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Published in: Diversity

DOI: 10.3390/d14110909

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Document Version
Publisher's PDF, also known as Version of record

Publication date: 2022

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):

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New Records of the Cryptogenic Soft Coral Genus *Stragulum* (Tubiporidae) from the Eastern Caribbean and the Persian Gulf

Kaveh Samimi-Namin 1,2,3,*, Leen P. van Ofwegen 2,†, Bert W. Hoeksema 2,4,5, Lucy C. Woodall 1,6, Melanie Meijer zu Schlochtern 6 and Catherine S. McFadden 7

1 Department of Zoology, University of Oxford, Oxfordshire, Oxford OX1 3SZ, United Kingdom
2 Taxonomy, Systematics and Geodiversity Group, Naturalis Biodiversity Center, P.O. Box 9517, 2300 RA Leiden, The Netherlands
3 Natural History Museum, Cromwell Road, London SW7 5BD, United Kingdom
4 Groningen Institute for Evolutionary Life Sciences, University of Groningen, P.O. Box 11013, 9700 CC Groningen, The Netherlands
5 Institute of Biology Leiden, Leiden University, P.O. Box 9505, 2300 RA Leiden, The Netherlands
6 Nature Foundation Sint Maarten, Wellsburg Street 1A Unit 25-26, Cole Bay, Sint Maarten, The Netherlands
7 Department of Biology, Harvey Mudd College, Claremont, CA 91711, United States

* Correspondence: kaveh.samimi@naturalis.nl
† Deceased.

Abstract: The monotypic soft coral genus *Stragulum* van Ofwegen and Haddad, 2011 (Octocorallia: Malacalcyonacea: Tubiporidae) was originally described from Brazil, southwest Atlantic Ocean. Here, we report the first records of the genus from the eastern Caribbean and the Persian Gulf in the northwest Indian Ocean. We compare the morphological features of specimens, together with molecular data from three commonly used barcoding markers (COI, mtMutS, 28S rDNA) and 308 ultraconserved elements (UCE) and exon loci sequenced using a target-enrichment approach. The molecular and morphological data together suggest that specimens from all three localities are the same species, i.e., *Stragulum bicolor* van Ofwegen and Haddad, 2011. It is still not possible to establish the native range of the species or determine whether it may be an introduced species due to the limited number of specimens included in this study. However, the lack of historical records, its fouling abilities on artificial substrates, and a growing number of observations support the invasive nature of the species in Brazilian and Caribbean waters and therefore suggest that it may have been introduced into the Atlantic from elsewhere. Interestingly, the species has not shown any invasive behaviour in the Persian Gulf, where it has been found only on natural, rocky substrates. The aim of the present report is to create awareness of this taxon with the hope that this will lead to new records from other localities and help to establish its native range.

Keywords: Octocorallia; phylogeny; DNA barcoding; ultraconserved elements; target-enrichment sequencing; Iran; Kuwait; Sint Maarten

1. Introduction

In recent years, a number of non-native and potentially invasive octocoral species have been reported from locations in the southwestern Atlantic [1–5]. Six species can be traced to three separate introductions confirmed or presumed to have come from the international marine aquarium trade [2–5]. Two of these introduced species, both xeniid soft corals native to the Indo-Pacific, have already caused widespread ecological damage to coral reefs in Venezuela and southeast Brazil, where they have overgrown native corals and other benthos, and are now dominant species on the reef [6,7]. Several additional new species of octocorals belonging to Indo-Pacific lineages have been discovered in the western Atlantic but their origins (source of introduction as well as native range) remain unknown. These include *Chromonephthea braziliensis*, a soft coral belonging to a genus and family that is
otherwise restricted to the Indo-Pacific [8], and *Stragulum bicolor*, an encrusting octocoral related to the organ-pipe coral genus *Tubipora* [1]. It has been suggested but not confirmed that both of these cryptogenic species may have been introduced to Brazil by fouling on oil rigs or other artificial structures [1,9].

