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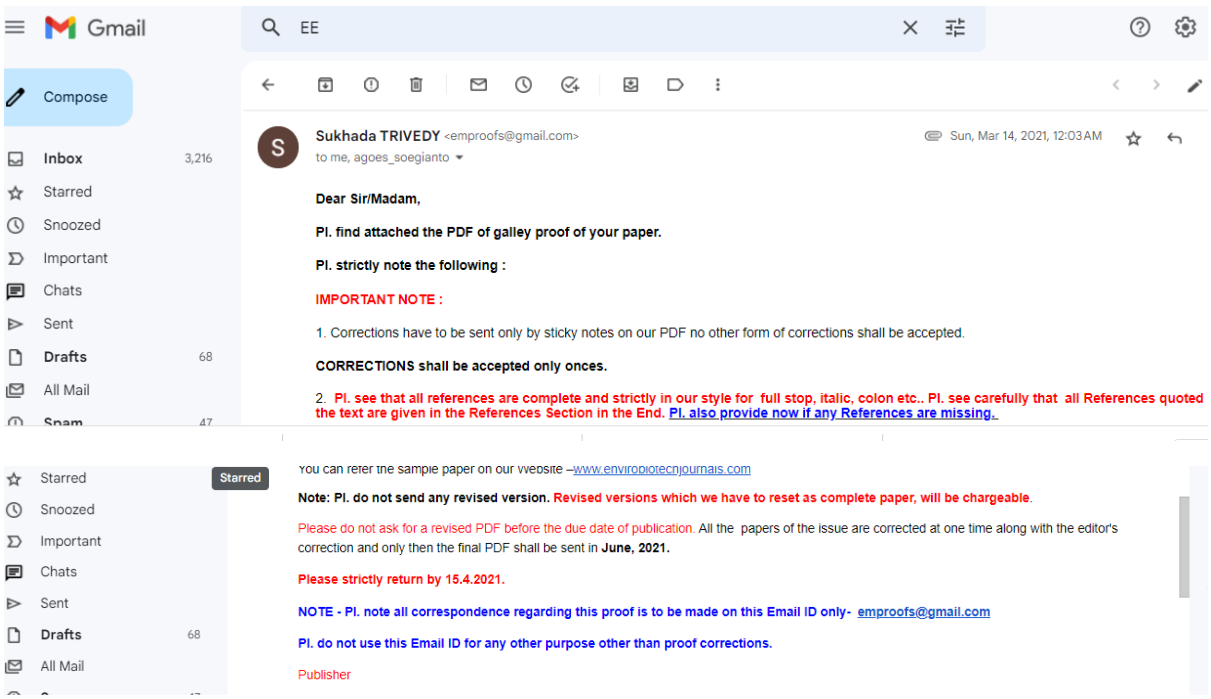

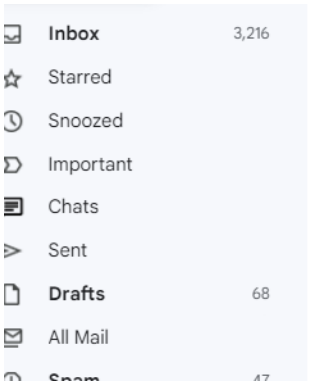

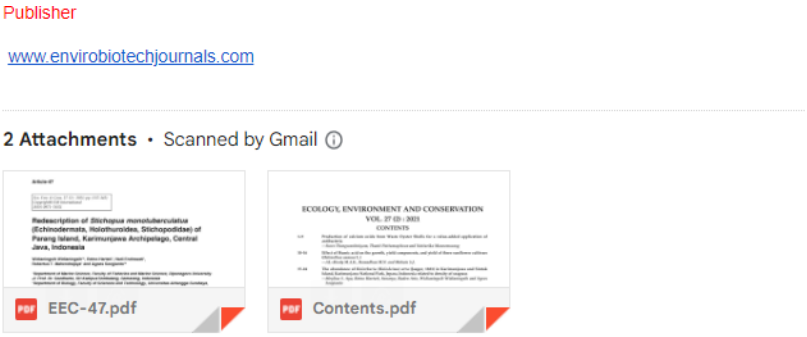

Redescription of *Stichopus monotuberculatus* (Echinodermata, Holothuroidea, Stichopodidae) of Parang Island, Karimunjawa Archipelago, Central of Java, Indonesia.

