

Article

Seawater eDNA and Underwater Visual Surveys Reveal Genus-Level Reef-Building Coral Communities in the Wanshan Archipelago, South China Sea

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Abstract

Environmental DNA (eDNA) metabarcoding is increasingly used as a complementary tool for coral biodiversity assessment, but its performance in subtropical, estuary-influenced reef systems remains insufficiently tested. This study combined seawater eDNA metabarcoding with concurrent underwater visual surveys and historical records to assess reef-building scleractinian corals in the Wanshan Archipelago, Pearl River Estuary. In June 2022, surface and bottom seawater samples were collected from seven sites, yielding 14 eDNA samples, and dive-based photographic surveys were conducted concurrently. A universal 18S rRNA assay was used for high-throughput sequencing, and eDNA detections were compared with visual and historical survey data at the genus level. eDNA detected 13 families and 21 genera, with *Porites* and *Echinopora* as dominant genera. eDNA recovered more genera than concurrent visual surveys in all five regional comparisons, although the two methods yielded overlapping but partly distinct genus inventories. Surface-water eDNA showed a slight, site-dependent advantage in genus recovery and overlap with concurrent visual surveys, whereas correspondence with historical records varied among regions; surface and bottom samples shared most genera but also contributed complementary layer-specific detections. Community composition showed spatial clustering broadly consistent with geographic proximity. These findings indicate that seawater eDNA provides complementary genus-level information and support paired surface- and bottom-water sampling for a more complete exploratory characterization of coral diversity in complex subtropical coastal reef systems.

Keywords: molecular biomonitoring; Pearl River Estuary; underwater visual survey; coral biodiversity; subtropical coral reef communities; 18S rRNA; metabarcoding



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Key Contribution: This study integrates seawater eDNA, concurrent underwater visual surveys, and historical records to evaluate reef-building coral communities in a subtropical, estuary-influenced reef system. It shows that eDNA can provide complementary genus-level information and highlights the value of vertically stratified sampling.

1. Introduction

Coral reefs are among the most diverse marine ecosystems and are fundamentally supported by reef-building scleractinian corals. By forming complex carbonate frameworks, these corals provide feeding, shelter, nursery, and spawning habitats for reef-associated fishes and other organisms, thereby contributing to marine biodiversity, coastal protection, and the maintenance of biological resources [1–3]. However, coral reefs are undergoing rapid degradation under the combined effects of climate change and local human disturbances. Global monitoring data show that the world’s coral reefs lost approximately 14% of their coral between 2009 and 2018 [4]. More recently, the fourth global coral bleaching event, which began in 2023 and likely ended in mid-2025, became the largest event on record: from January 2023 to September 2025, bleaching-level heat stress affected approximately 84.4% of the world’s coral reef area, and mass bleaching was documented in at least 83 countries and territories [5]. These global trends highlight the increasing vulnerability of coral reef ecosystems and the urgent need for accurate baseline surveys of reef-building coral diversity to support reef assessment, conservation, and management.

Traditional coral surveys mainly rely on underwater visual methods, such as line-intercept transects, video transects, and photographic surveys [6]. These methods can directly record coral cover, colony condition, and benthic habitat structure, and they remain essential for coral reef ecological assessment. However, visual surveys also have limitations. Field observations can be affected by water clarity, light conditions, depth, topographic complexity, and diver accessibility. Taxonomic identification may also be difficult when corals are damaged, rare, cryptic, or morphologically similar. In addition, colony morphology can vary under different environmental conditions, and reliable identification of some taxa may require skeletal characters or microscopic structures [7]. Therefore, rare or inconspicuous corals may be overlooked or misidentified during visual surveys. These limitations highlight the need for complementary, efficient, and non-invasive methods for coral biodiversity assessment.

Environmental DNA (eDNA) metabarcoding provides a molecular approach for detecting organisms from DNA released into environmental samples such as water, sediment, or soil. The general workflow includes DNA extraction from environmental matrices, PCR amplification of target gene regions, high-throughput sequencing, and taxonomic assignment by comparison with reference databases [8]. Because this method does not require direct observation or destructive sampling of target organisms, it has been widely applied in aquatic biodiversity monitoring, invasive species detection, and ecological assessment [8,9]. In marine ecosystem surveys, eDNA can improve detection efficiency and provide complementary molecular evidence for taxa that are difficult to observe directly. This advantage is particularly relevant under challenging underwater conditions, such as low visibility, strong currents, complex reef topography, or limited diver accessibility, where conventional visual surveys may overlook rare, cryptic, or inconspicuous organisms. However, eDNA results can also be influenced by marker choice, primer bias, DNA degradation, hydrodynamic transport, reference database completeness, contamination control, and detection thresholds. Therefore, eDNA results should be interpreted together with traditional ecological survey data, especially when they are used for coral community assessment.

Although eDNA has been used most widely in fish and other aquatic animal monitoring, its application to reef-building corals has developed rapidly in recent years. In 2018, Shinzato et al. [10] provided one of the earliest demonstrations that seawater eDNA combined with high-throughput sequencing could be used to document coral and Symbiodiniaceae diversity, opening a new direction for non-invasive coral reef monitoring. In 2019, Nichols and Marko [11] examined Hawaiian coral reefs and found that differences in the relative abundance of dominant coral genera inferred from seawater eDNA were broadly consistent with traditional visual survey results. In 2020, Alexander et al. [12] developed a multi-assay approach using CoralITS2 and CoralITS2_acro primers and showed that the combined use of primer sets increased the detection rate of scleractinian corals by 28% compared with a single-assay strategy. Subsequently, Shinzato et al. [13] designed more specific mitochondrial markers for scleractinian corals and demonstrated that eDNA metabarcoding could reveal differences among coral communities located less than 1 km apart. In 2022, Dugal et al. [14] detected 37 genera and 40 species of scleractinian corals from 56 seawater eDNA samples collected at the Rowley Shoals in northwestern Australia and reported substantial overlap with visual survey data when a curated reference database was used. More recently, McCartin et al. [15] developed and validated nuclear 28S rRNA primers for anthozoan coral biodiversity assessment, extending the potential application of eDNA to shallow-water, mesophotic, and deep-sea coral groups. Together, these studies indicate that eDNA metabarcoding is a promising complementary approach for coral biodiversity monitoring, and that its performance continues to improve through primer development, multi-assay strategies, and curated reference databases.

