**Title: Sanger-based genetic profiling of Pocilloporidae-Symbiodiniaceae symbioses using bleach-treated Hawaiian corals**

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## ABSTRACT

Scleractinians (stony corals) are the principal reef-building organisms in archipelagic environments, which generate structurally complex and biologically rich habitats through a physiological process of bio-calcification. As a holobiont, scleractinians maintain symbiotic associations with dinoflagellate algae (Symbiodiniaceae), which supply photosynthetically fixed carbon, recycled nutrients, and enhanced calcification in exchange for coral-metabolic waste products. These mutualistic exchanges serve to drive reef growth and biological productivity, resulting in an aragonitic ($CaCO_3$) reef framework that provides essential structure and shelter, supporting trophic interactions sustaining both local reef function and global marine biodiversity. However, escalating pressures from climate change, pollution, and overharvesting, necessitate rapid high-resolution assessments of holobiont biodiversity before global reef degradation continues to intensify. Therefore, we evaluated the performance of the E.Z.N.A. Tissue DNA Kit (Omega Bio-tek) at recovering both coral and algal DNA from *Pocillopora acuta* corals collected from a patch reef in Kāneʻohe Bay, Oʻahu, Hawaiʻi. These corals had definitive species-level assignments, providing a unique opportunity to validate DNA recovery, with a particular emphasis on specimens treated with sodium hypochlorite (bleach), a preservative widely employed in both academic and museum curation. Sodium hypochlorite, while effective at removing tissue and surface contaminants, is known to degrade DNA and inhibit enzymatic activity, thereby posing a significant barrier to downstream genetic analysis. To evaluate this effect, corals in this study were selected to be either water-rinsed, bleach-treated, or left in their natural state, with the bleach simulating its curatorial role aimed at eliminating organic material associated from the freshly collected coral skeletons. PCR amplification was performed using primers targeting two coral mitochondrial loci: *cytochrome oxidase subunit I* (CO1) and *12S*

*ribosomal RNA* (12S), as well as the algal nuclear non-coding region: *internal transcribed spacer unit 2* (ITS2). Despite low DNA yields in bleach-treated specimens, both *Pocillopora* and *Symbiodinium* extracts were successfully amplified, sequenced, and validated using GenBank. To our knowledge, this is the first standalone study to recover and sequence both coral and algal DNA from bleach-treated coral skeletons. The workflow presented demonstrated that DNA can be effectively extracted and sequenced from contemporary bleach-treated *P. acuta* specimens using an inexpensive, cost-effective DNA extraction kit.

## INTRODUCTION

Early ship-based expeditions surveying coral reefs began in the late 18th century as part of broader voyages of exploration and scientific discovery. These voyages led by early European explorers often included naturalists who documented various types of marine life across the Indo-Pacific and Caribbean regions (Anderson, 2008; Hoeksema et al., 2011; McCalman, 2014). Many of these journeys resulted in the collection of numerous scleractinian species which have contributed significantly to our understanding of coral biodiversity through the study of coral skeletons (McCalman, 2014). Today, ongoing efforts to survey biodiversity continue, and collections of coral in museums have grown substantially since early scientific exploration began centuries ago (Barnes & Lough, 1996; Connelly et al., 2024; Smithsonian, 2025).

Curated collections of coral are harbors of past and present biodiversity, providing insights into evolutionary change and environmental impacts shaping coral physiology (Connelly et al., 2024; Roth, 2014; Wehi et al., 2012). These collections serve as archives for genetic and phenotypic information, enabling the quantification of historical baselines and shifts in species compositions (Hoeksema et al., 2011). Such analyses are essential, for documenting biodiversity loss and guiding effective conservation strategies which aim to enhance reef resilience amidst the current and future challenges facing coral reefs (Knowlton et al., 2010).

Coral reefs, which collectively support an estimated 25% of all known marine species (Mies et al., 2020), have experienced global decline due to the escalating impacts of climate change and human activity (El-Naggar, 2021). The survival of corals is contingent upon a delicate symbiosis with photosynthetic algae, which depend on a narrow and stable range of environmental conditions required to maintain holobiont homeostasis. Even slight changes in sea temperature or pH have shown to destabilize this relationship, ultimately leading to large-scale

mortality events in coral reef ecosystems worldwide (Descombes et al., 2015). Notably, a recent global analysis reported an approximate 50% decline in coral reef cover between 1957 and 2007 (Eddy et al., 2021), highlighting the significance of curated collections to preserve biodiversity information from the past, even after the loss of living coral tissue had occurred.

In both academic and museum curation, sodium hypochlorite (5-12% NaClO) is routinely used to eliminate exogenous organic material associated with a freshly collected coral skeleton (Clode & Marshall, 2003; DeMartini et al., 2013; Kuffner et al., 2012; Le Tissier, 1988; Maragos & Jokiel, 1986; McLachlan et al., 2021; Rey et al., 2025). This preservation strategy serves to enhance the visibility of fine skeletal features, enabling detailed comparison between specimens, and allowing for dry and space-efficient storage inside of collection spaces (Smithsonian, 2025). As a strong oxidizing agent, sodium hypochlorite produces reactive chlorine species such as hypochlorous acid (HOCl), which cleave phosphodiester bonds and chemically alter nitrogenous bases, resulting in extensive degradation of nucleic acids. This process serves to effectively remove organic tissue and exogenous DNA, yielding clean coral specimens suitable for downstream morphological analysis (Hawkins & Davies, 2002; Osinnikova et al., 2019; Prütz, 1996). Historically, taxonomists have relied on subtle differences in skeletal features in conjunction with taxonomic keys to delineate coral species from one another (Zawada et al., 2019). Yet, this approach has proven unreliable for genus and species-level identification due to high phenotypic plasticity and absence of comprehensive field keys (Neigel et al., 2007; Todd, 2008; Zawada et al., 2019).

