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PHYLOGEOGRAPHY OF THE TRUMPETFISHES (*AULOSTOMUS*): RING SPECIES COMPLEX ON A GLOBAL SCALE

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Abstract.—The distribution of circumtropical marine species is limited by continental boundaries, cold temperate conditions, and oceanic expanses, but some of these barriers are permeable over evolutionary time scales. Sister taxa that evolved in separate ocean basins can come back into contact, and the consequences of this renewed sympatry may be a key to understanding evolutionary processes in marine organisms. The circumtropical trumpetfishes (*Aulostomus*) include a West Atlantic species (*A. maculatus*), an Indian-Pacific species (*A. chinensis*), and an East Atlantic species (*A. strigosus*) that may be the product of a recent invasion from the Indian Ocean. To resolve patterns of divergence and speciation, we surveyed 480 bp of mitochondrial DNA cytochrome *b* in 196 individuals from 16 locations. Based on a conventional molecular clock of 2% sequence divergence per million years, the deepest partitions in a neighbor-joining tree ($d = 0.063$–0.082) are consistent with separation of West Atlantic and Indian-Pacific species by the Isthmus of Panama, 3–4 million years ago. By the same criteria, trumpetfish in the East Atlantic were isolated from the Indian Ocean about 2.5 million years ago ($d = 0.044$–0.054), coincident with the advent of glacial cycles and cold-water upwelling around South Africa. Continental barriers between tropical oceans have only rarely been surmounted by trumpetfishes, but oceanic barriers do not appear to be substantial, as indicated by weak population partitioning ($\phi_{ST} = 0.093$) in *A. chinensis* across the Indian and Pacific Oceans. Finally, morphological and mitochondrial DNA data indicate hybridization of *A. strigosus* and *A. maculatus* in Brazil. After 3–4 million years and a globe-spanning series of vicariant and dispersal events, trumpetfish lineages have come back into contact in the southwest Atlantic and appear to be merging. This ring species phenomenon may occur in a broad array of marine organisms, with clear implications for the production and maintenance of biodiversity in marine ecosystems.

Key words.—Biogeography, cytochrome *b*, dispersal, marine fish, mitochondrial DNA, population structure, vicariance.

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There are many definitions of species, but almost all include some level of reproductive isolation (reviewed in Avise 2000). In marine organisms, high dispersal potential may retard the accumulation of reproductive barriers and promote evolutionary stasis by connecting widely separated populations (Palumbi 1996). In this context, there has been much attention given to the evolutionary significance of dispersal and retention of early life-history stages of marine animals (Hedgecock 1986; Sinclair 1988; Sinclair and Iles 1989; Cunningham and Collins 1994; Doherty et al. 1995; Jones et al. 1999; Bernardi 2000). Veron (1995) proposed that coral species may diverge in isolation for millions of years, but subsequently coalesce when currents, climate, or geography bring them back into contact through pelagic larval dispersal. Recent molecular tests tentatively support this hypothesis (Hatta et al. 1999). Many other marine organisms have distributions that are sundered by continental (or oceanic) barriers for millions of years (Hubbs 1952; Grant 1987; Stepien and Rosenblatt 1996; Bowen et al. 1998). Do some of these sister taxa subsequently come back into contact and coalesce via hybridization? Patterns of genetic diversity in cosmopolitan marine fish and marine turtles are consistent with such coalescence (Bowen et al. 1994; Graves 1998). This phenomenon may be essential for understanding speciation in the sea.

The trumpetfishes (*Aulostomus*) are at an evolutionary junction that is especially relevant to the issue of renewed sympatry. Three species are recognized (Wheeler 1955): *A. chinensis* occurs in the Indian and Pacific Oceans from the Gulf of Panama to South Africa (Smith and Heemstra 1986; Allen and Robertson 1994), although it is apparently absent from the Red Sea and rare in adjacent areas of the Indian Ocean (Randall 1983; J. E. Randall pers. comm.); *A. strigosus* occurs in the East Atlantic from Madeira to at least the Gulf of Guinea (Wheeler 1955); and *A. maculatus* is found in the West Atlantic from Bermuda to Brazil (Bohlke and Chaplin 1993; Randall 1996; Floeter et al. 2001). On the islands of the mid-Atlantic ridge, *A. strigosus* is identified from Ascension and St. Helena (Lubbock 1980; Edwards and Glass 1987), whereas *A. maculatus* is believed to inhabit St. Paul's Rocks off the Brazilian coast (Lubbock and Edwards 1981; Edwards and Lubbock 1983).

Wheeler (1955) published the only appraisal of trumpetfish relationships. This morphological analysis showed that the Indian-Pacific and West Atlantic species are distinguished by fixed differences in a number of meristic characters. However, the East Atlantic species is nearly indistinguishable from the Indian-Pacific species, as morphological characters (fin ray counts, scale counts, and vertebral counts) overlap. In terms of morphology, *A. strigosus* in the East Atlantic and *A. chinensis* in the Indian-Pacific Ocean are ''incompletely differentiated species'' (Wheeler 1955).

In the vast majority of Atlantic species, taxonomic affinities are between the East and West Atlantic, as geography would indicate (Briggs 1974). If trumpetfishes in the East and West Atlantic are not sister taxa, what biogeographic

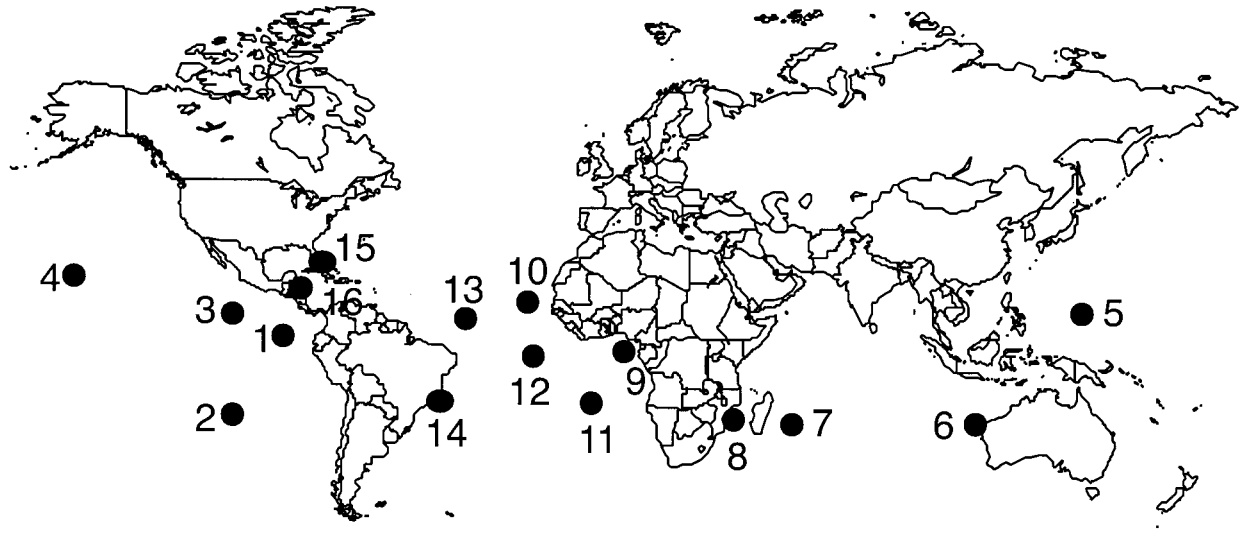

FIG. 1. The 16 sample locations for *Aulostomus* species. By conventional distributions, *A. maculatus* inhabits the West Atlantic, *A. strigosus* inhabits the East Atlantic, and *A. chinensis* inhabits the Indian and Pacific Oceans. *Aulostomus chinensis* was collected from: (1) Isla del Coco (Pacific Costa Rica, $n = 9$); (2) Easter Island ($n = 5$); (3) Clipperton Island ($n = 5$); (4) Hawaii ($n = 15$); (5) Guam ($n = 18$); (6) Ningaloo Reef (West Australia, $n = 7$); (7) Reunion Island ($n = 7$); and (8) Punta del Orro (Mozambique, $n = 2$). *Aulostomus strigosus* was collected from: (9) São Tome (Gulf of Guinea, West Africa, $n = 16$); (10) Cape Verde ($n = 16$); (11) St. Helena ($n = 19$); (12) Ascension Island ($n = 19$); and, based on a revision of species distributions (13) St. Paul's Rocks (Brazil, $n = 14$) and (14) Espirito Santo (Brazil, $n = 22$). *Aulostomus maculatus* was collected from: (15) Florida Keys (U.S.A., $n = 15$); and (16) Belize Barrier Reef ($n = 7$).

