Gene 590 (2016) 270–277

Contents lists available at ScienceDirect

Gene

journal homepage: www.elsevier.com/locate/gene

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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ARTICLE INFO

Article history:
Received 8 April 2016
Received in revised form 17 May 2016
Accepted 23 May 2016
Available online 25 May 2016

Keywords:
Sun-coral
Exotic species
South Atlantic
mt genome

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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http://dx.doi.org/10.1016/j.gene.2016.05.034
0378-1119/

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,

1999). Mitochondrial genome sequences are available on GenBank (NCBI accession numbers KX024566 and KX024567, for *T. coccinea* and *T. tagusensis*, respectively).

2.3. Phylogenetic analyses

Nucleotide sequences of all protein-coding and ribosomal genes from 57 scleractinians representing the "Basal" (1), "Complex" (39), and "Robust" (17) clades, in addition to sequences from Corallimorpharia (12), Actiniaria (2), Antipatharia (1), Zoantharia (1), and Octocorallia (1) were used to reconstruct anthozoan evolutionary history. The octocoral *Antillogorgia bipinnata* (Verrill, 1864) was used as outgroup. The final alignment comprised a total of 14,953 bp, of which 9714 positions were phylogenetically informative. Maximum likelihood analyses were conducted using PhyML (Guindon et al., 2010) under the *GTR + I + G* nucleotide evolutionary model, selected as the most appropriate for the complete dataset using MEGA 6. Phylogenetic analysis of the dendrophyllids was also conducted based on the COX1 gene using maximum likelihood analyses under the *GTR + I + G* nucleotide evolutionary model. The final alignment comprised a total of 1577 bp, of which 131 positions were phylogenetically informative.

3. Results and discussion

3.1. Organization and gene content

At 19,094 bp, the mt genomes of *T. coccinea* and *T. tagusensis* were at the high end of the size range of "Complex" scleractinians (see Kitahara et al., 2014). In general, *Tubastraea* mt genomes were intermediate in size between those of "Basal" (~19.5 kb) and "Robust" scleractinians (typically ~17 kb), but were among the longest "Complex" scleractinian mt genomes determined to date (see Medina et al., 2006; Lin et al., 2011; Chuang and Chen, 2015). The intermediate size of *Tubastraea* mt genomes is consistent with a relatively early divergence of the family Dendrophylliidae, implied by molecular phylogenetic reconstructions (Romano and Cairns, 2000; Fukami et al., 2008; Kitahara et al., 2010; Stolarski et al., 2011).

Whereas in two other azooxanthellate scleractinians (Embelm et al., 2011; Lin et al., 2012), mt gene organization differs, *Tubastraea* spp. follow the canonical scleractinian pattern (van Oppen et al., 2002; Fukami and Knowlton, 2005; Tseng et al., 2005; Medina et al., 2006; Flot and Tillier, 2007; Chen et al., 2008). As most hexacorallians (see Chen et al., 2008), *T. coccinea* and *T. tagusensis* mt genomes comprised each 13 protein-coding genes, 2 rRNAs, and 2 tRNAs, all transcribed from the same strand (Fig. 1). However, unlike most scleractinians, there were no overlaps between genes (Table 1). *Tubastraea* mt genome also has a COX1 group I intron, observed in some "Complex" corals and all Corallimorpharia, but absent in "Robust" scleractinians (Lin et al., 2014). Moreover, the COX1 group I intron, as well as the rRNAs and IGSs, were slightly larger in *Tubastraea* than those previously published for other scleractinians. The ND5 gene was also interrupted by another group I intron that contains 11 genes (Fig. 1).

The sense strand of the mt genomes from *T. coccinea* and *T. tagusensis* were composed of 25.3% A, 13.6% C, 23.7% G, and 37.4% T, and 25.4% A, 13.6% C, 23.6% G and 37.5% T, respectively. Within different regions, the (A + T)-content ranges from 45.1% in *trnM* to 69.7% in ND4L. This (A + T)-bias is common for hexacorallians (Kitahara et al., 2014) as for other metazoans (e.g.: Lavrov et al., 2008; Perseke et al., 2010). Overall, the (A + T)-content in *Tubastraea* was ~62.8%, which is similar to the mean value observed from other "Complex" scleractinians, but significantly lower if compared to "Robust" coral representatives (e.g.: *Pocillopora damicornis* (Linnaeus, 1858) [~70.2% - Chen et al., 2008], *Astrangia* sp. Milne Edwards & Haime, 1848 [68.3% - Medina et al., 2006]). The higher values observed in "Robust" corals has been related with a reduced efficiency of a putative DNA repair systems (Kitahara et al., 2014).

