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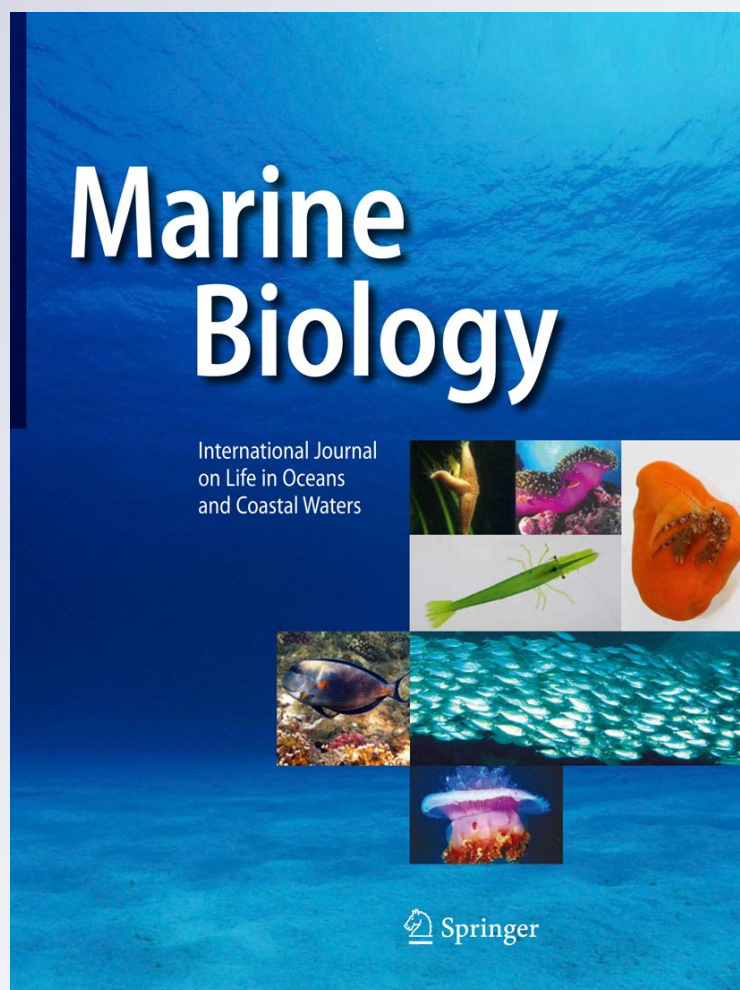Springer

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The surgeonfish, *Acanthurus bahianus,* has crossed the Amazon–Orinoco outflow barrier

**Jessy Castellanos-Gell · Aymée Robainas-Barcia ·
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Abstract Dispersal varies among species according to different biological and environmental factors. It is known that there is strong genetic division between the Ocean Surgeonfish (*Acanthurus tractus*) and the Barber Surgeonfish (*Acanthurus bahianus*) in the Caribbean and southern Atlantic biogeographic provinces with relation to the Amazon–Orinoco outflows. We analyzed *cyt*b gene sequence diversity from 149 individuals collected at five localities around Cuba between October 2006 and February 2010. As expected, most individuals had haplotypes identical or closely related to those previously reported for the Caribbean. However, south Atlantic lineage haplotypes were also found in all surveyed localities with frequencies around 5 %. This finding suggests that *A. bahianus* has dispersed in recent times across the Amazon–Orinoco barrier, probably because environmental perturbations have aided dispersal.

Introduction

The ability of marine species to disperse during different life stages, and how this influences the geographic structure of natural populations, has been one of the most important issues in marine ecology and biodiversity studies (Muss et al. 2001; Lubchenco et al. 2003; Rocha et al. 2008). However, direct and accurate estimates of dispersal are hard to obtain because many physical and biological factors impede observations of this process (Hellberg 2007). In this sense, population genetic analyses have been of the utmost importance in inferring the dynamics, dispersal patterns and connectivity of populations (Palumbi 2003; Purcell et al. 2006; Rocha et al. 2008).