processes could explain this curious set of relationships? Wheeler's analysis prompted us to propose a historical scenario with two components: (1) Atlantic and Indian-Pacific forms have been isolated through recent evolutionary history, possibly by the closure of the Isthmus of Panama 3–4 million years ago (Coates and Obando 1996; see Lessios et al. 1999), giving rise to *A. maculatus* and *A. chinensis*; and (2) the Indian-Pacific form subsequently invaded the East Atlantic, giving rise to *A. strigosus.*

To test this scenario, we analyzed mitochondrial DNA (mtDNA) cytochrome *b* sequences from 196 trumpetfishes collected at 16 locations across the global distribution of *Aulostomus* (Fig. 1). With the exception of one genus of damselfishes (Bermingham et al. 1997), tropical reef-associated species have not been surveyed at this geographic scale. However, this approach has proven informative for other globally distributed marine animals, including tunas (Graves and Dizon 1989), bluefish (Graves 1998), billfish (Graves and McDowell 1995), bonefish (Colborn et al. 2001), mackerel (Scoles et al. 1998), swordfish (Alvarado-Bremer et al. 1996), anchovies (Grant and Bowen 1998), mullet (Crosetti et al. 1994), sea turtles (Bowen et al. 1998; Dutton et al. 1999), marine mammals (Baker et al. 1994; Rosel et al. 1995; Dizon et al. 1997), and sea birds (Kidd and Friesen 1998; Avise et al. 2000). Most of these species migrate extensively. In contrast, the trumpetfish is a weak swimmer that depends on ambush tactics and cryptic coloration to capture prey and avoid predators (Kaufman 1976).

A second (related) goal of this study was to define the limits of population connectivity within the three trumpetfish species, especially the widely distributed *A. chinensis.* The range of this species spans 70% of the Earth's circumference, but is marked by extensive oceanic expanses in which reef habitat is absent. In particular, the broad oceanic zone (4000–7000 km wide) between the central Pacific Islands (Hawaii and eastern Polynesia) and the eastern Pacific coastlines is believed to be a formidable barrier to dispersal of coastal marine organisms (Briggs 1961). Rosenblatt and Waples (1986) and Lessios et al. (1998) presented genetic evidence that early life-history stages of some species can bridge this gap, but debate continues on the geographic limits of larval dispersal and the corresponding scale of population structure (Shulman and Bermingham 1995; Muss et al. 2001).

A common measure for speciation is that reproductive isolation evolves on a scale of the Pleistocene era (2 million years; Avise et al. 1998), although the variance around this benchmark is huge (Avise and Walker 2000). In this paper, we present evidence that the trumpetfishes have completed a globe-spanning series of colonization events, followed by contact and hybridization. Our evidence from the southwest Atlantic indicates that trumpetfish species have not developed strong reproductive barriers after 3–4 million years of geographic isolation. When brought into contact by their enormous dispersal potential, the terminal ends of this ring species complex have fused via introgression.

MATERIALS AND METHODS

To assess phylogeographic patterns, 196 individuals were collected from 16 locations (Fig. 1, Table 1) including two locations for *A. maculatus* ($n = 22$), six locations for *A. strigosus* ($n = 106$), and eight locations for *A. chinensis* ($n = 68$). Tissue samples (gill filament, muscle, gonad, heart, or liver) were taken from fish collected with spears between 1990 and 1999. Fifteen samples from Ascension Island were purified with CsCl density gradients (Lansman et al. 1981).

TABLE 1. Distribution of trumpetfish (*Aulostomus*) haplotypes and diversity values for each location. Haplotype diversity (*h*) and nucleotide diversity (π) for each sample location are shown at the ends of the rows ($\pm$ standard devations). Haplotype abbreviations: AC, *A. chinensis;* AM, *A. maculatus;* AST, *A. strigosus.*

	Haplotypes														
Location	AC 1	AC 2	AC 3	AC 4	AC 5	AC 6	AC 7	AC 8	AST 1	AST 2	AST 3	AM 1	AM 2	*h*	π
Pacific Costa Rica	8							1						0.222 ± 0.166	0.00047 ± 0.00069
Easter Island	5													0.000	0.000
Clipperton Island	5													0.000	0.000
Hawaii	6			1	1	1	1	5						0.762 ± 0.081	0.00215 ± 0.00170
Guam	10	1						7						0.569 ± 0.071	0.00130 ± 0.00120
West Australia	5		1	1										0.524 ± 0.209	0.00121 ± 0.00126
Reunion Island	6							1						0.286 ± 0.196	0.00061 ± 0.00082
Mozambique								2						—	—
São Tome									16					0.000	0.000
Cape Verde									14		2			0.125 ± 0.106	0.00027 ± 0.00005
St. Helena									18	1				0.105 ± 0.092	0.00044 ± 0.00063
Ascension Island									6	13				0.456 ± 0.085	0.00192 ± 0.00155
St. Paul's Rocks									14					0.000	0.000
Espirito Santo, Brazil									22					0.000	0.000
Florida, U.S.A.												14	1	0.133 ± 0.112	0.00028 ± 0.00050
Belize												7		0.000	0.000

All other samples were stored in a saturated salt buffer without refrigeration (Amos and Hoelzel 1991) prior to DNA isolations with a standard phenol chloroform protocol (Hillis et al. 1996). Whole genomic DNA was ethanol precipitated, resuspended in TE buffer, and stored at −20°C.

The polymerase chain reaction (PCR) was used to amplify a fragment of approximately 550 bp at the 5′ end of the mtDNA cytochrome *b* gene. PCR reactions were conducted with a 50-μl reaction containing 10 mM dNTPs, 3.0 mM $MgCl_2$, 0.26 μM of each primer, and 1.0 unit of *Taq* DNA polymerase in a 10 mM Tris-HCL, 50 mM KCl, 0.1% Triton X-100 reaction buffer (Promega, Madison, WI). A heavy-strand primer (5′-GCC AAC GGC GCA TCC TTC TTC TT-3′ [H15020]) from Meyer (1994) and a light-strand primer (5′-AAT AGG AAG TAT CAT TCG GGT TTG ATG-3′ [L15573]) from Taberlet et al. (1992) were used in all reactions. Cycling parameters were as follows: initial denaturation at 94°C for 2 min; 35 cycles of 94°C (45 sec), 52°C (30 sec), 72°C (45 sec); and a final extension at 72°C for 2 min before termination of the reaction at 4°C. Excess dNTPs and reagents were removed using 30,000-MW Millipore (Bedford, MA) filters.

Double-stranded cycle sequencing was conducted using dye-labeled terminators (ABI Prism technology) and the resulting products were separated on an ABI Prism 377 DNA Sequencer (Applied Biosystems, Inc., Foster City, CA) in the DNA Sequencing Core at the University of Florida. Sequences that matched known haplotypes were collated by population, whereas unique (or dubious) fragments were sequenced in both the forward and reverse directions to verify the haplotype designation. After verification of haplotypes, sequences were aligned using an exhaustive search algorithm in the program Sequencher 3.0 (Gene Codes, Inc., Ann Arbor, MI).

Genetic distances were generated using maximum-likelihood settings in PAUP 4.0b2 (Swofford 1998). The HKY85 model of substitution (Hasegawa et al. 1985) was used because it allows transitions and transversion to occur at different frequencies and allows base composition to vary (Page and Holmes 1998; Nei and Kumar 2000). Transition:transversion ratio and base frequencies were empirically estimated, and among-site rate variation was assumed to have a gamma distribution of 0.5, the default setting in PAUP.

Haplotype (*h*) and nucleotide (π) diversities were estimated using the program Arlequin (ver. 1.1; Schneider et al. 1997), which implements equations (8.4) and (10.5) of Nei (1987). An analysis of molecular variance (AMOVA; Excoffier et al. 1992) in Arlequin was used to test for population structure within species. AMOVA generates an F_{ST} analog (ϕ_{ST}) based on the frequency of haplotypes and the genetic distance between haplotypes. Two AMOVAs were conducted: (1) an overall treatment of diversity within and between species; and (2) a treatment of diversity within and between localities for each species.

