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When morphology cannot identify species: high morphological diversity of Pocillopora corals (Anthozoa: Scleractinia) in the Eastern Tropical Pacific

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When morphology cannot identify species: high morphological diversity of *Pocillopora* corals (Anthozoa: Scleractinia) in the Eastern Tropical Pacific

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Abstract

Accurate species identification is fundamental to biological diversity assessments and critical for understanding ecological and evolutionary dynamics. Nonetheless, the high levels of morphological variation in many scleractinian corals have blurred the recognition of species boundaries. The genus *Pocillopora* is widely distributed and exhibits high levels of colony level phenotypic plasticity and polymorphism, leading to the description of over 40 species worldwide. In the Eastern Tropical Pacific (ETP), nine "morphospecies" have been recorded, but only four genetically distinct species are now recognized in the area, based initially on variation in mitochondrial DNA and then supported by genomic data. This study aimed to identify useful molecular and morphological traits to recognize and describe the *Pocillopora* diversity in the ETP. We found that Histone and mitochondrial markers could identify discrete lineages, while most of the nuclear genes analyzed offered little power for delineation. Neither colonial morphology nor corallite micro-morphology reliably corresponded to the lineages identified. Based on mitochondrial and nuclear genetic data, we found that at least four distinct lineages occur in the ETP. The species names associated with these mitochondrial haplotypes in other studies are most often *P.* cf. *effusa*, *P. lacera*, and two putative cryptic species: *P. capitata* and *P. meandrina*.

Keywords: cryptic diversity; mitochondrial lineages; morphometrics; reef corals

Resumen

La identificación precisa de especies es fundamental para la evaluación de la diversidad biológica así como para entender la dinámica su ecológica y evolutiva. No obstante, los altos niveles de variación morfológica en muchos corales escleractinios, han complicado el reconocimiento de los límites entre especies. El género *Pocillopora* está ampliamente distribuido y exhibe altos niveles de plasticidad fenotípica y polimorfismo a nivel de colonia, lo que ha llevado a la descripción de más de 40 especies en todo el mundo. En el Pacífico Tropical Oriental (PTO), se han registrado nueve "morfoespecies", pero, a la fecha, solo cuatro especies genéticamente distintas son reconocidas en la zona, basándose inicialmente en la variación del ADN mitocondrial y luego respaldadas por datos genómicos. Este estudio tuvo como objetivo identificar rasgos moleculares y morfológicos útiles para reconocer y describir la diversidad de *Pocillopora* en el PTO. Descubrimos que las regiones de las Histonas sumada a la información mitocondrial, podían identificar linajes discretos, mientras que la mayoría de los genes nucleares analizados mostraron baja resolución para la delimitación de especies. Ni la morfología colonial ni la micromorfología de los coralitos correspondieron de manera confiable a los linajes identificados. Con base en datos genéticos mitocondriales y nucleares, encontramos que al menos cuatro linajes distintos ocurren en el POT. Los nombres de las especies asociadas con estos haplotipos mitocondriales suelen ser *P.* cf. *effusa*, *P. lacera* y dos especies crípticas potenciales: *P. capitata* y *P. meandrina*.

Palabras clave: diversidad críptica; linajes mitocondriales; morfometría; arrecife coralino

1. Introduction

Species identification is the cornerstone of biodiversity studies and is critical for interpreting ecological and evolutionary processes. Morphological traits have been used as the main criteria for species delimitation and recognition in tropical reef corals (Todd 2008; Veron 2011) and are often the only data source for ecological field studies, comparisons with established historical records, and applicability to the fossil record. However, the presence of cryptic species and extensive environmentally driven intraspecific morphological variation (phenotypic plasticity) often leads to blurred species boundaries in stony corals (Flot et al. 2011; Schmidt-Roach et al. 2013; Arrigoni et al. 2016).

Molecular approaches have helped to delineate species when morphological traits fail (Grupstra et al. 2024; Riginos et al. 2024), but these tools have their own set of limitations and, alone, will lack practical, field identifiable traits. Thus, integrative taxonomy that uses molecular data to guide the evaluation and identification of diagnostic physical features is advocated as a more rigorous way forward in resolving taxonomic hypotheses (e.g. Emerson 2025). While the number of studies that integrate various, independent lines of evidence for species delimitation of corals has increased in recent years, they are biased geographically (e.g. to the western Pacific) (Schmidt-Roach et al. 2013; Oury et al. 2020, 2022; Aurelle et al. 2022). To date, those studies that have analyzed the diversity of the Eastern Tropical Pacific (ETP) reef corals using molecular data (Combosch et al. 2008; Pinzón and LaJeunesse 2011; Pinzón et al. 2013; Voolstra et al. 2023; Millán-Márquez et al. 2024; Connelly et al. 2026) have only rarely integrated morphological analysis (Paz-García et al. 2015a, b; Johnston et al. 2017), hampering rigorous contextualization of taxa uniqueness.

The ETP realm spans from Mexico to Ecuador and comprises several oceanic islands, including Clipperton, Revillagigedo, Galapagos, Coco, and Malpelo (Glynn et al. 2017). This realm is characterized by the geographic isolation from the central and western Pacific by the Eastern Pacific Barrier (EPB) and by severe environmental conditions that also exhibit broad interannual fluctuations (Baums et al. 2012; Fiedler and Lavín 2017; Glynn et al. 2017; Romero-Torres et al. 2018). These adverse conditions represent significant challenges for coral growth and reef development. As a result, the coral reefs in the ETP exhibit high morphological variation and lower diversity that in turn promotes lower competition by space between species in smaller reef structures compared to other regions such as the Great Barrier Reef. Coral communities in the ETP form patchy coral communities dominated by a few species of the genera *Porites*, *Pavona,* and especially *Pocillopora* (Glynn et al. 2017). Despite these limitations, ETP reefs are ecologically relevant, serving as critical recruitment zones and primary habitats for numerous marine species (Ricart et al. 2016; Cortés et al. 2017).

Pocillopora (Lamarck, 1816) exemplifies the historical and biological factors that have affected coral classification and systematics. The genus is widely distributed in tropical areas and exhibit high polymorphism in corallum morphology, which has led to the description of more than 40 species worldwide (Hoeksema and Cairns 2019). However, recent systematic and genetic studies suggest that these named species do not necessarily reflect genetically distant taxa (Pinzón et al. 2013; Marti-Puig et al. 2014; Paz-García 2015; Oury et al. 2023). Indeed, Oury and collaborators (2023) undertook integrative analyses incorporating genomic data (17,465 SNPs from ultra-conserved elements), morphological traits (including corallum and corallite structures), and symbiotic algae diversity, to suggest that the genus *Pocillopora* comprises at least 13

well-supported species hypotheses worldwide. Several of these either await formal description or could represent species complexes.

In the ETP, nine "morphospecies" have been recorded (Glynn et al. 2017)*: P. capitata* Verrill, 1864*, P. effusa* Veron, 2000*, P. elegans* Dana, 1846*, P. damicornis* (Linnaeus, 1758)*, P. inflata* Glynn, 1999*, P. meandrina* Dana, 1846*, P. grandis* Dana, 1846*, P. verrucosa* (Ellis and Solander, 1786), and *P. woodjonesi* Vaughan, 1918. DNA sequence data (mainly from the mitochondrial open reading frame, mtORF), however, suggest just three biological species are present in the ETP (Table 1): *Pocillopora* Type 1, 2, and 3a-3b (sensu Pinzón and LaJeunesse 2011; Pinzón et al. 2013). Most recently, Connelly *et al.* (2026) used genome "skimming" to reveal the existence of four distinct *Pocillopora* species-level genomic clusters (and one pair of population-level subcluster) in the ETP. *Pocillopora* species complexes persist that cannot yet be reliably resolved into species-level lineages based on current knowledge.

Table 1 Corresponding nomenclature of *Pocillopora* lineages described for the ETP

mtORF lineage (Pinzon et al. 2013)	**Genomic Species Hypothesis (GSH, Oury et al. 2023; Connelly et al. 2026)**	**Consensus name by Oury et al. (2023)**	**Consensus name by Connelly et al. (2026)**	**This study based on mtORF**	**This study based on mt ORF plus Histone**
Type 1	GSH09b	*P. meandrina*	*P. meandrina*	*P. grandis-meandrina* complex	*P. meandrina*
Type 1	GSH09c, $GSH09c_{ETP}$	*P. grandis*	*P. capitata*	*P. grandis-meandrina* complex	*P. capitata*
Type 2	GSH01	*P. effusa*	*P. effusa*	*P.* cf. *effusa*	*P.* cf. *effusa*
Type 3a	GSH13b, $GSH13c_{ETP}$	*P. villosa nomen nudum*	*P. lacera*	*P. lacera*	*P. lacera*
Type 3b	GSH13c, $GSH13c_{ETP}$	*P. verrucosa*	*P. lacera*	*P. lacera*	*P. lacera*

Type 3d	GSH13c, GSH13c$_{ETP}$	*P. verrucosa*	*P. lacera*	*P. lacera*	*P. lacera*

One example of such a species complex is the Type 1 mitochondrial lineage (hereafter *P. grandis-meandrina* complex; see Table 1) which includes up to five hypothetical species (GSH09a, GSH09b, GSH09c$_{WIO}$, GSH09c$_{TSP}$ and GSH09c$_{ETP}$) (Oury et al. 2023; Connelly et al. 2026). These authors suggest that GSH09a may represent an undescribed species closely related to *P. meandrina*, GSH09b could represent *P. meandrina sensu stricto*, and that GSH09c from the WIO and TSP corresponds *P. grandis*. Meanwhile GSH09c is found exclusively in the ETP and correspond to a different species close to of *P. grandis*: *P. capitata* (Connelly et al. 2026). Interestingly, and despite sharing the same mitochondrial lineage, *P. meandrina* is not recovered as a sister species to other species in the complex according to the genomic-based phylogeny. Previous studies also suggest that the *P. grandis* and *P. meandrina* could be distinguished based on corallite micro-morphology (Schmidt-Roach et al. 2014; Oury et al. 2023), specifically, by the shape of the columella.

