



Phylogenetic relationships of tropical western Atlantic snappers in subfamily Lutjaninae (Lutjanidae: Perciformes) inferred from mitochondrial DNA sequences

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Phylogenetic relationships among 20 nominal species of tropical lutjanine snappers (Lutjanidae) (12 from the western Atlantic, one from the eastern Pacific, and seven from the Indo-Pacific) were inferred based on 2206 bp (712 variable, 614 parsimony informative) from three protein-coding mitochondrial genes. Also included in the analysis were DNA sequences from two individuals, identified initially as *Lutjanus apodus*, which were sampled off the coast of Bahia State in Brazil (western Atlantic), and from three individuals labelled as ‘red snapper’ in the fish market in Puerto Armuelles, Panama (eastern Pacific). Bayesian posterior probabilities and maximum-likelihood bootstrap percentages strongly supported monophyly of all lutjanines sampled and the hypothesis that western Atlantic lutjanines are derived from an Indo-Pacific lutjanine lineage. The phylogenetic hypothesis also indicated that oceans where lutjanines are distributed (western Atlantic, eastern Pacific, and Indo-Pacific) are not reciprocally monophyletic for the species distributed within them. There were three strongly supported clades that included all western Atlantic lutjanines: one included six species of *Lutjanus* from the western Atlantic, two species of *Lutjanus* from the eastern Pacific, and the monotypic genera *Rhomboplites* and *Ocyurus* (western Atlantic); one that included three, probably four, species of *Lutjanus* in the western Atlantic; and one that included *Lutjanus cyanopterus* (western Atlantic), an unknown species of *Lutjanus* from the eastern Pacific, and three species of *Lutjanus* from the Indo-Pacific. Molecular-clock calibrations supported an early Miocene diversification of an Indo-Pacific lutjanine lineage that dispersed into the western Atlantic via the Panamanian Gateway. Divergent evolution among these lutjanines appears to have occurred both by vicariant and ecological speciation: the former following significant geographic or geological events, including both shoaling and closure of the Panamanian Gateway and tectonic upheavals, whereas the latter occurred via phenotypic diversification inferred to indicate adaptation to life in different habitats. Taxonomic revision of western Atlantic lutjanines appears warranted in that monotypic *Ocyurus* and *Rhomboplites* should be subsumed within the genus *Lutjanus*. Finally, it appears that retail mislabelling of ‘red snapper’ in commercial markets extends beyond the USA. © 2011 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2011, 102, 915–929.

ADDITIONAL KEYWORDS: biogeography – ecological speciation – phylogeography – vicariance.

INTRODUCTION

Snappers of the perciform family Lutjanidae are perch-like marine fishes found worldwide in tropical

and subtropical waters (Allen, 1985). Most lutjanids are carnivorous and occur in reef- or other structure-associated habitats where they feed primarily on fishes, crustaceans, molluscs, and pelagic urochordates (Anderson, 2003). In addition, most lutjanines are long-lived, slow-growing, and extremely important to artisanal fisheries, providing a significant food

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resource for developing countries (Russ & Alcalá, 1989; Blaber *et al.*, 2005). Larger species in the family, primarily in the genus *Lutjanus*, also are highly sought by recreational fishers, particularly in the Caribbean region, including the Gulf of Mexico (Allen, 1985). The family itself consists of more than 120 species, the majority of which are found in the Indo-Pacific region and in the subfamily Lutjaninae (Allen, 1985). Eighteen lutjanid species (in six genera, three subfamilies) occur in tropical and subtropical waters of the western Atlantic Ocean. Most of these (three genera, 13 species) are placed in the subfamily Lutjaninae, which includes two monotypic genera (*Ocyurus* and *Rhomboplites*) and 11 species in the genus *Lutjanus*. The other subfamilies occurring in the region are Etelinae (two genera, four species) and Apsilinae (one genus, one species).

Phylogenetic relationships among western Atlantic lutjanines are not well established, in part because of morphological and behavioral similarities within the group (Sarver, Freshwater & Walsh, 1996), and in part because of occurrence of both interspecific and intergeneric hybrids (Domeier & Clarke, 1992). Rivas (1966) and Vergara (1980), based on morphological characters, proposed three phenetic groups within the assemblage: the *Lutjanus griseus* group (*L. griseus*, *Lutjanis apodus*, *Lutjanis jocu*, and *Lutjanis cyanopterus*); the *Lutjanis synagris* group (*L. synagris* and *Lutjanis mahagoni*); and the *Lutjanus analis* group (*L. analis*, *Lutjanis campechanus*, *Lutjanis purpureus*, and *Lutjanis vivanus*). They differed on placement of *Lutjanis buccanella*, with Rivas (1966) placing it in the *L. analis* group, whereas Vergara (1980) placed it in the *L. synagris* group. Chow & Walsh (1992) used allozymes and skull morphometry to assess similarities among seven of these species. Using the unweighted pair group method with arithmetic mean, clustering of the allozyme data supported phenetic similarity between *L. griseus* and *L. apodus* and between *L. analis* and *L. vivanus*, with *L. synagris* being more similar to the *L. analis*/*L. vivanus* pair; monotypic *Ocyurus* (*chrysurus*) was more similar to the species of *Lutjanus* than was monotypic *Rhomboplites* (*aurorubens*). A comparison of skull morphometry, however, indicated that *L. synagris* was more similar to the *L. griseus*/*L. apodus* pair. Sarver *et al.* (1996) employed 640 (combined) bp of the mitochondrially encoded 12S rRNA and cytochrome *b* (*cyt b*) genes to examine relationships among 12 of the lutjanine species and two species (*Pristipomoides aquilonaris* and *Etelis oculatus*) placed in the lutjanid subfamily Etelinae (Johnson, 1980). Strict consensus of three, equally parsimonious trees from maximum-parsimony analysis of unweighed sequences revealed strong support (100% bootstrap, 250 replicates) for an *L. griseus*

clade (*L. griseus* (*L. apodus*, *L. jocu*)) and weak to moderate support (76% bootstrap) for a clade containing *L. campechanus* and *L. vivanus*. Equally weak to moderate support (70% bootstrap) was obtained for a group that contained 13 of the 14 lutjanids surveyed; *Lutjanus cyanopterus* fell outside this group. Different relationships were suggested by a search that used weights derived from average consistency indices for characters. Rodríguez-Santiago (2008) used 405 characters (base pairs and gaps) of the 12S rRNA gene to generate both Neighbour-joining (NJ) and Bayesian topologies for 15 lutjanids found in the Caribbean Sea, including representatives from all three subfamilies. Strong support (93% bootstrap, 5000 replicates, in the NJ topology; 97% posterior probability in the Bayesian topology) was recovered only for the *L. griseus* group (*L. griseus*, *L. jocu*, *L. apodus*). Weak to moderate support (77% bootstrap, NJ topology; 69% posterior probability, Bayesian topology) was recovered for a group that included the nine species of *Lutjanus* (including *L. cyanopterus*) examined, *Rhomboplites*, and *Ocyurus*. Other less well supported relationships were suggested.

