CHAPTER I

GENERAL INTRODUCTION

Aquatic environment is broadly divided into two major domains namely, pelagic and benthic realm. Pelagic zone includes the entire ocean water column and mainly consists of phytoplankton (microscopic organisms constituting the food base for marine food chain); zooplankton (microscopic animals rely on water motion for transport) and nekton (animals who are free swimmer mainly dominated by decapods, fishes, mollusks etc.). Benthic environment is the most valuable habitat on earth that support high faunal diversity and play key ecosystem services. Benthos are small, size-based assemblages of organisms that live on or within the sediment. Based on size range, benthic domain are classified as: macrobenthos (> 500 µm; e.g., polychaete worms, crustaceans, gastropods etc.), meiobenthos (63 to 500 µm) and microbenthos (< 63 µm; e.g., bacteria, blue-green algae, diatoms, dinoflagellates, yeast, fungi etc.) (Giere, 2009). In 1942, Moly F. Mare first coined the word 'meiobenthos' (or 'meiofauna'), defining an assemblage of microscopically small sized aquatic invertebrates, distinguished from macro and microbenthos. Although the lower limit of their size boundaries is standardized as 63 µm howbeit International Association of Meiobenthologists (IAM) defines their size range from 45 to 500 µm. Some deep-sea meiobenthologists, on the other hand, use 31 µm as lower limit in order to get even smallest animals, especially nematodes (SCOR Working Group 76, 1994). Out of 34 multicellular phyla from animal kingdom 20 phyla are represented as meiobenthic organisms along with one important group of unicellular organisms, foraminifera (Higgins and Thiel, 1988; Giere, 2009). Free-living nematodes (phylum Nematoda) are typically the abundant component of meiobenthos followed by harpacticoid copepods (phylum Arthropoda). Apart from these, kinorhynchs, ostracods, turbellaria, tardigrades, gastrotrichs, loricifera and foraminifera are exclusive meiofaunal representatives and juveniles of macrofauna (mostly polychaete worms, bivalves and molluscs) are also considered as temporary meiofauna (Figure 1).

Meiobenthos serve as excellent candidate to perform a range of ecosystem services. Due to their mobility through the sediment (bioturbation), they maintain reworking of active sediment particles (Cullen, 1973) and generate vertical conveyors within the sediments (Coull, 1999), which modify sediment hydrodynamics, enhancing oxygen and carbon di-oxide diffusion and altering biogeochemical fluxes. Alkemade *et al*., (1992) demonstrated how increasing bioturbation activities by free-living nematodes had a triggering effect on sediment oxygen availability, which, on the other hand, stimulated associated microbial activities. Many meiobenthic animals, especially free-living nematodes are believed to secrete extracellular polymeric substances (EPS), which might have a significant impact on stability of surficial sediment (Chandler and Fleeger, 1984; Nehring, 1993). Additionally, meiobenthos have a potential impact on the structure and function of microbial communities. EPS, being a rich source of polysaccharides, are known to quickly consumed by bacterial colony, thereby stimulating the growth of bacterial population and directly or indirectly enhance organic matter mineralization (Nascimento *et al*., 2012). Moreover, they may break down detrital particles mechanically so that it could be more easily accessible to bacterial communities for degradation (Coull, 1973; 1999).

Meiobenthos also contribute to the diet of some predators, in particular, juvenile of benthivorous fishes (Gee, 1989; Coull, 1990) and some bottom-feeding shore birds (migratory sand pipers, flamingos, small waders and ducks) (Sutherland *et al*., 2000; Lee and Mayorga-Dussarrat, 2016). Several studies have reported that a number of demersal fishes like solenette, goby, scaldfish, dab etc. were reliant on meiofaunal prey throughout the year (Schückel *et al*., 2013; Carpentier *et al*., 2014). The authors also have shown that the recruitment, growth and survivability of these fishable stocks are dependent on meiofaunal densities. Other predators include many macroinvertebrates such as, polychaetes, amphipods, shrimps, crabs, echinoderms etc. Early stages of brown shrimps and shore crabs were revealed to feed upon preferentially harpacticoid copepods and ostracods (Pihl and Rosenberg, 1984; Gee, 1987, 1989). However, in this way they play an integral role in food web, by providing a short term compelling energy budget to higher trophic level and are used as important sentinels of fishery potential pertaining to a particular area. They utilize a wide array of food resources including microphytobenthos (epiphytic and epipelic diatoms, cyanobacteria etc.), bacteria, detrital particles, dissolved organic matter and other meiobenthic animals (Moens and Vincx, 1997; Coull, 1999; Moens *et al*., 1999). As a consequence, these tiny creatures acquire a variety of feeding mechanisms such as deposit and suspension feeding, grazing, scavenging, cuticular absorption and predation (Feller and Warwick, 1988). Variations in mouth morphology, in addition, are also attributed for their food selection and trophic niche partitioning.