Given that bio-calcification in corals involves the deposition of aragonitic calcium carbonate (Constantz, 1986), and that aragonite exhibits both a denser crystalline structure and higher organic content than calcite in mollusk shells (Martin et al., 2021), it is hypothesized that

47 trace amounts of coral and algal DNA could become entrapped during skeleton formation.
48 During deposition, coral epithelial cells and their algal symbionts release nucleic acids into the
49 calcifying medium (Hohn & Reymond, 2019), potentially permitting surface sterilization whilst
50 preserving the internal DNA which remains accessible following specimen pulverization.

51 Building on this hypothetical framework, strategic locus selection consequently becomes
52 critical for maximizing PCR success when DNA is degraded and or scarce, facilitating increased
53 taxonomic resolution of both coral-hosts and their algal symbionts. However, molecular-based
54 approaches for coral species identification have long been hindered by low inter- and
55 intraspecific genetic variation, a consequence of slow evolutionary rates and limited sequence
56 divergence observed in anthozoans (Keshavmurthy et al., 2013). Despite these limitations, the
57 development of genetic markers remains an active area of research driven by their utility in
58 advancing coral systematics and informing coral reef conservation strategies. For scleractinians,
59 mitochondrial loci such as *cytochrome oxidase subunit I* (CO1) and *12S ribosomal RNA* (12S)
60 are frequently employed in phylogenetic studies due to their high cellular copy count and
61 conserved sequence regions, which enable consistent primer binding, albeit with marginal
62 resolution for species identification (Shinzato et al., 2021). In Symbiodiniaceae, the nuclear
63 ribosomal marker *internal transcribed spacer unit 2* (ITS2) has widely been used given its
64 capacity to distinguish *Symbiodinium* lineages within various algal clades (Shi et al., 2021;
65 Quigley et al., 2014). While CO1 and 12S present relative constraints, both loci along with ITS2
66 have proven to be effective for the genetic identification of coral-hosts and their symbiotic algae,
67 supporting phylogenetic analysis of holobiont biodiversity across various types of coral reef
68 systems globally.

To date, studies have shown success in extracting and sequencing DNA from a variety of coral-skeletal material, including Symbiodiniaceae and scleractinian DNA from ancient cores and fossils (del Carmen Gomez Cabrera et al., 2019; Rey et al., 2025; Waller et al., 2007), and alcyonacean DNA from non-bleach treated coral jewelry (Lendvay et al., 2020; Lendvay et al., 2022, Lendvay et al., 2025). To our knowledge, no standalone study has recovered and sequenced both coral and algal DNA from bleach-treated coral specimens, an effort particularly relevant given the low genetic divergence among anthozoans and the known degradative effects sodium hypochlorite has on DNA.

In this proof-of-concept study, we evaluated the performance of the E.Z.N.A. Tissue DNA Kit (Omega Bio-tek) at extracting and sequencing Pocilloporidae and Symbiodiniaceae DNA from Hawaiian corals that were treated with sodium hypochlorite. Our objectives in this study were to: (a) assess the performance of a standardized DNA extraction kit, (b) utilize Sanger sequencing to identify coral-hosts and their symbiotic algae, and (c) establish preliminary results for the subsequent development of a protocol for genotyping sodium hypochlorite-treated specimens to enhance taxonomic precision in archived coral collections. The establishment of a workflow for genotyping archived corals represents a novel opportunity to incorporate molecular tools into curated collections, thereby enhancing the scientific utility of preserved coral skeletons in museum and academic spaces around the world.

## MATERIALS AND METHODS

To assess the performance of the E.Z.N.A Tissue DNA Kit (Omega Bio-tek), DNA was extracted from pulverized and intact *P. acuta* specimens. Coral fragments (58.5 mg – 169 mg) were divided into three treatment groups: positive control, water-rinsed, and bleach-treated, simulating common pre-extraction strategies used in the curation of coral skeletons. Samples originated from a single parent colony and were subjected to DNA extraction, PCR amplification, gel electrophoresis, spectrophotometric analysis, and Sanger sequencing. Efforts were taken to ensure spatial separation between protocols within a single shared laboratory space to minimize the risk of cross-contamination, including the use of dedicated equipment, sterilization procedures, and separate work-benches for each step.

### *Sample selection and collection*

A colony of *P. acuta* (n = 1), sourced from a patch reef in Kāne‘ohe Bay, O’ahu, Hawai’i, was subdivided into six individual fragments (n = 6), transported to Texas A&M University - Corpus Christi, briefly maintained in aquaria, harvested, and subsequently transferred to the Genomics Core Laboratory, Texas A&M University - Corpus Christi for genetic analysis.

