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Podia histology and ultrastructure of tropical sea cucumber *Phyllophorus* sp. (Phyllophoridae) and *Colochirus quadrangularis* (Cucumaridae) from Madura Strait, Indonesia

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Abstract. *Winarni D, Eridianti SA, Suhargo L, Khaleyla F, Wirawati I. 2023. Podia histology and ultrastructure of tropical sea cucumber* Phyllophorus *sp.* (*Phyllophoridae*) *and* Colochirus quadrangularis (*Cucumaridae*) *from Madura Strait, Indonesia. Biodiversitas 24: 5685-5692. Phyllophorus* sp. and *Colochirus quadrangularis* are sea cucumber species found in Madura Strait, East Java, Indonesia. Both species possess distinctive podia structures, which are protrusions on their body walls resembling spines. These structures serve as tube feet for movement (ventral podia) and sensory organs (dorsal papillae). This research aimed to compare the podia structures of *Phyllophorus* sp. and *Colochirus quadrangularis*. Five samples of each species were collected from the Madura Strait. Histological slides were prepared for the papillae (dorsal) and podia (ventral) on the anterior, median, and posterior body sections. Simultaneously, an examination of the ultrastructure was conducted using a scanning electron microscope (SEM). Analysis revealed differences between the podia of the two species. *Phyllophorus* sp. papillae had semi-flat tips, were non-suckered (SFNS), and exhibited squamous to short columnar epithelia, while their tube feet had flat tips, were non-suckered, and had columnar epithelia. Papillae were denser than tube feet. Conversely, *C. quadrangularis* papillae were pointed and non-suckered (PNS), and tube feet were flat and non-suckered (FNS). Notably, ossicle-dense areas in *C. quadrangularis* papillae had a pyramidal shape. In summary, the histology and ultrastructure of podia in these species displayed significant differences, highlighting their unique characteristics. They also imply to the adaptation biology of sea cucumber in Indonesian waters.

Keywords: *Colochirus quadrangularis*, histology, marine invertebrate, *Phyllophorus*, podia, sea cucumber, ultrastructure

INTRODUCTION

The sea cucumber (Holothuroidea) is a class of Echinodermata with cylindrical, elongated body with protrusions that resemble spines. As a deposit feeder that maintains the balance of benthic microenvironments, sea cucumbers play important role in the ecosystem (Baker-Médard and Ohl 2019). Sea cucumbers act as a bioturbator, which increases the oxygen content in sediment, nutrient recycling, and sediment cleaning in the ecosystem (Purcell et al. 2016). In addition to contributing to the ecology, sea cucumber also provides many benefits for humans, such as collagen, anticancer, antimicrobial, and antioxidant properties (Desmelati et al. 2020; Setianingsih et al. 2021; Hossain et al. 2022; Ru et al. 2022). Therefore, it also plays an important role as a commercial commodity. Commercial sea cucumbers are commonly referred as *teripang* in Indonesia (Setyastuti et al. 2019). Currently, lack of management caused sign of sea cucumber in Indonesia being over-exploited (Wirawati et al. 2021).

Various sea cucumber species can be found in the waters of Madura Strait. *Phyllophorus* sp. and *Colochirus quadrangularis* are the most common, along with *Acaudina rosettis* (Winarni et al. 2014). *Phyllophorus* sp. and *C. quadrangularis* are both suspension feeders and dendrochirotids. *Phyllophorus* sp. is an edible sea cucumber known locally as *terung laut* or ball sea cucumber because of its spherical shape. This species burrows through the substrate of the seabed and uses its tentacles to collect nutrients from the water (Khatulistiani et al. 2022). Previous research found that in the Madura Strait, *Phyllophorus* sp. had 44.4% abundance with a distribution index of 1.9062, suggesting that they live in groups (Winarni et al. 2014). *Phyllophorus* sp. has brownish-grey body color, and the body surface is covered in small papillae and tube feet.

The second species, *C. quadrangularis,* or prickly sea cucumber, is abundant in Madura Strait at 11.62% (Winarni et al. 2014). This species is commonly collected as aquarium decoration. The body of *C. quadrangularis* is elongated and cylindrical, with pointed oral and anal parts. *Colochirus quadrangularis* has a bright red or orange body color with yellow to orange tentacles. *Colochirus quadrangularis* has four longitudinal ridges along its body length, with some distinctive projections called papillae along these ridges. Unlike *Phyllophorus* sp., *C. quadrangularis* dwells on the seabed, usually clings to seagrass.

