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Mapping Taiwanese corals, including the endangered, Polycyathus chaishanensis, using environmental DNA metabarcoding

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Research Article

Keywords: Polycyathus chaishanensis, environmental DNA, endangered coral species, Taiwan

Posted Date: June 3rd, 2026

DOI: https://doi.org/10.21203/rs.3.rs-9748634/v1

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Additional Declarations: No competing interests reported.

Abstract

Background

The scleractinian coral, *Polycyathus chaishanensis*, first discovered in the Chaishan region of southern Taiwan, is an endangered species covered by the Taiwan Wildlife Protection Act (2017). At present, its population continues to decline due to environmental pressures. To develop and implement effective conservation strategies, better understanding of the distribution of this species is essential. However, because of its low population density, *P. chaishanensis* has been difficult to locate using conventional field surveys. In this study, we conducted an environmental DNA metabarcoding (eDNA-M) survey for scleractinian corals to infer the distribution of *P. chaishanensis* in Taiwan. Surface seawater was collected at 35 coastal sites along the main island of Taiwan and surrounding offshore islands. Coral eDNA were detected by PCR amplification of mitochondrial 12S rDNA fragments followed by amplicon sequencing.

Results

An eDNA-based database of scleractinian corals at 35 coastal sites in Taiwan was developed. eDNA signals matching *P. chaishanensis* mitochondrial sequences were detected at 13 sites, including several locations with previous records of this species. Notably, putative *P. chaishanensis*-derived eDNA signals were also detected at multiple sites where the species had not been previously reported, suggesting a broader potential distribution than currently recognized.

Conclusions

Although direct field verification at these suggested sites will be required in the future, our results demonstrate that eDNA-M is a viable approach for estimating distributions of rare and endangered corals. By enabling more targeted ecological surveys, this method is expected to support development of effective conservation and protection measures for *P. chaishanensis*.

Background

Polycyathus chaishanensis Lin, Kitahara, Tachikawa, Keshavmurthy & Chen, 2012 **(**Fig. 1a **and b)** is a scleractinian coral belonging to the family Caryophylliidae. It was first discovered in shallow waters of the Chaishan region south of Kaohsiung City, southern Taiwan, and reported in 2012 [1]. At the time of its description, no records of this species existed outside Taiwan, and its known distribution in Taiwan was restricted to a single population comprising approximately 50 small colonies in the Chaishan region. Owing to this highly localized distribution and limited population size, *P. chaishanensis* was proposed as a rare species and considered likely endemic to Taiwan [1]. Subsequently, concerns were raised regarding habitat degradation and population decline caused by coastal development and pollution in the Chaishan area, leading to its designation as an endangered species under the Taiwan Wildlife Protection Act in 2017 [2]. More recent ecological surveys have expanded its known distribution, confirming the presence of approximately 180 colonies in algal reefs in Guan-Shan, Taoyuan City, in northwestern Taiwan [3, 4, 5]. Although not yet documented in peer-reviewed literature, additional surveys have reported its occurrence in eastern Taiwan (Taitung) and on western offshore islands such as Xiaoliuqiu. Furthermore, *P. chaishanensis* is gradually being discovered outside Taiwan [6,7].

Currently, populations of *P. chaishanensis* appear to be declining, likely as a result of both global and local environmental stressors. In particular, colonies inhabiting algal reefs along the coast of Guan-Shan, Taoyuan City, Taiwan, have experienced bleaching during summer periods when seawater temperatures exceeded 34°C **(**Fig. 1c**)**. Global climate change models predict that the frequency and intensity of coral bleaching events will continue to increase over the next century [8]. In addition, increased sedimentation on coral reefs has been observed in recent years **(**Fig. 1d**)**, possibly associated with local ocean currents, nearby coastal development, and the presence or absence of typhoon. To effectively protect and conserve *P.*

chaishanensis under these circumstances, a comprehensive understanding of the species life history and distribution are needed.

Since the 2000s, environmental DNA (eDNA) methods have emerged as powerful tools for monitoring aquatic organisms [9–12], including scleractinian corals [13–16]. In particular, eDNA is highly effective for detecting rare species at large spatial scales with reduced sampling effort [17–19]. Generally, the number of *P. chaishanensis* colonies in the wild is very small, making direct observation and specimen collection challenging. Therefore, we hypothesized that eDNA techniques could be effective for investigating its distribution in Taiwanese coastal waters.

Taiwan is located in the western Pacific Ocean and comprises one main island and more than 100 islets **(**Fig. 2**)** [20]. Along the coast of the main island, stony corals are widely distributed, except along the sandy western coast [21]. The southern part of Taiwan is tropical and experiences relatively high seawater temperatures throughout the year, supporting well-developed coral reefs [21,22]. In contrast, northern Taiwan is subtropical, and seawater temperatures decrease to below 18°C during winter due to monsoonal influences, limiting corals to non-reefal coral communities. Consequently, coral species richness differs markedly between regions, with approximately 300 species in southern Taiwan and about 100 species in the north [23]. To date, no study has applied environmental DNA (eDNA) methods to investigate scleractinian coral distributions in Taiwanese waters.

In our previous study, we developed an analytical method, a scleractinian-specific environmental DNA metabarcoding (Scl-eDNA-M), capable of detecting and identifying 83 genera of zooxanthellate scleractinian corals recorded in Japan [24]. Here, we applied this methodology to construct an eDNA-based database of scleractinian corals at 35 coastal sites around Taiwan. We then used this framework to estimate the distribution of *P. chaishanensis*.

