Genetic Analysis of Queen Conch *Strombus gigas* from the Southwest Caribbean

Análisis Genético de Caracol Pala *Strombus gigas* del Caribe Suroccidental

Analyse Genetique du Lambi *Strombus gigas* dans Sud-Ouest de Caraibe

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ABSTRACT

Genetic connectivity among populations is of crucial importance in conservation and management of commercial threatened species. Here, we explored genetic connectivity and diversity from 490 queen conch *Strombus gigas* from nine oceanic atolls within the San Andres archipelago and three coastal islands closer to the colombian continental shelf (separated by more than 600 kilometers from the Archipelago), in the Southwestern Caribbean. Genetic differentiation was analyzed using the statistic Φ_{ST} provided by an analysis of molecular variance (AMOVA) and by a spatial analysis of molecular variance (SAMOVA). Correlation between genetic and geographic distance was explored by using Mantel test. All *loci* were polymorphic with high number of alleles per *locus* and showed deficit of heterozygosity departing from Hardy-Weinberg equilibrium. We found evidence for up to four different genetic stocks without indication of isolation by distance. Based on these results, the recovery of *S. gigas* in the Southwestern Caribbean should require management considerations that address local and regional actions.

KEY WORDS: Queen conch, *Strombus gigas,* conservation genetics, population genetics, Seaflower Reserve, stock restoration

INTRODUCTION

Queen Conch *Strombus gigas* is a large gastropod of significant economic importance across the Greater Caribbean region (Theile 2005, Prada et al. 2009). Its natural populations are nowadays declined in most of its distribution range due to over exploitation, loss of habitats, and additional biological conditions such as low growth, low maturity rate, and susceptibility to Allee effect at small population densities (Stoner and Ray-Culp 2000, Berec et al. 2006). Hence, *S. gigas* was considered a commercially threatened species and thus included in the Appendix II of CITES in 1992 to regulate its international trade. Despite this control, it remains to be seen a substantial recovery of the queen conch stocks at a regional basis (Shawl and Davis 2004, Ballesteros et al. 2005, Gómez 2005, Prada et al. 2009).

S. gigas larvae (veligers) live on surface waters and so they can be transported by currents, with the potential to travel up to 900 km during the first three weeks of their development (Davis et al. 1993). Depending on the environmental conditions, veligers can remain in the plankton for two months (Stoner et al. 1992, Brito et al. 2006), thus suggesting extensive gene flow through larval dispersal. However, previous studies using allozymes have revealed the opposite, showing genetic structure either at isolated sites or at micro-scales for queen conch populations in Bermuda, Alacranes reef in Yucatán Peninsula, Gros Islet and Vieux Fort in St. Lucia Island, Turks and Caicos, Grenadines, and St. Kitts and Nevis. Moreover, these studies have also shown absence of panmixia within similar genetic populations (Mitton et al. 1989, Campton et al. 1992, Tello-Cetina et al. 2005).

The population structure of *S. gigas* in the Southwestern Caribbean region is unknown, although this information is crucial to select adequate management and conservation strategies for this species, and furthermore prevent disturbances of genetic background of stocks. The region is affected by both the Panamá-Colombia oceanographic gyre and by the Central Caribbean current, which generate strong oceanic conditions and permanent eddies (Richardson 2005) that may induce genetic structuring in the queen conch populations. This idea is supported by studies based on molecular markers, which have found genetic differences among populations of species with different life strategies (Galindo et al. 2006, Acosta et al. 2008, Puebla et al. 2008, Landínez-García et al. 2009, Salas et al. 2010), and biophysical modeling studies of larval dispersal of reef fish (Cowen et al. 2006) and Caribbean corals (Galindo et al. 2006). In this work, microsatellite analysis was used to test the hypothesis that *S. gigas* populations across a broad area in the colombian southwestern Caribbean are genetically structured in more than a single stock.

MATERIALS AND METHODS

Sampling and Study Site

A total of 490 individuals of *S. gigas* were collected in 12 coral reef locations across the colombian continental and oceanic shelfs, in the southwestern Caribbean. Sampling locations were selected *a priori* based on predictions of biophysical models of larval dispersal (Cowen et al. 2006, Galindo et al. 2006) and genetic studies (Galindo et al. 2006, Acosta et al.

