Taxonomic analysis and abundance study of two invasive corals of recent appearance in the Canary Islands: *Tubastraea* sp. and *Oculina* sp.

Análisis taxonómico y estudio de abundancia de dos corales invasores de reciente aparición en las Islas Canarias: *Tubastraea* sp. y *Oculina* sp.

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CERTIFICAN

Que la alumna **Dña. Irene Moltó Martín** ha llevado a cabo bajo su dirección el trabajo titulado: "Taxonomic analysis and abundance study of two invasive corals of recent appearance in the Canary Islands: *Tubastraea* sp. and *Oculina* sp."

Autorizando su depósito, defensa y evaluación

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SUMMARY

In 2016 it is registered for the first time in the Canary Islands two non-native sclerantinians of the genus *Tubastraea* and Oculina. Both species have been introduced through the the two main Canary ports (Las Palmas de Gran Canaria harbor and Santa Cruz de Tenerife harbor), through the shipping of large offshore oil rigs. The morphological data allow to make an identification as *T. coccinea* (Cairns, 2001; Cairns & Kitahara, 2012; Creed et al., 2017), the *Oculina* responds to the morphological type of *O. Patagonian* described by Zibrowius (1976 and 1980) a species native to the Mediterranean (Leydet y Hellberg; 2015; Terrón-sigler et al., 2016). In this study, we will analyze the distribution and abundance of *Tubastraea* sp. and *Oculina* sp., in the sport port of Santa Cruz de Tenerife to see the current state of the population and stablish a base-line for future studies. We will also identify molecularly both species and we will try to determine its possible origin. We will also include species of *Tubastraea* sp. collected in Cabo Verde in order to clarify the taxonomy of these corals in the Macaronesia.

Palabras clave: *Tubastraea*, *Oculina*, oil platforms, invasión, , Canary Islands.

Resumen

En 2016 se registra por primera vez en las Islas Canarias dos escleractinias no nativas de los géneros Tubastraea y Oculina. Ambas especies han sido introducidas a través de los dos principales puertos canarios (el Puerto de Las Palmas de Gran Canaria y el Puerto de Santa Cruz de Tenerife), a través de grandes plataformas petrolíferas. Los datos morfológicos permiten hacer una identificación como *T. coccinea* (Cairns, 2001; Cairns y Kitahara, 2012; Creed et al., 2017), la *Oculina* responde al tipo morfológico de *O. patagonica* descrita por Zibrowius (1976 y 1980) una especie nativa del Mediterráneo (Leydet y Hellberg, 2015; Terrón-sigler et al., 2016). En este estudio, analizaremos la distribución y abundancia de *Tubastraea* sp. y *Oculina* sp., en el puerto de Santa Cruz de Tenerife para ver el estado actual de la población y establecer una línea de base para futuros estudios. Analizaremos molecularmente ambas especies e intentaremos determinar su posible origen. Incluiremos también especies de *Tubastraea* sp. recolectadas en Cabo Verde para aclarar la taxonomía de estos corales en la Macaronesia.

Keywords: *Tubastraea*, Oculina, oil platforms, invasion, Canary Islands.

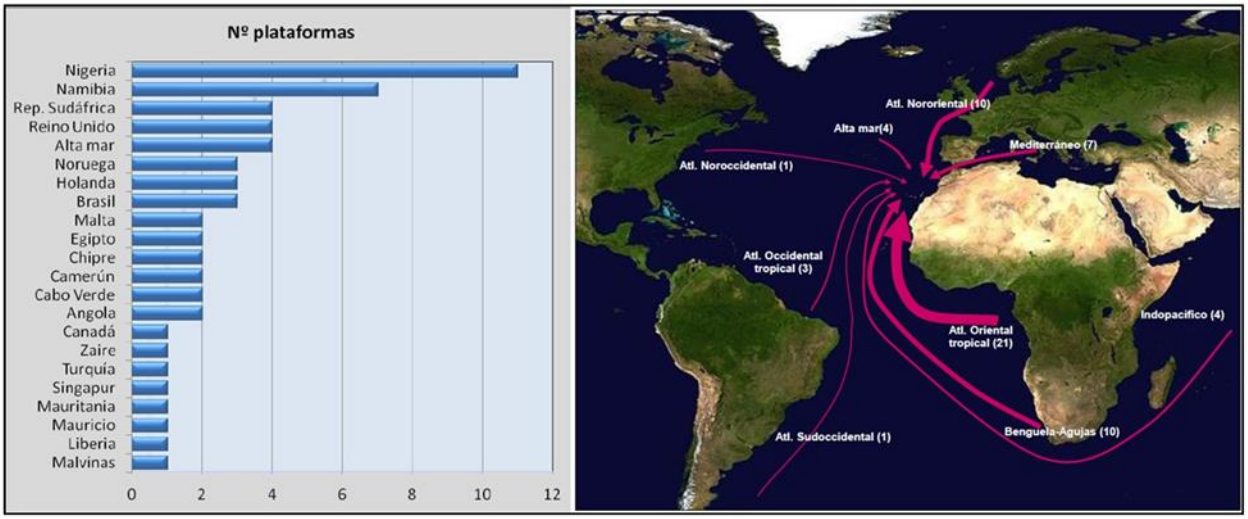
1. INTRODUCTION

1.1. Biota tropicalization

Currently ocean warming, due to the effect of climate change, facilitates the expansion of many marine organisms beyond their normal limit of distribution especially through oil platforms, vessels and anthropogenic flotsam (Nunes et al 2011, Hoeksema et al 2012). It has facilitated tropical species to establish populations towards highest latitudes (tropicalization) (Brito et al., 2005; Horta et al, 2014; Falcon, 2015) and, on the other hand, native species with warm affinity are expanded their populations to warmer sites within their own biogeographic region (meridionalization) (Yapici et al 2016). The development of these invasive species or potentially invasive tropical species to template regions can led to negative impacts in marine communities (Leydet et al., 2016; Creed et al., 2016).

The marine biodiversity of the Canary Islands is currently affected by a notable tropicalization process (Brito et al., 2005; Sangil et al., 2010; Falcon et al., 2015; Falcon, 2015; Riera et al., 2015; Gonzalez et al., 2017; Brito et al., 2017) Most of the species appear sporadically but some of them have already stable populations in the Archipelago, e.g. the ocean triggerfish *Canthidermis sufflamen* or the goldspot goby *Gnatholepis thompsoni* (Brito et al 2005). Not only pelagic but also sessile organisms have been able to settle down, such as the reef-building coral *Millepora alcicornis* (Clemente et al., 2011) or the algae *Penicillus capitatus* (Sangil et al., 2010). However, in recent years the main force of introduction is through maritime traffic, especially by oil platforms in the two main ports of the Canary Islands, Las Palmas de Gran Canaria and Santa Cruz de Tenerife (Brito et al.,2011; Clemente et al., 2011; Falcon et al.,2015; Falcon, 2015; Triay-Portella et al., 2015; Pajuelo et al., 2016).The fouling organisms are strongly associated with oil and gas platforms worldwide which are thus primary vectors for new introductions (Wanless et al., 2009; Creed et al.,2016). They come mainly from diverse tropical zones, both of the Atlantic as of other enclaves (Falcón, 2015; Pajuelo et al., 2016) and they remain moored for a long time. These platforms accumulate and transport a large number of organisms embedded in their hulls, forming true reef structures (Ferreira et al., 2006; Wanless et al., 2009; Sanmarcos et al., 2012; Kolian et al., 2013; Friedlander et al., 2014; Creed et al., 2017; Brito et al., 2017; Creed et al., 2017).

Figure. 1. The left graph represents the number of platforms arriving to the Port of las Palmas from different regions until March 2014. The map from the right represents the regions of origin (in a broad sense) of the platforms arriving at the port of Las Palmas de Gran Canaria until March 2014. The thickness of the arrows is proportional to the number of platforms. (Figure from Falcón, 2015)



1.2. *Tubastraea* and *Oculina* species as potential invaders

For several years we have known that some of the platforms that arrive at the Canary ports carry corals of the *Tubastraea* genus, in some cases forming large masses embedded in the hull (Brito et al., 2017). But only recently, in 2016, it was possible to confirm the presence of colonies of non-native scleractinian from the *Tubastraea* and *Oculina* genus developing in the Port of Las Palmas de Gran Canaria, specifically in the docking dock of the oil platforms, and also on artificial structures of Santa Cruz de Tenerife, not far from the berthing area of the platforms (Pajuelo et al., 2016; Brito et al., 2017). The primary coastal introduction points are always associated with nearby coastal port facilities, used by oil and gas industry associated shipping. This vector has also been reported in Brazil (Ilha Grande Bay) where shipping traffic (ships and oil platforms) probably brought these corals to the region (De Paula & Creed, 2004) during 1980′ s.

Nowadays, corals of the genus *Tubastraea* Lesson, 1829 (Dendrophyllidae, Anthozoa) are considered one of the most invasive species worldwide (Costa et al., 2014, Sammarco et al., 2015). Seven species of *Tubastraea* are currently recognized; *T. coccinea* Lesson, 1829, *T. diaphana* Dana, 1846; *T. faulkneri* Wells, 1982; *T. floreana* Wells, 1982; *T. micranthus*, Ehrenberg, 1834; *T. tagusensis*, Wells, 1982; *T. caboverdiana*, Ocaña and Brito 2015.

Tubastraea genus is thought to originate in the Indo-Pacific (Cairns 2000, 2001, Creed et al., 2016) and there are currently three species present in the western Atlantic (*T. coccinea*, *T. tagusensis* and *T. micranthus*) and are considered to be introduced by colonies embedded in the hull of boats. *T. coccinea* and *T. tagusensis* were introduced into the south-western Atlantic on oil platforms (De Paula & Creed, 2004, 2005; Creed, 2011; De Paula et al., 2014) and they are listed as powerful invaders. (Creed, 2006; Silva et al., 2011; Riul et al., 2013; Miranda et al., 2016; Creed et al., 2017; Global Invasive Species Database, 2018). The third specie, *T. micranthus*, is also expanding (Sammarco et al., 2014; Sanmarco et al., 2017; Creed et al., 2016; Fofonoff et al., 2018; Brito et al., 2017).

In the eastern tropical Atlantic there are ancient citations attributable to the genus *Tubastraea* sp., *T. coccinea* and *T. aurea* (Chevalier, 1966; Laborel, 1974; Boekschoten & Best, 1988; Morri et al., 1995, 2000; Friedlander et al., 2014; Brito et al., 2017) and in 2015 it was described a new species from the islands of Cabo Verde, *T. caboverdensis* Ocaña and Brito, 2015, based on morphological characteristics (Ocaña et al., 2015). The presence of *Tubastraea* is also mentioned in the fossil record of Quaternary deposits from several areas of the Cabo Verde Islands (Boekschoten & Best, 1988; Baarli et al., 2012; Mayoral et al., 2013).

Tubastraea coccinea is the most widely distributed azooxanthellate species in tropical and subtropical regions of the Pacific and Atlantic oceans (Cairns, 1994; Glynn et al., 2007). This species was first described from Bora Bora Island with a natural range throughout the Indo-Pacific. This azooxanthellate coral successfully invaded the Western Atlantic Ocean in the 1930s through shipping activities in the eastern Caribbean Sea (Cairns 2000; Glynn et al., 2007; Batista et al., 2016). However, *T. coccinea* seems to be an opportunist (Creed, 2004) and it is considered as a fouling and reef species (Fenner, 2001; Creed et al., 2016).

The invasive process of *Tubastraea* spp. has been profoundly studied both its geographical expansion (Creed et al., 2016) and the impact in the coralline communities (Creed, 2006; Lages et al., 2011; Dos Santos et al., 2013; Miranda et al., 2016). Due to the highly competitive and invasive properties of the members in this genus, it also has attracted public and media attention worldwide (Capel et al., 2016).

These azooxanthellate scleratinian corals are able to cover nearly 95% of the available surface (Vermeij, 2005; Sammarco et al., 2015; Capel et al., 2016; Miranda et al., 2016; Creed et al., 2016; Leydet & Hellberg, 2015) because of their fast growth, high fertility and larval dispersion rates, chemical defences and competitive aggressiveness, early reproductive maturity, sexual and asexual reproduction and absence of natural predators in the Atlantic Ocean (Cairns, 2000; Fenner & Banks, 2004; De Paula & Creed, 2004; Glynn et al., 2007; Lages et al., 2010; De Paula et al., 2014; Capel et al., 2016; Creed et al.,com 2017; Brito et al., 2017)

These biological invasions constitute one of the biggest threats to marine biodiversity because when established, exotic species could precipitate changes in community structure or composition and ecosystem functioning, mostly by out-competing and displacing native species (Grosholz, 2002; Wanless et al., 2009; De Paula et al., 2014; Sammarco et al., 2015; Capel et al., 2016; Leydet & Hellberg, 2016). In the Tropical East Atlantic the presence of *Tubastraea* is also known, both in the natural environment (Laborel, 1974; Boekschoten & Best, 1988; Morri et al., 2000; Ocaña et al., 2015) as in oil rigs (Friedlander et al., 2014), but it is considered that at least the origin of one of the species, *T. caboverdiana,* is uncertain (Creed et al., 2017), and could probably be considered as a native species (Ocaña et al., 2015).

The species attributed to *Oculina* genus are distributed by the Atlantic Western tropical and the Mediterranean. In the Mediterranean, *O. patagonica* De Angelis, 1908 (Oculinidae), has been introduced using the shipping traffic and oil rigs from South America where it was described based on fossils and where until now it has not been recorded alive (Zibrowius, 1974 and 1980; Fine et al., 2001). Recent genetic studies by Leydet & Hellbeger (2015) question that possibility and pose the hypothesis of a recent colonization from some unknown area of the eastern Atlantic, where they mention that there are also fossils from the same gender. This species is experiencing a rapid expansion favoured by climate change and the intense anthropization of the Mediterranean basin (Fine et al., 2001; Sartoretto et al., 2008; Serrano et al., 2012; Salomidi et al., 2013; Leydet, et al., 2016). *O. patagonica* is being able to successfully respond to changing environmental conditions in its native range, via symbiont switching and host adaptation,

which includes high growth rates, early reproduction, and high stress tolerance (Leydet et al., 2016).

1.3. *Tubastraea* and *Oculina* species identification

Features such as colony form, color, size of the corallites, number and relative size of their septal cycles, and columella morphology pointed at *Tubastraea coccinea* (Figure 1A) (Creed et al. 2016, Brito et al 2017). Regarding the other species, colonies´ morphology, their color, type of budding, size and density of the corallites' size and density, the number and relative size of their septal cycles and the type of columella responded to the characteristics of *Oculina patagonica* (Figure 1B), as described by Zibrowius (1974 and 1980). However, the taxonomy of both genders is complex and even now molecular studies question the validity of some species (Eytan et al., 2009; Arrigoni et al., 2014).

Scleractinian taxonomy has traditionally relied on skeletal morphology, which is often highly plastic and uninformative and prompted the quest for alternatives (Veron 1995;2000; Eytan, et al., 2009; Arrigoni et al., 2014). Corals are notoriously difficult to identify at the species level, several regions of the mitochondrial and nuclear genomes have been evaluated in terms of their potential for coral species identification, with limited success (Forsman et al., 2015; Capel et al., 2016). Widely used molecular markers appear to have insufficient levels of polymorphism for resolution below the genus level. DNA barcoding, based on a standardized sequence of the mitochondrial cytochrome c oxidase subunit I gene (COX1), has gained recognition as a tool for species delimitation (Ratnasingham & Hebert , 2007). In general, it evolves at a slow rate in corals relative to other metazoans (Shearer et al. 2002; Hellberg et al., 2006; Fornsman et al., 2006; Eytan, et al., 2009) but it is the most variable region in the *Tubastraea* spp. and can be used as markers for genus or in specific cases, species level (Kitahara et al., 2010; Capel et al., 2016). The advantage of using COI sequence data for coral phylogeny is that, unlike the 16S rDNA, 12S rDNA, and 28S rDNA genes, COI has a lower nucleotide substitution rate and is valid only for deeper nodes of the phylogenies.

Internal Transcribed Spacer of ribosomal DNA region (ITS-rDNA) is the most widely used molecular marker to reconstruct phylogenetic relationships among scleractinian

corals (Fornsman et al., 2006; Vollmer et al., 2015; Capel et al., 2016). The spacers are useful for species identification and examining hybridization between species by studying ITS-rDNA sequence divergence. (Fornsman et al., 2003; Fornsman et al., 2006). These molecular markers are hard to align with different species or even within the same species (between individuals). Despite this we will use them because they present a huge variation, we will analyse both the COI region and the ITS-rDNA, and we will compare the similarities/differences amongst them.