Despite these advances, several issues remain unresolved in coral eDNA applications. The taxonomic resolution of eDNA results depends strongly on marker selection and the completeness of reference databases, and genus-level interpretation is often more robust than species-level inference. In addition, eDNA read abundance cannot be directly interpreted as coral cover or colony abundance without further validation. Hydrodynamic transport, DNA persistence, sample depth, seasonal conditions, and sample preservation may also affect the relationship between detected eDNA signals and local coral assemblages [14–18]. These uncertainties are particularly important in subtropical coastal reef systems influenced by estuarine processes and human activities. Therefore, more case studies are needed to evaluate how seawater eDNA represents reef-building coral communities in such complex environments, especially when eDNA results can be compared with concurrent visual surveys and historical records.

The Wanshan Archipelago, located near the Pearl River Estuary in Guangdong Province, is an important component of China's subtropical coral reef resources. Since 2005, reef-building coral communities in this region have been affected by tourism development, coastal sewage discharge, and other human disturbances [19]. Previous surveys have reported the distribution and degradation of reef-building coral communities in the Wanshan Archipelago and the waters around Miaowan Island, indicating an urgent need for updated biodiversity assessment and conservation support [19–21]. In this study, we focused on the main coral reef distribution areas of the Wanshan Archipelago and applied seawater eDNA metabarcoding together with concurrent underwater visual surveys and historical survey records. Specifically, we aimed to identify the genus-level composition of reef-building corals detected by eDNA, compare eDNA detections with visual and historical records, evaluate the performance of surface and bottom seawater samples, and assess spatial differences in coral community composition. This study provides complementary genus-level information for coral reef assessment in the Wanshan Archipelago and further evaluates the applicability of eDNA metabarcoding in subtropical, estuary-influenced coral reef environments.

2. Materials and Methods

2.1. Study Area and Sampling Design

The study was conducted in the Wanshan Archipelago near the Pearl River Estuary, Guangdong Province, southern China. Based on published records of coral distribution in this region [20–22] and the locations of the main reef-building coral communities, seven eDNA sampling sites were established in June 2022 across the Dangan-Erzhou, Beijian, Miaowan-Qianchongwei, Pingzhou, and Wailingding Island areas. These sites were selected to cover the main known coral distribution areas of the archipelago and represented settings ranging from Pearl River estuary-influenced waters to more oceanic reef environments. At each site, paired surface and bottom seawater samples were collected, yielding 14 seawater eDNA samples in total. Bottom seawater was collected approximately 50 cm above the reef flat or benthic substrate. The sample groups included Dangan Island (DG01), Erzhou Island (DG02), Beijian Island (BJD01), Miaowan Island (MW01), Qianchongwei Island (QCW01), Pingzhou Island (PZ01), and Wailingding Island (WLD02), with surface and bottom samples distinguished by sample codes. Geographic coordinates and water-quality parameters, including temperature, salinity, pH, and dissolved oxygen, are provided in Table S1. Approximate straight-line distances among the seven eDNA sampling sites were calculated from geographic coordinates using geodesic distance and ranged from 1.78 to 43.02 km (Table S2).

This sampling design was intended as an exploratory genus-level assessment of reef-building coral eDNA signals across the major coral distribution areas of the Wanshan Archipelago. Because field replicate samples were not collected within each depth layer, the results should be interpreted as an exploratory assessment rather than as a fully replicated quantitative estimate of within-site coral diversity. For comparisons with historical survey records, sites were grouped into broader geographic areas following previous regional coral survey frameworks. Miaowan Island and Qianchongwei Island were pooled as the Miaowan–Qianchongwei area, and Dangan Island and Erzhou Island were treated as the Dangan–Erzhou area where appropriate. This grouping was used for regional comparison with historical records, whereas sample-level eDNA analyses were conducted using individual surface and bottom samples.

2.2. Underwater Visual Surveys and Video-Transect Interpretation

Concurrent underwater visual surveys were conducted using an underwater video-transect protocol adapted from established line-transect and coral video-transect methods [23,24]. In addition, an additional survey site designated WLD01 was established on Wailingding Island. At each visual survey station, two 50 m transect lines were laid along the reef area. Scuba divers recorded continuous underwater video footage along each transect from 0 to 50 m. In addition to the transect videos, close-up quadrat images of representative coral colonies and benthic substrates were recorded to assist subsequent taxonomic identification.

The video transects were interpreted in the laboratory using a point-intercept approach. For each 50 m transect, the organism or substrate directly beneath the transect line was recorded at 10 cm intervals, yielding up to 500 point observations per transect and up to 1000 point observations per station. Valid point observations were assigned to live scleractinian corals, dead corals, reef rock, rubble, sand, mud, algae, soft corals, sponges, anemones, or other benthic categories. Live scleractinian corals were identified to the lowest possible taxonomic level based on external morphology, and close-up quadrat images were used to assist genus- and species-level identification where possible. The coral nomenclature of the present study follows the World List of Scleractinia (WoRMS) [25].

Total live scleractinian coral cover at each station was calculated as the number of valid point observations assigned to live scleractinian corals divided by the total number of valid interpreted points. Genus- and species-level cover values were calculated as the number of points assigned to each coral genus or species divided by the total number of valid interpreted points. For the bubble-pie maps in Figure 1, pie size represents total live scleractinian coral cover, and pie segments represent the proportional composition of dominant genera or species within the live scleractinian coral assemblage. The underlying genus- and species-level visual survey data are provided in Tables S3 and S4, respectively, in the Supplementary Materials.

