Molecular Ecology (2005) doi: 10.1111/j.1365-294X.2005.02698.x

FAST-TRACK

Recent invasion of the tropical Atlantic by an Indo-Pacific coral reef fish

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Abstract

The last tropical connection between Atlantic and Indian–Pacific habitats closed *c.* 2 million years ago (Ma), with the onset of cold-water upwelling off southwestern Africa. Yet comparative morphology indicates more recent connections in several taxa, including reef-associated gobies (genus *Gnatholepis*). Coalescence and phylogenetic analyses of mtDNA cytochrome *b* sequences demonstrate that *Gnatholepis* invaded the Atlantic during an interglacial period ~145 000 years ago (d = 0.0054), colonizing from the Indian Ocean to the western Atlantic, and subsequently to the central (~100 000 years ago) and eastern Atlantic (~30 000 years ago). Census data show a contemporary range expansion in the northeastern Atlantic linked to global warming.

Keywords: Agulhas leakage, biogeography, dispersal, mtDNA, phylogeography

Received 30 March 2005; revision received 21 June 2005; accepted 11 July 2005

Introduction

The boundaries of the present-day tropical Atlantic Ocean were formed by two geological events: (i) the closure of the eastern Tethys Sea as Africa collided with Eurasia 15–20 million years ago (Ma) (Smith *et al*. 1995) and (ii) the more recent (3.1–3.5 Ma) uplift of the Isthmus of Panama (Duque-Caro 1990). Subsequent faunal exchanges of tropical organisms between the Atlantic and Indian oceans through the sole remaining gateway, around South Africa, were strongly curtailed after the late Pliocene/early Pleistocene (~2 Ma) establishment of the Benguela upwelling system, which forms a cold-water barrier along the Atlantic coast of southern Africa (Shannon 1985). However, morphological similarities between Indian Ocean and Atlantic Ocean molluscs indicate successful late Pleistocene invasions via this route (Vermeij & Rosenberg 1993).

Four criteria can distinguish a recent invader: (i) morphology: the invader is similar to a sister taxon in another region (Vermeij & Rosenberg 1993); (ii) biogeography: the invader is the sole representative of a genus that attains high diversity elsewhere (Vermeij 1991; Briggs 1995); (iii) palaeontology: the stratigraphy of fossil forms indicates recent colonization (Vermeij & Rosenberg 1993); and (iv) genetics: molecular clock estimates support a recent dispersal event (Bowen *et al*. 1994, 2001; Lessios *et al*. 2001). While conditions 1 and 2 can be evaluated for many shore fishes, condition 3 is very difficult to test due to an uneven fossil record. Condition 4 has rarely been tested on marine organisms.

The Atlantic goldspot goby, *Gnatholepis thompsoni*, is a prime candidate for a recent natural invader based on the first two conditions: it is morphologically very similar to a sister taxon in the Indian Ocean (*Gnatholepis scapulostigma*), and is the only representative of the genus *Gnatholepis* in the Atlantic, while there are eight species and subspecies in the Indo-Pacific (Randall & Greenfield 2001; Thacker 2004a).

To determine whether genetic data indicate a recent invasion of the Atlantic Ocean by *Gnatholepis*, we analysed 774 bp of the mtDNA cytochrome *b* gene in 133 *G. thompsoni* from the western, central, and eastern Atlantic (Fig. 1), 12 *G. scapulostigma* from the Indian and Pacific oceans

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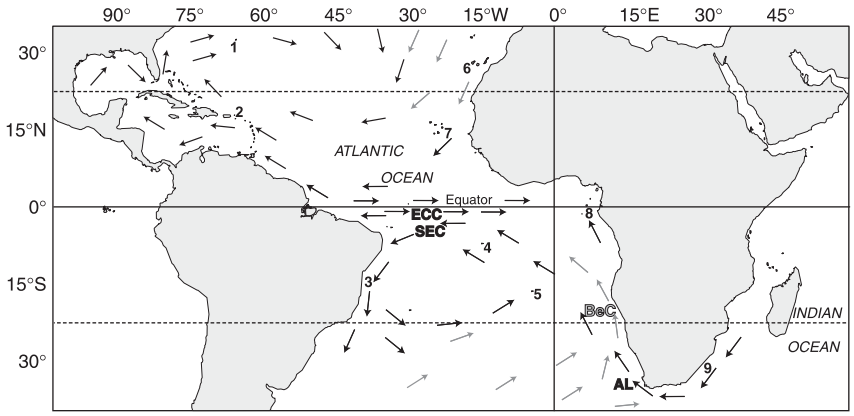

