Morphology of Spicules of Phyrella sp. (Sea Cucumber) from Demak Waters, Central Java, Indonesia

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Abstract

Phyrella is a genus of sea cucumbers that holds significant economic value and is widely exported for human consumption. Despite its importance, knowledge regarding the species diversity of Phyrella in Indonesian waters, particularly in Demak Waters, remains limited. This study aims to identify the Phyrella species found in this region through morphological analysis of spicules. The identification process involved examining the spicules present in the calcareous ring and various body parts, including the anterior, posterior, ventral, and dorsal regions. Spicules were observed using a light microscope at 100x magnification after dissolving body tissues with a sodium hypochlorite (NaOCl) solution to isolate their structure. The findings revealed four distinct spicule shapes: buttony, mensal, barbed, and barbed wire rod. These spicule characteristics are essential in distinguishing Phyrella species. Based on these morphological features, the sea cucumber species found in Demak Waters was identified as Phyrella fragilis. The results contribute to taxonomic studies and provide insights into the ecological role of Phyrella fragilis in marine ecosystems. Furthermore, this species has potential applications in the food, pharmaceutical, and cosmetic industries due to its rich nutritional content and bioactive compounds. Understanding its morphology and distribution can aid conservation efforts and sustainable resource management.

Keywords: *Phyrella fragilis; spicules; Demak Waters; morphology; species identification*

INTRODUCTION

Demak waters are located in the northern part of Central Java Province and are an aquatic ecosystem rich in biodiversity from benthic and pelagic groups of organisms. Various types of organisms have been found in these waters, including crabs (*Portunus pelagicus*), various types of shellfish, blue crabs, sea cucumbers, mollusks, gastropods, and various types of fish that have important ecological and economic value for the surrounding community (Hartati *et al*., 2023). One organism that attracts attention regarding ecology and its benefits is the sea cucumber, which belongs to the *Phyrella* genus.

Sea cucumber *Phyrella* sp. is often found together with several other types of sea cucumbers, such as *Phyllophorus* sp., *Acaudina* sp., and *Paracaudina* sp., all of which are members of the Holothuroidea class that live on the seabed (Widianingsih *et al*., 2021). The primary habitat of *Phyrella* sp. is generally in areas with muddy sand substrates rich in organic matter, with water temperatures ranging from 28.6 to 29.9 degrees Celsius and salinity levels ranging from 28 to 35 ppt, which are optimal conditions for the survival and growth of this sea cucumber (Widianingsih *et al*., 2023).

Various species of the genus *Phyrella* have been found in Indonesia, including *Phyrella fragilis* and *Phyrella tenera*, which have a wide distribution throughout Indonesian waters. In addition, there is also the *Phyrella bedoti* species, which has a more limited distribution and is only found in

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the waters of eastern Indonesia (Michonneau *et al*., 2014). Ecologically, the presence of *Phyrella* sp. in an aquatic ecosystem has a vital role, especially in increasing the fertility of the seabed substrate through bioturbation activities carried out by sea cucumbers when looking for food (Shiell *et al*., 2006). In addition, these sea cucumbers also contribute to the food chain by being a source of food for various natural predators in the sea, thus maintaining the balance of the aquatic ecosystem (Widianingsih *et al*., 2023).

Phyrella sp. also has economic value and benefits in the health sector, making it one of the most sought-after marine commodities (Purcell *et al*., 2014). Various studies have shown that *Phyrella* sp. has a relatively high nutritional content, such as protein in the form of collagen, various types of essential minerals, unsaturated fatty acids such as omega-3 and omega-6, and other healthy fats that are beneficial for the human body (Zhao *et* al., 2019; Apriliani *et al*., 2024). With this diverse nutritional content, *Phyrella* sp. has been widely used in the food, pharmaceutical, and cosmetic industries and is one of the fishery products with high export potential due to increasing demand in the international market (Lee *et al*., 2017).

Taxonomically, *Phyrella* sp. is classified into the Holothuroidea class, Dendrochirotida order, Phyllophoridae family, and *Phyrella* genus. To identify *Phyrella* sp., researchers use various methods, both through morphological identification and DNA analysis, to confirm the species found (Patantis *et al*., 2019; Sun *et al*., 2022). One of the identification methods often used in research is to observe the shape of the spicules found on its body (Kinch *et al*., 2008). Spicules are microscopic structures composed of lime and have a distinctive shape in each sea cucumber species (Byrne, 2015). They can be used as diagnostic characters in the identification process. Observation of the shape of the spicules is critical in taxonomic studies and species identification because it can provide more detailed information about the characteristics of the body tissue of *Phyrella* sp. (Widianingsih *et al*., 2023).

