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## First Record of Epizoic Diatom Presence on *Poricellaria ratoniensis* (Bryozoa, Cheilostomata) from Java Sea, Indonesia

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**Abstract:** Indonesian waters cover a high diversity of marine organisms (micros- and macros-) of interest to scientists. Among this diversity, the neglected bryozoan had been scarcely reported due to lack of information from this area, especially on the epizoic diatoms since they are favorable as their dietary. This preliminary study aimed to investigate and witness the presence of epizoic diatom on bryozoan *Poricellaria ratoniensis*. Our data revealed an assemblage of diatoms attached to bryozoan surfaces and exhibiting taxonomic diversity. In total, members of five genera *Amphora*, *Cocconeis*, *Neodetonia*, *Staurophora*, and *Thalassiosira* were found, including the measurement of their cell size, respectively. The attached diatoms were mainly within the bryozoan operculum (op) range, functioning as feeding organs. However, further study is needed to understand the interaction between bryozoan and diatoms aiming for ecological services.

**Keywords:** diatom, bryozoan, *Poricellaria*, epizoic, biodiversity.

### Introduction

Diatoms are single-celled photosynthetic organisms that play a vital role in the marine ecosystem, responsible for about 20% of the global primary production [1]. Various species of diatoms are commonly found in marine invertebrates, including bryozoans [2]. Bryozoan provides a favorable microenvironment for a diverse range of epizoic diatoms [3]–[6]. The presence of diatoms on bryozoan and other marine invertebrates can have significant impacts including hosts' feeding, growth, and reproduction [5]. Dahms *et al.* [3] showed that various diatoms helped the settlement of *Bugula neritina*'s larvae by forming of natural biofilms (NBF).

Moreover, diatoms can also have an impact on the larger marine ecosystem. Their presence on the exoskeletons of bryozoan and other invertebrates can affect their ability to sink to the ocean floor, which can impact

sedimentation rates and carbon storage [2], [7]. Additionally, diatoms can contribute to the formation of marine snow, a term used to describe the sinking of particulate organic matter to the ocean floor [8]. Marine snow plays a crucial role in the biogeochemical cycling of carbon in the ocean [9], [10]. It performs as the biological pump process, carrying organic carbon produced by photosynthesis in the well-lit surface ocean to the darker deep ocean [10], [11].

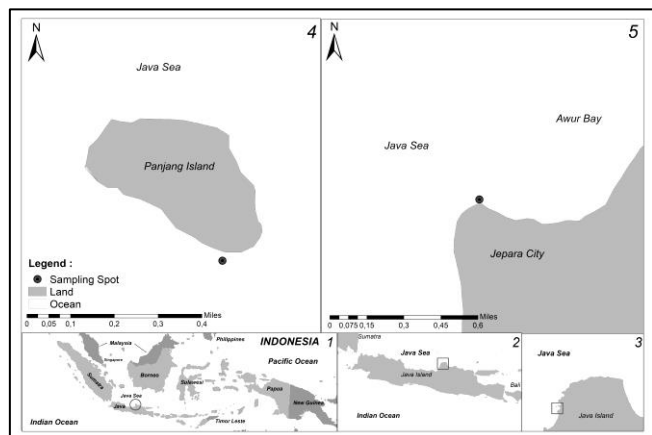
However, few studies have focused on the settlement of diatoms on bryozoan. There needs to be more information on the diversity of diatoms attached to bryozoans. Thus, investigating the presence of epizoic diatoms on bryozoans is essential for the evidence of mini-ecological systems occurring in marine environments.



## Methods

### Sampling and morphological identification of bryozoan

Bryozoan *Poricellaria ratoniensis* was sampled and identified in previous study [12], which were at Panjang Island, Java Sea, with a depth of 2-3 m (**Figure 1**). The specimen was transferred into sterile plastic bags to avoid attaching alien organisms from the air (terrestrial) and was brought by a cool box (-4 C) to the laboratory for further treatment.



**Figure 1.** Map of sampling area in the Java Sea. 1–3, enlargement of the Java Sea. Bryozoans were collected at Panjang Island (4) indicated by black dots.

### Sample preparation and microscopy

The bryozoan sample was fixed with 3% (v/v) glutaraldehyde in phosphate buffer saline (PBS). Bryozoan materials were passed through with PBS three times before further preparations. The sample was dehydrated with gradient ethanol series (30, 50, 70, 90, and 100%; v/v) and sputter-coated with a gold film of approximately 11- 15 nm thickness (Edwards SC06). Samples were viewed in Jeol JSM- 6460LV scanning electron microscope. Furthermore, pictures were stored digitally and processed using JSM Software (JEOL, USA).