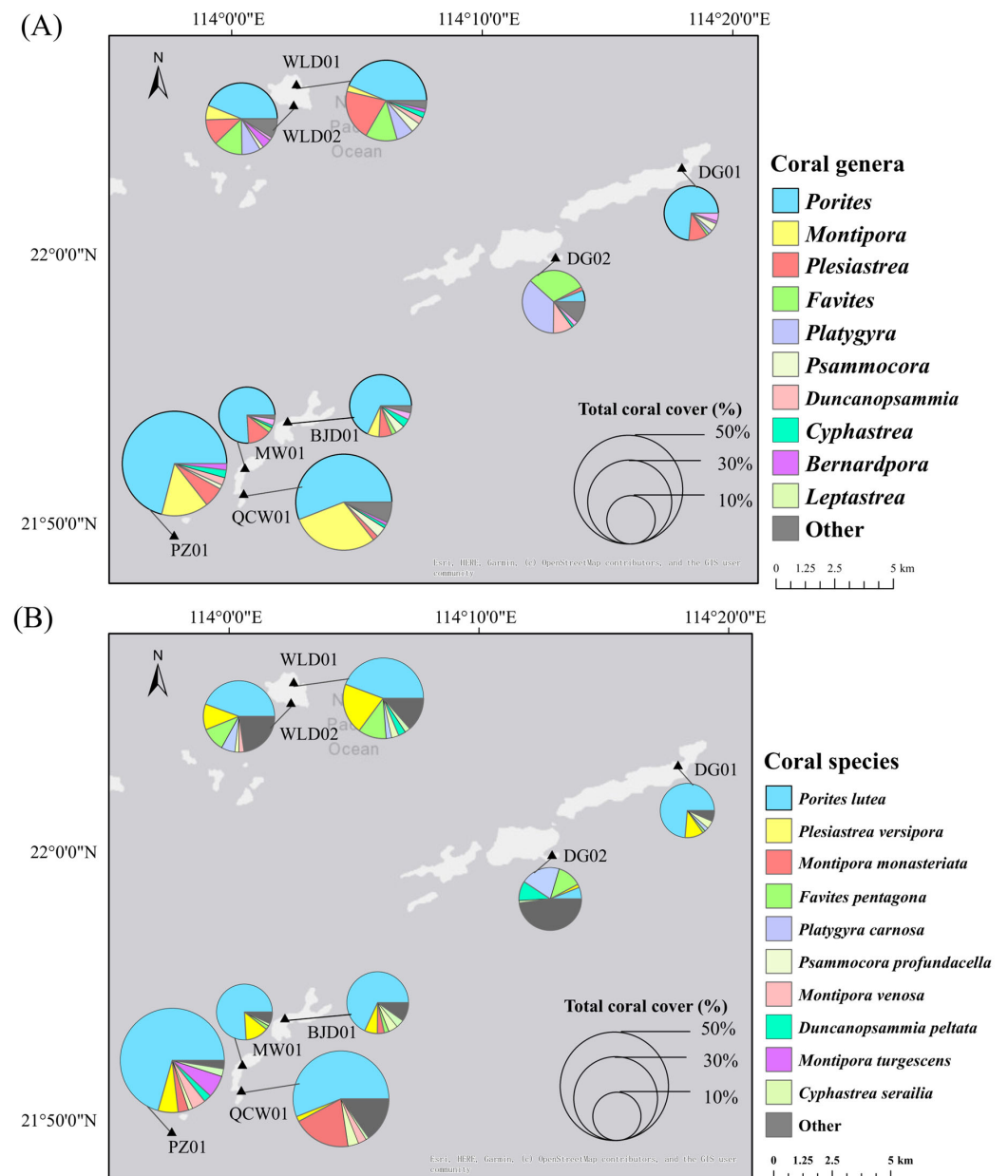


Figure 1. Spatial distribution of scleractinian coral communities recorded by underwater visual surveys in the Wanshan Archipelago: (A) genus-level composition; (B) species-level composition. Pie size represents coral cover, and pie segments represent the dominant taxa. Black triangles indicate underwater visual-survey stations. Site abbreviations: WLD01, Wailingding Island station 01; WLD02, Wailingding Island station 02; DG01, Dangan Island; DG02, Erzhou Island; BJD01, Beijian Island; MW01, Miaowan Island; QCW01, Qianchongwei Island; and PZ01, Pingzhou Island.

2.3. Seawater Collection and eDNA Extraction

At each eDNA sampling site, 2 L of surface seawater and 2 L of near-bottom seawater were collected using sterile sampling bottles. Near-bottom seawater was collected approximately 50 cm above the reef flat or benthic substrate. During sampling and filtration, personnel wore new disposable gloves, and sampling bottles and filtration equipment were handled carefully to reduce exogenous contamination. Before each sampling event, reusable sampling equipment was disinfected with bleach solution for 10 min and rinsed with site seawater before sample collection.

After collection, seawater samples were immediately stored at 4 °C and filtered within 2 h using 0.45 µm mixed cellulose ester membranes. Before each filtration, the filtration apparatus was cleaned and disinfected with 10% sodium hypochlorite solution to reduce cross-contamination among samples. After filtration, each eDNA-enriched membrane was placed in a nuclease-free sterile centrifuge tube, stored in a liquid nitrogen dewar, and transported to the laboratory under frozen conditions.

Environmental DNA was extracted from the 14 filter membrane samples using the E.Z.N.A.TM Mag-Bind Soil DNA Kit (Omega Bio-tek, Norcross, GA, USA) following the manufacturer's protocol. DNA integrity was checked using agarose gel electrophoresis, and DNA concentration was quantified using a Qubit fluorometer (Thermo Fisher Scientific, Waltham, MA, USA) before PCR amplification.

Dedicated field blanks, filtration blanks, and extraction blanks were not included during the 2022 field survey and extraction workflow. Therefore, potential low-level contamination during field sampling, filtration, or DNA extraction could not be directly evaluated using negative controls. This limitation is acknowledged in the Discussion, and eDNA detections were interpreted conservatively at the genus level.

2.4. PCR Amplification and High-Throughput Sequencing

The universal eukaryotic 18S rRNA primers 18S_uni_1F (5'-GCCAGTAGTCATATGCT TGTCT-3') and 18S_uni_400R (5'-GCCTGCTGCCTTCCTT-3') were used for PCR amplification [26]. PCR amplification and library preparation were performed by Sangon Biotech Co., Ltd. (Shanghai, China), following its standard 18S amplicon sequencing workflow.

Before amplification, genomic DNA was quantified using the Qubit dsDNA HS Assay Kit (Thermo Fisher Scientific, Waltham, MA, USA). The first-round PCR was performed in a 30 µL reaction system containing 15 µL of 2× Hieff[®] Robust PCR Master Mix (Yeasen Biotechnology (Shanghai) Co., Ltd., Shanghai, China), 1 µL of Bar-PCR primer F, 1 µL of primer R, 10–20 ng of template DNA, and 9–12 µL of nuclease-free water. The first-round PCR cycling program was as follows: initial denaturation at 94 °C for 3 min; five cycles of 94 °C for 30 s, 45 °C for 20 s, and 65 °C for 30 s; 20 cycles of 94 °C for 20 s, 55 °C for 20 s, and 72 °C for 30 s; and a final extension at 72 °C for 5 min, followed by a hold at 10 °C.

A second-round PCR was then performed to introduce Illumina bridge PCR-compatible primers and sample indices. Each 30 µL reaction contained 15 µL of 2× Hieff[®] Robust PCR Master Mix, 1 µL of primer F, 1 µL of Index-PCR primer R, 20–30 ng of first-round PCR product, and 9–12 µL of nuclease-free water. The second-round PCR cycling program was as follows: initial denaturation at 95 °C for 3 min; five cycles of 94 °C for 20 s, 55 °C for 20 s, and 72 °C for 30 s; and a final extension at 72 °C for 5 min, followed by a hold at 10 °C. The two-step PCR protocol and cycle numbers followed the service provider's standard 18S amplicon library preparation workflow.