Podia vary greatly among different classes of echinoderms, in terms of number, localization and function. Sea cucumbers use podia for mobility and sensory function. Podia are related to the ambulacral water vascular system; therefore, its function is controlled hydraulically by water flow in the vascular system, in addition to the muscle layer (Clark et al. 2017). Podia can be classified into two types based on its location; ventral and dorsal. In the dorsal section, podia form papillae or non-locomotor podia, which operate primarily as sensory organs, while in the ventral part, podia form tube feet that are necessary for sea cucumber's mobility (Zhou et al. 2016). Papillae of sea cucumber also gives additional protection when they stiffen their bodies (Mohsen and Yang 2021). The quantity and appearance of papillae contribute to the economic value of sea cucumbers (Zhan et al. 2019; Ru et al. 2019; Zhu et al. 2022). Podia in ventral body wall forms tube feet, referred as locomotor podia, which are used for movement and attachment to substrate.

While podia are noticeable in Elasipodida and Molpadiida, it is unnoticeable in Dendrochirotida, Apodida, and Molpadiida (Mohsen and Yang 2021). Both *Phyllophorus* sp. and *C. quadrangularis* belong to the order Dendrochirotida, so podia are not very prominent in the body wall. Thus, to analyze its structure in detail, histology or electron microscopy methods can be used. Study on the tissue structure of sea cucumber podia has only currently conducted on several species (Guerrero and Forero 2018). Furthermore, research on the biology of Indonesian sea cucumbers is still very limited. This study aimed to describe the histology and ultrastructure of podia from two different species of Indonesian sea cucumber, *Phyllophorus* sp. and *C. quadrangularis* collected from Madura strait, East Java. By doing so, the adaptation of both species in their ecosystem can be inferred, thus contributing insights for conservation of both species in their respective habitat.

MATERIALS AND METHODS

Sample collection

The samples of *Phyllophorus* sp. and *C. quadrangularis* were obtained from the Madura Strait in East Java, Indonesia (Figure 1). Five samples from respective species were taken randomly. The sea cucumbers were then immersed in 5% $MgCl_2$, and their body length was measured. The viscera were removed from all samples, and the fresh body wall was weighed. After that, the body wall was fixed in 10% neutral buffered formalin for at least 24 hours.

Species was identified using characteristic of external morphology and ossicles from body wall of the samples. The species description was based on Clark and Rowe (1971), Amin et al. (2020) and Hartati et al. (2021). *Phyllophorus sp.* was identified based on its firm, spherical body with irregular papillae. The color of the body is brown to grey. *Phyllophorus sp.* has more than 10 tentacles arranged in one to three rings that they use for feeding and cleaning (Hartati et al. 2021). On the other hand, *Colochirus quadrangularis* can be identified based on its quadrangular or rectangular body shape, with podia spread regularly throughout the body. The body color is brilliant red or orange with shades of grey and green or bluish line running along its length. Body length of adults is about 11-14 cm with diameter at about 1.5-4.88 cm. Papilla are found on the dorsal surface (bivium) and tube feet are on the ventral surface (trivium). It has ten large dendritic feeding tentacles and 2 smaller tentacles ventrally in oral part. Tentacle base has yellowish color, while the tip is red (Amin et al. 2020; Hartati et al. 2021).

Decalcification

The body wall was cut at papillae (dorsal) and tube feet (ventral) from each sea cucumber sample's anterior, median, and posterior, measured 1 x 1cm. Podia samples were then decalcified by soaking them in 13% Ethylenediaminetetraacetic Acid (EDTA) solution for 6 (six) weeks until the calcium content had adequately dissolved. The EDTA solution was replaced every 3 (three) days during the course of decalcification procedure.

Slide preparation

Decalcified samples were processed histologically. The samples were cleaned in tap water, dehydrated in a series of ethanol (4x70%, 2x80%, 1x96%, 1x100%; 30 minutes each), and then cleared in xylene for overnight. After that, the samples were soaked in paraffin: xylene 1:1 solution for 1 (one) hour before infiltrating with paraffin for 3x3 hours. The samples were then embedded into a block of paraffin and longitudinal sections were made in a serial section of 5 m thickness at 20 m intervals. Sections were stained using Hematoxylin and Eosin (HE) stain.

