The origins of marine biodiversity: Speciation and diversification in Caribbean corals (family Faviidae) and Indo-Pacific parrotfishes (genus *Scarus*).

by

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Committee in charge:

Professor George Roderick, Chair
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Professor Patrick O'Grady
Professor Rasmus Nielsen

Abstract

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Species-rich coral reef systems present a paradox to evolutionary biologists. Many reef invertebrates and fish have enormous dispersal potential via a planktonic larval stage, presumably leading to widespread patterns of genetic panmixia at large spatial scales. In the context of such high connectivity, how can we begin to get diversification? In this dissertation, I examine this question in two different systems, Caribbean corals and Indo-Pacific parrotfishes, and in two dimensions - time and space. The timing and tempo of species diversification can tell us how geological and environmental change is correlated to species formation. At the same time, the spatial distribution of genetic variation can reveal how contemporary processes may have contributed to population splitting and eventually speciation.

In Chapter 1, I examine the history of reef systems in the Caribbean basin by looking at macroevolutionary patterns in faviid coral. Combining a newly compiled fossil stratigraphy with a time-calibrated molecular phylogeny of extant Caribbean Faviidae, I show that these Caribbean corals are not related to their Mediterranean confamilials, and that all living species appear to have originated during a period of rapid environmental change in Mio-Pliocene. In Chapter 2, I examine cryptic speciation and the patterns of diversity in the parrotfish, *Scarus ghobban*. Though this species was thought to have a pan-Indo-Pacific distribution, a new mitochondrial phylogeny shows deep divergence between Indian and Pacific Ocean clades. Furthermore, these clades form a species complex with a Panamanian endemic species, *Scarus compressus*, and a newly described Western Australian endemic species. Finally, in Chapter 3, I use population genetics methods to take a deeper look at the history of these fishes in the Pacific and assess the spatiotemporal structuring of diversity. My results suggest those Pleistocene sea level fluctuations, peripheral isolation, and hybridization all played significant roles in creating and maintaining diversity in this complex.

By assessing taxonomic and population dynamics in the framework of historic environmental processes, I begin to answer fundamental questions about evolution in both Caribbean and Indo-Pacific tropical marine systems. The linking of patterns of diversity over multiple time scales can provide clues about processes that are important to ecology of marine taxa both in the past and in the present. Further, understanding the distribution of basic evolutionary units (e.g. species or

ESUs) identifies fundamental units for conservation. With coral reef ecosystems currently highly threatened by anthropogenic change, this knowledge will be essential in future conservation efforts.

This dissertation is dedicated to my mother, Teena Schwartz, who taught me to never give up on what I believe in, to always be generous and kind, and that sometimes you just really need to jump in a puddle. I only hope I can live up to her example.

TABLE OF CONTENTS

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As I put the final touches on this dissertation, I look back and realize I never would have made it to this point without the help and support of many people.

I would like to express deep gratitude to my co-advisors and committee, without whom this dissertation would not have been possible. Dave Carlon not only helped me come up with the ideas that brought life to these projects, but with endless patience, provided guidance and feedback throughout the research process. George Roderick generously took me into his lab when I had nowhere else to go (and despite the fact that my study organisms had some pesky backbones) and has given me all of the resources and help needed to complete this dissertation. I would also like to acknowledge and thank my other committee members, Patrick O'Grady and Rasmus Nielsen for their feedback and support.

The research in this dissertation covers a large area, both theoretically and geographically. Getting through it would not have been feasible without critical help from my collaborators. My co-authors for the first chapter, Nancy Budd and Dave Carlon, were essential to my work on all things coral, from providing samples, data, editing, and key intellectual contributions to this paper. Many samples of parrotfish for the second and third chapters were graciously provided by Howard Choat at James Cook University, Ross Robertson at STRI, and the Carlon lab at the University of Hawaii, Manoa. Field collections and sample identification in Western Australia were greatly assisted by Kendall Clements of the University of Auckland.

Of course, I would like to thank my labmates and peers for their help as I slogged through endless hours in the lab and analyzing data. I am especially grateful to my URAP apprentices, Jennifer Hoey, Sabina Lau, Sam Leiboff, and Lauren Williams, whose enthusiasm and assistance helped drive this project forward. I am also deeply indebted to Rick Lapoint and Pete Croucher, who were infinitely patient as I pestered them endlessly with questions about data analysis. Not least, I am thankful to Evolab as a whole for providing me with such a supportive environment in which to work and grow academically.

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LIST OF FIGURES

Figure **Page**

LIST OF TABLES

Table **Page**

Chapter 1: Molecules and fossils reveal punctuated diversification in Caribbean "faviid" corals

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Introduction:

Explaining rapid diversification and speciation remains a central challenge to evolutionary biology [1, 2]. Much work has focused on either understanding the ecology and phylogenetic history of species-rich systems that have recently diversified along ecological axes (e.g. adaptive radiations) [3], or looking for patterns of species change in the fossil record [4-8]. Taking the molecular phylogenetic/ecological approach alone, however, excludes information about extinct lineages that may substantially bias our ability to identify cases of rapid diversification [9]. Conversely, relying on the fossil record alone limits our ability to detect evolutionary relationships between fossil taxa and some shifts in ecological function that may not be apparent from fossil character states. Ultimately, a more complete understanding of the processes that drive rapid diversification will require historical information from both molecular and fossil data. By examining systems that show recent speciation within monophyletic groups, ecological differentiation, and a strong fossil record, we can begin to link past to present processes in the understanding of the evolution of diversity.

The marine Caribbean fauna provides rare examples of diversification of monophyletic lineages within in the context of well-understood changes in biogeography, oceanography, and climate. The isolation of Caribbean populations from their Indo-Pacific counterparts started ~15-17 Ma when the closure of the Tethys Sea cut off connections between the Mediterranean and Indo-Pacific [10]. Isolation was complete ~3.45 - 4.25 Ma when the rise of the Isthmus of Panama severed all Caribbean connections to the Indo-Pacific [11]. The period leading up to closure of the isthmus during the late Miocene to late Pliocene was characterized by changing global oceanographic circulation patterns, leading to drastic environmental, ecological, and taxonomic shifts within the Caribbean basin. Not only did the cessation of gene flow between the Pacific and Atlantic Oceans lead to widespread vicariant speciation across the newly formed isthmus[12-14], but on the Caribbean side, the accompanying geological and oceanographic changes caused an overall decrease in depth, primary productivity and turbidity and an increase in salinity, temperature, and local environmental heterogeneity [11, 15]. Fossil records of many marine taxa during this period show elevated levels of taxonomic turnover [11, 16-21], suggesting that climatic and geological variables drove elevated rates of cladogenesis and extinction.

The scleractinian corals of the Caribbean basin are an excellent system for using phylogenetic and fossil perspectives for examining this faunal shift as they have a fossil record that is well sampled in both space and time dating back to the early Neogene [22-26]. Furthermore, the origin of almost all living species can be traced back through time in this record. Many of the environmental shifts seen during the time period of the fossil record, such as increase in water

clarity and decrease in depth, favor the development of reef-building corals [16], predicting an increased potential for evolutionary radiation. Correspondingly, several studies of origination and extinction rates detail a massive species turnover between 4-1.5 Ma [21, 27, 28] where 80% of the Mio-Pliocene coral fauna went extinct, while more than 60% of modern coral species originated.

This taxonomic turnover is particularly striking in corals of the family Faviidae, where an examination of the stratigraphic ranges shows that all extant species originated nearly simultaneously during the Mio-Pliocene [29]. Moreover, for faviids, this recent radiation has resulted in impressive diversification of ecological and life-history traits [30-32]. Modern species of *Manicina* are representative of a free-living lifestyle adapted to sediment-rich seagrass habitats that expanded during the Miocene then contracted during the Plio-Pleistocene [15]. In contrast, species of the brain coral genus *Diploria* tend to be reef-builders, dominating shallow water reef platforms in Pleistocene and modern times [33-36]. These two "sediment" and "reef" clades appear to share a common ancestor and ecological diversification seems to have occurred over a short period of geological time, suggesting it is tied to the contemporaneous increase in environmental heterogeneity [28]. Yet this punctuated diversification event is inferred from a fossil record, which may be incomplete or contain uncertainties in dating and taxonomic relationships that may influence our interpretation of past patterns.

Molecular data combined with well sampled fossil records provide opportunities to test existing evolutionary hypotheses and extend our understanding of both the tempo and mode of evolutionary diversification. In the Scleractinia, deep divergences between coral orders, suborders and families are increasingly well understood[37-40]. Yet a recent series of phylogenies exploring relationships at the familial level and below have demonstrated pervasive polyphyly and paraphyly at the generic level [41-46]. In addition, these studies have shown that between ocean basins, species group geographically rather than taxonomically [42, 45, 46]. In particular, Atlantic lineages of Faviidae and Mussidae appear to be more closely related to other Atlantic lineages than to congeners or even confamilials in other ocean basins. This geographic split supports the evidence from the fossil record of a radiation of the Caribbean coral fauna before complete isolation from the Pacific. However, the failure to resolve species relationships within the traditional coral family Faviidae, and a long history of taxonomic difficulties in identifying and classifying corals [39, 43, 47] demands an independent assessment of trends apparent in the fossil record.

To explore the tempo and mode of this evolutionary diversification, we unite a new multi-locus phylogeny of the Caribbean Faviidae with new stratigraphic compilations from the fossil record. Our well-sampled phylogeny allows Bayesian approaches to place these relationships into a temporal context by dating divergence times based on molecular data and fossil calibrations. We compare our time-calibrated phylogeny to temporal patterns of origination and extinction revealed by the Neogene fossil record, and find remarkable congruence between data sets. The timing of events revealed by this analysis strongly implicates paleoenvironmental changes as drivers of diversification in scleractinian corals.

Methods

Taxon sampling

We sampled six of the seven nominal species from the genera *Favia*, *Diploria*, and *Manicina* that form a monophyletic group within the Caribbean Faviidae [42, 46]. The single missing taxon is *Favia gravida*, closely related to *Favia fragum,* but with a distinct non-Caribbean distribution in that it has been only described from Brazil and West Africa [48, 49]. We used the genus *Colpophyllia* as the outgroup because it has previously been shown to be a stem taxon to the ingroup species [42, 46]. Extensive sampling within each species was conducted at two reef systems in the Caribbean Sea: the Bocas del Toro, Panama, and the Florida Keys, USA with additional *F. fragum* sampled from St. Croix, USVI. The complete list of samples analyzed for this paper with collection localities is provided in **Appendix 1**. Morphological vouchers (skeletal) are deposited in the University of Iowa Paleontology Repository (http://geoscience.clas.uiowa.edu/paleo/index).Samples were collected, preserved, processed and genomic DNA extracted as described in Carlon and Lippé [50]. Skeletal vouchers were processed by bleaching in a 50% hypochlorite/water solution overnight, rinsing in DI water, and thoroughly drying. Species identification was conducted by D. Carlon in the field and confirmed by A. Budd from vouchers. Complete descriptions of taxa, photos, and references are available from the Neogene Marine Biota of Tropical American (NMITA) database (http://eusmilia.geology.uiowa.edu/)

Laboratory Protocols

For this study we chose to focus on nuclear markers, since rates of mitochondrial DNA evolution have been shown to be very slow in corals, limiting the ability to detect more recent speciation events[51]. We amplified three single-copy nuclear regions with primers listed in **Table 1**. *Pax-C* and *CaM* primers target introns located within the Pax protein and calmodulin binding protein respectively [52, 53], while *MaSC-1* is an anonymous region originally sequenced in *Montastraea annularis* [54]. For PCR amplification of all three loci, we combined: 1 µl of 1x to 100x diluted genomic DNA with a 24 µl PCR master mix consisting of: 0.3 µl of each primer (10 µM), 1 µl dNTPs (2.5 mM each), 2.5 µl 10x reaction buffer, 1 µl $MgCl_2$ (25 mM), 1 µl BSA (10 mg/ml), 0.3 µl Taq polymerase (Bioline), and 17.6 µl of H_20. Each reaction was run at 95°C for 10 min, 30 cycles of 94°C for 30s, Ta for 40s and 72°C for 1 min, with a final extension of 72°C for 10 minutes. Purified PCR products were sequenced on ABI 3731 XL 96 capillary DNA analyzers at the University of

Table 1. Coral PCR primers sequence & annealing temperatures

Primer	Sequence	(T_a)
PaxC-F	5' GGAGGAGCTTGCGAATAAGA 3'	62 °C
PaxC-R	5' CCCGGCGATTTGAGAACCAAACCT 3'	
CaM-F	5' GGAACAGTAATGCGATCTCTTGGA 3'	60 °C
CaM-R	5' TGTCTTTCATTTTCCGCGCCATCA 3'	
MaSC1-F	5' TGCTGTGAGAAATAGAACACCTG 3'	60 °C
MaSC1-R	5' CTGCCAAGAAGGATCGATTG 3'	

Hawaii at Manoa and chromatograms were then analyzed and edited using Sequencher 4.5 (Gene Codes). For heterozygous individuals, the PCR product was cloned using a TOPO TA cloning kit (Invitrogen) and sequenced using standard M13 primers.

Phylogenetic analyses

For all loci, alleles were called directly from homozygous individuals or from sequences of clones for heterozygotes. Allele sequences were aligned automatically using MAFFT v6 [55, 56], and corrected by eye in MacCladev4.08 [57]. Indels were coded as missing data. Models for molecular evolution for Bayesian analysis were selected using the Akaike Information Criteria (AIC) in MrModelTestv2.2 [58]. The HKY+G model was chosen for *CaM*, and the GTR model was chosen for *Pax-C* and *MaSC-1*. Bayesian trees for all three loci were generated in MrBayes v3.1 (5,000,000 generations, nruns=2, nchains=4) [59, 60]. Trees were sampled every 100 generations and 5,000 trees were discarded as burn-in. Maximum likelihood analysis was performed using RaxML v7.2.6 [61, 62] with 1000 rapid bootstraps and the default GTR+G model for all loci at the recommendation of the programmers.

Individuals sequenced at all three loci were used for the construction of a combined partitioned tree. For heterozygotes, SNPs were coded as ambiguous data using standard IUPAC nucleotide ambiguity codes. Partitions were aligned and evolutionary models were chosen for this new data set using the methods discussed above. For Bayesian analysis, the HKY+G model was used for the *CaM* and *MaSC-1* partitions, and the HKY+I model for the *Pax-C* partition. Bayesian trees were generated using MrBayes v3.1 as in gene trees (20,000,000 generations, nruns=4, nchains=4). Trees were sampled every 1000 generations and 5000 trees were discarded as burn-in. Maximum likelihood analysis was performed using RaxML v7.2.6 with 1000 rapid bootstraps on the Cipres Web Server [63]. For this analysis, the default GTR+G model was used as above. As a rough test for support of generic monophyly, trees retained from the Bayesian analysis after burn-in were filtered in PAUP [64] using a constraint tree with monophyletic genera.

Divergence Dating

The program BEAST v1.6.1 [65] was used to estimate divergence dates at species nodes using available fossil data for calibration. Beauti v1.5.4 was used on the partitioned alignment file to generate a phylogenetic tree with divergence dates. We used a Yule process speciation prior for branching rates along with an uncorrelated lognormal model for a relaxed molecular clock. Substitution models for each partition were identical to those used for the partitions of the concatenated Bayesian analysis above and base frequencies were estimated throughout the analysis. Based on the phylogenetic analysis, all species nodes were constrained to be monophyletic except for *M. areolata* and *M. mayori,* which were constrained at the genus node. Shape parameter priors were taken from MrModeltest v2.2 and priors for rates of evolution and Yule birth rates were chosen based on defaults narrowed from preliminary runs.

Stratigraphic ranges of extinct and living Caribbean Faviidae were compiled from the literature and unpublished sources (**Appendix 2**). Fossil stratigraphic ranges for extant species were used to calibrate species nodes for *Diploria spp., Favia fragum* and *Colpophyllia natans* and the genus node for *Manicina* (**Table 2**).

Table 2. Faviidae stratigraphic ranges and BEAST calibrations

Species	**Fossil Date of 1st occurrence (Ma)**	**Node Calibration** (mean, standard deviation, offset)	**Median** (*95% interval*)
Colpophyllia natans	5.1-5.3	0.5,1.0,5.1	6.7 (*5.3-16.8*)
Favia fragum	3.0-5.6	1.1,0.8,3.0	6.0 (*3.8 - 17.4*)
Diploria clivosa	4.6-5.9	0.6,1.0,4.6	6.4 (*4.9-17.5*)
Diploria labyrinthiformis	2.9-3.1	0.7,1.0,2.9	4.9 (*3.2-17.2)*
Diploria strigosa	4.6-5.9	0.6,1.0,4.6	6.4 (*4.9-17.5*)
Manicina areolata	3.0-5.6	1.1,0.8,3.0*	6.0 (*3.8 - 17.4)*
Manicina mayori	2.9-3.1	n/a*	

Ranges in fossil dates of first occurrence reflect accuracy of section dating. References for dates can be found in **Appendix 2**. *Calibration for *Manicina is* at genus node since species not well supported in phylogeny.

Calibrations of nodes were done following the guidelines of Ho and Phillips [66]. For all date priors, we used a lognormal distribution with a hard minimum bound set at youngest possible date of first appearance in the fossil record. The mode of the distribution was set to be slightly older than the oldest possible date of first appearance. Finally, the 95% probability distribution was set to encompass a soft maximum bound at the time of the closure of the Tethys (~17 Ma). These distributions incorporate the best-known estimates for origination dates of these taxa, but are wide enough to allow for shifts in dates that may reflect errors due to interpretation or incompleteness of the fossil record.