### *Sample processing*

Positive-control samples were placed immediately in storage at -80 °C and remained unaltered until lysis after initial harvesting. Water-rinsed samples were chilled for 1 h at -80 °C, immersed for 2 min in sterile Milli-Q water held at 70 °C, dried overnight in a Centrivap (Labconco,

7310021), and returned to storage at -80 °C. Bleach-treated samples were soaked for 24 h in a 10% bleach solution (0.75% sodium hypochlorite), then pre-warmed to 70 °C, rinsed with Milli-Q water and dried overnight using a Centrivap and stored at -80 °C. Negative-control samples were incorporated and composed of Milli-Q water and Omega Bio-tek reagents to assess the effectiveness of contamination control during DNA extraction.

Pairs of coral fragments assigned to the water-rinsed and bleach-treated groups were processed using a TissueLyser II (Qiagen, 85300). One sample from the treatment pair was placed in a pre-chilled stainless-steel grinding jar (Qiagen, 69985) at -80 °C at 25 Hz for 60 s. The coupled counterpart was placed in a 2 mL microcentrifuge tube containing a 5 mm stainless-steel bead (Qiagen, 69989), cooled to -80 °C, and shaken for 60 s at 25 Hz for three cycles. Lysis buffer was added after the first cycle whilst using the stainless-steel beads, ensuring the adapter plate was being inverted between cycles to distribute shear forces evenly. Samples designated as positive controls were not mechanically homogenized to preserve the natural integrity of the coral fragment (Figure 1).

***DNA extraction***

Genomic DNA was extracted using the E.Z.N.A Tissue DNA Kit (Omega Bio-tek) with slight modifications to the manufactures protocol. Specifically, ~100 mg of coral was used in place of the recommended 25 mg of tissue. Samples were incubated at 55 °C for 24 h at 800 rpm on a Thermomixer R (Eppendorf, 5355) with the addition of the optional RNase (100 mg/mL, 4 µl, 24 °C for 2 min) step performed. DNA was recovered in single elutions, each carried out with 100 µL of pre-warmed Elution Buffer (Omega Bio-tek).

### *Confirmation of Pocilloporidae and Symbiodiniaceae DNA*

DNA extracts were amplified using a Mastercycler Pro PCR System (Eppendorf, 950040025), targeting the mitochondrial CO1 and 12S regions of Pocilloporidae and nuclear ITS2 region of Symbiodiniaceae (Shinzato et al., 2021; Pochon et al., 2001; Quigley et al., 2014). Thermocycling parameters included an initial denaturization step of 95 °C at 3 min, followed by 20 s at 95 °C, with marker-specific annealing temperatures for 30 s. Extension occurred at 72 °C for 60 s and was followed by a final extension step of 72 °C for 5 min (Table 1).

PCR amplification was performed in 15 µL reactions containing 0.2 µM of each forward and reverse primer, 1x DreamTaq Green PCR Mastermix (Thermo Fisher Scientific, K1081), and 2.7 - 96.3 ng $\mu L^{-1}$ of template DNA. The used primer pairs (Table 1) targeted three loci: Scle_12S (mitochondrial 12S rRNA), Scle_CO1 (mitochondrial cytochrome c oxidase subunit I), and ITS2 (nuclear internal transcribed spacer 2).

### *DNA size distribution and quantification*

Gel electrophoresis (1x TAE) was employed on a 1% agarose (Thermo Fisher Scientific, BP1356-500) gel to qualitatively visualize the size distribution of amplified DNA template from all treatment groups (Figure 2). Specifically, 1 µL of PCR product was ran alongside 0.6 µL of 100 bp HyperLadder (Bioline, BIO-33056) for sizing. DNA was stained with GelStar (Lonza; 0.05X final concentration) and electrophoresed for 38 min at 90V. Visualization of DNA size was achieved using the Universal Hood II Gel Doc XR+ Imaging System (Bio-Rad, 170-8170). The

concentration of recovered template DNA was quantified using a NanoDrop 2000 (Thermo Fischer Scientific, ND-2000) and tabulated (Table 2).

### *Sanger sequencing and bioinformatics*

Sequencing was performed at the Genomics Core Laboratory, Texas A&M University - Corpus Christi, using an ABI 3730xl capillary Sanger sequencer (Thermo Fisher Scientific, A41046). Raw Sanger chromatograms were inspected in 4Peaks v1.8 and Unipro UGENE v51.0, ensuring the retention of only high-quality regions, with ambiguous base calls, primer sites, and low-quality tails trimmed. Curated reads were queried against the National Center for Biotechnology Information's (NCBI) GenBank Nucleotide Database using BLAST v2.8.1+. High sequence matches were aligned using MUSCLE v3.8.31 (Edgar, 2004). Locus-specific maximum-likelihood phylogenies were generated in R v4.5.0, employing the following evolutionary substitution models: TPM2u + G4 for CO1 and 12S loci, and Jukes-Cantor for ITS2 (https://github.com/Ph-IRES/phylogenetic_tools), and subsequently constructed using FigTree v1.4.4. Genus-level taxonomic assignments for this study required BLAST hits with a 100% query coverage and $\geq$ 97% sequence identity.

## RESULTS

All genomic isolates extracted using the E.Z.N.A. Tissue DNA Kit (Omega Bio-tek) yielded clean locus-specific amplicons producing high-quality Sanger sequences and confirming the recovery of both coral-host and algal-symbiont DNA. BLAST and phylogenetic analysis identified the coral specimens belonging to the family Pocilloporidae, genus *Pocillopora*, and its symbiotic algae as a member of the family Symbiodiniaceae, genus *Cladocopium*.

