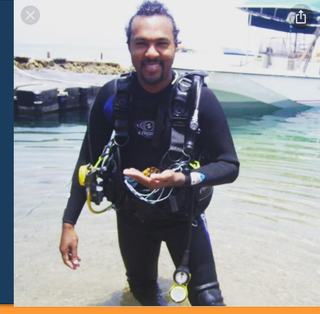
Molecular identification of zoantharians (Cnidaria: Hexacorallia: Zoantharia) from Trinidad, southern Caribbean

Stanton G. Belford, Ph.D.

University of Tennessee-Southern, 433 West Madison Street, Pulaski, Tennessee 38478. U.S.A.

sbelfor2@utsouthern.edu



Abstract & Introduction

Abstract

Shallow-water zooxanthellate zoantharians are colonial cnidarians commonly found in the Caribbean Sea, and are biodiverse throughout its global distribution. Although *Palythoa* and *Zoanthus* spp. occur at Toco, Trinidad, in the southern Caribbean, species identification is challenging due to unclear morphological features, such as: oral disc diameter, number of tentacles, oral disc and tentacle colors. Molecular techniques, such as DNA barcoding have standardize the use of molecular markers, where an applicable range of markers can accurately identify species. This study compares results from mitochondrial cytochrome oxidase subunit I (COI) and mitochondrial 16S ribosomal DNA (16S rDNA) genes for zoantharian identification. Results for 20 zoantharian samples showed differentiation of species to be: *Palythoa caribaeorum*, *Palythoa grandiflora*, *Zoanthus pulchellus*, *Zoanthus sociatus*, and *Zoanthus sansibaricus*. This is the first molecular analyses of zoantharians in this region of the Caribbean Sea, and both markers are considered to be reliable for future zoantharian identification, which will assist in coral reef surveys on zoantharian distribution in this region.

Introduction

Coral reef habitats along the north-eastern coast of Toco, Trinidad have variable cnidarian and invertebrate densities and distributions (Belford & Phillip 2011; Belford *et al*. 2019; Belford 2020; Belford 2021). Although identification of common benthic species is mostly easy relative to morphology, many species require molecular analyses in conjunction with morphological data for accurate identification. For example, zoantharians, such as *Palythoa* and *Zoanthus* spp. have various color morphotypes on coral reef ecosystems at Toco, Trinidad (Belford *et al*. 2019; Belford 2021). Morphs appear brown, green, blue, orange, and grey, making it difficult to identify specific species due to intraspecific variation (Reimer et al. 2004). Since zoantharian identification studies are scant in this region, molecular markers will add knowledge for this species.

Methods

Fig. 1. Map of the northeastern coast of Trinidad, showing the major bays along Toco.

DNA extraction, PCR, and Sequencing

Deoxyribonucleic acid (DNA) was extracted from 30-50 mg of tissue, followed by the manufacturer's protocol using an E.Z.N.A. Tissue DNA Kit (Omega. U.S.A.).

Mitochondrial cytochrome oxidase subunit 1 (COI) was amplified using a universal primer, zoantharian-specific, and 16S rDNA primers (Folmer et al. 1994; Sinniger et al. 2005).

Aliquots from Polymerase Chain Reaction (PCR) amplification were checked by 1.7% agarose gel electrophoresis, then enzymatically purified with 10 µl ExoSAP. PCR products were sequenced in both directions at Eurofins Genomics (Kentucky, U.S.A.).

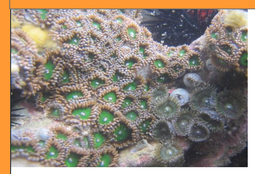
Specimen Collection

Zoantharian specimens were collected along the northeastern coast of Toco, Trinidad from May 2019-February 2020 (Fig. 1) at sites: Toco Bay and Baptiste Bay (Grande L´Anse), Straight Bay, Salybia, Peckel Bay, and at Toco Light House, during extreme low tides. A steel hand-held scapula and tweezers were used to excise 3-5 zoantharian polyps per colony. All specimens were placed in 1.5 ml vials containing 95% ethanol, and stored in a freezer at -20 °C.

Phylogenetic Analysis

DNA sequences were inspected by eye and manually edited using Molecular Evolutionary Genetics Analysis (MEGA-X). New sequences were deposited in GenBank (accession numbers OL310189-OL310195) and were used to align with publicly accessible sequences for other zoantharians (GenBank accession JX119160, JX119164, JX119165, JX119167, JX119157, JX119156, JX119154, JX119159, JX119168, KT454365, AB214177, KF499705, KF499712). Alignments were inspected and errors in nucleotide sequences that were low quality trimmed. Analysis used maximum likelihood and maximum parsimony with bootstrap percentages from 1000 trees .

Results



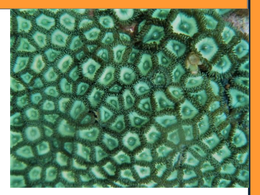


Fig. 2. Maximum likelihood tree of 16S rDNA sequences for zoantharian specimens: *Palythoa* and *Zoanthus* spp. for Salybia Bay (SB), Toco Lighthouse (TLH), and Grande L'Anse Bay (GA) respectively. Values at branches represent maximum likelihood bootstrap percentages of 1000 trees/maximum parsimony bootstrap percentages from 1000 trees. GenBank accession numbers are shown in parentheses.

Fig. 3. Phylogenetic maximum likelihood tree generated from the mitochondrial COI sequence alignment. GenBank accession numbers for species are shown in parentheses. Numbers above branches represent maximum likelihood and maximum parsimony bootstrap percentages from of 1000 trees. In situ photographs of zoantharian colonies along the northeastern coast of Toco, Trinidad showing brown color morphotype of *Palythoa caribaeorum* specimen P3-br-SB, green color morphotype in *Zoanthus pulchellus* specimen Z5-gr-SB, Z6-gr-SB.

Results & Conclusions

- Reef-building corals (*Porites* sp.) and zoantharians cover large areas of the reefs at Salybia, Pequelle and Grande L'Anse Bays, as well as at the Toco Lighthouse. In the past, zoantharians were surveyed and identified only as *Zoanthus* and *Palythoa* spp., however we can now accurately identify zoantharians using both mitochondrial COI and 16S rDNA (Fig 2 and 3).
- Genetic analyses of zoantharians for this region highlights the diversity of these benthic organisms on the reefs. *Palythoa* and *Zoanthus* spp. were identified as *Palythoa caribaeorum* Duchassaing & Michelotti, 1860 *Palythoa grandiflora* (Verrill, 1900), *Zoanthus sociatus* (Ellis, 1768), and *Zoanthus pulchellus (*Duchassaing & Michelotti, 1860), and *Zoanthus sansibaricus* Carlgren 1900. I plan to collect morphological and phylogenetic data to compare zoantharian species throughout the Caribbean Sea, and Atlantic, relative to species found at Toco, Trinidad. Data will publish new information on zoantharian species identity and phylogeny species in the Caribbean.

References

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