Finally, we used two nuclear DNA (nDNA) markers (fatty acid elongase and tachylectin-2 motif) to assess differentiation and genetic distances among named morphospecies of *Oculina*, among geographically distant sites. (Eytan et al. 2009; Leydet & Hellberg, 2015; Leydet, 2016). Here, we ask whether *Oculina* spp. from the Canary Islands have been recently introduced from the Mediterranean or from the North Western Atlantic or whether it is an eastern Atlantic native only newly become invasive.

1.4. Objectives

As morphology is a poor delineation of coral species in general (Fukami et al., 2004; Pinzón & Lajeunesse, 2011; Eytan et al., 2009) and widely used molecular markers appear to have insufficient levels of polymorphism for resolution below the genus level, scleratinian corals are notoriously difficult to identify at the species level. *Tubastraea* and *Oculina* are currently expanding its distribution due to the shipping traffic and oil rigs. The objective of this study is to identify these species through molecular analyses, establish a putative origin and know the current situation of these species in the harbour areas from the Canary Islands linked to oil platforms and maritime traffic.

The goals of this study are:

i. Study the distribution and abundance of *Tubastraea* sp. and *Oculina* sp., that are currently undergoing a range expansion related to human activities, in the port of Santa Cruz de Tenerife to see the current state of the population and stablish a base-line for future studies.
ii. Identify the species of *Tubastraea* spp. that have arrived in the Canary Islands and determine its possible origin using molecular markers COI (mDNA) and ITS

(rDNA) and identify at a molecular level the *Tubastraea* samples from Cabo Verde.

iii. Identify the species of *Oculina* spp. that have arrived in the Canary Islands and determine its possible origin using molecular (nDNA) fatty acid elongase and tachylectin-2.

2. MATERIALS AND METHODS

2.1. Populations of *Tubastraea* sp. and *Oculina* sp. in Tenerife

2.1.1. Sampling designed

The number of colonies of *Tubastraea* sp. and *Oculina sp*. were recorded in order to evaluate the abundance and distribution of each species inside the port of Santa Cruz de Tenerife (Figure 2a). *Tubastraea* spp. are characterized for its rapid expansion, therefore single individuals of this species were also registered. Six docks along the harbour, two in the entrance (A and B), two in the middle (D and E) and two inside the port (H and I) were sampled (Figure 2b). The arms of the jetty were numbered from 1 to 4 as an interval scale from its proximity to land, where one was the closest to the port infrastructure (Figure 2c). The specimens registered were part of the fouling of the floating blocks that sustain the docks. Orientation of the blocks was also taken into consideration, the different sides were named (a, b, c, d, e) (Figure 2d) depending on their exposure to light these were labelled as 1 (illuminated), 2 (shaded) and 3 (dark). Each dock had different dimensions and that is why the sizes of the blocks differed between them for this reason, fouling surface area of each block was also calculated (Table 1).

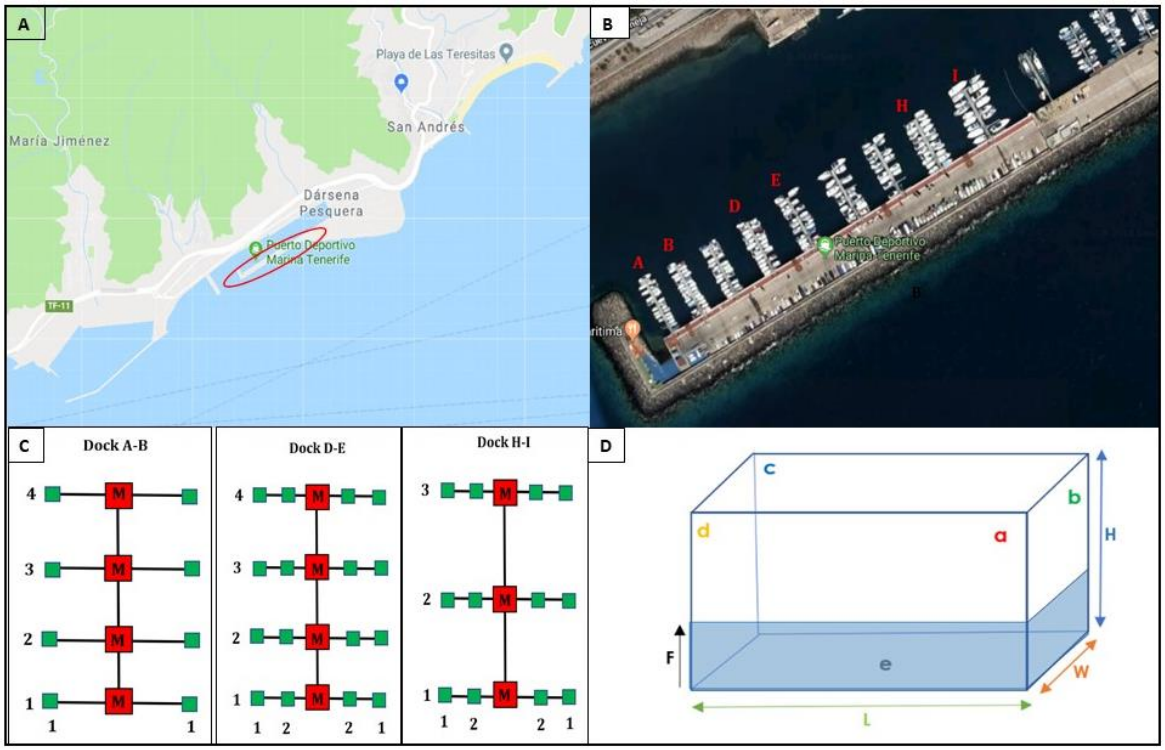


Figure. 2 Location of Sport Port Marina Santa Cruz de Tenerife (A). Docks sampled (B). Distribution of the docks: arms 1-4 (proximity to the pier), lateral blocks 1-2 (outer to inner blocks), M (central blocks of the dock that had a larger area than the lateral ones). Block measures: length (L), height (H) and width (W), fouling surface area (shaded area corresponds to the surface submerged in the water) and sides of the blocks (a,b,c, d,e) (D).

Table 1. Fouling surface area of the blocks from the docks of Santa Cruz de Tenerife Sport Port sampled. Each dock had different dimensions and that is why the sizes of the blocks differ in size. Lateral blocks (L block) were smaller than the central blocks (M blocks). Docks I-H were the largest ones and had three different types of blocks M1, M2 and M3 (measure 1-3).

FOULING SURFACE AREA										
Block		**Dock A-B**		**Dock E**		**Dock D**		**Dock I-H**		
side	**área**	**L block**	**M block**	**L block**	**M block**	**L block**	**M block**	**M1**	**M2**	**M3**
a	F*L	0,2574	0,2835	0,44	0,486	0,3	0,396	0,308	0,374	0,308
b	F*W	0,099	0,0255	0,11	0,324	0,125	0,264	0,143	0,187	0,099
c	F*L	0,2574	0,2835	0,44	0,486	0,3	0,396	0,308	0,374	0,308
d	F*W	0,099	0,0255	0,11	0,324	0,125	0,264	0,143	0,187	0,099
e	L*W	0,5265	0,3213	1	2,16	0,6	2,16	0,91	1,445	0,63

2.2 Statistical analyses

The abundance data set of the species of *Tubastraea* and *Oculina* registered in the Port of Santa Cruz de Tenerife were analysed by means of a multivariate analysis of the variance executed by permutations (PERMANOVA), using a total of 9999 permutations (Anderson & Ter Braak, 2003). No logarithmic transformation was applied to the original matrix and the Euclidian distance was calculated (Anderson, 2004; Anderson & Millar, 2004; Legendre & Legendre, 2012) A one-way analysis was carried out to assess the effect of the fixed factor "gradient" and the aleatory factor "dock" nested in the factor "gradient". Subsequent two-to-two comparisons were made by permutations of the levels of the factors that were significant (Anderson, 2004). Finally, the values of abundance found between *Oculina* and *Tubastraea* were represented in a bar-chart to visualize the differences found amongst them.

2.3 Study sites

2.3.1 Tubastraea samples

To identify the species of *Tubastraea* spp. that could have arrived with the oil platforms, we took samples from port areas, closely related with the shipping of these platforms, both in Tenerife and Gran Canaria. In addition, samples from other localities around the East Atlantic, proposed as possible populations source of the Canary samples for being the closest areas where the *Tubastraea* spp. are found naturally were included in the analysis to check if the species could have arrived by its own means.

Tubastraea specimens were collected from a total of 7 sites around the East Atlantic (Figure 3): four in the Cabo Verde Islands (Laginha, Ilhèu dos Passaros, Ponta de São João and Macario, Sao Vicente Island), one from Gabón (Pongara National Park) and two from the Canary Islands (Santa Cruz de Tenerife harbour, Tenerife and Jinamar, Gran Canaria).

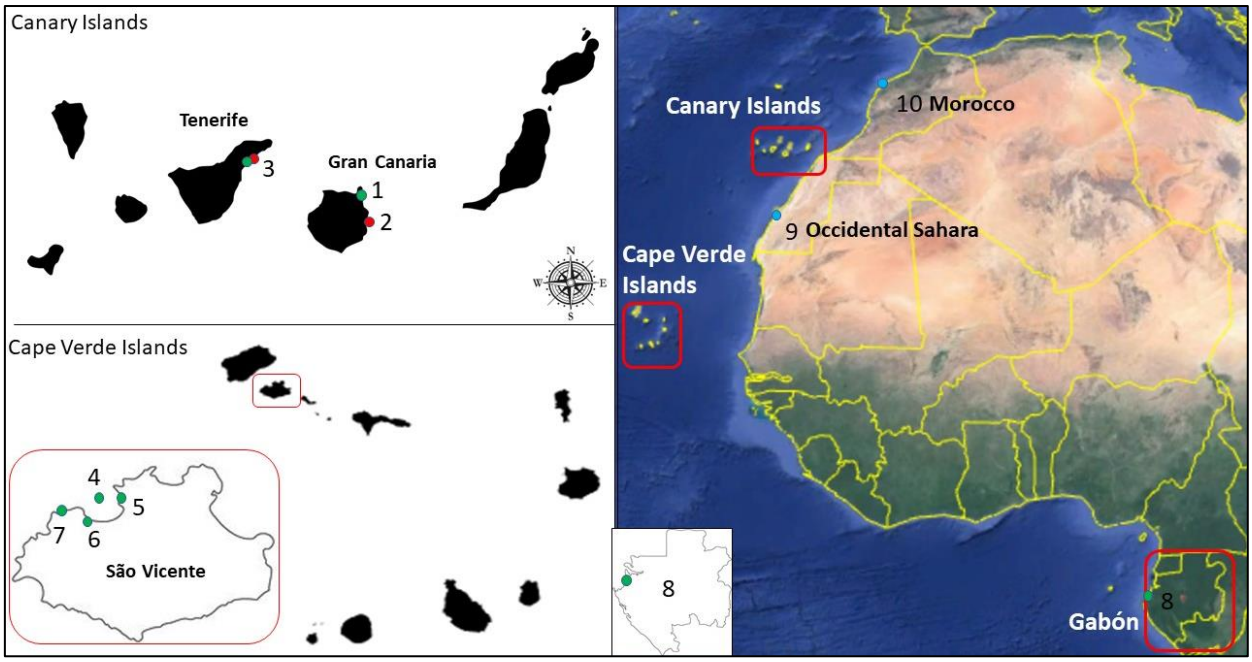


Figure 3. Study sites where *Tubastraea* sp. (green) and *Oculina* sp. (red) sp. were collected by means of scuba diving and snorkeling for molecular analyses; 1. Alcaravaneras, 2. Jinamar, 3. Santa Cruz de Tenerife harbour, 4. Ilhèu dos Passaros; 5. Laginha; 6. Macario; 7. Ponta de São João; 8. Gabón; 9. Dahkla (Occidental Sahara) 10. Oualidia (Morocco).

2.3.2 Oculina sp. samples

In the same way *Oculina* sp. specimens were collected in the Canary Islands, two samples from the Santa Cruz harbour (Tenerife) and one sample from Las Alcaravaneras (Gran Canaria) (Figure 3) (Table2).

Given the scarce morphological differentiation between *Astrangia* and *Oculina* species, and a possible colonization from the African coast, two specimens of *Astrangia* from Dakhla (Occidental Sahara) and one from Oualidia (Morocco) were included in the study (Table 2).

All the specimens were collected by snorkeling or scuba diving between 0,5 m down to 20 m depth (Table 2) and preserved in absolute ethanol for molecular analyses.

Table 2. Samples of *Tubastraea* sp., *Oculina* sp. and *Astrangia* sp. examined in this study, their collection details and molecular marker amplified for species.

Nº	Specie	Site	Region	Coordinates	Depth (m)	Date	LC2COI/ HCO2198	A18S/ITS4	P14F/R	P302F/R
301	*Oculina* sp.	Alcaravaneras	Las Palmas de Gran Canaria, Canary Islands	28° 7'43.11"N; 15°25'33.52"O	2	jul-17			×	×
302	*Oculina* sp.	Santa Cruz de Tenerife	Santa Cruz de Tenerife, Canary Islands	28°29'39.10"N; 16°12'34.70"O	1	jan-17			×	×
303	*Oculina* sp.	Santa Cruz de Tenerife	Santa Cruz de Tenerife, Canary Islands	28°29'39.10"N; 16°12'34.70"O	1	dec-17			×	×
304	*Tubastraea* sp.	Laginha	São Vicente, Cabo Verde	16°53'44.95"N ; 24°59'36.46"O	0,5-2	oct-17	×	×		
305	*Tubastraea* sp.	Ponta de São João	São Vicente, Cabo Verde	16°53'20.91"N; 24°59'59.71"O	20	nov-17	×	×		
306	*Tubastraea* sp.	Santa Cruz de Tenerife	Santa Cruz de Tenerife, Canary Islands	28°29'39.10"N; 16°12'34.70"O	1	dec-17	×	×		
308	*Tubastraea* sp.	Macario	São Vicente, Cabo Verde	16°53'1.15"N; 25° 1'1.44"O	8-10	nov-17	×	×		
309	*Tubastraea* sp.	Gabón	Pongara National Park, South-East Africa	2° 5'8.72"S; 9° 3'55.84"E	NA	nov-17	×	×		
315	*Tubastraea* sp.	Macario	São Vicente, Cabo Verde	16°53'1.15"N; 25° 1'1.44"O	7	nov-17	×	×		
316	*Tubastraea* sp.	Ilhéu	São Vicente, Cabo Verde	16°54'36.33"N; 25° 0'42.60"O	14	nov-17	×	×		
320	*Tubastraea* sp.	Santa Cruz de Tenerife	Santa Cruz de Tenerife, Canary Islands	28°29'39.10"N; 16°12'34.70"O	1	dec-17	×	×		
321	*Tubastraea* sp.	Santa Cruz de Tenerife	Santa Cruz de Tenerife, Canary Islands	28°29'39.10"N; 16°12'34.70"O	1	dec-17	×	×		
322	*Astrangia* sp.	Dakhla	Occidental Sahara, Africa	23°43'17.42"N; 15°52'11.73"O	NA	feb-12			×	
323	*Astrangia* sp.	Oualidia	Morocco, Africa.	32°44'34.99"N; 9° 2'31.75"O	0.5-1	feb-12				×
324	*Tubastraea* sp.	Jinamar	Las Palmas de Gran Canaria, Canary Islands	28° 2'31.91"N;15°24'33.47"O	8-12	mar-18	×	×		
325	*Tubastraea* sp.	Jinamar	Las Palmas de Gran Canaria, Canary Islands	28° 2'31.91"N;15°24'33.47"O	8-12	mar-18	×	×		

2.4 Molecular analyses

2.4.1 DNA extraction

A total of 16 individuals were analysed. Genomic DNA was extracted from coral samples using phenol/chloroform extraction protocol (Sambrook et al., 1989) Fifty mg of scraped tissue from the skeletal surface of each coral individual was deposited in 250 µl of a solution containing 30 mM Tris–HCl pH 8.0, 10 mM EDTA and 0.4 % SDS. Five µl of proteinase K (20 mg/ml-1) was added to the sample and then incubated at 56 °C overnight. Then, samples were centrifuged at 7000 rpm for 1 minute and 500 µl of phenol was added, mixed thoroughly and subsequently incubated 5 min at room temperature. Then the mix was spun at 7000 rpm for 5 minutes. The aqueous phase was then transferred to a fresh eppendorf and 250 µl of phenol and 250 µl of chloroform were added. The sample was then mixed thoroughly and later centrifuged at 7000 rpm for 5 minutes. Once again, the aqueous phase was transferred to a new fresh tube and 500 µl of chloroform added, mixed and incubated 5 min at room temperature. Then the mix was spun for 5 minutes at 7000 rpm and subsequently the aqueous phase transferred to a fresh tube. DNA was recovered by standard precipitation with AcNa 0.3 M (final concentration) and two volumes of absolute Ethanol. The mix was spun for 15 min at 13,000 rpm in order to precipitate the DNA. The supernatant was discarded, and the pellet cleaned with 200 µl ethanol 70%, then mix was spun 5 minutes at 13000 rpm, left to dry for 15 minutes at room temperature. Finally, the pellet was resuspended in 100 µl of 1X TE (10 mM Tris–HCl pH 8, 1 mM EDTA) according to the amount of pellet obtained. DNAs were quantified using a spectrophotometer Nanodrop ND- 1000 (Thermo Scientific, USA), and the concentration was adjusted to 10 ng /µl.

