

# Molecular analysis of zoantharians located at the southernmost region of the Caribbean Sea

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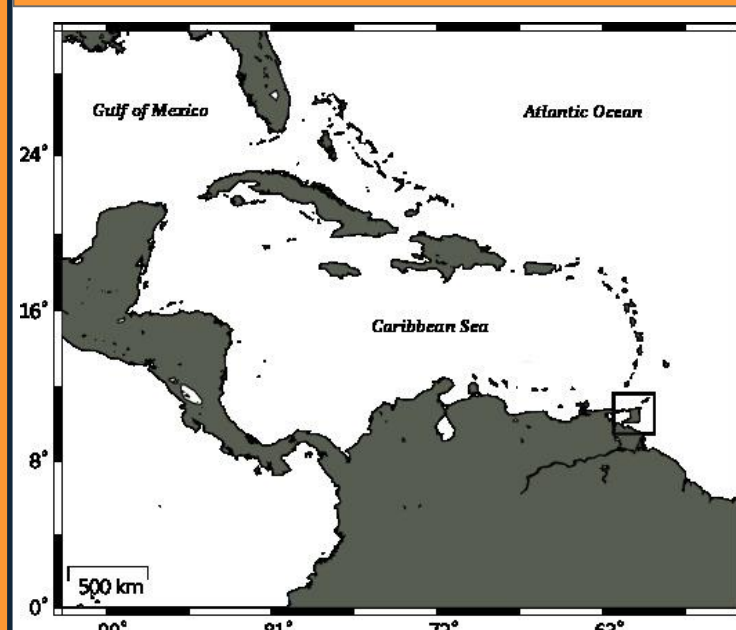
## Abstract

Zoantharians are colonial cnidarians that are globally distributed throughout various tropical and subtropical marine ecosystem. Despite their high abundance and vital ecological role in these marine waters, few studies have accurately identified zoantharians in Trinidad, which is part of the southernmost part of the Caribbean Sea, where *Palythoa* and *Zoanthus* spp. are common. Morphological differences, such as oral disc and tentacle color have made identification of zoantharians difficult. We used molecular and phylogenetic analyses to gain a more comprehensive understanding of zoantharian diversity in Trinidad. We sequenced the mitochondrial 16S ribosomal DNA (16S rDNA) and cytochrome oxidase subunit I (COI) genes of zoantharian polyps collected from this region. Zoantharians were identified as *Zoanthus pulchellus*, *Zoanthus sociatus*, and *Palythoa caribaeorum* using both 16S and COI gene markers. Since molecular information regarding zoantharians in Trinidad is limited, further research is necessary to attain knowledge of zoantharian species diversity in this region.

## Introduction

Zoantharians are colonial cnidarians that commonly contribute to an integral portion of benthos in tropical and subtropical reefs worldwide (Reimer *et al.*, 2014; Belford, 2021). In Trinidad, which is located near the southernmost region of the Caribbean, *Palythoa* and *Zoanthus* spp. are prevalent; in fact, these genera are more abundant than stony corals (Order: Scleractinia) in relation to benthic coverage in the region (Belford, 2021). Nevertheless, studies focusing on zoantharian species identification in Trinidad are lacking despite their high abundance and significant ecological function in these waters. This limited knowledge of zoantharian diversity not just in the southern Caribbean but also throughout coral reefs worldwide is partly due to difficulty with zoantharian species identification (López *et al.*, 2019). Zoantharians exhibit high levels of intraspecific morphological variation, rendering reliable identification challenging (Reimer *et al.*, 2014; Noori Koupaei *et al.*, 2018; Lopez *et al.*, 2019; Belford, 2021). In many ways, these morphological differences, such as differences in oral disk and tentacle color, can often cause zoantharians to be overlooked in ecological surveys (Santos *et al.*, 2015; Belford, 2021). Considering the current lack of information in the region and the difficulties associated with phenotypic plasticity, molecular and phylogenetic analyses in combination with morphological data are needed to achieve a more comprehensive understanding of species diversity in Trinidad. The knowledge that this study contributes is especially important not only because this molecular and morphological data is novel for the region but also because coral reefs in the area face the threat of development. In 2019, a development plan for a port that could provide various services, such as offshore bunkering and oil industry servicing, was proposed by the government. The port is set to be located in Grande L'Anse of Toco, Trinidad, destroying 100% of the coral reef ecosystems in the area.