Fig. 1 Map of the Atlantic Ocean and the western Indian Ocean. Sampling sites in the Atlantic are numbered as follows: 1, Bermuda; 2, St Croix, USVI; 3, southeastern Brazil; 4, Ascension; 5, St Helena; 6, Canary Islands; 7, Cape Verde; 8, Sao Tome; 9, South Africa. Warm (black arrows) and cold (grey arrows) currents are indicated. Abbreviations of currents mentioned in the text: SEC, South Equatorial Current; ECC, Equatorial Counter Current; BeC, Benguela Current; AL, Agulhas leakage.

(eight from South Africa and four from the Cook Islands in the South Pacific), and four *Gnatholepis anjerensis* from the eastern Indian Ocean (Cocos Keeling Islands) for outgroup comparisons. This sampling design was intended to test the age of a putative Atlantic invasion, as well as dispersal pathways within the tropical Atlantic.

Materials and methods

Sample collection and sequencing

Fish were collected using microspears, hand nets, or rotenone while scuba diving, between 1996 and 2004. Gills, muscle or fin tissues were preserved in 1.5 mL tubes containing a saturated salt-DMSO buffer, or 100% ethanol. Total genomic DNA was extracted by standard phenol–chloroform methods or using QIAGEN DNeasy extraction kits following the manufacturer's protocol. Extracted DNA was frozen in TE or AE buffer and archived at –20 °C. Primer names indicate the DNA strand (H = heavy and L = light strand) and the position of the 3′ end of the oligonucleotide primer relative to the human mitochondrial DNA sequence. A segment of 802 bp of the mtDNA cytochrome *b* gene was amplified with the primers L14725 (5′-GTGACTTGAAAAACCACCGTTG-3′) and H15573 (5′-AATAGGAAGTATCATTCGGGTTTGATG-3′) (Meyer 1993).

Thermal cycling in polymerase chain reactions (PCR) consisted of an initial denaturing step at 94 °C for 1 min 20 s, then 30 cycles of amplification (30 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.).

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.). All samples were sequenced in the forward direction with the primer L14725, but to ensure accuracy of nucleotide designations a subset of haplotypes were sequenced in both directions. Sequences of representative haplotypes have been deposited in GenBank. Copies of the complete data set are available from L.A.R. upon request.

Taxonomy of Indo-Pacific species

Although we follow the most recent nomenclatural rearrangement by Thacker (2004a), readers should be aware that the species she names *Gnatholepis scapulostigma* (formerly *Gnatholepis cauerensis*) is under investigation, and name changes are possible (J. Randall, D. Greenfield & H. Larson, personal communication). We refrain from using the name *G. thompsoni* for Pacific populations of putative *G. scapulostigma* (*sensu* Thacker 2004a) because the former is not defined by either geography, morphology or nuclear genes. Due to the instability of nomenclature in this group, we maintain voucher specimens and tissue of our South Africa samples at the Scripps Institution collection, with numbers SIO 04-51, 04-58, 04-59, 04-61, 04-63 and 04-64.

Mutation rates and coalescent analyses

Mutation rates in many tropical marine organisms can be estimated using the divergence of sister species that were formed by isolation due to the closure of the Isthmus of Panama, 3.1–3.5 Ma (Bermingham *et al*. 1997; Knowlton & Weigt 1998). As *Gnatholepis* lacks a species in the eastern Pacific (Robertson & Allen 2002) we could not directly estimate the mutation rate in this genus. Here we use a proxy estimate derived from sequences of the same fragment from an Atlantic and eastern Pacific pair of sisters species in the genus *Evorthodus (*two specimens of *Evorthodus lyricus* from the Caribbean Panama and Florida and two specimens of *Evorthodus minutus* from the eastern Pacific Panama and Mexico, the only members of this genus), which is closely affiliated with *Gnatholepis* in the subfamily

