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# Connectivity of the sea urchin *Diadema mexicanum* A. Agassiz, 1863 (Echinoidea, Diadematidae) in the Pacific coast of Costa Rica

# Conectividad de la población del erizo de mar *Diadema mexicanum* A. Agassiz, 1863 (Echinoidea, Diadematidae) en el Pacífico de Costa Rica

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**ABSTRACT**

*Diadema mexicanum* is essential for controlling algae and maintaining coral dominance on coral reefs. Despite its importance as a key grazing species, little is known about the genetic structure and connectivity of its populations. Molecular markers are particularly sensitive to genetic differences between disjunct populations, providing insight into their resilience to environmental changes. This study seeks to genetically characterize *D. mexicanum* populations on coral reefs along the Pacific coast of Costa Rica. Sampling took place between May and October 2019. DNA was extracted from each sample, and microsatellite markers were subsequently amplified using primers designed for *D. antillarum* and *Strongylocentrotus nudus*. Data analysis was performed using GeneMarker, R Studio, and Structure. The analysis revealed lower genetic diversity than previously reported for these microsatellites, resulting in high inbreeding coefficient values. This could be attributed to several factors, such as high reproductive success variation and null alleles. A weak genetic structure was found among sampling sites, but this structure was independent of the region where samples were collected. No isolation by distance was detected, suggesting

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genetic connectivity and gene flow within populations. Future studies would benefit from analyzing a wider range of molecular markers and ensuring more equitable sampling across sites.

**Keywords:** echinoid, gene flow, genetic markers, microsatellites, population genetics

**RESUMEN**

*Diadema mexicanum* es esencial para el control de algas y el mantenimiento del dominio coralino en los arrecifes de coral. A pesar de su importancia como especie clave de pastoreo, se conoce poco sobre la estructura genética y la conectividad de sus poblaciones. Los marcadores moleculares son particularmente sensibles a las diferencias genéticas entre poblaciones disjuntas, lo que proporciona información sobre su resiliencia frente a los cambios ambientales. Este estudio busca caracterizar genéticamente las poblaciones de *D. mexicanum* en arrecifes de coral a lo largo del Pacífico de Costa Rica. El muestreo se realizó entre mayo y octubre de 2019. Se extrajo ADN de cada muestra y, posteriormente, se amplificaron los marcadores microsatélites utilizando cebadores diseñados para *D. antillarum* y *Strongylocentrotus nudus*. El análisis de datos se realizó con GeneMarker, R Studio y Structure. El estudio reveló una diversidad genética menor que la reportada previamente para estos microsatélites, lo que resulta en altos valores de coeficiente de endogamia. Esto podría atribuirse a varios factores, incluyendo la alta variación en el éxito reproductivo y la presencia de alelos nulos. Se encontró una estructura genética débil entre los sitios de muestreo, pero esta estructura era independiente de la región donde se recolectaron las muestras. No se detectó aislamiento por distancia, lo que sugiere conectividad genética y un flujo génico dentro de las poblaciones. Estudios futuros se beneficiarían del análisis de una gama más amplia de marcadores moleculares y de asegurar un muestreo más equitativo entre los sitios.

**Palabras clave:** echinoidea, flujo génico, genética de poblaciones, marcadores genéticos, microsatélites

## INTRODUCTION

The sea urchin *Diadema mexicanum* A. Agassiz, 1863 (Echinodermata: Diadematidae) has a wide distribution in the Eastern Tropical Pacific (ETP) (Alvarado *et al*. 2015a; Paz-García *et al*. 2018), inhabiting a wide variety of environments such as coral reefs, rocky bottoms, mangrove roots, seagrass, and sandy bottoms (Birkeland, 1989). *Diadema mexicanum* is predominant in the coral reefs of the Pacific of Costa Rica (Alvarado *et al*. 2015b; Alvarado *et al*. 2016a; Alvarado *et al*. 2018), where they strongly influence algal biomass, diversity, and community structure, and


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are key determinants of the carbonate balance (Alvarado *et al*. 2015a; Alvarado *et al*. 2016b).

The population density of *D. mexicanum* is regulated by factors related to habitat complexity, the availability of algae, and the presence of predators (Alvarado *et al*. 2016c). In Costa Rica, the population density of *D. mexicanum* has increased following El Niño events due to the loss of coral cover and the subsequent increase in algae, which implies greater food availability due to its role as herbivores. A decrease in the population density of *D. mexicanum* has also been observed in Marine Protected Areas (MPAs), likely due to increases in the abundance of predatory fish that feed on urchins, keeping their abundance under control (Alvarado & Chiriboga, 2008; Alvarado *et al*. 2012). Therefore, the population density of *D. mexicanum* is closely related to the environmental conditions of its habitat and the abundance of its predators (Alvarado & Chiriboga, 2008; Alvarado *et al*. 2012; Alvarado *et al*. 2015a). Moreover, to better understand the communities of this species, it is also important to study the connectivity between subpopulations. Given *D. mexicanum*'s importance as a key herbivore and ongoing alterations to the ecological and environmental processes that determine their abundance, it is important to study the connectivity between subpopulations.

Connectivity is essential for the persistence and resilience of marine populations, as it facilitates the natural processes that sustain the health of marine ecosystems. In the absence of connectivity, these ecosystems may become more vulnerable to disturbances, experience a loss of genetic diversity, and face greater challenges in terms of recovery and long-term sustainability (Cowen *et al*. 2007; Cowen & Sponaugle, 2009). The connectivity between *D. mexicanum* populations could occur through the dispersal of its pelagic larvae, which can survive up to 42 days under laboratory conditions (Emlet, 1995).

Molecular markers such as microsatellites constitute very useful tools for studying the connectivity between populations (Amiteye, 2021). Microsatellites or simple sequence repeats (SSR) are known for their numerous advantages, including high polymorphism, evolutionary neutrality, a high mutation rate, codominance, multiallelic nature, reproducibility, and transferability between related species. These attributes make microsatellites a valuable tool for genetic mapping, population structure analysis, and evolutionary investigations. They enable the precise discrimination of genotypes within a population and offer insights into connectivity among nearby populations (Vieira *et al*. 2016).

