BIODIVERSITAS
Volume 26, Number 8, August 2025
Pages: 3939-3951

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d260824

Applications of environmental DNA for marine biodiversity monitoring in Sumberkima Village, Bali, Indonesia

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Manuscript received: 27 June 2024. Revision accepted: 16 August 2025.

Abstract. *Prasetia IND, Amelia JM, Sitepu GSB, Maharani MDK, Wulandari D, Ampou EE, Malik MDA, Nursalim N, Kholilah N, Cahyani NKD. 2025. Applications of environmental DNA for marine biodiversity monitoring in Sumberkima Village, Bali, Indonesia. Biodiversitas 26: 3939-3951.* Sumberkima Village in North Bali, Indonesia, is known for its high marine diversity, yet it increasingly faces ecological stress from fishing and tourism activities. This study aims to assess the biodiversity of coral reef ecosystems in Sumberkima using a combination of visual census and environmental DNA (eDNA) metabarcoding. A Line Transect Method was conducted through SCUBA for a visual census to detect coral diversity and coral cover. Then, eDNA samples were collected from three stations (i) coral reef ecosystems, (ii) the transitional zone between seagrass and coral reef ecosystems, and (iii) coral reef ecosystems near the bay close to the mainland and analyzed using the COI locus to detect invertebrates and vertebrates. Seawater was taken for 1 L per station. A total of 200,709 reads and 662 Amplicon Sequence Variants (ASVs) of Eukaryotes, enabling the detection of cryptic and hard-to-observe species. Meanwhile, visual census using the Line Transect Method assessed coral diversity and cover, identifying 39 genera with conditions ranging from poor to medium. The integration of eDNA with traditional visual census techniques provides a more comprehensive biodiversity assessment, particularly in ecologically rich yet threatened marine environments such as Sumberkima, North Bali, Indonesia.

Keywords: Coral reef ecosystems, COI, eDNA, invertebrates, visual census, vertebrates

INTRODUCTION

Indonesia is one of the areas within the coral reef triangle with the most diverse organisms (Veron et al. 2011; Linggi and Burhanuddin 2019; Anugrah et al. 2020), including coral reef ecosystems (Parravicini et al. 2013; Cowman and Bellwood 2013). Biodiversity within coral reef ecosystems can provide as much as 80% of daily protein needs for local communities (Moberg and Folke 1999) and support the local community's well-being through sustainable livelihoods and income. However, this ecosystem is one of the most threatened (Veron et al. 2009) because of various natural (disasters, bleaching, disease outbreaks, and crown-of-thorns starfish) and anthropogenic (overfishing, pollution, and irresponsible tourism activities) threats (O’Hara et al. 2021; Muir et al. 2022).

Understanding life within coral reef ecosystems is essential with the increasing threat to marine ecosystems (Sembiring et al. 2023). Information covering the composition of taxa, ecological processes that shape local biodiversity, and anthropogenic stresses affecting biodiversity is essential and could shape the dynamic of current and future biodiversity trajectory (Edinger et al. 1998; Whittaker et al. 2005). Bali is one of the coral triangle areas known for its beautiful marine diversity with several popular tourist areas like diving and snorkeling (Karim 2019; Widiastuti and Faiqoh 2020), especially in North Bali (Sembiring et al. 2023).

One way to find out changes in the biodiversity of an ecosystem is to carry out regular monitoring (Branchini et al. 2015). However, many areas in the Indonesian region, including North Bali, are still monitored for their marine biodiversity in coral ecosystems through visual census methods (Habibi et al. 2007; Doherty et al. 2013; Desvianti and Choesin 2015). There are several laxities in the visual census method. For example, it could not determine all the species found in the ecosystem (Pilliod et al. 2013). Then, due to limited observation time with visual censuses, which are mostly carried out from morning to afternoon, nocturnal fish will not be assessed.

Sumberkima Village as part of Buleleng District, Bali, Indonesia, has complete coastal diversity, including coral reefs, mangrove areas, seagrass beds and beaches with both black and white sand. One example of a natural destination in this village is Gili Putih Island, which provides stretches of white sand throughout its area. Gili Putih Island is the result of sedimentation from a combination of natural activities of the coral reef ecosystem, seagrass beds, and mangroves found in this area. The emergence of this island has had a direct and indirect impact on the rise of tourism in Sumberkima Village. However, the information on the diversity of eukaryotes in marine ecosystems in Sumberkima Village remains incomplete due to the limitations of the current method used in visual censuses, which focus only on targeted organisms such as fish and invertebrates (Aglieri et al. 2021).

Environmental DNA (eDNA) technology has revolutionized biomonitoring (Chen et al. 2024) in marine biodiversity research (Buxton et al. 2021; Cai et al. 2024). eDNA can detect DNA traces left by animals in their surroundings environment, such as soil, water, and sediment (Deiner et al. 2017; Ruppert et al. 2019). It is emerging as a tool for rapid biodiversity monitoring (Bessey et al. 2021; Thalinger et al. 2021; Sanchez et al. 2022; Naputo et al. 2024), not cause environemntal damage (Sahu et al. 2022), cost-effective (Munian et al. 2024), and is commonly used for resource monitoring (Jia et al. 2023) to examine fish assemblages (Allan et al. 2021; Oka et al. 2021; Rourke et al. 2021; Li et al. 2022; Wu et al. 2023; Parikh et al. 2024; Malik et al. 2025) and non-invasive biodiversity assessments (Formel et al. 2021).

Additionally, eDNA has been widely used to compile biodiversity assessments and combined with traditional visual census (Gold et al. 2021; Marwayana et al. 2022; Malik et al. 2024). Then, eDNA could potentially give data that has never been detected by visual census (Polanco Fernández et al. 2021; Muenzel et al. 2024). One currently utilized locus for eDNA is Cytochrome Oxidase Subunit 1 (COI), which can detect various eukaryotic taxa in marine environments (Andriyono et al. 2021; Madduppa et al. 2021). Through this, eDNA could potentially provide a comprehensive view of eukaryote diversity in this location. This study aimed to provide an overview of biodiversity assessment in coral reef ecosystems in Sumberkima, Bali, Indonesia, using a visual census (coral coverage) and the eDNA approach.

MATERIALS AND METHODS

Study site

The study was conducted from May to December 2023 in Sumberkima Village, Gerokgak Sub-district, Buleleng District, Bali, Indonesia. Three sampling stations were selected as they represent distinct locations (i) a coral reef ecosystem; (ii) a coral ecosystem near a seagrass bed; and (iii) a coral reef ecosystem near the mainland (Table 1 and Figure 1).

