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Diversity of marine tunicate from waters of Pannikiang Island and Badi Island of South Sulawesi, Indonesia

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Abstract. *Litaay M, Piri R, Jabir NB, Priosambodo D, Putra AW. 2023. Diversity of marine tunicate from waters of Pannikiang Island and Badi Island of South Sulawesi, Indonesia. Biodiversitas 24: 1431-1437.* The number of tunicates is currently known for approximately 3000 species, but it is estimated that there are still 1000-2000 more species that have not been described globally. The Spermonde Archipelago in South Sulawesi is a habitat for various tunicates, but not all islands have been well explored. This study aims to expand the distribution map of tunicates in Spermonde Archipelago, especially in the two study islands (Pannikiang Island and Badi Island). The diversity of tunicates between two islands, which have different ecological conditions, is compared in this study. This study used a line transect method along 20 m with a combination of plots at a depth of 5 meters. A transect was applied at four different locations according to wind direction. All tunicates inside the transect were recorded, counted, and sub-sample were collected for identification purposes. The data obtained were analyzed using the diversity index (Chao-1, Shannon-wiener, Uniformity, and Dominance). There are a total of 35 species (24 species at Pannikiang Island and 18 at Badi Island). *Nephtheis fascicularis*, *Didemnum molle*, *Clavelina robusta*, and *Polycarpa aurata* can be found on both islands. The levels of diversity of the two islands differ with little in common.

Keywords: Ascidian, biodiversity, Urochordate, Sangkarang, Wallacea region

INTRODUCTION

Tunicata is a marine animal belonging to the Urochordata group; most live attached to hard substrates on the seabed. There are 3000 species of tunicates recorded from four classes (Ascidiacea, Sorberacea, Thaliacea, and Appendicularia) (McClintock and Baker 2001; Santhanam and Ramesh 2020). Tunicata can be found on the seabed at depths of 3 m (Litaay et al. 2018) and inhibit habitat to more than 200 m depth (Ruppert et al. 2004). According to Ali et al. (2014), tunicates were found alive in the ship's hull. This unique ability makes several species of tunicates have a wide distribution globally with a pattern that follows the ship's path (Locke 2009). Approximately 40+17 tunicates are recorded as invasive species (Kelleher et al. 1995; Hayes et al. 2004) in most countries connected by sea transport (Locke 2009). Species such as *Styela piccata* and *Didemnum candida* distributed in Indonesia are now recorded to have been found in Canada (Locke 2009).

In addition to having invasive traits and fast-growing ability, tunicates are also known as marine animals producing bioactive compounds. Generally, bioactive compounds produced by various tunicates are believed to be part of nature's ecological defense. Those bioactive compounds have been widely used and benefit in different fields. For example, research has proven that tunicates have bioactive compounds that can be used as breast cancer inhibitors (Gonzales-Santiago et al. 2006; Michaelson et al. 2012; Atmaca et al. 2013; Kalimuthu 2014), prostate cancer inhibitors (Kalimuthu 2014) anti-cancer (Shaala and Youssef 2015). In addition, it can produce anti-bacterial, especially the methicillin-resistance *Staphylococcus aureus* (MRSA) (Litaay 2019a) and anti-fungal compounds (Karthikeyan et al. 2009; Christine et al. 2015; Litaay et al. 2015; Nurfadillah et al. 2015; Sardiani et al. 2015; Tahir et al. 2016) and a potential anti-virus (Murti and Agrawal 2010). One of the bioactive ingredients produced by tunicates can be used to treat soft tissue sarcoma (Sinko et al. 2012). The methanol fraction from *Pyura* sp. and *Polycarpa aurata* tends to be toxic for *Artemia salina* (Litaay et al. 2019). Apart from the present study area, some research has been conducted in other parts of Indonesia, especially on marine tunicate bioprospecting. Such as the study conducted by Manoppo et al. (2019); Ayuningrum et al. (2020); Rompas et al. (2022); Sibero et al. (2022).

