



<https://doi.org/10.15517/h7pzq822>

Identifying resilient individuals of *Pocillopora verrucosa* (Ellis & Solander, 1786): insights from diversity assessments for coral restoration

María Teresa Macedo Rodríguez¹; <https://orcid.org/0009-0004-0210-4273>

Eric Bautista-Guerrero¹; <https://orcid.org/0000-0002-4975-1767>

Jeimy Denisse Santiago-Valentín²; <https://orcid.org/0000-0003-2152-7875>

Amílcar Leví Cupul-Magaña¹; <https://orcid.org/0000-0002-6455-1253>

Alma Paola Rodríguez Troncoso^{1*}; <https://orcid.org/0000-0003-3958-6283>

1. Laboratorio de Ecología Marina, Centro Universitario de la Costa, Universidad de Guadalajara, México; teresa.macedo@alumnos.udg.mx, eric.bautista0177@academicos.udg.mx, levi.cupul@academicos.udg.mx, alma.rtroncoso@academicos.udg.mx (*Correspondence)
2. Departamento de Estudios para el Desarrollo Sustentable de Zonas Costeras, Universidad de Guadalajara, México, denisse.santiago@academicos.udg.mx

Received 30-X-2025. Corrected 05-III-2026. Accepted 13-IV-2026.

ABSTRACT

Introduction: The genus *Pocillopora* comprises coral species distributed throughout tropical and subtropical regions. In the Central Mexican Pacific region, *Pocillopora verrucosa* is the main reef-building species. In response to the recent decline in coral coverage, restoration protocols have been implemented over the past decade. However, to date, no genetic diversity records are available as potential markers to evaluate the effect of assisted recruitment on site dynamics in the area.

Objective: Determine the genetic diversity of *P. verrucosa* in an insular (Islas Marietas National Park) and a coastal (Punta de Mita) restoration site within the Central Mexican Pacific.

Methods: A 2 cm² fragment from 15 colonies per site was collected. Mitochondrial markers for the COI and ATP6 genes were amplified. A total of 40 sequences of the COI (n = 19) and ATP6 (n = 21) genes were obtained, and the haplotype and nucleotide diversity were determined.

Results: For the COI gene, two haplotypes shared between the sites were identified, with H1 being the most abundant. For the ATP6 gene, one exclusive haplotype was detected in Islas Marietas National Park, and one more abundant haplotype was shared between the two sites. The AMOVA results revealed a homogeneous pattern with *Fst* values of 0.21603 ($p < 0.10655$) for COI and *Fst* = 0.04174 ($p < 0.3753$) for ATP6.

Conclusions: The low genetic diversity suggests that, as previously reported, asexual reproduction has been the predominant mode throughout the site's history, and that the assisted propagation implemented may promote the maintenance of the individuals that have historically shown resistance to thermal stressors. However, it is essential to explore alternative propagation techniques in future restoration initiatives, as the long-term success of restoration also relies on reducing the vulnerability of these ecosystems to future environmental stressors.

Key words: haplotype; branching coral; Eastern Tropical Pacific; molecular markers; resistance.



RESUMEN

Identificación de individuos resilientes de *Pocillopora verrucosa* (Ellis y Solander, 1786): perspectivas de las evaluaciones de la diversidad para la restauración de corales

Introducción: El género *Pocillopora* comprende especies de coral distribuidas en regiones tropicales y subtropicales. En la región del Pacífico central mexicano, *Pocillopora verrucosa* es la principal especie constructora de arrecifes. En respuesta a la reciente disminución de la cobertura coralina, se han implementado protocolos de restauración en la última década. Sin embargo, hasta la fecha, no se dispone de registros de diversidad genética como marcadores potenciales para evaluar el efecto del reclutamiento asistido en la dinámica del sitio en el área.

Objetivo: Determinar la diversidad genética de *P. verrucosa* en un sitio de restauración insular (Parque Nacional Islas Marietas) y en uno costero (Punta de Mita) dentro del Pacífico central mexicano.

Métodos: Se recolectó un fragmento de 2 cm² de 15 colonias por sitio. Se amplificaron los marcadores mitocondriales de los genes COI y ATP6. Se obtuvo un total de 40 secuencias de los genes COI (n = 19) y ATP6 (n = 21), y se determinó la diversidad de haplotipos y nucleótidos.

Resultados: Para el gen COI, se identificaron dos haplotipos compartidos entre los sitios, siendo H1 el más abundante. Para el gen ATP6, se detectó un haplotipo exclusivo en el Parque Nacional Islas Marietas y un haplotipo más abundante compartido entre ambos sitios. Los resultados del AMOVA revelaron un patrón homogéneo con valores de *Fst* de 0.21603 ($p < 0.10655$) para COI y *Fst* = 0.04174 ($p < 0.3753$) para ATP6.

Conclusiones: La baja diversidad genética sugiere que, como se informó previamente, la reproducción asexual ha sido el modo predominante a lo largo de la historia del sitio, y que la propagación asistida implementada puede promover el mantenimiento de los individuos que históricamente han mostrado resistencia a estresores térmicos. Sin embargo, es esencial explorar técnicas de propagación alternativas en futuras iniciativas de restauración, ya que el éxito a largo plazo de la restauración también depende de reducir la vulnerabilidad de estos ecosistemas frente a futuros factores de estrés ambiental.

Palabras clave: haplotipo; restauración; coral ramificado; Pacífico Tropical Oriental; marcadores moleculares; resistencia.

INTRODUCTION

Coral reefs are considered ecosystems with high ecological and economic value (Souter et al., 2020). Their ecological importance lies in their role as one of the largest reservoirs of marine biodiversity (Sheppard et al., 2010; Suggett & Van Oppen, 2022). Additionally, they are economically important as a source of livelihood for local communities, in activities such as coastal fisheries and tourism (Alquezar & Boyd, 2007). Each coral colony can be considered an individual, and as a result of its growth, the polyps clone into identical genetic organisms that build the biogenic foundation of the entire ecosystem (Knowlton & Jackson, 2001; Sheppard et al., 2010) and, what characterizes a coral community or reef is the live coral cover, as well as its abundance and even the geo-ecological development of the ecosystem (González-Barríos et al., 2021; Suggett et al., 2023). Within the Eastern Tropical Pacific (ETP) region, coral communities are

characterized as monospecific, being dominated by branching corals belonging to the genus *Pocillopora* Lamarck, 1816 (Glynn & Ault, 2000), with changes in the percentage of live coral cover in each locality or region but maintaining a benthic structure in the key species along the ETP (González-Barríos et al., 2021). Given their high abundance, pocilloporids represent a key component of coral communities and have shown differential response to stressors considered natural, such as thermal anomalies known as ENSO (El Niño Southern Oscillation), as well as climate change, and anthropogenic stressors, most of which are local in scale (Glynn et al., 2017; Knowlton & Jackson, 2001). That said, a high capacity for recovery after disturbance has been observed in the ETP (Romero-Torres et al., 2020). Even in sites with massive coral loss, there are records of recovery attributed to both natural processes and the implementation of restoration techniques, also known as assisted propagation (Martínez-Castillo et al., 2022a).

