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THE EVOLUTIONARY ENIGMA OF BONEFISHES (*ALBULA* SPP.): CRYPTIC SPECIES AND ANCIENT SEPARATIONS IN A GLOBALLY DISTRIBUTED SHOREFISH

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Abstract.—Many examples of cryptic marine species have been demonstrated with biochemical and molecular studies. In most cases, a broadly distributed taxon is actually a group of sibling species that can be distinguished (upon closer examination) by ecological or morphological characters. Fishes of the family Albulidae constitute a notable exception. Bonefish (*Albula* spp.) morphology and ecology are highly conserved around the globe, and their extended pelagic larval stage could allow population connections on a vast geographic scale. Based on this perceived homogeneity, bonefishes were classified as a single pantropical species, *A. vulpes.* However, allozyme studies of Hawaiian populations indicated that two sympatric species (*A. glossodonta* and *A. neoguinaica*) are included in the synonymy of *A. vulpes.* To ascertain the number and distribution of evolutionary partitions in *Albula,* we surveyed 564 bp of mitochondrial DNA (mtDNA) cytochrome *b* from 174 individuals collected at 26 locations. Sequence comparisons reveal eight deep lineages (d = 5.56–30.6%) and significant population structure within three of the four lineages that could be tested (ϕ_{ST} = 0.047–0.678). These findings confirm the genetic distinctiveness of the three species noted above and invoke the possibility of five additional species. Clock estimates for mtDNA indicate that these putative species arose 4–20 million years ago. Distinct evolutionary lineages coexist in several sample locations, yet show little morphological or ecological differentiation in sympatry. Thus, bonefish species seem to defy the evolutionary conventions of morphological differentiation over time and ecological displacement in sympatry. Despite multiple cases of sympatry, sister-taxa relationships inferred from mtDNA indicate that divergence in allopatry has been the predominant speciation mechanism in *Albula.* Stabilizing selection in the homogeneous habitat occupied by bonefishes (tropical sand flats) could promote the retention of highly conserved morphology and ecology.

Key words.—Cytochrome *b,* leptocephalus, marine fish, mitochondrial DNA, phylogeography, speciation, stabilizing selection, sympatry.

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Speciation and corresponding ecological and morphological radiations embody the central riddle of modern evolutionary biology. Much progress has been made in understanding speciation mechanisms and consequences (Barton and Charlesworth 1984; Carson and Templeton 1984; Giddings et al. 1989; Otte and Endler 1989; Avise and Ball 1990; Smith et al. 1997), but most of these advances are based on terrestrial organisms. Speciation in marine organisms is not extensively documented, and there is a growing recognition that processes of evolutionary radiation may be different in the sea (Palumbi 1992; Knowlton 2000). For example, vicariant separations due to habitat discontinuities (and corresponding allopatric divergences) are a mainstay of terrestrial speciation, but are habitat discontinuities relevant for oceanic species? The mechanisms of speciation may be different in marine systems, due to the size and connectivity of marine habitats and to the high potential for dispersal in a transglobal aquatic medium. Alternately, the same rules may apply, but on vastly different geographical and temporal scales.

Molecular and biochemical methods have already proven useful for illuminating aspects of marine speciation. One of the first generalizations to emerge from these studies is that cosmopolitan (or widely distributed) ''species'' are often a taxonomic blanket for multiple evolutionary partitions (Grassle and Grassle 1976; Knowlton 1993; Garcia-Rodriguez et al. 1998). Upon closer examination, these cryptic taxa usually show some measure of morphological or ecological differentiation (see examples in Palumbi 1996; Lessios et al. 1999).

Bonefishes (*Albula* spp.) are one of the few examples of a cosmopolitan distribution in shorefishes (Briggs 1960); they are common denizens of shallow sand flats and grass flats in all tropical seas. Following the original description of *A. vulpes* by Linnaeus (1758), 23 nominal species were described; all of which were synonymized under *A. vulpes* by 1940 (reviewed in Whitehead 1986). In addition to the circumtropical *A. vulpes,* the genus *Albula* also includes the geographically restricted and morphologically distinct *A. nemoptera,* formerly in the genus *Dixonina* (for a discussion of the poorly known *A. nemoptera,* see Rivas and Warlen 1967). This study is restricted to the globally distributed *A. vulpes* (*sensu lato*).

Shaklee and Tamaru (1981) first challenged the validity of a single worldwide species, based on a Nei's $D \approx 1.2$ between sympatric bonefishes in Hawaii. Subsequently, Pfeiler (1996) found a distance of D = 0.19 between bonefishes in the Caribbean and Gulf of California. Both studies indicated the presence of cryptic species within the circumtropical *A. vulpes.* Three species are presently recognized within this complex: *A. glossodonta* (Forsskål 1775) and *A. neoguinaica* (Valenciennes 1847 in Cuvier and Valenciennes 1847) with uncertain distributions in the Pacific (Shaklee et al. 1982) and *A. vulpes* in the Atlantic. (Randall and Bauchot [1999] make a compelling argument that *A. neoguinaica* is a junior synonym of *A. forsteri.* To avoid confusion with previous literature, the former name is used throughout this paper.)

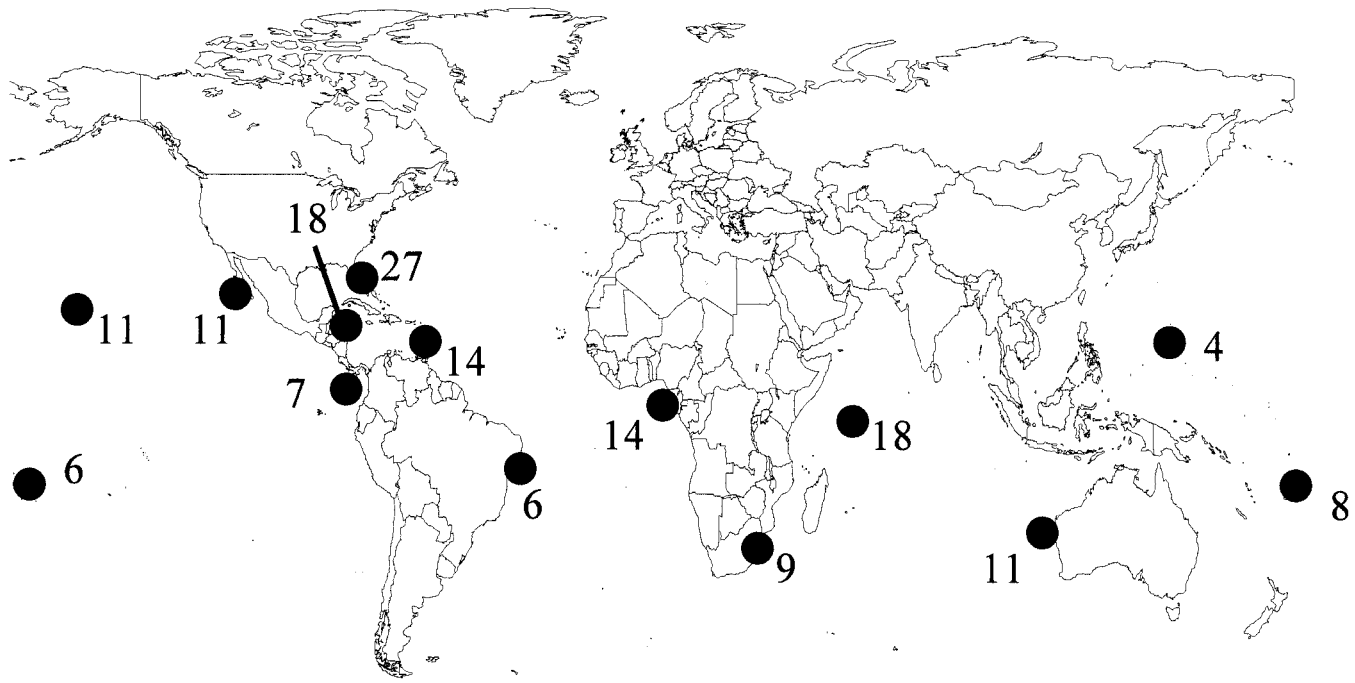

FIG. 1. Collection sites and corresponding sample sizes for *Albula* spp., for all sites having sample sizes greater than $n = 3$. Different collection sites in the same region were pooled on the map (see Appendix).