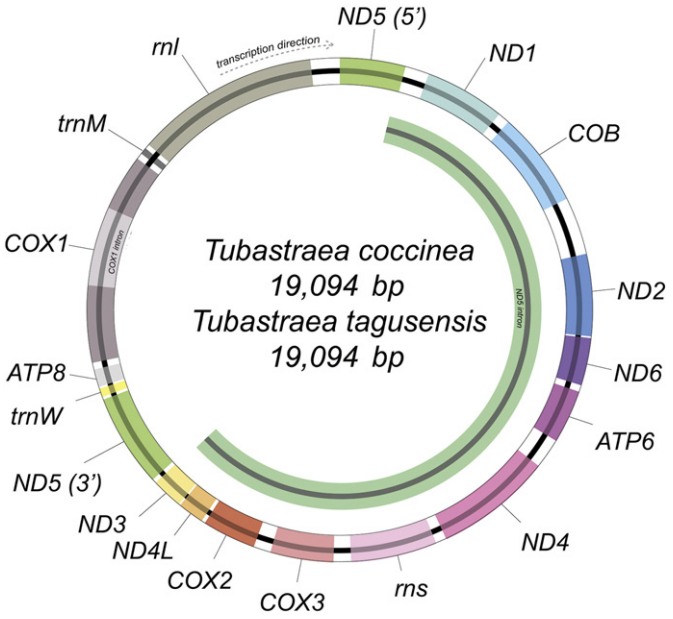

Fig. 1. Mitochondrial gene map of the scleractinians *Tubastraea coccinea* and *T. tagusensis*. Scaling is approximate only. Protein-coding, tRNA, and rRNA genes were abbreviated as in the text. Blank regions between genes represent intergenic spacers. The ND5 intron is indicated by the inner green line.

3.2. Codon usage

The 13 mt protein-coding genes of *T. coccinea* and *T. tagusensis* comprise 3945 codons, with all 62 amino acid codons but one (cysteine TGC) being used. Leucine (15.1%) and cysteine (0.9%) were the most and least frequent amino acids, respectively, while the most and least frequently used codons were phenylalanine (TTT) and arginine (CGC) (Table 2). In general, there was a strong bias towards codons ending with thymine (44%), a trend also observed in other scleractinians, especially those belonging to the "Robust" clade (Kitahara et al., 2014).

As with the majority of anthozoans (van Oppen et al., 2002; Chen et al., 2008), nine of the 13 mt protein-coding genes of *T. coccinea* and *T. tagusensis* use methionine (ATG) as start codon, while ND3–ND5 and ND4L use valine (GTG) and ND6 isoleucine (ATA). The usage of GTG and ATA as start codons has already been documented in other scleractinians (e.g.: *Acropora tenuis* (Dana, 1846); *Pocillopora damicornis*; *Seriatopora hystrix* Dana 1846 - van Oppen et al., 2002; Chen et al., 2008). Additionally, as in other Anthozoa (e.g.: Flot and Tillier, 2007), for both *Tubastraea* species, all protein-coding genes have complete (TAG or TAA) stop codons, with TAA being more frequently observed.

3.3. RNA genes and non-coding regions

The boundaries of *T. coccinea* and *T. tagusensis* rRNA genes were deduced by comparison with data from other anthozoans. As observed in other corals, the *rns* and *rnl* were located almost opposite to each other (Fig. 1). Also, as reported in most scleractinians, only two tRNAs have been found in *Tubastraea*: *trnM* (methionine) and *trnW* (tryptophan) (Fig. 2), which differs from the two species of *Seriatopora* (*S. caliendrum* Ehrenberg, 1834 and *S. hystrix*) that possess three mt tRNAs (Chen et al., 2008).

The *T. coccinea* and *T. tagusensis* mt genomes have each 18 IGSs, totaling 2404 and 2406 bp respectively, or 12.6% of the total mt genome size. Four of the 18 IGSs (IGS-1, -3, -6, and -8) account for >60% of the non-coding regions. van Oppen et al. (2002) argued that the IGS between *rns* and COX3 of *Acropora tenuis* has several features characteristic of control regions of higher animals (i.e.: repetitive sequences,

Table 1
Mt genome organization in *Tubastraea coccinea* and *T. tagusensis*.

