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Research paper

Complete mitochondrial genome sequences of Atlantic representatives of the invasive Pacific coral species *Tubastraea coccinea* and *T. tagusensis* (Scleractinia, Dendrophylliidae): Implications for species identification

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ABSTRACT

Members of the azooxanthellate coral genus *Tubastraea* are invasive species with particular concern because they have become established and are fierce competitors in the invaded areas in many parts of the world. Pacific *Tubastraea* species are spreading fast throughout the Atlantic Ocean, occupying over 95% of the available substrate in some areas and out-competing native endemic species. Approximately half of all known coral species are azooxanthellate but these are seriously under-represented compared to zooxanthellate corals in terms of the availability of mitochondrial (mt) genome data. In the present study, the complete mt DNA sequences of Atlantic individuals of the invasive scleractinian species *Tubastraea coccinea* and *Tubastraea tagusensis* were determined and compared to the GenBank reference sequence available for a Pacific "*T. coccinea*" individual. At 19,094 bp (compared to 19,070 bp for the GenBank specimen), the mt genomes assembled for the Atlantic *T. coccinea* and *T. tagusensis* were among the longest sequence determined to date for "Complex" scleractinians. Comparisons of genomes data showed that the "*T. coccinea*" sequence deposited on GenBank was more closely related to that from *Dendrophyllia arbuscula* than to the Atlantic *Tubastraea* spp., in terms of genome length and base pair similarities. This was confirmed by phylogenetic analysis, suggesting that the former was misidentified and might actually be a member from the genus *Dendrophyllia*. In addition, although in general the COX1 locus has a slow evolutionary rate in Scleractinia, it was the most variable region of the *Tubastraea* mt genome and can be used as markers for genus or species identification. Given the limited data available for azooxanthellate corals, the results presented here represent an important contribution to our understanding of phylogenetic relationships and the evolutionary history of the Scleractinia.

1. Introduction

Human activity has been responsible for unprecedented connectivity in the marine environment, particularly by the accidental transport of many species of crustaceans, mollusks, fishes, algae, cnidarians and ctenophores (Molnar et al., 2008; Ghabooli et al., 2013). When established, exotic species may cause dramatic changes in the new environment by altering community structure and displacing native species (Molnar et al., 2008). *Tubastraea* Lesson, 1829 is an azooxanthellate scleractinian genus originally described in the Pacific Ocean inhabiting tropical shallow waters (Cairns, 2000), but has recently attracted intense public and media concern due to the highly competitive and invasive properties of the members in this genus (Costa et al., 2014; Silva et al., 2014; Sammarco et al., 2015). With fast growth,

Abbreviations: A, adenine; aa, amino acid(s); ATP6, ATP synthase F0 subunit 6; ATP8, ATP synthase F0 subunit 8; bp, base pair(s); C, cytosine; COB, cytochrome *b*; COX1–3, cytochrome oxidase subunit 1–3; G, guanine; IGS, intergenic spacer; indel, insertion or deletion; ITS, internal transcribed spacer; Kb, kilobase; m, meter; min, minute; mt, mitochondrial; ND1–5, NADH dehydrogenase subunits 1–5; ND4L, NADH dehydrogenase subunit 4L; nt, nucleotide(s); *rnl*, 16S ribosomal RNA; *rns*, 12S ribosomal RNA; rRNA, ribosomal RNA; s, second(s); T, thymine; tRNA, transfer RNA; *trnM*, tRNA-Met (methionine); *trnW*, tRNA-Trp (tryptophan).

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early reproductive maturity and absence of natural predators in the Atlantic Ocean, *Tubastraea* species are able to cover nearly 95% of the available surface, out-competing native endemic species (Creed, 2006; Mantellato et al., 2011; Santos et al., 2013; Hennessey and Sammarco, 2014; Sammarco et al., 2015; Miranda et al., 2016).