Mitochondrial markers are commonly used due to their maternal inheritance, low recombination rates, and the fact that their mutations represent the unique genetic diversity observed within the population (Avisé, 2009). Particularly, the use of mitochondrial DNA (mtDNA) sequence data, as the cytochrome c oxidase subunit I (COI) gene, has proven to be a robust and widely applied approach for inferring recent evolutionary history, including founder events, population bottlenecks, and phylogeographic patterns in marine organisms (Liu et al., 2020; Moussa et al., 2022; Park et al., 2020). In the study of coral reef systems, this approach may provide a tool to characterize not only the genetic pool of a particular area, but have the potential to reveal new mechanisms of adaptation and assess the vulnerability of a particular location, which is currently highly relevant as a tool to contribute to implementing conservation and management actions, particularly for, coral restoration programs (Suggett et al., 2023; Van Oppen & Gates, 2006).

Previous studies have shown that *Pocillopora* populations with low genetic diversity can be considered demographically unique (Chizzari et al., 2019; Oury et al., 2021). This is particularly relevant in marginal regions, such as the ETP, where high genetic diversity within reefs has been recorded, but significant genetic structure among regions and even within meters of reefs, with the potential for local acclimatization responses along environmental gradients considered non-optimal (Combosch & Vollmer, 2011). A different scenario in the same region may be observed, in which entire sites can be a single clone, resulting in low genetic diversity in sites with high coverage of live coral (Baums et al., 2014). Therefore, *Pocillopora* can be structured by eco-regions, with exclusive genotypes in northern and southern populations, indicating restricted gene flow and localized genetic clusters (Dennis et al., 2024). Across the region, barriers created by ocean currents prevent connections between different populations, but changes in oceanographic conditions during El Niño Southern Oscillation (ENSO) events

intermittently promote the exchange of propagules (Paz-García et al., 2012).

In the Central Mexican Pacific (CMP), mass coral bleaching events have been primarily associated with thermal anomalies driven by ENSO events. However, differential mortality has been documented, with elevated temperatures during El Niño events resulting in catastrophic mass mortality, up to a 90% loss of live coral cover during the 1997-98 event (Carriquiry et al., 2001; Reyes-Bonilla et al., 2002). In this scenario, it is also important to consider La Niña events, which induce anomalous decreases in seawater temperature, trigger bleaching responses, and are usually followed by substantial recovery, with observed mortality as low as 1% (Cruz-García et al., 2020). Additionally, anthropogenic pressures have further negative effects, with urban development among the primary threats (Martínez-Castillo et al., 2020), but also non-regulated tourism has also been shown to be detrimental for the coralline areas (Burroughs & Rodríguez-Troncoso, 2024), promoting differential recovery response even among nearby sites (Carriquiry et al., 2001; Martínez-Castillo et al., 2022a). In response to tackle the loss of coral coverage, a science-based coral restoration program has been implemented over the past decade along the CMP, utilizing direct asexual propagation of coral fragments with positive results, increasing live coral cover and survival, with bleaching but not mortalities even during the 2014-2016 El Niño event (Tortolero-Langarica et al., 2025). Along the years, the restoration program have use as a toolbox to determine the success, the colonies survival, growth and increase in live coral cover (Tortolero-Langarica et al., 2019); also, the sexual reproduction capacity of the translocated colonies has been assessed by demonstrating the presence of gametes in adults resulting from both natural and assisted recruitment (Martínez-Castillo et al., 2023). However, the use of molecular tools as part of the restoration markers has not been implemented in the CMP nor in any restoration protocol along the ETP. Therefore, in order to increase the toolbox, the present study

represents a first approach to assess the genetic structure and haplotypic diversity of *Pocillopora verrucosa* (Ellis & Solander, 1786) in two survivor communities that have been subjected to historical mass coral bleaching events and recurrent restoration efforts: an insular site, Islas Marietas National Park, with restricted but constant touristic pressure, and a nearby coastal site, Punta de Mita, which is a highly impacted area with the presence of high-end hotels and golf courses. Given that both sites have shown positive results with the asexual propagation technique (Tortolero-Langarica et al., 2019), our goal is to include genetic diversity as an additional marker to assess the possible implications of asexual propagation on the structure of the restored community.

MATERIALS AND METHODS

Study site: The Mexican Central Pacific is a region characterized by an average salinity of 35 psu and a pH range of 7.72 to 8.03 (Comisión Nacional de Áreas Naturales Protegidas

[CONANP], 2007; Cupul-Cortés et al., 2018). The average sea surface temperature (SST) is 26.4°C, with a minimum of 23°C in March and a maximum of 30°C in September. These conditions are annually influenced by three currents: the California Current, the Gulf of California Current, and the Costa Rica Current, which converge with the North Equatorial Current (CONANP, 2007; Wyrтки, 1966). In addition, local processes such as the presence of internal waves (Plata-Rosas & Filonov, 2007) and water transport processes caused by upwellings, which occur periodically and seasonally, promote vertical movements of deep, cold-water masses, enriching the water column with inorganic nutrients (Portela et al., 2016; Wyrтки, 1966).

Pocillopora verrucosa samples were collected from two sites within the Mexican Central Pacific: Islas Marietas National Park (IMNP), an insular area (20°41'56" N, 105°35'06" W), and Punta de Mita (PM), a continental site (20°41'51.9" N, 105°34'07.9" W), both separated by 8 km (Fig. 1). The IMNP is categorized

