Journal of Marine Biology and Aquaculture (J Marine Biol Aquacult)

OPEN ACCESS

Research Article

OMMEGA Publishers

ISSN 2381-0750

DOI: 10.15436/2381-0750.19.2576

Studies on Diversity of Venomous Marine Invertebrates in Coasts of Dakshina Kannada and Udupi Districts

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Abstract

Marine conservation is of primary concern, due to rise in pollution and resulting deleterious variation. Venomous marine invertebrates secrete various biologically active molecules few are toxins and contribute significantly to enhance knowledge of pharmacology. Venomous marine invertebrates are important firstly due to their poisonous stings, secondly reports on them are scanty, as they are not hunted for food.

The objective of present study is to assess the diversity of venomous marine invertebrates along the coasts of Dakshina Kannada and Udupi districts to provide baseline data. The subset of marine invertebrates like phylum Mollusca, Cnidaria members were collected from different coasts during study periods of six months (Aug 2018-Jan 2019).

During our study, we found cone snails, jelly fishes, sea anemones. Cone snails were more in St mary,s followed by Kaup, Someshwara and Surathkal, Jellyfishes in St mary's, Sea anemones in Surthkal and Kaup shores. We collected 46 individuals of cone snails of five different species, which were distributed in most of the sites selected for study as cone snails are confined to shallow waters. Few were collected live and anatomy of their venomous structures was studied. Due to increased salinity and rise in temperature in Phalguni river we collected Jellyfishes during January second week of 2019.

This is the first report in Karnataka coasts and this study of baseline data on distribution of venomous invertebrates especially on cone snails provides platform for creating awareness on venomous stings of these animals and their sensitivity to pollution and climate changes. Information regarding the Anatomy of venomous structures and mechanisms of toxin action has enabled a more scientific approach towards the treatment of their intoxications.

Keywords: Marine conservation; Diversity; Invertebrates; Cone snails; Jellyfishes; Sea anemones; Venomous; Toxins.

Introduction

Animal toxins and venoms are deliberately known to have major sources of bioactive molecules. The venom is a substance produced in an animal by a specialized gland in order to defend them or to kill their prey. This secretion contains such molecules that alter the physiology of the victim. Venom is a complex mixture of bioactive substances that causes a wide array of symptoms. The venom secreted contains mucopolysaccharides, hyaluronidase, phospholipase, serotonin, histamines, enzyme inhibitors and certain peptides; the toxicity of these molecules has a striking effect on some of the vital systems of animal body such as central nervous system (CNS), cardiovascular system and skeletal muscle system. On other hand same toxin has great pharmacological use. The toxins hold promise to cure the out breaking cancer and other fatal diseases. Some of the venomous marine invertebrates that come under this group include the members of sponges such as red-beard sponge (*Micronia prolifera*), fire sponge (*Tedania ignis*), poison-bun sponge (*Fibulila sp*.) which secretes Crinitotoxins. Members of Cnidaria such as Hydrozoa (plume-like hydroids, medusae and Siphonophora), Scyphozoa (free swimming jellyfish), Cubozoa

Received date: August 05, 2019
Accepted date: August 30, 2019
Published date: August 31, 2019

Citation: Sumangala, R., et al. Studies on Diversity of Venomous Marine Invertebrates. (2019) J Marine Biol Aquacult 5(1): 17-24.

(box shaped medusa) and Anthozoa (hard and soft corals and anemones). Hydroids and jellyfish possess nematocysts, on other hand sea anemones and corals possess spirocysts. Members of most important venomous Mollusks include Gastropods such as genus Conus that produce conotoxins and Cephalopods such as blue-ringed octopus (*Hapalochlaena maculate* and *Hapalochlaena lunulata*) that produces maculotoxins[1].

The members of molluscan family Conidae is one of most important group of animals under order Gastropoda that constitute the super family Toxoglossa which are predominantly characterized by presence of venomous apparatus such as poisonous gland and radular tooth. All over the world nearly 500 *Conus* species are distributed, which belong to single genus Conus and which are highly Carnivorous in feeding habits. The taxonomy and distribution of Conidae in India is as early as the half of the 19th century by Ferdinand Stoliczka (1867,1868) and Ronald Winckworth (1943,1945). The Manual of living Conidae[2]. provides a recent, detailed and concise summary of 316 valid species along with several subspecies and forms, from tropical Indo-Pacific region[3]. Earlier literature on diversity of cone snails studied[2,4-6]. recorded 60 species from the Tamilnadu coast, 10 species of cone snails along Kerala coast, 16 species in Tuticorin Coast at Gulf of Mannar from Tamilnadu coasts, 49 species from Lakshadeep islands.