Gobionellinae (Thacker 2003). As *Everthodus* spp. live in estuaries (e.g. Robertson & Allen 2002), they should have been separated during the final closure of the Isthmus of Panama (3.1–3.5 Ma). The mutation rate (λ) in *Evorthodus* was estimated by solving the equation $\lambda = d/2T$, where T is the time since divergence (in years) between the lineages and d the genetic distance. The d of 0.135 was obtained when comparing cytochrome *b* sequences from the two species using a nucleotide substitution model (TrN), optimized for our data set using a maximum-likelihood (ML) approach calculated in MODELTEST (Posada & Crandall 1998). With a T = 3.1 to 3.5 million years (Myr), we calibrated the mutation rate within lineages at 1.93–2.17% per Myr ($\lambda = 1.93$ to 2.17×10^{-8}). While this rate must be regarded as provisional for *Gnatholepis*, it is within the range accepted for mtDNA protein-coding regions of bony fishes (Bermingham *et al*. 1997; Bowen *et al*. 2001; Muss *et al*. 2001).

Genetic distances within *Gnatholepis* were calculated under the Tamura–Nei (Tamura & Nei 1993) nucleotide substitution model (TrN + γ, with $\gamma = 0.9756$), optimized for our data set using an ML approach calculated in MODELTEST (Posada & Crandall 1998). The minimum-spanning network was built using TCS version 1.13 (Clement *et al*. 2000). Population differentiation was calculated through an analysis of molecular variance (AMOVA), conducted using the program ARLEQUIN version 2.000 (Schneider *et al*. 2000).

Two coalescence analyses were applied to our data set and used to estimate the time frame for population events in the Atlantic Ocean, both assuming the mutation rate of 1.93 to 2.17×10^{-8} substitutions per site per year, and a generation time of 1 year. Both coalescence methods assume that the populations are undergoing a continuous expansion that persists today. In order to test if this assumption was acceptable, we calculated mismatch distributions using DNASP (Rozas *et al*. 2003), and observed that all populations fitted the model of rapid expansion with an $\alpha = 0.05$.

Putative population ages on both methods were also calculated using a range of molecular clock rates from 1% to 4% per Myr to accommodate potential errors in the timing of speciation events in *Evorthodus* and the geological event used to calibrate the clock. The first method involves the calculation of the mutation rate per haplotype $v = m\mu$, where m is the sequence length and μ, the mutation rate in years. The coalescence time was estimated by solving the equation $t = \tau/2v$ (Harpending *et al*. 1993). The parameter τ was estimated using the computer program DNASP (Rozas *et al*. 2003). Fu's F_S test (Fu 1997), implemented in the same program, was used to test for evidence of recent population expansion.

In the second method, the ML estimate of the growth parameter (G) was calculated for the Atlantic populations using FLUCTUATE version 1.4 (Kuhner *et al*. 1998). This analysis was performed 10 times, using randomly generated seeds, a search strategy of 20 short and 5 long Monte Carlo chains of 200 and 20 000 steps, respectively, and a sampling increment of 20. The mean of G and its standard deviation from the 10 runs were used to estimate the time to most recent common ancestor.

Results

Within the Atlantic, we found 62 haplotypes among 133 individuals of *Gnatholepis thompsoni*. Overall haplotype diversity was higher in the western Atlantic ($h = 0.96 \pm 0.018$) and St Helena ($h = 0.95 \pm 0.037$), followed by the eastern Atlantic ($h = 0.62 \pm 0.081$), and Ascension Island ($h = 0.46 \pm 0.128$; Table 1). Genetic distances among populations (pairwise Φ_{ST}) demonstrate strong subdivisions within the Atlantic, corresponding to the western, central, and eastern regions (overall $\Phi_{ST} = 0.474$; Table 2). No significant population structure was observed among locations within each of the three regions, except between the mid-Atlantic islands (Ascension and St Helena; $\Phi_{ST} = 0.392$, $P < 0.001$). Three shallow Atlantic lineages were resolved (Fig. 2), which correspond to the western, central, and eastern subdivisions detected with AMOVA. Significant negative values of Fu's F_S were observed in all Atlantic populations (–23.9, –5.4 and –10.3, for western, central and eastern Atlantic respectively; all $P < 0.01$).

