

Phylogenetic relationships of tropical eastern Pacific snappers (Lutjanidae) inferred from mitochondrial DNA sequences

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A total of 2,206 base pairs of coding sequences of mitochondrial DNA from nine of 10 lutjanine (Lutjanidae) species endemic to the eastern Pacific Ocean were used in a phylogenetic analysis to infer species relationships among lutjanines on either side of the Isthmus of Panama. Monotypic *Hoplopagrus (guentherii)* appears to be at the base of all lutjanines assayed to date and represents the first of five, eastward dispersal events of lutjanines from the Indo-Pacific to the Americas. Diversification of lutjanines in the Americas appears to have occurred primarily in the western Atlantic Ocean (Caribbean Sea) and been aided by an early Miocene eastward-flowing current and by more optimal lutjanine habitat in the warmer Caribbean waters. Five geminate species pairs in five, strongly supported clades were identified. In three, the trans-isthmian geminate species in the eastern Pacific belonged to clades in which basal and other members of the clade are endemic to the western Atlantic, suggesting that the ancestor to each species geminate pair dispersed westward into the eastern Pacific prior to closure of connections between the two oceans; the opposite occurred in one of the clades. The fifth clade contained only two species, one on either side of the Isthmus. Diversification in life-history and other traits in each geminate species pair appears to have been slight. Five lutjanine species possessing horizontal blue stripes (the blue-lined complex) and distributed from the western Indian Ocean to the eastern Pacific formed a strongly supported monophyletic clade. The lone blue-lined species in the eastern Pacific (*Lutjanus viridis*) could be conspecific with its highly dispersive sister species (*Lutjanus kasmira*) from the Indo-Pacific. The chronology of lutjanine diversification and closure of seawater exchange between the two oceans is briefly discussed.

Key words: dispersal, Isthmus of Panama, Lutjanidae, phylogenetics, phylogeography

Introduction

Snappers of the perciform family Lutjanidae are distributed worldwide in tropical and subtropical, and occasionally temperate, waters (Allen, 1985; Hastings, Walker, & Galland, 2014) and constitute an important food and economic resource, particularly in developing countries (Blaber et al., 2005; Russ & Alcalá, 1989). Most snapper species occur in reef or other structure-associated habitats and exhibit a suite of life-history traits (e.g., slow growth, site fidelity, seasonal spawning migrations) that make them especially susceptible to overexploitation (Coleman et al., 2000; Musick, 1999), particularly given advances in fishing technology (Dayton, Thrush, Agardy, & Hofman, 1995). The family Lutjanidae comprises approximately

110 species (Eschmeyer & Fong, 2015), most of which are found in the Indo-Pacific (IP) biogeographic region and in the subfamily Lutjaninae (Allen, 1985).

Gold, Voelker, & Renshaw (2011) inferred phylogenetic relationships among 20 nominal species of lutjanines, 12 from the western Atlantic Ocean (WA), one from the eastern Pacific Ocean (EP), and seven from the IP, based on 2206 base pairs of coding mitochondrial (mt) DNA. Monophyly of all lutjanines sampled was strongly supported as were three clades that included all lutjanines from the WA. The phylogenetic hypothesis was consistent with the hypothesis of Miller and Cribb (2007) that lutjanines in the WA were derived from an IP lineage; the phylogenetic hypothesis also indicated that all lutjanines sampled are not reciprocally monophyletic in regions (IP, EP, and WA) in which they are found.

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In this study, we acquired sequences from the same mtDNA genes used by Gold et al. (2011) from eight of the 10 lutjanines endemic to the EP and from one species (*Lutjanus notatus*) from the southwestern Indian Ocean (IO). We sought to answer the following questions. First, what are the phylogenetic relationships within Lutjanidae of endemic EP lutjanines, including monotypic *Hoplopagrus (guentherii)*? In the study by Gold et al. (2011), relationships of three unknown, EP lutjanine species, sold as ‘red snapper’ in a market in Puerto Armuelles, Panama, were shown to belong to three different clades that included WA lutjanines. A second set of questions consequently involved whether there were additional lutjanine clades with species on either side of the Isthmus of Panama, and if so, how many contained geminate species pairs (*sensu* Jordan, 1908) and which species and clades were involved? Third, assuming there are multiple geminate species pairs, how much diversification in life-history and other traits in each species pair has occurred since the rise of the Isthmus and/or closure of the Panamanian Gateway and how much diversification has occurred among lutjanines on the western side of the Isthmus? Finally, we included *L. notatus* in the study because the horizontal blue stripes in this species are very similar to the horizontal, blue stripes found in *Lutjanus viridis*, one of the species endemic to the EP. The two species have limited distributions and are at opposite ends of the Indian and Pacific oceans. Also included in phylogenetic analysis were orthologous mtDNA sequences from two other species, *Lutjanus bengalensis* (IP) and *Lutjanus kasmira* (IP) that possess horizontal blue stripes, and mitochondrially encoded cytochrome *b* (*cytb*) sequences of *Lutjanus quinquelineatus* (IP) and *Lutjanus fulvus* (IP). The former possesses horizontal blue stripes and is related phylogenetically to *L. kasmira* (Miller & Cribb, 2007); the latter does not possess horizontal blue stripes but was the closest relative to *L. kasmira* and *L. quinquelineatus* in the study by Miller & Cribb (2007). A fourth question was whether species with horizontal blue stripes (the ‘blue-lined complex’ *sensu* Miller & Cribb, 2007) formed a monophyletic clade.

