Identification coral reef diatom using scanning electron microscope at Nature Reserve Pulau Sempu (NRPS), East Java

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ARTICLE INFO

Keywords:
- Hard coral
- Cocconeis
- Psammodictyon
- Pulau Sempu
- Diatom

DOI: 10.13170/depik.13.1.29516

ABSTRACT

Diatom, Bacillariophyceae, has an important role in environmental support because these algae contribute to 25% of the oxygen supply globally and each year represent 40% of primary production by fixed carbon using photosynthesis. Diatom samples were obtained from 3 different sites in around NRPS. Cleaning diatom analyzed using SEM for describe ultrastructure in diatom valves. Three dominant diatoms Cocconeis and Bacillariaceae from the south Java Sea (Indian Ocean). Basic information regarding the diversity of diatoms, and the success of photosynthetic micro-algae that can be found both in marine and freshwater habitats, is very important to describe the role of each taxon from an ecology perspective in the future. Samples are taken from the substrate of 3 stations in Nature Reserve Pulau Sempu and prepared in the laboratory for scanning electron microscopy photographs. Clear and detailed features diatom frustule of SEM photograph reveal a schild diatom (Cocconeis) and panduriform diatom (Psammodictyon) are dominating taxa in NRSP areas.

Introduction

Coral reef ecosystem is a forest in marine environment that supports another biota by providing shelter, food and breeding sites. This complex ecosystem also become important for protecting the coast from abrasion, providing goods and services, protein sources and also economic revenue for tourism activity (Bellwood et al., 2019; Harborne et al., 2017; Riniwati et al., 2019). This ecosystem builds by various marine organisms that scleractinian coral as a major structure since their deposit carbonate as a building block (DeVantier et al., 2006; Waheed et al., 2015). Coral reefs in East Java can be found scattered throughout the South and North coasts of East Java. On the south coast, which is directly adjacent to the Indian Ocean, coral can be found from the Banyuwangi, Jember, Prigi areas, to the Malang area. The average coral cover in southern East Java is 25-30% (Luthfi & Rendy, 2018; Luthfi et al., 2019). Coral reefs in the south are mostly found in the reef flat to reef crest areas. The coral reefs in these waters are composed of massive corals including Porites, Goniastrea, Favia and Favites. Also found Acropora, Pocillopora, Seriatopora and Montipora. Meanwhile, on the north coast of East Java, corals as the main constituent of coral reefs can be found in Banyuwangi, Situbondo and the Madura Strait. Coral cover in northern East Java also showed poor performance, with an average live coral cover of 31.6% (Asadi & Andrimida, 2017; Herawati et al., 2022; Hidayah & Nuzula, 2019; Samuel et al., 2021).

Coral can live well and requires appropriate environmental support, such as high-water clarity, pH 7.9 - 8.3 (Gagliano et al., 2010), salinity 29-35 ppt (Coles & Jokiel, 2018), DO > 4.8 mg/L (Altieri et al., 2017) and low nutrients (D’Angelo & Wiedenmann, 2006).
2014). This is in contrast to the water conditions required by algae in general. So that only certain algae and microalgae can survive in the coral reef ecosystem. Likewise with diatoms.

Research on diatoms from coral reef ecosystems is still not too much, from Réunion and Rodrigues Islands Indian Ocean (Al-Handal et al., 2016; Riaux-Gobin et al., 2012), Basra coast, Iraq (Al-Handal et al., 2018), Juan de Nova coral island Western Indian Ocean and Tuamotu archipelago, Pacific (Witkowski et al., 2022), Great Barrier Reef, Australia (Gottschalk et al., 2007), Guam (Christopher S Lobban et al., 2012), and Indonesia (Risjani et al., 2021). Recent research from Kryk et al. (2021) described a new species of diatom from Tiga Warna, E. Java. From the Thousand Islands a new species has also been described (Prasetiya et al., 2019). There is also a new genus described in the early 20th century by naturalists as a result of expeditions such as those carried out by Hustedt (1931-1959) who gave the species name Mastogloia lata from the island of Miang Besar, Borneo (East Kalimantan) (Pennesi et al., 2013, 2016).