Procedures

There were two approaches used in this study (i) the visual census for coral coverage and (ii) environmental DNA from one litre of seawater per sampling location. Water quality assessment in Sumberkima Village was conducted following the guidelines of SNI 6964.8:2015 regarding the sampling method for seawater testing, which included parameters, such as temperature, salinity, acidity level, dissolved oxygen (DO), conductivity, and turbidity. Samples were collected and subsequently analyzed at the Analytical Laboratory of Universitas Udayana, Denpasar, Bali, Indonesia.

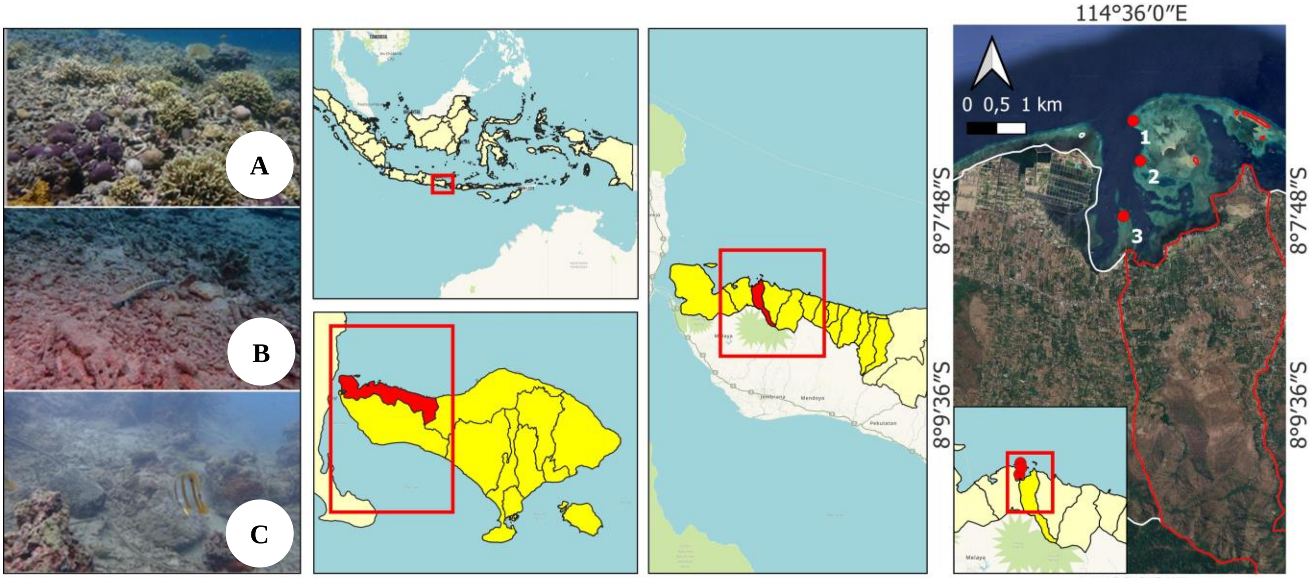

Figure 1. Three sampling locations in Sumberkima Village, Gerokgak, Buleleng, Indonesia. A. Station 1 with sample ID S2710, B. Station 2 with sample ID S2711, C. Station 3 with sample ID S2712

Table 1. Description of each station sampling

Station	**Coordinate**		**Description**
	S	**E**	
Station 1	8° 7' 43.66"	114° 35' 9.85"	Coral reef ecosystems with depths of 3 to 40 m.
Station 2	8° 8' 3.73"	114° 35' 15.20"	A combination of seagrass ecosystems to a depth of 3 m and coral reef ecosystems with depths of up to 20 m.
Station 3	8° 8' 26.87"	114° 36' 32.09"	Coral reef ecosystem near Sumberkima Bay and mangrove is close to the mainland, with fisheries activities in the form of floating net cages, and has a coral reef ecosystem with low live coral cover conditions.

Coral coverage observation

The coral reef ecosystem research utilized the line transect method conducted through SCUBA diving to gather data on (i) coral cover; (ii) substrate type cover within the observed coral reef ecosystem; (iii) observation of coral reef indicator organisms; (iv) coral diversity, uniformity, and dominance values; and (v) coral mortality index (English et al. 1994).

Environmental DNA

Water sampling

One liter of seawater was collected per sampling location with a sterile sampling bottle at a depth of 10 m from the sea surface from each sampling point. Water samples were then filtered using a vacuum pump with 0.45 µL filter paper (Pall Corporation). Filter paper was then preserved in RNA/DNA Shield (Zymo Research) and stored at -20°C.

Molecular analysis

DNA was extracted from filter paper using Quick-DNA™ Fecal/Soil Microbe MiniPrep Kit from ZymoBIOMICS™ following the manufacturer's protocol. DNA was quantified using Nanodrop (Thermo NanoDrop 2000 Spectrophotometer). Thirty µl of DNA extraction was sent to the sequencing facility for library prep and sequencing using the Next Generation Sequencing platform. Cytochrome Oxidase Subunit 1 (COI) genes were targeted using the mlCOIintF (5'-GGW ACW GGW TGA ACW GTW TAY CCY CC-3') and dgHCO2198 (5'-TAA ACT TCA GGG TGA CCA AAR AAY CA-3') primers (Meyer 2003; Leray et al. 2013). Library preparation for COI amplicons followed a single indexing approach where barcodes were incorporated into the forward primer to facilitate multiplexing of up to 96 samples per run. Sequencing was conducted with the MiSeq Illumina platform. The library preparation was conducted by PT. Genetika Science Indonesia. Raw sequences were deposited at NCBI as SRA (Sequence Read Archive) with accession numbers SAMN47192544, SAMN47192545, and SAMN47192546.

Data analysis

The analysis of coral cover using the line transect method was performed employing the formula proposed by English et al. (1994), which is:

$$\text{Percentage cover of lifeform } i = \frac{\text{Transect length of lifeform } i}{\text{Total transect length}} \times 100\%$$

Based on Decree of the State Minister for the Environment No. 04/2001, a high percentage (75-100%) of live coral cover indicates the presence of a healthy coral reef ecosystem in that area. This is supported by the high diversity index in the region.

For eDNA analysis, forward and reverse FASTQ sequences were cleaned and analyzed using the Quantification Insights into Microbial Ecology 2 tool (https://qiime2.org/) (Bolyen et al. 2019). The DADA2 software (Divisive Amplicon Denoising Algorithm 2) was used for the quality filter, trim, de-noise, and merge of the data (Callahan et al. 2016). Sequences were clustered into Amplicon Sequence Variants (ASVs). Taxonomical identification was produced by training a feature classifier in *QIIME2* against CRUX database (Curd et al. 2019). To summarize the taxonomic composition of each sample, we used *phyloseq* (McMurdie and Holmes 2013) in R Studio (R development core team).

