeder (FF).

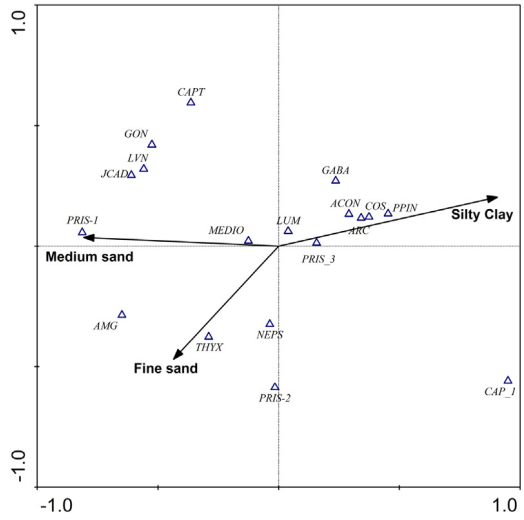

Fig. 5. Canonical correspondence analysis (CCA) showing polychaete species correlated with sediment type. CAPT: *Capitella capitata*, GON: *Goniadides* sp., LVN: *Levinsenia* sp., JCAD: *Jasmeneira caudate*, PRIS-1: *Prionospio aucklandica*, MEDIO: *Midiomastus capensis*, LUM: *Scoletoma funchalensis*, GABA: *Glycera alba*, ACON: *Sigambra constricta*, COS: *Cossura delta*, PPIN: *Paraprionospio cordifolia*, ARC: *Aricidea* sp., PRIS_3: *Prionospio cirrifera*, AMG: *Amage* sp., THYX: *Aphelochaeta multifilis*, NEPS: *Inermonephtys inermis*, PRIS_2: *Prionospio pygmaea*, CAP_1: *Capitellethus dispar*.

favoured by silt whereas the group III species favoured by a higher percentage of clay in NR. The species indicated in group I preferred the low percentage of clay and those in group III preferred lower silt content (Fig. 5).

4. Discussions

The west coast of India provides a unique environmental condition to support the benthic polychaetes community response to sediment texture occurring at spatial scale. The sediment texture delineated three distinct areas along the west coast of India (Jayaraj et al., 2008; Ingole et al., 2010; Sivadas and Ingole, 2016). The fine sand is dominant in the SR, whereas CR has mixed sediments of

Table 3
The abundance of polychaete (nos/m^{-2}) in the sub-tidal area along the South-eastern Arabian Sea.

	Assemblage	Southwest coast			Central west coast		Northwest coast	
S. no	Species	Trivandrum	Cochin	Mangalore	Karwar	Goa	Ratnagiri	Mumbai
1	*Aglaophamus uruguayi* Hartman, 1953	44	44	0	0	0	0	0
2	*Alitta succinea* (Leuckart, 1847)	0	0	0	44	88	44	44
3	*Amphicteis posterobranchiata* Fauvel, 1932	0	0	0	0	0	0	44
4	*Amage* sp.	44	44	0	0	0	0	0
5	*Aphelochaeta multifilis* (Moore, 1909)	1643	1511	1820	1332	132	44	44
6	*Sigambra constricta (Southern, 1921)*	44	44	44	44	0	0	0
7	Aphroditidae Malmgren, 1867	0	0	44	88	44	88	44
8	*Arabella (Arabella) iricolor* (Montagu, 1804)	0	0	0	0	0	44	0
9	*Aricidea* sp.	44	0	0	0	0	0	44
10	*Armandia sampadae* Gopal, Jaleel, Parameswaran & Vijayan, 2016	44	44	88	44	0	132	0
11	*Axiothella obockensis* (Gravier, 1905)	44	0	37	0	0	0	0
12	*Aonides* sp. Claparède, 1864	44	44	0	0	0	0	0
13	*Bylgides sarsi* (Kinberg in Malmgren, 1866)	0	0	0	88	88	44	0
14	*Capitella capitata* (Fabricius, 1780)	44	44	44	0	0	0	44
15	*Timarete dasylophius (Marenzeller, 1879)*	0	0	44	0	0	0	44
16	*Cirratulus spectabilis* (Kinberg, 1866)	44	88	44	0	0	0	0
17	*Cirriformia grandis* Verrill, 1873	0	0	0	0	0	0	44
18	*Cossura delta* Reish, 1958	44	88	132	266	710	844	577
19	*Capitellethus dispar* (Ehlers, 1907)	44	44	88	0	0	0	132
20	*Diopatra neapolitana* Delle Chiaje, 1841	0	0	44	0	0	0	0
21	*Trochochaeta* Levinsen, 1884	44	0	0	0	0	0	0
22	*Eunice indica* Kinberg, 1865	0	44	0	44	44	0	0
23	*Hypereteone heteropoda (Hartman, 1951)*	44	0	0	0	0	0	0
24	*Euclymene annandalei* Southern, 1921	0	88	0	0	0	0	0
25	*Goniadides* sp. Hartmann–Schröder, 1960	44	0	44	0	0	0	0
26	*Glycera capitata* Setosa Örsted, 1843	44	0	0	44	44	0	44
27	*Glycera alba* (O.F. Müller, 1776)	88	44	88	0	0	0	178
28	*Hesione* sp. Savigny in Lamarck, 1818	0	0	0	0	0	0	0
29	*Hesione picta* Müller in Grube, 1858	0	0	44	44	132	132	176
30	*Idanthyrsus* sp. Kinberg, 1876	44	0	0	0	44	0	0
31	*Jasmineira caudata* Langerhans, 1880	0	0	0	0	44	0	0
32	*Levinsenia* sp. Mesnil, 1897	44	88	88	0	0	0	0
33	*Scoletoma funchalensis* Kinberg, 1865	132	44	132	0	0	0	44
34	*Magelona* sp. F. Müller, 1858	0	0	132	44	44	88	44
35	*Magelona cincta* Ehlers, 1908	44	44	44	0	0	0	44
36	*Maldanella harai* (Izuka, 1902)	311	84	222	0	0	0	0
37	*Mediomastus capensis* Day, 1961	7	0	0	0	44	0	0
38	*Micronereides capensis* Day, 1963	0	0	0	44	0	0	0
39	*Micronephthys sphaerocirrata* (Wesenberg-Lund, 1949)	0	0	0	0	0	44	0
40	*Inermonephtys inermis (Ehlers, 1887)*	44	0	0	44	44	0	0
41	*Ninoe nigripes* Verrill, 1873	0	44	132	0	0	0	44
42	*Neanthes glandicincta (Southern, 1921)*	0	0	0	44	0	0	0
43	*Nereis sandersi* Blake, 1985	44	132	88	178	88	44	355
44	*Notomastus aberans* Day, 1957	44	0	0	0	0	0	0
45	*Onuphis eremita* Audouin & Milne Edwards, 1833	0	44	0	0	44	0	44
46	*Phyllodoce* sp. Lamarck, 1818	0	0	0	44	0	44	0
47	*Lagis koreni* Malmgren, 1866	44	0	44	0	44	0	44
48	*Paraprionospio patiens* (Yokoyama 2007)	0	44	88	132	44	132	222
49	*Prionospio aucklandica* Augener, 1923	44	44	0	0	0	0	0
50	*Prionospio pygmaeus* Hartman, 1961	0	0	0	88	0	0	0
51	*Prionospio (Minuspio) cirrifera* Wirén, 1883	88	44	0	44	44	132	132
52	*Paraprionospio cordifolia* Yokoyama, 2007	44	44	44	132	222	178	266
53	*Polydora ciliate* (Johnston, 1839)	0	44	0	44	0	0	0
54	*Paraonides lyra capensis* (Day, 1955)	0	88	0	0	0	0	0
55	*Potamilla leptochaeta* Southern, 1921	0	44	0	44	44	0	44
56	*Polyphysia crassa* (Örsted, 1843)	0	44	44	0	0	0	0
57	*Protodorvillea egena* (Ehlers, 1913)	44	0	0	88	44	0	0
58	*Poecilochaetus serpens* Allen, 1904	0	44	0	44	0	44	44
59	*Paralacydonia paradoxa* Fauvel, 1913	0	0	0	0	44	0	0
60	*Linopherus abyssalis (Fauchald, 1972)*	44	88	0	0	0	0	0
61	*Schistomeringos neglecta* (Fauvel, 1923)	0	88	44	44	0	0	0
62	*Sternaspis scutata* (Ranzani, 1817)	0	0	44	44	0	0	44
63	*Spiochaetopterus costarum* (Claparède, 1869)	44	0	0	0	0	0	0
64	Sabellidae Latreille, 1825	0	0	0	44	0	0	0
65	*Scoloplos (Scoloplos) marsupialis (Southern, 1921)*	44	44	44	0	0	0	0
66	*Scoloplos armiger* (Müller, 1776)	44	0	0	88	0	0	0
67	*Schistocomus* sp. hiltoni Chamberlin, 1919	0	44	0	0	0	0	0
68	*Synelmis* sp. Chamberlin, 1919	44	0	0	44	0	0	0
69	*Syllis cornuta* Rathke, 1843	44	0	88	0	0	0	0
70	*Terebellides stroemi* Sars, 1835	0	44	0	0	0	44	88
71	*Thormora jukesii* Baird, 1865	0	0	0	88	44	0	0

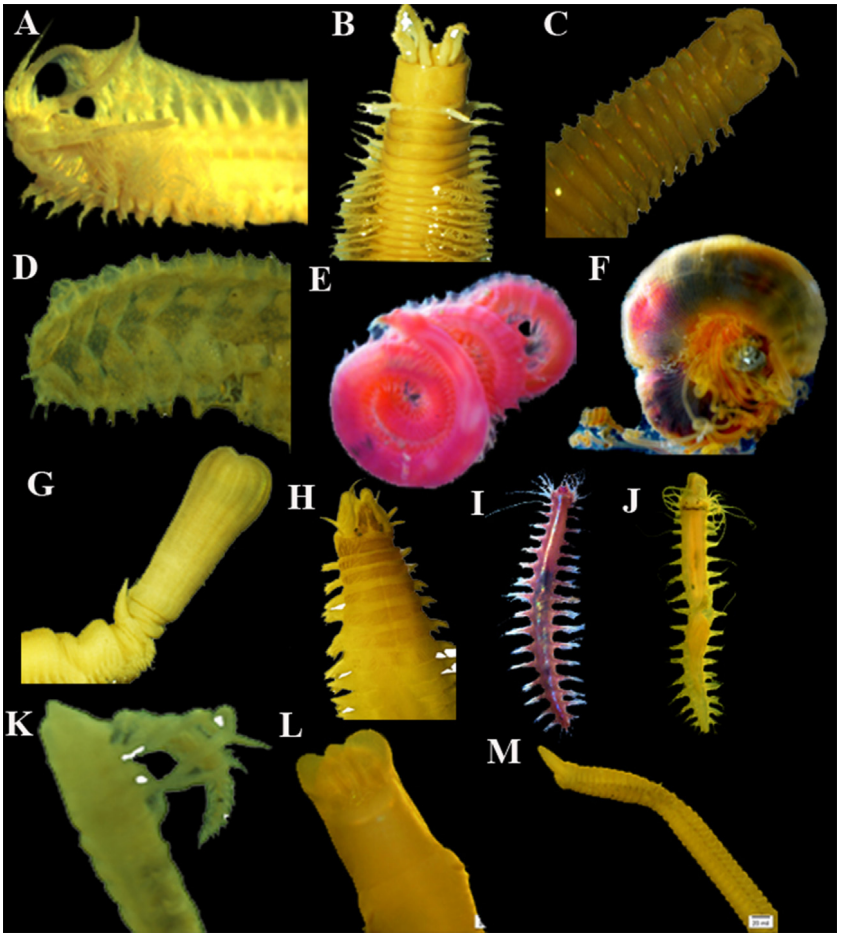

Fig. 6. Commonly occurring polychaete species- A: *Diopatra neapolitana*, B: *Eunice indica*, C: *Eunice* sp., D: *Thormora jukesii*, E and G: *Glycera capitata*, F: *Aphelochaeta multifilis*, H: *Nereis glandicincta*, I: *Hesione picta*, J: *Hesione* sp., K: *Paraprionospio cordifolia*, L: *Euclymene annandalei*, M: *Ninoe nigripes*.

fine sands. The silty clay was prevailing in the NR (Fig. 3). The SR region showed a higher percentage of polychaetes species off Trivandrum, Cochin, and Mangalore and their contribution to the total polychaetes species was 36.6%. Similar findings were also made by earlier workers from the west coast of India (Joydas and Damodaran, 2009; Musale and Desai, 2011). Harkantra et al. (1982) also observed that fine sandy substrate harbour high macrobenthic diversity in the southern region, while the silty clay showed low diversity in the northern region. Thus, the specificity of polychaetes feeding types largely depends upon the type of substratum. Fine particles of sand might result in clogging of feeding apparatus of filter feeders hence they avoid fine sandy substratum although the adequate supply of food is available (Jayaraj et al., 2008). Studies from tropical and temperate regions have shown that the grain size plays an important role in structuring the polychaetes community and high polychaete abundance is generally associated with fine sand (Defeo and McLachlan, 2005; McLachlan and Dorvlo, 2005).

The polychaetes species have more important role in the breakdown of the organic matter, and they provide recycling of nutrients into the pelagic system. Generally, the grain sizes increase from clayey to finer sand, with an increase in wave action towards the Southwest coast of India. *A. multifilis* dominate towards fine sand which can able to hold the organic matter in southern region; while *C. delta* and second opportunistic species of *P. cordifolia* were dominant towards the higher silt clayey content of Northwest coast of India. Yokoyama and Sukumaran (2012) reported that the *Paraprionospio* specimens found from the northwest coast of India, and identified them as *P. cordifolia* Yokoyama, 2007, *P. cristata* Zhou, Yokoyama and Li 2008, and *P. patiens* Yokoyama, 2007. Some of the most dominance polychaete species identified during the present study are shown in Fig. 6.

The species richness and polychaete abundance were high in the SR having fine sandy substratum. The proficiency of fine sand to detain more organic carbon is well documented. Gray (1976) observed the relationship between the macrofauna diversity on the continental shelf region and structural complexity in the sediment type. He concluded that faunal diversity in heterogeneous sediments tends to be higher than homogeneous sediments. However, Palacin et al. (1991) studied the benthic infauna of the Mediterranean Bay and reported the highest density in the sandy sediment. Robert (1979) also noticed increased species richness with increasing sand content, which was independent of bathymetric position. However, Bergen et al. (2000) and Ellingsen (2002) observed that water depth was the primary factor organizing benthic communities followed by sediment grain size in the Southern California and Norwegian.

Most of the dominant and widespread polychaete species belonged to the surface and sub-surface deposit feeding families Spionidae, Cirratulidae, Cossuridae, and Capitellidae in deep depths where other families have low representation or even absent showed their ability to survive in adverse conditions, especially with a reduced amount of sediment grain size. Carnivorous species belonging to the Eunicidae and Glyceridae were also represented in terms of abundance and dominance. Mirza and Gray (1981) attributed low diversity of benthos and dominance of polychaetes (viz., *Capitella* and *Polydora*) to the oxygen-poor mud in Norway. The present study showed an increased density, richness and polychaete diversity in the southern region with the dominance

of sandy substrate which could hold high organic carbon matter. Stable benthic conditions allow many specialized species such as *C. delta* and *A. multifilis* to be present in the study area, but competition for food is probably higher, leading to low densities (Duineveld et al., 1991). The biological factors include competition, predator–prey relations, and a larval settlement which could be the important factors in polychaetes assemblage.

The significant spatial variation in polychaetes assemblage was directly related to change in the occurrence of the dominant species and their present/absent at each station. Overall, abundance was higher for sub-surface deposit feeder *C. delta* species in the northern stations. *C. delta* is considered as a shallow water muddy species. Polychaete assemblages along the South-eastern Arabian Sea were characterized by high species richness, density, and environment related characteristics. Recently Sivadas and Ingole (2016) reported that the macrofaunal assemblages in diverse communities were dependent on the substratum type. Therefore, knowledge on the substratum type, species diversity, and species assemblages are necessary to understand the ecosystem functioning.

Our study suggests that the polychaete species response to substrate composition in the study area. Differences in the distribution of polychaete species between SR and NR habitats may be supported statistically in the present study, however, the variability of polychaete species distribution between central parts with fine sandy sites reflects the increased heterogeneity and complexity of substrate when compared with clay silty substrate. The sediment composition has mainly influenced the distribution and diversity of polychaetes species, with diversity increasing in the sandy sediment (Hernández-Arana et al., 2003). The data generated through this study provides valuable baseline information of polychaete assemblage from an ecologically important area with differentiate polychaete community structure and use to focus on future benthic research. It is necessary to monitor the nature of polychaete species—relation with environmental changes and estuary-related coastal environment. As described by Gambi and Giangrande (1986) the macrobenthic distribution to test the efficacy of polychaetes as 'markers' of different ecological conditions. The distribution of polychaetes species between habitats has also been observed earlier by Ingole et al. (2010), Joydas and Damodaran (2009) and Sivadas et al. (2010). The present study shows the substratum composition determine the type of polychaetes assemblage.