PCR products and sequencing libraries were checked using 2% agarose gel electrophoresis. Library concentrations were measured using a Qubit fluorometer, and qualified libraries were sequenced using paired-end 300 bp sequencing on the Illumina MiSeq platform (Illumina, Inc., San Diego, CA, USA).

PCR technical replicates, no-template PCR controls, and positive controls were not included in this PCR workflow. Therefore, PCR-stage stochasticity, potential PCR contamination, or amplification failure could not be directly evaluated using replicate or control reactions. This limitation is acknowledged in the Discussion, and low-abundance or eDNA-only detections were interpreted conservatively at the genus level.

2.5. Bioinformatic Processing and Taxonomic Assignment

Raw paired-end FASTQ reads were processed by Sangon Biotech Co., Ltd. using its 18S amplicon sequencing workflow. Sequencing adapters were removed using cutadapt v1.18 [27], and paired-end reads were merged according to the overlap between forward and reverse reads using PEAR v0.9.8 [28]. Samples were demultiplexed according to barcode and primer sequences, and sequence orientation was corrected. Quality filtering was performed using PRINSEQ v0.20.4 [29]. Bases with quality scores below 20 were trimmed from the 3' end of reads, and a 10 bp sliding window was used to truncate reads when the mean quality score within the window was below 20. Reads containing ambiguous bases, short sequences, and low-complexity sequences were removed.

Quality-filtered sequences were dereplicated, and singleton sequences were removed before operational taxonomic unit (OTU) clustering. OTUs were clustered at 97% sequence similarity using USEARCH v11.0.667 [30], with chimeric sequences removed during OTU construction. Quality-filtered sequences were subsequently mapped back to representative OTU sequences at 97% similarity to generate the OTU abundance table.

Representative OTU sequences were taxonomically assigned by NCBI BLAST+ v2.10.0 against the SILVA 18S rRNA database [31,32]. Assignments with sequence similarity > 90% and query coverage > 90% were retained; sequences not meeting these criteria were classified as unclassified. Coral-related assignments were extracted from the complete taxonomic table, after which non-scleractinian, non-reef-building coral, unclassified, and other non-target records were removed.

Species-level assignments were examined; however, the amplified 18S region did not provide sufficient sequence differentiation to consistently distinguish several closely related scleractinian coral species. Taxonomic interpretation was therefore conservatively restricted to the genus level to avoid uncertain species-level assignments. For presence-absence comparisons, a genus was operationally considered detected when its filtered sequence count was greater than zero. Because field, extraction, and PCR negative controls were unavailable, low-read detections were interpreted conservatively and were not treated as independently validated occurrence records. Detailed per-sample sequence-processing statistics and genus-level eDNA relative-abundance and presence-absence data are provided in Tables S5, S6, and S7, respectively. Representative coral-associated OTU sequences are provided in Supplementary_Representative_OTU_Sequences.fasta, with the corresponding OTU index listed in Table S8.

2.6. Statistical Analyses and Comparative Assessment

Alpha diversity indices, including observed richness (Sobs), Chao1, ACE, Shannon, and Gini-Simpson indices, were calculated using R v4.1.3 [33]. A Bray-Curtis dissimilarity matrix was generated based on genus-level sequence abundance, and principal coordinate analysis (PCoA) was used to visualize differences in community composition among samples. Community analyses and visualizations were conducted using R and relevant community ecology packages.

Historical records from surveys conducted in 2005, 2008, and 2016 were taxonomically standardized according to currently accepted nomenclature and pooled within each region as the union of all recorded genera [20–22]. For each region, surface- and bottom-water

overlap rates were calculated separately as the number of genera shared between the eDNA and historical inventories divided by the corresponding historical genus richness. For comparisons with concurrent visual surveys, the overlap rate for each water layer was calculated as the number of genera shared between the eDNA and visual-survey inventories divided by the number of genera recorded by the visual survey. Because each site–depth combination was represented by a single field sample, all layer-specific comparisons were descriptive, and no significance tests were performed.

3. Results

3.1. Sequencing Output and Quality Control

A total of 969,538 sequences were obtained from the 14 seawater eDNA samples. After quality filtering, 963,834 clean sequences were retained, corresponding to an overall retention rate of 99.41%. The number of clean sequences per sample ranged from 42,398 to 81,510, and the mean sequence length ranged from 374.81 to 379.41 bp. Detailed per-sample sequencing statistics are provided in Table S5.

3.2. Visual Survey Patterns and Dominant Genera Detected by eDNA

Underwater video-transect surveys revealed clear spatial heterogeneity in coral cover and community composition among the eight stations (Figure 1). Mean total coral cover ranged from 12.5% at DG01 to 46.3% at PZ01. *Porites*, represented mainly by *Porites lutea*, dominated seven stations, whereas DG02 showed a distinct assemblage, with *Platygyra* and *Platygyra carnosa* as the dominant named genus and species, respectively, together with a relatively large contribution from other taxa. Overall, the surveyed reefs were broadly *Porites*-dominated but exhibited pronounced local compositional differences.

Across the 14 seawater eDNA samples, the dominant genera were *Porites* and *Echinopora* (Figure 2a). *Porites* accounted for approximately 49% of the relative abundance of the top genera, while *Echinopora* accounted for approximately 37%. Other genera, including *Leptoseris*, *Pavona*, *Pachyseris*, *Psammocora*, *Oulastrea*, *Pseudosiderastrea*, *Goniastrea*, and *Favites*, contributed smaller proportions. Site-level composition showed that *Echinopora* was particularly dominant in the Wailingding Island area, whereas *Porites* was generally dominant in most other surveyed areas (Figure 2b).