Cone snails usually inhabit different habitats from shallow waters to depths more than 600m[4]. Also some species are associated with the attached algae of coral reefs, others are found to reside beneath the coral head and few burry themselves in sandy or coral rubble substrate[7]. Based on their feeding mechanism they can be classified into vermivorous(feed on worms), piscivorous(feed on fishes) and molluscivorous(feed on small mollusks)[8]. Piscivorous is the most dangerous compared to other types it can swallow the prey of similar size. The piscivorous venom is considered to be fatal for humans[9].

Cone snails are nocturnal carnivores, feeding mainly on mollusks, worms and fish. To capture and immobilize their prey they use venom secreted by poisonous gland. The venom apparatus mainly comprises of muscular venom bulb, tubular venom duct and a hallow radular tooth. The identification of species in genus *Conus*, is exclusively based on colour pattern and its shell morphology, this leads to lot of disagreement between taxonomists. Development of the concept of radular tooth and the qualitative and quantitative characters of radular tooth are immensely useful in describing species and geographic subspecies into different taxonomic groups. Cone snail capture their prey by injecting paralytic, neurotoxic venom by single, hallow, detachable, barbed, chitinous radular tooth[10].

Each radular row consists of one pair of hallow, harpoon like marginal teeth, each tooth functions independently. During prey capture mechanism one tooth is held within the extreme end of long, tubular proboscis. As the hydrostatic pressure increases in proboscis it propels down the tooth, followed by penetrating and catch holding the victims and releasing the potent venom into wound of target victim which causes paralysis of victim[11].

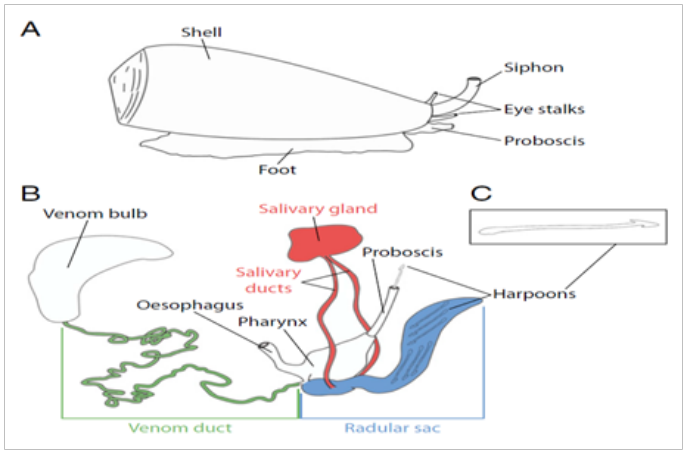


Figure 1: Macroscopic anatomy of a cone snail (A), its venom apparatus (B) Venom bulb (C) Radula tooth[12].

Conotoxins of cone snails contain tremendous and diverse peptides which plays a potential role in pharmacology and drug discovery. The five important conotoxins are α-(alpha) conotoxins, δ-(delta) conotoxins, κ-(kappa) conotoxins, μ-(mu) conotoxins, and ω-(omega) conotoxins[13]. Though, there are 500 cone snails present in world, venom of each species is distinct, complex and peptide rich. The venom in general used for defensive, competitive and probably for other biological purpose. There are more than 100 different venom components in each species, So far only 100 out of 50,000 toxins have been extracted and analyzed. There are potential pharmacologically active components in venom of all living cone snails which has diverse therapeutic use[14].

Jellyfish are marine invertebrates distributed throughout the world under class scyphozoa phylum cnidaria. Phylum Cnidaria (corals, sea pens, sea anemones, jellyfish and hydroids) contains about 13,000 diverse, complicated and colorful marine organisms living in marine habitats worldwide. They range in size from the tiny hydra *Psammohydra nanna* with a height of only 0.02 in (0.5 mm) to the massive Lion's Mane Jellyfish *Cyanea arctica*, which has a bell diameter of up to 1.7 meters and a tentacle length of 36.6m[15].