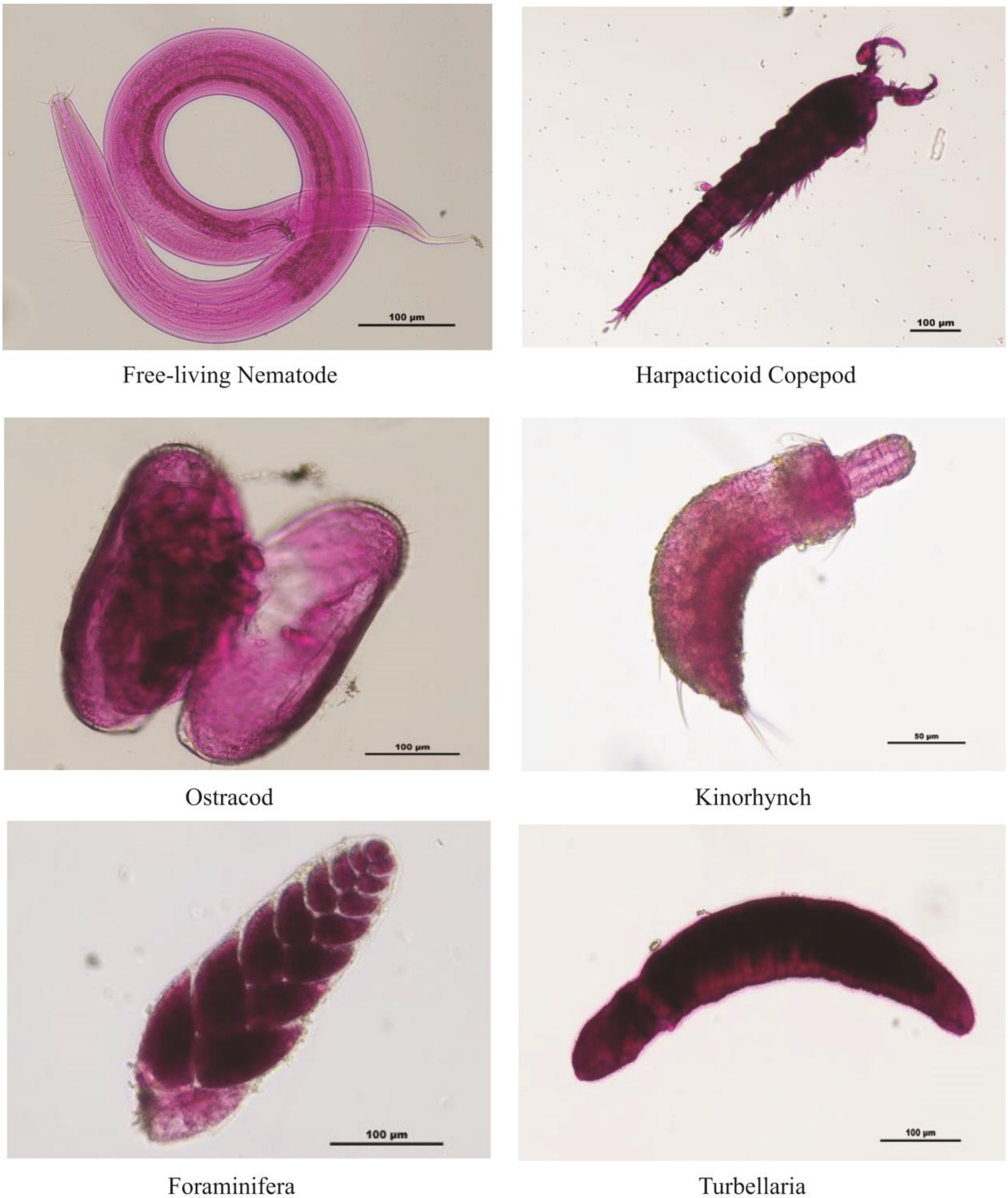

Figure 1: Representative meiobenthic taxa.

