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Adult habitat preferences, larval dispersal, and the comparative phylogeography of three Atlantic surgeonfishes (Teleostei: Acanthuridae)

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Abstract

Although many reef fishes of the tropical Atlantic are widely distributed, there are large discontinuities that may strongly influence phylogeographical patterns. The freshwater outflow of the Amazon basin is recognized as a major barrier that produces a break between Brazilian and Caribbean faunas. The vast oceanic distances between Brazil and the mid-Atlantic ridge islands represent another formidable barrier. To assess the relative importance of these barriers, we compared a fragment of the mitochondrial DNA (mtDNA) cytochrome *b* gene among populations of three species of Atlantic surgeonfishes: *Acanthurus bahianus*, *A. chirurgus* and *A. coeruleus*. These species have similar life histories but different adult habitat preferences. The mtDNA data show no population structure between Brazil and the mid-Atlantic islands, indicating that this oceanic barrier is readily traversed by the pelagic larval stage of all three surgeonfishes, which spend ~45–70 days in the pelagic environment. The Amazon is a strong barrier to dispersal of *A. bahianus* ($d = 0.024$, $\Phi_{ST} = 0.724$), a modest barrier for *A. coeruleus* ($\Phi_{ST} = 0.356$), and has no discernible effect as a barrier for *A. chirurgus*. The later species has been collected on soft bottoms with sponge habitats under the Amazon outflow, indicating that relaxed adult habitat requirements enable it to readily cross that barrier. A limited ability to use soft bottom habitats may also explain the low (but significant) population structure in *A. coeruleus*. In contrast, *A. bahianus* has not been collected over deep sponge bottoms, and rarely settles outside shallow reefs. Overall, adult habitat preferences seem to be the factor that differentiates phylogeographical patterns in these reef-associated species.

Keywords: Acanthurus, biogeography, Brazil, Caribbean, central Atlantic islands, dispersal, reef fishes

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Introduction

Tropical coral and rocky reef habitats of the Atlantic are widely distributed, with large gaps between reefs in the North, South, central and East Atlantic. Some of these gaps are probably substantial barriers to dispersal, and may influence evolutionary processes in reef organisms (Bernardi *et al*. 2000; Muss *et al*. 2001). For example, the northeastern coast of South America is influenced by the freshwater discharge of several rivers that drain the rain forest region: the Amazon, Pará and Orinoco are the largest. Consequently, the shallow coastal waters are very turbid, and the bottom is soft, with mobile mud banks (Briggs 1974; Longhurst 1998). This 2300 km wide area represents a major barrier to the dispersion of corals and is responsible for the Brazilian region having one of the highest levels of endemism (~32%) in the world for corals (Veron 1995). Recently several endemic reef fish species have also been described from Brazil (e.g. Rocha & Rosa 1999; Gasparini *et al*. 2001; Rocha & Rosa 2001), indicating that this barrier is also effective in isolating fishes.

Another barrier to dispersal of coastal marine organisms in the Atlantic Ocean is the vast distance between oceanic

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islands and the mainland. One of the most isolated islands is Trindade, 1100 km from the southeastern coast of Brazil. Based on recent faunal surveys, three endemic fish species are being described (Floeter & Gasparini 2000; J. L. Gasparini, personal communication). Ascension Island is located in the central Atlantic, 1540 km from the coast of Africa, 2261 km from Brazil, and 25% of its fish fauna are endemics (Lubbock 1980). Saint Helena is one of the most isolated islands in the world, located 3540 km from Brazil, 1870 km from Africa; the level of endemism among its shorefishes is similar to that at Ascension (~22%, Edwards & Glass 1987).

The surgeonfishes, family Acanthuridae, are herbivorous fishes, found primarily in tropical coral or rocky reef habitats (Lawson *et al*. 1999). They spawn positively buoyant planktonic eggs that hatch after approximately one day, and their pelagic larval stage is relatively long, up to 75 days (Thresher 1984).

Four species of *Acanthurus* have been described from the western Atlantic, *Acanthurus bahianus*, *A. chirurgus*, *A. coeruleus* and *A. randalli*, *A. bahianus*, the ocean surgeonfish, is distributed throughout the tropical western Atlantic from North America to southern Brazil. It also occurs in the oceanic islands of Bermuda in the North Atlantic and Fernando de Noronha, Atol das Rocas, Trindade, Ascension and St. Helena, in the South and Central Atlantic (Randall 1956; Floeter *et al*. 2001). *A. chirurgus*, the doctorfish, has a similar distribution, but occurs only at Ascension and not St Helena (Lubbock 1980; Edwards & Glass 1987; Robertson, whose collections represent the first record of this species at Ascension). It is also absent from Trindade Island (Floeter & Gasparini 2000). *A. coeruleus*, commonly known as the blue tang, ranges from New York to São Paulo, southeastern Brazil, and occurs in the same oceanic islands as the ocean surgeonfish, except for St. Helena (Edwards & Glass 1987; Robertson, personal observations). In contrast to the other three species, *A. randalli* has a restricted distribution in the eastern Gulf of Mexico. Morphologically it is very similar to *A. bahianus*, from which it differs only in the shape of the caudal fin (Briggs & Caldwell 1957). This species currently is the subject of a detailed morphological examination and will probably be treated as a junior synonym of *A. bahianus* (W. F. Smith-Vaniz, personal communication). It was not included in our analysis.