Results

Phylogenetic analysis of Caribbean "Faviidae"

We sequenced three single copy nuclear loci for six ingroup and one outgroup Caribbean faviid species. A total of 43 unique alleles were identified for *CaM* (alignment length=590 bp), 30 alleles were identified for *MaSC-1* (alignment length=490 bp), and 55 alleles were identified for *Pax-C* (alignment length=419 bp) (**Table 3**). Maximum likelihood and Bayesian analysis of gene trees showed little support for structure above the species level with no conflict between trees at highly supported nodes (**Appendix 3**). A total of 80 individuals with unique genotypes were successfully sequenced at all three loci and used for a concatenated analysis. See **Appendix 1** for genotype data of all individuals in study.

Table 3. *CaM*, *Masc-1*, and *Pax-C Alleles* identified in Caribbean Faviidae.

Species	Individuals sequenced	Number of alleles		
		CaM	*MaSC-1*	*Pax-C*
C. natans	23	7	1	8
D. clivosa	18	4	2	5
D. labyrinthiformis	19	6	5	9
D. strigosa	19	10	8	13
F. fragum	135	6	4	6
M. areolata	29	7	5	13
M. mayori	5	1	2	0
Manicina spp.	34	10	10	14

Number of individuals sequenced per species and alleles isolated per locus per species. Allele counts for *M. mayori* and *M. areolata* only include alleles that were isolated solely from that species. Combined number of alleles that were isolated from both species, including shared alleles, are listed under *Manicina spp.*

Bayesian and maximum likelihood trees had identical topologies at all major nodes with support values (Bayesian/ML bootstrap) indicated in **Figure 1**. The ingroup node was well supported (100/100) as well as species nodes for *C. natans* (100/100), *D. clivosa* (100/100), *D. labyrinthiformi*s (100/96), *D. strigosa* (100/99) and *F. fragum* (100/100). *Manicina mayori* and *Manicina areolata* failed to form monophyletic groups, though support was high at the genus node for *Manicina* (100/84). The genus *Diploria* failed to form a monophyletic group. *Diploria clivosa* formed a clade with *Manicina spp.* and *D. strigosa* formed a clade with *Favia fragum*. Support for these nodes, however, was low (64/37 and 76/55 respectively). Filtering of Bayesian trees to test for generic monophyly resulted in retention of only 333 out of the 60004 trees retained after burn-in (0.6%), indicating very low support for that topology.

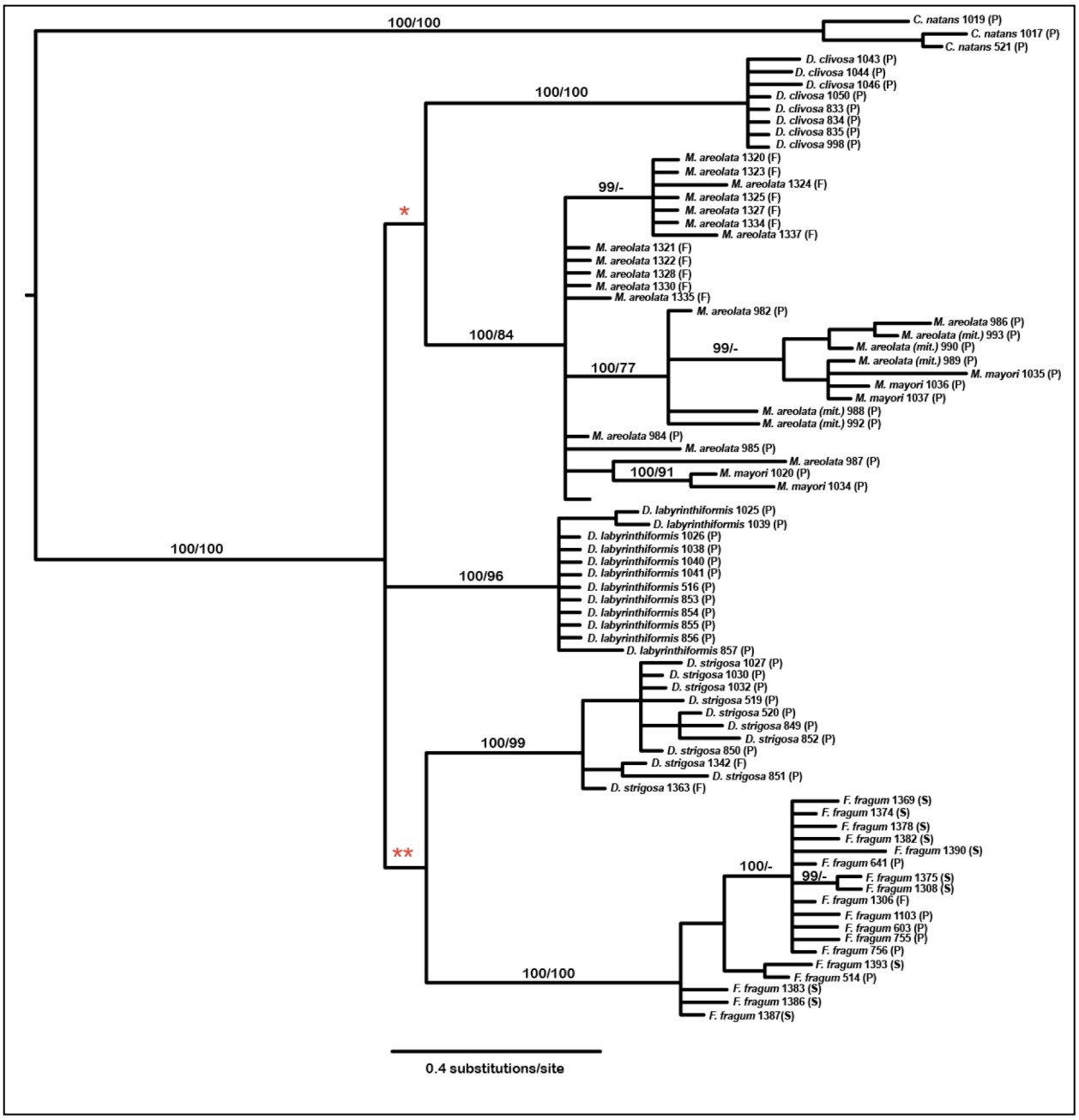

Figure 1. Phylogenetic tree of the Caribbean Faviidae

Tree based on a partitioned analysis of individual genotypes at the *CaM, MaSC-1*,and *Pax-C* loci. Terminal taxa are individuals of each species. Letters after sample names indicate coarse geographic sampling information **(F=**Florida, **P=**Panama, **S =** St. Croix). Further sampling and genotype information can be found in **Appendix 1**. Trees shown were created using MrBayes v3.1. Maximum likelihood trees from RaxML yielded a similar topology. Posterior probabilities (>95%) and bootstrap support (>75%) (Bayesian/ML) are indicated for each node. Dashes indicate nodes unsupported in ML analysis. Several deeper nodes in the tree indicated by asterisks were poorly supported in this analysis (*=64/37, **=76/55). All *Diploria* and *Colpophyllia* species are reef-building, while *Favia* and *Manicina* species are also free-living.

Timing of Divergence

BEAST analysis of the data produced a tree topologically consistent with those of the MrBayes and RaxML analyses. Visual analysis of plots in Trace v1.5 showed rapid convergence of the analysis and narrowing of priors. The mean rate of substitution was 7.10×10^{-4} per site (95% Highest Posterior Density (HPD) interval): 4.73×10^{-4} - 9.63×10^{-4}) with a coefficient of variation of 1.25 (95% HPD interval: 0.71- 1.82) indicating significant heterogeneity in substitution rates across the tree. A list of selected posterior estimates and 95% HPD intervals can be found in **Table 4.**

Table 4. BEAST posterior estimates from analysis of Caribbean faviid corals.

Statistic	Mean	Median	95% HPD
Posterior	-3570.9	-3570.3	-3603.5- -3539.1
Likelihood	-3880.3	-3380.0	-3397.1 - -3364.1
Mean rate	7.098×10^{-4}	7.024×10^{-4}	4.727×10^{-4} - 9.634×10^{-4}
Coefficient of Variation	1.246	1.217	0.712 - 1.817

Effective sample size (ESS) of all parameters > 200

Mean ages of species, ingroup, and root nodes with 95% HPD intervals are shown in **Figure 2** and listed in **Table 5**. The posterior mean of the time of the most recent common ancestor (TMRCA) of the *Manicina* group, which was calibrated from species fossil data, shifted several MY from the prior distribution, indicating that the sequence data is influencing divergence dates. For *D. clivosa*, D. *strigosa,* and *F. fragum*, mean estimated ages fell close to the earliest possible dates of their appearance in the fossil record. For the *D. labyrinthiformis* and *Manicina* nodes, fossil dates were closer to the youngest part of the 95% HPD interval. Mean origination time of *D. labyrinthiformis* is pushed back approximately 1.6 MY earlier than previously seen in the fossil record, putting it closer to the origination times of the other species. All mean species origination dates occur shortly prior to the final closure of the Central American Isthmus at 4.25 - 3.45 Ma [11], but we note that the youngest part of 95% HPD for *F. fragum* and *D. labyrinthiformis* overlap with this estimated age of final closure. The timing of the *Manicina* node is considerably earlier than the appearance of the *Manicina areolata* in the fossil record, indicating that this genus diverged earlier than the first appearance of *M. areolata*. Deeper nodes in the tree had significantly larger HPD confidence intervals, due to the lack of fossil calibrations for earlier taxa. The estimate of origination time for the ingroup was 13.79 Ma (95% HPD interval: 8.41-19.88), while origination of the entire Caribbean Faviidae group is indicated by the root node at 16.98 Ma (95% HPD: 9.83-25.68). These dates coincide with the timing of the closure of the Tethys Sea in the eastern Mediterranean [10].

Table 5. Extant Faviidae divergence dates estimated from BEAST

	Date of Origination - Ma		
Taxa	**Mean**	**Median**	**95% Highest Posterior Density (HPD) interval**
C. natans	6.26	6.01	5.15-8.05
F. fragum	5.68	5.47	3.51-8.21
D. clivosa	5.61	5.42	4.55-7.05
D. labyrinthiformis	4.70	4.41	2.99 - 7.10
D. strigosa	5.99	5.73	4.68-8.01
Manicina	8.20	7.96	4.71-12.13
Ingroup	13.79	13.38	8.41-19.88
Root	16.98	16.27	9.83-25.68

Overlay of the molecular phylogeny onto the fossil stratigraphy reveals three striking patterns (**Figure 3**). First, older and extinct *Diploria* and *Favia* cannot be reconciled with this molecular tree, suggesting these genera are not monophyletic. Second, the origination and diversification of a clade of sediment dwelling corals (particularly *Thysanus* and *Manicina*) is confirmed by both the fossil record and molecular phylogeny. Lastly, the appearance of new reef dwelling species of *Favia* and *Diploria* is simultaneous in the fossil record around 5 Ma.

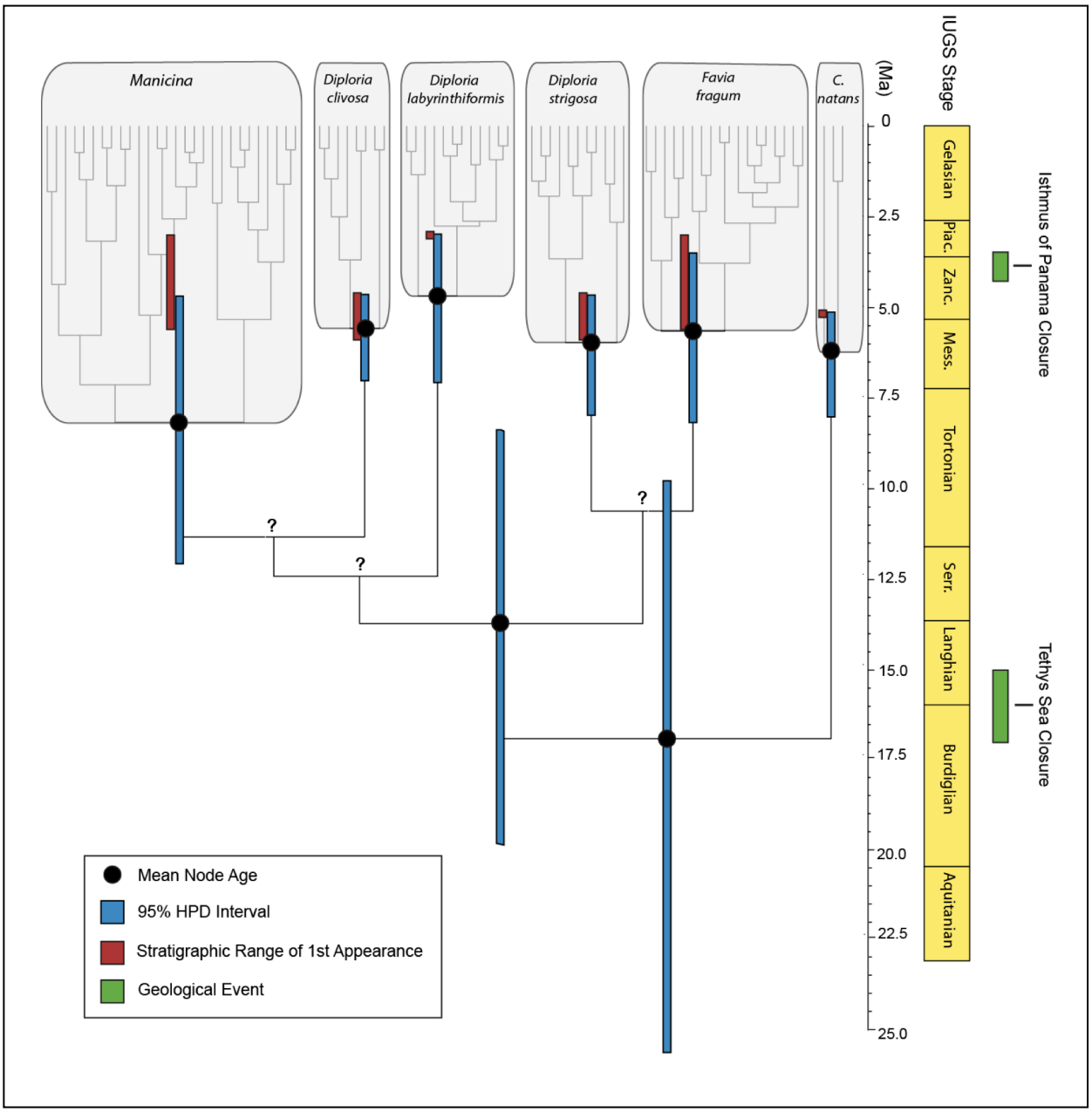

Figure 2. Caribbean Faviidae chronogram

Divergence dates of terminal (species) and internal nodes of a phylogeny of the Caribbean Faviidae are shown. Original chronogram and tree generated in BEAST. Grey boxes indicate species or genera as labeled. Black circles and blue bars correspond to mean node age (Ma) and 95% HPD intervals produced by BEAST analysis. Red bars indicate the stratigraphic age range of the first appearance of that taxon in the fossil record. Green bars next to the time axis are used to indicate major geological events in the isolation of the Caribbean Sea including the closure of the Central American Isthmus at 4.25-3.5 Ma and the closure of the Tethys Sea at 17-15 Ma. Nodes marked with a '?' are poorly supported in this analysis. Detailed information about dates and node calibration can be found in **Tables 2** and **5.**

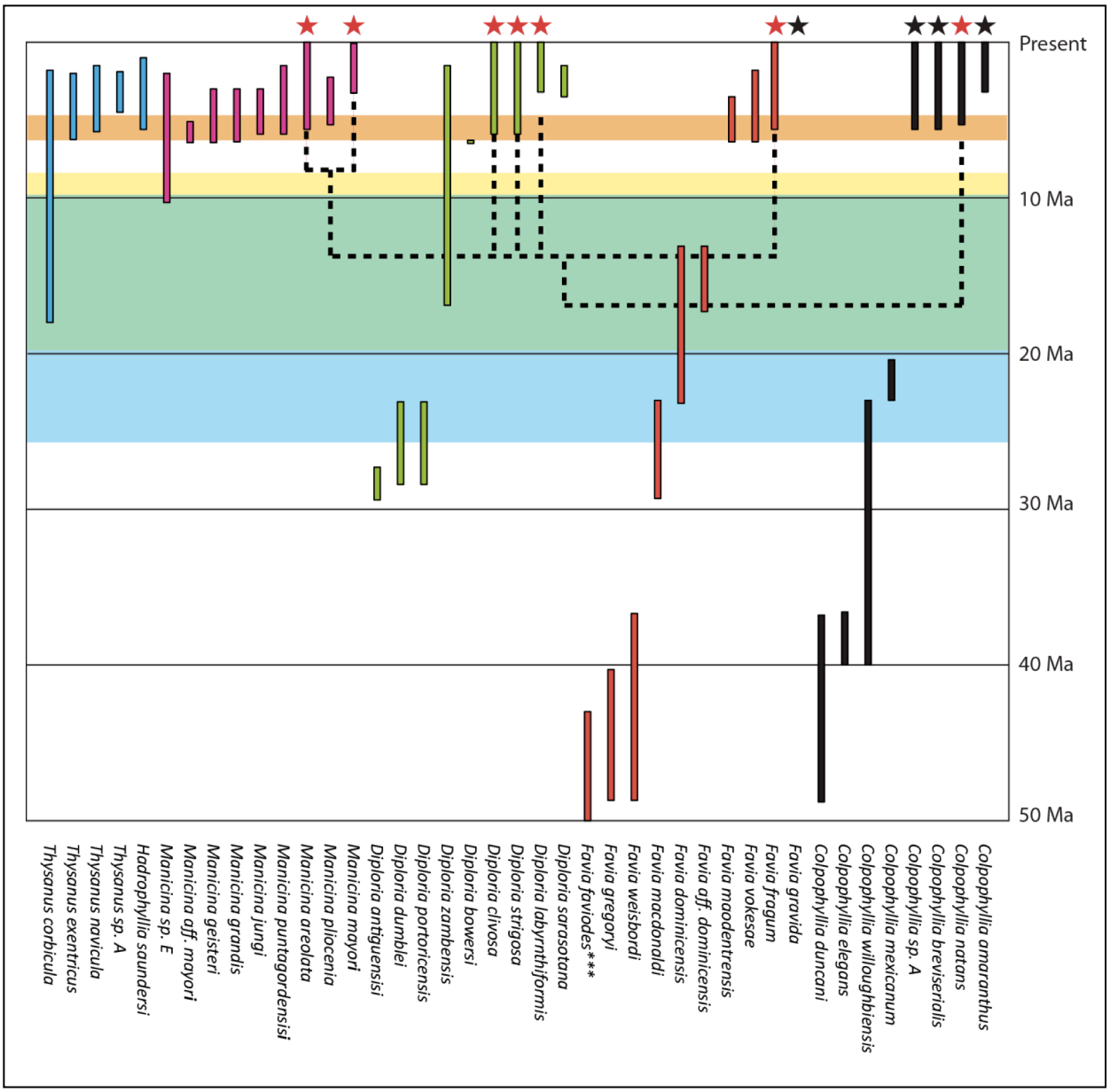

Figure 3. Phylogeny on updated stratigraphy of living and extinct Caribbean Faviidae species. Stratigraphic range bars are color-coded by genera, listed on the x axis. Green + blue shading are 95% highest posterior density (HPD) intervals for the ingroup node, and green + yellow shading are 95% HPD intervals for the root node as seen on the chronogram. Orange shading indicates the range of origination dates in the fossil record for all living taxa. Species within genera are ranked by earliest origination date, left to right. The genera *Thysanus* and *Hadrophyllia* are free living, as are all the extinct species of *Manicina*. See **Appendix 2** for stratigraphic references.