	Position		Length (bp)	AT%	Start-Stop codon	IGS
Region	*T. coccinea*	*T. tagusensis*	*T. coccinea*/*T. tagusensis*	*T. coccinea*/*T. tagusensis*	*T. coccinea*/*T. tagusensis*	*T. coccinea*/*T. tagusensis*
ND5 (5′)	168–887	168–887	720/720	62.4%/62.4%	GTG-/GTG-	430/430 (IGS 18)
Group I intron	888–12,210	888–12,213	11,323/11,324	63.2%/63.1%	–	0/0
ND1	1220–2203	1220–2203	984/984	62.0%/62.0%	ATG-TAA/ATG-TAA	332/332 (IGS 1)
COB	2331–3494	2331–3494	1164/1164	61.1%/61.0%	ATG-TAA/ATG-TAA	127/127 (IGS 2)
ND2	4133–5230	4133–5230	1098/1098	62.5%/62.7%	ATG-TAA/ATG-TAA	638/638 (IGS 3)
ND6	5264–5857	5264–5857	594/594	64.8%/64.8%	ATA-TAA/ATA-TAA	33/33 (IGS 4)
ATP6	5940–6638	5940–6638	699/699	64.2%/64.2%	ATG-TAG/ATG-TAG	82/82 (IGS 5)
ND4	6940–8415	6940–8415	1476/1476	63.6%/63.7%	ATG-TAA/ATG-TAA	301/301 (IGS 6)
rns	8529–9593	8529–9593	1065/1065	58.9%/58.9%	–	113/113 (IGS 7)
COX3	9813–10,601	9814–10,602	789/789	61.9%/62.1%	ATG-TAG/ATG-TAG	219/220 (IGS 8)
COX2	10,639–11,382	10,640–11,383	744/744	63.2%/63.2%	ATG-TAG/ATG-TAG	37/37 (IGS 9)
ND4L	11,436–11,735	11,437–11,736	300/300	69.7%/69.7%	GTG-TAA/GTG-TAA	53/53 (IGS 10)
ND3	11,754–12,110	11,756–12,112	357/357	65.0%/65.0%	GTG-TAG/GTG-TAG	18/19 (IGS 11)
ND5 (3′)	12,210–13,325	12,212–13,327	1116/1116	62.5%/62.8%	TAG/-TAG	99/99 (IGS 12)
trnW	13,365–13,434	13,367–13,436	70/70	50.0%/50.0%	–	39/39 (IGS 13)
ATP8	13,468–13,683	13,470–13,685	216/216	68.5%/68.5%	ATG-TAA/ATG-TAA	33/33 (IGS 14)
COX1 (5′)	13,807–14,700	13,809–14,702	894/894	60.3%/60.3%	ATG-/ATG-	123/123 (IGS 15)
COX1-intron	14,701–15,664	14,703–15,666	964/964	63.9%/64.4%	–	–
COX1 (3′)	15,665–16,348	15,667–16,350	684/684	63.6%/63.3%	TAA/-TAA	–
trnM	16,470–16,540	16,472–16,542	71/71	45.1%/45.1%	–	121/121 (IGS 16)
rnl	16,577–18,831	16,579–18,831	2255/2253	61.3%/61.3%	–	36/36 (IGS 17)

conserved sequence blocks and secondary structure potentially associated with the initiation of heavy-strand replication). To identify candidate control regions in the *Tubastaea* mt genomes, searches were conducted for tandem sequence repeats in IGS-1, -3, -6, and -8 but none were detected. Interestingly, among the IGS checked, the smallest one (IGS-8), also found between *rns* and COX3, had a high degree of similarity to the putative mt control region of some other scleractinians (*Enallopsammia rostrata* (Pourtalmm, 1878) and *Porites* spp. Link, 1807) (data not shown), indicating that this region in *Tubastraea* may, in fact, correspond to the mt control region. In other coral species, different IGS regions may function as control regions; that between ATP6 and ND4 in pocilloporids (Flot and Tillier, 2007; Chen et al., 2008) and the IGS between COB and ND2 for the Dendrophylliid *Turbinaria peltata* (Esper, 1794) (Shi et al., 2014). For the sponge *Amphimedon queenslandica* Hooper & van Soest, 2006, the longest IGS is thought to contain the control region on the basis that it contains repeated sequences and resembles the control region of higher Metazoa (Erpenbeck et al., 2006).

