Assessing Biodiversity, Evolution, and Biogeography in Bonefishes (Albuliformes): Resolving Relationships and Aiding Management

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Abstract

Bonefishes (Albuliformes: *Albula*) are tropical marine fishes that are the focus of a valuable recreational fishery. Despite substantial research interest, their global diversity, fishery composition, and phylogenetic relationships remain uncertain. Bonefishes exhibit significant morphological conservatism; cryptic species have been identified, complicating conservation efforts. My research addresses these lingering questions to ensure effective management. In the first chapter of this dissertation, I resurrect *Albula goreensis* (Valenciennes 1847) from synonymy with *Albula vulpes* (Linnaeus 1758) and apply the name to a cryptic sympatric congener based on an integrated taxonomic approach, using multilocus molecular, ecological, and behavioral evidence. Genetic assignment tests based on microsatellites correctly diagnose *A. goreensis* from congeners (posterior probabilities=0.97-1.0). Phylogenetic analyses based on cytochrome *b* yielded substantial divergence among Atlantic lineages (d=0.08-0.10), and five nucleotides were diagnostic for *A. goreensis*. Microhabitat use, pelagic larval duration, and growth rate differences have been documented between *A. goreensis* and *A. vulpes*. Phylogenetic distinctiveness in sympatry, and ecological and behavioral differences are considered strong evidence that *A. goreensis* is valid and distinct from congeners.

In regions with intensive fisheries, information is needed on species ranges and stock structure. In Chapter 2, I explore the distributions, genetic structure, and occurrence of hybrids among three members of the Atlantic bonefish (*Albula* sp.) complex, using 19 microsatellite loci. Samples were analyzed from 14 locations across the Caribbean Sea and western Atlantic region, with one external sample from the eastern Atlantic. The species in the complex were broadly sympatric across the region; though local level overlap was variable, likely due to habitat partitioning. Analyses identified *Albula vulpes* as the species predominantly supporting the coastal flats recreational fishery. Unexpected population partitioning was identified within all three species, but the partitions co-occurred within most geographic locations. No clear geographic or temporal patterns were revealed. A strong, consistent identification of two *A. vulpes* genetic populations

was further supported by hypothesis testing for migration patterns between them. Hybrids occurred at low frequency, and results suggested a combination of intrinsic and extrinsic semi-permeable barriers to gene flow exist among these divergent species.

Lastly, advanced phylogenetic methods can clarify evolutionary relationships and offer insights into the potential geographic drivers of diversity. In the third chapter I present a multilocus phylogenetic assessment of Albuliformes. The results support recognition of all known members of *Albula* as distinct species, including several morphological cryptics that occur in sympatry. The broader phylogenetic relationships inferred between Albuliformes and other elopomorphs suggest the genus *Pterothrissus* is not a member of Albuliformes, warranting additional revisions to the order. Estimated dates of divergence within Albuliformes further suggest up to four invasions of the Atlantic Ocean through multiple historical routes. Two pairs of transisthmian geminate species were identified, though divergence well predates final closure of the Isthmus of Panama.

Each chapter of this dissertation will be submitted to a specific scientific journal. Variations in formatting within chapters, such as citation styles, reflect the different journals requirements.

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Introduction

No one definition has as yet satisfied all naturalists; yet every naturalist knows vaguely what he means when he speaks of a species.

~Charles Darwin (1859)

One of the most fundamental concepts in biological research is that of a species. While seemingly a simple designation it remains the subject of debate among systematists, though there is agreement that lineage diversification is a continual process (Hausdorf, 2011). The theoretical concept also has very real practical applications, particularly in the areas of conservation and management. In essence, the species is the unit of "currency" for biological science (Agapow et al., 2004).

In the genetic sense, isolation of organisms occurs along a temporal continuum. The phylogenetic species concept (*sensu* Cracraft, 1983) recognizes species as distinct earlier along this continuum, while the biological species concept (*sensu* Mayr, 1942) recognizes taxa later. Molecular phylogenetic studies frequently reveal hidden diversity by identifying evolutionarily distinct lineages. Yet the appropriate cutoff between what constitute distinct populations within a species versus distinct species is not always readily apparent. As a result, the act of defining a population (or group of closely related populations), and what warrants species status is not a simple matter. Traditionally species delimitation has relied heavily on morphological characterization to distinguish taxa. However, this method can fail to adequately reflect distinct evolutionary lineages and may result in underestimates of biodiversity (Knowlton, 2000). Species delimitation is especially challenging for organisms with highly conserved morphology, so-called cryptic species (*sensu* Bickford et al., 2007). Consequently, modern methods of species delimitation use objective criteria and incorporate data from multiple sources including ecological, geological, morphological, phylogenetic, and behavioral attributes (Yang and Rannala, 2010).

In addition to the theoretical and practical importance of species status, the issues of taxonomy and nomenclature also play a critical role in biology. Estimates suggest that

only about 10% of the world's biodiversity has been described (Wilson, 2000). Many taxonomic groups also have undergone revisions or are in need of revision, on the basis of new information from ongoing systematic research. However, the pace of new species descriptions has been described as glacial (Scotland et al., 2003), and many species remain in a taxonomic limbo due to the lack of formal description. This noted problem has been termed the "Linnean shortfall" (Brown and Lomolino, 1998). Although the ostensibly arbitrary designator of a name should not dictate management or conservation of a species, in reality name recognition can have social, political, and legal influence (Beheregaray and Caccone, 2007). Ambiguous species status can severely hamper conservation efforts for threatened cryptic taxa (Niemiller et al., 2013). Consequently, formal descriptions and correct nomenclature for recognized species remain valuable and relevant aspects of biology.

Within a species, the number and geographic distribution of distinct populations are basic, though vital, pieces of information. These population features determine species sensitivity to local overharvest and the appropriate geographic scope for management and conservation actions. Particularly for species of concern, information on the number of distinct populations and their ranges are necessary for effective management and conservation actions (Waples, 1998). However, for many species this information is lacking. Obtaining population structure information is further complicated when dealing with morphologically cryptic species. Under such circumstances, genetic methods are able to evaluate population structure when other field-based methods are ineffective (Waples and Gaggiotti, 2006). Genetic connectivity occurs through gene flow, and may limit or prevent populations from diverging. Low numbers of migrants can maintain sufficient gene flow to prevent the formation of genetically distinct populations (Avise, 2000). For species with broad distributions, population demographic information enables the accurate delineation of geographic boundaries for conservation or management units. This is important for species resiliency and the maintenance of genetic diversity and local adaptations. Yet, genetic divergence may be revealed in the absence of morphological divergence.

Exploring hybridization between species can also provide important insights into the formation and maintenance of species boundaries. Genotypic data permits investigation into the hybridization patterns between related species beyond observable phenotypic differences. Multilocus genotypic data allows for the categorization of individuals into distinct classes such as first or second-generation hybrids. This information is useful for establishing the level of past or present gene flow between taxa. Sympatric species will generally accrue intrinsic isolating barriers (such as hybrid sterility), as extrinsic barriers may break down over time (Orr and Smith, 1998). In the absence of such barriers, introgression may lead to distinct hybrid zones or eventual species fusion between closely related organisms (Roques et al., 2001). Thus, an understanding of hybridization can be useful for establishing species limits, especially for cryptic species complexes.

Evolutionary relationships among species and the mechanisms responsible for those relationships are topics of considerable interest to biologists. This area of inquiry draws from multiple disciplines: phylogenetics, phylogeography, population genetics, systematics, and biogeography. The ultimate goal is to reconstruct the tree of life and understand the processes that have led to the biodiversity we see today. Information on species boundaries is lacking for many taxonomic groups, and limits our understanding of biodiversity and evolution (Fouquet et al., 2014). However, recent advances in molecular phylogenetic methods have substantially increased our understanding of the timing and pattern of evolution (Near et al., 2012). The ability to simultaneously estimate species relationships and the timing of diversification allows inference into the likely mechanisms for speciation (Reece et al., 2010). Analytical tools such as multispecies coalescent phylogenetic approaches increase our ability to delimit species, and are particularly useful for morphologically cryptic species complexes (Giarla et al., 2014).

My research interests include the evaluation and description of species diversity, genetic population structure, and phylogenetic relationships. The broad goal is to understand the evolutionary patterns and processes that have led to current biodiversity, and further to use these tools to meet conservation and management needs. In this study, I explore these topics within the marine fish order Albuliformes (bonefishes) and their

relatives. Albuliformes was placed in the basal teleost lineage Elopomorpha by Greenwood *et al.* (1966). Monophyly of Elopomorpha was initially based on a single uniting character (the leptocephalus larvae), and remains the subject of active debate among systematists (Filleul and Lavoue, 2001; Inoue et al., 2004; Obermiller and Pfeiler, 2003). However, mounting evidence supports monophyly of this taxon and its placement as the earliest diverging teleost group (Chen et al., 2014; Near et al., 2012). The leptocephalus is a nearly transparent, leaf or ribbon-like planktonic larval form that is unique to elopomorphs. The most striking feature of this larval stage may be its protracted duration. Leptocephalus duration in eels is up to 3 years (Albert et al., 2006), and estimates in bonefishes range from 41 days to 7 months (Mojica et al., 1995; Pfeiler et al., 1988). This extended pelagic larval stage results in high dispersal potential for these fishes, which may drive genetic connectivity across large geographic ranges.

Bonefishes are interesting subjects in which to explore species diversity, delineation, and evolutionary relationships. Albuliformes is in an active state of revision, driven by the recent identification of morphologically cryptic taxa. The order contains one family (Albulidae) that includes two genera, *Albula* and *Pterothrissus* (*sensu* Eschmeyer and Fong, 2014). *Albula* exhibits substantial morphological conservatism across its global tropical range and was once considered monotypic (Whitehead, 1986). The genus now includes 11 recognized species (Eschmeyer and Fong, 2014), and some members support economically valuable commercial and recreational fisheries. Bonefishes are iconic denizens of coastal flats ecosystems; prized for their wariness, speed, and strength. The recreational fishery supports destination tourism and is a major economic driver in many tropical coastal communities. Two species were recently assigned to the IUCN Red List. Thus, bonefishes are the focus of substantial management and conservation interest (Adams et al., 2013).

Bonefishes have a lengthy and complicated taxonomic history. Linnaeus described the first species, *Albula vulpes*, in 1758. Later up to 23 scientific names were applied to members of the group (Whitehead, 1986). Of the 11 currently recognized species, one does not yet have a formal description. In addition, a 12th recently identified taxon remains to be fully evaluated. Taxonomic and nomenclatural issues surrounding

these fishes have led to substantial confusion. In the recent literature, a single bonefish taxon has been referred to by as many as four different names. Due to the importance of accurate taxonomy and to clarify its status for future research, the first chapter of this dissertation focuses on the formal description of the 11th recognized bonefish.

The greater Caribbean Sea and Western Atlantic Ocean have an economically and culturally valuable fishery targeting bonefish. Until quite recently, the diversity of bonefishes in the region was uncertain. Molecular studies have identified four distinct taxa in this region (Adams et al., 2008; Bowen et al., 2008; Colborn et al., 2001; Pfeiler et al., 2006; Wallace and Tringali, 2010). However the distributions of these taxa, and the degree of overlap, remain unknown. Which species of bonefish the fishery targets is also unknown. Within the species supporting the fishery, information on genetic population structure will aid in effective management. In addition, evaluation of hybridization rates among the evolutionarily distinct lineages will assist in species delineation. The second chapter addresses these deficiencies in order to inform sound conservation and management.

Significant questions still exist regarding evolutionary relationships among members of Albuliformes and their placement within Elopomorpha. Previous molecular phylogenetic studies focused on broad relationships within Elopomorpha or among the major teleost lineages have included few members of Albuliformes (Broughton et al., 2013; Chen et al., 2014; Near et al., 2012) and thus are unable to directly address relationships within it. In particular, representatives of the genus *Pterothrissus* were absent from these evaluations. Previous studies on the 12 recognized and proposed species in *Albula* have consisted almost exclusively of single locus mitochondrial genetic data, and as a result inadequately address species level relationships. To address these questions, the third chapter consists of a comprehensive multilocus phylogenetic assessment of Albuliformes. The inclusion of an absolute time calibrated phylogeny further allows exploration of diversification among bonefishes and inference of potential geographic drivers.

Chapter 1

Redescription of *Albula goreensis* Valenciennes, 1847 (Albuliformes: Albulidae): a cryptic species of bonefish in the *A. vulpes* complex, with designation of a neotype

1.1 Introduction

Taxonomy has historically been based largely on morphological differences. This presents challenges for determining species status in groups of organisms with highly conserved morphology. Species biodiversity within the genus *Albula* has been enigmatic due to highly conserved morphology, contributing to a complex taxonomic history. After early descriptions resulting in 23 nominal species, in the mid 20th century all were subsumed into the monotypic *Albula vulpes sensu lato* (reviewed in Whitehead 1986), despite the resultant circumtropical distribution. However, beginning with the work of Shaklee and Tamura (1981), species diversity in this problematic genus has been re-examined with molecular systematic methods. As a result, there have been a number of taxonomic revisions stemming from the identification of cryptic species (*sensu* Bickford *et al*. 2007).

Originating during the late Cretaceous (100-65 MYA), members of *Albula* exhibit significant morphological conservatism despite millions of years of geographic and genetic separation (Greenwood *et al*. 1966). The first molecular phylogenetic examination of global bonefish diversity identified deep divergence among *Albula* lineages (Colborn *et al*. 2001). Four species recently have been described or resurrected through molecular characterization alone or in combination with subtle morphological characters (Kwun & Kim 2011; Pfeiler *et al*. 2011; Pfeiler 2008). At present, *Albula* is comprised of 12 species that are distributed across the global tropics in shallow marine waters (Table 1). Of these, *Albula* sp. B (Colborn *et al*. 2001) and *Albula* sp. cf. *vulpes* remain to be formally described (Wallace & Tringali 2010).

Accurate assignment of species status to evolutionarily and functionally distinct taxonomic groups remains a cornerstone of biological science, and is critical for successful management and conservation. Traditionally, species descriptions have relied on morphological characterization to distinguish taxa. However, in many cases, this

method fails to adequately reflect distinct evolutionary lineages and results in underestimates of biodiversity that can have profound consequences for management and conservation (Knowlton 2000; Pfenninger & Schwenk 2007). Often true in recently diverged taxonomic groups, this also is the case among albulids despite their basal phylogenetic position among teleosts. Central to species recognition is the concept applied and operational criteria for delimitation (Bernardo 2011). The phylogenetic and biological species concepts overlap for sympatric taxa, and agreement of independent genetic markers provides strong evidence for distinctiveness in sympatry (Knowlton 2000). There is widespread acceptance and use of modern molecular systematic techniques for species delineation (Abraham *et al*. 2013; Fermin *et al*. 2012; Wiens & Penkrot 2002). Thoughtful examination suggests recognition of cryptic species through genetic assessment is valid (Bernardo 2011).

The original description of *A. goreensis*, based on two syntypes, is in *Histoire Naturelle des Poissons* by Cuvier & Valenciennes (1847) (Figure 1). These syntypes (MNHN 0000-3586 and MNHN 0000-3587) were confirmed by Bertin (1940) and are held at the Museum National d'Histoire Naturelle, Paris (MNHN) (reviewed in Whitehead 1986). In that review, *Albula goreensis* was deemed available and is redescribed here. All other available nominal albulid species were considered. *Albula goreensis* is designated for the bonefish considered here on the basis of principle of priority, article 23, of the International Code for Zoological Nomenclature (ICZN) and verified occurrence in the syntype locality (Gorée, Senegal). Only 1 extant albulid species is known from the west coast of Africa; modern specimens have been identified as *Albula* sp. B (hereafter *A. goreensis*) by molecular analysis (Colborn *et al*. 2001; Wallace 2014). No other albulids are known from these waters.

Due to the critical importance (and general lack) of positively identified types for Atlantic albulids, a neotype and a series of paraneotypes are designated here. They provide a molecular characterization for this species and will facilitate future comparative work. Despite efforts to collect a neotype from the original type locality, none could be obtained from the waters off of Senegal. The neotype and paraneotypes were collected from Florida, USA.

1.2 Materials and methods

Field collections.

A total of 28 *Albula goreensis* voucher specimens were collected via shoreline seine or hook and line. The neotype JFBM 47419 and paraneotype JFBM 47420 were collected by shoreline beach seine. Paraneotype JFBM 47421 was collected by hook and line. Tissues samples were preserved in Puregene® cell lysis buffer or 70% ethanol. Whole specimens were frozen and later transferred to 70% ethanol. Type specimens are deposited in the James Ford Bell Museum of Natural History [JFBM] at the University of Minnesota.

Molecular phylogenetics.

Genomic DNA was isolated from fin clips or muscle tissue with the Gentra® Puregene® tissue kit. Individuals were screened with a previously characterized bonefish specific library of 19 microsatellite loci to determine species identification (Seyoum *et al*. 2008; Wallace & Tringali 2010). The multiplex microsatellite PCR reactions followed Wallace & Tringali (2010), were screened on an Applied Biosystems 3730XL genetic analyzer, and alleles scored in Genemapper®. Allele frequencies and expected and observed heterozygosities were calculated in Genepop version 4.2 (Rousset 2008). Species assignment analyses were conducted through three-dimensional clustering in GENETIX (Belkhir *et al*. 2000) and Bayesian assignment tests in NewHybrids version 1.1 (Anderson & Thompson 2002), comparing *A. goreensis* individuals against sympatric congeners *A. vulpes* (N= 209) and *A.* sp. cf. *vulpes* (N= 75). The NewHybrids analyses were run with Jeffreys-like priors for at least 5 million generations, following a burn-in of 100,000 MCMC simulations, in pairwise comparisons for all possible species combinations.

A reduced set of 6 *A. goreensis* was screened for a 502 nucleotide fragment of the mitochondrial cytochrome b gene, using bonefish specific primers alba-2 & alba-3 (Colborn *et al*. 2001). Sequences were edited and aligned in Geneius version 6.1 (Drummond *et al*. 2010). Heterozygous sites within sequences were coded according to

the IUPAC ambiguity code. In two cases the heterozygous sites altered the encoded amino acids (in *A. esuncula* and *A. glossodonta*). Previously published cytochrome *b* sequences were downloaded from GenBank and combined with new data from the present study (Table 2). However, due to known identification errors for some bonefish in Genbank and the presence of a cytochrome b-like pseudogene, sequences were also obtained directly from tissues to ensure correct species assignments. Sequence divergence values (K2P *d*) were calculated in Mega version 5.2 (Tamura *et al*. 2011). The optimal partitioning scheme and models of molecular evolution for codon positions were simultaneously derived in PartitionFinder using AICc to assess likelihood scores (Lanfear *et al*. 2012). Molecular phylogenetic analyses were conducted via the CIPRES science gateway (Miller *et al*. 2011). Maximum likelihood phylogenies were estimated in RAxML (Stamatakis *et al*. 2008), and Bayesian phylogenies were estimated in BEAST (Drummond *et al*. 2012). These phylogenies were then used comparatively to explore inferred relationships among albulids. Individual BEAST runs were assessed in Tracer (Rambaut & Drummond 2007) for convergence, and resultant post burn-in phylogenies combined into a maximum credible clade tree in TreeAnnotator (Rambaut & Drummond 2010). Phylogenetic relationships among albulids from the RAxML and BEAST phylogenies were compared for congruence in FigTree (Rambaut 2009).

Morphology.

Morphometric characters in 28 preserved subadult and adult specimens of *Albula goreensis* were examined for this study (Table 3). In addition, 2 genetically identified *A. vulpes* vouchers were examined for comparative purposes. An initial examination of *A. goreensis* morphology in subadult specimens was conducted previously (Wallace & Tringali 2010), and my methods followed theirs for counts and measurements. All measurements were taken on the left side with digital calipers (accurate to +/- 0.03 mm), and are reported as a percentage of standard length (SL). Body depth measurement was often estimated, due to abdominal incisions in the specimens. Estimated body depth was measured from the same landmark (origin of the dorsal fin) as with actual measurements; the inward curling of tissue at the abdominal incisions may result in slight

underestimates. In a few cases, counts were estimated (by counting scale pockets) due to missing scales. Additionally dorsal height, length of last dorsal ray, and length of last anal ray were approximated due to frequent fin tip fraying.

1.3 Results

Molecular phylogenetic relationships

The microsatellite loci were able to correctly distinguish *A. goreensis* from the sympatric *A. vulpes* and *A.* sp. cf. *vulpes*. The spatial analyses in GENETIX displayed three well separated, distinct clusters representing *A. goreensis*, *A. vulpes*, and *A.* sp. cf. *vulpes* (Figure 2). The species assignments in NewHybrids for all *A. goreensis* specimens yielded significant posterior probabilities (p= 0.97 to 1.0) in comparisons against both sympatric congeners in multiple independent runs. Allelic diversity within *A. goreensis* averaged 7 alleles per locus. The mean observed heterozygosity (H_o) was 0.347 and expected heterozygosity (H_e) was 0.468.

The 502 base segment of the mitochondrial cytochrome *b* gene sequenced was located between nucleotides 14,677 and 15,178 of the mitochondrial genomes of *Pterothrissus gissu*, *Elops saurus*, *Megalops cyprinoides*, and *Megalops atlanticus* (downloaded from Genbank). This segment corresponded to the first reading frame in the translated vertebrate mitochondrial protein code, beginning with Alanine and ending with Valine. At 2 residues each for *A. esuncula* and *A. glossodonta*, the heterozygous nucleotide sites changed the amino acid encoded. This resulted in either Alanine or Glycine at residue 76, and Serine or Cysteine at residue 162 in *A. esuncula*. In *A. glossodonta*, the heterozygous sites resulted in Proline or Serine at residue 100, and Isoleucine or Valine at residue 167. None of the heterozygous sites within *A. goreensis* altered the amino acid sequence.

A total of 5 sites in the cytochrome *b* alignment were diagnostic for *A. goreensis* among all other albulids (sympatric congeners shown in Table 4). An additional 2 sites (60 and 384) were heterozygous in *A. goreensis*, whereas they were fixed in all other albulids. One site (441) was further diagnostic for *A. goreensis* across all taxa considered

(all albulids, *P. gissu*, *E. saurus*, *E. hawaiensis*, *E. smithi*, *M. cyprinoides*, and *M. atlanticus*). *Albula goreensis* exhibited substantial sequence divergence (K2P d= 8-10%) between the sympatric *A. vulpes*, *A.* sp. cf. *vulpes*, and *A. nemoptera*, while intraspecific d≤ 0.01 for all albulids.

The phylogenies recovered from multiple maximum likelihood and Bayesian simulations did not differ substantially with respect to terminal clades. Similarly, terminal clades recovered in Bayesian phylogenies under HKY or GTR substitution models, and exponential or lognormal speciation models did not vary substantially. Although nodal support varied among simulations, in all cases the clade placement for *A. goreensis* was consistent. There was weak (i.e. <0.95) node support (posterior probability=0.89) for the clade containing *A. goreensis*, *A. vulpes*, and *A. glossodonta* in the BEAST tree under a GTR substitution model and exponential speciation model (Figure 3). However, most other terminal clades received significant support (posterior probability≥0.95). Based on the cytochrome *b* sequence data, *A. goreensis* is sister to a clade containing the sympatric *A. vulpes* and *A. glossodonta* from the Pacific and Indian oceans. These results are consistent with phylogenetic relationships recovered in previous studies (Bowen *et al*. 2008; Wallace & Tringali 2010). These relationships also strongly suggest that *A. goreensis*, *A. vulpes*, *A. nemoptera*, and *A.* sp. cf. *vulpes* are currently sympatric due to secondary contact and not through sympatric speciation.

Morphology

External measurements and counts were completed for the 28 available voucher specimens previously identified through genetic analysis as *A. goreensis* (Table 3). All meristic counts fell within the range reported in Wallace & Tringali (2010) for *A.* sp. B or were overlapping. Similarly, almost all morphology measurements from the present study were within the range, or overlapping, of those from Wallace & Tringali. The exception was mandible length, which was lower in all examined specimens than reported in Wallace & Tringali despite similarity in SL among specimens. However slightly different body landmarks might have been used between studies and that could underlie the few differences observed.

Almost all of the meristic counts for *A. goreensis* overlapped (or nearly) those of *A. vulpes* in the present study, as well as *A. vulpes* and *A.* sp. cf. *vulpes* in Wallace & Tringali (2010). Diagnostic differences for the number of scales around the caudal peduncle and vertebrae may exist for *A.* sp. cf. *vulpes*. However, these counts are overlapping for *A. goreensis* and *A. vulpes*. Similarly, all morphology measurements overlapped (or nearly) those of *A. vulpes* and *A.* sp. cf. *vulpes*. The length of last dorsal ray may be greater in *A.* sp. cf. *vulpes*, however this feature should be viewed with caution as it is based on a single specimen. Additionally, the measurements for length of last dorsal ray overlapped for *A. goreensis* and *A. vulpes*. Although body depth, dorsal height, and length of last dorsal and anal rays were estimated on some specimens due to damage; these features are not generally known to distinguish any members of *Albula* other than the significantly extended last dorsal ray in *A. nemoptera*. Actual measurements (i.e. not estimates) were still overlapping among *A. goreensis* and sympatric congeners for body depth, dorsal height, and length of last anal ray. The average values for most counts are slightly below those for *A. vulpes* in Rivas & Warlen (1967), however the ranges are overlapping. It is important to note that the *A. vulpes* specimens examined by Rivas & Warlen (1967) were not positively identified through molecular analysis. It is possible the fish used in their study consisted of *A. vulpes*, *A. goreensis*, *A.* sp. cf. *vulpes*, or some combination thereof. Additionally, Crabtree *et al*. (2003) did not find any morphometric characters to distinguish *A. goreensis* from *A. vulpes*. Based on the available evidence, it is not possible to diagnose *A. goreensis* solely on the basis of external characteristics.

Other morphological characters reported to discriminate among Pacific Ocean bonefishes include lower jaw shape, tooth patch patterns on the mesopterygoid and parasphenoid bones, and pelvic fin tip relative to the anal vent. Representative specimens of *A. goreensis* and the sympatric *A. vulpes* were compared for these features, however no differences between them were observed.

In this study sample sizes were small, due limited availability of genetically identified voucher specimens. It is presumed available vouchers are representative of *A. goreensis*. Although larger sample sizes may have revealed some differences, they would likely be

subtle and not useful for field identification.

