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Histological Study of *Phyllidia coelestis* (Nudibranch) Epidermal Tissue from Pasir Putih, Situbondo

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Abstract

Nudibranch are a mollusk which can be found in all zones of ocean. Further, they are distributed widely from subtropical to tropical areas. The Nudibranch is characterized by lack of a shell and usually have a bright shiny color. Nudibranch has a potency to be used in the medical field because they have chemical compound inside their body. These chemical compound are the result of secondary metabolites from symbiosis with zooxanthellae in nudibranch tissues. This research aims to study the structure of epidermal tissue of the Phyllidia coelestis collected from Pasir Putih, Situbondo, East Java Indonesia. The results showed that the epithelial structure of the Phyllidia coelestis composed of cuboidal epithelium with vacuoles spread regularly along the mantle layer below the epidermal layer. Gland cells are rarely found in the outer epithelial layer. The foot epidermal layer consisted of simple ciliated columnar epithelial cells, with goblet-shaped mucous secreting glands among the epithelium. Gill tissue structure was composed of cell layers of cuboidal ciliated epithelial cells resting on a basement membrane and connective tissues

1. INTRODUCTION

Indonesia's marine wealth is known to be very diverse, one of which is marine invertebrate. Marine invertebrates in the food chain sistem are the herbivores, dominant predators and determinant of the food pyramid sytem [Murniasih, 2005]. Various marine invertebrates found in many coastal areas such as sponge, jellyfish, nudibranch, and many more.

Nudibranchia is a member of the Gastropod class and is commonly called the naked sea snail, characterised by its lack of a shell and is well known for its huge number of species, which are widely spread all over the world [Rudman, 1982]. However, the Indo-Pacific region has the highest diversity of Nudibranchia, containing more than 80 percent of species, whereby Indonesia is home to a significant number. The shell-less soft body of Nudibranchia is an easy target for predators. Therefore, Nudibranchia should have special defence mechanisms to survive from obstacles and predation.

Self-protection mechanism in Nudibranch comes from the color of the body which is prominent and the active substance contained in it. The active substance is thought to be the result of a nudibranch symbiolsis with zooxanthellae [Kevin, *et al*., dalam Bold and Wynne, 1985]. To find out more, it is necessary to carry out preliminary research regarding the histological structure of epidermal tissue in Nudibranchia.

2. RESEARCH METHODS

a. Sampling

Sample collection was conducted by scuba diving at Pasir Putih Beach, Situbondo, East Java. Each sample species was taken from their original habitat and was then documented. Meanwhile, environmental parameters of the area of collection were observed, measured and noted.

b. Morphological Examination of the Sample

Visual observations of a sample's morphological characteristics was conducted for several parameters of the study, specifically: colour, size, and shape. After identification and data

collection, the samples were kept for further analysis.

c. Histological Preparation and Observation

The detailed structure of the foot, mantle and gills were histologically examined in this study which was conducted at a laboratory. The tissues were dissected and fixed in 4% formalin, followed by histological preparation using the Paraffin method following Humason [1972], and then stained with Hematoxylin Eosin (HE) and Mallory Acid Fuchsin (MAF).

d. Identification of histological structure

Identification of the histological structure Nudibranch tissue was conducted with the aid of light microscope. The data was descriptively analysed.

3. RESULTS AND DISCUSSION

a. Morphological Characteristic of *Phyllidia coelestis*

Phyllidia coelestis were collected during the sampling period from Pasir Putih Beach, Situbondo, East Java with low tide and waves. The water temperature was measured and ranged between 28-29^{o}C. Phyllidia were found at depths of 5-15 metres. Nudibranchs were found feeding on sponge (Fig. 1).

