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## Phylogeography of two Atlantic squirrelfishes (Family Holocentridae): exploring links between pelagic larval duration and population connectivity

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**Abstract:** Genetic surveys of reef fishes have revealed high population connectivity within ocean basins, consistent with the assumption that pelagic larvae disperse long distances by oceanic currents. However, several recent studies have demonstrated that larval retention and self-recruitment may be higher than previously expected. To assess connectivity in tropical reef fishes, we contribute range-wide mtDNA surveys of two Atlantic squirrelfishes (family Holocentridae). The blackbar soldierfish, *Myripristis jacobus*, has a pelagic juvenile phase of about 58 days, compared to about 71 days (~22% longer) in the longjaw squirrelfish, *Holocentrus ascensionis*. If the pelagic duration is guiding dispersal ability, *M. jacobus* should have greater population genetic structure than *H. ascensionis*. In comparisons of mtDNA cytochrome *b* sequences from 69 *M. jacobus* (744 bp) and 101 *H. ascensionis* (769 bp), both species exhibited a large number of closely related haplotypes ( $h=0.781$  and  $0.974$ ,  $\pi=0.003$  and  $0.006$ , respectively),

indicating late Pleistocene coalescence of mtDNA lineages. Contrary to the prediction based on pelagic duration, *M. jacobus* has much less population structure ( $\phi_{ST}=0.008$ ,  $P=0.228$ ) than *H. ascensionis* ( $\phi_{ST}=0.091$ ,  $P<0.001$ ). Significant population partitions in *H. ascensionis* were observed between eastern, central and western Atlantic, and between Brazil and the Caribbean in the western Atlantic. These results, in combination with the findings from 13 codistributed species, indicate that pelagic larval duration is a poor predictor of population genetic structure in Atlantic reef fishes. A key to understanding this disparity may be the evolutionary depth among corresponding taxonomic groups of “reef fishes”, which extends back to the mid-Cretaceous and encompasses enormous diversity in ecology and life history. We should not expect a simple relationship between pelagic larval duration and genetic connectivity, among lineages that diverged 50–100 million years ago.

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### Introduction

Reef-resident fishes typically do not relocate over large distances as adults, so population connections may be accomplished primarily by dispersal of the pelagic larvae (Sale 1978; Leis 1991). Thus the “pelagic larval duration” (PLD, which here includes the duration of all pelagic larval and juvenile stages) is the primary vehicle of both local replenishment and long-distance dispersal. Roberts and Hawkins (1999) estimated that 76% of reef fishes have ranges exceeding 800,000 km<sup>2</sup>, yet less than 1% of that range is appropriate reef habitat. In these circumstances, dispersal across great distances is essential, yet self-recruitment is necessary to prevent extirpation of local populations, especially at remote oceanic islands (Robertson 2001; Irisson et al. 2004). An emphasis on dispersal would promote large populations and broad range: Bonhomme and Planes (2000) observed a positive correlation between PLD and the occurrence of reef fish species on isolated islands (see

also Brothers and Thresher 1985). In contrast, an emphasis on local larval retention is likely to promote speciation, but at the expense of dispersal and colonization: Hourigan and Reese (1987) observed that Hawaiian endemics are predominantly from fish families with relatively short larval periods. Each species of reef-associated fishes must strike a balance between local recruitment and long-distance dispersal, and this balance will be influenced by a variety of phylogenetic and ecological constraints.

A relationship between PLD and population genetic structure was initially predicted by Scheltema (1968) and Crisp (1978) but has proven difficult to characterize precisely (Shulman 1998). Riginos and Victor (2001) documented a rank order agreement between PLD and population structure in eastern Pacific blennies; two species with PLDs of 18–24 days exhibited significant population structure at distances of 100–350 km, while the species with a PLD of 50 days did not. Other recent studies have reported population structure at scales far smaller than the theoretical limit of larval dispersal (Rocha et al. 2005a; Taylor and Hellberg 2005). Population structure is high in the damselfishes with demersal eggs and short pelagic stages (PLDs, < 40 days; Doherty et al. 1995; Planes et al. 1998) but very low in two of three trumpetfishes (PLD = 71–116 days; Bowen et al. 2001). Hence a brief PLD seems to promote population structure in some cases, and a long PLD seems to diminish genetic structure in other cases, but the generality of this relationship is uncertain.

The squirrelfishes (family Holocentridae) are ubiquitous and abundant members of the coral reef fish fauna in all tropical seas (Wyatt 1983). The blackbar soldierfish *Myripristis jacobus* and the longjaw squirrelfish *Holocentrus ascensionis* are representatives of two subfamilies that diverged at least 50 MY BP (Greenfield 1974). Both species occur on shallow reefs of the Atlantic, residing beneath structures during the day and moving into adjacent foraging habitats at night (Thresher 1980; Randall 1996). Both species have pelagic egg and larval stages, followed by a spiny “rhynchichthys” pelagic stage that is unique to the family Holocentridae. The corresponding pelagic intervals are 40–58 and 43–56 days, for *M. jacobus* and *H. ascensionis*, respectively (Tyler et al. 1993). However, *Holocentrus* species can manifest an additional “meeki” stage after the rhynchichthys stage. The meeki is a large (47–60 mm) streamlined prejuvenile stage which is capable of both extended pelagic duration and rapid transformation into a benthic juvenile, and may last for a mean of 13 additional days after the approximate 48 days of egg-to-rhynchichthys pelagic existence. *Holocentrus* spp. in meeki form have been collected at substantially larger sizes than recruited juveniles, indicating accelerated growth and/or protracted pelagic duration (Tyler et al. 1993). The optional meeki stage allows a longer pelagic interval in *Holocentrus* species, believed to be at least 22% longer in *H. ascensionis* relative to *M. jacobus* (Tyler et al. 1993). Hence one question we address here

is whether this longer pelagic stage confers greater dispersal ability and higher gene flow among locations. We test this prediction with mitochondrial DNA (mtDNA) cytochrome *b* sequences and samples from biogeographic provinces across the ranges of both species. Beyond the immediate comparison of two Atlantic squirrelfishes, we assemble data from 13 co-distributed species, to assess the relationship between PLD and genetic connectivity on a range-wide scale.