The research about bioprospecting of tunicate usually begins with a biodiversity study. This preliminary study needs to be executed to know the species and its abundance in the research area. Researchers should avoid exploiting one species of tunicate in certain areas so that this species still exists naturally. On the other hand, there is only a few research on tunicate biodiversity in Indonesia (Gittenberger 2015; Tapilatu and Ginzel 2018; Sabrina et al. 2019; Saputri et al. 2019). Spermonde archipelago is located Southwest of Makassar, the capital city of South Sulawesi province. The most popular published research about tunicates comes from the Spermonde islands. In this study, both locations are situated within the Spermonde archipelago. Although the Badi island has been researched before by Fikruddin (2013) and Litaay et al. (2018), the Pannikiang island's waters are rarely explored. Pannikiang Island and Badi Island have different distances from the mainland. If referring to the Spermonde zoning by Hutchinson (1945) in Suriamihardja (2011), Pannikiang island is in zone I or the *inner zone*, while Badi island is in zone III or *the middle-outer zone.* Zonation in this archipelago is based on ecological factors due to the influence of the mainland on the east side and the Makassar Strait on the west. It is argued that these two major pressures can create a unique condition contributing to the distribution and abundance of marine biota. Therefore, assessing the species composition of marine fauna, particularly ascidian origin from different locations, is valuable to prove this hypothesis. This study aims to compare the diversity of marine ascidian between Pannikiang and Badi islands, improve biodiversity data, and expand the distribution map of tunicates in Indonesia.

MATERIALS AND METHOD

Study area

This research was conducted from October 2020 to November 2021 on Pannikiang Island, Barru District (4°20'20.1"S, 119°35'40.8"E) and Badi Island, Makassar City (4°57'53.9"S, 119°17'14.5"E). Each island is part of Spermonde island. Pannikiang is located in the Northeast of the Spermonde archipelago, while Badi is in the south. There are four sites on each island named by the site's position. Pannikiang island is coded PN, with PNN (North Pannikaing) site, PNE (East Pannikiang) site, PNS (South Pannikiang) site, and PNW (West Pannikiang) site. Badi island is coded BD, with BDN (North Badi), BDE (East Badi), BDS (South Badi), and BDW (West Badi) (Figure 1).

Procedures

This study used a line transect method with a combined plot of 20 m long with three repetitions. Data retrieval of the Pannikiang tunicate was carried out at 4 station points in the cardinal directions, namely north, east, south, and west, using a combination plot transect method at a depth of 5 meters' (following the island contour) with 20 meters transect and 2 meters width. The tunicate inside the transect line is recorded, photographed, and sampled for further identification. The tunicate samples were preserved using 70% alcohol for identification. Samples were identified based on morphological characteristics referring to the tunicate identification reference, including books and related articles. Species identification was carried out at the Environmental and Marine Sciences Laboratory, Faculty of Mathematics and Natural Sciences, Hasanuddin University.

Data analysis

Analysis used in this study includes the Chao-1 and rarefaction, which is used to estimate the suitability of the number of species and individuals sampled with the estimated number of samples in nature. In addition, diversity analysis, such as the Shanon-Wiener Index (H'), is used to calculate the level of diversity of each location, and uniformity index (e) and Dominance Index (D) are used to calculate the level of species dominance. A clad tree was analyzed using the Bray-Curtis method on species composition data of each location. All data analysis was carried out using the PAST 4.03 application. The result of each analysis was then compared to show the difference or similarities between both islands.