Systematics

Albula goreensis (Valenciennes, 1847)

Channel bonefish

(Figs. 1-3, Tables 1-4)

Albula vulpes (not of Linnaeus): Alexander 1961

Albula garcia: Adams *et al*. 2008; Valdez *et al*. 2010

Albula sp. B: Colborn *et al*. 2001; Bowen *et al*. 2008; Seyoum *et al*. 2008; Wallace & Tringali 2010

Albula nova sp.: Crabtree *et al*. 2003

Each of the studies listed above included specimens of what we recognize here as *A. goreensis* on the basis either of collection locations (West African coast) or molecular data.

Neotype. JFBM 47419: Field ID 05180510-29, sex U juvenile, 210.7 mm SL, USA: Bahia Honda, Florida, 24° 66.424' N, 81° 25.759' W, collected by A. Adams, May 18, 2005.

Paraneotypes. JFBM 47420: Field IDs 05180510-01 & 05180510-12, sex U juvenile, 209.9 & 215.6 mm SL. Same collection information as neotype.
JFBM 47421: Field ID BP117, sex U juvenile, 271.6 mm SL, USA: St. Lucie Inlet, Florida, collected by B. Pelosi November 2007.

Diagnosis. The microsatellite library previously developed for studies on Atlantic *Albula* species is able to distinguish *A. goreensis* from sympatric *A. vulpes* and *A.* sp. cf. *vulpes* and the allopatric *A. glossodonta* through allelic differences in Bayesian assignment tests ($p \geq 0.95$), and through spatial cluster analyses. Further, *A. goreensis* can be distinguished from all other albulids by five diagnostic nucleotides in its cytochrome b sequence (Table 4).

The number of scales around the caudal peduncle may distinguish *A. goreensis* from the sympatric *A.* sp. cf. *vulpes* (15-17 versus 14, respectively), but not from *A. vulpes*. Similarly, the number of vertebrae may also distinguish *A. goreensis* from *A.* sp. cf. *vulpes* (71-72 versus 69), but not from *A. vulpes*. The lack of an extended dorsal threadfin distinguishes *A. goreensis* (as well as *A. vulpes* and *A.* sp. cf. *vulpes*) from the fourth Atlantic species, *A. nemoptera*.

Description.

Morphometric data for the neotype, three paraneotypes, and 24 non-type specimens are listed in Table 3. See Figure 1 for general body appearance. Body elongate, slightly laterally compressed, with greatest body depth approximately at dorsal fin origin, and least caudal peduncle depth just anterior to caudal fin base; conical snout with inferior mouth; head free of scales; protruding upper jaw; lower jaw rounded; anterior edge of tooth patch on mesopterygoid in front of parasphenoid patch; anal vent located nearer pelvic fins than anal fin; fins containing only soft rays; dorsal and anal fin rays tapered posteriorly, but last ray slightly elongated; caudal fin highly forked.

Distribution. This species has a broad distribution across the Caribbean Sea, Gulf of Mexico, and tropical Atlantic Ocean. However, it may be patchily distributed at smaller geographic scales. Adult *A. goreensis* are generally found in nearshore waters at greater depths than *A. vulpes*. It is presumed that East- West Atlantic connectivity is being maintained by larval transport. This connectivity is likely aided by prevailing westerly currents and tropical storm patterns.

Conservation status. *Albula goreensis* is currently listed as data deficient according to the International Union for the Conservation of Nature (IUCN) Red List, following an assessment for bonefishes (family Albulidae) (Adams *et al.* 2013). Concern for this species, as well as other global bonefishes, stems primarily from habitat loss and degradation. Bonefishes inhabit nearshore tropical waters that are often targeted for coastal development. An economically and culturally important fishery exists for these fishes in many areas, however in many regions there are currently no regulations in place to prevent overharvest. An economic impact study in the Bahamian recreational bonefish

fishery was valued at $141 million USD (Fedler 2010). The eastern Atlantic population along the Senegal coast may be at increased risk of overharvest due to illegal fishing. The West African coast has among the highest levels of illegal catch in the world.

Etymology. *Albula:* from the Latin albus meaning white. The specific epithet is derived from the island of Gorée off the coast of Senegal where the syntypes were collected, in combination with the Latin suffix ensis, which means of or from a place.

1.4 Discussion

The degree of cytochrome *b* sequence divergence (K2P *d*) observed between *A. goreensis* and sympatric congeners *A. vulpes*, *A.* sp. cf. *vulpes*, and *A. nemoptera* is within the range previously reported for albulids (Bowen *et al*. 2008; Colborn *et al*. 2001; Pfeiler 2011). This divergence range is similar to that observed between sister species *M. atlanticus* and *M. cyprinoides* (K2P d= 8%). It is also substantially greater than the 2-3% reported between *E. saurus* and *E. smithi*, recently recognized Elopomorpha sister species (McBride *et al*. 2010).

As previously discussed, it is not possible to diagnose *A. goreensis* solely on the basis of external morphological or meristic characters (Crabtree *et al*. 2003; Wallace & Tringali 2010). Those characters proposed by other authors as potentially diagnostic between *A. goreensis* and its sympatric congeners (eye diameter and tooth patch patterns) have not held up to scrutiny based on the available voucher specimens.

Ecological and developmental differences from A. vulpes. While broadly sympatric with *A. vulpes* across the western Atlantic Ocean and Caribbean Sea, *A. goreensis* exhibits microhabitat use differences at multiple life stages. As settlement stage larvae and juveniles, *A. goreensis* prefers shallow sandy coastal habitat with higher exposure and wave energy than *A. vulpes* (Adams *et al*. 2008; Crabtree *et al*. 2003; C. Haak- unpublished data). As adults, it appears that *A. goreensis* prefers slightly deeper water habitat (Crabtree *et al*. 2003; Wallace & Tringali 2010). The majority of *A. goreensis* voucher specimens were collected from an inlet slightly offshore in waters >1m

deep. Directed sampling from the onshore flats recreational fishery (typically in waters <1m deep) was almost exclusively *A. vulpes* (Wallace & Tringali 2010; Wallace- submitted).

There are also significant developmental differences between *A. goreensis* and *A. vulpes*. Pelagic larval duration (PLD) has been estimated via measurement of otolith daily growth increments from bonefish collected in The Bahamas. Although based on only a few individuals, these data indicate that PLD is approximately 3 months for *A. goreensis*, while PLD estimate for *A. vulpes* is roughly 2 months (C. Haak- unpublished data). There is also preliminary evidence that growth rate is slower at juvenile stages in *A. goreensis* than *A. vulpes*. Similarly, juvenile behavioral and developmental differences have been noted between the elopomorphs *E. saurus* and *E. smithi* (McBride & Horodysky 2004).

Genetic incompatibilities. In a comprehensive study of hybridization between *A. goreensis* and *A. vulpes* across the species ranges less than 1.5% of individuals assessed were hybrids (Wallace- submitted). The hybrids identified were almost exclusively assigned to a combined F1/F2 first category, suggesting barriers to gene flow between these species such as hybrid sterility or reduced hybrid fitness. Adult reproductive behavior, such as spatiotemporal spawning differences, may also contribute to the low frequency of hybridization between these species.

Management implications. There is considerable management emphasis on population dynamics and recruitment for Atlantic and Caribbean bonefishes resulting from the valuable recreational fishery. Consistent recruitment is important for population maintenance, and low recruitment may be affecting limiting some populations, such as in the Florida Keys (Adams et al. 2013). This study suggests previous research on recruitment dynamics may involve multiple species with different ecologies and population dynamics, not just *A. vulpes* as previously believed. However, *A. vulpes* appears to be supporting the coastal flats fishery (Wallace 2014). Determination of recruitment dynamics for this species remains a high research priority, and will ensure management efforts are focused at the appropriate geographic scale.

Other materials examined. *Albula goreensis* syntypes: MNHN 0000-3586 & 3587, syntypes, both 540mm TL, coll. P. Rang from waters near Gorée, Senegal, Africa, received by MNHN in 1830. These 2 specimens were screened for species identification with the microsatellite library. Due to the age and poor condition of the specimens, data was only obtained from a few microsatellite loci. Species assignment tests conducted in NewHybrids were unable to correctly assign the syntypes to *A. goreensis*. However, based on the collection location they are most likely *A. goreensis*.

Other *Albula goreensis*: BP112-141, 124.3-344.4mm SL, coll. B. Pelosi from St. Lucie Inlet, Florida, USA 11/2007.
Albula vulpes: BP129, 298.41mm SL, coll. B. Pelosi 11/2007 in St. Lucie Inlet, Florida, USA.--Ozello-01, 158.43mm SL, coll. unknown from Ozello, Florida, USA.

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Table 1. Species diversity and species complexes within *Albula*.

Species	**Other applied names**	**Distribution**
Albula vulpes complex		
Albula vulpes (Linnaeus)		Western Atlantic & Caribbean
Albula goreensis (Valenciennes)	*Albula* sp. B, *A. garcia, A. nova sp.*	Tropical Atlantic & Caribbean
Albula sp. cf. *vulpes* Wallace & Tringali	*Albula* sp. F	Western Atlantic & Caribbean
Albula esuncula (Garman)	*Albula* sp. C, *A. neoguinaica*	Tropical Eastern Pacific
Albula gilberti (Pfeiler)	*Albula* sp. A	Eastern Pacific, Gulf of California
Albula glossodonta (Forsskål)		Indian, Western & Central Pacific
Albula koreana (Kwun & Kim)		Western Pacific (East China Sea)
Albula argentea complex		
Albula argentea (Forster *in* Block & Schneider)	*A. forsteri, A. neoguinaica*	Western & Central Pacific
Albula oligolepis (Hidaka, Iwatsuki & Randall)	*Albula* sp. D	Indian & Western Pacific
Albula virgata (Jordan & Jordan)	*A. neoguinaica*	Hawaii
Albula nemoptera complex		
Albula nemoptera (Fowler)	*Albula* sp. E & *Dixonina nemoptera*	Western Atlantic & Caribbean
Albula pacifica (Beebe)	*A. nemoptera*	Tropical Eastern Pacific

*Amended from Pfeiler *et al*. (2011)

Table 2. Specimens sequenced, including collection numbers, locality information, and Genbank accession numbers.

Species	Voucher	Locality	GenBank accession numbers
A. argentea	-	Fiji	HQ683755.1 – 683761.1
A. esuncula	-	Gulf of Panama & Mexico	AF311760.1 – 311762.1 & EF602158.1 – 602160.1
A. glossodonta	-	Hawaii, Tahiti, Guam, Seychelles	AF311767.1 – 311769.1
A. gilberti	-	California	JF803969.1 & 803971.1
A. goreensis	JFBM 47419-47421	Florida	KJ910038-KJ910040
A. virgata	-	Hawaii	KJ910045
A. koreana	-	Korea & Taiwan	HM119396.1- 119400.1
A. nemoptera	-	Brazil	AF311754.1 & 311755.1
A. oligolepis	JFBM 47242	South Africa	KJ910041-KJ910043
A. pacifica	-	Mexico	DQ272657.1 – 272659.1
A. sp. cf. vulpes	-	Florida, Honduras, Virgin Islands	KJ910044
A. vulpes		Florida	KJ910046-KJ910047

Table 3. Meristic and morphometric comparisons of *A. goreensis* and 2 sympatric congeners.

Character	*A. goreensis* Neotype JFBM 47419	*A. goreensis* N=27	Wallace & Tringali *A.* sp. B N=5	*A. vulpes* N=2	Wallace & Tringali *A. vulpes* N=1	Wallace & Tringali *A.* sp. cf. *vulpes* N=1
Meristic counts						
Dorsal rays	17	17-19	18-19	17-18	18	19
Anal rays	7	8-9	8-10	8	9	8
Pectoral rays	-	-	16-19	-	20	19
Pelvic rays	-	-	10	-	10	11
Lateral line scales	74	70-76	72-78	70-72	76	70*
Pre-dorsal scales	17	14-19	17-21	16-18	20	14*
Scales above lateral line	8	8-9	9-10	8	9	8
Scales below lateral line	5	5-6	5-6	5	7	5
Scales around caudal peduncle	16	15-17	15-16	16	15	14
Vertebrae	-	-	71-72	-	71	69
Standard length (mm)	210.7	124.3-344.4	148.3-212.3	158.4-298.4	192.0	129.0
Measurements as %SL						
Head length (mm)	26.0	24.7-29.5	26.2-27.6	27.5-28.7	26.8	27.7
Body depth	22.4	19.0-24.5*	20.1-21.6	21.5-25.9	22.1	21.7
Least depth of caudal peduncle	7.3	5.1-8.7	6.8-7.5	7.6-8.4	7.7	7.7
Anal base length	5.5	3.9-6.4	5.1-6.0	4.6-5.5	5.5	5.3
Dorsal base length	14.6	13.1-18.1	13.8-17.2	13.4-15.8	16.0	16.7
Dorsal insertion to anal origin	29.1	25.3-30.3	24.4-27.7	26.0-29.0	27.4	27.8
Eye diameter	5.9	4.0-6.6	5.3-5.6	5.5-5.7	5.7	5.2
Bony interorbital width	6.7	5.1-8.2	6.0-7.0	6.4-7.5	6.5	6.4
Snout length	10.3	8.3-11.8	9.9-11.5	10.3-11.6	10.2	11.4
Tip of snout to rear of maxillary	8.3	7.4-10.1	8.6-9.1	8.4-9.2	9.1	9.7

Maxillary length	6.9	5.7-7.4	6.5-7.3	6.6-6.9	7.7	7.8
Mandible length	6.0	4.7-7.0	8.1-9.0	4.8-6.2	9.1	9.5
Preoral length	2.8	1.2-3.8	2.8-3.4	2.8-3.6	3.5	3.6
Dorsal height	16.4*	14.2-19.3*	13.5-17.0	18.6-20.3*	18.6	17.0
Length of last dorsal ray	5.0*	4.2-6.2*	5.4-6.4	6.2-6.5	6.0	7.0
Length of last anal ray	4.9	4.1-5.7*	5.0-6.4	6.2	5.6	6.4

* Estimated counts/ measurements due to missing elements

Table 4. Diagnostic nucleotides in the mitochondrial cytochrome b gene fragment in *Albula goreensis* and sympatric congeners *Albula vulpes*, *A. nemoptera*, and *A.* sp. cf. *vulpes*.

Cyt b position	***A. goreensis***	***A. vulpes***	***A. nemoptera***	***A.* sp. cf. *vulpes***
198	C	A	A	G
216	A	T	T	T
369	C	T	T	T
441	C	A	A	G
447	A	C	T	T

Figure 1. A) syntype MNHN-3586 (540 mm TL) of *Albula goreensis* B) syntype MNHN-3587 (540 mm TL) of *Albula goreensis* C) neotype JFBM 47419 (211 mm SL) of *Albula goreensis*

A

B

C

Figure 2. Microsatellite spatial analyses in GENETIX for *A. goreensis* and congeners *A. vulpes* and *A.* sp. cf. *vulpes*.

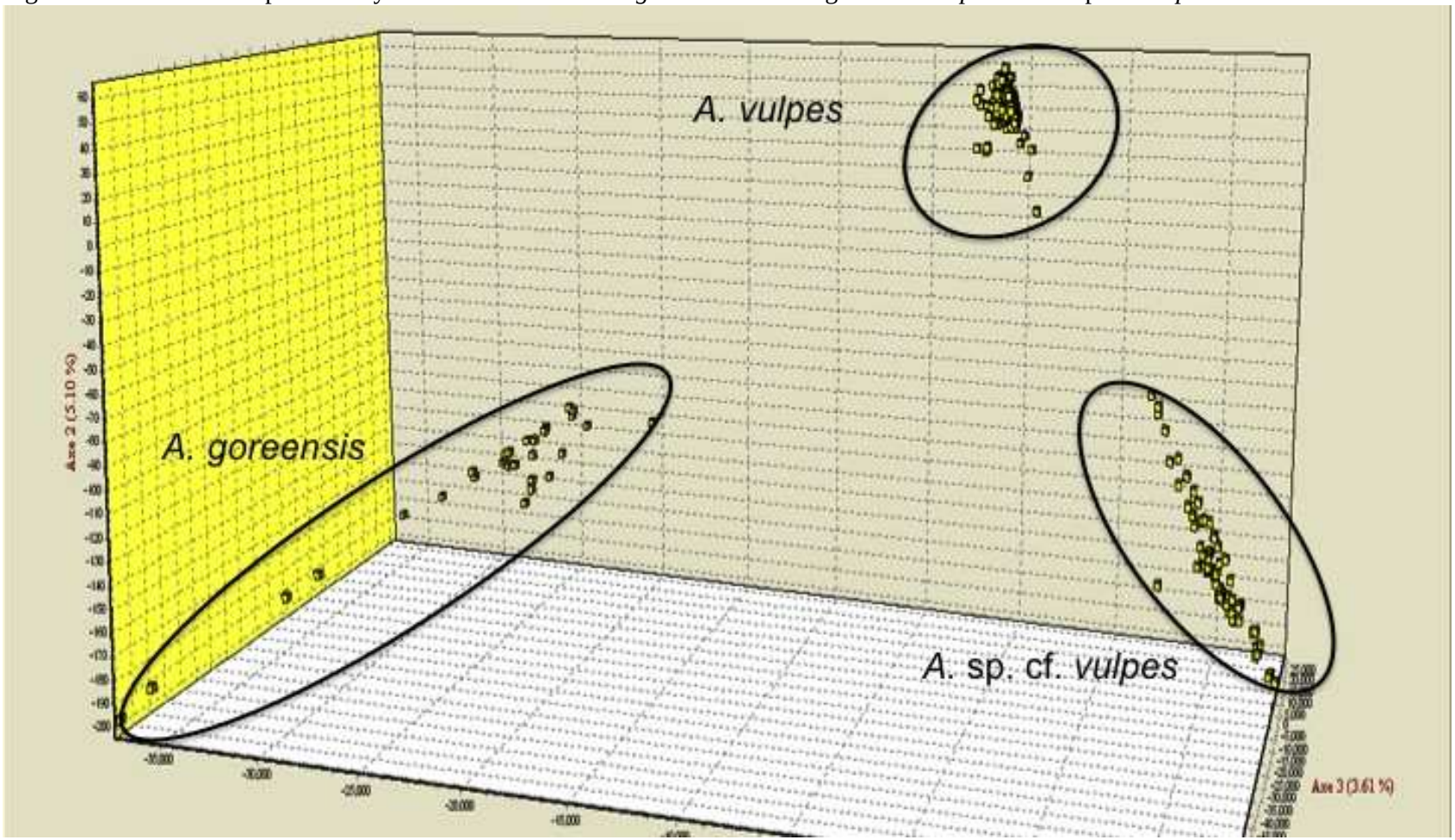
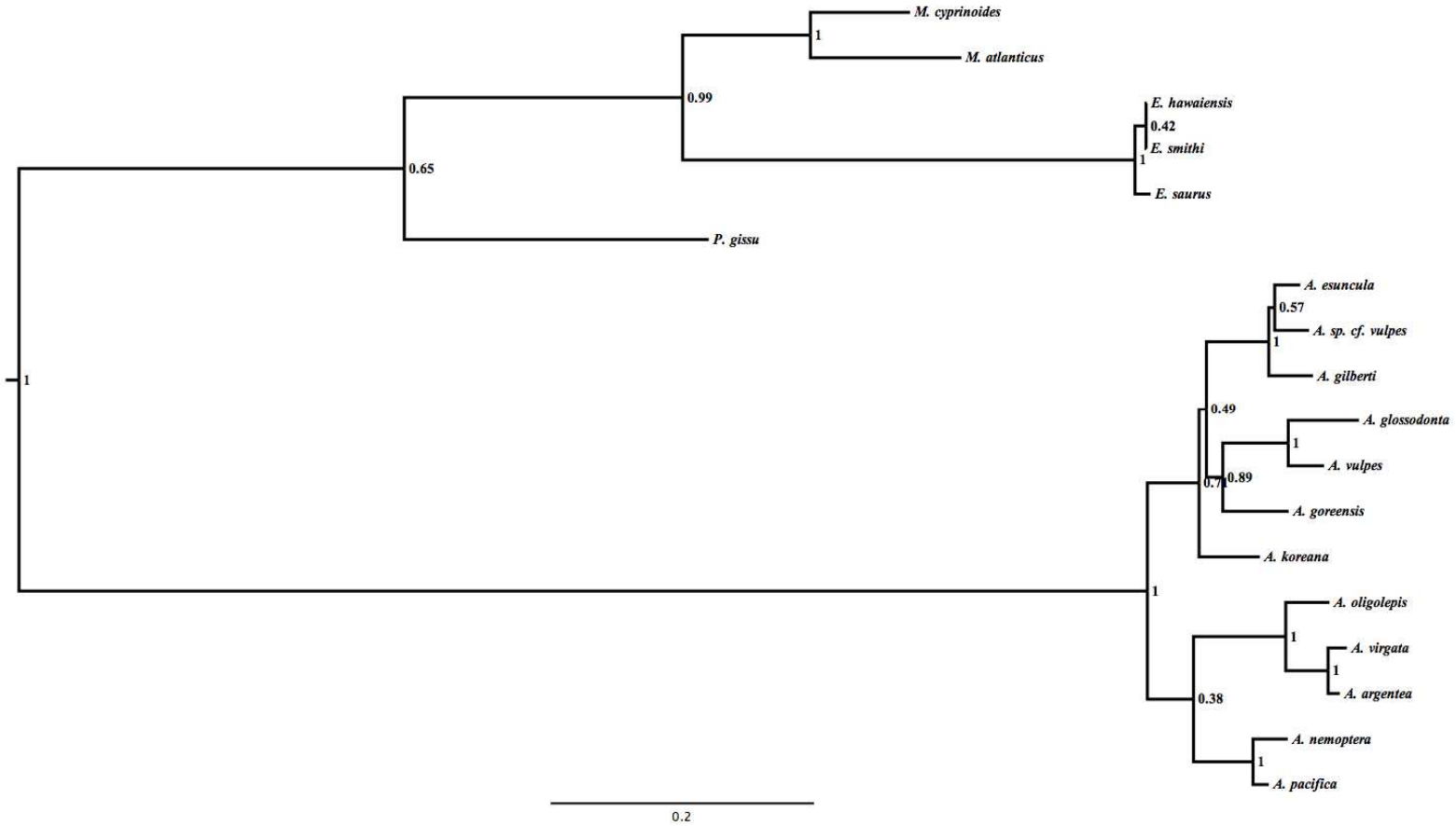


Figure 3. Phylogenetic relationships among all known *Albula* species based on BEAST analysis of cytochrome b sequence data. Branch lengths represent sequence divergence and node support values are Bayesian posterior probabilities.



Chapter 2

Fishery Composition and Evidence of Population Structure and Hybridization in the Atlantic Bonefish Species Complex (*Albula* sp.)

2.1 Introduction

Fundamental species characteristics such as geographic ranges and population structure are required for effective management and conservation. However in bonefishes of the Western Atlantic Ocean and Caribbean Sea (ATL-CAR) this information is lacking. As a result, the species composition of the recreational fishery across the region is unknown. Although information voids are common in fisheries research, it is unusual to lack such basic knowledge for species supporting popular fisheries as they generally garner considerable research attention. The bonefish recreational fishery is an important source of income for many coastal communities (Danylchuk et al. 2008). The economic impact of the flats fishery (focused on bonefish, tarpon, and permit) is substantial: estimated at $427 million annually in the Florida Keys and $25 million in Belize (Fedler and Hayes 2008; Fedler 2013). For bonefishes globally, research on basic life history and population demographics have been confounded by the presence of morphologically cryptic sympatric species. The genus remains in an active state of taxonomic revision. Within the ATL-CAR region a 4-member species complex exists: *Albula vulpes*, *A. goreensis*, *A. nemoptera*, and *A.* sp. cf. *vulpes* (Pfeiler et al. 2006; Bowen et al. 2008; Wallace & Tringali 2010; Wallace- in review). Of these only *A. nemoptera* is visually distinct; the remaining species form a morphologically cryptic complex. *Albula nemoptera* also appears to have a restricted southern Caribbean range and is rarely encountered.

This study focused on the three remaining species in the complex. An International Union for the Conservation of Nature (IUCN) Red List assessment listed *A. vulpes* as near threatened under Criterion A (population declines) (Adams et al. 2013). *Albula goreensis* (referred to as *A.* sp. B) was listed as data deficient and *A.* sp. cf. *vulpes* could not be evaluated due to the limited information available for this recently identified

species. Of primary interest is the determination of species ranges across the region, and the potential contribution of each species to the fishery. This information is essential for the development of comprehensive management and conservation plans.

In addition to identification of fishery species composition in a cryptic complex, molecular data are useful for intraspecific stock identification. The number and geographic distribution of distinct genetic populations is essential to determine the correct scale for management and conservation actions. The marine environment is typically considered to support high levels of connectivity, thereby minimizing potential population structuring within species. However, there is growing recognition of the difference between potential and realized dispersal in the marine environment. Generally fishes with lengthy pelagic larval duration (PLD) have been predicted to have high dispersal potentials that affect range and population structure (Lester and Ruttenberg 2005). Indeed, many population genetic studies on marine fishes have found limited discernible structure across species ranges (Palumbi 2003). However, this pattern may not be as common as previously thought, and could reflect historical processes rather than current demographics (Weersing and Toonen 2009; Eble et al. 2011). An ecological perspective on drivers of population structure, the member-vagrant hypothesis, emphasizes the interaction of early life history traits and physical oceanographic conditions as regulating population structure in marine fishes (Sinclair and Iles 1988). The ecological and physical processes that influence realized dispersal can be complex, resulting in lower dispersal than predicted (Patarnello et al. 2007; Eble et al. 2011). Oceanographic currents are highly dynamic, varying both spatially and temporally. As a result, physical oceanographic features may serve to aid dispersal or increase local retention. Even in fishes with extended PLD, local oceanographic conditions can result in decreased realized dispersal (Papetti et al. 2012). Bonefishes present an excellent case study in potential versus realized gene flow through larval dispersal. They are broadly-distributed, aggregate-broadcast spawners, and their leptocephalus larvae exhibit a PLD of 2-3 months in the ATL-CAR species. If dispersal is mainly current driven, PLD in bonefishes is sufficient to carry leptocephalus > 2400km. The life history and ecology of bonefishes result in an expectation of panmixis within species.