Many reef fishes have little population genetic structure within biogeographic provinces such as the Caribbean Sea (Shulman and Bermingham 1995) and phylogenetic partitions appear on the broader scale of ocean basins (McMillan and Palumbi 1995; Bernardi et al. 2001; Planes and Fauvelot 2002; Rocha et al. 2002). Both *M. jacobus* and *H. ascensionis* occur in the four disjunct biogeographic provinces of the tropical Atlantic (Fig. 1): (1) the eastern tropical Atlantic; (2) the mid-Atlantic islands of St. Helena and Ascension; (3) the Brazilian coastline and associated islands; and (4) the greater Caribbean region (including Bermuda, the Gulf of Mexico, the West Indies and continental coastline from Florida to Venezuela; Briggs 1995). The eastern, central and western Atlantic provinces are separated by gaps of oceanic habitat ranging from 1,500 to 3,500 km, with the most isolated Atlantic reefs at Ascension and Saint Helena on the mid-Atlantic ridge (Edwards 1990). The Brazilian and Greater Caribbean provinces are separated by the Amazon-Orinoco outflows, which prevent the accumulation of reef-building corals along 2,300 km of coastline (Briggs 1974; Floeter and Gasparini 2000; Rocha 2003). These outflows of low-salinity turbid water may represent a substantial impediment to dispersal of reef organisms. However, this barrier does not affect all species uniformly. Rocha et al. (2002, 2005a) note a pattern of strong genetic isolation across the Amazon-Orinoco barrier in fishes (surgeonfishes genus *Acanthurus* and wrasse genus *Halichoeres*) that are strict shallow reef affiliates, and low-to-no isolation in congeners with broader habitat and depth preferences. Squirrelfishes are associated with reefs, but both *M. jacobus* and *H. ascensionis* have been captured in deep sponge beds underlying the Amazon outflow (Collette and Rützler 1977) and in trawls off the (soft-bottom) coasts of Suriname and Guiana (Uyeno et al. 1983). These species therefore represent appropriate subjects to test the prediction that broad habitat and depth preference may allow some reef-associated species to disperse underneath the Amazon-Orinoco barrier, sufficient to homogenize Greater Caribbean and Brazilian populations.

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## Materials and methods

We sampled *M. jacobus* and *H. ascensionis* using polespears and microspears during 1990–1997. In the Caribbean Sea, we collected *M. jacobus* from the Florida Keys and Grenada, and *H. ascensionis* from Bermuda,

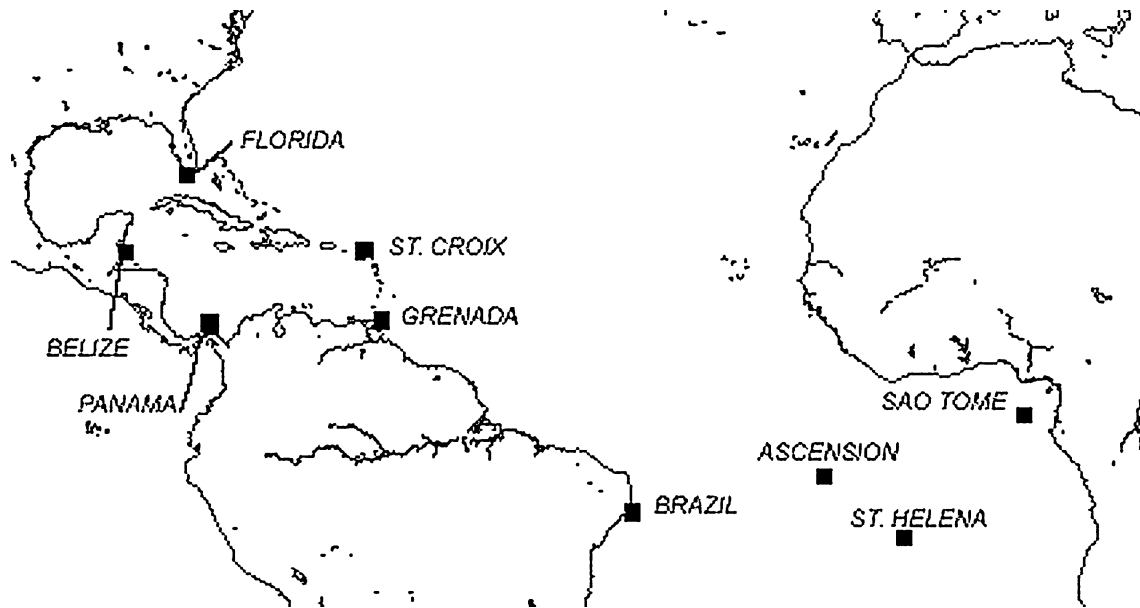


Fig. 1 Collection localities for the blackbar soldierfish *Myripristis jacobus* and the longjaw squirrelfish *Holocentrus ascensionis*. See Table 1 for details

Florida Keys, Panama and the Virgin Islands (Saint Croix). We obtained both species from Brazil (combined collections from João Pessoa and Cabo Frio, approximately 1,912 km apart), Ascension Island on the mid-Atlantic ridge, and from São Tomé in the Gulf of Guinea, East Atlantic (Fig. 1). Additional samples of *H. ascensionis* were collected at St. Helena on the mid-Atlantic ridge. Gill and/or muscle tissue was sampled and stored in a saturated-salt DMSO buffer (Amos and Hoelzel 1991).

Total genomic DNA was isolated via organic extraction (phenol:chloroform:isoamyl alcohol), precipitated with sodium acetate in a 95% ethanol solution, and resuspended in 100  $\mu$ l TE (10 mM Tris and 1 mM EDTA, pH 8.0). Using primers Cyb-09H (5'-GTGAC TTGAAAACCCAC CGTTG-3'; Song et al. 1998) and Cyb-07L (5'-AATAGGAAGTATCATTCGGGTTG ATG-3'; Taberlet et al. 1992), we amplified approximately 850 base pairs (bp) of the mitochondrial cytochrome *b* gene via the polymerase chain reaction (PCR; Saiki et al. 1985). The amplification reaction mix for both species contained 3.0 mM MgCl<sub>2</sub>, 20 nM of each primer, 17.5 nM of each dNTP, 0.40  $\mu$ l of *Taq* DNA polymerase (Promega Inc., Madison WI) and 5.0  $\mu$ l of 10 $\times$  PCR buffer (Promega, Inc., Madison WI) in 50  $\mu$ l total volume. PCR reactions utilized the following cycling parameters: initial 94 C denaturation and 72 C final extension (3 min each), with an intervening 25 cycles of 30 s at 94 C, 1 min at 52 C, and 1 min at 72 C. Excess primers were removed with either 30,000 MW Ultrafree-MC centrifugal filter units (Millipore Corp., Bedford MA) or by simultaneous incubation of PCR reaction with exonuclease I and shrimp alkaline phosphatase (USB Corp., Cleveland OH).

DNA sequencing reactions with fluorescently-labeled dideoxy terminators were conducted according to manufacturer's recommendations, and the labeled extension products were analyzed with ABI models 373A and 377 (PE Applied Biosystems, Foster City CA), at the University of Florida Sequencing Core. Our sequencing reactions contained Cyb-07L or Cyb-09H, or in some instances the internal primers Cyb-02H (5'-AAA CTGCAGCCCCTCAGAATGATATTTGTCCTCA-3'; Kocher et al. 1989) and Cyb-05L (5'-GCCAACGGCG CATCCTTCTTCTT-3'; Meyer 1993). Forward and reverse sequences were obtained in selected cases to assure the accuracy of nucleotide assignments. We aligned and edited resulting chromatograms using Sequencher ver. 3.0 (Gene Codes Corp., Ann Arbor MI). For clarity of discussion, we designated *M. jacobus* haplotypes with letter designations, and *H. ascensionis* haplotypes with numerals. Thus, "haplotype A" denotes the most common *M. jacobus* sequence and "haplotype 3" denotes the third most common *H. ascensionis* sequence.