Jellyfish venom is mixture of toxic antigenic polypeptides and pathogenic enzymes that show adverse effect on human physiology, which can lead to local cutaneous reactions, systemic or fatal responses[16]. Hemotoxicity of jellyfish venom is mainly restricted to hemolysis. Jelly fish venom has antibacterial, antimycotic, antioxidant, anticoagulant and cytotoxic activities.

Studies revealed that the tentacle extract from barrel jellyfish *Rhizostoma pulmo* showed Anticoagulant activity[17]. Antioxidant and cell adhesion properties of collagen from jellyfish *Acromitus flagellatus* was studied[18]. Anticancer effect of nematocysts venom from jelly fish *Acromitus flagellatus*, on human breast cancer cell line was analysed[19].

Sea anemones are distributed in intertidal to deep oceans and live attached with rocks, sea floor, shells and some forms burrow in the mud or sand. Sea anemone captures their prey by means of specialized structures known as nematocysts. This are microscopic capsule (length ranging from 20–200 μm) secreted by the Golgi apparatus of a cell specialized for this function. In general nematocyst is also known as cnidocyst. The

cells that make the capsules are cnidoblast (cnidae), it is the capsule made by the cell that delivers the sting. Upon receipt of an appropriate chemical or mechanical stimulus, as nematocyst discharges, the tubule that had been coiled and twisted inside the capsule evokes, and delivers the venom into the victim. This discharge is among the fastest cellular processes. The cindae is present throughout the body conformation including tentacles, column, actinopharynx, and acontia filaments[20].

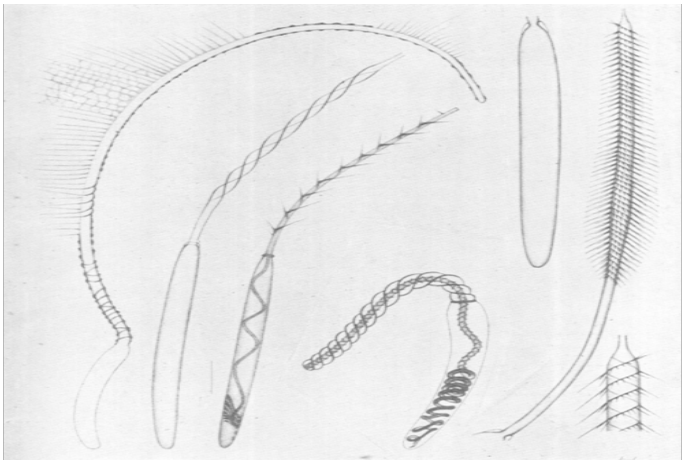


Figure 2: Different types of nematocysts (stinging capsule) present in sea anemone[21].

Present study is to evaluate and explore the diversity of venomous marine invertebrates along the coasts of Dakshina Kannada and Udupi districts to provide baseline data and to interpret relationship between fauna and its habitats due to their sensitivity towards pollution and climate change.

Materials and Methods

Biodiversity Studies

The present study on biodiversity of venomous marine invertebrates in coasts of Dakshina Kannada and Udupi districts was under taken in five different study sites viz. Surathkal beach, Thannir Bavi and Bunder (fish landing center) located in Dakshina Kannada district, St. Mary's Island and Kaup beach located in Udupi district. The survey was conducted for the period of six months (Aug 2018-Jan 2019).

Study Area

St. Mary's Island and Kaup beach (N 13°20'41.64"; E74°40'59.73", N13° 13'28.50"; E74°44'10.47") located in Udupi district; NITK beach, Thannir Bavi and Bunder (N13°0'16.05"; E74°47'21.66", N12°54'9.04"; E74°48'42.18", N12°51'20.09"; E74°49'58.") located in Dakshina Kannada district, Karnataka.

