Studies on the inter-tidal rocky shore crabs (Decapoda: Crustacea: Brachyura) from Goa, West coast of India

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by

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II *Shri Ganesh* **II**

Dedicated to

My Family for their love

and endless support

Statement

As required by the University ordinance 0.19.8 (vi), I state that the present thesis entitled "***Studies on the inter-tidal rocky shore crabs (Decapoda: Crustacea: Brachyura) from Goa, West coast of India***" is my original contribution and the same has not been submitted on any previous occasion. To the best of my knowledge the present study is the first comprehensive work of its kind from the area mentioned.

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

J. Vijaylaxmi

Certificate

This is to certify that the thesis entitled "***Studies on the inter-tidal rocky shore crabs (Decapoda: Crustacea: Brachyura) from Goa, West coast of India***", submitted by **Miss. J. Vijaylaxmi** for the award of Doctor of Philosophy in School of Earth, Ocean and Atmospheric Sciences is based on her original studies carried out by her under my supervision. The thesis or any part thereof has not been previously submitted for any degree or diploma in any Universities or Institutions.

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Chapter 1

General Introduction

1.1. Background information

The coastal environment endowed with habitat heterogeneity support rich and diverse life forms (Venkatraman and Wafer, 2005). Among these, rocky shore comprises of sub-tidal and intertidal zones, and the latter is a transition zone between the sea and the terrestrial habitats, which is subjected to alternate tidal inundation. This rocky intertidal region is significant site for investigation due to its diverse species composition and ecosystem processes. These intertidal rocky shore habitats are biologically rich ecosystems owing to diverse niches those offer shelter to wide array of organisms that are well adapted to extreme tidal fluctuations (Nybakken and Bertness, 2005). The intertidal rocky areas due to its zonation patterns influencing morphology and colour play vital role in diverse fauna, subjected to interest in ecologists (Coleman, 1933; Connell, 1972; Underwood and Denley, 1984). Larval settlement, predation and competition, food availability, wave stress and dehydration in these habitats contribute to diverse species distribution. These complex and dynamic forces determine the diversity of the organisms in these environments through provision of quantity and variety of food (Newell, 1970; Reise, 1985; Peterson, 1991). The organisms inhibiting in such zones include sedentary animals such as barnacles, mussels, chitons and anemones as well as mobile organisms such as small fishes, crustaceans, echinoderms and gastropods (Nybakken and Bertness, 2005).

Among these, brachyuran crabs belonging to infraorder Brachyura are one of the most common and relatively diverse groups of organisms, which comprise of more than 7000 species worldwide, about 910 species of crabs from Indian waters and around 150 species are known to be commercially important (Suseelan, 1996; Ng *et al.,* 2008; Trivedi *et al.*, 2018). These crabs are known to have complex behavioural pattern in association with other invertebrates as they play significant role in maintenance of ecosystem by demonstrating the prey and predator role in marine food web and also enable regeneration of nutrients through

scavenging (Boudreau and Worm, 2012; Arya *et al.,* 2014), hence considered as one of the key organisms due to their rich diversity, besides ecological and economic importance. The crabs inhabiting rocky shores mainly belong to families Xanthidae, Oziidae, Pilumnidae, Portunidae, Grapsidae and Sesarmidae. They inhabit various niches such as rock crevices, underneath rocks, on moist sandy-muddy substratum and even tidal pools. Moreover, they exhibit diverse strategies for defense (Todd *et al.,* 2009), habitat prospecting (Cannicci *et al.,* 1998; Sivasothi, 2000) and social behavioral patterns (Latruffe *et al.,* 1999; Negreiros-Fransozo *et al.,* 2003). Their adaptability to intertidal fluctuations (Nybakken and Bertness, 2005), accumulation of metals (Szefer *et al.,* 1997) and PSP toxins (Koyama *et al.,* 1983) make them potential indicators of water and sediment quality.

Brachyuran crabs are ecologically important organisms for their abundance and species richness. They are known to be prominent in both biomass and species diversity (Bas *et al.,* 2009; Pandya *et al.,* 2010; De Lima *et al.,* 2014a). The assessment on spatial and temporal is essential for the better understanding of ecology of brachyuran crabs and their interaction with physical environment. On the other hand, species in the intertidal habitat experiences distinct environmental conditions and survival rates throughout their distribution range which generally influence their abundance (Ciannelli *et al.,* 2008; Triay-Portella, 2017). The distribution of crabs varies depending on various factors like type of substrate, habitat type and physical parameters effecting individual survival (Kareiva, 1990; Ciannelli *et al.,* 2007). However, the dynamics of a given community is influenced by various processes affecting survival and emaciated biological processes those determine community structure with regard to environmental anomaly. Hence, the understanding of ecological mechanisms those regulate species distribution and composition at different time is mandatory.

Population dynamics represent estimation of parameters, such as growth, fecundity and mortality that determines the patterns of population abundance and structure (Miller and

Smith, 2003). It is well documented that reproduction is the main mechanism to uphold and regulate species proliferation and continuity (Soundarapandian *et al.,* 2013). Therefore, in order to understand the population dynamics of crabs in natural ecosystems the study of reproductive biology with spawning season and fecundity is essential (Safaie *et al.,* 2013). Crustacean biology provides an insight across a wide array of biological problems due to their complex physiology with respect to growth, metabolism and reproduction resulting various patterns of life history and reproductive strategies. Published literature on the growth and reproduction in decapod crustaceans are described (Pillay and Nair, 1971; Somerton, 1980; Mantelatto and Fransozo, 1999; Leme, 2002; Brian *et al.,* 2006; Marochi *et al.,* 2013). The reproductive cycle in Decapoda may take place around a year or restricted for few months depending upon the seasonal and environmental factors as these abiotic factors work as metabolic or biochemical regulators and hormonal modulators which triggers the processes of mating and gonad development (Sastry, 1983; Batoy *et al.,* 1987). Reproduction in crabs is commonly associated with effort or the proportion of body energy partitioned for reproduction (Comparada & Biologicas, 1999).

Fecundity is the number of eggs produced by a female in an egg batch or during a period of its life cycle, is one of the important parameter which determines the reproductive potential in crab population, however a considerable variation in fecundity is observed among brachyuran crabs due to various factors such as age, size, nourishment, ecological conditions of the water body (Hines, 1988; Mantelatto & Fransozo, 1997; Przemyslaw & Marcello, 2013). Therefore, study of fecundity is essential in order to understand the potential of number of spawns a female can produce over life time. Brachyuran crabs have reproductive strategy for the survival of their offspring and also to maintain the population at adequate level (Hartnoll & Gould, 1988). Hence, study of reproductive aspects is essential to have a better understanding of its biology and population dynamics.

Phylogenetics is the study of relationships among the individual organisms and their evolutionary history. It can be used in both molecular and morphological data in order to classify organisms. However, large numbers of undiscovered species also need to be resolved. In this backdrop, the use of DNA has the potential to revitalize taxonomy (Moritz, 1994; Hebert *et al.,* 2003). Conventional taxonomy has its own limitations in identification of organisms which has been addressed sufficiently and suggested to be supplemented by a molecular based technique known as DNA barcoding or molecular taxonomy. The use of mitochondrial gene as a DNA barcode for species identification has strengthened the documentation of global diversity (Hebert *et al.,* 2003). Earlier, to understand the evolutionary aspects and phylogenetic relationships of diverse group of crustacean phylogeny, adult morphology was the only means to approach (Spears *et al.,* 1992). However, the applications of molecular techniques in modern study, the evaluation of morphological and phylogenetic relationships are carried out (Rice, 1980) to enable to provide better insight to taxonomic issues.

Brachyuran crabs are known to be extremely diverse morphologically and ecologically containing approximately 18,000 living and extinct species (De Grave *et al.,* 2009). Published literature (Xin *et al.,* 2018) suggests controversy regarding relationships among the major decapod taxa based on complete mitochondrial genome sequences. In phylogenetic studies, the molecular markers have become extremely important in recent times (Patwardhan *et al.,* 2014). However, due to the high morphological characteristics and diverse group the relationships of these brachyuran families are scanty (Tsang *et al.,* 2014). Hence, various attempts have been done to study the morphological, behavioural, and physiological diversity in decapods (Spears *et al.,* 1992; Hultgren and Stachowicz, 2008).

Studies on intertidal rocky communities have been intensely carried out due to their accessibility and richness of natural resources. Much of the studies conducted in such

ecosystems have been focused on sedentary communities, zonation pattern and associated ecological processes that determine their composition and abundance. The present study area along the west coast of India is marked with rocky promontories along northern parts, and is characterized by conglomeratic laterite rocks that are exposed to form wave-cut terraces in the intertidal zone (Wagle, 1993). In recent times, due to anthropogenic activities such as waste discharge, accelerated tourism activity, shipping, fishing activities, etc. have resulted significant alteration in coastal habitats.

Hence, the ecological studies of these organisms are important in restoring their populations through appropriate management strategies. The present scenario makes it pertinent to study crab communities and their environments in a holistic manner to have a better insight on their role in ecosystem function and response to anthropogenic pressure in view of the threat to their habitats. Therefore, a continuous assessment of the brachyuran community of the rock shores is mandatory to elucidate specific issues for management of these resources.

1.2. Literature review

The Indian coast and its adjoining shelf waters harbour diverse assemblage of crabs and the brachyuran crab of India has been studied since the mid of 1700s. Earlier the crab samples of Indian waters were collected through the ships from the deep sea. Initially, the collection was started by Linnaeus (1758) from Indian waters. Thereafter, Fabricius (1775, 1787, and 1792) was the next to record the brachyuran crabs while, several other species were described by Herbst (1782). Earlier works (Kemp, 1917, 1919; Chopra and Das, 1930, 1937) were mostly carried out in east coast of India. The studies on west coast of India (Chopra & Das, 1930, 1937; Pillai 1951; Chhapgar 1957a, b, 1958, 1969; Chhapgar *et al.,* 2004; Sankarankutty, 1961, 1966, 1975; Prasad and Neelakantan, 1989) made an attempt to provide

an insight on species identification and composition. The comprehensive work on the brachyuran fauna was carried out by Pillai (1951) from Travancore waters of Kerala coast and Chhapgar (1957a,b) studied the taxonomy of marine crabs and published a series of key articles of Bombay presidency state, presently covering the area of Gujarat, Maharashtra and the northern part of Karnataka. 13 species were recorded from the mangrove ecosystem of Uran, Maharashtra (Pawar, 2012). Haragi *et al.,* (2010) and Bandekar *et al.,* (2011) worked on the diversity of brachyuran crabs in sub-littoral zones and mangrove regions of Karwar estuary in Karnataka. The diversity on brachyuran crabs of Gujarat state was studied by Trivedi *et al.,* (2012) and reported 19 brachyuran crab species suggesting the dominancy of species based on habitat types from Gulf of Kutch. Trivedi and Vachhrajani (2012) from Saurashtra coast reported 67 species observed in four zones among which algal zone was diverse with highest number of species. Shukla *et al.,* (2013) from Mahi and Dhadar estuaries recorded 14 species of brachyuran crab and reveal mangrove as diverse habitat due to maximum diversity of species. Crab communities on the west coast of India were studied in detail by Dev Roy (2013), reporting 226 species and revealed highest diversity of crabs in Kerala coasts with 93 crab species.

Considerable work and factors responsible for the distribution of intertidal organisms and their habitat have been of major concern for the marine ecologist. The infraorder Brachyura which consists of 7000 species belonging to 93 families is one of the most species-rich decapod groups. Several publications have listed about 808 species in Indian coasts (Ng *et al.,* 2008; Dev Roy, 2013; Trivedi *et al.,* 2018). Dev Roy & Bhadra (2008) listed the occurrence of 82 species of brachyuran crabs providing a checklist of marine brachyuran crab fauna of Goa coast. Studies on the faunal checklist are of great importance for the conservation purpose as these lead to further aspect like the structure, for better understanding of ecological processes and the problem of the ecosystem.

Available literature (De Haan, 1833-1849; Edwards, 1834; Heller, 1868; Henderson, 1893) along Indian coast revealed that naturalists have described numerous species from this region. The most comprehensive study of brachyuran fauna has been undertaken by Alcock (1895-1900). He classified these crabs into five main groups namely Oxyrhyncha, Oxystomata, Cyclometopa, Dromiacea and Grapsoidea. Subsequently, several carcinologists including Borradaile (1903), Gravely (1927), Chopra (1935), Chopra and Das (1937), Sankarankutty (1961, 1962, 1966), Rao and Rath (2013), Balasubramanian *et al.,* (1998), Balasubramanian & Suseelan (2001), Dev Roy and Das (2000), Jeyabaskaran *et al.,* (2000), Ravichandran *et al.,* (2010), Varadharajan and Soundarapandian (2014) and Josileen *et al.,* (2018) undertook faunistic studies on a regional scale. It is apparent from the above literature that most studies have dealt only with the taxonomic aspects of brachyuran crabs, and recently, only few studies (Dineshbabu *et al.*, 2011; Josileen, 2011, 2013) have dealt with biological aspects of few commercially important crabs.

The taxonomy of the brachyuran crabs in Indian waters was carried out by most of the workers (Edwards, 1852; Alcock, 1899; De Man, 1888). Later the studies on other aspects like habitat structure, crab abundance, distribution, and environmental parameters and also larval development aspects in crabs were examined by several other carcinologists (Naidu, 1953; Balasubramanian, 1966; Dev Roy and Das, 2000; Ravichandran *et al.,* 2001; Josileen, 2001; Khan and Ravichandran, 2009; Trivedi and Vachhhrajani, 2012). Community structure of commercially important brachyuran crab species of Pondicherry coast with respect to season was studied by Satheeshkumar (2012). The study on diversity of crabs and their community structure was undertaken by Kumaralingam *et al.,* (2013) and reported 402 specimens of brachyuran crabs in North Andaman. Sen *et al.,* (2014) conducted studies pertaining with the effect of different habitat attributes on the crab diversity of Sundarban mangrove. Vartak *et al.,* (2018) studied the morphometric and phylogenetic relationship of

genus Portunus using the COI gene along Konkan coast of Maharashtra. However, few studies have been carried out on the commercially important crabs by Mandal *et al.,* (2014), Balasubramanian *et al.,* (2016), Ravichandran *et al.,* (2017).

The literature available on brachyuran crabs from different parts of the world reveals that initially most of the work was carried out on the taxonomic aspects by Linnaeus (1758), Fabricius (1798), De Haan (1833), Dana (1852a, b, c), Stimpson (1871), Boas (1880), Miers (1886), Ortmann (1892), Bouvier (1896), Borradile (1907), Balss (1957), Glaessner (1969), Guinot (1977, 1978), Barnwell & Thurman (1984), Apel & Spiridonov (1998). Crane (1975) focused mostly on the study of fiddler crabs. Stephenson (1972) with respect to the Indo-west Pacific region provided a checklist on the portunid crabs. A diverse nature of work was carried out on taxonomy of undescribed crabs species by Manning & Holthuis (1989), Zmarzly (1992), Manning (1993), Jakobsen & Collins (1997), Relini *et al.,* (2000), Ho *et al.,* (2004), Asakura & Watanabe (2005), Ng & Santos (2007), Shih *et al.,* (2010). Sakai (1976) provided the systematic of brachyuran crabs of Japan and Guinot (1977, 1978) based on the genital openings proposed a taxonomy classification of brachyuran crabs. Lucas (1980) and Davie (1982) dealt with Australian mangrove crabs species further a review on crabs of mangrove ecosystem was provided by Jones (1984) and Abele (1992). Tan and Ng (1994) carried out work based on mangrove habitats from the regions of Malaysia and Singapore and proposed the annotated checklist of brachyuran crabs. Later the annotated checklist of the brachyuran crabs of the world was provided by Ng *et al.,* (2008) which comprised of around 6,793 valid species. Crane (2015) proposed a systematic revision on fiddler crabs of the world based on phylogeny of the morphological comparisons also evidence from social behavior, biogeography and ecology.

The species of family Portunidae *Carupella banlaensis* Tien, 1969, a rare inshore crab species of genus *Carupella* (Crosnier, 1962; Zarenkov, 1970; Vannini & Innocenti, 2000)

was recorded from India. The genus is represented by three species, namely *Carupella banlaensis* (Tien, 1969), *C. epibranchialis* (Zarenkov, 1970), and *C. natalensis* (Lenz & Strunck, 1914). The species is distributed from the geographical range from China to eastern Africa.

The species *Dotilla myctiroides* (H. Milne Edwards, 1852) is small-sized crab inhabiting in sand with high percentage of silt clay fraction. This species is known to either occur throughout the shore or along the lower water line and in some cases it also occurs with another dotillid species namely *Copimera proxima* (Silas and Sankarankutty, 1967). Its intense burrowing and filter feeding activities regulate recycling of organic matter and nutrients in the ambient environment (Takagi *et al.,* 2010). This species is known to overcome the extreme environmental temperature by burrowing and igloo-construction that allows it to reach below the water level (Nguyen *et al.,* 2011). It is also capable of aerial respiration and controls osmoregulation through its tympanic membrane (Matsumasa *et al.,* 2001). The original description of this species given by Edwards, (1852) however lacked illustrations. Alcock (1900) provided a brief description of this species. Kemp (1915) reported *D. myctiroides* from Chilka Lake, east coast of India and described along with diagrams of male and female abdomens. Chhapgar (1957b) provided a short description and illustrated with diagrams of carapace and G1. Sankarankutty (1961) provided a short description of *D. myctiroides* complemented with diagrams of pterygostomial grooves and spoon-tipped setae of second maxillipeds. Vogel (1984) provided SEM photographs of spoon-tipped setae of second maxillipeds. Allen (2010) attempted a revision of the genus *Dotilla*, and provided a diagnostic description of *D. myctiroides* supported with diagrams of carapace of male abdomen, cheliped, pereiopod and G1.

It is important to understand the ecological variables influencing the abundance and distribution of crabs which are associated with spatial and temporal variation (Mantelatto and

Fransozo, 1999 and De Lima *et al.,* 2014a). Earlier Hartnoll (1963) noted size and maturity of individual male and female crabs. Later Hartnoll *et al.,* (1993) provided the factors determining the size and difference in the size distribution in matured crabs and the effect on the population structure. Various aspects like seasonal abundance, sex ratio, size composition, molting and spawning periods, individual growth rates, and migratory behavior was reported by Carroll (1982) from the coast of California. Dahdough-Guebas (1994) studied the feeding and behavioral ecology of few crab species from the Kenyan mangroves. The study on distribution patterns based on the range of salinity and the habitats of estuarine crab species was carried out by Sánchez & Raz-Guzman (1997) in the Gulf of Mexico. Flores & Paula (2001) and Flores *et al.,* (2002) provided species composition of marine intertidal brachyuran crabs along with the temporal and spatial variations with respect to the settlement of megalopa larvae from the rocky shore coast of Portugal. The comparison of brachyuran crab community was provided by Ashton *et al.,* (2003) from the coast of Malaysia and Thailand. It is imperative to note that the spatial and temporal variations play an important role to regulate distribution and abundance of these crabs. Lim *et al.,* (2005) examined the density and diversity of two species of fiddler crabs from the coast of Singapore and revealed that the species *Uca annulipes* preferred sandy habitats and *Uca vocans* are mud associated. Bezzera *et al.,* (2006) studied the factors affecting the spatial distribution and types of setae in the second maxilliped of fiddler crabs from the tropical mangroves of Brazil. The studies on various aspects like population structure, sex ratio, growth and reproduction of fiddler crab species *Uca annulipes* was reported by Mokhtari *et al.,* (2008) and revealed low production and mortality in the species. Xue-lei *et al.,* (2009) studied the larval settlement of mud crab species *Scylla paramamosain* from the coast of china and suggested the selection of substratum depends on the adaptation of flood tide transport mechanism. Aschenbroich *et al.,* (2016) studied the differences in crab assemblage in relation to microhabitat and sediment

characteristics in the young mangroves from French Guiana suggesting, the microhabitat which is formed by the biological sediment mainly depends on the age of the mangrove. The study also suggests that crabs abundance was observed high in young mangroves due to larval survival rates and juvenile recruitment areas. Nóbrega & Lemos (2016) reported 14 crab species in Marapanim estuary along Amazonian coast, and examined the variation in species composition with difference in habitats and proved salinity as important parameter for species composition.

The biological aspects of crab species are restricted however, the sex ratio, population density, size and breeding proportion influence the reproductive strategy and some of these effects have studied by ecologists. Hines (1982) examined the reproductive effort in 20 brachyuran crab species of North America using allometric relationships. Further, Hines (1992) studies tested the reproductive variables between the crab species of family Pinnotherid and other families depending on the space available for accumulation of yolk in the cephalothorax of females and suggested that Pinnotherids are able to produce larger broods than other crabs. González-Gurriarán & Freire (1985) examined the sexual maturity of crabs by assessing on molting of puberty based on the external morphological variation and the development in gonads. Fecundity and relative growth were studied by Carsen *et al.,* (1996) by using the CL of the crab as the reference dimension from the fishery areas of Argentina. Further, Lee (2004) studied the factors determining the post larvae settlement and examined the habitats of juvenile in swimming crab species *Necora puber* based on different substrate types which depends on the hydrodynamic conditions and also suggested that the distribution of megalopa was low in abundance during the day. Castiglioni *et al.,* (2004) described some reproductive aspects of species *Armases rubripes* in different mangrove habitats in Sao Paulo coast of Brazil. The study suggested males with larger size at maturity and the species showed high rate of fecundity in the region. While, De Lima *et al.,* (2014b)

studied the reproductive biology of species *Hepatus pudibundus* including sexual maturity and reproductive period along the Sao Paulo coast of Brazil. Lardies & Wehrtmann (1996) examined the aspect related to the reproductive biology by selecting the ovigerous females of porcellanid crabs and examined the egg production and chemical composition during incubation period. Further, similar studies were carried out by Elner & Beninger (1992) on the reproductive biology of snow crab which included reproductive systems of both sex interpreting the mating pathways and suggested the spermatophore storage in male crab and also delay in fertilization. Choy (1988) observed the moulting and copulation period of Portunid crab species. Lardies & Castilla (2001) demonstrated the significant differences in the egg number, volume, and dry weight of egg and studied the reproductive output of ovigerous females of Pinnotheridae species. Pinho *et al.,* (2001) studied sex composition, size distribution and morphometric relationships and determined of maturity stages by observing the ovary colour of the deep water crab *Chaceon affinis.* Mantelatto *et al.,* (2003) examined the population structure of crab species *Mithraculus forceps* from Brazil and reported the species was a continuous breeder as ovigerous females were observed in all the seasons. Litulo (2005) studied the population structure and reproductive biology which included the embryonic development, gonadosomatic index and juvenile recruitment of species *Uca inversa*. Zairion *et al.,* (2015) examined the fecundity of *Portunus pelagicus* female and determined the size at which females became potential to reproduce based on the relationships with body size and egg mass with respect to season. Edritanti *et al.,* (2016) explained the reproductive aspects including fecundity, egg volume and reproductive output of ovigerous females of crab species *Emerita emeritus*. Wilson (1980) studied the larval stages of species *Euchirograpsus americanus* in the laboratory conditions and provided complete description of development stages and also compared and provided similarities of the first zoel stage of other subfamilies namely, Varuninae, Grapsinae, Sesarminae, and Plagusiinae from Florida.

Anger (1981) studied the difference and effects in the starvation period in the laboratory in zoeal development of brachyuran crabs. Hines (1986) described the developmental and larval pattern in the life histories of brachyuran crabs using the data available from the literature of 47 crab species and larval description of 154 species. Paula & Hartnoll (1989) established the larval and post-larval development of crab species *Percnon gibbesi* by describing the larval characters. Fransozo *et al.,* (1990) analysed four zoeal stages and one megalopa stage of the crab species *Hexapanopeus paulensis* along the Brazilian coast. De Souza *et al.,* (2013) examined the larval stages and provided detail description of 12 estuarine crab species providing the identification keys of larval stage of Amazonian region. The reproduction ecology was examined by Fukui & Wada (1986) among four estuarine crab species and revealed the relation between habitat and life history attributing these crabs along the estuary of Japan. Henmi & Kaneto (1989) studied reproductive traits of three Ocypodid crabs species and revealed that the large broods incubate in burrows and small broods feed actively while incubating. Zimmerman & Felder (1991) examined various aspects like molting period, ovarian development and seasonal variation in egg laying, egg hatching, fecundity and larval release was observed in individual females of Sesarmid species from the Gulf of Mexico. Flores & Fransozo (1998) reported that the external factors influencing the seasonal breeding and also observed reproductive pattern in species *Pachygrapsus transverses.* Ituarte *et al.,* (2004) studied the female reproductive cycle of estuarine crab including breeding period, egg laying, hatching and larval release.

In recent years, several efforts have been attempted to address phylogeny using the morphological as well as molecular approaches. From the 20th century, Edwards (1834) and Balss (1957) worked on the brachyuran classification and its implied phylogeny based on the carapace type and buccal frame. Hence, the molecular studies have been used in order to deal with the crustacean phylogeny by various workers. However, Boyden (1926) refined the

serological techniques in crustacean systematic and Vaughn and Traeger (1976) revealed a close association between the hybridized DNA and evolutionary difference from the fossil record for the selected decapod crustaceans. Spears *et al.,* (1992) with a molecular approach tested a hypothesis regarding the phylogeny of the brachyuran crab species. The use of molecular methods for specimen identification and classification has become most common in recent years (Ortea *et al.,* 2009; Laakmann *et al.,* 2013). The DNA molecular study with the utility of DNA barcodes has the potential to regenerate the number of comprehensive studies with respect to taxonomy of crustacean species which is still incomplete (Hebert *et al.,* 2003; Lefébure *et al.,* 2006). Xin *et al.,* (2018) conducted the phylogenetic analysis of the molecular data of cytochrome oxidase I (CO1) genes on the evolutionary relationships among crab species of Grapsoidea based on analysis. The DNA sequence of 16s r*RNA* gene of crab genus Brachynotus of Mediterranean and northeastern Atlantic was sequenced by Schubart *et al.,* (2001). Tang *et al.,* (2003) resolved the phylogenetic relationships of mitten crabs corresponding to the COI. Similar works with respect to 16s r*RNA* and COI gene sequencing was carried out by Schubart *et al.,* (2000) between two crab species of genera Panopeus and Eurypanopeus belonging to family Panopeidae resulting they are not monophyletic however, the genes resulting sister-species relationships between them. Kitaura *et al.,* (2002) examined the ecological and morphological similarities between family Grapsoid and Ocypodoid crabs with reference to crab species of the genus Metaplax and Macrophthalmus indicating polyphyletic relationship between them. Chu *et al.,* (2003) studied the phylogenetics of the mitten crab species showing the affinities between them and revealed that three species of genus Eriocheir are similar and monophyletic. Weinberg *et al.,* (2003) studied the commercially important deep-sea red crab species of North America and examined greatest genetic difference among geographical groups. Harrison (2004) explored the evolutionary relations among species of the Pinnotherid crab of genus Austinixa using the molecular data.