Materials and methods

Tissues, primarily fin clips from the caudal fin, of seven species (*H. guentherii*, *Lutjanus aratus*, *Lutjanus argentiventris*, *Lutjanus colorado*, *Lutjanus guttatus*, *Lutjanus novemfasciatus*, and *L. viridis*) were obtained in August 2010 at fish markets in La Paz, Baja California Sur. Most except *L. aratus* (sampling location unknown) were caught by artisanal fishers working in the Sea of Cortez near El Sargento and La Ventana in the Municipality of San Juan de los Planes. Fin clips were stored in DMSO buffer (Seutin, White, & Boag, 1991) and returned to the

laboratory. Tissue (muscle) preserved in 95% ETOH from *Lutjanus inermis* (EP) and *L. notatus* was obtained from the Marine Vertebrate Collection, Scripps Institution of Oceanography, U.C., San Diego. Sample locations and GenBank accession numbers for all individuals sequenced in this study and for two species (one pomacanthid and one chaetodontid) used as outgroups in phylogenetic analysis are given in Table 1. GenBank numbers and provenance of mtDNA sequences of other lutjanid species and of other species outside of Lutjanidae (used as outgroups in initial phylogenetic analyses) are given in Table 1 of Gold et al. (2011), as are GenBank numbers for mtDNA sequences of one individual of *L. guttatus* (identified then as *L. sp. #1*), one individual of *L. colorado* (identified then as *L. sp. #2*), and four individuals of *Lutjanus peru*, one individual of which was then identified as *L. sp. #3*. Methods of DNA extraction, polymerase-chain-reaction (PCR) amplification of three, protein-coding mtDNA genes – cytochrome *c* oxidase subunit 1 (COI), cytochrome *b* (*cyt b*), and NADH-dehydrogenase subunit 4 (ND-4) – and DNA sequencing of PCR-amplified fragments were the same as in Gold et al. (2011), with the exception of the *cyt b* gene in *H. guentherii* where non-specific binding of the original reverse primer required the use of internal, species-specific primers HGCBF1- TGGCAAGCCTACGCAAACC and HGCBR2 ATGAATGGGTGTTTCGACAGGTAT. Sequences were edited with Sequencher, v. 3.0 (Gene Codes) and aligned across all taxa; resulting consensus fragments were 652 bases (COI), 964 bases (*cyt b*), and 590 bases (ND-4).

As in Gold et al. (2011), orthologous sequences from two species of Haemulidae, two species of Lethrinidae, and four species of Sparidae were used as outgroups in initial phylogenetic analysis to identify the appropriate root to infer relationships within Lutjanidae. A second analysis, based on a recent classification of bony fishes (Betancur et al., 2013), employed the two species of Haemulidae, one species of Pomacanthidae, and one species of Chaetodontidae as outgroups. The species *Pterocaesio tile* (Caesioninae, Caesionidae) was included in both datasets as two prior phylogenetic studies (Chu, Rizman-Idid, & Ching, 2013; Miller & Cribb, 2007) that employed mtDNA sequences placed *P. tile* firmly within Lutjanidae. Bayesian and maximum-likelihood analyses of concatenated sequences of all three mtDNA coding genes, with haemulids, lethrinids, and sparids as outgroups (90 terminals), and with the haemulids, the pomacanthid, and the chaetodontid as outgroups (86 terminals) were implemented. Bayesian analysis employed MrBayes 3.2.2. Following recommendations in Brandley, Schmitz, and Reeder (2005), data were partitioned by 1st, 2nd, and 3rd codons of *cyt b*, ND-4, and COI, and given a separate mutation model as follows: HKY+I+G, HKY+I, GTR+I+G, HKY+G, HKY+I, GTR, HKY+I, HKY, HKY+G, respectively (Hasegawa, Kishino, & Yano,

Table 1. Collection localities and GenBank accession numbers for species examined in the study: SIO = Scripps Institution of Oceanography.

Species	ID	Location	Genbank ND-4	GenBank COI	GenBank cyt <i>b</i>
Lutjanidae					
Eastern Pacific Ocean					
<i>Hoplopagrus guentherii</i>	1	Baja California Sur ¹	KJ570944	KJ557476	KJ570968
	2	Baja California Sur ¹	KJ570945	KJ557447	KJ570969
	3	Baja California Sur ¹	KJ570946	KJ557448	KJ570970
<i>Lutjanus aratus</i>	1	La Paz Market ²	KJ570938	KJ557440	KJ570962
	2	La Paz Market ²	KJ570939	KJ557441	KJ570963
	3	La Paz Market ²	KJ570940	KJ557442	KJ570964
<i>Lutjanus argentiventris</i>	1	Baja California Sur ¹	KJ570929	KJ557431	KJ570953
	2	Baja California Sur ¹	KJ570930	KJ557432	KJ570954
	3	Baja California Sur ¹	KJ570931	KJ557433	KJ570955
<i>Lutjanus colorado</i>	1	Baja California Sur ¹	KJ570936	KJ557438	KJ570960
	2	Baja California Sur ¹	KJ570937	KJ557439	KJ570961
<i>Lutjanus guttatus</i>	1	Baja California Sur ¹	KJ570923	KJ557425	KJ570947
	2	Baja California Sur ¹	KJ570924	KJ557426	KJ570948
	3	Baja California Sur ¹	KJ570925	KJ557427	KJ570949
<i>Lutjanus inermis</i> (SIO 08-135) (SIO 09-372) (SIO 09-137)	1	Baja California Sur ³	KJ570926	KJ557428	KJ570950
	2	Baja California Sur ¹	KJ570927	KJ557429	KJ570951
	3	Baja California Sur ¹	KJ570928	KJ557430	KJ570952
<i>Lutjanus novemfasciatus</i>	1	Baja California Sur ¹	KJ570941	KJ557443	KJ570965
	2	Baja California Sur ¹	KJ570942	KJ557444	KJ570966
	3	Baja California Sur ¹	KJ570943	KJ557445	KJ570967
<i>Lutjanus viridis</i>	1	Baja California Sur ¹	KJ570932	KJ557434	KJ570956
	2	Baja California Sur ¹	KJ570933	KJ557435	KJ570957
	3	Baja California Sur ¹	KJ570934	KJ557436	KJ570958
Indo Pacific					
<i>Lutjanus fulvus</i>	1				DQ784756.1
<i>Lutjanus quinquelineatus</i>	1				DQ784760.1
Western Indian Ocean					
<i>Lutjanus notatus</i> (SIO 04-51)	1	South Africa	KJ570935	KJ557437	KJ570959
Caesionidae					
<i>Pterocaesio tile</i> (Indo-Pacific)			AP004447 (complete mtDNA sequence)		
Chaetodontidae					
<i>Chaetodon auripes</i> (Western Pacific)			AP006004.1 (complete mtDNA sequence)		
Pomacanthidae					
<i>Centropyge loriculus</i> (Pacific Ocean)			AP006006.1 (complete mtDNA sequence)		

¹Sea of Cortez (Gulf of California).²Sampled at fish market; exact location unknown.³Pacific Ocean.