Genetic variation is described with nucleotide diversity ( $\pi$ ; equation 10.5 in Nei 1987), and haplotype diversity ( $h$ ; equation 8.5 in Nei 1987). Genetic distances ( $d$  values) are calculated with the substitution model of Tamura and Nei (1993) using a 3:1 transition/transversion ratio. Genetic variance among sampling locations is described with  $\phi_{ST}$  values and an analysis of molecular variance (AMOVA; Excoffier et al. 1992). To determine if the relationships among haplotypes are consistent with large stable populations (Rogers and Harpending 1992; Rogers 1995) the distribution of pairwise differences are used to generate a mismatch distribution, with a raggedness index ( $r$ , Harpending 1994) calculated from the sum of squared differences in frequency at each mis-

match class. The observed distribution is compared to an expected distribution of pairwise differences using population expansion parameters estimated by a least squares nonlinear regression. These calculations were performed using Arlequin ver. 2.0 (Schneider et al. 2000). If the cytochrome *b* locus examined here is both neutral and has been transmitted under equilibrium conditions, then a multimodal distribution of haplotypes should result. Alternately a unimodal distribution (i.e., a large number of closely related haplotypes) could indicate non-equilibrium conditions, especially population expansion. Fu's  $F_S$  test was used to detect an excess of low-frequency mutations, arising from either selection or rapid population growth (Fu 1997). These calculations were performed using Arlequin ver. 2.0 (Schneider et al. 2000).

Coalescence analyses require estimates of generation time and molecular clock rates. The mutation rates for cytochrome *b* in fishes has been estimated at 2%/MY (Bowen et al. 2001), near the benchmarks for protein coding regions in vertebrates (1–3%/MY; see Bermingham et al. 1997). We provisionally apply this benchmark to estimate divergence dates in the evolutionary history of squirrelfishes, with the recognition that such dates must be interpreted with caution. Generation time in squirrelfishes is unknown, but these species can live at least a decade in captivity. As a first approximation, we chose three years as the average generation time for both species.

Networks of haplotype relationships were produced with TCS version 1.13 (Clement et al. 2000). PAUP\* ver. 4.0 $\beta$ 5 (Swofford 1999) was used to construct a neighbor-joining (NJ) tree of haplotypes for each species using Tamura and Nei (1993) distances with a gamma correction of 0.50 (Yang 1996). Additional cytochrome *b* sequences were available from GenBank, including representatives from the soldierfish subfamily (Myripristinae) and squirrelfish subfamily (Holocentrinae). *Myripristis berndti* and *M. violacea* were available to represent the Myripristinae, as defined by Randall and Greenfield (1996). Nine species from the two Holocentrinae genera, *Sargocentron* (*S. diadema*, *S. microstoma*,

*S. punctatissimum*, *S. spiniferum*, *S. tiere* and *S. xantherythrum*) and *Neoniphon* (*N. argenteus*, *N. aurolineatus* and *N. sammara*), were available to represent Holocentrinae. All those accessions (GenBank U57525–U57535) were submitted by Toller et al. (1996).

The influence of PLDs on the range-wide population structure of various Atlantic reef fishes was tested using a simple linear regression analysis. Two comparisons were conducted; all available species ( $n=15$ ) and only those species without cryptic evolutionary partitions ( $n=7$ ), a surprisingly common outcome in phylogeographic surveys of the Atlantic reef fishes. A  $t$  test with  $n-2$  degrees of freedom was used to determine significance (Sokal and Rohlf 1981). All the sequences produced in this study are available at Genbank accession numbers DQ379998–DQ380084.

## Results

### *Myripristis jacobus*

We surveyed 69 blackbar soldierfish specimens from five localities (Table 1; Appendix 1), using 744 bp of cytochrome *b*. The 37 variable sites defined 28 haplotypes (overall  $h=0.781$ ;  $\pi=0.003$ ; Table 2). Close relationships among haplotypes (mean  $d=0.005$ ; range 0.001–0.014) indicate a relatively recent (late Pleistocene) coalescence. The observed mismatch distribution is unimodal (raggedness index  $r=0.014$ ,  $P=0.98$ ). This analysis indicates a population expansion in the Atlantic basin at approximately 175,000 years BP, with an initial (female) effective population size of  $N_{f0}=112$  (Table 3).

The dataset was dominated by a single common haplotype (A; frequency = 0.463), which was observed in all four biogeographic provinces (Appendix 1). Statistical parsimony (TCS analysis) indicates haplotype A as the ancestral haplotype (Fig. 2a). The next most abundant haplotype (B; frequency = 0.072) was observed in both the Brazilian and Caribbean provinces, with each of the remaining 28 haplotypes restricted to single biogeographic provinces (if we treat the eastern Atlantic

**Table 1** Genetic variation in the blackbar soldierfish *Myripristis jacobus* and the squirrelfish *Holocentrus ascensionis*

| Locality   | <i>Myripristis jacobus</i> |          |               |               | <i>Holocentrus ascensionis</i> |          |               |               |
|------------|----------------------------|----------|---------------|---------------|--------------------------------|----------|---------------|---------------|
|            | <i>N</i>                   | <i>n</i> | <i>h</i>      | $\pi$         | <i>N</i>                       | <i>n</i> | <i>h</i>      | $\pi$         |
| Florida    | 10                         | 9        | 0.978 ± 0.054 | 0.004 ± 0.002 | 7                              | 7        | 1.000 ± 0.076 | 0.005 ± 0.003 |
| St. Croix  | –                          | –        | –             | –             | 6                              | 6        | 1.000 ± 0.096 | 0.005 ± 0.004 |
| Belize     | –                          | –        | –             | –             | 8                              | 8        | 1.000 ± 0.063 | 0.006 ± 0.004 |
| Panama     | –                          | –        | –             | –             | 6                              | 6        | 1.000 ± 0.096 | 0.007 ± 0.005 |
| Grenada    | 16                         | 8        | 0.892 ± 0.048 | 0.004 ± 0.002 | –                              | –        | –             | –             |
| Brazil     | 10                         | 3        | 0.378 ± 0.181 | 0.001 ± 0.001 | 20                             | 17       | 0.979 ± 0.024 | 0.006 ± 0.003 |
| Ascension  | 13                         | 4        | 0.615 ± 0.136 | 0.002 ± 0.002 | 20                             | 14       | 0.947 ± 0.034 | 0.005 ± 0.003 |
| St. Helena | –                          | –        | –             | –             | 17                             | 12       | 0.956 ± 0.033 | 0.006 ± 0.003 |
| São Tomé   | 20                         | 10       | 0.758 ± 0.101 | 0.003 ± 0.002 | 17                             | 9        | 0.868 ± 0.068 | 0.006 ± 0.003 |
| Total      | 69                         | 28       | 0.781 ± 0.053 | 0.003 ± 0.002 | 101                            | 59       | 0.974 ± 0.008 | 0.006 ± 0.003 |

The number of specimens (*N*), the number of haplotypes observed (*n*), the haplotype diversity (*h*) and nucleotide diversity ( $\pi$ ) for each locality and in total are listed. A n-dash (–) indicates that a species was not collected from the locality

**Table 2** *Myripristis jacobus* population pairwise  $\phi_{ST}$  values (below diagonal) and associated  $P$  values (above diagonal)

|           | Florida | Grenada | Brazil | Ascension | São Tomé |
|-----------|---------|---------|--------|-----------|----------|
| Florida   |         | 0.358   | 0.753  | 0.137     | 0.302    |
| Grenada   | 0.004   |         | 0.760  | 0.118     | 0.402    |
| Brazil    | -0.015  | -0.026  |        | 0.280     | 0.966    |
| Ascension | 0.037   | 0.037   | 0.024  |           | 0.134    |
| São Tomé  | 0.008   | 0.000   | -0.042 | 0.030     |          |

locations as a single biogeographic province). The genetic variation in this species is illustrated by a parsimony network (Fig. 2a). The AMOVA revealed no significant population structure for blackbar soldierfish (overall  $\phi_{ST}=0.008$ ;  $P=0.252$ ), and no pairs of populations were significantly different (range  $\phi_{ST}=-0.042$  to 0.037; Table 2). Additional AMOVA's considering other possible combinations of localities resulted in similar or lower  $\phi_{ST}$  values (e.g., when St. Helena and Ascension samples were combined).