3.4. Comparison among Tubastraea genomes

In total, there were 54 nt differences between the *T. coccinea* and *T. tagusensis* mt genomes reported here, distributed across 9 of the 13 protein-coding genes, *rnl* and the IGS regions, of which 34 were transitions, 16 transversions, and 4 indels (Fig. 3) (sequence diversity [p-distance] = 0.0026 ± 0.0005). For 6 of the protein coding genes, nucleotide divergence resulted in changes at the amino acid level. The COX1 and *rnl* loci were the most variable, with 8 and 6 variable sites (0.33% and 0.27% of differences) respectively (Table 3). The average difference between genomes was 0.28%, similar to what has been observed between other scleractinians congeners (e.g.: 0.18% between *Pocillopora damicornis* and *Pocillopora eydouxi* Milne Edwards, 1860 [Flot and Tillier, 2007]; and 0.48% between *Seriatopora caliendrum* and *S. hystrix* [Chen et al., 2008]).

At 19,070 bp, the database *Tubastraea coccinea* mt genome sequence (GenBank accession number NC026025) is 24 bp smaller than the *T. coccinea* mt genome presented herein, the difference being accounted for by a 23 bp indel in *rns* (Fig. 4) and a 1 bp indel in *rnl*. Additionally, 117 nt differences were found between the genomes, which is about two times higher than the difference between *T. coccinea* and *T. tagusensis* presented here (54 nt). Previous studies have already indicated the anomalously high similarity between the GenBank database *T. coccinea* and *Dendrophyllia arbuscula* (accession number KR824937) sequences relative to other *Tubastraea* species (Luz et al., 2015). The sizes of the *T. coccinea* NC026025 and *D. arbuscula* KR824937 mt genomes were very similar (19,070 and 19,069 bp respectively) and differ only at

Table 2
Number of occurrences of each codon in the 13 protein-coding genes from *Tubastraea coccinea* (Tc) and *T. tagusensis* (Tt) mt genomes.

		Tc/Tt			Tc/Tt			Tc/Tt			Tc/Tt
Phe	UUU	312/314	Ser	UCU	113/113	Tyr	UAU	148/148	Cys	UGU	37/37
	UUC	23/22		UCC	20/20		UAC	11/11		UGC	0/0
	UUA	278/278		UCA	33/33	End	UAA	8/8	Trp	UGA	39/40
	UUG	161/160		UCG	37/37		UAG	5/5		UGG	59/58
Leu	CUU	92/91	Pro	CCU	68/67	His	CAU	68/69	Arg	CGU	12/13
	CUC	18/17		CCC	25/26		CAC	11/10		CGC	5/4
	CUA	33/35		CCA	31/31	Glu	CAA	57/57		CGA	20/20
	CUG	13/13		CCG	28/28		CAG	17/17		CGG	12/12
Ile	AUU	180/181	Thr	ACU	79/79	Asn	AAU	75/75	Ser	AGU	70/71
	AUC	36/37		ACC	18/18		AAC	22/22		AGC	11/11
	AUA	117/116		ACA	38/38	Lys	AAA	63/62	Arg	AGA	41/41
Met	AUG	118/118		ACG	28/28		AAG	38/39		AGG	12/12
Val	GUU	198/196	Ala	GCU	128/127	Asp	GAU	64/65	Gly	GGU	90/91
	GUC	30/31		GCC	47/49		GAC	22/22		GGC	34/32
	GUA	71/71		GCA	43/43	Glu	GAA	55/55		GGA	58/58
	GUG	89/88		GCG	61/61		GAG	65/64		GGG	150/150