In contrast to the widely distributed *P. grandis-meandrina* complex (i.e. Type 1 mitochondrial lineage), Type 2 represents a single, highly genetically divergent species, which may correspond to *P. effusa* (hereafter *P.* cf. *effusa*) (Schmidt-Roach et al. 2014; Oury et al. 2023). Finally, mitochondrial lineage Type 3 is the most diverse and complex, with over 10 closely related mitochondrial haplotypes reported (Types 3a to 3j) (Pinzón et al. 2013). These lineages represent up to three hypothetical species that are not monophyletic and could include different mtORF haplotypes (Oury et al. 2023). *Pocillopora* GSH13a (Types 3c, e, g, j) was considered an undescribed species, whereas *Pocillopora* GSH13b (Type 3a) corresponds to *P. villosa nomen nudum* (not formally described) (Oury et al. 2024), and *Pocillopora* GSH13c (Type 3b, d, f, h, i) that may

correspond to *P.* cf. *verrucosa.* However, according to Connelly et al. (2026), mitochondrial lineages Type 3a, b and d from the ETP form a distinct, sister lineage to GSH13c (GSH13c$_{ETP}$) which was formally redescribed as *P. lacera* Verril, 1869.

Based on these genomic analyses, Connelly et al. (2026) indicated the presence of four species in the central and south region of the ETP: *P. capitata* (Type 1), *P.* cf. *effusa* (Type 2), *P. lacera* (Type 3a, 3b, 3d) and *P. meandrina* (Type 1).

To date, there has been no comprehensive systematic assessment of pocilloporids across the entire ETP that combines genetic and morphometric analyses. Assessing species diversity and gene flow patterns is crucial for developing effective conservation strategies and restoration efforts, particularly following severe bleaching events, such as those in 2023 that caused up to 90% mortality in some Mexican reefs (López-Pérez et al. 2024; Nava et al. 2025). This study aims to: (1) identify diagnostic traits for recognizing species of *Pocillopora* in the ETP by comparing specimens from South-east Pacific and Western Pacific regions, and (2) characterize *Pocillopora* species diversity in the ETP using integrated molecular and morphological approaches.

2. Materials and methods

2.1 Sampling

Coral samples were collected from 36 localities within the ETP, including Clipperton Atoll, Mexico, Costa Rica, Panama, and Colombia. Additional samples from Easter Island, Japan, Palau, and the Philippines were also collected and included in this study (Supporting Information, Table S1). At any given locality, colony selection for samples was targeted to represent variation either a) within or b) among purported morphospecies, such that the latter protocol also provides an estimate of local *Pocillopora* diversity. Coral fragments were preserved in 95% ethanol for subsequent genetic and morphological analyses.

2.2 DNA extraction and amplification

Genomic DNA was isolated from 1 cm^2 coral fragments using the PureLink™ Genomic DNA Mini Kit following the manufacturer's instructions. To determine the mitochondrial lineages, we amplified the ORF and the Control Region using PCR (Flot and Tillier 2007). Additionally, we amplified and sequenced seven nuclear regions: histone 3-like and histone 2B-like genes (denoted here as Histone 2 and 3), two regions of the heat shock protein 70 gene (Hsp70A and Hsp70B), the ATP synthase β subunit gene (ATP-β), the membrane carbonic anhydrase (MBSα-CA), and the β-Tubulin region. The information on primer sequences and the annealing temperature for the seven nuclear regions is in Table S2, as well as the primer design for histone regions used in this study.

2.3 Genetic analyses

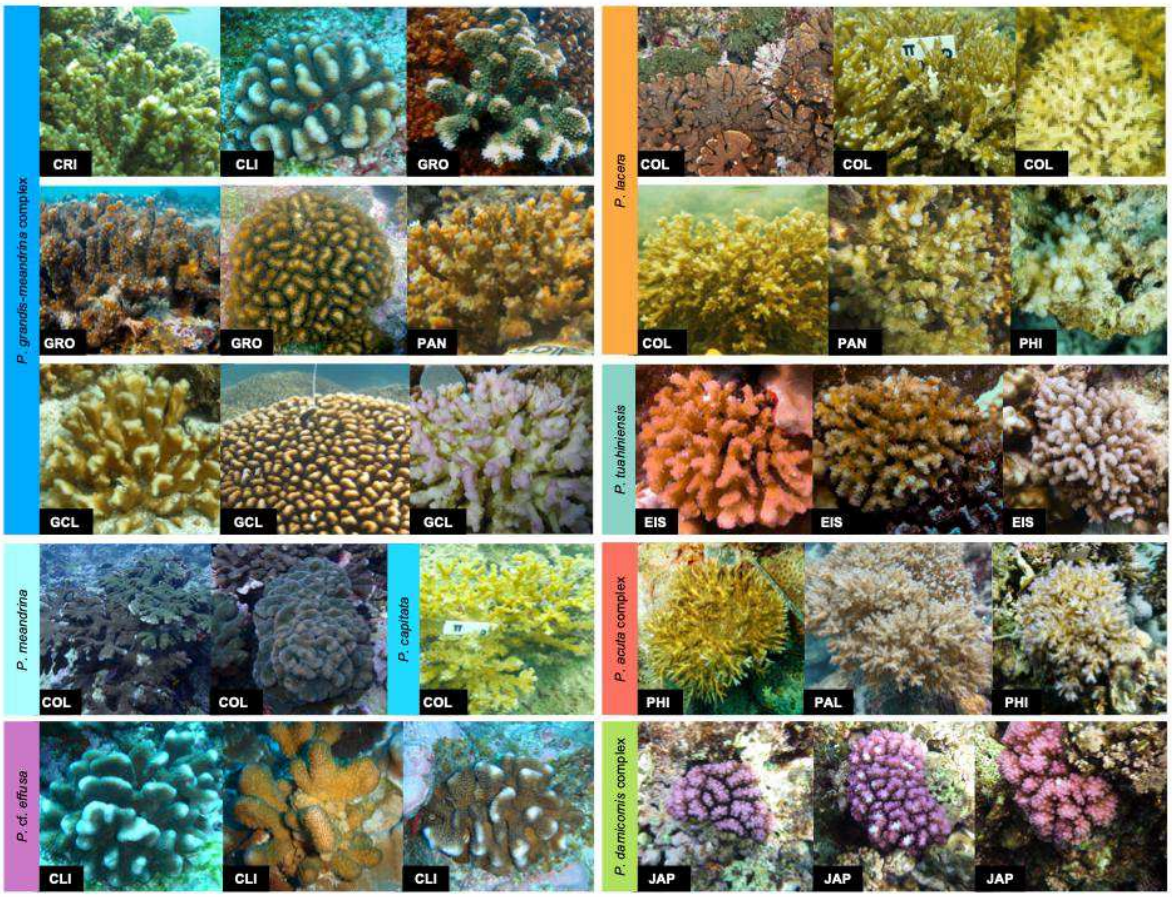
We analyzed 1,509 new sequences from nine loci (two mitochondrial and seven nuclear), obtained from over 700 colonies across all locations. To comprehensively assess genetic diversity, we incorporated 770 previously published sequences from individuals throughout the ETP and other regions for subsequent analyses (Pinzón and LaJeunesse 2011; Pinzón et al. 2013; Schmidt-Roach et al. 2013, 2014) (Supporting Information, Table S1 and S2).

All new DNA sequences were cleaned and aligned using the PhyDE® program (Müller et al. 2010). For nuclear loci, sequences were phased to reconstruct haplotypes using DNAsp (Rozas et al. 2003) with default parameters. Finally, sites subject to recombination were identified and removed using IMgc (Woerner et al. 2007).

Samples were assigned to lineages based on comparisons with previously published sequences of mtORF haplotypes (Fig. 1), using the naming conventions of Oury et al. (2023), Pinzón *et al*. (2013) and Connelly et al. (2026) (Table 1). Haplotype networks for each locus were then estimated using statistical parsimony in Hapsolutely (Vences et al.

2024). Finally, genetic diversity indices for each locus were calculated using the R package *pegas* (Paradis 2010).

Fig. 1 Colony level variation in *Pocillopora*. Samples are colored according to the consensus lineage name based on mtORF (and Histone 3 when available). Locality codes: CLI: Clipperton, COL: Colombia, CRI: Costa Rica, EIS: Easter Island, GCL: Gulf of California, GRO: Guerrero, JAP: Japan, PAN: Panama, PAL: Palau, PHI: Philippines



2.4 Morphological characterization

To characterize morphological variation across the entire distribution of lineages for the first time, we analyzed scanning electron microscopy (SEM) images for 146 skeletal samples collected from the ETP (Mexico, Clipperton, Costa Rica, Panama, and Colombia), Easter Island, Palau, and the Philippines and further integrated data from an

additional 211 colonies that were sourced from recent published taxonomic revisions of *Pocillopora* (Schmidt-Roach et al. 2014; Oury et al. 2023). Since species names are inconsistent throughout the literature, all SEM images from samples of published data were classified and sorted according to mtORF haplotype nomenclature and/or the Genomic Species Hypothesis (GSH) (Supporting Information, Table S1).

New skeletal samples were bleached in a 10% sodium hypochlorite solution, rinsed with distilled water, air-dried, and then scanned with an electron microscope. Images were edited for contrast and brightness using ImageJ2 (Rueden et al. 2017). Eight morphological traits were used to construct a qualitative data matrix for each colony (Supporting Information, Table S3). The selection and coding of these traits followed previous taxonomic and molecular systematic studies of Pocilloporidae (Marti-Puig et al. 2014; Schmidt-Roach et al. 2014). SEM images of *Stylophora pistillata* (Esper, 1792) were used as an outgroup for all traits, either by establishing observed states (0,1,2,3,4) or non-applicable (-) (Stefani et al. 2011; Keshavmurthy et al. 2013). Samples with skeletal damage or micro-residual tissue that obscured micro-morphological traits were excluded from the analysis.