Relationships among haplotypes were assessed with PAUP, using a neighbor-joining algorithm (Saitou and Nei 1987) and weighted parsimony with default settings and a 3:1 transition:transversion ratio. Nodes in the neighbor-joining tree were assessed with 100 bootstrap resamples. A hand-drawn parsimony network was constructed to elucidate phylogeographic patterns.

The phylogenetic trees were created with midpoint rooting. The nearest outgroups for trumpetfishes (Aulostomidae) would be the cornetfishes (Fistulariidae), but we were unable to obtain a specimen during this project. A BLAST search in Genbank revealed homology of trumpetfish cytochrome *b* with corresponding sequences from many bony fishes, but at divergences greater than 25%, too distant to provide informative comparisons. Thus, midpoint rooting was used for all phylogenetic analyses.

In the course of this study, questions arose about the species identity of trumpetfish samples from Brazil, specifically whether they are *A. maculatus* (as all prior literature indicates) or *A. strigosus* (as the mtDNA data indicates). Trumpetfish are absent from most reef habitats along the Brazilian coast (Fowler 1942; Rocha et al. 1998; L. A. Rocha, pers. obs.)

and were found by our team at St. Paul's Rocks and at Isla Esclava, Espirito Santo, near the southern end of the tropical zone. To address species identity, two diagnostic characters identified by Wheeler (1955), number of anal fin rays and rows of scales above the lateral line, were surveyed in available specimens from Brazil, including those collected at Espirito Santo for molecular analysis.

Results

A cytochrome *b* fragment was amplified from 196 individuals, and 480 bp were utilized in this analysis (38 bp from the 5′ end and 29 bp from the 3′ end were removed due to uncertainties in nucleotide designations). Sequence comparisons reveal 48 variable sites, with 38 transitions and 12 transversions (Table 2). (Position 15 had both a transition and a transversion, and one instance of homoplasy is apparent at site 336.) In sum, these polymorphic sites defined 13 haplotypes. In all three taxa, a single species-specific haplotype dominated samples across the range (Table 1). Within species, haplotypes were distinguished by one to three mutations (d = 0.002–0.006; Tables 1, 2).

Haplotype diversity was moderate to low, and nucleotide diversity was low at every location, with no variation observed at seven of the 16 sample sites (Table 1). Although this result may be attributed to small sample sizes in some cases (Mozambique, n = 2; Clipperton Island, n = 5; and Easter Island, n = 5), well-sampled locations in Espirito Santo, Brazil (n = 22), and Cape Verde (n = 16) also have low levels of mtDNA diversity.

One unexpected finding was that all samples from coastal Brazil and St. Paul's Rocks, locations that were identified as *A. maculatus* habitats in previous treatises (Lubbock and Edwards 1981; Robins et al. 1986; Randall 1996), contained the most common haplotype from *A. strigosus* at 100% frequency. Therefore, these sample locations are grouped with *A. strigosus* in all phylogenetic and AMOVA analyses.

Aulostomus chinensis

Seven polymorphic sites were observed in *A. chinensis*, defining eight haplotypes in 68 individuals from eight locations (Tables 1, 2). All haplotypes were differentiated from adjacent haplotypes by single point mutations, and all changes were transitions. Haplotypes AC-1, AC-4, and AC-8 were found at multiple sampling locations, and the remaining haplotypes were restricted to single locales. The Hawaiian population exhibited the highest haplotype and nucleotide diversities (Table 1) with decreasing diversity moving to the west or east from this location. Population structure was shallow but statistically significant in *A. chinensis* (ϕ_{ST} = 0.093, P = 0.033).

Aulostomus strigosus

Three haplotypes were observed in 106 individuals from six locations (Tables 1, 2). The two polymorphic sites that differentiated haplotypes AST-1 and AST-2 were transversions (Table 2), whereas no transversions were observed within the other two species. Three of the six samples were fixed for haplotype AST-1. In the other three locations (As-

TABLE 2. Polymorphic sites observed in 480 bp of cytochrome *b* in trumpetfishes (*Aulostomus*). The location of each site is indicated at the top of the column. A nucleotide designation of N indicates an uncertian assignment. Complete sequences for AC-1, AM-1, and AST-1 are available under Genbank accession numbers AF327455–AF327457. AC, *A. chinensis;* AM, *A. maculatus;* AST, *A. strigosus.*

	1 2 3	1 3 4	1 5 4	2 4 8	3 0 9	4 6 5	6 7 4	7 8 0	8 9 5	1 0 4	1 1 2	1 3 5	1 3 8	1 5 3	1 6 0	1 6 8	1 7 1	1 7 7	1 8 9	2 0 7	2 3 4	2 3 7	2 4 9	2 5 5	2 6 2	2 7 6	2 8 9	3 0 2	3 1 7	3 2 6	3 3 8	3 4 0	3 7 4	3 8 9	4 0 1	4 2 2	4 3 6	4 3 9	4 5 5	4 6 8	4 7 5	4 8 9	4 9 5	5 1 1
AC-1	A	G	G	T	G	T	G	G	T	C	A	A	C	A	T	T	G	C	A	A	G	G	C	T	G	C	A	T	A	T	G	G	G	G	G	A	C	T	C	T	T	T	C	T	T	C	A
AC-2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
AC-3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
AC-4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	A	-	-	-	-	-	-	-	-	-	-
AC-5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-
AC-6	-	-	-	-	-	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
AC-7	-	-	-	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
AC-8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	A	-	-	-	-	-	-	-	-	-	-	-	-
AST-1	G	-	C	C	-	-	-	A	C	-	-	G	-	-	C	C	A	-	-	-	-	-	-	A	A	-	-	-	-	-	A	C	-	A	-	-	-	-	C	C	-	C	C	C	-	C
AST-2	G	-	C	C	C	-	-	A	C	-	-	G	-	-	C	C	C	A	-	-	-	-	-	A	A	-	-	-	-	-	A	C	-	A	-	-	-	-	C	C	-	C	C	C	-	C
AST-3	G	-	C	C	-	-	-	A	C	-	-	G	-	-	C	C	C	A	-	-	-	-	A	A	-	-	-	-	-	-	A	C	-	A	-	-	-	-	C	C	-	C	C	C	-	C
AM-1	-	A	A	C	N	C	A	-	-	G	C	-	-	-	C	C	C	-	-	G	G	-	A	T	-	A	-	C	C	T	-	A	A	-	-	-	T	C	-	-	T	-	C	C	G	-
AM-2	-	A	A	C	N	C	A	-	-	G	C	-	-	-	C	C	C	-	-	G	G	-	A	T	-	A	-	C	C	T	-	A	A	-	-	-	T	C	-	-	T	-	C	C	G	-

cension Island, St. Helena, and Cape Verde) trumpetfish exhibited moderate haplotype diversities (h = 0.105–0.456). Due to a haplotype endemic to samples from the mid-Atlantic ridge (Ascension and St. Helena) and the dearth of variation elsewhere, population structure was relatively high in *A. strigosus* (ϕ_{ST} = 0.585, $P < 0.001$).

Aulostomus maculatus

Two haplotypes, distinguished by a single transition, were observed in 22 individuals from two locations (Tables 1, 2). The Florida Keys (n = 15) exhibited low haplotype and nucleotide diversity (Table 1), and no variation was observed in the samples from Belize (n = 7). Population structure of *A. maculatus* in the Caribbean (as tested by AMOVA) was not significant ($P > 0.30$), but this test was hampered by low diversity and modest sample size. As noted above, samples of this species from coastal Brazil and St. Paul's Rocks revealed an *A. strigosus* haplotype.

Evolutionary History

The divergences between species are typical of intrageneric comparisons of marine fishes (Johns and Avise 1998); d = 0.044–0.054 between *A. chinensis* and *A. strigosus,* d = 0.063–0.069 between *A. chinensis* and *A. maculatus,* and d = 0.077–0.082 between *A. strigosus* and *A. maculatus.* An AMOVA, measuring the proportion of diversity within species versus between species yielded ϕ_{ST} = 0.984. Neighbor-joining and weighted parsimony analyses generated nearly identical tree topologies, with bootstrap support of 98–100% for species-level relationships. *Aulostomus maculatus* represents the deepest lineage in this genus, relative to a sister relationship between *A. chinensis* in the Indian-Pacific and *A. strigosus* in the Atlantic (Fig. 2).

