



## Skipping across the tropics: The evolutionary history of sawtail surgeonfishes (Acanthuridae: *Prionurus*)



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### ABSTRACT

Fishes described as “anti-equatorial” have disjunct distributions, inhabiting temperate habitat patches on both sides of the tropics. Several alternative hypotheses suggest how and when species with disjunct distributions crossed uninhabitable areas, including: ancient vicariant events, competitive exclusion from the tropics, and more recent dispersal during Pliocene and Pleistocene glacial periods. Surgeonfishes in the genus *Prionurus* can provide novel insight into this pattern as its member species have disjunct distributions inhabiting either temperate latitudes, cold-water upwellings in the tropics, or low diversity tropical reef ecosystems. Here the evolutionary history and historical biogeography of *Prionurus* is examined using a dataset containing both mitochondrial and nuclear data for all seven extant species. Our results indicate that *Prionurus* is monophyletic and Miocene in origin. Several relationships remain problematic, including the placement of the Australian *P. microlepidotus*, and the relationship between *P. laticlavus* and *P. punctatus*. Equatorial divergence events between temperate western Pacific habitats occurred at least twice in *Prionurus*: once in the Miocene and again in the late Pliocene/early Pleistocene. Three species with tropical affinities, *P. laticlavus*, *P. punctatus*, and *P. biafraensis*, form a clade that originated in the Pliocene. These results suggest that a variety of mechanisms may regulate the disjunct distribution of temperate fishes, and provide support for both older and younger equatorial crossing events. They also suggest that interspecific competitive exclusion may be influential in fishes with “anti-equatorial” distributions.

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### 1. Introduction

A variety of terms describe related species that are distributed outside of, and on opposing sides of, the tropics: “amphitropical,” “bipolar,” “bitemperate,” and “biantitropical” are some examples. Many of these terms are specific to certain latitudes, but for marine fishes all temperate regions (warm-temperate, temperate, and polar) are encapsulated by the term “anti-tropical” (Hubbs, 1952). Several authors have summarized this distribution pattern over time for fishes (Hubbs, 1952; Randall, 1981; Briggs, 1987; Burrige, 2002). Hubbs’ (1952) original list included mostly pelagic species and was later expanded by the inclusion of many coastal and shallow water species by Randall (1981). Furthermore, Randall (1981) included several species that occurred within

tropical latitudes, but which are restricted to cooler waters (such as those found in upwelling areas) whose temperatures are more typical of warm-temperate regions. As these examples did not strictly fit within the term “anti-tropical” as defined by Hubbs (1952), Randall (1981) coined the more inclusive term “anti-equatorial.”

The timing and mechanisms responsible for creating these disjunct distributions restricted to colder waters are poorly understood. However, several hypotheses have been suggested, including: (1) dispersal during cooler interglacial periods (Berg, 1933; Ekman, 1953; Lindberg, 1991) or via deeper, cooler waters (Hubbs, 1952; Poortvliet et al., 2013); (2) competitive exclusion of once widespread species by younger species in the tropics isolating “relict” populations at higher latitudes (Théel, 1885; Briggs, 1987); and (3) vicariance – either from the separation of larger supercontinents, or from former continuous habitats being divided during sea-level shifts (Crame, 1993). Importantly, it is possible to distinguish between the likelihood of these various hypotheses by examining the timing of divergence events. Glacial dispersal events

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would date to either Pliocene or Pleistocene glacial events. Alternatively, vicariance or competitive exclusion from the tropics would be expected to occur at older intervals, as they either must coincide with the breakup of supercontinents, or reflect sufficient time for species to originate in the tropics and subsequently exclude anti-equatorial taxa. A review by Burrige (2002) applied strict molecular clocks to a variety of pelagic and coastal fish species and found evidence for Pleistocene or Pliocene equatorial crossing events in most species, suggesting a possible influence of glacial cycles in these crossing events. However, to date, this phenomenon has not been examined with a fossil-calibrated, taxonomically complete phylogeny of any fish lineage.