*Stragulum bicolor* was first described in 2011 subsequent to its sudden appearance and rapid spread along the coastline of southeast Brazil. The earliest known reports of this species date from approximately 2000 in Paranaguá Bay, where it was first noticed growing on rocks, loose littoral material, mangrove roots, stems of algae and gorgonians, and other natural substrates [1]. By 2008 it had been reported from sites in Santa Catarina and Rio de Janeiro and was dominating artificial surfaces (e.g., piers and other port structures, ropes, acrylic and polyethylene settling plates) in the ports and marinas of Paranaguá Bay [1]. It has subsequently reached northeast Brazil, with reports from Pernambuco to Ceará [10]. *Stragulum bicolor* has been documented to grow on a wide range of artificial and natural substrates including at least 27 taxa of other sessile organisms [10]. To date there is little evidence, however, that *S. bicolor* kills the organisms it overgrows or that its presence in the community negatively impacts local biodiversity [11,12].

The sudden appearance and rapid spread of *S. bicolor* combined with its frequent occurrence on artificial surfaces in ports have led to the assumption that this species was introduced to Brazil. This assumption has been supported by ecological studies that suggest it meets eight of the ten criteria proposed [13] to characterize a species as introduced [12]. Nonetheless, some authors have noted that *S. bicolor* is found in cryptic habitats such as underneath rocks and on mangrove roots and shells, and it is therefore possible that it is indeed native to Brazil but had simply been overlooked until recently [10]. In theory, it is also possible that it had a much deeper depth range, like the Caribbean scleractinian *Cladopsammia manuelensis*, originally only known from deep water but recently discovered in shallow reef zones [14,15]. Such uncertainty about the geographic origins of this monotypic genus and the scattered records have led to its classification as a cryptogenic species, i.e., one “that is not demonstrably native or introduced” [16].

Here we present new records of the genus *Stragulum* from the Caribbean Sea and the Persian Gulf, and use both morphological and genomic data to confirm their identity as the species *S. bicolor*. We further discuss the distribution range and ecological implications of this discovery and provide suggestions for further studies to clarify the geographical origins and distribution of this species.

2. Materials and Methods

2.1. Sampling Collection

Specimens were collected using SCUBA in the Persian Gulf (Iran) and Caribbean Sea (Sint Maarten, Dutch Caribbean) (Figure 1). In situ photographs were taken using a compact underwater camera and the depth recorded using a depth gauge. The specimens were preserved in 96% ethanol for morphological and molecular study.

Material from Iran was collected at 11 m depth from sandy gravelly substrate. It was overgrowing a possible gorgonian stem as well as a colony of the bryozoan genus *Conopeum* (P. Taylor pers. comm.; see also [17]) (Figure 2A,B). A fragment of a second specimen was sent to us by H. Mahmoud (University of Kuwait) from Kuwait, in 2012, collected from natural substrate at 8–15 m depth (Figure 2C,D).

Material from Sint Maarten was collected at 1–3 m depth from the hull of a decommissioned tugboat, the 27 m long *MV Marion* (Figure 2E,F). This ship was temporarily anchored at the southern half of Simpson Bay Lagoon, inside the Dutch Caribbean territory, just south of the border with the French, northern part of the island. The boat was salvaged after it grounded almost four years earlier during hurricane Irma (6 September 2017) in another part of the lagoon. Since no comprehensive marine biodiversity surveys have ever been carried out around the island, there is no information on how long the species may have been present. Sint Maarten is well known for its harbours, which provide shelter to large yachts, cruise ships and commercial vessels.
species may have been present. Sint Maarten is well known for its harbours, which provide shelter to large yachts, cruise ships and commercial vessels.

Figure 1. Distribution of *Stragulum bicolor*. Colour shades in the background represent different marine realms. TA = Tropical Atlantic; TNA = Temperate Northern Atlantic; TSAM = Temperate Southern America; TSA = Temperate Southern Africa; WI-P = Western Indo-Pacific; CI-P = Central Indo-Pacific; and TEP = Tropical Eastern Pacific. Green circles = locations of examined materials. Red triangles = literature records. PG = Persian Gulf; CAR = Caribbean Sea. The lines with different thicknesses represent major, medium, and minor worldwide shipping routes (Shipping data modified after [18]).

