Journal of Sustainability Science and Management
Volume 19 Number 1, January 2024: 12-19
eISSN: 2672-7226

CHECKLIST OF MARINE SPONGES OFF BIDONG ARCHIPELAGOS BASED ON COI GENE SEQUENCES

KAMARIAH BAKAR, NUR AMIRA JAMALUDIN AND JASNIZAT SAIDIN*

Institute of Climate Adaptation and Marine Biotechnology, Universiti Malaysia Terengganu, 21030 Kuala Nerus, Terengganu, Malaysia.

**Corresponding author: ijaxzt@umt.edu.my*
Submitted final draft: 22 February 2023 *Accepted: 10 September 2023* http://doi.org/10.46754/jssm.2024.01.002

Abstract: Sponges are sessile marine animals under the phylum Porifera. Conventionally, the identification of sponges is based on two criteria: morphology (shape, colour, surface characteristics and consistency) and macro-morphology (spicules, sponging fibres and their arrangement). This identification technique is sometimes unreliable due to the plasticity of sponges' characteristics, which causes misidentification. We utilised universal barcode COI primers to identify sponges in this study. This study shows the results that have been collected through the study of the following methods. Out of 83 samples, only 25 sponges were successfully identified. They were from Class *Demospongia* and were under subclass *Heteroscleremorpha*. The majority of the two main orders are *Haplosclerida* (28%) and order *Tetractinellida* (24%). The rest were from order *Clionida* and *Scopalinida* (12%), *Suberiterida* (8%), while order *Axinellida*, *Tethyida, Poeciloscelerida* and *Agelasida* 4%. Among the identified samples 16 were identified to species level while the rest were only identified up to genus level. A significant limitation of this study is that the sequences in the database are not well populated and have low diversity, making it challenging to conduct molecular identification. It also impacted the accuracy of the classification, making a single method of identification less reliable.

Keywords: Marine sponges, identification, cytochrome c oxidase subunit I, COI gene sequence.

Introduction

Sponge (phylum Porifera) are sessile, benthic filter feeders' evolutionary oldest multicellular animals (Yang *et al.,* 2017). They have essential roles in biogeochemical cycling, the spatial structure of the seafloor, and the benthic-pelagic coupling of nutrient transfer within the ocean ecosystem (Vargas *et al.,* 2012). In addition, they are also significant to the pharmaceutical and biomaterials industries as they participate in complex biotic interactions with a wide variety of macrobiotic taxa and microbiological communities to produce up to 30% of all active marine metabolites (Yang *et al.,* 2017).

The World Porifera Database reported over 9083 described species in the phylum in 2018, most of them belonging to the Class *Demospongiae* (Vargas *et al.,* 2012). Sponges are ancient metazoans that exist long on earth that serve as a food source for marine organisms, provide a wide range of associations with other organisms such as providing homes for a variety of marine habitats such as snapping shrimps, polychaetes and groups of small fishes, and even giving protection to organisms from their prey (Buhl-Mortensen *et al.,* 2010; Verdín, 2010). However, identifying the species is particularly difficult because the available characters used for classification are limited (Federwisch *et al.,* 2020). The common characteristics are their organic and inorganic skeletons, including skeletal size, form, structure and composition. The arrangement of the skeletal parts can be inconsistent, and our knowledge of the evolution of skeletal features is limited. The traditional morphological identification methods can be incorrect, and species' true diversity and distribution may be underestimated (Sethmann & Wörheide, 2008).

However, due to their unique morphological traits and intraspecific variability in colour and

shape, many marine sponges are challenging to identify using conventional taxonomic techniques (Pons *et al.,* 2017). Thus, methods of identification based on molecular approaches such as DNA barcoding, were used as complementary data for sponge classification. In this study, the identification of the sponges has proceeded to the molecular method, where the DNA of the sponge samples are extracted and amplified using a mitochondrial cytochrome c oxidase subunit I (COI) gene marker. This DNA region is the most frequently used to differentiate genetic variability between metazoan species and between species (Bucklin et al., 2011).

