RESEARCH ARTICLE

PHYLOGENETIC ANALYSIS AND SPECIES IDENTIFICATION OF 11 GORGONIAN CORALS (OCTOCORALLIA: ALCYONACEA) IN THE NORTH CENTRAL COAST OF VIETNAM BASED ON MSH1 mtDNA AND 28S rDNA MARKERS

Nguyen Chi Mai1,2, Ninh Thi Ngoc1,2, Nguyen Xuan Cuong1,2, Nguyen Hoai Nam1, Nguyen Tuong Van3, Le Quang Trung4, Pham Thi Hoe1, Nguyen Quang Hung4 and Tran My Linh1,2

1. Institute of Marine Biochemistry, Vietnam Academy of Science and Technology (VAST), 18 Hoang Quoc Viet, Cau Giay, Hanoi, Vietnam. 
2. Graduate University of Science and Technology, VAST, 18 Hoang Quoc Viet, Cau Giay, Hanoi, Vietnam.
3. Institute of Biotechnology, VAST, 18 Hoang Quoc Viet, Cau Giay, Hanoi, Vietnam.
4. VNTEST Institute for Quality Testing and Inspection, 7/161/28 Nguyen Xien, Thanh Xuan, Hanoi, Vietnam.

Abstract

Vietnam contains diverse marine ecosystems with the high biodiversity of marine organisms, including gorgonian corals of Alcyonacea order. In order to support traditional classification of these corals, in this study mitochondrial barcoding markers msh1 and nuclear 28S rDNA were developed for analysis of 11 specimens collected in 2015 and 2016 from different islands and bays along the North Central coast of Vietnam. Phylogenetic analyses based on msh1 and 28S sequence polymorphism showed that all specimens belonged to Anthozoa class, Octocorallia sub-class and Alcyonacea order. At lower taxa levels, they were divided into 4 sub-orders, 7 families and 7 genera according to 7 distinct clades with bootstrap values from 99-100%. The identifications of 7 out of 11 specimens including Sinularia brassica (2 specimens) and Sinularia leptoclados, Dichotellagemmacea, Annella reticulata, S. conferta and S. nanolobata were in concordance between morphological and molecular methods. The other 4 specimens were only identified at genus levels of Astrogorgia sp., Melithaea sp., Scleronephthya sp. and Muricella sp. by either msh1-morphology or msh1-28S markers. These results highlight the importance of molecular markers to elucidate patterns of biodiversity and species identification of soft coral.

Introduction:

Vietnam contains diverse marine ecosystems harbouring the high biodiversity of marine organisms, including gorgonian corals of Alcyonacea order. Until 1952, Dawydoff had first reported about soft coral species in Vietnam and classified them into 6 families including Alcyonidae, Fasciculariidae, Xaniidae, Telestaceidae, Tubiporidae and Nephtheidae. Tixier-Durivault (1970) conducted the comprehensive study on soft coral samples in the Museum of Oceanography (Nha Trang, Vietnam) and reported 94 soft coral species belonging to 15 genera and 5 families. Among them, 18 species of Sinularia genus were recorded as new species. From 1990s to
2010s, investigations on biodiversity and distribution of soft coral communities in some islands such as Phu Quoc, Con Co, Truong Sa, Ly Son, Cat Ba, Cu Lao Cham and coastal areas such as Nha Trang Bay, Ha Long Bay were carried out. The results revealed that soft coral species in Vietnam belong to 22 genera and 9 families (Vo et al., 1997; Dautova and Savinkin, 2009; Ben and Dautova, 2010; Dautova et al., 2010; Thao and Ngai, 2013; Dautova and Savinkin, 2013; Ben et al., 2018; Ben and Quang 2020). Molecular DNA studies on soft coral species in Vietnam are still very poor. Recently, Lien et al. (2015) analyzed phylogenetic relationship of Sinularia, Sarcophyton, and Lobophytum genera in Nha Trang Bay (South Central coast of Vietnam) using 696bp sequences of msh1 genes and 866bp fragment of irg-c Cox1 gene. There has not been, however, research on this approach of soft corals in the North Central coast of Vietnam. Accurate identification of soft coral species is fundamental for genetics, physiology, ecology, particularly applied pharmacology. Molecular identification has been used in biodiversity and conservation, but it also has great potential for applications in taxonomy. Recently, based on nucleotide sequences, several coral species-specific molecular markers from ribosomal subunits 16S, 18S, 28S and genes coding for proteins on mitochondrial DNA (mtDNA) have been found (France et al., 1996; McFadden et al., 2006a, 2006b; Shearer et al., 2002; Herrera et al., 2009). Among molecular markers on mtDNA, msh1 gene encoded for MSH1 protein, has been used widely in molecular taxonomy of soft coral. It is known that the genetic diversity of msh1 gene is as twice as other coding genes on mtDNA. Moreover, msh1 gene is present in mtDNA of almost all soft corals published in the present GenBank (van der Ham et al., 2009). The diversity of the msh1 gene also occurs among species in a genus and has been used to phylogenetic analysis of soft coral species in the Asia-Pacific (Thoma et al., 2009).

