Mar Biol (2016) 163:165
DOI 10.1007/s00227-016-2935-6

ORIGINAL PAPER

Genetic diversity of reef fishes around Cuba: a multispecies assessment

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Received: 17 December 2015 / Accepted: 9 June 2016

Abstract We aimed to identify biotic and abiotic factors underlying genetic structure and diversity of reef fish around Cuba. For three species, *Stegastes partitus*, *Haemulon flavolineatum* and *Acanthurus tractus*, we investigated the effects of shared environmental factors, such as the geography of the Cuban Archipelago, and specific characteristics, such as life history traits, on genetic structure and diversity. Samples were collected at five locations around Cuba. For *S. partitus* and *H. flavolineatum*, mitochondrial DNA and microsatellite loci were examined, whereas only mitochondrial DNA polymorphism was analyzed for *A. tractus*. All three species showed high genetic diversity. Mismatch distribution analyses suggest past population expansion in all species, but at different times in each species. Haplotype network and population genetic analyses suggest that: (1) *S. partitus* went through a recent population bottleneck in the late Pleistocene, (2) *H. flavolineatum* went through a population bottleneck but earlier, in the mid-Pleistocene, and (3) *A. tractus* has had a large and stable population size with coalescence times that go back to the late Pliocene. Genetic polymorphism in *H. flavolineatum* and *A. tractus* is homogeneous throughout the archipelago, whereas there is significant genetic structure in *S. partitus*. Genetic differentiation among *S. partitus* populations is most likely the result of the combined effects of egg type and oceanic current patterns along the Cuban coast.

Introduction

At first glance, we would expect high connectivity between populations of reef fish species around Cuba because there are no obvious physical barriers in the ocean to hamper long-range dispersal. Even if the dispersal ability of the adults itself is low, most reef fishes have planktonic larvae with high dispersal potential (Bowen et al. 2013; Palumbi 2003). However, even when there are widely distributed suitable

Responsible Editor: O. Puebla.

Reviewed by C. A. Villegas Sanchez and undisclosed experts.

Electronic supplementary material The online version of this article (doi:10.1007/s00227-016-2935-6) contains supplementary material, which is available to authorized users.

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Published online: 28 June 2016

habitats, physical barriers such as landmasses, river outflows and geographic distance may prevent the dispersal of eggs, larvae and adults, resulting in genetic isolation in less mobile species (D'Aloia et al. 2014; Damerau et al. 2014; Galarza et al. 2009; Planes et al. 1996; Taylor and Hellberg 2006). Oceanographic factors such as marine currents, eddies and gyres may also be barriers to dispersal (Cowen et al. 2000; Taylor and Hellberg 2006). Genetic differentiation in fishes has been observed not only at large but also at small geographic scales (DiBattista et al. 2012; Hepburn et al. 2009; Salas et al. 2010) comparable to the Cuban Archipelago size.

The main factors involved in the genetic differentiation of several tropical reef fish species have been identified using comparative phylogeography (Rocha et al. 2008). Pelagic larval duration (PLD) and geographic distance have been frequently identified as factors influencing spatial genetic variation (Lessios and Robertson 2006; Selkoe and Toonen 2011). Other biological factors such as reproductive behavior (Portnoy et al. 2013), egg type (benthic or pelagic; Bradbury et al. 2008; Riginos et al. 2011), variation in reproductive success (Hedgecock 1994) and adult dispersal ability (Sponaugle et al. 2003) can also affect genetic structure of reef fish populations. It has also been suggested that adaptation to different habitats may produce genetically divergent lineages within a species, independent of both oceanographic factors and PLD (Riginos and Nachman 2001; Rocha et al. 2005).

Various degrees of population connectivity have been identified in the Caribbean region. Genetic isolation occurs between populations of reef fishes (*Acanthemblemaria aspera* and *A. spinosa*, Eytan and Hellberg 2010; *Elacatinus spp*. Taylor and Hellberg 2003, 2006), corals (*Acropora palmata*, Baums et al. 2005; *Orbicella* (=*Montastraea*) annularis, Foster et al. 2012; *A. cervicornis*, Vollmer and Palumbi 2007) and a gastropod (*Cittarium pica*, Diaz-Ferguson et al. 2010) consistent, in greater or lesser extent, with the forecast of a high-resolution biophysical model used to predict larval dispersal scales (Cowen et al. 2006). Among regions, restricted gene flow is more evident between the Bahamas, the eastern and western Caribbean and the region of the Colombia–Panama Gyre. This is suggested to be the result of marine circulation at different scales, limited larval dispersal, as well as historical events (Cowen et al. 2006). Conversely, wide connectivity has been detected through the Caribbean, between populations of the sea urchin, *Echinometra lucunter* (McCartney et al. 2000), the reef-building coral *Orbicella* (=*Montastraea*) *faveolata* (Severance and Karl 2006) and the reef fishes *Halichoeres bivittatus* (Rocha et al. 2005), *Holocentrus ascensionis* (Bowen et al. 2006) and *Thalassoma bifasciatum* (Purcell et al. 2006). Larval transport by marine currents, extended pelagic larval phase and larval behavior are the most common reasons given for this wide connectivity. However, contradictory patterns may often appear in species with similar life history traits, suggesting that a fluctuating hydrodynamic regime (Robainas et al. 2005; Severance and Karl 2006) and the highly stochastic nature of larval transportation through large distances (McCartney et al. 2000) may also influence the genetic connectivity of marine populations.

Information on the population structure of marine species around Cuba, particularly fishes, is limited. However, the morphology of the island of Cuba, as well as its size and its geographic position in the Caribbean suggests that reef fish populations would be genetically differentiated, a hypothesis worth testing. The Cuban Archipelago is composed of a main island surrounded by thousands of keys and islets. The submerged shelf is the largest in the insular Caribbean and can be divided into four relatively wide regions separated by long stretches of narrow shelf areas. The shelf is bordered, for most of its length, by extensive reefs and drops steeply to 400 m or more (Claro et al. 2001). In addition, there are three main marine current systems affecting Cuba: one moving westward in the south, one moving northeastward in the northwest and the third moving westward in the northeast (Fig. 1).

Local current patterns and habitat availability have been shown to affect the population genetic structure of two Cuban penaeid shrimp species, *Farfantepenaeus notialis* and *Litopenaeus schmitti*. Significant population differentiation at a small geographic scale (20–50 km) as well as significant genetic isolation between the southern gulfs, separated by 350 km, was identified (Borrell et al. 2004; Espinosa et al. 2003; García-Machado et al. 2001; Robainas et al. 2005). Nevertheless, the only study on a Cuban reef fish species, *Acanthurus tractus* Poey, 1860, has shown no population differentiation in the archipelago using partial cytochrome b sequences (Castellanos-Gell et al. 2012). Paris et al. (2005) used realistic intra-annual marine current patterns variations to simulate the larval transport of five commercial snapper species around Cuba. They found considerable levels of self-recruitment, particularly in the southern and north-central regions. Based on the scenarios proposed by Paris et al. (2005), we decided to study the genetic structure of three reef fishes [*Stegastes partitus* (Poey 1868), *Haemulon flavolineatum* (Desmarest 1823) and *Acanthurus tractus*] with contrasting life history traits, to explore the possible influence of reproductive behavior, egg type, larval and adult dispersal and marine currents on population structuring and history.

