BIODIVERSITAS
Volume 23, Number 2, February 2022
Pages: 922-927

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d230234

Genetic diversity of commercial sea cucumbers *Stichopus* (Echinoderm: Stichopodidae) based on DNA Barcoding in Karimunjawa, Indonesia

BAMBANG SULARDIONO[1,♥], AGUS HARTOKO[1], ARIFAH NUR AINI[3], DYAH WULANDARI[3], ANTO BUDIHARJO[2,3,♥♥]

[1]Department of Aquatic Resources, Faculty of Fisheries and Marine Science, Universitas Diponegoro. Jl. Prof. Sudharto SH, Semarang 50275, Central Java, Indonesia. Tel.: +62-24-7474698, ♥♥email: bambangsulardiono@gmail.com

[2]Program of Biotechnology, Faculty of Science and Mathematics, Universitas Diponegoro. Jl. Prof. Sudharto SH, Semarang 50275, Central Java, Indonesia. Tel./fax.: +62-24-7474754, ♥♥email: anto.budiharjo@fulbrightmail.org

[3]Molecular and Applied Microbiology Laboratory, Central Laboratory of Research and Service, Universitas Diponegoro. Jl. Prof. Sudharto SH, Semarang 50275, Central Java, Indonesia

Manuscript received: 24 December 2021. Revision accepted: 24 January 2022

Abstract. *Sulardiono B, Hartoko A, Aini AN, Wulandari D, Budiharjo A. 2022. Genetic diversity of commercial sea cucumbers* Stichopus *(Echinoderm: Stichopodidae) based on DNA Barcoding in Karimunjawa, Indonesia. Biodiversitas 23: 922-927.* DNA barcoding has proven a sufficient tool for species identification in varied groups of marine invertebrates, including crustaceans, mollusks, polychaetes, and echinoderms. The outdated system of morphological identification of the *Stichopus* spp. group is generally based on color patterns, causing confusion in identification. This study aimed to investigate genetic diversity, confirm the species identification, and examine phylogenetic relationships of the *Stichopus* genus (Echinoderm: Stichopodae) in the Karimunjawa Sea, by analyzing DNA sequence variation at cytochrome C oxidase subunit I (COI) in mitochondrial DNA and internal transcribed spacers (ITS). This genetic information is very helpful in inventorying and mapping commercial sea cucumber resources in Karimunjawa to support conservation efforts. Three types of sea cucumbers from the genus *Stichopus* were found, with different local names, namely Crengek gamete, Pace gamete, and Kuning gamete. The results of DNA extraction from the three samples showed that the DNA from soft bone tissue had a better level of purity and concentration than body tissue. The COI phylogenetic tree analysis showed that these samples with different local names were closely related to *Stichopus monotuberculatus*. Therefore, it is necessary to conserve the resources of the *S. monotuberculatus* in the management of Karimunjawa sea.

Keywords: COI, ITS, molecular phylogenetic, sea cucumber taxonomy

INTRODUCTION

Sea cucumbers are a group of echinoderms (Conand 2004), which generally have important economic value, among them are species from the genus *Stichopus*. However, of all sea cucumbers species, not all have commercial value and the high commercial value of each species is highly dependent on the values of its chemical content. As it is known, sea cucumbers have been known to contain bioactive compounds (Lawrence et al. 2009) that scientifically has benefited as antioxidant properties, anti-inflammatory, and antiaging.

Sea cucumbers live in the habitat of seagrass ecosystems and coral reefs, ranging from shallow waters to depths of more than 3000 meters (Purcell et al. 2012). Sea cucumbers are included in the class Holothuroidea and belong to the phylum Echinodermata. There are seven orders in this class, namely Dendrochirotida, Synallactida, Molpadida, Persiculida, Holothuriida, Elasipodida, and Apodida, which can be distinguished based on their ossicles and tentacle shape (Miller et al. 2017). Indonesia has 24 commercial sea cucumbers from the Holothuriidae (Holothuriida) and Stichopodidae (Synallactida) families scattered in the waters coral reefs and associations.