1985; Tavaré, 1986). Models for each partition were chosen using the Akaike Information Criterion in MrModeltest (Nylander, 2004). Each partition was allowed to have its own proportional mutation rate and branch lengths were unconstrained except by the exponential prior with a mean of 10. Two simultaneous cold chains were run for 10⁷ generations, and each was paired with 10 heated chains, with 10 swap attempts between chains per generation. Trees were sampled every 1000 generations.

Convergence of the chains was assessed using average standard deviations of split frequencies; 50% of trees were discarded as burn-in. Maximum-likelihood analysis employed PhyML 3.0 (Guindon *et al.*, 2010), with the GTR+I+G model (Tavaré, 1986) and SPR/NNI branch swapping. Branch support was assessed with 1,000 bootstrap replicates. Results of initial phylogenetic analysis confirmed that the two haemulids were the closest available outgroup (root) for Lutjanidae. Bayesian and

maximum-likelihood analyses were then rerun (as above) using only the two haemulids as outgroups (84 terminals). Finally, to incorporate two additional taxa (*L. fulvus* and *L. quinquelineatus*) for which only *cytb* sequence data were available, additional MrBayes and PhyML analyses were run as above, using the haemulids as outgroups (86 terminals).

The geographic distribution of ancestral taxa was estimated on the consensus topology from phylogenetic analysis, with the haemulids as outgroups, and using a parsimony optimization in Mesquite v2.6 (Maddison & Maddison, 2001). Branches were only allowed a single character state and characters were treated as unordered. Of the resulting optimizations and given the taxonomic sampling, we estimated the number of dispersals between regions by assuming that the most basal branches of the lutjanine clade were in the IP.

Results

DNA sequence characterization

A total of 2,206 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 clade that included all lutjanines sampled (Fig. 1), and without considering taxa with missing data (*L. fulvus* and *L. quinquelineatus*), there were 247 variable sites (229 parsimony informative) in ND-4, 219 variable sites (206 parsimony informative) in COI, and 345 (325 parsimony informative) in *cyt b*. Mean nucleotide frequency for ND-4 was A = 25.3%, C = 36.1%, G = 12.9%, T = 25.7%, values for COI were 25.1%, 28.5%, 18.7%, 27.6%, and values for *cyt b* (25.0%, 33.0%, 14.5%, 27.5%).

Inferred phylogenetic relationships

The phylogenetic hypothesis generated from the lutjanid dataset is presented in Fig. 1. Inferred relationships within the basal clade (*Apsilus* (*Etelis*, *Pristipomoides*)) were the same as in Gold et al. (2011) and are consistent with current placement (Allen, 1985; Johnson, 1980) of *Pristipomoides* within the subfamily Etelinae. The one exception to this was the analysis, using MrBayes, that included the two taxa (*L. fulvus* and *L. quinquelineatus*) with missing data; *Pristipomoides* was recovered with low posterior probability (0.65%) as the sister to all other lutjanids. The remaining species in the lutjanid dataset were placed in a strongly supported clade (Clade A, Fig. 1) that included 34 species placed by Johnson (1980) and Allen (1985) in Lutjaninae (monotypic *Hoplopagrus*, *Ocyurus*, and *Rhomboplites*, and 31 species of *Lutjanus*) and the caesionine *Pterocaesio tile*. There was moderate to strong support

(99% posterior probability, 62% bootstrap) for placement of *Hoplopagrus* at the base of Clade A (Fig. 1). Within the remainder of Clade A, there was strong support for the clade (*P. tile*, (*L. sebae* (*L. erythropterus*, *L. malabaricus*))) as sister to a strongly supported clade that contained *L. russelli* as the sister to a strongly supported clade (Clade B) that contained all the lutjanines from the EP and WA. This clade also included *L. rivulatus* and *L. fulvus* (both IP) and members of the blue-lined complex (viz., *L. bengalensis*, *L. kasmira*, *L. notatus*, *L. quinquelineatus*, and *L. viridis*). Basal relationships within Clade B remained relatively unchanged from those reported in Gold et al. (2011). There were two strongly supported clades (C and D) and a third clade (E) with moderate to strong support (99% posterior probability, 73% bootstrap). There also were two, strongly supported subclades within Clade E: one with *L. colorado* at the base and one with *L. rivulatus* at the base. As in Gold et al. (2011), support for a sister-group relationship between Clades D and E remained relatively weak, effectively collapsing Clades C, D, and E into an unresolved trichotomy.

Inferred relationships within Clade C (Fig. 1) were essentially the same as in Gold et al. (2011), with five strongly supported clades or lineages essentially forming an unresolved polytomy. Three of the species from the EP were placed in Clade C as geminate species pairs with a species in the WA: *L. peru* as sister to *L. campechanus* (as in Gold et al., 2011), *L. guttatus* as sister to *L. synagris*, and *L. inermis* as sister to *O. chrysurus*. Comparison of available morphological data for *L. inermis* (Tavera & De la Cruz-Agüero, 2006) is consistent with a sister-species relationship with *O. chrysurus* as both have slender bodies, a small mouth, a long anal fin, nearly the same fin-ray counts (dorsal, anal, pectoral), and a deeply forked caudal fin. In addition, one of the few available colour photos of *L. inermis* (<http://www.discoverlife.org/mp/20q?search=Lutjanus+inermis>) reveals an intense, bright yellow area on the caudal peduncle that extends as a mid-lateral stripe to roughly the posterior base of the dorsal fin; in *O. chrysurus* the same intense yellow, mid-lateral stripe extends to the snout. The only notable difference apparent was the number of gill rakers on the lower limb of the first arch (21–22 in *O. chrysurus*, 14–15 in *L. inermis*), possibly suggesting slightly different feeding habits. Inferred relationships within Clade D were the same as in Gold et al. (2011) except for inclusion of *L. argentiventris* (EP) and its placement as sister to *L. jocu* (WA).