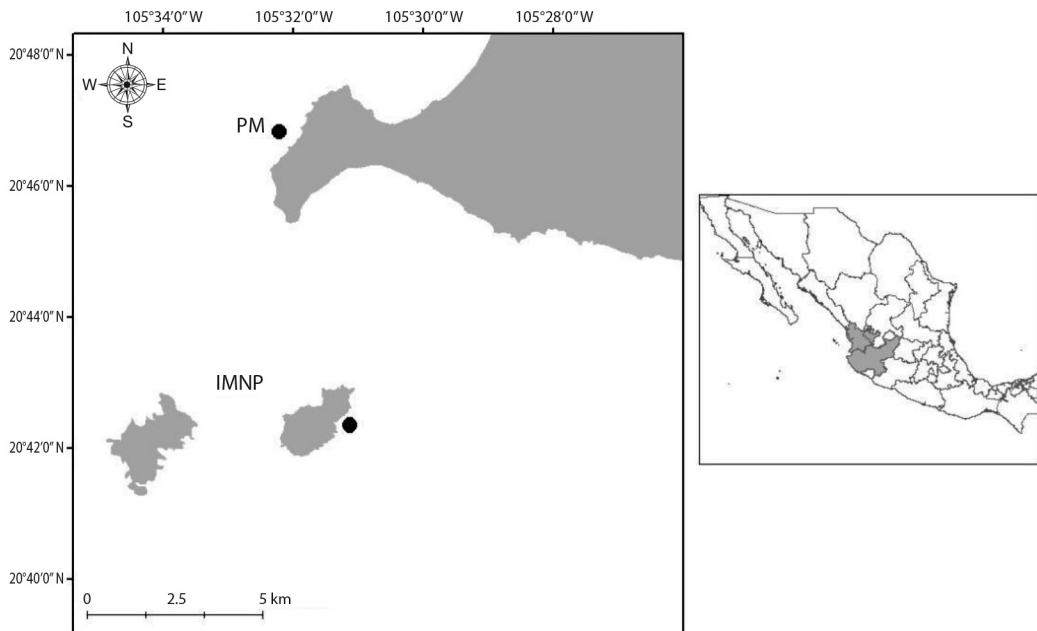


Fig. 1. Study area location. An insular restoration site: Islas Marietas National Park (IMNP), and a coastal restoration site: Punta de Mita (PM), located in the Central Mexican Pacific Region.

as a Natural Protected Area (NPA) comprised by Isla Redonda, Isla Larga, and two islets, with a benthic structure composed primarily of hermatypic corals, but also of rocky and sandy bottoms ranging in depth from 6 to 30 m (CONANP, 2007). The coral community has been characterized as a biodiversity hotspot at the regional level (Glynn & Ault, 2000), and due to their value for the local population, regulated tourism and recreational activities are permitted, the most popular being SCUBA diving or free diving, which exerts moderate pressure on the park's natural resources (Cupul-Magaña & Rodríguez-Troncoso, 2017).

The Punta de Mita site has experienced minimal direct pressure from tourism uses in its marine area; however, its terrestrial area has experienced high anthropogenic pressure from tourism derived from the development of the Nuevo Vallarta-Punta de Mita tourist corridor, with an important urbanization due to the construction of hotels and golf courses (ECOPLAMB, S.A. DE C.V., 2004). Despite this, a patch-shaped coral community is found along the coast, home to corals of the genus *Pocillopora*, which have demonstrated a high capacity for resilience and recovery in the face of both natural and anthropogenic stressors (Martínez-Castillo et al., 2022a).

Sample Collection and Processing: At each location, fragments of the *P. verrucosa* colonies were collected by SCUBA diving. A sample of approximately 2 cm² was obtained from each colony using a chisel and hammer, with a total of 15 colonies sampled at each site. To mitigate the collection of clones, each sampled colony was separated by at least 5 m. The collected samples were preserved in 96 % alcohol and stored at -20 °C until further processing.

Tissue was extracted individually from each coral fragment using a needle and forceps, and a Zeiss® Stemi 508 stereomicroscope for visualization. The resulting tissue was stored in 1.5 ml tubes with 500 µl of 96 % ethanol. Subsequently, genomic DNA was extracted using the salt-out method (Aljanabi & Martínez, 1997). Partial sequences of the mitochondrial

genes cytochrome oxidase subunit 1 (COI) and ATP synthase subunit 6 (ATP6) were amplified by polymerase chain reaction (PCR). The use of these molecular markers stems from the fact that COI and ATP6 are generally considered complementary and therefore primary mitochondrial markers, owing to their strong species-level resolution, and, particularly for *Pocillopora*, both genes have extensive reference databases. Primers were designed based on the *Pocillopora verrucosa* reference genome project (PRJNA551401) available at the National Center for Biotechnology Information (NCBI). The COI fragment (~1500 bp) was amplified using FCOIPv: 5'-CCCCGTCTA-AATGCTTTCTG-3' and RCOIPv: 3'-CGG-TATATAAACCCGGCACT-5', while the primers FATP6Pv: 5'-GAGGCCTTAGGGCA-GATTTT-3' and RATP6Pv: 3'-ACAT-GAGCCATCATCCCTTC-5' were used for amplification of the ATP6 (~678 bp). Each PCR reaction included 7.23 µl of nuclease-free H₂O, 0.75 µl of MgCl₂, 0.66 µl of dNTPs, 2.5 µl of 10x buffer, 0.13 µl of each primer, 0.10 µl of Taq polymerase (Promega®), and 1 µl of DNA.

Amplification for the sequences was carried out in an Applied Biosystems® thermal cycler using the following conditions: an initial denaturation step at 95 °C for 2 minutes, 35 cycles comprising 50 seconds at 95 °C, 50 seconds at 58 °C, and 1 minute at 72°C, and a final extension cycle at 72 °C for 10 minutes. PCR products were visualized on a 2 % agarose gel in TAE buffer (Tris-acetate-EDTA; 40 mM Tris base, 20 mM glacial acetic acid, 2 mM EDTA, pH 8.3). Reactions were classified as positive amplification (single band on the agarose gel) or negative (no apparent band). Only positive reactions were purified using a commercial DNA purification kit (Promega®) and then sent to Macrogen Inc.® in Seoul, Korea for sequencing.

Bioinformatic and Statistical Analysis: The sequences were manually edited in Geneious Prime®, and multiple alignments were performed with ClustalW in Mega11®. To confirm species identity, consensus sequences were



queried against the National Center for Biotechnology Information (NCBI; <https://www.ncbi.nlm.nih.gov/nuccore>) database using the Basic Local Alignment Search Tool (BLAST). Also, the COI and ATP6 sequences obtained from the two sites were deposited in the GeneBank database, and the accession numbers are included as supplementary material (Table S1).

For each location, genetic diversity levels were calculated using estimates of haplotype diversity (Hd) and nucleotide diversity (Pi). This estimate was performed using the DnaSP software v. 6 to analyze DNA sequence polymorphisms (Rozas et al., 2003). Genetic differentiation between sites was assessed using an analysis of molecular variance (AMOVA), and comparisons were made based on estimates of the fixation index *Fst* with 10 000 permutations using the Arlequin® v3.5.2 software (Excoffier & Lischer, 2010).