The sequences of the sister species pair *G. thompsoni* and *Gnatholepis scapulostigma* were highly divergent from those of the outgroup *Gnatholepis anjerensis* ($d = 0.11–0.14$). *G. scapulostigma* specimens contained two lineages ($d = 0.03$), one of which (representing all individuals from eastern South Africa and one from the Pacific Ocean) was very

Table 1 Sample sizes (N), haplotype number (H), haplotype diversity (h) and nucleotide diversity (π) for *Gnatholepis thompsoni* in the Atlantic Ocean and *Gnatholepis caurensis* in the Indian Ocean (South Africa). In the text, West Atlantic locations refer to Bermuda, St Croix, and SE Brazil, central Atlantic locations include the islands of Ascension and St Helena, and East Atlantic locations include Sao Tome, Cape Verde, and Canary Islands. Location numbers correspond to those in Fig. 1

Location	N	H	h	π
1. Bermuda	20	16	0.97 ± 0.028	0.007 ± 0.004
2. St Croix	30	21	0.96 ± 0.023	0.008 ± 0.004
3. SE Brazil	3	2	0.89 ± 0.160	0.001 ± 0.001
4. Ascension	23	7	0.46 ± 0.128	0.002 ± 0.001
5. St Helena	14	10	0.95 ± 0.037	0.004 ± 0.003
6. Canary Islands	24	5	0.62 ± 0.091	0.001 ± 0.001
7. Cape Verde	12	6	0.68 ± 0.148	0.001 ± 0.001
8. Sao Tome	7	3	0.52 ± 0.208	0.001 ± 0.001
9. South Africa	8	8	0.99 ± 0.062	0.004 ± 0.002

Table 2 Pairwise Φ_{ST} among Atlantic Ocean locations. Significant values ($P < 0.01$) indicated with an asterisk (*)

	Pairwise Φ_{ST}						
Location	1	2	3	4	5	6	7
1. Bermuda	—						
2. St Croix	0.001	—					
3. SE Brazil	0.001	0.001	—				
4. Ascension	0.595*	0.557*	0.561*	—			
5. St Helena	0.420*	0.395*	0.397*	0.392*	—		
6. Canary Islands	0.477*	0.426*	0.432*	0.797*	0.602*	—	
7. Cape Verde	0.395*	0.356*	0.362*	0.771*	0.522*	0.017	—
8. Sao Tome	0.353*	0.323*	0.328*	0.774*	0.488*	0.042	0.001