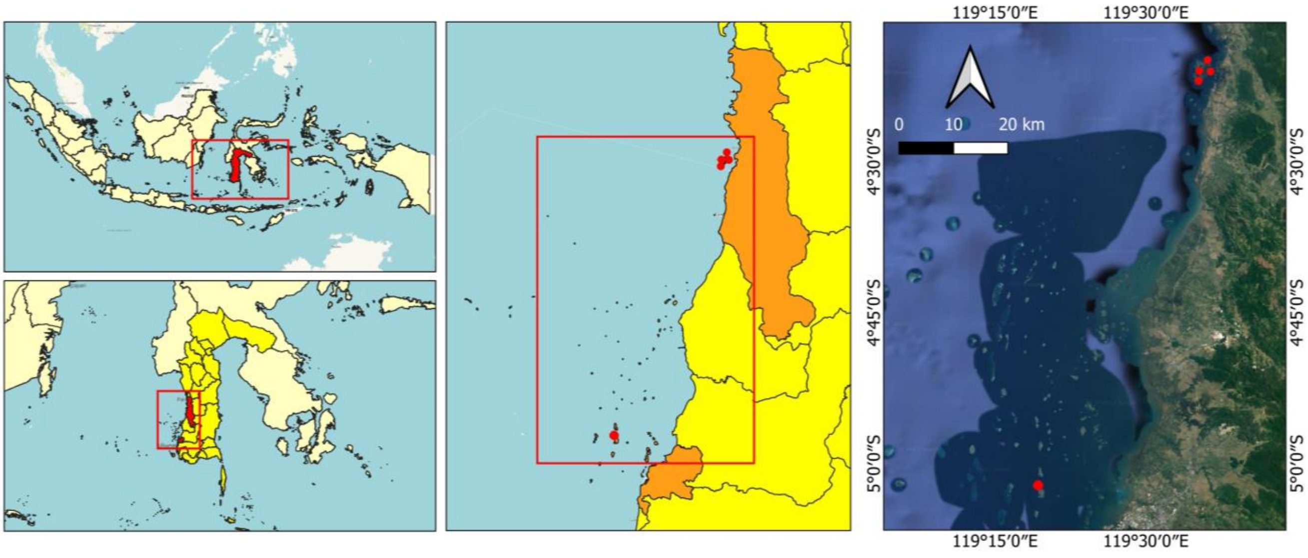


Figure 1. Research map in Pannikiang Island, Barru District and Badi Island, Makassar City, South Sulawesi, Indonesia

RESULTS AND DISCUSSION

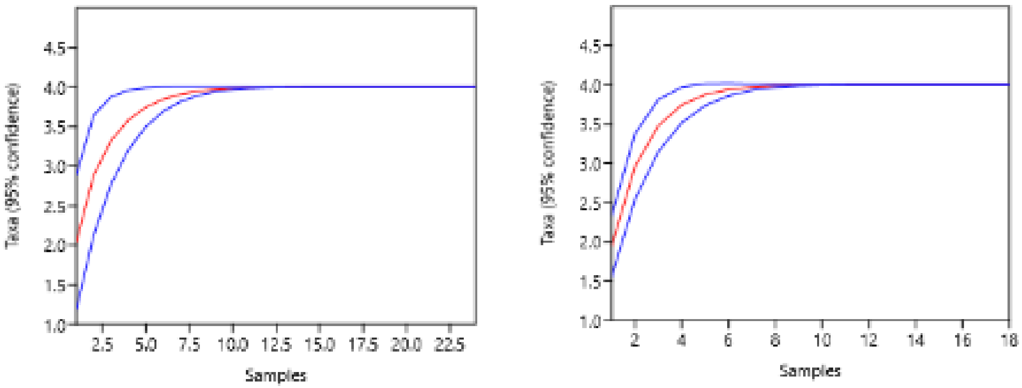
There are 24 species of tunicate found on Pannikiang island and 18 on Badi island. A total of 1416 individuals were recorded from Badi island and 2942 individuals from Pannikiang island (Table 1). The PNE has the highest species number (20 species), and PNN has the highest individual number (1,017). Both *Didemnum molle* and *Polycarpa aurata* have been recorded from every study site. These two species are known to be easily found around the Spermonde archipelago. The *Didemnum molle* number found on Pannikiang island (723) is greater than on Badi island (601). Same as the *Polycarpa aurata* number found on Pannikiang island, which is 159 individuals, while Badi island is only 99 individuals. We can observe that although both species can be found on both islands, their preferred habitat is likely on Pannikiang island.

The sites with the highest individual number (North Pannikiang and West Pannikiang) seem to have the same substrate types with sand and massive substrates. At the same time, the site with fewer individual numbers tends to have branching coral substrate. From this data, we know that ascidian's most proffered substrate is Massive coral (then the sand substrate is too hard to attach).