The waters in the west of Karimunjawa Island, Indonesia, are part of the Karimunjawa National Park area, which is included in the rehabilitation zone (Sulisyati et al. 2018). These waters store natural wealth, where one of them is a sea cucumber. The high number of commercial sea cucumbers, such as *Holothuria scabra, Holothuria nobilis, Holothuria fuscogilva, Thelenota ananas*, and other high commercial species in this area have been exploited for years. As a result of overexploitation, now the presence of these species are rare and hard to find. Fishermen in this area now switch to catching the other sea cucumbers with lower commercial value, such as Stichopodidae family, including the *Stichopus* genus (*Stichopus vastus, Stichopus variegatus, Stichopus horrens, Stichopus chloronotus, Stichopus ocellatus, Stichopus herrmanni* and others). In Karimunjawa, these types of sea cucumbers are one of the important commodities and as a source of livelihood for the local community. The local fishermen call the *Stichopus* group with the term "gamete" (Kamarudin et al. 2015). There are 1250 species identified in Indonesia (Oh et al. 2017), among them are *Stichopus herrmanni* (Semper 1868), *Stichopus horrens* (Selenka 1867), and *Stichopus monotuberculatus* (Quoy and Gaimard 1833).

The high demand for sea cucumbers encourages fishermen to do large-scale fishing, especially the high

commercial types. In Karimunjawa, sea cucumber fishing has been carried out excessively. This is supported by Sulardiono (2016) that the biomass potential of sea cucumber population in Karimunjawa of all species utilized in the study area up to a depth of 10 m is 44,641,789 individuals with a sustainable potential value (MSY) of 24,590,814 individuals year^{-1}, the allowable retrieval quota of 20,277,981 individuals year^{-1}, where for the *Stichopus* group taken such as *Stichopus vastus* and *Stichopus quadrifasciatus* each had a Maximum Sustainable Yield (MSY) value of 4,161,541.23 and 2,080,736, 56 individuals year^{-1} whereby the catch quota of each species should not exceed 3,329,232.98 and 1,664,589.25 individuals year^{-1}. Furthermore, Sulardiono (2011) said that the two species have a relatively long lifespan, namely for *S. vastus* with values of L∞: 315.8 mm and K: 0.55 years^{-1} and for *S. quadrifasciatus* with values L∞: 387.25 mm and K: 0.34 years^{-1}.

In order to protect the *Stichopus* caught in Karimunjawa from extinction, conservation measures are needed. The conservation efforts are focused on protecting genetic diversity, which causes biota to adapt to new conditions. Biological factors in nature make sea cucumbers vulnerable to low genetic diversity because, (i) sea cucumbers are sedentary and slow-moving (Friedman et al. 2008); (ii) low recruitment rates, with relatively far distances between males and females; (iii) reproductive failure often occurs due to low-density levels (Uthicke et al. 2004; Bell et al. 2008); (iv) at the larval level it is very vulnerable to predatory threats. Sea cucumbers are solitary and depend on the availability of food and habitat conditions. If there is overfishing, it will cause a decline in population or stock (Sulardiono 2011).

Another problem that exists in the community is the inconsistency in giving local names, especially for species belonging to *Stichopus*, because it often occurs with the same name to designate different species. *Stichopus* is one type of sea cucumber which is abundant in Karimunjawa waters. Morphologically, the *Stichopus* group has several forms of various patterns and colors, so local fishermen give it different names such as Crengek gamete, Pace gamete, and Kuning gamete. Therefore, we need a study on genetic diversity. Knowing the distribution of species through a genetic approach will be very helpful in the management of commercial sea cucumber resources, especially in providing information through the approach of systematic, ecological and evolutionary aspects. Knowing the biological and environmental aspects of sea cucumbers is needed in the management of resource conservation. So far, sea cucumber resource management has only emphasized a narrow definition, namely the diversity and size of sea cucumbers to be caught. Based on this, the biological perspective of sea cucumber resource management is more dominated by understanding population dynamics and ecology than by understanding population genetics. The genetic aspect of sea cucumbers is one of the most important parts in knowing the kinship and genetic variants of a sea cucumber population in an area.