Based on the importance of the ecological, economic, and health benefits of *Phyrella* sp., further research is needed on this species, especially in terms of its morphological identification and characterization. Therefore, this study was conducted to identify and morphologically analyze *Phyrella* sp. by observing spicules on various parts of its body to provide a deeper understanding of the characteristics and potential of this species.

MATERIALS AND METHODS

Sampling of *Phyrella* sp. in this study was conducted in Demak Waters, Central Java, a coastal area with a muddy sand substrate rich in organic matter. The method used was bottom dredge, which was applied randomly at several predetermined points. Bottom dredge was chosen because this tool effectively captures benthic organisms that live on the bottom of the waters without causing significant disturbance to the surrounding environment. After being successfully captured, *Phyrella* sp. samples were separated from other organisms, stored in containers filled with seawater, and taken to the laboratory for further analysis.

In this study, various equipment was used to support the identification and morphological analysis process of *Phyrella* sp. The leading equipment used includes a camera for sample documentation before and after analysis, surgical instruments such as scalpels and tweezers for tissue collection, sample bottles for specimen storage, a light microscope for observing spicules with a magnification of 100x, as well as object glass and dropper pipettes to facilitate the extraction and observation process. In addition, several chemicals were also used, such as 70% alcohol for sample preservation and sodium hypochlorite (NaOCl) solution, which dissolves body tissue so that only the spicule structure remains to be observed.

Morphological observations of *Phyrella* sp. were carried out with a focus on the structure of the spicules that play a role in the species identification process. Spicules were taken from various

body parts, such as the calcareous ring, ventral, dorsal, anterior, and posterior parts. This process begins by taking a piece of tissue measuring about 1 cm, which is then soaked in NaOCl solution for 15 to 20 minutes until all soft tissue dissolves. After that, the spicules were washed using distilled water seven times to remove residual chemical solutions and dirt that sticks. The clean spicules were then placed on a glass object and allowed to dry before being observed under a light microscope with a magnification of 100x.

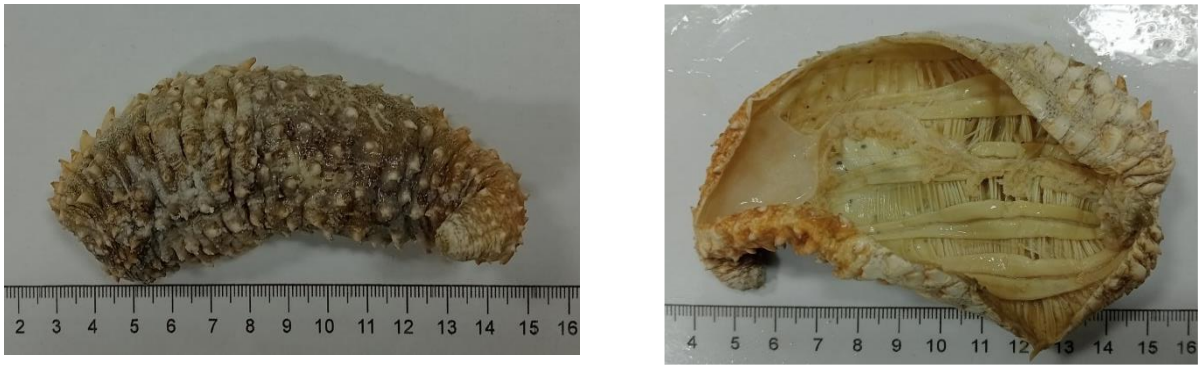
Overall, the method used in this study allows for systematic and accurate identification of *Phyrella* sp. Using NaOCl solution proved effective in separating spicules from body tissue without damaging them, while observation using a light microscope with 100x magnification provided a detailed picture of the spicule structure. The results of this study are expected to be a reference in taxonomic and ecological studies of *Phyrella* sp. and provide further insight into its distinctive morphological characteristics.

RESULTS AND DISCUSSION

Demak waters, located on the northern coast of Central Java, are natural habitats for various benthic organisms, including sea cucumbers of the genus *Phyrella*. Research conducted in this area has successfully identified the presence of *Phyrella* sp., which has distinctive morphological characteristics that can be used for taxonomic identification.

