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Short Communication

Genetic differences of *Acropora palmata* populations of the Mexican Atlantic

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ABSTRACT. The populations of *Acropora palmata* have decreased over the last four decades, and although there are several studies on their genetic diversity in the Caribbean, no studies have been published about the reefs from the southern Gulf of Mexico. This research aimed to determine, using five microsatellite markers, the genetic variation of three *A. palmata* populations in the Mexican Atlantic located in the southwest Gulf of Mexico, in the Campeche Bank, and in the Caribbean. The mean of genetic richness (N_g/N) in the studied reefs was 0.583; the lowest estimated value corresponded to the Campeche Bank reef. A low genetic diversity was registered in the studied reefs (reef mean $H_e = 0.315 \pm 0.052$). The significant genetic structure observed among studied populations could be related to ocean currents from the region and specific biological characteristics, mainly, short larvae phase, high mortality, and high rate of self-recruitment. These results may be particularly useful for designing management strategies, considering the lack of studies of this type in the region.

Keywords: *Acropora palmata*; elkhorn coral; genetic structure; microsatellites; Campeche Bank; southwest Gulf of Mexico; Mexican Caribbean

Elkhorn coral (*Acropora palmata*) is a species found on coral reefs of the tropical Caribbean and Gulf of Mexico; large colonies of great sizes provide critical protection to the coastline by reducing incoming wave energy. From the ecological point of view, *A. palmata* populations provide habitat and protection for many organisms in the reef (Harborne et al. 2006). Species belonging to this genus have undergone a drastic decline in their coverage in the last decades due to many factors, including diseases, hurricanes, predation, pollution, and climate change (Precht et al. 2002, Porto-Hannes et al. 2014). *A. palmata* is listed as critically endangered by the International Union for Conservation of Nature Red List criteria (Porto-Hannes et al. 2014). Nevertheless, there are currently no specific actions to protect and conserve the populations of *A. palmata* in Mexico (SEMARNAT 2018).

Several studies have been carried out using molecular analysis on *A. palmata.* Baums et al. (2005) and Porto-Hannes et al. (2014) detected a restricted gene flow of this species between populations geographically separated by hundreds of kilometers in the western Caribbean, while Japaud et al. (2019) registered isolation by distance in the Lesser Antilles (eastern Caribbean). Japaud et al. (2015) found extremely low genotypic richness for the populations of *A. palmata* and *A. cervicornis* from Guadalupe, French Lesser Antilles; this suggests long periods of clonal growth without sexual recruitment which may lead to the extinction of these populations. For the reefs of the southwest Gulf of Mexico, there are no studies evaluating the genetic structure and diversity. Based on that background, the present study analyzed the population structure of *A. palmata* of three populations in the Mexican Atlantic.

Considering the three major reef subregions identified by Jordán-Dahlgren & Rodríguez-Martínez (2003), one reef from each subregion was sampled in the Mexican Atlantic. For the southwest Gulf of Mexico subregion, samples were obtained from Isla de

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Enmedio Reef in the Veracruz Reef System (VE; 19°06'3.6"N, 95°56'20.4"W); samples from the Campeche Bank subregion were obtained from a site located inside Alacranes Reef (AL; 22°22'55.2"N, 89°40'58.8"W); and in the Caribbean subregion, Mahahual Reef (MA; 18°42'07.2"N, 87°42'32.4"W) was sampled (Fig. 1). All reefs are within Marine Protected Areas, VE and AL having National Park status, while MA is located within the Mexican Caribbean Biosphere Reserve, decreed in 2016.

Samples from VE and MA were obtained between September and October 2010, while the AL reef was sampled in February 2011. A total of 60 colonies were sampled (20 per site). With a chisel and a hammer, fragments of 5 cm^2 were snipped off from each colony.