Shih *et al.,* (2009) described a new species of fiddler crab based on the morphological and molecular data using 16S r*RNA* and COI genes. Reuschel & Schubart (2006) determined the DNA sequence of the *Scylla paramamosain* a mud crab species. Schubart *et al.,* (2006) studied a molecular phylogeny of grapsoid crabs based on DNA sequences of smaller and larger ribosomal subunits in mitochondria and suggest that Grapsoidea and Ocypodoidea as monophyletic superfamilies. Ma *et al.,* (2015) described the complete mitochondrial genome for *Charybdis feriata* and suggested that the genus Charybdis should be classified into subfamily Portuninae rather than into subfamily Thalamitinae.

The works on brachyuran crabs has been undertaken worldwide and also within the country, however few studies have been carried on the brachyuran crabs of Goa. Review of literature on crabs from Goa coast suggest that emphasis has been laid on the taxonomy of brachyuran crabs using morphometric analyses (Padate *et al.,* 2010, 2013, 2015; Kaullysing, 2015; Velip and Rivonker, 2013; Vijaylaxmi *et al.,* 2015; Komarpant *et al.,* 2018). Moreover, preliminary attempts have been made to study phylogenetic relationships among rock crabs using phenotypic characters (Kaullysing, 2015). However, no comprehensive information on the eco-biological aspects of crabs inhabiting rocky shores is available.

It is apparent from the above-cited studies that the information on various aspects of rocky shore crabs along the coast is scanty. Hence, a comprehensive assessment of rocky shore crabs with population ecology and reproductive behavior is essential to understand their role in the ecosystem function. Hence, in view of above it is proposed to address the following objectives.

1.3. Objectives

1. Taxonomy and diversity of rocky shore crabs with detailed description of new report.
2. Seasonal variations in density of few species of crabs at different habitats.

3. To study some of the biological aspects of selected species of crabs.
4. To study phylogenetic relationships among these crabs.

Chapter 2

Materials and Methods

2.1. Study area

The study area comprised of five selected estuarine beaches along the coast of Goa (Figure 2.1) namely Verem (15°29.747'N, 073°48.200'E), Odxel (15°27.255'N, 073°49.534'E), Cacra (15°27.059'N, 073°50.243'E), Siridao (15°26.584'N, 073°51.465'E) and Cortalim (15°24.525'N, 073°54.466'E). The study was located along the Mandovi-Zuari estuarine system, which exhibit annual cycle of varying hydrographical features (Dalal and Goswami, 2001). It is influenced by semi-diurnal tides with a range of 0.5 to 2.9 m (Shetye *et al.,* 2007). Among the five stations, Cacra, Verem, Odxel and Siridao are rocky in nature whereas, Cortalim is inhabited with mangrove vegetation. Among five stations, four are situated in one estuary while Verem is in the adjoining estuary. The distance between them vary from 800 m to 3 km. The stations are located on the northern bank of Zuari estuary while Verem is located on the northern bank of the Mandovi estuary, Goa, central west coast of India.

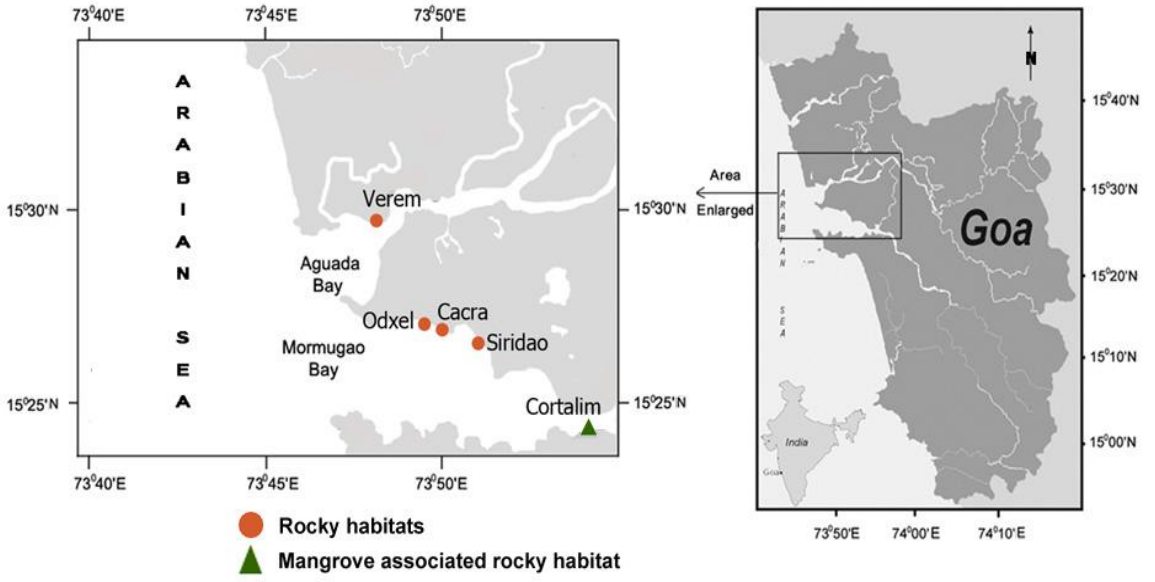

Figure 2.1. Map of the study area indicating the sampling sites

The stations Cacra, Verem, Odxel and Siridao are made up of several habitats like weathered laterite rocks with sandy and partly muddy substrate and discoloured broken shell pieces are strewn throughout the beach on high tide level. The mid and low tide level were marked with barnacles, oyster and algal growth on the laterite rocks, intersperse with tidal pools that are submerged during high tide. The dominant vegetation on the beach consists of algal mats. However, the stations Verem and Siridao were observed with patchy mangrove vegetation. At Cortalim the beach was marked with thick mangrove vegetation with sandy, muddy and clay substrate with small broken algal deposited laterite rocks and mangrove litter. In addition, there are palms and other terrestrial vegetation along the upper boundary of the beach.

2.2. Sample collection and preservation

The sampling was designed during the period from March, 2014 – August, 2015 along all the five sampling sites during low tide. To study the density of crabs, $1m^2$ quadrate sampling method was employed at three tidal levels viz. high, mid and low tide level at Cacra, Verem and Odxel and number of individual crabs were counted per square meter. Whereas at Cortalim, a small beach exists wherein high tide level is limited by human habitation and encompasses with sandy surface. Siridao comprised of fixed laterite boulders at mid and low tide and with sandy beach containing washed out broken shells at high tide level. Hence, at these two stations the sampling was done only at high tide level. To avoid any bias in sampling, random sampling method was carried involving regions such as tide pools, areas underneath the rocks as well as rock crevices so as to study crab species diversity.

Preliminary observations of the sampling sites revealed that the crabs inhabited rock crevices and close gaps between laterite boulders. In view of this, crab collection was carried out on pre-determined days at mid and low tide levels (Table 2.1; Tides at Goa, 2014; Tides at

Goa, 2015). The geographical position of the sampling site was recorded using the Geographical Positioning System (GPS-12 Channel). Sample collection was done by overturning the laterite stones to expose the crabs. In addition, large boulders were pounded with hammer to expose the crabs. The collected specimens were then brought to the laboratory, washed under running tap water to remove any debris and or sediment attached to the body parts. Crab specimens were preserved in 5 % buffered formalin (buffered with hexamethylenetetramine to prevent fragmentation of appendages) or 95% ethanol at -20^{0}C as per the requirement. These are stored in pre-labelled transparent plastic bottles and deposited as reference vouchers at the Marine Biology laboratory, Department of Marine Sciences, Goa University. Type specimens (*Carupella banlaensis*) with ZSI/FPS voucher specimen number ZSI C 6458/2 and ZSI C 6459/2 are are deposited in the type collections of the Crustacea section (C) of the National Zoological Collections at the Zoological Survey of India, Kolkata (ZSI/FPS/Kolkata; FPS-Fire Proof Spirit Building).

Table 2.1. Details of sampling surveys carried out in the study area

S.No	Date	Tide level data		Time of sampling
		Lowest Tide Level (m)	Time (hrs)	
Station/ Geographical position - Cacra (15° 27.059' N 073°50.243' E)				
1	12-03-2014	0.75	14:16	14:00-15:30
2	08-04-2014	0.85	11:06	10:45-13:00
3	07-05-2014	0.85	09:59	09:45-12:00
4	05-06-2014	0.8	09:03	08:45-11:00
5	17-07-2014	0.43	08:04	08:00-11:00
6	11-08-2014	0.53	17:20	17:00-19:30
7	25-09-2014	0.35	17:22	17:00-19:00
8	20-10-2014	0.7	14:49	14:30-16:30

9	18-11-2014	0.66	14:05	13:45-15:30
10	03-12-2014	0.34	14:23	14:00-16:30
11	25-01-2015	0.58	08:33	08:15-10:45
12	12-02-2015	0.71	10:13	10:00-12:30
13	28-03-2015	0.74	11:15	11:00-13:30
14	27-04-2015	0.89	11:33	11:15-13:45
15	05-05-2015	0.94	17:47	17:30-19:00
16	15-06-2015	0.98	16:04	15:45-17:45
17	15-07-2015	0.92	16:04	15:45-17:30
18	27-08-2015	0.95	14:58	14:45-17:00
Station/ Geographical position - Verem (15° 29.742' N 073°48.200' E)				
19	13-03-2014	0.69	15:03	14:45-17:15
20	09-04-2014	0.89	12:09	11:30-13:00
21	21-05-2014	0.72	10:04	09:45-12:00
22	06-06-2014	0.93	09:59	09:45-12:30
23	16-07-2014	0.21	07:14	07:00-09:00
24	09-08-2014	0.89	15:41	14:30-16:30
25	20-09-2014	0.89	14:53	14:45-17:00
26	19-10-2014	0.84	14:12	14:00-16:30
27	17-11-2014	0.82	13:23	13:00-15:30
28	02-12-2014	0.49	13:30	13:00-15:00
29	26-01-2015	0.57	09:27	09:00-11:00
30	13-02-2015	0.68	11:18	11:00-13:00
31	27-03-2015	0.61	10:04	09:45-11:45
32	28-04-2015	0.94	12:41	12:30-14:45
33	26-05-2015	0.92	10:33	10:00-12:30
34	11-06-2015	0.91	11:37	11:00-13:00
35	09-07-2015	0.84	10:06	09:45-11:00
36	12-08-2015	0.92	15:49	15:30-17:30
Station/ Geographical position - Odxel (15° 27.255' N 073° 49.534' E)				
37	11-03-2014	0.79	13:17	13:00-15:00
38	10-04-2014	0.88	13:24	13:10-14:30

39	08-05-2014	0.94	11:12	11:00-13:30
40	04-06-2014	0.67	08:19	08:00-10:00
41	18-07-2014	0.65	08:56	08:45-11:00
42	10-08-2014	0.71	16:31	16:15-18:00
43	24-09-2014	0.44	16:54	16:45-18:30
44	21-10-2014	0.56	15:21	15:00-17:00
45	19-11-2014	0.51	14:23	14:00-16:00
46	04-12-2014	0.22	15:11	15:00-17:30
47	20-01-2015	0.13	16:28	16:00-18:00
48	16-02-2015	0.38	14:30	14:00-16:30
49	13-03-2015	0.58	09:24	09:15-11:30
50	13-04-2015	0.64	11:13	11:00-13:30
51	13-05-2015	0.79	12:04	11:45-14:00
52	09-06-2015	0.59	09:21	09:00-11:00
53	18-07-2015	0.8	18:42	18:30-19:00
54	14-08-2015	0.77	17:08	16:45-18:00
Station/ Geographical position - Cortalim (15° 24.525' N 073° 54.466' E)				
55	14-03-2014	0.65	15:42	15:30-17:30
56	25-04-2014	0.76	13:26	13:00-15:30
57	28-05-2014	0.98	16:54	16:00-18:00
58	18-06-2014	0.37	08:20	08:00-10:00
59	25-07-2014	0.98	16:28	16:00-18:30
60	22-08-2014	0.97	15:27	15:00-17:30
61	21-09-2014	0.78	15:27	15:27-17:30
62	18-10-2014	0.97	13:24	13:00-17:30
63	20-11-2014	0.37	15:15	15:00-17:00
64	06-12-2014	0.14	16:34	16:15-18:30
65	19-01-2015	0.19	15:43	15:30-18:00
66	14-02-2015	0.6	12:28	12:00-14:00
67	18-03-2015	0.42	15:03	14:45-17:00
68	17-04-2015	0.64	15:36	15:15-17:30
69	16-05-2015	0.87	15:19	15:00-17:00

70	10-06-2015	0.77	10:28	10:00-12:00
71	23-07-2015	0.86	08:39	08:15-10:30
72	13-08-2015	0.83	16:33	16:15-18:30
Station/ Geographical position - Siridao (15° 26.584' N 073° 51.465' E)				
73	10-03-2014	0.8	12:07	12:00-14:30
74	11-04-2014	0.85	14:20	14:00-15:30
75	23-05-2014	0.86	11:53	10:45-12:00
76	19-06-2014	0.59	09:17	09:00-11:00
77	19-07-2014	0.87	09:52	09:45-11:00
78	25-08-2014	0.72	16:59	16:30-18:00
79	22-09-2014	0.6	15:56	15:45-17:30
80	24-10-2014	0.21	16:51	16:45-18:00
81	21-11-2014	0.24	15:49	15:30-17:00
82	05-12-2014	0.16	15:55	15:45-18:00
83	21-01-2015	0.12	17:13	17:00-18:30
84	17-02-2015	0.27	15:24	15:00-16:30
85	19-03-2015	0.39	15:55	15:45-17:00
86	29-04-2015	0.95	13:42	13:30-15:00
87	14-05-2015	0.85	13:13	13:00-14:30
88	24-06-2015	0.89	09:28	09:15-10:30
89	30-07-2015	0.95	16:09	16:00-18:30
90	28-08-2015	0.77	15:46	15:30-17:30

Among the collected crab samples, four species namely *Leptodius exaratus, Epixanthus frontalis, Metopograpsus frontalis* and *Heteropanope glabra* were most abundant and commonly occurring in the study sites, hence were selected for further studies. The species *L. exaratus* and *E. frontalis* are euryhaline, rock dwellers residing underneath the rocks and moist sand at high, mid and low tidal region. These species preferred the areas like sandy patches along rocky habitats of stations namely Cacra, Odxel, Verem and Siridao. *M. frontalis* occurred in all the five sampling stations crawling on algal encrusted large laterite boulders

and under the loose laterite rocks at mid and low tide region and also in the muddy substratum and mangrove habitats. Due to their wide range of feeding habits makes these crab species possible to live in diversified habitats (Nishihira, 1984; Lee, 1998; Poon *et al.,* 2010; Kent & McGuinness, 2010). However, the species *H. glabra* mostly occurs in mangrove mudflats suggesting that this species exhibit high degree of tolerance to low saline condition (Davie, 1989; Trivedi *et al.,* 2015; Naderloo, 2017) and was dominant with high density in Cortalim as the station is covered with mangrove vegetation and small broken laterite rocks. The species was also found in other three rocky stations (Cacra, Odxel and Siridao) with least abundance in patches of muddy substrate.

2.3. Statistical analysis

A statistical technique of Cluster analysis was used to assign data into groups (clusters) to reveal difference among the groups. The software programs employed for the analysis of data includes PRIMER version 6.1.10 software (Plymouth Routines in Multivariate Ecological Researches version 6) (Clarke & Gorley, 2006) for analysing the species composition, spatial and temporal wise using Bray - Curtis coefficients (Bray – Curtis, 1957). Further, the significance of the cluster was tested by similarity profile SIMPROF analysis and similarity percentage by SIMPER analysis.

Species diversity indices were carried out using the following three indices to determine the intertidal brachyuran crab diversity along Goa. The data was analysed for Species diversity indices by Shannon-Wiener diversity, Margalef's species richness and Pielou's species evenness measures. ANOVA for two-factor without replication was analysed for crab population among station and season were computed using PRIMER version 6.1.10 software.

The overall male and female sex ratio was tested using Chi square test (X^2). The data was analysed using a two way ANOVA in excel without replication to study the crab

population among station and season. The analysis of variance was also statistically analysed for CW and fecundity of crabs (Gupta, 2009). The histogram plots for size frequency distribution graph in CW of male, female and ovigerous female was plotted using Grapher - version 8.4.696 software.

Chapter 3

Taxonomy and diversity of rocky shore crabs

3.1. Introduction

Studies pertaining to the diversity of brachyuran crabs from the estuarine regions of Goa were focused considering the lacunae in the available information on the occurrence and distribution of rocky shore crab species from this region. The estuarine shores in this region are characterized by mixed habitats comprising mangrove vegetation alternating with laterite rocky shores and sandy shores (Hegde *et al.,* 2013), which provide ambient environment for diverse brachyuran assemblage. Estuarine intertidal areas comprise of high abundant and diverse macrobenthic invertebrates, which attribute to a high concentration of organic matter. Crabs play an important role in detritus formation and nutrient recycling in the ecosystem and remain association with other organism like algae, seaweeds, barnacles, bivalves and holothurians (Khan and Ravichandran, 2009).

Present study on the taxonomic identification and diversity of brachyuran crab species, primarily attempts to provide baseline information, through intensive sampling and subsequently creating an inventory of brachyuran crab occurring along the five different estuarine habitats of Goa. Among the reported species, *Carupella banlaensis* is re-described as the rare crab from Goa, additionally also providing preliminary information on the occurrence morphological description of *Dotilla myctiroides* along the west coast of India. A preliminary reporting on the tropical sandy shore benthic life of Goa was provided by Ingole (2003). The studies carried on the brachyuran crabs of Goa were mostly on the taxonomy aspects by Padate *et al.,* (2010, 2013, 2015), Kaullysing (2015), Velip and Rivonker (2013), Vijaylaxmi *et al.,* (2015) and Komarpant *et al.,* (2018). It is important to create a baseline data of epibenthic fauna from estuarine habitat and therefore present study envisages the density, distribution, occurrence, spatio-temporal variation and few biological aspects of marine brachyuran crabs along few regions of Goa coasts.

3.2. Methodology

3.2.1. Taxonomic identification and Morphometry

At the laboratory, representative specimen of each species was washed thoroughly and body parts (dorsal carapace, abdomen, sternum, gonopods, chelipeds, pereiopods 2-5 and maxillipeds) of one male and female crab representing each morphological variant were photographed using a digital camera of 7.2 mega pixel (SONY DSC S750, 3X optical zoom) to elucidate the distinguishing morphological characteristics. In addition, minute morphological details were studied and recorded with the help of Camera Lucida diagrams using an Olympus PEN E-PL1 attached to Olympus SZX16 stereomicroscope. Taxonomic identification was carried out using methods describing morphology, colour, texture patterns, meristic counts, morphological measurements and gonopod structure of male.

Taxonomic identification was aided by published taxonomic literature for brachyuran crabs. (Alcock 1895, 1896, 1899, 1900; Leene, 1938; Chhapgar, 1957a, b; Fischer & Whitehead, 1974; Sakai, 1976; Wee & Ng, 1995; Jeyabaskaran *et al.,* 2000) Ng *et al.,* (2008); Stephenson (1972); *Carupella* species (Lenz & Strunck, 1914; Barnard, 1950; Crosnier, 1962; Tien, 1969; Zarenkov, 1970; Moosa, 1981). In addition, internet websites such as Fishbase (Froese & Pauly, 2010), Sealifebase (Palomares & Pauly, 2010), were referred for species identification. Standard morphometric parameters were measured down to the nearest 0.01 cm using digital vernier calipers (Absolute Digimatic Digital Caliper, 150 mm). The morphometric parameters measured and abbreviations derived are shown in Table 3.1. Subsequently, line diagrams of diagnostic morphological characters were drawn using Adobe Photoshop CS5 software. The terminology used in the taxonomic description of the specimens follows Wee & Ng (1995) and Velip & Rivonker (2015).

Table 3.1. List of morphological parameters and abbreviations used in the present study (Keenan *et al.,* 1998)

S. No.	Body part	Morphological parameter	Abbreviation
1	Carapace	Carapace width	CW
2		Carapace length	CL
3		Frontal width of carapace	FW
4		Abdomen width	AW
5		Sternal width	SW
6	Chelipeds	Meral length	ML_{3m}
7		Meral width	MW_{3m}
8		Dactylus length	DL_{ch}
9		Propodal length	PL_{ch}
10	Pereiopods (2-5)	Overall cheliped length	ChL
11		Overall periopodal length	PrL

S. No.	Abbreviation	Meaning
1	SIMPER	Similarity percentage
2	SIMPROF	similarity profile
3	BLAST	Basic Local Alignment Search Tool
4	PCR	Polymerase chain reaction
5	BOLD	Barcode of Life Data
6	NCBI	National Center for Biotechnology Information
7	DNA	Deoxyribonucleic acid
8	16srRNA	16S ribosomal ribonucleic acid
9	COI	Cytochrome c oxidase subunit I
10	EDTA	Ethylenediaminetetraacetic acid

3.2.2. Scanning Electron Microscopy (SEM)

Scanning Electron Microscope photography (SEM) was carried out to ascertain the identity and distinctiveness of the crab species. Initially, the specimens like spoon-tipped setae of second maxillipeds, first gonopods from male crabs and cheliped dactylus were removed, preserved in 70 % alcohol and air dried for three days and mounted on small screws for gold coating. The samples were then coated with gold at 6-8 mbar pressure with Quorum sputter coater (Model SC7620) and placed into the specimen chamber of the SEM. The

chamber was then pumped, and the specimen moved into a vacuum. In this vacuum, the SEM creates a beam of electrons which scans across the surface of the mounted object. The object, being coated in gold, emits secondary electrons that are detected by a scintillation material which produces flashes of light. Finally, an image is produced by correlating the scanning position and the consequent light signal with Roberts, 2000 and Carl Zeiss Electron Microscopy GmbH, 2016. SEM photographs were taken at the National Institute Oceanography, Goa using a JOEL JSM–5800 LV Scanning Electron Microscope at 15 or 20 KV accelerating voltage at various magnifications depending on the size of the samples. The photographs were also taken with EVO-18 Carl Zeiss Electron Microscope at 10 KV accelerating voltage at 38 X and 100 X magnifications at the University Science Instrumentation Centre (USIC), Goa University.

3.3. Results.

A total of 29 species of crabs belonging to 21 genera and 12 families were collected from inter tidal rocky shore habitats along Goa during the present study (Table 3.2). The data collected on crab density revealed maximum number of species from Family Portunidae (7 species) followed by the Family Ocypodidae and Sesarmidae (4 species each), Family Dotillidae, Grapsidae, Oziidae, Pilumnidae, Varunidae (2 species each), Families Hymenosomatidae, Menippidae, Macrophthalmidae and Xanthidae (1 species each). Here, an attempt has been made to re-describe *Carupella banlaensis* of family Portunidae from Goa as a new geographical record for the entire southern Asian region. Also, preliminary information on the occurrence and morphological description of species *Dotilla myctiroides* of family Dotillidae is provided. Further, a brief description of all other 27 species along with their habitat specificity is elucidated.