5. Conclusion and future perspectives

This study provides imminent into the effects of substratum composition on benthic polychaete communities. Changes in assemblage patterns were due to the physical forcing (coastal current) together with changes in sediment texture and its potential role towards ecosystem understanding. Altogether 71 polychaete species belonging to 16 families were identified in the study area. Increased richness and diversity patterns in the SR were in contrast with northern region. The west coast of India is an ecologically productive region which promotes higher biodiversity in the Arabian Sea. The regional species assemblage dominated by *C. delta* in the Northwest coast of India, whereas *A. multifilis* dominated in the Southwest coast of India. Therefore long-term studies could be directed towards the demographic, behavioural, population genetics and ecological responses of species assemblages. Further considering the seasonal monsoonal variability and reversing surface current in the region, studies on Oceanic connectivity and gene flow could be very helpful in establishing the possible east–west larval dispersal of benthic species.

Acknowledgements

The authors are grateful to Director, CSIR-National Institute of Oceanography, for encouragement and permission to publish this work. We also express our gratitude to Ministry of Earth Science (MOES), Govt. of India for financial support and ship board facilities on FORV *Sagar Sampada*. The entire team of PSC0206 project for all the help rendered during the field and laboratory study. Financial support for PR received from the MOES, Govt. of India under the COMAPS project (GAP-2741) and KD acknowledges financial help from the DST-INSPIRE Fellowship.

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Journal of Coastal Zone Management

Baban et al., J Coast Zone Manag 2016, 19:4
DOI: 10.4172/2473-3350.1000436

Research Article OMICS International

Macrobenthic Community Structure Response to Coastal Hypoxia off Southeastern Arabian Sea

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Abstract

The analysis of changes in macrobenthic community using multivariate statistical techniques has been applied to find the structure by the environmental condition. The aim of the study was to evaluate macrofaunal community patterns between natural occurrence of coastal hypoxia condition (30 to 100 m depth) and normoxic bottom waters over the Southeastern Arabian Sea (SEAS). The macrofaunal communities patterns were analyzed by using various statistical methods (e.g. rank correlation, hierarchical clustering, nMDS, BIO-ENV). A clear seasonal difference was found in macrofaunal abundance, biomass, taxonomic composition, diversity and their relation to environmental conditions. Multivariate analysis of Non Multidimensional Scaling (nMDS) showed two major groups macrofaunal communities and ANOSIM results showed a significant difference between macrofaunal community structure in between nornaxia and hypoxia conditions (R=0.913). Spearman rank correlation (using BIO-ENV procedure included in PRIMER, V.6) showed the highest correlation of dissolved oxygen (R=0.678) with community structure. The SIMPER analysis illustrated community pattern changed seasonally with Paraprionospia cordifolia (20.03%) dominated during hypoxia whereas Tharyx sp. (22.63%) dominated in nornaxia conditions. The macrofaunal community patterns revealed contrasting pattern with two seasons, perhaps due to the dissolved oxygen (DO).

Keywords: Hypoxia; Normoxia; Macrobenthos; Community structure; Dissolved oxygen

Introduction

Changes in the structure and composition of macrobenthic communities driven by environmental condition may have marked effects on biogeochemical cycles and benthic ecosystem processes and functions. They are sedentary and trophically diverse [1] and their communities mix the effects of water and sediment changes over time. In addition, macrobenthic fauna play an important ecological role within food webs. They are a direct and indirect food source for many animals, including large crustaceans, fishes, marine birds and marine mammals [2]. Macrobenthic communities can also alter physical and chemical conditions at the sediment–water interface, promote decomposition of sediment organic matter (OM), and are important mediators in nutrient recycling from the sediments to the water column through bioturbation and suspension feeding activities [3,4]. Therefore, changes in macrobenthic community composition, abundances and diversity can affect the functioning of the entire ecosystem [5].

Macrobenthic communities are composed of sedentary organisms capable of integrating long-term environmental conditions at a particular site [6]. Large areas of high productivity induced by natural upwelling and limited mixing led to decrease in the Dissolved Oxygen (DO) concentration at coastal regions [7,8]. Studies defined that DO concentration at normoxia is >2.8 mgL^{-1}, mild hypoxia 2.1-2.8 mgL^{-1}, and hypoxia is ≤ 2 mgL^{-1}. The lower concentration of DO has a major impact on structure and functioning of biogeochemical processes such as the carbon, nitrogen cycles [9,10] and benthic ecology [11,12]. During the southwest monsoon the southward movement of the West Indian coastal current influences the upwelling and it causes the hypoxia condition in the Arabian Sea [13]. During southwest monsoon the coastal upwelling occurs along west coast of India between 7°N and 14°N [14-16].

Hypoxia is the most intense marine environments based on harshness conditions in sediment and water flux; also it alters the marine benthic communities. The effects of hypoxia condition on biological community are frequent and related to different levels of dwelling and tolerance. Such responses may change to the feeding habit and also reduced the predator population [17,18]. Hypoxia conditions leads to changes in macrofaunal community structure marine benthic ecosystem due to physiological changes such as stratification and mixing [7,19-23]. Hypoxic events will increase the susceptibility of coastal marine ecosystems to further hypoxia through alteration of ecosystem functioning of the sediments and show that this has already occurred in a number of coastal marine ecosystems [22].

The effect of hypoxia conditions on macrobenthic community have been studied by many researchers [24-26]. The macrobenthic abundance has been reduced in the Arabian Sea due to deceased levels of DO during winter. Many of the inshore regions exhibit poor water quality due to extensive domestic and industrial waste disposal; very low dissolved oxygen occurs during post monsoon in fall, which is mainly due to anoxia developing along the open coastal [23]. There is no study so far in the coastal SEAS explaining the effect of very low dissolved oxygen on macrofauna. However, it is known that macrofaunal communities may response in a different way to the normoxia and hypoxia condition and thus DO play a vital role in benthic ecosystem functioning. The aim of the present study is to assess the macrofaunal structural changes between normoxia and hypoxia conditions and to predict spatio-temporal variation of benthic biodiversity.

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Received December 12, 2016; **Accepted** December 26, 2016; **Published** December 30, 2016

Citation: Baban SI, Periasamy R, Kalyan D (2016) Macrobenthic Community Structure Response to Coastal Hypoxia off Southeastern Arabian Sea. J Coast Zone Manag 19: 436. doi:10.4172/2473-3350.1000436

Materials and Methods

Study area

The study was carried out at two fixed Transects, off Cochin (9°56'N and 76°12'E) and off Trivandrum (8°28'N and 76°54'E) along the coastal water of SEAS perpendicular to western Ghats which receives bulk of rain fall during tropical South-West monsoon regime (Figure 1). The sampling was carried out with the CORV *Sagar Sukti* at SIM and on FORV *Sagar Sampada* during for coastal upwelling during the peak of the South-West monsoon season. In each transect, three stations (bottom depth 30, 50 and 100 m) were chosen for studying the various environmental parameters and macrofauna community structure.

Physio-chemical characteristics

In order to measure the bottom dissolved oxygen concentration, a modified Niskin type water sampler which is capable of collecting bottom water above 20 cm from the surface sediment was used. Dissolved oxygen was analyzed by Winkler's method [27]. Water depth, salinity and temperature of water column were measured using a CTD meter (SBE-19, Sea-Bird Electronics).

Macrofaunal sampling and analysis

The sediment samples were collected using Smith-McIntyre grab of 0.2 sq.m surface areas, triplicate grab samples were collected, and sieved through a 0.5 mm mesh screen, and the retained organisms were preserved in 10% buffered formalin with Rose Bengal solution in plastic bags. Once the samples brought to laboratory, macrofauna were carefully washed again and sorted into major taxonomic groups (phylum, order or class) and preserved in 5% buffered formalin. The faunal counts from individual grabs were averaged and converted to individual per sq. meter. The faunal counts from the water overlying the grabs were divided by the number of sub-cores taken. Biomass (g/m^2) was determined by using the wet weight method after blotting. The biomass (shell on) was estimated similarly and converted to $g.m^{-2}$ (wet weight). As polychaeta were dominated taxa, then were identified up to species [28,29] level if possible and their number was counted as individual per square meter under stereo-microscope.

Statistical analysis

Macrobenthos data were subjected to univariate analyses to study community structure using Margalef's index [30] for species richness (d), Pielou's index [31] for species evenness (*J'*), and the Shannon-Wiener index [32] for species diversity (*H'* by using $\log^2$). For multivariate analysis, a square-root transformation of biological abundance data was carry out and contributed most to the observed differences among groups were found by means of SIMPER (similarity percentage) and cluster analysis and nMDS (Non-metric multidimensional scaling) ordination stand on the Bray-Curtis similarity matrix were attained using the PRIMER 6 package (Plymouth Routines in Multivariate Ecological Research) [33]. Similarity profile (SIMPROF) test was carried out to detect the significant of the clusters. The null hypothesis of no inside group structures of occupied samples was rejected when significance level of $P<0.05$. ANOVA analysis was carried to find out the significance of spatial and temporal variation on the environmental and biological parameters. Types of feeding were assigned to polychaetes based on the previous reports.

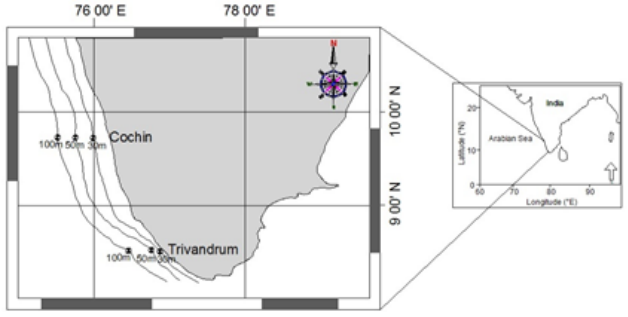

Figure 1: Map showing the study area and sampling locations for macrobenthos in Southeastern Coast of Arabian Sea.

Results

Environmental characteristics

Physico-chemical characteristics such as temperature, salinity and DO (DO saturation %) along SEAS during SM and SIM conditions are shown in Table 1. The results showed that cold and low oxygen condition in the bottom water during SM. Notable feature was observed that the bottom water salinity did not vary along different water depths, whereas temperature showed variation between seasons range from 20.3 to 30.4°C in SIM and 19.6 and 23.5°C during SM (ANOVA, $P<0.05$). DO deficient of near bottom-water during the SM showed ranged from 0.038 to 0.804 $mg.L^{-1}$, while oxygen saturated conditions during SIM DO ranged from 4.38 to 5.5 $mg.L^{-1}$ (Figure 2) and significantly differed between both season (ANOVA, $P<0.05$).

Macrofaunal composition

The highest number of taxa (68) was identified in the SIM, and Polychaeta was dominated group, contributing 86.21% to total fauna abundance. Macrofaunal abundance decreased from depths 30-100 m in Cochin (5556 - 3520 individuals m^{-2}), then increased from shallow depth to deep on Trivandrum (1529-2493 individuals m^{-2}) and average abundance 3412 individuals m^{-2} were in the SIM (Table 2). Moreover, 25 polychaete families were found in the SIM, in which Cirratulidae family were showed highest contribution (39.81%) followed by Spionidae (14.2%) and Capitellidae (5.37%). The SIMPER analyses showed that benthic community was dominated by *Tharyx* sp. (22.63%) and *Mediomastus* sp. (10.48%) at SIM (Table 3). On the other hand *P. cordifolia* (20.03%) and *Cirriformia* sp. (12.19%) showed major dominance in benthic community during SM. The overall mean abundance was 3383 individuals' m^{-2} with minimal value Cochin 100 m depth (650 individuals m^{-2} and 8 taxa) and polychaeta groups were contributing 86.8% on benthic faunal abundance. Among these families, highest contributions of Spionidae and Crustacean were dominated (56.45% and 11.58% respectively) followed by Chaetopteridae (5%), Orbiniidae (4.63%), Sabellidae (4.17%) and Glyceridae (3.41%) (Table 4). The Amphipoda was most abundance group among the crustaceans, contributing 8.05% to total macrofaunal diversity. However, the low abundance of Echinodermada and Fish larva were observed at the low oxygen conditions. The average biomass showed that the higher biomass value (16.5 $g.m^{-2}$) found at low oxygen environmental conditions (Figure 3).

Diversity indices

Margalef's index (d) showed that species richness (d) was varied from 2.4 to 7.4 during SIM, while hypoxia zone (SM) was recorded lower d value from 1.7 to 4.98 (Figure 4). The species evenness (J) varied from 0.87 to 0.95 in high oxygen conditions, whereas in low oxygen conditions species evenness range of 0.93 to 0.98. However, highest value of Shannon diversity index (H') varied from 3.72 to 5.19

Transect	Depth (m)	Temp (˚C)		Salinity PSU		Dissolved oxygen (ml.L^{-1})		Dissolved oxygen (saturation %)	
		SIM	SM	SIM	SM	SIM	SM	SIM	SM
Cochin	30	30.46±0.5	22.77±0.8	35.71±0.4	35.05±0.3	4.70±0.7	0.04±0.8	91	1
	50	28.47±0.7	21.72±0.5	35.48±0.3	35.03±0.9	4.45±0.8	0.14±0.7	83	2
	100	20.30±0.3	19.59±0.3	35.37±0.4	34.95±0.5	5.13±0.3	0.16±0.9	82	3
Trivandrum	30	29.74±0.8	23.46±0.9	34.76±0.9	35.01±0.4	4.38±0.3	0.80±0.7	84	14
	50	27.60±0.3	22.61±1.2	35.27±1.1	35.01±0.8	5.50±0.4	0.36±0.4	101	6
	100	24.63±0.9	20.71±1.5	35.34±0.7	34.93±1.3	4.75±0.9	0.21±0.4	82	3

Note: Mean ± SD (n=3)

Table 1: Physico-chemical characteristic of habitats study (mean± SD) on the southeastern Arabian Sea.

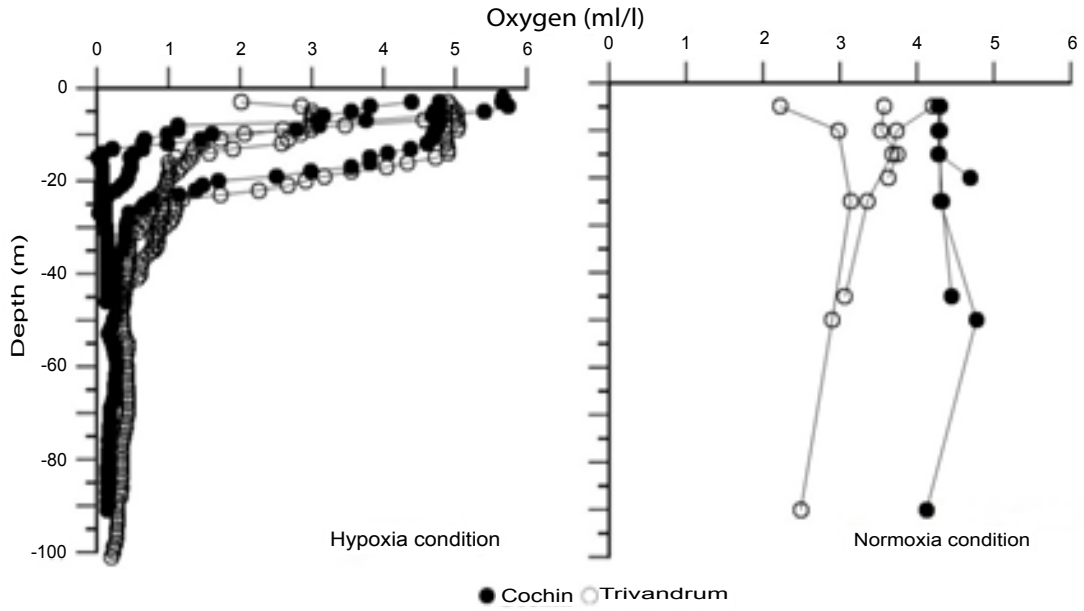

Figure 2: Season-wise distribution of DO (mL^{-1}) during SIM and SM conditions.

