Diversity of marine algae assessed by environmental DNA metabarcoding: A case study from Gili Genting, Gili Labak, and Karang Takat-Indonesia

Insafitri Insafitri[1*], *Wahyu* Andy Nugraha[1], *Ni* Kadek Dita Cahyani[2,3], *Muhammad* Danie al Malik[4,5], and *Ambariyanto* Ambariyanto[4,5]

[1]Department of Marine Science, Faculty of Agriculture, Universitas Trunodjoyo Madura. Jl. Raya Telang, Bangkalan 69162, East Java, Indonesia,
[2]Department of Biology, Faculty of Science and Mathematics, Universitas Diponegoro. Jl. Prof. Sudarto, S.H., Tembalang, Semarang 50275, Central Java, Indonesia
[3]Yayasan MERO Foundation, Banjar Dinas Muntig, Tulamben, Kubu, Karangasem 80853, Bali, Indonesia
[4]Diponegoro Marine Biodiversity Project Laboratory, Universitas Diponegoro. Jl. Prof. Sudarto, S.H., Tembalang, Semarang 50275, Central Java, Indonesia
[5]Department of Marine Science, Faculty of Fisheries and Marine Sciences, Universitas Diponegoro. Jl. Prof. Sudarto, S.H., Tembalang, Semarang 50275, Central Java, Indonesia

Abstract. Environmental DNA (eDNA) metabarcoding has emerged as a powerful tool for detecting and monitoring marine biodiversity, particularly for taxa that are difficult to identify using traditional methods. The objective in this study is to assess the diversity of marine algae, including Chlorophyta, Phaeophyta, and Rhodophyta, in three coastal sites of Indonesia: Gili Genting, Gili Labak, and Karang Takat. eDNA samples were collected and sequenced to identify algal taxa and estimate their relative abundance across locations. The results revealed clear spatial variations in algal communities. Gili Genting showed dominance of *Micromonas pusilla* and other Chlorophyta species associated with nutrient-enriched waters. Karang Takat exhibited higher Rhodophyta diversity, reflecting a stable and mature benthic environment. Gili Labak displayed lower algal diversity and abundance, possibly due to limited substrate availability or lower DNA yield. These findings highlight the effectiveness of eDNA metabarcoding in detecting fine-scale spatial patterns of algal diversity and provide valuable insights for monitoring and managing tropical coastal ecosystems in Indonesia.

1 Introduction

Use Marine macroalgae, including Chlorophyta, Phaeophyta, and Rhodophyta, are fundamental primary producers in coastal ecosystems, providing habitat structure, food resources, and regulating nutrient cycles [1]. Monitoring algal diversity is critical for detecting ecological shifts, eutrophication, and the spread of invasive or harmful taxa [2].

Conventional survey methods such as morphological identification and quadrat sampling remain important, but they are time-consuming, labor-intensive, and often limited in detecting cryptic, microscopic, or rare taxa [3]. In contrast, environmental DNA (eDNA) metabarcoding has become a promising tool for biodiversity assessment because it enables the detection of a wide range of taxa from water or sediment samples without direct observation [4]. Recent applications show that eDNA metabarcoding reveals community composition and fine-scale spatial variation that may otherwise be overlooked [5].

The accuracy of eDNA detection depends on methodological choices, including sampling protocols, DNA extraction, primer design, and sequencing platform, although taxonomic resolution can vary depending on primer sets and reference databases [6], yet large knowledge gaps remain for tropical algal taxa [7].

Globally, eDNA metabarcoding has been successfully applied to monitor harmful algal blooms [8], early detection of green tide events [9], and to complement coral reef biodiversity surveys [10]. However, in Indonesia—recognized as one of the global centers of marine biodiversity—applications of eDNA metabarcoding for algal diversity assessment remain scarce [11]. This gap is significant because Indonesian coastal waters are undergoing increasing anthropogenic pressures, including fisheries, aquaculture, and tourism, that can alter community structure of primary producers.

The present study aims to apply eDNA metabarcoding to assess the diversity of Chlorophyta, Phaeophyta, and Rhodophyta in three Indonesian coastal sites: Gili Genting, Gili Labak, and Karang Takat. Specifically, we (1) documented algal taxonomic composition and relative abundance per site, (2) compared community differences to identify spatial

* Corresponding author: insafitri@trunojoyo.ac.id

variation, and (3) evaluated the effectiveness of eDNA metabarcoding for algal surveys in tropical ecosystems. Findings from this study provide important baseline data for monitoring and conservation of Indonesian coastal biodiversity.

2 Material and methods

Seawater samples were collected from coral reef sites around Madura Island, Indonesia. Approximately 1–2 liters of seawater were filtered on-site using sterile filtration units with 0.45 µm pore-size membrane filters (Millipore, USA) to capture microbial and extracellular DNA particles. The filters were preserved in 100% molecular-grade ethanol and transported on ice to the laboratory for further processing [12].

