ZOANTHIDS (CNIDARIA: VERRILL, 1865) DWELLING THE FOUR ROCKY HABITATS ALONG THE PAKISTAN COAST

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ABSTRACT

Field observations as well as laboratory rearing experiment were carried out. During the study four (4) different species of zoanthids dwelling rocky intertidal platforms of Sindh coast belonging to three (3) genera were collected and identified from four (4) different localities. Other than that laboratory, rearing experiment was also performed on trial basis for all four species. Identified species were *Zoanthus sansibaricus* (Carlgren, 1900), *Z. vietnamensis* (Pax and Müller, 1957), *Epizoanthus scotinus* (Wood, 1957) and *Palythoa tuberculosa* (Esper, 1791). Except *Zoanthus sansibaricus* aforementioned three species have never been reported previously from Pakistan. Collection sites are Buleji, Manora, Paradise point and Sunera beach. Field surveys made during September and October 2016. During laboratory trials *Zonthus sansibaricus Z. vietnamensis* and *Epizoanthus scotinus* were reproduced successfully. However, *Palythoa tuberculosa* did not reproduce sexually in aquarium although it was also maintained successfully.

Key-words: Zoanthids, diversity, Rocky shores, Karachi, Pakistan, Arabian Sea.

INTRODUCTION

Commonly known button polyps or zoanthids are soft bodied most vibrant invertebrates belong to phylum Cnideria. They may be found single or in colonial form attached to the intertidal rocks, some on the sponges or other corals from shallow water or deep seas (Trivedi and Vachhrajani, 2014; Reimer and Miyake, 2009; Irei *et al.*, 2011; Gul, 2013).

To date about 354 known species holding different morphological characters belonging to zoanthids have been reported worldwide (Trivedi and Vachhrajani, 2014; Appeltans *et al.*, 2012). Genus *Acrozoanthus* and *Isaurus* encrust sediments into their tissues except *Zoanthus* (Khushali *et al.*, 2014; Reimer, 2007). Zoanthids contain symbiotic relationship with Zooxanthellae "*Symbiodinium*" (Algae or dinoflagellate) for photosynthesis but some species also feed on Planktons, brine shrimps, small fishes or invertebrate food solutions. They require calcium and some amount of Iodine for an excellent growth. They reproduce by budding and fragmentation (Trivedi and Vachhrajani, 2014; Reimer *et al.*, 2006; Sinniger *et al.*, 2010; Sinniger, 2011). Zoanthids act as epizoic organisms i.e. they live in association with other flora and fauna without harming them. For instance crabs like *Brachyuran*, *Atergatis* and *Eriphia*, *Ulva sp* are found in association with *Zoanthus sansibaricus* and Xanthid crabs in association with Palythoa sp. (Reimer *et al.*, 2010; Khushali, 2015). Recently from American Samoa yellow zoanthids found colonizing the base of a dead golden octocoral have been recorded (NOAA, 2017). Moreover, some zoanthids have toxicological effects as well. Farooq *et al.* (2017) found corneal toxicity associated with aquarium coral palytoxin species.

In Pakistan, only cursory information is available over Zoanthids. Some taxonomic work has been done along the Karachi coast which only documented the presence of *Zoanthus sansibaricus* at Manora of Karachi coast (Gul, 2013) while earlier another study discovered a new alkaloid zoanthaminone isolated form a *Zoanthus* sp. (Atta-ur-Rahman, 1989).

They are difficult to identify morphologically hence, their taxonomy is neglected to be explained (Reimer *et al.*, 2006; Sinniger *et al.*, 2010; Reimer and Miyake, 2009; Irei *et al.*, 2011; Gul, 2013; Trivedi and Vachhrajani, 2014) and there is an immense need of molecular investigations to appropriately identify them, in spite the taxonomic classification (Van der Land and Hartog, 2001). Therefore, from Pakistan in-depth studies are also very much essential to explore zoanthid fauna on appropriate level as well as to safeguard local species from various climatic and anthropogenic threats.

Aim of the study was to investigate the species diversity of Zoanthids along the Karachi coast, Pakistan as there is any pertinent record of Zoanthids is not available in published literature from Pakistan, therefore preliminary studies were taken that will be helpful in future wild stock management and conservation perspective.