### *DNA recovery and PCR amplification*

DNA was recovered from all coral samples using each preservation and processing method described. Extractions yielded DNA template concentrations ranging from 2.7 – 96.3 ng $\mu L^{-1}$. Positive control and water-rinsed specimens produced the highest yields, whereas the sodium hypochlorite-treated samples and negative controls resulted in the lowest recovery (Table 2). The Negative control yielded 3.0 ng $\mu L^{-1}$ of detectable DNA, suggesting cross-contamination introduced during laboratory procedures (Table 2).

PCR amplification produced clear bands corresponding to the expected amplicon sizes for each locus (ITS2: 350 bp; CO1: 296 – 302 bp; 12S: 366 – 465 bp) as previously reported (Quigley et al., 2014; Shinzato et al., 2021), confirmed by gel electrophoresis (Figure 2) and quantified with spectrophotometry (Table 2). Notably, extracts from sodium-hypochlorite treated samples despite containing as little as 2.7 ng $\mu L^{-1}$ of DNA yielded clear bands, indicating that chemically treating coral specimens with sodium hypochlorite reduces DNA yield but does not inhibit downstream amplification. No amplification was observed in the negative control.

### *BLAST confirmation and phylogenetic assignments*

High-quality raw bidirectional Sanger reads were obtained for each locus (Table 1). Subsequent processing generated curated read lengths of 300 bp (ITS2), 265 bp (CO1), and 301 bp (12S). BLAST analysis confirmed locus-specific amplification with strong sequence identity matches across the curated read lengths. The nuclear ITS2 sequence showed 100% identity to the algal genus *Cladocopium*, whilst mitochondrial CO1 and 12S sequences matched the coral genus *Pocillopora* with 98.11% and 93.69% identity. These results confirmed successful amplification of both coral-host and algal-symbiont DNA, aligning with expected taxonomic assignments. Maximum-likelihood phylogenetic trees (Figure 3-5) produced branch support values $\geq 50\%$, indicating statistically significant tree topologies.

## DISCUSSION

This proof-of-concept study demonstrated that DNA can be successfully recovered and sequenced from live *P. acuta* corals following treatment with sodium hypochlorite and using the E.Z.N.A. Tissue DNA Kit (Omega Bio-tek).

### *Efficacy of DNA recovery from bleach-treated corals*

Notably, the sodium hypochlorite concentration used in this study of 0.75% was substantially lower than concentrations typically employed in coral skeleton studies, which often exceed 5%. Despite reduced DNA recovery from bleach-treated samples, successful PCR amplification and Sanger sequencing were achieved across all three treatment scenarios. No amplification was observed in the PCR negative control; however, spectrophotometric analysis of the original DNA extract revealed 3.0 ng $\mu L^{-1}$ of detectable DNA, suggesting low-level cross-contamination from non-scleractinian or Symbiodiniaceae DNA introduced during laboratory handling. Although minimal, this highlights the importance of stringent contamination control measures, such as physically separating extraction and amplification workflows in different lab spaces, applying UV irradiation and ethanol for decontamination, and using dedicated consumables, especially when working with rare or irreplaceable museum material (Fulton, 2011).

### *Methodological constraints using archived coral*

Given that our current protocol requires ~100 mg of skeletal material, we advocate for the development and validation of minimally destructive alternatives, such as micro-drilling or

chiseling, to reduce specimen damage from valuable and or historically significant coral specimens (Fox, 2020; Lendvay et al., 2020). This material requirement imposes curatorial constraints and inherently limits the broader applicability of the presented method to be used with precious coral collections.

### *Optimizing DNA extraction workflows in museum-scale genomics*

Subsequent research should prioritize optimizing DNA extraction methods across all common preservation strategies and holobiont taxa, with a particular emphasis on long-term EDTA-mediated calcium carbonate dissolution and use of high-throughput sequencing platforms (Fredd & Fogler, 1998; Lendvay et al., 2020; McLachlan et al., 2021; Niu et al., 2016; Shi et al., 2021). To support conservative bench-top and sampling strategies, future work should also conduct serial-dilution experiments on bleach-treated extracts to determine the minimum DNA template required for successful PCR amplification in both coral-hosts and their algal symbionts. This should be done in conjunction with identifying the minimal starting mass of skeletal material necessary for consistent DNA recovery.

### *Natural history collections and conservation*

As high-throughput sequencing technologies continue to advance and become more accessible, skeletal DNA extraction is positioned to serve as a foundational method for accessing genomic data from archived coral specimens (Nakahama, 2020). Contemporary approaches such as shotgun genome sequencing and target-capture strategies can now generate genomic assemblies from trace DNA inputs, enabling high-resolution phylogeographic profiling of holobiont

communities (Liu & Cheng, 2018; Voolstra et al., 2021). When integrated with geographic collection data, these molecular datasets can inform spatiotemporal models of reef connectivity and resilience, thereby guiding conservation strategies amid accelerating environmental change (Carpenter et al., 2011).

