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DNA barcode of seven species coral from Sepulu, Madura Island, Indonesia

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Abstract. *Insafitri, Nursalim N, Kholilah N, Kurniasih EM, Cahyani NKD, Nugraha WA, Ambariyanto A. 2023. DNA barcode of seven species coral from Sepulu, Madura Island, Indonesia. Biodiversitas 24: 317-323.* Diversity of coral species is needed because it affects the disruption of coral reef ecosystems, nutrient recycling, coral reef growth and the habitat of biota that live in these ecosystems. The main constituents of coral reef ecosystems are coral animals that belong to the Anthozoa class. Sepulu waters (Madura Island), Bangkalan District, East Java Province, Indonesia, has a percentage of coral cover that is in the bad category, management of coral reefs in this area has not been the main focus and it is feared that the situation will get worse so efforts are needed to maintain coral reefs to be sustainable and better. This management effort requires an analysis of species identification. DNA barcoding of corals has never been done on corals of Sepulu waters. The purpose of this study is to find out the DNA of barcoding coral species on Sepulu waters. Genetic marker Cytochrome Oxidase I of the mitochondrial genome DNA (mtDNA) was used to analyze genetic diversity. Reconstruction of phylogenetic tree and genetic diversity were done by using software MEGA X. Research results showed that sample DBP011101 closely related to *Dipsatraea maxima* 99.55%, DBP011102 closely related to *Porites rus* 99.4%, DBP011103 closely related to *Acropora hyacinthus* 99.11%, DBP011104 closely related to *Porites cf lichen* 98.66%, DBP011106 closely related to *Dipsastraea rotumana* 95.37%, DBP011107 closely related to *Porites horrisoni* 98.81%, and DBP011108 closely related to *Acropora valida* 98.38%. The results of the study are the first coral barcode DNA on Sepulu waters that can help in determining coral species that are useful in managing the sustainability of coral resources, especially on Sepulu waters.

Keywords: Biodiversity, coral species, Cytochrome C Oxidase I, mitochondrial gene, phylogenetic

INTRODUCTION

Coral reef is a coastal ecosystem which contains important and beneficial natural resources. Coral reefs have many good functions in ecology that act as biota habitats for many biotas such as octopus (Kholilah et al. 2021), giant clam (DeBoer et al. 2012), sea cucumber (Widianingsih et al. 2021; Sulardiono et al. 2022), and lobsters (Haryono and Ambariyanto 2018). Even the research of Pertiwi et al. (2015), states that dead corals can become habitats for decapods. Coral animals as the main constituents of coral reefs have benefits that are now widely studied, including in the pharmaceutical world, which has the potential to be an antibacterial source (Radjasa 2004; Sabdono and Radjasa 2006; Radjasa et al. 2007; Radjasa et al. 2008; Radjasa et al. 2013; Sabdono et al. 2015; Cristianawati et al. 2016; Trianto et al. 2017), bioactive (Radjasa 2004), antifungal (Riyanti et al. 2016), polyester biodegradation (Widyananto et al. 2022), and biodegradation of herbicide compounds (Radjasa and Sabdono 2005).

Coral reefs have high species diversity which is the result of ecological and evolutionary processes (Hughes et al. 2008). Coral reefs are habitat to 25 percent to 33 percent of species on this earth (Plaisance et al. 2011) even though it occupies 0.1% of the area on earth (Bouchet 2006). There are 590 species of hard coral in Indonesia which are included in 80 genera (Suharsono 2010). This number of species illustrates that Indonesian waters contain 69.42% of the world's coral, the number species is 850 species (Hidayati et al. 2007) and is the center of world coral species diversity.