The rarefaction curves were created with the Ranacapa package using the *grade* command (Kandlikar et al. 2018). Stacked bar plots summarizing taxonomic composition and sequence abundance were generated using *ggplot2* (Wickham 2009) in R Studio (R development core team) based on the total abundance in the sample's reads or sequences. For each sample from each location, we used http://bioinformatics.psb.ugent.be/webtools/Venn/ to create a set of Venn diagrams to determine how many ASVs were shared between sampling locations. Additionally, Alpha diversity from Shannon and Simpson indices in each station were analyzed in R.

This study utilized the QIIME2 pipeline *align-to-tree-mafft-fasttree* to construct a phylogenetic tree. The pipeline begins by performing a multiple-sequence alignment using MAFFT, which aligns the input sequences. Then, columns in the alignment that are phylogenetically uninformative or ambiguously aligned are masked, producing a masked alignment. The masked alignment is then used to infer an unrooted phylogenetic tree using FastTree in QIIME2. Finally, the unrooted tree is rooted at its midpoint to generate a rooted phylogenetic tree. Additionally, ggtree package (Yu et al. 2017) was used to edit a phylogenetic tree in R from a representative sequence of the eDNA results from the presence of sessile macrofauna in eDNA data at each station.

RESULTS AND DISCUSSION

Water quality

The research showed that the water temperature ranges between 28.7°C and 29.2°C, still meeting sea water standard for marine tourism and marine life according to Governor Regulation of Bali No. 16 of 2016, which specifies a required temperature range of 28°C to 30°C. The salinity value of the water ranges between 32.3 and 32.5 parts per thousand (‰) (Table 2). This falls within the normal conditions of seawater, according to Decree of the State Minister for the Environment (KepMenLH) No. 51 of 2004 concerning Seawater Quality Standards, which range from 30 to 35 ‰, conducive to the growth of coral reefs and marine life inhabiting the waters.

The waters of Sumberkima Village exhibit a stable acidity level, ranging from 8.7 to 9.3, slightly exceeding the ideal range for marine tourism water quality, which typically falls between 7 and 8.5 based on Decree of the State Minister for the Environment (KepMenLH) No. 51 of 2004. The Dissolved Oxygen (DO) levels ranging from 0.03 to 0.08 in the waters of Sumberkima Village clearly indicate conditions well below the water quality standard required to support marine life, which typically necessitates DO levels above 5 mg/L based on Decree of the State Minister for the Environment (KepMenLH) No. 51 of 2004. The conductivity value ranges between 49.2 and 49.5 (Table 2). The disparity in turbidity values, with 10 in the marine waters indicates significant variation in water quality between the two environments.

Coral coverage

Studies at the three stations showed that abiotic cover dominates at all stations ranging from 58.4 to 89%, consisting of rubble, sand, and rock. Station 1, which is the outermost part of the bay, has 34.2% live coral cover consisting of *Acropora* and non-*Acropora* groups, while Station 2 has 17.4% coral cover, and the lowest is Station 3 with 4.8% coral cover (Figure 2). The low live coral cover at Station 3 is because this area is close to the coast which has high sedimentation and there is very intense fishing activity in the form of floating net cages.

This study observed a total of 39 coral genera, namely *Acropora, Alveopora, Anacropora, Astreopora, Caulastrea, Coscinaraea, Ctenactis, Cycloseris, Duncanopsammia, Echinophyllia, Echinopora, Euphyllia, Favia, Favites, Fungia, Galaxea, Goniastrea, Goniopora, Heliofungia, Herpolitha, Hydnopora, Leptoria, Leptoseris, Lobophyllia, Isis, Montastrea, Montipora, Pachyseris, Palauastrea, Paraclavarina, Pavona, Physogyra, Pocilopora, Porites, Psammocora, Seriatopora, Stylopora, Tubipora*, and *Turbinaria* in three sampling stations. Coral life forms from the genus *Acropora* are found in the forms of branching, digitata encrusting, submassive, and tabulate. The life forms from non-*Acropora* include encrusting, branching, foliose, massive, submassive, and mushroom.

Table 2. Sumberkima Village water quality test results

Parameter	Station 1	Station 2	Station 3	Standard based on Decree of the State Minister for the Environment No. 51 of 2004
Temperature (°C)	28.7	29.4	29.2	Natural
Salinity (‰)	32.3	32.5	32.3	Natural
Degree of Acidity (pH)	9.19	9.3	8.7	7 - 8.5
DO (mg/L)	0.03	0.08	0.03	> 5
Conductivity value	49.2	49.5	49.3	
Turbidity (ntu)	10	10	10	5

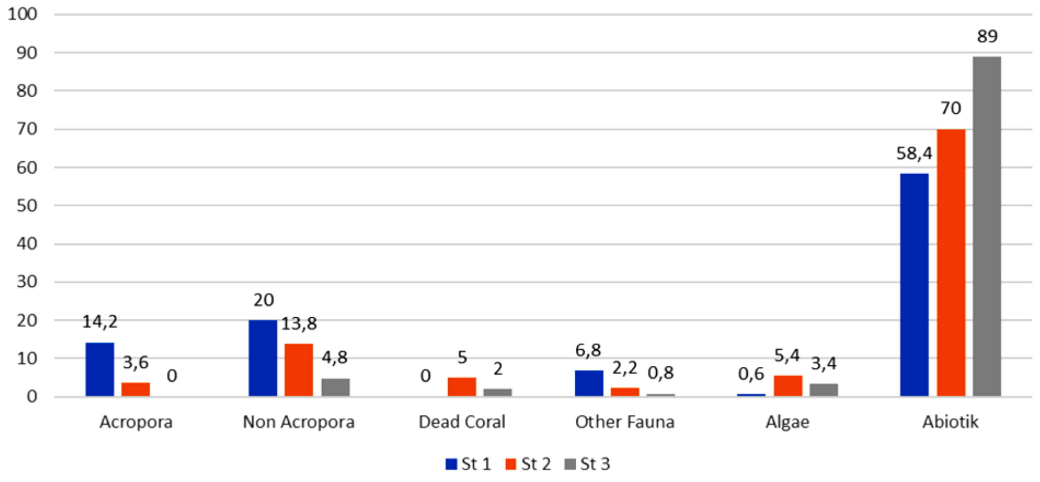

Figure 2. Coral coverage in Sumberkima Village, Pemuteran, Bali, Indonesia

The other fauna group (soft coral, sponges, and zoanthids) in station 1 is observed at 6.8%, station 2 is 2.2%, and station 3 is 0.8%. Meanwhile, the algae group was most commonly found at Station 2 with 5.4% and the lowest was at Station 1 with 0.6% consisting of algae assemblage, coralline algae, halimeda, macro algae, and turf algae (Figure 2).