In contrast to the genetic results, there is a dearth of diagnostic morphological characters to distinguish bonefish populations worldwide (Briggs 1960; Alexander 1961; Whitehead 1986). Differences between *A. glossodonta* and *A. neoguinaica* are slight, with external diagnostic differences only in the length of the maxilla (upper jaw; Shaklee and Tamaru 1981; Randall and Bauchot 1999). *Albula vulpes* and an undescribed species in the Caribbean are also difficult to distinguish (R. E. Crabtree, pers. obs.). In both cases, these sympatric species are routinely captured in the same habitats, sometimes in the same net haul, so that ecological differences are not apparent. These observations define the evolutionary enigma of bonefishes: morphological and ecological homogeneity in a cosmopolitan species complex.

Here we apply molecular methods to bonefish populations on a global scale, with the objective of resolving evolutionary relationships and patterns of species distributions. Recent allozyme studies have aided in the verification of three bonefish species, two more are in the process of being described (R. E. Crabtree and E. Pfeiler, unpubl. data) and additional species may exist in unsampled areas. We have chosen mitochondrial DNA (mtDNA) cytochrome *b* sequence comparisons to complement the allozyme studies. This geneological approach, combined with available knowledge of ecology and morphology, provides the foundation to address several questions about the evolution of bonefishes and speciation in marine systems: (1) What are the limits of larval dispersal, and how does this influence opportunities for allopatric speciation? Here we hypothesize that dispersal is more restricted in the bonefish than the extended larval stage (Pfeiler et al. 1988; Mojica et al. 1995) would indicate, enhancing opportunities for alloptric speciation. (2) Can phylogeographic patterns provide clues to the origins of species in the genus *Albula*? The evolutionary relationships among coexisting species are of primary interest, especially to test for the possibility of sympatric speciation. (3) Is the morphological and ecological homogeneity of this circumtropical group due to recent divergences or to retention of conserved traits across long evolutionary time scales? The available evidence supports the latter explanation. If this explanation holds up, then what restricts morphological and ecological differentiation between (often sympatric) bonefish species that diverged many millions of years ago?

MATERIALS AND METHODS

Most of the samples used for this project were obtained for allozyme work (J. B. Shaklee and C. S. Tamaru, unpubl. data). A few fish were purchased in coastal fish markets, but most were caught on hook-and-line or in cast nets and beach seines in water less than 1 m deep (Appendix). Additional specimens were caught on hook-and-line in 10–30 m, and a small number were obtained from head boats working at depths greater than 30 m. Most specimens were frozen prior to sampling (some as long as 17 years), but all samples were placed into saturated salt buffer (Amos and Hoelzel 1991) for storage prior to mtDNA studies. Tissues from individual fish came from fin clips, gill raker, muscle, heart, or liver. The total sample size consists of 174 individuals from 26 locations, including every major ocean basin where bonefishes live except the Red Sea (see Fig. 1 for representative locations).

Details about collection locations, tissue types, and sample sizes are provided in the Appendix. Vouchers for a subset of specimens are housed in the Florida Museum of Natural History, Florida Marine Research Institute, the Australian Mu-

seum, the Northern Territory Museum, the Arizona State University fish collection, and the University of Washington fish collection. Tissue samples used in this analysis are stored at the Department of Fisheries and Aquatic Sciences at the University of Florida.

DNA was extracted from tissue following either a standard phenol-chloroform recipe (Hillis et al. 1996) or a lithium chloride DNA isolation protocol (Colborn 1999). The isolated DNA was then pelleted by centrifugation, rinsed with ethanol, dried in a vacuum, and resuspended in 200 μl of TE buffer.

Preliminary trials with universal cytochrome *b* primers (Kessing et al. 1989) yielded inconsistent results, so primers were designed specifically to amplify bonefish DNA. In total, three primer pairs were employed, yielding at least 650 bp of sequence information from the cytochrome *b* gene: alba-1 (5′-GTCTCCAAGAAGGTTAGGCGA-3′) is a light-strand primer that begins at site L15526 on the human genome, and amplifies mtDNA from *A. glossodonta, A. vulpes,* and *Albula* sp. *E*; alba-2 (5′-CCAAGAAGATTGGGAGAGAA-3′ is a light-strand primer that begins at site L15522 and amplifies *Albula* sp. *A, Albula* sp. *B,* and *Albula* sp. *C*; alba-3 (5′-TGCTAGGGTTGTGTTTAATTA-3′) is a heavy-strand primer that begins at site H14803 and amplifies successfully in all *Albula* spp; alba-6 (5′-GACAAACCCTAACAAGTC-3′) is a light-strand primer that begins at site L15468 and amplifies *A. neoguinaica* and *Albula* sp. *D.*

Polymerase chain reactions (PCRs) employed standard conditions (annealing temperature: 50–53°C) for amplification of vertebrate mtDNA (Hillis et al. 1996). Single-stranded DNA sequencing reactions were performed with a robotic work station (Applied Biosystems model 800, Foster City, CA) and the labeled extension products were analyzed with an automated DNA sequencer (Applied Biosystems model 373A and 377). All sequencing was accomplished at the DNA Sequencing Core, University of Florida. Cytochrome *b* fragments were aligned and edited with Sequencher version 3.0 (Gene Codes Corp., Ann Arbor, MI). Those mtDNA sequences that matched known haplotypes were collated for analysis, whereas ambiguous haplotypes were resequenced to assure the accuracy of nucleotide sequence designations.

Edited DNA sequences were analyzed using PAUP version 4.0b1 (Swofford 1998). Genetic distances were calculated using a Kimura two-parameter (K2P), maximum-likelihood (ML), Jukes-Cantor, HKY85, and Tamura-Nei models with an empirically derived 6:1 transition:transversion (ti:tv) ratio (Jukes and Cantor 1969; Kimura 1980; Felsenstein 1981; Hasegawa et al. 1985; Tamura and Nei 1993; Swofford 1998). Phylogenetic trees were constructed using maximum-parsimony (MP) and the neighbor-joining (NJ) method (Saitou and Nei 1987). The trees produced by these various methods were evaluated with log-likelihood scores using a ML model. Substitution probabilities were assigned with three criteria: unweighted, empirically derived, and a general time reversible (GTR) model. Because a parsimony tree using the full dataset was computationally intractable, MP analyses were conducted on a representative subset of the sequence data.

The closest relative to and logical outgroup for *A. vulpes* (*sensu lato*) is *A. nemoptera*; other members of the superorder Elopomorpha are too distantly related to provide useful comparisons. However, samples of *A. nemoptera* proved impossible to obtain during this study, so the NJ tree was rooted using the midpoint rooting option in PAUP. Bootstrap resampling was accomplished with PAUP using 100 pseudoreplicates (Felsenstein 1985).

Genetic diversity within populations and species was estimated with haplotype and nucleotide diversities (Nei 1987, eqs. 8.5 and 10.5) using Arlequin version 1.1 (Schneider et al. 1997). The distribution of variation within and between populations was calculated with analysis of molecular variance (AMOVA) in Arlequin using the K2P distances (Excoffier et al. 1992).

