Diversity of holothuroidea by spicule type in the intertidal zone of Sepanjang Beach, Gunungkidul, Yogyakarta

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Abstract. Holothuroidea are common individuals of benthic communities in numerous marine biological systems. They work as supplement recyclers and bioturbators of delicate bottoms and are preyed upon by fishes, birds, mollusks, crustaceans, and other taxa. Sepanjang Beach has a coral community composed of coral reefs and dominated by algae in the surface, which is a habitat for a variety of complex and diverse benthic invertebrates, one of which is holothuroidea. However, little is known about the diversity of holothurians in the area. From 2014 to 2015 there were 5 species found on Sepanjang coast, namely Holothuria leucospilota, Actinopyga echinites, Stichopus horren, Holothuria scabra, and Holothuria hilla. Samples were collected on June 9, 2021 at Sepanjang Beach using the purposive random sampling method and identified based on the type of spicules taken on the dorsal and ventral integument. The results obtained found 7 species, namely Holothuria papilifera, Holothuria cinerascens, Holothuria impatiens, Holothuria pardalis, Actinopyga miliaris, Holothuria hilla, and Actinopyga sp. The absence of previous records of these species may be associated with their low densities and cryptic habits. This research acts to fill knowledge about distribution and diversity of holothurians in the south coast of java island, especially Sepanjang beach, Gunungkidul, Yogyakarta.

1. Introduction

Holothuroidea is a class of Echinoderms. Holothuroidea have long or cylindrical bodies with soft bodies. These animals have podia feet on the ventral side and reduce spines. Its body is composed of lime or CaCO3, this causes Holothuroidea to have quite thick skin. Holothuroidea has a crown of tentacles anteriorly or its mouth, and anus posteriorly. Some species can spray a sticky liquid Cuvieri an Tubules (CT) to protect from predators [1]. In the sea Holothuroidea acts as a nutrient decomposition and food chain [2].

Holothuroidea can be found in the intertidal zone to the deep sea. In Indonesia, about 300 species of Holothuroidea have been identified [1, 3, 4, 5, 6, 7]. Holothuroidea can be identified by taking small parts of their bodies and identifying them microscopically. Spicules are lime substances that can precipitate when dissolved in sodium hypochlorite (NaClO). Spicules have various shapes so that one species will have differences with other species [8].

One of the beaches that has the potential for Holothuroidea is Sepanjang Beach, according to research by Amalia et al. [8] there are three species of Holothuria leucospilota, Actinopyga echinites, and
Stichopus horrens found on Sepanjang Coast. According to research conducted by Yusron [9], three species were also found, namely Holothuria scabra, Holothuria hilla, and Holothuria leucospilota. Holothuroids are less popular compared to the other members of Echinoderms, for example Asteroids or sea star. The research carried out about the diversity of this class is also less in number compared to Echinoids and Ophiuroids, especially in Sepanjang Beach. This research was conducted to determine the diversity of Holothuroidea on Sepanjang Coast and as data and at the same time annual monitoring.

2. Materials and methods
This research was conducted in the intertidal zone of Sepanjang beach, Gunung Kidul Yogyakarta, Indonesia, situated in 8°8’15”S latitude and 110°33’59”E logitude at June 9th 2021. The area is characterized by sandy and rocky substrate with various species of algae and some sea grass on it. Sample were collected using purposive sampling methods by walking through the coast line and take a random sample of Holothuria. The sample of Holothuria is placed in a millimeter block with a comparison of tweezers and then taking picture of the dorsal, ventral and perifer part of obtained sample using digital camera for further identification. Identification of holothurians requires examination of various features including the morphologic characteristic and the form of calcareous spicules. Holothuria that has been taken randomly is placed on a tray. After that, relaxed with MgCl2. The spicules extracted by using a method adapted from Rowe and Doty [10]. This was done by getting 1 cm tissue of the dorsal and ventral epidermis. Then, the extracted tissue is placed in a flacon containing NaOCl and left for 15 – 20 minutes. After obtaining lime deposit or spicules, NaOCl is taken carefully with a dropper. The lime deposit or spicule were rinsed with clean water and allowed to stand for 5 minutes. Afterwards, clean water is taken with a dropper until leave the lime precipitate. The lime precipitate or spicule was given 70% alkohol and examined using compound microscope. The morphological character analysis using taxonomix classification keys as a guide based on Clark and Rowe [3] and Purcell et al [11].