Connectivity in the population genetic context occurs through gene flow and represents a historical process. Low numbers of migrants (as few as 1 effective migrant per generation) can provide sufficient gene flow to prevent distinct population genetic structure from forming (Waples 1998; Avise 2000). Connectivity in the contemporary ecological context occurs through dispersal events between local populations, and thus can affect functional metapopulations and dictates the proper geographic scope for management and conservation (Kritzer and Sale 2004). As a result, the lack of genetic population structure across a species range does not necessarily preclude contemporary self-recruitment occurring within locations (Eble et al. 2011). Within the region, major oceanographic currents follow a clock-wise path from the southern Caribbean into the Gulf of Mexico (via the Caribbean and Loop currents) and then connect with the Florida Current and Gulf Stream. Thus contemporary connectivity among locations across the region is expected to follow a unidirectional, clock-wise pattern for marine species with protracted PLD. An East-West barrier has been noted in some previous genetic and oceanographic modeling studies (Galindo et al. 2006). For Caribbean corals, this break is located between the Netherland Antilles and Columbia in the West and extends northeasterly approximately between the Dominican Republic and Dominica (Foster et al. 2012). In a previous study of ATL-CAR bonefishes, phylogenetic analyses of mitochondrial lineages (with small sample sizes) suggested population structure might exist in *A. goreensis*, but was not detected in *A. vulpes* (Colborn et al. 2001).

Lastly, genotypic data permits investigation into hybridization patterns between related species. The prevalence of aggregate-broadcast spawning behaviors in many marine fishes provides ample opportunity for genetic exchange. In sympatry, species are expected to accrue intrinsic barriers to gene flow (such as hybrid sterility or reduced fitness) as extrinsic barriers (abiotic factors like unsuitable habitat) can break down over time (Orr and Smith 1998). Introgressive hybridization can lead to persistent hybrid zones, distinct hybrid swarms, or the potential for species fusion over evolutionary timescales (Roques et al. 2001). Within the ATL-CAR, instances of hybridization have been found between species supporting important fisheries (Tringali et al. 2011) leading to difficulties for management such as enforcement of catch limits (Allendorf et al.

2001). Exploration of hybridization patterns can provide meaningful insights into the generation and maintenance of species biodiversity at local and regional scales. However, hybridization is rarely considered in marine genetic studies beyond the identification of putative hybrid individuals.

In this study, the prevalence and patterns of hybridization among ATL-CAR bonefishes is explored to further inform management and conservation efforts for these species. The objectives of the current study were to: (1) establish the species composition in the coastal flats recreational fishery, (2) determine ranges for the ATL-CAR bonefishes across the region, (3) identify intraspecific stock structure, and (4) determine rates of hybridization among ATL-CAR bonefishes.

2.2 Materials and Methods

Fin clips were obtained from across the ATL-CAR with the assistance of a network of scientific collaborators and volunteer anglers to determine the geographic ranges of each bonefish species (Table 1). Adult fish were collected by hook and line, seining, or from fish markets. Juveniles and larvae were collected by shoreline seining, light traps, or channel nets. Fin clips were preserved in Gentra Puregene® lysis buffer, 70% ethanol, or by drying.

Genomic DNA was isolated from fin clips using the Puregene tissue kit and protocol. Individuals were screened with 19 microsatellites (previously characterized in Seyoum et al. 2008 and Wallace and Tringali 2010) by PCR amplification and genotyped on an Applied Biosystems® 3130XL genetic analyzer. Thermal cycling conditions for PCR reactions followed Wallace & Tringali (2010). Alleles were assessed in Genemapper®.

All samples were initially treated as unknowns. Genetic species identification was conducted through correspondence analyses against known individuals (previously identified through cytochrome *b* sequence analysis) in *Genetix 4.05* (Belkhir et al. 2000). Suspected hybrids were excluded from intraspecific population analyses and hybrid classifications were later confirmed in *NewHybrids 1.1* (Anderson and Thompson 2002).

Intraspecific genetic diversity indices (allele frequencies, observed and expected heterozygosities, F_{st}, Hardy-Weinberg equilibrium departures, and genotypic linkage disequilibrium) were assessed in *GENEPOP 4.2* (Rousset 2008) (Table 2, 3, S1 and S2). Pairwise exact G tests were also calculated in *GENEPOP* to identify population differentiation across locations (Table 3). Locations with small sample sizes (N≤12) are not reported. *Cervus 3.0* was used to calculate polymorphism information content (PIC) (Kalinowski et al. 2007). Allele calling errors due to null alleles, stuttering, or large allele dropout were investigated with *Micro-Checker* (Van Oosterhout et al. 2004).

Intraspecific population genetic partitioning was explored with the Bayesian Markov chain Monte Carlo (MCMC) framework in *STRUCTURE 2.3* (Pritchard et al. 2000); simulations were conducted on the University of Minnesota Supercomputing Institute or the University of Oslo Bioportal (Kumar et al. 2009). Each *STRUCTURE* simulation was carried out for 10 million steps, following a burn-in of 100,000 steps. Consistency of population inference was assessed through multiple runs. The maximum number of genetically distinct populations (Pritchard's K) was determined by treating each geographic location as unique. Parameters, such as the inclusion of geographic origin, use of the general or admixture models, correlated allele frequencies, and the presumed migration rate, were varied and the resultant population structure assignments compared. The results of multiple runs under the varied model parameters were compared to provide support that observed partitioning was not the result of undue influence of any prior assumptions. Individual population assignments were examined under the inferred K to investigate spatial and/or temporal partitioning. *STRUCTURE HARVESTER 0.6.93* was used to infer the number of genetic populations for *STRUCTURE* simulations by plotting the log probabilities of the data across all K, and the Delta K method (Evanno et al. 2005; Earl and vonHoldt 2012). Assignments of individuals to genetic cluster were checked in *CLUMPP 1.1.2* to verify label switching had not occurred across multiple *STRUCTURE* runs (Jakobsson and Rosenberg 2007).

Potential migration patterns between the genetic clusters identified for *A. vulpes* were explored in *MIGRATE-n 3.6* using Bayesian inference (Beerli 2006; Beerli and Palczewski 2010). Four hypotheses were considered: panmixis, one-way migration from

A to B, one-way migration from B to A, and a full migration model (two populations with bidirectional migration) (Table 5). Individuals identified as admixed in STRUCTURE (approximately 50% membership in both cluster A and B) were included in the dominant genetic population (cluster A). *MIGRATE-n* runs were conducted for 1,000,000 generations following a 100,000 generation burn-in using 4 MCMC chains (1 heated) and uniform priors. Convergence was assessed by examining the parameter effective sample sizes, histograms, and comparison of median and mean values (similar and within 50% credibility intervals). Model fit comparisons were conducted by evaluation of Bayes Factors to determine support for the hypotheses regarding connectivity between the observed population partitions.

Hybridization rates among ATL-CAR bonefishes were assessed through Bayesian assignment tests in *NewHybrids 1.1*. Hybrid assignments were initially run in pairwise comparisons among all possible combinations between bonefish species. Because there was evidence for genetic population structure within species (a violation of NewHybrids model assumptions) as well as coincident erroneous assignments, simulations were further partitioned to assess the *STRUCTURE* inferred populations within species individually. *NewHybrids* MCMC simulations were run for 5 million generations with a burn-in of 100,000 generations and Jeffreys priors for the mixing proportions and allele frequencies. Hybrid individuals were further examined manually and in *Genetix*. Some F1/F2 individuals were heterozygous for locus *AspB12*, but homozygous or had missing data for the other semi-diagnostic loci. The possible genotypic distributions of later generation hybrids overlap that of an F1, and can result in classification errors with low numbers of loci (Epifanio and Philipp 1997). The presence of null alleles could also give an F1 the appearance of a later generation hybrid. As a result, these hybrids are considered as belonging to a combined F1/F2 hybrid category.

2.3 Results

The microsatellite assays identified a total of 1,897 *A. vulpes*, 620 *A. goreensis*, 87 *A.* sp. cf. *vulpes*, and 40 hybrids from the region-wide collections (Table 1 and Table 4). Of the 19 microsatellite loci, two were semi-diagnostic (*AspB*12 for *A. goreensis*, *AspB*18 for *A.* sp. cf. *vulpes*) among the ATL-CAR bonefish species (Table 6). Locus *Avu*02 was nearly diagnostic, exhibiting extreme frequency differences among all three bonefishes. Locus *Avu*11 was nearly diagnostic for *A. vulpes*, and *Avu*12 for *A.* sp. cf. *vulpes*. Three others (*Avu*16, *AspB*01, *AspB*15) exhibited low allelic diversity, which may have been the result of selecting for markers that cross-amplified among these divergent species. The polymorphic information content (PIC) of the loci ranged from 0.010 to 0.971 (Table 2).

Although all 3 bonefishes were broadly sympatric across the ATL-CAR, they were patchily distributed within locales (Figure 1). In order to determine the species composition of the recreational fishery, most adult sampling occurred in coastal flats habitats. However, in a few locations some tissues were obtained from fish markets (i.e. Brazil, Honduras) or by directed sampling of deeper water habitat to investigate species habitat partitioning (Florida). Juvenile and larval collections were only available in a few locations where studies by colleagues occurred. *Albula vulpes* was collected widely across the region: from Venezuela in the southern Caribbean to coastal Central America and Florida and The Bahamas in the western Atlantic (Table 1; Figure 1 and 2). Adult *A. vulpes* were exclusively collected from inshore flats habitat, and thus accounted for 98% of collections from the fishery (Figure 3). *Albula goreensis* occurred from Brazil in the southern Caribbean to coastal Central America and Florida and The Bahamas in the Atlantic (Table 1; Figure 1 and 4). This species also occurred in the tropical eastern Atlantic; along the west coast of Africa near Sao Tome and Principe, though its full African coastal range remains unknown. The majority of *A. goreensis* samples (N=550) were juveniles, collected in windward beach habitats. Almost all adult *A. goreensis* were collected from deeper water habitats (>1m) occurring slightly offshore and in channel areas; only four were collected from the flats fishery. Few *A.* sp. cf. *vulpes* were collected. This species occurred from the Virgin Islands in the Caribbean Sea, along the

Central American coast, and North to Florida and The Bahamas in the Atlantic (Table 1; Figure 1). Within sampling locations, this species predominantly occurred in protected lagoon/estuarine habitats.

Genetic population analyses revealed unexpected patterns in two ATL-CAR bonefishes (*A. vulpes* and *A. goreensis*). Within each of these species, *STRUCTURE* inferred two often co-occurring genetic clusters across their range. Though for each species the population proportions were variable among geographic locations, no clear geographic or temporal (i.e. cohorts, determined by collection years) partitioning was apparent. In both species datasets, locations consisting of only one of the *STRUCTURE* populations may have been artifacts due to small sample sizes in those areas. For both the *A. vulpes* and *A. goreensis* datasets, Hardy-Weinberg (HW) departures were observed in most loci and within most populations (*STRUCTURE* inferred and geographic) due to heterozygote deficits (Table 7). The observed deficiencies in some loci may have been the result of null alleles, as determined in *Micro-Checker*. However, no errors due to large allele dropout or stuttering were identified. Additional *STRUCTURE* simulations with the deficient loci removed did not alter the inferred K for *A. vulpes* or *A. goreensis*. Within each locus F_{st} (W&C) was generally low except *Avu*14 in *A. vulpes*, four loci (*Avu*02, *Avu*11, *Avu*26, *AspB*01) in *A. goreensis*, and five loci (*Avu*02, *Avu*12, *Avu*16, *Avu*27, *AspB*03) in *A.* sp. cf. *vulpes* (Table 2). The inflated F_{st} values for some loci in *A. goreensis* and *A.* sp. cf. *vulpes* may have been due to different proportions of the two identified populations across sites, or may have been due to null alleles or evidence of selection.

One predominant genetic cluster (A; N=1,086) was identified by *STRUCTURE* in *A. vulpes*, which contained the majority of samples from across the region (The Bahamas, Belize, Cuba, Florida, Honduras, Mexico, Puerto Rico, Turks & Caicos, Venezuela, and Virgin Islands) (Figure 2). Membership of this main cluster consisted of all life stages, and individuals collected across a wide range of years. The second genetic cluster (B; N=492) consisted of samples from The Bahamas, Belize, Cayman Islands, Cuba, Florida, Honduras, Turks & Caicos, Mexico, Panama, Puerto Rico, and Virgin Islands. The majority of *A. vulpes* individuals were assigned to either cluster A or B with strong

posterior probabilities (>0.95). An additional 138 fish were identified as admixed individuals (posterior probabilities of approximately 0.5 for both genetic clusters). The admixed individuals were collected over a number of years and from several geographic locations. Only 182 (9.6%) individuals showed inconsistent assignments (population assignment varied with run conditions). No clear cause for the observed inconsistencies was apparent. These individuals included adults and juveniles, and were collected across a number of locations and years. Exact G tests indicated differentiation among geographic locations, but this may have been due to geographic structure or variable representation of the two *STRUCTURE* inferred clusters (Table 4). To isolate geographic effects, G tests were conducted among locations within clusters. Cluster A yielded significant differentiation in 14 comparisons mostly between pairs including The Bahamas, Mexico, and the Turks and Caicos Islands. Cluster B yielded 11 significant comparisons, between pairs including The Bahamas, Belize, Florida, Mexico, and the Virgin Islands. To further explore support for the two co-occurring *A. vulpes* genetic clusters, hypothesis testing for all possible migration patterns was conducted in *MIGRATE-n*. The results of these simulations supported a uni-directional model of migration from population A to B, as inferred through Bayes Factor comparisons (Table 5).

Albula goreensis also had one predominant cluster (A; N=514) in *STRUCTURE* simulations with individuals collected from Belize, Florida, Mexico, Panama, and the Virgin Islands (Figure 4). Members of this cluster consisted of all life stages and were collected over a number of years. A second cluster (B) contained 97 individuals from across the region (The Bahamas, Brazil, Florida, Panama, and Sao Tome). Nine individuals collected in Florida (1.5%) were admixed, as identified by roughly equal assignment probabilities to both genetic clusters. Exact G tests indicated geographic differentiation in 4 location pairs containing The Bahamas or Belize (Table 4). However after isolating geographic effects by testing within *STRUCTURE* inferred clusters, no differentiation was indicted.

Population partitioning was less clear in *A.* sp. cf. *vulpes*, with *STRUCTURE* simulations inferring two or three genetic partitions under various model and priors

specifications. This was likely due to the small sample size for this species, which may have increased the effect of allelic outliers. However the genetic partitions may reflect temporal rather than spatial variation, due to substantial time elapsing between some of the collections within a geographic location. Samples collected in Florida during 2010-2012 were in a separate cluster from samples collected in 2004.

Hybrids were identified among all 3 ATL-CAR bonefishes (N=40) at low density, resulting in an overall hybridization rate of 1.5% (Table 5; Figure 5). The majority of the identified hybrids (N=34) were F1/F2 *A. vulpes* x *A. goreensis*. Further, most of these (N= 28) were collected from Bahamian and Florida waters. Only one potential backcross *A. vulpes* x *A. goreensis* individual was identified with non-significant posterior probabilities (0.82-0.91). Other hybrids were rare. Five *A. goreensis* x *A.* sp. cf. *vulpes* F1/F2 hybrids were collected from Florida and Mexico. One *A. vulpes* x *A.* sp. cf. *vulpes* F1/F2 hybrid was collected from The Bahamas. For the individuals identified as F2 in *NewHybrids*, confirmation of F1 or F2 status was not possible with the available data.

2.4 Discussion

Recent studies have identified a cryptic species complex of albulids exists in the ATL-CAR. However species ranges and species composition of the recreational fishery were unknown. Genetic population structure within these species, and the existence of hybrids were also unknown. In this study, the microsatellite data reveal three broadly sympatric species of bonefishes in the ATL-CAR. The geographic extent of the fourth ATL-CAR bonefish, *A. nemoptera*, remains uncertain. *Albula goreensis* has the greatest geographic distribution, occurring in the tropical eastern Atlantic as well as ATL-CAR. Targeted flats sampling determined one species, *A. vulpes*, mostly supports the recreational fishery. Genetic population structure was identified within each of these species, and low occurrence of hybrids was observed.

The apparent patchy distributions of ATL-CAR albulid species among locales may be partially due to variable sampling effort across coastal habitats. The study emphasis was on determining which species in this complex support the recreational fishery. Thus, sampling effort focused mainly on the coastal flats habitat targeted by

anglers. Collections in other habitats occurred opportunistically through volunteers and colleagues. Adult *A. vulpes* predominantly utilize inshore flats, and thus appear to support most of the recreational fishery. However, *A. goreensis* is caught occasionally by anglers targeting deeper water habitats. Likewise, *A.* sp. cf. *vulpes* is likely caught occasionally by anglers within estuaries. Previous juvenile habitat use studies in the Florida Keys found the majority of individuals collected along exposed beaches were *A. goreensis*, (Adams et al. 2008; Crabtree et al. 2003). These results support other studies that found distinct habitat partitioning among species (Crabtree et al. 2003; C. Haak-unpublished; Wallace 2014). A similar pattern of depth partitioning in sympatry has been found in African cichlids (Kerschbaumer et al. 2014). Among the ATL-CAR bonefishes *A. goreensis* has the greatest range, which spans the tropical Atlantic Ocean. Limited availability of East ATL specimens precludes determination of its full African coastal extent. The greater range of *A. goreensis* may result from a higher dispersal potential, due to a longer PLD than *A. vulpes* (*A.* sp. cf. *vulpes* PLD remains unknown) (C. Haak-unpublished). However realized contemporary dispersal from the Eastern ATL into the ATL-CAR is likely infrequent. Cross-Atlantic dispersal may be driven by events such as hurricanes, which originate off the West African coast.

The intraspecific results indicating the existence of population structure within each of the ATL-CAR bonefishes were unexpected due to the high dispersal potential of bonefishes (via lengthy PLD). Given the lack of clear temporal or spatial patterns to the observed partitioning, the biological validity of these population clusters must be considered. Within each species, generally low F_{st} values for individual loci suggest high background levels of gene flow (Table 3). The two population clusters (A and B) identified for *A. vulpes* and *A. goreensis* may not be homogeneous (within clusters). Detectable levels of geographic differentiation via F_{st} were found for some location pairs in both *A. vulpes* and *A. goreensis* (Table 4). Admixed individuals were excluded from the F_{st} analyses, thus results represent likely biased maximum F_{st} values. The F_{is} estimates for the *A. vulpes* and *A. goreensis* datasets each revealed substantial heterozygote deficiencies for most loci. When the datasets were grouped into the *STRUCTURE* inferred A and B populations significant F_{is} remained, suggesting the heterozygote

deficiencies were not solely due to a Wahlund effect. In addition, significant differentiation was revealed between several geographic location pairs by the exact G tests for both the *A. vulpes* and *A. goreensis* datasets (Table 4). The differentiation between some geographic locations remained for *A. vulpes* within the *STRUCTURE* inferred A and B clusters. However, these results may reflect a combined spatial and temporal effect. The observed pattern of heterozygote deficiencies may result from the mixing of genes in variable proportions (via gene flow during overlapping spawning events), or fish migrating between the two populations. Within both *A. vulpes* and *A. goreensis*, admixed individuals were identified (shared ancestry from both intraspecific populations). The assumptions of population genetic analyses imply that heterozygote deficiencies due to a Wahlund effect can be distinguished from null alleles. In reality that may not be accurate (ex. cryptic population structure), and discarding non-equilibrium loci may reduce the ability to discern population patterns by eliminating the most informative markers (Dharmarajan et al. 2013). However, in the present study the inferred *STRUCTURE* population clusters remained consistent even with reduced datasets (following removal of non-equilibrium loci). The *MIGRATE-n* results for potential migration patterns between the *A. vulpes* genetic clusters also supported the existence of two discrete populations over a single panmictic population. A similar pattern was observed between two genetic clusters (coastal and offshore) in the European anchovy (Oueslati et al. 2014).

In marine systems there has often been an expectation for substantial connectivity due to dispersal via oceanographic currents. The potential for current driven larval transport between geographic locations has led to a prediction of panmixis for many species (Fauvelot and Planes 2002). This is particularly true for species with extended PLD, such as bonefishes. Yet oceanographic currents are often complex and highly dynamic, varying temporally and spatially (Paris et al. 2002). Additionally, larval ecological and behavioral traits can play a significant role in larval dispersal (Woodson and McManus 2007). As a result, there is growing recognition of the distinction between potential and realized dispersal, and even species with long PLDs may exhibit lower dispersal than predicted (Weersing and Toonen 2009).

The time scale of the processes under consideration is also an important variable. Regional population genetic structure is the product of historical evolutionary processes, whereas larval dispersal is a contemporary ecological process. These processes act simultaneously on species and may result in conflicting patterns of connectivity. In ATL-CAR bonefishes, an unexpected pattern of genetic population structure emerged in this study. If these patterns represent real biological populations, it is unclear what is causing the observed co-occurring genetic stocks. The patterns may be due to historical or contemporary influences. If the observed patterns arise from contemporary causes, they may be the result of spatial and/or temporal separation of spawning groups. Spawning in ATL-CAR bonefishes may occur nearly year-round, however there are seasonal peaks (Mojica et al. 1995). The locations of spawning aggregations remain largely unknown, with the exception of The Bahamas (Danylchuk et al. 2011). Although the definitive cause of the two genetic populations is uncertain, each of the stocks spans the region for *A. vulpes* and *A. goreensis*. This broad geographic connectivity within genetic units may reflect historical connectivity among locations but may also suggest ongoing contemporary larval dispersal among sites.

Studies on population structure in other elopomorphs have not found consistent patterns: some species exhibit panmixis (*Anguilla rostrata*, *A. japonica*, *Conger conger*), whereas others may consist of multiple stocks (Correia et al. 2012; Minegishi et al. 2012; Cote et al. 2013). Studies have identified 2-5 stocks in the giant mottled eel (*A. marmorata*) in the Indo-Pacific, which may be due to infrequent long distance dispersal (Gagnaire et al. 2011; Donovan et al. 2012). The Polynesian longfinned eel (*A. megastoma*) may consist of eastern and western populations in the South Pacific (Watanabe et al. 2011). In the European eel (*A. Anguilla*) population structure is apparently driven by female philopatry in their Sargasso Sea spawning grounds (Baltazar-Soares et al. 2014). Genetic structure has not been explored in the other economically important ATL-CAR elopomorphs: tarpon (*Megalops atlanticus*) or ladyfishes (*Elops saurus*, *E. smithi*). Tarpon are highly migratory as adults, thus genetic stock structure is unlikely within the ATL-CAR. Similar population genetic patterns to ATL-CAR bonefishes have been observed in other marine organisms. Two co-occurring

mtDNA lineages were observed in the spiny lobster (*P. argus*), with one dominant around Florida and The Bahamas (Naro-Maciel et al. 2011); however no significant structure was detected. Fine scale genetic stock structure has been observed in the queen conch (*Strombus gigas*), though PLD in conch (16-28 days) is somewhat less than bonefishes (Stoner et al. 2012; Stoner and Banks 2014).

Hybridization is common among many marine fishes, due to broadcast spawning that allows for substantial mixing of gametes. The observed hybridization rate among bonefishes is within the reported range for freshwater basses (*Micropterus* sp.) (1-5%), but substantially lower than the 15% reported between the marine species weakfish (*Cynoscion regalis*) and sand seatrout (*C. arenarious*) (Tringali et al. 2004; Bolnick 2009). Distinct hybrid zones may exist where ranges overlap between closely related species. A hybridization rate of 51% was observed in a Florida coastal zone between weakfish and sand seatrout (Tringali et al. 2011). In other elopomorphs, hybridization rates of 15.5% have been reported (between *A. rostrata* and *A. anguilla* in a hybrid zone) (Albert et al. 2006). Among ATL-CAR bonefishes, low hybridization rates (1.5%) suggest that barriers to gene flow exist between these species. Almost all hybrids were assigned F1/F2 status. As *NewHybrids* is unable to assign hybrid individuals beyond the second generation, it is possible for advanced generation hybrids to be misclassified as pure species. However the almost complete absence of backcross individuals, despite intensive sampling, suggests semipermeable intrinsic barriers exist (reduced hybrid fitness and/or hybrid sterility). Half of all hybrid individuals were larvae or juveniles. However, this is possibly an artifact due to unequal sampling effort among life stages. Extrinsic factors such as spatiotemporal spawning differences and assortative mating likely also exist between these species. Introgressive hybridization is expected in the absence of barriers to gene flow, either extrinsic or intrinsic. Moderate levels of introgression could eventually lead to species fusion. In sympatric species, intrinsic barriers to gene flow are expected to evolve, as extrinsic barriers may break down over time. In the ATL-CAR bonefishes, there is little apparent introgression. Phylogenetic studies have revealed substantial levels of genetic divergence between these species (Adams et al. 2008; Bowen et al. 2008; Wallace and Tringali 2010; Wallace 2014). The

phylogenetic reconstructions in those studies all displayed reciprocal monophyly, and suggested distant, non-sister taxa, relationships among the ATL-CAR bonefishes. Further, the genetic data in the present study generally yielded accurate species differentiation with robust (≥0.95) assignment probabilities.

Most hybrids were *A. vulpes* x *A. goreensis*, and occurred at low frequency across the region. One hypothesis is that there is more spawning habitat and timing overlap between these two species than between either and *A.* sp. cf. *vulpes*. Although habitat constraints may result in more overlap in a particular location, timing of spawning is not expected to vary substantially within bonefish species across their range. In fact, the larval and juvenile data suggest spawning occurs nearly year round. However, there are peak pulses during Fall and Spring. The region-wide presence of hybrids may further suggest that most larvae settle locally, as current driven external recruitment might result in most hybrids occurring in downstream locations. Alternatively, the observed hybrids may be an artifact due to lower overall sampling of *A.* sp. cf. *vulpes* (i.e., limited presence of *A.* sp. cf. *vulpes* hybrids strictly due to smaller number collected). In other Atlantic elopomorphs (*Anguilla rostrata* and *Anguilla anguilla*), hybrid larvae have an intermediate PLD that determines settlement location (Albert et al. 2006; Gagnaire et al. 2009). However, data are not yet available to evaluate the possibility of intermediate PLD in hybrid bonefishes.

Management Implications:

Species delimitation is the biological foundation upon which management and conservation efforts are ideally based. Accurate identification of species, particularly in cases of cryptic complexes (such as ATL-CAR bonefishes), becomes of critical importance. Range determination and identification of potential areas of overlap become especially important for species that are targeted by recreational or commercial fisheries. The results presented here confirm a broadly sympatric bonefish species complex occurs throughout the region. However, *A. vulpes* largely supports the recreational flats fishery. Species habitat partitioning was observed at local scales. The existence of hybrids should have little effect on management of these species, as they are rare.

Within fisheries targeted species, the accurate identification of population genetic stocks is vital for appropriate management actions. The number and spatial distribution of genetic stocks define the geographic management scale, and sensitivity of the species to local overharvest. Further, identification of stocks can assist in the maintenance of genetic diversity across a species' range. Within each of the ATL-CAR bonefishes examined here (*A. vulpes*, *A. goreensis*, and *A.* sp. cf. *vulpes*) multiple stocks were identified. However these stocks co-occur across the region, and no discernible geographic partitions were found. It is possible the observed stocks reflect a combined geographic and temporal genetic variability. The temporal component could be due to fluctuations in recruitment dynamics, as a result of the wide sampling timeframe. There was a general trend for the second genetic stock to consist of far fewer individuals than the first. Highly variable recruitment is common in many fisheries, and can lead to temporal genetic differences among cohorts (Christie et al. 2010).