Among its representatives, *Tubastraea coccinea* Lesson, 1829 and *Tubastraea tagusensis* Wells, 1982 (described from Bora Bora of French Polynesia and Galapagos of Ecuador, respectively) are spreading fast throughout the Atlantic, where they have been first reported in Puerto Rico and Curaçao (Vaughan and Wells, 1943; Boschma, 1953). In Brazilian waters, the presence of this genus had been documented since 1980′s (Castro and Pires, 2001), although they were first identified to species level in 2004 (de Paula and Creed, 2004). Since then, the genus has spread over 3000 km along the Brazilian coast (e.g.: de Paula and Creed, 2004; Mantellato et al., 2011; Capel, 2012; Sampaio et al., 2012; Costa et al., 2014) and predictions indicate that there is a high risk that *T. coccinea* could colonize the entire coast of Brazil (Riul et al., 2013).

Tubastraea belongs to the order Scleractinia, approximately half (~706 species) of the representatives of which do not host the symbiotic dinoflagellate *Symbiodinium* (zooxanthellae) (Cairns, 2007). Despite being highly diverse, azooxanthellate scleractinians are under-represented in terms of available molecular data. Complete mitochondrial (mt) genome sequence data are available for 55 scleractinians (see Tseng et al., 2005; Medina et al., 2006; Chen et al., 2008; Lin et al., 2011; Arrigoni et al., 2014; Kitahara et al., 2014; Zeng et al., 2014) and only nine of these are azooxanthellate species. A complete mt genome sequence for *Tubastraea coccinea* has been lodged in GenBank (NCBI accession number NC026025), but phylogenetic analyses indicate that this sequence has a higher similarity with that from *Dendrophyllia arbuscula* van der Horst, 1922 than with other *Tubastraea* species (Luz et al., 2015), raising the possibility of misidentification. Although widely dispersed, *Tubastraea* has poorly defined taxonomic characters with several unidentified morphotypes (e.g.: Fenner, 2005; Arrigoni et al., 2014), which highlights the challenges of species identification in this genus. Indeed, many shallow-water scleractinians exhibit high intraspecific morphological variation (Todd, 2008) that frequently challenges taxonomy based exclusively on morphology.

Anthozoa mt genomes are atypical in terms of the presence of only 2 tRNAs compared to >20 in Bilateria (Beagley et al., 1998; Boore, 1999; see also Chen et al., 2008 that reported a *trnW* duplication in *Seriatopora* spp. Lamarck, 1916). In addition, they have relatively loose gene packing, especially those species that belongs to the "Basal" and "Complex" clades (van Oppen et al., 2002; Kitahara et al., 2014; Lin et al., 2011). Despite an extremely low rate of evolution (van Oppen et al., 1999; Shearer et al., 2002; Huang et al., 2008), mt genome data have been extensively exploited to investigate phylogenetic and evolutionary relationships within the Scleractinia and related groups (Park et al., 2012; Kitahara et al., 2014; Lin et al., 2014). Furthermore, DNA barcoding methods based on mt genes, such as Cytochrome Oxidase subunit I (COX1), have recently been developed for coral genus (Hsu et al., 2014) or species (Keshavmurthy et al., 2013) identification.

Despite the limited data available for azooxanthellate corals, there are some intriguing differences between zooxanthellate and azooxanthellate taxa in terms of mt genome characteristics. For instance, although mt gene order is highly conserved among zooxanthellate corals (Medina et al., 2006; Chen et al., 2008; Kitahara et al., 2014), two gene rearrangement events have occurred across the nine azooxanthellate corals that have so far been examined (Embelm et al., 2011; Lin et al., 2012). Intriguingly, a similar trend has also been observed in corallimorpharians, the anthozoan order most closely related to Scleractinia (Lin et al., 2014). In brief, all of the zooxanthellate corallimorphs for which data are available (10 species) have the same mt gene organization (which differs from the scleractinian norm), while in *Corynactis californica* Carlgren, 1936 and *Corallimorphus profundus* Moseley, 1877, the two azooxanthellate corallimorphs for which data are available, mt gene organization differs substantially (Lin et al., 2014). A shared characteristic of the azooxanthellate corals and corallimorphs that differ in mt genome organization to the respective canonical patterns is that they inhabit temperate and/or deep-water environments (Cairns, 2007; Fautin et al., 2009). By contrast, *Tubastraea* species inhabit tropical shallow waters; mt genome organization in representatives of this genus is therefore of particular interest in terms of the apparent correlation between the mt genome structure and the presence/absence of symbiotic dinoflagellates.