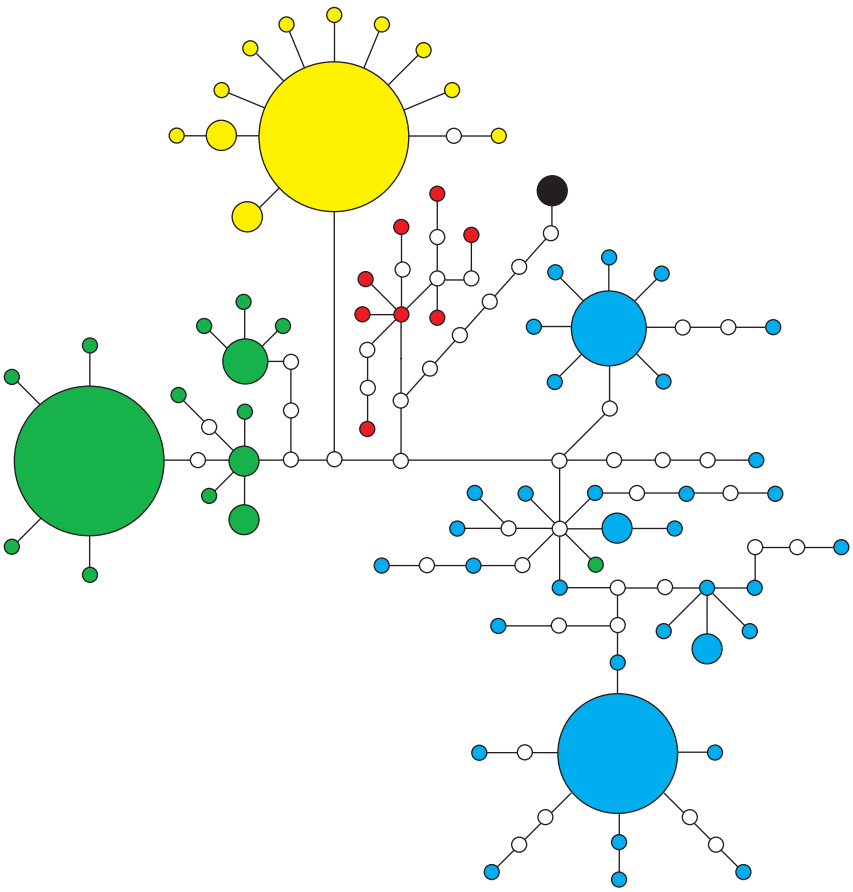

Fig. 2 Minimum-spanning network showing relationships among *Gnatholepis* mtDNA haplotypes. Haplotypes observed in the western, central and eastern Atlantic are indicated with blue, green and yellow branches, respectively. Red circles represent the closely related *Gnatholepis scapulostigma* from South Africa, and black represents *G. scapulostigma* from the central Pacific. The sizes of the coloured circles are proportional to haplotype frequencies. Small, uncoloured circles represent intermediate haplotypes not observed in our survey.

closely related to the Atlantic *G. thompsoni* ($d = 0.0054$). These two lineages were also observed in studies of Pacific Ocean *Gnatholepis* using mtDNA ND2 sequences (Thacker 2004a, b). Using the two coalescent methods, we estimated the time to the most recent common ancestor (TMRCA) between the Atlantic *G. thompsoni* and the Indo-Pacific *G. scapulostigma* at ~130–155 thousand years ago (kyr BP). Even if we use the most conservative mutation rate, which produces an estimate of 268 kyr for the age of the Atlantic population (Table 3), this represents the most recent date for a natural invasion of the Atlantic by a tropical reef fish (Bowen *et al*. 2001). Our coalescent estimates also demonstrate that the western Atlantic population is the oldest (115–139 kyr), followed by the central Atlantic island of St Helena (83–115 kyr), while Ascension and the eastern Atlantic are the youngest (both less than 30 kyr; Table 3).

Table 3 Results of the coalescent analyses. Location names are: EA, eastern Atlantic; Asc, Ascension; StH, Saint Helena; WA, western Atlantic; AO + IO, Atlantic Ocean + Indian Ocean. Age estimates are calculates with mutation rates of 1.93–2.17% per million years, the range (in parenthesis) is calculated with mutation rates from 1% to 4% per million years. Age estimates for AO + IO (in bold) represent the time to the most recent common ancestor between Atlantic and Indian Ocean populations

Locations	Tau (τ)	τ Age estimate (range)	Growth parameter (*G*)	*G* Age estimate (range)
EA	0.844	25 125–30 289 (13 630–54 521)	8 412.5 ± 292.9	25 228–28 365 (13 686–54 745)
Asc	0.512	15 241–18 374 (8 268–33 074)	10 000.0 ± 322.8	21 221–23 860 (11 512–46 051)
StH	2.821	83 354–100 488 (45 219–160 878)	2 063.2 ± 59.9	102 869–115 661 (55 806–188 227)
WA	4.012	116 100–139 965 (62 984–192 937)	1 848.2 ± 55.2	115 211–129 538 (62 502–206 009)
AO + IO	**4.397**	**130 687–146 938 (70 897–219 491)**	**1 446.4 ± 33.7**	**146 763–155 013 (79 616–268 476)**

Discussion

Both the star-shaped haplotype network (Fig. 2) within each of the three lineages, and significant negative values of Fu's F_S for each lineage indicate recent population expansions at all Atlantic locations (Fu 1997; Avise 2000). Fu's F_S test is designed to detect genetic signatures of selection, but it is also sensitive to population expansion. The latter explanation has strong independent corroboration for Atlantic reef organisms. Reef habitats were 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 & Wainwright 2002). Thus recent population expansions by *Gnatholepis thompsoni* probably occurred during the current interglacial period, in response to greatly increasing habitat availability.