MATERIALS AND METHODS

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A total of five (5) field surveys made during September 2016 and October 2016 on selected rocky ledges which are Buleji (24° 50′ 356″ N 066° 49′ 368″ E), Paradise (39.7596° N, 121.6219° W) and Manora (24° 47′ 869″ N 066° 58′ 030″ E) and Sunera beach of (24° 50′ 559″ N 066° 46′ 528″ E) along the Karachi coast (Fig. 1). During field surveys photographs of zoanthid colonies were made and GPS device used to record geographic position of the subjected location. Other than that hand held refract meter, PH meter, digital and mercury thermometers were also used to record in situ and in aquarium (ex situ) physical parameters like Salinity, PH, air and water Temperature respectively. Polyp collection also made from the wild stock by hand for laboratory rearing purpose. Some features of study areas are given below:

Study Area (Fig. 1)

Manora Rocky Ledge (24° 47' 869" N 066° 58' 030" E, Elevation: 10 feet): Located southwest to Arabian Sea.

Buleji (24° 50° 356″ N 066° 49° 368″ E, Elevation: 12 feet): Located southwest to the Arabian Sea comprised of about 8 km long rocky platform. Buleji is sandy from eastern side while mainly rocky from its western side. It contains some muddy patches in eastern flank while tidal pools in western side (Saifullah, 2009). Its rocky platform is triangular in appearance and highly diversified in Flora and Fauna (Afsar *et al.*, 2012).

Paradise Point (39.7596° N, 121.6219° W): It comprises of rocky sandstones and it is an eroding beach which is under the influence of strong wave action and high tides that is why its natural arch or zero point has vanished now that was once exist.

Sunera Beach (24° 50′ 559″ N 066° 46′ 528″ E): Sunera beah is next to Sonari and situated at the boundary between Sindh and Balohistan provinces near Hub River. Benthic profile is rocky with predominately occupied with large and small boulders, caves, shelters and a parallel prominently uplifted rocky platform containing fossil records of oyster beds present along the beach.

Collection identification and maintenance

During surveys, patch sizes recorded at three intertidal zones of each site. Collected samples of Zoanthids transported alive to the laboratory for preliminary cultural trials where placed in aquarium safely by maintaining required Salinity and PH. Total number of polyps counted before placing in aquarium. Periodically Lugol's iodine solution used to boost the Zoanthid's growth in aquarium. Moreover, some collected material also preserved in 4% formaldehyde solution as earlier described by Gul (2013).

Laboratory rearing trials carried out during September 2016 to January 2017 and for this 60-80 liter glass aquaria filled with sea water used with adequate aeration and light system. After maintaining physical parameters, collected polyps of all four species placed in aquarium. Light and aeration provided by using a robust system in aquarium. Data recorded on weekly basis i.e., PH, salinity, temperature, No. of polyp for each species to record growth pattern and possible mortalities.

All of the collected material was identified by the help of certain published literature and collection available in the online libraries i.e., Borneman, 2001; Cairns *et al.*, 2003; Reimer *et al.*, 2004 and 2006; Gotshall, 2005; Reimer, 2007; Reimer and Hickman, 2009; Sinniger, 2011; Appeltans *et al.*, 2012; Gul, 2013; Trivedi and Vachhrajani, 2014; Khushali *et al.*, 2013; Khushali, 2014; Morandini *et al.*, 2015; IUCN, 2016.

RESULTS

Identified Species

A total of four (4) species belonging to three genera have been collected and identified from rocky intertidal ledges of Sindh coast which are Manora, Buleji, Paradise and Sunera beach (Figure 1 and 2). Identified species are *Palythoa tuberculosa* (Esper, 1791) *Zoanthus sansibaricus* (Carlgren 1900) *Zoanthus vietnamensis* (Pax and Müller, 1957) and *Epizoanthus scotinus* (Wood 1957). Species were identified on the basis of external taxonomic characteristic features. Systematic classification of identified species is as given below:

Kingdom Animalia
Phylum Cnidaria (Verrill, 1865)
Class Anthozoa (Ehrenberg, 2834)
Subclass Hexacorallia (Haeckel, 1896)
Order Zoantharia or Zoanthiniaria (Gray, 1832)
Suborder Brachycnemina (Haddon & Shackleton, 1891)
Family Sphenopidae (Hertwig, 1882)