In summary, our results support the hypothesis that bleach-treated corals retain recoverable genetic material entrapped within their aragonitic skeletons. Moreover, future refinements of this approach may extend its applicability to other bio-calcifying marine taxa such as mollusks, echinoderms, and coralline algae, that similarly deposit calcium carbonate capable of entrapping DNA during the process of bio-calcification (Nienhuis, 2009; Wörheide & Jackson, 2011). Collectively, our findings highlight the potential for extracting genetic information from bleach-treated coral specimens preserved in museum and academic collections (Connelly et al., 2024; Nakahama, 2020). The presented study serves as a steppingstone to significantly enhance researchers' capacity to recover DNA from sodium hypochlorite-treated scleractinians. In doing so, it elevates the scientific utility of century old expedition-collected corals, effectively bridging the gap between historical taxonomy and contemporary molecular biology (Smithsonian, 2025; Todd, 2008; Zawada et al., 2019).

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# TABLES & FIGURES

**Table 1.** PCR primer sets and target amplicon lengths used in this study. Coral-host primers targeted the mitochondrial 12S and CO1 regions of Pocilloporidae corals, whereas symbiont-specific primers amplified the algal nuclear ITS2 region of Symbiodiniaceae.

| Locus | Target taxa | Genome | Oligonucleotide sequence (5'–3') | Target length (bp) | Annealing temperature (°C) | Primer citation |
|---|---|---|---|---|---|---|
| ITS2 | Symbiodiniaceae | Nuclear | ITS2-F: GTGAATTGCAGAACTCCGTG | 350 | 57 | Pochon et al. (2001) |
| | | | ITS2-R: CCTCCGCTTACTTATATGCTT | | | |
| 12S | Pocilloporidae | Mitochondrial | Scle_12S_Fw: CCAGCMGACGCGGTRANACTTA | 366–465 | 60 | Shinzato et al. (2021) |
| | | | Scle_12S_Rv: AAWTTGACGACGGCCATGC | | | |
| CO1 | Pocilloporidae | Mitochondrial | Scle_CO1_Fw: ATTGTNTGRGCNCAYCATATGTTTA | 296–302 | 55 | Shinzato et al. (2021) |
| | | | Scle_CO1_Rv: CCCATAGARAGNACATARTGAAA | | | |

**Table 2.** Comparative DNA quantity and spectral ratios across extraction treatments with corresponding gel identifiers (Gel ID) that match lane numbers shown in Figure 2. The positive control provided the highest DNA recovery and the cleanest A260/280 and A260/230 values; water-rinsed samples were intermediate; bleach treatments and the negative control (extraction blank) yielded the lowest DNA recovery. All measurements were obtained using a NanoDrop 2000 spectrophotometer (Thermo Fischer Scientific, ND-2000).

| Sample ID | Gel ID | Elution | DNA ($ng\,\mu L^{-1}$) | A260/280 | A260/230 |
|---|---|---|---|---|---|
| Positive Control | 1 | 1 | 96.3 | 1.85 | 1.90 |
| Water – I | 2 | 1 | 12.6 | 1.71 | 1.03 |
| Water – II | 3 | 1 | 20.6 | 1.77 | 1.10 |
| Bleach – I | 4 | 1 | 2.7 | 1.31 | 0.56 |
| Bleach – II | 5 | 1 | 4.3 | 1.46 | 0.61 |
| Negative Control | 6 | 1 | 3.0 | 1.17 | 0.64 |