In the present study, phylogenetic analysis and species identification of 11 soft coral specimens collected in the North Central region of Vietnam were investigated based on the 639bp on their msh1 mtDNA and the 704bp fragment on 28S rDNA gene. The congruence between morphological analysis and molecular markers in species identification could offer an alternative taxonomic method for further biodiversity studies of soft corals in Vietnam.

Materials and Methods:

Materials
Soft coral specimens were collected in different islands and bays of the North Central region of Vietnam in 2015-2016 using SCUBA diving equipment. A map of sampling location and specimen name are shown in Fig 1. The specimens were washed several times with fresh water to remove sand, algae and other surface organisms and then 5 times with distilled water. One portion of each sample was stored in 70% ethanol for morphological analysis and the smaller portion was kept at -80°C for DNA extraction. All vouchers were deposited at Institute for Marine Biochemistry, Vietnam Academy of Science and Technology (VAST). Morphological characters of specimens were identified by Prof. Do Cong Thung (Institute of Marine Resources and Environment, VAST) right after sampling and cleaning.

PCR and DNA Sequencing:
Genomic DNA of each specimen was isolated using DNeasy® Tissue Kit (Qiagen) according to the manufacturer’s instruction. The concentration of DNA was quantified using a NanoDrop 1000 instrument (Thermo Scientific, USA) and by electrophoresis in 0.8% agarose.

The 639 bp DNA fragment of msh1 gene and 704bp - DNA fragment of 28S rDNA gene were amplified using genomic DNA of soft coral specimens as templates and the specific primer pairs MSHF (5'-ATGAACCAGATACTAGTGC-3') and MSHR (5'-AGGGAACCAGCTACTAGATG-3') and 28SF (5'-CGTTGAAAGGGAAGCGAATG -3') and 28SR (5'-AGGGAAACCAGCTACTAGATG-3'). PCR components were 5 μl 10X PCR Buffer, 1 mM dNTPs, 2 mM primers, 50 ng of genomic DNA, 1 unit of Taq Polymerase and 2.5 mM of MgCl2 and H2O up to total 50 μl. The PCR cycle were 94°C for 3 min, followed by 30 cycles of 1 min at 94°C, 30s at an annealing temperature of 52°C for MSHF/R primers or 55°C for 28SF/R primers, DNA synthesis for 1 min at 72°C, and then a final extension of 10 min at 72°C. Amplification products were verified by 1.5% agarose gel electrophoresis in TAE buffer. PCR products were purified using QIAquick PCR purification kit (Qiagen, Germany), cloned into pTZ57R/T Vector (Thermo, USA) and sequenced by 1st Base (Singapore). Nucleotide sequences of studied soft coral specimens registered to GenBank as accession numbers were listed in Table 1.

DNA Analysis:
Phylogenetic relationship of the 11 soft coral samples (Table 1) was analyzed based on referred sequences from Gene Bank using ClustalW method of Mega 3.1 (Kumar et al., 2004). Phylogeny reconstruction was done with tree
inferences using Neighbor Joining (NJ) method from Mega 3.1 with Bootstrap test of 5000 replicates. For species determination, homology level after aligning DNA sequences of the studied samples and references in the same clades on NJ trees obtained from phylogeny reconstruction analysis was estimated using Multiple Sequence Alignment method of DNAMAN 4.15 (LynnonBioSoft). Specific point mutations (SNPs) within msh1 and 28S sequences were also indicated using Align of ClustalW and Sequence Data Explorer methods from Mega3.1.