The limited information regarding the genus of commercial sea cucumber in Karimunjawa has prompted an assessment of management efforts to use these resources sustainably. This research is also a genetic database of commercial sea cucumber resources of *Stichopus* in Indonesia so that resources can be mapped based on their genetic kinship level. One method that can be used to determine genetic variants and kinship in animals is to use the gene coding for cytochrome oxidase subunit I (COI) and internal transcribed spacers (ITS). The study aimed to identify commercial sea cucumber species from the genus *Stichopus* (Echinoderm: Stichopodidae) harvested by fishermen from Karimunjawa, Central Java Province, Indonesia based on the DNA barcoding approach.

MATERIALS AND METHODS

Sample collection

Samples of sea cucumbers from the genus *Stichopus* with different local names were collected from 3 fishermen operating in the waters west of Karimunjawa Island, Central Java province, Indonesia. Samples were taken as many as 100 individuals consisting of three sea cucumber types (30 Crengek gamete, 36 Pace gamete, and 34 Kuning gamete) were obtained from the catch of fishermen in the eastern waters of Karimunjawa Island with a depth of 10-15 m. The samples were then observed for morphological characterization and chosen one individual sample each type and taken to the laboratory for further examination. The tissue was preserved in 96% ethanol until DNA extraction. DNA extraction was carried out by taking 20 mg of sea cucumber body tissue (body wall) and mashing it. The refined samples were then put in a micro-centrifuge tube and DNA extraction was carried out using the Promega Genomic DNA Purification Wizard kit following company protocols. Two molecular marker genes used in this study are COI (Cytochrome C Oxidase subunit I) and ITS (Internal Transcribed Spacers).

DNA amplification and sequencing

Amplification was carried out by setting the temperature according to Byrne et al. (2010) in a total volume of 50 µL consisting of My Taq HS Red Mix (Bioline, UK), ddH2O, 10µM each primer, and 100 ng of template DNA. The primer pairs used in this study were COIe-F 5'-ATA ATG ATA GGA GGR TTT GG-3'/ COIe-R 5'-GCT CGT GTR TCT ACR TCC AT-3' for the COI gene and ITS_F1 5'-GTA GGT GAA CCT GCG GAA GGA-3/ITS_R1 5- GTT GGT TTC TTT TCC TCC GCT-3 for ITS gene (Itskovich et al. 2015). The Polymerase Chain Reaction (PCR) program for COI gene was set as follows: initial denaturation at 95°C for 4 min, 40 cycles of denaturation (95°C for 30 sec), annealing (53°C for 30 sec), and extension (72°C for 1 min) with the final extension step at 72°C for 10 min. The ITS gene was amplified following the program set as: 2 min initial denaturation at 94°C following 35 cycles of denaturation (94°C for 30 sec), annealing (49°C for 30 sec), and extension (72°C for 1 min 30 sec) with the final extension step at 72°C for 8 min. The PCR products were visualized by electrophoresis in 1% 100V agarose gel for 30 minutes.

After purification, the PCR products were then sent to 1st Base Malaysia for sequencing.

Data analysis

Sequence data were contig using BioEdit v.7.0.5.3 (Hall 2005). Additional sequences from GenBank were added as a dataset. Alignment was conducted with MUSCLE3.6 software (Edgar 2004). BLAST searches were used to compare the DNA sequences in this study with GenBank database to determine the closest matches with *Stichopus*. The final alignment was used for building a phylogenetic tree using maximum parsimony analyses. MP analyses used PAUP4.0a116 (Swofford 2019). Heuristic search was performed with 100 replications of random addition of sequences and the TBR (tree bisection reconnection swapping) algorithm. Bootstrap analysis was performed using the maximum parsimony criterion with 1000 replications and the addition of 10 sequences at random for each replication.