In recent times, meiobenthos emerge as promising auxiliaries for the assessment of ecosystem health. A number of scientific literatures have been demonstrated their role in detecting anthropogenic perturbations (Schratzberger *et al*., 2002; Moreno *et al*., 2008a, b; 2011; Balsamo *et al*., 2012; Irizuki *et al*., 2015). Many undisputable ecological advantages make them very good descriptors in pollution studies: high abundance and taxonomic diversity within a small area, rapid growth and short generation time, lack of planktonic larval phases in their life cycles and limited mobility; as a result, they are consistently exposed to contamination (Coull and Chandler, 1992; Giere, 2009). Within meiobenthic groups, harpacticoids, ostracods, tardigrades and foraminifera have been positively impacted by stressful environment, leaving nematode assemblages to be very tolerant or opportunistic organisms. They are often used as ecological indicators as they can provide information about their surrounding environment. A long term monitoring program using meiobenthic components could provide better and accurate information on the state of biodiversity and formulating conservation and mitigation tool for sustainable ecosystem development.

As most of the constituent species of meiobenthic communities have limited mobility, they are also potential contributors to ongoing climate change research. Benthic environment is a reservoir of biodiversity, thus any alteration or shift in their abundance would decipher useful information on benthic ecosystem functioning. Increasing amount of human activities has profound potential consequences on marine ecosystem with change in sea level, water temperature and pH, eutrophication, oil pollution, chemical contamination and hypoxia. A number of experimental studies, both *in situ* and *ex situ*, with meiobenthic counterparts have shown significant alteration in their communities and diversities. Furthermore, different ecosystem modeling approaches are being used to forecast the possible putative effect of climate change using meiobenthic fauna as proxies (Wernberg *et al*., 2011).

Meiobenthos have ubiquitous distribution from highest water mark to deep oceanic ridge (Danovaro *et al*., 2002; Vanhove *et al*., 2004; Gaever *et al*., 2009; Wang *et al*., 2019); from coastal lagoon (Doulgeraki *et al*., 2006) to fresh water lake (Anderson and De Henau, 1980; Rundle *et al*., 2000; Majdi *et al*., 2017) through estuaries (Hodda and Nicholas, 1985; Nozais *et al*., 2005; Alves *et al*., 2009) up to polar ice (Vanhove *et al*., 2000; Zeppilli and Luduc, 2018). Most of the studies have shown that meiobenthic populations are distributed in aggregations, developing patches on the sea floor. The community structure of meiobenthic invertebrates is determined by several interacting factors. Biotic factors (oxygen, food and nutrients supply, predation, competition etc.) and abiotic factors (temperature, pH, salinity, redox potential, water flow, sediment characteristics etc.) are important drivers for their horizontal and vertical distribution (McIntyre, 1969). A complex interplay among different factors enhances habitat patchiness at different spatial scales. Other factors such as depth of water column and tidal velocity have also been examined as influencing factors for their distribution pattern by many researchers (Fleeger *et al*., 1984; Fegley, 1987). Habitat heterogeneity is considered as one the valuable characteristics in modifying sea floor biota along with an array of physico-chemical dynamics as multiple factors over different geographical regions (Snelgrove *et al*., 1997). Sediment particle size, oxygen penetration, organic matter deposition throughout the sediment can shape vertical distribution of meiobenthos (Modig and Ólafsson, 1998; Majdi *et al*., 2017). In finer sediments, more than 90% of fauna are confined within upper few centimeters to millimeters (Coull and Bell, 1979) of sediment; while in coarse-grained sediment they are abundant in deeper layer, even up to 50 cm depth (McLachlan, 1978) due to high porosity of sediment, which helps in easy transportation of food and oxygen. Their densities show patchiness in distribution with some hundreds to thousands of individuals 10 cm^{-2} sediment surfaces (Findlay, 1981). In benthic environment, they occupied a diverse array of habitats; apart from sandy and muddy sediments they inhabit on various aquatic vegetation like sea grasses and algae as well as different animal structures such as worm tubes, crab burrows, coral crevices, echinoderm spines and sea turtles carapaces (Dittmann, 1996; dos Santos *et al*., 2018). They also exhibit facultative associations with ciliate protozoans (epibionts), known as epibiosis (Fisher, 2003; Dovgal *et al*., 2009; Fernandez-Leborans *et al*., 2012; Padmakumar *et al*., 2014; Ghosh and Mandal, 2019a).