Since *A. vulpes* supports the recreational fishery, stock identification may be most important for this species. The source of the observed genetic partitioning remains unclear. Spatial or temporal separation of spawning groups could account for this pattern, such as occurs in some Pacific salmonids (Marshall et al. 2000). Separation would need to be maintained across generations for these genetic stocks to persist. Even if spawning occurs in the same location throughout the year, seasonally variable local currents may alter recruitment patterns between spawning groups (Paris et al. 2002). This effect could result in higher levels of self-recruitment in one season. Whatever the cause for separation into distinct populations, it is imperfect, as evidenced by the identification of admixed individuals.

Population genetic studies in other elopomorphs have also found unexpected patterns of structure, despite expectations of panmixis; significant population structure was found in three species of eels. Notably, spatial and temporal patterns of genetic structure have been observed even under simulated conditions of panmixis in the European eel (Baltazar-Soares et al. 2014). Genetic structure was not due to isolation by distance, and was most prominent under low recruitment scenarios. The implication that these ephemeral genetic patterns may reflect low recruitment provides reason for

concern. An understanding of recruitment dynamics is vital in fisheries management, but we lack that information for ATL-CAR bonefishes. We urgently need such data for *A. vulpes*, as this species supports the recreational fishery in many parts of the region. Data on recruitment sources will aid restoration efforts for local populations experiencing declines, such as the Florida Keys.

Local demographics may play a role in stock identification and should also be incorporated into management plans. In some cases, demographic and genetic stock data may conflict, leading to challenges for fishery managers (Tringali et al. 2008). In some coastal recreational fisheries, such as common snook (*Centropomus undecimalis*), adult fish utilize discrete home ranges and may never venture beyond a specific estuary (Muller and Taylor 2006). Marine population genetic studies have often inferred panmixis across broad geographic scales, due to substantial gene flow from larval dispersal (Palumbi 2003). However the absence of genetic population structure may result from very few effective migrants (Avise 2000). These processes operate on evolutionary timescales. However, management plans focus on present day connectivity. Self-recruitment within local stocks may be common in many species, but genetic structure is not found due to occasional migration. The analysis of genetic structure in *A. vulpes* suggests gene flow occurs on a region-wide scale. However these results do not specifically address whether recruitment is static or dynamic, and dominated by local or distant sources. This information will dictate specific management actions and sites that will be most effective for conservation and restoration of local populations, and thus remains an urgent need.

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Table 1. Collection locations for tissues samples and sample sizes for albulids in the Atlantic Ocean and Caribbean Sea.

	A. vulpes		*A. goreensis*		*A. sp. cf. vulpes*	
Location	**Adults**	**Juveniles/Larvae**	**Adults**	**Juveniles/Larvae**	**Adults**	**Juveniles/Larvae**
Bahamas	576	362	~	37	1	~
Belize	247	5	1	41	~	~
Brazil	~	~	1	~	~	~
Cayman Islands	6	~	~	~	~	~
Cuba	39	~	~	~	~	~
Florida	301	34	67	457	14	25
Honduras	11	1	~	~	10	~
Mexico	195	4	~	6	~	30
Panama	~	2	~	4	~	~
Puerto Rico	44	~	~	~	~	~
Sao Tome	~	~	1	0	~	~
Turks and Caicos Islands	24	~	~	~	~	~
Venezuela	6	~	~	~	~	~
Virgin Islands	3	37	~	5	~	7
Totals	**1452**	**445**	**70**	**550**	**25**	**62**

Table 2. Observed (Ho) and expected (He) heterozygosities, Fst and Fis (Weir & Cockerham), and polymorphism information content (PIC) per locus for 19 microsatellite loci in three species of Atlantic bonefish.

	A. vulpes					*A. goreensis*					*A. sp. cf. vulpes*				
Locus	Ho	He	W&C Fst	W&C Fis	PIC	Ho	He	W&C Fst	W&C Fis	PIC	Ho	He	W&C Fst	W&C Fis	PIC
Avu01	0.562	0.764	-0.001	0.270	0.732	0.652	0.867	0.030	0.208	0.857	0.722	0.754	0.003	0.041	0.711
Avu02	0.002	0.015	-0.006	0.880	0.015	0.030	0.510	**0.447**	0.923	0.436	0	0.233	**1.000**	0.000	0.195
Avu04	0.033	0.129	0.018	0.740	0.122	0.681	0.683	0.020	-0.028	0.622	0.5	0.707	0.081	0.252	0.648
Avu09	0.476	0.513	-0.003	0.070	0.397	0.756	0.780	0.004	0.015	0.756	0	0.089	-0.015	1.000	0.084
Avu11	0.524	0.554	-0.001	0.050	0.456	0.102	0.531	**0.474**	0.776	0.306	0.108	0.418	0.161	0.714	0.396
Avu12	0.016	0.034	0.007	0.550	0.034	0.346	0.514	0.068	-0.033	0.340	0	0.029	**0.560**	1.000	0.029
Avu14	0.04	0.341	**0.219**	0.860	0.291	0.439	0.597	0.044	-0.006	0.456	0	0.033	-0.065	1.000	0.032
Avu16	0.212	0.229	-0.001	0.080	0.207	0.029	0.059	-0.028	0.457	0.065	0	0.211	**0.289**	1.000	0.200
Avu17	0.263	0.275	-0.001	0.040	0.259	0.810	0.882	0.007	0.049	0.878	0.509	0.639	0.077	0.162	0.582
Avu18	0.848	0.874	0.004	0.030	0.861	0.536	0.803	0.042	0.323	0.797	~	~	~	~	~
Avu25	0.832	0.855	0.001	0.030	0.845	0.934	0.976	0.001	0.021	0.971	0.606	0.595	-0.018	-0.006	0.513
Avu26	0.911	0.933	0.000	0.020	0.929	0.038	0.100	**0.587**	0.656	0.226	0.7	0.835	0.043	0.140	0.806
Avu27	0.555	0.673	0.001	0.175	0.606	0.203	0.622	0.193	0.643	0.678	0.031	0.031	**0.316**	-0.311	0.031
AspB01	0.002	0.01	-0.008	0.800	0.010	0.228	0.672	**0.273**	0.624	0.593	~	~	~	~	~
AspB03	0.08	0.118	0.000	0.320	0.117	0.57	0.587	-0.009	0.040	0.578	0.586	0.433	**0.240**	-0.648	0.343
AspB05	0.671	0.752	0.017	0.097	0.729	0.809	0.922	0.003	0.097	0.918	0.515	0.685	0.079	0.205	0.652
AspB12	0.003	0.006	-0.006	0.501	0.006	0.004	0.021	-0.053	0.751	0.027	0.273	0.654	~	0.595	0.574
AspB15	0.012	0.031	0.000	0.602	0.031	0.01	0.069	-0.065	0.752	0.117	~	~	~	~	~
AspB18	0.008	0.026	-0.005	0.684	0.026	0.002	0.158	-0.034	0.008	0.099	~	~	~	~	~

Table 3. Population differentiation among albulids for all location pairs. Values in the upper half are Fst, and the lower half are G test p-values.

A) *A. vulpes*

Location	Bahamas	Belize	Cuba	Florida	Honduras	Mexico	Puerto Rico	Turks & Caicos	Virgin Islands
Bahamas	~	**0.00**	0.00	**0.01**	-0.01	**0.02**	0.00	0.00	**0.11**
Belize	**P<0.01**	~	**0.01**	0.00	0.01	**0.01**	0.01	0.01	**0.07**
Cuba	0.97	**P<0.01**	~	**0.02**	0.00	**0.03**	0.00	0.01	**0.14**
Florida	**P<0.01**	0.01	**P<0.01**	~	0.01	**0.01**	0.01	0.02	**0.06**
Honduras	1.00	0.86	0.89	0.97	~	0.03	-0.01	0.01	**0.15**
Mexico	**P<0.01**	**P<0.01**	**P<0.01**	**P<0.01**	0.42	~	**0.03**	0.04	**0.04**
Puerto Rico	0.76	0.10	0.70	0.00	1.00	**P<0.01**	~	0.00	**0.13**
Turks and Caicos Islands	0.68	0.07	0.43	0.05	0.61	0.00	0.69	~	**0.14**
Virgin Islands	**P<0.01**	**P<0.01**	**P<0.01**	**P<0.01**	**P<0.01**	**P<0.01**	**P<0.01**	**P<0.01**	~

B) *A. goreensis*

Location	Bahamas	Belize	Florida	Mexico	Virgin Islands
Bahamas	~	**0.20**	**0.08**	0.11	0.16
Belize	**P<0.01**	~	**0.14**	**0.20**	0.08
Florida	**P<0.01**	**P<0.01**	~	0.00	0.03
Mexico	0.03	**P<0.01**	0.96	~	0.10
Virgin Islands	P<0.01	0.15	0.70	0.17	~

*significant values after Bonferroni corrections are in **bold**.

Table 4. Occurrence and classification for hybrid *Albula* individuals from the Atlantic Ocean and Caribbean Sea.

Location	Generation	Life Stage	Hybrid Type		
			A. vulpes x A. goreensis	***A. vulpes x A. sp. cf. vulpes***	***A. goreensis x A. sp. cf. vulpes***
Bahamas	F1/F2	Adult	6	1	~
		Juvenile	14	~	~
Florida	F1/F2	Adult	6	~	1
		Juvenile	2	~	2
		Larvae	~	~	~
Mexico	F1/F2	Adult	2	~	2
Panama	F1/F2	Larvae	1	~	~
Puerto Rico	F1/F2	Adult	1	~	~
Virgin Islands	F1/F2	Larvae	1	~	~
Belize	Backcross *A. goreensis*	Adult	1	~	~
		Total	**34**	**1**	**5**

Table 5. Migration model comparisons, conducted in MIGRATE-n, between two *Albula vulpes* populations using Bayes Factors.

Model	**Log-prob of the data under the model**	**Bayes Factor**	**Rank**
Full	-20878.41	0.00	3
A to B	**-20574.98**	19278276346516.60	1
B to A	-20605.57	0.00	2
Panmictic	-21879.22	0.00	4

Table 6. Allele frequencies, listed as the fragment length, for western Atlantic Ocean and Caribbean Sea bonefishes at 19 microsatellite loci.

A) *Albula vulpes*

Location	Bahamas	Belize	Cayman Is.	Cuba	Florida	Honduras	Mexico	Panama	Puerto Rico	Turks & Caicos	Venezuela	Virgin Is.
Locus												
Avu01												
199	0.009	0.005	0.000	0.056	0.027	0.000	0.000	0.000	0.012	0.000	0.000	0.000
203	0.088	0.079	0.100	0.069	0.087	0.182	0.075	0.000	0.093	0.130	0.083	0.000
205	0.016	0.028	0.000	0.000	0.025	0.046	0.031	0.000	0.035	0.000	0.167	0.041
211	0.171	0.204	0.200	0.111	0.178	0.136	0.204	0.500	0.209	0.283	0.083	0.203
213	0.010	0.005	0.000	0.000	0.003	0.000	0.004	0.000	0.000	0.000	0.000	0.014
217	0.034	0.019	0.000	0.000	0.010	0.000	0.031	0.000	0.012	0.000	0.000	0.000
221	0.387	0.357	0.400	0.389	0.375	0.409	0.363	0.500	0.372	0.348	0.167	0.351
223	0.210	0.206	0.200	0.306	0.215	0.227	0.257	0.000	0.186	0.196	0.333	0.324
225	0.071	0.097	0.100	0.069	0.080	0.000	0.035	0.000	0.081	0.044	0.167	0.068
227	0.001	0.002	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Avu02												
140	0.989	0.698	0.000	0.969	0.993	0.333	0.992	0.000	1.000	0.947	1.000	0.4
142	0.002	0.296	1.000	0.031	0.000	0.667	0.008	1.000	0.000	0.053	0.000	0.6
144	0.002	0.005	0.000	0.000	0.000	0.000	0.112	0.000	0.000	0.000	0.000	0.000
150	0.000	0.000	0.000	0.000	0.003	0.000	0.000	0.000	0.000	0.000	0.000	0.000
152	0.005	0.000	0.000	0.000	0.003	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Avu04												

208	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.014	0.000	0.000	0.000
212	0.003	0.000	0.000	0.000	0.004	0.000	0.000	0.000	0.000	0.000	0.000	0.000
214	0.077	0.053	0.000	0.028	0.002	0.000	0.116	0.000	0.083	0.105	0.000	0.000
216	0.915	0.947	1.000	0.972	0.992	1.000	0.884	1.000	0.903	0.895	1.000	1.000
218	0.002	0.000	0.000	0.000	0.002	0.000	0.000	0.000	0.000	0.000	0.000	0.000
222	0.004	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Avu09												
144	0.004	0.000	0.000	0.000	0.003	0.000	0.000	0.000	0.000	0.000	0.000	0.000
148	0.000	0.000	0.000	0.000	0.003	0.000	0.000	0.000	0.000	0.000	0.000	0.000
158	0.001	0.000	0.000	0.000	0.008	0.000	0.016	0.000	0.000	0.000	0.000	0.000
160	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.015
162	0.003	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
164	0.001	0.003	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.015
166	0.001	0.000	0.000	0.000	0.000	0.000	0.011	0.000	0.000	0.000	0.000	0.000
170	0.533	0.506	0.625	0.516	0.531	1.000	0.532	0.500	0.458	0.667	0.500	0.591
172	0.449	0.485	0.375	0.484	0.453	0.000	0.432	0.500	0.542	0.333	0.500	0.394
174	0.004	0.003	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Avu11												
139	0.000	0.000	0.000	0.000	0.007	0.000	0.000	0.000	0.000	0.000	0.000	0.000
143	0.004	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
153	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
155	0.054	0.060	0.167	0.061	0.067	0.042	0.044	0.000	0.046	0.044	0.083	0.080
157	0.415	0.440	0.250	0.409	0.410	0.500	0.496	0.000	0.386	0.348	0.583	0.420
159	0.524	0.500	0.583	0.530	0.514	0.458	0.460	0.000	0.546	0.609	0.333	0.480
161	0.002	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.023	0.000	0.000	0.020

163	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
169	0.000	0.000	0.000	0.000	0.002	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Avu12												
157	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
171	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
173	0.001	0.000	0.000	0.000	0.009	0.000	0.000	0.000	0.000	0.000	0.000	0.000
175	0.001	0.002	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
177	0.000	0.000	0.000	0.000	0.002	0.000	0.000	0.000	0.000	0.000	0.000	0.000
179	0.985	0.991	1.000	1.000	0.978	1.000	0.944	1.000	1.000	1.000	1.000	1.000
181	0.011	0.007	0.000	0.000	0.011	0.000	0.056	0.000	0.000	0.000	0.000	0.000
183	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Avu14												
126	0.000	0.004	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
128	0.006	0.009	0.000	0.014	0.009	0.000	0.031	0.000	0.000	0.083	0.000	0.000
130	0.893	0.736	0.000	0.987	0.667	1.000	0.580	0.000	0.969	0.917	0.667	0.066
132	0.101	0.251	1.000	0.000	0.313	0.000	0.389	1.000	0.031	0.000	0.333	0.934
134	0.000	0.000	0.000	0.000	0.012	0.000	0.000	0.000	0.000	0.000	0.000	0.000
136	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
140	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Avu16												
108	0.000	0.004	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
110	0.006	0.000	0.000	0.000	0.002	0.000	0.041	0.000	0.014	0.000	0.000	0.000
112	0.876	0.853	0.875	0.878	0.869	0.875	0.862	1.000	0.878	0.864	0.750	0.838
114	0.116	0.138	0.125	0.122	0.129	0.125	0.097	0.000	0.095	0.091	0.250	0.162

116	0.002	0.004	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
118	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.014	0.000	0.000	0.000
120	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.046	0.000	0.000
Avu17												
110	0.000	0.000	0.000	0.000	0.002	0.000	0.000	0.000	0.000	0.000	0.000	0.000
120	0.001	0.002	0.000	0.000	0.000	0.000	0.003	0.000	0.000	0.000	0.000	0.000
122	0.039	0.034	0.000	0.068	0.062	0.100	0.043	0.000	0.048	0.046	0.083	0.035
124	0.845	0.864	1.000	0.824	0.838	0.800	0.847	1.000	0.838	0.841	0.667	0.931
126	0.095	0.087	0.000	0.108	0.092	0.100	0.098	0.000	0.040	0.114	0.250	0.035
128	0.009	0.005	0.000	0.000	0.002	0.000	0.006	0.000	0.059	0.000	0.000	0.000
130	0.005	0.007	0.000	0.000	0.005	0.000	0.003	0.000	0.000	0.000	0.000	0.000
134	0.006	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
136	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.015	0.000	0.000	0.000
Avu18												
142	0.000	0.009	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
144	0.002	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
146	0.000	0.000	0.000	0.000	0.003	0.000	0.000	0.000	0.000	0.000	0.000	0.000
148	0.000	0.009	0.000	0.000	0.003	0.000	0.000	0.000	0.000	0.000	0.000	0.000
150	0.000	0.000	0.000	0.000	0.003	0.000	0.000	0.000	0.028	0.000	0.000	0.029
152	0.003	0.004	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
154	0.004	0.000	0.000	0.000	0.000	0.000	0.000	0.500	0.000	0.000	0.000	0.000
156	0.108	0.172	0.000	0.238	0.134	0.500	0.168	0.000	0.306	0.000	0.167	0.058
158	0.089	0.043	0.625	0.143	0.033	0.000	0.038	0.000	0.167	0.357	0.167	0.071
160	0.158	0.185	0.125	0.071	0.189	0.000	0.161	0.000	0.056	0.071	0.000	0.143
162	0.093	0.047	0.000	0.143	0.058	0.000	0.034	0.000	0.083	0.071	0.000	0.086

164	0.064	0.082	0.000	0.000	0.055	0.000	0.068	0.000	0.028	0.000	0.083	0.100
166	0.050	0.099	0.125	0.095	0.107	0.500	0.119	0.500	0.167	0.214	0.250	0.100
168	0.071	0.026	0.000	0.024	0.019	0.000	0.026	0.000	0.000	0.071	0.000	0.014
170	0.005	0.052	0.125	0.119	0.089	0.000	0.088	0.000	0.056	0.071	0.000	0.100
172	0.108	0.043	0.000	0.024	0.031	0.000	0.027	0.000	0.028	0.071	0.083	0.057
174	0.008	0.009	0.000	0.143	0.013	0.000	0.008	0.000	0.056	0.071	0.250	0.029
176	0.061	0.047	0.000	0.000	0.070	0.000	0.065	0.000	0.000	0.000	0.000	0.086
178	0.041	0.052	0.000	0.000	0.046	0.000	0.027	0.000	0.000	0.000	0.000	0.000
180	0.042	0.013	0.000	0.000	0.039	0.000	0.000	0.000	0.028	0.000	0.000	0.014
182	0.053	0.051	0.000	0.000	0.036	0.000	0.049	0.000	0.000	0.000	0.000	0.043
184	0.016	0.017	0.000	0.000	0.024	0.000	0.061	0.000	0.000	0.000	0.000	0.029
186	0.003	0.013	0.000	0.000	0.006	0.000	0.023	0.000	0.000	0.000	0.000	0.014
190	0.001	0.000	0.000	0.000	0.021	0.000	0.015	0.000	0.000	0.000	0.000	0.013
192	0.008	0.022	0.000	0.000	0.012	0.000	0.011	0.000	0.000	0.000	0.000	0.014
194	0.004	0.004	0.000	0.000	0.009	0.000	0.008	0.000	0.000	0.000	0.000	0.000
196	0.003	0.000	0.000	0.000	0.000	0.000	0.004	0.000	0.000	0.000	0.000	0.000
202	0.005	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Avu25												
210	0.000	0.000	0.000	0.000	0.002	0.000	0.000	0.000	0.000	0.000	0.000	0.000
212	0.011	0.023	0.000	0.000	0.012	0.000	0.022	0.000	0.017	0.068	0.000	0.000
214	0.035	0.023	0.000	0.014	0.044	0.000	0.022	0.000	0.067	0.046	0.000	0.028
218	0.051	0.078	0.000	0.056	0.053	0.050	0.084	0.500	0.083	0.023	0.250	0.069
220	0.053	0.073	0.100	0.069	0.051	0.100	0.044	0.000	0.033	0.046	0.000	0.028
222	0.337	0.339	0.100	0.361	0.306	0.300	0.316	0.000	0.283	0.432	0.083	0.264
226	0.016	0.008	0.000	0.000	0.019	0.000	0.016	0.000	0.033	0.023	0.083	0.028
228	0.025	0.008	0.000	0.000	0.009	0.050	0.009	0.000	0.017	0.023	0.000	0.000

230	0.079	0.098	0.300	0.083	0.102	0.100	0.100	0.000	0.117	0.068	0.167	0.153
234	0.086	0.057	0.000	0.042	0.082	0.100	0.088	0.500	0.150	0.114	0.000	0.014
238	0.112	0.101	0.200	0.125	0.109	0.200	0.106	0.000	0.067	0.046	0.250	0.181
242	0.018	0.013	0.100	0.028	0.027	0.000	0.034	0.000	0.017	0.023	0.000	0.000
244	0.029	0.029	0.000	0.028	0.026	0.000	0.022	0.000	0.050	0.000	0.000	0.028
248	0.031	0.036	0.100	0.014	0.046	0.000	0.041	0.000	0.000	0.000	0.000	0.069
250	0.013	0.023	0.000	0.042	0.019	0.000	0.009	0.000	0.000	0.023	0.000	0.014
252	0.012	0.010	0.000	0.014	0.007	0.000	0.000	0.000	0.000	0.000	0.083	0.028
256	0.006	0.010	0.000	0.000	0.009	0.000	0.016	0.000	0.000	0.000	0.000	0.000
258	0.023	0.023	0.000	0.042	0.022	0.050	0.013	0.000	0.033	0.000	0.000	0.028
262	0.006	0.005	0.000	0.000	0.002	0.000	0.003	0.000	0.000	0.000	0.083	0.000
266	0.016	0.008	0.100	0.042	0.019	0.000	0.009	0.000	0.017	0.023	0.000	0.000
268	0.013	0.016	0.000	0.014	0.012	0.000	0.019	0.000	0.000	0.046	0.000	0.028
270	0.012	0.005	0.000	0.000	0.014	0.000	0.003	0.000	0.017	0.000	0.000	0.000
272	0.011	0.005	0.000	0.014	0.007	0.000	0.009	0.000	0.000	0.000	0.000	0.028
276	0.004	0.003	0.000	0.014	0.000	0.050	0.009	0.000	0.000	0.000	0.000	0.000
278	0.003	0.005	0.000	0.000	0.002	0.000	0.006	0.000	0.000	0.000	0.000	0.014
284	0.000	0.000	0.000	0.000	0.002	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Avu26												
227	0.000	0.000	0.000	0.000	0.005	0.000	0.000	0.000	0.000	0.000	0.000	0.000
229	0.007	0.010	0.000	0.000	0.010	0.000	0.003	0.000	0.013	0.000	0.000	0.000
237	0.007	0.000	0.000	0.000	0.000	0.000	0.003	0.000	0.000	0.000	0.000	0.015
241	0.002	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
243	0.000	0.000	0.000	0.000	0.000	0.000	0.003	0.000	0.013	0.000	0.000	0.000
245	0.007	0.000	0.000	0.000	0.008	0.000	0.000	0.000	0.000	0.000	0.000	0.000
247	0.026	0.019	0.000	0.029	0.018	0.050	0.018	0.000	0.026	0.000	0.000	0.000

249	0.007	0.002	0.000	0.000	0.013	0.000	0.006	0.000	0.013	0.000	0.000	0.000
253	0.030	0.029	0.000	0.043	0.039	0.000	0.047	0.000	0.013	0.031	0.000	0.059
255	0.071	0.062	0.000	0.086	0.062	0.150	0.047	0.000	0.118	0.031	0.000	0.000
257	0.052	0.052	0.125	0.071	0.077	0.050	0.067	0.000	0.053	0.063	0.000	0.088
259	0.053	0.043	0.000	0.057	0.046	0.100	0.059	0.000	0.040	0.125	0.000	0.059
261	0.087	0.131	0.125	0.014	0.095	0.150	0.070	0.000	0.053	0.031	0.167	0.118
263	0.022	0.031	0.000	0.014	0.023	0.000	0.015	0.000	0.040	0.031	0.167	0.015
265	0.042	0.062	0.125	0.071	0.039	0.000	0.064	0.500	0.066	0.000	0.083	0.044
267	0.054	0.041	0.000	0.071	0.041	0.100	0.053	0.000	0.105	0.063	0.000	0.044
269	0.062	0.067	0.000	0.057	0.062	0.000	0.053	0.000	0.013	0.031	0.083	0.029
271	0.096	0.102	0.375	0.100	0.105	0.000	0.108	0.000	0.092	0.063	0.167	0.132
273	0.077	0.083	0.000	0.086	0.054	0.000	0.061	0.000	0.053	0.125	0.000	0.059
275	0.020	0.017	0.000	0.014	0.039	0.000	0.032	0.500	0.000	0.031	0.000	0.059
277	0.039	0.036	0.000	0.043	0.041	0.100	0.029	0.000	0.026	0.063	0.000	0.044
279	0.131	0.138	0.250	0.100	0.115	0.200	0.120	0.000	0.158	0.156	0.250	0.177
281	0.046	0.036	0.000	0.043	0.056	0.000	0.061	0.000	0.013	0.094	0.000	0.000
285	0.040	0.031	0.000	0.086	0.039	0.100	0.023	0.000	0.079	0.031	0.083	0.015
287	0.008	0.007	0.000	0.000	0.008	0.000	0.038	0.000	0.013	0.000	0.000	0.029
289	0.009	0.002	0.000	0.000	0.005	0.000	0.015	0.000	0.000	0.031	0.000	0.015
291	0.002	0.000	0.000	0.014	0.003	0.000	0.006	0.000	0.000	0.000	0.000	0.000
293	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Avu27												
206	0.002	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
208	0.235	0.236	0.100	0.177	0.259	0.250	0.191	0.500	0.270	0.425	0.167	0.167
210	0.003	0.000	0.000	0.000	0.000	0.000	0.004	0.000	0.000	0.000	0.000	0.000
228	0.000	0.000	0.000	0.000	0.004	0.000	0.000	0.000	0.000	0.000	0.000	0.000