Based on the Chao-1 estimation method, the species number obtained in the field is the same as estimated. This analysis proves that the number of samples already represents each research location. The rarefaction graph also supports this result (Figure 2), which has reached a stationary (fixed) line. The fixed line in this figure means that it does not matter how many samples we took; the number of recorded taxa would not be increased.

The diversity analysis results showed that all locations showed a low level of tunicate dominance (<0.50). The highest level of diversity (H') was found in the western part of Pannikiang island (2.31), while the location with the lowest value was the southern part of Badi island (1.20). Therefore, all research sites are classified as areas with moderate diversity (1.0<H'<3.33). Based on the uniformity analysis, only the eastern part of Badi island (0.48) is classified as a depressed community (0<e<0.50). Apart from the eastern part of Badi Island, all research sites were classified as unstable communities (0.50<e<0.75).

Similarity analysis done by using the clustering method based on the presence of species in each location (Figure 3) shows a grouping by island (two clades). This difference indicates that each island has a different species composition between each site in Badi island; the North, West, and East site are grouped in a clade, with the North and west sides as the sites with the highest similarity in Badi island (67% similarity). Grouping in of clade in Pannikiang island seems to have the same composition as Badi island. North, West, and East are grouped in one clad with West and North Pannikiang as the sites with the highest similarity (75% similarity). Furthermore, this similarity is also the highest among other sites. The polygon pattern formed in the *non-Metric Multidimensional Scaling* (nMDS) analysis using the number of species shows low similarity (intersecting polygons) between the two islands. Therefore, the environmental factors at each location influence the similarity of both locations.



A B

Figure 2. Rarefaction curve of Pannikiang island (A) and Badi island (B). Both curves showed a fixed-line

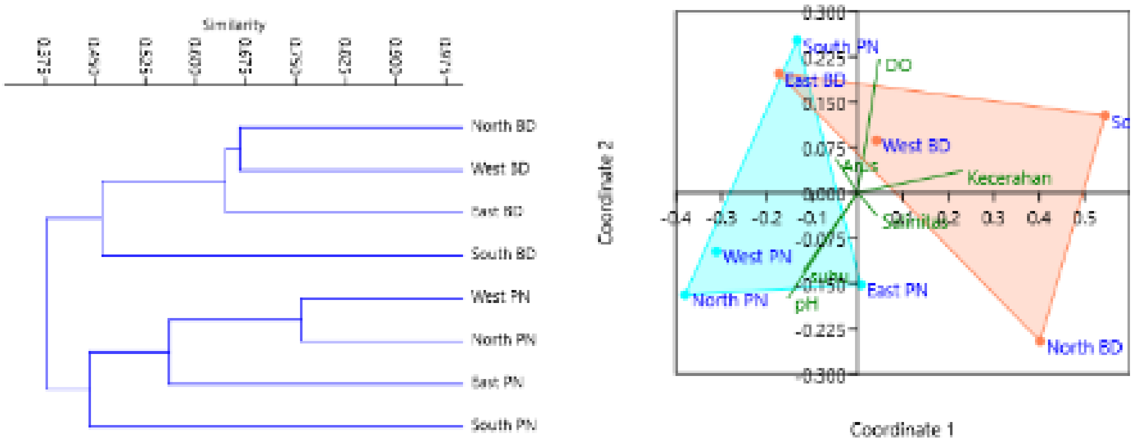


Figure 3. Results of clustering analysis (A) and *nMDS* (B) tunicate Badi Island (BD) and Pannikiang Island (PN)