In this paper we examine how the Amazon freshwater outflow and oceanic distances between central Atlantic islands and the mainland influence the population structure of three Atlantic species of the genus *Acanthurus* that occur on both sides of each barrier. To assess the phylogeography of *A. bahianus*, *A. chirurgus* and *A. coeruleus* we compared sequences of the cytochrome *b* gene from the mitochodrial DNA (mtDNA) of these species. This gene is widely used in studies of intrageneric divergence in fishes, and generally yields results consistent with morphological variations (Wiley & Hagen 1997). By assessing the degree of population separation within these three species of *Acanthurus* in the Atlantic, we hope to illuminate the influence of potential barriers and habitat preferences on microevolutionary processes in reef species.

Materials and methods

A total of 112 specimens from six locations were obtained for *Acanthurus bahianus*, 82 from four locations for *A. coeruleus*, and 48 from three locations for *A. chirurgus* (Table 1, Fig. 1). The fish were collected with polespears while scuba diving or snorkelling, between 1990 and 1999. Most tissue samples (muscle and/or gill) were stored in a saturated salt-DMSO buffer (Amos & Hoelzel 1991).

Total genomic DNA was extracted by standard phenol/chloroform methods (Sambrook *et al*. 1989). Extracted DNA was frozen in TE buffer and archived at −20 °C. A segment of approximately 700 bp of the mtDNA cytochrome *b* gene was amplified using a heavy strand primer (5′-GTGACTTGAAAAACCACCGTTG-3′) and a light strand primer (5′-AATAGGAAGTATCATTCGGGTTT-GATG-3′), designed by Song *et al*. (1998) and Taberlet *et al*. (1992), respectively.

Thermal cycling in polymerase chain reactions (PCR) consisted of an initial denaturing step at 94 °C for 1 min 20 s, then 35 cycles of amplification (40 s of denaturation at 94 °C, 30 s of annealing at 52 °C, and 55 s of extension at 72 °C), and a final extension of 2 min 30 s at 72 °C. Excess oligonucleotide primers were removed through simultaneous incubation of PCR product with exonuclease I and shrimp alkaline phosphatase (USB Corp., Cleveland OH).

Sequencing reactions with fluorescently labelled dideoxy terminators (BigDye) were performed according to manufacturer's recommendations, and analysed with an ABI 377 or 310 automated sequencer (Applied Biosystems, Inc., Foster City, CA). All samples were sequenced in the forward direction, but to ensure accuracy of nucleotide designations, rare and questionable haplotypes were sequenced in both directions. Sequences of representative haplotypes (Appendix I) have been deposited in GenBank with accession numbers AY029304–AY029311. Copies of the complete data set are available from L.A. Rocha on request.

Sequences were aligned and edited with SEQUENCHER version 3.0 (Gene Codes Corp., Ann Arbor, MI). Population structure and gene flow were assessed with an analysis of molecular variance (AMOVA, Excoffier *et al*. 1992), which generated Φ_{ST} values (a molecular analogue of F_{ST} that considers sequence divergence among haplotypes). Genetic variation is described with nucleotide diversity (π; equation 10.19 in Nei 1987) and haplotype diversity (h; equation 8.5 in Nei 1987) within each location. Sequence

Table 1 Sample size (n), haplotype diversity ($h \pm$ standard error) and nucleotide diversity ($\pi \pm$ standard error) for the three species of *Acanthurus* surveyed

	A. bahianus			*A. chirurgus*			*A. coeruleus*		
Location	*n*	*h*	π	*n*	*h*	π	*n*	*h*	π
St. Helena	14	0.81 ± 0.09	0.0041 ± 0.002	—	—	—	—	—	—
Ascension	23	0.77 ± 0.07	0.0035 ± 0.0022	5	0.0	0.0	19	0.75 ± 0.05	0.0023 ± 0.0016
Brazil	37	0.90 ± 0.03	0.0041 ± 0.0025	24	0.98 ± 0.01	0.0053 ± 0.0031	25	0.73 ± 0.07	0.0019 ± 0.0014
Grenada	17	0.90 ± 0.05	0.0053 ± 0.0032	—	—	—	20	0.89 ± 0.04	0.0039 ± 0.0025
Florida	15	0.69 ± 0.12	0.0064 ± 0.0038	—	—	—	18	0.94 ± 0.02	0.0042 ± 0.0027
Virgin Islands	6	0.80 ± 0.17	0.0040 ± 0.0029	—	—	—	—	—	—
Panama	—	—	—	19	0.99 ± 0.02	0.0049 ± 0.0029	—	—	—
Total *n*	112			48			82		