Review of Literature

Zoological investigation and taxonomic description of meiobenthic animals were first introduced in the middle of 19th century with the inventory of Kinorhyncha by Dujardin (1851). With the development of different sampling instruments and methods, for example, grab (Petersen, 1913) and dredge (Mortensen, 1925), studies on abundance and community structure reported from different part of the world. In the history of meiobenthic literature, Swedmark (1964) and Mclntyre (1969) unmasked their ecological importance for the first time. Soft-bottom meiobenthos was first investigated by Coull (1970), which gave valuable insights into their characteristics using field experimental methods. In 1980s, a series of investigations started on various aspects like; meiofauna-macrofauna interaction (Bell, 1980), marine environmental quality assessment using meiobenthos (Heip, 1980; Austen and Warwick, 1989a; Warwick, 1989), organic matter uptake by them and their relationships with bacteria (Reil and Faubel, 1980; Tietjen, 1980;

Alongi, 1985), distribution and dispersal of meiofauna (Arlt *et al.*, 1982; Palmer, 1988). In 1990s, meiofaunal research gained popularity and major efforts were taken into consideration to acquire baseline information on their community structure from different geographical areas (Shirayama and Ohta, 1990; Huys *et al.*, 1992; Vanaverbeke *et al.*, 1997; Vanhove *et al.*, 1997). Modern statistical approaches were started to demonstrate responses of meiobenthos to varying environmental conditions (Somerfield and Clarke, 1995; Schratzberger and Warwick, 1999a; De Troch *et al.*, 2001). Considerable research interests have aroused to understand the relationship between meiofaunal diversity with a stable and resilient ecosystem through direct consequences on global biogeochemical cycles (Covich *et al.*, 2004; Leduc *et al.*, 2013; Pusceddu *et al.*, 2014).

Increasing human activities, presently, contribute to depauperate ecosystems, with potential consequences on their essential services. Natural as well as man-made activities may create stressful conditions, including, acidification, rises in temperature and deoxygenation. Discovery of well-adapted meiobenthic animals, particularly foraminifera, nematodes and copepods have provided a new insight into their behavioral, ecological and physiological adaptations in changing environment (Danovaro *et al.*, 2010; Fontaneto *et al.*, 2015) and can sheds light on evolutionary pathways and phylogenetic relationships. A number of experimental evidences on ocean warming and acidification have revealed negative consequences on meiobenthos, including mortality of dominant species, which in turn might have severe consequences for benthic food web and altered biogeochemical cycles (Danovaro *et al.*, 2004b; Widdicombe *et al.*, 2009; Gingold *et al.*, 2013; McIntyre-Wressnig *et al.*, 2013). Temperature-induced stress strongly impacted fecundity, development time and reproductive mode of certain nematode species (Warwick, 1981; Moens and Vincx, 2000). Other anthropogenic disturbances such as heavy metal, hydrocarbon and thermal pollution from nuclear power plant have also marked influence on meiobenthic abundance and species diversity (Mahmoudi *et al.*, 2005; Ruiz *et al.*, 2005; Arieli *et al.*, 2011; Kang *et al.*, 2014). However, a growing body of research articles unequivocally considered meiobenthos a dynamic component of the marine environment and an integral part of benthic ecosystem. Nevertheless, their taxonomic identification still remains a challenging task due to their small size and lack of expertise. Howbeit, illustrated online identification keys and development of rapid molecular approaches help to strengthen traditional taxonomy.

