Journal of FISH BIOLOGY
Published for the Fisheries Society of the British Isles

Journal of Fish Biology (2010) **76**, 1972–1983
doi:10.1111/j.1095-8649.2010.02639.x, available online at www.interscience.wiley.com

Identification of a novel member in the family Albulidae (bonefishes)

E. M. Wallace*† and M. D. Tringali‡

**University of Minnesota, 100 Ecology, 1987 Upper Buford Circle, St Paul, MN 55108, U.S.A. and ‡Florida Fish and Wildlife Conservation Commission, Fish and Wildlife Research Institute, 100 Eighth Avenue Southeast, St Petersburg, FL 33701, U.S.A.*

(Received 12 August 2009, Accepted 25 February 2010)

A novel Caribbean species *Albula* sp. *cf. vulpes* in the family Albulidae (bonefishes) was diagnosed through genetic and morphometric study. Phylogenies derived from 16S rRNA sequences revealed deeply separated lineages among Caribbean bonefishes. Mitochondrial DNA sequence divergences indicated a separation between 3·0 and 5·2 million years before present (B.P.). Cytochrome *b* phylogenies further supported the classification of *A*. sp. *cf. vulpes* as a novel albulid. Morphological variability revealed several differences between *A*. sp. *cf. vulpes* and other Caribbean species. A microsatellite library was developed to discern hybridization rates among the species. Microsatellite analyses revealed low levels of hybridization between some members in the complex. One instance of backcrossing was found between *A. vulpes* × *A*. sp. *B* and a pure *A*. sp. *B*, indicating that hybrids may have reduced fitness or may be reproductively isolated due to temporal–spatial spawning habitat differences.
© 2010 The Authors
Journal compilation © 2010 The Fisheries Society of the British Isles

Key words: Albulidae; bonefish; cryptic species; fish taxonomy; genetic species identification.

INTRODUCTION

The family Albulidae is composed of two genera, *Albula* and *Pterothrissus*, with an interesting dichotomy within the former genus. *Albula* is the focus of a popular and valuable sport fishery across much of its range, yet despite its popularity these fishes have been little studied until recently. Many aspects of the basic biology of *Albula* remain elusive, including spawning times and location, larval ecology and species diversity. The genus has a complex taxonomic history. In the century following the description of *Albula vulpes* (L.) in 1758, as many as 23 specific names were assigned to *Albula*. More recently, most of these names have been synonymized under *A. vulpes*, based on a lack of obvious distinguishing morphological characters warranting separation (Bowen *et al*., 2008). Exceptions include *Albula nemoptera* (Fowler), *Albula neoguinaica* (Valenciennes), and *Albula glossodonta* (Forsskål). The genus *Pterothrissus* is composed of two species, *Pterothrissus belloci* (Cadenat) and *Pterothrissus gissu* (Hilgendorf), which live in deep waters off the coast of Africa

†Author to whom correspondence should be addressed. Tel.: +1 612 6247225; fax: +1 612 6246777; email: walla296@umn.edu

© 2010 The Authors
Journal compilation © 2010 The Fisheries Society of the British Isles

and Japan, respectively. Little is also known of their biology, specimens are lacking and these species are not considered in the present study.

The shafted bonefish (*A. nemoptera*) has a limited range (known only from tropical Pacific and Atlantic coasts of North and Central America) and is easily distinguishable by an elongated ray at the base of the dorsal fin. *Albula neoguinaica* and *A. glossodonta* were identified through morphological (vertebral counts, lower jaw and otolith shape and tooth-patch patterns) as well as molecular analyses (Shaklee & Tamura, 1981). These species occur in the tropical Indo-Pacific from the Red Sea to Hawaii, Southern Japan, Australia and Micronesia.

Recent molecular studies have identified a multispecies complex within the Caribbean, where three species are known to occur, such as *A. vulpes*, *A. nemoptera* and *A*. sp. *B* (Colborn *et al*., 2001; Pfeiler *et al*., 2006; Adams *et al*., 2008; Bowen *et al*., 2008; Seyoum *et al*., 2008). The latter taxon is a cryptic species closely resembling *A. vulpes* and was first identified by Colborn *et al*. (2001) through analysis of mitochondrial (mt) DNA cytochrome *b* sequences. Another possible Caribbean species, *A*. sp. *E*, was also identified solely through genetic analysis (Colborn *et al*., 2001).

The present study provides genetic and morphological evidence for the existence of an additional cryptic species of bonefish in the genus *Albula* in the Caribbean and western Atlantic, referred to here as *Albula* sp. *cf. vulpes*. This study is ongoing, and a formal description of this species will be completed following the collection and analysis of adult voucher specimens.

MATERIALS AND METHODS

Bonefish specimens were collected from all four major biogeographic regions of the Caribbean (northeast, western, southern and eastern) as identified in Galindo *et al*. (2006). Juvenile and sub-adult specimens were collected by shoreline seining, and adult specimens were collected by angling from August 2003 to June 2007. Total length (T_L), measured from snout tip to the longest compressed caudal fin ray, was recorded for whole specimens retained as vouchers. Tissue from an *A. glossodonta* specimen from the Seychelles (Indian Ocean) was obtained and was included in genetic analyses as a sister taxon to the Caribbean bonefishes.