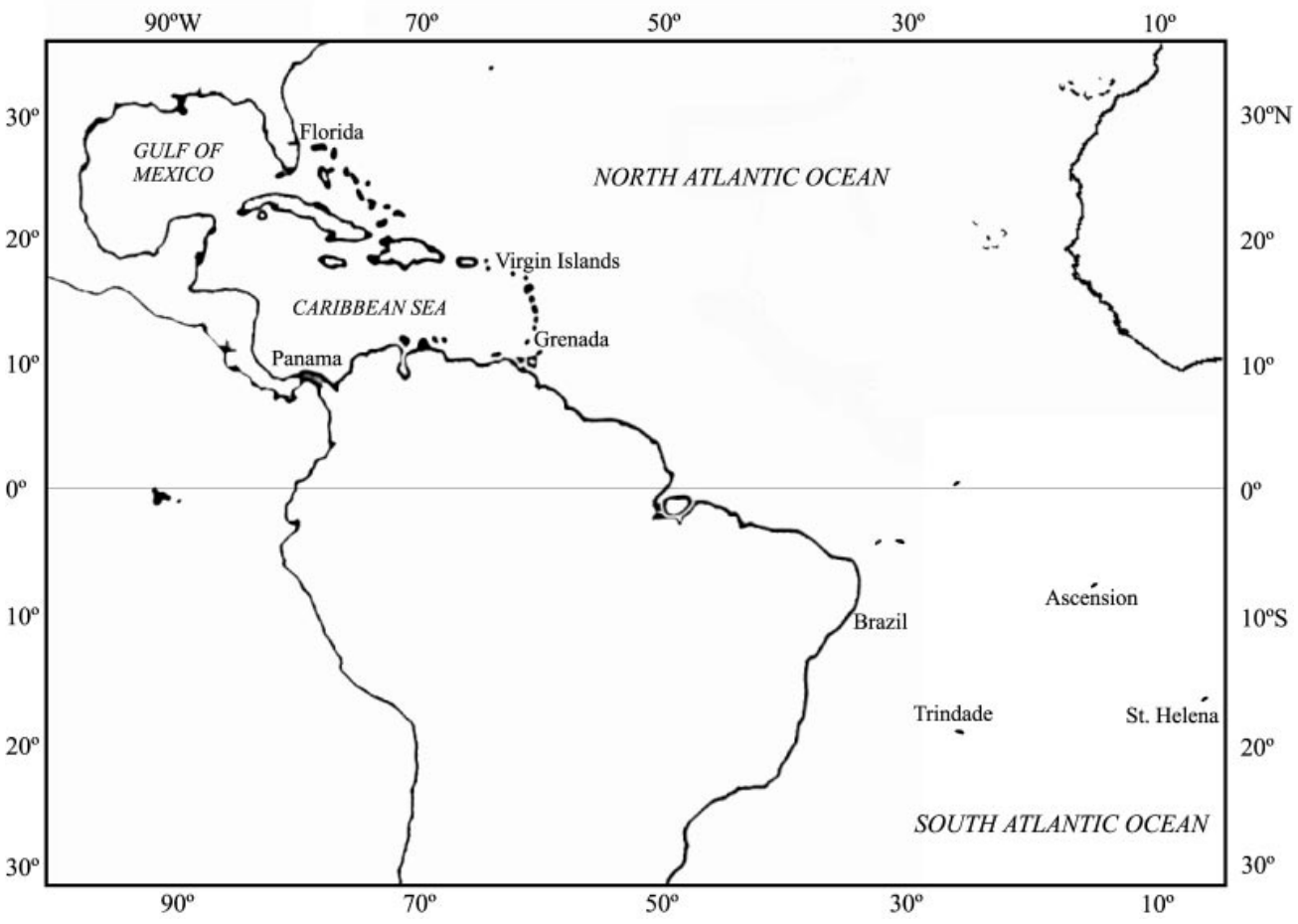

Fig. 1 Sample locations for *Acanthurus bahianus*, *A. chirurgus* and *A. coeruleus*.

divergences (d-values) between haplotypes were estimated with the Tamura & Nei's (1993) substitution model and equal weighting of transitions, transversions and all three codon positions. All the genetic structure calculations were performed with ARLEQUIN version 2.0 (Schneider *et al*. 2000). A Mantel nonparametric test (Mantel 1967) was performed to test the hypothesis of association between gene flow and geographical distance among populations using the software MANTEL version 2.0, Nonparametric Test Calculator (Liedloff 1999). Evolutionary relationships between haplotypes were estimated using maximum parsimony analysis (including 500 bootstrap replicates) performed with the software PAUP*, version 4.0b8 (Swofford 2001).