Sepulu is a sub-district in Bangkalan District, East Java Province, Indonesia. This area is located on Madura Island. The development of hard corals currently is quite concerning, because coral reefs in this area are currently experiencing a lot of pressure, both from nature and from humans (Ariyanti et al. 2022). Based on data, Sepulu, Bangkalan, Madura coast has coral reef potential with a percent cover of 11.37% (Ariyanti et al. 2022), categorized as bad. Total percentage obtained live coral cover categorized based on Gomez and Yap (1988), as follows;

0-24.9% (bad), 25-49.9% (moderate), 50-74.9% (good) and 75-100% (very good). The causes of damage to hard corals are caused by several factors, including boat or ship anchors, household waste and other factors. In addition, hard coral has also used for house building materials, so there is concern that damage will occur as a result of taking this type of coral, and there is even a tendency for certain types of coral to disappear. Until now the management of coral reefs in Sepulu, Bangkalan has not become an important concern. While the level of damage is feared to be more severe and human activities on coral reef ecosystems are getting out of control. Therefore, research on species diversity is very important to know the real conditions. Data and information on species diversity form the basis for management and conservation policies for coral reef ecosystems in this area. Accurate data on coral species will support government programs related to the conservation and rehabilitation of coastal and marine ecosystems as a focus area for maritime research. To be able to manage a coral reef area properly and correctly, we must really know the condition and health of the existing ecosystem, what natural resources can be utilized and what are their conditions.

The diversity of life in the oceans can be analyzed and understood at many levels of organization, including higher taxonomic levels, functional groups, species, populations and individuals. Careful processes of collecting, classifying, and identifying species and describing new species are still urgently needed to manage coral resources in a sustainable manner. Species identification is the most time-consuming, difficult, and often creates obstacles in data collection and analysis. Identification of coral species can be done based on coral morphology but it is quite complicated to do so a reliable and efficient method is needed one method of coral identification that is now developing is using molecular analysis of DNA barcoding (Wijayanti et al. 2004, 2018). Hebert et al. (2003) stated that DNA barcodes based on the mitochondrial gene of Cytochrome C Oxidase I (COI) are very effective because they are cost-effective, fast, and accurate for species identification and can generate new insights into genetic diversity and molecular evolution making it very useful for the preservation of marine resources. Furthermore, DNA barcoding can detect errors in the involvement of species with very few morphological differences and it is difficult to know the differences and complex morphological characteristics, thus adding genetic data, ecological data, morphological data, geographic data, and behavioral data (Bucklin and Frost 2009). Until now research in Sepulu, Bangkalan on the identification of coral is carried out only at the level of coral growth forms and not up to species. The purpose of this study was to determine the DNA barcoding of coral species in Sepulu waters (Madura Island), Bangkalan District, East Java Province, Indonesia. This research is the first attempt to identify species using molecular analysis of DNA barcoding and is expected to be useful for coral resource management.

MATERIALS AND METHODS

Study area

Sampling was carried out in July 2022 in the Sepulu Waters of Madura Island, Bangkalan District, East Java Province, Indonesia at a depth of 1 m (Figure 1).