Discussion

Phylogenetic relationships within modern Caribbean corals

Thorough sampling of individuals within species in our combined phylogenetic analysis confirms that most modern Caribbean species form well-supported monophyletic lineages (**Figure 1**). This allows us to reject the idea that widespread hybridization on ecological time scales [67] is important to the evolution of Caribbean faviids, though limited introgression not detected by this data set might have played a creative role in adaptive processes [68].The exception lies within the two modern species of *Manicina*, *M. areolata* and *M. mayori*, where extensive allele sharing between species might indicate that either reproductive isolation is of recent origin or that these species may actually represent a hybridizing species complex. With high levels of phenotypic plasticity within *M. areolata* [69] and some coral biologists proposing *M. mayori* as a form of *M. areolata* [70], the status of these two species remains an open question. Further sampling and analysis with a coalescent-based model of isolation and migration[71] could resolve this issue.

Above the species level, we could not further resolve the branching order of species within the larger clade. Previous single locus phylogenies using mitochondrial and nuclear genes that have included this group have shown a similar lack of resolution within the Caribbean faviids [42, 43]. Another study by Nunes *et al.* [46] shows some supported structure within this group. However, as this paper was looking mainly at broader scale phylogeographic relationships, sampling was done on only few individuals per species within the Caribbean faviids and using only mitochondrial markers. For examining relationships below the familial level, the low rates of mtDNA evolution in corals [51], combined with the use of a single genealogical history might limit our ability to detect more complex topologies amongst these species. With the increased sampling sizes of multiple loci with higher levels of variation (**Appendix 3**), we found little evidence for monophyly within genera, and branch lengths tended to be long (**Figure 1**). Therefore, our inability to resolve relationships among species might be driven by rapid diversification and short internal branch lengths deeper in the tree. With the rapidly declining cost of high throughput sequencing, a phylogenomic approach [72, 73] for this set of taxa is likely to improve topological resolution

Fossils and molecules reveal the tempo and mode of Caribbean coral diversification

Molecular divergence dating indicates extant Caribbean "faviid" corals radiated rapidly during the late Miocene to early Pliocene (**Figures 2** & **3**). This ecological radiation coincides with a series of biological and physical changes in the structure of shallow marine habitats during the early geological development of the Isthmus of Panama. During the Late Miocene, shallow marine habitats were dominated by broader and more gently sloping sedimentary shelves [74], while productivity in the water column above was much higher compared to the modern productivity of the Caribbean Sea [75]. Klaus *et al.* [15] hypothesize that these extensive mesophotic sedimentary bottoms may have selected for free-living coral species with large tentacle morphologies that were efficient at heterotrophic feeding. Interestingly, our node age for the clade containing the two living *Manicina* species is 8.20 Ma, which coincides with the appearance of other sibling *Manicina* species in the fossil record that have since gone extinct

[29]. Thus, it appears we are sampling the evolutionary remnants of a once more diverse and ecologically dominant clade. As the Miocene transitions into the Pliocene, the increasingly isolated Caribbean Sea becomes more oligotrophic and the once broad shelf habitats are now dominated by steeper reef platforms, ideal conditions for primarily photoautotrophic reef species. Our time-calibrated phylogeny shows repeated speciation events of *Diploria* and *Favia* species between ~ 6 – 4 Ma that are either reef specialists or are limited to very shallow (< 5 m) seagrass habitats. Thus the fossil record and molecular data broadly agree on the timing of these ecological radiations, which are temporally correlated with changes in habitat structure and productivity.

Deeper in the tree, node ages for the stem groups of the Caribbean faviids correspond to the isolation from the Mediterranean during the closure of the Tethys Sea (**Figure 2**). While these dates support the widely accepted notion of divergence driven by increased isolation of the region, the radiation of the stem group is much later than indicated by the fossil record (**Figure 3**). The origination of the *Favia-Diploria-Manicina* (FDM) clade is in the early Miocene, but older Oligocene *Diploria* fossils and Eocene *Favia* fossils are more distantly related and suggesting that both genera are para- or polyphyletic. Ken Johnson reached a similar conclusion based on morphological differences [29], hypothesizing that early *Diploria* and *Favia* are unrelated to their modern morphological counterparts. Morphological convergence appears to be a common theme in coral evolution [42] and our analysis points out some of the difficulties in determining the systematic positions of extinct taxa. The use of more informative micro-structural characters that can be quantified in both living and fossil species may be a promising approach to this problem [37].

Congruence of morphology, stratigraphy, and estimates of node ages can be used to include fossil taxa into potentially monophyletic lineages. For example, the diverse members of living and fossil taxa of the genus *Manicina* form a well-supported monophyletic group in Johnson's morphological phylogeny with all fossil origination dates falling within the lower confidence interval for the molecular *Manicina* node age (**Figure 2**). Superimposing the age-calibrated molecular phylogeny onto stratigraphy significantly alters the interpretation of the speed of evolution in this group (**Figure 3**), indicating rapid diversification of sediment dwelling corals in the late Miocene.

Are punctuated patterns driven by adaptation?

Our time calibrated phylogeny confirms fossil evidence that extant Caribbean coral species originated during a period of lineage diversification between 4 and 6 Ma (**Figures 2 & 3**). This diversification event corresponds with ecological radiation into the three main ecological niches seen in modern Caribbean faviids [21]: (i) small, free living morphologies adapted to sedimentary environments, (ii) attached species that live in shallow rubble beds and patch reefs, and (iii) massive colonies the build forereef slopes. During the same period, we also see diversification of reproductive strategy [30], from tightly synchronized annual mass-spawning events and broadcasting larvae typical of *Diploria* [76] to multiple lunar cycles of reproduction and brooding development found in *Favia* and *Manicina* [77, 78].

The changes in morphology and life history coupled with widespread environmental changes are suggestive that diversification of Atlantic "faviid" coral might be driven by the evolution of adaptive traits. Using our current phylogeny as a stepping stone, increased genomic and taxonomic sampling of Atlantic corals should allow us to take advantage of several promising new approaches to estimate rates of diversification and evaluate models of adaptive radiation [79, 80].

Conclusions

By combining data from the fossil record with molecular phylogenetic techniques for the first time, this study has given us extensive insight into the tempo of diversification in an ecologically diverse group of Caribbean corals. Two separate lines of evidence now verify the existence of a Mio-Pliocene radiation, while we have been able to additionally confirm species identity, verify origination dates, and understand taxonomic relationships in this diverse and ecologically important group. These findings give us the tools to re-interpret trends seen in the fossil record, allowing us to begin to link patterns of macroevolution to paleoenvironmental changes and gain a new comprehension into the origins and drivers of diversity in the Caribbean.

Besides clarifying evolutionary history, this study has broader contemporary implications. With global change currently causing a rapid decline in coral reef populations around the world [81, 82], understanding the processes that generated diversity in coral species will be key to predicting future changes and directing conservation efforts [83]. It has been suggested that species that evolved in a more heterogeneous environment and survived past climatic fluctuations will be more resistant to current global change [28]. Understanding patterns of Caribbean coral evolution during the Pleistocene may be key to understanding the potential outcomes of current environmental impacts.

Chapter 2: mtDNA reveals deep divergence between Indian and Pacific populations of the parrotfish *Scarus ghobban* and a newly discovered Western Australian endemic

Introduction

With global change threatening ocean ecosystems, the origin of the marine biodiversity hotspot in the tropical Indian and Pacific Oceans is still a looming question in evolutionary biology. In this region, maximum diversity is centered in the "Coral Triangle" across the Indo-Australian Archipelago (IAA) and declines in both the eastward and westward directions [84]. Much debate on how this pattern arose has focused on the relative importance of allopatry versus sympatry in marine speciation [85], the role of ecological speciation [86, 87], and biogeographic models for the generation of diversity in that place differing emphases on the role of the IAA as a refuge or source for new species [88]. While it has long been suggested that is unlikely that any of these categorical models are strictly correct [89-91], critical questions still remain in understanding both the patterns of diversity and the historical context in which they developed.

The tropical Indian and Pacific oceans can be divided into three major biogeographic provinces based on patterns of endemism in many invertebrates and reef-associated fish [92, 93]: the Indian Ocean, the Central & West Pacific, and the Tropical Eastern Pacific. Between the Indian and Western Pacific Oceans, the Indo-West Pacific Break (IWPB) is believed to be driven by fluctuating sea-levels in the Pleistocene periodically exposing land bridges across the broad Sunda Shelf which connects southeast Asia to Australia [94], creating a strong vicariant barrier to dispersal. The Tropical Eastern Pacific and Central & West Pacific are separated by the Tropical Eastern Pacific Barrier (EPB), a >5000 kilometer stretch of deep-water with a mean larval transport time beyond the pelagic larval duration of most species and lack of intermediate habitat for shallow coastal marine species [95, 96]. The prevalence of studies showing genetic discontinuities within populations [88, 97-101] and phylogeographic breaks [102, 103] between sibling species across these barriers highlights their significance in driving diversity patterns.

Despite their clear importance in structuring many taxa, these barriers vary temporally with climate and current fluctuations, and many tropical reef fish with long distance dispersal capabilities have wide geographical ranges that span them [104, 105]. With the advent of more advanced molecular techniques, however, recent studies in many of these broadly distributed species have revealed complex patterns of cryptic diversity in what were once thought to be homogenous populations [106, 107]. These divisions can occur both between traditional biogeographic provinces [108], and within peripheral populations [109]. While some of these new species pairs are found to exhibit strong ecological or morphological differences [87, 110], in other cases divergence does not seem to correspond to morphological or ecological lines [111]. Furthermore, while it was once believed that much Indo-Pacific marine diversity was of Pleistocene to recent origin [90], mounting fossil and molecular data show evidence of deeper taxon-specific divergences in many groups [112-114] and a complex interplay between patterns of extinction and speciation over time [115, 116]. Clearly, diversification is being driven by far more complicated geographic and ecological processes than simple models can account for, and the relative importance of these processes may be dependent on the group being studied. By

examining widespread taxa which can test both ecological and biogeographic bounds, we can begin to tease apart these factors [117].

The parrotfishes (family Scaridae) are a diverse group of keystone reef herbivores consisting of over 90 described species in 10 genera worldwide [118]. In addition to their ecological significance, these fish are a culturally and economically important resource in many parts of the Pacific and Indian Oceans [119]. The largest parrotfish genus, *Scarus,* has an origin in the late Miocene (~10 Ma) [120, 121], followed by an apparent burst in evolutionary rate potentially driven by sexual selection linked to the development of sexual dichromatism and associated life history characteristics [122]. Despite relatively high pelagic larval durations [123], within the Indo-Pacific group of parrotfish, there is a wide range of distribution patterns with most species endemic to only one or two biogeographic provinces. Only three species, *Scarus ghobban, Scarus rubroviolaceus*, and *Calotomus carolinus* are currently thought to span all three provinces [118]. However, with recent genetic studies showing considerably more structure in parrotfish populations than previously believed [97, 99, 124], these distribution patterns merit a closer examination.

Scarus ghobban (the blue-barred parrotfish) is a common species on reefs across the Indo-Pacific. Its putative range extends from the western Americas to the Red Sea and eastern coast of Africa [118], with a modern invasion into the Mediterranean through the Suez canal [125]. Several recent developments have cast doubt on this distribution and hint at a more complex pattern of diversity. A study of the Lessepsian invasion by Bariche and Bernardi [126], suggests a deep split between Indian and Pacific clades of *S. ghobban*. In addition, a potentially new species endemic to Western Australia has recently been discovered which shares all of the meristic features of *S. ghobban,* with only a dramatic shift in coloration (J.H. Choat, personal communication). Finally, a recent phylogeny of Indo-Pacific parrotfish by J.H. Choat *et al*. (in preparation), has shown that the Eastern Pacific endemic, *Scarus compressus*, falls within the *Scarus ghobban* clade. Combined, these studies cast doubt on taxonomic relationships both within and between these species and hint at diversification both across biogeographic boundaries and in peripheral populations.

In this study, we aim to 1) assess the taxonomic relationships between *S. ghobban, S. compressus*, and the new Western Australia species by creating an mtDNA phylogeny based on widespread geographic sampling and 2) estimate divergence times between groups by creating a time-calibrated species tree. By examining the geographic and temporal patterns of diversity in this complex, we can then generate hypotheses on possible drivers of diversification.

Methods

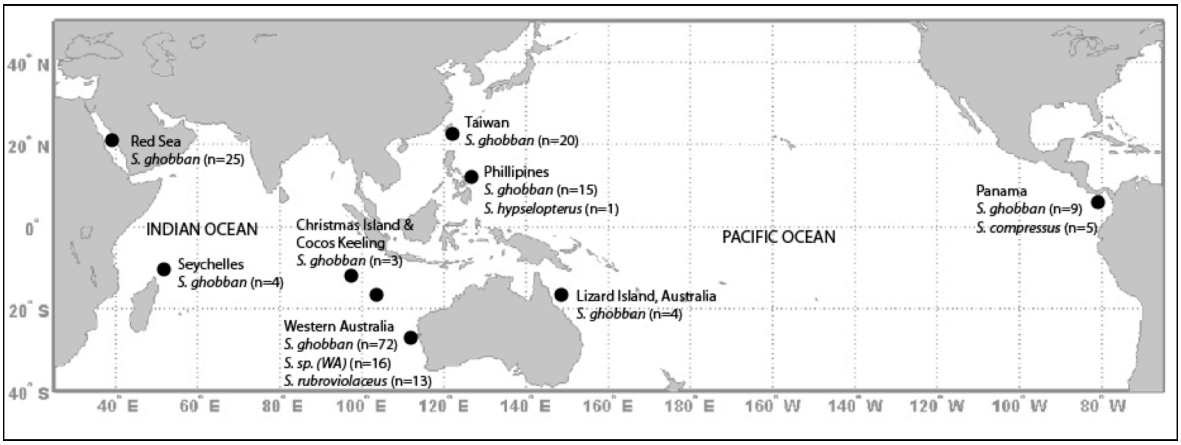

Figure 4. Map of parrotfish sampling localities.
Site names are given, followed by the number of samples of each species obtained from that locality. Indian Ocean localities include the Seychelles and the Red Sea. Pacific Ocean localities include Panama, Taiwan, the Philippines, and Lizard Island. The western coast of Australia and Christmas and Cocos Keeling Islands are in the Indian Ocean, but are often considered part of the Pacific biogeographic province.

Sampling

Tissue samples were obtained from individual fish collected by selective spearing or artisanal fish market sampling. Pectoral fin clips or liver tissue were removed and immediately placed in 95% ethanol for storage and preservation. Voucher photos were taken of each specimen to confirm species identification. A total of 152 samples of *Scarus ghobban* were collected from 9 sites in the Indian and Pacific Ocean. 16 samples of a newly discovered endemic species, *Scarus sp.* (WA), were collected from Western Australia, and 5 samples of the Eastern Pacific endemic, *Scarus compressus* were collected from Panama. For outgroup sampling, we collected 13 samples of *Scarus rubroviolaceus* from Western Australia, and 1 sample of *Scarus hypselopterus* from the Philippines **(Figure 4).**

Extractions & PCR

Genomic DNA was extracted from all samples using DNeasy Blood & Tissue kits (Qiagen) and DNA quality was checked by either gel electrophoresis or using a Nanodrop spectrophotometer (Thermo Scientific). Two mitochondrial genes, *16S* and the control region, were amplified using the polymerase chain reaction (PCR). Sequences of the *16S* rRNA subunit were obtained with primers *16Sar (*5'-CGCCTGTTTATCAAAAACAT-3'), and *16Sbr* (5'-CCGGTCTGAACTCAGATCACGT-3') [127]. For the control region, primers were redesigned from flanking tRNA regions to amplify a longer portion of the d-loop. Primers used were *tRNA-Phe* (5'-TGTTGTCGGGACTTTTAAGG-3') and *tRNA-Pro* (5'-TCCACCTCTAGCTCCCAAAG - 3').