Ecological research on meiobenthos of the Indian coasts started in the early nineties with the work of Anandale (1907), who paved the way for benthos research from West Bengal Gangetic delta region. Later on, preliminary observations on benthic fauna were carried out from Chennai coast (Panikkar and Aiyar, 1937; Samuel, 1944) and northeast coast (Ganapati and Rao, 1959) as well as from west coast of India (Kurien, 1953; Seshappa, 1953; Thiel, 1966; Kurien, 1967; McIntyre, 1968; Sanders, 1968; Kurien, 1972). First comprehensive study was conducted by Damodaran (1973) emphasizing seasonal variations, interlinking with prawn fishery from Kerala coast and quantitative efforts were made in relation with environmental factors from Goa (Parulekar *et al.*, 1976). Extensive surveys from a lagoonal ecosystem of Chillika along with Orissa coast were carried out on meiobenthic distribution (Sarma and Rao, 1980; Sarma *et al.*, 1981; Rao, 1987a, 1989). By using multivariate analyses approach Harkantra and Parulekar (1989) revealed that the community structure of meiobenthic assemblages were controlled by numerous ecological factors (dissolved oxygen, salinity, sediment grain size etc.). During 1990s, major efforts were taken into accounts towards various ecological aspects including effects of pollution on meiobenthic communities (Ansari and Parulekar, 1993; Ansari *et al.*, 1996; Ansari and Gauns, 1996; Ansari and Parulekar, 1998; Ingole and Parulekar, 1998). In the progress of 21st century, substantial research efforts have been added to the meiobenthology with the emerging advances of technologies and modern statistics. The increasing use of meiobenthic organisms have rapidly gained popularity in the field of taxonomy, diversity and seasonal dynamics to assess their role in benthic ecosystem functioning (Ingole and Goltekar, 2004; Chinnadurai and Fernando, 2007a; Nanajkar and Ingole, 2007; Sajan and Damodaran, 2007; Nanajkar and Ingole, 2010; Sajan *et al.*, 2010; Annapurna *et al.*, 2012; Ansari *et al.*, 2013, 2015; Sen *et al.*, 2016; Singh and Ingole, 2016; Ghosh *et al.*, 2018). Observations on the meiobenthos in the Andaman and Nicobar Islands as well as Lakhshadeep were paid attention by Rao (1980), Ansari and Parulekar (1981), Rao (1986, 1987b), Wells and Rao (1987) and Rao (1988, 1991). Vertical profiling of meiofauna have also been analyzed which suggests that more than 80% of fauna inhabit upper 0-4 cm of sediment (Sahoo *et al.*, 2013; Ghosh and Mandal, 2019b). Mesocosm experiments have become a major trend to observe community structure and physiological response as well within controlled environmental conditions. Laboratory culture experiments of marine nematode and benthic foraminiferal species have been started to understand reproductive behavior and to examine their responses upon long-term exposure of multiple stressors (Saraswat *et al*., 2004; Nigam *et al*., 2008, 2009; Singh *et al*., 2009; Saraswat *et al*., 2015; Ghosh and Mandal, 2021).

Regardless the efforts being taken in the research of meiobenthology, benthic studies suffer persistently from dearth of information on species diversity due to very time consuming morphological identification associated with rare taxonomical skill (Derycke *et al*., 2005; Fonseca *et al*., 2008). In parallel, many organisms encountered during microscopic analyses are in juvenile stages, thereby rendering their identification very difficult as some superficial characteristics are observed merely in adults. The innovations of DNA sequence based techniques holds promising aspects to overcome such limitations, especially in the field of marine nematode communities. However, such taxonomic intractability has lead marine ecologists to develop molecular tools (DNA sequencing and PCR clone library) elucidating nematode inventories worldwide (Bhadury *et al*., 2006, 2008; Derycke *et al*., 2010; Neres *et al*., 2010; Kumar *et al*., 2015).

Free-living nematodes are the most numerically dominant and species-rich meiofaunal component of aquatic sediments with ubiquitous distribution inhabiting all climatic conditions and can exceed their densities up to million individuals m^{-2} (Heip, 1980; Platt and Warwick, 1980; Lambshead *et al*., 2003; Balsamo *et al*., 2010). They present several advantages for considering ideal model organisms as elegant indicators of ecosystem health (Schratzberger *et al*., 2000, 2006a; Alves *et al*., 2013). Nevertheless, little is known on how such functional characteristics depend on their species composition and diversity, especially in tropical regions. In India, a copious amount of research papers have dealt with quantitative aspects of meiofauna, however, systematic databases on aquatic nematodes are insufficient and inadequate. An updated taxonomic list of nematode species from Indian coasts based on previous literature has been compiled by Ghosh and Mandal (2018). To our knowledge, data sets on marine nematode communities have sporadically been collected from different mangrove dominated estuarine sediment of Indian coasts (Ansari and Parulekar, 1993; Chinnadurai and Fernando, 2006a, 2007a; Anila Kumary, 2008; Baliarsingh *et al*., 2015).

