ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

Phylogenetic variation of eleven soft corals (Anthozoa, Octocorallia) in Nha Trang bay of Vietnam

Le Quynh Lien[1*], Tran My Linh[1], Vu Huong Giang[1], Nguyen Chi Mai[2], Phan Minh Tuan[2], Le Quang Trung[2], Ninh Khac Ban[1], Chau Van Minh[1], Tatyana N. Dautova[3]

1. Institute of Marine Biochemistry, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet Rd. , Cau Giay, Ha Noi, Viet Nam
2. High Technology Development Centre, Vietnam Academy of Science and Technology (VAST), 18 Hoang Quoc Viet Rd. , Cau Giay, Ha Noi, Viet Nam
3. A.V. Zhirmunsky Institute of Marine Biology, Far East Branch, Russian Academy of Sciences

Manuscript Info

Manuscript History:

Received: 15 June 2015
Final Accepted: 16 July 2015
Published Online: August 2015

Key words:

genetic diversity, irg-cox1, molecular markers, msh1, Nha Trang bay, soft corals

Corresponding Author

Le Quynh Lien

Abstract

Coral reef as well as soft corals (Cnidaria: Octocorallia) play an important role in marine environment. Most soft corals belong to order Alcyonacea with three major genera Sinularia, Lobophytum and Sarcophyton. In Vietnam, the Alcyoniidae is the most diverse with 124 species. Up to date, identification of soft coral to species level mostly based on morphological characters. Molecular approaches on determination of soft corals based on partial or complete 18S, partial 28S or partial 16S of ribosomal sequences have been applied recently. Discovery of a unique protein-coding gene, msh1, which is homolog of the bacterial mismatch repair gene mut-S provides an efficient tool for molecular phylogenetic studies of soft corals. msh1 has been found in all octocoral families, with approximately as twice variation as most other protein-coding regions in the octocoral mitochondrial genome. In addition, mitochondrial gene cytochrome c oxidase subunit 1 (cox1) and intergenic regions (IGRs) had been used in several soft coral phylogenetic studies. The combination of cox1, igr1 and msh1 gene markers obviously were more effective than single one. In this study, we investigated for the first time in Vietnam the genetic diversity of soft corals in Nha Trang bay based on 696bp of msh1 gene and 866bp of irg-cox1 fragments. The result indicated that eleven studied specimens were grouped into four different clades including Sinularia, Sarcophyton, Lobophytum and a mixed with Sarcophyton and Lobophytum genera. Both msh1 and irg-cox1 characterized some specific substitutions in each clade to distinguish them from each other. Initial results of this study should facilitate conservation of the soft corals in Vietnam.

INTRODUCTION

Soft corals (Cnidaria: Octocorallia) are important structural components of coral reef communities and contributors to coral reef biomass. Most soft corals belong to the order Alcyonacea, which is comprised of the families Xeniidae, Nephtheidae, and Alcyoniidae. Alcyonacean soft corals are abundant and ecologically important members of coral

reef communities throughout the Indo-West Pacific, often equaling or exceeding scleractinian corals in percent cover of available primary space (Tursch & Tursch, 1982; Dinesen, 1983; Dai, 1988; Riegl et al., 1995; Fabricius, 1997). In term of species diversity of alcyonacean soft corals, species belonging to the alcyoniid genera Sarcophyton LESSON 1834, Lobophytum VON MARENZELLER 1886, and Sinularia MAY 1898 are particularly common and conspicuous in shallow nearshore reef flat habitats where they often form large mono specific aggregations (Benayahu & Loya 1977; Benayahu 1995; Fabricius 1998; McFadden et al., 2006a).

Despite the abundance and importance, only few ecological studies of soft corals have been done (Fabricius, 1995, 1997, 1998; Fabricius & Dommisse, 2000; Bastidas et al., 2004; Aratake et al., 2012), partly due to the difficulties in correctly identifying species in the field. Colony morphology is highly variable (Benayahu, 1998), and species classification usually be distinguished by microscopic examination of the CaCO3 sclerites found within the coenenchymal tissue. In a series of taxonomic revisions Verseveldt (1980, 1982, 1983) indicated that many species of all three soft coral genera remain described. Recently, DNA molecular marker techniques have been providing more evident to solve such problems. Partial or entire several gene sequences from mtDNA were employed and considered as DNA barcodings for several soft coral species, such as the Folmer region of cytochrome oxidase I gene, coxI; a fragment of the octocoral-specific mitochondrial protein-coding gene, msh1; and an extended barcode of msh1 plus cox with a short adjacent intergenic region (igr1). One of above markers, msh1, a homolog to the bacterial DNA repair protein MutS that is found unique in the octocoral mitochondrial genome (Pont-Kingdon et al., 1995, 1998; France & Hoover, 2001), is sufficiently variable to discriminate at species level in some octocoral genera. McFadden et al. (2006a; 2009) examined the phylogenetic and taxonomic relationships among species of Sarcophyton and Lobophytum genera based on the polymorphism of partial msh1 gene and reveal that 103 octocoral genera of the Indo-Pacific Sinularian belonged to 28 families and supported Bayer's three order system in division octocorals. The other analysis of msh1 also indicated the phylogenetic relationship between two tropical soft coral genera Sarcophyton and Lobophytum (McFadden et al., 2006b), in which 92 specimens were of 19 species of Lobophytum and 16 species of Sarcophyton. In addition, the variation in sequences of cox1 gene was reconfirmed at species level of Sinularia genus (McFadden et al., 2013). Although, most mitochondrial genes evolve very slowly in octocorals and genetic variation within genera is not always available, the cox1, igr1and msh1 genes together have provide an addition value to octocoral taxonomy (McFadden et al., 2001).