Table 3.2. List of Brachyuran crab taxa observed during the study period

S. No.	Family/ Name of the Species	Reports from Goa	Literature Source
	Portunidae		
1	*Carupella banlaensis* (Tien, 1969)	Vijaylaxmi *et al*., 2015	Crosnier, 1962; Vannini & Innocenti, 2000
2	*Portunus (Portunus) sanguinolentus* (Herbst, 1783)	Dev Roy & Bhadra, 2008; Dev Roy, 2013; Present study.	Chhapgar,1957; Alcock, 1899; Forest & Guinot, 1961; Stephenson, 1976; Guinot, 1985
3	*Portunus (Portunus) pelagicus* (Linnaeus,1758)	Dev Roy & Bhadra, 2008; Dev Roy, 2013; Present study.	Alcock, 1899; Laurie, 1906; Gravely, 1927; Chopra, 1935; Pillai, 1951; Chhapghar, 1957
4	*Scylla olivacea* (Herbst, 1796)	Hegde *et al*., 2013; Padate *et al*., 2013; Present study.	Joel & Raj, 1980; Keenan *et al*., 1998; Padate *et al*., 2013; Trivedi and Vachhrajani, 2013
5	*Charybdis (Charybdis) hellerii* (A. Milne Edwards, 1867)	Present study	Stephenson *et al.,* 1957; Crosnier, 1962; Sankarankutty, 1966; Stephenson, 1972; Wee & Ng, 1995
6	*Charybdis (Charybdis) lucifera* (Fabricius, 1798)	Dev Roy & Bhadra, 2008; Dev Roy, 2013; Present study.	Stephenson *et al*., 1957; Wee & Ng, 1995
7	*Thalamita crenata* (Latreille, 1829)	Dev Roy & Bhadra, 2008; Dev Roy, 2013; Present study.	Alcock, 1899; Nobili, 1906; Klunzinger, 1913; Stephenson & Hudson, 1957; Crosnier, 1962
	Ocypodidae		
8	*Ocypode pallidula* (Hombron & Jacquinot, 1846)	Present study	Sakai & Turkay, 1976; Jones, 1988
9	*Uca (Gelasimus) vocans* (Linnaeus, 1758)	Present study	Barnard, 1950; Crosnier, 1965; Serène, 1973
10	*Uca (Paraleptuca) annulipes* (H. Milne Edwards, 1837)	Present study	Alcock, 1900; Crosnier, 1965; Rathbun, 1910; Sakai, 1936; 1939;1940; Barnard, 1950; Tweedie, 1950; Sankarankutty, 1961; Forest & Guinot, 1961; Ng & Davie, 2002; Litulo, 2004a, 2005
11	*Uca* sp.(Leach, 1814)	Present study	Leach, 1815; Rathbun, 1897; Barnard, 1950; Nobili,1903
	Sesarmidae		
12	*Nanosesarma andersonii* (De Man, 1888)	Dev Roy & Bhadra, 2008; Dev Roy, 2013; Present study.	De Man, 1888; Alcock, 1900; Tesch, 1917; Tweedie, 1950; Serène & Soh, 1970
13	*Nanosesarma batavicum* (Moreira, 1903)	Present study	Moreira, 1903; Tweedie, 1936, 1940, 1950; Kemp, 1915; Serène & Soh, 1970

14	*Nanosesarma minutum* (De Man, 1887)	Present study	Rathbun, 1910; Tesch, 1917; Tweedie, 1936; 1950; Chhapgar, 1957; Crosnier, 1965; Serène & Soh, 1970
15	*Nanosesarma pontianacense* (De Man, 1895)	Present study	De Man, 1895; Nobili, 1903; Tweedie, 1940
	Dotillidae		
16	*Dotilla myctiroides* (H. Milne Edwards, 1852)	Alcock, 1900; Kemp, 1919; Ingole, 2003; Dev Roy, 2013; Padate *et al*., 2015; Present study.	Stimpson, 1907
17	*Illyoplax* sp. (Stimpson, 1858)	Present study	Stimpson, 1858
	Grapsidae		
18	*Grapsus albolineatus* (Milbert, 1812)	Alcock, 1900; Dev Roy, 2013; Present study.	Lamarck, 1818; Banerjee, 1960; Sankarankutty, 1961; Crosnier, 1965; Sakai, 1976
19	*Metopograpsus frontalis* (H. Milne Edwards, 1834)	Present study	Banerjee, 1960; Sankarankutty, 1961
	Oziidae		
20	*Epixanthus frontalis* (H. Milne Edwards, 1834)	Dev Roy & Bhadra, 2008; Dev Roy, 2013; Kaullysing *et al*., 2015; Present study.	Edwards, 1834; Heller, 1861; Alcock, 1898; Stimpson, 1907; Sakai, 1939; Chhapgar, 1957; Chopra and Das, 1937; Sankarankutty, 1962
21	*Ozius rugulosus* (Stimpson, 1858)	Present study	Stimpson, 1907; Sakai, 1939; Chhapgar, 1957
	Pilumnidae		
22	*Heteropanope glabra* (Stimpson, 1858)	Kaullysing *et al*., 2015; Present study.	Stimpson, 1907, 1858; Takeda & Iwasaki, 1983
23	*Pilumnus* sp. (Leach, 1815)	Present study	Alcock, 1898; Rathbun, 1923; Balss, 1938; Barnard, 1950; Sakai, 1939
	Varunidae		
24	*Pseudograpsus elongates* (A. Milne Edwards, 1873)	Present study	Crosnier, 1965
25	*Pseudograpsus intermedius* (Chhapgar, 1955)	Present study	Chhapgar, 1955, 1957; Tesch, 1918
	Hymenosomatidae		

26	*Neorhynchoplax demeloi* (Kemp, 1917)	Kemp, 1917; Dev Roy & Bhadra, 2008; Present study.	Kemp, 1917
	Menippidae		
27	*Myomenippe hardwickii* (Gray, 1831)	Dev Roy & Bhadra, 2008; Dev Roy, 2013; Present study.	De Man, 1887; Alcock, 1898
	Macrophthalmidae		
28	*Macrophthalmus convexus* (Stimpson, 1858)	Present study	Stimpson, 1907; De Man, 1888, 1902; Rathbun, 1910; Tesch, 1915; Kemp, 1919; Sakai, 1939
	Xanthidae		
29	*Leptodius exaratus* (H. Milne Edwards, 1834)	Dev Roy & Bhadra, 2008; Dev Roy, 2013; Present study.	Klunzinger, 1913; Chopra & Das, 1937; Guinot, 1964; Serène, 1968; 1984; Guinot & Cleva, 2009

3.3.1. Description of Brachyuran crab taxa observed during the present study.

3.3.1.A. Family Portunidae

3.3.1.A.1. *Portunus (Portunus) sanguinolentus* (Herbst, 1783);

The species (N=1) was observed in station Siridao, occurred in the tidal pool of laterite boulders at low tide level, inhabiting in the sandy substrates, occurred during post-monsoon season. The species is distributed in Indo-West Pacific (Carpenter and Angelis, 2002), East Africa to French Polynesia and north to Japan and south to Australia (Carpenter, 1997).

3.3.1.A.2. *Portunus (Portunus) pelagicus* (Linnaeus, 1758)

The species (N=1) was observed in Siridao, during pre-monsoon season. The crab was observed inhabiting underneath the loose rock over the moist sand, slightly burrowed in the sediment at the low tidal level. The species is distributed throughout Indo-West Pacific Ocean (Corsini-Foka *et al*., 2004). China, Japan, Korea, Indonesia, Philippines, Red sea, African coast, Mediterranean sea, Persian Gulf (Lai *et al.*, 2010).

3.3.1.A.3. *Scylla olivacea* (Herbst, 1796)

The species (N=11) was observed in Verem, Cortalim and Siridao stations during monsoon and post monsoon seasons, inhabiting soft sandy and muddy bottoms remaining slightly burrowed in the sediment at low tide level, prefers rocky and mangrove habitats. The species is distributed in Karachi, Thailand, China, Taiwan, Malaysia, Philippines, Indonesia, and Australia (Keenan *et al.,* 1998, Alberts-Hubatsch *et al.,* 2016).

3.3.1.A.4.. *Charybdis (Charybdis) lucifera* (Fabricus, 1798)

The species (N=1) was observed in Verem and occurred during pre-monsoon season. The crab was observed inhabiting in the large tidal pool of laterite boulders at low tide level and preferred sandy substrates. The species is distributed in Australia, Southern coast of China, Hong Kong, Japan, Madagascar, Malay Archipelago, Philippines, Red Sea, Singapore, South Africa, Sri Lanka and Taiwan (Mizzan and Vianello, 2009).

3.3.1.A.5. *Charybdis (Charybdis) hellerii* (A. Milne Edwards, 1867)

The species (N=2) was observed in Siridao and occurred during pre-monsoon season. The crab was observed inhabiting in the large tidal pool of laterite boulders at low tide level and preferred sandy substrates. The species is distributed from South Africa/Madagascar to Japan/Hawaii regions (Ferry *et al.,* 2017).

3.3.1.A.6. *Thalamita crenata* (Latreille, 1829)

The species was abundant (N=412) and commonly occurring in all the five sampling stations during post-monsoon, monsoon and pre-monsoon seasons. The crab was observed inhabiting in the soft sandy and muddy bottoms remaining slightly burrowed in the sediment or underneath the laterite rocks at mid tide and low tide level, prefers moist rocky regions and

mangrove areas. The species is found mostly in China, Indonesia, Malaysia, Singapore, Australia, Tuamotu, Tonga, French Polynesia, and Hawaii **(**Carpenter and Angelis, 2002).

3.3.1.B. Family Ocypodidae

3.3.1.B.1. *Ocypode pallidula* (Hombron & Jacquinot, 1846)

The species (N=1) was observed in Siridao and occurred during pre-monsoon season. Mostly a sand dwelling crab observed in the high tidal zone of intertidal rocky shore. Prefer sandy substratum and keeps burrowing in to the sand. The species is distributed in Hawaii Islands in the Central Pacific, Australia (Fellows, 1975), Southern Pacific, Indonesia and Madagascar and Mauritius in the western Indian Ocean (Sakai and Türkay, 2013).

3.3.1.B.2. *Uca (Gelasimus) vocans* (Linnaeus, 1758)

The species (N=43) commonly occurred in all the five sampling stations during pre-monsoon season. The crab was observed to remain burrowed in the sandy or muddy substratum in mangrove as well as rocky habitat at high and mid tidal region. The species is distributed in China, Burma, Thailand, Indonesia, Philippines, Malaysia (Shih *et al.,* 2016).

3.3.1.B.3.. *Uca (Paraleptuca) annulipes* (H. Milne Edwards, 1837)

The species (N=33) commonly occurred in four sampling stations except Cacra during pre-monsoon season. The crab was observed to remain burrowed in the sandy or muddy substratum in mangrove as well as rocky habitat like that of *U, vocans.* The species was observed inhabiting in high tide and mid tide zones. The species is distributed in southern China, Philippines, Indonesia, Malaysia (Shih *et al.,* 2016).

3.3.1.B.4.. *Uca* sp.

The species (N=1) occurred at station Verem during pre-monsoon season at mid tide zone resting above its burrow on sandy substratum. The genus Uca is mostly distributed in the regions of Western Atlantic, Eastern Pacific and Indo-West Pacific (Alcock, 1900; Nabout *et al.,* 2010; Rosenberg, 2014; Crane, 2015).

3.3.1.C. Family Sesarmidae

3.3.1.C.1. *Nanosesarma andersonii* (De Man, 1888)

The species (N=43**)** occurred in abundance at Cortalim and least in Odxel in all the three seasons. Observed in moist sandy substratum underneath laterite boulders of mentioned estuarine shores at mid tide level. The species scamper for protection from predicators. The species *N. andersonii* is distributed in Mergui Archipelago (Alcock, 1900), Malaysia, Singapore (Tweedie, 1950), Japan (Komai *et al.,* 2004).

3.3.1.C.2. *Nanosesarma batavicum* (Moreira, 1903)

The species (N=1) was observed at Verem during pre-monsoon season. Preferred moist sandy substratum underneath laterite boulders of estuarine shore at mid tide level. *N. batavicum* is found in Indonesia, Malaysia and Singapore (Tweedie, 1950).

3.3.1.C.3. *Nanosesarma minutum* (De Man, 1887)

The species (N=384) commonly occurred in all the five sampling stations during post-monsoon, monsoon and pre-monsoon seasons. The species was highly abundant in Verem. Observed in the moist sandy and clayey substrates underneath loose pebbles and small laterite boulders of estuarine shores at mid tide level, also found inside crevices of oyster, encrusted and algal-covered laterite rocks. The species *N. minutum* is reported from Indonesia, Malaysia,

Singapore, Gulf of Thailand, South China Sea (Tweedie, 1936, 1950), Madagascar (Crosnier, 1965), Tanzania (Hartnoll, 1975).

3.3.1.C.4. *Nanosesarma pontianacense* (De Man, 1895)

The species (N=45) occurred in three sampling stations namely Cacra, Verem and highly abundant in Cortalim during post-monsoon, monsoon and pre-monsoon seasons. Observed in moist sandy and clayey substrates underneath loose pebbles, small laterite boulders and crevices of oyster-encrusted laterite rocks of estuarine shores at mid tide level, scamper for protection on being disturbed. The species is distributed from the western coast of India through Southeast Asia to Japan (Alcock, 1900; Tweedie, 1940, 1950; Komai *et al.,* 2004).

3.3.1.D. Family Dotillidae

3.3.1.D.1. *Illyoplax* sp.

The species (N=3) occurred in Cortalim during monsoon season. The species inhabit the intertidal mudflat habitat and has burrowing nature. The species of Genus Ilyoplax distributed across the temperate to tropical Indo-West Pacific region (Kitaura & Wada, 2006).

3.3.1.E. Family Grapsidae

3.3.1.E.1. *Grapsus albolineatus* (Milbert, 1812)

The species (N=34) commonly occurred in all the five sampling stations in all the three seasons. The crab was observed in sandy bottom and rocky intertidal areas mostly crawling on the laterite boulders in the low tidal region, scampering quickly when disturbed. Young crabs were found hiding underneath the laterite rocks and partially remain buried. The species *G. albolineatus* belonging to family Grapsidae is found to be distributed in Indo-Pacific region

from Red Sea and west coast of Africa to Japan, Polynesian Islands and Hawaii (Meiyappan and Kathirvel, 1978**;** Carpenter and Angelis, 2002).

3.3.1.E.2. *Metopograpsus frontalis* (H. Milne Edwards, 1834)

The species (N=619) is highly abundant commonly occurred in all the five sampling stations post-monsoon, monsoon and pre-monsoon seasons. The crab was observed in high, mid and low tidal zones, feeding and crawling on laterite boulders in mid and low tidal region. In high tide region they were mostly hiding underneath the rocks. The species is distributed in Sri Lanka, Malaya, Singapore, Indonesia, East Indies and Australia. West Pacific, from Singapore to southern China (Fratini *et al.,* 2018).

3.3.1.F. Family Oziidae

3.3.1.F.1. *Epixanthus frontalis* (H. Milne Edwards, 1834)

The species is a marine rock crab (N=994) occurring in four stations except Cortalim and appeared in all the three seasons. The crab was found in the high and mid tidal regions underneath the rocks and rock crevices and camouflaged with the laterite rocks. The species is distributed from the Indo-West Pacific and Atlantic regions (Alcock, 1898; Serène, 1984; Poupin, 1996).

3.3.1.F.2. *Ozius rugulosus* (Stimpson, 1858)

The species *O. rugulosus* (N=30) is marine rock crab occurred in abundance in Cacra and least in Verem and Odxel during post-monsoon, monsoon and pre-monsoon seasons. The crab was observed in high tide and mid tide regions, underneath the rocks, preferred sandy substratum and mostly camouflaged with the rocks. Distributed in the Red Sea, South Africa,

Madagascar, Mauritius, Sri Lanka; Andaman Islands; Arakan coast, Nicobar Islands, Japan, Taiwan, China and Australia. (Alcock, 1898; Heller, 1865; Forest & Guinot, 1961).

3.3.1.G. Family Pilumnidae

3.3.1.G.1. *Heteropanope glabra* (Stimpson, 1858)

The species is mud associated (N=1029) occurred in four stations except Verem. It was exclusively found in Cortalim and appeared in all the three seasons. Mostly preferred muddy substratum and resides underneath the moist rocks in high and mid tidal regions. *H. glabra* is distributed in Indo-West Pacific and eastern Atlantic regions (Chhapgar, 1957; Alcock, 1898; Crosnier, 1967).

3.3.1.G.2. *Pilumnus* sp.

The species (N=1) occurred in Cortalim during post-monsoon season. Preferred muddy substrate and kept burrowed underneath the rock in mid tide region. The genus is distributed in Indo Pacific and Atlantic regions (Alcock, 1898).

3.3.1.H. Family Varunidae

3.3.1.H.1. *Pseudograpsus elongates* (A. Milne Edwards, 1873)

The species (N=25) was observed in four stations except Cacra and occurred in post-monsoon and monsoon seasons. Prefers sandy and muddy substrate and resides under the rocks in the mid tide region. The species is distributed in Western Central Pacific, the Red Sea to Natal, Madagascar, Seychelles and New Caledonia (Vannini & Valmori, 1981).

3.3.1.H.2. *Pseudograpsus intermedius* (Chhapgar, 1955)

The species (N=2) was observed in Cortalim and occurred during post-monsoon season. The crab preferred sandy and muddy substrate and resides under the rocks in the mid tide region. The species *P. intermedius* is distributed in Indo- Pacific regions (Selvakumar & Khan, 1993).

3.3.1.I. Family Hymenosomatidae

3.3.1.I.1. *Neorhynchoplax demeloi* (Kemp, 1917)

The species (N=10) was observed in Cortalim and occurred during post-monsoon and pre-monsoon seasons. A small size crabs found in the holes of laterite and oyster rocks in the muddy substrate at mid and low tide region. The species is found in western Indian ocean and Persian Gulf Andaman Islands; West Bengal, Sri Lanka, China, South Africa, Australia (Kemp, 1917).

3.3.1.J. Family Menippidae

3.3.1.J.1. *Myomenippe hardwickii* (Gray, 1831)

The species (N=9) was observed in Cortalim and Siridao and occurred during post-monsoon and pre-monsoon seasons. Found on sandy and muddy substrate inhabiting in mangrove and rocky areas. *M. hardwickii* is mostly distributed in Akyab, Bangladesh, East coast of Africa, Mergui Archipelago, Singapore and throughout Southeast Asia, reaching the Philippines **(**Alcock, 1898; Carpenter and Angelis 2002).

3.3.1.K. Family Macrophthalmidae

3.3.1.K.1. *Macrophthalmus convexus* (Stimpson, 1858)

The species (N=2) was observed in Cortalim during post-monsoon season. Inhabit the intertidal mudflat habitat and remains burrowed partially into the sediment. Existing record of

the species suggest that the species is distributed in Indo-West Pacific ranging from the Red Sea eastward to New Caledonia and northward to the Ryukyu Islands, Japan, and southward to Queensland, Australia (Komai *et al.,* 1995).

3.3.1.L. Family Xanthidae

3.3.1.L.1. *Leptodius exaratus* (H. Milne Edwards, 1834)

The species (N=1694) is a rock crab, commonly occurred in four sampling stations except Cortalim during post-monsoon, monsoon and pre-monsoon seasons. Observed in rocky habitat, underneath the laterite rocks and in crevices of rocks, inhabit in sandy substrate in high, mid and low tidal region. The species *L. exaratus* is distributed in Western Indian Ocean, Africa, Madagascar, Red Sea and the Persian Gulf (Lee *et al.,* 2013).

In view of the observation made on the rocky shore crabs, apart from a brief description of above referred species, the following two species namely *Carupella banlaensis* reported first time from India and *Dotilla myctiroides* from Goa were re-described.

3.2.A. Description of rare portunid crab *Carupella banlaensis* Tien, 1969 from India.

The species belong to family Portunidae which represents a group of highly diverse crabs divided into seven subfamilies (Ng *et al.,* 2008) based on variations in exoskeletal structures (Stephenson, 1972). *Carupella* is a genus of mostly rarely occurring, inshore crabs (Crosnier, 1962; Zarenkov, 1970; Vannini & Innocenti, 2000) within the subfamily Portuninae (Ng *et al.,* 2008). This genus is known to be represented by three species, namely *Carupella banlaensis* Tien, 1969, *C. epibranchialis* Zarenkov, 1970, and *C. natalensis* Lenz & Strunck, 1914. The genus *Carupella* Lenz & Strunck, 1914 was initially assumed to morphologically resemble *Carupa* and *Lupocyclus* owing to the slender basal antennal

segment, which does not extend transversely and lacks any anterolateral extension. Hence, it was placed in the alliance Lupocycloida within the subfamily Lupinae (Lenz & Strunck 1914). Subsequently, Stephenson (1972) placed the genera *Carupella* and *Lupocyclus* within the subfamily Portuninae owing to a 'relatively broader carapace' and 'chelipeds longer than walking legs'. However, *Carupella* possesses 'nine slightly alternate large and small anterolateral teeth, the ninth being the largest' and clearly differs from *Lupocyclus* with 'distinctly alternate large and small anterolateral teeth'. Recently, De Grave *et al.,* (2009) placed both the above genera in a separate sub-family Lupocyclinae.

3.2.A.1. *Diagnosis*

Carapace subhexagonal, broader than long; metagastric and epibranchial ridges granulated, interrupted, forming the cardiac groove. Four frontal lobes, median and lateral lobes slightly subequal; lateral frontal lobes continuous with supraorbital margin. Nine anterolateral teeth, first eight teeth alternating large and small; ninth tooth longest, projecting laterally. Cheliped dactylus with large blunt tooth proximally; propodus armed with two distal and one proximal spine on the upper surface; merus with two large distal spines followed by three proximal spinules on the anterior margin.

3.2.A.2. *Description*

Carapace subhexagonal, broader than long (CW/CL = 1.3 ± 0.02). Dorsal surface of carapace longitudinally and transversely convex, microscopically granulated and sparsely pubescent; regions well marked; meso-gastric ridge granulated, interrupted in middle; meta-gastric and epibranchial ridge granulated, interrupted at cardiac groove. Frontal margin narrow (FW/CW = 0.4 ± 0.01). Margin of carapace granular, slightly in advance of external orbital teeth, divided into four lobes; median frontal lobes less prominent; notch separating

median and lateral frontal lobes indistinct (Figure 3.1A). Anterolateral margin granulated, divided into nine teeth; first eight teeth alternating large and small; ninth tooth largest, projecting laterally. Postero-lateral junction slightly curved, postero-lateral margins granulated. Posterior margin of carapace narrow, sinuous, granulated. Antennules long and cylindrical, extending beyond the frontal margin, folded obliquely in the antennulary fossae, antennules with distal tuft of hairs.

Orbits interrupted dorsally by two sutures, partially open at inner margin; orbital margin microscopically granulated, except at external orbital margin; roof and floor of orbit pubescent. Eyes globular, eye peduncle broad, short. Basal antennal segment located within orbital hiatus, antennal flagellum with 13 segments (Figure 3.1B). Pterygostomium region smooth, slightly pubescent.

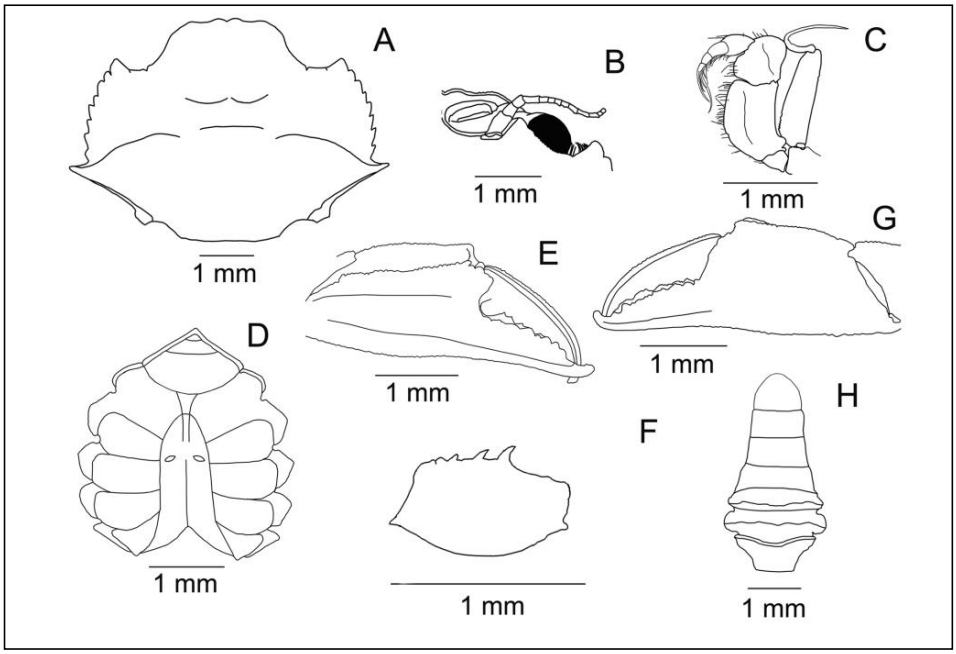

Figure 3.1. *Carupella banlaensis*. Line diagrams of (A) the dorsal surface of the carapace; (B) the ventral surface of the orbit and antennulary fossa; (C) the ventral surface of the third maxilliped; (D) the ventral surface of the sternum; (E) the outer surface of the dactylus and the propodus of the right cheliped; (F) the ventral surface of the merus of the left cheliped; (G) the outer surface of the dactylus and the propodus of the left cheliped; (H) the ventral surface of the abdomen (female).

Buccal cavity broader than long, wider anteriorly; epistome broad, with median projection into buccal cavity. Third maxillipeds not gaping, mouth parts not visible in closed position. Ischium 1.3 times longer than broad, with parallel sides, broader than merus; inner margin pubescent. Merus roughly hexagonal, as long as broad; inner margin completely pubescent. Palp short, reaches to half level of ischium, covered with long setae; distal segments progressively smaller. Exopod slender, with long flagellum (Figure 3.1C).

Thoracic sternum broad (SW/CW = 0.5 ± 0.03), its surface smooth, pitted; lateral margins microscopically granulated. First three thoracic sternites narrow, sutures 1/2 and 2/3 prominent. Suture 3/4 wide and shallow. Sternite 4 widest, medially divided by shallow anterior extension of sterno-abdominal cavity. Abdominal cavity commences from middle of sternite 4 and covers sternites 5-8. Deep groove along abdominal cavity medially divides sternites 5-8. Pair of gonopores located at sternite 5 (Figure 3.1D).

Lengths of chelipeds of larger female heterodonts less than two times CL. Dactylus of right cheliped thick, glossy, pitted, curved distally with blunt tip; dorsal surface with two ridges; cutting edge proximally armed with large blunt tooth followed by six small, sharp teeth; distal one-third portion unarmed (Figure 3.1E). Pollex thick, glossy, pitted, slightly curved distally; cutting edge with proximal depression to accommodate corresponding dactyl tooth. Fingers crossed in closed position, not gaping. Propodus granulated, its length more than two times its depth (PL/PD = 2.6); outer surface bears two granulated costae; upper surface sparsely pubescent, bears two blunt spines on distal margin trailed by granulated costae; third spine anterior to articulation with carpus (Figure 3.1E). Carpus with granulated upper surface, bears one large spine at its inner angle and two small spines at outer angle; five ridges on outer surface. Merus glossy, granulated, sparsely pubescent, anterior margin serrated, bears three large spines followed by small proximal granules; posterior margin serrated, bears distal spine (Figure 3.1F). Ischium, basis and coxa with granulated outer

margins, glossy lower margins; inner margins pubescent. Dactylus of left cheliped devoid of proximal blunt tooth (Figure 3.1G); PL/PD = 2.8. Structures and ornamentations of left dactylus, propodus, carpus, merus, ischium, basis and coxa similar to those of right cheliped. Chelipeds of smaller female in deteriorated condition.

Pereiopods slender, dorsoventrally flattened, pubescent, shorter than chelipeds. Pereiopod 2 longest, its length less than two times CL; pereiopods 3 and 4 sub-equal, pereiopod 5 smallest. P2-P4 dactyli subcylindrical, pubescent, with pointed tips, their lengths subequal to propodi; propodi subcylindrical, pubescent; carpi pubes-cent; meri longest, subcylindrical, pubescent. P5 paddle-like, its dactylus oval-shaped, dorsoventrally flattened, thickly pubescent, bearing serrated margins and ending distally in spine; propodus dorsoventrally flattened, thickly pubescent, bearing serrated margins; carpus and merus subcylindrical, pubescent. Pereiopods 2-5 of smaller female in deteriorated condition.

Abdomen narrow (AW/CW = 0.3 ± 0.04), of six somites and telson, its surface glossy and pitted. First somite narrower than second, second somite widest, somites 3-6 free, progressively narrow, sixth somite with slightly converging lateral margins; somites 2-3 with prominent transverse carinae; telson bluntly triangular (Figure 3.1H).

3.2.A.3. *Colour*

Carapace of fresh specimens light brown dorsally with dark greyish blotches on the anterior median portion; scattered dark blotches on the cardiac region (Figure 3.2A), light brown ventrally, dotted with black melanophores (Figure 3.2B).