Estuaries are the most hydrodynamically active and prevalent transitional zone, where indispensable biogeochemical processes take place (Costanza *et al*., 1997; Bauer *et al*., 2013). They are regarded as vital conduits for the transport of sediments, nutrients and organic materials from rivers to oceans making them an essential carbon sink. The amenities estuaries offer, render them as the most precious national treasures; which may impinge on the health and vibrancy of human society and economy. Due to their important ecological functions, such as high productivity, nutrient enrichment, ephemeral habitats for reproduction, feeding and nurseries for a variety of animals (Beck *et al*., 2001; Gili, 2002), interactions between the environment of this zone and human societies are very pronounced. Hence, estuarine resource management has become imperative for nutritional, environmental and economic reasons. In estuarine sediment, meiobenthos contribute in energy flow by serving as food material to a variety of predators and facilitating the biomineralization of nutrient regeneration (Coull, 1999). These naturally stressed ecosystems are characterized by high degree of physico-chemical variabilities, which might play a pivotal role in shaping heterogeneous distribution of meiobenthos (Adão *et al*., 2009; Alves *et al*., 2009; Giere, 2009). As yet, monitoring the status of estuarine meiobenthic components and their key ecosystem functions from Sundarbans Estuarine System (SES) is still remain discrete and limited to selected sites, which are not sufficient to exhaustively ensure ecological goals of sustainability. The major hindrance to speed up the process of evaluating the ecological status is the difficulty to capitalize on existing data, which are often fragmented in meiofuanal scientific literatures (Rao and Mishra, 1983; Sinha *et al*., 1987; Dey *et al*., 2012; Sen *et al*., 2016). Hence, formulation of a systematic and reliable database for estuarine meiobenthic fauna, focusing on nematode species composition, from fragile and ecologically important SES is need of the hour in the milieu of biodiversity loss and conservation.

Study Area

Sundarbans Estuarine System (SES), the largest contiguous deltaic mangrove ecosystem of the world, is situated on the delta created by successive sediment accumulation of Ganges, Meghna and Bhramaputra rivers covering coastal belt of India and Bangladesh (UNEP-WCMC 1987; Papa *et al*., 2010). This tropical coastal estuary is situated between 21˚40'04"N - 22˚09'21"N latitude and 88˚01'56"E - 89˚06'01"E longitude and inscribed as UNESCO world heritage site for its rich biodiversity. On its west, the estuary is bordered by Bhagirathi-Hooghly River, Raimangal River in the east, an imaginary Dampier–Hodges line as northern limit and Bay of Bengal (BoB) as southern boundary. This coastal ecosystem with its lush mangroves is sculptured by numerous rivers and several tidal-fed rivulets, creeks and canals forming a sprawling archipelago with hundreds of islands. The major estuaries of SES lying from west to east are the

Hooghly, Muriganga, Saptamukhi, Thakuran, Matla, Bidya, Gosaba, Gona, Haribhanga and Raimangal. Except Hooghly and Raimangal, remaining rivers are saline and tidally influenced rivers. The inward freshwater flow of the rivers and the tidal inundation result in salinity gradient that varies both spatially and seasonally. Generally, salinity decreases from west (polyhaline) to eastern part (oligohaline) the estuary. The SES is the largest macro-tidal and monsoonal estuarine system in India with average amplitude of tide ranging between 2 - 5 m. The mean temperature of this tropical mangrove system ranges from 11 to 34°C with humid air from BoB carrying 70 to 88 % humidity. SES experienced considerable precipitation in summer monsoon rainfall, ranging approximately from 1500 to 2500 mm $year^{-1}$ (Attri and Tyagi, 2010). SES is also prone to seasonal cyclonic events, mainly frequent during pre-monsoon (March - May) period, which are another sources of freshwater flow during dry season (Gopal and Chauhan, 2006). The elevation of Sundarbans delta ranges from 0.9 - 2.1 m above sea level (Allison *et al*., 2003). The ubiquitous mangrove vegetation is the most prominent feature of this landscape. SES comprised approximately 85% of total mangrove habitats found in India, among which 63-69 species coexist together in this delta (Kathiresan and Bingham, 2001). These mangroves perform a plethora of ecosystem services. The release of huge amount of litter by mangrove swamps contributes to enrich sediment nutrients, which support fisheries for commercially important species and sustains the marine fisheries of northern BoB (Mandal *et al*., 2012). Mangrove invaluably acts as nature's shield against natural disasters (cyclones and storm surges) and spawning-nursery ground, migratory routes and refuge areas for a variety of marine animals. Different hydrological regimes such as freshwater input and tidal ingress, topography and salinity result in habitat heterogeneity and thereby ensure a rich floristic and faunistic diversity. Over time, commercial exploitation of mangrove vegetation have led to habitat contraction leading to extirpations of many mangrove associates. Therefore, the knowledge of organisms inhabiting this environment along with their ecological interactions is a pre-requisite for planning and ecosystem management.

There is relatively little reliable information on the status of benthic population in Indian Sundarbans in terms of distribution and ecology due to a dearth of proper taxonomic literature from tropical water as well as lack of trained taxonomists. In this study, we explore for the first time the structure, biodiversity and functional traits of the meiobenthic and nematode assemblages inhabiting the bottom of Sundarbans.