Members of the surgeonfish genus *Prionurus* are easily recognized by the presence of three to seven fixed, keeled midlateral or peduncular bony plates (bestowing them the common name “sawtail surgeonfishes”), while all other surgeonfishes have either two fixed, keeled peduncular bony plates or one retractable caudal spine (Randall, 2002). While most surgeonfishes are widespread and distributed in diverse tropical regions, sawtail surgeonfishes are restricted in their distributions and commonly found in less diverse or cooler regions (Randall, 2002). While *Prionurus* was originally classified as anti-equatorial by Randall (1981), he noted that the eastern Pacific and African species inhabit lower tropical latitudes. The description of the Indonesian species, *P. chrysurus* (Randall, 2001), raised the number of lower latitude species to four, or more than half of the species in the genus. These low-latitude taxa inhabit environments that are either cooler than is typical for the tropics or less diverse than well-developed coral reef regions. For example, *P. laticlavus* and *P. punctatus* are distributed in the eastern Pacific, and *P. biafraensis* is found in the Gulf of Guinea; together these regions have some of the lowest coral and fish diversity of major coral reef regions in the world (Kulbicki et al., 2013). The fourth species that is found in lower latitudes is the Indonesian *P. chrysurus*, which is only known from the southern coastlines of the Lesser Sunda Islands (from Bali to Alor) in areas of strong cold-water upwellings (Randall, 2001; Allen and Erdmann, 2012). Furthermore, when these upwellings weaken seasonally this species appears to retreat to cooler, deeper waters (MVE pers. obs.). Only two other distantly related species of Acanthuridae are anti-equatorially distributed: *Acanthurus leucopareius* and *Naso maculatus* (Randall, 2002). Thus, it appears that *Prionurus* evolved this habitat preference independently.

Based on its distribution, *Prionurus* clearly cannot be considered a strictly anti-equatorial lineage. However, when the distribution of all species is taken into account, it is clear that this lineage has a disjunct distribution and an affinity for temperate, low diversity waters (Fig. 1). The distributional pattern of *Prionurus* is unique

compared to other anti-tropical fishes (Hubbs, 1952; Randall, 1981), and may provide notable insight into the evolutionary history of disjunct marine fishes. Here our goals are to determine the evolutionary relationships among species of *Prionurus*, and to examine the historical biogeography of this clade in relation to several biogeographical hypotheses. By reconstructing the evolution and biogeography of this genus we hope to gain a better understanding of the mechanisms responsible for the creation of disjunct distribution patterns in marine fishes.

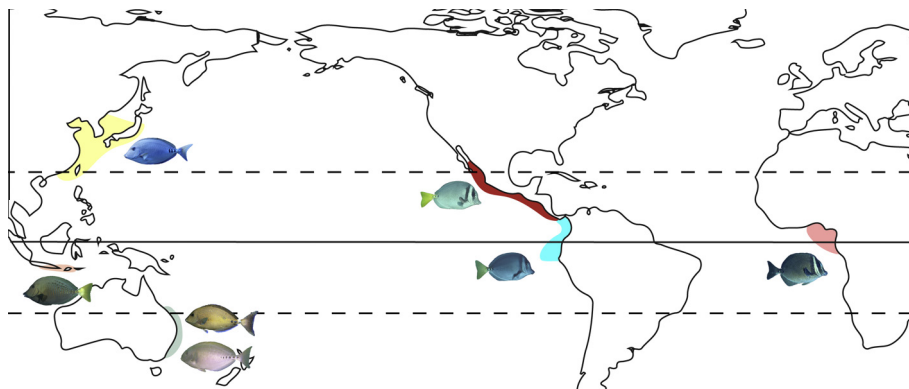
## 2. Materials and methods

### 2.1. Taxonomic sampling and extraction

Tissue samples were acquired by museum tissue loans or from our collecting efforts for all species within *Prionurus* (Table 1). Multiple individuals of each species were obtained when possible. Fin clips were stored in 95% ethanol at  $-80^{\circ}\text{C}$  prior to lab work. Genetic material was extracted from tissue samples using DNeasy Blood and Tissue extraction kits (Qiagen) following the manufacturers protocols and stored at  $-23^{\circ}\text{C}$  prior to PCR amplification. We supplemented our dataset with genetic data downloaded from GenBank for outgroup taxa. Outgroup selection followed previous publications (Klanten et al., 2004; Sorenson et al., 2013) and include representatives of Acanthuroidei including, *Luvaris imperialis* (Luvaridae), *Zanclus cornutus* (Zanclidae), and representatives of all other Acanthuridae genera: *Naso brevirostris*, *N. lituratus*, *Paracanthurus hepatus*, *Acanthurus blochii*, *Ctenochaetus striatus*, and *Zebrasoma flavescens*. These species were also chosen based on the availability of their sequences on GenBank, our goal being to have the most complete genetic data matrix possible.

