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ORIGINAL ARTICLE

Fish and coral assemblages of a highly isolated oceanic island: The first eDNA survey of the Ogasawara Islands

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Abstract

The Ogasawara Islands are a highly isolated oceanic archipelago in the Pacific Ocean that possess unique faunal and floral biodiversity with a high level of endemism. As historically more focus has been put on the terrestrial realm in examining diversification and evolutionary processes on oceanic islands, publicly accessible and spatially resolved data of marine reef ecosystems remain scarce. To address this issue, we conducted the first environmental DNA (eDNA) metabarcoding surveys of the actinopterygian (ray-finned) and elasmobranch fishes and of Scleractinia coral assemblages in the waters of the Ogasawara Islands. We detected a total of 124 unique taxa of fish and 38 unique taxa of scleractinian corals. Overall, our eDNA results confirmed that the Ogasawara Islands host a rich variety of coral and fish fauna and underline the strength of eDNA surveys in rapidly obtaining targeted multi-taxa data using seawater samples, requiring comparatively little effort and a lack of requirement for in situ taxonomic expertise. We anticipate that continued biomonitoring using eDNA with high sampling effort will add to and complement the body of knowledge regarding species distributions, invasive species, and biodiversity hotspots within oceanic archipelagos.

KEYWORDS
coral reefs, environmental DNA, fish, marine biodiversity, metabarcoding, oceanic islands

1 | INTRODUCTION

The Ogasawara Islands, also known as the Bonin Islands, are an archipelago in the north-western Pacific Ocean located approximately 1000 km south of Tokyo and mainland Japan (Figure 1). There are more than 30 islands, clustered in three groups, which form a highly isolated oceanic island ecosystem away from the path of any major current, formed by volcanic activities (Neall & Trewick, 2008) along the Izu–Bonin–Mariana arc (Figure 1). The isolated islands host unique and rich assemblages of flora and fauna with high levels of endemism (Chiba, 1999; Ito, 1998; Nakano et al., 2009; Sugai et al., 2019), and their unique environments act as natural laboratories to study evolutionary processes (Darwin, 1859). The Ogasawara Islands were awarded UNESCO Natural World Heritage status and have been colloquially dubbed the "Galápagos of the Orient" (World Heritage Convention, 2018). Ecotourism is an integral part of the economic activities and marketing of the islands, (Cunningham, 2005), supporting the very small human population of approximately 2600 on Chichi and Haha Islands. Residents abide by local rules and national and municipal laws in efforts to conserve and not negatively impact the island's natural environment (Ogasawara Rulebook, 2015).

The nearshore fish assemblages of the Ogasawara Islands have been generally well-described over the years (Randall et al., 1997), but spatiotemporally resolved biomonitoring data across the islands are extremely limited. The most recent multi-site report on fish included data from two separate surveys of Ani-jima in 2012 and of Chichi-jima in 2013. These surveys resulted in confirmation of a total of 321 fish species belonging to 15 orders, 59 families, and 169 genera (Sasaki et al., 2014). The authors noted Futami Bay as an important candidate area for conservation because of concentrated occurrences of fishes inhabiting the inner bay and underlined the importance of also considering inner bay fishes in addition to the reef-associated and pelagic fishes in assessments of biodiversity (Sasaki et al., 2014). The levels of endemism of fishes and marine taxa in general are thought to be lower than those of terrestrial groups due to the nature comparatively fewer barriers in marine connectivity (Palumbi, 1994). Among fish, *Scarus obishime*, *Genicanthus takeuchii*, *Rhinogobius ogasawaraensis*, and a *Pempheris* species have been reported as endemic to the islands (Koeda & Motomura, 2018; Randall et al., 1997; Randall & Earle, 1993; Suzuki et al., 2011), and there are more marine species endemic to the wider region including the Izu, Daito, and Mariana Islands (Koeda & Motomura, 2018; Randall & Yasuda, 1979). The fish assemblage consists of temperate species from the Pacific coast of mainland Japan and central Pacific. Many of these species are currently found within Japan only in the Ogasawara Islands (Kuriiwa et al., 2014; Murase et al., 2009; Senou, 1997; Senou et al., 1995; Tatsuta et al., 2014). Comparatively speaking, the fish assemblage is more similar to the Izu and Pacific Coast of southern Japan than to the Ryukyu Islands (Matsuura & Senou, 2012; Senou et al., 2006). The fish data include some elasmobranch species, but information on these taxa is very scarce. Randall et al. (1997) listed 26 species from six shark families and three ray families, and Sasaki et al. reported two shark and two ray species from four families in total (Sasaki et al., 2014). One notable species is the *Carcharias taurus* (Odontaspididae), the sand tiger shark, which is critically endangered (Rigby et al., 2020) and found only in the Ogasawara Islands within Japan and is a touristic diving attraction. A ray species of note is *Neotrygon kuhlii* (Dasyatidae), the bluespotted stingray, which has been reported as being common in the archipelago with a broad range (Randall et al., 1997), being recorded in multiple study sites across Ani-jima and Chichi-jima (Sasaki et al., 2014).

The scleractinian coral assemblages of the Ogasawara Islands have been considered important with regards to their isolated location, diversity, and richness, resulting in unique relative species abundances and distinct genetic populations. The Ogasawara Islands' coral assemblages are an outlier among the regional patterns of coral distributions in Japan, as most coral communities in

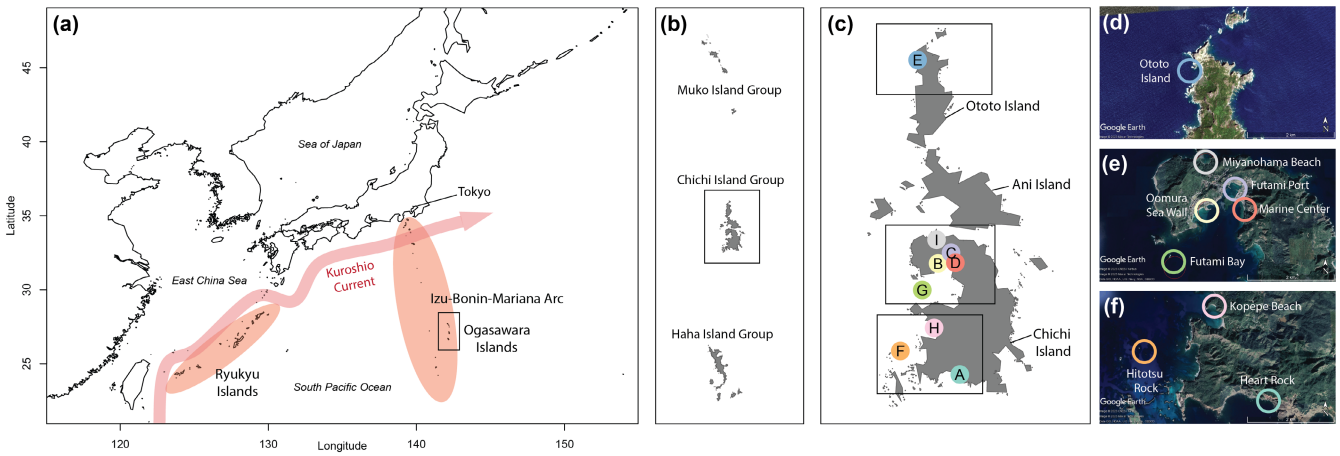

FIGURE 1 (a and b) Location of the Ogasawara Islands within Japan and the Pacific Ocean, with insets of the (c). Chichi Island group and (d–f). Google Maps images of the sampling sites labeled by circles representing the 200 m radius around the sampling point. See the Figure S1 for closer images of each sampling site.

Japan are strongly influenced by the Kuroshio Current that flows from the Ryukyus northward along the Pacific coast of Japan (Tsuchiya & Omori, 2004; Veron et al., 2015), while the Ogasawara Islands are removed from the region of influence of the Kuroshio Current (Figure 1). This strong oceanographic isolation has been implied as a driver of the genetic differentiation of its coral populations, giving rise to its distinct Scleractinia coral populations (Wepfer et al., 2022). When compared to the two closest, roughly equidistant coral ecoregions, the corals of Ogasawara Islands are more diverse (186 species from 48 genera) than those of high-latitude mainland Japan (115 species from 44 genera), but less diverse compared to those of the Ryukyu Islands (402 species from 73 genera), with which they share a common latitudinal range and similar climatic conditions (Veron et al., 2023). The Ogasawara Islands host sparsely developed fringing and patch reefs, albeit with coral cover at some locations comparable to that of the reefs of the Ryukyu Islands (Ministry of Environment, 2023). It has been reported that unlike these other areas, tabulate and staghorn *Acropora* spp. colonies are relatively scarce, and only a few *Acropora* species are among the main constituents of the coral reefs around Ogasawara, and instead *Galaxea* and *Porites* spp. are more common (Inaba, 2003; Tsuchiya & Omori, 2004). Futami Bay was noted as an exception, hosting large, *Acropora*-dominant communities (Inaba, 2003). However, such past datasets should be examined carefully, as major taxonomic revisions of Scleractinia have been ongoing (Kitahara et al., 2016). For example, the coral family Oculinidae, which Inaba (2003) assigned a majority of the coral cover to in one site from the eastern coast, has had many species revised to become members of families Acroporidae, Agariciciidae, Euphylliidae, and Lobophylliidae (WoRMS, 2023). Additionally, some of past members of the family Faviidae are now classified as belonging to Merulinidae (Fukami, 2013).

