

# Molecular identification, abundance and distribution of the coral-killing sponge *Terpios hoshinota* in Bengkulu and Seribu Islands, Indonesia

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**Abstract.** *Utami RT, Zamani NP, Madduppa HH. 2018. Molecular identification, abundance and distribution of the coral-killing sponge Terpios hoshinota in Bengkulu and Seribu Islands, Indonesia. Biodiversitas 19: 2238-2246.* Coral killing sponge *Terpios hoshinota* is one of threats to coral reefs. The outbreaks of *T. hoshinota* has been reported in the Indo-Pacific region. However, the current distribution of this species in Western of Sumatera Island is unknown, compared to Seribu Islands. This study aimed to identify coral-killing sponge molecularly and to compare the distribution and abundance of *T. hoshinota* in Bengkulu (Western of Sumatera) and in Seribu Islands (Northern of Java Island) and to record the preferences of coral substrate of *T. hoshinota*. Coral reefs and *T. hoshinota* data were collected using underwater photo transect method with 0.5x0.5m<sup>2</sup> quadrat transect. Coral reefs covered by *T. hoshinota* was analyzed by using Correspondence Analysis, while the determination of biophysical and chemical environment was analyzed by Principal Component Analysis. The identity of the sponge was *T. hoshinota*. *T. hoshinota* has been expanding in many reefs across Indonesia especially in Tikus Island and Belanda Island. The most prevalent coral genera in the Seribu Islands infected by *T. hoshinota* was *Acropora* while those in Bengkulu were *Porites* and *Pocillopora*. Pearson correlation between the live coral cover and *T. hoshinota* cover was revealed not significant, while *T. hoshinota* cover and orthophosphate was significant. This study suggests that orthophosphate may play a role invasion of *T. hoshinota* outbreaks.

**Keywords:** invasive sponge, urban reefs, DNA barcoding, correspondent analysis, principal component analysis

## INTRODUCTION

Coral reefs are marine ecosystems formed by the structure of calcium carbonate secreted by coral. Most coral reefs are built by rocky corals consisted of grouped polyps. Most coral reefs can grow in warm, shallow and clear waters. Even though coral reefs occupy less than 0.1% of the sea level in the world, they provide homes for at least 25 % of all marine species, including fish, mollusks, worms, crustaceans, echinoderms, sponges, tunicates and other cnidarians (Spalding and Grenfell 1997; Spalding et al. 2001; Mulhall 2009). However, coral reefs have been damaged around 20% over the past few decades in the world (Wilkinson 2004, Wilkinson 2008, Goatley and Bellwood 2011). While the condition of coral reefs in Indonesia according to Giyanto et al. (2017) has a very good status in which only 6.39% and 35.15% in poor conditions. The coral reef damage is caused by the increasing human pressure, the use of destructive fishing gear, the increased pollution, the global climate change that causes coral bleaching, as well as coral disease and predation (Wilkinson 2004).

*Terpios hoshinota* is one of the killer sponges that attack coral reef ecosystem. This sponge belongs to the Porifera Phylum with the Demospongiae Class. This sponge has a very thin tissue ( $\leq 1$  mm), encrusting, black or dark brown and usually grayish in shallow water. It can grow quickly both on living and on dead corals, which is living in waters along with several types of cyanobacteria

in a symbiotic manner (Rützler and Muzik 1993). *Terpios hoshinota* is known as a strong competitor for coral reefs to grow and live because it can envelop and cover live corals. Moreover, it causes damage and even death to live corals (Bryan 1973; Plucer-Rosario 1987; Wang et al. 2015). *T. hoshinota* is firstly identified in Tokunoshima Island, Japan (Rützler and Muzik 1993). The first record found that *T. hoshinota* attacked Guam Mariana Islands and American Samoa (Bryan 1973; Plucer-Rosario 1987) and then some records stated it also attacked coral in Green Island, Taiwan (Liao et al. 2007); Lizard Island, Australia (Fujii et al. 2011); Okinoerabu Island, Japan (Reimer et al. 2011a; 2011b); Yongxing Island, South China Sea (Shi et al. 2012); Tioman Island and Peninsular, Malaysia (Hoeksema et al. 2014); Maldives (Montano et al. 2015); Mauritius (Elliot et al. 2016) and Taiping Island, Taiwan (Yang et al. 2018). *T. hoshinota* are found in several regions in Indonesia, including Seribu (Thousand) Islands, Jakarta; Spermonde Islands, South Sulawesi; Riau Islands; Banten Bay, West Java; Balikpapan Bay, East Kalimantan; and Babar Island, South Maluku (de Voogd et al. 2013; van der Ent et al. 2016; Madduppa et al. 2017).