2008, Puebla et al. 2008, Landínez-García et al. 2009, Salas et al. 2010) across four regions: Region I. Colombian coastal areas (San Bernardo and Rosario archipelagos, and Tortugas Bank); Region II. San Andres archipelago-southern section (South-South-West and East-South-East atolls); Region III. San Andres archipelago-northern section (Old Providence island, Roncador, Queena and Serrana atolls); and Region IV. San Andres archipelago-most northern section, near to Jamaica (Serranilla atoll, Alice shoal and Bajo Nuevo Bank) (Figure 1). At each of these sampling locations, 30 (Regions I-III) and 60 (Region IV) queen conchs were collected by scuba diving in 2007.

Atolls of the San Andres archipelago are on a discontinuous submarine platform, influenced by both the Central Caribbean current and by the Panamá-Colombia oceanographic gyre. In this scenario, the superficial currents are non-uniformly distributed among the atolls (Richardson 2005) and additionally, bathymetric studies have shown a deep groove between San Andres and Old Providence islands (Andrade 2001). On the other hand, the sites on the colombian coast are influenced by the Panamá-Colombia oceanographic gyre, and are considered as good candidates to have elevated self-recruitment and partial isolation (Cowen et al. 2006, Galindo et al. 2006). In these areas, a sample of 3 cm^2 of tissue from intersection mantle-foot was taken from each specimen collected and preserved in DMSO (Carlo Erba, Milan, Italy) until DNA extraction in the laboratory.

Microsatellite Genotyping

Approximately 25 mg of preserved tissue were washed with distilled water and then grounded using liquid nitrogen. The DNA was extracted using DNAeasy Blood & Tissue Kit (Qiagen, Duesseldorf, Germany). The quantification of DNA was done with the reference commercial Fluorescent DNA Quantification Kit (Bio-Rad, Philadelphia, USA). Quality of DNA was inspected in agarose gels 0.8%, buffer TBE 1X (Sambrook et al. 1989) ran at 80 V per 30 minutes and colored with Ethidium Bromide (Amresco, Ohio, USA).

Eight primers and amplification conditions previously reported by Zamora et al. (2007) were used with modifications in the annealing temperature and $MgCl_2$ concentrations as described below. Amplification reactions were performed with 2.75 mM $MgCl_2$, 100 µM dNTPs (Invitrogen, California, USA), 0.5 µM of each primer, 5 ng/µl of template DNA, 1X PCR buffer and 0,04 U/µl recombinant Taq DNA polymerase (Invitrogen, California, USA). Each forward primer was fluorescently labeled with Cy 5.0 or Cy 5.5 (MWG Biotech, Atlanta, USA).

Amplification reactions were programmed in a PTC-200 thermocycler (M.J. Research Inc, Massachusetts, USA) with a slope of 2 seconds and the following PCR thermal cycling: one cycle at 90°C for 2 minutes, 32 cycles at 94ºC for 15 seconds, 10 seconds at specific annealing temperature for each primer (59ºC for *Sgig*-1, *Sgig*-3; 61ºC