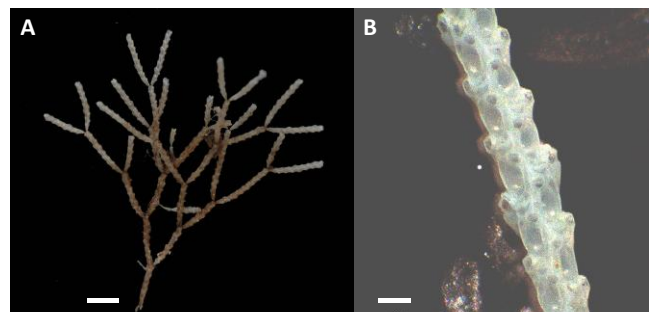
### Identification and measurement of diatoms

Diatoms were identified by morphological approach (morpho-species). All captured pictures from SEM results were selected by showing the clear diatoms attached to the bryozoan. All attached diatom were measured at the apical and transapical axis ( $\mu\text{m}$ ) by Fiji Software.

## Results And Discussion

### Morphological identification of bryozoan

Bryozoan *Poricellaria ratoniensis* was characterized as an erect colony with a specification of dichotomously branched (**Figure 2**). *P. ratoniensis* was featured on adventitious avicularium for protection from their predators [12]. Interestingly, this genus was the only known recent species that occurred in shallow tropical waters [13]. This erect bryozoan is common and outspread on tropical on-shore. It is likely on intertidal zones where their establishment was preserved from high waves.



**Figure 2.** (A) Preserved sample of bryozoan *Poricellaria ratoniensis* Waters, 1887 from Java Sea, Indonesia. (B) Close-up general view of the colony showing orifice. Scale bars: A = 2 mm, B = 200  $\mu\text{m}$ .

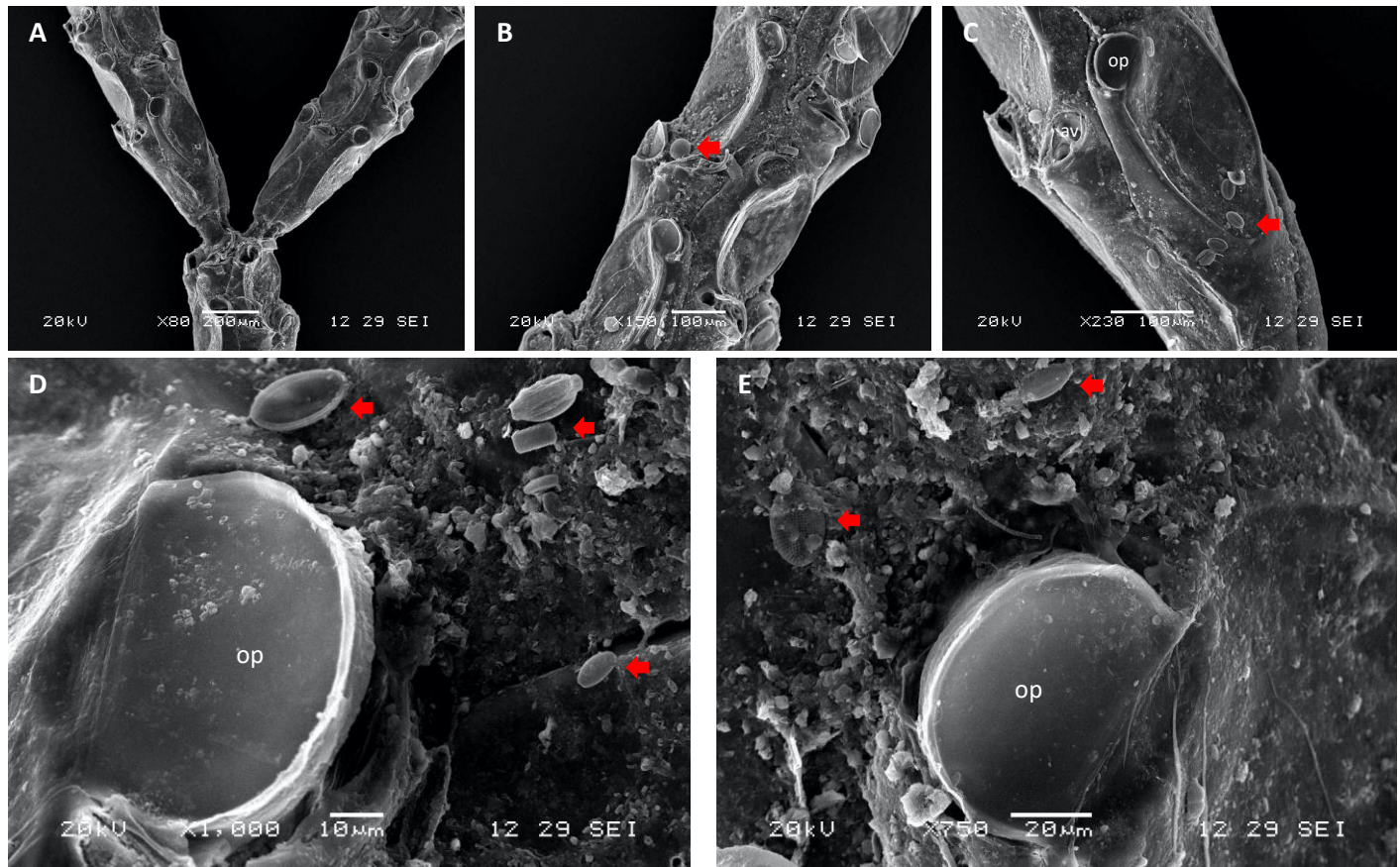
### Scanning electron microscopy of sample surfaces