Genomic DNA was extracted with an alkaline method based on treatment with sodium ethylenediaminetetraacetic acid (EDTA) and tris hydrochloric acid (Tris-HCl; Baums et al. 2009). The microsatellite loci used were 585, 513, 1490, 2637, and 5047, since these amplified correctly for most of the samples, following the protocol proposed by Baums et al. (2009). The PCR products were separated on 6% polyacrylamide gels (19:1 acrylamide:bis; 7.5 M urea; 1x TBE buffer). Electrophoresis was performed using a Sequi-Gen II GT Nucleic Acid Electrophoresis Cell (Bio-Rad), between 1400 and 1600 V for approximately 2 h. Amplified fragments were visualized with a silver stain following the methodology described by Bassam et al. (1991).

Estimates of genetic diversity and population structure were performed with unique genotypes (Baums et al. 2006). The number of unique genotypes (genets; Ng) and their proportion concerning the number of colonies sampled (genotypic richness; Ng/N) were calculated. For each reef, genetic diversity was calculated as the number of alleles (N_a) and the effective number of alleles (A_E), which is defined as the alleles with the capacity of passing to the next generation (Kimura & Crow 1964). Observed heterozygosity (H_o) and expected heterozygosity (H_e) were also calculated. Wright's fixation index (F_{IS}) was used to test if the allelic frequencies conformed to the Hardy-Weinberg equilibrium (*HWE*; Weir & Cockerham 1984). The Wright index (F_{ST}; Weir & Cockerham 1984) evaluated genetic differences between reef pairs. The false discovery rate (BY-FDR; Benjamini & Yekutieli 2001) was applied to multiple tests of F_{ST}. An analysis of molecular variance (AMOVA) evaluated the genetic variation at different hierarchical levels, with total genetic variation partitioned into three levels: among reefs, among individuals, and within individuals (Excoffier et al. 2005). These analyses were carried out with GenAlex 6.5 (Peakall & Smouse 2012) and Arlequin 3.5 (Excoffier et al. 2005).

The software Structure version 2.2 (Pritchard et al. 2000) was used to estimate the optimal number of homogeneous genetic units (K) with admixture model for K = 1-10, using 10 iterations, a burn-in length of 5×10^5, and 10^6 of Markov chain Monte Carlo (MCMC) replications, with correlated allele frequency. We also used an *ad-hoc* ΔK measure which provided a more robust estimate of K (Evanno et al. 2005).

Thirty-five genets and 25 clones from a total of 60 colonies were registered. The values of N_g/N ranged from 0.4 to 0.7, where the lowest value was registered at AL (Table 1). The most polymorphic locus was 1490, with four alleles at VE. The highest N_a mean was observed in MA (N_a = 2.6), followed by VE (N_a = 2.2). The locus 585 was monomorphic in AL, while loci 5047 and 513 were monomorphic in VE. H_e mean values for the reefs varied from 0.296 (VE) to 0.330 (AL). Although values for H_e and H_o were low, the results of the F_{IS} index (average = -0.232) did not indicate a departure to the *HWE*. The lowest F_{IS} mean was observed in AL (F_{IS} = -0.419; Table 1).

Pairwise values of F_{ST} ranged from 0.058 to 0.214. The highest F_{ST} value was observed between VE and AL and the lowest between VE and MA. The F_{ST} value between MA and AL was 0.116. All pairwise comparisons of F_{ST} values showed significant genetic differences. AMOVA results also suggested significant differences, only among reefs (F_{ST} = 0.177; P = 0.001), with a percentage of variation of 17.73.

The clustering analysis also suggested the existence of differences among reefs and showed that there is no genetic sub-structuring within each of them. According to ΔK, the most optimal value for K in this study was 3 (Fig. 2a). Structure software showed that the percentages of group membership for AL, MA, and VE in clusters reefs were 97, 88, and 93%, respectively (Fig. 2b).