RESULTS AND DISCUSSION

Three types of sea cucumbers from 100 individual samples were obtained from fishermen's catch in the eastern waters of Karimunjawa Island with a depth of 10-15 m. The samples have different local names, Crengek gamete Pace gamete, and Kuning gamete. Based on the initial identification of the genus *Stichopus* from marine species identification portal, it generally has three rows of well-defined tube feet beneath, with the body wall having prominent papillae, these samples belonged to the genus *Stichopus*. However, they have different morphological characteristics, both pattern and color. According to Clark (1922) and Massin et al. (2002), the external morphology of the genus *Stichopus* is biased and highly deceptive with interspecific similarity and intraspecific variation. Furthermore, it is said that the genus *Stichopus* is taxonomically very confusing because of the similarity between each species in external morphology and the presence of distinctive color variations and patterns. The samples used this depicted in Figure 1.

Based on observations, the three types of gamete samples from fishermen's catches have varied pattern and color characteristics, although they have almost the same morphological similarities, namely trapezoidal cross-section, thick, dense, soft, and smooth body walls. Crengek gamete has a yellowish-brown dorsal color pattern with papillae projections in the form of brown pointed spines, and when under stress conditions, the spines appear to be weakened and flattened against on the body surface. In addition, the ventral part has legs like brownish-white feathers. Local people call it a Crengek gamete because it has pointed protrusions. Pace gamete has characteristic folds of skin with variations in greenish-yellow and green with black folds. Papillae with large protrusions with brown color scattered along the dorsal part, while the ventral part is brownish in color with white feather-like legs that have a striped pattern on the surface. People call the Pace gamete because it looks like a pace fruit. Kuning gamete has almost the same morphology as the pace gametes but with different color variations. The dorsal part of the Kuning gamete is dominated by brown to yellowish in color with mattress-like indentations, and there are brown spots that are prominent but not as sharp as the Crengek gamete. Local people call it Kuning gamete because it has a yellow color evenly along the dorsal side. The habitats of these three types of gametes are scattered between coral reefs associated with seagrass therefore, many call them coral gametes.

DNA extraction was carried out by taking soft bone tissue and body tissue from each sample of sea cucumbers. The quality of the extracted DNA qualitatively can be determined by electrophoresis, which is depicted in Figure 2. The genomic DNA size was found to be in the high molecular weight, which is above 10kb. From this gel electrophoresis, the concentration of genomic DNA could be estimated. Then use the concentration estimate to calculate the total DNA available from this sample compared with their marker.

DNA samples were successfully isolated. The purity of the isolated DNA can be determined quantitatively. Quantitative DNA quality can be determined by measuring the purity and concentration of DNA. The value of DNA purity was measured based on comparing the absorbance values of the wavelengths of 260 µm and 280 µm (A260/A280) on spectrophotometric readings. The results of measuring the concentration of DNA in this research sample are depicted in Table 1.

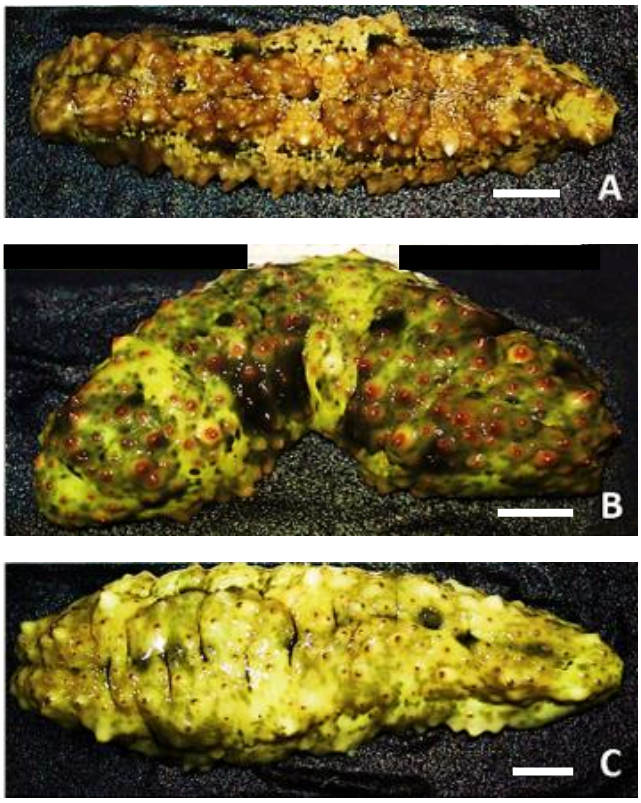

Figure 1. Samples of sea cucumbers (dorsal). A. Crengek gamete; B. Pace gamete; C. Kuning gamete. Bar = 3 cm