2.5 PCR amplification and sequencing

For *Tubastraea* specimens, a fragment of the COI gene from mtDNA was amplified by the polymerase chain reaction (PCR) using the primers designed by Folmer et al. (1994) (Table 3). Because coral mtDNA has a low rate of nucleotide substitution, we also sequenced the internal transcribed spacer of nuclear ribosomal DNA (ITS-rDNA) using primers A18S (Takabayashi et al., 1998) and ITS4 (White et al. 1990), exclusively designed for *Tubastraea* sp. (Arrigoni et al., 2014) (Table 3).

Table 3. Summary of the organisms and primers used in this study for genetic analyses. bp= base pair. Tm= melting temperature. Ta= optimum annealing temperature, it is calculated by subtracting four degrees from the lowest Tm of the pair of primers used.

Organism	Region	Length (bp)	Primer	Primer sequence	Tm (ºC)	Ta (ºC)
Tubastraea	COI (Mitochondrial cytochrome oxidase c subunit I)	595	LC2COI	5´-CGT-TAT-TTT-AGT-ATT-TGG-GAT-TGG-3'	64	60
			HCO2198	5'-TAA-ACT-TCA-GGG-TGA-CCA-AAA-AAT-CA-3'	70	
Tubastraea	ITS-rDNA (nuclear)	750	A18S	5'-GAT-CGA-ACG-GTT-TAG-TGA-GG-3'	60	54
			ITS4	5'-TCC-TCC-GCT-TAT-TGA-TAT-GC-3'	58	
Oculina and *Astrangia*	(Tachylectin-2 motif)	226–229	P302F*	5'-TTA-TAC-GGC-GTC-ACA-AAC-GA-3'	58	54
			P302R	5'-TCG-TCA-TCA-CCC-TTT-TAT-TCC-3'	60	
Oculina and *Astrangia*	(Fatty acid elongase)	206	P14F*	5'-TGT-ACC-ACT-TGG-GAT-GAA-CG-3'	60	56
			P14R	5'-TCA-AGC-TTC-CAG-TCT-TGT-GAA-A-3'	62	

In the case of *Oculina* specimens due to the scarce nucleotide substitution rate for mtDNA markers (Leydet & Hellberg, 2015), we amplified single-copy nuclear genes (Table 3) with a moderate variation at population level. The two genes used were p302F/R, (tachylectin-2, Schwarz *et al*. 2008; Leydet & Hellberg 2015) and p14F/R, (fatty acid elongase, Putnam *et al.* 2007; Leydet & Hellberg 2015) (Table 3).

PCR amplifications were performed in a total volume of 25 µl, containing T1X buffer (GeneAll Biotechnology, South Korea), dNTP (0.150 mM), 1 µl of BSA (10mg/ml), primers (0.4 µM of each), 1 U of Taq DNA polymerase (GeneAll Biotechnology, South Korea) and 20 ng of genomic DNA as template.

Initially, a PCR temperature gradient was completed to evaluate the optimal annealing temperature for each pair of primers.

PCR cycle conditions consisted of an initial denaturation cycle at 95 °C 2 min followed by 40 cycles at 95 °C 15 s, an annealing temperature specific to each primer (see Table X) for 20 seconds and 72°C 30 s with a final extension at 72 °C for 5 min and 22°C for 5min. The amplifications were carried out in a PTC-100 MJ Research thermocycler (MJ Research, USA). Reaction efficiencies were estimated running 5 µl of amplification products in an agarose electrophoresis (1.7%).

The unincorporated primers and nucleotides were removed with ExoSAP-IT (GE Healthcare, Illustra). Seven µl of the previously amplified DNA was added into 1,5 µl of ExoSAP-IT and incubated at 37°C for 15 minutes and subsequently at 80°C for 15 minutes to inactivate ExoSAP-IT™ reagent. Finally, the samples were sequenced at Macrogen Inc. (Seoul, South Korea).

2.6 Phylogenetic analyses of *Tubastraea* sp.

DNA sequences were edited and assembled using MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets (Kumar, Stecher, & Tamura 2016). Sequence alignment was performed using CLUSTAL W (Thompson et al. 1994) as implemented in MEGA7 and then revised by eye. Basic Local Alignment Search Tool (BLAST) (Altschul et al., 1990) was used to find the closest homologous sequences available in GenBank online database (Benson et al., 2011), optimizing for highly similar sequences (megablast). A total of 33 sequences of COI and 33 sequences of ITS-rDNA were obtained from GenBank (Annex I-Table 2) in order to include a representative number of Dendrophyllidae species. *Goniopora columna* was selected as outgroup for both COI and ITS-rDNA trees due the sister relationships of the family Poritidae (Gray, 1842), to the family Dendrophylliidae (Arrigoni et al., 2014).

DnaSP software v6: DNA Sequence Polymorphism Analysis of Large Datasets (Rozas et al., 2017) was used to concatenate sequences from *Tubastraea* COI and ITS4. The resulting concatenated file was used to generate 4 data sets: 1^{st}, 2^{nd} and 3^{rd} codon positions of mtDNA COI, and the nuclear noncoding region (ITS4) using MEGA7 software. The best-fitting DNA substitution model for each data set was determined according to the Bayesian Information Criterion (Schwarz, 1978) implemented in jModelTest (Darriba et al., 2012) (Table 4).

Table 4. Optimal evolutionary models determined with JModelTest for each data set of *Tubastraea* sp. nst= number of substitution types.

***Tubastraea* sp. concatenate DNA**			
Codon position	**Model**	**BIC**	**nst**
1st	HKY+I+G	K80	2
2nd	F81+I+G	F81	1
3rd	TPM1uf+I+G	HKY	2
Noncoding	TPM1uf+I+G	K80+I+G	2
***Tubastraea* sp. COI**			
Codon position	**Model**	**BIC**	**nst**
1st	F81+I+G	JC + I	1
2nd	F81+I+G	F81	1
3rd	F81+I+G	HKY	2

CIPRES Science Gateway V. 3.3: Cyberinfrastructure for Phylogenetic Research (Miller et al., 2010), was used to generate phylogenetic trees. Phylogenetic relationships between the taxa analysed were inferred by maximum likelihood (ML) using RAxML software (Stamatakis *et al.,* 2005) with the GTR+CAT approximation to accommodate heterogeneity rate among partitions and 1,000 replicates of bootstrap. In addition Bayesian inference of phylogenetic tree was obtained using MrBayes software (Ronquist & Huelsenbeck,2003).

Additional COI phylogenetic trees were constructed separately because some species of *Tubastraea* spp. were not present in the GenBank for both genes. (Annex I-Figure 1).

FigTree v1.4.3: Molecular Evolution, Phylogenetics and Epidemiology, was used to edit the resulting trees of RAxML and MrBayes.

2.7 Phylogenetic analyses of *Oculina* sp.

DNA sequences were edited and assembled using MEGA7 (Kumar, Stecher, & Tamura 2016). Sequence alignment was performed using CLUSTAL W (Thompson et al. 1994) as implemented in MEGA7 and then revised by eye. In addition, a total of 27 sequences of fatty acid elongase (P14F/R) and 27 sequences of tachylectin-2 motif (P302F/R) from GenBank (Eytan et al., 2009 and Leydet & Hellberg, 2015) (Annex II-Table 1) were

included. *Oculina* sequences for both gene fragments were concatenated and *Solenastrea hyades* selected as outgroup.

Haplotype network was constructed by means of the median-joining algorithm (Bandelt et al., 1999) as implemented in Network 5.0.0.3 software (http://www.fluxus-engineering.com) using the concatenated and individual genes of the *Oculina* samples.

Estimates of differences between populations was calculated with MEGA7 using K80 as distance between groups of individuals (populations) from four different geographic regions: Mediterranean, Canary Islands, America and Bermudas. Dendrogram was built using MEGA7, based on the distance matrix from the concatenated sequences.

3. RESULTS

3.1 Morphological identification

In this figure we can see images of the studied specimens of *Tubastraea* of Santa Cruz de Tenerife and Cabo Verde identified at morphological level as *Tubastraea coccinea* and *Tubastraea caboverdiana* respectively. We can also see a specimen identified as *Oculina patagonica* from the outside dock of the Port of Las Palmas de Gran Canaria (Figure 4).

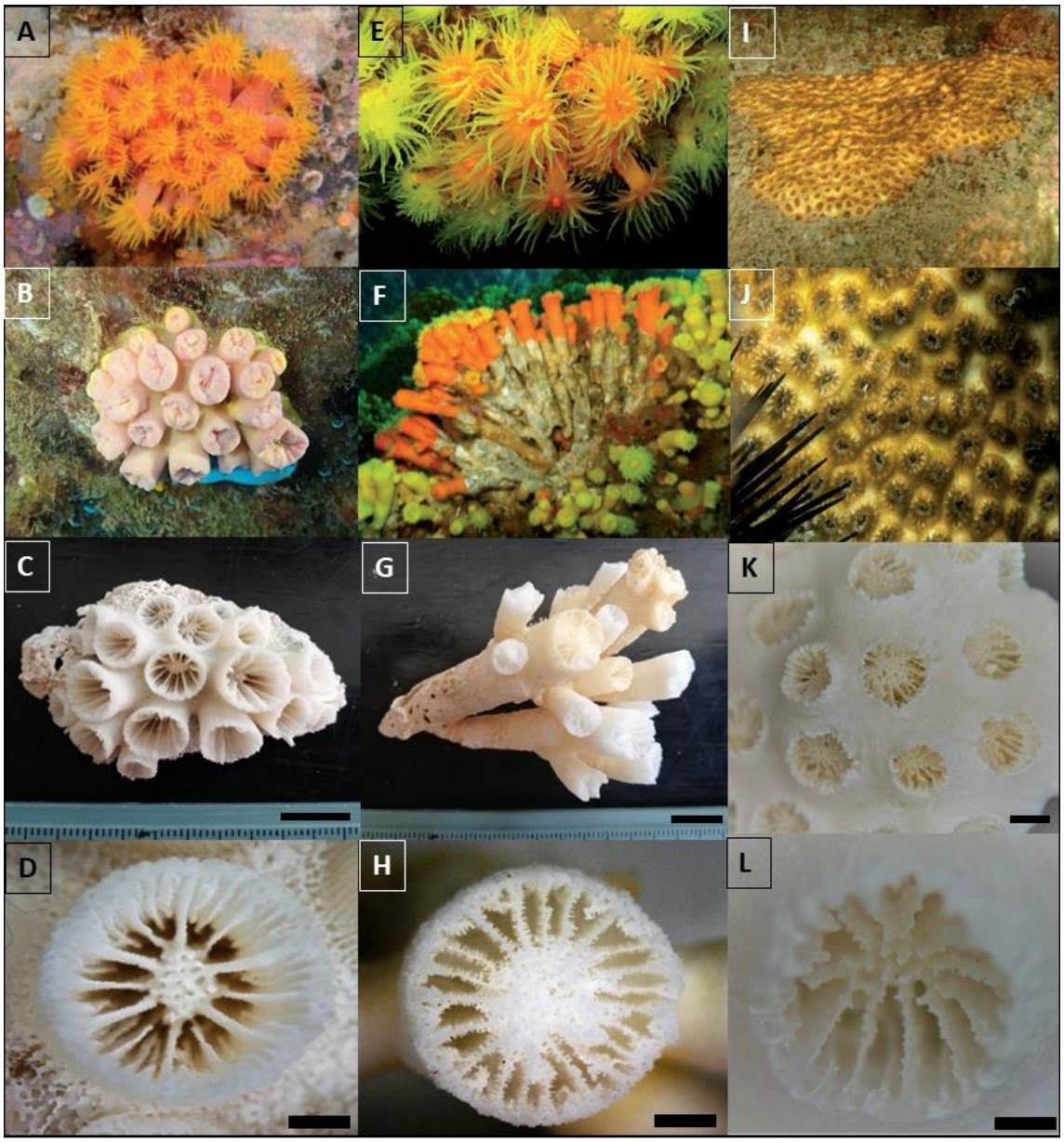


Figure 4. *T.coccinea* (A-D); *T. caboverdiana*, (E-H) *O. patagonica* (I-L). Images (A, B, E, F, I, J) courtesy of Dr. Alberto Brito Hernández (Ocaña & Brito., 2015; Brito et al., 2017). Image (E and F) from Tarrafal, Sao Tiago (Cabo Verde). P. Wirtz. Images (C, D, G, H, K, L) of detail of the calcareous structure.

3.2 Statistical analysis

Figures X -X show the abundances or number of colonies m^2 of *Tubastraea* and *Oculina* in the sport port of Santa Cruz de Tenerife. The first graph (Figure 5) represents the average of the total number of *Tubastraea* (colonies and solitary polyps) versus the average number of *Oculina* found in the pontoons of the sport port of Santa Cruz de Tenerife. Figure 6 is a comparison between the average number of colonies and individual polyps of *Tubastraea*. Figure 7 is a graphic representation of the average number of the total *Tubastraea* (colonial and solitary polyps) and *Oculina* and how these are distributed according to the exposition to light in three levels (illuminated, shaded and dark).

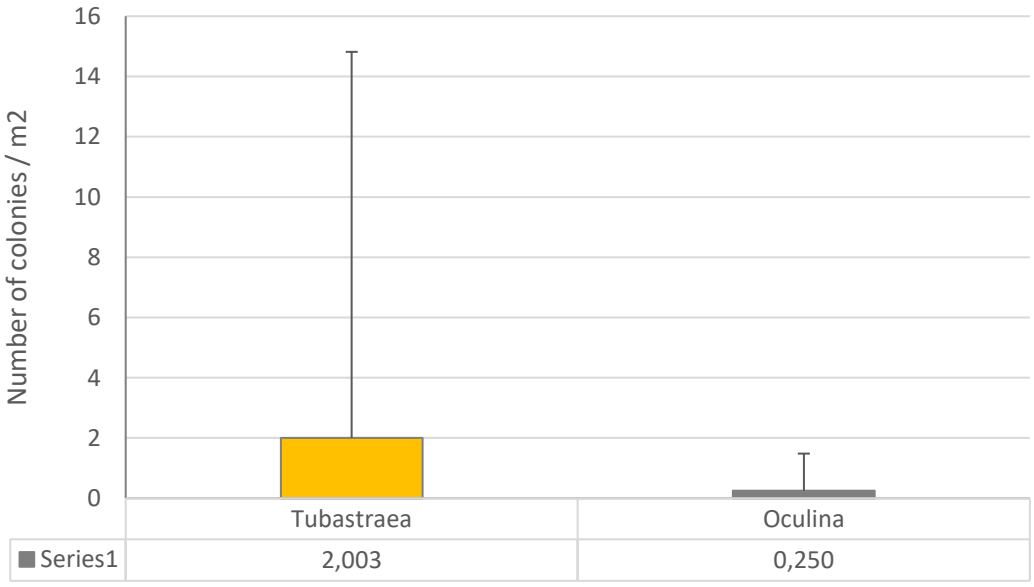


Figure 5. Abundances (number of colonies/m^2) of *Tubastraea* sp. (colonies and solitary polyps) and *Oculina* sp. in the docks of the sport port of Santa Cruz de Tenerife and standard deviation for each group.