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For example, a study of three Atlantic surgeonfishes (*Acanthurus bahianus*, *A. coeruleus,* and *A. chirurgus*), distributed throughout the tropical western Atlantic from North America to southern Brazil, showed that different habitat preferences and sensitivities to ecological conditions could explain the contrasting levels of population genetic subdivision in these three species across the Amazon–Orinoco outflow barrier (Rocha et al. 2002). *A. chirurgus* shows unrestricted gene flow between both regions, while *A. coeruleus* has an intermediate condition (i.e., statistically significant genetic differentiation but no reciprocal monophyly). In contrast, there is strong genetic subdivision within *A. bahianus* with endemic haplotypes in the Caribbean and south Atlantic biogeographic province. Based on caudal and dorsal fin color differences and genetic distances, Bernal and Rocha (2011) proposed that the Caribbean and the south Atlantic lineages of *A. bahianus* are different species, and they suggested the use of the name *A. tractus* proposed by Poey (1860) for the Caribbean lineage.

We have analyzed the genetic diversity of *A. tractus* around Cuba. In this study, we provide evidence for the occurrence of the south Atlantic lineage (i.e., *A. bahianus* Bernal and Rocha 2011) on Cuban reefs.

Materials and methods

Sample collection and DNA processing

A total of 149 adult fish (males and females) previously recognized as *Acanthurus bahianus* Castelnau 1855 (Castelnau 1855) (but recently *Acanthurus tractus* Poey 1860) were collected from five localities around Cuba between October 2006 and February 2010. The sample sites were the following: Avalos Key, Los Canarreos Archipelago ($n = 42$); La Bajada, Guanahacabibes peninsula ($n = 26$); Baracoa beach, northwest Havana ($n = 23$); Breton Key, Jardines de la Reina Archipelago ($n = 25$); and Coco Key, Jardines del Rey Archipelago ($n = 33$) (Fig. 1). Samples were collected using a polespear while snorkeling or scuba diving. Immediately after capture, fin clips were stored in 95 % ethanol.

Total DNA was extracted by proteinase K digestion and phenol/chloroform extraction using the Phase Lock Gel kit (Eppendorf). The primers Glufish 5′-CCAATGACTTGA ARAACCAYCGTTG-3′, modified from GluDGL (Meyer et al. 1990), and CB3 5′-GCCAAATAGGAARTATCAT TC-3′ (Palumbi 1996) were used to amplify 830 bp of the cytochrome b gene (*cyt*b). Polymerase chain reactions (PCRs) were carried out in 50 μl reaction volume with 5 ng to 100 ng of genomic DNA containing 1 unit of GoTaq DNA polymerase (Promega), 0.2 μM of each primer, 0.2 nM of dNTPs, and 1.5 mM $MgCl_2$. The reaction profile was 94 °C for 3 min for initial denaturing, followed by 40 cycles at 94 °C for 45 s, 50 °C for 1 min, 72 °C for 1:30 min and a final extension at 72 °C for 5 min. PCR products were purified using the NucleoSpin Extract II kit (Macherey–Nagel) and cycle-sequenced in both directions using an ABI Prism Big Dye terminator sequencing kit V.3 (Applied Biosystems). The fragments were resolved with an ABI 3100 automated sequencer (Applied Biosystems). The sequences of selected haplotypes were deposited in GenBank: accession numbers FJ905179 and FJ905180. The complete data set is available from the authors on request.

DNA data analysis

Sequences were edited using Bioedit 7.0.8.0 (Hall 1999) and aligned with the ClustalX alignment method using
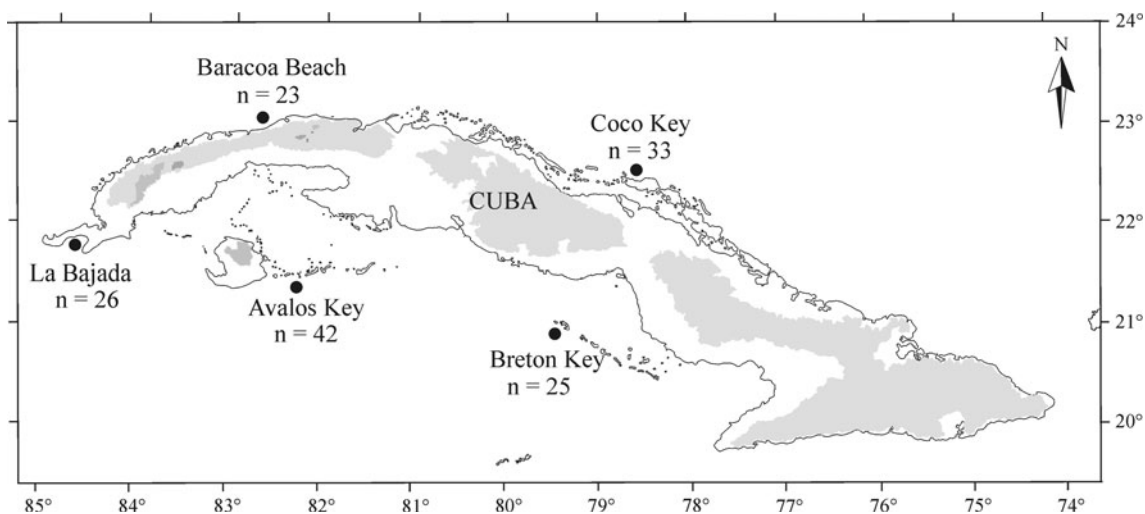
Fig. 1 Location of sampling sites of *Acanthurus tractus* around Cuba