Figure 3.2. *Carupella banlaensis*. Colour photographs of (A) the dorsal surface of the carapace and (B) the ventral surface of the carapace

Chelipeds light brown dorsally with scattered darker blotches on dactylus and propodus; inner surfaces of dactyli and propodi yellow with conspicuous reddish blotch at the base of fingers, finger tips whitish; outer surfaces of propodi, carpi and meri greyish to light brown, dotted with black melanophores; inner surfaces of meri yellowish with distal red blotch (Figure 3.2A,B). Pereiopods 2-5 light brown coloured, dotted with black melanophores and bear greyish bands on the distal four segments (Figure 3.2A, B). Colouration of formalin-preserved specimens is uniform light brown.

3.2.A.4. *Distribution and habitat*

Crabs of this genus are sporadically distributed across the Indo-West Pacific regions, namely the Gulf of Tonkin–China, Somalia (*Carupella banlaensis*), southern Japan (*C. epibranchialis*), South Africa and Madagascar (*C. natalensis*). The present observation of *C. banlaensis* along Goa, on the west coast of India, is a new distributional record for the entire South Asian region. *Carupella banlaensis* specimens were collected from clayey substratum underneath loose oyster-covered rocks in the vicinity of mangrove vegetation at low tide.

3.2.A.5. *Comparisons*

The Carupella banlaensis specimens superficially resembled juveniles of the mud crab *Scylla*, and a comparison between the two revealed the following differences.

1. Carapace of *Carupella banlaensis* specimens opaque (Figure 3.3A) as compared to the translucent carapace of *Scylla* juvenile (Figure 3.3B).
2. Low RL: CL ratio (0.2) in Carupella banlaensis specimens as compared to the *Scylla* juvenile (0.36) (Figure 3.4).
3. The frontal lobe in *Carupella banlaensis* specimens is much less prominent (Figure 3.4A) compared to that of the *Scylla* juvenile (Figure 3.4B).

Comparison of the *Carupella banlaensis* specimens with an immature *Scylla* female collected from the study area revealed that the former specimens differed from the latter by virtue of 'narrow basal antennal segment devoid of distal lobule' (Figure 3.5A), as compared to 'broad basal antennal segment possessing conspicuous distal lobule' (Figure 3.5B).

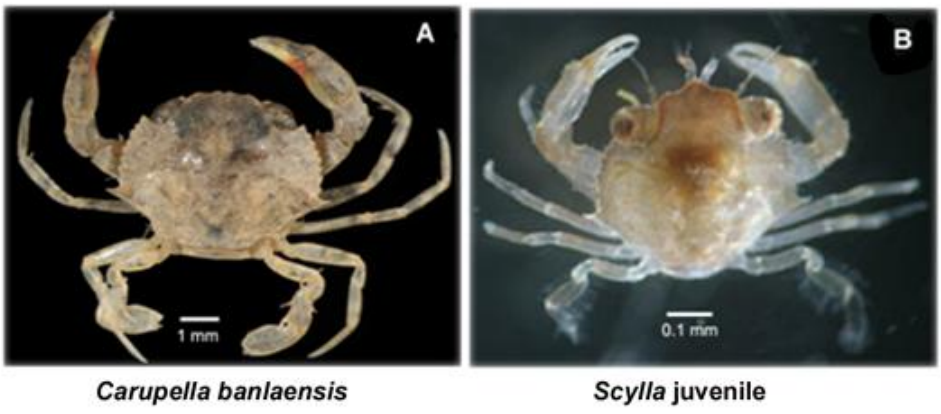

Figure 3.3. Colour photographs (live specimens) of the carapace and the appendages of (A) *Carupella banlaensis* and (B) *Scylla* juvenile

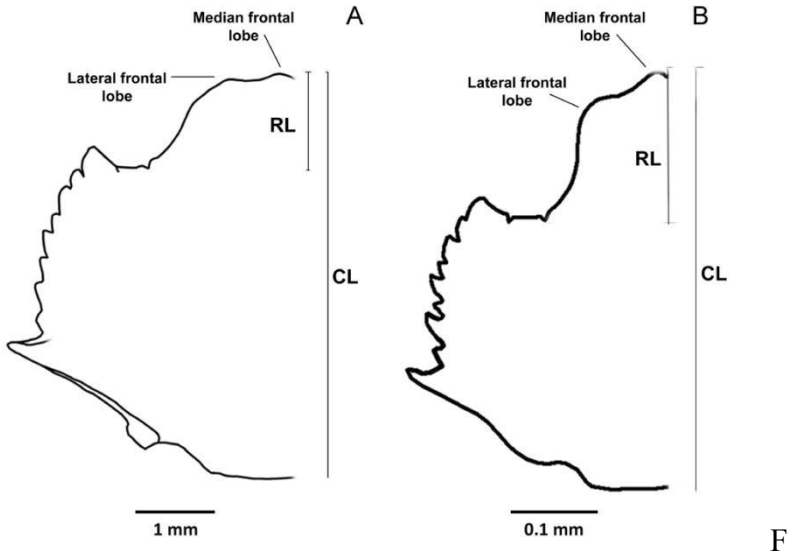

Figure 3.4. Line diagrams of dorsal surface of carapace of (A) *Carupella banlaensis* and (B) *Scylla* juvenile indicating the rostral length (RL) and the carapace length (CL)

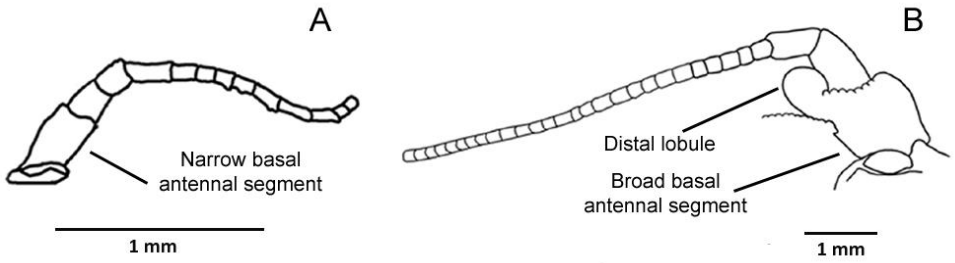

Figure 3.5. Line diagrams of the antenna (including the basal antennal segment) of (A) *Carupella banlaensis* and (B) immature *Scylla* female

The present specimens were compared with existing descriptions of three known species of the genus *Carupella* (Crosnier, 1962; Tien, 1969; Zarenkov, 1970).

1. The present specimens (Figure 3.6A) resembled Tien's (1969) diagrams of *C. banlaensis* (Figure 3.6B) by virtue of less-prominent median frontal lobes, which are separated from the lateral frontal lobes by a very shallow notch. Alternatively, the median frontal lobes of *C. epibranchialis* (Figure 3.6C) and *C. natalensis* (Figure 3.6D) are more prominent and separated from the lateral frontal lobes by a deep notch.

2. The present specimens possess ‘bluntly triangular anterolateral teeth’ (Figure 3.6A) as in *C. banlaensis* (Figure 3.6B). In *C. epibranchialis*, the first eight antero-lateral teeth are spine-like, distinctly alternating long and short (Figure 3.6C). In *C. natalensis*, the first eight anterolateral teeth decrease in size posteriorly (Figure 3.6D). The ninth anterolateral tooth is directed laterally in the present specimens (Figure 3.6A) as in *C. banlaensis* (Figure 3.6B) and C. *epibranchialis* (Figure 3.6C), whereas in *C. natalensis*, it is directed anterolaterally (Figure 3.6D).

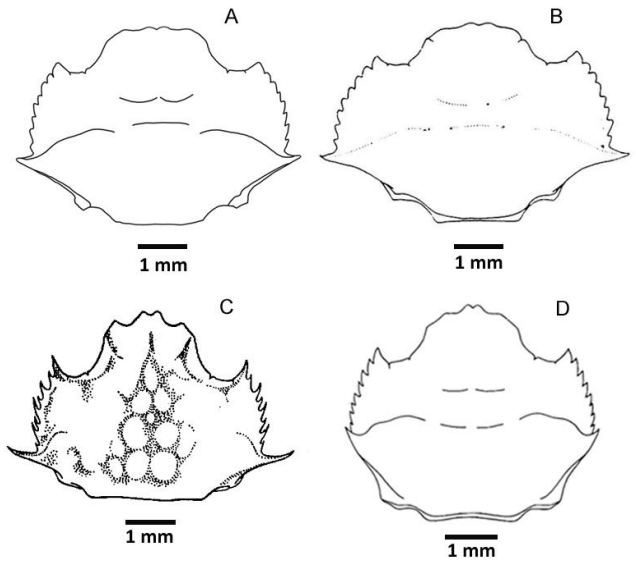

Figure 3.6. Line diagrams of the dorsal surface of the carapace of (A) *Carupella banlaensis* (present specimen), (B) *Carupella banlaensis* (type specimen – China; Tien, 1969), (C) *Carupella epibranchialis* (type specimen – East China Sea; Zarenkov, 1970) and (D) *Carupella natalensis* (Madagascar; Crosnier, 1962)

3. The present specimens possess mesogastric, meta-gastric and epibranchial ridges on the dorsal surface of the carapace (Figure 3.6A), which is similar to *C. banlaensis* (Figure 3.6B) and *C. natalensis* (Figure 3.6D); the metagastric ridge is interrupted in *C. natalensis* (Figure 3.6D). Alternatively, only a short epibranchial ridge is present in *C. epibranchialis* (Figure 3.6C).

The above comparisons revealed that the present specimens were morphologically most

similar to *C. banlaensis*. The present specimens were observed to be morphologically similar to Tien's (1969) original description of *C. banlaensis*, with the exception of the internal orbital tooth almost merging with the frontal margin, and two spines on the posterior margin of the merus of the cheliped. In the present specimens, the internal orbital tooth is indistinguishable from the frontal margin and there is one distal spine on the posterior margin of the merus of the cheliped.

The present observations describe a new distributional record of the rare portunid crab *Carupella banlaensis* from India and provide a detailed morphological description of this species complemented with illustrations and morphometric ratios. These observations indicate that *C. banlaensis* has an extremely patchy distribution within its known geographical range from China to eastern Africa.

3.2.B. Morphological Description of *Dotilla myctiroides* (H. Milne Edwards, 1852) along Goa coast.

Dotilla myctiroides (H. Milne Edwards, 1852) is small-sized crab inhabiting in sand which have high percentage of silt-clay fraction. This species is known to either occur throughout the shore, or along the lower water line and in some cases it also co-occurs with another dotillid species namely *Scopimera proxima* (Silas and Sankarankutty, 1967). The available literature reveals that Alcock (1900) and Kemp (1919) carried out the most comprehensive studies on dotillid crabs of Indian coasts and reported seven out of eight valid Dotilla species including *D. myctiroides*. Altevogt (1957) studied the biological and behavioural aspects of *D. myctiroides* from Mumbai and revealed its characteristic burrowing pattern. Chhapgar (1957) and Sankarankutty (1961, 1966) reported this species from coasts of Indian mainland and Andaman Islands and illustrated diagnostic characters including gonopod and spoon-tipped setae of second maxillipeds. Ingole (2003) reported this species

and its burrowing habit from Miramar beach, Goa. Varadharajan and Soundarapandian (2014) reported its occurrence and distribution along Tamil Nadu coast. Ali *et al.,* (2014) studied its abundance and spatio-temporal distribution patterns along Aksa beach (Mumbai) and revealed size-related differentiation in spatial distribution. From the above literature survey it is apparent that no study has been carried out on dotillid crabs along the beaches of Goa except Ingole (2003). In this view, the present study was focused at providing preliminary information on the occurrence and taxonomy of *D. myctiroides* from the region.

3.2.B.1. *Diagnosis*

Carapace as long as broad, except for the lateral grooves practically devoid of sculpture and chelipeds were at least three times of CL. Sternum with tympana on all segments. Abdomen with setal band on distal margin of fourth somite. Cheliped dactyli twice the length of propodi. Fourth pereiopod with tympanum on dorsal surface. First gonopod slightly sinuous, with blunt tip, furnished with numerous unbranched setae.

3.2.B.2. *Description*

Carapace oval, almost as broad as long (CW/CL = 0.97 ± 0.07) with rugose dorsal surface (except cardiac regions). Dorsal surface of carapace longitudinally and transversely convex, with scattered microscopic granules. Frontal margin of carapace narrow (FW/CW = 0.24 ± 0.06), unilobed, deflexed anteriorly (Figure 3.7A), its deflexed tip separates the antennulary fossa of either side. Antennules short, concealed beneath frontal margin. Antennae short, thin with eight segments. Basal antennal segment large, flagellum extremely thin, tapering comprises of seven segments (Figure 3.7B). Orbits wide, occupy approximately three-fourths of anterior carapace margin. Eyes (cornea) elongated, ocular peduncle slightly longer than cornea (Figure 3.7B). Lateral margins of carapace divergent, pubescent,

granulated in anterior half, and possess longitudinal folds along their entire length. Raised area corresponding to posterior branchial, cardiac, and intestinal regions membranous (Figure 3.7A). Posterior margin of carapace straight, broad (Figure 3.7A).

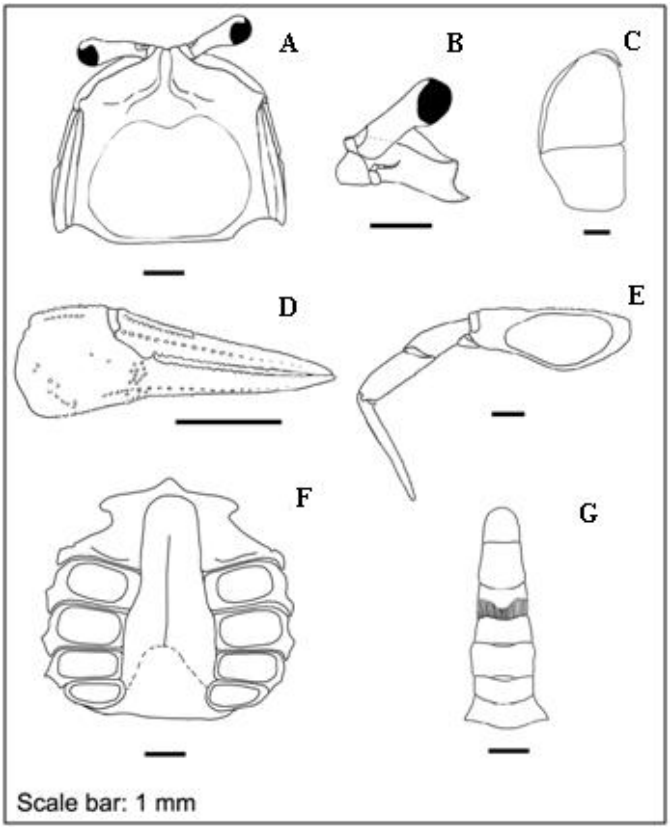

Figure 3.7. *Dotilla myctiroides* (Line diagrams): (A) Frontal margin of carapace; (B) Orbit; (C) Third maxilliped; (D) Major cheliped – dactylus and propodus; (E) Pereiopod (F) Male sternum; (G) Male abdomen

Buccal cavern broader than long, rounded anterolaterally, wider posteriorly. Epistome narrow. Third maxillipeds large, oval shaped, do not leave gape when closed; merus bluntly triangular, longer than ischium, with finely pitted glossy surface (Figure 3.7C), its length approximately twice its width (ML_{3m}/MW_{3m} = 1.86 ± 0.34); exopod extremely slender; palp articulates at middle of anterior margin of merus (Figure 3.8C). Second maxillipeds with numerous characteristic spoon-tipped setae on inner surface of merus (Figure 3.9A). Each seta

with three lobes at distal tip (Figure 3.9B), among which the middle lobe is further split into median petalloid shape lobe and two slightly curved lobes (Figure 3.9C).

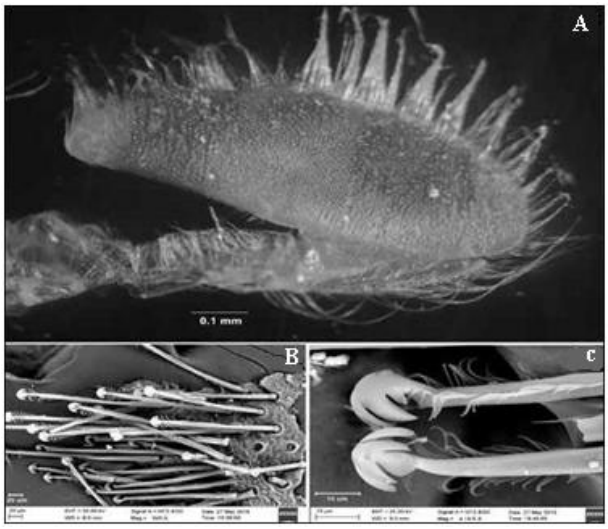

Figure 3.8. (A) Photograph of entire second maxilliped *Dotilla myctiroides*, (B) SEM photograph of section of second maxilliped showing numerous setae, (C) SEM photograph of distal portion of spoon-tipped setae

Chelipeds (P1) slender, compressed, subequal with glossy surface; their length greater than twice of CL (left cheliped ChL = 2.35 ± 0.38; right cheliped ChL = 2.38 ± 0.44). Fingers slender, slightly curved and tapering, leave a narrow gap between them (Figure 3.7D). Dactylus is longer than propodus (DL_{ch}/PL_{ch} (left) = 1.24 ± 0.41; DL_{ch}/PL_{ch} (right) = 1.25 ± 0.43)); dorsal surface covered with two longitudinal rows of serrations separated by a shallow groove; cutting edge with short serrations (Figure 3.7D). Pollex with short serrations on cutting edge. Propodus (including pollex) with single serrated ridge on dorsal surface, ventral surface with two serrated ridges, which converge at distal tip of pollex (Figure 3.7D). Inner surface of propodus smooth and glossy, outer surface granular (Figure 3.7D). Carpus large, with smooth glossy surface and serrated ridge on upper surface flanked with silken setae, outer surface granular. Merus larger than carpus, ornamentation similar to that of carpus; its inner surface with tympanum. Ischium, basis and coxa fused.

P2-P5 slender, shorter than chelipeds. P2 longest, approximately twice CL (PrL/CL = 2.08 ± 0.34), P3-5 subequal. Pereiopod dactyli slender, normal, shorter than propodi, terminate distally in acute tip (Figure 3.7E). Pereiopod meri with characteristic elongated tympana (Figure 3.7E). Margin of tympanic membrane marked with depressed granules.

Thoracic sternum smooth, glossy, narrow (SW/CW = 0.44 ± 0.08), eight segmented, all segments possess tympana (Figure 3.7F). Sternal sutures continuous (Figure 3.7F). Sterno-abdominal cavity present (Figure 3.7F). Male abdomen narrow (AW/CW = 0.40 ± 0.05), comprised of seven distinct, narrow segments; fourth segment overlapping fifth, with a thick brush of hairs at distal end (Figure 3.7G).

G1 slightly sinuous, lacks ornamentation (Figure 3.9A). Its proximal half covered with long unbranched setae on outer margin (Figure 3.8A). Distal tip blunt, covered with tuft of numerous long unbranched setae at outer margin (Figure 3.9B). Details of morphological measurements (μ ± σ) and morphometric ratios (μ ± σ) are provided in Tables 3.2 and 3.3, respectively.

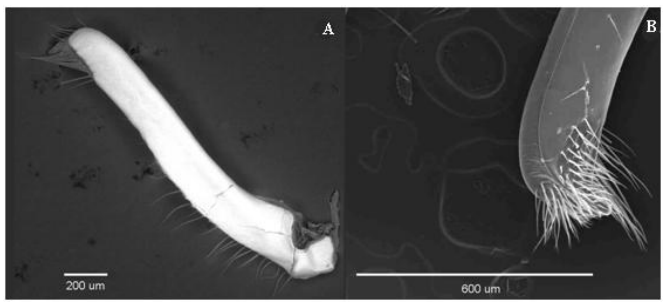

Figure 3.9. SEM photographs of (A) entire G1 (B) distal half of G1 of *Dotilla myctiroides*

Table 3.3. Morphological measurements for *Dotilla myctiroides* (N=49)

S. No.	Body part	Morphological parameters	Data (μ ± σ)
1	Carapace	CW	0.53 ± 0.05
		CL	0.55 ± 0.05
		FW	0.13 ± 0.04
		SW	0.24 ± 0.07
		AW	0.21 ± 0.01

2	Third Maxillipeds	ML3m	0.48 ± 0.10
		MW3m	0.26 ± 0.05
3	Chelipeds	DL_{ch}(Left)	0.41 ± 0.10
		PL_{ch}(Left)	0.37 ± 0.13
		ChL(Left)	1.28 ± 0.19
		DL_{ch}(Right)	0.41 ± 0.10
		PL_{ch}(Right)	0.37 ± 0.14
		ChL(Right)	1.29 ± 0.22
4	Pereiopods	PrL2	1.13 ± 0.17
		PrL3	1.04 ± 0.05
		PrL4	1.03 ± 0.07
		PrL5	1.06 ± 0.03

Table 3.4. Morphometric ratios for *Dotilla myctiroides* (N = 49)

S. No.	Body part	Morphometric ratios	Data (μ ± σ)
1	Carapace	CW/CL	0.97 ± 0.07
		FW/CW	0.24 ± 0.06
		AW/CW	0.40 ± 0.05
		SW/CW	0.44 ± 0.08
2	Third Maxillipeds	ML3m/MW3m	1.86 ± 0.34
3	Chelipeds	ChL(left)/CL	2.35 ± 0.38
		DLch(left)/PLch(left)	1.24 ± 0.41
		DLch(right)/PLch(right)	1.25 ± 0.43
4	Pereiopods	PrL2/CL	2.08 ± 0.34
		PrL3/CL	1.92 ± 0.19
		PrL4/CL	1.89 ± 0.23
		PrL5/CL	1.95 ± 0.17

3.2.B.3. *Colour*

Colouration of fresh specimens is dirty yellow dorsally with scattered melanophores (Figure 3.10A), raised area corresponding to posterior branchial, cardiac, and intestinal regions brown coloured (Figure 3.10A); ventral surface of sternum and abdomen whitish with scattered melanophores (Figure 3.10B). Fingers off-white, with scattered white, orange,

yellow speckles and melanophores (Figure 3.10A, B). Colouration of pereiopods similar to that of carapace (Figure 3.10A, B). Formalin preserved specimens appear whitish yellow.

Figure 3.10. Coloured photograph of (A) dorsal view, (B) ventral view of fresh specimen of *Dotilla myctiroides*

3.2.B.4. *Habitat and habit*

D. myctiroides was observed to inhabit in sandy substratum that was subjected to alternate submergence and exposure. This species was observed to live in small sized burrows. Further, observations on its behaviour revealed that it undertook foraging movements in the close vicinity of burrow, and left characteristic pseudofaecal pellets radially around the burrow. The mean density of occurrence was 24 individuals /m^2.

3.3. Discussion

The present study attempts to develop a strengthened scientific database on the occurrence of crabs from the region through continuous monitoring of crabs in five different habitats through sampling. The sampling was conducted along diverse habitats such as intertidal rocky shores and mangrove associated habitats with various substrates like soft sand, pebbles mixed sand, mud, clay and also oyster with algal encrusted rocks. From the collected samples, a total of 28 crab species were identified from intertidal regions of Goa coast.

A new report *Carupella banlaensis* (Tien, 1969) recorded for the first time from India and *Dotilla myctiroides* (H. Milne Edwards, 1852) reported from Goa coast suggesting the importance of the region with respect to diversity. The species above mentioned are reported in the present study for the first time in intertidal zones of Goa coast. The data collected during the study period with respect to new crab species enables to create a stronger database of the region. Hence, continuous assessment of these habitats is required to have a better insight of these crabs.

The present study has made an attempt to provide a comprehensive morphological description of new distributional record of rare portunid crab *Carupella banlaensis* from India. The observations indicate that *C. banlaensis* has an extremely patchy distribution within its known geographical range from China to eastern Africa.

Also an attempt was made to provide morphological description of species *Dotilla myctiroides* from Goa coast. The present observations on the morphology of carapace and pereiopods suggested that body shape enables the crab to dig burrows efficiently and protect itself from extreme environmental conditions influencing its habitat (Takeda *et al.,* 1996). Moreover, the spoon-tipped setae on the second maxillipeds in combination with hairs on first maxillipeds enable sorting of organic matter from sand particles for ingestion (Vogel, 1984). Observations on the habitat of this species suggested that it inhabited the well-drained sandy

substratum at mid and low tide level. This observation is also in agreement with the previous published literature (Silas and Sankarankutty, 1967).

Chapter 4

Spatial and temporal variations of few selected species of crabs

4.1. Introduction

The estuarine intertidal zones are considered as one of the gradient of physical and chemical parameters. The variations in such zones mainly occur due to the inputs of organic matter, estuarine hydrology and environmental factors influencing species distribution and abundance (Bergamino & Richoux, 2015). However, intertidal zones are recognized owing to various types of habitats formed across the shore due to the rise and fall of tides. Crustaceans are one of the significant groups of tropical benthic communities in these intertidal zones that are adapted to harsh environmental conditions. Among these, crabs are conspicuous and most abundant epibenthic organisms in shallow coastal waters. These organisms either crawl or burrow into the soft moist substratum (Mathieson and Berry, 1997). These brachyuran crabs are bio-energetically important faunal group which play important role in maintaining the ecosystem and also one of the most diverse groups of organisms inhabiting intertidal habitats (Ravichandran *et al.,* 2007). Brachyuran crabs among the estuarine fauna are important due to their abundance and species richness. Inhabiting various niches such as rock crevices, underneath algal encrusted rocks on moist sandy or muddy substratum and tidal pools and have important role in recycling nutrient, detritus formation and dynamics of ecosystem (Khan and Ravichandran, 2008). They are dominant and inhabit various estuarine habitats where salinity and temperature fluctuates dramatically (Ng *et al.,* 2008).

A total of 226 brachyuran crab species have been reported from the west coast of India belonging to 130 genera and 39 families (Josileen, 2015). Published litereture (Chhapgar, 1957a,b; Haragi *et al.,* 2010; Trivedi *et al.,* 2012; Pawar, 2017) suggest that efforts have been made to evaluate the brachyuran crab fauna of west coast of India. In Goa, much of the work has been carried out on taxonomy aspects by Padate *et al.,* (2010, 2013); Velip and Rivonker (2014); Kaullysing *et al.,* (2015); Vijaylaxmi *et al.,* (2015); Komarpant *et al.,* (2018). Moreover, the available literature reveals that most of the work support taxonomic

aspects of brachyuran crabs in Goa. Hence, the study on these organisms is significant to understand their role in ecosystem in view of the threat to their habitats.