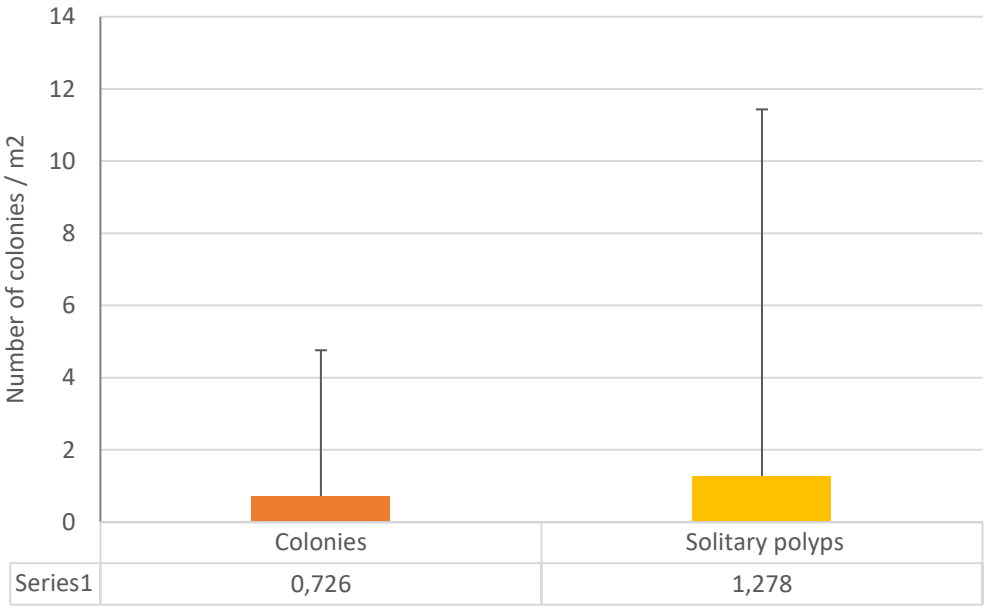


Figure 6. Abundances (number of colonies/solitary polyps)/m^2 of *Tubastraea* sp. found in the docks of the sport port of Santa Cruz de Tenerife.

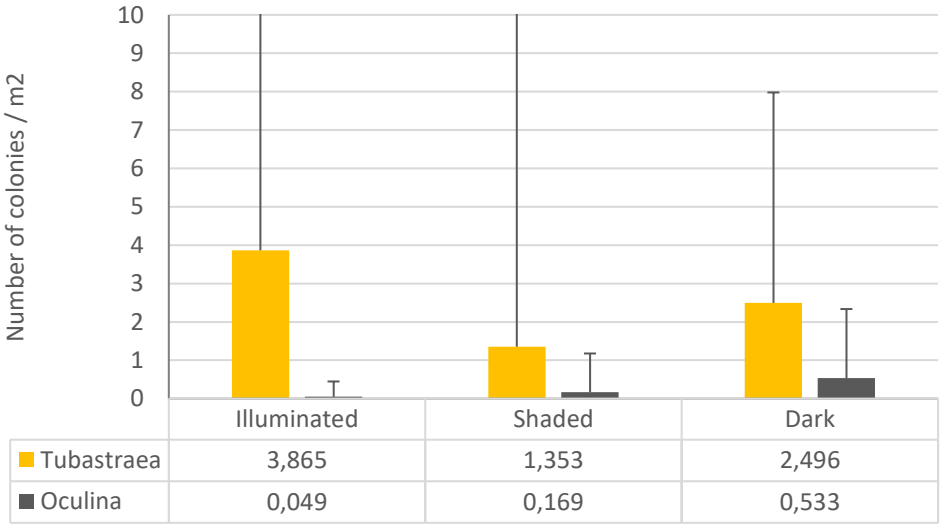


Figure 7. Abundances (number of colonies/m^2) of *Tubastraea* sp. and *Oculina* sp. in the docks of the sport port of Santa Cruz de Tenerife depending on their exposure to light with three levels (illuminated, shaded and dark).

The number of colonies per m^2 found in the docks of the Sport Port of Santa Cruz de Tenerife was greater in *Tubastraea* sp. than in *Oculina* sp. Furthermore, the number of *Tubastraea* polyps was slightly higher than that of the colonial form. Moreover, *Tubastraea* specimens were more abundant in environments exposed to light but were also found in dark and shaded areas. On the contrary, the *Oculina* was found in greater numbers in the dark areas and they were rare in areas exposed to light.

3.2.1. Primer analysis

Abundance data set for *Tubastraea* sp. was analysed with Primer and no significant value was found when exposition to light was evaluated for its three levels (light, shadow and dark) (Table 5). Similarly, no significant value was obtained when studying factor gradient and factor pantoon nested in factor gradient (Table 6).

Table 5. Results of the univariate analysis of the variance executed by one-way permutations (PERMANOVAs), based on the Euclidian distance matrix of the abundance data of Tubastraea sp. from the sport port of Santa Cruz de Tenerife. Three levels of exposition to light are tested (illuminated, shaded and dark).

Abundance of *Tubastraea* (Exposure to light)						
Source	**df**	**SS**	**MS**	**Pseudo-F**	**P(perm)**	**P(MC)**
Expo.	2	380,53	190,27	1,1595	0,3	0,315
Res	477	78275	164,1			
Total	479	78655				

Table 6. Results of the univariate analysis of the variance executed by one-way permutations (PERMANOVAs), based on the Euclidian distance matrix of the abundance data of *Tubastraea* sp. from the sport port of Santa Cruz de Tenerife. Factor gradient (GR) and factor pantoon (PA) nested in factor gradient.

Abundance of *Tubastraea*						
Source	**df**	**SS**	**MS**	**Pseudo-F**	**P(perm)**	**P(MC)**
GR	2	350	175	0,56825	0,621	0,65
PA(GR)	3	933,14	311,05	1,9056	0,12	0,125
Res	474	77371	163,23			
Total	479	78655				

The same statistical analysis was applied to the abundance data set for *Oculina* and this time significant value was found when studying the influence of exposure to light (p-value=0,006) (Table 7). A pair-wise analysis was done to test the three levels of exposure to light (illuminated, shaded and dark) (Table 5) Illuminated *vs.* dark (p-value=0,028) and shaded *vs.* dark (p-value=0,008) were significant. Besides, no significant value was found when studying factor gradient and factor pantoon nested in factor gradient (Table 9).

Table 7. Results of the univariate analysis of the variance executed by one-way permutations (PERMANOVAs), based on the Euclidian distance matrix of the abundance data of *Oculina* sp. from the sport port of Santa Cruz de Tenerife. Three levels of exposition to light are tested (illuminated, shaded and dark).

Abundance of *Oculina* (Exposure to light)						
Source	**df**	**SS**	**MS**	**Pseudo-F**	**P(perm)**	**P(MC)**
Expo.	2	14,809	7,4046	4,9527	0,006	0,008
Res	477	713,15	1,4951			
Total	479	727,96				

Table 8. Pair-wise comparisons for the levels of the significant factor "Shadow" obtained in the ANOVA by permutations of the abundance of Oculina. The statistical values (t-Student) and the level of significance for the comparison between different expositions to light are included.

PAIR-WISE TESTS		
Groups	**t**	**P(perm)**
Illuminated vs. shaded	0,94589	0,385
Illuminated vs. dark	2,1512	0,028
Shaded vs. dark	2,6232	0,008

Table 9. Results of the univariate analysis of the variance executed by one-way permutations (PERMANOVAs), based on the Euclidian distance matrix of the abundance data of *Oculina* sp. from the sport port of Santa Cruz de Tenerife. Factor gradient (GR) and factor pantoon (PA) nested in factor gradient.

Abundance of *Oculina*						
Source	**df**	**SS**	**MS**	**Pseudo-F**	**P(perm)**	**P(MC)**
GR	2	11,161	5,5806	4,8555	0,075	0,114
PA(GR)	3	3,4252	1,1417	0,75851	0,532	0,534
Res	474	713,48	1,5052			
Total	479	727,96				

3.3 Molecular analyses

A total of 14 samples were able to be analyzed, 11 from the genus *Tubastraea* and 3 from the *Oculina genus*, while for two samples of *Astrangia* sp. (322 and 323) the PCR amplification failed for the tachylectin-2 and fatty acid elongase genes respectively. Sequences 308, 309 and 325 of *Tubastraea* sp. were discarded because the sequences were "dirty" and uninterpretable for the ITS (Fig 8a). Some sequences were taken from GenBank in order to contrast ours.

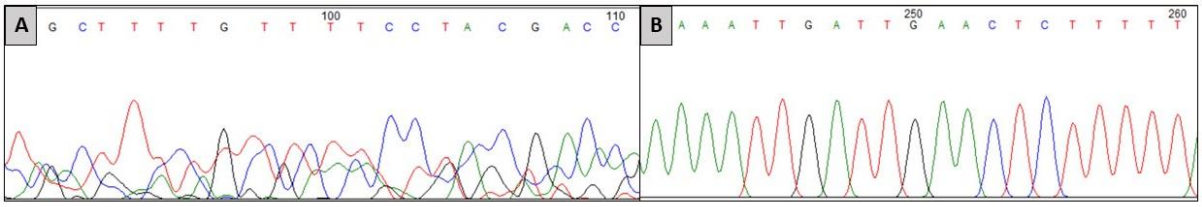


Figure 8. Sequence 325 *Tubastraea* sp. rDNA (uninterpretable) (A). Sequence 301 *Oculina* sp. homozygous.

3.3.1 Tubastraea sp. molecular analysis

The concatenated sequence alignment of *Tubastraea* sp. with 41 samples (8 from this study and 3 from GenBank) included 1281 positions, 576 base pairs (bp) for COI and 705 bp for ITS. Precisely was this fragment the most variable locus with 203 bp variable positions (172 phylogenetically informative and 31 singleton), yielding a moderate-good phylogenetic resolution at specie level. On the contrary, a total of 29 bp were variable in COI (all of them phylogenetically informative), 5 in the 1st codon and 24 in the 3rd codon.

In order to perform an analysis of our sequences in contrast to those of the species from which there was no ITS sequence available, we decided to do an exclusive analysis for the COI and for this 7 new GenBank sequences belonging to *T. coccinea* (3), *T.micranthus* (1), *T.tagusensis* (1), *B.elegans* (1) and *D. arbuscula* (1) were included (Annex I-Table 2). Samples 308, 309 and 325 of *Tubastraea* sp. from this study were also included. In this case 576 positions were analyzed, 39 were variable (29 phylogenetically informative and 10 singleton), 10 in the 1st codon, 1 in the 2nd codon and 28 in the 3rd codon.

3.3.2 Tubastraea sp. phylogenetic analysis

No substantial discordances or general patterns of conflicts were detected between Mr.Bayes and Maximum Likelihood (ML) trees (Figure 9 and Annex I-Figure 1).The topology of both trees was identical and so only the ML tree is shown with support of both methods (bootstrap and Bayesian posterior probability) on branchs. Similar topologies were obtained from individual COI sequences alignment, however both trees were less informative than those from the concatenate.

Within the *Tubastraea* clade, all species represented by more than one sample were monophyletic). At specie level, based on the combined tree samples 306 and 321 from Santa Cruz de Tenerife are more closely related to *Tubastraea micranthus* (Ehrenberg, 1834) and to *Tubastraea coccinea* (Lesson, 1829). Moreover, sample 321 from Santa Cruz de Tenerife and 324 from Gran Canaria seem to be more closely related to *Tubastraea aurea* (Quoy & Gaimard, 1833). Besides, *Tubastraea* samples from Cabo Verde cluster together in a well-supported monophyletic clade.

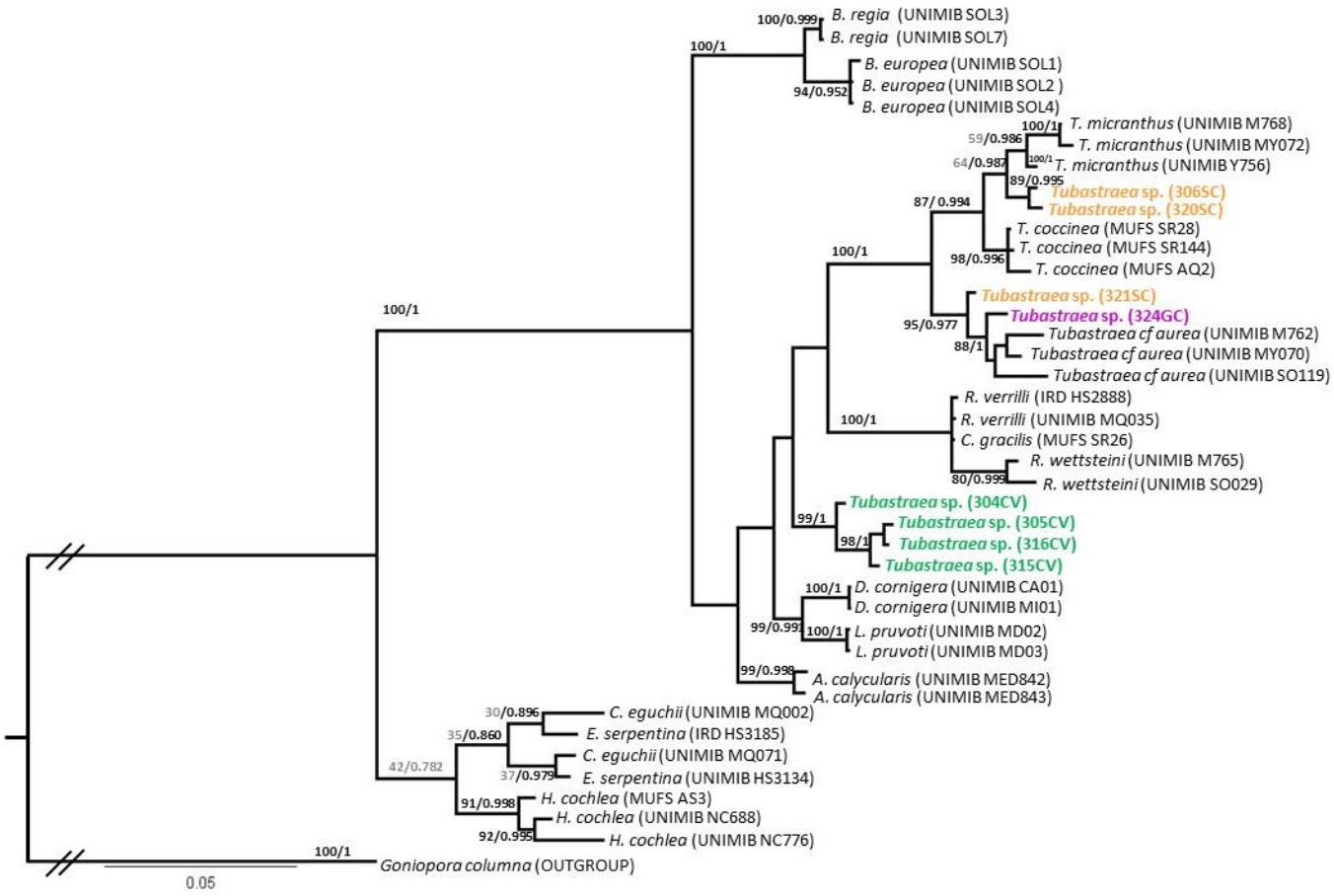


Figure 9. Combined gene tree for *Dendrophylliidae* based on two molecular loci (COI and ITS4) with outgroup *Goniopora*. Support values are posterior probability and maximum parsimony bootstrap values, respectively. Significant bootstraps are in bold and non-significant in grey.

3.3.3 Oculina sp. molecular analysis

We genotyped 3 samples of *Oculina* from the Canary Islands and 2 samples of *Astrangia* sp. from Oualidia and Dakhla. No ambiguities were observed on the electropherograms

obtained for these samples. For the analysis two or more samples from the different geographic regions were included (Eytan *et al.* 2009 and Leydet & Hellberg, 2015) (Annex II- Table 1,2 and 3).. In order to construct the haplotype networksand estimate distances between populations Western North Atlantic populations included North Carolina, Georgia, Daytona Beach, Cape Florida, Panama City, and Bermuda. Mediterranean Populations of *O. patagonica* included Spain, Italy, Greece, Lebanon, and Israel.

Individual fatty acid elongase sequence alignment with our 4 samples included 434 positions, 16 were variable (10 phylogenetically informative and 6 singleton), 8 in the 1st codon, 3 in the 2nd codon and 5 in the 3rd codon. Individual tachylectin-2 sequence alignment for our 4 samples included 376 positions, 24 of them variable (8 phylogenetically informative and 16 singleton), 10 in the 1st codon, 7 in the 2nd codon and 7 in the 3rd codon.