Together, the coral and fish assemblages of the Ogasawara Islands are important biodiversity assets from an ecotourism standpoint for the local community and from a national standpoint of diversity in Japan. However, as data on coral and fish assemblage structures and distributions within the Ogasawara Islands informed by recent taxonomy are comparatively limited, further surveys and reporting are necessary as global and local stressors continue to threaten Ogasawara's marine biodiversity.

Environmental DNA (eDNA) metabarcoding is sequencing-based identification of biodiversity and is rising in usage due to its non-invasive and time-effective approach to biomonitoring (Baird & Hajibabaei, 2012; Creer et al., 2016; Porter & Hajibabaei, 2018). Here, we conducted the first multi-taxa eDNA metabarcoding surveys targeting Actinopterygii and Elasmobranchii fish and Scleractinia coral assemblages at different sites around the Chichi-jima group in the Ogasawara Islands to assess the assemblage characteristics and identify potential biodiversity hotspots within the islands. Our dataset is a valuable baseline for continuous, spatiotemporally resolved biomonitoring efforts to better understand and conserve unique marine ecosystems, protect endangered species, and inform sustainable management practices for the benefit of both the environment and future generations at local and global scales.

2 | MATERIALS AND METHODS

2.1 | Sampling

Seawater was collected from nine sampling sites around Chichi-jima Island (sites A–I; Figure 1c,d), over a span of 5 days across 23–27 September 2021 (Table 1), all during the daytime. Sampling sites were chosen from a diverse set of locations with different levels of coastal development and natural environments, with the aim to cover different habitats and obtain a full and representative dataset of the islands (Figure 1, Table 1, Figure S2). Sites A, E, F, and G were accessed via boat with seawater sampled from the deck, while sites B, D, H, and I were reached by snorkeling from the beach and site C directly sampled from port docks (Table 1). All seawater samples were taken from the surface of seawater. For boat samples, seawater was collected with a bucket, 1.5 L per replicate, with three replicates for each site, which were transferred immediately to a Mighty Pack (Maruemu) and then sealed and placed in a dark cooler with ice packs until return to land. In the cases of beach entry and snorkeling, water was directly sampled with Mighty Packs. In between sampling sites, the bucket was bleached and rinsed with water and then rinsed again with seawater at the next sampling site. Water samples, field blanks (=1 L of ultrapure water processed on site as above), and filter blanks (=1 L of ultrapure water processed at filtering) were filtered within 4 h after sampling at the end of each respective sampling day. Water was filtered through a Sterivex cartridge filter (pore size 0.45 μm; Merck Millipore) generally following the protocol of Miya et al. (2020) with modifications to allow for multiple samples to be processed at the same time. Filters were then filled with 2 mL 99% RNALater with a disposable syringe and stored at −80°C until eDNA extraction.

2.2 | eDNA extraction and metabarcoding

eDNA was extracted from the Sterivex filters with the Qiagen DNeasy Blood and Tissue kit. Two milliliters of the lysis buffer were injected into the Sterivex filters from their outlets and incubated at 56°C for 1 h (Wong et al., 2020). The lysate was extracted in 15-mL Falcon tubes by centrifuging at 2500 *g* for 10 min. One milliliter of 99% ethanol was added to the lysates, and the lysate–ethanol mixture was transferred to the spin columns (from the Blood & Tissue kit) attached to the QIAvac manifold via adapters. Washing steps were performed on the spin columns with 800 μL of AW1 and AW2 with a subsequent dry spin at 17,000 *g* for 2 min. Finally, the eluates were collected in 150 μL of TE buffer by centrifuging the spin columns in their collection tubes at 6000 *g* for 1 min. Separate aliquots of each sample were taken and processed in parallel for metabarcoding for fish and scleractinian corals with different PCR protocols.

TABLE 1 Sampling site codes and their names, description, distance from the shore of the sampling point, code for site characteristics, coordinates, and sampling dates.

Sample code	Name	Description	Distance from the shore	Code for site characteristics	Latitude	Longitude	Sampling date
A	Heart Rock	Sheer cliff	25	Nearshore – rocky cliff	27.043788	142.205956	23-Sep-21
B	Oomura Sea Wall	Shallow water with concrete sea wall reached from the sandy beach, situated in Futami Bay	20	Nearshore – sandy beach	27.093103	142.195097	24-Sep-21
C	Futami Port	Inside the port on the most coastally developed area of Chichi Island, situated in Futami Bay	0	Nearshore – port	27.097844	142.201586	24-Sep-21
D	Marine Center	Rocky beach situated in Futami Bay	7	Nearshore – sandy beach	27.093406	142.203749	24-Sep-21
E	Ototo Island	Sheer cliff	300	Offshore	27.183361	142.184917	24-Sep-21
F	Hitotsu Rock	Sheer cliff with rock formations	975	Offshore	27.054252	142.176306	25-Sep-21
G	Futami Bay	At the open mouth of Futami Bay	630	Offshore	27.081289	142.187241	26-Sep-21
H	Kopepe Beach	Shallow water, reached from sandy beach	10	Nearshore – sandy beach	27.0645	142.193148	27-Sep-21
I	Miyanohama Beach	Shallow water, reached from sandy beach	10	Nearshore- sandy beach	27.103605	142.194352	27-Sep-21

Fish amplicons were generated with primers from Miya et al. (2020) (Table 2), with eight PCR replicates per sample (Minamoto et al., 2021). Each reaction replicate consisted of 2 μL of the eDNA sample and 10 μL of master mix (6.0 μL KAPA HiFi, 1.2 μL Milli-Q Direct UltraPure Water, and 2.8 μL of the primer mixture with a 1:2:1 ratio of MiFish-E-F/R-v2, MiFish-U-F/R, and MiFish-U2-F/R, respectively). Thermocycler conditions also followed the suggestions of Minamoto et al. (2021), set as initial denaturation at 95°C for 3 min, 38 cycles of (1) denaturation at 98°C for 20 s, (2) annealing at 65°C for 15 s 35 cycles, and (3) extension at 72°C for 15 s, finally followed by final extension at 72°C for 5 min. The eight PCR replicates were pooled together and then purified and concentrated with the GeneRead Size Selection Kit to remove primer and adapter dimers. Products were then quantified with TapeStation 2200, and TruSeq DNA CD Indexes 96 Indexes were used to index the pooled sample PCR products with second PCR. Metabarcoding for Scleractinia corals using the same eDNA samples was performed as described by Shinzato et al. (2021) and Nishitsuji et al. (2023) with their Scle-12S primers (Table 2).

Scleractinian coral amplicons were generated with Tks GflexTM DNA Polymerase (Takara). Thermocycler conditions were set as 1 min at 94°C, followed by 35 cycles of 10 s at 94°C, 15 s at 60°C, and 30 s at 68°C, with an extension of 5 min at 68°C in the final cycle. PCR products were extracted and cleaned with a FastGene Gel/PCR Extraction Kit (NIPPON Genetics). Amplicon sequencing libraries of cleaned PCR products were prepared using a KAPA Hyper Prep Kit (NIPPON Genetics Co., Ltd.) without fragmentation. Sequencing of both fish and coral sample sets was performed on MiSeq with a MiSeq v3 600 cycle cartridge, at paired-end sequencing at 2×150 bp for MiFish products, and 2×300 bp for Scle-12S products on separate runs.

2.3 | Data processing and analyses

Reads were quality-filtered, and primers were trimmed using CutAdapt (Martin, 2011). DADA2 (ver. 1.24.0) (Callahan et al., 2016) in RStudio (RStudio Team, 2020) using R (ver. 4.2.2) (R Core Team, 2022) was used to merge, denoise, and remove chimeras from the reads to generate one Amplicon Sequence Variant (ASV) table for each dataset. BLAST (Altschul et al., 1990) search of fish ASVs was performed against a custom database constructed from MitoFish sequences (Sato et al., 2018) with 'blastn' command. The most reliable taxa classification was chosen with the Last Common Ancestor (LCA) script (Mousavi-Derazmahalleh et al., 2021). These classifications were then searched for and converted to the World Register of Marine Species (WoRMS) (Ahyong et al., 2023) classifications using the worrms (ver. 0.4.2) and obistools (ver. 0.0.10) libraries in R (Chamberlain & Vanhoorne, 2023; Provoost & Bosch, 2023).

Scleractinia coral data were processed as described by Shinzato et al. (2021) and Nishitsuji et al. (2023). Briefly, after removal of low-quality bases (Phred quality score less than 20) and Illumina sequencing adapters, the remaining sequences were merged using USERCH (ver. 11.0.667). Then, de-noised (error-corrected) operational taxonomic units, called ZOTUs (zero-radius operation taxonomic units),

TABLE 2 Primer names and their sequences.