In the present study, we present phylogenetic analyses of 20 nominal lutjanine species (12 from the western Atlantic, one from the eastern Pacific, and seven from the Indo-Pacific) based on DNA sequence data from the mitochondrial protein-coding genes NADH dehydrogenase 4 (ND-4), cytochrome *c* oxidase I (COI), and *cyt b*. Three other lutjanids from the western Atlantic, *Etelis oculatus*, *Pristipomoides aquilonaris* (Etelinae), and *Apsilus dentatus* (Apsilinae), were included in the analysis as outgroups to Lutjaninae, based on the phylogenetic hypothesis (Etelinae (Apsilinae, Lutjaninae) of Johnson (1980). Sequences from species in the Indo-Pacific were obtained from GenBank. Also included in the analysis were DNA sequences from two individuals, identified as *L. apodus*, which were sampled in 1998 off the coast of Bahia State in Brazil, and from three individuals labelled as 'red snapper' in the fish market in Puerto Armuelles, Panama. Puerto Armuelles is a city on Panama's Pacific Coast in the western province of Chiriqui. One described lutjanine from the western Atlantic not sampled in the present study was *L. purpureus*, a species considered to be a close relative of the red snapper, *L. campechanus*. Recently, Gomes *et al.* (2008) presented convincing evidence, based on both phylogenetic and population-genetic analyses of DNA sequences from the mitochondrial D-loop, that there is only a single, widespread species of red snapper in the western Atlantic. In addition to phylogenetic assessment of western Atlantic lutjanines, we also sought to assess the hypothesis of Miller & Cribb (2007) that all western Atlantic lutjanines arose from an Indo-Pacific lutjanine lineage.

MATERIAL AND METHODS

Tissues, primarily clips from the caudal fin, for all species examined from the western Atlantic and eastern Pacific oceans were procured from fish markets, artisanal fishers or by angling, and stored in either 95% ethanol or a DMSO buffer (Seutin, White & Boag, 1991) before transport to Texas A&M University. Sample locations, dates of sampling, and GenBank accession numbers for all individuals sequenced in our laboratory are given in Table 1. Except for *E. oculatus*, *L. mahagoni*, the '*L. apodus*' (hereafter *L. sp. cf. apodus*) from Bahia State in Brazil, and the three unidentified species sampled from a market in Puerto Armuelles, Panama, sequences were obtained from three individuals of each species. Also included in Table 1 are GenBank accession numbers for seven lutjanine species from the Indo-Pacific, two species of Lethrinidae, two species of Haemulidae, and four species of Sparidae.

Genomic DNA was extracted from each sample using a DNeasy Blood and Tissue Kit (Qiagen). Fragments containing sequences of three mitochondrial protein-coding genes were generated using polymerase chain reaction (PCR) amplification. Genes and PCR primers were: *cyt b*, primers CB12F and CB13R (Marko *et al.*, 2004); NADH-dehydrogenase subunit 4 (ND-4), primers NAP-2 (Arevalo, Davis & Sites 1994) and ND4LB (Bielawski & Gold, 2002); and COI, primers FishF1, FishF2, FishR1, and FishR2 (Ward *et al.*, 2005). For all three fragments, the 30- μ L PCR mixture comprised: 0.5 μ M of each primer, 1 \times PCR buffer (5 \times Colorless GoTaq Flexi Buffer, Promega), 2 mM MgCl₂, 200 μ M of each dNTP, 1.5 U of GoTaq Flexi DNA Polymerase (Promega), and 3 μ L of DNA (unknown concentration). The PCR protocol was initial denaturation at 95 °C for 3 min, 38 cycles of denaturation at 95 °C for 30 s, annealing at 53 °C (*cyt b* and COI) or 48 °C (ND-4) for 45 s, elongation at 72 °C for 2 min, and final elongation at 72 °C for 20 min. PCR products were electrophoresed on a 2% agarose gel; successful amplifications were band-cut and cleaned with QIAquick Gel Extraction Kits (Qiagen). Fragments were sequenced in both directions, using appropriate forward and reverse primers and ABI BigDye TERMINATOR, version 1.1. Products were electrophoresed on an ABI 3100 automated DNA sequencer (Applied Biosystems) and sequences edited with SEQUENCHER, version 3.0 (Gene Codes). Sequences were aligned at each gene across all taxa sampled, resulting in consensus fragments of 964 bases (*cyt b*), 590 bases (ND-4), and 652 bases (COI).

Sequence data were analyzed using maximum-likelihood (ML) criteria in PAUP*, version 4.0b10; Swofford, 2002). Initially, sequences from two species of Haemulidae (Haemuloidea), two species of

Lethrinidae (Sparoidea), and four species of Sparidae (Sparoidea) were used as outgroups to Lutjanidae, based on the hypothesized close relationship of Haemuloidea and Sparoidea to Lutjanoidea (Johnson, 1980). The inclusion of these sequences in initial analysis aimed to insure both monophyly of the species of Lutjaninae sampled and that the species of Etelinae and Apsilinae were appropriate outgroups to a monophyletic Lutjaninae. An ML search was initiated from a LogDet NJ starting tree, using GTR+I+ Γ as the best fit model determined in JMODELTEST (Guindon & Gascuel, 2003; Posada, 2008). This search was allowed to run to completion (58 134 rearrangements); new GTR+ Γ +I parameters were then estimated based on the resulting tree and a second ML search was initiated. This second search ran for over 12 000 rearrangements without a change in likelihood score or tree topology.

Results of the initial analysis strongly supported monophyly of the lutjanids and lutjanines sampled and that the genera *Etelis*, *Pristipomoides*, and *Apsilus* were basal in Lutjanidae to the lutjanines. The non-lutjanid outgroups were then pruned to include only the taxa (*E. oculatus*, *P. aquilonaris*, and *A. dentatus*) most closely related to the focal clade (Lutjaninae). An ML search, based on GTR+ Γ +I parameters estimated on this reduced dataset, was then initiated from a LogDet NJ starting tree. This ML search was allowed to run to completion (41 550 rearrangements). The GTR+ Γ +I parameters were re-estimated based on the resulting tree and a second ML search initiated. This second search ran for over 19 000 rearrangements without a change in likelihood score.

Branch support was assessed via Bayesian posterior probabilities in MrBayes (Huelsenbeck & Ronquist, 2001) and via bootstrap in a 'Randomized Accelerated Maximum-Likelihood for High Performance Computing' (RAxML-VI-HPC) algorithm (Stamatakis, 2006). Four runs of four Markov chain Monte Carlo (MCMC) chains of five million generations each, with sampling every 100 generations, were initiated in MrBayes. In each run, genes were unlinked and the software was allowed to estimate GTR+ Γ +I parameters independently. Each run resulted in 50 000 trees and converged on the same topology. The first 150 000 generations (15 000 trees) from each analysis were removed as 'burn-in'; the remaining 140 000 trees were used to create a majority rule consensus tree. A GTR+ Γ +I model by gene partition with 100 bootstrap pseudo-replicates was used in RAxML.