### 2.2. Laboratory procedures

All specimens of *Prionurus* were amplified for two mitochondrial (16S and COI) and three nuclear (MyH6, Rag1, and Zic1) genes. Amplification of mitochondrial 16S followed procedures and primers outlined in Klanten et al. (2004), while COI was amplified following procedures and primers outlined in Ludt et al. (2012). Amplification of MyH6 and Zic1 used primers and methods outlined in Li et al. (2007), while Rag1 protocols were adapted from López et al. (2004). PCR products were verified with a 1% agarose gel using electrophoresis with SYBR Safe DNA gel stain (Invitrogen) and 6x blue/orange loading dye (Promega). All gel runs also contained a negative control (no DNA) to ensure a lack of contamination in laboratory work. Samples were sent to Beckman Coulter



**Fig. 1.** Map showing the distribution of all species of *Prionurus*. Both *P. microlepidotus* and *P. punctatus* are shown in green, *P. chrysurus* in orange, *P. scalprum* in yellow, *P. laticlavus* in dark red, *P. punctatus* in blue, and *P. biafraensis* in light red. Distributions are estimated from descriptions in Randall (2002) and museum records published on Fishnet2 (Fishnet2.net). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

**Table 1**  
Specimen information for the species used in this study. The following museum acronyms are used: KU (University of Kansas), MZB (Museum Zoologicum Bogoriense), NSMT (National Science Museum, Tokyo), ROM (Royal Ontario Museum), and SIO (Scripps Institute of Oceanography). \*denotes which individuals were used in the BEAST and \*BEAST analyses.

Species	Specimen/Tissue voucher	16S	COI	myh6	Rag1	Zic1
<i>Prionurus biafraensis</i>	N/A*	KP280474	KP280486	KP280498	KP280508	KP280516
	N/A	KP280475	KP280487	no data	KC623856	KP280517
<i>Prionurus chrysurus</i>	MZB22132	KP280476	KP280488	KP280499	KP280510	KP280518
<i>Prionurus laticlavus</i>	N/A*	KP280477	KP280489	KP280500	KP280511	KP280519
	SIO 07-188	KP280479	KP280491	KP280502	KC623857	KC623930
<i>Prionurus maculatus</i>	ROM 68379/KU:KUIT:1835	KP280480	KP280492	KC623792	no data	KC623932
<i>Prionurus microlepidotus</i>	N/A*	KP280481	KP280493	KP280503	KP280513	no data
	N/A	KP280482	KP280494	KP280504	no data	KP280522
<i>Prionurus punctatus</i>	SIO 05-56	KP280483	KP280495	KP280505	no data	no data
	SIO 07-188*	KP280484	KP280496	KP280506	KC623858	KC623931
	SIO 08-165 <sup>a</sup>	KP280478	KP280490	KP280501	no data	no data
<i>Prionurus scalprum</i>	NSMT-P 101309/NSMT-DNA 16614*	KP280485	KP280497	KP280507	KP280515	KP280524
<i>Acanthurus blochii</i>	N/A*	DQ898095	HM034180	KC623767	KC623821	KC623906
<i>Ctenochaetus striatus</i>	N/A*	EF210161	KC623675	EF536296	EF530096	EF533919
<i>Naso brevirostris</i>	N/A*	AY264607	KC623679	EF536293	EF530093	EF533916
<i>Naso lituratus</i>	N/A*	DQ532915	HM034247	EF536292	EF530092	EF533915
<i>Paracanthurus hepatus</i>	N/A*	DQ532928	KC623683	EF536297	EF530097	EF533920
<i>Zebrafish flavescens</i>	N/A*	DQ532985	KC623689	KC623795	KC623861	KC623935
<i>Zanclus cornutus</i>	N/A*	DQ532984	KC623652	EF536300	EF530100	EF533923
<i>Lavarus imperialis</i>	N/A*	DQ532902	KC623653	EF536299	EF530099	EF533922

<sup>a</sup> This specimen was cataloged as *P. laticlavus*. It was identified as *P. punctatus* after personal examination of the voucher by WBL.

Genomics for purification and sequencing on a BigDye Terminator v3.1 sequencer. All PCR products were sequenced using both the forward and reverse primers.

### 2.3. Phylogenetic inference

Sequences were edited and aligned in Geneious 6.0.5 (Biomatters) using the MUSCLE alignment plugin (Edgar, 2004). All alignments were then checked by eye. Aligned sequences were combined into files based on the individual genes, and also as a concatenated gene file with all markers present. The program jModelTest 2.1.3 (Darriba et al., 2012; Guindon and Gascuel, 2003) was used to determine the most appropriate nucleotide substitution model based on AIC scores for all individual genetic markers.