While the majority of collections used in the present study consisted of tissue samples obtained only for genetic analyses, nine whole specimens were recovered from Florida collections (Jacksonville and Anclote Key). Seven of these specimens (one *A. vulpes*, five *A*. sp. *B* and one *A*. sp. *cf. vulpes*) were used for the morphometric and meristic analyses. All nine specimens were catalogued in the Florida Fish and Wildlife Research Institute (FWRI) collection as voucher specimens; however, two adult *A. vulpes* specimens were excluded from the analyses due to their extreme size difference from all of the other sub-adult vouchers.

Purified genomic DNA was extracted from tissues with the Puregene isolation kit (Qiagen, Inc.; www.qiagen.com), following the methods in Adams *et al*. (2008). Sequencing polymerase chain reaction (PCR) assays were conducted with Hybaid thermocyclers under conditions described in Adams *et al*. (2008). Genetic species identification (GSI) assays, run on an Applied Biosystems 3130XL genetic analyser (www.appliedbiosciences.com), were conducted using the highly conserved 16S rRNA region. mtDNA cytochrome *b* sequences were also obtained from representative specimens to determine phylogenetic placement of Caribbean albulids. Sequence data for other bonefishes were obtained from GenBank.

The 16S rRNA sequences for all voucher specimens and additional representatives of each species from each biogeographic zone were edited and aligned in BioEdit, and then used to generate a consensus neighbour-joining (NJ) phylogram in MEGA 3 (Hall, 1999; Kumar *et al*., 2004). Per cent sequence divergences between species were also calculated with MEGA.

© 2010 The Authors

Representative 16S sequences for all three species have been submitted to GenBank (accession numbers EU693337, AY857934 and AY857935). Cytochrome *b* sequences were edited and aligned in Sequencher 4.7 (Gene Codes; www.genecodes.com). Maximum likelihood phylogenetic reconstructions were conducted in MrBayes 3 (Ronquist & Huelsenbeck, 2003).

An albulid-specific microsatellite library was developed through the slightly modified (Seyoum *et al*., 2005) PCR-based isolation of microsatellite arrays (PIMA), as described in Lunt *et al*. (1999). The primer sequences for five novel microsatellites are available from GenBank (accession numbers given in Table I). Multiplex microsatellite screening PCR assays were performed with Hybaid thermocyclers under standard conditions. Genotypes were obtained by an Applied Biosystems 3130XL genetic analyser using a customized ROX-labelled size standard (DeWoody *et al*., 2004) and scored in Genemapper.

Observed (H_O) and expected (H_E) heterozygosities were estimated with GENEPOP 4.0 (Rousset, 2008). Hardy–Weinberg expectations (HWE), linkage disequilibrium and standard diversity indices for microsatellite loci were estimated in GENEPOP. Three-dimensional cluster analysis of microsatellite data was conducted in GENETIX (Belkhir *et al*., 2000).

Radiographic images were obtained for the nine voucher specimens, which were previously identified through GSI screening and were deposited in the FWRI collection. Standard morphometrics and meristics were measured from radiographs or the specimens directly, following methods described by Rivas & Warlen (1967) with slight modifications. Standard length (L_S) was measured from the tip of the snout to the middle of the caudal base. All the dorsal and anal-fin rays were counted, including rudiments. Vertebral counts included the urostyle. It was necessary to estimate body depth due to abdominal incisions on many of the specimens. Dorsal height, length of last dorsal-fin ray and length of last anal-fin ray were also estimated, as fins were generally frayed at the tips.

Measurements of maxillary length in this study differed from those given by Rivas & Warlen (1967). There are three possible explanations for this. First, measurement methods in the present study may have differed. Second, the specimens examined by Rivas & Warlen (1967) may have included *A. vulpes* cryptic species. Third, the morphometric data were collected from sub-adult specimens in the present study, while Rivas & Warlen (1967) analysed adult specimens. The discrepancies are likely to be due to methodology or specimen age as the maxillary lengths reported by Rivas & Warlen (1967) are longer for all albulids than those in the present study.

RESULTS

A total of 52 *A*. sp. *cf. vulpes* specimens were collected from all four Caribbean biogeographic zones (Fig. 1). The 16S rRNA sequences revealed well-defined, deep separations among the Caribbean albulids. A 3·0% sequence divergence separated *A. vulpes* and *A*. sp. *B*., a 4·9% divergence separated *A. vulpes* and *A*. sp. *cf. vulpes*, and a 5·2% divergence separated *A*. sp. *B* and *A*. sp. *cf. vulpes*. *Albula vulpes* and *A*. sp. *cf. vulpes* each had one unique 16S haplotype that was found across the study area (Fig. 2). Two haplotypes were identified in *A. species B*. Haplotype I was present in the northeast (Florida and the Bahamas) and western zones (Mexico and Belize), whereas haplotype II was present in all biogeographic zones.