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Abstract

Stichopus monotuberculatus (Quoy & Gaimard, 1833) belong to family Stichopodidae, has high commercial value in fisheries trade world. Most of the sea cucumbers belong to family Stichopodidae have long been used for nutritional food and medicine. The objective of the study was to redescribe this species phenotype through morphological keys, review of their ossicles and genotype. The samples were collected from Parang Island and preserved in alcohol 96%. Commercial bleach were used to remove tissue and take the ossicles, then they were examined under light microscope. For genotype characteristic (DNA mapping) extraction DNEasy of tissue (Qiagen) was used for extraction of DNA. Primer CO1 was used to amplify the genom. Samples showed that morphologically they were grey-green colour with numerous small dark patches ventrally and grey green to orange-brown with dark green to black patches dorsally with square thick integument/body wall. The ossicles were rosette, table-shape, rod shape, rod C- shape, rod J-shape, rod S-shape, irregular rod shape and perforated plate shape which is the characteristic of *S. monotuberculatus*. DNA sequencing showed that Stichopus 1 and 3 have 99% similirity with *Stichopus monotuberculatus* haplotype4, 5, and 9. It is concluded the presence of S. *monotuberculatus* in Parang Island, Karimunjawa Archipelago.

Keywords: *Stichopus monotuberculatus*, phenotype, genotype, Parang Island.

Introduction

Stichopus monotuberculatus (Quoy & Gaimard, 1833) belongs to Class Holothuroidea (Echinodermata), the marine benthic invertebrate which has high economical value. It is inhabitat at coral reef, seagrass and deep seas ecosystem across the ocean. Ecologically, it has important role in benthic ecosystem through its bioturbation activity in benthic ecosystem (Hartati *et al.,* 2009). Study on Genus Stichopus from Karimunjawa has been conducted by Hartati *et al*. (2009), Purwati *et al*. (2010), and Widianingsih *et al.* (2013). Widianingsih *et al.* (2015) has tried to examine three Stichopodid samples, but has not been concluded the name of the species. Setyastuti and Pradina (2015) added list of Purwati *et al*. (2010) with *Stichopus* cf. *monotuberculatus* (Quoy and Gaimard, 1833).

The external morphology, internal organs, and spicules/ossicles has been widely used to identify sea cucumber. These characteristics may indicate a high level of similarity for particular families, including family Stichopodidae (Wirawati and Purwati, 2016). The molecular methods have been used to identify various marine organisms such as sea cucumbers (Byrne et al., 2010; Amin *et al*. 2016, Madduppa *et al*., 2017; Patantis *et al*., 2019). Therefore this research was aimed to redescribe *Stichopus monotuberculatus* (Family Stichopodidae) from Parang Island, Karimunjawa Archipelago, Central of Java, Indonesia through its phenotype (morphology and ossicle characteristic) and genotype characteristic with barcoding technique.

Material and Method

Morphological examination and ossicle observation

Fresh sample of sea cucumbers were collected from intertidal zone of Parang Island, Karimunjawa Archipelago and were examined morphologically for its overall shape, body wall, the position of mouth and anus, tentacle shape, tube feet, rete mirabile, pedicels, respiratory tree, gonad (if any) based on Samyn *et al*., (2006).

Ossicles were extracted from tissues of the mid part of dorsal body, papilla tips, tentacles, tube feet, and cloacal wall. A small piece (about 2 × 2 mm) of tissue is cut and placed on a clean glass slide. A few drops of weak commercial bleach were added using a pipette to dissolve the tissue. Tissues were left to dissolve for about 5 minutes. The ossicles were then washed several times with deionized water and finally with absolute alcohol. The spicules were then observed under a light microscope. The terms used to describe different types of ossicles followed Massin (1999) and Samyn *et al.* (2006)..

DNA extraction, PCR, and sequencing

Total genomic DNA were extracted from approximately 10–20 mg of tissue from the tube feet using DNeasy Blood & Tissue Kit (QIAGEN GmbH, Hilden, Germany) following the manufacturer's specification. Sections of the cytochrome *c* oxidase subunit I (COI) and the small subunit 16S ribosomal DNA (16S rDNA) genes were amplified using the primers COIe-F and COIe-R (Arndt *et al.* 1996) and 16Ss-F (5'GTAGCATAATCACTTGTCTCTTAAA-3') and 16Ss-R (5'TTATTTAGAAGAT AGAAGCTGACCT-3'), respectively. PCR amplification was conducted following Byrne *et al.* (2010).