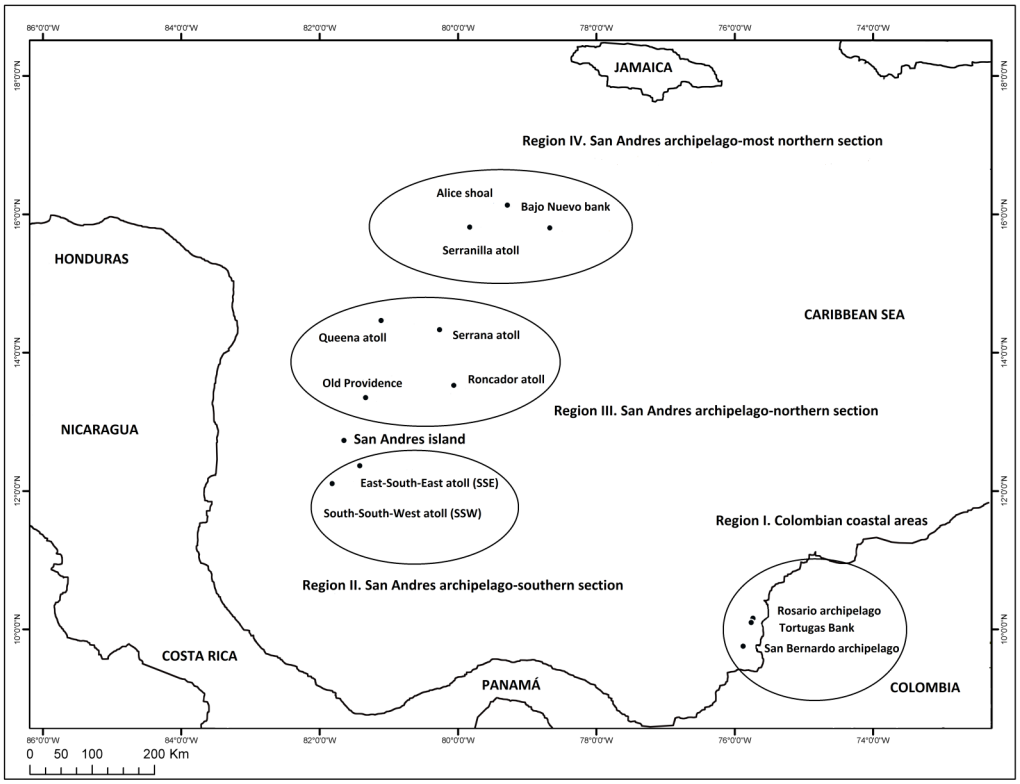

Figure 1. Geographic localization of sampling sites of queen conch *S. gigas* from Colombian Southwest Caribbean.

for *Sgig*-2, *Sgig*-7, and 63ºC for *Sgig*-5, *Sgig*-6), and 10 seconds at 72ºC, followed by a final extension at 72ºC for 10 minutes.

Denaturation of the amplified products was performed by incubation at 80ºC for 3 minutes in a 1:1 mix of the PCR product with denaturing buffer: 99% formamide (Amresco, Ohio, USA) and 0.1% bromophenol blue (Sigma, St. Louis, USA). Fragments were separated in the automated sequencer Long-Read Tower™ System (Siemens, Munich, Germany) under the following conditions: 1500 V, 58ºC gel temperature, 50% laser power, one second for reading, and running for 45 minutes in the presence of TBE 1X. Fragment sizes were determined using the software Gene Objects V3.0 (Siemens, Munich, Germany) with three internal controls of 102, 137 and 216 bp., which co-migrated with each sample. A sample was considered heterozygote when the lower peak represented more than 70% of the higher peak, while in the opposite case or when only one peak was observed it was considered homozygote (Butler 2001).

Data Analysis

Detection of possible genotyping errors was made with Micro-Checker V2.2.3 (Oosterhout et al. 2004). Genetic diversity was estimated for the total population and for each subpopulation by calculating the average number of alleles per *locus*, number of polymorphic *loci*, observed average heterozygosity, expected average heterozygosity, and fixation index. Departures from Hardy-Weinberg and Linkage equilibriums were estimated with Fisher Probability Test described by Raymond and Rousset (1995) using Genepop 4 (Rousset 2008). Given that assumptions of Hardy-Weinberg equilibrium were not satisfied, the genetic differentiation between samples according to geographical origin (regions, among and within populations) was tested using the statistic Φ_{ST} and analysis of molecular variance, AMOVA (Excoffier et al. 1992).

To explore alternative grouping of populations that maximize the among group variance component, we calculate F_{CT} for all possible numbers of distinct groups from K = 2 to K = 11 using the spatial analysis of molecular variance, SAMOVA V1.0 (Dupanloup et al. 2002). This group structure was subsequently tested for significance *a posteriori* in Arlequin V3.11. Genetic isolation by distance was evaluated by calculating the Spearman rank correlation coefficient using Mantel Test (Mantel 1967) following Smouse et al. (1986) procedure. Statistical significance of tests was carried out with 10000 permutations at a 95% level. Multiple comparisons were adjusted by Bonferroni Test (Dunn-Sidak) at 95% (Sokal and Rohlf 1995). All calculations of genetic diversity indexes, the statistic Φ_{ST}, AMOVA and Mantel Test were performed on GenAlEx V6.0 (Peakall and Smouse 2006).