Cytochrome *b* sequences further clarified the high levels of divergence between Caribbean bonefishes and provide clear evidence of the identity of *A*. sp. *cf. vulpes* as a novel albulid. The major nodes in the NJ tree had strong bootstrap support ($\geq$70%). This tree topology indicated that *A*. sp. *cf. vulpes* is a sister taxon to *A. esuncula* and *A*. sp. *A* from the eastern Pacific, whereas *A. vulpes* and *A*. sp. *B* are sister taxa to *A. glossodonta* from the western Pacific (Fig. 3). The maximum likelihood reconstructions also placed *A. nemoptera* as a sister taxon to the rest of the genus *Albula*.

© 2010 The Authors
Journal compilation © 2010 The Fisheries Society of the British Isles,

TABLE I. Characterization of five newly developed microsatellite loci for bonefishes *Albula* spp.

Locus	Primer sequence (5′–3′)	Repeat motif	K[a]	Allele size range	H_O[b]	H_E[c]	GenBank accession no.
*AspB*03	F: CAGCATGTCGTTCTGAGTCTTC-FAM R: ACTTCGCAAGCAGTCTAACCTT	$(AGAGAGG)_2$ (A) $(AG)_{11}$ (AAA) $(AGG)_3$	2	242–262	40	20·7	EU693332
*AspB*05	F: GCACCACCCTATGCCGTA-NED R: CCCACACACACGCACAGT	$(TG)_9 \ldots (GT)_{10}$ (AT) $(GT)_2 \ldots$ $(TG)_5$ (GG) $(TG)_5$	6	149–163	26	28·2	EU693333
*AspB*12	F: CAATATTCGTACAGGCTGCATTAG-HEX R: AGGAAGTAAGTTCAACTGGAATGG	$(AC)_3$ $(AG)_2$ $(AC)_2$ $(AT)_2 \ldots (AC)_4$	3	118–148	3	3·4	EU693334
*AspB*15	F: GTGATCACATAGACCAACAGGAAG-FAM R: CAGACTTTTCACCTTTCAGAGACA	$(CT)_3 \ldots (GT)_2$ GCC $(GA)_3$ GC $(GA)_3$	1	195	—	—	EU693335
*AspB*18	F: TGAGAGCGTGATGGAGATTG-NED R: GAATTCGATTACGGGAGCAA	$(GA)_2 \ldots (GA)_2 \ldots (GA)_2 \ldots (AG)_5$	1	112	—	—	EU693336

[a]Number of alleles.
[b]Observed heterozygosity.
[c]Expected heterozygosity.

© 2010 The Authors
Journal compilation © 2010 The Fisheries Society of the British Isles,

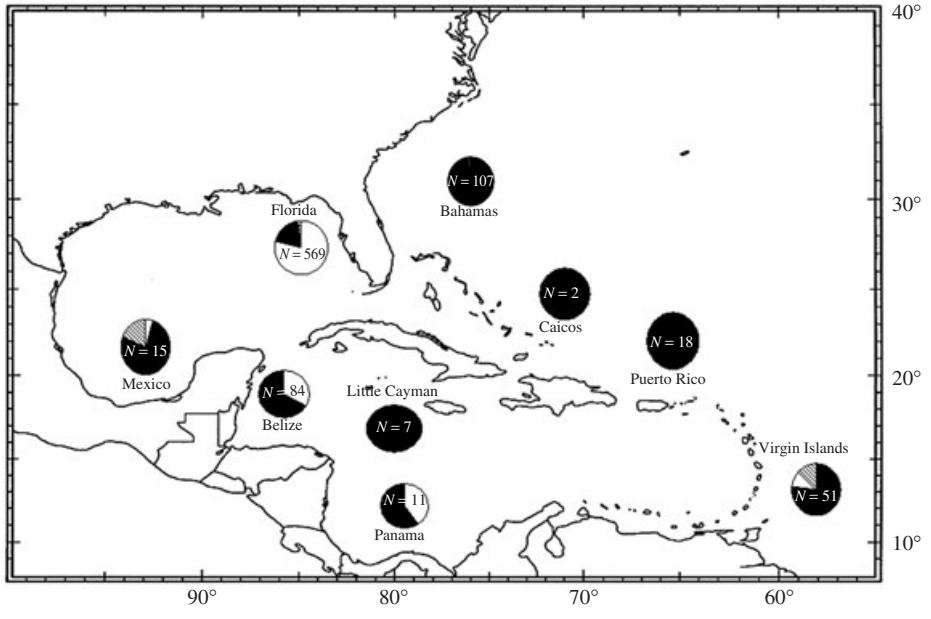

FIG. 1. Bonefish species composition in Caribbean collections. The number of individuals collected from each location is indicated inside the species composition chart. The composition of adult, juvenile and larval study specimens was as follows: Florida 338:31:200, Bahamas 84:15:8, Caicos 2:0:0, Puerto Rico 18:0:0, Virgin Islands 0:0:51, Panama 0:0:11, Little Cayman 7:0:0, Belize 38:46:0 and Mexico 0:0:15. ●, *Albula vulpes*; ○, *A*. sp. *B*; ▨, *A*. sp. *cf. vulpes*.