Materials and Methods

Samples Collection

Sponges were collected in 2016 at seven different geographic locations in the archipelago of Bidong Island, Terengganu (Figure 1). The sites are Pasir Cina (537.316'N 1033.516'E), Christmas Garden (537.526'N 1034.301'E), Pantai Vietnam (536.949'N 1033.557'E.), Terumbu Kerisi (535.832'N 1033.720'E), Christmas Garden 2 (537.480'N 1034.366'E), Pulau Karah (535.886'N 1033.794'E) and Batu Rusa (5.5576623 N, 102.988711 E). In each sampling site, sponge photographs were taken using an underwater camera. Subsequently, the tissue of the sponges was collected and kept in a portable fridge with iceboxes filled with ice cubes to keep the sponge chilled during transportation. For long-term storage and following analysis, the samples were kept at –80 °C in sealed sample bags or plastic containers. Several sponge pieces were stored in a sample jar submerged in 70% (v/v) ethanol for morphological observation based on Ngwakum *et al.* (2021). The voucher specimens were submitted to the South China Sea Repository and Reference Centre (RRC), Institut Oseanografi dan Sekitaran (INOS), Universiti Malaysia Terengganu.

Benthic Condition

Station A and B: the station is composed of branching corals with sandy and dead coral bottoms. Depth of the sampling location from 5–15 m. Station C: the location has a depth of 15 to 18 m with boulders, ravines, crevices, holes, and walls on the island side. The benthic communities were sparse branching, encrusting and tabulated corals attached to the boulders. Station D: the depth of this location was 19 to 20 m, with fine sand and silt on the bottom and sparse stones littering the ocean floor. Most of the benthic communities were whip corals. Station E: the depth of the sampling location was 5–18 m, and the benthic community consisted of branching corals. The deeper part is dotted with sand and boulders. Station F: the depth of this location was 19 to 20 m with a group of boulders and surrounded by the sandy bottom. The benthic community on the boulders is mostly branching corals, while whip corals dominate the surrounding sandy bottom. This location is affected by swift currents during low and high tides. Station G: the sampling location was a small rocky island, Batu Rusa (40 m^2), with a lighthouse, and the surrounding water composed of branching corals. The depth of the sampling location was 18 to 20 m.

DNA Extraction, Amplification and Sequencing

Sponge tissue (25 mg) was used as a sample for the total DNA extraction using the DNeasy Tissue kit (QIAGEN, Germany) following the manual provided by the manufacturer. The purified DNA was resuspended in 200 µL elution buffer and immediately used amplification or stored in a freezer at –20º***C. The purity and quantity of DNA were determined with a*** Nanodrop 1000 Spectrophotometer (Thermo Scientific, Wilmington, DE, USA). Only high-quality DNA was used for polymerase chain reaction (PCR) amplification, as described by Yang *et al.* (2017) using a thermocycler (Bio-Rad, USA). The universal COI primers LCO1490 (5'-GGTCAACAAATCATAAAGATATTTGG-3') and CO2198 (5'-TAAACTTCAGGGTGACCA AAAAATCA-3') were used as templates. The targeted fragments were amplified as followed: initial denaturation at 94ºC for 1 min, five cycles at 94ºC for 30 s, 45ºC for 90 s, 72ºC for 1 min, 35 cycles of 94ºC for 30 s, 51ºC for 40 seconds and 72ºC for 1 min, with a final 72ºC extension

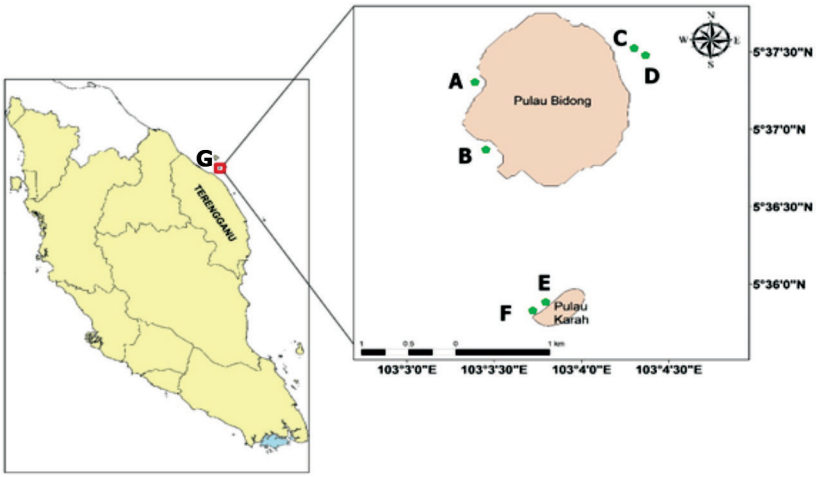


Figure 1: Sampling locations. Seven sampling locations (A-G). A- Pasir Cina (5°37.316'N 103°3.516'E, B- Pantai Vietnam (5°37.526'N 103°4.301'E), C- Christmas Tree Garden (5°36.949'N 103°3.557'E, D- Terumbu Kerisi (5°35.832'N 103°3.720'E), E-Anemone Garden 2 (5°37.480'N 103°4.366'E), F-Pulau Karah (5°35.886'N 103°3.794'E) and G-Batu Rusa (5.5576623 N, 102.988711 E)