Calibration of a molecular clock for *Aulostomus* is based on the closure of the Isthmus of Panama, with a final separation at 3.1–3.5 million years ago (Coates and Obando 1996). The division between Atlantic and Pacific trumpetfish species (d = 0.063–0.069) yields an estimated sequence divergence rate of 1.8–2.2%/million years, a value that encompasses the original benchmark for mtDNA divergence of 2%/million years between lineages (Brown et al. 1979). This conventional rate is used for subsequent discussions, with the recognition that departures from this rate (such as a slower clock and divergences prior to closure of the isthmus) would deepen the age (but not the pattern) of global dispersal and vicariant events.

The observation of an *A. strigosus* haplotype at 100% frequency in coastal Brazil and St. Paul's Rocks (reported previously as *A. maculatus* habitat) prompted us to examine the morphology of available specimens from this region. Based on two diagnostic morphological characters, we identified specimens from Brazil as *A. strigosus* (n = 2), *A. maculatus* (n = 23), and intermediate (n = 5; Table 3). Notably, 24 of these specimens from Saint Paul's Rocks and Espirito Santo were diagnosed with mtDNA as *A. strigosus.* This constitutes substantial evidence of introgression between the two Atlantic species in Brazil.

Discussion

Population Structure: Oceanic Barriers and Pelagic Dispersal

Genetic diversity was low overall in trumpetfish, although a few oceanic locations (Ascension and Hawaii) had high haplotype diversities. Population structure was also low in two of the three species. The test for population partitions within the Caribbean *A. maculatus* was not especially robust, but other surveys of marine fishes indicate that genetic structuring on this geographic scale is low or absent (Shulman and Bermingham 1995; Colborn et al. 2001; Muss et al. 2001). The widespread Atlantic *A. strigosus* had strong population structure (ϕ_{ST} = 0.585), due to the presence of a unique haplotype on the mid-Atlantic ridge islands of Ascension (13 of 19 individuals) and St. Helena (one of 19 individuals). This is the single exception to an overall pattern of low population structure.

The genetic distinction of Ascension Island, the most isolated reef habitat in the Atlantic, is observed in other reef species (Bermingham et al. 1997; Muss et al. 2001). These findings invoke a long-standing dilemma over how species with pelagic larvae persist on isolated islands. Either these populations are primarily self-seeding, with high larval retention in the vicinity of natal location, or they must be continuously replenished with larvae from elsewhere (Shultz and Cowen 1994). Recent research supports the larval retention model, with self-recruitment estimates of 15–89% for reef fish populations (Jones et al. 1999; Swearer et al. 1999). These levels of self-recruitment are consistent with the genetic isolation of Ascension. However, other oceanic locations are not genetically distinct (for trumpetfish or other species; Lessios et al. 1998), and self-recruitment rates of 15–89% leave plenty of room for dispersal, which require only a few colonizers per generation to genetically homogenize populations.

The population structure of *A. chinensis* (ϕ_{ST} = 0.093) is notably low for a reef-associated species distributed across two-thirds of the planet, at sites separated by large expanses of open ocean. The 4000–7000 km of deep water between the central and eastern Pacific is recognized as a prominent barrier to dispersal of shallow-water forms (Briggs 1961), but trumpetfish samples from Pacific Costa Rica (n = 9) and Hawaii (n = 16) share two of six haplotypes, and the corresponding population separation is not significant (ϕ_{ST} = 0.003). Clearly, these locations have been connected in recent evolutionary time, which is consistent with genetic surveys of other shallow-water coastal organisms (Rosenblatt and Waples 1986; Lessios et al. 1998).

The early life history of trumpetfish is nearly unknown (Fritzsche 1984), but may include an extended pelagic phase that would enhance opportunities for dispersal (Leis and Truski 1989). The evidence for this is scattered through published and unpublished accounts, but is based on three general observations: (1) Juveniles can occupy deep-water habitat. Smith-Vaniz et al. (1999) report juvenile trumpetfish at 1800 m depth off Bermuda, an unprecedented depth for species associated with shallow reef habitat. Bohlke and Chaplin (1993) also note that William Beebe ''collected the young in deep water off Bermuda.'' (2) Early life history includes

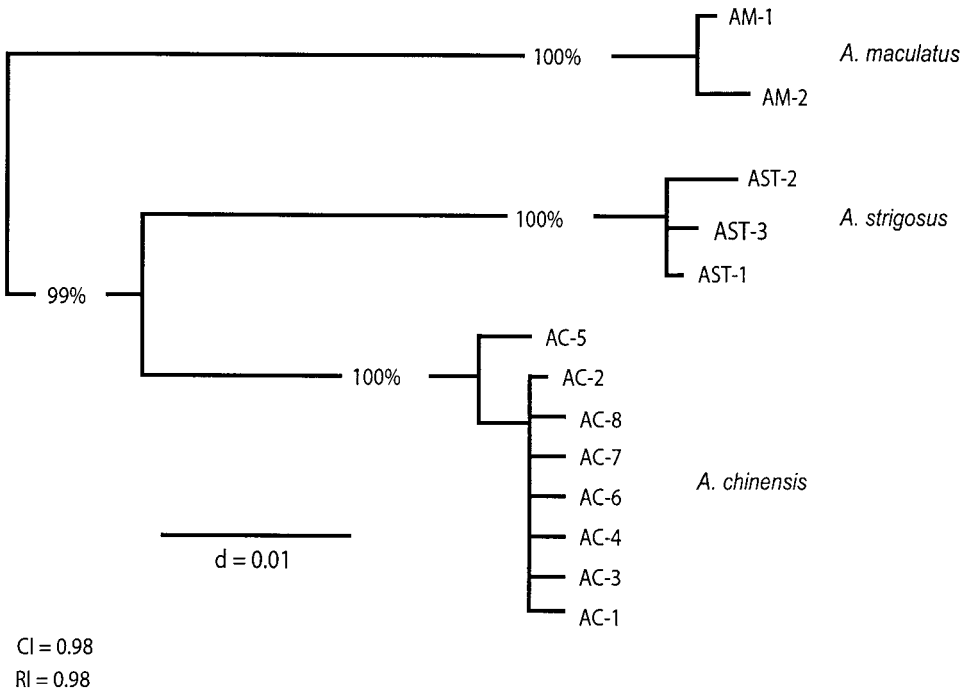

FIG. 2. Neighbor-joining tree for mitochondrial DNA haplotypes in trumpetfishes (*Aulostomus*). Bootstrap support is indicated on primary branches. The bar below the tree provides a distance scale. Weighted parsimony yielded an identical topology for species-level relationships and differed only in a minor rearrangement for haplotype AC-5. Consistency index (CI) and retention index (RI) are based on the unweighted parsimony analysis.

an extended pelagic stage. Juveniles of all three species (based on geographical considerations) have been collected in midoceanic plankton surveys (K. Hartel and A. Everly, pers. comm.), and juveniles of two species have been aged with otoliths. *Aulostomus maculatus* juveniles settling in Caribbean Panama measured 8.9–10.4 cm standard length and were aged at 80–94 days (D. Wilson, pers. comm.). Pelagic (or postpelagic) specimens of *A. chinensis* were measured at 11.6–15.1 cm standard length and aged at 71–116 days (A. Loyat, pers. comm.). (3) Adults are not restricted to shallow reefs. Large specimens of *A. chinensis* have been observed in caves deeper than 200 m in the Indian Ocean (H. Fricke and P. Heemstra, pers. comm.).

TABLE 3. (Top) Two of the diagnostic characters reported by Wheeler (1955) to distinguish the Atlantic species of trumpetfishes. (Below) The fish examined from locations in Brazil to determine species identifications. Museum voucher numbers are indicated in parentheses where applicable. MZUSP, Museum of Zoology at University of São Paulo, Brazil; USNM, U.S. National Museum.