Figure 1: Collection sites of 11 soft coral specimens in the North Central of Vietnam.

Results and Discussion:–
Phylogenetic analysis
After sequencing and extracting, partial msh1mtDNA of 11 specimens and 28S rDNA of 10 specimens respectively ranged from 707-719bp and 739-753bp, of which fragments of 627-639bp msh1 and 695-709bp 28S of the specimens were used for analysis due to the available length of related referred sequences from GenBank. Phylogenetic analyses based on msh1mtDNA sequence polymorphism of 11 specimens and referred taxa (Fig2) showed that all samples of this research belonged to Anthozoa class, Octocorallia Sub-class and Alcyonacea order. At lower taxa levels, they were divided into 4 sub-orders, 7 families and 7 genera according to 7 distinct clades (1 to 7) with bootstrap values from 99-100%. Phylogenetic tree of 28S rDNA (Fig3) were similar to that of msh1mtDNA, but only with 6 genera (without clade 4) because sequence of 28S fragment of NL-07 was not available. Of 11 specimens, 5 were clustered into clades 2a and 2b with referred taxa of Sinularia genus. The results of phylogenetic analyses indicated the high taxonomical diversity of Alcyonaceancorals in sampling regions. Clade order between msh1 and 28S was not the same possibly because of difference in evolution rate between mtDNA and nuclear rDNA. Basically, mitochondrial genes evolve 50–100 times slower than nuclear genes in anthozoans (Hellberg, 2006; Chen et al., 2009). Some publications (McFadden et al., 2014, Prada et al., 2014, van Oppen et al., 2001) revealed that lack of concordance among different molecular markers in phylogenetic analyses is not uncommon in corals.
Figure 2: Phylogenetic Neighbor Joining tree reconstructed based on DNA polymorphisms of 627-639bp msh1 fragments of 11 soft coral specimens and related referred taxa. Numbers in open circles: clade number corresponding to taxa in particular families and sub-orders on the right of the tree. Numbers after referred taxa: accession no. from GenBank. Only bootstrap values more than 70% were represented next to the nodes of the tree.

On phylogenetic trees instructed by msh1 and 28S markers, taxa of 6 genera, including Annella, Astrogorgia, Dichotella, Melithaea, Muricella and Scleronephthya were monophyletic, while those of Sinularia were found polyphyletic with 2 separate clades 2a and 2b (Fig 2 and 3). The polyphyletic sub-clades of 2a and 2b may be resulted from the hybridization among taxa in this genus, which was mentioned in some previous studies. Chemical markers of five new terpenoids isolated from Sinularia maxima and S. polydactyla elucidated the hybridization between these two soft coral species (Kamel et al., 2009). More recently, DNA barcode markers, mtMutS and 28S rDNA, proved the hybridization occurring within Sinularia genus (Quattrini et al., 2019). Analyses of Sinularia hybridization for clade 2b (Fig 3) based on 695-719bp 28S rDNA sequences of 10 soft coral specimens and related reference sequences resulted in seven SNPs along the aligned sequences. These SNPs could, therefore, add more evidence of hybridization of soft corals, which was one of the important motivations for evolution of Sinularia genus and Anthozoa class (Willis et al., 2006).
Species determination:
Analysis of homology levels