Based on observations, *Phyrella* sp. found in Demak waters has a body color that varies from white to brown, with brown tentacles contrasting with its body color. Its body is covered by tube feet for movement and attachment to the seabed substrate (Anderson *et al*., 2020). In addition, the number of tentacles owned by *Phyrella* sp. ranges from 14 to 20, arranged in two rings on the anterior part of its body. Its body structure is cylindrical with curved and tapered ends. This body shape helps *Phyrella* sp. adapt to the muddy substrate, its primary habitat (Michonneau *et al*., 2014).

The observation results show that the morphological characteristics of *Phyrella* sp. found in Demak waters are very similar to the *Phyrella fragilis* species. This is supported by the results of previous studies conducted by Heding *et al*. (1954) and Michonneau *et al*. (2014), which explained that *Phyrella fragilis* has a white-brown body color, several tentacles between 14 and 20 and a body structure that resembles a cylinder with a tapered tip. Therefore, considering the similarities in these morphological characteristics, it can be concluded that the *Phyrella* sp. species found in Demak waters is most likely *Phyrella fragilis*.



a b

Figure 1. *Phyrella fragilis* Fresh body (a); longitudinal muscle (b)

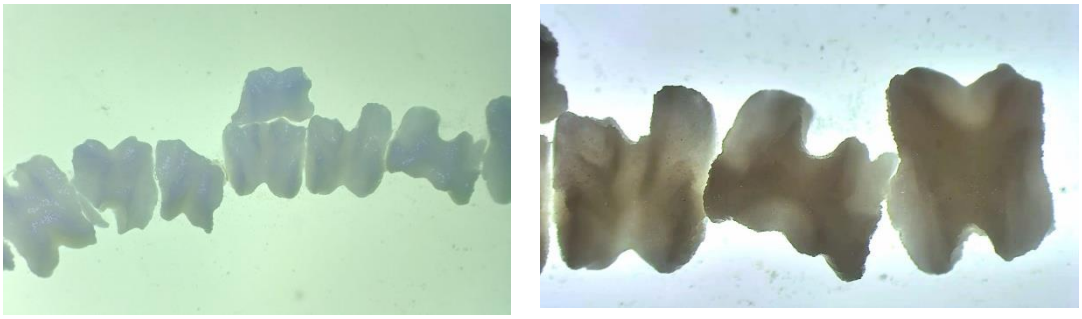


Figure 2. Calcareous ring of *Phyrella fragilis*

As one of the sea cucumber species that live in coastal areas, *Phyrella fragilis* has a primary habitat in tidal areas, especially in muddy sand substrates and between rock crevices. This environment provides ideal conditions for *Phyrella fragilis* to breed and survive. One of the unique characteristics of this species is its ability to cover its body with a layer of sand or use shells from other organisms as a form of protection from predators (Heding *et al*., 1954). This behavior is a common adaptation strategy found in several sea cucumbers living in open environments.

In addition, *Phyrella fragilis* can burrow into mud or sand substrates to avoid threats from predators and unstable environmental conditions (Hauksson, 2020). This ability makes it more resistant to changes in water conditions that can be influenced by environmental factors such as temperature, ocean currents, and oxygen levels in the water (MacTavish *et al*.,2012).

Not only does *Phyrella fragilis* function as a benthic organism that maintains the balance of the aquatic ecosystem, but it also has a very important ecological role in the nutrient cycle process in the marine environment (McElroy *et al*., 2020). As a detritus eater, this sea cucumber helps decompose organic matter at the bottom of the waters, ultimately contributing to the fertility of marine sediments. With the presence of this species, the quality of the aquatic substrate can be maintained, thus supporting the survival of other organisms in the coastal ecosystem (Morgan, 2000).

The geographical distribution of *Phyrella fragilis* is extensive compared to other *Phyrella* species (Sloan, 1984). This species has been found in various waters ranging from Western Australia, across the waters of Indonesia and the Philippines to Taiwan and Okinawa, Japan (Clark, 1932; Clark, 1938). This wide distribution shows that *Phyrella fragilis* is highly adaptable to various aquatic environmental conditions, including differences in temperature, salinity, and seabed substrates (Conand, 2004).

In the Philippines, *Phyrella fragilis* is commonly found in the tidal waters of Ilocos Norte Province. Local communities have utilized this species commercially, primarily as food. Locals call this species "absorber," referring to its characteristic behavior of expelling its guts when removed from its habitat (Michonneau *et al*., 2014). This mechanism is a common form of self-defense found in some sea cucumber species, where they expel their internal organs in response to threats from the external environment (Dulvy *et al*., 2003).