The bicolor damselfish, *S. partitus*, is a highly territorial species, dwelling in very small areas (<2 m^2). Females lay benthic eggs, in cavities or under the stones, and males take care of the nest (Robertson et al. 1988). The eggs hatch 3 days after laying, and the pelagic larvae are transported to the ocean where they spend from 27 to 31 days in the plankton (Robertson et al. 1988; Wellington and Victor 1989). Genetic connectivity studies among geographic

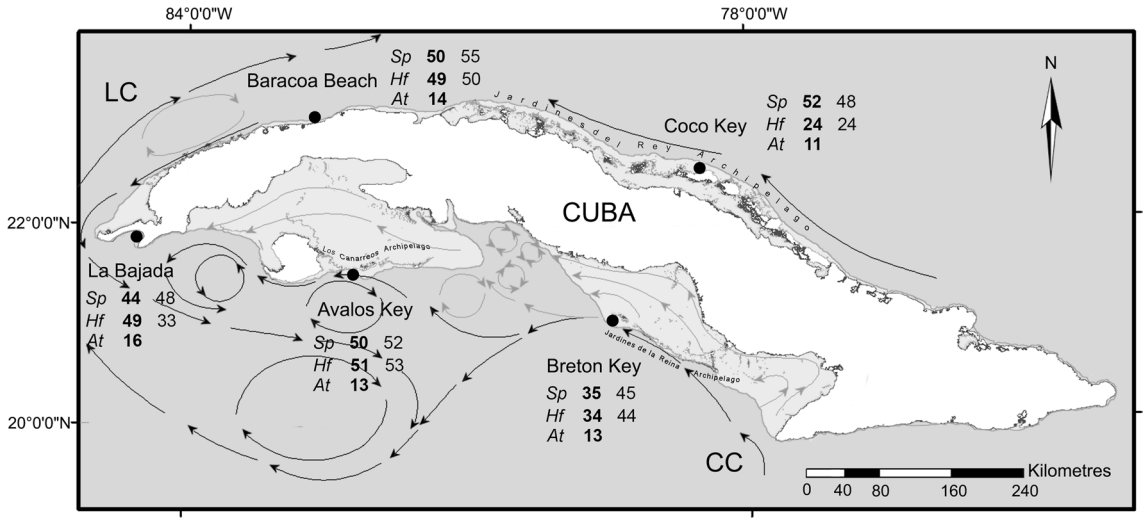

Fig. 1 Sampling sites around Cuba for *S. partitus* (*Sp*), *H. flavolineatum* (*Hf*) and *A. tractus* (*At*). *Arrows* indicate major (*black*) and secondary (*gray*) oceanic and shelf circulation (reviewed in Claro et al. 2001, chapter 1; Arriaza et al. 2012). Sampling sizes are indicated for each species and locality: in *bold* for mtDNA and regular font for microsatellite DNA. *Lighter gray areas* indicate the Cuban insular shelf. *LC* Loop Current, *CC* Caribbean Current

populations of *S. partitus* across the Caribbean region have identified patterns of connectivity, depending on the scale of the region examined, marine circulation and other factors. Weak genetic structure has been found within the Mesoamerican Barrier Reef System (MBRS, Hogan et al. 2010), although this was unstable across seasons and years (Hepburn et al. 2009). Within the MBRS, Hogan et al. (2012) detected high variability in self-recruitment among years and sampling sites, highlighting the random nature of connectivity. Weak but significant genetic differentiation was found in the MBRS and the Costa Rica–Panama regions with restricted gene flow within the Costa Rica–Panama region, associated with differences in habitat availability (Salas et al. 2010). Likewise, Villegas-Sánchez et al. (2010) found strong genetic structuring in the Mexican Caribbean (200 km) as a result of a sweepstake-chance effect and oceanographic factors. Finally, Purcell et al. (2009) detected a pattern of genetic isolation by distance across the Caribbean basin over <1000 km.

The French grunt, *H. flavolineatum,* is a supra benthic species with nocturnal habits. The species migrates to spawn from the inner waters of Cuba shelf regions to the slope, where spawning takes place (García-Cagides et al. 1994). The eggs are pelagic, and larvae spend only 15 days in the water column, which is among the shortest periods reported for reef fishes (Lindeman et al. 2001). Purcell et al. (2006) studied this species at several localities over the Caribbean region and found a significant yet modest isolation by distance.

Finally, the ocean surgeonfish *A. tractus* is a nomadic and highly mobile species (Valdés-Muñoz and Mochek 1994) that is frequently found in groups (Robertson 1985). Individuals spawn in couples or large groups producing pelagic eggs (Colin and Clavijo 1988). The pelagic larvae hatch after 24 h (Leis and Rennis 1983; Randall 1961) and spend 69 days in the water column before settling (Sponaugle and Cowen 1996). As mentioned above, genetic homogeneity around Cuba has been reported for this species (Castellanos-Gell et al. 2012).

Given the contrasting reproductive characteristics of these three species and the shared environmental factors that could influence larval dispersal and recruitment, we intended to shed light upon several questions regarding population connectivity around Cuba: Is the island itself a barrier to dispersal between the southern and northern populations? Is the current system a factor determining connectivity patterns around the island? Do life history traits affect population connectivity around the island? To address these questions, we sampled individuals of these three reef fish species at five localities representing the major regions of the Cuban insular shelf. The genetic variation and level of population genetic differentiation were evaluated using mtDNA and microsatellite markers.

Materials and methods

Sample collection

Stegastes partitus, *H. flavolineatum* and *A. tractus* adults were collected at five locations around Cuba: Breton Key (21°06′39.0″N, 79°26′55.6″W), Jardines de la Reina

Archipelago (December 2008), Avalos Key (21°32′55.6″N, 82°22′14.9″W), Los Canarreos Archipelago (December 2007 and October 2008), La Bajada (21°54′51.8″N, 84°29′05.8″W), Guanahacabibes Peninsula (June 2005 and February 2010), Baracoa Beach (23°03′36.2″N, 82°33′01.3″W), northwest Havana (February 2007 and November 2009) and Coco Key (22°32′30.6″N, 78°29′51.3″W) at Jardines del Rey Archipelago (March 2007 and February 2008; Fig. 1). Samples were collected using pole spears and hand nets while snorkelling or scuba diving. Immediately after capture, fin clips were stored in 95 % ethanol.

Molecular procedures

Total DNA extraction was carried out using proteinase K digestion and a standard phenol/chloroform protocol (Sambrook et al. 1989) with Phase Lock Gel tubes (Eppendorf). A fragment of the mtDNA noncoding region (NCR) was amplified for all three species with the primers CR-A 5′-AATTCTCACCCCTAGCTCCCAAAG-3′ and CR-E 5′-CCTGAAGTAGGAACCAGATG-3′ (Lee et al. 1995). For those DNA samples for which there was no amplification using these primers, (1) a second set of primers was designed to amplify a shorter internal region or (2) internal primers were designed to amplify two shorter and overlapping fragments corresponding to the original target fragment (Online Resource 1).