Environmental DNA was extracted from each filter using the DNeasy PowerWater it (Qiagen, Germany) following the manufacturer's protocol. DNA quantity and purity were verified using a NanoDrop spectrophotometer (Thermo Fisher Scientific, USA) and stored at −20 °C until amplification [13].

The mitochondrial cytochrome c oxidase subunit I (COI) gene region was amplified using the JG primer pair (jgLCO1490/jgHCO2198), which is widely used for metabarcoding marine biodiversity (Geller et al. 2013). PCR amplification was performed in 25 µL reactions containing 12.5 µL of PCR Master Mix (Thermo Fisher Scientific), 1 µL of each primer (10 µM), 2 µL of DNA template, and nuclease-free water to volume. Thermal cycling conditions consisted of an initial denaturation at 95 °C for 3 min, followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 48 °C for 45 s, extension at 72 °C for 60 s, and a final extension at 72 °C for 10 min [14].

Amplicons were purified using AMPure XP beads (Beckman Coulter, USA) and quantified with a Qubit fluorometer (Invitrogen, USA). Sequencing libraries were prepared following Illumina MiSeq library preparation protocols with dual-indexing adapters. Paired-end sequencing (2 × 300 bp) was carried out on an Illumina MiSeq platform (Illumina, USA) at a commercial sequencing facility.

Raw sequence reads were processed using QIIME2 and DADA2 pipelines for quality filtering [15], chimera removal, and operational taxonomic unit (OTU) clustering at 97% similarity. Taxonomic assignment was performed against the NCBI and BOLD COI reference databases using BLASTn [16].

3 Result and discussion

3.1 Main findings of the present study

A total of 40 operational taxonomic units (OTUs) belonging to three algal divisions were detected across the study sites: Chlorophyta, Phaeophyta, and Rhodophyta. Chlorophyta exhibited the highest richness and relative abundance, followed by Rhodophyta, whereas Phaeophyta showed the lowest diversity (Table 1).

Table 1. Relative abundance (%) of algal divisions detected by eDNA metabarcoding.

Division	Gili Genting (%)	Gili Labak (%)	Karang Takat (%)	Average (%)
Chlorophyta	60.07	60.76	43.22	54.68
Phaeophyta	18.78	1.84	3.11	7.91
Rhodophyta	21.15	37.40	53.67	37.41

Chlorophyta dominated in Gili Genting and Gili Labak, with *Micromonas pusilla* and as key contributors ini all sites. *Ramicrusta sp.* (invasive and competitor for coral) also as key contributor for Karang Takat and Gili Genting. Karang Takat exhibited the highest relative proportion of Rhodophyta, suggesting a more structurally complex habitat (Table 2, Table 3, and Table 4).

Table 2. Abundance of Chlorophyta in the study area.

No	Species	Gili Genting	Gili Labak	Karang Takat
1	*Micromonas pusilla*	1294	531	795
2	*Dolichomastix tenuilepis*	109	70	0
3	*Chloropicon roscoffensis*	38	50	81
4	*Pseudoscourfieldia marina*	74	0	0
5	*Chloropicon sieburthii*	56	41	66
6	*Prototheca wickerhamii*	63	0	0
7	*Pseudoscourfieldia marina*	34	0	10
8	*Elliptochloris subsphaerica*	11	0	0
9	*Dolichomastix tenuilepis*	0	0	7
Total		1679	692	959
Percentage		60.07	60.76	43.22
Average Percentage		54.68		

Table 3. Abundance of Phaeophyta in the study area.

No	Species	Gili Genting	Gili Labak	Karang Takat
1	*Acinetosporaceae sp. 1 AP-2014*	74	0	0
2	*Punctaria latifolia*	62	0	0
3	*Petalonia fascia*	97	0	11
4	*Chordariaceae sp. 5 AP-2014*	40	0	8
5	*Punctaria occidentalis*	39	0	0
6	*Pogotrichum sp.*	70	0	18
7	*Ectocarpus siliculosus*	36	16	0
8	*Chordariaceae sp. 5 AP-2014*	30	0	0
9	*Laminariocolax tomentosoides*	24	0	0
10	*Laminarionema elsbetiae*	18	0	21
11	*Scytosiphon shibazakiorum*	35	0	0
12	*Cladosiphon zosterae*	0	0	11
13	*Colpomenia sp. 1WA*	0	5	0
Total		525	21	69
Percentage		18.78	1.84	3.10
Average Percentage			7.91	

Table 4. Abundance of Rodhophyta in the study area.

