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Biogeography and the evolution of coral reef fish species

Thesis submitted by

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Statement on the Contribution of Others

This thesis includes some collaborative work with my supervisors, Professor David Bellwood and Dr. Lynne van Herwerden, and their former PhD candidate Charmaine Read. While undertaking these collaborations, I was responsible for the project concept and design, data collection, analysis and interpretation, and the final synthesis of results into a form suitable for publication. My supervisors provided intellectual guidance, equipment, access to their sample collections, financial support, and editorial assistance. My collaborator, Charmaine Read, performed laboratory analysis and provided the resultant molecular sequence data I analysed in the first two data chapters of this thesis. These chapters form collaborative publications and all authors contributed to earlier drafts of the papers.

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Abstract

This thesis examines the evolution of coral reef fish species, specifically the chronology and geography of extant species divergence, and the evolutionary processes that have shaped contemporary patterns of biodiversity. The evolutionary histories of species belonging to multiple genera from four common coral reef fish families: the Chaetodontidae (butterflyfishes), Labridae (wrasses), Pomacanthidae (angelfishes), and Epinephelidae (groupers) were reconstructed based on molecular data. Resultant phylogenies were temporally calibrated using palaeontological data. The reconstructed chronograms were combined with detailed distributional data to determine how closely related species are geographically distributed, and to explore the processes responsible for contemporary patterns of reef fish diversity. First, the diversification of endemic species was explored by considering a case study of the wrasse genus *Anampses*. A second case study assessed the evolution of sympatric species within the angelfish genus *Pomacanthus*. Finally, a multi-family phylogenetic hypothesis was constructed to broaden the generality of conclusions drawn from the case studies. This expanded phylogenetic hypothesis was used to critically evaluate traditional methods of phylogenetic age estimation; to compare the ages of species from different biogeographical areas; and to evaluate the role of geography in the speciation of coral reef fishes. Together, these studies have identified common evolutionary and biogeographical patterns among reef fish species, and begun to unravel potential processes involved in species divergence and maintenance.

A chronogram of the genus *Anampses* identified diversification of extant species from the mid-Miocene onward. Evolutionarily, this resulted in a high proportion of endemic species with varied divergence times and distributions largely restricted to the range edges of Indo-Pacific coral reefs. Evolutionary relationships within the genus, combined with limited spatial and temporal concordance among endemics, suggest that successive peripheral speciation, or peripheral budding, may have generated substantial species diversity within this genus. The findings highlight the importance of peripherally isolated locations in creating and maintaining endemic species.

Extant species in the genus *Pomacanthus* showed similar timing in their divergence, from the mid-Miocene onward. In contrast to *Anampses*, this genus consists of species that are largely sympatric, where 80% of sister-species demonstrated complete or substantial (> 85%) distributional overlap. Splits between lineages within the phylogeny corroborated key biogeographical events including the Terminal Tethyan Event and the rise of the Isthmus of Panama, suggesting that allopatric speciation impacted the early evolutionary history of the genus. Age-range correlation analyses revealed no significant relationship between the degree of distributional overlap and divergence time, demonstrating that exceptional sympatry is not restricted to evolutionarily older species. This work emphasizes the need to disentangle process from pattern by demonstrating that a number of speciation modes, including sympatry and peripatry, likely led to the divergence of species with exceptionally high distributional overlap.

Commonly, divergence time estimates from the nodes of a temporally-calibrated phylogeny are used as indicators of extant species' ages. However, this method can sometimes produce misleading age estimates, specifically in the presence of extinction and ancestral persistence. A method to minimize the impacts of extinction and ancestral persistence on divergence time estimation was established. The method focuses on recent divergences (using a sister-species approach) and involves the combination of minimum divergence time estimates (as indicators of species' ages) with the minimum geographical range area between two sister-species, for all sister-species pairs. When applied to coral reef fishes, this method revealed a general pattern of geographical range expansion with increasing evolutionary age. The differences in the trends recovered from excluding potential biases associated with ancestral persistence (i.e. maintaining a large geographic range over time) suggest that ancestral persistence may be prevalent among coral reef fishes, with successive peripheral speciation impacting age-area relationships. The described method may reveal the occurrence of successive peripheral speciation events across a broad range of taxa.

The multi-family phylogeny revealed similar temporal patterns of coral reef fish divergence among major marine realms and regions, despite differing geological histories. The evolutionary age of most coral reef fish species ranges from 1 to 5 Ma. Notable differences were recorded in the timing of divergence and spatial relationships of endemic species in the Red Sea and Hawaiian Islands. Red Sea endemics have diverged consistently throughout the past 16 Myr, whereas endemic species colonized the Hawaiian Islands in two distinct waves (0–3 Ma and 8–12 Ma). These results suggest that markedly different processes have shaped patterns of diversification in two prominent, peripherally isolated locations.

Important areas of common overlap and vicariance were identified through the continued application of a sister-species approach using the multi-family phylogeny. Congruent vicariance was detected across six previously described biogeographical barriers: the Amazon and Orinoco barriers, Isthmus of Panama, Hawaiian Archipelago, Indo-Pacific, and a previously unnamed barrier I term the Mid-Indian Ocean Barrier (MIOB). The MIOB is hypothesized to be driven by the unusually high sediment content of the Ganges and Indus river systems and the resultant impacts on physical oceanography. A high concentration of distributional overlap was strikingly concordant with the Coral Triangle. This may suggest that the Coral Triangle harbours sufficiently complex environments to facilitate reproductive isolation through niche specialisation that permits closely related species to co-occur. However, the significantly lower than expected degree of distributional overlap among sister-species in this region indicates that it is, more frequently, an area of secondary contact between species that largely occupy adjacent ocean basins, supporting the Centre of Overlap hypothesis. This information helps to illuminate the roles various biogeographical regions and boundaries play in creating and maintaining extant species diversity.

In summary, coral reef fish species have an intricate evolutionary history involving a combination of evolutionary processes that have led to the establishment of complex contemporary biogeographical patterns. Fluctuations in soft biogeographical barriers, founder events and potential divergence in sympatry appear to have driven present day biodiversity. The Coral Triangle not only harbours a unique richness of species but it also supports the co-existence of numerous sister-species. As a whole, this thesis provides a detailed description of the temporal evolution of coral reef fish species, their contemporary geographical distributions, and the evolutionary processes that have likely shaped their distinctive patterns of biodiversity.

Table of Contents

List of Figures

List of Tables

Page

Chapter 1: General Introduction

Understanding the complex history of earth's endless forms, how they came to be and how they are sustained, has been a fundamental facet of biology since the dawn of evolutionary theory (Darwin, 1859; Wallace, 1889). Over the past several decades, the use of molecular data has advanced the exploration of evolution and biogeography (Mantooth & Riddle, 2011; Posadas *et al.*, 2013; Morlon, 2014). Molecular based phylogenetic hypotheses provide a window into the evolutionary history of living forms, detailing organismal relationships and the temporal aspect, or chronology, of divergence. In the process of unravelling complex biogeographical histories the chronology of divergence is a vital component because it provides an opportunity to identify palaeogeographical events and their likely influence on the divergence of particular taxa. Together, the chronology and geography of evolution form a spatiotemporal framework that facilitates further investigation into the mechanistic processes of evolution.

Because evolution is a dynamic, continuous process, phylogenetic methods can be applied to a range of taxonomic levels. Biogeography can also be considered on an array of spatial scales. However, the diversity of life is most often described using species as the focal unit. It is therefore critically important to establish an evolutionary biogeographical framework at the species level to fully understand key factors that shape diversity and the processes through which diversity is generated. Successful projection of the biogeographical consequences of global environmental change critically hinge on this foundational knowledge (Pimm & Raven, 2000; Hughes *et al.*, 2003).

Geographical areas of concentrated diversity, or biodiversity hotspots, provide ideal opportunities to study evolution and biogeography. In the marine realm, the most prominent biodiversity hotspot, often referred to as the Coral Triangle, is centred in the Malay Archipelago between the Indian and Pacific Oceans (Rosen, 1984; Hoeksema, 2007; Bellwood & Meyer, 2009). The area is a hotspot of diversity for many marine organisms including plants, invertebrates, and coastal reef-associated fishes (reviewed in Hoeksema, 2007; Renema *et al.*, 2008; Bellwood *et al.*, 2012). Of the organisms aligned with this hotspot, the reef fishes are among the most diverse and have well-described, interesting distributional patterns, providing an exemplary system to study the biogeography and evolution of tropical species.

Early evolutionary studies of reef fishes used traditional morphological systematics to establish important biogeographical patterns among closely related taxa (Vari, 1978; Woodland, 1983; Winterbottom *et al.*, 1984; Springer, 1988; Blum, 1989; Hastings, 1990). Methodological advances in the collection and analysis of molecular sequence data led to the implementation of molecular phylogenies as a tool to investigate the evolutionary history of reef fishes. Initial molecular based studies were limited to a small number of taxa in select genera (Lacson & Nelson, 1993; McMillan & Palumbi, 1995; Streelman *et al.*, 2002; Crow *et al.*, 2004). However, they confirmed that the results of molecular phylogenetic approaches often aligned with predictions made by morphological studies. Even when the two approaches differed, these early studies reinforced their complementarities and demonstrated the effectiveness of their combined usage to investigate biogeographical patterns.

Further phylogenetic studies clarified the taxonomic structure and distinction of reef fishes, providing the foundational knowledge necessary to approach questions of biogeography (Streelman & Karl, 1997; Kuriiwa *et al.*, 2007; Chiba *et al.*, 2009; Cooper *et al.*, 2009; Near *et al.*, 2013; Sorenson *et al.*, 2013). The development of analytical methods that combined molecular data with palaeontological records created the opportunity to use fossils and palaeogeographical events as calibration tools to establish a temporal framework for phylogenetic hypotheses. While time-trees only provide estimates of the timing of divergence events surrounded by a certain level of uncertainty, they have successfully described the tempo and mode of divergence among reef fishes and corroborated the timing of lineage divisions with key palaeogeographical events such as the closure of the Tethys Seaway and the rise of the

Isthmus of Panama (Bellwood *et al.*, 2004; Barber & Bellwood, 2005; Robertson *et al.*, 2006; Fessler & Westneat, 2007; Teske *et al.*, 2007; Cowman & Bellwood, 2011; Lin & Hastings, 2013). Temporal estimates for dominant reef fish families showed that most generic lineages were in place by the early Miocene; however, these studies provided few direct estimates of the timing of species divergence.

Recent comparative phylogenetic studies have modelled ancestral lineages to infer patterns of origination and dispersal (Cowman & Bellwood, 2013a). They revealed that the Indo-Australian Archipelago has played a number of roles in accumulating, maintaining and exporting reef fish lineages through time. Reconstructing ancestral vicariance showed that both hard and soft barriers could have strong effects on the evolution of reef fish lineages (Cowman & Bellwood, 2013b). These findings illuminate the processes important in lineage diversification among reef fishes and provide a vital frame of reference for further investigation into their evolutionary history. However, to clarify the processes responsible for patterns of contemporary species diversity it is necessary to develop a framework that describes the evolution and biogeography of reef fish species.

Equally important findings have come from studies of population-level relationships within species. These studies have highlighted key biogeographical barriers to genetic connectivity of populations (Muss *et al.*, 2001; Rocha, 2003; Bernardi & Lape, 2005; Gaither *et al.*, 2010), areas of population overlap (Bowen *et al.*, 2001; Gaither *et al.*, 2011a), and potentially important modes of speciation for reef fishes (Rocha *et al.*, 2005; Craig *et al.*, 2006; Winters *et al.*, 2010). Comparative phylogeographic studies have constructively generalised intra-specific biogeographical patterns (Hellberg, 2009; Drew & Barber, 2012; Altman *et al.*, 2013; DiBattista *et al.*, 2013). If similar processes are responsible for driving genetic diversification within and between species, comparative phylogeographic studies may serve as a tool for predicting evolutionary and biogeographical patterns among species.

Phylogenetic studies have detailed the evolution of species for a growing number of reef fish genera (McCafferty *et al.*, 2002; Bernardi *et al.*, 2004; Klanten *et al.*, 2004; Read *et al.*, 2006; Bernardi *et al.*, 2008; Crow *et al.*, 2010; Choat *et al.*, 2012; Gaither *et al.*, 2014). However, few studies have considered the evolutionary history of reef fish species within a temporal and spatial framework to explore their biogeographical patterns (but see Bernardi *et al.*, 2008). Furthermore, comparative phylogenetic studies of reef fish species have been limited by methodological inconsistencies of estimated divergence times among closely related species, or the lack of temporal estimates for some taxa. Without a temporal framework describing the chronology of divergence, it is difficult to evaluate the importance of palaeogeographical events in generating and maintaining species. However, these comparative studies have established broad biogeographical patterns and highlighted potentially important processes that enable specific questions pertaining to the evolution and biogeography of coral reef fish species to be addressed.

With the increasing availability of molecular sequence data and the acquisition of previously un-sampled taxa it is now possible to explore the evolutionary and biogeographical history of species within a number of reef fish genera. Within the context of the evolutionary biogeography of ancient reef fish lineages and present-day populations, the research herein seeks to identify common phylogenetic and distributional patterns among species to uncover those that are likely to have shared a common biogeographical history, and to explore the potential evolutionary and biogeographical processes involved in species divergence and maintenance.

Aims

The primary objective of this thesis was to establish the chronology of extant species divergence and combine it with detailed contemporary biogeographical data to form the spatiotemporal framework necessary to investigate evolutionary processes. This was accomplished through the reconstruction of the evolutionary histories of reef fish species using molecular phylogenetic methods. Molecular sequence data for species within multiple coral reef fish genera were generated or mined from existing data. A range of recent phylogenetic reconstruction and age estimation programs were used to analyse the molecular data in combination with palaeontological data. The resultant phylogenetic hypotheses, along with further biogeographical approaches, were used to address specific objectives relating to the following key questions:

1. When did reef fish species diverge?
2. How are closely related species geographically distributed? and
3. What does this suggest about the processes responsible for contemporary patterns of reef fish diversity?

These key questions are addressed in five distinct chapters outlined below. **Chapters 3, 4,** and **6** correspond directly to publications arising from this thesis (see Appendix G).

Thesis outline

Question 1 was addressed in all five chapters by employing the latest Bayesian inference methods to reconstruct temporally-calibrated phylogenetic hypotheses based on molecular and palaeontological data for genera with complete (or near complete) sampling of extant, nominal species. **Question 2** involved the geographical analysis of contemporary distributions of species in conjunction with their estimated phylogenetic relationships and divergence times. Together, this information provided the framework necessary to explore variations in **Question 3**.

Chapter 3 investigated the role of peripheral isolation in the diversification of reef fish species in a case study of the wrasse genus *Anampses* (Family: Labridae). **Chapter 4** examined the evolution of sympatric species within the angelfish genus *Pomacanthus* (Family:

Pomacanthidae). To explore the evolutionary history of reef fish species more broadly, **Chapters 5–7** examined inter-specific relationships of 53 genera from four major coral reef fish families (Chaetodontidae, Labridae, Pomacanthidae, and Epinephelidae). **Chapter 5** critically evaluated methods commonly used to analyse the relationship between species age and geographical range area by addressing the inherent effects of phylogenetic age estimation using coral reef fishes as an example. **Chapter 6** explored global patterns in the temporal evolution of reef fish species, with particular focus on patterns of divergence among species restricted to isolated locations. **Chapter 7** analysed the geography of speciation in reef fishes where common areas of sympatry and vicariance among sister-species were identified.

Finally, **Chapter 8** summarised the preceding chapters, providing an overview of the evolutionary history of coral reef fish species and the processes potentially important in generating such exceptional species diversity.

Chapter 2: General Methods

2.1 Case Studies

Data selection

Data for both case studies (Chapters 3, 4) were collected in the form of tissue samples taken from all extant, nominal species corresponding to the applicable genera, *Anampses* and *Pomacanthus* (12 and 13 species, respectively; nomenclature following Randall, 1972, 1986; Allen *et al.*, 1998; Bellwood *et al.*, 2004). Tissues of *Anampses viridis* were unavailable. This species, endemic to Mauritius, has not been observed in recent years, despite dedicated sampling efforts. It is thought to be extinct (Hawkins *et al.*, 2000; Dulvy *et al.*, 2003), or to have been taxonomically confused (Russell & Craig, 2013). The closest sister groups to *Anampses* and *Pomacanthus* are not known with certainty; however, recent phylogenetic studies of the family Labridae (Westneat & Alfaro, 2005; Kazancıoğlu *et al.*, 2009; Cowman & Bellwood, 2011) and Pomacanthidae (Bellwood *et al.*, 2004) were consulted to identify putative sister taxa. These studies identified *Macropharyngodon* and *Halichoeres* as sister to *Anampses*, and *Chaetodontoplus*, *Pygoplites*, and *Holacanthus* as sister to *Pomacanthus*. Representative species from each sister genus were included in the phylogenetic analyses, as well as more distant species used to root the phylogenies and provide nodes for fossil calibration (see Tables S2.1 and S2.2 in Appendix A, and subsection ***Age estimation*** for details). Where possible, two individuals of each species were sampled from widely spaced geographical locations to improve the representation of intraspecific genetic variation.

Laboratory procedures

Genomic extraction methods were the same for both case studies. Standard salt-chloroform procedures (Sambrook *et al.*, 1989) were used to extract genomic DNA from ethanol preserved muscle tissue of recently euthanized individuals. One nuclear and three mitochondrial gene fragments for each case study were amplified using polymerase chain reaction (PCR) (Table S2.1 and S2.2, Appendix A). Partial sequences of the cytochrome c oxidase subunit 1 (CO1), 12S rRNA and 16S rRNA genes were obtained using previously published PCR and sequencing procedures and conditions (Read *et al.*, 2006). The first intron of the S7 gene was amplified using the primers S7RPEX1F (5′-TGG CCT CTT CCT TGG CCG TC-3′) and S7RPEX2R (5′-AAC TCG TCT GGC TTT TCG CC-3′) following Chow and Hazama (1998). Each 20 μl reaction volume contained 2.5 mM Tris–Cl pH 8.7, 5 mM KCl and $(NH_4)_2SO_4$, 1.5–4.5 mM $MgCl_2$, 200 μM each dNTP, 10 μM each primer, 0.75U of *Taq* polymerase (Qiagen) and 10 ng template DNA. Thermocycling profiles were carried out under the following conditions: an initial denaturation at 94°C for 2 min, 35 cycles each consisting of denaturation at 94°C for 45 s, annealing at 50°C for 45 s, and extension at 72°C for 1 min, and a final extension at 72°C for 10 min. Following quantification by electrophoresis on 1.5% agarose gel, PCR products were purified by isopropanol precipitation and directly sequenced in forward and reverse directions using dye terminator chemistry, then cleaned following manufacturer protocols (Applied Biosystems). Labelled sequenced products were analysed on an automated ABI3730XL DNA analyser (Macrogen, Seoul, South Korea).

Sequence data

For the *Anampses* case study (Chapter 3), all specimens were used as separate taxa for parsimony, likelihood and Bayesian analysis to establish whether or not evolutionarily significant units (ESUs) were equivalent to species designations. Consensus sequences (50% strict) were computed for age estimation following evaluation of the phylogenetic results. For the *Pomacanthus* case study (Chapter 4), consensus sequences were constructed and used for all analyses. Sequence assembly, editing and alignment were completed in GENEIOUS PRO v5.1 (Biomatters, Auckland, New Zealand; available at: http://www.geneious.com/) using default settings. All alignments were manually adjusted through the insertion or deletion of gaps and trimmed in order to minimize missing data. Care was taken to ensure that both alignments analysed in the *Anampses* case study, the alignment used for phylogenetic analysis and the consensus alignment used for age estimation, contained the same information. No gaps were present in CO1 sequences, which also lacked stop codons when translated. Sequences obtained from both case studies are available from GenBank (Table S2.1 and S2.2, Appendix A). Appropriate substitution models were identified for each alignment and partition in JMODELTEST (Posada, 2008). The corrected Akaike information criterion (AICc) (Sugiura, 1978; Hurvich & Tsai, 1989) was used to select models for further analysis because it allows sample size to be specified and is the preferred method for small alignments.

Phylogenetic analysis

All analyses were performed on a dual core processor MacBook Pro or via the Cyberinfrastructure for Phylogenetic Research (CIPRES) project (Miller *et al.*, 2010), a browser that provides access to TeraGrid resources for phylogenetic tree inference. Phylogenetic relationships were assessed based on concatenated alignments using parsimony, likelihood, and Bayesian inference analyses for each case study. PAUP* v4.0b10 (Swofford, 2003) was used to perform maximum parsimony (MP) analysis for which five independent heuristic searches were executed using a tree bisection–reconnection (TBR) branch-swapping algorithm with 100 random sequence addition replicates. Analyses were run with and without weights assigned to transversions relative to transitions (2:1 for the *Anampses* dataset; 5:1 for the *Pomacanthus* dataset). The weighted stepmatrix was calculated based on estimates of substitution rates obtained from JMODELTEST (Posada, 2008). Nonparametric bootstrapping was also implemented, with 1000 bootstrap pseudo-replicates using heuristic searches and TBR branch-swapping with two random sequence additions per pseudo-replicate, to determine the level of support for each clade. For each case study, a majority-rule consensus tree was produced from all topologies recovered from the bootstrap analysis. The final weighted MP tree was chosen based on consistency with other phylogenetic analyses.

Maximum likelihood (ML) analysis, implemented in GARLI v1.0 (Zwickl, 2006), was used to search for the tree topology, branch lengths and substitution model parameters with the highest log-likelihood (–ln*L*) score based on the maximum likelihood criterion. For each case study, 10 independent iterations of the analysis were performed using default settings for algorithm parameters. Of the ten best trees obtained, the tree with the best –ln*L* score was considered the best overall topology. Since the program simultaneously estimates substitution model parameters, the analyses were run with and without the substitution model specified (as per AIC_c). This served to further ensure the analyses were converging on similar solutions for each respective genus and not arriving in a suboptimal area of tree space. The final trees for each genus used in further analysis were chosen based on the best overall –ln*L* scores. Bootstrap analysis was also performed with 100 bootstrap replicates and the best ML tree specified as the starting tree. For both *Anampses* and *Pomacanthus*, a majority-rule consensus tree was constructed in PAUP* to determine the level of support for the clades recovered.

Bayesian inference (BI) methods were implemented in MRBAYES v3.1.2 (Ronquist & Huelsenbeck, 2003) using a partition mix model on TG, a large NSF Tera-Grid Resource accessed via CIPRES. Partitions were assigned according to gene region and the general substitution model structure was specified (as per AICc) for each partition. Parameters were unlinked across partitions and each was allowed to evolve under different rates using a flat Dirichlet prior. For the *Anampses* case study, posterior probabilities of clades were calculated following four 50 million generation Markov Chain Monte Carlo (MCMC) analyses, each with four chains sampling every 1000 generations. For the *Pomacanthus* case study, posterior probabilities were calculated following two 10 million generation MCMC analyses, each with four chains sampling every 500 generations. Convergence was assessed using AWTY (Wilgenbusch *et al.*, 2004) and TRACER v1.5 (Rambaut & Drummond, 2007). Upon examination of the trace files, a conservative burn-in of 10% (5 million generations, or 5000 trees for *Anampses*; 1 million generations, or 2000 trees for *Pomacanthus*) was discarded from each run and a 50% majority-rule consensus tree was computed for each respective genus using the remaining sampled trees.

Age estimation

Age estimation was performed using the BEAST v1.6.1 package (Drummond & Rambaut, 2007). Substitution models were set accordingly for each gene partition. A strict molecular clock was applied to the CO1 partition in the *Anampses* analysis and to the 16S partition in the *Pomacanthus* analysis following preliminary tests, which indicated a strict molecular clock could not be rejected because the marginal posterior distribution of the standard deviation of the substitution rate included zero. All other gene partitions rejected a strict molecular clock and a relaxed uncorrelated lognormal clock was employed (Drummond *et al.*, 2006). The birth–death process was specified as the tree prior to account for speciation and extinction (Gernhard, 2008). Starting trees were randomly generated for each run.

Time calibrations were made based on fossil evidence, specifically for *Anampses*, the minimum fossil age of *Eocoris bloti* (Bannikov & Sorbini, 1990) was used to place an exponential prior on the root node (50 Ma hard lower bound; 95 Ma soft 95% upper bound) following Cowman *et al.* (2009). For *Pomacanthus*, the fossil ages of *Avitoluvarus dianae*, *Avitoluvarus mariannae*, *Kushlukia permira* and *Luvarus necopinatus* (Bannikov & Tyler, 1995) were used to date the most recent common ancestor (MRCA) of *Luvarus*, *Zanclus* and Acanthuridae (*Acanthurus* and *Naso*) using an exponential prior (55.8 Ma hard lower bound; 63.9 Ma soft 95% upper bound) following Near *et al.* (2012). Posterior samples from four independent MCMC analyses, each with 10 million generations sampling every 1000th generation, were assessed for convergence and appropriate burn-in using AWTY and TRACER v1.5. Tree files were combined, after removal of 10% burn-in, using LOGCOMBINER v1.6.1 (Drummond & Rambaut, 2007) and a maximum clade credibility tree was constructed for each genus using TREEANNOTATOR v1.6.1 (Drummond & Rambaut, 2007) to display median ages and 95% highest posterior density (HPD) intervals (upper and lower) for each node.

2.2 Multi-Family Phylogeny

Data selection and handling

Sequence data for analyses used in Chapters 5–7 were obtained from GenBank for those coral reef fish families with distribution maps available from the IUCN Red List spatial database (IUCN, 2011). At the time of data collection this included genera belonging to the families: Acanthuridae, Chaetodontidae, Labridae, Pomacanthidae, and Epinephelidae. Species designations were based on the IUCN Red List (IUCN, 2011) and FishBase (http://www.fishbase.org). Only loci with the most coverage across all families were considered. Among the mitochondrial loci, these included 16S rRNA, 12S rRNA, CO1, and cytochrome b. Nuclear loci included TMO-4C4 and S7 intron 1. Sequence coverage was also assessed within each of the reef fish families to maximize taxon sampling within genera and minimize missing sequence data within the alignment. Only those genera where a minimum of 70 % of constituent species had sequences available for any of the four mitochondrial loci, and at least 50 % sequence coverage across all six loci were considered. This resulted in the inclusion of 53 genera within four coral reef fish families: Chaetodontidae, Labridae, Pomacanthidae, and Epinephelidae (Table S2.3, Appendix A). Additional sequences were also included for taxa used to root the phylogeny (*Opsanus pardus* and *Porichthys notatus*) and to provide additional nodes for fossil calibration (Tables S2.3 and S2.4, Appendix A).

Sequences were aligned in GENEIOUS PRO v6.1.2 (Biomatters, Auckland, New Zealand; available at: http://www.geneious.com/) using default settings for each locus. All alignments were manually adjusted through the insertion or deletion of gaps, and trimmed to minimize the amount of missing data. PARTITIONFINDER v1.0.1 (Lanfear *et al.*, 2012) was used to simultaneously select an appropriate partitioning scheme for the concatenated alignment and the best fitting models of molecular evolution for each locus based on the Bayesian Information Criterion (BIC) (Schwarz, 1978).

Spatial data were compiled from the IUCN Red List spatial database for all species included in the phylogenetic analysis (except *Aprops bilinearis*, *Grammistes sexlineatus*, *Niphon spinosus,* and *Zalanthias kelloggi*, which had not been assessed by the IUCN Red List at the time of sampling). Spatial analyses were conducted in GRASS (GRASS Development Team, 2011) and QGIS (QGIS Development Team, 2012). The geographical range area of each species was calculated as the total area of all constituent polygons in their respective distribution maps (IUCN, 2011).

Phylogenetic analysis and age estimation

A time-calibrated phylogeny was constructed based on partitioned Bayesian analysis in BEAST v1.7.5 (Drummond & Rambaut, 2007) using nine fossil calibrations (Table S2.4, Appendix A). Partitioning and models of molecular evolution were specified according to those identified by PARTITIONFINDER (Lanfear *et al.*, 2012) (Table S2.5, Appendix A). Divergence times were estimated under a relaxed uncorrelated lognormal clock model (Drummond *et al.*, 2006) following preliminary analyses that rejected a strict molecular clock for all partitions. The birth–death process was specified as the tree prior to account for speciation and extinction (Gernhard, 2008), and starting trees were randomly generated for each run. Temporal calibrations were made based on fossil evidence used in previous phylogenetic studies of fishes (Cowman *et al.*, 2009; Near *et al.*, 2012) (Table S2.4, Appendix A). Posterior samples from six independent MCMC analyses, each with 40 million generations and sampling every 2000th generation, were assessed for convergence and appropriate burn-in using TRACER v1.5

(Rambaut & Drummond, 2007). Tree files were combined using LOGCOMBINER v1.6.1 (Drummond & Rambaut, 2007) following the removal of 20 % burn-in and re-sampling every 4000 states. A maximum clade credibility tree was constructed using TREEANNOTATOR v1.6.1 (Drummond & Rambaut, 2007) to display median ages and 95 % HPD intervals for each node.

Chapter 3: The role of peripheral endemism in species diversification: evidence from the genus *Anampses* (Family: Labridae)

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3.1 Introduction

One of the central topics in marine evolutionary biogeography focuses on understanding and explaining the pattern of increased species richness in the area known as the Indo-Australian Archipelago (IAA). This area is often referred to as a biodiversity hotspot because it encompasses the world's largest concentration of marine biodiversity (Reaka *et al.*, 2008; Renema *et al.*, 2008; Bellwood *et al.*, 2012). Numerous ecological factors including habitat diversity, reef area and geographical location correlate with patterns of species richness in and around the hotspot (Bellwood *et al.*, 2005; Hoeksema, 2007). Though insightful, correlative ecological factors are based primarily on present day species' distributions and ecologies, and therefore contribute little to our understanding of how the diversity was generated. Large-scale patterns of biodiversity, such as that documented in the IAA hotspot, result from interactions between current ecological and environmental conditions, as well as the biogeographical history of the area. The study of evolutionary lineages in relation to their geographical distributions has played a crucial role in elucidating patterns of historical biogeography (Lessios *et al.*, 2001; Rocha *et al.*, 2007; Floeter *et al.*, 2008; Riddle *et al.*, 2008; Leray *et al.*, 2010). Recent analytical advances have permitted the incorporation of historical evidence in phylogenetic reconstructions, which has enabled the key issue of timing of species origination to be addressed through the construction of detailed chronograms (Alfaro *et al.*, 2007; Hurley *et al.*, 2007; Azuma *et al.*, 2008; Frey & Vermeij, 2008; Cowman *et al.*, 2009; Kazancıoğlu *et al.*, 2009; Santini *et al.*, 2009; Bellwood *et al.*, 2010). As a result, we are now able to tackle novel questions regarding the evolution of peripherally isolated endemic species and to explore the role they may play in shaping the diversity of this distinctive region.

Endemism has long been a topic of interest among marine researchers. The conventional biogeographical hypotheses put forth to explain the bulls-eye pattern of diversity concentrated in the IAA, typically referred to as the ‘Centre-of’ models (Hoeksema, 2007; Bellwood *et al.*, 2012), make explicit predictions about the ages and geographical locations of endemic species. Under the Centre of Origin model, young endemics should lie in the IAA, while under the Centre of Overlap and particularly, the Centre of Accumulation models, they should be peripheral to the IAA. Early contributions to the study of endemism in the marine realm equated hotspots to major centres of endemism, largely based on patterns known from terrestrial systems (Roberts *et al.*, 2002). Further work demonstrated that the majority of corals and reef fish found to occur within the IAA are not endemics (Hughes *et al.*, 2002). It is now broadly recognised that most coral reef associated endemism is concentrated along the periphery of the hotspot (Bellwood & Wainwright, 2002; Hughes *et al.*, 2002; Jones *et al.*, 2002; Connolly *et al.*, 2003; Meyer *et al.*, 2005; Allen, 2008; Briggs, 2009; Budd & Pandolfi, 2010; Hobbs & van Herwerden, 2010). Yet, previous molecular work has provided no consistent pattern with regard to the ages of peripheral endemic species in favour of any one ‘Centre of’ model over another. Furthermore, it has been acknowledged that no single explanation is likely to account for the presence of the IAA hotspot (Rosen, 1984; Palumbi, 1997; Bellwood & Wainwright, 2002; Cowman & Bellwood, 2011).

By their very nature, peripherally isolated endemic species do not add to the diversity of species found within the central hotspot. However, by examining the evolutionary relationships and ages of peripheral endemics and their closest relatives we may better understand what speciation mechanisms are implicated in generating coral reef fish biodiversity at range edges. To do this, I have focused on a widespread tropical Indo-Pacific reef fish genus with a relatively high proportion (42%) of peripherally isolated endemic or restricted range species, several of which have shared distributions. The genus *Anampses* (family: Labridae) is a relatively small group consisting of 12 extant species, all of which were sampled. There are few distinguishing morphological and meristic features among *Anampses* species and previous attempts to reconstruct their evolutionary history have relied almost exclusively on the highly varied colour forms present in this sexually dichromatic group of fishes (Randall, 1972). Knowledge of the evolutionary history of *Anampses* will enable us to identify sister relationships of endemic species and estimate when they diverged. By combining this information with species distributions I am able to consider the speciation processes responsible for generating coral reef fish diversity at range edges. Temporal concordance among endemic species will be of particular interest for those species with shared distributions and may identify certain times or events that facilitated diversification of peripheral endemic species.

With such a high proportion of endemic species within *Anampses*, I am are afforded an opportunity to consider the relative risk of extinction faced by vulnerable, isolated species. Older endemic species that may have experienced range contraction and remain as relictual isolates could face an increased risk of extinction compared to younger endemic species whose range has yet to reach its full extent. One of only two documented marine fish extinctions has already occurred within the genus *Anampses*, namely *Anampses viridis* (Dulvy, 2006). The species' small geographical range, which included only coastal waters of Mauritius, is widely acknowledged to have contributed significantly to its fate (Hawkins *et al.*, 2000; Dulvy *et al.*, 2003). Recently, Russell & Craig (2013) have shown that *A. viridis* had been taxonomically confused and that it is actually the adult male (terminal phase) colour form and a junior synonym of *Anampses caeruleopunctatus*. Nevertheless, by estimating the ages of endemic species I am able to inform conservation efforts, particularly in areas susceptible to habitat degradation and loss associated with increasing stresses on contemporary coral reefs.

This study aims to examine the genus *Anampses* with recently developed analytical tools to provide an overview of their evolutionary history and a detailed chronological understanding of peripherally isolated endemic species. The specific objectives of this study were:

1) To construct a phylogeny of the genus *Anampses* using maximum parsimony, maximum likelihood and Bayesian inference, as well as a fossil calibrated chronogram using BEAST (Drummond & Rambaut, 2007) to identify the age of the genus and its constituent species; and
2) To identify possible models of diversification responsible for generating endemic species at range edges; and
3) To explore concordant spatial and temporal patterns among endemic species within the *Anampses* clade; and finally
4) To discuss the role of peripheral endemism in shaping the diversity of the IAA and evaluate the probable threats endemic species face in relation to their age.

3.2 Materials and Methods

Methods of ***Data selection***, ***Laboratory procedures***, ***Sequence data***, ***Phylogenetic analysis*** and ***Age estimation*** for this data chapter follow those detailed in **Chapter 2: General Methods**, section **2.1 Case Studies**.

Biogeographical analysis

Geographical range and occurrence data were compiled for *Anampses* species (Choat *et al.*, 1988; Hughes *et al.*, 2002; Kuiter, 2010). These data were used to classify species into three biogeographical range categories: endemic, mid-range and widespread. An endemic is traditionally defined as a taxon restricted, or peculiar, to a locality or biogeographical province. Here, endemics were defined as species with distributions restricted to peripheral bio-geographical regions including islands, archipelagos and continental margins, with geographical ranges less than 5×10^6 km^2. I acknowledge that this definition encompasses some species with distributions that span multiple islands and/or archipelagos. Mid-range species were defined as those with geographical ranges between 5 × 10^6 and 20 × 10^6 km^2, while widespread species were defined as those with ranges exceeding 20 × 10^6 km^2. Geographical ranges of closely related species were mapped and used to explore possible models of diversification responsible for generating endemic species.

3.3 Results

Phylogenetic analysis

The combined data matrix consisted of 1666 bp and yielded a strong phylogenetic signal with 500 parsimony informative characters (30.0%). The 12S mitochondrial rRNA fragment had 415 bp, CO1 contributed a further 53 bp and intron 1 of the nuclear fragment S7 contributed 720 bp. Models selected using the corrected Akaike information criterion (AICc) criterion varied among loci as summarised in Table 3.1. For species with more than one available sequence there was a mean pairwise identity of 97.1%.

Table 3.1 Gene-specific models of evolution of *Anampses* fishes selected under the corrected Akaike information criterion (AICc) in JMODELTEST. Single gene alignments were analysed separately and the appropriate model applied for further partitioned analysis of the concatenated alignment. N_{ST} specifies the number of substitution parameters.

Data Set	**AICc Model**	**Invariable Sites**	**Gamma Shape (γ)**	***N*st**
12s	TPM3uf + I + G	0.439	0.342	6
CO1	TIM1 + I + G	0.633	1.565	6
S7	TPM3uf + G	—	1.643	6
Complete	TIM2 + I + G	0.265	0.491	6

The best maximum likelihood (ML) tree was selected based on the $-\ln L$ scores and resulted from the analysis without model specification in which parameters were estimated from the data during the analysis. This analysis consistently returned the same tree topology ($-\ln L$ =

−12,032.916) from all 10 independent runs, suggesting that the overall optimal tree was recovered every time. Bayesian inference (BI) analysis was assessed for convergence by plotting pairwise comparisons of node (split) frequencies of all four independent runs (Fig. S3.1, Appendix B). This diagnostic is assessed by the strength of the correlation between two runs and demonstrated moderate convergence for the analyses performed here. Cumulative posterior probabilities for the 20 most variable nodes (splits) for each Markov Chain Monte Carlo (MCMC) run were also examined and confirmed relatively stable clade frequencies indicating that the subsamples of trees were describing similar distributions (Fig. S3.2, Appendix B).

The phylogeny of the genus *Anampses*, represented by the best ML tree, was the most well resolved phylogeny consistent with all other analyses and showed strong bootstrap support [maximum parsimony (MP) and ML] and posterior probability support (BI) for the nodes (Fig. 3.1). All analyses confirmed the monophyly of *Anampses* with 100% support and placed *Macropharyngodon* and *Halichoeres* in a clade sister to *Anampses*. Species delineations were consistent with evolutionarily significant units (ESUs) based on the specimens and collection locations. The five endemic species within *Anampses* were spread throughout the two most speciose clades. Interestingly, those with shared geographical distributions (*Anampses chrysocephalus* and *Anampses cuvier* in Hawaii; *Anampses elegans* and *Anampses femininus* in the south Pacific) arose in separate clades.

Age estimation

Log files from four independent BEAST analyses showed high effective sample sizes (posterior ESS values > 200 for four combined analyses), which indicated valid estimates based on independent samples from the posterior distribution of the MCMC. Assessment of convergence in TRACER and AWTY indicated that all analyses had converged. Pairwise

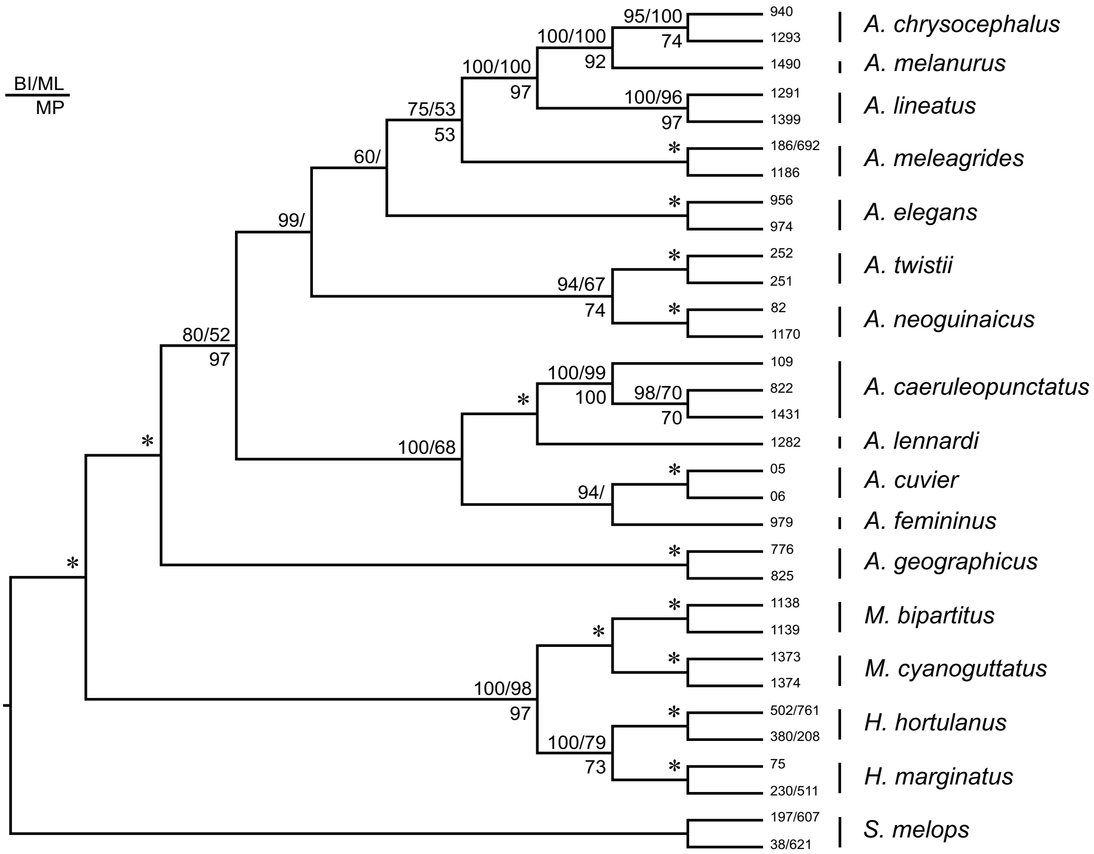


Figure 3.1 Inferred phylogeny of *Anampses* obtained by Bayesian inference (BI), maximum likelihood (ML) and maximum parsimony (MP) analyses of three loci (CO1, 12S and S7) with multiple sequences for each species (see Table S2.1 in Appendix A for locality information). The topology shows the best ML tree with bootstrap support (> 50%) from MP and ML (1000 and 100 bootstrap replicates respectively) and posterior probabilities from BI (consensus of 10,401 trees). Asterisks denote 100% support for the node across all three analyses.

comparisons of node (split) frequencies among all four independent runs demonstrated correlation (Fig. S3.3, Appendix B). Examination of the cumulative posterior probabilities of the 15 most variable nodes (splits) from each of the four independent runs revealed stable clade frequencies (Fig. S3.4, Appendix B).

The maximum clade credibility chronogram was compiled in TREEANNOTATOR from 36,004 post-burnin trees (36,004,000 generations) returned from the BEAST analyses. Ages are presented as median node heights with the 95% highest posterior density (HPD) intervals indicated by horizontal bars at each node. Strong posterior probabilities were obtained for all nodes (Fig. 3.2).

The *Anampses* lineage dates back to the mid-Eocene when it shared a most recent common ancestor (MRCA) with *Macropharyngodon* and Indo-West Pacific *Halichoeres* 46.2 Ma (95% HPD: 33.1–64.0 Ma). Subsequent divergence within the genus began in the early Miocene 19.6 Ma (95% HPD: 12.3–28.8 Ma) when *Anampses geographicus* emerged as the earliest extant species. Speciation continued throughout the Miocene and a substantial portion of species diversification (42%) occurred during the Pliocene (2.6–5.3 Ma) and Pleistocene (0.01–2.6 Ma) epochs.

Whilst all species of *Anampses* occur in the Indo-Pacific realm (Fig. 3.3), five of the 12 extant species have peripherally isolated distributions, while only four species, *Anampses meleagrides*, *Anampses twistii*, *Anampses caeruleopunctatus* and *A. geographicus* are widespread throughout the Indo-West Pacific. An apparent Indo-Pacific split occurred between two species (*Anampses lineatus*, which is widespread throughout the Red Sea and Indian Ocean, and *Anampses melanurus*, which is widespread throughout the West Pacific) at approximately 3.6 Ma (95% HPD: 2.0–5.7 Ma). The two Hawaiian endemics, *A. cuvier* and *A. chrysocephalus* revealed distinct divergence times, 9.6 Ma (95% HPD: 6.0–14.1 Ma) and 1.72 Ma (95% HPD: 0.9–2.9 Ma), respectively. In contrast, the two endemic species whose distributions include Lord Howe Island, *A. femininus* and *A. elegans*, have similar ages with overlapping 95% HPDs, 9.6 Ma (95% HPD: 6.0–14.1 Ma) and 11.2 Ma (95% HPD: 7.2–16.2 Ma), respectively. *Anampses lennardi* has a sympatric distribution with *A. caeruleopunctatus* and is the youngest endemic species with an estimated age of 0.6 Ma (95% HPD: 0.2–1.1 Ma).

3.4 Discussion

This is the first study to present a comprehensive and fully resolved molecular phylogeny of the genus *Anampses*. All analyses strongly supported the monophyly of *Anampses*, with 12 distinct species. The phylogeny identifies evolutionary relationships within the genus and presents a foundation for the estimation of ages. The diversification of *Anampses* during the early Miocene is consistent with published estimates for other, closely related Indo-Pacific reef fish genera (Bernardi *et al.*, 2004; Barber & Bellwood, 2005; Read *et al.*, 2006). Endemic *Anampses* species varied in their ages, ranging from 11.2 Ma (*A. elegans*) to 0.6 Ma (*A. lennardi*), and in their evolutionary relationships. No universal pattern was found. Most extant endemic species occur at the range edges of Indo-Pacific coral reefs; they have arisen throughout the evolutionary history of the genus, and display temporal concordance for some isolated locations, but not for others.

Phylogenetic structure

The presence of three main clades within a monophyletic *Anampses* was consistently supported by all analytical methods. Most relationships among species were consistent across methods, with only minor differences in the relationships among Clade 3 taxa (specifically, the relative placement of *A. meleagrides*, *A. elegans*, *A. twistii* and *Anampses neoguinaicus* to each other in the inferred phylogeny versus the chronogram). The sister relationship between *Anampses* and the clade containing *Macropharyngodon* and Indo-West Pacific *Halichoeres* is concordant with previous molecular studies of the Labridae (Westneat & Alfaro, 2005; Cowman *et al.*, 2009; Kazancıoğlu *et al.*, 2009; Cowman & Bellwood, 2011).