RESULTS

Among the eight pairs of primers examined in this work, *Sgig*-5 and *Sgig*-8 showed complex peaks difficult to interpret; therefore they were excluded from the analysis. Allelic size ranged from 105 to 224 bp with *Sgig*-4 and *Sgig*-3 exhibiting the highest polymorphism level (Table 1). The average number of alleles per *locus* per subpopulation was 10 (ranging from 8 to 11) (Table 2). All microsatellite *loci* showed heterozygosity deficit with a consequent departure from Hardy-Weinberg equilibrium ($p < 0.001$; Tables 1, 2) and no linkage disequilibrium was detected. The observed heterozygosity ranges from 0.332 to 0.510 showing the highest values in the populations from Bajo Nuevo bank, Serranilla atoll, Roncador atoll and Alice shoal, whereas the lowest values were observed in the South-South-West atoll, San Bernardo archipelago and East-South-East atoll populations, a north-south gradient.

On the other hand, *S. gigas* exhibited significant population structure at a regional scale defined *a priori* ($\Phi_{RT(3,458)} = 0.039$; $p = 0.001$), among ($\Phi_{RT(8,458)} = 0.021$; $p = 0.001$), and within populations ($\Phi_{RT(447,458)} = 0.059$; $p = 0.001$). The AMOVA analysis also showed that variations among regions (4%) and among populations (2%) were lower than within populations (94%). The pairwise comparisons among regions (Table 3) showed a slight significant differentiation among populations from colombian continental areas (Region I) and the populations from the oceanic ones (Regions II-IV), whereas in the San Andres archipelago, south atolls (Region II) showed moderate significant differentiation with north atolls (Region III) and those from most northern near to Jamaica atolls (Region IV). In general, within regions I and II, the populations were genetically different, whereas within region III and IV the populations were genetically similar (Table 3). On the other hand, the SAMOVA analysis indicated the maximal variance among groups at K = 4 ($F_{CT} = 0.041$, P = 0.000) with the following clustering:

i) San Bernardo archipelago,
ii) South-South-West and East-South-East atolls,
iii) Old Providence island, Serrana and Roncador atolls, and
iv) Rosario archipelago, Tortugas Bank, Queena atoll, Serranilla atoll, Alice shoal and Bajo Nuevo bank.

In addition, no correlation was found between genetic and geographic distances ($R^2 = 0.004$, $Rxy = 0.064$, $p = 0.0001$).

DISCUSSION

Microsatellite analyses suggest that populations examined in this work are not panmictic and exhibit a global deficit of heterozygosity. This latter result may be attributed to an underestimation of heterozygotes because of stutter products (Shinde et al. 2003), dominance of short alleles (Wattier et al. 1998), or presence of null alleles (Shaw et al. 1999). Micro-checker analysis did not detect

stutter products or dominance of short alleles in data set, but suggested the presence of null alleles in all *loci* examined. However, this explanation seems unlikely because these primers showed abilities to amplify a high number of *alleles* per *locus* (11 to 31, Table 1) and thus, they must permit the amplification of their respective heterozygotes. Moreover, no evidence of null alleles was found when these primers were used in two populations from Yucatán, México (Zamora-Bustillos et al. 2011). Furthermore, the deficit of heterozygosity has been also found in population studies based on allozymes in others Caribbean areas suggesting that the results obtained in this work could not be explained by technical problems with the molecular marker used.

Some biological process such as the Wahlund effect, assortative mating or inbreeding can cause heterozygote deficit. Wahlund effect does not seem to explain these results because heterozygote deficit at global and regional level remain significant in each geographic population (Tables 2 and 3). However, the coexistence in the same sites of different stocks cannot be disregarded given that the genetic heterogeneity within each geographic population was greater than among populations. Alternatively, the assortative mating seems unlikely in this species given the female polyandry behavior of *S. gigas* (D'Asaro 1965), but this aspect was not addressed in this study. Finally, the critically small population densities of those sites (Ballesteros et al. 2005, Gomez et al. 2005, Prada et al. 2009), suggest a reduced effective population size (adults that bring their gametes to populations) and susceptibility of these populations to a high rate of inbreeding and loss of genetic variation. This idea is supported by the observation that queen conch from East-South-East atoll (8.7 adults/ha; Prada et al. 2011) showed a lower number of *alleles* per *locus* in a relatively short period (Landínez-García et al. 2011).