2.2. Morphological Analyses

In order to identify the specimens, sclerites were obtained by dissolving the tissue in 10% sodium hypochlorite, followed by rinsing in fresh water. For scanning electron microscopy (SEM), the sclerites were carefully rinsed with double-distilled water, dried at room temperature, mounted on a stub with double-sided carbon tape, then coated with gold-palladium (AuPd), and examined using a Jeol 6480LV SEM operated at 10 kV. All specimens are deposited and catalogued at Naturalis Biodiversity Center, Leiden (formerly Rijksmuseum van Natuurlijke Historie, Leiden, The Netherlands).
2.3. Molecular and Phylogenetic Analyses

DNA was extracted from ethanol-preserved tissue using either a modified CTAB protocol [19] or Qiagen DNEasy Blood and Tissue kit following the manufacturer’s recommendations. Three gene regions commonly used as molecular barcodes in octocorals were amplified by polymerase chain reaction (PCR) and Sanger-sequenced using previously published protocols [20]. These included the octocoral-specific mitochondrial mtMutS (primers mut2761F and mut3270R; [21]); COI (primers COII-8068F and COI-OctR; [22,23]); and 28S rDNA (primers 28S-Far and 28S-Rar; [24]). The L-INS-i method in MAFFT [25] was used to
align the sequences to a reference dataset consisting of previously published sequences for a wide range of octocoral taxa including other stoloniferans (e.g., [26]). Pairwise measures of genetic distance (uncorrected p) among sequences were computed using MEGA v.7 [27]. PhyML [28] was used to construct a maximum likelihood tree (GTR + I + G, 100 bootstrap replicates) to explore the phylogenetic relationship of *Stragulum* to other octocorals. A Bayesian analysis was conducted using MrBayes v. 3.2.1 [29] with a GTR + I + G model of evolution, run for \(4 \times 10^6\) generations (until standard deviation of split partitions <0.01) with a 25% burn-in and default Metropolis coupling parameters.

In addition, ultraconserved elements (UCEs) and exonic regions from one specimen each from Brazil (RMNH.COEL.39694) and Iran (RMNH.COEL.42125) were sequenced using a target-enrichment approach [30] with the octocoral baitset of Erickson et al. [31]. Reads were processed using the Phyluce pipeline [32] as described in detail in [30,33]. Pairwise genetic differences between the two *Stragulum* specimens were compared to pairwise differences calculated for: (a) specimens of the octocoral *Alcyonium coralloides* from a single location in France; (b) specimens of *A. coralloides* from different geographic locations; and (c) *A. coralloides* and its two most closely related sister taxa, *A. bocagei* and an undescribed Mediterranean species, *Alcyonium* sp. M2 (all data from [31]). Briefly, for each pairwise comparison, fasta files were generated using the Phyluce commands *phyluce_assembly_match_contigs_to_probes* followed by *phyluce_assembly_get_match_counts* and *phyluce_assembly_get_fastas_from_match_counts* [32]. Paired sequences were aligned using a modification of *phyluce_align_seqcap_align* and *phyluce_align_get_informative_sites* was used to calculate the number of phylogenetically informative differences between sequences.

3. Results

3.1. Morphological and Systematic Account

**Sub-phylum Anthozoa Ehrenberg, 1831**

**Class Octocorallia Haeckel, 1866**

**Order Malacalcyonacea McFadden, van Ofwegen and Quattrini, 2022**

**Family Tubiporidae Ehrenberg, 1828**

*Stragulum* van Ofwegen and Haddad, 2011

**Diagnosis.** Colonies are encrusting, polyps fully retractile into closely set, low calyces, visible as star-shaped openings. Sclerites are crosses, capstans, radiates, branched spindles, and tuberculate spheroids in the upper layer of the coenenchyme; fused sclerites in the basal layer. Calyces with flattened sclerites that can extend into the base of the introvert. Colonies are red with magenta sclerites or white with colourless sclerites. Live colonies are magenta or white with translucent polyps.