Traditional morphology-based evolutionary hypotheses for *Anampses* suggested similar relationships to those presented herein (Randall, 1972). Earlier taxonomic debate has highlighted *A. geographicus*, and several authors have suggested that the genus be divided into two sub-genera: *Pseudoanampses*, containing just *A. geographicus*, and *Anampses* containing the remaining 11 species (Randall, 1972). This proposal was based primarily on differences in lateral-line scale counts (*Pseudoanampses* with 48–50 lateral-line scales and *Anampses* with 26 or 27). The results presented herein provide partial support for this suggestion, identifying *A.*

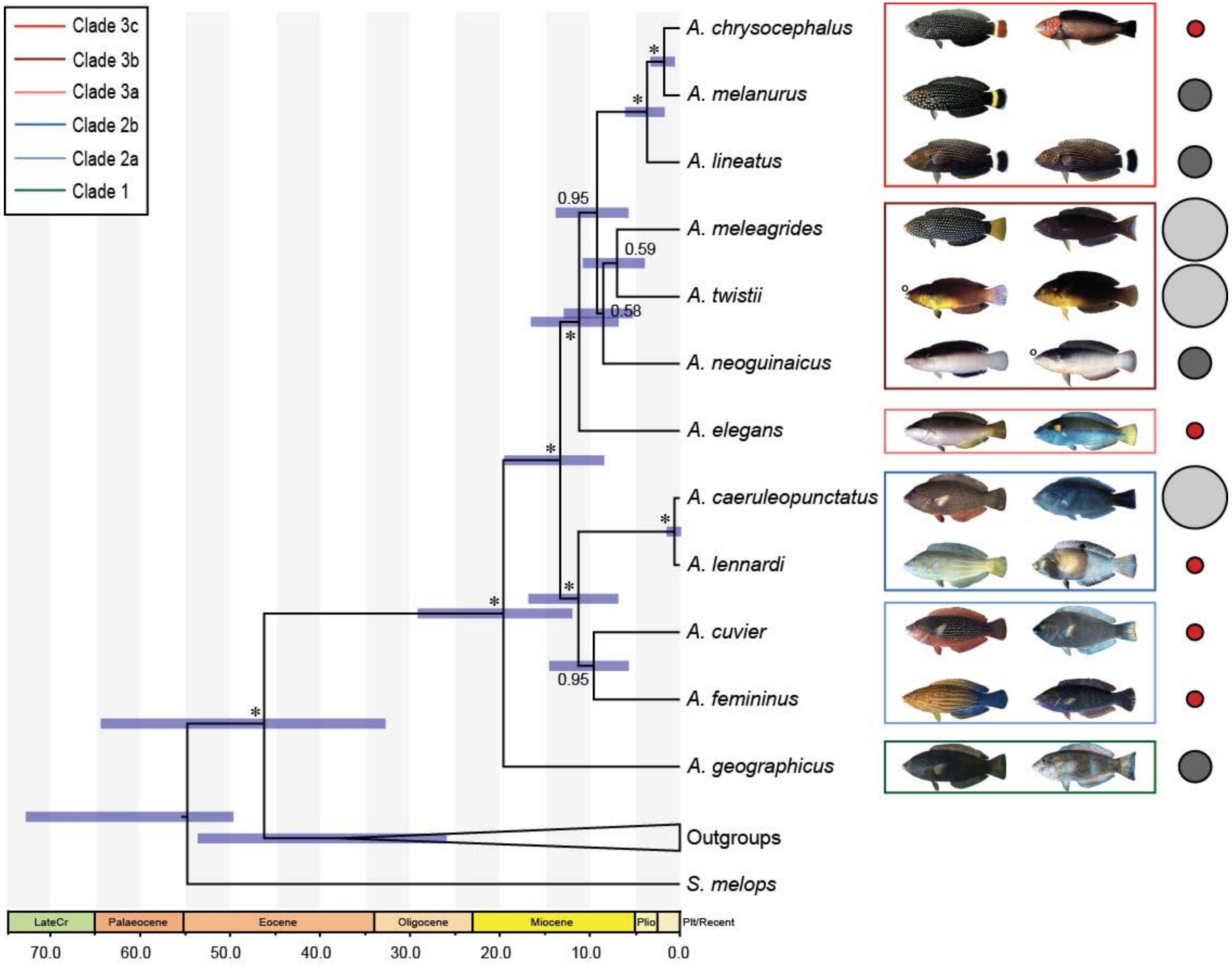

Clade 3c
Clade 3b
Clade 3a
Clade 2b
Clade 2a
Clade 1
A. chrysocephalus
A. melanurus
A. lineatus
A. meleagrides
A. twistii
A. neoguinaicus
A. elegans
A. caeruleopunctatus
A. lennardi
A. cuvier
A. femininus
A. geographicus
Outgroups
S. melops
*
0.95
0.59
0.58
LateCr
Palaeocene
Eocene
Oligocene
Miocene
Plio
Plt/Recent
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0

Figure 3.2 A fossil-calibrated chronogram of *Anampses*. Ages are represented as median node heights of the 95% highest posterior density (HPD) interval from a maximum clade credibility tree complied from post-burnin topologies of four independent Bayesian Markov Chain Monte Carlo (MCMC) analyses (10×10^6 generations per run) implemented in BEAST. Asterisks indicate 100% posterior probability for the node. Photos, by Randall (Unpublished), depict the initial phase (left panel) and terminal colour phase (right panel). ° indicates possible intermediate phase; a comparable terminal phase photo of *Anampses melanurus* was not available. Circles indicate relative biogeographical range size based on three separate categories; red circles represent peripherally isolated endemic species with geographical ranges $< 5 \times 10^6$ km^2.

geographicus as an early diverging lineage, within a single monophyletic *Anampses* genus. However, *A. geographicus* as a monotypic sub-genus may be paraphyletic and subgeneric divisions appear to be unwarranted.

Age estimation and the origin of endemics

The *Anampses* lineage began diversifying in the early Miocene and underwent further expansion during the Pliocene and Pleistocene, a time when other coral reef fish genera were also diversifying (Bernardi *et al.*, 2002; McCafferty *et al.*, 2002; Streelman *et al.*, 2002; Klanten *et al.*, 2004; Alfaro *et al.*, 2007). Indeed, the age of the MRCA of *Anampses* (~19.6 Ma) is similar to the ages of other, closely related coral reef fish genera with comparable sampling including *Macropharyngodon* (Read *et al.*, 2006), *Thalassoma* (Bernardi *et al.*, 2004) and *Halichoeres* (Barber & Bellwood, 2005; see Cowman & Bellwood, 2011 for an overview). For many modern day reef fish families, a shift onto coral reef habitats following the K/T boundary mass extinction event (65 Ma) was associated with an increase in species richness (Bellwood & Wainwright, 2002; Alfaro *et al.*, 2007; Bellwood *et al.*, 2010) particularly during the Miocene (Cowman & Bellwood, 2011). Once established on coral reefs, the Labridae underwent a wave of innovation during the Miocene resulting in the origination of many specialized feeding

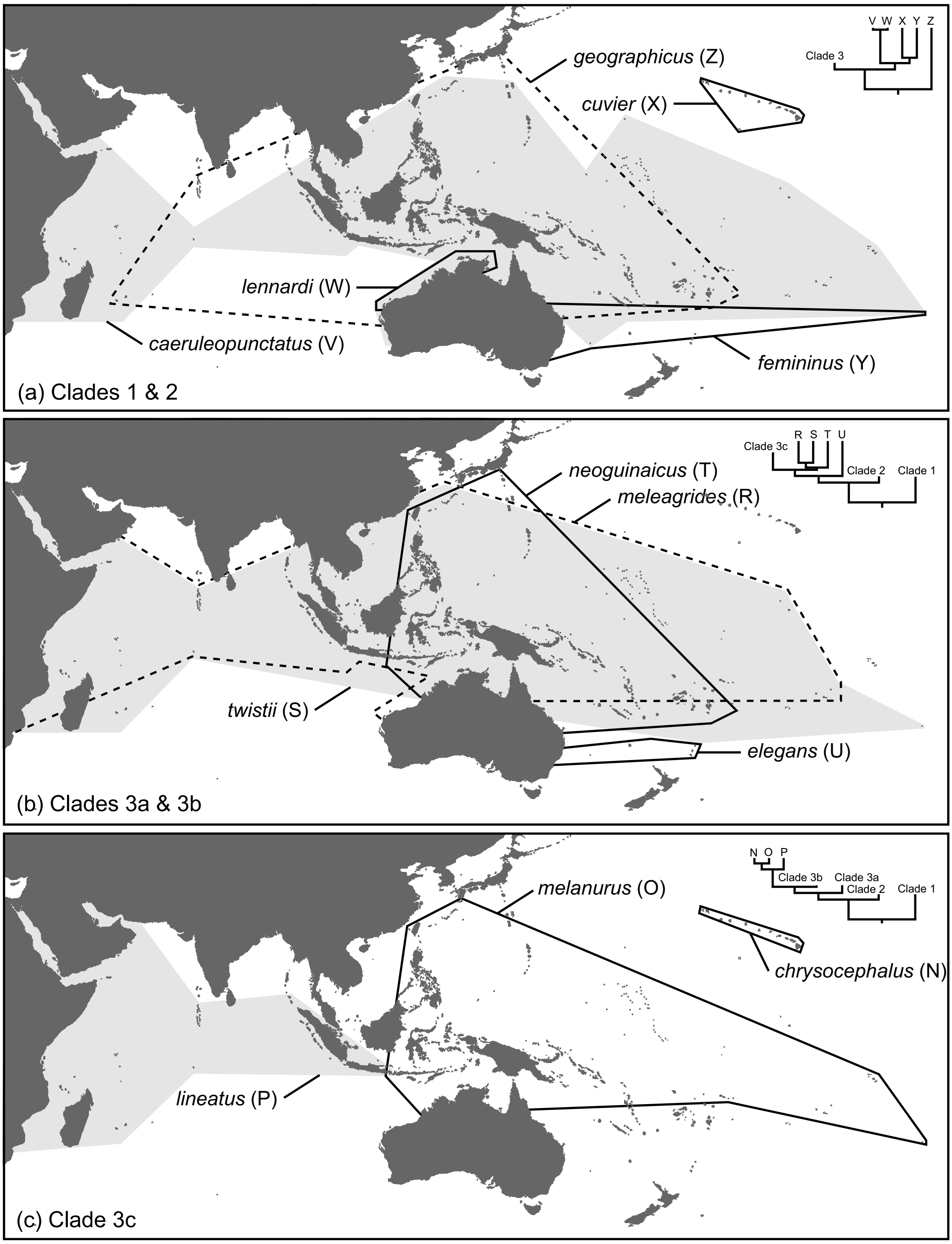


Figure 3.3 Maps showing the biogeographical distribution of *Anampses* species. Clade notations correspond to those in Figure 3.2. Species' geographical ranges and occurrences were compiled from Hughes *et al.* (2002) and Kuiter (2010).

modes (Cowman *et al.*, 2009). The expansion of *Anampses* in the early Miocene was no exception and signified the emergence of another highly specialized feeding mode. The genus characteristically possesses a single pair of broad projecting incisiform teeth at the front of the jaws (Randall, 1972). They use these highly modified teeth to help suck invertebrate prey, mainly crustaceans, from the epilithic algal matrix. This feeding mode appears to be unique among labrids (Wainwright *et al.*, 2004) and probably played a key role in the ecological success of the genus.

Discerning the biogeographical history of a given group of organisms from a phylogenetic hypothesis is difficult (Losos & Glor, 2003; Heads, 2009) and there are often multiple modes of speciation that can be invoked to explain observed patterns of relatedness and present-day distributions (Kirkendale & Meyer, 2004). The four main modes of speciation are: allopatry, which occurs when the range of a species is split into isolated populations (Mayr, 1942); peripatry, which is simply allopatry along the periphery of a species' distribution (Mayr, 1963); sympatry, which is driven by factors such as natural selection and competitive exclusion and occurs without geographical isolation (Coyne & Orr, 2004); and parapatry, which occurs when there is restricted gene flow between two diverging populations (Gavrilets, 2000). Evidence for all four modes of speciation has been reported for marine organisms (Lessios *et al.*, 2001; Munday, 2004; Rocha *et al.*, 2005; Bernardi *et al.*, 2008; Leray *et al.*, 2010; Malay & Paulay, 2010; Bird, 2011; Fitzpatrick *et al.*, 2011; Ingram, 2011). The peripheral location and timing of endemic species divergence within the *Anampses* clade revealed possible differences in the underlying modes of speciation. Although it is difficult to separate the major modes based on phylogenetic data, there are three models of diversification that may help us understand which modes are operating. These three models are the: (a) 'successive division', (b) 'successive colonization' and (c) 'peripheral budding' models (Fig. 3.4). The relationship between *A. lineatus, A. melanurus* and *A. chrysocephalus* in Clade 3c appears to have arisen from 'successive division' or 'successive colonization', or some combination of the two. Both models involve species' divergences coinciding with a directional shift in their distributions.

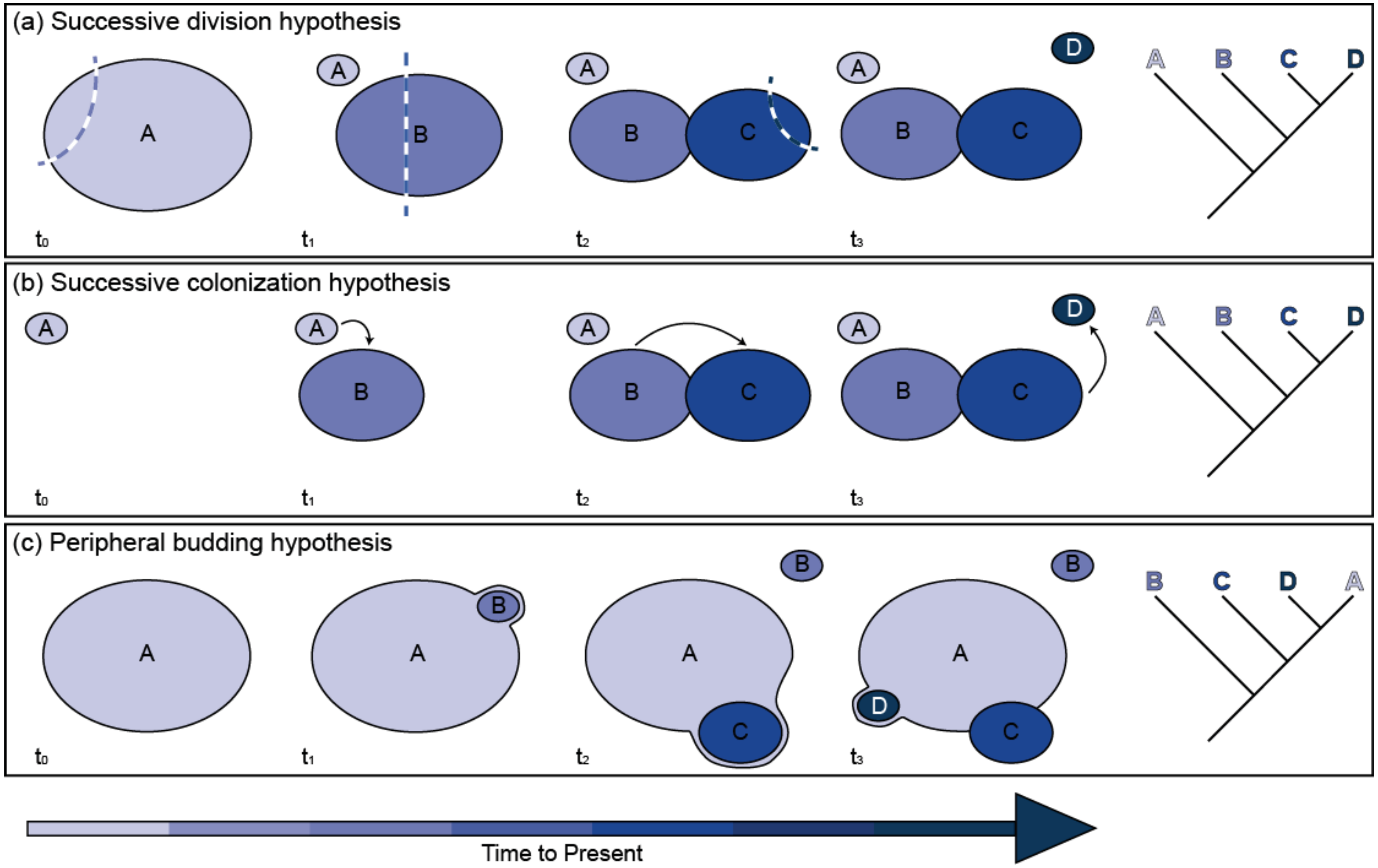
However, in ‘successive division’, vicariance events underpin allopatric speciation, whereby a series of divisions results in the fragmentation of a widespread distribution. In contrast, peripatric or parapatric speciation modes are behind ‘successive colonization’, whereby individuals from the ancestral population enter a new environment in an adjacent or isolated location.

The ‘peripheral budding’ model is less obvious from observed ages and topological relationships among species. Nevertheless, the species in Clade 2 appear to serve as an example of this model whereby it appears that peripatric, parapatric or sympatric speciation from a relatively widespread species has led to several peripheral isolates throughout the evolutionary history of the lineage, with the most recent bud displaying a sister relationship to the widespread species from which it diverged. Thus, the ages and topological relationships in the chronogram may be deceiving because the widespread species will appear much younger than it actually is due to the fact that it is genetically most similar to the most recently diverged bud. Similar models of diversification have also been proposed for gastropods and hermit crabs (Kirkendale & Meyer, 2004; Malay & Paulay, 2010), with support from phylogeographic data reported for the parrotfish species, *Scarus psitticus* (Winters *et al.*, 2010). It is important to note that without additional information from the fossil record it is not possible to substantiate the model. Still, the implication of the ‘peripheral budding’ model for *A. caeruleopunctatus* is that rather than being ~600 Ka, it is more likely older than 9.6 Ma, having given rise to budded species *A. cuvier, A. femininus*, and *A. lennardi* (9.6 Ma, 9.6 Ma and 600 Ka, respectively).

The alternative hypothesis would suggest that *A. caeruleopunctatus* has acquired a geographical range spanning the entire Indo-Pacific in less than 600 thousand years. Pleistocene sea-level changes may be invoked to explain such rapid range expansion. Under this scenario, peripheral isolates characteristically occupy reef slopes along the edges of continental margins or oceanic islands and suffer weak bottlenecks and isolation during stands of low sea level. Their populations would persist through fluctuations in sea-level due to the flexible nature of the reef slope habitat and its ability to track changes in sea-level. In contrast, lagoonal species would suffer stronger bottlenecks during periods of low sea level with substantial loss of habitat. Following sea-level rise, the opportunity would exist for lagoonal species to rapidly expand their ranges due to the re-appearance of suitable habitat (*sensu* Fauvelot *et al.*, 2003). *Anampses caeruleopunctatus* generally occupies inshore, protected reefs and lagoons (Randall, 1972; Kuiter, 2010), and while its age (~600 Ka) does coincide with a period of sea-level rise (Lisiecki & Raymo, 2005), this was only one of many fluctuations in sea-level during the Pleistocene. Population genetic data of these three endemic species and their widespread sister/putative ancestor are required to critically evaluate these alternate hypotheses.

Temporal discordance among co-occurring endemics

The age estimates revealed both similarities and differences in the ages of endemic species with shared isolated distributions. *Anampses cuvier* is a relatively old endemic (~9.6 Ma) found only in the Hawaiian Islands and Johnston Atoll. It shares part of this isolated distribution with another endemic species, *A. chrysocephalus*, which emerged more recently, ~8 Ma later. The Hawaiian Islands are part of the Hawaiian-Emperor seamount chain, which formed over the last 85 million years (Clague & Dalrymple, 1987; Rooney *et al.*, 2008). The chain formed as a result of volcanic outpourings onto the Pacific lithospheric plate as it moved in a northwesterly direction over a geographically fixed hotspot in the asthenosphere (Wilson, 1963; Rotondo *et al.*, 1981). Thus, the islands are ordered linearly by age, with the oldest island in the northwest and the youngest in the southeast (5.1–0.43 Ma, respectively); over time, these volcanoes eroded to form coral atolls and seamounts (Fleischer *et al.*, 1998). Given the seamount chain’s extensive geological history, *A. cuvier* could have colonized islands or seamounts in the area ~9.6 Ma, and subsequently expanded its range to other reef habitat on surrounding islands and atolls once it became available. As the island chain progressed this created more habitat, which would have allowed *A. cuvier* to stretch its distribution further from older, now submerged atolls as they moved northward, to the newly formed islands in the southeast.


(a) Successive division hypothesis

(b) Successive colonization hypothesis

(c) Peripheral budding hypothesis

Figure 3.4 A schematic diagram of three alternate models of diversification observed among *Anampses* species, the: (a) ‘successive division’, (b) ‘successive colonization’, and (c) ‘peripheral budding’ models. In the first, sequential allopatric speciation leads to an apparent directional shift in species’ distributions. In the ‘successive colonization’ model peripatric or parapatric speciation also leads to a directional shift in species divergence and, in this example, results in the same phylogenetic topology as (a). In the ‘peripheral budding’ hypothesis peripatric, parapatric or sympatric speciation leads to the divergence of several peripheral isolates throughout evolutionary history. This results in the ancestral species ‘A’ appearing as one of the youngest species due to its genetic similarity with the most recently diverged bud (species ‘D’).

Anampses cuvier is sister to *A. femininus*, a peripherally isolated species whose range is restricted to the area between the southern Great Barrier Reef and Easter Island. *Anampses femininus* probably arose as a peripheral bud from the ancestral lineage of *A. caeruleopunctatus*. The data suggest that *A. femininus* subsequently gave rise to its own bud, *A. cuvier*, via its eastern most populations. Similar phylogenetic relationships are known for other coral reef fish genera, specifically the *Dascylus trimaculatus* species complex where *Dascylus albisella* in Hawaii is sister to *Dascylus strasburgi* in the Marquesas, rather than to *Dascylus auripinnis* in the geographically closer Line and Phoenix Islands (Leray *et al.*, 2010). Additional evidence for genetic links between Hawaii and Easter Island has been reported in the sea urchin genus *Diadema* (Lessios *et al.*, 2001). *Diadema paucispinum*, a species considered endemic to Hawaii, actually extends as far south as Pitcairn and Easter Islands. Further exploration of extralimital sister-group relationships in the context of evolutionary time may provide more support for this route of Hawaiian colonization.

Anampses chrysocephalus is a young species (1.7 Ma) that occurs in partial sympatry with *A. cuvier*. These two species have not only arisen at different times, but they have also arisen in different clades. Thus, the results offer no evidence for the dispersal of species from older to younger islands as in some terrestrial fauna (Wagner & Funk, 1995) and no support for secondary endemism (Rotondo *et al.*, 1981; Bird *et al.*, 2011). Rather, there appears to have been an independent colonization by *A. chrysocephalus*. Multiple species' colonization of the Hawaiian Archipelago has occurred in other coral reef fish genera including the butterflyfish genus *Chaetodon* (Bellwood *et al.*, 2010; Craig *et al.*, 2010), and the wrasse genus *Thalassoma* (Bernardi *et al.*, 2004). Evidence from the fossil record of corals and gastropods also supports continuing, sporadic immigration to the Hawaiian Islands (Kay & Palumbi, 1987). *Anampses chrysocephalus* is sister to *A. melanurus*, which is distributed throughout the west Pacific. Thus, *A. chrysocephalus* may have utilized any number of corridors to colonize Hawaii, including dispersal from Easter Island.

Temporal concordance among co-occurring endemics

Another isolated distribution is shared by *A. elegans* and *A. femininus*. The former, *A. elegans* (~11.2 Ma) is endemic to Lord Howe Island, with temporary pseudo-populations in northern New South Wales and the island region of northern New Zealand (Choat *et al.*, 1988). *Anampses femininus* (~9.6 Ma) also occurs at Lord Howe Island, but has a larger distribution than *A. elegans*, occurring as far east as Easter Island. While the estimated ages of these species may seem disparate, the 95% HPDs surrounding them overlap considerably. This raises the prospect of peripherally isolated species evolving in two separate clades, at roughly the same time and possibly in the same isolated location. Although the more widespread distribution of *A. femininus* suggests that dispersal and range expansion could have allowed this species to colonize Lord Howe Island at any time throughout its ~10 million year history. When the ages of these two species were compared to the age of Lord Howe Island, which formed during a short interval in the late Miocene (6.4–6.9 Ma) (McDougall *et al.*, 1981), there is a mismatch; the lineages leading to the endemics apparently arose prior to the island.

At first it may be assumed that these species are relictual, particularly with the placement of *A. elegans* as the first to diverge in its lineage. However, Lord Howe Island is an eroded shield volcano, which, like the Hawaiian Islands, is part of a chain of volcanic seamounts that formed due to the northward movement of the Australian plate over a stationary mantel hotspot during the Oligocene and Miocene (Wellman, 1983; Quilty, 1993; Exon *et al.*, 2004). Following their formation, the volcanoes subsided and were fringed by reefs, some of which have persisted until the present day (McDougall & Duncan, 1988; Exon *et al.*, 2006; Przeslawski *et al.*, 2011). Lord Howe Island is an example of such a volcano that lies at the southern end of the chain.

The geological history of the region combined with the age estimates suggests that both *A. elegans* and *A. femininus*, as peripherally isolated species, occupy part of an island chain, the current configuration of which is the presence of Lord Howe Island. Furthermore, it appears that the age of an island is not a reliable indicator of the ages of its inhabitants. The Lord Howe seamount chain is an example of how islands come and go (Woodroffe *et al.*, 2010) and the presence of *A. elegans* and *A. femininus* as peripheral isolates is similar to the pattern of peripheral isolation observed in Hawaii. The creation of new habitat in the Lord Howe region due to tectonic uplift and volcanism would have allowed ancestral *Anampses* species to expand their ranges or found new populations on emerging islands during intervals of spatially extended tropical/subtropical conditions (Quilty, 1993). For *A. elegans*, Lord Howe Island is now the only stable population base, for *A. femininus* it is merely one of many. Other studies of marine organisms within and nearby the Lord Howe Region have emphasised the importance of isolation and limited dispersal (Samadi *et al.*, 2006; Castelin *et al.*, 2010) as drivers of genetic divergence among populations living in this patchy habitat. Overall, the results demonstrate that the divergence of peripherally isolated restricted range or endemic species may be facilitated by the presence of fragmented habitat provided by seamount and island chains.

The role and relative extinction risk of peripheral endemics

As is the case for most coral reef associated endemics, the majority of endemic *Anampses* are peripherally isolated relative to the IAA hotspot. Only one of the five endemic species studied here, *A. lennardi*, has a distribution that may be considered part of the IAA. While endemic species can represent newly diverged species (neo-endemics), or relatively old, relictual species that may have suffered significant range contraction, it seems that regardless of their age, peripherally isolated endemic species appear to have limited ability for range expansion and possibly limited ability to undergo speciation events. This is most likely a consequence of their occurrence on the periphery, or along the edges of the biogeographical domain. Expansion outward is most likely restricted by abiotic physical and environmental factors, while expansion inward may be limited by biotic interactions such as competitive exclusion and introgression (Brown & Lomolino, 1998).

However, recent phylogeographic studies have highlighted the potential importance of peripheral island populations in exporting larvae inward toward the IAA (Lessios *et al.*, 2001; Drew & Barber, 2009; Eble *et al.*, 2011; Fitzpatrick *et al.*, 2011; Skillings *et al.*, 2011). This suggests that some peripheral endemics may export diversity. Determining the extent to which peripheral endemics contribute to the biodiversity of species within the IAA will require further species-level phylogenetic studies. Population genetic studies may also be useful to estimate effective population sizes through time, provided coalescence and divergence times are comparable (Bernardi & Lape, 2005; Bowen *et al.*, 2006b; van Herwerden *et al.*, 2006; Klanten *et al.*, 2007). Studies of this nature will likely reveal cryptic patterns of peripheral speciation (Budd & Pandolfi, 2010; Gaither *et al.*, 2010; Hobbs & van Herwerden, 2010; Leray *et al.*, 2010; Fitzpatrick *et al.*, 2011) and provide more detail about the role of peripheral isolation at range edges in the evolution of Indo-Pacific coral reef biodiversity.

Regardless of their role, peripheral endemics are no doubt valuable within their ranges and face the possibility of being overlooked in terms of conservation initiatives simply because of their isolated locations relative to the bulk of coral reef biodiversity. The consequences of isolation may have already been realized by *A. viridis*, a species once thought to be endemic to the island of Mauritius (Hawkins *et al.*, 2000; Dulvy *et al.*, 2003; but see Russell & Craig, 2013). Randall (1972) suggested that *A. viridis* might have been a close relative of *A. caeruleopunctatus*. This seems likely in light of the ‘peripheral budding’ hypothesis. It may be that peripheral speciation is quite common and results in more extinction than previously realized. Peripheral endemic and restricted range species are more susceptible to extinction threats as a result of their limited ranges and relatively small population sizes, particularly if they are specialists (Hawkins *et al.*, 2000; Munday, 2004). Of the endemics considered here, *A. elegans* has the smallest range and the oldest estimated age. These factors, combined with the fact that its distribution is a narrow margin on the interface between tropical and subtropical environments, could put it at increased risk of extinction. It is no doubt vulnerable to any number of local impacts, which could cause global loss. Hopefully more consideration will be given to peripheral areas with high proportions of endemic species when designing future conservation initiatives.

Chapter 4: Evolution of sympatric species: a case study of the coral reef fish genus *Pomacanthus* (Pomacanthidae)

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4.1 Introduction

The study of the geographical distribution of organisms has made fundamental contributions to our understanding of evolutionary phenomena, including speciation. Recently, focus has shifted away from attempts to label divergence events according to speciation mode (i.e. allopatric or sympatric) and is moving towards identifying the processes and isolating barriers affecting divergence (Fitzpatrick *et al.*, 2008; Bird *et al.*, 2012; Santini *et al.*, 2012). Before one can investigate these processes and isolating barriers, it is necessary to establish biogeographical patterns in conjunction with robust evolutionary hypotheses (Coyne & Orr, 2004; Butlin *et al.*, 2008; Mallet *et al.*, 2009). This study therefore focuses on patterns of eventual sympatry, rather than sympatric speciation as a process, and considers the multiple modes of speciation that can lead to sympatry among species.

Age–range correlation (ARC) analysis has been used as a comparative method to quantify the relative importance of allopatric versus sympatric speciation (Barraclough & Vogler, 2000). The method involves assessing the degree of sympatry (contemporary range overlap) against species (node) age (Fitzpatrick & Turelli, 2006). It assumes that the extent of overlap between ranges is dependent on the geography of speciation, but becomes randomized over time through independent range changes (Barraclough & Vogler, 2000; Fitzpatrick & Turelli, 2006). If the mode of speciation within a group of taxa has been predominantly allopatric, then sister-species should have limited range overlap, and range overlap should increase with time and become more prevalent among distantly related sister clades. The opposite pattern presents if speciation has been predominantly sympatric, with recently diverged sister-species exhibiting greater range overlap than more distantly related sister clades.

ARC analysis has been questioned on the basis that substantial changes to species geographical ranges can effectively eliminate the relationship between the geography of speciation and contemporary geographical distributions (Barraclough & Vogler, 2000; Losos & Glor, 2003), especially in highly mobile species (Phillimore *et al.*, 2008). Despite this, ARC analysis has been shown to be capable of detecting when the predominant mode of speciation is sympatric (Phillimore *et al.*, 2008). Further improvements have been made as a result of simulation studies, including: independent comparisons to determine overlap between clades, and Monte Carlo methods to test the relationship between phylogenetic relatedness and geographical range overlap (Fitzpatrick & Turelli, 2006); and the proportion of species showing zero or complete range overlap as a more reliable indicator of the geography of speciation (Phillimore *et al.*, 2008). All of these methods require a robust phylogeny within a temporal framework (Fitzpatrick & Turelli, 2006) and generally work best when applied to geographical relationships among sister-species (Phillimore *et al.*, 2008; Fitzpatrick *et al.*, 2009).

Despite these advances, few studies have used ARC analysis to investigate the geography of speciation in the marine realm (Williams & Reid, 2004; Frey, 2010; Krug, 2011; Quenouille *et al.*, 2011). Most subsequently concluded that the majority of sister-species pairs have allopatric distributions and that allopatric speciation is likely to have been the predominant mode (but see Krug, 2011). Upon application of ARC analysis to several genera of coral reef organisms, Quenouille *et al.* (2011) found that most sister-species with contemporary sympatric ranges were no younger than 4 Myr. They concluded that the predominant mode of speciation among these genera was allopatry followed by range expansion. By applying ARC analysis, I can investigate whether the same patterns are more widely applicable to coral reef fishes by assessing evidence from the genus *Pomacanthus*.

Pomacanthus is a relatively small genus, with 13 extant species. This enabled complete sampling of extant taxa, which is essential when analysing relationships of sister-species (Barraclough & Nee, 2001; Santini *et al.*, 2012). *Pomacanthus* species occur throughout the Indo-Pacific and Caribbean, with extensive range overlap among species (92% of species occur in sympatry), facilitating meaningful analyses of the relationship between geography and evolution. This is the first study to use ARC analysis to investigate the relationship between geography and speciation for coral reef fishes in the context of a fossil-calibrated phylogeny. Previous studies have extrapolated the temporal scale of phylogenetic hypotheses using a range of methods, not all of which have been consistent (Quenouille *et al.*, 2011). Caution must be exercised when analyses rely heavily on a temporal component, to ensure that the methods used to determine such a context are both reliable and comparable.

This is also one of the first studies to employ relatively accurate geographical range maps that limit the extent to which geographical ranges of species may be overestimated. Overestimating geographical ranges by including areas with unsuitable habitat can potentially skew spatial analyses such as ARC. Species distribution maps were obtained from the recently compiled IUCN database (IUCN, 2011). These maps were used in conjunction with a fossil-calibrated phylogenetic hypothesis to establish a spatial and temporal framework within which I investigated the role of geography in the evolution of *Pomacanthus* species. My specific objectives were:

1) To determine the phylogenetic relationships among *Pomacanthus* species within a temporal context; and
2) To test whether the degree of sympatry among sister taxa correlates with their age using a range of methods and indices; and
3) To evaluate the relative contribution of the three main speciation modes in explaining the production of sympatric distributions.

4.2 Materials and Methods

Methods of ***Data selection***, ***Laboratory procedures***, ***Sequence data***, ***Phylogenetic analysis*** and ***Age estimation*** for this data chapter follow those detailed in **Chapter 2: General Methods**, section **2.1 Case Studies**.

Biogeographical analysis

Spatial data were compiled for all *Pomacanthus* species (IUCN, 2011) and analysed in GRASS (GRASS Development Team, 2011) to determine the degree of sympatry among sister taxa using the definition of Barraclough & Vogler (2000). Several methods were applied to investigate geographical patterns of speciation. The relationship between geography and species age was examined through ARC analysis by plotting the degree of sympatry against node age across all nodes within the genus. Geographical ranges of higher nodes in the phylogeny were reconstructed by summing the ranges of all species subtending the node (Barraclough & Vogler, 2000). Arcsine-transformed degree of sympatry was regressed onto node age to estimate an intercept and slope. A negative slope with an intercept > 0.5 is suggestive of sympatric speciation, where recently diverged sister-species exhibit high levels of range overlap compared with more distantly related sister clades (Barraclough & Vogler, 2000). In contrast, an intercept of < 0.5 and a positive slope suggests allopatric speciation as the predominant mode, where sister-species have low levels of range overlap and range overlap becomes more prevalent with time among distantly related sister clades (Barraclough & Vogler, 2000).

Independent comparisons (equivalent to independent contrasts; see Felsenstein, 1985) were also used in the form of nested averages of pairwise overlap between species as an alternative method of ancestral range reconstruction. This method provides an estimate of the average range overlap between species, or clades at each node since divergence. Average range overlap was regressed onto estimated node age. The null hypothesis of no relationship between divergence time and mean overlap was tested using 100,000 Monte Carlo simulations and a

Mantel test (Dietz, 1983) implemented in R (R Development Core Team, 2011). The same pattern of high intercept and negative slope indicative of sympatric speciation described above for ARC analysis also applies to the independent comparisons analysis (Fitzpatrick & Turelli, 2006). The proportion of sister-species pairs with zero or complete range overlap was also calculated and compared to the simulations of Phillimore *et al.* (2008).

Finally, the degree of range-size symmetry at each node was calculated following Barraclough & Vogler (2000), to determine whether peripatric speciation may have been a dominant mode within the genus *Pomacanthus*. The degree of range-size symmetry is bounded between zero and 0.5, with the latter representing sister clades with equal-sized ranges. Intercepts below 0.25 suggest the possible importance of small ranges in speciation. Post-speciation range movements determine the slope of the relationship between range-size symmetry and node age, where range movements within a finite area will lead to older clades occupying a greater proportion of the total area and range-size symmetry may increase with node age.

4.3 Results

Phylogenetic analysis

The combined data matrix yielded a strong phylogenetic signal with 301 parsimony informative characters (18.5%). The models selected using corrected Akaike information criterion (AICc) varied among loci (Table 4.1). No lack of convergence among Bayesian inference (BI) analyses was detected by AWTY (Figs S4.1 and S4.2, Appendix C).

The phylogeny of the genus *Pomacanthus*, represented by the best BI tree, was the most well resolved phylogeny consistent with all other analyses, and showed strong bootstrap support [maximum parsimony (MP) and maximum likelihood (ML)] and posterior probability support (BI) for most nodes (Fig. S4.3, Appendix C). All analyses confirmed the monophyly of

Pomacanthus with nearly 100% support and placed *Holacanthus*, *Pygoplites* and *Chaetodontoplus* in a clade sister to *Pomacanthus* (Fig. 4.1, Fig. S4.3, Appendix C). There is a clear, well-supported split between west and east Tethyan *Pomacanthus* species, with the west Tethyan species restricted to the Caribbean and East Pacific, and east Tethyan species distributed throughout the Indo-Pacific and West Indian Ocean. A further split within the east Tethyan clade separated the Indo-Pacific species from the West Indian Ocean clade, with the exception of *Pomacanthus semicirculatus*. *Pomacanthus semicirculatus* does not appear as a basal lineage within the West Indian Ocean clade, but rather as a crown species, albeit ambivalently supported at the crown within an otherwise totally supported crown clade of five sister-species. Finally, there is another split within the west Tethyan clade, which separates the two Caribbean species from *Pomacanthus zonipectus* in the East Pacific.

Table 4.1 Models of evolution of *Pomacanthus* fishes selected by JModelTest under the corrected Akaike information criterion (AIC_c). Single gene alignments were analysed separately and the appropriate model applied for further partitioned analysis of the concatenated alignment. N_{ST} specifies the number of substitution parameters.

Data Set	AICc Model	Invariable Sites	Gamma Shape (γ)	*N*st	Data Set
12S	350	TIM2ef + I + G	0.372	0.789	6
16S	556	TIM3 + I + G	0.394	0.383	6
S7	705	TVM + G	—	2.164	6
Complete	1611	SYM + I + G	0.158	0.638	6

Timing of speciation

Log files from four independent BEAST analyses showed high effective sample sizes (posterior ESS values > 200 for four combined analyses), which indicated valid estimates based on independent samples from the posterior distribution of the Markov chain. Assessment of convergence in AWTY indicated that all analyses had converged (Figs S4.4 and S4.5, Appendix C). The maximum clade credibility chronogram was compiled from 36,004 post-burn-in trees

(36,004,000 generations). Ages are presented as median node heights with the 95% highest posterior density (HPD) intervals indicated by horizontal bars at each node. Strong posterior probabilities were obtained for the majority of nodes (Fig. 4.1).

The *Pomacanthus* lineage dates back to the mid-Eocene when it shared a most recent common ancestor (MRCA) with *Holacanthus* and *Pygoplites c.* 40 Ma. Subsequent divergence within the genus began in the late Oligocene, around 24.5 Ma (95% HPD: 17.3–32.8 Ma), when species in the east Tethys split from those in the west Tethys. Diversification continued throughout the Miocene, with all extant lineages present by 5 Ma (minimum bound of 95% HPD: 2.6 Ma).

Biogeographical patterns of speciation

Although the geographical separation of east and west Tethyan species is maintained, most species within each of the four clades have a high degree of sympatry (Figs 4.2 and 4.3). Two of the three west Tethyan species have partially sympatric distributions (66.7%), as do all 10 of east Tethyan species (100%) (Fig. 4.4). The ARC analysis revealed a negative relationship between geographical range overlap and species age (r = –0.09) (Fig. 4.5a). However, the resulting values were not statistically significant (n = 12; d.f. = 1, 10; F = 0.04; P = 0.84). Furthermore, Spearman’s rank correlation coefficient showed that the relationship between degree of sympatry and node age was not significant (n = 12; d.f. = 10; t = 0.14; P = 0.66). Despite the non-significant results, the intercept was higher than 0.5 (0.81, +0.19/–0.15 SE untransformed; note that standard errors are asymmetrical because linear regressions were performed on arcsine-transformed values for both standard ARC and range-size symmetry analyses).

The ARC analysis using independent comparisons also yielded a negative relationship between average geographical range overlap and time since divergence, but showed a steeper

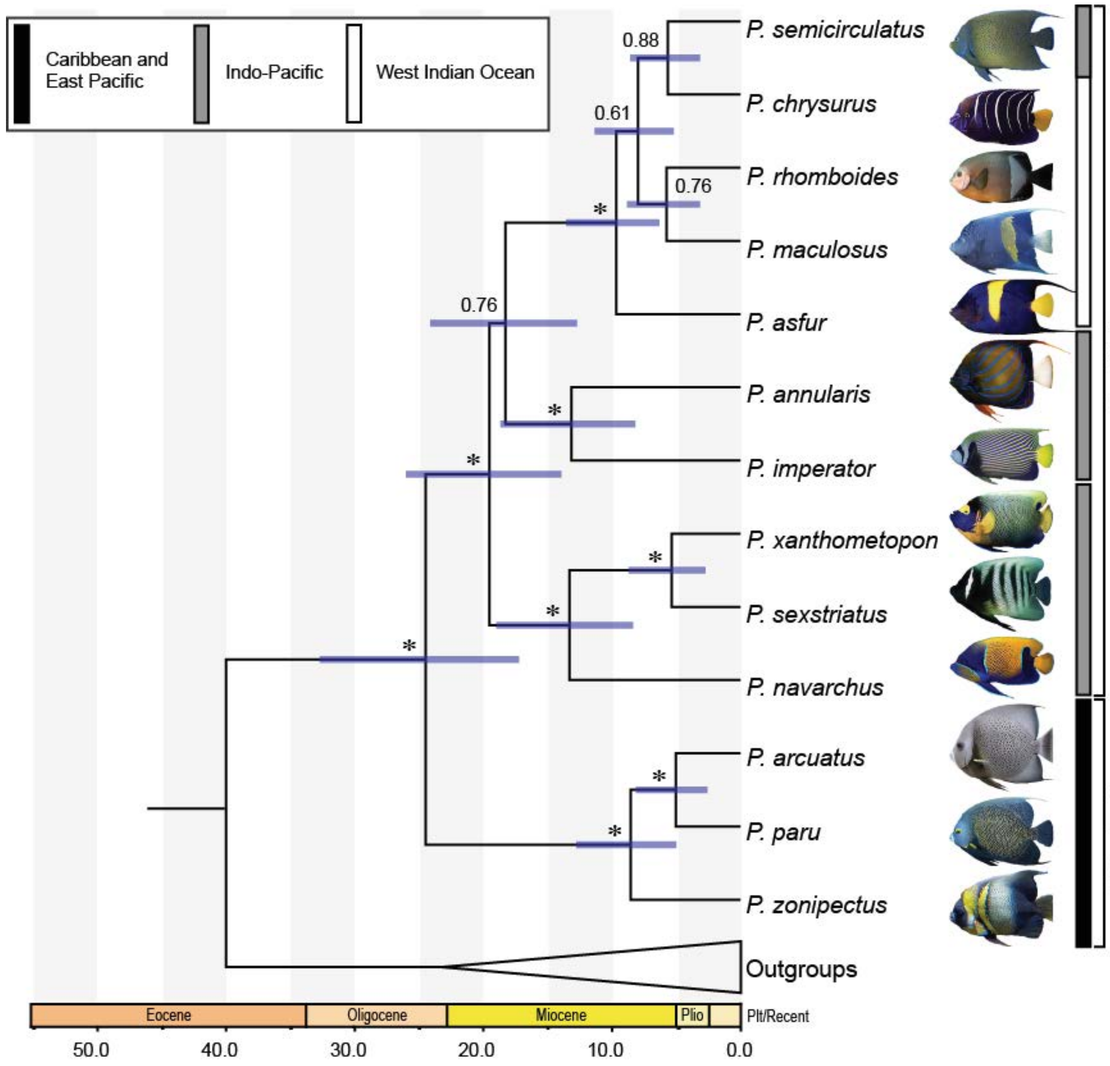

Figure 4.1 Fossil-calibrated chronogram of *Pomacanthus*. Ages are represented as median node heights of the 95% highest posterior density (HPD) interval from a maximum clade credibility tree compiled from post-burn-in topologies of four independent Bayesian Markov Chain Monte Carlo analyses (10 million generations per run), implemented in BEAST. Asterisks at nodes indicate 100% posterior probability. Photographs were obtained from FishBase (http://www.fishbase.org/).

decline in range overlap with increasing time since divergence compared to the standard ARC analysis ($r = –0.38$; Fig. 4.5b). Monte Carlo simulations of average overlap failed to reject the null hypothesis of no relationship between divergence time and degree of sympatry ($n = 12$; $r = 0.038$; $P = 0.25$). The independent comparisons ARC analysis generated an intercept of 0.79, slightly lower than that generated by the standard ARC analysis, but still greater than 0.5.

The majority of sister-species comparisons showed complete or high range overlap (> 0.85); only 20% of sister-species pairs showed zero range overlap. Furthermore, the ages of sister-species with sympatric distributions span the evolutionary history of the genus, ranging from 5 to 13.2 Ma (95% HPD: 2.6–18.7 Ma). The average age of sympatric sister-species (7.3 Ma; ± 3.9 SD) was similar to the age of the only sister-species pair found in allopatry (*P. rhomboides–P. maculosus*; 5.8 Ma; 95% HPD: 3.1–8.8 Ma), and to the age of the only species that does not occur in partial sympatry with any other *Pomacanthus* species, *P. zonipectus* (8.6 Ma; 95% HPD: 5–12.8 Ma).

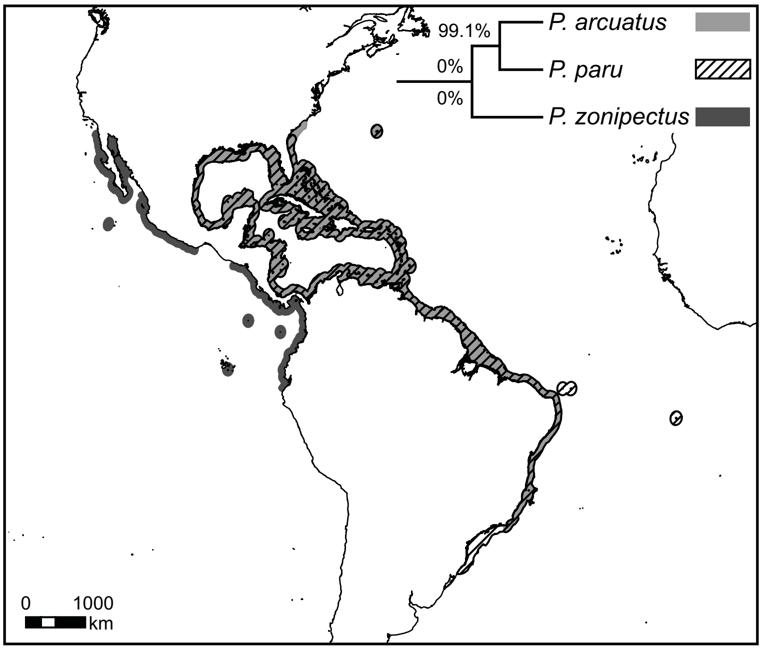

Figure 4.2 Geographical distributions of *Pomacanthus* species in the west Tethyan clade. Species' distributions were obtained from IUCN Red List maps (IUCN, 2011). The degree of sympatry is shown above each node as a percentage, where the geographical range of higher clades was reconstructed by combining the ranges of all constituent species such that all areas where at least one species is found were included. The average percentage overlap is shown below each of the higher nodes, where independent comparisons were used in the form of nested averages of pairwise overlap between species. Hatched areas overlaid onto shaded regions indicate sympatry among sister-species. The map uses the Mollweide projection.

No consistent pattern among sister-species pairs was recovered from the analysis of range-size symmetry (Fig. 4.6). The intercept was lower than 0.25 (0.17, +0.16/–0.02 SE untransformed) and range-size symmetry increased with node age ($r = 0.12$), although this was not statistically significant ($n = 12$; d.f. = 1, 10; $F = 6.4 \times 10^{-4}$; $P = 0.98$). In addition, Spearman's rank correlation coefficient showed that the relationship between range-size symmetry and node age was not significant ($n = 12$; d.f. = 10; $t = -0.15$; $P = 0.88$).

4.4 Discussion

The results reveal that *Pomacanthus* is a monophyletic genus, within which a number of speciation modes are likely to have been operating throughout evolutionary history. This is the first study to produce a robust temporal phylogenetic hypothesis for all species of *Pomacanthus* fishes. The phylogenetic reconstruction is consistent with previously published hypotheses and age estimates based on DNA evidence (Bellwood *et al.*, 2004). Several splits within *Pomacanthus* correspond to major historical vicariance events such as the Terminal Tethyan Event (TTE), and the rise of the Isthmus of Panama (IOP). There are an exceptionally high proportion of sympatric sister-species and clades whose divergence times span the evolutionary history of the genus. I find evidence consistent with sympatric speciation, based on the relationship between degree of sympatry and species (node) age, using several methods and indices. The results of the analysis of range-size symmetry, in combination with the general patterns of lineage division within the genus, suggest that peripatric speciation in the form of peripheral budding (cf. Chapter 3) may have also contributed to the richness of sympatric species, especially in the West Indian Ocean.