A haplotype network was created for each molecular marker using the algorithm implemented by Templeton et al. (1992), which employs statistical parsimony (TCS), with a 95 % confidence interval (Clement et al., 2000). The calculations were carried out using the Population Analysis with Reticulate Trees PopArt® software, as described by Leigh & Bryant (2015).

RESULTS

A total of 13 IMNP colonies and 8 PM colonies were successfully processed. In total, 40 sequences of the COI (*n* = 19) and ATP6 (*n* = 21) genes were obtained due to difficulties

during amplification. For the IMNP site, 12 COI gene sequences were obtained, and two haplotypes were detected. Regarding the ATP6 gene, 13 sequences were obtained, with two haplotypes. At the Punta de Mita site, seven COI gene sequences with two haplotypes were detected, whereas at the same site, eight ATP6 gene sequences were obtained, with only one haplotype detected (Table 1).

In the COI gene sequences analyzed, only one polymorphic site was found in position 437, corresponding to two haplotypes. Also, for the ATP6 gene, only one polymorphic site in position 374 was identified, and represented by two distinct haplotypes. Haplotype diversity values were low for both genes from both localities. In IMNP, the haplotype diversity values were 0.167 for COI and 0.153 for ATP6, whereas at the coastal site of PM, they were 0.571 (COI) and 0.000 (ATP6). Nucleotide diversity was also evaluated, with values of 0.00011 for the COI gene and 0.00022 for ATP6 at IMNP. Meanwhile, for the coastal site, Punta de Mita, the values were 0.00039 for the COI gene and 0.00000 for the ATP6 gene (Table 1).

The COI gene analysis allowed the construction of a haplotype network revealing a main group composed of two haplotypes connected by a single mutational step. Both haplotypes were present in the two sampled locations, with the COI H1 haplotype being the most abundant in each locality (Fig. 2a). Similarly, the ATP6 gene network comprised two haplotypes separated by one mutational step. The ATP6 H1 haplotype was the most frequent and occurred in both locations, whereas the

Table 1
Haplotype and nucleotide diversity in the coral *Pocillopora verrucosa*.

Site	Gene	n	H	Hd	Pi
IMNP	COI	12	2	0.167 ± 0.134	0.00011 ± 0.00009
IMNP	ATP6	13	2	0.153 ± 0.126	0.00022 ± 0.00037
PM	COI	7	2	0.571 ± 0.119	0.00039 ± 0.00008
PM	ATP6	8	1	0.000	0.000000

Sample size- colonies successfully amplified (*n*), number of haplotypes (H), haplotype diversity (Hd), and nucleotide diversity (Pi). Sites: Islas Marietas National Park (IMNP) and Punta de Mita (PM).

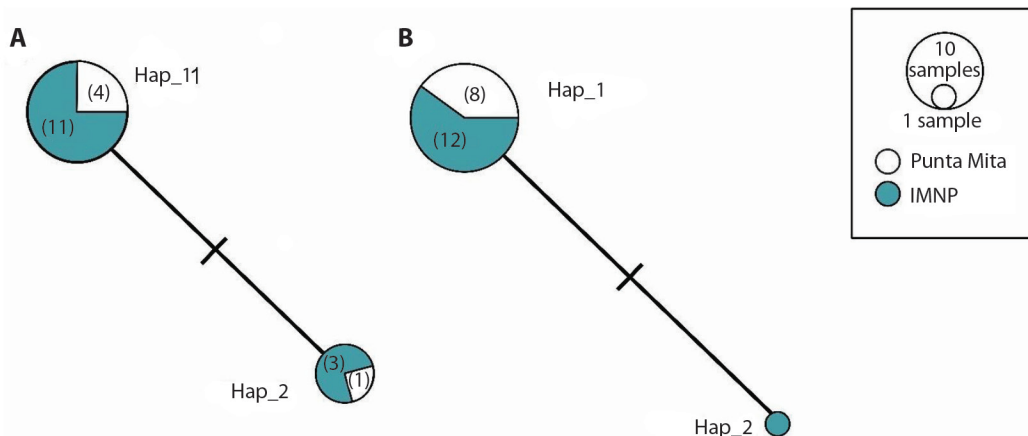


Fig. 2. TCS haplotype network of the **A)** COI and **B)** ATP6 gene in *Pocillopora verrucosa*. The locations are represented by colors; IMNP = Islas Marietas National Park (green) and Punta de Mita (white). Horizontal segments connecting the network nodes represent nucleotide substitutions. Numbers in parentheses indicate the abundance of individuals (n) associated with each haplotype.

ATP6 H2 haplotype was detected exclusively in IMNP and was represented by a single individual (Fig. 2b). Finally, the Analysis of Molecular Variance (AMOVA) revealed a homogeneous pattern across both gene loci, with overall F_{st} values of 0.21603 ($p < 0.10655$) for COI and $F_{st} = 0.04174$ ($p < 0.3753$) for ATP6, indicating no significant genetic differences between the studied sites.

DISCUSSION

Pocillopora is the most widespread reef-building coral, but also considered a highly vulnerable species complex, as historical records have shown massive bleaching and mortality events during extreme heat waves caused by ENSO events (Carriquiry et al., 2001), with contrasting ability to recover in the ETP region (Romero-Torres et al., 2020). The high restoration potential of this coral genus, particularly in the Mexican Pacific, can be attributed to its capacity for acclimatization and resilience, as well as to its rapid growth rates and branching morphology, which may enhance habitat availability and structural complexity over short temporal scales (García-Medrano et al., 2023;

Tortolero-Langarica et al., 2019; Tortolero-Langarica et al., 2025). In this context, our evaluation of the genetic structure and haplotypic diversity of two coral sites that have historically experienced mass mortality events provides valuable insights into the mechanisms underlying resilience to environmental stress. These findings contribute to assessing the long-term feasibility of restoration strategies and support informed decisions on incorporating this genus into coral reef restoration toolkits.

In this context, mitochondrial DNA (mtDNA) analyses provide critical insights into recent evolutionary dynamics, including founder events and population bottlenecks (Liu et al. 2020; Moussa et al., 2022; Park et al., 2020) and have been previously proven in marine invertebrates (Hellberg et al., 2002; Muller et al., 2003). The mitochondrial genome plays a fundamental role in cellular energy metabolism and is therefore closely associated with organismal responses to ecological pressures; accordingly, genetic variation is reflected not only in the nuclear genome but also in mitochondrial genes, which may contribute to observed patterns of population persistence and resilience (Xing et al., 2025). For the management and



conservation of coralline areas, this information is particularly relevant under contemporary scenarios marked by recurrent mass mortality events, as it helps to identify genetic signatures associated with persistence and recovery in reefs that have remained resilient despite ongoing local stressors (Souter et al., 2020).