Microsatellites have been used to describe different populations of

echinoderms. For example, they have been employed in studies of sea cucumbers such as *Holothuria leucospilota* (Yang *et al*. 2024) and *Apostichopus japonicus* (Dong *et al*. 2018), sea stars such as *Coscinasterias tenuispina* and *Echinaster sepositus* (García-Cisneros *et al*. 2013), and sea urchins such as *Paracentrotus lividus* (Calderón *et al*. 2009), *Arbacia lixula* (García-Cisneros *et al*. 2013), and *Strongylocentrotus droebachiensis*. To date, microsatellites have not been used to study sea urchin *D. mexicanum* populations.

Lessios *et al*. (2001) indicated that populations of *D. mexicanum* in the Galápagos and Cocos Islands are the same genetically as those in Panama and Mexico. Lessios *et al*. (2001) used mitochondrial DNA as a molecular marker; however, it has been demonstrated that microsatellites can detect isolation by distance when mitochondrial DNA fails to do so (Teske *et al*. 2018). The other molecular marker used by Lessios *et al*. (2001) was isozymes. When compared to microsatellites, it was observed that the SSRs can more effectively detect genetic differences (Becerra & Paredes, 2000).

Given the ecological relevance of *D. mexicanum* and the opportunity to test new molecular techniques for this species, we genetically characterized the population of *D. mexicanum* in the coral reefs from the Pacific coast of Costa Rica using microsatellites. The goal is to determine if there is connectivity between the populations, describing the genetic diversity and structure of the populations and assessing the genetic connectivity.

## MATERIAL AND METHODS

### Sampling sites

The sampling was conducted at various coral reef locations along the Pacific coast of Costa Rica between May and October 2019. Collection was carried out using SCUBA gear at depths ranging from 0 to 10 m. Additionally, three specimens from the Museum of Zoology of the Universidad de Costa Rica, collected in Punta Ulloa, Cocos Island in 2006 and 2011, were included. The study was divided into three regions: 1) The oceanic Cocos Island, 2) the north mainland Pacific, and 3) the southern mainland Pacific (Fig. 1).

### DNA extraction

DNA extraction was performed using tissue from the ambulacral feet stored in 90% alcohol following the protocol of the NucleoSpin Tissue Kit (Macherey-Nagel, 2020). The quality of the extracted DNA was verified through electrophoresis on a 1% agarose gel, and its concentration was quantified using a NanoDrop™ 2000 (Thermo Scientific).

### Microsatellites (SSR)

A total of 48 sea urchins were analyzed. There was no report of


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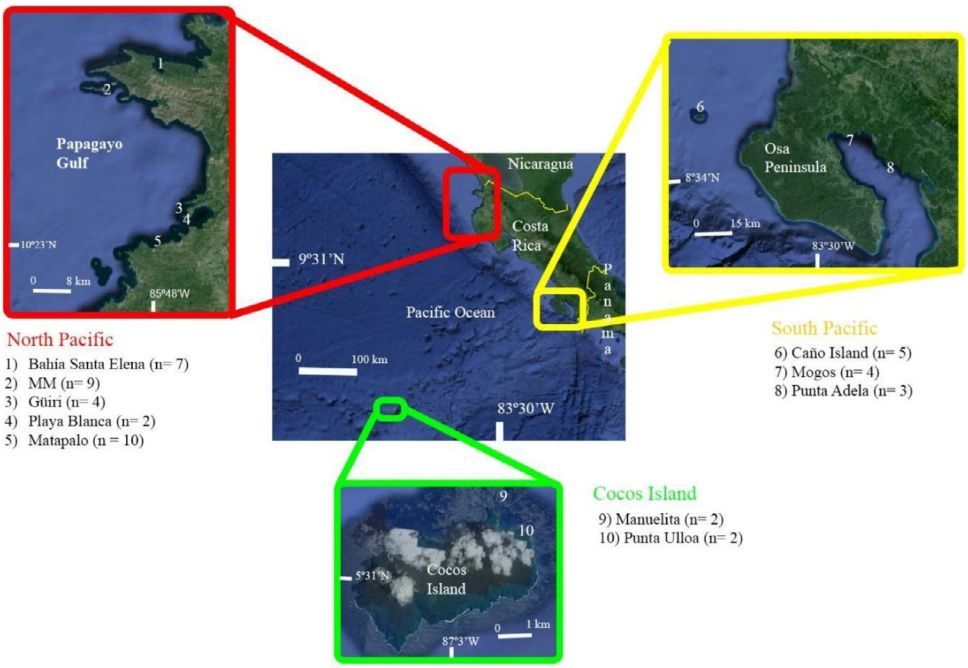


Fig. 1. Sampling locations of individuals of *Diadema mexicanum* and number of individuals (n) collected along the Pacific coast of Costa Rica, including two sites at the oceanic Cocos Island (Manuelita and Punta Ulloa), five sites in the northernmost mainland of the Pacific, along the coast of Guanacaste (Northern Pacific: Bahía Santa Elena, Murciélago Island (MM), Güiri, Playa Blanca, Matapalo) and three sites along the southernmost mainland of the Pacific (Southern Pacific: Caño Island, Mogos and Punta Adela)

Fig. 1. Localizaciones de muestreo de individuos de *Diadema mexicanum* y número de individuos (n) colectados a lo largo de la costa del Pacífico de Costa Rica incluyendo: dos sitios en la oceánica Isla del Coco (Manuelita y Punta Ulloa), cinco sitios en la parte más norte del Pacífico, en la costa de Guanacaste (Pacífico Norte: Bahía Santa Elena, Isla Murciélago (MM), Güiri, Playa Blanca, Matapalo) y tres sitios en la parte más sur del Pacífico costarricense (Pacífico Sur: Isla del Caño, Mogos y Punta Adela)

microsatellite primers specific for *D. mexicanum*. Therefore, 10 microsatellite primers developed for taxonomically close species, such as *Diadema antillarum* (Chandler *et al*. 2017) and *Strongylocentrotus nudus* (Li & Li, 2008), were chosen and tested on *D. mexicanum*. However, only four out of the ten SSRs were amplified in *D. mexicanum*