Methods

Seawater sampling

Sampling of coral environmental DNA (eDNA) from surface seawater was conducted at 35 coastal sites around Taiwan between 19 June 2023 and 9 July 2025, primarily through two survey campaigns **(Table 1)**. The first survey (Survey ID_I) was conducted between 2023 and 2024 at 27 sites located around the main island of Taiwan and at the southwestern island of Xiaoliuqiu **(Table 1; Fig. 2 a-f)**. As a positive control, surface seawater was also collected from an outdoor coral culture pond at the Coral Farm [25]. The second survey (Survey ID_II) was carried out in 2025 at 1 site in northwestern Taiwan **(Fig. 2 d)** and at 7 sites in the western offshore Penghu Islands **(Fig. 2 g)** using the same sampling procedures. Sampling dates, site names, and geographic coordinates (latitude and longitude) are summarized in **Table 1**. At each site, 2 L of surface seawater were collected using two Whirl-Pak® sterile sampling bags (1 L per bag; Filtration Group, Pleasant Prairie, WI, USA) and individually filtered through 0.45 µm Sterivex™ filters (Merck) soon after collection, typically within 10 min. Following filtration, 2 mL of RNAlater (Thermo Fisher Scientific) were added to each filter to prevent DNA degradation. Filters were temporarily stored at 4°C, and then transferred to a −20°C freezer at the Institute of Marine Environment and Ecology, National Taiwan Ocean University (NTOU), Taiwan. Frozen samples were subsequently shipped to the Marine Genomics Unit, Okinawa Institute of Science and Technology (OIST) for eDNA sequencing and downstream bioinformatic analyses.

DNA extraction, PCR amplification, and sequencing

Environmental DNA (eDNA) retained on Sterivex™ filters was extracted following the protocols described in the Environmental DNA Sampling and Experiment Manual v.2.1 [26]. Duplicate eDNA samples were processed independently through DNA extraction, PCR amplification, library preparation, sequencing, and metabarcoding analyses, and the resulting data were combined only at the final step to calculate average values. The primer set used for amplification was Scle_12S_Fw2 (5' ACACTCTTTCCCTACACGACGCTCTTCCGATCTCCAGCMGACGCGGTRANACTTA-3') and Scle_12S_Rv2 (5'-GTGACTGGAGTTCAGACGTGTGCTCTTCCGACTAAWTTGACGACGGCCATGC-3'). For the first PCR, primers were added to a final concentration of 0.2 µM in a reaction containing 2 µL of extracted eDNA. PCR amplification was performed using Tks

Gflex DNA Polymerase (Takara) under the following thermal cycling conditions: initial denaturation at 94°C for 1 min, followed by 40 cycles of denaturation at 98°C for 10 s, annealing at 60°C for 15 s, and extension at 68°C for 30 s, with a final extension step at 68°C for 5 min. First-round PCR products were purified using Sera-Mag™ Select Beads (Cytiva) at a 1:1.8 bead-to-PCR product ratio, following the manufacturer's instructions. PCR amplicons were evaluated by agarose gel electrophoresis. *Acropora tenuis* genomic DNA was used as a positive control, whereas surface seawater collected offshore at depths greater than 50 m and distilled water were used as negative controls. A 100-bp DNA ladder (N0559S, New England Biolabs) was included to determine fragment sizes. The positive control yielded a distinct band of approximately 470 bp, whereas no amplification was detected in the negative controls (data not shown).

To prepare amplicon sequencing libraries, a second indexing PCR was performed. For this reaction, 0.5 ng of the purified first-round PCR product was used as a template. TruSeq-compatible indexing primers (Integrated DNA Technologies, IDT) were added to a final concentration of 0.2 μM to incorporate Illumina adapters and unique dual indices. Indexing PCR was conducted using Tks Gflex DNA Polymerase (Takara) under the following thermal cycling conditions: initial denaturation at 94°C for 1 min, followed by 10 cycles of denaturation at 98°C for 10 s and combined annealing/extension at 68°C for 1 min, with a final extension step at 68°C for 5 min. Products of the second indexing PCR were purified using Sera-Mag™ Select Beads (Cytiva) at a 1:1 bead-to-PCR product ratio, following the same procedure used for first-round PCR products. Purified amplicon libraries were then quantified and quality-checked prior to sequencing. Libraries were multiplexed and sequenced as 300-bp paired-end reads on an Illumina NextSeq 1000 using a NextSeq 1000/2000 P2 300M Reagent Cartridge (600 cycles). Raw sequence data have been deposited in the DNA Data Bank of Japan (DDBJ) under BioProject ID PRJDB40393 and DRR Run No. DRR919038-DRR919143. Numbers of sequence reads, total base-pair counts, and average and maximum read lengths for each sample are summarized in **Supplementary Table S1**.

Samples collected from 27 sites in the first survey were subjected to DNA extraction, PCR amplification, and sequencing in 2024. Samples collected from 8 sites in the second survey were processed using the same procedures in 2025. Accordingly, samples from the first and second surveys were considered independent datasets and analyzed separately, as described below.

Scl eDNA-M system and bioinformatic analyses

The scleractinian-specific eDNA metabarcoding analytical system (Scl-eDNA-M) [24], which is capable of detecting and identifying 83 genera of zooxanthellate scleractinian corals in Japan, was used in this study. In order to detect our target species, *P. chaishanensis*, the Scl-eDNA-M pipeline was extended by incorporating the complete mitochondrial genome sequence of *P. chaishanensis* [27] (GenBank accession no. JF825140), including the mitochondrial 12S rDNA sequence information. For the analysis, low-quality reads (quality cutoff = 30; minimum length = 300 bp) and Illumina adapter sequences were trimmed using CUTADAPT v4.5 [28]. Zero-radius operational taxonomic units (ZOTUs) were employed for scleractinian genus identification following the protocol of Hisata et al. 2025 [24]. ZOTU generation and downstream analyses were conducted on a sample-by-sample basis using USEARCH v11.0.677 (Edgar 2010). ZOTUs showing BLAST e-values ≤ 1×10^{-20} against mitochondrial sequences of scleractinian corals were retained for further analyses. The number of reads mapped to each ZOTU was quantified using the USEARCH "otutab" command with a sequence identity threshold of 100% (–id 1.00).

