Determining the Drivers of Anti-Tropical Distributions Across the Fish Tree of Life

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DETERMINING THE DRIVERS OF ANTI-TROPICAL DISTRIBUTIONS ACROSS THE FISH TREE OF LIFE

A Dissertation

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
In partial fulfillment of the
Requirements for the degree of
Doctor of Philosophy

in

The Department of Biological Sciences

by

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B.S., University of Arizona, 2005
M.S., University of Texas at Austin, 2011
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To my parents, Bill and Cydnee Ludt, for their guidance and unwavering support.
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ABSTRACT

Anti-tropical distributions are those where populations of a single species, or multiple closely related taxa, are distributed outside of, and on opposing sides of, the tropics. These latitudinally disjunct distributions have been noted for over a century. Despite this long history of interest, little has been concluded regarding the actual mechanisms that drive this pattern, with several prominent hypotheses competing with one another in the literature. Here I review the proposed drivers of anti-tropicality, and subsequently test them using fishes with a variety of life history and taxonomic differences. This includes (1) a temperately restricted family with anti-tropical distributions – Cheilodactylidae, (2) a tropical reef fish family with a single temperate anti-tropical genus – Prionurus, and (3) a variety of fishes from across the fish tree of life that have populations split by the tropics. Using complete taxonomic sampling, and phylogenomic approaches coupled with fossil calibration points, I find evidence for recent equatorial divergence events in the Pleistocene and Pliocene, as well as divergence events dating to the Miocene for both Cheilodactylidae and Prionurus. Furthermore, taxonomic issues were detected, and explored within both of these groups. To disentangle the multiple hypotheses that can explain recent transitions, I used ecological niche models coupled with extant distributional data for a variety of species across the fish tree of life that exhibit intra-specific anti-tropicality. These data reveal distinct support for both glacial dispersal, and biotic exclusion from the tropics. These results are then interpreted in a comprehensive framework to determine what drives anti-tropical distributions in marine systems. Overall, multiple mechanisms seem responsible that act in concert over time to produce these distributions. Certain equatorial divergence events are recovered in time periods currently not associated with any anti-tropical hypotheses. It seems
likely that stochastic crossing events may be important in the initial colonization of a new hemisphere.
CHAPTER 1
INTRODUCTION

As one travels the world, it is intriguing to come across the same, or closely related, species at two locations separated by thousands of kilometres. Immediately, questions begin to form in one’s mind: How did this species get here? Was it transported naturally, or artificially? Was this species once distributed across a wider area than these two locations? Disjunct distributions have raised these, and many other questions, and have puzzled biogeographers since naturalists expanded the scope of their research past local regions. One of the most striking disjunct distributions is that of species which are found in the temperate zones of each hemisphere, but which are absent from the tropics (Fig. 1). This distribution has gone by several names in the past, including “bipolar,” “bitemperate,” “anti-equatorial” and “amphitropical.” However, collectively these names are commonly encompassed by the term “anti-tropical” (Hubbs, 1952). The anti-tropical pattern is observed at multiple taxonomic scales, including individual species, sister-species, genera, or families separated by the tropics. While this pattern is found in terrestrial systems (for example, in plants: Thorne, 1972; Smissen et al., 2003; Villaverde et al., 2015), it is more common in marine taxa, with examples found across the tree of life (see Briggs, 1995).

It is perhaps the aquatic medium of the world’s oceans that allows for such a distribution to form repeatedly. Many marine organisms disperse by means of a pelagic, larval stage, where larvae can stay in the water column anywhere from days, to months on end, traveling with the currents. Throughout the Cenozoic there have been few absolute barriers to gene flow and movement of these larvae, especially latitudinally. In concert this lack of barriers gives many marine taxa extremely high dispersal potentials over evolutionary time scales, allowing them to
either disperse across the tropics, or to expand and occupy large latitudinal ranges that could be subsequently split into anti-tropical distributions.

This striking biogeographic pattern has been noted for over a century, with numerous hypotheses proposed for its formation. These hypotheses generally fall into two categories: dispersal and vicariance. They are further distinguished by the timing of disjunction, or by the mechanisms operating within these two broader categories. However, it is whether or not species disperse from one hemisphere to the other, or whether species were once widespread, and have subsequently been extirpated from lower latitudes that mainly distinguish these hypotheses. Here I review seven central hypotheses regarding anti-tropical distributions (Table 1). Historical background for each hypothesis, along with associated assumptions and expectations, are outlined below with examples of taxa that could fit each scenario. Longstanding questions, and open avenues for research are given at the end. This chapter is not meant to be an exhaustive explanation of all potential hypotheses that have been proposed over the past century, but is
meant to lay a general framework of popular explanations that have been recurrent throughout the literature over time. Ultimately, it is my goal that the reader understand the history behind this pattern, and gain enough background to assess future studies regarding this fascinating distribution.

Table 1. Proposed hypotheses associated with the formation of anti-tropical distributions. These hypotheses are separated into vicariant and dispersal categories, and each hypothesis is listed with expected time scales of equatorial divergence times and assumptions. DL = dispersal limited, PR = physiologically restricted to cooler waters.

<table>
<thead>
<tr>
<th>Hypothesis</th>
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<tr>
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<td>Continental Fragmentation</td>
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<td>~21 – 14 mya</td>
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<td>Biotic Exclusion</td>
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<tr>
<td>Isothermal Submergence</td>
<td>No specific time</td>
<td>Species PR</td>
</tr>
<tr>
<td>Glacial Dispersal</td>
<td>Pleistocene</td>
<td>Tropics cooled significantly during the Pleistocene; Species PR</td>
</tr>
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*Fails to explain anti-tropical distributions associated with continental margins.

**VICARIANCE AND ANTI-TROPICALITY**

**Continental Fragmentation**

The hypothesis operating at the oldest time scales for anti-tropicality is continental fragmentation, which posits that anti-tropical species were restricted to the continental margins of the supercontinent Pangea (Nur & Ben-Avraham, 1982; Nelson, 1985). Then, as this continent separated during the early Mesozoic, species moved with the continents and were split by the tropics. Evidence for this hypothesis is limited primarily due to the fact that many of the extant anti-tropical species, genera, and families are from the Cenozoic, and the fossil record is rather incomplete for most marine lineages. Therefore, the timing of this splitting event does not
correspond to many current anti-tropical distributions (Briggs, 1987a; Lindberg, 1991). However, Crame (1993) proposed this as a possible explanation for the distribution of extinct molluscs of the Mesozoic for which there is a decent fossil record.

Continental fragmentation assumes that evolutionary lineages are dispersal limited, even over the time period of 175 million years (myr). While this may be true for taxa restricted to continental landmasses, over these time periods most marine lineages are unlikely to be dispersal limited. Furthermore, strong latitudinal thermal gradients were not established until ice-sheets formed in the Southern hemisphere during the late Eocene/early Oligocene (Liu et al., 2009; Zachos et al., 2001). Continental fragmentation implicitly assumes that anti-tropical species are not restricted by thermal tolerances, or at least were not originally restricted due to thermal tolerances. Fragmentation occurred over such a long period that under this hypothesis, anti-tropical lineages should only be found at higher taxonomic levels. If lineages restricted to coastlines could not disperse between hemispheres, then these lineages would have over 100 myr to diversify in their respective hemispheres. Further, fossil evidence for this explanation may be spurious, with seemingly closely related, anti-tropical taxa actually being distantly related and subject to convergent evolution (see Lee et al., 2016).

**Island Integration**

Islands have been used as natural experiments over the last century. Understanding the impact that isolation, colonization, and geological history have on island biotas has resulted in seminal papers in biogeography (for example the theory of island biogeography; MacArthur & Wilson, 1967). Dispersal is prominent in discussions of island biogeography, but came under scrutiny when vicariance regained popularity in the latter half of the 20th century. Originally postulated by Rotondo et al. (1981) as a vicariant counter-point to the founder principle (the
dispersal and colonization of remote islands), island integration suggests that species need not undergo long-distance dispersal to reach isolated areas. Instead, this theory suggests that the islands themselves are dispersal vectors, that follow the movements of the oceanic tectonic plates (Rotondo et al., 1981). If the paths of these islands intersect other island arcs, then colonization can happen without the need for long distance dispersal.

Rotondo and colleagues (1981) originally used island integration to explain the taxonomic similarities between the Hawaiian islands and islands in southeast Oceania by noting that some of the seamounts surrounding the Hawaiian islands are substantially older than their nearby Hawaiian counterparts. These ages suggest that they originated elsewhere, and moved with the Pacific plate to their current location, intersecting the islands formed by the Hawaiian anomaly zone (hot spot). The authors also suggest that, based on the ages estimated for these older islands and sea mounts that they could have originated south of the equator near the East Pacific Rise and travelled approximately 30º in latitude to their current locations (Figure 2 in Rotondo et al., 1981). Springer (1982) later explicitly used this hypothesis to explaining antitropicality in the Pacific. A community forming on an isolated island could conceivably travel this distance between 33 – 66 myr (assuming a rapid plate movement of 10 cm year⁻¹ or the average oceanic plate movement of 5 cm year⁻¹), respectively placing endemic biota in radically different ecosystems (Ali, 2017).

However, there are still several assumptions associated with island integration. Primarily, it assumes that anti-tropical taxa are dispersal limited across evolutionary time scales, and that these species are not physiologically restricted to cooler environments, but could cross the warmer tropics without extirpation. It also assumes that the islands could traverse this distance while either remaining subaerial, or staying shallow enough to support many of the anti-tropical
species that do not inhabit deeper waters. On the scale of 71 – 77.6 myr (Clague & Dalrymple, 1975; the original age estimates used in Rotondo et al. (1981)), Wentworth sea mount and Necker Island would have experienced significant erosion and sinking due to lithospheric cooling (Parsons & Sclater, 1977; Stein & Stein, 1992; Hillier & Watts, 2005). Updated age estimates for this sea mount of 90 myr (Pringle & Dalrymple, 1993) place it on the older range of time frames for the island integration hypothesis. This proposal addresses anti-tropical species between Hawaii and the southeast Pacific, but does not explain anti-tropical taxa in the western Pacific that occur along continental margins. Divergence estimates between Hawaiian and southeast Pacific taxa dating between the late Eocene/early Oligocene and the onset of the Cenozoic (or older), approximately 33 – 66 million years ago (mya) would provide some support for this hypothesis.

Island Submergence

Island submergence, another island-centric vicariant scenario, suggests that widespread oceanic species are split into northern and southern hemisphere populations when suitable habitat in the tropics, represented by islands, are submerged and lost. Originally proposed by Rehder (1980), this hypothesis does not have any defined time period on which it is acting, and therefore there are no expectations about which taxonomic level we would expect to be anti-tropical. However, this hypothesis implies several things. First, it assumes again that species are restricted due to a lack of available habitat, not due to physiological restrictions or species interactions. Secondly, this hypothesis assumes that there is a current lack of tropical oceanic habitat associated with islands. However, numerous examples of tropical islands can be found. In the Indian Ocean, the Seychelles, Chagos, and Maldives all occur within tropical latitudes. In the Pacific Ocean some examples include the Caroline islands, the Line Islands, the Marshall
Islands, the Solomon Islands, and the Cook Islands. In the Atlantic Ocean the Antilles, St. Paul’s
Rock, and Sao Tome and Principe occur within tropical waters. All of these examples contradict
a current lack of tropical habitat assumed by this hypothesis.

Mid-Miocene Warming

Climate has varied considerably throughout the Cenozoic, with several peaks in global
temperatures. From an early Eocene maximum ~52 mya when ocean temperature were 4° – 10°
warmer than current values (Miller et al., 1987; Zachos et al., 1994, 2003), temperatures
gradually cooled until the early Oligocene when Antarctic ice sheets formed ~34 mya,
strengthening the latitudinal thermal gradient (Liu et al., 2009; Zachos et al., 1994, 2001).
Following the cooler temperatures in the Oligocene, temperatures gradually rose from the late
Oligocene until the mid-Miocene climatic optimum ~17–15mya, when temperatures reached
their highest since the early Eocene (Flower & Kennet, 1994; Zachos et al., 2001). Following
this peak in global temperatures, sea surface temperatures (SST) cooled throughout the
remainder of the Miocene, Pliocene, and Pleistocene (Zachos et al., 2001; Zhang et al., 2014).

This thermal history likely helped shape the distribution of organisms across the planet,
including anti-tropical marine taxa. Links between anti-tropicality and Cenozoic temperature
fluctuations were first proposed by Valentine (1984), who suggested that many species may have
adapted to cooler temperatures during the Oligocene, only to be split into disjunct distributions
by rapid warming during the mid-Miocene climatic optimum. This was further expanded and
championed by White (1986) as the single solution to anti-tropicality due to the fact that it
“requires nothing unusual of modern anti-tropical taxa, only a mutual intolerance of modern
tropical conditions.” In this view, even the most distantly related anti-tropical taxa with
contradictory life history strategies or habitat requirements could be explained by the same mechanism.

However, this all-encompassing hypothesis has received criticism. Anti-tropical distributions are found across a variety of taxonomic scales, from populations of the same species separated by the tropics, to sister-species, genera, and higher taxonomic levels (Briggs, 1987a). The assortment of taxonomic scales for this pattern suggests that the mechanisms driving it span a variety of time-scales as well, and that they are not centred around a single event, such as the mid-Miocene warming period (Briggs, 1987b). Furthermore, advances in our understanding of paleoclimate have revealed that temperatures at the beginning of the Oligocene were not as cold as previously thought, and while there was substantial cooling at higher latitudes, the tropics only cooled ~3º C when Southern hemisphere ice sheets formed (Liu et al., 2009). This thermal regime does not create a scenario where there were wide-spread temperate taxa prior to global warming that occurred in the late Oligocene and continued to the mid-Miocene envisioned by White (1986). The discrepancies between temperature reconstructions, and between the taxonomic levels of anti-tropicality suggest that the mid-Miocene climatic optimum did not drive this pattern for all anti-tropical taxa. However, shifting temperature regimes and the strengthening of latitudinal thermal gradients before and after the mid-Miocene optimum likely influenced the strength and direction of surface currents, possibly affecting the distribution of species in the oceans.

**Biotic Exclusion from the Tropics**

A central tenet in evolution is the observation that every species has a center of origin from which they expand (Darwin, 1859). This principle forms the basis of the ‘Center of Origin’ hypothesis for marine species, which explains the latitudinal, and longitudinal, biodiversity
gradients in the Indo-Pacific by stating that the tropics, and in particular the Indo-Australian Archipelago (IAA), is where species originate and later expand their ranges, outcompeting other species (Ekman, 1953; Briggs, 1999; Briggs, 2000). If new species originate in the tropics, and out-compete older, widespread species that have expanded into the temperate zones of both hemispheres, then this could result in anti-tropical distributions. Originally coined the “relict theory” (Théel, 1885), this hypothesis was later refined and championed vociferously by Briggs (1987a).

Unlike many of the other hypotheses proposed for anti-tropical species, this is not contingent on any one time period. An older, wide-spread species could be excluded from the tropics to form a disjunct distribution at any period. This flexibility has been used as support for the relict theory, as anti-tropical distributions occur at multiple taxonomic scales, and therefore conceivably form at different time periods (Briggs, 1987a). However, what excludes these species from the tropics? Is it competition from younger species, as originally proposed? If so, then are they out-competed by younger species in the same family, or unrelated, but functionally equivalent species? Biotic exclusion could also not be related to competition, but could be the result of some other biotic interaction, such as the loss of food sources or mutualists, or the introduction of predators, parasites, or diseases in the tropics.

As such, biotic exclusion is notoriously difficult to test, but it does provide two central predictions. First, it assumes that anti-tropical species are old, and should not have formed recently. Second, it assumes that species are not restricted to temperate areas due to physiological constraints. That is, they are excluded from the tropics by biotic factors (competition, predation, disease, etc), but if only abiotic factors are considered, then they should
have available habitat. Younger coalescent times for anti-tropical taxa, or physiological restrictions to colder temperatures would question the validity of this hypothesis.

**DISPERSAL AND ANTI-TROPICALITY**

**Isothermal Submergence**

Dispersal hypotheses for anti-tropicality involve either individuals dispersing across the tropics, or communities dispersing during cooler time periods. Anti-tropical species are found in warm-temperate or temperate zones and are often assumed to be physiologically restricted to these regions (although this is often untested; see Chapter 6). These thermal limits presumably prevent anti-tropical taxa from dispersing across the warmer, tropical zones. One parsimonious way to explain the inter-hemisphere connections between anti-tropical taxa, is that they merely cross the tropics in deeper, cooler water. This avenue of dispersal was perhaps first suggested by Sir James Ross during the Ross expeditions to the Antarctic Ocean, where Ross noticed certain species that resembled species he had personally seen in the artic, and postulated that they must have migrated in abyssal waters (Murray et al., 1897). This idea was later formalized into the ‘migration theory’ of Ekman (1953), and is still considered a plausible explanation for certain taxa (Hubbs, 1952).

Anti-tropicality is a scenario of latitudinal discontinuity across the tropics. For isothermal submergence to be a valid mechanism that could lead to this distribution, it is still imperative that a species range be disjunct. If a species uses deeper, cooler water to traverse the tropics, they cannot have established populations in deeper, equatorial waters. These intermediate tropical populations would create a continuous distribution, which would therefore not be anti-tropical. This is potentially an issue for deep-water taxa in regions that are under-sampled; they may appear to be anti-tropical in distribution only because tropical populations have not been
discovered. For species that are adapted to shallow-water habitats, Ekman (1953) considered this processes as a historic one, rather than an ongoing connection between hemispheres. Randall (1981) furthered this by suggesting that shallow water species may only disperse in deeper waters during glacial periods when they would not have to go as deep to be in cooler water. This restriction makes isothermal submergence similar to glacial dispersal (see below), and could be an explanation if sea surface temperatures during the Pleistocene were not cool enough in the tropics for temperate taxa to cross.

It is important to note that isothermal submergence either needs entire populations to disperse in deeper water between hemispheres (with a subsequent extirpation of populations in the tropics), or for individuals to migrate across the tropics in deeper water. While this is plausible for species that occupy a wide depth range or that disperse long distances as adults (e.g. Veríssimo et al., 2010; Poortvilet et al., 2013; ), there are many shallow-water anti-tropical taxa that do not occupy deep water habitats, and do not disperse far as adults. However, it is still plausible that larvae of these species may use deeper waters to cross the tropics during a pelagic larval phase, as many of the larval characteristics of anti-tropical taxa are unknown.

**Glacial Dispersal**

The Pleistocene epoch is defined by the onset of repeated glacial cycles. These cycles altered global temperatures, and shifted sea levels. During glacial periods, sea levels were up to ~125m lower, and shallow water habitat was reduced to narrow bands of the continental shelves, in many cases reducing the population sizes, and restricting gene flow among marine taxa (Ludt & Rocha, 2015). While these fluctuations may have negatively impacted many marine species, SST during glacial maxima may have been cool enough to allow anti-tropical species to disperse between hemispheres (Darwin, 1859; Berg, 1933).
Glacial dispersal is a natural conclusion to make in marine systems, where there are not many barriers to dispersal and many species have a dispersive larval stage that can last for weeks to months. One may think that the only thing preventing gene-flow across the tropics currently would be physiological constraints that may disappear during cooler glacial periods. However, this conceptual model has been challenged with critics stating that SST did not cool enough during glacial periods to allow temperately restricted species to cross the tropics (Briggs, 1987a). SST during the Last Glacial Maximum (LGM) were cooler at higher latitudes, but tropical temperatures did not dramatically change (Fig. 2). This likely compressed temperate communities into narrower latitudinal bands, but even during the coldest month of the year, it does not appear that tropical temperatures were cool enough to allow species to cross in surface waters.