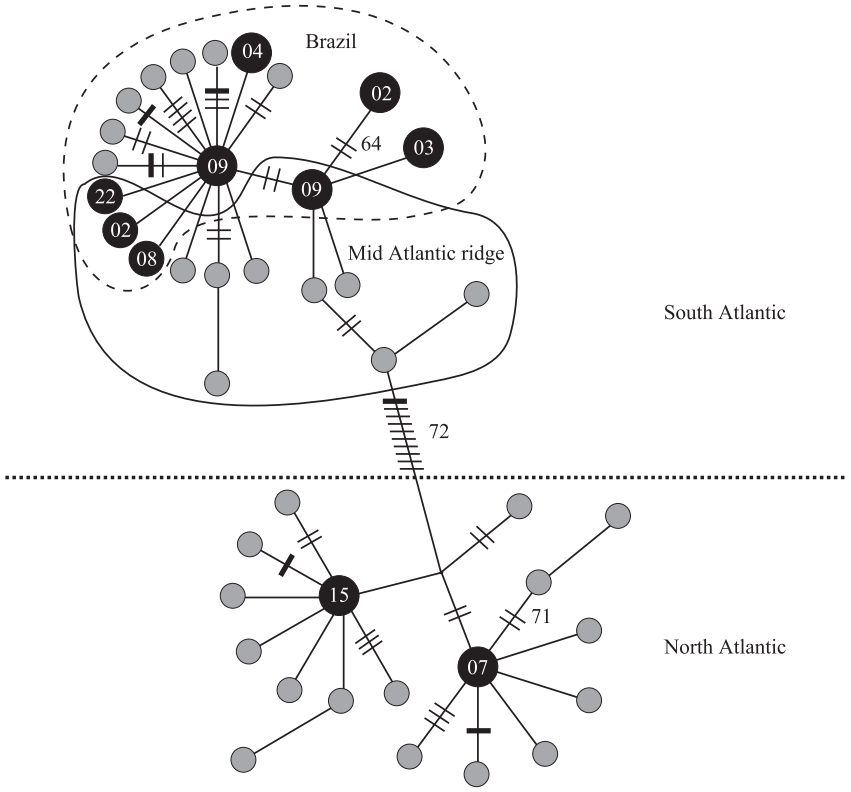

Fig. 2 Maximum parsimony mtDNA network of 41 haplotypes of *Acanthurus bahianus*. All bootstrap values of 50% or more are indicated over the branches (based on 500 replicates generated on PAUP). Network branches are of unit length except when indicated by slashes, which correspond to the number of mutations; narrow slashes correspond to transitions, wide slashes to transversions. Grey circles represent unique haplotypes, those in black represent common haplotypes with the number of individuals indicated.

Results

A 608 bp segment from the cytochrome *b* gene was analysed for *Acanthurus bahianus*, 663 bp for *A. chirurgus* and 584 bp for *A. coeruleus*. A total of 56 polymorphic sites distributed among 41 haplotypes were identified for *A. bahianus*, 32 polymorphic sites among 30 haplotypes for *A. chirurgus* and 18 polymorphic sites among 20 haplotypes for *A. coeruleus*. Samples of *A. bahianus* and *A. coeruleus* collected in southeast and northeast Brazil and at the oceanic island of Trindade (Fig. 1) were grouped into a single population labelled 'Brazil', because no population genetic difference was found between them. Haplotype diversities were high ($h = 0.69–0.99$) and nucleotide diversities were low ($\pi = 0.0019–0.0064$) for all species at all locations, except for *A. chirurgus* at Ascension (Table 1).

A. bahianus populations in the South Atlantic (represented by Brazil, St. Helena and Ascension) and North Atlantic (Caribbean) were separated by a fixed difference of 11 mutations (10 transitions and one transversion), corresponding to an average sequence divergence of $d = 0.024$ (range $d = 0.018–0.029$). Φ_{ST} values between northern and southern populations ranged from 0.805 to 0.836 ($P < 0.001$), indicating deep population structure (overall Φ_{ST} value 0.724; $P < 0.001$). The Φ_{ST} values for comparisons within each of the two major subdivisions (North and South Atlantic) were not significantly different from zero, except for a marginally significant difference between Brazil and St. Helena ($\Phi_{ST} = 0.064$; $P < 0.06$), and between Florida and Grenada ($\Phi_{ST} = 0.104$; $P < 0.06$) (Table 2). St. Helena, Ascension and Brazil shared four haplotypes, and Grenada, US Virgin Islands (USVI) and Florida shared two haplotypes (Fig. 2, Appendix I). A Mantel test revealed significant ($P < 0.005$) positive correlation ($r = 0.753$) between geographical distance and Φ_{ST} in *A. bahianus*.

A. coeruleus showed moderate levels of population structure and no fixed differences between North and South Atlantic populations with an overall Φ_{ST} of 0.356 ($P < 0.001$). Pairwise Φ_{ST} values for comparisons between northern and southern samples ranged from 0.443 to 0.459 (Table 2). Haplotype diversities were highest in the Caribbean (Florida and Grenada), and lowest in Brazil and Ascension (Table 1). A positive correlation ($r = 0.6296$) was observed between geographical distance and Φ_{ST} but it was not significant ($P > 0.05$). Two haplotypes were shared between the southern and northern populations, while 16 were unique to the northern and five to the southern region (Fig. 3). The most common haplotype in Brazil (12 out of 25 individuals) was also the most common in Ascension (seven out of 19 individuals), but it was uncommon in the Caribbean (two individuals out of 20 in Grenada and two out of 18 in Florida).