A molecular-clock approach was used to provide approximate evolutionary time frames for phylogenetic branching events. Conserved morphology is linked to a slow clock in other vertebrates, including turtles and sharks, and may be a function of long generation times and low metabolic rate (Avise et al. 1992; Martin et al. 1992). *Albula vulpes* (and presumably other *Albula* species) become sexually mature at 3.5 years or less (Crabtree et al. 1997; Pfeiler et al. 2000), are strong swimmers, and have moderate to high metabolic rates after larval metamorphosis (Pfeiler and Govoni 1993). In terms of the life-history traits that are thought to affect mtDNA mutation rates, bonefish are typical bony fishes. Therefore, we rely on the rate proposed by Bermingham et al. (1997), based on the divergences in the cytochrome oxidase I gene between 19 marine fish pairs separated by the Isthmus of Panama (approximately 1.5% [range 0.3–2.5%] per million years). In this study, the clock is based on the observation that cytochrome *b* and cytochrome oxidase evolve at similar rates in marine fishes (Muss et al. 2001; our unpubl. data). Given these several assumptions, the molecular clock must be regarded as provisional.

Results

A total of 564 bp of cytochrome *b* sequence was resolved in 174 specimens. Eighty-eight haplotypes were observed, characterized by K2P genetic distances of $d = 0.002–0.306$. Sequences for all 88 haplotypes, and a list of their distributions among the 26 locations, are available from the authors. Twenty-three representative sequences are available in Genbank under accession numbers AF311751–311773.

All phylogenetic analyses indicate that the haplotypes were distributed among eight deep lineages, distinguished by divergence values of $d = 0.056–0.306$ (Fig. 2). For the purposes of this analysis, we define deep lineages as those that are distinguished by K2P distances of $d > 0.056$ in cytochrome *b,* which is the average value for sister species of fishes in a survey of 81 genera (Johns and Avise 1998). Three of these deep lineages correspond to described species: *A glossodonta, A. neoguinaica,* and *A. vulpes.* Two additional lineages in the eastern Pacific will be formally described in the near future (E. Pfeiler, unpubl. ms.). The three remaining lineages are not (to our knowledge) the subjects of imminent species descriptions. Because of the depths of evolutionary separations and the concordance with recognized (and suspected) bonefish species, we will hereafter refer to these five novel lineages as *Albula* sp. *A, B, C, D,* and *E* (Table 1).

A ML test was used to determine which model of DNA sequence evolution provided the best fit to the data used to

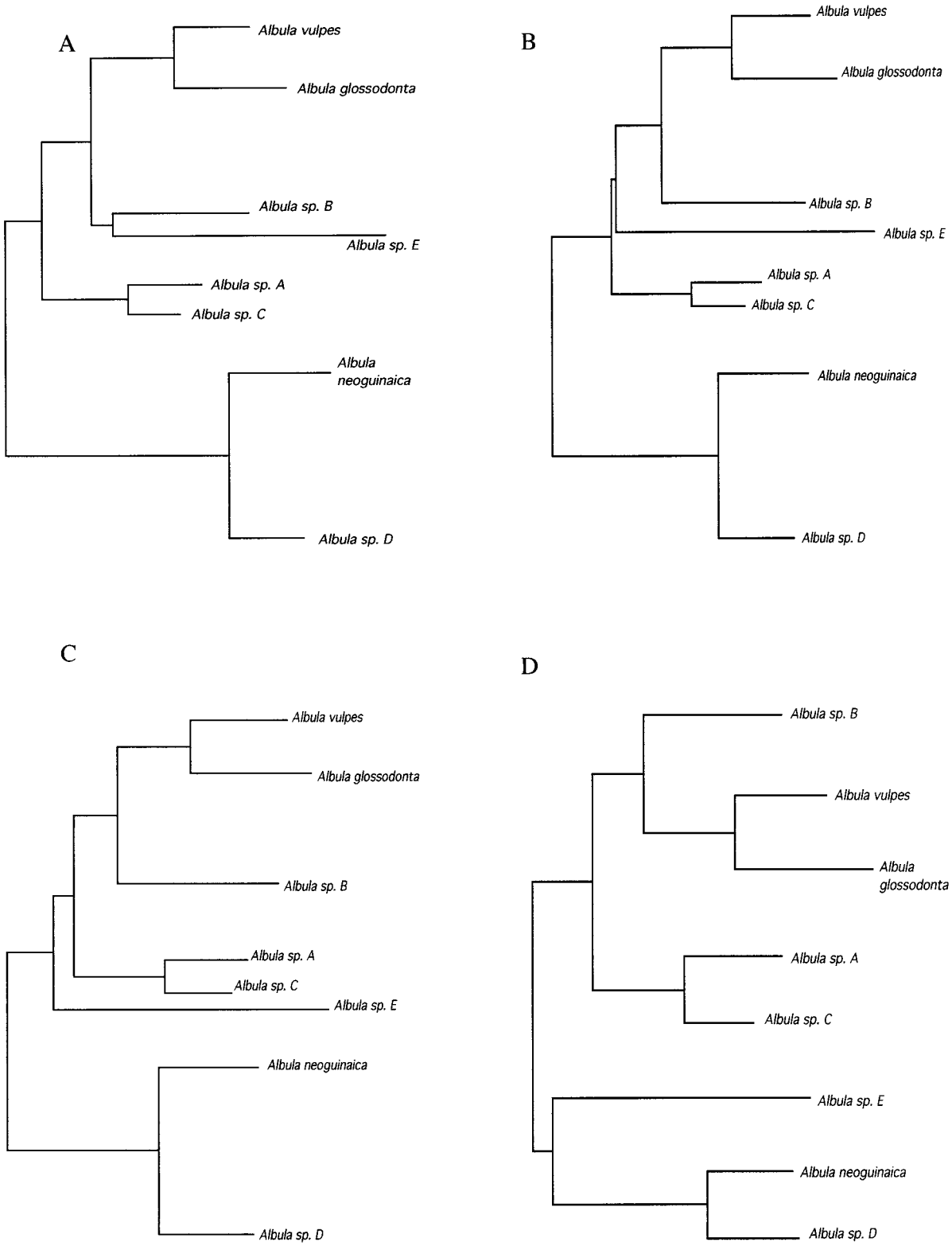

FIG. 2. Four tree topologies recovered for *Albula* spp. under a variety of assumptions and tree-building algorithms. Tree A is based on the neighbor-joining (NJ) algorithm with Kimura two-parameter (K2P) distances and a gamma distribution of substitution rates (−lnL = 2844.8). Tree B is based on the NJ algorithm with Jukes-Cantor distances and equal substitution rates (−lnL = 3057.0). Tree C is based on the NJ algorithm with K2P distances and equal substitution rates (−lnL = 2826.9). Tree D is based on weighted parsimony criteria (−lnL = 2289.4). Trees A–C use the entire dataset (88 terminal branches not shown), and tree C showed the best fit to the maximum-likelihood model. Tree D is based on the reduced dataset of 23 representative haplotypes (parsimony analysis on 88 haplotypes was computationally impractical), so −lnL scores are not comparable between tree D and trees A–C.

TABLE 1. Distribution of samples among recognized and putative *Albula* species.

Lineage	Collection location	Haplotypes
A. glossodonta	Hawaii, Tahiti, Guam, Seychelles	ALB57–ALB64 ($n = 33$)
A. neoguinaica	Hawaii, Fiji, Northern Territory	ALB45–AL56 ($n = 15$)
A. vulpes	Bahamas, Belize, Grenada, Florida	ALB1–ALB9 ($n = 47$)
Albula sp. *A*	Gulf of California	ALB32–ALB39 ($n = 11$)
Albula sp. *B*	Brazil, Florida, São Tome	ALB10–ALB21; ALB24–ALB31 ($n = 30$)
Albula sp. *C*	Gulf of Panama	ALB40–ALB44 ($n = 7$)
Albula sp. *D*	South Africa, Western Australia, Northern Territory, Papua New Guinea, Queensland, New Caledonia, Lord Howe Island	ALB65–ALB88 ($n = 29$)
Albula sp. *E*	Brazil	ALB22, ALB23 ($n = 2$)

construct phylogenetic trees. The likelihood model employed either a 6:1 ti:tv ratio or a GTR model, both with empirically derived substitution probabilities. This model always assumed a gamma distribution with a shape of $\alpha = 0.5$ for substitution rates and used empirically derived base frequencies (A: 0.196, C: 0.207, G: 0.234, T: 0.363). Four tree topologies emerged from the phylogenetic analysis, differing primarily in the placement of *Albula* sp. *E.* (Fig. 2). For trees based on the entire dataset, the NJ tree with K2P distances and an equal distribution of substitution rates (tree C in Fig. 2) provided the best fit to the ML model (Fig. 3).