Amplicon sequences generated by the primer set were aligned on a genus-by-genus basis. Because the amplified sequence region was generally identical within genera, congeneric species were clustered into single representative sequences to reduce database size and to improve computational efficiency. When sequence variation was observed within a genus, all distinct sequences were retained in the custom reference database. In addition, species lacking complete mitochondrial genome information, but possessing reported 12S rDNA regions compatible with the primer set were also included. Taxonomic assignment of ZOTUs was performed using Assign-Taxonomy-with-BLAST [29] against a custom database, and the most likely genus was selected. Because several genera shared identical amplicon sequences, discrimination at the generic level was not possible in these cases. Such ZOTUs were grouped into combined categories, e.g.,

Montipora/*Anacropora* (see **Supplementary Table S2 and S3**). Seawater samples were collected in duplicate using two sampling bags. As ZOTU counts were generally consistent between paired samples, values were averaged for downstream analyses. Due to the lack of available mitochondrial 12S rDNA sequences from closely related genera, e.g., *Paracyathus*, species-level specificity of the assay could not be fully validated. Therefore, in this study, eDNA sequences matching the mitochondrial reference sequence (GenBank accession no. JF825140) of *Polycyathus chaishanensis* were operationally defined as putative *P. chaishanensis* eDNA and used for all downstream analyses. This definition was adopted to ensure a conservative interpretation of detection signals in the absence of reference sequences from phylogenetically related taxa.

Results

Regional patterns of generic diversity, and dominant scleractinian genera revealed by scleractinian-specific environmental DNA metabarcoding in Taiwan

To construct an eDNA-based database of scleractinian corals in Taiwan, including our target species, *Polycyathus chaishanensis*, surface seawater was collected from 35 coastal sites around Taiwan between 2023 and 2025 **(Fig. 2 a-g, Table 1)**. Sampling and analyses conducted in 2023–2024 were designated as the first survey, whereas those performed in 2025 were treated as the second survey.

In the first survey, surface seawater was collected at 27 sites located around the main island of Taiwan **(Fig. 2 a–f)**, as well as on the southwestern offshore island of Xiaoliuqiu. Surface seawater was also collected from an outdoor pond at the Coral Farm, which cultures approximately 25 species of local scleractinian corals, as a positive control. As expected, seawater collected from the Coral Farm (Site ID 1) yielded eDNA signals corresponding to corals cultured there, including *Acropora*, *Stylophora*, and *Galaxea* **(Fig. 3 a)**. These results confirm that the Scl-eDNA-M system is also effective for detecting coral taxa in Taiwanese coastal environments.

In the Northeast region (NE, Site IDs 1–13), ZOTU composition and relative proportions varied markedly among sites. At Guihou and Wanghaixiang (Site IDs 2 and 3), no single dominant genus was observed, and several genera, including *Porites*, *Acropora*, and *Favites*, each accounted for relatively similar proportions (approximately 15–20%). In contrast, *Cyphastrea* and *Acropora* dominated sites CPC-SH1, CPC-SH2, and CPC-SH3 (Site IDs 5–7), each comprising 40–50% of the composition. At Nanya (Site ID 8), *Leptoria*/*Platygyra* and *Favites* were dominant, whereas *Porites* was predominant at Bitou and Tofu Cape (Site IDs 9 and 12) **(Fig. 3a**). Generic diversity was highest at Nanya (Site ID 8), with 39 detected genera, and lowest at Waiao Beach (Site ID 11), with only three genera detected where coral eDNA was present (**Fig. 3b**).

In the East and Southeast regions (E & SE, Site IDs 14–17), ZOTU composition was comparatively consistent among sites, with *Pocillopora*, *Acropora*, and *Porites* being the dominant genera (**Fig. 3a**). Generic diversity ranged from 11 to 36, with the highest diversity at Jihui (Site ID 15; 36 genera) and the lowest at Shanyan (Site ID 16; 11 genera) (**Fig. 3b**). Coral eDNA signals were not detected at Xuhai (Site ID 17).

In the South region (S, Site IDs 18–21), ZOTU composition was primarily dominated by *Montipora*/*Anacropora*. At Water Outlet (Site ID 20), *Pavona* and *Leptoria*/*Platygyra* also contributed substantially to the community composition (**Fig. 3a**). Generic diversity was highest at South Bay (Site ID 18), with 42 detected genera (**Fig. 3b**).

In the Southwest region, generic diversity at Xiziwan (Site ID 22) was the lowest among all surveyed sites, with only four genera detected, and southwestern sites were largely dominated by *Porites*. In contrast, at Xiaoliuqiu Island (SW, Site IDs 23–25), generic diversity ranged from 25 to 28, and ZOTU composition was relatively similar among sites, with *Porites*, *Leptoria*/*Platygyra*, *Montipora*/*Anacropora*, and *Cyphastrea* representing major components (**Fig. 3a, b**).

In northwestern Taiwan, including Taoyuan Algal Reef G1 and G2-1 (Site IDs 26-27), ZOTU counts were only detected at Taoyuan Algal Reef G1 (Site ID 26), and these were lower compared with other Taiwanese coastal regions **(Fig. 3 a)**. Genuericdiversity was also lower, with only 2 genera detected **(Fig. 3 b)**.

In the second survey conducted in 2025, surface seawater was collected at one site in northwestern Taiwan (Taoyuan Algal Reef G2-2) and seven sites in the western offshore Penghu Islands (Site IDs 28–35) (**Fig. 2 a, d, g**). At Taoyuan Algal Reef G2-2 (Site ID 27), generic diversity was notably low, with only two genera detected (**Fig. 3 c, d**).

In contrast, the Penghu sites were dominated by *Acropora*, *Porites*, and *Montipora*/*Anacropora* in terms of ZOTU composition (**Fig. 3 c**). Generic diversity across the Penghu sites varied substantially, ranging from 13 to 37 genera, with the highest diversity recorded at Huangwan-2 (Site ID 33; 37 genera) and the lowest at Qitou (Site ID 29; 13 genera) (**Fig. 3 d**).