The condition of coral reefs in the Belanda Island and Dapur Island, part of the Seribu Islands is often associated with anthropogenic activity, sedimentation and climate change (Rachello-Dolmen and Cleary 2007; Fadilah and Idris 2009). However, information about the influence of coral reef conditions in the area of *T. hoshinota* is limited. Many studies related to this had not to be done in

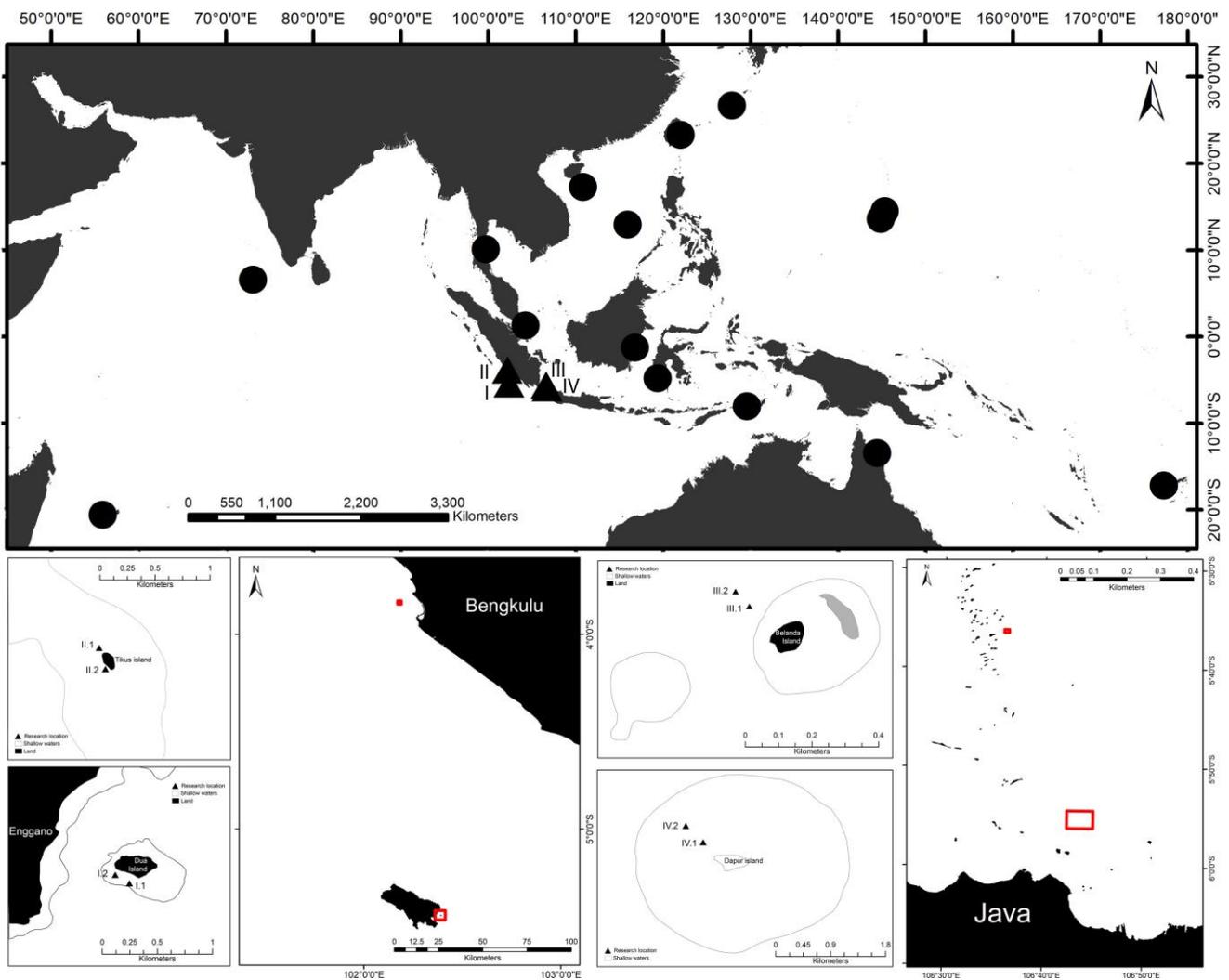
Indonesia, especially in the Dua Island (Enggano) and Tikus Island, Bengkulu and the Belanda and Dapur Island, Seribu Islands, Jakarta. Therefore, this study aimed to identify *T. hoshinota* based on molecular analysis; analyze abundance of *T. hoshinota* and describe distribution of *T. hoshinota* and aquatic habitat characteristic.