Previous palaeontological and phylogenetic research has demonstrated colonizations of the Atlantic from the central Pacific before the closure of the Isthmus of Panama (3.1–3.5 Ma), and possibly more recent colonizations from the Indian Ocean via southern Africa (Vermeij & Rosenberg 1993). Three lines of evidence indicate that *Gnatholepis* colonized the Atlantic from the Indian Ocean rather the central Pacific: (i) genetic divergence between Atlantic and Indian Ocean *Gnatholepis* taxa is one order of magnitude shallower than values observed in fishes isolated by the Isthmus of Panama (Bermingham *et al*. 1997; Craig *et al*. 2004); (ii) *Gnatholepis thompsoni* probably did not occur throughout the Neotropics prior to the isthmian closure, as *Gnatholepis* does not currently occur in the eastern Pacific (Robertson & Allen 2002); (iii) *Gnatholepis thompsoni*'s virtually identical sister species, *Gnatholepis scapulostigma*, is widely distributed in the Indian Ocean, including SE Africa, from where the Agulhas leakage feeds pulses of tropical water into the south Atlantic during warm interglacial periods (Peeters *et al*. 2004). Thacker (2004a) also suggested that *G. thompsoni* may have invaded the Atlantic coming from around South Africa, but stressed that more samples (especially from the Indian Ocean) were necessary to test this hypothesis.

Today, cold temperate conditions that predominate around southern Africa strongly obstruct exchanges of tropical organisms between the Atlantic and Indian oceans (Vermeij & Rosenberg 1993; Briggs 1995). However, our analysis supports the hypothesis that such exchanges can occur during interglacial periods warmer than today. Our estimates for the origin of the Atlantic populations, between ~130–155 kyr BP, closely overlaps an interglacial period at 120–145 kyr BP, characterized by tropical water intrusions from the Indian Ocean to the Atlantic Ocean that were stronger than intrusions observed today (Peeters *et al*. 2004).

After breaching the barrier between the Indian and Atlantic oceans, warm Agulhas eddies either mix with the cold Benguela Current along the coast of Africa, which is probably lethal to tropical organisms, or move northwest with the warm South Equatorial Current (Gordon 2003). Thus, the western and south-central Atlantic are the most likely destinations for tropical invaders from the Indian Ocean (Fig. 1). Crossing from equatorial Africa to Brazil would take an average of 70 days based on oceanic current velocities (Scheltema 1971). Even increasing this by 50% to allow for transport time between South Africa and lower latitudes, this dispersal interval is well within the pelagic larval duration of *G. thompsoni*, up to 122 days (Sponaugle & Cowen 1994). Other Pleistocene invaders, including molluscs and sea turtles, appear to have colonized from the Indian Ocean directly to the western Atlantic (Vermeij & Rosenberg 1993; Bowen *et al*. 1994), indicating a general dispersal path for natural invasions of the tropical Atlantic.

The descending ages (based on coalescence) of population expansions in western Atlantic (~130 kyr BP), St Helena in the central Atlantic (~100 kyr BP) and eastern Atlantic (~28 kyr BP) are subject to two explanations: first, in the period

since *Gnatholepis* colonized the Atlantic, the most benign region has been the western shoreline, and *G. thompsoni* probably established its largest and most stable population in the western Atlantic. The eastern shorelines have been subject to strong temperature fluctuations caused by cold-water upwelling events, especially during glacial periods (Sachs *et al*. 2001). Consequently, eastern Atlantic populations could have been established soon after the Atlantic invasion by immigration directly from the western Atlantic, and the cline in population age and genetic diversity may reflect bottlenecks rather than colonization events.

Alternatively, the cline could indicate a pathway of intra-oceanic colonization, from the western shores to the mid-Atlantic ridge and subsequently to the eastern Atlantic. Migration in this direction would be accomplished via eastward-flowing currents, such as the Equatorial Counter-Current and the southern branch of the Brazil current. In this case, the mid-Atlantic ridge island of St Helena may have acted as a stepping stone for colonization of shallow-water fauna across the Atlantic (Muss *et al*. 2001). The presence of one individual in St Helena containing a sequence that nests within the western Atlantic lineage (green circle within blue group in Fig. 2) indicates recent west–east migration and supports the stepping-stones hypothesis. Moreover, with the lowest genetic diversity of the data set (Table 1), Ascension Island shares a most common haplotype (in 17 of its 23 individuals) with St Helena, possibly indicating a recent colonization (< 30 000 years ago according to the coalescent analyses; Table 3) from the latter. If this hypothesis is supported by other taxa, the diminutive islands of the mid-Atlantic ridge may prove to be an important aid to trans-Atlantic dispersal, and conservation initiatives aiming to protect evolutionary mechanisms should have high priority on such evolutionary crossroads (Bowen & Roman 2005).