Nha Trang bay of South-central Vietnam has internationally important coral reefs with the highest coral biodiversity recorded in Vietnam. According to the estimation of Ben and Dautova (2010), more than 200 soft coral species belonging to 45 genera and 14 families were observed in the region, in which Sinularia genus is the most diverse followed by Lobophytum and Sarcophyton. However, there are, numerous species of these three genera difficult to identify if based on morphological characteristics and field observation. The reason is that the distinctions between Sarcophyton and Lobophytum less obvious possibly due to occurrence of hybridization between members of these genera in aquaria (McFadden et al., 2010). Although, molecular methods have been applied to solve this problem, however, thay have not yet employed to clarify genetic relationships of soft corals in Vietnam. Therefore, in this study, genetic diversity of soft corals around Nha Trang Island of Vietnam is investigated based on msh1 gene and cox1 gene plus irg1. Objectives of the research included: (1) to delineate phylogenetic variation of 11 soft coral collected around the island based on DNA polymorphism of their 866bp of irg1 region and cox1, and 696bp fragment of msh1; (2) to characterize the phylogenetic lineages by comparative alignment of the two mitochondrial fragments between 11 specimens and relevant known soft coral taxa from GenBank; and (3) to consider congruence between current morphological analysis and molecular employment in identification of soft coral specimens. Molecular approach in this study could be applied for research on genetic diversity of soft coral in other regions so as to fulfill a picture of their biodiversity in Vietnam, which is essential for conservation and sustainable use of soft corals latterly.

MATERIALS AND METHODS

Materials

Eleven soft coral specimens were collected at different sites in Nha Trang bay, Khanh Hoa province, Vietnam in May, 2013 using SCUBA diving equipment (Figure 1). Fresh specimens were stored in 70% ethanol for morphological analysis and -20^{o}C for DNA extraction. All specimens were deposited at Department of Biological resources, Institute for Marine Biochemistry (Vietnam Academy of Science and Technology) and A.V. Zhirmunsky Institute of Marine Biology (IMB) FEB RAS, Vladivostok, Russia. Specimens were morphologically identified as described by Dautova et al (2010).

Methods

DNA extraction

Genomic DNA of each specimen was isolated using DNeasy® Tissue Kit (Qiagen) according to the manufacturer's instruction. The concentration and purify of DNA was analyzed by electrophoresis in 0.8% agarose gel.

PCR and DNA sequencing

PCR amplifications was carried out using genomic DNA of 11 soft coral specimens as templates and the 2 primer pairs, including COIF: 5'-CAGCCGCGTC ACGTAGGAGC GAG-3' and COIR: 5- GGTATAATTT GAGATACCAT AC-3' to amplify irg1+COI region, including 112bp of the entire irg1 region and 782bp of partial COI gene, and msh1F: 5'- CCTATGCAAT ATTTCAACTT AGC-3' and msh1R: CATAACTTCA ATTTTAGCAT TGG-3'. Both PCR components were 5 µl 10X PCR Buffer, 10 mM dNTPs, 2 mM primers, 50 ng of genomic DNA, 1 unit of Taq Polymerase and 2 mM of MgCl2 and H20 up to total 50 µl. The reaction mixtures were heated to 94°C for 5 min, followed by 30 cycles of 1 min at 94°C, 30 s at an annealing temperature of52°C for both primer pairs, DNA synthesis for 1 min at 72°C, and then a final extension of 10 min at 72°C. Size of amplicons was verified by 1.5% agarose gel electrophoresis in TAE buffer using known standards. PCR products were purified using QIAquick® PCR purification kit (Qiagen), cloned in TA® cloning vector and sequenced byMacrogen Inc. (Korea). Nucleotide sequences of 11 soft coral specimens, whose names and sites of collection shown in Figure 1, were registered to GenBank accession numbers from KP057893 to KP057903 for msh1 fragments and from KP057904 to KP057914 for irg-cox 1 fragments.

Phylogenetic reconstruction

Related nucleotide sequences to irg-cox1 and msh1 fragments of studied samples were blasted using http://www.ncbi.nlm.nih.gov/BLAST to obtain the reference sequences, which were selected separately because the availability of each targeted fragment in GenBank. Phylogenetic tree of the aligned DNA sequences for each fragment set was separately reconstructed by Neighbor Joining (NJ) method with Kimura 2-parameter model of 1000 replicates using Mega3.1 (Kumar et al., 2004). Phylogenetic variation was estimated with bootstrap values, which indicated confidence interval between phylogenetic lineages of the studied samples on the tree.

Sequence analysis

Gene fragment sequences of taxa from two sets (one from irg-cox1and the other from msh1 sequences) of phylogenetic lineages, which included those of the studied samples and referred taxa clustered in the NJ trees, were aligned using Clustal W and Sequence Data Explorer in MEGA3.1 (Kumar et al., 2004). Specific nucleotide substitutions when comparatively aligning the sequences among lineages were recorded to interpret phylogenetic variation of the studied sponges. Congruence between morphological analysis and molecular approach in identification of the 11 soft corals was considered on the basis of homology level resulted from aligning sequences of the studied sponge and closest referred taxon clustered in each lineage using DNAMAN4.15.

RESULTS AND DISCUSSION

Molecular phylogenetic analysis

Based on the available length of known sequences from Genbank, 866bp of irg-cox1 fragments including 112bp of the entire irg1 and 754bp of cox1 and 696bp of msh1 fragments were analyzed. Neighbor-joining trees based on polymorphism of both fragments showed high phylogenetic variation of 11 studied soft corals. All sequences were reconstructed into 4 phylogenetic clades for 3 different genera in family Alcyoniidae, Order Alcyonacea, Class Octocorallia (Figure 2; Figure 3).