Environmental DNA (eDNA)

Three individual samples from 3 L of seawater (1 L per sample) were successfully sequenced in this study. A total of 440,133 reads and 1,442 ASVs were obtained from this study, with the mean reads per sample being 146,711 reads. This study found two major kingdoms, namely Bacteria and Eukaryotes, with Eukaryota having 722 ASVs and 274,934 reads and Bacteria with 80 ASVs and 5,217 reads. The unidentified group was detected in 159,982 reads (36%). After excluding the *Homo sapiens* Linnaeus 1758 sequences, all eukaryotic taxa were used for further analysis. Sequences were rarefied to an equal number of reads (66,903 reads) to minimize bias during analysis. After rarefying, a total of 200,709 reads and 662 ASVs of Eukaryotes were used for downstream analysis. The rarefaction curve result indicates that the sample from Station 1 has the highest species richness of the two other samples (Figure 3). However, it also shows that the curves for all the samples did not reach a plateau, indicating that the study needs more sequencing depth to take account of most of the amplified taxa. Raw sequences were deposited at NCBI as SRA (Sequence Read Archive) with accession numbers SAMN47192544, SAMN47192545, and SAMN47192546.

In total, 112 ASV's (16.9% of total ASV's) were found in all three samples (Figure 4). In addition, the most ASV's shared between two samples were between location 1 and location 2, with a total of 91 ASV's or 13.7% of total ASV's. However, the highest number of unique ASV's was found in location 1, with a total of 226 ASV's or 34.1% of total ASV's, followed by location 2 with 132 ASV's or 19.9% of total ASV's and location 3 with 80 ASV's or 12.1% of total ASV's (Figure 4).

The alpha diversity, assessed using Simpson and Shannon indices, revealed that Station 2 is the most diverse, with a Shannon value of 2.74 and a Simpson value of 0.82, indicating the highest species richness and evenness among the other stations. Station 1 follows with a Shannon value of 2.54 and a Simpson value of 0.75. Station 3 has the lowest diversity, with a Shannon value of 1.58 and a Simpson value of 0.58, suggesting lower species richness and evenness.

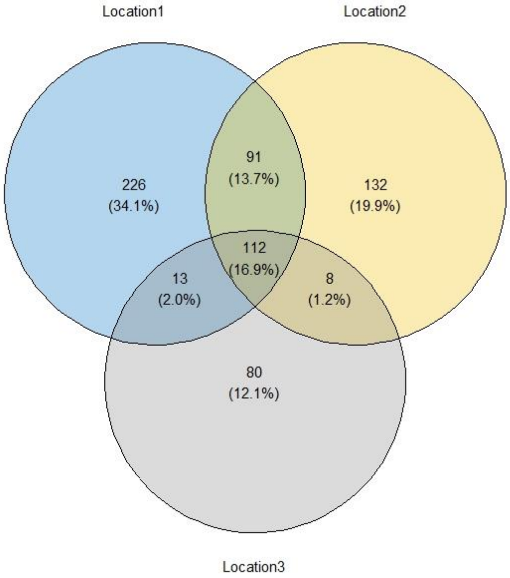

Figure 4. The number and distribution of ASVs revealed from COI metabarcoding obtained from three locations/stations

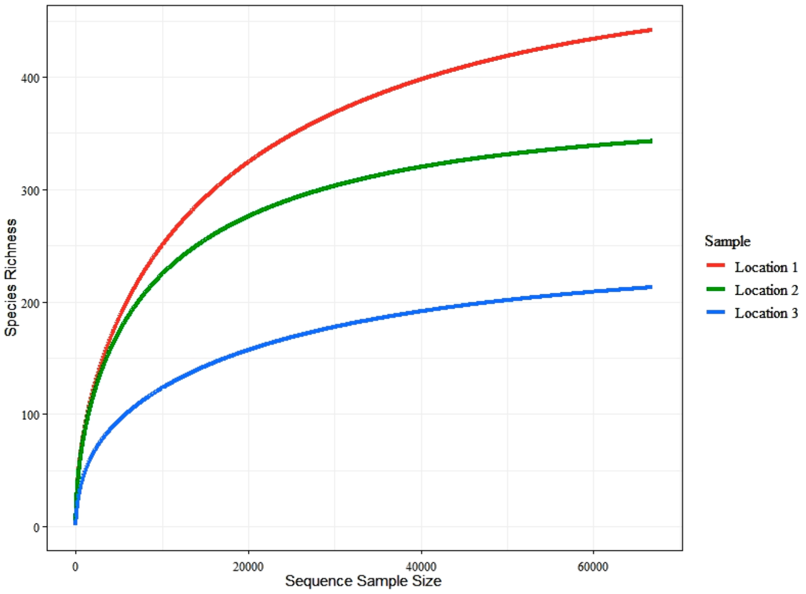

Figure 3. Rarefaction curve from three stations examined in this study. Species richness (left axis) plotted against sequencing depth (bottom axis)

Each sample has a different dominant taxon composition at the phylum level. The sample from station 1 was dominated by Chlorophyta (47%), Arthropoda (18%), Basidiomycota (14%), Ochrophyta (5%), and Bacillariophyta

(2%). Station 2 was dominated by Chlorophyta (37%), Arthropoda (34%), Basidiomycota (8%), Ochrophyta (7%), and Bacillariophyta (2%). In addition, station 3 was dominated by Arthropoda (71%), Chlorophyta (21%), and 1% each for Bacillariophyta, Cnidaria, Mollusca, and Ochrophyta (Figure 5).

The eDNA method is able to detect biodiversity at species level with 141 species in the three locations (see Table S1). Some of the phyla have low abundance in the samples. The phylum Annelida is only detected in station 3 and has low reads abundance (less than 1%). Only two species were identified, namely *Syllis vittata* Grube 1840 and *Pygospio elegans* Claparède 1863. Other than *H. sapiens*, there are two other species identified from Phylum Chordata, *Pateobatis jenkinsii* and *Brevitrygon walga* rays. Phylum Echinodermata is only detected from Station 1 with less than 1% reads abundance and one ASV from genus *Echinaster* (starfish). Phylum Gastrotricha, Nemertea, and Porifera also identified single ASVs from genus *Chaetonotus*, *Tubulanus*, and *Clathria* (*Clathria cancellaria*), respectively.