PCR reaction with Kapa (2x Kapa Taq Extra Hotstart Ready Mix Dye (2mM MgCl2 at 1x) consisted of one cycle initial denaturation at 95 °C for 3 x 60 s followed by 40 cycles of denaturation at 95 °C for 30s, annealing Tm at -5°C for 30 s, extension at 72 °C for 80 s, and final extension at 72 °C for 1 min. All nucleotide sequences were determined using BigDye Terminator Kit ver. 3.1 and ABI 3730 Genetic Analyzer (Life Technologies, California, USA).

The PCR products were visualized to check DNA quality on the Mufid-EXU (Submarine electroforesis system) specification with a duration of 30 minutes and 100 volts. The positive PCR products were sent to a sequencing facility and loaded into an ABI 3130xl automated sequencer (Applied Biosystems). Sequences editing and alignment will be conducted using MEGA 5 (Tamura *et al*. 2011; Lucas *et al.*, 2012). Sample identification was performed using the Basic Local Assignment Search Tool (BLAST; Altschul *et al*. 1990).

For phylogenetic analysis, one or more reference sequences from GenBank sequence database with the highest maximum identity to each amplicon sequence were downloaded. In the phylogenetic tree, *Portunus pelagicus* (accession number KP976342) was used as an out-group comparison (Madduppa *et al*. 2017). A neighbor-joining phylogenetic tree using Kimura-2 parameter models (Kimura, 1980) was reconstructed with 1000 replicates of bootstrap value. The genetic distance within and between species was investigated.

Result and Discussion

Family Stichopodidae belonged to Ordo Aspidochirotida Class Holothuroidea (Echinodermata) in which consisted mostly the commercially trade sea cucumber in Indonesia and high commercial value due to its medicinal and edible properties (Maryam *et al*., 2012). One of Stichopodidae species found in Karimunjawa Archipelago was *Stichopus monotuberculatus.* It was widely distributed in the Indo-Pacific Ocean (Massin, 1996) as well as in Indonesia and had synonym name of *Holothuria monotuberculata* Quoy & Gaimard, 1833 (Massin, 1996).

Phenotype (morphology and ossicles characteristics)

Twelve fresh samples of *S. monotuberculatus* were observed their morphological characters. It is usually medium to large-sized sea cucumbers, the length of the samples were in the range of 100-320 mm. Their body colour were grey-green with numerous small dark patches ventrally and grey green to orange-brown with dark green to black patches dorsally. During preservation, the colour was changed became light green with light brown patches. According to Massin (1996) this species appeared to exhibit a wide colour range from green/bluish-green to orange-brown. His living specimens colour were pale brown to rust coloured dorsally and ventrally. Dorsally, there were numerous white and deep brown dots. The white dots often clumped together forming a whitish surface.

As other member of Family Stichopodidae, it was usually square in cross section, and distinctly flattened below (Pradina *et al*., 2010). Ventral side of the body of the samples were flattened with swollen dorsal side. Mouth was in anterior ventral position surrounded by 20 large tentacles in which encircled by a large papillae as found by Massin (1996) in *S. monotuberculatus* samples. Anus position was terminal and there was no anal teeth or papillae. Large conical papillae were distributed in bivium. There was a distinct fringe of 8-10 larger papillae laterally. This character was similar found by Maryam et al. (2012) in Gulf of Persian. In the samples of Massin (1996), the numerous papillae were also found

in the dorsal with a white apex. There was one row of 9-10 very large papillae on each side of the body, posterior ones being the largest.

The tube feets of the samples in present studies were large and cylindrical shape, their colour were yellowish brown. According to Massin (1996), the tube feet of *S. monotuberculatus* were well developed and abundant along the three ventral ambulacra (trivium). They were located on 3-6 rows along the lateral ambulacra and on 8-14 rows along the median ambulacrum. The tube feet were translucent, ending in a large deep brown sucker.

Examination of the calcareous ring and ossicles found that the calcareous ring of the samples had large radial pieces and narrow interradial pieces. The radial pieces had a posterior notch and four short anterior points whereas the interradial pieces had a long anterior tooth. This character also found in Massin's sample (1996) of *S. monotuberculatus.* After calcareus ring, there was one large polian vesicle and one contorted stone canal, embedded in the dorsal mesentery.

