Molecular analyses and benthic distribution of shallow-water zoantharians (Cnidaria: Hexacorallia) and their symbionts Symbiodiniaceae along the north-eastern coast of Toco, Trinidad.

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INTRODUCTION

- Coral reef habitat in Trinidad, southern Caribbean have a biodiverse assemblage of cnidarian and invertebrate abundance along the north-eastern coast of Toco (Belford & Phillip 2012; Belford *et al*. 2019; 2020). Although similar species may exist throughout the Caribbean, many species require molecular analyses and morphological data for accurate identification.
- Zoantharians, such as *Palythoa* and *Zoanthus* spp. have variable color morphotypes on coral reef ecosystems in the Caribbean (Belford & Phillip 2011; 2012; Belford *et al*. 2019; Belford 2020; Belford 2021). Colors range from brown, green, blue, orange, and grey in zoantharians, making it difficult to accurately identify them in marine habitat.
- Symbiotic marine dinoflagellates in the family Symbiodiniaceae form mutualistic association with cnidarian hosts. As global climate change trends towards elevated sea surface temperature, the cnidarian-Symbiodiniaceae relationship may be key to determine cnidarian response to heat stress, hence Symbiodiniaceae biodiversity will determine species sensitivity to heat stress.

AIMS

- Compare annual cnidarian and zoantharian abundance and distribution, and explore any patterns at sites along the northeastern coast of Toco, Trinidad.
- Add molecular data on zoantharian and symbiont species identification.

METHODS

- A 50 meter open reel fiberglass measuring tape was placed in a 500 m² area parallel to the shoreline, and benthic components recorded at 0.5 meter intervals on the measuring tape.
- DNA was extracted from 1-2 polyps, followed by the manufacturer's protocol using an E.Z.N.A. Tissue DNA Kit (Bio-Tek, Omega. U.S.A.).
- Mitochondrial cytochrome oxidase subunit 1 (mt COI), and 16S zoantharian-specific primers (LCOant, COIantr, and 16 SmoH, 16Sant1a were used to amplify genes (Folmer *et al*. 1994; Sinniger *et al*. 2005).
- Symbiodiniaceae DNA extraction used the (Cetyl Trimethyl Ammonium Bromide (CTAB) method (Baker 1999) for the internal transcribed spacer 2 (ITS2) region, and sequenced using protocols mentioned in LaJeunesse *et al*. 2003).
- Aliquots from PCR amplification were checked by 1.0% agarose gel electrophoresis, then enzymatically purified with 1-3 µl ExoSAP-IT. PCR products were sequenced in both directions at Eurofins Genomics (Kentucky, U.S.A.).

CONCLUSIONS

- This is the first recorded genetic analysis of zoantharians for this region highlighting the diversity of these benthic organisms on these reefs. *Zoanthus* species remain challenging to identify in the field due to intraspecific variation from phenotypic plasticity seen in morphology (Reimer *et al*. 2004).
- Both mitochondrial COI and 16S markers are sufficient to identify zoantharians (Reimer *et al*. 2006), hence will be used to 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 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.

RESULTS

1

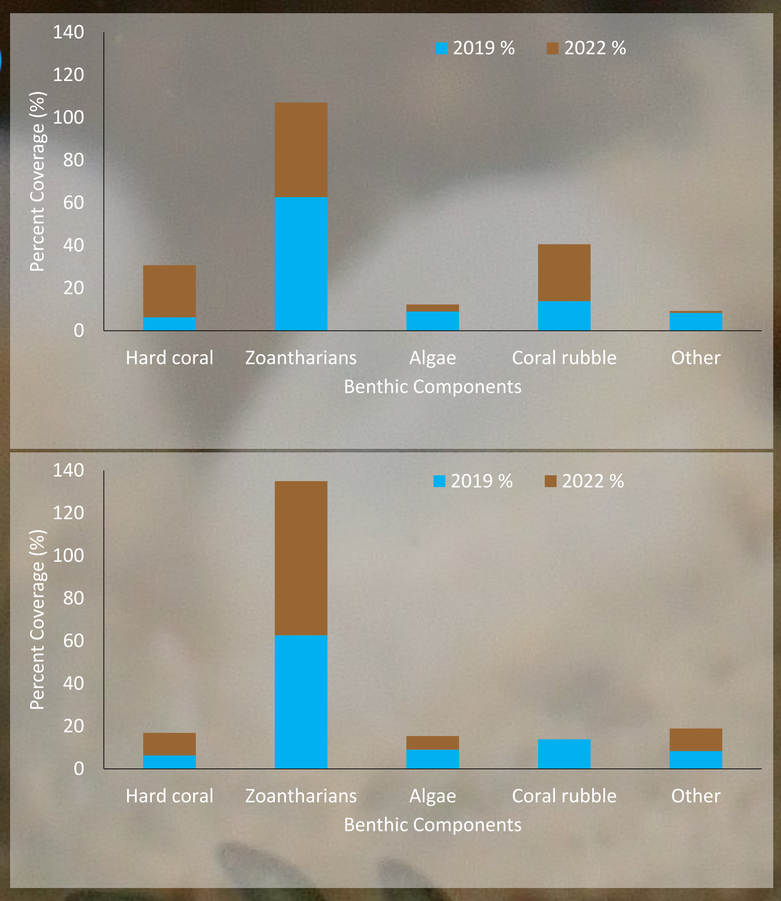

Fig. 1 Percentage benthic coverage of cnidarians, algae, coral rubble, and invertebrates for Salybia Bay (top), and Grande L'Anse (bottom).