## Methods



### Study site and Sampling

A 50-meter open reel fiberglass measuring tape was placed in a 500 m<sup>2</sup> area parallel to the shoreline, and benthic components recorded at every 0.5 m interval on the measuring tape. Benthic components were reef-building included corals, zoantharians, macroalgae, coral rubble, and other invertebrates, such as sea urchins, fire worms, and sea cucumbers.



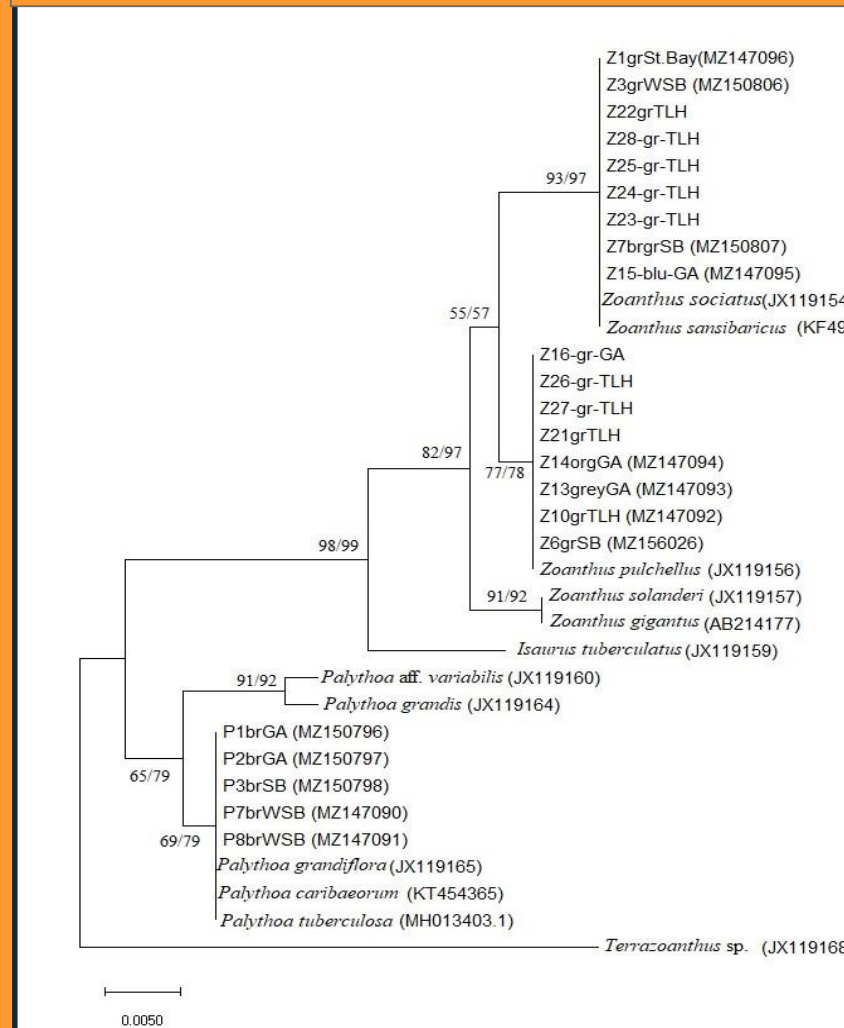
### DNA Extraction, PCR, and ITS

DNA was extracted from 30-50 mg of the specimen's tissue per the manufacturer's protocol of the E.Z.N.A. Tissue DNA Extraction Kit (Omega U.S.A.) A universal primer and the zoantharian specific primers (Folmer *et al.* 1994; Sinniger *et al.* 2005) were used to enhance the mitochondrial cytochrome oxidase subunit I (COI) and 16S ribosomal DNA (rDNA) genes before putting the extracted DNA in the PCR. The PCR was run to amplify the DNA. The aliquots were checked by 1.7% agarose gel electrophoresis to ensure amplification and then were enzymatically purified using 10 ml ExoSAP. The PCR products were sent to Eurofins Genomics (Kentucky, U.S.A) to be sequenced in both directions.