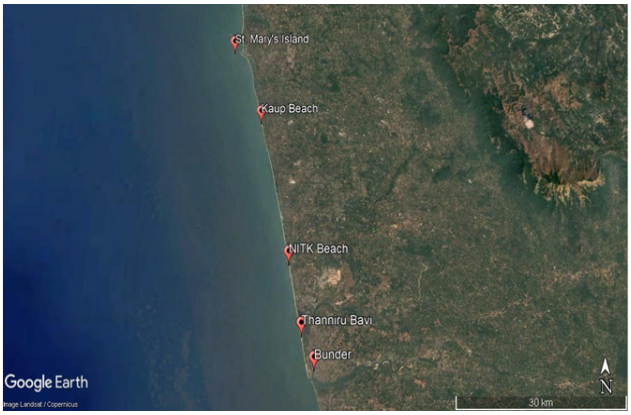


Figure 3: Map of Dakshina Kannada and Udupi districts showing different study sites.

Collection of Cone Snails

Both live and dead specimens were collected from different study sites, once in a month for the period of six months. The animal was separated from shell by means of forceps, boiling in water or by deep frosting. Shells with clear surfaces were cleaned with clean fresh water and periostracum of the shell was cleaned by dipping them in a solution containing, one portion of concentrated HCL and three portion of distilled water for few seconds. Then the surface of cone shells was gently brushed out to remove the periostracum completely. The cleaned specimens of *Conus* species were identified, by using the guide manual[2]. As these guides have figurative keys, specimens were identified based on their morphological characters and colour pattern without any ambiguity. Then the shells were photographed and preserved for further studies. The animal from *C.milneedwardsii* was dissected and the morphology of venomous apparatus was studied.

Collection of Jellyfish

Jelly fish were collected from different study sites. Both adult and juvenile scyphozoans are mainly obtained by drag nets, bag nets, and scoop nets and also by buckets operated by local fisherman. The specimens collected by bag net and scoop net were relatively in good condition and least damaged. Specimens were photographed immediately after capture to record their live colouration. Initial identification of species was based on live specimens as well as photographs taken in the field.

Collection of Sea Anemones

Sea anemones were collected from different study sites, anemones attached firmly to the rock crevices of intertidal rocky shores (microhabitats) were detached by means of forceps or by scrapper. The live specimens were brought to lab and were narcotized or relaxed to expand their polyps, by using magnesium chloride or menthol crystals as narcotizing agents. This provides easy accesses for photographing of the specimen and for preparation of tentacle squash for nematocysts study.

Cnidae Preparations

The microscopic study of Nematocysts (stinging capsule) or *Cnidae* from tentacle and column squash of sea anemone *Actinia equina* were conducted. The simple tissue squashes were made from small (<1 mm^2) pieces of tissue placed on a clean grease

free glass slide with a drop of distilled water and squashed by placing a thin cover slip and a gentle pressure, observed in light microscope under 40x.

Biodiversity Assessment

Biodiversity index for cone snails distributed in different study sites were calculated using PAST soft ware version 2.17E. Total number of individuals was recorded for the period of six months (Aug 2018-Jan 2019).

Results

In present assessment, a detailed survey was undertaken to study the biodiversity of different marine venomous invertebrates along the coasts of Daksnina Kannada and Udupi districts. The detailed investigation was carried out, for the period of six months from (Aug 2018-Jan 2019). The study area was divided into five sites viz., Surathkal beach, Thannir Bavi and Bunder (fish landing center) located in Dakshina Kannada district, St. Mary's Island and Kaup beach located in Udupi district. During the study period 46 individuals of cone snails of five different species viz., *C.milneedwardsi* (Jousseaume,1894),*C.inscriptus* (Reeve,1843), *C.balteatus* (Sowerby,1833), *C.biliosus* (Roding, 1798) and *C.lentiginosus* (Reeve, 1844) were collected. (Figure 4). 87 individuals of jellyfish of two different species of order Rhizostomeae were collected which includes *Rhizostoma pulmo* (Macri,1778), *Acromitus flagellatus* (Mass, 1903) and 40 individuals of sea anemones of two different species of order Actanaria were collected which includes *Actinia equina* (Linnaeus,1758) and *Anthopleura species* (Fonbressin & Michelotti,1860).