There were numerous tables-shaped ossicles in the anterior tissue of the bodywall (Fig. 1A) but very few the C- (Fig. 1H), Rosette- (Fig.1D), and plate-shape rods (Fig. 1E). It were also very numerous in the posterior tissue (Fig. 1B) but no rosette-shape. The modified-rods also were found in this part of the body (Fig.1J). In the tube feet there are reduced tables and spiny rods with an enlarged central process, very often perforated

In the ventral tissue of the body wall, there were only tables (Fig. 1G, N) which are very small. At the base of the dorsal papillae, the ossicles are the same as in the body wall whereas at the apex there were large perforated plates (Fig. 1F), smooth or spiny or modified rods (Fig. 1J), a few tables and C-shaped rods (Fig. 1H). In the dorsal tissue of the body wall, there were only few table- (Fig. 1C) and C-shape rods (Fig. 1H), but the rossete (Fig. 1I) were numerous as found by Cherbonnier, 1988). The tables were small (39-61mm in diameter) with a squarish disc perforated by 4 large central holes and 4-10 small peripheral holes. The four short pillars are united by one cross beam and end in a crown of spines which is often as wide as the disc. The rosettes are sparse and irregular (Fig. 1J). The C-shaped rods are long and smooth or with a few excrescences (Fig. 1H). The character of ossicles found in present study similar to that Massin (1996).

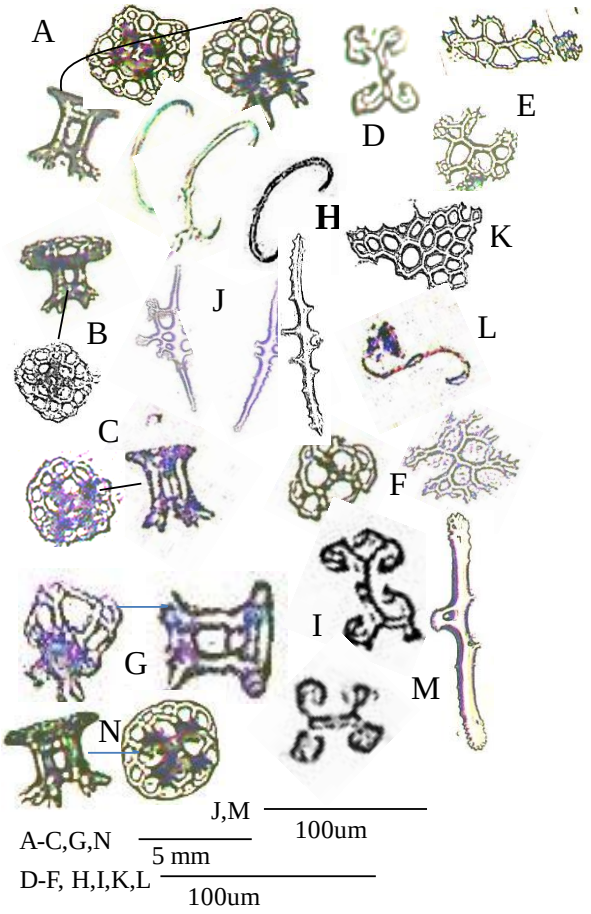

Figure 1. Ossicles of *Stichopus monotuberculatus* (Quoy & Gaimard, 1833) form Parang Island, Karimunjawa Archipelago, Jepara

A : tables of anterior, B : tables of posterior, C : tables of dorsal bodywall, D: rossete of anterior, E: plate-shape anterior, F: plate of dorsal, G: tables of ventral bodywall, H: plate of ventral bodywall, H: C-shape rods of bodywall, I: rossete shape of dorsal bodywall, J : rods of bodywall, K: plate of posterior, L: S-rod shape of dorsal, N : plate of vetral bodywall, M: rod shape, N: table of ventral bodywall.

The result of present study revealed that he general aspect of the specimen, morphology and of the remaining ossicles of the body wall and the tentacles matched with *S. monotuberculatus* (Quoy & Gaimard, 1833).

Genotype Character with DNA barcoding

Twelve samples of sea cucumber from Parang Island, Karimunjawa Archipelago which had been morphology and ossicle examined as *S. monotuberculatus* were then analyzed for their molecular identification. COI gene mtDNA of four sea cucumbers samples (S2-OK-F, S2-OK-R, S3-OK-F, S3-OK-R) have been successfully amplified using universal primers of the COI gene. Amplification results obtained DNA fragments with a length of about 667 bp. Blast analysis of this sea cucumber COI gene sequence showed that sample of sea cucumber S2-OK-F and S2-OK-R had similarity with *Stichopus monotuberculatus* H9 and H4 with acc

No. KC424504.1 and KC424499.1 NCBI Genbank at 99%. While S3-OK-F and S3-OK-R had similarity with *Stichopus monotuberculatus* H5 with acc No. KC424500.1 NCBI Genbank at 99%.