Genus *Palythoa* (Lamouroux, 1816) **1. Species** *Palythoa tuberculosa* (Esper, 1791)

Family Zoanthidae (Rafinesque, 1815)

Genus Zoanthus (Lamarck, 1801)

- **2. Species** Zoanthus sansibaricus (Carlgren, 1900)
- 3. Species Zoanthus vietnamensis (Pax and Müller, 1957)

Suborder Macrocnemina (Haddon & Shackleton, 1891)

Family Epizoanthidae (Delage & Hérouard, 1901)

Genus Epizoanthus (Gray, 1867)

4. Species Epizoanthus scotinus (Wood, 1957)

Identifications of above mentioned four (4) species were made in the light of description provided by earlier workers. Generally, *Palythoa tuberculosa* polyps aggregate to form colony together and a single polyp has been described as a thick and short body column, containing a wide oral disk on top with surrounding tentacles comprised in two rows. When these polyps are out of water, the tentacles and oral disks are folded back into the body column. Colours scheme includes brown, cream and yellow radiate (Ryland and Babcock, 1991; Mather and Bennet, 1993; Delbeek and Sprung, 1997; Borneman, 2001; Reimer, 2007; Deeds *et al.*, 2011).

Zoanthus sansibaricus also occur in colonies in intertidal and shallow waters mostly in strong water currents locations or areas exposed to strong wave action. In general outside of the polyp is consistent, exhibit light to dark gray-blue shades with no noteworthy markings on exterior. Tentacles up to 40–58 besides extensive variation in oral disk colors, patterns, and in colors of tentacles (Reimer *et al.*, 2004, 2006; Reimer and Hickman, 2009; Gul, 2013).

Zoanthus vietnamensis olonies may extend up to 10-15cm. Outside of a polyp's body column grayish blue in color. Bright pink loop around the top of the body column. Pink loop is most apparent when the tentacles are folded back into the body (Reimer, 2007; Khushali *et al.*, 2014).

Table 1. *In-situ* observations and polyp collection details from survey sites.

Site	Date of Collection	Species	Tidal level	Patch size	No. of polyp collected
Buleji	9/16/2016	Palythoa tuberculosa	Mid-Tide	12 x 9	38
		"	Mid-Tide	5 x 6	18
		"	Mid-Tide	3.1 x 1.3	
		Zoanthus sansibaricus	Low Tide	13 x 5	6
		"	Low Tide	8 x 5	53
Paradise	9/16/2016	Zoanthus sansibaricus	Low Tide	5.5 x 3.5	60
		Palythoa tuberculosa	Low Tide	2 x 1.5	
Manora	9/30/2016	Epizoanthus scotinus	Low Tide	13 x 8	40
		и	Low Tide	5 x 3	21
		и	Low Tide	15 x 8	57
		Zoanthus sansibaricus	Low Tide	7.5 x 2	73
		и	Mid-Tide	1.5 x 1.5	50
		u	Low Tide	6 x 2	60
Buleji	9/30/2016	Zoanthus vietnamensis	Mid-Tide	3 x 1.5	28
		u	Low Tide	18 x 15	200
		Zoanthus sansibaricus	Low Tide	10 x 7	130
		"	Mid-Tide	9 x 4	100
		Palythoa tuberculosa	Low Tide	6 x 3.5	180
Sunera	10/18/2016	Zoanthus vietnamensis	Low Tide	8 x 4	>500
		Palythoa tuberculosa	Low Tide	-	
			Mid-Tide	-	
		Zoanthus sansibaricus	Low Tide	-	

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Epizoanthus scotinus with bright orange colour zoanthids found on rocks, from lower intertidal to 54 meters. Reproduce sexually and asexually producing dense aggregations of clones (Cairns *et al.*, 2003; Gotshall, 2005; Sinniger, 2011).

Status and Growth Patterns of Zoanthids along the collection Sites:

Field surveys revealed that there are plenty of Zoanthids present along the rocky beaches of Pakistan related to different genera specifically *Zoanthus*, *Epizoanthus* and *Palythoa*. An excellent growth is seen along the low tidal regions of the coasts but sparse growth is also seen at the mid tidal regions while at high tidal regions there is very little or negligible growth of Zoanthids. *Zoanthus and Epizoanthus sp* are found extremely abundant at low tides while sparse at mid tides while that of *Palythoa* their growth patterns are slightly different to *Zoanthus* species and found in all three intertidal zones (Table 1). In situ observations taken on zoanthid populations inhabiting all four aforementioned sites plus notes taken to keep records of occurrence at these sites as mentioned below and shown in Table 1.