Primer name	Primer sequence (5′-3′)		Length
MiFish-E-F-v2	FW	5′-ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNNNRGTTGGTAAATCTCGTGCCAGC-3′	61 bp
	REV	5′-GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTNNNNNNGCATAGTGGGGTATCTAATCC TAGTTTG-3′	68 bp
MiFish-U	FW	5′-ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNNNGTCGGTAAAACTCGTGCCAGC-3′	60 bp
	REV	5′-GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTNNNNNNCATAGTGGGGTATCTAATCCCAGTTTG-3′	67 bp
MiFish-U2	FW	5′-ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNNNGCCGGTAAAACTCGTGCCAGC-3′	60 bp
	REV	5′-GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTNNNNNNCATAGGAGGG TGTCTAATCCCCGTTTG-3′	67 bp
Scle-12S	FW	5′-CCAGCMGACGCGGTRANACTTA-3′	22 bp
	REV	5′-AAWTTGACGACGGCCATGC-3′	19 bp

were prepared for each sample. ZOTU sequences from all samples were concatenated and clustered using CD-HIT-EST (ver. 4.6) with 100% nucleotide identity. Clustered, unique ZOTU sequences were used for the database for mapping. Merged sequences from each sample were mapped to the clustered ZOTUs, and the numbers of mapped sequences for each ZOTU were counted using USERCH 'otutab' command with 99% identity (-id.0.99). ZOTUs originating from Scleractinia corals were identified based on criteria described in Shinzato et al. (2021). After selecting scleractinian ZOTUs, mapped reads from the same genera were combined. Scleractinia taxa with best matches to classifications that are strictly Atlantic were annotated as having a high affinity for these taxa (e.g., "Scleractinia aff. *Meandrina*"). Next, respective tables of ASV with their WoRMS classification data and read abundance across the samples were obtained for fish and Scleractinia corals. Multiple ASVs matched to the same classifications were combined under a representative ASV with the 'taxa_glom' function of the phyloseq library (McMurdie & Holmes, 2013). Sunburst diagrams were created with Krona tools (Ondov et al., 2011) and edited in Adobe Illustrator for visual clarity.

All subsequent analyses and visualizations including read abundance and presence–absence heatmaps and richness bar plots were generated with R in RStudio (RStudio Team, 2020). Read abundances were normalized with cumulative-sum scaling (CSS) using the metagenomeSeq library, and heatmaps were generated and annotated with the ComplexHeatmap package (version 2.12.1 (Gu, 2022)) using the Ward D clustering method to find and compare groups of eDNA replicates and samples with high affinity. Finally, we discuss the potential causes for the differences in the variability of the replicates between coral and fish datasets.

3 | RESULTS AND DISCUSSION

We detected 38 Scleractinia coral and 124 fish taxa. While most fish ASV classifications were to species level, some were identified at genus or family levels (Table S1) due to the delineation limitations of the primers and database incompleteness. Almost all Scleractinia corals were identified at the genus level, with a single family-level identification (Table S2). Overall makeup of the taxonomic identifications can be seen in the Supplementary Figure (Figure S1). We were able to describe the most frequent and potentially abundant taxa and observed potentially novel detections in the eDNA datasets both for coral and fish. Through hierarchical clustering with the Ward-D method, we confirmed that the Chichi-jima Island group hosts a variety of distinct coral habitats with an even richer overall fish biodiversity that our sampling effort could not cover, further confirming the importance of this oceanic island region as a marine biodiversity asset and the need for spatially resolved biomonitoring to ensure its protection.

For Scleractinia coral taxa, the seven genera *Acropora*, *Astreopora*, *Dipsastraea*, *Montipora*, *Platygyra*, *Plesiastrea*, and *Pocillopora* were detected at all sampling sites, pointing to their higher abundance and overall prevalence across the archipelago (Figure 2a). This disagrees with the previous reports that generalized the coral assemblage characteristics of Ogasawara Islands as *Acropora* spp. being minor constituents and *Galaxea* and *Porites* spp. being more common constituents (Inaba, 2003; Tsuchiya & Omori, 2004). However, the highest read abundances of *Acropora* were detected from sites C and D (Futami Port and Marine Center), which agrees with the previous report of *Acropora*-dominated assemblages found along the coast of Futami Bay. Though *Galaxea* reads were found in all sites except for A, C, and E (Heart Rock, Kopepe Beach and Miyanohama Beach), assigned reads were not in particularly higher abundance compared to *Acropora* and other more prevalent genera (Figure 2). *Porites* was detected in sites A, H, and I (Heart Rock, Kopepe Beach, and Miyanohama Beach) at roughly equal levels of abundance with *Acropora* and in site D (Marine Center) at very low abundance. It is important to reemphasize that the past data were obtained with visual censuses only at the family level and were based on taxonomy that was subsequently revised, making it difficult to interpret and directly compare past and present datasets. Nevertheless, our data underline that *Acropora* constituents of Ogasawara coral assemblages may have been underestimated in the past, due to either taxonomic revisions or recent increases in *Acropora* in the archipelago. Ideally, the acquisition of new visual data and comparisons with our eDNA dataset and past visual datasets should help further clarify the situation.

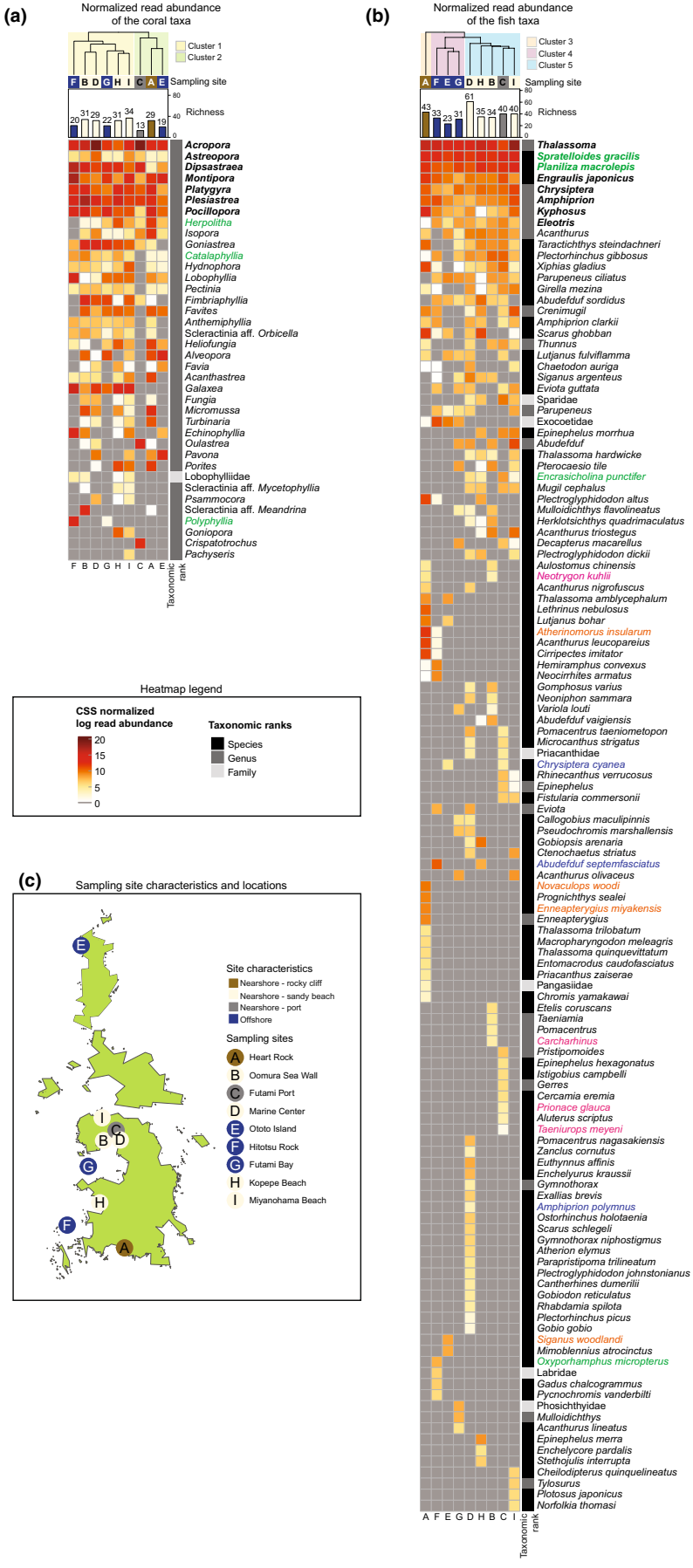

FIGURE 2 (a) Coral and (b) Fish datasets' heatmaps of cumulative sum scale (CSS) normalized read abundance of taxa (rows) across merged sample replicates (columns), and (c) the sampling sites and the codes for their site characteristics as described in Table 1. Taxa are ordered based on detection frequency from least detected (top) to most detected (bottom). Columns of the heatmap are clustered to show sampling site affinities. Bar plots show the richness of detected taxa at each sampling site. The top of the columns of the read abundance heatmap are annotated with colors coding for site characteristics of sampling sites. The right side of each row is annotated with colors coding for the taxonomic rank of the classification, namely, species, genus, or family, as generated by the pipeline. Taxa names are bolded for those detected in all of the sites. Additional font colors reflect novelty of records; green for those with no prior records from Ogasawara, orange for species considered to be endemic to other localities, and blue for potential seasonal vagrants or matches to close relatives. Pink represents Elasmobranch taxa to distinguish them from Actinopterygii taxa.