For PCR amplification of both loci, we combined 10-20 ng of genomic DNA in a 20 µl reaction mix consisting of 2.0 mM $MgCl_2$, 0.5 mM dNTPs, 0.5 mg/ml bovine serum albumin (BSA),

0.035 μM each forward and reverse primers, 1.5 units of DreamTaq DNA polymerase (Thermo Scientific), and 1X DreamTaq buffer. Thermocycler conditions for *16S* were as follows: initial denaturation at 95°C (2 min), followed by 30 cycles at 95°C (30s), 52°C (40s), 72°C (1 min), with a final extension of 72°C for 10 minutes. Thermocycler conditions for the control region reactions were: initial denaturation at 95°C (2 min), followed by 30 cycles at 95°C (30s), 53°C (1 min), 72°C (2 min), with a final extension of 72°C for 10 minutes. 5 μl of each reaction was run on a 1.2% agarose gel to check for amplification. For successful reactions, the remaining 15 μl of PCR product was purified for sequencing by adding 10 U Exonuclease 1 and 1.5 U of shrimp alkaline phosphatase (SAP) and incubated in the thermocycler at 37°C for 30 minutes, followed by an enzyme inactivation step of 80°C for 15 minutes. Purified PCR products were directly sequenced using the above primers on ABI 3731 XL 96 capillary DNA analyzers at the University of California, Berkeley. Sequences chromatograms were t analyzed and edited using Geneious v5.5.6 [128].

Alignments and outgroup verification

For both loci, sequences were aligned automatically using MAFFT v6 [55] with corrections made by eye in Geneious. Final alignments for each locus were trimmed to minimize missing information at the ends of sequences. In previous phylogenies (Choat *et al.*, unpublished), *S. rubroviolaceus* has been shown to be the sister taxa to *S. ghobban* and *S. compressus*. However, since this earlier phylogeny did not include *Scarus sp.* (WA), further verification was needed to ensure that our ingroup species were monophyletic with respect to *S. rubroviolaceus.* We performed a maximum likelihood analysis for both loci using the more distantly related *S. hypselopterus* as an outgroup. Analyses were run for 1000 bootstrap replications in RaxML-HPC2 v7.3.0 [61] on the Cipres Web server [63]. Support values for tree topologies were examined in Figtree v1.3.1 [129].

Phylogenetic analyses

To assess the resolving power of each locus, separate gene trees were created for *16S* and the control region using *Scarus rubroviolaceus* as an outgroup. For both trees, duplicate haplotypes were removed from the analysis. Models for molecular evolution for Bayesian analysis were selected from 24 models using the Akaike Information Criteria (AIC) in jmodeltest v0.1.1 [130, 131]. Both Bayesian and maximum likelihood analysis were performed on the CIPRES server [63]. Bayesian gene trees were generated in MrBayes v3.1.2 (10,000,000 generations, samplefreq=1000,nruns=4, nchains=4, burnin=25%) [59, 60]. Maximum likelihood trees were generated using RaxML-HPC2 v7.3.0 with 1000 bootstrap replicates.

Since the mitochondrial genome is non-recombining, we concatenated the sequences for each individual successfully sequenced at both loci make a combined mtDNA tree. Model selection and maximum likelihood tree generation were performed as described for individual gene trees above. Bayesian gene trees were generated in MrBayes v3.1.2 (20,000,000 generations, samplefreq=1000, nruns=4, nchains=4) [59, 60]. Output files from Bayesian analysis were examined in Tracer v1.5 [132] to assess for convergence of all runs, and a burnin of 10,000,000 generations was removed. Following phylogenetic runs, to examine the degree of divergence

within and between identified clades, pairwise distances were calculated separately for each locus in Arlequin v3.5 [133].

Divergence Dating

The program BEAST v1.7 [65] was used to estimate divergence dates between species and clades identified in our phylogenetic analysis. Since our data includes many samples of individuals from highly divergent clades, we used the *BEAST method described in Heled and Drummond [134]. This implementation using the multispecies coalescent, which models the rates of divergence within species using a coalescent population model and divergence between species using a speciation model. For our analysis, coalescent constant and Yule speciation tree priors were used.

Because no fossil data is available for closely related taxa, we calibrated the root of our tree using the posterior probabilities of divergence dates between *S. rubroviolaceus* and *S. ghobban* from a larger scale species level phylogeny done by Choat *et al*. (unpublished). Substitution models and clocks were unlinked between loci for the analysis, while tree models were kept linked since the mitochondrial genome is non-recombining. Substitution model priors were identical to those used in the Bayesian run. Based on an assessment of convergence in preliminary runs, we used the relaxed uncorrelated lognormal clock model for both genes that allows rate heterogeneity between lineages. The medians of priors for mean clock rates were set for each gene based on literature estimates of substitution rates for *16S* (0.32%/MY) [135] and the control region (3.6%/MY±0.46% SE) [136] in fish.

Table 6. BEAST date & rate calibrations - *Scarus* spp.

Prior Name	Calibration	95% range
tMRCA (All)	Normal (3.43,0.9)	1.95-4.91
16S.ucld.mean	Lognormal (0.0016, 0.25)	$1.03E^{-3}$ - $2.34E^{-3}$
CR.ucld.mean	Lognormal (0.03,1.0)	$3.5E^{-3}$ - $9.4E^{-2}$

Calibrations for the root node and mean clock rates for both mitochondrial loci are shown. The calibration gives the type of distribution used followed by the mean and standard deviation. Means for lognormal distributions are given in real space. The 95% range for the prior distribution is also given as calculated in Beauti 1.7.1.

Since recent work done on ancient DNA samples in *Scarus rubroviolaceus* has calculated much higher rates of substitution in the control region, set our prior distributions fairly wide to account for this heterogeneity. Priors for TMRCA and clock rates for *BEAST analysis can be found in **Table 6**. All other priors were kept at their default values. *BEAST was run 4 times (generations= 50,000,000, samplefreq=1000) on the Bioportal webserver at the University of Oslo [137]. Log files were examined in Tracer v.1.5 to assess convergence of each run. After a burn-in was removed, logs and trees for all runs were combined in LogCombinerv1.7.1 and species trees were generated with TreeAnnotater v1.7.1.

Results

Sequencing

A total of 187 individuals were successfully genotyped at the 16S with a total alignment length of 542 base pairs. 157 Individuals were genotyped for the control region with a total trimmed alignment length of 938 bp (**Table 7)**. Differences in amplification between loci were most likely due to template quality.

A total of 30 unique haplotypes for 16S were recovered from the data. No haplotypes were shared between the outgroup species, *Scarus sp*. (WA) and *Scarus ghobban*. Within *Scarus ghobban,* no haplotypes were shared between Pacific populations (including Western Australia, Cocos Keeling & Christmas Island), and Indian Ocean populations. *Scarus compressus* shared all of its haplotypes with the Pacific *Scarus ghobban* populations. A total of 155 unique haplotypes for the control region were recovered. Two *S. rubroviolaceus* samples shard a haplotype and two *S. ghobban* (Pacific) samples from the Philippines and W. Australia shared a haplotype.

Table 7. Parrotfish sample size and haplotype information.

Species	Region	Locality	16S # of individuals (haplotypes)	Control region # of individuals (haplotypes)
Scarus hypselopterus (Outgroup)	Pacific	Philippines	1 (1)	1(1)
Scarus rubroviolaceus (Outgroup)	Pacific	W. Australia	13(3)	13 (12)
Scarus compressus	Pacific	Panama	5(2**)	5 (5)
Scarus sp. (WA)	Pacific	W. Australia	16(4)	15 (15)
Scarus ghobban	Pacific	All sites	123(15)	102 (101)
		Taiwan	20	14
		W. Australia	72	69
		Philippines	15	8
		Lizard I.	4	2
		Panama	9	9
		C. Island	2	0
		Cocos Keeling	1	0
Scarus ghobban	Indian	All sites	29(6)	21 (21)
		Red Sea	25	20
		Seychelles	4	1
Totals			187(29)	157(155)

***Scarus compressus* shares both 16S alleles with Pacific *Scarus ghobban*. This number is not included in the total allele count.

Outgroup, model selection and haplotype trees

Maximum likelihood trees rooted with *S. hypselopterus* for *16S*, the control region, and the combined mtDNA data set all indicated monophyly of the ingroup containing *S. ghobban*, *S. compressus* and *Scarus sp.* (WA), with *Scarus rubroviolaceus* as a sister taxa. Bootstrap support values for the ingroup node were high (*16S*: 87, control region: 100, combined: 100). Further analyses were done using *Scarus rubroviolaceus* as the outgroup. Bayesian model selection in jmodeltest v0.1.1 using the Akaike Information Criterion (AIC) selected the HKY+I model for 16S and the GTR+I+G with 6 gamma parameters for the control region. These models were used for all downstream Bayesian analyses. Haplotype trees for both loci (**Appendix 4**) separate the ingroup into 3 clades, though support and resolution were much greater for the more variable control region.

Phylogenetics

A total of 156 individuals with unique mitochondrial haplotypes were successfully genotyped at both loci for a total alignment length of 1480bpwhich was used for both MrBayes and RaxML analysis. Bayesian and maximum likelihood trees had identical topologies at all major nodes (**Figure 5**). The ingroup node was well supported (100/100) and within the ingroup, three highly supported clades (100/100) were seen, one corresponding to *Scarus sp.* (WA), one corresponding to *S. ghobban* samples from the Indian Ocean, and one corresponding to both *S. ghobban* and *S. compressus* from Pacific biogeographic province. Some structure was seen within clades, but branch lengths were short and no geographic patterns are readily apparent. Between clades, both the Bayesian tree and ML tree showed *Scarus sp.* (WA) and *S. ghobban* (Pacific) as sister taxa, but support values for this node were low (62/76), indicating an overall lack of resolution between clades. Pairwise differences between clades (**Table 8**) indicate much higher divergence at the faster evolving control region locus. For both loci, divergence between clades was notably higher than that within clades.

Table 8: Pairwise differences within and between *Scarus ghobban* complex clades.

	Scarus rubroviolaceus	***Scarus sp.* (WA)**	***S. ghobban* (Indian)**	***S. ghobban* (Pacific)**
Scarus rubroviolaceus	*(0.002,0.013)*	0.154	0.135	0.123
***Scarus sp.* (WA)**	0.012	*(0.001,0.037)*	0.145	0.127
***S. ghobban* (Indian)**	0.010	0.006	*(0.001,0.028)*	0.106
***S. ghobban* (Pacific)**	0.014	0.009	0.007	*(0.002, 0.030)*

Numbers below the diagonal represent the corrected pairwise difference between clades for 542 bp of *16S*. Numbers above the diagonal represent the corrected pairwise difference between clades for 919 bp of the control region. Numbers on the diagonal are the within clade pairwise differences for *16S* and the control region.

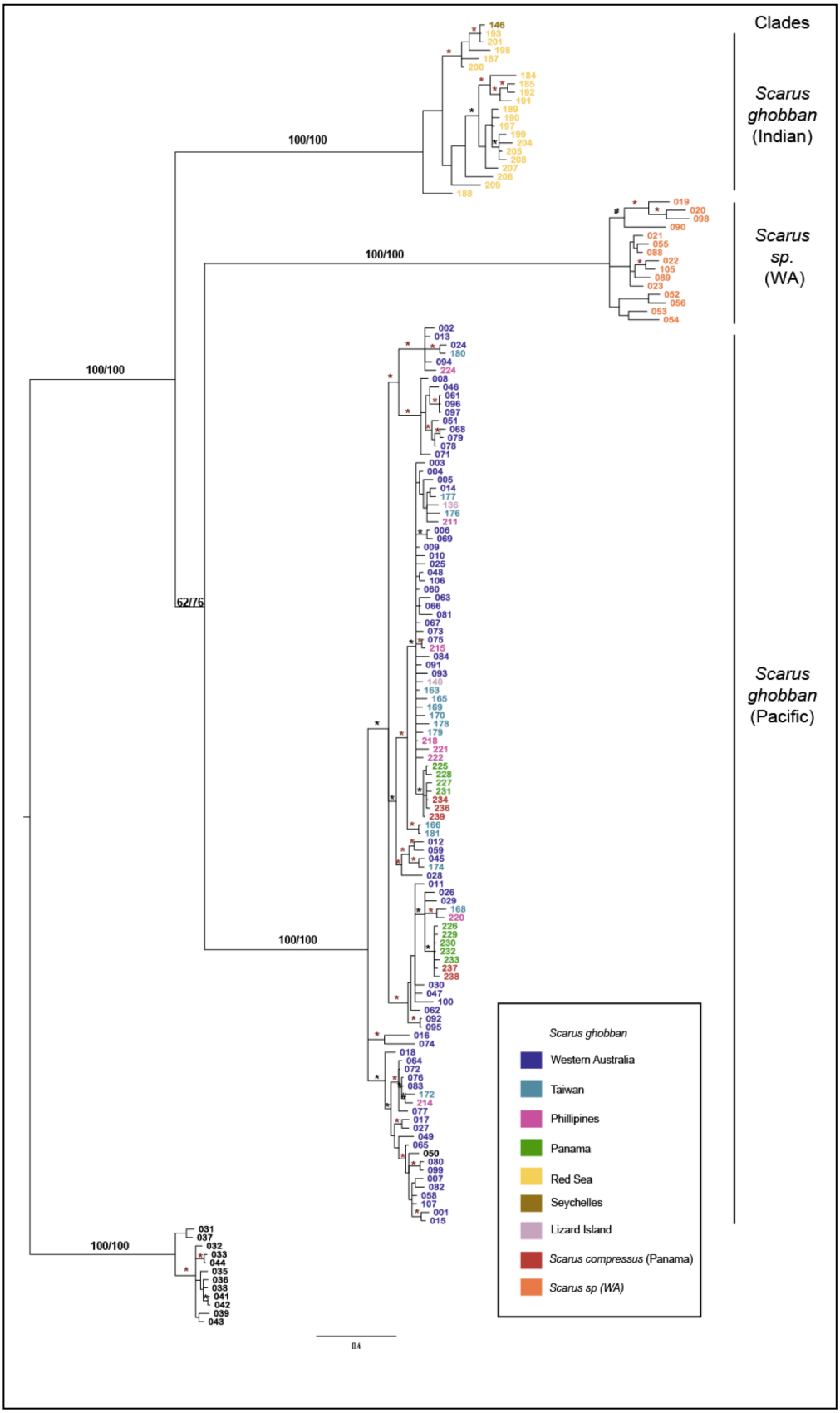

Figure 5. Parrotfish mtDNA Tree based on *16S* and control region. Terminal taxa are individuals color-coded by species & locality. Tree shown is the consensus tree generated by MrBayes 3.1.2. Maximum likelihood (ML) trees generated in RaxML had similar topology. Numbers on clade branches indicate Bayesian posterior/ ML bootstrap support. Within clades, nodes supported by both analyses (>95% posterior or >80% bootstrap) are indicated by red stars, nodes supported only by Bayesian analysis are indicated by black stars, and nodes supported only by ML are indicated by a hash symbol. Well-supported clades are indicated right of the tree.

Species tree and Divergence dating

Visual analysis of BEAST logs in Tracer v1.5 showed rapid convergence of the analysis and narrowing of priors. Effective samples sizes (ESS) for the posterior, prior, and likelihood were all high (>450), indicating good sampling of parameter space. Posterior values for rates and divergence times can be found in **Table 9**. The mean rate of substitution for *16S* was 1.61×10^{-3} substitutions/site/MY, corresponding to a between lineage rate of 0.32%/ MY. The coefficient of variation was 0.482 however, indicating variation between lineages. The mean rate of substitution for the control region was 4.44×10^{-2} substitutions/site/MY respectively, corresponding to a between lineage rate of 8.8%/MY. The coefficient of variation, however, was 0.148 indicating some variation between lineages.

The species tree produced by *BEAST analysis (**Figure 6**) differed from the Bayesian tree in that *S. ghobban* (Indian) and *S. ghobban* (Pacific) were found to be sister taxa. Again, however, support for this node was very low. The date for the divergence of the ingroup was estimated at ~2.3 Ma, while the date for the split between the two *Scarus ghobban* clades was ~1.0 Ma placing both splits in the early to mid-Pleistocene. 95% HPD intervals on these nodes were large, however, indicating significant uncertainty around these dates. In addition, visual analysis of posterior estimates of dates for the ingroup split showed a slightly bimodal distribution which could be due to a potential conflict within the data for this estimate.

Table 9. Selected posterior estimates from *Scarus* BEAST analysis.