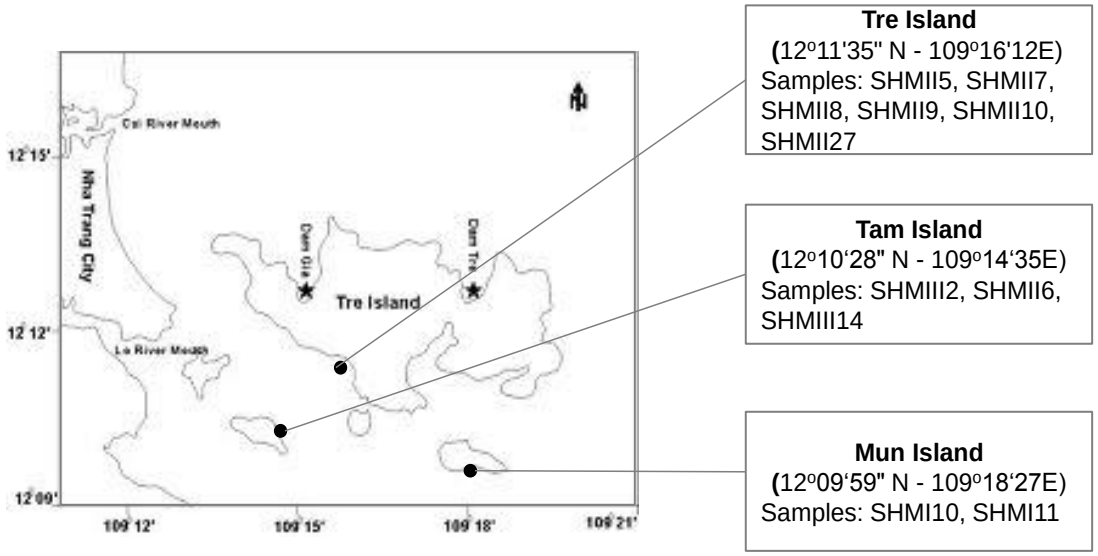

Figure 1: Collect sites of 11 soft coral specimens in Nha Trang bay, Vietnam. Specimens SHMII5, SHMII7, SHMII8, SHMII9, SHMII10 and SHMII27 were collected near Tre island (12°11'35" N - 109°16'12E); Specimens SHMI10 and SHMI11 were collected near Mun island (12°09'59" N - 109°18'27E); Specimens SGMIII2, SHMIII6 and SHMIII14 were collected near Tam island (12°10'28" N - 109°14'35E)

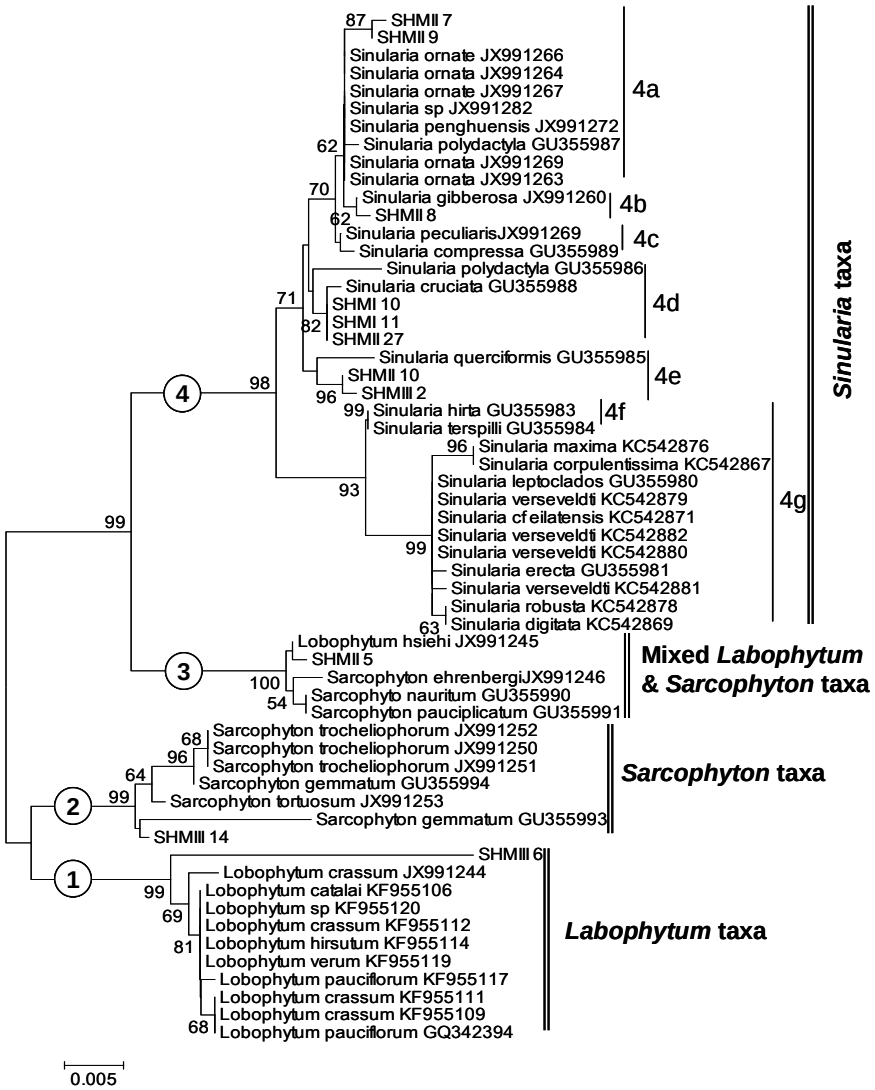

Figure 2: Phylogenetic Neighbor Joining tree reconstructed using 866bp-*irg1* and COI fragments on mtDNA of soft corals. Numbers in open circles: clade number corresponding to taxa in particular genus on the right of the tree. 4a-4b, sub-clade of Sinularia taxa in clade 4. SHMIII_6 to SHMII_7, names of specimens in this study. Numbers after referred taxa: accession no. from the GeneBank. Only bootstrap values more than 50 were represented next to the nodes of the tree