The NJ tree (Fig. 3) shows three pairs of sister species, including *Albula* sp. *A* and *Albula* sp. *C* ($d = 0.056$–0.070), *A. glossodonta* and *A. vulpes* ($d = 0.077$–0.094), and *A. neoguinaica* and *Albula* sp. *D* ($d = 0.083$–0.133). In all cases these groupings are supported by NJ bootstrap values of 98–100%. Within the Atlantic, *A. vulpes* and *Albula* sp. *B* are distinguished by $d = 0.120$–0.148. All other pairwise divergence among species exceeded $d = 0.130$, and the highest values were observed between sympatric Pacific species *A. neoguinaica* and *A. glossodonta* ($d = 0.264$–0.306).

Three lineages, corresponding to *A. vulpes, Albula* sp. *B,* and *Albula* sp. *E,* were observed in the Atlantic Ocean. *Albula vulpes* was collected exclusively in the Caribbean, whereas *Albula* sp. *B* was collected in the Caribbean, Bahia (Brazil), as well as São Tome in the eastern Atlantic. *Albula* sp. *E* was collected only in Bahia (the sole collection site in the South Atlantic). Two lineages were collected in the eastern Pacific only: *Albula* sp. *A* (Gulf of California) and *Albula* sp. *C* (Gulf of Panama). The mtDNA lineage corresponding to *A. glossodonta* was collected at several locations throughout the Indo-Pacific, from the Seychelles on the western boundary of the Indian Ocean to the Hawaiian Archipelago and Tahiti. *Albula neoguinaica* was collected only in samples from Hawaii, Fiji, and one individual from the Northern Territory (Australia). *Albula* sp. *D* was collected at scattered locations from the Coral Sea to South Africa. The distribution of haplotypes among these putative species and locations is described in Table 1 and Figure 3.

Genetic diversity indices are summarized in Table 2. Nucleotide diversity values ranged from 0.001 to 0.018. Haplotype diversities were consistently high ($h = 0.86$–0.97) with the exception of *A. glossodonta* ($h = 0.39$) and *A. vulpes* ($h = 0.54$). The ϕ_{ST} values were used to test for population structure within putative species (Table 2). In *A. vulpes,* populations were defined simply as different collecting locations (with all samples from Florida grouped as a single location). However, due to small sample sizes for *A. glossodonta, Albula* sp. *B,* and *Albula* sp. *D,* locations were grouped within regions. For *A. glossodonta* and *Albula* sp. *D,* regional populations corresponded to the Pacific and Indian Oceans, and for *Albula* sp. *B* they corresponded to eastern and western Atlantic.

In only three cases, corresponding to *A. vulpes, A. glossodonta,* and *Albula* sp. *B,* are haplotypes shared among sample locations. Population structure, as defined by ϕ_{ST} values, was significant in *Albula* sp. *B, Albula* sp. *D, A. glossodonta,* but not in (Caribbean-restricted) *A. vulpes* (Table 2). For *A. vulpes* the two most common haplotypes are ubiquitous in the Florida Keys, Beliz, and Grenada. This pattern of low or no population structure within the Caribbean is concordant with mtDNA surveys of reef associated species on the same geographic scale (Shulman and Bermingham 1995; Muss et al. 2001). In general, population structure was only detected at geographic scales greater than the Caribbean (eastern vs. western Atlantic or Indian vs. Pacific Oceans).

For *A. glossodonta,* the Indian Ocean sample (two locations in the Seychelles) and the Pacific Ocean sample (Hawaii, Guam, and Tahiti) share a haplotype (ALB57) observed at 100% frequency in the Seychelles. All other haplotypes were endemic to single sample locations: four in Hawaii, one in Tahiti, and one in Guam. The two ocean basins are distinguished by a ϕ_{ST} of 0.05 ($P < 0.003$), indicating shallow but significant population structure between Pacific and Indian Oceans.

For *Albula* sp. *B* in the Atlantic a common haplotype was shared between Florida and Brazil (ALB20); the other haplotypes (10 in Florida, three in Brazil, and six in São Tome) are endemic. The western Atlantic sample (Florida and Brazil) is separated from the eastern Atlantic sample by a fixed genetic difference ($d = 0.007$–0.017) and $\phi_{ST} = 0.678$ ($P < 0.001$), a deep population separation.

Albula sp. *D,* the fourth species with multiple population samples, has the highest haplotype diversity ($h = 0.97$), moderate nucleotide diversity ($\pi = 0.008$), and significant population partitioning ($\phi_{ST} = 0.14$, $P < 0.001$). The remaining four lineages were not analyzed for intraspecific patterns. *Albula* sp. *A* (Gulf of California), *Albula* sp. *C* (Gulf of Panama), and *Albula* sp. *E* (Bahia, Brazil) are represented by single sampling locations. *Albula neoguinaica* contains two divergent branches ($d = 0.029$–0.044): one observed exclusively in Fiji and the other represented by Hawaii and a single individual from the Northern Territory, Australia. The two distinct lineages induce a high nucleotide diversity value (π