The most significant findings of this study were: a) a low dominance of clones for MA and VE reefs, while a high dominance for AL, b) a low genetic diversity in the studied reefs, c) heterozygote excess in the three studied populations, and d) a significant genetic structure between the three populations.

The general mean ratio of genets found (0.583 ± 0.093) is similar to that reported by Baums et al. (2006) for the same species, both for the western Caribbean (N_g/N = 0.43), as well as for the wider Caribbean (N_g/N = 0.51). Nevertheless, other studies have reported higher values; for example, Mège et al. (2015) carried

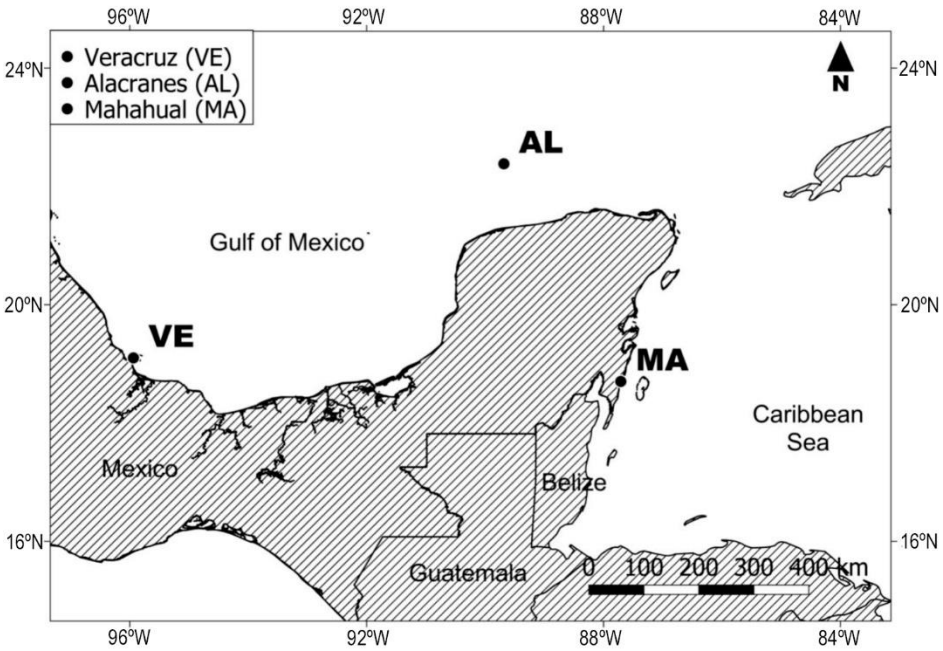

Figure 1. Geographic location of the three sampled reefs.

Table 1. Number of alleles (N_a), number of effective alleles (A_E), observed heterozygosity (H_o), expected heterozygosity (H_e), and fixation index (F_{IS}) by locus for *Acropora palmata* populations in the Mexican Atlantic. Also, n: sample size, number of genets (N_g ± standard error), and genotypic richness (N_g/N) of *A. palmata* populations. SE: standard error. AL: Alacranes, MA: Mahahual, and VE: Veracruz.

	Locus	N_a	A_E	H_o	H_e	F_{IS}
AL	585	1.000	1.000	0.000	0.000	-
(n = 8)	1490	2.000	1.600	0.500	0.400	-0.333
N_g = 8 ± 0.681	2637	2.000	1.969	0.875	0.525	-0.778
N_g/N = 0.400	5047	2.000	1.438	0.375	0.325	-0.231
	513	2.000	1.600	0.500	0.400	-0.333
Mean		1.800	1.521	0.450	0.330	-0.419
SE±		0.200	0.157	0.140	0.089	0.109
MA	585	3.000	1.266	0.231	0.218	-0.099
(n = 13)	1490	3.000	1.587	0.462	0.385	-0.248
N_g = 13 ± 0.215	2637	3.000	1.610	0.462	0.394	-0.219
N_g/N = 0.650	5047	2.000	1.451	0.385	0.323	-0.238
	513	2.000	1.352	0.308	0.271	-0.182
Mean		2.600	1.453	0.369	0.318	-0.197
SE±		0.245	0.066	0.045	0.033	0.027
VE	585	2.000	1.415	0.357	0.304	-0.217
(n = 14)	1490	4.000	3.213	0.429	0.714	0.378
N_g = 14 ± 0.291	2637	3.000	1.806	0.571	0.463	-0.280
N_g/N = 0.700	5047	1.000	1.000	0.000	0.000	-
	513	1.000	1.000	0.000	0.000	-
Mean		2.200	1.687	0.271	0.296	-0.040
SE±		0.583	0.410	0.116	0.138	0.162
All population						
Mean		2.200	1.554	0.364	0.315	-0.232
SE±		0.223	0.139	0.061	0.052	0.066