**MATERIALS AND METHODS**

**Study area and data collection**

The study was conducted in different four locations namely Dua Island, Tikus Island, Dapur Island, and Belanda Island. The Dua (Enggano) and Tikus Islands are located in Bengkulu, while the Dapur and Belanda Island

are part of Seribu Islands, Jakarta (Figure 1). Belanda Island belongs to the region of North Seribu Islands District and it is a core coral reef preservation zone, while Dapur Island is included in the South Seribu Islands District and it is currently submerged due to sea sand exploitation (BPS 2017a; BPS 2017b). The Enggano Island is one of the most populous outer islands located in the Indian Ocean (BPS 2017c), while the Tikus Island is located in west of Bengkulu City at a distance of 10 km from the center of Bengkulu City and it is directly connected to the Indian Ocean (BPS 2018). This research was divided into two zones, namely the inshore zone which was less than 20 km from the mainland and offshore zone with a distance of more than 60 km from the mainland.



**Figure 1.** Research location of coral-killing sponge *Terpios hoshinota* at small islands in Indonesia. (▲): I. Dua Island, Enggano; II. Tikus Island; III. Belanda Island and IV. Dapur Island. Location covered by *Terpios hoshinota* (●) reported by other studies

Data collection was carried out from January to February 2018 in the coral reefs ecosystem. A total of 8 observation points, spread over 4 islands at small island in Indonesia, were thoroughly surveyed by scuba diving for the presence of *T. hoshinota*. The sessile benthic community of the surveyed reefs and patches of *T. hoshinota* were collected using the UPT (Underwater Photo Transect) method with a 0.5x0.5 m<sup>2</sup> transect area (Giyanto et al. 2014). The observation was carried out at two observation points. At each observation point 50 m transect were laid down, at ≤ 5 m and ≤ 10 m (Seribu Islands) and at 1 m and 1.5 m (Bengkulu). Identification of coral genus was based on the Coral Finder guidebook (Kelley 2011) and Suharsono's report (2008). Sponge identification in situ was based on the morphological characteristics given by Rützler and Muzik (1993). Specimen of *T. hoshinota* were preserved in 96% ethanol. Tissue was collected to confirm the identity of the encrusting sponge *T. hoshinota* by using DNA analysis.

### Molecular identification

The *T. hoshinota* samples obtained in the field were preserved using 96% alcohol and put into a sample bottle. Extraction of DNA from a sample of sponge tissue used a Geneaid kit. The primers used in this study were ITS2Fwd (5' GCA GAC-GAC GGA CAG CCT CA-3') and ITS2Rev (5' TTT GCA GCA CCC CTC TCAG-3'). The PCR condition was carried out as: 35 cycles, each cycle consisted of pre-denaturation at 94°C for 3 minutes denaturation at 94°C for 30 seconds, annealing at temperature 53°C for 30 seconds, extension at 72°C for 30 seconds and final extension at 72°C for 10 minutes (Yang et al. 2018). The next stage was electrophoresis started with making agar. The PCR product was inserted into the wells contained in agar. Electrophoresis results were visualized in GelDoc to see the presence of the amplified region. If the product had a positive DNA content, the PCR product could be sequenced to know the order of the ingredients. The DNA sequencing process was carried out by FirstBase sequencing facilities found in Malaysia.

### Data analysis

#### Molecular analysis

The obtained results of *T. hoshinota* sequencing were then analyzed using the Mega 6.0 (Molecular Evolutionary Genetic Analysis) program (Tamura et al. 2013). The obtained sequence was aligned first using the Clustal W menu contained in the program to know the level of nucleotide diversity. Nucleotide alignment data obtained were then matched to the available data on GenBank at NCBI (National Center for Biotechnology Information).

#### Abundance of *T. hoshinota*

Analysis data of *T. hoshinota* area and coral genus attacked by *T. hoshinota* were based on photos taken using a digital camera of Canon G16 analyzed using a computer

and Image J software. Moreover, the Microsoft Excel 2016 software was used for processing displays of graphics data (Abramoff et al. 2004; Munro 2013).

#### Distribution of *T. hoshinota* and aquatic habitat characteristic

Analysis of the coral reefs condition was based on photos taken using a digital camera of Canon G16 and analyzed using computers and devices CPCE (Coral Point Count with Excel extensions) software (Kohler and Gill 2006). A total of 30 random point samples was selected for each photo frame and for each point coded according to the code of each category, biota, and substrate at the random point. Furthermore, the percentage of cover for each category, biota, and substrate were calculated for each photo frame using the formula:

$$\text{Coral cover percentage} = \frac{\text{number of points on each categories}}{\text{number of random points}} \times 100\%$$

In this study, a descriptive statistical analysis was used to describe the data that corresponded to the actual conditions of the observed location. The obtained data were analyzed by simple calculations using Excel 2016 software, and Xlstat Version 2015.1 Distribution of *T. hoshinota* composition was analyzed using a multivariate statistical model based on Correspondence Analysis (CA). Whereas, determination of environmental characteristic among observed stations was carried out by multivariate statistical approaches based on the Principal Component Analysis (PCA) (Bengen 2000).