There were detections of coral genera with ranges that do not include the Ogasawara Islands. These include *Mycetophyllia*, *Meandrina*, and *Orbicella*, genera endemic to other distant ecoregions in the Atlantic, specifically the Caribbean Sea and the Gulf of Mexico, and these were annotated in our dataset as "aff." (=affinis) to indicate some uncertainty in our identification. Scleractinia aff. *Mycetophyllia* was detected at sites B, H, and I (Oomura Sea Wall, Kopepe Beach, and Miyanohama Beach), and Scleractinia aff. *Meandrina* in all of the site B replicates (Oomura Sea Wall) and a replicate from site A (Heart Rock), and Scleractinia aff. *Orbicella* from all sites except C and E (Futami Port and Ototo Island). Such identifications are very likely attributable to the limitations of incomplete scleractinian databases where absence of exact barcodes led to results including the closest available relative in the database, underlining the need for continued barcode collection efforts and manual checks of pipeline outputs based on geographical information and taxonomic knowledge.

Our coral eDNA dataset also included potentially novel detections of *Cataphyllia*, *Herpolitha*, and *Polyphyllia* (Figure 2, green taxa). We detected the monotypic genus *Cataphyllia* from all sampling sites except C (Futami Port). *Cataphyllia jardinei* is a small stony coral that prefers sandy areas of shallow reefs exposed to strong currents, and it is a species overexploited by the aquarium trade for its unique and colorful anemone-like appearance, leading to it being listed as vulnerable on the IUCN Red List (*IUCN Red List of Threatened Species*, 2008). *Cataphyllia jardinei* was previously considered to have a discontinuous distribution in Japan and thought to not be present in the Ryukyus (Nishihara & Veron, 1995) but was recently recorded in the region (Fujii et al., 2020). The recent discovery in the Ryukyus was attributed to the insufficiency of environmental surveys, as well as the species' preference for turbid, sheltered, and upper mesophotic environments (Fujii et al., 2020). These recent past findings give credence to our new detection of this species in the Ogasawara Islands. Based on this, we believe our detection of *Cataphyllia* in our eDNA samples represents a reliable first record of *C. jardinei* from the Ogasawara Islands. Additionally, we detected two Fungiidae mushroom coral genera, *Herpolitha* and *Polyphyllia*, outside of their recorded range. *Herpolitha* was detected from all sampling sites except site F (Hitotsu Rock) and until now has been known from the Ryukyus and the Marianas but not from the Ogasawara Islands. *Polyphyllia* was only detected at sites G (Futami Bay) and F (Hitotsu Rock) sites, and *P. talpina* has previously been recorded from the Ryukyus and the Marianas but until now not from Ogasawara, while *P. novaehiberniae* is restricted to the South Pacific Ocean. The widespread distribution of the family Fungiidae in the Pacific including the neighboring Marianas and the ubiquity of detection throughout our sampling sites suggest true positive detection, and therefore we believe these represent two more new coral genera records of the Ogasawara Islands. These eDNA records remain to be confirmed by visual surveys. Such detections contribute baseline data and lines of evidence to reliably assess the large-scale spatial assemblage patterns of scleractinian corals across the Pacific and should help strengthen our understanding of the role(s) of the oceanic islands as sources of coral and other pelagic larvae. Overall, these findings will help better understand marine connectivity of the Pacific. In a broader context, these discoveries underscore the importance of integrating eDNA into ecological research and conservation practices to address the challenges posed by human activities to biodiversity and ecosystem health such as coastal development and aquarium trade and safeguard coral diversity and resilience.

Similarly, for fish, there were a range of read abundance values and detection frequencies across the sampling sites, from detection in a single sample replicate to detection of some species in all sampling sites. The most abundant and frequently detected taxa (Figure 2b) were primarily reef-associated (*Thalassoma*, *Chrysiptera*, *Amphiprion*, *Kyphosus*, *Parupeneus ciliatus*, *Plectorhinchus gibbosus*, *Abudefduf sordidus*, *Acanthurus*, *Girella mezina*, *Scarus ghobban*, *and Amphiprion clarkii*) and pelagic, oceanodromous taxa (*Spratelloides gracilis*, *Engraulis japonicus*, *Xiphias gladius*, *and Taractichthys steindachneri*), reflecting a combination of oceanic properties and coral reefs that is characteristic of the Ogasawara Islands. A few freshwater or estuarine taxa were also prevalent such as *Eleotris* (family: Eleotridae, order: Gobiiformes), which are often the major predators of stream systems on oceanic islands and have a marine planktonic larval stage and *Planiliza macrolepis*. As a whole, the detected fish taxa consisted of reef-associated (77.8%), pelagic (pelagic-neritic 4.6%, pelagic-oceanic 4.6%), benthopelagic (6.5%), and demersal (6.5%) species.

The detected fish assemblage consisted of fishes with distributions in the Indo-Pacific. However, while most were confirmed to have been reported from the Ogasawara Islands in the past (GBIF.Org User, 2023; Hidenori, 2002; Randall et al., 1997; Sasaki et al., 2014), there were a few species that have not been previously recorded from the islands, making our detections potential novel records. The probable explanations for these detections include gaps in the distribution data, seasonal vagrancy, and intraspecific variation in the DNA barcode region, leading to the ASV inadvertently matching to close relatives, all of which have implications for metabarcoding efforts in understudied habitats.

Some detections are possible additions to distribution data of widespread, oceanodromous or catadromous species (Figure 2, green taxa). *Spratelloides gracilis* (silver-stripe round herring) and *Planiliza macrolepis* (largescale mullet) were both detected in all of the sites and have widespread distributions across mainland Japan, with the nearest records being from the Izu Islands. *Oxyporhamphus micropterus* (bigwing halfbeak) was detected at site F (Hitotsu Rock). This species is a pelagic and oceanodromous species that has been recorded in Iwo Jima (GBIF Backbone Taxonomy, 2023b), part of the Ogasawara Archipelago approximately 260 km south from our site. *Encrasicholina punctifer* (buccaneer anchovy), detected in sites C, D, H, and I (Futami Port, Marine Center, Kopepe Beach, Miyanohama Beach), is likewise a widespread oceanodromous species in the Indo-Pacific and can occur at distances of over 1000 km away from land. The nearest record of *E. punctifer* is from Agrihan in the Northern Mariana Islands (GBIF Backbone Taxonomy, 2023a, 2023b, 2023c), less than 200 km away. These detections add to the growing body of knowledge of the distribution ranges of these species.

Some detections call into question previous reports of endemicity (Figure 2, orange taxa). *Atherinomorus insularum* (Hawaiian silverside) was detected in sites A and F (Heart Rock and Hitotsu Rock), but as its name suggests, it is considered endemic to Hawaii, where it is commonly found schooling near the surface in tidepools and coastal waters. However, the genus *Atherinomorus* has a complicated history of taxonomic revisions that include additions and synonymizations on the basis of high intra- and inter-specific morphological variability of the proposed species (Ivantsoff & Crowley, 1991), and a proposed subspecies (= *Pranesus insularum*, currently accepted as synonymous to *A. insularum*) has been reported from Saipan, Northern Mariana Islands. Our detection, which was a 100% match to the barcode of *A. insularum*, implies that at least a close relative from this potential species complex is found in the Ogasawara Islands, which is not improbable considering the relative proximity of the islands and the reported fish assemblage similarities and connectivity with the Mariana Islands. Similarly, *Novaculops woodi* (Hawaiian sandy or Wood's wrasse) is considered endemic to Hawaii (Yeeting & Russell, 2010) and yet has previously been detected in other localities within Japan (Motomura, 2023) and now by our eDNA survey, at site A (Heart Rock). Interestingly, a previous record of *N. woodi* from Ogasawara Islands was retracted on the basis of misidentification (Froese & Pauly, 2023). Another similar detection is of *Siganus woodlandi* (family: Siganidae), considered by some sources to be endemic to New Caledonia (Carpenter & Smith-Vaniz, 2016). However, very similar specimens have been documented and described from mainland Japan (Randall & Kulbicki, 2005), and the GBIF repository currently recognizes these Japanese records under this species (GBIF Backbone Taxonomy, 2023a, 2023b, 2023c). Finally, *Enneapterygius miyakensis* (Izu Islands triplefin) was detected from site A (Heart Rock) but has previously been reported with apparent endemicity to the Izu Islands where it has common occurrence in the tidepools (Fricke, 1987). This species was reported from the Ogasawara Islands by Randall et al. (1997), implying that either this species or a close congener is found in the Ogasawara Islands. These detections add to our knowledge of marine connectivity of Pacific Ocean oceanic islands and the coasts of mainland Japan. Initially reported levels of endemism are subject to change as our knowledge of fishes and their distributions continues to expand (Randall, 1998). It remains to be confirmed whether or not these species above are successfully settled and reproducing in the Ogasawara Islands, and this can ultimately can only be confirmed by in situ observations. For now, we posit that metabarcoding eDNA efforts can contribute lines of evidence to support or reject endemicity.