This study also identified phylum Ascomycota, Basidiomycota, and Blastocladiomycota. There is one species from phylum Ascomycota detected from the station 1 sample with <1% reads abundance (*Leohumicola verrucosa*). Other than that, the study also identified class Saccharomycetes. Basidiomycota was most abundant in sample station 1 (14% reads abundance), followed by Station 2 with 8% reads abundance, and sample Station 3 with less than 1% reads abundance. The sequences are only identified at the genus level (*Pleurotus* and *Pluteus*). Lastly, phylum Blastocladiomycota is only detected in sample Station 3 with less than 1% reads abundance. The species identified is *Clavochytridium emersonii*.

The more abundant phyla are Arthropoda, Bacillariophyta, Chlorophyta, Cnidaria, Mollusca, and Ochrophyta. The Arthropoda consists of 63 species, including Arachnida, Insecta, Malacostraca, Maxillopoda, and Merostomata. Most of the species from phylum Bacillariophyta are marine species (*Coscinodiscus* spp., *Pseudo-nitzschia sabit*, *Ditylum brightwellii*, *Haslea ostrearia*, *Chaetoceros socialis*, *Pseudo-nitzschia hasleana*, *Gomphonema parvulum*, *Minutocellus polymorphus*), and some are freshwater species (*Cyclotella* sp., *Sellaphora capitata*, *Sellaphora pupula*, and *Pinnularia subcommutata*). There are 11 species identified from phylum Cnidaria and consists of three classes, i.e. Hydrozoa, Anthozoa, and Scyphozoa. Phylum Mollusca consists of eight species from classes Bivalvia, Cephalopoda, and Gastropoda. Class Ochrophyta identified 21 species with the highest reads abundance from station 2 (7%), station 1 (5%), and station 3 (1%) (Figure 5). There are three species identified from phylum Chlorophyta **(***Micromonas pusilla*, *Dolichomastix tenuilepis*, and *Nannochloris* sp. MBTD-CMFRI-S048). The reads are abundant in station 1 (47%), station 2 (37%), and station 3 (21%).

The sessile macrofauna found in all the samples consists of three phyla, namely Cnidaria, Porifera, and Mollusca, with a total of four classes, namely Anthozoa and Hydrozoa (Phylum: Cnidaria), Demospongiae (Phylum: Porifera), and Bivalvia (Phylum: Mollusca). In addition, only family Oculinidae from phylum Cnidaria (Class: Anthozoa) was found in three samples (Figure 6).

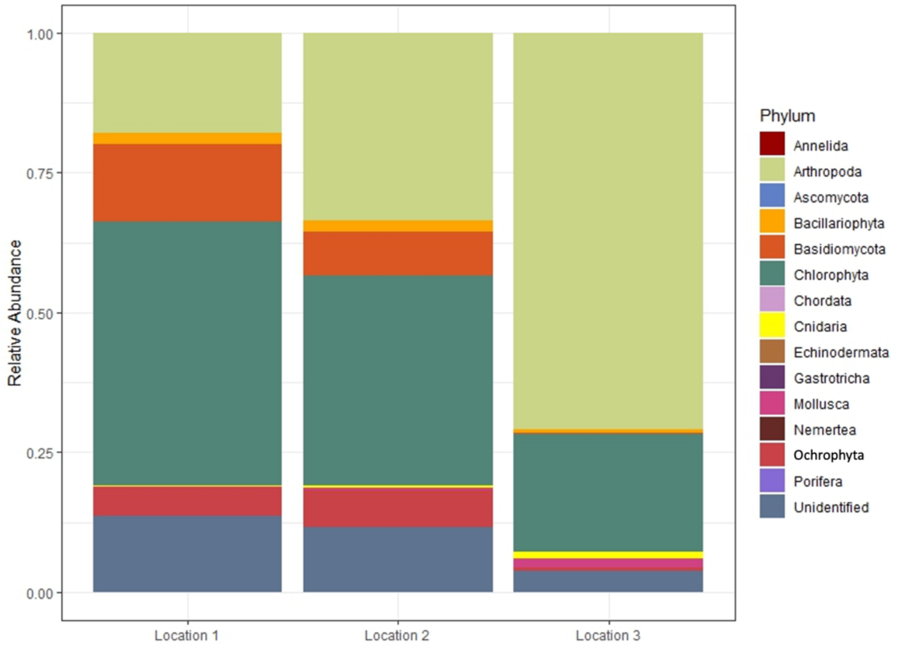

Figure 5. Taxa composition on the phylum level between three sample locations/stations

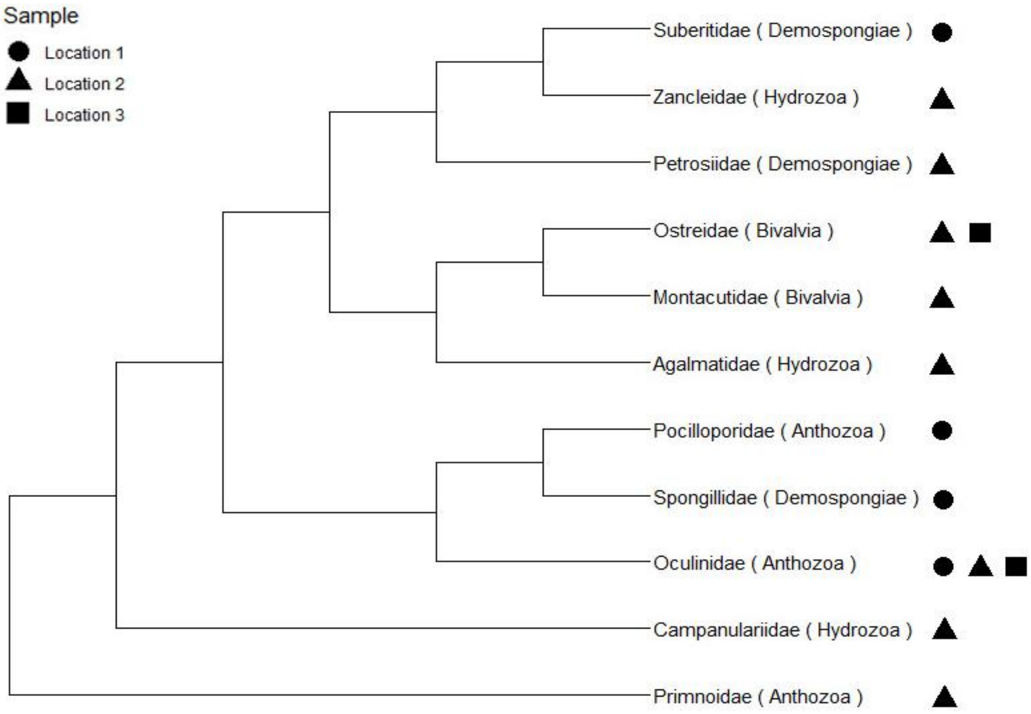

Figure 6. A phylogenetic tree from present-absent sessile macrofauna in all three locations/stations. All tips were labeled with a family (Class) name

Discussion

Based on Bali Provincial Regulation No. 3 of 2020, Sumberkima Village, located in Gerokgak Sub-district, Buleleng District, Bali, Indonesia, holds strategic economic importance and ecological uniqueness due to its diverse coastal habitats, including coral reefs, mangroves, and seagrass beds, making it an ideal site for marine spatial planning and biodiversity baseline development. This marks a modification to Regional Regulation No. 16 of 2009 concerning the spatial planning of Bali Province for the period 2009-2029. Consequently, the water quality in Sumberkima Village must adhere to the water quality standards for marine ecosystems as well as for tourism. This study demonstrates that the water continues to meet the seawater quality standards required for marine tourism and marine life.