Total genomic DNA, 5–100 ng, was used as template in a 50-µl PCR with one unit of GoTaq DNA polymerase (Promega), 0.2 µM of each primer, 0.2 nM of dNTPs and 1.5 mM of $MgCl_2$. The reaction started with denaturation at 94 °C for 3 min, followed by 40 cycles of denaturation for 45 s at 94 °C, annealing for 1 min at 50 °C and extension for 1:30 min at 72 °C. Ten minutes at 72 °C were added for final extension. PCR products were purified using the NucleoSpin Extract II kit (Macherey–Nagel) and cycle-sequenced in both directions, using the ABI Prism Big Dye terminator sequencing kit V.3 (Applied Biosystems). Fragments were resolved on an ABI 3100 automated sequencer (Applied Biosystems). The sequences were deposited in GenBank with the accession numbers: KU207911–KU207992 (*S. partitus*), KU207820–KU207910 (*H. flavolineatum*) and KU207747–KU207819 (*A. tractus*).

Four microsatellite loci described by Williams et al. (2003) were analyzed in *S. partitus* (*Sp*GATA16, *Sp*AAT40, *Sp*AAC41 and *Sp*GATA40T), six microsatellite loci (Williams et al. 2004) in *H. flavolineatum* (*Hf*AAC3, *Hf*AAC10, *Hf*AAC37, *Hf*AAC41, *Hf*AAC43 and *Hf*AAC46) and none in *A. tractus*. PCRs were carried out in a 10-µl volume using the following conditions: 5–20 ng of genomic DNA diluted in TE 1X, 0.5 µM of each primer, 0.2 nM of dNTPs, 1.5 mM of $MgCl_2$ and 0.5 units of GoTaq DNA polymerase (Promega). The reaction started with a denaturation at 94 °C for 3 min, followed by 34 cycles of denaturation for 45 s at 94 °C, annealing for 45 s at 53 °C and extension for 1 min at 72 °C; 10 min at 72 °C was added for final extension. Genotypes were scored using a ABI 3130 XL Genetic Analyzer with GS500(-250)LIZ size standard and the software Genemapper 3.0 (Applied Biosystems).

Mitochondrial DNA sequence analysis

Sequences were aligned with ClustalW using MEGA 6.06 (Tamura et al. 2013). Alignments were optimized by eye. The model of nucleotide substitution best fitting the data was selected using the Bayesian information criterion (Schwarz 1978) as implemented in jModelTest version 0.1.1 (Guindon and Gascuel 2003; Posada 2008).

Estimation of population differentiation and genetic diversity using mtDNA

Sample sets collected in different years were checked for haplotype frequency homogeneity with the Chi-squared test using the Monte Carlo simulation method (Roff and Bentzen 1989) as implemented in the program CHIRXC (Zaykin and Pudovkin 1993). Haplotype networks were constructed to represent haplotype relationships and examine geographic partitioning using the median-joining network algorithm (Bandelt et al. 1999) and post-processed using maximum parsimony (Polzin and Daneschmand 2003) as implemented in Network 4.6.1.3 (Fluxus-engineering.com).

Pairwise differentiation between localities was estimated using the nearest-neighbor statistic S_{nn} of Hudson (2000) implemented in DnaSP 5.10 (Librado and Rozas 2009). This statistic is more powerful than other sequence-based statistics described by Hudson et al. (1992) when variation is weak to moderate. Population genetic diversity was estimated by nucleotide diversity (π) and haplotype diversity (h) (Nei 1987), as well as the number of haplotypes, polymorphic sites, and transitions and transversions. The sequence divergence between haplotypes was estimated using the TrN + Γ substitution model (Tamura and Nei 1993). Calculations were carried out with Arlequin 3.5 (Excoffier and Lischer 2010).

Demographic inferences using mtDNA

Departure from the neutral Wright–Fisher model was assessed using Tajima's D (Tajima 1989) and Fu's F_S (Fu 1997). The significance of Fs and D was evaluated by comparing the observed values with a null distribution generated under selective neutrality and population equilibrium with the coalescent simulation algorithm in DnaSP 5.10

(Librado and Rozas 2009). To identify population growth, we used the statistic R2 (Ramos-Onsins and Rozas 2002) which is based on differences between the number of singletons and the average number of nucleotide differences and is known to perform well with small sample sizes. 10,000 coalescent simulated samples were used to test for statistical significance using DnaSP 5.10.

Expansion events were identified by mismatch distribution analysis (Rogers and Harpending 1992) using Arlequin 3.5 (Excoffier and Lischer 2010). The fit of observed nucleotide differences to the expected mismatch distribution was evaluated by confidence interval as proposed by Schneider and Excoffier (1999). Population parameters such as theta (θ) before demographic expansion (θ_0) as well as the time since the expansion measured in mutational units (τ) were estimated. The parameters *Ne* (effective population size) and μ (mutation rate) were estimated using the generalized nonlinear least-square approach and confidence intervals calculated using a parametric bootstrap method (Schneider and Excoffier 1999). Coalescence time was estimated from $\tau = 2ut$ and $u = \mu k$ (Rogers and Harpending 1992), where t is the number of generations since coalescence, k is the sequence length, and u and μ are the mutation rates for the entire sequence and at each nucleotide position, respectively. An overall mutation rate (u) was calculated using 12.9 % substitutions per site per million years (Myr) for variable sites and 1.1 % substitutions per site per Myr for the conserved control region (Alvarado-Bremer et al. 1995) and dividing the total by k. Coalescence time in generations (t) was then transformed into years by multiplying by generation time. Generation time for *S. partitus* and *A. tractus* was calculated using $T = (\alpha + \omega)/2$ where α is the age at first reproduction and ω is the age at last reproduction (Pianka 1978). For *S. partitus*, α and ω were obtained from Robertson (1990) and Robertson (personal communication), and for *A. tractus*, they were based on age estimations of samples from the Bahamas and Belize (Robertson et al. 2005). Finally, generation time for *S. partitus* and *A. tractus* was estimated to 1 and 6.75 years, respectively. Generation time of *H. flavolineatum* was estimated to 3 years based on data for *Anisotremus davidsonii,* another member of the Haemulidae family (Bernardi and Lape 2005).

Population growth, theta (θ) and migration rates were computed by Bayesian analysis implemented in LAMARC v2.1.9 (Kuhner 2006). Population growth was estimated by the exponential growth rate "g". Positive values of g indicate growth of the population, and negative values indicate shrinkage. When g is almost zero or zero, the population size is assumed to be constant through time. Estimations of g are not symmetrical in order of magnitude, a value of $g = 10$ indicates weak growth, but $g = -10$ indicates significant decrease in population size for most theta values (Kuhner 2006). LAMARC estimates the migration rate as $M = m/\mu$, where m is the probability of immigration of a lineage per generation and μ is the mutation rate per site per generation. To convert this value into migrants per generation, it was multiplied by the theta value of the recipient population (Kuhner 2006). Two runs with different seed numbers were carried out for checking for convergence in results. Each one included 0 initial chains and 1 final chain (50,000 recorded items per chain, interval between them set to 50 and 10,000 samples to be discarded).

Microsatellite loci analysis

Each microsatellite allele was assigned to a size range bin using FLEXIBIN (Amos et al. 2007). Micro-Checker 2.2.3 (Van Oosterhout et al. 2004) was used to detect null alleles and large allele dropout and to check for stuttering.

Estimation of population differentiation using microsatellites

Sample sets collected in different years were checked for allele frequencies homogeneity using the same methods used for mitochondrial sequence (see estimation of population differentiation and genetic diversity using mtDNA). All pairs of loci were tested for linkage disequilibrium using the log likelihood ratio G-statistic in FSTAT 2.9.3 (Goudet 2001). Deviations from Hardy–Weinberg proportions (HWE) were evaluated by estimating the exact probability value of the F_{IS} statistic (Wright 1965) modified by Weir and Cockerham (1984) using the Markov chain method in GENEPOP 4.0 (Rousset 2008). In case of multiple comparisons, the rejection zone was adjusted with the Bonferroni correction (Rice 1989) using FSTAT 2.9.3 (Goudet 2001).