Ultrastructure observation

Fixed podia tissue was first dehydrated in a series of ethanol (4x70%, 2x80%, 1x96%, 1x100%; 30 minutes each). The tissue was attached to a carbon tip and then coated in gold and palladium (Au-Pd) using a sputter coater (Quorum SC7620). The coated tissue was examined with a scanning electron microscope, Inspect S50 FEI. One papillae and tube feet respectively was evaluated from each species.

Data analysis

Data was descriptively and statistically analyzed. Microscope imaging of slides was used to examine podial components, water vascular system structure, ossicle structure, and podial surface. To determine the difference between the ventral and dorsal parts of the same species, the data of podial height (*C. quadrangularis* and *Phyllophorus* sp.), also podial diameter and muscle thickness of *Phylloporus* sp were analyzed by Analysis of Varians (ANOVA), followed by Duncan test whereas the data of *C. quadrangularis's* podial diameter, muscle thickness, and density, and the podial density of *Phyllophorus* sp. were statistically analyzed using Kruskal Wallis followed by Mann Whitney test (α = 0.05).

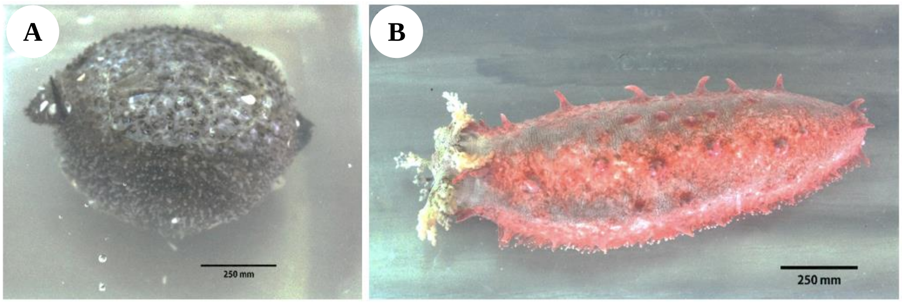

Figure 1. Sample of A. *Phyllophorus* sp. and B. *Colochirus quadrangularis*

RESULTS AND DISCUSSION

Histological structure of *Phyllophorus* sp. and *C. quadrangularis* podia

Based on the observation, both podia of *Phyllophorus* sp. comprised of a thin cuticle, epidermis with epithelial cells, an ossicle-dense area, a dermis layer consisting of solid connective tissue and loose connective tissue, muscle fiber, and a water vascular system (Figure 2). A layer containing a large number of ossicles, known as the ossicle-dense area, is found underneath the *Phyllophorus* sp. epidermal tissue. As a protective barrier for the tissue layers below, the ossicle-dense area of *Phyllophorus* sp. was found near the body's outer surface. The papillae protrusion was discovered to have the exterior epithelial layer, a water vascular system in the lumen, a wall coated with layers of mesothelium, muscle tissue, and dense connective tissue, with a radial nerve cord at the base of the papillae protrusion.

The tube feet of *Phyllophorus* sp. are similar in structure to the papillae (Figure 3). With a thin layer of cuticle and epithelium on the outside, the body wall around the tube feet was primarily made up of dense connective tissue. Despite not being as prominent as papillae, tube feet protrusion also had a water vascular system, layers of mesothelium and muscle, and a radial nerve cord. The difference between tube feet and papillae could be observed in their epithelium types. In the tube feet, the epithelium was columnar. In contrast, the papillae tip of *Phyllophorus* sp. was squamous in some places and short columnar epithelia in others.

In comparison to *Phyllophorus* sp., the protrusion of papillae and tube feet of *C. quadrangularis* were more evident on its body surface. Figure 4 depicts a portion of the papillae, whereas Figure 5 depicts a section of the tube feet. The columnar epithelium was discovered in the papilla and tube feet of *C. quadrangularis*. Similar to the epidermal tissue of *Phyllophorus* sp., an ossicle-dense area was found in *C. quadrangularis* tube feet beneath the outer epidermal tissue, while in papillae, the ossicle-dense area formed a pyramidal structure. The ossicle-dense area formed a dense lattice-like structure shaped like an ellipse in various sizes that grew smaller to the tip, known as pyramidal structure. The papillae of *C. quadrangularis* were sturdy because of this structure.