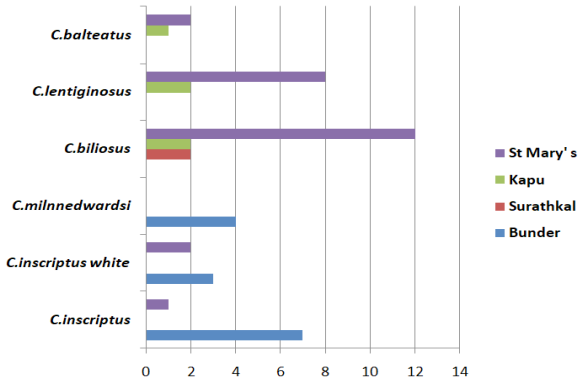
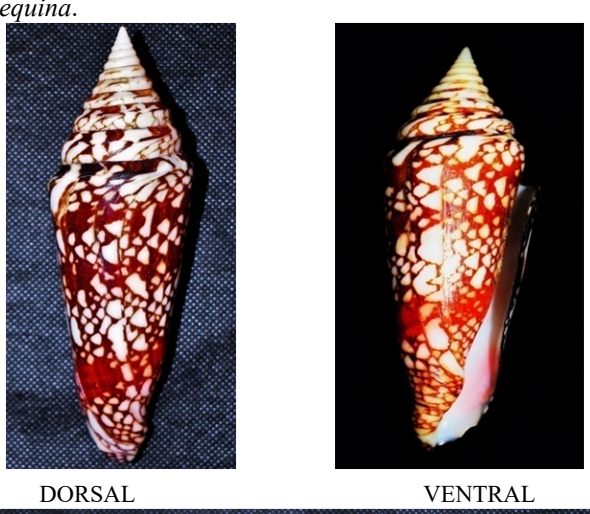


Figure 4: Distribution of cone snails in different study sites.

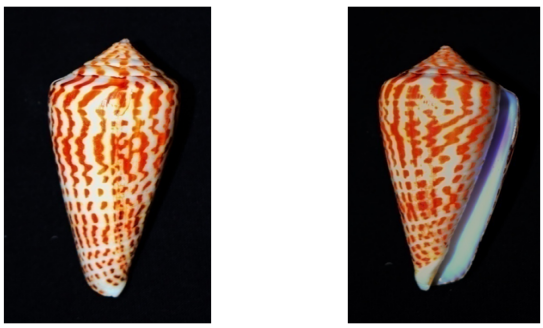
The venom apparatus of *C.milneedwarsi* mainly comprises of following parts such as muscular venomous bulb and tubular venomous duct. The production of venom takes place in a long, convoluted venom duct. The proximal end of the duct is equipped with a muscular bulb and distally the duct enters into the pharynx. The muscular bulb is generally thought to take little or no part in the secretion of venom, the important function of venom bulb is to provide force for final venom ejection and delivery into victim.

Different types of Nematocysts (stinging capsule) or *Cnidae* were observed in tentacle and coloum squash of sea anemone *Actinia equina*. Which involves, the Basitrichous isorhizas (''basitrichs'') and (spirocysts) from tentacles and (microbasic p-mastigophore) from coloum of the sea anemone *Actinia equina*.



DORSAL VENTRAL

PLATE: 1 *Conus milneedwardsi* (jousseaume, 1894)

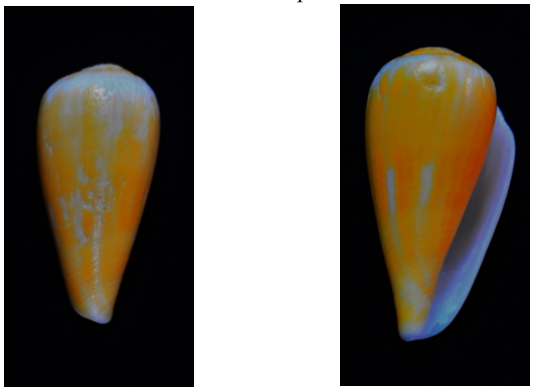


DORSAL VENTRAL

PLATE: 2 *Conus inscriptus* (Reeve, 1843)