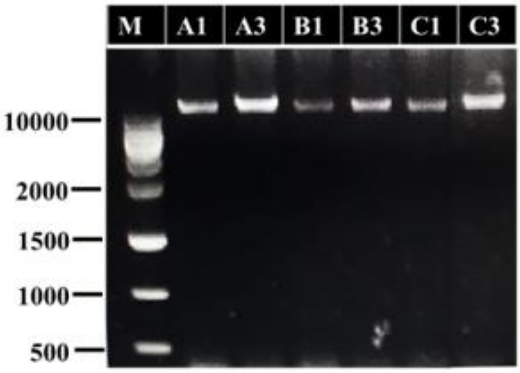

Figure 2. Results of sea cucumber genomic DNA electrophoresis. M. Marker; A. Crengek gamete; B. Pace gamete; C. Kuning gamete; 1. Body tissue; 3. Soft bone tissue

According to Sambrook et al. (1989), DNA purity standards ranged from 1.8 to 2.0. A purity value below 1.8 indicates that DNA is still contaminated by protein, while a purity value above 2.0 indicates that DNA is contaminated by RNA. Based on the results of measurements of DNA with nanodrops, it was known that the DNA extraction of the sample was still contaminated with a protein. DNA from soft bone tissue (A3, B3 and C3) had a higher level of purity and concentration than DNA from body tissue (A1, B1 and C1) of sea cucumbers. However, the DNA concentration in this study was sufficient for amplification.

Amplification was performed to multiply the COI and ITS loci. The samples used for amplification were samples from soft bone tissue (A3, B3, and C3) because they had a higher concentration. The amplification results were then visualized with 1% agarose in TAE 1X buffer. DNA ladder was added to determine the size of the product. The gel was stained using GelRed so that the product PCR band could be seen in UV light. The results of electrophoresis can be seen in Figure 3.

The PCR products were sent for sequencing at 1st Base Malaysia. A total of 6 new sequences from three samples were obtained in this study (Table 1). Each gene was analyzed individually to create a phylogenetic tree using the Maximum Parsimony method with the PAUP4.0a116 program (Swofford 2019). The COI dataset included 31 taxa consisting of 753bp. The dataset included 753 characters, of which 384 characters were constant, 32 were parsimony-uninformative, and 337 were parsimony-informative. Gaps were treated as missing data. The maximum parsimony analyses resulted in 10 equally most parsimonious trees, of which one is shown in Figure 4 (tree length: 790 steps). *Holothuria leucospilota* was used as the outgroup for this dataset. The dataset for ITS included 14 taxa consisting of 1647bp. The dataset included 1647 characters, of which 816 characters were constant, 204 were parsimony-uninformative, and 627 were parsimony-informative. Gaps were treated as missing data. The maximum parsimony analyses resulted in 4 equally most parsimonious trees, of which one is shown in Figure 5 (tree length: 1073 steps). *Holothuria edulis* and *Holothuria atra* were used as the outgroup for this dataset.

Table 1. The results of quantitative DNA concentration measurements using nanodrop

Sample	A260	A280	A260/A280	Concentration (ng/µL)
A1	0.178	0.621	1.16	35.9
A3	1.009	0.619	1.63	50.4
B1	0.433	0.386	1.12	21.6
B3	1.571	0.939	1.68	78.6
C1	0.368	0.311	1.18	17.1
C3	0.341	0.273	1.25	18.4

Note: A. Crengek gamete; B. Pace gamete; C. Kuning gamete; 1. Body tissue; 3. Soft bone tissue