230	0.328	0.377	0.500	0.250	0.329	0.417	0.375	0.000	0.297	0.225	0.250	0.375
238	0.000	0.000	0.000	0.000	0.002	0.000	0.000	0.000	0.000	0.000	0.000	0.000
240	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
242	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
244	0.386	0.387	0.400	0.456	0.406	0.333	0.426	0.500	0.432	0.350	0.583	0.458
246	0.003	0.000	0.000	0.000	0.000	0.000	0.004	0.000	0.000	0.000	0.000	0.000
248	0.042	0.000	0.000	0.118	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
AspB01												
167	0.003	0.000	0.000	0.000	0.012	0.000	0.000	0.000	0.000	0.000	0.000	0.000
169	0.994	1.000	1.000	1.000	0.988	0.000	1.000	1.000	1.000	1.000	0.000	1.000
171	0.003	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
AspB03												
228	0.007	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
230	0.006	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.016	0.000	0.000	0.000
232	0.017	0.000	0.000	0.000	0.008	0.000	0.000	0.000	0.000	0.000	0.000	0.014
234	0.003	0.029	0.000	0.016	0.016	0.000	0.017	0.000	0.063	0.031	0.000	0.029
238	0.004	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
240	0.005	0.006	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
242	0.947	0.959	1.000	0.952	0.959	1.000	0.962	0.000	0.922	0.906	1.000	0.957
244	0.001	0.000	0.000	0.016	0.006	0.000	0.014	0.000	0.000	0.063	0.000	0.000
246	0.010	0.006	0.000	0.016	0.008	0.000	0.007	0.000	0.000	0.000	0.000	0.000
248	0.000	0.000	0.000	0.000	0.002	0.000	0.000	0.000	0.000	0.000	0.000	0.000
268	0.000	0.000	0.000	0.000	0.002	0.000	0.000	0.000	0.000	0.000	0.000	0.000
AspB05												

155	0.000	0.000	0.000	0.000	0.000	0.000	0.018	0.000	0.000	0.000	0.000	0.000
157	0.001	0.000	0.000	0.000	0.000	0.000	0.193	0.000	0.000	0.000	0.000	0.014
159	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.014
217	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
221	0.001	0.002	0.000	0.000	0.002	0.000	0.000	0.000	0.000	0.000	0.000	0.000
223	0.002	0.000	0.000	0.000	0.003	0.000	0.000	0.000	0.000	0.000	0.000	0.000
225	0.090	0.086	0.200	0.132	0.088	0.042	0.092	0.000	0.114	0.065	0.000	0.095
227	0.471	0.451	0.200	0.588	0.384	0.458	0.276	0.500	0.546	0.674	0.583	0.216
229	0.182	0.175	0.600	0.088	0.231	0.292	0.211	0.000	0.125	0.130	0.000	0.338
231	0.043	0.049	0.000	0.029	0.034	0.000	0.026	0.000	0.046	0.022	0.000	0.054
233	0.026	0.021	0.000	0.029	0.037	0.083	0.022	0.000	0.034	0.000	0.000	0.095
235	0.002	0.002	0.000	0.000	0.003	0.000	0.000	0.000	0.000	0.000	0.000	0.027
237	0.000	0.000	0.000	0.000	0.002	0.000	0.000	0.000	0.000	0.000	0.000	0.027
239	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
243	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
255	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.011	0.000	0.000	0.000
265	0.001	0.002	0.000	0.000	0.003	0.000	0.000	0.000	0.000	0.000	0.000	0.000
269	0.012	0.019	0.000	0.029	0.022	0.042	0.018	0.000	0.023	0.000	0.000	0.000
271	0.001	0.000	0.000	0.000	0.002	0.000	0.000	0.000	0.000	0.000	0.000	0.000
273	0.056	0.054	0.000	0.044	0.052	0.000	0.053	0.000	0.011	0.022	0.167	0.027
275	0.055	0.075	0.000	0.044	0.054	0.042	0.044	0.000	0.068	0.087	0.083	0.054
277	0.014	0.016	0.000	0.000	0.027	0.000	0.013	0.000	0.000	0.000	0.000	0.027
279	0.001	0.002	0.000	0.000	0.003	0.000	0.000	0.500	0.000	0.000	0.000	0.000
283	0.015	0.019	0.000	0.000	0.012	0.000	0.013	0.000	0.023	0.000	0.000	0.000
287	0.012	0.019	0.000	0.000	0.025	0.042	0.013	0.000	0.000	0.000	0.083	0.014
289	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
295	0.010	0.007	0.000	0.015	0.012	0.000	0.009	0.000	0.000	0.000	0.083	0.000

299	0.002	0.000	0.000	0.000	0.003	0.000	0.000	0.000	0.000	0.000	0.000	0.000
301	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
305	0.000	0.000	0.000	0.000	0.002	0.000	0.000	0.000	0.000	0.000	0.000	0.000
AspB12												
148	0.000	0.000	0.000	0.000	0.003	0.000	0.006	0.000	0.000	0.000	0.000	0.000
150	0.997	1.000	1.000	1.000	0.997	1.000	0.994	1.000	1.000	1.000	1.000	1.000
152	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
156	0.002	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
AspB15												
188	0.004	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
192	0.007	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
194	0.969	1.000	1.000	1.000	0.987	1.000	1.000	1.000	1.000	1.000	1.000	1.000
196	0.018	0.000	0.000	0.000	0.013	0.000	0.000	0.000	0.000	0.000	0.000	0.000
198	0.002	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
AspB18												
101	0.009	0.003	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
103	0.000	0.003	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
109	0.978	0.994	1.000	1.000	0.986	1.000	0.997	1.000	1.000	1.000	1.000	1.000
111	0.013	0.000	0.000	0.000	0.014	0.000	0.003	0.000	0.000	0.000	0.000	0.000

B) *Albula goreensis*

Location	Bahamas	Belize	Brazil	Florida	Mexico	Panama	Sao Tome	Virgin Is.

Locus								
Avu01								
198	0.000	0.000	0.000	0.022	0.000	0.000	0.000	0.000
200	0.036	0.031	0.000	0.082	0.200	0.000	0.000	0.333
202	0.107	0.156	0.000	0.145	0.200	0.250	0.000	0.167
204	0.179	0.406	0.000	0.470	0.600	0.000	0.000	0.167
208	0.000	0.094	0.000	0.051	0.000	0.250	0.000	0.167
218	0.036	0.063	0.000	0.062	0.000	0.000	0.000	0.000
216	0.071	0.047	0.000	0.043	0.000	0.000	0.000	0.167
220	0.071	0.141	0.000	0.092	0.000	0.250	0.000	0.000
224	0.000	0.031	0.000	0.007	0.000	0.250	0.000	0.000
226	0.036	0.016	0.000	0.011	0.000	0.000	0.000	0.000
228	0.286	0.016	0.000	0.010	0.000	0.000	0.000	0.000
230	0.179	0.000	0.000	0.005	0.000	0.000	0.000	0.000
Avu02								
138	0.000	0.000	0.000	0.008	0.000	0.000	0.000	0.000
140	0.167	0.089	0.000	0.015	0.000	0.500	0.000	0.000
148	0.833	0.054	0.000	0.736	1.000	0.500	0.000	1.000
150	0.000	0.857	0.000	0.242	0.000	0.000	0.000	0.000
Avu04								
208	0.000	0.000	0.000	0.001	0.000	0.000	0.000	0.000
212	0.517	0.385	0.000	0.457	0.583	0.500	1.000	0.500
214	0.017	0.000	0.000	0.010	0.000	0.000	0.000	0.000
218	0.433	0.615	0.000	0.516	0.417	0.500	0.000	0.250
220	0.000	0.000	0.000	0.007	0.000	0.000	0.000	0.250

222	0.033	0.000	1.000	0.010	0.000	0.000	0.000	0.000
Avu09								
138	0.000	0.000	0.000	0.003	0.000	0.000	0.000	0.000
142	0.040	0.059	0.000	0.036	0.000	0.000	0.000	0.000
146	0.300	0.353	0.000	0.410	0.400	0.125	0.000	0.600
148	0.320	0.324	0.500	0.273	0.100	0.250	0.000	0.200
152	0.220	0.147	0.500	0.154	0.400	0.125	0.000	0.200
154	0.000	0.000	0.000	0.006	0.000	0.000	0.000	0.000
156	0.000	0.000	0.000	0.014	0.100	0.000	0.000	0.000
160	0.020	0.059	0.000	0.026	0.000	0.000	0.000	0.000
162	0.080	0.029	0.000	0.023	0.000	0.000	0.000	0.000
164	0.020	0.029	0.000	0.042	0.000	0.000	0.000	0.000
168	0.000	0.000	0.000	0.010	0.000	0.250	0.000	0.000
172	0.000	0.000	0.000	0.000	0.000	0.250	0.000	0.000
178	0.000	0.000	0.000	0.001	0.000	0.000	0.000	0.000
186	0.000	0.000	0.000	0.001	0.000	0.000	0.000	0.000
Avu11								
137	0.811	0.000	1.000	0.210	0.000	0.000	1.000	0.000
139	0.189	0.044	0.000	0.715	1.000	0.000	0.000	0.750
141	0.000	0.000	0.000	0.008	0.000	0.000	0.000	0.000
145	0.000	0.000	0.000	0.001	0.000	0.000	0.000	0.000
147	0.000	0.000	0.000	0.024	0.000	0.000	0.000	0.000
151	0.000	0.000	0.000	0.004	0.000	0.000	0.000	0.000
155	0.000	0.087	0.000	0.001	0.000	0.000	0.000	0.000
157	0.000	0.000	0.000	0.004	0.000	0.000	0.000	0.000

159	0.000	0.000	0.000	0.001	0.000	1.000	0.000	0.000
167	0.000	0.826	0.000	0.031	0.000	0.000	0.000	0.250
169	0.000	0.044	0.000	0.001	0.000	0.000	0.000	0.000
Avu12								
170	0.042	0.000	0.000	0.010	0.000	0.000	1.000	0.000
172	0.889	0.833	1.000	0.822	0.667	0.375	0.000	0.750
174	0.069	0.150	0.000	0.145	0.250	0.125	0.000	0.250
176	0.000	0.017	0.000	0.023	0.083	0.000	0.000	0.000
180	0.000	0.000	0.000	0.000	0.000	0.500	0.000	0.000
Avu14								
130	0.000	0.000	0.000	0.000	0.000	0.125	0.000	0.000
134	0.210	0.061	0.000	0.059	0.000	0.500	0.000	0.000
136	0.581	0.697	0.500	0.729	0.583	0.250	0.000	1.000
138	0.032	0.000	0.000	0.003	0.000	0.000	0.000	0.000
140	0.177	0.242	0.500	0.209	0.417	0.125	0.000	0.000
Avu16								
112	0.971	0.987	1.000	0.972	1.000	1.000	1.000	1.000
114	0.029	0.013	0.000	0.028	0.000	0.000	0.000	0.000
Avu17								
113	0.016	0.000	0.000	0.001	0.000	0.125	0.000	0.000
115	0.000	0.000	0.000	0.001	0.000	0.000	0.000	0.000
119	0.048	0.000	0.000	0.004	0.000	0.000	0.000	0.000
121	0.274	0.353	0.500	0.303	0.300	0.250	0.000	0.167

123	0.016	0.000	0.000	0.008	0.000	0.500	0.000	0.000
127	0.081	0.147	0.500	0.125	0.100	0.125	0.500	0.000
131	0.113	0.000	0.000	0.035	0.000	0.000	0.000	0.000
133	0.048	0.177	0.000	0.068	0.100	0.000	0.500	0.000
137	0.097	0.118	0.000	0.093	0.000	0.000	0.000	0.000
139	0.097	0.118	0.000	0.191	0.300	0.000	0.000	0.333
143	0.032	0.000	0.000	0.053	0.100	0.000	0.000	0.167
145	0.000	0.000	0.000	0.007	0.000	0.000	0.000	0.000
147	0.113	0.059	0.000	0.059	0.100	0.000	0.000	0.000
151	0.032	0.029	0.000	0.049	0.000	0.000	0.000	0.333
155	0.016	0.000	0.000	0.002	0.000	0.000	0.000	0.000
159	0.016	0.000	0.000	0.002	0.000	0.000	0.000	0.000
Avu18								
138	0.250	0.000	0.000	0.003	0.000	0.000	0.000	0.000
146	0.000	0.361	0.000	0.412	0.167	0.000	0.000	0.375
148	0.750	0.194	0.000	0.158	0.167	0.000	0.000	0.125
150	0.000	0.028	0.000	0.086	0.167	0.000	0.000	0.000
154	0.000	0.000	0.000	0.007	0.000	0.000	0.000	0.000
156	0.000	0.000	0.000	0.003	0.000	0.125	0.000	0.000
160	0.000	0.056	0.000	0.024	0.000	0.250	0.000	0.000
162	0.000	0.028	0.000	0.069	0.000	0.125	0.000	0.125
166	0.000	0.139	0.000	0.078	0.167	0.250	0.000	0.250
168	0.000	0.028	0.000	0.019	0.000	0.000	0.000	0.125
172	0.000	0.000	0.000	0.035	0.000	0.000	0.000	0.000
174	0.000	0.000	0.000	0.014	0.000	0.000	0.000	0.000
176	0.000	0.000	0.000	0.010	0.000	0.250	0.000	0.000

178	0.000	0.056	0.000	0.029	0.000	0.000	0.000	0.000
182	0.000	0.000	0.000	0.010	0.000	0.000	0.000	0.000
184	0.000	0.028	0.000	0.006	0.083	0.000	0.000	0.000
188	0.000	0.028	0.000	0.003	0.083	0.000	0.000	0.000
192	0.000	0.000	0.000	0.007	0.000	0.000	0.000	0.000
194	0.000	0.000	0.000	0.007	0.000	0.000	0.000	0.000
202	0.000	0.000	0.000	0.006	0.000	0.000	0.000	0.000
204	0.000	0.028	0.000	0.003	0.083	0.000	0.000	0.000
208	0.000	0.028	0.000	0.004	0.000	0.000	0.000	0.000
216	0.000	0.000	0.000	0.001	0.000	0.000	0.000	0.000
220	0.000	0.000	0.000	0.001	0.000	0.000	0.000	0.000
224	0.000	0.000	0.000	0.001	0.083	0.000	0.000	0.000
226	0.000	0.000	0.000	0.003	0.000	0.000	0.000	0.000
Avu25								
218	0.000	0.000	0.000	0.002	0.000	0.000	0.000	0.000
220	0.000	0.000	0.000	0.001	0.000	0.000	0.000	0.000
222	0.000	0.000	0.000	0.002	0.000	0.375	0.000	0.000
226	0.000	0.000	0.000	0.002	0.000	0.000	0.000	0.000
228	0.000	0.000	0.000	0.001	0.000	0.000	0.000	0.000
230	0.000	0.019	0.000	0.006	0.000	0.000	0.000	0.000
234	0.019	0.000	0.000	0.001	0.000	0.000	0.000	0.000
236	0.058	0.000	0.000	0.022	0.000	0.000	0.000	0.000
240	0.019	0.019	0.500	0.013	0.100	0.000	0.000	0.000
244	0.000	0.037	0.000	0.024	0.000	0.000	0.000	0.000
246	0.019	0.000	0.000	0.020	0.000	0.000	0.000	0.000
248	0.019	0.037	0.000	0.023	0.000	0.000	0.000	0.000

250	0.019	0.000	0.000	0.048	0.000	0.000	0.000	0.125
252	0.019	0.056	0.000	0.039	0.000	0.000	0.000	0.000
256	0.019	0.056	0.000	0.060	0.000	0.125	0.000	0.000
258	0.019	0.037	0.000	0.060	0.100	0.000	0.500	0.000
260	0.077	0.056	0.000	0.066	0.000	0.000	0.000	0.000
264	0.077	0.074	0.000	0.068	0.100	0.125	0.000	0.000
268	0.173	0.074	0.000	0.069	0.000	0.000	0.000	0.250
272	0.115	0.130	0.000	0.109	0.200	0.125	0.000	0.125
276	0.019	0.056	0.000	0.060	0.300	0.000	0.000	0.125
280	0.096	0.074	0.000	0.094	0.100	0.000	0.000	0.125
282	0.058	0.093	0.500	0.048	0.000	0.000	0.000	0.000
286	0.077	0.093	0.000	0.044	0.000	0.125	0.500	0.000
288	0.019	0.037	0.000	0.019	0.000	0.000	0.000	0.125
290	0.019	0.019	0.000	0.020	0.100	0.125	0.000	0.000
294	0.000	0.000	0.000	0.007	0.000	0.000	0.000	0.125
298	0.000	0.019	0.000	0.009	0.000	0.000	0.000	0.000
300	0.000	0.019	0.000	0.012	0.000	0.000	0.000	0.000
302	0.000	0.000	0.000	0.007	0.000	0.000	0.000	0.000
306	0.000	0.000	0.000	0.009	0.000	0.000	0.000	0.000
308	0.000	0.000	0.000	0.006	0.000	0.000	0.000	0.000
312	0.000	0.000	0.000	0.010	0.000	0.000	0.000	0.000
316	0.000	0.000	0.000	0.002	0.000	0.000	0.000	0.000
318	0.019	0.000	0.000	0.009	0.000	0.000	0.000	0.000
322	0.000	0.000	0.000	0.006	0.000	0.000	0.000	0.000
324	0.019	0.000	0.000	0.000	0.000	0.000	0.000	0.000
328	0.000	0.000	0.000	0.001	0.000	0.000	0.000	0.000
330	0.019	0.000	0.000	0.000	0.000	0.000	0.000	0.000

Avu26								
212	0.500	0.000	0.000	0.002	0.000	0.000	0.000	0.000
216	0.000	0.000	0.000	0.005	0.000	0.333	0.000	0.000
226	0.000	0.929	0.000	0.976	1.000	0.333	0.000	1.000
228	0.000	0.000	0.000	0.013	0.000	0.000	0.000	0.000
234	0.000	0.071	0.000	0.000	0.000	0.000	0.000	0.000
254	0.250	0.000	0.000	0.000	0.000	0.000	0.000	0.000
258	0.000	0.000	0.000	0.005	0.000	0.000	0.000	0.000
272	0.250	0.000	0.000	0.000	0.000	0.000	0.000	0.000
274	0.000	0.000	0.000	0.000	0.000	0.167	0.000	0.000
280	0.000	0.000	0.000	0.000	0.000	0.167	0.000	0.000
Avu27								
206	0.150	0.046	0.000	0.000	0.000	0.000	0.000	0.000
208	0.350	0.227	0.000	0.012	0.000	0.250	0.000	0.000
210	0.050	0.000	0.000	0.000	0.000	0.000	0.000	0.000
212	0.050	0.000	0.000	0.000	0.000	0.000	0.000	0.000
226	0.350	0.182	0.000	0.730	1.000	0.500	0.000	1.000
228	0.050	0.227	0.000	0.089	0.000	0.250	0.000	0.000
230	0.000	0.046	0.000	0.003	0.000	0.000	1.000	0.000
234	0.000	0.000	0.000	0.003	0.000	0.000	0.000	0.000
238	0.000	0.046	0.000	0.006	0.000	0.000	0.000	0.000
244	0.000	0.136	0.000	0.015	0.000	0.000	0.000	0.000
246	0.000	0.000	0.000	0.009	0.000	0.000	0.000	0.000
250	0.000	0.000	0.000	0.009	0.000	0.000	0.000	0.000
252	0.000	0.000	0.000	0.006	0.000	0.000	0.000	0.000

256	0.000	0.091	0.000	0.009	0.000	0.000	0.000	0.000
260	0.000	0.000	0.000	0.012	0.000	0.000	0.000	0.000
262	0.000	0.000	0.000	0.003	0.000	0.000	0.000	0.000
264	0.000	0.000	0.000	0.003	0.000	0.000	0.000	0.000
268	0.000	0.000	0.000	0.022	0.000	0.000	0.000	0.000
270	0.000	0.000	0.000	0.012	0.000	0.000	0.000	0.000
274	0.000	0.000	0.000	0.012	0.000	0.000	0.000	0.000
276	0.000	0.000	0.000	0.015	0.000	0.000	0.000	0.000
278	0.000	0.000	0.000	0.006	0.000	0.000	0.000	0.000
292	0.000	0.000	0.000	0.003	0.000	0.000	0.000	0.000
300	0.000	0.000	0.000	0.006	0.000	0.000	0.000	0.000
304	0.000	0.000	0.000	0.009	0.000	0.000	0.000	0.000
312	0.000	0.000	0.000	0.003	0.000	0.000	0.000	0.000
AspB01								
164	0.000	0.013	0.000	0.002	0.000	0.000	0.000	0.000
166	0.814	0.026	0.500	0.600	0.900	0.500	1.000	0.000
168	0.157	0.895	0.000	0.350	0.100	0.500	0.000	0.900
172	0.029	0.066	0.500	0.048	0.000	0.000	0.000	0.100
AspB03								
224	0.027	0.000	0.000	0.024	0.000	0.000	0.000	0.000
226	0.054	0.052	0.000	0.024	0.000	0.000	0.000	0.000
230	0.689	0.655	1.000	0.639	0.833	0.750	1.000	0.500
232	0.000	0.000	0.000	0.002	0.000	0.000	0.000	0.000
234	0.014	0.000	0.000	0.002	0.000	0.000	0.000	0.000
238	0.000	0.035	0.000	0.013	0.000	0.000	0.000	0.000

240	0.041	0.035	0.000	0.050	0.083	0.000	0.000	0.100
244	0.108	0.138	0.000	0.122	0.000	0.000	0.000	0.100
248	0.027	0.035	0.000	0.026	0.000	0.250	0.000	0.100
250	0.014	0.017	0.000	0.044	0.000	0.000	0.000	0.100
252	0.000	0.000	0.000	0.004	0.000	0.000	0.000	0.000
256	0.000	0.000	0.000	0.001	0.000	0.000	0.000	0.000
258	0.014	0.000	0.000	0.002	0.000	0.000	0.000	0.000
262	0.000	0.000	0.000	0.003	0.000	0.000	0.000	0.000
264	0.000	0.035	0.000	0.024	0.000	0.000	0.000	0.000
268	0.000	0.000	0.000	0.012	0.083	0.000	0.000	0.000
270	0.014	0.000	0.000	0.004	0.000	0.000	0.000	0.100
272	0.000	0.000	0.000	0.001	0.000	0.000	0.000	0.000
AspB05								
151	0.000	0.000	0.000	0.001	0.000	0.000	0.000	0.000
209	0.000	0.000	0.000	0.001	0.000	0.000	0.000	0.000
217	0.000	0.026	0.000	0.037	0.100	0.000	0.000	0.000
221	0.081	0.105	0.000	0.083	0.100	0.167	0.000	0.000
225	0.297	0.145	0.500	0.201	0.200	0.167	0.000	0.250
227	0.189	0.079	0.000	0.130	0.100	0.000	0.000	0.125
229	0.162	0.316	0.000	0.261	0.200	0.167	0.000	0.375
233	0.068	0.026	0.000	0.005	0.100	0.167	0.000	0.000
235	0.027	0.053	0.000	0.077	0.000	0.000	1.000	0.125
239	0.027	0.026	0.000	0.035	0.200	0.000	0.000	0.125
241	0.000	0.000	0.000	0.020	0.000	0.000	0.000	0.000
243	0.054	0.066	0.500	0.040	0.000	0.167	0.000	0.000
247	0.014	0.000	0.000	0.007	0.000	0.000	0.000	0.000

249	0.000	0.013	0.000	0.018	0.000	0.000	0.000	0.000
253	0.000	0.013	0.000	0.006	0.000	0.000	0.000	0.000
259	0.041	0.026	0.000	0.025	0.000	0.000	0.000	0.000
261	0.014	0.000	0.000	0.000	0.000	0.000	0.000	0.000
263	0.000	0.026	0.000	0.008	0.000	0.167	0.000	0.000
267	0.000	0.026	0.000	0.015	0.000	0.000	0.000	0.000
271	0.000	0.026	0.000	0.007	0.000	0.000	0.000	0.000
275	0.014	0.000	0.000	0.008	0.000	0.000	0.000	0.000
279	0.000	0.000	0.000	0.005	0.000	0.000	0.000	0.000
281	0.000	0.013	0.000	0.001	0.000	0.000	0.000	0.000
285	0.014	0.000	0.000	0.002	0.000	0.000	0.000	0.000
287	0.000	0.000	0.000	0.003	0.000	0.000	0.000	0.000
291	0.000	0.000	0.000	0.002	0.000	0.000	0.000	0.000
295	0.000	0.000	0.000	0.002	0.000	0.000	0.000	0.000
297	0.000	0.013	0.000	0.002	0.000	0.000	0.000	0.000
AspB12								
148	1.000	1.000	1.000	0.990	1.000	1.000	1.000	1.000
150	0.000	0.000	0.000	0.010	0.000	0.000	0.000	0.000
AspB15								
191	0.000	0.000	0.000	0.002	0.000	0.000	0.000	0.000
193	1.000	1.000	1.000	0.992	1.000	1.000	1.000	1.000
197	0.000	0.000	0.000	0.006	0.000	0.000	0.000	0.000
AspB18								
101	0.000	0.000	0.000	0.001	0.000	0.000	0.000	0.000

109	1.000	1.000	1.000	0.999	1.000	1.000	1.000	1.000

C) *Albula sp. cf. vulpes*

Location	Bahamas	Florida	Honduras	Mexico	Virgin Is.
Locus					
Avu01					
207	0.000	0.275	0.278	0.250	0.357
219	0.000	0.300	0.556	0.417	0.500
221	0.000	0.300	0.111	0.222	0.143
225	0.000	0.075	0.056	0.028	0.000
229	0.000	0.050	0.000	0.083	0.000
Avu02					
142	1.000	1.000	0.000	0.000	0.000
Avu04					
203	0.000	0.283	0.438	0.000	0.000
205	1.000	0.283	0.000	0.444	0.500
207	0.000	0.083	0.063	0.083	0.071
209	0.000	0.317	0.500	0.472	0.429
215	0.000	0.033	0.000	0.000	0.000
Avu09					
162	1.000	1.000	1.000	1.000	1.000

Avu11					
139	0.000	1.000	1.000	1.000	0.595
155	0.000	0.000	0.000	0.000	0.054
157	0.000	0.000	0.000	0.000	0.176
159	0.000	0.000	0.000	0.000	0.176
Avu12					
145	0.500	1.000	1.000	1.000	1.000
183	0.500	0.000	0.000	0.000	0.000
Avu14					
131	1.000	0.957	1.000	1.000	1.000
145	0.000	0.044	0.000	0.000	0.000
Avu16					
111	1.000	0.944	0.000	1.000	1.000
115	0.000	0.056	0.000	0.000	0.000
Avu17					
116	0.000	0.023	0.000	0.000	0.000
122	0.000	0.068	0.000	0.000	0.000
124	0.000	0.227	0.000	0.000	0.000
128	1.000	0.068	0.000	0.000	0.071
130	0.000	0.250	0.000	0.304	0.357
132	0.000	0.364	0.000	0.696	0.571
Avu25					

220	0.000	0.000	0.000	0.048	0.000
224	1.000	0.857	0.889	0.929	0.900
226	0.000	0.086	0.056	0.024	0.100
228	0.000	0.057	0.056	0.000	0.000
Avu26					
230	0.000	0.036	0.000	0.000	0.000
232	0.000	0.393	0.000	0.409	0.125
236	0.000	0.214	0.000	0.273	0.250
240	0.000	0.000	0.000	0.046	0.125
244	1.000	0.000	0.000	0.000	0.000
258	0.000	0.036	0.000	0.136	0.000
262	0.000	0.036	0.000	0.046	0.125
266	0.000	0.000	0.000	0.046	0.375
268	0.000	0.000	0.000	0.046	0.000
272	0.000	0.036	0.000	0.000	0.000
274	0.000	0.143	0.000	0.000	0.000
296	0.000	0.107	0.000	0.000	0.000
Avu27					
217	0.000	0.016	0.000	0.000	0.000
231	0.500	0.984	1.000	1.000	1.000
245	0.500	0.000	0.000	0.000	0.000
AspB03					
226	0.000	0.017	0.000	0.000	0.000
242	0.000	0.167	0.000	0.521	0.500

262	0.000	0.817	1.000	0.479	0.500
AspB05					
149	0.000	0.000	0.000	0.017	0.000
155	0.000	0.150	0.150	0.183	0.000
157	0.000	0.425	0.050	0.250	0.071
161	0.000	0.425	0.800	0.533	0.857
163	0.000	0.000	0.000	0.017	0.071
223	0.500	0.000	0.000	0.000	0.000
227	0.500	0.000	0.000	0.000	0.000
AspB12					
118	0.000	0.227	0.000	0.000	0.000
132	0.000	0.136	0.000	0.000	0.000
148	0.000	0.091	0.000	0.000	0.000
150	0.000	0.546	0.000	0.000	0.000
AspB15					
195	1.000	1.000	0.000	1.000	1.000
AspB18					
112	0.000	1.000	0.000	1.000	1.000

Table 7. Hardy Weinberg U-test p-values (<0.05 in **BOLD**) for heterozygote deficiencies for Atlantic bonefishes. Tildes denote monomorphic loci within a sampled location.