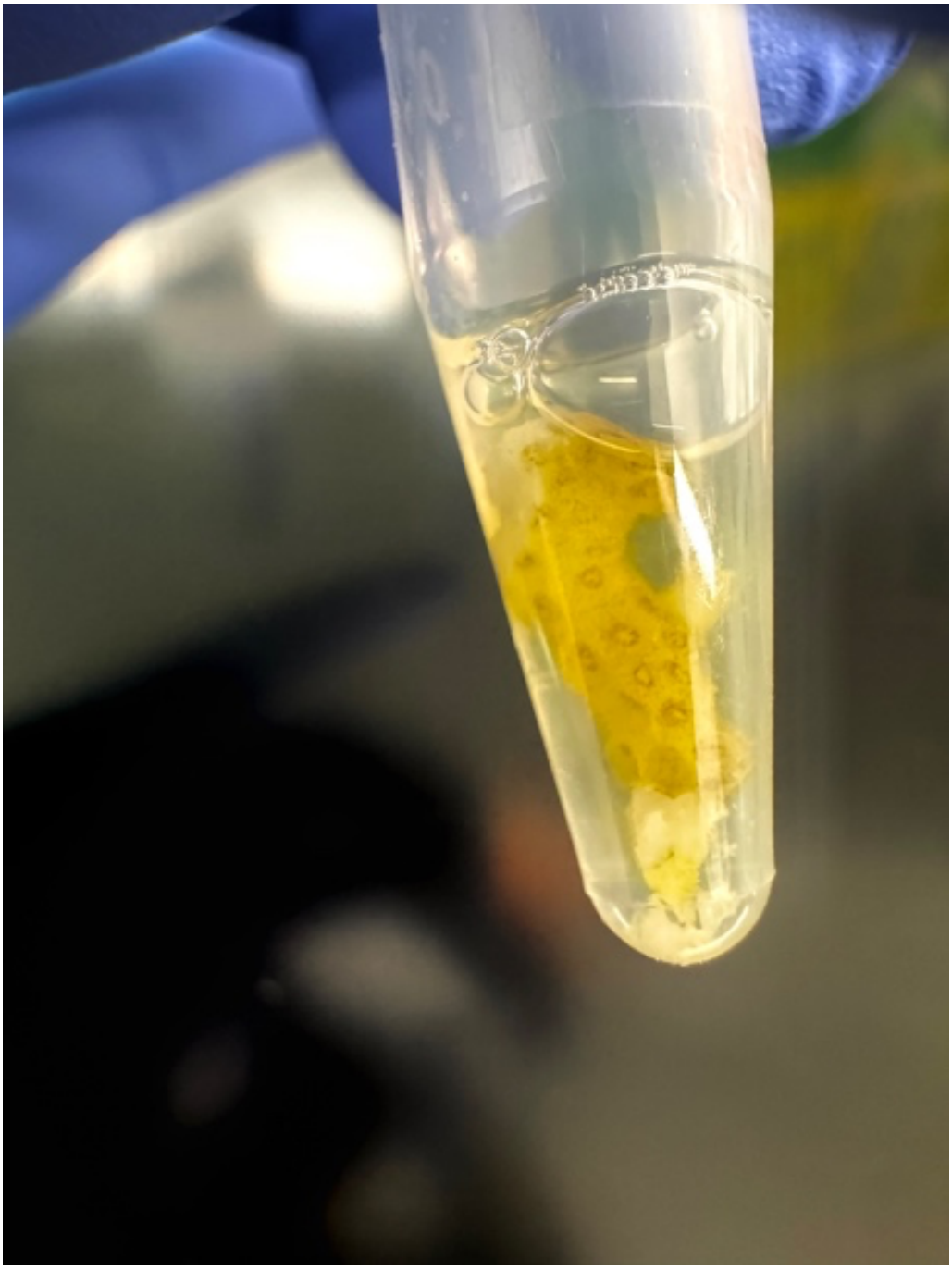

**Figure 1.** Fragment of *Pocillopora sp.* submerged in lysis buffer during DNA extraction using the E.Z.N.A. Tissue DNA Kit (Omega Biotek).

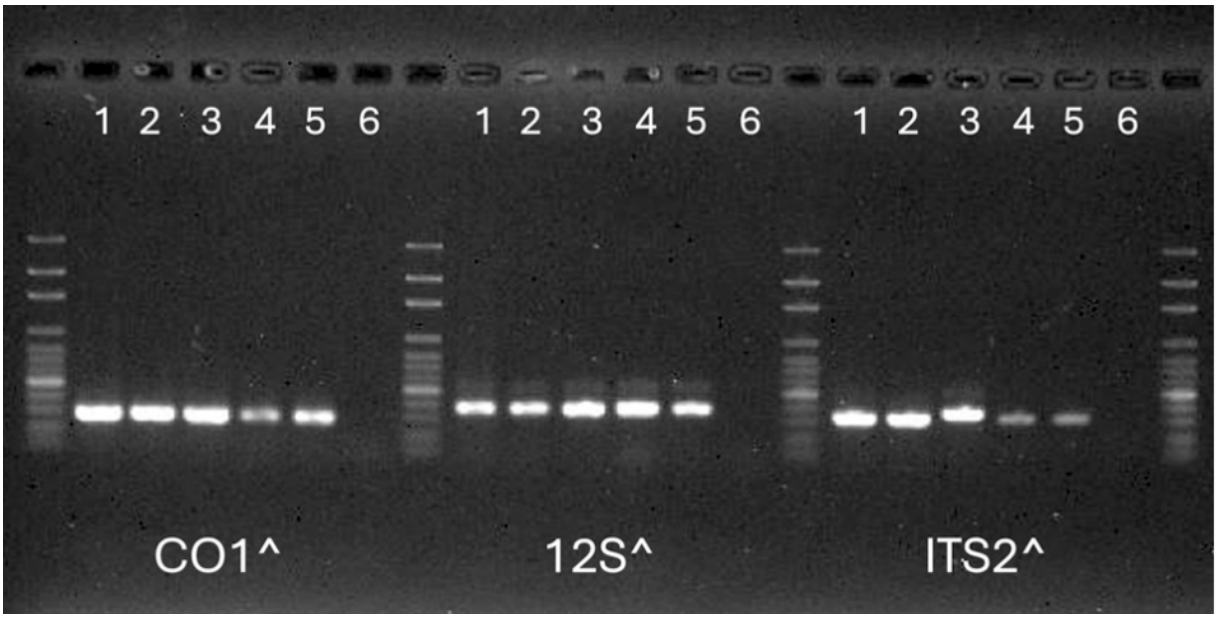


**Figure 2.** Agarose-gel profile of PCR products from *Pocillopora sp.* extracts. Locus-specific bands for the coral mitochondrial CO1 and 12S genes and the symbiont nuclear ITS2 region appear at the expected literature cited sizes listed in Table 1, confirming successful amplification of DNA across all samples and treatments. Lane assignments (Gel ID) correspond to samples listed in Table 2: 1 – Positive Control, 2 – Water I, 3 – Water II, 4 – Bleach I, 5 – Bleach II, 6 – Negative Control.