## RESULTS AND DISCUSSION

### Molecular identification

The results of ribosomal DNA sequencing showed that the species of sponge was actually *T. hoshinota*. Moreover, the similarity percentage and Genetic bank sources were shown in Table 1. Based on BLAST analysis in genetic banks, it was known that the percentage of similarity ranging from 96% to 98%. It means that the similarity of *T. hoshinota* in each observed station was quite high.

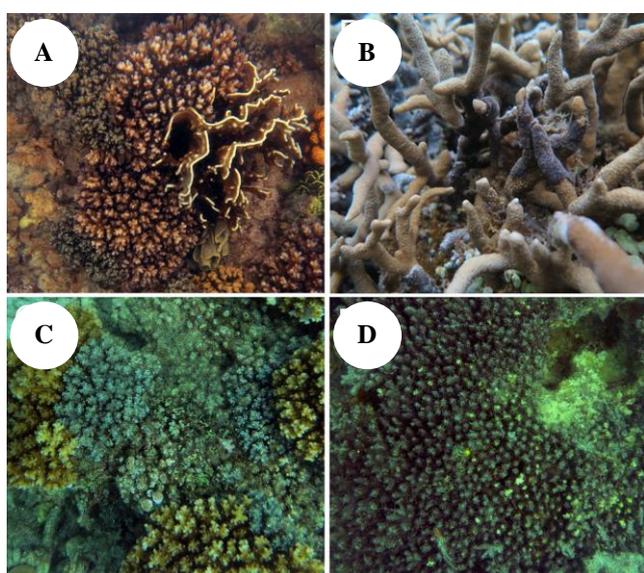
*Terpios hoshinota* is one of the killer sponges that attacks coral reef ecosystems. Figure 2 presents *T. hoshinota* in the observed regions.

**Table 1.** Identification data of invasive species using BLAST

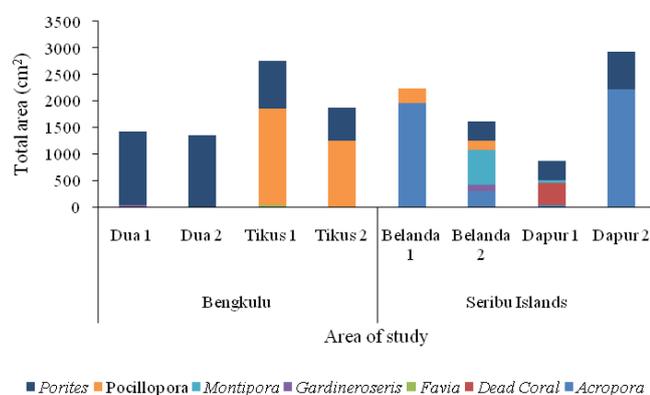
Sample ID	BLAST Analysis	Similarity (%)	GeneBank Accession
ITK_TIK1_SP1	<i>Terpios hoshinota</i>	97	MH048888.1
ITK_DUA1_SP1	<i>Terpios hoshinota</i>	98	MH048888.1
ITK_BLD1_SP1	<i>Terpios hoshinota</i>	96	MH048888.1
ITK_DPR2_SP1	<i>Terpios hoshinota</i>	97	MH048888.1

**Table 2.** Measurement data of coral reefs area covered by *Terpios hoshinota* at each point of the study location

Locality	Coordinate	Distance from Bengkulu/Jakarta (km)	Depth of patches (m)	Size of patch (cm <sup>2</sup> )	
Bengkulu	Dua 1	5°26'52,44" S 102°23'37,824" E	±140	±1	1426.49
	Dua 2	5°26'52,44" S 102°23'37,824" E	±140	±1,5	1355.98
	Tikus 1	3°50'12,624" S 102°11'47,65" E	±10	±1	2763.50
	Tikus 2	3°50'12,624" S 102°11'47,65" E	±10	±1,5	1874.07
Seribu Islands	Belanda 1	5°36'13,77" S 106°36'11,00" E	±65	±3	2247.77
	Belanda 2	5°36'13,77" S 106°36'11,00" E	±65	±6	1628.83
	Dapur 1	5°55'23,07" S 106°43'21,03" E	±15	±5	880.694
	Dapur 2	5°55'23,07" S 106°43'21,03" E	±15	±8	2927.224
Sum				15,104.55	



**Figure 2.** Morphology of *Terpios hoshinota*. A) Colonies found in the Tikus Island, Bengkulu; B) Colonies found in the Dua Island (Enggano ), Bengkulu C) Colonies found in the Belanda Island, Jakarta; D) Colonies found in the Dapur Island, Jakarta



**Figure 3.** Distribution of coral genus covered by *Terpios hoshinota* in the coral reef ecosystem

**Abundance of *Terpios hoshinota***

The total area covered by *T. hoshinota* at the research location was 15,104.55 cm<sup>2</sup>. The area of coral reefs covered by *T. hoshinota* varied at each station. The highest area covered by *T. hoshinota* was found in Tikus Island and Belanda Island was 4,637.57 cm<sup>2</sup> and 3,876.60 cm<sup>2</sup>. While the lowest area of coral reefs covered by *T. hoshinota* was found in Dua Island, with an area of 2,782.46 cm<sup>2</sup>. The result of coral reef area covered by *T. hoshinota* was shown in Table 2.