using end-point Polymerase chain reactions (PCR), and therefore, they were used in this study. These four markers were fluorescently labeled with either Applied Biosystems™ 6-FAM or NED (Table 1). PCR were performed following the protocols specified in the references for each of the primers (Chandler *et al*. 2017; Li & Li, 2008). Briefly, for KS03, KS09 and KS29, the reactions took place using 1µL DNA, 2µL 10x PCR buffer, 1.3µL 25mM MgCl2, 1.6µL 10mM dNTPs, 1µL 10µM reverse primer, 0.3µL 10µM forward primer, 0.2µL DreamTaq DNA Polymerase, and 1µL 10mM fluorescent dye. PCRs included an initial denaturation step at 94°C for 4 min, 35 cycles at 94°C for 30 s., annealing at 55°C for 35 s., and 72°C for 45 s., followed by a final extension period at 72°C for 7 min. For SN225, the PCR contained 0.25 U DreamTaq DNA Polymerase 1X PCR buffer (10 mM Tris–HCl, 50 mM KCl, pH 8.3), 0.2 mM dNTP mix, 1 uM of each primer set, 1.5 mM MgCl2 and and 1µL 10mM fluorescent dye. The PCR was performed as follows: 3 min at 94°C; 35 cycles of 1 min at 94°C, annealing for 1 min, 72°C for 1 min per cycle; followed by 5 min at 72°C. The PCR reactions were performed in a Veriti™ Thermal Cycler, 96-well Fast. The PCR products were analyzed by capillary electrophoresis using an Applied Biosystems® ABI3130 and genotyped with the software GeneMarker® 2.6.4 (Soft Genetics, 2021).

**Data analysis**

Once all the samples were genotyped, the Hardy-Weinberg equilibrium test was performed using 1,000 replicates for the Monte Carlo procedure (Guo & Thompson, 1992). Linkage disequilibrium (LD) was evaluated by calculating the Standardized Index of Association rbarD for the SSR (Kamvar *et al*. 2015). Statistical significance of LD was assessed by conducting a one-sided permutation test, where the observed LD values were compared against those generated from 999 simulated datasets. The percentage of null alleles was calculated using the FreeNA software with 10000 replicates (Chapuis & Estoup, 2007). To assess the genetic diversity, observed ($H_o$) and expected ($H_e$) heterozygosity, allelic richness (AR), number of private alleles, and number of effective alleles ($N_e$) were estimated per locus, location, and region. The reduction in individual heterozygosity within the locations and regions was calculated through the inbreeding coefficient ($F_{is}$). To investigate if there is a genetic structure in the established regions (i.e., if they behave as isolated populations), two tests were conducted: 1) Nei´s genetic differentiation ($G_{st}$) and 2) a discriminant analysis of principal components


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Table 1. Sequence of the Forward (F) and Reverse (R) primers, fluorescent dyes, species in which the primers were designed, and reference of the article of each primer used in this investigation. ND= No data available in the original publication

Cuadro 1. Secuencia de los cebadores Forward (F) y Reverse (R), colorantes fluorescentes, especies en las que se diseñaron los cebadores y referencia del artículo de cada cebador utilizado en esta investigación. ND= No hay información disponible en la publicación de referencia

| Locus | Primer sequences (5´-3´) | Ta (ºC) | Dyes | Species | Repeat motif | Size range (bp) | Reference |
|---|---|---|---|---|---|---|---|
| KS03 | F: TGTAAAACGACGGCCAGTCTTCCCGTTTTGTTGCATTT | 57 | 6-Fam | *Diadema antillarum* | *ND* | *ND* | Chandler *et al.* 2017 |
| | R: CCGAACATGGATCCCTAAAA | | | | | | |
| KS09 | F: TGTAAAACGACGGCCAGTTTTGCCAATGAGCTGTCAAG | 62 | NED | | | | |
| | R: CCACCTCAACCACATCTGAG | | | | | | |
| KS29 | F: TGTAAAACGACGGCCAGTCTTCCCGTTTTGTTGCATTT | 62 | 6-Fam | | | | |
| | R: AGTTGGAAGGGACGATGTTG | | | | | | |
| SN225 | F: 5'TATTTTGGTTCCGATTTCA 3' | | 6-Fam | *Strongylocentrotus nudus* | (GA)10 | 253-261 | Li & Li 2008 |
| | R: 5'TCGTGTCAAGTCGCTGTC 3' | 49 | | | | | |

(DAPC) (Jombart *et al*. 2010). Additionally, to estimate genetic differences between Pacific regions and sampling location, a global molecular analysis of variance (AMOVA) was performed. To determine the presence of genetic isolation among the sampling sites is associated with geographical distance, a Mantel test was conducted, which uses geographic distances between collection sites and Nei's genetic differentiation ($G_{st}$) (Nei, 1973; Nei & Chesser, 1983). The mentioned test and statistics were conducted using R studio software (R Core Team, 2021), using the following libraries: poppr (Kamwar *et al*. 2015), hierfstat (Goudet, 2005), adegenet (Jombart & Ahmed, 2011), ade4 (Dray *et al*. 2007), mmod (Winter, 2012), and pegas (Paradis, 2010). To conclude, a Bayesian-based clustering method was performed with the program STRUCTURE v2.3.4 (Pritchard *et al*. 2000), to determine the most likely number of clusters (K) supported by the data, with 10 runs for each cluster from 1 to 10 (10 000 iterations with 10 000 burn-in period). StructureSelector program (Li & Li, 2018) was used to process the data from the previous analysis.

## RESULTS

### Diversity estimates per molecular marker

A total of 48 sea urchins were genotyped with four microsatellite markers; among these, only the SN225 marker exhibited a significant deviation from HWE (Table 2); consequently, this marker was excluded from analyses of population strata. Overall, the global null allele frequency was 9.5%, while the estimated frequency was 15%. There was no evidence of linkage disequilibrium among the markers (rbarD = 0.03, $P$ = 0.46). Marker KS03 showed the greater number of alleles and effective alleles $N_a$ = 47 and $N_e$ = 6.44, while SN225 had the lowest number of alleles and fewer effective alleles $N_a$ = 18 and $N_e$ = 2.91. All the SSRs presented a lower observed heterozygosity than expected (Table 2). The marker with more genetic diversity was KS03 $H_o$= 0.83; on the other hand, SN225 was the least diverse $H_o$ = 0.54. The marker KS29 had the highest heterozygotes deficit ($F_{is}$ = 0.37), while KS09 had the lowest ($F_{is}$ = 0.18).