The results of nuclear sequences of two microsatellite loci (*AspB*15 and *Avu*27) supported those of the mtDNA sequences, indicating distinct, deep separations between these species (Figs 4 and 5). Numerous diagnostic sites were identified within the flanking regions of both microsatellite loci.

A total of 16 polymorphic loci were identified, which amplified appropriately sized fragments for all three species. These data further support the identification of *A*. sp. *cf. vulpes* as a novel albulid. Three alleles per locus appeared on average, mean H_O was 0·320 and mean H_E was 0·299. After applying sequential Bonferroni corrections (Rice, 1989), no significant departures from HWE, nor non-random associations between locus pairs, were identified (table-wide $\alpha = 0{\cdot}05$). Characterization of five

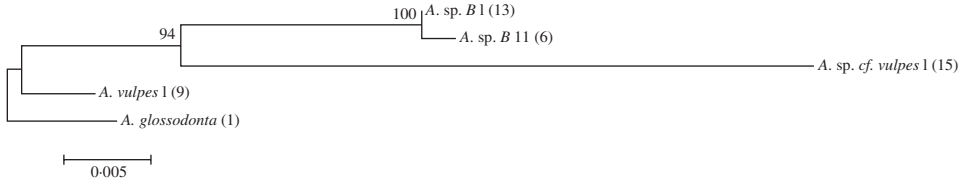

FIG. 2. Consensus neighbour-joining phylogram (500 bootstrap replicates) for Caribbean bonefish mitochondrial DNA 16S rRNA sequences. The number of specimens for each haplotype is listed in parentheses. The Pacific species, *Albula glossodonta*, is included as a sister taxon.

© 2010 The Authors
Journal compilation © 2010 The Fisheries Society of the British Isles,

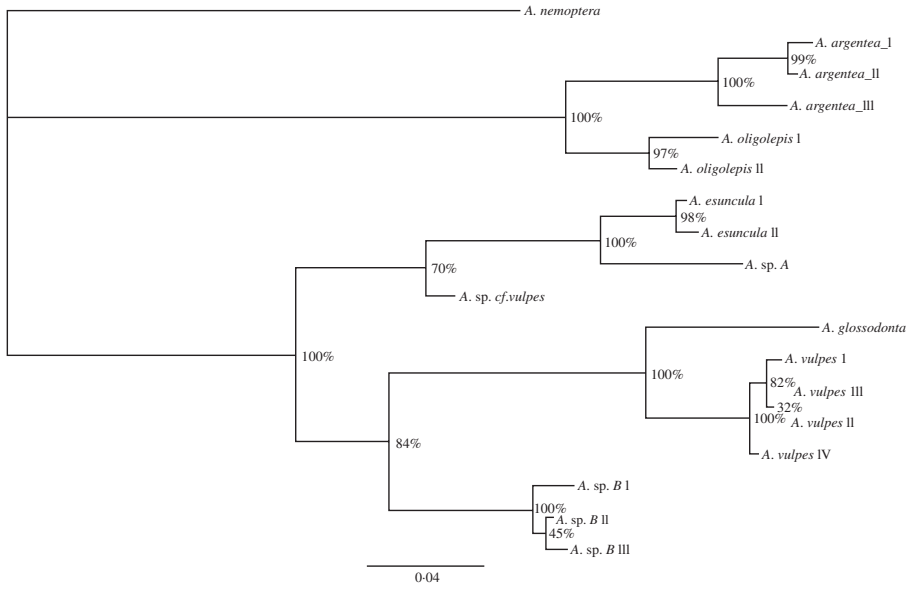

FIG. 3. Consensus maximum likelihood phylogram (500 bootstrap replicates) for global albulid cytochrome *b* mtDNA sequence data.

novel microsatellites is described in Table I. The remaining loci were characterized in Seyoum *et al*. (2008). Microsatellite analyses revealed well-separated, tight clusters for each species, with evidence of low levels of hybridization (Fig. 6). One apparent F1 *A. vulpes* × *A*. sp. *B* hybrid was identified, as well as one backcross specimen (to a pure *A*. sp. *B*), and these specimens were collected in Florida (Biscayne National Park and Key West, respectively). No evidence was found for a backcross *A*. sp. *B* × *A. vulpes* hybrid to a pure *A. vulpes*. Five likely *A*. sp. *B* × *A*. sp. *cf. vulpes* hybrids were also discovered. These F1 hybrids were collected at St Lucie, Bahia Honda, and Key West, FL and Veracruz, Mexico. No evidence of backcrossing between the two species, however, was found. Also, no evidence of hybridization was found between *A. vulpes* and *A*. sp. *cf. vulpes*.