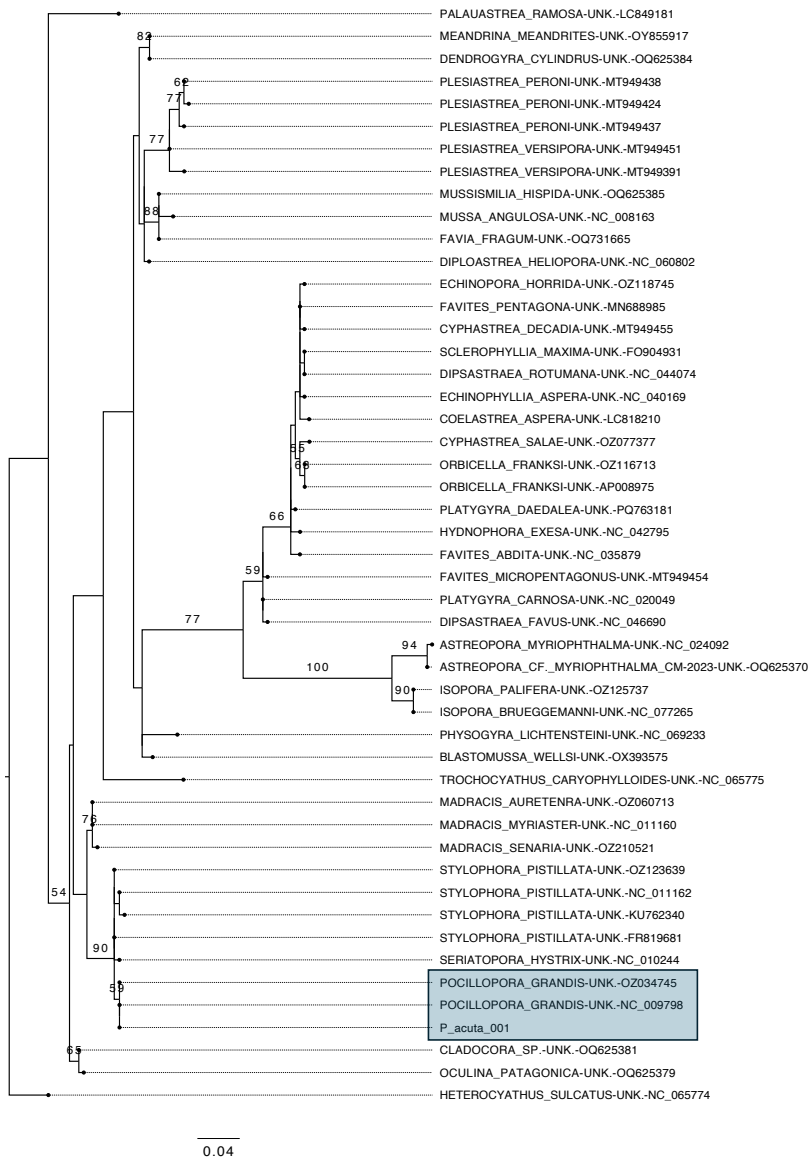


**Figure 3.** Maximum-likelihood phylogeny of 49 scleractinian coral mitochondrial *cytochrome c oxidase subunit I* (CO1) sequences generated using GenBank and a curated 265 bp *Pocillopora sp.* sample sequence (P_acuta_001) in R v4.5.0 using the TPM2u + G4 substitution model. *Heterocyathus sulcatus* (NC_065774) served as the outgroup. Bootstrap-support values from 100 replicates are shown; the blue-shaded clade corresponds to the genus *Pocillopora* with a 59% bootstrap-support value confirming the hypothesized sample sequence placement in the Pocilloporidae lineage and a statistically significant tree topology.