Alignments between sequences of msh1 mtDNA and 28S rDNA fragments of 11 specimens and reference taxa in each clade on Fig 2 and Fig 3 showed the high homology level (99.7-100.0%) for both markers (Table 1). Five specimens were classified as Sinularia brassica (HV-03, NL-06), S.leptoclados (SLE-05), Dichotellagemmacea (NL-03) and Annella reticulata (VM-02) based on msh1 marker with 99.7-100% of identity and 28S marker with 99.9-100% of identity), which was also in agreement with previously morphological identification. In case of unclassified by morphological criteria due to samples damaged, NL-08 and NL-05 specimens were still diagnostic at the genus level as Astrogoriasp. and Melithaea sp., respectively. Msh1 markers with identity to referred taxa from 99.8-100% were in accordance with morphological analysis to identify specimens of SN-04 and SCO-05 to be Sinulariananolobata and Sinulariaconferta, while 28S rDNA sequences of these two species were not available from GenBank. Though 28S fragments of HM-09 and Scleronephthyacorymbosawas aligned with 100% of identity, this specimen was still decided to be Scleronephthya sp. because morphological diagnosis classified this specimen only at genus level and msh1 sequence of S. corymbosawas unavailable in GenBank. Similarly, NL-07 was unclassified at species level in Muricella genus due to morphological identification to be Muricella sp. and identity of msh1 sequence of this specimen and Muricella sp. referred in GenBankto be 100%. In short, among 11 specimens, seven of HV-03 and NL-06 (Sinularia brassica), SLE-05 (S.leptoclados), NL-03 (Dichotellagemmacea), VM-02 (Annella reticulata), SN-04 (S. conferta) and SCO-05 (S. nanolobata) were classified at species level, of which the first five wereby three markers (msh1, 28S and morphology) and the other two by msh1 and morphology. The other four of NL-08, NL-05, HM-09 and NL-07 were only identified at genus level to be Astrogoriasp., Melithaea sp., Scleronephthya sp. and Muricella sp. by either msh1-morphology or msh1-28S markers.

Figure3:- Phylogenetic Neighbor Joining tree reconstructed based on DNA polymorphisms of 695-709bp 28S fragments of 10 soft coral specimens and related referred taxa. Numbers in open circles: clade number corresponding to taxa in particular families and sub-order on the right of the tree. Numbers after referred taxa: accession no. fromGenBank. Only bootstrap values more than 70% were represented next to the nodes of the tree.
Table 1: Combination of molecular markers and morphological characters for species identification of soft coral specimens in this study.

| C* | Homology levels of 627-639bp msh1 mtDNA** | Homology levels of 609-709bp 28S rDNA** | Morphological classification for species level*** |
|----|----------------------------------------|----------------------------------------|-----------------------------------------------|
|    | Reference taxa | Taxa in this study | Reference taxa | Taxa in this study | % | Reference taxa | Taxa in this study | % |                                           |
|    | Taxa | Acc. No. | Taxa | Acc. No. | % | Taxa | Acc. No. | Taxa | Acc. No. | % |                                           |
| C1 | Scleronephthya sp. | DQ3028 25 | HM-09 | MW0778 99 | 100.0 | Scleronephthya corymbosa | JX1243 50 | HM-09 | MW0778 89 | 100.0 | Scleronephthya sp. (Studer, 1887) |
| C2 | Sinularia brassica | HG9700 90 | HV-03 | MW0779 00 | 100.0 | Sinularia brassica | KP915 491 | HV-03 | MW0778 90 | 100.0 | Sinularia brassica (May, 1898) |
|    | Sinularia brassica | MH5166 94 | NL-06 | MW0779 03 | 99.7 | Sinularia brassica | MF817 931 | NL-06 | MW0778 93 | 100.0 | Sinularia brassica (May, 1898) |
| C2 | Sinulariacofer ta | JF62138 9 | SCO-05 | MW0779 07 | 100.0 | Sinularia sp. | KC542 826 | SCO-05 | MW0778 97 | 99.7 | Sinulariacofer ta (Dana, 1846) |
| C2 | Sinularia leptoc lados | KC542 57 | SLE-05 | MW0779 06 | 99.8 | Sinularia leptoc lados | MF817 912 | SLE-05 | MW0778 96 | 99.9 | Sinularia leptoc lados (Ehrenberg, 1834) |
| C2 | Sinulariananol obata | JF62145 1 | SN-04 | MW0779 08 | 99.8 | Sinularia sp. | KP915 519 | SN-04 | MW0778 98 | 99.9 | Sinulariananol obata (Verseveldt, 1977) |
| C3 | Astrogorgia sp. | KP9155 66 | NL-08 | MW0779 04 | 100.0 | Astrogorgia sp. | KP915 326 | NL-08 | MW0778 94 | 99.8 | NA |
| C4 | Muricella sp. | KP8561 97 | NL-07 | MW0779 09 | 100.0 | Muricella sp. | NA | NL-07 | NA | NA | Muricella sp. (Verrill, 1888) |
| C5 | Dichotellagem maceae | KF8036 69 | NL-03 | MW0779 01 | 100.0 | Dichotellagem maceae | JX2037 01 | NL-03 | MW0778 91 | 100.0 | Dichotellagem maceae (Milne Edwards & Haime, 1857) |
| C6 | Anella reticulata | KP7139 84 | VM-02 | MW0779 05 | 100.0 | Anella reticulata | KP915 314 | VM-02 | MW0778 95 | 100.0 | Anella reticulata (Ellis & Solander, 1796) |
| C7 | Melithaea sp. | JX20379 9 | NL-05 | MW0779 02 | 99.8 | Melithaea sp. | KC845 912 | NL-05 | MW0778 92 | 100.0 | NA |