The trees of both fragments showed that clade 1 included species in Lobophytum genus while all Sarcophyton species were grouped in clade 2; clade 3 was a mixture of species in Lobophytum and Sarcophyton genera and clade 4 was joined by species in Sinularia genus. These four clades were genetically separated with high confidence intervals from 98-100% with irg-cox1 fragments (Figure 2) and 99-100% with msh1 fragments (Figure 3). Of 11 current soft corals, SHMIII6 was grouped with Lobophytum crassum together to other Lobophytum species in clade 1. In clade 2 of taxa in Sarcophyton genus, there existed SHMIII14 specimen. Being different from the other 10 studied specimens, SHMII5 was positioned in clade 3 together with a mixture of known taxa in both Lobophytum and Sarcophyton genera. Current specimens and referred taxa of Sinularia genus indicated the most phylogenetic divergence in clade 4, in which 8 out of 11 studied soft corals clustered into 4-7 different sub-clades of 4a-4gwith significant genetic distance of bootstrap values from 62-96% (Figure 2) and 79-99% (Figure 3) when based on DNA polymorphism of irg-cox1 and msh1 fragments, respectively. In these clusters, SHMII7 and SHMII9 was closely to Sinularia ornate in sub-clade 4a and SHMII8 to S. gibberosa in 4b, while SHMI10, SHMI11 and SHMII27 in sub-clade 4d, and SHMII10 and SHMIII2 in sub-clade 4e were cladded with different Sinularia taxa. As a result, DNA polymorphism of irg-cox1 and msh1 gene fragments of the examined soft corals revealed almost similar phylogenetic patterns of 4 clades, except that clade 1, 2 and 3 were rooted with clade 4 when employing the msh1 fragments (Figure 2) whereas clade 3 was clustered close to clade 4 when recruiting the irg-cox1 sequences (Figure 3).

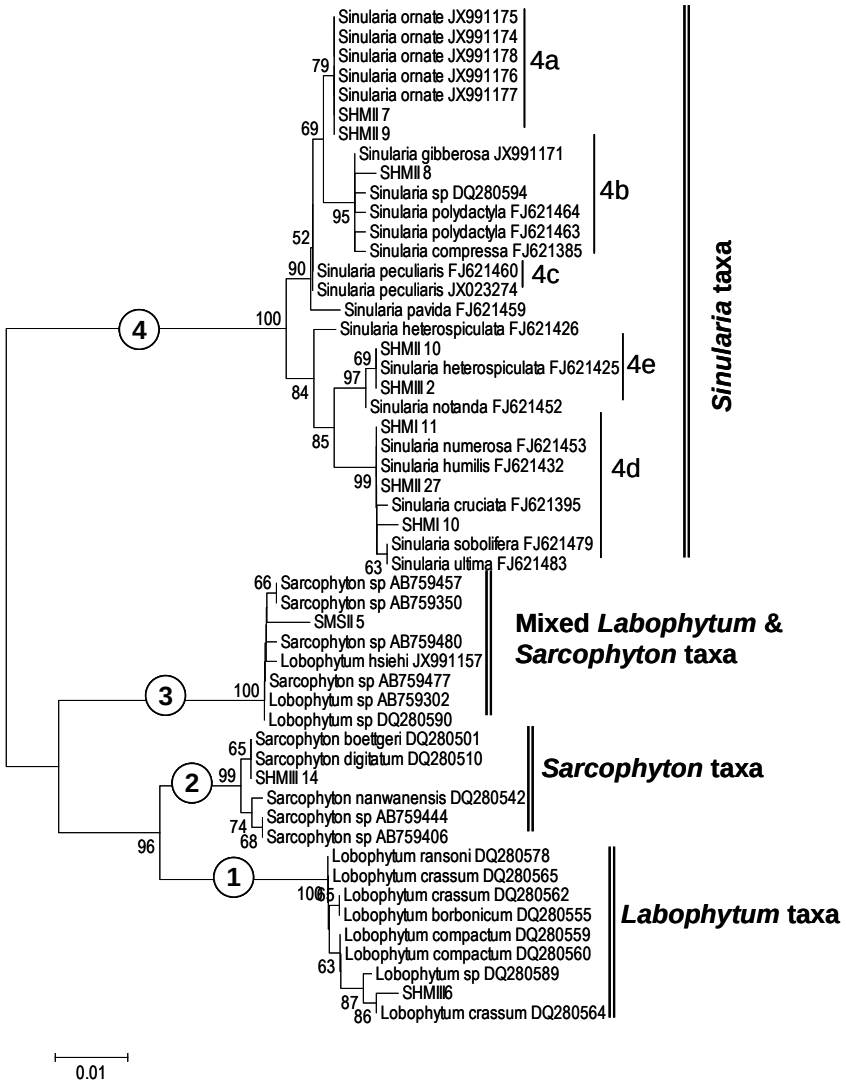

Figure 3: Phylogenetic Neighbor Joining tree reconstructed using 696bp –*msh*1 fragments on mtDNA of soft corals. Numbers in open circles from 1 to 4, clade number corresponding to taxa in particular genus on the right of the tree. 4a-4b, sub-clade of Sinularia taxa in clade 4. SHMIII6 to SHMII7, names of specimens in this study. Numbers after referred taxa: accession no. from the GeneBank. Only bootstrap values more than 50 were represented next to the nodes of the tree