Nucleotide and haplotype diversity analyses of both molecular markers (COI and ATP6) showed low genetic diversity. It has been observed that, unlike other marine invertebrates, cnidarian mtDNA evolves up to 20 times more slowly than in most metazoans (France & Hoover, 2002; Shearer et al., 2002); still, our results reveal a diagnostically informative pattern. Specifically, we detected extremely low haplotypic diversity and negligible genetic differentiation between localities. While the comparatively slow evolutionary rate of mitochondrial markers in anthozoans has often been cited as a limitation for resolving fine-scale genetic structure, in this context, such conservatism constitutes a key diagnostic advantage. Rather than reflecting insufficient marker resolution, the observed genetic homogeneity provides strong evidence of a pronounced historical genetic bottleneck and suggests that a metapopulation dynamic shaped by sustained connectivity among local coral communities, along with other traits (e.g., reproduction plasticity), must be considered to understand the high resilience of this group.

Branching corals, particularly the genus *Pocillopora*, are distinguished by a prevalence of asexual reproduction through fragmentation (Chávez-Romo & Reyes-Bonilla, 2007; Whitaker, 2006), which generates clones that recruit in proximity to the progenitor colonies. This could explain the low haplotypic diversity observed between the two genes across both studied locations and the high frequency of the H1 haplotype in both locations, resulting in genetic homogeneity at the site level and suggesting that all individuals are genetically identical. This could be considered as negative, as low genetic diversity is often associated with low fitness; however, it has been shown to be a successful strategy that increases cover

at a faster rate, while also maintaining and stabilizing species populations and maintains within the population the genotypes that currently prove to be the most resistant (Adjeroud & Tsuchiya, 1999; Miller & Ayre, 2008; Stoddart, 1984). However, it should not be overlooked that, although asexual reproduction has been a successful strategy thus far, one of its consequences is the prevalence of clonal organisms, resulting in genetic homogeneity within the population. Interestingly, the H1 haplotype was shared at both locations, indicating that at some point there was a connection or exchange of propagules between the sites. However, unique haplotypes were also evident, indicating that *P. verrucosa* can reproduce both sexually and asexually at both locations, as has been observed throughout the region (Santiago-Valentín et al., 2020), with an evident high rate of self-recruitment and sporadic subsidiary recruitment.

Although the results showed no significant genetic differentiation between *P. verrucosa* colonies from both locations, this does not indicate the absence of genetic diversity in other parts of the genome. The conceptualization that low diversity is synonymous with poor health should be taken with caution, since, in pocilloporids, as in the rest of the hermatypic corals, their capacity for resistance and resilience is not only attributed to the genetic diversity of the coral but to the entire holobiont (Berkelmans & Van Oppen, 2006; Howells et al., 2012). Corals are characterized by their symbiotic relationships with other groups, such as their endosymbiont dinoflagellates of the family *Symbiodineaceae*, as well as assemblages of bacteria, fungi, and other viruses (Herre et al., 1999); therefore, the coral physiology, and their susceptibility or tolerance, is the response to the whole holobiont (Rowan et al., 1997; Warner et al., 1996). In particular, *P. verrucosa* in the study region harbors only *Durusdinium glynni* algal endosymbiont (Martínez-Castillo et al., 2022b), which is considered a high-thermo tolerant dinoflagellate (Baker et al., 2017; Berkelmans & Van Oppen, 2006). In addition, its bacterial assemblage (microbiome) has been found to

respond to changes in local conditions, primarily high concentrations of inorganic nutrients (Ostria-Hernández et al., 2022). Therefore, the response, as well as the persistence of *P. verrucosa* genotypes in the region, results from the holobiont, not just from the cnidarian's genetic identity (Van Oppen & Gates, 2006).

This approach leads to the need to implement and conduct new comprehensive studies that allow us to determine the composition and dynamics of the holobiont, as the theory establishes that effective coral restoration depends on preserving genetic diversity by using multiple, genetically distinct local donor colonies and ongoing genetic monitoring as an approach to enhance resilience and support long-term reef recovery (Banaszak et al., 2023; Blanco-Pimentel et al. 2023; Chan et al., 2018; Rios et al., 2024; Van Oppen & Gates, 2006). The observed genetic homogeneity reflects low genetic variability and minimal differentiation among sites. This pattern suggests that the surviving coral colonies exhibiting resistance to thermal stress do not represent a random subset of the population but rather belong to a shared evolutionary lineage. Consequently, this genetic coherence may reduce the risk of outbreeding depression when such colonies are considered for restoration or propagation efforts, and therefore, assisted asexual propagation has promoted the maintenance of haplotypes that have historically shown resistance to thermal stressors. Additionally, it has provided us with other initial insights, such as that the genetic resources available for natural recovery may be limited and unstable. Consequently, reduced genetic diversity could constrain the capacity for natural recovery, supporting the need for timely implementation of active restoration strategies.

In this context, our findings provide a rationale for considering assisted gene flow and the translocation of coral fragments among localities as potential measures to enhance fertilization success while maintaining adaptive potential in these vulnerable assemblages. Nevertheless, further research incorporating both mitochondrial and nuclear markers (e.g.,

internal transcribed spacers or microsatellites) is necessary to achieve a more comprehensive understanding of the underlying evolutionary processes and to more accurately assess population structure in this species.

Ethical statement: The authors declare that they all agree with this publication and made significant contributions; that there is no conflict of interest of any kind; and that we followed all pertinent ethical and legal procedures and requirements. All financial sources are fully and clearly stated in the acknowledgments section. A signed document has been filed in the journal archives.

ACKNOWLEDGMENTS

The present study was funded by the National Geographic grant NGS-100354C-23. We thank the authorities of Islas Marietas National Park for the logistical support provided during the field trips and sampling. The collection was conducted under the permit PPF/DGOPA-88/22.