Table 1. Tunicate from Badi and Pannikiang Island

Familia	Species	Location							
		Pannikiang				Badi			
		North	East	South	West	North	East	South	West
Ascidiidae	*Phallusia julinea*	0	0	0	4	0	0	0	0
	Phallusia arabica	0	0	0	2	0	0	0	0
	Ascidia sp. (morph transparent white)	0	0	0	0	2	0	0	0
	Ascidia sp.	0	0	0	0	0	0	1	0
Clavelinidae	*Clavelina lepadiformis*	134	0	0	109	0	0	0	0
	Clavelina arafuensis	224	58	0	176	0	0	0	0
	Clavelina robusta	13	4	0	8	5	47	0	17
	Nephtheis fascicularis	134	27	49	102	0	149	0	0
Diazonidae	*Diazona* sp.	94	18	24	49	0	0	0	0
	Rhopalea sp. (morph yellow spot)	2	0	0	1	0	0	0	0
	Rhopalea crassa	10	0	0	18	0	0	0	3
Didemnidae	*Didemnum albidum*	0	0	0	0	0	18	0	0
	Didemnum molle	114	84	327	198	30	322	47	202
	Didemnum carnulentum	0	0	0	11	0	0	0	0
	Trididemnum solidum	0	0	0	0	12	114	0	0
	Diplosoma sp.	0	0	136	0	0	0	0	0
	Diplosoma simile	0	0	0	0	0	0	89	0
	Didemnum sp.	0	0	118	0	0	0	0	0
	Diplosoma sp 2	0	0	0	8	0	0	0	0
	Atriolum robustum	0	0	0	82	0	0	0	0
Holozoidae	*Sigillina signifier*	0	0	0	126	0	0	0	0
Perophoridae	*Ecteinascidia bandaensis*	216	0	0	52	0	0	0	0
Polyclinidae	*Aplidium breviventer*	0	0	0	0	1	0	0	0
	Polyclinadae (morph black)	0	0	0	0	0	0	0	96
Pseudodistomaidae	*Pseudodistoma fragile*	28	0	0	0	0	0	0	0
Pyuridae	*Pyura chilensis*	1	2	0	0	0	0	0	0
	Pyura sp.	0	0	0	0	8	2	0	14
	Halocynthia dumosa	0	1	0	3	0	0	0	0
	Herdmania pallida	2	0	3	2	4	0	0	0
	Herdmania sp.	0	0	0	0	5	0	0	1
Styelidae	*Polycarpa aurata*	42	48	23	46	24	16	9	50
	Polycarpa sp. (morph brown)	0	0	0	0	0	13	8	34
	Polycarpa contecta	0	0	0	0	34	0	0	0
	Polycarpa sp. (morph white dots)	3	0	0	2	0	0	0	0
	Polycarpa sp.	0	0	0	4	13	3	10	13
	Individual count	1017	242	680	1003	138	684	164	430
	Species count	14	8	7	20	11	9	6	9
	Chao-1	14.00	8.00	7.00	20.00	11.00	9.00	6.00	9.00
	Dominance_D	0.15	0.24	0.31	0.12	0.16	0.30	0.39	0.29
	Shannon_H	2.04	1.60	1.42	2.31	2.02	1.47	1.20	1.53
Indices	Evenness_e^H/S	0.55	0.62	0.59	0.50	0.69	0.48	0.55	0.52

Table 2. Table of environmental data on Pannikiang and Badi islands, South Sulawesi, Indonesia

	pH	Salinity	Temp.	DO	Current (m/s)	Clarity	Substrate
Pannikiang							
North: 4°20'20.1"S 119°35'40.8"E	8.6	30	28	5.8	0.18	3.2	Sand, massive coral
East: 4°20'58.2"S 119°36'10.4"E	8.5	28	29	5.4	0.14	3.9	Sand, massive coral, Branching coral
South: 4°21'43.7"S 119°35'48.4"E	8.3	27	28	5.8	0.15	2.4	Sand, massive coral rubble
West: 4°20'54.8"S 119°35'42.2"E	8.6	28	28	5.8	0.22	3	Sand, massive coral
Badi							
North: 4°57'53.9"S 119°17'14.5"E	8.1	30	27	5.8	0.11	9.7	Sand, massive coral
East: 4°58'13.9"S 119°17'15.6"E	7.6	30	26	5.9	0.09	8	Sand, massive coral
South: 4°58'18.5"S 119°17'01.5"E	7.9	30	27	5.8	0.16	8.5	Sand, branching coral
West: 4°58'00.6"S 119°16'55.6"E	7.8	30	26	5.8	0.1	10.5	Sand, rubble