Sites inhabited by the stony coral, Polycyathus chaishanensis, as assessed by eDNA analysis

Among the 27 sites in the first survey in 2023–2024 **(Fig. 4 a)**, putative *P. chaishanensis*-eDNA, was detected at 8 sites, including Shitiping and Jihui (Site IDs 14 and 15) along the eastern and southeastern coast of Taiwan; South Bay, Houbihu, and Water Outlet (Site IDs 18-20) in southern Taiwan; and Yuchengwei and Houshi on Xiaoliuqiu Island (Site IDs 24-25) in southwest Taiwan **(Fig. 4 a)**. Putative *P. chaishanensis*-derived eDNA was also detected at Taoyuan Algal Reef G1 in northwestern Taiwan (Site ID 26), where several dozen colonies had been confirmed by previous ecological surveys; however, no corresponding eDNA was detected at the adjacent G2 site during the first survey. No putative *P. chaishanensis*-derived eDNA was detected at any of the sites in northeastern Taiwan **(Fig. 4 a)**.

In the second survey, conducted in 2025, surface seawater samples were collected from Taoyuan Algal Reef G2-2 (Site ID 28), where several dozen *P. chaishanensis* colonies had been previously confirmed, as well as at seven sites in the Penghu Islands (Site IDs 29-35) **(Fig. 4 a and b)**. Among those sites, putative *P. chaishanensis*-derived eDNA was detected at 5 sites, including Taoyuan Algal Reef G2-2 (Site ID 28), Hangwan-1, Hangwan-2, Yueli Bay, and Woniu in the Penghu Islands (Site IDs 32-35) **(Fig. 4 b)**.

Discussion

In this study, we successfully constructed an eDNA database of scleractinian corals at 35 coastal sites around Taiwan and estimated the probable distribution of the endangered scleractinian coral, *Polycyathus chaishanensis*. In analyses of surface seawater collected from an outdoor pond at the Coral Farm [25], the Scl-eDNA-M system successfully detected eDNA signals corresponding to coral taxa cultured in the pond, including *Acropora*, *Stylophora*, *Galaxea*, and *Fimbriaphyllia*. Notably, because *Acropora*, *Stylophora*, and *Galaxea* were the most abundant genera in the farm pond [25], their corresponding ZOTU counts were higher than those of other coral genera. These results demonstrate that the Scl-eDNA-M method is effective for detecting coral taxa in Taiwanese coastal environments. Furthermore, eDNA-based analyses revealed pronounced regional differences in dominant genera at geographically defined coastal regions of Taiwan. These spatial patterns are broadly consistent with results of previous ecological surveys [20, 21]. Taken together, these findings indicate that the eDNA-based database of Taiwanese scleractinian corals established in this study is reliable and can serve as a valuable foundation for future ecological surveys and long-term monitoring programs.

To efficiently protect an endangered scleractinian coral, *Polycyathus chaishanensis*, it is crucial to precisely determine its distribution. However, due to its low numbers in the wild, it has been difficult to locate this species using conventional methods, such as short, limited-range surveys via diving or snorkeling. In this study, we demonstrated that our eDNA metabarcoding approach [24] is capable of detecting *P. chaishanensis*. Successful detection of *P. chaishanensis* eDNA at Taoyuan Algal Reef sites, where several dozen colonies have been confirmed by previous ecological surveys, provides strong validation of the applicability of the eDNA-M system for this species. This study suggests that the Scl-eDNA-M approach could accelerate the distribution survey of *P. chaishanensis*. By identifying areas where putative *P. chaishanensis*-derived eDNA is present, it will be possible to conduct more focused and efficient diving and snorkeling surveys to find the species in the future.

It is noteworthy that putative *P. chaishanensis*-derived eDNA were detected at multiple coastal sites around Taiwan where its presence had not been reported previously. These sites included Shitiping and Jihui on the eastern coast, South Bay,

Houbihu, and Water Outlet in southern Taiwan, Yuchengwei and Houshi on Xiaoliuqiu Island, and several sites in the Penghu Islands. These findings suggest that the distribution of *P. chaishanensis* may be broader previously currently recognized, based on conventional surveys alone. In coastal systems, eDNA has detected scleractinian coral communities at spatial scales of approximately 700 m [14]. Based on this detection range, our results suggest the possibility that *P. chaishanensis* is likely to occur within approximately 700 m of seawater sampling sites. However, interpretation of eDNA signals requires careful consideration of eDNA production, degradation, and persistence in coastal environments [19]. *P. chaishanensis* is expected to release eDNA primarily through passive processes such as mucus secretion [30] and tissue sloughing, as well as through transient reproductive processes such as gamete and/or larval release [31]. Environmental stressors, including UV irradiation and sedimentation, may further influence eDNA production by altering mucus release [32,33]. In addition, eDNA degradation in shallow coastal waters is likely influenced by environmental factors such as high temperature, ultraviolet radiation, pH, and microbial activity, potentially limiting the temporal window of detectability [34–40]. Conversely, eDNA may persist for extended periods when adsorbed onto sediments or organic particles, raising the possibility that some detections reflect historical rather than contemporary presence [39,41–43]. Therefore, eDNA detection at sites lacking visually confirmed *P. chaishanensis* colonies may be explained by resuspension of sediment-bound eDNA originating from past populations, particularly in dynamic coastal systems subject to wave action and seasonal disturbance. Thus, integrating repeated temporal eDNA surveys with targeted field observations will be essential in the future to disentangle these processes and refine our understanding of the current distribution of *P. chaishanensis*.

Taoyuan Algal Reef sites G1 and G2 both harbor more than several dozen visually confirmed *P. chaishanensi*s colonies [4]. Therefore, they were designated as positive control sites in this study. During the first survey in 2023–2024, *P. chaishanensis* eDNA was detected from seawater collected at G1 as expected, whereas no eDNA signal was detected at G2. Two possible explanations were considered for the failure to detect *P. chaishanensis* eDNA at the G2 site in the first survey in 2023–2024. First, a delay of several hours between sampling and filtration occurred at G2 in the first survey due to a malfunction of the filtration apparatus. This may have resulted in eDNA degradation. Second, sea conditions at G2 were relatively rough on the sampling day, and collected seawater contained a high load of suspended sediment, potentially reducing eDNA recovery efficiency. In contrast, during the second survey in 2025, a clear *P. chaishanensis* eDNA signal was detected at site G2 under calm sea conditions, with filtration and preservation completed within 10 min after sampling. Although it is not possible to identify the primary cause at present, these results suggest that the failure to detect *P. chaishanensis* eDNA at G2 during the first survey is more likely attributable to sample handling conditions than to limitations of the detection system itself.