The waters in Nature Reserve Pulau Sempu and its surroundings have not received the attention of researchers in Indonesia, so far there have only been 2 publications providing information on diatoms in the southern Malang region. This study aims to provide basic information about the type or genus of diatoms in the Sempu Island nature reserve and its surroundings as well as information on the diversity of coral reef species in the southern Malang area.

The research was conducted June 2022 in Nature Reserve Pulau Sempu which 3 sampling stations, they were Pulau Sempu, Kondang Buntung and Tiga Warna. The sampling point can be seen in Figure 1. The local name of station in Pulau Sempu is Teluk Semut which has short beach with montiporids and acroporids dominate in the sea water. Tiga warna and Kondang Buntung located in adjacent of Teluk Semut, both of sites had wider beach and more diverse of scleractinian coral.

**Diatom sampling**

Samples were taken from coral reef habitat, namely sand, dead coral and macro algae, using a 50 ml falcon bottle. In order for diatom metabolism to be maintained while in the sample bottle, ¼ falcon bottles are emptied for air space. Furthermore, the samples were stored in the laboratory at room temperature and not exposed to direct sunlight. For cleaning calcium carbonate, the samples then soak into a beaker glass of 250 ml and added 10% of HCl. After the bubbles were gone, the samples were washed 3 times using distilled water. Furthermore, samples were boiled using peroxide (H2O2) 37% for 5 hours in total to remove organic material in the samples in a hot plate at 250°C. After the samples were cleaned, then were washed with distilled water 5 times. The clean sample materials kept in 15 ml bottles samples (Battarbee & Berglund, 1986; Bodén, 1988; Gersonde & Wefer, 1987).

For SEM, suspensions of clean material were dropped into Whatman Nucleopore filter membrane size 5 µm and mounted into SEM specimen stubs 12.5 nm. Furthermore samples were coated with 20 nanometer of gold using a Quorum Q 1500OT ES Turbo-Pumped Sputter Coater. Diatoms were observed using a Hitachi SU 8010 scanning electron microscope (SEM) at the University of Rzeszów.

**Results**

The results showed that there were 3 diatoms identified using SEM, namely *Coconeis* sp. cf. *mascarenica*, *Psammodictyon* sp1 and *Psammodictyon* sp2. *Coconeis* sp. cf. *mascarenica* was found in Kondang Buntung with valve length and width of 15.57 x 8.15 µm (Figure 2A) and 14.33 x 7.83 µm (Figure 2B). *Psammodictyon* sp 1 was found in Pulau Sempu and *Psammodictyon* sp 2 was found in Tiga Warna with valve length and width of 14.3 x 7.12 and 14.45 x 3.62 µm (Figure 2 C, D).

*Coconeis* sp. cf. *mascarenica* (Figure 2 A, B) is seen sternal valve (SV) of diatoms. The sternum is elliptical in shape with not too wide in size and the striae tend to be towards the apices. No poles found in apex. Figure 2C is an internal view of *Psammodictyon*

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**Materials and Methods**

**Location and time of research**

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**Figure 1.** Research location in NRPS. Sampling sites were Tiga Warna (1), Kondang Buntung (2) and Pulau Sempu (3)
sp 1, showing the areolae of the diatom valves in the form of confined pores with a size of 200-300 nm. Valve in oblique view showing epithecal valve, hypothecal valve, raphe, valvocopula, and cingulum (Figure 2D).

![Figure 2](image-url)

**Figure 2.** External view of diatom valve Cocconeis diatom lacking raphe and uniseriate striae show on white arrows (A, B); oblique view of internal valve of Psammodictyon diatom with clear areolae (arrow) and broken cingulum (arrow) (C); and tilt view of diatom frustule show ornamental characters of valve and girdle band. The bigger valve is called as epivalve (EV) and smaller valve is known as hypovalve (HV). Valvocopula is direct cingulum attach valve and copula is silica band made. The position of raphe is eccentric (D).