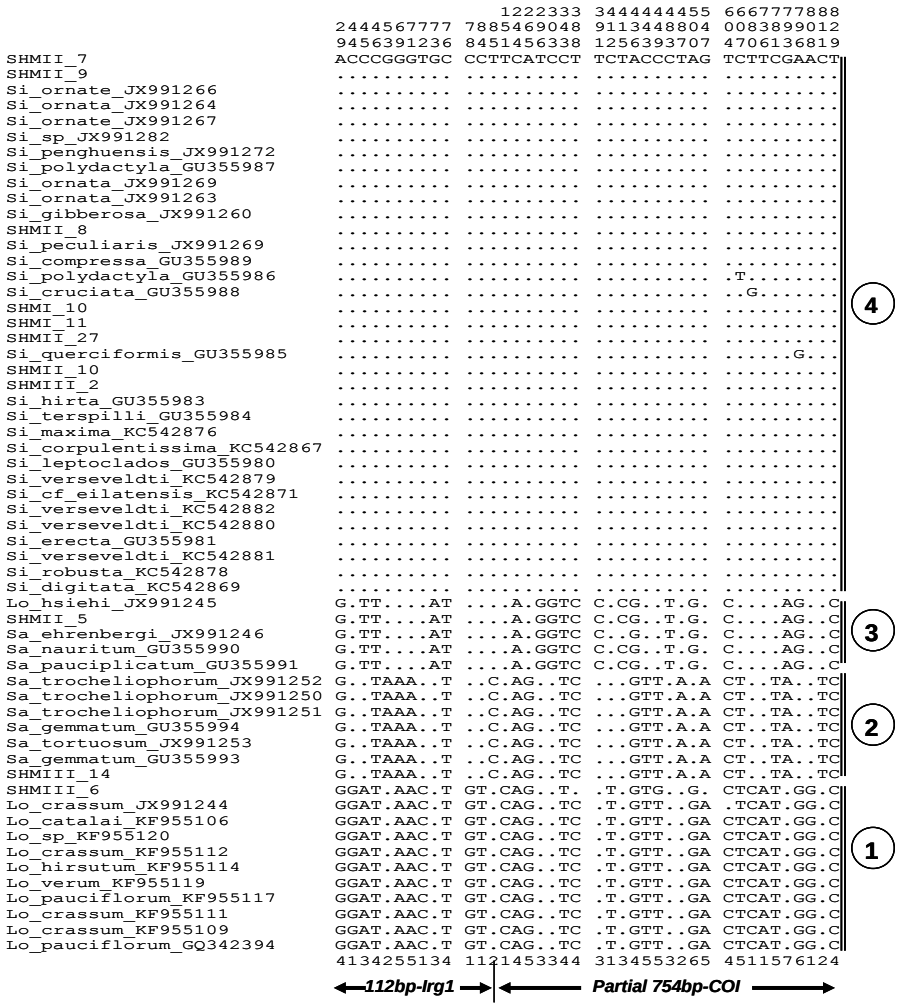

Figure 4: Alignment of *irg*1 and COI fragments of taxa between four clades in Figure 2. Abbreviated genera in the first column: Si: Sinularia; Sa: Sarcophyton; Lo: *Lobophytum*. Dotted sign (-): same as nucleotide in column in the first row. Number in vertical in 3 first rows: site number of nucleotide along 866bp of irg1 region and *cox1*. Numbers in last row: 1 typical for clade1 (C1), 2 for Clade2 (C2), 3 for clade3 (C3), 4 for C1, C2 and C3, 5 for C1 and C2, 6 for C1 and C3, 7 for C2 and C3

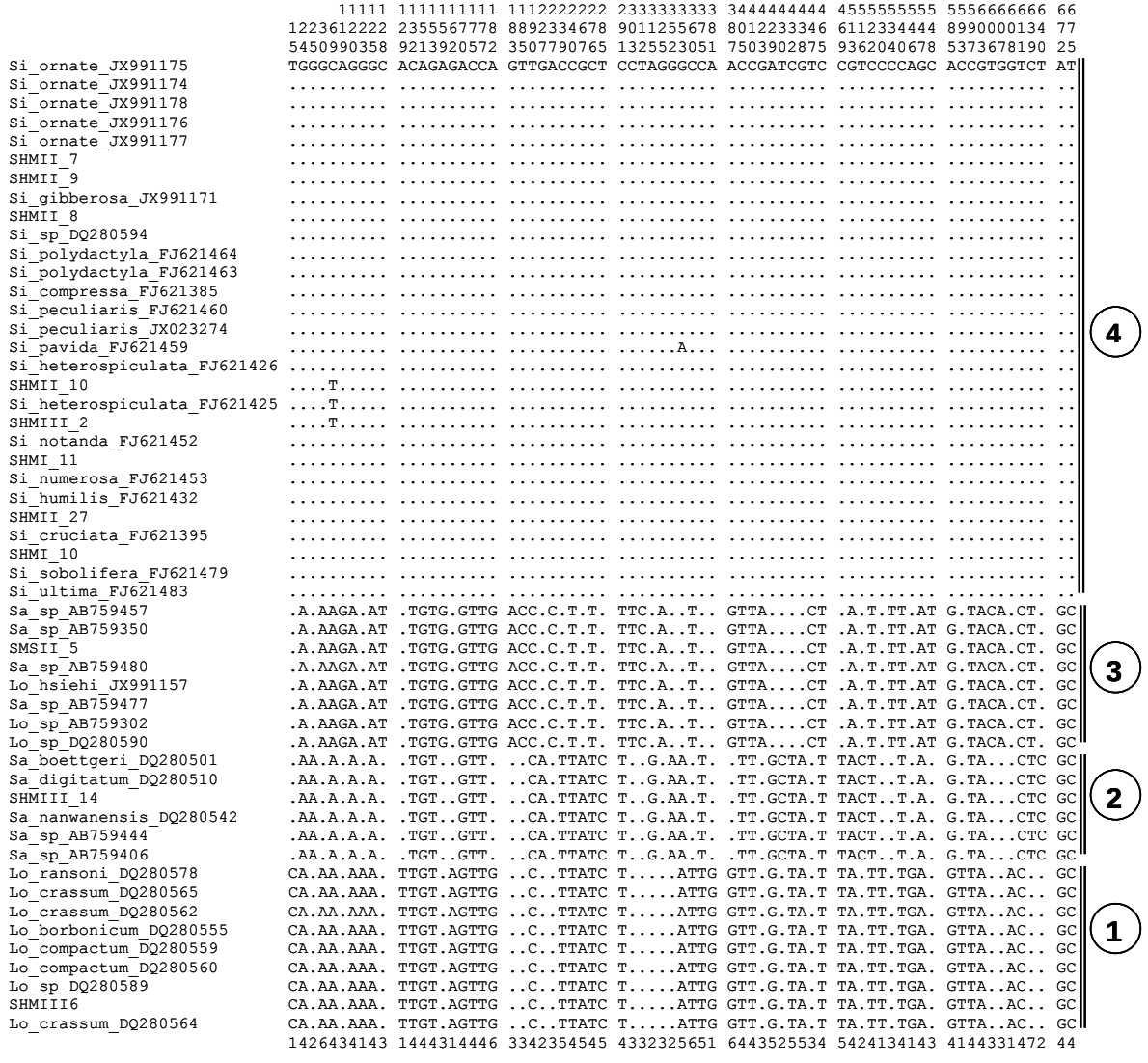

Figure 5: Alignment ofmsh1 sequences of taxa between 4 clades in Figure 3. Abbreviated genera in the first column: Si: Sinularia; Sa: Sarcophyton; Lo: *Lobophytum*. Dotted sign (-): same as nucleotide in column in the first row. Number in vertical in 3 first rows: site number of nucleotide along 696bp fragment of *msh1* Numbers in last row: 1 typical for clade1 (C1), 2 for Clade2 (C2), 3 for clade3 (C3), 4 for C1, C2 and C3, 5 for C1 and C2, 6 for C1 and C3, 7 for C2 and C3