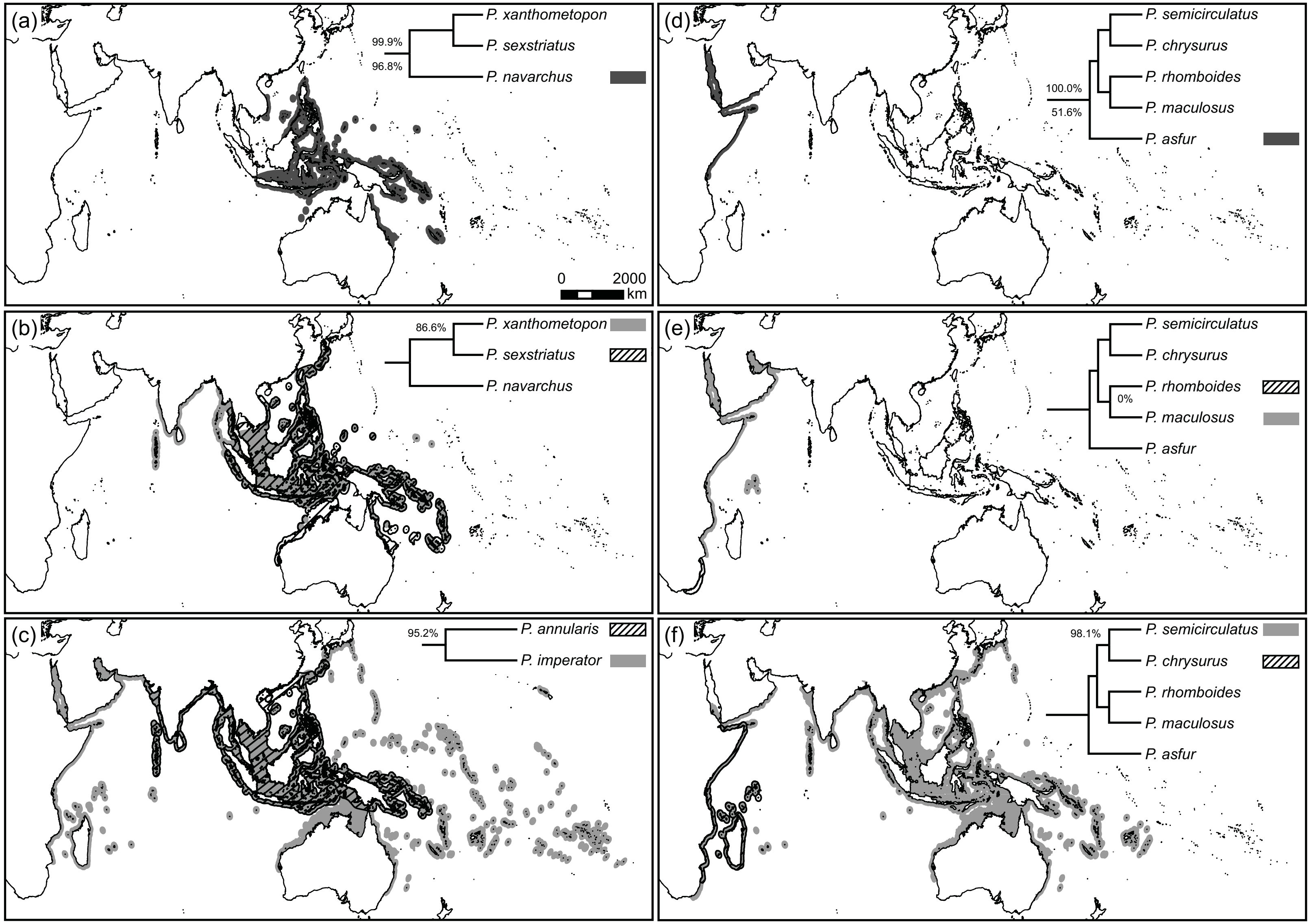
(a)
99.9%
96.8%
P. xanthometopon
P. sexstriatus
P. navarchus
0
2000
km
(b)
86.6%
P. xanthometopon
P. sexstriatus
P. navarchus
(c)
95.2%
P. annularis
P. imperator
(d)
100.0%
51.6%
P. semicirculatus
P. chrysurus
P. rhomboides
P. maculosus
P. asfur
(e)
0%
P. semicirculatus
P. chrysurus
P. rhomboides
P. maculosus
P. asfur
(f)
98.1%
P. semicirculatus
P. chrysurus
P. rhomboides
P. maculosus
P. asfur

Figure 4.3 Geographical distributions of *Pomacanthus* species in the east Tethyan clade. Species' distributions were obtained from IUCN Red List maps (IUCN, 2011). The degree of sympatry is shown above each node as a percentage, where the geographical range of higher clades was reconstructed by combining the ranges of all constituent species such that all areas where at least one species is found were included. The average percentage overlap is shown below each of the higher nodes, where independent comparisons were used in the form of nested averages of pairwise overlap between species. Hatched areas overlaid onto shaded regions indicate sympatry among sister-species. Maps use the Mollweide projection.

Allopatry

There are few well-known vicariance events that have led to simultaneous divergence and allopatric speciation across many groups of marine organisms. One well-documented example is the TTE, which involved the collision of the African and Eurasian continental plates and the subsequent loss of shallow marine habitats and marked the end of the connection between the tropical Indian and Atlantic oceans (Bellwood & Wainwright, 2002). This took place over millions of years, with the final closure of the Tethyan seaway between 12 and 18 Ma (Steininger & Rögl, 1984). The TTE has been associated with a division between Caribbean and Indo-Pacific taxa for many groups of marine organisms, including coral reef fishes (Bellwood *et al.*, 2004; Bernardi *et al.*, 2004; Bellwood *et al.*, 2010). The split of the *Pomacanthus* lineage from its sister taxa *c.* 40 Ma pre-dates the final closure of the Tethyan seaway, but remains consistent with other generic divisions of coral reef fishes (Bellwood *et al.*, 2004; Cowman & Bellwood, 2011). The divergence of the west Tethyan *Pomacanthus* species from those in the east Tethys *c.* 25 Ma suggests that barriers to dispersal and panmixia in the central Tethys may have facilitated allopatric divergence prior to the final closure of the seaway. Although the 95% HPD intervals surrounding the deeper age estimates within the *Pomacanthus* phylogeny span approximately 15 Myr, the results support previous suggestions (Bellwood *et al.*, 2004) that the marine influences of this major biogeographical boundary considerably pre-dated the final closure of the Tethyan seaway at 12–18 Ma.

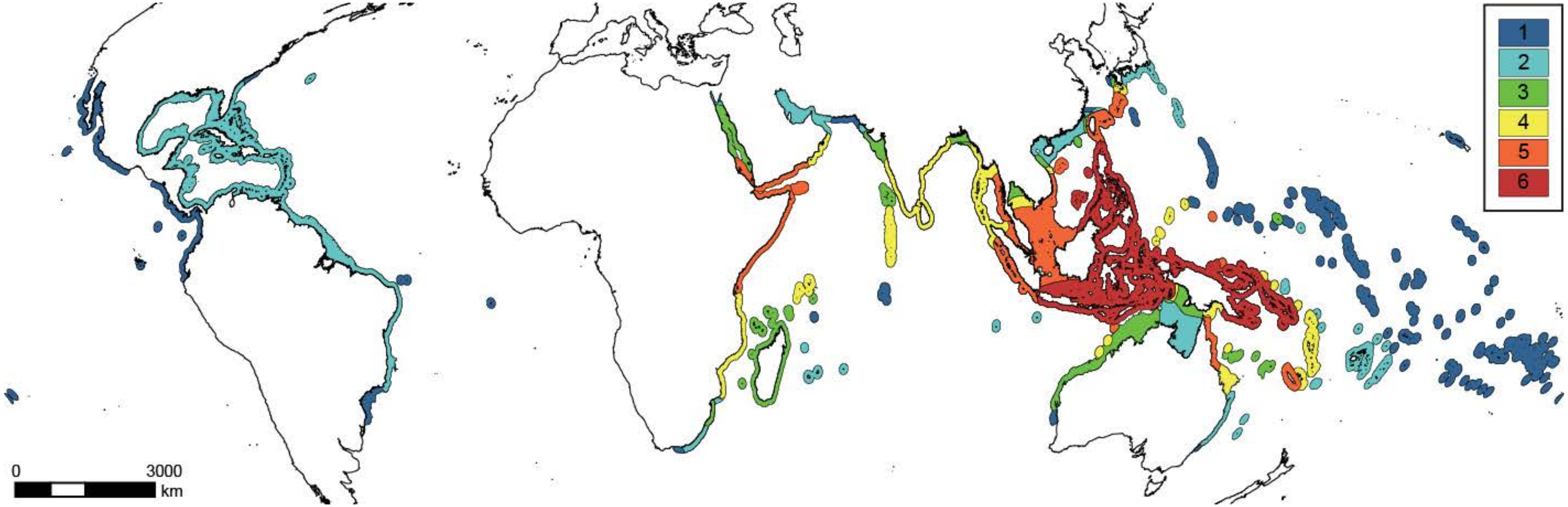

Figure 4.4 Areas of species' range overlap within the *Pomacanthus* genus. The key in the upper-right corner details the corresponding number of species that occupy each coloured region. The map uses the Mollweide projection.

Another biogeographical event that physically divided marine populations was the rise of the IOP. The rise began about 16 Ma, with the final closure separating the Atlantic Ocean and Pacific Ocean basins at 3.1 Ma (Coates & Obando, 1996). This provides an absolute minimum age for geminate species divergence (Coates *et al.*, 1992), although many divisions among reef associated taxa pre-date the final closure, as ecological and genetic barriers were in place much earlier (reviewed by Lessios, 2008). Bellwood *et al.* (2004) discussed the rise of the IOP in relation to their estimated divergence of *P. paru*/*P. arcuatus* from *P. zonipectus* at 19.9 Ma, highlighting that this estimate is considerably older than the minimum age of 3.1 Ma. The age of this split estimated herein (8.6 Ma; 95% HPD: 5–12.3 Ma) is much younger and closer to the final closure, but still pre-dates it. The older age estimate in the previous study may have arisen from limited taxon sampling (Milne, 2009), a lack of nuclear markers (Brandley *et al.*, 2011), and/or different age estimation methods (Hug & Roger, 2007). The age estimated herein falls well within the period of historical vicariance associated with the rise of the IOP (Lessios, 2008), providing further independent geological support of the fossil-calibrated phylogenetic hypothesis.

The consideration of the age estimates in conjunction with contemporary geographical distributions provides evidence in favour of two key biogeographical events driving allopatric divergence at least twice during the evolutionary history of *Pomacanthus*.

Sympatry

Most *Pomacanthus* species (92%) occur in partial sympatry with at least one other congeneric species. The results revealed that 80% of *Pomacanthus* sister-species also display a high proportion of sympatry. For all sympatric sister-species, range overlap was above 85%. In comparison to other marine taxa, this is one of the highest proportions of sympatric sister-species, with only one other taxon (*Haemulon*/*Inermia*) reported to have a greater proportion (Fig. 4.7). Furthermore, to my knowledge, intercepts as high as those obtained from the ARC analyses have not been reported before. Such a high proportion of sympatric distributions among sister-species suggests that sympatric speciation may have played an important role in the evolutionary history of the genus. Similarly, the low proportion of *Pomacanthus* sister-species pairs that showed zero range overlap suggests that sympatric speciation could account for 70–100% of speciation events within the genus. This estimate is based on a simulation study where varied levels of sympatric speciation and parameters were used to investigate the relationship between range overlap and species age (Phillimore *et al.*, 2008). The study found that even when all simulated speciation was sympatric, a considerable proportion (approximately 20%) of sister-species still showed zero range overlap. Therefore, the large proportion of recent splits that exhibit patterns consistent with models of sympatric speciation suggest that this mode of speciation may be common within *Pomacanthus*.

Despite the extent of range overlap among sister-species, it is not possible to discount the notion that allopatric speciation followed by range changes could have produced the same results. As with most coral reef fishes, *Pomacanthus* species have great potential for dispersal. Although highly site-attached as adults, they are broadcast spawners whose larvae spend extended periods of time in the plankton (ranging from 17 to 24 days for Indo-Pacific species; Thresher & Brothers, 1985) with the ability to swim considerable distances (Stobutzki & Bellwood, 1997), which clearly demonstrates the potential for shifting ranges. Furthermore, large-scale historical biogeographical range shifts among coral reef fishes and other marine organisms are well documented (Renema *et al.*, 2008; Cowman & Bellwood, 2013a). Contemporary geographical ranges can, however, be informative with respect to the past if range shifts occur in such a way that spatial relationships are conserved (Fitzpatrick & Turelli, 2006).

Evidence from mammals, given their more complete fossil record, suggests the relative position of contemporary geographical ranges may provide some information about the geographical mode of speciation, even though ranges have shifted. Specifically, Fitzpatrick & Turelli (2006) found that 97% of the 62 co-occurring congeneric species they examined in the

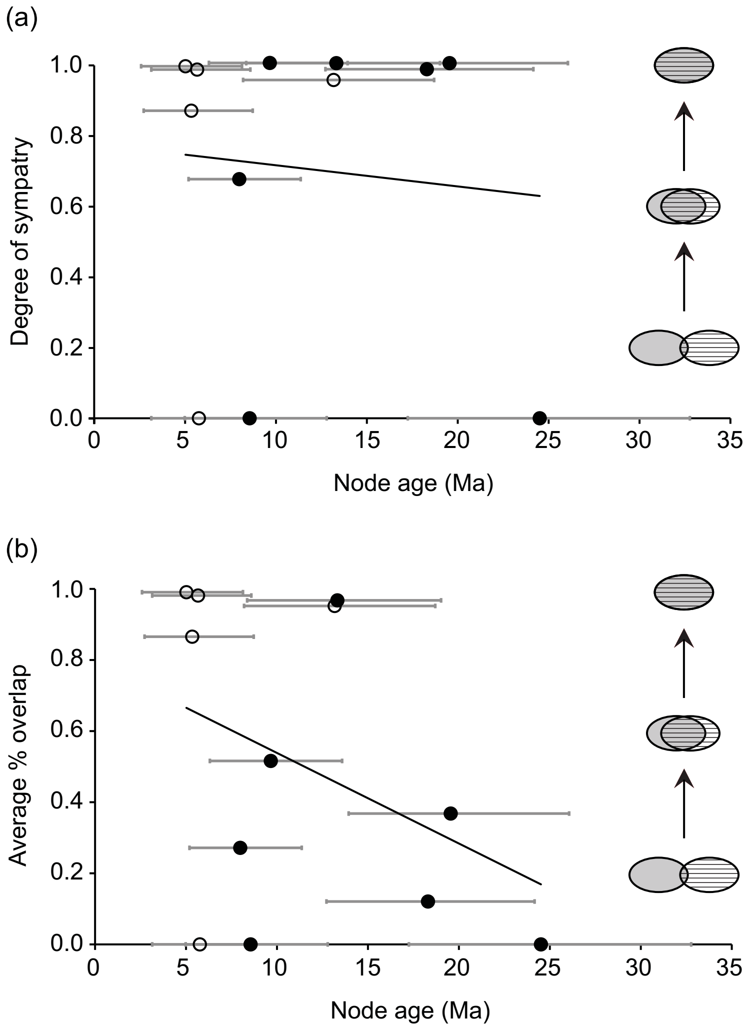

Figure 4.5 Plots of the degree of sympatry versus node age in the *Pomacanthus* phylogeny. Grey bars represent the 95% highest posterior densities of node ages. Open circles denote sister-species pairs. Diagrams to the right of each plot represent the gradient of range overlap along the y-axis. (a) Filled circles mark nodes of higher clades where geographical ranges were obtained by summing the area where at least one constituent species is found. (b) Filled circles mark nodes of higher clades where independent comparisons, or nested averages of pairwise overlap between species in each clade were used as an alternative to the union method above.

fossil record also show range overlap today. If there has been long-term maintenance of sympatry within *Pomacanthus*, this would explain the non-significant relationship between range overlap and species age. Unfortunately, it is not possible to substantiate whether or not spatial relationships have been conserved through time for *Pomacanthus* species, or coral reef fishes in general, due to the scarcity of appropriate fossils. However, all the evidence presented herein suggests that sympatry is a common and sustained feature in pomacanthids.

I found no species, sympatric or otherwise, substantially younger than 4 Myr, with the youngest species pair – *P. paru* and *P. arcuatus* – estimated to have diverged 5 Ma (95% HPD: 2.6–8.1 Ma). Indeed, there are few species pairs of coral reef fishes younger than 4 Ma (but see Bellwood *et al.*, 2010; Cowman & Bellwood, 2011; Choat *et al.*, 2012 for exceptions), making it potentially difficult to quantify the amount of time required for sister-species to acquire sympatric distributions. Furthermore, in contrast to the findings of Quenouille *et al.* (2011), no sharp transition from sister-species in allopatry to those in sympatry was detected in the more recent evolutionary history of *Pomacanthus*. The results do not, however, disagree with the suggestion made by Malay & Paulay (2010) in their study of speciation in hermit crabs, that a substantial amount of time (> 2 Ma) may be required for sister-species to develop sympatric distributions following allopatric divergence.

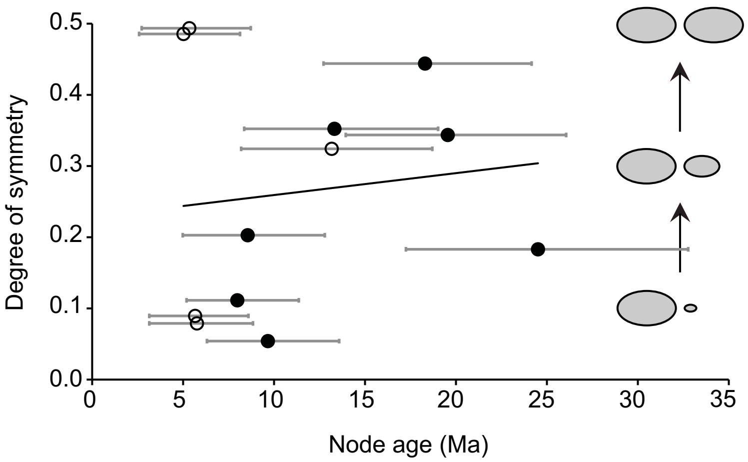

Figure 4.6 Plot of the degree of symmetry versus node age in the *Pomacanthus* phylogeny. Grey bars represent the 95% highest posterior densities of node ages. Open circles denote sister-species pairs. Diagrams to the right of each plot represent the gradient of symmetry along the y-axis. Filled circles mark nodes of higher clades where geographical ranges were obtained by summing the area where at least one constituent species is found.

Ecological knowledge can often inform the relative likelihood of sympatric speciation within a group of taxa (reviewed by Rocha & Bowen, 2008; Puebla, 2009). The ecological data currently available for *Pomacanthus* species provide little information on how processes of sympatric speciation may have taken place, although more detailed data may yet reveal ecological differentiation.

Overall, the best evidence obtained for sympatric speciation was the high proportion of sister-species pairs with substantially overlapping contemporary geographical distributions. Although this could be due to secondary contact as a result of shifting or expanding ranges, or simply the increased survival of taxa whose ranges encompass the IAA and thus accumulate in the IAA (Cowman & Bellwood, 2013a), the extent of overlap among sister-species pairs detected herein is comparable with models of high levels of sympatric speciation. The results therefore lend support to the possible importance of sympatric speciation in the evolutionary history of *Pomacanthus*.

Peripatry

The intercept and slope of the analysis of range-size symmetry are consistent with simulations of peripatric speciation within a finite area (Barraclough & Vogler, 2000), suggesting that peripatry may also be an important mode of speciation within *Pomacanthus*. This is particularly apparent when focusing on the topological relationships among species in the West Indian Ocean clade. *Pomacanthus semicirculatus* is the only widespread species with a distribution spanning the Indo-Pacific; all of the other Indian Ocean species have fairly restricted distributions centred at varying locations along the east coast of Africa. Peripatric speciation may have dominated in this African clade in the form of peripheral budding (*sensu* Chapter 3). Under this scenario, a relatively widespread *P. semicirculatus* would have given rise to several peripherally isolated species through peripatry, parapatry or sympatry. Maintaining the order of lineage divergence observed within the clade, these species would have been successively isolated along the east coast of Africa (with initial isolation of *Pomacanthus asfur*, then *P. rhomboides*/*P. maculosus*, and later *P. chrysurus*). If this occurred primarily through founder events, subsequent range expansion must have led to contemporary sympatry among all constituent species.

Evidence for peripheral budding has been reported for other marine organisms (Pandolfi, 1992; Kirkendale & Meyer, 2004; Budd & Pandolfi, 2010; Malay & Paulay, 2010; Winters *et al.*, 2010; Chapter 3) and is likely to be more common than previously recognized. Unfortunately, it can confound many of the inferences made from phylogenetic hypotheses, including topological relationships among species and age estimation (Chapter 3). Therefore, the consideration of all possible speciation scenarios when interpreting phylogenetic hypotheses is advised, especially when the hypotheses are then applied to other questions and used in analyses such as ARC.

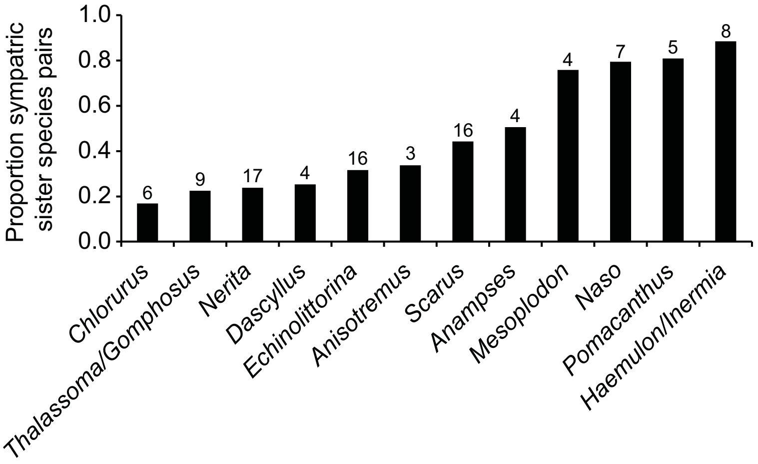

Figure 4.7 Proportion of sympatric sister-species among marine taxa for which there exists a species-level phylogeny with near complete taxon sampling (> 85%) and more than two sister-species pairs. Sympatry is defined here as > 10% overlap. Numbers above bars indicate the total number of sister-species pairs within each taxon. Phylogenies were obtained from the following studies: *Chlorurus*, Choat *et al.* (2012); *Thalassoma*/*Gomphosus*, Bernardi *et al.* (2004); *Nerita*, Frey (2010); *Dascyllus*, McCafferty *et al.* (2002); *Echinolittorina*, Williams & Reid (2004); *Anisotremus*, Bernardi *et al.* (2008); *Scarus*, Choat *et al.* (2012); *Anampses*, Chapter 3; *Mesoplodon*, Dalebout *et al.* (2008); *Naso*, Klanten *et al.* (2004); *Pomacanthus*, this study; *Haemulon*/*Inermia*, Rocha *et al.* (2008).

4.5 Conclusions

This study explored potential patterns of speciation among all 13 *Pomacanthus* species by comparing extant geographical distributions in the context of a fossil-calibrated phylogenetic hypothesis. The lack of a direct relationship between the degree of sympatry and node age can be explained by the likelihood of multiple speciation modes operating throughout the evolutionary history of this genus. In order for a significant relationship between range overlap and node age to be detected, there must have been a single predominant geographical mode of speciation in the data set (Fitzpatrick & Turelli, 2006). Furthermore, if sympatric speciation has been the dominant mode and *Pomacanthus* species have maintained sympatric distributions over long periods of time, this would also explain the lack of a relationship between range overlap and node age. Nevertheless, I found support for all three major modes of speciation and evidence of a lengthy and evolutionarily complex history in pomacanthid fishes.

Chapter 5: On the relationship between species age and geographical range in reef fishes: are widespread species older than they seem?

In press *Global Ecology and Biogeography* (2014)

5.1 Introduction

Identifying the evolutionary dynamics of species' geographical ranges is a central goal of macroecological studies. Three processes shape geographical ranges: speciation, range-size transformation (which can be influenced by factors such as dispersal ability and habitat connectivity), and extinction (Gaston, 1998). The resultant relationship between the age of a species and its geographical range depends on the probability that species with different range sizes will go extinct or speciate, and the way in which ancestral geographical ranges are partitioned between daughter species (Webb & Gaston, 2000). Four basic theoretical models describe how geographical ranges may change through time. The 'age and area' model predicts constant geographical range expansion over time, producing larger ranges with increased evolutionary age (Willis, 1922; Hubbell, 2001). The 'stasis' or 'post-expansion stasis' model states that species achieve their maximum geographical ranges rapidly and maintain a uniform trajectory thereafter (Jablonski, 1987; Gaston, 1998; Gaston, 2003). Both of these models describe instantaneous range crashes corresponding to the geological timing of species' extinctions.

In contrast, the 'taxon cycle' model predicts that species will expand their initially restricted ranges to become maximally dispersed for a period of time until ranges begin to decline towards extinction or the beginning of a new cycle (Wilson, 1961; Ricklefs & Cox, 1972; Ricklefs & Bermingham, 2002). The 'taxon cycle' model can produce symmetrical (cf. Foote,

2007) or asymmetrical trajectories (cf. Webb & Gaston, 2000) depending upon the rate of range expansion relative to range decline. All three of the aforementioned models predict initial geographical range expansion, but discriminating between them is difficult because factors such as extinction and sampling bias can influence the likelihood of detecting newly diverged or near-extinct species and their respective geographical ranges (Pigot *et al.*, 2012). Biologically, the ‘taxon cycle’ model is arguably the most logical because species with smaller distributions are generally at greater risk of extinction (Jablonski & Hunt, 2006). However, there need not be a general pattern in the evolution of species’ geographical ranges through time. A fourth theoretical model, the ‘idiosyncratic’ model proposes that species exhibit individual trajectories, producing limited similarity in the range sizes of closely related species and the potential for no over-arching pattern in range size evolution through time among a given group of taxa (Gaston, 2003).

Studies from a wide variety of groups have shown a positive relationship between species age and geographical range size including, aquatic beetles (Abellan & Ribera, 2011), bats (Weber *et al.*, 2014), birds (Webb & Gaston, 2000; Böhning-Gaese *et al.*, 2006), frogs (Wollenberg *et al.*, 2011), minnows (Taylor & Gotelli, 1994), marine molluscs (Jablonski, 1987; Miller, 1997), and tropical plants (Paul & Tonsor, 2008; Paul *et al.*, 2009). However, the relationship between species age and range size is often variable and can be clade specific (Webb & Gaston, 2000). Studies on large, diverse taxonomic groups such as New World bird species (Gaston & Blackburn, 1997), mammals (Jones *et al.*, 2005), and coral reef fishes (Mora *et al.*, 2012) found no consistent relationship between species age and geographical range.

To examine how species age relates to geographical range it is essential to have accurate estimates of both parameters. While there has been much recent success in phylogenetic age estimation techniques, conceptual synthesis of the inherent problems with these techniques has been limited. For example, the use of divergence time estimates, obtained from the nodes of a temporally-calibrated phylogeny (or relative node position e.g. Taylor & Gotelli, 1994), as an indication of species’ ages is common, but beyond examining uncertainty in the tree or calibrations, few other issues have been examined. Here I address the effects of phylogenetic age estimation on the relationship between species age and geographical range. I argue that estimates of divergence time are not always indicative of species age, and if considered as such, they may mask any underlying relationship between species age and geographical range. I highlight an example of this using coral reef fishes and explore the insights these methods bring to light for models of reef fish geographical range evolution.

5.2 Phylogenetic Methods of Age Estimation

Zuckerkandl and Pauling (1965) first proposed the idea of dating evolutionary divergences using calibrated differences among proteins. Their idea was based on evidence that sequence differences between proteins among species varied as a function of the time since their evolutionary separation. This central idea gave way to the development of molecular clock methods for time-calibrating phylogenetic hypotheses (reviewed by Kumar, 2005). Since their development, phylogenetic methods of age estimation have been increasingly applied to a myriad of studies as a means of investigating the general mechanisms and processes of evolution. In general, these methods involve converting the pairwise percentage of sequence divergence between two species (or clades) into an estimate of the time since the most recent divergence (Nei, 1987), hereafter referred to as divergence time. This provides a divergence time estimate (relative or absolute, depending on the methods used to calibrate the tree) for every node in the phylogeny, which represents the most recent common ancestor (MRCA) between two taxa. To examine the relationship between species age and geographical range, divergence times for all nodes in the phylogeny subtended by extant species are typically considered to reflect species age (cf. Taylor & Gotelli, 1994; Gaston & Blackburn, 1997; Webb & Gaston, 2000; Böhning-Gaese *et al.*, 2006; Paul & Tonsor, 2008; Paul *et al.*, 2009; Abellan & Ribera, 2011; Mora *et al.*, 2012; Weber *et al.*, 2014). Therefore, divergence times that are shared between two subtending sister-species (defined by their close genetic relationship) are assigned to both daughter species and plotted against each of their corresponding geographical ranges (Fig. 5.1a, b). To illustrate how extinction and certain models of diversification can bias the potential relationship between species age and geographical range using these methods, and how to account for the potential biases, I consider an example using coral reef fishes.

5.3 Quantifying Species Age in Reef Fishes

The traditional phylogenetic approach

A comprehensive genetic dataset of coral reef fish genera with near-complete sampling of described species was constructed and combined with available fossil data to simultaneously infer the phylogeny and estimate divergence times of species from four of the major coral reef fish families. Sequence data were obtained from GenBank for those coral reef fish families with distribution maps available from the IUCN Red List spatial database (IUCN, 2011). Molecular data were assessed and selected to maximize coverage across all fish families, to maximize taxon sampling within genera, and to minimize the amount of missing sequence data in the alignment (see **Chapter 2: General Methods**, section **2.2 Multi-Family Phylogeny** for specific selection criteria and potential limitations). This resulted in the inclusion of four mitochondrial loci (16S rRNA, 12S rRNA, CO1, cytochrome b), and two nuclear loci (TMO-4C4, S7 intron 1) for 53 genera within four coral reef fish families: Chaetodontidae, Labridae, Pomacanthidae, and Epinephelidae (Table S2.3, Appendix A). Sequences were also included for additional taxa to root the phylogeny and provide nodes for fossil calibration. A time-calibrated phylogeny was constructed based on six independent partitioned Bayesian analyses in BEAST v1.7.5 (Drummond & Rambaut, 2007) using nine fossil calibrations (Table S2.4, Appendix A). Log files showed high effective sample sizes (posterior ESS values > 200 for six combined analyses), which indicated valid estimates based on independent samples from the posterior distribution of the Markov chain. The maximum clade credibility chronogram was compiled from 48,003 post-burn-in trees (48,003,000 generations) (Table S5.1 and Fig. S5.1, Appendix D). Biogeographical data were also compiled and used to quantify species'

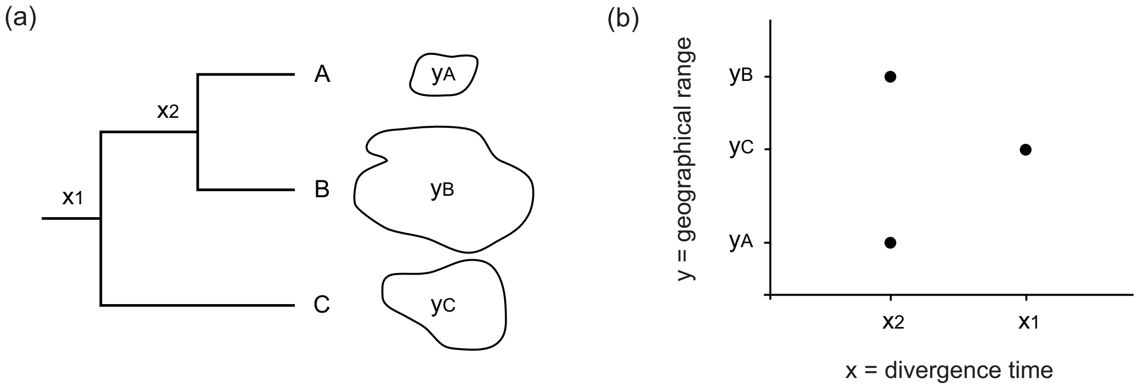

Figure 5.1 (a) Methods for time-calibrating nodes from a phylogeny using molecular data involve converting the pairwise percentage of sequence divergence between two species (A and B) into an estimate of the time since the most recent divergence. This is done the same way for deeper nodes in the phylogeny, except the mean of the pairwise divergence between A and C, and between B and C is used. These methods provide a divergence time estimate (x_2 and x_1) for every node in the phylogeny, which represents the most recent common ancestor between two taxa. (b) The relationship between species age and geographical range is typically examined by considering the estimated divergence times for all nodes in the phylogeny subtended by extant species as representative of species age. Thus, divergence times that are shared among two subtending sister-species are assigned to both daughter species and plotted against each of their corresponding geographical ranges as (x_2, y_A), (x_2, y_B) and (x_1, y_C).

geographical range areas following the general methods outlined in **Chapter 2**, section **2.2 Multi-Family Phylogeny**.

First, divergence time estimates for all nodes in the phylogeny subtended by extant species were considered as an indication of species age (as described in Fig. 5.1a, b). I tested the theoretical ‘age-area’ model of geographical range evolution using linear regression and found no significant linear relationship between divergence time and geographical range for species included in the phylogenetic analysis ($r^2 = 0.00789$; $n = 293$; d.f. = 1, 291; $F = 2.31$; $P = 0.129$) (Fig. 5.2). The ‘stasis/stasis post-expansion’ and ‘taxon cycle’ models predict species’ range expansion from initially restricted ranges in a curvilinear manner. To test these curvilinear theoretical models I fitted a quadratic model to our reef fish data, but found no evidence for a cyclical form of range evolution ($r^2 = 0.00942$; $n = 293$; d.f. = 2, 290; $F = 1.38$; $P = 0.254$). The majority of reef fish species sampled were estimated to have diverged ~3.3 Ma (mean = 5.4 Ma) during the Pliocene. The oldest extant lineage was 59 Ma.

Phylogenetic methods are only able to estimate the age of the MRCA between two species when data for both species are present in the analysis. Therefore, taxa missing from the phylogeny, whether extinct or extant, will cause the ages of sister taxa to be over-estimated (Fig. 5.3). If the rate of extinction through time has remained relatively constant, then older lineages are more susceptible to extinction (Rabosky & Lovette, 2008). Furthermore, the potential degree of divergence time overestimation increases with branch length (i.e. the degree of divergence time overestimation is potentially greater for species D compared with species B in Fig. 5.3, given extinct species *z* and *x*, because species D has a longer branch and therefore a greater range of time across which it could have shared a common ancestor with species *z*). It is worth noting that if an extinct (or missing extant) species is not sister to a single extant species

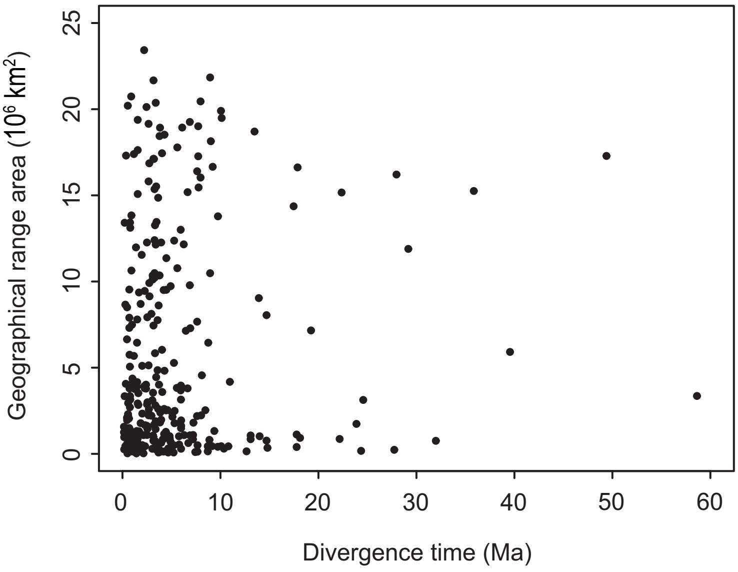

Figure 5.2 Relationship between species age and geographical range plotted as described in Figure 5.1, where the estimated divergence times for all nodes in the phylogeny subtended by extant species are considered as species' ages. This analysis included 293 reef fish ingroup species from four families: Chaetodontidae, Labridae, Pomacanthidae, and Epinephelidae (Table S2.3, Appendix A). Mean estimated species age was 5.4 Ma; modal species age was 3.3 Ma; and the range in species age was 58.5 Ma.

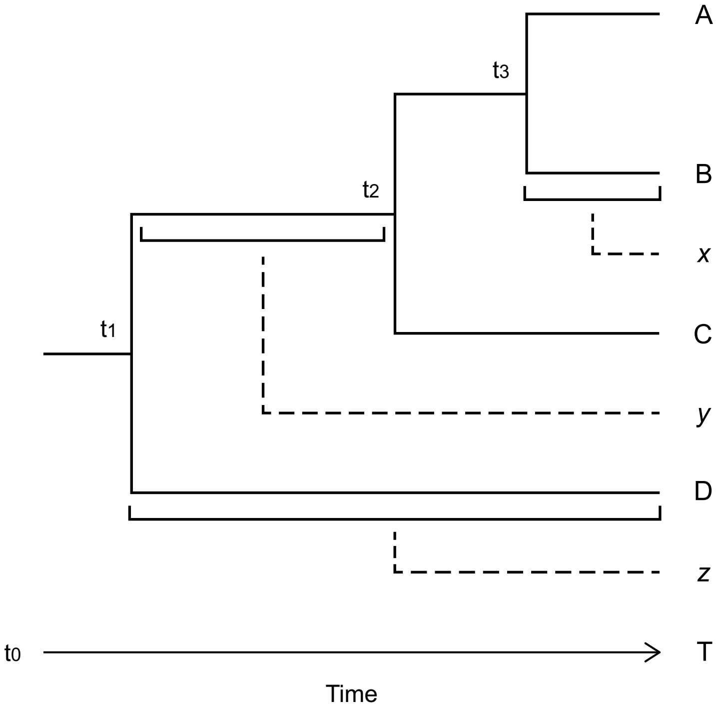

Figure 5.3 Effects of extinction, or missing extant taxa, on phylogenetic estimates of divergence time. The letters A, B, C, and D subtending lineages with solid lines denote sampled extant species. Extinct, or missing extant species, are denoted by *x*, *y*, and *z* subtending dashed lineages. Excluding species from the phylogeny, whether extinct or extant, will cause the ages of sister-species to be overestimated. For example, species *x* is sister to B, but missing from the phylogeny. Therefore, the divergence time estimated for species B (t_3) is older than it would otherwise be if *x* had been included. Assuming a relatively constant rate of extinction, older lineages are more susceptible to effects of extinction. Furthermore, older lineages have greater potential range for overestimation of divergence times compared to more recent lineages, simply because they have longer branches. For example, there is a greater range of time for missing species *z* to have diverged from D then there is for missing species *x* to have diverged from B. Thus, it is likely that the estimated divergence time obtained for species D (t_1) will be more greatly overestimated than that of B (t_3), given the existence of extinct or un-sampled species. If an extinct or missing species is not sister to a single extant species, as in missing species *y*, this results in a missing data point when examining the relationship between divergence time and geographical range, rather than an overestimation of divergence time.

this results in a missing data point when examining the relationship between divergence time and geographical range (e.g. extinct species *y* in Fig. 5.3), rather than an overestimation of divergence time.

The impacts of extinction on the estimation of divergence times have been reviewed previously (Harvey *et al.*, 1994; Ricklefs, 2007), and approaches to minimize these impacts have provided valuable insights into processes associated with divergence, such as rates of phenotypic change (Seddon *et al.*, 2013). One way to avoid these problems is by focusing on sister-species. When robust sampling of extant species is achieved, sister-species relationships can be reconstructed with confidence. Focusing on recent divergences between pairs of sister-species minimizes the impact of extinct taxa on divergence time estimation, although it may not be eliminated entirely. This technique narrows the focus of the question to address how geographical ranges have evolved among the most recently diverged species. The impact of missing extant taxa on estimates of divergence time will thus depend on the shape of the phylogeny and sampling bias; and a sister-species approach will eliminate some of the impact that missing extant taxa may have on estimates of divergence time.

A sister-species approach

Using the phylogeny of reef fishes, I considered divergence time estimates for all sister-species pairs, excluding monotypic genera, as indicators of species age. This approach minimizes the effects missing extant taxa may have on species age estimation, but additional steps are required to prevent the pseudoreplication of divergence time estimates among sister-species (as in Fig. 5.1a, b). To overcome this issue I first took the average of each sister-pair's geographical range and compared it with their estimated divergence time. Average geographical range increased linearly with increasing species age ($r^2 = 0.0481$; $n = 91$; d.f. = 1, 89; $F = 4.50$; $P = 0.0368$) (Fig. 5.4). The relationship can also be described using a curvilinear model ($r^2 = 0.0713$; $n = 91$; d.f. = 2, 88; $F = 3.38$; $P = 0.0386$). However, model comparison using ANOVA showed that the curvilinear model was not a significant improvement upon the linear model ($n = 91$; d.f. = 2, 88; $F = 2.20$; $P = 0.142$).

Another way to overcome the pseudoreplication of divergence time estimates among sister-species is to randomly assign one of two potential geographical ranges to each sister-species' divergence time estimate. The advantages of this approach are that it eliminates the problem of pseudoreplication of divergence time estimates, and it selects geographical range data from only one of the two sister-species without making any prior assumptions of the models under which species have diversified. In addition, when the randomisation approach is repeated, it can illustrate the likelihood, or percentage, of a significant relationship. I performed 1000 bootstrap replicates of geographical range randomisation; for each replicate I fitted linear and curvilinear models to the data. A significant linear relationship between species age and geographical range was detected in 13% of the randomised replicates (the range of statistical values across all 130 significant replicates: $r^2 = 0.111$–0.0426; $n = 91$; d.f. = 1, 89; $F = 11.12$–3.96; $P = 0.00125$–0.0497; plot not shown, see the vertical grey bars in Fig. 5.4 for the differences in sister-species' geographical ranges).

The relationship between species age and geographical range could also be described by a curvilinear model in 29% of the randomised replicates (the range of statistical values across all 290 significant replicates: $r^2 = 0.157$–0.0495; $n = 91$; d.f. = 2, 88; $F = 8.17$–2.29; $P = 0.000885$–0.0499). However, model comparison performed using ANOVA revealed that that the curvilinear model was only a significant improvement over the linear model 4.8% of the time (the range of statistical values across all 48 significant replicates: $n = 91$; d.f. = 2, 88; $F = 7.94$–3.96; $P = 0.00598$–0.0498). These applications of a sister-species approach help to overcome some of the problems associated with estimating species' ages from phylogenetic hypotheses and they have unveiled the potential for a positive relationship between species age and geographical range in reef fishes.

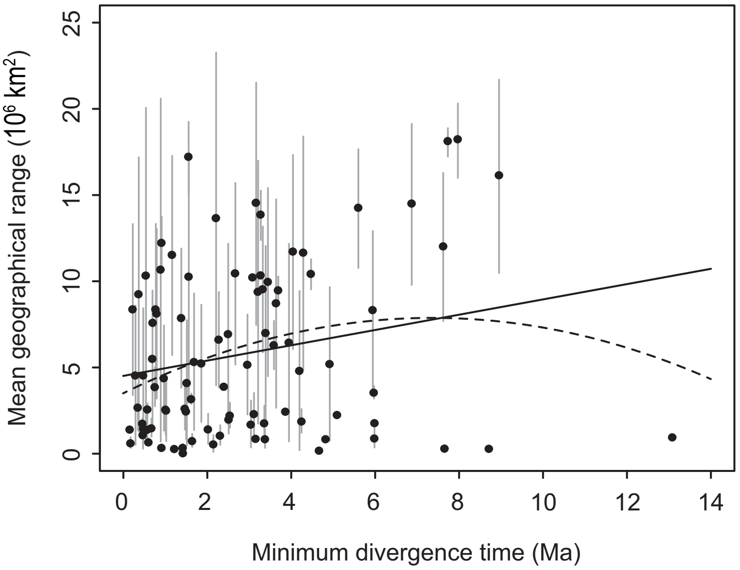

Figure 5.4 Minimum divergence times are used as indicators of species age for all sister-species pairs, excluding monotypic genera, and plotted against the pairs' mean geographical range area. Grey vertical bars show the difference in geographical range areas between sister-pairs. Mean geographical range area increases linearly with increasing species age ($r^2 = 0.0481$; $n = 91$; d.f. = 1, 89; $F = 4.50$; $P = 0.0368$). The relationship can also be described by a curvilinear model ($r^2 = 0.0713$; $n = 91$; d.f. = 2, 88; $F = 3.38$; $P = 0.0386$); however this model was not a significant improvement upon the linear model following model comparison in ANOVA ($n = 91$; d.f. = 2, 88; $F = 2.20$; $P = 0.142$).

However, even when utilizing a sister-species approach it is important to consider the spatial component of speciation when evaluating divergence time estimates in a phylogenetic hypothesis, as certain models of diversification can confound them. For instance, under some models of peripatric speciation such as peripheral budding (Chapter 3), a centrally distributed, widespread species successively gives rise to multiple species along the periphery of its geographical range (Fig. 5.5a). As a result, topologically the widespread species will appear to be sister to the most recently diverged (budded) species and its age will thus be grossly underestimated (Fig. 5.5b). Evidence for this mode of diversification has been reported for marine (Pandolfi, 1992; Kirkendale & Meyer, 2004; Budd & Pandolfi, 2010; Malay & Paulay, 2010) and terrestrial taxa (Hoskin *et al.*, 2011; Garrigan *et al.*, 2012; Toussaint *et al.*, 2013). If this is a common occurrence in the diversification of coral reef fishes, as increasing evidence suggests (Winters *et al.*, 2010; Fitzpatrick *et al.*, 2011; Choat *et al.*, 2012; Chapters 3, 4), it would mask any relationship between species age and geographical range when analysed using age estimates for all extant species in the phylogeny. It may also mask the relationship when analysed using a sister-species approach, depending on the methods used to assign geographical ranges to species age estimates. If we consider this potential bias in the light of the initial results of this relationship for reef fishes, it may help explain the presence of numerous species with relatively young ages and large geographical ranges (Fig. 5.2). These large-range species may appear to be young only because they have recently budded a peripheral species. They may be much older.

Alternatively, young species may be able to attain large ranges given the considerable potential for larval dispersal of most coral reef fish species (Strathmann *et al.*, 2002). For example, young species with restricted distributions would have the potential to expand their geographical ranges into areas of suitable habitat over several successive generations. However, many recent studies suggest that the level of self-recruitment among coral reef fish populations is much higher than expected given this dispersal potential (Swearer *et al.*, 2002; Bowen *et al.*, 2006a; Priest *et al.*, 2012). Thus, although fishes have great dispersal potential it may not be consistently realized and dispersal alone may not be substantial enough to decouple any prospective relationship between species age and geographical range area.

Comparing minimum divergence time with minimum geographical range

To overcome the potential problem of underestimating the divergence times of widespread species that have successively given rise to multiple species through time, I suggest using a sister-species approach and considering the estimated minimum divergence time as an indication of species age only for the species with the smaller of the two geographical ranges. This method therefore examines the relationship between the minimum divergence time (species age) and the minimum geographical range area. This approach also eliminates the

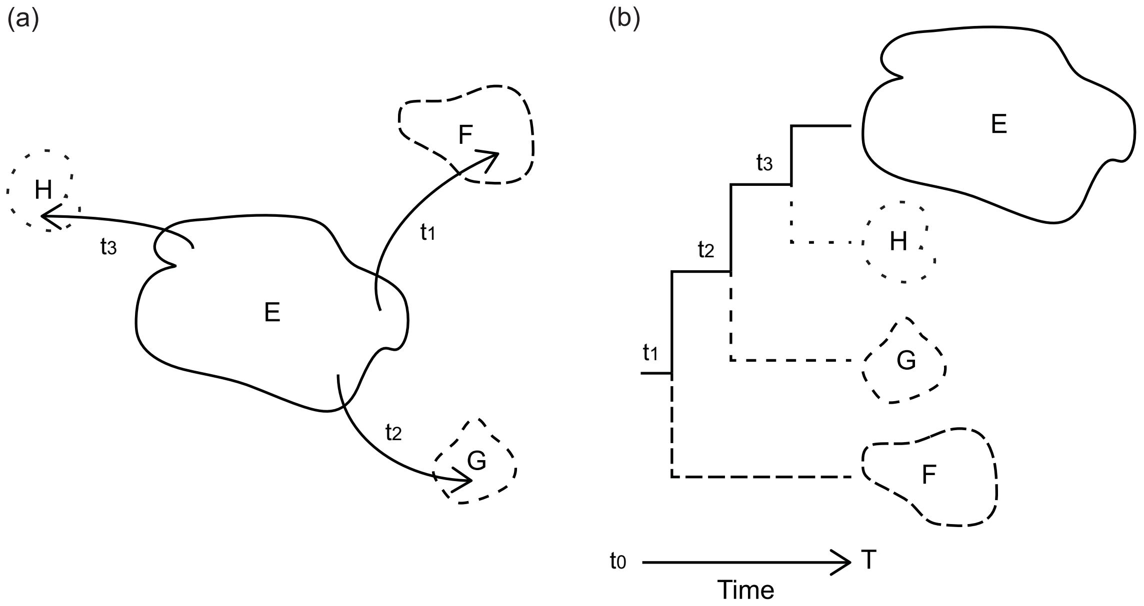

Figure 5.5 (a) Illustrates the peripheral budding model (*sensu* Chapter 3), also known as ancestral persistence. A centrally distributed, widespread species E successively gives rise to multiple species along the periphery of its geographical range from time t_0 to time t_3. (b) The topological relationships resolved from a molecular phylogeny of species that have diverged under the peripheral budding model. Lineage dashing corresponds to range outline in part (a). Species E and H are sister because they diverged most recently and are molecularly most similar. However, the successive peripheral splitting, or budding, of species causes the divergence time for species E to be grossly underestimated as t_3 rather than t_0. Thus masking any potential relationship between species age and geographical range when analysed using the methods described in Figure 5.1. This can be overcome by using a sister-species approach and considering the estimated minimum divergence time as indicative of species age only for the species with the smaller of the two distributions (e.g. plot t_3 against the geographical range size of species H). This method examines the relationship between minimum divergence time and minimum geographical range area. This approach also eliminates the problem of pseudoreplication of species' ages where a single (shared) age is plotted against multiple geographical range areas (as in Fig. 5.1b).

problem of pseudoreplication of species' ages where a single (shared) age is plotted against multiple geographical range areas (as in Fig. 5.1a, b).