	Mean	95% High Posterior Density (HPD) interval	Effective sample size (ESS)
tMRCA (ingroup)	2.296	(0.604,4.148)	12274
tMRCA (root)	3.479	(1.836,5.063)	10181
***16S* mean rate**	1.61×10^{-3}	(8.93×10^{-4}, 2.35×10^{-3})	8472
***16S* coefficient of variation**	0.482	(7.75×10^{-7}, 1.22)	3283
Control region mean rate	4.44×10^{-2}	(2.18×10^{-2}, 6.89×10^{-2})	3457
Control region coefficient of variation	0.148	(9.15×10^{-7}, 0.31)	1597

tMRCA (time of most recent common ancestor) for the ingroup and the root node are given in million years ago (Ma). Rates for *16s* and control region evolution are given in substitutions/site/million years

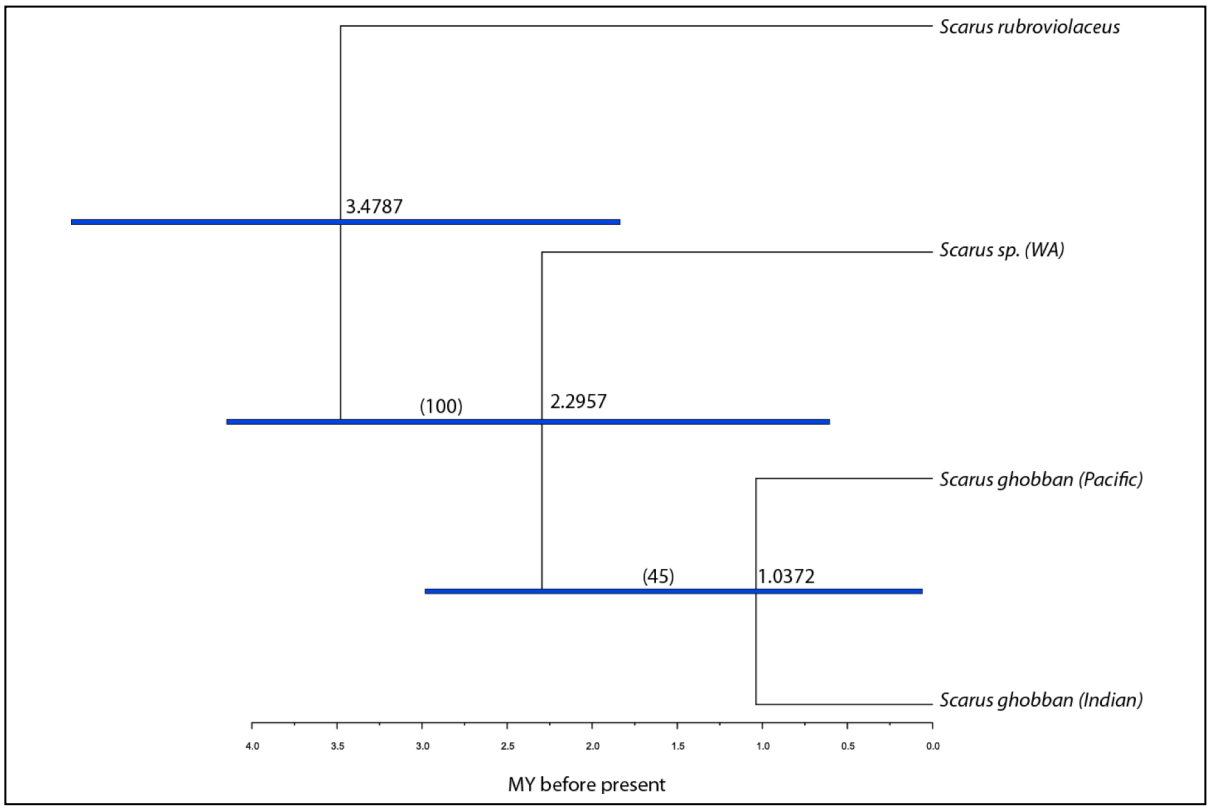

Figure 6. Parrotfish species chronogram from *BEAST.

Time-calibrated species tree produced by Beast v1.7.1. Posterior support for nodes are indicated by the numbers in parentheses. Numbers at nodes are estimates of ages for those nodes with blue bars to indicate 95% Highest Posterior Density (HPD) intervals on those estimates.

Discussion

Patterns of Diversity in the S. ghobban complex

Phylogenetic analysis of mitochondrial DNA indicates that *Scarus ghobban*, *Scarus sp.* (WA), and *Scarus compressus* may actually represent a species complex, with an intricate history of divergence along geographic and morphological lines. Reciprocal monophyly (**Table 7, Figure 5**) and high pairwise distances (**Table 8**) at the *16S* and control region loci indicate deep divergence between the newly discovered Australian species, *Scarus sp*. (WA), and Indian and Pacific clades of *S. ghobban.* Despite its location in the Indian Ocean, the Western Australia population falls within the Pacific clade. This pattern has been observed in both fish [97] and marine invertebrates [138, 139] and is most likely a result of the Indonesian flow-through bringing Pacific waters into Western Australia, coupled with an upwelling along that coast maintains isolation from the rest of the Indian Ocean. While it is possible that the depth of the Indian/Pacific split is due to poor sampling of intermediate populations in the Indian Ocean, a previous study showing this division [126], plus a lack of genetic structure within the Indian Ocean[140], make it unlikely that adding more localities will alter this pattern.

Within the Pacific clade of *S. ghobban*, the mtDNA tree (**Figure 5**) does indicate some genetic structure, but no geographic pattern is readily apparent from our data. The lack of separation between Eastern and Central Pacific populations implies that the Tropical Eastern Pacific Barrier (EPB) is not an important driver of diversification for *Scarus ghobban.* This study fails to distinguish, however, whether this is truly a panmictic population or whether rare migration events over the EPB are causing a pattern of incomplete lineage sorting. Further population genetic analyses of this clade will be required to test between these alternate hypotheses.

For fish, color pattern is often one of the primary means of species identification and its role in the creation and maintenance of reproductive isolation in both sympatric and allopatric speciation is a topic of much debate [111, 141-143]. The relationship between color and species/clade boundaries appears to be similarly complicated in the *S. ghobban* complex. The deep divergence between *S. ghobban* (Pacific) and *S. ghobban* (Indian) is accompanied by only subtle changes in color (H. Choat, personal communication), while *Scarus sp.* (WA) differs drastically from the other clades in color pattern, but not meristic characteristics (K. Clements, personal communication). Thus divergence appears to occur with varying degrees of morphological change, although whether this difference is related to speciation mode is unclear.

To further muddy the waters, *Scarus compressus*, which is morphologically distinct from *Scarus ghobban* in both morphology and coloration, falls paraphyletically within the *Scarus ghobban* (Pacific) clade in our analysis (**Figure 5**). This result is surprising considering *S. compressus* has been described for nearly a century [144] and is commonly observed on Eastern Pacific reefs [145]. While this pattern may be the result of incomplete lineage sorting between *S. ghobban* and *S. compressus*, several intermediate features in *S. compressus* color patterning indicate possible hybrid origin (B. Victor, personal communication). Hybrid speciation or stable hybrid zones [135, 146, 147] are common in some fish groups. Since our data only includes mitochondrial DNA, it is possible that *S. compressus* may actually represent a recent hybrid between *S. ghobban* and another species. Alternatively, an older "mitochondrial capture" event [148-150]

between *S. compressus* and *S. ghobban* could lead to a lack of divergence at mtDNA genes with divergence at nuclear loci consistent with morphological differences. Further work using multiple nuclear markers for admixture analysis [151, 152] and models of isolation and migration [153] and will be needed to conclusively evaluate the status of *S. compressus* in the Eastern Pacific.

Relationships and Timing of Diversification

While divergence between the three clades is well-supported in all phylogenies, there is a notable lack of support for the order of splitting events in Bayesian, maximum likelihood, and BEAST phylogenies **(Figures 5 & 6)**. This lack of resolution could be due to nearly simultaneous speciation events creating an unresolved "star" phylogeny as seen during an adaptive radiation, or due to long branch attraction [154] in the rapidly evolving control region locus. In addition to this lack of resolution, further uncertainty is introduced in the BEAST analysis with the estimation of substitution rates for both loci. While estimates of *16S* rate appear to agree closely with literature values [135], our estimates for control region evolution are considerably higher than those reflected in the literature on fish evolution [136] and approach the maximum rates described in Avise [155]. Whether this increased rate is the result of a rapid burst of diversification in the *Scarus* genus as hinted at by [122] or is an artifact of our analysis remains to be seen and would require harder calibration points. Preliminary results of ongoing work on ancient DNA samples of *Scarus rubroviolaceus* in Hawaii (Chan *et al.*, unpublished) suggest that our estimates may reflect real rates of control region evolution in these taxa. A secondary issue is that the high levels of rate heterogeneity in the control region [156, 157] could lead to problems in accurately estimating divergence times [158]. Finally, the smaller effective population size of the mitochondrial genome loci can lead to faster coalescent times [159], possibly biasing estimates of divergence dates. Overall, the addition nuclear loci to this data set is likely to be of great help in resolving the relationships and timing of splits between clades.

Given these caveats, the divergence date estimates from BEAST (**Figure 6, Table 9**) reflect rough approximations of splitting times between these groups. Divergence between the *S. ghobban* (Indian) and *S. ghobban* (Pacific) clades occurred approximately 1 Ma, possibly due to a vicariant event across the Indo-West Pacific Barrier (IWPB). The timing and location of this division is similar to patterns seen in many reef fish [90, 108], and supports models of Indo-Pacific diversity that propose the Indo-Australian Archipelago as a center of origination (COO) due to Pleistocene sea level changes [84]. Given the temporal ephemerality of the IWPB, however, it is unclear what mechanisms are maintaining isolation of these two clades during glacial minima. Since post-zygotic barriers are unlikely to develop over the time period of separation, it is possible that assortative mating based on subtle morphological differences could play a role.

The split between *Scarus sp.* (WA) and *Scarus ghobban* seems to have taken place in the early Pleistocene (~2.3 Ma) (**Figure 6, Table 9**). At first glance, this seems to be a perfect example of peripheral endemism promoted by peripatric isolation [160]. In this case, however, the pattern is slightly more complicated since *Scarus sp.* (WA) exists sympatrically with *S. ghobban* throughout its range. Three plausible models for this pattern exist. First, *Scarus sp.* could be the result of sympatric ecological speciation followed by a reinforcement of isolation by a shift in

color pattern [86]. Second, *S. sp.* could be the result of Pleistocene vicariant isolation. The cut-off of the Indonesian flow through during glacial maxima could have resulted in allopatric speciation in the newly isolated Western Australian population. By the time the current returned and *S. ghobban* was able to recolonize, a mechanism for reproductive isolation could have already evolved between the two species. Finally, the Western Australian coast may have served as a refuge for tropical marine fauna during times of environmental stress accompanying Pleistocene climate fluctuations [161]. *Scarus sp.* (WA) could therefore be a relict population of a formerly more widespread Indo-Pacific species. It is possible that alternate topologies for the placement of *Scarus sp.* (WA) within the *Scarus ghobban* complex (see **Figure 5** versus **Figure 6)** could lead to alternate divergence depths that would affect the specific mechanisms of these hypotheses. However, since the basal node of the group is still of Pleistocene origin, we can assume the same types of processes were at play. Distinguishing between these alternatives will require both a thorough assessment of life history of the new species to look for evidence of ecological differentiation, plus an examination of genetic diversity to look for signatures of past population expansion and bottleneck that would indicate a formerly wider range.

Conclusions

The *Scarus ghobban* complex examined in this study serves as a microcosm for the complexity of marine speciation. Within just this small set of taxa and populations, we see evidence of Pleistocene vicariance events across major biogeographic borders, peripheral endemism, shifts in morphology that could be a hallmark of ecological speciation, and potential hybridization. While more data and analyses will be needed to tease apart the many processes at work in this system, these data highlight role of chance and plurality of mechanisms in generating biodiversity in such an open medium as the ocean. Is the emerging pattern in marine speciation that there is no real pattern? More studies aimed at a fine-scale examination of taxa will clearly be key in answering this question and protecting our important and increasingly threatened marine resources.

Chapter 3: Genetic structure and demographic history of the *Scarus ghobban* complex highlight the role of Pleistocene sea level fluctuations, isolation by distance, and divergence in periphery populations

Introduction

Phylogenetic reconstruction and divergence dating of closely related taxa can tell us about large scale patterns of diversity over time and tie them to major periods of geological change (Chapters 1 and 2). However, when examining speciation events from the distant past, it becomes challenging to infer the geographic, ecological, and genetic context of the initial separation [162-164]. Part of this gap in understanding can be closed by investigating dynamics within populations to deduce ongoing processes that may be key in both past and future diversification. Combined with phylogenetic results, we can then begin to piece together an "evolutionary animation" of the different stages of speciation [165, 166].

Our phylogenetic analysis of the *Scarus ghobban* complex in the Indo-Pacific shows a rich evolutionary history dating back to the early to mid-Pleistocene (Chapter 2). While strong divisions are seen between monophyletic clades in this complex, within the Pacific basin, there is no apparent phylogeographic structure over a >10,000 kilometer range. Though such wide species-level biogeographic patterns are common in marine taxa with high dispersal capability [85, 167], over such large heterogeneous areas, diversity is often nested at several spatial scales [168]. In fact, population genetic studies in the Pacific have shown evidence of structure both within central regions [88, 169] and on the periphery [99, 124, 160, 170, 171]. These divergences are likely being driven by processes such as population contractions and expansion, isolation by distance, and natural selection and hybridization in peripheral populations [172].

Phylogeographic studies that examine the genetic structure of populations can infer both contemporary connectivity and signatures of events from the recent past, allowing us to distinguish between competing hypotheses of diversification [99, 101, 173, 174]. Analyses of Indo-Pacific fish have revealed complex genetic structure and colonization history throughout the ocean basin [175-177]. In many cases, there appears to be a heavy influence of Pleistocene fluctuations in sea level on current population-level patterns [97, 101, 124, 140, 178], but the extent of this effect also seems to vary by taxa. For example, closely related species may have very different demographic histories, which may be explained by the interaction between niche divergence and the effects of sea level change [179, 180].

In addition to providing a historical perspective, understanding the phylogeography of marine species can help in their conservation [107]. How to assess connectivity in species with a pelagic larval dispersal stage is still a central question in marine ecology and reserve design [181]. By looking at genetic structure and history, we can identify evolutionarily significant units (ESUs) [182] and the spatial scale of processes that generated and maintain them. Obtaining this information is an essential first step in management of marine biodiversity and resources [183].

In this study, we build upon our previous phylogenetic work (Chapter 2) by using population genetics to answer lingering questions about the structure and history of the *Scarus ghobban* complex: i) do dispersal limitations play any role in the structuring of genetic diversity in these

species? ii) how do populations respond to environmental fluctuations over time? and iii) how do we explain the origins of peripheral endemics in Western Australia and the Eastern Pacific? With this information, we seek not only to link present to past mechanisms for diversification of this group, but also to examine the future impacts of contemporary connectivity.

Methods

Population structure

Sampling and molecular data collection for the control region and *16S* loci are described in Chapter 2. To look for geographic structure in *Scarus ghobban* (Pacific) populations, a haplotype network of *16S* was constructed using TCS v1.2.1[184]. This program uses statistical parsimony to create a median-joining network of related haplotypes. Haplotype counts and sampling localities were then added by hand to the network. Preliminary population analyses using the *16S* locus showed little power at this level, most likely due to very low diversity. Further structural and demographic analyses were performed using only control region data.

To quantify variation within clades and populations, molecular diversity indices for the control region, including number of haplotypes, number of variable sites, haplotypic diversity and nucleotide diversity[185], were calculated for all Pacific clades and geographic populations in the *Scarus ghobban* complex using Arlequinv3.5 [133]. Haplotypic diversity measures the uniqueness of haplotypes in populations, while nucleotide diversity measures the level of genetic variation in a population by examining the average number of differences per site between any two sequences. Genetic distances and structure of populations within the Pacific were then compared using pairwise F_{ST} values calculated in Arlequin v3.5 with 10,000 Monte Carlo permutations to assess significance. To test for a pattern of isolation by distance (IBD) [186, 187], we calculated the reduced major axis (RMA) regression of genetic distance versus geographic distance in population pairs using Isolation By Distance Web Service (IBDWS) v3.2.3 [188]. A Mantel test with 1,000 permutations was used to assess the significance of the relationship. For genetic distances, values of $F_{ST}/(1-F_{ST})$ were used as described in Rousset [186]. Negative values were entered as 0.000001.Geographic distances between populations were calculated by obtaining approximate coordinates for sampling sites in Google Earth™ (http://earth.google.com). We used the web server at http://www.movable-type.co.uk/scripts/latlong.html to calculate great circle distances between points using the haversine formula [189]. Due to small sample sizes, Lizard Island and *S. compressus* populations were removed from this analysis.

The spatial structure of *S. ghobban* (Pacific) populations was then further examined using three sets of hierarchical AMOVA analyses in Arlequin v3.5 [190]. For the first set, to assess the level of geographic structuring, we examined the amount of variance seen within versus between geographic populations. For the second set, we tested the hypothesis of divergence across the Eastern Pacific Barrier by dividing the population into two groups. The Eastern Pacific (EP) group consisted of samples of both *S. ghobban* and *S. compressus* from Panama. The Central-West Pacific (CWP) group consisted of samples of *S. ghobban* from Taiwan, the Philippines, Lizard Island, and Western Australia. The third analysis tested for further structure within the CWP, by dividing this region into a Central Pacific group (Taiwan, Philippines, Lizard Island) and the W. Australian population. Though F_{ST} values indicated no genetic differences between

Panama samples of *S. ghobban* and *S. compressus*, to test for potential bias in grouping different morphological species, analyses were repeated excluding the *S. compressus* samples.

Demographic analysis

To test for population expansion, we performed mismatch distributions based on observed and modeled pairwise differences between individuals [191, 192]. Distributions were calculated in Arlequin v3.5 for a pooled samples of all *Scarus ghobban* (Pacific) individuals, and separate geographic populations of both *Scarus ghobban* (Pacific) and *Scarus sp.* (WA). Goodness-of-fit of the data to the sudden demographic expansion model was tested using the sum-of-squares deviation (SSD) and Harpending's raggedness index (r). Signs of recent bottleneck and population expansion were also tested by calculating Tajima's D [193] and Fu's F [194] using Arlequin v3.5. These tests are designed to look for departures from neutrality due to selection on the number of segregating sites (Tajima's D) or number of alleles (Fu's F). Significant negative values of these statistics, however, can be a sign of bottleneck and expansion [194, 195].