REFERENCES

- Adjeroud, M., & Tsuchiya, M. (1999). Genetic variation and clonal structure in the scleractinian coral *Pocillopora damicornis* in the Ryukyu Archipelago, southern Japan. *Marine Biology*, 134, 753–760. <https://doi.org/10.1007/s002270050592>
- Aljanabi, M. S., & Martinez, I. (1997). Universal and rapid salt-extraction of high-quality genomic DNA for PCR-based techniques. *Nucleic Acids Research*, 25(22), 4692–4693. <https://doi.org/10.1093/nar/25.22.4692>
- Alquezar, R., & Boyd, W. (2007). Development of rapid, cost-effective coral survey techniques: tools for management and conservation planning. *Journal of Coastal Conservation*, 11(2), 105–119. <https://doi.org/10.1007/s11852-008-0011-1>
- Avise, J. C. (2009). Phylogeography: retrospect and prospect. *Journal of Biogeography*, 36(1), 3–15. <https://doi.org/10.1111/j.1365-2699.2008.02032.x>
- Banaszak, A., Marhaver, K., Miller, M., Hartmann, A., Albright, R., Hagedorn, M., Harrison, P., Latijnhouwers, K., Quiroz, S., Pizarro, V., & Chamberland, V. (2023). Applying coral breeding to reef restoration: best practices, knowledge gaps, and priority actions



- in a rapidly-evolving field. *Restoration Ecology*, 31(7), e13913. <https://doi.org/10.1111/rec.13913>
- Baker, A. C., Correa, A. M. S. & Cunning, R. (2017). Diversity, distribution and stability of *Symbiodinium* in reef corals of the Eastern Tropical Pacific. In: P. Glynn, D. Manzello, & I. Enochs (Eds.), *Coral Reefs of the Eastern Tropical Pacific*. Coral Reefs of the World, (Vol 8, pp. 405–420). Springer. https://doi.org/10.1007/978-94-017-7499-4_13
- Baums, I., Devlin-Durante, M., Laing, B., Feingold, J., Smith, T., Bruckner, A., & Monteiro, J. (2014). Marginal coral populations: the densest known aggregation of *Pocillopora* in the Galápagos Archipelago is of asexual origin. *Frontiers in Marine Science*, 1, 1–11. <https://doi.org/10.3389/fmars.2014.00059>
- Berkelmans, R., & van Oppen, M. J. (2006). The role of zooxanthellae in the thermal tolerance of corals: a 'nugget of hope' for coral reefs in an era of climate change. *Proceedings of the Royal Society B: Biological Sciences*, 273, 2305–2312. <https://doi.org/10.1098/rspb.2006.3567>
- Blanco-Pimentel, M., Kenkel, C., Kitchen, S., Calle-Triviño, J., Baums, I., Cortés-Useche, C., & Morikawa, M. (2023). Overcoming barriers to reef restoration: field-based method for approximate genotyping of *Acropora cervicornis*. *Restoration Ecology*, 32(3), e14073. <https://doi.org/10.1111/rec.14073>
- Burroughs, C., & Rodríguez-Troncoso, A. P. (2024). Contrasts in ecological assessment and tourism sector perceptions of coral reefs: a case study at Islas Marietas National Park. *Discover Oceans*, 1(1), 10. <https://doi.org/10.1007/s44289-024-00014-9>
- Chan, W., Peplow, L., Menéndez, P., Hoffmann, A., & Van Oppen, M. (2018). Interspecific hybridization may provide novel opportunities for coral reef restoration. *Frontiers in Marine Science*, 5, 160. <https://doi.org/10.3389/fmars.2018.00160>
- Carriquiry, J. D., Cupul-Magaña, A. L., Rodríguez-Zaragoza, F. A., & Medina-Rosas, P. (2001). Coral bleaching and mortality in the Mexican Pacific during the 1997–98 El Niño and prediction from a remote sensing approach. *Bulletin of Marine Science*, 69(1), 237–249.
- Chávez-Romo, H. E., & Reyes-Bonilla, H. (2007). Reproducción sexual del coral *Pocillopora damicornis* al sur del Golfo de California, México. *Ciencias Marinas*, 33(4), 495–501.
- Chiazzari, B., Magalon, H., Gélín, P., & Macdonald, A. (2019). Living on the edge: assessing the diversity of South African *Pocillopora* on the margins of the Southwestern Indian Ocean. *PLOS One*, 14(8), e0220477. <https://doi.org/10.1371/journal.pone.0220477>
- Clement, M., Posada, D., & Crandall, K. A. (2000). TCS: a computer program to estimate gene genealogies. *Molecular Ecology*, 9(10), 1657–1660. <https://doi.org/10.1046/j.1365-294x.2000.01020.x>
- Comisión Nacional de Áreas Naturales Protegidas (2007). *Programa de conservación y manejo Parque Nacional Islas Marietas* [Management program]. Comisión Nacional de Áreas Naturales Protegidas. https://www.conanp.gob.mx/que_hacemos/pdf/programas_manejo/marietas.pdf
- Cruz-García, R., Rodríguez-Troncoso, A. P., Rodríguez-Zaragoza, F. A., Mayfield, A., & Cupul-Magaña, A. L. (2020). Ephemeral effects of El Niño–Southern Oscillation events on an eastern tropical Pacific coral community. *Marine and Freshwater Research*, 71(10), 1259–1268. <https://doi.org/10.1071/MF18481>
- Cupul-Cortés, M., Hernández-Ayón J. M., Cupul-Magaña A. L., & Rodríguez-Troncoso A. P. (2018). Variabilidad del sistema de CO₂ en el Parque Nacional Islas Marietas (PNIM), Bahía de Banderas. Nayarit. *Simposio Internacional del Carbono en México*, Sonora, pp 235–242.
- Cupul-Magaña, A. L., & Rodríguez-Troncoso, A. P. (2017). Tourist carrying capacity at Islas Marietas National Park: An essential tool to protect the coral community. *Applied Geography*, 88, 15–23. <https://doi.org/10.1016/j.apgeog.2017.08.021>
- Combosch, D., & Vollmer, S. (2011). Population genetics of an ecosystem-defining reef coral *Pocillopora damicornis* in the Tropical Eastern Pacific. *PLOS One*, 6(8), e21200. <https://doi.org/10.1371/journal.pone.0021200>
- Dennis, L., Favoretto, F., Balart, E., Munguía-Vega, A., Sánchez-Ortiz, C., & Paz-García, D. (2024). Isolation by disturbance: a pattern of genetic structure of the coral *Pocillopora grandis* in the Gulf of California. *Marine Ecology Progress Series*, 733, 43–57. <https://doi.org/10.3354/meps14553>
- ECOPLAMB, S.A. DE C.V. (2004). *Plan parcial de desarrollo urbano turístico "Punta de Mita" del Municipio de Bahía de Banderas, Nayarit* [Technical report]. Manifestación de Impacto Ambiental. Bahía de Banderas. Nayarit, México
- Excoffier, L., & Lischer, H. E. (2010). Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources*, 10(3), 564–567. <https://doi.org/10.1111/j.1755-0998.2010.02847.x>
- France, S. C., & Hoover, L. L. (2002). DNA sequences of the mitochondrial COI gene have low levels of divergence among deep-sea octocorals (Cnidaria: Anthozoa). *Hydrobiologia*, 471(1), 149–155. <https://doi.org/10.1023/A:1016517724749>
- García-Medrano, D., López-Pérez, A., Guendulain-García, S., Valencia-Méndez, O., Granja-Fernández, R., González-Mendoza, T., & Torres-Hernández, P. (2023).