Source	Sample size	Soft anal fin rays	Longitudinal scales	Species diagnosis
Wheeler (1955)	14–15[1]	22–25	220–240	*maculatus*
Wheeler (1955)	14	26	247–262	*strigosus*
Fernando de Noronha (MZUSP 14617, 52035)	2	24	247, 251	intermediate
Espirito Santo	8	25	—	*maculatus*[2]
	2	26, 27	—	*strigosus*[2]
(MZUSP 44,671)	1	24	262	intermediate
''Probably São Paulo'' (MZUSP, uncat.)	1	24	—	*maculatus*
Rio de Janeiro (USNM 34680)	1	25	270	intermediate
Cabo Frio	1	27	239	intermediate
St. Paul's Rocks	14	24	—	*maculatus*[2]

[1] Includes one specimen from Bahia, Brazil.
[2] Specimens used in the present study, containing the *A. strigosus* haplotype.

Evidently the trumpetfish is a versatile animal that uses epipelagic habitats as larvae and juveniles and deep substrates (as well as shallow reefs) as juveniles and adults. With habitats that extend to the continental slope and midoceanic currents, it is understandable that population structure is low in *A. chinensis.* Notwithstanding the genetic isolation of Ascension Island, we conclude that oceanic expanses and deep water are not substantial impediments to dispersal of trumpetfishes.

Phylogeography: Continents, Currents, and Climates

The mtDNA divergences between species are entirely consistent with our biogeographic hypothesis based on the morphological findings of Wheeler (1955). The deepest separation in mtDNA sequence divergence is between the populations in the West Atlantic and the other regions, and the closest relative of *A. strigosus* is not *A. maculatus,* as geography would indicate, but the Indian-Pacific *A. chinensis.* Together, these data indicate vicariant isolation between the trumpetfishes in the West Atlantic and Indian-Pacific, followed by isolation between the Indian-Pacific and the East Atlantic (Fig. 3). Whereas oceanic barriers are highly permeable to trumpetfishes, continental barriers have an indelible influence on the history of this group.

Under a conventional mtDNA mutation rate of 2%/million

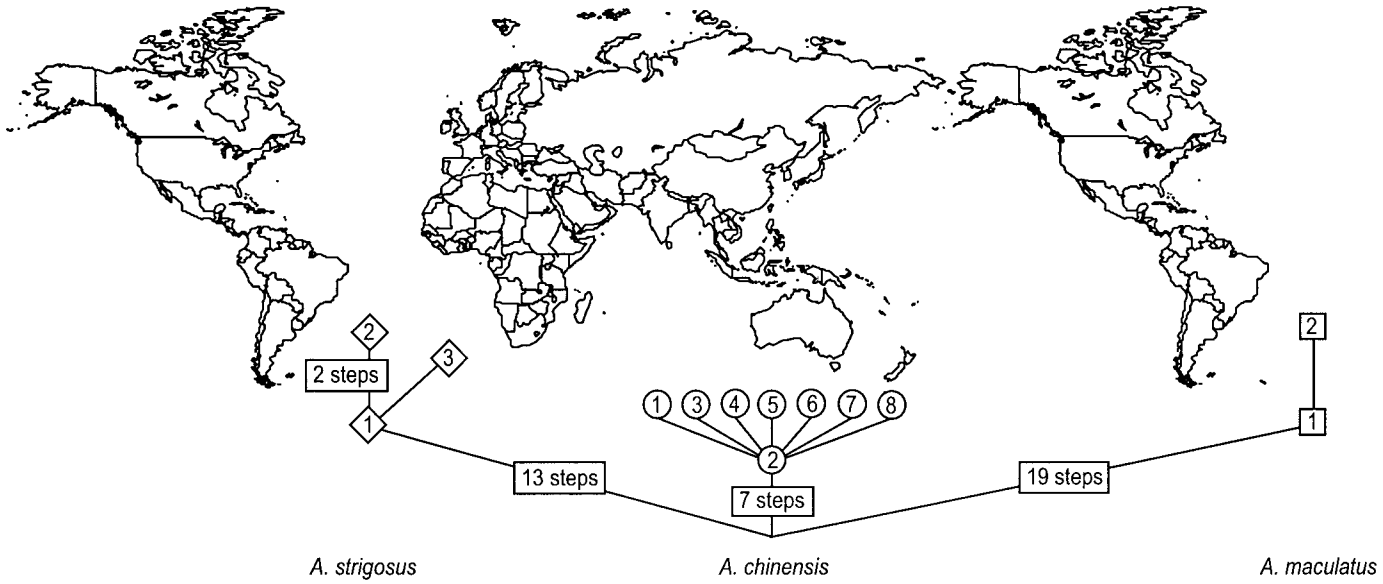

FIG. 3. A parsimony network describing relationships among ocean basins for *Aulostomus* based on 480 bp of mitochondrial DNA cytochrome *b*. These relationships indicate a vicariant separation of West Atlantic and Indian-Pacific populations, followed by colonization (or vicariant separation) of the South Atlantic from the Indian-Pacific. Squares indicate *A. maculatus* haplotypes (AM-1, 2), circles indicate *A. chinensis* haplotypes (AC-1–8), and diamonds indicate *A. strigosus* haplotypes (AST-1–3). Haplotype distributions within each ocean are described in Table 1.

years (Brown et al. 1979), the divergence between *A. maculatus* and the other species (d = 0.063–0.082) is consistent with a vicariant separation at the closure of the Isthmus of Panama (Lessios 1981; Bermingham and Lessios 1993; Coates and Obando 1996). Based on this calibration, the divergence of East Atlantic and Indian Ocean populations (d = 0.044–0.054) is estimated at about 2.5 million years ago. It is notable that the divergence between *A. maculatus* and *A. chinensis* 3–4 million years ago corresponds to a fixed difference in morphological characters, whereas the divergence between *A. chinensis* and *A. strigosus* 2.5 million years ago does not (see Wheeler 1955).

The connection between the Indian Ocean and East Atlantic was probably via the Cape of Good Hope. Smith (1949) favored this route to explain the distribution of coastal species that live on both sides of Africa. The Agulhas current, a narrow, fast-flowing body of tropical water, flows westward around the continental shelf of southern Africa, feeding Indian Ocean water into an Agulhas-Atlantic mixing area below South Africa (Shannon 1970). This current system promotes the transportation of Indian Ocean biota into the South Atlantic, as indicated by the observation of southern African kelp mats and associated fauna at St. Helena, the southernmost subtropical outpost on the mid-Atlantic ridge (Edwards 1990).

The *A. chinensis–A. strigosus* split, estimated at about 2.5 million years ago, coincides with the beginning of cold-water upwelling in South Africa (2.5 million years ago; Shannon 1985), and the onset of glacial cycles in the Pliocene (2.6–2.8 million years ago; Dwyer et al. 1995; Williams et al. 1997). This association raises a subtle point about vicariance and dispersal models. Perhaps the divergence of *A. chinensis* and *A. strigosus* was not due to a rare dispersal event that introduced Indian Ocean colonists into the Atlantic, but was caused by the vicariant sundering of a warm-water connection between the South Atlantic and Indian Oceans. The evolutionary consequences are indistinguishable here: allopatric isolation 2.5 million years ago. Dispersal and vicariance models are not always the stark alternatives they appear to be (Bowen and Grant 1997). In this case, expectations under either model converge on the same outcome.

The presence of an *A. strigosus* haplotype at 100% frequency in coastal Brazil and St. Paul's Rocks contradicts the accepted distribution of trumpetfish species. However, given the low level of population structure observed elsewhere, it is not surprising that the East Atlantic species has colonized Brazil. It is possible that this colonization was mediated by mid-Atlantic ridge islands such as Ascension and the westward flowing South Equatorial Current. Regardless of colonization route, all sampled individuals of *A. strigosus* share a common ancestor in very recent evolutionary time: The most divergent haplotypes are separated by only three nucleotide substitutions. While this could indicate recent colonization of Brazil, it could alternately reflect an ongoing genetic connection between East, Central, and West Atlantic.