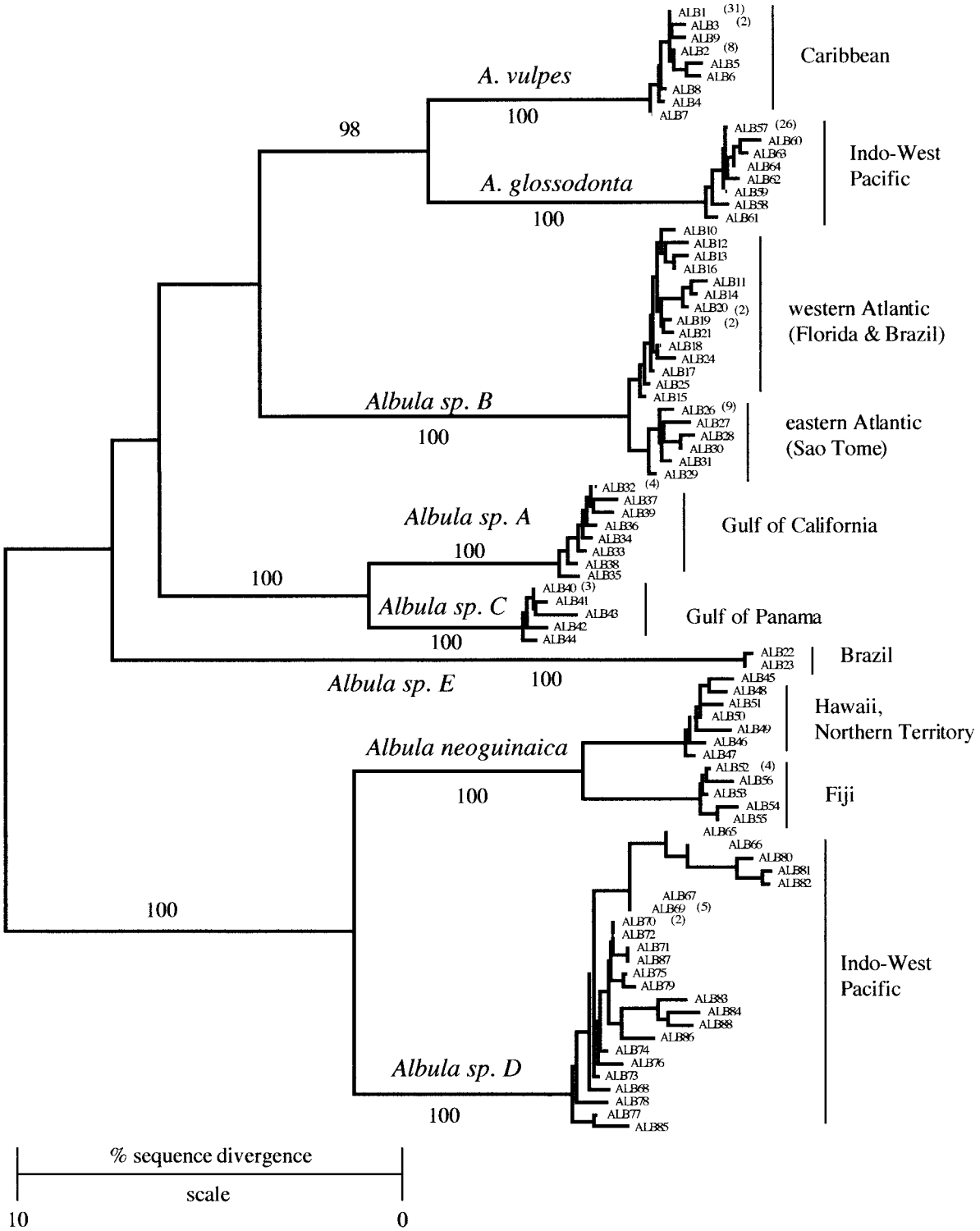

FIG. 3. Neighbor-joining tree (tree C in Fig. 2) showing all haplotypes for all *Albula* lineages. Bootstrap values are indicated on branches. Values in parentheses indicate the number of specimens with particular haplotypes. Haplotypes without numbers were observed in only one individual.

= 0.018) in *A. neoguinaica*, but the small sample sizes, especially from Australia, preclude population analyses.

Based on the provisional molecular clock of 1.5%/million years between lineages, divergence times for the eight deep lineages range from about 4–20 million years. The upper value applies to the two sympatric Hawaiian species (*A. glossodonta* and *A. neoguinaica*). Notably, Shaklee and Tamaru (1981) estimated that these two species diverged approxi-

TABLE 2. Summary of population statistics for the eight major *Albula* lineages. Under the column labeled ''Number of populations,'' a dash indicates that the sampling regime was insufficient for a test of population structure in that species. Under the same column, ns indicates that the sampling regime was sufficient for a test of population structure, but the sample locations were not significantly different.

Lineage	π	h	n	Number of haplotypes	Number of populations	ϕ_{ST}
A. glossodonta	0.001 ± 0.001	0.39 ± 0.08	33	8	2	0.05 $P < 0.003$
A. neoguinaica	0.018 ± 0.009	0.94 ± 0.05	15	12	—	—
A. vulpes	0.001 ± 0.001	0.54 ± 0.08	47	9	ns	0.007 $P < 0.33$
Albula sp. *A*	0.004 ± 0.003	0.89 ± 0.09	11	8	—	—
Albula sp. *B*	0.008 ± 0.005	0.91 ± 0.05	30	20	2	0.68 $P < 0.001$
Albula sp. *C*	0.004 ± 0.003	0.86 ± 0.13	7	5	—	—
Albula sp. *D*	0.008 ± 0.005	0.97 ± 0.02	29	24	2	0.14 $P < 0.001$
Albula sp. *E*	—	—	2	2	—	—
Totals			174	88		

mately 20–30 million years ago, based on allozyme differences. We conclude that divergence dates derived from mtDNA and allozyme data are approximately concordant, at least in indicating ancient (Pliocene-Miocene) separations among bonefish lineages.

DISCUSSION

Bonefishes are members of the primitive superorder Elopomorpha, along with tarpons, ladyfishes, anguilliform eels, deep-sea halosaurs and notacanths, and saccopharyngoid gulper eels. The most distinctive commonality of this group is the leptocephalus larval stage (Greenwood et al. 1966). Leptocephali are transparent, ribbon-like in shape, and pass through an unusually long premetamorphic phase in which (for bonefishes) they may grow up to 70 mm while planktonic and shrink to 20 mm at metamorphosis (Alexander 1961). Members of the Elopomorpha have some of the longest reported larval durations for marine fishes, which may extend from 2 to 24 months depending on species and region (Castle 1984; Pfeiler et al. 1988; Mojica et al. 1995).

The mtDNA sequence data revealed eight major lineages and significant population structure within three of the four lineages that allowed such tests. In the following sections, we consider arguments that these eight lineages represent distinct species, and how the mtDNA data can elucidate aspects of marine speciation. The discovery of cryptic species in *Albula* is especially notable when considered with two aspects of their life history. First, the ancient genetic differentiations indicated by the mtDNA data contrast sharply with the weak differentiation in morphological and general ecological characters. Second, the extended larval period of bonefishes would seem to reduce opportunities for allopatric speciation. In the absence of morphological differentiation, and geographic opportunities for speciation, why then are there so many bonefish species?

Morphological and Ecological Differentiation

The otolith-based fossil record for Albulidae indicates that the family reached a peak of diversity and abundance in the mid-Cretaceous (approximately 100 million years ago) and that modern bonefish morphologies were present by the mid-Miocene (approximately 15 million years ago; Frizzell 1965). The presence of a leptocephalus larva has traditionally attracted more attention than most other aspects of bonefish biology (Gill 1907; Fitch 1950). All leptocephalus larvae were initially placed in the genus *Leptocephalus.* It was not until Delage (1886) raised a leptocephalus through metamorphosis, nearly 100 years after their discovery, that it became clear they were the larvae of a diverse group of primitive bony fishes. Now well studied, the leptocephali of different members of the Elopiformes (bonefishes, tarpons, and ladyfishes) can easily be distinguished by differences in morphology and in the number of myomeres (Greenwood 1977; Smith 1989). However, Alexander (1961) examined bonefish larvae collected from the mid-Pacific, Indonesia, East and West Africa, and the West Indies and found no significant differences in external morphology, coloration, myomere counts, or allometric growth patterns. Geographical considerations indicate that Alexander (1961) compared larvae from at least four *Albula* species, and they were indistinguishable.

Juveniles have likewise proven difficult to distinguish morphologically or ecologically. In the Atlantic, immature *A. vulpes* and *Albula* sp. *B* have been identified (based on mtDNA markers) in the same tidal flats, sometimes schooling together (J. Colborn, R. E. Crabtree, and D. Snodgrass, unpubl. ms.). In the Pacific, *A. glossodonta* and *A. neoguinaica* juveniles are commonly collected in the same beach habitat at the same time (Shaklee and Tamaru 1981).

Adults are also difficult to distinguish. Admittedly, some of these difficulties may be due to gaps in scientific coverage, but bonefishes in Hawaii, the Gulf of California, and the Florida Keys have been well studied, and the differences that have been discovered between species are subtle. In the Florida Keys, *A. vulpes* is incrementally larger and is found in shallower water than *Albula* sp. *B* (Bruger 1974; Crabtree et al. 1996), but the two species (separated by $d = 0.120–0.148$) cannot yet be distinguished by conventional external morphology (R. E. Crabtree, unpubl. ms.). Furthermore, the shallow water form in the Florida Keys (*A. vulpes*) is probably present in deep water elsewhere in the Caribbean (J. B. Shaklee and J. Colborn, pers. obs.) and the putative deepwater form (*Albula* sp. *B*) was collected in shallow water in the eastern Atlantic and Brazil. Thus, the bathic segregations of

adult forms, as observed in the Florida Keys, is not a diagnostic feature of these species.