*Stragulum bicolor* van Ofwegen and Haddad, 2011

**Figures 2–8**


**Description.** Encrusting colonies. Most polyps are retracted, with calyces up to 1 mm high and 1–2 mm wide; closely set to each other, every 1–2 mm on the surface of the colony (Figure 2).

The upper layer of the coenenchyme and most of the calyces have sclerites in the form of crosses, capstans, radiates, branched spindles, and tuberculate spheroids. Transitional
forms between spindles and crosses also occur. The sizes of the sclerites become smaller towards the interior of the coenenchyme. Polyps do not have any sclerites.

The tops of the calyces have smaller, less tuberculate radiates, capstans, and flattened sclerites up to 0.10 mm long (Figures 3A, 5A and 6A), with simple tubercles. Some of these sclerites branch at one or both ends. These sclerites sometimes can extend into the basal part of the introvert of the polyp. The radiates and capstans are up to 0.05 mm long (Figures 3B, 5B and 7B); crosses and radiates up to 0.15 mm long (Figure 3C, Figure 4B, Figure 5B,C, Figure 6A,B and Figure 7A,B), spindles up to 0.20 mm long with side branches (Figures 3C and 6C), and tuberculate spheroids up to 0.25 mm in diameter (Figure 4A).

The interior layer of the coenenchyme has fused sclerites, forming a network that does not extend into the surface of the coenenchyme (Figures 3D, 5D and 6D).

Sclerites are either all magenta or colourless. After preservation in ethanol, the colonies and the sclerites retain the colour.

**Remarks.** All studied material shared consistent, general morphological features of the sclerites and the colonies. They all have flattened spindles on the distal sides of the calyces. The rest of the upper layer of the coenenchyme includes capstans, some branched spindles, and radiates with differing degrees of tuberculation. The base layer consists of fused sclerites forming a network.

Some variations in sclerite form were observed among the studied specimens. Some radiate sclerites from the Iran material (RMNH.COEL.42125) are highly tuberculate and appear as tuberculate spheroids (Figure 4A). The specimen from Kuwait lacks the tuberculate spheroids (Figure 5) that are observed in the specimen from Iran. This could be simply due to the size of the fragment we received. Since we no longer have access to this material, we only presented the sclerite features. The radiates in the Brazil and Sint Maarten specimens have longer branches and less tuberculation (Figures 6B and 7A).

The colour patterns of the live colonies of the Sint Maarten and the Persian Gulf material are similar, i.e., magenta with translucent polyps (Figure 2) and magenta sclerites. However, the Brazil colonies have two colour patterns, magenta and white. One colony of RMNH.COEL.39666, two colonies of RMNH.COEL.39700, and one colony of RMNH.COEL.39701 are white, with colourless sclerites. There are no published photographs of in situ colonies from Brazil; images presented in the original description of the species [1] (p. 45) were made under stereomicroscope and not in the natural environment. It is worth mentioning that *Canarya canariensis* (Clavulariidae), reported from the East Atlantic, has a very similar colony appearance, but it has different types of sclerites and also does not have any fused sclerites [34].

The specimens from the Persian Gulf, Iran (RMNH.COEL.42125) were overgrowing a possible gorgonian stem and a *Conopeum* bryozoan (P. Taylor pers. comm.; see [17]). The colonies observed in Kuwait were mainly on natural rocks. However, colonies from Brazil and Sint Maarten were growing on man-made substrates and structures.