Zones	Spring Inter Monsoon						Summer Monsoon					
Transects	Cochin			Trivandrum			Trivandrum			Cochin		
Water Depth (m)	30	50	100	30	50	100	30	50	100	30	50	100
Total abundance(Ind.m^{-2})	1499	2443	2261	4814	5506	3954	1675	4475	650	7450	5025	1025
Total number of species	38	39	27	21	14	22	15	29	15	25	27	22
Total Biomass (wet wt. g.m^{-2})	12.66	4.82	1.34	10.7	166.2	1.30	6.06	20.02	7.45	42.03	14.03	9.39
Most dominant species* (comprising >10%) of the density	1802	2289	1311	311	1111	955	1550	3550	325	275	1375	200
Dominant feeding types	SDF	SDF	SDF	SDF	SDF	SDF	SDF	SDF	SDF	SDF	SDF	SDF
d	4.46	4.45	3.25	2.75	1.67	2.75	1.89	3.34	1.99	2.67	3.08	2.94
J'	0.55	0.68	0.69	0.80	0.74	0.70	0.90	0.68	0.92	0.79	0.73	0.85
H'(log^{2})	2.89	3.61	3.29	3.55	2.81	3.12	3.54	3.28	3.61	3.65	3.46	3.78

Note: (Dominant species*1: *Paraprinospia cordifolia,* 2: *Prinospia cirrifera*, 3: *Tharyx* sp.), SDF: Surface deposit feeder

Table 2: Comparison of community parameters studied along the two transects during two different seasons at SIM and SM.

in SIM, whereas in SM value ranged from 2.93 to 4.52.

Linking macrofauna community structure to environmental variables -bio-env

The BIO-ENV procedure was explained on a species assemblage similarity matrix attuned for two sites and the resemblance matrices created using one various transformations of primary environmental 106-by-3 matrix (Temperature, salinity and DO are log-transformed prior to the normal transformation). The Spearman correlation coefficient (r) was selected as a rank correlation measure. For the normal transformed environmental matrix, DO revealed the best association with the abundance, (r=0.709). It was followed by J (r=0.590) (Figure 5). Those variables were liable for most of the similarity between the biotic and abiotic matrices (Table 5). The highest correlation (r=0.597)

Hypoxic intolerant species (SIM)	Composition (%)	Hypoxic tolerant species(SM)	Composition (%)
Tharyx sp.	22.63	*Paraprionospio cordifolia*	20.03
Mediomastus sp.	10.48	*Prionospio pygmaea*	13.04
Lumbrineris sp.	6.34	*Cirriformia* sp.	12.19
Prinospia cirrifera	5.45	*Prinospia cirrifera*	10.86
Paraprinospia cordifolia	3.74	*Glycera alba*	10.26
Prionospio aucklandica	3.3	*Prionospio steenstrupi*	4.89
Aricidea sp.	3.18	*Magelona* sp.	4.16
Nepthys sp.	2.73	*Lumbrineris* sp.	1.45

Table 3: Comparison of similarity of macro fauna observed among the dominant species from normoxic and hypoxic condition.

Hypoxic intolerant (SIM)	%	Hypoxic tolerant (SM)	%
Cirrtulidae	39.81	Spionidae	56.45
Spionidae	14.20	Crustacea	11.58
Capitellidae	5.37	Chaetopteridae	5.00
Paraonidae	4.21	Orbiniidae	4.63
Gastropoda	2.85	Sabellidae	4.17
Bivalvia	2.80	Glyceridae	3.41
Nereididae	2.56	Cirrtulidae	3.17

Table 4: Dominant macrobenthic taxa tolerating from SIM and SM seasons.

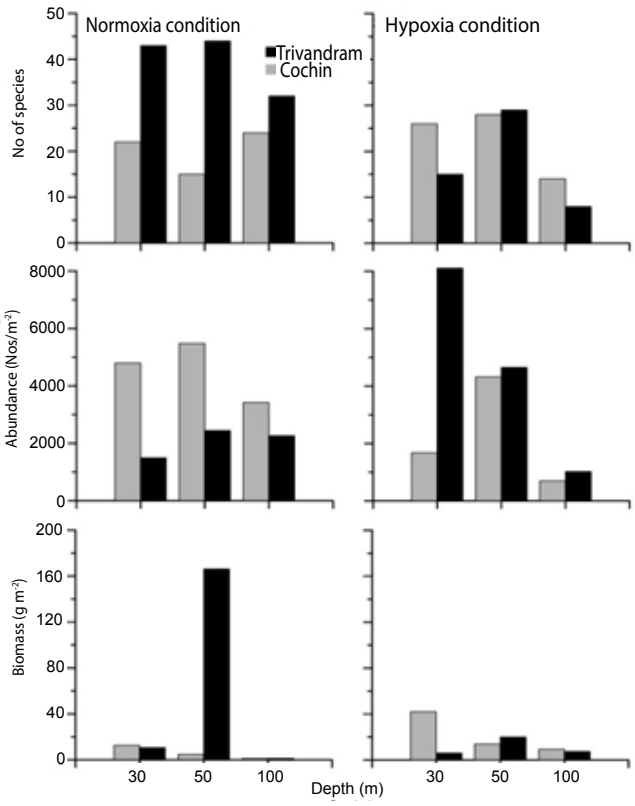

Figure 3: Season-wise distributions of macrofaunal species, abundance and biomass at the southeastern Arabian Sea.

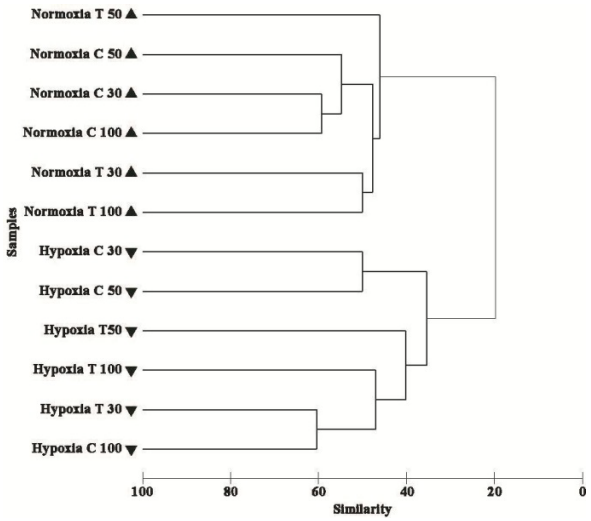

Figure 4: Dendrograms and MDS plots based on the macrobenthos abundance data.

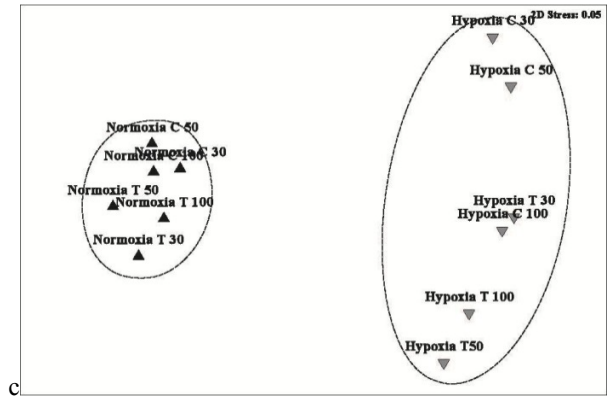

Figure 5: Bray-Curtis similarity for seasonal conditions (normoxia and hypoxia).

is found for a combination of factors: Abundance; Temperature; DO.

Multivariate (MDS) analysis of macrofaunal community structure

The MDS plot based on the abundance of macrofauna communities shows two different groups at dissimilarity (84.59%) in this study area (Figure 6). The SIM group was differentiated by *Tharyx* sp. and *Mediomastus* sp. whereas SM group were dominated by *P. cordifolia* and *Cirriformia* sp. (Table 3). An ANOSIM (R=0.913) test shows the significant differences between season (SIM and SM) and Bray-Curtis analysis explains two distinct clusters at 20% similarity (Figure 7). Most of the surface and subsurface deposit feeder in cluster was mainly from the oxygen saturated conditions and they were also showed low abundance in the hypoxia conditions. The surface deposit feeder of polychaete such as *Tharyx* sp. and subsurface deposit feeder of *Mediomastus* sp. were abundant along with carnivore species

Number of variables	Best variable combinations	Correlation(ρ_w)
1	Dissolved Oxygen	0.633
2	Abundance-Dissolved Oxygen	0.709
	J'-Dissolved Oxygen	0.590
	d-Dissolved Oxygen	0.588
	No of species-Dissolved Oxygen	0.570
	H'(log2)-Dissolved Oxygen	0.550
3	Abundance-J'-Dissolved Oxygen	0.599
	Abundance-Temperature-Dissolved Oxygen	0.597
	Abundance-salinity-Dissolved Oxygen	0.588
	Abundance- H'(log2)-Dissolved Oxygen	0.582

Table 5: BIO-ENV procedure results showed that highest Spearman rank correlation coefficients (ρ) evaluated between square root of transformed biotic similarity matrix and abiotic matrix (ρ_{normal}).

Lumbrineris sp. Another cluster was formed due to the abundance of *P. cordifolia, Prionospio pygmaea* and *Cirriformia* sp. included carnivore polychaeta such as *Glycera alba*.

Distribution of polychaeta feeding type

The surface deposit feeder was dominant feeding type (dwelling polychaeta) 78.5% with carnivores (17%) in the hypoxia zone (SM). The both surface and subsurface feeder existed 74.3% with carnivore (12%) in the normoxia condition. *P. cordifolia* was highest dominated as well as it also feeder tolerated at low oxygen condition; consequently the suspension of deposit feeder or filler feeders (4.6%) were uncommon in low oxygen zone (SM) (Table 6). There is also a common propensity for suspended feeders to be replaced by deposit feeders, in contest that second order opportunistic species were dominated during SM. The low oxygen condition showed highest representation of carnivores (17%) on the surface of bottom than carnivores (12%) at SIM conditions.

Discussion

The seasonal upwelling may have influence on the biological productive region in and around the Arabian Sea. The nutrient rich in upwelled water causes oxygen shortage in the subsurface water along the SEAS during SM. The observed high DO values on the normoxia zone and low DO values in the hypoxic zone were in agreement with earlier studies of the west coast of India (Muni Krishna 2008). The lowest DO value ranged from 0.04-0.8 ml.L^{-1} SM, whereas high DO values (4.38-5.5 ml.L^{-1}) observed along the normoxia conditions SIM and high tolerance in low oxygen levels has moderately connected with continuously low temperature and oxygen deficiency apt to promoted with decreasing temperature [34-36]. During the SM (June to September) the wind pattern has favored the upwelling along the west coast of Indian. However, the end of the SM indicates that the process cannot be driven by winds alone, but may be remotely forced to a large extent [10]. Studies implied increased intensity of upwelling processes in Cochin during July and creating the drop in sea level as well as surface temperature.

P. cordifolia and *Tharyx* sp. are opportunistic species belonging to the families of Spionidae and Capitellidae. Both the species are surface deposit feeder and propagate in high organic enrichment sediments [37]. However, Spionidae and Capitellidae contributed to 68% of the total polychaete species during SM and rich organic content of sediment can support the tolerant species and reduce sensitive species [37]. Among the Polychaetes, two species namely *P cordifolia* and *Prionospio pygmaea,* belonging to the Spionidae family and one species of *Cirriformia* sp. under the Cirratulidae family were abundant on the low oxygen conditions (SM). These surface deposit feeders were replaced by more carnivorous species including *Lumbrineris* sp., *Ancistrosyllis* sp., *Syllis* sp., *Notomastus* sp. and *Cirratulis* sp. [38]. Echinoderms are typically more sensitive to hypoxia with lower oxygen thresholds, than annelids, Sipuncula, Molluscs and Cnidarians. Moreover, as shown by the SIMPER analyses, the Spionidae contributed most of the difference between the hypoxic and normoxic conditions. Our results showed that the highest density of the subsurface feeder *Mediomastus* sp. and Oligochaeta at the normoxia conditions SIM, further the presence of the suspension feeder *Megalomma* sp. was restricted to the hypoxia conditions.

Although many omnivores are opportunistic and capable to switch prey depending on food availability, thus it is expected that they will balance their diet as a result of nutritional needs, food quality and availability of alternate foods [39]. On contrary, *Tharyx* sp. and *Mediomastus* sp. were found at low density during hypoxia conditions. The *P. cordifolia* was dominant macrobenthic species in this DO (≤ 2 mg L^{-1}) depleted areas [12,40,41]. The macrobenthos presented in normoxia condition did not cluster with hypoxia group, because of high abundance of *P. cordifolia* (Figure 8). This species is well-known to tolerated hypoxia conditions [42,43]. In addition, study reported that the dominance of *P.cordifolia* within OMZ off concepection. The rich organic matter in study area was strongly influence on evenness and dominance of macrofauna community. Therefore, it is often complicated to eminent effects of oxygen depletion from those of decreased pH on taxonomic composition [11].

The low density of macrofauna was observed at Cochin (30 and 100 m) during SM, which falls within the site of seasonal sulphate reduction [10]. Typically, the first disappear of crustaceans and echinoderms, with annelids and selected molluscs exhibiting greatest tolerance to hypoxia [19,44]. The important taxa of Spionidae and Cossuridae were found in low oxygen level [26]. However, coastal systems are become saturated through organic matter which leads to develop hypoxia condition and reduction in the biomass [45]. The present studies shows that macrofaunal species were interrelated to surroundings conditions and various environmental factors which are playing major role for the changes on macrofauna structural. The surface deposit feeders and low-oxygen tolerant species are dominated over the suspension feeders. However, the low biodiversity were observed mostly sensitive to the hypoxia conditions. According to studies classification of polychaete feeding types during SIM were dominated by surface deposit-feeding with subsurface deposit-feeding fauna playing a major role in the normoxia conditions.

The macrofauna community structure showed the evidence of low biomass supported by lowest oxygen levels during SM. Rowe reported the reduced biomass within low oxygen condition was stress induced small but found that this low biomass involves high macrofaunal densities, indicating small body size. Mobile vertebrate and invertebrate taxa were observed to avoid hypoxic condition and less mobile invertebrate taxa try to escape low-oxygen conditions or even die and if they cannot escape [7]. Further, Spionidae contributed (52.8%) of the total polychaete family and responses to the hypoxia conditions depend on the duration. The low oxygen conditions to support metazoans, small size of organisms, soft-bodied invertebrates (naturally annelids) and often time of the generation short and intricate branchial structures [7,46]. In addition, the polychaetes have high gill surface area enhances respiratory surface and morphological adaptation. Moreover, the

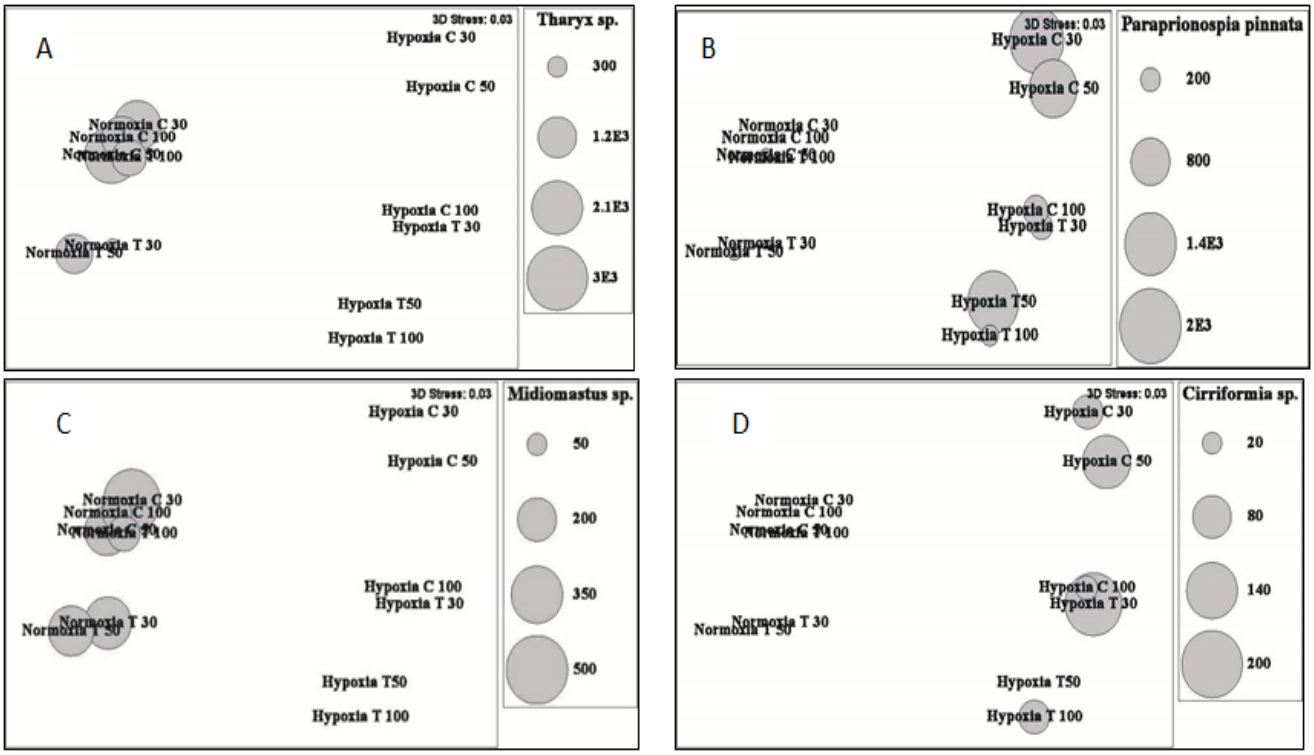

Figure 6: Seasonal distribution of species and abundance pattern.