COI sequences were used to estimate divergence times within the dataset, using BEAST, version 1.5.3 (Drummond *et al.*, 2006; Drummond & Rambaut, 2007). A Yule process speciation prior and an uncor-

Table 1. Collection localities and sampling dates for lutjanids from the western Atlantic and eastern Pacific

Species	Sample ID	Location	Sample date	GenBank # ND-4	GenBank # COI	GenBank # cyt <i>b</i>
Lutjanidae						
Western Atlantic Ocean						
Apsilinae						
<i>Apsilus dentatus</i>	1	Puerto Rico (west coast)	January 2010	HQ162358	HQ162408	HQ162458
	2	Puerto Rico (west coast)	January 2010	HQ162359	HQ162409	HQ162459
	3	Puerto Rico (west coast)	January 2010	HQ162360	HQ162410	HQ162460
Etelinae						
<i>Etelis oculatus</i>	1	Puerto Rico (west coast)	November 2006	HQ162319	HQ162369	HQ162419
	2	Puerto Rico (west coast)	November 2006	HQ162320	HQ162370	HQ162420
<i>Pristipomoides aquilonaris</i>	1	Galveston, Texas	October 2004	HQ162352	HQ162402	HQ162455
	2	Galveston, Texas	October 2004	HQ162353	HQ162403	HQ162456
	3	Galveston, Texas	October 2004	HQ162354	HQ162404	HQ162457
Lutjaninae						
<i>Lutjanus analis</i>	1	Puerto Rico (east coast)	March 2007	HQ162330	HQ162374	HQ162430
	2	Puerto Rico (east coast)	March 2007	HQ162331	HQ162375	HQ162431
	3	Puerto Rico (east coast)	March 2007	HQ162332	HQ162376	HQ162432
<i>Lutjanus apodus</i>	1	Puerto Rico (northwest coast)	November 2007	HQ162333	HQ162377	HQ162433
	2	Puerto Rico (east coast)	November 2006	HQ162334	HQ162378	HQ162434
	3	St. Thomas (USVI)	February 2009	HQ162335	HQ162379	HQ162435
<i>Lutjanus buccanella</i>	1	Puerto Rico (northwest coast)	November 2007	HQ162336	HQ162380	HQ162436
	2	Puerto Rico (west coast)	November 2006	HQ162337	HQ162381	HQ162437
	3	Puerto Rico (west coast)	November 2006	HQ162338	HQ162382	HQ162438
<i>Lutjanus campechanus</i>	1	Brownsville, Texas	October 2005	HQ162321	HQ162371	HQ162421
	2	Brownsville, Texas	October 2005	HQ162322	HQ162372	HQ162422
	3	Brownsville, Texas	October 2005	HQ162323	HQ162373	HQ162423
<i>Lutjanus cyanopterus</i>	1	St. Thomas (USVI)	February 2009	HQ162339	HQ162383	HQ162439
	2	Puerto Rico (northwest coast)	November 2007	HQ162340	HQ162384	HQ162440
	3	Key West, Florida	May 2006	HQ162341	HQ162385	HQ162441
<i>Lutjanus griseus</i>	1	Long Key, Florida	July 2005	HQ162324	HQ162386	HQ162424
	2	Jupiter Island, Florida	July 2005	HQ162325	HQ162387	HQ162425
	3	Jupiter Island, Florida	July 2005	HQ162326	HQ162388	HQ162426
<i>Lutjanus jocu</i>	1	Puerto Rico (northwest coast)	November 2007	HQ162342	HQ162389	HQ162442
	2	St. Thomas (USVI)	February 2009	HQ162343	HQ162390	HQ162443
	3	St. Thomas (USVI)	February 2009	HQ162344	HQ162391	HQ162444
<i>Lutjanus mahogoni</i>	1	Puerto Rico (northwest coast)	November 2007	HQ162345	HQ162392	HQ162445
<i>Lutjanus synagris</i>	1	Puerto Rico (east coast)	March 2007	HQ162327	HQ162393	HQ162427
	2	Puerto Rico (east coast)	March 2007	HQ162328	HQ162394	HQ162428
	3	Puerto Rico (east coast)	March 2007	HQ162329	HQ162395	HQ162429

<i>Lutjanus vivanus</i>	1	Puerto Rico (west coast)	January 2010	HQ162346	HQ162396	HQ162446
	2	Puerto Rico (northwest coast)	November 2007	HQ162347	HQ162397	HQ162447
	3	Puerto Rico (west coast)	November 2006	HQ162348	HQ162398	HQ162448
<i>Lutjanus</i> sp. (cf <i>apodus</i>)	1	Siriba Island, Brazil	January 1998	HQ162367	HQ162417	HQ162467
	2	Siriba Island, Brazil	January 1998	HQ162368	HQ162418	HQ162468
<i>Ocyurus chrysurus</i>	1	Key West, Florida	April 2002	HQ162349	HQ162399	HQ162449
	2	Key West, Florida	April 2002	HQ162350	HQ162400	HQ162450
	3	Key West, Florida	June 2002	HQ162351	HQ162401	HQ162451
<i>Rhomboplites aurorubens</i>	1	St. Thomas (USVI)	February 2009	HQ162355	HQ162405	HQ162452
	2	St. Thomas (USVI)	February 2009	HQ162356	HQ162406	HQ162453
	3	St. Thomas (USVI)	February 2009	HQ162357	HQ162407	HQ162454
Eastern Pacific Ocean						
Lutjaninae						
<i>Lutjanus peru</i>	1	La Paz, Mexico	November 2009	HQ162361	HQ162411	HQ162461
	2	La Paz, Mexico	November 2009	HQ162362	HQ162412	HQ162462
	3	La Paz, Mexico	November 2009	HQ162363	HQ162413	HQ162463
<i>Lutjanus</i> sp. #1	10	Puerto Armuelles, Panama	January 2010	HQ162364	HQ162414	HQ162464
<i>Lutjanus</i> sp. #2	11	Puerto Armuelles, Panama	January 2010	HQ162365	HQ162415	HQ162465
<i>Lutjanus</i> sp. #3	12	Puerto Armuelles, Panama	January 2010	HQ162366	HQ162416	HQ162466
Indo-Pacific Ocean						
Lutjaninae						
<i>Lutjanus bengalensis</i>	FJ171339					
<i>Lutjanus erythropterus</i>	GQ265897					
<i>Lutjanus kasmira</i>	FJ416614					
<i>Lutjanus malabaricus</i>	FJ824741					
<i>Lutjanus rivulatus</i>	AP006000					
<i>Lutjanus russelli</i>	EF514208					
<i>Lutjanus sebae</i>	FJ824742					
Haemulidae						
<i>Diagramma pictum</i>	AP009167					
<i>Parapristipoma trilineatum</i>	AP009168					
Lethrinidae						
<i>Lethrinus obsoletus</i>	AP009165					
<i>Monotaxis grandoculis</i>	AP009166					
Sparidae						
<i>Pagellus bogaraveo</i>	AB305023					
<i>Pagrus auriga</i>	AB124801					
<i>Pagrus major</i>	AP002949					
<i>Paragyrops edita</i>	EF107158					

GenBank accession numbers for each fragment of NADH dehydrogenase 4 (ND-4), cytochrome *c* oxidase I (COI), and cytochrome *b* (cyt *b*) are given. GenBank accession numbers for DNA sequences of the Indo-Pacific lutjanids and outgroups (Haemulidae, Lethrinidae, and Sparidae) were obtained from whole mitochondrial DNA sequences available in GenBank, aligned (using SEQUENCHER) with sequences acquired in our laboratory, and nonconsensus parts removed.

related lognormal model of rate variation were implemented in each BEAST analysis. Best-fit models of nucleotide substitution for the data were the same as those identified above as part of the phylogenetic analyses. Because of the absence of an acceptable calibration point within western Atlantic lutjanines, a 1.2% per million year divergence rate (and therefore a lineage substitution rate of 0.006 per site/million years) was employed. This rate has been calibrated from several geminate fish lineages, including two species of *Lutjanus*, based on final uplift of the Isthmus of Panama (Bermingham, McCafferty & Martin, 1997).

Two separate MCMC analyses were run for 10 000 000 generations (burn-in at 10%), with parameters sampled every 1000 steps. Independent runs were combined using LOGCOMBINER, version 1.5.3 (Drummond & Rambaut, 2007). TRACER, version 1.5 (Rambaut & Drummond, 2004), was used to measure effective sample size of each parameter (all resulting effective sample sizes exceeded 200) and to calculate the mean and upper and lower bounds of the 95% highest posterior density interval for divergence times. Tree topologies were assessed using TREEAN-NOTATOR, version 1.5.3 (Drummond & Rambaut, 2007), and FIGTREE, v.1.3.1 (Rambaut, 2008).