The *Myripristis* species available here and in GenBank were compared in a NJ tree based on 307 bp of aligned sequence (Fig. 3). The three species were separated by  $d=0.133-0.161$ . Hence the divergences among species were an order of magnitude greater than the diversity within *M. jacobus*. Overall, *M. jacobus* samples exhibited high haplotype diversity coupled with low intraspecific divergence. Common haplotypes are shared extensively across biogeographic provinces, especially between the West Atlantic provinces.

### *Holocentrus ascensionis*

Our analysis of 101 longjaw squirrelfish from eight localities revealed 65 variable sites in 769 bp of cytochrome *b*. A total of 59 haplotypes were observed, yielding high haplotype diversities ( $h=0.868-1.000$ ; overall  $h=0.974$ ; Table 1, Appendix 2). Like *Myripristis jacobus*, most *H. ascensionis* haplotypes differed by only a single transition mutation (Fig. 2b), yielding similar estimates of nucleotide diversity (overall  $\pi=0.006$ ) and sequence divergence (mean  $d=0.005$ ; range 0.001–0.026). The maximum sequence divergence ( $d=0.026$ ; between haplotypes from Panamá and São Tomé) was larger than that observed in *M. jacobus* ( $d=0.014$ ), but mtDNA lineages within both species coalesce to a common ancestor in the late Pleistocene (Table 3). As in the blackbar soldierfish, the close relationship among longjaw squirrelfish haplotypes created a mismatch distribution ( $\tau=4.508$ ) with a (marginally) unimodal distribution ( $r=0.029$ ,  $P=0.052$ ). This analysis indicates a population expansion at approximately 293,000 years BP, with an initial (female) effective population size of about  $N_{f0} = 455$  (Table 3).

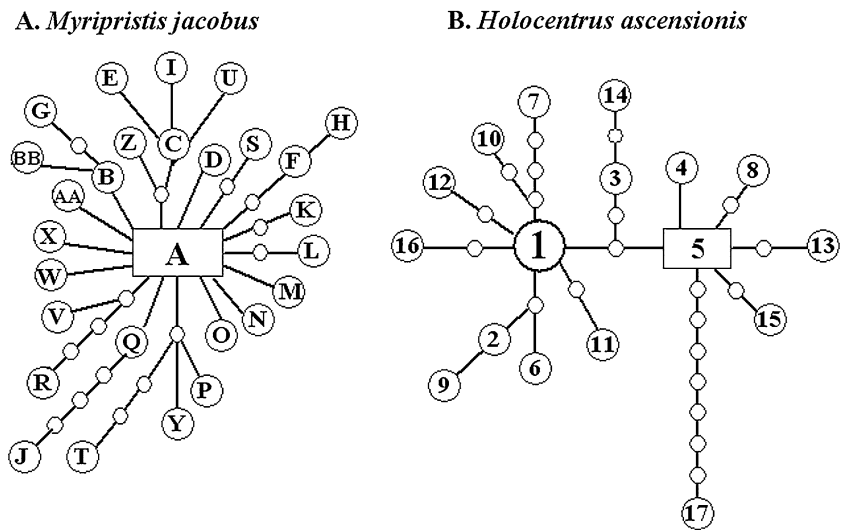
In contrast to *M. jacobus*, no single *H. ascensionis* haplotype dominated in sample collections. Seven haplotypes (1, 2, 3, 5, 6, 11 and 12; combined total

**Table 3** Fu's  $F_s$  test (with  $P$  value) for selection or population expansion (Fu 1997), and mismatch distribution parameters for the blackbar soldierfish (*Myripristis jacobus*) and longjaw squirrelfish (*Holocentrus ascensionis*)

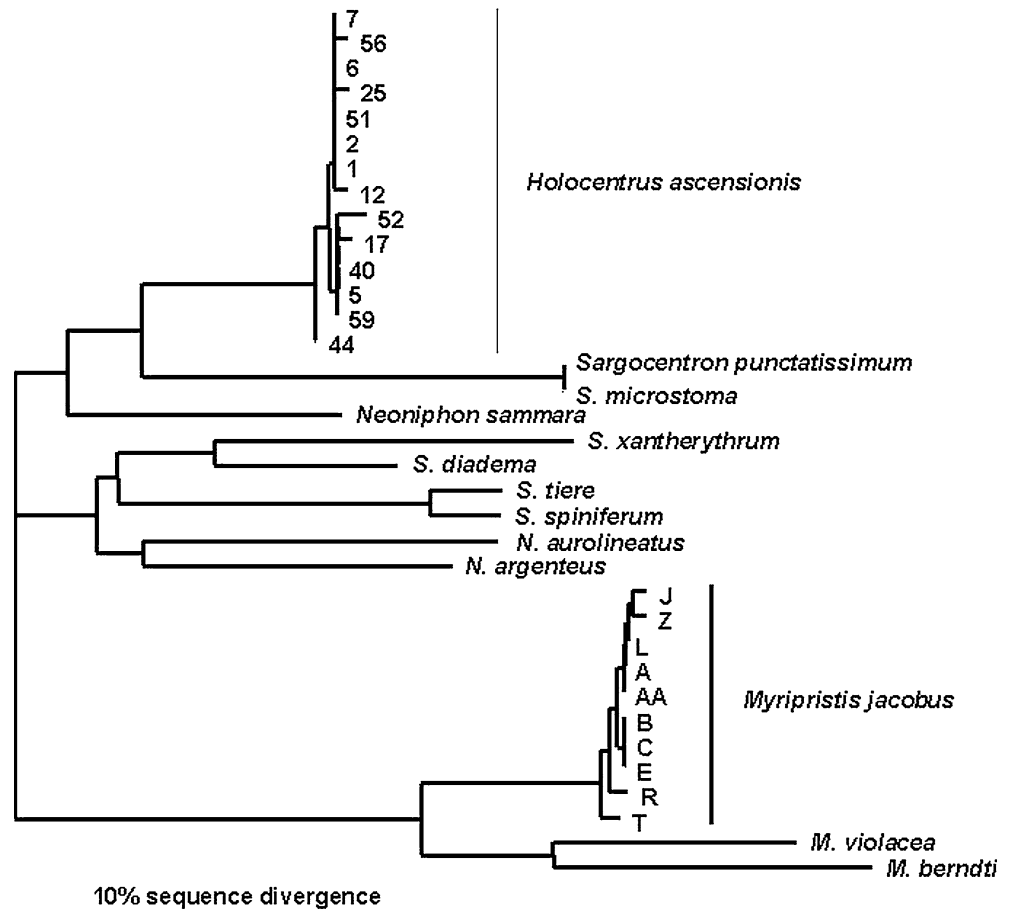
| Species               | $F_s$ test                 | $r$                      | $\tau$                 | Age                          | $\theta_0$             | $N_{f0}$          | $\theta_1$                    | $N_{f1}$                          |
|-----------------------|----------------------------|--------------------------|------------------------|------------------------------|------------------------|-------------------|-------------------------------|-----------------------------------|
| <i>M. jacobus</i>     | -13.108<br>( $P < 0.001$ ) | 0.014<br>( $P = 0.98$ )  | 2.606<br>(0.402–6.518) | 175,000<br>(27,000–438,000)  | 0.005<br>(0.000–1.710) | 112<br>(0–38,000) | 4.668<br>(0.583–5.380)        | 105,000<br>(13,000–128,000,000)   |
| <i>H. ascensionis</i> | < -100<br>( $P < 0.002$ )  | 0.029<br>( $P = 0.052$ ) | 4.508<br>(2.513–5.890) | 293,000<br>(163,000–382,000) | 0.021<br>(0.000–2.162) | 455<br>(0–47,000) | 116.592<br>(20.231–8.762.842) | 2,523,000<br>(44,000–190,000,000) |