The two lineages (subclades) in Clade E (Fig. 1) were strongly supported: one (*L. colorado* (*L. aratus* (*L. novemfasciatus*, *L. cyanopterus*))) contained three species from the EP and one species (*L. cyanopterus*) from the WA. This clade is the only terminal lineage in the dataset that contains more than one species from the EP. All four species are large (>50 cm in total length), especially *L. novemfasciatus* and *L. cyanopterus*, and all four are

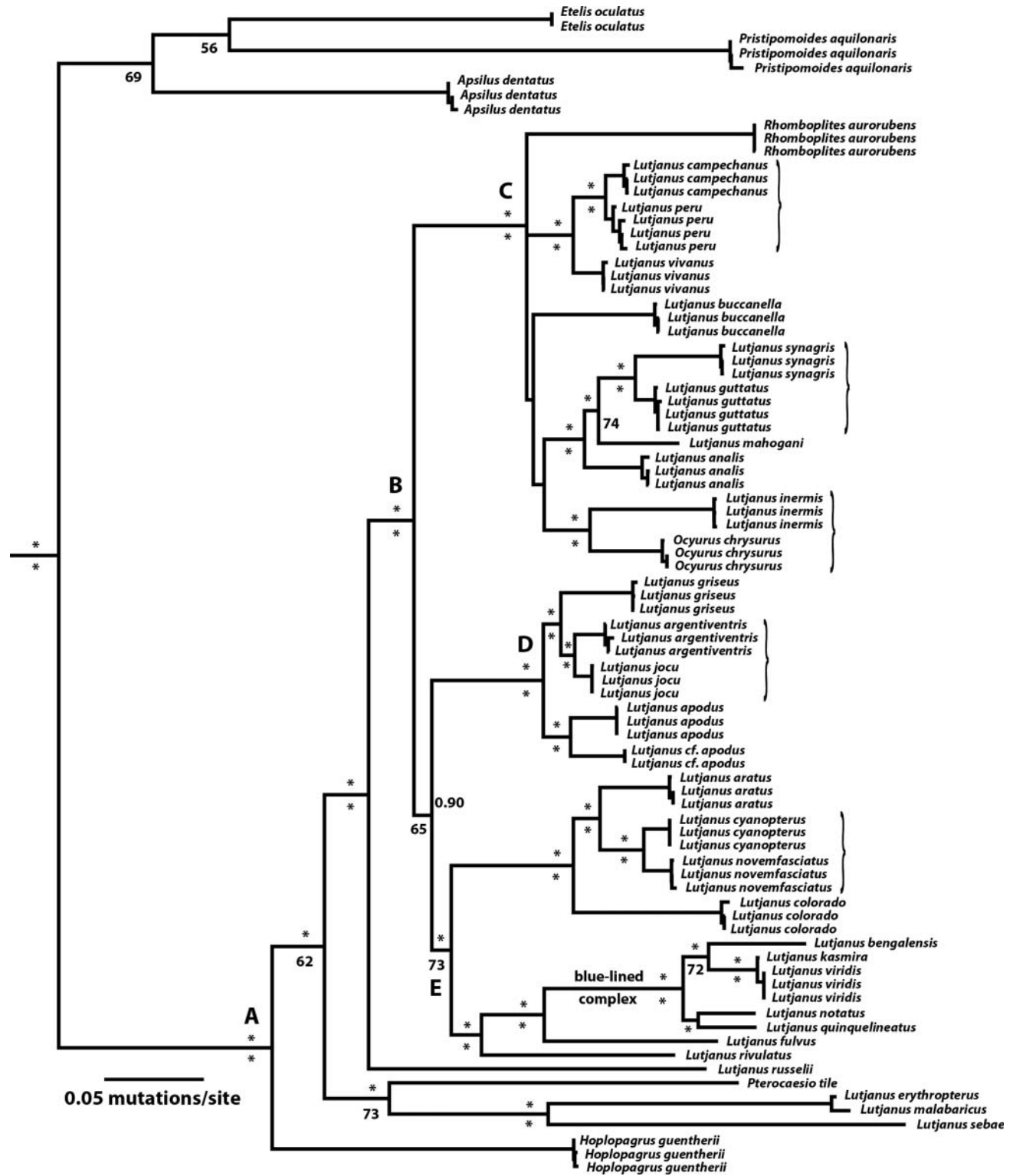


Fig. 1. Phylogenetic hypothesis (from PhyML) for eastern Pacific (EP) and western Atlantic (WA) Lutjaninae. Numbers above nodes are Bayesian posterior probabilities; numbers below nodes are maximum-likelihood, bootstrap-support percentages; asterisks indicate posterior probabilities of 0.95% or greater and bootstrap percentages of 75% or greater. Letters at nodes indicate major clades discussed in text. Brackets denote geminate species pairs on either side of the Isthmus.

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relatively drab in life colour; *L. aratus* is the most derived morphologically, differing from the other three in scale rows on the back (parallel to the lateral line in *L. aratus*, rising obliquely in the others), number of dorsal-fin spines and soft rays (11-12, 12 in *L. aratus*; 10, 13-14 in the others), and number of pectoral-fin rays (15 in *L. aratus*, 16-17 in the others). The other lineage within Clade E contained *L. rivulatus*, *L. fulvus*, and a strongly supported clade of five species ((*L. bengalensis* (*L. kasmira*, *L. viridis*) (*L. notatus*, *L. quinquelineatus*)), each possessing conspicuous blue to blueish-white stripes (the blue-lined complex *sensu* Miller & Cribbs (2007)). When examining the initial topologies, we noticed that the relative length of the branch separating *L. kasmira* from *L. viridis* was considerably shorter than branches separating any other terminal sister-species pairs in the dataset. Estimates of the nucleotide sequence difference between the five geminate species pairs in the dataset (*L. peru/L. campechanus*, *L. inermis/O. chrysurus*, *L. guttatus/L. synagris*, *L. argentiventris/L. jocu*, and *L. novemfasciatus/L. cyanopterus*) set ranged from 0.018 to 0.072 and averaged 0.037, almost an order of magnitude greater than the average nucleotide sequence difference (0.004) between haplotypes of *L. kasmira* and *L. viridis*. We then used Network v. 4.6.11 (<http://www.fluxus-engineering.com/>) to construct a median-joining network, based on 475 base pairs of homologous *cyt b* sequence, which contained the one haplotype found in *L. viridis* and 83 haplotypes of *L. kasmira* sampled from throughout most of the species' range by Gaither, Toonen, Robertson, Planes, & Bowen (2010). The *cyt b* haplotype from *L. viridis* occurred well within the distribution of *cyt b* haplotypes observed in *L. kasmira* except for those found in the Marquesas Islands (Fig. S1, see online supplemental material, which is available from the article's Taylor & Francis Online page at <http://dx.doi.org/10.1080/14772000.2015.1078857>). The latter were distinct phylogenetically from other *L. kasmira*, possibly due to unusual local conditions (Gaither et al., 2010).