The estuarine systems are known as the nursery grounds of many marine organisms, however due to the human activities these habitats are threatened and exposed to wide range of anthropogenic impacts. In recent days, the development of harbour results in increasing of artificial rocky shores, owing to such drastic changes in the intertidal ecosystem attracts many ecologists. Artificial habitats in comparison to the natural habitats provide different types of environment within the habitat like rock crevices, tidal pools due to tidal influence (Cha *et al.,* 2013). Moreover, the abundance and distribution of crab species diversity may vary in different habitats and within the habitats due to the often fluctuating tide and seasons (Kent and McGuinness, 2010).

Species diversity and richness are associated with the constancy of a community, and ecologists have often examined the benthic communities as ecological indicators in order to measure natural and anthropogenic impacts (Lui *et al.,* 2007). These benthic organisms in the intertidal rocky shore are mainly influenced by the environmental factors like the temperature, substratum and hydrodynamic forces. Benthic invertebrates are represented as an important group influencing the energy flow in the ecosystem (Nordhaus *et al.,* 2009). The community structure of the species provides the information based on the relationship with environmental variables and the distribution patterns. Hence, the spatio-temporal studies are essential for understanding of the biodiversity of a habitat, also known as the major characteristics of ecological systems (Kumar & Wesley, 2010). Hence, the present work is undertaken to study the spatio-temporal variations of these crabs from few selected habitats of Goa.

4.2. Methodology

4.2.1. Data analysis

The data were analysed by using PRIMER version 6.1.10 software software (Plymouth Routines in Multivariate Ecological Researches version 6) (Clarke & Gorley 2006) for analysing the species composition, spatial and temporal wise using Bray - Curtis coefficients (Bray - Curtis, 1957). Further, the significance of the cluster was tested by similarity profile SIMPROF analysis and similarity percentage by SIMPER analysis. The data was also analysed for Species diversity indices reflected by Shannon-Wiener diversity (H'), Margalef's species richness (S) and Pielou's species evenness (J') measures. ANOVA for two-factor without replication was analysed for crab population among station and season were computed using PRIMER version 6.1.10 software.

4.3. Results

4.2.1. *Similarity of species composition between the study sites*

The analysis of habitat with respect to species composition of 29 identified taxa was examined using Bray - Curtis similarity index. The station wise cluster analysis revealed 2 groups (Group 1 and 2) composed of 5 sampling stations (Figure 4.1). Group 1 showed below 50% similarity with Group 2.

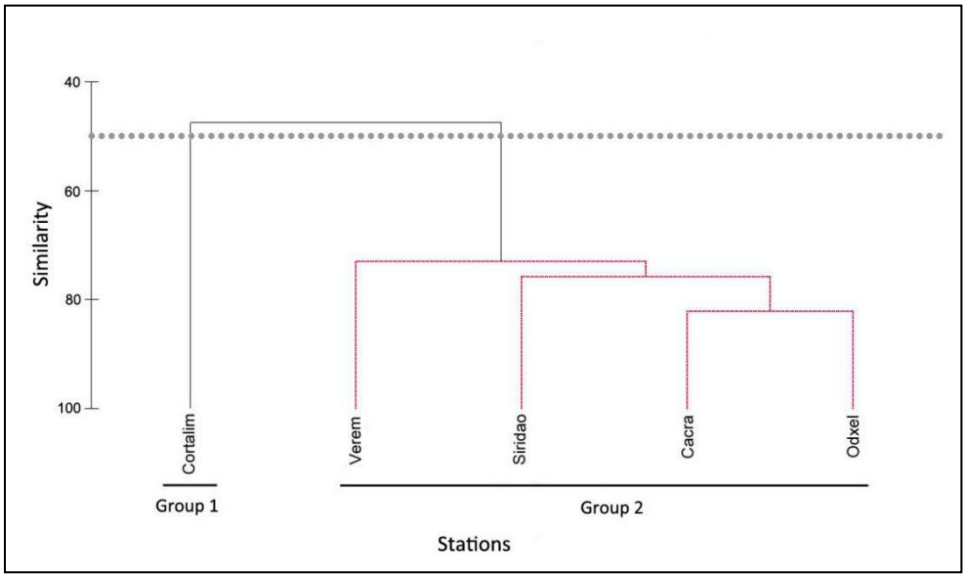

Figure 4.1. Cluster dendrogram of five sampling stations using Bray – Curtis similarity coefficient

Group 1 comprised of one station which is mangrove associated, formed of several habitats such as sandy, muddy, clayey areas, loose rocky patch, algal deposition, oyster rocks and mangrove vegetation forming a highly heterogeneous habitat and frequent fluctuation in environmental conditions. Hence, Group 1 was found to be highly productive with high number of mud associated species due to the presence of mangrove vegetation. However, Group 1 does not support rock crabs species (Table 4.1). As some of the crab species are site specific like *L. exaratus, E. frontalis* and *O. rugulosus* and are restricted to marine rocky habitat.

Group 2 comprised of four rocky stations representing large laterite boulders and sandy substratum throughout the beach stretch. Among these two stations namely Verem and Siridao comprised of partial mangrove vegetation with sandy and muddy substrate while, the station Cacra and Odxel covered with only sandy substratum. The stations in Group 2 are similar in terms of the habitat heterogeneity limiting with 5 rare species which are adapted to mangrove habitats and muddy substrate (Table 4.1).

Table 4.1. Contribution of species in Group 1 and Group 2

S. No	Species	Group 1	Group 2	Average dissimilarity	Standard Deviation	Contribution %
		Average abundance	Average abundance			
1	*Leptodius exaratus*	0	5.97	7.36	7.75	14.06
2	*Epixanthus frontalis*	0	4.7	5.75	2.91	10.98
3	*Heteropanope glabra*	6.86	2.3	5.55	2.98	10.58
4	*Nanosesarma andersonii*	3.76	0.17	4.4	12.26	8.41
5	*Nanosesarma pontianacense*	3.78	0.35	4.24	6.74	8.09
6	*Neorhynchoplax demeloi*	2.4	0	2.95	17.52	5.63
7	*Thalamita crenata*	2.64	4.56	2.38	4.54	4.54
8	*Ozius rugulosus*	0	1.59	1.95	1.19	3.72

9	*Scylla olivacea*	2.08	0.52	1.94	2.17	3.7
10	*Pseudograpsus elongatus*	2.2	1.04	1.81	2.05	3.45
11	*Illyoplax sp.*	1.39	0	1.71	17.52	3.26
12	*Nanosesarma minutum*	2.56	3.95	1.66	1.29	3.16
13	*Macrophthalmusconvexus*	1.1	0	1.35	17.52	2.58
14	*Carupella banlaensis*	1.1	0	1.35	17.52	2.58
15	*Pseudograpsus intermedius*	1.1	0	1.35	17.52	2.58
16	*Myomenippe hardwickii*	1.1	0.52	1.32	11.81	2.51
17	*Uca (Paraleptuca) annulipes*	2.2	1.57	1.11	0.92	2.11

Further analysis of numerical data using Bray - Curtis similarity index of Group 1 revealed 13 crab species as cluster in Group 1 and 16 species in Group 2 (Figure 4.2). The crab species belonging to Group 1 are the rarely occurring crab species with 100% similarity, among which 7 species occurred during pre-monsoon at Verem and Siridao, 3 species during post-monsoon at Cortalim and Siridao and 3 species during monsoon at Cortalim (Figure 4.3A,B).

Further, Group 2 was divided into subgroup A and B. Subgroup A comprised of 6 species which are most abundant and frequently occurring throughout the year whereas subgroup B with 10 species which are relatively low in abundance and occurrence and showed more than 60% similarity among each other (Figure 4.2).

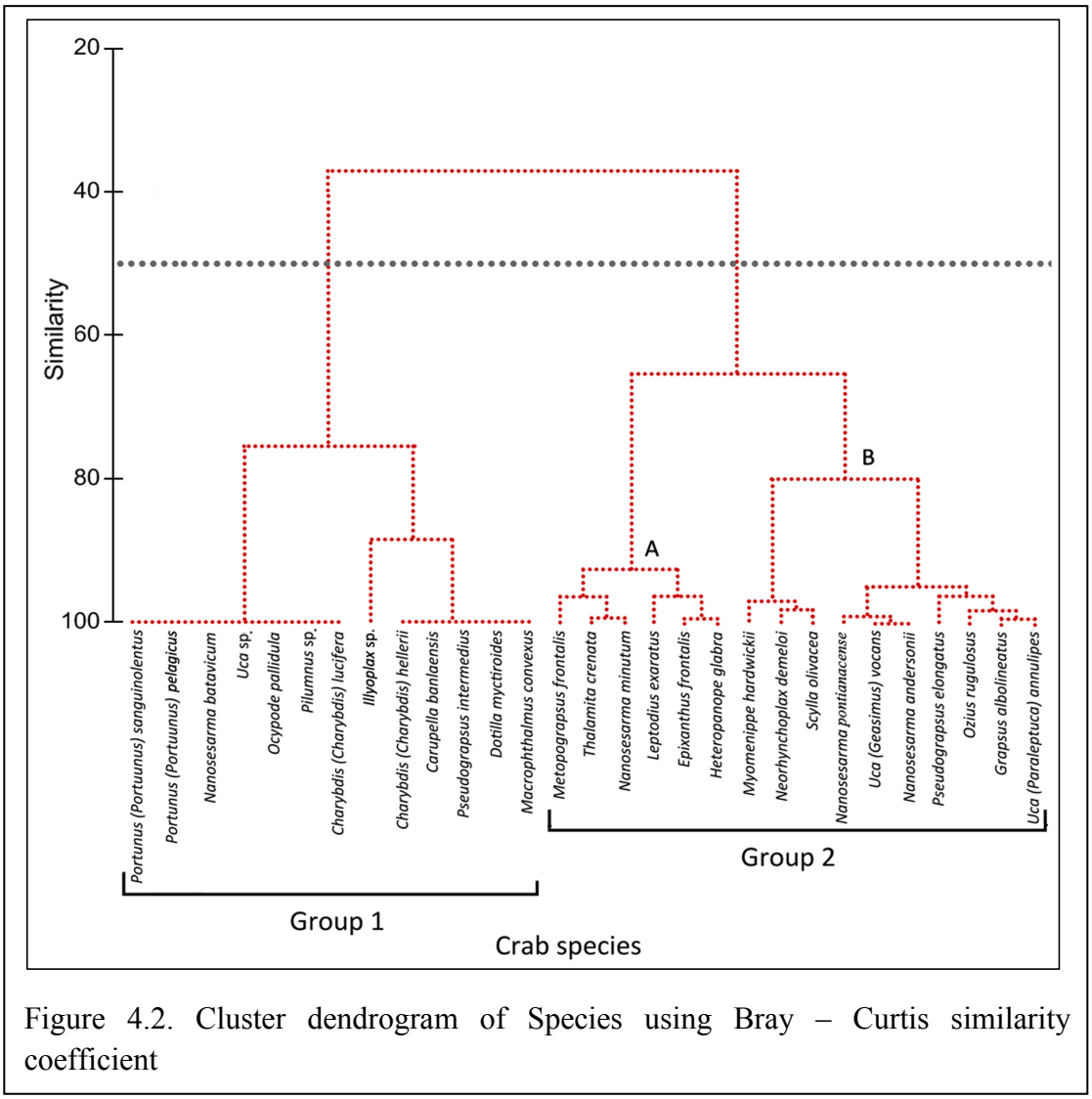

Figure 4.2. Cluster dendrogram of Species using Bray – Curtis similarity coefficient

The abundant and commonly occurring species of subgroup A namely *Metopograpsus frontalis*, *Thalamita crenata* and *Nanosesarma minutum* was observed in all the 5 stations whereas, *Leptodius exaratus*, *Epixanthus frontalis* and *Heteropanope glabra* occurred only at 4 stations (Figure 4.4A,B).

However, the species of subgroup B namely *Myomenippe hardwickii* (2 stations), *Neorhynchoplax demeloi* (1 station) and *Scylla olivacea* (3 stations) were least abundant and occurred in two seasons (post-monsoon and pre-monsoon). The species *Nanosesarma pontianacense* (3 stations), *Nanosesarma andersonii* (2 stations), Ozius *rugulosus* (3 stations),

Grapsus albolineatus (5 stations) occurred in all the 3 seasons whereas *Uca (Gelasimus) vocans* (5 stations) and *Uca (Paraleptuca) annulipes* (4 stations) occurred only during pre-monsoon and species *Pseudograpsus elongatus* (4 stations) only during monsoon season (Figure 4.5A,B).

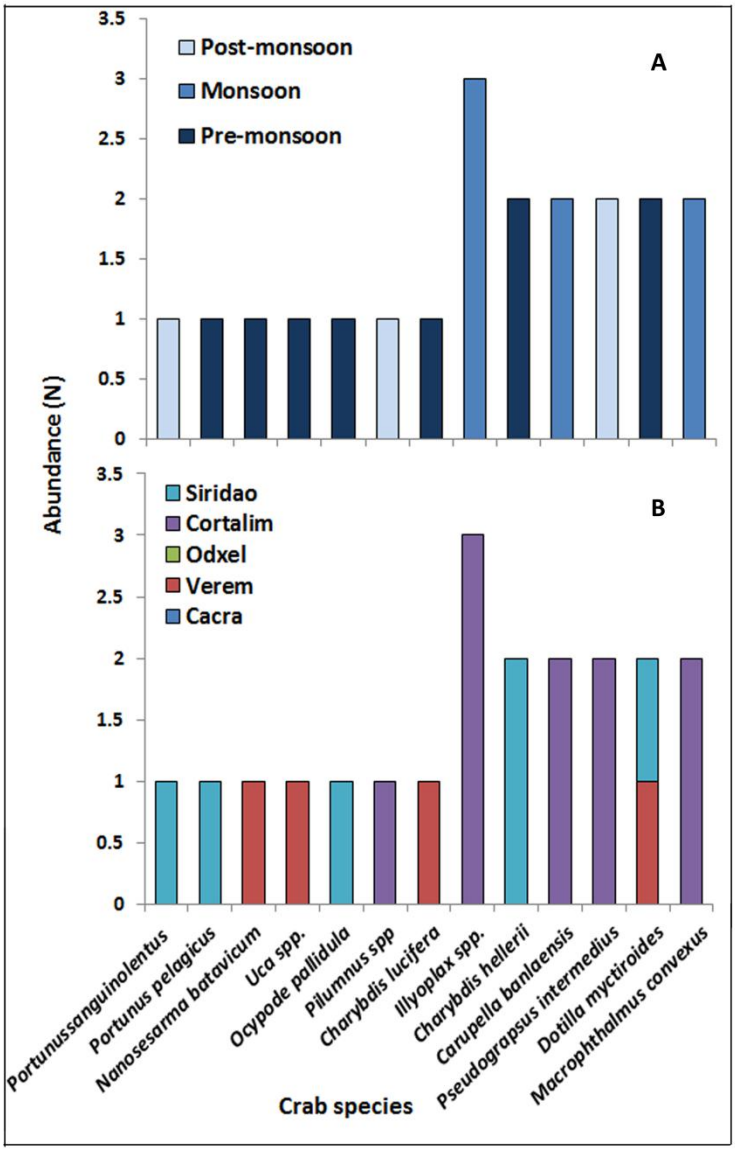

Figure 4.3. (A) Seasonal and (B) Spatial variations of rarely occurring crab species

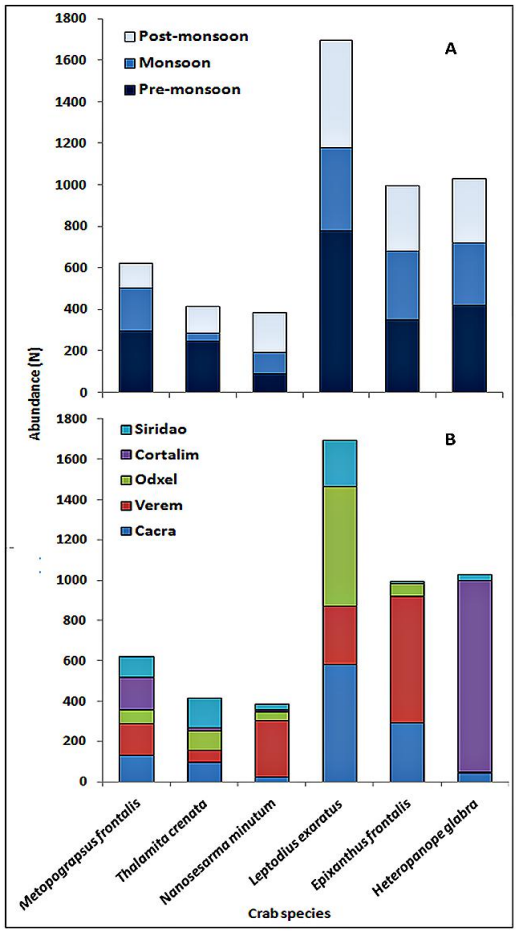

Figure 4.4. (A) Seasonal and (B) Spatial variations of frequently and commonly occurring crab species

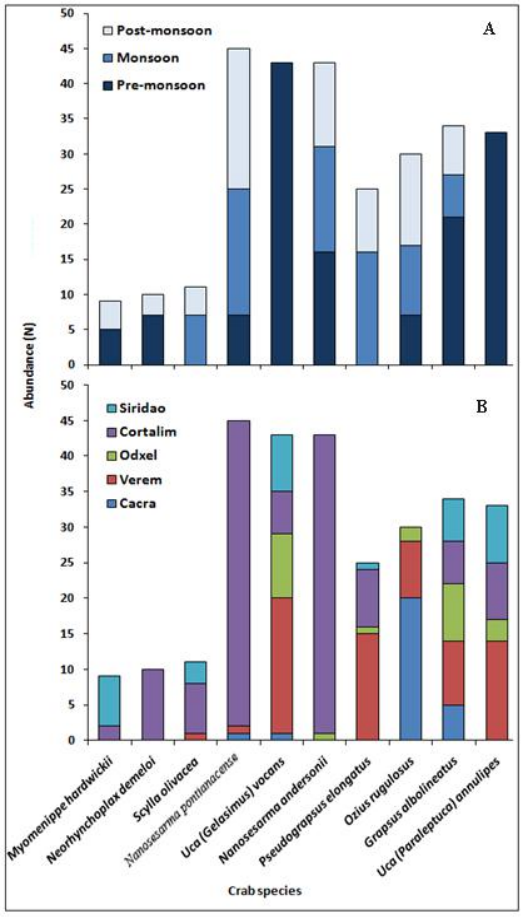

Figure 4.5. (A) Seasonal and (B) Spatial variation of less abundance crab species

4.3.1. *Species Diversity Indices*

A change in crab community structure was reflected by species diversity indices (diversity, richness and evenness) between stations and seasons.

4.3.1.a. *Species Diversity*

The computation of species diversity based on the data obtained is depicted in Figure 4.6a. A higher value of species diversity was observed at Verem during pre-monsoon season where as Cortalim showed minimum diversity in monsoon season.

4.3.1.b. *Species Richness*

The computation of species richness based on the data obtained is depicted in Figure 4.6b. Among stations, pre-monsoon season displayed a higher value of species richness at Siridao whereas minimum richness was observed in Cortalim in the same season.

4.3.1.c. *Species Evenness*

The computation of species evenness based on the data obtained is depicted in Figure 4.6c. Among stations, a higher value of species evenness was found at Siridao during pre-monsoon whereas post-monsoon season showed minimum evenness at Cortalim.

Highest diversity, richness and evenness value was witnessed amongst sites (Table 4.2). The station wise numerical abundance at Verem (N=1490) was observed to be the highest compared to other stations. The observation also revealed that pre-monsoon season is favourable, supporting high species diversity indices.

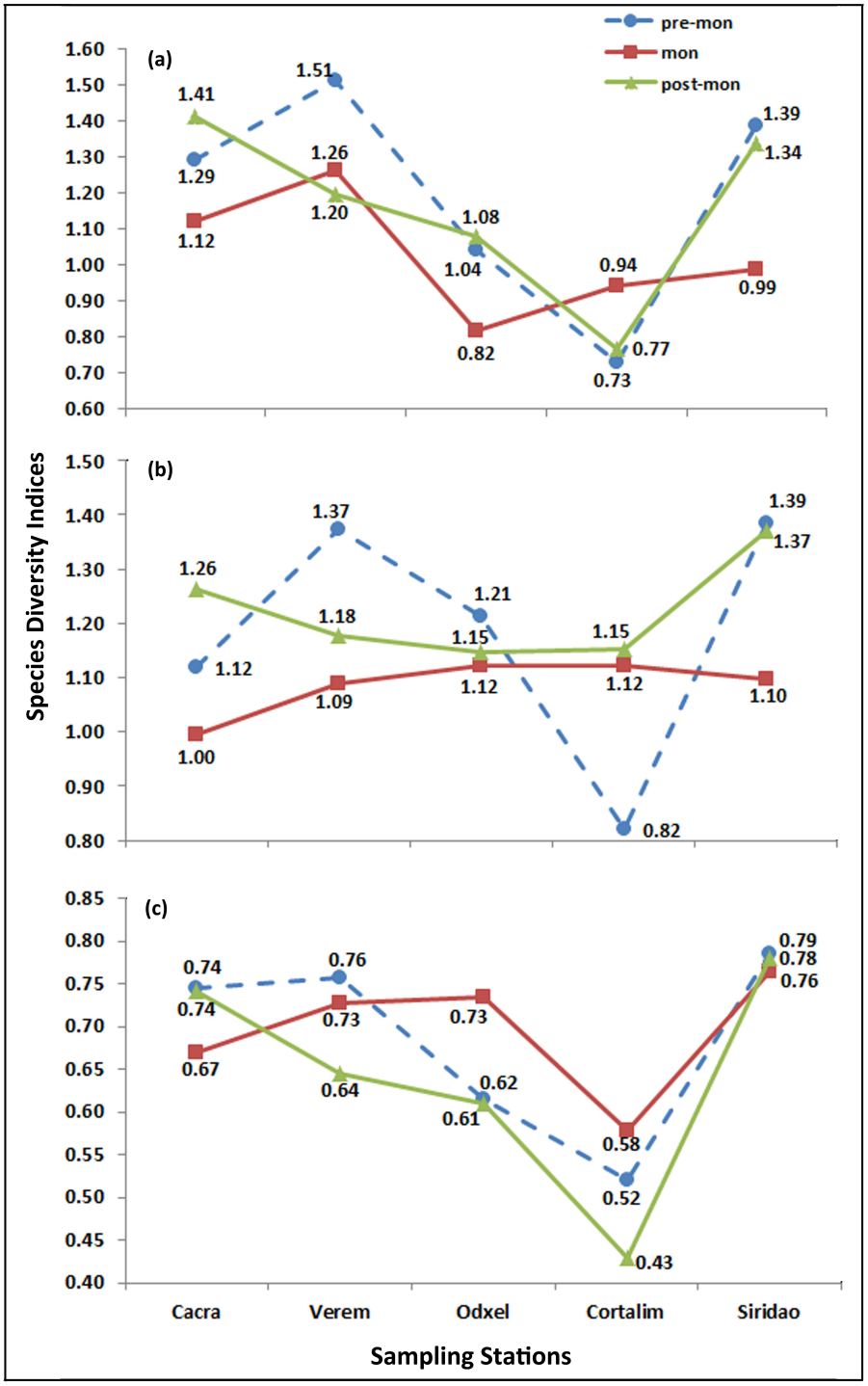

Figure 4.6 Spatial and temporal variations at five stations in (a) Shannon-Wiener diversity, (b) Margalef's species richness and (c) Pielou's species evenness measures

Table 4.2. Abundance of rocky shore Brachyuran crabs at five sampling stations

S. No.	Family/ Species	Cacra	Verem	Odxel	Cortalim	Siridao	N
	Portunidae						
1	*Carupella banlaensis*	0	0	0	2	0	2
2	*Portunus (Portunus) sanguinolentus*	0	0	0	0	1	1
3	*Portunus (Portunus) peagicus*	0	0	0	0	1	1
4	*Scylla olivacea*	0	1	0	7	3	11
5	*Charybdis (Charybdis) hellerii*	0	0	0	0	2	2
6	*Charybdis (Charybdis) lucifera*	0	1	0	0	0	1
7	*Thalamita crenata*	96	58	99	13	146	412
	Ocypodidae						
8	*Ocypode pallidula*	0	0	0	0	1	1
9	*Uca (Gelasimus) vocans*	1	19	9	6	8	43
10	*Uca (Paraleptuca) annulipes*	0	14	3	8	8	33
11	*Uca* sp.	0	1	0	0	0	1
	Sesarmidae						
12	*Nanosesarma andersonii*	0	0	1	42	0	43
13	*Nanosesarma batavicum*	0	1	0	0	0	1
14	*Nanosesarma minutum*	21	283	41	12	27	384
15	*Nanosesarma pontianacense*	1	1	0	43	0	45
	Dotillidae						
16	*Dotilla myctiroides*	0	1	0	0	1	2
17	*Illyoplax*sp.	0	0	0	3	0	3
	Grapsidae						
18	*Grapsus albolineatus*	5	9	8	6	6	34
19	*Metopograpsus frontalis*	132	156	66	161	104	619
	Oziidae						
20	*Epixanthus frontalis*	290	631	61	0	12	994
21	*Ozius rugulosus*	20	8	2	0	0	30
	Pilumnidae						
22	*Heteropanope glabra*	42	0	6	949	32	1029
23	*Pilumnus* sp.	0	0	0	1	0	1
	Varunidae						
24	*Pseudograpsus elongatus*	0	15	1	8	1	25
25	*Pseudograpsus intermedius*	0	0	0	2	0	2
	Hymenosomatidae						
26	*Neorhynchoplax demeloi*	0	0	0	10	0	10

	Menippidae						
27	*Myomenippe hardwickii*	0	0	0	2	7	9
	Macrophthalmidae						
28	*Macrophthalmus convexus*	0	0	0	2	0	2
	Xanthidae						
29	*Leptodius exaratus*	580	291	591	0	232	1694
Station wise abundance		1188	1490	888	1277	592	5435
No. of species station wise		10	16	12	18	17	-

4.3.2. *Temporal variation*

The temporal plot reveals station wise abundance of overall brachyuran crab population (Figure 4.7). The highest crab abundance was observed during pre-monsoon season in Verem whereas minimal abundance was observed during monsoon season at Odxel. The relationships between stations and seasons were further analyzed statistically using analysis of variance (ANOVA) two-factor without replication. The results for crab populations showed significant variations with respect to stations and seasons ($P<0.05$) (Table 4.3).