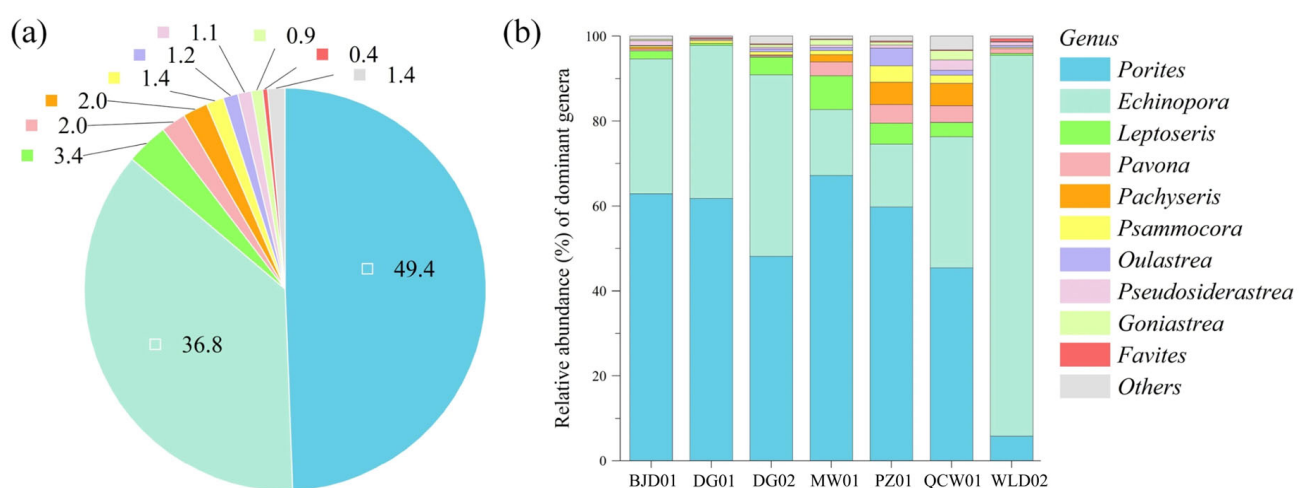


Figure 2. Dominant scleractinian coral genera detected by seawater eDNA: (a) overall relative abundance of the top genera across all samples; (b) relative abundance of dominant genera in each sampling area. Abbreviations: BJD, Beijian Island; DG01, Dangan Island; DG02, Erzhou Island; MW, Miaowan Island; PZ, Pingzhou Island; QCW, Qianchongwei Island; WLD, Wailingding Island.

3.3. Comparison of eDNA Detections with Visual and Historical Survey Records

To resolve taxonomic inconsistencies among historical surveys and facilitate comparison with the eDNA dataset, historical species names and family assignments were standardized according to the current World List of Scleractinia before aggregation to the genus level. The standardized historical inventories and taxonomic standardization records are provided in Tables S9 and S10, respectively. Seawater eDNA metabarcoding detected 13 families and 21 genera of reef-building scleractinian corals in the Wanshan Archipelago (Table 1). After taxonomic standardization and application of the reef-building target-scope filter, the compiled historical records comprised 13 families and 25 genera (Table 2). Six eDNA-detected genera—*Coscinaraea*, *Echinopora*, *Leptoseris*, *Micromussa*, *Pachyseris*, and *Stylocoeniella*—were absent from the standardized historical inventory. Conversely, ten historically recorded genera—*Astrea*, *Bernardpora*, *Coelastrea*, *Dipsastraea*, *Duncanopsammia*, *Galaxea*, *Hydnophora*, *Montipora*, *Plesiastrea*, and *Turbinaria*—were not detected in the present eDNA dataset. Thus, the eDNA detections and standardized historical inventory were overlapping but not identical and provided complementary genus-level information on regional coral diversity.

Table 1. Genus-level distribution of reef-building scleractinian corals detected by seawater eDNA in the Wanshan Archipelago. A plus sign indicates detection; blank cells indicate no detection.

Family	Genus	MW-QCW Surface	MW-QCW Bottom	WLD Surface	WLD Bottom	BJD Surface	BJD Bottom	PZ Surface	PZ Bottom	DG01 Surface	DG01 Bottom	DG02 Surface	DG02 Bottom
Acroporidae	<i>Acropora</i>	+	+	+		+	+					+	+
	<i>Cyphastrea</i>	+	+	+	+	+	+	+	+	+	+	+	+
	<i>Echinopora</i>	+	+	+	+	+	+	+	+	+	+	+	+
Merulinidae	<i>Goniastrea</i>	+	+	+	+	+	+	+	+	+	+	+	+
	<i>Favites</i>	+	+	+	+	+	+	+	+	+	+	+	+
	<i>Platygyra</i>				+		+		+	+		+	
Leptastreaeidae	<i>Leptastrea</i>	+	+	+	+	+	+	+	+	+	+	+	+
	<i>Porites</i>	+	+	+	+	+	+	+	+	+	+	+	+
Poritidae	<i>Goniopora</i>	+	+	+	+	+	+	+		+	+	+	+
	<i>Pseudosiderastrea</i>	+	+	+	+	+	+	+	+	+	+	+	+
Psammocoridae	<i>Psammocora</i>	+	+	+	+	+	+	+	+	+	+	+	+
Astrocoeniidae	<i>Stylocoeniella</i>	+	+	+	+	+		+	+	+	+	+	+
Pocilloporidae	<i>Pocillopora</i>	+	+	+	+	+	+	+	+	+	+	+	+
	<i>Acanthastrea</i>			+								+	+
Lobophylliidae	<i>Micromussa</i>	+	+	+	+	+	+	+	+	+	+	+	+
	<i>Echinophyllia</i>	+	+	+	+	+	+	+	+	+	+	+	+
Coscinaraeidae	<i>Coscinaraea</i>	+	+		+			+	+			+	+
Pachyseridae	<i>Pachyseris</i>	+	+	+	+	+	+	+	+	+	+	+	+
Agariciidae	<i>Leptoseris</i>	+	+	+	+	+	+	+	+	+	+	+	+
	<i>Pavona</i>	+	+	+	+	+	+	+	+	+	+	+	+
Oulastreidae	<i>Oulastrea</i>	+	+	+	+	+	+	+	+	+	+	+	+
Total	Total	19	19	19	19	19	18	18	18	18	17	21	20

At the regional scale, eDNA detected 19 genera in the Miaowan–Qianchongwei area, 21 genera at Wailingding Island, 20 genera at Beijian Island, 19 genera at Pingzhou Island, and 21 genera in the Dangan–Erzhou area. eDNA recovered more genera than the concurrent visual surveys in all five regional comparisons, although the two methods yielded overlapping but non-identical inventories.

Table 2. Historical records of reef-building scleractinian coral genera in the Wanshan Archipelago and adjacent waters compiled from previous surveys. A plus sign indicates a recorded genus; blank cells indicate no record in the corresponding survey.