Discussion

Basal relationships

Johnson (1980) considered Apsilinae to occupy an intermediate evolutionary position between Etelinae and Lutjaninae and suggested the hypothesis (Etelinae (Apsilinae (Paradicichthyinae, Lutjaninae))), with Etelinae as basal to the other three subfamilies within Lutjanidae. Based on derived characters of larval morphology, Leis (2005) hypothesized that Etelinae and Apsilinae formed a monophyletic group that was basal to all other lutjanids. The hypothesis based on analysis of mtDNA sequences (Fig. 1) is more consistent with Leis's (2005) hypothesis. However, as there are at least another 17 species of

Etelinae, eight species of Apsilinae, and two species in the subfamily Paradicichthyinae (Allen, 1985), delineation of subfamilies and their relationships within Lutjanidae awaits further study.

Several lines of evidence, including 16S, *cyt b*, and COI mtDNA sequences (Chu et al., 2013; Miller & Cribb, 2007), jaw musculature (Johnson, 1993), and larval development (Leis, 1987; Reader & Leis, 1996) indicate species currently placed in the subfamily Caesioninae (Caesionidae) may belong in Lutjanidae, and both Miller and Cribb (2007) and Chu et al. (2013) placed species of *Pterocaesio* and *Caesio* into a monophyletic Caesioninae embedded within a paraphyletic Lutjaninae. Also, Betancur et al. (2013) placed a clade of Lutjanidae and Caesionidae as sister to Haemulidae. Given that both *Ocyurus* and *Rhomboplites* should be subsumed with *Lutjanus* (Gold et al., 2011), Clade A appears to define a monophyletic Lutjaninae that includes *Hoplopagrus*, *Lutjanus*, and *P. tile* (and perhaps other caesionines).

Historical dispersal

The three species of *Lutjanus* in the clade with *P. tile* (and *P. tile*), *L. russelli*, *L. rivulatus*, *L. bengalensis*, *L. kasmira*, *L. fulvus*, and *L. quinquelineatus* are endemic to the IP; *L. viridis* is endemic to the EP, while *L. notatus* is endemic to the IO. The three lutjanine species in the WA examined by Miller & Cribb (2007) formed a strongly supported clade that was nested within clades of IP lutjanines. Miller & Cribb (2007) hypothesized that these WA lutjanines were derived from an IP ancestor and raised the question of whether all WA lutjanines arose from a single IP lineage. Assuming that Lutjaninae arose in the IP, which seems likely as the majority of lutjanines and lutjanids occur in the region (Allen, 1985), the parsimony optimization of the geographic distribution of ancestral taxa (Fig. 2; Fig. S2, see supplemental material online) is consistent with at least five different, eastward dispersal events from the IP: one leading to or involving *Hoplopagrus*, one involving the ancestor to Clade C, one involving the ancestor to Clade D, one involving the ancestor to the clade with *L. colorado* at the base, and one involving *L. viridis*. A map of the regions involved and arrows indicating general pathways of dispersal are shown in Fig. 3. We inferred two eastward dispersal events in Clade E given that *L. rivulatus*, *L. fulvus*, and three of the species in the blue-lined complex (*L. viridis* and *L. notatus* excepted) are endemic to the IP. Because *Hoplopagrus* is presently known only from the EP (from Mexico to Columbia), it seems plausible that there may be (or were) species referable to *Hoplopagrus* in the IP.

Eastward movement of lutjanines into the WA fits well with the inferred EP/Caribbean trans-isthmian track (Rosen, 1975). However, in three of the five lutjanine

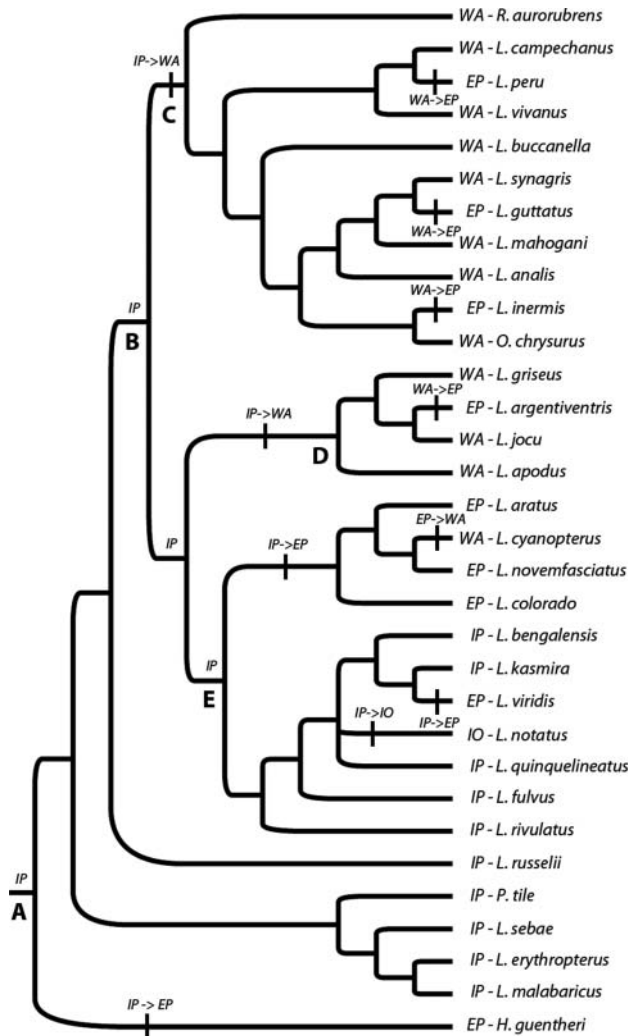


Fig. 2. Parsimony optimization of the geographic distribution of ancestral taxa; branches of the lutjanine clade preferentially resolved to be in the Indo-Pacific are indicated by IP above the branch. Bars indicate inferred dispersal events. Bold letters at nodes indicate major clades discussed in text. Area codes: IP, Indo-Pacific; EP, eastern Pacific; WA, western Atlantic; IO, western Indian Ocean.