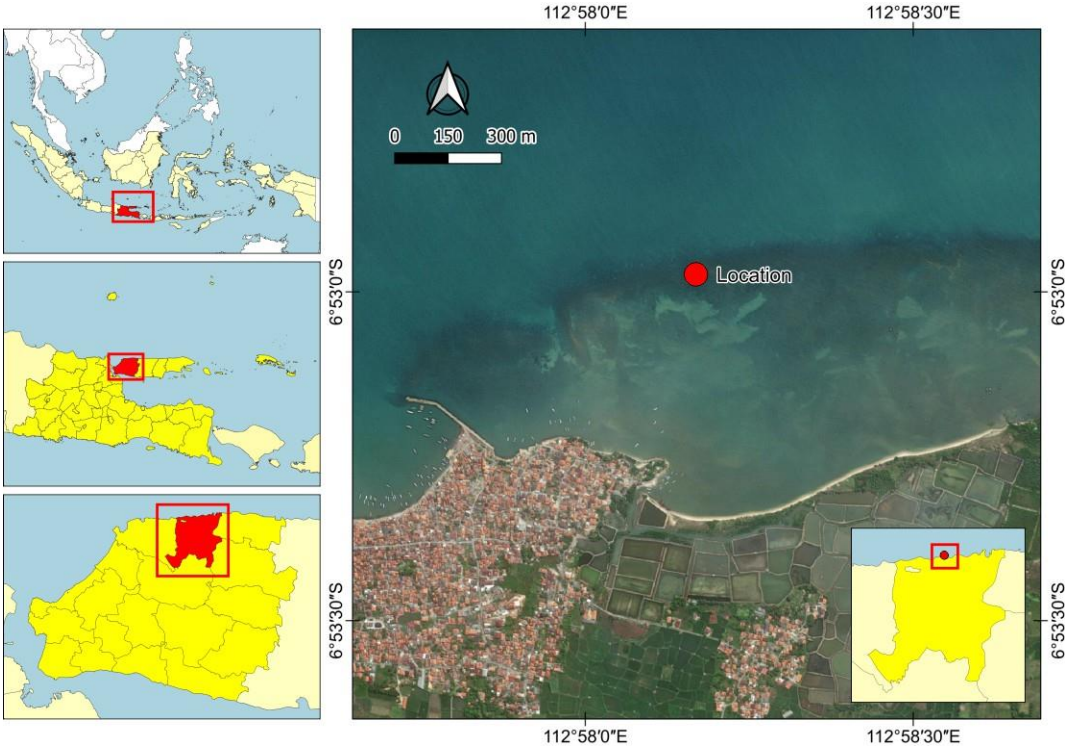

Figure 1. Location of Sepulu, Bangkalan District, East Java Province, Indonesia indicating the sampling sites of coral sample

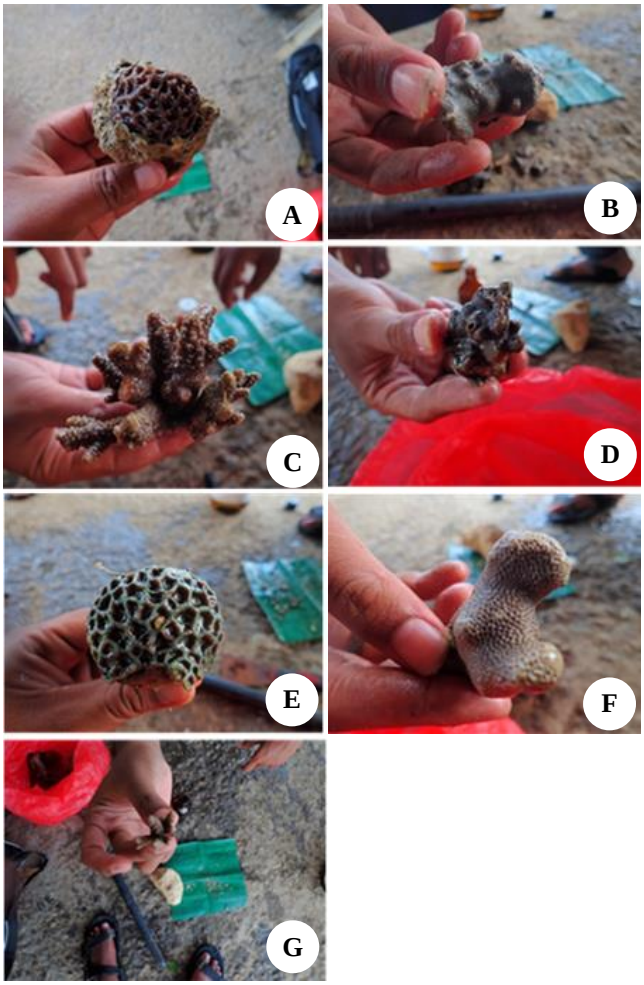

Figure 2. Photos of coral sample from Sepulu waters, Bangkalan District, East Java Province, Indonesia. A. DBP011101. B. DBP011102. C. DBP011103. D. DBP011104. E. DBP011106. F. DBP011107. G. DBP011108

