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Report of a *Zoanthus* Zone from the Cabo Verde Islands (Central Eastern Atlantic)

Lopez Cataixa ^{1,2,*}, Freitas Rui ³, Magileviciute Edita ⁴, Ratao Sara S. ⁵, Brehmer Patrice ⁶,
Reimer James D. ^{2,7}

¹ ULL, Fac Ciencias, Dept Biol Anim Edafol & Geol, Ave Astrofis Francisco Sanchez S-N, San Cristobal De La Lagu 38206, Spain.

² Univ Ryukyus, Fac Sci, Dept Biol Chem & Marine Sci, Mol Invertebrate Systemat & Ecol Lab, 1 Senbaru, Nishihara, Okinawa 9030213, Japan.

³ Univ Cabo Verde Uni CV, Fac Engn & Ciencias Mar, Mindelo 163, CP, Cape Verde.

⁴ ECOCV, Assoc Cabo Verdiana Ecoturismo, Santiago Isl 7600, Cape Verde.

⁵ FMB, Cidade Do Porto Ingles 6110, Maio Island, Cape Verde.

⁶ CRODT Pole ISRA Hann, Inst Rech Dev IRD France, UMR 195 Lemar, Dakar, Senegal.

⁷ Univ Ryukyus, Trop Biosphere Res Ctr, 1 Senbaru, Nishihara, Okinawa 9030213, Japan.

* Corresponding author : Cataixa Lopez, email address : clopezba@ull.edu.es

ruifreitas@docente.unicv.edu.cv ; edita.magileviciute@icloud.com ; ratao.sara@gmail.com ;
patrice.brehmer@ird.fr ; jreimer@sci.u-ryukyu.ac.jp

Abstract :

Zoanthus is a genus of colonial cnidarians very common in shallow subtropical and tropical rocky and coral reef ecosystems around the world. Some species, such as *Zoanthus solanderi* and *Z. sociatus*, can coexist and cover extensive areas in the subtidal zone of some areas in the Caribbean. We report on a *Zoanthus* zone' in Western Africa in a bay in the north of Maio Island in the Cabo Verde Archipelago, discovered in October 2014. Molecular analyses showed that the Maio Island carpet is formed by *Z. sociatus* and *Z. solanderi*. Similar aggregations have been reported from the other side of the Atlantic Ocean in the Caribbean. This work demonstrates the presence of the *Zoanthus* zone' in the Central East Atlantic and adds two new records of *Zoanthus* species in the Cabo Verde Islands.

Keywords : Marine biodiversity, Maio Island, Molecular analyses, West Africa, *Zoanthus sociatus*, *Zoanthus solanderi*

INTRODUCTION

Zoantharians (Anthozoa: Hexacorallia) are an order of colonial cnidarians with species found in most marine environments from temperate to tropical areas and from the intertidal zone to the deep sea (Ryland and Lancaster 2003). In particular, the zooxanthellate genera *Zoanthus* and *Palythoa* are common in subtropical and tropical shallow rocky and coral reef ecosystems (Karlson et al. 1981; Irei et al. 2011) usually as discrete colonies formed by hundreds of polyps. However, some zoanthids species, such as *P. caribaeorum*, are frequently found covering large areas in the subtidal zones (Sebens 1982; Acosta 2001). In 1981 Karlson and collaborators discovered, on the north coast of Jamaica, the unique place where it is known that local populations of *Zoanthus solanderi* (LeSueur 1818 *sensu* Karlson 1980) and *Z. sociatus* (Ellis 1768) can cover a huge portion of the intertidal zone. The area occupied by these two species is so huge that this portion of the intertidal has been called the ‘*Zoanthus zone*’ (Karlson et al. 1981). In October 2014 a huge *Zoanthus* aggregation was accidentally discovered in Porto Cais Bay, one of the northernmost and sheltered areas of Maio Island, Cabo Verde. This bay is characterized by being a flat area of warm shallow waters affected by semi-diurnal microtidal low current regimes with relatively low transparency due to terrigenous material accumulation in the water column from leaching. Porto Cais Bay is included in the protected Nature Park of the North of Maio Island (PNNM). This Nature Park is part of a network of protected areas implemented in 2003 with a significant expansion of marine area and final boundaries including a marine area were set in 2014 (Anonymous, 2014).

It is known that several coral species, including zoanthids (Pax and Müller 1956; Reimer et al. 2010), have an amphi-atlantic distribution (Nunes et al. 2011), however, *Z. solanderi* and *Z. sociatus* have never been found in Cabo Verde. In fact, only one species of *Zoanthus* has been reported from the Archipelago until now, *Z. aff. pulchellus* (Duchassaing and Michelotti 1864) (Reimer et al. 2010), and there is no record that this species has the ability to grow disproportionately. On the other hand, *Z. aff. pulchellus* is characterized by having polyps partially

or completely embedded in a well-developed coenenchyme (Reimer et al. 2010) and the species located in Maio had liberate polyps.