out a study in the continental shelf of Puerto Rico and reported a ratio of genets of 0.71; on the other hand, Porto-Hannes et al. (2014), reports a ratio of N_g/N = 0.852 for the Caribbean Sea. The difference in results

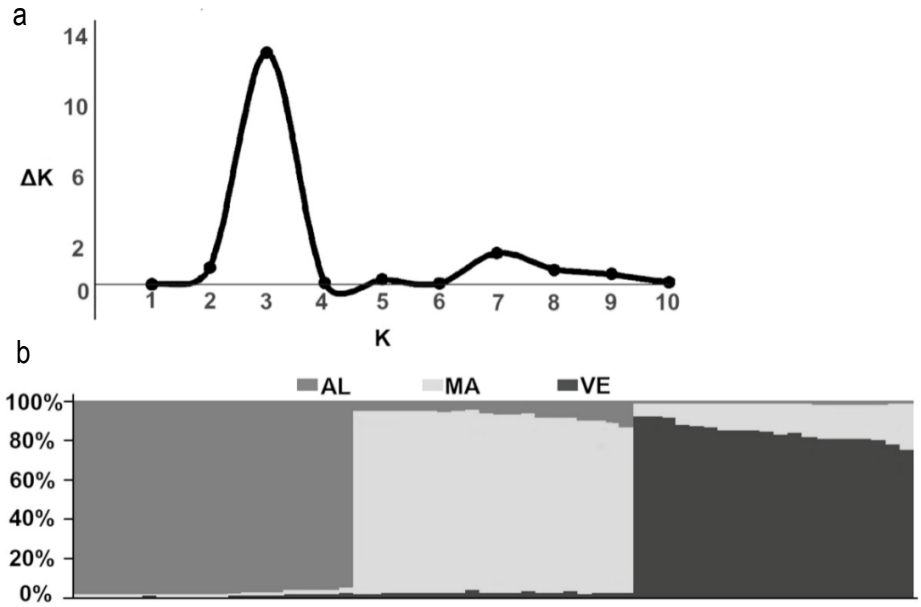

Figure 2. Structure analysis results: a) modal value of the likelihood differences (ΔK) standardized with the standard deviation of the data. The highest ΔK value was registered for three homogeneous genetic units (K = 3); b) structure-generated bar-plot. Cluster identifications are: Alacranes (AL), Mahahual (MA), and Veracruz (VE).

can be attributed to various factors, such as sample size, environmental factors, and the type of genetic marker used (Porto-Hannes et al. 2014, Mège et al. 2015).

The differences among the genotypic richness of the three reefs in this study can be associated with hurricane disturbance, ocean-current dynamics and competition, and reef orientation and inclination (Highsmith et al. 1980). The lowest genotypic richness was observed in AL, which suggests that the maintenance of the *A. palmata* population could be related to asexual reproduction for this reef. Baums et al. (2006) mention that certain environmental conditions such as an extensive and shallow continental shelf trigger a higher presence of clones in this genus. Additionally, it has been reported that high wave energy promotes the asexual reproduction of the *Acropora* population (Japaud et al. 2015). Similar conditions to those mentioned above are found in AL, since it is located within an extensive continental platform, 135 km off the coast, and usually presents high wave intensity; within the zone, also, a complex ocean dynamic exists (with strong eddies and a complex vertical profile), due to the Yucatan Current (Athié et al. 2011).