Nine voucher specimens have been added to the FWRI collection. High resolution radiographic images were used to count vertebrae: representative images for members of the *A. vulpes* complex appear in Fig. 7. Standard morphometric and meristic data for each species are listed in Table II. Ranges are reported for *A*. sp. *B* ($n = 5$); only an individual *A. vulpes* and *A*. sp. *cf. vulpes* of similar size was available for measurement. Dorsal height was greatest in *A. vulpes*, but overlapped between *A*. sp. *B* and *A*. sp. *cf. vulpes*. All of the other standard metrics overlapped, or nearly overlapped, between *A. vulpes* and *A*. sp. *B*., making solely morphologic diagnosis impossible. Possible diagnostic differences were found in pelvic fin ray, lateral line scale, pre-dorsal scale, scales above lateral line, scales around caudal peduncle and vertebral counts for *A*. sp. *cf. vulpes* (Table II). Additional whole specimens, however, are needed to verify count ranges within the species. Whole specimens of *A. nemoptera* were unavailable for direct measurement; however, data from Rivas & Warlen (1967) are reported for comparison in Table II.

© 2010 The Authors
Journal compilation © 2010 The Fisheries Society of the British Isles,

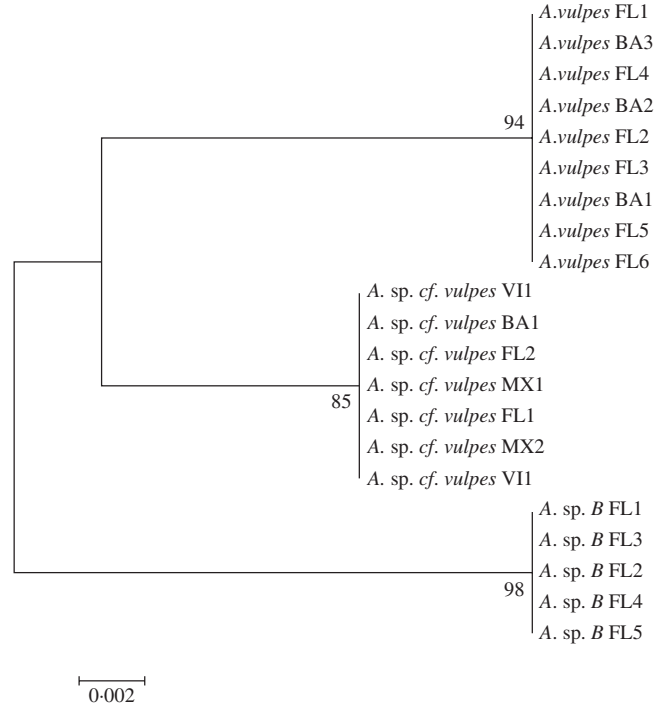

FIG. 4. Consensus neighbour-joining phylogram (500 bootstrap replicates) for Caribbean albulid *AspB*15 microsatellite sequences. Locations are as follows: FL, Florida; BA, Bahamas; VI, Virgin Islands; MX, Mexico. Individuals are identified by the number following the location code.

Proportions of adult, juvenile and larval specimens varied among collection locations and analyses. Representative specimens sequenced for 16S rRNA consisted of 18 adults, seven juveniles and 19 larvae. Representatives in the cytochrome *b* analyses contained five adults, three larvae and 10 individuals of undetermined maturity (probably adult specimens) from GenBank. Nuclear microsatellite sequence data were obtained from eight adult, six juvenile and seven larval specimens (*AspB*15) and six larvae (*Avu*27). The overall composition was 487 adult, 92 juvenile and 285 larval specimens in the present study.

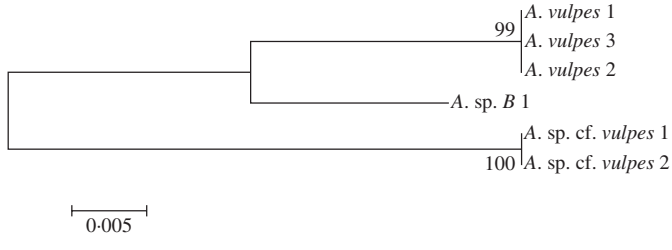

FIG. 5. Consensus neighbour-joining phylogram (500 bootstrap replicates) for Caribbean albulid *Avu*27 microsatellite sequences. Individuals are identified by number following the species name.

© 2010 The Authors
Journal compilation © 2010 The Fisheries Society of the British Isles,

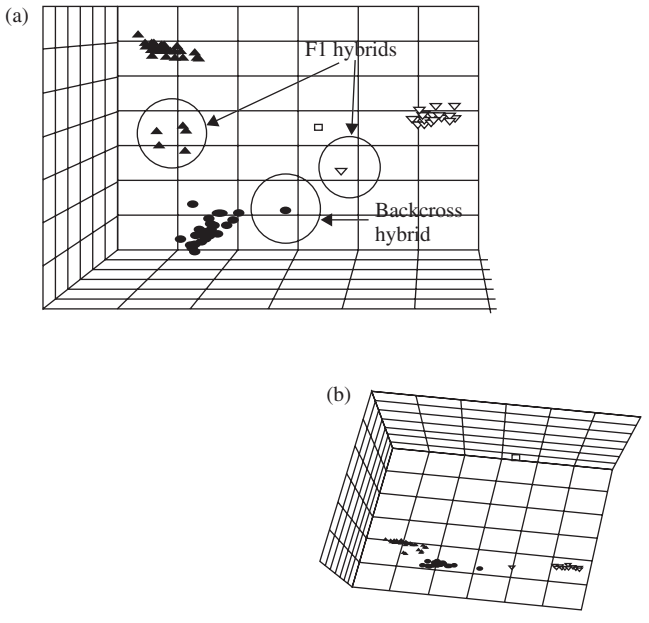

FIG. 6. Three-dimensional (a) elevation, (b) plan, cluster analysis results for bonefish microsatellite loci. *Albula glossodonta* is included as a sister taxon. □, *A. glossodonta*; ●, *A.* sp. *B*; ▲, *Albula* sp. *cf. vulpes*; ▽, *A. vulpes.*