RESULTS AND DISCUSSION

DNA SEQUENCE CHARACTERIZATION

A total of 2206 bp (590 from ND-4 gene, 652 from COI, and 964 from *cyt b*) were aligned without issue (i.e. no insertions or deletions), indicating that the sequences amplified were most likely of mitochondrial origin. Within the core clade that included all western Atlantic lutjanines sampled (Clade A, Fig. 1), there were 225 variable sites (200 parsimony informative) in ND-4, 188 variable sites (166 parsimony informative) in COI, and 299 (254 parsimony informative) in *cyt b*. Mean nucleotide frequency for ND-4 was A = 25%, C = 36.5%, G = 12.8%, T = 25.6%, with generally similar values for COI (25.4%, 28.5%, 18.7%, 27.4%) and *cyt b* (25%, 33.2%, 14.4%, 27.4%).

BASAL RELATIONSHIPS AND DIVERGENCE DATES

The phylogenetic hypothesis generated from the entire mitochondrial dataset (2206 bases) is presented in Figure 1. There was strong support for monophyly of Lutjanidae (Etelinae, Apsilinae, and Lutjaninae) and for monophyly of Lutjaninae. Within Lutjanidae, a sister relationship between *Etelis* and *Pristipomoides* (Etelinae) was weakly supported (0.74 posterior probability), whereas a sister-group relationship between that clade and *Apsilus* (Apsilinae) was strongly supported (1.0 posterior probability).

As discussed by Johnson (1980), Apsilinae has not been recognized as a natural group and *Apsilus* has been placed variously in Etelinae and Lutjaninae because it possesses characteristics of both sub-families. Johnson (1980) hypothesized ultimately that Apsilinae occupied an evolutionary position intermediate between Etelinae and Lutjaninae and settled on the hypothesis (Etelinae (Apsilinae, Lutjaninae)). A possible sister-group relationship between Etelinae (*Etelis* and *Pristipomoides*) and Apsilinae (*Apsilus*), as indicated by the mitochondrial-based phylogeny (Fig. 1), may suggest that *Apsilus* should be subsumed within Etelinae. Further study, however, is warranted because, globally, there are nine additional species currently placed in Apsilinae and 17 additional species in Etelinae (Allen, 1985), and it is possible that broader taxon sampling may refute a sister-group relationship between Etelinae and Apsilinae.

All of the western Atlantic lutjanines sampled were placed in a strongly supported clade (Clade A, Fig. 1). Three Indo-Pacific species of *Lutjanus* (*Lutjanus erythropterus*, *Lutjanus malabaricus*, and *Lutjanus sebae*), were basal to Clade A, whereas another Indo-Pacific species (*Lutjanus russelli*) was basal to the remaining lutjanines (Clade B, Fig. 1). This finding is consistent with and fully supportive of the hypothesis (Miller & Cribb, 2007) that Western Atlantic lutjanines are derived from an Indo-Pacific lutjanine lineage. The phylogenetic hypothesis also indicates that: (1) oceans where lutjanines are distributed (western Atlantic, eastern Pacific, and Indo-Pacific) are not reciprocally monophyletic for the species distributed within them (Fig. 2) and (2) there clearly have been several instances of biotic dispersion/speciation events between oceans, both deep within the phylogeny (e.g. Clade B), as well as more recently (see below).

Divergence of Clade A was dated to approximately 23 Mya, whereas divergence of Clade B was dated to approximately 20 Mya (Fig. 2). The time period of approximately 19–23 Mya (Late Oligocene to Early Miocene) was a significant period of palaeoceanographic change (Keller & Barron, 1983) that resulted in alterations in oceanic circulation, sedimentation, and biogeographic distributions. Major palaeogeographic events occurring during this period and affecting the global distribution of lutjanids and other marine species include the closure of the Tethys Sea (Dercourt *et al.*, 1986; Vrielynck, Odin & Dercourt, 1997; Adams, Bayliss & Whittaker, 1999), when modern patterns of atmospheric and ocean circulation were formed, and the opening of the Panamanian Gateway, a 'conveyor-belt' (Sumata *et al.*, 2004) of free and active water circulation between the tropical and subtropical Pacific and Atlantic oceans (Duque-Caro,

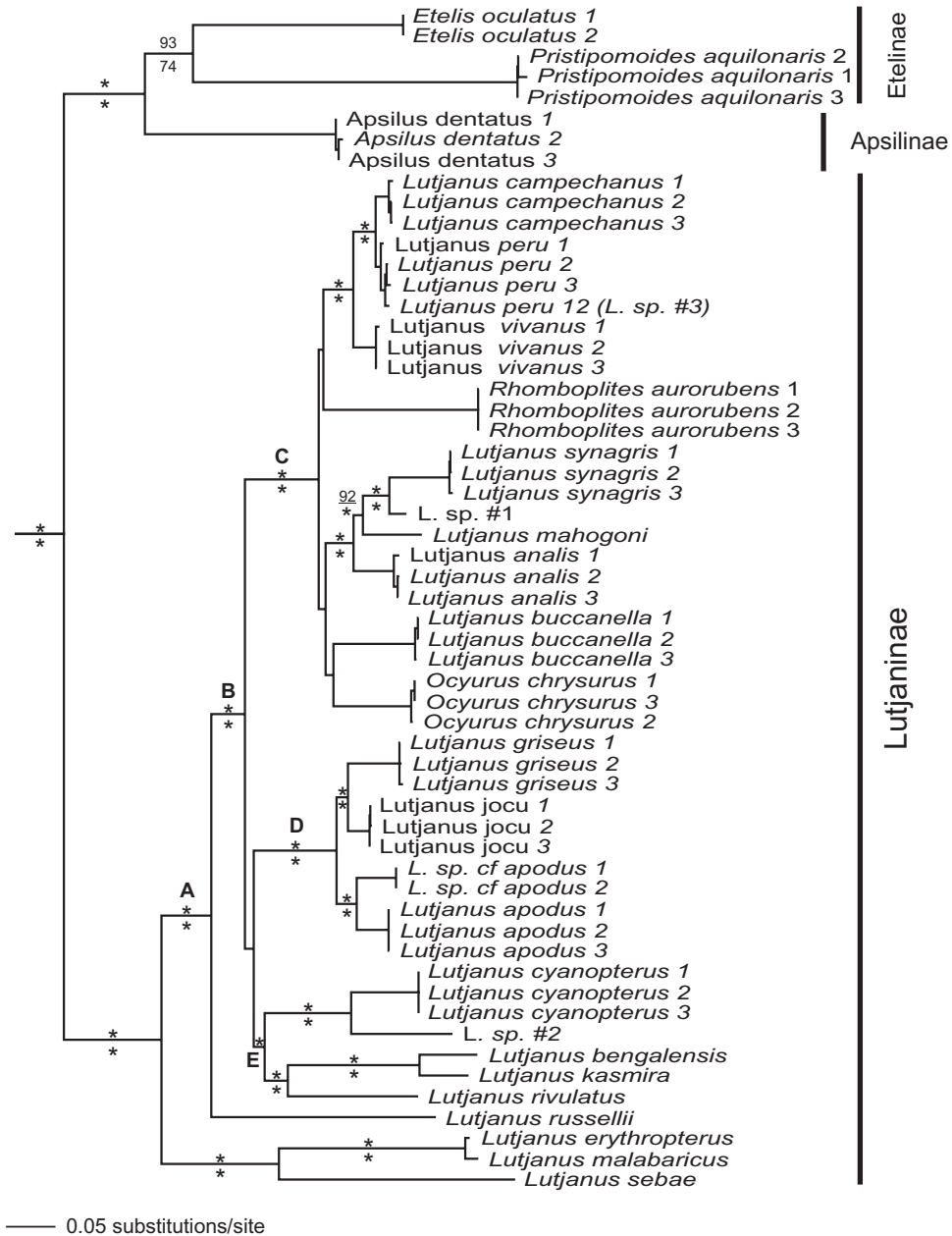


Figure 1. Phylogenetic hypothesis for western Atlantic Lutjaninae. Numbers above nodes are Bayesian posterior probabilities, whereas numbers below nodes are maximum-likelihood, bootstrap-support percentages; asterisks indicate posterior probabilities of 0.95 or greater and bootstrap percentages of 75% or greater. Letters at nodes indicate the major clades discussed in text. The phylogeny is rooted to eight additional outgroup taxa (Table 1). Samples labelled as ‘L. sp.’ are discussed in the text.