Population differentiation was estimated by calculating pairwise F_{ST} (FSTAT 2.9.3). These values were then used to estimate standardized F_{ST} (GST; Hedrick 2005) using RecodeData 0.1 (Meirmans 2006). This calculation is strongly recommended with high within-population diversity (Heller and Siegismund 2009). To represent graphically in a bidimensional space the genetic relationships between sampling locations as defined in a Nei's genetic distances matrix (Nei 1978), following Lessa (1990), a non-metric multidimensional scaling approach was applied using XLSTAT version 2012.2.01 (http://www.xlstat.com).

The software STRUCTURE 2.3.3 (Pritchard et al. 2000) was used to determine genetic differentiation of the populations. We used the "admixture model" and LOCPRIOR models which use the sampling locations as prior information to determine genetic structure. According to Hubisz et al. (2009), this model can be used to accurately estimate the population structure when the signal of structure is too weak (as provided by pairwise F_{ST}) to be detected using standard methods. "Correlated allele frequencies" were

also set. Nineteen iterations were carried out for K (number of estimated populations) ranging from 1 to 8 with a burn-in period of 10^4 and a number of MCMC repetitions after burn-in of 10^6. Evanno's test (Evanno et al. 2005) was used to determine the parameter ΔK, an ad hoc quantity based on the rate of change of the mean log likelihood of the data between successive K values. The true value of K is the mode of the distribution of ΔK, generated by STRUCTURE HARVESTER version 0.6 (Earl and vonHoldt 2012).

A Mantel test (Mantel 1967) was carried out to test for correlation between genetic differentiation (standardized F_{ST}) and geographic distance using XLSTAT version 2012.2.01. ArcGis 9.3 (Environmental Systems Research Institute, Redlands, CA, USA) was used to determine the shortest geographic distance between sampling locations. Two distance matrices were estimated: the first following the direction of the main current pattern (along Cuba from southeast to west and then from west to northeast) and the second based on the shortest distance between the southeastern (Breton Key) and northeastern (Coco Key) shelf (see Fig. 1).

Estimation of genetic diversity using microsatellites

Genetic diversity was assessed for each species and for each sampling location. Estimated parameters were as follows: number of alleles per locus (Na) and observed (Ho) and expected (He) heterozygosity calculated after Nei (1978) using ARLEQUIN 3.5 (Excoffier and Lischer 2010).

Demographic inferences using microsatellites

Microsatellite data were analyzed as described for mitochondrial DNA analysis using Bayesian methods implemented in LAMARC v2.1.9 (Kuhner 2006).

Results

Mitochondrial DNA

A fragment of the first hypervariable domain of the mtDNA noncoding region was amplified for individuals of each species. The size of the fragments varied according to the species and the primers used: 379 bp in *S. partitus*; 367 bp in *H. flavolineatum*; and 274 bp in *A. tractus*. In most cases, haplotype frequencies did not vary significantly in the 2 years sampled. The exception was *H. flavolineatum*, which had significantly different ($\chi^2 = 47.4$, $p = 0.04$) haplotype frequencies at Baracoa Beach between 2007 ($N = 32$) and 2009 ($N = 17$). Population differentiation analysis (S_{nn}) was performed with these two samples separately as well as pooled together, and results were not significantly different. We therefore pooled individuals from both sampling years and locality for further analysis.

Table 1 Pairwise estimates of genetic differentiation (S_{nn}) between sampling localities of *S. partitus*, *H. flavolineatum* and *A. tractus* in the Cuban Archipelago

	Breton Key	Avalos Key	La Bajada	Baracoa Beach
S. partitus				
Avalos Key	0.56*			
La Bajada	0.51	0.52		
Baracoa Beach	0.53	0.51	0.51	
Coco Key	0.57*	0.55*	0.55*	0.50
H. flavolineatum				
Avalos Key	0.57			
La Bajada	0.52	0.54		
Baracoa Beach	0.48	0.43	0.45	
Coco Key	0.50	0.55	0.51	0.46
A. tractus				
Avalos Key	0.47			
La Bajada	0.61	0.49		
Baracoa Beach	0.41	0.37	0.43	
Coco Key	0.56	0.33	0.51	0.45

Analyses were performed using the mtDNA noncoding region sequences

* Statistically significant values, $p < 0.05$ after 1000 permutations

Population structure inferences using mtDNA

Pairwise sequence differentiation values between localities (S_{nn} statistic) are shown in Table 1. In *S. partitus*, the Coco Key sample showed statistically significant differences with all other sampling localities except Baracoa Beach. Differentiation was also significant between the southern localities, i.e., the Breton and Avalos Keys. In contrast, no statistically significant differentiation between any localities was found in *H. flavolineatum* and *A. tractus*, indicating genetic homogeneity (Table 1). To further evaluate significant differences in S_{nn} between localities, we estimated pairwise Φ_{ST} values (Excoffier et al. 1992) (Online Resource 2). For *S. partitus*, the comparisons of Avalos Key with Breton Key, Coco Key and La Bajada were statistically significant. The Φ_{ST} values for *S. partitus* are concordant with S_{nn} statistic for Avalos Key vs. Breton Key and Coco Key, but not La Bajada. For the other two species, pairwise estimates were not significant between all localities.

Genetic diversity and haplotype relationships

Overall molecular diversity indices estimated for the three fish species are shown in Table 2. The lowest diversity estimates were found in *S. partitus* and the highest in *A. tractus*. All three species show high values of haplotype and nucleotide diversity: *S. partitus* ($h = 0.763$, $\pi = 0.004$; model TrN + Γ, $\alpha = 0.44$), *H. flavolineatum* ($h = 0.960$, $\pi = 0.012$; model TrN + Γ, $\alpha = 0.40$) and *A. tractus* ($h = 1.00$, $\pi = 0.057$; model TrN + Γ, $\alpha = 0.526$).

Figure 2 shows the relationships among haplotypes within each species. In *S. partitus*, the network has a star-like topology, with one high-frequency haplotype ($n = 111$) from which most of the other variants, with either low frequencies or as singletons, are derived (Fig. 2a). Similar proportions of shared and unique haplotypes were found (mean 0.45) among localities. In *H. flavolineatum,* the haplotype network has many singletons and several other haplotypes with relatively high to low frequencies present in all or most localities (Fig. 2b). In *A. tractus,* haplotypes were all singletons and the network shows an arrangement of relationships with many haplotypes differentiated by a relatively high number of mutations (Fig. 2c).

Demographic inferences using mtDNA

The Tajima's D and Fu's F_S statistics produced negative values significantly different to those expected under neutrality and at population equilibrium (Table 2). Highly significant and large negative values of F_S and significant negative values of Tajima's D statistic suggest population expansion in all three species (Schmidt and Pool 2002).