**Distribution of *T. hoshinota* and aquatic habitat characteristic**

Figure 3 shows the total area of the coral genus covered by *T. hoshinota* at the study location. The types of coral covered by *T. hoshinota* at the study location consisted of six genera namely *Acropora*, *Favia*, *Gardineroseris*, *Montipora*, *Pocillopora*, and *Porites*. Belanda Island is the location where the most common species of coral genus covered by *T. hoshinota* was found which consisted of five genera. While the other one was in Dua Island and Tikus Island with three coral genera in each island. The types of the coral genus found in the Belanda Island were *Acropora*, *Gardineroseris*, *Montipora*, *Pocillopora*, and *Porites*. The types of the coral genus found in Dua Island were *Acropora*, *Gardineroseris* and *Porites*, while the genera found in Tikus island were *Favia*, *Pocillopora* and *Porites*.

The results showed that the number of corals predominantly used as a substrate by invertebrates to grow and attach was the genera of *Porites*, *Acropora* and *Pocillopora* compared to other genera. The genera of *Porites*, *Acropora* and *Pocillopora* are the most dominant genera that easy to be attached by invertebrates because they have a gap among coral branches. So, it is easy for invertebrates to live on it.

The spatial distribution of *T. hoshinota* was shown in Figure 4. This diagram showed that four groups of research stations have linked between observed stations and *T. hoshinota* cover. The first group consisted of the Belanda 1 and Dapur 2 Islands identified by Scleractinia coral with genus of *Acropora*. The second group was the Belanda 2 identified by Scleractinia coral with a genera *Montipora* and *Gardineroseris*. The third group consisted of Dapur 1 Island identified by dead coral. The fourth group was the

Tikus 1, Tikus 2, Dua 1 and Dua 2 Islands identified by Scleractinia coral with the genera of *Favia*, *Porites*, and *Pocillopora*.

Water quality measurement was done to find out the actual condition of the waters as the research location. The characteristics of the coastal waters of the four research sites were classified as a feasible area for coral reefs to live and grow (Table 3).

Correlation matrix of Pearson at each point of the study location was shown in Table 4. This table showed that *T. hoshinota* has a negative correlation with the percentage of live coral cover, temperature, salinity, pH, ammonia, nitrate, and nitrite. Meanwhile, it has a positive correlation with DO and orthophosphate.

The characteristics of the biophysical conditions of the chemical environment were analyzed by the main component analysis approach called Principal Component Analysis (PCA). It consisted of percentage of live coral cover (%Lc), Total area of *T. hoshinota*, temperature, salinity, pH, dissolved oxygen (DO), ammonia, nitrate, nitrite, and orthophosphate data. The results indicated that the information describing the correlation among parameters was focussed on the two main axes F1 and F2. The reliance percentage of information quality for two axes were 45.92% and 19.28%, respectively, so the range of environmental characteristics of Scleractinian corals in the observed station could be explained from the total range of

the two main axes that was 65.20%. For more details, could be seen in the diagram (Figure 5).

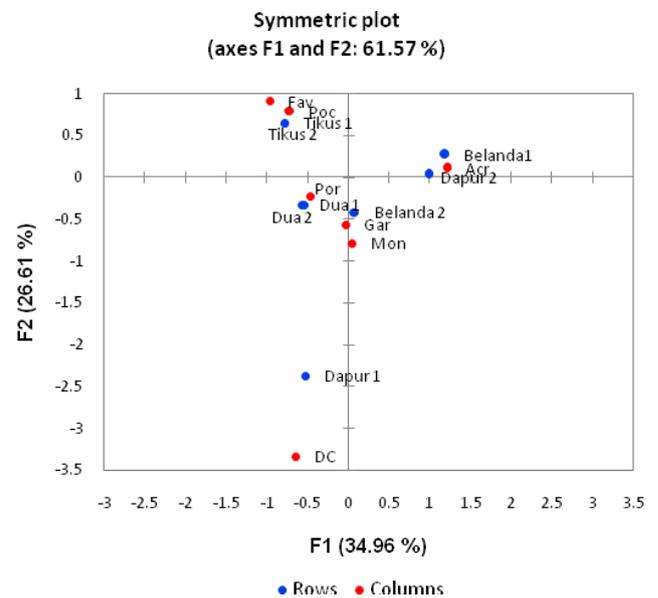


Figure 4. Correspondent analysis of genus invaded by *Terpios hoshinota* at each station