A limitation of this study is that putative *P. chaishanensis*-derived eDNA signals cannot be unequivocally attributed to this species. Thus, species-level specificity of the eDNA assay could not be fully validated due to the absence of mitochondrial 12S rDNA reference sequences from closely related taxa, such as *Paracyathus* [7]. Consequently, it remains possible that the detected sequences originate from phylogenetically related, yet uncharacterized species. To resolve this limitation, future studies should prioritize the generation of comprehensive mitochondrial reference datasets for family Caryophylliidae, including closely related and sympatric taxa, to enable rigorous in silico and empirical validation of marker specificity. In addition, integration of multiple genetic markers and application of phylogenetic frameworks will be essential. Nevertheless, findings of this study provide important insights for establishing a systematic framework for surveying *P. chaishanensis*, and represent a critical first step toward its effective monitoring and conservation.

The present study did not carry out seawater sampling along the western coast of mainland Taiwan, a region generally reported to support few coral assemblages due to low water clarity and sediment-heavy conditions [20, 21]. Given that *P. chaishanensis* has persisted and formed colonies in environments characterized by elevated turbidity and sedimentation, it is possible that it also inhabits western Taiwanese coastal waters. In addition, seawater sampling was not performed at offshore islands on the eastern and southern sides of Taiwan, including Green Island and Lanyu Island, where the presence of zooxanthellate scleractinian corals has been documented [44–47]. Considering the spatial pattern of eDNA signals corresponding to *P. chaishanensis* observed in this study, it is possible that *P. chaishanensis* may also occur in these regions. Increasing the number of sampling sites and enhancing survey density across currently unsampled coastal and offshore areas will enable us not only to construct a more comprehensive eDNA-based database of Taiwanese scleractinian corals,

but also to achieve a more accurate assessment of the habitat range of *P. chaishanensis* along Taiwan's coast in future studies.

Conclusions

This study provides new insights into the distribution of *P. chaishanensis* in Taiwan, based on eDNA analysis. Information derived from eDNA-based surveys, together with ongoing efforts to establish ex situ propagation and aquaculture techniques, will contribute to safeguarding this species by enabling development of ex situ backup populations to reduce extinction risk and by supporting future restoration initiatives.

Abbreviations

eDNA-M: environmental DNA Metabarcoding

eDNA: environmental DNA

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

Raw sequence data supporting the conclusions of this article have been deposited in the DNA Data Bank of Japan (DDBJ) under BioProject ID PRJDB40393 and DRR Run numbers DRR919038–DRR919143.

Competing interests

We have no competing interests.

Funding

This research was supported by grant from CPC Corporation, Taiwan (LHF1100005) (to SS).

Author contributions

SS and NS conceived and designed the study. YCC, EC, and YCC collected the water samples. CCH contributed to the selection and design of the sampling sites. HN and KH analyzed the data. YLC, SS, CS, and NS wrote the manuscript.

Acknowledgments

We also thank Dr. Steven Aird for editing the manuscript.

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Table

Table 1. Survey and sampling information

Survey ID	Site ID	Sampling date	Region	Site name	Latitude	Longitude
I	1	2024/02/15	Northeast	Coral Farm	25.02127012	121.9870131
I	2	2023/06/30	Northeast	Guihou	25.19904096	121.6922094
I	3	2023/07/14	Northeast	Wanghaixiang	25.13602802	121.8034475
I	4	2023/07/05	Northeast	Taipower SH	25.12706604	121.8188158
I	5	2024/02/20	Northeast	CPC SH-1	25.12457912	121.8275812
I	6	2024/02/20	Northeast	CPC SH-2	25.12363249	121.8287014
I	7	2024/02/20	Northeast	CPC SH-3	25.12621221	121.8287408
I	8	2023/06/19	Northeast	Nanya	25.1298343	121.8901585
I	9	2023/06/19	Northeast	Bitou	25.12536521	121.9147623
I	10	2024/02/15	Northeast	Maoao Bay	25.01793695	121.9903567
I	11	2023/06/26	Northeast	Waiao Beach	24.87781609	121.8436552
I	12	2023/06/26	Northeast	Tofu Cape	24.58440146	121.8728102
I	13	2023/06/26	Northeast	Nanao	24.43668162	121.803395
I	14	2023/06/27	East	Shitiping	23.48511457	121.512684
I	15	2023/06/27	East	Jihui	23.11675826	121.3966051
I	16	2023/06/27	East	Shanyuan	22.83109282	121.1868112
I	17	2023/06/28	Southeast	Xuhai	22.18847268	120.8908006
I	18	2023/06/28	South	South Bay	21.95908046	120.7628562
I	19	2023/06/28	South	Houbihu	21.93909449	120.7457392
I	20	2023/06/28	South	Water Outlet	21.93215757	120.7450787
I	21	2023/06/28	South	Wanlitong	21.99518491	120.7063107
I	22	2023/06/28	Southwest	Xiziwan	22.62531198	120.2632711
I	23	2023/06/29	Southwest	Xiaoliuqiu Island - Beauty Beach	22.35287482	120.3708769
I	24	2023/06/29	Southwest	Xiaoliuqiu Island - Yuchengwei	22.3459572	120.3888752
I	25	2023/06/29	Southwest	Xiaoliuqiu Island- Houshi	22.32571163	120.36616
I	26	2024/03/09	Northwest	Taoyuan Algal Reef G1	25.04505308	121.0572136
I	27	2024/03/11	Northwest	Taoyuan Algal Reef G2-1	25.0377755	121.0503512
II	28	2025/06/24	Northwest	Taoyuan Algal Reef G2-2	25.03900401	121.0502284
II	29	2025/07/08	West	Penghu islands - Qitou	23.65467376	119.6096513
II	30	2025/07/08	West	Penghu islands - Chixi	23.60402051	119.5027707
II	31	2025/07/08	West	Penghu islands - Neian	23.57180151	119.4958129
II	32	2025/07/05	West	Penghu islands - Hangwan-1	23.51664321	119.6056412
II	33	2025/07/08	West	Penghu islands - Hangwan-2	23.51629703	119.6069906
II	34	2025/07/09	West	Penghu islands - Yueli Bay	23.19673451	119.4301724