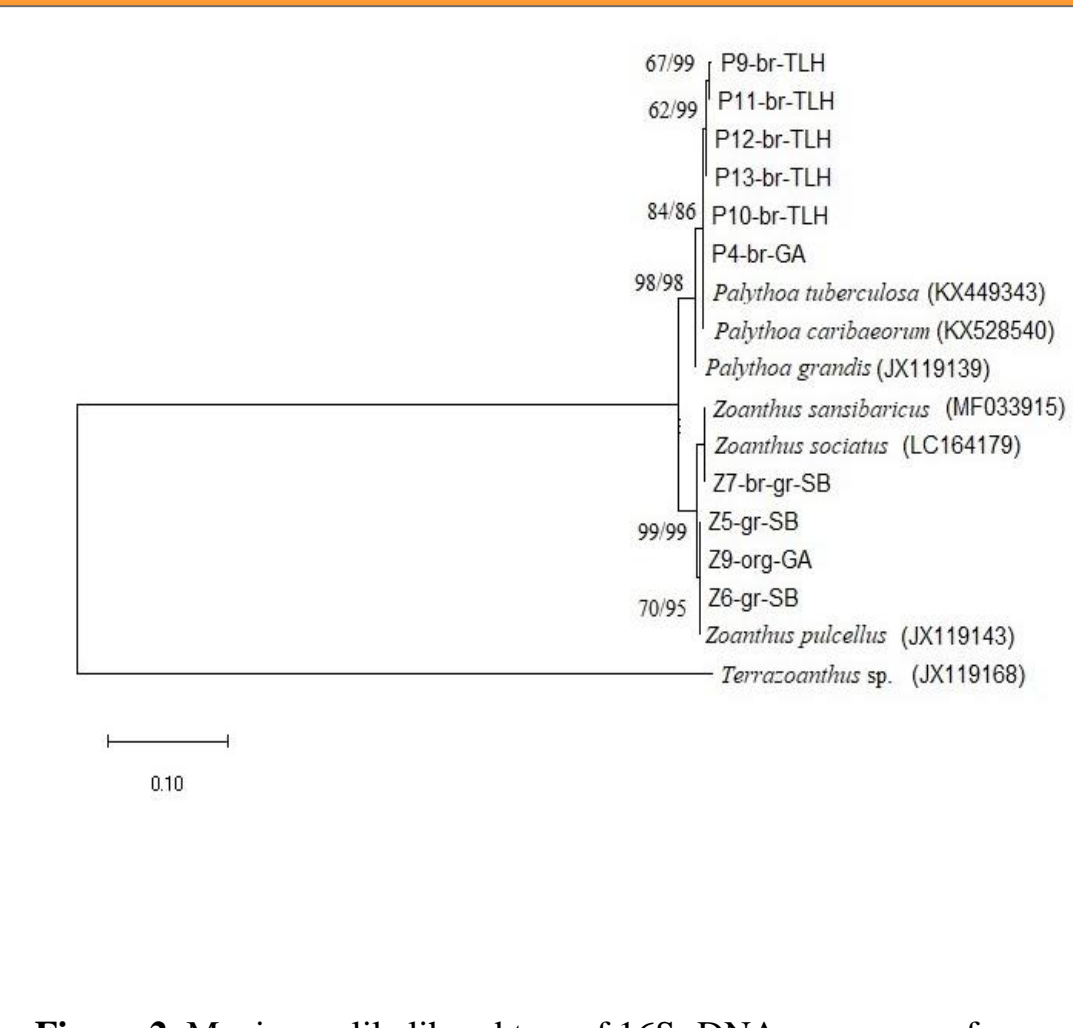
### Specimen Collection

The zoantharian samples were collected from the northeastern coastline of Toco, Trinidad between May 2019-February 2020. The specimens were from sites at Toco Bay, Salybia, Peckel Bay (Grande L'anse), Straight Bay, and Toco Light House during low tides. Each sample from collected using tweezers to excise 3-5 polyps per each zoantharian colony. The specimens were placed in 1.5ml vials with 95% ethanol. Each was stored in a -20 C freezer at the University of Tennessee Southern, Pulaski, Tennessee, U.S.A