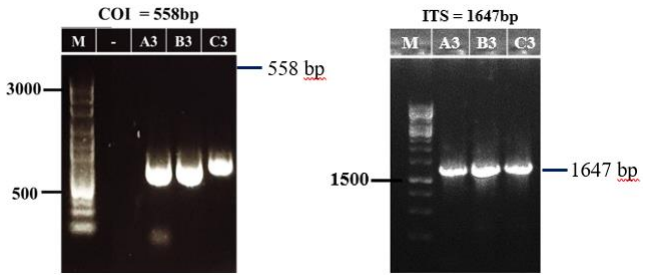

Figure 3. COI and ITS electrophoresis results. A. Crengek gamete; B. Pace gamete; C. Kuning gamete

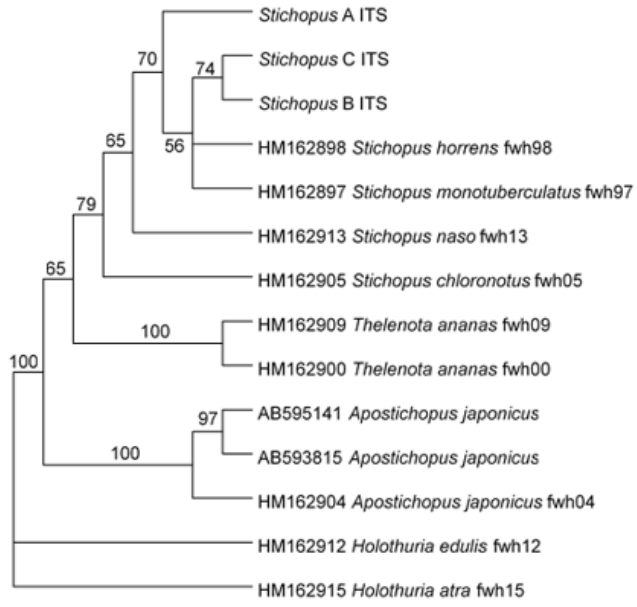

Figure 4. ITS phylogenetic tree. A. Crengek gamete; B. Pace gamete; C. Kuning gamete

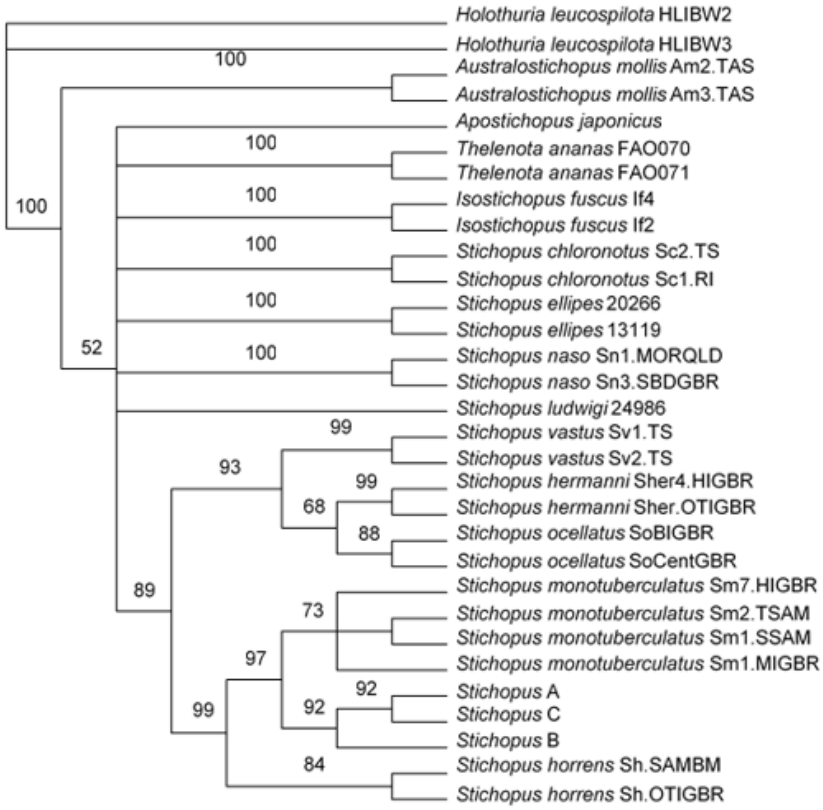

Figure 5. COI Phylogenetic tree: A. Crengek gamete; B. Pace gamete; C. Kuning gamete