On the other hand, this study found evidence that the populations of *S. gigas* in colombian southwestern Caribbean are genetically structured in four groups, reflecting heterogeneous spatial mosaics of marine dispersion. This genetic diversity pattern contrasts with the general idea that the extended larval development of this species promotes the extensive gene flow among different Caribbean sites (Mitton et al. 1989, Campton et al. 1992, Tello-Cetina et al. 2005). However, the heterogeneous spatial mosaics have also been found in *S. gigas* populations from other Caribbean regions at macro and micro spatial scales (Mitton et al. 1989, Campton et al. 1992, Tello-Cetina et al. 2005). They seem to be shared by other marine invertebrates with planktonic larvae such the mantis shrimp *Haptosquilla pulchella* (Barber et al. 2002), the stag horn coral *Acropora cervicornis* (Vollmer and Palumbi 2007) and the seastars *Linckia laevigata* and *Protoreaster nodosus* (Crandall et al. 2008).

The genetic structure of *S. gigas* found in this work may have several non-exclusive explanations. First, given the critically reduced populations, the effect of genetic drift and the potential larval retention and self-recruitment may promote genetic differentiation among sites. This explanation is supported by the deficit of heterozygosity

Table 1. Genetic diversity detected in six microsatellite *loci* of *S. gigas* from the Southwest Caribbean (Colombia). N: number of specimens examined; Na: total number of alleles per *locus*; Ra: range of allele size in base pairs; He: expected average heterozygosity; Ho: observed average heterozygosity; F: fixation index. Values in bold denote statistical significance after sequential *Bonferroni* adjustment.

LOCI	N	Na	Ra	He	Ho	F
AY707889 (*Sgig4*)	384	24	155-203	0.906	0.286	**0.685**
DQ533624 (*Sgig3*)	425	24	105-173	0.873	0.641	**0.267**
DQ533622 (*Sgig1*)	434	14	192-224	0.807	0.404	**0.501**
AY707892 (*Sgig7*)	451	11	107-127	0.683	0.541	**0.205**
AY707891 (*Sgig6*)	443	10	152-172	0.658	0.166	**0.749**
DQ533623 (*Sgig2*)	431	11	112-134	0.626	0.538	**0.116**

Table 2. Genetic diversity of populations of *Strombus gigas* from the Colombian Southwest Caribbean. N: number of specimens examined; Na: average number of alleles per *locus*; He: expected average heterozygosity; Ho: observed average heterozygosity; F: fixation index. Values in bold denote statistical significance after sequential *Bonferroni* adjustment.

Population	N	Na	He	Ho	F
New Bank	61	11	0.789	0.51	**0.356**
Serranilla	53	10	0.779	0.509	**0.349**
Roncador	29	11	0.782	0.493	**0.373**
Alice Bank	64	11	0.804	0.452	**0.44**
Queena	28	10	0.808	0.426	**0.478**
Serrana	29	10	0.762	0.419	**0.454**
Tortugas Bank	31	9	0.795	0.419	**0.477**
Rosario archipelago	33	9	0.763	0.418	**0.455**
Providence Island	30	8	0.742	0.402	**0.462**
East-South-East	29	9	0.753	0.398	**0.476**
San Bernardo archipelago	30	9	0.769	0.39	**0.496**
South-South-West	31	9	0.722	0.332	**0.543**

found in all loci and populations above-mentioned and it is also concordant with the changes in allelic frequencies exhibited by East-South-East atoll population in a relatively short period (Landínez-García et al. 2011).

Second, the congruence among distribution patterns of different species with different life history (Acero 1985, Acosta et al. 2008, Landínez-García et al. 2009) suggests that the oceanographic scenario may explain the genetic structuring of populations that inhabit this southwest Caribbean Sea area. This idea is supported by the observation that genetic differences between colombian Caribbean coast sites (San Bernardo and Rosario archipelagos) was also found in populations of snapper *Lutjanus synagris* from the colombian Caribbean coast (Capurganá and Rosario archipelago) (Landínez-García et al. 2009). Another genetic study also found a weak but significant genetic differentiation among *Stegastes partitus* populations along Costa Rica and Panamá coasts that are influenced by the Panamá-Colombia oceanographic gyre (Salas et al. 2010). Based on the predictions of elevated self-recruitment and partial isolation in this area (Cowen *et al.* 2006, Galindo et al. 2006), the Panamáa-Colombia oceanographic gyre may explain the isolation of San Bernardo archipelago from other sites of San Andres archipelago.