### Diversity estimates per region

Out of the 48 genotyped samples, 32 belong to the North Pacific region, four to Cocos Island, and 12 to the South Pacific region. The North Pacific showed a higher number of alleles


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Table 2. Estimates of genetic diversity and allelic richness per SSR for *D. mexicanum* collected in three regions of the Pacific coast of Costa Rica: Number of alleles (Na), number of effective alleles (Ne), heterozygosity (Ho) observed, heterozygosity expected (He), inbreeding coefficient (Fis), observed (Obs) and estimated (Est) frequency of null alleles, Hardy-Weinberg equilibrium test, and allelic richness (AR) per region (North Pacific, South Pacific, and Cocos Island) using the sample size (N) for each region

Cuadro 2. Estimación de la diversidad genética y riqueza alélica por SSR para *D. mexicanum* colectado en tres regiones de la costa Pacífica de Costa Rica: Número de alelos (Na), número de alelos efectivos (Ne), heterocigosidad observada (Ho), heterocigosidad esperada (He), coeficiente de endogamia (Fis), frecuencia observada (Obs) y estimada (Est) de alelos nulos, prueba de equilibrio Hardy-Weinberg y riqueza alélica (RA) por región (Pacífico Norte, Pacífico Sur e Isla del Coco) utilizando el tamaño de muestra (N) para cada región

| | | | | | | | | | North Pacific | | South Pacific | | Cocos Island | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **SSR** | **Na** | **Ne** | **Ho** | **He** | **Fis** | **Null Alelle Obs** | **Null Alelle Est** | **HWE test** | **N** | **AR** | **N** | **AR** | **N** | **AR** |
| **KS03** | 47 | 6.44 | 0.83 | 0.97 | 0.23 | 0.03 | 0.05 | chi2=1102.3589, df=1081, $P > 0.05$ | 31 | 5.69 | 12 | 5.63 | 4 | 4.93 |
| **KS09** | 40 | 5.61 | 0.73 | 0.96 | 0.18 | 0.05 | 0.1 | chi2=930.9167, df=780, $P > 0.05$ | 30 | 5.51 | 12 | 5.73 | 3 | 6 |
| **KS29** | 40 | 5.64 | 0.72 | 0.96 | 0.37 | 0.11 | 0.19 | chi2=1023.4341, df=780, $P > 0.05$ | 32 | 5.57 | 12 | 5.62 | 3 | 4 |
| **SN225** | 18 | 2.91 | 0.54 | 0.73 | 0.25 | 0.16 | 0.25 | chi2=348.184, df=153, $P < 0.05$ | 31 | 3.28 | 12 | 3.98 | 3 | 4 |
| **Mean** | 36.25 | 5.15 | 0.71 | 0.91 | 0.26 | 0.09 | 0.15 | ---- | 31 | 5 | 12 | 5.24 | 3.25 | 4.73 |
| **SD** | 12.61 | 1.54 | 0.12 | 0.12 | 0.08 | 0.06 | 0.09 | ---- | 0.82 | 1.18 | 0 | 0.84 | 0.5 | 0.95 |

$N_a$ = 64, effective alleles $N_e$ = 22.76 (± 2.95) and private alleles (21 ± 2.86). The region with the lowest number of alleles $N_a$ = 5 and effective alleles $N_e$ = 4.97 (± 1.01) and private alleles (1.67 ± 0.47) was Cocos Island (Table 3).

The South Pacific region presented the highest genetic diversity ($H_o$ = 0.80 ± 0.09) and the Cocos Island region had the lowest ($H_o$ = 0.61 ± 0.34). Regarding the inbreeding coefficient, Cocos Island showed the highest $F_{is}$ (0.38 ± 0.35) and the South Pacific the lowest ($F_{is}$ = 0.18 ± 0.10). It was found that the region with the highest allelic richness is the South Pacific (AR= 5.24 ± 0.84), while the North Pacific showed the lowest (AR = 5.00 ± 1.18; Table 2).

**Diversity estimates per sampling location**

The sampling location that showed the highest genetic diversity was Güiri ($H_o$ = 0.92 ± 0.14), while Punta Ulloa had the lowest genetic diversity ($H_o$ = 0.36 ± 0.48). It was found that MM had many private alleles (20), whereas Playa Blanca had the minority (2). The location with the highest inbreeding coefficient was Santa Elena Bay ($F_{is}$ = 0.35 ± 0.06), while Mogos presented the lowest ($F_{is}$ = 0.11 ± 0.18; Table 3).

**Genetic structure**

Among the regions, no evidence of genetic structure was found (GstNei = 0.0563, $P > 0.05$), whereas a global small but significant structure was detected between the sampling locations (GstNei = 0.031, $P < 0.05$, Table 4). Significant genetic structure was found among the sampling sites within regions according to AMOVA (Table 5). The biggest source of variation (80.46%) comes from differences within the samples ($P < 0.01$), followed by 18.31% of variation between the samples within the locations ($P < 0.01$) and 2.19% variation is coming from between the regions ($P < 0.01$). According to the DAPC results (Fig. 2), three distinct groups were identified: 1) Isla del Caño and Matapalo, 2) Mogos, Bahía Santa Elena, Playa Blanca, and Manuelita and 3) Güiri and MM Islas Murciélago. The sampling site Punta Adela is entirely separate from these groups, while Punta Ulloa is positioned nearby but does not overlap with any of the groups mentioned. In addition, the data showed no evidence of isolation by geographic distance ($P$ = 0.49) (Fig. 3). Bayesian clustering analyses performed revealed the presence of two genetic groups across populations (K = 2, ΔK = 4.63) (Fig. 4). The mean likelihood values remained low and showed no significant improvement with increasing K, suggesting that two clusters are the most biologically relevant grouping for the data.