In the mismatch analysis,  $r$  is the raggedness index (with  $P$  value),  $\tau$  is the mutational timescale, Age is measured in years (with 90% confidence intervals),  $\theta_0$  and  $\theta_1$  are the average pairwise mutational distances before and after population expansion (with 90% confidence intervals), and  $N_{f0}$  and  $N_{f1}$  are the female effective population size estimates (with 90% confidence intervals) before and after population expansion. See Materials and methods for details

**Fig. 2** Parsimony networks for (A) *Myripristis jacobus* and (B) *Holocentrus ascensionis*. Haplotype A in *M. jacobus* was observed in all biogeographic provinces. Due to the complexity of the *H. ascensionis* network (including 59 haplotypes), only the 17 haplotypes that were detected in more than one individual are included here. Most of the remaining 42 haplotypes differ from these common types by single mutations



**Fig. 3** Neighbor-joining tree of holocentrid haplotypes, using Tamura-Nei distances after gamma correction ( $\alpha = 0.50$ ). Intraspecific trees for *M. jacobus* and *H. ascensionis* include representative haplotypes



frequency=0.347) were found in multiple biogeographic provinces. The most common, haplotype 1, was observed in one individual each from Florida, St. Croix and Panamá (Caribbean); two individuals from Brazil; four individuals from Ascension and three from St. Helena (frequency=0.119; Appendix 2). The three most common *H. ascensionis* haplotypes were absent

from the East Atlantic, while the most common São Tomé haplotype (4) was not detected outside of the East Atlantic. Haplotype 5 was the only one shared between the eastern Atlantic and other biogeographic province (one specimen each from Ascension and Brazil), indicating less trans-oceanic dispersal than in *M. jacobus*.

**Table 4** *Holocentrus ascensionis* population pairwise  $\phi_{ST}$  values (below diagonal) and associated  $P$ -values (above diagonal)

|            | Florida | St. Croix | Belize | Panama | Brazil | Ascension | St. Helena | São Tomé |
|------------|---------|-----------|--------|--------|--------|-----------|------------|----------|
| Florida    |         | 0.150     | 0.681  | 0.789  | <0.001 | 0.156     | 0.003      | 0.003    |
| St. Croix  | 0.071   |           | 0.136  | 0.583  | 0.422  | 0.133     | 0.139      | 0.087    |
| Belize     | -0.029  | 0.057     |        | 0.696  | <0.001 | 0.021     | 0.025      | 0.002    |
| Panama     | -0.046  | -0.026    | -0.035 |        | 0.095  | 0.354     | 0.451      | 0.033    |
| Brazil     | 0.189   | -0.002    | 0.141  | 0.056  |        | 0.046     | 0.165      | 0.008    |
| Ascension  | 0.109   | 0.054     | 0.097  | 0.006  | 0.036  |           | 0.563      | <0.001   |
| St. Helena | 0.153   | 0.057     | 0.103  | -0.007 | 0.019  | -0.010    |            | <0.001   |
| São Tomé   | 0.223   | 0.073     | 0.212  | 0.146  | 0.080  | 0.150     | 0.143      |          |

Population genetic structure for longjaw squirrelfish was low but significant, as indicated by an overall  $\phi_{ST}=0.091$  ( $P<0.001$ ). No significant differences were found in pairwise comparisons among Caribbean locations (Belize, Florida Keys, Panama, or Saint Croix; Table 4). Shulman and Bermingham (1995) compared mtDNA sequences of *H. ascensionis* between six locations in the Caribbean and obtained low population differentiation between locations and low genetic diversity estimates ( $h=0.94$ ,  $\pi=0.004$ ) similar to those obtained when the four Caribbean localities surveyed here are combined ( $h=0.989$ ,  $\pi=0.006$ ). When we repeated the AMOVA considering only five populations (Brazil, Ascension, St. Helena, São Tomé, and a combined Caribbean), we found the same magnitude of population structure ( $\phi_{ST}=0.094$ ,  $P<0.001$ ) as found when all localities were considered separately ( $\phi_{ST}=0.091$ ). Because Briggs (1995) included both St. Helena and Ascension within a single mid-Atlantic biogeographic province, we conducted another AMOVA considering these as a single population and found a slightly larger  $\phi_{ST}$  value ( $\phi_{ST}=0.104$ ,  $P<0.001$ ), essentially supporting the proposed classification. Most of the population structure was explained by separations between the western, central, and (especially) eastern Atlantic (Table 4).

Most of the 59 haplotypes were a single transition mutation removed from a haplotype in a different biogeographic province or region (Fig. 2b). We compared the haplotypes of *H. ascensionis* to those of other holocentrines utilizing 307 bp of cytochrome *b* (Fig. 3). The genera were separated by  $d=0.126$ – $0.251$ , with the lowest interspecific divergence between (*Sargocentron microstoma* + *S. punctatissimum*) and *H. ascensionis*. This contrasts with a mean intraspecific divergence of  $d=0.006$ . The gene tree of *H. ascensionis* haplotypes was an unresolved polytomy (after 500 bootstraps) of over 50 branches, and for that reason only a subset of 17 haplotypes (those that occurred in more than one individual) is depicted Fig. 2a, and representative haplotypes are included in the phylogeny (Fig. 3).

The regression analyses of PLDs versus population structure in 15 Atlantic reef species (Table 5) yielded non-significant negative linear relationships with corresponding  $r$  values explaining less than 20% of the variation (all species,  $r=0.4039$ ,  $t=0.643$ ,  $P>0.25$ ; subset of

species without evolutionary partitions,  $r=0.1233$ ,  $t=0.277$ ,  $P>0.25$ ).