Procedures

Coral sample and preparation

Each of the coral colonies was documented and identified morphologically using Veron (2000) (Figure 2). Coral colonies are cut by 1-2 cm using cutting pliers. Small pieces were then put into a 2 mL labeled cryovial. To avoid resampled, each colony that was already cut was tagged using the small plastic number and cable ties. The Samples were preserved in 95 % ethanol until used. In total, 7 corals (which is abundant in this area) were further processed that have different morphologies by followed coral finder protocol. We observed the coral texture, shape, and lifeform to decide key group from the main page of coral finder, follow the prompts & select the similar page of "best bets" for what coral samples look like by confirmed some characters of the coralite form and size, type of wall, form of septa, form of costae, axial coralite.

DNA extraction

DNA extraction was carried out using a quick and simple DNA extraction method with Chelex 100 resin (BioRad). A 0.2 mm section of tissues were scraped using sterile spatula and place into 1.5 mL tubes filled with 250 uL Chelex 10%. The samples were then vortexed in Chelex slurry for 10-15 seconds briefly at high speed and centrifuged for 10-15 seconds. Incubated for 45 minutes at 95°C (Akbar and Aris 2018). The Chelex then centrifuge again for 10-15 seconds to ensure that all contents are in the bottom of the microcentrifuge tube. The supernatant was directly used for Polymerase Chain Reaction (PCR) amplification.

PCR amplification

The PCR reaction was carried out in 25 µL volumes, using 1.25 µL of template. Universal primer for COI gene, consisted of forward primer JgLCO1490: 5'-TITCIACI AAYCAYAARGAYATTGG-3' and reverse primer JgHCO2198: 5'-TAIACYTCIGGRTGICCRAARAAYCA-3' (Geller et al. 2013).

The PCR reaction was carried out in 25 µL volumes, using 1.25 µL of DNA template. Each reaction included 12.5 µL MyTeqTM Red Mix (Bioline), 1 µL of each primer and 9.25 µL ddH_2O. The thermocycling profile included an initial denaturation of 95°C for 4 min, 40 cycles of 95°C for 30s, 50°C for 30s, and 72°C for 1 min, with a final extension of 72°C for 10 min. The PCR reactions were checked on 1% agarose gels stained with Florosafe.

Forward and reverse sequences were proofread using MEGA X then aligned using ClustalW (Kumar et al. 2016). Individual sequence were compared to National Center for Biotechnology Information (NCBI; https://www.ncbi.nlm.nih.gov/) data using Program Basic Local Alignment Search *Tool*; https://blast.ncbi.nlm.nih.gov/Blast.cgi (BLAST). Neigbor Joining (NJ) methods were used to generate phylogenetic reconstructions The NJ analysis based on genetic distance, was conducted in MEGA X with the Kimura 2-Parameter model using 1000 bootstrap replicates to assess clade support.

RESULTS AND DISCUSSION

Coral species sequence

Diversity of coral species is needed because it affects the disruption of coral reef ecosystems, nutrient recycling, coral reef growth and the habitat of biota that live in these ecosystems. In total, there are seven sequences obtained from Sepulu waters, Indonesia (Table 1). The BLAST results for COI sequences homology shows three genus and seven species with 98.38-99.55% similarity (Table 1). Sample DBP011102 is closely related to *Dipsatraea maxima* (99.55%), DBP011102 is closely related to *Porites rus* (99.4%), DBP011103 is closely related to *Acropora hyacinthus* (99.11%), DBP011104 is closely related to *Porites cf lichen* (98.66%), DBP011106 is closely related to *Dipsastraea rotumana* (95.37%), DBP011107 is closely related to *Porites horrisoni* (98.81%), and DBP011108 is closely related to *Acropora valida* (98.38%). These similarities between the sample sequences and the NCBI data is quite high, range from 99.55% to 98.38%. The observations of coral morphology in this study are only

