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 & Alcala, 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.
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 generic species pairs (sensu Jordan, 1908) and which species and clades were involved? Third, assuming there are multiple generic 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 argenteiventris, 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 (cytb), 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 cytb gene in H. guentherii where non-specific binding of the original reverse primer required the use of internal, species-specific primers HGCBF1- TGGCAAGCCTACGCAAAACC and HGCBR2 ATGAATGGGTGTTCGACAGGTAT. Sequences were edited with Sequencher, v. 3.0 (Gene Codes) and aligned across all taxa; resulting consensus fragments were 652 bases (COI), 964 bases (cytb), 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, lethriniids, 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 cytb, 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, 1985).
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 $10^7$ 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+$\Gamma$+$I$ 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

**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 b |
|--------------------------|------|---------------------------|--------------|-------------|--------------|
| **Lutjanidae**           |      |                           |              |             |              |
| **Eastern Pacific Ocean**|      |                           |              |             |              |
| *Hoplopagrus guentherii* | 1    | Baja California Sur$^{1}$ | KJ570944     | KJ557476    | KJ570968     |
|                          | 2    | Baja California Sur$^{1}$ | KJ570945     | KJ557447    | KJ570969     |
|                          | 3    | Baja California Sur$^{1}$ | KJ570946     | KJ557448    | KJ570970     |
| *Lutjanus aratus*        | 1    | La Paz Market$^{2}$       | KJ570938     | KJ557440    | KJ570962     |
|                          | 2    | La Paz Market$^{2}$       | KJ570939     | KJ557441    | KJ570963     |
|                          | 3    | La Paz Market$^{2}$       | KJ570940     | KJ557442    | KJ570964     |
| *Lutjanus argentiventris*| 1    | Baja California Sur$^{1}$ | KJ570929     | KJ557431    | KJ570953     |
|                          | 2    | Baja California Sur$^{1}$ | KJ570930     | KJ557432    | KJ570954     |
|                          | 3    | Baja California Sur$^{1}$ | KJ570931     | KJ557433    | KJ570955     |
| *Lutjanus colorado*      | 1    | Baja California Sur$^{1}$ | KJ570936     | KJ557438    | KJ570960     |
|                          | 2    | Baja California Sur$^{1}$ | KJ570937     | KJ557439    | KJ570961     |
| *Lutjanus guttatus*      | 1    | Baja California Sur$^{1}$ | KJ570923     | KJ557425    | KJ570947     |
|                          | 2    | Baja California Sur$^{1}$ | KJ570924     | KJ557426    | KJ570948     |
|                          | 3    | Baja California Sur$^{1}$ | KJ570925     | KJ557427    | KJ570949     |
| *Lutjanus inermis* (SIO 08-135) | 1 | Baja California Sur$^{1}$ | KJ570926     | KJ557428    | KJ570950     |
| (SIO 09-372)             | 2    | Baja California Sur$^{1}$ | KJ570927     | KJ557429    | KJ570951     |
| (SIO 09-137)             | 3    | Baja California Sur$^{1}$ | KJ570928     | KJ557430    | KJ570952     |
| *Lutjanus noveofasciatus*| 1    | Baja California Sur$^{1}$ | KJ570941     | KJ557443    | KJ570965     |
|                          | 2    | Baja California Sur$^{1}$ | KJ570942     | KJ557444    | KJ570966     |
|                          | 3    | Baja California Sur$^{1}$ | KJ570943     | KJ557445    | KJ570967     |
| *Lutjanus viridis*       | 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**         |      |                           |              |             |              |
| *Lutjanus fulvus*        | 1    |                           |              |             |              |
| *Lutjanus quinquelineatus*| 1   |                           |              |             |              |
| **Western Indian Ocean** |      |                           |              |             |              |
| *Lutjanus notatus* (SIO 04-51) | 1 | South Africa             | KJ570935     | KJ557437    | KJ570959     |
| **Caesionidae**          |      |                           |              |             |              |
| *Pterocaesio tile* (Indo-Pacific) |   |                           |              |             |              |
| **Chaetodontidae**       |      |                           |              |             |              |
| *Chaetodon auripes* (Western Pacific) |  |                           |              |             |              |
| **Pomacanthidae**        |      |                           |              |             |              |
| *Centropyge loriculus* (Pacific Ocean) | |                           |              |             |              |