Maximum likelihood and Bayesian approaches were used to generate phylogenetic hypotheses using Garli 2.0 (Zwickl, 2006) and MrBayes (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003), respectively. A partitioned model analysis where each gene in the dataset was assigned to its own optimal nucleotide substitution model was used for each analysis using a concatenated dataset. Multiple runs were performed to ensure convergence on an optimal species tree. For the maximum likelihood analysis a bootstrap analysis was performed with 1000 replicates. The program SumTrees 3.3.1 of the DendroPy 3.8.0 package (Sukumaran and Holder, 2010) was used to summarize the bootstrap support onto the maximum likelihood tree. For the MrBayes analysis all priors were kept on default settings, and three runs were performed using a Markov Chain Monte Carlo (MCMC; Geyer, 1991) search algorithm with a chain length of 10,000,000 and four chains with sampling frequency of 500. Chain heat and swapping frequencies were kept on default settings. For each run the parameters and trees were summarized and marginal densities, effective sample sizes and run convergence were checked in Tracer 1.5 (Rambaut and Drummond, 2007). Burnin was also determined while examining trace plots. Pairwise posterior distributions of branch support were plotted using AWTY (Wilgenbusch et al., 2004) to further ensure that MrBayes analyses had run long enough to allow proper mixing. Once confidence was attained for proper run length and mixing, a maximum clade credibility tree was summarized from the program outputs. All trees for the Bayes-

ian and maximum likelihood methods were imported into FigTree 1.4 for further manipulation and final editing.

A multispecies coalescent analysis was also performed using the \*BEAST function in the BEAST 1.7.5 software package (Drummond et al., 2012). This model estimates a species tree while taking into account variation among gene trees (Heled and Drummond, 2010). Input files were created using BEAUti v1.7.5 setting nucleotide substitution model, clock model, and partition tree model to be separate for each gene, except for the mitochondrial 16S and COI genes, as they are from a single locus. Starting trees in BEAST must be strictly bifurcating and cannot contain polytomies, which were present in both the ML and Bayesian trees; therefore, a simplified tree was produced using only one individual per species to remove all unresolved nodes in the tree. We used a relaxed lognormal clock model for each gene, with a Yule process species tree prior, and a piecewise linear and constant root population size model. We performed three independent runs, each for 500,000,000 generations using a random start tree, sampling every 50,000 iterations. Each run was checked for convergence using ESS values with the program Tracer as per the MrBayes analysis above. Runs were then combined using LogCombiner and visualized with TreeAnnotator (Drummond and Rambaut, 2007) prior to final manipulation in FigTree.

### 2.4. Time calibrated phylogenies and fossil calibrations

To determine the timing of coalescent events within *Prionurus*, a time-calibrated tree was constructed with BEAST 1.7.5 (Drummond and Rambaut, 2007) using a random starting tree made from the simplified dataset that had single representatives from each species (to ensure a strictly bifurcating tree was found). All BEAST runs were conducted with appropriate substitution models partitioning the concatenated dataset. Each run was set with a Yule speciation prior, as well as an uncorrelated relaxed log-normal molecular clock. Two lognormal prior fossil calibration points were used for all runs: the fossil Luvarid, †*Kushlukia permira* (55.8 mya; Bannikov and Tyler, 1995), and the basal Nasinae, †*Sorbinithurus sorbinii* (50 mya; Papazzoni and Trevisani, 2006). These fossils were chosen as per previous studies (Klanten et al., 2004; Sorenson et al., 2013). Each analysis ran for 500,000,000 generations, sampling every 50,000 iterations. Runs were checked for

convergence and proper mixing using Tracer as mentioned above for the MrBayes runs. As for the \*BEAST analysis, runs were then combined using LogCombiner and visualized with TreeAnnotator. This time-calibrated tree was then exported to FigTree for final manipulation.

### 2.5. Determining ancestral ranges

To determine probable ancestral ranges for *Prionurus*, and to estimate possible equatorial crossing events, we used the program RASP 2.1, which implements a Bayesian Binary MCMC analysis to determine ancestral ranges on phylogenies (Yu et al., 2013). Extant species of *Prionurus* were coded using marine biogeographic provinces described by Briggs and Bowen (2012). This program requires a time-calibrated phylogeny input file. We used our majority rule consensus tree from our BEAST analysis as our input for this analysis. A null root distribution model, which states that the ancestor to the lineage did not inhabit any currently inhabited location, was chosen over the ‘widespread distribution’ option where the ancestor is assumed to have been present at all current descendent localities. This option was favored over a circumglobal distribution due to the restricted nature presently seen in members of *Prionurus*. The program ran for 1,000,000 cycles with 10 chains swapping every 100 cycles. Rates of change were estimated with equal among site variation within the data. Each analysis was checked for convergence by comparing the split frequencies between different runs.