The concatenated sequence alignment of *Oculina* sp. with 30 samples (3 from this study and 27 from GenBank) included 432 positions. Examining the individual gene data sets, alignments lengths were 206bp for fatty acid elongase and 226 bp for tachylectin-2. Tachylectin-2 was the most variable locus. A total of 5 positions were variable in fatty acid elongase (4 phylogenetically informative), 4 in the 1st codon and 1 in the 3rd codon 15 positions were variable in tachylectin-2 (14 phylogenetically informative and 1 singleton), 6 in the 1st codon, 5 in the 2nd codon and 4 in the 3rd codon. Note that the sequences available in GenBank limited the length of the aligned sequences these were shorter than those obtained in this study.

Sample data set was categorized in four different groups according to its geographic region. Mediterranean, Canary Islands and America this last includes all North western localities except Bermuda which was classified separately due to its position further north regarding the American locations and for being the closest North Western region to the Canary Islands. Estimates of differences was calculated with MEGA 7 using K80 as distance between groups of individuals (populations) of the before mentioned geographic regions (Annex II- Table 1).

Distances between and within groups between the Mediterranean and the Canary Islands are smaller (Table 10), results also indicate that distances for the Mediterranean region

are lower than those observed within any other region. Samples from the Canary Islands seem to be more closely related to *O. patagonica* from the Mediterranean (Figure 10).

Table 10. Sequence variation between and within groups from different geographic regions.

Distance Between groups (concatenate)					**Distance within groups**
Region	Canary Islands	Bermuda	America	Mediterranean	**Concatenate**
Canary Islands	0				Canary Islands 0,012
Bermuda	0,020	0			Bermuda 0,014
America	0,013	0,020	0		America 0,012
Mediterranean	0,008	0,020	0,012	0	Mediterranean 0,007
Distance Between groups (fatty acid elongase)					**Distance within groups (fatty acid elongase)**
Region	Canary Islands	Bermuda	America	Mediterranean	
Canary Islands	0				Canary Islands 0,652
Bermuda	0,007	0			Bermuda 0,816
America	0,009	0,009	0		America 0,838
Mediterranean	0,005	0,008	0,007	0	Mediterranean 0,434
Distance Between groups (tachylectin-2)					**Distance within groups (tachylectin-2)**
Region	Canary Islands	Bermuda	America	Mediterranean	
Canary Islands	0				Canary Islands 0,018
Bermuda	0.033	0			Bermuda 0,021
America	0.018	0.030	0		America 0,021
Mediterranean	0.012	0.031	0.016	0	Mediterranean 0,011

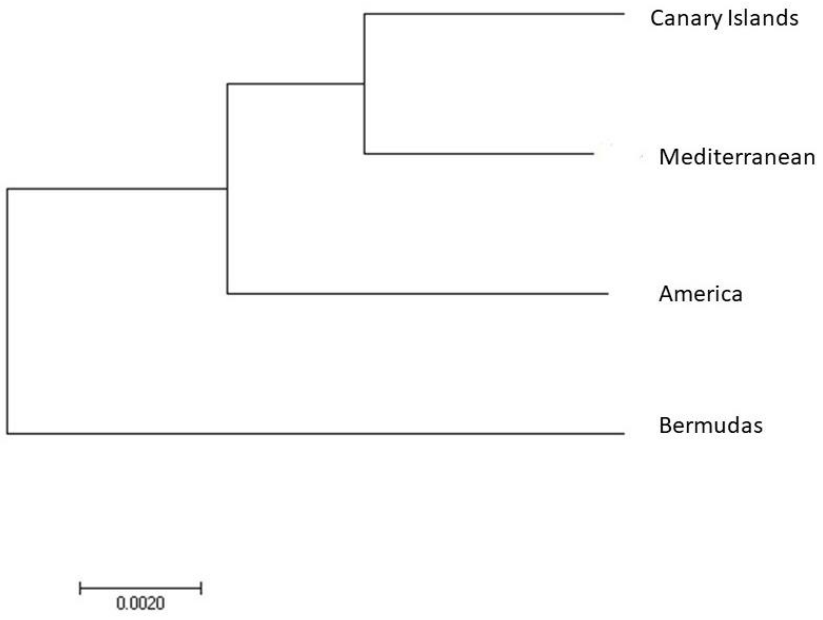


Figure 10. Phylogenetic tree based on distances between groups from the *Oculina* sp. data set for the concatenated sequences of two molecular loci (fatty acid elongase and tachylectin-2).

3.3.4 *Oculina* sp. haplotype Network (concatenate)

Figure 11 show the haplotype network based on the concatenated sequences showing the close relationships of samples from the Canary Island seem to have with those from the Mediterranean area. Haplotype 3 is constituted by sample 303 from Santa Cruz de Tenerife and is closely related with a *O. patagonica* from Cabo de Palos, Murcia, Spain differing from those in only one position. Sample 302 from Santa Cruz de Tenerife shared haplotype with two specimens of *O. patagonica* from Israel and Lebanon (Mediterranean)while sample 301 from Gran Canaria belong to haplotype 1 present also in *O. patagonica* specimen from Greece.

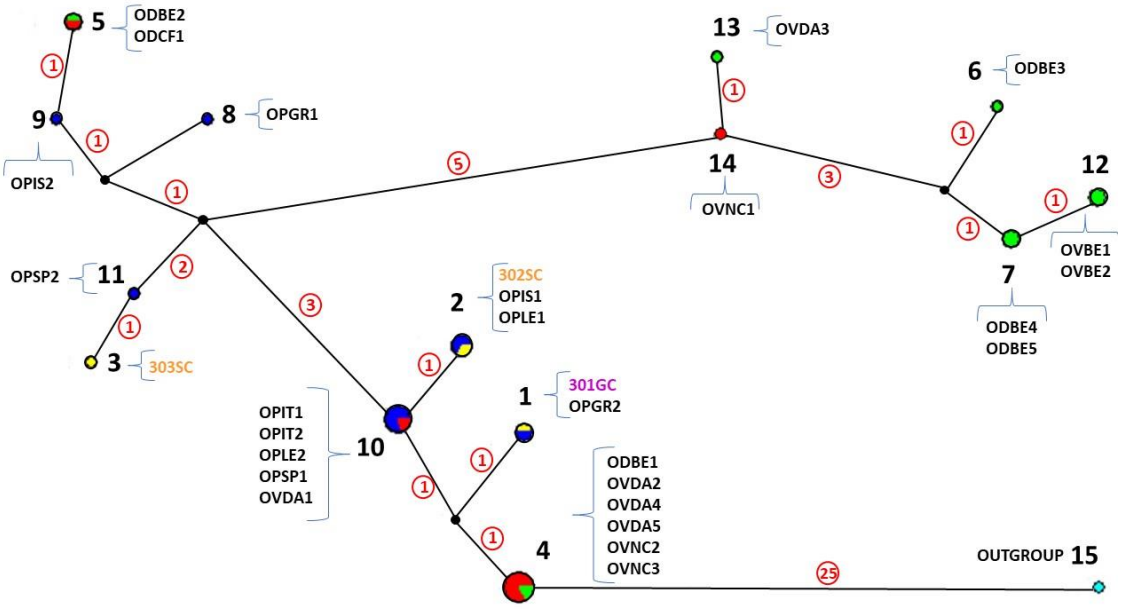


Figure 11. Haplotype network based on the concatenated sequences of *Oculina* spp. from different geographic regions: Canary Islands (yellow), Mediterranean (blue), America (red) and Bermudas (green). Haplotypes 1-15; circled numbers represent the number of mutations.

3.3.5 Oculina sp. fatty acid elongase haplotype network

Once more samples from the Canary Islands seem to be more closely related to those from the Mediterranean in the fatty acid elongase haplotype network (Figure 12). The haplotype network shows how sample 301 from Gran Canaria and sample 303 from Santa Cruz de Tenerife share the cosmopolitan haplotype 1 with four samples from the Mediterranean, two from Americas and one from Bermudas. Sample 302 from

Santa Cruz de Tenerife share haplotype with the *Astrangia* sp. specimen, (sample 322 from Oualidia, Morocco) and other two samples from the Mediterranean (haplotype 2).

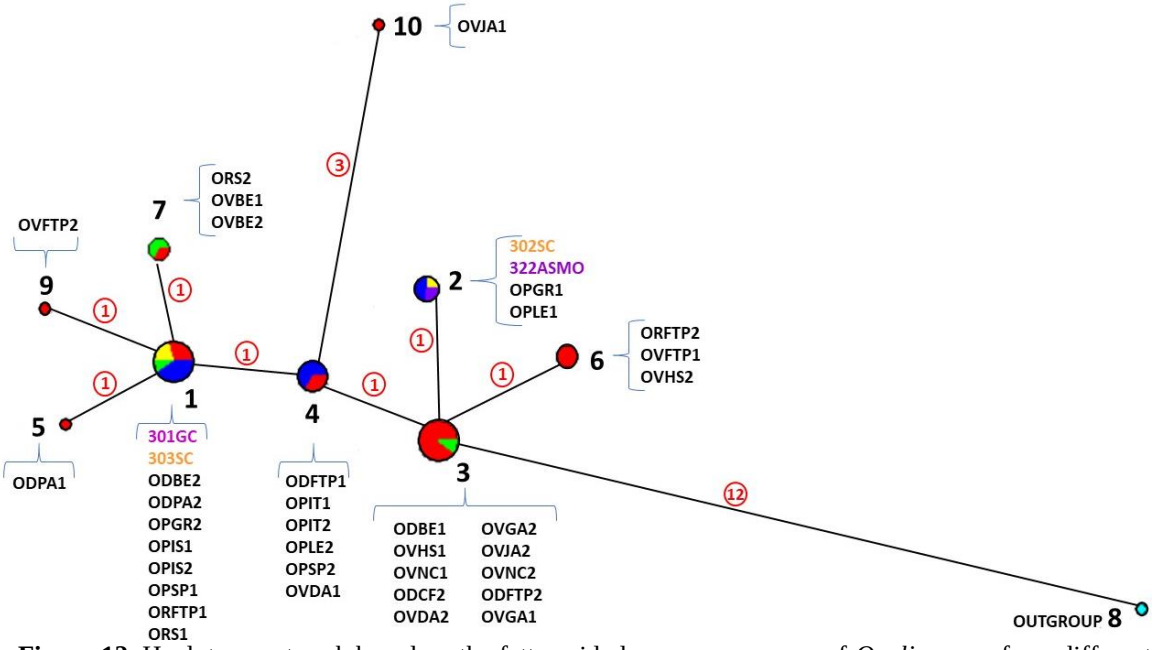


Figure 12. Haplotype network based on the fatty acid elongase sequences of *Oculina* spp. from different geographic regions: Canary Islands (yellow), Mediterranean (blue), America (red) and Bermudas (green). Haplotypes 1-10; circled numbers represent the number of mutations. *Astrangia* sp from Morocco, Oualidia (322ASMO) is represented in purple.

3.3.6 Oculina sp. tachylectin-2 haplotype network

In the individual haplotype network for tachylectin-2, (Figure 13) samples from the Canary Island showed to be closely related to those from the Mediterranean area. Sample 302 from Santa Cruz de Tenerife belongs to haplotype 2 together with most of the Mediterranean samples but also with samples from Bermudas and America. The other sample from Santa Cruz de Tenerife share haplotype 3 with a specimen of *O. patagonica* from Cabo de Palos, Murcia, Spain. The sample from Gran Canaria 301 share haplotype 1 with one specimen of *O. patagonica* from Greece and from America. *Astrangia* sp. from Occidental Sahara, Dahkla constitutes an exclusive haplotype and it moves away 8 mutations of haplotype 3 that show the sample 323 from Santa Cruz de Tenerife.

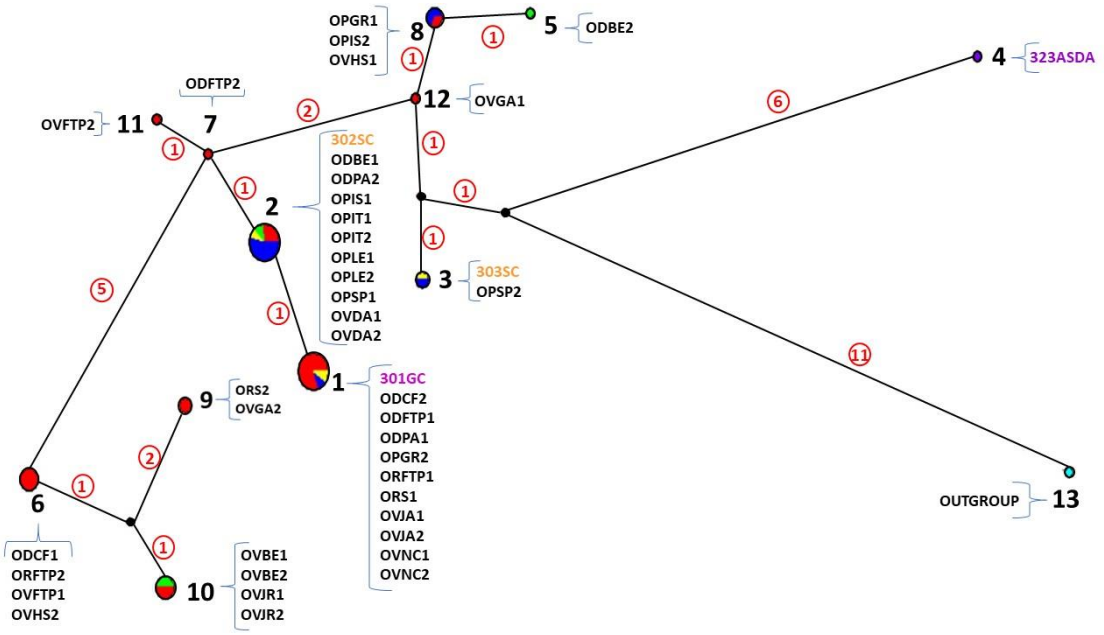


Figure 13. Haplotype network based on the tachylectin-2 sequences of *Oculina* spp. from different geographic regions: Canary Islands (yellow), Mediterranean (blue), America (red) and Bermudas (green). *Astrangia* sp. from Occidental Sahara, Dahkla (323ASDA) is represented in purple. Haplotypes 1-13; circled numbers represent the number of mutations. Black circles are middle points.

In the three networks we observe that the sample 301 from Gran Canaria share haplotype with the *O. patagonica* specimen from Greece (OPGR2). Sample 302 from Santa Cruz de Tenerife in a similar way share haplotype with the *O. patagonica* from Lebano (OPLE1). In the case of sample 303 from Santa Cruz de Tenerife for the concatenate sequences (Figure 11) as for the fatty acid elongase (Figure 12) this specimen constitutes an exclusive haplotype, but it is closely related to a specimen of *O. patagonica* from Cabo de Palos, Murcia, Spain.whereas for the tachylectin-2 haplotype network both specimens constitute the same haplotype. Regarding the two specimens of *Astrangia* sp. we observe that they present a different behavior. In the fatty acid elongase network sample 322 from Oualidia, Morocco shared haplotype with sample 302 from Santa Cruz de Tenerife (Figure 13) while that of Occidental Sahara, Dahkla, it is more related to sample 303 from Santa Cruz de Tenerife

4. DISSCUSION

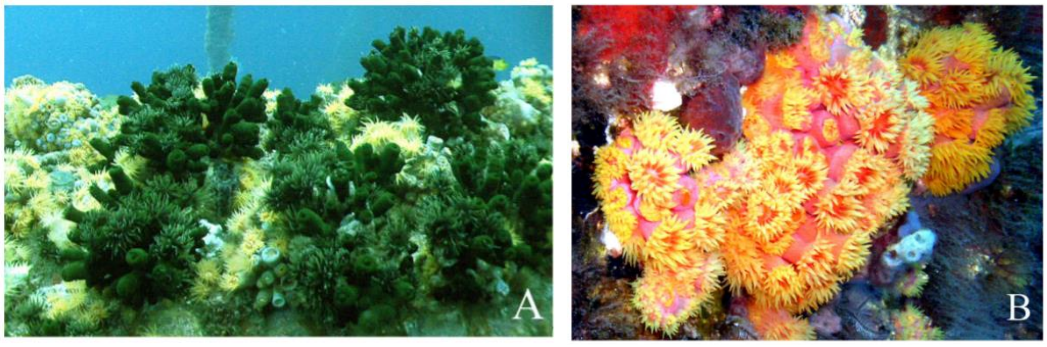
In this study we establish the first base-line for subsequent studies of the invasion and dispersal processes of *Tubastraea* and *Oculina* in the sport port of Santa Cruz de Tenerife. In the case of *Tubastraea* no distribution pattern was found. This means that the species is distributed throughout the harbour and no scattering focus is detected; a possible interpretation is that *Tubastraea* planulae have a very high dispersive capacity and are able to remain competent up to 100 days (Richmond 1997; Fenner, 2001; Glynn et al., 2018) this makes it difficult to locate the dispersion focus. Furthermore, in this study we detected a greater number of solitary polyps of small size and a smaller number of colonies. This indicates that it is a recent colonization process and what we observe is the initial stage of settlement posterior to the dispersion phase. Likewise, we found a greater number of *Tubastraea* in the areas most exposed to light, however, they were also present in shaded and dark areas. Conversely, the *Oculinas* were located mostly in the dark and shaded areas. *Oculina patagonica* is a zooxanthellated coral (with symbiotic algae), the presence of these algae favors the growth and assimilation of carbon faster than corals that lack them. For this reason, they do not need to be so exposed to light or water currents to feed themselves. *Tubastraea* on the other hand is an azoxantelated scleractinia, an active predator that depends on the capture of zooplankton, so it is not surprising to see specimens located in more lighted areas and exposed to water currents actively feeding on the zooplankton.