No	Species	Gili Genting	Gili Labak	Karang Takat
1	*Crusticorallina painei*	0	0	211
2	*Ramicrusta sp.*	338	159	333
3	*Asterfilopsis sp*	0	0	183
4	*Nothocladus theaquus*	0	224	50
5	*Hypnea wynnei*	82	0	116
6	*Stirnia prolifera*	0	0	162
7	*Jania rubens*	52	0	0
8	*Beda leptophylla*	49	0	0
9	*Pterocladiella media*	25	0	37
10	*Metacallophyllis laciniata*	34	0	0
11	*Dasysiphonia japonica*	0	11	27
12	*Corallina vancouveriensis*	0	0	23
13	*Sporolithon sp. 3*	0	0	22
14	*Lithophyllum sp. 1*	0	0	21
15	*Corallina officialis*	0	19	0
16	*Bryotamnion seaforthii*	0	13	0
17	*Lithophyllum sp. 6*	11	0	0
18	*Titanophycus valids*	0	0	6
Total		591	426	1191
Percentage		21.14	37.40	53.67
Average Percentage		31.47		

Environmental DNA (eDNA) metabarcoding successfully revealed the diversity and composition of marine algae (Chlorophyta, Phaeophyta, and Rhodophyta) from three reef sites around Madura Island—Gili Genting, Gili Labak, and Karang Takat. A total of 40 algal taxa were identified, comprising 9 Chlorophyta, 13 Phaeophyta, and 18 Rhodophyta.

Total read abundance varied among sites, with Gili Genting (2795 reads) showing the highest overall detection, followed by Karang Takat (2219 reads) and Gili Labak (1139 reads). Among the phyla, Chlorophyta dominated at Gili Genting (1679 reads), whereas Rhodophyta dominated at Karang Takat (1191 reads). Phaeophyta contributed relatively fewer reads across all sites, particularly at Gili Labak (21 reads).

The most abundant Chlorophyta taxa included Micromonas pusilla, Dolichomastix tenuilepis, Chloropicon roscoffensis, and Chloropicon sieburthii. Within Phaeophyta, Pogotrichum sp., Acinetosporaceae sp., and Petalonia fascia were dominant, while Rhodophyta was primarily represented by Ramicrusta sp., Nothocladus theaquus, Crusticorallina painei, and Hypnea wynnei.

Species richness also differed among sites. For Chlorophyta, richness was highest at Gili Genting (8 species); for Phaeophyta, at Gili Genting (11 species); and for Rhodophyta, at Karang Takat (12 species).

Overlap analysis revealed few shared taxa among all sites. Chlorophyta shared two taxa across all sites, whereas Phaeophyta showed none, and Rhodophyta exhibited one shared species. These results demonstrate clear spatial heterogeneity of algal communities across the three reef systems, like found in other researches elsewhere [17, 18].

3.2 Comparison with other studies

The metabarcoding data revealed distinct spatial differences in marine algal diversity among Gili Genting, Gili Labak, and Karang Takat. Gili Genting, characterized by proximity to inhabited areas and likely influenced by coastal runoff, showed a dominance of Chlorophyta, particularly *Micromonas pusilla*. This species is a cosmopolitan picoplanktonic alga associated with nutrient-enriched and mixed coastal waters [19]. Its prevalence may reflect eutrophic conditions and higher environmental variability typical of nearshore reefs [20].

In contrast, Karang Takat, likely more offshore and less anthropogenically influenced, was dominated by Rhodophyta, including *Ramicrusta* sp. and *Crusticorallina painei*. Red algae are known to colonize stable reef substrates and contribute significantly to reef framework formation [21].

Phaeophyta (brown algae) displayed moderate diversity but lower read abundance overall, indicates the coexistence of multiple taxa with relatively low abundance, possibly reflecting microhabitats in shallow reef flats or rocky substrates exposed to varying light and wave conditions [22].

3.3 Implication and explanation of findings

The low number of shared species across sites underscores the localized nature of algal assemblages, influenced by substrate type, hydrodynamics, and human activities [23, 24]. The spatial heterogeneity detected here aligns with previous studies showing that tropical reef algal assemblages are highly site-specific and sensitive to microenvironmental gradients [25].

3.4 Strengths and limitations

From a methodological standpoint, this study demonstrates the power of eDNA metabarcoding to detect hidden algal diversity across different reef environments. However, it should be noted that sequence read abundance does not equate to biomass or cover [26]. PCR bias, gene copy number variability, and DNA degradation can influence read proportions [27, 28]. Thus, while metabarcoding provides a valuable molecular snapshot, future work should integrate morphological surveys and environmental data (e.g., nutrient levels, turbidity, and substrate composition) to link molecular diversity with ecosystem structure.

4 Conclusion

The application of eDNA metabarcoding revealed clear spatial variation in the composition and diversity of marine algae across Gili Genting, Gili Labak, and Karang Takat reefs. Gili Genting showed dominance of *Micromonas pusilla* and other Chlorophyta species associated with nutrient-enriched waters. Karang Takat exhibited higher Rhodophyta diversity, reflecting a stable and mature benthic environment. Gili Labak

displayed lower algal diversity and abundance, possibly due to limited substrate availability or lower DNA yield.

These findings highlight the ecological uniqueness of each reef system and demonstrate the potential of eDNA metabarcoding as a non-invasive tool for assessing marine algal biodiversity in tropical reef ecosystems of Indonesia. To enhance accuracy for future work, comparative studies combining eDNA and traditional morphological identification are needed for method validation and calibration.

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