Despite this, there are several lines of evidence suggesting that many equatorial divergence events occurred during this time period. By dating molecular data in a variety of fishes using molecular clocks, Burridge (2002) found that many intra-specific, and sister-species equatorial divergences dated to either the Pleistocene or Pliocene epochs. Using a different approach, Lindberg (1991) demonstrated that a variety of fossil marine invertebrates in the
Eastern Pacific also show bidirectional movement across the tropics during the Pleistocene and Pliocene epochs. Furthermore, many of these fossil taxa first appear in temperate areas, suggesting that these are not once widespread tropical taxa that were split into two populations through competition, or other factors (Lindberg, 1991).

While the timing of these findings do link divergence events to the Pleistocene, they also find evidence for Pliocene divergences as well. However, the tropics during the Pliocene extended to higher latitudes than modern limits, and lacked high-amplitude climate cycles (Dowsett et al., 1994). If the tropics did not decrease in temperature during this time period, then how could temperately restricted species cross this barrier? Both the Pliocene and Pleistocene have been regarding as periods of dramatic change in marine systems due to the closure of the Isthmus of Panama, which impacted global currents, and altered regional ecosystems (Karas et al., 2017). These changes may have created cold-water upwelling zones along continental margins, allowing anti-tropical species to cross the tropics in a stepping stone manner (Lindberg, 1991). While the tropics maintained warm temperatures during these periods, the tropical eastern Pacific had higher cooling rates during the Pliocene and Pleistocene than the western tropical Pacific and had a shallow thermocline and active upwelling (Zhang et al., 2014). While this does not strictly fit in with glacial dispersal, it is worth noting that this supports the notion that the eastern Pacific may be an important corridor for anti-tropical taxa (Lindberg, 1991; Bowen & Grant, 1997; Burridge, 2002; Grant & Bowen, 2006).

**DETERMINING THE DRIVERS OF THIS PATTERN**

The variety of hypotheses proposed for this distributional pattern all have different levels of support and criticism. To identify the hypotheses that could potentially drive anti-tropicality a multi-faceted approach must be taken. Perhaps most importantly, one needs to show that species
are indeed anti-tropical. Many early lists of anti-tropical species included species that were later found to be distantly related, or widespread in deeper waters (Ekman, 1953). Strongly supported systematic hypotheses with complete taxonomic sampling are required, therefore, to test anti-tropical distributions. If taxa are missing, or relationships are unclear, then it is impossible to tell if the species of interest are actually anti-tropical. Once a strongly supported phylogeny is obtained, many of these proposed hypotheses can be separated by the timing of anti-equatorial divergences (Table 1). Therefore, phylogenetic hypotheses should be rigorously dated, ideally with fossil calibration points (as opposed to molecular clocks) from multiple fossil formations (to avoid any bias associated with the dating of a single formation). These time-calibrated phylogenies can then be examined within a historical biogeographic context to estimate how species shifted their distributions over time.

However, there are several hypotheses listed above that could operate on the same temporal scales, and that cannot be distinguished solely by using a dated phylogeny. Therefore, interdisciplinary studies that use phylogenetic data in conjunction with physiological or environmental data are needed to tease these hypotheses apart. Here I test these hypotheses using a variety of approaches (both phylogenetic and ecological) in marine fishes from across the fish tree of life to determine which mechanisms, if any, drive anti-tropicality in marine systems.
CHAPTER 2
SYSTEMATICS AND ANTI-TROPICALITY WITHIN A TEMPERATE FISH FAMILY

INTRODUCTION

To understand how anti-tropical distributions are formed, the first step is to determine which species are anti-tropical. In most cases this involves understanding the phylogenetic relationships of species that have representatives split by the tropics. For fishes, the past decade has seen several large-scale molecular phylogenies that can be used to confidently find anti-tropical taxa (Chen et al., 2003, 2014a; Smith & Craig, 2007; Near et al., 2012a, 2013; Betancur-R et al., 2013, 2017; Alfaro et al., 2018). These studies, while not specifically targeting anti-tropical species, have produced a multitude of hypotheses for ray-finned fish relationships based on different numbers of taxa or loci, and have questioned previous phylogenetic hypotheses based on morphology (Johnson & Paterson, 1993; Wiley & Johnson, 2010). While the resulting molecular hypotheses differ from one another in some regards, similarities among these studies have begun to shift our thinking towards the evolutionary history of fishes. One consistent finding between many of these molecular studies is a lineage not previously recognized by morphology, containing a variety of temperate freshwater and marine species now recognized as the order Centrarchiformes (Near et al., 2012b; Betancur-R et al., 2017, but see Smith & Craig, 2007). Interestingly, this order contains a variety of anti-tropical distributions, found across multiple different taxonomic scales (intra-specific, inter-specific, and inter-familial).

Five suborders have been placed within Centrarchiformes, with taxonomic confusion regularly occurring in the suborder Cirrhitioidei — a clade containing Cirrhitidae, Chironemidae, Aplodactylidae, Cheilodactylidae and Latridae (Betancur-R et al., 2017). The close affinity of these families has been long recognized based on the presence of thickened and elongated
unbranched pectoral-fin rays (Gill, 1886), and several systematic revisions have focused on relationships within these five marine families. However, uncertainty in the relationships between, and within, these families persists. One recurring taxonomic issue involves the Cheilodactylidae, and how it relates with the other four cirrhitoid families. The Cheilodactylidae currently comprises 27 species and four genera (Nelson et al., 2016). The majority of these species inhabit temperate regions of the Southern Hemisphere. Diversity is highest along the Australian coastline (Kuiter, 1993), but species occur in South Africa, along both coasts of South America, around several oceanic islands in the Southern Hemisphere, and around the coasts of Japan, Korea, China, Taiwan and Hawaii in the Northern Hemisphere – a distribution that is notably anti-tropical (Randall, 2005; Nelson et al., 2016). This distribution has led to regional studies with limited taxonomic sampling that have only exacerbated taxonomic confusion within the family. To understand how anti-tropicality formed in this family, a well-resolved phylogeny is needed.

Much of the confusion regarding cheilodactylid taxonomy stems from the genus *Cheilodactylus*, which is the most speciose and widely distributed group in the family. The type species of this genus (and of the family), *Cheilodactylus fasciatus* Lacépède, is quite distinct morphologically from all other species in the genus (apart from *C. pixi* Smith), which historically led to the description of various new genera. However, Allen & Heemstra (1976) noted “the differences between these various type-species [of these genera] and *C. fasciatus* are no greater than those between *C. fasciatus* and any other species of *Cheilodactylus,*” and placed many of these genera in synonymy with *Cheilodactylus*. While this suggestion simplified the taxonomy of the family, it had the unintended consequence of making *Cheilodactylus* a ‘catch-all’ name for a variety of unique fishes, and may not accurately reflect their evolutionary history. The
uniqueness of the type species, *C. fasciatus*, likely has led to the overall taxonomic confusion within the Cheilodactylidae.

Recent studies have recovered a polyphyletic Cheilodactylidae, with two South African species, *Cheilodactylus fasciatus* and *C. pixi*, forming a clade distantly related to the other members of the family, which have been recovered within the Latridae (Burridge and Smolenski, 2004; Sanciangco et al., 2016). As the type species for *Cheilodactylus*, and the Cheilodactylidae, is *C. fasciatus*, this result would restrict Cheilodactylidae *sensu stricto* to these two South African species, and the remaining cheilodactylids should be placed within the Latridae, a classification which echoes the original proposed relationships of cirrhitoid fishes (Gill, 1886). However, despite recent studies repeatedly finding evidence that Cheilodactylidae is polyphyletic, no formal taxonomic changes have been made either due to low topological support values (Burridge and Smolenski, 2004) or limited taxonomic sampling (Sanciangco et al., 2016). Here we use ultraconserved elements (UCEs) to help resolve the relationships among the cirrhitoid families, with particular focus on the complex relationships involving the Latridae and Cheilodactylidae. We then use this resolved phylogeny to test different hypotheses regarding anti-tropicality within this group.

**MATERIALS AND METHODS**

**Sampling Strategy**

Museum specimens were examined for all possible species in Cirrhitoida with standard meristic counts and measurements. Radiographs were taken for select key taxa to examine predorsal bone arrangement, which was scored following Ahlstrom et al. (1976). For genomic work, when possible, tissue samples from Burridge & Smolenski (2004) were used, allowing for a direct comparison between studies. However, some tissues used in that study were either
exhausted, or could not render enough genomic material for the sequencing approaches used here. In these cases, and for certain key-taxa, we supplemented our dataset with tissues obtained from vouchered museum specimens. Our sampling design included species from all five cirrhitoid families, as well as outgroup taxa that have been consistently recovered within the Centrarchiformes (Near et al., 2012b, Betancur-R et al., 2013, 2017, Chen et al., 2014b, Lavoué et al., 2014).

**Laboratory Protocols**

Genomic material was extracted from tissues using a DNeasy Blood & Tissue kit (Qiagen) following manufacturer’s protocols. Extracts were stored at -23°C prior to DNA quantification and library preparations. DNA was quantified with a Qubit® 2.0 Fluorometer using a dsDNA BR assay kit following manufacturer’s protocols (Life Technologies). Quality of DNA was superficially assessed by running pure genomic extracts on a 1% agarose gel with SYBR® safe DNA gel stain (Life Technologies) and 6x Blue/Orange loading dye (Promega). Approximately 0.5–1.0µg of DNA was combined with custom solid-phase reversible immobilization beads (following protocols outlined in Rohland & Reich, 2012) to remove small fragments present in each extract. These were then eluted in 30µL of TE buffer, and then sonicated using an Episonic Multi-Functional Bioprocessor to an average length of 600bp. All samples were then examined on a 1% agarose gel to ensure that the sonication process was successful, and the process was repeated if necessary.

Illumina libraries were constructed using the Kapa Hyper Prep Kit (Kapa Biosystems) with dual-indexing barcodes. All reactions followed manufacturer protocols, except reaction sizes were scaled to half the volume indicated by the manufacturer. After library amplification, samples were pooled in equimolar ratios in sets of six to eight samples. Target enrichment of
UCE loci was performed on each pool using the MYbaits 0.5k Actinopterygian UCE capture kit (MYcroarray), originally described in Faircloth et al. (2013), following manufacturer’s protocols. Pools were then amplified and cleaned using 16–18 PCR cycles following procedures outlined in Faircloth et al. (2013). All pools were combined in equimolar proportions, and were sequenced either at the University of Georgia Genomics Institute, or the Oklahoma Medical Research Institute, using an Illumina HiSeq or MiSeq Sequencer. Sequences in demultiplexed fastq files were then trimmed of unique indexes and low quality base calls using trimmomatic (Bolger et al., 2014), as part of the program Illumiprocessor (Faircloth, 2013). De novo assembly of UCE sequences was completed using Trinity v.2.0.6 (Grabherr et al., 2011) with default settings. Using the Phylouce v1.5.0 repository (Faircloth, 2015) we constructed a 75% complete concatenated data matrix, which we analyzed using both likelihood and Bayesian phylogenetic approaches.

**Phylogenomic Analyses**

Maximum likelihood trees were constructed using RAxML v8.1.24 (Stamatakis, 2014) on the CIPRES scientific gateway portal (Miller et al., 2010). All analyses were completed using the GTRGAMMA model for bootstrapping, with 1000 bootstrap iterations, with the rapid bootstrapping option (-x). Bayesian topologies were constructed using the program ExaBayes (Aberer et al., 2014) implemented on the supercomputer clusters at Louisiana State University. By default, this program uses the GTR substitution model. Four separate chains were run for 1,000,000 generations, sampling every 500 generations. Chains were then combined using LogCombiner v.1.8.2 (Drummond et al., 2012), and trace plots and ESS values were examined to ensure stationarity and convergence using Tracer v1.6 (Drummond et al., 2012). In addition to concatenated analyses, two multi-species summary coalescent methods were used to take
variation among gene trees into account. First, gene trees were estimated independently using RAxML with the GTRGAMMA substitution model and 10 alternative runs. A species tree was then estimated with ASTRAL v5.4.4 (Mirarab & Warnow, 2015) using a mapping file to specify which species had multiple individuals sequenced. Second, SVDquartets was implemented in PAUP* 4.161 (Swofford, 2003) using 50,000 quartets and the bootstrapping option.

We compared results from our analyses to alternative topologies with the likelihood based approximately unbiased (AU) and Shimodaira-Hasegawa (SH) tests (Shimodaira, 2002) using the program Consel v0.2 (Shimodaira & Hasegawa, 2001). This enabled comparisons between our output trees and constrained topologies. Two constrained trees were constructed using the –g option in RAxML; the first enforced cheilodactylid monophyly, and the second allowed *Cheilodactylus fasciatus* and *C. pixi* to form their own clade (see results below), and also restricted the remaining species of *Cheilodactylus* to be monophyletic. Per-site log likelihood scores were then estimated using the –f g option in RAxML to create Tree-Puzzle-type input files. Consel was then used to generate 10,000 hierarchical bootstrap replicates to test between alternative topologies.

**Time Calibration**

As our concatenated and coalescent based trees largely agreed with one another, a time-calibrated tree was constructed from the more strongly supported concatenated tree in BEAST v2.5 (Bouckaert et al., 2014). BEAST has difficulty starting with UCE data if no start tree is given. Therefore, we created a time-calibrated start tree using the program Chronos in the R package ape with a relaxed substitution model and four calibration points (Paradis et al., 2004). This is a penalized maximum likelihood method that can quickly produce a time-calibrated phylogeny (Sanderson, 2002; Kim & Sanderson, 2008; Paradis, 2013). All calibration points
were from Centrarchidae, which was used as an outgroup for this study, and is a closely related family to Cirrhitoida (Near et al., 2012; Betancur-R et al., 2013; Sanciangco et al., 2016). Fossil calibrations were chosen based on rigorous testing in a previous Centrarchidae study (Near et al., 2005). These fossil calibrations were used to set minimum ages for *Archoplites/Ambloplites* (Smith & Miller, 1985), *Pomoxis* (Wilson, 1968), and *Lepomis* (Matthew & Thomson, 1924). A fourth calibration point was added to the base of Centrarchidae based on the confidence intervals for this node in Near et al. (2005). Running BEAST on the full UCE dataset was not computationally feasible, so five subsets of 50 randomly chosen loci from the 75% complete data matrix were concatenated for each BEAST run. We ran these subsets with a constraint tree (the concatenated 75% topology above), the penalized-likelihood chronogram starting tree, an HKY model of nucleotide substitution, an estimated strict clock, and a speciation birth-death process tree prior for 50,000,000 iterations, sampling every 1000 iterations. The same fossil constraints from the penalized maximum likelihood chronogram were used for BEAST, with a lognormal distribution and a standard deviation of 1. The constraint on the age of Centrarchidae was input with a uniform prior spanning the confidence interval for this node in Near et al. (2005). All subsequent runs were combined and checked for convergence with Tracer (Drummond et al., 2012) after a burnin of 25,000 trees, and a maximum clade credibility tree was produced using TreeAnnotator (Drummond et al., 2012).

**Biogeographic Analyses**

To determine when equatorial divergence events occurred within Cheilodactylidae, we implemented a Dispersal-Extinction-Cladogenesis (DEC) biogeographic model (Ree & Smith, 2008) using the biogeobears package in R (Matzke, 2013). This method reconstructs ancestral areas using a maximum likelihood framework, and can subsequently allow for testing between
different models that restrict dispersal at various time points. For these analyses we used the time
calibrated tree from BEAST, and coded each species based on which marine biogeographic
provinces and regions the majority of their extant range was in. Biogeobears performs more
efficiently with fewer areas; therefore, when multiple species occupied several provinces within
a major biogeographic region, the region was used instead of listing each province individually.
Provinces and regions used were those described by Briggs and Bowen (2012), and resulted in
five major biogeographic areas for our models. Two of these areas (the Hawaiian province, and
the Sino-Japanese/Oriental provinces) reside in the Northern hemisphere, three areas (New
Zealand-Australia region, South America region, and the Benguela province) reside in the
Southern hemisphere, and the Indo-West Pacific region occupies the tropics. This configuration
allows us to test the historical biogeography of these families in general, and to specifically test
when anti-tropical species pairs diverged across the tropics. Many of the hypotheses regarding
anti-tropicality can be distinguished by the timing of equatorial divergence events (see Chapter
1). Therefore, to test these we constrained connections between Southern hemisphere and
Northern hemisphere locations during specific time intervals that correspond to various
hypotheses. This resulted in an unconstrained model (dispersal between any biogeographic area
at any time), and six other models (see Fig. B.1). Furthermore, each model was tested with the
conventional DEC model, and with a +J parameter, which allows for long distance dispersal
between areas. In total, this amounted to 14 models that were then compared using likelihood
ratio tests and AIC values to determine the most likely anti-tropical scenario.

While the DEC model comparisons can test between various biogeographic hypotheses,
they will not directly show how frequently, or when specifically, equatorial crossing events
occurred. Furthermore, they are generally based on a single topology. As distributions in this
family can be reduced to two states (Northern hemisphere vs Southern hemisphere), we used stochastic character mapping to determine when equatorial divergence events specifically occurred. Stochastic character mapping used in this way (with two discrete states) are essentially a simplified biogeographic model that assumes an ancestor can only be in one location at a time (i.e. assuming that there isn’t a widespread species occurring in both hemispheres at the same time). This method allows us to quickly determine crossing times across multiple trees instead of a single tree. While all posterior trees from BEAST share the same topology due to our topology constraint, the posterior set of trees all contain varying branch lengths. Testing crossing events with a variety of these trees, therefore, will give us a better approximation of when these events may have occurred. Stochastic mapping was conducted using the program SIMMAP in the Phytools R package (Revelle, 2012). One hundred stochastic iterations were done on 100 randomly chosen posterior post-burnin trees with equal weighting on north-to-south and south-to-north transitions. The timing and direction of these transitions were then quantified.

RESULTS

Sequencing Summary and Phylogenomic Relationships

Our UCE data matrix contained 439 loci, totaling 277,505bp with an average of 618bp per locus. The species included, source, total number of sequencing reads, and number of UCE loci per sample can be found in Table B.1. All phylogenetic analyses recovered near identical results, including a monophyletic Cirrhitoidi, and a polyphyletic Cheilodactylidae (Fig. 3; Fig. B.2–B.4). Concatenated data generally produced results with higher support values than the species-tree approaches. Two South African species, Cheilodactylus fasciatus and C. pixi, form a clade that is the sister group to the Chironemidae. This clade in turn was recovered as the sister group to Aplodactylidae, and together, all three of these clades are the sister group to a clade
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comprising the Latridae and remaining cheilodactylids. The Cirhitidae is the sister group to all
other cirrhitoid families, consistent with previous analyses (Greenwood, 1995; Sanciangco et al.,
2016; Betancur-R et al., 2017). The ASTRAL species-tree approach differs from the
concatenated analyses only in the placement of ‘Cheilodactylus’ vittatus Garrett and
Nemadactylus gayi (Kner); however, for both species the nodes subtending these branches were
weakly supported (Fig. B.3). The SVDquartets coalescent tree additionally deviated from the
concatenated trees in the placement of Dacylophora nigricans and ‘Cheilodactylus’ francisi,
although with low support (Fig. B.4).
Cheilodactylus as recognized here is polyphyletic. The two aforementioned South African species are distantly related to the remaining species of Cheilodactylus. Furthermore, the remaining Cheilodactylus is also non-monophyletic. A strongly supported clade containing ‘Cheilodactylus’ spectabilis Hutton and ‘C.’ variegatus Valenciennes is recovered as the sister group to all Chirodactylus. All species of ‘Cheilodactylus’ that have historically been placed within the subgenus Goniistius are recovered as non-monophyletic, with ‘Cheilodactylus’ nigripes Richardson consistently recovered as the sister group to Nemadactylus. Furthermore, ‘Cheilodactylus’ rubrolabiatus Allen & Heemstra is recovered within the remaining species of Goniistius. Topological comparisons using AU and SH tests comparing our results to a tree constraining cheilodactylid monophyly, and to a tree constraining Cheilodactylus without C. fasciatus and C. pixi as monophyletic, found statistically significant greater log likelihood values for the observed tree over all constrained trees (all p values < 0.01).