II	35	2025/07/09	West	Penghu islands - Woniu	23.19052408	119.4317515

Figures

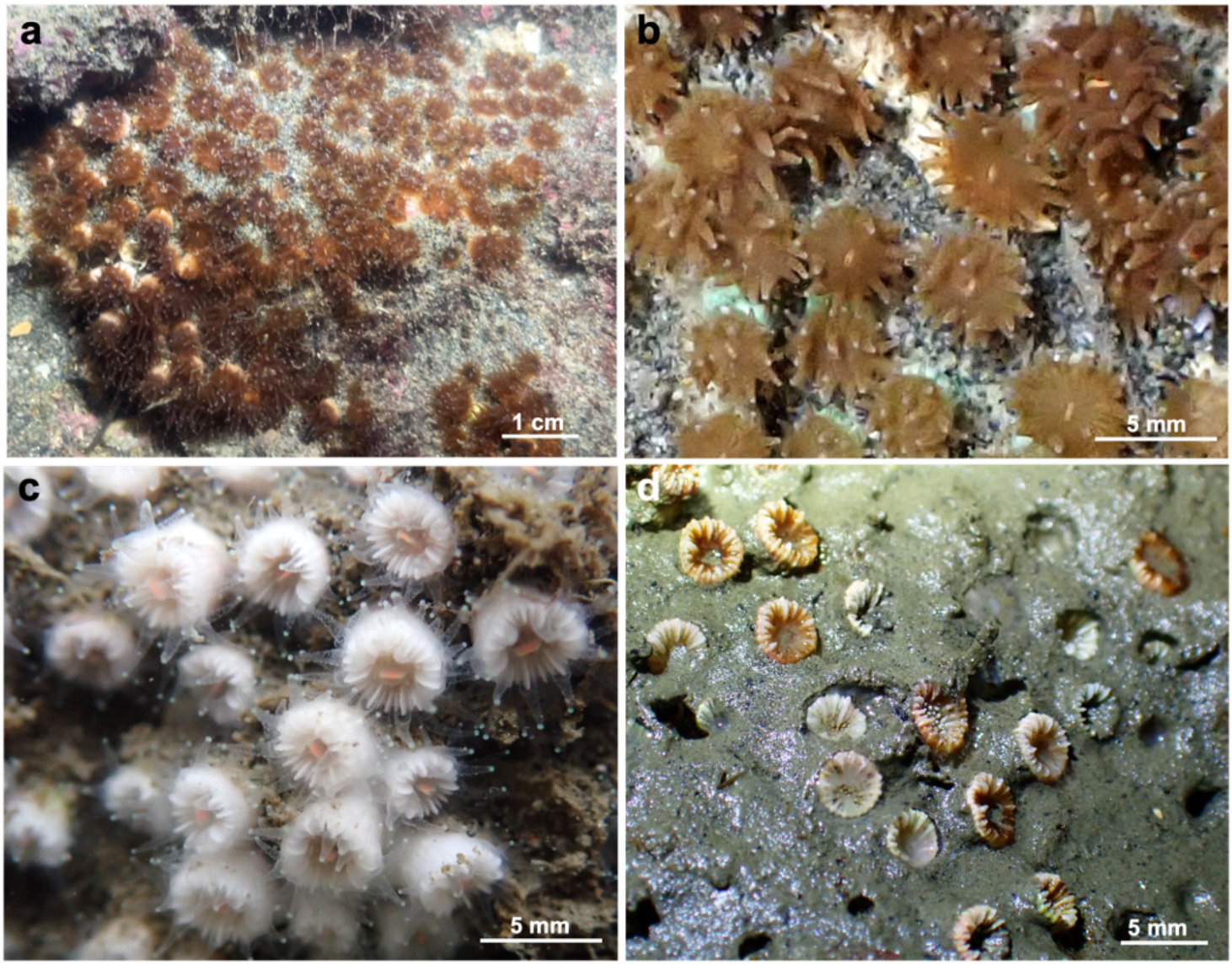


Figure 1

The scleractinian coral, *Polycyathus chaishanensis*, observed in algal reef habitats along the coast of Guan-Shan, Taoyuan City, Taiwan. **a** Colony morphology of *P. chaishanensis*. **b** Representative image of healthy polyps. **c** Bleached polyps. **d** Polyps exposed to sedimentation.