Table 3. Pairwise Φ_{ST} and P-value among regions and populations within regions. Values in bold denote statistical significance after sequential *Bonferroni* adjustment

Pairwise comparisons		Φ_{ST}	P
Among Regions			
Region I	Region II	0.042	**0.001**
Region I	Region III	0.042	**0.001**
Region II	Region III	0.097	**0.001**
Region I	Region IV	0.022	**0.001**
Region II	Region IV	0.056	**0.001**
Region III	Region IV	0.044	**0.001**
Within Regions, among populations			
Region I			
San Bernardo archipelago	Rosario archipelago	0.066	**0.001**
San Bernardo archipelago	Tortugas Bank	0.046	**0.001**
Rosario archipelago	Tortugas Bank	0.023	**0.013**
Region II			
South-South-West	East-South-East	0.032	**0.011**
Region III			
Queena	Providence	0.027	0.015
Queena	Roncador	0.025	0.019
Queena	Serrana	0.019	0.035
Providence	Serrana	0.009	0.147
Providence	Roncador	0.007	0.172
Serrana	Roncador	0.006	0.207
Region IV			
Alice Bank	New Bank	0.013	0.005
Serranilla	New Bank	0.006	0.087
Serranilla	Alice Bank	0.002	0.277

Furthermore, the genetic differences between northern and southern atolls on San Andres archipelago may result from the patterns of distribution of the Central Caribbean current, the superficial currents non-uniformly distributed among the atolls (Richardson 2005) and the presence of a deep groove between San Andres and Old Providence islands (Andrade 2001). The genetic differences between northern and southern atolls on San Andres archipelago are concordant with the lack of larval dispersion of *S. gigas* in this area, where the larvae of the northern atolls move towards the Cayman Basin, whereas the southern atolls stay in the Panamá-Colombia oceanographic gyre (Lonin et al. 2010). Moreover, a zoogeographical study based on fish endemic species (Acero 1985) proposed two biogeographic provinces (north and south) in the San Andres archipelago separated at San Andres island (N 12.5°) suggesting that these differences are not a recent event. This idea is additionally supported by genetic differences based on analysis of Internal Transcribed Sequence (ITS) between populations of the zoanthids *Palythoa caribaeorum* from Old Providence island (San Andres archipelago-northern section) and San Andres Island (San Andres archipelago-southern section) (Acosta et al. 2008).

Finally, the genetic relationship among populations near to Jamaica (Serranilla atoll, Bajo Nuevo bank and Alice shoal) and those from oceanic and coastal locations provided an experimental support to simulation studies that predict a low larval survival of reef fishes in this area (Cowen et al. 2006). In general, the populations near to Jamaica, which exhibit the highest genetic diversity found in this study, seem to play a weak role in the reinforcement of the other populations from San Andres archipelago. Only Alice shoal showed gene flow with Queena atoll population and the continental Rosario archipelago suggesting a potential source of genetic diversity. This genetic relationship may explain the clustering suggested by SAMOVA analysis among populations near to Jamaica, Queena atoll (San Andres archipelago-northern section), Rosario archipelago and Tortugas Bank (colombian Caribbean coast). The genetic similarities between San Andres archipelago and colombian coast were also found in *P. caribaeorum* from Grande Island (Rosario archipelago) and Old Providence island (San Andres archipelago-northern section) (Acosta et al. 2008).

Our results suggest that the conservation of critically reduced populations from of *S. gigas* from Rosario and San Andres (southern section) archipelagos should be a priority in the conservation plans. The recovery of these populations must be extremely poor because they are highly susceptible to the synergistic effect of inbreeding, genetic drift, Allee effect, fishing pressure, anthropogenic impacts (e.g. pollutants, disturbed habitat quality), and likely to larval dispersion to other Caribbean areas (McCarthy et al. 2002, Delgado et al. 2004, Gascoigne and Lipcius 2004). The potential role of Allee effect in the poor recovery of population densities in those sites is supported by declining

reproductive events registered for both atolls South-South-West and East-South-East (Castro et al. 2007), although this issue has not been studied in Rosario archipelago.

In conclusion, *S. gigas* populations from colombian southwest Caribbean are not panmictic, exhibit a global deficit of heterozygosity, and are structured in four stocks, which will require independent management. Our study suggests that the populations near to Jamaica play a weak role in the reinforcement of the other populations of the San Andres archipelago and colombian coast. This outcome highlights the importance of additional genetic studies in Central America and other Caribbean sites upstream of the San Andres archipelago to complement the genetic connectivity in this region and support the management and conservation of this resource focused on a local and regional level.

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