Family	Genus	MW-QCW 2005 [22]	MW-QCW 2008 [20]	MW-QCW 2016 [21]	WLD 2008 [20]	BJD 2005 [22]	BJD 2008 [20]	DG 2008 [20]	PZ 2005 [22]	PZ 2008 [20]
Acroporidae	<i>Acropora</i>	+		+	+	+	+		+	
	<i>Montipora</i>	+	+	+	+	+	+	+		+
Merulinidae	<i>Astrea</i>			+	+					
	<i>Coelastrea</i>			+						
	<i>Cyphastrea</i>	+	+	+	+	+	+	+	+	+
	<i>Dipsastraea</i>	+		+	+	+		+	+	
	<i>Favites</i>	+	+	+	+	+	+	+		+
	<i>Goniastrea</i>		+		+		+	+		+
	<i>Hydnophora</i>			+	+		+			+
	<i>Platygyra</i>	+	+	+		+		+	+	+
	<i>Bernardopora</i>		+		+					+
Poritidae	<i>Goniopora</i>	+		+	+					+
	<i>Porites</i>	+	+	+	+	+	+	+	+	+
Lobophylliidae	<i>Acanthastrea</i>			+						
	<i>Echinophyllia</i>	+		+		+			+	
Leptastreidae	<i>Leptastrea</i>		+	+	+			+		
Oulastreidae	<i>Oulastrea</i>		+	+			+			+
Plesiastreidae	<i>Plesiastrea</i>		+	+	+			+		
Dendrophylliidae	<i>Duncanopsammia</i>	+	+	+		+		+	+	+
	<i>Turbinaria</i>	+		+		+	+			
Psammocoridae	<i>Psammocora</i>	+	+	+	+	+		+		+
Agariciidae	<i>Pavona</i>	+	+	+		+		+	+	
Rhizangiidae	<i>Pseudosiderastrea</i>				+					
Pocilloporidae	<i>Pocillopora</i>			+			+			
Euphylliidae	<i>Galaxea</i>	+	+	+		+				
Total	Total	14	14	22	15	13	10	12	8	12

3.4. Layer-Specific Comparisons with Concurrent Visual and Historical Surveys

At the seven matched stations, surface-water overlap with concurrent visual surveys was higher at DG01 (71.4% vs. 57.1%) and DG02 (54.5% vs. 45.5%) and identical between layers at the other five stations, where overlap rates ranged from 50.0% to 83.3% (Table S11). At both DG stations, the difference was attributable to *Platygyra*, which was recorded visually and detected in surface water but not in bottom water. This genus had low visual cover at DG01 but was visually dominant at DG02. After taxonomic standardization and pooling of historical records within regions, surface-water overlap rates were 50.0%, 60.0%, 58.8%, 56.3%, and 66.7% for the Miaowan–Qianchongwei, Wailingding Island, Beijian Island, Pingzhou Island, and Dangan–Erzhou areas, compared with 50.0%, 53.3%, 64.7%, 56.3%, and 58.3% for bottom water, respectively (Table 3, detailed calculations are provided in Table S12). Surface-water overlap was higher in two regions, bottom-water overlap was higher in one, and the two layers were equal in the remaining two regions; these differences were driven mainly by layer-specific detections of *Acropora* or *Platygyra*.

Table 3. Genus-level overlap between eDNA detections and historical survey records in different regions of the Wanshan Archipelago. A plus sign indicates overlap between eDNA and historical records; blank cells indicate no overlap.

Genus	MW-QCW Surface	MW-QCW Bottom	WLD Surface	WLD Bottom	BJD Surface	BJD Bottom	PZ Surface	PZ Bottom	DG Surface	DG Bottom
<i>Acropora</i>	+	+	+		+	+				
<i>Cyphastrea</i>	+	+	+	+	+	+	+	+	+	+
<i>Goniastrea</i>	+	+	+	+	+	+	+	+	+	+
<i>Leptastrea</i>	+	+	+	+					+	+
<i>Favites</i>	+	+	+	+	+	+	+	+	+	+
<i>Platygyra</i>						+		+	+	
<i>Porites</i>	+	+	+	+	+	+	+	+	+	+
<i>Goniopora</i>	+	+	+	+			+			
<i>Pseudosiderastrea</i>			+	+						
<i>Psammocora</i>	+	+	+	+	+	+	+	+	+	+
<i>Pocillopora</i>	+	+			+	+				
<i>Echinophyllia</i>	+	+			+	+	+	+		
<i>Pavona</i>	+	+			+	+	+	+	+	+
<i>Oulastrea</i>	+	+			+	+	+	+		
Total	12	12	9	8	10	11	9	9	8	7

Paired surface- and bottom-water samples shared 17–20 genera per station, and surface water recovered 89.5–100% of the genera detected in bottom water (Table S11). Surface samples contained more genera at four of the seven stations, bottom samples contained more genera at one station, and equal numbers were detected at two stations. Most layer-specific detections represented low-read signals, whereas the dominant eDNA genera *Porites* and *Echinopora* were consistently detected in both layers.

Overall, the two layers were highly similar, but surface-water eDNA showed a slight, site-dependent advantage in genus recovery and survey correspondence, while both layers contributed complementary detections.

3.5. Diversity and Spatial Differentiation of eDNA-Detected Coral Communities

In situ measurements showed a pronounced salinity gradient across the survey area (Table S1). WLD02 had one of the lowest surface salinities, with 17.67 PSU in surface water and 23.90 PSU in near-bottom water, indicating marked vertical stratification. In contrast, the more oceanic Dangan stations showed higher and more vertically uniform salinities. Temperature and pH varied less strongly among sites and water layers, whereas dissolved oxygen showed some vertical variation.

A Venn diagram based on the 14 eDNA samples showed that 14 reef-building scleractinian coral genera were shared across all samples, namely *Echinopora*, *Cyphastrea*, *Oulastrea*, *Favites*, *Goniastrea*, *Leptastrea*, *Leptoseris*, *Micromussa*, *Pachyseris*, *Pavona*, *Pocillopora*, *Porites*, *Psammocora*, and *Pseudosiderastrea* (Figure 3a). PCoA based on Bray–Curtis dissimilarity showed that samples from Beijian Island and the Dangan Islands were relatively similar in genus-level coral community composition, while samples from Qianchongwei, Miaowan Island, and Pingzhou Island also showed compositional proximity. In contrast, the Wailingding Island samples were separated from most other samples (Figure 3b). The Wailingding samples also contained a relatively high proportion of reads assigned to *Echinopora*, which contributed to their distinctive genus-level eDNA profile (Figure 2b).

Alpha diversity indices were calculated for each sampling site and visualized as box plots, as shown in Figure 3c–e. The Chao1 and ACE indices were generally higher than the observed richness (Sobs) across the samples, suggesting that some rare or low-abundance coral taxa may not have been fully captured by the sequencing effort. In the bottom water sample from Qianchongwei, both the Shannon and Gini–Simpson indices were relatively high, whereas the Chao1 and ACE indices were low. This pattern suggests that this sample contained a relatively limited number of detected coral taxa, but that their sequence abundances were more evenly distributed.