Some fish detections can be explained by seasonal vagrancy or database incompleteness (Figure 2, blue taxa). *Abudefduf septemfasciatus* (banded sargeant) was detected from sites F and H (Hitotsu Rock and Kopepe Beach), but its distribution in the Indo-Pacific does not include the Ogasawara Islands, and the closest records are from Guam, Mariana Islands. However, the species can be observed in the Izu Islands during the summer and autumn months as seasonal vagrants carried by the Kuroshio Current. Seasonal vagrancy or "ineffective dispersal" is a phenomenon documented annually along the eastern coastline of Japan where the Kuroshio Current, after an increase in its flow rate in the East China Sea during the summer months, transports subtropical fish larvae and juveniles to temperate mainland sites in the autumn months, including many pomacentrids (damselfishes and clownfishes) and chaetodontids (butterflyfishes) (Kai et al., 2022). Our detection of *A. septemfasciatus* could potentially have been of migrants of such vagrants from the Izu Islands, facilitated by the Kuroshio and Zunan Islands as stepping stones (Kai et al., 2022; Kuriiwa et al., 2014). However, we consider this unlikely as it would require an exceptional reach by the Kuroshio or significant mobility by immature fish. Another explanation is intraspecific variability of the DNA barcode sequence across distant geographic regions. Under-sampled areas, including isolated oceanic islands such as Ogasawara, can host genetically distinct lineages represented by a pool of barcode sequences that may exceed the maximum known intraspecific threshold for species assignment and cause incorrect matching of the query sequence to close congeners instead. In the case of *A. septemfasciatus*, the next closest matches in the database for this specific ASV were *A. sordidus* and *A. notatus*, which are commonly recorded in the Ogasawara Islands (Randall et al., 1997; Sasaki et al., 2014) and are placed in the same phylogenetic clade as *A. septemfasciatus* according to the most recent and comprehensive analyses (Campbell et al., 2018). *A. septemfasciatus*, *A. sexfasciatus*, *A. sordidus*, and *A. notatus* are all morphologically and behaviorally distinguishable from each other, and we consider a misidentification in the previous records to be unlikely. Hence, divergent lineages of *A. sordidus* or *A. notatus* with barcode sequences that are more similar to those of *A. septemfasciatus* in the database may be inhabiting the Ogasawara Islands. Similarly, *Chrysiptera cyanea* (sapphire devil) was detected at sites C and E (Futami Port and Ototo Island), and the closest records are from the Marianas and Micronesia. Its congeneric species reported in the Ogasawara Islands are *C. brownrigii*, *C. starcki*, and *C. tricincta*. *Amphiprion polymnus* (saddleback anemonefish) was detected at site D (Marine Center), but the Ogasawara Islands are outside of this species' distribution range of Indo-Malayan Archipelago northward to the Ryukyu Islands; and its host anemones *Stichodactyla haddoni* and *Radianthus crispa* have also not been reported from the Ogasawara Islands. While we could speculate that juvenile *A. polymnus* drifted to the Ogasawara Islands by chance events but never settled due to the lack of host anemones, the close congener explanation stands as another potentially acceptable explanation.

Our investigated sites well represent the heterogeneous coastal habitats of Ogasawara Islands, ranging from beaches within bays to rocky reefs along sheer cliffs facing the open ocean, with different levels of coastal development and potential anthropogenic impact. On one end of the anthropogenic impact spectrum are relatively untouched sites which include the sandy beaches of Kopepe and Miyanohama (sites H and I), the sites along the sheer cliffs in Heart Rock and Ototo Island (sites A and E), and the offshore locations of Hitotsu Rock and the mouth of Futami Bay (sites E, F, and G), while

on the other end are sandy beaches with sea walls and other wave-breaking constructions on the coast of Futami Bay (sites B and D) and finally, the inner area of Futami Port (site C).

These nine sampling sites clustered differently in Ward-D analyses when comparing the fish and the Scleractinia coral datasets (Figure 2). In the coral dataset, the port and nearshore rocky cliffs were clustered separately from the sandy beaches and offshore locations (Clusters 1 and 2). On the other hand, the fish dataset delineated the only nearshore sampling site along a rocky cliff from the other sites (Cluster 3), while the rest of the sites were further clustered into two groups, separating all of the offshore locations from all of the sandy beaches and the port (Clusters 4 and 5). Both datasets separated nearshore and offshore sampling sites along rocky cliffs (sites A and E) from sandy beaches (sites B, D, H, and I) in the main clusters. These patterns in the clustering of the sites can be explained by a range of environmental constraints that independently govern the composition and richness of the coral and fish assemblages and the difference in active selection of the habitats by the organisms. Overall, the detected richness of coral taxa was higher in sites that are nearshore and lower in offshore or extensively coastally developed sites. Exact differences between the assemblages can be seen in the constituents and their relative abundances. A major difference was seen for *Galaxea*, which was relatively abundant in the sites of Cluster 1 (sites B, D, F, G, H, and I) but completely absent from the sites of Cluster 2 (sites A, C, and E). Another property of the Cluster 2 sites was the shared absence or lower abundance of relatively rarely detected taxa as seen toward the bottom of the heatmap. This can be interpreted that the southeast coastline of Chichi Island is marked by sandy beaches within bay structures of varying scales that host coral assemblages characterized by *Galaxea* and high diversity that develop toward offshore but with diversity attenuated by depth and distance from shore. This contrasts with the sites A (Heart Rock) and E (Ototo Island) along the sheer cliffs on the northern or southern margins of the island group, where *Galaxea* was absent in both sampled assemblages, of which site A (Heart Rock) was much more diverse than site E (Ototo Island).

An interesting outlier in this pattern was site C, Futami Port, which is part of the continuous coastline of Futami Bay, closely situated near sites B and D. Sites B and D are on the opposite sides of the Futami Bay, and their assemblages showed very close affinity within Cluster 1, strongly separated from site C. Within Cluster 2, site C (Futami Port) was further separated from sites A and E, as it had one of the highest abundances of *Acropora* reads, with the lowest number of detected genera, missing four of the genera that were detected in all other sites *Cataphyllia*, *Hydophora*, *Lobophyllia*, and *Pectinia* along with other commonly detected genera such as *Favites*, *Anthemiphyllia*, and *Heliofungia*. All genera detected from site C, except *Crispatotrochus*, were detected also from neighboring sites B and D, making it a much less diverse subsample of the closest neighboring sites' scleractinian coral assemblages. This high degree of nestedness of the three closely situated sites within Futami Bay, all within 500 m of one another, could be the result of local reduction of diversity by coastal development. Futami Port has seen the most extensive coastal development of any coastline across the Ogasawara Islands, which may have drastically altered the marine assemblages, which were originally similar to those of sites B and D. Futami Port also possesses a long breakwater that could decrease chances of dispersal and settlement, and active boat traffic and regular port management activities including dredging could work to keep the assemblage in its altered state, causing it to closely cluster with similarly depauperate and less-developed assemblages situated further away along sheer rocky cliffs at the sampling sites A and E. A very close and more recent example of well-developed coral communities that "disappeared" are those from eastern Hahajima Island in the Ogasawara Islands, which vanished due to harbor development (Tsuchiya & Omori, 2004). Connected to this, the coral genus *Oulastrea* was detected from sites A, B, C, and D, with the highest read abundance from site C, Futami Port (Figure 2a and Figure S3a). Cosmopolitan *Oulastrea crispata* is a low-temperature and turbidity-tolerant species able to settle on a variety of substrates (Röthig et al., 2020), which may explain its concentrated detection from the most coastally developed area of the Ogasawara Islands.

When the unique situation of Futami Port is considered, the differences between coral assemblages of Clusters 1 and 2 can be generally explained by differences in geophysical and environmental properties between the sampled sandy beaches and the sheer cliffs. The sandy beaches of sites B, D, H, and I possess soft bottoms with gentle slopes that extend from the shore, while sites A and E with sheer cliffs show a more rapid decline over a rocky substrate. Such differences in adjacent coastal structures and depth gradients could result in a set of ranges of watershed sizes, turbidity, wave exposure, and available light and nutrient input levels, all impacting the growth and cover of individual coral species and ultimately resulting in distinct compositions with varying richness (Houk & Van Woesik, 2010). The sites in Cluster 1 may have more accommodating habitats that can host richer coral assemblages compared to those in Cluster 2, which may constrain the growth of specific species such as *Galaxea* and thus limit the overall richness.

The fish dataset showed a different pattern of clustering of the sites (Figure 2b). Unlike the coral assemblages, the fish assemblages in the offshore sites were not attenuated subsamples of the high-diversity assemblages of the nearshore sites. Rather, nearshore and offshore sites were highly differentiated due to distinct assemblages arising from their habitat characteristics, as fishes actively select for habitat or activity. Site A was highly distinct from the rest of the sites with the second-highest number of unique detections (11) and high relative abundance of certain rare detections such as the previously discussed *Neotrygon kuhlii*, *Atherinomorus insularum*, and *Novaculops woodi*. These unique detections also included the blennioid taxa *Enneapterygius miyakensis*, the genus *Enneapterygius*, and *Entomacrodus caudofasciatus*. The only other blennioid taxa in the dataset, *Mimoblennius atrocinctus*, was detected from site E, which is also along a rocky cliff albeit offshore, reflecting the habitat preference of these blennioid species for hard substrates of rocky shorelines, mainly rocks with rough surfaces (Patzner, 2009). On the other hand, there was a diversity of gobioid species that were detected

from other sites including the port, sandy beaches, and their offshore sampling locations but not in either of these sites along rocky cliffs. These included *Eviota guttata*, which are associated with shallow reefs; *Gobiopsis arenaria*, a cryptic inhabitant of sand-rubble bottoms near coral reefs; *Gobiodon reticularis*, which lives on coral as a coral-commensal species, and *Istigobius campbelli*, which are associated with shallow, sandy bottoms (Patzner et al., 2011). Another notable taxon was the genus *Exocoetidae* (flying fishes), highly active epipelagic and oceanodromous fishes known for their enlarged pectoral fins that give them gliding abilities. *Exocoetidae* were detected only in sites in Clusters 3 and 4, including all of the offshore and sheer cliff sites that face the open ocean. These contrasting species compositions with different habitat preferences for sandy versus rocky bottoms reflect the distinct benthic characteristics of site A and E compared to B, C, D, H, and I. Sites A, E, F, and G show open ocean characteristics with greater depths; and sites A and E in particular have additional properties that result in hosting a rich rocky, intertidal assemblage.