A) *Albula vulpes*

Location	Bahamas	Belize	Cayman Is.	Cuba	Florida	Honduras	Mexico	Panama	Puerto Rico	Turks & Caicos	Venezuela	Virgin Is.
Locus												
Avu01	**0.000**	**0.000**	0.676	**0.000**	**0.000**	**0.000**	**0.000**	~	**0.003**	**0.001**	1.000	**0.036**
Avu02	**0.000**	~	~	~	**0.000**	~	**0.005**	~	~	~	~	~
Avu04	**0.000**	**0.000**	~	1.000	1.000	~	**0.000**	~	**0.000**	0.161	~	~
Avu09	**0.000**	0.099	0.429	0.159	**0.001**	~	**0.000**	1.000	0.312	0.828	0.870	0.123
Avu11	**0.000**	**0.009**	0.760	0.561	**0.000**	0.230	0.898	~	0.135	0.890	0.511	0.628
Avu12	**0.000**	**0.006**	~	~	**0.000**	~	**0.000**	~	~	~	~	~
Avu14	**0.000**	**0.000**	~	~	**0.000**	~	**0.000**	~	**0.016**	1.000	**0.031**	0.132
Avu16	**0.000**	**0.000**	~	1.000	0.611	1.000	**0.000**	~	0.427	**0.025**	1.000	0.654
Avu17	**0.001**	**0.006**	~	0.486	0.159	0.306	0.612	~	1.000	0.440	0.513	**0.033**
Avu18	**0.000**	0.555	0.421	0.122	0.690	0.600	0.549	~	0.153	1.000	1.000	0.090
Avu25	**0.003**	0.180	0.133	0.135	0.209	0.035	**0.007**	~	0.482	0.995	1.000	0.059
Avu26	**0.000**	0.719	0.463	0.065	**0.000**	1.000	0.429	~	0.536	0.399	1.000	0.176
Avu27	**0.000**	**0.002**	0.241	**0.012**	**0.000**	0.071	**0.023**	~	0.129	**0.001**	0.334	0.701
AspB01	**0.000**	~	~	~	**0.000**	~	~	~	~	~	~	~
AspB03	**0.000**	**0.002**	~	1.000	**0.000**	~	**0.000**	~	1.000	**0.032**	~	**0.016**
AspB05	0.278	0.459	0.623	0.625	0.056	0.067	**0.000**	~	0.205	0.439	0.752	0.328
AspB12	1.000	~	~	~	**0.002**	~	**0.003**	~	~	~	~	~
AspB15	**0.000**	~	~	~	**0.026**	~	~	~	~	~	~	~
AspB18	**0.000**	1.000	~	~	**0.000**	~	~	~	~	~	~	~

B) *Albula goreensis*

Location	Bahamas	Belize	Brazil	Florida	Mexico	Panama	Sao Tome	Virgin Is.
Locus								
Avu01	**0.027**	**0.032**	~	**0.000**	0.207	1.000	~	0.194
Avu02	~	**0.010**	~	**0.000**	~	0.331	~	~
Avu04	0.920	0.569	~	0.079	0.753	0.598	~	1.000
Avu09	0.068	0.938	~	**0.000**	1.000	0.078	~	0.605
Avu11	**0.000**	**0.000**	~	**0.000**	~	~	~	~
Avu12	0.172	0.496	~	0.566	0.519	0.088	~	1.000
Avu14	0.192	0.859	~	0.184	1.000	0.137	~	~
Avu16	1.000	~	~	**0.000**	~	~	~	~
Avu17	**0.022**	0.052	~	**0.020**	1.000	**0.027**	~	0.333
Avu18	0.144	0.134	~	**0.000**	1.000	1.000	~	0.080
Avu25	0.251	0.409	~	**0.000**	1.000	0.422	~	1.000
Avu26	0.330	**0.037**	~	**0.000**	~	0.074	~	~
Avu27	**0.000**	**0.000**	~	**0.000**	~	0.303	~	~
AspB01	**0.000**	**0.002**	~	**0.000**	~	0.601	~	~
AspB03	0.441	0.330	~	0.157	1.000	~	~	0.877
AspB05	0.244	**0.023**	~	**0.000**	0.295	1.000	~	0.497
AspB12	~	~	~	**0.000**	~	~	~	~
AspB15	~	~	~	**0.000**	~	~	~	~
AspB18	~	~	~	~	~	~	~	~

C) *Albula sp. cf. vulpes*

Location	Bahamas	Florida	Honduras	Mexico	Virgin Is.
Locus					
Avu01	~	0.564	0.573	0.292	0.491
Avu02	~	~	~	~	~
Avu04	~	0.065	**0.048**	0.175	0.845
Avu09	~	~	~	~	~
Avu11	~	**0.000**	~	~	~
Avu12	0.335	~	~	~	~
Avu14	~	**0.024**	~	~	~
Avu16	~	**0.031**	~	~	~
Avu17	~	**0.010**	~	0.694	0.294
Avu18	~	~	~	~	~
Avu25	~	0.272	1.000	**0.027**	~
Avu26	~	**0.014**	~	0.973	1.000
Avu27	~	~	~	~	~
AspB01	~	~	~	~	~
AspB03	~	1.000	~	1.000	1.000
AspB05	~	**0.034**	0.160	0.227	1.000
AspB12	~	**0.006**	~	~	~
AspB15	~	~	~	~	~
AspB18	~	~	~	~	~

Figure 1. Total species composition of bonefishes within collection locations across the western Atlantic and Caribbean Sea, including identified hybrids.

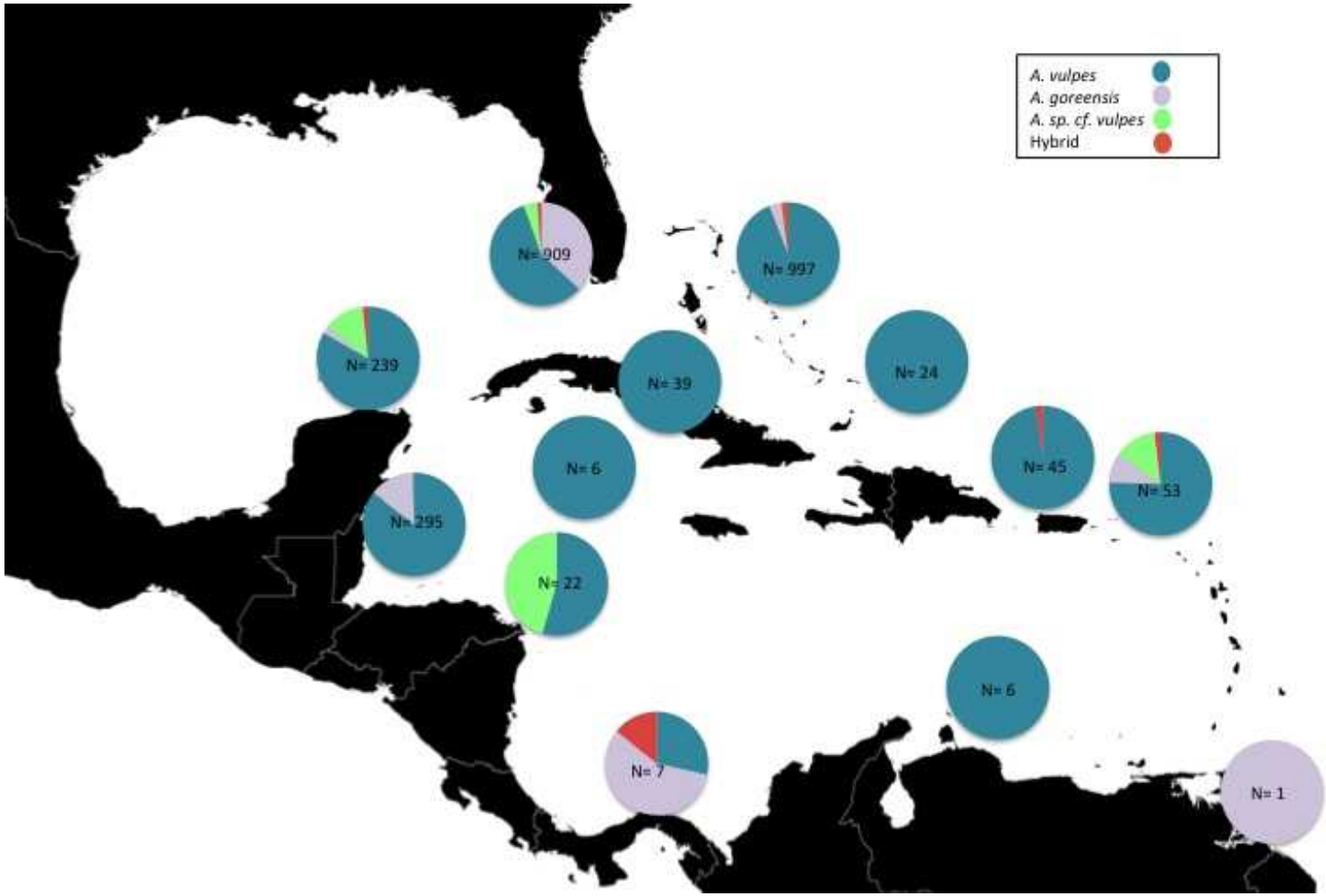


Figure 2. Regional occurrence of two *Albula vulpes* genetic populations (as inferred in *STRUCTURE*). Total sample sizes are indicated for each regional collection location.

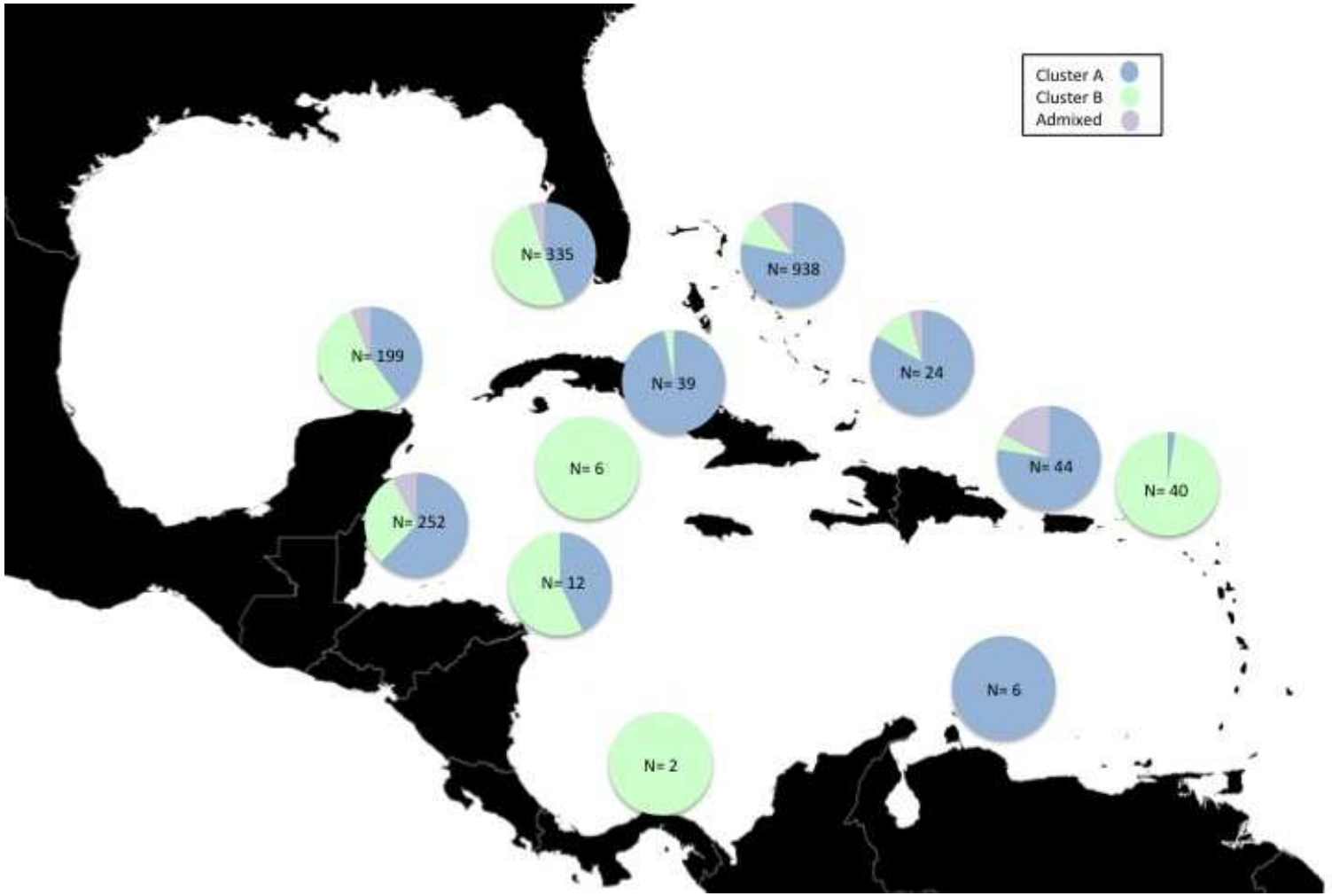


Figure 3. Species composition for adult bonefishes collected from the fishery in coastal flats habitat.

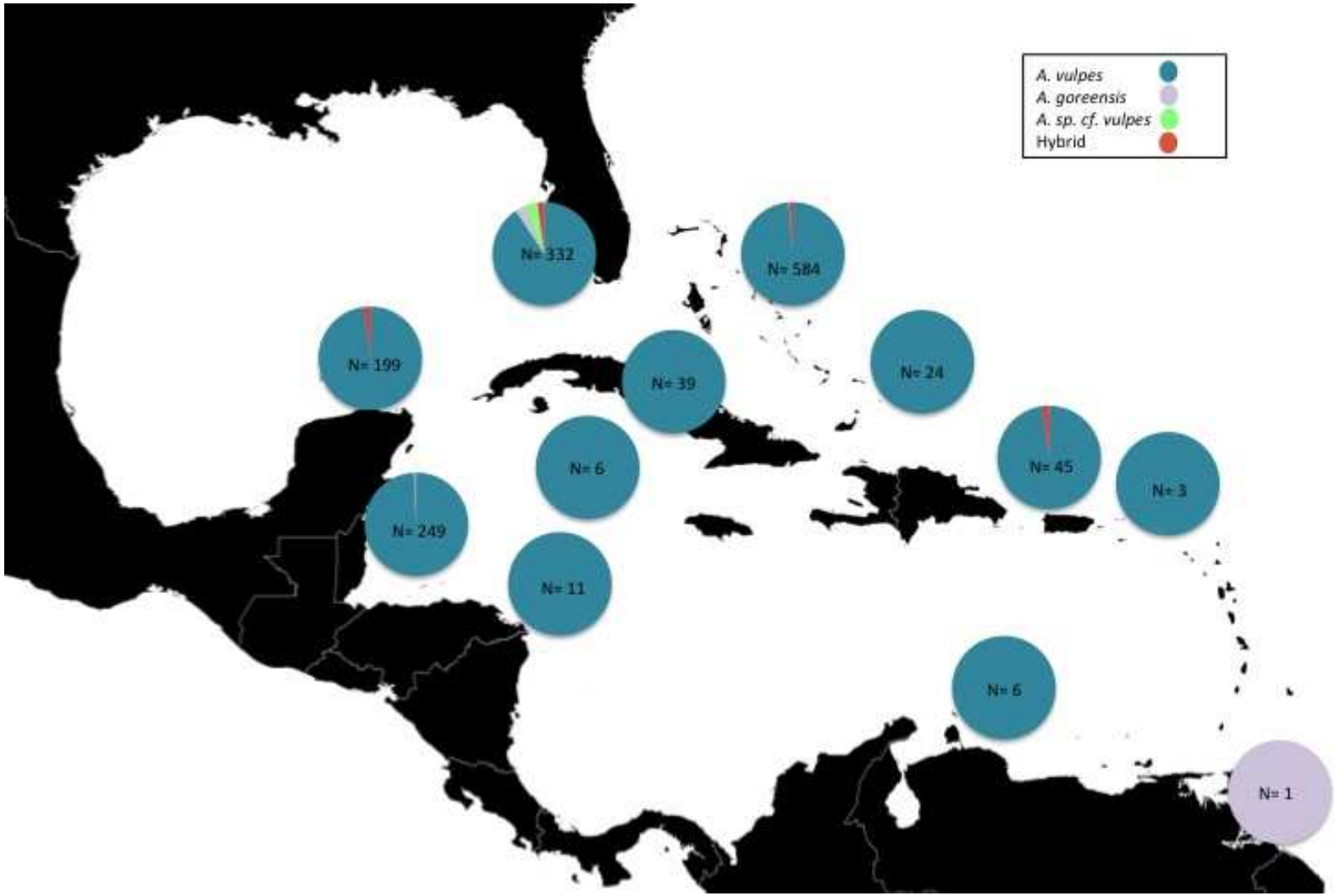


Figure 4. Regional occurrence of two *Albula goreensis* genetic populations (as inferred in *STRUCTURE*). Not pictured is the sample from the west coast of Africa. Total sample sizes are indicated for each regional collection location.

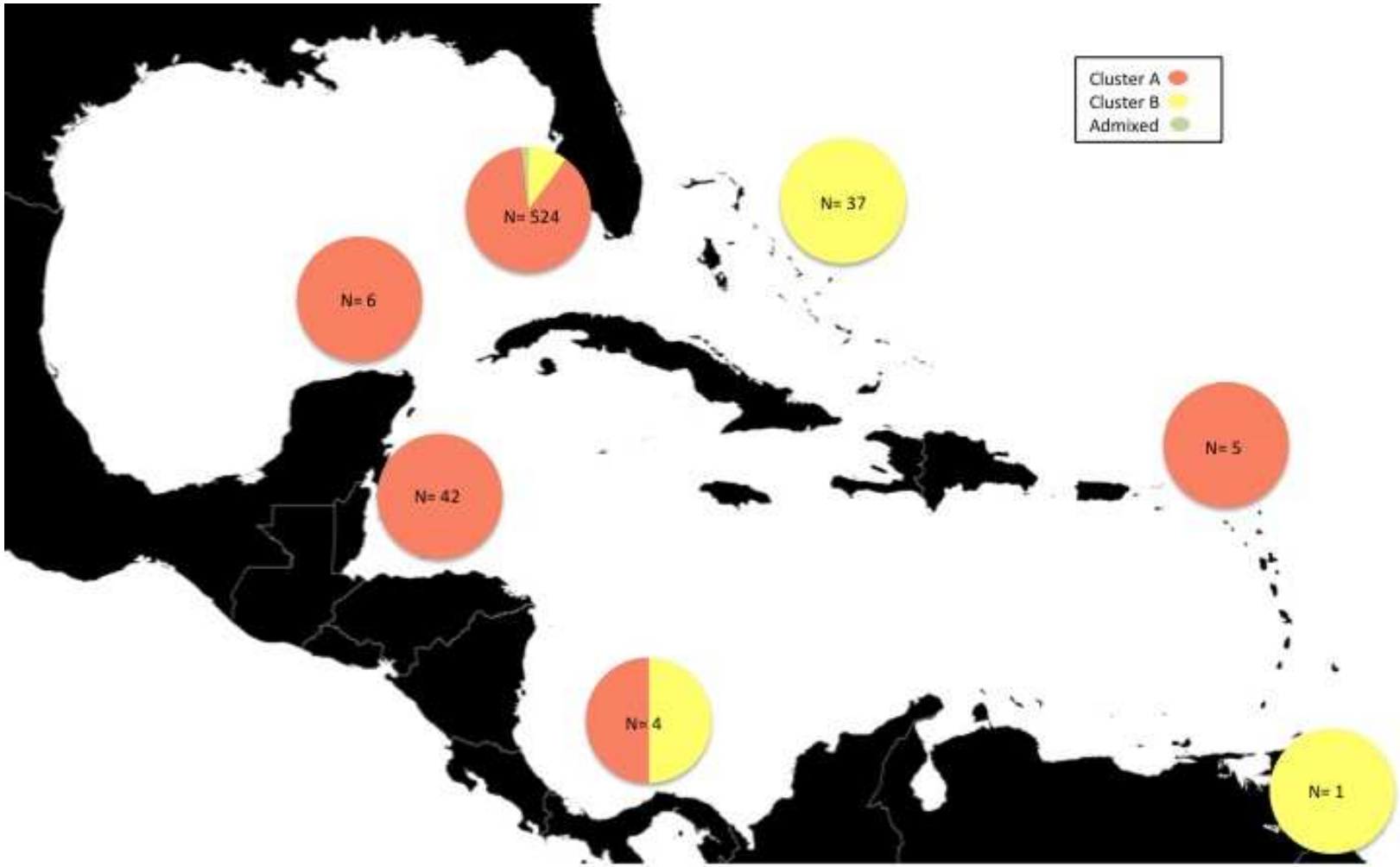
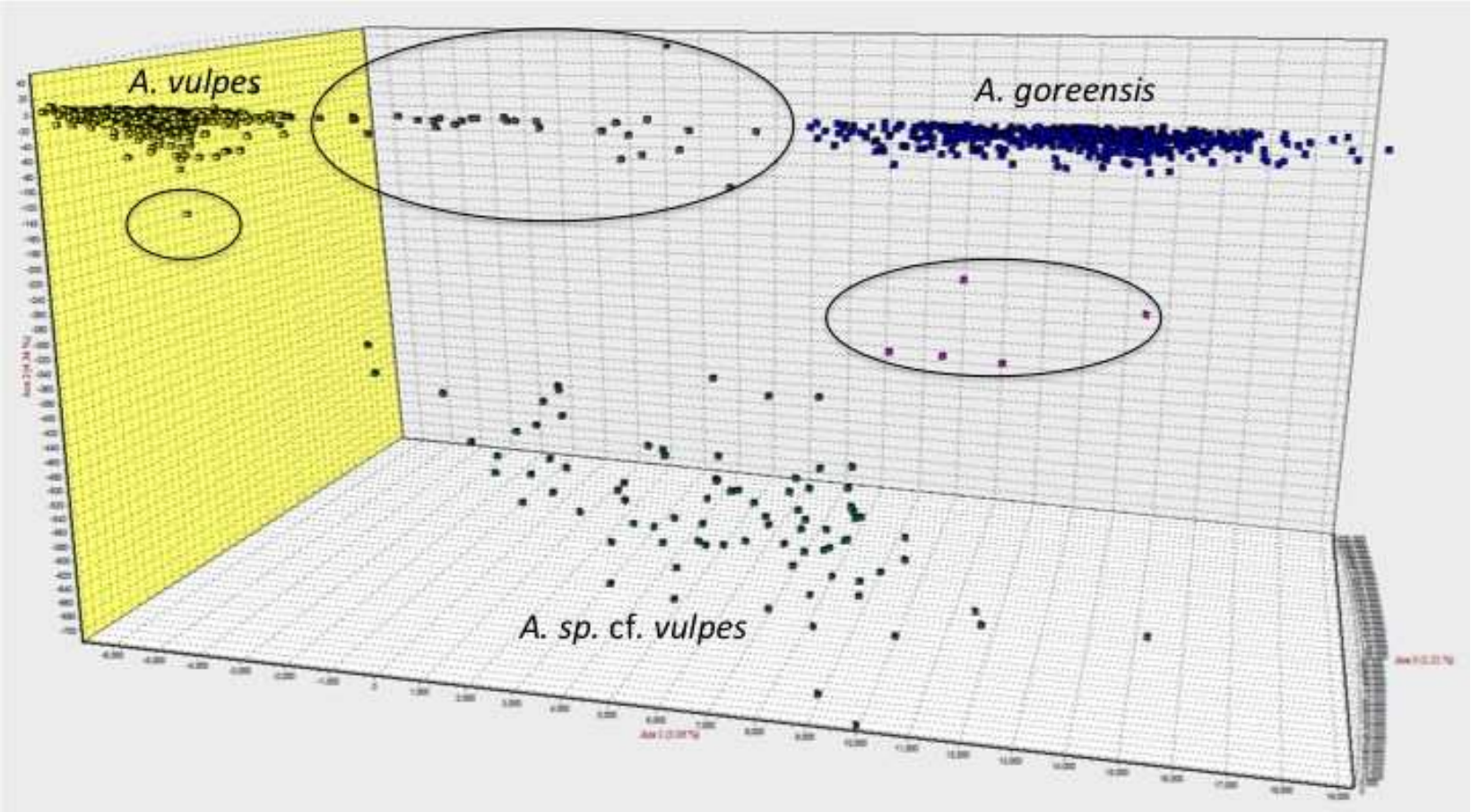


Figure 5. Spatial correspondence analysis for *Albula vulpes*, *A. goreensis*, *A.* sp. cf. *vulpes*, and their hybrids. Identified hybrid individuals are circled.