In Hawaii, *A. glossodonta* and *A. neoguinaica* (d = 0.264–0.306) are also difficult to distinguish. Except for a small difference in the length of the maxilla (Randall and Bauchot 1999), no external morphological characters distinguish these species, but they can be diagnosed with vertebral counts, dentition, and multivariate analysis of the jaw and adjacent structures (Shaklee and Tamaru 1981). These differences include the shape of the lower jaw, shape of the molariform tooth patches, the numbers of teeth, the numbers of tooth patches on the gill arches, and the numbers of branchiostegal rays. Shaklee and Tamaru (1981) suggested that differences in prey selection may be a mechanism that allows the two morphologically similar species to coexist in sympatry. This suggestion, however, has not been directly tested because no information (to our knowledge) is available on feeding habits of bonefishes in Hawaii. Localized difference in habitat preference may exist between the two species: Only one has been commonly found in fish ponds (semi-open impoundments; J. B. Shaklee and C. S. Tamaru, unpubl. data).

In conclusion, there appears to be little difference between bonefish species in terms of morphology or gross aspects of ecology. Multiple species are commonly observed in the same habitat both as juveniles and adults, and species separated since the Pliocene or Miocene are difficult to distinguish with external anatomy. Admittedly the ecological information is fragmentary, and it is possible that subtle differences exist in feeding preference. Nonetheless, these data support and extend the conclusions of Shaklee and Tamaru (1981) that rates of morphological (and perhaps ecological) divergence among bonefish species are extremely slow, whereas evolution at the nucleotide sequence level appears to proceed at or near a conventional pace.

Genetic Evidence for Distinct Species

In contrast to bonefish morphology and ecology, eight deep genetic lineages were found in mtDNA surveys, and several of these partitions are supported by previous allozyme surveys (Shaklee and Tamaru 1981; Pfeiler 1996). Based on the depth of these separations, we hypothesize that these partitions represent previously unrecognized species. However, it is possible that the disparity between ecology, morphology, and genetics is because bonefish genomes evolve rapidly in terms of nucleotide substitutions. We provisionally discount this explanation based on four lines of evidence. First, bonefish are an ancient group of fishes (dating to the Cretaceous; Frizzell 1965; see also Greenwood 1977) and such ''living fossils'' have not been previously characterized by rapid genomic evolution. To the contrary, accumulated evidence indicates that genomes evolve more slowly in ancient, morphologically conserved groups of vertebrates (Avise et al. 1992; Martin et al. 1992).

Second, the depth of molecular and biochemical partitions in *Albula* are consistent with conventional rates of genomic evolution. The divergence between *A. neoguinaica* and *A. glossodonta* is estimated at about 20–30 million years ago with a conventional allozyme clock (Shaklee and Tamaru 1981), and at about 20 million years ago with a conventional mtDNA clock. These dates are concordant with the first appearance of modern bonefish in the fossil record of the middle-Miocene (Frizzell 1965).

Third, putative bonefish species have typical levels of intraspecific genetic diversity. The allozyme heterozygosities (H = 0.005, 0.022, and 0.031 for *A. neoguinaica*, *A. glossodonta*, and *Albula* sp. *A*, respectively) are within the range of values reported for teleost fishes (Nevo 1978; Shaklee and Tamaru 1981; Shaklee et al. 1982; J. Colborn and E. Pfeiler, unpubl. data). The mtDNA haplotype diversities within putative species (Table 2) are also typical for marine fishes (Grant and Bowen 1998).

Fourth, the smallest sequence divergences between putative *Albula* species (d = 0.056–0.070 for *Albula* sp. *A* and *C*) are at or above the average divergence between sister species (d = 0.056) reported by Johns and Avise (1998). Notably, two divergent lineages in *A. neoguinaica* (d = 0.029–0.044) are below the 5.6% cutoff point for this study, but fall within the range of values reported for sister species by Johns and Avise (1998).

The available evidence indicates that the protein-coding loci surveyed with mtDNA sequences and allozymes do not evolve at an unusually fast rate and that the deep genetic partitions reflect ancient divergences, on a time scale (4–20 million years) that would indicate species-level separations. However, genetic distances are not the only aspect of mtDNA and allozyme data that indicate cryptic species. One powerful test of species integrity is the maintenance of genetic isolation in sympatry. As noted by Knowlton (2000), ''In sympatry, the biological and phylogenetic species concepts are equivalent.'' (See also Avise and Wollenberg 1997; McCune and Lovejoy 1998.) In Hawaiian waters, *A. neoguinaica* and *A. glossodonta* maintain diagnostic molecular characters, including mtDNA sequence divergence (d = 0.264–0.306) and 70% fixation of allozyme loci (Shaklee and Tamaru 1981), providing compelling evidence that they are distinct species. Furthermore, field assignments based on the subtle morphological differences matched genetic assignments with 100% precision (Shaklee and Tamaru 1981; J. B. Shaklee, pers. obs.). Based on the distribution of mtDNA lineages, putative bonefish species are sympatric at three additional sampling locations: *A. neoguinaica* and *Albula* sp. *D* in northern Australia, *Albula* sp. *B* and *A. vulpes* in the Florida Keys, and *Albula* sp. *B* with *Albula* sp. *E* in Brazil. However, only in one of these three locations (Florida Keys) were field identifications of cryptic species attempted (*Albula* sp. *B* vs. *A. vulpes*). In this case, genetic partitions (d = 0.120–0.148) exactly matched provisional assignments in a sample of 20 individuals. Therefore, the evidence from sympatric forms in Hawaii and Florida provide robust support for species designation.

We conclude that the differentiation in allozymes and cytochrome *b* indicates eight species in the globally distributed *A. vulpes* complex. It is possible that genetic differentiation within *A. neoguinaica* indicates additional geminate species, and new species may await discovery in undersampled areas such as the South Atlantic, Indian Ocean, or Red Sea.

Models of Speciation in Albula

Processes of speciation that are important in terrestrial environments may be less important in aquatic environments

(Knowlton 1993; Palumbi 1994; Schluter 1996; Bernatchez and Wilson 1998; Briggs 1999). In marine systems, populations of shallow-water invertebrates and fishes are generally large, widespread, highly fecund, and have tremendous potential for dispersal (Palumbi et al. 1997; Graves 1998; Shulman 1998). These conditions may promote evolutionary stasis, and opportunities for allopatric speciation may only occur on vast geographic scales (Palumbi 1992; Knowlton 2000; but see Burton 1998). Here we consider three general models for speciation in bonefishes: (1) sympatric speciation; (2) rare, long-distance dispersal; and (3) vicariant isolation.

Of the four instances of sympatry documented for bonefishes previously and in this study, only one can support an argument for sympatric speciation. For *A. glossodonta*/*A. neoguinaica* in Hawaii and *A. vulpes*/*Albula* sp. *B* in Florida, sympatric speciation is ruled out simply because neither species is the other's closest relative (Fig. 3). *Albula* sp. *B* and *Albula* sp. *E* are sympatric in Brazil, but the uncertain phylogenetic position of *Albula* sp. *E* precludes the robust conclusion of a sister relationship for this pair. These three instances of sympatry are probably the result of secondary geographic overlap following speciation (see Hellberg 1998). (The two eastern Pacific forms are monophyletic, but there is no evidence that these putative species occur in sympatry.)

Albula neoguinaica is sympatric with its sister species, *Albula* sp. *D,* in Northern Territory, Australia, and these two lineages could be used to argue for sympatric speciation. However, the region of overlap is adjacent to the Sunda shelf, which has functioned as a terrestrial barrier between Pacific and Indian Oceans during low sea levels associated with glacial maxima (Valentine and Jablonski 1983; Valentine 1984). The Sunda shelf is a biogeographic boundary for many marine organisms (Springer 1982; Chenoweth et al. 1998; Benzie 1999) and is likely important in structuring other bonefish lineages as well (see below). Thus, the sympatry of *A. neoguinaica* and *Albula* sp. *B* is likely the result of secondary contact. We conclude that sympatric speciation, as a general mechanism for generating new lineages (see Schluter 1996; Smith et al. 1997), is not supported by the bonefish phylogeny.