## 3. Results

### 3.1. Sampling and laboratory procedures

All seven species of *Prionurus* were obtained for this study, and sequences for all non-*Prionurus* surgeonfishes were obtained from GenBank. A 512 bp sequence of 16S was obtained along with 535 bp of CO1, 1154 bp of Rag1, 706 bp of Zic1, and 536 bp of MyH6. All new sequences were deposited in GenBank, and accession numbers for each sequence, along with museum voucher information for each sample of *Prionurus* can be found in Table 1.

### 3.2. Phylogenetic inference and time calibration

All analyses recovered *Prionurus* as monophyletic and the topology of species within Acanthuridae was consistent with previous osteological (Guiasu and Winterbottom, 1993), myological (Winterbottom, 1993), and molecular (Klanten et al., 2004; Holcroft and Wiley, 2008; Sorenson et al., 2013) analyses (Figs. S1 and S2). Estimates obtained from the maximum likelihood analysis, and Bayesian analysis, placed *P. microlepidotus* as the sister lineage to a clade containing *P. biafraensis* + *P. laticlavus* + *P. punctatus*, albeit with low bootstrap support and posterior probabilities (Fig. 2a). Counter to this, species tree analysis using \*BEAST, as well as our time-calibrated phylogeny using BEAST, recover *P. microlepidotus* as the sister lineage to all other species of *Prionurus* with strong support (Fig. 2b), which is consistent with previous analyses (Sorenson et al., 2013). The posterior distribution of the Bayesian analysis also recovered this topology 30% of the time. There are known issues with concatenating datasets for phylogenetic analyses (reviewed in Degnan and Rosenberg, 2009), and because the BEAST and \*BEAST results are also recovered in 30% of the posterior distribution of the MrBayes trees, the topology with *P. microlepidotus* as the sister lineage to all other species of *Prionurus* was used for our ancestral range reconstruction analysis.

A Miocene (12.1 mya) crown age (the split between *P. microlepidotus* and all other congeners) of *Prionurus* was recovered. The

remaining six species of *Prionurus* were distributed between two main clades which diverged approximately 9.4 mya: one containing the western Pacific *P. chrysurus*, *P. maculatus*, and *P. scalprum*, and a second clade containing the eastern Pacific *P. laticlavus* and *P. punctatus*, as well as the eastern Atlantic *P. biafraensis* (Fig. 3). Both clades are Pliocene in origin, with the crown age of the eastern Pacific/Atlantic clade dating to 3.1 mya, and the western Pacific clade crown age dating to 4.7 mya. Estimated divergence times and 95% high posterior distributions (HPDs) can be found in Table 2.

The shallowest divergence between any two species of *Prionurus* was found between the two eastern Pacific species, *P. laticlavus* and *P. punctatus* (Fig. 3). These two species have ranges that overlap in some areas (Fig. 1) and the most recent common ancestor (MRCA) for them was estimated to be only 492 kya. Notably, when multiple individuals of each species were included in analyses the species were no longer recovered as reciprocally monophyletic (Fig. 2a), and a shared mtDNA COI haplotype was found between them. In contrast, the western Pacific *P. chrysurus* and *P. maculatus* share a MRCA at approximately 2.2 mya. It should be noted that we only had single individuals representing these two species, but given their older divergence time, and non-overlapping ranges, their species status was not in question.

### 3.3. Ancestral range reconstruction

All independent RASP runs recovered similar results, and all average standard deviation split frequencies between runs were less than 0.01, suggesting ample convergence between runs. The MRCA of all species of *Prionurus* was estimated to be southern hemisphere and eastern Australian in origin, as was the MRCA of the western Pacific and eastern Pacific/eastern Atlantic clades. The MRCA of the eastern Pacific/Atlantic clade was estimated to be either African or eastern Pacific in origin. The MRCA of the western Pacific clade was hypothesized to be Japanese in origin, while the MRCA of *P. chrysurus* and *P. maculatus* was estimated as being eastern Australian.