1Sea of Cortez (Gulf of California).
2Sampled at fish market; exact location unknown.
3Pacific Ocean.
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 (Apislius (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 Hoplopogrus, Ocyrurus, 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 Hoplopogrus 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 gerninate 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. argenteiventris (EP) and its placement as sister to L. fasciatus (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.
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. bangalensis* (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 gennmate species pairs in the dataset (*L. peru*/*L. campechanus, L. inermis/*O. chrysurus, L. guttatus/*L. synagris, *L. argentiventris*/*L. joco*, and *L. novemfasciatus*/*L. cyanoperus*) 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 Paradichthyinae (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 Lutjani-

**Historical dispersal**

The three species of *Lutjanus* in the clade with *P. tile* (and *P. tile*), *L. russelli*, *L. rivulatus*, *L. bangalensis*, *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 lutja-
nids 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 Hoplopag-

rurs, 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 Hoplopagrurs is presently known only from the EP (from Mexico to Columbia), it seems plausible that there may be (or were) species refer-

able 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
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. argentiventris/L. jocu: parallel in L. argentiventris 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. argentiventris 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
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 shallow 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 (CO1) 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 Hoplopragus 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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No potential conflict of interest was reported by the author(s).

**Supplemental data**

Supplemental data for this article can be accessed here.

**References**

Allen, G. R. (1985). Snappers of the world: An annotated and illustrated catalogue of lutjanid species known to date. In FAO Fisheries Synopsis No. 125 (vol. 6, pp. 1–208). Rome: Food and Agriculture Organization of the United States.

Bacon, C. D., Silvestro, D., Jaramillo, C., Smith, B. T., Chakrabarty, P., & Antonelli, A. (2015). Biological evidence supports an early and complex emergence of the Isthmus of Panama. In Proceedings of the National Academy of Sciences USA, http://www.pnas.org/content/112/19/6110.full.pdf (accessed 15 May 2015).

Banford, H. M., Bermingham, E., & Collette, B. B. (2004). Molecular phylogenetics and biogeography of transisthian and amphi-Atlantic needlefishes (Belonidae: Strongylura and Tylosurus): Perspectives on New World marine speciation. Molecular Phylogenetics and Evolution, 31, 833–851.

Banford, H. M., Bermingham, E., Collette, B. B., & McCafferty, S. S. (1999). Phylogenetic systematics of the Scomberomorus regalis (Teleostei: Scombriidae) species group: Molecules, morphology and biogeography of Spanish mackerels. Copeia, 1999, 596–613.

Bartoli, G., Santhein, M., Weinelt, M., Erlenkeuser, H., Garbe-Schönberg, D., & Lea, D. W. (2005). Final closure of Panama and the onset of northern hemisphere glaciation. Earth and Planetary Science Letters, 237, 33–44.

Bermingham, E., McCafferty, S. S., & Martin, A. P. (1997). Fish biogeography and molecular clocks: Perspectives from the Panamanian Isthmus. In T. D. Kocher, & C. A. Steppein (Eds.), Molecular systematics of fishes (pp. 113–128). San Diego, CA: Academic Press.

Betancur, R. R., Broughton, R. E., Wiley, E. O., Carpenter, K., López, J. A., Li, C., … Ortí, G. (2013). The tree of life and a new classification of bony fishes. Public Library of Science Currents Tree of Life Edition, 1. doi:10.1371/currents.tol.53ba26640df0ccaee75bb165c8c26288

Blaber, S. J. M., Dichmont, C. M., Buckworth, R. C., Badrudin, Sumiono, B., Nurhakim, S., Iksandar, B., Fegan, B., … Salini, J. P. (2005). Shared stocks of snappers (Lutjanidae) in Australia and Indonesia: Integrating biology, population dynamics and socio-economics to examine management scenarios. Reviews in Fish Biology and Fisheries, 15, 111–127.

Brandley, M. C., Schmitz, A., & Reeder, T. W. (2005). Partitioned Bayesian analyses, partition choice, and the phylogenetic relationships of scincid lizards. Systematic Biology, 54, 373–390.

Brasier, M. D. (1975). An outline history of seagrass communities. Paleontology, 18, 681–702.

Chu, C., Rizman-Idid, M., & Ching, C. V. (2013). Phylogenetic relationships of selected genera of Lutjanidae inferred from mitochondrial regions, with a note on the taxonomic status of Pinjalo pinjalo. Ciencias Marinas, 39, 349–361.

Coates, A. G., Collins, L. S., Aubry, M. P., & Berggren, W. A. (2004). The geology of the Darien, Panama, and the Miocene-Pliocene collision of the Panama arc with northwestern...
South America. Geological Society of America Bulletin, 116, 1327–1344.

Coleman, F. C., Koenig, C. C., Huntsman, G. R., Musick, J. A., Eklund, A. M., McGovern, J., … Grimes, C. B. (2000). Long-lived reef fishes: The grouper-snapper complex. Fisheries, 25, 14–21.