Isolation by chance, long-distance dispersal has been proposed to explain microevolutionary structure in widespread marine species. Benzie and Williams (1997), Palumbi et al. (1997), and Williams and Benzie (1997) argue that changes in the direction and magnitude of ocean currents, especially during low sea-level stands, are the most likely explanation for patterns of long-distance gene flow in Indo-Pacific sea urchins, giant clams, and starfishes. This dispersal pattern shares some important features with Carson's (1968) founder flush model of speciation. Both require dispersal by a few founders, followed by evolution of reproductive isolation. What is not required in the long-distance dispersal model is a genetic revolution resulting in rapid morphological change, an important criteria for Carson's founder flush model.

Two cases provisionally support long-distance dispersal as a mechanism for speciation in *Albula.* First, *Albula* sp. *B* in the eastern and western Atlantic (separated by more than 3500 km) are characterized by a shallow but diagnostic difference of $d = 0.007$–0.017. Applying the provisional molecular clock, eastern and western Atlantic haplotypes coalesce at about 0.5–1.1 million years ago, well within the period of Pleistocene glaciations and associated climatic fluctuations. Thus, *Albula* sp. *B* has evidently made a rare jump across the Atlantic during the Pleistocene, with no evidence of subsequent contact between eastern and western Atlantic populations. The second possible case for long-distance dispersal involves the two putative species in the eastern Pacific, a monophyletic pair of lineages separated from *A. glossodonta* (nearest Pacific relative) by $d = 0.157$–0.179. The oceanic gap between central Pacific islands and eastern Pacific shores is a formidable 4000 km and is believed to be a substantial barrier to dispersal of shorefishes (Briggs 1961; but see Lessios et al. 1998). However, this example is provisional because the observed separations predate the Isthmus of Panama, and it is possible that the eastern Pacific lineages are derived from Atlantic ancestors rather than Pacific ancestors (Fig. 2). Regardless, central and eastern Pacific populations of bonefishes show no evidence of contact since the Pliocene. We conclude that rare, long-distance dispersal may promote speciation in at least some instances.

Vicariant separations in marine species and corresponding allopatric species can result from plate tectonics, shifts in current patterns and climate, or corresponding sea-level changes. The influence of sea-level change on the genetic structure of marine organisms is dramatic on the Sunda Shelf, at the broad shallow interface between the Indian and Pacific Oceans (Springer 1982; Brown and Lomolino 1998). Tropical oceanic pathways between the two oceans have repeatedly been constricted during Pleistocene glaciations, when sea levels dropped to 130 m below contemporary positions (Haq et al. 1987). For marine species distributed through the Indo-Pacific, haplotype shifts, allele frequency shifts, and deeper separations across the Sunda Shelf are common. Organisms that show such partitions include butterflyfishes (McMillan and Palumbi 1995), sea bass (Chenoweth et al. 1998), coconut crab (Lavery et al. 1996), prawns (Benzie et al. 1992), starfishes (Benzie 1998), corals (McManus 1985; Potts 1985), and bonefishes (this study). Three *Albula* lineages are distributed adjacent to this region; we have multiple samples for two. Within the *A. glossodonta* lineage, Pacific samples are distinguished from those in the Indian Ocean by a ϕ_{ST} of 0.05 ($P < 0.003$). This population structuring is due to a shift in the number of haplotypes, which taper from six in the Pacific to one in the western Indian Ocean. *Albula* sp. *D* also shows significant differentiation across the Sunda Shelf ($\phi_{ST} = 0.14$, $P < 0.001$). Given the multiple observations of genetic structure associated with the Sunda Shelf, the most likely explanation for the observed structure in *A. glossodonta* and *Albula* sp. *D* is the transient isolation of Indian and Pacific populations during low sea-level stands.

The phylogeographic survey of bonefishes reinforces the evidence that changes in sea level associated with glacial intervals have had major effects on the intraspecific variation of marine creatures in the Indo-Pacific region (and possibly in the Atlantic Ocean). Given that the same isolating mechanism likely was operating periodically over long temporal scales (throughout the Pleistocene, Pliocene, and to a lesser degree since the Miocene), the phylogeographic structure within *A. neoguinaica* may be a glimpse of the eventual fate of the more recent structure in *A. glossodonta* and perhaps

in *Albula* sp. *B.* Within *Albula* lineages there are a range of divergences, from shallow population separations (within *A. glossodonta*) to paraphyly (within *Albula* sp. *B*) to reciprocal monophyly and the possibility of incipient species (within *A. neoguinaica*) . Most of the divisions within *Albula* lineages are plausibly attributable to geological barriers such as the Sunda Shelf or oceanic barriers such as the mid-Atlantic expanse. Although there is certainly evidence for dispersal across such barriers, these geological and oceanographic boundaries seem to be a primary force in shaping genetic architectures within *Albula* species. If these microevolutionary processes can be extrapolated to macroevolutionary scales, then allopatric speciation is the predominant mode of diversification in bonefishes.

Cryptic Species

Many cases of cryptic species are attributed to stabilizing selection, which can maintain a constant phenotype across the range of the group (Williamson 1987). However, a distinction must be made between morphological conservatism resulting from stabilizing selection and morphological conservatism in living-fossil lineages, which arguably could include bonefishes. Living fossils are generally considered to be examples of bradytely (extremely slow evolution), both in morphological and DNA sequence evolution (Eldredge and Stanley 1984). These ancient lineages can remain relatively unchanged in gross morphology for more than 100 million years (Smith 1939; Avise et al. 1994; King and Hanner 1998), but usually demonstrate diagnostic morphological differentiation associated with divergences of less than 5 million years (Bowen et al. 1993; Avise et al. 1994; Erdmann et al. 1998). The deepest divergences within the *Albula* genus (d = 0.264–0.306 between *A. glossodonta* and *A. neoguinaica*) corresponds to about 20 million years, yet only one external morphological characters is known to discriminate these species. The split between *A. vulpes* and *Albula* sp. *B* (d = 0.120–0.148) can be provisionally dated to about 8–10 million years, and no external morphological characters have been found to discriminate these species (R. E. Crabtree, unpubl. data). In this case, ancient separations do not correspond to morphological divergences. Because of the extreme levels of anatomical conservatism, even by the criteria of living fossil, other factors such as stabilizing selection or developmental constraints must be considered to explain the extremely slow morphological evolution in bonefishes.

The large number of cryptic species in the ocean (Knowlton 1993) can be attributed to a relatively stable marine environment or to the homeostatic qualities of marine communities on evolutionary time scales (Jackson 1994). The location of individual habitats may change as a result of climatic and sea-level fluctuations, but these habitats exist on a broad geographic scale for millions of years (Jackson 1994). Mature bonefishes are most frequently found on sand and sea-grass flats, a nearly ubiquitous feature of nearshore tropical and subtropical marine environments. Notably, few obvious physical or ecological differences exist between tropical sand flats around the world (Alongi 1990). The infaunal communities are dominated by polychaetes, gastropods, amphipods, bivalves, and decapods (Vargas 1987; Alongi and Christoffersen 1992; Dittmann 1995; Wijnsma et al. 1999). However, there can be considerable geographic variation in the relative abundances of the chief players, and the diet of bonefish populations can vary greatly between locations, sometimes over very short distances (Warmke and Erdman 1963; Colton and Alevizon 1983; Crabtree et al. 1998). Geographic differences in composition of invertebrate communities might prompt changes in the prey-handling structures of bonefishes, and this could explain why jaw structure and dentition are among the few characters known to distinguish *Albula* species (Shaklee and Tamaru 1981; Randall and Bauchot 1999). Beyond this prey handling, there appear to be relatively few opportunities for ecological diversification in flats habitat. The morphological and ecological conservatism in bonefishes may therefore be an example of stabilizing selection on a circumtropical scale, in response to a homogeneous habitat.