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Table 3. Genetic diversity estimates for *D. mexicanum* across regions (North Pacific, South Pacific, and Cocos Island) and sampling locations: Number of individuals (n), percentage and standard deviation of number of alleles (Na), number of effective alleles (Ne), observed heterozygosity (Ho), expected heterozygosity (He), inbreeding coefficient (Fis), private alleles, and observed (Obs) and estimated (Est) frequencies of null alleles
Cuadro 3. Estimaciones de la diversidad genética de *D. mexicanum* en las distintas regiones (Pacífico Norte, Pacífico Sur e Isla del Coco) y lugares de muestreo: Número de individuos (n), porcentaje y desviación estándar del número de alelos (Na), número de alelos efectivos (Ne), heterocigosidad observada (Ho), heterocigosidad esperada (He), coeficiente de endogamia (Fis), alelos privados, y frecuencias observadas (Obs) y estimadas (Est) de alelos nulos

| | Na | Ne | Ho | He | Fis | Private Alleles | Location | n | Na | Ne | Ho | He | Fis | Private Alleles | Null Alelle Obs | Null Alelle Est |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **North Pacific** | 64 | 22.76 ± 2.95 | 0.76 ± 0.07 | 0.97 ± 0.05 | 0.21 ± 0.06 | 21 ± 2.86 | **Santa Elena Bay** | 7 | 31 | 6.34 ± 3.51 | 0.63 ± 0.06 | 0.98 ±0.01 | 0.35 ± 0.06 | 11 | 0 | 0 |
| | | | | | | | **Islas Murciélago (MM)** | 9 | 45 | 8.66 ± 3.10 | 0.70 ± 0.13 | 1 ± 0.02 | 0.27 ± 0.12 | 20 | 0.04 | 0.14 |
| | | | | | | | **Güiri** | 4 | 21 | 4.72 ± 2.01 | 0.92 ± 0.14 | 0.94 ± 0.02 | 0.30 ± 0.17 | 5 | 0 | 0.24 |
| | | | | | | | **Playa Blanca** | 2 | 10 | 2.42 ± 1.26 | 0.83 ± 0.29 | --- | --- | 2 | 0.17 | 0.17 |
| | | | | | | | **Matapalo** | 10 | 48 | 9.19 ± 4.77 | 0.83 ± 0.21 | 1 ± 0.03 | 0.13 ± 0.19 | 18 | 0.56 | 0.61 |
| **South Pacific** | 19 | 15.75 ± 0.84 | 0.80 ± 0.09 | 0.98 ± 0.008 | 0.18 ± 0.10 | 6 ± 2.62 | **Caño Island** | 5 | 27 | 5.80 ± 2.48 | 0.80 ± 0.2 | 0.97 ± 0.01 | 0.17 ± 0.21 | 8 | 0 | 0.05 |
| | | | | | | | **Mogos** | 4 | 24 | 5.33 ± 0.00 | 0.83 ± 0.14 | 0.9 ± 0.02 | 0.11 ± 0.18 | 6 | 0 | 0 |
| | | | | | | | **Punta Adela** | 3 | 21 | 4.88 ± 0.75 | 0.78 ± 0.19 | 1 ± 0 | 0.22 ± 0.19 | 8 | 0 | 0.08 |
| **Cocos Island** | 5 | 4.97 ± 1.01 | 0.61 ± 0.34 | 1 ± 0.00 | 0.38 ± 0.35 | 1.67 ± 0.47 | **Manuelita Coral Garden** | 2 | 12 | 2.73 ± 0.98 | 0.67 ± 0.29 | 1 ± 0 | 0.33 ± 0.29 | 3 | 0 | 0 |
| | | | | | | | **Punta Ulloa** | 2 | 8 | 1.92 ± 0.69 | 0.50 ± 0.5 | --- | --- | 4 | 0.13 | 0.28 |

Table 4. Genetic distance matrix based on Nei's Gst among the sampling sites of the sea urchin *D. mexicanum* from 10 sampling sites along the Pacific coast: North Pacific (PN), South Pacific (PS), and Cocos Island (IC)

Cuadro 4. Matriz de distancias genéticas según Gst de Nei entre los sitios de muestreo del erizo de mar *D. mexicanum* provenientes de 10 sitios de muestreo a lo largo de la costa pacífica: Pacífico Norte (PN), Pacífico Sur (PS) y la Isla del Coco (IC)

| | PN_PlayaBlanca Bahía Culebra | PN_Bahía Santa Elena | PS_Isla del Caño | IC_Manuelita Coral Garden | IC_Punta Ulloa | PN_Islas Murciélago (MM) | PS_Mogos Golfo Dulce | PN_Matapalo Guanacaste | PS_Punta Adela Golfo Dulce | PN_Güiri Bahía Culebra |
|---|---|---|---|---|---|---|---|---|---|---|
| **PN_Playa Blanca Bahía Culebra** | 0 | 0.023 | 0.024 | 0.033 | 0.104 | 0.028 | 0.018 | 0.025 | 0.011 | 0.031 |
| **PN_Bahía Santa Elena** | 0.023 | 0 | 0.013 | 0.038 | 0.075 | 0.016 | 0.014 | 0.014 | 0.025 | 0.022 |
| **PS_Isla del Caño** | 0.024 | 0.013 | 0 | 0.028 | 0.072 | 0.022 | 0.03 | 0.011 | 0.019 | 0.022 |
| **IC_Manuelita Coral Garden IC** | 0.033 | 0.038 | 0.028 | 0 | 0.125 | 0.017 | 0.02 | 0.03 | 0.04 | 0.006 |
| **IC_Punta Ulloa IC** | 0.104 | 0.075 | 0.072 | 0.125 | 0 | 0.083 | 0.105 | 0.088 | 0.097 | 0.101 |
| **PN_Islas Murciélago (MM)** | 0.028 | 0.016 | 0.022 | 0.017 | 0.08 | 0 | 0.008 | 0.016 | 0.017 | 0.009 |
| **PS_Mogos Golfo Dulce** | 0.018 | 0.014 | 0.03 | 0.02 | 0.1 | 0.008 | 0 | 0.022 | 0.027 | 0.015 |
| **PN_Matapalo Guanacaste** | 0.025 | 0.014 | 0.011 | 0.03 | 0.09 | 0.016 | 0.022 | 0 | 0.019 | 0.016 |
| **PS_Punta Adela Golfo Dulce** | 0.011 | 0.025 | 0.019 | 0.04 | 0.1 | 0.017 | 0.027 | 0.019 | 0 | -0.002 |
| **PN_Güiri Bahía Culebra** | 0.031 | 0.022 | 0.022 | 0.006 | 0.1 | 0.009 | 0.015 | 0.016 | -0.001 | 0 |