The emergence of Gili Putih Island has significantly influenced tourism development in Sumberkima Village, both directly and indirectly. Directly, the community has been able to leverage the island as a tourist attraction, contributing to the village's income. Indirectly, the rise in tourism has stimulated other sectors to support and enhance tourism activities. To harness the ecological and ecotourism potential of Gili Putih Island and Sumberkima's surrounding waters, it is critical to integrate biodiversity assessments like eDNA into sustainable marine monitoring planning efforts. Given the relatively recent discovery of Gili Putih Island, these efforts should be prioritized to ensure long-term benefits for both the environment and the community. Some basic efforts that can be implemented to support sustainable management are (i) inventory of the ecological, economic, and social potential of the aquatic ecosystem in Sumberkima Village; (ii) synergy of stakeholders in determining strategies and management models for tourist areas; and (iii) community empowerment in increasing the potential for managing cultivated products to support tourism.

The acidity level in Sumberkima Village ranges from 8.7 to 9.3, which slightly exceeds the typical ideal range of 7 to 8.5 for marine tourism, and contrasts with the findings in Gili Air, where pH was recorded at 6.3, below the ideal range (Melinda and Nurhidayah 2023). Additionally, Dissolved Oxygen (DO) levels in Sumberkima Village between 0.03 and 0.08 mg/L, fall significantly below the water quality standard needed to support marine life, which typically requires DO levels above 5 mg/L. In comparison, phosphate levels in Gili Air were found to exceed the standard for marine tourism at 0.1 mg/L, highlighting the importance of monitoring both physical and chemical parameters across various locations (Melinda and Nurhidayah 2023).

The coral reef ecosystem in Sumberkima faced significant pressures before 2000, such as explosive fishing, potassium cyanide capture for ornamental fish, coral mining, uncontrolled aquaculture, and general neglect. Research conducted by Burke et al. (2012) showed that 86% of coral reefs in Indonesia face medium or high threat levels. These activities have contributed to a global decline in coral reef biodiversity, abundance, and habitat structure (Hughes et al. 2018). Public awareness of the importance of conserving coral reef ecosystems has increased since 2000 (Boakes et al. 2022).

Currently, live coral cover at Station 1 is 34.2%, categorized as medium according to the Decree of the

Minister of Environment No. 4 of 2001. There are also 6.8% other fauna groups and 0.6% algae. Stations 2 and 3 are classified as poor, with 17.4% and 4.8% live coral cover, respectively. Station 2 has a large seagrass bed at a depth of 1-3 m. Station 3 is dominated by abiotic cover, ranging from 58.4-89%, consisting of rubble, sand, and rock. The high percentage of rubble is due to past impacts on the coral reef ecosystem, but new coral recruitment is beginning. Overall, Station 1, which is farthest from land, has better coral cover than Station 3, which is closer to land. eDNA monitoring revealed high biodiversity at Station 1, especially among marine invertebrates, reinforcing its value as a sensitive tool for capturing biological complexity in ecologically important areas like Sumberkima. Improving water quality and regular biodiversity conservation and monitoring activities are essential.

The integration of eDNA with visual census methods provided a complementary assessment of marine biodiversity in Sumberkima. eDNA successfully detected a range of taxa, including sessile macrofauna, that were consistent with visual observations. However, eDNA results showed limitations in capturing the full spectrum of marine biodiversity, particularly for certain sessile groups. For instance, only five sessile macrofauna taxa were detected (Hydrozoa, Bivalvia, Anthozoa, Bryozoa, and Porifera), whereas foundational literature (Sara 1986) lists additional important groups like Serpulida, Cirripedia, and Ascidiacea, which were not detected. Such discrepancies likely reflect methodological biases, including limited primer efficiency, rapid eDNA degradation, and DNA persistence variability in tropical marine environments.

Additionally, marine fish detection via eDNA using COI primers was limited to only two species (Family: Dasyatidae), while the visual census identified 20 fish families. This underscores a detection bias likely due to the non-targeted nature of universal COI primers, which are less efficient for fish compared to the more specific 12S rRNA primers (Collins et al. 2019). Primer selection thus remains critical for target taxa, and the integration of specific primers like 12S for fish (Miya et al. 2015) and 16S for invertebrates (Komai et al. 2019) could significantly improve detection accuracy.

A large proportion of ASVs remained taxonomically unassigned, likely due to gaps in reference databases. The possible reason for the unidentified presence of taxa implies that many of the taxa uncovered in this study have not yet been documented in the databases used in this research. Madduppa et al. (2021) reported some unidentified ASVs may constitute marine species that have yet to be formally identified by science. This indicates that a substantial portion of Indonesia's rich biodiversity remains unexplored, underscoring the inadequate representation of taxa from the coral triangle and neighboring regions (including this site study) in global COI databases. The results obtained in this study are closer to the results abroad. Based on previous research, one of the challenges in eDNA research is that the database is incomplete (Juhel et al. 2020). Another challenge is the occurrence of non-native species in bioinformatic outputs, often due to limited regional reference sequences, which leads to misassignments to closely related species outside Indonesia. There are several assumptions that can explain this, including the incomplete Indonesian biodiversity database. This can result in the analysis results only identifying the closest species, even though the population is outside Indonesia (non-native). This is why there is still a need to explore the bioinformatics used (Cahyani et al. 2024).

Metabarcoding using universal primers like COI is currently best suited for broad, rapid biodiversity assessments in diverse aquatic environments (Bakker et al. 2019). However, for more definitive species identification or long-term ecological monitoring, it is essential to adopt a multi-marker approach with targeted primers. Integrating eDNA surveys with traditional methods (e.g., visual census) into a standardized, long-term monitoring framework can offer a more robust and scalable biodiversity assessment strategy (Clarke et al. 2017; Casey et al. 2021), particularly for data-limited regions like Sumberkima.