1990). The early Miocene also was a time of global warming and sea grass expansion (Brasier, 1975), both of which would have greatly increased suitable habitat for lutjanines dispersing from the Indo-Pacific to the eastern Pacific and through the gateway into the western Atlantic. The early Miocene also was a fairly stable period tectonically (Duque-Caro, 1990), which could have allowed lutjanine and other

structure-associated fish to colonize the various islands and reefs in the eastern arc of the Lesser Antilles (Caribbean Sea) that were formed during the volcanic periods between the Eocene and the beginning of the Miocene (Draut, Clift & Scholl, 2008). The phylogenetic hypothesis and estimates of lineage divergence (Figs 1, 2) thus suggest an early Miocene diversification of an Indo-Pacific lutjanine lineage

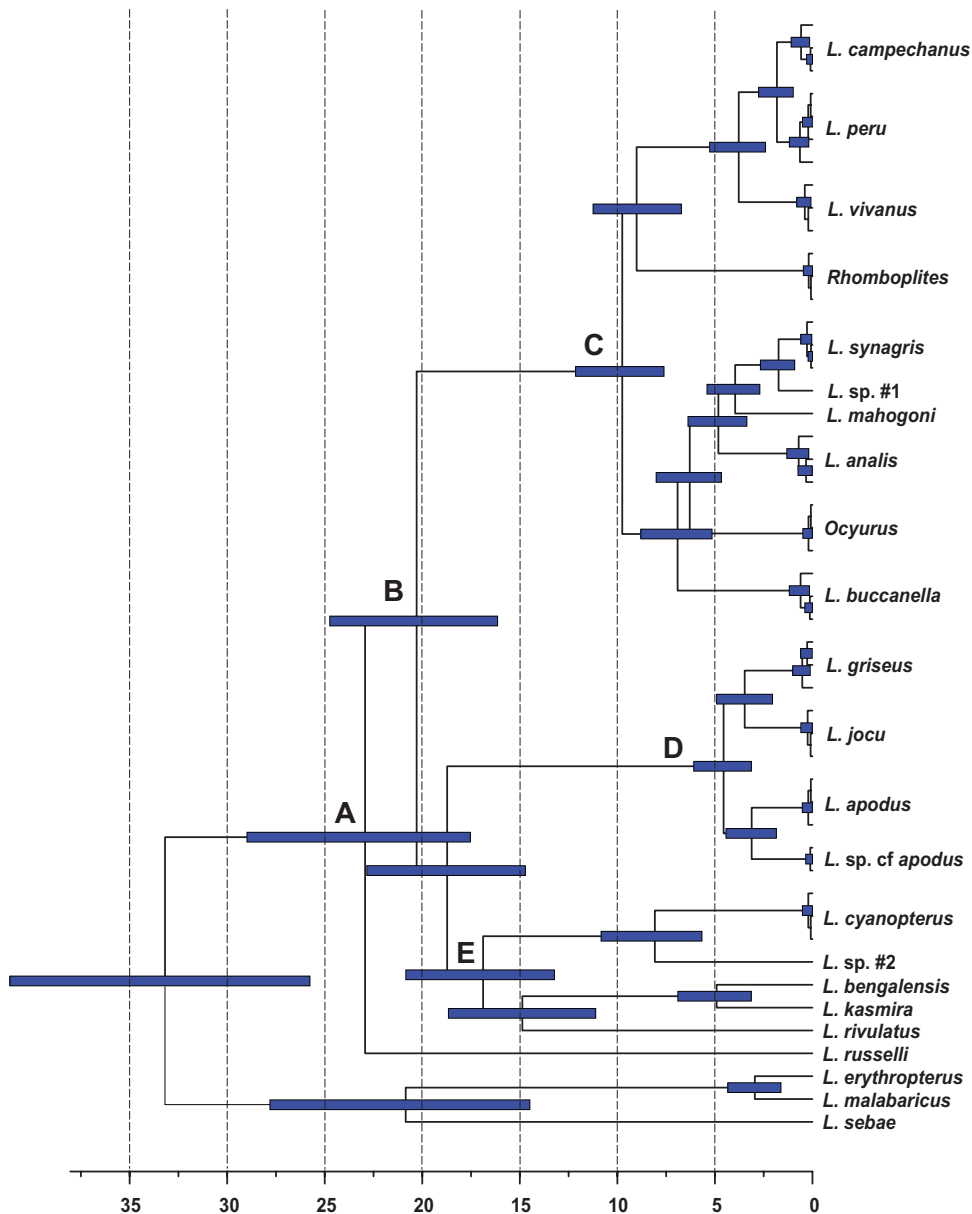


Figure 2. Molecular clock calibrations, based on sequences of the mitochondrially encoded cytochrome *c* oxidase I (COI) gene and a divergence rate of 1.2% per million years, for lutjanine species examined in the present study. Bars at nodes reflect 95% confidence intervals. Letters at nodes indicate major clades discussed in text. The scale (abscissa) represents millions of years before present.

that dispersed into the western Atlantic via the Panamanian Gateway.

PHYLOGENY AND DIVERGENCE DATES OF WESTERN ATLANTIC LUTJANINAE

All western Atlantic lutjanines were included in a well-supported clade (B) that also includes three Indo-Pacific species (*Lutjanus rivulatus*, *Lutjanus kasmira*, and *Lutjanus bengalensis*), and *Lutjanus peru* and three unidentified species, *L. sp. #1*, *L. sp.*

#2, and *L. sp. #3* from the eastern Pacific. On the basis of the phylogenetic hypothesis and estimates of sequence divergence, the closest relatives (in this dataset) to the unidentified species from the eastern Pacific would appear to be *L. synagris* (*L. sp. #1*), *L. cyanopterus* (*L. sp. #2*), and *L. peru* (*L. sp. #3*). The phylogenetic position and estimated sequence divergence (0.45–0.73%) between *L. sp. #3* (sampled from western Panama) and known samples of *L. peru* (sampled from Baja, Mexico) indicates that *L. sp. #3*

is *L. peru* (considered as such hereafter). By similar logic and considering the phylogenetic hypothesis, *L. sp. #1* and *L. sp. #2* (also from western Panama) would appear to be distinct species, related to the Atlantic-distributed *L. synagris* and *L. cyanopterus*, respectively. On the basis of estimates of sequence divergence, *L. sp. #1* and *L. synagris* appear to have diverged more recently than *L. sp. #2* and *L. cyanopterus* (4.4% versus 8.0%).