Collins, L. S., Budd, A. F., & Coates, A. G. (1996). Earliest evolution associated with closure of the Tropical American Seaway. Proceedings of the National Academy of Sciences USA, 93, 6069–6072.

Collins, L. S., Coates, A. G., Berggren, W. A., Aubry, M.-P., & Zhang, J. (1996). The late Miocene Panama isthmian strait. Geology, 24, 687–690.

Cowman, P. F., & Bellwood, D. R. (2011). Coral reefs as drivers of cladogenesis: Expanding coral reefs, cryptic extinction events, and the development of biodiversity hotspots. Journal of Evolutionary Biology, 24, 2534–2562.

Cronin, T. M. (2011). Geographical isolation in marine species: Evolution and speciation in Ostracoda, I. In N. Ikeya, K. Ishizaki, & T. Hanai (Eds.), Evolutionary biology of Ostracoda: Its fundamentals and applications (pp. 871–889). Amsterdam: Elsevier.

Dayton, P. K., Thrush, S. F., Agardy, M. T., & Hofman, R. J. (1995). Environmental effects of marine fishing. Aquatic Conservation: Marine and Freshwater Ecosystems, 5, 205–232.

Domning, D. P. (2001). Sireniens, seagrasses, and Cenozoic ecological change in the Caribbean. Palaeogeography, Palaeoclimatology, Palaeoecology, 166, 27–50.

Duque-Caro, H. (1990). Neogene stratigraphy, paleoceanography, paleobiogeography in northwest South America and the evolution of the Panama Seaway. Paleogeography, Palaeoclimatology, and Palaeoecology, 77, 203–234.

Eschmeyer, W. N., & Fong, J. D. (2015). Species by family/subfamily in the Catalog of Fishes. http://researcharchive.calacademy.org/research/ichthyology/catalog/SpeciesByFamily.as. (accessed 13 May 2015).

Fisher-Reid, M. C., & Wiens, J. J. (2011). What are the consequences of combining nuclear and mitochondrial data for phylogenetic analysis? Lessons from Plethodon salamanders and 13 other vertebrate clades. BioMed Central Evolutionary Biology, 11, 300.

Gaither, M. R., Toonen, R. J., Robertson, D. R., Planes, S., & Bowen, B. W. (2010). Genetic evaluation of marine biogeographic barriers: Perspectives from two widespread Indo-Pacific snappers (Lutjanus kasmira and Lutjanus fulvus). Journal of Biogeography, 37, 133–147.

Glynn, P. W. (1972). Observations on the ecology of the Caribbean and Pacific coasts of Panama. Bulletin of the Biological Society of Washington, 2, 13–30.

Gold, J. R., Voelker, G., & Renshaw, M. A. (2011). Phylogenetic relationships of tropical western Atlantic snappers in Subfamily Lutjaninae (Lutjanidae; Perciformes) inferred from mitochondrial DNA sequences. Biological Journal of the Linnean Society, 102, 915–929.

Gomes, G., Sampaio, I., & Schneider, H. (2012). Population structure of Lutjanus purpureus (Lutjanidae - Perciformes) on the Brazilian coast: Further existence evidence of a single species of red snapper in the western Atlantic. Anais da Academia Brasileira de Ciências, 84, Retrieved from http://dx.doi.org/10.1590/S0001-37652012000400013 (accessed 15 May 2015).

Gomes, G., Santos, S., Vallinoto, M., Schneider, H., Orti, G., & Sampaio, I. (2008). Can Lutjanus purpureus (south red snapper) be “legally” considered a red snapper (Lutjanus campechanus)? Genetics and Molecular Biology, 31, 372–376.

Guindon, S., Dufayard, J. F., Lefort, V., Anisimova, M., Hordijk, W., & Gascuel, O. (2010). New algorithms and methods to estimate maximum-likelihood phylogenies: Assessing the performance of PhyML 3.0. Systematic Biology, 59, 307–321, 2010.

Hasegawa, M., Kishino, H., & Yano, T. (1985). Dating of the human-ape splitting by a molecular clock of mitochondrial DNA. Journal of Molecular Evolution, 22, 160–174.

Hastings, P. A., Walker, H. J. Jr., & Galland, G. R. (2014). Fishes: A guide to their diversity. Oakland, CA: University of California Press.

Haug, G. H., Tiedemann, R., Zahn, R., & Ravelo, A. C. (2001). Role of Panama uplift on oceanic freshwater balance. Geology, 29, 207–210.

Ho, S. Y. W. (2009). An examination of phylogenetic models of substitution rate variation among lineages. Biology Letters, 5, 421–424.