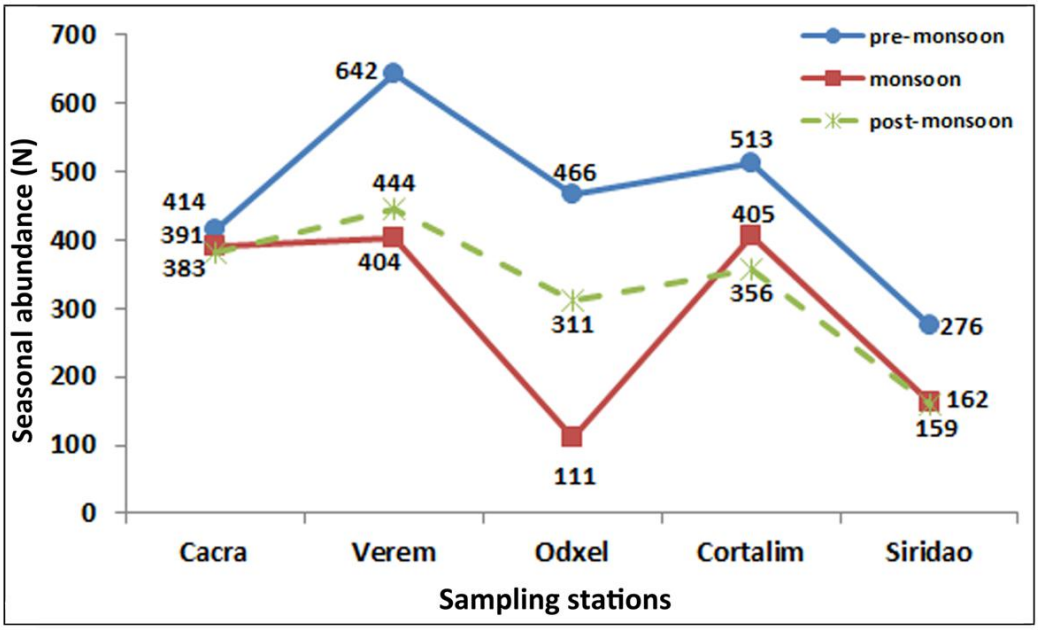

Figure 4.7. Overall seasonal abundance of five sampling stations

Table 4.3. Seasonal and spatial difference in crab population using two - way ANOVA

Source of Variation	*Sum of squares (SS)*	*Degrees of freedom (d.f.)*	*Mean squares (MS)*	*F- value*	*P- value*	*F critical*
Months	36568	17	2151.06	5.5493	1.48E-07	1.77518
Stations	27462.6	4	6865.65	17.712	5.28E-10	2.50662
Error	26358.6	68	387.627	-	-	-
Total	90389.2	89	-	-	-	-

4.3.3. *Size frequency*

The size frequency plot revealed that the CW of male and female size group from 0.5-2.5 cm were most abundant and active group of crabs occurred in abundance during pre-monsoon and least during monsoon season mainly represented by adult males, ovigerous females and non- ovigerous females (Figure 4.8).

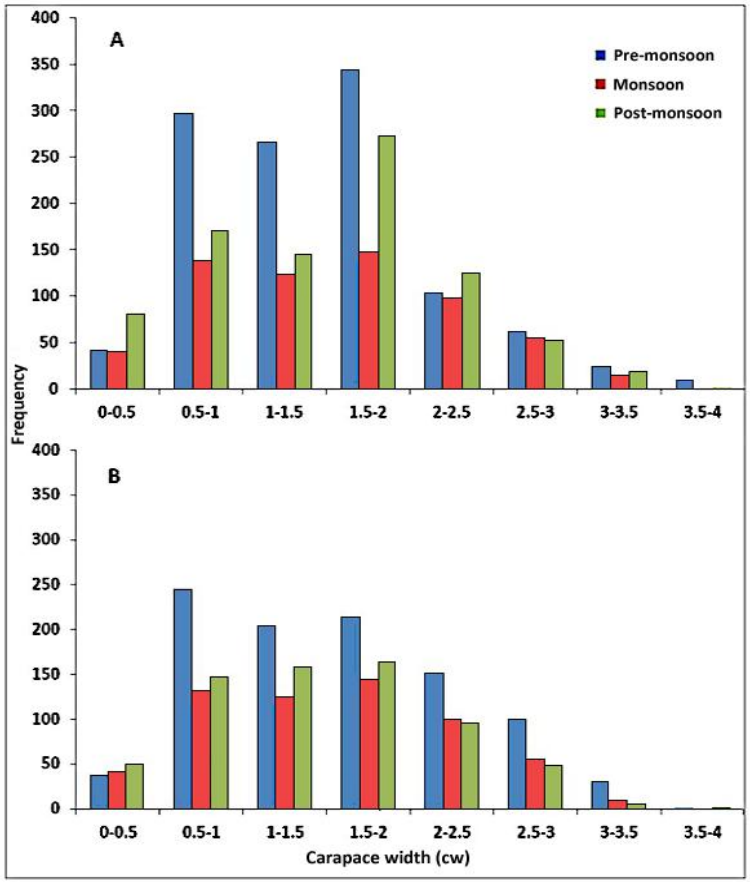

Figure 4.8. Seasonal variation in overall size frequency of (A) male and (B) female crab species

The larger size crabs of size range 2.5-4.00 cm CW were least in abundance as they were observed hidden in the large rock crevices or underneath the rocks, tidal pools or at sub tidal regions or remain borrowed in the sediment. The plot also revealed that male (Figure 4.8A) were dominant and larger in size compared to females (Figure 4.8B). The family wise length range (cm) of total sample of 29 crabs species containing male, female and ovigerous females reported in the present study is provided in the Table 4.4.

Table.4.4. Length range of male, female and ovigerous female crab species used in the present study.

S. No.	Family/ Species	**Length (cm)**		
		M	**F**	**F(G)**
	Portunidae	-	-	-
1	*Carupella banlaensis*	-	0.26-0.44	-
2	*Portunus (Portunus) sanguinolentus*	-	0.59*	-
3	*Portunus (Portunus) pelagicus*	2.57*	-	-
4	*Scylla olivacea*	1.00-3.57	1.07-3.08	-
5	*Charybdis (Charybdis) hellerii*	1.79*	2.59*	-
6	*Charybdis (Charybdis) lucifera*	5.07*	-	-
7	*Thalamita crenata*	0.44-3.58	0.60-3.93	2.10*
	Ocypodidae	-	-	-
8	*Ocypode pallidula*	0.90*	-	-
9	*Uca (Gelasimus) vocans*	0.30-1.47	0.26-1.39	-
10	*Uca (Paraleptuca) annulipes*	0.31-1.78	0.51-0.62	-
11	*Uca* sp.	-	0.66*	-
	Sesarmidae	-	-	-
12	*Nanosesarma andersonii*	0.28-1.37	0.31-0.55	0.42-0.6
13	*Nanosesarma batavicum*	0.53*		
14	*Nanosesarma minutum*	0.22-1.5	0.25-1.32	0.22-0.55
15	*Nanosesarma pontianacense*	0.27-1.49	0.28-1.42	0.4-0.69
	Dotillidae	-	-	-
16	*Dotilla myctiroides*	0.28-0.80	-	-
17	*Illyoplax* sp.	0.23-0.39	-	-
	Grapsidae	-	-	-
18	*Grapsus albolineatus*	1.07-5.30	0.96-4.77	2.14*
19	*Metopograpsus frontalis*	0.41-1.99	0.45-1.94	0.75-1.95
	Oziidae	-	-	-
20	*Epixanthus frontalis*	0.44-2.62	0.09-2.58	1.19-2.00
21	*Ozius rugulosus*	1.62-3.50	1.20-3.50	2.71-3.30

	Pilumnidae	-	-	-
22	*Heteropanope glabra*	0.26-1.93	0.30-1.69	0.27-1.66
23	*Pilumnus* sp	1.40*	-	-
	Varunidae	-	-	-
24	*Pseudograpsus elongatus*	0.60-0.94	0.46-0.96	0.63-0.99
25	*Pseudograpsus intermedius*	1.04*	1.01*	-
	Hymenosomatidae	-	-	-
26	*Neorhynchoplax demeloi*	0.27-0.40	0.38-0.44	0.47*
	Menippidae	-	-	-
27	*Myomenippe hardwickii*	1.60-2.92	1.30-3.68	-
	Macrophthalmidae	-	-	-
28	*Macrophthalmus convexus*	0.90-1.30	-	-
	Xanthidae	-	-	-
29	*Leptodius exaratus*	-	0.50-1.98	0.40-1.99

*Only one specimen was available/ collected during the study period.

4.4. Discussion

Bray-Curtis similarity index among stations clustered into rocky, partially mangrove and mangrove habitat. Among five sampling stations Cacra and Odxel were made up of weathered laterite rocks, steep rocky cliffs and rock pool with sandy substratum and discoloured broken shells strewed throughout the beach. Also, large number of gastropods and barnacles were attached on lateritic boulders. Verem and Siridao station showed similar habitat characteristics as of Cacra and Odxel. In addition, there is patchy mangrove vegetation defining partially mangrove habitat. Cortalim sampling station was marked with thick mangrove vegetation, mangrove litter, sandy muddy and clay substratum with algal deposits on oyster rocks that perfectly defines mangrove habitat.

The index among species showed cluster of rare species, less frequently and frequently occurring group of species. The sediment composition, characteristic of habitat, physical parameters and availability of food are the determining factors for distribution and abundance of crabs (Jones, 1976; Ravichandran *et al.,* 2007, Pereira *et al.,* 2014). The abundance and occurrence of crab population is probably due to the availability of the habitat which

influences the survival and fitness of the organisms in several ways like, structurally complex habitat significantly provides appropriate shelter which reduces desiccation and osmotic fluctuations (Lohrer *et al.,* 2000). Further, organisms with suitable shelter are less susceptible to predation. In addition, complex habitats tend to provide greater quantity of food and accumulate organic detritus and play an important role in numerous processes like competition in organisms, wave stress, growth, moulting and larval settlement (Connell, 1972; Peterson, 1991; Moksnes *et al.,* 1998; McGaw, 2001). The heterogeneity of rocky habitats is formed due to the numerous microhabitats for organisms to inhabit, as the suitable habitat and shelter reduces the physiological stress of the animal, and protects from the predators (Kennish, 1996; Smith, 2013). The limit of distribution in most of the benthic organisms is mainly influenced by habitat characteristics and environmental factors like temperature, salinity, organic matter, and sediment composition and also by intra specific and inter specific relationships of species (Meireles *et al.,* 2006; Bertini *et al.,* 2010; Pandya and Vachhrajani, 2010).

A variation in the species diversity indices among five stations showed high diversity at Verem whereas, high richness and evenness at Siridao. The stations (Verem and Siridao) encompass with combination of rocky and mangrove associated habitat with sandy, muddy and clayey substratum including numerous microhabitats. Moreover, these stations also composed of algal and seaweed vegetation resulting in heterogeneous habitat. Fonseca (1992) suggested that high species diversity and abundance is associated with algal and seaweed vegetation. Myers (1997) suggested that diversity is influenced by distribution of organisms which are composed of transitory, temporary and permanent residents and also due to the fluctuation of environmental conditions (Sanders, 1968).

Seasonally, the species diversity, richness and evenness were observed to be higher during pre-monsoon while, monsoon season showed a lower species diversity and species

richness. Whereas, species evenness was seen to be lower only during post-monsoon season. Salinity is an important factor in the distribution of marine organisms, hence observed to be high in pre-monsoon and post-monsoon seasons and least in monsoon (Satheeshkumar, 2012). The overall crab assemblage was observed highest during pre-monsoon in all the five sampling stations whereas, least during monsoon season. In Goa, the estuaries have high salinity, temperature, dissolved oxygen and nutrients during pre-monsoon and post-monsoon season however, during monsoon the influence of fresh water remains dominated due to precipitation and land run off leading to variabality in the environmental parameters (Qasim & Gupta, 1981; Shenoy & Patil, 2003; Patil and Anil, 2008). Further, pertaining to above observations the analysis of ANOVA at 5% significant level revealed that the crab population has significant variation between the stations and seasons, indicating that the biological as well as physical factors have considerable influence on crab abundance and distribution (Buchanan & Stoner, 1988).

The data on the size frequency of crab population revealed that the males and females were abundant during pre-monsoon and post-monsoon seasons. However, male crabs were observed to be dominating females due to the ability of tolerating low salinity conditions compared to females and also larger in size than females for the purpose of copulation. The most abundant and active group of crabs were observed from 0.5-2.5 cm size class. The species composition showed that distributions of crab species were site-specific (Frusher *et al.,* 1994). The species distribution clearly delineates strong influence of season as well as substratum. The seasonal influence on crabs is associated with various life stages like maturity, mating, spawning and development (Kumar and Wesley, 2010). The variations in the environmental parameters due to changing monsoonal sequences and anthropogenic input leading to habitat degradation are some of the significant aspects which determine the distribution, indicating that crab diversity mainly depends on the environmental factors and

thus influence the biological processes (Brown *et al.,* 2001; Varadharajan *et al.,* 2013). The salinity and temperature are vital factors for intertidal crabs to stimulate breeding and spawning (Prasad & Neelakantan, 1989; DevRoy and Das, 2000). As higher salinity and temperature helps in the spawning, development of the gonads, ripening of eggs, larval development and survival while, warm waters enhance the nutrient contents for the growth of plankton leads to abundant food supply which reduces stress in production of eggs (Orton, 1920; Chandran, 1968; Bert *et al.,* 2016). Further, the low abundance of females was also observed as the ovigerous crabs tend to hide themselves in the rock crevices and deep burrows in order to avoid loss of eggs and desiccation (Oliveira *et al.,* 2005). Published reports (Garth & Abbott, 1980; Fletcher *et al.,* 1990; Ryer *et al.,* 1997; Donahue *et al.,* 2009) suggest that such behaviour of crabs enables to protect themselves during moulting from potential predators for increased survivorship.

Chapter 5

Reproductive biology of few selected species of rock crabs

5.1. Introduction

The population biology determines the understanding of sexual maturity, reproductive period, fecundity and several other aspects of organism (Wenner, 1972). A structural representation of the population is necessary for the conservation of natural assets (Gregati & Negreiros-Fransozo, 2009). Crabs are one of the ecologically important assets of littoral environment. Biological aspects of these organisms are less understood and few studies have been assessed in estuaries and along the coast. These areas are significant for biology and life habits, owing the impacts of ecological effects on the population of crabs. Hence study of the reproductive aspect is essential for better understanding of the population biology (Henmi, 1989; Araújo *et al.,* 2014; Watanabe *et al.,* 2014). Brachyuran crabs inhabiting in the inter-tidal ecosystems live in stressful environment and still demonstrate a great diversity due to their reproductive strategies that maximize survivorship of offspring and maintain population stocks at adequate levels (Hartnoll and Gould, 1988). Hence, it is imperative that the studies pertaining to the reproductive aspect of the above referred species is important for better understanding of its population dynamics.

Fecundity is an integral part of the reproductive biology which determines the reproductive potential of species and stock size of population, however it varies within and between the species and is influenced by various environmental factors and population processes such as food and feeding habits, size, growth, etc. (Mantelatto & Fransozo, 1997; Przemyslaw and Marcello, 2013). The present study attempts to focus on the population biology of four brachyuran crab species occurring along the different habitats of Goa with an emphasis on the size frequency distribution of male, female and ovigerous females, their seasonal fluctuation sex ratio and fecundity.

Brachyuran crab population along the intertidal rocky shores of estuarine regions displays high diversity in their occurrence and abundance. These crabs play an important role

in the eco-biological processes those regulate the ecosystem functions to facilitate the life processes and determine the population density. The present study attempts to emphasise the reproductive habits of few selected brachyuran crab species from the heterogeneous habitats (Sandy, rocky and muddy) of Goa.

5.2. Methodology

5.2.1. Reproductive traits

The fecundity of brachyuran crabs is defined as the number of eggs produced per female in each clutch (Reid and Corey, 1991). Among the collected crab samples, four crab species namely *L. exaratus*, *E. frontalis*, *M. frontalis* and *H. glabra* were selected due to their abundance and occurrence throughout the year. The CW of the specimens was measured using vernier caliper (0.01mm). Weight of the crabs along with eggs was obtained in grams using chemical balance (0.01 g). Later the eggs were separated from the pleopods of crabs, weighed and sub-sampled. The sub-samples were then weighed separately, placed on the counting slide and the eggs were counted under Olympus IX51 inverted fluorescence microscope (Andres *et al.,* 1996; Oliveira *et al.,* 2005). The reproductive period was determined on basis of monthly examination of existence of ovigerous females during the course of the sampling period. To study the fecundity weight of the ovigerous crabs along with eggs was obtained in grams using Sartorius CP124S analytical balance (0.01 g). Absolute fecundity was calculated using the formula given by Gaikwad *et al.,* (2009). Number of eggs produced per crab was estimated by the following equation:

$$\text{Fecundity (F)} = \frac{\text{No. of eggs in sub-sample} \times \text{Total weight of eggs (g)}}{\text{Sub-sample weight (g)}} = \text{Total no. of eggs}$$

5.3. Results

A total of 4068 crabs were collected at monthly intervals over a period from March, 2014 to August, 2015. The observations revealed that among the collected crab samples, 1865 were males (46%), 2203 were total females (54%) and among the total females 904 were ovigerous females (22%). It is also observed from the data (Table 5.1) that males dominated the females and ovigerous female population.

The overall size frequency distribution in CW of male, female and ovigerous female (Figure 5.1) was also assessed among the different species collected from the study area. It was observed that males exceeded the total females up to 3.29 cm in the species *L. exaratus* (Figure 5.1A). In *E. frontalis* (Figure 5.1B), the males and females showed no size variations whereas, in the species *M. frontalis* (Figure 5.1C) and *H. glabra* (Figure 5.1D) the overall CW of males exceeded that of females up to 3.55 cm and 2.0 cm respectively.

The largest CW was observed in *E. frontalis* compared to other species, with average size of 2.16 cm (male) and 2.41 cm (female) whereas *H. glabra* showed minimum size range with an average of 0.9 cm (male) and 0.83 cm (female). Comparing between the males and females, in *L. exaratus*, *M. frontalis* and *H. glabra* showed maximum size in males compared to female whereas, *E. frontalis* showed no distinct size variation among males and females (Table 5.2).

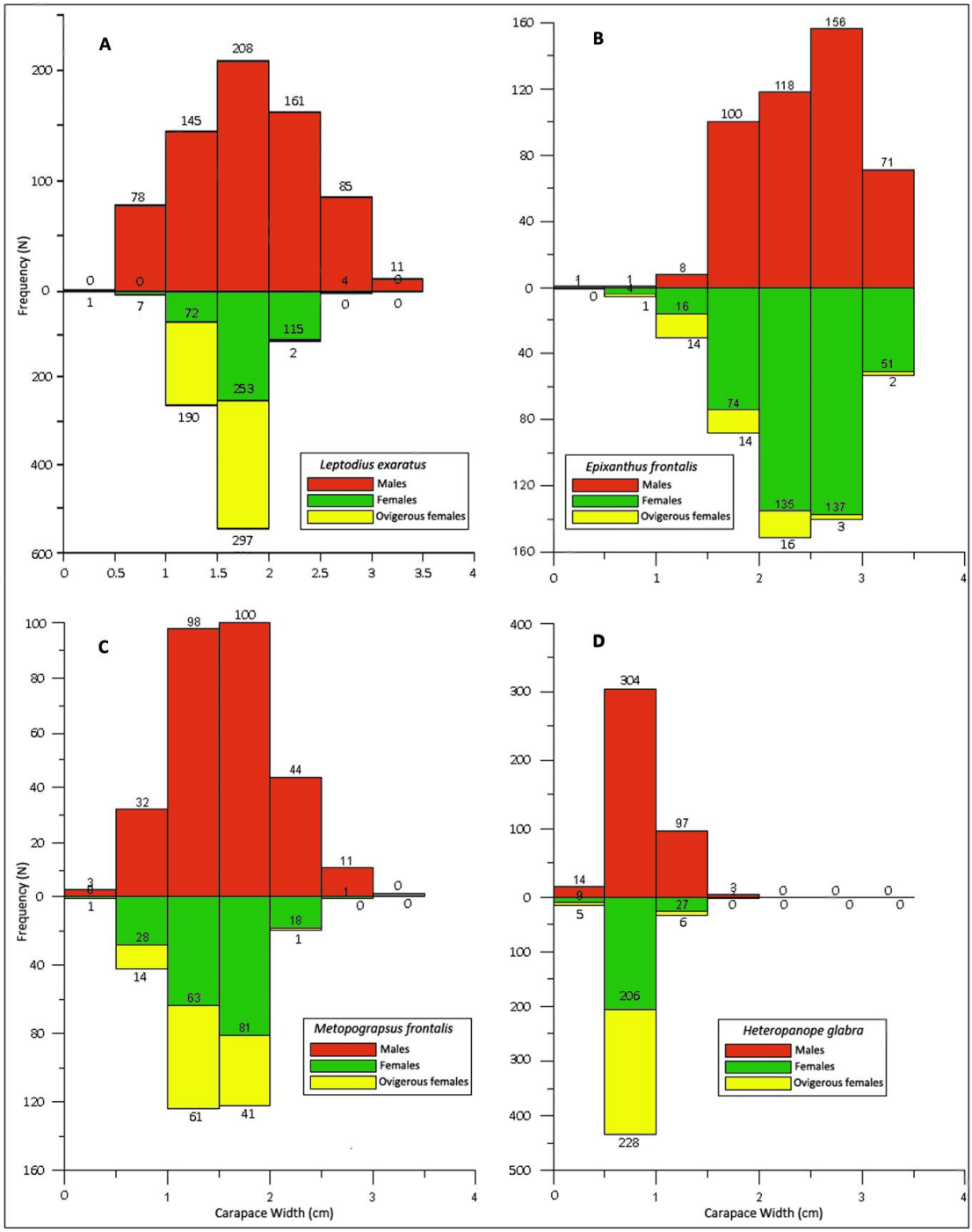

Figure 5.1. Size frequency distribution of male, female and ovigerous female of (A) *L. exaratus*, (B) *E. frontalis*, (C) *M. frontalis* and (D) *H. glabra*

Table 5.1. Abundance of Male, Female, Ovigerous females, percentage of ovigerous females and sex ratio of four crab species

S. No	Crab species	Abundance (N)			Total females	% Ovigerous females	Sex ratio %
		Males	Females	Ovigerous females			
1	*Leptodius exaratus*	689	444	496	940	53	73
2	*Epixanthus frontalis*	469	418	50	468	11	100
3	*Metapograpsus frontalis*	289	191	118	309	38	94
4	*Heteropanope glabra*	418	245	241	487	50	86

Table 5.2. CW of male and female of four crab species

S.No	Crab species	Males (cm)				Females (cm)			
		Minimum	Maximum	Average	Standard Deviation	Minimum	Maximum	Average	Standard Deviation
1	*Leptodius exaratus*	0.29	3.29	1.8	0.61	0.48	2.54	1.66	0.3
2	*Epixanthus frontalis*	0.32	3.53	2.16	0.57	0.21	3.55	2.41	0.58
3	*Metapograpsus frontalis*	0.39	3.50	1.42	0.47	0.34	2.55	1.4	0.41
4	*Heteropanope glabra*	0.35	2.0	0.9	0.21	0.39	1.52	0.83	0.18

The data obtained on the seasonal occurrence and abundance of male, female and ovigerous females of the species *L. exaratus* (Figure 5.2A), *M. frontalis* (Figure 5.2C), *H. glabra* (Figure 5.2D) indicated high values during pre-monsoon however, *E. frontalis* was observed to be abundant during post-monsoon season. On the contrary, low abundance was noticed in *L. exaratus, H. glabra* and *E. frontalis* during monsoon, whereas in *M. frontalis*, low value was observed during post-monsoon. Secondly, the ovigerous females in all the four species were observed to be less abundant during monsoon.

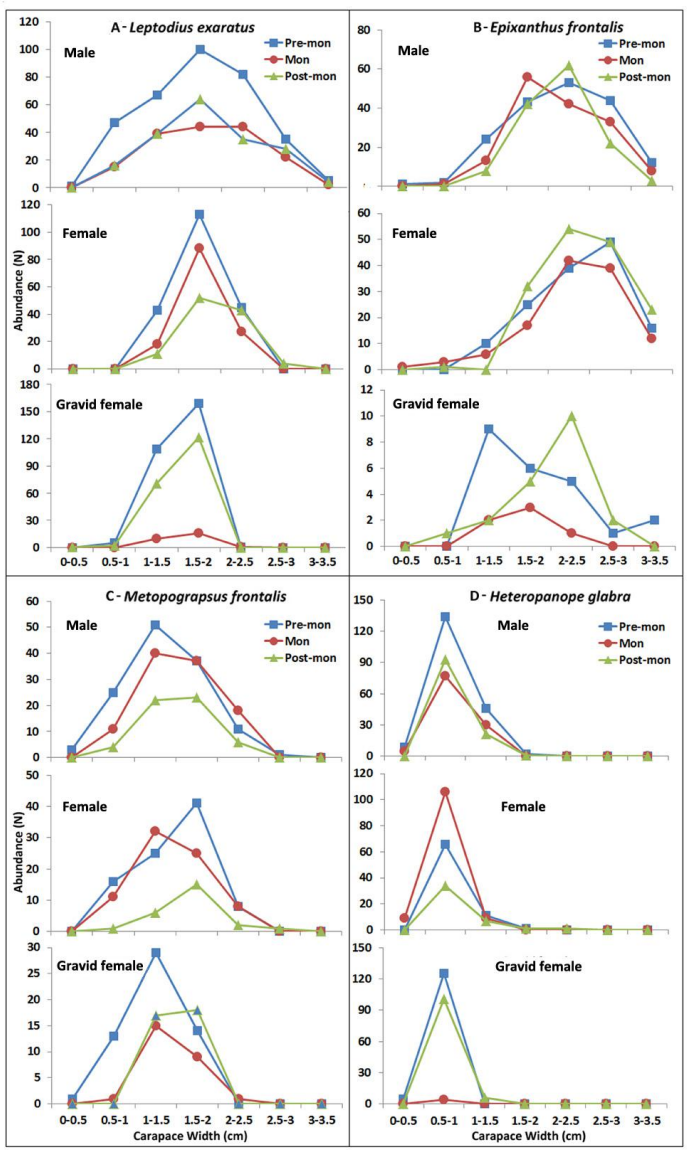

Figure 5.2. Seasonal fluctuation of male, female and ovigerous female of (A) *L. exaratus*, (B) *E. frontalis*, (C) *M. frontalis* and (D) *H. glabra*

The sex-ratio was computed to investigate the temporal variations in the male, female and ovigerous female in all the species. The analysis revealed that overall sex ratio among *L. exaratus* (Figure 5.3A), *M. frontalis* (Figure 5.3C) and *H. glabra* (Figure 5.3D) showed that males dominated during monsoon season, whereas females were high during pre-monsoon and post-monsoon seasons. The non ovigerous females with respect to ovigerous females of species *L. exaratus* and *H. glabra* were higher in abundance during monsoon, whereas in *M. frontalis* such an observation was evident during pre-monsoon and monsoon seasons. In *E. frontalis* (Figure 5.3B), no distinct seasonal relationship on dominance of males was seen. The ratio of ovigerous female with respect to female showed dominance of females, probably due to hiding of ovigerous females in the rock crevices, holes of laterite rocks, and may even remain burrowed in the sediments.