limited to genus identification, morphology up to the species level is very necessary but very difficult to do because we must observe complicated coral morphological details (Wallace 2008) such as colony form, branch type, radial corallite, coral wall, and coenosteum. The form of colony growth interpreted can be observed when sampling in the field. For branch types are described as terete or tapered. The shape of the radial corallite is a key characteristic for identifying species and groups of species; The description includes the shape of the corallite, the shape of the calice (round, oblong or dimidiate), the indication of the size (the same size, graded or two different sizes) and the density of the coralite on the branches (in contact or touch). Radial coral walls and coenosteal architecture (axial coral walls) are described, based on the type of spinulae and the organization of the spinulae as costate, reticulate or reticulocostate.

Corals are one of a large family of marine life that has a sting or better known as the phylum Cnidaria (cnida = nettle). In recent developments what is meant by cnidarians are lime-producing biota only (Suharsono 2008). Cnidaria is divided into two, namely Hydrozoans and Anthozoa which are biota that have skeletons in their bodies. Hard corals are in the order Scleractinia. In this study there is the genus *Acropora*, by far the most diverse genus of living reef-forming corals, *Acropora* is distinguished from other extant coral genera with a unique polyp structure shape. Corallites are small, divided into two types of axial and radial. Corallite has septa in two cycles or less and columellae rarely develop (Veron 2000). According to Suharsono (2008) a feature of the genus *Acropora* is the branching form varies greatly from corymbose, arborescent, caepitose and others. A distinctive feature of this genus is that it has axial corallite and radial corallite. The radial shape of the corallite also varies from the tubular form to nariform, and sinking. This genus has about 113 species, spread throughout Indonesian waters. The species in the genus Acropora found in this study are the species *A. valida* and *A. hyacnthus*, according to Wallace (1999) the morphological feature of *A. valida* is that radial corallites are evenly distributed, flattened into partially closed tubular with nariform openings. Coenosteum reticulated, simple spinules or laterally flat. *A. hyacinthus* according to Suharsono (2008) has morphological characteristics of a wide table-shaped colony that can reach a size of 4 meters. Vertical branches are relatively small with small coral axials but can still be distinguished from bowl-shaped radial corallites, with color dark brown or gray.

The genus *Porites* was also identified in this study. Suharsono (2008) stated that the characteristic of the genus *Porites* is that colony has a form of massive change, encrusting, branching and sheets, small cereoid corallites. Septa unite with each other and form a very distinctive structure that is used for identification. This characteristic includes the presence of three septas that merge into one called a triplet with one pali. The species in the genus Porites found in this study are *Porites lichen*, *P. rus, P. horrisini.* The *P. lichen*, according to Suharsono (2008) has a morphology of creeping colonies with various irregular protrusions or short branching. Corallites are scattered irregularly and form small irregular trenches. Irregular septa with irregular teeth. Characteristics of *P. rus* Colonies are massive, sheets or branches that are united with each other. Corallites are small and clustered. The surface of the colony is rough irregular and notched. The species *P. horrisoni* seems to be new in this study reported to exist in Indonesia.

The genus *Dipsastrea* described in this study is a species of *Dipsastraea maxima* which according to Veron et al. (1977) its real name is *Favia maxima* which has the characteristics of the genus *Favia,* according to Suharsono (2008) is a massive colony with varying sizes. Corallites tend to be placoid in shape with intratentacular budding. Corallites tend to be rounded to varying sizes. Septa is well developed with regular teeth. This clan has about 20 types, spread throughout Indonesian waters. This species has the characteristic of a massive colony usually with a small size. Corallite has perfect walls. Septa thickens near the wall and has a pali that forms a crown near the columella. Fawn color sometimes with a dark green tint.