Biogeographic Reconstructions

The BEAST phylogeny recovered a crown age for the Cirrhitoida of 102mya (95% HPD interval 96.5mya–108.5mya). Crown ages for the families within Cirrhitoida, and their associated 95% HPD intervals, can be found in Appendix B (Fig. B.5; Table B.2). For each individual biogeobears model, the +J version allowing for long-distance dispersal was always favored (p values for likelihood ratio tests all < 0.001). The most strongly supported model was one which allowed equatorial crossing after the mid-Miocene climatic optimum with long distance dispersal (Post Miocene Warming +J model; Table 1). The unconstrained model with long distance dispersal allowing movement at any time period was the second most supported model (ΔAIC = 2.9). Ancestral area reconstructions for this model support a southern hemisphere origin for the most recent common ancestor (MRCA) of
Latridae/Cheilodactylidae/Aplodactylidae/Chironemidae near Australia and New Zealand (Fig. 4). At some point between the late Eocene and Early Miocene there was a transition for the ancestor of *Cheilodactylus fasciatus* and *C. pixi* to South Africa. The MRCA of *Chirodactylus* also dispersed to South Africa and South America during the Miocene, as did the MRCA of *Nemadactylus*. Transitions from South Australia/New Zealand to the Northern hemisphere likely occurred in the late Miocene for the MRCA of *C. quadricornis* and *C. zonatus*, and in the Pliocene and Pleistocene for *C. zebra* and *C. vittatus*. This is corroborated by the stochastic character mapping analysis, which recovered a distinctly bimodal distribution for equatorial crossing times occurring in the Pleistocene/Pliocene, and in the late Miocene (Fig. 5).

Furthermore, the vast majority of these simulated transitions were from the southern hemisphere to the northern hemisphere, and not in the opposite direction (33716 vs. 2811 simulated transitions).

<table>
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<th>D</th>
<th>E</th>
<th>J</th>
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**SYSTEMATIC ACCOUNTS**

**Family Cheilodactylidae Regan**

**DIAGNOSIS** – Diagnosis follows that of Smith (1980) for *Cheilodactylus*. The family can be diagnosed by the following combination of characters: body compressed and ovoid, with small, terminal to sub-terminal mouth with large lips; eyes moderate size; two pairs of nostrils with cirri on the lower pair of nostrils; no bony processes on frontal bone or maxilla; teeth small, villiform in several rows, absent from vomer and palatines. Dorsal-fin elements XVII–XX, 19–25; anal-fin elements III, 9–11; pectoral-fin rays 14 with ventral 4–5 thickened and unbranched. Dorsal-fin continuous with no division between spinous and soft portions; spines increasing in length to sixth spine, and decreasing thereafter; second dorsal ray longest. Gas bladder absent; three pre-
dorsal bones, with first pre-dorsal preceding first neural spine and second and third pre-dorsal between the first and second neural spines in the arrangement of 0/0+0/2+1/1/1. Lateral-line scales 78–85; scales small and cycloid; scaly sheath present at base of dorsal and anal-fins. Cheilodactyliidae can be further differentiated from Cirrhitidae by dorsal spines lacking cirri (versus present), and from both Chironemidae and Aplodactyliidae by higher anal-fin ray counts and a more laterally compressed, deeper body. Cheilodactyliidae can be further differentiated from Latridae by the absence of a gas bladder, by late-stage larvae lacking a ‘paperfish’ stage (Dudnik, 1977), and by the arrangement of pre-dorsal bones with the first neural spine (see family diagnosis for Latridae below).

HABITAT AND DISTRIBUTION – Cheilodactyliidae is only known from the coasts of Namibia and South Africa. Species can be found in tide pools and rocky reefs to 90m, and generally stay close to the benthos where they hide among rocks and other rubble (Smith, 1980).

**Genus Cheilodactylus Lacépède**

INCLUSIVE SPECIES – *Cheilodactylus fasciatus* Lacépède (type species), *C. pixi* Smith

DIAGNOSIS – As per family diagnosis.

MATERIAL EXAMINED – *C. fasciatus*, ROM 050995 [n=6, South Africa: Port Alfred]; *C. pixi*, AM I.37729 [n=5, South Africa: Tsitsikama], ANSP 97464 [n=1, Mozambique: Maputo Bay], CAS 45331 [n=1 (paratype), South Africa: Algoa Bay], USNM 221144 [n=1 (paratype), South Africa: Algoa Bay], USNM 385232 [n=6, South Africa: Tsitsikama].

**Family Latridae Gill**

DIAGNOSIS – Latridae can be diagnosed by the following combination of characters: body ovoid to elongate and compressed or round in cross-section; dorsal-fin elements XV–XXV, 22–44; anal-fin elements III, 7–37; pectoral-fin rays 14 with ventral rays thick and unbranched. Gas
bladder present; predorsal bones never in arrangement of Cheilodactylidae—all genera except *Mendosoma* with two pre-dorsal bones prior to first dorsal pterygiophore in arrangement of 0+0/2; no cirri on dorsal-fin elements. Latridae can be distinguished from all other cirrhitoids by having two pre-dorsal bones preceding first neural spine, except for *Mendosoma*, which can be distinguished by having a single dorsal-fin spine articulating with first dorsal pterygiophore (as opposed to two in all other families within Cirrhitoidae). While not all larvae have been described, Latridae remains the only family in Cirrhitoidae to exhibit a late-larval ‘paperfish’ stage where larvae have deep bodies with a strong ventral keel adapted for pelagic life.

**Genus Chirodactylus Gill**

**Inclusive Species** – *C. variegatus* (Valenciennes) (type species), *C. brachydactylus* (Cuvier), *C. grandis* (Günther), *C. jessicalenorum* Smith, *C. spectabilis* (Hutton)

**Diagnosis** – *Chirodactylus* can be diagnosed by the following combination of characters: dorsal-fin elements XVII–XVIII, 22–31; anal-fin elements III, 7–10; pectoral-fin rays 14 with ventral 6–7 unbranched and thickened; lateral-line scales 46–56. Body ovoid and compressed; dorsal profile of head slight to moderate; dorsal-fin increasing gradually in length to 5th or 6th spine, decreasing thereafter; no bony processes on frontal bones medially to orbit or anterior to maxilla.

**Habitat and Distribution** – *Chirodactylus brachydactylus*, *C. grandis*, and *C. jessicalenorum* occur off the coast of South Africa to 240m (Smith, 1980). *Chirodactylus variegatus* occurs in the southeast Pacific off the coast of Chile and Peru, and *C. spectabilis* occurs in the north island of New Zealand, Tasmania, and occasionally in southern mainland Australia.

**Comments** – Smith (1980) noted the convoluted taxonomic history of the genus, which is briefly described here. Gill (1862) erected *Chirodactylus* to include *C. antonii* Valenciennes 1833 (type
 species), C. variegatus Valenciennes 1833, and C. grandis Günther 1860. Barnard (1927) later described Palunolepis with P. grandis as the type species. Chirodactylus variegatus was later considered a senior synonym to C. antonii (de Buen 1959). In a review of Australian cheilodactylids, Allen and Heemstra (1976) regarded several genera, including Chirodactylus (but not Palunolepis), as junior synonyms to Cheilodactylus. Chirodactylus was later resurrected in a comparison of South African morwongs by Smith (1980), who included C. brachydactylus, C. jessicalenorum, C. grandis, and C. variegatus. However, the latter species was not recognized by all (see list of recognized species in Eschmeyer et al., 2017). The genus is expanded here to include C. variegatus (senior synonym of C. antonii, type species) and C. spectabilis based on strongly supported molecular evidence and morphological characters. Chirodactylus is superficially similar to Goniistius, but can be distinguished by a shallower dorsal head profile, a lack of bony processes on the frontal bones and maxilla, and a lack of a greatly enlarged 4\textsuperscript{th} dorsal-fin spine.

**MATERIAL EXAMINED** – C. brachydactylus, USNM 93652 [n=1, South Africa: Western Cape], USNM 153508 [n=2, South Africa: Western Cape], ANSP 97440 [n=1, Mozambique: Maputo Bay]; C. jessicalenorum, USNM 221145 [n=3, South Africa: Natal]; C. spectabilis, NMV A22205 [n=1, Australia: New South Wales: Green Cape], NMV A14 [n=1, Australia: Victoria], NMV A44 [n=1, Australia: Victoria: Welshpool], NMV A24816 [n=1, Australia: Victoria: Little Ram Head Point]; C. variegatus, CAS 8447 [n=4, Peru: Lima: Bay of Callao], USNM 77517 [n=1], USNM 128061 [n=4].

**Genus Dactylophora De Vis**

**INCLUSIVE SPECIES** – Dactylophora nigricans (Richardson) (type by monotypy)
Diagnosis – *Dactylophora* can be diagnosed by the following combination of characters: dorsal-fin elements XV–XVI, 24–26; anal-fin elements III, 9–10; pectoral-fin rays 14 with ventral 5 unbranched and thickened; lateral-line scales 45–55. Height of soft dorsal fin roughly equal to height of spinous portion. Elongate body with shallow dorsal head profile; body cylindrical in cross section; scales cycloid and large on body; eyes moderate size; no bony processes on frontal bones or maxilla.

Habitat and Distribution – Found by rocky reefs and weeds and seagrasses to 30m (Kuiter, 1993). Distributed along the southern coast of Australia and northern Tasmania.

Comments – Distinguished from all other latrids by a long, cylindrical body that lacks both a pointed snout and high anal-fin ray counts. Can acquire large adult sizes, reaching 1.2m TL (Kuiter, 1993).

Material Examined – *D. nigricans*, LACM 52122 [n=1, Australia], NMV A17775 [n=1, Australia: Victoria: Port Phillip Bay], NMV A13967 [n=1, Australia: Victoria: Port Phillip Bay], NMV A25379-001 [n=1, Australia: Victoria: Port Phillip Bay], USNM 440480 [n=1, Australia: Tasmania].

Genus *Goniistius* Gill

Inclusive Species – *Goniistius zonatus* (Cuvier) (type species), *G. francisi* (Burridge), *G. gibbosus* (Richardson), *G. plessisi* (Randall), *G. quadricornis* (Günther), *G. rubrolabiatu*s (Allen & Heemstra), *G. vestitus* (Castelnau), *G. vittatus* (Garrett), *G. zebra* (Döderlein)

Diagnosis – Diagnosis as in Randall (1983) using the following combination of characters: dorsal-fin elements XVI–XVIII, 29–35; anal-fin elements III, 8–12; lateral-line scales 54–71; pectoral-fin rays 14 with ventral 6 thickened and unbranched; pectoral-fin rays not extending to anal-fin origin. Body ovoid and compressed; lips large and fleshy; bony processes commonly
found on frontal bone medially to orbit or anteriorly on maxilla except for *G. rubrolabiatus* and *G. zonatus*; dorsal profile of head steep and resulting in a deep body for all species except *G. rubrolabiatus*. All species with more than two angled bars along the body and head, which are black and white in most species (reddish brown in *G. rubrolabiatus*, and yellow in *G. zonatus*).

**Habitat and Distribution** – This genus has an anti-tropical distribution in the Pacific (Randall, 1983). In the Southern Hemisphere they are found in the temperate waters off eastern and western Australia and two species occur among south Pacific islands, including Easter Island. Members of this genus also occur in the Northern Hemisphere in Japan, Korea, China, Taiwan, and Hawaii. Members of *Goniistius* are commonly found in rocky reef areas consuming invertebrates from the substrate.

**Comments** – In their revision of Australian cheilodactylids, Allen and Heemstra (1976) placed several genera, including *Goniistius*, in synonymy with *Cheilodactylus* because many of these genera were erected due to morphological differences with the type species, *C. fasciatus*. Since then, *Goniistius* has been treated as a valid subgenus of *Cheilodactylus* by many authors (Randall, 1983, Burridge & White, 2000), and several have suggested re-elevating *Goniistius* (Randall, 2005). Here we distinguish *C. fasciatus* and *C. pixi* as entirely distinct from all Australian morwongs, and elevate *Goniistius* as a genus within the Latridae. Of all species in this genus, *G. rubrolabiatus* appears to be the most phenotypically distinct, lacking the elevated dorsal head profile, the elongated 4<sup>th</sup> dorsal-fin spine, and the black and white coloration. However, molecular evidence strongly supports its placement within the genus (Fig. 3.).

**Material Examined** – *G. francisi*, AM I27139-006 [n=1, Australia: Tasman Sea: Middleton Reef], AM I42728-001 [n=1, Australia: Lord Howe Island], AM I27134-003 [n=1, Australia: Tasman Sea: Middleton Reef], USNM 47814 [n=1]; *G. gibbosus* WAM P25999-001 [n=1,
Australia: Western Australia: Point Peron], WAM P24836 [n=1, Australia: Western Australia: Irwin Inlet], WAM P21780-001 [n=1, Australia: Western Australia: Swan River], WAM P25270-001 [n=1, Australia: Western Australia: Hardy Inlet], WAM P25072 [n=1, Australia: Western Australia: Harding River], USNM 84377 [n=1]; G. plessisi CAS 47908 [n=1 (paratype), French Polynesia: Easter Island], USNM 226553 [n=1 (paratype), French Polynesia: Easter Island], USNM 378135 [n=1, French Polynesia: Easter Island]; G. rubrolabiatus WAM 25225 [n=1 (holotype), Australia: Western Australia: Fremantle], WAM P22580 [n=1 (paratype), Australia: Western Australia: Rockingham], WAM P5562 [n=1, Australia: Western Australia: Rottnest Island], WAM P5925 [n=1, Australia: Western Australia: Trigg Island], USNM 214831 [n=1 (paratype), Australia: Western Australia: Cockburn Sound]; G. vestitus AM I41831-003 [n=1, Australia: New South Wales: Iron Peg Point], AM I4858-005 [n=1, Australia: New South Wales: Clarence River], CAS 20400 [n=1, Australia: Queensland: Moreton Bay], NMV 54113 [n=1, Australia: New South Wales: Port Jackson]; G. vittatus CAS 20386 [n=2, United States: Hawaii: Oahu: Honolulu], USNM 126514 [n=1, United States: Hawaii]; G. zebra CAS 23483 [n=1, Japan: Kanagawa Prefecture: Misaki], USNM 56431 [n=1]; G. zonatus CAS 13996 [n=3, China: Hong Kong: Cape D’Aguilar], USNM 71062 [n=1, Japan: Osaka Prefecture: Misaki].

**Genus Latridopsis Gill**

**INCLUSIVE SPECIES** – *Latridopsis ciliaris* (Forster) (type species), *Latridopsis forsteri* (Castelnau)

**DIAGNOSIS** – *Latridopsis* can be diagnosed with the following combination of characters: dorsal-fin elements XVI–XVII, 37–43; anal-fin elements III, 31–37; pectoral-fin rays 16–19; pectoral-fin rays not greatly elongated, upper rays longer than lower rays, distal edges of fins rounded. Body moderately ovoid to elongate and highly compressed laterally; caudal peduncle thin; snout pointed with a terminal mouth; lips not as enlarged as other species in Latridae; strong notch
between spinous and soft dorsal fins; dorsal-fin spines not enlarged and none that are significantly longer than others; anal-fin long and reaching caudal peduncle. Body gray in appearance; scales cycloid.

**Habitat and Distribution** – Tasmania, southeastern Australia and New Zealand. Demersal species, generally found near rocky reefs to 160m (Roberts, 2015).

**Comments** – These species feed on a variety of benthic invertebrates. They are generally solitary, or in small groups, but migrate in large schools (Kuiter, 1993). Commercially harvested in parts of their range (Roberts, 2015).

**Material Examined** – *L. ciliaris* CAS 58777 [n=1, New Zealand: Cape Wanbrow]; *L. forsteri*, AM I17556-010 [n=1, Australia: Tasmania: Granville Harbor], USNM 226548 [n=1].

**Genus Mendosoma Guinchenot**

**Inclusive Species** – *Mendosoma lineatum* Guinchenot (type by monotypy)

**Diagnosis** – *Mendosoma* is diagnosed from all other latrids by having a combination of the following characters: dorsal-fin elements XXII–XXV, 23–27; anal-fin elements III, 17–21; pectoral-fin rays 16–19; vertebrae 42–46. Body elongate with a pointed snout and terminal mouth; mouth highly protrusible; eye moderate; no teeth on lower jaw; scales small and cycloid; pre-dorsal bones arranged 0/0/0/1+1/1+1/1.

**Habitat and Distribution** – Found throughout the temperate waters of the Southern Hemisphere from Tasmania, southern Australia, New Zealand and southern Chile. Commonly found in tide pools and in the water column near rocky reefs to 22m (Roberts, 2015).

**Comments** – Distinguished from all other latrids by the unique pre-dorsal bone arrangement with a single dorsal-fin spine articulating with the first dorsal pterygiophore, the elongate, tubular body, and the pointed, highly protrusible mouth. Feeds on zooplankton in the water
column. Five species of *Mendosoma* have been described in the literature, but here we take the conservative approach of only recognizing a single species based on the detailed results of Gon & Heemstra (1987).

**Material Examined** – *M. lineatum*, CSIRO H 2377-01 [n=1, Australia: Tasmania], CSIRO T 1119 [n=1, Australia: Tasmania: Maria Island], NVM A19874 [n=1, Australia], NVM A11395 [n=1, Australia].

**Genus Latris Richardson**

**Inclusive Species** – *Latris lineata* (Forster) (type species), *Latris pacifica* Roberts

**Diagnosis** – Diagnosis follows that of Roberts (2003) with the following combination of characters: elongate, compressed body; eye small; terminal mouth; caudal peduncle thin, with caudal fin strongly forked; dorsal-fin elements XVII–XX, 33–44; anal-fin elements III, 26–37; pectoral-fin rays 16–19 with 6–9 branched rays; pectoral-fin rays not reaching anal-fin origin; 98–125 lateral line scales; 37–43 vertebrae; scales small and cycloid.

**Distribution** – Found throughout the temperate Southern Hemisphere, with the exception of South Africa, to 300m in rocky regions (Roberts, 2003).

**Comments** – *Latris lineata* is popular in commercial fisheries, and can live to 43 years (Roberts, 2015). Less is known of *L. pacifica*, although it too may be harvested in large numbers but misidentified as *L. lineata*. Larvae are adapted to a long pelagic ‘paper fish’ stage that allow for long-distance dispersal. There is an extensive taxonomic history of this genus outlined in Roberts (2003).

**Material Examined** – *L. lineata* USNM 176770 [n=1, New Zealand: Auckland], CSIRO H 4944 [n=1, Australia: Tasmania:], CSIRO H 4945 [n=1, Australia].
Genus Morwong Whitley

INCLUSIVE SPECIES – *Morwong fuscus* (Castelnau) (type species), *M. ephippum* (McCulloch & Waite)

DIAGNOSIS – *Morwong* can be diagnosed by the following combination of characters: dorsal-fin elements XVI–XVIII, 30–35; anal-fin elements III, 8–9; lateral-line scales 59–66; pectoral-fin rays 13–14 with ventral 5–6 rays thickened and unbranched. Can be distinguished from *Goniistius* by a shallower dorsal head profile, and a shorter 4th dorsal-fin spine, and from *Chirodactylus* by a higher lateral-line scale count (59–66 in *Morwong* versus 46–56 in *Chirodactylus*) and higher dorsal-fin soft ray count (30–35 in *Morwong* versus 22–31 in *Chirodactylus*). Color generally brown to brownish red.