DISCUSSION

The determination of *A.* sp. *cf. vulpes* as a novel, previously unreported albulid species was established through genetic analyses and supported by the small amount of morphological data available. Alternative identifications as *A. nemoptera* or *A.* sp. *E* were excluded by the analyses.

Genetic screening and comparison with existing data for all other possible bonefishes revealed multiple diagnostic sites distinguishing this taxon as a distinct species considerably different from other Caribbean bonefishes. The 16S rRNA sequence divergences were largest for *A.* sp. *cf. vulpes* (4·9 and 5·2%), and the sequence data for two nuclear sites supported the distinct, well-separated lineages. Cytochrome *b* sequences further yielded strong support for deep divergences among Caribbean albulids. Phylogenetic reconstructions identified *A.* sp. *cf. vulpes* as a sister taxon to eastern Pacific bonefishes, whereas *A. vulpes* and *A.* sp. *B* were identified as sister taxa to western Pacific *A. glossodonta*. These data also indicate that *A. nemoptera* is distantly related to all other known bonefishes. The phylogeny also provides strong evidence for speciation due to multiple Panamanian Seaway crossings.

Microsatellite analyses revealed a few hybridizations between Caribbean albulids that were geographically restricted to Florida and Mexico. Notably, hybridization is not occurring between all of the species in the complex. Evidence of low levels of hybridization was found between *A.* sp. *B.* and both *A. vulpes* and *A.* sp. *cf. vulpes*. *Albula vulpes* and *A.* sp. *cf. vulpes*, however, apparently do not hybridize with each other. This may be due to temporal or spatial separation of *A. vulpes* and *A.* sp. *cf. vulpes* spawning groups, or may indicate genetic incompatibility or low hybrid

© 2010 The Authors

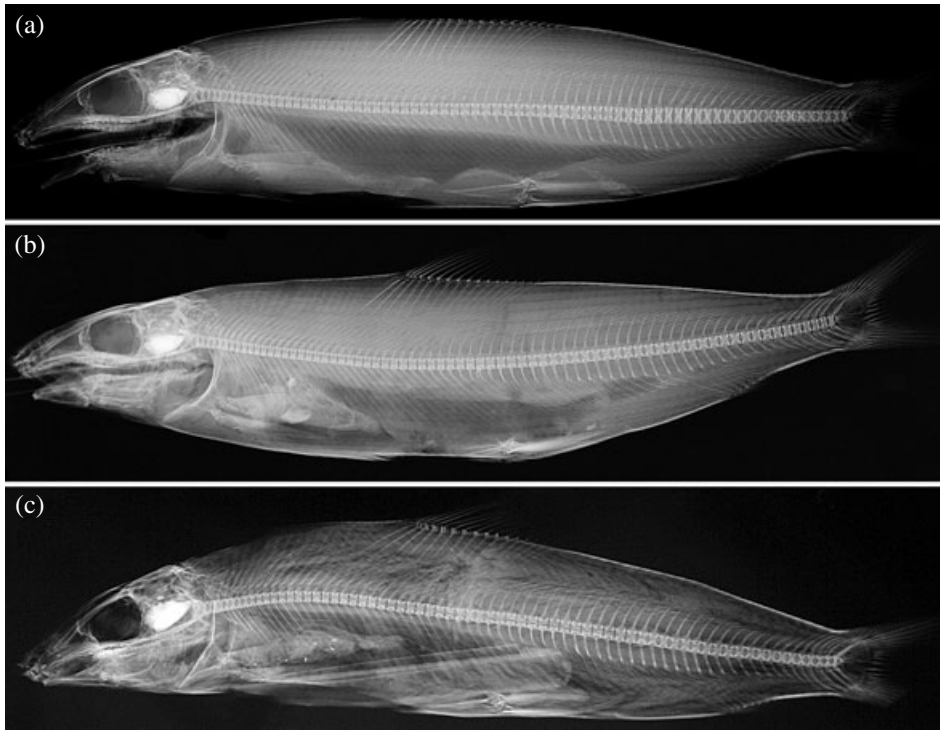

FIG. 7. Radiographic images of representative specimens for three species of Caribbean albulid: (a) *Albula vulpes*, $T_L = 242$ mm; (b) *A*. sp. *B*, $T_L = 260$ mm and (c) *A*. sp. *cf. vulpes*, $T_L = 167$ mm.

fitness. The F1 *A. vulpes* × *A*. sp. *B* hybrid and the backcross specimen were larvae collected in Florida. The five F1 *A*. sp. *B* × *A*. sp. *cf. vulpes* hybrids consisted of larval and adult specimens from Florida (2:1) and sub-adult–adult specimens from Mexico (two). Further adult collections will elucidate hybrid viability and fitness across the region. The limited evidence of hybridization may be due to spawning habitat specificity within each species or to temporal variations among species. Bonefish spawning aggregation sites have yet to be identified in the Caribbean for any species.