In the study of McFadden et al (2006b) the phylogenetic tree within Sarcophyton and Lobophytum species were clearly divided into three clades. One clade included only morphologically typical Sarcophyton species with a stalk distinct from the polypary, poorly formed slub-shaped sclerites in the colony surface, and large spindles in the interior of the stalk. A second clade included only morphologically typical Lobophytum colonies with lobes and ridges on the colony surface, poorly formed clubs in the colony surface, and interior sclerites consisting of oval forms with regular grids of ornamental warts. The third clade included a mixture of Sarcophyton and Lobophytum nominal species with intermediate morphologies. Similar result was also obtained in our study. In both phylogenetic tree reconstructed using msh1 or irg-cox1 fragments, the mixture clade of Sarcophyton and Lobophytum was still observed (Figure 2 and Figure 3). Additionally, msh1 or irg-cox1 alone in several cases is not enough to classify some Sarcophyton or Lobophytum species.

Polymorphism of msh1 sequences was also employed to investigate diversity of Sinularia genus[18] and Sarcophyton genus[1] and to distinguish Lobophytum and Sarcophyton species (McFadden et al., 2006b). In addition, polymorphism level ofmitochondrial cytochrome c oxidase subunit 1 gene (cox1) and intergenic regions (IGRs) were also successful to elucidate phylogenetic relationships between 77 specimens of bamboo corals (Octocorallia: Isididae) (van der Ham et al., 2009). It is the fact that two or more markers were used together could make

interpretation of genetic relationships between taxa more convincible. A combined barcode consisting of cox1, igr1 and msh1 was a more effective barcode than either cox1 or msh1 alone (McFadden et al., 2010). In our study, two molecular markers from irg1-cox1 and msh1 fragments exhibited powerful in phylogenetic analysis of soft coral specimens collected in Nha Trang bay of Vietnam. Their sequence polymorphism both cladded 11 studied specimens and referred taxa into four different groups of taxa with high confidence intervals from 98-100%, of which Sinularia genus was indicated the most diverse (Figure 2 and Figure 3).

Sequence analysis of irg-cox1 and msh1 gene fragments

Comparative analyses by alignment of irg-cox1 (866bp) and msh1 (696bp) sequences of 11 studied samples and other known referred taxa resulted in specific substitutions of particular lineage, which provided molecular characteristics of these 2 gene fragments for interpretation of phylogenetic confidence intervals between 4 lineages as exposed in Figure 2 and Figure 3. Four lineages of all studied alcyonacean soft corals were clearly separated with 40 substitutions when aligned their 866bp of irg-cox1 sequences (Figure 4), interpreting their genetic distance with 98-100% of bootstrap values (Figure 2). Irg-cox1 character sets of the soft coral taxa in lineage 1, 2, 3 and 4 were characterized with respectively 9, 4, 7 and 9 specific substitutions to distinguish from each other (Figure 4). Similarly, these four lineages of soft coral taxa were also separated with the same topology (Figure 3) with 72 particular different sites, of which 9, 7, 15 and 27 different sites on their msh1 fragments were particular to lineage 1, 2, 3 and 4, respectively (Figure 5). Characterization of amino acid residues of the 2 fragments resulted in 5 and 25 specific substitutions on irg-cox1 and msh1 fragments respectively to distinguish the 4 lineages of studied samples (Table 1), indicating the latter was more polymorphic than the former. Together, both studied specimens and referred taxa were cladded into 4 lineages with high confidence intervals representing by 112 nucleotide substitutions on their irg-cox1 and msh1 fragments. In addition, clade 1 and 2, clade 1 and 3 and clade 2 and 3 respectively shared the same substitutions 17, 6 and 2 (Figure 4 and 5 and Table 1), indicating their close phylogenetic relationships (Figure 2 and 3).

Table 1: Specific nucleotide (N.S.) and amino acid (A.S.) substitutions on irg1 and COI (866bp) and msh1 (696bp) fragments among 4 lineages of Alcyonacean soft corals as showed in Figure 4 and Figure 5.

Clade	irg-*cox1*		*msh1*		Total	
	No. of N.S.	No. of A.S.	No. of N.S.	No. of A.S.	No. of N.S.	No. of A.S.
1	9	0	9	5	18	5
2	4	0	7	4	11	4
3	7	4	15	4	22	8
4	9	0	27	9	36	9
1+2	8	0	9	2	17	2
1+3	2	1	4	1	6	2
2+3	1	0	1	0	2	0
Total	40	5	72	25	112	30

Table 2: Congruence between morphological analysis and molecular approach in determination of 11specimens in this study

Samples	Molecular taxonomy	Morphological taxonomy	Homology analysis			
			irg1-*cox1*	References	*msh1*	References
SHMII7, SHMII9	*Sinularia ornate*	*Sinularia ornata*	99.8%	JX991263, JX991264, JX991266, JX991267	100%	JX991174 to JX991178
SHMII8	*Sinularia gibberosa*	*Sinularia gibberosa*	99.9%	JX991260	99.7%	JX991171
SHMI10 SHMI11 SHMII27	*Sinularia cruciate*	*Sinularia cruciata*	99.9%	GU355988	99.6 – 99.9%	FJ621395
SHMII10	*Sinularia*	*Sinularia*	NA	NA	100%	FJ621425