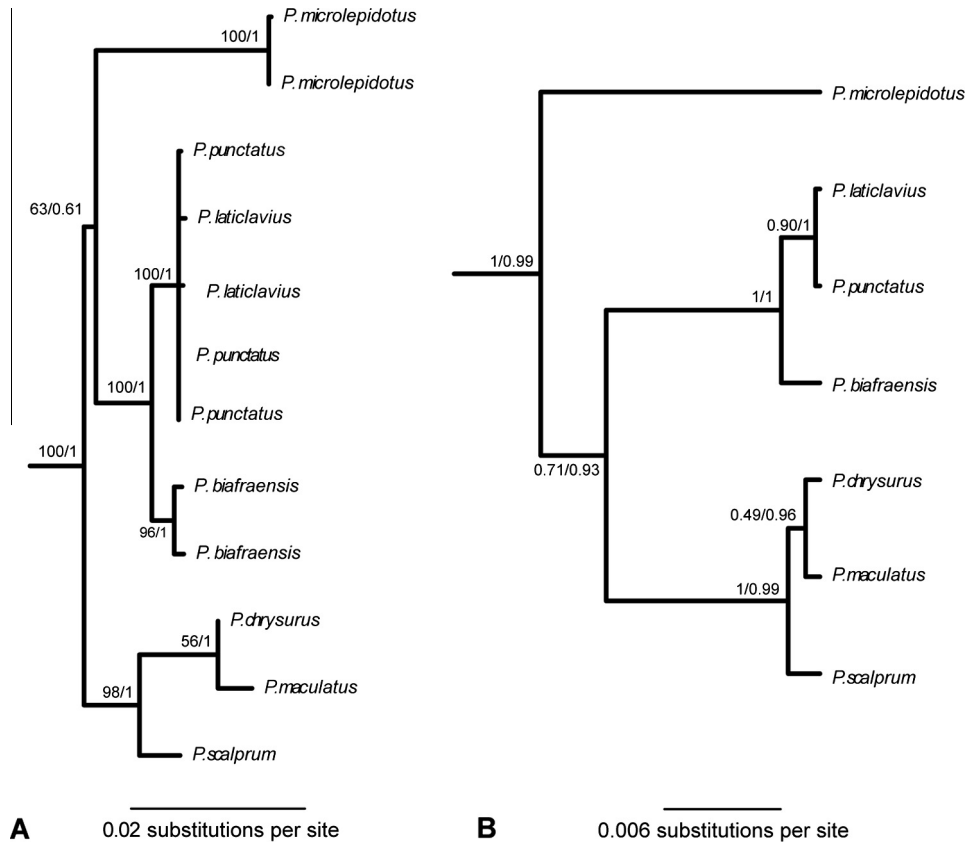
## 4. Discussion

*Prionurus* is one of several fish groups described as “anti-equatorial” that have a disjunct distribution, and here we present the first analyses of such a group with complete taxon sampling using fossil-calibrated phylogenies to test hypotheses of biogeography. This shift to temperate habitats is unique within surgeonfishes (which are mostly a tropical family with widespread species), and may provide us with a greater understanding of the timing and mechanisms responsible for speciation in disjunct coastal fishes. We focus our discussion on the general taxonomy, evolutionary timing and historical biogeography of this unique clade.

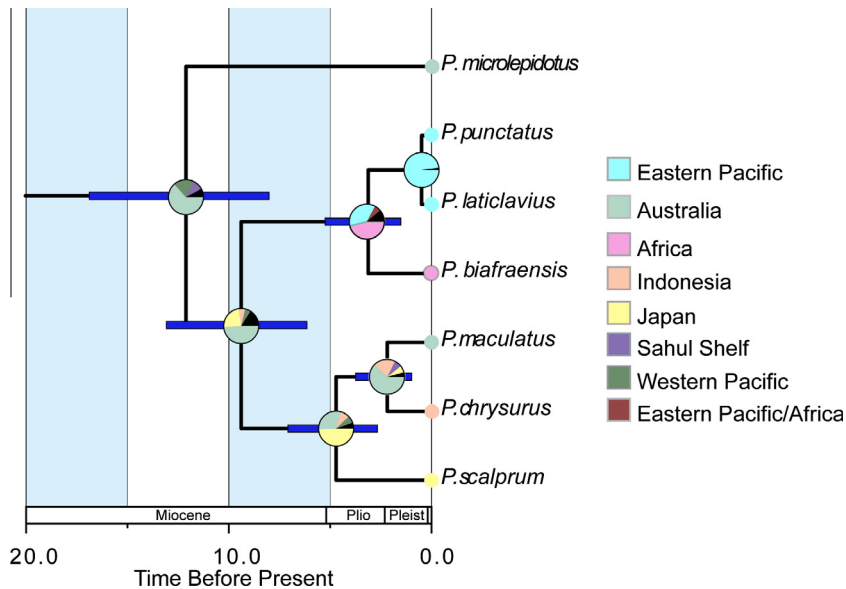
### 4.1. Uncertain relationships among several sawtail surgeonfishes