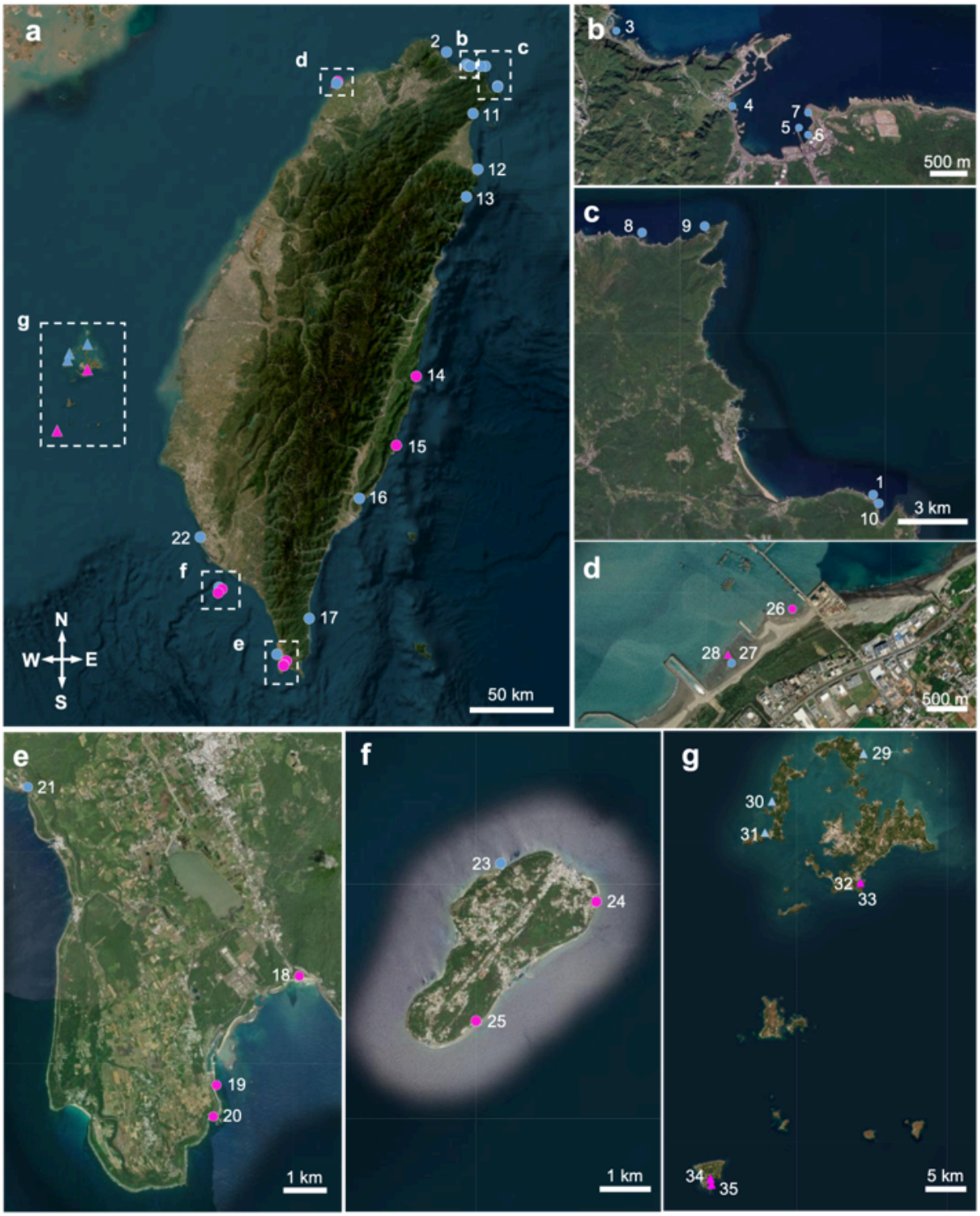


Figure 2

Map of Taiwan and survey sites investigated in this study. **a** Map of the main island of Taiwan and surrounding offshore islands. Circles indicate sites surveyed in the first survey, and triangles indicate sites surveyed in the second survey. Pink symbols denote sites where *P. chaishanensis* eDNA was detected. **b–g** Enlarged maps of the regions indicated by dashed boxes in panel **a**. **b** Five survey sites in the northeastern region of the main island. **c** Three survey sites in another northeastern region. **d** Three survey sites in the northwestern region. **e** Four survey sites in the southern region. **f** Three survey sites on Xiaoliuqiu Island. **g**Seven survey sites in the Penghu Islands. Base maps were provided by the Geospatial Information Authority of Japan.