No.	ID sample	Similarity	ID genbank	Length bp	Comment
1	S-1-O-F (Stichopus 1)	Stichopus sp. SF-2010	HM853683.2	16257	Conclussion ? S-1-O-F what is species?
2	Stichopus 1 (S-1-O-F)	*Stichopus monotuberculatus* (haplotype 9)	KC424504.1	667	
3	S-1-O-F	*Stichopus monotuberculatus* (haplotype 4)	KC424499.1|	667	
4	S-1-OK-R	Stichopus sp. SF-2010	HM853683.2	16257	idem
5	S-1-OK-R	*Stichopus monotuberculatus* (haplotype 9)	KC424504.1	667	
6	S-1-OK-R	*Stichopus monotuberculatus* (haplotype 4)	KC424499.1	667	
7	S3-OK-F	*Stichopus monotuberculatus* (haplotype 5)	KC424500.1	667	idem
8	S-3-OK-F	*Stichopus monotuberculatus* haplotype 13	KC424508.1	674	
9	S3-OK-R	*Stichopus monotuberculatus* (haplotype 13	KC424500.1	667	
10	S3-OK-R	*Stichopus monotuberculatus* (haplotype 13	KC424508.1	674	
11	S4-OK-F	*Stichopus monotuberculatus* haplotype 13	KC424508.1	674	

The molecular methods have been widely used to identify various marine organisms and their processed products, including sea cucumbers, because of their specificity. The result of molecular identification (COI gene mtDNA and Blast analysis) of samples of sea cucumber with morphological features of grey-green with numerous small dark patches ventrally and grey green to orange-brown

with dark green to black patches dorsally with square thick integument/body wall and had same spicules characteristics showed that they had similarities with *Stichopus monotuberculatus* at 99%. This method has been successfully identified one species of sea cucumber from Surabaya (Amin *et al*. 2016), seven species of sea cucumbers from Kepulauan Seribu (Madduppa *et al*., 2017), and 25 beche-de-mere from Boalemo, Pesawaran, Surabaya, and West Lombok (Patantis *et al*., 2019).

As other member of Family Stichopodidae, because of high demand and good economy value, *S. monotuberculatus* was very prone to overexploitation. Therefore it is need urgent management intention (Purcell *et al.* 2013). and effort to conservation effort. The idea of sea cucumber ranching has been initiated by Hartati *et al*., (2021 Inpress) with lower value sea cucumber of *Holothuria atra,* it might be applied for *S. monotuberculatus* since it has been demonstrated that this species can be reared in captivity, thus the farming of this species would provide an alternative to fisheries (Hu *et al*. 2010, Chen *et al*., 2015).

Conclussion

Twelve samples has been successfully identified through morphology and ossicle examination as *Stichopus monotuberculatus.* Based on genetic DNA barcoding analysis, for samples had 99% similarity with *Stichopus monotuberculatus* H4, H5 and H9. This was confirmed the existence of this species in Parang Island, Karimunjawa Archipelago, Jepara.

Acknowledgments

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ABSTRACT

Stichopus monotuberculatus (Quoy & Gaimard, 1833) belongs to family Stichopodidae, has high commercial value in fisheries trade world. Most of the sea cucumbers belong to family Stichopodidae have long been used for nutritional food and medicine. The objective of the study was to redescribe this species by its phenotype characteristics through morphological keys and review of their ossiclesas well as genotype by DNA mapping. The samples were collected from Parang Island andpreserved in alcohol 96%. Commercial bleach were used to remove tissue and take the ossicles, then they were examined under light microscope. For genotype characteristic (DNA mapping) extraction DNEasy of tissue (qiagen) was used for extraction of DNA. Primer CO1 was used to amplify the genom. Samples showed that morphologically they were grey-green colour with numerous small dark patches ventrally and grey green to orange-brown with dark green to black patches dorsally with square thick integument/body wall. Calcareous ring radial pieces had a posterior notch and four short anterior points whereas the interradial pieces had a long anterior tooth. The ossicles showed numerous tables-shaped ossicles in the anteriorand dorsal tissue of the bodywall, but no rossete-shape in dorsal body wall which is the characteristic of *S. monotuberculatus*. DNA sequencing of nine samples showed that all samples had got 93-99% similarity with *Stichopus monotuberculatus* haplotype 4, 5, 9, and 13. This result confirmed the identification through morphology and ossicles characters. It is approved the presence of *S. monotuberculatu* (Quoy and Gaimard, 1833) *in* Parang Island, Karimunjawa Archipelago, Jepara.