An overall phylogenetic analysis for COI and ITS genes (using maximum parsimony method and bootstrapping with 1000 replicates) indicated that all samples in this study belong to the genus *Stichopus*. The results of the COI phylogenetic tree analysis showed that all the samples were closely related to *Stichopus monotuberculatus*. This species comes from the stichopod family, which has high commercial value in today's trading world. In the phylogenetic tree, the sample and *Stichopus monotuberculatus* are in one branch with a bootstrap value of 97%. However, the sample still formed further branches in the *S. monotuberculatus* complex with a bootstrap value of 92%.

According to Rannala and Yang (1996) if the bootstrap value in parsimony analysis is more than 70%, the branch will be constant. However, the results of the ITS locus phylogenetic analysis still lead to bias. The sample of this research is included in the branching species of *S. monotuberculatus* and *S. horrens*. This happens because of the unavailability of a comparison sequence to be used as a data set in the ITS locus analysis. Bootstrap values ranging from 70%-90% showed that genetic characteristics between the samples were very close even though their physical appearances (pattern and color) were significantly different. From this study, it is shown that the differences in patterns and colors of sea cucumbers do not indicate they are different species.

In conclusion, three samples of commercial sea cucumbers used in this study have the local names Crengek gamete (sample A), Pace gamete (sample B), and Kuning gamete (sample C). Based on initial identification, these three samples belong to the genus Stichopus. Although the three samples have different morphological characters, the COI phylogenetic tree analysis results showed that the three samples with different local names were closely related to *Stichopus monotuberculatus*. This species is classified as a species that has commercial value in the world of trade. Based on this, the three samples with different local names (Crengek Gamete, Pace gamete, and Kuning gamete) have the closest relatives to the *Stichopus monotuberculatus* species that live in Karimunjawa waters, where the three species are often caught by fishermen in Karimunjawa must management efforts and conservation actions.

ACKNOWLEDGEMENTS

BS would like to thank Rector of Diponegoro University, Semarang, Indonesia c.q. the Chair of the Institute for Research and Community Service, for the funding RPI Grant no: 233-19/UN7.6.1/PP/2021.

REFERENCES

Bell JD, Purcell SW, Nash WJ. 2008. Restoring small-scale fisheries for tropical sea cucumbers. Ocean Coast Manag 51 (8): 589-593. DOI: 10.1016/j.ocecoaman.2008.06.011.

Byrne M, Rowe F, Uthicke S. 2010. Molecular taxonomy, phylogeny, and evolution in the family Stichopodidae (Aspidochirotida: Holothuroidea) based on COI and 16S mithocondrial DNA. Mol Phylogenet Evol 56 (3): 1068-1081. DOI: 10.1016/j.ympev.2010.04.013.

Clark HL. 1922. The holothurians of the genus *Stichopus*. Bull Mus Comp Zool 65 (3): 39-74.

Conand C. 2004. Present status of world sea cucumber resources and utilization: An international overview: 13-23. In: Lovatelli A, Conand C, Purcell S, Uthicke S, Hamel JF, Mercier A (eds). Advances in Sea Cucumber Aquaculture and Management. FAO, Rome.

Edgar RC. 2004. MUSCLE: Multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res 32 (5): 1792-1797. DOI: 10.1093/nar/gkh340.

Friedman K, Purcell S, Bell J, Hair C. 2008. Sea Cucumber Fisheries: A Manager's Toolbox. ACIAR Monograph No.135. Australian Centre for International Agricultural Research, Canberra.

Hall T. 2005. BioEdit, version 7.0.5.3. Department of Microbiology, North Carolina State University, Raleigh, North Carolina. http://www.mbio.ncsu.edu/bioedit.html.

Itskovich V, Kaluzhnaya O, Veynberg E, Erpenbeck D. 2015. Endemic Lake Baikal sponges from deep water, 1: Potential cryptic speciation and discovery of living species known only from fossils. Zootaxa 3990: 123-137. DOI: 10.11646/zootaxa.3990.1.7.