The calyces have been also called “coenenchymal mounds” for some stoloniferans in the past. Here for consistency, any layer of surface tissue that covers a part of the polyp that protrudes above the surface when retracted whether it contains specialized sclerites or not is called “calyx”. 
Figure 3. *Stragulum bicolor*. RMNH.COEL.42125 from the Persian Gulf: (A) calyx sclerites; (B,C) branched spindles, crosses and radiates of the upper layer; (D) fused sclerites from the basal part of the coenenchyme. Scale at (D), only applies to (D).
Figure 4. *Stragulum bicolor*. RMNH.COEL.42125 from the Persian Gulf. Sclerites of the upper layer of the coenenchyme: (A) tuberculate spheroids from the upper layer of the coenenchyme; (B) branched sclerites, crosses and radiates.
Figure 5. *Stragulum bicolor* from Kuwait (material no longer available): (A) Calyx sclerites; (B,C) branched sclerites, crosses and radiates of the upper layer; (D) fused sclerites from the basal part of the coenenchyme.
Figure 6. *Stragulum bicolor* from Sint Maarten, RMNH.COEL.44550: (A) Calyx sclerites; (B) branched sclerites, crosses and radiates from the upper part of the coenenchyme; (C) branched spindles; (D) fused sclerites from the basal part of the coenenchyme. Scale at (D) only applies to (D).
Figure 7. *Stragulum bicolor* from Sint Maarten, RMNH.COEL.44550: (A) coenenchymal sclerites; (B) branched sclerites, crosses and radiates of the upper layer.
Figure 8. Maximum likelihood phylogeny based on 462 bp of mtMutS showing the identity of all three specimens of *S. bicolor* and the position of the genus *Stragulum* in family Tubiporidae. Numbers at nodes are bootstrap values (% of 100 bootstrap replicates) followed by posterior probabilities (pp) from Bayesian analysis; only nodes with >70% bootstrap support or pp > 0.9 are labelled. ns = not supported.

3.2. **Phylogenetic Analyses**

DNA sequences were obtained from the specimens from Iran (RMNH.COEL.42125), Sint Maarten (RMNH.COEL.44550), and one of the specimens from Brazil (RMNH.COEL.39693). All three specimens had identical sequences for *mtMutS* (426 bp), confirming the identity of all material as *Stragulum*. The Brazil and Sint Maarten specimens also shared identical *COI* sequences (556 bp), but we were unable to amplify COI from the Iranian material for comparison. Specimens from Iran and Sint Maarten shared identical 28S rDNA sequences (790 bp), which differed from the Brazilian specimen by 3 nucleotide substitutions (uncorrected genetic distance, \( p = 0.004 \)). Phylogenetic analysis of the *mtMutS* locus (the marker for which the most taxon-comprehensive set of reference sequences is available [33]) confirmed that *Stragulum* belongs to the family Tubiporidae (Figure 8). This family has recently been recircumscribed to include seven genera of stoloniferous octocorals in which sclerites in the calyx or coenenchyme are inseparably fused, often giving rise to rigid tubes or branches; the fused sclerites of these genera are almost always red [33]. Neither maximum likelihood nor Bayesian analyses of *mtMutS* alone resolved the relationships among genera within Tubiporidae, but the monophyly of the family was strongly supported by both analyses. Previously published analyses of concatenated *mtMutS* + *COI* + 28S sequences suggested that *Stragulum* is sister to the organ-pipe coral genus, *Tubipora* [35].

Pairwise comparison of the assembled UCE and exon loci for the *Stragulum* specimens from Brazil and Iran found a total of 3084 differences at 85468 nucleotide sites for a total of 10.01 phylogenetically informative differences per locus. In comparison, pairwise differences per locus between individuals of *Alcyonium coralloides* (Pallas, 1766) from a single location in France were higher, ranging 16.89–27.33 (\( n = 5 \)) (Table 1). Pairwise differences between individuals of *A. coralloides* from different locations ranged 15.33–27.81 per locus (\( n = 7 \)), while pairwise differences between *A. coralloides* and two closely related species were >48.
Table 1. Number of phylogenetically informative differences per UCE or exon locus in pairwise comparisons of specimens of Stragulum from Iran vs. Brazil. For reference, specimens of Alcyonium coralloides (A. cor.) have been compared to (a) conspecifics collected from the same population (intrapopulation); (b) conspecifics collected from a different geographic location (intraspecific); and (c) closely related sister taxa (interspecific; A. boc. = A. bocagei; A. sp. M2 = Alcyonium sp. M2). Data for Alcyonium taken from [31].