Discussion

This research recorded 35 tunicates species from Pannikiang and Badi Island. The number of tunicates found on Pannikiang island (24 species) more than on Badi island (18 species), respectively. The number of tunicates recorded was higher than found in the waters of Jemeluk and Panuktukan of Bali, as indicated in the study by Saputri et al. (2018) (10 species). But those results are lower than the number of tunicates recorded from the South China sea, as indicated by the study by Lee et al. (2011) (50 species). Previous research on Samalona island, a Spermonde archipelago (Zone II) (Litaay et al. 2018), found 18 species of tunicates. Those research are the same number as Badi island in this study. In a previous study by Fikruddin (2013), also on Badi island only recorded ten species of tunicates. The number of species found on each island has been proven to have maximized based on Chao-1 estimation and rarefaction analysis.

Different environmental conditions influence the number of species at each location. As Saputri et al. (2018) observed, which has good brightness (92.5 m), tunicates found only ten species. Similar to the study of Litaay et al. (2018b), with a brightness of 8.2 meters, the number of species found was 18, the same number as found in this study (Badi island), with a brightness level of ~9.1 meters. A different result was observed on Pannikiang island with low brightness (~3.5 meters) but found 24 species of tunicates. Concerning the relationship between the number of species and seawater's brightness level, suppose we use seawater quality standards for marine tourism (> 6 meters) (UU No. 22 of 2021) as a benchmark. In that case, water areas with a brightness level above the standard quality should have fewer species than those with a brightness level below the water quality standard. The more turbid the water area, the more species of tunicates are found, which means that tunicates can be used as an indicator of the level of turbidity of the waters. Another study by Sala (2012) observed that the abundance of tunicate is also affected by depth. The highest amount of tunicate was found at 10 meters.

Another environmental factor that affects the life of the tunicate is pH. A pH of 7.8-8.1 is considered safe for the life of the tunicate (Piri et al. 2022), with the optimum pH range being between 6.7-8.6 (Radhalakshmi 2014). In this study, most recorded site pH was in the optimum range for tunicate growth. Based on Kott et al. (2009), the optimum salinity level for tunicates is 29-32‰ or 30-32‰ (Saputri 2019), but in some locations (East PN, South PN, West PN) with a salinity level of fewer than 29‰ tunicates are still found. This study's lowest salinity level for tunicates recorded was 27‰ (South PN), below the optimum range. The salinity of the environment also affects colony growth (McKenzie et al. 2017). A controlled experiment carried out in the natural environment by Bullard and Whitlatch (2009) showed that ascidian can grow higher and faster with salinity in the range of 26-30‰ (normal seawater salinity) than in 15-28‰ or 10-26‰. This study revealed that colonies tested in the lower salinities (<10‰) experienced die-offs during this experiment. That also proved tunicates are impossible to grow in brackish water like sponges.

Pannikiang island is known to have natural mangrove forests that grow in almost all of its territory (Paembonan 2020). This mangrove forest traps sediment, usually released when waves hit. This sediment affects the turbidity of the waters around the island of Pannikiang. In addition, the presence of rivers (Takkalasi river, Mangempang river, and Salo Binangae) which empties near Pannikiang, also brought organic material to the Pannikiang waters. This condition is in stark contrast to Badi island, which is located far from the mainland and is not overgrown by mangroves like Pannikiang island.

The different environmental conditions between the two islands also affect the species of tunicates that can live on the two islands. Based on the similarity analysis using the Bray-Kurtis method with clades, the sampling points are grouped by island (Figure 3). The two islands have only 37% similar species composition. Analysis *nMDS* also showed similar results, showing two separate polygons with little overlapping. The area that intersects means there are similarities between the two areas. The results of this analysis (similarities) are supported by the presence of 4 species of tunicates (*Nephtheis fascicularis*, *Didemnum molle*, *Clavelina robusta*, and *Polycarpa aurata*) which were observed on both Pannikiang and Badi islands.