The scanning electron microscopy results showed that diatoms were covered on bryozoan *Poricellaria ratoniensis* surfaces (**Figure 3**). Diatoms could be found on the skeletons, but not all the parts of the zooid (individual organisms) are covered. Diatom colonization was mainly detected close to the lateral part of the operculum (op) and avicularium (av) (**Figure 3C, D, E**). Avicularium is a modified zooid that actively moves for predatory defense, and it suggests adaptive functions like food-gathering, cleaning, nutrient storage, and the creation of water currents [14]. We determined that most diatoms were assembled close to those parts range. McKinney [15] also documented the diatoms as a food source for bryozoans.

We also found the diatom community on the distal part of zooids (**Figure 3C**). A biofilm might be formed at the bryozoan *P. ratoniensis* surface. Other data showed some zooids also covered by a thin biofilm which bacteria and pennate diatoms [16], [17]. It suggested the interaction between the systems of bryozoans and diatoms controlled by external environmental

conditions such as light, nutrients, depth, Etc. Anthropogenic activities can affect microbial communities. It could be additive, synergistic, or antagonistic [18], [19]. These effects could impair the

bryozoans' biofilm and fitness, affecting colonization on their surfaces. Regrettably, it was not provided for understanding of interactions in this study.



**Figure 3.** Scanning electron microscopy (SEM) of bryozoan *Poricellaria ratoniensis*. (A) General view of the colony showing branch bifurcation (B) rounded diatoms (red arrow) was shown on distal view of operculum (C) close-up view of operculum (op) and avicularia (av) and colony of diatoms (red arrow) on distal surface (D) close-up view of operculum showing various diatoms (red arrow) on different shape and size (E) close-up view of other operculum showing diatoms on distal side. Scale bars: A = 200  $\mu\text{m}$ , B = 100  $\mu\text{m}$ , C = 100  $\mu\text{m}$ , D = 10  $\mu\text{m}$ , E = 20  $\mu\text{m}$ .

### Identification and measurement of diatoms

Bryozoan *P. ratoniensis* investigated hosted a slightly diverse diatom, representing five genera *Amphora*, *Cocconeis*, *Neodetonia*, *Stauraphora*, and *Thalassiosira* (Figure 4). For some diatoms, identification was not possible because the frustules were partly covered by the exoskeleton of the bryozoan or broken or only visible in girdle view. In order to verify bryozoans as a habitat for diatoms and food sources, we classified the diatoms only at the genus level. We were also able to reveal a diatom size (Table 1).