The low levels of hybridization found in the present study are supported by ongoing research, and additional specimens ($n = 250$) from studies in the Bahamas and Florida have yielded no further hybrid albulids (V. Haley, pers. comm.; E. Wallace, unpubl. data). Hybridization rates among albulids are similar to those in centrarchids, which generally range from 1·3 to 5% for some members (Bolnick, 2009). In contrast, hybridization rates were 15% between two *Cynoscion* species (Sciaenidae) (Tringali *et al*., 2004).

Recent re-examination of *A*. sp. *E* has led to a consensus that it is the Atlantic form of *A. nemoptera* (Pfeiler *et al*., 2006; Bowen *et al*., 2008). The voucher specimen of *A*. sp. *cf. vulpes* lacked the diagnostic threadfin of *A. nemoptera*, which is evident at the juvenile life stage. Additionally, the lateral line scale count for *A*. sp. *cf. vulpes* (*c*. 70) is well under the reported range of 78–84 for *A. nemoptera*.

© 2010 The Authors

TABLE II. Comparison of morphometric and meristic data for *Albula vulpes*, *A.* sp. *B*, unknown and *A. nemoptera*

	A. vulpes	*A.* sp. *B*	*A.* sp. *cf. vulpes*	Rivas *A. vulpes*	Rivas *A. nemoptera*
Dorsal-fin rays	18	18–19	19	18–19	20–21
Anal-fin rays	9	8–10	8	—	—
Pectoral-fin rays	20	16–19	19	17–19	16–18
Pelvic-fin rays	10	10–10	11	9–11	9–9
Lateral-line scales	76	72–78	70*	68–77	78–84
Pre-dorsal scales	20	17–21	14*	14–24	17–22
Scales above lateral line	9	9–10	8	8–10	9–10
Scales below lateral line	7	5–6	5	6–7	7–8
Scales around caudal peduncle	15	15–16	14	16–16	16–17
Branchiostegal rays	13	11–12	11	10–14	13–15
Upper gill rakers	8	6–9	7	7–11	5–10
Lower gill rakers	12	11–12	12	11–13	9–11
Total gill rakers	20	18–20	19	18–23	15–20
Vertebrae	71	71–72	69	—	77–79
Standard length (mm)	192·0	148·3–212·3	129·0	204–387	234–341
Head length (mm)	26·8	26·2–27·6	27·7	26·7–29·6	28·9–31·2
Body depth (mm)	22·1	20·1–21·6	21·7	—	—
Least depth of caudal peduncle (mm)	7·7	6·8–7·5	7·7	7·0–7·8	6·0–6·8
Anal base length (mm)	5·5	5·1–6·0	5·3	—	—
Dorsal base length (mm)	16·0	13·8–17·2	16·7	13·8–17·6	17·3–19·0
Diagonal from dorsal insertion to anal origin (mm)	27·4	24·4–27·7	27·8	—	—
Eye diameter (mm)	5·7	5·3–5·6	5·2	—	—
Bony interorbital width (mm)	6·5	6·0–7·0	6·4	—	—
Snout length (mm)	10·2	9·9–11·5	11·4	—	—
Tip of snout to rear of maxillary (mm)	9·1	8·6–9·1	9·7	—	—
Maxillary length (mm)	7·7	6·5–7·3	7·8	9·1–10·3	13·3–14·2
Mandible length (mm)	9·1	8·1–9·0	9·5	8·3–9·7	11·8–12·8
Preoral length (mm)	3·5	2·8–3·4	3·6	2·6–3·5	4·3–4·9
Dorsal height (mm)	18·6	13·5–17·0	17·0	18·2–19·7	16·1–17·7
Length of last dorsal ray (mm)	6·0	5·4–6·8	7·0	5·4–5·6	15·2–19·3
Length of last anal ray (mm)	5·6	5·0–6·4	6·4	5·4–6·5	8·0–9·9

*Approximate counts due to missing scales.

In the present study, limited numbers of sub-adult whole specimens were available for morphometric and meristic analyses. Some individuals had small damaged areas where some scales were missing, necessitating an approximate count for these individuals. Some morphological characters, such as length, probably vary between juvenile and adult life stages. Many characters, however, are not expected to vary across life stages (such as fin-ray, gill-raker and vertebral counts). Verification of the results presented here will be conducted on additional specimens as they are collected. Morphometric analyses of albulids are complicated by the presence of cryptic species with high levels of character overlap.

© 2010 The Authors

The identification of *Albula* sp. *cf. vulpes* increases the total number of albulid species in the Caribbean complex to at least four, two of which have been formally described (*A. vulpes* and *A. nemoptera*). This discovery illustrates the complexity of conducting research on this fishery due to the presence of cryptic species. Genetic species identification will continue to be a necessary component of research on members of Albulidae, unless or until diagnostic morphological characters can be identified and verified. Further collaborative studies across the region will increase the understanding of this species complex and will provide answers to the many remaining questions about life history. This work may also provide additional insight into gene-flow patterns among populations of other presumably panmictic species with large dispersal potentials through protracted larval stages. The complete descriptions of the unnamed species in the complex are underway.