Key words: *Stichopus monotuberculatus, Phenotype, Genotype, Parang Island.*

Introduction

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Total genomic DNA was extracted from approximately 10–20 mg of tissue from the tube feet using DNeasy Blood and Tissue Kit (QIAGEN GmbH, Hilden, Germany) following the manufacturer's specification. Sections of the cytochrome *c* oxidase subunit I (COI) and the small subunit 16S ribosomal DNA (16S rDNA) genes were amplified using the primers COIe-F and COIe-R (Arndt *et al.,* 1996) and 16Ss-F (5'GTAGCATAATCACTTGTCTCTTAAA-3') and 16Ss-R (5'TTATTTAGAAGATAGAAGCTGACCT-3'), respectively. PCR amplification was conducted following Byrne *et al.* (2010).

PCR reaction with Kapa (2x Kapa Taq Extra Hotstart Ready Mix Dye (2mM MgCl2 at 1x) consisted of one cycle initial denaturation at 95 °C for 3 x 60 s followed by 40 cycles of denaturation at 95 °C for 30s, annealing Tm at -5°C for 30 s, extension at 72 °C for 80 s, and final extension at 72 °C for 1 min. All nucleotide sequences were determined using BigDye Terminator Kit ver. 3.1 and ABI 3730 Genetic Analyzer (Life Technologies, California, USA).

The PCR products were visualized to check DNA quality on the Mufid-EXU (Submarine electrophoresis system) specification with duration of 30 minutes and 100 volts. The positive PCR products were sent to a sequencing facility and loaded into an ABI 3130xl automated sequencer (Applied Biosystems). Sequences editing and alignment will be conducted using MEGA 5 (Tamura *et al*. 2011; Lucas *et al.*, 2012). Sample identification was performed using the Basic Local Assignment Search Tool (BLAST; Altschul *et al*. 1990).

For phylogenetic analysis, one or more reference sequences from GenBank sequence database with the highest maximum identity to each amplicon sequence were downloaded. In the phylogenetic tree, *Portunus pelagicus* (accession number KP976342)was used as an out-group comparison (Madduppa *et al*. 2017). A neighbor-joining phylogenetic tree using Kimura-2 parameter models (Kimura, 1980) was reconstructed with 1000 replicates of bootstrap value. The genetic distance within and between species was investigated.

Results and Discussion

Family Stichopodidae belonged to Ordo Aspidochirotida Class Holothuroidea (Echinodermata) in which consisted mostly the commercially trade sea cucumber in Indonesia and high commercial value due to its medicinal and edible properties

(Maryam *et al*., 2012). One of Stichopodidae species found in Karimunjawa Archipelago was *Stichopus monotuberculatus.* Itwas widely distributed in the Indo-Pacific Ocean (Massin, 1996) as well as in Indonesia and had synonym name of *Holothuria monotuberculata* Quoy and Gaimard, 1833 (Massin, 1996).

Phenotype (morphology and ossicles characteristics)

Nine fresh samples of *S. monotuberculatus* were observed their morphological characters. It is usually medium to large-sized sea cucumbers, the length of the samples was in the range of 100-320 mm. Their body colour were grey-green with numerous small dark patches ventrally and grey green to orange-brown with dark green to black patches dorsally. During preservation, the colour was changed became light green with light brown patches. According to Massin (1996) this species appeared to exhibit a wide colour range from green/bluish-green to orange-brown. His living specimens colour were pale brown to rust coloured dorsally and ventrally. Dorsally, there were numerous white and deep brown dots. The white dots often clumped together forming a whitish surface.

As other member of Family Stichopodidae, it was usually square in cross section, and distinctly flat- tened below (Purwati *et al*., 2010).Ventral side of the body of the samples wasflattened with swollen dor- sal side. Mouth was in anterior ventral position sur- rounded by 20large tentacles in which encircled by a large papilla as found by Massin (1996) in *S. monotuberculatus* samples. Anus position was termi- nal and there was no an anal tooth or papillae. Large conical papillae were distributed in bivium.There was a distinct fringe of 8-10 larger papillae laterally. This character was similar found by Maryam *et al.* (2012) in Gulf of Persian. In the samples of Massin (1996), thenumerous papillae were also found in the dorsal with a white apex. There was one row of 9-10 very large papillae on each side of the body, posterior ones being the largest.