2.5 Morphological and molecular phylogenetic analyses

The best-fit model of character evolution for the morphological data was selected using the ModelFinder tool (Kalyaanamoorthy et al. 2017) implemented in IQ-TREE. Although ModelFinder selected the MK model (assuming equal exchange probability among all trait states), the ORDERED model (restricting transitions to adjacent states) demonstrated superior performance across all information criteria. Phylogenetic analyses were performed under the ORDERED+ASC model with 1,000 bootstrap replicates. The resulting maximum likelihood consensus tree, comprising 184 individuals, was generated using IQ-TREE (Nguyen et al. 2015).

We also inferred the phylogenetic relationships among mtORF haplotypes with *S. pistillata* as the outgroup. The mutation model was determined with ModelFinder, and the phylogenetic tree was estimated under a maximum likelihood approximation with IQ-TREE. We also performed 10,000 bootstrap alignments using the ultrafast bootstrap method (Hoang et al. 2018). All these analyses were run in the IQ-TREE server (Trifinopoulos et al. 2016). The trees were visualized and edited with the *ggtree* v3.5.0.901 package (Yu et al. 2017).

2.6 Morphometric analyses

We analyzed SEM images from 187 samples using ImageJ2. We measured seven characters describing the micromorphology of corallites (Oury et al. 2023): maximum calice diameter (V1) (an approximation of the corallite size), maximum calice diameter perpendicular to V1 (V2), distance between the center of the corallite and the center of the closest adjacent corallite (V3), distance between denticles of the coenosteum (V4), height of septa or septal teeth (V5), maximum columella diameter (V6), and maximum columella diameter perpendicular to V6 (V7) (Supporting Information, Fig. S1). Each variable was measured for 2-10 corallites per sample and then averaged per colony. We also integrated two categorical variables: the shape of the septa (e.g. rudimentary, intermediate, long and thin, robust and spinulate) (V8) and the shape of the columella (e.g. flat and spinulate, oval-convex, styliform) (V9). Additionally, morphometric data from 170 samples measured in a previous study (Oury et al. 2023) were included.

To test differences in calice diameter (V1) among lineages, we performed Welch´s ANOVA followed by a Games-Howell post-hoc test using the *rstatix* R package (Kassambara 2021). To integrate both quantitative and qualitative morphological variables, we performed a factorial analysis of mixed data (FAMD) using the R library *FactoMineR* (Lê et al. 2008). The results of three principal dimensions that describe the

morphological spaces were plotted with the library *ggplot2* (Wickham 2011), highlighting the mitochondrial lineage of the samples.

3. Results

3.1 Mitochondrial lineages

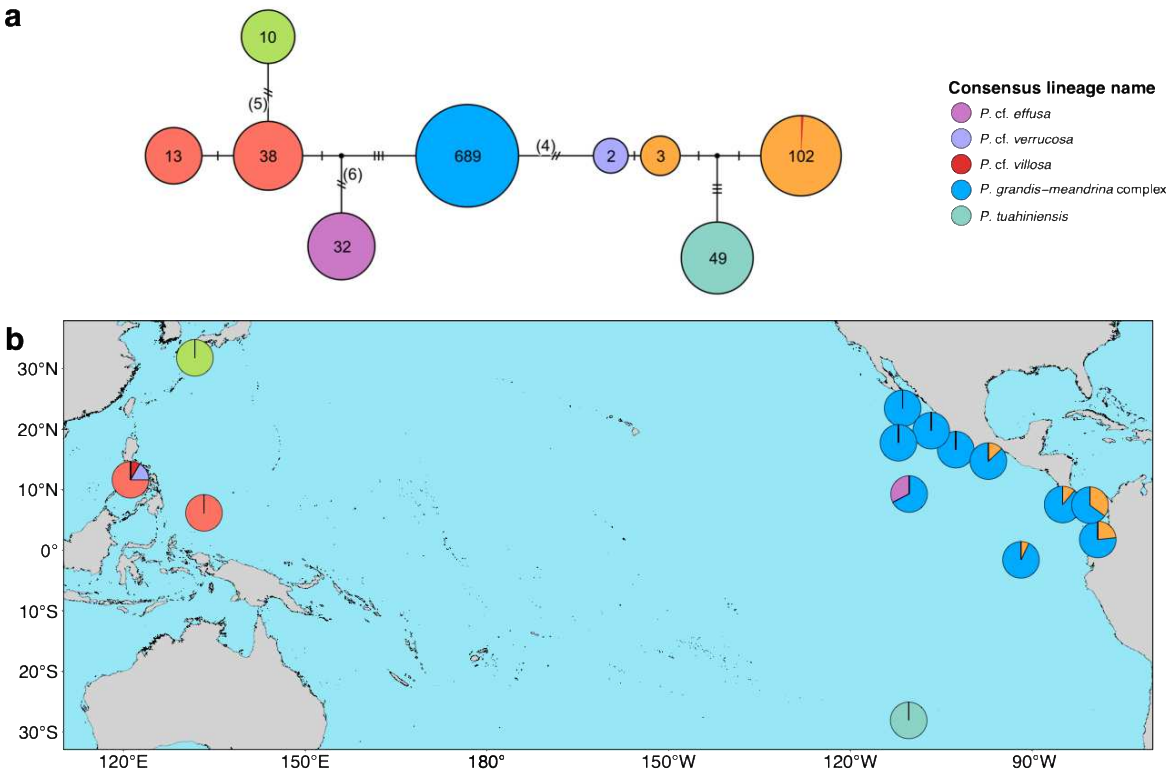
We found nine mtORF haplotypes among the 938 colonies genotyped (Table 2). In the ETP, the most common mitochondrial lineage was the one corresponded to the *P. grandis-meandrina* complex, which was detected at all sample localities (Fig. 2). The second most common lineage corresponded to *P*. *lacera*, and was found from Oaxaca southwards to Costa Rica, Panama, Colombia, and the Galapagos Islands. The *P.* cf. *effusa* lineage was restricted to the Clipperton Atoll.

Table 2 Genetic diversity statistics for mitochondrial and nuclear loci in *Pocillopora*. n = number of sequences included in the final analyses; h = haplotype number; Hd = haplotype diversity; π = nucleotide diversity

Loci	**n**	**h**	**Hd**	**π**
Mitochondrial				
mtORF	938	9	0.44	0.014
Control Region	314	24	0.61	0.004
Nuclear				
Histone 2	130	22	0.92	0.016
Histone 3	186	13	0.81	0.006
Hsp70A	339	28	0.61	0.001
Hsp70B	330	23	0.85	0.003
ATP-β	631	24	0.87	0.024
MBSα-CA	127	14	0.82	0.007
β-Tubulin	158	6	0.50	0.003

Fig. 2 mtORF lineage diversity and distribution of *Pocillopora* corals. **a**, Haplotype network based on 938 mtORF sequences. The haplotype frequency is shown in the center

of each circle. Consensus lineage names according to the latest taxonomic revisions of the genus. **b**, Map of haplotype distribution of the collection sites. Pie charts represent the frequencies of haplotypes found in each locality



We only found one mitochondrial lineage in the South-east Pacific (at Easter Island), corresponding to the recently described *P. tuahiniensis* (Johnston and Burgess 2023). In the western Pacific, we found the *P. acuta* complex in the Philippines and Palau. *Pocillopora* cf. *verrucosa* and *P.* cf. *villosa* were also present in the Philippines. At the northernmost locality, Japan, we only found the *P. damicornis* complex lineage.

For the Control Region, we identified four haplotypes in the ETP and one haplotype from Easter Island (Table 2; see Supplementary Information, Figure S2). These results were partially congruent with mtORF data: the *P. grandis-meandrina* complex and *P.* cf. *effusa* could be identified, but *P. lacera*, *P.* cf. *verrucosa,* and *P.* cf. *villosa* shared the same haplotype.

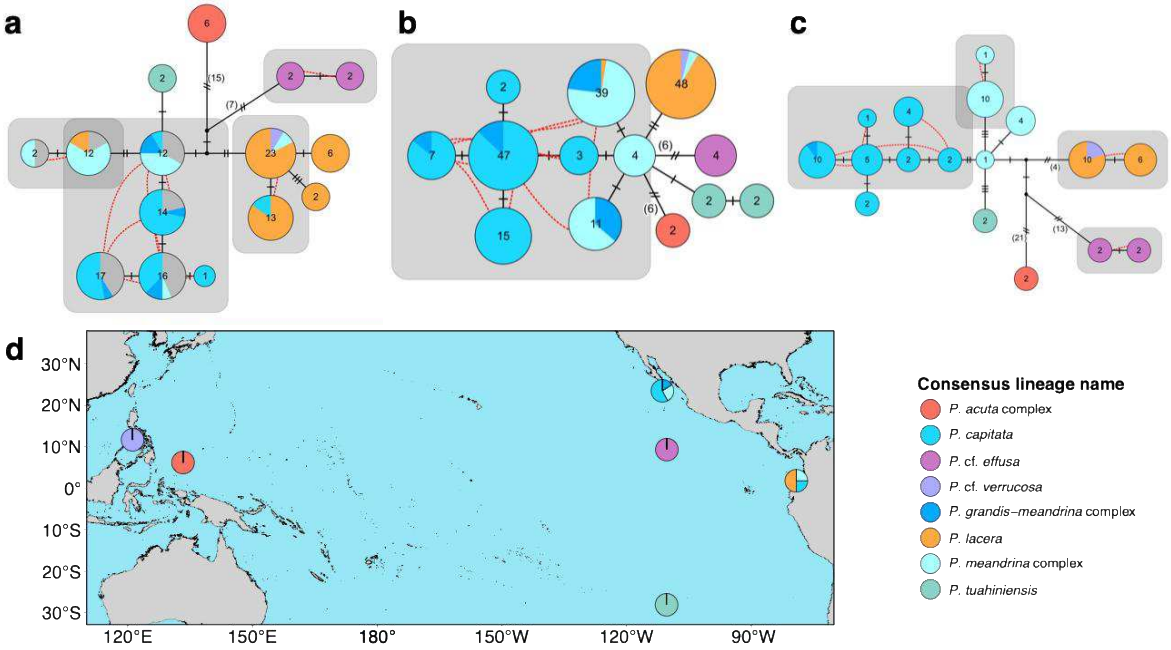
3.2. Nuclear genetic variation

High haplotypic and nucleotide diversity were found for most nuclear loci (Table 2). Five of the seven loci analyzed showed little congruence with mitochondrial data (Supporting Information, Figure S3). For example, at the Hsp70A locus, the most common haplotype was shared between most lineages (i.e. *P. damicornis* complex, *P.* cf. *verrucosa*, *P. tuahiniensis,* and *P. grandis-meandrina* complex). Some lineages possessed unique haplotypes, such as *P.* cf. *effusa* and *P. acuta* complex. The Hsp70B, ATP-β, MBSα-CA, and β-Tubulin loci presented a similar pattern (Supporting Information, Figure S3).