All analyses recovered a monophyletic *Prionurus* with strong support (Fig. 2). However, the relationship among several species remains unclear. In particular, the phylogenetic placement of *P. microlepidotus* varies depending on the analysis used. These results may be due to the amount of genetic variation in *P. microlepidotus* relative to all other *Prionurus*, where little genetic variation was found. Surprisingly, both Bayesian programs MrBayes and BEAST found alternative topologies. It may be that differences in some of the priors used by these analyses may also be influencing results. However, the placement of *P. microlepidotus* as the sister group to all other *Prionurus* (the topology used in the ancestral area reconstruction) was suggested previously (Sorenson et al., 2013)





**Fig. 2.** Phylogenetic estimates for *Prionurus* from four analyses. Maximum likelihood and Bayesian analyses are shown in A, and BEAST and \*BEAST multispecies coalescent analyses are shown in B. Node values represent bootstrap/posterior probabilities for the maximum likelihood and Bayesian analysis, respectively, in A, and for the posterior probabilities of the BEAST and \*BEAST analyses, respectively, in B. Discrepancies in the phylogenetic placement of *P. microlepidotus* can be seen when comparing A and B.



**Fig. 3.** Time-calibrated phylogeny of *Prionurus* using two fossil calibrations. Horizontal blue bars at nodes represent the 95% HPD intervals for each date estimate. Pie graphs at each node represent the estimated ancestral area of that node, with colors representing marine biogeographic provinces where extant species are found, or combinations of locations where species are found. Black represents areas outside of the defined locations. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

and is strongly supported in our multispecies coalescent analysis; this topology was also supported at various levels in all analyses.

Some additional ambiguity in species-level relationships was recovered in the maximum likelihood and Bayesian analyses

between the two eastern Pacific species, *P. laticlavus* and *P. punctatus*. These two species differ phenotypically by the presence or absence of dark spots on the body (Gill, 1862), and were not recovered as reciprocally monophyletic. This result may be indicative of

**Table 2**

Estimated crown ages of different clades generated from the BEAST analysis, and their associated 95% HPD intervals. All age estimates are in millions of years before present.

Clade contains	Estimated age	95% HPD
All <i>Prionurus</i>	12.12	8.01–16.88
All except <i>P. microlepidotus</i>	9.39	6.14–13.08
<i>P. chrysurus</i> , <i>P. maculatus</i> , and <i>P. scalprum</i>	4.71	2.67–7.09
<i>P. biafraensis</i> , <i>P. laticlavus</i> , and <i>P. punctatus</i>	3.13	1.51–5.25
<i>P. chrysurus</i> and <i>P. maculatus</i>	2.19	0.98–3.74
<i>P. laticlavus</i> and <i>P. punctatus</i>	0.49	0.07–1.21

three possible scenarios: (1) incomplete lineage sorting due to recent speciation, (2) recent hybridization and introgression, or (3) phenotypic variation within a single species. However, as this study only contained five individuals, greater sampling effort is needed to differentiate between these possibilities.

#### 4.2. Crossing the tropics

The timing of equatorial crossing events can differentiate between alternative hypotheses of evolutionary patterns in anti-tropical fishes, separating younger glacial crossing hypotheses (Berg, 1933; Ekman, 1953; Lindberg, 1991; Burrridge, 2002) from older vicariance or competitive exclusion hypotheses (Théel, 1885; Briggs, 1987; Crame, 1993). Glacial crossing events would coincide with Pliocene or Pleistocene glacial events, while vicariance would coincide with the breakup of supercontinents. Competitive exclusion by younger taxa would likely occur at older time scales that allow sufficient time for new species to originate in the tropics, and consequently expand their ranges. Our study finds evidence using ancestral area reconstructions that reveal two possible trans-equatorial divergence events in the western Pacific clade, with a more recent third tropical invasion involving *P. chrysurus* (a species currently restricted to cold-water upwellings in southeastern Indonesia; Fig. 3). The first divergence across the tropics by members of *Prionurus* occurred sometime in the late Miocene or early Pliocene between the southern hemisphere MRCA of the eastern and the western clades and the northern hemisphere MRCA of the western clade (Fig. 3). A second equatorial crossing likely occurred between the MRCA of the western hemisphere clade, and the MRCA of both *P. maculatus* and *P. chrysurus* in the late Pliocene or early Pleistocene (Fig. 3). Finally, there was also a Pleistocene speciation event within the last 2.19 million years that led *P. chrysurus* to inhabit cold-water upwellings within the tropics.