In addition to being used as a food source, *Phyrella fragilis* also has a relatively high economic value due to its rich nutritional content (Eriksson *et al*., 2015; Ebert, 2013; Ferdouse, 2004). This organism contains important nutrients such as protein, collagen, essential minerals, omega-3, omega-6, and healthy fats (Hasan *et al., 2021*). The high collagen content makes it one of the most sought-after ingredients in the health and beauty industry (Bordbar *et al*., 2011). Further research also shows that extracts from *Phyrella fragilis* have the potential to be used in the pharmaceutical field, especially in the development of supplement products and medicines based on natural ingredients (Liu *et al*., 2021).

In the calcareous ring of *Phyrella fragilis*, various spicules were found to play an important role in forming and strengthening its body structure (Dabbagh *et al*., 2013). The spicules successfully identified in this study include three main types: barbed wire rod, buttony, and mensal. Each spicule type has a different shape and function, but overall it supports the structure of the *Phyrella fragilis* body tissue. This study's results align with previous studies that found that the spicules in the calcareous ring have a distinctive shape that can be used to identify species more accurately.

Based on the observation results, the calcareous ring found in *Phyrella fragilis* consists of about 15 to 20 elements arranged regularly to form a solid structure. The structure of this calcareous ring is located in a fairly thick body membrane, providing additional protection to the inside of the body and supporting the flexibility and strength of the *Phyrella fragilis* body. The elements that make up the calcareous ring are divided into two main parts: radial and inter-radial elements, which are the primary support in maintaining this organism's body shape and movement (Michonneau *et al*., 2014).

Radial and inter-radial elements in the *Phyrella fragilis* calcareous ring tend to experience relatively high fragmentation, especially in individuals with larger body sizes (Gamboa *et al*., 2004). This fragmentation may play a role in increasing body flexibility, allowing this species to adapt to the substrate in which it lives. These radial and inter-radial elements are interconnected along most of their length, forming a sturdy yet elastic structure (Hamel *et al*., 2008). At the anterior edge, the radial elements are unevenly divided, creating a unique pattern that can be used in morphological identification. Meanwhile, the inter-radial elements at the anterior edge have a shape that resembles an arrowhead, providing a striking difference compared to the radial elements (Friedman *et al*., 2011).

As the size of the individual increases, the fragmentation of the elements that make up the calcareous ring increases. The radial elements can extend to the posterior part of the body, forming a long tail that tends to curve at the tip. This structural adaptation allows *Phyrella fragilis* to maintain its body shape in various environmental conditions, especially in the muddy and rocky habitats that are the main habitats of this species. This long-curved tail can also increase the organism's stability and movement when it is in the bottom substrate of the water (Heding *et al*., 1954; Michonneau *et al*., 2014).

The observations made resulted in the spicule shape found in the anterior, posterior, ventral and dorsal parts of *Phyrella fragilis* having a dominant shape in the form of a perforated plate. This study is in accordance with the study of *Phyrella fragilis* species identification, where the shape of the spicules that are often found are perforated plates such as buttony and mensal (Michonneau *et al*., 2014; Salari-Aliabadi *et al*., 2020).

Table 1. Calcareous Ossicles Observed in the Anterior and Posterior

Calcareous ossicles observed in the Anterior	
Form	Type
	Buttony
	Mensal
	Mensal

Calcareous ossicles observed in the Posterior	
Form	Type
	Buttony
	Mensal

Table 2. Calcareous Ossicles Observed in the Ventral and Dorsal

Calcareous ossicles observed in the Ventral	
Form	Type
	Mensal
	Barbed
	Mensal
	Buttony

Calcareous ossicles observed in the Dorsal	
Form	Type
	Mensal
	Buttony
	Barbed wire rod

The results of the study showed that the spicules found in the anterior, posterior, ventral, and dorsal tissues were perforated plates, such as buttony and mental. In addition, barbed and barbed wire rod shapes were found. This study is in line with previous studies, where the dominant spicule shapes in *Phyrella fragilis* were perforated plates and button shapes, while in some parts of the body, rod shapes were found (Heding *et al*., 1954; Michonneau *et al*., 2014; Salari-Aliabadi *et al*., 2020). The spicule shapes of the Sea Cucumber *Phyrella fragilis* have many similarities with other *Phyrella* species. The differences lie in the shape of the spicule stem and its number of tentacles (Cherbonnier, 1988).

CONCLUSION

The dominant spicules found in the calcareous, anterior, posterior, ventral, and dorsal ring tissues of *Phyrella* sp. are buttony, mensal, barbed, and barbed wire rods. Based on the shape of the spicules found in the body tissue, it can be concluded that the type of sea cucumber is *Phyrella fragilis*.

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