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Table 5. Analysis of molecular variance AMOVA of 48 samples of *D. mexicanum* from 10 sampling sites within 3 regions of the Pacific coast of Costa Rica obtained with four SSRs
Cuadro 5. Análisis de varianza molecular AMOVA de 48 muestras de *D. mexicanum* provenientes de 10 sitios de muestreo dentro de 3 regiones de la costa pacífica de Costa Rica obtenidas con cuatro SSRs

| Source of variation | d.f. | Sum of squares | Variance components | Percentage of variation | phi values | p value |
|---|---|---|---|---|---|---|
| **Between regions** | 2 | 6.67 | -0.03 | -0.96 | 0.2 | 0.96 |
| **Between locations within regions** | 6 | 24.23 | 0.06 | 2.19 | 0.19 | 0.003 |
| **Between samples within locations** | 34 | 116.22 | 0.53 | 18.31 | 0.02 | 0.0009 |
| **Within samples** | 43 | 101 | 2.35 | 80.46 | -0.01 | 0.0009 |
| **Total** | 85 | 248.12 | 2.92 | 100 | --- | |

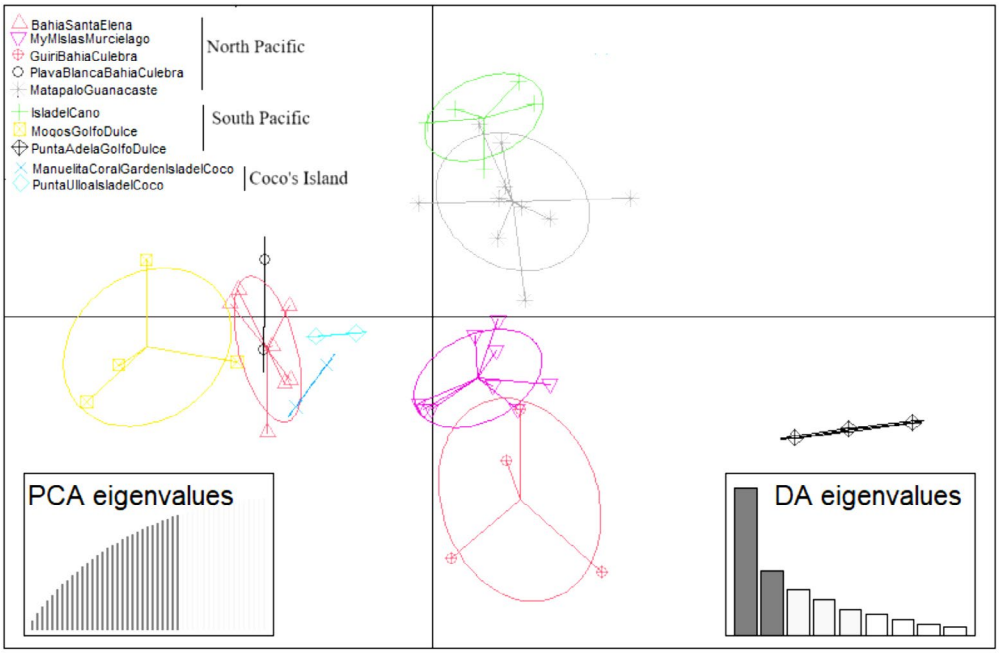


Fig. 2. DAPC analysis using sampling locations for *D. mexicanum* in the Pacific of Costa Rica as prior populations. Three distinct groups were identified based on overlap: 1) Isla del Caño and Matapalo, 2) Mogos, Bahía Santa Elena, Playa Blanca, and Manuelita, and 3) Güiri and MM Islas Murciélago. Punta Adela is entirely separate from these groups, while Punta Ulloa is positioned nearby but does not overlap with any of them

Fig. 2. Análisis DAPC utilizando los sitios de muestreo de *D. mexicanum* en el Pacífico de Costa Rica como poblaciones previas. Se identificaron tres grupos distintos basados en el traslape: 1) Isla del Caño y Matapalo, 2) Mogos, Bahía Santa Elena, Playa Blanca y Manuelita, y 3) Güiri y MM Islas Murciélago. Punta Adela está completamente separada de estos grupos, mientras que Punta Ulloa se encuentra cerca, pero no se traslapa con ninguno de ellos


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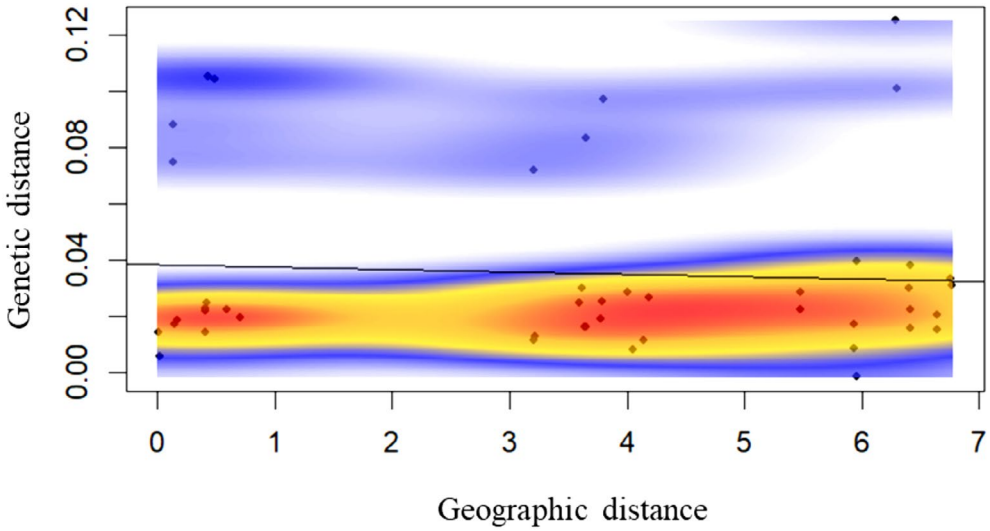