<table>
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<tr>
<th>Sample 1</th>
<th>Sample 2</th>
<th>Comparison</th>
<th>Total Sites</th>
<th>Total Differences</th>
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4. Discussions and Conclusions

The material collected from the Persian Gulf in the northwest Indian Ocean and Sint Maarten in the eastern Caribbean belong to the genus Stragulum, originally described from Brazil in 2011. Based on morphological and molecular evidence, this is the first report of the presence of this genus and the species S. bicolor in the Indo-Pacific and the Caribbean regions. Although there are some morphological differences among studied colonies from the different geographic regions, the molecular data suggest that the specimens from Iran and Sint Maarten both belong to S. bicolor. All specimens shared the same mitochondrial haplotypes at the mtMutS and COI loci, and the 0.4% sequence difference observed between S. bicolor from Brazil and the other material at 28S rDNA falls within the range of intraspecific variation often observed at that locus [36]. In addition, the number of phylogenetically informative differences observed between specimens from Brazil and Iran at the UCE and exon loci was less than that seen between individuals of A. coralloides from a single population in France. Together, the genetic evidence suggests that all Stragulum specimens belong to the same species. The original description of S. bicolor provided images of sclerites for only the holotype individual from Brazil, and only a single individual was available to us from both the Iran and Sint Maarten populations. Without data from additional specimens from those locations it is impossible to determine if the morphological differences we observed among specimens represent a typical range of intraspecific variation in this species.

The number of records of Stragulum from the Atlantic may not reflect its true distribution. The identity of the genus may have been confused with that of some other genera such as Erythropodium, another encrusting species with magenta sclerites. Altvater et al. [10] suggest that specimens of Erythropodium caribaeorum (Duchassaing and Michelotti, 1860) recorded recently from Brazil actually belong to S. bicolor. However, Carpinelli et al. [3] confirm the occurrence of Erythropodium caribaeorum (Duchassaing and Michelotti 1860) in southeast Brazil. It is worth mentioning that there are some discrepancies between the species’ images and descriptions provided in Altvater et al. [10] compared with ours. They reported colourless sclerites in the inner layer of the coenenchyme and red sclerites in the upper layer, small scales 0.08–0.12 mm in calyces, and tentacles with diminutive spindles. These characters do not match the S. bicolor material studied by us.
The discovery of *S. bicolor* in the Persian Gulf raises new questions and possibilities about the disjunct distribution of the genus *Stragulum* in the Atlantic and the Indian Ocean. Because the present morphological information and geographic records of the genus are still not adequate to determine and explain its distribution range and native origin, it remains cryptogenic. However, there are two possible scenarios: (1) the species has a wide, natural distribution but because of its rarity it has not been recorded in the Indo-Pacific until now; (2) the species has been transported as a fouling organism on ship hulls or oil platforms between the Atlantic and the Persian Gulf. The species grows on rocky, natural substrate in the Persian Gulf and as a fouling organism on all kinds of artificial and natural substrates in the Atlantic, such as ship hulls in Sint Maarten (Figure 2E,F) and polyethylene settling plates, piers and marinas, barnacle shells, and natural rock in Brazil [1,11,37,38]. The ability to settle on artificial substrates makes this species an ideal candidate for the use of ship hulls as a vector in range expansion and eventually for becoming invasive.

Transport through ballast water or on ship hulls, or via aquaculture, fisheries, and the aquarium trade are the primary vectors of marine invasions [39]. It has been estimated that over seven thousand species might be moving around in ballast water tanks in ships on the world’s oceans, a number that far exceeds background rates of natural invasions [40]. However, this is not a surprise, as not all translocated species become invasive. Instead, the establishment of the invader depends on the survival of the transported organism to form reproducing populations, influenced by the characteristics of the invader and the receiving ecosystem [39,41].