Kamarudin KR, Usup G, Hashim R, Rehan MM. 2015. Sea cucumber (Echinodermata: Holothuroidea) species richness at selected localities in Malaysia. Pertanika 38 (1): 7-32.

Lawrence AJ, Afifi R, Ahmed M, Khalifa S, Paget T. 2009. Bioactivity as an options value of sea cucumbers in the Egyptian Red Sea. Conserv Biol 24 (1): 217-225. DOI: 10.1111/j.1523-1739.2009.01294.x.

Massin C, Zulfigar Y, Hwai TS, Boss SZR. 2002. The genus *Stichopus* (Echinoderm: Holothuroidea) from the Johore Marine Park (Malaysia with the description of two new species. Bulletin de L'Institut Royal des Science Naturalles de Belgique, Biologie 72: 73-99.

Miller AK, Kerr AM, Paulay G, Reich M, Wilson NG, Carvajal JI, Rouse GW. 2017. Molecular phylogeny of extant Holothuroidea (Echinodermata). Mol Phylogenet Evol 111: 110-131. DOI: 10.1016/j.ympev.2017.02.014.

Oh GW, Ko SC, Lee DH, Heo SJ, Jung WK. 2017. Biological activities and biomedical potential of sea cucumber (*Stichopus japonicus*): A review. Fish Aquat Sci 20: 28. DOI: 10.1186/s41240-017-0071-y.

Purcell SW, Hair CA, Mills DJ. 2012. Sea cucumber culture, farming and sea ranching in the tropics: Progress, problems and opportunities. Aquaculture 368-368: 68-81. DOI: 10.1016/j.aquaculture.2012.08.053.

Quoy JRC, Gaimard JP. 1833. Voyage de la Corvette de l'Astrolabe, Exécuté par ordre du roi Pendant les Années 1826-1829 sous le Commandement de MJ Dumont d'Urville. J. Tastu, Paris. [French]

Rannala B, Yang Z. 1996. Probability distribution of molecular evolitionary trees: A new meuhod of phylogenetic inference. J Mol Evol 43 (3): 304-311. DOI: 10.1007/BF02338839.

Sambrook J, Fritsch EF, Manniatis T. 1989. Molecular Cloning: A Laboratory Manual. 2nd Edition. Cold Spring Harbour Laboratory Press, New York.

Selenka E. 1867. Beiträge zur anatomie und systematik der holothurien. Zeitschrift für wissenschafltiche Zoologie 17 (2): 291-374. [German]

Semper C. 1868. Reisen im Archipel der Philippinen, zweiter Theil, Wissenschaftliche Resultate, Erster Band, Holothurien. W. Engelmann, Leipzig.

Sulardiono B. 2011. Kematangan gonad teripang komersial *Stichopus vastus* (Holothuriidea: Stichopodidae) di perairan Karimunjawa, Kabupaten Jepara, Jawa Tengah. Jurnal Saintek Perikanan 7 (1): 24-31. [Indonesia]

Sulardiono B. 2016. Potensi pemanfaatan teripang (Holothurians) di Perairan Karimunjawa, Kabupaten Jepara, Provinsi Jawa Tengah. Buletin Oseanografi Marina 5 (1): 64-72. DOI: 10.14710/buloma.v5i1.11298. [Indonesia]

Sulisyati R, Prihatinningsih P, Mulyadi. 2018. Revisi zonasi Taman Nasional Karimunjawa sebagai upaya kompromi pengelolaan sumber daya alam. Seminar Nasional Geomatika 2018 3: 713. DOI: 10.24895/SNG.2018.3-0.1030. [Indonesia]

Swofford DL. 2019. PAUP: Phylogenetic Analysis Using Parsimony. Version 4.0a116. Sinauer Associates, Sunderland, Massachusetts.

Uthicke S, Welch D, Benzie JAH. 2004. Slow growth and lack of recovery in overfished holothurians on the Great Barrier Reef: Evidence from DNA fingerprints and repeated large-scale surveys. Conserv Biol 18 (5): 1395-1404. DOI: 10.1111/j.1523-1739.2004.00309.x.