HABITAT AND DISTRIBUTION – Occurs off the southeast coast of Australia, the northern island of New Zealand, and islands of the Tasman Sea, to 50m among rocky reef habitats.

COMMENTS – Originally erected by Whitley (1957), *Morwong* was described as distinct from other members of *Cheilodactylus* by the number of dorsal-fin elements and lateral line scales, as well as ‘transverse dark bars’ on the body. These diagnostic characters remain largely valid when compared to Cheilodactylidae as recognized herein (restricted to two species in South Africa). Both species of *Morwong* are largely brown to brownish red, a character only shared with *G. rubrolabiatus*, but absent from any other members of the family.

MATERIAL EXAMINED – *M. ephippium*, AM I20493-001 [n=1, Australia: New South Wales: Broughton Island], AM I20255-001 [n=1, Australia: New South Wales: Norfolk Island], AM I27891-026 [n=1, Australia: Tasman Sea: Elizabeth Reef], AM I24294-001 [n=1, Australia: New South Wales: Montague Island]; *M. fuscus*, AM I24982-001 [n=1, Australia: New South Wales: Manly], ANSP 122393 [n=1, Australia: Queensland: Bribie Island], CAS 20803 [n=1, Australia:...
New South Wales: Port Jackson], NMV 54265 [n=1, Australia: New South Wales: Port Jackson], USNM 59938 [n=1].

**Genus *Nemadactylus* Richardson**

**INCLUSIVE SPECIES** – *Nemadactylus macropterus* (Forster) (type species), *N. bergi* (Norman), *N. douglasii* (Hector), *N. gayi* (Kner), *N. monodactylus* (Carmichael), *N. rex* Roberts, *N. valenciennesi* (Whitley), *N. vema* (Penrith)

**DIAGNOSIS** – *Nemadactylus* can be diagnosed by the following combination of characters:

dorsal-fin elements XVI–XVIII, 24–31; anal-fin elements III, 11–19; pectoral-fin rays 14–16 with one greatly elongated ray extending past origin of anal-fin; body ovoid and compressed without any greatly elongated dorsal-fin spines; dorsal head profile shallow; spinous and soft dorsal-fin portions not separated by a large notch.

**HABITAT AND DISTRIBUTION** – Widely distributed throughout the temperate Southern Hemisphere. Occur in Australia, New Zealand, South America, and oceanic islands within the Southern Ocean. Typically found on rocky reefs, or sandy habitat near rocky reefs to 400m (Kuiter, 2003).

**COMMENTS** – Feed on a variety of benthic invertebrates. Some species targeted in both recreational and commercial fisheries.

**MATERIAL EXAMINED** – *N. bergi*, ANSP 102720 [n=1, Argentina: Buenos Aires]; *N. douglasii*, NMV A13196 [n=5, Australia: New South Wales: Merimbula]; *N. gayi*, USNM 176401 [n=3], USNM 176402 [n=1]; *N. macropterus*, CAS 58782 [n=2, New Zealand: Wellington Harbor], NMV A21603 [n=5, Australia: Tasmania: Flinders Island], USNM 39674 [n=1]; *N. valenciennesi*, NMV A12627 [n=2, Australia: Victoria: Cape Duquesne], WAM P21896 [n=1, Australia: Western Australia: Esperance].
Genus *Pseudogoniistius* Ludt, Burridge & Chakrabarty, gen. nov.

INCLUSIVE SPECIES – *Pseudogoniistius nigripes* (Richardson) (type by monotypy)

DIAGNOSIS – Diagnosis follows that of Randall (1983). Dorsal-fin elements XVII–XIX, 25–28; anal-fin elements III, 9–10; pectoral-fin rays 14 with ventral 5 or 6 thickened and unbranched; fifth pectoral-fin ray longest, extending past anal-fin origin; lateral line scales 63–69; scales cycloid; scaly sheath present at base of dorsal and anal fins; sheath is taller under soft portions of dorsal-fin than under the spinous portions. Dorsal-fin spines increasing in length to fifth, then decreasing slightly thereafter. Body compressed and ovoid with a steep head angle; fleshy, large lips present; two pairs of bony processes – one pair on frontal bones medial to orbit and one pair superior to maxilla. Body has a unique coloration for the family, with two wide, vertical dark bars intersecting the anal and pelvic fins, and a narrower dark bar intersecting the eye; caudal fin color is a reddish-brown. Only species in family that is known to rapidly change color by lightening the dark bars on the body.

HABITAT AND DISTRIBUTION – Found on shallow rocky reefs in Southern Australia to 25m. Recorded, but rare, in northern New Zealand.

ETYMOLOGY – Gender masculine. Named for the superficial similarity this species has with species of *Goniistius*, and for the confusion that this species has caused with morwong classification in the past (Randall, 1983).

COMMENTS – *Pseudogoniistius* has traditionally been allied with *Goniistius*. However, morphological (Randall, 1983) and molecular approaches (Burridge & White, 2000) clearly demonstrate that this species is not closely related to other species of *Goniistius*. Furthermore, the ability to rapidly change color appears unique for the family.
MATERIAL EXAMINED – *P. nigripes* NMV A2569 [n=1, Australia: Victoria: Leonard Bay], NMV A20553 [n=1, Australia: Tasmania: Flinders Island], NMV A11913 [n=1, Australia], YPM 005957 [n=1, Australia: South Australia: Kangaroo Island].

DISCUSSION

Taxonomic confusion has persisted in cheilodactylid fishes for over a century. Here the families Cheilodactylidae and Latridae are redefined with extensive taxonomic sampling, morphological characters, and strongly supported molecular data. Previous efforts to clarify the relationships of cheilodactylid fishes resulted in most genera being recognized as junior synonyms of *Cheilodactylus*, which became a catch-all for a variety of morphologically, geographically, and behaviorally distinct fishes. The previous classification did not reflect the evolutionary history of these fishes and seems to have aided in the confusion surrounding cheilodactylid relationships, and has confounded previous attempts at determining the history of anti-tropical distributions in these fishes (Burridge & White, 2000).

The relationships recovered here have been found by previous studies (Burridge & Smolenki, 2004; Sanciangco et al., 2016). The repeated recovery of a non-monophyletic Cheilodactylidae from a variety of studies, which have used different species, molecular loci, and analytical approaches, increases the confidence that our findings accurately reflects the evolutionary history of these fishes. This result is further corroborated by the osteological characters and larval characteristics included here. This new classification scheme highlights clades that are sufficiently unique to be recognized as separate genera. One of our goals was to achieve a monophyletic taxonomy with the fewest number of changes that can be supported by morphology. While both *M. fuscus* and *M. ephippium* could be placed within *Goniistius* to reduce the number of genera in the Latridae, these taxa have never been associated with
Goniistius in the past, and are quite distinct; in coloration, they are mostly red or brown while almost all Goniistius are striped with black and white bars, and the length of dorsal-fin spines gradually increase to the fourth or fifth spine whereas species in Goniistius have a distinctly elongated fourth dorsal-fin spine compared to the preceding spines. Likewise, *P. nigripes* could be placed within *Nemadactylus* instead of a new, monotypic genus, yet this grouping would be unsatisfactory as this species lacks diagnostic characters of *Nemadactylus*, and is noticeably distinct from all other species in the Latridae.

By re-describing the families Cheilodactylidae and Latridae, we have clarified their evolutionary history for future studies. The Cheilodactylidae is a small, but unique, family that is restricted to the temperate coastal waters of southern Africa. Conversely, the Latridae is a temperate family of 30 species that are extremely variable in diet, habitat, and body shape. This classification reflects the evolutionary history of this group and is a solid basis for studying antitropicality, which is now restricted to the genus *Goniistius* within the family Latridae.

The MRCA of all the temperate families within the Cirrhitoidea (Latridae, Cheilodactylidae, Aplodactylidae, and Chironemidae) appear to originate near Southern Australia and New Zealand in the early Eocene, approximately 56mya. The exception to this is the tropical family Cirrhitidae, which is fairly widespread in temperate and tropical habitats, worldwide, and which we lack the sampling necessary to discuss biogeographic patterns. Therefore this discussion will be limited to the remaining four families of the Cirrhitoidea. Crown ages for the temperate families all date to the Miocene, with the exception of Latridae, which is estimated from the Oligocene. Most diversification in these families was found in the Australia/New Zealand region, with several example of lineages dispersing to either South America or South Africa. The family Cheilodactylidae, now represented by two South African...
species, transitioned from Australia/New Zealand to its current location sometime between Eocene and Oligocene. Given the long branch that this family is on, it is possible that this family did this either by west wind drift (summarized in Waters, 2008), or by occupying Antarctic habitats that were not glaciated prior to the Oligocene (with subsequent extinction of species or populations found along these coasts). The other transitions from Australia/New Zealand to South America or South Africa all occurred during the Miocene or into the Pleistocene, and are likely driven by west wind drift, as previously hypothesized for several families with similar distributions (Burridge, 2000; Burridge et al., 2006).

![Transition Times](image)

**Figure 5.** Simulated equatorial transition times generated from 100 posterior BEAST trees.

While there has been considerable longitudinal dispersal throughout the evolutionary history of this group, there is also latitudinal dispersal underlying anti-tropicality within *Goniistius*. This seems to have happened at two distinct time periods: the late Miocene and the Pleistocene/Pliocene. The transition in the late Miocene saw the MRCA of *G. quadricornis* and *G. zonatus* transition into the Northern hemisphere, followed by a subsequent speciation event. These two species overlap entirely in their distribution in the northeast Pacific, but differ in the depths that they inhabit, with *G. quadricornis* occupying deeper water (Masuda et al., 1985).
Speciation by depth has been suggested in other temperate species (Ingram, 2011), and may have played a role in the divergence between these two species.

More recently, south to north transitions also occurred with the MRCA of *G. zebra*, which occurs in the northeastern Pacific, and with the MRCA of *G. vittatus*, which occurs in Hawaii. These two species are part of a clade along with *G. plessisi* (Easter Island), and *G. francisi* (South Pacific) that is difficult to resolve, even with the amount of genomic data here. Interestingly, we do not recover *G. francisi* to be sister to *G. vittatus*, despite the two being considered the same species at one point. However, support values within this clade are lower, and alternative topologies for this clade are recovered in each analysis conducted (Appendix B). Ultimately this does not affect our anti-tropical hypothesis testing, as there still must have been at least one (but likely two) transitions within this clade. This entire clade dates to 3.7mya (3.1–4.5 95% HPD), approximately at the onset of Pleistocene glaciations. This time period is coupled with the closure of the Isthmus of Panama, which altered ocean currents locally in the Eastern Pacific and Caribbean (O’Dea et al., 2016), and globally (Schneider & Schmittner, 2006). Current models suggest that the closure of the Isthmus of Panama likely increased throughflow in the Indo-Australian Archipelago (Schneider & Schmittner, 2006). These global current shifts could have influenced dispersal in the MRCA to this clade, resulting in short internodes between speciation events that are difficult to resolve.

Regardless, our biogeographic reconstructions support a scenario under which all anti-tropical divergences occurred after the mid-Miocene warming event. This precludes any of the anti-tropical hypotheses outlined in Chapter 1 that occur before the mid-Miocene, and suggests that global warming in the early Miocene did not split widespread, temperate species in anti-
tropical forms. Under this scenario only three potential hypotheses remain regarding anti-
tropicality: isothermal submergence, biotic exclusion and glacial dispersal.

The first hypothesis seems unlikely, as species of *Goniistius* are generally shallow water
species that don’t disperse very far as adults, especially in very deep, cold water (McCormick,
1989; Lowry & Suthers, 1998). Biotic exclusion is difficult to test, as identifying the excluding
agent can be challenging. However, one of the assumptions of biotic exclusion is that anti-
tropical species are older than species occupying the tropics (see Chapter 1). This assumption
may fit equatorial divergences dating to the Miocene, but fails to fit with anti-tropicality for *G.
zebra* and *G. vittatus*, which are in a clade of four species that seems to have diversified very
recently. Counter to biotic exclusion, glacial dispersal fails to explain equatorial divergence
events in the late Miocene, but can be used to possibly explain recent divergences. However,
simulated divergence events extend into the Pliocene, when global temperatures were warmer
than current values (Zachos et al., 2001), raising some doubts to glacial dispersal.

**Conclusions**

Taxonomic issues for Cheilodactylidae have been suspected for over a decade, yet
previous attempts at resolving these relationships were either poorly supported (Burridge &
Smolenski, 2004), or lacked the taxonomic sampling necessary to make changes (Sanciangco et
al., 2016). This subsequently also made estimating the historical biogeography of these fishes
difficult (Burridge & White, 2000). Using genomic analyses in concert with morphological
approaches we have shown that Cheilodactylidae *sensu stricto* is represented solely by two
species in South Africa. Furthermore, this suggests that Latridae is a large family of temperate
marine fishes with a variety of behaviors, body shapes, and niches. Anti-tropicality within
Latridae is restricted to the genus *Goniistius*, and seems to be the result of at least two, but
possibly three, equatorial transitions from the southern hemisphere to the northern hemisphere occurring in late Miocene and Pleistocene/Pliocene. These dates directly match up with previous anti-tropical estimates in other unrelated families (Ludt et al., 2015), and suggest that a multitude of drivers may cause this pattern. Finally, it must be noted that anti-tropicality may be the result of stochastic crossing events, unrelated to any of the previously proposed mechanisms.
INTRODUCTION

A variety of terms describe related species that are distributed outside of, and on opposing sides of, the tropics: “amphitropical,” “bipolar,” “bitemperate,” and “bianti-tropical” 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, 1987a; Burridge, 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”

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populations at higher latitudes (Théel, 1885; Briggs, 1987a); 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 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 Burridge (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. laticlavius* 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. 6).

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.
MATERIALS AND METHODS

Taxonomic Sampling and Extraction

Tissue samples were acquired by museum tissue loans or from our collecting efforts for all species within Prionurus (Table 2). Multiple individuals of each species were obtained when possible. Fin clips were stored in 95% ethanol at -80 °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 °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.

Figure 6. Map showing the distribution of all species of Prionurus. Both P. microlepidotus and P. maculatus are shown in green, P. chrysurus in orange, P. scalprum in yellow, P. laticlavius 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).
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.

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

**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 Bayesian 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.

**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.

**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.

**RESULTS**

**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 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. Estimates obtained from the maximum likelihood analysis, and Bayesian analysis, placed P. microlepidotus as the sister lineage to a clade containing P. biafraensis + P. laticlavius + P. punctatus, albeit with low bootstrap support and posterior probabilities (Fig. 7a). 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. 7b), 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 congener) 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. laticlavius and P. punctatus, as well as the eastern Atlantic P. biafraensis (Fig. 8). Both clades are Pliocene in origin, with the crown age of the eastern Pacific/Atlantic clade dating
Table 3. 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), and SIO (Scripps Institute of Oceanography). * denotes which individuals were used in the BEAST and *BEAST analyses.

<table>
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<tr>
<th>Species</th>
<th>Tissue Voucher</th>
<th>16S</th>
<th>COI</th>
<th>myh6</th>
<th>Rag1</th>
<th>Zic1</th>
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<td>no data</td>
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<tr>
<td></td>
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<td>KC623821</td>
<td>KC623906</td>
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<td>HM034247</td>
<td>EF536292</td>
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<td>EF533915</td>
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<td>KC623683</td>
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<td>KC623689</td>
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<td>KC623861</td>
<td>KC623935</td>
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<td>EF536300</td>
<td>EF530100</td>
<td>EF533923</td>
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<td>DQ532902</td>
<td>KC623653</td>
<td>EF536299</td>
<td>EF530099</td>
<td>EF533922</td>
</tr>
</tbody>
</table>

*This specimen was cataloged as *P. laticlavius*. It was identified as *P. punctatus* after personal examination of the voucher by WBL.
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 3.

The shallowest divergence between any two species of *Prionurus* was found between the two eastern Pacific species, *P. laticlavius* and *P. punctatus* (Fig. 8). These two species have ranges that overlap in some areas (Fig. 6) 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. 7a),

![Figure 7. 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.](image-url)
and a shared mtDNA COI haplotype was found between them. In contrast, the western Pacific \textit{P. chrysurus} and \textit{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.

\textbf{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 \textit{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 \textit{P. chrysurus} and \textit{P. maculatus} was estimated as being eastern Australian.

Table 4. 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.

<table>
<thead>
<tr>
<th>Clade Contains</th>
<th>Estimated Age</th>
<th>95% HPD</th>
</tr>
</thead>
<tbody>
<tr>
<td>All \textit{Prionurus}</td>
<td>12.12</td>
<td>8.01 – 16.88</td>
</tr>
<tr>
<td>All except \textit{P. microlepidotus}</td>
<td>9.39</td>
<td>6.14 – 13.08</td>
</tr>
<tr>
<td>\textit{P. chrysurus, P. maculatus, and P. scalprum}</td>
<td>4.71</td>
<td>2.67 – 7.09</td>
</tr>
<tr>
<td>\textit{P. biafraensis, P. laticlavius, and P. punctatus}</td>
<td>3.13</td>
<td>1.51 – 5.25</td>
</tr>
<tr>
<td>\textit{P. chrysurus} and \textit{P. maculatus}</td>
<td>2.19</td>
<td>0.98 – 3.74</td>
</tr>
<tr>
<td>\textit{P. laticlavius} and \textit{P. punctatus}</td>
<td>0.49</td>
<td>0.07 – 1.21</td>
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</table>

\textbf{DISCUSSION}

\textit{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.

**Uncertain Relationships Among Several Sawtail Surgeonfishes**

All analyses recovered a monophyletic *Prionurus* with strong support (Fig. 7). 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

![Figure 8. 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.](image-url)
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) 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. laticlavius* 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 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.

**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; Burridge, 2002) from older vicariance or competitive exclusion hypotheses (Théel, 1885; Briggs, 1987a; 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. 7). 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. 8). 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. 8). 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, 1987a), 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 (Burridge and White, 2000; Burridge, 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, 1987a), and it seems to suggest that some species of *Prionurus* may be physiologically restricted to cooler water habitats.

**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. laticlavius* and *P. punctatus*, seem more tolerant of warmer waters. These species form a clade that dates to the Pliocene (Fig. 8) 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.

**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. 6). All analyses found *Prionurus* to be monophyletic, and late Miocene in origin with a crown age estimated at 12.1mya (Fig. 8). 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 (Burridge and White, 2000; Burridge, 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. laticlavius* and *P. punctatus*. 
CHAPTER 4
SPECIES LIMITS OF TWO EASTERN PACIFIC SPECIES IN AN ANTI-TROPICAL GENUS

INTRODUCTION

Species are the fundamental unit of biology, and as such their proper identification is critical for a variety of disciplines, including phylogenetics, biogeography, population genetics, and conservation (De Queiroz, 2005). Traditionally species are diagnosed by one or more morphological difference (either fixed or in combination) between groups. In groups that generally display vibrant coloration patterns, such as tropical coral reef fishes, many species have been identified through subtle color differences (Taylor & Hellberg, 2005; Leray et al., 2010; Rocha, 2004). For many reef fishes, color or squamation patterns have been used to identify genetic breaks between major biogeographic provinces (DiBattista et al., 2013; Coleman et al., 2016), and to detect areas with high rates of endemism, such as Hawaii (Randall & Rocha, 2009) and the Marquesas (Gaither et al., 2015a). However, a number of studies have also shown that differences in color patterns are not always indicative of reduced gene flow (Ramon et al., 2003; Lin et al., 2009), and can be discordant with patterns of genetic structure (Leray et al., 2010; Gaither et al., 2014; DiBattista et al., 2015). Taken together, these studies indicate that color patterns alone are not well-suited for defining species limits, but should be used in concert with other measurements to ensure an accurate reflection of evolutionary history.