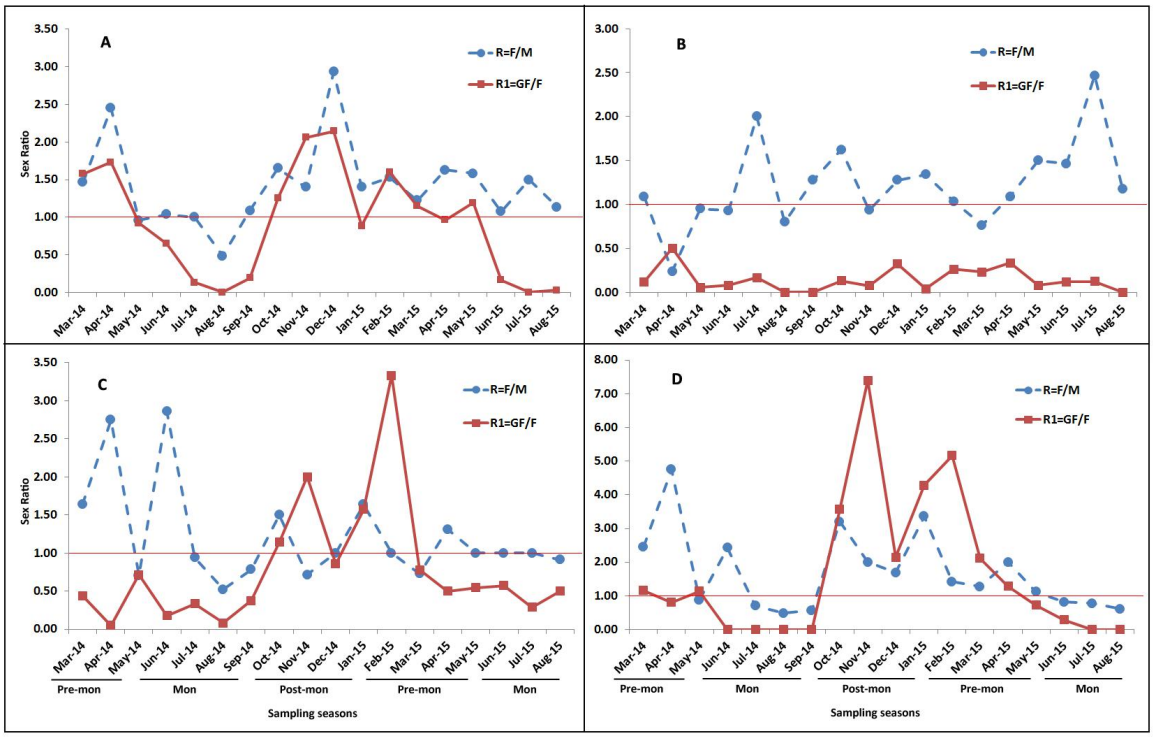

Figure 5.3. Ratio between male and female, and Ovigerous female and female of species (A) *L. exaratus*, (B) *E. frontalis*, (C) *M. frontalis* and (D) *H. glabra*

The Chi-square test carried out for species *E. frontalis* and *M. frontalis* showed that computed values are less than tabulated values at 1 *df* at 5% significance level. Hence, there was no significant difference in number of male and female. Whereas the sex ratio of species *L. exaratus* and *H. glabra* showed significant difference for 1 *df* at 5% level of significance (Table 5.3 and 5.4).

Table 5.3. Chi-square Test on Sex ratio of *Leptodius exaratus* and *Epixanthus frontalis*

	Leptodius exaratus				*Epixanthus frontalis*			
	Observed number	Expected number	Calculated X^2	Tabulated X^2	Observed number	Expected number	Calculated X^2	Tabulated X^2
Males	689	815	38.67	3.84	469	469	0.002	3.84
Females	940	815	-	-	468	469	-	-
Total	1629	1629	-	-	937	937	-	-

Table 5.4. Chi-square Test on Sex ratio of *Metapograpsus frontalis* and *Heteropanope glabra*

	Metapograpsus frontalis				*Heteropanope glabra*			
	Observed number	Expected number	Calculated X^2	Tabulated X^2	Observed number	Expected number	Calculated X^2	Tabulated X^2
Males	289	299	0.66	3.84	418	452	5.12	3.84
Females	309	299	-	-	486	452	-	-
Total	598	598	-	-	904	904	-	-

The fecundity analysis revealed that there was a linear relation with the fecundity and CW in all the four crab species (Figure 5.4). The observations made revealed maximum number (6038 ± 3656) of eggs in *M. frontalis* and minimum (822 ± 489) in *H. glabra* (Table 5.5). The analysis of fecundity index was carried out and was observed highest in *M. frontalis* (3630) and least in the species *H. glabra* (648) (Table 5.6). The coefficient of determined number in the leniar relationship among CW and fecundity in *L. exaratus* was R^2 = 0.3939 (Figure 5.4A) followed by *E. frontalis* (R^2 = 0.475) (Figure 5.4B), *M. frontalis (*R^2 = 0.2606) (Figure 5.4C) and *H. glabra* (R^2 = 0. 4484) (Figure 5.4D).

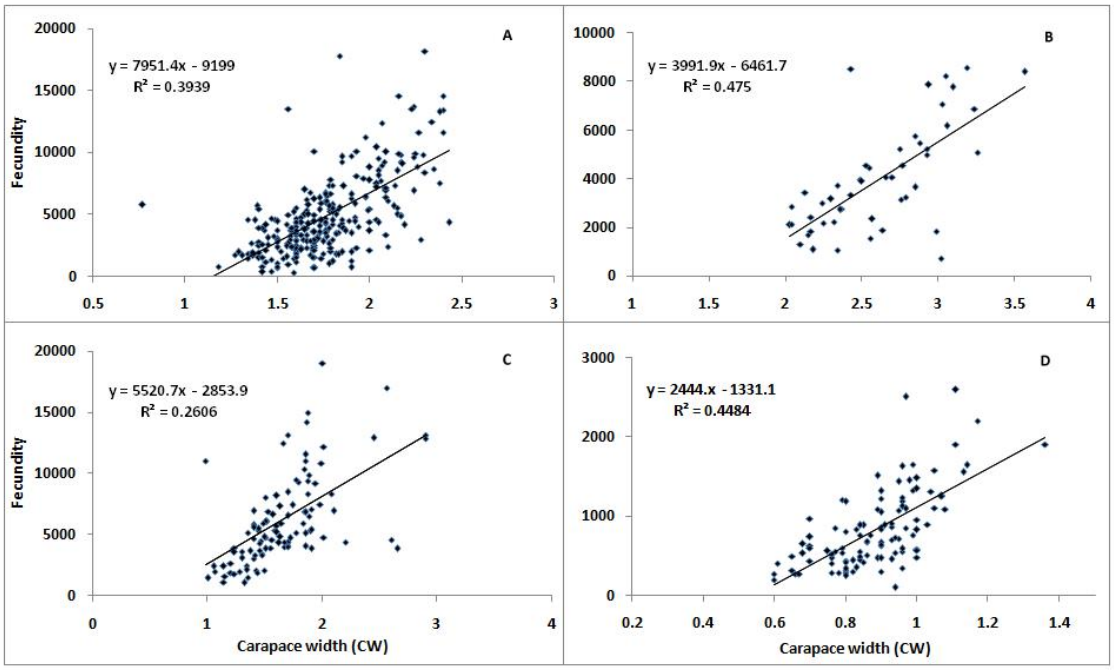

Figure 5.4. Relationship between CW and fecundity of (A) *L. exaratus*, (B) *E. frontalis*, (C) *M. frontalis* and (D) *H. glabra.*

Table 5.5. Abundance of ovigerous females, Egg range, Average number of eggs and mean fecundity of four crab species

S. No	Crab Species	Ovigerous female abundance (N)	Egg number	Average number of eggs	Number of eggs produced (X±SD)
1	*Leptodius exaratus*	275	318 – 18162	52147	4768 ± 3154
2	*Epixanthus frontalis*	50	721 – 8578	18645	3944 ± 2224
3	*Metopograpsus frontalis*	105	1090 – 19020	45946	6038 ± 3656
4	*Heteropanope glabra*	114	100 – 2590	41733	822 ± 489

Table 5.6. Fecundity index of four crab species

S. No	Crab species	Fecundity Index
1	*Leptodius exaratus*	2781
2	*Epixanthus frontalis*	1331
3	*Metapograpsus frontalis*	3630
4	*Heteropanope glabra*	648

The variance of CW and fecundity were further analyzed statistically using a one-way analysis of variance (ANOVA). The value of F computed for all the four species was higher when compared to critical value (α (2): 0.05). Therefore, the hypothesis that there is no significance in variance in fecundity with respect to carapace is rejected. Thus, variance in fecundity with respect to CW is significant and hence CW is related to fecundity (Table 5.7-5.10).

Table 5.7. Results of one-way analysis of variance (ANOVA) between CW and fecundity of *Leptodius exaratus*

Source of variance	*Degrees of freedom (d.f.)*	*Sum of squares*	*Mean sum of squares*	*Variance Ratio (F)*
Between CW size range	3	4.433718E+08	1.477906E+08	3.017794E+01
Error	81	396681814.3	4897306.35	-
Total	84	840053593.9	-	-

Table 5.8. Results of one-way analysis of variance (ANOVA) between CW and fecundity of *Epixanthus frontalis*

Source of variance	*Degrees of freedom (d.f.)*	*Sum of squares*	*Mean sum of squares*	*Variance Ratio (F)*
Between CW size range	3	96541637.3	32180545.8	10.2755071
Error	45	140929742	3131772.04	-
Total	48	237471379	-	-

Table 5.9. Results of one-way analysis of variance (ANOVA) between CW and fecundity of *Metopograpsus frontalis*

Source of variance	*Degrees of freedom (d.f.)*	*Sum of squares*	*Mean sum of squares*	*Variance Ratio (F)*
Between CW size range	3	3.124404E+08	1.041468E+08	1.290761E+01
Error	56	451843631.3	8068636.273	-
Total	59	764283997.8	-	-

Table 5.10. Results of one-way analysis of variance (ANOVA) between CW and fecundity of *Heteropanope glabra*

Source of variance	*Degrees of freedom (d.f.)*	*Sum of squares*	*Mean sum of squares*	*Variance Ratio (F)*
Between CW size range	3	3.457161E+06	1.152387E+06	5.137137E+00
Error	46	10318938.55	224324.7512	-
Total	49	13776099.7	-	-

5.4. Discussion

In order to study the population biology of crabs occurring along the study area, only four species namely *Leptodius exaratus*, *Epixanthus frontalis*, *Metopograpsus frontalis* and *Heteropanope glabra,* were examined due to their abundance at study sites. The analysis of the collected crab samples revealed 46% were males and 54% females, of which 22% were ovigerous females resulting the dominance of male over females population. The dominance of male is observed due the greater ability to adapt relatively low salinity conditions compared to females (Schaffner and Diaz, 1988; Novo *et al.,* 2005). Further, published reports (Tan and Van Engel, 1966) suggest that the osmoregulatory mechanism in the male crabs mediated through the blood is better expressed than the females. However, ovigerous females remain burrowed to avoid utilizing energy in osmoregulation which results in poor development of ovary suggesting that the differences in theses abilities could probably have resulted in dominance male population.

The overall size frequency distribution of CW reveals that males exceeded the total females and tend to reach larger in size to perform the function of mate guarding so as to have greater efficiency for successful female copulation. Published reports (Nakayama and Wada, 2015; Grafen and Ridley, 1983) suggest that the larger size males of these crab species

inhabiting in the inter-tidal habitats occupy larger size females that produces large broods, displaying size-assortative mating resulting in production of large clutches.

The maximum CW (2.16 cm) was observed in *E. frontalis* and minimum (0.9 cm) in *H. glabra.* Seasonal variation revealed that the species were abundant during pre-monsoon and post-monsoon. However, the ovigerous females of these species were least during monsoon. The observations made here suggest that salinity play an important role in the abundance of these species in the inter-tidal habitats. It is noteworthy to mention that during the pre-monsoon period, high salinity is known to prevail along this region suggesting that saline waters are preferred by the species. The species showed high abundance during pre-monsoon and post-monsoon due to the prevalence of more saline waters during these seasons. Along this region, high salinity is known to occur during pre-monsoon and post-monsoon with decrease in salinity during monsoon season due to the fresh water influence through precipitation (De Sousa, 1977; Patil and Anil, 2008). Low density of these crab during the monsoonal period suggest that influx of fresh water coupled with much of the sestonic material in suspension affecting the food availability may be a constraint for decreased abundance of these species (Varadharajan *et al.,* 2013).

The low abundance of females was due to low osmoregulatory mechanism leading to low tolerance in changing salinity conditions. Further, it was also observed that these egg bearing females hide in deep burrows in order to prevent egg loss during low saline conditions (Oliveira *et al.,* 2005). However, the non ovigerous females of *H. glabra* showed high abundance during monsoon. It appears that this species exhibit high degree of tolerance to low saline condition as it inhabits in the mangroves habitat (Trivedi *et al.,* 2015; Naderloo, 2017). Published literatures (Satheeshkumar, 2012; Saher *et al.,* 2018a) suggest that the changes in the seasonal conditions like temperature and salinity during different seasons affects the abundance of crab seasonally. As in low salinity, the development of ovary is

delayed as the crustaceans have to spend much of its energy in osmoregulation, which leads to poor gonadal development (Long *et al.,* 2017). However, temperature plays an important role in incubation of egg, larval development and survival of offspring's. Bert *et al.,* (2016) suggested that warm water and abundant food reduces the stress of production of eggs and stimulates physiological maintenance.

The analysis of temporal variations in sex-ratio of males and females revealed dominance of male during monsoon and females during pre-monsoon and post-monsoon seasons. The non ovigerous females with respect to ovigerous females were observed high in abundance during monsoon. The highest occurrence of males and females during monsoon season suggests that the reproductive activity of these species coincides with monsoon (Litulo, 2004b). Ovigerous females showed their occurrence during post-monsoon as the oocytes maturity is low during low temperature which need longer period of incubation (Yamaguchi, 2001).

The fecundity analysis reveals maximum number of eggs in *M. frontalis* and low in *H. glabra* due to the result of its smaller body size (Doi *et al.,* 2008). However, the fecundity index was also observed highest in *M. frontalis* and least in *H. glabra.* The variation in egg production in these intertidal species depend on several factors which influence the development of egg such as food availability, incomplete fertilization, multiple spawning and water temperature (Fahimi *et al.,* 2017 and Litulo, 2005). The observation made in present study suggests that such tropical habitats largely influenced by the spatio-temporal variations alter the food availability and environmental conditions. Such variable condition prevailing in tropical habitats play a key role for the wide spectrum in the number of egg produced. Moreover, it is also important to note that much of the inter-tidal tropical habitats, there exists anomaly in the environmental factors, causing stressful conditions at different times resulting in the resource partitioning of the energy affecting the fecundity.

Chapter 6

Phylogenetic relationships among few selected species of rock crab

6.1. Introduction

Phylogenetics is the study based on similarity traits and also the possible mechanism of the evolutionary history and relationships among individuals or groups of organisms. Molecular phylogenetic mostly reveals the genetic change occurring in species over time based on the structural and functional change in molecules which support the evolutionary relationships (Dowell, 2008). A German biologist named William Hennig in 1950's had proposed phylogenetic systematic as he recommend that the evolutionary history of lineage should be reflected through systematics. Hence, phylogenetics study was to deals with the better understanding of evolutionary relationships and identification of organisms (Unda, 2005). Therefore, it is used in both molecular and morphological data in order to classify organisms. In the phylogenetic studies of various organisms the molecular markers have gained importance (Patwardhan *et al.,* 2014). However, due to the highest morphological diversity, the relationships among these brachyuran families are scanty (Tsang *et al.,* 2014). Brachyuran crabs have undergone extensive variation and are extremely diverse both morphologically and ecologically (Spears *et al.,* 1992).

In phylogenetic analyses the sequences length varies among the genes. In the total mitochondrial genome, the sequences in GenBank database ranges from 400 base pairs to 600 base pairs (Laemmli, 1975; Unda, 2005). The analyses of mitochondrial gene sequences greatly support the species identification and phylogenetic assumption, as the process of recombination is not observed in the mitochondrial genes and they are inherited maternally. In phylogenetics studies the molecular and morphological characters are important to trace the evolution of morphology, behavior and physiological diversity in decapods. Many studies have been conducted to examine the phylogenic relationship in these decapod groups (Hultgren and Stachowicz, 2008). The mitochondrial COI (Cytochrome c oxidase subunit I) and 16S r*RNA* (16S ribosomal ribonucleic acid) gene is a widespread tool in the field of DNA

taxonomy and barcoding for resolving the species classifications (Oliveira-Biener *et al.,* 2010). DNA barcoding has been mainly successful in the identification and delimitation of new species from various groups (Kerr *et al.,* 2009). DNA barcode is a short section of DNA sequence itself comprises of a 648 base pair region from the 5'- end of the COI. It is assumed that a unique DNA barcode is present mostly in every kind of species therefore the genetic variation is observed between the species which results in the exceeding variations within the species. The gene occurring in the mitochondria of all eukaryotic organisms and the preliminary studies suggest consistent resolving ability at the species level for many animals.

The information published on brachyuran crabs along the Goa coast is scanty, attempts has been made to study the phylogenetic relationships among rock crabs using phenotypic characters (Kaullysing, 2015). The present study attempts to elucidate the phylogenetic relationships among 11 species belonging to 9 genera and 7 families. A molecular approach was used to test the phylogeny of selected brachyuran crabs using 16S r*RNA* and COI sequences and compare its relationship. The reason for using 16S r*RNA* genes is because they are ubiquitous and contains highly conserved regions for designing of primer and to identify phylogenetic characteristics (Lane *et al.,* 1985; Yang *et al.,* 2016) and COI as universal marker for species identification and effective for resolving the phylogenetic relationships among closely related species (Lu *et al.,* 2011). Further, the software named Molecular Evolutionary Genetics Analysis (MEGA) X (Kumar *et al.,* 2018) was used for analysis of phylogenetic time tree to understand the evolutionary patterns of species over time.

6.2. Methodology

The phylogenetic analysis was done employing appropriate phylogenetic software to elucidate ancestral relationships and affinities between various crab species. For the extraction of DNA (Deoxyribonucleic Acid) and subsequent analysis, whole crab or the pereiopods were

preserved in 95% ethanol at -20^{0}C. The bottles containing samples were coded as GUMS (Goa University Marine Sciences) followed by a sample number. Further, the molecular analysis was carried out at the laboratory of Central Aquaculture Genetics Laboratory (CAGL), Rajiv Gandhi Centre for Aquaculture (RGCA), Sirkali, Tamil Nadu.

6.2.1. *Extraction of Total Genomic DNA*

Eleven crab samples belonging to nine genera and six families were examined and used for the extraction of total genomic DNA using Phenol Chloroform method. The molecular phylogenetic analysis of tissue extraction was initially carried out for fifteen brachyuran crab samples with sample code GUMS-01, GUMS-02, GUMS-03, GUMS-04, GUMS-05, GUMS-6, GUMS-7, GUMS-8, GUMS-9, GUMS-10, GUMS-11, GUMS-12, GUMS-13, GUMS-14 and GUMS-15. Among these, DNA of four samples (GUMS-01, GUMS-03, GUMS-6 and GUMS-8) was found to be degraded. Hence, the remaining samples were processed for further extraction. The extraction of DNA was carried out by scraping out the muscle tissue from the walking legs (pereiopods) of the crab and subjected to further analysis as shown in Table 6.1 using DNA isolation kit by following the manufacturer's protocol and amplification of 16s r*RNA* (16S ribosomal ribonucleic acid) and COI (cytochrome oxidase) was done using Polymerase Chain Reaction (PCR) technique using Universal Forward & Reverse primers. The source of primers used in phylogenetic studies was from Sigma-Aldrich Chemical Company, Bangalore.

Table 6.1. Protocol for sequencing

DNA extraction using Phenol Chloroform method
↓
Polymerase chain reaction(PCR)- 16S r*RNA* /COI gene
↓

Purification of 16S r*RNA* /COI PCR products
↓
Cycle sequencing of purified 16S r*RNA* /COI PCR products
↓
Clean-up/purification of 16S r*RNA* /COI Cycle sequencing product
↓
Denaturation and snap chilling of purified Cycle sequencing product
↓
Sequencing 16S r*RNA* /COI gene (forward/reverse)

The PCR was carried out using various cycling conditions (Table 6.2), the purification and sequencing of PCR product was carried out using PCR purification kit. Through the National Center for Biotechnology Information (NCBI) Gene Bank website, The Basic Local Alignment Search Tool (BLAST) was used to obtain the sequence alignment and phylogenetic. Quality of the genomic DNA was assessed using 0.7 % agarose gel and the quantity of the genomic DNA was assessed in Biophotometer (Eppendorf). Genomic DNA was observed in all the samples.

Table 6.2. The PCR cycling conditions

Reaction steps	Temperature	Time	No of Cycles
Initial denaturation	94^0C	1min	1
Denaturation	93^0C	1min	35
Annealing	50^0C	30 sec	
Extension	72^0C	1min	
Elongated extension	72^0C	7min	1
Hold	4^0C	∞	

Amplification of 16S r*RNA* and COI gene was carried out using Universal 16S r*RNA* forward and reverse primer and universal COI forward & reverse primers (Table 6.3). A band

between 524-572bp (product length may vary between the samples) was amplified for 16S r*RNA* and 604-711bp (product length may vary between the samples) was amplified for COI.

Table 6.3. List of Forward and Reverse Universal primers of 16S *rRNA* and COI

Primers used	Sequence (5' to 3')
Universal *16S rRNA* (Forward)	CGCCTGTTTAACAAAAACAT
Universal *16S rRNA* (Reverse)	CCGGTCTGAACTCAGATCATGT
Universal *COI* (Forward)	GGTCAACAAATCATAAAGATATTGG
Universal *COI* (Reverse)	TAAACTTCAGGGTGACCAAAAAATCA

6.2.2. *PCR testing for 16S rRNA amplification*

Amplification of 16S r*RNA* gene was carried out using Universal 16S r*RNA* Forward & Reverse primer for all the 07 samples. The amplification was performed in 25µl reaction volume containing 1X standard Buffer, 1.5mM $MgCl_2$, 0.2mM dNTP, 0.2µM each primer, 50 ng of genomic DNA and 0.5 unit Taq polymerase. A band between 524-572bp (product length may vary between the samples) was amplified in all the samples. PCR-generated amplicons were confirmed and purified with Gene JET PCR purification kit (Thermo Scientific, EU-Lithuania) to remove the primer dimer and other carryover contaminations. The quality was assessed using 2% agarose gel and was found to be good for sequencing. The amplified products were separated in 2.0% agarose gels with 100bp ladder as size standard (Table 6.4).

Table 6.4. Detail of 16S r*RNA* amplification and sequencing

S. No.	Sample code	Species	Sequence length	Remarks
01	GUMS-02	*Heteropanope glabra*	554bp	The sequence length mentioned here is after the alignment
02	GUMS-04	*Uca annulipes*	524bp	
03	GUMS-05	*Metopograpsus frontalis*	569bp	

04	GUMS-07	*Macrophthalmus convexus*	563bp	and editing (Tamura *et al.,* 2011).
05	GUMS-09	*Epixanthus frontalis*	570bp	
06	GUMS-12	*Nanosesarma andersonii*	572bp	
07	GUMS-15	*Scylla olivacea*	562bp	

6.2.3. *PCR testing for COI gene amplification*

Amplification of *COI* gene was carried out for 04 samples using Universal Forward & Reverse primers. A band between 604-711bp (product length may vary between the samples) was amplified in all the samples. PCR-generated amplicons were confirmed and purified with Gene JET PCR purification kit (Thermo Scientific, EU-Lithuania) to remove the primer dimer and other carryover contaminations. The quality was assessed using 2% agarose gel and was found to be good for sequencing (Table 6.5).

Table 6.5. Detail of *COI* amplification and sequencing

S. No.	Sample code	Species	Sequence length	Remarks
01	GUMS-05	*Metopograpsus frontalis*	674bp	The sequence length mentioned here is after the alignment and editing (Tamura *et al.,* 2011).
02	GUMS-10	*Portunus pelagicus*	711bp	
03	GUMS-11	*Nanosesarma pontianacense*	604bp	
04	GUMS-12	*Nanosesarma andersonii*	709bp	
05	GUMS-13	*Nanosesarma minutum*	626bp	
06	GUMS-14	*Portunus pelagicus*	709bp	
07	GUMS-15	*Scylla olivacea*	709bp	

6.2.4. *Sequencing*

Purified PCR products were prepared for Cycle sequencing using the Big Dye® Terminator 3.1 sequence kit (Applied Biosystems, Foster City, California, USA). After cycle

sequencing, the products were purified using Ethanol-EDTA (Ethylenediaminetetra acetic acid) purification protocol to remove the un-incorporated dNTP's (Deoxynucleoside triphosphate), ddNTP's and primer dimer**.** After purification the products were dissolved in 12µl Hi-Di formamide and the samples were subjected for denaturation at 95^0C for 5 mins. Denatured products were used for sequencing in forward and reverse directions using Genetic Analyzer 3500 (Life Technologies Corporation, Applied Biosystems®, California 94404, USA) as per manufacture's instruction. Sequences were aligned, edited, and analyzed using ClustalW and MEGA software version 5 (Tamura *et al.,* 2011).

The phylogenetic tree was constructed by using the Maximum Likelihood method based on the General Time Reversible model (Nei and Kumar, 2000). The percentage of trees is shown next to the branches in which the associated taxa clustered together. Further, the evolutionary study was conducted using the Molecular Evolutionary Genetics Analysis (MEGA) X (Kumar *et al.,* 2018).