In contrast, Histone 2 and 3 loci provided a higher resolution between species (Fig. 3). Histone 3 shows an *Xho*I restriction site, which, according to Johnston *et al.* (2017), allows the separation of the lineages of *P. grandis* and *P. meandrina* (in this case *P. grandis* from ETP corresponds to *P. capitata* according to Connelly *et al.* 2026). Based on this method, it was possible to recognize the two lineages in our Histone data, especially when combined (Fig. 3c). However, we found an important proportion of heterozygous specimens at this restriction site (G or T) (Fig. 3b). Both the "*P. capitata*" and "*P. meandrina*" lineages were found in the Gulf of California as well as in Colombia; the heterozygous organisms were also distributed in both localities (Fig. 2d). Moreover, these loci revealed unique haplotypes for *P.* cf. *effusa*, the *P. acuta* complex, and *P. tuahiniensis,* whereas *P.* cf. *verrucosa* and *P. lacera* shared a common haplotype (Fig. 3).

Fig. 3 Histone loci diversity and distribution. **a**, haplotype network based on 130 Histone 2 sequences. **b**, haplotype network for 186 Histone 3 sequences and genotype frequency for the *Xho*I restriction cut site. **c**, haplotype network based on 66 concatenated Histone sequences. The frequency of each haplotype is displayed in the center of each circle. Consensus lineage names according to the mtORF and *Xho*I restriction site

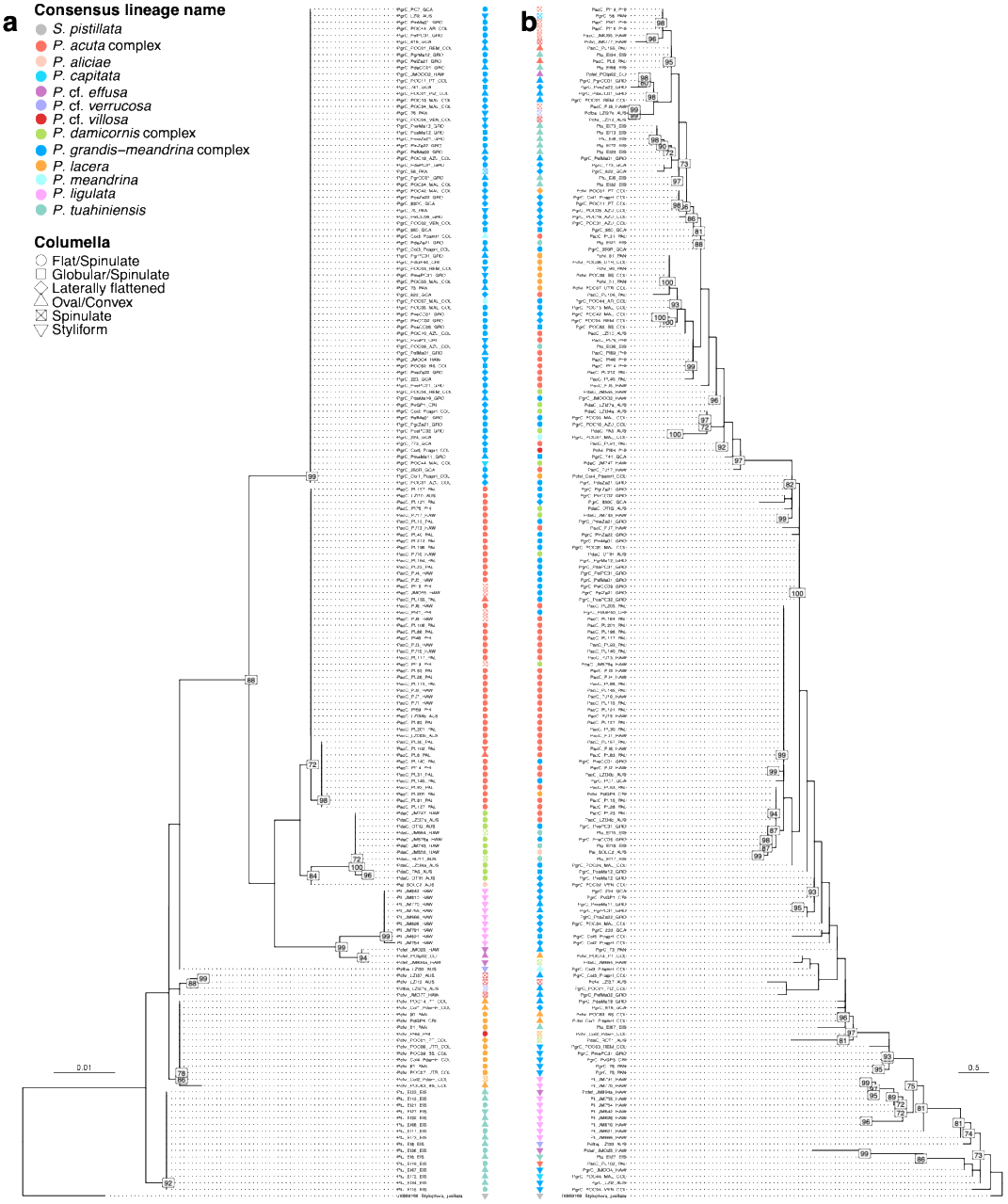
haplotypes for each sample; gray color represents samples with unknown mitochondrial lineage. Red dotted lines connect haplotypes from heterozygote samples. Gray rectangles represent fields for recombination. **d**, map of haplotype distribution. Pie charts represent the frequencies of haplotypes found in each locality



3.3. Phylogenetic relationships and morphological convergence

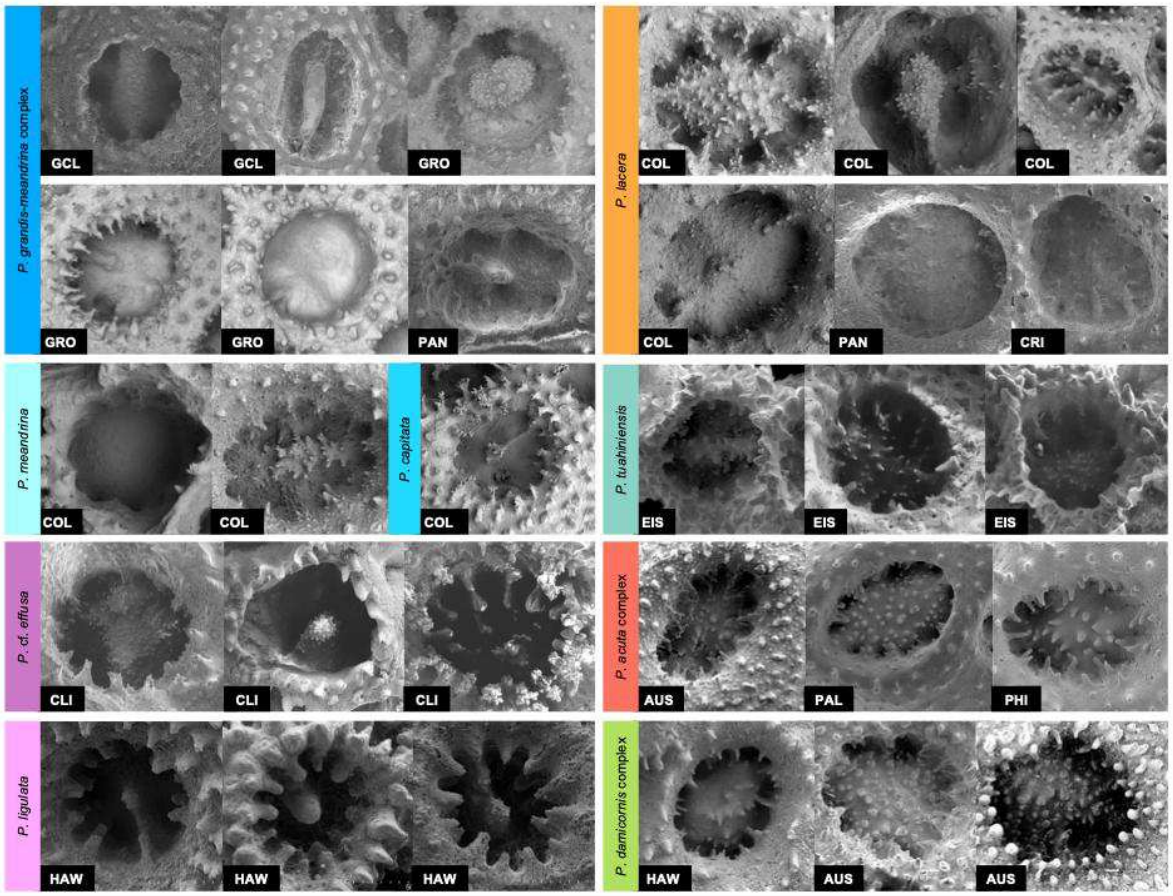
Phylogenetic analysis based on micro-morphological diagnostic traits resulted in a very poorly resolved topology (Fig. 4). Most nodes had low support, with a high trait similarity between distantly related taxa (e.g. *P. lacera,* the *P. grandis-meandrina* complex, and *P. tuahiniensis*, etc.). Most specimens did not conform to published morphological descriptions and, therefore, were overtly dispersed in different groups with styliform or oval/convex columella (Fig. 4). Furthermore, we identified additional columella types not previously described, including globular/spinulate and laterally flattened forms (e.g. *P. grandis-meandrina* complex from GLC and GRO and for *P. lacera* from COL; Fig. 5).