Finally, to more explicitly model demographic history of populations, we used the Bayesian skyline method [196] in BEAST v1.7 [65]. This approach uses the coalescent to model effective population size over time under a given mutation model. For each population, we performed four independent runs of 10,000,000 generations each. Based on phylogenetic analysis (Chapter 2), we used GTR+I+G as a substitution model. Preliminary runs, however, indicated problems with sampling small relative substitution rates, so prior shapes for these parameters were reset to a uniform distribution between 0 and 5. Mean substitution rate was fixed at 8.8% based on parameters calculated during phylogenetic analysis (Chapter 2, **Table 9**). Preliminary runs were performed using both strict clock model, which assumes that rate is constant amongst all lineages, and a relaxed uncorrelated lognormal clock models, which allows rates to vary between lineages by drawing from a lognormal distribution [197]. Results for all parameters were similar between models and Bayes factor comparisons of the marginal likelihoods of each model were inconclusive. Since the phylogenetic analysis did indicate some variation in rate between lineages (Chapter 2, **Table 9**), we chose to use the uncorrelated relaxed lognormal clock for our final runs. For skyline analysis, a piece-wise constant model was used with a group size of 10 for all analyses except for the Panama population, where group size was reduced to 6 due to small sample size. For each skyline analysis, logs of each run were visually inspected in Tracer v1.5 [132] to assess for convergence and that effective sample size (ESS) values were adequate (>150). A 10% burn-in was then removed and logs of all runs for each sample were combined using LogCombiner 1.7.1. Bayesian skyline plots of the combined logs were then generated using Tracer v.1.5 [132].

S7 data analysis

An additional locus, the first intron of the *S7* protein, was amplified by PCR using primers *S7RPEX1F (*5'- TGGCCTCTTCCTTGGCCGTC-3'), and *S7RPEX2R* (5'-AACTCGTCTGGCTTTTCGCC-3') [198]. PCR reaction conditions were identical to those described for the control region, with a change in annealing temperature to 60°C. All subsequent verification, purification, and sequencing protocols remained the same. Chromatograms were edited in Geneious v5.5.6 [128] and double peaks at heterozygous sites were re-coded using IUPAC ambiguity codes. Sequences without insertions or deletions were aligned in MAFFT v6 [55] with corrections made by eye in Geneious. Sequences with insertions or deletions were assembled against the alignment. Indels were detected and reconstructed by visual parsing of overlapping chromatograms.

While our mtDNA phylogeny indicates that *Scarus compressus* is part of the *Scarus ghobban* (Pacific) clade (Chapter 2), morphological and ecological differences between these two nominal species raise the possibility that *S. compressus* may be a hybrid. Since mtDNA is matrilineally inherited, it can only tell us about the taxonomic relationships of one parent. To accurately identify a hybrid individual or species, an analysis of biparentally inherited nuclear genes is necessary. Therefore, to assess the status of *Scarus compressus*, we used PHASE v2.1 [199, 200] to reconstruct haplotypic phase for all individuals at the *S7* intron. Since phasing algorithms run under the assumption that individuals are sampled from freely mixing populations, based on phylogenetic results, alignments were separated into separate files for *Scarus sp.* (WA), *S. ghobban* (Pacific), *S. ghobban* (Indian), and *S. rubroviolaceus.* Individuals with multiple indels were removed from the alignment due to high amounts of missing sequence data. Input files were then generated using the SeqPHASE web tool [201] and run using PHASE v2.1 with the S algorithm. This algorithm does not calculate recombination rates, but can give higher posterior probabilities for haplotypes. For our analysis, the default 0.90 probability cut off was used for accepting phase calls. Output files were reconstructed into FASTA alignments using SeqPHASE.

All phased and partially phased individuals were then pooled into a single alignment along with *Scarus ghobban* S7 sequences retrieved from Genbank from Bariche and Bernardi[126]. Duplicate sequences were removed, and a consensus Neighbor Joining Tree was generated in Geneious v5.5.6 [128] using Tamura-Nei distances with 1000 bootstrap replicates.

Results

Population Structure

A *16S* haplotype network of the *Scarus ghobban* (Pacific) populations revealed no geographic structure (**Figure 7**). Most haplotypes differed by only a single base pair indicating low population-level variation at this locus. The most common haplotype was seen in 55 individuals and shared amongst all geographic populations.

The control region locus showed high haplotype and nucleotide diversity in all clades and populations (**Table 10**). Haplotype diversity was close to 1.0 in all samples, while nucleotide diversity ranged from a high of 3.8% in *Scarus sp.* (WA) to between 1.1 to 3.2% in *S. ghobban* (Pacific). Diversity was lower in Eastern Pacific populations than Central and Western Pacific populations.

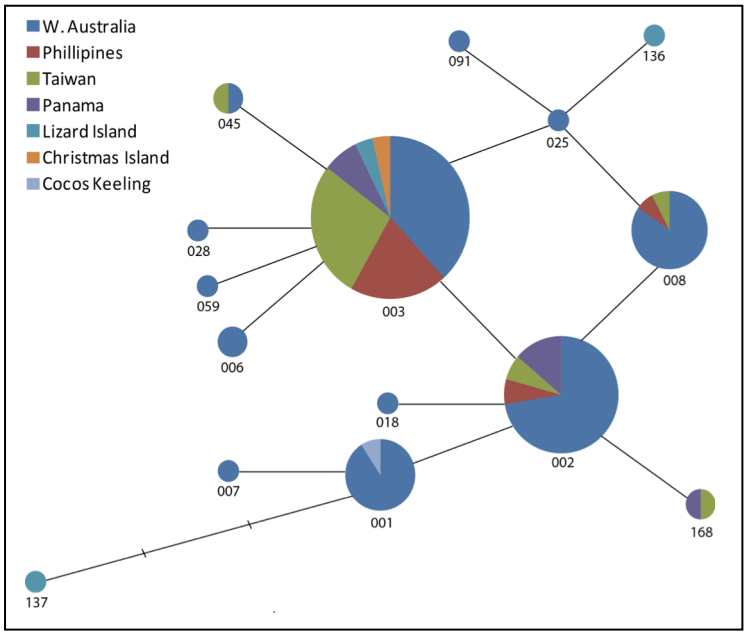

Figure 7. *Scarus ghobban* (Pacific) *16S* haplotype network. Circles represent *16S* haplotypes as numbered in genealogy in **Appendix 4.** Size and color of circles indicate number of individuals and sampling location for that haplotype. Connections between circles represent a median-spanning network as calculated in TCS v1.2.1 Each line segment (divided by ticks) indicates a single bp difference between haplotypes.

Table 10. Control region sampling & diversity data -*Scarus sp.* (WA) and *S. ghobban* (Pacific)

	Region	n	n_h	# sites (# var.)	H±S.D	π±S.D
Scarus sp. (WA)		15	15	901 (132)	1.0000 ±0.0243	0.0381 ± 0.0197
Scarus ghobban (Pacific)		106	102	919 (224)	0.9989 ±0.0015	0.0300 ± 0.0146
Panama	EP	9	9	896 (39)	1.0000± 0.0524	0.0205 ±0.0114
S. compressus	EP	5	5	895(30)	1.0000 ±0.1265	0.0192 ± 0.0121
Lizard Island	CWP	2	2	670 (8)	1.0000 ±0.5000	0.0119 ± 0.0127
Philippines	CWP	8	8	894 (86)	1.0000 ±0.0625	0.0297 ± 0.0166
Taiwan	CWP	14	13	896 (106)	0.9890 ± 0.0314	0.0246 ± 0.0130
W. Australia	CWP	69	66	899 (189)	0.9983 ±0.0030	0.0323 ±0.0159

Sampling sites, region (EP=Eastern Pacific, CWP=Central West Pacific), sample size (n), number of haplotypes (n_h), number of total and variable sites, haplotypic diversity (h+/-S.D), and nucleotide diversity (π±S.D) as calculated in Arlequin v3.1 using 1000 permutations. Top two rows represent total clade estimates, while bottom six rows show a breakdown of the *S. ghobban* (Pacific) into sampled populations.

Pairwise comparisons of populations using F_{ST} show some significant structuring between Pacific populations (**Table 11)**. Intermediate values of F_{ST} (0.10-0.19) between populations from the Eastern Pacific (Panama and *S. compressus*) and Central-West Pacific (Taiwan and W. Australia) regions indicate divergence between these two regions. Aside from a low, but significant, result between W. Australia and Taiwan, within these regions, F_{ST} values were non-significant. Lack of significance for comparisons including Lizard Island or the Philippines are most likely due to small sample sizes at these localities.

Table 11. F_{ST} values for *Scarus ghobban* (Pacific) populations.

	Panama	*S. compressus*	Philippines	Taiwan	Lizard I.	W. Australia
Panama	-----	0.29082	0.06169	0.00436	0.28407	0.00119
S. compressus	-0.08195	-----	0.23686	0.03214	0.10355	0.02896
Philippines	0.12566	0.03664	-----	0.88713	0.93255	0.21881
Taiwan	**0.19343**	**0.10649**	-0.04212	-----	0.99226	0.03293
Lizard Island	0.25485	0.17437	-0.21345	-0.24665	-----	0.8171
W. Australia	**0.1454**	**0.10264**	**0.01782**	0.04561	-0.08525	-----

Pairwise F_{ST} values are shown below the diagonal while significance values (p) are shown above the diagonal. Significant values of F_{ST} ($p<0.05$) are indicated in bold. All values calculated in Arlequin v3.1 with 10,000 permutations.

Reduced major axis regression and a Mantel test showed a strong and significant correlation between the log of pairwise genetic distance and the log of geographic distance ($r = 0.9199$, $p= 0.0390$) (**Figure 8**), indicating a pattern of isolation by distance across the entire range of *Scarus ghobban* (Pacific).

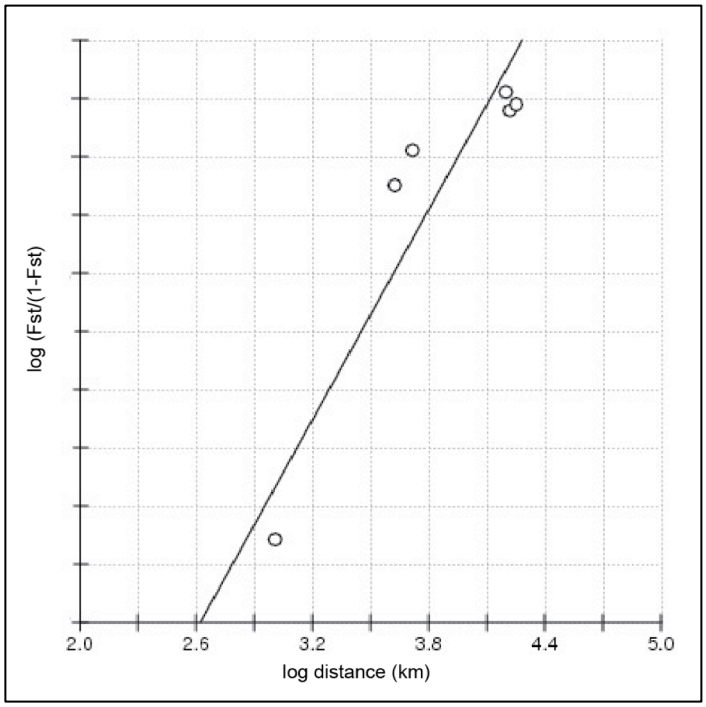

Figure 8. Isolation by distance analysis of *Scarus ghobban* (Pacific) populations. Plot of the log of genetic distance between populations versus of the log of the geographic distance.

The results of the AMOVA analysis indicate that the highest level of genetic variation (85-93%) is seen within populations rather than between geographic populations or regions (**Table 12**). Splitting the populations into two groups (Table 12B) showed that significant variation (14%) can be accounted for by partitioning between the Eastern and Central Western Pacific populations. Separating out the Western Australian population (**Table 12C**) did not account for much more variation and led to negative variance components and loss of significance, possibly due to smaller sample size. Overall results are congruent with pairwise comparisons.

Table 12. Results of hierarchical AMOVA for *Scarus ghobban* (Pacific)

Source of variation	Df	Sum of squares	Variance components	% variation	statistic (P)
A) One group					
Among populations	5	123.406	0.971	6.84	
Within Populations	101	1334.968	13.218	93.16	**0.0684** (0.001)
Total	106	1458.374	14.188		
B) Two groups: Eastern Pacific vs. Central West Pacific					
Among regions	1	67.082	2.171	14.04	**0.0057** (0.002)
Among populations in regions	4	56.324	0.076	0.49	0.1453 (0.282)
Within populations	101	1334.968	13.218	85.47	0.1404 (0.062)
Total	106	1458.374	15.464		
C) Three groups: Eastern Pacific vs. Central Pacific vs. W. Australia					
Among regions	2	108.058	2.373	16.55	**-0.1045**(0.001)
Among populations in regions	3	15.348	1.2517	-8.72	0.0782(0.990)
Within populations	101	1334.968	13.218	92.18	**0.1655**(0.013)
Total	106	1458.374	14.339		

Significant values (p<0.05) in bold. Probability values obtained using 5,000 permutations in Arlequin 3.1.

Demographic history

Results of the mismatch analysis are shown in **Table 13** and **Figures 3** & **4.**The sum of squares deviation **(**SSD) measures the goodness-of-fit to the mismatch curve and Harpending's raggedness index measures variation around the curve. Both test against a null model of population expansion, therefore low and insignificant values suggest a good fit to the expansion model.

Table 13. Demographic model estimates for *Scarus ghobban* (Pacific) and *Scarus sp*. (WA)

	τ	θ_0	θ_1	SSD (p value)	Raggedness (p value)	Tajima's D (p value)	Fu's F (p value)
S. ghobban							
All Pacific	33.9 (24.4-38.4)	0.0035	94	0.00269 (0.534)	0.00128 (0.948)	-1.10421 (0.102)	**-23.95042 (0.001)**
Panama	0.5 (0.0-27.7)	19.5	99999	0.07438 (0.305)	0.06944 (0.543)	1.57127 (0.975)	-1.51147 (0.133)
Philippines	35.6 (21.0-43.5)	0	70	0.0351 (0.642)	0.07143 (0.514)	-1.03914 (0.183)	-0.48791 (0.233)
Taiwan	6.7 (3.2-40.0)	18.6188	99999	0.0196 (0.473)	0.039 (0.157)	-1.46845 (0.061)	-1.67054 (0.165)
W. Australia	34.0 (24.9-38.4)	0	109.6	0.00305 (0.473)	0.00148 (0.959)	-0.90237 (0.177)	**-24.07824 (0.000)**
Scarus sp.	36.9 (26.4-51.1)	5.79551	134.86	0.02071 (0.122)	**0.05043 (0.045)**	-0.67619 (0.256)	-2.64103 (0.057)

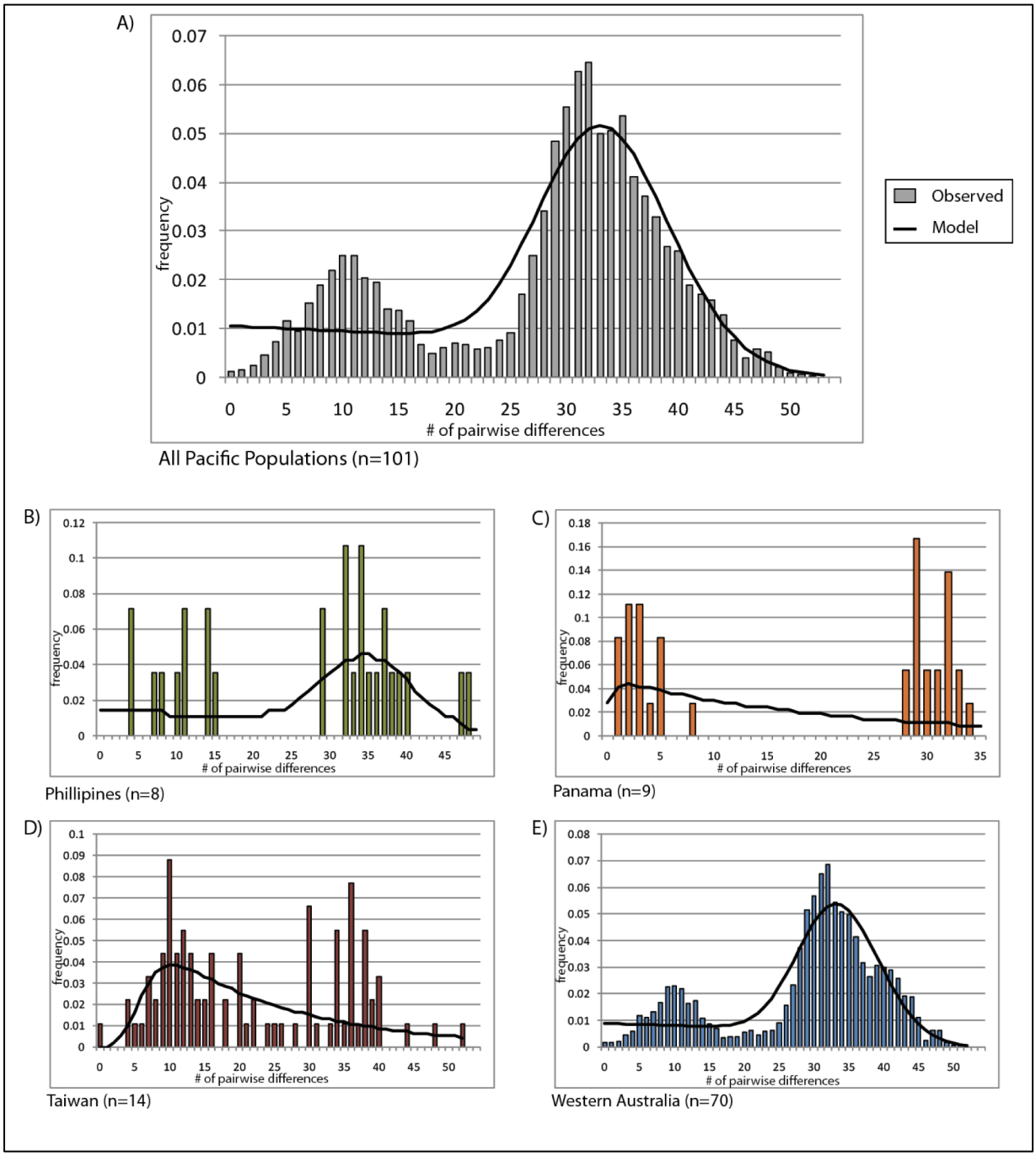

Figure 9. Mismatch distributions for *S. ghobban* (Pacific) populations. A) Pooled samples from all individuals. B-E) Individual populations. Sample sizes are indicated with each plot.