Ramos-Onsins and Rozas statistic R2 was also significant in all three surveyed species, and the mismatch distribution was unimodal (SSD < 0.001; $p > 0.05$; Table 3a) with the mean pairwise number of mutations between haplotypes gradually increasing from *S. partitus* (1.6) to *H. flavolineatum* (5.5) and then to *A. tractus* (15.6; Online Resource 3). Using mismatch distribution analysis, estimations of τ indicate that *S. partitus* experienced the most recent population expansion ($\tau = 1.66$; Table 3a). Estimated control region mutation rate (μ) was 3.4 % per Myr for *S. partitus* (12.9 % × 74 variable sites + 1.1 % × 305 conserved sites divided by 379 bp), 2.9 % per Myr for *H. flavolineatum* (12.9 % × 56 variable sites + 1.1 % × 311 conserved sites divided by 367 bp) and 7.3 % per Myr for *A. tractus* (12.9 % × 144 variable sites and 1.1 % × 130 conserved sites divided by 274 bp), which indicates a coalescence time back to the Pleistocene for both *S. partitus* (64,000 yr) and *H. flavolineatum* (845,000 yr) and the late Pliocene (2.7 Myr) for *A. tractus*.

LAMARC's Bayesian estimates of theta ($\theta = 2N_e\mu$) ranged from 0.45 (*S. partitus*) to 4.05 (*A. tractus*). Estimates of g indicate exponential growth in all three species, but lower in *A. tractus* than in the other species (Table 3b). Pairwise migration rates in *S. partitus* showed high levels of exchange among all sampling locations, which in terms of migrants per generation ranged from $Nm_{\text{Breton to Avalos Key}} = 7.80$ to $Nm_{\text{Avalos Key to Baracoa}} = 13.11$ (Online Resource 4). However, migration rate between Coco Key and Breton Key was notably lower in both directions ($Nm_{\text{Coco to Breton Key}} = 1.41$, $Nm_{\text{Breton to Coco Key}} = 0.70$). The confidence intervals were large for all these estimations; however, this low gene flow is between the two localities most isolated by the direction of the currents around Cuba (Fig. 1).

Microsatellite DNA

Four and six microsatellite loci were analyzed for 248 and 204 individuals of *S. partitus* and *H. flavolineatum*, respectively. No significant difference in allelic frequency between sampling years was identified using the frequency homogeneity test, except for *H. flavolineatum* locus *Hf*AAC3 in Avalos Key ($\chi^2 = 15.51$, $p < 0.01$). Despite this exception, data from different years were pooled together. Null alleles were detected for *H. flavolineatum* locus *Hf*AAC46; therefore, corrected allelic and genotype frequencies were estimated using Micro-Checker (Van Oosterhout et al. 2004). All analyzed loci were at linkage

Table 2 Molecular diversity indices and neutrality statistics estimated for the mtDNA noncoding region of *S. partitus*, *H. flavolineatum* and *A. tractus*

	S. partitus	*H. flavolineatum*	*A. tractus*
Number of sequences	231	207	67
Number of haplotypes	82	91	67
Transitions	53	52	127
Transversions	24	18	28
Indels	6	17	9
Number of polymorphic sites	74	80	144
Haplotype diversity (h) ± SD	0.763 ± 0.031	0.960 ± 0.0069	1.00 ± 0.0026
Nucleotide diversity (π) ± SD	0.004 ± 0.003	0.012 ± 0.0083	0.057 ± 0.034
Tajima D (p value)	−2.61 (0.000)	−1.58 (0.024)	−1.62 (0.024)
Fu F_S (p value)	−27.76 (0.000)	−24.46 (0.0003)	−24.20 (0.0002)

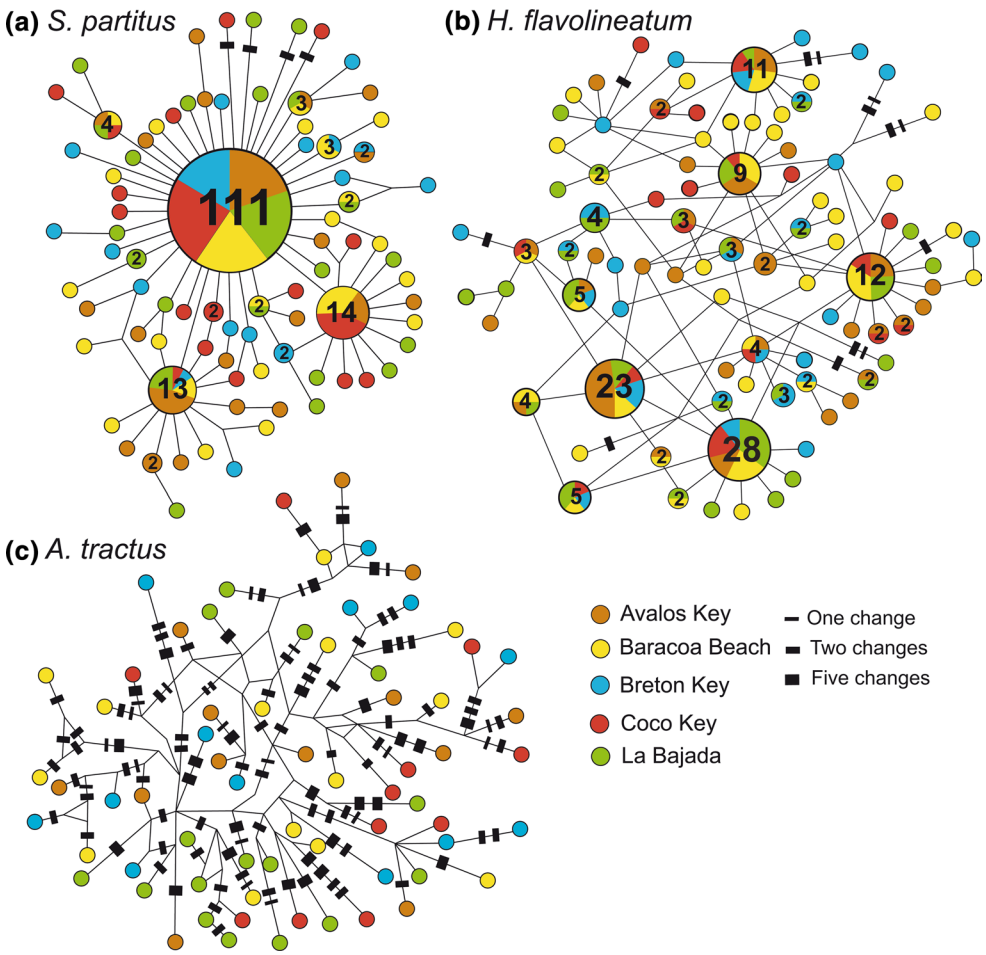

Fig. 2 Haplotype networks representing the relationships among the haplotypes found in *S. partitus*, *H. flavolineatum* and *A. tractus*. The networks were obtained using the median-joining algorithm and post-optimized using parsimony. Haplotypes are connected by one mutational step unless indicated by *crosswise bars*. The size of the *crosswise bars* corresponds to the number of mutations. The number of individuals is indicated inside the *circles*, except for haplotypes that are present in only one individual

Table 3 Demographic parameters from the mtDNA noncoding region of *S. partitus* (*Sp*), *H. flavolineatum* (*Hf*) and *A. tractus* (*At*)

	τ (CI 95 %)	θ_0 (CI 95 %)	SSD (prob)	R2 (prob)
(a) Mismatch distribution estimates				
Sp	1.66 (0.18–3.77)	0.26 (0.0–0.68)	0.00001 (0.99)	0.01 (0.001)
Hf	6.00 (2.94–9.55)	0.49 (0.0–2.51)	0.0007 (0.96)	0.033 (0.027)
At	15.91 (11.48–18.28)	<0.001 (0.0–3.38)	0.001 (0.55)	0.045 (0.006)
	θ (CI 95 %)	g (CI 95 %)		
(b) Bayesian estimates using LAMARC v2.1.9				
Sp	0.45 (0.32–0.63)	993 (715.4–1003.3)		
Hf	1.32 (0.74–2.10)	965.6 (647.4–996.6)		
At	4.05 (2.30–7.88)	97.8 (69.6–129.7)		

τ time since expansion, measured in mutational units, *CI* confidence interval, θ_0 theta before the demographic expansion, *prob* exact probability, *SSD* sum of square deviations, *R2* Ramos-Onsins and Rozas statistic, *g* exponential growth rate estimate

equilibrium, with Bonferroni correction with the threshold, $\alpha = 0.0083$ for *S. partitus* and $\alpha = 0.0033$ for *H. flavolineatum*.