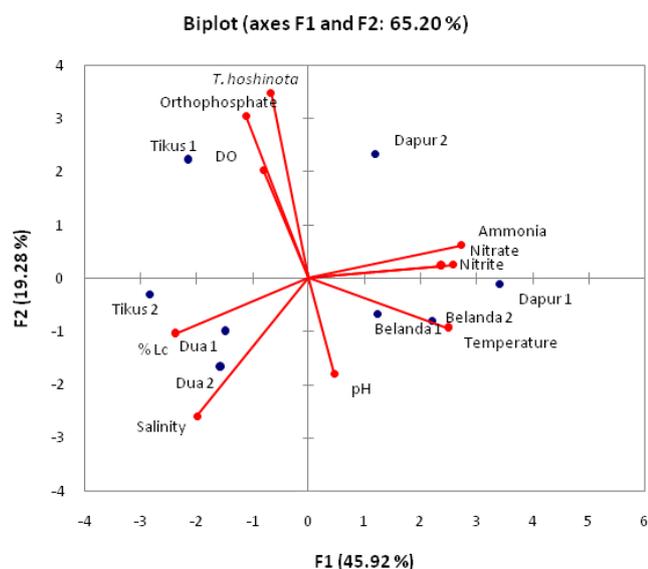
Table 3. Results of physical-chemical-biological parameters measurement of the waters at each point of the study location

Parameters	Bengkulu				Seribu Islands, Jakarta			
	Dua 1	Dua 2	Tikus 1	Tikus 2	Belanda 1	Belanda 2	Dapur 1	Dapur 2
Percentage of live coral cover (%)	38.27	36.27	39.67	66.73	31.80	26.47	17.67	15.53
Total area of <i>T. hoshinota</i> (cm <sup>2</sup> )	1426.49	1355.98	2763.50	1874.07	2247.77	1628.83	880.69	2927.22
Temperature (°C)	28.8	28.7	28.3	28.3	29.4	29.3	29.1	29.1
Salinity (psu)	34.6	34.6	32.2	32.2	31.8	31.8	29.1	29.2
pH	8.3	8.4	8.1	8.3	8.3	8.4	8.2	8.4
DO (mg/L)	7.6	7.2	7.4	7.6	6.8	6.6	7.6	7.8
Ammonia (mg/L)	0.085	0.034	0.037	0.045	0.183	0.174	0.311	0.221
Nitrate (mg/L)	0.071	0.071	0.071	0.065	0.072	0.087	0.098	0.08
Nitrite (mg/L)	0.004	0.004	0.005	0.004	0.006	0.007	0.006	0.005
Orthophosphate (mg/L)	0.004	0.004	0.007	0.004	0.003	0.005	<0.002	0.006

Note: \* Ammonia Quality Standard 0.3 mg/L; Nitrate 0.008; Orto Phosphate 0.015 mg/L (Ministry of Environment Decree 51 2004)

Table 4. Correlation matrix of Pearson at each point of the study location

Variables	% Lc	Temperature	Salinity	pH	DO	Ammonia	Nitrate	Nitrite	Ortho phosphate	<i>T. hoshinota</i>
% Lc	1	-0.737	0.543	-0.156	0.077	-0.771	-0.762	-0.577	0.040	-0.003
Temperature	-0.737	1	-0.384	0.442	-0.486	0.763	0.586	0.727	-0.385	-0.133
Salinity	0.543	-0.384	1	0.136	-0.230	-0.813	-0.669	-0.543	0.043	-0.235
pH	-0.156	0.442	0.136	1	-0.250	0.058	-0.025	0.000	-0.084	-0.093
DO	0.077	-0.486	-0.230	-0.250	1	0.008	-0.064	-0.632	0.089	0.102
Ammonia	-0.771	0.763	-0.813	0.058	0.008	1	0.862	0.675	-0.427	-0.174
Nitrate	-0.762	0.586	-0.669	-0.025	-0.064	0.862	1	0.702	-0.318	-0.398
Nitrite	-0.577	0.727	-0.543	0.000	-0.632	0.675	0.702	1	-0.109	-0.028
Orthophosphate	0.040	-0.385	0.043	-0.084	0.089	-0.427	-0.318	-0.109	1	0.765
<i>T. hoshinota</i>	-0.003	-0.133	-0.235	-0.093	0.102	-0.174	-0.398	-0.028	0.765	1



**Figure 5.** Principal Component Analysis between chemical biophysical parameters and the distribution of research stations on the F1 and F2 axes

Based on the circular diagram, the intersection between the axes F1 and F2 (Figure 5) showed that there was a correlation among temperature, ammonia, nitrate, nitrite, and percentage of live coral cover forming a positive F1 axis, while salinity contributed to forming a negative F1 axis. Orthophosphate and *T. hoshinota* contributed to forming a positive F2 axis.