I compared species age with minimum geographical range area to illustrate the potential impact successive peripheral speciation, or peripheral budding, may have on the relationship between these two variables for reef fishes. A significant positive linear relationship between species age and minimum geographical range areas was found using this approach ($r^2 = 0.104$; $n = 91$; d.f. = 1, 89; $F = 10.35$; $P < 0.01$) (Fig. 5.6). While the model still has fairly low predictive power, it is one of the strongest linear relationships obtained overall (only one out of the 1000 randomised replicates had a higher r^2 value). Furthermore, the difference in the trend recovered after excluding potential biases associated with ancestral persistence suggests that ancestral persistence may be prevalent among coral reef fishes, with successive peripheral speciation events impacting area-age relationships.

Minimum geographical range area also increased in a curvilinear fashion with increasing species age ($r^2 = 0.124$; $n = 91$; d.f. = 2, 88; $F = 6.25$; $P = 0.00289$; Fig. 5.6). However, the results of model comparison using ANOVA showed that the curvilinear model was not a significant improvement upon the linear model ($n = 91$; d.f. = 2, 88; $F = 2.03$; $P = 0.157$). The task of discriminating between theoretical models of range evolution remains difficult; however, the results for reef fishes suggest that the ‘age-area’ model may sufficiently describe the early range expansion dynamics of species.

Because both variables, geographical range area and species’ age, were positively skewed all of the preceding analyses were repeated on log-transformed data. A significant log-log linear relationship would suggest that as species age, their geographical ranges increase exponentially. Such a pattern may be consistent with the ‘stasis post-expansion’ or ‘taxon cycle’ theoretical models of geographical range evolution if range expansion occurs rapidly. No significant linear relationships were detected between the transformed variables in the preceding analyses, except for the randomisation approach, for which there was a significant log-log relationship between geographical range and species age in 9.3% of replicates (the range of statistical values across all 93 significant replicates: $r^2 = 0.0993$–0.0427; $n = 91$; d.f. = 1, 89; $F = 9.82$–3.97; $P = 0.00234$–0.0495) (Fig. S5.2c, Appendix D). The statistical results of all of the log-transformed analyses are reported and discussed in Appendix D.

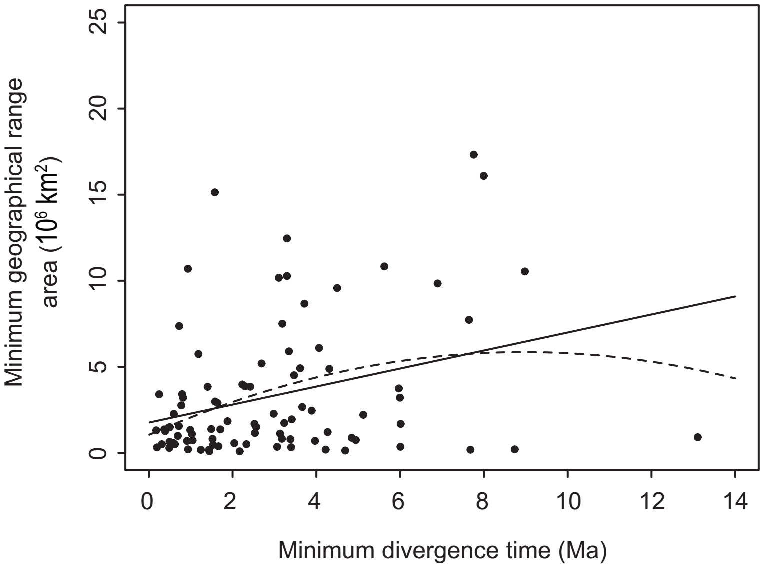

Figure 5.6 A sister-species approach where minimum divergence time (as an indication of species age) is plotted against minimum geographical range area (i.e. the smaller of two geographical range areas for all sister-species pairs, excluding monotypic genera). As minimum divergence time increases, minimum geographical range area also tends to increase. The relationship can be described by a linear model (r^2 = 0.104; n = 91; d.f. = 1, 89; F = 10.35; P = 0.00181). While the correlation is not strong, it is the strongest linear relationship I obtained overall. The relationship can also be described by a curvilinear model (r^2 = 0.124; n = 91; d.f. = 2, 88; F = 6.25; P = 0.00289); however, this model was not a significant improvement upon the linear model according to our ANOVA model comparison (n = 91; d.f. = 2, 88; F = 2.03; P = 0.157). Because both variables, geographical range area and species' age, were positively skewed I repeated all of the preceding analyses on log-transformed data. The statistical results of the log-transformed analyses are reported in Appendix D.

Species as sampling units are non-independent because they share an evolutionary history. Therefore, the relationship between species age and geographical range recovered from the sister-species approaches could be an artefact of the phylogenetic relationships among species. Phylogenetically independent contrasts (PICs) were used to evaluate the consistency of this relationship when accounting for the phylogenetic topology (Felsenstein, 1985). For variables, species age and geographical range area, I optimised an OU model of character evolution and performed linear regression analyses using PICs in R (R Development Core Team, 2011; packages "geiger" and "ape"). Only the approaches that recovered previous significant results were tested. PICs for the randomisation approach were calculated using the average geographical range across all 1000 randomisation replicates. For this analysis, averaging across bootstrapped replicates approximates the sister-species approach where geographical ranges were averaged for each sister-pair. Thus, the two approaches produced similar results. When tested for consistency using PICs the significant relationships between species age and geographical range were not recovered (mean geographical range: $r^2 = 0.0252$; $n = 91$; d.f. = 1, 89; $F = 2.30$; $P = 0.133$; randomised replicates: $r^2 = 0.0245$; $n = 91$; d.f. = 1, 89; $F = 2.24$; $P = 0.138$; Fig. 5.7a, b, respectively). Therefore, phylogenetic structure may explain the original relationship recovered from these two sister-species approaches (although it may also reflect a loss of power as a result of the PIC approach). In contrast, the relationship between species age and geographical range was consistent following the PIC analysis of the sister-species approach comparing species age with minimum geographical range ($r^2 = 0.0599$; $n = 91$; d.f. = 1, 89; $F = 5.67$; $P = 0.0194$; Fig. 5.8). Thus in the context of phylogenetic relationships, only the minimum geographical range area between two sister-species was significantly related with species age in our reef fish example. It is likely that detailed information on geographical range dynamics was lost through the randomisation or averaging of geographical ranges in the other two sister-species approaches.

Geographical range symmetry

To further explore the dynamics of geographical range evolution among reef fishes I investigated whether species age also effects the distribution of range size symmetry among sister-pairs. I have shown that as species age increases, on average, the minimum geographical range between sister-pairs also increases. Given this relationship, changes in range symmetry with species age can be described using three possible scenarios of range dynamics. Firstly, if the rate of range expansion were greater for the smaller-ranged species among sister-pairs, then range size symmetry would increase, on average, with increasing species age. Range

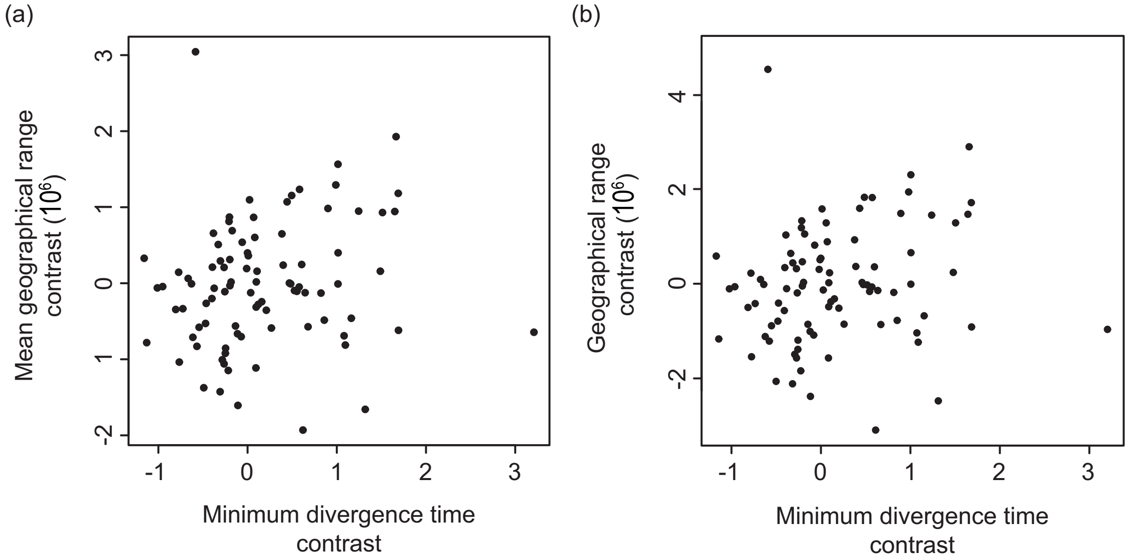

Figure 5.7 The results of phylogenetically independent contrasts (PICs), which were performed to evaluate the consistency of the relationship between species age and geographical range when accounting for the phylogenetic topology (Felsenstein, 1985). An OU model of character evolution was optimised for both variables and linear regression analyses using PICs were conducted in R (R Development Core Team, 2011; packages "geiger" and "ape"). Only approaches that recovered previous significant results were tested. (a) The PIC analysis of the average geographical range area between sister-pairs showed a non-significant relationship with species age ($r^2 = 0.0252$; $n = 91$; d.f. = 1, 89; $F = 2.30$; $P = 0.133$). (b) Similarly, the sister-species approach where geographical ranges between sister-pairs were randomised and averaged across 1000 bootstrap replicates recovered a non-significant result when the phylogeny was considered ($r^2 = 0.0245$; $n = 91$; d.f. = 1, 89; $F = 2.24$; $P = 0.138$).

contraction of the large-ranged species could also produce an increase in range symmetry with species age. Secondly, if both species in a sister-pair expand their ranges at the same rate, then minimum geographical range size would still increase with species age, but range symmetry would remain constant. Finally, if the rate of range expansion were slower for the smaller-ranged species, then range symmetry would decrease with species age. Substantial range contraction of the large-ranged species could also produce a decrease in range symmetry with species age.

Any of the three aforementioned scenarios of range dynamics may result if peripheral budding has occurred. However, peripheral speciation events would generally result in sister-pairs with asymmetrical ranges, thus one may expect asymmetry to be more prevalent among younger sister-pairs if peripheral speciation is common in reef fishes. However, the occurrence of young, asymmetric sister-pairs does not require diversification to have proceeded under the peripheral budding model, it may instead reflect single, rather than successive, peripheral speciation events.

The degree of geographical range symmetry among sister-pairs was incorporated into the linear model describing the relationship between species age and minimum geographical range area using multivariate analysis of variance (MANOVA) and Pillai's test. Symmetry was calculated following Barraclough & Vogler (2000), where the smallest geographical range area between two sister-species was divided by the sum of their geographical range areas. Symmetry values range from zero to 0.5 and higher numbers indicating greater symmetry.

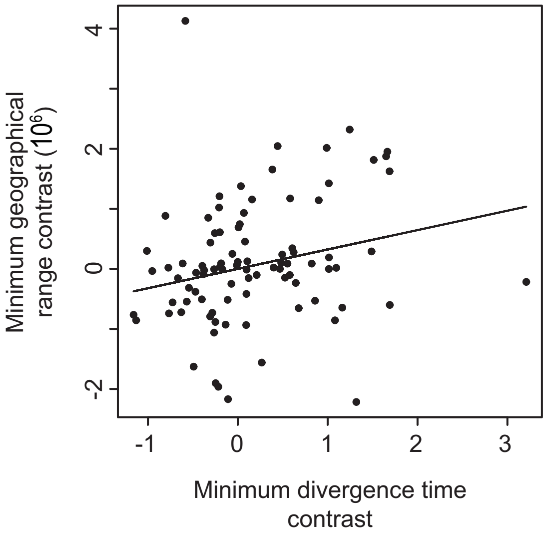

Figure 5.8 The relationship between species age and geographical range was consistent following the PIC analysis when I tested the sister-species approach comparing species age with minimum geographical range ($r^2 = 0.0599$; $n = 91$; d.f. = 1, 89; $F = 5.67$; $P = 0.0194$).

As species age increases, the minimum geographical range between sister-pairs increases and their ranges become more symmetrical (*n* = 91; d.f. = 2, 88; Pillai's statistic = 0.116; *P* = 0.00442). To visualise the relationship between species age and both dependent variables, I divided range symmetry into three categories, asymmetrical species were those with symmetry values ≤ 0.1, symmetrical species were those with symmetry values ≥ 0.4, and those species with symmetry values between 0.1 and 0.4 were defined as having medium symmetry. Symmetry categories were added to the plot of species age and minimum geographical range (Fig. 5.9). Over half of the asymmetrical species (53.8%, *n* = 13) diverged less than 2 Ma, compared to 44.4% of those with medium symmetry (*n* = 54) and 37.5% of symmetrical species (*n* = 24). Furthermore, I found a significant difference in the mean ages of asymmetrical (mean = 2.0 Ma) and symmetrical (mean = 3.7 Ma) sister-species using a Welch two-sample t-test (*n* = 36; d.f. = 34.0; *t* = 2.18; *P* = 0.0367) (Figure 5.9). This result persisted when symmetry extremes were defined at 10% (graph not shown; asymmetric mean species age = 1.9 Ma; symmetric mean species age = 3.7 Ma; *n* = 21; d.f. = 14.2, *t* = 2.2; *P* = 0.0429).

I performed univariate tests of linear (r^2 = 0.0688; *n* = 91; d.f. = 1, 89; *F* = 6.58; *P* = 0.0120; Fig. 5.10) and curvilinear models (r^2 = 0.0690; *n* = 91; d.f. = 2, 88; *F* = 3.26; *P* = 0.0431; Fig. 5.10) to visualise the relationship between species age and the degree of geographical range symmetry. The results of model comparison using ANOVA showed the curvilinear model was not a significant improvement over the linear model (*n* = 91; d.f. = 2, 88; *F* = 0.0123; *P* = 0.912).

The patterns of geographical range symmetry observed among reef fishes support peripheral speciation as a significant factor in the generation of reef fish biodiversity. The results suggest that as species age, they expand their geographical ranges in a way that produces greater symmetry between the geographical ranges of sister-pairs.

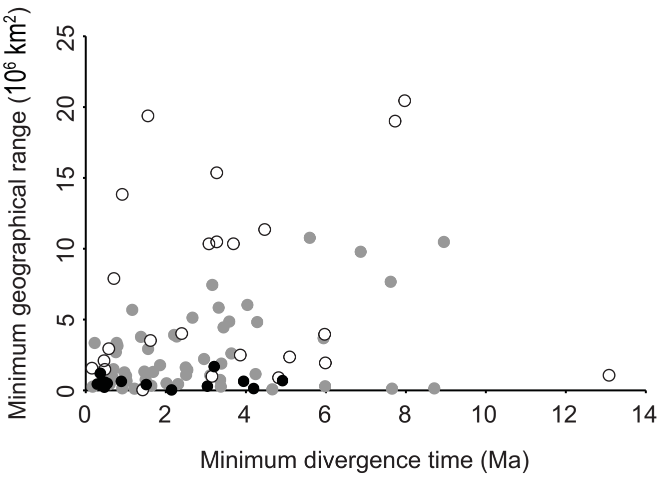

Figure 5.9 Incorporates geographical range symmetry into the model relating minimum geographical range to species age. Geographical range symmetry was calculated following Barraclough and Vogler (2000), where the smallest geographical range area between two sister-species was divided by the sum of their geographical range areas. Symmetry values range from zero to 0.5 and higher numbers indicate greater symmetry. Symmetry was divided into three categories: asymmetrical sister-species with symmetry values ≤ 0.1 (black circles), symmetrical sister-species with symmetry values ≥ 0.4 (white circles), and sister-species with symmetry values between 0.1 and 0.4 were defined as having medium symmetry (grey circles). As species age increases, the minimum geographical range between sister-pairs increases and their ranges become more symmetrical (*n* = 91; d.f. = 2, 88; Pillai's statistic = 0.116; *P* = 0.00442). Over half of the asymmetrical species (53.8%, *n* = 13) diverged less than 2 Ma, compared to 44.4% of those with medium symmetry (*n* = 54) and 37.5% of symmetrical species (*n* = 24). Furthermore, a significant difference in the mean ages of asymmetrical (mean = 2.0 Ma) and symmetrical (mean = 3.7 Ma) sister-species pairs was detected using a Welch two-sample t-test (*n* = 36; d.f. = 34.0; *t* = 2.18; *P* = 0.0367).

5.4 Applications and Implications

The method I present herein narrows the question of species' geographical range evolution to the early dynamics of expansion. Because the method focuses on recent divergences between pairs of sister-species, the detection of range decline or crash preceding extinction is less likely. Nevertheless, our sister-species approach of comparing minimum divergence time (as an indication of species' age) with the minimum geographical range area between sister-pairs is

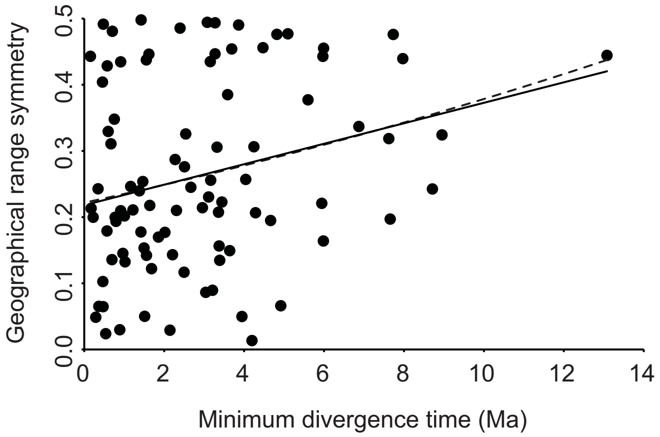

Figure 5.10 The relationship between species age and the degree of geographical range symmetry can be described by linear (r^2 = 0.0688; *n* = 91; d.f. = 1, 89; *F* = 6.58; *P* = 0.0120) and curvilinear models (r^2 = 0.0690; *n* = 91; d.f. = 2, 88; *F* = 3.26; *P* = 0.0431); however, the results of model comparison using ANOVA showed the curvilinear model was not a significant improvement over the linear model (*n* = 91; d.f. = 2, 88; *F* = 0.0123; *P* = 0.912).

applicable to any diversification scenario – it need not be used only in cases where peripheral budding or ancestral persistence is suspected. Even if species diverged in allopatry, our method simply re-phrases the question to explore the minimum geographical range area attainable by species given their age. It provides a logical means of removing the pseudoreplication of species' ages and does not require the use of averaging geographical range areas.

I advocate the use of this method as a tool to explore the dynamics of range evolution and note that its use in comparison with other approaches can potentially reveal interesting patterns. The example I presented using reef fishes was based on contemporary geographical range data. I acknowledge that the results may change given future additions of geographical range data or nominal species. All of the methods examined in the present study assume that range size transformations occur over periods of time concordant with the time-scale of the analyses. Relatively short-term, idiosyncratic, cyclical range transformations may confound the results of any phylogenetic analysis of geographical range dynamics. Comparative phylogeographic studies may be better suited to detect these range transformations over shorter time-scales.

However, successive peripheral isolation of populations or lineages within species can produce the same issues with divergence time estimation as those discussed herein. Thus, our sister-species method can be adapted into a sister-lineage method when examining range dynamics within-species or populations.

Overall, the observations on how phylogenetic age estimation can affect the relationship between species age and geographical range size will have important implications in macroecology and biogeography. The methods I present herein provide a new way of exploring the evolutionary dynamics of species' geographical ranges. By establishing methods to minimize the impacts of extinction and successive peripheral speciation on divergence time estimation, I hope to stimulate further investigation into the prevalence of this model of diversification across a broad range of taxa.

Chapter 6: Temporal evolution of coral reef fishes: global patterns and disparity in isolated locations

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6.1 Introduction

Coral reef fishes are exceptionally diverse throughout the world's tropical oceans. To understand the foundation of such high biodiversity, and to help predict the response of contemporary patterns of diversity to future environmental change, we study the distribution of organisms through space and time. Over the past 150 years, many authors have described spatial patterns of marine diversity (reviewed by Bellwood *et al.*, 2012; Briggs & Bowen, 2012; Kulbicki *et al.*, 2013). However, the molecular techniques and analytical tools to assess distributional patterns through evolutionary time have only been developed recently. These tools allow us to examine the impacts of differing geological histories on the age structure of fauna from distinct biogeographical areas, or within isolated locations.

Spatial patterns of diversity are generally described by the delineation of areas with unique biotas. Biogeographical divisions of marine environments have been based on the concentration of endemic species (Ekman, 1953; Briggs, 1974; Briggs & Bowen, 2012), shared biotic and environmental characteristics (Longhurst, 1998; Spalding *et al.*, 2007), or quantitative assessments of community composition (Floeter *et al.*, 2008; Kulbicki *et al.*, 2013). These methods have produced varying degrees of division and identified areas that often differ in boundary placement and scale. However, most workers have recognised the importance of historical isolation in separating two major biogeographical realms: the Indo-Pacific and the Atlantic. Each realm has experienced a distinctive history that has shaped its constituent fauna – from ongoing speciation as part of the expanding biodiversity hotspot that has developed in the central Indo-Pacific since the Miocene (Renema *et al.*, 2008; Cowman & Bellwood, 2013a), to substantial loss of many marine taxa in the Atlantic during a period of faunal turnover in the Plio-Pleistocene (Bellwood, 1997; O'Dea *et al.*, 2007). Given such different evolutionary histories, one may expect to find distinct realm-specific temporal patterns in the evolution of coral reef fishes. My first aim was to investigate these realm-specific patterns.

With increased taxon sampling and increasingly congruent molecular sequence data, I am also able to evaluate the predictions of the four main models of diversification (Centre of Origin, Overlap, Accumulation or Survival) used to explain the hotspot of biodiversity in the central Indo-Pacific [referred to as the Indo-Australian Archipelago (IAA) or Coral Triangle] (the ‘Centre of’ hypotheses are reviewed briefly in Palumbi, 1997; Barber & Bellwood, 2005; and in detail in Bellwood *et al.*, 2012). There are two contrasting scenarios. If the IAA were a Centre of Accumulation or Overlap of species ranges (Gaither & Rocha, 2013), one would expect to find younger species in peripheral locations outside the IAA. These hypotheses suggest that species arise in isolated, peripheral locations (such as the island arcs in the West Pacific) and remain there as endemics until they disperse to the IAA. Conversely, if the IAA were the Centre of Origin, one would expect to find the youngest species within this region. The Centre of Survival hypothesis suggests that species can arise anywhere, but they survive better in the IAA.

If species arise at the same rate across all biogeographical regions, then there should be no pattern in the distribution of young endemics. But if species arise in proportion to regional species richness, then most endemics would be located in the IAA. Consequently, the geographical distribution of young endemics can help evaluate these alternate hypotheses. My second aim therefore was to examine the extent of peripheral speciation as a mode of diversification in reef fishes. To specifically examine peripheral locations, I compared the ages of endemic species from two disparate locations with high coral reef fish endemism, the Red Sea and the Hawaiian Islands (Allen, 2008). If either location has been a significant area of recent species origination, I would expect endemic species to be younger than species in the larger adjacent regions (the Western Indian and Central Pacific regions, respectively).

To address my aims, I constructed the most comprehensive assemblage of coral reef fish genera with near-complete taxon sampling to-date, and combined it with available fossil data to simultaneously infer the phylogeny and estimate divergence times of species from four major coral reef fish families: Chaetodontidae, Labridae, Pomacanthidae and Epinephelidae. I used this phylogenetic hypothesis in conjunction with recent distributional data to investigate how the age structure of coral reef fish species varies in response to differing geological histories among marine biogeographical areas, and test the predictions of the 'Centre-of' hypotheses. My specific questions were:

1) How old are coral reef fish species and do their ages differ among major marine realms or regions?
2) Do peripheral, isolated locations of high endemism support greater numbers of young endemics; are peripheral locations a source of 'new' species?

6.2 Materials and Methods

Methods of ***Data selection and handling*** and ***Phylogenetic analysis and age estimation*** for this data chapter follow those detailed in **Chapter 2: General Methods**, section **2.2 Multi-Family Phylogeny**.

Biogeographical analysis

The recent work of Kulbicki *et al.* (2013) was used to classify species' distributions by realm: Indo-Pacific, and Atlantic; by region: Western Indian (WI), Central Indo-Pacific (CIP), Central Pacific (CP), Eastern Tropical Pacific (ETP), Western Atlantic (WA), and Eastern Atlantic (EA); and to identify those species with distributions restricted to the Hawaiian Islands and the Red Sea. Kulbicki *et al.* (2013) quantitatively delineated biogeographical patterns based on species composition. They described the ETP as both a realm and region; however, I considered it only as a region outside of the Indo-Pacific and Atlantic realms.

Age comparison

Phylogenetic reconstruction is based on a bifurcating process that produces an exponential distribution of age estimates, I thus log-transformed the data to normalise the distribution. I used two approaches for defining species age with the aim of comparing ages between realms and regions. First, I considered the estimated divergence time for all nodes in the phylogeny subtended by extant species as reflective of species age, excluding fossil calibration and outgroup species. I refer to this as the full-phylogeny approach where the advantage was achieving a large sample size for comparison among biogeographical areas. There were several limitations to this approach including the co-variance of ages shared among sister-species, which may have a levelling effect on any potential differences in age structure if sister-species are found in adjacent, or different areas.

Another limitation of the full-phylogeny approach was the impact of missing taxa on the estimation of species' ages. Phylogenetic methods are only able to estimate the age of the most recent common ancestor between two species if both species are present in the analysis. Therefore, taxa missing from the phylogeny, whether extinct or extant, will cause the ages of sister taxa to be over-estimated. Genera were selected with the aim of minimising the amount of missing extant species; however, the proportion of missing extant species in the analysis varied by genus (Table S6.1, Appendix E). Extinct species will have a greater impact on the age estimation of older lineages if the rate of extinction through time has remained relatively constant (Rabosky & Lovette, 2008).

To minimize the effects of missing taxa on species age estimation my second approach considered only sister-species pairs, excluding monotypic genera. I refer to this as the sister-species approach where I assigned estimated divergence times to the species with the smallest geographical range and did not include its sister in the analysis. This approach eliminated the levelling effects of co-varying sister-species' ages and minimized the likelihood of underestimating the ages of widespread species that may have persisted through successive peripheral divergence events (i.e. peripheral budding; Chapters 3, 5).

For both approaches, I used bootstrap re-sampling to test whether the mean age of species from each biogeographical area differed from a random distribution of mean ages. Bootstrap re-sampling was used because the data did not meet assumptions of independence required by parametric analyses. Furthermore, the bootstrap analyses could be applied to both age-estimation approaches. For each realm, region and province I sampled the corresponding number of species (for all biogeographical areas with more than two constituent species) randomly without replacement from the full phylogeny and from the full pool of resultant species following the sister-species approach. I also compared the mean ages of Red Sea and Hawaiian Island endemic species to corresponding random samples of species restricted to the WI and CP regions, respectively, to resolve whether endemic species are young relative to non-endemic species from the same region and thus potentially mark locations of species origination. Random sampling was repeated 1000 times for each case. I performed a two-tailed significance test where the observed mean age was considered significantly different from random if it was outside of the central 95% of the resampled distribution. I repeated the same randomisations using the mean upper and lower 95% HPD intervals of species age.

Peripheral speciation

To further explore the frequency of peripheral speciation events I fitted a model to the distribution of untransformed species' ages across the entire phylogeny and compared it to models fitted to distributions of untransformed species' ages for both Red Sea and Hawaiian Island endemics. All models were fitted to density distributions of 2 Myr intervals. Finally, to gain insight into potential processes underlying endemism and establish patterns of geographical range distribution among peripherally isolated species, I determined whether endemic species are contemporarily allopatric or sympatric with regard to their respective sister-species (Barraclough & Vogler, 2000) or clade (using independent contrasts *sensu* Felsenstein, 1985). All statistical analyses were performed using R (R Development Core Team, 2011).

6.3 Results

Phylogenetic analysis

See **Chapter 5**, section **5.2 Phylogenetic Methods of Age Estimation** for results of the multi-family phylogenetic analysis (see also Table S5.1 and Fig. S5.1, Appendix D).

Age comparisons

Species with distributions confined to the Indo-Pacific and Atlantic realms had strikingly similar mean ages and variances (using both the full-phylogeny and sister-species approach; Table 6.1 and Fig. 6.1). Mean species age was expectedly younger under the sister-species approach (2.9 Myr versus 5.4 Myr for species sampled using the full-phylogeny approach), but with comparably little variability between realms. No significant differences in the mean ages of species from the Indo-Pacific and Atlantic realms were detected when compared to distributions of means from 1000 resampled permutations, for both the full-phylogeny and sister-species approach.

Little variation was detected in mean species age among regions under both approaches (Table 6.1 and Fig. 6.1). No significant differences in mean species age compared to the randomly resampled distributions for all six regions using the full-phylogeny approach, and for all five bootstrapped regions using the sister-species approach were found (note that bootstrapping was not performed for the CIP region using the sister-species approach because it did not have more than two constituent species). Overall, both approaches produced similar patterns (Table 6.1 and Fig. 6.1). The most notable distinction between the two approaches was observed in the mean age of widespread species in the Indo-Pacific realm. Under the full-phylogeny approach, the mean age did not differ significantly from the bootstrapped distribution, whereas the mean age was significantly older than the central 95% of the resampled distribution using the sister-species approach. A shift in the age structure of species in the Atlantic realm was detected along with a slight increase in regional variation of mean species age using the sister-species approach. In general, the sister-species approach produced ages 0.6–2.7 Myr younger than the full-phylogeny approach, except for the WA region where the mean age was 4.1 Myr younger using the sister-species approach.

Species endemic to the Red Sea and Hawaiian Islands had similar mean ages to those restricted to the WI and CP regions, respectively (Table 6.1). The two oldest species in the WI region are endemic to the Red Sea (oldest species: *Larabicus quadrilineatus*; median age: 14.7 Ma; 95% HPD: 7.4–23.8 Ma). However, Hawaiian endemic species did not include the oldest species in the region. No significant difference in the mean age of species endemic to the Red Sea or Hawaiian Islands was found when compared to the bootstrapped distributions of species across the full phylogeny, or compared to the bootstrapped distributions of species restricted to the corresponding regions. These results persisted under the sister-species approach.

Peripheral speciation

Phylogeny-wide age estimates were randomly distributed under a negative binomial model ($r = 0.2$, $p = 3.2$; $n = 293$; d.f. = 1, 292; $\chi^2 = 2.72$; $P = 0.10$) (Fig. 6.2). However, Red Sea endemic species have diverged steadily through time and fit a Poisson model ($\lambda = 1$; $n = 16$; d.f. = 1, 15; $X^2 = 0.09$; $P = 0.76$) (Fig. 6.3a), while Hawaiian endemic divergences fit neither a

Table 6.1 Sample sizes and resulting mean species age for two approaches used to compare the ages of coral reef fish species between marine biogeographical realms and regions. (a) The full-phylogeny approach that considered the ages of all extant species, excluding fossil calibration and outgroup species. (b) The sister-species approach that considered the age of only the species with the smallest geographical range (per sister-species pair, excluding monotypic genera). Contemporary spatial data were used to classify species by realm and region, and to identify those species endemic to the Red Sea and Hawaiian Islands (IUCN, 2011) following the biogeographical delineations of Kulbicki *et al.* (2013). Regions included the: Western Indian (WI), Central-Indo Pacific (CIP), Central Pacific (CP), Eastern Tropical Pacific (ETP), Western Atlantic (WA), and Eastern Atlantic (EA). Numbers in parentheses corresponding to the WI and CP indicate the number of species from that region endemic to the Red Sea and Hawaiian Island provinces, respectively. Region-restricted species combined with widespread species (WS) in the Indo-Pacific and Atlantic realms to give the total number of species for each realm. Bootstrap re-sampling was performed for all biogeographical areas with more than two constituent species. Asterisks indicate a significant difference ($P < 0.05$) in the mean age of species compared to 1000 resampled permutations.

		Mean (Ma)				**Mean (Ma)**	
Realm	**No. Extant Species**	**Age**	**95% HPD**	**Region**	**No. Extant Species**	**Age**	**95% HPD**
(a) Full-phylogeny approach							
Indo-Pacific	204	5.5	3.1–8.4	WI	29 (16)	3.9 (4.2)	2.0–6.2 (2.2–6.8)
				CIP	6	2.9	1.4–4.9
				CP	18 (9)	5.3 (4.8)	2.9–8.6 (2.7–7.4)
				WS	151	5.9	3.4–9.0
—	—	—	—	ETP	22	4.6	2.7–6.9
Atlantic	57	5.6	3.3–8.5	WA	34	6.3	3.8–9.2
				EA	21	5.0	2.6–7.8
				WS	2	1.9	0.6–3.9
(b) Sister-species approach							
Indo-Pacific	66	3.0	1.3–5.3	WI	15 (9)	2.6 (3.0)	1.1–4.7 (1.2–5.3)
				CIP	2	0.5	0.1–1.2
				CP	9 (4)	2.6 (1.2)	0.9–4.9 (0.4–2.2)
				WS	40	3.3*	1.4*–5.9*
—	—	—	—	ETP	8	2.1	0.9–3.8
Atlantic	17	2.9	1.3–5.0	WA	10	2.2	0.9–4.0
				EA	6	4.4	2.1–7.0
				WS	1	1.2	0.3–2.4

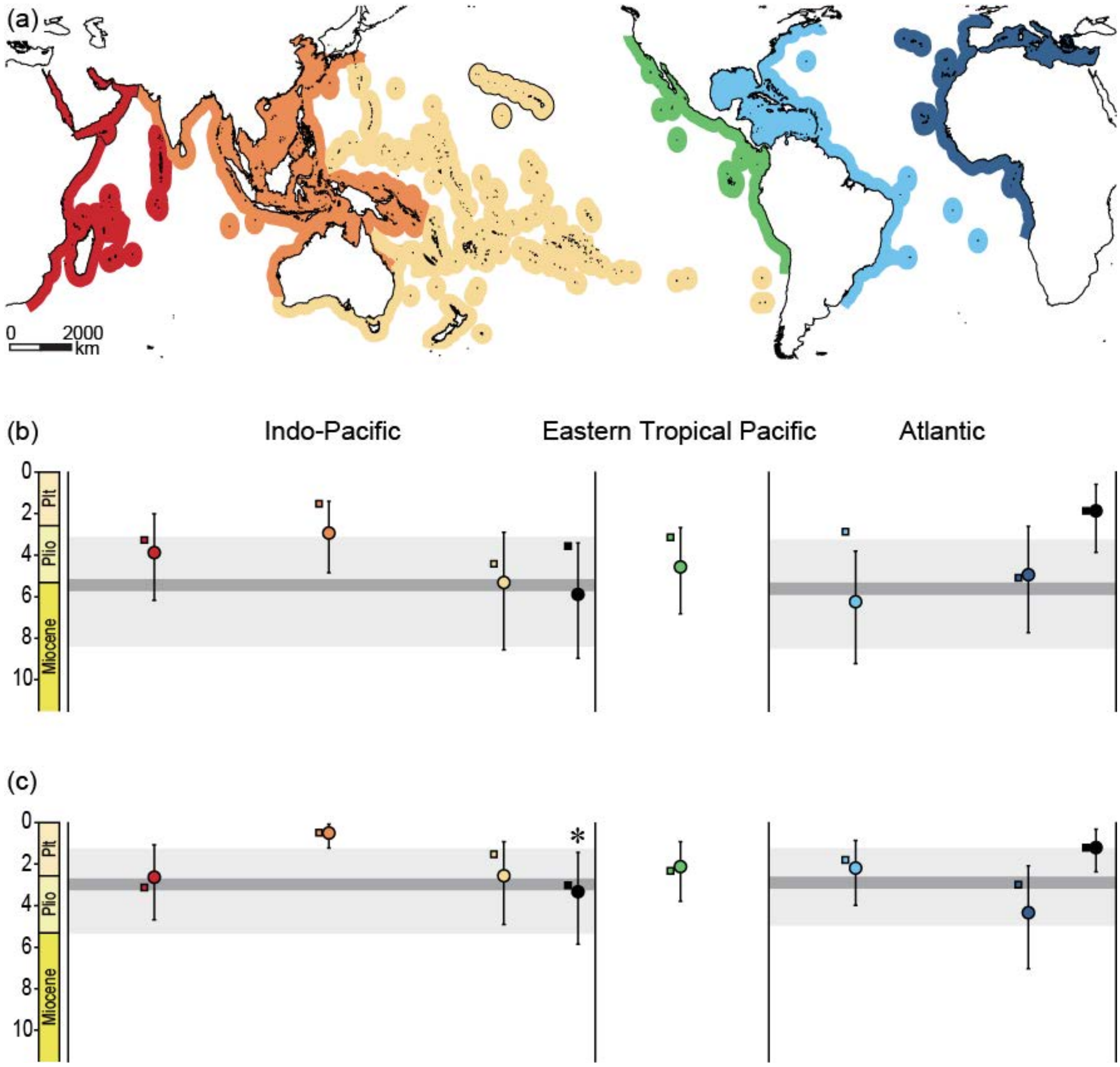


Figure 6.1 Biogeographical variation in the ages of coral reef fish species. (a) Map showing two marine realms, the Indo-Pacific and Atlantic, and the regions within each realm differentiated by colour. Western Indian (WI) (red), Central Indo-Pacific (CIP) (orange), Central Pacific (CP) (light orange), Eastern Tropical Pacific (ETP) (green), Western Atlantic (WA) (light blue), and Eastern Atlantic (EA) (dark blue). Areas follow those outlined by Kulbicki *et al.* (2013). Two peripherally isolated provinces within the Indo-Pacific realm, the Hawaiian Islands and the Red Sea, are outlined in black. The map uses a Behrmann projection. Below the map are the results of age comparisons of coral reef fish species among marine realms and regions using (b) a full-phylogeny approach, and (c) a sister-species approach. Mean species age (dark grey horizontal bars) and 95% highest posterior density (HPD) intervals (light grey shading) are displayed for species in the Indo-Pacific and Atlantic realms as well as for species restricted to regions (corresponding coloured circles). Black circles indicate mean ages (and corresponding 95% HPD intervals) of non region-restricted species for both the Indo-Pacific and Atlantic realms. Corresponding coloured squares represent median species age. Asterisks indicate a significant difference ($P < 0.05$) in the mean age of species compared to 1000 resampled permutations. Sample sizes for each biogeographical area are listed in Table 6.1.

negative binomial, nor a Poisson model. Instead, the age estimates of Hawaiian endemic species fit a bimodal distribution with distinct peaks between 0–3 Ma and 8–12 Ma (Fig. 6.3b), which conforms to a mixture of two normal distributions [$D = 20.58$; $D > 2$ required for clear separation; (Ashman *et al.*, 1994)]. Furthermore, the mean 95% HPD intervals of both peaks do not overlap (95% HPD early peak: 5.7–13.9 Ma; late peak: 0.4–2.3 Ma) (Fig. 6.4). The separation of Hawaiian Island endemic species' divergences is apparent despite ongoing divergence within the CP throughout this period. It also holds true for multiple species within genera. For example, two of the three endemic *Chaetodon* species arose in the late peak (between 0–3 Ma), while the third endemic *Chaetodon* species arose in the early peak (between 8–12 Ma) (Fig. 6.4).

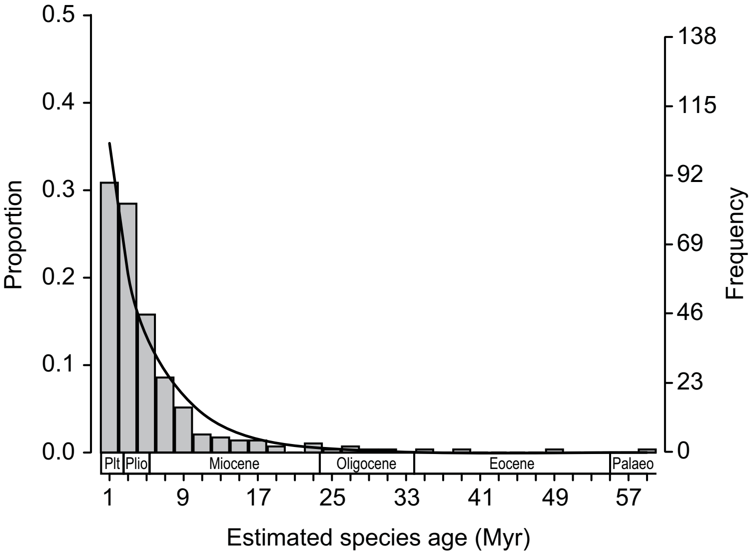
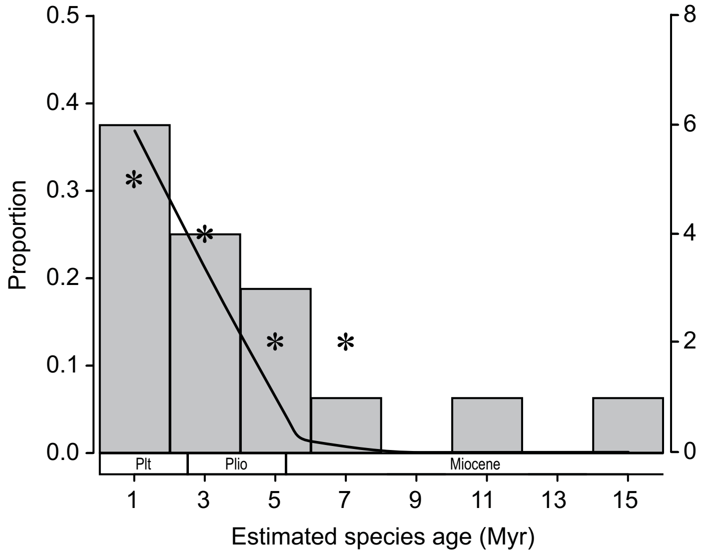


Figure 6.2 Frequency distribution of age estimates for coral reef fish species across the entire mulit-family phylogeny plotted as the proportion of total species (left side) and as a frequency (right side) on the y-axis. This age distribution fits a negative binomial model ($r = 0.2$, $p = 3.2$; $n = 293$; d.f. = 1, 292; $\chi^2 = 2.72$; $P = 0.10$), represented by the black line.

(a) Red Sea and WI



(b) Hawaiian Islands and CP

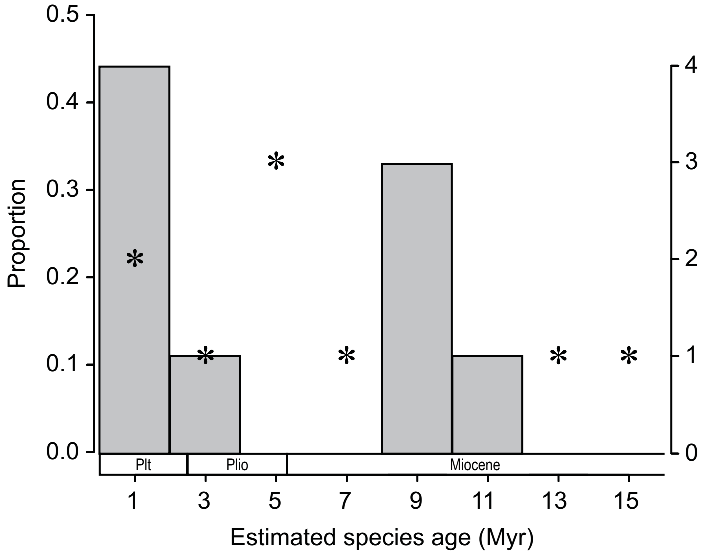


Figure 6.3 Frequency distributions of age estimates for coral reef fish species plotted as the proportion of total species (left side) and as a frequency (right side) on the y-axis. (a) Species endemic to the Red Sea (grey bars) fit a Poisson model ($\lambda = 1$; $n = 16$; d.f. = 1, 15; $X^2 = 0.09$; $P = 0.76$), represented by the black line; asterisks denote the age distribution of species restricted to the Western Indian region. (b) Species endemic to the Hawaiian Islands (grey bars) have a bimodal age distribution (Ashman's $D = 20.58$); asterisks denote the age distribution of species restricted to the Central Pacific region.

To explore peripheral speciation processes I determined whether species endemic to the Red Sea and Hawaiian Islands are contemporarily allopatric or sympatric relative to their sister taxon. Endemics in these two regions differed in the proportion of allopatrically and sympatrically distributed sister-species (Fig. 6.4). Seven of the nine Hawaiian endemic species (78%) have allopatric distributions with their sister-species or clade, while the remaining two species have sympatric distributions (22%). Six of the nine endemic species are sister to a single species and the remaining three are sister to a clade. Interestingly, both sympatric species are sister to a clade and were among the early wave of speciation in the Hawaiian Islands (between 8–12 Ma) with an average degree of overlap less than 10%. The average degree of sympatry for Hawaiian endemic species was 1%, compared to an average of 19% sympatry among species restricted to the CP region (where four of nine species were sympatric). In contrast, half of the Red Sea endemic species (eight of 16 species) have allopatric distributions, and half have sympatric distributions. Of the 16 endemic species, 11 are sister to a single species, and the remaining five are sister to a clade. Species that are sister to a clade are distributed throughout the age range of endemic Red Sea species, as are allopatric and sympatric species. The average degree of sympatry for Red Sea endemics was 23%, similar to the average degree of sympatry for species in the WI region (19%, eight of 13 species were sympatric), but substantially larger compared to Hawaiian endemics.

6.4 Discussion

Despite differing geological and evolutionary histories between the Indo-Pacific and Atlantic biogeographical realms, the results show limited variation in the ages of their constituent coral reef fish faunas. The CIP and its peripheral regions have all experienced recent divergence events, with no detectable difference in the mean ages of reef fish species among regions in the Indo-Pacific. Atlantic regions have also experienced recent divergence events with mean ages similar to those of the Indo-Pacific. Furthermore, ages of endemic species do not differ from more widespread species. Therefore, the results do not support the indiscriminate use of endemic species as markers of species origination. Interesting patterns of diversification in isolated locations provide insights into the processes underlying endemism and peripheral speciation. Specifically, I report notable differences between the Red Sea and Hawaiian Islands in the timing of divergence events and in patterns of contemporary geographical distribution of endemic species.