Fig. 4 Morphological and molecular phylogenetic analyses of 184 *Pocillopora* colonies. **a**, molecular phylogenetic tree based on mtORF. **b**, morphological phylogenetic tree based on micro-morphological characters. Consensus lineage identification (colors) and columella shape (shapes) are mapped on the trees. Bootstrap supports for nodes > 70% are shown. The branch length of the outgroup was scaled



In contrast to the morphological tree, the molecular phylogenetic tree based on the mtORF haplotypes showed six well-supported clades under the TPM3+F+G4 substitution model. *Pocillopora tuahiniensis* is monophyletic and sister to the rest of the mitochondrial lineages (Fig. 4), although with low support. *Pocillopora* cf. *villosa*, *P.* cf. *verrucosa* and *P. lacera* lineages formed a paraphyletic group. The *P. grandis-meandrina* complex was monophyletic and sister to a diverse clade that includes the *P. acuta* complex, *P.* cf. *damicornis* complex, *P. ligulata,* and *P.* cf. *effusa* lineages, all monophyletic.

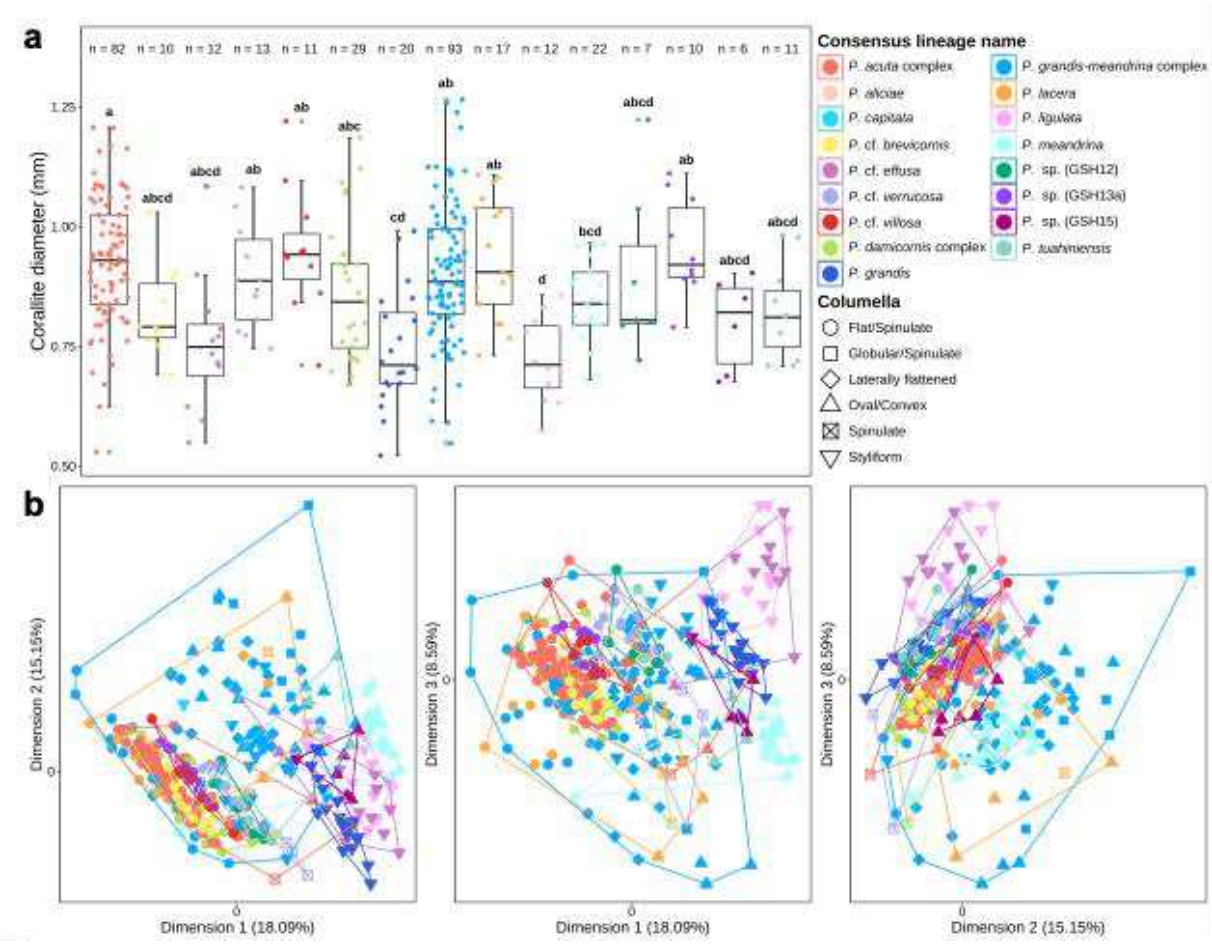
Fig. 5 Corallite variability for *Pocillopora* lineages. Samples are colored according to the consensus lineage name based on mtORF lineage (and Histone 3 when available). Locality codes: AUS: Australia, CLI: Clipperton, COL: Colombia, CRI: Costa Rica, EIS: Easter Island, GCL: Gulf of California, GRO: Guerrero, HWA: Hawaii, PAN: Panama



3.4 High micro-morphology variation

We found high morphological variation within most of the genetic lineages analyzed (Fig. 5 and 6). Welch's ANOVA revealed significant differences in corallite diameter between lineages (W = 6.8; *p*-value = 0.000). Specifically, *P. ligulata* corallites were smaller than those from the *P. acuta* complex, the *P. damicornis* complex, the *P. grandis-meandrina* complex, *P. lacera* and *P.* cf. *villosa* (Fig. 6; see Supporting Information, Table S3). The *P. acuta* complex and the *P. grandis-meandrina* complex exhibited the greatest variation in corallite diameter, resulting in no clear differentiation in corallite size among most lineages (Fig. 6a).

Fig. 6. Morphometric variation of the micro-morphology in *Pocillopora*. **a**, corallite diameter variation. Box-whiskers indicate median and top-bottom 95% quartiles. White spots are outliers. **b**, plots of the first three principal dimensions from the FAMD analysis of 357 *Pocillopora* samples. Samples are colored according to the consensus lineage name based on mtORF or/and nuclear data



The three principal dimensions of FAMD analysis, which explained 41.8% of the total variation, showed that the mitochondrial lineages did not form distinct morphological groups. While some lineages showed less variation in the morphospace, such as *P. ligulata*, *P.* sp (GSH12), *P.* sp (GSH13a), and *P.* sp (GSH15), these still presented overlap with other lineages (Fig. 6b). The *P. grandis-meandrina* complex exhibited the greatest morphological variation in both size and shape, overlapping extensively with all other lineages (Fig. 6b).

4. Discussion

Delineating species in the genus *Pocillopora*, and in scleratinian corals in general, has been difficult due to large macro- and micro-morphological diversity and plasticity, recent diversification, wide distribution, low mitochondrial substitution rate, and the

presence of cryptic species. Here, we analyzed several molecular markers as well as micro-morphological data to elucidate the diversity of pocilloporids in the ETP.

4.1 How many Pocillopora *species are distributed in the ETP?*

We found three mtORF lineages in the ETP that could represent up to four species: *Pocillopora* cf. *effusa*, *P. capitata*, *P. lacera*, and *P. meandrina*. The consistent delineation between the different methods of our results and those recently reported by Connelly et al. (2026), provides further confidence that these are robustly distinct lineages. All these lineages showed a high variation in the corallum morphology with multiple morphospecies present, highlighting the difficulty of distinguishing them (Fig. 1). The first species that can be identified with most of the loci analyzed is *P.* cf. *effusa* (Type 2/GSH01). This species is restricted to Clipperton Atoll in the ETP, which is also the type locality of *P. effusa* (Veron 2002). This species has been reported from the Tropical South Pacific (TSP: Moorea, Ducie, Gambier, Chesterfield), Hawaii, and to a lesser extent in the Western Indian Ocean (WIO) (Gélin et al. 2017; Johnston et al. 2022; Voolstra et al. 2023). Although there is no evidence of genetic structure suggesting cryptic species within this lineage, populations from the full distribution should be sampled to rule out this possibility. Worth noting, *P.* cf. *effusa* from WIO, TSP, and Hawaii present corallites with a styliform columella and developed septa, while the specimens analyzed from Clipperton showed greater variation in micro-morphology, from styliform to flat and spinulate columella with rudimentary septa (Fig. 5).

The second species found was that recently redescribed by Connelly *et al.* (2026): *P. lacera* (Type 3a/GSH13c$_{ETP}$). This species presents a unique combination of mitochondrial and Histone loci genotypes in the ETP. Also, this species has a restricted distribution in the ETP, occurring only in southern Mexico (Oaxaca), Costa Rica, Panama, Colombia, and the Galapagos; this is the first record of this species in Mexico

and second for Costa Rica (Paz-García 2015; Connelly et al. 2026). Although this species is frequently found in sympatry with the *P. grandis-meandrina* complex, it is consistently less common. The limited distribution of this species could be associated with climate variation within the ETP, specifically, it seems to be associated with warmer and more thermally stable water masses (i.e. eastern Pacific Warm Pool, Bolaños-Durán *et al.* 2025). Nevertheless, it is necessary to carry out analyses that allow us to discern whether the distribution pattern of this species is associated with dispersal capacity, local adaptation or even interspecific competition processes. Globally, this mitochondrial lineage (Type 3a) has a wide distribution from the WIO and the Central Indo-Pacific (CIP) to several areas of the TSP. Yet, the genomic analyses directed by Connelly et al. (2026) suggested that the colonies distributed in the ETP represents a different species from those in the WIO, CIP and TSP despite sharing the same mtORF lineage. Regarding the micro-morphology, *P. lacera* is described to present a weakly developed columella and rudimentary or absent septa. However, we found more morphological variation in the ETP, including globular, oval/convex, and spinulated columella (Fig. 5).