Table 1 shows the differences in podial variables between the two species, including podia height, diameter, muscle thickness, and podia density. Podia heights were found to be identical across the body in *Phyllophorus* sp., but significantly different in *C. quadrangularis* in the median-posterior dorsal and posterior ventral podia. Similarly, the diameter of the body surface of *Phyllophorus* sp. didn't vary considerably. The diameter of the dorsal podia in 3 (three) sites differ considerably from the median-posterior ventral podia in *C. quadrangularis*. All areas of the body wall that were studied showed that the muscle tissue thickness was similar between the two species. The density of podia in *Phyllophorus* sp. differ significantly between dorsal and ventral areas. In *C. quadrangularis*, however, a substantial difference was identified between the anterior and median dorsal, as well as between the median dorsal and the three ventral parts.

Podial tissue ultrastructure of *Phyllophorus* sp. and *C. quadrangularis*

The ultrastructure of *Phyllophorus* sp. podia are depicted in Figure 6. *Phyllophorus* sp. had tube feet that were flat and non-suckered (FNS), while the papillae were semi-flat and non-suckered (SFNS). Macroscopically, both were identical in shape and were difficult to distinguish. Ossicles were discovered in both the papillae and the tube feet. The papilla ossicles were classified as knobbed button forms with or without perforation, while the tube feet ossicles were identified as non-perforated knobbed button and star shapes.

Figure 7 depicts the ultrastructure of the podia of *C. quadrangularis*. The papillae of *C. quadrangularis* had a pointed tip non-suckered (PNS) type, different from *Phyllophorus* sp., while the tube feet had the FNS shape, similar to *Phyllophorus* sp. The papillae of *Phyllophorus* sp. were found to be semi-flat non-suckered (SFNS), whereas the tube feet were flat non-suckered (FNS). Ossicles were found in both papillae and tube feet. The papilla ossicles were knobbed button types with or without perforation, while the tube feet ossicles were non-perforated knobbed button and spinous edges shapes. This type of ossicles is in accordance with previous study (Amin et al. 2020).

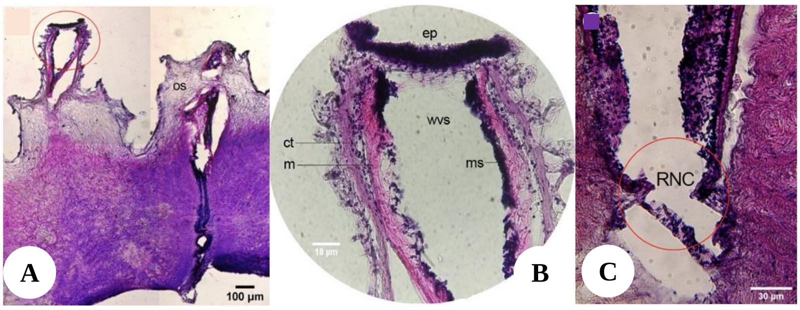

Figure 2. Longitudinal section of *Phyllophorus* sp. papillae. A. Section of body wall with papillae, os: Dense ossicles layer in loose connective tissue, red circle: Papillae, B. Magnification of papillae from figure A., ep: Epithelium, wvs: Water vascular system, ct: Dense connective tissue, m: Podial muscle, ms: Mesothelium, C. Magnification of the base of papillae, RNC: Radial nerve cord

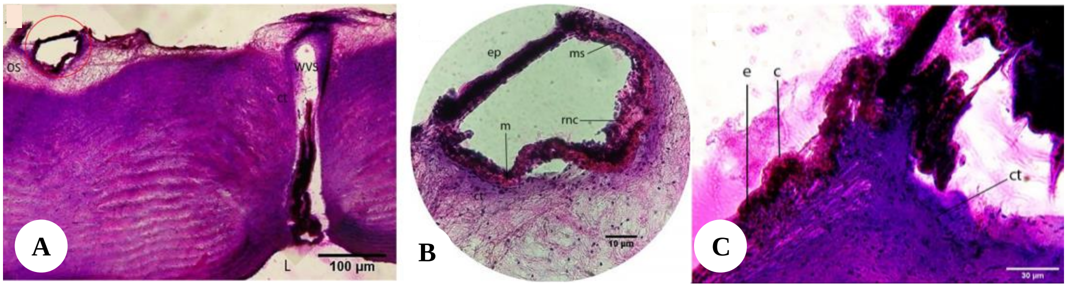

Figure 3. Longitudinal section of sea cucumber *Phyllophorus* sp. tube feet, stained with HE. A. Body wall section with tube feet (red circle), os: Ossicle-dense layer, wvs: Water vascular system, ct: Dense connective tissue, L: Lumen, B. Magnification of tube feet, ep: Podial epithelium, ms: Mesothelium, rnc: Radial nerve cord, m: Muscle, C. Magnification of lumen, ct: Dense connective tissue, c: Cuticle, e: Body wall epithelium