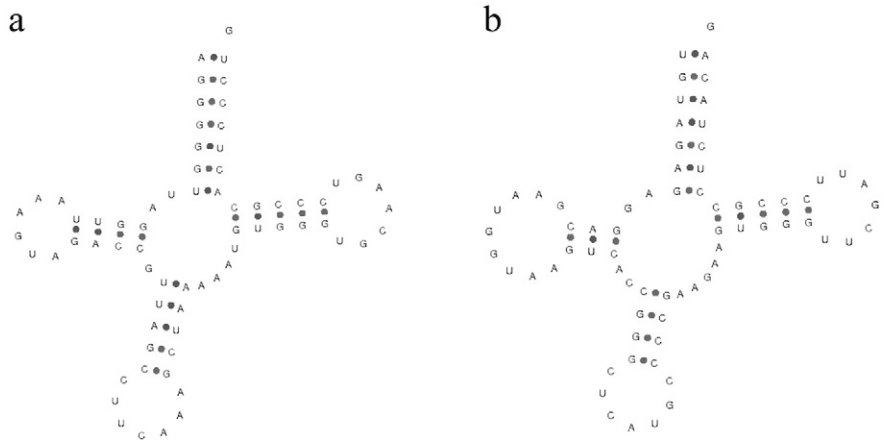

Fig. 2. Predicted tRNA secondary structures from the mt genomes of *Tubastraea coccinea* and *T. tagusensis*: (a) *trnW* (*tryptophan*); (b) *trnM* (*methionine*).

48 nt positions. Furthermore, *D. arbuscula* KR824937 and *T. coccinea* NC026025 share a 23 bp gap in the *rns* that is not present in the *Tubastraea* sequences presented here or in other dendrophyllids, including *Dendrophyllia cribrosa* Milne Edwards & Haime, 1851 (Fig. 4).

Given the extremely low rate of mt genome evolution in Scleractinia relative to other Metazoa (van Oppen et al., 1999; Shearer et al., 2002; Huang et al., 2008), the level of sequence divergence between the database (NC026025) and novel (reported here) *T. coccinea* sequences is unprecedented and highly unlikely. Altogether, these similarities suggest that *T. coccinea* NC026025 might be a misidentified member of the genus *Dendrophyllia*. Several *Dendrophyllia* species that inhabit tropical shallow-waters are deceptively similar to *Tubastraea* in terms of colony morphology and have a *Tubastraea*-like reddish-orange coenosarc, so the potential for mis-identification is high.

3.5. Phylogenetic analyses

Data from mt genomes from 57 anthozoans, representing two subclasses and six orders, were used to reconstruct the phylogenetic relationship using maximum likelihood methods (Fig. 5). The recovered topology was largely consistent with those previously published (Fukami et al., 2008; Kitahara et al., 2010; Kitahara et al., 2014). In general, the phylogenetic reconstruction presented herein recovered the three main scleractinian clades, "Complex", "Robust" and "Basal", supports the monophyly of the family Dendrophylliidae, and places the dendrophylliids as sister group of Poritidae (Medina et al., 2006; Fukami et al., 2008; Kitahara et al., 2010; Arrigoni et al., 2014). The

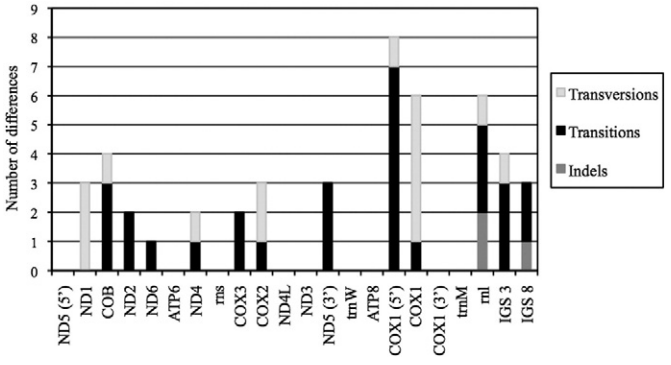

Fig. 3. Repartition of the sequence differences observed between *Tubastraea coccinea* and *T. tagusensis* mitochondrial genomes. All intergenic regions except the two with three or more differences (IGS 3 and IGS 8) have been omitted from the graph.