The species collected in the sport port of Santa Cruz de Tenerife were identified to prior as *Tubastraea coccinea* and *Oculina patagonica* based on its morphological characters. The combined use of the molecular marker COI with the ITS showed that two of our samples (321 and 324 from Santa Cruz de Tenerife and Gran Canaria, respectively) were more closely related to *Tubastrea aurea* (Quoy & Gaimard, 1833) which is considered a junior synonym of *Tubastraea coccinea*. Regarding the other two samples from Santa Cruz de Tenerife (306 and 320) these specimens were more related to *Tubastraea micranthus* (Ehrenberg, 1834) with a significant value of posterior probability (0.99) but with a non-significant bootstrap value (64%) (Figure 9). This group in turn seems to be related to other *T. coccinea* sequences (Figure 9).

Tubastraea micranthus (Figure 14) is an azooxanthellate coral commonly known as the Black Sun Coral. It is native to the tropical Indo-Pacific. It grows in bush or tree-like

colonies and is adapted to environments with strong currents. Colonies can grow on exposed bottom surfaces, on ledges, shipwrecks, and oil platforms. The corallites (calcareous wall around a single polyp) flare outward, and project in a loosely branching fashion. The only known location for *T. micranthus* introduced on an oil platform was Louisiana, if confirmed this would mean the second record of invasion of this species (Sammarco et al. 2010) a fact that does not surprise us since this species is in expansion and present in the oil platforms. At a morphological level, *T. micranthus* species differs from *T. coccinea*, which suggests that the sequences raised in the GenBank could be misidentified. The *Tubastraea* genus presents a certain degree of confusion and some of the described species have not yet been able to be confirmed with molecular methods (Arrigoni et al., 2014; Ocaña et al., 2015; Capel et al., 2016; Creed et al., 2017) needing a review. The widespread incongruence between molecular findings and the traditional systematics of Scleractinia has stimulated the search for new micromorphological and microstructural characters that are evolutionary informative (Arrrigoni et al., 2014).

Figure 14. *Tubastraea micranthus* colonies, identified by their dark tissue coloration and extended vertical growth, shown at 20m depth on a horizontal support of the oil platform GI-93 in the northern Gulf of Mexico. The coral species surrounding these colonies are *T. coccinea* (photo by SAP); (B) *Tubastraea coccinea* colonies observed on an oil platform in the High Island (HI) lease area. Note the more compressed habit and lighter, brighter tissue color (photo by PWS). (Images from Sanmarco et al., 2010)



Morphological characters used for the reconstruction of phylogenetic relationships among shallow-water scleratinian are largely uninformative (Cairns1984, 1997, 2001; Hoeksema 1989, 1993a; Wallace et al. 1991; Wallace 1999; Arrigoni et al., 2014) and an example of this is what we can observe with the newly described specie *Tubastraea caboverdiana* (Ocaña & Brito, 2015). Our phylogenetic tree (Figure 9) indicates that all the samples from Cabo Verde form a monophyletic cluster and that these belong to a different genus other than *Tubastraea*.

To identify the *Oculina* species of the sport port of Santa Cruz we used molecular markers (nDNA) tachylectin-2 and fatty acid elongase. Unfortunately, samples could not be

identified at the species level because this genus presents a high degree of taxonomic confusion and many misidentified specimens (Leydet & Hellberg, 2015). It should be noted that unlike the heterozygosity observed in the samples of *Oculina* sp. from Leydet & Hellberg, 2015, samples from the Canary Islands, Occidental Sahara and Morocco did not present this heterozygosity being therefore homozygous (Figure 8b). It would be recommendable to increase the number of samples to detect if heterozygosity exists in the specimens from the Canary Islands and also to be able to study genetic differences within and between the population of the Canary Islands. At least a minimum of twenty samples from each locality studied are necessary.

Regarding the samples of *Astrangia* sp. the one belonging to Morocco, is more closely related to the one of the Canary Islands (302 from Santa Cruz de Tenerife) and two *O. patagonica* from the Mediterranean for the fatty acid elongase network. This result agrees with the suspicions of Dr. Alberto Brito Hernández that this species has been misidentified and it belongs to *Oculina* genus. However, the other sample of *Astrangia* sp. from Dahkla (Occidental Sahara) seems to be more distant with respect to the *Oculina* samples.

The haplotype network generated from the concatenated sequences of both molecular markers indicated that *Oculina* samples in this study were more closely related to the Mediterranean species *Oculina patagonica*. This fact is also supported by the distance matrix calculated (Table 10) with very low distances between the Mediterranean and the Canary Islands. This result can be observed too in the phylogenetic tree based on these distances (Figure 10).

This decade has seen a dramatic rise in oil prices, which in turn has driven an unprecedented rise in the rate of semi-submersible rig transport (Wanless et al., 2009). Biofouled structures across biogeographic boundaries present unexcelled opportunities for invasion to a wide diversity of marine species. The primary coastal introduction points are always associated with nearby coastal port facilities, used by oil and gas industry associated shipping. In the case of oil platforms, the slow navigation of these vessels allows the associated fish and other fouling organisms to reach far from their original distribution areas. Better control and management of this vector is required urgently (Wanless, et al., 2009; Creed et al., 2016).

Marine environments around isolated islands are typically also depauperate but high in endemism, and this makes them vulnerable to biological invasions (Wanless et al., 2009). Biological invasion of *Tubastraea* and *Oculina* may be more worrisome in those areas of great ecological interest (Marine Protected Areas or Sites of Community Interest) and / or where threatened and / or endemic species exist as is the case of the Canary Islands. Colonization of *Tubastraea* and *Oculina* in the Canary Islands seems a recent process since the average number of both species in the sport port of Santa Cruz de Tenerife sampled in this study is still low. Colonization of these two species in the Canary Islands is very likely related to the arrival, especially from 2011, of oil platforms to the two main Canary ports (Las Palmas de Gran Canaria harbor and Santa Cruz de Tenerife harbor), most of them from different tropical areas of the Atlantic (Brazil, the Caribbean, Gulf of Guinea), but also of the Indo-Pacific and the Mediterranean (Falcón, 2015; Pajuelo et al., 2016; Brito et al., 2017)(Figure 1).

Current environmental conditions in the Canary Islands, where temperatures rise have increased with climate change (Vélez et al., 2015), with an actual range between 17 and 25 °C and an average of 21° C, have favored the settlement and expansion of *T. coccinea*, a thermophilic species with a wide range of thermal tolerance (Batista et al., 2017). The Canary Islands marine biodiversity has undergone a process of tropicalization since the mid-1990s that seems directly related to this increase in temperature (Brito et al., 2005, Sangil et al., 2010, Hernández et al., 2010; Falcón et al., 2015, Falcón, 2015, Riera et al., 2015, González et al., 2017). In many cases this is the natural expansion of the species' distribution area, since the Canary Islands are not far from the thermal front of Cabo Blanco (Mauritania) and the biogeographic frontier in which there is already a notable discontinuity between warm-temperate and tropical faunas (Spalding et al., 2007; Almada et al., 2013). In other cases, particularly in the case of species that are located in ports and their surrounding area, one can suspect on the basis of an introduction with maritime transport as a vector (ballast water, fouling, rafting under oil platforms) (Brito & Falcon, 1996, Brito et al., 2005, Brito et al., 2011, Clemente et al., 2011, Falcón et al., 2015, Falcón, 2015, Triay-Portella et al., 2015, Pajuelo et al. al., 2016; Brito et al., 2017).

Regarding the risk of invasion in the Canary Islands, in principle and in the short-medium term we can suspect it of the *T. coccinea* for its demonstrated ability to expand rapidly and cover the bottoms in high densities, due to its type of sexual and asexual reproduction, high fecundity and larval dispersion rate, rapid growth, chemical defences and

competitive aggressiveness (De Paula et al., 2014; Lages et al., 2010 a and b; Creed et al. 2017; Brito et al., 2017). Since its settlement in Ihla Grande, Brazil *T. coccinea* has been able to expand its population very fast and current predictions indicate that there is a high risk that *T. coccinea* could colonize the entire coast of Brazil (Riul et al., 2013) this could also happen in the Canary Islands if preventive measures are not taken.

O. patagonica is a species native to the Mediterranean (Leydet & Hellberg, 2015) that, due to climate changes that are occurring globally, could be spreading through the Mediterranean basin. The propagation of *O. patagonica* in the Mediterranean has a marked East-West component. Kružić et al. (2012), confirmed that there is a gradual warming of the waters of the Northwest of the Mediterranean. This unusual sea surface warming events in the last two decades could be allowing the establishment of this species in subtropical zones, probably through the oil platforms. *Oculina* sp. has been considered as an invasive species due to its behavior, since it colonizes rocky substrates excluding the community present in them (Serrano et al., 2012; 2013) and, in the last decade, is spreading rapidly along the coastline of the Iberian Peninsula (Coma et al., 2011). *Oculina* sp. is rapidly expanding mainly due the presence of anthropogenic structures, being able to survive even to the effects of contaminated and polluted waters. Population studies of *Oculina* coincide in that it mainly inhabits artificial structures such as harbours and dykes, colonies of the species have been found in the proximity of this man-made structures (Fine *et al*. 2001, Serrano *et al*. 2013, Rubio-Portillo *et al*. 2014, Salomidi *et al*. 2014; Terrón-sigler et al., 2016). Nevertheless, it has been also found in abundance in natural habitats (Fine et al. 2001, Coma et al. 2011, Serrano et al. 2013, Serrano et al. 2012). *Oculina* has the ability to live and reproduce under varying and diversified environmental conditions, such as a wide range of water temperatures, salinity, UV radiation, turbidity and strong wave energy (Fine *et al*. 2001). It is considered an "opportunistic dominant settler" that overgrows the calcareous structures of serpulids, vermetids, barnacles, etc., competing with algae and other soft organisms. (Sartoretto et al. 2008).

Periodic samplings are vital to know the progress of this type of species with invasive or opportunistic behavior, in order to adopt the necessary management measures. The question arises as to whether a rapid response is called for on the part of government to eradicate this potential invasive in its early stages of colonization, rather than waiting too long. In the early stages of colonization, successful complete eradication of an invasive species is possible. Delay of eradication of invasive species, however, is much more

difficult to achieve (Simberloff 2000; Hewitt et al. 2005) and potentially problematic. A community may have reached a new stable equilibrium, integrating the new species. Under such conditions, if the species is removed, the community can become destabilized and rapidly thrown into disequilibrium. If *Tubastraea micranthus*, *Tubastraea coccinea*, *Oculina patagonica* have to be eradicated from the Canary Islands, such action should be taken soon (Sanmarco et al., 2010).

5. CONCLUSION

- Abundance of *Tubastraea* sp. in the sport port of Santa Cruz de Tenerife is still low, with a larger number of solitary polyps distributed throughout the port indicating a recent colonization process. Eradication of the invasive species is recommended in its initial stages and such actions should be taken as soon as possible.
- *Oculina patagonica* colonies are located in shaded to dark areas, abundance is still low indicating a recent colonization. Similar preventive policies to that of *Tubastraea* sp. should be applied with this specie.
- Two *Tubastraea* species are identified in the Canary Islands *Tubastraea coccinea* and *Tubastraea micranthus.*
- The species identified morphologically as *Tubastraea caboverdiana* are actually a new genus.
- *Oculina* specimens from the Canary Islands are more closely related to the species *Oculina patagonica* from the Mediterranean.

Astrangia sp. specimen from Oualidia, Morocco were closely related to *Oculina patagonica* from the Mediterranean but also to a specimen from Santa Cruz de Tenerife,Canary Island therefore it is not ruled out that this specimen has been misidentified and it is actually an *Oculina* sp. On the other hand, the *Astrangia* sp. from Dahkla (Occidental Sahara) is less related to the species of Santa Cruz de Tenerife. It will be necessary to review the morphological identification analysis and complete the molecular one.

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ANNEX I: *Tubastraea* sp.

Table 1. Samples of *Tubastraea* sp. examined in this study, their collection details and molecular marker amplified. Concatenate data set. NA (Not Available)

Species	ID	**GenBank**	**COI**	**rDNA**	**Region**	**Site**
Tubastraea sp.	**304CV**	NA	NA	NA	São Vicente, Cape Verde	Laginha
Tubastraea sp.	**305CV**	NA	NA	NA	São Vicente, Cape Verde	Ponta de São João
Tubastraea sp.	**306SC**	NA	NA	NA	Santa Cruz de Tenerife, Canary Islands	Santa Cruz de Tenerife
Tubastraea sp.	**315CV**	NA	NA	NA	São Vicente, Cape Verde	Macario
Tubastraea sp.	**316CV**	NA	NA	NA	São Vicente, Cape Verde	Ilhéu
Tubastraea sp.	**320SC**	NA	NA	NA	Santa Cruz de Tenerife, Canary Islands	Santa Cruz de Tenerife
Tubastraea sp.	**321SC**	NA	NA	NA	Santa Cruz de Tenerife, Canary Islands	Santa Cruz de Tenerife
Tubastraea sp.	**324GC**	NA	NA	NA	Las Palmas de Gran Canaria, Canary Islands	Jinamar
*Astroides calycularis**	AC1	UNIMIB MED842	HG965307	HG965371	Mediterranean Sea	NA
*Astroides calycularis**	AC2	UNIMIB MED843	HG965308	HG965372	Mediterranean Sea	NA
Balanophyllia europaea	BE1	UNIMIB SOL1	HG965309	HG965373	Mediterranean Sea	NA
Balanophyllia europaea	BE2	UNIMIB SOL2	HG965310	HG965374	Mediterranean Sea	NA
Balanophyllia europaea	BE3	UNIMIB SOL4	HG965311	HG965375	Mediterranean Sea	NA
Balanophyllia regia	BR1	UNIMIB SOL3	HG965314	HG965378	Mediterranean Sea	NA
Balanophyllia regia	BR2	UNIMIB SOL7	HG965315	HG965379	Mediterranean Sea	NA
Cladopsammia eguchii	CE1	UNIMIB MQ002	HG965317	HG965381	Marquesas, French Polynesia	NA
Cladopsammia eguchii	CE2	UNIMIB MQ071	HG965318	HG965382	Marquesas, French Polynesia	NA
Cladopsammia gracilis	CG	MUFS SR26	HG965320	HG965385	Japan	NA
Dendrophyllia cornigera	DC1	UNIMIB CA01	HG965322	HG965387	Mediterranean Sea	NA
Dendrophyllia cornigera	DC2	UNIMIB MI01	HG965323	HG965388	Mediterranean Sea	NA
Eguchipsammia serpentina	ES1	UNIMIB HS3134	HG965327	HG965392	New Caledonia1	NA
Eguchipsammia serpentina	ES2	IRD HS3185	HG965328	HG965393	New Caledonia1	NA
*Heteropsammia cochlea**	HC1	MUFS AS3	HG965329	HG965394	Australia	NA
*Heteropsammia cochlea**	HC2	UNIMIB NC688	HG965330	HG965395	New Caledonia4	NA

*Heteropsammia cochlea**	HC3	UNIMIB NC776	HG965331	HG965396	New Caledonia4	NA
Leptopsammia pruvoti	LP1	UNIMIB MD02	HG965332	HG965397	Mediterranean Sea	NA
Leptopsammia pruvoti	LP2	UNIMIB MD03	HG965333	HG965398	Mediterranean Sea	NA
Rhizopsammia verrilli	RV1	UNIMIB MQ035	HG965334	HG965400	Marquesas, French Polynesia	NA
Rhizopsammia verrilli	RV2	IRD HS2888	HG965336	HG965402	New Caledonia2	NA
Rhizopsammia wettsteini	RW1	UNIMIB M765	HG965338	HG965404	Maldives	NA
Rhizopsammia wettsteini	RW2	UNIMIB SO029	HG965340	HG965406	Socotra Island, Yemen	NA
Tubastraea cf aurea	TA1	UNIMIB M762	HG965341	HG965407	Maldives	NA
Tubastraea cf aurea	TA2	UNIMIB MY070	HG965342	HG965408	Mayotte Island6	NA
Tubastraea cf aurea	TA3	UNIMIB SO119	HG965343	HG965409	Socotra Island, Yemen7	NA
*Tubastraea coccinea**	TC1	MUFS AQ2	HG965344	HG965410	Japan	NA
*Tubastraea coccinea**	TC2	MUFS SR144	HG965345	HG965411	Japan	NA
*Tubastraea coccinea**	TC3	MUFS SR28	HG965346	HG965412	Japan	NA
Tubastraea micranthus	TM2	UNIMIB M768	HG965348	HG965416	Maldives	NA
Tubastraea micranthus	TM3	UNIMIB MY072	HG965349	HG965417	Mayotte Island6	NA
Tubastraea micranthus	TM4	UNIMIB Y756	HG965350	HG965418	Yemen	NA
Goniopora columna	OUT	NA	AB906954.1	AB907031	NA	NA

Table 2. Samples of *Tubastraea* sp. examined in this study, their collection details and molecular marker amplified. COI data set.