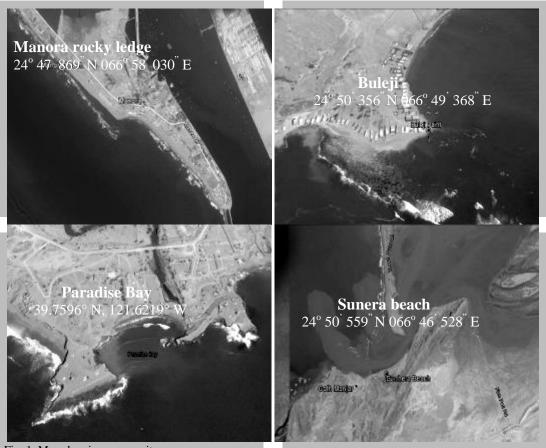


Fig. 1. Map showing survey sites.

Buleji:- Three species of *Zoanthus* and *Palythoa* have been recorded at Buleji which are namely *Zoanthus* vietnamensis, *Zoanthus* sansibaricus and *Palythoa* tuberculosa. **Paradise: -** Patches of *Zoanthus* sansibaricus and *Palythoa* tuberculosa were found at the Paradadise during September 2016.

Manora:- We have only seen *Zoanthus sansibaricus* at Manora but unique patches of *Epizoanthus scotinus* with bright orange colors and low densities have also been observed in lower tidal zone which are not found to any other survey sites except Manora.

Sunera:- Zoanthus vietnamens, Zoanthus sansibaricus and Palythoa tuberculosa species were found at Sunera at low and mid tidal levels.

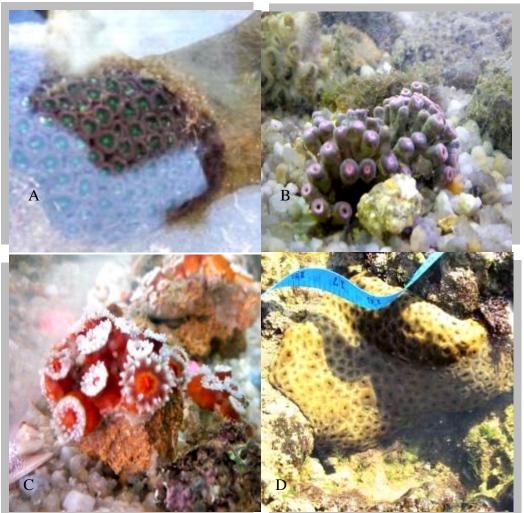


Fig. 2. Identified Zoanthid species maintained in aquarium for laboratory culture trials: Zoanthus sansibaricus (A), Zoanthus vietnamensis (B), Epizoanthus scotinus (C), Palythoa tuberculosa (D).

Growth patterns

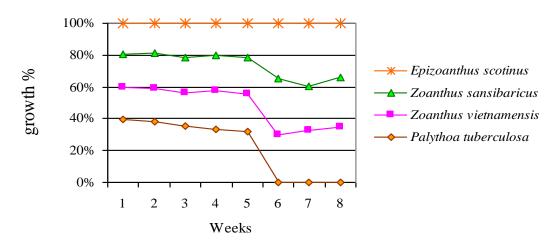


Fig. 3. Trend lines showing growth patterns of Zoanthids over eight weeks; maintained in aquarium for laboratory culture trials in optimum conditions.

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Ex situ growth of collected Zoanthids in aquarium:

The growth of collected Zoanthids judged during September 2016 to January 2017 (Figure 3). Zoanthids have successfully maintained under special observations. Zoanthids related to genus *Zoanthus* and *Epizoanthus* have shown stability under artificial environmental conditions as well as reproduced sextually. In contrast only few which are related to *Palythoa* genus survived for about a month and then died. No sign of polyp multiplication shown by *P. tuberculosa*. Whereas an increase in reared polyps of *Zoanthus vietnamensis*, *Zoanthus sansibaricus* and *Epizoanthus scotinus* was recorded after two weeks that continued over eight weeks. Best growth seen in *Epizoanthus scotinus* that undergo multiplication and persistent growth over eight weeks as shown in Figure 3. While other two zoanthus species shown slight declining trend over a month and *P. tuberclusa* completely declined after four weeks under optimal conditions (Fig. 3).