In conclusion, this study highlights the effectiveness of integrating eDNA with visual census methods to enhance biodiversity assessments in Sumberkima Village. A total of 200,709 reads and 662 ASVs representing 14 phylum and 141 species from both vertebrate and invertebrate (eukaryotic) taxa were identified. The visual census approach using the line transect method assessed coral diversity and cover, identifying 39 coral genera with conditions ranging from poor to moderate. The eDNA approach enhances marine ecosystem monitoring by detecting a broader spectrum of eukaryotic taxa, including rare, cryptic, and undocumented taxa, supporting the development of biodiversity baselines for strategic sites like Sumberkima. The results underscore the effectiveness of combining molecular and observational techniques to provide a comprehensive overview of eukaryotic marine biodiversity.

ACKNOWLEDGEMENTS

This research was supported by the RIIM Indonesia Endowment Fund for Education Agency (LPDP) grant and National Research and Innovation Agency (BRIN) grant number 64/IV/KS/05/2023 entitled The Model of Sustainable Aquaculture-Based Minawisata Development in Sumberkima Village, Buleleng, Bali, Indonesia. We thank the Universitas Pendidikan Ganesha, Singaraja and Yayasan Restorasi Karang Buleleng for their support and cooperation in this program.

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Table S1. Presence-absence from eukaryotic species that detected from eDNA data in the three locations (1: present and 0: absence)

Phylum	**Family**	**Species**	**Location_1**	**Location_2**	**Location_3**
Annelida	Spionidae	*Pygospio elegans*	0	0	1
Annelida	Syllidae	*Syllis vittata*	0	0	1
Arthropoda	Culicidae	*Anopheles albitarsis*	1	1	1
Arthropoda	Culicidae	*Anopheles darlingi*	0	0	1
Arthropoda	Culicidae	*Anopheles subpictus*	1	1	0
Arthropoda	Limoniidae	*Antocha saxicola*	1	1	1
Arthropoda	Limoniidae	*Antocha* sp.	1	1	1
Arthropoda	Liocranidae	*Apostenus fuscus*	1	1	1
Arthropoda	Chrysomelidae	*Aspidimorpha furcata*	1	1	0
Arthropoda	Paracalanidae	*Bestiolina similis*	1	1	1
Arthropoda	Caligidae	*Caligus cheilodactylus*	1	1	1
Arthropoda	Calliphoridae	*Calliphora terraenovae*	1	0	0
Arthropoda	Calliphoridae	*Calliphora vicina*	1	1	1
Arthropoda	Noctuidae	*Callopistria mollissima*	1	0	0
Arthropoda	Ceratopogonidae	*Ceratopogonidae* sp.	1	1	0
Arthropoda	Tabanidae	*Chlorotabanus inanis*	1	0	0
Arthropoda	Calliphoridae	*Chrysomya megacephala*	1	1	1
Arthropoda	Sciaridae	*Claustropyga refrigerata*	0	0	1
Arthropoda	Zopheridae	*Colydium elongatum*	1	0	0
Arthropoda	Scytodidae	*Dictis denticulata*	1	1	1
Arthropoda	Tachinidae	*Drino* sp.	1	0	0
Arthropoda	Tettigoniidae	*Ducetia japonica*	0	0	1
Arthropoda	Noctuidae	*Feltia jaculifera*	1	0	0
Arthropoda	Linyphiidae	*Floronia bucculenta*	1	0	0
Arthropoda	Cicadidae	*Hadoa texana*	0	1	0
Arthropoda	Chironomidae	*Kiefferulus* sp.	0	0	1
Arthropoda	Pontellidae	*Labidocera acuta*	1	1	1
Arthropoda	Muscidae	*Limnophora* sp.	1	0	0
Arthropoda	Potamidae	*Longpotamon siguqiaoense*	0	1	0
Arthropoda	Phoridae	*Megaselia rufipes*	1	0	0
Arthropoda	Nitidulidae	*Meligethes coracinus*	1	1	0
Arthropoda	Buthidae	*Mesobuthus eupeus*	0	1	0
Arthropoda	Cyclopidae	*Mesocyclops pehpeiensis*	1	1	1
Arthropoda	Mycetophilidae	*Mycetophilidae* sp.	1	1	0
Arthropoda	Asilidae	*Neoitamus* sp.	1	0	0
Arthropoda	Nephilidae	*Nephila inaurata*	1	0	0
Arthropoda	Nolidae	*Nola laguncularlae*	1	1	0
Arthropoda	Geometridae	*Odontopera bidentata*	0	1	0
Arthropoda	Oithonidae	*Oithona attenuata*	1	1	0
Arthropoda	Oithonidae	*Oithona simplex*	1	1	1
Arthropoda	Squillidae	*Oratosquilla oratoria*	1	0	0
Arthropoda	Paracalanidae	*Paracalanus aculeatus*	1	1	1
Arthropoda	Paracalanidae	*Paracalanus indicus*	1	1	0
Arthropoda	Paracalanidae	*Paracalanus quasimodo*	0	1	0
Arthropoda	Sesarmidae	*Perisesarma guttatum*	1	1	1
Arthropoda	Pionodesmotidae	*Pionodesmotes domhainfharraigeanus*	1	1	1
Arthropoda	Cicadidae	*Platypleura* sp.	1	0	0
Arthropoda	Carabidae	*Pterostichus minor*	1	1	1
Arthropoda	Agelenidae	*Sinocoelotes luoshuiensis*	0	0	1
Arthropoda	Stephidae	*Stephos longipes*	1	1	1
Arthropoda	Limulidae	*Tachypleus gigas*	1	0	0
Arthropoda	Cicadidae	*Tettigettalna afroamissa*	1	0	0
Arthropoda	Theridiidae	*Theridion glaucescens*	1	0	0
Arthropoda	Osmylidae	*Thyridosmylus langii*	0	0	1
Arthropoda	Plutellidae	*Tonza purella*	0	1	0
Arthropoda	Chironomidae	*Ubatubaneura atlantica*	0	0	1
Arthropoda	Calanidae	*Undinula vulgaris*	1	1	1
Arthropoda	Carabidae	Unidentified	1	0	0
Arthropoda	Unidentified	Unidentified	0	1	0
Arthropoda	Unidentified	Unidentified	0	0	1
Arthropoda	Linyphiidae	*Walckenaeria cucullata*	0	1	1
Ascomycota	Unidentified	*Leohumicola verrucosa*	1	0	0
Bacillariophyta	Chaetocerotaceae	*Chaetoceros socialis*	1	0	0