3. Result and discussion
Based on sampling and data analysis of Echinoderms species from the intertidal zone of Sepanjang Beach, Gunung Kidul, Yogyakarta, the result obtained from this study is there are 8 species of echinoderms with various types of spicules.

| Table 1. Diversity species of Holothuroidea in Sepanjang Beach. |
|-------------------------------------|
| **Family** | **Genus** | **Species** | **Type of spicules** |
| Holothuriidae | Holothuria | Holothuria papillifera | Table, button, rod, and perforate plates |
| | | Holothuria chinerascens | Table, button, rod, and perforated plates |
| | | Holothuria impatiens | Table, button and rod |
| | | Holothuria pardalis | Table, button, rod |
| | | Holothuria hilla | Button, tables, tables, and rod |
| Actinopyga | Actinopyga miliaris | | Perforated plates and rod |
| | Actinopyga sp. | | Rosettes and rod |
| | Actinopyga sp. | | Rosettes and rod |
**Figure 1.** Morphological *Holothuria papillifera* dorsal view (a) ventral view (b) perifer view (c).

**Figure 2.** Spicules of *Holothuria papillifera* with (a) table top-view shaped (b) button shaped (c) rod shaped of dorsal tegument (d) perforated plates shaped of ventral tegument.

**Figure 3.** Morphological *Holothuria cinerascens* dorsal view (a) ventral view (b) perifer view (c).

**Figure 4.** Spicules of *Holothuria cinerascens* with (a) table side-view shaped of dorsal tegument (b) perforated plates shaped (c) rod shaped (b) button shaped of ventral tegument.

**Figure 5.** Morphological *Holothuria impatiens* dorsal view (a) ventral view (b) perifer view (c).
Figure 6. Spicules of *Holothuria impatiens* with (a) table side-view shaped (b) rod shaped of dorsal tegument (c) button shaped of ventral tegument.

Figure 7. Morphological *Holothuria pardalis* dorsal view (a) ventral view (b) perifer view (c).

Figure 8. Spicules of *Holothuria pardalis* with (a) table top-view shaped of dorsal tegument (b) button shaped (c) rod shaped of ventral tegument

Figure 9. Morphological *Holothuria hilla* dorsal view (a) ventral view (b) perifer view (c).

Figure 10. Spicules of *Holothuria hilla* with (a) button shaped (b) tables-slide view shaped (c) rod shaped of dorsal tegument(d) tables top-view shape of ventral tegument.
Figure 11. Morphological *Actinopyga miliaris* dorsal view (a) ventral view (b) perifer view (c).

Figure 12. Spicules of *Actinopyga miliaris* with (a) perforate plates shaped of dorsal tegument (b) rod shaped of ventral tegument.

Figure 13. Morphological *Actinopyga sp* (sp 1) dorsal view (a) ventral view (b) perifer view (c).

Figure 14. Spicules of *Actinopyga sp.* (sp 1) with (a) rosettes shaped (b) rod shaped of dorsal tegument.

Figure 15. Morphological *Actinopyga sp* (sp 2) dorsal view (a) ventral view (b) perifer view (c).
All sea cucumbers are ocean dwellers, though some inhabit the shallows and others live in the deep ocean. Holothuroidea are a benthic animal that moves slowly, alive on sand substrate, mud, reef environment coral and seagrass beds are often encountered on the vote in Indonesia. Holothuroidea have been used as a food and for various medicinal purposes such as inflammatory conditions in Asia (especially in East Asia, such as China, Japan, and South Korea) and the Middle East for many years [8]. This aquatic animals are often deposit and suspension feeder as benthic nutrient recyclers feed large volumes of benthic sediments, and assimilate fungal, bacterial and detrital organic matter (OM) [12]. Also, they play an important role in coral reef, food chains as detritus feeders, suspension feeders and help to increase coral reef production [13].

Based on the results of this study, eight species of Holothuroids was found in this location, there are *H. papillifera*, *H. chinerascens*, *H. impatiens*, *H. pardalis*, *H. hilla* from the genus Holothuria and *A. miliaris*, Actinopyga sp. from the genus Actinopyga. Identification is done based on its calcareous spicules and morphological characteristics. Each species has a specialized character of calcareous spicules on their ventral, dorsal, and perifer body. Ventral, dorsal, and perifer spicules were collected and preserved. The morphological spicules observed and compared to existing literatures (*FAO Species Identification Guide for Fishery Purposes Vol. 2*).