We thank A. Adams and the Mote field staff, M. Lara, L. Vasquez, M. Valdez, B. Victor, B. Pelosi, FWRI's Jacksonville FIM crew and M. Larkin for providing study specimens. The morphometric and meristic data could not have been obtained without the assistance of R. Ruiz-Carus and E. Matheson of FWRI. We also thank A. Simons as well as an anonymous reviewer for providing constructive comments which improved the quality of this manuscript. This work was supported financially by the State of Florida and the Federal Sportfish Restoration Act, Department of the Interior, U.S. Fish and Wildlife Service, grant F-69.

References

Adams, A. J., Wolfe, R. K., Tringali, M. D., Wallace, E. M. & Kellison, G. T. (2008). Rethinking the status of *Albula* spp. biology in the Caribbean and western Atlantic. In *Biology and Management of the World Tarpon and Bonefish Fisheries* (Ault, J. S., ed.), pp. 203–214. Boca Raton, FL: CRC Press.

Belkhir, K., Borsa, P., Chikhi, L., Raufaste, N. & Bonhomme, F. (2000). GENETIX, logiciel sous WindowsTM pour la génétique des populations. *Laboratoire Génome, Populations, Interactions CNRS UMR 5000*. Montpellier: Université de Montpellier II.

Bolnick, D. I. (2009). Hybridization and speciation in centrarchids. In *Centrarchid Fishes: Diversity, Biology, and Conservation* (Cooke, S. & Philipp, D. J., eds), pp. 39–41. Oxford: Blackwell.

Bowen, B. W., Karl, S. A. & Pfeiler, E. (2008). Resolving evolutionary lineages and taxonomy of bonefishes (*Albula* spp.). In *Biology and Management of the World Tarpon and Bonefish Fisheries* (Ault, J. S., ed.), pp. 147–154. Boca Raton, FL: CRC Press.

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

DeWoody, J. A., Schupp, J., Kenefic, L., Busch, J., Murfitt, L. & Keim, P. (2004). Universal method for producing ROX-labelled size standards suitable for automated genotyping. *Biotechniques* **37**, 348–352.

Galindo, H. M., Olson, D. B. & Palumbi, S. R. (2006). Seascape genetics: a coupled oceanographic–genetic model predicts population structure of Caribbean corals. *Current Biology* **16,** 1622–1626.

Hall, T. A. (1999). BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* **41,** 95–98.

Kumar, S., Tamura, K. & Nei, M. (2004). MEGA3: integrated software for molecular evolutionary genetics analysis and sequence alignment. *Briefings in Bioinformatics* **5,** 150–163.

Lunt, D., Hutchinson, W. & Caravalho, G. (1999). An efficient method for PCR-based isolation of microsatellite arrays (PIMA). *Molecular Ecology* **8,** 891–894.

Pfeiler, E., Bitler, B. G. & Ulloa, R. (2006). Phylogenetic relationships of the shafted bonefish *Albula nemoptera* (Albuliformes: Albulidae) from the eastern Pacific based on cytochrome b sequence analyses. *Copeia* **2006,** 778–784.

© 2010 The Authors

Rice, W. R. (1989). Analyzing tables of statistical tests. *Evolution* **43,** 223–225.
Rivas, L. R. & Warlen, S. M. (1967). Systematics and biology of the bonefish, *Albula nemoptera* (Fowler). *Fishery Bulletin* **66,** 251–258.
Ronquist, F. & Huelsenbeck, J. P. (2003). MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* **19,** 1572–1574.
Rousset, F. (2008). GENEPOP '007: a complete re-implementation of the GENEPOP software for Windows and Linux. *Molecular Ecology Resources*, **8,** 103–106.
Seyoum, S., Tringali, M. D. & Sullivan, J. G. (2005). Isolation and characterization of 27 polymorphic microsatellite loci for the common snook, *Centropomus undecimalis*. *Molecular Ecology Notes* **5,** 924–927.
Seyoum, S., Wallace, E. M. & Tringali, M. D. (2008). Twelve polymorphic microsatellite markers for the bonefish, *Albula vulpes* and two congeners. *Molecular Ecology Resources* **8,** 354–356.
Shaklee, J. B. & Tamura, C. S. (1981). Biochemical and morphologic evolution of Hawaiian bonefishes (*Albula*). *Systematic Zoology* **30,** 125–146.
Tringali, M. D., Seyoum, S., Wallace, E. & Higham, M. (2004). The distribution of weakfish (*Cynoscion regalis*), sand seatrout (*C. arenarius*), and their hybrids in Florida Atlantic waters. A special in house report to the Florida Fish and Wildlife Conservation Commission, St. Petersburg. *Report number* **IHR2004-018**. St Petersburg, FL: Florida Marine Research Institute.

© 2010 The Authors