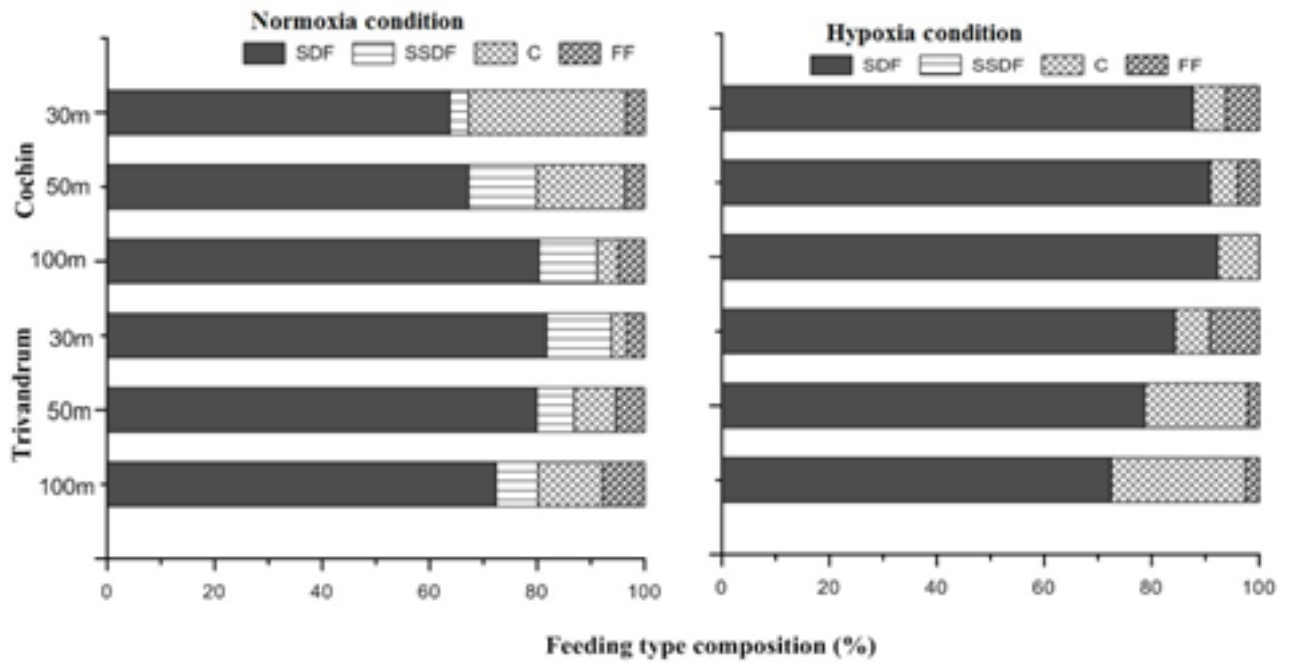

Figure 7: Seasonal distributions of feeding types of macrofauna occurred in southwest coast of Arabian Sea (SDF: surface deposit feeder, SSDF: subsurface deposit-feeder, C: Carnivores and FF: suspension feeder or filter feeder).

Group	Family	Species name	Normoxia zone (SIM)						Hypoxia zone (SM)					
			Cochin			Trivandrum			Trivandrum			Cochin		
			Water depth (m)											
			30	50	100	30	50	100	30	50	100	30	50	100
Polychaeta														
	Pilargidae													
		Synelmis sp.	5	0	0	0	0	22	0	0	0	0	0	0
		Ancystrocyllis sp.	47	22	0	15	89	0	25	50	0	0	0	25
	Ampharetidae													
		Amphicteis sp.	0	22	0	0	0	0	0	0	0	0	0	0
	Opheliidae													
		Armandia sp.	0	22	0	0	0	0	0	0	0	0	0	0
	Maldanidae													
		Axiothella sp.	0	67	0	0	0	0	0	0	0	0	0	0
	Capitellidae													
		Midiomastus sp.	425	156	0	89	267	133	0	0	0	0	0	0
	Chaetopteridae													
		Chaetopterus sp.	2	22	0	0	44	0	0	1025	0	0	0	0
	Cirrtulidae													
		Cirratulidae sp.	0	0	0	44	0	22	0	0	0	0	0	0
		Cirriformia sp.	0	0	0	0	0	0	175	0	50	50	125	25
		Cirratulus sp.1	0	0	0	0	44	0	0	0	0	0	0	0
		Cirratulus sp.2	0	0	22	0	0	0	0	0	0	0	0	0
		Cirratulus sp.3	17	22	156	0	0	0	0	25	125	0	0	0
		Caulleriella sp.	0	0	0	0	0	0	0	0	0	50	0	0
		Tharyx sp.	1802	2289	1311	141	1111	955	0	0	0	25	0	0
	Cossuridae													
		Cossura sp.	0	0	0	44	0	0	0	25	75	0	0	0
	Dorvilleidae													
		Dorvillidae sp.	7	44	22	0	0	0	0	0	0	0	0	0
		Ophryotrocha sp.	0	0	0	0	0	0	25	0	0	0	0	0
	Polynoidae													
		Euphione sp.	0	0	0	0	0	0	0	0	0	0	75	0
		Eunoe sp.	0	0	0	0	0	0	0	0	0	0	50	0
	Eunicidae													
		Eunice sp.	2	111	0	0	0	0	0	0	0	25	0	0
	Glyceridae													
		Glycera alba	15	89	44	0	0	0	75	0	50	275	175	75
	Goniadidae													
		Glycinde sp.	131	0	0	0	0	22	0	0	0	50	0	0
	Goniadidae													
		Goniada sp.	0	0	0	0	0	0	0	0	0	0	0	50
	Hesionidae													
		Ophiodromus sp.	0	0	0	0	0	0	0	0	0	0	0	25
		Hesionidae sp.	0	0	22	0	0	0	0	0	0	0	0	0
	Amphinomidae													
		Pseudeurythoe sp.	15	133	89	89	0	0	0	0	0	0	0	0
		Harmothos sp.	0	0	0	0	0	0	0	0	0	0	25	0
	Sabellidae													
		Euchone sp.	0	0	0	0	0	0	0	0	0	0	75	0
		Sabellidae	0	0	0	0	0	44	0	0	0	0	0	0
		Chone sp.	0	0	0	0	0	0	0	0	0	0	0	0
		Hydroides sp.	0	0	0	0	0	0	0	25	0	0	0	0
		Megalomma sp.	0	0	0	0	0	0	0	0	0	705	0	0
		Jasmineira sp.	7	89	0	0	89	22	0	25	0	0	0	25
	Paraonidae													
		Aricidae sp.	3	111	22	9	89	22	0	25	0	0	0	0
		Levinsenia sp.	7	333	178	22	0	44	0	0	0	0	0	0
	Lumbrinereidae													
		Lumbrineris longifolia	30	44	111	89	178	44	0	125	0	0	25	25

		Ninoe nigripes	0	0	0	0	0	0	0	50	0	0	75	0
	Ampharetidae													
		Melinna sp.	0	0	0	0	0	0	0	25	0	0	0	0
		Amage sp.	12	89	0	17	0	111	0	25	0	0	0	0
	Magelonidae													
		Magelona cincta	10	44	0	0	0	133	75	175	0	100	0	25
	Nephtyidae													
		Nepthys inermis	0	89	0	0	0	0	0	0	0	0	0	0
		Nepthys sp.	0	22	0	267	133	0	0	0	0	0	0	0
	Nereididae													
		Micronereides sp.	0	89	0	0	0	0	0	0	0	0	0	0
		Neries sp.	0	0	0	0	0	0	150	0	0	0	0	0
	Onuphide													
		Onuphis sp.	79	0	22	22	0	0	0	25	0	0	0	0
	Pisionidae													
		Pisionidens sp.	0	0	0	0	0	0	0	0	0	0	50	0
	Phyllodocidae													
		Eteone sp.	0	0	0	0	0	0	0	25	0	25	50	0
		Phyllodoce sp.	40	44	0	2	0	22	0	0	0	75	75	0
	Spionidae													
		Prionospia steenstrupi	0	0	0	0	0	0	150	350	0	0	0	200
		Prionospia cirrifera	706	44	178	178	0	89	275	25	200	950	1350	25
		Prionospia pygmaea	10	0	22	22	0	22	125	25	75	600	350	125
		Paraprionospia cordifolia	5	67	22	22	89	22	250	1375	50	1550	1200	325
		Prionospia cirrobranchiata	2	0	0	22	0	0	0	450	50	0	0	0
		Prionospio sp.	168	178	311	311	44	22	0	0	0	0	0	0
		Prionospio aucklandica	0	44	22	22	44	133	0	0	0	0	0	0
		Scolelepis sp.	0	0	0	0	0	0	0	0	0	0	200	0
		Spiophanes sp.	0	0	0	0	0	0	0	0	0	1000	0	0
		Streblospio sp.	0	0	0	0	0	0	175	0	0	125	0	0
	Orbiniidae													
		Scoloplos sp.	0	22	0	0	0	0	0	0	0	900	50	0
	Syllidae													
		Syllids sp.	12	0	0	0	0	0	0	0	0	0	0	0
		Exogone sp.	15	200	22	0	0	0	0	0	0	0	0	0
	Trichobranchidae													
		Trichobranchus sp.	0	0	0	0	0	0	0	25	0	0	0	0
	Terebellidae													
		Terebellides sp.	5	22	0	0	0	0	0	75	0	0	25	0
		Lanice conchilega	0	0	0	0	0	0	0	50	0	0	50	0
		Amphitrite sp.	0	0	0	0	0	0	0	0	0	25	0	0
Crustacea														
		Isopoda	0	0	0	0	0	0	0	0	0	75	50	0
		Mystidea	0	0	0	0	0	0	0	0	0	0	50	0
		Ostropoda	0	22	0	0	0	0	0	25	0	0	0	50
		Shrimp larva	32	22	0	0	0	0	0	0	25	0	75	0
		Sand dollar	0	0	0	0	0	0	0	0	0	0	225	0
		Tanaidacea	15	89	0	0	0	22	0	25	0	0	0	0
		Decapoda larva	0	0	0	0	0	0	0	0	0	0	0	25
		Cumacea	7	22	22	0	89	0	0	50	0	50	0	0
		Caperlloidea	12	0	0	0	0	22	50	50	0	775	50	0
		Ampelisca sp.		0	0	0	0		25	50	0	350	50	0
		Byblis sp.	0	0	0	0	0	0	0	0	0	150	0	0
		Lijeborgiidae	0	0	0	0	0	0	25	25	0	50	0	0
Bivalvia														
		Bivalvia	99	0	178	0	0	0	75	75	0	100	50	0
		Gnathia cerina	0	0	22	0	0	0	0	0	0	0	0	0
		Arca sp.	0	0	0	0	0	22	0	0	0	0	0	0
		Babylonia	0	22	0	0	0	0	0	0	0	0	0	0
		Corbiculidae	0	0	0	0	0	0	0	0	0	0	0	0

		Cylichna	0	0	22	0	0	0	0	0	0	0	0	0
		Glycymeridae	2	0	0	0	0	0	0	0	0	0	0	0
		Mesodesmatidae	7	67	22	0	0	0	0	0	0	0	0	0
		Nuculena	0	0	22	0	0	0	0	0	0	0	0	0
		Nucula	7	0	0	0	0	0	0	0	0	0	0	0
		Tellina	0	0	22	0	0	0	0	0	0	0	0	0
		Naticidae	0	22	0	0	0	0	0	0	0	0	0	0
		Trochidae	0	22	0	0	0	0	0	0	0	0	0	0
		Gastropoda	10	0	0	0	0	0	0	0	0	25	75	0
Minor phyla														
		Nematoda	721	244	356	53	0	178	0	0	0	0	0	0
		Nematinea	97	244	89	0	89	22	0	0	0	0	0	0
		Sipuncula	111	111	22	4	44	0	0	0	0	0	0	0
Oligochaeta														
		Oligocheata	96	67	67	15	0	111	0	0	0	0	0	0
Total abundance(Ind.m^{-2})			4795	5484	3420	1499	2443	2261	1675	4325	700	8105	4650	1025

Table 6: Mean abundances of macrofauna (ind.m^2) at normoxia (SIM) and hypoxia (SM) conditions.

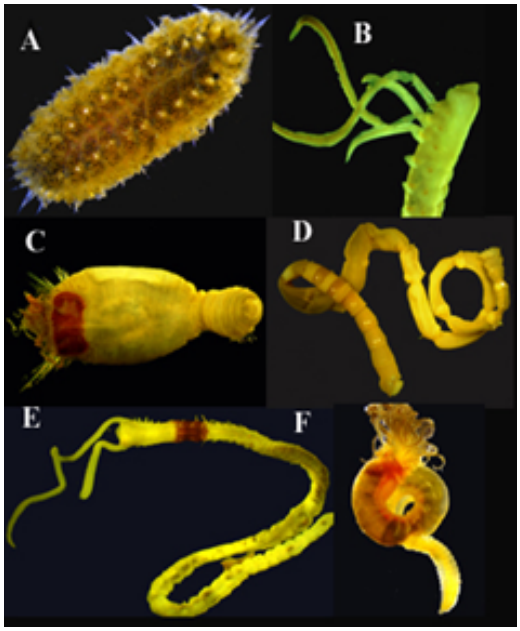

Figure 8: A: *Thormora jukesii*, B: *Paraprionospio cordifolia*, C: *sternaspis scutata,* D: *Maldanella* sp. E: *Magelona cincta* F. *Terebellides stroemi.* In figure B, E and F respiratory surface gills improved in hypoxia condition, photograph of anterior part of polychaetes with well-developed branchia.

species with an expanded branchial structure appeared due to the adaptations at low oxygen conditions. It also suggested that branchial are importance for feeding rather than for gas exchange.

Conclusions

The present study reveals that macrofaunal community structure changes by natural occurrence of coastal hypoxia in the SEAS and event of hypoxia repeatedly in the SM. The species diversity showed the seasonal variation and the *P. cordifolia* tolerated and respond to hypoxic condition and heavy recruitment that could be tolerated a wide range of low oxygen conditions. Most recruited macrofauna in fall were composed of opportunistic species and they disappeared again with the next normoxia condition. Further, we observed that *Tharyx* sp. and *P. cordifolia* could be second order opportunistic species in surface deposit-feeder. The DO and environmental variables may influence the changes in macrofauna community structural with alteration of food webs. The surface deposit feeder and hypoxic tolerant species were highly dominant when compared to suspension feeder and hypoxic intolerant species.

Acknowledgements

The authors are grateful to Director, CSIR-National Institute of Oceanography, for his encouragement and permission to publish this work. We also express our gratitude to Ministry of Earth Science, India for their financial support and for providing CVR *Sagar Sukti* and FORV *Sagar Sampada* for sample collection and onboard analysis. The entire team of SIP project for all the help rendered during the field study. Financial support for Mr. Kalyan De and Mr. Perisamy R received from the MOES under the COMAPS project (GAP-2740).

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Citation: Baban SI, Periasamy R, Kalyan D (2016) Macrobenthic Community Structure Response to Coastal Hypoxia off Southeastern Arabian Sea. J Coast Zone Manag 19: 436. doi:10.4172/2473-3350.1000436

Phylogeny and genetic variation within population of *Tachypleus gigas* (Müller, 1785)

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Isolated population of Indian horseshoe crabs, *Tachypleus gigas* plays an important role in the ecology of several marine organisms, and is being scrutinized for its abundance and morphology by many researchers. However, limited information is available about its genetic variability and evolution. Samples of horseshoe crab were collected from the east coasts of India and analysed for their phylogenetic relationship, genetic variability and structure within population based on the cytochrome oxidase I (*COI*) gene sequence. Analysis of molecular variance revealed two groups with significant genetic differentiation indices (F_{ST} = 0.544, $p < 0.001$), and the number of migrants (*Nm*) was estimated as 0.11 individuals per generation. Maximum likelihood results revealed two distinct clusters, showing that the evolution of the Indian population was genetically diverse forming a separate clade from other Southeast Asian populations, moderately with a low gene flow. Considering the ecological, economic and evolutionary significance of *T. gigas* and its declining population, there is a pressing need for conservation measures.

Keywords: *COI* gene, genetic variation, horseshoe crab, phylogeny.