The relationships between trumpetfish species has a parallel in the circumtropical ridley sea turtles (*Lepidochelys*). Based on morphological and molecular comparisons, a West Atlantic species (*L. kempi*) was separated from an Indian-Pacific congener (*L. olivacea*) by the rise of the Isthmus of Panama (Pritchard 1969; Bowen et al. 1998). Subsequently, *L. olivacea* invaded the Atlantic via southern Africa and has spread up the African coast at least to Guinea Bissau and up the South American coast to the Guyanas (Pritchard and Trebbau 1984). Unlike the trumpetfish, this Atlantic invasion appears to be relatively recent (within a few tens of thousands of years; Bowen et al. 1998). This sea turtle invasion also has progressed north of the Amazon plume, which represents

a strong barrier to interconnections between reef fish populations of the Caribbean and Brazil (e.g., Muss et al. 2001). Like the trumpetfish, globe-spanning ridley lineages appear to be at the point of imminent contact in the West Atlantic. Many marine species groups have a history of vicariant separations due to the rise of the Isthmus of Panama (Lessios 1981; Knowlton et al. 1993; Bermingham et al. 1997), and many species groups have a record of exchange between Atlantic and Indian-Pacific basins via southern Africa (Briggs 1974; Graves 1998). Both these phenomena create allopatric distributions and corresponding opportunities for speciation. Combining these two processes invokes another possibility: renewed contact between sister lineages that have been isolated for millions of years.

Evolution: Do Marine Species Coalesce?

The process by which marine populations diverge, speciate, and possibly coalesce has received little attention until recently (Sinclair and Iles 1989; Cunningham and Collins 1994; Palumbi 1996). Several global mtDNA phylogenies for tropical marine taxa show evidence of long-term isolation between Atlantic and Indian-Pacific Oceans (Bowen et al. 1992; Baldwin et al. 1998; Grant and Bowen 1998; Graves 1998). In at least some species, these periods of isolation are punctuated by sporadic gene flow between Indian and Atlantic Ocean basins, a phenomenon apparent in fishes (Grant 1987; Grant and Bowen 1998; Graves 1998; the present study), marine turtles (Bowen et al. 1994, 1998; Dutton et al. 1999), marine mammals (Baker et al. 1994), and sea birds (Avise et al. 2000).

The ring species was originally conceived as a group of subspecies with a circuitous distribution, in which the terminal taxa overlap and retain reproductive isolation (Cain 1954; Dobzhansky 1958). This pattern was invoked to argue for gradual allopatric divergence as a predominant mode of speciation (Mayr 1963, 1982; Frost and Hillis 1990). However, few examples of ring species have been documented, and this phenomenon is most thoroughly examined in salamanders of western North America (Stebbins 1949; Wake et al. 1986; Moritz et al. 1992). Are trumpetfishes a ring species complex? This case departs from the original ring species model because the most divergent taxa appear to hybridize in sympatry (Table 3). Nonetheless, evolutionary separations are indicated by both morphology and genetics, and these separations have a ringlike distribution. Indeed, the trumpetfish more closely fit the ring imagery than previous examples, because this ring is closed by introgressive hybridization. We suggest that the ring species concept should include the taxa that hybridize in renewed contact, where the ring closes. This pattern of isolation and episodic reconnection may be relatively common in circumtropical marine organisms and may be an important element of evolutionary processes in the sea.

Veron (1995) hypothesized that coral species are isolated by oceanic barriers for millions of years, but can subsequently coalesce when currents, climate, or geography reconnect these geminate species. The same phenomenon occurs in marine vertebrates. The Isthmus of Panama isolated entire suites of tropical marine taxa 3–4 million years ago (Lessios 1981; Knowlton et al. 1993; Lessios et al. 1998), but some groups have come back into contact via a globe-spanning series of dispersal events. How do these groups respond to reconnections? Some large oceanic migrants show clear evidence of introgression of Indian-Pacific lineages into Atlantic populations (Bowen et al. 1994; Graves 1998). In these species, episodic contact between Atlantic and Indian-Pacific is frequent enough (over tens to hundreds of thousands of years) to reduce opportunities for allopatric speciation (see Dutton et al. 1999). In other groups, colonization between Atlantic and Indian-Pacific Oceans appears to proceed at a slower pace (over millions of years), and this longer time frame may allow sister species to retain reproductive integrity in sympatry (Grant 1987; Grant and Leslie 1993; Colborn et al. 2001).

After 3–4 million years of isolation, trumpetfishes are at the critical junction of renewed sympatry. In this case, the terminal ends of the phylogeny appear to be fusing, a prospect that defies conventional systematic treatment. This appraisal would benefit from investigations of the nuclear genome (Karl et al. 1995; Quattro and Jones 1999) to more thoroughly characterize the suspected introgression in Brazil. In these circumstances of renewed sympatry, other species may retain their integrity, some will vanish due to competition, and some will fuse via hybridization. Documenting how sister evolutionary lineages respond to contact and renewed sympatry is essential to understanding how marine biodiversity is produced and maintained. Veron's (1995) phenomenon is probably an important regulator of marine biodiversity, and global ring species complexes may manifest this process at the largest geographic scale.

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Literature Cited

Allen, G. R., and D. R. Robertson. 1994. Fishes of the tropical eastern Pacific. Univ. of Hawaii Press, Honolulu, HI.

Alvarado-Bremer, J. R., J. Mejuto, T. Greig, and B. Ely. 1996. Global population structure of the swordfish (*Xiphias gladius L.*) as revealed by analysis of the mitochondrial DNA control region. J. Exp. Mar. Biol. Ecol. 197:295–310.

Amos, B., and A. R. Hoelzel. 1991. Long-term preservation of whale skin for DNA analysis. Rep. Int. Whal. Comm. (Special Issue) 13:99–103.

Avise, J. C. 2000. Phylogeography: the history and formation of species. Harvard Univ. Press, Cambridge, MA.

Avise, J. C., and D. Walker. 2000. Abandon all species concepts? A response. Conserv. Gen. 1:77–80.

Avise, J. C., D. Walker, and G. C. Johns. 1998. Speciation durations and Pleistocene effects on vertebrate phylogeography. Proc. R. Soc. Lond. B 265:1702–1712.

Avise, J. C., W. S. Nelson, B. W. Bowen, and D. Walker. 2000. Phylogeography of colonially nesting seabirds, with special reference to global matrilineal patterns in the sooty tern (*Sterna fuscata*). Mol. Ecol. 9:1783–1792.

Baker, C. S., R. W. Slade, J. L. Bannister, R. B. Abernethy, M. T. Weinrich, J. Lein, J. Urban, P. Corkeron, J. Calmabokidis, O. Vasquez, and S. R. Palumbi. 1994. Hierarchical structure of mitochondrial DNA gene flow among humpback whales, *Megaptera novaeangliae,* world-wide. Mol. Ecol. 3:313–328.

Baldwin, J. D., A. L. Bass, B. W. Bowen, and W. H. Clark Jr. 1998. Molecular phylogeny and biogeography of the marine shrimp genus *Penaeus.* Mol. Phylogenet. Evol. 10:399–407.

Bermingham, E., and H. A. Lessios. 1993. Rate variation of protein and mtDNA evolution as revealed by sea urchins separated by the Isthmus of Panama. Proc. Natl. Acad. Sci. USA 90: 2734–2738.

Bermingham, E., S. S. McCafferty, and A. P. Martin. 1997. Fish biogeography and molecular clocks: perspectives from the Panamanian Isthmus. Pp. 113–128 *in* T. D. Kocher and C. A. Stepien, eds. Molecular systematics of fishes. Academic Press, New York.

Bernardi, G. 2000. Barriers to gene flow in *Embiotoca jacksoni,* a marine fish lacking a pelagic larval stage. Evolution 54:226–237.

Bohlke, J. E., and C. C. G. Chaplin. 1993. Fishes of the Bahamas and adjacent tropical waters. 2d ed. Univ. of Texas Press, Austin, TX.

Bowen, B. W., and W. S. Grant. 1997. Phylogeography of the sardines (*Sardinops* spp.): assessing biogeographic models and population histories in temperate upwelling zones. Evolution 51: 1601–1610.

Bowen, B. W., A. B. Meylan, J. P. Ross, C. J. Limpus, G. H. Balazs, and J. C. Avise. 1992. Global population structure and natural history of the green turtle (*Chelonia mydas*) in terms of matriarchal phylogeny. Evolution 46:865–881.