MEGA 5.04 (Tamura et al. 2011). The length of the analyzed sequences was restricted to 608 bp in order to identify the haplotypes with respect to those previously defined by Rocha et al. (2002). The substitution model used in the phylogenetic analysis was the Tamura and Nei (1993) substitution matrix with a gamma distribution of substitution rate variation across sites (TrN + Γ with $\alpha = 0.259$) as suggested by the Bayesian Information Criterion (BIC) (Schwarz 1978) implemented in jModelTest version 0.1.1(Guindon and Gascuel 2003; Posada 2008). The neighbor joining (NJ) method was used to estimate the phylogenetic tree. The robustness of the nodes was assessed by bootstrap with 1,000 replicates. The available sequences of the species *A. bahianus* (accession numbers AY029306 and AY029307) and *A. tractus* (accession numbers AY029308 and AY029309) were included in the analysis. Additionally, the sequences of *A. chirurgus* (AY029304 and AY029305) and *A. coeruleus* (AY029311) were included as outgroups.

Haplotype relationships were represented by a network constructed using the program Network 4.5.1.6 software (Fluxus Technology). The network was obtained by applying the Median Joining (MJ) network algorithm (Bandelt et al. 1999) and was postprocessed using maximum parsimony calculations to reduce the number of superfluous network links.

Results

The 608 bp of the mtDNA *cyt*b gene fragment analyzed from Cuban samples had a total of 74 polymorphic sites, all at third codon positions, and a total of 61 haplotypes for the 149 sequenced individuals.

The NJ tree inferred with the *cyt*b sequences is shown in Fig. 2a. The Cuban sequences belong to two clades. The first is comprised of most of the individuals ($n = 142$) as well as the Caribbean haplotypes ABA1 and ABA2 representing *A. tractus*. The second one is comprised of seven sequences and the haplotypes ABA3 and ABA4 representing the species *A. bahianus*, which previous studies have only detected in the south Atlantic (Rocha et al. 2002; Bernal and Rocha 2011).

The relationships among the *cyt*b haplotypes from Cuban localities further support the existence of two major haplotype groups separated by nine mutational changes (eight transitions and one transversion) (Fig. 2b). The first group contains two common haplotypes that correspond to haplotypes ABA1 and ABA2. These haplotypes were found in 50 and 19 individuals, respectively, (Supplementary material 1) and appear to represent the diversification cores of *A. tractus* mtDNA haplotypes. Among the

other haplotypes, eight were found in two to nine individuals and 48 were found in only one individual. The average number of nucleotide differences within this cluster was $k = 2.93 \pm 0.034$. The haplotypes from this group were identified as At-C1 to At-C58.

The second Cuban group comprised three haplotypes connected by one to four transitions substitutions to haplotypes ABA3 and ABA4 (Fig. 2b). Of the three haplotypes, one was present in five of the seven individuals (Supplementary material 1) and was distributed among the sampled localities. The average number of nucleotide differences within this cluster (excluding haplotypes ABA3 and ABA4) was $k = 1.143 \pm 0.182$.

The mean genetic distance estimated between groups ($d = 0.020 \pm 0.006$) was roughly the same as estimated previously by Rocha et al. (2002). Haplotype and nucleotide diversity estimates for *A. tractus* were relatively high $h = 0.856 \pm 0.026$ and $\pi = 0.0048 \pm 0.0003$. On the other hand, the *A. bahianus* like clade was represented by only three haplotypes ($h = 0.524 \pm 0.209$ and $\pi = 0.0019 \pm 0.0009$) in Cuba.