We also found *P.* cf. *verrucosa* (Type 3b, d) according to Oury et al. (Type 3b/GSH13b), which is restricted to the Galapagos Islands (Pinzón et al. 2013), although according to Connelly *et al.* (2026) this lineage also corresponds to *P. lacera*.

Finally, the most common and widely distributed lineage in the ETP corresponded to the *P. grandis-meandrina* complex (Type 1/GSH09a, b, c). This group shows high morphological variation in the corallum morphology, including the nine "morphospecies" recorded in the ETP. Additionally, there is evidence of temporal switch between morphospecies from *P. inflata* to *P. damicornis*-like and variation of corallum morphology according to shift of environmental conditions and water flow (Paz-García et al. 2015a, b). In this regard, the genetic lineage of *P. damicornis* was present in Japan

and but not present in samples from the ETP (Fig. 2). A corallum morphology resembling *P. damicornis* is commonly present in the ETP, belonging to *P. grandis-meandrina* complex and *P. lacera* and may be result of convergence driven by low current condition (Paz-García et al. 2015b).

Globally, the *P. grandis-meandrina* complex is distributed in the WIO, TPS, and the Central Pacific in Hawaii (Marti-Puig et al. 2014; Johnston et al. 2018). However, this lineage may consist of up to five distinct species that, possibly due to incomplete lineage sorting, present the same mitochondrial lineage (Johnston et al. 2017; Oury et al. 2023). Thus, the distribution of putative species is still unclear.

Although different methods have been suggested to identify the cryptic species of this complex, such as the variation at Histone 3 loci as well as the micro-morphology of corallites (Schmidt-Roach et al. 2014; Johnston et al. 2017, 2018; Oury et al. 2023), our results showed that both the genetic and micro-morphological variation in the complex is greater than previously recorded, complicating the identification of cryptic species. Based on the restriction enzyme site suggested by Johnston *et al.* (2017), we recovered "*P. capitata"* (genotype C'TCGAG) and "*P. meandrina"* (genotype C'GCGAG) lineages, however, the number of samples heterozygous for the restriction site (C'KCGAG) was considerable (20% of the samples; Fig. 3a). This suggests either the method was only applied to homozygous individuals or it cannot identify cryptic species, since histone loci often occur in multiple copies (Noël et al. 2023).

Nevertheless, by using the concatenated Histone loci (2 and 3), we found higher resolution, with unique haplotypes to each hypothetical species. Unfortunately, the number of samples with information for both Histone loci was low (Fig. 3c). The two Histone lineages are found at both limits of geographical distribution of *Pocillopora* corals in the ETP, from the far north of the Gulf of California to southern Colombia,

suggesting that heterozygous samples may occur across the distribution in the ETP or, alternatively, that heterozygous samples occur at the edges of the distribution.

For the micro-morphological traits, the *P. grandis-meandrina* complex is the most variable lineage (and best sampled), with up to six types of columellae, most found in the ETP (Fig. 5). Even samples that could be identified as "*P. capitata*" or "*P. meandrina* complex" based on the Histone loci presented different morphotypes from those suggested in the taxonomic reviews of the species (an oval/convex columella for *P. meandrina* according to Schmidt-Roach *et al.* 2014 and Oury et al. 2023) (Fig. 5 and 6). Determining whether these lineages and morphotypes represent different species will require genomic analyses that encompass the entire distribution of the mitochondrial lineage *P. grandis-meandrina* complex.

4.2 Are there informative genetic and/or morphological traits for species identification of pocilloporids?

The use of molecular markers to determine species boundaries has grown exponentially in recent decades. For *Pocillopora*, the most widely used marker has been the mtORF region, which has helped to elucidate patterns of diversity (Pinzón and LaJeunesse 2011; Pinzón et al. 2013; Schmidt-Roach et al. 2013, 2014). However, with the accessibility of next-generation sequencing techniques (e.g. RADseq, UCEs, LcGWS), the picture of species boundaries in *Pocillopora* has changed again. To date, several species complexes that share the same mitochondrial lineage are recognized (Oury et al. 2023, 2024, 2025; Connelly et al. 2026). This mito-nuclear discordance seems to be common in anthozoans and may be due to introgression (Oury et al. 2025), but principally to low rates of mitochondrial genome evolution (Hellberg 2006; Quattrini et al. 2023). Therefore, basing the identification of *Pocillopora* specimens only on mtORF

could underestimate the number of species and obscure underlying patterns of biodiversity.

Consequently, alternative approaches must be explored to accurately identifying specimens, particularly through the analysis of nuclear genetic variation. However, our results showed that most of the nuclear loci analyzed have high diversity but low divergence between mitochondrial lineages and potential species (Fig. 3; Supporting Information, Fig. S3). The exception to this pattern was found in the Histone 2 and 3 loci, especially when they were used together (Fig. 3c). These markers recovered the *P. acuta* complex, *P.* cf. *effusa*, *P. lacera,* and *P. tuahiniensis* as different and divergent lineages. However, individuals in the *P. grandis-meandrina* complex were highly heterozygous, making it difficult to identify the cryptic species of this complex.

On the other hand, with respect to morphology as a diagnostic trait, skeletal and colonial macroscopic features of corals deemed as diagnostic for many species are indeed highly variable and could be a response to certain environmental gradients instead of evidence of evolutionary divergence (Todd 2008). For example, several experimental settings with differential water flow, exposure to predators, and *in situ* observations of morphological changes through different seasons and sites have demonstrated how colony morphology in *Pocillopora* is strongly driven by environmental factors (Nakamura and Yamasaki 2005; Mass and Genin 2008; Palacios et al. 2014; Paz-García et al. 2015a, b).

Our results suggest that the micro-morphology of *Pocillopora* is widely variable, and although certain lineages, such as *P. ligulata* (Fig. 5 and 6), have some morphological cohesion, most lineages overlap morphologically, especially within the *P. grandis-meandrina* complex. We showed evidence of the high variation of the corallite morphology, and the analysis of the traditional diagnostic traits used for identification of

Pocillopora species resulted in a very poorly resolved topology. Further, more targeted re-examination of physical features is needed to reevaluate and potentially uncover nuanced, yet currently unperceived diagnostic morphological traits.

5. Conclusions

The diversity of pocilloporid corals in the ETP may be represented by up to four species: *P.* cf. *effusa*, *P. lacera*, and two cryptic species of the *P. grandis-meandrina* complex: *P. capitata* and *P. meandrina*. Most of the nuclear loci tested proved phylogenetically uninformative, but the concatenated region of Histone 2 and 3 allows the recognition of most of the ETP species as well as *P. acuta, P. tuahiniensis* (from other geographical regions). On the other hand, we showed that the macro- and micro-morphology of corallites does not allow the identification of the species neither in the ETP nor in other geographical regions. Finally, it is necessary to perform a novel global integrative analysis to determine if the ETP *Pocillopora* species represent entities with a wide distribution in other marine biogeographic realms, or if the ETP species are different and undescribed species.

Supplementary information

Table S1. Specimen collected and published data included in the analyses.

Table S2. List of primers used for PCR amplification. Fragment sizes in base pairs. T°m = melting temperature. n = sequences obtained in this study; total = sequences included in the analysis.

Table S3. Morphological diagnostic traits states used for phylogenetic analysis. All traits were selected according to taxonomic and systematic studies of Pocilloporidae.

Table S4. Games Howell test for pairwise comparisons of corallite diameter between lineages. P-value: adjusted p-values by Tukey HSD method. Significance: P-values level of significance: ****= $p < 0.0001$; ***= $p<0.001$; ** = $p<0.01$; * =$p< 0.05$; ns= not significance.

Figure S1. Corallite variables measured following Oury *et al*. (2023).

Figure S2. Control region diversity and distribution. A, haplotype network based on 314 sequences. The haplotype frequency is in the center of each haplotype. Mitochondrial lineage names according to the mtORF lineages of the samples. B, map of haplotype distribution. Pie charts represent the frequencies of haplotypes found in each locality.

Figure S3. Nuclear genetic variation in Pocillopora. A, Hsp70A locus haplotype network based on 339 sequences. B, Hsp70B locus network based on 330 sequences. C, ATP-β locus network based on 631 sequences. D, MBSα-CA haplotype network based on 127 sequences. E, β-Tubulin network based on 158 sequences. The haplotype frequency number is in the center of each haplotype. Lineage names according to the mtORF and *Xho*I restriction site haplotypes for each sample; gray color represents unknown mitochondrial lineage samples. Red dotted lines connect haplotypes from heterozygote samples.

Statements and declarations

Conflict of interest

The authors declare that they have no conflicts of interest in relation to this work.

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Author contributions

Yesenia M. Vega-Sánchez (conceptualization, analysis, data curation, SEM images work, field and laboratory work, original draft writing), Rebecca Albright (field and laboratory work, SEM images work), Ana M. Millan-Marquez (field and laboratory work, SEM images work), Jean Francois Flot (field and laboratory work, review), Yuko F. Kitano (field and laboratory work, review), Michael E. Hellberg (field and laboratory work, review), García de León, FJ (funding, field work, review), Eduardo F. Balart (funding, field work, review), Héctor Nava (funding, field work, review), Antonio González-Rodríguez (funding, field work, review), Nina Yasuda (funding, field work, review), Fernando A. Zapata (funding, field work, review), Jorge Cortés (field work and review), Juan J. Alvarado (field work and review), Evie A. Wieters (field work and review), Andrés López-Pérez (field work and review), P. Puig-Martí (SEM images work and funding), Zac

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Data availability statement

Sequence and morphological data used in this study are all available at the Supporting Information; Table S1.