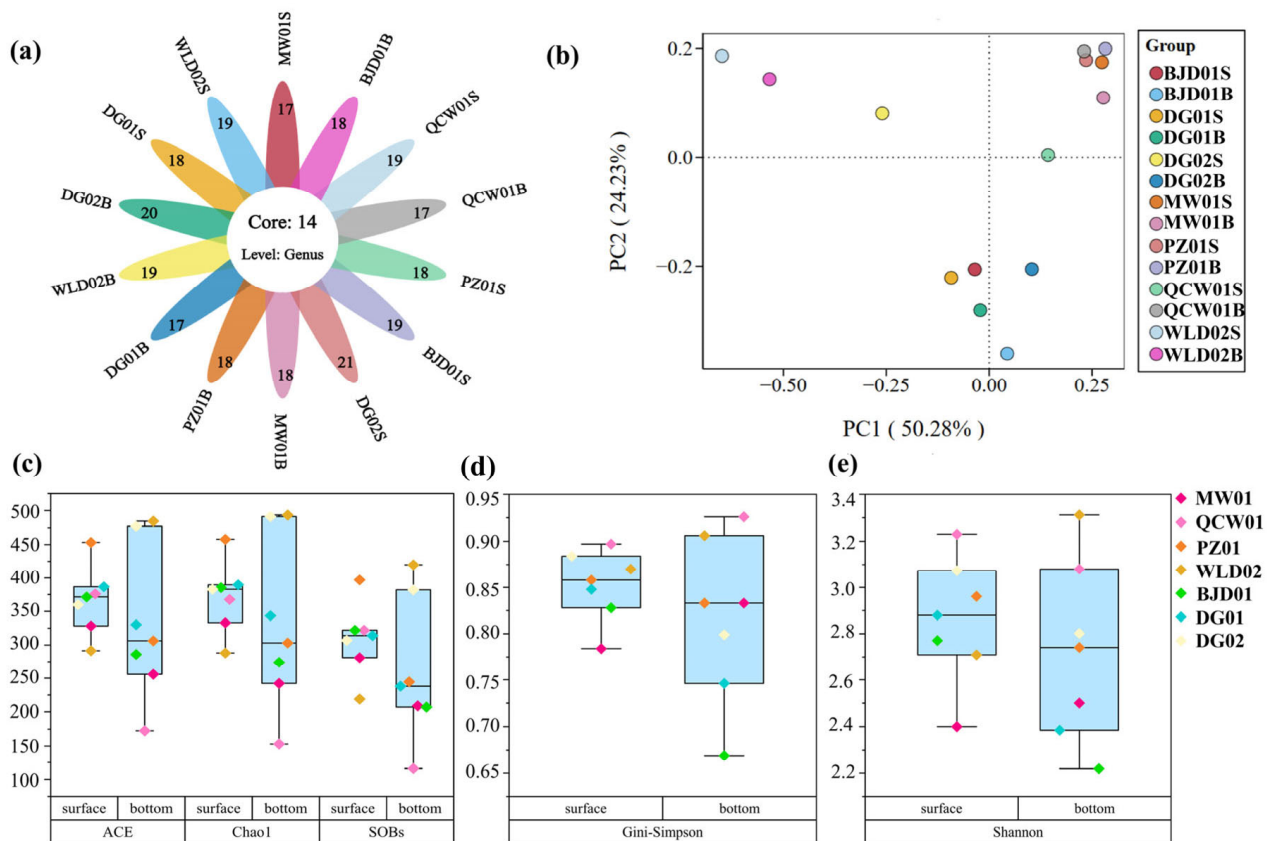


Figure 3. Diversity and community structure of scleractinian corals detected by eDNA: (a) Venn diagram showing shared and unique genera among samples; (b) PCoA based on Bray–Curtis dissimilarity; (c) ACE, Chao1, and observed richness (Sobs) indices; (d) Gini–Simpson index; (e) Shannon index. In panels (c–e), boxes represent the interquartile range, horizontal lines indicate the median, whiskers indicate the data range defined by the plotting method, and colored points represent individual samples. Site abbreviations: BJD, Beijian Island; DG01, Dangan Island; DG02, Erzhou Island; MW, Miaowan Island; PZ, Pingzhou Island; QCW, Qianchongwei Island; and WLD, Wailingding Island. The suffixes B and D denote surface- and bottom-water samples, respectively. BJD02D and MW02D are the original sequencing identifiers for the bottom-water samples collected at stations BJD01 and MW01, respectively.

The Dangan Islands and Pingzhou Island showed relatively high values across multiple alpha diversity indices, indicating comparatively high detected richness and evenness of reef-building coral genera in these areas. These results suggest that the coral communities in these waters may retain relatively complex and balanced genus-level structures. In contrast, Beijian Island exhibited relatively low Shannon and Gini–Simpson indices but relatively high Chao1 and ACE indices. This pattern indicates that although more taxa may have been detected or estimated in this area, their relative abundances were uneven and dominated by a limited number of genera.

4. Discussion

4.1. eDNA Provides Complementary Genus-Level Information for Coral Surveys

This exploratory study demonstrates that seawater eDNA metabarcoding can provide complementary genus-level information on reef-building scleractinian corals in the Wanshan Archipelago. The filtered coral-focused dataset contained 13 families and 21 genera and showed substantial, but incomplete, overlap with concurrent visual surveys and compiled historical records. This pattern is consistent with previous coral eDNA studies in

which molecular and visual methods recovered overlapping but non-identical taxonomic inventories [11,14,16].

The broader genus inventories recovered by eDNA across the surveyed regions may reflect differences in the spatial scales and detection processes represented by the two survey approaches. Visual transects directly document colonies within a restricted surveyed area, whereas seawater eDNA may integrate biological signals from nearby reef patches. Consequently, the two approaches recovered overlapping but non-identical inventories. The present eDNA dataset should therefore be viewed as complementary rather than as a substitute.

4.2. Implications of Surface and Bottom Water Sampling

The three comparisons indicated a slight but site-dependent advantage of surface-water eDNA. Surface samples more frequently contained a greater number of genera and showed higher overlap with concurrent visual surveys at DG01 and DG02, whereas correspondence with historical inventories varied among regions. The differences between water layers were generally small and were often attributable to a single layer-specific genus.