HORSESHOE crabs play an integral role in sustaining ecological food web for migrating shorebirds, finfish, including loggerhead turtles[1]. They are a unique group of animals in maintaining their genotype almost unchanged for millions of years[2,3]. They are marine chelicerate arthropods under the class Merostomata, a sister group to scorpions and ticks followed by spiders and crabs[2]. Four extant species, *Tachypleus tridentatus*, *Tachypleus gigas*, *Carcinoscorpius rotundicauda* and *Limulus polyphemus* were distributed worldwide[4]. Based on the global distribution, the Atlantic horseshoe crab (*L. polyphemus*) is commonly found in the Gulf of Mexico, and *T. gigas* is predominantly found along the east coast of India (Odisha), Indo-China, North Vietnam, Borneo and Celepes. *T. tridentatus* occurs from the Northern shores of Japan to South Vietnam and along the western islands of the Philippines. Mangrove horseshoe crab *C. rotundicauda* is found from the northern shores of the Bay of Bengal to the southern coast of the Philippines. *C. rotundicauda* and *T. gigas* inhabit the coastal waters of the Bay of Bengal (India) and the continental shelf region within 48 km up to 312 km (refs 5, 6). However, they exhibit restricted distribution in the west coast of India.

Molecular studies have widely been used to determine generic variation as well as predicting their phylogeny of population structure[7]. The highly conserved region of Mitochondrial Cytochrome Oxidase subunit 1 (*mtCOI*) gene is considered as a powerful marker at the lower level of species due to high mutation rates making population subdivisions[8]. Hence highly variable region of *mtDNA* gene is useful for phylogenetic studies and genetic variation[9]. The genetic integrity between the northern and southern populations of American horseshoe crabs (*L. polyphemus*) was observed along the Florida coast using *mtDNA* gene. High turbidity and strong currents would appear to make a potential barrier to movement[10]. Similarly, genetic variation within the population of *T. tridentatus* was found in East Malaysia[11,12]. The aim of this study was to determine the phylogenetic relationship and genetic variation within the *T. gigas* population using *mtCOI* gene along the east coast of India.

Materials and methods

Sample collection

Three samples were collected from the east coast of India (Odisha) during 2008 (Figure 1). Specimens were identified, weighed, sexed and morphometric characteristics were noted[13]. A part of walking leg was removed from horseshoe crabs using sterilized scissors and the internal soft tissue was preserved in 95% ethanol. All horseshoe crabs were released back to the beach in live condition to ensure their sustainability[14].

Laboratory procedure

The genomic DNA was extracted from muscle tissues using Qiagen DNA Easy Blood and Tissue kit according to the manufacturer's instruction. DNA concentration was quantified spectrophotometrically using NanoDrop (Thermo Scientific, DE, USA) and the partial *COI* gene sequence was amplified by PCR using universal primers LCO1490 (5′-GGTCAACAAATCATAAAGATATTGG-3′)

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and HCO2198 (5′-GTCTAACCGCGGTAGCTGGCAC-3′)[15]. The amplification reaction was carried out in a 50 µl containing ~30 ng genomic DNA, 0.5 U Sigma *Taq* DNA polymerase, 1 × *Taq* buffer, 2.5 mM $MgCl_2$, 400 µM of each deoxynucleotide triphosphate (dNTP) and 0.4 µM of forward and reverse primers. PCR reactions were performed on a ABI thermal cycler with an initial denaturing step at 94°C for 2 min, 39 amplification cycles (94°C for 30 sec, 50°C for 60 sec, 72°C for 90 sec), and a final elongation step at 72°C for 5 min, followed by a 4°C, and the amplified DNA was separated through electrophoresis in 1% agarose gel and purified (QIAGEN PCR Purification kit, Qiagen, USA). Final DNA product was sequenced (ABI 3130xl sequencer) and the obtained chromatogram was edited using ABI sequence scanner software 1.0v. The sequences were analysed using BLAST to find similar sequences in the NCBI database; finally identified sequences were deposited in NCBI GenBank (accession numbers KJ825847–KJ825849) and other DNA sequences were taken from the NCBI website (Table 1).

The *mtDNA* gene sequences were edited using BioEdit version 7.0.1 (ref. 16) and aligned with CLUSTAL-W using MEGA6 (Molecular Evolutionary Genetics Analysis) software[17]. Genetic variation of *T. gigas* was calculated using Arlequin 3.0v for analysis of molecular variance (AMOVA) with 1000 permutations as implemented[18]. Phylogenetic relationship within populations was inferred using maximum likelihood (ML; 2000 bootstrap replicates) approaches to construct in a MEGA6 phylogram[17]. *L. polyphemus* was used as the outgroup, and best tree was selected and imported into Tree view to produce a 50% consensus tree with ML support values added to the tree nodes. Unique haplotypes (*h*), nucleotide diversity (π)[19] and pairwise *F*-statistics (F_{ST}) were calculated as genetic distances based on pairwise differences between populations. The demographic history of *T. gigas* was inferred by mismatch distribution analyses using DnaSP software 4.50.3v (ref. 20). Gene flow was estimated based on the equation $Nm = 0.5 \times [(1/F_{ST}) - 1]$, where N is the effective number of females and m is the migration rate by GENALEX 6 (ref. 21). Percentage of AT and GC content was calculated using BioEdit software[16].

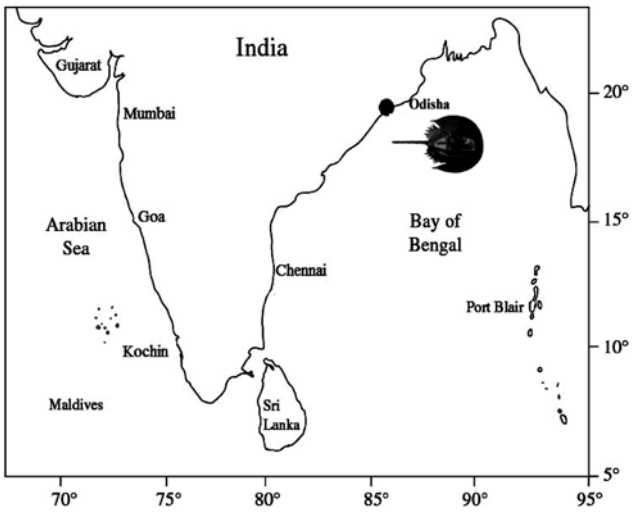

Figure 1. Map showing the location of sample collection.

Results and discussion

Phylogenetic analysis

Comparative analysis of the sequences from different geographic regions resulted in two distinct clusters based on ML data from Malaysia, Thailand and Southeast Asia. The data from these three regions were clustered in one group whereas samples from Indian formed as a separate group (Figure 2). Phylogenetic analysis showed two clades within the population and geographic structure, suggesting migration between historically isolated populations. Phylogeny represents a topology where the two form a separate branch from *T. gigas*. In India, the population of *T. gigas* was significantly better than Southeast Asia ($P < 0.05$), indicating that the mtDNA lineage is certainly divergent from the two bristle row lineage. The numbers at each node in Figure 2 represent percentage of bootstrap values based on 2000 pseudo replications of ML analyses. *T. tridentatus*, *C. rotundicauda* and *L. polyphemus* used as an outgroup were clearly clustered in a separate branch to prove the reliability of the constructed phylogram. The reconstructed phylogenetic tree clearly indicates that Indian horseshoe crabs are related to the Southeast Asian population. The *L. polyphemus* species from the east coast of North America has

Table 1. Detailed information of the sampling location

Species	Origin	*COI* gene sequence
India		
	Odisha	KJ825847
		KJ825848
		KJ825849
Southeast Asian		
Tachypleus gigas	Malaysia	JF896114
		JF896108
		JF896107
	Thailand	HQ588748
		HQ588749
	Malaysia	JF896111
		JF896109
Tachypleus tridentatus		JN018216
	Thailand	HQ588752
Carcinoscorpius rotundicauda		AF370828
	Malaysia	JF896106
		JF896105
	Thailand	HQ588754
		HQ588746
		HQ588750
Limulus polyphemus	Atlantic	HQ588751
		EU834780

Table 2. Analysis of molecular variance for *COI* gene within *T. gigas* populations

Source of variation	Degree of freedom	Sum of squares	Contribution of variation (%)	Percentage of variation	F_{ST}	*P*
Among populations	3	442.143	31.708	54.45	0.544	0.001
Within populations	20	623.200	31.160	45.55		
Total	23	1177.000	68.402	100		

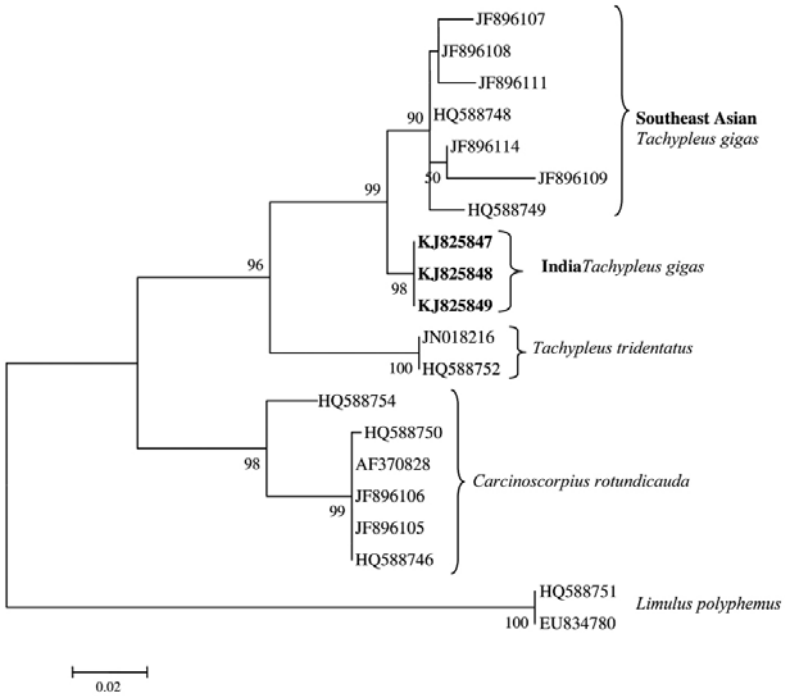

Figure 2. Phylogenetic tree showing the relationship between Indian and Southeast Asian *Tachypleus gigas*. (Inference based on *COI* gene and bootstrap values >50% are shown in the nodes. Scale bar 0.02 nucleotide substitutions per nucleotide positions.)

genetically isolated population differentiated from the three other species[22]. Another interesting point emerged from the phylogenetic relationship is that *T. gigas* is closer to *T. tridentatus* followed by *C. rotundicauda*. Xia[23] reported that partial mtDNA sequence analysis show high similarity index between *T. gigas* and *T. tridentatus*.

Gene flow and genetic differentiation

The fixation index (F_{ST}) was estimated to be 0.635 between the Indian and Southeast Asian populations. This indicates isolated and low gene flow between populations. It also implies lower migratory rate per generation (*Nm*: 0.12) between the Indian and Southeast Asian samples. The limited migration pattern of horseshoe crab population clearly proved that a geographical barrier to gene flow was highly restricted between populations. However, AMOVA within population yielded F_{ST} = 0.544, ($P < 0.001$), indicating a significant level of genetic variation among populations (Table 2). In fact, adults of *T. tridentatus* stay in the deeper waters and migrate to shallow areas for reproduction[5]. The trilobite larvae settle right after hatching and juveniles spend their life stages at or near the natal beach for feeding[24]. These characteristics indicate that larvae and juveniles have a limited migratory pattern, but adults can travel long distances by swimming with the sea current. The geographical barriers may be an important factor limiting the gene flow. For instance, the small geographic range in the Bay of Bengal was the fundamental cause of population subdivision, that is semi-closed, and as a consequence the population might have limited genetic exchange with those outside the Bay[25]. Similarly, the genetic break of *L. polyphemus* species was also observed between the Gulf of Mexico and Atlantic populations which suggest that Florida Peninsula might be the barrier to gene flow[26].

Genetic and haplotype diversity

The trimmed mtCOI dataset consists of 650 bp and three individuals from localities on the east coast of India (Odisha). Of those, three mtDNA sequences were taken

Table 3. Variation in the composition of nucleotides

Populations	*T. gigas*		*T. tridentatus*	*C. rotundicauda*	*L. polyphemus*
	India	Southeast Asia			
Length (bp)	653	538	588	569	633
A%	27.81	27.69	29.35	30.32	28.59
C%	21.84	21.77	18.71	20.63	23.7
G%	15.97	15.85	15.63	15.81	16.9
T%	34.39	34.69	36.32	33.2	30.81
G + C content (%)	37.81	37.62	34.33	36.44	40.6
A + T content (%)	62.19	62.38	65.67	63.53	59.4

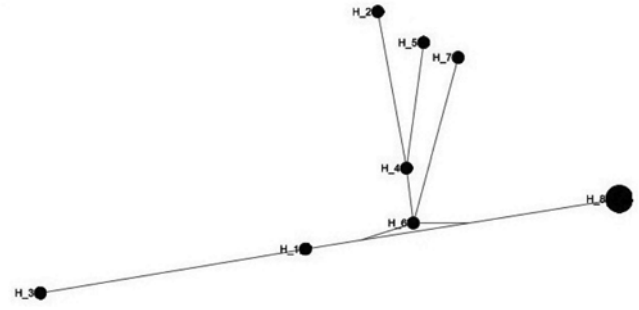

Figure 3. Median-joining network showing the phylogenetic relationships among partial mtDNA *COI* gene haplotypes of *T. gigas*. Numbers crossing lines characterize sites of nucleotide substitutions; circled areas show the proportion of haplotypes.

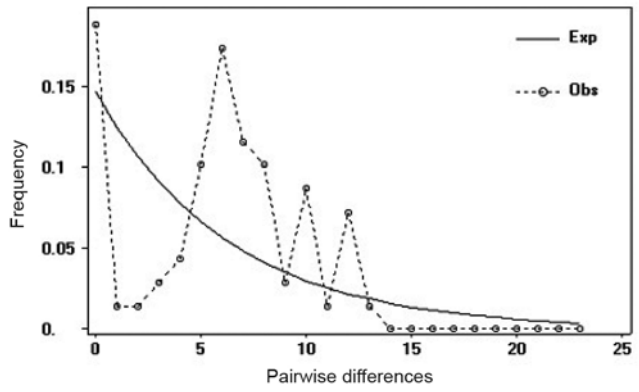

Figure 4. Mismatch analyses of eight haplotypes of *T. gigas.*

for average nucleotide base composition and adenine–thymine bias (T for 34%, A for 28%, C for 22% and G for 16%). AT content (62%) was much higher than that of GC (38%) (Table 3). Eight haplotypes (Figure 3) (designated as H1–H8) were defined from all partial mtCOI sequences. Intra-population genetic diversity varied among populations with haplotype diversity (*h*) being 0.019, whereas nucleotide diversity (π) was 0.88 and showed the high genetic diversity. Among the diverse eight haplotypes, H8 was observed as dominant type in the Indian locality, and H6 and H7 were shared by Thailand. H4 was observed in Malaysia, and others sample-specific haplotypes. Five haplotypes (H1, H2, H4, H5 and H6) were one mutational step away from H3, which was most likely an ancestral type. H2 and H4 were also one mutational step away from H1. The prolonged star-like network indicated the stable existence of the historic population of horseshoe crab. Figure 3 also shows the topology of the median-joining network of eight haplotypes. Figure 4 shows the mismatch frequency spectra for the eight populations.

The genetic variation in horseshoe crab indicated that expansion of refugial populations occurred in less genetically diverse species living in the recently colonized population[7,27]. Similar observations were made in other aquatic organisms such as bivales[27,28]. The geographically isolated population showed a restricted gene flow and high genetic variations between the populations[8,29,30]. The nucleotide diversity of *T. tridentatus* populations from the closer geographical area of Taiwan coast showed similar results to the restricted gene flow within the population[25]. The loss of suitable spawning and feeding grounds was due to coastal development and population explosion[31]. Furthermore, the appropriate protected areas could be planned to conserve the horseshoe crab and its habitats.

Conclusion

The phylogenetic analysis of *mtDNA* gene sequence showed that it clustered with sister species in the individual group, proving evolution of the species. Reconstruction of phylogenetic tree and genetic variation data of *T. gigas* species clearly proved that the Indian and Southeast Asian populations are more genetically related to *T. tridentatus* other than horseshoe crab species. Interestingly, restricted gene flow was observed between India and Southeast Asian populations. However, the distribution of Indian population was geographically isolated. Therefore, the high genetic variation indicated that measures need to be taken to protect the rare and threatened marine species. The available genetic information on Indian horseshoe crab could be used in different conservation strategies for their sustainable fishery management along the east coast of India (Odisha). This study also suggests the need for further monitoring genetic changes within the population of Indian horseshoe crab.

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ACKNOWLEDGEMENTS. We thank the Director, CSIR-National Institute of Oceanography (NIO), Goa, for permission to publish this work; and Dr N. Ramaiah (Biology Division, NIO, Goa) for providing sequencing facilities. We also thank CSIR, New Delhi for financial support through a project (No. PSC0206) and MoES, GoI for providing a fellowship to R.P. under COMAPS project (No.GAP-2741). This is CSIR-NIO contribution number 5974.