The tropical Eastern Pacific (TEP) is a marine biogeographic region that spans 29° of latitude from Magdalena Bay, Mexico, to the Gulf of Guayaquil, Ecuador (Robertson & Cramer, 2009). Numerous studies have categorized the TEP into three to five biogeographic provinces based on the distribution records of fishes (Briggs, 1974; Hastings, 2000; Spalding et al., 2007; Robertson & Cramer, 2009; Briggs & Bowen, 2012). This region has been partially isolated from
the Indo-Pacific since the Miocene, and completely separated from the Atlantic since the closure of the Isthmus of Panama. The biodiversity of the TEP pales in comparison to that of its neighboring Central/West Pacific region, and it has consequently been discussed as having “reduced speciation capacity”, particularly in several iconic reef-fish families (Cowman & Bellwood, 2013). Still, speciation within the TEP is facilitated by the limited connectivity between the offshore islands and the continental coast (Allen & Robertson, 1994). Examples include the high rates of fish endemism of the Galapagos (~17% endemic species), Clipperton atoll (~7% endemic species), Cocos Island (~4%), and the Revillagigedos (~8%; Roberston & Cramer, 2009; Cortés, 2012). Many of these offshore endemics are distinguished by coloration differences from their continental congeners, and for some groups, multiple offshore islands have their own endemic species. For example, in Holocanthus angelfishes, H. clarionensis and H. limbaughi occur on the Revillagigedos and Clipperton Islands, respectively, and diverged from their widespread mainland sister species, H. passer, approximately 1.4 mya (Alva-Campbell et al., 2010; Tariel et al., 2016). Divergences between oceanic and continental species have been detected at a variety of time scales, suggesting that not one oceanographic event led to isolation of coastal and oceanic populations, and that limited connectivity between these ecosystems repeatedly promotes speciation (Craig et al., 2006; Alva-Campbell et al., 2010; Tariel et al., 2016; Wainwright et al., 2017).

However, not all speciation in the TEP is between offshore islands and the mainland, as sister species are also distributed latitudinally along the continental coast (Hastings, 2000; Riginos, 2005). In many cases, coastal speciation is observed in fishes with reduced dispersal capabilities, such as those with demersal eggs or short pelagic larval durations (e.g. blennies; Lin & Hastings, 2011; Eytan et al., 2012; Miller et al., 2016). However, this is not always the case, as
fishes with high dispersal potential are hypothesized to have diverged *in situ* in coastal habitats, such as grunts (Bernardi et al., 2008; Rocha et al., 2008; Tavera et al., 2012; Bernal et al., 2016), wrasses (Wainwright et al., 2017), and *Prionurus* surgeonfishes (Ludt et al., 2015).

The present study focuses on two species of *Prionurus* distributed latitudinally throughout the TEP: *P. punctatus* occurs from the Gulf of California to Costa Rica, while *P. laticlavius* extends from Costa Rica to Ecuador, also occupying offshore islands of the TEP (Fig. 9; Robertson & Allen, 2015). This pattern of distribution is somewhat unexpected, as surgeonfishes have extremely high dispersal potentials (Doherty et al., 1995), and several species lack any population structure across entire ocean basins (Eble et al., 2009, 2011; Dibattista et al., 2011). In fact, while seven surgeonfish species regularly occur in the TEP (Allen & Robertson, 1994), the two species of *Prionurus* are the only surgeonfishes in the region that aren’t also present in the Indo-Pacific. Furthermore, these two species are nearly identical phenotypically. In the description of *P. punctatus*, Gill notes that “it widely differs from the previously known [*P. laticlavius*] by its spotted body; in other respects it is most nearly allied to the *Prionurus laticlavius* from the Galapagos Islands” (Gill, 1862). The situation is further complicated by a recent phylogenetic analysis of the genus, where a multi-locus approach did not recover these two species as reciprocally monophyletic (Chapter 3; Ludt et al., 2015). However, that particular study was based on three individuals of *P. punctatus* and two of *P. laticlavius*, and it is possible that the loci used were not appropriate for resolving shallow divergences (Ludt et al., 2015).

Considering their distribution across the continental waters of the TEP, as well as their morphological and phylogenetic similarities, it would be interesting to discover potential differences at the genomic level that could diagnose *P. punctatus* and *P. laticlavius*, to clarify the species status of these two TEP species.
Here we expand upon the results of Chapter 3 (Ludt et al., 2015) by including individuals from several locations across the TEP and by adding genomic analyses between the two species. In addition to genetic data, we gathered traditional morphological and meristic data for both species across their ranges and compared it to original species descriptions and type material. We then examined if ecological factors may be responsible for any divergences between these species in order to assess possible speciation drivers along the coastal TEP.

Figure 9. Distribution of two species of TEP surgeonfishes. *Prionurus punctatus* (upper left) is shown in blue, and *P. laticlavius* (lower left) is shown in red. Yellow stars show the sampling locations for this study. The offshore islands are previously only thought to be occupied by *P. laticlavius*. However, two vouchered specimens of *P. punctatus* have been verified from the Revillagigedos (upper left group of islands).
METHODS

Phenotypic and Morphological Comparisons

To assess species limits in this system, both molecular and specimen-based approaches were used. An in-depth morphological comparison of these two species has never been conducted, and potentially could reveal more characters consistent with species diagnoses than just squamation patterns. For this purpose, specimens for *P. punctatus* and *P. laticlavius* were examined from across their distributions for examination of phenotypic and morphological variation. Standard measurements and meristic counts were taken for each specimen following those reported in Randall (2002). This included counting the spines and rays of the dorsal, anal, and pectoral fins, and measuring the body depth, pre-dorsal length, pelvic-fin and anal-fin lengths in proportion to standard length. The two species mainly differ in the presence or absence of dark spots covering the body, thus photographs of all specimens were taken to determine how consistent of a character spotting pattern is across the entire TEP. All measurements were made with digital calipers, and averages were calculated for each species.

Molecular Sampling and Extraction

To have a better understanding of the genetic divergence between *P. punctatus* and *P. laticlavius* along the mainland TEP, we sampled at three localities: San José del Cabo, Baja California, Mexico, Cuajiniquil, Guanacaste, Costa Rica, and Las Perlas Islands, Panama. This sampling scheme targets two extreme locations, where only a single species is reported in the literature (Mexico for *P. punctatus*, and Panama for *P. laticlavius*), as well as one location where the two species overlap in their recorded distributions (Guanacaste, Costa Rica). Samples were obtained between 2012 and 2015 using either nets along the shore, or pole spears while scuba diving. Tissue samples were taken from pectoral fins, gills, or muscle tissue and stored in 95%
EtOH. Once in the laboratory, tissue samples were stored in a -80ºC freezer prior to sample preparation. When possible, voucher specimens were fixed in formalin and deposited at the Louisiana State University Museum of Natural Science.

Genomic material was extracted from each sample using Qiagen DNeasy Blood and Tissue extraction kit following manufacturers protocols. Extracts were then quantified using a Qubit 2.0 fluorometer with a dsDNA BR Assay Kit (Life Technologies). Quality of genomic extractions was assessed via gel electrophoresis, with a 1% agarose gel using SYBR Safe DNA gel stain (Invitrogen) and 6x blue/orange loading dye (Promega). All extracts were then kept in a -20ºC prior to library preparation and amplification.

**Mitochondrial Sequencing and Analysis**

To determine if our increased sampling effort was enough to resolve the relationships of these two species we amplified all samples for the mitochondrial COI barcoding region. Primers and PCR reactions protocols were identical those described in Ludt et al. (2015) and can be found in Appendix C. All samples were purified and sequenced in both forward and reverse directions using the Genomic Sequencing and Analysis Facility at the University of Texas at Austin. Sequencing was performed on an Applied Biosystems 3730 sequencer. All sequences were edited and aligned using Geneious 6.0.5 (Biomatters) and all alignments were checked manually. Haplotype networks were created using the TCS networks option in PopART (Clement et al., 2000). Summary statistics (haplotype and nucleotide diversities, \( \Phi_w \)), and Fu’s F statistic (Fu, 1997) were calculated using Arlequin 3.5 (Excoffier et al., 2005). An AMOVA was conducted to test for population structuring between the two species, as well as between sampling localities, using 50,000 permutations in Arlequin. These summary statistics were calculated for both species and for all sampling locations.
Genomic Library Preparation, Sequencing, and Analysis

For each sample, approximately 0.5–1ug of DNA was sonicated to approximately 600bp using an Episonic 1000E sonicator with 15 second pulse intervals. Fragmentation was verified on a 1% agarose gel, and the process was repeated as necessary. Library preparation was conducted using a KAPA Hyper Library Prep Kit (KAPA Biosciences) using 10bp TruSeq-style oligonucleotide dual-indexing barcodes (Faircloth & Glenn, 2012). Library preparation followed manufacturers protocols, with the exception that the reaction sizes were scaled to 0.5x. Pre-amplification and post-library amplification values were quantified before equimolar pooling of samples in batches of eight. A target capture approach was then used to amplify ultraconserved elements (UCEs; Faircloth et al., 2012). Pooled libraries were enriched for 1300 UCE loci using a custom probe set (Arbor Biosciences) originally designed by McGee et al. (2016), following manufacturers protocols. Pools were then amplified and cleaned using 16-18 PCR cycles following procedures outlined in Faircloth et al. (2013). These pools were then combined in equimolar ratios and paired-end fragments of 150bp were sequenced on a single lane of an Illumina HiSeq Sequencer at the University of Oklahoma Medical Research Institute.

The sequenced libraries were de-multiplexed, and barcodes, low-quality base calls, and reads shorter than 40bp were removed using Trimmomatic (Bolger et al., 2014) as part of the program Illumiprocessor (Faircloth et al., 2013). Sequences were then assembled into *de novo* contigs using Trinity 2.0.6 with default parameters (Grabherr et al., 2011), and these were mapped to UCE probes using the Phyluce 1.5 pipeline (Faircloth, 2015). Sequence data was then processed in two ways optimized for phylogenomic or population genomic analyses.

For phylogenomic analyses, contigs were first aligned in Phyluce pipeline using Mafft (Katoh & Stanley, 2013) with the *--no-trim* option. Internal trimming using gblocks (Castresana,
was then conducted on this alignment prior to outputting a final 70% complete data
matrix. These alignments were then concatenated, and a maximum-likelihood phylogenomic tree
was then constructed using RAxML v8.1.24 (Stamatakis, 2014) on the CIPRES scientific
gateway portal (Miller et al., 2010). Two samples of *P. biafraensis* were included as outgroups
for rooting the tree, as a previous study indicates this is the sister clade to the TEP species (Ludt
et al., 2015). All analyses were completed using the GTRGAMMA model for bootstrapping,
with 1000 bootstrap iterations, and the rapid bootstrapping option (-x) selected. All nodes with a
bootstrap value less than 50 were then collapsed.

Meanwhile, for the population genomic analyses a reference dictionary was created to
assist in SNP alignment using Picard (http://broadinstitute.github.io/picard/). This dictionary was
created using the sample that recovered the most UCE loci. The reference was then indexed
using SAMtools (Li et al., 2009). All samples were then aligned to this reference using BWA (Li
& Durbin, 2009), and the maximal exact matches (MEM) command, with two threads, and the
-M option for downstream Piccard compatibility. Outputs were converted to BAM formats using
SAMtools. The software Piccard was used for trimming, adding reading groups, and removing
duplicated reads. All alignments were then merged, and sequences were re-aligned around indels
using the genome analysis toolkit (GATK; McKenna et al., 2010). Indels were then called and
masked, and SNPs with a quality score above Q30 were kept and outputted to a VCF file with
GATK. In order to minimize the influence of linkage-disequilibrium in our statistical estimates,
only one randomly chosen SNP per UCE locus was kept for all subsequent analyses. The
resulting file was then converted to various formats for downstream analyses using the scripts of
the seqcap_pop pipeline (https://github.com/mgharvey/seqcap_pop/).
A discriminant analysis of principle components (DAPC) was conducted to identify clusters in the SNP data with the package adegenet in R (Jombart et al., 2010). This was conducted both with, and without outgroup samples of *P. biafraensis*. Since a DAPC that supports a single group cannot be graphed, the UCE SNP data was also examined with a principal components analysis using the dudi.PCA command in the R package ade4 (Dray & Dufour, 2007). The program STRUCTURE v2.3.4 (Pritchard et al., 2000) was used to assign, and assess the fit of individuals to pre-determined numbers of populations (*K*). An admixture model was used with correlated allele frequencies and no *a priori* populations information was given. Populations ranging between one and five (*K* = 1 – 5) were tested using 500,000 MCMC iterations with a burn-in of 25,000. Five replicates were performed for each *K* to ensure convergence. Results were summarized with Structure Harvester (Earl, 2012) using the Evanno method (Evanno et al., 2005). Summary statistics of population genomic parameters (*F*$_{ST}$, observed and expected heterozygosity, effective number of alleles, and Hardy-Weinberg equilibrium) were calculated using GenoDive v2 (Meirmans & Van Tienderen, 2004). An AMOVA analysis was performed with 1,000 permutations to test for genetic structure between the two species, as well as between all sampling locations. The package PEGAS (Paradis, 2010) was used to examine the distribution of *F*$_{ST}$ values across all loci in the dataset containing a single SNP per UCE locus, as well as across all SNPs.

**Ecological Comparisons**

Considering the broad geographic range occupied by these sister-species, it is quite possible that they are occupying ecologically distinct habitats, which could drive speciation even in the presence of gene flow (Rocha et al., 2005; Rocha & Bowen, 2008; Bernardi, 2013). To test this, niche equivalency and similarity tests were conducted to determine if these two species are
occupying similar habitats in the TEP (Broennimann et al., 2012). This approach uses kernel
density smoothing to compare the density of species occurrence in environmental space using
occurrence and environmental data. Occurrence data for both species was acquired from the
Global Biodiversity Information Facility (GBIF) using the R package RGBIF (Chamberlain,
2017). Locality information was checked manually for any mistakes, verifying species
assignments with vouchered museum specimens or photographs. Eleven environmental layers
that summarize bathymetry and annual properties of sea surface salinity (SSS) and sea surface
temperature (SST) for the TEP were downloaded from the MARSPEC database (Sbrocco &
Barber, 2013; http://www.marspec.org). These included: distance to shore, depth, mean annual
range, and annual variance of SSS and SST, as well as the SSS of the wettest and driest months,
and SST of the coldest and warmest month of the year. The comparison tests used here are
bivariate, thus a principal components analysis was conducted using all 11 environmental layers,
and the top two axes were kept for subsequent analyses. Niche equivalency and similarity tests
were conducted in the R package ENMTools (Warren et al., 2010).

RESULTS

Phenotypic and Morphological Data

In total 169 vouchered museum specimens (100 P. punctatus specimens, 64 P. laticlavius
specimens) were examined from the Scripps Institute of Oceanography, Los Angeles County
Museum, California Academy of Sciences, and Louisiana State University Museum of Natural
Sciences. This included specimens distributed across the entire TEP, including offshore islands
(Table C.1).

Overall, type specimens exhibited spotting patterns that were in agreement with the
literature records of “pure” individuals (i.e., those that were not of mixed phenotypic traits).
However, five of the measured specimens had an intermediate phenotype of faint dark spots, suggesting a possible lack of reproductive isolation between the two groups or plasticity of squamation patterns (Figure C.1. & C.2.). These specimens mainly came from Costa Rica where the two species overlap, although intermediate phenotypes were also found in Panama. Further, our morphological observations suggest all meristic counts and measurements overlapped for the two species. Dorsal-fin rays were VII–VIII, 24–28, anal-fin rays II–III, 22–24, and pectoral-fin rays were 15–17 for both species. Body depth ranged from 1.6–2.1, pre-dorsal fin length was 2.4–4.3, pre-pelvic fin length was 2.2–3.6, and pre-anal fin length was 1.3–3 in standard length for both species. The only perceivable difference was the modal number of pectoral-fin (16 in *P. punctatus* and 17 in *P. laticlavius*) and dorsal-fin rays (27 in *P. punctatus*, and 26 in *P. laticlavius*), but the ranges of these counts overlapped between the two species (Table 4).

Table 5. The averages and ranges of meristic and morphological measurements of the two species. All morphological measurements are in comparison to standard length. Modes are reported for meristic counts, and means are reported for measurement comparisons.

<table>
<thead>
<tr>
<th></th>
<th><em>P. punctatus</em></th>
<th><em>P. laticlavius</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Doral-fin spines</td>
<td>VIII (VII–VIII)</td>
<td>VIII (VII–VIII)</td>
</tr>
<tr>
<td>Dorsal-fin rays</td>
<td>27 (25–28)</td>
<td>26 (24–28)</td>
</tr>
<tr>
<td>Pectoral-fin rays</td>
<td>16 (15–17)</td>
<td>17 (15–17)</td>
</tr>
<tr>
<td>Anal-fin spines</td>
<td>III (II–III)</td>
<td>III (II–III)</td>
</tr>
<tr>
<td>Anal-fin rays</td>
<td>23 (22–24)</td>
<td>23 (22–24)</td>
</tr>
<tr>
<td>Pre-dorsal length</td>
<td>3.2 (2.4–4.3)</td>
<td>3.3 (2.5–4.2)</td>
</tr>
<tr>
<td>Pre-pelvic length</td>
<td>2.9 (2.4–3.6)</td>
<td>3 (2.2–3.7)</td>
</tr>
<tr>
<td>Pre-anal length</td>
<td>2 (1.8–2.7)</td>
<td>2 (1.3–3)</td>
</tr>
<tr>
<td>Body Depth</td>
<td>1.8 (1.6–2.1)</td>
<td>1.9 (1.6–2.1)</td>
</tr>
</tbody>
</table>

**Mitochondrial COI and Sampling**

In total, 53 individuals were collected, including 25 *P. punctatus*, 23 *P. laticlavius*, and 5 individuals with intermediate phenotypes that had faint spots restricted to certain regions of their bodies. The analyses reported here used all collected individuals, including fishes with
intermediate phenotypes; the presence of these individuals in the analyses did not change the observed results.

A 546bp portion of the mitochondrial COI gene was successfully amplified for all individuals. Regardless of how the data was analyzed, all results revealed low haplotype and nucleotide diversity. In total, nine haplotypes were recovered: one main haplotype shared between 46 individuals and eight singleton haplotypes (Fig. 10). There was no genetic structure between either species, or between any of the localities (ΦST = 0, for all comparisons). Furthermore, Fu’s F statistic was negative in all comparisons (F = -9.1, p < 0.001 for all samples; F = -4, p = 0.001 for P. punctatus; F = -2.5, p = 0.006 for P. laticlavius). Overall haplotype diversity was 0.282 for all samples, and was 0.342 for P. punctatus and 0.222 for P. laticlavius, while nucleotide diversity was 0.001 for all comparisons. All COI summary statistics can be found in Table 5.

Figure 10. Mitochondrial COI haplotype network for both species of Prionurus across all sampling sites. Each circle represents a unique haplotype, and the size of the circle corresponds to the number of individuals that have that haplotype. Perpendicular dashes on connecting lines represent missing haplotypes.

UCE Phylogenomics and Population Genomics

Ultraconserved elements were successfully sequenced for 49 individuals: 23 P. punctatus, 24 P. laticlavius, as well as two individuals of P. biafraensis used as outgroups. The average number of sequencing reads per individual was 2.8 million, and ranged from approximately 941,000 – 4.7 million. A data matrix with completeness of 70% was assembled
for phylogenomic analyses, which contained 866 UCE loci, with an average UCE locus length of 963bp. The resulting phylogenomic hypothesis failed to recover the two species as monophyletic, with overall low support throughout the tree (Fig. 11a).