Species	ID	**GenBank**	**COI**	**Region**	**Site**
Tubastraea sp.	**304CV**	NA	NA	São Vicente, Cape Verde	Laginha
Tubastraea sp.	**305CV**	NA	NA	São Vicente, Cape Verde	Ponta de São João
Tubastraea sp.	**306SC**	NA	NA	Santa Cruz de Tenerife, Canary Islands	Santa Cruz de Tenerife
Tubastraea sp.	**308CV**	NA	NA		
Tubastraea sp.	**309GA**	NA	NA	South East Africa	Gabón
Tubastraea sp.	**315CV**	NA	NA	São Vicente, Cape Verde	Macario
Tubastraea sp.	**316CV**	NA	NA	São Vicente, Cape Verde	Ilhéu

Tubastraea sp.	**320SC**	NA	NA	Santa Cruz de Tenerife, Canary Islands	Santa Cruz de Tenerife
Tubastraea sp.	**321SC**	NA	NA	Santa Cruz de Tenerife, Canary Islands	Santa Cruz de Tenerife
Tubastraea sp.	**324GC**	NA	NA	Las Palmas de Gran Canaria, Canary Islands	Jinamar
Tubastraea sp.	**325GC**	NA	NA	Las Palmas de Gran Canaria, Canary Islands	
*Astroides calycularis**	AC1	UNIMIB MED842	HG965307	Mediterranean Sea	
*Astroides calycularis**	AC2	UNIMIB MED843	HG965308	Mediterranean Sea	
B.elegans	BEL1	NA	DQ445805	NA	
Balanophyllia europaea	BE1	UNIMIB SOL1	HG965309	Mediterranean Sea	
Balanophyllia europaea	BE2	UNIMIB SOL2	HG965310	Mediterranean Sea	
Balanophyllia europaea	BE3	UNIMIB SOL4	HG965311	Mediterranean Sea	
Balanophyllia regia	BR1	UNIMIB SOL3	HG965314	Mediterranean Sea	
Balanophyllia regia	BR2	UNIMIB SOL7	HG965315	Mediterranean Sea	
Cladopsammia eguchii	CE1	UNIMIB MQ002	HG965317	Marquesas, French Polynesia	
Cladopsammia eguchii	CE2	UNIMIB MQ071	HG965318	Marquesas, French Polynesia	
Cladopsammia gracilis	CG	MUFS SR26	HG965320	Japan	
D.arbuscula	DA1		KR824937	Brasil	Sao Paulo
Dendrophyllia cornigera	DC1	UNIMIB CA01	HG965322	Mediterranean Sea	
Dendrophyllia cornigera	DC2	UNIMIB MI01	HG965323	Mediterranean Sea	
Eguchipsammia serpentina	ES1	UNIMIB HS3134	HG965327	New Caledonia1	
Eguchipsammia serpentina	ES2	IRD HS3185	HG965328	New Caledonia1	
*Heteropsammia cochlea**	HC1	MUFS AS3	HG965329	Australia	
*Heteropsammia cochlea**	HC2	UNIMIB NC688	HG965330	New Caledonia4	
*Heteropsammia cochlea**	HC3	UNIMIB NC776	HG965331	New Caledonia4	
Leptopsammia pruvoti	LP1	UNIMIB MD02	HG965332	Mediterranean Sea	
Leptopsammia pruvoti	LP2	UNIMIB MD03	HG965333	Mediterranean Sea	
Rhizopsammia verrilli	RV1	UNIMIB MQ035	HG965334	Marquesas, French Polynesia	
Rhizopsammia verrilli	RV2	IRD HS2888	HG965336	New Caledonia2	
Rhizopsammia wettsteini	RW1	UNIMIB M765	HG965338	Maldives	

Rhizopsammia wettsteini	RW2	UNIMIB SO029	HG965340	Socotra Island, Yemen	
T. tagusensis	TT1		KX024567.1		
Tubastraea cf aurea	TA1	UNIMIB M762	HG965341	Maldives	
Tubastraea cf aurea	TA2	UNIMIB MY070	HG965342	Mayotte Island6	
Tubastraea cf aurea	TA3	UNIMIB SO119	HG965343	Socotra Island, Yemen7	
*T. coccinea**	TC1	MUFS AQ2	HG965344		
*T.coccinea**	TC2	MUFS SR144	HG965345		
*T.coccinea**	TC3	MUFS SR28	HG965346		
T. coccinea	TC4	NA	KX024566		
T. coccinea	TC5	NA	NC026025		
T.coccinea	TC6	NA	DQ445806		
T.micranthus	TM1	IRD HS3129	HG965347		
T.micranthus	TM2	UNIMIB M768	HG965348		
T.micranthus	TM3	UNIMIB MY072	HG965349		
T.micranthus	TM4	UNIMIB Y756	HG965350		
Goniopora columna	OUT	NA	AB906954.1		

Figure 1. Mr.Bayes tree from the COI sequence alignment for *Tubastraea* sp. Support values are posterior probability and maximum parsimony bootstrap values, respectively. Significant bootstraps are in bold and non-significant in grey.

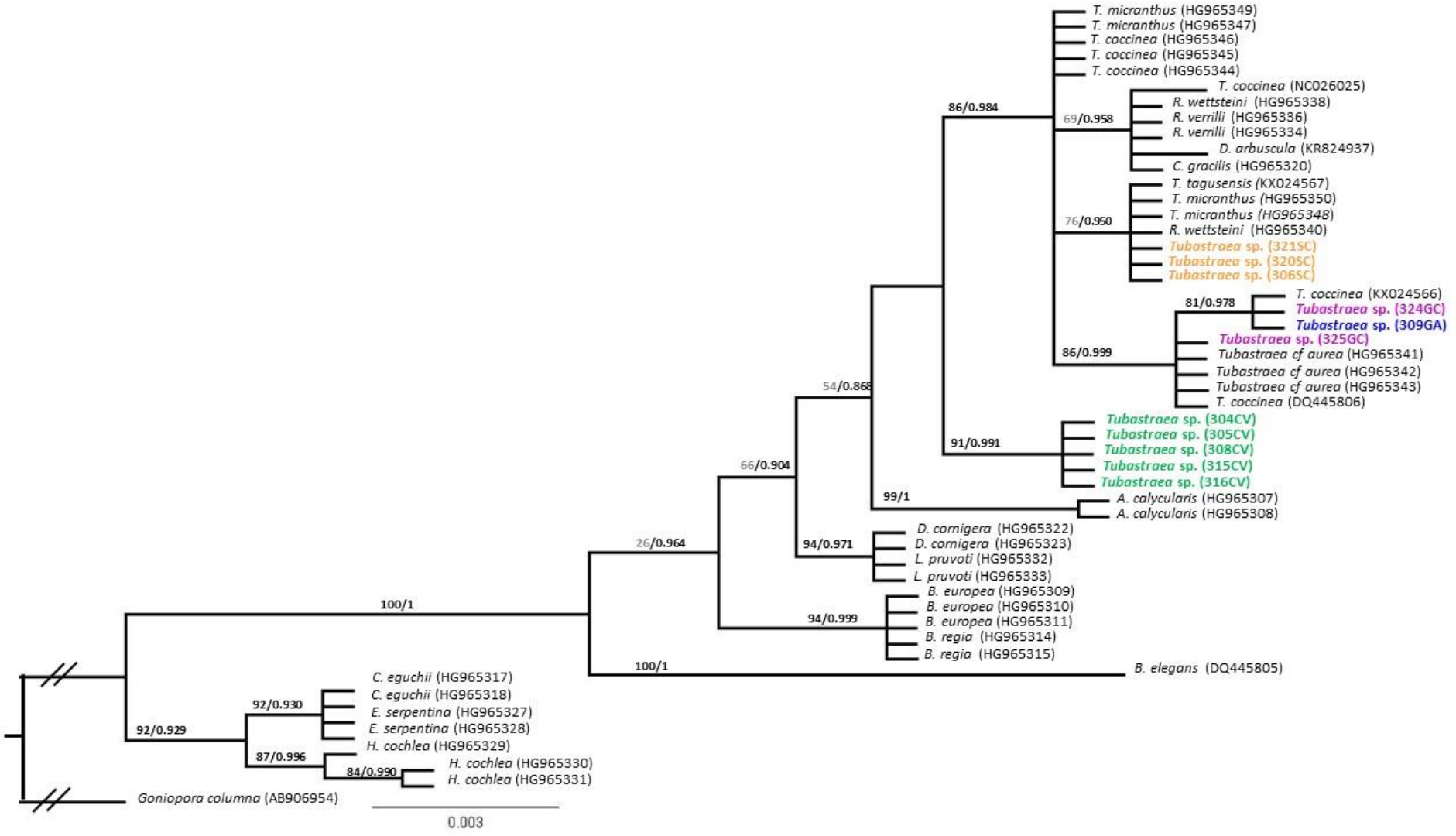


Figure 2. Maximum Likelihood tree from the COI sequence alignment for *Tubastraea* sp. Support values are posterior probability and maximum parsimony bootstrap values, respectively. Significant bootstraps are in bold and non-significant in grey.

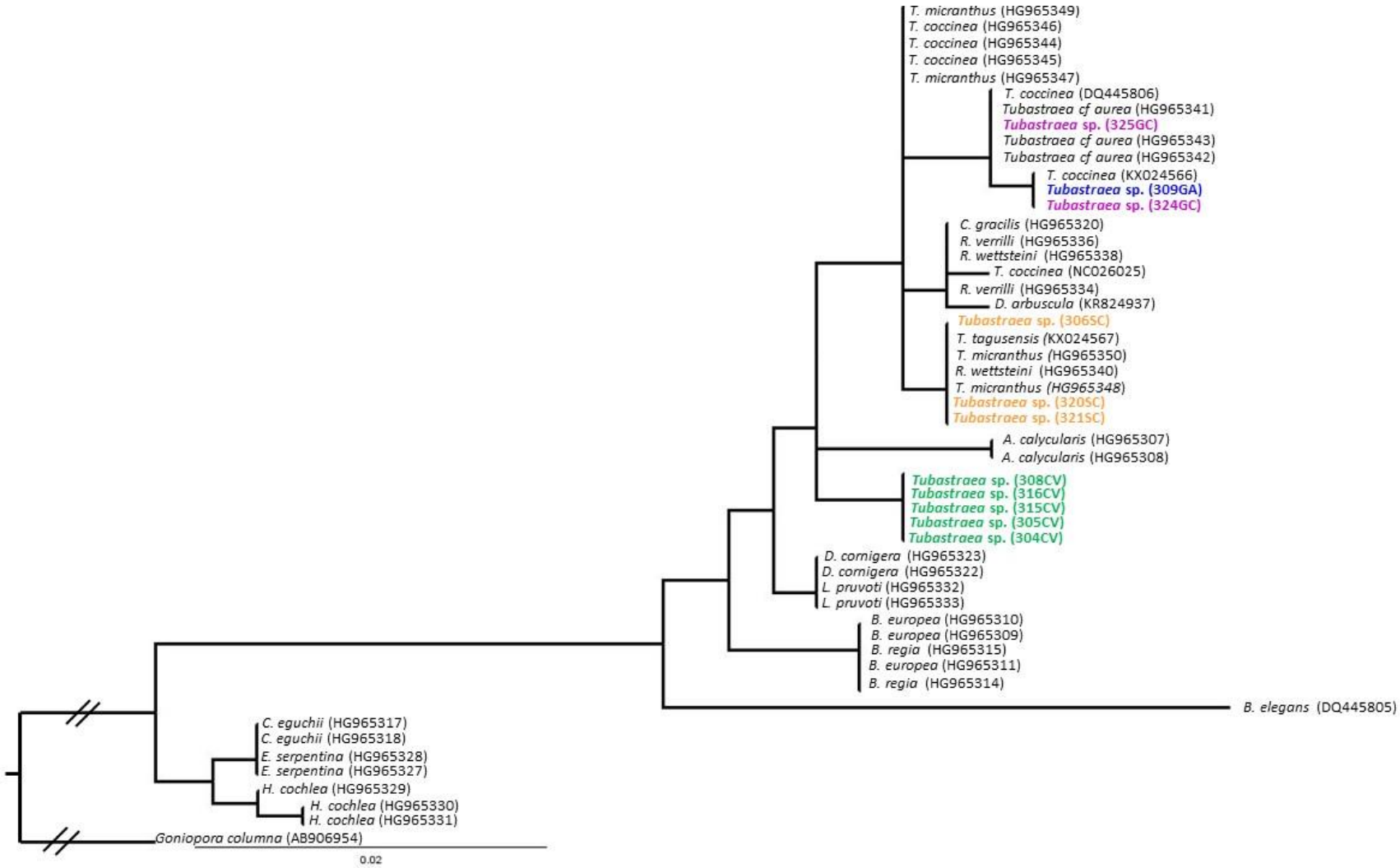
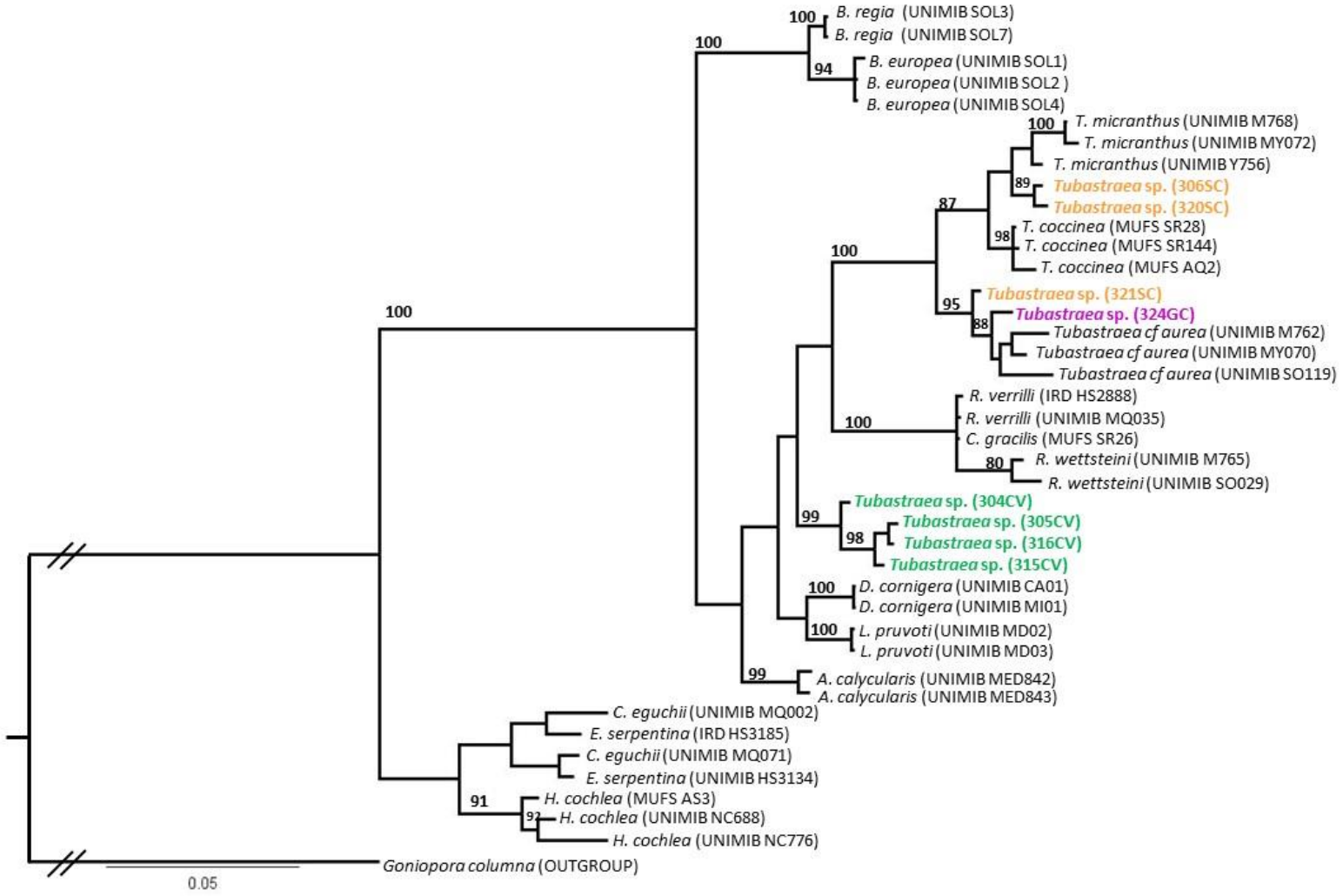


Figure 3. Mr. Bayes for the concatenate sequence alignment for *Tubastraea* sp. Support values are posterior probability and maximum parsimony bootstrap values, respectively. Significant bootstraps are in bold and non-significant in grey.