**Table 1.** Individual size of attached diatoms on bryozoan *Poricellaria ratoniensis*

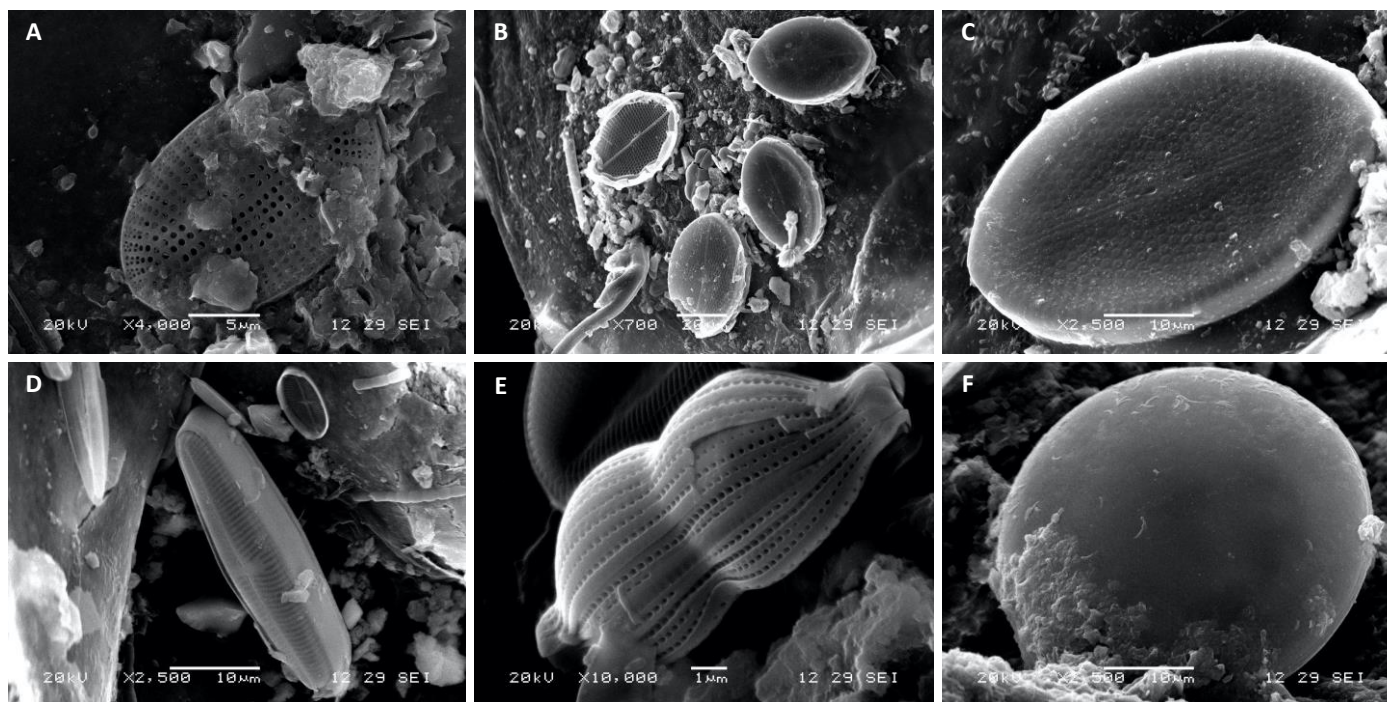
No.	Genera	size ( $\mu\text{m}$ )	
		Apical Axis	Transapical Axis
1	<i>Amphora</i>	12,22 $\pm$ 0,2	5,36 $\pm$ 0,2
2	<i>Cocconeis</i>	47,91 $\pm$ 0,7	29,17 $\pm$ 0,2
3	<i>Neodetonia</i>	20,33 $\pm$ 0,2	12,95 $\pm$ 0,2
4	<i>Stauraphora</i>	32,17 $\pm$ 0,3	5,62 $\pm$ 0,5
		Diameter ( $\mu\text{m}$ )	
5	<i>Thalassiosira</i>	40,85 $\pm$ 0,9	

Our data showed that the diatom genus *Cocconeis* was abundant and dominantly settled on some bryozoan parts instead of other four genera. Genus *Cocconeis* is hardly surprising to attach to other marine hosts,



especially bryozoan. The dominance of genus *Cocconeis* was investigated on bryozoan *Electra* [5]. All laminary bryozoans were also densely colonized by the genus *Cocconeis* [4]. This genus dominance was revealed because of its herbivore properties on other diatom competitors. Thus many grazer organisms avoid genus *Cocconeis* [9], [20].

Genus *Amphora* and *Thalassiosira* were also found on bryozoan *Membranipora membranacea* [5] and *Celleporaria hyalina* [4]. The presence and abundance differences of epizoic diatoms on bryozoan were affected by the light supply, seasonal change, and the richness of nutrients in their living environments due to the photosynthetic systems they had.



**Figure 4.** Various diatoms shown on the surface of bryozoan *Poricellaria ratoniensis*, (A) *Neodetonia* (B) colony view of *Cocconeis* (C) Close-up view of *Cocconeis* (D) *Staurophora* (E) *Amphora* (F) *Thalassiosira*. Scale bars: A = 5 µm, B = 20 µm, C = 10 µm, D = 10 µm, E = 1 µm, F = 10 µm.

## Conclusions

Our study first recorded the evidence of diatoms attached to bryozoan *Poricellaria ratoniensis*. The data resulted in the diversity, characteristics, and morphology of diatoms. This record is hardly surprising, considering that Indonesia has a large diversity of marine organisms. However, this preliminary study also indicates that the epizoic diatoms on bryozoan are crucial to be more fully explored. Further research is needed to understand the interactions between epibiont diatoms and bryozoans for the ecological dynamics of benthic communities and the functioning of aquatic ecosystems.

## Conflicts of interest

The authors have declared that no conflict interests exist.

## Acknowledgements

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