(*) C1-C7: clade 1-clade 7 in Fig. 2 and 3; **) H: homology level (%); *** By Prof. Do Cong Thung

Single nucleotide polymorphism analysis

Separate alignment of msh1 fragments (Fig 2) and 28S fragments (Fig 3) of soft coral taxa respectively resulted in 174 and 188 single nucleotide polymorphisms (Fig 4 and 5). These diagnostic SNPs distinguished each of 7 specimens to be classified at species level and each of 4 being identified at genus level (Table 1). Number of SNPs along msh1 fragments of 11 taxa ranged from 0-50 (Fig 4) while 28S fragments of 10 taxa varied from 1-85 along them (Fig 5), revealing lower substitution rates of msh1 mtDNA than those of 28S rDNA in corals as estimated by Hellberg (2006). Moreover, nucleotide substitution rate of the same coral taxon also varied depending on particular genomes. In this study, Melithaea sp. has the highest number of 50SNPs on msh1 fragment, while the taxon is the second position with 18 SNPs on 28S fragment. In contrast, D. gemmacea has the highest number of 84 SNPs on its 28S fragments but was the second of 38 SNPs on msh1 fragments of the species (Fig 4 and 5). The lowest number of SNPs on msh1 (0-9) and 28S fragments of Sinularia taxon in clade 2b again implied their hybridization. Difference in nucleotide substitution rate between msh1 mtDNA and 28S rDNA could interpret the difference in order of 7 clades between msh1 and 28S phylogenetic trees of 11 specimen and related referred sequences (Fig 2 and 3).
Msh1 and 28S markers supporting morphological classification in determination of soft coral taxa:

Recently, classification of corals still has been based on morphological characters of colony morphology and/or sclerites comparison. In fact, the first approach was unable to determine at species level in some genera for mixture of morphological characters among them, while their sclerite were so varied that many species were not identified if applied skeleton composition comparison (van Ofwegen and Groenenberg, 2007). Similarly, both approaches were unable to identify some octocoral taxa or were incongruent with morphospecies identification due to high variation, especially taxa in such genus as *Sinularia*, within which the hybridization between closely related taxa happened (Benayahu et al., 2012; McFadden et al., 2014; 2017; Quattrini et al., 2019). Molecular data such as mtDNA and rDNA could support to overcome such limitation in coral classification (Reijnen, 2015; Reijnen and Van der Meij, 2017).

Besides nuclear DNA, loci from mtDNA such as *msh1*, *MutS*, *ND2*, *16S* ... were available in GenBank and widely used as markers for identification of numerous octocoral taxa (McFadden et al., 2010). However, in some cases, sequences of mitochondrial loci were not informatively polymorphic enough to identify coral taxa at species level due to their slow rates of evolution (Shearer et al., 2002; Huang et al., 2008). Only 80% of *Sinularia* species were in agreement with morphospecies when identified using *MutS* mtDNA (McFadden et al., 2014). They also concluded that a single DNA marker, e.g., *mtMutS* was not powerful enough to discriminate most closely related species such
as *Sinularia spp*. Loci from rDNA genome, i.e., *18S, ITS, 5.8S* and *28S* were shown to be more powerful than those from mtDNA to resolve relationships of corals below genus level (van Ofwegen and Groenenberg, 2007). Therefore, markers from mtDNA could be combined with those from rDNA to discriminate coral taxa at species level, especially closely-related species within most coral genera (Benayahu et al., 2018). In this study, results of determination of 11 soft corals by *msh1* and *28S* markers were in agreement to those by morphological classification (Table 1), again indicating the strong support of the two DNA markers to morphospecies of these soft coral species. In spite of powerfulness in discrimination of coral taxa, including closely-related species in a genus, molecular markers including *msh1* and *28S* loci showed some disadvantages. Of all specimens, five were identified at species level by all *msh1*, *28S* and morphological markers. However, the other 6 specimens were determined at species level or only genus level with the pair marker of *msh1*- morphospecies, *msh1-28S* due to unavailability of related *msh1* mtDNA and *28S* rDNA in GenBank. When related sequences of these two markers are available, these genus-level taxa will be resolved.