Ho, S. (2008). The molecular clock and estimating species divergence. Nature Education, 1, 168.

Ivany, L. C., Patterson, W. P., & Lohmann, K. C. (2000). Cooler winters as a possible cause of mass extinctions at the Eocene/Oligocene boundary. Nature, 407, 887–890.

Jackson, J. B. C., & Herrera Cubilla, A. (2000). Adaptation and constraint as determinants of zooid and ovicell size among encrusting ascophoran cheilostome Bryozoa from opposite sides of the Isthmus of Panama. In A. Herrera, J. B. C., & Cubilla, & J. B. Jackson (Eds.), Proceedings of the 11th international bryozoology association conference (pp. 249–258). Balboa, Republic of Panama: Smithsonian Tropical Research Institute.

Jackson, J. B. C., Jung, P., Coates, A. G., & Collins, L. S. (1993). Diversity and extinction of tropical American mollusks and emergence of the Isthmus of Panama. Science, 260, 1624–1626.

Jackson, J. B. C., & O’Dea, A. (2013). Timing of the oceanographic and biological isolation of the Caribbean Sea from the tropical eastern Pacific Ocean. Bulletin of Marine Science, 89, 779–800.

Johnson, G. D. (1980). The limits and relationships of the Lutjaninae. Journal of Evolutionary Biology, 24, 133–205.

Johnson, G. D. (1993). Perciform phylogeny: Progress and problems. Bulletin of Marine Science, 52, 3–28.

Jordan, D. S. (1908). The law of geminate species. American Naturalist, 42, 73–80.

Keller, G., & Barron, J. A. (1983). Paleogeographic implications of Miocene deep-sea hiatuses. Geological Society of America Bulletin, 94, 590–613.

Leis, J. M. (1987). Review of early life history of tropical groupers (Serranidae) and snapper (Lutjanidae). In J. Polovina, &
S. Ralston (Eds.), Tropical snappers and groupers: Biology and fisheries management (pp. 189–237). Boulder, CO: Westview Press.

Leis, J. M. (2005). A larva of the eteline lutjanid, Randallichthys filamentosus (Pisces: Perciformes), with comments on phylogenetic implications of larval morphology of basal lutjanids. Zootaxa, 1008, 57–64.

Lessios, H. A. (1990). Adaptation and phylogeny as determinants of egg size in echinoderms from the two sides of the Isthmus of Panama. American Naturalist, 135, 1–13.

Lessios, H. A., & Weinberg, J. R. (1994). Genetic and morphological divergence of the isopod Excirolana on the two sides of the Isthmus of Panama. Evolution, 48, 530–548.

Maddison, W. P., & Maddison, D. R. (2001). Mesquite: A modular system for evolutionary analysis. Version 0.98. Retrieved from http://mesquiteproject.org (accessed 15 May 2015).

Marko, P. B., & Jackson, J. B. C. (2001). Patterns of morphological diversity among and within arctic bivalve species pairs separated by the Isthmus of Panama. Journal of Paleontology, 75, 590–606.

Mayr, E. (1970). Populations, species, and evolution. Cambridge, MA: Harvard University Press.

Mihaljevic, M., Renema, W., Welsh, K., & Pandolfi, J. M. (2014). Eocene-Miocene shallow-water carbonate platforms and increased habitat diversity in Sarawak, Malaysia. Palaios, 29, 378–391.

Miller, K. G., Fairbanks, R. G., & Mountain, G. S. (1987). Terrestrial oxygen isotope synthesis, sea level history, and continental margin erosion. Paleoceneography, 2, 1–19.

Miller, T. L., & Cribb, T. H. (2007). Phylogenetic relationships of some common Indo-Pacific snappers (Perciformes: Lutjanidae) based on mitochondrial DNA sequences, with comments on the taxonomic position of the Caesionidae. Molecular Phylogenetics and Evolution, 44, 450–460.

Montes, C., Cardona, A., Jaramillo, C., Pardo, A., Silva, J. C., Valencia, V., … Niño, H. (2015). Middle Miocene closure of the Central American Seaway. Science, 348, 226–229.

Msicuk, J. A. (1999). Ecology and conservation of long-lived marine animals. American Fisheries Society Symposium, 23, 1–10.

Muths, D., Gouwsb, G., Mwale, M., Tessiere, E., & Bourjea, J. (2012). Genetic connectivity of the reef fish Lutjanus kasmira at the scale of the western Indian Ocean. Canadian Journal of Fisheries and Aquatic Sciences, 69, 842–853.

Near, T. J., & Cheng, C.-H. C. (2008). Phylogenetics of nototheniid fishes (Teleostei: Acanthomorpha): Inferences from mitochondrial and nuclear gene sequences. Molecular Phylogenetics and Evolution, 47, 832–840.