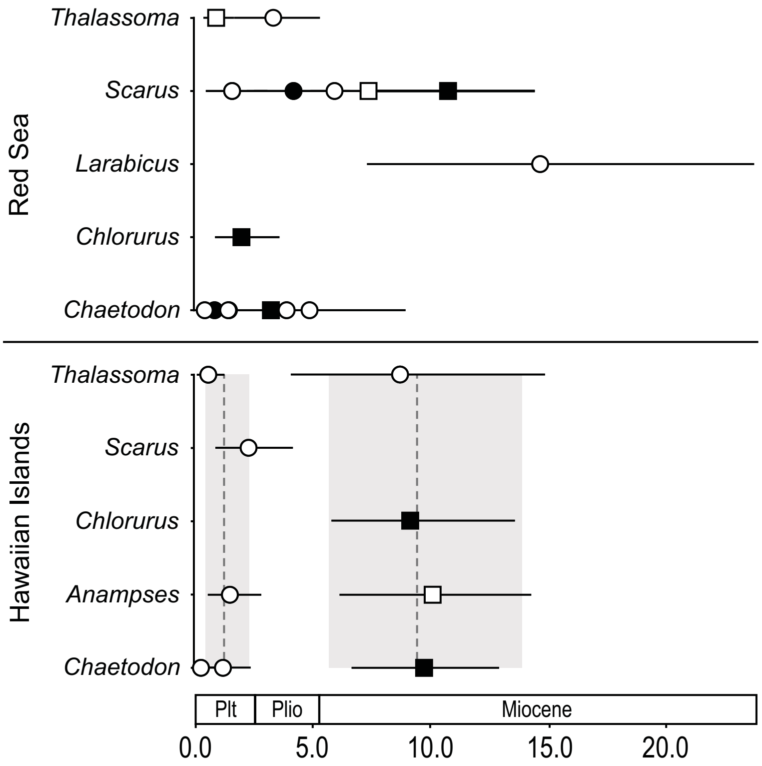


Figure 6.4 Ages of Red Sea and Hawaiian endemic coral reef fish species [circles and squares; 95% highest posterior density (HPD) intervals indicated by horizontal black lines]. Circles represent endemic species sister to a single species; squares represent endemic species sister to a clade. White shapes indicate allopatric endemic species; black shapes indicate sympatric endemic species (i.e. degree of sympatry above zero). Degree of sympatry was calculated following Barraclough & Vogler (2000) and using independent contrasts (Felsenstein, 1985) for endemic species sister to a clade. The ages of Hawaiian endemics conforms to a mixture of two normal distributions according to Ashman's *D* statistic [$D = 20.58$; $D > 2$ required for clear separation; (Ashman *et al.*, 1994)]. Dashed vertical lines show the mean of the two separate periods of divergence and the grey shaded area indicates the mean 95% HPD intervals, which do not overlap (95% HPD early peak: 5.7–13.9 Ma; late peak: 0.4–2.3 Ma).

Age comparisons

The Indo-Pacific and Atlantic realms are undoubtedly characterised by unique assemblages of coral reef fishes (Briggs & Bowen, 2012; Kulbicki *et al.*, 2013). While some species have attained circum-tropical distributions, most species are restricted to a particular realm and share the influence of its historical periods of isolation (Kulbicki *et al.*, 2013). Vicariance events such as the closure of the Tethys seaway (Steininger & Rögl, 1984) and the rise of the Isthmus of Panama (Coates & Obando, 1996) have produced diffuse signals of vicariance among reef fish lineages, while soft hydrological barriers to dispersal can result in tightly concordant vicariant speciation (Cowman & Bellwood, 2013b). Furthermore, each region has a markedly different amount of space for reef fishes to occupy, which has varied through time, and may have influenced the rate of species divergence or extinction (Bellwood & Wainwright, 2002; Renema *et al.*, 2008). Despite the potential for temporally diffuse or concentrated vicariance events, and variable habitable area within each realm, few significant differences among the ages of constituent species were found using two approaches for age comparison. On average, recent divergence of extant species has occurred throughout the past 1–5 Myr in the Indo-Pacific and Atlantic realms, suggesting similar timing in the divergence of reef fishes, despite different geological histories and different contemporary patterns of biodiversity.

Differences between the two approaches in the ages of widespread species in the Indo-Pacific realm suggest that extinction may have resulted in substantial overestimation of species' ages using the full-phylogeny approach. While the larger magnitude of change in mean species age in the WA region, relative to other regions, suggests that extinction may have had the greatest influence on the ages of extant species in this region. The Atlantic has experienced substantial loss of marine taxa during a period of faunal turnover in the Plio-Pleistocene (Bellwood, 1997; O'Dea *et al.*, 2007), which may explain the differences observed when comparing full-phylogeny and sister-species approaches.

In the Atlantic, widespread species are young compared to their region-restricted counterparts. However, I found just two species with distributions spanning the Eastern and Western Atlantic regions. The low number of widespread species in the Atlantic may be due in part to the relatively low overall coral reef fish biodiversity of this realm (Bellwood, 1997; Kulbicki *et al.*, 2013), but it may also indicate that regional spatial structure is an important characteristic of Atlantic species (Bender *et al.*, 2013). In comparison to the Indo-Pacific, the Atlantic has fewer centrally located islands available to facilitate range expansion, which may produce more defined spatial structure among its faunal constituents.

More variation in the ages of reef fishes was detected between regions than among realms, with younger ages in the CIP compared to the WI and CP. Younger ages of reef fish species in the CIP, although not significantly different from random permutations, lends some support to the Centre of Origin or Centre of Survival hypotheses. Recent models of coral reef fish evolution and dispersal over the last 65 Myr demonstrate that the IAA (located within the greater CIP region) has played a number of different roles, supporting the accumulation, survival, origination and export of species (Barber & Bellwood, 2005; Barber, 2009; Halas & Winterbottom, 2009; Bowen *et al.*, 2013; Cowman & Bellwood, 2013a). More specifically, models suggest that since the Miocene (23 Ma) the IAA has been characterised by exceptionally high rates of species origination (Cowman & Bellwood, 2013a). This may explain the slightly younger species’ ages obtained for the CIP relative to adjacent regions that, according to the models, were colonized by lineages that originated in the IAA during this time (Cowman & Bellwood, 2013a).

If vicariance has played a major role in recent species diversification, it would have a neutralising effect on any potential patterns between vicariant regions when comparing species’ ages using the full-phylogeny approach. For example, the rise of the Isthmus of Panama produced geminate species pairs with a shared age that are now distributed either side of the barrier. Therefore, when comparing the ages of species from the WA and the ETP, the ages of geminate species pairs are considered for both regions and neutralize any differences in age structure between them. However, patterns of relative age between realms and regions remained constant, with the exception of species in the WA region, when such co-variance of ages was accounted for using the sister-species approach.

The methods employed here considered only extant species and their lineages, and therefore were not capable of resolving historical evolutionary differences that may have distinguished biogeographical areas. The applied protocols to maximize sampling of extant species meant that generic sampling achieved within families was limited and I was not able to resolve deeper splits in the phylogeny with confidence, precluding an assessment of historical differences among lineages. Instead, I have focused on recent speciation events because it is unlikely that many older species have survived to the present day. If older species have survived and also given rise to other species through time (i.e. peripheral budding; Chapters 3, 5), their true age may be masked by recent speciation events. Thus, historical signals are largely overwhelmed by recent speciation. Different geological and evolutionary histories among regions have probably shaped lineages, but speciation has largely been shaped by events in the past 1–5 Myr.

Peripheral speciation

Endemic species in the Red Sea and Hawaii displayed different age distributions when compared to each other and to the underlying age distribution of the full phylogeny, suggesting that distinctive processes of diversification have operated at these peripherally isolated locations. The topological and chronological hypotheses of Red Sea and Hawaiian endemic species agree with previous phylogenetic hypotheses for *Chaetodon* (Fessler & Westneat, 2007; Bellwood *et al.*, 2010; Craig *et al.*, 2010), *Anampses* (Chapter 3), *Chlorurus* (Choat *et al.*, 2012), *Larabicus* (Westneat & Alfaro, 2005; Cowman *et al.*, 2009; Kazancıoğlu *et al.*, 2009), *Scarus* (Choat *et al.*, 2012), and *Thalassoma* (Bernardi *et al.*, 2004), offering additional confidence in the chronogram.

Red Sea endemics appear to have arisen steadily throughout the past 16 Myr, roughly 10 Myr after the sea first appeared (Bosworth *et al.*, 2005). They include the oldest extant lineage in the WI region, *Larabicus quadrilineatus*, which is estimated to have diverged 14.7 Ma, around the time when the Red Sea was becoming increasingly isolated from the Mediterranean (Bosworth *et al.*, 2005) and the Arabian hotspot was dwindling (Renema *et al.*, 2008). In the recent geological past, the Red Sea has experienced volatile changes in temperature and salinity (Biton *et al.*, 2008). The effects of these environmental fluctuations have reportedly caused mass extirpation of marine organisms including planktonic foraminifera (Hemleben *et al.*, 1996). The results, as well as other phylogeographic studies of reef fishes (DiBattista *et al.*, 2013), suggest that the Red Sea, or the adjacent Gulf of Aden, has sustained coral reef fish lineages (and presumably coral reefs) throughout these environmental fluctuations. Older endemic lineages, like *L. quadrilineatus*, are likely to have survived outside of the Red Sea during extreme environmental periods and subsequently re-invaded when the Red Sea opened up to the WI region *c.* 5 Ma and the environment became suitable for the maintenance of coral reefs (Siddall *et al.*, 2003; Bosworth *et al.*, 2005). The majority of Red Sea endemics (75% of those studied herein) diverged after this time. The continuity of the continental shelf to the east and south of the Red Sea, in combination with its relative close proximity to the IAA, has likely facilitated the ongoing divergence of lineages throughout the past 16 Myr.

In contrast to the Red Sea, the Hawaiian Archipelago is located in the central Pacific Ocean and is part of the larger Hawaiian-Emperor seamount chain, a series of volcanic islands and atolls separated by oceanic channels. The data suggest that colonization of the Hawaiian Islands has occurred independently for multiple species belonging to the genera *Chaetodon*, *Anampses*, and *Thalassoma*. This is consistent with previous phylogenetic hypotheses (Bernardi *et al.*, 2004; Fessler & Westneat, 2007; Craig *et al.*, 2010; Chapter 3). Multiple models of diversification have likely led to the divergence of Hawaiian endemics including, but not limited to, successive colonization/division and peripheral budding (Chapter 3). For example, Craig *et al*. (2010) proposed that a closely related *Chaetodon* species complex containing

Chaetodon punctofasciatus, *Chaetodon pelewensis*, and *Chaetodon multicinctus*, originated in the western Pacific, spread through the South Pacific Islands and finally colonized the Hawaiian Islands. With the inclusion of the closely related WI species, *Chaetodon guttatissimus*, I found that the diversification of species in this clade could fit a successive division or colonization model, with initial separation between the Indian Ocean and the CIP/CP, followed by separation between the CIP/CP and the Hawaiian Islands. However, the region of origination of this lineage remains unclear.

In contrast to the ongoing cladogenesis in the Red Sea, I found evidence for two distinct waves of divergence among Hawaiian endemics. The two waves of divergence (0–3 Ma and 8–12 Ma) occurred either side of a broad period of increased primary productivity (Dickens & Owen, 1999) that coincided with increased cladogenesis for a wide range of taxa in the Indo-Pacific, including reef fishes, between 3.5 and 9.0 Ma (Renema *et al.*, 2008; Reid *et al.*, 2010; Cowman & Bellwood, 2013a, b). The first wave coincides with the late Miocene-Pliocene (9–12 Ma) when deep-water circulation reorganisation occurred as a result of the reduction in deep-water exchange between the Atlantic and Pacific Oceans through the Panamanian gateway prior to the emergence of the isthmus (Lyle *et al.*, 1995). These changes in deep-water circulation caused disruption to large-scale ocean circulation patterns (Butzin *et al.*, 2011). Further evidence suggests that atmospheric and oceanic circulation intensified about 10 Ma (Rea & Bloomstine, 1986) and during the glacial periods of the past 1.2 Myr (Hall *et al.*, 2001). These changes in ocean circulation and intensity may have produced more favourable conditions that allowed founder populations of reef fishes to reach the Hawaiian Islands and establish themselves, ultimately leading to the divergence of peripatric populations and the formation of endemic species.

Different proportions of allopatric and sympatric sister-species among Red Sea and Hawaiian endemics provides additional support for the operation of distinctive processes of diversification at these peripherally isolated locations. Red Sea endemics have equal proportions of allopatrically and sympatrically distributed sister-species, and approximately one-third appeared as sister to a clade. Secondary endemism, where a primary endemic gives rise to one or more subsequent endemics (Rotondo *et al.*, 1981), appears likely to have operated in the Red Sea province given the sympatric distribution of the well-supported sister-species pair: *Scarus persicus* and *Scarus ferrugineus*, both Red Sea/Persian Gulf endemics (Choat *et al.*, 2012). The results, in combination with knowledge of the dynamic geological and environmental past of the Red Sea, suggests that a number of speciation modes may have operated through time and that both allopatric and sympatric speciation have likely played a role in generating Red Sea endemics.

While a number of speciation modes may have also led to the diversification of Hawaiian endemics, the results revealed potential key differences between the two isolated provinces. As in the Red Sea, one-third of Hawaiian endemic species are sister to a clade. However, allopatric distributions constitute the bulk (78%) of endemic species' distributions in the Hawaiian Islands. With such a low level of sympatry it seems unlikely that either sympatric speciation or secondary endemism have been important in the evolution of Hawaiian endemic reef fishes. Rather, allopatric speciation, likely in the form of peripatric speciation, appears to have been the dominant mode in generating Hawaiian reef fish endemics. The topological relationships and estimated ages of sympatric endemic species (between 8–12 Ma), combined with the low levels of average overlap (< 10%), suggest that peripheral budding (*sensu* Chapters 3, 5) may be a key model under which Hawaiian endemic reef fishes have diverged.

6.5 Conclusions

Both marine realms and all six biogeographical regions show similar patterns of recent species divergence. Two peripherally isolated locations with high levels of endemism, the Hawaiian Islands and the Red Sea, show contrasting patterns. The age structure of Red Sea endemics is comparable with the larger WI region, with a steady increase in species over the past 16 Myr. In contrast, the Hawaiian Islands showed two distinct periods of divergence,

between 0–3 Ma and 8–12 Ma. Spatial distributions of endemic species relative to their closest sister taxon reveal that allopatric and sympatric speciation are likely important processes in the divergence of endemic species, with peripatry especially important in the divergence of Hawaiian Island endemics. Taken together, the differences in age structure and spatial patterns between Red Sea and Hawaiian Island endemic species point to markedly different processes of diversification at two peripherally isolated locations.

Chapter 7: The geography of speciation in coral reef fishes

Submitted for publication (2014)

7.1 Introduction

Present-day patterns of biodiversity result from evolutionary processes of genetic divergence, speciation, range-size transformation and extinction. The influence of geography on these processes has intrigued biologists and biogeographers for centuries (Darwin, 1859; Mayr, 1954). Geographical areas of concentrated diversity, or biodiversity hotspots, provide ideal opportunities to study evolutionary history and examine the role of geography in speciation. In the marine realm, a prominent biodiversity hotspot, often referred to as the Coral Triangle, is found in the central Indo-Pacific (Rosen, 1984; Hoeksema, 2007). The area is a hotspot of diversity for many marine organisms including plants, invertebrates, and coastal reef-associated fishes (reviewed in Hoeksema, 2007; Renema *et al.*, 2008; Bellwood *et al.*, 2012). Of the organisms aligned with this hotspot, the reef fishes are exceptionally diverse with well-described, interesting distributional patterns.

Many researchers have evaluated the influence of geography on the divergence of ancestral reef fish lineages (Renema *et al.*, 2008; Cowman & Bellwood, 2013a, b) and the foundations of reef fish biodiversity are generally well established (Bellwood *et al.*, 2012). Studies of population connectivity have also illuminated the role of geography in producing and preserving genetic diversity within species (reviewed in Carpenter *et al.*, 2011; Bowen *et al.*, 2013). Yet, comparatively little is known about the role of geography in generating and maintaining diversity at the species level (but see Rocha & Bowen, 2008; Gaither & Rocha, 2013). Because the diversity of life is most often described using species as the focal unit, it is critically important to establish key factors that influence the diversity of species and the processes by which they diverge. Our ability to predict patterns that may result from global environmental change crucially hinges on this knowledge.

Uncertainty surrounding sister-species relationships and their associated timing of divergence is a fundamental reason why the geography of speciation is not yet fully understood. Without these two key pieces of information, it is difficult to eliminate the importance of past geological and geographical events in generating and maintaining new species. Traditionally, sister-species have been defined based on morphological characteristics, including differences in colour patterns and morphometrics. However, inter-specific relationships are not always as clear as morphology or colour patterns suggest (Gaither *et al.*, 2014). Molecular phylogenetics has proved to be a useful tool in establishing sister relationships among taxa; although, phylogenetic methods require complete sampling of all or most extant, nominal taxa to establish robust sister relationships (Barraclough & Nee, 2001). Few phylogenies exist for reef fishes with such complete sampling at the species level (but see Klanten *et al.*, 2004; Bernardi *et al.*, 2008; Rocha *et al.*, 2008; Crow *et al.*, 2010; Chapters 3, 4 for examples).

Fortunately, molecular data are becoming increasingly available and it is now possible to evaluate the geography of speciation in reef fishes using a newly constructed phylogenetic hypothesis that incorporates genera with near-complete sampling of extant, nominal species across four major reef fish families: Chaetodontidae, Labridae, Pomacanthidae and Epinephelidae (methods detailed in Chapter 2). The aim of this chapter is to combine robust sister-species relationships from these phylogenies with precise data detailing species geographical distributions to investigate the geography of recent speciation events and identify areas that are important in the generation and maintenance of coral reef fish diversity.

The Central Indo-Pacific (CIP) is a broad area (approximately 90°–165° E) that was recently quantified as a biogeographical region based on faunal similarity among reef fishes (Kulbicki *et al.*, 2013). The CIP contains a long-recognised biodiversity hotspot that has been ascribed various names and delineations dependent upon diversity patterns of different organismal groups and different methods of assessing diversity (Bellwood *et al.*, 2012; Briggs & Bowen, 2013). Most of the delineations generally describe the area bounded by the Philippines, the Malaysian Peninsula and New Guinea, with designations including the Coral Triangle (Allen, 2008; Veron *et al.*, 2009), among others (reviewed in Hoeksema, 2007). The Indo-Australian Archipelago (IAA) (Bellwood & Hughes, 2001; Renema *et al.*, 2008), however, encompasses a larger area that is inclusive of the majority of the other delineated areas, but extends considerably north and south (approximately 90°–160° E, and 10° S–15° N; Connolly *et al.*, 2003). To consider the largest range of potential processes and factors that could explain the high level of species richness within the hotspot, this work uses the CIP region to describe its general location.

Multiple hypotheses have been put forth to explain the bull's-eye pattern of biodiversity in the CIP (reviewed in Bellwood *et al.*, 2012). Together, they are often referred to as the 'Centre of' hypotheses. They include the Centre of: (1) Origin, (2) Accumulation, (3) Survival, and (4) Overlap. Each hypothesis explicitly predicts the evolutionary processes responsible for the concentration of species richness in the CIP. The Centre of Origin hypothesis, initially proposed by Ekman (1953) and expanded upon by others (Potts, 1985; Briggs, 1999), asserts that species originate in the CIP and radiate outward. Species origination is thought to be driven by the geological complexity of the region, its dynamic history, and the heterogeneous nature of its environments (McManus, 1985; Hoeksema, 2007; Rocha & Bowen, 2008). The Centre of Accumulation hypothesis contrasts with the aforementioned by proposing speciation in locations peripheral to the hotspot and subsequent accumulation of species via inward range movements (Ladd, 1960) or tectonic collisions (Rosen, 1984). The Centre of Survival hypothesis places less emphasis on the location of species origination and instead, emphasizes the CIP as an area of increased species survival (Heck & McCoy, 1978; Barber & Bellwood, 2005). As a consequence, areas outside of the CIP are predicted to have higher rates of extinction (Potts, 1985; Paulay, 1990; Bellwood & Hughes, 2001). Finally, the Centre of Overlap hypothesis arose from the recognition of congruent biogeographical boundaries separating closely related taxa (Woodland, 1986; Blum, 1989; Wallace *et al.*, 2000). This hypothesis argues that distinctive Indian and Pacific Ocean faunas overlap in the CIP, creating local species enrichment. It invokes changes in sea level and the formation of land barriers as isolating mechanisms. Importantly, the Centre of Overlap hypothesis predicts distributional overlap among sister-species within the CIP as a result of secondary contact following periods of isolation. This differs from the Centre of Accumulation hypothesis, which makes no predictions about distributional relationships among sister-species.

Testing these hypotheses has proved difficult because in many cases they make identical biogeographical predictions (Pandolfi, 1992), although they need not be mutually exclusive. Biogeographical modelling has shown that the CIP has acted sequentially and simultaneously as a centre of accumulation, survival, and origin of reef fish lineages throughout evolutionary history (Cowman & Bellwood, 2013a). Equipped with a phylogenetic hypothesis detailing the evolutionary history of reef fish species, it is now possible to explore factors that have shaped patterns of total species richness in different biogeographical areas, including the CIP. One approach is through an evaluation of sister-species and their corresponding biogeographical distributions.

Sister-species may be either sympatric (i.e. their geographical distributions have some degree of overlap) or allopatric. If the distributional overlap of sympatric sister-species were concentrated in the CIP this would support the Centre of Overlap or Centre of Origin hypotheses. Similarly, if allopatric sister-species have shared vicariant signals associated with previously described biogeographical barriers in the CIP, this would lend further support to the suggestion that Pacific and Indian Ocean reef fishes were isolated as a consequence of divisions within the CIP. The biogeographical analysis of sister-species will therefore enable the identification of areas important in the generation and maintenance of modern coral reef fish biodiversity.

Specifically, this study addressed the following questions:

1) What is the geographical pattern of overlap among sympatric sister-species? and
2) What is the relative importance of previously described biogeographical barriers in the divergence of contemporary reef fish species?

7.2 Materials and Methods

Methods of ***Data selection and handling*** and ***Phylogenetic analysis and age estimation*** for this data chapter follow those detailed in **Chapter 2: General Methods**, section **2.2 Multi-Family Phylogeny**.

Biogeographical analysis

Sister-species distributions were classified as either contemporarily allopatric with non-overlapping distributions (degree of sympatry = 0), or sympatric with some amount of distributional overlap (degree of sympatry > 0). Degree of sympatry was calculated following Barraclough and Vogler (2000), where the area of overlap was divided by the smallest range area between two sister-species. This value extends from zero to one, with increasing values signifying increasing overlap. Overlapping areas were mapped for all sympatric sister-species pairs by intersecting their distributional areas and isolating all areas of overlap using GRASS (GRASS Development Team, 2011) and QGIS (QGIS Development Team, 2012) (Fig. 7.1a). A heat-map was created using the isolated areas to show locations of concentrated overlap.

Isolated areas of overlap were also grouped according to the quantitatively delineated biogeographical areas defined by Kulbicki *et al.* (2013). These included the Indo-Pacific and Atlantic realms, and the Western Indian (WI), Central Indo-Pacific (CIP), Central Pacific (CP), Eastern Tropical Pacific (ETP), Western Atlantic (WA), and Eastern Atlantic (EA) regions. Bootstrap re-sampling was performed in R (R Development Core Team, 2011) to test whether the mean degree of sympatry for species with overlapping distributions in different biogeographical areas differed from random distributions of mean values. For each realm and region, the corresponding number of overlapping sister-species pairs was sampled randomly without replacement from the full set of sympatric sister-species pairs (for all biogeographical areas with more than two constituent overlapping areas).

The nearest linear distance separating allopatric sister-species’ distributions was also calculated and mapped. The line was then rotated 90 degrees and mapped as an indication of the location of vicariance between the allopatric sister-species (Fig. 7.1b). Hereafter these lines are referred to as splits. Splits were compared to previously described biogeographical barriers (Bellwood & Wainwright, 2002; Rocha *et al.*, 2007; Floeter *et al.*, 2008; Luiz *et al.*, 2012) to identify locations important in recent vicariance events. Only those previously described barriers with three or more corresponding splits were considered for further analysis. All maps were created using a Behrmann projection.

Distributional symmetry was also calculated for all sister-species pairs (allopatric and sympatric) as the smallest distribution divided by the sum of the two sister-species’ distributions (Barraclough & Vogler, 2000). This value ranges from zero to 0.5, with increasing values signifying increasing distributional symmetry. Further bootstrapping was used to test whether the mean degree of distributional symmetry among allopatric and sympatric sister-species differed from random distributions of mean values generated from the full pool of sister-species, and from the respective pools of allopatric and sympatric sister-species. Symmetry values for sympatric sister-species were grouped by biogeographical region (as in the previous bootstrap analysis of sympatry), while symmetry values for allopatric sister-species were classified based on common areas of vicariance. Random sampling was repeated 1000 times for each case. A two-tailed significance test was performed where the observed mean was considered significantly different from random if it was outside of the central 95% of the resampled distribution.

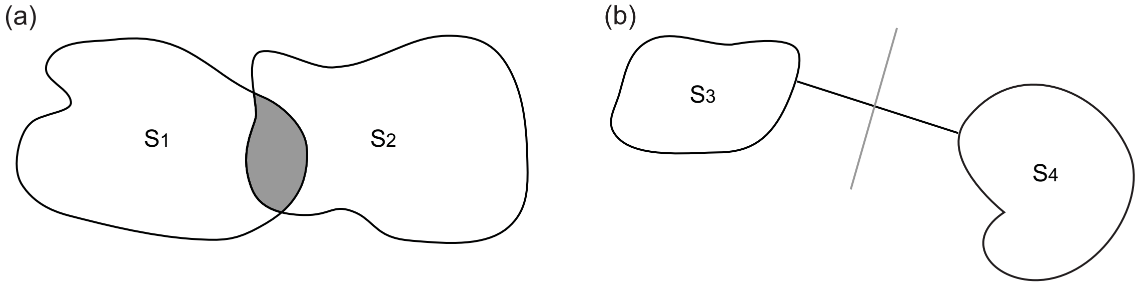


Figure 7.1 Schematic showing how overlap and vicariance were mapped for sister-species pairs. (a) Sister-species with a degree of overlap greater than zero were considered sympatric (species S_1 and S_2). Degree of overlap was calculated following Barraclough and Vogler (2000), where the area of overlap (shaded grey) was divided by the smallest range area between the two sister-species. This value extends from zero to one, with increasing values signifying increasing overlap. Overlapping areas were mapped for all sympatric sister-species pairs by intersecting their distributional areas and isolating all areas of overlap. A heatmap was created using these isolated areas to show locations of concentrated overlap. (b) The nearest linear distance separating allopatric sister-species' distributions (S_3 and S_4) was calculated and mapped as a straight line (black). The line was rotated 90 degrees (grey) and mapped as an indication of the location of vicariance between the two sister-species. Grey lines were assessed for commonness among themselves and among known biogeographical barriers to determine locations important in recent vicariance events.

7.3 Results

Phylogenetic analysis

See **Chapter 5**, section **5.2 Phylogenetic Methods of Age Estimation** for results of the multi-family phylogenetic analysis (see also Table S5.1 and Fig. S5.1, Appendix D). The resultant chronogram contained 93 pairs of sister-species, excluding monotypic, fossil calibration, and outgroup taxa. Thirty-three sister-pairs had allopatric distributions and the remaining 60 sister-pairs were sympatric.

Vicariance

Multiple splits between allopatric sister-species corresponded to six distinct biogeographical barriers (Fig. 7.2a, b). These included the Amazon and Orinoco barriers, Isthmus of Panama (IOP), Hawaiian Archipelago, Indo-Pacific, and a previously unnamed barrier I term the Mid-Indian Ocean Barrier (MIOB; f in Fig. 7.2a, b). In total, splits at these barriers accounted for ~61% of all allopatric sister-species. The remaining splits (n = 13) did not show congruence and often did not align with previously described barriers (Fig. S7.1, Appendix F). The area of likely vicariance was ambiguous for most of these remaining splits because the distance between their sister-species distributions was large enough to span multiple biogeographical barriers. However, some of the remaining splits did solely correspond to previously described biogeographical barriers, including: II, n, o, and q (see Fig. 7.2a for location of previously described barriers, remaining splits mapped in Fig. S7.1, Appendix F).

Allopatric sister-species (n = 33) had significantly lower than expected distributional symmetry compared to the bootstrapped distribution of mean values drawn from all sister-species pairs. This appeared to be driven largely by sister-species that occur either side of the MIOB, as these were the only pairs that showed significantly less than expected distributional symmetry when compared to random sampling from the pool of allopatric sister-species (Fig. 7.2c). Sister-species either side of the Hawaiian Archipelago biogeographical barrier had similarly low distributional symmetry; however, they were fewer in number and not significantly different from the bootstrapped distribution of mean values.

Sympatry

The concentration of overlap among sympatric sister-species forms a striking pattern. The highest concentration of overlap was found in the CIP region in an area concordant with the biodiversity hotspot (Fig. 7.3a, b). The concentration of overlap declines markedly in all outward directions in a similar manner to the concentration of biodiversity. The CIP region also contained the largest number of overlapping distributions restricted to a single region (15 of 39), followed by the WI region with seven. However, most distributional overlap was not restricted to any one biogeographical region, but spanned the Indo-Pacific realm. Sister-species pairs with realm-wide overlap had significantly greater than expected distributional overlap and symmetry compared to the full pool of sympatric sister-species (Fig. 7.3c). Sister-species pairs with overlapping distributions in the Atlantic regions were similar in their comparatively high degree of sympatry (significantly greater than expected in the WA) and distributional symmetry (significantly greater than expected in the EA). In contrast, sister-species with overlapping distributions in the CIP had significantly lower than expected distributional overlap and symmetry (Fig. 7.3c). The WI region also showed significantly low distributional symmetry and a similarly low degree of sympatry, although, the latter was not significantly different from the bootstrapped distribution.

Overall, distributional symmetry among sympatric sister-species (n = 60) was significantly higher than the bootstrapped distribution of mean values drawn from all sister-species pairs. Sympatric sister-species with a high degree of sympatry generally also had high distributional symmetry and vice versa.

7.4 Discussion

The present study identified important areas of common overlap and vicariance for sister-species pairs of coral reef fishes. This information is consistent with suggestions that biogeographical boundaries have played an important role in creating and maintaining patterns of extant species diversity. A high concentration of sympatric distributions restricted to the CIP region and concordant with hotspot of biodiversity suggests that this area may harbour sufficiently complex environments to facilitate reproductive isolation that allow closely related species to co-occur. However, the significantly lower than expected degree of sympatry among sister-species in this region suggests that it may, more frequently, be an area of secondary

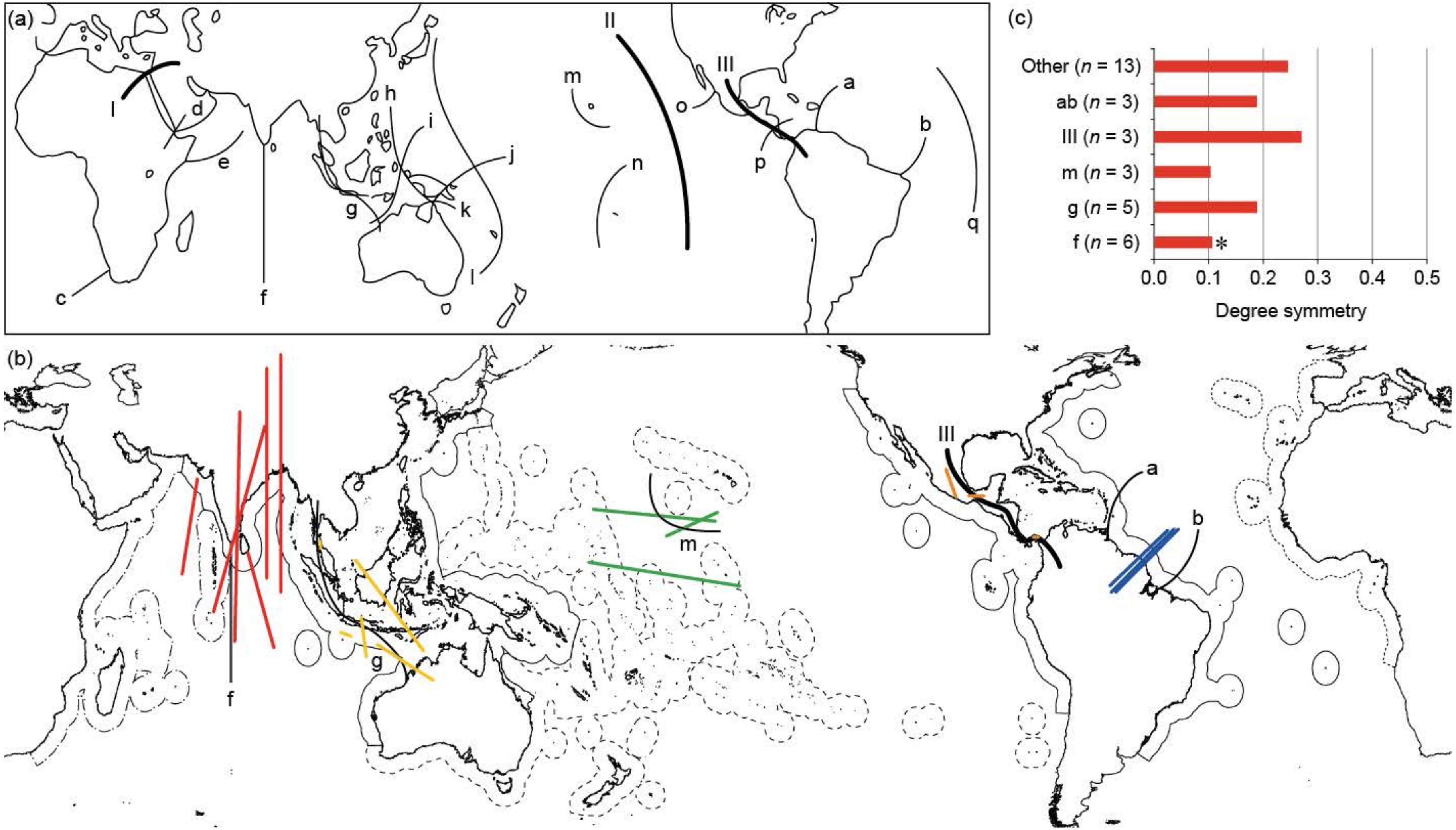

(a)
I
II
III
a
b
c
d
e
f
g
h
i
j
k
l
m
n
o
p
q
(b)
III
a
b
m
g
f
(c)
Other (n = 13)
ab (n = 3)
III (n = 3)
m (n = 3)
g (n = 5)
f (n = 6)
*
0.0
0.1
0.2
0.3
0.4
0.5
Degree symmetry

Figure 7.2 Patterns of vicariance among sister-species of coral reef fishes. (a) Previously described biogeographical barriers used to assess splits obtained from mapping the nearest linear distance between allopatric sister-species (figure modified after Bellwood & Wainwright, 2002; Rocha *et al.*, 2007; Floeter *et al.*, 2008; Luiz *et al.*, 2012). (b) Three or more concordant splits between allopatric sister-species are mapped as coloured lines in relation to corresponding biogeographical barriers. Colours corresponded to six distinct biogeographical barriers including the: Mid Indian Ocean (f, red lines), Indo-Pacific (g, yellow lines), Hawaiian Archipelago (m, green lines), Isthmus of Panama (III, orange line), and the Amazon and Orinoco (b and a, blue lines) barriers. The remaining allopatric splits (n = 13) are shown in Figure S7.1, Appendix F. (c) Average degree of distributional symmetry for allopatric sister-species that occur either side of each biogeographical barrier shown in part (b). The average degree of symmetry for allopatric splits mapped in Figure S7.1, Appendix F were grouped and plotted as ‘other’. Bootstrapping was used to test whether the mean degree of distributional symmetry differed from random distributions of mean values drawn from the full pool of allopatric sister-species. Asterisks denote significant values.

contact between species that primarily occupy adjacent ocean basins. It thus appears to act more as a Centre of Overlap (and accumulation and survival) than a Centre of Origin for recently diverged species. Interestingly, allopatric sister-species that were separated by the Indo-Pacific biogeographical barrier showed similarly low levels of distributional symmetry compared to sympatric sister-species in the CIP. The WI region and the MIOB were also associated with highly asymmetrical sister-species pairs, be they allopatric or sympatric, suggesting that these areas might be important in peripatric speciation. The most prominent vicariant barrier, however, seems to lie outside of the IAA prompting a re-evaluation of the factors that may influence the nature and strength of the MIOB in separating reef fish species.

Vicariance

Of the three major biogeographical barriers, and 17 other previously described barriers, the results of this biogeographical analysis highlight the influence of six barriers on recent reef fish divergence. These include one of the three major barriers, the IOP, and five soft barriers, the: MIOB, Indo-Pacific, Hawaiian Archipelago, and the Amazon and Orinoco barriers (Fig 7.2b).

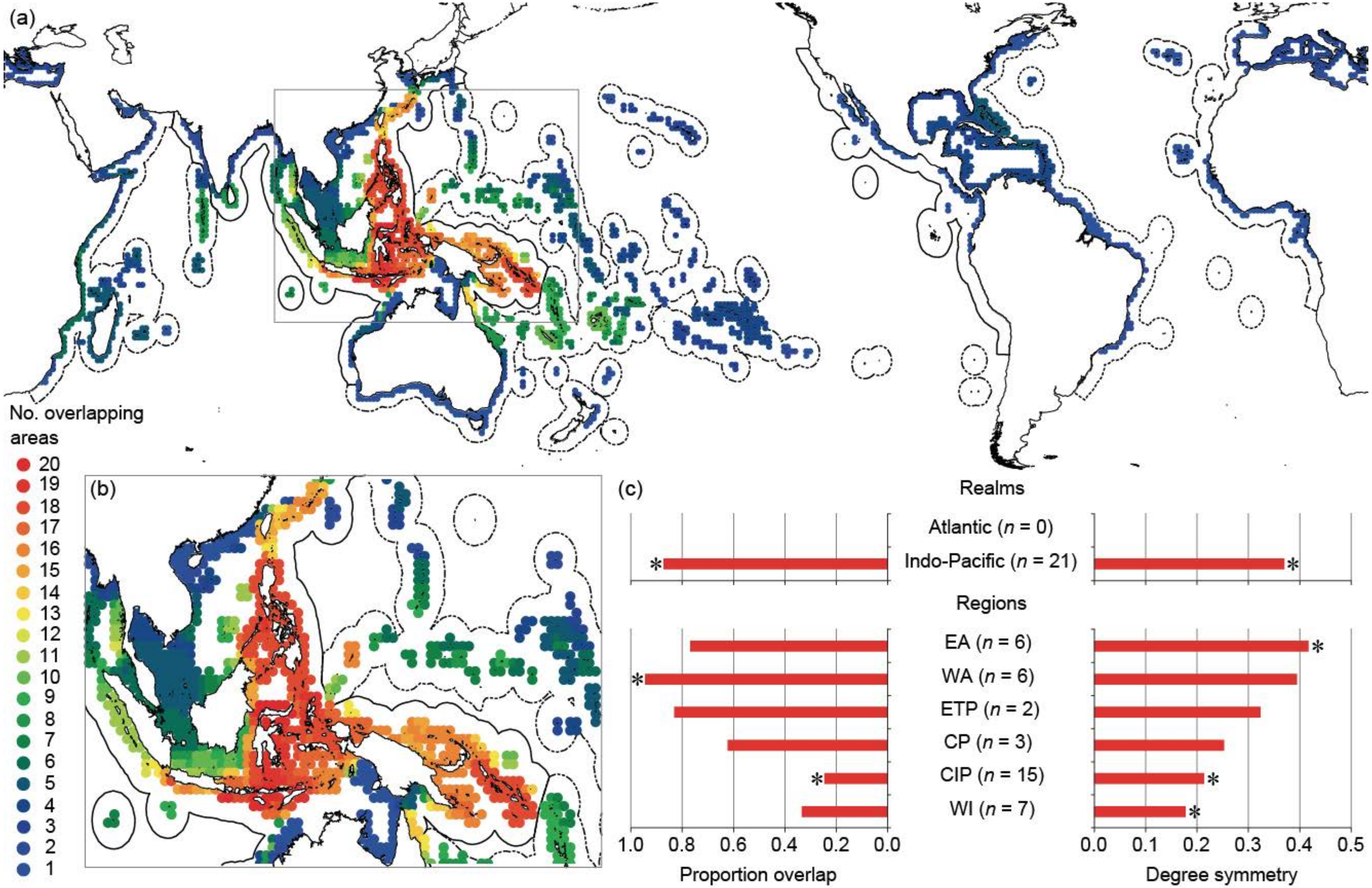

(a)
No. overlapping areas
20
19
18
17
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
(b)
(c)
Realms
Atlantic (*n* = 0)
Indo-Pacific (*n* = 21)
Regions
EA (*n* = 6)
WA (*n* = 6)
ETP (*n* = 2)
CP (*n* = 3)
CIP (*n* = 15)
WI (*n* = 7)
1.0
0.8
0.6
0.4
0.2
0.0
Proportion overlap
0.0
0.1
0.2
0.3
0.4
0.5
Degree symmetry
*
*
*
*
*
*
*
*

Figure 7.3 Patterns of sympatry among sister-species of coral reef fishes. (a) Heatmap showing the concentration of overlap among sympatric sister-species. Biogeographical areas are outlined following Kulbicki *et al.* (2013) and included the Atlantic and Indo-Pacific realms; and the Western Indian (WI), Central Indo-Pacific (CIP), Central Pacific (CP), Eastern Tropical Pacific (ETP), Western Atlantic (WA), and Eastern Atlantic (EA) regions. (b) Enlarged view of the CIP region showing the highest concentration of overlapping areas. (c) Average degree of sympatry and distributional symmetry for sympatric sister-species with overlapping distributions restricted to different regions. Those sister-species with overlapping distributions not restricted to a particular region were grouped by realm. Bootstrap re-sampling was used to test whether the mean degree of sympatry and the mean degree of distributional symmetry differed from random distributions of mean values drawn from the full pool of sympatric sister-species. Asterisks denote significant values.

Despite the repeated significance of the Terminal Tethyan Event (TTE; ‘I’ in Fig. 7.2a) and the East Pacific Barrier (EPB; ‘II’ in Fig. 7.2a) in the evolution and biogeography of marine organisms, including reef fishes (Bellwood *et al.*, 2004; Bernardi *et al.*, 2004; Lessios & Robertson, 2006; Bellwood *et al.*, 2010; Cowman & Bellwood, 2013b; Chapter 4), these barriers did not appear to play a major role in the vicariance of recently diverged extant species. The timing of the TTE [12–18 Ma (Steininger & Rögl, 1984)] places its influence much deeper in the evolutionary history of reef fishes and away from vicariance of contemporary species whose ages typically range between 1 and 5 Ma (Chapter 6). Consequently, the influence of the TTE was in dividing genera and major clades of reef fishes and its lack of influence on the divergence of present-day species was not surprising.

Unlike the TTE and IOP, the EPB does not have an associated specific time during which it is expected to have influenced vicariance. Rather than hard, physical landmasses to impede dispersal, the EPB consists of a large area of deep water (~5000 km) separating the tropical eastern Pacific from the central Pacific Ocean. It is considered to be the widest marine biogeographical barrier. Yet, this vast expanse of ocean had little apparent impact on vicariance of extant species, with only one split solely associated with this barrier (Fig. S7.1, Appendix F). Because of its soft nature, the EPB is semipermeable and acts as a filter to dispersal and genetic connectivity in a largely unidirectional manner from west to east (Lessios *et al.*, 1998; Lessios & Robertson, 2006). Thus, it primarily creates geographical range boundaries for some species and populations, but not in a way that consistently produces isolated populations on either side that would eventually lead to vicariant species. If populations do become isolated in such a way that may lead to vicariance, those to the east of the barrier are isolated in a region with comparatively low species richness (Kulbicki *et al.*, 2013), suggesting that the survival of a species in this region may be more difficult, or that the dispersion of fauna from the CP has been sporadic.

The IOP is the most recent hard barrier to have separated populations of marine organisms, and the timing of its rise [3.1–16 Ma (Coates & Obando, 1996)] falls within the range of extant reef fish species divergence (Chapter 6). The IOP has been implicated in clade divergence for a number of reef fish genera (Barber & Bellwood, 2005; Alva-Campbell *et al.*, 2010; Chapter 4). Thus, the detection of a vicariant signal across this barrier was not unexpected. However, considering this is a hard barrier to dispersal, the strength of its vicariant signal was surprisingly weak relative to other soft barriers. This may be the result of subsequent events. Previous studies of deeper phylogenetic lineages of reef fishes have shown that hard barriers, including the IOP, can produce temporally diffuse vicariance (Cowman & Bellwood, 2013b). If subsequent species divergence within the Atlantic or ETP occurred after geminate species pairs were separated by the land bridge, the present analysis of sister-species pairs would not detect the IOP as a vicariant barrier. Thus, it appears that although the IOP has influenced extant species diversity, given the timing and duration of its rise, it likely had a greater impact on the divergence of clades within reef fish genera than on recent species divisions.

Of the 17 other previously identified biogeographical barriers (Bellwood & Wainwright, 2002; Rocha *et al.*, 2007; Floeter *et al.*, 2008; Luiz *et al.*, 2012), five were concordant with vicariance between sister-species in the present study. The most prominent barrier detected herein separates the WI region from the CIP region. It is one of the least discussed barriers in the literature. The Mid-Indian Ocean Barrier (MIOB) offers few indications of the physical forces underlying vicariance in this location, but it is clear from the results herein, and the work of others (Winterbottom, 1986; Springer, 1988; Blum, 1989; Springer & Williams, 1994; Randall, 1998), that it is important in separating sister-species.

The northward movement of the Indian sub-continent has been suggested as the establishing mechanism underlying the MIOB (Springer, 1988). This movement may have caused vicariance among ancient lineages of reef fishes, but as noted by Briggs (1989), it was unlikely to have influenced separation at the species level. Based on the age estimates now available for contemporary reef fish species (1–5 Ma; Chapter 6), it is conclusive that the movement of the Indian continent between 50 and 148 Ma cannot explain vicariance among recently diverged species. What, then, drives the separation of sister-species at this location?

Previous studies have noted that India’s continental shelf areas are largely devoid of coral reefs (Springer, 1988; Bakus *et al.*, 2000). The scarcity of reefs in this region is generally attributed to its inhospitable nature as a result of extensive sandy coasts, numerous river deltas and consequent freshwater plumes that decrease salinity and generate intense siltation (Springer, 1988; Bakus *et al.*, 2000). It follows that coral reef fishes may also be inhibited by these same physical factors (Vivekanandan *et al.*, 2012), or are generally scarcer due to the lack of suitable habitat. Any of these environmental factors may cause a barrier to the dispersal and genetic connectivity of reef fishes. However, it is proposed herein that the physical consequences of large sediment loads may be primarily responsible for the strength of this biogeographical barrier.

The Ganges-Brahmaputra-Megna River delta spans approximately 350 km, discharging into the Bay of Bengal and contains an unusually high amount of sediment compared to other major rivers of the world (Curray *et al.*, 2003). A large portion of the sediments are derived from the fast-eroding Himalaya and have formed an extensive alluvial plain, a large delta system and the largest submarine fan in the world, the Bengal Fan (Singh, 2008). The Bengal Fan was formed by turbidity currents where sediment-laden water moves rapidly down a slope because of its higher density relative to the surrounding water. These currents can move exceptionally fast and velocity estimates for turbidity currents in the Bengal Fan range from 4–10 m/s (Curray *et al.*, 2003). Current speeds of this nature could easily sweep away any viable marine larvae that happened to be caught at the surface, in the vicinity of discharge onto the continental shelf. Thus, if a propagule managed to survive the generally inhospitable nature of the water due to low salinity and high sediment content, it would likely not survive being swept to depth in a turbidity current. The influence of turbidity currents as a barrier to dispersal may be exacerbated if the pelagic larval phase of newly spawned reef fishes coincides with the expansion of the river plume during warmer monsoonal months.

Sedimentary analysis of the Bengal Fan traced the first deposition of sediments to the early Eocene; around the time the Indian continent collided with Asia (Curray *et al.*, 2003). The same study also revealed that the fan is still active and received large volumes of sediment during periods of lowered sea level in the Pleistocene (Curray *et al.*, 2003), a time when many extant reef fish species were diverging (Chapter 6) and taxa in the CIP were expanding into the Indian Ocean (Cowman & Bellwood, 2013a). Most of the splits between sister-species detected in the present study span the Arabian Sea, Bay of Bengal and the Andaman Sea, encompassing the entire coastline of India. Therefore, it may be inappropriate to attribute the vast distance between sister-species solely to turbidity currents on India’s far eastern coast. Rather, there may be a number of barriers acting independently, or in concert, that contribute to this vicariance.

Interestingly, the Indus River, which flows through Pakistan, India and China and drains into the Arabian Sea over an area of approximately 210 km, is also one of the most sediment-laden river deltas on earth. Like the Ganges, the Indus now derives the bulk of its sediment load from the Himalaya, and has formed an associated submarine fan (Clift & Blusztajn, 2005). However, approximately five million years ago major rivers of the Punjab rerouted into the Indus, increasing its drainage and erosional products from the Himalaya (Clift & Blusztajn, 2005). Around the same time (over the past 4 Myr), the Himalaya have experienced faster erosion as a result of a stronger monsoon (Zhisheng *et al.*, 2001). Turbidity currents in these two sediment-laden river systems, in combination with the increased strength of the monsoon over the past 4 Myr may have underpinned the strength and magnitude of this biogeographical barrier.