All *S. partitus* microsatellite loci alleles were present in Hardy–Weinberg proportions, after Bonferroni correction for multiple tests ($\alpha = 0.0025$, 400 randomizations; Online Resource 5). All loci but one (*Hf*AAC41) of *H. flavolineatum* followed Hardy–Weinberg proportions (Online Resource 5).

Population structure inferences using microsatellites

The analysis with the program STRUCTURE (Pritchard et al. 2000) was performed (1) using the LOCPRIOR

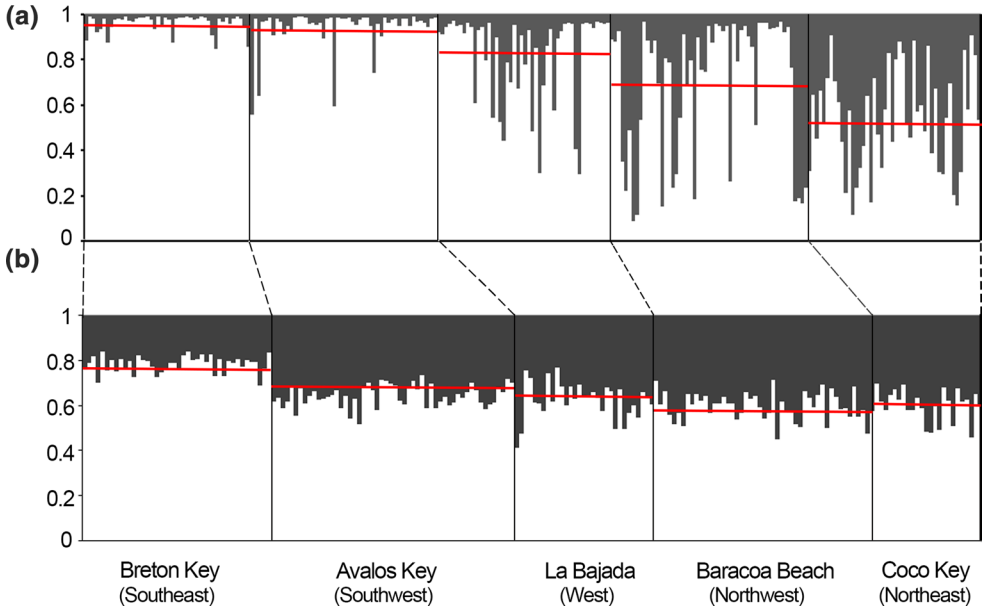

Fig. 3 Estimated membership in each of the two defined clusters (*gray* and *white*) obtained with STRUCTURE. Nineteen iterations, 10^4 burn-ins and 10^6 MCMC after burn-ins were carried out. The individuals are represented by *vertical lines* with membership coefficients indicated by *colors* **a** *S. partitus*, **b** *H. flavolineatum.* The *red lines* represent the mean assignment value of individuals to one of the clusters (*white color*) in each sampled localities. The geographic position of the sampled localities appears in parentheses. Sampling locations are presented following the direction of the main current patterns along Cuba: from southeast to west and then from west to northeast

model (Fig. 3) and (2) with no a priori information of sampling locations (Online Resource 6a), and the results were similar. In both figures, the sampling locations are presented following the direction of the main current around Cuba: from southeast to west and then from west to northeast. According to Evanno's test, individuals of *S. partitus* are grouped into two main clusters ($\Delta K = 2$, see Online Resource 7), that may be interpreted as including the southern localities (Breton and Avalos Keys; in white) and western-northern localities (La Bajada, Baracoa Beach and Coco Key; in gray; Fig. 3a). However, this subdivision seems also to show a tendency to a progressive assignment of the individuals to a different cluster (cluster white to gray in Fig. 3) in the direction Breton Key (southeastern; average = 96.2 %) to Coco Key (northeastern; average = 54.4 %). This trend is better observed when individuals are arranged inside each locality according to their estimated membership coefficients to each of the two clusters (Online Resource 6b).

Pairwise F_{ST} statistics among *S. partitus* populations are presented in Table 4. They show statistically significant genetic differentiation between all but La Bajada localities. The multidimensional scaling approach based on Nei genetic distances shows a pattern consistent with the pattern obtained with STRUCTURE (Online Resource 8). Mantel test based on pairwise genetic differentiation (F_{ST}) and geographic distances did not revealed any pattern of isolation by distance, independent of the geographic direction: either following the direction of the main Caribbean Current ($r = 0.513$, $p = 0.124$) or the minimum distance between Breton and Coco Keys ($r = 0.539$, $p = 0.109$).

For *H. flavolineatum*, the analysis using STRUCTURE suggested a single population around the Cuban Archipelago, although a subtle differentiation was observed in the same direction observed in *S. partitus* (Fig. 3b). Genetic homogeneity was further supported by the absence of genetic differentiation (F_{ST}) between populations (Table 4).

Genetic diversity using microsatellites

Genetic diversity was assessed according to the pattern observed of genetic differentiation (F_{ST}): (1) per locus and sampling location for *S. partitus*; (2) per locus and pooled sampling locations for *H. flavolineatum* (Online Resource 9).

The four loci studied in *S. partitus* showed high variability with a mean of 22.7 alleles per locus. Observed and expected heterozygosities per locus were high overall as well as at each sampling locality. However, observed values were lower than expected values. In *H. flavolineatum*, moderate numbers of alleles per locus were found, with values ranging from 15 to 30 (*locus Hf*AAC3 and *Hf*AAC46, respectively; Online Resource 9). Observed and expected

Table 4 Pairwise standardized F_{ST} (Hedrick 2005), using microsatellites, among sampling localities of *S. partitus* (below the diagonal) and *H. flavolineatum* (above the diagonal) around Cuba

	Breton Key	Avalos Key	La Bajada	Baracoa Beach	Coco Key
Breton Key		0.044	0.005	0.043	0.018
Avalos Key	0.142*		0.003	−0.001	0.007
La Bajada	0.038	0.007		0.001	−0.013
Baracoa Beach	0.137*	0.137*	0.093		−0.004
Coco Key	0.159*	0.198*	0.101	0.180*	

* Significant values, $p < 0.05$

heterozygosities per locus were >0.75. However, the locus *Hf*AAC3 showed much lower estimates due to the occurrence of a high-frequency allele.