Nylander, J. A. A. (2004). MrModeltest v2. Program distributed by the author. Evolutionary Biology Centre, Uppsala University.

Reader, S. E., & Leis, J. M. (1996). Larval development in the lutjanid subfamily Caesionidae (Pisces): The genera Caesio, Dipterygonotus, Gymnoctes and Petcroctes. Bulletin of the Marine Science, 59, 310–369.

Roch, L. A., Lindeman, K. C., Rocha, C. R., & Lessios, H. A. (2008). Historical biogeography and speciation in the reef fish genus Haemulon (Teleostei: Haemulidae). Molecular Phylogenetics and Evolution, 48, 918–928.

Rosen, D. E. (1975). A vicariance model of Caribbean biogeography. Systematic Zoology, 24, 431–464.

Russ, G. R., & Alcala, A. C. (1989). Effects of intense fishing pressure on an assemblage of coral reef fishes. Marine Ecology Progress Series, 56, 13–27.

Schneider, B., & Schmittner, A. (2006). Simulating the impact of the Panamanian seaway closure on ocean circulation, marine productivity and nutrient cycling. Earth and Planetary Science Letters, 246, 367–380.

Sepulchre, P., Arsoz, T., Donnadieu, Y., Dutay, J.-C., Jaramillo, C., Le Bras, J., Martin, E., Montes, C., & Waite, A. J. (2014). Consequences of shoaling of the Central American Seaway determined from modeling Nd isotopes. Paleoceanography, 29, 176–189.

Seutin, G., White, B. N., & Boag, P. T. (1991). Preservation of avian blood and tissue samples for DNA analysis. Canadian Journal of Zoology, 69, 82–90.

Soubrier, J., Steel, M., Lee, M. S. Y., Sarkissian, C. D., Guindon, S., Ho, S. W. Y., & Cooper, A. (2012). The influence of rate heterogeneity among sites on the time dependence of molecular rates. Molecular Biology and Evolution, 29, 3345–3358.

Steelman, J. T., Alfaro, M., Westneat, M. W., Bellwood, D. R., & Karl, S. A. (2002). Evolutionary history of the parrotfishes: Biogeography, ecomorphology, and comparative diversity. Evolution, 56, 961–971.

Sumata, H., Minobe, S., Motoi, T., & Chan, W-L. (2004). Impact of Panamanian Gateway opening on the global ocean circulation. In S. F. Mawatari & H. Okada (Eds.), Neo-science of natural history: Integration of geoscience and biodiversity studies. Proceedings of the International Symposium on 'Dawn of a New Natural History' (pp. 93–101). Sapporo: Hokkaido University.

Tavera, S. (1986). Some probabilistic and statistical problems in the analysis of DNA sequences. Lectures in Mathematics in the Life Sciences, 17, 57–86.

Tava, J. J., & De la Cruz-Agüero, J. C. (2006). Rediscovery of the golden snapper Lutjanus inermis (Peters, 1869) (Perciformes: Lutjanidae) in the Gulf of California. Ocean Science Journal, 41, 191–193.

Teske, P. R., Hamilton, H., Matthias, C. A., & Barker, N. P. (2007). Signatures of seaway closures and founder dispersal in the phylogeny of a circumglobally distributed seahorse lineage. BioMed Central Evolutionary Biology, 7, 138.

Tringali, M. D., Bert, T. M., Seyoum, S., Beringham, E., & Bartolacci, D. (1999). Molecular phylogenetics and ecological diversification of the transisthmian fish genus Centropomus (Perciformes: Centropomidae). Molecular Phylogenetics and Evolution, 13, 193–207.

USCFF (United States Commission of Fish and Fisheries). (1893). Report of the Commissioner for 1889-1891. Washington, D.C.: Government Printing Office.

van der Heydt, A., & Dijkstra, H. A. (2005). Flow reorganizations in the Panama Seaway: A cause for the demise of Miocene corals? Geophysical Research Letters, 32, L02609.

van der Heydt, A., & Dijkstra, H. A. (2006). Effect of ocean gateways on the global ocean circulation in the late Oligocene and early Miocene. Paleoceneography, 21, PA1011, doi:10.1029/2005PA001149.

Wellington, G. M., & Robertson, D. R. (2001). Variation in larval life history traits among reef fishes across the Isthmus of Panama. Marine Biology, 138, 11–22.

Zink, R. M., & Barrowclough, G. F. (2008). Mitochondrial DNA under siege in avian phylogeography. Molecular Ecology, 17, 2107–2121.

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