Most layer-specific detections had low read counts, while the dominant eDNA genera, *Porites* and *Echinopora*, were consistently recovered from both layers. *Platygyra* at DG02 was a notable exception: it was dominant in the visual survey but occurred as a low relative eDNA signal and was detected only in surface water. This discrepancy suggests that eDNA read abundance and layer-specific occurrence do not directly reflect local coral cover. Layer-specific differences may instead arise from patchy colony distributions, variation in sample proximity to coral colonies, water-column transport and mixing, particle settling and resuspension, or stochastic detection of low-concentration DNA. Because these processes were not directly measured, they should be regarded as plausible explanations rather than demonstrated mechanisms.

Overall, surface-water sampling captured most genus-level information detected in bottom water, but bottom-water samples still contributed complementary detections. Paired surface- and bottom-water sampling therefore remains preferable when the objective is to maximize genus-level inventory completeness.

4.3. Spatial Heterogeneity of Coral Communities in the Wanshan Archipelago

The distinctive coral eDNA profile at Wailingding Island may be associated with its stronger exposure to the Pearl River plume. WLD02 had one of the lowest surface salinities recorded during the survey and showed pronounced vertical stratification, with salinities of 17.67 PSU in surface water and 23.90 PSU in near-bottom water. Such estuarine conditions may influence coral eDNA through dilution, horizontal transport, vertical exchange, particle settling, local retention, and resuspension. In contrast, the more oceanic Dangan stations had higher and more vertically uniform salinities, indicating different hydrodynamic conditions for eDNA transport and persistence.

A notable feature of the Wailingding samples was the relatively high proportion of reads assigned to *Echinopora*, although this genus was not recorded in the concurrent video-transect surveys. This discrepancy may reflect colonies occurring outside the surveyed transects or taxa overlooked during visual identification. Alternatively, estuarine circulation and water-column stratification may have transported or retained *Echinopora*-derived DNA near the sampling site. Marker bias and taxonomic assignment uncertainty may also have contributed to the observed read proportion. Thus, the *Echinopora* signal may reflect a combination of local coral occurrence, hydrodynamic transport, and methodological effects rather than a direct indication of local coral abundance.

4.4. Limitations and Future Directions

The present study was designed as an exploratory, archipelago-scale assessment of reef-building corals at the genus level. The selected 18S assay provided broad eukaryotic coverage and has previously been used to recover scleractinian coral signals from seawater eDNA. In the present dataset, however, several closely related coral species could not be distinguished consistently; genus-level reporting was therefore adopted to maintain taxonomic reliability. The established 97% OTU workflow was applied consistently throughout the taxonomic and community analyses and was suitable for identifying broad genus-level patterns, although it was not intended to resolve fine-scale sequence variants or biological species.

The sampling design covered the principal known coral distribution areas and enabled paired comparisons between surface and near-bottom waters. However, each site–depth combination was represented by one field sample, preventing quantification of local-scale eDNA variability and sampling uncertainty. Dedicated stage-specific controls and PCR technical replicates were not included in the original workflow, limiting the independent evaluation of contamination and PCR stochasticity. Accordingly, coral-assigned read proportions should be interpreted as relative molecular signals rather than direct measures of coral cover or abundance, and eDNA-only detections should be interpreted cautiously. Comparisons with historical surveys should also consider differences in spatial coverage, sampling effort, methodology, and taxonomic practice.

Future studies should retain broad spatial coverage while incorporating replicated water samples, stage-specific controls, and PCR replicates. Coral-targeted multi-marker assays, ASV-based processing, curated regional reference databases, replicated visual surveys, and environmental and hydrodynamic measurements would further improve taxonomic resolution and ecological interpretation.

5. Conclusions

This exploratory study combined seawater eDNA metabarcoding, concurrent underwater visual surveys, and historical records to assess genus-level patterns of reef-building scleractinian corals in the Wanshan Archipelago, Pearl River Estuary. At the genus level, eDNA detected 13 families and 21 genera, with *Porites* and *Echinopora* as dominant genera. Seawater eDNA recovered broader genus-level information than concurrent visual surveys and provided information complementary to visual and historical survey records. Surface- and bottom-water samples showed high overlap, with surface water displaying a slight, site-dependent advantage in genus recovery and survey correspondence, while both layers provided complementary detections. These findings support integrating detections from both water layers within combined eDNA–visual survey frameworks for exploratory coral biodiversity assessment in subtropical, estuary-influenced reef systems. The results provide updated genus-level baseline information for coral reef conservation and monitoring in the Wanshan Archipelago. Replicated sampling, coral-specific multi-marker assays, and improved regional reference databases will be required for more reliable species-level assessment.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/fishes11080454/s1>, Supplementary_Tables.xlsx, containing sample metadata (Table_S1_Metadata), in situ water-quality parameters (Table_S1_Metadata), pairwise distances among eDNA sampling stations (Table_S2_Distances), per-sample sequencing statistics (Table_S3_VisualGenus), corrected genus- and species-level visual-survey coral-cover data, Table_S4_VisualSpecies), Table_S5_SeqStats, genus-level eDNA detection and abundance tables (Table_S6_eDNA_RA, Table_S7_eDNA_PA), source data for Figures 2 and 3 (Figure2a_Source,

Figure2b_Source, Figure3a_Source, Figure3b_BrayCurtis, Figure3b_PCoA, Figure3c-e_Alpha), standardized historical species and genus records (Table_S8_OTU_Index), pooled regional historical inventories (Table_S8_OTU_Index), eDNA–historical survey overlap calculations (Table_S9_Historical), and taxonomic standardization records (Table_S10_Taxonomy); the representative OTU index (Table_S11_LayerComparisons); paired surface- and bottom-water comparisons and overlap with concurrent visual surveys (Table_S12_Overlap), Supplementary_Representative_OTU_Sequences.fasta contains representative sequences of coral-associated OTUs.

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Institutional Review Board Statement: Ethical review and approval were not required for this study because the study involved only underwater visual surveys and environmental water sampling for eDNA analysis. No live fish or other animals were captured, handled, restrained, experimentally manipulated, or sacrificed during the study. Therefore, no ethics committee name, approval date, or approval code is applicable.

Data Availability Statement: The raw sequencing reads generated in this study have been deposited in the NCBI Sequence Read Archive (SRA) under BioProject accession number PRJNA1505780. The remaining data supporting the findings of this study are included in the article and its Supplementary Materials.

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Abbreviations

Abbreviation	Definition
eDNA	Environmental DNA
NGS	Next-generation sequencing
OTU	Operational taxonomic unit
PCR	Polymerase chain reaction
PCoA	Principal coordinate analysis
Sobs	Observed richness

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