Demographic inferences using microsatellites

Similar θ estimates were obtained for *S. partitus* ($\theta = 9.6$; IC 95 % 8.69–10.0) and *H. flavolineatum* ($\theta = 9.052$; IC 95 % 9.04–9.06). The populations of both species appear demographically stable around Cuba: $g = -0.002$ (IC 95 % −0.040 to 0.149) for *S. partitus* and $g = -0.103$ (IC 95 % −0.095 to 0.111) for *H. flavolineatum*. For *S. partitus,* pairwise migration rates and migrants per generation among all sampling locations were lower than those observed for the mitochondrial marker (Online Resource 4). Dispersal from Coco Key was low in all directions in contrast to a high value estimated in the Breton to Coco Key direction (Nm = 12.88).

Discussion

Here, we present the first comparative study of population genetic structure in Cuban reef fishes. The results indicate that the interplay between life history traits, geography and marine circulation can effectively influence connectivity patterns in marine fishes.

Different patterns of population differentiation

Our analyses show that only one of the three species analyzed, *S. partitus,* presents significant population genetic structure in the sampled area (Tables 1, 4). Both microsatellite and mtDNA markers indicate that the *S. partitus* population from Coco Key, at the northeastern platform, is the most genetically divergent. However, mtDNA also revealed significant differentiation between the two southern localities Breton and Avalos Keys, separated by a stretch of ocean about 350 km, and with this latter and La Bajada. In addition, the STRUCTURE assignment (Fig. 3) and the arrangement of localities according to pairwise genetic distances (Online Resource 8) clearly suggest that for this species there are different levels of gene flow between localities in the Cuban Archipelago. For *S. partitus*, isolation by distance at a regional scale (Purcell et al. 2009) and genetic differentiation at relatively small geographic scales—200–700 km—(Hepburn et al. 2009; Hogan et al. 2012; Villegas-Sánchez et al. 2010) have already been reported. However, at smaller geographic scales, this species appears to maintain enough genetic exchange to homogenize allele frequencies (Christie et al. 2010).

For *H. flavolineatum*, no genetic structure was observed at either the mtDNA or microsatellite loci. A similar pattern of population homogeneity, using allozyme loci, has been reported for Florida Keys (Schlueter 1998). However, an analysis of *H. flavolineatum* using microsatellite DNA in the Caribbean basin has showed a weak but significant pattern of isolation by distance (Purcell et al. 2006). In contrast to our findings, Purcell et al. (2006) also found that in some adjoining locations there was significant population structure. This suggests that local factors may be influencing the observed patterns.

The lack of genetic structure in *A. tractus* at the scale surveyed was not unexpected since Acanthurids have been described as nomads, moving freely across wide reef regions (Valdés-Muñoz and Mochek 2001). The results are congruent with previous analysis using *cyt*b sequences (Castellanos-Gell et al. 2012). At the level of the distribution area (Atlantic and Caribbean basins), other *Acanthurus* species, *A. chirurgus* and *A. coerulus*, also show genetic homogeneity (Rocha et al. 2002). However, gene flow in *A. bahianus* appears to be more restricted across the Amazon–Orinoco outflow barrier, probably as a consequence of habitat preference or larval dispersal capabilities (Castellanos-Gell et al. 2012). Similarly, gene flow is high among populations of different surgeonfish species over thousands of kilometers across the Indo-Pacific Ocean, from Hawaii to the eastern Indian Ocean (Eble et al. 2011), from the Indo-Pacific region to French Polynesia (Horne et al. 2008) and in the Hawaiian Archipelago (Eble et al. 2009).

Factors involved in population differentiation

The three fish species examined have different life history attributes. *S. partitus* is sedentary and lays benthic eggs just a few tens of meters from feeding areas (Robertson 1990). Pelagic larval duration is on average 28.8 days (Villegas-Hernández et al. 2008). We have no information for *S. partitus* in particular, but in general it has been reported that pomacentrid larvae are collected near the shore (Cowen

and Castro 1994; Leis 1991). In contrast, both *H. flavolineatum* and *A. tractus* are more vagile species with pelagic eggs spawned offshore and a PLD being half of the time and more than two times that of *S. partitus*, respectively.

The comparative analysis of the three fish species suggests that larval dispersal potential and random variation in recruitment are not factors affecting population differentiation. All three species have planktonic larval stages. *H. flavolineatum,* with the most ephemeral pelagic larval duration (about 15 days) did not show any population subdivision in our analysis, and at much larger geographic distances weak subdivision has been reported (Purcell et al. 2006). Similarly, our analysis of genetic variation over sampling years did not show any significant allele frequency changes. Thus, egg type emerges as the most probable factor involved in population structuring. While *H. flavolineatum* and *A. tractus* have pelagic eggs, *S. partitus* eggs are benthonic. This trait has been described as a significant predictor of genetic differentiation (Bradbury et al. 2008; Riginos et al. 2011, 2014). Riginos et al. (2014) and Riginos et al. (2011) found higher mean F_{ST} estimates in benthic compared to pelagic egg fish species, while a positive correlation was observed between the genetic structure and the proportion of fish species with demersal eggs (Bradbury et al. 2008).

Various models have been developed to predict larval dispersion and genetic structure based on increasingly accurate oceanographic data (e.g., Paris et al. 2005; White et al. 2010). Coastal circulation around the Cuban Archipelago has been described by Victoria del Río and Penié (1998). There is a complex pattern in the southwestern region (Claro et al. 2001), which includes gyres that may promote mixing of marine organism populations from the western and southern platforms (La Bajada, Avalos and Breton Keys) as well as the western and northwestern areas (La Bajada and Baracoa Beach) and to some extent the northeastern platform (Coco Key). On the other side of the island, the Loop Current in the Gulf of Mexico (Hofmann and Worley 1986) could carry individuals eastward, limiting mix with those from south, as indicated by a larval transport simulation in this area (Figure 2 from Paris et al. 2005). Finally, even though theoretically marine circulation should affect organisms in the same way, the different degrees of mobility of the three species studied probably exposes them to different local or oceanic currents on a small or medium scales (see Shulman and Bermingham 1995). Accordingly, a high proportion of self-recruitment has been previously detected in populations of *S. partitus* at Exuma Sound, Bahamas (Christie et al. 2010), associated with the effect of specific oceanographic features in the area.

Although the spatial pattern of connectivity identified by the microsatellite results does not fit a stepping stone model, the graphical arrangement of assignment probabilities by the STRUCTURE analysis on microsatellite data and the distinction between southern and northern localities reveal a fairly clear progression of genetic relationships around the archipelago coast, suggesting a stepping stone model of population differentiation (Fig. 3a).

The genetic structure observed in *S. partitus* most likely results from the combined effects of lower dispersal capacity, benthic eggs and ocean current patterns. Microsatellite and mitochondrial DNA markers appropriate for analyzing both ongoing and historical population processes were used (Wang 2010). Concordance in results from the two markers suggests therefore that the genetic structure in *S. partitus* is stable over time. This concordance also allows us to rule out natural selection as a factor. The subtle genetic differentiation found with microsatellite loci and the concordant and significant mtDNA genetic differentiation patterns at Coco Key suggest restricted gene flow. If ocean currents do play a role in *S. partitus* genetic differentiation, the combined action of the Loop Current flowing eastward near the Cuban northwestern coast and the northeastern current flowing in the opposite direction (Fig. 1) could produce a retention effect that may prevent the mixing of populations between this region and the rest of the ocean around Cuba. Finally, larval dispersal simulation results reported by Paris et al. (2005) are consistent with our *S. partitus* genetic structure results. While genetic differentiation has not yet been studied in Lutjanidae, the results obtained for *H. flavolineatum* suggest that there would be low or no differentiation in this species.