The final cluster of fish assemblages was represented by the port and the sandy beaches (sites B, C, D, H, and I), which are all situated along sheltered bays of varying sizes. This close clustering and therefore similarities in fish assemblages was contrasted with the nested coral assemblages of sites B, C, and D of Futami Bay. While the depauperate coral assemblage of site C may be unsuitable for habitation by certain fish species, neighboring sites B and D may accommodate them and act as sources of eDNA that diffuse over a wider area and were thus detected in site C. Also, fishes are highly mobile organisms compared to generally sessile corals (but see Fungiidae) and can move over great distances for foraging and spawning activities. With such mobility, fishes could easily move across these closely situated coral assemblages and deposit eDNA over the wider area, resulting in the close clustering as seen in Cluster 5.

Fishes are one the largest vertebrate groups with a diversity of life histories, diets, and behaviors. A variety of habitat characteristics such as sheltered bay structures versus open waters, soft, shallow bottoms versus rocky intertidal shorelines, connectivity with freshwater streams, and coral reef cover and composition can all influence the composition and richness of fish assemblages. This complexity is reflected by the fact that there was not a straightforward linear relationship between coral richness and fish richness in our dataset; higher richness in coral taxa did not necessarily co-occur with higher richness in fish, and vice versa. The detected fish taxa richness ranged from 23 at site E (Ototo Island) to 61 at site D (Marine Center), while for coral taxa, it ranged from 13 at site C (Futami Port) to 34 at site I (Miyanohama Beach). Sites C and I, which are on the opposite ends of detected coral taxa richness, showed equal richness in fish species at 40. However, the three sites within Futami Bay (B, C, and D) in total had 30 fish species detected, the highest being 18 from site D, which also boasted the highest diversity of gobioids. This number, compared to 27 site-specific detections in total from the six other sites (A, E, F, G, H, and I), which included well-developed coral reefs with similar coral assemblages at sites B and D (Figure 2), implies that Futami Bay is a hotspot of fish biodiversity within the island group. It is also worth noting that three out of the four detected Elasmobranch taxa, namely, *Taeniurops meyeni* and the apex predators *Carcarhinus* and *Prionace glauca*, were unique to sites from Futami Bay, and *Neotrygon kuhlii* was also detected in Futami Bay along with at one more site, suggesting that Futami Bay may be an important inshore habitat for sharks and rays. Finally, it is important to note that our survey was from a single time point and potentially captured only a snapshot of the seasonal variety in the biodiversity and abundances of its fish assemblages.

When sample replicates were kept separate and hierarchically clustered (Figure S3), a range of variability in the replicates taken from the same sampling site was observed. Some replicate groups showed exclusive clustering under a single node pointing to highly similar read abundance data from the replicates, while others were clustered with replicates from other samples across different nodes, pointing to more variable read abundance data obtained from the same site. For example, site C (Futami Port) replicates consistently clustered within a node for both fish and Scleractinia coral datasets; while site I (Miyanohama Beach) replicates clustered together for coral but not for fish, and finally, Site A (Heart Rock) replicates clustered for fish but not for corals. Interestingly, the coral dataset showed higher consistency in read abundance data of the replicates compared to the fish dataset, which showed mostly disjointed clustering of the replicates except at sites A (Heart Rock) and C (Futami Port).

Variation among replicates is to be expected with eDNA coverage limitations of a 1.5-L sample and the stochastic properties of sampling and amplicon sequencing (Bessey et al., 2020; Troth et al., 2021). On the other hand, the difference in this variation between the coral and fish dataset could have arisen from the differences in eDNA availability and homogeneity within a body of water as a combination of deposition rate and persistence by the two taxa each with very different zoological, biological, physiological, and morphological characteristics. Corals are sessile organisms with no to limited mobility, resulting in a biomass in an area that does not change significantly in short time frames, while fishes are highly mobile organisms with daily movements that can range from few kilometers to less than a few hundred meters within their home ranges. As such, repeated sampling from the same sampling site may capture a more similar mix of coral eDNA compared to a much more variable fish eDNA. Such differences in coverage should be considered when designing a multi-taxa metabarcoding survey to fully capture differences in biodiversity across sites so that the sampling effort can be adjusted accordingly.

The species accumulation curves for the two groups were markedly different (Figure 3), with the curve for the fish dataset showing a steeper slope and reaching a higher number of genera than the curve for the coral dataset, indicating that fishes had a higher rate of genus accumulation and higher overall genera richness than corals in the Ogasawara Islands. The steeper slope also indicated that additional sampling efforts with the current method, higher than that needed for the corals, would be required to fully capture the diversity of fishes. Agreeing with this, our eDNA survey data came

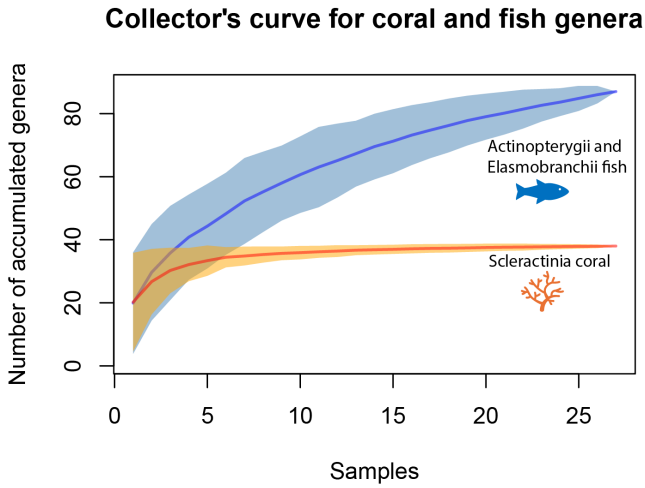

FIGURE 3 Mean species accumulation curves for fish and coral genera detected in our survey. Number of genera accumulated over samples selected in a random order, with 100 permutations for both fish and coral genera. Shading shows the confidence interval obtained by multiplying the standard deviation from random permutations of the data (standard error of the estimate) by 2.

closer to capturing the total known coral diversity of the Ogasawara Islands, detecting 37 out of the previously recorded 48 genera (Veron et al., 2023). The number of fish species recorded by Randall et al. (1997) in the Ogasawara Islands was 801, belonging to over 170 genera, which is much higher than what we observed in the fish eDNA dataset and much higher than the 48 recorded coral genera, explaining the differences in curve slopes and required sampling efforts.

4 | CONCLUSIONS

In summary, with our eDNA survey, we detected 38 Scleractinia coral and 124 fish taxa across nine sampling sites in the Chichijima Archipelago. We report likely novel records of the coral genera *Cataphyllia*, *Herpolitha*, and *Polyphyllia* as well as of fish species including *Oxyporhamphus micropterus*, *Encrasicholina punctifer*, *Atherinomorus insularum*, *Novaculops woodi* and *Siganus woodlandi*. While these detections have varying caveats and should ultimately be confirmed by in situ observations, our report provides more data for the growing body of knowledge of fish distributions and connectivity of the oceanic islands of the Pacific. We observed a high differentiation in the coral and fish assemblages across sampling sites, showing that the oceanic Ogasawara Islands host a variety of coral reef habitats with differing constituents and relative abundances across small distances, supporting their importance as a biodiversity reservoir. For future marine eDNA surveys around oceanic islands such as the Ogasawara Islands, we suggest a higher sampling effort adjusted to the taxa of interest focused on sites of interest and including different ecosystems for biomonitoring.

AUTHOR CONTRIBUTIONS

AHOA, JDR, and TR contributed to the conception or design of the study. AHOA, HN, KN, RH, JDR, and TR contributed to the acquisition, analysis, or interpretation of the data. AHOA wrote the manuscript, with review and editing by HN, RH, KN, NS, JDR, and TR.