**Divergence of gorgonian corals:**

The molecular analysis in this study indicated the high diversity of gorgonian corals in Alcyonacea order along the North Central coast of Vietnam (Fig 1). All 11 specimens were classified to be 10 species (Table 1) in 7 genera, 7 families and 4 suborders (Fig 2 and 3). Of 11 specimens, five belonged to *Sinularia*, indicating the richness of the species in this genus. Vietnam occupied as long as 3000 km of tropical coastal length, within which high diversity of marine animal including soft coral has been investigated. Ben et al. (2010) had found a total of 60 taxa including 10 genera and 5 families from 85 collected specimens in Ly Son Island (30 km from the South Central coast of Quang Ngai province), of which 14 species of *Sinularia*, 9 species of *Lobophytum*, 6 species of *Sarcophyton* genera were newly recorded in Vietnam. After that, a phylogenetic investigation grouped 11 soft corals in Nha Trang Bay (Khanh Hoa province) grouped into four different clades with different gorgonian coral genera of *Sinularia*, *Sarcophyton*, *Lobophytum* and a mixed between *Sarcophyton* and *Lobophytum* (Lien et al., 2015). More recently, Ben et al. (2020) also found species richness of soft corals along Cu Lao Cham Marine Protected Area (South Central coast of Quang Nam province), in which a total of 45 taxa belonging to 12 genera and 7 families were identified. Among them, *Sinularia* genus was the highest diversity group with 19 species, followed by *Sarcophyton* with 8 species and *Lobophytum* with 6 species. However, it is the fact that the above findings have been only spotty trying. Recently, molecular markers, especially DNA barcodes have been proved really powerful to resolve various problematic aspects of marine animals. An overall project on application of these tools to deal with phylogenetic patterns, species determination and diversity research on marine animals including soft corals along 3000 km coastal length of Vietnam, could be, therefore, programmed in order to effective conservation and sustainable development of these resources in the country.

**Conclusion:-**

In this study, the developed molecular markers clearly supported the classification of soft corals at species level. The identifications of 9 out of 11 soft coral specimens based on *msh1* mtDNA and *28S* rDNA polymorphism of the soft coral specimens and referred taxa was in concordance with the traditional taxonomy. There were 7 specimens to be classified at species level, of which 5 were identified by *msh*, *28S* and morphological markers and the other two by *msh1* and morphospecies. The other four were only identified at genus level by either *msh1*-morphology or *msh1-28S* marker pairs. All of the taxa belonged to Anthozoa class, Octocorallia sub-class and Alcyonacea order, from which they were divided into 4 sub-orders, 7 families and 7 genera according to 7 distinct clades on phylogenetic trees with high confidence intervals from 99-100%.

**Acknowledgments:-**

This study was supported by a grant from Vietnam Academy of Science and Technology (code: VAST.TD.DAB.02/16-18).

**References:-**

1. Ben, H. X. and Dautova, T. N. (2010): Diversity of soft corals (Alcyonacea) in Vietnam. Paper presented at the Proceedings of International Conference: Marine biodiversity of East Asia seas: Status, challenges and sustainable development.
2. Ben, H. X., Long, N. V., Tuyen, H. T., Hoang, P. K. and Quang, T. M. (2018): Biodiversity and characteristics of coral reef communities in Ly Son Marine Protected Area, Quang Ngai province. Vietnam Journal of Marine Science and Technology, 18(2): 150-160.
3. Ben, H. X. and Quang, T. M. (2020): Biodiversity and characteristic of octocoral communities (Octocorallia: Alcyonacea and Gorgonacea) in Cu Lao Cham Marine Protected Area, Quang Nam province. Vietnam Journal of Marine Science and Technology, 19: 589-599.