## Discussion

The range-wide survey of two Atlantic squirrelfishes reveals shallow gene genealogies, with low but statistically significant population structure in the longjaw squirrelfish (*H. ascensionis*), and no structure in the blackbar soldierfish (*M. jacobus*). The latter case indicates exceptionally high connectivity between widely separated locations, defining one end of the spectrum for mtDNA surveys of Atlantic reef fishes.

Prior to dissecting the intraspecific results, we address three caveats:

1. The differences in PLD between longjaw squirrelfish and blackbar soldierfish (mean duration 58 vs. 71 days) are modest and may not be sufficient to produce a corresponding difference in genetic connectivity. The strength of our conclusions lies not in the 22% difference in PLD, but in the dramatic difference in AMOVA results for these two species. Contrary to expectations, the species with the longer PLD had an order of magnitude greater population differentiation ( $\phi_{ST}=0.091$  vs.  $\phi_{ST}=0.008$ ). Indeed we can relegate the difference in PLD to sampling artifact, and the central conclusion remains: these two squirrelfishes have different population genetic structures, indicating that PLD is a poor predictor of dispersal.
2. The mutation rate and generation time used to estimate population parameters are uncalibrated approximations. In these circumstances, the corresponding estimates of coalescence time ( $t$ ) and population size ( $N_e$ ) should be regarded as provisional. However, any of the conventional mutation rates for cytochrome *b* (1–3%/MY between lineages) would yield the same qualitative conclusion: population expansions in the late Pleistocene for both species. We feel that the conclusions on this timescale are robust.
3. Estimates of coalescence time and effective population size are based on a model of recent population expansion, with a unimodal (Poisson) distribution of mismatches. The results of Fu's  $F$  test unequivocally

**Table 5** Comparison of pelagic larval duration and population structure in Atlantic reef fishes

| Species common name and scientific name             | Mean pelagic duration (days) | Population structure ( $\phi_{ST}$ ) | Citations for pelagic duration and citation for population structure   |
|---|------------------------------|--------------------------------------|--|
| Slippery dick <i>Halichoeres bivittatus</i>         | 24                           | 0.77*                                | Sponaugle and Cowen (1997), Rocha et al. (2005a)                       |
| Black-ear wrasse <i>Halichoeres poey</i>            | 25                           | 0.23                                 | Sponaugle and Cowen (1997), Rocha et al. (2005a)                       |
| Pudding wife <i>Halichoeres radiatus</i>            | 26                           | 0.83*                                | Sponaugle and Cowen (1997), Rocha et al. (2005a)                       |
| Clown wrasse <i>Halichoeres maculipinna</i>         | 29                           | 0.88*                                | Sponaugle and Cowen (1997), Rocha et al. (2005a)                       |
| Pygmy angelfish <i>Centropyge</i> spp.              | 33                           | 0.62*                                | Thresher and Brothers (1985), Bowen et al. (2006)                      |
| Redlip blenny <i>Ophioblennius atlanticus</i>       | 38                           | 0.93*                                | D. Wilson, personal communication, Muss et al. (2001)                  |
| Soapfish <i>Rypiticus saponaceus</i>                | 40                           | 0.87*                                | Lindeman et al. (2000), Carlin et al. (2003)                           |
| Rock hind <i>Epinephelus adscensionis</i>           | 40                           | 0.93*                                | Lindeman et al. (2000), Carlin et al. (2003)                           |
| Ocean surgeonfish <i>Acanthurus bahianus</i>        | 52                           | 0.72*                                | M. Bergenius, personal communication, Rocha et al. (2002)              |
| Blue tang <i>Acanthurus coeruleus</i>               | 52                           | 0.36                                 | B. Victor, personal communication, Rocha et al. (2002)                 |
| Doctofish <i>Acanthurus chirurgus</i>               | 55                           | 0.02 NS                              | Bergenius et al. (2002), Rocha et al. (2002)                           |
| Blackjaw squirrelfish <i>Myripristis jacobus</i>    | 58                           | 0.01 NS                              | Tyler et al. (1993), Present study                                     |
| Longjaw squirrelfish <i>Holocentrus ascensionis</i> | 71                           | 0.09                                 | Tyler et al. (1993), Present study                                     |
| Goldspot goby <i>Gnatholepis thompsoni</i>          | 89                           | 0.47                                 | Sponaugle and Cowen (1994), Rocha et al. (2005b)                       |
| Trumpetfish <i>Aulostomus strigosus</i>             | 93                           | 0.59                                 | H. Fricke and P. Heemstra, personal communication, Bowen et al. (2001) |

All estimates of population structure are based on mtDNA cytochrome *b* sequences except for the pygmy angelfish estimate, which is based on mtDNA control region sequences. The pelagic duration for trumpetfish, rock hind, soapfish, and pygmy angelfish are estimates based on other members of the genus or family. Asterisk (\*) indicates species with suspected evolutionary partitions within the Atlantic range

support a model of selective sweep or recent population expansion (Table 3). The observed mismatch distribution strongly supports this assumption for the blackbar squirrelfish (raggedness index  $r=0.014$ ,  $P=0.98$ ), but the corresponding distribution in longjaw squirrelfish verges on significantly different from unimodal ( $r=0.029$ ,  $P=0.052$ ). It is not clear how sensitive these analyses are to violations of the unimodal distribution, and so corresponding conclusions should be interpreted with appropriate caution.

With these cautions in mind, we examine the topics of genetic architecture, population structure, phylogeography, and pelagic dispersal.

### Genetic architecture

The blackbar soldierfish and the longjaw squirrelfish are characterized by a large number of closely related haplotypes, and sharing of haplotypes among biogeographic provinces. Shallow mtDNA genealogies, characterized by low nucleotide ( $\pi$ ) and high haplotype ( $h$ ) diversities, are a recurring feature of marine fishes (Grant and Bowen 1998). The squirrelfish data are consistent with this pattern: values of  $\pi$  (0.003 and 0.006 for *M. jacobus* and *H. ascensionis*, respectively) and  $h$  (0.781 and 0.974, respectively) are similar to those reported in other reef fishes ( $\pi=0.001-0.013$ ,  $h=0.417-1.000$ ; Muss et al. 2001; Rocha et al. 2002; Carlin et al. 2003). Notably, we see no evidence of an island effect (reduced genetic diversity resulting from isolation and small population size) at the isolated locations of Ascension and St. Helena on the mid-Atlantic ridge (Table 1).

The pattern observed here could be generated by (1) high variance in reproductive success, or (2) population fluctuations, especially those linked to sea level change (Grant and Bowen 1998; Skibinski 2000). Several studies have supported the argument that shallow genealogies in marine organisms are due to strong genetic drift as a result of high variance in reproductive success (Avise et al. 1984; Hedgecock 1994; Planes and Lenfant 2002; Lecomte et al. 2004). In this “sweepstakes recruitment” (Hedgecock et al. 1982), a tiny fraction of propagules attain sexual maturity even under relatively stable conditions. Strong genetic drift, resulting in shallow mtDNA genealogies, would be an expected consequence of this low survivorship.