clades containing geminate species on either side of the Isthmus, the trans-isthmian geminate species in the EP belong to clades where the basal species and other members of the clade are endemic to the WA (Fig. 2; Fig. S2, see supplemental material online), suggesting that the ancestor to each species geminate pair dispersed westward into the EP prior to cessation of seawater transfer between the two oceans. Based on the parsimony optimization (Fig. 2; Fig. S2, see supplemental material online), we inferred that *L. inermis* also dispersed eastward into the EP. Geminate species or species groups on either side of the Isthmus have been inferred from phylogenetic hypotheses of several marine fishes, including mackerels (Banford, Bermingham, Collette, & McCafferty, 1999),

centropomines (Tringali, Bert, Seyoum, Bermingham, & Bartolacci, 1999), parrotfishes (Streelman, Alfaro, Westneat, Bellwood, & Karl, 2002), needlefishes (Banford, Bermingham, & Collette, 2004), seahorses (Teske, Hamilton, Matthee, & Barker, 2007), and grunts (Roch, Lindeman, Rocha, & Lessios, 2008). The lutjanines, however, appear to be the only group to have originated in one of the ocean basins (Pacific or Atlantic), crossed through the Gateway, and then returned to the ancestral basin prior to closure of seawater exchange between the oceans. Last, the parsimony optimization (Fig. 2; Fig. S2, see supplemental material online) also is consistent with a westward dispersal from the IP involving an ancestor to *L. notatus*. As noted in Gold *et al.* (2011), ocean regions where lutjanines are distributed (IP, EP, and WA) are not reciprocally monophyletic for the species distributed within them.

Patterns of diversification

The five geminate species pairs inferred from phylogenetic analysis belonged to five different, well-resolved lutjanine lineages. All five species pairs are very similar if not identical in morphology, size, habitat, and life colours (Allen, 1985), and except for *L. inermis/O. chrysurus*, in latitudinal distribution (Allen, 1985). The morphological similarities in the pairs *L. peru/L. campechanus* and *L. novemfasciatus/L. cyanopterus* are particularly striking, suggesting that species status may, in part, be a function of geographic separation. Along similar lines, the species *Lutjanus purpureus*, previously thought to be closely related to (and often confused with) *L. campechanus*, appears merely to be a southern extension of *L. campechanus* (Gomes *et al.*, 2008, 2012). One interesting difference was in the pattern of scale rows along the lateral line in the species pair *L. argenteiventris/L. jocu*: parallel in *L. argenteiventris* but rising obliquely above the lateral line in *L. jocu*. The latter (scale rows rising obliquely along the lateral line) is found in most lutjanines except for *Hoplopagrus*, *L. aratus*, and *L. colorado* (scales parallel to the lateral line) and *L. griseus* and *L. apodus* (scale rows are parallel to the lateral line but rise obliquely posteriorly). We regard this character as homoplasious. The two species do differ in life colours; *L. argenteiventris* is rosy red anteriorly, becoming bright yellow or orange/yellow posteriorly, while *L. jocu* is olive brownish w/ copper tinge on back.

Patterns of divergence between presumed geminate species of other organisms on either side of the Isthmus vary, with divergence in life-history parameters observed in some invertebrate groups (Jackson, Jung, Coates, & Collins, 1993; Jackson & Herrera Cubilla, 2000; Lessios, 1990) but not in others (Lessios & Weinberg, 1994; Marko & Jackson, 2001). Data on vertebrates are sparse and limited primarily to a study between congeneric