The sample's tube feets in present studies were large and cylindrical shape, their colour were yellowish brown. According to Massin (1996), the tube feet of *S. monotuberculatus* were well developed and abundant along the three ventral ambulacra (trivium). They were located on 3-6 rows along the lateral ambulacra and on 8-14 rows along the media nambulacrum. The tube feet were translucent, ending in a large deep brown sucker.

Examination of the calcareous ring and ossicles found that the calcareous ring of the samples had large radial pieces and narrow interradial pieces. The radial pieces hada posterior notch and four short anterior points whereas the interradial pieces hada long anterior tooth. This character also found in Massin's sample (1996) of S. *monotuberculatus.* After calcareous ring, there was one large polian vesicle and one contorted stone canal, embedded in the dorsal mesentery.

There were numerous tables-shaped ossicles in the anterior tissue of the bodywall (Fig. 1A) but very few the C- (Fig. 1H), Rosette- (Fig.1D), and plate-shape rods (Fig. 1E). It was also very numerous in the posterior tissue (Fig. 1B) but no rosette-shape. The modified-rods also were found in this part of the body (Fig.1J). In the tube feet there are reduced tables and spiny rods with an enlarged central process, very often perforated.

In the ventral tissue of the body wall, there were only tables (Fig. 1G, N) which are very small. At the base of the dorsal papillae, the ossicles are the same as in the body wall whereas at the apex there were large perforated plates (Fig. 1F), smooth or spiny or

Table 1. COI gene mtDNA sequence blast analyses result of sea cucumbers samples from Parang Island, Karimunjawa Archipelago, Jepara

No.	Sample ID	Blast sequence	ID GenBank	Length bp	Similarity %
1	S1-OK-F1	*Stichopus monotuberculatus* haplotype 9	KC424504.1	667	99
2	S1-OK-F	*Stichopus monotuberculatus* haplotype 4	KC424499.1	667	99
3	S1-OK-R1	Stichopus monotuberculatus haplotype 9	KC424504.1	667	99
4	S1-OK-R	*Stichopus monotuberculatus*haplotype 4	KC424499.1	667	99
5	S3-OK-F2	*Stichopus monotuberculatus* haplotype 5	KC424500.1	667	99
6	S3-OK-F3	*Stichopus monotuberculatus* haplotype 13	KC424508.1	674	99
7	S3-OK-R1	*Stichopus monotuberculatus* haplotype 13	KC424508.1	674	99
8	S3-OK-R2	*Stichopus monotuberculatus* haplotype 13	KC424508.1	674	99
9	S4-OK-F 1	*Stichopus monotuberculatus* haplotype 13	KC424508.1	674	93

modified rods (Fig. 1J), a few tables and C-shaped rods (Fig. 1H). In the dorsal tissue of the body wall, there were only few table-(Fig. 1C) and C-shape rods (Fig. 1H), but the rossete (Fig. 1I) were numerous as found by Cherbonnier (1988). The tables were small (39-61mmin diameter) with a squarish disc perforated by 4 large central holes and 4-10 small peripheral holes. The four short pillars are united by one cross beam and end in a crown of spines which is often as wide as the disc. The rosettes are sparse and irregular (Fig. 1J). The C-shaped rods are long and smooth or with a few excrescences (Fig. 1H). The character of ossicles found in present study similar to that Massin (1996).