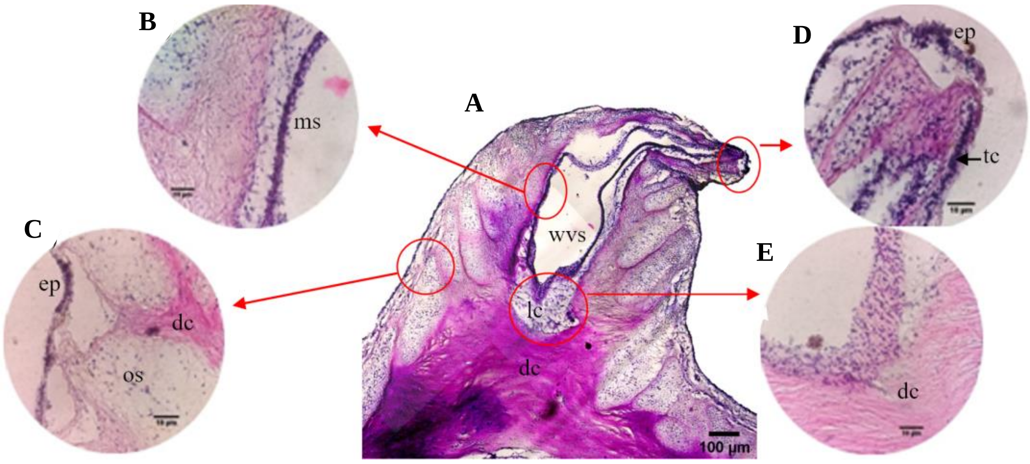

Figure 4. Longitudinal section of *Colochirus quadrangularis* papillae. A. Section of *C. quadrangularis* papillae, B. Detail on inner wall, C. Detail on the outer wall, D. Detail on papillae tip, E. Detail on the base of water vascular system. ms: Mesothelium, ep: Podial epithelium, dc: Dense connective tissue, os: Ossicles, wvs: Water vascular system, tc: Thin cuticle, lc: Loose connective tissue

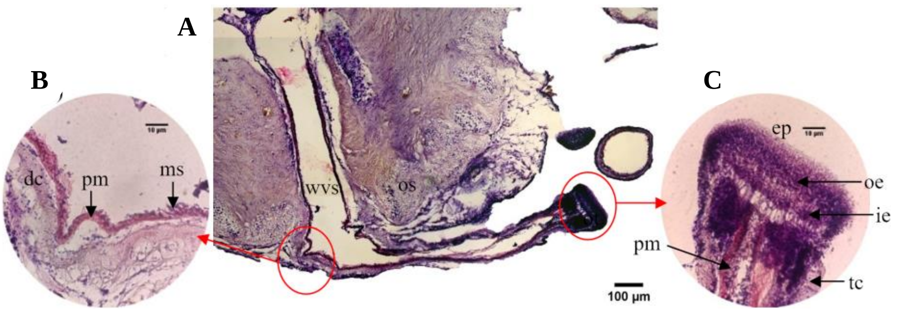

Figure 5. Longitudinal section of *Colochirus quadrangularis* tube feet. A. Section of *C. quadrangularis* tube feet, B. Detail on inner wall, C. Detail on the tip of tube feet. wvs: Water vascular system, os: Ossicle-dense layer, dc: Dense connective tissue, pm: Podial muscle, ms: Mesothelium, tc: Thin cuticle, ep: Podial epithelium, ie: Inner epidermis layer, oe: Outer epidermis layer