Table 3
Nucleotide (nt) and amino acid (aa) sequence identities (%) and numbers of variable sites (Vn) between *Tubastraea coccinea* and *T. tagusensis* mt genomes.

	nt		aa	
Locus	Identity	Vn	Identity	Vn
Protein-coding				
ND5 (5′)	100.0	0	100.0	0
ND1	99.7	3	99.1	3
COB	99.7	4	99.5	2
ND2	99.8	2	100.0	0
ND6	99.8	1	99.5	1
ATP6	100.0	0	100.0	0
ND4	99.9	2	99.8	1
COX3	99.7	2	100.0	0
COX2	99.6	3	99.2	2
ND4L	100.0	0	100.0	0
ND3	100.0	0	100.0	0
ND5 (3′)	99.7	3	100.0	0
trn-Trp	100.0	0	100.0	0
ATP8	100.0	0	100.0	0
COX1 (5′)	99.6	4	99.3	2
COX1 - intron	99.4	6	–	–
COX1 (3′)	99.4	4	99.1	2
trn-Met	100.0	0	100.0	0
rRNA				
rns	100.0	0		
rnl	99.7	6		
tRNA				
trnM	100.0	0		
trnW	100.0	0		
Intragenic spacer				
IGS 1	99.7	1		
IGS 2	100.0	0		
IGS 3	99.4	4		
IGS 4	100.0	0		
IGS 5	100.0	0		
IGS 6	100.0	0		
IGS 7	99.1	1		
IGS 8	98.6	3		
IGS 9	100.0	0		
IGS 10	100.0	0		
IGS 11	94.4	1		
IGS 12	100.0	0		
IGS 13	100.0	0		
IGS 14	100.0	0		
IGS 15	99.2	1		
IGS 16	99.2	1		
IGS 17	100.0	0		
IGS 18	99.5	2		

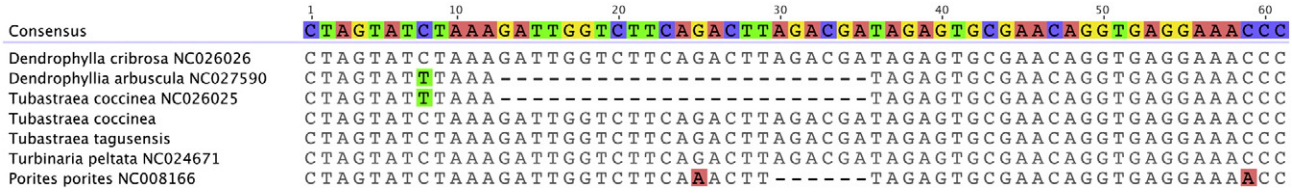

Fig. 4. Initial fragment of *rns* showing the 23 bp indel in the GenBank mt genome sequences for *Tubastraea coccinea* (NC026025) and *Dendrophyllia arbuscula* (NC027590).

recovered topology also supports the monophyly of Scleractinia and Corallimorpharia, placing them as sister groups.

The *T. coccinea* sequence retrieved from GenBank was included in analyses, *Dendrophyllia* and *Tubastraea* were the only scleractinian genera that were not recovered as monophyletic (Fig. 5). According to an extensive molecular phylogeny of the family, *Dendrophyllia* is a polyphyletic genus, while *Tubastraea* is monophyletic with low intrageneric distances (Arrigoni et al., 2014). The phylogenetic results presented here were consistent with the idea that *T. coccinea* NC026025 is more closely related to *D. arbuscula* KR824937 than to other *Tubastraea* species (Fig. 5).

3.6. Potential for species identification

The issue of molecular markers for coral identification is a challenging subject since COX1, the near-universal marker for DNA barcoding of metazoan species (Hebert et al., 2003), is highly conserved in Scleractinia (<2%, Shearer and Coffroth, 2008), as in other anthozoans (Hellberg, 2006). Additionally, the most variable regions often differ among coral genera, making it unlikely that a universal mt marker can be developed for coral identification. For example, in *Seriatopora* spp. ND5 (3′), ATP6 and IGS-9 (between *trnW* and a putative ATP8) were the most variable