Phylogenetic tree

Phylogenetic analysis of samples had been collected from Sepulu waters, Indonesia and gene bank using DNA barcoding (COI) was successful. Phylogenetic construction based on COI sequences found four distinct clusters of seven coral taken from Sepulu waters (Figure 3). *Thunnus albacares* sequence was used as outgroup in the phylogenetic reconstruction because *T. albacares* also using COI gene. Clade 1 was comprised of 2 samples (DBP011108 and DBP011103), clade 2 consisted each colony of DBP011102, BP011104, DBP011107, clade 3 consisted only calony DBP011106, while clade 4 sample DBP011101.

Table 1. BLAST results of seven sequences from Sepulu waters, Bangkalan District, East Java Province, Indonesia

Sample code	Percentage of sequences homologies closely related to
DBP011101	*Dipsatraea maxima* 99.55%
DBP011102	*Porites rus* 99.4%
DBP011103	*Acropora hyacinthus* 99.11%
DBP011104	*Porites cf lichen* 98.66%
DBP011106	*Dipsastraea maxima* 98.45%
DBP011107	*Porites horrisoni* 98.81
DBP011108	*Acropora valida* 98.38%.

Table 2. Genetic distance between clades

	Clade 1	Clade 2	Clade 3	Clade 4
Clade 1				
Clade 2	0.113			
Clade 3	0.317	0.300		
Clade 4	0.227	0.227	0.305	
Clade 5	0.479	0.516	0.517	0.476