The growth of Zoanthids boosted with Lugol's Iodine Solution that administered weekly in a small quantity. Recorded salinity range was 34 (minimum) to 38 (maximum) parts per thousand during experiment. Whereas temperature fluctuated between 19 (minimum) to 29 (maximum) and PH remained around eight (8) on average during the study period.

DISCUSSION

Information's on zoanthids from Pakistan are scarce only few published reports are available i. e., *Zoanthus sansibaricus* is reported from Manora rocky ledges, Karachi, Pakistan but it was not confirmed on appropriate level thus molecular identifications is required (Gul, 2013). There is no further work done about Zoanthids regarding their status and identification along the Pakistan Coasts and especially on their culture trials. The present study is not only the first step with respect to exploration of zoanthids at four sites and their status, besides it was the first ever laboratory rearing effort for culture trials of native Zoanthids species collected from the respective sites.

Zoanthids are abundant along the rocky ledges of Pakistan coast. Zoanthus sansibaricus, Z. vietnamensis, Epizoanthus scotinus and Palythoa tuberculosa that have been collected from the respective beaches i.e. Buleji, Paradise and Manora rocky ledges and Sunera belong to three (3) Genera: Palythoa, Zoanthus and Epizoanthus. From India Khushali and Pradeep (2013) found Z. vietnamensis at Veraval and Sutrapada coast. Other than that they have also reported Z. sansibaricus, P. mutuki, P. tuberculosa from India.

Some of the species of Zoanthids also contain stinging cells known as Nematocysts that containing poisonous chemical or toxin called the Palytoxin produced by *Palythoa* sp. while some species for instance, *Zoanthus* sp, contain biologically active compounds like norzoanthamine which is known to have leukemia and osteoporosis controlling properties (Mehra *et al.*, 2015; Genji *et al.*, 2010). Other than Ophthalmological constraints have been recorded caused by palytoxins (Farooq *et al.*, 2017; Vassallo 2017). Another study proved the presence of an acid α -Amino- β -phosphonopropionic in *Zoanthus sociatus* (Kittredge and Hughes, 1964). *Zoanthus* sp are recently recorded to have some fluorescent proteins like Zs Green (zFP506) and Zs Yellow (zFP538) (Krishna and Gophane, 2013; Khushali *et al.*, 2014).

The samples collected for growth purpose in aquarium resulted in efficient growth except *P. tuberculosa* that was not reproduced. While other three *Zoanthus* and *Epizoanthus* species have grown successfully under artificial environmental conditions whenever their Physico-Chemical parameters maintained up to their optimal requirements. Although *Epizoanthus scotinus* has been placed in IUCN red list of threatened species (IUCN, 2016) but during ex situ experiment species growth was remain 100% and *E. scotinus* found to be most persistent among all species. Zoanthid aquarium trade is very common in many countries thus; zoanthids are common in home aquariums. Apart from that in Pakistan still aquarium trade is not well known. Commonly these beautiful organisms are considered comparatively trouble-free to keep in home aquarium furthermore are often recommended to new aquarium keepers (Sud 2013). Thus, several aquarium shop staff and at home certainly several people had experienced palytoxin poisoning resulting in severe inception of clinically attuned symptom (Hamade *et al.*, 2015).

As aforementioned that aim of the study was to investigate the occurrence and species diversity of Zoanthids in and around the Karachi coast, Pakistan due to lack of pertinent record of Zoanthids in published literature from Pakistan, therefore preliminary studies were taken. It is concluded that above mentioned species were superficially identified and preliminary efforts been made to rear them in artificial environment to assess their stability and growth potential. However, it is suggested that critical taxonomic revision and identifications on molecular level may be carried out for confirmation. Thus, DNA bar-coding studies are required for authentic results.

Acknowledgement

Field surveys were managed by utilizing the financial support of DFS grant (DFS-16/ UOK) University of Karachi. Grant efficacy is hereby acknowledged.

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(Accepted for publication August 2018)