Fig. 3. Isolation by distance plot. Pairwise relationship between estimated genetic distance and geographic distance between the different sampling locations (km) of *D. mexicanum*. The color represents different correlation densities of genetic and geographic distances (red = high density, blue = low density). No pattern of data dispersion shows that the longer the distance, the greater the genetic difference. Also, the Mantel test does not show a significant relationship between the genetic distance and the geographical distance for these samples ($P > 0.05$)

Fig. 3. Gráfica de aislamiento por distancia. Relación por pares entre la distancia genética estimada y la distancia geográfica entre las diferentes ubicaciones de muestreo (km) de *D. mexicanum*. El color representa diferentes densidades de correlación de las distancias genéticas y geográficas (rojo = alta densidad, azul = baja densidad). No existe un patrón de dispersión de datos que muestre que cuanto mayor sea la distancia, mayor será la diferencia genética. Además, la prueba de Mantel no muestra una relación significativa entre la distancia genética y la distancia geográfica para estas muestras ($P > 0.05$)

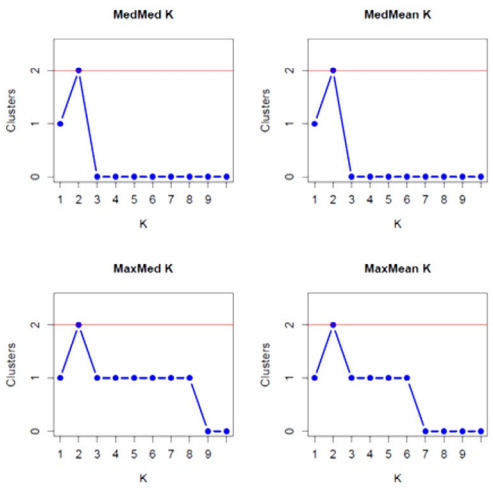


Fig. 4. Inferences of the number of genetic groups (K) based on Bayesian analysis in STRUCTURE with 10 runs for each cluster from 1 to 10 (10,000 iterations with 10,000 burn-in periods)
Fig. 4. Inferencias del número grupos genéticos (K) según análisis Bayesiano en STRUCTURE con 10 runs para cada cluster 1 to 10 (10 000 iteraciones con períodos de 10 000 burn-in)

## DISCUSSION

### Genetic diversity

Among the SSR markers analyzed, KS03, KS09, and KS29 exhibited lower heterozygosity, with KS03 showing the highest value. Both results are contrary to Chandler's findings (2017). For marker SN225, this study reports higher heterozygosity compared to Li & Li (2008); however, it was the only SSR that did not meet Hardy-Weinberg equilibrium. Correspondingly, SN225, as in Li & Li (2008), also exhibited the highest estimated null allele frequency, followed by KS29. The success of SSR cross-amplification from markers developed for other species is related to the evolutionary closeness between the species (Steinkellner *et al*. 1997). Marker SN225 was originally developed for the sea urchin *Strongylocentrotus nudus*, which is less related to *D. mexicanum* than *D. antillarum,* the species for which the KS markers were designed. The presence of null alleles can underestimate population diversity and bias genetic structure analyses (Chapuis & Estoup, 2007). As such, developing species-specific markers for the study organism is crucial. No evidence of linkage disequilibrium (LD) was found using the standardized index of association (rbarD) for the SSR markers. This supports the null hypothesis of recombination occurring across all loci and between populations (Kamvar *et al*. 2015), suggesting that alleles from these markers are not associated and should not influence the data analysis. The average allelic richness values show a general trend where the South Pacific population presents the highest genetic diversity, followed by North Pacific and Cocos Island. Allelic richness is a more sensitive measure than heterozygosity for detecting reductions in genetic diversity, as it is directly affected by genetic drift events and bottlenecks


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(Greenbaum *et al*. 2014). In marine populations, connectivity and gene flow can influence the distribution of genetic diversity. The lower allelic richness observed for *D. mexicanum* in Cocos Island could be related to its geographic isolation and reduced gene flow with continental populations.

Across all regions and locations, observed heterozygosity was consistently lower than expected heterozygosity, which is consistent with the high inbreeding coefficient ($F_{is}$) values obtained. While it is common to find higher levels of local retention than dispersal, such a deficit in heterozygotes typically should not result in high inbreeding if populations are genetically homogeneous. This suggests that the populations might be experiencing a degree of isolation or could be very small in size. Addison and Hart (2005) suggest that such a deficit in heterozygotes can occur in planktonic larvae due to factors such as the Wahlund effect, the presence of null alleles (commonly observed in free-spawning echinoderms), and high variance in reproductive success. Similarly, Hedgecock (1994) hypothesized that elevated inbreeding coefficients in marine free spawners could result from their inherently high variance in reproductive success. In this study, the low levels of genetic structure observed across regions and locations suggest that the Wahlund effect may be at play, and geographic distance or small population sizes could also influence these results. Furthermore, null alleles detected across all sampling sites and the potential combined effects of high reproductive variance could also explain these patterns.

As demonstrated by Alvarado *et al*. (2016c), *D. mexicanum* occurs at intermediate densities in the ETP, a critical factor for reproduction in this species. The Allee effect is particularly relevant in free-spawning organisms like *D. mexicanum*, where changes in population density can significantly impact reproductive success. At low densities, mate or sperm limitation can occur, while at high densities, polyspermy and increased competition for mates and resources may reduce fitness (Levitan, 2012). Additionally, reproduction in this species is closely linked to spatial and temporal oceanographic processes that influence gonad maturation, spawning, fertilization, larval survival, and recruitment (Hedgecock, 1994). It is known that *D. mexicanum* aligns its spawning period with environmental conditions, typically between April and November (Lessios, 1981; Olivares-González, 1986). This period is further shaped by oceanic dynamics and currents, which play a crucial role in larval survival (Ruiz-Bravo, 2013). Also, in Cocos Island, no synchrony has been observed hence organisms in all gametogenic stages can be found (Pearse, 1968).