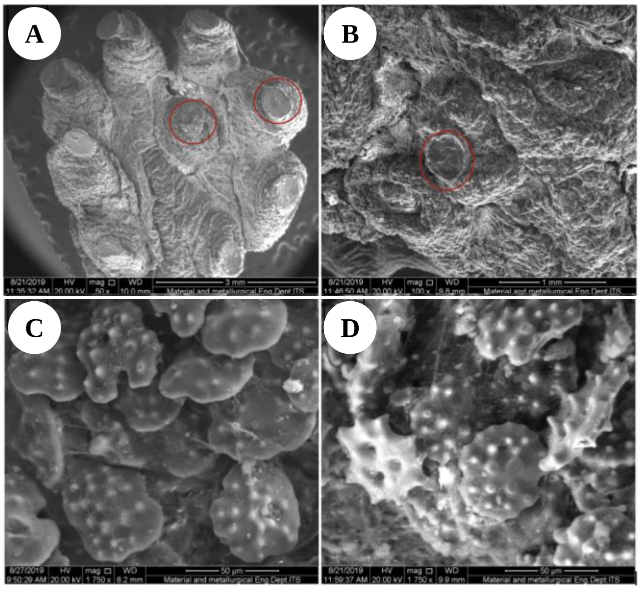

Figure 6. Image of *Phyllophorus sp*. body surface taken on scanning electron microscope (SEM). A. Semi-flat nonsuckered (SFNS) papillae tip (red circle), B. Flat non-suckered (FNS) *tube feet* (red circle), C. Ossicles in papillae; knobbed button shapes, both perforated and nonperforated, D. Ossicles in *tube feet*; nonperforated knobbed button and star shapes. Ossicles were found in loose connective tissue layer

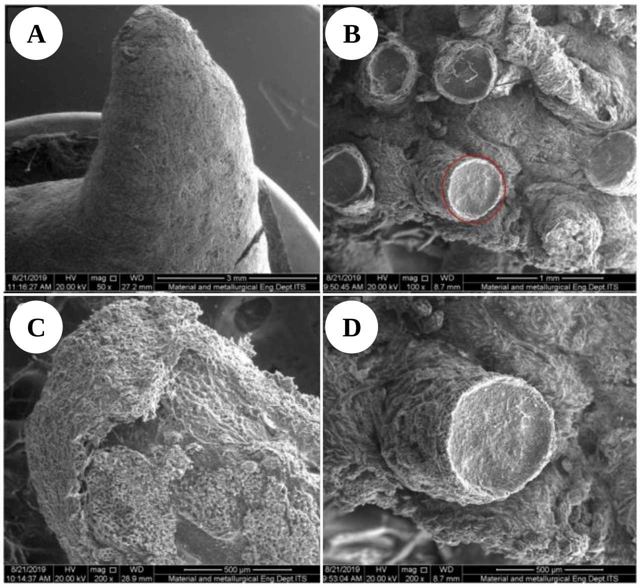

Figure 7. Scanning electron micrograph of *Colochirus quadrangularis* body surface. A. Pointed non-suckered (PNS) tip of papillae, B. Flat non-suckered (FNS) tip of tube feet (red circle), C. Ossicles in papillae; plate and perforated knobbed button shapes, D. Ossicles in tube feet; perforated knobbed button shape

Table 1. Difference of podial properties in different parts of *Phyllophorus* sp. and *Colochirus quadrangularis*

Properties	Location	*Phyllophorus* sp.*	p-value	*C. quadrangularis**	p-value
Podia height (µm)	Dorsal anterior	323.77±80.38[a]	0.142	859.35±389.96[ab]	0.048
	Dorsal median	400.84±215.97[a]		1,086.80±592.56[ab]	
	Dorsal posterior	506.08±231.80[a]		1,321.83±408.25[b]	
	Ventral anterior	199.44±85.59[a]		1,049.69±336.05[ab]	
	Ventral median	308.69±207.38[a]		578.60±174.39[a]	
	Ventral posterior	195.03±50.03[a]		674.15±246.09[a]	
Podial diameter (µm)	Dorsal anterior	426.56±94.07[a]	0.731	1,296.26±557.37[a]	0.005
	Dorsal median	445.02±154.47[a]		1,422.66±528.52[a]	
	Dorsal posterior	416.43±208.84[a]		1,580.83±459.58[a]	
	Ventral anterior	346.25±127.89[a]		1,051.26±629.28[ab]	
	Ventral median	361.98±78.89[a]		417.83±162.39[b]	
	Ventral posterior	394.34±53.59[a]		451.13±209.62[b]	
Muscle thickness (µm)	Dorsal anterior	22.48±5.86[a]	0.278	19.38±3.63[a]	0.375
	Dorsal median	25.14±5.27[a]		17.00±3.56[a]	
	Dorsal posterior	18.17±7.78[a]		21.17±3.23[a]	
	Ventral anterior	18.55±4.61[a]		27.16±14.56[a]	
	Ventral median	17.37±3.24[a]		20.54±4.90[a]	
	Ventral posterior	19.35±6.04[a]		21.41±4.67[a]	
Podial density (number of podia/cm^2)	Dorsal anterior	9±2.35[ac]	0.016	2.2±1.30[a]	0.000
	Dorsal median	15.4±4.67[b]		2.8±1.79[a]	
	Dorsal posterior	13.4±3.36[ab]		2±0.71[a]	
	Ventral anterior	8.4±1.14[c]		12±3.00[b]	
	Ventral median	10.2±2.77[abc]		12.4±4.72[b]	
	Ventral posterior	8.6±1.82[c]		16.6±7.33[b]	