for 5 min. The PCR product electrograph and the respective DNA band were purified using the Gel Purification Kit (BioRad). Sequencing was outsourced.

Sequences Analysis and Identification

The DNA sequence, with approximately 550 to 650 base pairs, was trimmed using MEGA 7 (Amiri *et al.,* 2018). Subsequently, the sequences were compared with the existing standard nucleotide database (nr/nt) using the online Basic Local Alignment Search Tool (BLAST) on the National Centre for Biotechnology Information (NCBI) website (https://blast.ncbi.nlm.nih.gov/Blast.cgi) for species identification.

Results and Discussion

Sequences Analysis and Identifications

In the present study, 25 sponge samples were identified based on DNA similarity and morphological observation. The photographs, identity, NCBI accession numbers and locality of the samples are tabulated in Table 1. All samples were classified in Class *Demospongia* subclass *Heteroscleremorpha*. The identification similarity ranged from 97% to 100% based on the NCBI database. Findings showed that two main orders of marine sponges were identified, which were order *Haplosclerida* (28%, 7 samples) and order *Tetractinellida* (24%, 6 samples) (Figure 2). This was followed by order *Clionida* and *Scopalinida* (12%, 3), *Suberiterida* (8%, 2), order *Axinellida*, *Tethyida*, *Poecilosclerida* and *Agelasida* (4%, 1 sample, respectively).

Among the sampling locations, station F is inhabited by unique sponges species. This location consists of a cluster of boulders surrounded by sand and affected by the swift current. On the sandy part of this location, only a few sponge species were found, this included *Stelletta clavosa,* and *Strongylacidon bermuda* (with sand in the sponge body). *Strongylacidon bermuda* can adapt to the sandy substrate and swift current by including sand in its body as the anchor. Simultaneously, *S. clavosa* used its sticky hook-like dermal spicule and round shape to adapt to the swift current; most of the samples of this species were found to be attached to seaweed or other sponges (Bacero *et al.,* 2012).

Despite being unnoticeable and unevolved like other species, marine sponges continue to produce vast secondary metabolites that have proven to be useful (Lee *et al.,* 2021). DNA barcoding emerged as one of the tools used to

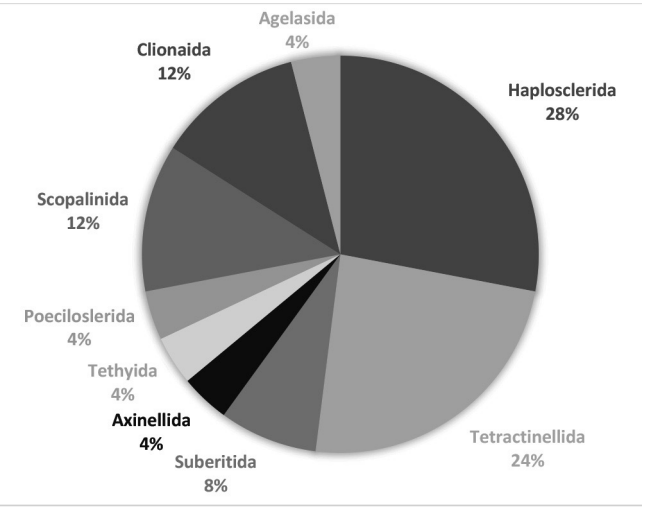


Figure 2: Proportion of sample identified Order under Subclass *Heteroscleromorpha*