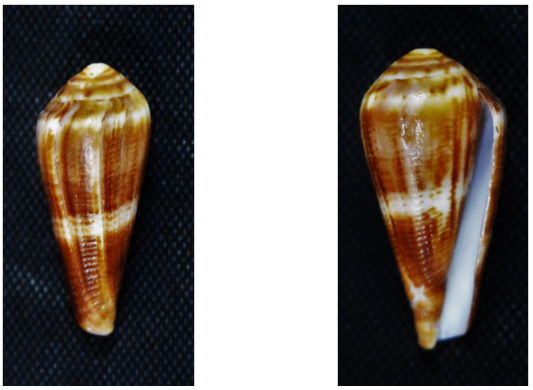
DORSAL VENTRAL

A :*Conus inscriptus* white



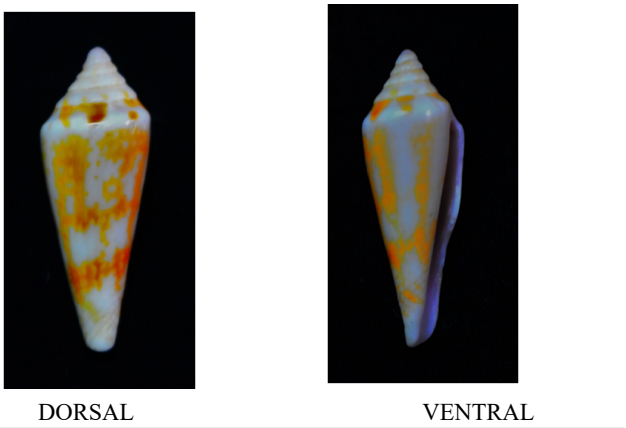
DORSAL VENTRAL

PLATE: 3 B: *Conus balteatus* (Sowerby, 1833)



DORSAL VENTRAL

PLATE: 4 *Conus biliosus* (RÖDING, 1798)



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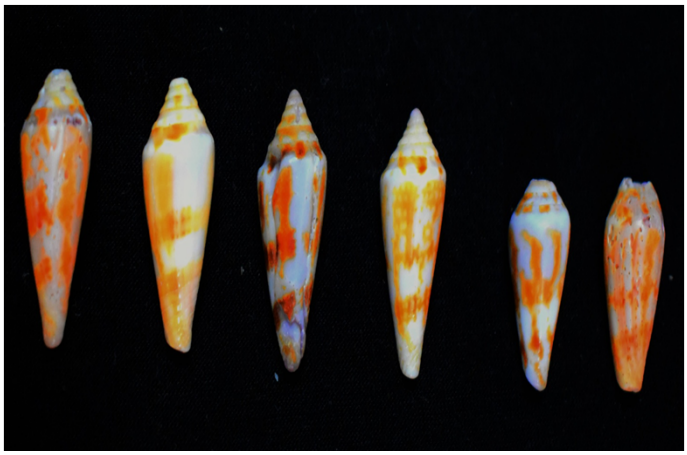
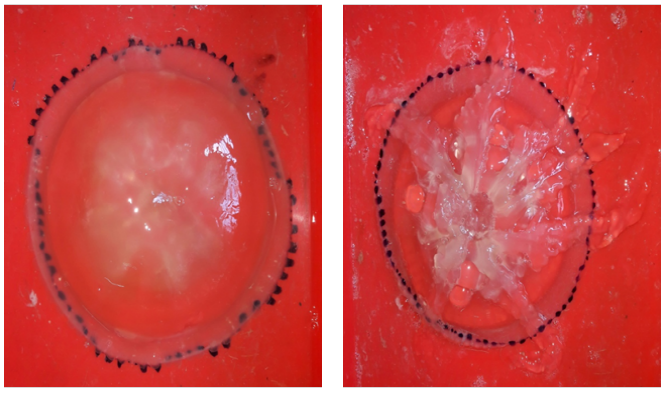


PLATE: 5 *Conus lentiginosus* (Reeve, 1844)

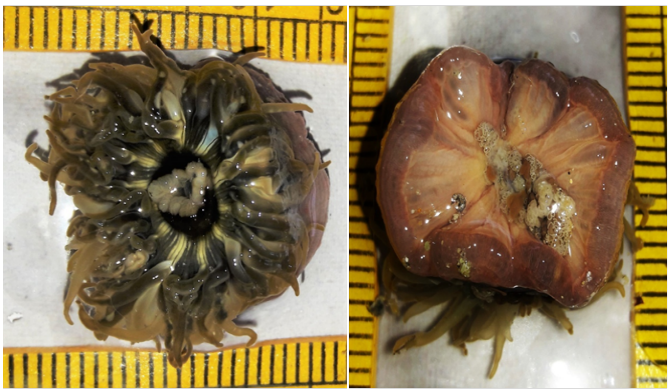


DORSAL VENTRAL

PLATE: 6 *Rhizostoma pulmo* (Macri, 1778)