Mismatch parameters for pooled and individual populations of *S. ghobban* (Pacific) all show non-significant SSD and raggedness indexes indicating possible demographic expansion **(Table 13)**. Tajima's D values were negative for all but the Panama population, but non-significant, possibly due to sensitivity of this index to small sample sizes [202]. Fu's F values were highly negative and significant for both the pooled and W. Australian samples, providing additional evidence for expansion.

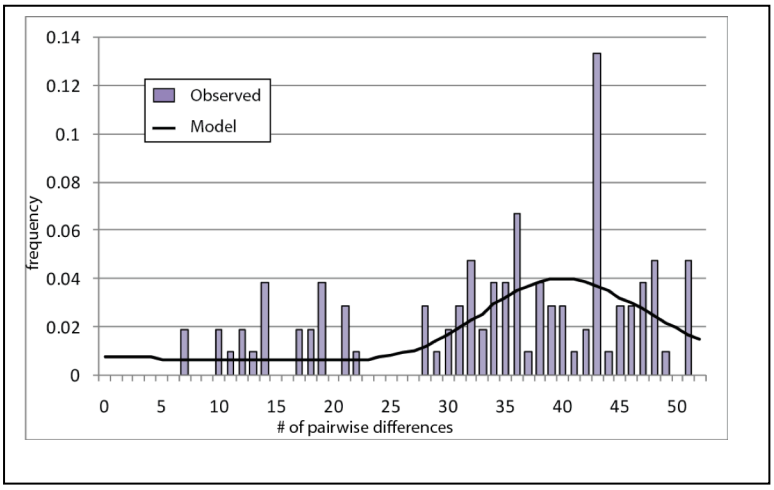

Figure 10. Mismatch distribution for *Scarus sp.* (WA).

An examination of mismatch curves (**Figure 9**) indicates a potentially more complex situation than simple population expansions. Populations that have been stable in size over time will show a bimodal distribution of sequence pairs with a high and low degree of mismatch, while rapidly expanding populations will show a unimodal distribution with a high frequency of pairs with a high degree of mismatches and a smaller number of pairs with a lower degree of mismatch [203]. A bimodal distribution, however, can also be a sign of admixture between two divergent populations [204, 205], where the first peak results from expansion within populations and accumulation of pairwise differences, while the second peak results from the admixture of multiple populations that have diverged in isolation for some period of time. Distributions for the pooled and W. Australian samples (**9A** and **9E**) appear to be intermediate between a bimodal and unimodal distribution, while Taiwan, Philippines, and Panama populations (**9B-9D**) appear more strongly bimodal, but with low sample sizes. Further analysis is necessary to distinguish between these possibilities. *Scarus sp.* (WA) also appears to have a different history than *S. ghobban.* Mismatch curves and SSD fit show weak evidence of expansion **(Figure 10, Table 13**), but a significant raggedness index and insignificant Tajima's D and Fu's F values contradict this scenario.

In the context of historical sea level changes, Bayesian skyline plots provide a more detailed look at populations over time (**Figure 11)**. The pooled Pacific population (**11A**) shows a strong and significant expansion starting ~125,000 years ago. This period coincides with the rise of sea level following a drop to a minima of 120 meters below present at ~135,000 years ago. Similar patterns are seen in both the Central Pacific and W. Australian populations (**11B** & **11C**). Most likely due to small sample sizes, confidence intervals for both Panama (**11D**) and *Scarus sp.* (WA) (**11E**) plots are large, limiting our ability to make conclusions about the significance of trends in the data. Despite this, the overall shape of these plots suggests that these populations experienced a different history than the CP and WA populations. The Panama plot shows a small expansion event occurring approximately 25k years ago, coinciding with the most recent sea level minima (**11D**), while the *Scarus sp.* (WA) population (**11E**) seems to have slowly expanded between 250-100,000 years ago, leveling off until present.

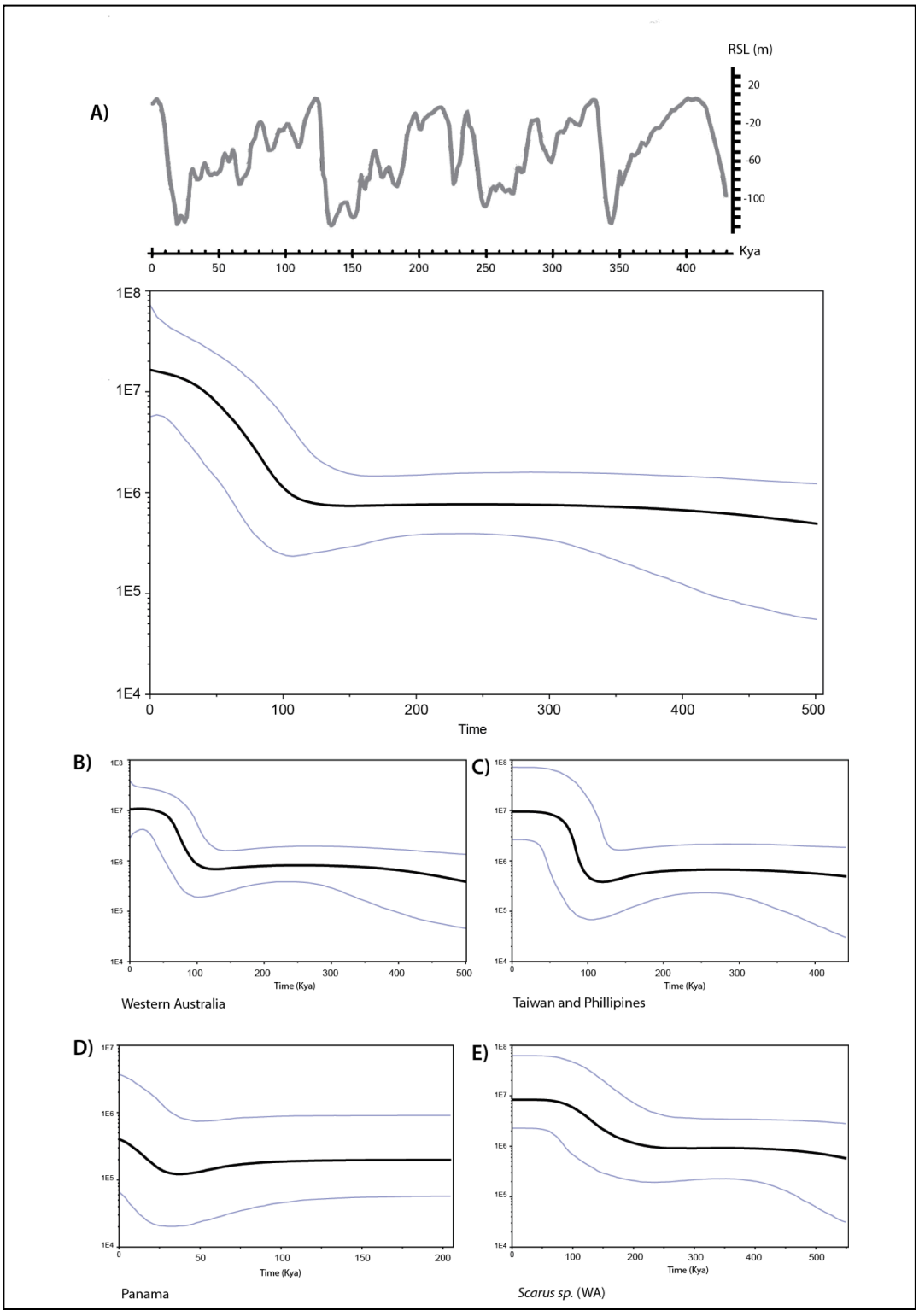

Figure 11. Bayesian skyline plots for *Scarus ghobban* (Pacific) and *Scarus sp.* (WA). **A)** Pooled sample of *Scarus ghobban*. **B-E**) Individual populations. For plots, X-axis shows the time in Ky before present. Y-axis represents the female effective population size x generation time in years. The top graph shows relative sea level over time and is modified from Figure 3 in Walbroeck *et al.* (2002)[40].

S7 analysis

The first intron of *S7* was successfully sequenced in 159 individuals for a total alignment of 520 bp. Heterozygosity at this locus was extremely high. Only 4 of 111 *S. ghobban* (Pacific) and 1 of 16 *Scarus sp*. (WA) individuals were homozygous. No homozygous individuals were found in *S. rubroviolaceus* (n=13) or *S. ghobban* (Indian) (n=19). Because of this high variability, phasing of haplotypes using PHASE v.2.1 was only moderately successful. After individuals with double indels and missing sequence data were removed, 21/72 and 4/15 heterozygous individuals were phased at all sites in *S. ghobban* (Pacific) and *S. ghobban* (Indian) respectively. No individuals were completely phased in *S. rubroviolaceus* or *S. sp.* (WA).

A neighbor-joining tree created from phased and partially phased *S7* haplotypes (**Figure 12)** clustered haplotypes into three well-supported groups. The first group (**Group A**) consists of all *S. ghobban* complex individuals plus one allele from each *S. compressus* individual (designated Allele 1). Despite high divergence in the mitochondrial DNA loci for the S. ghobban complex (Chapter 2), no further resolution is seen in this group. The second group (**Group B**) consists of all *S. rubroviolaceus* individuals, the sister taxa to the *S. ghobban* complex. The third group (**Group C**) consists of the second allele from each S. compressus individual (Allele 2). This group is well-supported by bootstrap analysis and highly divergent from the other groups, differing from *Scarus ghobban* by 4.2% and from *S. rubroviolaceus* by 3.4%.

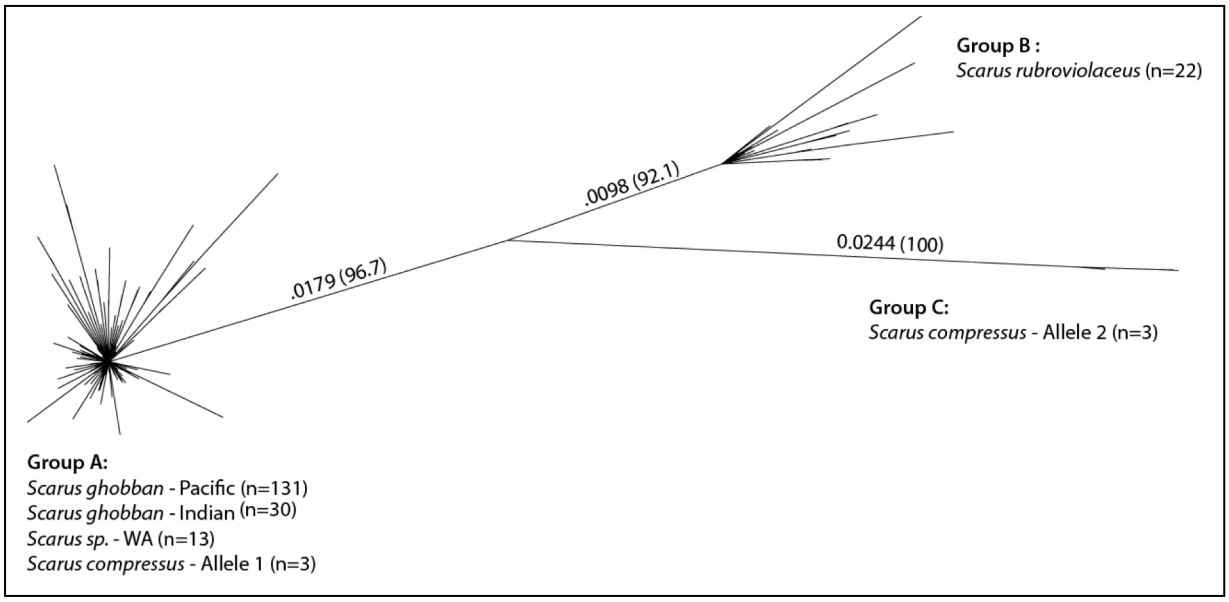

Figure 12. Neighbor joining tree of *S7* for *Scarus ghobban* complex and *S. rubroviolaceus*. Sample sizes indicate number phased and partially-phased *S7* intron 1 haplotypes. Branch labels show Tamura-Nei genetic distance and bootstrap support as calculated in Geneious v5.5.6.

Discussion

Population level analysis of *Scarus ghobban* (Pacific) and *Scarus sp.* (WA) reveal several striking patterns in the structure and demographic history of these clades. Despite no geographic pattern in the phylogenetic analysis of the *Scarus ghobban* (Pacific) clade, both F_{ST} and AMOVA indicate intermediate structure between the Eastern Pacific (EP) and Central-Western Pacific (CWP) populations, consistent with a pattern of isolation by distance. Within the CWP, including W. Australia, little structure is evident. Low *F*st values for the W. Australia and Taiwan comparison and a larger non-significant result between the Philippines and W. Australia indicate there may be some structure at the very far edges of the region, but this is not supported by the AMOVA analysis. More sampling of Central Pacific populations will be needed to more conclusively detect structure in this region. Overall, however, for *S. ghobban*, gene flow seems mostly to be limited only over large distances with no intermediate habitat. Population structure is thus most likely the result of exchange between adjacent populations as in the stepping stone model [206, 207], limited by the continuity of suitable habitat [208]. Previous studies on other Scaridae with similar larval duration and life histories show a congruent pattern across the Eastern Pacific Barrier [99] and in the Central West Pacific [97, 99, 124]. It is important to note, however, that these species have different overall ranges than *Scarus ghobban.* This difference supports theories that, for reef fish, there is a strong relationship between larval duration and degree of population subdivision [209], but not between larval duration and overall range [123, 210] (though see [211] for a counterexample).

Demographic analysis using Bayesian skyline analysis supports a model of rapid demographic expansion in *Scarus ghobban* in the Central West Pacific starting at about 125,000 years ago (**Figure 11**). This timing matches up closely to expansions seen in both *S. ghobban* (Indian) [140] and *Scarus psittacus* [124], indicating that these population changes are likely being driven by ocean-wide climactic fluctuations. During the glacial maxima ~135,000 years ago, sea levels dropped to over 120 m below present day levels [212]. A change of this magnitude would have serious negative impacts on reef systems, potentially creating a severe population decline in associated fish populations [140]. In addition, during this period, the range of *S. ghobban* would have been substantially fragmented by land bridges formed between adjacent islands along the Sunda shelf [94], effectively cutting off the potential for recruitment from even geographically proximate populations. As sea levels rose again in the next cycle, populations would be able to expand as more suitable habitat became available and eventually connections between isolated populations could be re-established. The bimodal mismatch distribution of the pooled and W. Australian samples (**Figure 9**) could be evidence of division of populations during the Pleistocene followed by subsequent admixture of populations. This period of separation could have been sufficient to create significant differentiation in the rapidly evolving control region, but the more slowly evolving nuclear genome did not diverge enough for reproductive isolation to be established. High levels of gene flow would then effectively wipe out most of the signature of geographic structure in the CWP, while retaining the temporal pattern in the mismatch distribution. A similar pattern of temporal, but not geographic, partitioning of populations has been described in surgeonfishes in the Indo-Pacific [177, 213], suggesting that a series of complex subdivision and admixture events during the Pleistocene may have been an important part of the history of many reef fish taxa. Further work with more loci and an isolation and migration model [153] could give us more power to explore these hypotheses [174].

Demographic history in the Eastern Pacific (EP) population is harder to discern, most likely due to small sample sizes from this region. A weak signal of population expansion around the time of the more recent sea level minima ~25,000 years ago (**Figure 11D**) suggests that the environmental forces structuring this population might be different from those at work in the CWP. Since the Eastern Pacific population is more likely structured by isolation and invasion than vicariance events [96], it may not be as sensitive to periods of subdivision during Pleistocene cycles. However, these same cycles coincide with shifts in sea surface temperature, nutrient levels, and currents in the Eastern equatorial Pacific [214], that may have led to local population declines. Furthermore, Eastern Pacific waters became cooler and more nutrient rich subsequent to the Isthmus of Panama closure [215]. As a result, environmental conditions differ substantially between the Eastern and Central Pacific, with the more marginal EP reef environment supporting far lower levels of biodiversity [92]. *S. ghobban* populations that are already on the edge of their suitable habitat could be far more sensitive to climactic fluctuations, leading to periodic population bottlenecks. This could drive the divergence of the EP populations both through genetic drift in reduced populations and across the environmental gradient due to ecological speciation processes [87].

The Western Australian endemic clade, *Scarus sp.* (WA), also shows a different demographic history than the central *S. ghobban* (Pacific) populations. Rather than a sudden expansion following the 135 Ka sea level minima, this clade slowly expanded throughout the mid-Pleistocene, leveling out in the late Pleistocene to modern times (**Figure 11E**). Since the Western Australian coast was relatively stable and even served as a refuge for corals during the Pleistocene climactic fluctuations [161], this clade may have been able to expand even while other Pacific populations were fragmented and contracting. Interestingly, *Scarus sp*. (WA) seems to slow in growth and stabilize during the period that the W. Australian S. *ghobban* population expanded, suggesting that competition may have played a role in structuring local community dynamics and structure.