There were three strongly supported clades within Clade B: one (C) that includes six species of western Atlantic *Lutjanus*, two (*L. peru* and *L. sp. #1*) from the eastern Pacific, and the monotypic genera *Rhomboplites* and *Ocyurus*; one (D) that includes three, possibly four (see below) species of *Lutjanus* found in the western Atlantic; and one (E) that includes *L. cyanopterus* (western Atlantic), *L. sp. #2* (eastern Pacific), and three species of *Lutjanus* from the Indo-Pacific. There was weak (0.81 posterior probability, 58% bootstrap) support for a sister-group relationship between Clades D and E, a hypothesis that certainly warrants further study.

Diversification within Clade C led to two primary lineages (Fig. 2). The first contains *Rhomboplites aurorubens* as sister to a strongly supported *L. vivanus*, *L. campechanus*, and *L. peru* clade. The second contains *Ocyurus chrysurus* + *L. buccanella* as sister to a strongly supported *L. synagris*, *L. sp. #1*, *L. mahagoni*, and *L. analis* clade. The positions of *Rhomboplites*, *Ocyurus*, and *L. buccanella* are not strongly supported (Fig. 1); this may be a result of short internodes (rapid speciation), which are difficult to support even with mitochondrial (mt)DNA that sorts comparatively faster than nuclear genes (Zink & Barrowclough, 2008).

The estimated time of diversification of the major lineages in Clade C (approximately 7–10 Mya; Fig. 2) coincided with a period of low sea levels (Haq, Hardenbol & Vail, 1987) that followed a Neogene Hiatus (11.8–12.9 Mya), which reflected Mid-Miocene tectonic disturbance in northwest South America (Duque-Caro, 1990). A hypothesized consequence of the ensuing tectonic uplift was a circulation barrier between the two oceans, which prevailed until a subsequent Neogene Hiatus occurring 6.3–7.0 Mya when surface-water circulation between the Caribbean and Pacific Ocean was re-established (Duque-Caro, 1990). Partial closures or shoaling of the gateway between the two oceans potentially intensifies the Gulf Stream and introduces warm and saline water masses into higher latitudes (Haug & Tiedemann, 1998). Such a situation possibly could accelerate adaptive divergence via emergence of new habitats to the north of the gateway. Martin & Dunn (2000) identified a number of otoliths from the Mid-Late Miocene (5.6–11.4 Mya) Gatun Formation in Central Panama as

belonging to the lutjanine genera *Lutjanus* and *Ocyurus*. This suggests that tropical waters were present around reefs and other structures during this time, and that the lineage leading to morphologically unique *Ocyurus* already had diverged.

Relationships among species in the two, well-supported lineages in Clade C have been controversial. Sarver *et al.* (1996) proposed a relationship between *L. vivanus* and *L. campechanus*, based on parsimony analyses of combined mitochondrial 12S rDNA and *cyt b* sequences (640 bp, 112 phylogenetically informative). Earlier studies based on morphology also had supported a *L. vivanus*–*L. campechanus* relationship, and had allied these species with *L. analis* (Rivas, 1966; Vergara, 1980). Similarities in allozyme phenotypes also supported a relationship between *L. analis* and *L. vivanus* (Chow & Walsh, 1992).

The phylogenetic hypothesis presented here (Fig. 2) strongly supported both: (1) *L. analis* as basal to the clade (*L. mahagoni* (*L. synagris*, *L. sp. #1*)), rather than near *L. vivanus* and *L. campechanus*; (2) the clade (*L. vivanus* (*L. campechanus*, *L. peru*)). We note that, although *L. analis* does share morphological similarities (e.g. a pointed anal fin and a small eye) with *L. vivanus* and *L. campechanus*, in other characters (e.g. overall body shape, preference for shallower water and, in adults, a prominent black spot below the soft portion of the dorsal fin), it shares similarities with *L. synagris* and *L. mahagoni*. Therefore, the conflict between the results obtained in the present study and those of previous studies may be a result of the phenetic-based approaches used in morphological and allozyme analyses (which could have incorporated primitive character states; Rivas, 1966; Vergara, 1980; Chow & Walsh, 1992) or to limited genetic data (Sarver *et al.*, 1996).

Estimated inter-specific divergence times within the *L. analis* and *L. vivanus* clades range from 1.7–4.8 Mya and 1.8–3.8 Mya, respectively. Divergence of the western Atlantic/eastern Pacific species pairs *L. synagris*/*L. sp. #1* and *L. campechanus*/*L. peru* almost certainly reflects vicariant isolation after final closure of the Panamanian Gateway during the Mid-Pliocene, approximately 3.1–3.5 Mya (Keigwin, 1978, 1982; Duque-Caro, 1990). All earlier divergences within Clade C, including lineages leading to *Rhomboplites*, *Ocyurus*, and *L. buccanella*, occurred earlier (7–10 Mya during the beginning of the Late Miocene). This period (Mid- to Late Miocene) was tectonically quiet and could have permitted resource partitioning, leading to species-level divergence. As an example, *L. analis* grows to a larger size, is slightly deeper in body shape, and has different dorsal, anal, and caudal fin shapes than do *L. synagris* and *L. mahagoni*, reflecting its preference for slightly deeper water over

sandy bottoms. Similarly, *L. vivanus* has a more lunate to forked caudal fin and is less deep-bodied than either *L. campechanus* or *L. peru*, reflecting its preference for deeper-water habitats over continental and island shelves and for vertical diel migrations to the surface. Both *Ocyurus* and *Rhomboplites* have adaptations for life in the water column and feeding on smaller prey items; these include more streamlined bodies, deeper to moderately forked tails, reduced canine teeth, and greater numbers of gill rakers on the lower limb of the first arch. These adaptations, however, are almost certainly convergent (homoplasious); *Ocyurus* occupies more pelagic habitats nearer to shore, whereas *Rhomboplites* inhabits rocky bottoms near the edge of continental and island shelves.

The last lineage within Clade C is *L. buccanella*, a relatively deep-bodied, reddish-coloured fish that inhabits fairly deep waters (80–200 m) over sandy or rock bottoms. In habitat preference, coloration, and many other characteristics, *L. buccanella* resembles *L. campechanus* and *L. peru*. These similarities led Rivas (1996) to place *L. buccanella* into a group that contained *L. campechanus*. Vergara (1980), however, allied *L. buccanella* with *L. synagris* and *L. mahagoni*. Maximum-parsimony analysis of mitochondrial 12S rDNA and *cyt b* sequences (Sarver *et al.*, 1996) failed to resolve relationships of *L. buccanella*, although a search using weights derived from the average consistency indices for characters suggested a relationship between *L. buccanella* and *Rhomboplites*. Maximum-likelihood analyses in this study placed *L. buccanella* as sister to *Ocyurus* (Fig. 2); however, this relationship was not strongly supported by bootstrap or posterior probabilities.

Clade D (Fig. 2) was a strongly supported group of three, probably four species of *Lutjanus*. On the basis of work by Moura & Lindeman (2007), it is likely that *L. sp. cf. apodus*, which were sampled in 1998 from waters off Bahia state in Brazil, is the species *Lutjanus alexandrei*. Moura & Lindeman (2007) described *L. alexandrei* and noted that the species had long been misidentified as either *L. griseus* or *L. apodus* and that presence of either *L. griseus* or *L. apodus* in Brazilian waters was 'highly doubtful' given that specimens in Brazilian museums, field surveys, and fishery landings were invariably *L. alexandrei*. Regardless, the posterior probability and bootstrap support (100%) for separation of *L. apodus* from *L. sp. cf. apodus*, sequence divergence between them (4.0%), and their estimated time of divergence (3.1 Mya), argue that the two are specifically distinct.