Moreover, species such as *Didemnum molle* and *Polycarpa aurata* can be found in all research stations. These two tunicates are species that are widely distributed in the Spermonde archipelago. At least five islands (Samalona, Barrang caddi, Barrang lompo, Lae-lae, Bone

Batang, and Badi) are recorded as habitats for *D. molle* and *P. aurata* (Fikruddin 2013; Litaay 2018a). But many more islands' biodiversity that is not published yet (writer experience) from the Spermonde archipelago. Furthermore, these two species are also found in Jemeluk and Penuktukan, Bali (Saputri 2019). The genus *Didemnum* is an invasive species at home and abroad (Shenkar and Swalla, 2011; Anzani et al. 2019). Two other species, *Nephtheis fascicularis,* previously known as *Oxycornia fascicularis* (WoRMS 2022) and *Clavelina robusta,* have also been found in the Spermonde archipelago (Litaay 2018a). *Polycarpa aurata* and *Didemnum molle* are found in the tropical eastern Indian and western Pacific oceans, including the Philippines, Indonesia, and northern Australia. Their habitat depth ranges are 5 to 50 m (Polycarpa) and 5 to 30 m (*Didemnum*) (Cornu 2009). Both species had the same lowest depth for their habitat and were the same as the sampling depth of this study. A recent inventory of coral reef organisms recorded species of *Polycarpa* and *Didemnum* among ascidians found off western Halmahera (Gittenberg et al. 2015), an island situated in northern Maluku.

The genus *Didemnum* is also known as colonial ascidians, and many of them are reported as introduced or invasive species (Lambert 2002; Oliviera et al. 2017; Anzani et al. 2019). The impact of introduced species like *Didemnum* is still not studied in-depth in Indonesia (Kartikasari et al. 2012), especially in the Spermonde archipelago. Still, from direct observation on Pannikiang island (the northernmost island of the Spermonde archipelago), there are already a few massive hard corals (*Porites* sp.) already colonized by hundreds of small-sized *Didemnum molle*. On the other hand, the study from Raja Ampat (Anzani et al. 2019) shows the same case where the ascidian colonies grew in relatively small mats and were patchy over larger areas. This kind of colonies coverage area is similar to the known invasive species such as *Didemnum vexillum* (McKenzie et al. 2017). Many seas squirt within the genus *Didemnum* have become widely introduced and invasive species around the world's marine waters (Anzani et al. 2019).

Some species of tunicates from the family of Styelidae, such as *Polycarpa,* are characterized by the brittle sand-embedded test and muscles to withdraw apertures and flatten the body and are adapted to live on the open sea floor (Kott 2005). *Polycarpa aurata* is commonly known as the ink-spot sea squirt (Charpin 2017). This species commonly lives solitary, unlike *Didemnum,* which lives within the colonies. That is also why this species number in this study is lower than *Didemnum.* Although this species lives in a solitary condition, these species tend to have the highest survival rate in harsh environments with high currents and tides due to the exitance of the muscle-like tissue mentioned before.

The presence of the native and introduced species on both islands is still unclear. Thus from this study, we can only present the actual diversity in the study area within the study time. For further information about any case of introduced species in both islands, we need to do a molecular study or at least compare the data from our study with older data from the available place. The biodiversity of ascidian in the Spermonde archipelago is still not fully mapped, considering there are still many islands that have not been explored yet (even in Indonesia, the same case of fewer studies also happens). So, Further research would be needed to confirm the species' biodiversity on every island so we can have baselines for subsequent studies or even for conservation on each island in the future.

In conclusion, the number of species found was 35 (24 from Pannikiang island and 18 species from Badi island). The levels of diversity of the two islands differ with little in common. Four species (*Nephtheis fascicularis, Didemnum molle, Clavelina robusta,* and *Polycarpa aurata*) can be found on both islands. More studies, especially on the exploration of marine tunicates, are suggested. That will provide baseline data to help the sustainable use of marine natural resources.

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