4. Benayahu, Y., Ofwegen, L., Dai, C., Jeng, M., Soong, K., Shlagman, A., McFadden, C. (2012): Diversity, Distribution, and Molecular Systematics of Octocorals (Cnidaria: Anthozoa) of the Penghu Archipelago, Taiwan. Zoological Studies, 51: 1529-1548.

5. Benayahu, Y., van Ofwegen, L. P., Dai, C. F., Jeng, M. S., Soong, K., Shlagman, A. and McFadden, C. S. (2018): The Octocorals of Dongsha Atoll (South China Sea): An Iterative Approach to Species Identification Using Classical Taxonomy and Molecular Barcodes. Zoot Stud, 57: e50.

6. Chen, I. P., Tang, C. Y., Chiou, C. Y., Hsu, J. H., Wei, N. V., Wallace, C. C. and Chen, C. A. (2009): Comparative analyses of coding and noncoding DNA regions indicate that Acropora (Anthozoa: Scleractina) possesses a similar evolutionary tempo of nuclear vs. mitochondrial genomes as in plants. Mar. Biotechnol. (NY), 11(1): 141-152.

7. Dautova, T., Ofwegen, L. and Savinkin, O. (2010): New species of the genus Sinularia (Octocorallia: Alcyonacea) from Nha Trang Bay, South China Sea, Vietnam. Zoologische Mededelingen, 84: 47-91.

8. Dautova, T. N. and Savinkin, O. V. (2009): New data on soft corals (Cnidaria: Octocorallia: Alcyonacea) from Nha Trang Bay, South China Sea. Zootaxa, 2027: 1-27.

9. Dautova, T. N. and Savinkin, O. V. (2013): Octocorallia: Alcyoniidae. Moscow: KMK.

10. Dawydoff, C. (1952): Contribution a l'étude des invertébrés de la faune marine benthique de l'Indochine. 37: 1-158.

11. France, S. C., Rosel, P. E., Agenbroad, J. E., Mullineaux, L. S. and Kocher, T. D. (1996): DNA sequence variation of mitochondrial large-subunit rRNA provides support for a two-subclass organization of the Anthozoa (Cnidaria). Mol. Mar. Biol. Biotechnol., 5(1): 15-28.

12. Hellberg, M. E. (2006): No variation and low synonymous substitution rates in coral mtDNA despite high nuclear variation. BMC Evol. Biol., 6(1): 24.

13. Herrera, S., Baco, A. and Sanchez, J. A. (2009): Molecular systematics of the bubblegum coral genera (Paragorgiidae, Octocorallia) and description of a new deep-sea species. Mol. Phylogenet. Evol., 55: 123-135.

14. Huang, D., Meier, R., Todd, P. A., and Chou, L. M. (2008): Slow mitochondrial COI sequence evolution at the base of the metazoan tree and its implications for DNA barcoding. J. Mol. Evol., 66(2): 167-174.

15. Kamel, H. N., Ding, Y., Li, X. C., Ferreira, D., Fronczek, F. R. and Slattery, M. (2009): Beyond polymaxenolide: Cembrane-africanane terpenoids from the hybrid soft coral Sinularia maxima x S. polydactyla. J. Nat. Prod., 72(5): 900-905.

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. Lien, L. Q., Linh, T. M., Giang, V. H., Mai, N. C., Tuan, P. M., Trung, L. Q. and Dautova, T. N. (2015): Phylogenetic variation of eleven soft corals (Anthozoa, Octocorallia) in Nha Trang bay of Vietnam. Int. J. Adv. Res., 3: 1-11.

18. McFadden, C., Sanchez, J. A. and France, S. (2010): Molecular Phylogenetic Insights into the Evolution of Octocorallia: A Review. Integr. Comp. Biol., 50: 389-410.

19. McFadden, C. S., Alderslade, P., Van Ofwegen, L. P., Johnsen, H. and Rusmevichientong, A. (2006b): Phylogenetic relationships within the tropical soft coral genera Sarcophyton and Lobophyton (Anthozoa,Octocorallia). Invertebr. Biol., 125(4): 288-305.