Semi-enclosed, shallow seas under human influence, such as those under discussion here, are particularly susceptible to biological invasions [42]. The health of the Persian Gulf ecosystem has declined rapidly in recent years due to human activities and climate change [42], to the point that there may be no natural “marine baseline” in the Gulf [43]. Although the Gulf is expected to be susceptible to the introduction of non-native marine species, so far only sixteen non-native sessile marine invertebrates have been reported from there, fifteen of which may have been introduced by hull fouling or other biofouling [44]. However, no invasive octocoral has been reported from Persian Gulf waters so far [45–47].

Brazil, with its open West Atlantic coastline, has a much greater reputation for harbouring non-indigenous marine species, with over sixty-five introduced benthic marine invertebrates having been recorded, especially in states with the densest maritime traffic [48]. There have been no comprehensive overviews of sessile marine invasives in the Caribbean, although there are detailed records of *Tubastraea* corals [49] and some reports of cryptogenic species [14,50]. One other possibly invasive octocoral species has been reported from Brazilian waters that may have been introduced by ship traffic, *Chromonephthea braziliensis*, first found in Rio de Janeiro in 1997 [8]. Most of the octocorals recently introduced to Brazil have instead been imported through the international aquarium trade [5]. Since *Stragulum* with its encrusting shape and small, translucent polyps is not conspicuous, it is probably not of interest for the aquarium industry. However, small, inconspicuous octocorals may still end up in the aquarium trade accidentally, imported on live rock.

Models of marine biological invasions suggest a growing shipping industry could raise the risk of invasions dramatically, and will have a far greater effect on the spread of non-native species than the environmental effects of climate change [51]. Vessel biofouling is a widespread vector of marine dispersal linked to biological invasions in coastal areas around the world [52–54]). Consequently, the rate of marine species introductions to non-native areas has increased dramatically with the recent growth of maritime traffic [54–56]. There is great potential for the transport of marine species between the Persian Gulf region and the Atlantic due to a high volume of marine traffic. Interestingly, all the localities of the specimens recorded in this study match closely with major worldwide shipping routes (Figure 1).

In summary, our molecular data suggest that all the material we examined belongs to *Stragulum bicolor*, although there was some morphological variation among specimens. The number of specimens studied, and the recorded geographical occurrence is too low to
confidently establish the natural range of this species. Additional material and observations are required from all known localities to study the genetic variation within and among populations in order to identify the source population, assuming that more genetic variation occurs in the native population than in those that have been introduced. We hope that the morphological and molecular data presented in this study will lead to further reports and observations of this potentially invasive species worldwide.


**Funding:** The research and partial fieldwork supported by the Martin-Fellowship programme (Naturalis Biodiversity Center), Alfred P Sloan Foundation, Richard Lounsbery Foundation, and Census of Marine Life are gratefully acknowledged for the research grant provided to the first author. Molecular work was supported by NSF DEB-1457817 to C. McFadden.

**Institutional Review Board Statement:** Not applicable.

**Data Availability Statement:** Not applicable.

**Acknowledgments:** J.H. Ausubel (Rockefeller University), M.R. Claereboudt (Sultan Qaboos University, Oman), N. D’Adamo (UNESCO, IOC, Perth), and L. Brown (Lounsbery Foundation) are greatly appreciated for their continued support and encouragement. We thank the staff of Nature Foundation Sint Maarten for the material from Sint Maarten. J. Goud and A.N. van der Bijl are appreciated for curatorial support, K. Erickson for laboratory support and A. Quattrini for assistance with data analysis. Four anonymous reviewers are appreciated for their constructive comments and suggestions, which helped improve the manuscript.

**Conflicts of Interest:** The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

**GenBank Accessions for Stragulum bicolor:** mtMutS: MW473941, OP157219, OP157220; COI: MW473942, OP137266285 rDNA: MW473676, OP137256, OP137257 UCEs-exons: SAMN27177439, SRR20862043

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