A shallow mtDNA genealogy could also be due to historical population crashes. Coral reef habitats have experienced massive fluctuations in distribution and quality due to glacial influences on sea level, especially on the shallow Caribbean plate (Daly 1915; Veron 1995; Shulman 1998). Reef habitats may have been reduced by 90% in the Caribbean during the last glacial maximum (20,000 years ago), when the sea level was at least 100 m below current levels (Bellwood and Wainwright 2002). Corresponding disruptions of oceanographic processes have been demonstrated in both the Pacific and Atlantic



Basins (Berggren and Hollister 1974; Lehman and Kiegwin 1992; Stenni et al. 2001). Thus the population expansions by Atlantic squirrelfishes (as indicated by mismatch distributions) probably occurred during interglacial periods, with rising sea level and corresponding increase in habitat availability. While we cannot link the most recent expansion in *M. jacobus* (approximately 175,000 years BP) with that in *H. ascensionis* (approximately 293,000 years BP), it is notable that both expansions occurred in the late Pleistocene, in two species that have probably occupied the Atlantic basin for a much longer interval (see below).

Both glacial era bottlenecks and sweepstakes recruitment are feasible explanations for the observed patterns of genetic diversity. These mechanisms are not mutually exclusive and may work in concert to produce the observed haplotype diversities and shallow genealogies in Atlantic squirrelfishes.

In principle, the shallow gene genealogies could also be due to a recent colonization of the Atlantic. Late Pleistocene colonizations from the Indian Ocean to the Atlantic have been demonstrated for pygmy angelfishes (genus *Centropyge*; Bowen et al. 2006), and goldspot goby (genus *Gnatholepis*; Rocha et al. 2005b). However, this explanation is unlikely for *H. ascensionis* because the genus *Holocentrus* is endemic to the Atlantic, indicating a long evolutionary history in this region. In contrast, *M. jacobus* is the sole Atlantic member of a genus that is widespread and diverse in the Indian-Pacific (Greenfield 1968; Randall and Greenfield 1996). This could be construed as evidence of recent colonization into the Atlantic, but *M. jacobus* is notably distinct from Pacific congeners in terms of morphology (Greenfield 1974), to the extent that Woods (1953) suggested a subgenus distinction for this species. Hence *M. jacobus* is unlikely to be a recent arrival. Consequently, population processes within the Atlantic must be invoked to explain the shallow genetic architecture in both species.

## Population structure

The overall population structure for both holocentrids was less than observed in other Atlantic reef fishes with similar PLDs. These include two of three surveyed Atlantic surgeonfishes (*Acanthurus bahianus*  $\phi_{ST}=0.724$ , *A. coeruleus*  $\phi_{ST}=0.356$ , although *A. chirurgus*  $\phi_{ST}=0.02$  N.S.), with PLDs of 45–70 days (Bergenius et al. 2002, and B. Victor, personal communication in Rocha et al. 2002). Even more extensive genetic differences are observed in the redlip blenny *Ophioblennius atlanticus* (Atlantic pairwise  $\phi_{ST}=0.134$ –0.951) despite a PLD of 40–50 days; D. Wilson, personal communication in Muss et al. 2001). Overall, the squirrelfishes have some of the lowest  $\phi_{ST}$  values in surveys of Atlantic reef fishes (Table 5).

In our comparison of longjaw squirrelfish and blackbar soldierfish, the holocentrid with a longer

pelagic stage (*H. ascensionis*) had significant population structure, while the species with a shorter pelagic duration (*M. jacobus*) did not. What might account for these counterintuitive differences? Perhaps the blackbar soldierfish has greater dispersive ability than indicated by previous research. Leis and Carson-Ewart (1997) captured pelagic juveniles of *Myripristis* spp. from the West Pacific up to 66 mm SL in size, compared to the seven *M. jacobus* rhychichthys (41–49 mm) captured by Tyler et al. (1993). Hence it is possible that blackbar squirrelfish have a longer dispersive stage than indicated by the modest sample in Tyler et al. (1993). However, differences in intraspecific genetic divergences among surgeonfishes, blenny, and squirrelfish (species with similar PLDs demonstrating considerable variation in population structure), indicate that factors other than PLD are strongly influencing larval dispersal and genetic connectivity. Larval behavior, rather than larval duration, may be the key to understanding connectivity in reef fishes.

## Phylogeography

The Caribbean and Brazilian reefs are separated by the freshwater outflows of the Amazon and Orinoco Rivers, and associated soft bottom habitats (Floeter and Gasparini 2000; Rocha 2003). The pairwise  $\phi_{ST}$  values reported here indicate that the Amazon barrier is less permeable to *H. ascensionis* than to *M. jacobus* (Tables 2, 4). However, only two of the four comparisons between Caribbean and Brazilian *H. ascensionis* were significant, indicating some low level of exchange between these biogeographic provinces.

Habitat preferences of demersal (post-pelagic) stages might explain some of the differences in connectivity across the Amazon barrier. Rocha et al. (2002) demonstrated that genetic partitions in Atlantic surgeonfishes were strongly correlated with adult habitat specificity. The reef specialist *Acanthurus bahianus* has a deep evolutionary separation (sequence divergence  $d=3.2\%$  in cytochrome *b*) across the Amazon barrier, while the habitat generalist *A. chirurgus* has no significant population structure across the same barrier. A similar pattern is observed in wrasses (genus *Haliichoeres*), another ubiquitous group of reef fishes. In the western Atlantic, the wrasse with the most specialized jaw morphology for reef feeding (*H. maculipinna*) has an ancient evolutionary separation across the Amazon barrier ( $d=6.5\%$  in cytochrome *b*), while the habitat generalists (*H. poeyi* and *H. bivittatus*) have only population-level separations across the same barrier ( $\phi_{ST}=0.25$ –0.59; Rocha 2004; Rocha et al. 2005a).

Both *M. jacobus* and *H. ascensionis* have been observed in sponge communities below the Amazonian freshwater plume (Collette and Rützler 1977), as well as soft-bottom between the mouths of the Amazon and Orinoco rivers (Uyeno et al. 1983). During a hyposaline fish-kill in the southeastern Caribbean, the list of mor-

talities included 15 fish families, but not Holocentridae (Siung-Chang and Lum-Kong 2001). Squirrelfishes appear to tolerate turbid, low salinity waters as well as soft-bottom habitats. Hence these species fit the prediction of Rocha et al. (2002), that reef denizens with broad habitat preferences can maintain population connectivity across the Amazon barrier.

The other major biogeographic barrier is the oceanic gap between eastern, central, and western Atlantic reefs. Chesher (1966) estimated that water masses could move from north-eastern Brazil to Africa in 43–70 days, within the PLD of both squirrelfish species. Yet the pairwise  $\phi_{ST}$  values reported here (Tables 2, 4) indicate that oceanic barriers separating the eastern and western Atlantic are much less permeable to *H. ascensionis* than to *M. jacobus*.

Like the soldierfish examined here, the soapfish *Rypticus saponaceus* can readily cross the Amazon barrier, as indicated by mtDNA sequence comparisons, probably due to tolerance of low salinity. However, *R. saponaceus* exhibits a deep genetic separation across the oceanic barrier of the mid-Atlantic (Carlin et al. 2003). Thus a barrier such as the Amazon, that is effective in one reef fish species may be transparent to another species with different habitat preferences and ecology (including salinity/temperature tolerances or larval biology).