A. chirurgus showed no population structure among Panama, Brazil and Ascension (overall Φ_{ST} not significantly different from zero, Table 2), and the Mantel test revealed no correlation between geographical distance and Φ_{ST}. The

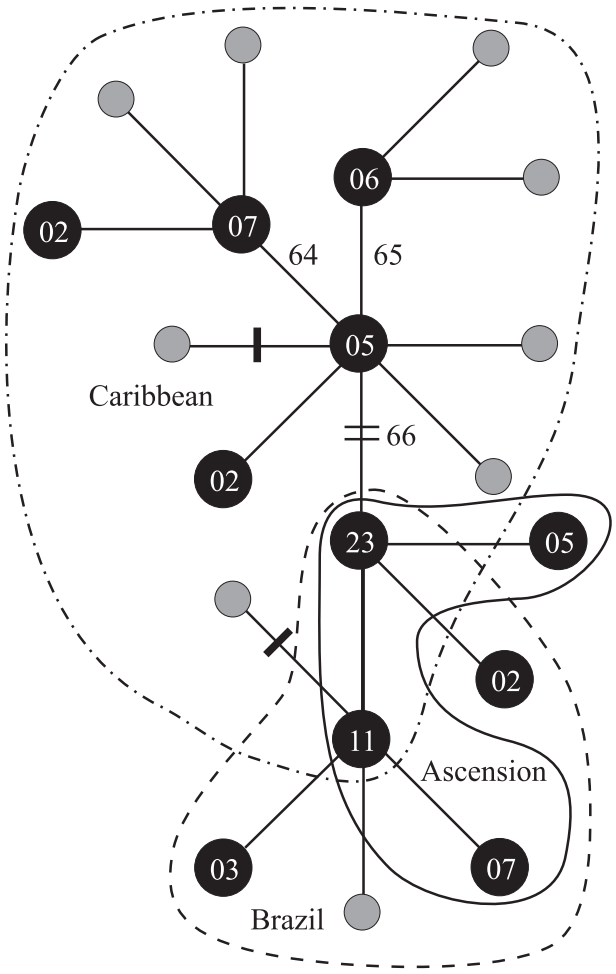

Fig. 3 Maximum parsimony mtDNA network of 30 haplotypes of *Acanthurus coeruleus*. Bootstrap values, branch length and number of individuals as in Fig. 2.

most widely distributed haplotype was also the only one found at Ascension ($n = 5$), and all the haplotypes that occurred in more than two individuals were observed in both Brazil and Caribbean (Panama) sample locations (Fig. 4). Panama showed the highest haplotype diversity and Ascension had the lowest value (Table 1).

Discussion

The mtDNA survey of *Acanthurus bahianus* revealed strong separation between the Brazilian and Caribbean provinces ($d = 0.024$; pairwise $\Phi_{ST} = 0.805–0.836$), and no significant population structure across the oceanic gap separating Brazil and the mid-Atlantic ridge. The genetic differentiation between the Brazilian and Caribbean populations is matched by a colour difference: individuals of *A. bahianus* collected in the North Atlantic typically show a distinguishing narrow, bluish white posterior margin on the caudal and dorsal fins (Humann 1994; Randall 1996), whereas South Atlantic *A. bahianus* have a bright yellow margin (Edwards 1990; observations by Robertson at Ascension, and Rocha at several locations along the Brazilian coast). As no other morphological characters are known to separate these two populations they have been treated as the same species. However, the genetic and colour differences indicate that a morphological reappraisal of geographical variation is warranted, to test the possibility that the wide-ranging *A. bahianus* is in fact a pair of sibling species.

The Φ_{ST} value between Brazil and Grenada, the closest North and South Atlantic sampling points, *c.* 2600 km apart, was 0.811 ($P < 0.001$), whereas only shallow, nonsignificant differences were observed between Brazil and the central Atlantic islands ($\Phi_{ST} = 0.026$ and 0.064; $P < 0.07$ and $P < 0.06$ for Ascension and St Helena, respectively) separated by *c.* 2300–3500 km of deep oceanic waters. This distance is shortened if we consider Trindade Island, off Brazil, as a stepping-stone for colonization of the Central Atlantic islands, but the distance between Trindade and St. Helena is still more than 2000 km, and major warm

Table 2 Estimate of geographical distance (km), genetic variation among population (Φ_{ST}), and the exact test (N.S. = not significant at $\alpha = 0.05$) of population differentiation for *Acanthurus bahianus*, *A. coeruleus* and *A. chirurgus*

Locations	Distance (km)	Φ_{ST}	Exact test
A. bahianus			
USVI – Grenada	705	0.13188	N.S. ($P < 0.09$)
Ascension – St. Helena	1095	0.01241	N.S. ($P < 0.40$)
Florida – USVI	1772	0.01333	N.S. ($P < 0.33$)
Brazil – Ascension	2261	0.02593	N.S. ($P < 0.07$)
Florida – Grenada	2462	0.10428	N.S. ($P < 0.06$)
Grenada – Brazil	2588	0.81086	$P < 0.001$
USVI – Brazil	3095	0.81333	$P < 0.001$
Brazil – St. Helena	3540	0.06392	N.S. ($P < 0.06$)
Florida – Brazil	4847	0.82454	$P < 0.001$
Grenada – Ascension	5638	0.81575	$P < 0.001$
USVI – Ascension	6209	0.82964	$P < 0.001$
Grenada – St. Helena	6710	0.80508	$P < 0.001$
USVI – St. Helena	7300	0.81790	$P < 0.001$
Florida – Ascension	8024	0.83660	$P < 0.001$
Florida – St. Helena	9200	0.83076	$P < 0.001$
A. coeruleus			
Brazil – Ascension	2261	0.05920	N.S. ($P < 0.07$)
Florida – Grenada	2462	0.00362	N.S. ($P < 0.83$)
Grenada – Brazil	2588	0.45926	$P < 0.001$
Florida – Brazil	4847	0.46128	$P < 0.001$
Grenada – Ascension	5638	0.44458	$P < 0.001$
Florida – Ascension	8024	0.44332	$P < 0.001$
A. chirurgus			
Brazil – Ascension	3540	0.04653	N.S. ($P < 0.22$)
Brazil – Panama	4600	0.00232	N.S. ($P < 0.96$)
Panama – Ascension	8700	0.02014	N.S. ($P < 0.26$)