A number of previous studies of morphology (Rivas, 1966; Vergara, 1980), allozymes (Chow & Walsh, 1992), and mitochondrial DNA sequences (Sarver *et al.*, 1996; Rodríguez-Santiago, 2008) have sup-

ported close relationships among *L. apodus*, *L. griseus*, and *L. jocu*. Sarver *et al.* (1996), however, found strong parsimony bootstrap support (100%, 250 replicates) for the relationship (*L. griseus* (*L. jocu*, *L. apodus*)). Differences between their study and the present study include number of phylogenetically informative characters (112 versus 620), phylogenetic hypothesis generation (maximum parsimony versus maximum-likelihood and Bayesian estimation), and the inclusion in the present study of an additional species (*L. sp. cf. apodus*). In addition, although *L. apodus*, *L. griseus*, and *L. jocu* are very similar in a number of morphological characters, dorsal anterior scale rows in *L. apodus* and *L. griseus* are parallel to the lateral line, rising obliquely posteriorly. In all other lutjanines in the dataset, these scales rows rise obliquely above the lateral line, both anteriorly and posteriorly. On the basis of the phylogenetic hypothesis (Fig. 1), this character state would need to have arisen independently in the two species.

Diversification of extant lineages within Clade D appears to have begun considerably more recently (approximately 4.6 Mya) than diversification within Clade C (Fig. 2). This time period corresponds to the Early Pliocene, another tectonically stable period following a phase of volcanism (Draut *et al.*, 2008) that gave rise to the western arc of islands in the Lesser Antilles (Haggett, 2002). In addition, continued shoaling of the Panamanian Gateway (Duque-Caro, 1990) probably further strengthened the Gulf Stream (Keigwin, 1982) and intensified the stream of warm water masses to higher northern latitudes (Haug & Tiedemann, 1998).

Separation of *L. griseus* from *L. jocu* and *L. apodus* from *L. sp. cf. apodus* are dated to 3.5 Mya and 3.1 Mya, respectively. All of the species in Clade D are found primarily in shallow, coastal waters, and juveniles of both *L. griseus* and *L. jocu* are often found in rivers. This apparent greater tolerance for low salinity would have made available juvenile habitat not generally occupied by most other lutjanids. The pair *L. griseus* and *L. jocu* are similar in other respects, including overall distribution, and differ primarily with respect to body depth and the length of the pectoral fin (*L. griseus* is more slender with a much shorter pectoral fin). This could suggest that resource partitioning via possible differences in side-to-side manoeuvrability relative to prey acquisition may have promoted divergence between the two. The species pair *L. sp. cf. apodus* and *L. apodus* occurs on either side of the discharge of the Amazon and Orinoco rivers, a barrier of some 2300 km of muddy coastline lacking coral reefs (Rocha *et al.*, 2005), which was established approximately 11 Mya (Hoorn *et al.*, 1995). Because of fluctuations in sea level, climate, and ocean currents, however, the effective-

ness of the barrier has varied through time (Floeter *et al.*, 2008), suggesting that divergence of *L. sp. cf apodus* could be a result of vicariance at some point subsequent to 11 Mya.

Clade E contains one species from the western Atlantic (*L. cyanopterus*), one species from the eastern Pacific (*L. sp. #2*), and three species from the Indo-Pacific (*L. bengalensis*, *L. kasmira*, and *L. rivulatus*). The lone representative from the western Atlantic (*L. cyanopterus*) has been phylogenetically enigmatic. Rivas (1966) and Vergara (1980) placed it in a morphological group with *L. griseus*, *L. apodus*, and *L. jocu*, whereas Sarver *et al.* (1996) had weak to moderate support (70% bootstrap, 250 replicates) for placement of *L. cyanopterus* outside of a group that included 11 other western Atlantic lutjanines, including *Ocyurus* and *Rhomboplites*, and two western Atlantic lutjanids, *Pristipomoides aquilonaris* and *Etelis oculatus*, currently placed in the Subfamily Etelinae (Johnson, 1980). Sarver *et al.* (1996) noted that the estimates of sequence divergence indicated that *L. cyanopterus* was as diverged from some of the species of *Lutjanus*, as were species of *Etelis* and *Pristipomoides*. Rodriguez-Santiago (2008), alternatively, found weak to moderate support (0.69 posterior probability, 77% NJ bootstrap) for monophyly of a group of nine species of *Lutjanus* (including *L. cyanopterus*), *Rhomboplites*, and *Ocyurus*, with representatives of Apsilinae (*Apsilus*) and Etelinae (*Etelis*, *Pristipomoides*) basal to that clade. The phylogenetic hypothesis generated in the present study (Fig. 1) clearly places *L. cyanopterus* within a monophyletic *Lutjanus*, yet distant from other lutjanines found in the western Atlantic (most species in Clades C and D). Separation of *L. cyanopterus* from its closest relative in this dataset (*L. sp. #2*) was dated to 8.1 Mya, well before the closing of the Panamanian Gateway. Whether *L. cyanopterus* in the western Atlantic diverged with the closing of the gateway is problematic because there may be other lutjanines in the eastern Pacific to which *L. cyanopterus* is more closely related.

DIVERGENCE AND SPECIATION IN WESTERN ATLANTIC LUTJANINAE

The phylogenetic hypothesis (Fig. 2) of western Atlantic Lutjaninae and estimates of divergence times within the lineage indicate the occurrence of major radiations after significant geographic or geologic events. Clades A and B date to the Late Oligocene–Early Miocene when there were major changes in oceanographic circulation, including the opening of the Panamanian Gateway. The origin of the two major lineages within Clade B appears to have occurred much later during relatively quiet periods,

each following tectonic upheaval that gave rise to the arc of islands of the Lesser Antilles. In addition, at both times, water exchange through the gateway was somewhat restricted (Duque-Caro, 1990), strengthening the Gulf Stream (Keigwin, 1982) and intensifying the movement of warm water masses to higher northern latitudes (Haug & Tiedemann, 1998). In general, lutjanines are relatively large, long-lived predatory fish that have relatively low rates of natural mortality, extended spawning, pelagic eggs and larvae, and the capability to live in habitats from shallow, inshore areas to depths greater than 500 m (Anderson, 2003). These life-history traits and the ability to occupy diverse habitats are highly conducive to dispersal, indicating that, once through the gateway, the ancestors of present-day Lutjaninae easily could have dispersed to the west and north.

Divergent evolution in the western Atlantic Lutjaninae appears to have occurred both by vicariant (Losos & Glor, 2003) and ecological (Schluter, 2009) speciation. The two, well-established geographic barriers in the region, the Isthmus of Panama that separated the western Atlantic from the eastern Pacific and the discharge of the Amazon and Orinoco rivers that separated Caribbean from Brazilian reef habits, are implicated in the divergence of at least four species pairs. Final closure of the isthmus is implicated in the divergence of *L. campechanus* from *L. peru*, *L. synagris* from *L. sp. #1* (present study), and *L. apodus* from *L. argentiventris* (Bermingham *et al.*, 1997), whereas the Amazon-Orinoco barrier is implicated in the divergence of *L. apodus* and *L. sp. cf apodus* (present study). Of interest is that *L. apodus* appears to be involved in both vicariant events, and, as might be expected based on the time of origin of each barrier, *L. apodus* and *L. argentiventris* are more similar in mitochondrial COI sequence (3.48% difference; Bermingham *et al.*, 1997) than are *L. apodus* and *L. sp. cf apodus* (4.0%; present study). Finally, there may well be other geminate species pairs of *Lutjanus* on either side of the isthmus as there six additional species of *Lutjanus* in the eastern Pacific (Allen, 1985).