Meanwhile, the population genomics approach identified a total of 14,723 SNPs in the data, which was reduced to 872 SNP-loci after randomly selecting a single SNP per UCE locus to reduce any linkage effects within UCE loci. These SNPs had an average sequence depth of 30x coverage, and were found to be in Hardy-Weinberg equilibrium. The AMOVA analysis found significant, albeit low, structuring between the two species ($F_{st} = 0.013, p < 0.001$). If genetic variation is examined by sampling location, significant structuring is found between Mexico and all other locations ($F_{st} = 0.014, p < 0.001$ for Costa Rica comparison, and $F_{st} = 0.018, p < 0.001$ for Panama comparison). However, no significant structure was found between Costa Rica and Panama ($F_{st} = 0.003, p = 0.198$). All pairwise comparisons can be found in Table 6.

DAPC analyses that included *P. biafraensis* recovered the most likely number of clusters to be two, with the sister-species pair *P. punctatus* and *P. laticlavius* together in a single group. This pattern might be driven by a large amount of difference between *P. biafraensis* and both TEP species, which could mask any subtle differences between the two TEP species.

Table 6. Mitochondrial DNA COI summary statistics with different groupings of individuals. Number of individuals (N), number of haplotypes (N$_h$), haplotype diversity ($h$), nucleotide diversity ($\pi$), and Fu’s F are given for each type of grouping. * designates significant p-values ($p < 0.02$; Fu 1997).

<table>
<thead>
<tr>
<th>GROUPING</th>
<th>N</th>
<th>N$_h$</th>
<th>$h$</th>
<th>$\pi$</th>
<th>Fu’s F</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>BY SPECIES</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>P. punctatus</em></td>
<td>27</td>
<td>6</td>
<td>0.342 ± 0.117</td>
<td>0.001 ± 0.001</td>
<td>-3.965*</td>
</tr>
<tr>
<td><em>P. laticlavius</em></td>
<td>26</td>
<td>4</td>
<td>0.222 ± 0.106</td>
<td>0.001 ± 0.001</td>
<td>-2.451*</td>
</tr>
<tr>
<td><strong>BY LOCALITY</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mexico</td>
<td>20</td>
<td>3</td>
<td>0.195 ± 0.15</td>
<td>0.001 ± 0.001</td>
<td>-0.626</td>
</tr>
<tr>
<td>Costa Rica</td>
<td>21</td>
<td>7</td>
<td>0.5 ± 0.133</td>
<td>0.001 ± 0.001</td>
<td>-5.074*</td>
</tr>
<tr>
<td>Panama</td>
<td>12</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>NA</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td>53</td>
<td>9</td>
<td>0.282 ± 0.082</td>
<td>0.001 ± 0.001</td>
<td>-9.099*</td>
</tr>
</tbody>
</table>
However, if the outgroup is removed, the most likely number of clusters recovered is one, with both TEP species still clustering together. This result can also be seen in a PCA of the SNP dataset, which reveals both species completely overlapping in 95% confidence intervals (Fig. 11b). These results are mirrored by our STRUCTURE results. When testing between \( K = 1 \)–\( 5 \), a comparison of model outputs with the Evanno method recovered \( K = 2 \) as the most likely result, with \( K = 1 \) the second most likely number of clusters (Table C.2.). However, the two populations recovered do not correspond to the two TEP species, but rather groups of individuals from both species with differences in allele frequencies for particular sets of loci (Figure 11c). Examining the distribution of individual locus \( F_{\text{ST}} \) values further reveal little to no divergence between the species, with most locus comparisons resulting in an \( F_{\text{ST}} \) of 0, and the highest individual \( F_{\text{ST}} \) for one locus was 0.24 (Fig. 11d). Even when the analyses were expanded to include all 14,723 SNPs, no single locus was found to be alternatively fixed between the two species.

Table 7. Pairwise comparisons between species and locations for mtDNA COI (\( \Phi_{\text{ST}} \) values reported below diagonal) and UCE SNPs (\( F_{\text{ST}} \) values reported above diagonal). * designates significant AMOVA \( p \)-values (\( p < 0.05 \)).

<table>
<thead>
<tr>
<th>BY SPECIES</th>
<th>( P. ) punctatus</th>
<th>( P. ) laticlavius</th>
<th>BY LOCALITY</th>
</tr>
</thead>
<tbody>
<tr>
<td>( P. ) punctatus</td>
<td>–</td>
<td>0.013*</td>
<td>Mexico –</td>
</tr>
<tr>
<td>( P. ) laticlavius</td>
<td>0</td>
<td>–</td>
<td>Costa Rica 0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Panama 0</td>
</tr>
</tbody>
</table>

**Ecological Niche Models**

After accounting for duplicates and filtering questionable locality points, we recovered 86 occurrence points for \( P. \) punctatus and 50 occurrence points for \( P. \) laticlavius. The PCA of the 11 environmental layers found that PC1 encompasses 48% of the environmental variation in these layers, and that PC2 encompasses 23% of remaining variation, together totaling approximately 71% of all variation in the environmental layers. Comparisons of niche
equivalency and similarity both failed to reject the null hypothesis that these species are occupying equivalent habitats (all $p$ values > 0.05; Fig. C.3.)

**DISCUSSION**

Slight differences in color patterns between populations can indicate that groups are following distinct evolutionary trajectories. However, even consistent differences in color patterns can sometimes be misleading, as contrasting phenotypes do not always correspond to distinct genetic clusters. The results from our study suggest that highly vagile *Prionurus* in the TEP are a clear example of this paradox: two taxa that have been recognized as distinct species for over 150 years by the presence or absence of dark spots show no morphological or genomic divergence.

This study represents the most comprehensive morphological analysis for *P. punctatus* and *P. laticlavius*, as it includes historical specimens of both taxa, as well as individuals collected from offshore islands (Galapagos and the Revillagigedos). Overall, our results are very clear in showing complete overlap of all meristic counts and measurements between the two species. Perhaps the most unique observation is that the spotting pattern is not discrete, as suggested by the type specimens of these species. Several individuals display faint spots on parts of their bodies (Fig. C.1. & C.2.), and while these phenotypic traits could be interpreted as evidence of hybridization without any other information, the lack of any genetic structuring between the species suggests that this is merely an intermediate phenotype between two populations.
Figure 11. Summary of nuclear UCE results. Maximum likelihood phylogeny inferred from 866 concatenated loci, with nodes collapsed that have a bootstrap support less than (caption cont’d)
Mitochondrial analyses revealed a single main haplotype distributed across the entire coastline of the TEP, resulting in low haplotype and nucleotide diversities. This genetic signature is typically observed in groups that have recently experienced a population bottleneck, or recent founder events (Grant & Bowen, 1998). A founder event seems unlikely given that these TEP Prionurus are the sister group to P. biafraensis from the eastern Atlantic and must have had a common ancestor in the Central American Seaway prior to the closure of the Isthmus of Panama, (Ludt et al., 2015). However, it is reasonable to expect that this group recently underwent a population bottleneck. Using fossil calibrations, Ludt et al. (2015) estimated a crown age for these two TEP species in the late Pleistocene, approximately 490,000 years before present (95% HPD intervals ranging from 70,000 years ago – 1.2 million years ago). This age is contemporary with the climatic shifts promoted by the Pleistocene glaciations, which impacted many other marine organisms in a similar way (Ludt & Rocha, 2015). These climate shifts also correspond with the appearance of upwelling areas and ENSO oscillations in the TEP (Cortes, 1997, 2003), which contributed to a period of rapid community turnover in the reef structure of the TEP from a community composed of Atlantic-related corals, to a community of sparsely distributed Pacific corals (Leigh et al., 2014; López-Pérez, 2017). This rapid turnover could easily result in population fluctuations and potential population bottlenecks.

In our comparison of nuclear loci, which are gathered from SNPs distributed throughout the entire genome, a similar pattern of little to no differentiation between the species was recovered. This dataset failed to reveal any alternatively fixed alleles between the two species, however, a low, but significant, $F_{st}$ was found between the two species. This value was
comparable to $F_s$ estimates of different collection sites (e.g. Mexico vs. other localities), and these results suggest a possible signature of isolation by distance, which has been previously reported for other fishes of the TEP (Bernal et al., 2016; Lessios & Baums, 2017). It would be tempting to suggest that SNPs gathered from UCEs lack sufficient signal to detect differentiation at this time scale given the conserved nature of these genomic regions. However, only the cores of these loci are conserved, and variation increases in the regions flanking this core (Faircloth et al., 2012; Gilbert et al., 2015). In fact, SNPs gathered from UCEs have been proven informative in detecting population structure at shallow timescales for various taxa (ex. in birds: Smith et al., 2013; Oswald et al., 2016; Harvey et al., 2017, and fishes: Burress et al., 2017). Thus, it is likely that the similarities between the mitochondrial and UCE loci reflect an actual shared history, and that this situation echoes one in which a single species displays color variation across its range.

We compared the abiotic habitats that these species occupy to test whether ecology could be a driving factor in speciation. Ecological speciation is considered an important mechanisms for speciation in coral reef fishes (Rocha et al., 2008), and in species of the TEP it could explain the observed diversity in the absence of strong physical barriers in the region. Using locality data from across the entire range of these species, we failed to detect any significant differences in the abiotic habitats that they occupy. However, these data are all associated with the regions abiotic habitat (e.g. temperature, salinity), and therefore we could not assess biological habitat factors (e.g. coral cover, species interactions), which could differ between provinces of the TEP. This is particularly relevant to the Central American faunal gap, a stretch of coastal habitat the lacks many corals and their associated communities (Springer, 1959; Briggs, 1974; Hastings, 2000). These two phenotypes are roughly separated by this gap, but the spotted phenotype does occur further south of this gap in El Salvador and Costa Rica, suggesting that this habitat
discontinuity is not sufficient for restricting gene flow along the continental coast, which is in agreement with studies on other species (Robertson & Cramer, 2009).

This study adds to the growing list of examples where phenotype, either coloration or patterning, does not correspond to genetic structure. Examples of this in reef fishes can be found in angelfishes (Schultz et al., 2007; DiBattista et al., 2012), butterflyfishes (DiBattista et al., 2015), damselfishes (Leray et al., 2010), groupers (Craig et al., 2006), and Caribbean hamlets (McCartney et al., 2003; Ramon et al., 2003; Garcia-Machado et al., 2004) among others. The latter is perhaps the most well studied example for reef fishes, where 11 distinct color phenotypes exist in a genetically homogeneous species complex (Puebla et al., 2008). Genome scans have thus far only detected a single outlier locus, which corresponds with a Hox gene that could be associated with coloration (Puebla et al., 2014). Something similar could be taking place in Prionurus; slight differences in squamation patterns controlled by a small number of loci. However, since these genomic regions were not caught with our targeted capture approach, this hypothesis remains elusive.

Our results support a scenario where a single wide-spread species has undergone a population bottleneck that led to the observed phenotypic variation. A scenario where a severe population bottleneck results in several distant, small populations could lead to fixing of alternative spotting patterns which can be rapidly fixed through genetic drift. In this case, incomplete dominance at a single locus could explain the prevalence of intermediate phenotypes, and this scenario could also explain the modal differences observed in the pectoral-fin and dorsal-fin ray counts between the two phenotypes.

These phenotypic difference could also be adaptive. However there are no known mechanisms of reproductive isolation or clear evidence for resource partitioning between the two
phenotypes, and where they do overlap they have been observed schooling together (WBL personal observation in Costa Rica). In a scenario with a highly dispersive species, as evidenced by a complete lack of structure in this system, any adaptive mutation would have to dramatically increase fitness to counteract the homogenizing influence of high gene-flow, making this scenario unlikely. An extended genomic approach that targets whole genomes, including samples from oceanic islands, could reveal the molecular underpinnings of the squamation patterns of *Prionurus*.

**Conclusions**

While this study found a lack of divergence among two TEP surgeonfishes, it does give insight into the diversification processes that takes place in this region, with regards to both speciation and extinction. Pleistocene glaciations resulted in the whole-scale community turnover in corals in the TEP, which may have adversely impacted all reef-dwelling species (López-Pérez, 2017). This study shows that a prominent, large-bodied, schooling herbivore underwent a dramatic population bottleneck recently, possibly as a result of TEP environmental fluctuations during, and after, the closure of the isthmus. The two phenotypes suggest that there were multiple refugia, which have dispersed and re-colonized much of the TEP since their Pleistocene bottleneck. In species that are more dispersal limited, or that have more rapid turnover rates with shorter generation times, these environmental fluctuations and corresponding population bottlenecks could result in isolated populations that ultimately form new species, suggesting a mechanism in which TEP *in situ* speciation can occur in allopatry. However, this study also highlights why *in situ* speciation along the TEP coastline may be uncommon in large bodied fishes, as these surgeonfishes are perhaps some of the best dispersers among reef fishes, and have long generations times (approximately 45 years for other species of this genus; Choat.
& Axe, 1996) allowing populations to regain connectivity after population bottleneck events. Additionally, such severe population crashes could also easily result in high extinction rates, contributing to the reduced diversification rates previously observed for this region (Cowman & Bellwood, 2012). Only further studies including a diverse set of endemic taxa in the TEP will ultimately shed light on how speciation occurs in one of the most distinctive tropical marine regions.
CHAPTER 5
INTRA-SPECIFIC ANTI-TROPICALITY ACROSS THE FISH TREE OF LIFE

INTRODUCTION

Disjunct distributions have long intrigued, and puzzled, biologists. Early biogeographers proposed that these distributions were the result of long distance dispersal from a center of origin (Prichard, 1826; Darwin, 1859; Forbes, 1859), but the widespread acceptance of plate tectonics and a non-static Earth led to the rise of vicariance biogeography in the latter part of the 20th century (Nelson & Platnick, 1980; Nelson & Rosen, 1981; Wiley, 1988). While it is generally accepted that both dispersal and vicariance occur in nature (Stace, 1989; Zink et al., 2000), it is still difficult in most cases to determine which mechanism may have played a driving role in forming disjunct distributions, and therefore the relative roles that each mechanism plays in speciation and biogeography.

In the marine realm, one of the most striking disjunct distribution patterns is that of anti-tropicality, where closely related populations or species occur on opposite sides of the tropics, but not within (Fig. 12a; Hubbs, 1952). This pattern is seen across a variety of marine organisms (e.g., fishes, cetaceans, crustaceans, gastropods), and is observed across multiple taxonomic scales (Randall, 1981; Chen et al., 2014; Ludt et al., 2015). For the most recent divergences across the tropics, wherein species maintain populations on both sides of the tropics, three mechanisms have been proposed: (1) dispersal between hemispheres in deeper, colder water (Ekman, 1953); (2) dispersal across the tropics during Quaternary glacial cycles (Hubbs, 1952); and (3) vicariance of a once widespread species by means of biotic exclusion from the tropics (Théel, 1885; Briggs, 1987a). While the first may apply to more vagile species, like sharks (Veríssimo et al., 2010) or bony fishes with long-range adult movement (Poortvliet et al., 2013),
many coastal marine taxa have small home ranges as adults, and only disperse during a pelagic larval phase (Sale, 2002). Therefore, most studies examining recent anti-tropicality invoke glacial dispersal or vicariance as the driving mechanism (Burridge, 2002).

Previous studies have not been able to distinguish between these two hypotheses because they have relied solely on time-calibrated phylogenies or molecular clocks (Burridge, 2002). Both dispersal and vicariance processes can operate on the same temporal scales, rendering this approach uninformative. However, these hypotheses do have distinct, testable biogeographic predictions (Fig. 12). The glacial dispersal hypothesis predicts that species are physiologically restricted to temperate regions, and that they can only cross the tropics during cooler, glacial cycles. Vicariance, alternatively, suggests that species are absent in the tropics due to competition or other biotic interactions. Here we test these two hypotheses by examining tropical suitable abiotic habitat (TSH). Vicariant species should in theory have current TSH, yet cannot establish populations due to biotic interactions (Fig. 12b). In contrast, glacial dispersers should not have current TSH, but dispersal corridors of TSH should emerge during glacial periods (Fig. 12c,d). Within-species anti-tropicality is well-documented in a variety of fishes (Hubbs, 1952; Randall, 1981), and recent molecular studies have shown that populations diverged across the tropics during the Quaternary (Burridge, 2002), making them an ideal model system for testing this pattern. Here, using distribution records of species from across the fish tree of life and climate information for current and glacial periods, we illuminate how frequently these two hypotheses drive recent disjunct distributions in the oceans.
METHODS

Species and Distribution Datasets

To test if dispersal or vicariance drive anti-tropical distributions in marine systems we used fishes described in Randall (1981) as a model system. This dataset comprises all known cases of intra-specific anti-tropicality in fishes, whose anti-tropical distributions have diverged recently. Species that may disperse as adults in deeper, cooler water were removed to constrain the analysis for the specific hypothesis tests of divergences due to glacial dispersal and vicariance. Species that recently underwent taxonomic splitting were also removed from the Randall (1981) species list because divergence times are not yet constrained to the timeline of our hypotheses. For the remaining taxa, distribution records were gathered from the Global Biodiversity Information Facility using the package ‘rgbif’ (Chamberlain et al., 2017) in R.

Figure 12. Expectations under glacial dispersal and biotic exclusion hypotheses. An example of a current anti-tropical distribution is shown in (a). Suitable abiotic habitat expectations differ between biotic excluders (b), and glacial dispersers (c). Additionally, glacial dispersal predicts suitable habitat in the tropics during glacial periods (d).
These data were filtered by comparing them to distribution records from the literature (Kuiter, 1993; Allen et al., 2007; Randall, 2005), and all erroneous occurrences were removed.

**Species Distribution Models and Hypothesis Testing**

Species that form anti-tropical distributions through vicariance or glacial dispersal have distinct predictions regarding contemporary TSH, which should be present in the former, and absent in the latter. Furthermore, candidate glacial dispersers are predicted to exhibit a corridor of TSH between hemispheres during the LGM across which they could disperse. This may be observed as an increase in TSH and/or a reduction in Least Cost Path estimates (LCP) during the LGM. LCP distances are measured as a function of distance weighted by quality of TSH across the tropics. Therefore, to test the hypotheses of vicariance versus dispersal in driving patterns of modern anti-tropicality, we estimated area and distribution of TSH for each species under contemporary and LGM climate conditions using ecological niche modeling (ENM).

ENM is a widely used tool to predict species’ suitable habitat through space and time (Elith & Leathwick, 2009; Peterson et al., 2011; Myers et al., 2015; Guisan et al., 2017). These models perform multivariate statistical correlation between species’ occurrences and the combinations of environments existing at those localities. In this way, ENMs attempt to estimate a species’ abiotic niche, which is defined as the suite of abiotic conditions within which a species may survive and reproduce (Soberón, 2007; Peterson et al., 2011). ENMs are constructed in an n-dimensional environmental space (e-space) reflecting the number of environmental input variables. ENMs may be translated into species distribution models by projecting e-space model predictions onto geography (g-space; Peterson et al., 2011).

In addition to spatially explicit species occurrences, ENMs require spatially continuous environmental layers that depict environmental gradients across space. Here we used ten abiotic
environmental layers gathered from the MARSPEC database for current, and LGM conditions. These layers summarize the mean, range, and variance in sea surface temperature and salinity across the oceans at a 5 arc-minute (~10km) resolution (Sbrocco & Barber, 2013; Table D.1.). The Maxent algorithm (Phillips, 2005) was used for all models, which has been shown to work well with presence-only occurrence data (as utilized in this study), as well as non-uniform and smaller sample sizes (Peterson, 2001; Hernandez et al., 2006; Guisan et al., 2007, Pearson et al., 2007; Jiménez-Valverde et al., 2008; Peterson et al., 2011). ENMs were evaluated using a partial-ROC analysis with 500 iterations, which minimizes evaluation bias from presence-only data (Peterson et al., 2007). ENMs with an AUC ≤ 0.70 indicate poor model fit (Elith et al., 2006; Franklin, 2009), however all models here were above this threshold.