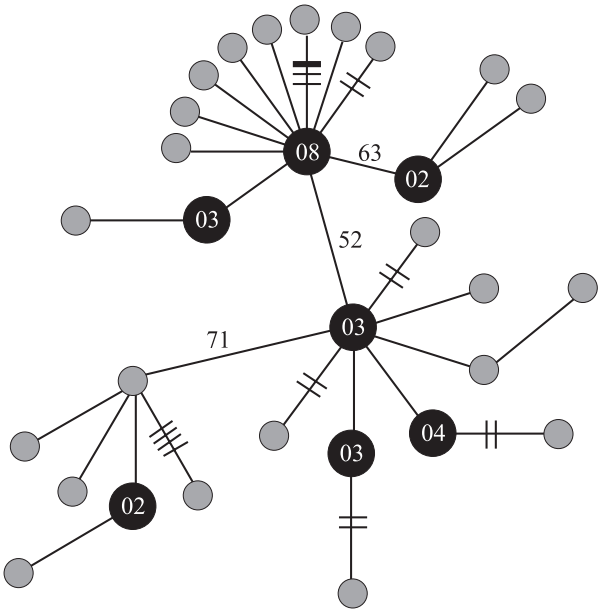

Fig. 4 Maximum parsimony mtDNA network of 20 haplotypes of *Acanthurus chirurgus*. Bootstrap values, branch length and number of individuals as in Fig. 2.

currents do not facilitate this crossing. The Φ_{ST} values within North and South Atlantic were nonsignificant, but those between Grenada and the other two North Atlantic locations were higher than the values within the South Atlantic. This may be due to the larger population size and the large number of discontinuous habitats in the Caribbean Sea, and it is also reflected in the higher nucleotide diversity within the North Atlantic.

In *A. coeruleus* a much lower, but still significant level of genetic structure (overall $\Phi_{ST} = 0.356$, $P < 0.001$) was observed between North and South Atlantic populations, suggesting limited dispersal across the Amazon barrier. Similarly to *A. bahianus*, no structure was observed within the North or South Atlantic, indicating an ability to disperse through oceanic distances that separate the Brazilian mainland and Ascension (*c*. 2261 km).

No significant differences were found among the *A. chirurgus* populations surveyed. Interestingly, very high haplotype diversity was observed in both Brazil and Panama ($h = 0.98–0.99$), higher than that observed for both *A. bahianus* ($h = 0.69–0.90$) and *A. coeruleus* ($h = 0.73–0.94$), which also supports our conclusion that Panama and Brazil are part of a large, panmictic population in the western Atlantic. In contrast, only one haplotype was observed in the five specimens of *A. chirurgus* from Ascension ($h = 0.00$). These genetic findings are especially interesting in light of a fish survey of Ascension, conducted almost two decades before our sampling at that island; Lubbock (1980) did not record *A. chirurgus* on this mid-Atlantic island. One of us (Robertson) found *A. chirurgus* present at Ascension in 1996 in relatively low numbers. The few individuals seen were mixed in with large schools of *A. coeruleus*. In those schools both species typically had a dark blue-grey colour pattern, which made it difficult to distinguish them. Perhaps *A. chirurgus* was present when Lubbock censused fishes, but eluded detection due to low abundance and difficulty in distinguishing them from *A. coeruleus*. Alternatively, it is possible that the population at Ascension is the product of a very recent colonization. These two explanations are not mutually exclusive, and either would be sufficient to explain the low haplotype diversity.

The populations sampled in the three species are separated by similar geographical distances but show different genetic structures with northern and southern populations of *A. bahianus* being highly structured, *A. coeruleus* moderately structured and *A. chirurgus* not structured. The general trend obtained in Mantel tests (strong, significant correlation between geographical distance and Φ_{ST} for *A. bahianus*, positive, marginally significant correlation for *A. coeruleus* and no correlation for *A. chirurgus*) strengthens the observation of contrasting genetic structure among the species surveyed. These results raise the following question: how could such contrasting phylogeographical patterns be present in three species that are very similar in general biology and distributed over approximately the same geographical area? Two classes of explanation may be invoked to address this question: pelagic larval duration and adult habitat preferences.