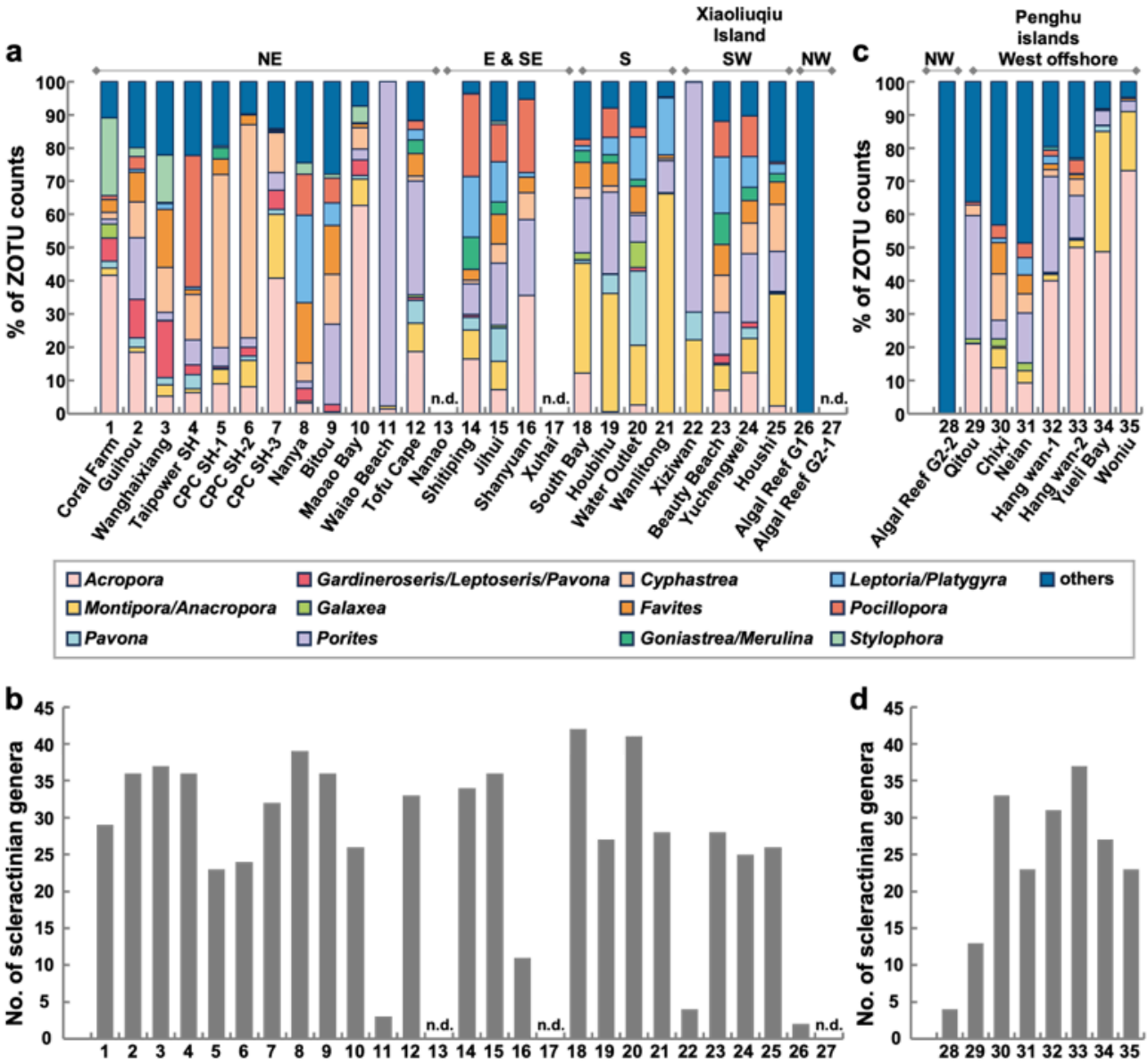


Figure 3

Detected eDNA counts, taxonomic composition, and diversity at 35 survey sites using a scleractinian-specific eDNA metabarcoding system. **a**Numbers of scleractinian ZOTU counts detected in the first survey. **b**Relative proportions (%) of detected scleractinian genera in the first survey. **c**Numbers of detected scleractinian genera in the first survey. **d** Numbers of scleractinian ZOTU counts detected in the second survey. **e** Relative proportions (%) of detected scleractinian genera in the second survey. **f**Numbers of detected scleractinian genera in the second survey. n.d., not detected. NE, northeast; E, east; SE, Southeast; S, South; SW, Southwest; and NW, Northwest.

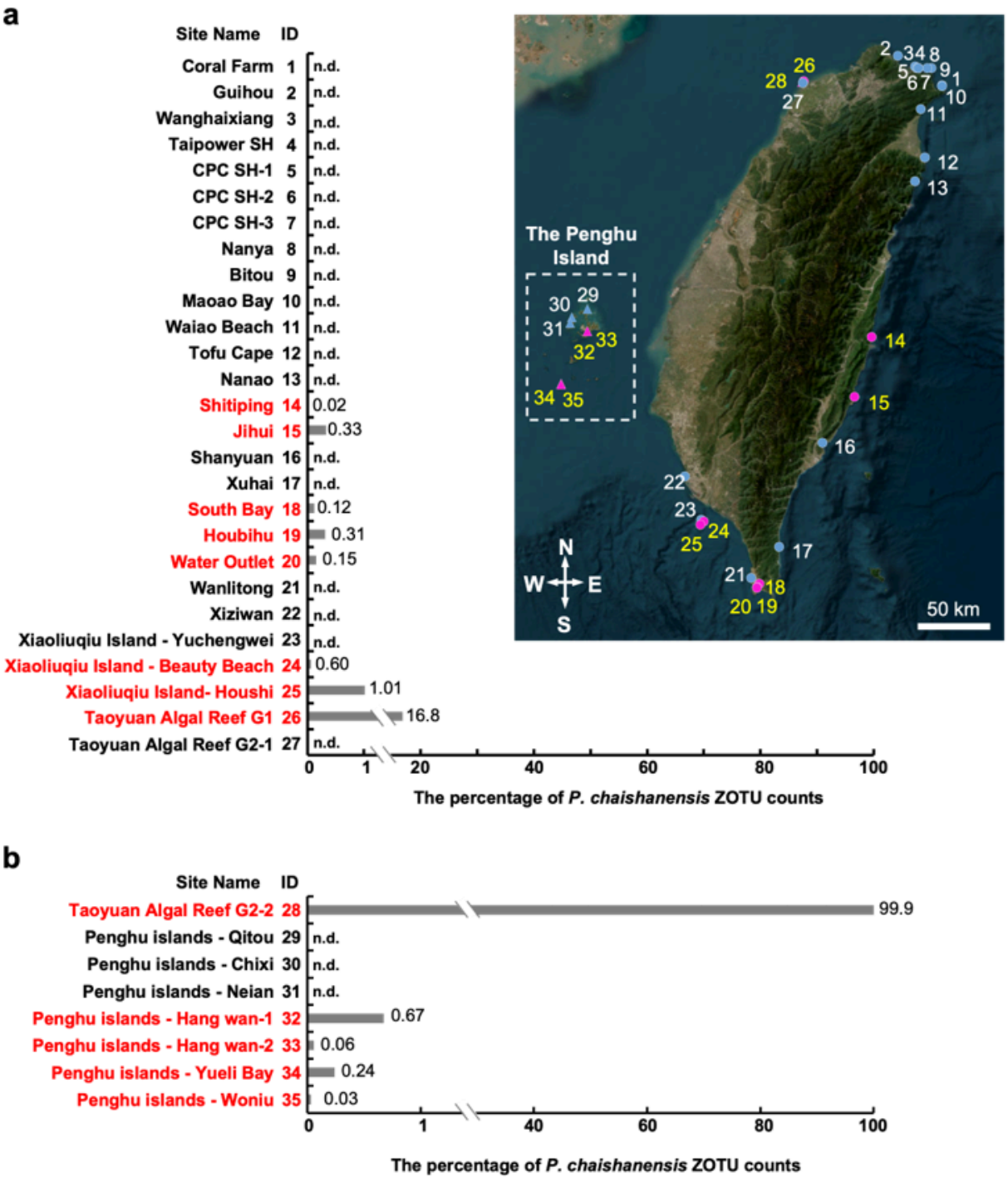


Figure 4

Numbers of ZOTU counts corresponding to *P. chaishanensis* detected at each survey site. **a** Results from the first survey. **b** Results from the second survey. n.d., not detected. Inset maps indicate locations of the survey sites. Circles represent sites surveyed in the first survey, and triangles represent sites surveyed in the second survey. Pink symbols labeled with yellow letters indicate sites where *P. chaishanensis* eDNA was detected.

Supplementary Files

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