Regarding the VE reef, which presented the highest genotypic richness, the sampled colonies were located from the windward slope to the inner frontal section with depths of almost 20 m. In this context, it has been reported that depth limits the capacity for asexual reproduction (Mège et al. 2015). Additionally, this reef is less affected by hurricanes, differentiating it from the AL reef (Salas-Monreal et al. 2018). On the other hand, sampled colonies in the MA reef (N_g/N = 0.650) were found between the surf zone and the reef lagoon.

In general terms, a low genetic diversity was observed when compared to other studies carried out in the Caribbean for the same species and using the same molecular markers, as the cases of Porto-Hannes et al. (2014; H_e = 0.797), Baums et al. (2009; H_e = 0.675) and Japaud et al. (2015; H_e = 0.710). The difference in the sample size and number of molecular markers used cannot be discarded as factors for the low values of H_e calculated. According to Porto-Hannes et al. (2014), a sample size between 15 and 24 individuals allows the characterization of less than 50% of the allelic richness. Also, registered H_e values could be associated with high levels of self-recruitment and the presence of clones (Porto-Hannes et al. 2014). Asexual reproduction (fragmentation) is very frequent in *A. palmata* (Baums et al. 2005) since it can occur throughout the year, unlike sexual reproduction, which is limited to one or two events annually (Jackson 1977). The loss of coverage of *A. palmata* in the last decades in the study area (Jordán-Dahlgren & Rodríguez-Martínez 2003, SEMARNAT 2018) is another factor to be considered for the low levels of genetic diversity reported, since it can reduce the viable populations and, in consequence, the connectivity among populations (Baums et al. 2005, Rodríguez Martínez et al. 2014).

Mean F_{IS} values for each reef were negative, suggesting a heterozygote excess, where asexual reproduction could be the main cause. The highest F_{IS} was reported in AL, a reef where the highest presence of clones was observed. The divergence between a locus's alleles tends to increase with asexual reproduction over time; previous studies with microsatellites showed high heterozygosity in asexual lineages (Delmotte et al. 2002, Stoeckel et al. 2006).

The existence of significant genetic differences between the three reefs suggests that the number of migrants between the reefs is not enough to homogenize the genetic variability of the loci. Similar results have been found in genetic studies carried out with the same species; Baums et al. (2005) concluded that the populations of *A. palmata* presented significant genetic discontinuity between east and west Caribbean populations, suggesting little or no gene flow between regions. Porto-Hannes et al. (2014) suggested that *A. palmata* subdivides into four sub-regions in the Caribbean, with significant genetic differences. They also reported genetic differences between the reefs of the Mesoamerican Barrier Reef System. Currents can be one of the variables influencing genetic differences. Galindo et al. (2006), who used an oceanographic genetic model of dispersion for larvae of *A. cervicornis*, suggested that the connectivity between Campeche Bank and the Caribbean is limited. Besides the predominant oceanic currents in the study area, the genetic differences reported in this study can be attributed to the biological characteristics of the species: short larvae period (3-7 days), high self-recruitment, and dispersion determined by the duration of the planktonic phase, with high mortality (>90%) caused by predation and natural mortality (Goreau & Hayes 1981, Harrison & Wallace 1990, Cetina-Heredia & Connolly 2011). The results here presented further the knowledge of *A. palmata* populations in the Mexican Atlantic; however, in order to better understand the genetic dynamics of this species, more studies with an increasing number of samples and reefs from the region would be needed to build conservation programs based on relevant genetic variation information for *A. palmata* populations in the Mexican Caribbean.

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