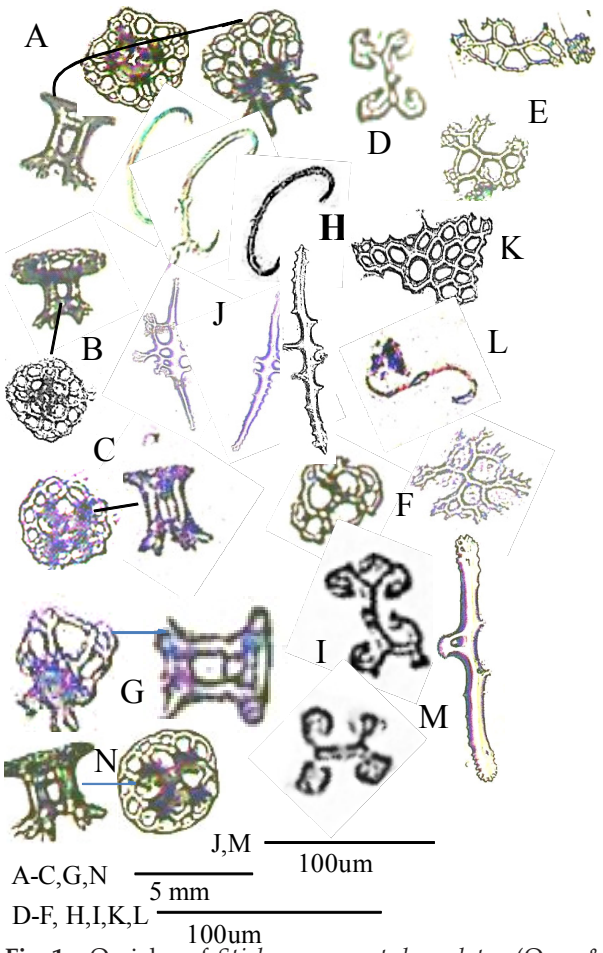

Fig. 1. Ossicles of *Stichopus monotuberculatus* (Quoy& Gaimard, 1833) form Parang Island, Karimunjawa Archipelago, Jepara, Indonesia

A : tables of anterior, B : tables of posterior, C : tables of dorsal body wall, D: rossete of anterior, E: plate-shape anterior, F: plate of dorsal, G: tables of ventral body wall, H: plate of ventral body wall, H: C-shape rods of body wall, I: rossete shape of dorsal body wall, J : rods of body wall, K: plate of posterior, L: S-rod shape of dorsal, N : plate of ventralbody wall, M: rod shape, N: table of ventral body wall.

The result of present study revealed that the general aspect of the specimen, morphology and of the remaining ossicles of the body wall and the tentacles matched with S.*monotuberculatus* (Quoy & Gaimard, 1833).

Genotype Character with DNA barcoding

Nine samples of sea cucumber from Parang Island, Karimunjawa Archipelago which had been morphology and ossicles examined as *S. monotuberculatus* were then analyzed for their molecular identification. COI gene mtDNA of all sea cucumbers samples have been successfully amplified using universal primers of the COI gene. Amplification results obtained DNA fragments with a length of about 674-667 bp. Blast analysis of this sea cucumber COI gene sequence showed that allsample of sea cucumber had similaritywith *Stichopus monotuberculatus* H haplotype4, 5, 9 and 13 with acc No. KC424499.1, KC424500.1, KC424504.1 and KC424508.1NCBI Genbank at 93-99% (Table 2). These results confirmed their identification through morphology and ossicles characters.

The molecular methods have been widely used to identify various marine organisms and their processed products, including sea cucumbers, because of their specificity. The result of molecular identification (COI gene mtDNA and Blast analysis) of samples of sea cucumber with morphological features of grey-green with numerous small dark patches ventrally and grey green to orange-brown with dark green to black patches dorsally with square thick integument/body wall and had same spicules characteristics showed that they had similarities with *Stichopusmonotuberculatus* at93-99%. This method has been successfully identified one species of sea cucumber from Surabaya (Amin *et al.* 2016), seven species of sea cucumbers from Kepulauan Seribu (Madduppa *et al*., 2017), and 25 beche-de-mere from Boalemo, Pesawaran, Surabaya, and West Lombok (Patantis *et al*., 2019).

As other member of Family Stichopodidae, because of high demand and good economy value, *S. monotuberculatus* was very prone to overexploitation. Therefore, it was need urgent management intention (Purcell *et al.* 2013) and effort to conservation effort. The idea of sea cucumber

ranching (releasing cultured/wild juvenile/young sea cucumber into unenclosed coastal environments for harvest at a larger size in 'put and take' operation) (Bartley and Bell, 2008) has been initiated by Hartati *et al*., (2021 Inpress) with lower value sea cucumber of *Holothuria atra,* it might be applied for *S. monotuberculatus* since it has been demonstrated that this speciescan be reared in captivity, thus sea ranching or farming of this species would provide an alternative to fisheries (Hu *et al*. 2010; Chen *et al*., 2015).

Conclusion

Nine samples have been successfully identified through morphology and ossicles examination as *Stichopus monotuberculatus.* Based on genetic DNA barcoding analysis, all samples had 99% similarity with *Stichopus monotuberculatus* haplotype 4, 5 and 9 and lower (93%) similarity with *Stichopus monotuberculatus* haplotype 13. These results confirmed the existence of species in Parang Island, Karimunjawa Archipelago, Jepara.

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