ENMs were used to predict anti-tropical species’ TSH under three different modeling scenarios for each species: (1) N-model: models trained only using the extent of northern populations; (2) S-model: models trained using the extent of southern populations; (3) All-model: models trained using the full species distribution (i.e., including northern and southern populations, and tropical habitats in between). To test the hypothesis of vicariance, N-models and S-models for each species were projected into modern tropical zones to predict the current area and distribution of TSH. Northern and southern populations were modeled independently in order to quantify TSH without making a priori assumptions that tropical habitat was not currently suitable. A training region that encompasses the full species distribution (and therefore includes the tropics), produces biased ENMs wherein the modeling algorithm assumes that the lack of tropical occurrence points as an indication of unsuitable habitat.

The All-model was utilized to interrogate the hypothesis that anti-tropical species dispersed across the tropics via TSH during the LGM. In this scenario, modern TSH is
hypothesized to be unsuitable, thus tropical environments are informative for training the model and generating predictions of abiotic habitat preferences. All-model predictions were projected to LGM climate layers, and area and distribution of TSH was calculated.

To quantify modern TSH, ENMs were thresholded using the mean model value (Freeman & Moisen, 2008; Liu et al., 2013), and TSH was calculated for each model as a percentage of available tropical habitat. The extent of tropical habitat was defined by areas exhibiting temperatures in which tropical hermatypic corals grow, i.e., temperature ≥ 20°C during the coolest period of the year (Briggs, 1974; Siqueira et al., 2016). To test for vicariance driving anti-tropical distributions we compared N-model and S-model outputs for each species using K-means clustering (Hartigan & Wong, 1979), which minimizes within group variation. This analysis identified natural clusters of species with similar quantities of contemporary TSH consistent across both northern and southern ENM population predictions. As there can be multiple ways to cluster multivariate data with equal support, this analysis was repeated 100 times to establish consistency in grouping patterns. Species with large amounts of contemporary TSH support the hypothesis of vicariance, whereas species with small contemporary TSH are candidates for the glacial dispersal hypothesis.

However, to support a hypothesis of glacial dispersal, candidate glacial dispersers are also expected to show either an increase of TSH and/or a decreased LCP between hemispheres during the LGM. To quantify the former, the change in proportional TSH between both northern and southern contemporary, and LGM projections was calculated. Notably, in defining the tropics by temperature, proportional TSH could increase between contemporary and glacial times merely as the result of a smaller tropical area during the LGM. To assess the effect of this
potential bias, analyses were re-run defining the tropics by latitude, wherein there is no change in area between contemporary and LGM periods.

In addition to examining the change between contemporary and LGM TSH, the LCP between populations in both hemispheres was also measured for all projections (i.e., N-model, S-model, and All-model projections). LCP requires a distance between starting and ending points as well as a “cost” matrix used to weight travel distance. Distance was determined using a minimum spanning polygon constructed in ArcMap (ESRI, 2010) covering the distribution of contemporary northern and southern populations. Cost matrices were defined by the un-thresholded model predictions (ranging from 0 – 1), such that higher model values were associated with less “cost” to traverse than low model values. Least cost paths were calculated in ArcMap, where shorter paths indicate greater continuity of suitable habitat across the tropics (i.e., lower “cost” for dispersal distance). Species demonstrating low modern TSH in combination with higher TSH during the LGM and/or low LCP distances across the tropics during the LGM support the hypothesis of glacial dispersal as a mechanism producing modern anti-tropicality.

**Detecting Phylogenetic and Ecological Signals**

Anti-tropical species supporting the hypotheses of vicariance versus glacial dispersal were tested for a correlation between biogeographic mechanism and evolutionary history or ecological/life history traits. Phylogenetic clustering was used to test for the effect of evolutionary history on biogeographic mechanism using a comprehensive molecular phylogeny representing the largest sampling of the fish tree of life (Betancur-R et al., 2015). Because some taxa in this study were missing from this phylogenetic tree, they were grafted into their respective genera or families as polytomies using the R package ape (Paradis et al., 2004). A
simplified birth-death process (Kuhn et al., 2011) was used to resolve polytomies for 1,000,000 generations in BEASTv 1.8 (Drummond et al., 2012), sampling every 1000 trees. After discarding a 10% burnin, Pagel’s $\lambda$ (Pagel, 1999) was calculated for 100 randomly chosen posterior trees using the R package phytools (Revell, 2012). Pagel’s $\lambda$ quantifies how dispersed a trait or characteristic is across a phylogeny, and can indicate whether shared evolutionary history influences the presence or absence of a trait, or if it is randomly distributed. This metric was used in two ways: first, to determine if there is any phylogenetic signal across all anti-tropical taxa in our dataset, and secondly, to determine if there was any signal associated with the pools of species identified in the k-means clustering analysis (see results).

To test for the influence of life history or ecological traits on biogeographic mechanism, trait data was compiled for each species using scientifically vetted databases such as: Fishbase (http://www.fishbase.org), the IUCN Redlist (http://www.iucnredlist.org), and other published guidebooks (Kuiter, 1993; Allen et al., 2007; Randall, 2005). The life history traits tested include reproduction mode and body size; the ecological traits summarized aspects of habitat, diet, schooling behavior, depth preference, nocturnal versus diurnal foraging behavior, and water column feeding preferences (benthopelagic versus epipelagic; Table D.2.). General linear models for multiple logistic regressions (GLMs) were used to test specific $a\ priori$ hypotheses of the influence of each trait independently, and all traits in concert, on predicted biogeographic mechanism driving anti-tropicality. GLMs were run using the stats package in R, and the likelihood of each model was determined using the Akaike Information Criterion (AIC; Akaike, 1974), in which models with higher likelihood receive lower AIC values, and models with similar likelihood have AIC values within two of each other (Burnham & Anderson, 2002).
Model explanatory power for each GLM was determined by comparison of null and residual deviances (Anderson & Burnham, 2002, Burnham & Anderson, 2002).

RESULTS

To comprehensively examine how anti-tropical distributions form, we gathered distribution and climate data for all identified species with intra-specific anti-tropical distributions across the fish tree of life (Randall, 1981). After removing highly vagile species that may continuously disperse in deeper, cooler water, and accounting for occurrence data sample size, model fit, and taxonomic changes, our dataset included 28 species that differ in life history, ecology, and phylogenetic relatedness (Table 7). Ecological niche models were constructed using modern populations and projected to modern tropical regions and those during the LGM. To account for implicit model assumptions, northern and southern hemisphere populations were treated separately for contemporary reconstructions, and together for glacial reconstructions. The resultant 84 models were well-supported and display varying amounts of TSH. Vicariant and dispersal hypotheses for marine anti-tropical taxa differ primarily in the predicted amount of contemporary TSH, which should be high in the former and low in the latter. Further, support for the glacial dispersal hypothesis depends on a lack contemporary TSH, in conjunction with an increase of TSH during glacial periods.

Clustering of TSH in model projections resulted in three, well-supported groups that distinctly support both hypotheses. Two of these groups (recovered 100% and 86% of the time) show high amounts of contemporary TSH that varied between northern and southern population projections (Fig. 13a). A third group is comprised of species with low amounts of contemporary TSH, regardless of whether the northern or southern population model is used for projection (recovered 100% of the time). Two species, Hemitaurichthys thompsoni and Aploactis aspera,
fluctuated in their group affinities and were removed from subsequent comparisons. The former two groups support a vicariance scenario, and the latter matches expectations under the glacial dispersal hypothesis for contemporary time periods.

When the tropics are defined by minimum annual temperature, the proportion of TSH to total tropical habitat increased for most species under LGM conditions, and candidate glacial dispersers tended to have a greater TSH increase than candidate vicariant species (as predicted). However, this pattern could reflect an overall decrease in tropical regions during glacial periods (leading to an increase in proportional TSH). Thus, changes in TSH were also tested when defining the tropics by latitude, which remains constant between contemporary and LGM time periods. A trend of increased TSH in candidate glacial dispersers was supported in both cases: mean increase of 108% when defined by temperature (Fig. 13b), mean increase of 132% when defined by latitude (Fig. 13c).

To identify potential dispersal corridors and test for continuity of LGM TSH, we examined the least cost path (LCP) between northern and southern hemisphere populations (Adriaensen et al., 2003). This approach simultaneously considers habitat suitability and distance between suitable habitats to find the shortest optimal route across the tropics. This “effective distance” is a metric of functional connectivity between the landscape and species’ abiotic niche traits, thus providing a quantitative measure of the difficulty to disperse across an area (Adriaensen et al., 2003). Interestingly, the LCP effective distance for candidate glacial dispersers did not differ between current and LGM conditions (mean percent path length increase of 0.28%). Conversely, the LCP effective distance for the two candidate vicariant groups greatly increased during glacial periods (mean percent increase 273% and 159%, respectively; Fig. 13d). These results support either an overall decrease in suitability of tropical habitats during the
LGM, or that TSH for these species is distributed in distinct, somewhat isolated clusters that may impede dispersal for the two candidate vicariant groups (as opposed to continuous dispersal corridors).

These models provide evidence that both dispersal and vicariance actively drive disjunct distributions in different marine taxa. Patterns of dispersal vs. vicariance in species may be driven by several factors, such as shared evolutionary history, life history or ecological traits. To identify possible heritability of anti-tropicality, we tested for phylogenetic signal in our focal species across the fish tree of life (Betancur-R et al., 2015). Overall phylogenetic signal is low across all species in this study ($\lambda = 0.11$), and absent when only glacial dispersers were examined ($\lambda = 0$). When the two vicariant groups were analyzed together, phylogenetic signal was marginally higher ($\lambda = 0.05$), however overall support for heritability is lacking (Fig. 14).

Generalized linear models relating life history and ecological traits to dispersal versus vicariance mechanism do not show strong support for any particular trait driving the observed patterns (Table 8.). In concert, these results suggest that dispersal and vicariance can act ubiquitously, and stochastically, across taxa, which likely leads to the lack of phylogenetic and ecological signal for either mechanism.

**DISCUSSION**

In total, these results shed new light onto the dispersal versus vicariance debate. Out of 28 focal taxa, we find evidence that disjunct distributions in 17 species were possibly formed through vicariance by means of biotic interactions. When examining the abiotic niche, all candidate vicariant species had ample amounts of current TSH when models were trained either on the northern or southern populations. However, no single candidate vicariant species had similar projections for both populations, suggesting that each of these species may occupy a
Table 8. Summary of species included in this study with information regarding their latitudinal ranges and amount of habitat in the tropics. Latitudinal ranges are given with lowest latitude, highest latitude, and total latitudinal span for each hemisphere.

<table>
<thead>
<tr>
<th>Species</th>
<th>Family</th>
<th>Latitudinal Range Northern Hemisphere</th>
<th>Latitudinal Range Southern Hemisphere</th>
<th>No. Samples North/South</th>
<th>% TSH Current (N/S)</th>
<th>% LGM Increase (N/S)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acanthurus leucopareius</td>
<td>Acanthuridae</td>
<td>19.4º–42.6º (23.2º)</td>
<td>0.3º–27.8º (27.7º)</td>
<td>46/33</td>
<td>38.7/37.2</td>
<td>108.5/116.9</td>
</tr>
<tr>
<td>Amblycirrhitus unimaculac</td>
<td>Cirrhitidae</td>
<td>20.4º–24.5º (4.1º)</td>
<td>9.8º–25.1º (15.3º)</td>
<td>8/26</td>
<td>53.6/92.2</td>
<td>39.2/–19.1</td>
</tr>
<tr>
<td>Arothron firmamentum</td>
<td>Tetraodontidae</td>
<td>24.8º–44º (19.2º)</td>
<td>16º–43º (26.5º)</td>
<td>29/76</td>
<td>41.4/16</td>
<td>-71.7/-26.9</td>
</tr>
<tr>
<td>Aulacocephalus temmincki</td>
<td>Serraniidae</td>
<td>22º–35.2º (13.2º)</td>
<td>20º–35.5º (15.5º)</td>
<td>28/55</td>
<td>44.1/21.3</td>
<td>-42.4/-19.2</td>
</tr>
<tr>
<td>Bodianus leucostictus</td>
<td>Labridae</td>
<td>21.9º–33.2º (11.3º)</td>
<td>4.1º–49.8º (45.7º)</td>
<td>20/21</td>
<td>27.8/79.5</td>
<td>47.5/–48.4</td>
</tr>
<tr>
<td>Bodianus tanyokidus</td>
<td>Labridae</td>
<td>16.7º–23.1º (6.4º)</td>
<td>12.2º–27º (14.8º)</td>
<td>5/11</td>
<td>24/82.5</td>
<td>257.1/3.9</td>
</tr>
<tr>
<td>Carangoides equula</td>
<td>Carangidae</td>
<td>9.5º–40.7º (31.2º)</td>
<td>2.8º–36.8º (34º)</td>
<td>200/580</td>
<td>26.8/22</td>
<td>41/71.8</td>
</tr>
<tr>
<td>Cheerodon fasciatus</td>
<td>Labridae</td>
<td>5.8º–23.1º (17.3º)</td>
<td>10.6º–32.1º (21.5º)</td>
<td>20/622</td>
<td>53.1/11.3</td>
<td>-58.2/96.5</td>
</tr>
<tr>
<td>Chromis chrysura</td>
<td>Pomacentridae</td>
<td>8.1º–34.1º (26º)</td>
<td>5.2º–34.7º (29.5º)</td>
<td>29/148</td>
<td>22.2/29.6</td>
<td>132.4/74.3</td>
</tr>
<tr>
<td>Chrysipitera starcki</td>
<td>Pomacentridae</td>
<td>14.3º–30.8º (16.5º)</td>
<td>14º–33.8º (19.8º)</td>
<td>12/32</td>
<td>52.3/31.9</td>
<td>-2.5/59.9</td>
</tr>
<tr>
<td>Chrysipitera tricincta</td>
<td>Pomacentridae</td>
<td>26.2º–27.1º (9.9º)</td>
<td>12.1º–33.8º (21.7º)</td>
<td>3/27</td>
<td>37.7/84.1</td>
<td>45.9/–34.6</td>
</tr>
<tr>
<td>Cirrhitops fasciatus</td>
<td>Cirrhitidae</td>
<td>18.9º–28.2º (9.3º)</td>
<td>16º–21.2º (5.2º)</td>
<td>60/23</td>
<td>19.9/57.9</td>
<td>203/4.1</td>
</tr>
<tr>
<td>Coradion altivelis</td>
<td>Chaetodontidae</td>
<td>1.2º–33.7º (32.5º)</td>
<td>5.3º–27.5º (22.2º)</td>
<td>35/194</td>
<td>23.8/11.8</td>
<td>-0.8/100</td>
</tr>
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<tr>
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Table 9. Ranking of the GLMs by AIC value to determine if any specific life history characteristic drove whether species were glacial dispersers or biotic excluders. For details of each model please see Table S1. Degrees of freedom (df), ΔAIC, and p-values are given for each model.

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Figure 13. Summary of TSH for all model outputs. Current TSH can be separated into three distinct groups (a). Glacial TSH for these groups varies slightly if the tropics are defined by temperature (b), or latitude (c). LCP distance between hemispheres are shown in (d).
somewhat different set of habitats in both hemispheres. Generally, one hemisphere demonstrates a geographic expanse of suitable habitat that includes TSH, compared to the other hemisphere, which is more restricted to temperate regions. This suggests some degree of niche divergence between populations that have been separated for some time. Moreover, populations with greater modern TSH area may have developed of larger niche breadth compared to their more restricted populations; this could reflect population niche expansion vs. pure divergence between populations (Petitpierre et al., 2012). During the LGM, candidate vicariant species have additional TSH, fitting the assumption that these were once widespread species present in the tropics prior to vicariance.

Candidate glacial dispersers, on the other hand, have similar northern and southern population model projections for current climate conditions, strongly supporting a lack of contemporary TSH. During the LGM these species have an increased amount of TSH, and do not have a substantial increase in their least cost path distance (as seen in the vicariant species). Taken together, these data support a movement across the tropics in an LGM dispersal corridor, and thus, restriction of these species to their current anti-tropical distributions by physiological limits, not biotic interactions. Four species consistently have shorter LCP distances in the LGM and may be considered the strongest candidates for glacial dispersal: *Chromis chrysura, Evistias acutirostris, Lepadichthys frenatus*, and *Microcanthus strigatus*. These four species vary in life history strategies (from broadcast spawners to demersal egg layers), habitat (rocky caves and reefs to intertidal areas), diet (herbivores to invertivores), and body size, further corroborating that this mechanism is not restricted to specific life history or ecological traits. While thermal tolerances have not been quantified in the candidate glacial dispersers here, physiological
experiments in the Eastern Pacific goose barnacle, *Pollicipes eligans*, highlight the role that physiological restrictions have in some anti-tropical taxa (Walther et al., 2013).

Figure 14. Distribution of species used in this study across the fish tree of life. Species are shown by the corresponding color of their grouping. Pagel’s λ is given for each group in the center (both biotic excluder groups have been combined).
Most of the species investigated here occur in the Indo-Pacific, and border the IAA – a marine biodiversity hotspot. Notably, no verified examples of anti-tropical Atlantic reef fish distributions have been documented (Rocha, 2003). The greater prevalence of anti-tropical species in the Indo-Pacific may relate to the processes that govern the IAA. How tropical marine biodiversity hotspots form is unknown, but several theories have been proposed over the last century (Bowen et al., 2013). One of these, the center of origin theory (Ekman, 1953), suggests that species form in the tropics and outcompete older species, which are then excluded to extratropical regions. The special case where a species is equally split between both hemispheres forms the basis for vicariant anti-tropicality (Théel, 1885; Briggs, 1987a), and is substantiated by the 17 vicariance-driven distributions recovered here. It is important to note that these data support vicariance through biotic interactions generally, and by deduction given our finding here that abiotic factors are not explanatory and dispersal abilities are similar across taxa. Biotic interactions driving tropical vicariance may include competitive exclusion, food availability, predation pressure, and/or distribution of commensalisms or mutualisms. Not all species herein support this distinction, however, and our data provide evidence that both glacial dispersal and vicariance have acted in concert to produce recent anti-tropical distributions in marine fishes. Overall, these results are in line with a growing body of literature supporting the idea that multiple mechanisms drive biogeographic patterns in marine systems, as opposed to a single process (Bowen et al., 2013; Cowman & Bellwood, 2013; Cowman, 2014).

The taxa included here represent all known species with intra-specific anti-tropical distributions that may be the direct result of dispersal or vicariance. However, it is likely that disjunct distributions occur more often than have been observed, where species are excluded from the tropics into marginal habitat at the edges of their range. Marginal temperate regions are
more environmentally variable than tropical habitats, and the signature of anti-tropicality can be easily lost if species are extirpated from either hemisphere (Grant & Bowen, 1998). This highlights the ephemeral nature of disjunct species; they are likely more susceptible to environmental changes that could lead ultimately to extirpation, which would erase the signature of anti-tropicality. Speciation, another byproduct of isolated populations, will also obscure the signal of anti-tropicality among intra-specific populations. However, in this case a record of sister-species (or sister genera) with anti-tropical distributions will preserve the evolutionary history of this biogeographic mechanism (Burridge, 2002).