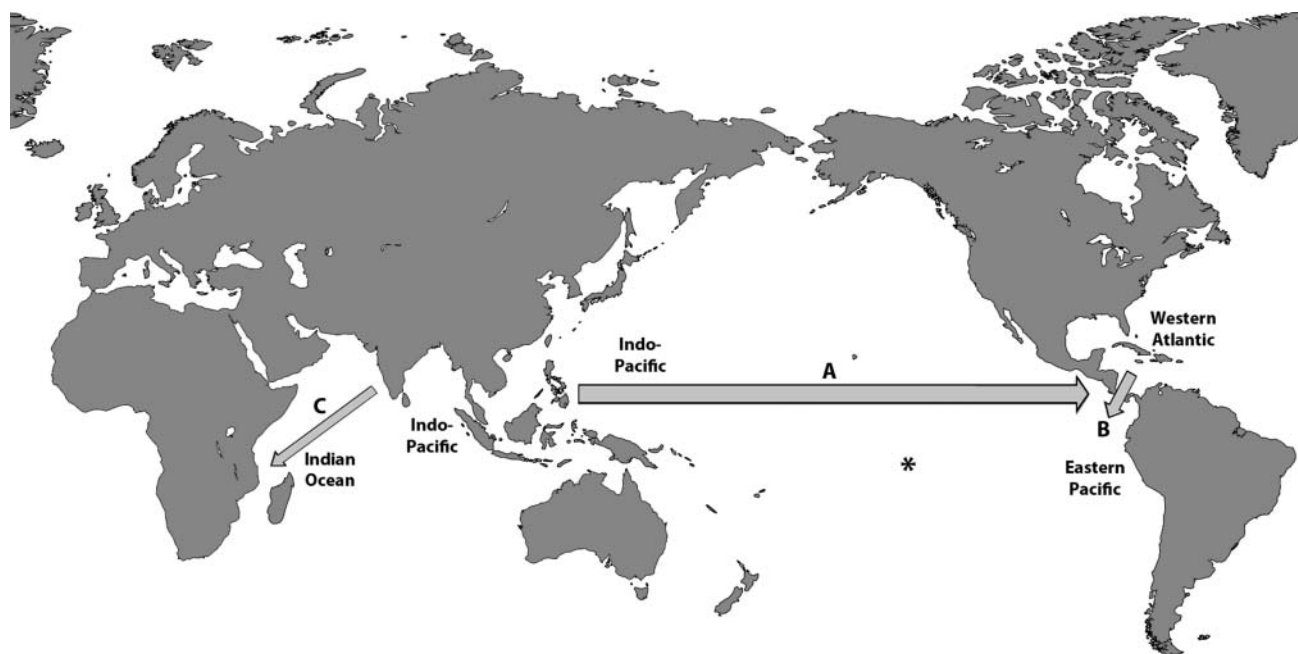


Fig. 3. Map indicating dispersal patterns of lutjanines. A, Eastward dispersals from the Indo-Pacific to the Americas, involving *Hoplopagrus*, the ancestor(s) to Clade C, the ancestor(s) to Clade D, the ancestor(s) to the clade with *L. colorado* at the base, and *L. viridis*. B, Westward dispersal from the western Atlantic (Caribbean Sea) to the eastern Pacific, involving *L. argentiventris*, *L. guttatus*, *L. inermis*, and *L. peru*. C, Westward dispersal of *L. notatus* or its ancestor from the Indo-Pacific to the Indian Ocean. Asterisk represents location of the Marquesas Islands.

species of damselfish and wrasses where differences in several life-history traits between species on either side of the Isthmus were inconsistent (Wellington & Robertson, 2001). In addition, as pointed out by Cronin (2011), a number of presumed geminate species on either side of the Isthmus have been described based on very minor differences, with similarities often ascribed to post-Isthmus exchange, raising the question of whether separation by a land barrier and a potential bias to the allopatric model of speciation (Mayr, 1970) led to separate species descriptions. This question also pertains to the morphological and mtDNA-sequence similarities between *L. kasmira* and *L. viridis* (see below), where species distinction may have been based in part of the ocean distance over which the two are separated.

The phylogenetic relationships inferred (Fig. 1) indicate considerably more divergence among lutjanines in the WA than in the EP. Gold et al. (2011) estimated that divergence within Clade B began near the end of the Oligocene and beginning of the Miocene, approximately 20 million years ago (Ma). During this time, a westward-flowing current through the Gateway was replaced by an eastward-flowing current (Bartoli et al., 2005; von der Heydt & Dijkstra, 2005, 2006; Schneider & Schmittner, 2006) that continued until the Early Pliocene (Collins, Budd, & Coates, 1996, Collins, Coates, Berggren, Aubry, & Zhang, 1996) and would have eased movement of planktonic larvae and even adults from the EP into the

WA (Caribbean Sea). The early Miocene also was a time of global warming and sea grass expansion (Brasier, 1975), and lutjanines entering the WA would have found both extensive seagrass beds in shallower water, large coral reefs, and hard bottoms in deeper water, habitats preferred by most lutjanines during various life-history phases (Allen, 1985) and not commonly found in the EP (Domning, 2001; Glynn, 1972; Larkum & den Hartog, 1989; Leigh, O’Dea, & Vermeij, 2014). The growing land bridge could then have tended to further separate EP habitats with productive, somewhat turbid, waters rich in plankton from WA (Caribbean) habitats, resulting in smaller, faster-growing, shorter-lived organisms in the EP and larger, slower-growing, longer-lived ones (e.g., lutjanids) in the WA (Leigh et al., 2014).

Blue-lined complex

The five species that possess blue stripes (*L. bengalensis*, *L. kasmira*, *L. notatus*, *L. quinquelineatus*, and *L. viridis*) clearly form a monophyletic clade that includes species distributed from the IO (*L. notatus*) to the EP (*L. viridis*). This clade, the blue-lined complex (*sensu* Miller & Cribb, 2007), also likely includes *Lutjanus coeruleolineatus* from the IO and seas surrounding the southern end of the Arabian Peninsula, as it too possesses the unusual blue stripes (Allen, 1985). Assuming an origin in the IP,

inferred relationships among the five species suggest dispersal westward to the IO (*L. notatus*) and eastward, ultimately to the EP (*L. viridis*). The genetic similarity of the *cyt b* haplotype of *L. viridis* (EP) to those of *L. kasmira* (IP) is curious, given that *L. kasmira* and *L. viridis* are typically restricted to coral reef habitat and that the open-ocean distance between the eastern-most locality where *L. kasmira* is found is 4,700 km to the west of the American mainland (Gaither *et al.*, 2010). The two species also are nearly identical in a number of morphological characters (e.g., well-developed pre-opercular notch and knob; number of dorsal-, anal-, and pectoral-fin rays; tongue without teeth; posterior profile of dorsal and anal fins; shape of caudal fin; shape of vomerine tooth patch) and in life colours (Allen, 1985). The only reported differences we could find between the two species were in a few morphometric measurements involving the snout, pre-orbital, maxilla, and second anal spine (USCFF, 1893). Based on the above, the question would seem to be not whether *L. kasmira* and *L. viridis* are close relatives but whether they are distinct species. Certainly, the open-ocean distance over which the two are separated suggests that gene flow should be limited. Alternatively, *L. kasmira* is regarded as a very highly dispersive species (Gaither *et al.*, 2010; Muths, Gouwsb, Mwale, Tessierc, & Bourjea, 2012).

Additional considerations

A number of deeper nodes in the phylogenetic hypothesis were not clearly resolved; included were the trichotomy of Clades C, D, and E, and the unresolved polytomy of five lineages within Clade C. Addition of nuclear (nDNA) sequences potentially could help resolve these nodes if the rate of mtDNA evolution led to recurrent substitutions at single-base positions (saturation) that obscured phylogenetic signal (Zink & Barrowclough, 2008). However, short internodes are expected if rapid divergence occurred, and the unresolved nodes in the inferred phylogeny appear to be near the point in time when diversification of lutjanines in the Americas began. In general, mtDNA sorts comparatively faster than nDNA and is more likely to provide resolution of nodes that diverged over short periods of time (Zink & Barrowclough, 2008). Phylogenies resulting from nDNA sequences also are not necessarily better resolved than those inferred from mtDNA sequences (Fisher-Reid & Wiens, 2011; Near & Cheng, 2008).