Discussion

The mtDNA evidence

Compared with the other two species of the genus within the same area (*A. chirurgus* and *A. coeruleus*), *A. bahianus,* and *A. tractus* appears highly sensitive to the ecological constraint imposed by the Orinoco–Amazon freshwater outflows (Rocha et al. 2002) and has been considered a textbook example of deep population structuring due to long evolutionary separation by a geographic barrier (Fig 17.1 in Helfman et al. 2009). Our analysis of *cyt*b haplotype relationships of *A. tractus* from Cuba grouped seven Cuban individuals within the southern lineage with the ABA3 and ABA4 haplotypes described as the south Atlantic *A. bahianus* (Rocha et al. 2002). These haplotypes were separated by nine mutations from the second cluster that defines the resurrected *A. tractus* for the Caribbean (Bernal and Rocha 2011).

While our results do not negate previous results showing deep population structuring, the presence of mtDNA haplotypes from the southern region in the Caribbean (i.e., Cuba) suggests that *A. bahianus* may be able to overcome the Orinoco–Amazon freshwater outflow barrier. Our survey sample size is about four times larger (149 vs. 38) than that of Rocha et al. (2002). The southern haplotype individuals represent only about 5 % of our sample; therefore, these haplotypes may not be detected in a much smaller sample despite being widespread in the Caribbean.
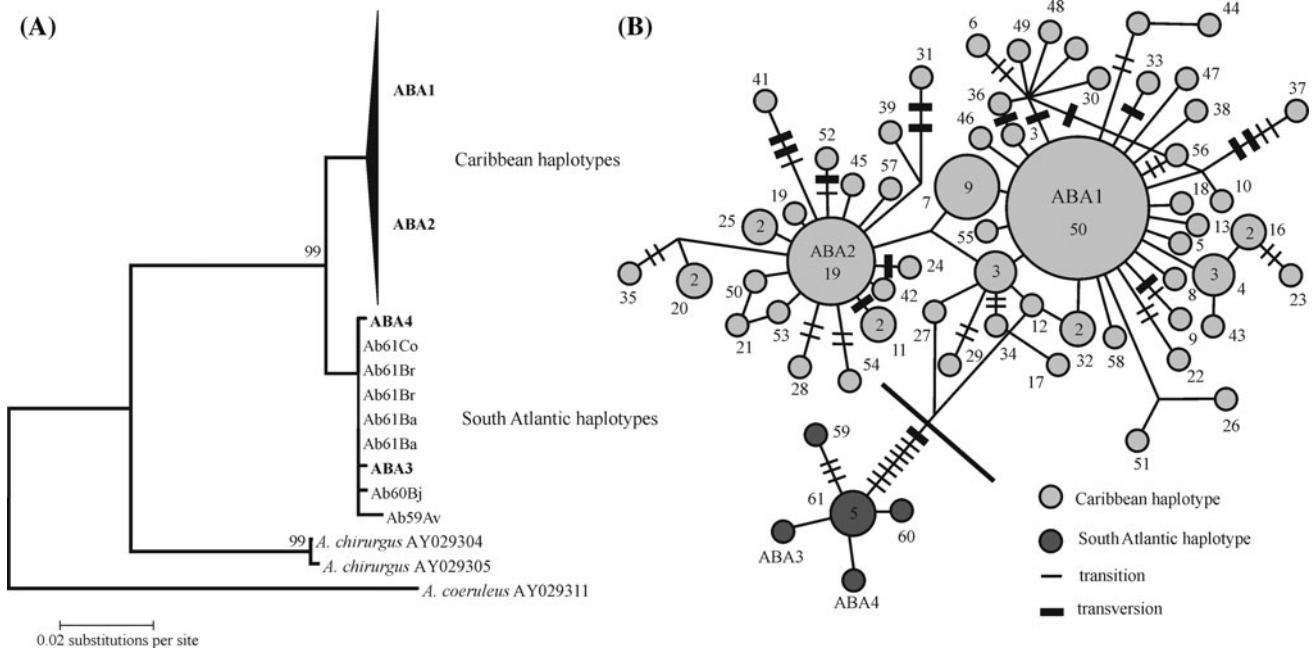
Fig. 2 a Phylogenetic tree showing the relationships among Caribbean and Brazilian haplotypes of *A. tractus* and *A. bahianus* with other atlantic acanthurids. *Av*: Avalos key; *Ba*: Baracoa beach; *Bj*: La Bajada; *Br*: Breton key; *Co*: Coco key. **b** *Acanthurus tractus* and *A. bahianus* Maximum Parsimony network of *cyt*b haplotypes found around Cuba. *Numbers inside circles* indicate number of individuals excepted for haplotypes found in only one individual. *Numbers outside circles* correspond to haplotype identification. ABA1, ABA2, ABA3, and ABA4 haplotypes were available from Genbank (Rocha et al. 2002). Network branches correspond to one mutation unit except when indicated by slashes. Southern haplotypes were distributed as follows: 59 from Av; 60 from Bj; 61 from Co, Br, and Ba