- Gardening *Pocillopora* spp. fragments and their potential for rebuilding reef systems in the southern Mexican Pacific. *Restoration Ecology*, 31(8), e14006. <https://doi.org/10.1111/rec.14006>
- Glynn, P. W., & Ault, J. S. (2000). A biogeographic analysis and review of the far eastern Pacific coral reef region. *Coral Reefs*, 19, 1–23. <https://doi.org/10.1007/s003380050220>
- Glynn, P. D., Voinov, A. A., Shapiro, C. D., & White, P. A. (2017). From data to decisions: Processing information, biases, and beliefs for improved management of natural resources and environments. *Earth's Future*, 5(4), 356–378. <https://doi.org/10.1002/2016EF000487>
- González-Barrios, F. J., Cabral-Tena, R. A., & Alvarez-Filip, L. (2021). Recovery disparity between coral cover and the physical functionality of reefs with impaired coral assemblages. *Global Change Biology*, 27(3), 640–651. <https://doi.org/10.1111/gcb.15431>
- Hellberg, M., Burton, R., Neigel, J., & Palumbi, S. (2002). Genetic assessment of connectivity among marine populations. *Bulletin of Marine Science*, 70(1), 273–290.
- Herre, E. A., Knowlton, N., Mueller, U. G., & Rehner, S. A. (1999). The evolution of mutualisms: exploring the paths between conflict and cooperation. *Trends in Ecology & Evolution*, 14(2), 49–53. [https://doi.org/10.1016/s0169-5347\(98\)01529-8](https://doi.org/10.1016/s0169-5347(98)01529-8)
- Howells, E. J., Beltran, V. H., Larsen, N. W., Bay, L. K., Willis, B. L., & Van Oppen M. J. H. (2012). Coral thermal tolerance shaped by local adaptation of photosymbionts. *Nature Climate Change*, 2(2), 116–120. <https://doi.org/10.1038/nclimate1330>
- Knowlton, N., & Jackson, B. C. (2001). The ecology of coral reefs. In M. D. Bertness, S. D. Gaines & M. E. Hay (Eds.), *Marine Community Ecology* (pp. 395–422). Sinauer Associates.
- Leigh, J. W., & Bryant, D. (2015). PopART: Full-feature software for haplotype network. *Methods in Ecology and Evolution*, 6(9), 1110–1116. <https://doi.org/10.1111/2F2041-210X.12410>
- Liu, B., Zhang, K., Zhu, K., Shafi, M., Gong, L., Jiang, L., Liu, L., Muhammad, F., & Lü, Z. (2020). Population genetics of *Konosirus punctatus* in chinese coastal waters inferred from two mtDNA genes (COI and Cytb). *Frontiers in Marine Science*, 7, 534. <https://doi.org/10.3389/fmars.2020.00534>
- Martínez-Castillo, V., Rodríguez-Troncoso, A. P., Bautista-Guerrero E., & Cupul-Magaña A. L. (2022a). Symbiont-coral relationship in the main reef building scleractinians of the Central Mexican Pacific. *Symbiosis*, 86, 315–323. <https://doi.org/10.1007/s13199-022-00848-x>
- Martínez-Castillo, V., Rodríguez-Troncoso, A. P., & Cupul-Magaña, A. L. (2023). Evidence of sexual reproduction in out-planted coral colonies. *Oceans*, 4(4), 350–359. <https://doi.org/10.3390/oceans4040024>
- Martínez-Castillo V., Rodríguez-Troncoso, A. P., Santiago-Valentín, J. D., & Cupul-Magaña, A. L. (2020). The influence of urban pressures on coral physiology on marginal coral reefs of the Mexican Pacific. *Coral Reefs*, 39, 625–937. <https://doi.org/10.1007/s00338-020-01957-z>
- Martínez-Castillo, V., Rodríguez-Troncoso, A. P., Mayfield, A. B., Rodríguez-Zaragoza, F. A., & Cupul-Magaña, A. L. (2022b). Coral recovery in the Central Mexican Pacific 20 years after the 1997–1998 El Niño event. *Oceans*, 3(1), 48–59. <https://doi.org/10.3390/oceans3010005>
- Miller, K. J., & Ayre, D. J. (2008). Protection of genetic diversity and maintenance of connectivity among reef corals within Marine Protected Areas. *Conservation Biology*, 22(5), 1245–1254. <https://doi.org/10.1111/j.1523-1739.2008.00985.x>
- Moussa, M., Choulak, S., Rhouma-Chatti, S., Chatti, N., & Said, K. (2022). First insight of genetic diversity, phylogeographic relationships, and population structure of marine sponge *Chondrosia reniformis* from the eastern and western Mediterranean coasts of Tunisia. *Ecology and Evolution*, 12(1), e8494. <https://doi.org/10.1002/ece3.8494>
- Müller, W., Brümmer, F., Batel, R., Müller, I., & Schroder, H. (2003). Molecular biodiversity. Case study: Porifera (sponges). *Naturwissenschaften*, 90, 103–120. <https://doi.org/10.1007/s00114-003-0407-6>
- Oury, N., Gélin, P., & Magalon, H. (2021). High connectivity within restricted distribution range in *Pocillopora* corals. *Journal of Biogeography*, 48(7), 1679–1692. <https://doi.org/10.1111/jbi.14104>
- Ostria-Hernández, M. L., Hernández-Zulueta, J., Vargas-Ponce, O., Díaz-Pérez, L. Araya, R., Rodríguez-Troncoso, A. P., Rios-Jara, E., & Rodríguez-Zaragoza, F. (2022). Core microbiome of corals *Pocillopora damicornis* and *Pocillopora verrucosa* in the northeastern tropical Pacific. *Marine Ecology*, 43(6), e12729. <https://doi.org/10.1111/maec.12729>
- Park, J. W., Park, K., & Kwak, I. S. (2020). Phylogenetic analysis using cytochrome c oxidase subunit I of silver croaker (*Pennahia argentata*) mitochondria DNA. *Korean Journal of Ecology and Environment*, 53(3), 265–274. <https://doi.org/10.11614/KSL.2020.53.3.265>
- Paz-García, D. A., Chávez-Romo, H. E., Correa-Sandoval, F., Reyes-Bonilla, H., López-Pérez, A., Medina-Rosas, P., & Hernández-Cortés, M. P. (2012). Genetic connectivity patterns of corals *Pocillopora damicornis* and *Porites panamensis* (Anthozoa: Scleractinia) Along the West Coast of Mexico. *Pacific Science*, 66(1), 43–61. <https://doi.org/10.2984/66.1.3>