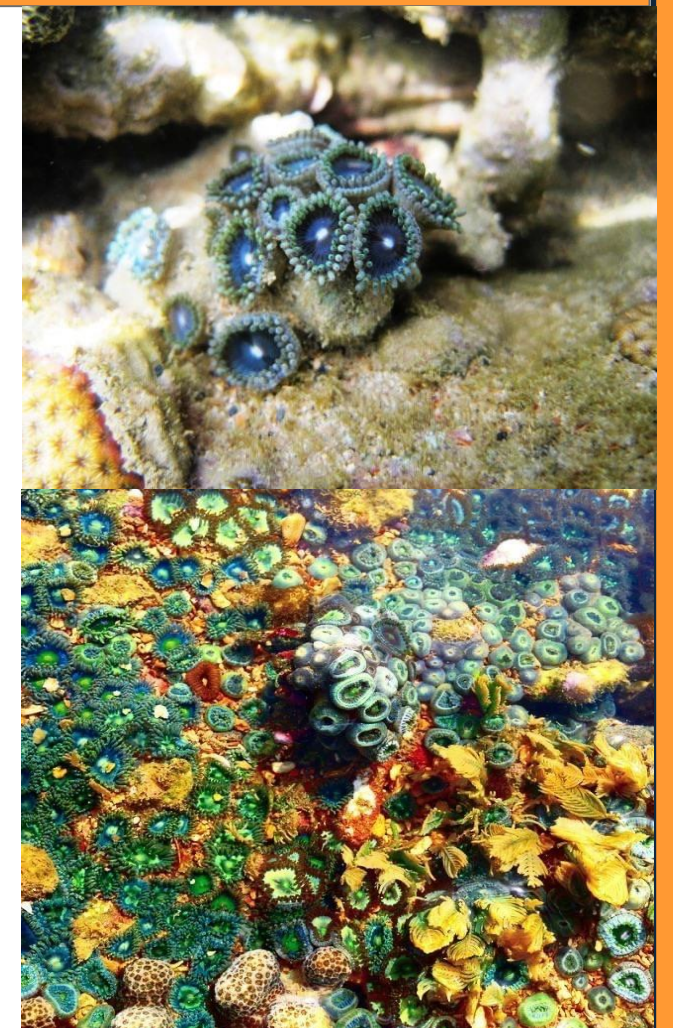
## Results



**Figure 1.** Maximum likelihood tree of COI sequences for zoantharian specimens. Values at branches represent maximum likelihood bootstrap percentages from 1000 trees/maximum parsimony bootstrap percentages from 1000 trees. Scale shows substitutions per site. Accession numbers from other studies shown in parentheses.



**Figure 2.** Maximum likelihood tree of 16S rDNA sequences of zoantharian species collected at Toco, Trinidad: Toco Lighthouse (TLH). Values at branches represent maximum likelihood bootstrap percentages from 1000 trees/maximum parsimony bootstrap percentages from 1000 trees. In this case, the 16S analysis confirms P9-P13 (our recent samples) to be *Palythoa caribaeorum*. Unfortunately, we did not get clear results for *Zoanthus* sp.



**Figure 3.** Photographs of zoantharians in Trinidad. Top photo: Photo of *Zoanthus sociatus*. Bottom photo: Photo of *Zoanthus* spp., including *Zoanthus sansibaricus*, *Zoanthus sociatus*, and *Zoanthus pulchellus*.

## Results & Conclusion

PCRs yielded 9 COI high quality amplicons, with lengths between 658-817 bp. BLAST results showed sequences belonging to the following species: *Zoanthus sociatus*, *Zoanthus pulchellus*, and *Zoanthus sansibaricus* (Figures 1 and 2). *Palythoa* samples did not reveal clear sequences.

Large areas of the reefs at Pequelle and Grande L'anse Bays are engulfed in various species of reef-building corals and zoantharians. Invertebrates were found in both low wave action areas such as rocky protected areas, and high wave action areas. Each species plays a vital ecological role within this region. Intraspecific morphological differences within zoantharians varies greatly in this benthic region. Genetic analyses revealed identification of *Palythoa caribaeorum* as well as *Zoanthus sociatus*, *Zoanthus pulchellus* (Figure 3), and *Zoanthus sansibaricus*. More morphological and phylogenetic data will be collected here and around the Caribbean and the Atlantic Ocean in an effort to better characterize the distribution of cnidarian species in these regions. This study is important because of the new morphological and molecular data obtained and to better understand and highlight the harmful effects of potential development in Trinidad, which will inevitably destroy vital coral reefs in the region.

## References

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