The diagram of main component analysis formed three station distribution groups based on their relationship to environmental biophysical characteristics. The first station group consisted of the Dapur 1, the Belanda 1 and the Belanda 2 Islands where the station was characterized by a high concentration of ammonia, nitrate, nitrite, and temperature compared to other stations. The high concentration of ammonia and nitrate in seawater at the study site came from the river flow from the mainland of Java entering the Jakarta Bay. The distance of the study location to the Jakarta bay ranged  $\pm 15$  km, so the proximity of the study location allowed the increase of nutrients in the Dapur Island water. The concentration of nitrite and the value of temperatures on the Dapur 1, the Belanda 1 Island and the Belanda 2 Island were still categorized as optimum conditions for coral growth. The second group consists of the Dua 1 Island and the Tikus 2 Island characterized by their salinity and percentage of live coral cover. Salinity concentration at this station is still at the optimum limit for coral reef to grow. While the third group consisted of the Dapur 2 Island and Tikus 1 Island identified by Orthophosphate and *T. hoshinota*.

## Discussion

The current study has confirmed the species of *T. hoshinota* using molecular approach. Molecular techniques have become essential tools for identification of marine species (Madduppa et al. 2014; Prehadi et al. 2015; Kusuma et al. 2016; Maulid et al. 2016). The ITS region of

the ribosomal DNA has been used to distinguish genera and species of a wide range of demosponge taxa. The examination of ribosomal DNA sequences confirmed that the identity of sponge was *T. hoshinota*. All sequences were submitted to NCBI GenBank under accession number MH048888.1. The ITS sequence of *T. hoshinota* obtained from GenBank, originates from Taiping Island, South China Sea, where the sponge has had outbreak event in 2017 (Yang et al. 2018).

In the present study, *T. hoshinota* was observed in many reefs across Indonesia. Eight patches of *T. hoshinota* were found varying in size between 880.694 cm<sup>2</sup> and 2927.224 cm<sup>2</sup> and a total cover of 15,104.55 cm<sup>2</sup>. *T. hoshinota* was most abundant on the reef of Tikus Island (distance from Bengkulu 10 km) and Belanda Island (distance from Jakarta 65 km). These findings are supported by a survey Spermonde Archipelago (van der Ent et al. 2016), where *T. hoshinota* was also found at various locations across the shelf. It indicated that *T. hoshinota* has been observed in all different environments and spatially.

All live coral substrates were identified to genera level. *T. hoshinota* patches were mostly found on corals of the family Acroporidae, Poritidae and Pocilloporidae. Correspondence Analysis (CA) showed that the most prevalent coral genera infected by *T. hoshinota* in Belanda 1 and Dapur 2 Islands was *Acropora* (Acroporidae), while those in Bengkulu were *Porites* (Poritidae) and *Pocillopora* (Pocilloporidae). Multiple studies on *T. hoshinota* have been carried out in various countries including research conducted by Liao et al. (2007) and Tang et al. (2011) in Green Island, Taiwanese. The research study found that the common genera infected by *T. hoshinota* were *Montipora*, *Acropora*, *Isopora*, *Millepora*, *Favia* and *Porites*. In addition, van der Ent et al. (2016) figured out that the infected genera in the Spermonde Islands, South Sulawesi were *Acropora*, *Isopora*, *Montipora*, *Pocillopora*, *Seriatopora* and *Pavona*. However, according to Wang et al. (2012), there was no specific coral reef species that could be infected by *T. hoshinota*.

Various studies on the distribution and condition of coral reefs in the Seribu Islands were widely carried out (Cleary et al. 2006; Rachello-Dolmen and Cleary 2007; Johan et al. 2012; Madduppa et al. 2012). The eight observation points where patches of *T. hoshinota* were discovered also varied distinctly from each other in benthic community structure. *T. hoshinota* were found at sites with good, fair and poor cover of live corals. It indicated that *T. hoshinota* can survive within different benthic community structures. These findings are supported by a survey of Ryukyu Archipelago (Reimer et al. 2011) and Spermonde Archipelago (van der Ent et al. 2016), where *T. hoshinota* was also found in pure reefs, as well as reefs that increasing human pressure. However, the present study showed no large variation in patch size between the eight observation points where *T. hoshinota* was observed. *T. hoshinota* outbreaks are probably not related to variation in benthic community structure.