Symmetry between allopatric sister-species across the MIOB was among the lowest detected. The barrier between the CP and the Hawaiian Archipelago had similarly low symmetry among its fewer allopatric sister-pairs. The boundary separating the Hawaiian Archipelago is likely to be maintained by the relative isolation of the islands compared to the bulk of reef fish and coral diversity in the CIP (Chapter 6, Randall, 1998; Allen, 2008; Gaither *et al.*, 2011b). However, the boundary is dynamic and has been breached, with founder events that can lead to peripatric speciation (Hourigan & Reese, 1987; Jokiel, 1987; Kay & Palumbi, 1987; Chapters 3, 6). Asymmetry in the distributions of species that inhabit the Hawaiian Islands and the WI region, to the west of the MIOB, suggests that both areas are likely important locations for peripheral speciation, but with differing temporal patterns of divergence (Chapter 6). Neither of these areas, however, exclusively receive peripatric species because genetic connectivity and dispersal also occurs from the Hawaiian Islands toward the CIP (Eble *et al.*, 2011) and from the isolated Red Sea province toward the larger WI region (DiBattista *et al.*, 2013).

The second most prominent vicariance signal was detected in the CIP. Barrier ‘g’, as it appears in Figure 7.2, was first delineated in Blum’s study of closely related chaetodontid species (1989). Later, Bellwood and Wainwright (2002) included this barrier in their discussion of the history and biogeography of reef fishes, but without referring to it by a particular name. Rocha *et al.* (2007) also identified a barrier in this location and referred to it as the Sunda Shelf barrier based on the work of Randall (1998). However, a plethora of other barriers have also been described within the broad CIP region including the Sunda shelf (Fleminger, 1986; Woodland, 1986; Springer, 1988; Springer & Williams, 1994; Barber *et al.*, 2000; Reid *et al.*, 2006). The area encompassing these barriers is often referred to generally as Wallacea (Mayr, 1944), and division in the area is attributed to the Indo-Pacific Barrier (Briggs, 1974; Gaither *et* *al.*, 2010). Barrier ‘g’ stands apart from these barriers in that it lies not within the biodiversity hotspot (regardless of its designation), but along its western boundary with the Indian Ocean. It may therefore be regarded as the Sunda Margin Barrier, reflecting the Sunda affinities highlighted by Rocha *et al.* (2007), but emphasising its marginal location, a position which points to different, and potentially older, biogeographical influences than those that supported vicariance within the broader IAA and Sunda Shelf regions.

Despite the array of described barriers within the CIP, the majority of their associated vicariance has been attributed to Plio-Pleistocene changes in sea level (reviewed in Hoeksema, 2007; Carpenter *et al.*, 2011; Bellwood *et al.*, 2012). Others have identified the northern movement of Australia [over the past 120 Ma; (Hall, 1998)] (Woodland, 1986), or regional tectonic activity (*c.* 8–16 Ma) that changed surface circulation patterns in the Indian and Pacific Oceans (Springer & Williams, 1994) as potential causes of vicariance within the CIP. However, these events are more likely to have influenced vicariance among ancient lineages of reef fishes. The ages of extant reef fish species (1–5 Ma; Chapter 6) suggest that recent events, such as sea level changes, may provide a more plausible explanation for extant species-level vicariance.

Barriers between sister-species mainly track the boundary between the Sunda and Sahul shelves and the Indian Plate. These shelves formed hard barriers during periods of lowered sea level and potentially restricted all but the most resilient species to their respective oceans (Bowen *et al.*, 2001; Horne *et al.*, 2008; Gaither *et al.*, 2010; Reece *et al.*, 2010). Recent sea-level oscillation has also been implicated in intra-specific genetic isolation (Bay *et al.*, 2004; Klanten *et al.*, 2007; Horne *et al.*, 2008; Leray *et al.*, 2010; Gaither *et al.*, 2011a), which signifies a degree of vicariance that may lead to geminate species. However, the oscillating nature of sea level also allows for the overlap of adjacent Indian and Pacific Ocean faunas (Rocha *et al.*, 2007; Briggs & Bowen, 2013). Evidence for this is discussed below in the subsection ***Sympatry***. Overall, it seems that the Sunda Margin Barrier (‘g’) has potentially operated across ecological and evolutionary time scales, with the divergence of extant reef fish species likely caused by events in the recent past.

In the Atlantic, the vicariant signal detected between allopatric sister-species lies between two previously defined barriers, the Amazon and Orinoco river outflows (b and a, respectively in Figure 7.2) (Blum, 1989; Hastings & Springer, 1994; Bellwood & Wainwright, 2002). This is consistent with recent studies, which have shown the freshwater outflows from the Amazon and Orinoco rivers act as a joint barrier to dispersal spanning 2300 km of the South American coastline (Rocha, 2003; Floeter *et al.*, 2008; Luiz *et al.*, 2012). The results of the sister-species analysis expand on the work of others in suggesting that the Amazon-Orinoco barrier has acted as a filter to dispersal between the north and south-western Atlantic throughout its 11 Myr history (Hoorn *et al.*, 1995), influencing vicariance between species. This may be particularly important during periods of low sea level (Kaneps, 1979; Rocha, 2003; Robertson *et al.*, 2006). However, at times the barrier also allows mixing between populations in the north and south (Floeter *et al.*, 2008; Rocha *et al.*, 2008). Furthermore, the barrier may impact species differently depending on their life history characteristics (Rocha *et al.*, 2002).

Overall, vicariance between coral reef fish sister-species has resulted from biogeographical barriers that have acted at some time over the past 5 Myr. Most of these barriers act as filters to dispersal, with only one hard barrier having caused detectable vicariance between sister-species. The permeability and dynamic nature of soft barriers, in combination with founder events have likely facilitated the majority of vicariance among sister-species leading to the complex patterns of biodiversity observed today.

Sympatry

For coral reef fishes, the concentrated area of overlap among sympatric sister-species bears remarkable resemblance to the hotspot of reef fish biodiversity (Allen, 2008), forming a striking bull’s-eye pattern with the highest concentration of overlap within the CIP and marked decline in all outward directions. This lends strong support to the Centre of Overlap model that predicts range expansion of isolated Pacific and Indian Ocean faunas following sea-level rise and subsequent overlap of sister-species within the CIP (Woodland, 1983). However, it may also be possible for the CIP region to have attained the highest concentration of overlap due to its position in the middle of three bounded Indo-Pacific regions (Connolly *et al.*, 2003), or because of the extent of coral reef habitat in the area (Bellwood & Hughes, 2001; Bellwood *et al.*, 2005), which would facilitate rapid expansion and overlap of ranges. Additionally, if overlap among sister-species is not largely restricted to the CIP, or if sister-species have widely overlapping distributions, this may further diminish support for the Centre of Overlap model.

However, the CIP contained the largest number of overlapping distributions restricted to a single biogeographical region, and these sister-species had significantly less than expected distributional overlap. This evidence, considered together with corresponding patterns of vicariance associated with the Sunda Margin Barrier, further highlights the importance of the CIP as an area of evolutionary dynamism where the separation of Indian and Pacific Ocean faunas and their subsequent range expansion has led to increased species overlap and higher regional biodiversity.

The average age of reef fish species, from 1 to 5 Ma (Chapter 6), suggests that tectonic rearrangements in the Miocene (cf. Renema *et al.*, 2008) need not be invoked as the mechanism of isolation responsible for separating extant Indian and Pacific Ocean reef fish species in the CIP (cf. Rocha & Bowen, 2008; Gaither & Rocha, 2013), although geological events of this nature likely contributed to some vicariance among reef fish lineages. Species diversification is temporally concordant with the initial suggestion proposed by Woodland (1983) that Pleistocene low sea level stands may have led to the isolation of populations in the adjacent ocean basins. The detection of a vicariant signal among allopatric sister-species across the Sunda Margin Barrier lends further support to the notion that the Sunda and Sahul shelves influenced species divergence as land barriers during low sea level stands.

Distributional asymmetry between sister-pairs whose overlap was restricted to the CIP region also supports the Centre of Overlap and Accumulation models. Such distributional patterns may have arisen from the expansion of Pacific or Indian Ocean species into the CIP region whereby vicariance may have caused peripheral divergence and produced sister-pairs with asymmetrical distributions. However, if the most geographically restricted species in the aforementioned example originated in the CIP, then this may also be considered as evidence for the Centre of Origin model. Sympatric sister-species in the WI region had similarly low levels of distributional symmetry. Comparatively, the WI region has limited, bounded habitat, which could account for asymmetry among sister-species whose distributions overlap in the region. Alternatively, peripatric speciation may be important in producing peripherally isolated species in this region (Chapters 4, 6). Overall, peripatric speciation appears to play a greater role in generating allopatric sister-pairs, given the significance of their asymmetric distributions. While it may also play a role in generating sympatric sister-species in certain regions, subsequent range expansion likely leads to increasing distributional overlap and symmetry among sympatric sister-pairs.

The concentration of overlap among sympatric sister-species in the CIP is not inconsistent with other hypotheses that invoke range contraction of previously widespread species, or range expansion of species that originated in the CIP region. Over time the dispersal ability of most reef fishes could produce the patterns detected herein under the latter scenario (Lynch, 1989). However, multiple lines of congruent evidence from both allopatric and sympatric sister-species pairs that highlight patterns unique to the CIP region, and the lack of a general explanation for why such common range contraction may have occurred, would favour the Centre of Overlap model. Alternate hypotheses are more difficult to evaluate without direct evidence of the location of species origination. While such records remain elusive, phylogeographic data may provide insights into processes of genetic isolation preceding species divergence. Indeed, divergent genetic lineages within many coral reef species complexes also overlap in a manner consistent with the Centre of Overlap hypothesis (Bay *et al.*, 2004; Craig *et al.*, 2007; Horne *et al.*, 2008; Gaither *et al.*, 2011a; DiBattista *et al.*, 2012).

The majority of sympatric sister-species in the present study had high levels of overlap and distributional symmetry with ranges often spanning large portions of the Indo-Pacific realm. This does not necessarily refute evidence from the Centre of Overlap model because high dispersal potential may be conserved between closely related species enabling them to vastly expand their geographical ranges during periods of connectivity and increased habitat availability (Bellwood & Wainwright, 2002). Furthermore, barriers to dispersal do not influence species in a uniform manner as certain life history characteristics may make species more or less susceptible to range expansion (Luiz *et al.*, 2012). However, widespread species are generally older than their regionally restricted counterparts (Chapter 6) and therefore are more likely to have been impacted by extinction. Thus, widespread, highly sympatric sister-pairs may be the only extant species remaining in a clade where other, more restricted, species went extinct or have not yet been described. Consequently, they may not actually be sister-species. For example, sympatric sister-pairs in the Atlantic show similar patterns of widely symmetrical overlapping ranges. However, faunal turnover and substantial loss of marine taxa in the Plio-Pleistocene has been documented in the Atlantic (Bellwood, 1997; O'Dea *et al.*, 2007) and may have played a key role in establishing the significantly high degree of overlap and distributional symmetry observed in extant sympatric species in this realm.

Regardless of the mechanisms responsible for the patterns of concentrated overlap among sister-species within the CIP (Centre of Overlap and other models), evolutionary theory stipulates that in areas of geographical overlap closely related species must differ in their ecological niche or mate selection to maintain reproductive isolation, which is required for their persistence as separate species (Brown & Wilson, 1956; Hardin, 1960). The CIP may encompass the geological complexity, habitat heterogeneity and reef area required to facilitate the co-occurrence of closely related genetic lineages (i.e. populations and species) while maintaining their separation through nice partitioning. Nevertheless, hybridization of reef fish species in and around the CIP (McMillan *et al.*, 1999; Yaakub *et al.*, 2006; Hobbs *et al.*, 2009), implies that niche partitioning or mate selection may take some time to develop and establish full reproductive isolation upon secondary contact of closely related species. Alternatively, these mechanisms may break down at times (Montanari *et al.*, 2012), leading to the production of hybrid species.

7.5 Conclusions

This work has helped elucidate areas important in the generation and maintenance of modern coral reef fish biodiversity. Focusing on well-established sister-species and their relative geographical distributions, I have shown that species vicariance has resulted from biogeographical barriers that have been in place over the past 5 Myr. The permeability and dynamic nature of most of these barriers suggest that they act as filters to dispersal in combination with founder events. Recent expansion of geographical ranges probably underpinned the distinctive concentration of overlap of sympatric sister-species in the CIP, a pattern that may be maintained by the geological complexity and extensive heterogeneous habitat of the region. Together, this evidence largely supports the Centre of Overlap hypothesis while highlighting the dynamic evolutionary history of reef fish species in the central Indo-Pacific region.

Chapter 8: Concluding Discussion

A framework detailing the chronology of extant species divergence combined with detailed biogeographical data is essential to investigate evolutionary processes that shape patterns of biodiversity. This thesis combined molecular phylogenetic approaches with biogeographical analyses to reconstruct the evolutionary history of extant reef fish species. The development of methods to minimize the impacts of extinction and successive peripheral speciation on age estimation have resulted in more precise age estimates for reef fish species (Chapters 5, 6). These methods are widely applicable to other taxonomic groups and their application may shed light on previously unrealized processes of divergence for some. Across all biogeographical areas throughout the world's tropical oceans, species of coral reef fishes have similar evolutionary ages (Chapter 6). A combination of evolutionary processes has shaped the distinctive biogeographical patterns of modern-day reef fishes, including fluctuations in soft biogeographical barriers (Chapter 7), founder events (Chapters 3–7), and potential divergence in sympatry (Chapters 4, 6 and 7). As a whole, this thesis has identified common evolutionary and biogeographical patterns among reef fish species and begun to unravel potential processes involved in species divergence and maintenance that can be further explored within the spatiotemporal framework it has provided.

When did reef fish species diverge?

The findings of this research emphasize the need to distinguish between the timing of species divergence and the evolutionary age of species. Phylogenetic methods considered within a temporal framework provide estimates of the most recent divergence events between extant taxa. Applied to two case studies, these methods revealed that species divergence within the reef fish genera *Anampses* and *Pomacanthus* occurred predominantly from the mid-Miocene onward (Chapters 3, 4). Across a broader range of reef fish species from four families (Chaetodontidae, Labridae, Pomacanthidae and Epinephelidae) temporally-calibrated phylogenetic hypotheses revealed that both the Atlantic and Indo-Pacific biogeographical realms experienced divergence events throughout the past nine million years, with two distinctive periods of divergence among species endemic to the Hawaiian Islands (Chapter 6).

Evolutionary ages of species can be estimated by assembling recently diverged sister-pairs and applying a biogeographically focused approach to minimize the impacts of historical events (Chapters 5, 6). The application of this method uncovered a general pattern of geographical range expansion with increasing evolutionary age among coral reef fish species (Chapter 5). General evolutionary theories that predict patterns of this nature (Willis, 1922; Wilson, 1961; Hubbell, 2001; Ricklefs & Bermingham, 2002) have limited supporting evidence from marine systems and the results presented herein lend them initial support. The evolutionary age of extant reef fish species ranges from 1 to 5 Ma, with markedly little variation in the ages of species distributed throughout the world’s biogeographical realms and regions (Chapter 6). Well-known vicariance events such as the Terminal Tethyan Event and the rise of the Isthmus of Panama had little to no impact on the divergence of extant reef fish species (Chapter 7), rather these events produced lineage divisions within genera (Chapters 3, 4).

The chronology of species divergence presented in this thesis contributes to the broad study of the evolutionary history of reef fishes (reviewed in Bellwood *et al.*, 2012). The evolutionary ages estimated for species within the Chaetodontidae, Labridae, Pomacanthidae and Epinephelidae add to the growing work on age estimation of coral reef fish species (McCafferty *et al.*, 2002; Klanten *et al.*, 2004; Read *et al.*, 2006; Bernardi *et al.*, 2008; Choat *et al.*, 2012; Gaither *et al.*, 2014). Both temporal components, the timing of species divergence and the evolutionary ages of species, provide distinct information necessary to eliminate the likely influence of palaeogeographical events on the creation and maintenance of biodiversity.

How are closely related species geographically distributed?

The geographical distribution of closely related species in conjunction with the chronology of their divergence suggests that successive peripheral speciation, or peripheral budding, may have generated substantial species diversity among reef fishes (Chapters 3-7). These results add to the mounting evidence that founder events and peripatric speciation are important in faunal enrichment within marine systems where few hard barriers to dispersal exist (Paulay & Meyer, 2002; Queiroz, 2005; Floeter *et al.*, 2008; Drew & Barber, 2009; Budd & Pandolfi, 2010; Gaither *et al.*, 2010; Malay & Paulay, 2010; DiBattista *et al.*, 2011; Fitzpatrick *et al.*, 2011). Successive peripatric speciation has the potential to generate closely related species with highly overlapping geographical distributions when the founded area is constrained (Chapters 3, 4, 6, 7). Common vicariance separating sister-species across the Mid-Indian Ocean Barrier (MIOB) confirmed the marked influence of this previously identified biogeographical barrier (Winterbottom, 1986; Springer, 1988; Blum, 1989; Springer & Williams, 1994; Randall, 1998; Bellwood & Wainwright, 2002) on the divergence of contemporary reef fish species (Chapter 7). Like most soft biogeographical barriers (Rocha, 2003; Floeter *et al.*, 2008), the MIOB is permeable and dynamic. The oscillation of permeable barriers appears particularly important in facilitating founder events during periods of favourable environmental conditions (Chapters 6, 7).

This thesis highlights the extensive nature of sympatry among closely related reef fish species (ranging from partial to complete distributional overlap) and emphasizes the necessary disentanglement of process from pattern by demonstrating that a number of speciation modes likely led to the divergence of sympatric species (Chapters 4, 7). Specifically, the high concentration of distributional overlap among sister-pairs concordant with the hotspot of biodiversity in the central Indo-Pacific supports this region as a Centre of Overlap, where vicariance followed by geographical range expansion has likely underpinned regional patterns of overlap and biodiversity (Chapter 7). These findings agree with a large number of studies on intra-specific genetic divergence of marine organisms (reviewed in Carpenter *et al.*, 2011;

Gaither *et al.*, 2011a; DiBattista *et al.*, 2012; Ackiss *et al.*, 2013; DeBoer *et al.*, 2014; Raynal *et al.*, 2014) and offer support for the notion that the central Indo-Pacific region is an evolutionarily dynamic area.

What does this suggest about the processes responsible for contemporary patterns of reef fish diversity?

Overall, this thesis combined a detailed description of the temporal evolution of coral reef fish species with biogeographical analyses to form a spatiotemporal framework that facilitated the exploration of evolutionary processes responsible for contemporary patterns of biodiversity. Rather than the traditional view of a single predominant evolutionary process, this work supports the notion that multiple, alternate processes have acted in concert to generate and maintain contemporary patterns of biodiversity. Modern-day reef fish species have temporally constrained divergence coupled with complex biogeographical patterns. The ability to investigate the mechanistic processes of evolution at the species level within a spatiotemporal framework is a necessary first step to the successful prediction of future patterns of biodiversity.

Every effort has been made to reduce potential confounding effects within this thesis; however, the results must be interpreted with care. The primary concern pertains to taxon sampling and the probable existence of cryptic, yet to be described species. Future studies may test the generality of the results by increasing taxon sampling and including any newly described, or probable biological species (Chapters 5–7). The temporal estimates made herein describe the occurrence of events along a continuous speciation process with particular reference to nominal species. Changing the focal unit will shift the order of magnitude of temporal estimates and may produce different biogeographical patterns. The inclusion of more sequence data and the implementation of future analytical methods may also expand on the work herein. Specifically, coalescent methods applied within the context of robust phylogenetic frameworks may provide a more thorough interpretation of the evolutionary history of coral reef fish species (Bowen *et al.*, 2001; Nichols, 2001; Bowen *et al.*, 2006b; van Herwerden *et al.*, 2006; Leray *et al.*, 2010; Eytan *et al.*, 2012; Harrington & Near, 2012; Lessios & Robertson, 2013). Experimental work and the incorporation of more detailed ecological data are also likely to provide new evidence to evaluate the processes of divergence proposed herein (Munday *et al.*, 2004; Bolnick *et al.*, 2006; Crow *et al.*, 2010). It will be of great interest to discover the ways through which sympatric sister-species arise and persist, and what role, if any, the Coral Triangle plays in their concentrated coexistence.

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Appendix A: Supplementary materials for Chapter 2

The following supplemental accompanies general methods for the articles

The role of peripheral endemism in species diversification: evidence from the coral reef fish genus *Anampses* (Family: Labridae)

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Molecular Phylogenetics and Evolution 62(2): 653–663 (2012)

Evolution of sympatric species: a case study of the coral reef fish genus *Pomacanthus* (Pomacanthidae)

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Journal of Biogeography 40(9): 1676–1687 (2013)

On the relationship between species age and geographical range in reef fishes: are widespread species older than they seem?

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Global Ecology and Biogeography (2014 in press)

Temporal evolution of coral reef fishes: global patterns and disparity in isolated locations

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Journal of Biogeography 41(11): 2115–2127 (2014)

The geography of speciation in coral reef fishes

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Submitted for publication

TABLES

Table S2.1 Locality information and GenBank accession numbers for specimens used in the *Anampses* case study (Chapter 3). Includes representative sister group and outgroup species.

Table S2.2 Collection localities and GenBank accession numbers for specimens used in the *Pomacanthus* case study (Chapter 4). Includes representative sister group and outgroup species.

Table S2.3 GenBank accession numbers for species included in the multi-family phylogenetic analyses (Chapters 5–7).

Table S2.4 Details of fossil data used for temporal-calibration of the multi-family phylogenetic analyses (Chapters 5–7) in BEAST.

Table S2.5 Partition scheme and models of evolution selected by PARTITIONFINDER under the Bayesian Information Criterion (BIC) used in the multi-family phylogenetic analyses (Chapters 5–7).

REFERENCE LIST

Table S2.1 Locality information and GenBank accession numbers for specimens used in the *Anampses* case study (Chapter 3). Includes representative sister group and outgroup species. (—) = location/sequence unavailable. Where possible individual specimens were sampled from different locations.

Specimen ID	**Genus**	**Species**	**Collection Locations**	**GenBank Accession Numbers**		
Ingroup taxa				**12s**	**CO1**	**S7**
109	*Anampses*	*caeruleopunctatus*	Moorea	JN935296	JN935324	JN935348
822			Lizard Island, GBR	JN935297	JN935325	JN935349
1431			Mauritius	JN935298	JN935326	JN935350
940	*Anampses*	*chrysocephalus*	Hawaii	JN935299	JN935327	JN935351
1293			Hawaii	JN935300	—	—
05	*Anampses*	*cuvier*	Hawaii	JN935301	JN935328	JN935352
06			Hawaii	JN935302	JN935329	JN935353
956	*Anampses*	*elegans*	Lord Howe Island, NSW	JN935303	JN935330	JN935354
974			Lord Howe Island, NSW	JN935304	JN935331	JN935355
979	*Anampses*	*femininus*	Lord Howe Island, NSW	JN935305	JN935332	JN935356
776	*Anampses*	*geographicus*	Day Reef, GBR	JN935306	JN935333	JN935357
825			Lizard Island, GBR	JN935307	JN935334	JN935358
1282	*Anampses*	*lennardi*	Gove Peninsula, NT	JN935308	JN935335	JN935359
1291	*Anampses*	*lineatus*	Bali	JN935309	JN935336	JN935360
1399			Mauritius	JN935310	JN935337	JN935361
1490	*Anampses*	*melanurus*	Vanuatu	JN935311	JN935338	JN935362
186	*Anampses*	*meleagrides*	Bird Island, Seychelles	JN935312	—	JN935363
692			Pohnpei, Micronesia	—	JN935339	—
1186			Cocos (Keeling) Islands	JN935313	JN935340	JN935364
252	*Anampses*	*neoguinaicus*	Yonge Reef, GBR	JN935314	JN935341	JN935365
251			Yonge Reef, GBR	JN935315	—	JN935366
82	*Anampses*	*twistii*	Moorea	JN935316	JN935342	JN935367
1170			Cocos (Keeling) Islands	JN935317	JN935343	JN935368
Representative sister group taxa						
502 [a]	*Halichoeres*	*hortulanus*	Indonesia	—	FJ583502	—
208 [b]			Indonesia	—	—	EF488031
380 [c]			Indonesia	AY850823	AY850761	—
761 [d]			—	AY279601	—	—
75	*Halichoeres*	*marginatus*	Trunk Reef, GBR	JN935318	JN935344	JN935369
230			Pelorus Island, GBR	JN935319	—	—

Specimen ID	**Genus**	**Species**	**Collection Locations**	**GenBank Accession Numbers**		
Representative sister group taxa cont.				**12s**	**CO1**	**S7**
511 [a]			—	—	FJ583511	—
1138	*Macropharyngodon*	*bipartitus*	Maldives	JN935320	JN935345	EF488039
1139			Maldives	JN935321	JN935346	EF488040
1373	*Macropharyngodon*	*cyanoguttatus*	Mauritius	JN935322	—	EF488041
1374			Mauritius	JN935323	JN935347	EF488042
Outgroup taxa						
38 [e]	*Symphodus*	*melops*	Portugal	AY092049	—	—
197 [e]			Portugal	AF414197	—	—
607 [f]			Scotland	—	—	—
621 [g]			—	—	GQ341604	GQ341621

Publication source for sequences obtained from GenBank: [a] Steinke et al. (2009); [b] Yaakub et al. (2007); [c] Barber and Bellwood (2005); [d] Westneat and Alfaro (2005); [e] Almada et al. (2002); [f] Hanel et al. (2002); [g] Kazancıoğlu et al. (2009).

Table S2.2 Collection localities and GenBank accession numbers for specimens used in the *Pomacanthus* case study (Chapter 4). Includes representative sister group and outgroup species. Analyses were preformed on consensus sequences; where individual specimens were sampled from different locations, each location is listed. (—) = location/sequence unavailable. GBR, Great Barrier Reef.

			GenBank accession numbers		
Genus	**Species**	**Collection locations**	**12S**	**16S**	**S7**
Ingroup taxa					
Pomacanthus	*annularis*	Sri Lanka/Indonesia	KC820895	KC820905	KC820915
Pomacanthus	*arcuatus*	Caribbean	KC820896	KC820906	KC820916
Pomacanthus	*asfur*	Red Sea	AY530826[a]	AY530854[a]	KC820917
Pomacanthus	*chrysurus*	—	KC820897	KC820907	KC820918
Pomacanthus	*imperator*	Sri Lanka	KC820898	KC820908	KC820919
Pomacanthus	*maculosus*	Red Sea	KC820899	KC820909	KC820920
Pomacanthus	*navarchus*	Indonesia	KC820900	KC820910	KC820921
Pomacanthus	*paru*	Belize	AY530824[a]	AY530852[a]	KC820922
Pomacanthus	*rhomboides*	South Africa	KC820901	KC820911	KC820923
Pomacanthus	*semicirculatus*	Indonesia/One Tree Island, GBR	AF108574[b]	AY530844[a]	KC820924
Pomacanthus	*sexstriatus*	Lizard Island, GBR	AF108575[b]	AY530858[a]	KC820925
Pomacanthus	*xanthometopon*	Philippines/Indonesia	KC820902	KC820912	KC820926
Pomacanthus	*zonipectus*	Sea of Cortez	AY530840[a]	AY530874[a]	KC820927
Representative sister group taxa					
Pygoplites	*diacanthus*	Moorea/Younge Reef, GBR	AF108577[b]	AY530873[a]	KC820928
Holacanthus	*africanus*	Cape Verde Islands/São Tomé	KC845393[c]	KC845330[c]	KC845372[c]
Holacanthus	*tricolor*	Ceará State, Brazil/Marathon Key, Florida	KC845399[c]	KC845336[c]	KC845378[c]
Holacanthus	*limbaughi*	Clipperton Atoll	KC845391[c]	KC845328[c]	KC845370[c]
Chaetodontoplus	*duboulayi*	Mackay, GBR	KC820903	KC820913	KC820929
Chaetodontoplus	*mesoleucus*	Philippines/Indonesia	KC820904	KC820914	KC820930
Outgroup taxa					
Acanthurus	*nigricans*	Hick's Reef, GBR/Cocos (Keeling) Island	AY057239[d]	AY057286[d]	EF648230[e]
Naso	*lituratus*	—/West Australia	AF055603[f]	AF055624[f]	EF648256[e]
Zanclus	*cornutus*	—	EF616898[g]	EF616980[g]	—

			GenBank accession numbers		
Genus	**Species**	**Collection locations**	**12S**	**16S**	**S7**
Outgroup taxa cont.					
Luvarus	*imperialis*	—/Australian Museum, Sydney	AF055601 [f]	AY264587 [h]	—
Platax	*orbicularis*	Philippines	AF055597 [f]/AY279562 [h]	AF055618 [f]/AY279665 [i]	FJ167848 [j]

Publication source for sequences obtained from GenBank: [a] Bellwood *et al.* (2004); [b] Nelson *et al.* (unpublished), location unavailable; [c] Alva-Campbell *et al.* (2010); [d] Clements *et al.* (2003); [e] Marie *et al.* (2007); [f] Tang *et al.* (1999), location unavailable; [g] Fessler & Westneat (2007); [h] Klanten *et al.* (2004); [i] Westneat & Alfaro (2005), location unavailable; [j] Bellwood *et al.* (2010).

Table S2.3 GenBank accession numbers for species included in the multi-family phylogenetic analyses (Chapters 5–7). (—) = unavailable sequence. * sequences courtesy of K. Ma.

		Mitochondrial loci				**Nuclear loci**	
Genus	**Species**	**16S**	**12S**	**CO1**	**CYTB**	**TMO-4C4**	**S7**
Chaetodontidae							
Amphichaetodon	*howensis*	AY530860	AY530830	—	FJ167682	—	FJ167793
Amphichaetodon	*melbae*	EF616904	EF616820	—	—	EF617151	—
Chaetodon	*adiergastos*	EF616942	EF616859	—	—	EF617190	—
Chaetodon	*argentatus*	—	AF108505	FJ583020	U23581	—	FJ167794
Chaetodon	*aureofasciatus*	—	AF108506	—	FJ167683	—	FJ167795
Chaetodon	*auriga*	EF616943	EF616860	JF434764	JF457933	EF617191	—
Chaetodon	*auripes*	AP006004	AP006004	FJ583027	FJ167684	—	FJ167796
Chaetodon	*austriacus*	EF616910	EF616826	—	AF108583	EF617157	—
Chaetodon	*baronessa*	EF616957	EF616874	—	FJ167685	EF617205	FJ167797
Chaetodon	*bennetti*	EF616911	EF616827	JF434768	JF457936	EF617158	FJ167798
Chaetodon	*blackburnii*	JF457269	—	JF434770	JF457938	—	FJ167799
Chaetodon	*burgessi*	EF616934	EF616851	FJ583031	—	EF617182	—
Chaetodon	*capistratus*	EF616914	EF616830	JQ839993	FJ167688	EF617161	FJ167800
Chaetodon	*citrinellus*	EF616950	EF616867	JF434772	JF457940	EF617198	FJ167801
Chaetodon	*collare*	EF616924	EF616840	FJ583043	FJ167689	EF617171	FJ167802
Chaetodon	*decussatus*	—	AF108512	FJ583045	AF108587	—	—
Chaetodon	*dolosus*	EF616938	EF616855	JF493091	—	EF617186	—
Chaetodon	*ephippium*	EF616926	EF616842	JF434773	FJ167690	EF617173	FJ167803
Chaetodon	*falcula*	EF616919	EF616835	JF434776	JF457943	EF617166	—
Chaetodon	*fasciatus*	EF616940	EF616857	—	—	EF617188	—
Chaetodon	*flavirostris*	—	AF108514	FJ583053	AF108589	—	—
Chaetodon	*fremblii*	EF616925	EF616841	—	FJ973160	EF617172	FJ167804
Chaetodon	*guentheri*	EF616951	EF616868	—	—	EF617199	—
Chaetodon	*guttatissimus*	JF457281	AF108515	JF434782	JF457949	—	—
Chaetodon	*hoefleri*	EF616908	EF616824	—	—	EF617155	—
Chaetodon	*humeralis*	HM778172	—	—	FJ167692	—	FJ167805
Chaetodon	*interruptus*	—	—	—	FJ167693	—	FJ167806
Chaetodon	*kleinii*	EF616909	EF616825	JF434785	JF457952	EF617156	FJ167807
Chaetodon	*larvatus*	EF616930	EF616847	—	AF108592	EF617178	—

		Mitochondrial loci				**Nuclear loci**	
Genus	**Species**	**16S**	**12S**	**CO1**	**CYTB**	**TMO-4C4**	**S7**
Chaetodontidae cont.							
Chaetodon	*lineolatus*	EF616939	EF616856	—	AF108593	EF617187	FJ167808
Chaetodon	*lunula*	EF616944	EF616861	JF434792	JF457959	EF617192	FJ167809
Chaetodon	*lunulatus*	JF457293	AJ748307	JF434794	JF457961	—	FJ167810
Chaetodon	*madagaskariensis*	—	AJ748308	JF493098	U23598	—	—
Chaetodon	*marleyi*	—	—	JF493101	—	—	—
Chaetodon	*melannotus*	EF616945	EF616862	JF434800	JF457967	EF617193	FJ167811
Chaetodon	*mertensii*	EF616958	EF616875	JF43480	JF457969	EF617206	—
Chaetodon	*mesoleucos*	—	AF108521	—	AF108596	—	—
Chaetodon	*meyeri*	EF616936	EF616853	JF434804	JF457971	EF617184	FJ167812
Chaetodon	*miliaris*	EF616918	EF616834	—	FJ973249	EF617165	FJ167813
Chaetodon	*mitratus*	—	—	JF493105	—	—	—
Chaetodon	*multicinctus*	EF616922	EF616838	FJ583067	FJ973301	EF617169	FJ167814
Chaetodon	*ocellatus*	EF616933	EF616850	JQ839994	FJ167698	EF617181	FJ167815
Chaetodon	*ocellicaudus*	EF616946	EF616863	FJ583069	AF108598	EF617194	—
Chaetodon	*octofasciatus*	EF616952	EF616869	—	AF108599	EF617200	—
Chaetodon	*ornatissimus*	EF616953	EF616870	JF434807	JF457974	EF617201	FJ167816
Chaetodon	*oxycephalus*	EF616929	EF616845	—	AF108601	EF617176	—
Chaetodon	*paucifasciatus*	EF616937	EF616854	—	U23607	EF617185	—
Chaetodon	*pelewensis*	EF616932	EF616849	JF434809	JF457976	EF617180	—
Chaetodon	*plebeius*	—	EF616846	JQ362391	AF108602	EF617177	FJ167817
Chaetodon	*punctatofasciatus*	EF616931	EF616848	FJ583074	U23619	EF617179	—
Chaetodon	*quadrimaculatus*	EF616923	EF616839	JF434811	JF457978	EF617170	FJ167818
Chaetodon	*rafflesii*	EF616947	EF616864	FJ583082	AF108604	EF617195	—
Chaetodon	*rainfordi*	EF616935	EF616852	—	FJ167699	EF617183	FJ167819
Chaetodon	*reticulatus*	EF616916	EF616852	JF434813	JF457980	EF617163	FJ167820
Chaetodon	*robustus*	EF616921	EF616837	—	FJ167701	EF617168	FJ167821
Chaetodon	*sanctaehelenae*	—	—	—	FJ167702	—	FJ167822
Chaetodon	*sedentarius*	EF616912	EF616828	—	FJ167703	EF617159	FJ167823
Chaetodon	*selene*	EF616948	EF616865	—	—	EF617196	—
Chaetodon	*semeion*	EF616913	EF616829	FJ583083	—	EF617160	—
Chaetodon	*semilarvatus*	EF616920	EF616836	—	AJ748305	EF617167	—
Chaetodon	*speculum*	EF616917	EF616833	FJ583084	AF108606	EF617164	—
Chaetodon	*striatus*	EF616915	EF616831	JQ840450	FJ167704	EF617162	FJ167824

		Mitochondrial loci				Nuclear loci	
Genus	**Species**	**16S**	**12S**	**CO1**	**CYTB**	**TMO-4C4**	**S7**
Chaetodontidae cont.							
Chaetodon	*tinkeri*	—	—	—	U23629	—	FJ167825
Chaetodon	*trichrous*	JF457314	AJ748312	JF434815	JF457982	—	—
Chaetodon	*tricinctus*	—	—	—	FJ167706	—	FJ167826
Chaetodon	*trifascialis*	EF616927	EF616843	JF434822	JF457989	EF617174	FJ167827
Chaetodon	*trifasciatus*	EF616954	EF616871	JF434830	JF457997	EF617202	FJ167828
Chaetodon	*ulietensis*	EF616928	EF616844	JF434832	JF457999	EF617175	FJ167829
Chaetodon	*unimaculatus*	EF616955	EF616872	JF434836	JF458003	EF617203	FJ167830
Chaetodon	*vagabundus*	EF616949	EF616866	JF434842	JF458009	EF617197	—
Chaetodon	*wiebeli*	—	—	FJ583110	—	—	—
Chaetodon	*xanthocephalus*	—	—	JF493107	—	—	—
Chaetodon	*xanthurus*	EF616956	EF616873	JF434845	JF458012	EF617204	—
Chaetodon	*zanzibarensis*	EF616941	EF616858	JF434846	JF458013	EF617189	—
Chelmon	*marginalis*	EF616960	EF616877	—	FJ167711	EF617208	FJ167831
Chelmon	*muelleri*	—	—	—	FJ167712	—	FJ167832
Chelmon	*rostratus*	EF616959	EF616876	FJ583134	AF108612	EF617207	FJ167833
Chelmonops	*curiosus*	—	—	—	FJ167713	—	FJ167834
Chelmonops	*truncatus*	EF616961	EF616878	—	FJ167714	EF617209	FJ167835
Coradion	*altivelis*	—	AF108538	—	AF108613	—	FJ167836
Coradion	*chrysozonus*	EF616963	EF616880	—	AF108614	EF617211	FJ167837
Coradion	*melanopus*	EF616962	EF616879	—	—	EF617210	—
Forcipiger	*flavissimus*	EF616964	EF616881	JF434973	JF458132	EF617212	FJ167838
Forcipiger	*longirostris*	EF616965	EF616882	JF434975	FJ167715	EF617213	FJ167839
Hemitaurichthys	*polylepis*	EF616906	EF616822	JF435020	JF458156	EF617153	FJ167840
Hemitaurichthys	*thompsoni*	JF457508	—	JF435022	JF458158	—	FJ167841
Hemitaurichthys	*zoster*	EF616966	EF616883	JF435024	JF458160	EF617214	—
Heniochus	*acuminatus*	EF616907	EF616823	JF435025	JF458161	EF617154	FJ167842
Heniochus	*chrysostomus*	JF457512	EF616884	JF435027	JF458163	—	FJ167843
Heniochus	*diphreutes*	EF616970	EF616888	JF435031	JF458167	EF617218	—
Heniochus	*monoceros*	EF616968	EF616886	JF435033	JF458169	EF617216	—
Heniochus	*pleurotaenia*	—	AF108545	—	AF108620	—	—
Heniochus	*singularius*	EF616967	EF616885	FJ583547	FJ167718	EF617215	FJ167844
Heniochus	*varius*	EF616969	EF616887	FJ583549	AF108621	EF617217	—
Johnrandallia	*nigrirostris*	EF616971	EF616889	—	FJ167719	—	FJ167845

		Mitochondrial loci				Nuclear loci	
Genus	**Species**	**16S**	**12S**	**CO1**	**CYTB**	**TMO-4C4**	**S7**
Chaetodontidae cont.							
Parachaetodon	*ocellatus*	—	AF108547	—	AF108622	—	FJ167847
Labridae							
Anampses	*caeruleopunctatus*	KJ866392	JN935296	JN935325	JF457906	AY279781	JN935348
Anampses	*chrysocephalus*	KJ866393	JN935299	JN935327	—	—	JN935351
Anampses	*cuvier*	KJ866394	JN935301	JN935328	—	—	JN935352
Anampses	*elegans*	KJ866395	JN935303	JN935330	—	—	JN935354
Anampses	*femininus*	KJ866396	JN935305	JN935332	—	—	JN935356
Anampses	*geographicus*	KJ866397	JN935306	JN935333	—	—	JN935357
Anampses	*lennardi*	KJ866398	JN935308	JN935335	—	—	JN935359
Anampses	*lineatus*	KJ866399	JN935309	JN935336	JF457908	—	JN935361
Anampses	*melanurus*	KJ866400	JN935311	JN935338	—	—	JN935362
Anampses	*meleagrides*	KJ866401	JN935312	JN935339	—	—	JN935363
Anampses	*neoguinaicus*	KJ866402	JN935314	JN935341	—	AY279782	JN935366
Anampses	*twistii*	KJ866403	JN935317	JN935343	JF457909	—	JN935367
Bolbometopon	*muricatum*	JX026453	AY081073	—	EU601357	AY081108	JX026592
Centrolabrus	*caeruleus*	AY092034	AF414192	—	—	—	GQ341613
Centrolabrus	*exoletus*	AY092041	EU601224	GQ341588	EU601403	EU601303	—
Centrolabrus	*trutta*	AY092035	AF414195	—	—	—	GQ341614
Chlorurus	*atrilunula*	JX026457	—	—	—	—	JX026596
Chlorurus	*bleekeri*	JX026458	EU601182	—	EU601361	EU601267	JX026597
Chlorurus	*bowersi*	EU601231	EU601183	—	EU601362	EU601268	JX026598
Chlorurus	*capistratoides*	JX026460	EU601184	—	EU601363	EU601269	JX026599
Chlorurus	*cyanescens*	JX026461	—	—	—	—	JX026600
Chlorurus	*enneacanthus*	JX026462	—	—	—	—	JX026601
Chlorurus	*frontalis*	JX026463	—	JQ431620	—	—	JX026602
Chlorurus	*gibbus*	JX026464	—	FJ237699	—	—	—
Chlorurus	*japanensis*	JX026465	EU601209	—	EU601388	EU601288	JX026603
Chlorurus	*microrhinos*	JX026466	EU601185	—	EU601364	EU601270	JX026604
Chlorurus	*oedema*	JX026467	EU601186	—	EU601365	AY081107	JX026605
Chlorurus	*perspicillatus*	JX026468	—	—	—	—	JX026606
Chlorurus	*rhakoura*	JX026469	—	—	—	—	JX026607
Chlorurus	*sordidus*	JX026471	EU601187	JQ349891	EU601366	AY081106	JX026609
Chlorurus	*spilurus*	JX026470	—	—	—	—	JX026608

		Mitochondrial loci				**Nuclear loci**	
Genus	**Species**	**16S**	**12S**	**CO1**	**CYTB**	**TMO-4C4**	**S7**
Labridae cont.							
Chlorurus	*strongylocephalus*	JX026472	—	—	—	—	JX026610
Cryptotomus	*roseus*	EU601235	AY279592	JQ841518	EU601367	EU601271	—
Diproctacanthus	*xanthurus*	AY279698	AY279595	—	—	AY279801	—
Epibulus	*brevis*	EF520654	—	—	—	—	—
Epibulus	*insidiator*	AY279699	AJ810132	JF434969	JF458128	EU601301	—
Gomphosus	*caeruleus*	JF457465	—	JF434979	JF458133	—	AY329640
Gomphosus	*varius*	JF457467	AY279597	JF434981	AY328858	AY279803	AY329641
Haletta	*semifasciata*	AY662708	AY279656	EF609368	—	AY279862	—
Hemigymnus	*fasciatus*	JF457502	AJ810136	JF435016	JF458152	—	—
Hemigymnus	*melapterus*	JF457504	AJ810137	JF435018	JF458154	AY279817	—
Hipposcarus	*harid*	JX026455	—	—	—	—	JX026594
Hipposcarus	*longiceps*	JX026456	EU601189	—	EU885924	AY081110	JX026595
Labrichthys	*unilineatus*	AY850875	AJ810138	JF493715	—	AY279819	—
Lachnolaimus	*maximus*	AY857946	AY279618	JQ839821	EU601404	AY662809	—
Larabicus	*quadrilineatus*	AY279722	AY279619	—	—	AY279825	—
Leptoscarus	*vaigiensis*	AY081094	EU601190	FJ583627	EU601369	AY081111	—
Neodax	*balteatus*	AY279760	AY279657	—	—	AY279863	—
Odax	*acroptilus*	AY279761	AY279658	—	—	AY279864	—
Odax	*cyanoallix*	AY279762	AY279659	—	—	AY279865	—
Odax	*cyanomelas*	AY279763	AY279660	4850752	*4850753*	AY279866	—
Odax	*pullus*	AY279764	AY279661	—	—	AY279867	—
Ophthalmolepis	*lineolata*	AY279731	AY279628	JQ839560	—	AY279834	—
Oxyjulis	*californica*	AY279735	AY279632	JN582150	DQ132499	AY279838	—
Pseudodax	*moluccanus*	JF457633	AY279636	JF435149	JF458248	AY279842	—
Scarus	*altipinnis*	JX026473	EU601192	JQ432095	EU601371	EU601273	JX026611
Scarus	*arabicus*	JX026474	—	—	—	—	JX026612
Scarus	*chameleon*	JX026475	EU601193	FJ237917	EU601372	EU601274	JX026613
Scarus	*coelestinus*	AY081084	EU601194	—	EU601373	AY081101	JX026614
Scarus	*coeruleus*	JX026476	—	—	—	—	JX026615
Scarus	*collana*	JX026477	—	—	—	—	JX026616
Scarus	*compressus*	JX026478	—	—	—	—	JX026617
Scarus	*dimidiatus*	JX026479	EU601195	—	EU601374	EU601275	JX026618
Scarus	*dubius*	JX026480	—	—	—	—	JX026619

		Mitochondrial loci				**Nuclear loci**	
Genus	**Species**	**16S**	**12S**	**CO1**	**CYTB**	**TMO-4C4**	**S7**
Labridae cont.							
Scarus	*falcipinnis*	JX026481	—	—	—	—	JX026620
Scarus	*ferrugineus*	JX026482	—	—	—	—	JX026621
Scarus	*festivus*	JX026483	EU601196	—	EU601375	EU601276	JX026622
Scarus	*flavipectoralis*	JX026484	EU601197	—	EU601376	AY081103	JX026623
Scarus	*forsteni*	JX026485	EU601198	JQ432097	EU601377	EU601278	JX026624
Scarus	*frenatus*	JX026486	EU601199	FJ237920	EU601378	AY081104	JX026625
Scarus	*fuscopurpureus*	JX026487	—	—	—	—	JX026626
Scarus	*ghobban*	EU601241	EU601200	EF609452	EU601379	EU601279	JX026629
Scarus	*globiceps*	JX026492	EU601201	JQ350330	EU601380	EU601280	JX026631
Scarus	*guacamaia*	EU601243	EU601202	JQ843039	EU601381	AY081102	JX026632
Scarus	*hoefleri*	JX026493	AY141393	—	—	—	JX026633
Scarus	*hypselopterus*	EU601245	EU601204	—	EU601383	EU601283	—
Scarus	*iseri*	JX026494	EU601203	JQ840986	EU601382	EU601282	JX026634
Scarus	*koputea*	JX026495	—	—	—	—	JX026635
Scarus	*longipinnis*	JX026496	—	—	—	—	JX026636
Scarus	*niger*	JX026497	EU601205	JQ350332	EU601384	EU601284	JX026637
Scarus	*oviceps*	JX026498	EU601206	JQ432107	EU601385	EU601285	JX026638
Scarus	*ovifrons*	JX026499	—	—	—	—	JX026639
Scarus	*perrico*	JX026500	—	—	—	—	JX026640
Scarus	*persicus*	JX026501	—	—	—	—	JX026641
Scarus	*prasiognathos*	JX026502	EU601207	—	EU601386	EU601286	JX026642
Scarus	*psittacus*	JX026503	EU601208	JQ432110	EU601387	EU601287	JX026643
Scarus	*quoyi*	JX026504	EU601210	—	EU601389	EU601289	JX026644
Scarus	*rivulatus*	JX026505	EU601211	—	EU601390	EU601290	JX026645
Scarus	*rubroviolaceus*	EU601253	EU601212	GU805008	EU601391	EU601291	JX026646
Scarus	*russelii*	JX026510	—	—	—	—	JX026650
Scarus	*scaber*	JX026511	—	JQ350334	—	—	JX026651
Scarus	*schlegeli*	JX026512	EU601213	JQ432114	EU601392	EU601292	JX026652
Scarus	*spinus*	JX026513	EU601214	—	EU601393	EU601293	JX026653
Scarus	*taeniopterus*	JX026514	EU601215	JQ842302	EU601394	EU601294	JX026654
Scarus	*tricolor*	JX026515	EU601216	JQ350335	EU601395	EU601295	JX026655
Scarus	*trispinosus*	JX026516	—	—	—	—	JX026656
Scarus	*vetula*	JX026517	—	FJ584084	—	—	JX026657