speed up identification up until species name, in this study, however, when only one method of DNA barcoding was used, the identification of marine sponges has been considered challenging to determine its specific species name fully. DNA barcoding of marine sponges in this study was directed to amplification and sequencing using the mitochondrial COI gene. The mitochondrial DNA of the sponge was used as the basis for sponge identification, but due to the complexity of sponge metagenome deriving with other diverse invertebrates associated with marine sponges, amplification attempts by using the COI gene failed at certain PCR conditions (Kurnia *et al.,* 2017). Consequently, this varying non-targeted microorganism appears to be a hold-up in marine sponge identification as the amplification of the primers favours them rather than the actual sponge mitochondrial DNA. This could be due to the COI gene that has diverged too little to be diagnosed for specific species in marine sponges where COI genes are predominantly paraphyletic (Neigel, 2007; Bucklin *et al.,* 2011). Some researchers solved this problem by separating invertebrates from marine sponge tissues. The extracted mitochondrial DNA that has been relatively free from associated invertebrates are amplified using the COI gene, where results from the amplification of the COI gene showed target PCR products of an average of approximately 600 bp on 1% of agarose gel for most of the marine sponges identified. It can be highlighted that removing the associate microbiome during the extraction of mitochondrial DNA gives a higher percentage of success rate in identification by using the COI gene in marine sponges.

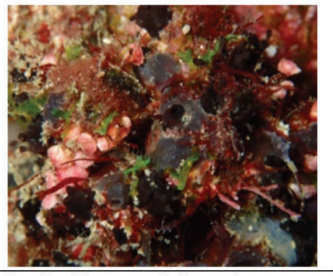
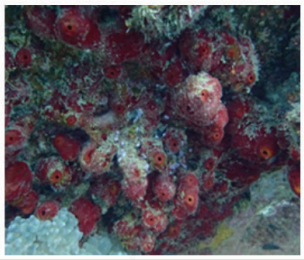
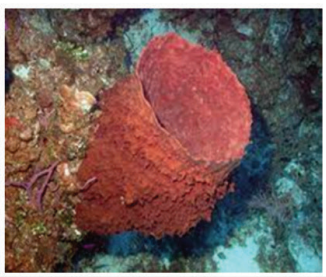
Conclusion

Regardless of the significant limitation, DNA barcoding can be utilised for marine biodiversity surveys and strategies. DNA sequence plays a critical role in biodiversity assessment in marine ecosystems. The main issue in the utilisation of the standard COI gene marker is that it does not reveal adequate variability for specific species diagnosis. In cases of low DNA sequence similarities, the marine sponges' identification should be based on data from morphological and histological analysis.

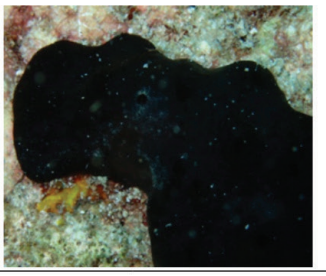
Acknowledgements

Authors would like to thank the Malaysian government for the Fundamental Research Grant (FRGS number 59384) research funding and sampling team of Institute of Marine Biotechnology Universiti Malaysia Terengganu, for samples collection.

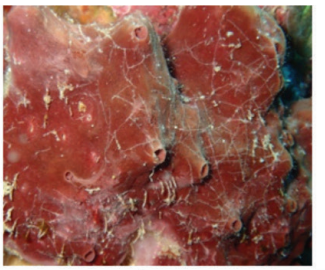
Table 1: Photographs, name of species, accession numbers and location of identified samples from this study

					
Species Name	*Paratetilla bacca*	Species Name	*Sollasipelta* sp.	Species Name	*Theonella cupola*
Accession Number	MK43885	Accession Number	MK43887	Accession Number	MK473904
Location	A	Location	B, C	Location	B
					
Species Name	*Theonella swinhoei*	Species Name	*Theonella mirabilis*	Species Name	*Stelletta clavosa*
Accession Number	MK903075	Accession Number	MK903074	Accession Number	MK473900
Location	B, C	Location	D	Location	D
					
Species Name	*Haliclona fascigera*	Species Name	*Haliclona amboinensis*	Species Name	*Xestospongia muta*
Accession Number	MK473891	Accession Number	MK43899	Accession Number	MK473903
Location	A, C, D	Location	D	Location	A, B, C, E, F, G

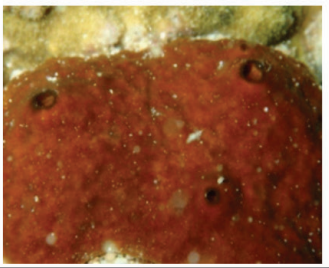
Species Name	*Xestospongia testudinaria*
Accession Number	MK903078
Location	A, B, E, F, G