The geological (vicariant) events associated with divergence and speciation in western Atlantic lutjanines have been acknowledged in other transisthmian marine groups as well. Tringali *et al.* (1999), for example, in their study of 12 species of centropomine fish, found that three of four species in the basal clade were from the eastern Pacific, suggesting a Pacific or Indo-Pacific centropomine origin, and that the four major divisions among the 12 species probably were in place by the mid- to late-Miocene (approximately 10 Mya). Similarly, Teske *et al.* (2007) also described a putative vicariant event that generated a basal Indo-Pacific versus western Atlantic distribution pattern in

seahorses (genus *Hippocampus*), likely occurring during the late early- to mid-Miocene, and was affected by the closure of the Tethys Seaway. Far better documented are putative vicariant events associated with the closing of the Panamanian Gateway. Geminant species of either side of the Panamanian Isthmus have been described in a number of marine fishes, including centropomines (Tringali *et al.*, 1999), parrotfishes (Streelman *et al.*, 2002), needlefishes (Banford, Bermingham & Collette, 2004), seahorses (Teske *et al.*, 2007), and grunts of the genus *Haemulon* (Rocha *et al.*, 2008), amongst others. Interestingly, as first described by Knowlton *et al.* (1993) in their study of snapping shrimp (genus *Alpheus*) and then by Streelman *et al.* (2002) in their study of parrotfishes, estimated divergence times of putative sister species on either side of the isthmus range from approximately mid-Miocene (10 Mya) to mid-Pliocene (3.1–3.5 Mya), suggesting that isolation across the isthmus was staggered temporally and possibly the result of varying periods of low sea levels (Haq *et al.*, 1987) and continued shoaling (Duque-Caro, 1990). Finally, the Amazon-Orinoco barrier also has been documented in other marine fishes, including parrotfishes (Robertson *et al.*, 2006), grunts (Rocha *et al.*, 2008), and wrasses (Rocha *et al.*, 2005; Beldade *et al.*, 2009). This barrier also appears to have been porous over time (Rocha, 2003; Floeter *et al.*, 2008).

The inference of ecological speciation in lutjanines is based in part on the absence of known geographic barriers aside from the two discussed above, and in part on phenotypic differences that indicate adaptation to life in different habitats. Notable examples include separation of *L. vivanus* from *L. campechanus*/*L. peru* (difference in body and caudal fin shape in *L. vivanus* reflecting a preference for deeper-water habitats and vertical diel migrations), *L. analis* from *L. mahagoni*/*L. synagris*/*L. sp. cf. synagris* (larger size, deeper body, and different fin shapes in *L. analis* reflecting preference for slightly deeper, more open water), *L. griseus* from *L. jocu* (differences in body shape and pectoral fin length reflecting possible differences prey acquisition), and separation of both *Ocyurus* and *Rhomboplites* from other lineages in Clade C (numerous changes reflecting life in the water column and feeding on smaller prey items). A major ecological shift also is suggested by the preference of species in Clade D for shallow, coastal waters, including estuaries and even lower reaches of rivers, because greater tolerance for low salinity would have made available habitats not generally occupied by most other lutjanines. There are two final points worthy of note. First, previous studies of tropical reef fishes in the Caribbean region (Streelman *et al.*, 2002; Rocha *et al.*, 2005, 2008)

have provided considerable evidence that morphological changes associated with ecological adaptation may be common in coral reef fishes and that vicariance may not be the only factor promoting speciation. Lutjanines appear to fit this pattern, although, strictly speaking, lutjanines are not coral reef fish *sensu stricto* but rather are bottom, structure-associated fishes that live over coral reefs or rocky outcrops. Second, the evidence for ecological speciation in lutjanines is inferential and will remain so until there is documented evidence that divergence and speciation were primarily a function of differing natural selection among habitats. This evidence would include the parallel evolution of reproductive isolation, trait-based assortative mating, and/or reproductive isolation via active selection (Schluter, 2009).

TAXONOMY OF WESTERN ATLANTIC LUTJANINAE

All assayed western Atlantic Lutjaninae were placed into Clade A, which also included three species (two unidentified) from the eastern Pacific and four species from the Indo-Pacific. All nominal species in Clade A except for monotypic *Ocyurus* and *Rhomboplites* currently are placed in the genus *Lutjanus*; inclusion of *Ocyurus* and *Rhomboplites* in Clade A thus renders *Lutjanus* paraphyletic in a cladistics-based taxonomy. The taxonomic status of *Ocyurus* has long been controversial. Domeier & Clarke (1992) and others have posited that *Ocyurus* should be reclassified as *Lutjanus* given the apparent frequency with which *Ocyurus* hybridizes with species of *Lutjanus*. Species known or suspected of hybridization with *Ocyurus* now include *L. synagris* and *L. griseus* (Domeier & Clarke (1992), *L. jocu* (Bortone & Williams, 1986), and *L. apodus* and *L. analis* (Carson & Gold, unpubl.). Placement of *Ocyurus* into *Lutjanus* also is consistent with, although not necessarily strongly supported by, the mtDNA phylogenies generated by Sarver *et al.* (1996) and Rodríguez-Santiago (2008), and with the cytogenetic studies of Nirchio *et al.* (2009). To our knowledge, no one has questioned whether *Rhomboplites* also should be included in *Lutjanus*, although inclusion of *Rhomboplites* within *Lutjanus* was reflected in, but not strongly supported by, the studies of Sarver *et al.* (1996) and Rodríguez-Santiago (2008). *Rhomboplites* does possess a unique chromosomal location of the 5S rDNA gene (Nirchio *et al.*, 2009) relative to the few other lutjanids examined; this character state, however, could simply be autapomorphic and uninformative in cladistic analysis (Bryant, 1995). Characters that distinguish *Ocyurus* and *Rhomboplites* from species of *Lutjanus* include the presence of ectopterygoid teeth, additional gill rakers on the lower limb of the first arch, and forked caudal

fins, which are all adaptations for feeding on smaller prey items in the water column. The two species differ from one another in pectoral fin length and ray number, eye size, shape of the vomerine tooth patch, and coloration. Most of these morphological differences, both between *Ocyurus/Rhomboplites* and *Lutjanus* and between *Ocyurus* and *Rhomboplites*, appear to be associated with ecological adaptations related to feeding and habitat. On the basis of all of the above, taxonomic revision of western Atlantic lutjanines appears warranted and we recommend that *Ocyurus* and *Rhomboplites* be subsumed within *Lutjanus*.

MARKETABILITY OF 'RED SNAPPER'

The three unidentified snappers from offshore of Puerto Armuelles, Panama, were sampled by a Panamanian colleague from Texas A&M. The person had no formal training in ichthyology and, at our request, took small caudal fin clips from three fish labelled as 'red snapper' that his mother had brought home. The three fish turned out to be three different species: one (*L. sp.* #3) was hypothesized to be *L. peru*, typically sold as huachinango or Pacific red snapper; one (*L. sp.* #1) was closely related to *L. synagris*; and one (*L. sp.* #2) was related, albeit distantly, to *L. cyanopterus*. These findings were reminiscent of the study by Marko *et al.* (2004) who found that 77% of fish sold as red snapper (ostensibly *L. campechanus*) from nine vendors in eight states in the USA were in fact other species. It appears that retail mislabelling of 'red snapper' extends beyond the USA.

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