SHMIII2	*heterospiculata*	*heterospiculata*				
SHMII5	*Lobophytum hsiehi*	*Lobophytum hsiehi*	99.9%	JX991245	NA	NA
SHMIII6	*Lobophytum crassum*	*Lobophytum crassum*	97.1%	JX991244	99.7%	DQ280564
SHMIII14	*Sarcophyton digitatum/S. boettgeri*	*Sarcophyton digitatum*	NA	NA	100.0%	DQ280510 DQ280501

Species determination

Nucleotide analysis based on msh1 and irg-cox1 provide alternative strategy for classification 11 specimens collected in Nha Trang bay, Vietnam. msh1 and irg-cox1 sequences of two specimens including SHMII7 and SHMII9 were 100% and 99.8% identical to the same sequences of Sinularia ornata (Table 2), suggesting three specimens are the same species. SHMII10 and SHMIII2 were identified as Sinularia heterospiculata with 100% homology in their msh1 sequences (irg-cox1 of this species in not available in Genbank). SHMII8 could be classified as Sinularia gibberosa since their irg-cox1 and msh1 sequences were 99.9% and 99.7% identical, respectively. The similarities between Sinularia cruciata and three specimens (SMHI10, SHMI11 and SHMII27) were 99% comparing their irg-cox1 sequences and 99.6%-99.9% comparing their msh1 sequences, implying they are one species. Although the absence of information about msh1 sequences in Genbank, SHMII5 was inferred to be Lobophytum hsiehi because of the 99.9% similarities in their irg-cox1. SHMIII6 could be identified in Lobophytum genus with 99.7% and 97.1% homology to msh1 and irg-cox1 sequences of Lobophytum crassum. The last samples SMHIII14 was in question when based on these molecular approaches. msh1 sequences of this specimen were similar to two different species in Sarcophyton genus including Sarcophyton digitatum and Sarcophyton boettgeri. Because irg-cox1 sequences of this species were not available in Genbank, SMHIII14 specimen was only concluded to be either Sarcophyton digitatum or Sarcophyton boettgeri.

In parallel, morphological analysis indicated that eight specimens belong to Sinularia genus, two are in Lobophytum genus and the last one is Sarcophyton genus (Table 2). Among genus Sinularia, SHMII7 and SHMII9 are S. ornata; SHMII8 is S. gibberosa; three others (SHMI10, SHMI11 and SHMII27) are S. cruciata and SHMII10 and SHMIII2 are S. heterospiculata. Specimens SHMII5 and SHMIII6 are Lobophytum hsiehi and Lobophytum crassum, respectively while SHMIII14 is Sarcophyton digitatum. In comparison, there was congruence between morphometric and molecular studies. Molecular markers can be an alternative strategy for species determination.

Table 2:

Nha Trang bay of the central-south Vietnam posse a range of coastal and marine habitat types, especially coral reefs and soft corals. In 1970, the significant studies for soft coral in Vietnam revealed 94 soft coral species found in Nha Trang bay (Tixier-Durivault, 1970). A latter survey indicated that soft coral in Nha Trang bay encompass of 76 species, belonging to 9 families and 20 genera, of which the Sinularia genus was the most diverse followed by Lobophytum and Sarcophyton (Dautova and Savinkin, 2009). Similarly, 11 studied specimens collected in Mun, Tre and Tam islands (Figure 1) belonged to three genera in the Alcyonacea family. Eight specimens were identified as Sinularia species, two were Lobophytum species and one was Sarcophyton species. Sequence polymorphism of the 866bp of irg-cox1 fragments and the 696bp of msh1 fragments separated 11 specimens into 4 clades including Sinularia, Lobophytum, Sarcophyton taxa and a mixture of Lobophytum and Sarcophyton taxa (Figure 2, 3). The clade 3 might be hybrids in nature between Lobophytum and Sarcophyton taxa. Alignment of their irg-cox1 and msh1 fragments revealed a share of substitutions between taxa in clade 1-3, i.e. clade 1 and 2, clade 1 and 3 and clade 2 and 3 respectively shared the same substitutions 17, 6 and 2 (Figure 3, 4 and Table 2), indicating their closely phylogenetic relationships (Figure 2 and 3).

CONCLUSION

The high phylogenetic variation of 11 soft coral in Nha Trang bay of Vietnam was revealed in this study. The polymorphism of partial msh1 and irg-cox1 sequences divided 11 samples into four clades of 3 genera, Sinularia, Lobophytum, Sarcophyton and a mixture of Lobophytum and Sarcophyton taxa. However in some cases, other markers are needed for morphometry of phylogenetic analysis and soft coral taxonomy at species level.

Acknowledgment

This work was financially supported by the bilateral corporation research project between Vietnam Academy of Science and Technology and A.V. Zhirmunsky Institute of Marine Biology Far East Branch, Russian Academy of Sciences - "Biodiversity research and application of molecular markers for taxonomy of key groups of the soft corals (Cnidaria: Alcyonacea) in Khanh Hoa, Vietnam" (grant number VAST.HTQT.NGA.09/12-13) to Dr. Le Quynh Lien.