Received 30 June 2016; revised accepted 13 December 2016

doi: 10.18520/cs/v112/i10/2029-2033

Mitochondrial DNA

http://informahealthcare.com/mdn
ISSN: 1940-1736 (print), 1940-1744 (electronic)

Mitochondrial DNA, Early Online: 1–2
 DOI: 10.3109/19401736.2015.1007368

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MITOGENOME ANNOUNCEMENT

Complete mitochondrial genome of the Spadenose shark *Scoliodon laticaudus* (Carcharhiniformes: Carcharhinidae)

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Abstract

The complete mitochondrial genome of the Spadenose shark *Scoliodon laticaudus* has been determined for the first time in this study. It was 16,695 bp in length and consisted of 37 genes with typical gene order in vertebrate mitogenome. The nucleotide base content of *S. laticaudus* mitogenome was 31.5% A, 23.7% C, 13.2% G and 31.6% T. Two start codons (GTG and ATG) and three stop codons (AGA, TAG and TAA/T) were used in the protein-coding genes. The 22 tRNAs ranged from 67 bp (tRNA-*Cys* and tRNA-*Ser2*) to 75 bp (tRNA-*Leu1*) in length. The tRNA-*Ser2* could not be folded into typical cloverleaf secondary structure by lacking the dihydrouridine (DHC) arm stem.

Keywords

Mitochondrial genome, scoliodon laticaudus, *Scoliodon macrorhynchos*

History

Received 10 January 2015
Accepted 10 January 2015
Published online 19 February 2015

The Spadenose shark *Scoliodon laticaudus* was first scientifically described by Müller & Henle in 1838 based on the specimen from India. It was considered the only species of the genus *Scoliodon* and widely distributed in the Indo-West Pacific oceans, from northeast Africa to southern Japan (Compagno et al., 2005). However, the recent evidences suggest that the population distributed in the West Pacific is a distinct species – *S. macrorhynchos* (Chen et al., 2014; White et al., 2010), which was first described based on the specimen collected in Jakarta, Indonesia (Bleeker, 1952), and was later synonymized as *S. laticaudus* (Günther, 1870). In this study, we present the mitochondrial genome of *S. laticaudus*, hoping it could contribute to the species identification and phylogenetic study.

One specimen was collected from Goa (Lat 15°19 N; Long 73°49 E), India. The experimental protocols and data analysis methods were followed as described in Chen et al. (2013). The whole mitogenome of *S. laticaudus* is 16,695 bp in length (GenBank Accession No. KP336547). It consists of 22 tRNA genes, 2 rRNA genes, 13 protein-coding genes (PCGs) and 1 control region with typical gene order in vertebrate mitogenome (Table 1). The nucleotide base content of *S. laticaudus* mitogenome was 31.5% A, 23.7% C, 13.2% G and 31.6% T.

Table 1. Characters of mitochondrial genome in *Scoliodon laticaudus*.

Gene	Position	Size (bp)	Start codon	Stop codon	Inter-genic spacer	Nucleotide homology with *S. macrorhynchos* (%)	Amino acids homology with *S. macrorhynchos* (%)
tRNA-*Phe*	1–70	70			0	100	
12S rRNA	71–1021	951			0	99.05	
tRNA-*Val*	1022–1093	72			0	98.61	
16S rRNA	1094–2763	1670			0	98.62	
tRNA-*Leu1* (TAA)	2764–2838	75			0	98.67	
ND1	2839–3813	975	ATG	TAA	0	96.72	97.22
tRNA-*Ile*	3814–3883	70			1	98.57	
tRNA-*Gln*	3885–3956	72			0	98.61	
tRNA-*Met*	3957–4025	69			0	100	
ND2	4026–5072	1047	ATG	TAG	−2	96.94	97.70
tRNA-*Trp*	5071–5141	71			1	94.37	
tRNA-*Ala*	5143–5211	69			0	98.55	
tRNA-*Asn*	5212–5284	73			0	100	
OL	5285–5322	38			−4	86.84	
tRNA-*Cys*	5319–5385	67			1	100	

(*continued*)

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Table 1. Continued.

Gene	Position	Size (bp)	Start codon	Stop codon	Inter-genic spacer	Nucleotide homology with *S. macrorhynchos* (%)	Amino acids homology with *S. macrorhynchos* (%)
tRNA-*Tyr*	5387–5455	69			1	100	
COI	5457–7013	1557	GTG	TAA	0	97.82	99.23
tRNA-*Ser1* (UGA)	7014–7084	71			3	97.18	
tRNA-*Asp*	7088–7156	69			7	98.55	
COII	7164–7854	691	ATG	T– –	0	97.83	99.57
tRNA-*Lys*	7855–7928	74			1	100	
ATP8	7930–8097	168	ATG	TAA	−10	100	100
ATP6	8088–8771	684	ATG	TAA	−1	97.37	98.24
COIII	8771–9556	786	ATG	TAA	2	98.09	100
tRNA-*Gly*	9559–9628	70			0	100	
ND3	9629–9979	351	ATG	TAG	−2	97.72	98.28
tRNA-*Arg*	9978–10,047	70			0	97.14	
ND4L	10,048–10,344	297	ATG	TAA	−7	99.66	100
ND4	10,338–11,718	1381	ATG	T– –	0	97.54	98.26
tRNA-*His*	11,719–11,787	69			0	98.55	
tRNA-*Ser2* (GCU)	11,788–11,854	67			0	98.51	
tRNA-*Leu2* (UAG)	11,855–11,926	72			1	97.22	
ND5	11,928–13,757	1830	ATG	TAA	−5	96.67	97.54
ND6	13,753–14,274	522	ATG	AGA	0	97.13	97.69
tRNA-*Glu*	14,275–14,344	70			2	100	
Cyt b	14,347–15,492	1146	ATG	TAG	−1	96.51	99.21
tRNA-*Thr*	15,492–15,561	70			2	100	
tRNA-*Pro*	15,564–15,632	69			0	98.55	
Control region	15,633–16,695	1063				98.03	

There was a total of 32 bp gene overlaps ranging from 1 to 10 bp, and was a total of 22 bp short inter-gene spaces ranging from 1 to 7 bp. Two start codons (GTG and ATG) and three stop codons (AGA, TAG and TAA/T) were used in the PCGs, the incomplete T could be extended to complete TAA through polyadenylation in transcriptions (Ojala et al., 1981). All start codons and stop codons of PCGs in the two *Scoliodon* species were identical except for the stop codon of the *ND6* gene, the *S. laticaudus* used AGA while the *S. macrorhynchos* used AGG (Chen et al., 2014). The nucleotide homology of PCGs between *S. laticaudus* and *S. macrorhynchos* were from 96.51% (*Cyt b*) to 100% (*ATP8*). However, the amino acids similarity of PCGs was from 97.22% (*ND1*) to 100% (*ATP8*, *COIII* and *ND4L*). The 22 tRNA genes ranged from 67 bp (tRNA-*Cys* and tRNA-*Ser2*) to 75 bp (tRNA-*Leu1*) in length, which formed three conserved clusters (IQM, WANCY and HSL). The origin of L-strand replication (OL, 38 bp) was identified between tRNA-*Asn* and tRNA-*Cys* genes, and formed a hairpin structure (13 bp stem and 12 bp loop). All tRNAs could be folded into typical cloverleaf secondary structure except for tRNA-*Ser2*, which lacked the dihydrouridine (DHU) arm stem by forming a simple loop. The nucleotide homology of tRNA genes between two *Scoliodon* species were extremely high, nine tRNA genes (40.9%) were completely identical. The highest nucleotide divergence of tRNA genes was between two tRNA-*Trp* (94.37%), which was also the highest nucleotide divergence in all mtDNA sections. The nucleotide composition of control region was 32.8% A, 19.5% C, 12.2% G and 35.5% T, showing the highest A + T content (68.3%) in the mitogenome. The termination-associated sequence (TAS) of *S. laticaudus* was identical to *S. macrorhynchos*.

Declaration of interest

This study was supported by the National Natural Sciences Foundation of China (41006080) and Council for Scientific & Industrial Research (project No. PSC0206), National Institute of Oceanography, Goa. The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the article.

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Regional Studies in Marine Science 7 (2016) 150–158

Contents lists available at ScienceDirect

Regional Studies in Marine Science

journal homepage: www.elsevier.com/locate/rsma

Community structure and coral health status across the depth gradients of Grande Island, Central west coast of India

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HIGHLIGHTS

- Grande Island is less explored for its biological diversity and this study provides the first comprehensive report on the coral community structure.
- Live coral cover and its diversity were high in the mid-shelf zone (5–8 m) compared to the shallow (<5 m) and deep zones (>8 m).
- Competition posed by turf algae and sponges was the predominant stressor affecting the live coral colonies.
- Coral diseases such as white plaque disease and trematodiosis were common among the live coral colonies.

ARTICLE INFO

Article history:
Received 29 March 2016
Received in revised form
20 May 2016
Accepted 31 May 2016
Available online 4 June 2016

Keywords:
Coral
Community structure
Grande Island
Sedimentation
Turf algae
Boring sponges

ABSTRACT

The Grande Island, located at the central west coast of India is one of the less studied coral reef systems in India. In this study, we provide a comprehensive description of the coral community structure and health status of corals across the spatial scales at different depths in Grande Island. Such descriptions provide a baseline for evaluating future community changes and effective conservation in the face of changing climate scenario. Individual benthic components and the coral health were quantified using line-intercept transects and belt transects respectively along three depth zones: shallow (<5 m); mid-shelf (5–8 m) and deep zone (>8 m). Average live coral cover was high in the mid-shelf zone (8.05 ± 3.98%) compared to the shallow (1.92 ± 2.01%) and deep zones (2.12 ± 0.05%). In total, 15 genera of corals were recorded in Grande Island of which 14 genera were present in the mid-shelf and shallow zone and six genera in the deep zone. *Turbinaria* and *Goniopora* spp. were dominant in the mid-shelf zone. Whereas, *Pseudosiderastrea* and *Porites* spp. were dominant in the shallow and deep zones. Potential threats to the corals in Grande Island include diseases and competition posed by algal turf and boring sponges. An average of 53.2% of the live corals was affected by algal turf intrusion; 2.7% by boring sponges and 2.6% by diseases that include white plaque disease and trematodiosis. Understanding the physical processes around Grande Island will reveal more about the distribution and colonization of coral communities and their vulnerability to changes in the future.

1. Introduction

Coral reefs are biodiversity hotspots that provide numerous ecological and economic benefits (Moberg and Folke, 1999). India comprises four major coral reef formations along its 7500 km vast coastline viz. Gulf of Kachchh and Lakshadweep archipelago on the west coast and Gulf of Mannar & Palk Bay and Andaman & Nicobar Islands on the east coast. Coral reefs cover an approximate area of 2375 km^{-2} in India. Lakshadweep reefs are the coral atolls, and the other major reefs are of fringing and barrier types. There are also many coral formations located near shore as a small detached patch or platform reefs along the central west coast of India comprising Maharashtra and Goa between 15°33′N and 73°27′E (Nair and Qasim, 1978; Qasim and Wafar, 1979). The biological structure and ecological status of these patch reefs remain relatively less studied compared to the other major reefs in India. In this study, we addressed the gap in knowledge by undertaking the first broad-scale survey of the patch reefs along Grande Island, an exposed rocky island, located off Marmagoa Port in Goa focusing on the coral community structure (diversity, density and taxonomic composition of scleractinian corals) and health status of reef building corals.

Sexual mode of reproduction and ensuing larval dispersal by the ocean currents had enabled the corals to colonize distant marine

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http://dx.doi.org/10.1016/j.rsma.2016.05.013
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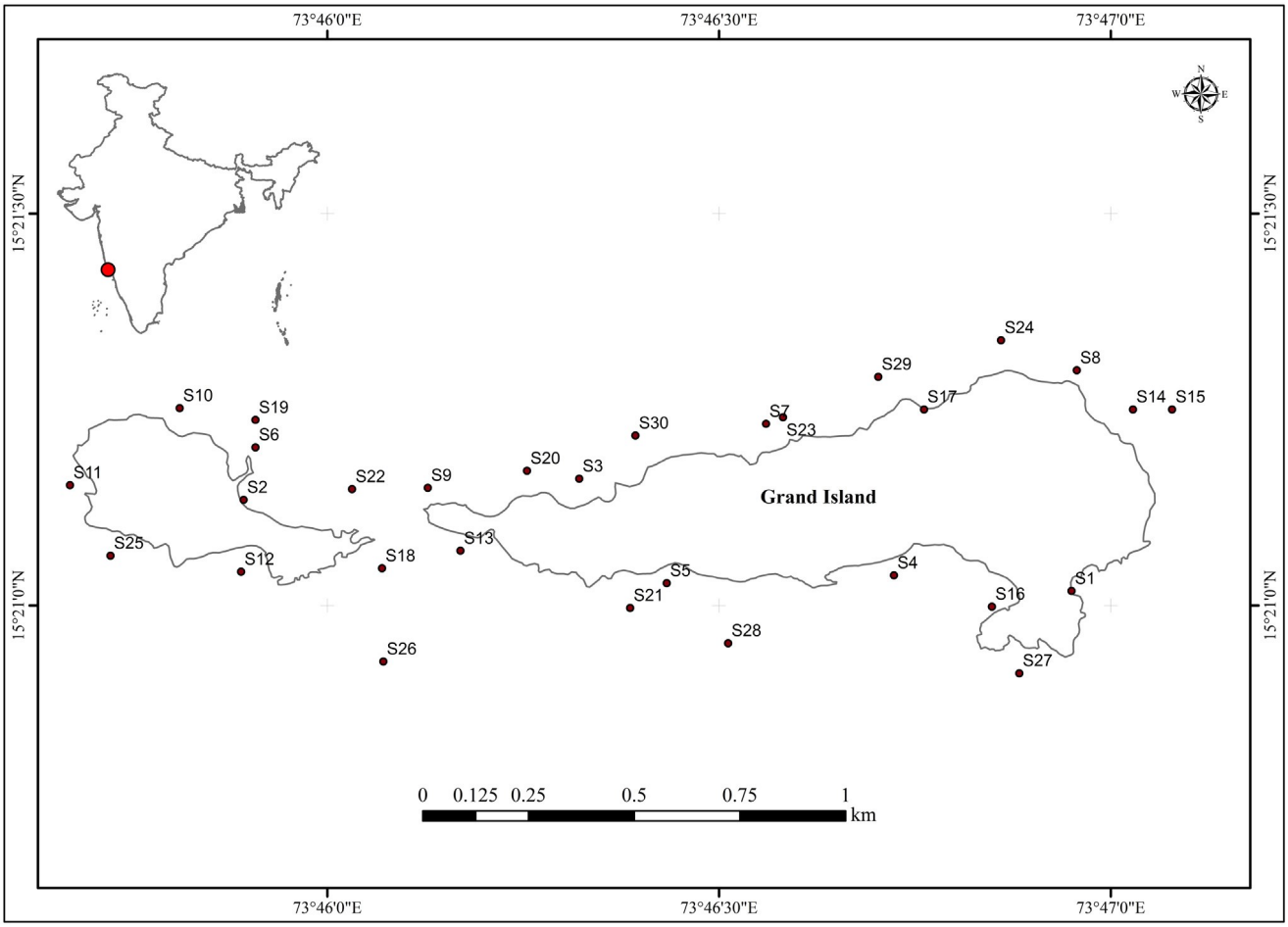

Fig. 1. Map showing the study locations around Grande Island at different depth zones. S1–S15 (shallow zone); S16–S25 (mid-shelf zone) and S26–S30 (deep zone).

habitats such as isolated islands (Richmond, 1987; Carlson and Olson, 1993; Torda et al., 2013) and establish their population. This process of larval dispersal from a parent reef and its subsequent settlement and growth in a remote habitat is termed as connectivity, and it determines the distribution, genetic structure and population dynamics of corals in distant shallow marine habitats (Cowen and Sponaugle, 2009; Jones et al., 2009). The Grande Island in Goa (15.352°N; 73.773°E) comprises natural habitats such as coral patches, submerged rocks encrusted with corals, sandy bottoms and artificial habitats such as shipwrecks. The complex habitat structure provided by the coral patches and submerged rocks acts as a habitat for breeding, feeding and shelter for variety of benthic organisms including reef fishes (Sreekanth et al., 2015).