*H. impatiens* have a light brown body color with 5 or more dark brown transverse bands on the dorsal surface which become spots posteriorly while ventral surface is beige. The body of this species has been described as bottle shaped and is rough to the touch. Podia are relatively sparse and the mouth contains 20 tentacles. Ventral and dorsal body wall with similar table and button spicules. Table spicule with round, smooth disc, perforated by 4 large and 4–8 peripheral holes, short spire ending in a spiny crown. Button spicules with a smooth rim and 3–4 pairs of holes, and sometimes with median Ine. Maximum body length is about 26 cm and average weight is 50 g. average body length probably <20 cm. It can be found under rocks in shallow waters between 0 and 2 m. However, it can be observed up to 30 m depth. It can be found from East Africa and the Indian Ocean to the western central Pacific including Hawaii, in the Pitcairn Islands group, and including much of the Pacific coast of Central America and Indonesia [14].

*H. papillifera* occupies the soft substrate, in sand, and seagrass areas and is never found at the reef slope or reef crest. They have medium size to large specimens, body club-shaped, narrow anteriorly, much larger posteriorly. Colour of living specimen uniform greyish brown. Colour in alcohol brown dorsally, beige to light brown ventrally, Dorsally, numerous, large conical papillae without alignment. Ventrally, tube feet densely crowded in the ambulacral as well as in the interambulacral areas. Habitat of *H. papillifera* was considered to be endemic to the Red Sea [15].

The physical structure of *H. cinerascens* includes retractable tube-feet (podia) and distinctive feeding tentacles often called primary tentacles or primary buccal podia, rough skin, and are void of arms and pedicellariae. *H. cinerascens* like other sea cucumbers have a decentralised nervous system, a simple reproduction system and a complete digestive system. The alimentary canal provides support for many organs such as gonad, respiratory trees and circulatory systems (rete mirabile). The cloaca participates in the respiration process by conducting impulses from the nervous system of the adjacent body wall to induce the pumping action of the cloaca that aerates the respiratory trees. The madreporite draws water from the coelom into the ring canal via the stone canal and directs the water into the ampullae. The
constriction of the top ampullae extends the tube feet (podia) for movement and the tentacles that surround the mouth (elongated podia) for feeding. *H. cinerascens* are suspension and non-selective feeders that feed by holding their tentacles in the water column to trap suspended particulate materials (microorganisms, phytoplankton, plant debris, solid particles, inorganic components) including microplastics, thus contributing to the transfer of great amounts of energy, nutrients and non-nutritious (microplastics) items from benthic-pelagic organisms to benthopelagic predator species [16].

*H. pardalis* found under coral rubble or hard stones, inhabits shallow waters over blocks of coral and buried in the coral rubble. The distribution of species from Madagascar and east Africa, to the Red Sea, Australia to China, and east across the Pacific to Hawaii, Cocos Island, Costa Rica, the Galapagos Islands and the countries in South America is also a native species of Pakistan. Body wall tables with rounded disc with four distinct central and several small marginal holes; spire of moderate height with about eight teeth; pseudo-buttons mostly complete, only few incomplete, always smooth. Ventral podia with curved rods with 1–5 perforations at each end, elongated plates with a double series of holes and a smooth undulating margin, never serrate [17].

Holothuria hilla is the next species that was found in Sepanjang Beach. Its known as the Sand Sifting Sea Cucumber. *H. hilla* widespread in the Indo-Pacific region [17] [18]. This species can be found in sandy, rocky and seagrass ponds hiding behind stone [19]. The maximum length and weight of *H. hilla* species is recorded 44 cm and 210 gram, respectively. *H. hilla* have a cylindrical body shape and has blunt ends with a thin and soft texture. The color is generally brown with white spots and numerous spiny papillae on the surface body. This Sand Sifting Sea Cucumber feed on bacteria, algae and surface particles. This species is very sensitive to high levels of copper-based medications. Its will not tolerate high nitrate levels. Cuverian tubules absent [20]. Spicules found on the body-wall are buttons tables, and perforated plate. While the rods occurs in the tentakel [4].

