7.1. SUMMARY:

The first objective of identifying the zoanthid under study has been achieved by a combination of morphological, histological and molecular analyses. Morphological analysis showed that the zoanthid has a green oral disc with pink ring, tough in nature, colonial intertidal life-style, polyp diameter (8~10mm), polyp height (6~8mm), tentacle count 52~54 and mesentry count 52~56. Histological analysis after decalcification and desilification treatment revealed the arrangement of the mesentries is brachycnemina as the fifth pair of mesentries from the dorsal directive is incomplete. In molecular analysis, from the DNA extracted of the zoanthid sample, 16S mt rDNA gene was amplified (~1200bp). The partial sequence obtained of this amplified gene showed >91% alignment to Z. sansibaricus (Zoanthidae) after BLAST. Though the next nearest alignment was Isaurus tuberculatus of the same family, morphological dissimilarity proved it otherwise.

The second objective of identifying a red-shifted fluorescent protein has been achieved by analysing the purified total protein extract, electrophoretically for its molecular weight and spectrally for its absorbance and fluorescence properties. Three different lysis buffers containing and not containing the enzyme chitinase have been used to extract the protein. These have revealed a better extraction protocol using chitinase as it is the protein present in the cell wall and is hard for lysis. For purification of the extract, ammonium sulphate
precipitation method was employed which resulted in almost 3-6 fold increase in the concentration of the protein extracted, which was estimated by the Bradford’s assay. The electrophoresis conducted in SDS-PA gels indicated a possible presence of one or two proteins in the molecular weight range of 27-28 KDa which is similar to the FPs so far reported. After the spectrophotometric analysis four major peaks were obtained at 670nm, 411nm, 277nm and 214nm respectively. The peak present in the red-range at 670nm was further considered for the fluorescent profile studies and it revealed that the component responsible for absorbance at 670nm has an emission at 678-680nm and excitation at 670-673nm.

7.2. CONCLUSION:
The published scientific reports made so far from India on zoanthids are few and merely deal with the genus level identification, perhaps due to its complicated morphological (internal and external) characteristics. This situation has been rescued by the advent of molecular studies and recent documentation of data world over. According to OBIS (Ocean Biogeographic Information System) database global distribution of *Z. sansibaricus* (Figure. 7) indicates its record mainly from the coast of Japan and along the tropics[107]. This is the first report of molecular and morphological data from India. All the results obtained concluded that the species under identification is *Zoanthus sansibaricus* (Zoanthidae).
Also the literature available so far on zoanthids from India has not revealed anything about their spectral properties. Hence this data being presented is a valuable contribution as it is the first report to this effect from India. Moreover, internationally, the data of fluorescent proteins from zoanthids, has not indicated any red-shifted protein from any specific zoanthid identified to the species level. This concludes that the value of the present work is further increased as it is reporting such a protein from a zoanthid, identified to the species level—*Zoanthus sansibaricus* (Zoanthidae).

As mentioned earlier, fluorescent proteins are not only excellent tags in proteomics but also help resolve phylogenetic analysis and so are zoanthids. Perhaps, this work will also open new frontiers to the scientific fraternity in India to start working in the field of zoanthid taxonomy and identification and isolation of fluorescent proteins from *Zoanthus sansibaricus*.
native sources and be in par with the international scientific community.

7.3. RECOMMENDATIONS FOR FUTURE WORK:

- As mentioned earlier in conclusion, this is the first work of its sort in zoanthid taxonomy and search for fluorescent proteins from India. The author itself, being suitably located at Goa and nearer to National Institute of Oceanography, intends to carry forward future research in this field.

- The work can be extended in both ways – taxonomically by collecting more samples from different shores of Goa and further to other parts of west coast and identifying them to the species level as well as biochemically by identifying, isolating and characterizing FPs from the same.