Figure 1. *Phyllidia coelestis* Bergh, 1905 (personal documentation)

Phyllidia coelestis with its distinctive character has three black lines extending from the head to the tip of the tail, and the form of the letter Y on the head. Notches are yellow with dorsal parts larger in size than the notum in the mantle margin (Fig. 2)

The rhinopores of *Phyllidia coelestis* had a more bright-shiny colour and consisted of more than two different colours such as green, blue and yellow. The special characters of *Phyllidia coelestis* consisted of having three black lines extending from head to tail, and a Y shape in the head.

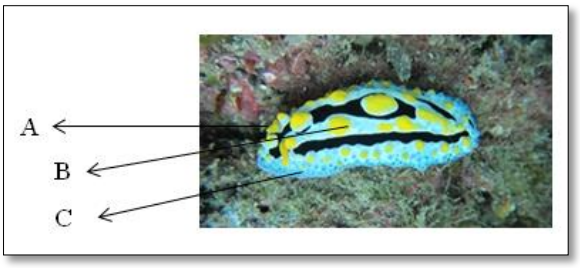

Figure 2. Morphology of *Phyllidia coelestis* (dokumentasi pribadi). Notes: A. Rhinophores; B. Tuberkula; C. Notum (basic color og body)

Phyllidia coelestis were found at the apical surface of the sponge, which suggests that they were most likely feeding on it. All members of the sub-order Doridacea, the species found in this study, are known as predators of sponge [Yasman, 2003; McDonald, GR and JW Nybakken, 1992; Arbi U.Y., 2011]. The Nudibranchia exhibited different preferences of microhabitat segmentation, which is probably linked to food availability, predatorship, and reproduction. *Phyllidia* inhabit shallow to middle depth water, where the sunshine reaches abundantly and temperature fluctuation is not very high.

b. Histological tissue Structure of *Phyllidia coelestis*

1) Mantle histology

The outer epithelial was generally composed of cuboidal epithelium with vacuoles spread regularly along the mantle layer below the epidermal layer. Gland cells are rarely found in the outer epithelial layer. *P. coelestis* showed the thick cuticle layer (Fig. 3).

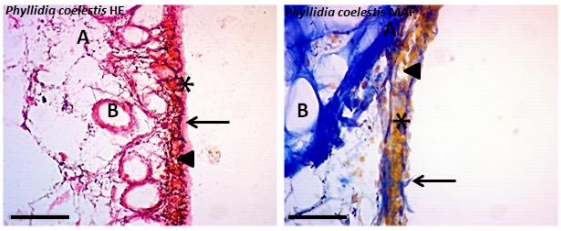

Figure 3. Histological structure of Epidermal tissue *Phyllidia coelestis* with HE and MAF staining. Notes: A. Connective tissue; B. Vacuole; *. Outer epithelium (mantel); ►. Gland cell; ←. Cuticle layer. magnification 40x10; bar = 50µm.

The outermost layer of the mantle was composed of a thin cuticle and epithelial cells. The outer epithelium consisted of single layer epithelial cells, which resided on connective tissue of the basal membrane. The epithelial cell nucleus was violet in colour with HE and red in color with MAF staining. The mantle showed many vacuoles that although colourless, were scattered among connective tissue. This result is in line with the reports of Edmunds [1968] and Graham [1975], which describes the basic epithelial of molluscs, and also with Wagele

and Cervera [2001], that the epithelium at the dorsal part or mantle of *Geniodoris castanea* consists of cuboidal to columnar cells and is characterized by large vacuoles.

The mantle vacuoles in some areas showed vessicle like structures that stained red-brown colour with MAF and brown colour with HE staining respectively.