**Discussion**

**Habitat and function diatom in ecology**

Diatom is a photosynthetic unicellular alga that has an internal skeleton made from SiO₂ (silica) (Mann, 2010; Williams & Kociolek, 2011). And now the diatom has been classified as class Bacillariophyceae. Diatom is easily be found in both fresh and marine habitats (Risjani et al., 2021), also in the maximum photic zone, 100 m (Schvarcz et al., 2022). Furthermore, diatoms are responsible for marine primary production of about 25% (Vardi, 2008), comprising food for herbivores since diatoms are rich in lipids (Hussein & Abdullah, 2020).

**Cocconeis sp. cf. mascarenica**

The genus Cocconeis was first described by Ehrenberg (1838) in his first compendium book entitled *Infusionsthierchen*. The name refers to the shape of the frustule diatom which is similar to a shield or schild in German (Romero & Jahn, 2013). In general, diatoms from this genus are categorized as monoraphid diatoms where only one of the valves has a raphe, or it is also called heterovalvar, where one of the valves has different ornamentation (Pappas & Gordon, 2021). Another common feature of these diatoms is their elliptic shape and sandle-like shape. On all sides the valve has striae grooves which are uniseriate in shape because they only have one column of areolae (Romero, 2011).

Cocconeis diatoms are epiphytic which means these diatoms live in other algae or higher plants. About 8 species were found living on the leaves of seagrass *Posidonia oceanica* at Ischia Island in Italy (De Stefano et al., 2000). Others report said some diatom such as *C. heteroidea* has lived on seaweed surface and *C. caulerpacola* was living on green alga *Caulerpa taxifolia* known as an algae killer (Car et al., 2012; Suzuki et al., 2001). Moreover, Cocconeis also known as epilithic and epipsammic diatom since also can be found in rock surface and attached to the grain of sand (Hafner et al., 2018; Riaux-Gobin et al., 2011). As monoraphid, the raphe valve (RV) side attaches to the substrate and sometimes made a single layer foul at the surface of the substrate (Molino et al., 2009; Sullivan, 2019).
Psammodictyon sp1 and sp2

The genus Psammodictyon was described by Round et al., (1990) for the extent the genus Nitzschia. The first species was Psammodictyon panduriforme based on Nitzschia sect. Panduriformes (Lobban, 2015). The Nitzschiodoid group has 11 genera which have main characteristics: bilateral symmetry both apical and transapical axes, raphe eccentric and some have keel (Round et al., 1990). The special features for this genus are the panduriform shape of valves as on genus Tryblionella, raphe arranged eccentric or in edge of the valve, the areolae are large in size with variations in shape such as round, polygonal and irregular in shape. The occlusions also are found in areolae (Yang et al., 2020).

Psammodictyon was reported originally from marine habitats (Round et al. 1990) and minor from freshwater (Yang et al., 2020). To date, 15 species belong to this genus which has 11 species from marine and 4 from freshwater (Kociolek et al., 2021). The differences in environments or habitat influence the size of marine diatoms which have larger comparing freshwater diatoms (Litchman et al., 2009). Nutrient and mixed layer depth (MLD) of water also had a role in diatom size. High phosphorus supply in the freshwater area, in general, and lack of MLD contribute to the small size of the diatom.

Conclusion

Diatoms in NR Pulau Sempu Island have not been revealed much by researchers. In this study, 3 species of pennate diatoms from the families Bacillariaceae and Cocconeidae were obtained. More photos of diatoms from SEM results are needed to be able to identify diatoms to the species level, because there are many details of the frustule ornament of each diatom species that must be observed. Future research will open up a lot of diatom biodiversity in NR Sempu Island.

Acknowledgments

We thank the member of Acropora Universitas Brawijaya for assistance during the fieldwork and also our sincere to the anonymous reviewers for their insightful comments on this manuscript.

References