Species Name	*Petrosia ficiformis*
Accession Number	MK473906
Location	D

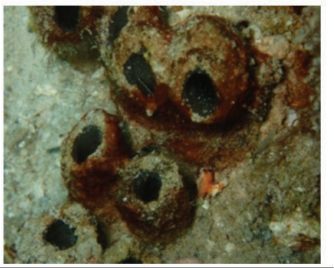


Species Name	*Cribrochalina vasculum*
Accession Number	MK473898
Location	D



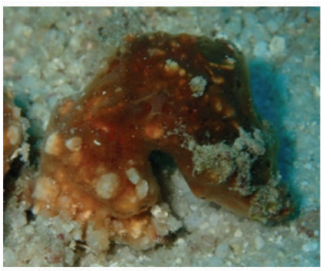
Species Name	*Hymeniacidon* sp.
Accession Number	MK473888
Location	B

Species Name	*Rhizaxinella* sp.
Accession Number	MK473901
Location	F

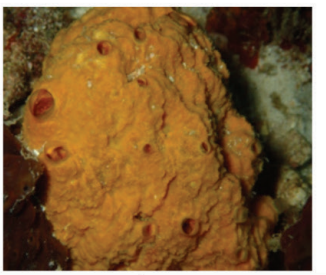


Species Name	*Axinella* sp.
Accession Number	MK473892
Location	C

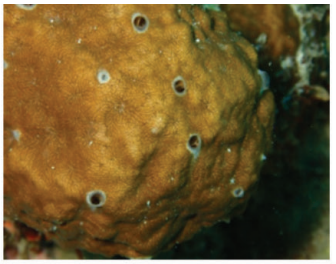
Species Name	*Hemiastrella* sp.
Accession Number	MK473905
Location	C, D



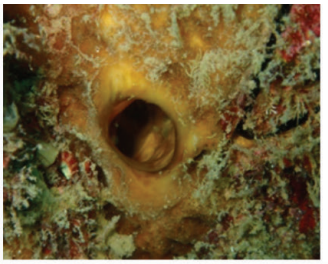
Species Name	*Strongylacidon bermuda*
Accession Number	MK473899
Location	D



Species Name	*Stylissa carteri*
Accession Number	MK473894
Location	B, E, G

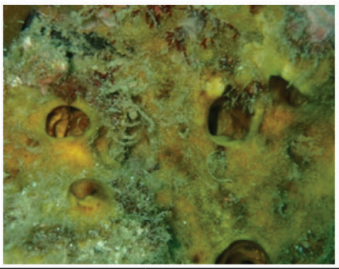


Species Name	*Cliona* sp.
Accession Number	MK473890
Location	G

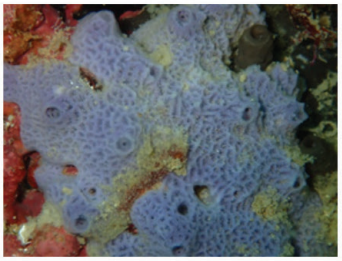


Species Name	*Cliona* sp.
Accession Number	MK473895
Location	D

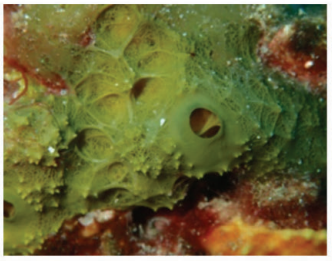
Species Name	*Agelas sventres*
Accession Number	MK473902
Location	F



Species Name	*Spheciospongia* sp.
Accession Number	MK903076
Location	D, F



Species Name	*Chalinidae* sp.
Accession Number	MK903080
Location	G



Species Name	*Subarea mollis*
Accession Number	MK903083
Location	G

References

Amiri, S., Ghavam Mostafavi, P., Nabavi, M. B., & Shahhosseini, M. H. (2018). DNA barcoding of Nereididae polychaetes based on COI gene barcoding in intertidal shores of Busher and Bandar Abbas provinces, Iran. *Iranian Journal of Fisheries Sciences*, *19*(3), 1193-1213. DOI: 10.22092/ijfs.2019. 120645.

Becerro, M. A., Uriz, M. J., Maldonado, M., & Turon, X. (2012). *Advances in marine biology: Advances in sponge science: Phylogeny, systematics, ecology*. Singapore: Elsevier. Pp 430.