Genetic diversity and demography

Genetic variability at the noncoding region of the mtDNA showed contrasting characteristics in the three species, ranging from closely related to extremely divergent haplotypes.

Genetic diversity in *S. partitus* is characterized by high haplotype (*h)* and low nucleotide (π) diversity, a pattern indicative of rapid population growth after a relatively recent population bottleneck. This is reflected in the haplotype network with one main, highly frequent haplotype and several new, low-frequency, and closely related (one or two mutations) haplotypes (Fig. 2a). This type of pattern has been repeatedly reported in marine fishes (Avise 2000; Grant and Bowen 1998).

In *H. flavolineatum*, moderate values of both *h* and π were identified, suggesting a large population that experienced an ancient bottleneck. In accordance with this hypothesis, the haplotype network showed several closely related highly frequent variants that are connected to many low-frequency haplotypes.

Very high *h* and high π were observed in *A. tractus*. The haplotype network had the peculiarity of having as many

haplotypes as individuals sampled. In addition, these haplotypes were linked by long branches (Fig. 2). This type of pattern appears to be common in Acanthurids (Horne et al. 2008; Klanten et al. 2007) where rapid diversification has been detected among lineages (Clements et al. 2003). The higher haplotype and nucleotide diversity in both *H. flavolineatum* and *A. tractus* could be the result of two different scenarios: (a) secondary contact between populations separated for a long period of time followed by splitting into several well-differentiated lineages, or (b) populations with large and stable effective sizes during long periods of their evolutionary history and up to the present (Avise 2000; Grant and Bowen 1998). However, since no highly divergent clades were identified, the second scenario is the most likely.

The negative Tajima's D and Fu's F_S values suggest population size expansion for all three species (Table 2). The much lower Tajima's D statistic in *S. partitus* indicates a past population bottleneck since the D value is highly divergent from the expected value at equilibrium (Schmidt and Pool 2002). Mismatch distribution analyses show, for all three species, a unimodal distribution of pairwise number of differences between haplotypes which would be expected under a rapid population size expansion. These expansion events appear to have commenced at different times for each species (Online Resource 3).

The evolutionary histories of *S. partitus* and *H. flavolineatum* around Cuba may have been influenced by sea level changes associated with Pleistocene climate oscillations (Bintanja and van de Wal 2008; Bintanja et al. 2005). However, the earlier coalescence time seen in *S. partitus* (64,000 compared to 845,000 years ago) suggests that territorial behavior and demersal eggs may make this species more vulnerable to reef habitat reduction associated with quaternary glacial cycles (Bellwood and Wainwright 2002). In contrast, the estimated control region mutation rate for *A. tractus* places the coalescent time of this lineage at 2.7 Myr ago. This date precedes Pleistocene sea level fluctuations (Pillans et al. 1998), but is consistent with two major events associated with Plio-Pleistocene coral reef faunal turnover that could have caused habitat alteration: first, the final closure of the Panamanian Isthmus, resulting in oceanic circulation changes (Haug and Tiedemann 1998), and second, the sea level drop caused by the onset of major glaciations in the Northern Hemisphere (Raymo 1994). Population expansion of *A. tractus* probably occurred posterior to these events.

Theta (θ) estimations differ among these three reef fishes, with values ten times higher for *A. tractus* ($\theta = 4.05$) than for *S. partitus* ($\theta = 0.45$). However, if we remove the effect of mutation rate, which is two times higher in *A. tractus* ($\mu = 7.3$ % per Myr) than in *S. partitus* ($\mu = 3.4$ % per Myr), then we would expect that population size be five times higher for *A. tractus* than for *S. partitus*. The three species examined are among the most common in Cuban reefs (Claro 1994), and *S. partitus* is the second most abundant reef fish in Cuban and the Lesser Antilles reefs (Claro et al. 1998) with population sizes of thousands of individuals in north Havana (González-Sansón and Aguilar 2003) and tens of millions in Exuma Sound, Bahamas (Christie et al. 2010), and the Mesoamerican Reef Barrier (Puebla et al. 2012). However, high reproductive success variability could lead to an effective population size decrease and early coalescence of extant lineages (Hedgecock 1994). This type of sweepstake effect has been previously reported for *S. partitus* in other Atlantic populations (Christie et al. 2010).

When microsatellite markers are analyzed, genetic diversity estimates of marine fishes are highly variable (DeWoody and Avise 2000). The results obtained here were similar, in terms of observed heterozygosities and mean number of alleles per locus, to those previously reported for *S. partitus* (Hepburn et al. 2009; Hogan et al. 2010) and *H. flavolineatum* (Purcell et al. 2006) and slightly lower to those described by Purcell et al. (2009), Christie et al. (2010) and Salas et al. (2010) for *S. partitus*. The high levels of genetic diversity in both species suggest large effective population sizes around Cuba for *S. partitus* and *H. flavolineatum* (McCusker and Bentzen 2010). Heterozygosities ranging from moderate to high values have been reported in marine fish due mainly to large evolutionarily effective sizes characterizing their populations (DeWoody and Avise 2000).

No past changes in population size was detected in *S. partitus* and *H. flavolineatum* using microsatellite markers. This apparent contradiction with the mtDNA results may be the result of the intrinsic characteristics of microsatellite and mtDNA, which means that in some cases there is no concordance between results from microsatellite and mitochondrial markers (Zink and Barrowclough 2008). While mtDNA provides information at intermediate past timescales and is useful to detect demographic events at that scale, the microsatellite loci only perform well for a recent past. The high mutation rates characteristic of microsatellites can rapidly reach saturation, and homoplasy, erasing any information about events occurring earlier.

General considerations

In the present study, we show, for the first time, that at the scale of the Cuban Archipelago, there is an interplay between biological and environmental factors that may promote marine fish population subdivision. Particularly, low dispersal capabilities at some stages of the life cycle, and possibly local sea current patterns may facilitate self-recruitment and population isolation. Comparing the

population differentiation patterns observed in the three species, any generalized tendency of population subdivision associated with the geographic locality is evident. *S. partitus* shows both population subdivision and a tendency to genetic differentiation corresponding to the major sea current direction. Coco Key locality in the northeast shows genetic differentiation with both nuclear and mtDNA, and two other localities, Breton Key and Avalos Key, show significant differentiation with mtDNA. In contrast, *H. flavolineatum* displays only slight population differentiation in the locality of Breton Key, while *A. tractus* represents a single population in the study area.

According to the present results, we would expect that the northeastern region of the archipelago, by its position and direction of the major marine current patterns, would show higher population isolation than other areas of the archipelago. However, it seems not applicable for all fish species and future work with low vagility species is needed to reveal the consistency of this pattern around the island.

Acknowledgments We would like to thank Oscar Valmaña and Luis Sánchez for essential support with field work, and Gaspar González Sansón and Consuelo Aguilar Betancourt for valuable discussions on the ecology of marine fish. We also acknowledge Roamsy Volta for assistance with figure production. We thank also the three reviewers and the editor for their valuable comments and suggestions. This study was partially financed by the Research Grant A4139-1 from the International Foundation for Science awarded to ARB and the Embassy of France in Cuba.

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