Note: *) Different letters indicated significant differences based on statistical tests within each species (α=0.05)

Discussion

The papilla and tube feet of *Phyllophorus* sp. and *C. quadrangularis* samples collected from the Madura Strait were constructed of epidermis with epithelial cells covered by thin cuticle, ossicle-dense layer, dermis layer which made up of solid and loose connective tissue, muscle fibers, and water vascular system. Similar structures were identified in the podia of *Isostichopus* sp. aff. *badionotus* (Guerrero and Forero 2018). Similar to *Luidia clathrate* (Asteroidea), the papillae and tube feet of *Phyllophorus* sp.

and *C. quadrangularis* had a thin layer of cuticle. The tube feet of *L. clathrata* were covered by thin cuticles. This cuticle acts as a protective coat for the tissue layers below it (McCurley and Kier 1995). The papillae of *Phyllophorus* sp. were discovered to be semi-flat non-suckered (SFNS), and the tube feet were flat non-suckered (FNS) based on electron microscope images. Both had nearly identical shapes and were difficult to differentiate. The measured diameter and thickness of the *Phyllophorus* sp. muscle between the papilla and tube foot were not significantly different. Thus, if the body of *Phyllophorus* sp. is turned upside down, its papillae could also be used for mobility like the tube feet. Podia with flat ends are used as supports for sea cucumber mobility. On the other hand, the papillae of *C. quadrangularis* were pointed non-suckered (PNS), and the tube feet were Flat non-suckered (FNS). Compared to podia or tube feet of Asteroidea, these variations are similar. Based on previous study, asteroids tube feet were categorized onto three types based on the histological structure; knob-ending, simple-disc ending, and reinforced disc-ending (Santos et al. 2005). Non-suckered tip ending of the tube feet in the present study is similar to the simple-disc ending of Asteroid in terms of structure and function for locomotion, but not for attachment to substrate. This is related to the habitat of *Phyllophorus* sp. and *C. quadrangularis* in Madura Strait, as both species lives in sandy substrate, thus attachment function is rarely needed.

The epithelium identified near the tip of *Phyllophorus* sp. papillae was both flat and columnar, but the epithelium found in the tube foot was columnar. The columnar epithelium was discovered in both papilla and tube foot of *C. quadrangularis*. This is similar to the columnar epithelium studied in the tube feet of *Luidia clathrata*. The epithelium around the podia of *Phyllophorus* sp. was squamous and the podia could be retracted into its body. This retraction movement indicates *Phyllophorus* sp. physiological response to stress. On the other hand, the papillae of *C. quadrangularis* do not retract into its body. The epithelium of its podia was found to be columnar, in contrast to the retractile podia of *Phyllophorus* sp. The different types of epithelium found in podia from both species confirm previous theory that tube feet that can be moved or folded have thinner epithelial cells and able to reduce the number of folds (McCurley and Kier 1995).

Ossicles were discovered under the outer epithelial layer in both species. The ossicles that are part of the ossicle-dense layer are intertwined and linked by thin loose connective tissue. Interossicular muscle can be discovered in the ossicle-dense layer. Blowes et al. (2017) defined the interossicular muscle as collagen tissue that surrounds and connects adjacent ossicles. Collagen tissue occupies space between ossicle and fibers, forming a dense lattice. This connective tissue called mutable collagenous tissue can be found in the body wall of all echinoderms (Mo et al. 2016).

Based on the ultrastructure imaging of *Phyllophorus* sp. podia, it was shown that both papillae and tube feet had stacks of knobbed, button-shaped ossicles, either with or without perforation. The interossicular muscle made of collagen was discovered between the stacks of ossicles. The interossicular muscle made the body wall of *Phyllophorus* sp. more flexible, enabling it to expand its body to twice its original size under stress. As a result, it is known locally as ball sea cucumber. A substantial layer of solid connective tissue was discovered in *Phyllophorus* sp. underneath ossicle-dense area. This tissue runs along the water vascular system all the way to the gastrovascular lumen. Due to the tissue's high collagen content, sea cucumbers are widely used in cosmetics (Siahaan et al. 2017; Li et al. 2020).