6.3. Results

The molecular phylogenetic for COI and 16S r*RNA* analysis of brachyuran crabs was carried out for eleven species (Table 6.6). Among which the species *Metopograpsus frontalis*, *Nanosesarma andersonii* and *Scylla olivacea* were sequenced for both COI and 16S r*RNA*. Four species namely *Thalamita crenata, Nanosesarma pontianacense, Nanosesarma minutum* and *Portunus pelagicus* were found to be degraded for COI while, *Heteropanope glabra, Uca annulipes, Macrophthalmus convexus* and *Epixanthus frontalis* were found to be degraded for 16S r*RNA*. Hence, the COI tree consists of 7 species belonging to 3 different families whereas 16S r*RNA* tree consists of 7 species belonging to 7 different families. The analysis involved 588 and 542 nucleotide positions in COI and 16S r*RNA* alignments, respectively for tree construction. All ambiguous positions were removed for each sequence pair. The COI tree

was drawn using 7 sequences produced in this study and 29 reference sequences (Table 6.7) extracted from BOLD (Barcode of Life Data) and NCBI (National Center for Biotechnology Information) databases. 16S r*RNA* tree was drawn using 7 sequences produced in this study and 18 reference sequences (Table 6.8) extracted from NCBI. Phylogenetic tree was constructed in MEGA X (Kumar *et al.,* 2018) and refined in iTOL (Interactive Tree Of Life) (Letunic and Bork, 2019). The confirmed sequences through the BLAST analysis for COI and 16S r*RNA* was submitted in the GenBank (NCBI). The accession numbers obtained from the NCBI are given in Table 6.6. The evolutionary history was inferred using the Neighbor-Joining method. The COI and 16S r*RNA* tree was drawn to scale with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distance or scale bar in the tree was found to be 0.1. The COI and 16S r*RNA* phylogenetic tree was drawn using the Neighbor-Joining method (Saitou and Nei, 1987) by computing Kimura 2-parameter (Kimura, 1980) represented in the units of the number of base substitutions per site.

Table 6.6. Brachyuran crab species used for phylogenetic analysis of COI and 16s r*RNA*

S. No	Species	COI code	Accession number	16srRNA code	Accession number
1	*Heteropanope glabra*	-	-	MT16SGUMS2	MN631208
2	*Austruca annulipes*	-	-	MT16SGUMS4	MG211745
3	*Metopograpsus frontalis*	MTCOIGUMS5	MN635595	MT16SGUMS5	MG211746
4	*Macrophthalmus convexus*	-	-	MT16SGUMS7	MG211747
5	*Epixanthus frontalis*	-	-	MT16SGUMS9	MG211748
6	*Thalamita crenata*	MTCOIGUMS10	MG356481	-	-
7	*Nanosesarma pontianacense*	MTCOIGUMS11	MN635596	-	-
8	*Nanosesarma andersonii*	MTCOIGUMS12	MN635597	MT16SGUMS12	MN631209
9	*Nanosesarma minutum*	MTCOIGUMS13	MN635598	-	-
10	*Portunus pelagicus*	MTCOIGUMS14	MG356482	-	-
11	*Scylla olivacea*	MTCOIGUMS15	MN635599	MT16SGUMS15	MG211749

Table 6.7. List of reference sequences used for the construction of COI tree

S. No	Species-strain	Accession number	Isolation source	via	Family
1	*Nanosesarma batavicum*	MG753569	Vellar mangrove crab	Genbank	Sesarmidae
2	*Nanosesarma minutum*	KY284644	Vellar mangrove crab	Genbank	Sesarmidae
3	*Sarmatium crassum*	MF564021	East African mangroves	BOLD	Sesarmidae
4	*Selatium brockii*	MG753568	Vellar mangrove crab	Genbank	Sesarmidae
5	*Perisesarma onychophorum*	KX400913	Singapore	BOLD	Sesarmidae
6	*Metopograpsus frontalis*	KY284642	Vellar mangrove crab	BOLD	Grapsidae
7	*Metopograpsus thukuhar*	MH298869	Kerala mangrove crab	BOLD	Grapsidae
8	*Thalamita crenata*	MG725241	Vellar mangrove crab	Genbank	Portunidae
9	*Thalamita crenata*	MG693215	Vellar mangrove crab	Genbank	Portunidae
10	*Thalamita crenata*	KX018514	Vellar mangrove crab	Genbank	Portunidae
11	*Thalamita crenata*	KT365763	Hawaii USA	Genbank	Portunidae
12	*Thalamita crenata*	ACB4758 280	Kosi bay South Africa	BOLD	Portunidae
13	*Thalamita crenata*	ACB4758 281	Kosi bay South Africa	BOLD	Portunidae
14	*Thalamita crenata*	ACB4758 282	Kosi bay South Africa	BOLD	Portunidae
15	*Thalamita crenata*	ACB4758 283	Kosi bay South Africa	BOLD	Portunidae
16	*Thalamita crenata*	ACB4758 32	Kenya	BOLD	Portunidae
17	*Scylla olivacea*	MF565487	Sundarbans Bangladesh	Genbank	Portunidae
18	*Scylla olivacea*	KT921346	Poompuhar coast Tamil Nadu	Genbank	Portunidae
19	*Scylla olivacea*	KC200563	Poompuhar coast Tamil Nadu	Genbank	Portunidae
20	*Scylla olivacea*	MH577539	Poompuhar coast Tamil Nadu	Genbank	Portunidae
21	*Scylla olivacea*	MF611599	Sundarbans Bangladesh	BOLD	Portunidae
22	*Portunus pelagicus*	MH577536	Poompuhar coast Tamil Nadu	Genbank	Portunidae

23	*Portunus pelagicus*	MF594621	Sundarbans Bangladesh	Genbank	Portunidae
24	*Portunus pelagicus*	KX381802	Mandapan TN India	Genbank	Portunidae
25	*Portunus pelagicus*	MG742722	Vellar mangrove crab	Genbank	Portunidae
26	*Portunus pelagicus*	KT159799	Gulf of Mannar TN India	Genbank	Portunidae
27	*Portunus pelagicus*	MK281258	Museums USA	Genbank	Portunidae
28	*Portunus pelagicus*	MG693212	Vellar mangrove crab	Genbank	Portunidae
29	*Portunus pelagicus*	KP666120	Andhra coast India	Genbank	Portunidae

Table 6.8. List of reference sequences used for the construction of 16S r*RNA* tree

S. No	Species-strain	Accession number	Isolation source	via	Family
1	*Epixanthus frontalis*	HM637960	East Malaysia	Genebank	Oziidae
2	*Epixanthus dentatus*	HM637958	Indonesia	Genebank	Oziidae
3	*Heteropanope glabra*	KJ132560	Taiwan	Genebank	Pilumnidae
4	*Pilumnus reticulatus*	MF504065	Brazil	Genebank	Pilumnidae
5	*Austruca annulipes*	AB471894	Tioman Island, Malaysia	Genebank	Ocypodidae
6	*Austruca annulipes*	MK348671	Maharashtra, India	Genebank	Ocypodidae
7	*Austruca annulipes*	AF481965	Singapore	Genebank	Ocypodidae
8	*Austruca annulipes*	MF673823	Karachi, Pakistan	Genebank	Ocypodidae
9	*Austruca annulipes*	HG515384	Kenya, Western Indian Ocean	Genebank	Ocypodidae
10	*Metopograpsus thukuhar*	KM510112	Japan	Genebank	Grapsidae
11	*Metopograpsus thukuhar*	AJ784027	Mozambique	Genebank	Grapsidae
12	*Metopograpsus thukuhar*	MF469805	Port Lunay, Seychelles	Genebank	Grapsidae
13	*Macrophthalmus convexus*	LC097094	Taiwan	Genebank	Macrophthalmidae
14	*Perisesarma samawati*	AJ621186	Kenya	Genebank	Sesarmidae
15	*Scylla olivacea*	AF109321	Taiwan	Genebank	Portunidae
16	*Scylla olivacea* voucher	KC154071	Kerala, India	Genebank	Portunidae
17	*Scylla olivacea*	KT984200	Bangladesh	Genebank	Portunidae
18	*Scylla olivacea*	AB857337	Tamil Nadu, India	Genebank	Portunidae

The COI tree (Figure 6.1) reveals 7 sequenced crab species clustered with reference species forming a group of 3 different families among which the strains in orange text label are the sequences produced in the present study. However, the members of family Grapsidae were represented by green clade, Sesarmidae in red clade and Portunidae in purple clade. In the family Grapsidae the species *Metopograpsus frontalis* (GUMS5) was observed to be clustered in a clade with Genbank species *M. frontalis* and *M. thukuhar*. However, the present sequenced species showed close resemblance with reference species *M. frontalis* (HM637960).

The species *Nanosesarma pontianacense* (GUMS11), *N. andersonii* (GUMS12) and *N. minutum* (GUMS13) of family Sesarmidae clustered in same clade with other species of Genbank namely, *Nanosesarma minutum, Sarmatium crassum, Perisesarma onychophorum, Selatium brockii* and *Nanosesarma batavicum* and showed no similarity among each other. However, the analysis revealed the present sequenced crabs species of family Sesarmidae were sequenced for COI gene for the first time as those sequences were absent in both the Genbank and BOLD databases. Similar patterns were also observed within the members of family Portunidae in which the species of the family grouped in 3 different clades with its respective sequenced species from Genbank. The species *Thalamita crenata* (GUMS10), *Scylla olivacea* (GUMS15) and *Portunus pelagicus* (GUMS14) corresponds well with the reference species used to construct the tree.

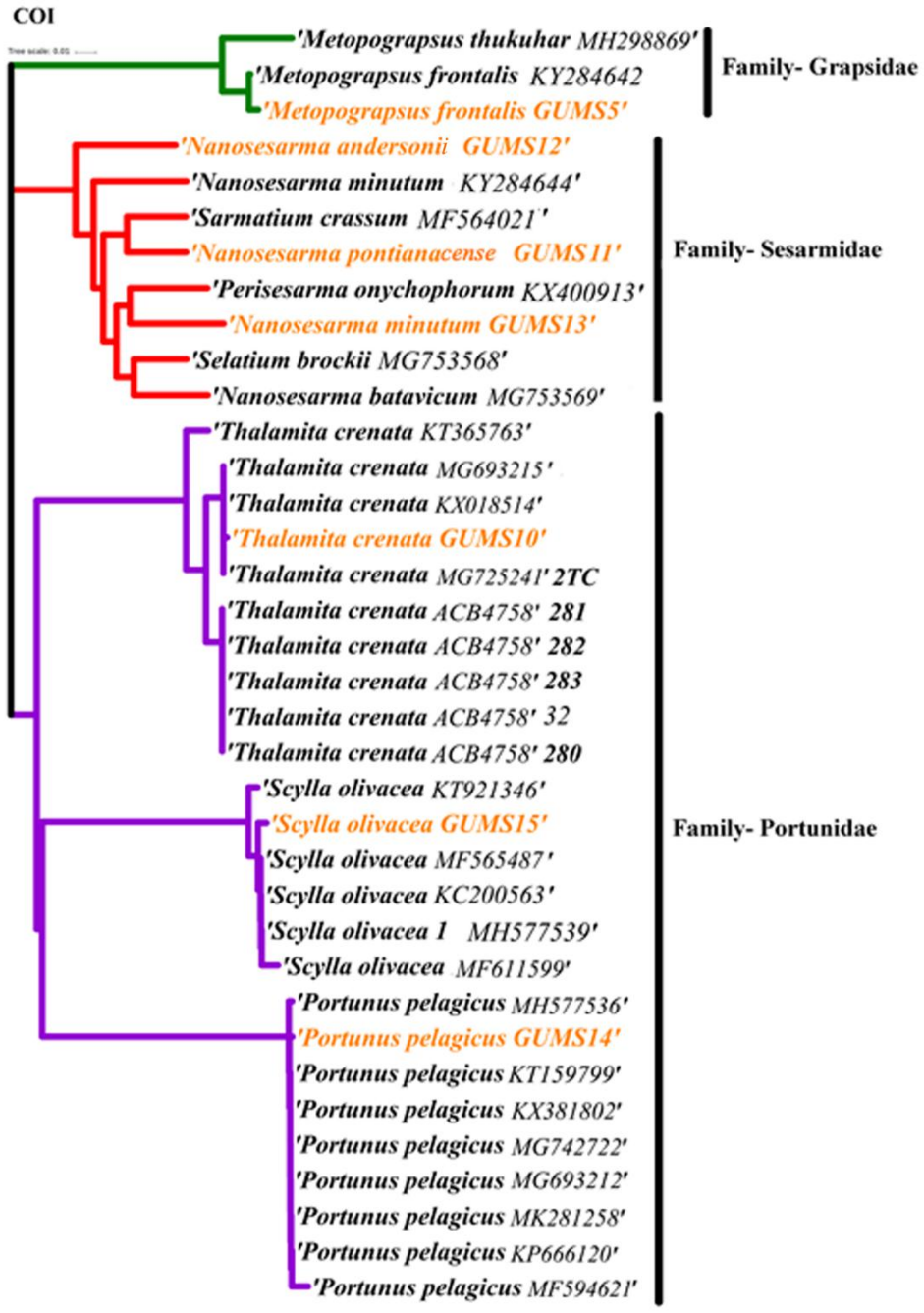

Figure 6.1. Phylogenetic consensus tree of COI gene of brachyuran crab taxa constructed with the Neighbor-joining method

The 16S r*RNA* tree (Figure 6.2) reveals 7 sequenced crab species clustered with reference species forming a group of 7 different families among which the strains in orange text label are the sequences produced in the present study. However, the purple clade represented family Macrophthalmidae and the species *Macrophthalmus convexus* (GUMS7) resembled well with reference species *M. convexus* (LC097094). The members of family Portunidae formed blue clade in which the species *Scylla olivacea* (GUMS15) showed similarity with elsewhere reference species *S. olivacea* (AF109321). The family Oziidae represented by red clade revealed the sequenced species *Epixanthus frontalis* (GUMS9) clustered with reference species *E. dentatus* and *E. frontalis* however, showed close resemblance with Genbank species *E. frontalis* (HM637960).

Further, family Pilumnidae representing dark green clade includes present sequenced species *Heteropanope glabra* (GUMS2) with Genbank species namely, *Pilumnus reticulates* and *H. glabra* however, the sequenced species showed similarity with Genbank species *H. glabra* (KJ132560). The family Grapsidae represented light green clade with cluster of reference species *Metopograpsus thukuhar* and sequenced species *Metopograpsus frontalis* (GUMS5) which did not show closeness among each other. In the family Sesarmidae representing gray clade formed cluster of sequenced species and Genbank species *Perisesarma samawat* (AJ621186), showing no similarity among each other and reveals that *N. andersonii* (GUMS12) was sequenced for the first time for 16S r*RNA* gene as the sequence was absent in Genbank. The family Ocypodidae representing black clade consists of sequenced species and Genbank species *Austruca annulipes* and revealed that the sequenced species *Austruca annulipes* (GUMS4) well resembled with reference crab species of *Austruca annulipes* (AB471894).

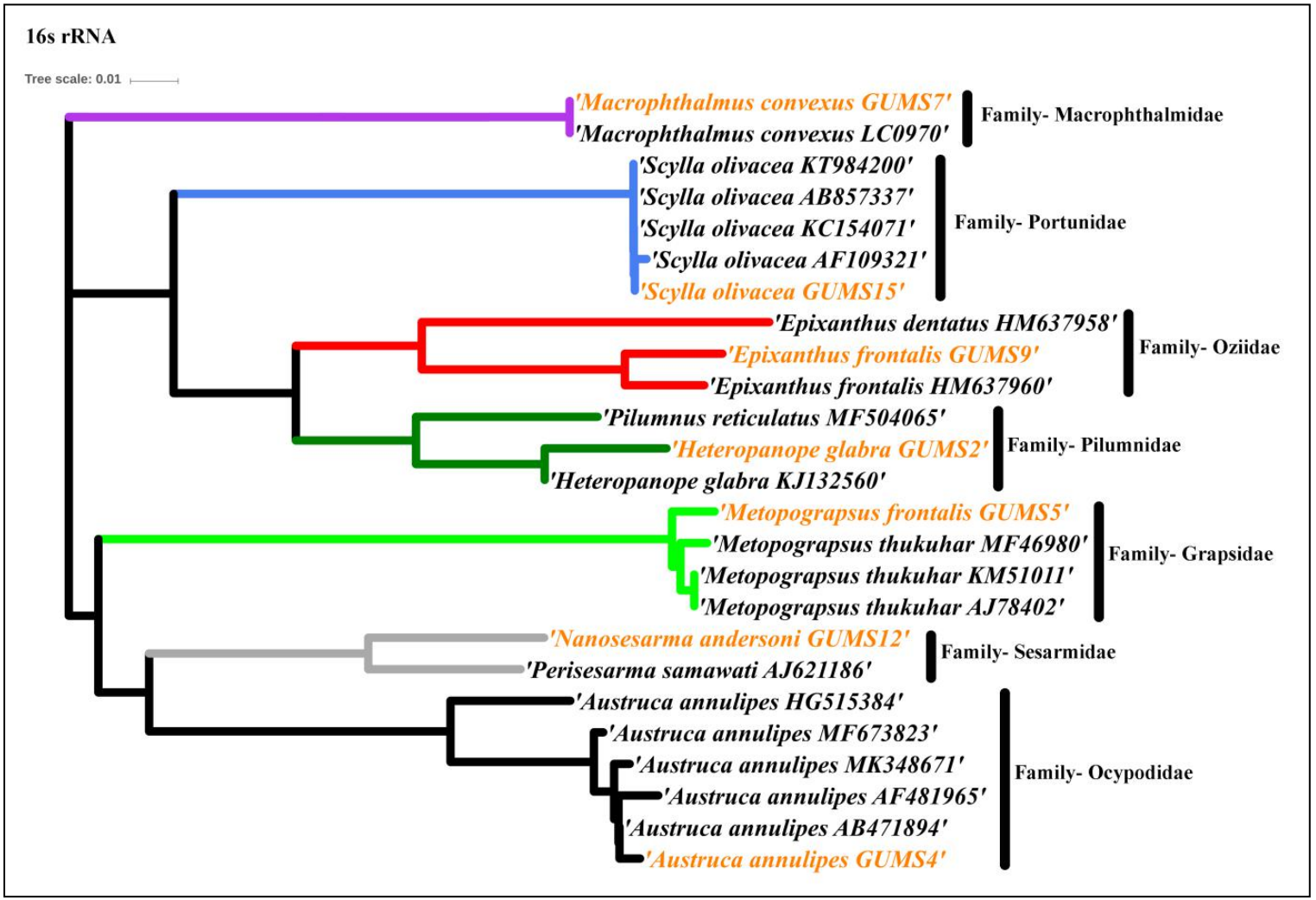

Figure 6.2. Phylogenetic consensus tree of 16s r*RNA* gene of brachyuran crab taxa constructed with the Neighbor-joining method

6.4. Discussion

In the present study the molecular phylogenetic for COI (Cytochrome c oxidase subunit I) and 16S r*RNA* (16S ribosomal ribonucleic acid) analysis of eleven brachyuran crabs species was carried out. The COI tree consists of 7 sequenced species belonging to 3 different families whereas 16S rRNA tree consists of 7 species belonging to 7 different families. There were a total of 588 and 542 nucleotide positions in COI and 16S r*RNA* alignments respectively for tree construction. The gene successfully amplified by using primers LCO-Forward and HCO-Reverse was used to study the evolutionary relationship of these brachyuran crabs. Previously Bhavan *et al.,* (2015) reported that LCO1490 and HCO2198 are the suitable universal primers for the purpose of amplification, delineation and identification of species. The sequence were used for confirmation of species in nucleotide BLAST and showed 99 – 100 % similarity with previously published COI and 16S r*RNA* genes, showing likely similar morphological species in BLAST-NCBI's nucleotide database. The confirmed sequences were submitted in the GenBank (NCBI) and the accession numbers were obtained (Table 6.6). The phylogenetic tree drawn using the Neighbor-Joining method (Saitou and Nei, 1987) showed three well defined clusters within the sequenced species for COI genes and seven well defined clusters for 16s r*RNA* genes. The closely resembled reference species in the present study for COI genes share close geographic distributions mostly from the regions of Bangladesh and Tamil Nadu whereas, the species in 16S r*RNA* genes were observed from Malaysia and Taiwan regions. The species clustered together in a tree are observed to be well separated from each other implies generic and morphologic distinction (Mantelatto *et al.,* 2007). The present findings support the previous work carried on most of the families namely Portunidae, Grapsidae, Sesarmidae and Macrophthalmidae (Schubart *et al.,* 2006; Robles *et al.,* 2007; Mantelatto *et al.,* 2007, 2009; Saher *et al.,* 2018b). Among the sequenced COI gene the crabs of family Sesarmidae namely *Nanosesarma pontianacense* (GUMS11), *N.*

andersonii (GUMS12)*, N. minutum* (GUMS13) were sequenced for the first time due to the absence of these sequences in both Genbank and BOLD databases. However, in 16S r*RNA* gene the species *N. andersonii* (GUMS12) of family Sesarmidae was observed to be sequenced for first time. The tree plotted for COI and 16s r*RNA* corresponds well with the reference sequences used to construct the tree, implying the genes were successful in identification of sequenced species in the present study. The result of the present study on the molecular data provides the evidence for gene flow among the 11 brachyuran crab species and further provides molecular identification and taxonomic status of brachyuran crabs. The genetic divergence obtained were enough to differentiate individuals of various species. Published literature (Pons *et al.,* 2006) suggests that within species, lineages expand more quickly than between the species. The present study clearly differentiated species examined based on the genetic divergence represented in the constructed phylogram suggesting sequenced brachyuran crabs are different genetically. It is evident from the results obtained that COI and 16s r*RNA* could act as a potential barcode for its identification.

Chapter 7

Summary

The present study attempted to examine the taxonomy, species composition, few biological aspects and phylogenetic relationship of brachyuran crabs from heterogeneous habitats based on the data collected. The sampling was carried out among five estuarine habitats along Goa, west coast of India.

1. The present observation revealed a total of 29 brachyuran crab species belonging to 21 genera and 12 families. Among these, 12 crab species were found to be reported for the first time in the intertidal habitats zones of Goa.
2. A new species *Carupella banlaensis* (Tien, 1969) was recorded for the first time from India. *Dotilla myctiroides* (H. Milne Edwards, 1852) was reported from Goa coast for the first time and a detailed morphological description of both the species is provided. Family Portunidae was dominant with highest contribution of species among all the families recorded in the study area.
3. The sampling stations comprised predominantly of rocky, partially mangrove and mangrove habitat representing heterogeneous habitat. The species among these stations were grouped as rare, less frequently and frequently occurring species. The crab communities showed high diversity, richness and evenness indices during post-monsoon and pre-monsoon seasons.
4. Seasonally, highest crab abundance was observed during pre-monsoon season at in all the five sampling stations whereas, least during monsoon season. Since, in Goa the estuaries have high salinity, temperature, dissolved oxygen and nutrients during pre-monsoon and post-monsoon and least during monsoon season. Further, analysis of variance showed significant variation between the stations and seasons. The male crabs were abundant and their sizes were larger compared to females. The size ranges of 0.5-2.5 cm were observed to be most abundant and active groups of crabs.

5. The reproductive biology of the species namely *Leptodius exaratus*, *Epixanthus frontalis*, *Metopograpsus frontalis* and *Heteropanope glabra* revealed 46% were males and 54% females, of which 22% were ovigerous females. The male crabs were dominating and exceeding female population due to their low salinity tolerance mechanism. The maximum and minimum CW was observed in *E. frontalis* (2.16 cm) and *H. glabra* (0.9 cm) respectively.
6. Seasonally, the *L. exaratus*, *M. frontalis* and *H. glabra* were abundant during pre-monsoon while, *E. frontalis* was abundant in post-monsoon. However, the sex-ratio revealed that males and females were abundant during monsoon since the reproductive activity coincides with rainfall. While ovigerous females were abundant during pre-monsoon and post-monsoon seasons as the oocytes prefers wamer environment for incubation and maturity. Fecundity index was highest in *M. frontalis* (3630) and least in *H. glabra* (648) due to its smaller body size.
7. The 16S r*RNA* and COI gene sequencing was carried out for 11 crab species reveal that the selected families of crab species differ genetically and the sequenced species were successful in identification. The species *Nanosesarma pontianacense, N. andersonii, N. minutum* were sequenced for first time for COI gene while, *N. andersoni* was sequenced for first time for 16S r*RNA* gene from Goa. The tree plotted for COI and 16s r*RNA* corresponds well with the reference sequences used to construct the tree. Further, the phylogenetic analysis support that the newly sequenced species of genus *Nanosesarma* belongs to family Sesarmidae as useful for further taxonomic status of brachyuran crabs.

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Papers Published

1. Vijaylaxmi, J., Padate, V. P., & Rivonker, C. U. (2015). First distributional record of *Carupella banlaensis* from India. *Marine Biology Research*, 12 (1), 104-111.
2. Padate, V. P., Vijaylaxmi, J., Maurya, S., & Rivonker, C. U. (2015). Morphology and morphometrics of *Dotilla myctiroides* (Decapoda: Brachyura: Dotillidae) from an impacted beach of Goa along west coast of India. *Journal of the Marine Biological Association of India*, 57 (2), 5-10.
3. Vijaylaxmi, J., Can, A. A., & Rivonker, C. U. (2020). Population biology of some species of crabs along Goa, west coast of India. *Indian Journal of Geo Marine Sciences*. 49(11), 1742-1749.

Research papers presented at the Conference

1. National Conference of Young Researchers 16-17 March 2017, Goa University, Goa, India.
2. National Conference on Mangrove Ecosystem in association with CSIR- National Institute of Oceanography (NIO), held at NIO, Dona Paula, Goa, India on 26th and 27th July 2017.

Workshop attended

1. Workshop on Scientific Writing and Effective Communication organised by Goa University, Goa on 05th - 06th January 2015.
2. Application of Molecular Markers in Fisheries and Aquaculture Management organised by Rajiv Gandhi Centre for Aquaculture, Sirkali, Tamil Nadu on O7-11 November 2016.

3. The Nobel Dialogue “Nobel Prize Series, India 2018” an International science event organized by Media AB, Department of Biotechnology, Govt. of India and Department of Science and Technology, Govt. of Goa at Panaji, Goa on February 2, 2018.
4. Intellectual Property Rights (IPR) Awareness Programme, organised by ASSOCHAM, Goa University and IP office at Goa University, Goa on 11th December 2018.