In this study we report on this large aggregation showing that the *Zoanthus* zone in Jamaica is not an isolated event and some zoanths species can overspread large areas. Moreover, we utilize both morphological and molecular analyses in order to identify the species of *Zoanthus*. Our aim is to demonstrate if the ability of cover huge areas is characteristic of *Z. solanderi* and *Z. sociatus* and therefore, we registered two new records of *Zoanthus* species in Cabo Verde, or if on the contrary, this massive grow is common in other Zoantharian species.

MATERIAL AND METHODS

Study site, specimen collection and initial *in situ* identification

This study was carried out around Maio Island, situated on the largest insular shelf in Cabo Verde Archipelago. The *Zoanthus* aggregation was discovered in Porto Cais Bay (15°18.95'N, 23°11.06'W) (Figure 1) at around 1.5 m depth in October 2014. For an accurate outer boundary of the *Zoanthus* aggregation a thorough survey was carried out in October 2016 by snorkeling around the outer edge while obtaining a total of 31 geo-referenced points (in UTM WGS 84) taken with a waterproof GPS receiver. The QGIS (2016) open source software was used to estimate the area as a polygon of the '*Zoanthus* zone' in Porto Cais Bay, Maio Island based on geo-referenced points.

Species were initially identified as *Zoanthus* spp. due to the lack of sandy or muddy encrustations in the body wall. However, the specimens had polyps well clear and free of the coenenchyme, indicating that the component species of the aggregation were not *Z. aff. pulchellus* (Figure 2). Underwater digital images taken *in situ* were used for subsequent gross morphological analyses including polyp shape, numbers of tentacles, coenenchyme development, and colour (n = 30 polyps analyzed). While there are historical records of zoantharians in the East Atlantic (e.g., Van der Land 1993; Morri et al. 2000), many species of *Zoanthus* remain hard to identify in the

field due to high levels of intraspecific variation resulting from phenotypic plasticity of polyp shapes, colours, sizes, and oral disk colours (Reimer et al. 2004; Ong et al. 2013). Therefore, we identified specimens from Maio Island by utilizing a DNA barcoding approach.

DNA extraction and PCR amplification

For molecular analyses, polyps from both *Zoanthus* morphotypes (n = four colonies) were collected (Figure 2). DNA was extracted from 50mg of body tissue following the manufacturer's protocol of a DNeasy Blood & Tissue Kit (Qiagen, Tokyo). The nuclear internal transcribed spacer region of ribosomal DNA (ITS-rDNA) was amplified by polymerase chain reaction (PCR) using primers designed by Swain (2009). Mitochondrial cytochrome oxidase subunit 1 (COI) was amplified using newly designed zoantharian-specific primers (COIZPF 5'-GAACTATGTTGGGGGACGAC 3', COIZPR 5'-GGCGGGGTCAAAGAAAGTAG - 3') based on a sequence obtained with the general COI primers of Folmer et al. (1994). Products obtained by PCR were checked by 1% agarose gel electrophoresis and positive reactions sequenced in both directions at Fasmac (Kanagawa, Japan).

Phylogenetic analyses

DNA sequences generated in this study were aligned with previously reported sequences in GenBank (Altschul et al. 1990) using ClustalW (Thompson et al. 1994) implemented in MEGA v.7 (Kumar, Stecher and Tamura 2015). The alignments were cleaned by eye and ambiguous sites were removed prior to analyses. A suitable outgroup to each alignment was added to the phylogenetic analyses.

The best-fitting DNA model of nucleotide substitution was determined for each data set according to the Bayesian Information Criterion (Schwarz 1978) in Jmodeltest (Darriba et al. 2012). Phylogenetic trees were inferred using MrBayes (Huelsenbeck and Ronquist 2001) and RAxML

(Ott et al. 2007) software through the Mobylye SNAP Workbench (Monacell and Carbone 2014). Posterior distributions of trees and parameters were estimated using Markov Chain Monte Carlo (MCMCs) for 5000000 generations sampled every 100 generations. The consensus trees were visualized with Fig.Tree v1.4.2 software (Rambaut 2009).

RESULTS

The zoantharian area was approximately estimated at 4 hectares with a perimeter of approximately 800 meters (Figure 1). The underwater video transect of the carpet (video available at <https://youtu.be/CduwshatD60>) revealed that below the colonies, the benthos was dominated by typical local stony coral species (i.e., *Siderastrea radians*, *Porites* spp., *Millepora alcicornis*) attached to isolated pavements (Moses et al. 2003).

We observed two morphotypes within the 'Zoanthus zone'. All *Zoanthus* polyps were 'liberae', i.e., well free and clear of coenenchyme (Pax 1910), connected by a poorly developed coenenchyme. This grow form differ to the *Zoanthus* species already registered in Cabo Verde, *Z. aff. pulchellus*, whose polyps are embedded in a well-developed coenenchyme (Reimer et al. 2010). The number of tentacles varied from 36 to 58 and colour patterns of an oral disk varied from green and fluorescent green to yellow. According to previous studies these characteristics are similar to both *Z. sociatus* and *Z. solanderi* found in the Caribbean Sea (Sebens 1982; Reimer et al. 2012).