Bucklin, A., Steinke, D., & Blanco-Bercial, L. (2011). DNA barcoding of Marine Metazoa. *Annu. Rev. Mar. Sci., 3*(1), 471-508. https://doi.org/10.1146/annurev-marine-120308-080950.

Buhl-Mortensen, L., Vanreusel, A., Gooday, A. J., Levin, L. A., Priede, I. G., Buhl-Mortensen, P., Gheerardyn, H., King, N. J., & Raes, M. (2010). Biological structures as a source of habitat heterogeneity and biodiversity on the deep ocean margins. *Marine Ecology-An Evolutionary Perspective*, *31*(1), 21-50. doi: 10.1111/j.1439-0485.2010. 00359.x

Imsiecke, G., Steffen, R., Custodio, M., Borojevic, R., & Muller, W. E. (1995). Formation of spicules by sclerocytes from the freshwater sponge Ephydatia muelleri in short-term cultures in vitro. *In Vitro Cell Dev Biol Anim, 31*(7), 528-535. doi: 10.1007/BF02634030

Kurnia, N. M., Uria, A. R., Kusnadi, Y., Dinawati, L., Zilda, D. S., Hadi, T. A., Setyahadi, S., & Felix, F. (2017). Metagenomic survey of

potential symbiotic bacteria and Polyketide Synthase Genes in an Indonesian Marine Sponge. *HAYATI Journal of Biosciences, 24*(1), 6-15. https://doi.org/https://doi.org/10.1016/j.hjb.2017.04.004

Lee, Y.-J., Cho, Y., & Tran, H. N. K. (2021). Secondary metabolites from the marine sponges of the Genus Petrosia: A literature review of 43 years of research. *Marine Drugs, 19*(3), 122. https://doi.org/10.3390/md19030122

Maldonado, M., Ribes, M., & van Duyl, F. C. (2012). Nutrient fluxes through sponges: Biology, budgets, and ecological implications. *Advances in Marine Biology, 62*, 113-182. https://doi.org/10.1016/B978-0-12-394283-8.00003-5

Neigel, J., Domingo, A., & Stake, J. (2007). DNA barcoding as a tool for coral reef conservation. *Coral Reefs*, *26*(3), 487. https://doi.org/10.1007/s00338-007-0248-4

Núñez-Pons, L., Calcinai, B., & Gates, R. D. (2017). Who's there? – First morphological and DNA barcoding catalogue of the shallow Hawai'ian sponge fauna. *PlosOne*, *12*(12), e0189357. https://doi.org/10.1371/journal.pone.0189357

Van Soest, R. W. M., Boury-Esnault, N., Vacelet, J., Dohrmann, M., Erpenbeck, D., De Voogd, N. J., Santodomingo, N., Vanhoorne, B., Kelly, M., & Hooper, J. N. A. (2012). Global diversity of sponges (Porifera). *PlosOne, 7*(4), e35105. doi: 10.1371/journal.pone.0035105

Vargas, S., Schuster, A., Sacher, K., Büttner, G., Schätzle, S., Läuchli, B., Hall, K., Hooper, J. N. A., Erpenbeck, D., & Wörheide, G. (2012). Barcoding sponges: An overview based on comprehensive sampling. *Plos One*, *7*(7), e39345. doi: 10.1371/journal.pone.0039345

Verdín Padilla, C. J., Carballo, J. L., & Camacho, M. L. (2010). A qualitative assessment of sponge-feeding organisms from the Mexican Pacific Coast. *The open Marine Biology Journal*, *4*, 39-46. DOI: 10.2174/1874450801004010039

Wörheide, G., Dohrmann, M., Erpenbeck, D., Larroux, C., Maldonado, M., Voigt, O., Borchiellini, C., & Lavrov, D. V. (2012). Chapter One - Deep phylogeny and evolution of sponges (Phylum Porifera). In M. A. Becerro, M. J. Uriz, M. Maldonado, & X. Turon (Eds.), *Advances in marine biology* (Vol. 61 pp. 1-78). Academic Press. https://doi.org/https://doi.org/10.1016/B978-0-12-387787-1.00007-6

Yang, Q., Franco, C. M., Sorokin, S. J., & Zhang, W. (2017). Development of a multilocus-based approach for the sponge (phylum Porifera) identification: Refinement and limitations. *Scientific Reports, 7*, 41422. https: //doi.org/10.1038/srep41422.