		Mitochondrial loci				Nuclear loci	
Genus	**Species**	**16S**	**12S**	**CO1**	**CYTB**	**TMO-4C4**	**S7**
Labridae cont.							
Scarus	*viridifucatus*	JX026518	—	—	—	—	JX026658
Scarus	*xanthopleura*	JX026519	—	—	—	—	JX026659
Scarus	*zelindae*	JX026520	—	—	—	—	JX026660
Scarus	*zufar*	JX026521	—	—	—	—	JX026661
Symphodus	*bailloni*	AY092037	AY092052	GQ341601	—	—	GQ341616
Symphodus	*cinereus*	AY092036	AJ810147	GQ341603	—	—	GQ341618
Symphodus	*doderleini*	AF517602	—	GQ341606	—	—	—
Symphodus	*mediterraneus*	AF517601	AJ810148	GQ341607	—	—	GQ341620
Symphodus	*melanocercus*	AF517595	AJ810149	GQ341600	—	—	—
Symphodus	*melops*	AY092038	AF414197	GQ341604	—	—	GQ341621
Symphodus	*ocellatus*	AF517603	AJ810150	—	—	—	—
Symphodus	*roissali*	AY092039	AJ810151	GQ341605	—	—	GQ341622
Symphodus	*rostratus*	AY092040	AF414198	GQ341608	—	—	GQ341617
Symphodus	*tinca*	AF517596	AJ810152	GQ341602	—	—	GQ341619
Tautoga	*onitis*	AF517588	AY279648	GQ341609	EU601402	AY662810	—
Tautogolabrus	*adspersus*	AF517585	AY279649	GQ341610	HM049968	AY279855	—
Thalassoma	*amblycephalum*	JF457667	—	JF435183	AY328860	—	AY329643
Thalassoma	*ascensionis*	AY328988	—	—	AY328861	—	AY329644
Thalassoma	*ballieui*	AY328989	—	DQ521017	AY328862	—	AY329645
Thalassoma	*bifasciatum*	AY279753	AJ810153	JQ839917	AY328863	AY279856	AY329646
Thalassoma	*cupido*	AY328991	—	—	AY328864	—	AY329647
Thalassoma	*duperrey*	AY328994	—	—	AY328865	—	DQ443832
Thalassoma	*genivittatum*	JF457672	—	JF435188	AY328866	—	DQ443833
Thalassoma	*grammaticum*	AY328993	—	JQ839619	AY328867	—	AY329650
Thalassoma	*hardwicke*	AY850865	AY850802	JF435196	AY328868	—	DQ443821
Thalassoma	*hebraicum*	JF457683	—	JF435199	AY328869	—	AY329652
Thalassoma	*jansenii*	AY328997	—	FJ459566	AY328870	—	DQ443820
Thalassoma	*loxum*	AY328998	—	—	AY328871	—	AY329654
Thalassoma	*lucasanum*	AY328999	—	JQ839621	AY328872	—	AY329655
Thalassoma	*lunare*	JQ178236	AJ810154	JF435203	AY328873	AY279857	DQ443836
Thalassoma	*lutescens*	AY850863	AJ810155	FJ584187	AY328874	—	DQ443835
Thalassoma	*newtoni*	AY329002	—	—	AY328875	—	AY329658
Thalassoma	*noronhanum*	AY329003	—	JQ839633	AY328876	—	AY329659

		Mitochondrial loci				**Nuclear loci**	
Genus	**Species**	**16S**	**12S**	**CO1**	**CYTB**	**TMO-4C4**	**S7**
Labridae cont.							
Thalassoma	*pavo*	AF517583	—	GQ341611	DQ198011	—	AY329660
Thalassoma	*purpureum*	JF457693	—	JF435209	AY328878	—	AY329661
Thalassoma	*quinquevittatum*	AY850864	AY850801	JF435210	AY328879	—	DQ443802
Thalassoma	*robertsoni*	AY329007	—	JQ839646	AY328880	—	AY329663
Thalassoma	*rueppellii*	AY329008	—	—	AY328881	—	AY329664
Thalassoma	*sanctaehelenae*	AY329009	—	—	AY328882	—	AY329665
Thalassoma	*septemfasciatum*	AY329010	—	—	AY328883	—	AY329666
Thalassoma	*trilobatum*	JF457696	—	JF435212	AY328884	—	AY329667
Thalassoma	*virens*	AY329012	—	JQ839654	AY328885	—	AY329668
Xiphocheilus	*typus*	AY279756	AY279653	—	—	AY279859	—
Pomacanthidae							
Holacanthus	*africanus*	KC845330	KC845393	—	KC845351	—	KC845372
Holacanthus	*bermudensis*	KC845337	KC845400	—	KC845358	EF617231	KC845379
Holacanthus	*ciliaris*	KC845332	KC845395	JQ841232	KC845353	—	KC845385
Holacanthus	*clarionensis*	KC845327	KC845390	—	KC845348	—	KC845369
Holacanthus	*limbaughi*	KC845328	KC845391	—	KC845349	—	KC845370
Holacanthus	*passer*	KC845329	KC845392	—	KC845350	—	KC845371
Holacanthus	*tricolor*	KC845336	KC845399	JQ840536	KC845357	—	KC845384
Pomacanthus	*annularis*	KC820905	KC820895	FJ583875	AF108646	—	KC820915
Pomacanthus	*arcuatus*	KC820906	KC820896	JQ841953	—	EF617229	KC820916
Pomacanthus	*asfur*	AY530854	AY530826	—	—	—	KC820917
Pomacanthus	*chrysurus*	KC820907	KC820897	—	—	—	KC820918
Pomacanthus	*imperator*	KC820908	KC820898	JQ350235	FJ424070	—	KC820919
Pomacanthus	*maculosus*	KC820909	KC820899	HQ149906	JN604372	—	KC820920
Pomacanthus	*navarchus*	KC820910	KC820900	FJ583883	AF108648	—	KC820921
Pomacanthus	*paru*	AY530852	AY530824	JQ840654	—	—	KC820922
Pomacanthus	*rhomboides*	KC820911	KC820901	HQ945884	FJ167723	—	KC820923
Pomacanthus	*semicirculatus*	AY530844	AF108574	JQ350237	AF108649	—	KC820924
Pomacanthus	*sexstriatus*	AY530858	AF108575	—	AF108650	—	KC820925
Pomacanthus	*xanthometapon*	KC820912	KC820902	—	AF108651	—	KC820926
Pomacanthus	*zonipectus*	AY530874	AY530840	GU440472	—	—	KC820927
Pygoplites	*diacanthus*	AY530873	AF108577	JQ350298	AF108652	EF617232	KC820928
Epinephelidae							

		Mitochondrial loci				**Nuclear loci**	
Genus	**Species**	**16S**	**12S**	**CO1**	**CYTB**	**TMO-4C4**	**S7**
Epinephelidae cont.							
Aethaloperca	*rogaa*	AY947565	AY949367	JX674944	EF213721	AY949225	—
Alphestes	*afer*	AY314003	AY313982	JQ840759	AY313996	AY313992	—
Alphestes	*immaculatus*	AF297290	AY313980	—	AY314002	AY313994	—
Alphestes	*multiguttatus*	AF297305	AY313981	—	AY313995	AY313991	—
Anyperodon	*leucogrammicus*	AF297306	AY949379	DQ107922	AY963557	EF517738	—
Dermatolepis	*dermatolepis*	AF297317	AY313984	HM032025	AY314000	AY313988	—
Dermatolepis	*inermis*	AY314005	AY313979	EU752075	AY314001	AY313987	—
Dermatolepis	*striolata*	AY314004	AY313983	—	AY313999	AY313989	—
Gracila	*albomarginata*	AY947582	AY949348	—	—	AY949250	—
Mycteroperca	*acutirostris*	AY947591	AY949411	GU702334	—	AY949251	—
Mycteroperca	*bonaci*	DQ267147	AY949449	JQ840175	—	AY949270	—
Mycteroperca	*fusca*	AY947597	AY949448	—	DQ197968	AY949252	—
Mycteroperca	*interstitialis*	AY947632	AY949359	FJ583668	—	AY949221	—
Mycteroperca	*jordani*	AF297329	AY949435	GU440412	—	AY949303	—
Mycteroperca	*microlepis*	AF297312	AY949373	JQ842598	—	AY949253	—
Mycteroperca	*olfax*	AF317512	AY949360	—	—	AY949276	—
Mycteroperca	*phenax*	AF297303	AY949450	—	—	AY949265	—
Mycteroperca	*prionura*	AY947583	AY949361	—	—	AY949254	—
Mycteroperca	*rosacea*	AF297300	AY949350	—	—	AY949268	—
Mycteroperca	*rubra*	AY947587	AY949364	—	DQ197969	AY949255	—
Mycteroperca	*tigris*	AY947574	AY949452	JQ839849	—	AY949217	—
Mycteroperca	*venenosa*	AF297291	AY949419	JQ839850	—	AY949273	—
Mycteroperca	*xenarcha*	AY947637	AY949445	GU440413	—	—	—
Niphon	*spinosus*	HQ731416	AY949420	EF143386	AB108493	AY949210	DQ864743
Paranthias	*colonus*	AF297301	AY949351	GU440449	HM049960	—	—
Paranthias	*furcifer*	AY947584	AY949372	JQ365486	—	AY949263	—
Plectropomus	*areolatus*	EF213706	AY949447	JN242595	EF213725	EF517750	*
Plectropomus	*laevis*	DQ067320	AY949444	JX675027	AY963554	EF517749	*
Plectropomus	*leopardus*	DQ067321	AY949352	JF750763	AY963555	EF517747	—
Plectropomus	*maculatus*	AF297320	AY949423	JF750764	EF503636	EF517751	—
Plectropomus	*oligacanthus*	AY947615	EU930863	*	EF213726	EF517748	*
Plectropomus	*pessuliferus*	—	—	—	AY963553	*	*
Plectropomus	*punctatus*	*	*	*	—	—	—

		Mitochondrial loci				Nuclear loci	
Genus	**Species**	**16S**	**12S**	**CO1**	**CYTB**	**TMO-4C4**	**S7**
Epinephelidae cont.							
Saloptia	*powelli*	AY947631	AY949375	JQ432090	—	—	KM
Variola	*albimarginata*	EF213708	AY949412	JX675035	EF213728	AY949261	—
Variola	*louti*	DQ067319	AY949412	JQ432218	AY786428	EF517745	—
Fossil Calibration Taxa							
Acanrthurus	*nigricans*	AY057286	AY057239	EF648266	AY264634	—	EF648230
Ambloplites	*rupestris*	AY742515	—	EU524414	AY225663	—	—
Archoplites	*interruptus*	AY742516	—	HQ557524	AY225665	—	—
Bodianus	*mesothorax*	AY279681	AY279578	JQ839406	—	AY279784	—
Calatomus	*carolinus*	AY081092	EU601179	JQ349815	EU601358	AY081109	—
Cetoscarus	*bicolor*	JX026454	EU601181	JQ349875	EU601360	AY081105	JX026593
Gazza	*minuta*	DQ648428	—	EU148511	—	—	—
Leiognathus	*equulus*	EU366341	DQ533223	EU381032	EU380961	—	—
Luvarus	*imperialis*	AF055622	AF055601	*5618975*	AB276966	—	—
Naso	*lituratus*	AF055624	AF055603	HM034244	AB276964	—	EF648255
Prognathodes	*aculeatus*	EF616972	EF616890	—	FJ167725	EF617219	FJ167852
Sparisoma	*chrysopterum*	AY279748	AY279645	JQ841012	DQ457033	AY081100	—
Zanclus	*cornutus*	AF055623	AY057235	JQ350417	AB276965	—	—
Outgroup Taxa							
Opsanus	*pardus*	DQ874745	AF165327	—	AF165347	DQ874850	—
Porichthys	*notatus*	EF119249	AF165333	JQ354294	AF165352	—	—

Table S2.4 Details of fossil data used for temporal-calibration of the multi-family phylogenetic analyses (Chapters 5–7) in BEAST. Calibrations were placed on nodes representing the most recent common ancestor (MRCA) of specified genera. $_{\mathrm{H}}$ indicates a hard lower bound on the prior distribution; $_{\mathrm{S}}$ indicates a soft 95% upper bound.

MRCA	**Minimum fossil age (Ma)**	**Parametric prior distribution**	**Prior bounds**	**Fossil evidence**
Archoplites/*Ambloplites*	15.5	Lognormal	$15.5_{\mathrm{H}} – 17.8_{\mathrm{S}}$	*Archoplites clarki* [1,2]
Bolbometopon/*Cetoscarus*	5	Lognormal	$5_{\mathrm{H}} – 36_{\mathrm{S}}$	*Bolbometopon* sp. [3]
Calotomus/*Sparisoma*	14	Lognormal	$14_{\mathrm{H}} – 50_{\mathrm{S}}$	*Calotomus preisli* [3]
Chaetodon/*Prognathodes*	7.1	Lognormal	$7.1_{\mathrm{H}} – 25.2_{\mathrm{S}}$	*Chaetodon ficheuri* [4]
Gazza/*Leiognathus*	11.6	Lognormal	$11.6_{\mathrm{H}} – 30.1_{\mathrm{S}}$	*Euleiognathus tottori* [5,6]
Hypsigenyines	50	Exponential	$50_{\mathrm{H}} – 95_{\mathrm{S}}$	*Phyllopharyngodon longipinnis* [7]
Labridae (–*Hypsigenyines*)	50	Lognormal	$50_{\mathrm{H}} – 100_{\mathrm{S}}$	*Eocoris bloti* [8], *Bellwoodilabrus landinii* [9]
Luvarus, *Zanclus*, and Acanthuridae (*Acanthurus* and *Naso*)	55.8	Lognormal	$55.8_{\mathrm{H}} – 63.9_{\mathrm{S}}$	*Avitoluvarus dianae*, *Avitoluvarus mariannae*, *Kushlukia permira*, and *Luvarus necopinatus* [10]
Pseudodax/*Bodianus*	14	Lognormal	$14_{\mathrm{H}} – 50_{\mathrm{S}}$	*Trigondon jugleri* [11]

Publications sources of fossil evidence: [1] (Smith & Elder, 1985); [2] (Smith & Miller, 1985); [3] (Bellwood & Schultz, 1991); [4] (Carnevale, 2006); [5] (Yabumoto & Uyeno, 1994); [6] (Yabumoto & Uyeno, 2011); [7] (Bellwood, 1990); [8] (Bannikov & Sorbini, 1990); [9] (Bannikov & Carnevale, 2010); [10] (Bannikov & Tyler, 1995); [11] (Schultz & Bellwood, 2004).

Table S2.5 Partition scheme and models of evolution selected by PARTITIONFINDER under the Bayesian Information Criterion (BIC). The data set consisted of molecular sequences for 312 Percomorph fishes found throughout the tropical Atlantic and Indo-Pacific; the data were used in the multi-family phylogenetic analyses (Chapters 5–7). Γ represents the gamma shape parameter.

Data set	Base pairs	Partition	BIC model
16S	569	1	TVM+I+Γ
12S	308		
CO1	477		
CYTB	92		
TMO-4C4	434	2	TrN+I+Γ
S7	611	3	TrN+I+Γ
Total	2491	—	—

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Appendix B: Supplementary materials for Chapter 3

The following supplemental accompanies the article

The role of peripheral endemism in species diversification: evidence from the coral reef fish genus *Anampses* (Family: Labridae)

Jennifer R. Hodge [1,2,3], Charmaine I. Read [1,3], Lynne van Herwerden [1,3], David R. Bellwood [1,2]

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Molecular Phylogenetics and Evolution 62(2): 653–663 (2012)

FIGURES

Figure S3.1 AWTY output from the phylogenetic analysis of *Anampses* showing pairwise comparisons of the posterior probabilities of nodes for each of four independent Bayesian inference analyses implemented in MRBAYES.

Figure S3.2 AWTY output from the phylogenetic analysis of *Anampses* showing the cumulative posterior probabilities of the 20 most variable nodes for trees searched by four independent Markov Chain Monte Carlo analyses implemented in MRBAYES.

Figure S3.3 AWTY output from the phylogenetic analysis of *Anampses* showing pairwise comparisons of the posterior probabilities of nodes for each of four independent Bayesian inference analyses implemented in BEAST.

Figure S3.4 AWTY output from the phylogenetic analysis of *Anampses* showing the cumulative posterior probabilities of the 15 most variable nodes for trees searched by four independent Markov Chain Monte Carlo analyses implemented in BEAST.

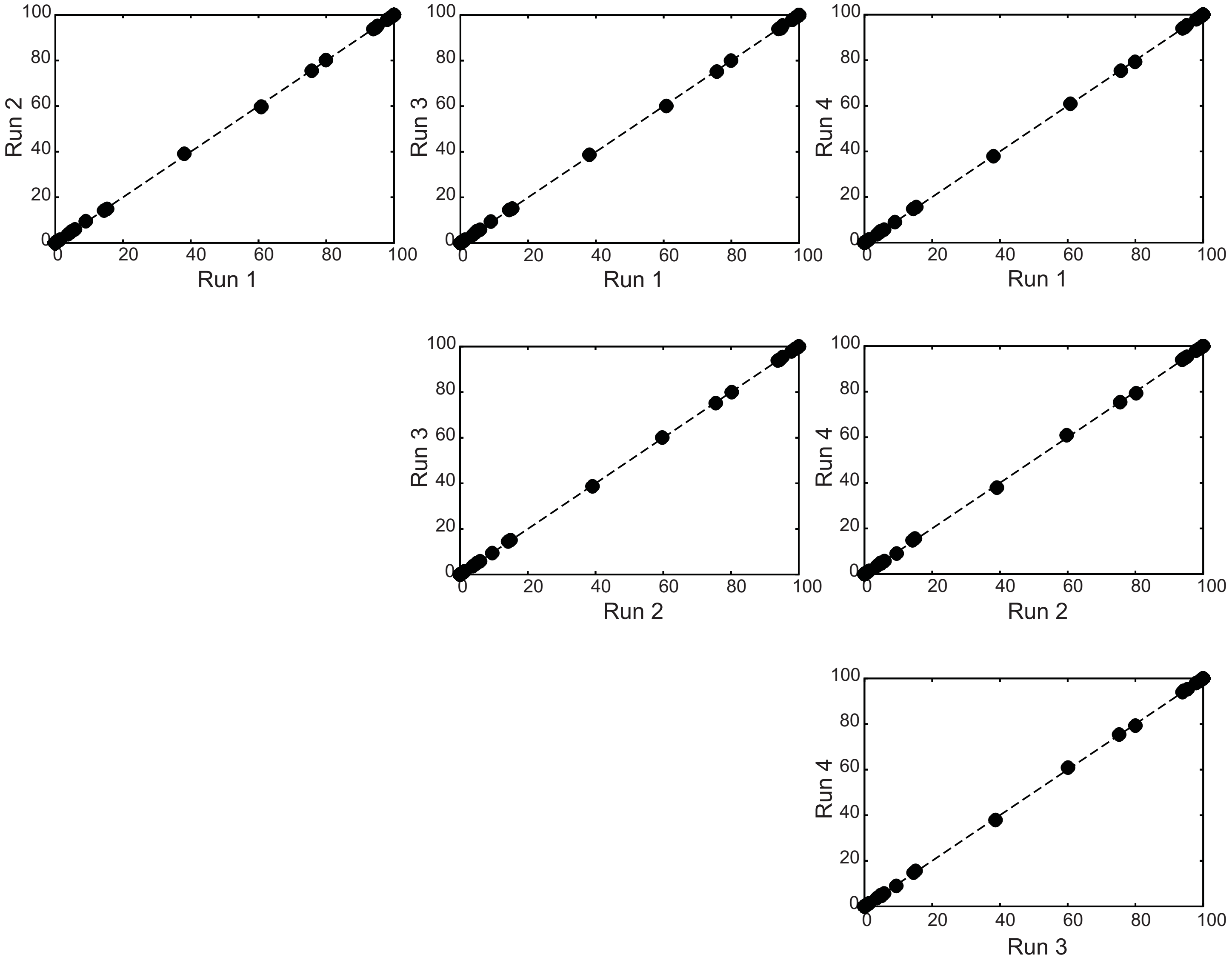

Figure S3.1 AWTY output from the phylogenetic analysis of *Anampses*. Each bivariate plot is a pairwise comparison of the posterior probabilities of nodes for each of four independent Bayesian inference analyses as implemented in MrBayes. Low correlation among runs would diagnose lack of convergence, however that was not detected here.

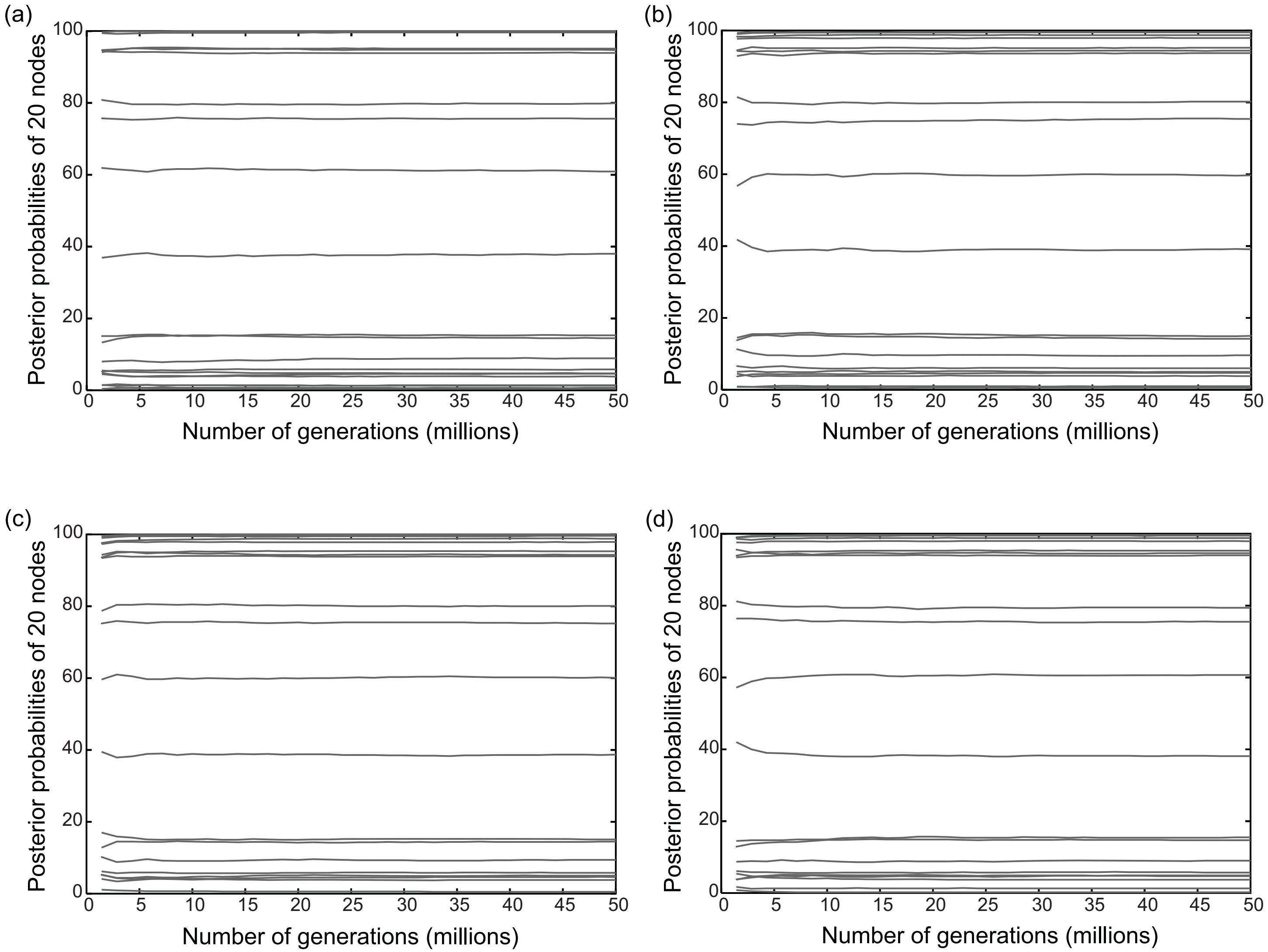

Figure S3.2 AWTY output from the phylogenetic analysis of *Anampses*. Each plot shows the cumulative posterior probabilities of the 20 most variable nodes for trees searched by four independent Markov Chain Monte Carlo analyses as implemented in MRBAYES; (a) analysis 1; (b) analysis 2; (c) analysis 3; and (d) analysis 4. A trend in the frequency of posterior probabilities for nodes diagnoses lack of convergence, but was not observed here.

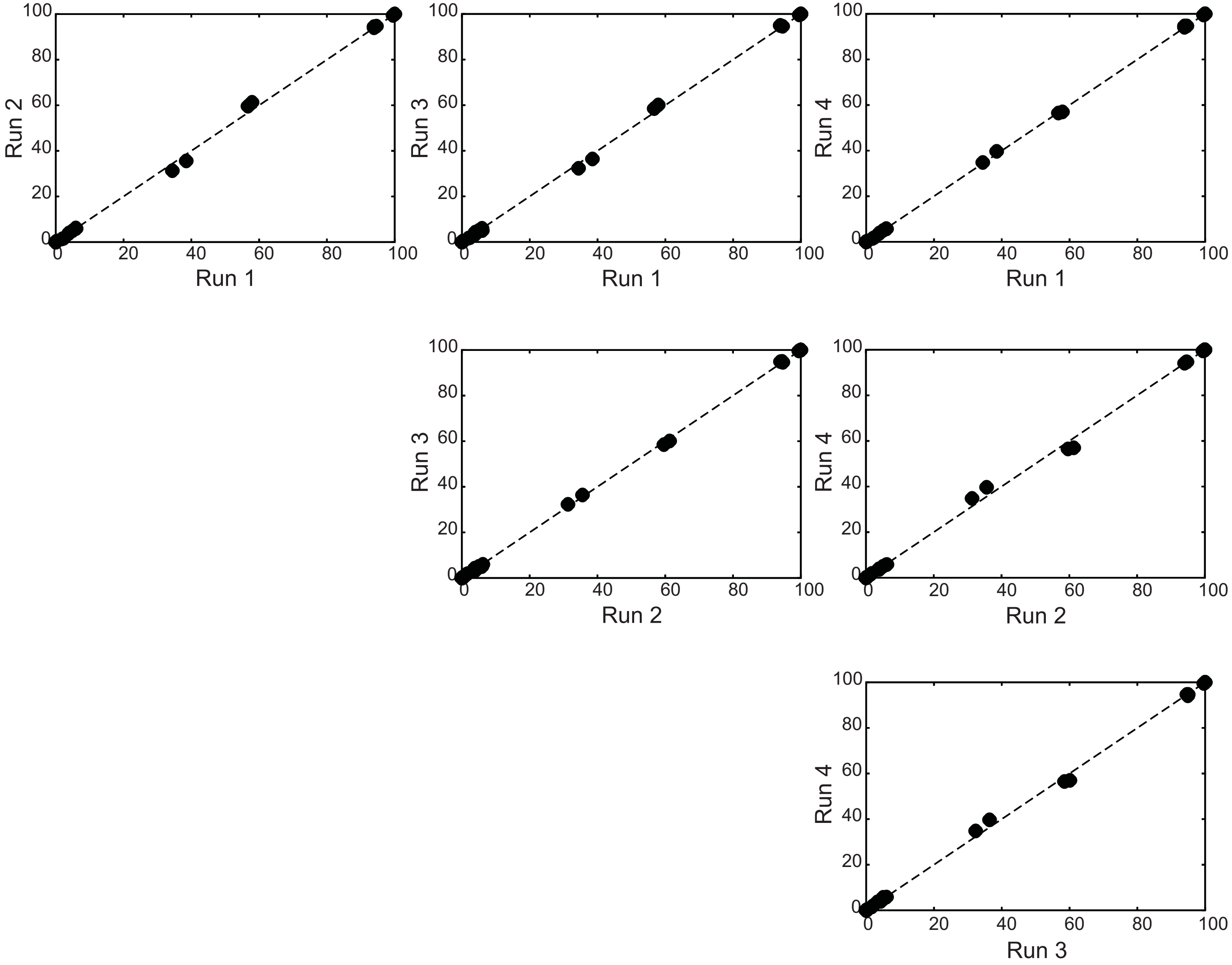

Figure S3.3 AWTY output from the phylogenetic analysis of *Anampses*. Each bivariate plot is a pairwise comparison of the posterior probabilities of nodes for each of four independent Bayesian inference analyses as implemented in BEAST. Low correlation among runs would diagnose lack of convergence, however that was not detected here.

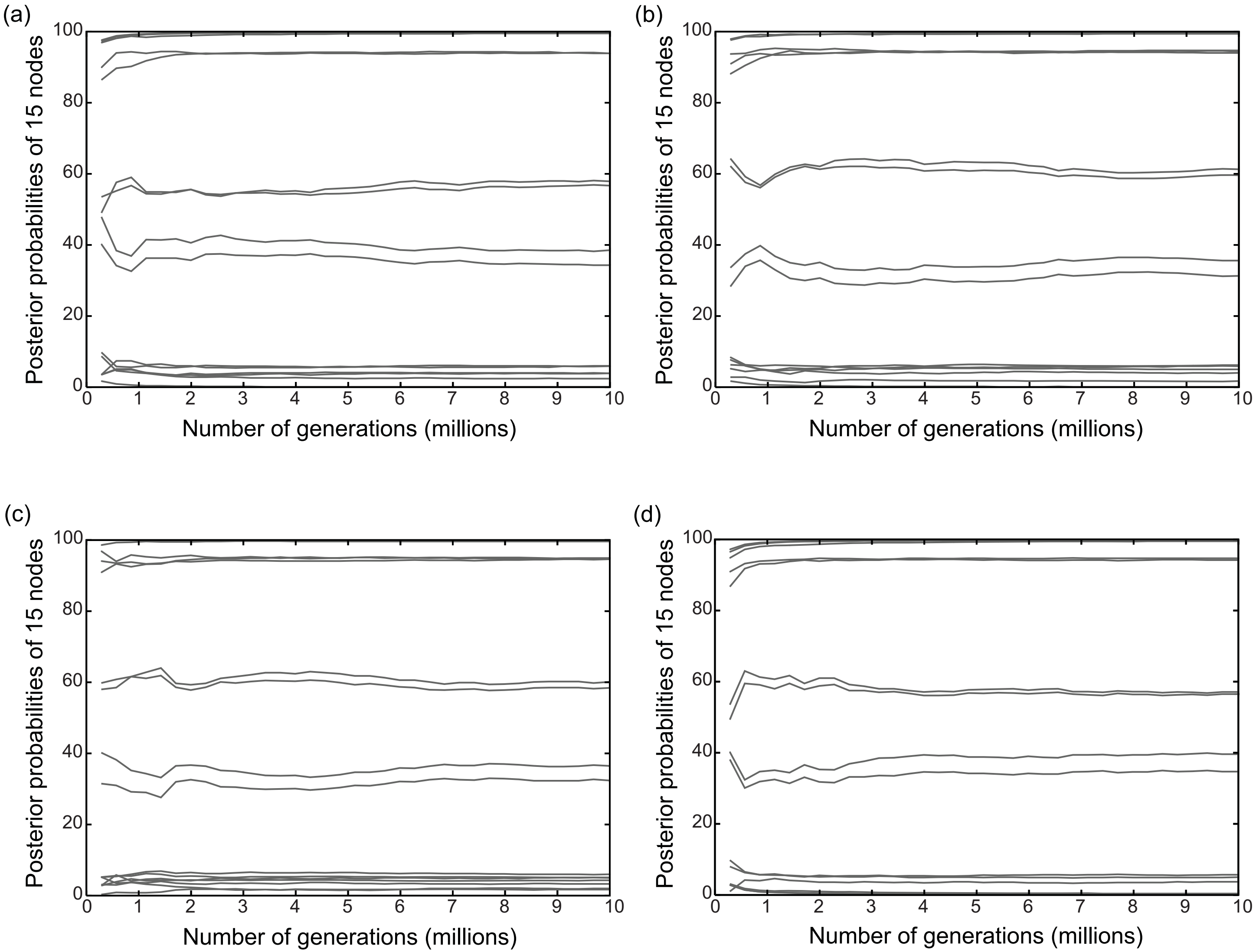

Figure S3.4 AWTY output from the phylogenetic analysis of *Anampses*. Each plot shows the cumulative posterior probabilities of the 15 most variable nodes for trees searched by four independent Markov Chain Monte Carlo analyses as implemented in BEAST; (a) analysis 1; (b) analysis 2; (c) analysis 3; and (d) analysis 4. A trend in the frequency of posterior probabilities for nodes diagnosis lack of convergence, but was not observed here.

Appendix C: Supplementary materials for Chapter 4

The following supplemental accompanies the article

Evolution of sympatric species: a case study of the coral reef fish genus *Pomacanthus* (Family: Pomacanthidae)

Jennifer R. Hodge [1,2,3], Charmaine I. Read [1,3], David R. Bellwood [1,2], Lynne van Herwerden [1,3]

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Journal of Biogeography 40(9): 1676–1687 (2013)

FIGURES

Figure S4.1 AWTY output from the phylogenetic analysis of *Pomacanthus* showing pairwise comparison of the posterior probabilities of nodes for two independent Bayesian inference analyses implemented in MRBAYES.

Figure S4.2 AWTY output from the phylogenetic analysis of *Pomacanthus* showing the cumulative posterior probabilities of the 18 most variable nodes for trees searched by two independent Markov Chain Monte Carlo analyses implemented in MRBAYES.

Figure S4.3 Inferred phylogeny of *Pomacanthus* obtained by Bayesian inference, maximum likelihood and maximum parsimony analyses of three molecular loci.

Figure S4.4 AWTY output from the phylogenetic analysis of *Pomacanthus* showing pairwise comparisons of the posterior probabilities of nodes for each of four independent Bayesian inference analyses implemented in BEAST.

Figure S4.5 AWTY output from the phylogenetic analysis of *Pomacanthus* showing the cumulative posterior probabilities of the 15 most variable nodes for trees searched by four independent Markov Chain Monte Carlo analyses implemented in BEAST.

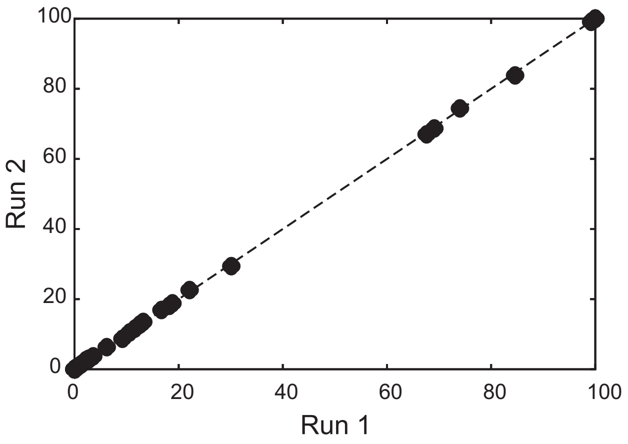

Figure S4.1 AWTY output from the phylogenetic analysis of *Pomacanthus*. The bivariate plot is a pairwise comparison of the posterior probabilities of nodes for two independent Bayesian inference analyses implemented in MRBAYES. Low correlation among runs would diagnose lack of convergence; however, that was not detected.

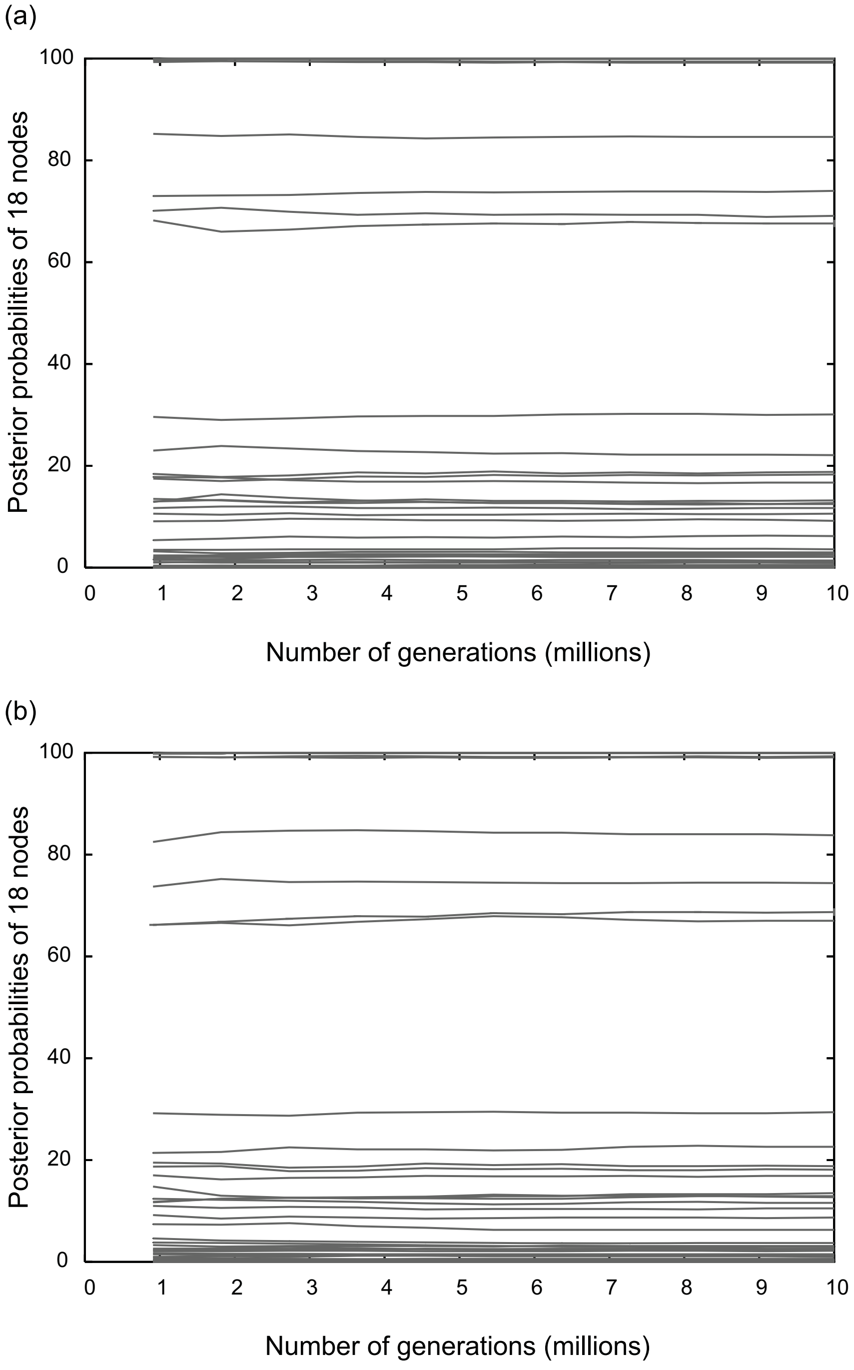

Figure S4.2 AWTY output from the phylogenetic analysis of *Pomacanthus*. Each plot shows the cumulative posterior probabilities of the 18 most variable nodes for trees searched by two independent Markov Chain Monte Carlo analyses implemented in MRBAYES: (a) analysis 1; and (b) analysis 2. A trend in the frequency of posterior probabilities for nodes diagnoses lack of convergence, but was not observed here.

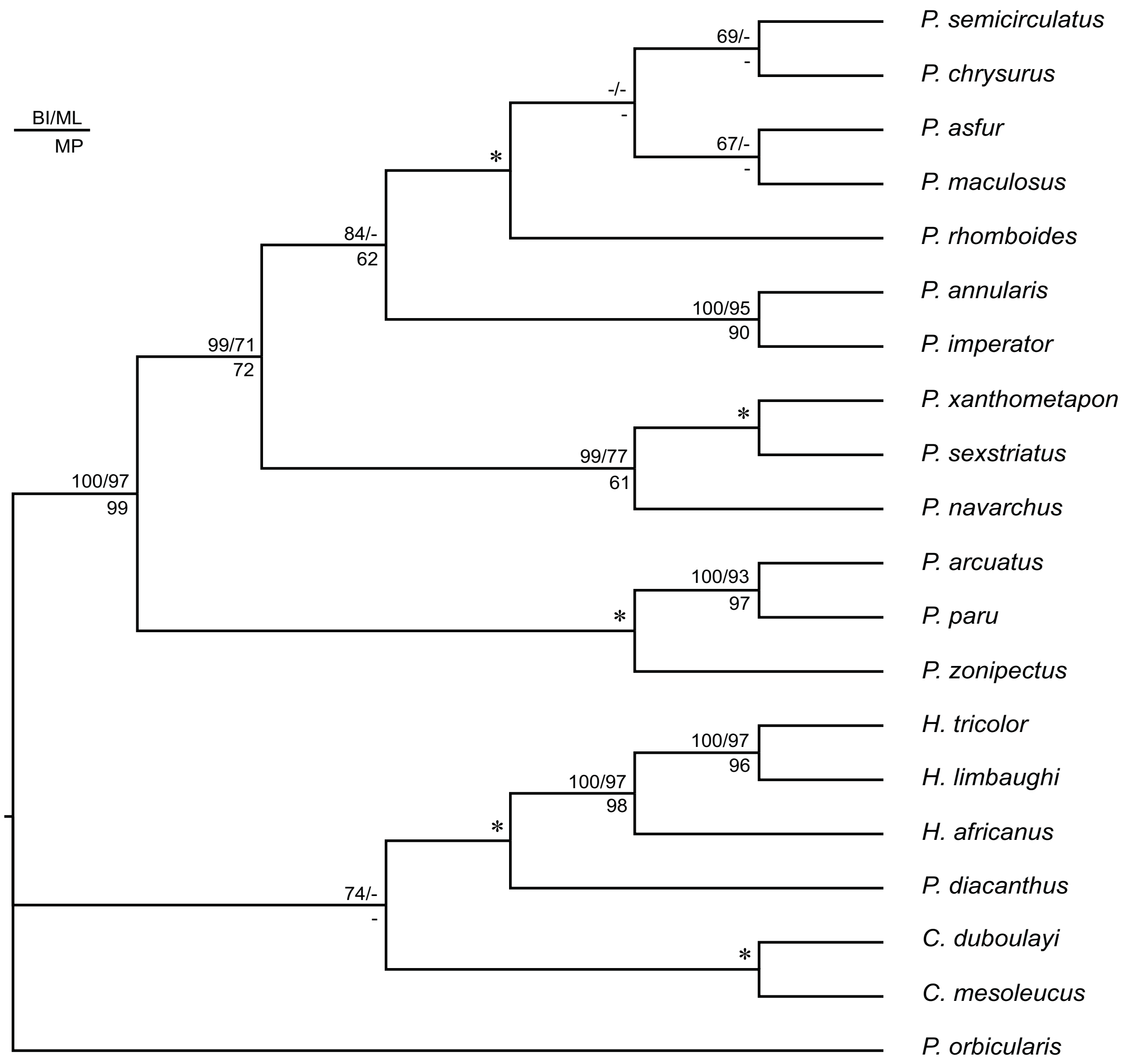

Figure S4.3 Inferred phylogeny of *Pomacanthus* obtained by Bayesian inference (BI), maximum likelihood (ML) and maximum parsimony (MP) analyses of three loci (12S, 16S and S7). The topology shown is the best Bayesian tree with bootstrap support values (> 50%) from MP and ML (1000 and 100 bootstrap replicates, respectively) and posterior probabilities from BI (consensus of 36,002 trees). Asterisks denote 100% support for the node across all three analyses. Sister taxa include *Chaetodontoplus mesoleucus*, *Chaetodontoplus duboulayi*, *Pygoplites diacanthus*, *Holacanthus tricolor*, *Holacanthus limbaughi* and *Holacanthus africanus*. *Platax orbicularis* was used to root the phylogeny.

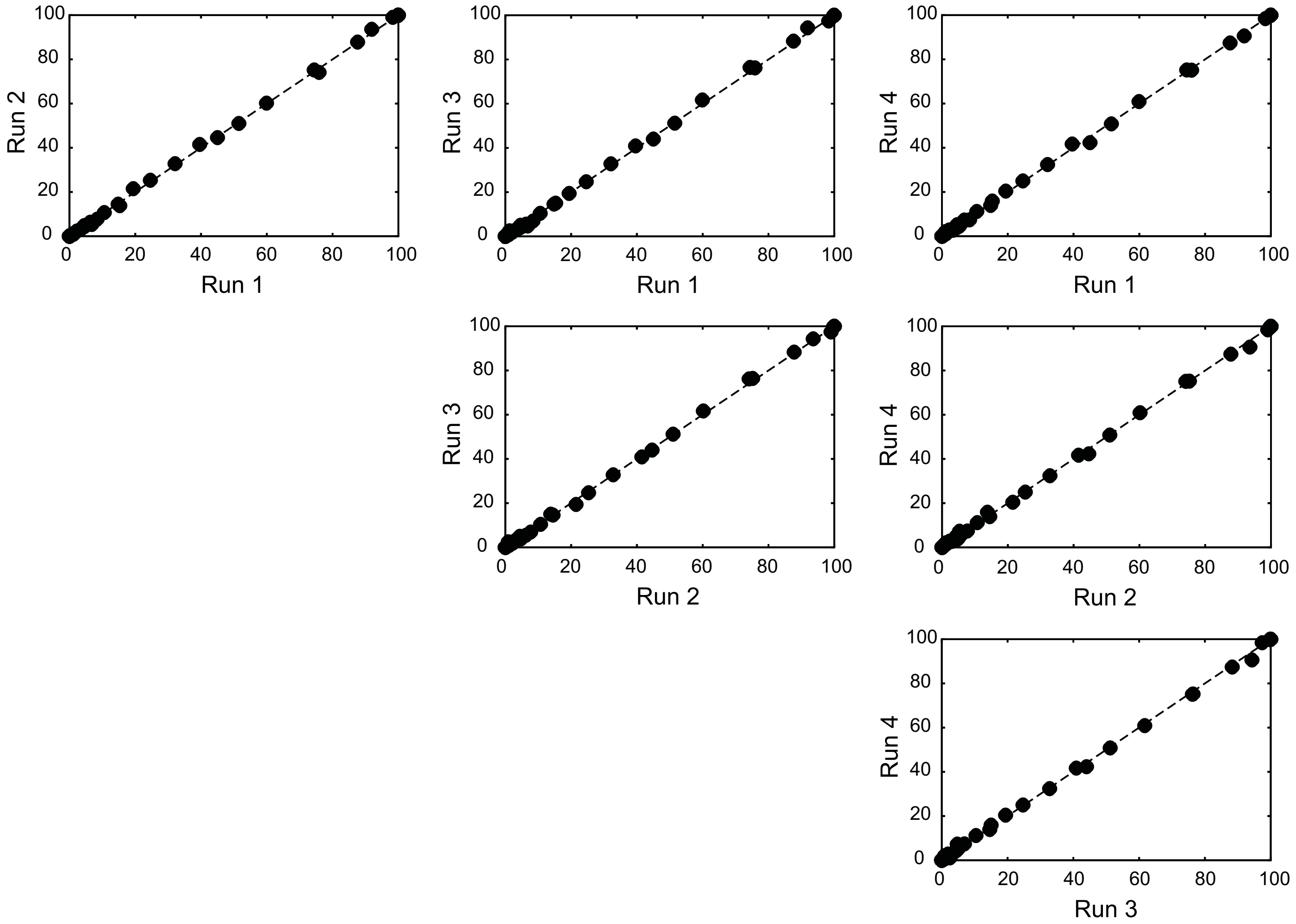

Figure S4.4 AWTY output from the phylogenetic analysis of *Pomacanthus*. Each bivariate plot is a pairwise comparison of the posterior probabilities of nodes for each of four independent Bayesian inference analyses implemented in BEAST. Low correlation among runs would diagnose lack of convergence; however, that was not detected here.