DORSAL VENTRAL

PLATE: 7 *Acromitus flagellatus* (Maas, 1903)



DORSAL VENTRAL

PLATE: 8 *Anthopluera. spp*

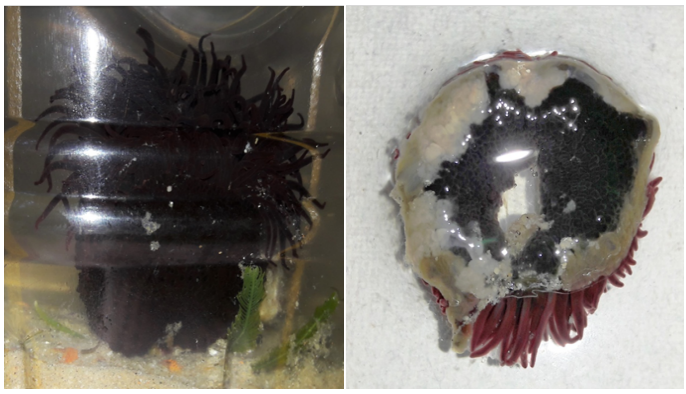
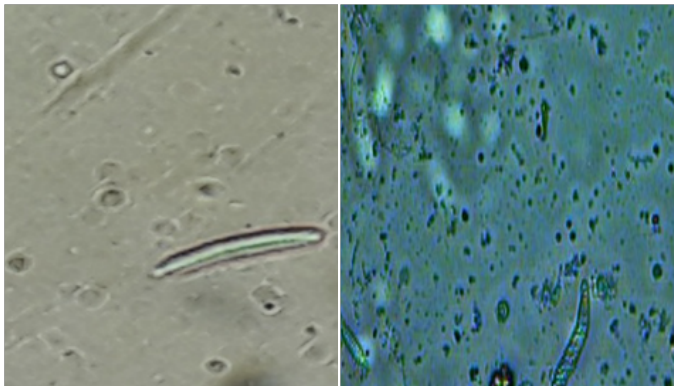
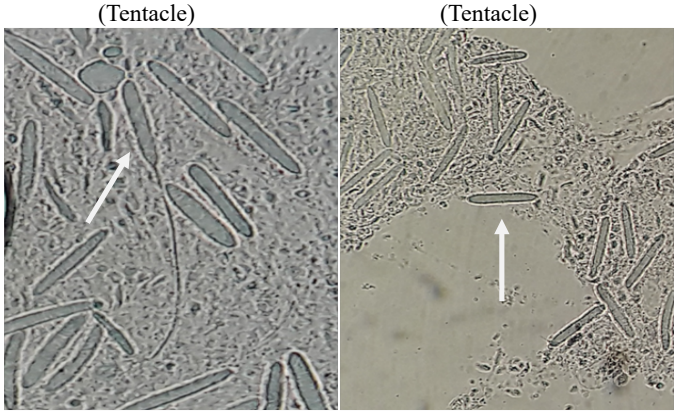



PLATE: 9 *Actinia equina*



Basitrichous isorhizas (“basitrichs”) (Tentacle) Spirocysts (Tentacle)



Microbasic p-mastigophore (DISCHARED) Microbasic p-mastigophore (Coloum)

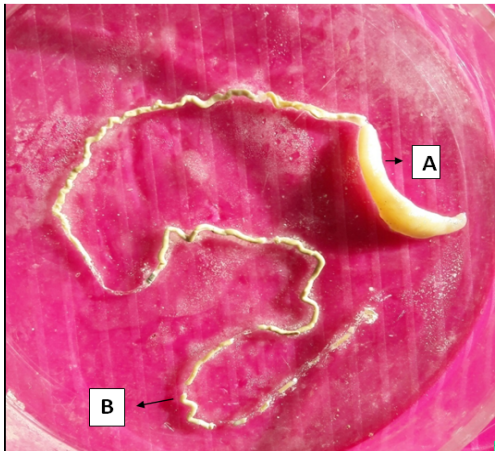


PLATE 11: VENOMOUS APPARATUS OF CONE SNAILS: (A) Muscular venom bulb, (B) Tubular venom duct

The biodiversity indices of cone snails were calculated for five different study sites along the coasts of Dakshina Kannada and Udupi districts. Species richness and species abundance were comparatively higher for St Mary's island than other sites. (Table 1-3)

Table 1: Diversity indicies of cone snails collected from different study sites.