*Actinopyga* is one of the five genera in the family Holothuriidae within the genus there are sixteen species generally recognized as valid: *A. agassizii*, *A. albonigra*, *A. bacilla*, *A. bannwarthi*, *A. caroliniana*, *A. crassa*, *A. echinites*, *A. flammaea*, *A. fusca*, *A. lecanora*, *A. miliaris*, *A. obese*, *A. palauensis*, *A. serradens*, *A. spinea* nd *A. mauritiana* [21]. *Actinopyga* sp. widespread in the Indian and Pacific Ocean also the Atlantic (Caribbean area). *Actinopyga* sp. species is associated with a well-developed coral reef ecosystem [22]. *Actinopyga* sp. have elongated body shape with tentacles 20-30. Anus is located at posterior end, while mouth at anterior part. Anus surrounded by 5 calcified anal papilae [23, 3]. Podia are found on the ventral side and usually irregularly arranged on the interradii or more regularly on the radii. On the dorsal side papillae are scattered. *Actinopyga* sp. body-wall have similarities with *Bohadschia* species, including size and calcareous ring. Spicules are found on dorsal and ventral side. Spinose rods and rosette are the most common types. Tables, buttons or elaborate plates type never present in *Actinopyga* sp. The species of *Actinopyga* are separate from each other by the form and complexity of the rods [23].

*Actinopyga miliaris* (Hairy Blackfish) distributions occur in the tropical Indo-Pacific, including the Red Sea, but excluding the Persian Gulf and Hawaii [24]. The Hairy Blackfish *A. miliaris* mostly common on the reef flat and lagoon habitat [11]. They live in moderately shallow waters and rarely found at a depth of more than 10 meters. *A. miliaris* about 35 cm in maximum length but commonly to about 25 cm, mean live weight about 0.4 kg–1 kg. The body-wall thickness about 6 mm. Actinopyga miliaris have an elongated body shape, cylindrical, slightly arched dorsally (bivium) and flattened ventrally (trivium). Dorsal side with characteristic covered by mucus and sediment with papillae long and slender [24]. Their dorsal color in general is black or dark brown but on the ventral side more lighter [11]. Long and thick podia on ventral side arranged more or less regularly in tight rows on the radii. Mouth ventral surrounded by 20 stout tentacles. Calcareous disc diameter of podia *A. miliaris* around 700 μm. Anus guarded by 5 triangular anal teeth. Calcareous ring with large radial pieces and narrow interradials. Cuvierian tubes not found. Spicules are found on dorsal and ventral side. Rosette type most occurs on dorsal side. A variety simple or more complicated rosettes spicules are found on the ventral side. Rosettes spicules present on the podia ventral dorsal. Large spicules found in the podia and papillae. Tentacles have many rods spicules [24].
Just like other organisms, sea cucumbers have certain environmental conditions to be able to live and breed in a location. Environmental factors that affect the presence of sea cucumbers include salinity, temperature, light, pressure, and food. The salinity required by sea cucumbers of the genus Holothuria is normal salinity of 30-37‰, this genus cannot survive at low salinity. Low salinity levels will cause the cells in the body of Holothuria to lyse so that they are unable to survive. The next factor is environmental temperature, hoturia lives at all depths of the sea so that it has the ability to tolerate a wide temperature range. Some researchers note that sea cucumbers are able to survive at temperatures of 25-35°C. More than 35°C the body of the sea cucumber will experience inactivity but its tentacles can still move. The genus Holothuria can be found at a depth of 0-10,750 m above sea level, this shows that the tolerance to pressure is very large. This genus is able to live in various pressures, but in some other genera pressure will affect its existence. Another major factor that supports the existence of sea cucumbers in a particular habitat is food. Sea cucumber food in the form of plankton, detritus and other organic substances contained in the mud or sand. Other types of food are small organisms, protozoa, algae, seaweed, and small pieces of marine animals and plants as well as sand particles. The results showed that sea cucumbers like habitats with clear waters and relatively calm water flow. They live in groups. In addition to habitat differences, the spread of Holothuria is also influenced by the abundance of available food in the form of plankton and detritus. Several studies have shown that there is a positive correlation between the distribution of several types of sea cucumbers and the type of habitat [25].

4. Conclusion
Based on the results of the research that has been done, it can be concluded that there are 7 species of Holothuroidea on the coast along Gunungkidul, namely Holothuria papillifera, Holothuria chinerascens, Holothuria impatiens, Holothuria pardalis, Actinopyga miliaris, Actinopyga sp., and Holothuria hilla. There are 7 spicules found in this study, namely table side view shaped, perforated plates shaped, rod shaped, button shaped, table top view shaped, table slide view shaped, and rosettes shaped.

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