20. McFadden, C. S., Brown, A. S., Brayton, C., Hunt, C. B. and van Ofwegen, L. P. (2014): Application of DNA barcoding in biodiversity studies of shallow-water octocorals: molecular proxies agree with morphological estimates of species richness in Palau. Coral Reefs, 33(2): 275-286.

21. McFadden, C. S., France, S. C., Sánchez, J. A., and Alderslade, P. (2006a): A molecular phylogenetic analysis of the Octocorallia (Cnidaria: Anthozoa) based on mitochondrial protein-coding sequences. Mol. Phylogenet. Evol., 41(3): 513-527.

22. McFadden, C. S., Haverkort-Yeh, R., Reynolds, A. M., Halász, A., Quattrini, A. M., Forsman, Z. H. and Toonen, R. J. (2017): Species boundaries in the absence of morphological, ecological or geographical differentiation in the Red Sea octocoral genus Ovabunda (Alcyonacea: Xeniidae). Mol. Phylogenet. Evol., 112: 174-184.

23. Prada, C., DeBiasse, M., Neigel, J., Yednock, B., Stake, J., Forsman, Z. and Hellberg, M. (2014): Genetic species delineation among branching Caribbean Porites corals. Coral Reefs, 33.

24. Quattrini, A. M., Wu, T., Soong, K., Jeng, M.-S., Benayahu, Y. and McFadden, C. S. (2019): A next generation approach to species delimitation reveals the role of hybridization in a cryptic species complex of corals. BMC Evol. Biol., 19(1): 116.
25. Reijnen, B. (2015): Molecular data for *Crenavolva* species (Gastropoda, Ovulidae) reveals the synonymy of *C. chiapponii*. ZooKeys, 501: 15.
26. Reijnen, B. T. and van der Meij, S. E. (2017): Coat of many colours-DNA reveals polymorphism of mantle patterns and colouration in Caribbean CyphomaRöding, 1798 (Gastropoda, Ovulidae) PeerJ, 5, e3018.
27. Shearer, T. L., Van Oppen, M. J., Romano, S. L. and Wörheide, G. (2002): Slow mitochondrial DNA sequence evolution in the Anthozoa (Cnidaria). Mol Ecol, 11(12): 2475-2487.
28. Thao, D. V. and Ngai, N. D. (2013): New data on the species composition of soft corals in Cat Ba, Hai Phong city. Collection of Marine Resources and Environment, 17: 178–182.
29. Thoma, J. N., Pante, E., Brugler, M. R. and France, S. C. (2009): Deep-sea octocorals and antipatharians show no evidence of seamount-scale endemism in the NW Atlantic. Mar. Ecol. Prog. Ser., 397: 25-35.
30. Tixier-Durivault, A. (1970): Les Octocoralliaires de Nouvelle Calédonie. In: Expedition francaiseRecifscoralliens de la Nouvelle-Caledonie, organisé sous l'égide de la fondation Singer-Polignac 1960–1963, 4: 171-350.
31. 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), 183-192. doi:10.1016/j.margen.2009.10.002.
32. van Ofwegen, L. P. and Groenenberg, D. S. J. (2007): A centuries old problem in nephtheid taxonomy approached using DNA data (Coelenterata: Alcyonacea). Contributions to Zoology, 76(3): 153-178.
33. van Oppen, M. J., McDonald, B. J., Willis, B. and Miller, D. J. (2001): The evolutionary history of the coral genus *Acropora* (Scleractinia, Cnidaria) based on a mitochondrial and a nuclear marker: reticulation, incomplete lineage sorting, or morphological convergence? Mol. Biol. Ecol. 18(7): 1315-1329.
34. Vo, S. T. and Hodgson, G. (1997): Coral reefs of Vietnam: recruitment limitation and physical forcing. Paper presented at the Proceedings of the 8th International Coral Reef Symposium, Smithsonian Tropical Research Institute, Panama.
35. Willis, B. L., van Oppen, M. J. H., Miller, D. J., Vollmer, S. V. and Ayre, D. J. (2006): The Role of Hybridization in the Evolution of Reef Corals. Annu. Rev. Ecol. Evol. Syst, 37(1): 489-517.