	Bunder	Surathkal	Kapu	St Mary's
Taxa-S	3	1	3	5
Individuals	14	2	5	25
Dominance-D	0.3776	1	0.36	0.4592
Simpson-1-D	0.6224	0	0.32	0.7456
Shannon-H	1.034	0	0.5004	1.467
Evenness-e^H/S	0.938	1	0.8247	0.8671

Table 2: List of cone shells collected during study period.

	Bunder (fish landing center)	Surathkal	Taneer Bhavi	Kapu	St Mary's island
C.inscriptus	7	0	0	0	1
C.inscriptus white	3	0	0	0	2
C.milneedwardsi	4	0	0	0	0
C.biliosus	0	2	0	2	12
C.lentiginosus	0	0	0	2	8
C.balteatus	0	0	0	1	2

Table 3: List of jellyfish collected during study period.

	Bunder (fish landing center)	Surathkal	Taneer bhavi	Kapu	St Mary's island
Rhizostoma pulmo	0	5	7	15	20
Acromitus flagellatus	0	0	25	0	15

Table 4: List of sea anemone collected during study period.

	Bunder (fish landing center)	St Mary's island	Kapu	Surathkal	Taneer bhavi
Actinia equine	0	0	6	10	0
Anthopleura spp.	0	0	12	12	0

Discussion

Marine conservation is of primary concern, due to rise in pollution and resulting deleterious variation. Venomous marine invertebrates secrete various biologically active molecules; few are toxins due to which they receive more attention for their potential uses in drugs and pharmaceuticals. These toxins pay a significant contribution to enhance knowledge in human physiology and pharmacology. Venomous marine invertebrates are important firstly due to their poisonous stings, secondly reports on them are scanty, as they are not hunted for food. Animal kingdom acquires numerous poisonous species that produce venoms (actively delivered) or toxins (passively delivered). Poisonous animals are found in most classes of the Animal Kingdom and inhabit both terrestrial and marine ecosystems.

In present investigation, a detailed survey was under taken to study biodiversity of different venomous marine invertebrates, 5 species of cone snails belonging to single genus *Conus* were recorded. These species were first time reported form the coasts of Dakshina Kannada and Udupi districts.

From the diversity indices calculated species richness, abundance was comparatively higher in St' Marys islands than other study sites; this reflects good health of ecosystem over there. Cone snails are brightly coloured, patterned and highly priced marine ornamental Gastropods so they are used as raw material for shell craft industry and these animals were over exploited.

Among the cone snails collected the most important one is *Conus milneedwardsi* because it is included in schedule list 1 of wild life protection Act 1972.

During study 2 species of jellyfish (Schyphozoans) of order Rhizostomeae belonging to two different genera and 2 species of intertidal sea anemones (Anthozoans) of order Actinaria belonging to two different genera were collected. As studies done by Parulekar (1990)[22] reported 40 species of sea anemones belonging to 33 genera under 17 families from India, of which 13 species were reported for the first time. Out of 40 species, 24 species inhabits in marine, 13 species in estuarine, while 3 species are common to both the habitats. In few study sites the populations of this Cnidarians was completely absent. This negative result of their distribution is probably due to their sensitivity towards exploitation as marine fishery resources, mass mortality in fishing nets, pollution and drastic climatic change.

The Cnidarians like jellyfish play crucial and essential role in marine ecosystem as they consume phytoplanktons and transport carbon to the benthos via faecal pellets, jellyfish are harvested for food and other uses, they serve as food for many invertebrates such as turtles and they provide habitats, refugia for a large variety of taxas. Some of them have potential use in pharmacology and drug discovery[23]. Where as Cnidarians such

as sea anemones form the symbiotic associations with clown fish and hermit crabs.

This study of baseline data on distribution of venomous marine invertebrates especially on cone snails provides platform for creating awareness on venomous stings of these animals and their sensitivity to pollution and climate changes. Conservation of these animals is a primary concern. Otherwise in future, some of the species will be threatened or endangered and ultimately will break the ecological balance in marine environment.

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