References

Arrigoni R, Benzoni F, Terraneo TI, Caragnano A, Berumen ML (2016) Recent origin and semi-permeable species boundaries in the scleractinian coral genus *Stylophora* from the Red Sea. Sci Rep 6:34612. https://doi.org/10.1038/srep34612

Aurelle D, Pratlong M, Oury N, Haguenauer A, Gélin P, Magalon H, Adjeroud M, Romans P, Vidal-Dupiol J, Claereboudt M, Noûs C, Reynes L, Toulza E, Bonhomme F, Mitta G, Pontarotti P (2022) Species and population genomic differentiation in *Pocillopora* corals (Cnidaria, Hexacorallia). Genetica 150:247-262. https://doi.org/10.1007/s10709-022-00165-7

Baums IB, Boulay JN, Polato NR, Hellberg ME (2012) No gene flow across the Eastern Pacific Barrier in the reef-building coral *Porites lobata*. Mol Ecol 21:5418-5433. https://doi.org/10.1111/j.1365-294X.2012.05733.x

Bolaños-Durán E, Muñiz-Castillo AI, Valenzuela-Quiñonez F, Paz-García DA (2025) Classifying the seascape of eastern tropical Pacific based on physicochemical variables. Mar Environ Res 210:107294. https://doi.org/10.1016/j.marenvres.2025.107294

Combosch DJ, Guzman HM, Schuhmacher H, Vollmer SV (2008) Interspecific hybridization and restricted trans-Pacific gene flow in the Tropical Eastern Pacific Pocillopora. Mol Ecol 17:1304-1312. https://doi.org/10.1111/j.1365-294X.2007.03672.x

Connelly MT, Glynn VM, Cornejo A, Villalobos-Cubero T, Kleypas J, Alvarado JJ, Brandt M, Logan CA, Palacio-Castro AM, Flot JF, Leray M, Connolly SR, Quattrini AM (2026) Genome skimming illuminates hidden species diversity and Symbiodiniaceae associations in east Pacific *Pocillopora* corals. Genome Biol Evol 18:evaf235. https://doi.org/10.1093/gbe/evaf235

Cortés J, Reyes-Bonilla H (2017) Human influences on Eastern Tropical Pacific coral communities and coral reefs. In: Glynn PW, Manzello DP, Enochs IC (eds) Coral reefs of the Eastern Tropical Pacific: persistence and loss in a dynamic environment. Springer, Dordrecht, pp 549-563. https://doi.org/10.1007/978-94-017-7499-4_20

Emerson BC (2025) Delimiting species—prospects and challenges for DNA barcoding. Mol Ecol 34:e17677. https://doi.org/10.1111/mec.17677

Fiedler PC, Lavín MF (2017) Oceanographic conditions of the Eastern Tropical Pacific. In: Glynn PW, Manzello DP, Enochs IC (eds) Coral reefs of the Eastern Tropical Pacific: persistence and loss in a dynamic environment. Springer, Dordrecht, pp 59-83. https://doi.org/10.1007/978-94-017-7499-4_3

Flot JF, Tillier S (2007) The mitochondrial genome of *Pocillopora* (Cnidaria: Scleractinia) contains two variable regions: The putative D-loop and a novel ORF of unknown function. Gene 401:80-87. https://doi.org/10.1016/j.gene.2007.07.006

Flot JF, Blanchot J, Charpy L, Cruaud C, Licuanan WY, Nakano Y, Payri C, Tillier S (2011) Incongruence between morphotypes and genetically delimited species in the coral genus *Stylophora*: Phenotypic plasticity, morphological convergence, morphological stasis or interspecific hybridization. BMC Ecol 11:22. https://doi.org/10.1186/1472-6785-11-22

Glynn PW, Alvarado JJ, Banks S, Cortés J, Feingold JS, Jiménez C, Maragos JE, Martínez P, Maté JL, Moanga DA, Navarrete S, Reyes-Bonilla H, Riegl B, Rivera F, Vargas-Ángel B, Wieters EA, Zapata FA (2017) Eastern Pacific coral reef provinces, coral community structure and composition: an overview. In: Glynn PW, Manzello DP, Enochs IC (eds) Coral reefs of the Eastern Tropical Pacific: persistence and loss in a dynamic environment. Springer, Dordrecht, pp 107-176. https://doi.org/10.1007/978-94-017-7499-4_5

Grupstra CGB, Gómez-Corrales M, Fifer JE, Aichelman HE, Meyer-Kaiser KS, Prada C, Davies SW (2024) Integrating cryptic diversity into coral evolution, symbiosis and conservation. Nat Ecol Evol 8:622-636. https://doi.org/10.1038/s41559-023-02319-y

Gélin P, Postaire B, Fauvelot C, Magalon H (2017) Reevaluating species number, distribution and endemism of the coral genus *Pocillopora* Lamarck, 1816 using species delimitation methods and microsatellites. Mol Phylogenet Evol 109:430-446. https://doi.org/10.1016/j.ympev.2017.01.018

Hellberg ME (2006) No variation and low synonymous substitution rates in coral mtDNA despite high nuclear variation. BMC Evol Biol 6:24. https://doi.org/10.1186/1471-2148-6-24

Hoang DT, Chernomor O, von Haeseler A, Minh BQ, Vinh LS (2018) UFBoot2: Improving the ultrafast bootstrap approximation. Mol Biol Evol 35:518-522. https://doi.org/10.1093/molbev/msx281

Hoeksema BW, Cairns S (2019) World list of Scleractinia. World Register of Marine Species (WoRMS). https://www.marinespecies.org/scleractinia

Johnston EC, Burgess SC (2023) *Pocillopora tuahiniensis*: a new species of scleractinian coral (Scleractinia, Pocilloporidae) from French Polynesia. Zootaxa 5369:117-124. https://doi.org/10.11646/zootaxa.5369.1.5

Johnston EC, Forsman ZH, Flot JF, Schmidt-Roach S, Pinzón JH, Knapp ISS, Toonen RJ (2017) A genomic glance through the fog of plasticity and diversification in *Pocillopora*. Sci Rep 7:5991. https://doi.org/10.1038/s41598-017-06085-3

Johnston EC, Forsman ZH, Toonen RJ (2018) A simple molecular technique for distinguishing species reveals frequent misidentification of Hawaiian corals in the genus *Pocillopora*. PeerJ 6:e4355. https://doi.org/10.7717/peerj.4355

Johnston EC, Cunning R, Burgess SC (2022) Cophylogeny and specificity between cryptic coral species (*Pocillopora* spp.) at Mo′orea and their symbionts (Symbiodiniaceae). Mol Ecol 31:5368-5385. https://doi.org/10.1111/mec.16654

Kalyaanamoorthy S, Minh BQ, Wong TKF, von Haeseler A, Jermiin LS (2017) ModelFinder: Fast model selection for accurate phylogenetic estimates. Nat Methods 14:587-589. https://doi.org/10.1038/nmeth.4285

Kassambara A (2021) Pipe-friendly framework for basic statistical tests. R Foundation for Statistical Computing, Vienna

Keshavmurthy S, Yang SY, Alamaru A, Chuang YY, Pichon M, Obura D, Fontana S, De Palmas S, Stefani F, Benzoni F, MacDonald A, Noreen AME, Chen C, Wallace CC, Pillay RM, Denis V, Amri AY, Reimer JD, Mezaki T, Sheppard C, Loya Y, Abelson A, Mohammed MS, Baker AC, Mostafavi PG, Suharsono BA, Chen CA (2013) DNA barcoding reveals the coral 'laboratory-rat', *Stylophora pistillata* encompasses multiple identities. Sci Rep 3:1520. https://doi.org/10.1038/srep01520

Lê S, Josse J, Husson F (2008) FactoMineR: An R package for multivariate analysis. J Stat Softw 25:1-18. https://doi.org/10.18637/jss.v025.i01

López-Pérez A, Granja-Fernández R, Ramírez-Chávez E, Valencia-Méndez O, Rodríguez-Zaragoza FA, González-Mendoza T, Martínez-Castro A (2024) Widespread coral bleaching and mass mortality of reef-building corals in Southern Mexican Pacific reefs due to 2023 El Niño warming. Oceans 5:196-209. https://doi.org/10.3390/oceans5020012

Marti-Puig P, Forsman ZH, Haverkort-Yeh RD, Knapp IS, Maragos JE, Toonen RJ (2014) Extreme phenotypic polymorphism in the coral genus *Pocillopora*; micro-morphology corresponds to mitochondrial groups, while colony morphology does not. Bull Mar Sci 90:211-231. https://doi.org/10.5343/bms.2012.1080

Mass T, Genin A (2008) Environmental versus intrinsic determination of colony symmetry in the coral *Pocillopora verrucosa*. Mar Ecol Prog Ser 369:131-137. https://doi.org/10.3354/meps07578

Millán-Márquez AM, Velasco-Montoya DA, Terraneo TI, Benzoni F, Bocanegra-Castaño C, Zapata FA (2024) Symbiodiniaceae diversity in *Pocillopora* corals in different environments of the Colombian Eastern Pacific: symbiont specificity in spite of coral-host flexibility. Coral Reefs 43:1581-1597. https://doi.org/10.1007/s00338-024-02552-2

Müller J, Müller K, Neinhuis C, Quandt D (2010) PhyDE—Phylogenetic Data Editor, version 0.9971

Nakamura T, Yamasaki H (2005) Requirement of water-flow for sustainable growth of pocilloporid corals during high temperature periods. Mar Pollut Bull 50:1115-1120. https://doi.org/10.1016/j.marpolbul.2005.06.025