In general, the isolated islands that lack any human impacts are characterized by rich biodiversity dominated by corals and fish communities. Any variation in the structure of reef communities is more likely to be attributed to the natural factors such as habitat availability, predation (Thorson, 1950), sedimentation (Hodgson, 1990; Gilmour, 1999), light availability (Mundy and Babcock, 1998), and species competition (Birrell et al., 2005). However Grande Island in Goa is a tourism hotspot witnessing an average of 6–7 boats comprising 7–12 tourists every day during the peak tourist season between October and April (Personal communication). The wealth of marine life had invited a variety of anthropogenic stressors such as uncontrolled tourism activities like recreational SCUBA diving, snorkelling and recreational fishing. In addition, overfishing, discarded fishing nets and littering also exaggerate the risk to corals in Grande Island.

In this study, our primary aim was to provide a comprehensive description of the coral community structure across depth gradients of Grande Island. Specifically, we compare and contrast the coral communities in the shallow (<5 m); mid-shelf (5–8 m) and deep zones (>8 m) around the Grande Island. Secondly, we examined the relationship between the depth and the average percent cover of various benthic components such as corals, soft corals and macroalgae. Furthermore, we assessed the health status of live corals and potential threats to the corals in Grande Island. Documentation of the diversity and community structure of corals in a region is essential to identify areas for conservation, recreation and evolving specific management principles. Also, such data will serve as a baseline for evaluating changes in the future due to the changing climate and environmental scenario.

2. Materials and methods

2.1. Study site

The study sites around Grande Island were selected randomly across different depths and the number of study sites at each depth zone was determined based on the visibility. The substratum around the Grande Island was categorized in to three distinct zones based on depth. The shallow zone (<5 m), that largely comprises rocks with intermittent sand patches. This was followed by a mid-shelf zone (5–8 m) that comprise a flat sandy substrate with small rocks and deep zone (>8 m) that comprise rocks further up to the depth of 14 m. In total, 15 sites were selected in the shallow zone; 10 sites in the mid-shelf zone and 5 sites in the deep zone (Fig. 1).

Each study site was parted by at least 50–100 m and located at an average distance of 10 m–50 m from the shore.

2.2. Benthic surveys

Underwater surveys were carried out using SCUBA diving at all the study sites. We used the line intercept transect (LIT) method (English et al., 1997) to determine the average percent cover of different benthic components. Two 20 m transect lines were laid, one parallel and the other perpendicular to the shore at each study site. Different benthic forms that fall under the transect points were recorded and the coral colonies were photographed in the underwater macro mode using Olympus underwater camera (Model: μ tough 8000) for identification. The corals were identified to the genus level following the standard keys (Veron, 2000). Species-level identification of corals was not possible due to the legal restrictions on the collection of coral samples in India. The density of corals was estimated following the quadrat method. Quadrats of 1 m^{-2} ($n = 10$) were placed randomly in each study site and the total number of colonies within each quadrat was counted. Each quadrat has been put at least 5 m apart. The coral density is reported as the average number of colonies m^{-2} in each study site. The taxonomic composition of the corals was calculated as the average percentage of corals in each genus relative to the total number of corals in the other genera.

2.3. Coral health assessment

Belt transect method was employed to determine the status of the live coral colonies in the study sites. A 30 m transect line was laid parallel to the shore at each study site and the observations were made following the belt transect survey, with a swath of 2.5 m on either side of the transect making the effective width of the transect as 5 m. Coral colonies, either live or partially alive that falls within the effective width of the transect were considered. The live coral colonies were categorized as healthy live corals (HLC), live corals colonized by turf algae (LC/TA); macroalgae (LC/MA); infested with diseases (LC/D) and boring sponges (LC/S). The status of live coral colonies was reported as the average percentage of corals in each of the above categories.

2.4. Analysis

The data was analysed by comparing the generic richness and density of corals between the study sites at each depth zone and also across different depth zones. The mean density of coral colonies at shallow, mid-shelf and deep zones were calculated by averaging the density of coral colonies across the study sites within each zone. Univariate indices such as Shannon diversity index (H') and species evenness (Pielou's J) (loge based) were calculated for each study site as a measure of determining the degree to which the corals of different genus at different depth zones are related to each other. One way Analysis of Variance (ANOVA) was done to test for significant differences in the average coral cover between the study sites in different depth zones. The data on average live coral cover was log transformed and checked for their normality and homogeneity of variance. The significance of differences in coral density between different depth zones has been individually compared using the two sampled t-test. K-dominance curve was plotted for species abundance data to determine the patterns of relative species abundance in different depth zones.

Bray–Curtis cluster analysis under the paired linkage was done to test the similarity in benthic composition between the study sites across different depth zones in Grande Island. The data was fourth root transformed to reduce the effect of dominant community forms on the data analysis. Analysis of Similarity (ANOSIM) was used to test for the significance of differences in benthic cover (percent cover) among the study sites in each depth zone and also between the different depth zones. The test was performed using the same similarity matrix generated for Bray–Curtis, calculated using fourth root transformed data. Similarity percentage (SIMPER) was used to know the average dissimilarity in the taxonomic composition of live coral colonies between the different depth zones and to determine the contribution of individual species to the observed differences in the taxonomic composition. All the analysis was performed using the PRIMER statistical software version 6.1.15 (Warwick and Clarke, 1991; Clarke, 1993).

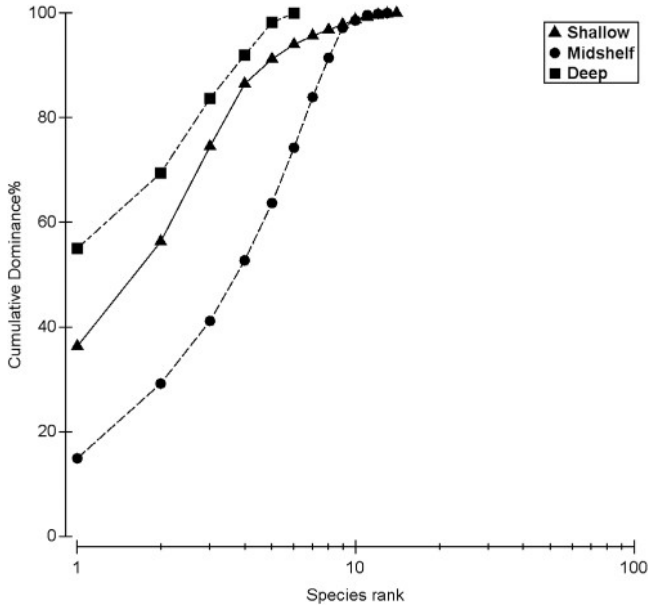

Fig. 2. K-dominance curve plot showing the difference in the generic richness across different depth zones in Grande Island. Highest generic diversity of corals was evident in the shallow and mid-shelf zone.

3. Results

3.1. Coral diversity

In total, 15 genera of corals were recorded from the Grande Island of which 14 genera were present in both shallow and mid-shelf zone and 6 genera in the deep zone (Table 1). Correspondingly, the diversity Index (H', loge based) was high in the mid-shelf zone ($H' = 1.86$) compared to the shallow and deep zones ($H' = 0.9$). *Pseudosiderastrea* and *Porites* spp. were abundant in the shallow zone. Dense patches of *Turbinaria* and *Goniopora* spp. dominated the mid-shelf zone. Different genera of corals were more evenly distributed in the mid-shelf zone (Evenness (J) = 0.89) compared to the shallow ($J = 0.86$) and deep zones ($J = 0.81$). Corals of *Pseudosiderastrea*, *Siderastrea*, *Favites*, *Plesiastrea*, *Coscinarea* and *Dendrophyllidae* spp. were present at all the depth zones. *Pocillopora* sp. was found only in the shallow zone. New recruits of *Dendrophyllidae* sp were abundant in the deep zone. Difference in the generic richness at different depth zones was shown in Fig. 2 where the cumulative percentage of dominance of different genus is ranked on a logarithmic scale. The highest generic diversity was evident in the mid-shelf and shallow zone.

3.2. Coral density

The mean density of corals (no. of colonies m^{-2}) were high in the mid-shelf zone (4.8 ± 0.13) (Mean ± SD) compared with

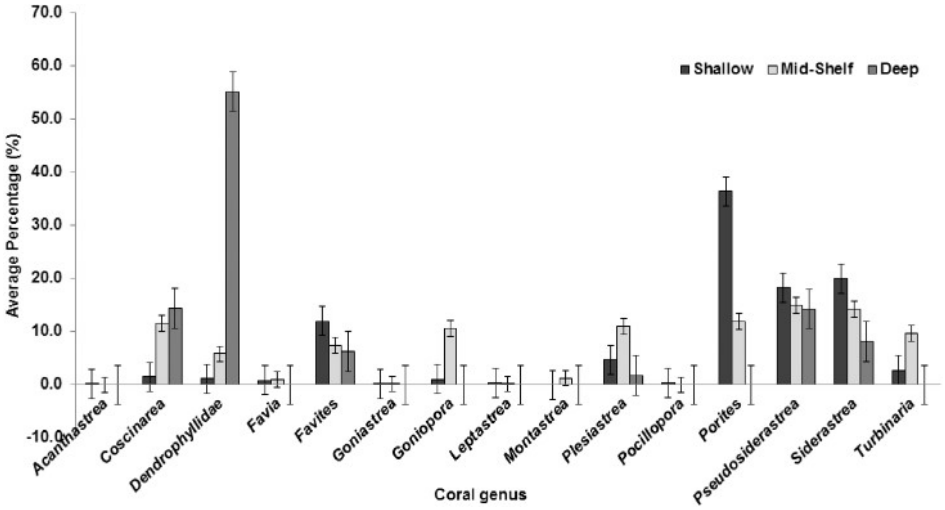

Fig. 3. Taxonomic composition of different genera of corals at different depth zone in Grande Island. Error bars indicate standard error.

Table 1
Distribution of corals of different genera across different depth zones in Grande Island. (+) indicates presence; (−) indicates absence.

S. No	Genus	Shallow	Mid-shelf	Deep
1	*Acanthastrea*	+	−	−
2	*Coscinarea*	+	+	+
3	*Dendrophyllidae*	+	+	+
4	*Favia*	+	+	−
5	*Favites*	+	+	+
6	*Goniastrea*	+	+	−
7	*Goniopora*	+	+	−
8	*Leptastrea*	+	+	−
9	*Montastrea*	−	+	−
10	*Plesiastrea*	+	+	+
11	*Pocillopora*	+	+	−
12	*Porites*	+	+	−
13	*Pseudosiderastrea*	+	+	+
14	*Siderastrea*	+	+	+
15	*Turbinaria*	+	+	−

the shallow (3.4 ± 1.26) and the deep zone (2.4 ± 1.13). The two sampled *t*-test revealed no statistically significant differences in coral density between the shallow and mid-shelf zone ($t_{stat} = -1.85 < t_{crit} = 2.07$) and between the shallow and deep zone ($t_{stat} = 1.24 < t_{crit} = 2.2$). However, the coral density differs significantly between the mid-shelf and deep zone ($t_{stat} = 2.98 > t_{crit} = 2.22$). The average percentage of contribution of different coral genera to the total generic composition at each depth zone was presented in Fig. 3. *Porites* sp. was dominant in the shallow zone contributing 36.3% to the total generic composition of corals followed by *Siderastrea* sp (20%) and *Pseudosiderastrea* sp. (18.3%). However, *Porites* sp was absent in the deep zone and is dominated by the *Dendrophyllidae* sp (55.2%) followed by *Coscinarea* sp (14.4%) and *Pseudosiderastrea* sp (14.3%). Corals of the genus *Pseudosiderastrea, Siderastrea, Coscinarea, Turbinaria, Plesiastrea* and *Goniopora* spp. contribute >10% to the total generic composition of corals in the mid-shelf zone.

3.3. Community structure

The average percent cover of different benthic forms around Grande Island was presented in the Fig. 4. The average live coral cover was low in the deep and shallow zones and they contribute 1.92% ± 2.18% and 2.12% ± 2.88% respectively to the total benthic composition. The live coral cover was high in the mid-shelf zone (8.05% ± 3.95%). The live coral cover differed significantly between different depth zones (One-way ANOVA, $df = 2$; $F = 9.62$; $F_{crit} = 3.35$; p-value $= 0.0006 < 0.05$). The live coral cover at the study sites at different depth zones was presented in the Fig. 5. Rocks form the major benthic component in the shallow zone accounting for 87.61% ± 37.2% followed by sand (8.3% ± 26.9%). The flat sandy substratum accounts for 46% ± 10.8% and 51.2% ± 16% in the mid-shelf and deep zones respectively. Rocks in the deep zone were smothered by a thick mat of turf algae. Whip corals were abundant in the deep zone contributing 22.2% ± 14% to the total benthic composition. However, it is scarce in the mid-shelf zone (2.5% ± 5.7%) and absent in the shallow zone.

As per the results of Bray–Curtis cluster analysis, the study sites at Grande Island were grouped in to nine individual clusters based on the benthic composition (Fig. 6). Benthic composition at the study sites within same depth zone are highly similar and are grouped under the same cluster with few exceptions. Study site 24 at mid-shelf zone is merged with the study sites 13 and 17 at the shallow zone with a mean similarity value of 93.9. Similarly, study sites 22 and 23 are merged with the study sites at deep zone with an average similarity value of 83.4 and 87.8 respectively. The difference in benthic composition among the study sites in the same depth zone was not significant as revealed by One-way ANOSIM (p value >0.05). SIMPER analysis showed that the benthic composition was highly dissimilar between the shallow and deep zone with an average dissimilarity value of 42.75. Sand and Rock are the major benthic components contributing 29.7% and 23.6% respectively to the observed dissimilarity values. Similarly, macroalgae and whip corals contribute 22.8% and 20.9% respectively to the observed dissimilarity value of 32.3 between the mid-shelf and deep zone. Average dissimilarity value between shallow and mid-shelf zone was 27.9 and sand and rock are the major contributors to the observed differences (Table 2).

3.4. Coral health assessment

The proportion of live coral colonies affected by various stressors across the depth zones in Grande Island was presented in the Fig. 7. Turf algae affects majority of the live corals compared to the other stress factors. In total, 68% ± 14.72% of the live coral colonies in the deep zone were smothered either partially or wholly by the turf algae. Similarly 48.8% ± 4.48% and 43.03% ± 10.25% of the live corals were affected by turf algal smothering in the shallow and mid-shelf zone respectively. Coral diseases were more prevalent in the mid-shelf and shallow zone. In total, 3.98% ± 1.32% of the live corals were disease affected in the mid-shelf zone; 3.1% ± 0.44% in the shallow zone and

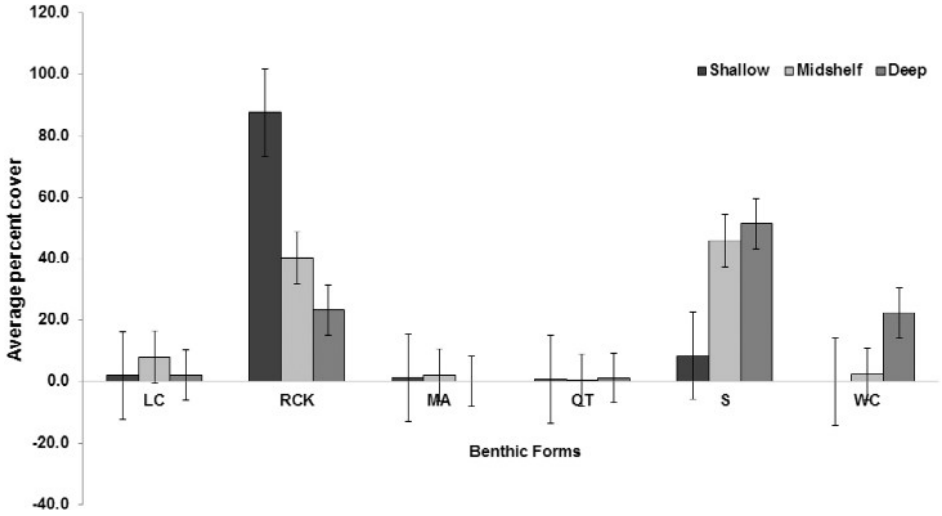

Fig. 4. Average percent cover of different benthic forms at different depth zones around Grande Island. LC—Live coral; RCK—Rock; MA—Macroalgae; OT—Others; S—Sand; WC—Whip coral. Error bars indicate standard error.