Contemporary global warming, a phenomenon known to affect marine fish distributions (Perry *et al*. 2005), may have facilitated the most recent range expansion of *G. thompsoni*: the colonization of subtropical islands of the northeastern Atlantic. The sea around the Canary Islands began warming in the 1980s, with the highest recorded water temperatures observed in 1996–1998 (International Research Institute for Climate Prediction website http://iri.ldeo.columbia.edu/). Annual surveys of shore fishes in the Canary Islands (by J. V. T.) that began in 1984 recorded no *G. thompsoni* (a conspicuous, easily recognizable shallow-water species) prior to the 1996–1998 high-temperature episode. Goldspot gobies appeared during this episode and have persisted in abundance to date. Despite the recent timing of this colonization, mtDNA diversity in the Canary Islands population is similar to that elsewhere in the eastern Atlantic ($h = 0.62$ vs. $h = 0.68$ in tropical Cape Verde). This indicates that colonizers arrived in great quantities, rather than as a few stragglers. The appearance of other tropical fish species at the Canaries during that same period (Brito *et al*. 2002 and references therein) strengthens our hypothesis that elevated sea temperatures influenced this recent range expansion in *G. thompsoni*.

Previous research indicates that the fish fauna in the tropical Atlantic has been isolated from that of the Indo-Pacific over the last 3 Myr, as would be expected in a closed ocean basin (Briggs 1995). Within the Atlantic, species distributions and recent phylogeographic studies have revealed evolutionary partitions that correspond to ecological discontinuities, particularly the Amazon barrier between tropical reef habitats in Brazil and the Caribbean Sea (Rocha *et al*. 2002; Rocha 2003, 2004), and the oceanic barrier between western, central, and eastern Atlantic (Lessios *et al*. 2001; Muss *et al*. 2001; Bernardi *et al*. 2004). Superimposed on these vicariant partitions are the episodic dispersal events (or range expansions) mediated by larval dispersal (Rocha *et al*. 2005; Lima *et al*. 2005).

In the genus *Gnatholepis*, large-scale range expansions in the late Pleistocene, including colonization of the Atlantic and subsequent spread into the NE Atlantic, are associated with global warming events. Marine and terrestrial species alike share the signatures of evolutionary processes mediated by climate change (Dynesius & Jansson 2000; Hellberg *et al*. 2001). The mtDNA data demonstrate that tropical Indian Ocean fishes can breach the Benguela barrier (which is opened intermittently by the warm Agulhas leakage) during warm interglacial periods and subsequently colonize the entire tropical Atlantic Ocean. Contemporary census data show a range expansion in *G. thompsoni* linked to elevated temperatures, consistent with the conclusions based on phylogeographic analyses. These observations of past and present trends may inform predictions about the future impacts of climate change in the marine realm.

Acknowledgements

We thank C. Ferreira, C. Thacker and P. Wirtz for samples; M. Courtney, S. Floeter, S. Grant, S. Karl, C. Moritz, D. Murie, G. Paulay, J. Roman and P. Taylor for advice and comments; and M. Steele for data on *Gnatholepis thompsoni*'s generation time. D. Greenfield, H. Larson and J. Randall provided taxonomic advice. H. Lessios critically read the manuscript. Fieldwork was facilitated by the Bermuda Biological Station for Research, the National Park Service at St. Croix, the Government of St Helena and Ascension, the Fisheries Department of Cape Verde, the Direccao das Pescas do Sao Tome, and P. C. Heemstra, South African Institute for Aquatic Biodiversity. Financial support came from the Brazilian Ministry of Education (to L. A. R.), National Science Foundation (DEB-9727048 to B. W. B.), and the Smithsonian Tropical Research Institute (to L. A. R and D. R. R.).

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