Mean zoantharian percentage cover between 2019 and 2022 was 53.5% and 67.5% for Salybia and Grande L'Anse Bays respectively (Fig. 1).

Zoanthus sociatus and *Zoanthus pulchellus* are the main species found along the coast of Toco (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. 2).

Variable zoantharian color morphotypes continue to be very diverse with many green, blue, orange, grey, and brown types observed (Fig 3).

Symbiodiniaceae species *Cladocopium* and *Symbiodinium* identified for *Palythoa* and *Zoanthus* spp. respectively.

2

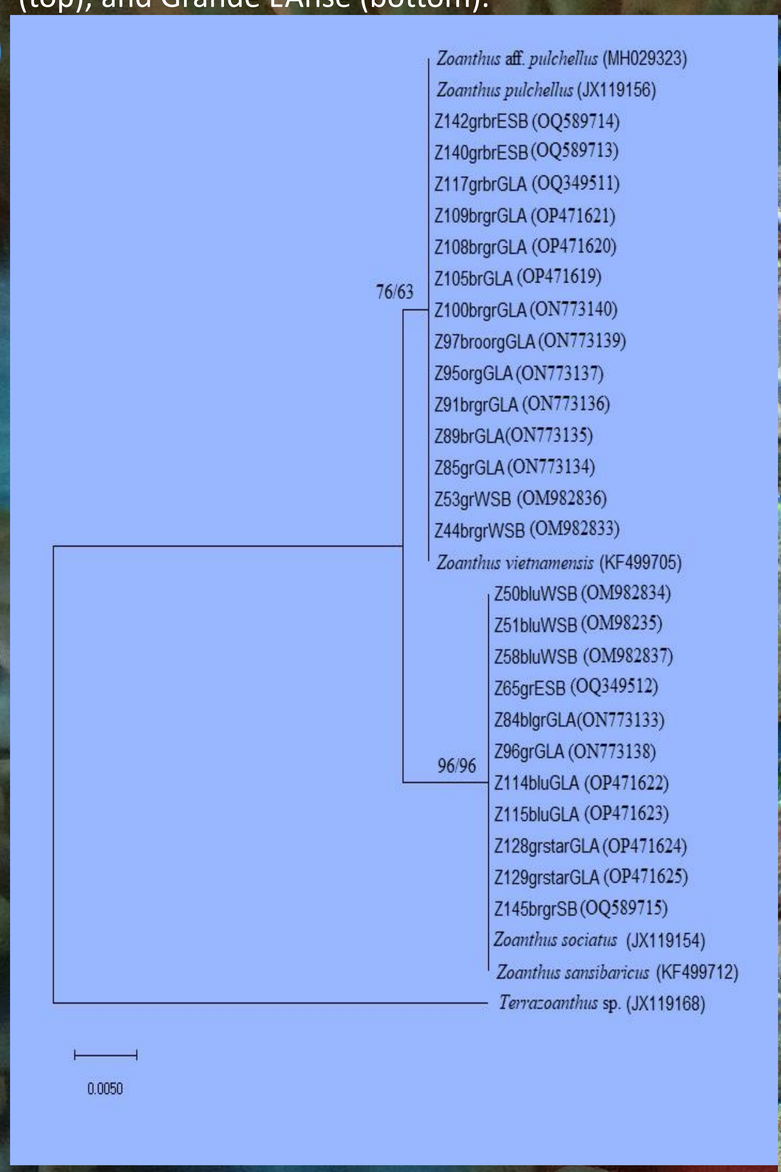

Fig. 2 (above) Phylogenetic maximum likelihood tree (top) generated from the mitochondrial COI sequences. GenBank accession numbers are shown in parentheses. Values above branches represent maximum likelihood and maximum parsimony for 1000 bootstraps.

3

Fig. 3 (right) *In situ* images showing various zoantharian color morphotypes observed along the north eastern coast of Toco, Trinidad: Top: *Zoanthus sociatus* blue color morph (Z114-blu-GLA), green-star (Z128-gr-star-GLA). Middle: *Zoanthus pulchellus* brown (Z88-br-GLA). Bottom: *Zoanthus* aff. *pulchellus* green morph (Z140-grbr-GLA). White bar scale = 1 cm.