Another implication for the structure of papillae and tube feet is the function of the hydraulic water vascular system (WVS). Sea cucumber has a branching WVS; all branches are linked to one another by a canal ring in the center. The movement of sea cucumber is caused by the hydraulic WVS assisted by muscle layer. The water vascular system is consisted of water ring canal channels which are linked to five radial channels. Because the tube feet are connected to the WVS through lateral branch of the channel, the water pump causes movement in the tube feet (Clark et al. 2017). Podia, particularly tube feet, is employed for protraction and retraction. Protraction is the movement away from the body's midline, while retraction is the movement toward the midline. Protraction and retraction of podia is controlled by the podia ampullae, which is connected to the hydrovascular system (Gianasi et al. 2021).

The WVS lumen was found to be lined with muscles and epithelium. These muscles are different from the muscles in the gastrovascular lumen that enable sea cucumbers to lengthen and contract their bodies. The muscles found in papillae and tube feet are used for the movement of *Phyllophorus* sp. and *C. quadrangularis*.

The density, diameter, and thickness of the muscle were concentrated in the middle part of the body based on *Phyllophorus* sp. papillae measurements. This indicated that the papillae in the median section might have a specific purpose, such as separating the individual from foreign objects, acting as a self-defense mechanism against predators, or assisting in movement due to the SFNS shape of the papilla tips. Although not significantly different, the posterior tube feet of *Phyllophorus* sp. had the largest diameter and muscle thickness, likely to assist the locomotion function for forward or backward movement. The thick muscle was also discovered in the anterior section, potentially used for locomotion. Contrary to the rear and anterior regions of the body, tube feet in the middle part had the largest density but the thinnest muscles, indicating that it is not involved in propelling movement of the body. The muscle thickness of papillae and tube feet of *Phyllophorus* sp. was not substantially different, indicating that every part of the podia can possibly contribute to movement function.

The papillae of *C. quadrangularis* seemed to be similar in almost every part. However, the height of the papilla was higher in the posterior part, while the muscle thickness was slightly higher in the anterior part. These could possibly indicate that the papillae in both parts have different functions, such as removing foreign substances or performing sensory activities, for example detecting threats or foreign objects. On the other hand, the tube feet in the anterior parts of *C. quadrangularis* showed higher podial height, diameter, and muscle thickness. Moreover, the density of tube feet was slightly higher in the posterior part compared to the anterior and median parts. The anterior tube feet support body movement, whether forward or backward. Higher height for tube feet in the anterior parts might likely indicate their function of lifting the body weight of *C. quadrangularis* to ensure that the individuals can direct their dendritic tentacles upward to collect any substrate since they are suspension feeders (Sun et al. 2018).

Based on the density of podia, the tube feet of *C. quadrangularis* were denser than papilla. In contrast, the height and diameter of the papilla were higher. Thus, the tube feet of *C. quadrangularis* was smaller in size but had a denser distribution on the surface of the body wall, while the papilla was larger in size but had a sparser density. The higher density of tube feet might help their movement on sandy or muddy ground.

Table 1 shows that the podia of *C. quadrangularis* were typically larger and wider than those on *Phyllophorus* sp., except for the median and posterior ventral podia. In those areas, the tube feet of both species were nearly similar in size. Muscle thickness in the podia was nearly the same in both species, with the exception of the anterior tube foot of *C. quadrangularis* and the median papillae of *Phyllophorus sp*, where it was significantly thicker. The thicker muscle on different points of the two species indicated a different function. In *C. quadrangularis*, it supported elevating the body to capture substrates better, while in *Phyllophorus* sp., it possibly supported the self-defense mechanism or movement.

The density of *C. quadrangularis* papillae was lower than the density of *Phyllophorus* sp. papillae and tube feet. However, the tube feet were a little bit denser than the tube feet of *Phyllophorus* sp. This may suggest that *C. quadrangularis* was faster to move across the same distance than *Phyllophorus* sp. because of that, as well as somewhat thicker muscle. However, further research is needed to confirm this.

In conclusion, due to their different use and nature, the papillae and tube feet of *Phyllophorus* sp. and *C. quadrangularis* were found to have different shapes, types of epithelium cells, composition of tissues and ossicles, podia height, podia diameter, and podia density.

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