ANNEX II: *Oculina* sp.

Table 1. Samples of *Oculina* sp. examined in this study, their collection details and molecular marker amplified. Concatenate data set. NA (Not Available)

Specie	**ID**	**GenBank**	**P14F**	**P302F**	**Country**	**Locality**	**Author**
Oculina sp.	301GC	NA	NA	NA	Canary Islands, Spain	Alcaravaneras, Las Palmas de Gran Canaria	Moltó-Martín, I. (2018)
Oculina sp.	303SC	NA	NA	NA	Canary Islands, Spain	Santa Cruz de Tenerife	Moltó-Martín, I. (2018)
Oculina sp.	302SC	NA	NA	NA	Canary Islands, Spain	Santa Cruz de Tenerife	Moltó-Martín, I. (2018)
O. difussa	ODBE 1	BER12b	LN613422.1	LN613738. 1	Bermuda	Tynes Bay	Eytan et al., 2009; Leydet and Hellberg, 2015
O. difussa	ODBE 2	BER9b	LN613442.1	LN613758. 1	Bermuda	Tynes Bay	Eytan et al., 2009; Leydet and Hellberg, 2015
O. difussa	ODBE 3	BER11b	LN613420.1	LN613736. 1	Bermuda	Tynes Bay	Eytan et al., 2009; Leydet and Hellberg, 2015
O. difussa	ODBE 4	BER3a	LN613429.1	LN613745. 1	Bermuda	Tynes Bay	Eytan et al., 2009; Leydet and Hellberg, 2015
O. difussa	ODBE 5	BER7a	LN613437.1	LN613753. 1	Bermuda	Tynes Bay	Eytan et al., 2009; Leydet and Hellberg, 2015
O. difussa	ODCF 1	CFL7.a1	FJ966403.1	FJ966717.1	United States	Cape Florida	Eytan et al., 2009
O. patagonica	OPGR 1	GREa11a	LN613477.1	LN613793. 1	Greece	Athens	Eytan et al., 2009; Leydet and Hellberg, 2015
O. patagonica	OPGR 2	GREa10a	LN613475.1	LN613791. 1	Greece	Athens	Eytan et al., 2009; Leydet and Hellberg, 2015
O. patagonica	OPIS1	ISRc9a	LN613573.1	LN613889. 1	Israel	Caesarea, Hadera,Sdot-Yam	Eytan et al., 2009; Leydet and Hellberg, 2015
O. patagonica	OPIS2	ISRb4a	LN613545.1	LN613861. 1	Israel	Caesarea, Hadera,Sdot-Yam	Eytan et al., 2009; Leydet and Hellberg, 2015

O. patagonica	OPIT1	ITAa1b	LN613472.1	LN613788.1	Italy	Savona	Eytan et al., 2009; Leydet and Hellberg, 2015
O. patagonica	OPIT2	ITAa2b	LN613474.1	LN613790.1	Italy	Savona	Eytan et al., 2009; Leydet and Hellberg, 2015
O. patagonica	OPLE1	LEBt1a	LN613511.1	LN613827.1	Lebanon	Tyre	Eytan et al., 2009; Leydet and Hellberg, 2015
O. patagonica	OPLE2	LEBt4b	LN613518.1	LN613834.1	Lebanon	Tyre	Eytan et al., 2009; Leydet and Hellberg, 2015
O. patagonica	OPSP1	SPAcp9b	LN613470.1	LN613786.1	Spain	Cabo de Palos	Eytan et al., 2009; Leydet and Hellberg, 2015
O. patagonica	OPSP2	SPAcp12a	LN613447.1	LN613763.1	Spain	Cabo de Palos	Eytan et al., 2009; Leydet and Hellberg, 2015
O. varicosa	OVBE1	BER2a	LN613427.1	LN613743.1	Bermuda	Tynes Bay	Eytan et al., 2009; Leydet and Hellberg, 2015
O. varicosa	OVBE2	BER2b	LN613428.1	LN613744.1	Bermuda	Tynes Bay	Eytan et al., 2009; Leydet and Hellberg, 2015
O. varicosa	OVDA1	DAYM3.a1	FJ966417.1	FJ966731.1	United States	Daytona Beach, Florida	Eytan et al., 2009
O. varicosa	OVDA2	DAYC8.HO	FJ966414.1	FJ966727.1	United States	Daytona Beach, Florida	Eytan et al., 2009
O. varicosa	OVDA3	DAYM7.HO	FJ966423.1	FJ966737.1	United States	Daytona Beach, Florida	Eytan et al., 2009
O. varicosa	OVDA4	DAYC2.HO	FJ966409.1	FJ966721.1	United States	Daytona Beach, Florida	Eytan et al., 2009
O. varicosa	OVDA5	DAYC7.HO	FJ966413.1	FJ966726.1	United States	Daytona Beach, Florida	Eytan et al., 2009
O. varicosa	OVNC1	NC1HO	FJ966488.1	FJ966794.1	United States	North Carolina	Eytan et al., 2009
O. varicosa	OVNC2	NC2HO	FJ966489.1	FJ966795.1	United States	North Carolina	Eytan et al., 2009
O. varicosa	OVNC3	NC3HO	FJ966492.1	FJ966798.1	United States	North Carolina	Eytan et al., 2009
Solenastrea hyades	OUT		FJ966563.1	FJ966866	NA	NA	Eytan et al., 2009; Leydet and Hellberg, 2015

Table 2. Samples of *Oculina* sp. examined in this study, their collection details and molecular marker amplified. Fatty acid elongase data set. NA (Not Available)

Specie	**ID**	**GenBank**	P14F	**Country**	**Locality**
Oculina sp.	301GC	NA	NA	Canary Islands, Spain	Alcaravaneras, Las Palmas de Gran Canaria
Oculina sp.	303SC	NA	NA	Canary Islands, Spain	Santa Cruz de Tenerife
Oculina sp.	302SC	NA	NA	Canary Islands, Spain	Santa Cruz de Tenerife
Astrangia sp.	322ASDA	NA	NA	Occidental Sahara, Africa	Dakhla
O. difussa	ODBE1	BER12b	LN613422	Bermuda	Tynes Bay
O. difussa	ODBE2	BER9b	LN613442	Bermuda	Tynes Bay
O. difussa	ODPA1	PAN2-2.a1	FJ966538	United States	Panama City
O. difussa	ODPA2	PAN1-2.a2	FJ966526	United States	Panama City
O. difussa	ODCF1	CFL10HO	FJ966395	United States	Cape Florida
O. difussa	ODCF2	CFL12HT	FJ966398	United States	Cape Florida
O. difussa	ODFTP1	OdFTP.A.HT	FJ966501	United States	Ft. Pierce
O. difussa	ODFTP2	OdFtP.B.HO	FJ966502	United States	Ft. Pierce
O. patagonica	OPIS1	ISRc8a	LN613571	Israel	Caesarea, Hadera,Sdot-Yam
O. patagonica	OPIS2	ISRb4a	LN613545	Israel	Caesarea, Hadera,Sdot-Yam
O. patagonica	OPSP1	SPAcp10a	LN613443	Spain	Cabo de Palos
O. patagonica	OPSP2	SPAcp12a	LN613447	Spain	Cabo de Palos
O. patagonica	OPIT1	ITAa1b	LN613472	Italy	Savona
O. patagonica	OPIT2	ITAa2b	LN613474	Italy	Savona
O. patagonica	OPGR1	GREa11a	LN613477	Greece	Athens
O. patagonica	OPGR2	GREa10a	LN613475	Greece	Athens
O. patagonica	OPLE1	LEBt1a	LN613511	Lebano	Tyre
O. patagonica	OPLE2	LEBt4b	LN613518	Lebano	Tyre
O. robusta	ORFTP1	OrFTP.B.a2	FJ966510	United States	Ft. Pierce

O. robusta	ORFTP2	OrFTP.B.a1	FJ966509	United States	Ft. Pierce
O. robusta	ORS1	SAR10.HO	FJ966547	United States	Florida, Sarasota
O. robusta	ORS2	SAR1.HT	FJ966546	United States	Florida, Sarasota
O. varicosa	OVDA1	DAYM3.a1	FJ966417	United States	Daytona Beach
O. varicosa	OVDA2	DAYM7.HO	FJ966423	United States	Daytona Beach
O. varicosa	OVHS1	HSH24.a1	FJ966451	United States	South Florida,Horseshoe Reef
O. varicosa	OVHS2	HSH24.a2	FJ966452	United States	South Florida,Horseshoe Reef
O. varicosa	OVBE1	BER2a	LN613427	Bermuda	Tynes Bay
O. varicosa	OVBE2	BER2b	LN613428	Bermuda	Tynes Bay
O. varicosa	OVGA1	GA-JR11HO	FJ966431	United States	Georgia
O. varicosa	OVGA2	GA-JR12.a1	FJ966432	United States	Georgia
O. varicosa	OVJA1	JAXpc8.a1	FJ966460	United States	Jacksonville
O. varicosa	OVJA2	JAXpm1.aA	FJ966463	United States	Jacksonville
O. varicosa	OVJR1	JR80-4.a1	FJ966478	United States	Jeff's Reef
O. varicosa	OVJR2	JR80-8.a2	FJ966484	United States	Jeff's Reef
O. varicosa	OVNC1	NC9HO	FJ966498	United States	North Carolina
O. varicosa	OVNC2	NC1HO	FJ966488	United States	North Carolina
O. varicosa	OVFTP1	OvFTP35.HO	FJ966519	United States	Ft. Pierce
O. varicosa	OVFTP2	OvFtP39.a1	FJ966521	United States	Ft. Pierce
S.hyades	OUT	SO25.38KHT	FJ966563	NA	NA

Table 3. Samples of *Oculina* sp. examined in this study, their collection details and molecular marker amplified. Tachylectin-2 elongase data set. NA (Not Available)

Specie	**ID**	**GenBank**	**P302**	**Country**	**Locality**
Oculina sp.	301GC	NA	NA	Canary Islands, Spain	Alcaravaneras, Las Palmas de Gran Canaria
Oculina sp.	303SC	NA	NA	Canary Islands, Spain	Santa Cruz de Tenerife
Oculina sp.	302SC	NA	NA	Canary Islands, Spain	Santa Cruz de Tenerife
Astrangia sp.	323ASMO	NA	NA	Morocco, Africa	Oualidia
O. difussa	ODBE1	BER12b	LN613738	Bermuda	Tynes Bay

O. difussa	ODBE2	BER9b	LN613758	Bermuda	Tynes Bay
O. difussa	ODPA1	PAN2-1HO	FJ966838	United States	Panama City
O. difussa	ODPA2	PAN1-1HO	FJ966829	United States	Panama City
O. difussa	ODCF1	CFL10.a1	FJ966708	United States	Cape Florida
O. difussa	ODCF2	CFL4HO	FJ966716	United States	Cape Florida
O. difussa	ODFTP1	OdFtPBaE	FJ966810	United States	Ft. Pierce
O. difussa	ODFTP2	OdFtPG.a1	FJ966813	United States	Ft. Pierce
O. patagonica	OPIS1	ISRc8a	LN613887	Israel	Caesarea, Hadera,Sdot-Yam
O. patagonica	OPIS2	ISRb4a	LN613861	Israel	Caesarea, Hadera,Sdot-Yam
O. patagonica	OPSP1	SPAcp10a	LN613759	Spain	Cabo de Palos
O. patagonica	OPSP2	SPAcp12a	LN613763	Spain	Cabo de Palos
O. patagonica	OPIT1	ITAa1b	LN613788	Italy	Savona
O. patagonica	OPIT2	ITAa2b	LN613790	Italy	Savona
O. patagonica	OPGR1	GREa11a	LN613793	Greece	Athens
O. patagonica	OPGR2	GREa10a	LN613791	Greece	Athens
O. patagonica	OPLE1	LEBt1a	LN613827	Lebano	Tyre
O. patagonica	OPLE2	LEBt4b	LN613834	Lebano	Tyre
O. varicosa	OVDA1	DAYM3.a1	FJ966731	United States	Daytona Beach
O. varicosa	OVDA2	DAYM4.HO	FJ966733	United States	Daytona Beach
O. varicosa	OVHS1	HSH24.a1	FJ966762	United States	Horseshoe Reef
O. varicosa	OVHS2	HSH24.a2	FJ966763	United States	Horseshoe Reef
O. varicosa	OVBE1	BER2a	LN613743	Bermuda	Tynes Bay
O. varicosa	OVBE2	BER2b	LN613744	Bermuda	Tynes Bay
O. varicosa	OVNC1	NC12HT	FJ966793	United States	North Carolina
O. varicosa	OVNC2	NC2HO	FJ966795	United States	North Carolina
O. varicosa	OVGA1	GA-JR11HO	FJ966742	United States	Georgia
O. varicosa	OVGA2	GA-JR7a2	FJ966746	United States	Georgia
O. varicosa	OVJA1	JAXPC10HO	FJ966767	United States	Jacksonville
O. varicosa	OVJA2	JAXPC7HO	FJ966770	United States	Jacksonville
O. varicosa	OVJR1	JR80-12HO	FJ966783	United States	Jeff's Reef
O. varicosa	OVJR2	JR80-4HO	FJ966786	United States	Jeff's Reef
O. varicosa	OVFTP1	OvFtP26HT	FJ966820	United States	Ft. Pierce
O. varicosa	OVFTP2	OvFtP38a1	FJ966825	United States	Ft. Pierce
O.robusta	ORS1	SAR10a2	FJ966844	United States	Sarasota

O.robusta	ORS2	SAR10a1	FJ966843	United States	Sarasota
O.robusta	ORFTP1	OrFtPA.a1	FJ966815	United States	Ft. Pierce
O.robusta	ORFTP2	OrFtPBHO	FJ966817	United States	Ft. Pierce
S.hyades	OUT	SolNC38k4	FJ966866	NA	NA

Table 4. Collection sites from Leydet & Hellberg, 2015 study.

APPENDIX B.
SUPPLEMENTARY MATERIAL FOR CHAPTER 3

Table 3.S1. Collection sites of all samples used in this study.

	Country	Locality	Latitude	Longitude	n^a	Depth (m)	Date collected
Western Atlantic	United States	North Carolina	34°42′ N	76°40′ W	8	2–4	August 2003
		Daytona Beach, Florida	29°15′ N	80°45′ W	11	21–23	July 2005
		Cape Florida, Florida	25°40′ N	80°09′ W	9	2	March 2004
		Panama City, Florida	30°03′ N	85°51′ W	11	28–29	January 2004
	Bermuda	Tynes Bay	32°18′ N	64°46′ W	13	3–10	October 2005
Mediterranean	Spain	Cabo de Palos	37°38′ N	00°41′ W	14	3–8	August 2011
	Italy	Savona	44°20′ N	08°30′ E	2	1–2	July 2013
	Greece	Athens	37°53′ N	23°43′ E	18	0.5–1	July 2011
	Lebanon	Tyre	33°16′ N	35°11′ E	4	4	August 2011
	Israel	Caesarea, Hadera, Sdot-Yam	32°28′ N	34°54′ E	27	2–5	July 2012

[a] number of samples (this final dataset included all individuals with no missing data)