2) Foot histology

The outermost epidermal layer consisted of simple ciliated columnar epithelial cells, with goblet-shaped mucous secreting glands among the epithelium. The vacuoles were spread regularly underneath the epidermal layer along the mantle tissues. Sub epithelial connective tissues of *P. coelestis* were dense and filled with fiber of connective tissue (Fig. 4)

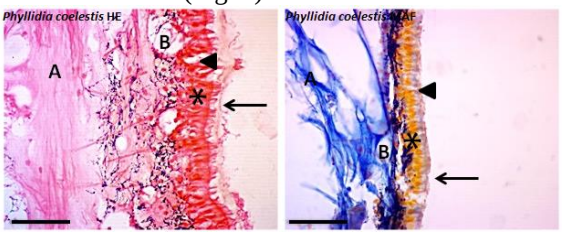

Figure 4. Histological structure of *Phyllidia coelestis* foot with HE and MAF staining. Notes: A. Connective tissue; B. Vacuole; *. Outer Epitelial; ►. Gland cell; ←. Cilia. Magnification 40x10; bar=50µm.

The molluscs foot structure is generally composed of long ciliated columnar epithelium, with the nucleus located at the basal cells. The epithelial layer rests on the basement membrane [Freeter and Graham, 1962], which forms sheets but this structure supports the body of the Nudibranch. Vacuole numbers at the foot are far less compared to the mantle.

The epithelial structure of the Phyllidia's foot is the same as the foot epithelium *in Archidoris pseudoargus* (Potts, 1981) which is composed of ciliated columner epithelium. The nucleus is located in the basal part of the cell. There are glands in the foot with goblets consisting of a single cell or a collection of cells. This gland can open directly to the surface of the foot being connected by a long channel between the foot - muscle fibers that extend to the basement membrane from the epithelium of the foot to the foot matrix. This causes local contractions to facilitate mucus secretion from the gland to the foot. This mucus secreted is very helpful in locomotion or nudibranch movement.

3) Gill histology

Gills on Phyllidia consist of sheets and are located in the area between the mantle and foot. A triangle shape with fusion length and ventral notum. Gill tissue structure was composed of cell layers of cuboidal ciliated epithelial cells resting on a basement membrane and connective tissues (Fig. 5).

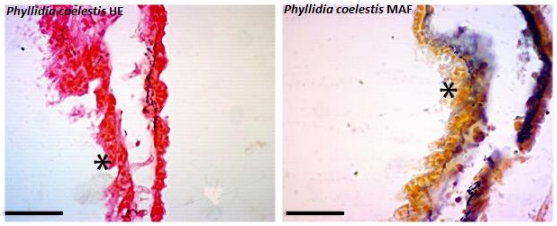

Figure 5. Histological structure of *Phyllidia coelestis* gill with HE dan MAF staining. Notes:*. Ephitel. Magnification 40x10; bar=50µm

Schrold [2001] explain that the gill structure in the *Corambe lucea*, there are globular gill glands and violet-colored cell. In *Corambe lucea* there are also retractile muscle fibers in each gill because the gills are in the dorsal part of the body and can be inserted into the body if there is danger around the environment. In *Phyllidia coelestis* there is no muscle fiber due to different gill structures.

The vacuole is not found in the gills and there is only a small amount of connective tissue in the gills. However, in the research of Wagele and Cervera [2001] on species *Goniodoris castanea* found globular gland glands which are composed of columner cells. This is what distinguishes gills from *Phyllidia coelestis.* However, the gill epithelium in *Goniodoris castanea* is the same as the *Phyllidia coelestis* epithelium which is composed of a cuboidal epithelium and there are few muscle fibers.

4. CONCLUSIONS AND RECOMMENDATIONS

Histological structure of Nudibranchia epidermal tissue in *Phyllidia coelestis* species seen from the mantle, legs and gills. The mantle part is composed of cuboidal epithelium with vacuoles spread regularly along the mantle layer below the epidermal layer. Gland cells are rarely found in the outer epithelial layer. The foot epidermal layer consisted of simple ciliated columnar epithelial cells, with goblet-shaped mucous secreting glands among the epithelium. Gill tissue structure was composed of cell layers of cuboidal ciliated epithelial cells resting on a basement membrane and connective tissues.

Based on the results of this study, further research is needed to determine the chemical compound found in Nudibranch *Phyllidia coelestis*.

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