**Genetic structure**

The results indicate a small but significant genetic structure, with the main sources of variation arising from within samples and within and between locations. However, no isolation by distance was detected, despite the geographical separation between locations along the Pacific coast and those on Cocos Island. The DAPC analysis further revealed that some locations form groups, but these are independent of the Pacific region to which they belong. Additionally, the results of the Bayesian analysis suggest that all samples can be grouped into two clusters. This implies a high level of connectivity, likely driven by gene flow and the presence of structure, which could indicate that the existing flow is not uniform. These findings align with those of Lessios *et al*. (2001), who, using allozymes and mitochondrial DNA, determined that *D. mexicanum* populations in the Galapagos Islands and Cocos Island are genetically indistinguishable from Panama and Mexico.

The AMOVA reveals that most genetic variation (80.46%) occurs within individual samples, with only a small amount attributed to differences between regions and locations. This suggests a high level of genetic homogeneity between broader regions, but more variation exists within specific sampling sites. The DAPC further supports this finding by showing no differences between regions but clear differences between sampling sites, with Punta Adela (Golfo Dulce) appearing the most distinct. Golfo Dulce, with its four major rivers flowing into the sea, presents a challenging environment for coral reef development (Cortés, 1990). These unique conditions may reduce genetic connectivity, as variations in habitat conditions can impact the fitness of dispersing individuals (Hendry, 2004).

Sampling sites from the southern region, such as Mogos, and the North Pacific region, including Santa Elena Bay, Playa Blanca, and Cocos Island, are grouped together, indicating high connectivity among these locations. Finally, we observe that Isla del Caño, located in the South Pacific, is grouped with Matapalo, which are not particularly close sites. In contrast, the sites of Güiri and MM, which are closer to each other, also group together, suggesting the presence of genetic flow and high connectivity between these populations. However, to obtain more robust conclusions and explain the presence of group populations that are difficult to explain biologically, it is essential to increase the sampling size to enhance the statistical power and accuracy (Puechamaille, 2016).

The circulation of the Golfo Dulce is very slow, primarily due to its basin's shape and geographic position (Quesada-Alpízar & Morales-Ramírez, 2004; Svendsen *et al*. 2006). During the dry season (January-May),


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the lack of rainfall results in the Gulf lacking an internal engine to drive water movement, which causes the waters to remain stagnant for longer periods. Additionally, at this time of year, the Intertropical Convergence Zone (ITCZ) shifts southward, weakening the Costa Rican Coastal Current (CRCC), which flows from south to north (Wyrtki, 1965; Brenes & Leon, 1988). By May, the trade winds begin to weaken, allowing the ITCZ to move northward, thus favoring the intensification of the CRCC. The dynamics of these currents and related oceanographic and atmospheric factors help explain the connectivity between populations along the coast, as the CRCC facilitates the potential movement of larvae from south to north, connecting Golfo Dulce with the North Pacific of Costa Rica. Furthermore, during the dry season, coastal upwelling intensifies, driving water from north to south (Amador *et al*. 2016; Fallas & Oviedo, 2003), which maintains high connectivity between areas as close as the Murcielago Islands and Culebra Bay. Although Cocos Island is located 500 km off the coast, it is influenced by the North Equatorial Countercurrent (NECC), which moves water from west to east, from the island toward the continent. During the dry season, this current weakens, and the flow from east to west becomes more prominent (Wyrtki, 1965; Lizano, 2006; Lizano, 2007; Amador *et al*. 2016). These ocean currents enhance the connection between Cocos Island and the mainland. The isolation of Punta Adela in the analysis may be attributed to its location in the inner eastern part of Golfo Dulce, where larval retention may be higher and movement to other regions is limited. In contrast, Mogos, located in the northern inner part of the Gulf near several rivers, may experience greater water movement during the rainy season.

Furthermore, species with resistant larvae such as those of *D. mexicanum* that can survive up to 42 days (under laboratory conditions) (Emlet, 1995) are expected to maintain this relatively panmictic population across large geographical scales (Binks *et al*. 2011). This can result in a widespread dispersion potential. As mentioned before, oceanographic currents may be an important contributor in facilitating the transport of larvae (Díaz *et al*. 2018). Cocos Island exhibits different oceanic dynamics throughout the year. During the first quarter, an anticyclonic circulation is identified, centered slightly south of the island. The intensity of this current is evident along the northern edge of the current, reaching the coast of Costa Rica. In the second quarter, the circulation pattern remains the same, but the current intensifies in a northeast direction due to the arrival of the NECC. By the third quarter, the NECC is well established around Cocos Island, flowing eastward. Finally,

in the fourth quarter, the current behavior remains like the previous quarter, but the NECC reaches its maximum intensity further east, reaching the Costa Rican coast (Lizano, 2008).

The upwelling phenomenon that reaches the North Pacific of Costa Rica between December and April causes a marked difference in biological productivity (Alfaro *et al*. 2012; Fernández-García *et al*. 2012). As observed in the DAPC, some sampling locations that experience upwelling are grouped, suggesting possible connectivity due the influence of the winds and currents characteristic of upwelling seasons. Additionally, it has been established that *D. mexicanum* reproduces between April and November, with reproduction influenced by winds currents and temperature. In upwelling areas, especially in the north Pacific it spawns just before the event to ensure that the currents do not disrupt larval settlement and movement (Alvarado *et al*. 2015a; Benítez-Villalobos *et al*. 2015).

## CONCLUSION

The species *D. mexicanum* has been ecologically well-studied in Costa Rica; however, there is a gap in genetic studies related to this keystone sea urchin. Through this research, we have studied the genetic diversity and connectivity of the *D. mexicanum* population in the Pacific of Costa Rica, generating novel results. We found that cross-amplifying microsatellite primers created for species related to *D. mexicanum* was advantageous in terms of cost and time. Our results suggest that, although there is a slight genetic structure along the Pacific coast of Costa Rica, evidence of connectivity and gene flow is also present. We consider that these findings are due to the characteristics of this species' larvae combined with the different currents that maintain our sampling locations connected. For future studies, we recommend increasing the number of SSR markers and ensuring a more uniform sample size across locations to enhance the robustness of genetic analyses. Additionally, incorporating samples from the Central Pacific region would provide a more comprehensive understanding of this species population dynamics. A more balanced sampling effort could lead to more representative and reliable conclusions about *D. mexicanum* connectivity and genetic diversity.

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