The two morphotypes could be clearly distinguished by the sizes of the polyps. One morphotype had polyps with an average oral disc diameter of 3.2 ± 0.6 mm (n = 6 polyps examined), while the other morphotype was smaller 2.64 ± 0.53 mm (n = 11 polyps). The larger morphotype had white striped vertical markings on the body walls of polyps, similar to as previously reported for *Z. solanderi* (Karlson 1980), and the oral disk varied in colour (green, brown, grey) with white dots present. The smaller morphotype matched well with *Z. sociatus*, as this species has many colour morphotypes. We observed a grey oral disk with yellow or green

fluorescent in the middle (Figure 2). The sizes of each morphotype also fit well within values previously reported for each species.

Both ITS-rDNA and COI sequences from the specimens each showed two different genotypes, one for each morphotype. ITS-rDNA sequences from specimens morphologically identified as *Z. solanderi* (ID 91, 92 and 93 on the phylogenetic tree) were identical to previously reported sequences of *Z. giganteus* from Japan (DQ442412, DQ442465), the Pacific sister species of Atlantic *Z. solanderi* (Reimer et al. 2012). COI sequences from these specimens were also similarly identical to *Z. giganteus* from Japan (AB252675, AB252673). Based on combined results, these three specimens were identified as *Z. solanderi*. Both ITS-rDNA and COI sequences from the other morphotype (specimen ID94 on the phylogenetic tree) grouped with previously reported *Z. sociatus* sequences (JX119131, JX119133, KT454373, KT454372) from Florida and Brazil, and thus this specimen was identified as *Z. sociatus*.

Similar *Zoanthus* aggregations were also subsequently observed in two more locations on more northerly Boavista Island in Cabo Verde (i.e., Derrubado and Gatas Bays; Carlos ML Afonso *Pers. Comm.*). However, field surveys of other sheltered bays off the western coast of Maio Island (Ponta Rica, Calhetinha, and two bays in Pau Seco) in October 2016 revealed no *Zoanthus* spp. (triangular arrows in Figure 1).

DISCUSSION

Our results showed that the *Zoanthus* aggregation in Porto Cais Bay, Maio Island was composed of two species: *Z. sociatus* and *Z. solanderi*. It has been previously observed that these two species can reach high densities (Karlson 1981) while covering extensive areas of reef flats in the Caribbean Sea, and such areas have been called the ‘*Zoanthus* zone’ (Sebens 1977). This work reports for the first time the presence of such a ‘*Zoanthus* zone’ in the central Eastern Atlantic. While it is not known in this ‘*Zoanthus* zone’ is a recent occurrence or not, it is known that

Zoanthus spp. can asexually reproduce (Burnett et al. 1995), and that they can expand their benthic coverage rapidly after disturbances (Wee et al. 2017). Polyclonal *Zoanthus* aggregations are theorized to be beneficial to success of sexual reproduction (Karlson 1988). Although the Pau Seco area is similar to Porto Cais Bay, both Pau Seco bays are more hydrodynamic (current and wind intensities) with steeper slopes and a more complex topography of the substrata (sand and rubble) in comparison to the ‘*Zoanthus* zone’ location found at Porto Cais Bay; these facts may explain the absence of *Zoanthus* spp. in Pau Seco. Detailed exploration around the Cabo Verde Archipelago and neighbouring islands is needed to confirm if the *Zoanthus* zone is a common occurrence in this region or a relatively unique ecosystem. However, until now, no such zone has been reported in any of the Cabo Verde Islands or along the West African coasts, and thus it is likely that this is either a relatively rarely occurring ecosystem and/or has appeared relatively recently in this part of the Atlantic Ocean. Long-term monitoring of the site at Porto Cais Bay is needed to understand if the ‘*Zoanthus* zone’ is expanding or not, and to elucidate the long-term dynamics of this phenomenon. Future investigations should try to elucidate if such ‘*Zoanthus* zones’ are native to Cabo Verde or rather recently arrived due to an anthropogenic effect (e.g., invasive species, phase shift (Cruz et al. 2015)).

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CONFLICT OF INTEREST

On behalf of all authors, the corresponding author states that there is no conflict of interest.

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FIGURE LEGENDS

Fig. 1: Location of the Cabo Verde Archipelago in the eastern Atlantic Ocean and the large aggregation (4 hectares) of *Zoanthus* spp. in Porto Cais Bay, Maio Island with nearby surveyed bays (black triangular arrows). The study area ‘*Zoanthus* zone’ is indicated by white area near the shoreline within the orthophotograph (40 cm/pixel; 2010 – UCCP MAHOT www.sit.gov.cv).

Fig. 2: Underwater picture of *Zoanthus sociatus* and *Z. solanderi* observed inside the ‘*Zoanthus* zone’ reported at Porto Cais Bay, Maio Islands (Cabo Verde, see Fig. 1).