REFERENCES

1. Aratake S., Tomura T., Saitoh S., Yokokura R., Kawanishi Y., Shinjo R., Reimer J.D., Tanaka J., Maekawa H. (2012): Soft Coral Sarcophyton (Cnidaria: Anthozoa: Octocorallia) Species Diversity and Chemotypes, PLoS ONE, 7(1): e30410
2. Bastidas C., Willis B.L, Fabricius K.E. (2004): Demographic processes in the soft coral Sinularia flexibilis leading to local dominance on coral reefs, Hydrobiologia, 530: 433-441
3. Ben H.X., Dautova T.N. (2010) Diversity of soft corals (Alcyonacea) in Vietnam. Proceedings of International Conference: Marine biodiversity of East Asia seas: Status, challenges and sustainable development: 82-87
4. Benayahu Y., Loya Y. (1977): Space partitioning by stony corals, soft corals and benthic algae on the coral reefs of the northern Gulf of Eilat (Red Sea), Helgoland Wiss Meeresunt, 30: 365-382
5. Benayahu Y. (1995): Species composition of soft corals (Octocorallia, Alcyonacea) on the coral reefs of Sesoko I., Ryukyu Archipelago, Japan, Galaxea, 12: 103-124
6. Benayahu Y. (1998) Lobe variation in the soft coral Sinularia nanolobata Verseveldt, 1977 (Cnidaria, Alcyonacea), Bull. Mar. Sci., 63: 229-240
7. Dai, C.-F. (1988): Coral communities of southern Taiwan, Proceedings of the 6th International Coral Reef Symposium, 2: 647–652
8. Dautova T.N., van Ofwegen L.P., Savinkin O.V.(2010) New species of the genus Sinularia, Vietnam, Zool. Med. Leiden, 84: 47-91
9. Dautova T., Savinkin O.(2009) New data on soft coral from Nha Trang Bay, Zootaxa, 2027: 1-27
10. Dinesen Z.D. (1983): Patterns in the distribution of soft corals across the central Great Barrier Reef. Coral Reefs, 1: 229–236
11. Fabricius K.., Alderslade P. (2001): Soft Corals and Sea Fans: A Comprehensive Guide to the Tropical Shallow-Water Genera of the Central- West PaciWc, the Indian Ocean and the Red Sea. Australian Institute of Marine Science, Townsville
12. Fabricius K.E. (1995): Slow population turnover in the soft coral genera Sinularia and Sarcophyton on mid- and outer-shelf reefs of the Great Barrier Reef, Mar. Ecol. Prog. Ser,, 126: 145-152
13. Fabricius K.E. (1997): Soft coral abundance on the central Great Barrier Reef: eVects of Acanthaster planci, space availability, and aspects of the environment, Coral Reefs, 16: 159–167
14. Fabricius K.E. (1998): Reef invasion by soft corals: which taxa and which habitats. In: Greenwood, J., Hall, N. (Eds.), Australian Coral Reef Society 75th Anniversary Conference. School of Marine Science, The University of Queensland, Brisbane, Heron Island: 77–90
15. France S., Hoover L. (2001): Analysis of variation in mitochondrial DNA sequences (ND3, ND4L, MSH) among Octocorallia (=Alcyonaria) (Cnidaria:Anthozoa), Bull. Biol. Soc. Wash., 10: 110–118
16. Kumar S., Tamura K., Nei M. (2004): MEGA3: integrated software for Molecular Evolutionary Genetics Analysis and sequence alignment, Brief. Bioinform., 5: 150-163
17. Mc Fadden C.S., Alderslade P., Ofwegen L.P., Johnsen H., Rusmevichientong A. (2006b): Phylogenetic relationships within the tropical soft coral genera Sarcophyton and Lobophytum (Anthozoa: Octocorallia), Invertebr. Biol., 125 (4): 288-305
18. Mc Fadden C.S., Ofwegen L.P., Beckman E.J., Benayahu Y., Alderslade P. (2009): Molecular systematics of the speciose Indo-Pacific soft coral genus, Sinularia (Anthozoa: Octocorallia). Invertebr. Biol ., 128 (4): 303-323
19. McFadden C.S., Donahue R., Hadland B.K., Weston R. (2001): A molecular phylogenetic analysis of reproductive trait evolution in the soft coral genus Alcyonium. Evolution, 55(1): 54-67.
20. McFadden C.S., van Ofwegen L.P. (2013): A second, cryptic species of the soft coral genus Incrustatus (Anthozoa: Octocorallia: Clavulariidae) from Tierra del Fuego, Argentina revealed by DNA barcoding, Hegel. Mar. Res., 67: 137-147
21. McFadden CS, Sánchez JA, France SC (2010) Molecular phylogenetic insights into the evolution of Octocorallia: a review. Integr Comp Biol. 50(3): 389-410

22. McFadden C.S., France S.C., Sánchez J.A., Alderslade P.(2006a): A molecular phylogenetic analysis of the Octocorallia (Cnidaria: Anthozoa) based on mitochondrial protein-coding sequences, Mol, Phylogenet, Evol., 41(3): 513-27
23. Pont-Kingdon G1, Okada NA, Macfarlane JL, Beagley CT, Watkins-Sims CD, Cavalier-Smith T., Clark-Walker G.D., Wolstenholme D.R. (1998): Mitochondrial DNA of the coral Sarcophyton glaucum contains a gene for a homologue of bacterial MutS: a possible case of gene transfer from the nucleus to the mitochondrion. J. Mol. Evol., 46: 419–431
24. Pont-Kingdon G.A., Okada N.A., Macfarlane J.L., Beagley C.T., Wolstenholme D.R., Cavalier-Smith T., Clark-Walker G.D. (1995): A coral mitochondrial MutS gene, Nature, 375:109–11
25. Riegl B., Schleyer M.H., Cook P.J., Branch G.M. (1995): Structure of Africa's southernmost coral communities. Bull. Mar. Sci., 56: 676–691
26. Tixier-Durivault A. (1970): Les octocoralliaires de Nha-Trang (Viet-Nam), Cahiers du Pacifique, 14: 115-236
27. Tursch B., Tursch A. (1982): The soft coral community on a sheltered reef quadrat at Laing Island (Papua New Guinea), Mar. Biol., 68: 321–332
28. van der Ham J.L., Brugler M.R., France S.C. (2009): Exploring the utility of an indel-rich, mitochondrial intergenic region as a molecular barcode for bamboo corals (Octocorallia: Isididae), Mar. Genomics, 2(3-4): 83-92
29. Verseveldt J. (1980): A revision of the genus Sinularia May (Octocorallia: Alcyonacea). Zool. Verh. Leiden, 179: 1-128
30. Verseveldt J. (1982): New species of Alcyonacea (Octocorallia) from the Great Barrier Reef, South-East Asia and the Red Sea. Zool. Meded. Leiden, 56: 143-151
31. Verseveldt J. (1983): A revision of the genus Lobophytum von Marenzeller (Octocorallia: Alcyonacea). Zool. Verh. Leiden, 200: 1-103