The highest area covered by *T. hoshinota* was found in Tikus Island and Belanda Island. Belanda Island has poor water quality with high nutrient concentrations. Cleary et

al. (2006) stated that the location of coastal waters was around 15-50 km influenced by fishing activities. In addition, it has poor water quality with high nutrient concentrations. During the dry season, the southeast wind blows, so that the flow of nutrients from the Jakarta Bay River flows to the Seribu Islands. The high level of nutrients in the water where coral reefs live and grow caused the fertile proliferation of algae and sponges which led to space competition (Cleary et al. 2006; Fadilah and Idris 2009; Madduppa et al. 2013; Fahlevy et al. 2018). As in Guam, *T. hoshinota* was found in polluted areas and the area experiences stress caused by coastal development (Plucer-Rosario 1987; Rützler and Muzik 1993). However, the lowest area covered by *T. hoshinota* was found in Dua Island, Enggano. Enggano Island is one of the outer Islands and far away from human activity. Dua Island has a clean aquatic environment quality remains infected by *T. hoshinota*. Similar situations were found in Green Island, Taiwan and Yonxing Island (Shi et al. 2012). An area located far away from human activity has a good water quality, however; it is also infected by *T. hoshinota*.

Coral reef degradation is a significant problem in the world. Competition among sessile organisms is one of the main ecological processes in coral reefs ecosystem and it can lead to changes in the diversity and abundance of coral species at spatial and temporal scales (Connel et al. 2004; Chadwick and Morrow 2011). Sponges are sessile organisms defending themselves by using chemicals. It causes them to become natural predators or parasites and they do this as an ecological function (Proksch 1994; Laport et al. 2009). *T. hoshinota* is a threat to coral reefs in Indo-Pacific Ocean. *T. hoshinota* make a symbiotic interaction with various microorganisms, such as bacteria and fungi (Bryan 1973; Plucer-Rosario 1987; Rützler and Muzik 1993; Hirose and Murakami 2011). Cyanobacteria have a very dense population on the spongy tissue. The composition of cyanobacteria in *T. hoshinota* sponges reaches 61% - 98% (Rützler and Muzik 1993; Hirose and Murakami 2011; Tang et al. 2011) and cyanobacterial photosynthesis are responsible for nutrient sources for *T. hoshinota* (Rützler and Muzik 1993; Soong et al. 2009). Some of these compounds are important in sponge ecological adaptations (Faulkner et al. 1994; Kobayashi and Kitagawa 1994; Guyot 2000; Proksch et al. 2003; Thakur and Müller 2004). The symbiosis between *T. hoshinota* and cyanobacteria helps *T. hoshinota* to be more competitive to attack coral reefs spatially and grow to cover coral reefs (Plucer-Rosario 1987).

In the current study, Principal Component Analysis (PCA) showed that at Belanda 1, Belanda 2 and Dapur 1 Islands, temperature, ammonia, nitrate and nitrite has high environmental characteristics correlation. At Dua 1 and Tikus 2 Islands, salinity and percentage of live coral cover have high environmental characteristics correlation. Meanwhile, at Tikus 1 and Dapur 2 Islands, orthophosphate has high environmental characteristics correlation. The result of the Pearson correlation between the live coral cover and *T. hoshinota* cover showed a negative linear relationship. It indicated that the higher live coral cover,

the lower *T. hoshinota* cover. But the value was not significant (-0.003).

Meanwhile, the result of the Pearson correlation between *T. hoshinota* cover and orthophosphate showed that positive linear relationship. This is indicated that the higher the orthophosphate, the higher *T. hoshinota* cover. The value was significant (0.765).

The cause of the spread of *T. hoshinota* was not yet clearly known. It was expected that the cause of the spreading *T. hoshinota* is due to environmental factors including availability of nutrients, host specificity, coral cover, ocean currents, and others (Shi et al. 2012). Ng et al. (2012) suspected that the increased concentrations of iron, phosphate, and nitrite lead to the blooming of cyanobacteria found in *T. hoshinota*. Meanwhile, according to Schils (2012), *T. hoshinota* infection was not only due to chemical components, but it might also due to human activities such as the sinking of the ship, burning forest and storm. Moreover, Averts (2000) suspected that the health of coral reefs might affect *T. hoshinota* infection. According to Elliot et al. (2016), there are three possibilities for *T. hoshinota* to attack coral reefs. First, *T. hoshinota* grow up covering the coral and rapidly expand to cover the coral. Second, the presence of allelopathic compounds that is able to produce cytotoxic compounds produced by sponges (Teruya et al. 2004). The last possibility is the presence of a bacterial community caused *T. hoshinota* to be more aggressive in killing corals (Wang et al. 2012; Tang et al. 2011).

This study concludes that the Coral-killing sponge *T. hoshinota* has been expanding in many reefs across Indonesia. *T. hoshinota* was most abundant on the reef of Tikus Island and Dapur Island. The most prevalent coral genera in the Seribu Islands infected by *T. hoshinota* was *Acropora* (Acroporidae) while those in Bengkulu were *Porites* (Poritidae) and *Pocillopora* (Pocilloporidae). Pearson correlation between the live coral cover and *T. hoshinota* cover was not significant, while *T. hoshinota* cover and orthophosphate was significant. This study suggests that orthophosphate may play a role invasion of *T. hoshinota* outbreaks.

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