Chapter 3

Multilocus Phylogenetic Assessment of Bonefishes (Teleostei: Elopomorpha: Albuliformes) Supports Recognition of Sympatric Cryptic Species and Further Revision to the Order

3.1 Introduction

Fishes are the subjects of significant phylogenetic research attention with good reason. Ray-finned fishes (Actinopterygii) represent roughly 50% of the world's vertebrate species (Vega and Wiens, 2012). The superorder (or cohort *sensu* Betancur et al., 2013) Elopomorpha is an early diverging lineage originating during the Late Triassic to mid Jurassic (Broughton et al. 2013; Near et al. 2012). Elopomorpha contains approximately 1000 species (almost exclusively marine) contained in four orders: bonefishes (Albuliformes), tarpons (Elopiformes), true eels (Anguilliformes + Saccopharyngiformes), and spiny eels and halosaurs (Notacanthiformes)(Chen et al., 2014; Eschmeyer and Fong, 2014). This fish lineage has been the subject of longstanding debate regarding placement and monophyly of the superorder, as well as relationships among its members (Arratia, 1997; Greenwood et al., 1966; Nelson, 2006). Recent molecular phylogenetic studies place Elopomorpha as the earliest teleost lineage, sister to all others, and provide further support for monophyly (Broughton et al., 2013; Chen et al., 2014; Near et al., 2012). A comprehensive review of previous hypothesized relationships within Elopomorpha is found in Chen et al. (2014). A few coastal species support economically valuable commercial and recreational fisheries. Among these, bonefishes in the genus *Albula* are notable as the focus of substantial management and conservation interest (Adams et al., 2013). Albuliformes is in an active state of revision, driven by the identification of morphologically cryptic taxa. The order contains a single family (Albulidae) consisting of two genera, *Albula* and *Pterothrissus* (*sensu* Eschmeyer and Fong, 2014). However the placement of *Pterothrissus* (deep water bonefishes) within Albuliformes is uncertain, and additional revisions to the order may be necessary. *Pterothrissus*, consisting of two geographically disjunct species, was not included in the

above recent phylogenetic treatments. Thus its placement within Elopomorpha remains undetermined. The genus *Albula* was once considered monotypic due to substantial morphological conservatism. Several previous molecular phylogenetic studies of *Albula* identified substantial divergence between mitochondrial lineages, suggesting previously unrecognized levels of diversity (Bowen et al., 2008; Colborn et al., 2001; Pfeiler et al., 2008). However these single locus studies excluded some taxa, and did not formally evaluate species limits or explore divergence dates on an absolute timescale.

The bonefish genus *Albula* now includes 11 recognized species (Eschmeyer and Fong, 2014; Whitehead, 1986). Five of these species were recently identified and a 12th species remains to be formally described (Kwun & Kim 2011; Pfeiler et al., 2011; Pfeiler, 2008; Wallace and Tringali, 2010; Wallace, 2014). This work has revealed cryptic species complexes in both the Tropical Eastern Pacific (TEP) and Caribbean-Western Atlantic (CAR-ATL) regions. However a multilocus phylogenetic assessment, inclusive of all known albulids, is lacking.

Central to the practical issue of species delimitation is the species concept used to identify taxonomic units warranting recognition. Though theoretical debate persists, it is accepted that lineage diversification is a continual process (Hausdorf, 2011). The phylogenetic species concept recognizes species temporally earlier along this continuum; however, for sympatric taxa the phylogenetic and biological species concepts overlap (Knowlton, 2000). A lack of information on species boundaries limits our understanding of biodiversity and evolution for many taxonomic groups (Fouquet et al., 2014). Different species delimitation approaches can have direct impacts on conservation and management efforts as species status has legal, political, and social ramifications (Beheregaray and Caccone, 2007). Explicit criteria are particularly important for problematic radiations, such as those with cryptic diversity. Globally distributed bonefishes (genus *Albula*) present an excellent case study in cryptic diversification in the marine environment. They also are the focus of conservation and management efforts, especially in the CAR-ATL. Two species (*A. vulpes* and *A. glossodonta*) were recently assigned to the IUCN Red List, due to population declines and habitat degradation (Adams et al., 2013). Other species may also warrant consideration; but currently data is

insufficient to allow their evaluation. Multi-species coalescent approaches are at present the only objective method for evaluating species limits (Carstens and Dewey, 2010; Fujita and Leache, 2011). They account for the inherent uncertainty in gene tree estimation, while simultaneously accounting for gene tree- species tree discord (Heled and Drummond, 2010). These methods are beneficial for aiding in the diagnosis of valid species in sympatric taxonomic groups consisting of morphological cryptics (Giarla et al., 2014).

In addition to the practical value of assessing species limits, advances in phylogenetic methods allow for simultaneous inference of evolutionary relationships and divergence estimation on an absolute timescale. A primary benefit is substantial progress toward reconstruction of an accurate Tree of Life. Our understanding of the evolution of fishes in particular has seen major recent gains. In addition, these methods have allowed significant progress in the field of historical biogeography. Unlike terrestrial biogeographic features, marine provincial "soft" barriers act as filters, with variable effects on different taxa, rather than hard boundaries (Floeter et al., 2008). Evidence suggests oceanic currents and barriers, as well as taxon specific dispersal potential and environmental tolerance may drive diversification in the marine environment (Boehm et al., 2013; Rocha et al., 2008). Diversification route inference often centers on the role of transient connections between the world's oceans in dispersal or vicariance. For extant coastal fishes, maximum global diversity occurs in the Western Pacific (Tittensor et al., 2010). The tropical Atlantic Ocean has been divided into four biogeographic provinces: Brazil, Greater Caribbean, Mid-Atlantic Ridge, and Eastern Atlantic (Briggs, 1974). Historically, three routes existed for dispersal into the Atlantic. The Tethys Seaway (closure 12-18 MYA) served as a corridor for tropical species from the Indian Ocean (Bellwood and Wainwright, 2002; Reese et al., 2010). The Central American Seaway was a critical link between the TEP and the CAR-ATL (closure 3 MYA), and has served as an important model in evolutionary studies (Craig et al., 2004; Lessios, 2008). Dispersal around the southern tip of Africa was also possible until approximately 2.5 MYA, when strengthening of the cold-water Benguela Current effectively closed this route to tropical species (Peeters et al., 2004; Shannon, 1985).

This study represents the first multilocus evaluation focused on the phylogenetic relationships and dates of divergence within Albuliformes. The timing of diversification in Albuliformes suggests several invasions of the CAR-ATL through multiple seaway corridors. Further, this study is the first to evaluate species limits in *Albula* through a Bayesian multi-species coalescent approach. The genus has been the subject of renewed attention due to the identification of cryptic species complexes in the TEP and CAR-ATL.

3.2 Materials and methods

DNA extraction, amplification, and sequencing

Tissues were obtained through field collections conducted by colleagues and through museum loans. Total genomic DNA was purified from tissues using the Gentra Puregene™ tissue kit (Qiagen Inc.) and manufacturer's protocol. All polymerase chain reaction (PCR) assays were performed in a 12.5µl reaction volume using 1µl DNA, 6.25µl GoTaq Green Master Mix (Promega Inc.), 0.5µl of each 10µM primer, and 4.25µl water. A portion of mitochondrial cytochrome *b* was sequenced using primers alba-1, alba-2, alba-3, and alba-6 of Colborn et al. (2001). These PCR reactions were amplified using the following thermal profile: 1x of 94° for 60s, 50° for 30s, 72° for 60s, 36x of 94° for 30s, 55° for 30s, 72° for 90s, and final extension at 72° for 8min. The nuclear introns EIF3C and STX5A were amplified with primers eif3cf13, eif3cr13, stx5a10f, and stx5a10r of Halas and Simons (2014). Two nuclear exons, SREB2 and ZIC1, were initially amplified with the primers of Li et al. (2007). Due to variable amplification among elopomorphs, the following internal primers were designed: sreb2intF [5' TTCCTCAAACTGACCTCCC 3'] sreb2intR [5' GCYTGGGCGAARCTCATC 3'], and zic1intF [5' AATCAACCACRGCACCCAC 3'] and zic1intR [5' TTCGCCGGTGTGTACTCTRATG 3']. The nuclear regions were all amplified using a touchdown thermal cycling profile: 1x of 95° for 3min., 5x at three annealing temperatures of 58-54° (decreasing in 2° increments): initial denaturation of 95° for 30s,

58-54° for 30s, 72° for 60s, 20x of 95° for 30s, 52° for 30s, 72° for 60s, and a final extension at 72° for 6min. Sequencing reactions were prepared using Big Dye terminator cycle sequencing, and run on an automated genetic analyzer at the University of Minnesota Genome Center or at Beckman Coulter Genomics. Sequences for each gene region were edited and aligned in Geneious version 7.1.4 (Drummond et al., 2010). Final sequence alignments were completed using the Muscle implementation in Geneious (Edgar, 2004). Genbank accession numbers for sequence data are provided in Table 1.

Data analyses

The appropriate models for sequence evolution and optimal partitioning schemes for each gene region were simultaneously determined in PartitionFinder version 1.1.0 and ranked according to BIC (Lanfear et al., 2012). Pairwise sequence divergence values (K2P *d*) were calculated in Mega version 5.2 for the cytochrome *b* dataset (Tamura et al., 2011). A maximum likelihood genealogy based on cytochrome *b* was estimated in RAxML version 8.0.9 under a GTR nucleotide substitution model (Stamatakis et al., 2008). The dataset was partitioned by codon position, five independent runs were conducted, and the best ML tree selected from them. Nodal support was determined through bootstrapping of 1000 pseudo-replicates from each run. All molecular phylogenetic analyses were conducted via the CIPRES science gateway (Miller et al., 2010).

In order to evaluate species limits within *Albula*, species trees were estimated in *BEAST version 1.8.0 under the multi-species coalescent on a combined nuclear dataset (EIF3C, SREB2, ZIC1)(Heled and Drummond, 2010). This approach requires the previous identification of operational taxonomic units (OTUs), and sampling of multiple individuals within each OTU. Thus individuals were assigned to putative species based on the relationships inferred by the ML cytochrome *b* phylogeny. However to avoid circular reasoning, the cytochrome *b* data was not included in the species tree analyses, nor was it used to constrain any taxonomic groupings. The three-locus nuclear dataset consisted of two to twenty individuals per locus. Three independent *BEAST analyses

were run for 100 million generations with sampling every 1,000 generations. The resultant 100,000 trees were pruned to 50,000 following a conservative burn-in of 50%. The species tree analyses were conducted using the HKY+I nucleotide substitution model for EIF3C and ZIC1, and HKY+I+G for SREB2, as indicated in PartitionFinder. The dataset was further partitioned into three (by codon position) for the SREB2 locus, and two (codon position 1 & 2, position 3) for ZIC1. *BEAST analyses were conducted under both strict and lognormal relaxed clock models, as well as linear and constant population sizes, in order to test model fit and for potential affects of prior assumptions. All analyses were run under the birth-death speciation model. Each set of runs were also compared to an empty analysis on the priors only to confirm the posterior distributions were not driven by prior assumptions for any parameters. The strict clock analyses were run with the conditional reference prior (after Ferreira and Suchard, 2008) for the SREB2 and ZIC1 clock rate priors. Convergence for each of the *BEAST analyses was assessed by manual examination of parameter acceptance ratios, and in Tracer version 1.5 by evaluation of effective sample size (ESS) values and likelihood plots (Rambaut and Drummond, 2007). Consistency across runs was further assessed by agreement of parameter estimates and overlapping marginal densities between independent runs. Evaluation of the clock and population models confirmed the strict clock and constant population size models best fit the data. The tree files from three independent runs were combined in LogCombiner version 1.8, and the maximum credible clade tree was created from the summarized trees in TreeAnnotator version 1.8 (Rambaut and Drummond, 2007; Rambaut and Drummond, 2010).

In order to investigate evolutionary relationships within Elopomorpha and the timing of divergence, an absolute time scaled phylogeny was reconstructed in BEAST. The combined multilocus dataset consisted of a portion of mitochondrial cytochrome *b*, nuclear introns EIF3C and stx5a, and nuclear exons SREB2 and ZIC1 for 30 taxa. An unconstrained maximum likelihood tree was estimated in RAxML under a GTR substitution model for the concatenated multilocus dataset, and used as a starting tree for the divergence analyses. The branch lengths were transformed in TreeEdit version 1.0 under non-parametric rate smoothing (Rambaut and Charleston, 2001). Based on the

PartitionFinder analyses, optimal partitioning for the combined dataset for divergence estimation was by gene and further by codon position within the 3 protein coding regions, for a total of 11 partitions. The XML files for the BEAST analyses were constructed in BEAUti. The root node and 3 internal nodes were calibrated based on the age of origin for Teleostei from the literature (early Jurassic) and available fossil data (Arratia, 2000; T. Near-personal communication). The root node prior was set as a normal distribution with a mean of 189 Ma and standard deviation of 4.69. The fossil calibration prior for Albuliformes was set as an exponential distribution with a mean of 66 Ma and offset of 132 Ma. The fossil calibration priors for both Anguilliformes and Pterothrissidae were set as uniform distributions with a lower bound of 96.6 Ma and an upper bound of 1000 Ma. Divergence estimates were run under a birth-death speciation model, and inferred under lognormal and strict clock models to examine the effect of rate correlation assumptions. Under the lognormal relaxed clock model, the mean rate prior was set as an exponential distribution with a mean of 10.0. Under the strict clock model, the clock rate prior was set as the conditional reference prior (Ferreira and Suchard, 2008). The run conditions and convergence assessment methods for each BEAST analysis followed those used in the *BEAST analyses. Comparison of results from the strict and lognormal clock models confirmed the lognormal clock model best fit the data. The resultant tree files from the 3 analyses were summarized in LogCombiner following discard of the first 50% as a conservative burn-in. The maximum clade credibility tree was constructed in TreeAnnotator from the combined trees LogCombiner output file.

3.3 Results

Sequence summary and mitochondrial gene tree results

Sequence data (2,766 bp in total) were obtained for a portion of the mitochondrial DNA gene cytochrome *b* and four nuclear loci (EIF3C, SREB2, stx5a, and ZIC1) for 30 taxa (29 elopomorphs and 1 osteoglossomorph as the outgroup). All new sequences were deposited in Genbank (accession numbers given in Table 1). The level of sequence

divergence observed among global albulids in cytochrome *b* was substantial. The lowest pairwise genetic distance (K2P *d*=0.01) was between the Pacific taxa *A. argentea* and *A. virgata* (Table 2). However average *d*=0.13 for Albuliformes and the highest for congenerics, *d*= 0.18, was between the Pacific species *A. glossodonta* and *A. argentea*. The levels of sequence divergence between members of *Albula* and *Pterothrissus gissu* were even greater (*d*= 0.24-0.31). The cytochrome *b* RAxML genealogy reflects relative divergence among members of *Albula*, *Pterothrissus gissu*, and *Anguilla rostrata*, as an outgroup taxon (Fig. 1). The relationships inferred in this phylogeny suggest *P. gissu* is more closely related to *A. rostrata* than to the bonefishes of *Albula*. Several clades within *Albula* received only weak support (< 90% bootstrap proportion [BP]). However, a strongly supported clade consisting of the Pacific *A. pacifica* and Atlantic *A. nemoptera* was recovered (92% BP). A clade consisting of Indo-Pacific species *A. argentea*, *A. virgata*, and *A. oligolepis* received strong support (99% BP). The clade consisting of Pacific taxa *A. gilberti*, *A. esuncula*, and the Atlantic *A.* sp. cf. *vulpes* was also strongly supported (92% BP). Also inferred was a clade containing the Atlantic taxa *A. goreensis*, *A. vulpes*, and Pacific *A. glossodonta*, although this received weak support.

Species tree analyses

Lognormal and strict clock model results were compared for the *Albula* species tree analyses in *BEAST. The marginal posterior distributions of the lognormal relaxed clock standard deviation included zero, thus a strict clock model was the best fit for the three nuclear gene dataset (EIF3C, SREB2, and ZIC1). Constant and linear population growth model results were also compared. The constant population model was determined to best fit the data following assessment of the growth rate marginal posterior distribution (included zero). The species tree estimate presented here thus assumes strict clock and constant population models. The maximum clade credibility tree summarized from the posterior set of post burn-in 150,000 trees was well resolved, with all but one node in the phylogeny supported by significant Bayesian posterior probabilities (BPP ≥0.95) (Fig. 2). The posterior parameter estimates from each of the three independent

*BEAST analyses exhibited high ESS (>200). Three *Albula* taxa (*A. koreana*, *A. nemoptera*, and *A. pacifica*) were excluded from the species tree analyses due to the lack of multiple intraspecific gene sequence data. The nine included members of the genus (as interpreted by the cytochrome *b* tree) were contained in well supported clades. Inferred relationships from the multi-species coalescent species tree analyses largely matched those estimated from the cytochrome *b* phylogeny. The clade containing Indo-Pacific species *A. virgata*, *A. argentea*, and *A. oligolepis* supported *A. virgata* as a distinct lineage from *A. argentea* (BPP=1.0). The results also support the Atlantic species *A. vulpes* and Pacific *A. glossodonta* as sister (BPP=0.95). The *BEAST species-tree results supported an alternate placement of *A.* sp. cf. *vulpes* as sister to the clade containing *A. esuncula* and *A. gilberti*, rather than as sister to *A. esuncula* (as in the ML cytb tree). However, that clade was also the only one to receive support just below the statistical significance threshold (BPP=0.94).

Elopomorpha relationships and divergence time estimates

Following comparisons of strict and uncorrelated lognormal relaxed clock models, the divergence time BEAST analyses supported the lognormal relaxed clock as the best fit for the five-locus dataset (cytb, EIF3C, SREB2, stx5a, and ZIC1). The marginal posterior distribution of the lognormal relaxed clock standard deviation was non-zero, rendering a strict clock model inappropriate. The results presented here thus assume a lognormal clock model. The phylogeny and divergence dates were estimated from three independent BEAST analyses. The posterior parameter estimates from each independent analysis displayed ESS>300. Following confirmation of individual run convergence, the results were combined to produce a summary file consisting of 150,000 trees. The inferred family level relationships were well supported (BPP=1.0), with the exception of the Anguilliformes clade (BPP=0.78)(Fig. 3). The placement of *Pterothrissus gissu* within Notacanthiformes received strong support (BPP=1.0), thus rendering Albuliformes (*sensu* Eschmeyer and Fong, 2014) paraphyletic. The divergence time estimates at the family level suggest Elopiformes, Notacanthiformes, and Anguilliformes arose during the

Jurassic (approximately 191-161 MYA). The divergence results further suggest Albuliformes arose approximately 138 MYA, during the early Cretaceous.

Within Albuliformes, almost all phylogenetic relationships were strongly supported (BPP=0.96-1.0). Relationships inferred in the multilocus BEAST analyses were also largely concordant with those of the ML cytb genealogy and *BEAST species tree. The split for the clade consisting of *A. virgata, A. argentea,* and *A. oligolepis* received strong support (BPP=0.96). Partial discord occurred for the sister relationship of *A.* sp. cf. *vulpes* and *A. esuncula,* rather than *A. esuncula* as sister to *A. gilberti* suggested by the *BEAST species tree. The clade containing *A.* sp. cf. *vulpes* and *A. esuncula* was concordant with the cytb genealogy, however it received weak support (BPP=0.61). Date estimates suggest bursts of diversification in albulid lineages beginning during the late Cretaceous. The estimates suggest the oldest sister species, *A. pacifica* and *A. nemoptera*, diverged roughly 16 MYA. *Albula vulpes* and *A. glossodonta* split from a common ancestor approximately 11 MYA, while *A.* sp. cf. *vulpes* and *A. esuncula* diverged about 9 MYA. The youngest time estimate was for the split between *A. virgata* and *A. argentea* approximately 6 MYA.

3.4 Discussion

Relationships within Albuliformes

The results presented here lend additional support to previous studies that found deep divergence among albulids and suggest further revision of Albuliformes is necessary. The observed levels of mitochondrial divergence was substantial, and generally in accord with those reported by Colborn et al. (2001) and Bowen et al. (2008). Especially striking was the substantial divergence between *P. gissu* and members of *Albula*. The current results support the revisions proposed by Chen et al. (2014) to exclude Notacanthiformes (which they recommended be elevated to order) from Albuliformes (*sensu* Forey et al., 1996). However the current results clearly show that Pterothrissinae is not a member of Albulidae. Chen et al. (2014) did not include a

member of Pterothrissinae in their evaluations of Elopomorpha. The phylogenetic placement of *P. gissu* as sister to the notacanthids *Polyacanthonotus challengeri*, *Notacanthus chemnitzii*, and *Halosauropsis macrochir* received strong support (BPP=1.0). While data were unavailable from the sister species, *P. belloci*, these results support revision excluding *Pterothrissus* from Albuliformes. Proposed revisions therefore include restriction of Albuliformes to include only Albulidae (*sensu stricto*), elevation of Pterothrissinae to Pterothrissidae, and Notacanthiformes to include Halosauridae, Notacanthidae, and Pterothrissidae.

The evolutionary relationships inferred among members of the genus *Albula* (true bonefishes) are mostly concordant with those presented in Colborn et al. (2001), Bowen et al. (2008), and Pfeiler et al. (2008). The proposed species *A.* sp. cf. *vulpes* was identified after the Colborn et al. (2001) study, and thus is absent from their analyses (Wallace and Tringali, 2010). In the current study, there was conflict between the species tree and the time tree analyses regarding inferred relationships among the ATL *A.* sp. cf. *vulpes* and the TEP *A. esuncula* and *A. gilberti*. The species tree relationships indicated *A. esuncula* and *A. gilberti* are sister, while the time tree suggested *A.* sp. cf. *vulpes* and *A. esuncula* are sister. A larger molecular dataset may be necessary to clarify the evolutionary relationships among the three species.

Among the CAR-ATL species (*A. nemoptera*, *A. vulpes*, *A. goreensis*, and *A.* sp. cf. *vulpes*), the phylogenetic evidence supports current sympatry as the result of secondary contact rather than sympatric or parapatric speciation. No sister relationships were recovered among any of these species. Pacific species reflect both sister relationships resulting from local diversification as well as secondary contact. The *A. argentea* species complex (*A. argentea*, *A. virgata*, and *A. oligolepis*) reflects *in situ* Indo-PAC diversification. *Albula virgata* and *A. glossodonta* are distantly related, however have partially overlapping PAC distributions, and co-occur in Hawaiian waters. The TEP bonefishes (*A. pacifica*, *A. esuncula*, and *A. gilberti*) reflect a combination of secondary contact and local diversification.

Relationships in Elopomorpha

Recent comprehensive re-examinations place Elopomorpha as sister to all other teleosts (Betancur et al., 2013; Near et al., 2012), and support earlier work by Arratia (1997, 1998). However these previous studies do not fully resolve relationships within the clade due to limited taxon sampling. A recent multilocus phylogenetic evaluation of Elopomorpha provides further support for its monophyly (Chen et al., 2014), but taxon sampling within Albulidae was limited to just two species. Further, *Pterothrissus* was absent from the earlier studies of Betancur et al. (2013), Chen et al. (2014), and Near et al. (2012), thus preventing inference of its phylogenetic position.

The emphasis of the present study was on phylogenetic relationships within Albuliformes and its placement among elopomorphs. Outgroup taxon sampling was limited to a representative member of Osteoglossomorpha, thus results cannot directly support monophyly of Elopomorpha nor its position among teleosts. However the results do support the relationships within Elopomorpha hypothesized by Chen et al. (2014) among most major clades. Importantly, Albuliformes and Notacanthiformes are not sister. The results place Albuliformes as sister to Anguilliformes rather than sister to a clade comprising Notacanthiformes + Anguilliformes as in Chen et al. (2014) and Forey et al. (1996). However, this relationship was weakly supported (BPP=0.78), as were relationships among Anguilliformes with the exception of the split between Synaphobranchidae and other anguillids (BPP=1.0). Interestingly, the current results place the “living fossil” eel *Protoanguilla palau* sister to other members of Anguilliformes (though with weak support, BPP=0.55), rather than Synaphobranchidae as in Chen et al. (2014). Taxonomic sampling within Anguilliformes was less dense than in Chen et al. (2014), which may have affected the inferred relationships within this diverse order.

The results presented here are in complete accord with all previous studies regarding Elopiformes as sister to all other elopomorphs (Chen et al., 2014; Forey et al., 1996; Inoue et al., 2004; Near et al., 2012; Obermiller and Pfeiler, 2003; Wang et al., 2003). The inferred relationships are also in accord with Tang & Fielitz (2013), with the

exception of Albuliformes *sensu lato* (including *Pterothrissus*) as sister to Notacanthiformes. Their dataset was exclusively mitochondrial and taxon sampling within *Albula* was limited to two species.

Divergence time estimates

The dates of divergence from the present study generally agree with previous molecular work on early teleost lineages. Recent estimates for the origin of Elopomorpha range from Late Triassic in Broughton et al. (2013) to Early Jurassic in Betancur et al. (2013), and mid Jurassic in Near et al. (2012) though 95% probability distributions overlapped. The dates estimated from the molecular phylogenetic studies are roughly in accord with the fossil record (Arratia, 2000; Nelson, 2006). Molecular derived date estimates are commonly older than those inferred from fossils, and may reflect gaps in the fossil record (Near et al., 2012). The sensitivity of molecular calibration to variations in taxon sampling, molecular markers screened, and fossil usage is also well known (Betancur et al., 2013; Warnock et al., 2012).