Bacillariophyta	Coscinodiscaceae	*Coscinodiscus* sp.	1	1	1
Bacillariophyta	Stephanodiscaceae	*Cyclotella* sp.	0	1	0
Bacillariophyta	Lithodesmiaceae	*Ditylum brightwellii*	1	1	0
Bacillariophyta	Gomphonemataceae	*Gomphonema parvulum*	0	1	0
Bacillariophyta	Naviculaceae	*Haslea ostrearia*	1	0	0
Bacillariophyta	Pinnulariaceae	*Pinnularia subcommutata*	1	0	0
Bacillariophyta	Bacillariaceae	*Pseudo-nitzschia hasleana*	1	0	0
Bacillariophyta	Bacillariaceae	*Pseudo-nitzschia sabit*	1	1	0
Bacillariophyta	Sellaphoraceae	*Sellaphora capitata*	1	1	1
Bacillariophyta	Sellaphoraceae	*Sellaphora pupula*	0	0	1
Chlorophyta	Dolichomastigaceae	*Dolichomastix tenuilepis*	1	1	1
Chlorophyta	Mamiellaceae	*Micromonas pusilla*	1	1	1
Chlorophyta	Chlorellaceae	*Nannochloris* sp.	1	0	0
Chordata	Dasyatidae	*Pateobatis jenkinsii*	0	1	0
Chordata	Dasyatidae	*Brevitrygon walga*	0	1	0
Cnidaria	Blackfordiidae	*Blackfordia virginica*	1	0	0
Cnidaria	Plexauridae	*cf. Thesea* sp.	1	0	0
Cnidaria	Campanulariidae	*Eucheilota maculata*	1	1	0
Cnidaria	Agalmatidae	*Halistemma amphytridis*	0	1	0
Cnidaria	Oculinidae	*Madrepora oculata*	1	1	1
Cnidaria	Nausithoidae	*Nausithoe atlantica*	0	1	0
Cnidaria	Bougainvilliidae	*Nemopsis bachei*	1	1	1
Cnidaria	Campanulariidae	*Orthopyxis crenata*	1	0	0
Cnidaria	Corynidae	*Sarsia tubulosa*	1	1	1
Cnidaria	Pocilloporidae	*Stylophora pistillata*	1	0	0
Cnidaria	Zancleidae	*Zanclea gallii*	0	1	0
Mollusca	Unidentified	*Alviniconcha kojimai*	1	1	0
Mollusca	Haminoeidae	*Haminoea japonica*	0	0	1
Mollusca	Cypraeidae	*Notocypraea hartsmithi*	0	0	1
Mollusca	Philinidae	*Philine scabra*	0	0	1
Mollusca	Stenothyridae	*Stenothyra gelasinosa*	1	0	0
Ochrophyta	Acinetosporaceae	*Acinetospora* sp.	0	1	0
Ochrophyta	Agaraceae	*Agarum clathratum*	1	1	0
Ochrophyta	Chordariaceae	*Chordaria flagelliformis*	1	1	0
Ochrophyta	Chordariaceae	*Chordariaceae* sp.	1	1	0
Ochrophyta	Durvillaeaceae	*Durvillaea antarctica*	1	1	0
Ochrophyta	Durvillaeaceae	*Durvillaea potatorum*	1	0	0
Ochrophyta	Ectocarpaceae	*Ectocarpus fasciculatus*	1	0	0
Ochrophyta	Ectocarpaceae	*Ectocarpus siliculosus*	1	1	1
Ochrophyta	Fucaceae	*Fucus distichus*	0	1	0
Ochrophyta	Chordariaceae	*Hecatonema maculans*	1	1	0
Ochrophyta	Chordariaceae	*Laminariocolax aecidioides*	1	1	0
Ochrophyta	Chordariaceae	*Laminariocolax tomentosoides*	1	0	0
Ochrophyta	Chordariaceae	*Microspongium tenuissimum*	0	1	0
Ochrophyta	Chordariaceae	*Myriotrichia claviformis*	1	1	0
Ochrophyta	Notheiaceae	*Notheia anomala*	1	1	0
Ochrophyta	Dictyotaceae	*Padina durvillei*	0	0	1
Ochrophyta	Scytosiphonaceae	*Petalonia fascia*	0	1	0
Ochrophyta	Chordariaceae	*Stilophora tenella*	1	1	0
Ochrophyta	Alariaceae	*Undaria pinnatifida*	1	1	0
Porifera	Microcionidae	*Clathria cancellaria*	0	1	0
Unidentified	Saprolegniaceae	*Achlya bisexualis*	1	0	0
Unidentified	Phyllophoraceae	*Asterfilopsis* sp.	1	1	0
Unidentified	Corallinaceae	*Bossiella orbigniana*	0	1	0
Unidentified	Chrysochromulinaceae	*Chrysochromulina* sp.	1	1	0
Unidentified	Noelaerhabdaceae	*Emiliania huxleyi*	1	1	0
Unidentified	Pythiaceae	*Halophytophthora bahamensis*	1	1	0
Unidentified	Peronosporaceae	*Hyaloperonospora dentariae*	1	1	1
Unidentified	Delesseriaceae	*Martensia jejuensis*	0	1	0
Unidentified	Hapalidiaceae	*Mesophyllum sphaericum*	1	1	0
Unidentified	Unidentified	*Nothophytophthora vietnamensis*	0	1	0
Unidentified	Unidentified	*Parvamoeba rugata*	1	0	0
Unidentified	Phaeocystaceae	*Phaeocystis pouchetii*	1	0	0
Unidentified	Unidentified	*Phytophthora austrocedrae*	1	1	1
Unidentified	Unidentified	*Phytophthora cinnamomi*	1	1	1
Unidentified	Unidentified	*Phytophthora citrophthora*	0	1	0
Unidentified	Unidentified	*Phytophthora frigida*	1	0	0

Unidentified	Unidentified	*Phytophthora nemorosa*	1	1	1
Unidentified	Unidentified	*Phytophthora palmivora*	1	1	1
Unidentified	Unidentified	*Phytophthora syringae*	1	0	0
Unidentified	Unidentified	*Pseudopedinella elastica*	1	0	0
Unidentified	Bangiaceae	*Pyropia haitanensis*	0	1	0
Unidentified	Pythiaceae	*Pythium heterothallicum*	1	1	1
Unidentified	Pythiaceae	*Pythium splendens*	0	1	0
Unidentified	Pythiaceae	*Pythium ultimum*	1	0	0
Unidentified	Thraustochytriaceae	*Schizochytrium* sp.	0	1	1
Unidentified	Apusomonadidae	*Thecamonas trahens*	0	1	0
Unidentified	Triparmaceae	*Triparma laevis*	1	1	0