Pelagic larval stage

The majority of the fishes that inhabit coral reefs have a pelagic, dispersing larval stage, followed by a relatively sedentary adult life. Due to the sedentary nature of the adult stage, it is accepted that the pelagic stage is responsible for most patterns of geographical distribution of adult fish (Leis 1991; Bonhomme & Planes 2000). However, in one of the few genetic studies of an *Acanthurus* species, Planes *et al*. (1998) concluded that even species with a long pelagic larval stage, such as the widely distributed *A. triostegus*, may have limited gene flow at small scales, indicating that other environmental factors may influence species distributions.

The pelagic larval stage duration (PLD) of the species herein surveyed is very similar: *A. bahianus* with a mean of 52.3 days, ranging from 42 to 68 days, and a standard deviation of 4.1 days ($n = 244$); *A. chirurgus* with a mean of 55.2 days, ranging from 45 to 71 days, and a standard deviation of 4.4 days ($n = 603$) (M. Bergenius, personal communication); and *A. coeruleus* with a mean of 51.6 days, ranging from 46 to 57 days ($n = 9$) (B. Victor, personal communication). All three species settle at very similar mean standard lengths: *A. bahianus* 26.9 mm (range 23–33,

$n = 400$), *A. coeruleus* 26.7 mm (24–30, $n = 133$) and *A. chirurgus* 26.9 mm (23–32, $n = 400$) (Robertson 1992).

The relatively long pelagic larval stage of the three species explains the lack of structure between populations at Brazil and the central Atlantic islands. However, the genetic structure found in *A. bahianus* and *A. coeruleus* is between populations subject to currents that are capable of carrying larvae across the Amazon barrier in ~25–50 days (Muss *et al*. 2001), well within the PLD of both species. The oceanic barrier between Brazil and the central Atlantic islands has a straight-line distance about the same as that between Brazil and the Caribbean but the later is a more effective barrier for *A. bahianus* and *A. coeruleus*. The simplest explanation for this pattern is that low salinity conditions around the Amazon barrier are more effective at preventing larval transport than current patterns *per se*. Based on this biological and oceanographic information we reject the hypothesis that differences in pelagic dispersal are responsible for the observed patterns of mtDNA distribution.

Adult habitat preferences

Adult habitat preferences may contribute to the observed phylogeographical pattern, and depending on the nature of the barrier, these may be more influential than the ability to disperse across oceanic distances. Tringali *et al*. (1999) presented a compelling example of how habitat preferences influence the phylogeography of transisthmian sister species of snook (the teleost fish genus *Centropomus*): the sequence divergence between species pairs separated by the Panama isthmus varies according to their adult habitat preferences; species that prefer strictly marine habitat have higher divergence than those that also inhabit estuarine, low salinity waters. Prior to the final closure of the isthmus, estuarine waters likely provided the last link between the eastern Pacific and western Atlantic oceans, and may have functioned as a bridge for species that tolerate low-salinity.

The ecological barrier imposed by the freshwater outflow of the Amazon strongly affects the population structure of *A. bahianus*, moderately affects *A. coeruleus* and has no effect on *A. chirurgus*. Due to the similarities of larval stage duration and size at settlement observed among Atlantic surgeonfish, one would expect to find similar phylogeographical patterns. Differences in habitat preferences among larvae and adults may explain why that does not occur; larvae of *A. coeruleus* and *A. chirurgus* may have a higher tolerance to low salinity and high sedimentation than *A. bahianus*, and/or adults of the first two species may have a greater ability to live within the Amazon barrier. We cannot test the larval habitat hypothesis, but the data on differences in the habitat preferences of adult surgeonfishes provides a compelling explanation for the observed differences in the population structure among *Acanthurus* species.

Collette & Rützler (1977) collected several reef fish species under the Amazon freshwater plume and suggested that deep-water sponge bottoms (50–70 m) may function as a corridor between Brazil and the southern Caribbean. *A. coeruleus* was not collected by Collette & Rützler (1977), but was subsequently found in association with deep sponge bottoms (50–60 m) off northeast Brazil (Rocha *et al*. 2000), indicating that it has the potential to use this corridor. In contrast, *A. bahianus* has never been recorded at these depths, and is associated with shallow reefs (max 25 m) during its entire postlarval life (Nagelkerken *et al*. 2000).

Among the three *Acanthurus* species, *A. chirurgus* is the least specific with regards to habitat. Thresher (1980) states that it is more tolerant to sediment in the water than the other two surgeonfish species and Rosa *et al*. (1997) found it in tide pools off northeast Brazil, often close to low salinity waters, where the other two species were absent. Nagelkerken *et al*. (2000) reported that *A. chirurgus* uses mangrove, seagrass beds and shallow reefs as nurseries, while juvenile *A. bahianus* are almost entirely restricted to shallow reefs. *A. chirurgus* was the only surgeonfish collected by Collette & Rützler (1977) in deep waters (50–70 m) under the Amazon freshwater plume and by Uyeno *et al*. (1983) in trawls over soft bottoms off the coast of Suriname and French Guyana. The capacity of *A. chirurgus* to tolerate turbid water and use deep sponge bottoms apparently enables it to regularly disperse across the Amazon barrier, resulting in a panmictic population in the western Atlantic.