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CONFLICT OF INTEREST STATEMENT

The authors have no conflicts of interest to declare.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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REFERENCES

Ahyong, S., Boyko, C. B., Bailly, N., Bernot, J., Bieler, R., Brandão, S. N., Daly, M., De Grave, S., Gofas, S., Hernandez, F., Hughes, L., Neubauer, T. A., Paulay, G., Boydens, B., Decock, W., Dekeyzer, S., Vandepitte, L., Vanhoorne, B., Adlard, R., … Zullini, A. (2023). *World Register of Marine Species* (*WoRMS*). WoRMS Editorial Board. https://www.marinespecies.org

Altschul, S. F., Gish, W., Miller, W., Myers, E. W., & Lipman, D. J. (1990). Basic local alignment search tool. *Journal of Molecular Biology*, *215*(3), 403–410. https://doi.org/10.1016/S0022-2836(05)80360-2

Baird, D. J., & Hajibabaei, M. (2012). Biomonitoring 2.0: A new paradigm in ecosystem assessment made possible by next-generation DNA sequencing. *Molecular Ecology*, *21*(8), 2039–2044. https://doi.org/10.1111/j.1365-294X.2012.05519.x

Bessey, C., Jarman, S. N., Berry, O., Olsen, Y. S., Bunce, M., Simpson, T., Power, M., McLaughlin, J., Edgar, G. J., & Keesing, J. (2020). Maximizing fish detection with eDNA metabarcoding. *Environmental DNA*, *2*(4), 493–504. https://doi.org/10.1002/edn3.74

Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., & Holmes, S. P. (2016). DADA2: High-resolution sample inference from Illumina amplicon data. *Nature Methods*, *13*(7), 7. https://doi.org/10.1038/nmeth.3869

Campbell, M. A., Robertson, D. R., Vargas, M. I., Allen, G. R., & McMillan, W. O. (2018). Multilocus molecular systematics of the circumtropical reef-fish genus *Abudefduf* (Pomacentridae): History, geography and ecology of speciation. *PeerJ*, *6*, e5357. https://doi.org/10.7717/peerj.5357

Carpenter, K. E., & Smith-Vaniz, W. F. (2016). *Siganus woodlandi* (errata version published in 2017). *The IUCN Red List of Threatened Species*. https://doi.org/10.2305/IUCN.UK.2016-3.RLTS.T69738962A69742654.en

Chamberlain, S., & Vanhoorne, B. (2023). *worrms: World Register of Marine Species* (*WoRMS*) *Client*.

Chiba, S. (1999). Accelerated evolution of land snails *Mandarina* in the oceanic Bonin Islands: Evidence from mitochondrial DNA sequences. *Evolution*, *53*(2), 460–471.

Creer, S., Deiner, K., Frey, S., Porazinska, D., Taberlet, P., Thomas, W. K., Potter, C., & Bik, H. M. (2016). The ecologist's field guide to sequence-based identification of biodiversity. *Methods in Ecology and Evolution*, *7*(9), 1008–1018. https://doi.org/10.1111/2041-210X.12574

Cunningham, P. A. (2005). *A chronology of ecotourism development in Ogasawara*.

Darwin, C. (1859). *On the origin of species*.

Fricke, R. (1987). *A New Species of* Enneapterygius *from Miyake-jima, Izu Islands, Japan*. https://www.researchgate.net/profile/Ronald-Fricke/publication/235679517_A_new_species_of_Enneapterygius_from_Miyake-jima_Izu_Islands_Japan_Teleostei_Tripterygiidae/links/55de598208ae45e825d39d09/A-new-species-of-Enneapterygius-from-Miyake-jima-Izu-Islands-Japan-Teleostei-Tripterygiidae.pdf

Froese, R., & Pauly, D. (2023). *All fishes reported from Ogasawara Islands* (*Bonin Islands*). https://www.fishbase.se/country/CountryChecklist.php?c_code=I556&vhabitat=all&csub_code=&cpresence=absent

Fujii, T., Kitano, Y., & Tachikawa, H. (2020). New distributional records of three species of Euphylliidae (Cnidaria, Anthozoa, Hexacorallia, Scleractinia) from the Ryukyu Islands, Japan. *Species Diversity*, *25*(2), 275–282. https://doi.org/10.12782/specdiv.25.275

Fukami, H. (2013). キクメイシ科およびオオトゲサンゴ科の分類体系の改変の理由. 日本サンゴ礁学会誌, *15*(1), 107–113.

GBIF Backbone Taxonomy. (2023a). Encrasicholina punctifer Fowler, *1938 in GBIF Secretariat*. GBIF. https://doi.org/10.15468/39omei

GBIF Backbone Taxonomy. (2023b). Oxyporhamphus micropterus (*Valenciennes*, *1847*) *in GBIF Secretariat*. GBIF. https://doi.org/10.15468/39omei

GBIF Backbone Taxonomy. (2023c). Siganus woodlandi Randall & Kulbicki, *2005 in GBIF Secretariat*. GBIF. https://doi.org/10.15468/39omei

GBIF.Org User. (2023). *GBIF.org GBIF Occurrence Download* [dataset]. The Global Biodiversity Information Facility. https://doi.org/10.15468/dl.smduw2

Gu, Z. (2022). Complex heatmap visualization. *iMeta*, *1*(3), e43. https://doi.org/10.1002/imt2.43

Hidenori, Y. (2002). Inland water fishes, shrimps and crabs of Chichijima and Haha-jima Islands, Ogasawara (Bonin) islands, Japan, with notes for inland water fish fauna of some subtropical islands. *Miscellaneous Reports of the Hiwa Museum for Natural History*, *41*, 1–30.

Houk, P., & Van Woesik, R. (2010). Coral assemblages and reef growth in the Commonwealth of the Northern Mariana Islands (Western Pacific Ocean). *Marine Ecology*, *31*(2), 318–329. https://doi.org/10.1111/j.1439-0485.2009.00301.x

Inaba, M. (2003). Ecological feature and status of hermatypic corals in the Ogasawara Islands. *Midoriishi*, *14*, 20–23.

Ito, M. (1998). Origin and evolution of endemic plants of the Bonin (Ogasawara) islands. *Population Ecology*, *40*(2), 205–212.

IUCN Red List of Threatened Species. (2008). https://www.iucnredlist.org/species/132890/3479919

Ivantsoff, W., & Crowley, L. (1991). Review of the Australian silverside fishes of the genus *Atherinomorus* (Atherinidae). *Marine and Freshwater Research*, *42*(5), 479–505. https://doi.org/10.1071/mf9910479

Kai, Y., Hiroyuki, M., & Keiichi, M. (Eds.). (2022). Fish diversity along the Kuroshio current. In *Fish diversity of Japan: Evolution, zoogeography, and conservation* (pp. 63–78). Springer Nature. https://doi.org/10.1007/978-981-16-7427-3_5

Kitahara, M. V., Fukami, H., Benzoni, F., & Huang, D. (2016). The new systematics of Scleractinia: Integrating molecular and morphological evidence. In S. Goffredo & Z. Dubinsky (Eds.), *The Cnidaria, past, present and future: The world of medusa and her sisters* (pp. 41–59). Springer International Publishing. https://doi.org/10.1007/978-3-319-31305-4_4

Koeda, K., & Motomura, H. (2018). A new species of *Pempheris* (Perciformes: Pempheridae) endemic to the Ogasawara Islands, Japan. *Ichthyological Research*, *65*, 21–28.

Kuriiwa, K., Arihara, H., Chiba, S., Kato, S., Senou, H., & Matsuura, K. (2014). Checklist of marine fishes of the Zunan Islands, located between the Izu and Ogasawara (Bonin) islands, Japan, with zoogeographical comments. *Check list*, *10*(6), 1479–1501. https://doi.org/10.15560/10.6.1479

Martin, M. (2011). Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet.Journal*, *17*(1), 1. https://doi.org/10.14806/ej.17.1.200

Matsuura, K., & Senou, H. (2012). Kuroshio and Fishes. *Matsuura Keiichi, Fishes of Kuroshio*, 3–16.

McMurdie, P. J., & Holmes, S. (2013). Phyloseq: An R package for reproducible interactive analysis and graphics of microbiome census data. *PLoS One*, *8*(4), e61217. https://doi.org/10.1371/journal.pone.0061217

Minamoto, T., Miya, M., Sado, T., Seino, S., Doi, H., Kondoh, M., Nakamura, K., Takahara, T., Yamamoto, S., Yamanaka, H., Araki, H., Iwasaki, W., Kasai, A., Masuda, R., & Uchii, K. (2021). An illustrated manual for environmental DNA research: Water sampling guidelines and experimental protocols. *Environmental DNA*, *3*(1), 8–13. https://doi.org/10.1002/edn3.121

Ministry of Environment. (2023). *Monitoring Sites 1000 Coral Reef Research Summary of Results 2022* (*In Japanese*). Ministry of Environment. https://www.biodic.go.jp/moni1000/findings/newsflash/pdf/coral_2022.pdf

Miya, M., Gotoh, R., & Sado, T. (2020). MiFish metabarcoding: A high-throughput approach for simultaneous detection of multiple fish species from environmental DNA and other samples. *Fisheries Science*, *86*, 939–970. https://doi.org/10.1007/s12562-020-01461-x

Motomura, H. (2023). *Fish collection of the Kagoshima University Museum. National Museum of Nature and Science, Japan*. [dataset]. https://doi.org/10.15468/vcj3j8

Mousavi-Derazmahalleh, M., Stott, A., Lines, R., Peverley, G., Nester, G., Simpson, T., Zawierta, M., De La Pierre, M., Bunce, M., & Christophersen, C. T. (2021). eDNAFlow, an automated, reproducible and scalable workflow for analysis of environmental DNA sequences exploiting Nextflow and singularity. *Molecular Ecology*

Resources, *21*(5), 1697–1704. https://doi.org/10.1111/1755-0998.13356

Murase, A., Uchino, H., Morita, Y., & Senou, H. (2009). Record of a blenniid fish, *Cirrisalarias bunares* (Teleostei: Perciformes), from the Ogasawara Islands, first ever from Japan. *Bulletin of the Biogeographical Society of Japan*, *64*, 47–55.