This study provides the complete mt genome sequences of Atlantic specimens of the invasive coral species *Tubastraea coccinea* and *T. tagusensis*. Together with all of the scleractinian data available in GenBank, these novel sequences were subjected to phylogenetic analysis, providing new perspectives on relationships within and between dendrophylliids and the evolution of mt genomes within the Scleractinia. Comparisons across the range of species suggest that the COX1 locus may provide markers useful for identification of *Tubastraea* at the genus or species level.

2. Materials and methods

2.1. DNA extraction and sequencing

Specimens of *Tubastraea coccinea and T. tagusensis* (specimen # MVK-CEBIMar 6 and # MVK-CEBIMar 43, respectively) were collected on May 2nd, 2013 from underneath a monobuoy (IMODCO 4) around 5 m depth in the São Sebastião channel (23°48′55″S/45°24′01″W), Brazil. Upon collection, total genomic DNA was extracted from the specimens and skeleton vouchers dried and deposited in the Cnidaria collection of the Center for Marine Biology (CEBIMar-USP). Species identification followed Wells (1982) and Cairns (1991, 2000).

Whole mesenteries were dissected from each species and total genomic DNA extracted using the DNeasy Tissue Kit (Qiagen, Seoul, Korea), following the manufacturer's instructions. Portions of all mt protein-coding and rRNA genes were amplified using the he DNeasy Tissue Kit (Qiagen, Seoul, Korea), following the manufacturer's instructions. Portions of all mt protein-coding and rRNA genes were amplified using the "Complex" scleractinian universal primers CS-1 to CS-21 under the polymerase chain reaction (PCR) conditions described by Lin et al. (2011), using the TopTaq polymerase master mix kit (Qiagen, Seoul, Korea). To obtain sequences from regions not covered by the universal primers, 26 specific primers were developed based on *T. coccinea* and *T. tagusensis* sequences (Supplementary Material, Table S1). For the specific primers, PCR were carried out using the same mix as for the universal primers and the following cycling conditions: One cycle at 95 °C for 3 min, followed by 30 cycles of 30 s at 94 °C, 45 s at 50 to 52 °C (depending on the primers annealing temperature) and 90 s at 72 °C, and ending with 4 min at 72 °C. Amplicons ranged in size between ~500 and 1500 bp, and were subjected to direct (Sanger) sequencing at Macrogen (South Korea).

2.2. Sequence analyses and annotation of the complete mitochondrial genomes

Sequences were verified, assembled and analyzed using Geneious v.6.1.6 (Biomatters) and Sequencher 5.1 (Gene Codes). Sequences were aligned to previously published data in MEGA 6 using a weighted matrix of Clustal W (Thompson et al., 1994) in order to identify protein-coding and ribosomal RNA genes. Examination of open reading frames (ORFs) and codon usage, as well as other DNA statistics, were performed using Dual Organelle Genome Annotator (Wyman et al., 2004), Sequence Manipulation Suite v.2 (Stothard, 2000), and MEGA 6 (Tamura et al., 2013). tRNAs were predicted using tRNAscan-SE search server v1.21 (Lowe and Eddy, 1997). Tandem repeat sections were searched in the five largest intergenic spacers (IGS-1, IGS-3, IGS-6, IGS-8 and IGS-18) using Tandem Repeat Finder (Benson,