Nava H, Vázquez-Peralta Á, Candelaria-Silva CF, González-Rodríguez A, Narchi NE, Mena-Castañeda AD (2025) El Niño's devastating legacy: Coral reef collapse and regime shift on the Mexican Pacific Coast. Mar Ecol 46:e70050. https://doi.org/10.1111/maec.70050

Nguyen LT, Schmidt HA, von Haeseler A, Minh BQ (2015) IQ-TREE: A fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. Mol Biol Evol 32:268-274. https://doi.org/10.1093/molbev/msu300

Noël B, Denoeud F, Rouan A, Buitrago-López C, Capasso L, Poulain J, Boissin E, Pousse M, Da Silva C, Couloux A, Armstrong E, Carradec Q, Cruaud C, Labadie K, Lê-Hoang J, Tambutté S, Barbe V, Moulin C, Bourdin G, Iwankow G, Romac S, Agostini S, Banaigs B, Boss E, Bowler C, de Vargas C, Douville E, Flores JM, Forcioli D, Furla P, Galand PE, Lombard F, Pesant S, Reynaud S, Sullivan MB, Sunagawa S, Thomas

OP, Troublé R, Thurber RV, Allemand D, Planes S, Gilson E, Zoccola D, Wincker P, Voolstra CR, Aury JM (2023) Pervasive tandem duplications and convergent evolution shape coral genomes. Genome Biol 24:123. https://doi.org/10.1186/s13059-023-02960-7

Oury N, Gélin P, Magalon H (2020) Cryptic species and genetic connectivity among populations of the coral Pocillopora damicornis (Scleractinia) in the tropical southwestern Pacific. Mar Biol 167:142. https://doi.org/10.1007/s00227-020-03757-z

Oury N, Gélin P, Rajaonarivelo M, Magalon H (2022) Exploring the *Pocillopora* cryptic diversity: a new genetic lineage in the Western Indian Ocean or remnants from an ancient one. Mar Biodivers 52:5. https://doi.org/10.1007/s12526-021-01246-0

Oury N, Noël C, Mona S, Aurelle D, Magalon H (2023) From genomics to integrative species delimitation? The case study of the Indo-Pacific *Pocillopora* corals. Mol Phylogenet Evol 184:107803. https://doi.org/10.1016/j.ympev.2023.107803

Oury N, Mona S, Magalon H (2024) Same places, same stories? Genomics reveals similar structuring and demographic patterns for four *Pocillopora* coral species in the southwestern Indian Ocean. J Biogeogr 51:754-768. https://doi.org/10.1111/jbi.14788

Oury N, Berumen ML, Paulay G, Benzoni F (2025) One species to rule them all: genomics sheds light on the *Pocillopora* species diversity and distinctiveness around the Arabian Peninsula. Coral Reefs 44:983-998. https://doi.org/10.1007/s00338-025-02659-0

Palacios MM, Muñoz CG, Zapata FA (2014) Fish corallivory on a pocilloporid reef and experimental coral responses to predation. Coral Reefs 33:625-636. https://doi.org/10.1007/s00338-014-1173-y

Paradis E (2010) Pegas: An R package for population genetics with an integrated-modular approach. Bioinformatics 26:419-420. https://doi.org/10.1093/bioinformatics/btp696

Paz-García DA (2015) Variación genética y morfológica de los corales *Pocillopora* (Anthozoa: Scleractinia) en el Pacífico Oriental. Doctoral dissertation, CIBNOR

Paz-García DA, Aldana-Moreno A, Cabral-Tena RA, García-De-León FJ, Hellberg ME, Balart EF (2015a) Morphological variation and different branch modularity across contrasting flow conditions in dominant *Pocillopora* reef-building corals. Oecologia 178:207-218. https://doi.org/10.1007/s00442-014-3199-9

Paz-García DA, Hellberg ME, García-de-León FJ, Balart EF (2015b) Switch between morphospecies of *Pocillopora* corals. Am Nat 186:434-440. https://doi.org/10.1086/682363

Pinzón JH, LaJeunesse TC (2011) Species delimitation of common reef corals in the genus *Pocillopora* using nucleotide sequence phylogenies, population genetics and symbiosis ecology. Mol Ecol 20:311-325. https://doi.org/10.1111/j.1365-294X.2010.04939.x

Pinzón JH, Sampayo E, Cox E, Chauka LJ, Chen CA, Voolstra CR, LaJeunesse TC (2013) Blind to morphology: Genetics identifies several widespread ecologically common species and few endemics among Indo-Pacific cauliflower corals (*Pocillopora*, Scleractinia). J Biogeogr 40:1595-1608. https://doi.org/10.1111/jbi.12110

Quattrini AM, Snyder KE, Purow-Ruderman R, Seiblitz IGL, Hoang J, Floerke N, Ramos NI, Wirshing HH, Rodriguez E, McFadden CS (2023) Mito-nuclear discordance within Anthozoa, with notes on unique properties of their mitochondrial genomes. Sci Rep 13:7443. https://doi.org/10.1038/s41598-023-34059-1

Ricart AM, Rodríguez-Zaragoza FA, González-Salas C, Ortiz M, Cupul-Magaña AL, Adjeroud M (2016) Coral reef fish assemblages at Clipperton Atoll (eastern tropical

Pacific) and their relationship with coral cover. Sci Mar 80:479-486. https://doi.org/10.3989/scimar.04301.12B

Riginos C, Popovic I, Meziere Z, Garcia V, Byrne I, Howitt SM, Ishida H, Bairos-Novak K, Humanes A, Scharfenstein H, Richards T, Briggs E, Clark V, Lei C, Khan M, Prata KE (2024) Cryptic species and hybridisation in corals: challenges and opportunities for conservation and restoration. Peer Community J 4:e106. https://doi.org/10.24072/pcjournal.492

Romero-Torres M, Treml EA, Acosta A, Paz-García DA (2018) The Eastern Tropical Pacific coral population connectivity and the role of the Eastern Pacific Barrier. Sci Rep 8:9354. https://doi.org/10.1038/s41598-018-27644-2

Rozas J, Sánchez-DelBarrio JC, Messeguer X, Rozas R (2003) DnaSP, DNA polymorphism analyses by the coalescent and other methods. Bioinformatics 19:2496-2497. https://doi.org/10.1093/bioinformatics/btg359

Rueden CT, Schindelin J, Hiner MC, DeZonia BE, Walter AE, Arena ET, Eliceiri KW (2017) ImageJ2: ImageJ for the next generation of scientific image data. BMC Bioinformatics 18:529. https://doi.org/10.1186/s12859-017-1934-z

Schmidt-Roach S, Lundgren P, Miller KJ, Gerlach G, Noreen AME, Andreakis N (2013) Assessing hidden species diversity in the coral *Pocillopora damicornis* from Eastern Australia. Coral Reefs 32:161-172. https://doi.org/10.1007/s00338-012-0959-z

Schmidt-Roach S, Miller KJ, Lundgren P, Andreakis N (2014) With eyes wide open: a revision of species within and closely related to the *Pocillopora damicornis* species complex (Scleractinia; Pocilloporidae) using morphology and genetics. Zool J Linn Soc 170:1-33. https://doi.org/10.1111/zoj.12092

Stefani F, Benzoni F, Yang SY, Pichon M, Galli P, Chen CA (2011) Comparison of morphological and genetic analyses reveals cryptic divergence and morphological

plasticity in *Stylophora* (Cnidaria, Scleractinia). Coral Reefs 30:1033-1049. https://doi.org/10.1007/s00338-011-0797-4

Todd PA (2008) Morphological plasticity in scleractinian corals. Biol Rev 83:315-337. https://doi.org/10.1111/j.1469-185X.2008.00045.x

Trifinopoulos J, Nguyen LT, von Haeseler A, Minh BQ (2016) W-IQ-TREE: a fast online phylogenetic tool for maximum likelihood analysis. Nucleic Acids Res 44:W232-W235. https://doi.org/10.1093/nar/gkw256

Vences M, Patmanidis S, Schmidt JC, Matschiner M, Miralles A, Renner SS (2024) Hapsolutely: a user-friendly tool integrating haplotype phasing, network construction, and haploweb calculation. Bioinformatics Adv 4:vbae083. https://doi.org/10.1093/bioadv/vbae083

Veron J (2002) New species described in Corals of the World. Australian Institute of Marine Science Monograph Series, Australian Institute of Marine Science

Veron J (2011) Scleractinia, evolution and taxonomy. In: Hopley D (ed) Encyclopedia of modern coral reefs. Springer, Dordrecht, pp 947-957. https://doi.org/10.1007/978-90-481-2639-2_78

Voolstra CR, Hume BCC, Armstrong EJ, Mitushasi G, Porro B, Oury N, Agostini S, Boissin E, Poulain J, Carradec Q, Paz-García DA, Zoccola D, Magalon H, Moulin C, Bourdin G, Iwankow G, Romac S, Banaigs B, Boss E, Bowler C, de Vargas C, Douville E, Flores M, Furla P, Galand PE, Gilson E, Lombard F, Pesant S, Reynaud S, Sullivan MB, Sunagawa S, Thomas OP, Troublé R, Thurber RV, Wincker P, Planes S, Allemand D, Forcioli D (2023) Disparate genetic divergence patterns in three corals across a pan-Pacific environmental gradient highlight species-specific adaptation. npj Biodivers 2:1-16. https://doi.org/10.1038/s44185-023-00020-8

Wickham H (2011) ggplot2. Wiley Interdiscip Rev Comput Stat 3:180-185. https://doi.org/10.1002/wics.147

Woerner AE, Cox MP, Hammer MF (2007) Recombination-filtered genomic datasets by information maximization. Bioinformatics 23:1851-1853. https://doi.org/10.1093/bioinformatics/btm253

Yu G, Smith DK, Zhu H, Guan Y, Lam TTY (2017) ggtree: an R package for visualization and annotation of phylogenetic trees with their covariates and other associated data. Methods Ecol Evol 8:28-36. https://doi.org/10.1111/2041-210X.12628

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