Here we present the first clear results distinguishing between dispersal and vicariance mechanisms in structuring anti-tropical distributions in fish species from across the fish tree of life. These results move beyond the limitations of molecular dating techniques and demonstrate support for both mechanisms in the absence of phylogenetic, life history, or basic ecological dependence. Given these findings, continued exploration of this biogeographic pattern should focus specifically on population genetic structure that may identify genetic bottlenecks supporting vicariance, and higher resolution investigation of genetic divergence times during the LGM that would support dispersal. Physiological studies that test species’ thermal limits and those on species’ dispersal systems will also shed further light on the history of species dispersal patterns. Ultimately this research demonstrates the species-specific nature of biogeography and its influence on patterns of evolution. In the face of current climate change, these results have sincere implications, wherein predicting “winners” and “losers” under changing biotic and abiotic conditions is increasingly challenging (Pacifici et al., 2015; Urban, 2015).
CHAPTER 6
CONCLUSIONS

Disjunct distributions are used by biogeographers to search for common patterns and possible underlying mechanisms that may explain the distribution of life. Anti-tropical distributions are a unique example of disjunct distributions that have been recognized for over a century, and have captivated the imaginations of many early biogeographers and natural historians. The presence of similar species separated by thousands of kilometers begs the questions of how these distributions formed. However, the close relationships of these initially proposed anti-tropical taxa was determined without the use of phylogenetic methods, and subsequent phylogenetic studies have questioned whether these represent actual anti-tropical taxa, or merely examples of convergent evolution.

Here I have used the most robust phylogenomic methods, coupled with complete taxonomic sampling and fossil calibration points, in an attempt to precisely evaluate previously proposed hypotheses regarding the formation of anti-tropicality. These methods were applied to two genera from vastly different families: Goniistius, a genus found in a family of entirely temperate species (Chapter 2), and Prionurus, a relatively small genus is a primarily tropical coral reef family (Chapter 3 and 4). In both situations, systematic inconsistencies were found, and taxonomic changes were made. In terms of anti-tropicality, both cases resulted in remarkably similar equatorial divergence estimates, despite the ecological dissimilarities and different fossil calibration points used. These estimates suggest that species either crossed, or were split by, the tropics in the late Miocene and in the Pliocene/Pleistocene. Furthermore, these divergences occur in distinct phases, rather than continuously since the late Miocene (see the distinct bimodal distribution of transition times in Fig. 5.).
While these results narrow the potential driving mechanisms behind anti-tropicality, independently they cannot distinguish between glacial dispersal or biotic exclusion from the tropics for recent divergence events (i.e. dispersal vs. vicariance). To distinguish between these alternative, ENMs were used on intra-specific anti-tropical taxa across the fish tree of life (Chapter 5). These models tested the underlying assumptions of these hypotheses and revealed that both mechanisms are potential drivers of anti-tropicality and that they make act stochastically for various taxa.

In conjunction, these results raise more questions than they answer. However, they do suggest against certain hypotheses. No evidence was found for continental fragmentation, island submergence, or island integration. These mechanisms operate on very long time scales (see Chapter 1), and would require anti-tropical divergence events to occur at time scales not observed in these studies. This is in agreement with other studies that have looked at anti-tropical fishes across a variety of taxa (Burridge, 2002). Even though evidence for these hypotheses were not found in these studies, it does not preclude them from happening. If they were occurring, however, divergence events would be expected to occur in the Paleogene or earlier, and would likely be represented at higher taxonomic scales than those included in these studies. However, few higher-level anti-tropical relationships have been proposed for fishes in general, and for marine fishes in particular.

The timing of recovered equatorial divergence events raises new questions. On the most recent time scales, both glacial dispersal and biotic exclusion could drive this pattern. Evidence in support of both is seen in Chapter 5. Similarly, isothermal submergence could operate on these times scales, and evidence from species not included here seem to support this as a viable hypothesis (Poortvliet et al., 2013). However, with the taxa selected for these projects, none are
likely candidates for isothermal submergence due to their life history characteristics. Support for Pliocene and late Miocene equatorial divergence events were supported by evolutionarily distinct fishes in Chapters 1 and 2, and have been supported by others (Burridge, 2002). Glacial dispersal does not apply to these time periods, and while biotic exclusion does, it also predicts that anti-tropical species are older relicts that were competitively excluded from the tropics. Both of these systems, however, demonstrate that anti-tropical species in these systems are quite young, counter to expectations under this hypothesis. What then, could drive this pattern?

It is possible that divergence events occur somewhat stochastically through time, and that they need not be tied to a single mechanism. Similarly, these species may react to environmental changes that occur too quickly for us to recover with current methods. For example, species may use patches of cold-water upwelling to cross the tropics. Lindberg (1991) suggested this as a mechanism in conjunction with glacial dispersal to explain anti-tropicality in the eastern Pacific for marine invertebrates in the Pleistocene and Pliocene. As sea levels shifted (such as the lows in the Pleistocene, or the sea level highs in the Pliocene), coastal topography was altered, likely influencing upwelling locations (Brink, 1983). While Lindberg (1991) used this to explain anti-tropicality in the eastern Pacific, it could apply to the Indo-West Pacific as well.

Anti-tropical species in the western Pacific could traverse the tropics, using upwelling zones as stepping stones. Currently, these upwelling zones vary significantly in their strength due to El Niño-Southern Oscillation cycles (Meyers, 1996), but some species are restricted to these colder waters. A great example of this is Prionurus chrysurus, which inhabits cold-water upwelling zones in Indonesia (Randall, 2001). Furthermore, while Pleistocene SST did not drop significantly in many equatorial localities, regions surrounding the Sunda Shelf did decrease by
several degrees (Fig. 2.), potentially allowing temperately restricted fishes to expand their ranges to lower latitudes.

As for mid to late Miocene crossing events, my results do not corroborate the mid-Miocene warming hypothesis, nor do they necessarily match other hypotheses. Biotic exclusion could apply to this time period, but other explanations could as well. In particular, this time period was geographically complex, as Australia moved further into lower latitudes, colliding with Asia and influencing oceanic dynamics in the western Pacific (Hall, 2002, 2011, 2013). This time period also coincides with increased biodiversity in mid-latitude species and the continued geological development of the Japanese archipelago (Ogasawara, 1994; Maruyama et al., 1997; Yasuhara et al., 2017). Mid-Miocene sea level shifts associated with global warming and glacial melt caused dramatic shifts in the current patterns of the region, and the Indo-Pacific throughflow is thought to have been its narrowest during the late Miocene (Kuhnt et al., 2004). These shifts in currents, coupled with a decreased distance needed to traverse the tropics and an increase in northern hemisphere habitat, could have caused stochastic shifts in species distributions, including those of anti-tropical species during these time periods.

Overall, anti-tropicality is a complex biogeographic pattern that is likely driven by multiple processes. On shallow time scales it seems that both biotic (biotic exclusion) and abiotic (glacial dispersal) processes can drive this pattern. At deeper time-scales, such as those in the mid to late Miocene, complex geologic processes in the western Pacific likely influenced shallow water habitat area, currents and upwelling zones, allowing species to cross this region. Further study is needed to examine the physiological constraints of anti-tropicality. This area of research has been largely neglected but is central to several of the operating hypotheses. The only system that this has been tested in is the barnacle *Pollicipes elegans*, which occurs in the eastern Pacific.
in upwelling areas (Marchant et al., 2015). Physiological studies of *P. elegans* larvae suggest that thermal tolerances prevent this species from dispersing into warmer waters, in line with the glacial dispersal hypothesis (Walther et al., 2013). However, to date, this is the only study that has specifically examined this with anti-tropical taxa. Additional studies on the thermal limits of anti-tropical fish larvae could provide valuable insight into the underlying processes driving this pattern. Ultimately, a greater understanding of anti-tropicality will hinge on a multi-faceted approach using well-resolved, taxonomically complete phylogenetic datasets coupled with physiological, environmental, and population genetic components.
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APPENDIX A
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To whom it may concern,

In 2015 I published the article titled "Skipping across the tropics: The evolutionary history of sawtail surgeonfishes (Acanthuridae: Prionurus)" in the journal *Molecular Phylogenetics and Evolution* (issue 84, pages 166–172). The research that went into this publication is part of my doctoral dissertation at Louisiana State University, which I will be defending this summer. For my dissertation to be accepted by the university I need permission to re-print this article in my dissertation. Under the copyright agreement for this article, do I have permission to do this? If so, I need an email that states that I have permission to do this. If not, who should I contact to gain permission for this? Thank you very much for your help with this matter. I hope to hear from you soon.

-Bill Ludt
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**APPENDIX B**

**SUPPLEMENTAL MATERIAL FROM CHAPTER 2**

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**Time Before Present (MYA)**

Figure B.1. Different models constraining movement between the Southern and Northern hemispheres used in the Biogeobears reconstructions. Cells show when movement is allowed (yes) or is restricted (no) across time. Protracted Mid-Miocene warming allows species to cross as the oceans were warming in the Miocene, whereas acute Mid-Miocene warming restricts movements at the onset of the Miocene right as global warming began in the late Oligocene.

1No specific hypotheses have been given for these models. 2Also includes older hypotheses, which are any that occur in the Paleogene or before as outlined in Chapter 1.
Figure B.2. Maximum likelihood phylogenomic hypothesis generated using a 75% complete concatenated data matrix with the program RAxML. Node values represent bootstrap support values, and are all 100, unless otherwise noted. Outgroups have been removed for simplicity.
Figure B.3. Multi-species coalescent tree generated from UCE data using ASTRAL III. Local posterior probabilities are given at nodes if the values for those nodes were less than 1. Outgroups have been excluded for simplicity.
Figure B.4. Multi-species coalescent phylogenetic hypothesis generated using SVDquartets. Bootstrap support values are given at nodes with support values less than 95. Outgroups have been removed for simplicity.
Figure B.5. Time calibrated phylogeny generated with BEAST and four calibration points. Calibration points are marked with an *, and 95% HPD intervals are given for each node.


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Table B.2. Estimated crown ages and their associated 95%HPD intervals for families within the Cirrhitoida. Ages are in millions of years before present.

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Figure C.1. Intermediate phenotypes found in this study. Faint spotting patterns can be found on various portions of the body, such as the head in LSUMNS 17879 (a), and the ventral portions of the body in LSUMNS 17801 (b). Both specimens are from Costa Rica.

Figure C.2. Individuals from Coiba Island, Panama displaying differing amounts of spots while foraging. Red arrows highlight the irregular spotting pattern for the region. Photograph credit Moisés A. Bernal.
Figure C.3. Maps showing the extent of the PC1 (A), and PC2 (B) layers used for abiotic niche comparisons, which span the entirety of the TEP and cover the realized region where these two surgeonfishes could disperse. Blue dots represent verified occurrence data for *P. punctatus*, and red represents occurrence points for *P. laticlavius*. 
Table C.1. Materials examined for morphological comparisons. Institution codes are as follows: CAS - California Academy of Sciences; CMN – Canadian Museum of Nature; LACM – Natural History Museum of Los Angeles County; LSUMZ – Louisiana State University Museum of Natural Science; MNHN – Muséum national d’Histoire naturelle; SIO – Scripps Institution of Oceanography. Some specimens in these lots exhibited intermediate phenotypes. Type material. Only photograph examined to determine spotting pattern. Specimens miss-identified, or lot contains a mixture of both phenotypes.

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Table C.1. cont’d.

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<th>INSTITUTION</th>
<th>CATALOG NUMBER</th>
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<th>COUNTRY</th>
<th>STATE/PROVINCE</th>
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<td>Isla Fernandina</td>
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<td>Todos Santos</td>
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</table>

Table C.2. Summary of STRUCTURE analyses for $K = 1$–$5$ ordered in decreasing likelihood values.

| $K$ | MEAN LnP($K$) | STDEV LnP($K$) | LN’($K$) | |LN’($K$)| | $\Delta K$ |
|-----|---------------|----------------|----------|----------|----------------|--------------|
| 2   | -14774.22     | 1.303          | 69.4     | 1380.04  | 1059.37752     |              |
| 1   | -14843.62     | 0.766          | –        | –        | –              | –            |
| 5   | -14922.76     | 133.425        | 206.38   | –        | –              | –            |
| 4   | -15129.14     | 771.405        | 955.72   | 749.34   | 0.971          |              |
| 3   | -16084.86     | 3075.458       | -1310.64 | 2266.36  | 0.737          |              |
Supplemental Methods – COI Amplification

All samples were sequenced for the mtDNA COI region following procedures outlined in Ludt et al. (2012). Briefly, COI segments were amplified with primers BOL-F1 (59 TCA ACY AAT CAY AAA GAT ATY GGC AC 39) and BOL-R1 (59 ACT TCY GGG TGR CCR AAR AAT CA 39) (Ward et al. 2005). Each 25 ml reaction was comprised of approximately 10 ng DNA, 3.5 mM MgCl2, 16 buffer, 0.18 mM of each primer, 2.5 mM DNTP, and 2 units of GoTaq DNA Polymerase (Promega). Polymerase chain reactions were conducted using a temperature profile of a one minute denaturing step at 95°C, followed by a 30 second annealing temperature of 45–52°C depending on the species, and completed with an extension of 45 seconds at 72°C, for 32 cycles. Samples were then purified and sequenced using both the forward and reverse primer.
### APPENDIX D
SUPPLEMENTAL MATERIAL FOR CHAPTER 5

Table D.1. Table S1. Environmental layers used in this study for ENMs. All layers were downloaded from the MARSPEC database. Sea surface salinity (SSS) is measured in psu, and sea surface temperature (SST) is measured in °C.

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<tr>
<th>LAYER NAME</th>
<th>DESCRIPTION</th>
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<tr>
<td>biogeo09</td>
<td>SSS of the freshest month</td>
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<tr>
<td>biogeo10</td>
<td>SSS of the saltiest month</td>
</tr>
<tr>
<td>biogeo11</td>
<td>Annual range in SSS</td>
</tr>
<tr>
<td>biogeo12</td>
<td>Annual variance in SSS</td>
</tr>
<tr>
<td>biogeo13</td>
<td>Mean annual SST</td>
</tr>
<tr>
<td>biogeo14</td>
<td>SST of the coldest month</td>
</tr>
<tr>
<td>biogeo15</td>
<td>SST of the warmest month</td>
</tr>
<tr>
<td>biogeo16</td>
<td>Annual Range in SST</td>
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<tr>
<td>biogeo17</td>
<td>Annual variance in SST</td>
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Table D.2. Life history data used in GLM analyses for all species. The following acronyms are used: BR = Broadcast spawning; DL = Demersal Egg Layer; R = Rocky or Coral Reef; CS = Continental Shelf; OR = Outer Reef; SZ = Surge zone, or tidepools; SL = Sandy Lagoon; S = Shallow; D = Deep; SM = Small; MD = Medium; L = Large; H = Herbivore; IN = Invertivore; P = Piscivore; PL = Planktivore; NC = Nocturnal; DI = Diurnal; EP = Epipelagic; BP = Benthopelagic.

<table>
<thead>
<tr>
<th>SPECIES</th>
<th>SPAWNING TYPE</th>
<th>HABITATS USED</th>
<th>MAX DEPTH</th>
<th>BODY SIZE</th>
<th>DIET</th>
<th>SCHOOLING BEHAVIOR</th>
<th>TEMPORAL ACTIVITY</th>
<th>FEEDING LOCATION</th>
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<tr>
<td><em>Acanthurus leucopareius</em></td>
<td>BR</td>
<td>R,SZ</td>
<td>S</td>
<td>MD</td>
<td>H</td>
<td>Yes</td>
<td>DI</td>
<td>BP</td>
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<tr>
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<td>BR</td>
<td>R,SZ</td>
<td>S</td>
<td>SM</td>
<td>IN,P</td>
<td>No</td>
<td>DI</td>
<td>BP</td>
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<tr>
<td><em>Arothron firmamentum</em></td>
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<td>CS</td>
<td>D</td>
<td>L</td>
<td>H,IN</td>
<td>Yes</td>
<td>DI</td>
<td>BP</td>
</tr>
<tr>
<td><em>Aulacocephalus temmincki</em></td>
<td>BR</td>
<td>CS</td>
<td>D</td>
<td>L</td>
<td>IN,P</td>
<td>No</td>
<td>DI</td>
<td>BP</td>
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<tr>
<td><em>Bodianus leucostictus</em></td>
<td>BR</td>
<td>R,OR</td>
<td>S</td>
<td>MD</td>
<td>P</td>
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<td>DI</td>
<td>BP</td>
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<td><em>Bodianus tanyokidus</em></td>
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<td>R,OR</td>
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<td>BP</td>
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<td><em>Choerodon fasciatus</em></td>
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<td>R,SL</td>
<td>S</td>
<td>MD</td>
<td>IN</td>
<td>No</td>
<td>DI</td>
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<td><em>Chrysiptera starcki</em></td>
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<td>R</td>
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(table cont’d)
Table D.2. cont’d.

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<th>Body Size</th>
<th>Diet</th>
<th>Schooling Behavior</th>
<th>Temporal Activity</th>
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<td>No</td>
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<td>EP</td>
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<td>D</td>
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<td>PL</td>
<td>Yes</td>
<td>DI</td>
<td>EP</td>
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</table>

**Glacial Dispersers**

| Carangoides equula           | BR            | CS                     | D         | MD        | P    | No                 | DI                | BP               |
| Chromis chrysura             | DL            | R                      | S         | MD        | PL   | No                 | DI                | BP               |
| Coradion altivelis           | BR            | R                      | S         | MD        | H, IN| No                 | DI                | BP               |
| Evistias acutirostris        | BR            | R, CS                  | D         | L         | IN   | Yes                | DI                | BP               |
| Gymnothorax eurostus         | BR            | R, SZ                  | S         | L         | IN, P| No                 | NC                | BP               |
| Lepadichthys frenatus        | DL            | R                      | S         | SM        | IN   | No                 | DI                | BP               |
| Limnichthys fasciatus        | BR            | SL                     | D         | SM        | PL   | No                 | DI                | BP               |
| Microcanthus strigatus       | BR            | R                      | D         | MD        | H, IN| Yes                | NC                | BP               |
| Ostichthys japonicus         | BR            | CS, OR                 | D         | L         | IN, P, PL| No   | NC                | BP               |
| Suezichthys gracilis         | BR            | R, SL                  | S         | MD        | IN   | No                 | DI                | BP               |
| Zenopsis nebulosa            | BR            | CS                     | D         | L         | P, PL| Yes                | DI                | BP               |

1. Shallow water max depth defined as ≤ 100m
2. Deep water maximum depth defined as ≥ 100m
3. Small body size defined as ≤10cm
4. Medium body size defined as 10 – 30cm
5. Large body size defined as ≥30cm
VITA

William Benton Ludt (“Bill”) grew up in Tucson, Arizona exploring the Sonoran desert. Growing up he loved to snorkel Mission Bay with his father and sisters during family vacations to Southern California. Eventually, this led to him becoming SCUBA certified in college, only to find out that he couldn’t identify any of the fishes he was looking at underwater on his checkout dive in San Carlos, Mexico. Shortly thereafter he purchased his first fish identification book and a love of ichthyology was born. This led to him volunteering in the only marine lab at the University of Arizona during his undergraduate degree, which gave him his first taste of field work in the northern Gulf of California near Puerto Peñasco, Mexico. After he graduated from the University of Arizona he received a Master’s degree in marine science at the University of Texas at Austin under the mentorship of Dr. Luiz Rocha, who introduced him to evolutionary biology, biogeography, and coral reef fishes. Following that he began working with Dr. Prosanta Chakrabarty at the LSU Museum of Natural Science who inspired him to pursue the world of museum sciences and global fieldwork. Bill plans to graduate in the Summer of 2018 to begin the Sara E. and Bruce B. Collette Postdoctoral Fellowship in Systematic Ichthyology at the Smithsonian Institution under the tutelage of Drs. Carole Baldwin and Bruce Collette.