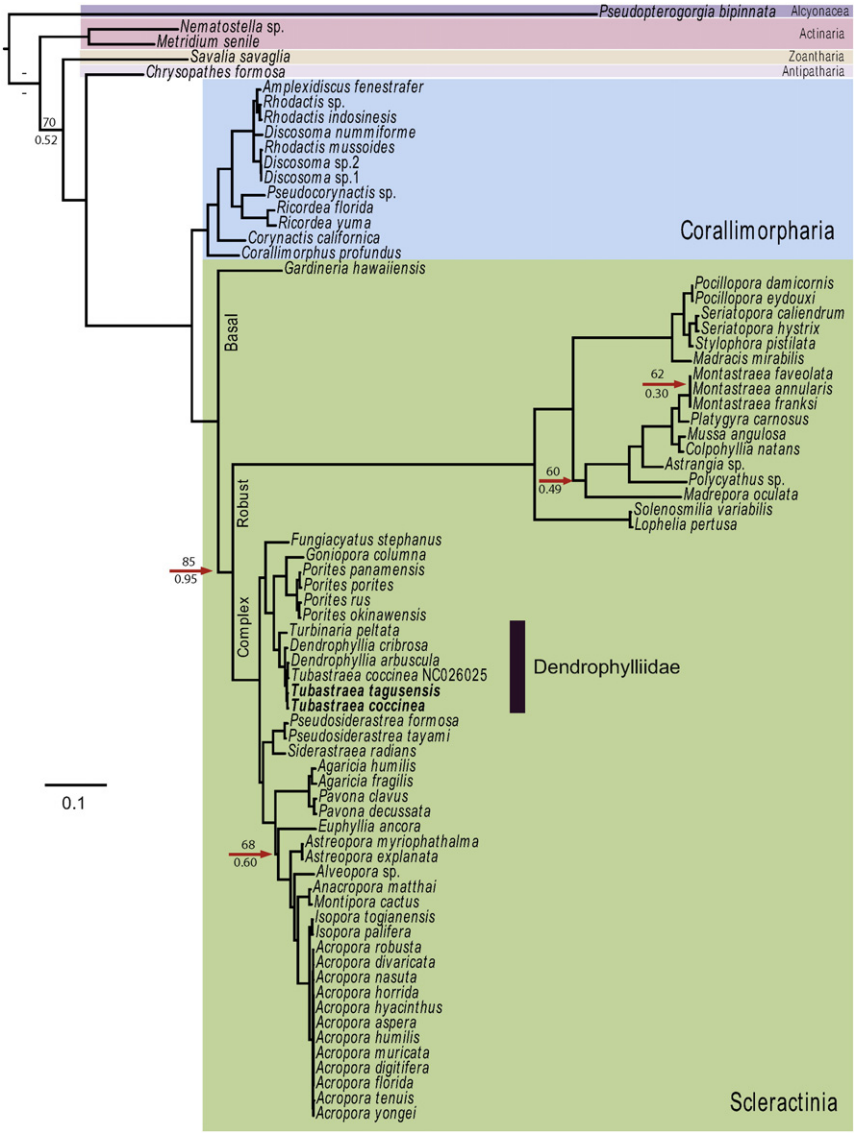

Fig. 5. ML phylogeny of scleractinian corals based on all 13 mitochondrial protein-coding genes, *rns* and *rnl* with ML bootstrap (upper) and Sh-Like (lower) node support values. Nodes without support numbers indicate bootstrap and Sh-Like support over ≥98.

Table 4
Matrix of mean difference between species based on COX1 sequences (substitutions per site and standard errors, calculated by the Kimura (1980) method). An asterisk (*) indicates the sequences obtained by the present work.

	*Tubastraea tagusensis**	*Tubastraea coccinea**	*Tubastraea coccinea* NC026025	*Dendrophyllia arbuscula* NC027590	*Dendrophyllia cribrosa* NC026026	*Turbinaria peltata* NC024671
*Tubastraea coccinea**	0.0051 ± 0.0018					
Tubastraea coccinea NC026025	0.0083 ± 0.0022	0.0108 ± 0.0025				
Dendrophyllia arbuscula NC027590	0.0076 ± 0.0023	0.0102 ± 0.0026	0.0019 ± 0.0011			
Dendrophyllia cribrosa NC026026	0.0114 ± 0.0027	0.0127 ± 0.0027	0.0133 ± 0.0028	0.0127 ± 0.0028		
Turbinaria peltata NC024671	0.0273 ± 0.0041	0.0298 ± 0.0043	0.0317 ± 0.0044	0.0311 ± 0.0045	0.0337 ± 0.0047	
Porites porites NC008166	0.0565 ± 0.0060	0.0590 ± 0.0059	0.0603 ± 0.0062	0.0610 ± 0.0062	0.0610 ± 0.0060	0.0578 ± 0.0062

regions (Chen et al., 2008), while in *Pocillopora* spp. (Flot and Tillier, 2007) the corresponding regions were ND3, ATP6 and IGS-11 (between COX1 and ATP8). Among these regions, only the ND5 (3′) and the IGS between ATP8 and COX1 (IGS-15) differed between *T. coccinea* and *T. tagusensis* (Table 3).