Summary and Prospectus

Bonefishes seem to defy a few of the basic tenets of organismal evolution. First, species are expected to develop diagnostic morphological characters, especially after isolation for millions of years. This is a fundamental premise for the morphological classification of organisms. Even living fossils are anatomically distinct after a few million years of separation, and so bonefishes represent an extreme example of morphological stasis (bradytely). Second, congeneric species are expected to be recognizably different when they occur in sympatry. This observation, originally made by Brown and Wilson (1956), stands as one of the fundamental principles of evolutionary ecology (Schluter 1994). Cases abound in which congeneric species coexist in the same habitat, but they invariably exhibit differences in terms of morphology, ecology, or coloration. In contrast, ecological character displacement seems to be weak or undetectable in bonefishes, despite evidence of sympatry among lineages that are millions of years old.

Bonefishes, as members of the Elopomorpha, also defy expectations for larval dispersal. This ancient taxonomic order contains species (such as eels of the genus *Anguilla*) that routinely cross entire oceans during their extended leptocephalus stage. Bonefishes are not ocean migrants as juveniles and adults, but the pelagic larval stage of *Albula* spp. lasts about two to six months, depending on the species (Pfeiler et al. 1988; Mojica et al. 1995). This is at least twice the average for widely distributed reef fishes. Thus, bonefishes should be world-class dispersers, but the genetic evidence argues to the contrary. *Albula* spp. have evidently colonized across the eastern Pacific barrier and mid-Atlantic barriers in the distant past, but not in the last 1 million years (for the mid-Atlantic) to 10 million years (for the eastern Pacific). We also detected no interchange between Atlantic and Indian Oceans on a scale of at least 5 million years, despite the presence of *Albula* sp. *D* in Natal, South Africa. Certainly part of the explanation for limited dispersal is historical: Paleocirculation patterns created windows for colonization that are occluded by contemporary currents and climate (Paulay 1990; Palumbi 1996; Muss et al. 2001). However, larvae of coastal species, most with pelagic periods of 20–50 days,

are able to cross the mid-Atlantic and eastern Pacific barriers on a contemparary scale (Lessios et al. 1998; Bowen et al., in press; B. W. Bowen, unpubl. data). So why don't *Albula* larvae make these crossings? The answer likely includes specialized behaviors that promote retention in nearshore waters.

For decades, experienced bonefish anglers have voiced suspicions that more than one *Albula* species enters the recreational fisheries, and these observations were a primary motivation for genetic studies. So what have we learned about bonefishes from the mtDNA data? There are more species than expected, and more population structure than expected given the extended pelagic larval period. These findings stand in stark contrast to other fish species with an extended pelagic phase, in which mtDNA surveys show little population structure across vast oceanic expanses (Graves 1998; Bowen et al., in press). Attenuation of larval dispersal would certainly enhance opportunities for allopatric isolation and ultimately may explain patterns of speciation in *Albula*. In this regard, bonefishes seem to follow the conventional evolutionary pattern of speciation through vicariant separations (Mayr 1954). However, limited larval dispersal would also seem to enhance opportunities for morphological differentiation, and this is demonstrably not the case. In terms of morphological and ecological differentiation (especially in sympatry), the evolutionary enigma of bonefishes remains intact and the ultimate answers will require additional research. Ecological studies are surprisingly sparse for a group of fishes that commands a dedicated rod-and-reel fishery. This group could also benefit from genus-wide comparisons of morphology and additional nuclear DNA loci, to test the phylogenetic conclusions based on allozymes and mtDNA. Studies of other cosmopolitan marine species will determine whether the genetic patterns observed in bonefishes are routine elements of marine speciation. Finally, ongoing genomic projects (on a variety or organisms) will reveal the operons that control morphological structure and development in bonefishes. Studies of these key loci may demonstrate whether the morphological conservatism of bonefishes is due to genetic constraints, developmental limitations, stabilizing selection, or some combination of influences. By looking inward to the genome and outward to the living organism and its habitat, biologists may yet solve the evolutionary enigma of bonefishes.

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APPENDIX

Summary of sampling locations, sample size, and archival information. Abbreviations: AM, Australian Museum (Sydney); ASU, Arizona State University Fish Collection; FAS, Department of Fisheries and Aquatic Sciences, University of Florida; FLMNH, Florida Museum of Natural History; FMRI, Florida Marine Research Institute; NTM, Northern Territory Museum; UW, University of Washington Fish Collection.

Region	Location	*n*	Providers	Method	Tissue type	Collection notes	Archival notes
Eastern Atlantic	São Tome (Gulf of Guinea)	14	A. Muss	beach seine	fin clips	caught on sand flat, November 1997	1 voucher with FLMNH
Western Atlantic	Florida Keys	15	R. Crabtree, C. Harnden	beach seine, hook and line	heart, liver, muscle	collections from shallow water	8 vouchers with FMRI
	Key West, Florida	12	R. Crabtree	hook and line	heart, liver	head boat catch, 10–30 m depth	12 vouchers with FMRI
	Glovers Reef Atoll Lagoon, Belize	18	G. Sedberry	hook and line	fin clips	caught in shallow lagoon	no vouchers
	Windward, Carriacou, Grenada, (West Indies)	14	J. Colborn	hook and line	gill	caught in shallow lagoon, 15 June 1998	no vouchers
	Bahia, Brazil	6	L. Rocha	market	gill		no vouchers
Eastern Pacific	Guaymas, Mexico (Gulf of California)	11	E. Pfeiler	beach seine	muscle	caught in shallow lagoon	13 vouchers with ASU
	Panama City, Panama (Gulf of Panama)	7	R. Robertson	market	gill		7 vouchers with FLMNH
Central Pacific	Kailua Beach, Hawaii	11	J. Shaklee	beach seine	muscle	collected between September 1980 and December 1997	12 vouchers with UW
	Tahiti	6	B. Bowen	market	heart, liver		no vouchers
Indo-West Pacific	Fiji	8	J. Shaklee	?	muscle		8 vouchers with UW
	Mangiloa, Guam	4	B. Tibbats	hook and line	muscle	caught in shallow water	no vouchers
	Noumea, New Caledonia	3	J. Shaklee	market	muscle	Noumea market, 8 June and 20 July 1983	3 vouchers with UW
	Lord Howe Island	1	J. Shaklee	?	muscle		voucher with AM
	Moreton Bay, Queensland	2	J. Shaklee	?	muscle	caught in shallows, November 1984	3 vouchers with UW
	Port Moresby, Papua New Guinea	1	J. Shaklee	market	muscle	Koki fish market, 1 June 1983	2 vouchers with UW
	Lynedoch Bank, Northern Territory, Australia	1	S. Brown, G. White	hook and line	muscle	caught from the *Fianne*, 100 m depth, 19 July 1998	voucher with NTM
	Northern Territory, Australia	1	J. Shaklee	?	?		voucher with UW
	18°52′2″S, 118°23′8″E (Northwestern Australia)	1	CSIRO cruise FRV Seola	?	muscle	cruise 0283, station 45, 102–104 m depth	voucher with UW
	Exmouth Gulf, Western Australia	9	P. Greenham	hook and line	muscle	caught in shallows, 3 October 1984	9 vouchers with UW
	Coral Bay, Western Australia	2	N. Dixon	cast net	muscle	netted over sand, 27 June 1985	2 vouchers with UW
Western Indian Ocean	St. Joseph's Atoll, Seychelles	2	J. Mortimer	hook and line	muscle	caught in shallows, November 1996	2 vouchers with FAS
	Aldabra Atoll, Seychelles	16	J. Mortimer	hook and line	heart, liver	caught in shallows, May–June 1997	no vouchers
	Durban, South Africa	1	R. van der Elst, S. Fennesy	?	muscle	16 October 1998	no voucher
	Kosi Bay, South Africa	2	J. Shaklee	?	?	11 December 1984	2 vouchers with UW
	Sodwana Bay, South Africa	6	R. van der Elst, S. Fennesy	?	fin clips	17–26 November 1998	no vouchers
Total		174					