Conclusions

Several patterns are apparent from the comparative phylogeography of Atlantic surgeonfishes. First, these fish are characterized by clusters of closely related mtDNA haplotypes, high haplotype diversity, and low nucleotide diversity overall, a recurring pattern in marine fishes (Grant & Bowen 1998; Muss *et al*. 2001). Second, oceanic distances as long as 3500 km do not seem to be a major obstacle to larval dispersal in *Acanthurus* species. Trindade, Ascension, and St. Helena, located 1100, 2261, and 3540 from the Brazilian mainland, respectively, have low or no population separations from continental reefs, and haplotype distributions that are very similar to those in southeast Brazil. These findings are consistent with the extended pelagic larval stage (up to 70 days) in Atlantic surgeonfishes, which may translate into enhanced dispersal abilities. Third, all populations of *A. bahianus* and *A. coeruleus* separated by more than 2500 km have significantly different haplotype frequencies, with the exception of the marginally significant difference between Brazil and St. Helena (*c*. 3500 km apart) for *A. bahianus* (Table 2). These findings, combined with the absence of the

three species in the eastern Atlantic, provide an approximate yardstick for the limits of maintenance of homogenizing gene flow across open ocean distances in Atlantic surgeonfishes.

Finally, the genetic separations between Brazil and the Caribbean show a strong rank order agreement with the habitat specificity of adults of the three surgeonfishes, with a fixed genetic difference and evolutionary distinctions in the reef-associated *A. bahianus* ($d = 0.024$; $\Phi_{ST} = 0.805–0.836$), moderate population structure in the reef-and-sponge associate *A. coeruleus* ($\Phi_{ST} = 0.356$), and no significant differences in *A. chirurgus*, the surgeonfish that has been repeatedly collected under the Amazon plume. These findings prompt the conclusion that riverine outflows and associated soft-bottom zones are a major barrier to shallow reef obligates, but not to species with broader habitat preferences, and that river outflows have an added effect beyond those simply due to distance.

Recent studies, and the data gathered here, indicate that the barriers between Brazilian and Caribbean reef fauna are only rarely surmounted (Bowen *et al*. 2001; Colborn *et al*. 2001; Muss *et al*. 2001). Reef fauna in either province may be isolated for thousands to millions of years before stochastic events or oceanographic conditions bring them back into contact. In renewed sympatry, some reef organisms may retain genetic (and reproductive) integrity, while others will hybridize and coalesce into a single evolutionary entity. These processes may be a key to understanding evolutionary diversity in Atlantic reef species.

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Appendix I

Distribution of haplotypes among *Acanthurus* and sampling locations across the Atlantic. Abbreviations: GR, Grenada; VI, U.S. Virgin Islands; FL, Florida; PA, Panama; AS, Ascension; BR, Brazil; SH, Saint Helena

A. bahianus							*A. chirurgus*				*A. coeruleus*				
	Location							Location				Location			
Haplotype	GR	VI	FL	AS	BR	SH	Haplotype	BR	PA	SH	Haplotype	FL	GR	BR	AS
ABA1[a]	3	3	9				*ACH1*[e]	1			*ACC1*[g]	2	2	12	7
ABA2[b]	5		2				*ACH2*[f]	2	1		*ACC2*[h]	2	2	5	2
ABA3[c]				10	6	6	*ACH3*	1			*ACC3*			2	5
ABA4[d]				4	2	2	*ACH4*	1			*ACC4*	2	5		
ABA5				4	3	2	*ACH5*	2			*ACC5*	3	2		
ABA6				1	1		*ACH6*	2	2		*ACC6*	2	4		
ABA7				1			*ACH7*	1			*ACC7*				5
ABA8				1			*ACH8*	2	1		*ACC8*			3	
ABA9				1			*ACH9*	1			*ACC9*			1	
ABA10				1			*ACH10*	2	1	5	*ACC10*			2	
ABA11					2		*ACH11*	1	2		*ACC11*		1		
ABA12					3		*ACH12*	1			*ACC12*		1		
ABA13					4		*ACH13*	1			*ACC13*		2		
ABA14					1		*ACH14*	1			*ACC14*		1		
ABA15					1		*ACH15*	1			*ACC15*	1			
ABA16					1		*ACH16*	1			*ACC16*	2			
ABA17					1		*ACH17*	1			*ACC17*	1			
ABA18					1		*ACH18*	1			*ACC18*	1			
ABA19					1		*ACH19*	1			*ACC19*	1			
ABA20					9		*ACH20*		1		*ACC20*	1			
ABA21					1		*ACH21*		1						
ABA22	1						*ACH22*		1						
ABA23	1						*ACH23*		1						
ABA24	1						*ACH24*		1						
ABA25	1						*ACH25*		1						
ABA26	1						*ACH26*		1						
ABA27	1						*ACH27*		1						
ABA28	1						*ACH28*		1						
ABA29	1						*ACH29*		1						
ABA30	1						*ACH30*		2						
ABA31		1													
ABA32		1													
ABA33		1													
ABA34			1												
ABA35			1												
ABA36			1												
ABA37			1												
ABA38						1									
ABA39						1									
ABA40						1									
ABA41						1									

GenBank accession numbers as follows: [a]AY029306; [b]AY029307; [c]AY029308; [d]AY029309; [e]AY029304; [f]AY029305; [g]AY029310; [h]AY029311.