Larval or adult dispersal

The juvenile and adult stages of both *A. bahianus* and *A. tractus* are highly vagile (Robertson et al. 2005). However, its paucity in deep and muddy sea beds and its occurrence almost exclusively in shallow reefs have been considered major factors in explaining the isolation between the Caribbean and south Atlantic lineages of these species (Rocha et al. 2002). On the other hand, it lays pelagic eggs and the larvae have a planktonic phase of up to 69 days (Sponaugle and Cowen 1996). Both these characteristics are known to enhance opportunities for dispersal driven by marine currents (Muss et al. 2001). The widespread occurrence of *A. bahianus* from Brazil to the mid-Atlantic ridge, with no genetic differentiation, suggests that low salinity conditions around the Amazon–Orinoco outflow barrier are more effective at preventing larval dispersal than current patterns (Rocha et al. 2002).

Lessios and Robertson (2006) and Floeter et al. (2008) shed considerable light on this issue when they compared population structure and gene flow in several fish species from the Pacific and Atlantic Ocean. Both studies showed that there is no single shared pattern of genetic divergence and separation times between the populations of different species. This suggests that fish larvae may sporadically disperse through a barrier as a result of environmental fluctuations that eventually modify the effectiveness of the barrier. This appears also to apply for the Amazon–Orinoco barrier in the case of *A. bahianus*. The dispersal of *A. tractus* to the south Atlantic cannot be ruled out but it is less likely as the main current runs from south to the Caribbean. Nonetheless, this needs to be verified, as it is known that other species migrate across both sides of the barrier (Floeter et al. 2008; Rocha et al. 2008).

One or more dispersal events

The available data are insufficient to estimate the approximate time of the dispersal event to the Caribbean. The haplotypes identified in the present study have terminal and internal positions in the network (Fig. 2b) but they do represent just a small fraction of the diversity revealed by the previous study. However, as the number of nucleotide substitutions between the southern haplotypes found in Cuba and the available sequences of ABA3 and ABA4 haplotypes are small (one to four changes), it suggests that the migration of *A. bahianus* into the Caribbean region has occurred quite recently. The current haplotype distribution also suggests that at least one ancient colonization event occurred much earlier in the *A. bahianus/A. tractus* evolutionary history. If we assume the widely used divergence rate of 2 % per million years for estimating evolutionary time (Brown et al. 1979), the sequence divergence of $d = 2.0$ % for the *cyt*b gene between these two species

indicates that they diverged about 1 million years ago, well after the origin of the present configuration of the Amazon–Orinoco basin (*c.* 10 million years ago, Lovejoy et al. 1998). This suggests that a colonization event mediated by restricted dispersal across the established freshwater barrier and probably associated with or followed by demographic reduction, explains the reciprocal monophyly and population divergence on each side of the barrier.

Final considerations

The occurrence of *A. bahianus* haplotypes in the Greater Caribbean biogeographic province gives further support to the faunal enrichment hypothesis (Robertson et al. 2006; Rocha et al. 2008). In our study, the color pattern identifying *A. bahianus* was not noticed. However, its expected low frequency at the surveyed localities and its natural variation may have led to it remaining undetected. New samples and a careful examination of the color differences that separate *A. tractus* and *A. bahianus* are needed in order to study the correlation between this character and the genetic patterns. The study of nuclear DNA markers is now necessary to analyze the isolation between *A. bahianus* and *A. tractus*. In particular, we need to test whether *Acanthurus bahianus* is really present in the Caribbean, or whether the presence of some mtDNA haplotypes is the result of an ancient hybridization that led to introgression. Assessing whether these two species are interbreeding or simply coexisting in overlapping areas is the main goal of future work on these fishes.

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