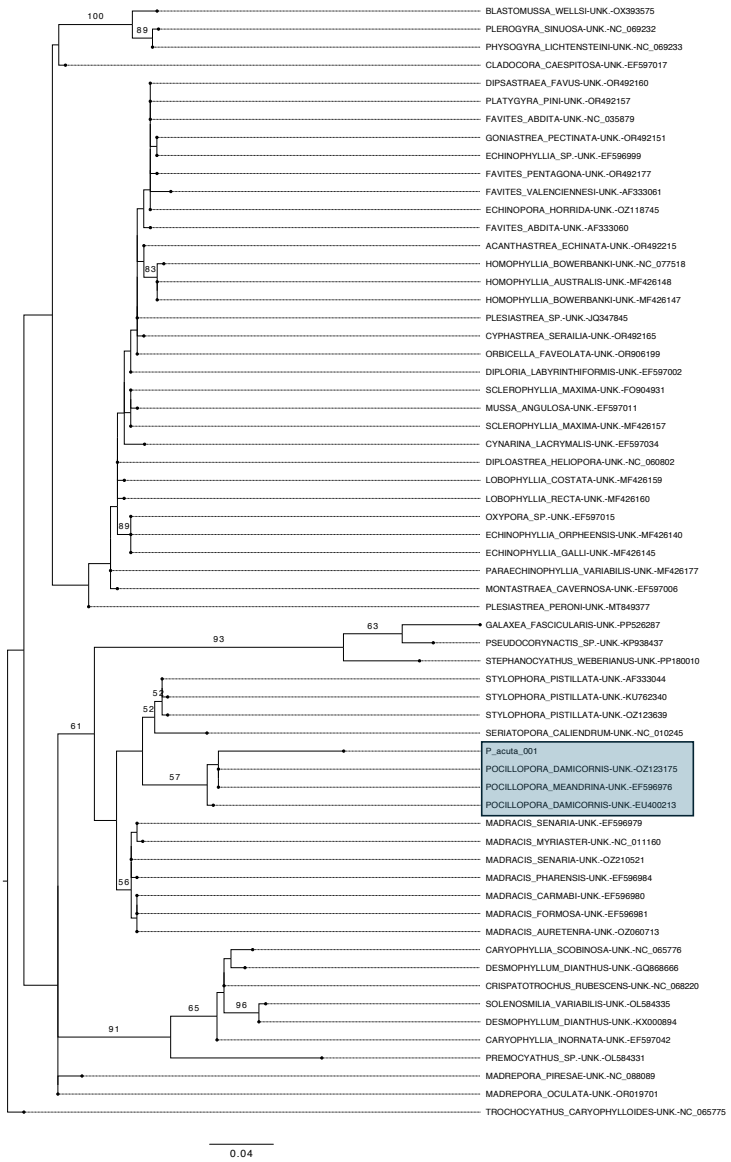


**Figure 4.** Maximum-likelihood phylogeny of 62 scleractinian coral mitochondrial *12S ribosomal RNA* (12S) sequences generated using GenBank and a curated 301 bp *Pocillopora sp.* sample sequence (P_acuta_001) in R v4.5.0 using the TPM2u + G4 substitution model. *Trochocyathus caryophylloides* (NC_065775) served as the outgroup. Bootstrap-support values from 100 replicates are shown; the blue-shaded clade corresponds to the genus *Pocillopora* with a 57% bootstrap-support value confirming the hypothesized sample sequence placement in the Pocilloporidae lineage and a statistically significant tree topology.

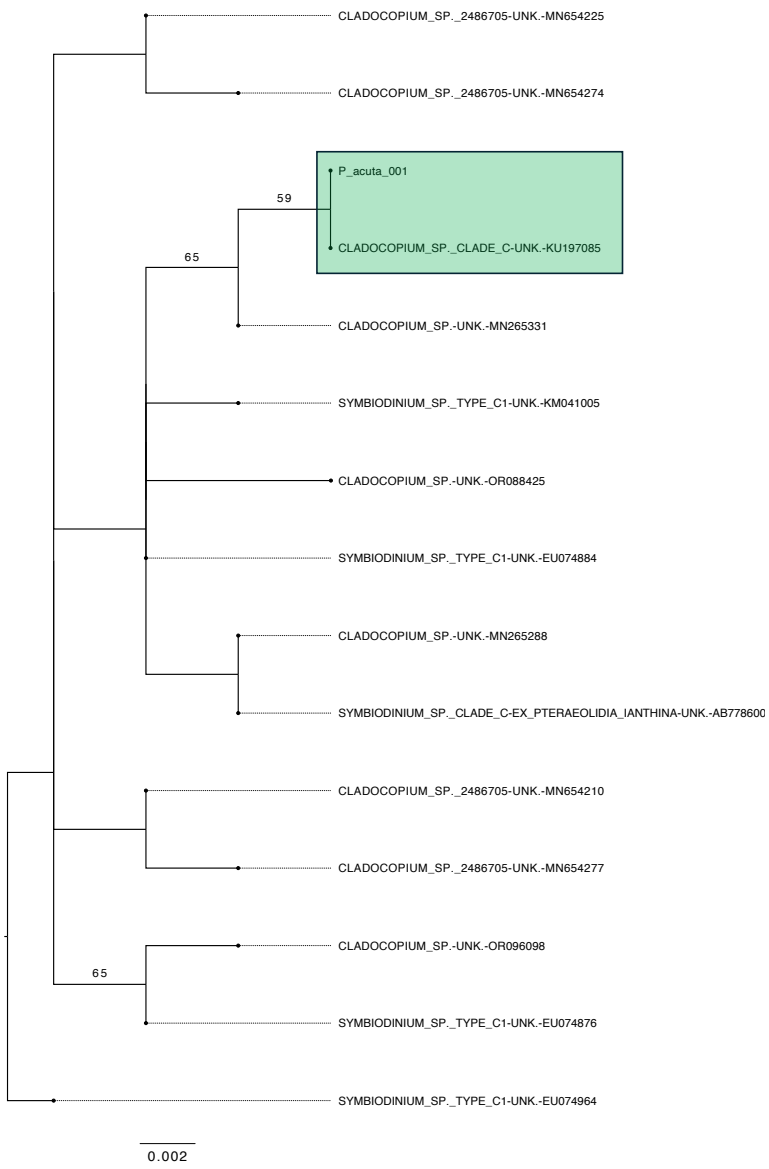


**Figure 5.** Maximum-likelihood phylogeny of 15 Symbiodiniaceae nuclear *internal transcribed spacer 2* (ITS2) sequences generated using GenBank and a curated 300 bp *Pocillopora sp.* experimentally derived sequence (P_acuta_001) in R v4.5.0 using the Jukes-Cantor substitution model. *Symbiodinium sp.* Type C1 (EU_074964) served as the outgroup. Bootstrap-support values from 100 replicates are shown; the green-shaded clade corresponds to the genus *Symbiodinium* with a 59% bootstrap-support value confirming the hypothesized sample sequence placement in the Symbiodiniaceae lineage and a statistically significant tree topology.