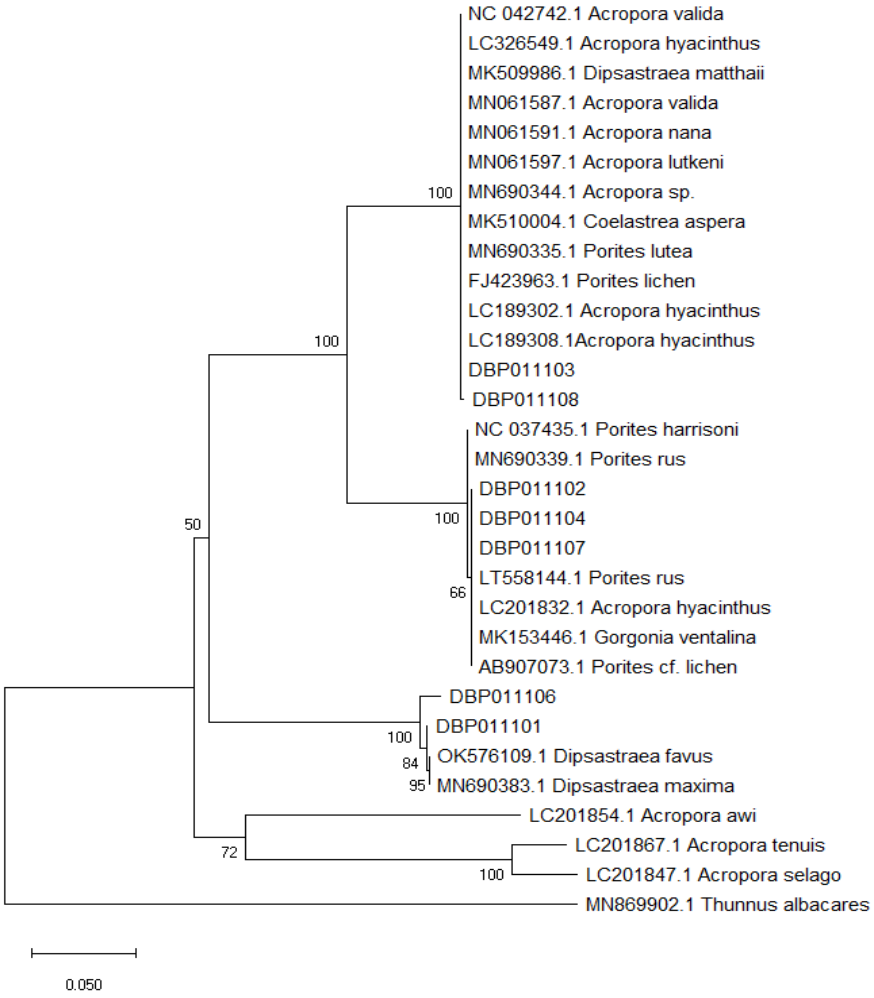

Figure 3. Phylogenetic tree construction of seven corals (squared) collected from Sepulu waters (Madura Island), Bangkalan District, East Java Province, Indonesia. Samples from this tudy are highlighted. There are five different clades observed from the tree. *Thunnus albacares* was used as outgroup. The genetic distance between clades can be seen in Table 2

The results showed that in one clade, judging from the homology sequences, it is indeed in the same genus as clade 1 which consists of samples DBP011108 and DBP011103, the sequences are close to the same genus, namely Acropora as well as clade 2 which consists of samples DBP011102, BP011104, DBP011107 sequences are closely related to the same genus, namely Porites. This is in accordance with the Clade 3 study, namely sample Clade 3, namely sample DBP011106, even though clades 3 and 4 are still one group. Furthermore, the phylogenetic tree samples were divided into two main clade complexes, namely Clade 1 and 2 which are in one group (consisting of the genera *Acropora*, *Porites*, *Coelastrea*, *Gorgonia*, and *Dipsastraea matthaii*) and groups of clades 3 and 4 which consist of the genus *Dipsastraea.* This is in accordance with the study by Sadek et al. (2018) that Acroporidae is in the same major clade as Poritidae. When viewed from morphological characteristics, all samples in clade 1 show the same characteristics, namely having axial corallite and radial corallite, so it makes sense to be one clade. While all samples in clade 2 have small corallite 0.5-1.5 mm, walls that share and have walls that are perfectly formed so that it is normal if they are absent in one clade. This is appropriate with Veron (2000) clade 1 has axial and radial corallite which is the main feature of the genus *Acropora* (Veron 2000). Meanwhile, clade 2 has the main characteristic of the genus *Porites* has shared walls with a corallite diameter of less than 2 mm and perfectly formed walls. Clades 3 and 4 have the main characteristics of the genus *Dipsastraea* which has a thick share wall and septa.

In one clade there are many species similar to other studies such as the studies of Min-Hsu et al. (2014) and Sadek et al. (2018). Clade 1 consisting of samples DBP011108 and DBP011103, *A. valida, A. hyacinthus, A. nana, A. lutkeni, P. lutea, P. lichen, C. aspera,* and *D. matthai.* Clade 2 consists of *P. horrisoni, P. rus* together with samples DBP011102, BP011104, DBP011107. What is interesting is that the same genus *Porites* is in a different clade. This is also the same as Sadek's research (2018) which states that *P. horrisoni* is in a different clade than

others *Porites* species. Within one clade there are many different species, this is because the length of the COI sequence cannot differentiate species (Bucklin et al. 2011). It is possible that the GenBank sequence used in this study has a longer sequence to differentiate species, while the species used in this study are shorter.

To conclude, COI can be used for molecular identification of corals, but for more precision, longer sequences are needed. In Sepulu waters, Indonesia, this is the first study of phylogenetic analysis to seven scleractinian corals species. Further research is required on Sepulu, Indonesia in order to understand coral genetic diversity in order to manage the coral resources. Sample DBP011102 is closely related to *D. maxima* (99.55%), DBP011102 is closely related to *P. rus* (99.4%), DBP011103 is closely related to *A. hyacinthus* (99.11%), DBP011104 is closely related to *P. cf lichen* (98.66%), DBP011106 is closely related to *D. rotumana* (95.37%), DBP011107 is closely related to *P. horrisoni* (98.81%), and DBP011108 is closely related to *A. valida* (98.38%).

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