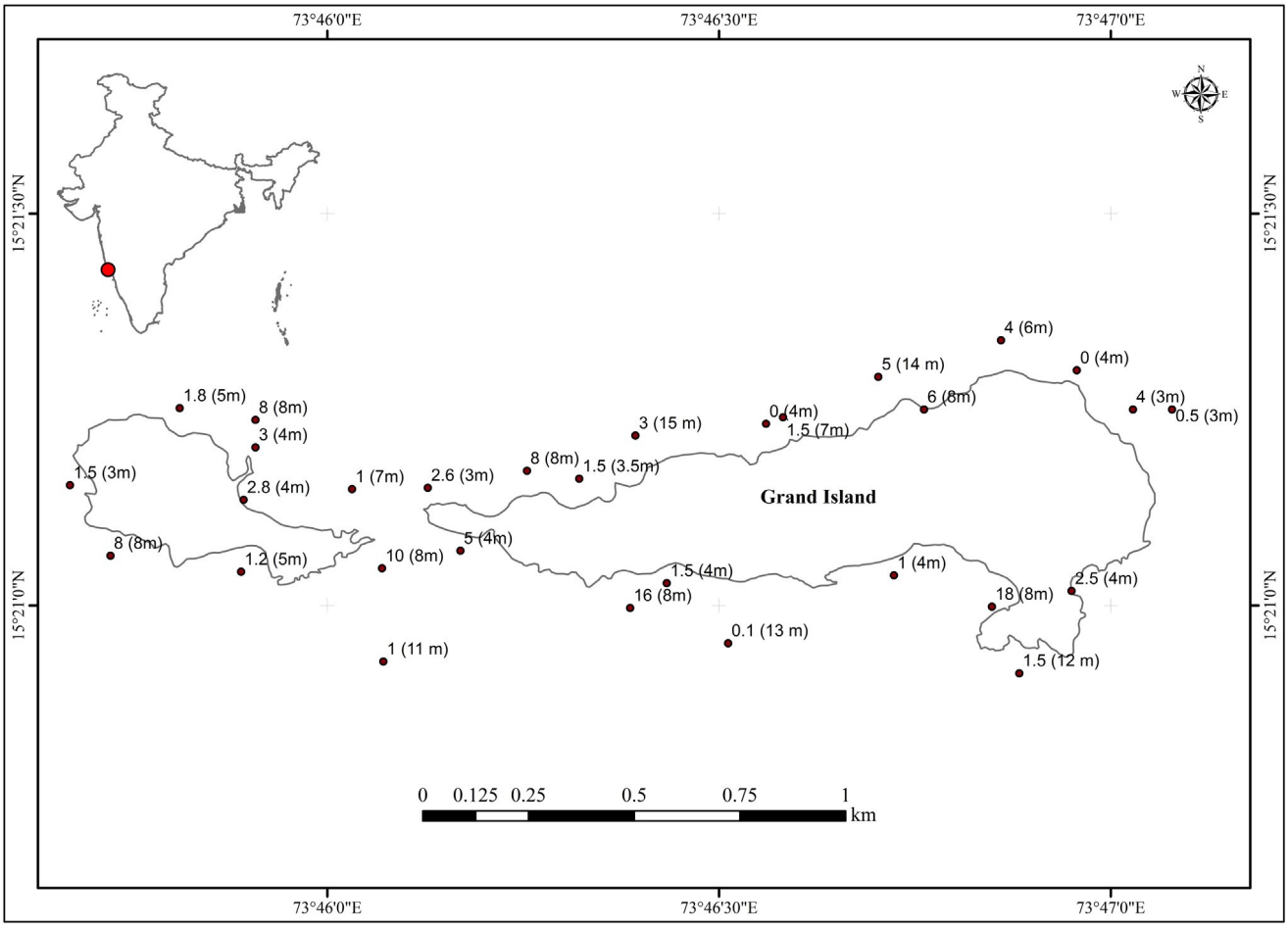

Fig. 5. Map of Grande Island showing the average percent cover of corals at the study sites corresponding to the depth (in parenthesis).

0.9% ± 1.76% in the deep zone. Trematodiosis was common among the *Plesiastrea* sp (Fig. 8(a)) and White plaque disease was common with *Pseudosiderastrea* (Fig. 8(b)) and *Coscianrea* sp (Fig. 8(c)). Boring sponges smother 5.06% ± 2.36% of the live coral colonies in the shallow zone and 3.03% ± 0.33% in the mid-shelf zone. Corals of the genera *Pseudosiderastrea* (Fig. 9(a)) and *Favites* sp (Fig. 9(b)) were predominantly affected by the boring sponges.

4. Discussion

The generic richness of corals in Grande Island is comparatively lower than the other major reefs along the west coast of India. In total, 36 species of 20 genera and 91species of 34 genera were recorded in Gulf of Kachchh and Lakshadweep Islands respectively (Venkataraman, 2003). The coral formations around Grande Island

Table 2
Results of SIMPER analysis and One-way ANOSIM (*R* value and Significance level) on the abundance (percent cover) of different benthic forms at different depth zones in Grande Island. (S- Sand; RCK- Rock; LC- Live coral; MA- Macroalgae; OT- Others; WC- Whipcoral).

Shallow vs. Mid-shelf zone					
R value = 0.354; Level of significance (%) = 0.1; Avg. dissimilarity = 27.95					
Benthic component	Average abundance		Average dissimilarity	% contribution	Cum %
	Shallow	Mid-shelf			
S	0.96	2.49	11.09	39.69	39.69
RCK	3.04	2.28	5.63	20.13	59.82
LC	1.04	1.60	4.21	15.05	74.87
MA	0.74	1.09	4.06	14.52	89.39
OT	0.81	0.82	1.53	5.48	94.87
Shallow vs. Deep zone					
R value = 0.741; Level of significance (%) = 0.1; Average dissimilarity= 42.75					
Benthic component	Average abundance		Average dissimilarity	% contribution	Cum %
	Shallow	Deep			
S	0.96	2.44	12.73	29.77	29.77
RCK	3.04	1.77	10.12	23.67	53.44
WC	0	1.09	7.2	16.85	70.29
MA	0.74	0	5.31	12.42	82.71
OT	0.81	0.63	4.28	10.02	92.73
Mid-shelf vs. Deep zone					
R value = 0.543; Level of significance (%) = 0.2; Average dissimilarity= 32.34					
Benthic component	Average abundance		Average dissimilarity	% contribution	Cum %
	Mid-shelf	Deep			
MA	1.09	0	7.14	22.08	22.08
WC	0.22	1.09	6.76	20.9	42.98
RCK	2.28	1.77	6.72	20.77	63.75
S	2.49	2.44	4.36	13.48	77.23
LC	1.6	1.1	3.77	11.66	88.89
OT	0.82	0.63	3.59	11.11	100

are uneven in their distribution, as the maximum live coral cover was observed in the mid-shelf zone. There is no continuous reef-like structure and dense patches of corals were found in very few locations around Grande Island. Variation in the spatial distribution of corals is largely determined by the settlement choice of coral larvae, availability of hard substratum and other physic–chemical parameters (Richmond, 1997).

Various physical factors such as light, sedimentation, salinity and substratum availability can be attributed to the variation in the taxonomic composition of corals between different depth zones in Grande Island. In general, distribution of corals in a particular depth zone largely depends on their substrate (Baird et al., 2003) and light preferences (Mundy and Babcock, 1998). Changing with its intensity and spectral quality with depth (Falkowski et al., 1993), light is an important environmental factor that determines the optimal depth range for the hermatypic corals. In Grande Island, live coral cover and coral density was low in the shallow zone despite the maximum availability of light and hard substratum. However it was high in the mid-shelf zone despite low light availability and low hard substratum cover. This might be due to the photosensitive nature of the coral larvae. Various studies reported the photosensitive response of coral larvae (Gleason and Hofmann, 2011) and experimental evidence also indicate that the optimal light regime for the settlement and metamorphosis of coral larvae could be species specific (Mundy and Babcock, 1998; Mason and Cohen, 2012). A photo-positive larva, sensitive to the changes in the light intensity can revert to a photo-negative one and tend to aggregate in the deep zone.

Live corals mostly occurred as small patches on the vertical substrate of the rocks in the shallow zone of Grande Island. This can be attributed to the competition posed by turf algae, macroalgae and zoanthids for space. Other factors such as excessive sedimentation, increased temperature and reduced

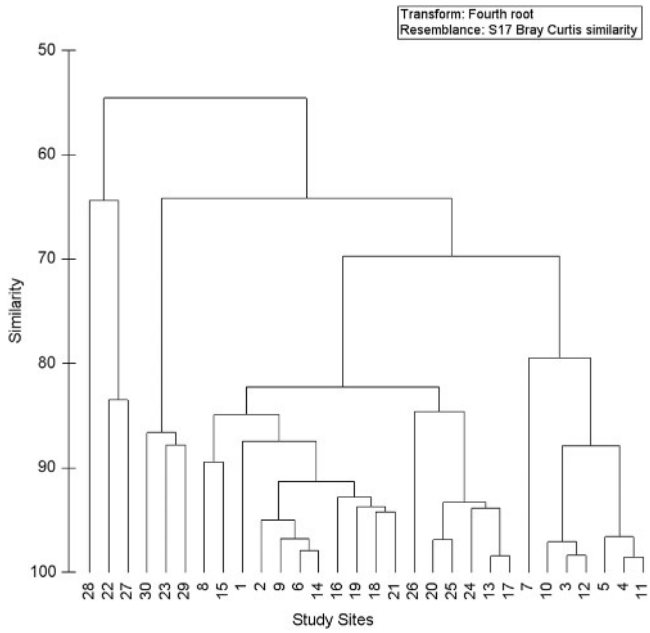

Fig. 6. Bray–Curtis cluster analysis of the study sites around Grande Island. Shallow zone (1–15); Mid-shelf zone (16–25); Deep zone (26–30).

salinity also affect the settlement and behaviour of coral larvae (Thorson, 1964; Richmond, 1996). There are no reports on the physicochemical nature of the waters of Grande Island. However, the coastal waters of Goa have been reported to experience low salinity during the southwest (SW) monsoon (Qasim, 1982; Sarma et al., 2001). The elevated topography of the Grande Island and the immediate discharge of rain runoff waters in to the shallow

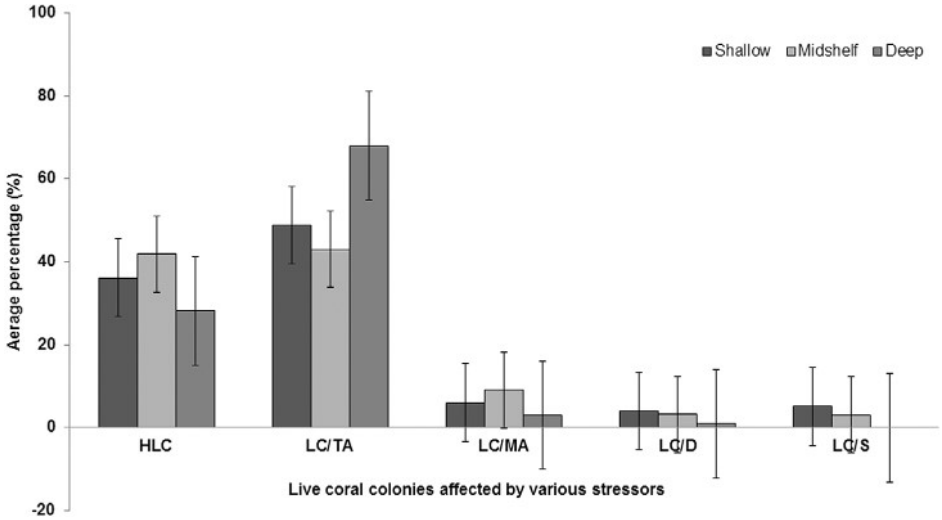

Fig. 7. Average percent composition of live corals existing in different states across different depth zones in Grande Island. HLC—Healthy live coral; LC/TA—Live coral colony affected by algal turf smothering; LC/MA—Live coral colony affected by Macroalgae overgrowth; LC/D—Live coral colony infested with disease; LC/S—Live coral colony affected by sponge intrusion. Error bars indicate standard error.

Fig. 8. Figure plates showing corals affected by Trematodiosis (8a—*Plesiastrea* sp) and White plaque disease (8b—*Pseudosiderastrea* sp; 8c—*Coscinarea* sp).

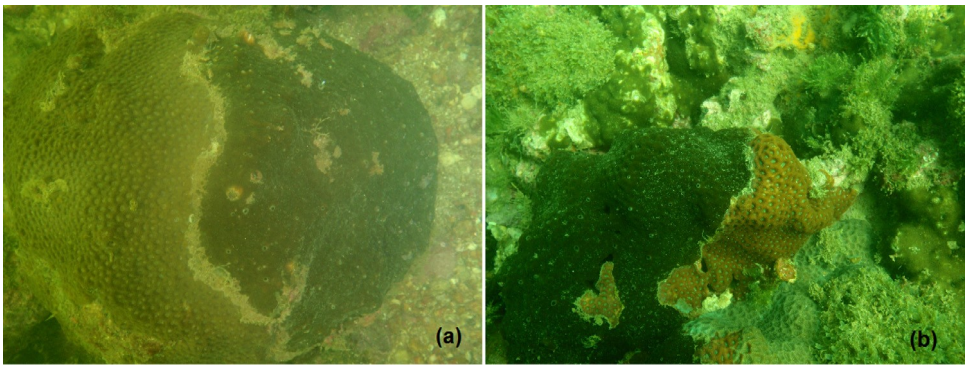

Fig. 9. Live corals of *Pseudosiderastrea* sp (9a) and *Favites* sp (9b) encrusted by sponges.

zone during SW monsoon will further reduce the salinity of the waters around Grande Island. The coral density and its species richness have been reported to be low under conditions of reduced salinity (Lirman et al., 2003). The immediate discharge of runoff waters from the Grande Island also increase the sediment load in the shallow zone which could smother the corals and hinder the light available for performing photosynthesis. Corals in the Grande Island are affected by high sedimentation as evident from the deposition of thick layer of sediment over the coral colonies. Excessive sedimentation can disrupt the ability of the coral larvae to settle and survive and also tends the larvae to settle in a vertical substrate (Rogers, 1990).

Analysis of the health status of live coral colonies revealed that the corals are under stress mainly due to algal turf smothering that in turn lead to the deposition of a fine layer of silt and sediment. In general, algal turf colonizes the corals under the conditions of nutrient enrichment and reduced herbivory (Smith et al., 2001; Gorgula and Connell, 2004). Once established, the turf algae can overgrow corals rapidly reducing their photochemical efficiency (Vermeij et al., 2010), reproductive output (Birrell et al., 2005) and their recovery potential after a stress event (Ravindran et al., 2012). Certain corals like *Porites* sp are reported to be successful against turf algae under the influence of nutrient enrichment (McCook, 2001). However, the response of a particular coral species cannot be generalized to the other coral species.

The boring and excavating sponges can affect the corals in a variety of ways leading to their mortality and permanent loss. Sponges that encrust the carbonate substrate compete with live corals for space (Glynn, 1997; Rützler, 2002). In Grande Island, the boring and excavating sponges spread as a thin mat over the live corals and covers them completely. The coral polyps lying adjacent to the vicinity of sponges were dead and covered with a thick layer of sediment and turf algae. Though the average proportion of coral colonies colonized by sponges appeared to be low compared to the colonies affected by other stressors, it needs much attention. Once established, these sponges can occupy the available substratum aggressively dominating the corals and displace the live corals tissue (López-Victoria and Zea, 2005). Recent studies have shown that the sponges secrete toxic metabolites that kill the live coral tissue (Chaves-Fonnegra et al., 2008). Colonization of sponges has been reported to occur more commonly on the reefs with high coral mortality (López-Victoria and Zea, 2005). However, the scenario was different in Grande Island as the sponges were observed to colonize the healthy live corals. In another way, physical contact between a coral fragment colonized by sponge and a healthy coral, induced by an external force like storm also paves the way for massive sponge colonization over corals in a reef (López-Victoria and Zea, 2004).

The presence of corals and its associated fishes made Grande Island, a tourism hot spot in Goa. Various recreational activities like SCUBA diving, snorkeling, and other water sports activities were practised in Grande Island. Intensive tourism is often associated with physical damage to the corals (Zakai and Chadwick-Furman, 2002). However, no physical damage was observed among the corals in Grande Island. Intensive tourism activities in coral reef areas also found to elevate the prevalence of diseases among corals (Lamb and Willis, 2011; Lamb et al., 2014). In Grande Island, white plaque and trematodiosis disease were prevalent among the corals. The link between tourism activities and prevalence of these diseases among corals in Grande Island needs further investigation.

In summary, the distribution of coral communities around Grande Island differs considerably between different depth zones, with high proportion of live coral cover in the mid-shelf zone followed by the shallow and deep zones. Though numerous biotic and abiotic factors would act synergistically in shaping the community structure of corals and their distribution in Grande Island, the relationship between them is unclear. Such factors include light and substratum availability, sedimentation, level of tourism activities and pollution, monsoonal effects and biological competition. Future research should focus on the physic-chemical and biological processes around Grande Island that determine the patterns of distribution of coral communities. Understanding the above processes around the Grande Island will reveal more about the distribution and colonization of coral communities and their vulnerability to change in the face of global warming and its associated impacts in future.

Acknowledgements

The authors thank the Director, CSIR-National Institute of Oceanography for the facilities and support. The first author thanks the Department of Science and Technology, Government of India for the award of INSPIRE fellowship (Grant No: IF110663).

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