The estimated dates of diversification within *Albula* are generally in accord with those previously suggested by mitochondrial clock-based analyses, and the fossil record (Colborn et al., 2001; Frizzell, 1965). They also strongly suggest up to four CAR-ATL invasions from both TEP and Indo-PAC sources. Bonefishes exhibit site fidelity as adults, making brief local migrations to spawning grounds (Danylchuk et al., 2011). However as with all elopomorphs, bonefish leptocephalus larvae have a relatively long pelagic larval duration (from 2 to 7 months) (Mojica et al., 1995). As a result, diversification could have occurred by dispersal through adult range expansion or larval connectivity followed by vicariant events. The oldest Atlantic cladogenesis (during the late Oligocene/Early Miocene boundary in the MRCA of CAR-ATL *A. goreensis*, *A. vulpes*, and Indo-PAC *A. glossodonta*) may have occurred via the Tethys Seaway or around Southern Africa (Fig. 4). While the closure of the Tethys represents a hard boundary for tropical marine species, the separation of the Atlantic from the southern Indian Ocean is a soft barrier that may be breeched by some taxa. The TEP *A. pacifica*

and ATL *A. nemoptera* may represent transisthmian geminates (*sensu* Jordan 1908). The estimate for this divergence during the mid Miocene well pre-dates the final closure of the Central American Seaway. However, earlier episodic partial closures likely affected TEP-ATL marine connectivity (Elmer et al., 2013; Lessios, 2008). Taxon specific ecological tolerance may have driven early separation. Due to intensive recent molecular study of the TEP and CAR-ATL bonefishes, it is unlikely that undetected taxa exist thus creating an erroneous sister relationship. The results suggest diversification in the MRCA of the CAR-ATL *A. vulpes* and Indo-PAC *A. glossodonta* during the mid Miocene was likely via dispersal around Southern Africa. The most recent ATL invasion (also during the mid Miocene in the MRCA of ATL *A. sp. cf. vulpes* and TEP *A. esuncula*) may represent a second pair of geminate species. This splitting event also pre-dates the final closure of the Central American Seaway. However that node was weakly supported in the divergence time analyses. It is also possible that the TEP-ATL split occurred at the previous node approximately 13 MYA, and was followed by speciation within the TEP resulting in sister species *A. esuncula* and *A. gilberti*. That relationship was supported by the species tree analysis (BPP =0.94).

A similar pattern was found among Moray eels, with diversification estimates supporting multiple invasions into the ATL from the Indo-PAC during the early to mid Miocene (Reece et al., 2010). That study also identified a geminate species pair that dated to the final closure of the Isthmus of Panama. Studies of diversification in other marine fishes have found evidence for multiple ATL invasions (butterflyfishes, wrasses, damselfishes, parrotfishes), as well as a single colonization event (gobies, angelfishes, wrasses) (reviewed in Rocha and Bowen, 2008). In general there is less opportunity for allopatric speciation the marine environment, thus ecological boundaries likely play an increased role in divergence (Bowen et al., 2013) These patterns may also reflect variable realized dispersal potential among these taxonomic groups.

Conclusions

Our understanding of the timing and pattern of evolution in early teleost lineages has increased substantially. The evidence presented here [and elsewhere] suggests taxonomic revisions are needed for Albuliformes. Proposed changes include elevation of Pterothrissinae to Pterothrissidae and its inclusion in Notacanthiformes. These modifications accurately reflect evolutionary history, and render Albuliformes monotypic for both family Albulidae and genus *Albula*.

Organisms exhibiting morphological conservatism, such as bonefishes, make interesting subjects for exploring diversification. Other examples of morphological stasis include horseshoe crabs, plethodontid salamanders, lampreys, and African butterfly fishes (Lavoué et al., 2011). Phylogenetic studies frequently identify cryptic lineages, complicating the practical need to accurately delimit species. Comprehensive approaches incorporate multiple criteria such as phylogenetic, ecological, behavioral, and geographic information. Coalescent phylogenetic methods can aid in species delimitation for cryptics that occur in sympatry (Yang and Rannala, 2010). In the current study 9 of 12 recognized and putative bonefish species were considered in a species-tree analysis. The remaining three species exhibit clear (though subtle) morphological differences, and already have formal descriptions. All 9 putative species considered displayed substantial divergence, including the most recently diverged *A. virgata* from its sister species *A. argentea*. The cryptic species in the CAR-ATL are broadly sympatric, however current sympatry is due to secondary contact as none are sister. The complex phylogenetic relationships among the ATL species stem from up to four invasions that occurred through multiple historical routes. In addition, ecological and behavioral evidence further support their recognition as distinct species (Wallace, 2014). The identification of cryptics can directly affect conservation as presumed widespread species may in reality consist of several species with smaller ranges and populations (Niemiller et al., 2013). In addition, cryptic species often remain in a taxonomic limbo without formal descriptions (termed the Linnean shortfall) (Brown and Lomolino, 1998). While the seemingly arbitrary designator of a name should not affect conservation actions, a species taxonomic status can in fact have

political and legal implications (Beheregaray and Caccone, 2007). This study has clarified species recognition and phylogenetic relationship questions as well as nomenclatural confusion that have hampered bonefish conservation efforts.

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Table 1. List of taxa examined in this study, tissue sources, and NCBI Genbank accession numbers.

Taxon	Tissue Source[1]	Genbank accession numbers				
		Cyt *b*	EIF3C	SREB2	stx5a	ZIC1
Albula oligolepis	BMNH, SAIAB & QM	KJ910041-KJ910043	KJ910260	KJ910107-KJ910116	KJ910278	KJ910229-KJ910238
Albula argentea	BMNH	KJ910027- KJ910028	KJ910271	KJ910048-KJ910051	KJ910279	KJ910162-KJ910166
Albula virgata	BMNH	KJ910045	KJ910275	KJ910117-KJ910124	KJ910280	KJ910239-KJ910246
Albula nemoptera	HIMB	AF311754.1-AF311755.1	~	KJ910105-KJ910106	KJ910276-KJ910277	KJ910227-KJ910228
Albula pacifica	Genbank	DQ272657.1-DQ272659.1	~	~	~	~
Albula gilberti	BMNH	KJ910029- KJ910032	KJ910264, KJ910266 & KJ910273	KJ910062-KJ910073	KJ910285	KJ910183-KJ910194
Albula sp. cf. *vulpes*	BMNH & new collections	KJ910044	KJ910270	KJ910052-KJ910060	KJ910283	KJ910167-KJ910180
Albula esuncula	new collections	AF311760.1-AF311762.1 & EF602158.1	KJ910265 & KJ910272	KJ910061	KJ910284	KJ910181-KJ910182
Albula glossodonta	BMNH & new collections	KJ910033- KJ910037	KJ910267 & KJ910274	KJ910074-KJ910084	~	KJ910195-KJ910206
Albula vulpes	BMNH & new collections	KJ910046-KJ910047	KJ910262 & KJ910268	KJ910125-KJ910139	~	KJ910247-KJ910259
Albula goreensis	new collections	KJ910038-KJ910040	KJ910261 & KJ910263	KJ910085-KJ910103	KJ910281	KJ910207-KJ910226
Albula koreana	PNU	HM119396.1-HM119400.1	~	KJ910104	~	~
Anguilla rostrata	JFBM	AB021767.1	~	KJ910147	~	EU001889.1
Echidna nebulosa	JFBM	HQ122482.1	~	KJ910140	~	KJ910152
Elops saurus	TCWC & new collections	NC005803.1	~	EU002123.1	KJ910282	KJ910159

[1] BMNH: Burke Museum of Natural History; SAIAB: South African Institute for Aquatic Biodiversity; QM: Queensland Museum; HIMB: Hawaii Institute of Marine Biology; PNU: Pukyong National University; JFBM: James Ford Bell Museum; TCWC: Texas A & M Biodiversity Research and Teaching Collection; VIMS: Virginia Institute of Marine Science; AMNC: Australia Museum of Nature and Culture; NHMI: National History Museum and Institute Chiba; NMNS: National Museum of Nature and Science Tokyo.

Elops hawaiensis	Genbank	HQ616667.1	~	~	~	~
Elops smithi	Genbank	GQ183886.1	~	~	~	~
Eurypharynx pelecanoides	VIMS	AB046473.2	~	KJ910146	~	KJ910150
Halosauropsis macrochir	VIMS	~	~	KJ910142	~	KJ910157
Hiodon tergisus	JFBM	NC015082.1	~	JX191059.1	KJ910286	JX191258.1
Histiobranchus bathybius	VIMS	AF120951.1	~	KJ910148	~	KJ910153
Megalops atlanticus	new collections	AP004808.1	~	~	~	KJ910160
Megalops cyprinoides	AMNC	AB051110.1	KJ910269	~	~	KJ910161
Notacanthus chemnitzii	VIMS	AP002975.2	~	KJ910143	~	FJ906631.1
Polyacanthonotus challengeri	VIMS	~	~	KJ910144	~	KJ910158
Protoanguilla palau	NHMI	AP011809.1	~	~	~	KJ910155
Pterothrissus gissu	NMNS	NC005796.1	~	KJ910145	KJ910287	KJ910156
Scolecenchelys macroptera	JFBM	~	~	KJ910141	~	KJ910151
Synaphobranchus affinis	VIMS	~	~	KJ910149	~	KJ910154
Synaphobranchus kaupii	Genbank	AP002977.2	~	~	~	~

Table 2. Pairwise mitochondrial sequence divergence (K2P d) among global albulids based on a portion of the cytochrome b gene.

	A. oligolepis	*A. virgata*	*A. argentea*	*A. pacifica*	*A. nemoptera*	*A. gilberti*	*A. esuncula*	*A.* sp. cf. *vulpes*	*A. glossodonta*	*A. vulpes*	*A. goreensis*	*A. koreana*	*P. gissu*
A. oligolepis	~												
A. virgata	0.06	~											
A. argentea	0.06	0.01	~										
A. pacifica	0.13	0.15	0.15	~									
A. nemoptera	0.15	0.15	0.16	0.04	~								
A. gilberti	0.12	0.15	0.14	0.11	0.13	~							
A. esuncula	0.12	0.14	0.14	0.12	0.12	0.04	~						
A. sp. cf. *vulpes*	0.12	0.14	0.14	0.12	0.12	0.04	0.04	~					
A. glossodonta	0.14	0.17	0.18	0.16	0.16	0.10	0.09	0.08	~				
A. vulpes	0.14	0.16	0.16	0.13	0.14	0.10	0.09	0.09	0.06	~			
A. goreensis	0.15	0.17	0.17	0.10	0.10	0.08	0.09	0.09	0.09	0.08	~		
A. koreana	0.12	0.13	0.13	0.12	0.12	0.08	0.09	0.09	0.11	0.09	0.08	~	
P. gissu	0.29	0.31	0.31	0.29	0.28	0.30	0.28	0.30	0.24	0.26	0.26	0.29	~

Figure 1. Relationships among all known members of *Albula*, recognized and proposed species, inferred in RAxML based on a portion of the cytochrome *b* mitochondrial gene. Nodal ML bootstrap support values greater than 90% are shown, and branch lengths represent sequence divergence between taxa.

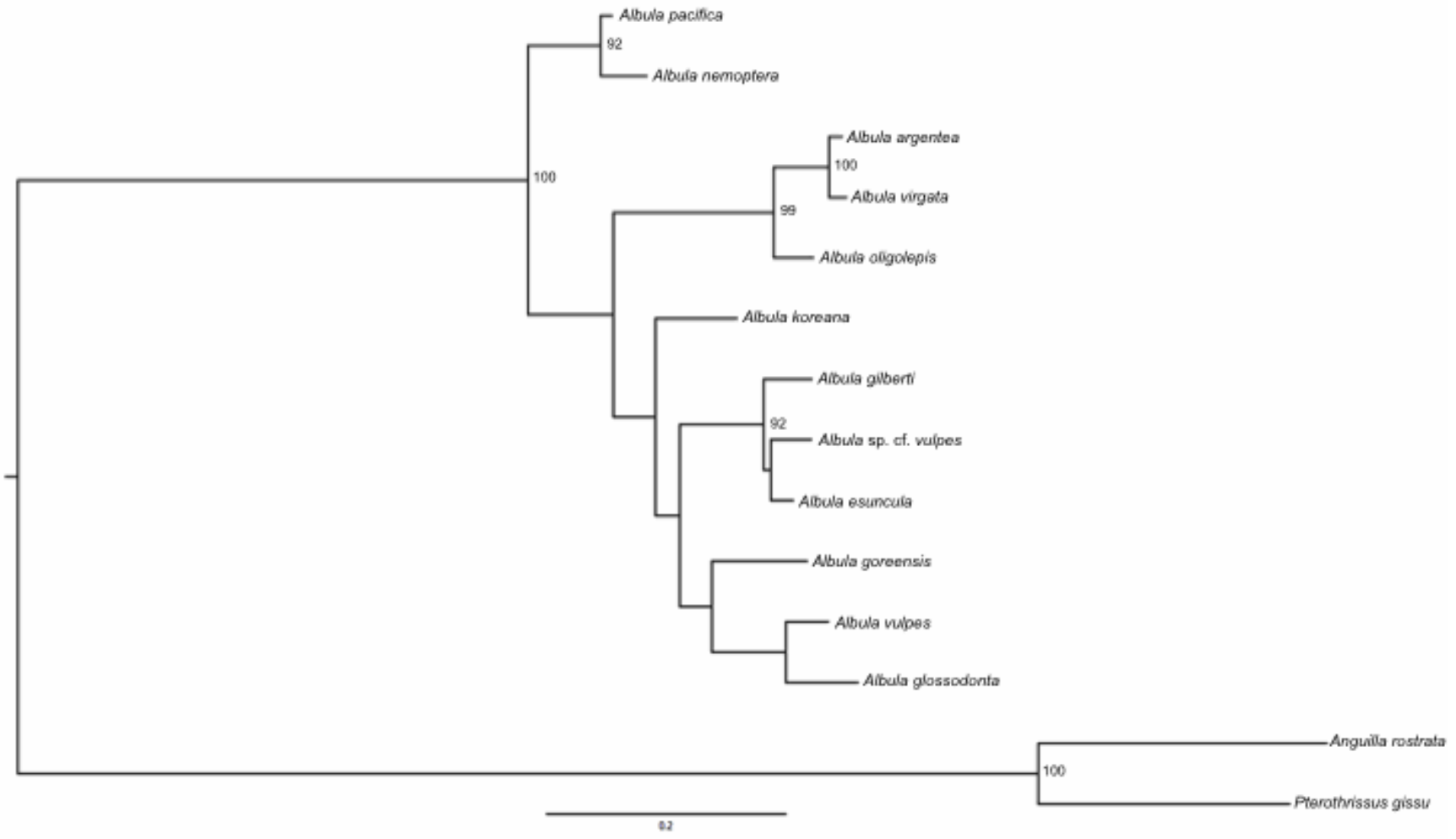


Figure 2. *Albula* species tree inferred in *BEAST based on a multilocus nuclear dataset. The nodal support values shown are Bayesian posterior probabilities (BPP), and branch lengths reflect substitutions per site.

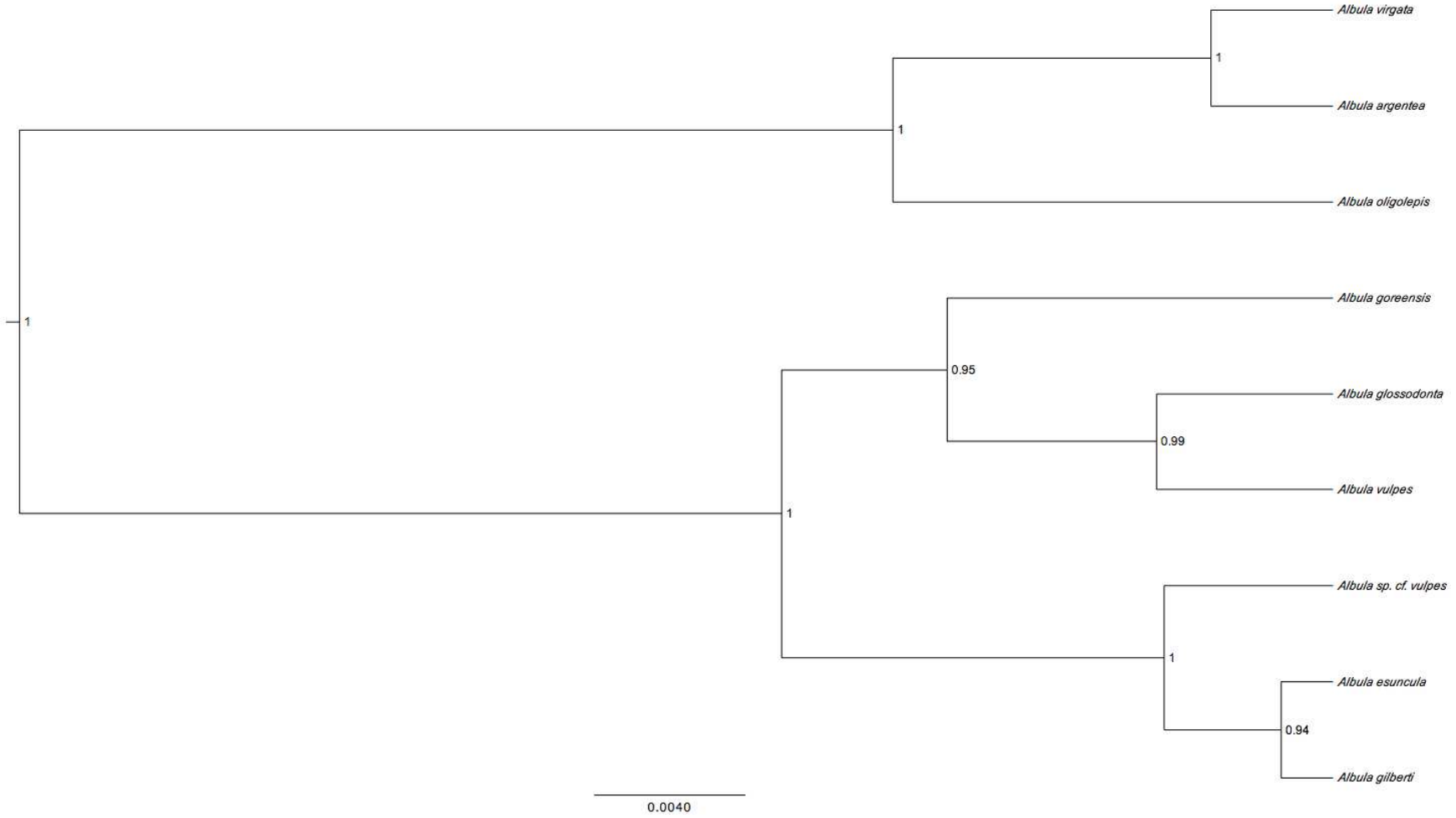


Figure 3. Multilocus time calibrated Elopomorpha phylogeny constructed in BEAST. Albulid species occurring in the Atlantic Ocean are denoted by ATL. Nodal support values in Bayesian posterior probabilities (BPP≥0.75) are indicated by circles, and fossil calibrations are displayed as numbered inverted triangles.

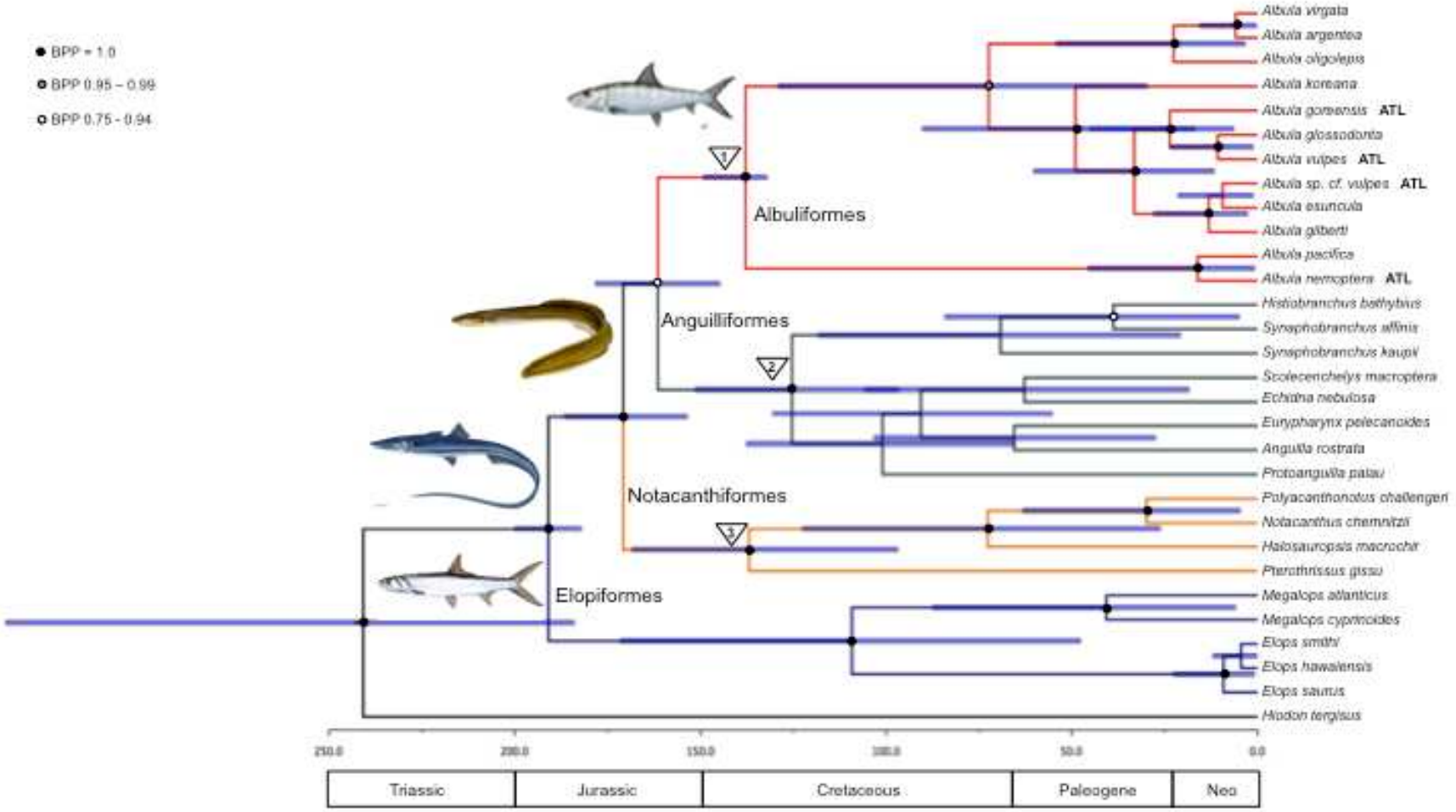
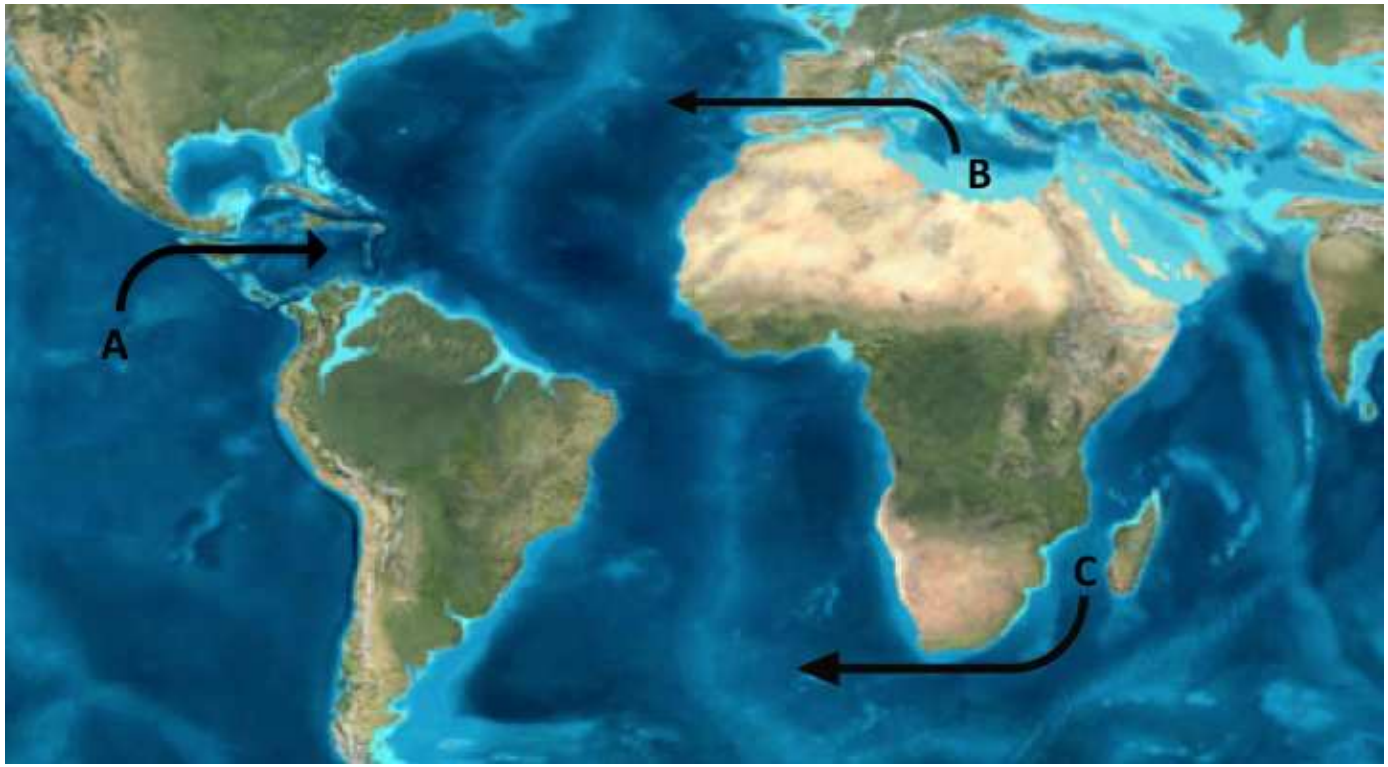


Figure 4. Hypothesized routes of bonefish dispersal into the Atlantic Ocean. A) Central American Seaway-likely route for MRCA of *A. pacifica* and *A. nemoptera* (split ~ 16 MYA) and MRCA of *A.* sp. cf. *vulpes*, *A. esuncula*, and *A. gilberti* (split ~ 9 MYA). B) Tethys Seaway- possible route for MRCA of *A. goreensis*, *A. vulpes*, and *A. glossodonta* (split ~ 24 MYA). C) southern Africa- likely dispersal route for MRCA of *A. vulpes* and *A. glossodonta* (split ~ 11 MYA), also alternate route for MRCA of *A. goreensis*, *A. vulpes*, and *A. glossodonta*. The map displays hypothesized ocean levels and land mass arrangements during the Oligocene, 38-24 MYA (map ©Ron Blakey 2011, used with permission).



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