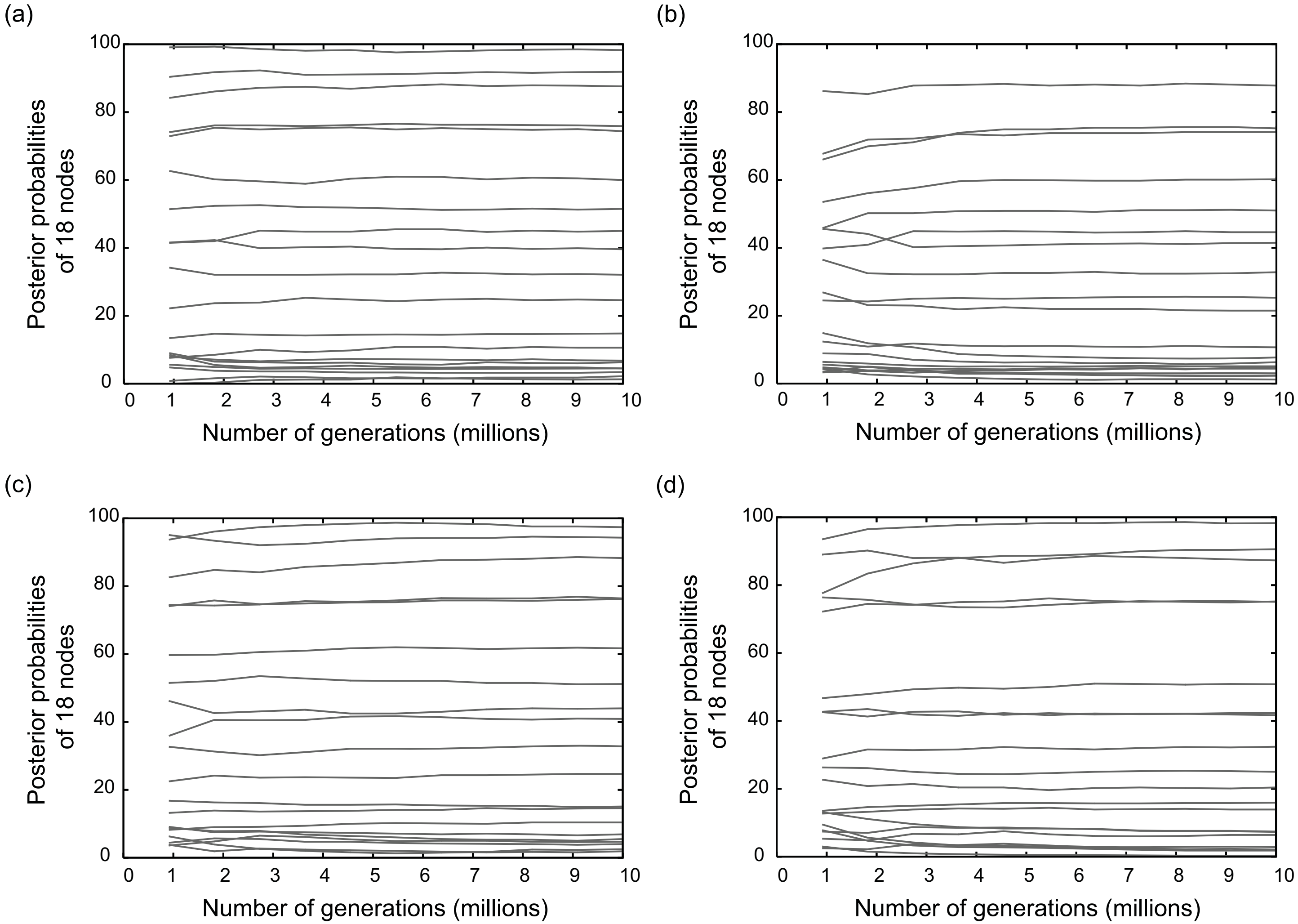

Figure S4.5 AWTY output from the phylogenetic analysis of *Pomacanthus*. Plots show the cumulative posterior probabilities of the 18 most variable nodes for trees searched by four independent Markov Chain Monte Carlo analyses implemented in BEAST: (a) analysis 1; (b) analysis 2; (c) analysis 3; and (d) analysis 4. A trend in the frequency of posterior probabilities for nodes diagnoses lack of convergence, but was not observed here.

Appendix D: Supplementary materials for Chapter 5

The following supplemental accompanies the article

On the relationship between species age and geographical range in reef fishes: are widespread species older than they seem?

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Global Ecology and Biogeography (2014 in press)

TABLES

Table S5.1 Median node age estimates and corresponding 95% highest posterior density intervals from a maximum clade credibility tree constructed from post-burn-in topologies of six independent Bayesian analyses of molecular data using nine fossil calibrations implemented in BEAST.

FIGURES

Figure S5.1 Time-calibrated phylogeny compiled from post burn-in topologies of six independent Bayesian analyses of molecular data using nine fossil calibrations implemented in BEAST.

Figure S5.2 Plots of the log-transformed correlations for (a) all nodes in the phylogeny and using a sister-species approach with minimum divergence time versus (b) averaged; (c) randomised; and (d) minimum geographical range.

Table S5.1 Median node age estimates with corresponding 95% highest posterior density (HPD) intervals from a maximum clade credibility tree constructed from post-burn-in topologies of six independent Bayesian Markov Chain Monte Carlo analyses (40×10^6 generations per run) of molecular data and nine fossil calibration points implemented in BEAST. The data set consisted of molecular sequences (16S rRNA, 12S rRNA, CO1, cytochrome b, TMO-4C4 and S7 intron 1) for 312 Percomorph fishes used in the multi-family phylogenetic analyses (Chapters 5–7).

Genus	**Species**	**Median age (Ma)**	**95 % HPD (Ma)**	
Chaetodontidae			**Lower bound**	**Upper bound**
Amphichaetodon	*howensis*	4.664	1.225	10.41
Amphichaetodon	*melbae*	4.664	1.225	10.41
Chaetodon	*adiergastos*	0.7242	0.0001546	1.985
Chaetodon	*argentatus*	6.302	3.674	9.631
Chaetodon	*aureofasciatus*	1.857	0.3916	3.964
Chaetodon	*auriga*	2.209	0.8929	3.856
Chaetodon	*auripes*	2.540	0.9352	4.240
Chaetodon	*austriacus*	1.497	0.4979	2.896
Chaetodon	*baronessa*	4.918	1.839	8.988
Chaetodon	*bennetti*	6.649	4.105	9.618
Chaetodon	*blackburnii*	7.202	4.531	10.05
Chaetodon	*burgessi*	1.006	0.2109	2.342
Chaetodon	*capistratus*	5.971	3.039	9.322
Chaetodon	*citrinellus*	4.041	1.851	6.537
Chaetodon	*collare*	3.729	2.484	5.217
Chaetodon	*decussatus*	2.209	0.8929	3.856
Chaetodon	*dolosus*	1.466	0.5252	2.602
Chaetodon	*ephippium*	3.640	1.330	6.668
Chaetodon	*falcula*	0.7941	0.2603	1.554
Chaetodon	*fasciatus*	0.8894	0.2672	1.741
Chaetodon	*flavirostris*	0.7242	0.0001546	1.985
Chaetodon	*fremblii*	9.718	6.654	12.89
Chaetodon	*guentheri*	2.013	0.9684	3.329
Chaetodon	*guttatissimus*	1.249	0.4980	2.425
Chaetodon	*hoefleri*	13.08	6.662	20.19
Chaetodon	*humeralis*	9.373	6.673	12.55
Chaetodon	*interruptus*	1.558	0.4509	3.229
Chaetodon	*kleinii*	0.3655	0.06169	0.9483
Chaetodon	*larvatus*	4.918	1.839	8.988
Chaetodon	*lineolatus*	2.445	1.312	3.809
Chaetodon	*lunula*	0.8894	0.2672	1.741
Chaetodon	*lunulatus*	3.170	1.559	5.338
Chaetodon	*madagaskariensis*	0.7523	0.1787	1.598
Chaetodon	*marleyi*	10.35	6.048	15.50
Chaetodon	*melannotus*	1.162	0.3786	2.433
Chaetodon	*mertensii*	0.4726	0.03071	1.275
Chaetodon	*mesoleucos*	3.946	1.410	7.141
Chaetodon	*meyeri*	3.476	1.808	5.695
Chaetodon	*miliaris*	1.219	0.3221	2.389
Chaetodon	*mitratus*	1.006	0.2109	2.342
Chaetodon	*multicinctus*	0.2885	0.06392	0.6570
Chaetodon	*ocellatus*	10.96	7.928	14.49
Chaetodon	*ocellicaudus*	1.162	0.3786	2.433
Chaetodon	*octofasciatus*	1.857	0.3916	3.964
Chaetodon	*ornatissimus*	2.732	1.325	4.617
Chaetodon	*oxycephalus*	1.486	0.6583	2.556
Chaetodon	*paucifasciatus*	0.4726	0.03071	1.275
Chaetodon	*pelewensis*	0.4576	0.1276	0.9471
Chaetodon	*plebeius*	5.250	2.913	8.133

Genus	Species	Median age (Ma)	95 % HPD (Ma)	
Chaetodontidae cont.			**Lower bound**	**Upper bound**
Chaetodon	*punctatofasciatus*	0.2885	0.06392	0.6570
Chaetodon	*quadrimaculatus*	4.041	1.851	6.537
Chaetodon	*rafflesii*	3.946	1.410	7.141
Chaetodon	*rainfordi*	5.243	3.013	7.965
Chaetodon	*reticulatus*	2.732	1.325	4.617
Chaetodon	*robustus*	13.08	6.662	20.19
Chaetodon	*sanctaehelenae*	1.219	0.3221	2.389
Chaetodon	*sedentarius*	1.466	0.5252	2.602
Chaetodon	*selene*	8.100	4.438	12.28
Chaetodon	*semeion*	6.247	3.105	9.816
Chaetodon	*semilarvatus*	3.276	1.820	4.940
Chaetodon	*speculum*	3.390	1.503	5.799
Chaetodon	*striatus*	5.971	3.039	9.322
Chaetodon	*tinkeri*	2.135	0.3973	5.768
Chaetodon	*trichrous*	0.3655	0.06169	0.9483
Chaetodon	*tricinctus*	12.66	9.282	16.33
Chaetodon	*trifascialis*	10.06	6.835	13.59
Chaetodon	*trifasciatus*	1.497	0.4979	2.896
Chaetodon	*ulietensis*	0.7941	0.2603	1.554
Chaetodon	*unimaculatus*	1.558	0.4509	3.229
Chaetodon	*vagabundus*	3.389	1.731	5.255
Chaetodon	*wiebeli*	2.540	0.9352	4.240
Chaetodon	*xanthocephalus*	3.640	1.330	6.668
Chaetodon	*xanthurus*	0.7523	0.1787	1.598
Chaetodon	*zanzibarensis*	3.390	1.503	5.799
Chelmon	*marginalis*	1.684	0.6321	3.121
Chelmon	*muelleri*	4.425	2.133	7.628
Chelmon	*rostratus*	1.684	0.6321	3.121
Chelmonops	*curiosus*	1.418	0.2735	3.619
Chelmonops	*truncatus*	1.418	0.2735	3.619
Coradion	*altivelis*	3.079	1.229	5.467
Coradion	*chrysozonus*	3.079	1.229	5.467
Coradion	*melanopus*	5.550	2.901	8.946
Forcipiger	*flavissimus*	7.971	3.649	13.26
Forcipiger	*longirostris*	7.971	3.649	13.26
Hemitaurichthys	*polylepis*	0.2232	0.01580	0.6504
Hemitaurichthys	*thompsoni*	4.429	1.967	7.732
Hemitaurichthys	*zoster*	0.2232	0.01580	0.6504
Heniochus	*acuminatus*	3.164	1.396	5.277
Heniochus	*chrysostomus*	9.746	6.567	13.10
Heniochus	*diphreutes*	3.164	1.396	5.277
Heniochus	*monoceros*	3.274	1.503	5.633
Heniochus	*pleurotaenia*	2.498	0.5871	5.329
Heniochus	*singularius*	3.274	1.503	5.633
Heniochus	*varius*	2.498	0.5871	5.329
Johnrandallia	*nigrirostris*	14.00	9.730	18.95
Parachaetodon	*ocellatus*	13.91	10.43	17.68
Labridae				
Anampses	*caeruleopunctatus*	0.5373	0.1307	1.204
Anampses	*chrysocephalus*	1.512	0.5776	2.841
Anampses	*cuvier*	10.08	6.142	14.25
Anampses	*elegans*	14.80	10.67	19.73
Anampses	*femininus*	7.198	3.787	10.98
Anampses	*geographicus*	19.24	13.59	26.28
Anampses	*lennardi*	0.5373	0.1307	1.204
Anampses	*lineatus*	4.058	2.211	6.378
Anampses	*melanurus*	1.512	0.5776	2.841
Anampses	*meleagrides*	7.761	4.929	11.04

Genus	**Species**	**Median age (Ma)**	**95 % HPD (Ma)**	
Labridae cont.			**Lower bound**	**Upper bound**
Anampses	*neoguinaicus*	7.621	4.353	11.39
Anampses	*twistii*	7.621	4.353	11.39
Bolbometopon	*muricatum*	17.47	12.91	22.58
Centrolabrus	*caeruleus*	0.9127	0.2128	1.873
Centrolabrus	*exoletus*	0.4775	0.08054	1.268
Centrolabrus	*trutta*	0.9127	0.2128	1.873
Chlorurus	*atrilunula*	0.6688	0.08743	1.813
Chlorurus	*bleekeri*	2.756	1.402	4.429
Chlorurus	*bowersi*	3.839	2.278	5.862
Chlorurus	*capistratoides*	0.6688	0.08743	1.813
Chlorurus	*cyanescens*	1.510	0.4230	3.209
Chlorurus	*enneacanthus*	0.9664	0.1759	2.315
Chlorurus	*frontalis*	0.9664	0.1759	2.315
Chlorurus	*gibbus*	2.030	0.9095	3.641
Chlorurus	*japanensis*	6.915	4.924	9.370
Chlorurus	*microrhinos*	0.7725	0.09398	2.067
Chlorurus	*oedema*	0.4659	0.04009	1.258
Chlorurus	*perspicillatus*	9.132	5.801	13.56
Chlorurus	*rhakoura*	0.4659	0.04009	1.258
Chlorurus	*sordidus*	2.668	1.425	4.512
Chlorurus	*spilurus*	2.668	1.425	4.512
Chlorurus	*strongylocephalus*	0.7725	0.09398	2.067
Cryptotomus	*roseus*	24.57	15.80	32.88
Diproctacanthus	*xanthurus*	14.70	7.351	23.78
Epibulus	*brevis*	4.286	1.035	9.545
Epibulus	*insidiator*	4.286	1.035	9.545
Gomphosus	*caeruleus*	3.444	1.420	7.054
Gomphosus	*varius*	3.444	1.420	7.054
Haletta	*semifasciata*	4.118	1.595	7.680
Hemigymnus	*fasciatus*	7.732	3.154	14.29
Hemigymnus	*melapterus*	7.732	3.154	14.29
Hipposcarus	*harid*	5.944	2.535	11.62
Hipposcarus	*longiceps*	5.944	2.535	11.62
Labrichthys	*unilineatus*	22.36	13.80	32.22
Lachnolaimus	*maximus*	58.63	50.00	69.86
Larabicus	*quadrilineatus*	14.70	7.351	23.78
Leptoscarus	*vaigiensis*	35.86	28.45	44.49
Neodax	*balteatus*	4.118	1.595	7.680
Odax	*acroptilus*	22.16	15.09	30.34
Odax	*cyanoallix*	7.656	3.293	13.30
Odax	*cyanomelas*	18.12	11.26	25.44
Odax	*pullus*	7.656	3.293	13.30
Ophthalmolepis	*lineolata*	31.98	24.79	41.79
Oxyjulis	*californica*	27.74	18.66	37.36
Pseudodax	*moluccanus*	49.39	38.73	60.77
Scarus	*altipinnis*	2.529	1.26	4.048
Scarus	*arabicus*	5.984	2.570	10.36
Scarus	*chameleon*	0.7034	0.1547	1.596
Scarus	*coelestinus*	2.682	1.203	4.621
Scarus	*coeruleus*	3.863	1.324	6.899
Scarus	*collana*	10.80	7.544	14.48
Scarus	*compressus*	1.021	0.2543	2.100
Scarus	*dimidiatus*	3.780	2.068	5.603
Scarus	*dubius*	2.308	0.9034	4.183
Scarus	*falcipinnis*	0.6932	0.1212	1.539
Scarus	*ferrugineus*	1.640	0.5244	3.139
Scarus	*festivus*	0.7034	0.1547	1.596
Scarus	*flavipectoralis*	3.588	1.489	6.071

Genus	Species	Median age (Ma)	95 % HPD (Ma)	
Labridae cont.			**Lower bound**	**Upper bound**
Scarus	*forsteni*	4.472	2.565	6.562
Scarus	*frenatus*	6.094	3.660	8.572
Scarus	*fuscopurpureus*	4.245	2.259	6.637
Scarus	*ghobban*	1.021	0.2543	2.100
Scarus	*globiceps*	1.021	0.2543	2.100
Scarus	*guacamaia*	0.5693	0.08921	1.409
Scarus	*hoefleri*	4.820	2.456	7.700
Scarus	*hypselopterus*	3.588	1.489	6.071
Scarus	*iseri*	8.033	5.490	10.66
Scarus	*koputea*	4.197	1.734	7.079
Scarus	*longipinnis*	2.308	0.9034	4.183
Scarus	*niger*	3.780	2.068	5.603
Scarus	*oviceps*	1.380	0.4725	2.723
Scarus	*ovifrons*	5.984	2.570	10.36
Scarus	*perrico*	4.820	2.456	7.700
Scarus	*persicus*	1.640	0.5244	3.139
Scarus	*prasiognathos*	0.6932	0.1212	1.539
Scarus	*psittacus*	9.023	6.350	12.32
Scarus	*quoyi*	4.197	1.734	7.079
Scarus	*rivulatus*	0.9119	0.2255	1.991
Scarus	*rubroviolaceus*	2.669	1.132	4.502
Scarus	*russelii*	4.245	2.259	6.637
Scarus	*scaber*	1.380	0.4725	2.723
Scarus	*schlegeli*	5.276	3.152	7.714
Scarus	*spinus*	2.274	0.8596	4.215
Scarus	*taeniopterus*	2.019	0.7149	3.791
Scarus	*tricolor*	4.472	2.565	6.562
Scarus	*trispinosus*	0.5693	0.08921	1.409
Scarus	*vetula*	3.863	1.324	6.899
Scarus	*viridifucatus*	2.274	0.8596	4.215
Scarus	*xanthopleura*	6.467	3.824	9.364
Scarus	*zelindae*	2.019	0.7149	3.791
Scarus	*zufar*	7.428	4.960	10.10
Symphodus	*bailloni*	6.715	4.505	9.375
Symphodus	*cinereus*	7.608	5.265	10.57
Symphodus	*doderleini*	5.989	2.426	10.02
Symphodus	*mediterraneus*	5.096	2.914	7.792
Symphodus	*melanocercus*	0.4775	0.08054	1.268
Symphodus	*melops*	0.5752	0.19	1.146
Symphodus	*ocellatus*	5.989	2.426	10.02
Symphodus	*roissali*	0.5752	0.19	1.146
Symphodus	*rostratus*	5.317	3.255	7.692
Symphodus	*tinca*	5.096	2.914	7.792
Tautoga	*onitis*	17.78	8.090	28.94
Tautogolabrus	*adspersus*	17.78	8.090	28.94
Thalassoma	*amblycephalum*	3.829	2.105	5.873
Thalassoma	*ascensionis*	1.423	0.4163	3.025
Thalassoma	*ballieui*	8.712	4.094	14.84
Thalassoma	*bifasciatum*	3.359	1.432	5.991
Thalassoma	*cupido*	3.375	1.720	5.356
Thalassoma	*duperrey*	0.5995	0.1121	1.285
Thalassoma	*genivittatum*	1.539	0.8012	2.572
Thalassoma	*grammaticum*	0.5995	0.1121	1.285
Thalassoma	*hardwicke*	6.871	3.871	10.19
Thalassoma	*hebraicum*	5.328	2.895	8.484
Thalassoma	*jansenii*	6.871	3.871	10.19
Thalassoma	*loxum*	3.375	1.720	5.356
Thalassoma	*lucasanum*	2.145	0.9822	3.569

Genus	**Species**	**Median age (Ma)**	**95 % HPD (Ma)**	
Labridae cont.			**Lower bound**	**Upper bound**
Thalassoma	*lunare*	10.12	7.312	13.37
Thalassoma	*lutescens*	1.961	1.027	3.172
Thalassoma	*newtoni*	3.673	1.686	6.158
Thalassoma	*noronhanum*	3.359	1.432	5.991
Thalassoma	*pavo*	8.459	5.079	12.42
Thalassoma	*purpureum*	1.554	0.5377	2.961
Thalassoma	*quinquevittatum*	9.213	5.945	12.66
Thalassoma	*robertsoni*	2.145	0.9822	3.569
Thalassoma	*rueppellii*	0.9532	0.4111	1.715
Thalassoma	*sanctaehelenae*	1.423	0.4163	3.025
Thalassoma	*septemfasciatum*	8.712	4.094	14.84
Thalassoma	*trilobatum*	1.554	0.5377	2.961
Thalassoma	*virens*	5.219	3.187	7.454
Xiphocheilus	*typus*	39.55	29.81	49.99
Pomacanthidae				
Holacanthus	*africanus*	8.880	5.560	13.05
Holacanthus	*bermudensis*	0.3457	0.03093	0.9602
Holacanthus	*ciliaris*	0.3457	0.03093	0.9602
Holacanthus	*clarionensis*	0.1722	0.001940	0.5250
Holacanthus	*limbaughi*	0.5038	0.1057	1.187
Holacanthus	*passer*	0.1722	0.001940	0.5250
Holacanthus	*tricolor*	6.649	3.905	10.16
Pomacanthus	*annularis*	8.952	4.934	13.31
Pomacanthus	*arcuatus*	2.398	0.9885	4.268
Pomacanthus	*asfur*	5.759	3.645	8.297
Pomacanthus	*chrysurus*	3.210	1.527	5.376
Pomacanthus	*imperator*	8.952	4.934	13.31
Pomacanthus	*maculosus*	3.948	2.210	5.884
Pomacanthus	*navarchus*	8.757	4.776	13.51
Pomacanthus	*paru*	2.398	0.9885	4.268
Pomacanthus	*rhomboides*	4.870	3.007	7.040
Pomacanthus	*semicirculatus*	3.210	1.527	5.376
Pomacanthus	*sexstriatus*	3.274	1.340	6.018
Pomacanthus	*xanthometapon*	3.274	1.340	6.018
Pomacanthus	*zonipectus*	4.808	2.544	7.890
Pygoplites	*diacanthus*	13.47	8.760	18.97
Epinephelidae				
Aethaloperca	*rogaa*	27.97	20.32	37.86
Alphestes	*afer*	4.590	2.322	7.403
Alphestes	*immaculatus*	3.154	1.291	5.508
Alphestes	*multiguttatus*	3.154	1.291	5.508
Anyperodon	*leucogrammicus*	17.87	11.90	25.36
Dermatolepis	*dermatolepis*	4.319	2.055	7.225
Dermatolepis	*inermis*	3.112	1.268	5.564
Dermatolepis	*striolata*	3.112	1.268	5.564
Gracila	*albomarginata*	29.17	17.90	41.51
Mycteroperca	*acutirostris*	0.4565	0.004133	1.553
Mycteroperca	*bonaci*	1.618	0.6392	2.948
Mycteroperca	*fusca*	5.787	2.247	9.765
Mycteroperca	*interstitialis*	2.689	1.201	4.351
Mycteroperca	*jordani*	3.580	1.899	5.512
Mycteroperca	*microlepis*	6.888	4.758	9.403
Mycteroperca	*olfax*	3.776	2.028	5.635
Mycteroperca	*phenax*	0.1549	0.000001878	0.7431
Mycteroperca	*prionura*	3.776	2.028	5.635
Mycteroperca	*rosacea*	3.040	1.554	4.836
Mycteroperca	*rubra*	0.4565	0.004133	1.553
Mycteroperca	*tigris*	3.040	1.554	4.836

Genus	Species	Median age (Ma)	95% HPD (Ma)	
Epinephelidae cont.			**Lower bound**	**Upper bound**
Mycteroperca	*venenosa*	1.618	0.6392	2.948
Mycteroperca	*xenarcha*	0.1549	0.000001878	0.7431
Niphon	*spinosus*	51.32	29.49	69.90
Paranthias	*colonus*	2.509	0.8494	5.361
Paranthias	*furcifer*	2.509	0.8494	5.361
Plectropomus	*areolatus*	3.691	1.579	6.102
Plectropomus	*laevis*	3.322	1.571	5.680
Plectropomus	*leopardus*	3.691	1.579	6.102
Plectropomus	*maculatus*	2.957	1.485	4.681
Plectropomus	*oligacanthus*	3.322	1.571	5.680
Plectropomus	*pessuliferus*	2.957	1.485	4.681
Plectropomus	*punctatus*	7.243	4.597	10.26
Saloptia	*powelli*	23.88	13.84	36.50
Variola	*albimarginata*	5.599	2.352	10.43
Variola	*louti*	5.599	2.352	10.43
Fossil Calibration Taxa				
Acanrthurus	*nigricans*	45.34	22.60	57.48
Ambloplites	*rupestris*	16.58	15.80	17.96
Archoplites	*interruptus*	16.58	15.80	17.96
Bodianus	*mesothorax*	52.08	41.61	64.12
Calatomus	*carolinus*	30.64	24.42	38.03
Cetoscarus	*bicolor*	17.47	12.91	22.58
Gazza	*minuta*	17.57	12.23	27.83
Leiognathus	*equulus*	17.57	12.23	27.83
Luvarus	*imperialis*	45.34	22.60	57.48
Naso	*lituratus*	40.99	20.01	55.92
Prognathodes	*aculeatus*	28.45	22.91	34.71
Sparisoma	*chrysopterum*	24.57	15.80	32.88
Zanclus	*cornutus*	40.99	20.01	55.92
Outgroup Taxa				
Opsanus	*pardus*	43.56	13.93	79.73
Porichthys	*notatus*	43.56	13.93	79.73

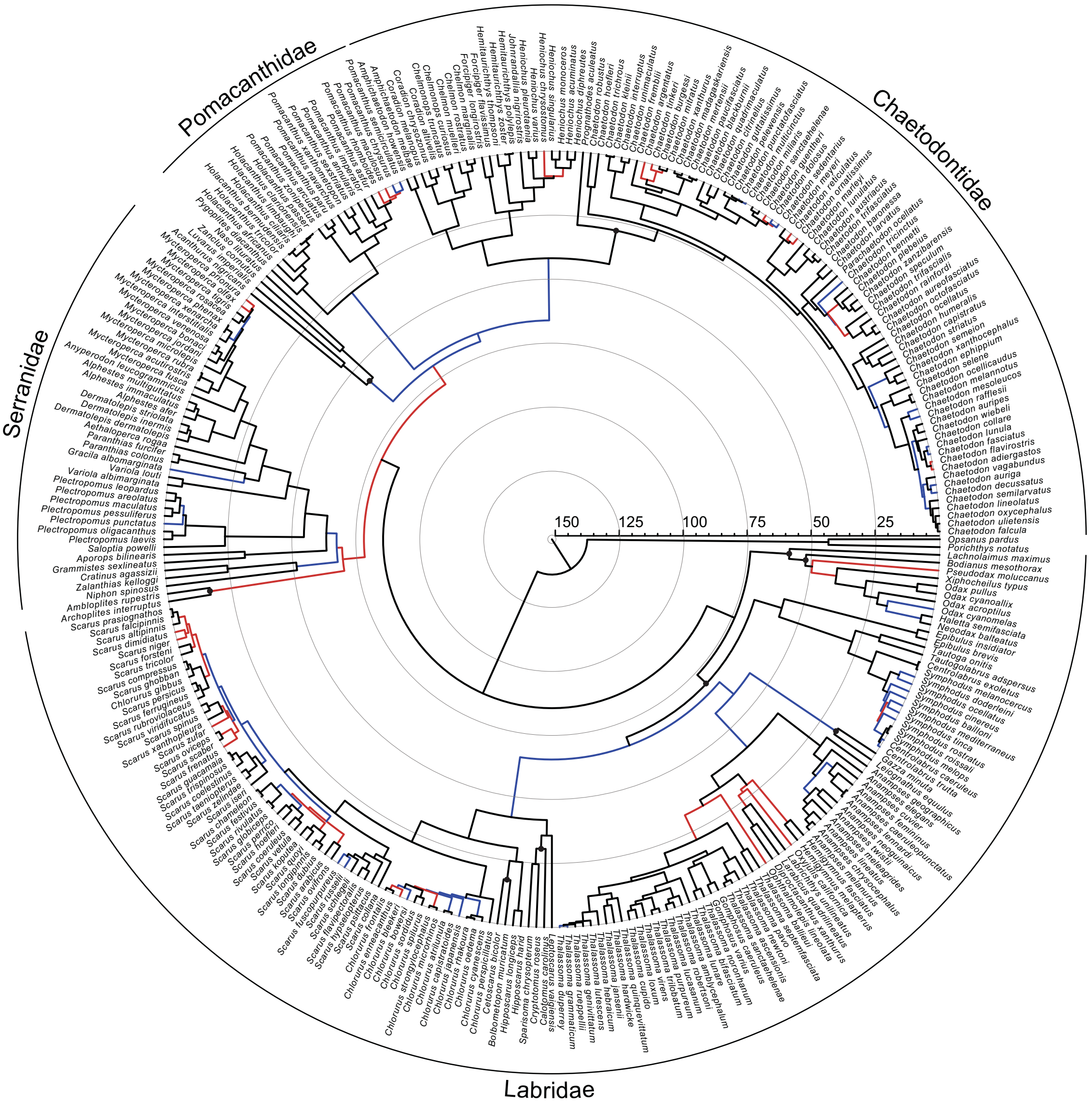

Figure S5.1 Time-calibrated phylogeny of 312 Percomoph fishes compiled from post burn-in topologies of six independent Bayesian Markov Chain Monte Carlo analyses (40 × 10^6 generations per run) using nine fossil calibration points implemented in BEAST. Molecular data included four mitochondrial loci (16S rRNA, 12S rRNA, CO1, cytochrome b) and two nuclear loci (TMO-4C4, S7 intron 1); see Table S2.5 in Appendix A for partitioning scheme. Nodes represent median ages from a maximum clade-credibility tree (values provided in Table S5.1 above, along with corresponding 95% highest posterior density intervals). Branch colours subtending nodes indicate posterior probability for the node: black > 75%; blue 50–74%; and red < 49%. • indicates nodes calibrated with priors based on fossil data (Table S2.4, Appendix A). Time scale is in millions of years before present.

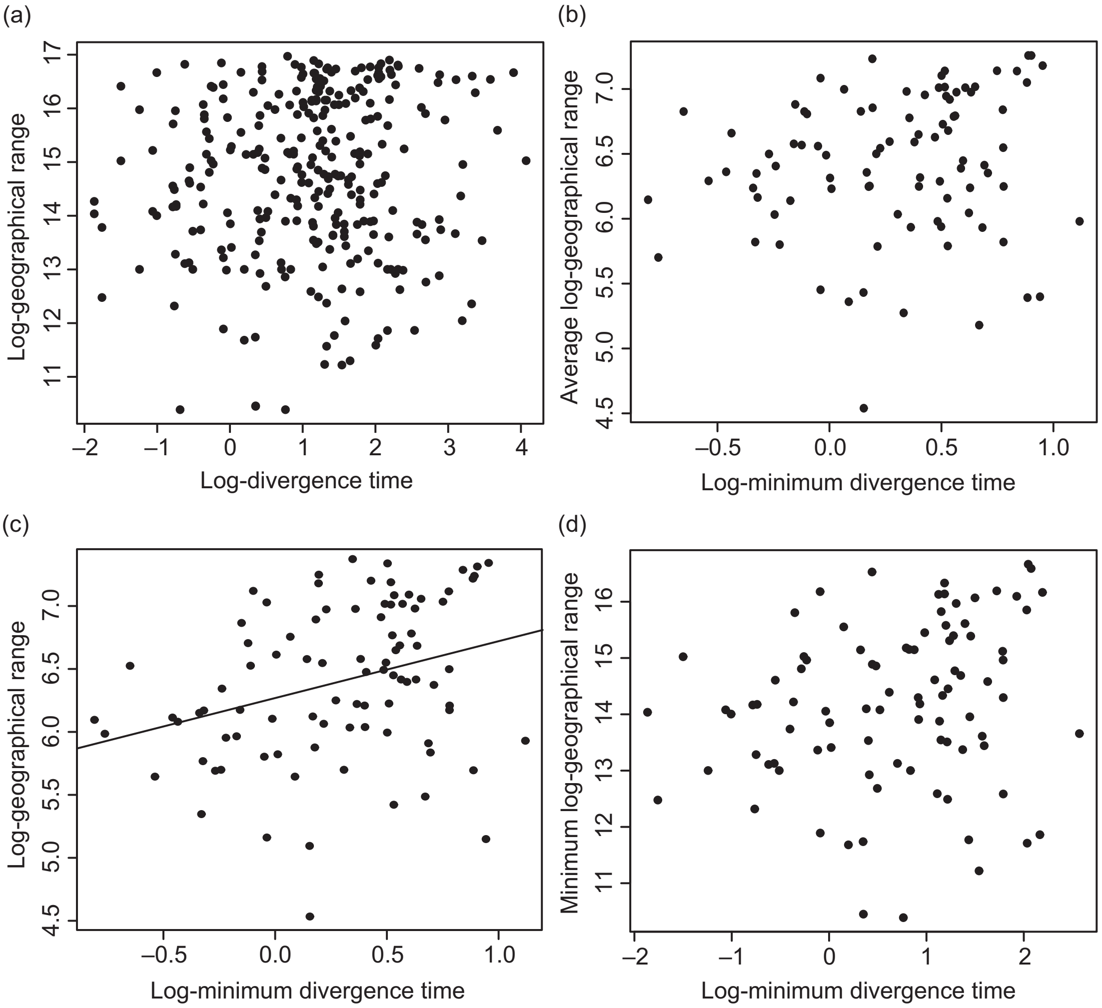

Figure S5.2 All analytical approaches were repeated using log-transformed data and linear regression. Linear models were not plotted unless the relationship was significant. (a) No significant linear log-log relationship was detected between geographical range and species age across all nodes in the phylogeny ($r^2 = 0.00544$; $n = 293$; d.f. = 1, 291; $F = 1.59$; $P = 0.208$). (b) No significant linear log-log relationship was detected between average geographical range area (calculated as the mean of two sister-species ranges) and species' age for all sister-pairs ($r^2 = 0.0247$; $n = 91$; d.f. = 1, 89; $F = 2.26$; $P = 0.137$). (c) Using a sister-species approach and randomly selecting one of two geographical ranges per sister-pair, this plot shows the relationship between the log-transformed variables for the most significant randomised replicate (9.3% of 1000 bootstrapped replicates were significant; the range of statistical values across all 93 significant replicates: $r^2 = 0.0993$–0.0427; $n = 91$; d.f. = 1, 89; $F = 9.82$–3.97; $P = 0.00234$–0.0495). (d) A marginally non-significant linear log-log relationship was detected between minimum geographical range and species age ($r^2 = 0.0419$; $n = 91$; d.f. = 1, 89; $F = 3.89$; $P = 0.0516$). If a type II error produced a false non-significant result in this analysis and there were a significant relationship between the log-transformed data, it would suggest that as species age their geographical ranges increase exponentially. Such a pattern may be consistent with the 'stasis post-expansion' or 'taxon cycle' theoretical models of geographical range evolution if range expansion occurs rapidly.

Appendix E: Supplementary materials for Chapter 6

The following supplemental accompanies the article

Temporal evolution of coral reef fishes: global patterns and disparity in isolated locations

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Journal of Biogeography 41(11): 2115–2127 (2014)

TABLES

Table S6.1 The number of species per genus and the proportion of missing extant species in the multi-family phylogenetic tree.

REFERENCE LIST

Table S6.1 The total number of extant, nominal species for each genus included in the multi-family phylogenetic analyses; the number of extant, nominal species missing from the multi-family phylogenetic analyses, and the resultant proportion of missing extant, nominal species for each genus. Species descriptions were obtained from the IUCN Red List (IUCN, 2011) and FishBase (http://www.fishbase.org/).

Genus	Total No. constituent species	No. missing extant species	Percent missing extant species
Chaetodontidae			
Amphichaetodon	2	0	0
Chaetodon	87	15	17.2
Chelmon	3	0	0
Chelmonops	2	0	0
Coradion	3	0	0
Forcipiger	2	0	0
Hemitaurichthys	4	1	25.0
Heniochus	8	1	12.5
Jonhrandallia	1	0	0
Parachaetodon	1	0	0
Labridae			
Anampses	12	0	0
Bolbometopon	1	0	0
Centrolabrus	3	0	0
Chlorurus	18	2	11.1
Cryptotomus	1	0	0
Diproctacanthus	1	0	0
Epibulus	2	0	0
Gomphosus	2	0	0
Haletta	1	0	0
Hemigymnus	2	0	0
Hipposcarus	2	0	0
Labrichthys	1	0	0
Lachnolaimus	1	0	0
Larabicus	1	0	0
Leptoscarus	1	0	0
Neodax	1	0	0
Odax	4	0	0
Ophthalmolepis	1	0	0
Oxyjulis	1	0	0
Pseudodax	1	0	0
Scarus	52	6	11.5
Symphodus	10	0	0
Tautoga	1	0	0
Tautogolabrus	1	0	0
Thalassoma	28	2	7.1
Xiphocheilus	1	0	0
Pomacanthidae			
Holacanthus	8	1	12.5
Pomacanthus	13	0	0
Pygoplites	1	0	0
Epinephelidae			
Aethaloperca	1	0	0
Alphestes	3	0	0
Anyperodon	1	0	0
Aporops	1	0	0
Dermatolepis	3	0	0
Cratinus	1	0	0
Gracila	1	0	0

Genus	**Total number of constituent species**	**Number of missing extant species**	**Percentage of missing extant species**
Epinephelidae cont.			
Grammistes	1	0	0
Mycteroperca	15	1	6.7
Niphon	1	0	0
Paranthias	2	0	0
Plectropomus	7	1	14.3
Saloptia	1	0	0
Variola	2	0	0
Zalanthias	1	0	0

References

IUCN (2011) *IUCN Red List of Threatened Species*. Version 2011.2. Available at: http://www.iucnredlist.org/.

Appendix F: Supplementary materials for Chapter 7

The following supplemental accompanies the article

The geography of speciation in coral reef fishes

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Submitted for publication

FIGURES

Figure S7.1 Map showing allopatric spits that did not show congruence and/or align with a single previously described biogeographical barrier.

REFERENCE LIST

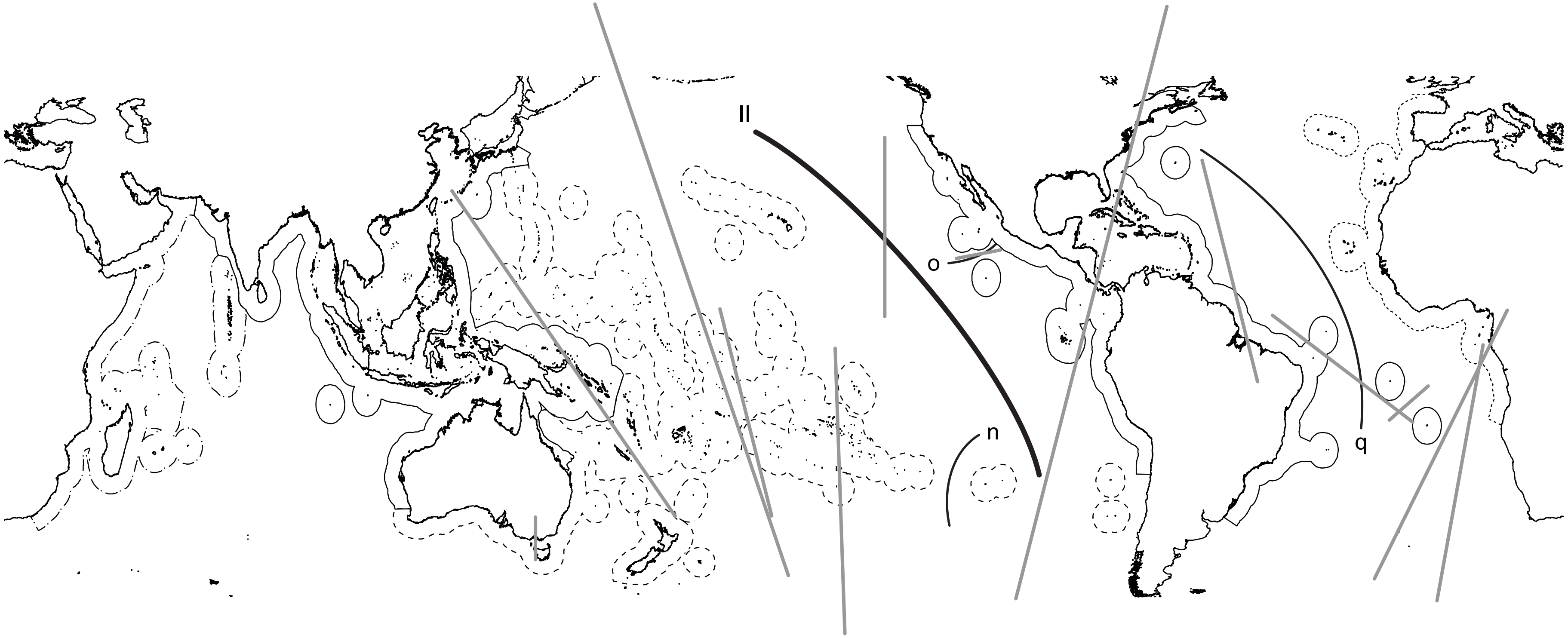

Figure S7.1 Allopatric spits classified as ‘other’ (*n* = 13) that did not show congruence and/or align with a single previously described biogeographical barrier are mapped as grey lines. The area of likely vicariance was ambiguous for most of these splits because the distance between their sister-species distributions was large enough to span multiple biogeographical barriers (see Figure 7.2a for location of all previously described barriers considered herein). However, some of the remaining splits did solely correspond to previously described barriers, including: II, n, o, and q (cf. Bellwood & Wainwright, 2002; Rocha *et al*., 2007; Floeter *et al*., 2008; Luiz *et al*., 2012). The average degree of symmetry for these splits is plotted in Figure 7.2c, and was not significantly different from a random distribution of mean values drawn from the full pool of allopatric sister-species.

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Appendix G: Publications arising from thesis

Hodge, J. R., Read, C. I., van Herwerden, L. & Bellwood, D. R. (2012) The role of peripheral endemism in species diversification: evidence from the coral reef fish genus *Anampses* (Family: Labridae). *Molecular Phylogenetics and Evolution* **62**(2), 653–663. doi: 10.1016/j.ympev.2011.11.007

Hodge, J. R., Read, C. I., Bellwood, D. R. & van Herwerden, L. (2013) Evolution of sympatric species: a case study of the coral reef fish genus *Pomacanthus* (Family: Pomacanthidae). *Journal of Biogeography* **40**(9), 1676–1687. doi: 10.1111/jbi.12124

Hodge, J. R., van Herwerden, L. & Bellwood, D. R. (2014) Temporal evolution of coral reef fishes: global patterns and disparity in isolated locations. *Journal of Biogeography* **41**(11), 2115–2127. doi: 10.1111/jbi.12356

Hodge, J. R., & Bellwood, D. R. (2014 in press) On the relationship between species age and geographical range in reef fishes: are widespread species older than they seem? *Global Ecology and Biogeography*.

Hodge, J. R., & Bellwood, D. R. (2014 submitted) The geography of speciation in coral reef fishes.

Molecular Phylogenetics and Evolution 62 (2012) 653–663

Contents lists available at SciVerse ScienceDirect

Molecular Phylogenetics and Evolution

journal homepage: www.elsevier.com/locate/ympev

The role of peripheral endemism in species diversification: Evidence from the coral reef fish genus *Anampses* (Family: Labridae)

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A R T I C L E I N F O

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Molecular phylogeny
Age estimation
Successive division
Successive colonisation
Peripheral budding

A B S T R A C T

We examined how peripherally isolated endemic species may have contributed to the biodiversity of the Indo-Australian Archipelago biodiversity hotspot by reconstructing the evolutionary history of the wrasse genus *Anampses*. We identified three alternate models of diversification: the vicariance-based 'successive division' model, and the dispersal-based 'successive colonisation' and 'peripheral budding' models. The genus was well suited for this study given its relatively high proportion (42%) of endemic species, its reasonably low diversity (12 species), which permitted complete taxon sampling, and its widespread tropical Indo-Pacific distribution. Monophyly of the genus was strongly supported by three phylogenetic analyses: maximum parsimony, maximum likelihood, and Bayesian inference based on mitochondrial CO1 and 12S rRNA and nuclear S7 sequences. Estimates of species divergence times from fossil-calibrated Bayesian inference suggest that *Anampses* arose in the mid-Eocene and subsequently diversified throughout the Miocene. Evolutionary relationships within the genus, combined with limited spatial and temporal concordance among endemics, offer support for all three alternate models of diversification. Our findings emphasise the importance of peripherally isolated locations in creating and maintaining endemic species and their contribution to the biodiversity of the Indo-Australian Archipelago.

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ORIGINAL ARTICLE

Evolution of sympatric species: a case study of the coral reef fish genus *Pomacanthus* (Pomacanthidae)

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ABSTRACT

Aim To establish a spatial and temporal framework within which we can begin to investigate the role of geography in the evolution of *Pomacanthus* species. This study examines the phylogenetic relationships among *Pomacanthus* species, tests whether the degree of sympatry among sister taxa correlates with their age, and explores potential modes of diversification.

Location Pan-tropical coral reef systems.

Methods Three gene regions (12S rRNA, 16S rRNA and nuclear S7) from all 13 *Pomacanthus* species were used in conjunction with fossil calibration to reconstruct a chronogram. IUCN maps were used to evaluate geographical range overlap. Various age–range correlation (ARC) analyses were used to test for a correlation between range overlap and node age. Range-size symmetry and node age were also examined.

Results Biogeographical splits within the phylogeny corroborated key biogeographical events well, suggesting a potential role for allopatric speciation in the evolutionary history of the genus. ARC analyses suggested that speciation leading to sympatry is widespread in *Pomacanthus*, with 80% of sister species showing complete or substantial (> 85%) range overlap. No significant relationship between degree of sympatry and node age was recovered, which demonstrates that, for this group, exceptional sympatry is not necessarily a result of relatively old lineages. Range-size symmetry analysis was consistent with models of peripatric speciation within a finite area.

Main conclusions We present evidence consistent with allopatric speciation, while our ARC results are consistent with simulations of sympatric speciation, with the possibility of peripheral budding having led to the richness of sympatric species in the West Indian Ocean. We discuss how these processes can affect the interpretation of temporal and spatial analyses, and provide a basis for future investigations into the processes driving speciation.

Keywords
Age–range correlation, allopatry, coral reefs, divergence dating, fossil calibration, marine biogeography, pan-tropical, peripheral budding, speciation mode, sympatry.

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doi:10.1111/jbi.12124

Journal of Biogeography (*J. Biogeogr.*) (2014) **41**, 2115–2127

Journal of Biogeography

ORIGINAL ARTICLE

Temporal evolution of coral reef fishes: global patterns and disparity in isolated locations

Jennifer R. Hodge[1,2,3]*, Lynne van Herwerden[1,3] and David R. Bellwood[1,2]

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ABSTRACT

Aim Many coral reef regions have a history of isolation and extinction. Our aim was to test whether the disparate evolutionary and biogeographical histories of the world's coral reef regions have significantly impacted temporal patterns of speciation within regions. In essence, do assemblages in peripheral locations contain the youngest coral reef fish species?

Location Pan-tropical coral reef systems.

Methods Molecular data (mitochondrial 16S rRNA, 12S rRNA, *CO1*, cytochrome *b*; nuclear *TMO-4C4*, *S7* intron 1) were assembled for genera with near-complete taxon sampling (minimum 70% nominal species) from four major coral reef fish families (Chaetodontidae, Labridae, Pomacanthidae and Serranidae). This was combined with fossil data to simultaneously infer the phylogeny and estimate species' divergence times. Species' distributions were quantified using IUCN maps and the ages of species with different biogeographical extents were compared. Model fitting was used to compare the distribution of species' ages across the whole phylogeny with age distributions of species restricted to the Red Sea and Hawaiian Islands.

Results Temporal patterns of coral reef fish divergence were similar among major marine realms and regions. However, notable differences were recorded between the Red Sea and Hawaiian Islands. Red Sea endemics have diverged consistently throughout the last 16 Myr, whereas endemic species colonized the Hawaiian Islands in two distinct waves (0–3 Ma and 8–12 Ma). Differences in the proportions of allopatric and sympatric sister-species between Red Sea and Hawaiian endemics were also detected.

Main conclusions Despite differing geological histories, marine realms and regions have all experienced comparable and relatively recent divergences of extant coral reef fish species. Differences in age distributions and spatial relationships of endemic species in the Red Sea and Hawaiian Islands suggest that markedly different processes have shaped patterns of diversification in these peripherally isolated locations.

Keywords

Biodiversity, coral reef fishes, Coral Triangle, endemism, evolutionary age, Hawaiian Islands, marine biogeography, peripatric speciation, Red Sea, sister-species.

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doi:10.1111/jbi.12356