The earliest of these events supports older anti-equatorial hypotheses such as competitive exclusion by younger tropical species causing once widespread species to become isolated in temperate habitats (Théel, 1885; Briggs, 1987), but is too recent an event to support ancient supercontinent vicariance hypotheses (Crame, 1993). However, due to the uncertainty regarding the phylogenetic placement of *P. microlepidotus*, this ancestral state (and age estimates associated with it) should be regarded with caution. More recent events and tropical invasions occurred in the Pliocene or early Pleistocene, supporting the younger glacial crossing hypothesis regarding anti-equatorial species (Berg, 1933; Ekman, 1953; Lindberg, 1991), and supporting other studies that found similar younger crossing events in temperate disjunct coastal species (Burrridge and White, 2000; Burrridge, 2002; Mabuchi et al., 2004; Poortvliet et al., 2013). Furthermore, one species in this genus, *P. chrysurus*, is restricted to cold-water upwellings within the tropics. The isolation of one of the youngest species in this genus to cold water upwellings in the tropics argues against the relict theory that states that disjunct taxa are merely what remains of once widespread species that have been competitively excluded from the tropics (Théel, 1885; Briggs, 1987), and it seems to sug-

gest that some species of *Prionurus* may be physiologically restricted to cooler water habitats.

#### 4.3. Relicts and competitive exclusion

While Théel's (1885) relict theory does not seem to be able to explain the distribution of all *Prionurus*, the competitive exclusion underpinnings of the theory do have some resonance. *Prionurus* is unique among “anti-equatorial” groups, as three species, *P. biafraensis*, *P. laticlavus* and *P. punctatus*, seem more tolerant of warmer waters. These species form a clade that dates to the Pliocene (Fig. 3) and are only found in the relatively depauperate eastern Pacific and eastern Atlantic (Kulbicki et al., 2013). This clade originated prior to the closure of the Isthmus of Panama (assuming a closure date approximately 3 mya; Leigh et al., 2014). Directly following the closure of the Isthmus these regions became isolated from the rest of the western Pacific (Cowman and Bellwood, 2013). Periods of faunal turnover (Bellwood and Wainwright, 2002), coupled with a lack of species replenishment, have resulted in what has been deemed “reduced speciation capacity” in these areas (Cowman and Bellwood, 2013). A reduced speciation capacity may result in decreased levels of competition in these regions, possibly allowing this clade to reinvade tropical waters. The absence of *Prionurus* from tropical regions with high species richness in the western Pacific and the Caribbean, and the presence of several species in tropical regions of low biodiversity, provides support that interspecific competition may play a role in the disjunct distribution pattern of this genus.

#### 4.4. Conclusions

Sawtail surgeonfishes are restricted to the temperate waters bordering the tropics, in areas of cold-water upwelling in the lesser Sunda Islands of southeastern Indonesia, or in low diversity areas such as those found in the eastern Pacific and the Gulf of Guinea (Fig. 1). All analyses found *Prionurus* to be monophyletic, and late Miocene in origin with a crown age estimated at 12.1 mya (Fig. 3). There is evidence for both older and more recent equatorial divergence events. While the relict theory may not strictly apply to this group, competition may play an important role in the re-colonization of warmer habitats, showing that a mixture of mechanisms regulates these disjunctly distributed species. However, we also find evidence that crossing events possibly occurred during glacial cycles in the Pliocene or early Pleistocene, as other recent studies have found (Burrridge and White, 2000; Burrridge, 2002; Mabuchi et al., 2004). Whether crossing events were due to dispersal or vicariance of widespread species is difficult to discern with molecular data alone (Parenti and Ebach, 2013). However, it is possible that during glacial periods when sea levels dropped significantly, circulation patterns may have changed and formed pockets of cold-water upwelling in tropical latitudes, resulting in a “stepping stone” which would allow cool water species to cross the tropics (Lindberg, 1991). Ultimately, only an in-depth physiological approach may determine what restricts these species to their current ranges and explain what would limit or allow the equatorial crossing and colonization of new areas. Ecological, physiological, and total evidence approaches with combined molecular and morphological data would be useful in understanding the complete evolutionary history of this group, particularly for resolving the placement of *P. microlepidotus* and the relationship between *P. laticlavus* and *P. punctatus*.

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## Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.ympev.2014.12.017>.

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