Bowen, B. W., N. Kamezaki, C. J. Limpus, G. H. Hughes, A. B. Meylan, and J. C. Avise. 1994. Global phylogeography of the loggerhead turtle (*Caretta caretta*) as indicated by mitochondrial DNA haplotypes. Evolution 48:1820–1828.

Bowen, B. W., A. M. Clark, F. A. Abreu-Grobois, A. Chavez, H. Reichart, and R. J. Ferl. 1998. Global phylogeography of the ridley sea turtles (*Lepidochelys* spp.) inferred from mitochondrial DNA sequences. Genetica 101:179–189.

Briggs, J. C. 1961. The East Pacific barrier and the distribution of marine shore fishes. Evolution 15:545–554.

———. 1974. Marine zoogeography. McGraw Hill, New York.

Brown, W. M., M. George Jr.,and A. C. Wilson. 1979. Rapid evolution of animal mitochondrial DNA. Proc. Natl. Acad. Sci. USA 76:1967–1971.

Cain, A. J. 1954. Animal species and their evolution. Hutchinson University Library, London.

Coates, A. G., and J. A. Obando. 1996. The geologic evolution of the Central American isthmus. Pp. 21–56 *in* J. Jackson, A. F. Budd, and A. G. Coates, eds. Evolution and environment in tropical America. Univ. of Chicago Press, Chicago, IL.

Colborn, J., R. E. Crabtree, J. B. Shaklee, E. Pfeiler, and B. W. Bowen. 2001. The evolutionary enigma of bonefish (*Albula* spp.): cryptic species and ancient separations in a globally distributed shorefish. Evolution 55:807–820.

Crosetti, D., W. S. Nelson, and J. C. Avise. 1994. Pronounced genetic structure of mitochondrial DNA among populations of the circumglobally distributed grey mullet (*Mugil cephalus*). J. Fish Biol. 44:47–58.

Cunningham, C. W., and T. M. Collins. 1994. Developing model systems for molecular biogeography: vicariance and interchange in marine invertebrates. Pp. 405–433 *in* B. Shierwater, B. Streit, G. P. Wagner, and R. DeSalle, eds. Molecular ecology and evolution: approaches and applications. Birkhauser Verlag, Berlin.

Dizon, A. E., S. J. Chivers, and W. F. Perrin, eds. 1997. Molecular genetics of marine mammals. Society for Marine Mammology, Spec. Pub. no. 3.

Dobzhansky, T. 1958. Species after Darwin. Pp. 19–55 *in* S. A. Barnett, ed. A century of Darwin. Heinemann, London.

Doherty, P. J., S. Planes, and P. Mather. 1995. *Acanthochromis polyacanthus,* a fish lacking larval dispersal has genetically differentiated populations at local and regional scales on the Great Barrier Reef. Mar. Biol. 121:11–21.

Dutton, P. H., B. W. Bowen, D. W. Owens, A. Barragan, and S. K. Davis. 1999. Global phylogeography of the leatherback turtle, *Dermochelys coriacea.* J. Zool.: 248:397–409.

Dwyer, G. S., T. M. Cronin, P. A. Baker, M. E. Raymo, J. S. Buzas, and T. Correge. 1995. North Atlantic deepwater temperature change during late Pliocene and late Quaternary climatic cycles. Science 270:1347–1351.

Edwards, A. 1990. Fish and fisheries of Saint Helena Island. Center for Tropical Coastal Management Studies, Univ. of Newcastle upon Tyne, U.K.

Edwards, A. J., and C. W. Glass. 1987. The fishes of Saint Helena Island, South Atlantic Ocean. I. The shore fishes. J. Nat. Hist. 21:617–686.

Edwards, A. J., and R. Lubbock. 1983. Marine zoogeography of St. Paul's rocks. J. Biogeogr. 10:65–72.

Excoffier, L., P. E. Smouse, and J. M. Quattro. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics 131:479–491.

Floeter, S. R., J. L. Gasparini, L. A. Rocha, R. Z. P. Guimarães, C. E. L. Ferreira, C. A. Rangel, B. M. Feitoza, and G. W. Nunan. 2001. Brazilian reef fish fauna: checklist and remarks. BioBase Project: http://www.biobase.org/BCF/Index.html

Fowler, H. W. 1942. A list of fishes known from the coast of Brazil. Aquivos Zool. Estado Sao Paulo 3:115–184.

Fritzsche, R. A. 1984. Gasterosteiformes: development and relationships. Pp. 398–405 *in* H. G. Moser, W. J. Richards, D. M. Cohen, M. P. Fahay, A. W. Kendall Jr., and S. L. Richardson, eds. Ontogeny and systematics of fishes. American Society of Ichthyologists and Herpetologists, Spec. Pub. no. 1.

Frost, D. R., and D. M. Hillis. 1990. Species in concept and practice: herpetological application. Herpetologica 46:87–104.

Grant, W. S. 1987. Genetic divergence between congeneric Atlantic and Pacific Ocean fishes. Pp. 225–246 *in* R. Ryman and F. M. Utter, eds. Population genetics and fishery management. Univ. of Washington Press, Seattle.

Grant, W. S., and B. W. Bowen. 1998. Shallow population histories in deep evolutionary lineages of marine fishes: insights from the sardines and anchovies and lessons for conservation. J. Hered. 89:415–426.

Grant, W. S., and R. W. Leslie. 1993. Biochemical divergence and biogeography of anglerfish of the genus *Lophius* (Lophiiformes). J. Zool. Lond. 231:465–485.