Two recent studies (Bacon *et al.*, 2015; Montes *et al.*, 2015) have reopened the debate regarding the chronology of closure of the Panamanian Gateway (also referred to as the Central American Seaway or CAS) and cessation of exchange of biota between the EP and WA. Montes *et al.* (2015) found a unique, Panamanian-Eocene detrital zircon fingerprint in mid-Miocene fluvial and shallow marine

strata in the northern Andes that was absent in underlying lower Miocene and Oligocene strata, and suggested existence of a mid-Miocene (15–13 Ma) fluvial connection between the Panama arc and South America; they hypothesized that the CAS had vanished by that time. Bacon *et al.* (2015) used a migration-rates model, based on molecular and fossil data in light of geological evidence, to identify significant waves of dispersal of terrestrial organisms and suggested that corresponding events separating marine organisms between the two oceans occurred at ~23 and ~7 Ma. They also found that migration rates in marine organisms decreased by 50% after a third inferred shift at ~2 Ma, suggesting complete closure between the oceans may have been near the end of the Pliocene. Bacon *et al.* (2015) hypothesized that full closure of the CAS occurred by 10 Ma but acknowledged evidence (e.g., Coates, Collins, Aubry, & Berggren, 2004; Haug, Tiedemann, Zahn, & Ravelo, 2001; Sepulchre *et al.*, 2014) that continued water exchange between the Pacific and Atlantic (Caribbean) could have continued for many millions of years along channels other than the main gateway. Suggested dates when seawater exchange between the oceans ceased generally occur around the late Miocene to early Pliocene (Coates *et al.*, 2004; Collins, Budd, & Coates, 1996; Collins, Coates *et al.*, 1996; Haug *et al.*, 2001; Jackson & O’Dea, 2013).

In our prior paper (Gold *et al.*, 2011) we used a divergence rate for cytochrome oxidase 1 (COI) sequences of 1.2% per million years, a rate calibrated from 19 geminate fish species pairs, including lutjanines, under the assumption of a ~3.0 Ma closing of the Isthmus (Bermingham, McCafferty, & Martin, 1997). Divergence within Clade B and the ancestors to most of the lutjanine species in the Americas was estimated to begin ~20 Ma (early Miocene), a time of significant palaeoceanographic change that impacted oceanic circulation and biogeographic distributions (Keller & Barron, 1983) and included opening of the Gateway (Sumata, Minobe, Motoi, & Chan, 2004) and active water circulation between the two oceans (Duque-Caro, 1990). Arrival of these lutjanines into the EP and WA in the early Miocene and the habitat changes that accompanied or promoted lutjanine radiation in the WA were discussed above. Estimated divergence times within clades containing primarily lutjanines in the WA were ~10 Ma (Clade C) and ~4.5 Ma (Clade D). These estimates coincide roughly with the hypothesized ~10 Ma closure of the CAS (Bacon *et al.* (2015) and the suggested dates (late Miocene-early Pliocene) when seawater exchange between the oceans ceased.

The above is not intended to be in support of cessation of seawater exchange between the two oceans at ~3.0 Ma. The most critical consideration in using molecular data to estimate divergence times is calibration (Ho, 2008). Assuming, for example, that the Miocene-Pliocene border (~5.3 Ma) was the time when seawater exchange

between the oceans ceased, and decreasing the sequence divergence rate used in Gold et al. (2011) accordingly (to 0.67%/million years, adjusting from 3.0 to 5.3 Ma), divergence within Clades B, C, and D would be further back in time at ~30 Ma, ~15 Ma, and 6.8 Ma, respectively. These divergence times within Clades C and D are not discordant with an open gateway at ~15 Ma (Clade C) and the ~7.0 Ma separation date (Clade D) proposed by Bacon et al. (2015). The divergence time within Clade B (~30 Ma) is early- to mid-Oligocene, a period of major global cooling when marine biotic provinces became fragmented and there was a worldwide decline in marine invertebrates (Ivany, Patterson, & Lohmann, 2000; Miller, Fairbanks, & Mountain, 1987). Even older dates would then be implied for divergence of other IP lutjanines and for the inferred dispersal of *Hoplopagrus* to the EP. However, a marine biodiversity hotspot in the Indo-Pacific appears to have originated during the Oligocene, possibly as a function of increased habitat diversity (Mihaljević, Renema, Welsh, & Pandolfi, 2014), and may have precipitated an increase in cladogenesis among coral-reef fishes during Oligocene/Miocene times (Cowman & Bellwood, 2011). In short, either rate of COI evolution (0.67% or 1.2%/million years) in lutjanines is not inconsistent with available palaeoceanographic or palaeogeological data. One last point to note is that rates of base-pair substitution are not necessarily consistent over time and can vary considerably among nucleotide sites, genes, species, and lineages (Ho, 2009; Lanfear, Welch, & Bromham, 2010). It also appears that base-pair substitution rates can be related to the time-scale over which they are measured (Soubrier et al., 2012 and references therein). Regardless of the present caveats and uncertainties, lutjanines and other marine groups with geminate species pairs on either side of the Isthmus are fertile ground in the debate over the timing of geographic events surrounding final closure of seawater connections between the two oceans. Continued palaeoceanographic and palaeogeological efforts and acquisition of larger numbers of independently evolving DNA sequences over a broader swath of taxa are to be encouraged.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

Supplemental data

Supplemental data for this article can be accessed here.

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Figure S1. Median-joining network, based on 475 base pairs of homologous *cyt b* sequence, of 83 haplotypes of *Lutjanus kasmira* and one haplotype of *Lutjanus viridis*. Each node (circle) represents a unique haplotype; sizes of nodes are scaled to reflect the relative frequency of each haplotype. Lengths of lines connecting haplotypes reflect number of single-base substitutions between haplotypes; the shortest line is one base-pair substitution. Small nodes indicated by small, black circles are inferred mtDNA haplotypes.

Figure S2. Parsimony optimization of geographic distribution of ancestral lineages on the consensus cladogram, generated using MESQUITE (see text). The most parsimonious optimization has eleven steps; and nodes with multiple equally optimal states are indicated with multiple colors.