Several regions of the mitochondrial (Keshavmurthy et al., 2013; Wares, 2014) and nuclear genomes (Flot et al., 2008; Hsu et al., 2014; Forsman et al., 2015) have been evaluated in terms of their potential for coral species identification, with limited success. The ITS (Internal Transcribed Spacer) regions of the (nuclear) ribosomal RNA transcription unit appeared to be promising marker for identification of *Porites* spp. However, its multicopy status and the consequent need for cloning makes its use expensive and time consuming (van Oppen et al., 2000; Chen et al., 2004; Forsman et al., 2006; Forsman et al., 2015). The potential of COX1 for coral identification has not been widely explored, largely because of its documented low diversity. Nevertheless, COX1 may enable resolution at genus (Fukami et al., 2008; Kitahara et al., 2010) and, in specific cases, species level (Keshavmurthy et al., 2013).

Among the dendrophylliid species examined, the smallest estimated evolutionary divergence in COX1 observed (sequence diversity [p-distance] = 0.0019 ± 0.0011; Table 4) was that between the database sequences for *T. coccinea* (NC026025) and *D. arbuscula* (KR824937). Furthermore, when reconstructing the phylogeny of *Dendrophyllia* using only COX1 data, these two species were also clustered together with high statistical support (Fig. 6), suggesting that these might be congeners. These results also indicate that despite the low evolutionary rate, COX1 may be useful to confirm *Tubastraea* identification, although more samples are necessary to corroborate this assumption.

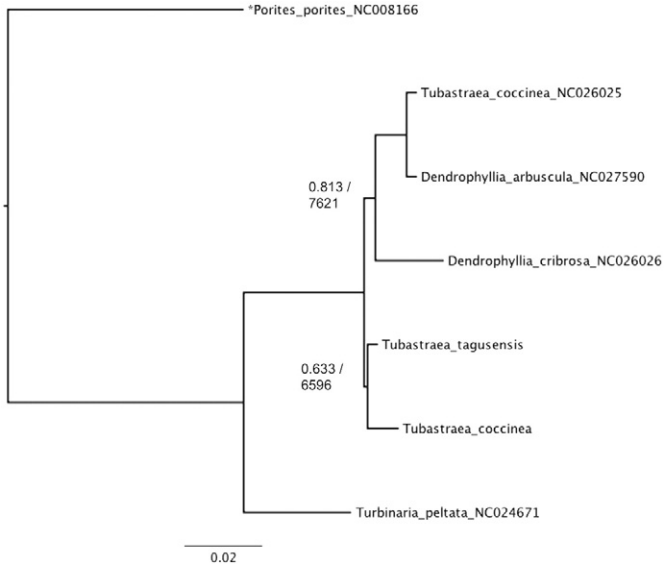

Fig. 6. ML phylogeny of dendrophyllid corals based on COI with ML bootstrap (upper) and Sh-Like (lower) node support values. An asterisk (*) indicates the outgroup. Nodes without support numbers indicate bootstrap and Sh-Like support over ≥98.

4. Conclusion

The complete mt genome sequences (19,094 bp) of two shallow water invasive coral species, *Tubastraea coccinea* and *T. tagusensis* have been determined, adding data for ecologically important species to the rather limited body of mt genome sequences for azooxanthellate corals. The results also call into question the identity the source of the database mt genome sequence NC026025, which is nominally *T. coccinea* but is more likely to have been a member of the genus *Dendrophyllia*. The analyses presented also suggest that the mt COX1 gene may be useful for *Tubastraea* identification at the genus or species level.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.gene.2016.05.034.

Acknowledgements

This is a contribution of the Center for Marine Biology of the University of São Paulo (CEBIMar/USP) and the Center for Marine Biodiversity Research (NP-BioMar/USP). This research was supported by the São Paulo Research Foundation (FAPESP), granted to M.V. Kitahara (grant # 2011/17537-1), A.E. Migotto (grant # 2012/21583-1), and K.C.C. Capel (grant # 2013/02696-2). This paper is part of the D.Sc. Requirements of Kátia Cristina Cruz Capel at the Biodiversity and Evolutionary Biology Graduate Program of the Federal University of Rio de Janeiro.

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