———. 1996. Late Pleistocene dispersal of Indian-Pacific sardine populations in an ancient lineage of the genus *Sardinops*. Mar. Biol. 126:133–142.
Graves, J. E. 1998. Molecular insights into the population structure of cosmopolitan marine fishes. J. Hered. 89:427–437.
Graves, J. E., and A. E. Dizon. 1989. Mitochondrial DNA sequence similarity of Atlantic and Pacific albacore tuna (*Thunnus alalunga*). Can. J. Fish. Aquatic. Sci. 46:870–873.
Graves, J. E., and J. R. McDowell. 1995. Inter-ocean genetic divergences of istiophorid billfishes. Mar. Biol. 122:193–203.
Hasegawa, M., H. Kishino, and T.-A. Yano. 1985. Dating of the ape-human splitting by a molecular clock of mitochondrial DNA. J. Mol. Evol. 22:160–174.
Hatta, M., H. Fukami, W. Wang, M. Omori, K. Shimoike, T. Hayashibara, Y. Ina, and T. Sugiyama. 1999. Reproductive and genetic evidence for a reticulate evolutionary history of mass-spawning corals. Mol. Biol. Evol. 16:1607–1613.
Hedgecock, D. 1986. Is gene flow from pelagic larval dispersal important in the adaptation and evolution of marine invertebrates? Bull. Mar. Sci.: 39:550–564.
Hillis, D. M., B. K. Mable, A. Larson, S. K. Davis, and E. A. Zimmer. 1996. Nucleic acids. IV. Sequencing and cloning. Pp. 321–381 *in* D. M. Hillis, C. Moritz, and B. K. Mable, eds. Molecular systematics. 2d ed. Sinauer Associates, Sunderland, MA.
Hubbs, C. L. 1952. Antitropical distribution in fishes and other organisms. Pp. 324–329 *in* Proceedings of the seventh Pacific Science Congress Vol. 3. Bishop Museum Press, Honolulu, HI.
Johns, G. C., and J. C. Avise. 1998. A comparative summary of genetic distances in the vertebrates from the mitochondrial cytochrome *b* gene. Mol. Biol. Evol. 15:1481–1490.
Jones, G. P., M. J. Milicich, M. J. Emslie, and C. Nunow. 1999. Self-recruitment in a coral reef fish population. Nature 402: 802–804.
Karl, S. A., B. W. Bowen, and J. C. Avise. 1995. Hybridization among the ancient mariners: identification and characterization of marine turtle hybrids with molecular genetic assays. J. Hered. 86:262–268.
Kaufman, L. 1976. Feeding behavior and functional coloration of the Atlantic trumpetfish, *Aulostomus maculatus*. Copeia 1976: 377–378.
Kidd, M. G., and V. L. Friesen. 1998. Patterns of control region variation in populations of *Cepphus* guillemots: testing microevolutionary hypotheses. Evolution 52:1158–1168.
Knowlton, N., L. A. Weigt, L. A. Solorzano, D. K. Mills, and E. Bermingham. 1993. Divergence in proteins, mitochondrial DNA, and reproductive compatibility across the Isthmus of Panama. Science 260:1629–1632.
Lansman, R. A., R. O. Shade, J. F. Shapira, and J. C. Avise. 1981. The use of restriction endonucleases to measure mitochondrial DNA sequence relatedness in natural populations. III. Techniques and potential applications. J. Mol. Evol. 17:214–226.
Leis, J. M., and T. Truski. 1989. The larvae of Indo-Pacific shorefishes. Univ. of Hawaii Press, Honolulu, HI.
Lessios, H. A. 1981. Divergence in allopatry: molecular and morphological differentiation between sea urchins separated by the Isthmus of Panama. Evolution 35:618–634.
Lessios, H. A., B. D. Kessing, and D. R. Robertson. 1998. Massive gene flow across the world's most potent marine biogeographic barrier. Proc. R. Soc. London B 265:583–588.
Lessios, H. A., B. D. Kessing, D. R. Robertson, and G. Pauly. 1999. Phylogeography of the pantropical sea urchin *Eucidia* in relation to land barriers and ocean currents. Evolution 53:806–817.
Lubbock, R. 1980. The shore fishes of Ascension Island. J. Fish Biol. 17:283–303.
Lubbock, R., and A. Edwards. 1981. The fishes of St. Paul's rocks. J. Fish Biol. 18:135–157.
Mayr, E. 1963. Animal species and evolution. Belknap Press, Cambridge, MA.
———. 1982. Processes of speciation in animals. Pp. 1–19 *in* C. Barigozzi, ed. Mechanisms of speciation. Alan R. Liss, New York.
Meyer, A. 1994. DNA technology and phylogeny of fish: molecular phylogenetic studies of fish. Pp. 219–249 *in* A. R. Beaumont, ed. Genetics and evolution of aquatic organisms. Chapman and Hall, New York.
Moritz, C., C. J. Schneider, and D. B. Wake. 1992. Evolutionary relationships within the *Ensatina eschscholtzii* complex confirm the ring species interpretation. Syst. Biol. 41:273–291.
Muss, A., D. R. Robertson, C. A. Stepien, P. Wirtz, and B. W. Bowen. 2001. Phylogeography of the genus *Ophioblennius*: the role of oceanic currents and geography in reef fish evolution. Evolution 55:561–572.
Nei, M. 1987. Molecular evolutionary genetics. Columbia Univ. Press, New York.
Nei, M., and S. Kumar. 2000. Molecular evolution and phylogenetics. Oxford Univ. Press, Oxford, U.K.
Page, R. D. M., and E. C. Holmes. 1998. Molecular evolution; a phylogenetic approach. Blackwell Science, Oxford, U.K.
Palumbi, S. R. 1996. Macrospatial genetic structure and speciation in marine taxa with high dispersal abilities. Pp. 101–118 *in* J. R. Ferraris and S. R. Palumbi, eds. Molecular zoology; advances, strategies, and protocols. Wiley-Liss, New York.
Pritchard, P. C. H. 1969. Studies of the systematics and reproductive cycles of the genus *Lepidochelys*. Ph.D. diss., University of Florida, Gainesville, FL.
Pritchard, P. C. H., and P. Trebbau. 1984. The turtles of Venezuela. Contrib. Herpetol. no. 2, Society for the Study of Amphibians and Reptiles, Fundacion de Internados Rurales, Caracas, Venezuela.
Quattro, J. M., and W. J. Jones. 1999. Amplification primers that target locus-specific introns in Actinopterygian fishes. Copeia 1999:191–196.
Randall, J. E. 1983. Red Sea reef fishes. Immel, London.
———. 1996. Caribbean reef fishes. 3rd ed. T. F. H. Publications, Neptune City, NJ.
Robins, C. R., G. C. Ray, and J. Douglass. 1986. A field guide to Atlantic coast fishes of North America. Houghton Mifflin, Boston.
Rocha, L. A., I. L. Rosa, and R. S. Rosa. 1998. Peixes recifais da costa da Paraiba, Brasil. Rev. Bras. Zool. 15:553–566.
Rosel, P. E., A. E. Dizon, and M. G. Heygood. 1995. Variation of the mitochondrial control region in populations of the harbour porpoise, *Phocoena phocoena*, on interoceanic and regional scales. Can. J. Fish. Aquatic. Sci. 52:1210–1219.
Rosenblatt, R. H., and R. S. Waples. 1986. A genetic comparison of allopatric populations of shore fish species from the eastern and central Pacific Ocean: dispersal or vicariance? Copeia 1986: 275–284.
Saitou, N., and M. Nei. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol. 4:406–425.
Schneider, S., J.-M. Kueffer, D. Roessli, and L. Excoffier. 1997. Arlequin ver. 1.1: a software for population genetic data analysis. Genetics and Biometry Laboratory, Univ. of Geneva, Switzerland.
Schultz, E. T., and R. K. Cowen. 1994. Recruitment of coral reef fishes to Bermuda: local retention or long-distance transport? Mar. Ecol. Prog. Ser. 109:15–28.
Scoles, D. R., B. B. Collette, and J. E. Graves. 1998. Global phylogeography of the mackerels of the genus *Scomber*. Fish. Bull. 96:823–842.
Shannon, L. V. 1970. Ocean circulation off South Africa. Fish. Bull. S. Afr. 6:27–33.
———. 1985. The Benguela ecosystem. I. Evolution of the Benguela physical features and processes. Oceanogr. Mar. Biol. Annu. Rev. 23:105–182.
Shulman, M. J., and E. Bermingham. 1995. Early life histories, ocean currents, and the population genetics of Caribbean reef fishes. Evolution 49:897–910.
Sinclair, M. 1988. Marine populations: an essay on population regulation and speciation. Univ. of Washington Press, Seattle, WA.
Sinclair, M., and T. D. Iles. 1989. Population regulation and speciation in the oceans. J. Cons. Int. Explor. Mer 45:165–175.
Smith, J. L. B. 1949. Sea fishes of southern Africa. Central News Agency, Cape Town, South Africa.

Smith, M. M., and P. C. Heemstra. 1986. Smith's sea fishes. Springer-Verlag, Berlin.

Smith-Vaniz, W., B. Collette, and B. Luckhurst. 1999. Fishes of Bermuda. American Society of Ichthyologists and Herpetologists, Spec. Pub. no. 4.

Stebbins, R. C. 1949. Speciation in salamanders of the plethodontid genus *Ensatina.* Univ. Calif. Publ. Zool. 48:377–526.

Stepien, C. A., and R. H. Rosenblatt. 1996. Genetic divergence in antitropical marine fishes (*Trachurus, Merluccius,* and *Scomber*) between North and South America. Copeia 1996:586–598.

Swearer, S. E., J. E. Caselle, D. W. Lea, and R. R. Warner. 1999. Larval retention and recruitment in an island population of coral-reef fish. Nature 402:799–802.

Swofford, D. 1998. PAUP* 4.0: phylogenetic analysis using parsimony. Beta vers. 4.0b2. Sinaeur Associates, Sunderland, MA.

Taberlet, P., A. Meyer, and J. Bouvet. 1992. Unusually large mitochondrial variation in populations of the blue tit, *Parus caeruleus.* Mol. Ecol. 1:27–36.

Veron, J. E. N. 1995. Corals in space and time: the biogeography and evolution of the Scleractinia. Comstock/Cornell Univ. Press, Ithaca, NY.

Wake, D. B., K. P. Yanev, and C. W. Brown. 1986. Intraspecific sympatry in a ''ring species,'' the plethodontid salamander *Ensatina eschscholtzii,* in southern California. Evolution 40: 866–868.

Wheeler, A. C. 1955. A preliminary revision of the fishes of the genus *Aulostomus.* Ann. Mag. Nat. Hist. Zool. Bot. Geol. (12)8: 613–623.

Williams, D. F., J. Peck, E. B. Karabanov, A. A. Prokopenko, V. Kravchinsky, J. King, and M. I. Kuzmin. 1997. Lake Baikal record of continental climate response to orbital insolation during the past five million years. Science 278:1114–1117.

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