- Plata-Rosas, L., & Filonov, A. (2007). Internal tide in the northwestern part of Banderas Bay, Mexico. *Ciencias Marinas*, 33(2), 197–215. <https://doi.org/10.7773/cm.v33i2.1013>
- Portela, E., Beier, E., Barton, E. D., Castro, R., Godínez, V., Palacios-Hernández, E., Fiedler, P. C., Sánchez-Velasco, L., & Trasviña, A. (2016). Water masses and circulation in the tropical Pacific off central Mexico and surrounding areas. *Journal of Physical Oceanography*, 46(10), 3069–3081. <https://doi.org/10.1175/JPO-D-16-0068.1>
- Reyes-Bonilla, H., Carriquiry, J. D., Leyte-Morales, G. E., & Cupul-Magaña, A. L. (2002). Effects of the El Niño-Southern Oscillation and the anti-El Niño event (1997–1999) on coral reefs of the western coast of Mexico. *Coral Reefs*, 21, 368–372. <https://doi.org/10.1007/s00338-002-0255-4>
- Rios, D., Torrado, H., Lemer, S., Drury, C., Burdick, D., Raymundo, L., & Combosch, D. (2024). Population genomics for coral reef restoration—a case study of staghorn corals in Micronesia. *Evolutionary Applications*, 18(6), e70115. <https://doi.org/10.1111/eva.70115>
- Romero-Torres, M., Acosta, A., Palacio-Castro, A. M., Tremblay, E. A., Zapata, F. A., Paz-García, D. A., & Porter, J. W. (2020). Coral reef resilience to thermal stress in the Eastern Tropical Pacific. *Global Change Biology*, 26(7), 3880–3890. <https://doi.org/10.1111/gcb.15126>
- Rowan, R., Knowlton, N., Baker, A., & Jara, J. (1997). Landscape ecology of algal symbionts creates variation in episodes of coral bleaching. *Nature*, 388(6639), 265–269. <https://doi.org/10.1038/40843>
- Rozas, J., Sánchez-DelBarrio, J. C., Messeguer, X., & Rozas, R. (2003). DnaSP, DNA polymorphism analyses by the coalescent and other methods. *Bioinformatics*, 19(18), 2496–2497. <https://doi.org/10.1093/bioinformatics/btg359>
- Santiago-Valentín, J. D., Rodríguez-Troncoso, A. P., Bautista-Guerrero, E., López-Pérez, A., & Cupul-Magaña, A. L. (2020). Settlement ecology of scleractinian corals of the Northeastern Tropical Pacific. *Coral Reefs*, 39, 133–146. <https://doi.org/10.1007/s00338-019-01872-y>
- Shearer, T. L., Van Oppen, M. J. H., Romano, S. L., & Wörheide, G. (2002). Slow mitochondrial DNA sequence evolution in the Anthozoa (Cnidaria). *Molecular Ecology*, 11(12), 2475–2487. <https://doi.org/10.1046/j.1365-294X.2002.01652.x>
- Sheppard, C. R. C., Davy, S. K., & Pilling, G. M. (2010). *The biology of coral reefs* (1st Ed.) Oxford University Press. <https://doi.org/10.1093/acprof:oso/9780198566359.001.0001>
- Souter, D., Planes, S., Wicquart, J., Logan, M., Obura, D., & Staub, F. (2020). *Status of coral reefs of the world: 2020 report* [Technical report]. Global Coral Reef Monitoring Network (GCRMN) and International Coral Reef Initiative (ICRI). <https://doi.org/10.59387/WOTJ9184>
- Stoddart, J. A. (1984). Genetic differentiation amongst populations of the coral *Pocillopora damicornis* off Southwestern Australia. *Coral Reefs*, 3, 149–156. <https://doi.org/10.1007/BF00301959>
- Suggett, D. J., Edwards, M., Cotton, D., Hein, M., & Camp, E. F. (2023). An integrative framework for sustainable coral reef restoration. *One Earth*, 6(6), 666–681. <https://doi.org/10.1016/j.oneear.2023.05.007>
- Suggett, D. J., & Van Oppen, M. J. (2022). Horizon scan of rapidly advancing coral restoration approaches for 21st century reef management. *Emerging Topics in Life Sciences*, 6(1), 125–136. <https://doi.org/10.1042/etls20210240>
- Tortolero-Langarica, J. J. A., Rodríguez-Troncoso, A. P., & Nava H. (2025). Advances in coral reef restoration in the Mexican Pacific: Active interventions and scaling approaches. *Restoration Ecology*, 33(8), E70176. <https://doi.org/10.1111/rec.70176>
- Tortolero-Langarica, J. J. A., Rodríguez-Troncoso, A. P., Cupul-Magaña, A. L., Alarcón-Ortega, L. C., & Santiago-Valentín, J. D. (2019). Accelerated recovery of calcium carbonate production in coral reefs using low-tech ecological restoration. *Ecological Engineering*, 128, 89–97. <https://doi.org/10.1016/j.ecoleng.2019.01.002>
- Templeton, A. R., Crandall, K. A., & Sing, C. F. (1992). A cladistic analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping and DNA sequence data. III. Cladogram estimation. *Genetics*, 132(2), 619–633. <https://doi.org/10.1093/genetics/132.2.619>
- Van Oppen, M. J. H., & Gates, R. D. (2006). Conservation genetics and the resilience of reef building corals. *Molecular Ecology*, 15(13), 3863–3883. <https://doi.org/10.1111/j.1365-294X.2006.03026.x>
- Warner, M. E., Fitt, W. K., & Schmidt, G. W. (1996). The effects of elevated temperature on the photosynthetic efficiency of zooxanthellae in *hostite* from four different species of reef coral: a novel approach. *Plant Cell Environment*, 19(3), 291–299. <https://doi.org/10.1111/j.1365-3040.1996.tb00251.x>



Whitaker, K. (2006). Genetic evidence for mixed modes of reproduction in the coral *Pocillopora damicornis* and its effect on population structure. *Marine Ecology Progress Series*, 306, 115–124. <http://doi.org/10.3354/meps306115>

Wyrski, K. (1966). Oceanography of the Eastern Equatorial Pacific Ocean. *Oceanographic and Marine Biological Annual Reviews*, 4, 33–68.

Xing, B., Lin, L., & Wu, Q. (2025). Application of mitochondrial genomes to species identification and evolution. *Electronic Journal of Biotechnology*, 76, 39–48. <https://doi.org/10.1016/j.ejbt.2025.04.001>