Another relevant factor may be feeding strategies in reef residents. Robertson et al. (2004) note that pelagic-feeding reef fishes tend to have broad distributions across the central and eastern Pacific, indicating greater dispersal ability. The pelagic-feeding *M. jacobus* has no population structure, whereas the benthic-feeding *H. ascensionis* has significant population structure, consistent with the dispersal trend noted by Robertson and colleagues.

What then are the relative influences of larval biology, adult ecology, and habitat preference on dispersal and isolation of these and other reef fish species? The answer is likely specific to the organism. For instance, the holocentrids examined here, and surgeonfishes examined by Rocha et al. (2002), differ by more than  $\phi_{ST}$  and PLD. Holocentrid larvae are much larger than those of surgeonfishes, and size may be a good predictor of swimming ability (Bellwood and Fisher 2001). In addition to the large size of pelagic holocentrids, larval behavior is an important determinant of vertical and horizontal larval assemblage structure (Leis 1993). The dispersive stages in holocentrids may include behavioral mechanisms that ensure some gene flow at moderate (for longjaw squirrelfish) and large (for blackbar soldierfish) geographic scales. Clearly life history factors in addition to PLD must be considered in understanding the phylogeography of reef fishes.

#### Pelagic dispersal

Debate continues over the role of PLD in population genetic separations and evolutionary divergences

(Bonhomme and Planes 2000; Barber et al. 2002; Mora and Sale 2002; Swearer et al. 2002; Marko 2004). From our survey of two squirrelfishes and 13 other Atlantic reef fishes (Table 5), we did not find a significant correlation between PLD and population genetic structure, with PLD accounting for less than 20% of variation.

The pioneer surveys of PLD and genetic connectivity in marine fishes include comparisons of ten species in the eastern Pacific (Waples 1987), seven species on the Great Barrier Reef (Doherty et al. 1995), and eight species in the Caribbean Sea (Shulman and Bermingham 1995). The first two studies found a correlation between PLD and population genetic structure. The third did not, consistent with recent surveys of three reef fishes in New Caledonia (Planes et al. 1998) and eight species on the Great Barrier Reef (Bay et al. 2006).

What can reconcile these contradictory results? The explanation likely includes the recognition that “reef fishes” is not a cohesive category in the phylogenetic, ecological, or taxonomic sense. This grouping includes lineages that diverged 50–100+ MY BP (Streelman and Karl 1997; Bellwood and Wainwright 2002). Is it reasonable to expect a simple relationship between PLD and genetic connectivity across this evolutionary interval? The corresponding time depth in other taxonomic groups would mandate comparisons across placental mammals, or passerine birds.

The two studies that report a significant correlation between PLD and genetic connectivity (Waples 1987; Doherty et al. 1995) are anchored by species that lack a pelagic dispersive stage, and the significant relationship is weakened without these examples (Bohonak 1999; Bay et al. 2006). In gauging the function of PLD in reef fish dispersal, perhaps it is most informative to survey phylogenetically-affiliated species (Riginos and Victor 2001; Rocha et al. 2002, 2005a; Bay et al. 2006), as this provides a means of controlling (at least in part) the other life-history variables that contribute to differences in population connectivity.

As more data sets become available, it may be possible to resolve dispersal trends for taxonomic families of reef fishes (Planes 2002). Candidates for high larval dispersal include the squirrelfishes surveyed here (family Holocentridae), trumpetfishes (family Aulostomidae; Bowen et al. 2001), and parrotfishes (family Scaridae; Bernardi et al. 2000; Streelman et al. 2002; Bay et al. 2004). Candidates for limited pelagic dispersal include gobies (family Gobiidae; Shulman and Bermingham 1995; Dawson et al. 2002; Lima et al. 2005; Rocha et al. 2005b; Taylor and Hellberg 2005); blennies (suborder Blennioidei; Muss et al. 2001; Riginos and Victor 2001), cardinalfishes (family Apogonidae; Hoffman et al. 2005), and seahorses and pipefishes (family Sygnathidae; Chenowith et al. 2002; Jones et al. 2003; Lourie et al. 2005). Families with a broad range of dispersal capabilities may include surgeonfishes (Acanthuridae; Planes et al. 1998; Rocha et al. 2002), wrasses (Labridae; Shulman and Bermingham 1995; Rocha et al. 2005a), groupers (Serranidae; Planes et al. 1998; Carlin et al.

2003; McCartney et al. 2003; Rivera et al. 2004; Craig et al. 2006), damselfishes (family Pomacentridae; Doherty et al. 1995; Shulman and Bermingham 1995; Planes et al. 1998; Bernardi et al. 2001; Bay et al. 2006), and likely many others. As the survey of squirrelfishes indicates, population genetic structure of co-distributed reef fishes can vary by an order of magnitude even within a single taxonomic family. Hence the evolutionary depth of reef fish lineages, and the diversity of larval strategies embedded therein, preclude sweeping generalities about pelagic larval dispersal and population genetic structure.

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## Appendix 1

Haplotype distribution of 69 specimens of *Myripristis jacobus*.

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| Haplotype | Florida (N=10) | Grenada (N=16) | Brazil (N=10) | Ascension (N=13) | São Tomé (N=20) | Total (N=69) |
|-----------|----------------|----------------|---------------|------------------|-----------------|--------------|
| A         | 2              | 4              | 8             | 8                | 10              | 32           |
| B         | 1              | 3              | 1             | –                | –               | 5            |
| C         | –              | 3              | –             | –                | –               | 3            |
| D         | –              | 2              | –             | –                | –               | 2            |
| E         | –              | –              | –             | 2                | –               | 2            |
| F         | –              | –              | –             | 2                | –               | 2            |
| G         | –              | –              | –             | –                | 2               | 2            |
| H         | –              | –              | –             | 1                | –               | 1            |
| I         | –              | –              | 1             | –                | –               | 1            |
| J         | 1              | –              | –             | –                | –               | 1            |
| K         | 1              | –              | –             | –                | –               | 1            |
| L         | 1              | –              | –             | –                | –               | 1            |
| M         | 1              | –              | –             | –                | –               | 1            |
| N         | 1              | –              | –             | –                | –               | 1            |
| O         | 1              | –              | –             | –                | –               | 1            |
| P         | 1              | –              | –             | –                | –               | 1            |
| Q         | –              | 1              | –             | –                | –               | 1            |
| R         | –              | 1              | –             | –                | –               | 1            |
| S         | –              | 1              | –             | –                | –               | 1            |
| T         | –              | 1              | –             | –                | –               | 1            |
| U         | –              | –              | –             | –                | 1               | 1            |
| V         | –              | –              | –             | –                | 1               | 1            |
| W         | –              | –              | –             | –                | 1               | 1            |
| X         | –              | –              | –             | –                | 1               | 1            |
| Y         | –              | –              | –             | –                | 1               | 1            |
| Z         | –              | –              | –             | –                | 1               | 1            |
| AA        | –              | –              | –             | –                | 1               | 1            |
| BB        | –              | –              | –             | –                | 1               | 1            |

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