

Combining molecular analyses with morphology to identify zoantharians at Toco, Trinidad, southernmost region of the Caribbean Sea

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Abstract

Annual coral reef surveys recorded cnidarian and invertebrate abundance off the northeastern coast of Toco, Trinidad. However, some species were difficult to accurately identify due to morphological differences. One such cnidarian species are zoantharians, therefore molecular and phylogenetic analyses in this study gained a more comprehensive understanding of zoantharian diversity. Species morphology and molecular analyses were used for species identification. Benthic surveys at Saline Bay (SB) recorded zoantharian mean cover as 60.5 %, with *Palythoa* and *Zoanthus* spp. being most widely distributed. Mitochondrial cytochrome oxidase subunit I (mt COI) and mitochondrial 16S rDNA markers identified zoantharian specimens as *Z. pulchellus*, *Z. sociatus*, *P. caribaeorum*, and *P. grandiflora*. Specimens sequenced also added for the first time *Z. aff. pulchellus*.

Introduction

Zoantharians along the northeastern coast of Toco, Trinidad are cnidarian invertebrates that form a biodiverse assemblage unique to the area (Belford et al. 2019; 2021; 2023).

Variations in color morphotypes among zoantharians: *Palythoa* and *Zoanthus* spp. can hamper accurate species identification (Belford 2021).

Molecular and morphological analyses aid in accurate identification of zoantharian species, which will assist in future species distribution surveys.

Methods

Study Site and Sampling

The line intercept transect (LIT) method was used to collect benthic abundance data. A 50 m open reel fiberglass measuring tape was placed parallel to the shoreline in each area, and benthic components were recorded at every 0.5 m interval.

DNA Extraction and PCR Amplifications

DNA extraction was done using the E.Z.N.A tissue DNA kit.

PCR amplification of mitochondrial cytochrome oxidase subunit 1 (COI) and 16S ribosomal (rDNA) were done using zoantharian-specific primers.

A 1% gel agarose gel electrophoresis was used to check aliquots from PCR amplification.

PCR products were sequenced in both directions at Eurofins Genomics (Louisville, KY, USA).

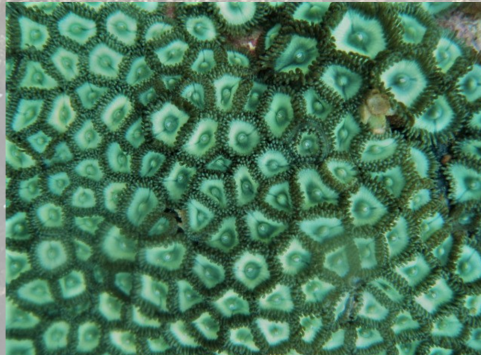
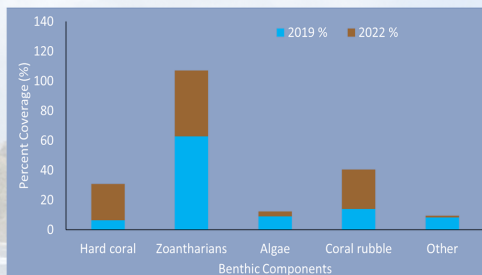


Fig. 3 (top) *In situ* images showing zoantharian color morphotypes observed along the coast of Toco, Trinidad: Top: (1) *Palythoa caribaeorum* (brown), (2) *Palythoa grandiflora* (green), (3) *Zoanthus sociatus* (blue color morph). Bottom: *Zoanthus* aff. *pulchellus* green morph.

Fig. 1 (left) Percentage benthic coverage of cnidarians, algae, coral rubble, and invertebrates for Saline Bay.

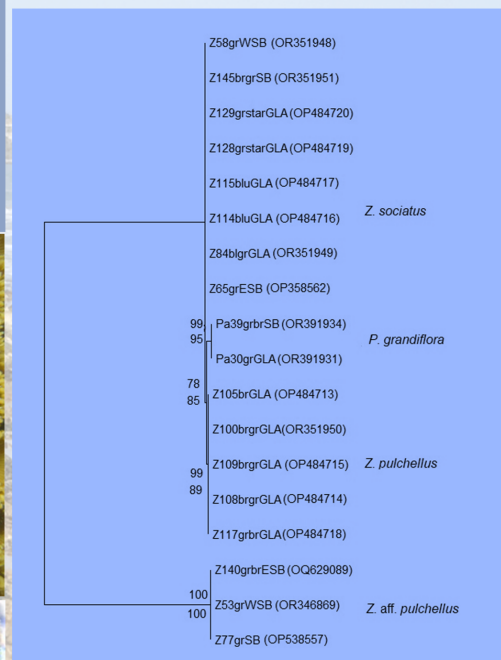


Fig. 2 (top) Maximum likelihood tree of 16S rDNA sequences for zoantharian specimens collected from the northeastern coast of Toco, Trinidad. Values at branches represent maximum likelihood bootstrap percentages from 1000 trees respectively.

Results

Mean zoantharian percentage cover between 2019 and 2022 was 67.5% and 53.5% for Saline Bay (Fig. 1).

Phylogenetic analyses identified zoantharian specimens as *Z. pulchellus*, *Z. sociatus*, *P. caribaeorum*, and *P. grandiflora*, and *Zoanthus* aff. *pulchellus*.

Palythoa caribaeorum only has a brown color morph with a well developed coenenchyme, whereas *Palythoa grandiflora* has brown and green color morphs lacking a well-developed coenenchyme: confirmed by 16 S molecular analysis (Fig. 2).

This also is the first documentation of *Zoanthus* aff. *pulchellus* using 16S zoantharian-specific primers, which allows for more species-specific benthic surveys of zoantharians in the future (Fig. 3).

Conclusions

Both mitochondrial COI and 16S markers are sufficient to identify zoantharians (Reimer et al. 2006), hence we will continue genetic analyses for samples in this area. The goal is to now use information from these analyses to conduct future surveys with accurate zoantharian species distribution.

The immediate future goal is to continually collect morphological data, which can be used to compare genetic analysis of other zoantharian species, and other benthic organisms located at these southern Caribbean reefs.