Nakano, T., Yazaki, I., Kurokawa, M., Yamaguchi, K., & Kuwasawa, K. (2009). The origin of the endemic patellogastropod limpets of the Ogasawara Islands in the northwestern Pacific. *Journal of Molluscan Studies*, *75*(1), 87–90.

Neall, V. E., & Trewick, S. A. (2008). The age and origin of the Pacific islands: A geological overview. *Philosophical Transactions of the Royal Society, B: Biological Sciences*, *363*(1508), 3293–3308. https://doi.org/10.1098/rstb.2008.0119

Nishihara, M., & Veron, J. E. N. (1995). *Hermatypic corals of Japan*. Kaiyusha Publishers Co.

Nishitsuji, K., Nagata, T., Narisoko, H., Kanai, M., Hisata, K., Shinzato, C., & Satoh, N. (2023). An environmental DNA metabarcoding survey reveals generic-level occurrence of scleractinian corals at reef slopes of Okinawa Island. *Proceedings of the Royal Society B*, *290*(1995), 2023002620230026. https://doi.org/10.1098/rspb.2023.0026

Ogasawara Rulebook. (2015). Ogasawara Ecotourism Council. https://www.vill.ogasawara.tokyo.jp/wp-content/uploads/sites/2/files1/27rulebook.pdf

Ondov, B. D., Bergman, N. H., & Phillippy, A. M. (2011). Interactive metagenomic visualization in a web browser. *BMC Bioinformatics*, *12*(1), 385. https://doi.org/10.1186/1471-2105-12-385

Palumbi, S. R. (1994). Genetic divergence, reproductive isolation, and marine speciation. *Annual Review of Ecology and Systematics*, *25*(1), 547–572.

Patzner, R. (2009). *The biology of blennies*. CRC Press.

Patzner, R., Van Tassell, J. L., Kovacic, M., & Kapoor, B. (2011). *The biology of gobies*. CRC Press.

Porter, T. M., & Hajibabaei, M. (2018). Scaling up: A guide to high-throughput genomic approaches for biodiversity analysis. *Molecular Ecology*, *27*(2), 313–338. https://doi.org/10.1111/mec.14478

Provoost, P., & Bosch, S. (2023). *obistools: Tools for data enhancement and quality control*. Ocean Biodiversity Information System. Intergovernmental Oceanographic Commission of UNESCO. https://cran.r-project.org/package=obistools

R Core Team. (2022). *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing. https://www.R-project.org/

Randall, J. E. (1998). Zoogeography of shore fishes of the indo-Pacific region. *Zoological Studies*, *37*(4), 227–268.

Randall, J. E., & Earle, J. L. (1993). *Scarus obishime*, a new parrotfish (Perciformes: Scaridae) from the Ogasawara Islands. *Japanese Journal of Ichthyology*, *39*(4), 287–293.

Randall, J. E., Ida, H., Richard, L. P., & John, L. E. (1997). Annotated checklist of the inshore fishes of the Ogasawara Islands. *National Science Museum Monographs*, *11*, 1–74.

Randall, J. E., & Kulbicki, M. (2005). *Siganus woodlandi*, new species of rabbitfish (Siganidae) from New Caledonia. *Cybium: International Journal of Ichthyology*, *29*, 185–189.

Randall, J. E., & Yasuda, F. (1979). *Centropyge shepardi*, a new angelfish from the Mariana and Ogasawara Islands. *Japanese Journal of Ichthyology*, *26*(1), 55–61.

Rigby, C. L., Carlson, J., Derrick, D., Dicken, M., Pacoureau, N., & Simpfendorfer, C. (2020). IUCN Red List of Threatened Species: *Carcharias taurus*. *IUCN Red List of Threatened Species*. https://dx.doi.org/10.2305/IUCN.UK.2021-2.RLTS.T3854A2876505.en

Röthig, T., Bravo, H., Corley, A., Prigge, T.-L., Chung, A., Yu, V., McIlroy, S. E., Bulling, M., Sweet, M., & Baker, D. M. (2020). Environmental flexibility in *Oulastrea crispata* in a highly urbanised environment: A microbial perspective. *Coral Reefs*, *39*(3), 649–662. https://doi.org/10.1007/s00338-020-01938-2

RStudio Team. (2020). *RStudio: Integrated Development Environment for R*. RStudio, PBC. http://www.rstudio.com/

Sasaki, T., Senou, H., Yamada, T., & Uchino, H. (2014). Marine fish fauna in Ani-jima and Chichi-jima islands, the Ogasawara (Bonin) Islands, Japan. 小笠原研究.

Sato, Y., Miya, M., Fukunaga, T., Sado, T., & Iwasaki, W. (2018). MitoFish and MiFish pipeline: A mitochondrial genome database of fish with an analysis pipeline for environmental DNA metabarcoding. *Molecular Biology and Evolution*, *35*(6), 1553–1555. https://doi.org/10.1093/molbev/msy074

Senou, H. (1997). 小笠原諸島から得られた日本初記録の魚類. *IOP Diving News*, *8*(2), 2–7.

Senou, H., Matsuura, K., & Shinohara, G. (2006). Checklist of fishes in the Sagami Sea with zoogeographical comments on shallow water fishes occurring along the coastlines under the influence of the Kuroshio current. 国立科学博物館専報 = *Memoirs of the National Museum of Nature and Science*, *41*, 389–542.

Senou, H., Morita, Y., & Arai, K. (1995). Two new Records of Lizardfishes (Pisces: Synodontidae) from Japan. 魚類学雑誌, *42*(1), 93–95.

Shinzato, C., Narisoko, H., Nishitsuji, K., Nagata, T., Satoh, N., & Inoue, J. (2021). Novel mitochondrial DNA markers for Scleractinian corals and generic-level environmental DNA metabarcoding. *Frontiers in Marine Science*, *8*, 1791. https://doi.org/10.3389/fmars.2021.758207

Sugai, K., Mori, K., Murakami, N., & Kato, H. (2019). Strong genetic structure revealed by microsatellite variation in Callicarpa species endemic to the Bonin (Ogasawara) Islands. *Journal of Plant Research*, *132*(6), 759–775. https://doi.org/10.1007/s10265-019-01144-4

Suzuki, T., Chen, I.-S., & Senou, H. (2011). A new species of *Rhinogobius* Gill, 1859 (Teleostei: Gobiidae) from the Bonin Islands, Japan. *Journal of Marine Science and Technology*, *19*(6), 693–701. https://doi.org/10.51400/2709-6998.2213

Tatsuta, N., Imamura, H., Nakaya, K., Kawai, T., Abe, T., Sakaoka, K., Takagi, S., & Yabe, M. (2014). Taxonomy of mesopelagic fishes collected around the Ogasawara Islands by the T/S Oshoro-Maru. *Memoirs of the Faculty of Engineering, Kyushu University*, *56*, 1–64.

Troth, C. R., Sweet, M. J., Nightingale, J., & Burian, A. (2021). Seasonality, DNA degradation and spatial heterogeneity as drivers of eDNA detection dynamics. *Science of the Total Environment*, *768*, 144466. https://doi.org/10.1016/j.scitotenv.2020.144466

Tsuchiya, M., & Omori, M. (2004). *Coral reefs of Japan*. Ministry of the Environment.

Veron, J. E. N., Stafford-Smith, M., DeVantier, L., & Turak, E. (2015). Overview of distribution patterns of zooxanthellate Scleractinia. *Frontiers in Marine Science*, *1*(81). https://www.frontiersin.org/articles/10.3389/fmars.2014.00081

Veron, J. E. N., Stafford-Smith, M. G., Turak, E., & DeVantier, L. M. (2023). *Corals of the World*. http://www.coralsoftheworld.org/coral_geographic/interactive_map/

Wepfer, P. H., Nakajima, Y., Fujimura, A., Mikheyev, A. S., Economo, E. P., & Mitarai, S. (2022). The oceanographic isolation of the Ogasawara Islands and genetic divergence in a reef-building coral. *Journal of Biogeography*, *49*(11), 1978–1990. https://doi.org/10.1111/jbi.14475

Wong, M. K.-S., Nakao, M., & Hyodo, S. (2020). Field application of an improved protocol for environmental DNA extraction, purification, and measurement using Sterivex filter. *Scientific Reports*, *10*(1), 1. https://doi.org/10.1038/s41598-020-77304-7

World Heritage Convention, U. (2018). Ogasawara Islands. In *Unesco.org*. https://whc.unesco.org/en/list/1362/

WoRMS. (2023). *Oculinidae Gray, 1847*. WoRMS. https://www.marinespecies.org/aphia.php?p=taxdetails&id=135078

Yeeting, B., & Russell, B. (2010). Xyrichtys woodi. *The IUCN Red List of Threatened Species*. https://doi.org/10.2305/IUCN.UK.2010-4.RLTS.T187408A8527804.en

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