Despite the intriguing patterns seen in our demographic analysis, it is important to consider that Bayesian skyline models, mismatch analysis, and inference of expansion from Fu's F and Tajima's D all assume selective neutrality. Recurrent selective sweeps, in which a neutral locus is linked to a beneficial mutation at a nearby site, can produce similar patterns of genetic diversity and inferred coalescence to those seen after bottleneck and expansion [216]. Our results are therefore also consistent with the alternate scenario that, rather than a series of expansions and contractions due to Pleistocene climate change, we are seeing the effects of a series of selective sweeps in response to changing habitat conditions during that same time period. Differentiation between these alternatives will require the use of more independent loci and analytical methods designed to test for selective sweeps using linkage disequilibrium [217] or "boosting" of summary statistics [218] combined with approximate Bayesian computation [219]. Such an approach allows tests of alternative demographic scenarios that differentially weight isolation and natural selection.

Finally, our analysis of the *S7* intron yielded two surprising results. First, no structure between *S. ghobban* complex clades is seen at this locus, contradicting our mtDNA phylogenetic results (Chapter 2). The mitochondrial genome generally evolves faster than nuclear DNA [220], both due to smaller effective population size and higher rates of replication error [221]. Therefore, in

recently evolved species, reciprocal monophyly could be established in the mtDNA before nuclear genes have sorted completely [159]. It is also possible that the lack of resolution is a spurious result produced by the high levels of variability at the *S7* intron, along with an inability to determine haplotype phase. An earlier study by Bariche and Bernardi [126] that found structure in *S7* between Indian and Pacific *S. ghobban* lends some support to the latter explanation. Sequencing of more nuclear genes and cloning of sequences to determine phase will be necessary to conclusively assess the phylogenetic history of these clades.

Our *S7* analysis also suggests that *Scarus compressus*, the Eastern Pacific endemic, is likely the hybrid offspring of a cross between a female *S. ghobban* parent and a male parent of unknown identity. No difference is seen between *S. compressus* and *S. ghobban* in the matrilineally inherited mtDNA (**Table 11** and Chapter 2 phylogeny) and one *S7* allele from each S. compressus individual clusters with the *S. ghobban* complex. Yet, the second *S7* allele is highly divergent. Only two congeneric species to the *Scarus ghobban* complex are present in the Eastern Pacific: its sister taxa, *Scarus rubroviolaceus,* and the more distantly related *Scarus perrico* [222]. While the N-J tree seems to exclude *S. rubroviolaceus* as the male parent, all samples for *S. rubroviolaceus* used were from the geographically distant Western Australian population. Since this species does show considerable structure across that range [99], further sampling of *S. rubroviolaceus* and *S. perrico* from the Eastern Pacific will be needed to more accurately assess the parentage of *S. compressus.* Reef fish are generally thought to hybridize in times of rarity or upon secondary contact of closely related species [223]. The divergence between the two parental allele types makes the latter explanation unlikely for *S. compressus*. However, demographic analysis has shown the Eastern Pacific population of *S. ghobban* may have been historically more sensitive to climactic disturbance than elsewhere in its range. Contemporarily, conditions in the tropical eastern pacific have been shown to vary widely with the El Nino Southern Oscillation (ENSO) [224]. During these time periods, as habitat quality decreases, EP populations could be under enough stress to drive continuous hybridization between parrotfish species.

From present to past

As would be expected over a wide geographic region, our analysis reveals that different forces influence genetic structure in different parts of the Pacific. Populations in the central part of the range have been heavily shaped by late Pleistocene climactic fluctuations, while eastern Pacific demography and structure are also affected by isolation by distance and possibly by marginal environmental conditions. The severity of the impact of glacial cycles on these populations also explains why the demographic history of these populations only shows Pleistocene and later events, while the clades themselves may have a much older origin [225].

From the contemporary patterns, we begin to see what processes played key roles in the more distant past. First, *Scarus ghobban* (Pacific) shows both dispersal limitation over long distances and a strong response to climate fluctuations. These factors may have been driving forces in the separation of the Pacific from the Indian Ocean clade in the *S. ghobban* complex. While reproductive isolation failed to develop in the late Pleistocene fragmentation within the Pacific, the increased distance to the Indian Ocean could have resulted in a longer period of isolation in which enough genetic differences accrued to prevent remixing of populations upon secondary

contact. Second, the dissimilar demographic histories of the Eastern Pacific population of *S. ghobban* and *Scarus sp.* (WA), suggest that populations on the edge of the range experienced different conditions than central regions, both in terms of connectivity and possibly paleoecology. These differences suggest that periphery isolation and perhaps hybridization may have played an important role in the generation of diversity in this complex.

From present to future

Coral reef ecosystems are currently highly threatened by anthropogenic change [82]. Conservation of these taxa will require planning that not only takes into account present connectivity of populations, but also the processes that generate biodiversity. Our study of the *Scarus ghobban* complex suggests several considerations in management of this and related reef fish species in the wake of changing global conditions. First, populations that have experienced different histories are likely undergoing different evolutionary trajectories and should be managed separately. Second, maintaining connections between populations will be key to their health. Pleistocene history shows that to keep populations healthy throughout the range we need to maintain enough intermediate populations in Central West Pacific to allow for effective dispersal and recruitment. This will require the linking of management plans over international lines. Finally, as keystone reef herbivores, parrotfish are closely ecologically tied to the health of the coral themselves [226] and these taxa will likely track with each other to some extent. Even in the case of severe declines, recovery of fish populations could be possible as we saw after the Pleistocene contractions, but not if there is no reef substrate to which they could return. Therefore, any management plans need to account for multiple taxa with multiple histories. The challenges in implementing any kind of cohesive plan for conservation are huge, but with rapid advances in molecular technology giving us more and more information about the structure and history of marine populations, we move closer to these important goals.

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Appendix 1. Sampling and genotype data for all individual corals.

Homozygous state indicated by one allele. A '?' Indicates that a genotype was obtained at the given locus, but allelic phase was unknown. Individuals in bold were used for the partitioned analysis.

Species	Region	Locality	Catalog #	*Cam*	*MaSC-1*	*Pax-C*
Colpophyllia natans	**Panama**	**Cayos Tigres**	**521**	**76**	**?**	**?**
Colpophyllia natans	Panama	Crawl Cay	824			?
Colpophyllia natans	Panama	Crawl Cay	827		?	
Colpophyllia natans	Panama	Colon	843		?	?
Colpophyllia natans	Panama	Colon	844			35
Colpophyllia natans	Panama	Colon	846			?
Colpophyllia natans	Panama	Colon	847			36
Colpophyllia natans	Panama	Cayo Agua	1012	80		
Colpophyllia natans	Panama	Cayo Agua	1013			38
Colpophyllia natans	Panama	Cayo Agua	1014			35
Colpophyllia natans	Panama	Cayo Agua	1015			35
Colpophyllia natans	**Panama**	**Cayo Agua**	**1017**	**76/81**	**?**	**39**
Colpophyllia natans	Panama	Cayo Agua	1018			36/37
Colpophyllia natans	**Panama**	**Cayo Agua**	**1019**	**77**	**?**	**36**
Colpophyllia natans	Panama	Isla Popa	1022			40
Colpophyllia natans	Panama	Isla Popa	1023	79		
Colpophyllia natans	Panama	Isla Popa	1024			41
Colyphillia natans	Florida	Marathon Key	1344	79/82		
Colyphillia natans	Florida	Marathon Key	1345			
Colyphillia natans	Florida	Marathon Key	1346	76	40	38/42
Colyphillia natans	Florida	Key West	1350	78	?	
Colyphillia natans	Florida	Key Largo	1358	77/78		
Colyphillia natans	Florida	Key Largo	1359	?		
Diploria clivosa	**Panama**	**Zapatilla**	**833**	**1**	**?**	**1**
Diploria clivosa	**Panama**	**Zapatilla**	**834**	**3/5**	**?**	**1/4**
Diploria clivosa	**Panama**	**Zapatilla**	**835**	**1**	**1**	**1**
Diploria clivosa	Panama	Colon	997			1
Diploria clivosa	**Panama**	**Colon**	**998**	**1**	**?**	**1/7**
Diploria clivosa	**Panama**	**Cristobol**	**1043**	**?**	**1**	**1/2**
Diploria clivosa	**Panama**	**Cristobol**	**1044**	**1**	**1**	**?**
Diploria clivosa	Panama	Cristobol	1045	1	1	1
Diploria clivosa	Panama	Cristobol	1046	1	1	1/3
Diploria clivosa	Panama	Cristobol	1047	1	1	1
Diploria clivosa	Panama	Cristobol	1048	1	1	
Diploria clivosa	Panama	Cristobol	1049	1	1	1
Diploria clivosa	**Panama**	**Cristobol**	**1050**	**1/2**	**1/2**	**?**
Diploria clivosa	Florida	Marathon Key	1338	1		1
Diploria clivosa	Florida	Key West	1352	1		
Diploria clivosa	Florida	Key West	1355	1		
Diploria clivosa	Florida	Key West	1356	1/3		
Diploria clivosa	Florida	Key West	1357	1		
Diploria labyrinthiformis	**Panama**	**Cayos Tigres**	**516**	**6/7**	**5/9**	**11/14**
Diploria labyrinthiformis	**Panama**	**Colon**	**853**	**8**	**5/6**	**11**
Diploria labyrinthiformis	**Panama**	**Colon**	**854**	**6/9**	**5**	**10/15**
Diploria labyrinthiformis	**Panama**	**Colon**	**855**	**8/10**	**5**	**8/13**
Diploria labyrinthiformis	**Panama**	**Colon**	**856**	**7/9**	**5**	**11/12**
Diploria labyrinthiformis	**Panama**	**Colon**	**857**	**7/10**	**7/8**	**11/16**
Diploria labyrinthiformis	**Panama**	**Isla Popa**	**1025**	**7/8**	**5/6**	**?**

Diploria labyrinthiformis	**Panama**	**Isla Popa**	**1026**	**8/9**	**5**	**8/9**
Diploria labyrinthiformis	Panama	Isla Popa	1033		?	
Diploria labyrinthiformis	**Panama**	**Colon**	**1038**	**8/10**	**5**	**11/15**
Diploria labyrinthiformis	**Panama**	**Colon**	**1039**	**8/10**	**5**	**10**
Diploria labyrinthiformis	**Panama**	**Colon**	**1040**	**7/8**	**5/6**	**?**
Diploria labyrinthiformis	**Panama**	**Colon**	**1041**	**?**	**5/6**	**?**
Diploria labyrinthiformis	Panama	Colon	1042	8	5	11
Diploria labyrinthiformis	Florida	Marathon Key	1339	7/8		11
Diploria labyrinthiformis	Florida	Marathon Key	1340			11
Diploria labyrinthiformis	Florida	Marathon Key	1341			?
Diploria labyrinthiformis	Florida	Key West	1351	9/12		
Diploria labyrinthiformis	Florida	Key Largo	1365	7/8		
Diploria strigosa	Panama	Cayos Tigres	518	16/17	?	
Diploria strigosa	**Panama**	**Cayos Tigres**	**519**	**15/16**	**?**	**27/28**
Diploria strigosa	**Panama**	**Cayos Tigres**	**520**	**15**	**11/15**	**21/26**
Diploria strigosa	**Panama**	**Colon**	**849**	**15/16**	**11**	**23**
Diploria strigosa	**Panama**	**Colon**	**850**	**18/19**	**11/18**	**24**
Diploria strigosa	**Panama**	**Colon**	**851**	**15**	**13**	**25**
Diploria strigosa	**Panama**	**Colon**	**852**	**16/20**	**13**	**19/29**
Diploria strigosa	**Panama**	**Isla Popa**	**1027**	**15/16**	**10/11**	**19**
Diploria strigosa	Panama	Isla Popa	1028	16/21	12/13	20/21
Diploria strigosa	Panama	Isla Popa	1029	15/22		?
Diploria strigosa	**Panama**	**Isla Popa**	**1030**	**15/23**	**14/?**	**19/21**
Diploria strigosa	Panama	Isla Popa	1031	?	14	22/21
Diploria strigosa	**Panama**	**Isla Popa**	**1032**	**?**	**14**	**19/30**
Diploria strigosa	**Florida**	**Marathon Key**	**1342**	**15/28**	**13/17**	**21**
Diploria strigosa	Florida	Key West	1353			19/21
Diploria strigosa	**Florida**	**Key Largo**	**1363**	**15**	**?**	**19/21**
Diploria Strigosa	Florida	Key Largo	1364			21
Diploria strigosa	Texas	West Flower Gardens	1402	?	11/11	
Diploria strigosa	Texas	West Flower Gardens	1403	?	16/16	
Favia fragum	Panama	Cayos Tigres	513		?	66
Favia fragum	**Panama**	**Cayos Tigres**	**514**	**71**	**35**	**66**
Favia fragum	Panama	Cayos Tigres	515		37	66
Favia fragum	**Panama**	**STRI Point**	603	**71**	**37**	**67**
Favia fragum	Panama	STRI Point	613	71	?	66
Favia fragum	Panama	STRI Point	614	71	?	66
Favia fragum	Panama	STRI Point	615	71	37	66
Favia fragum	Panama	STRI Point	616	71	?	66
Favia fragum	Panama	STRI Point	617	71	37	66
Favia fragum	Panama	STRI Point	618	71	37	66
Favia fragum	Panama	STRI Point	619	71	37	67
Favia fragum	Panama	STRI Point	620	71	37	66
Favia fragum	Panama	Colon	640	71		66
Favia fragum	**Panama**	**Colon**	**641**	**71**	**38**	**66**
Favia fragum	Panama	Colon	642	71	38	66
Favia fragum	Panama	Colon	643	71	38	66
Favia fragum	Panama	Colon	645	71		66
Favia fragum	Panama	Colon	646	71	38	66
Favia fragum	Panama	Colon	647	71	38	66
Favia fragum	Panama	Colon	648	71	38	66
Favia fragum	Panama	Colon	649	71	38	66

Favia fragum	Panama	Colon	650	71	37	66
Favia fragum	Panama	Colon	651	71	38	66
Favia fragum	Panama	Pastores	652	71	?	66
Favia fragum	Panama	Pastores	653	71	?	66
Favia fragum	Panama	Pastores	654	71	37	66
Favia fragum	Panama	Pastores	655	71	37	66
Favia fragum	Panama	Pastores	656	71	?	66
Favia fragum	Panama	Pastores	657	71	37	66
Favia fragum	Panama	Pastores	658	71	37	
Favia fragum	Panama	Pastores	659	71	37	66
Favia fragum	Panama	Pastores	660	71	37	66
Favia fragum	Panama	Pastores	661	71	37	66
Favia fragum	Panama	Valiente	719	71	37	
Favia fragum	Panama	Valiente	720	71	37	66
Favia fragum	Panama	Valiente	721		37	66
Favia fragum	Panama	Valiente	722	71	37	66
Favia fragum	Panama	Valiente	723	71	37	66
Favia fragum	Panama	Valiente	724	71	37	
Favia fragum	Panama	Valiente	725	71	37	
Favia fragum	Panama	Valiente	726	71	37	
Favia fragum	Panama	Valiente	727	71	37	
Favia fragum	Panama	Valiente	728	71	37	
Favia fragum	Panama	Valiente	729	71	37	
Favia fragum	Panama	Zapatilla	748	71	37	
Favia fragum	Panama	Zapatilla	749	71	37	66
Favia fragum	Panama	Zapatilla	750		37	66
Favia fragum	Panama	Zapatilla	751	71		66
Favia fragum	Panama	Zapatilla	752	71	37	66
Favia fragum	Panama	Zapatilla	753		?	66
Favia fragum	Panama	Zapatilla	754	71	37	66
Favia fragum	**Panama**	**Zapatilla**	**755**	**71**	**37**	**69**
Favia fragum	**Panama**	**Zapatilla**	**756**	**71**	**37**	**71**
Favia fragum	Panama	Zapatilla	757	71	37	66
Favia fragum	Panama	Zapatilla	758	71	37	66
Favia fragum	Panama	Zapatilla	759	71	37	66
Favia fragum	Panama	Zapatilla	760	71	37	66
Favia fragum	Panama	Zapatilla	761	71	37	66
Favia fragum	Panama	Zapatilla	762	71	37	66
Favia fragum	Panama	Colon	929	71	37	66
Favia fragum	Panama	Colon	930	71	?	66
Favia fragum	Panama	Colon	931	71	37	66
Favia fragum	Panama	Colon	933	71	37	66
Favia fragum	Panama	Colon	934	71	37	66
Favia fragum	Panama	Colon	935	71	37	66
Favia fragum	Panama	Colon	936	71	37	66
Favia fragum	Panama	Colon	937	71	37	66
Favia fragum	Panama	Colon	938	71	37	66
Favia fragum	Panama	Colon	943	71	37	66
Favia fragum	Panama	Colon	944	71	37	66
Favia fragum	Panama	Colon	945	71	37	66
Favia fragum	Panama	Colon	952	71	37	66
Favia fragum	Panama	Colon	1093	71	?	66

Favia fragum	Panama	Colon	1094	71	?	66
Favia fragum	Panama	Colon	1095	71	?	66
Favia fragum	Panama	Colon	1096	71	?	66
Favia fragum	Panama	Colon	1097	71	37	66
Favia fragum	Panama	Colon	1098	71	37	66
Favia fragum	Panama	Colon	1099	71	37	66
Favia fragum	Panama	Colon	1100	71	37	66
Favia fragum	Panama	Colon	1101	71	37	66
Favia fragum	**Panama**	**Colon**	1103	**71**	**38**	**68**
Favia fragum	Panama	Colon	1109			66
Favia fragum	Panama	Pastores	1187			66
Favia fragum	Florida	Tavernier	1304		37	66
Favia fragum	**Florida**	**Tavernier**	**1305**	**71**	**37**	**66**
Favia fragum	Florida	Tavernier	1306	71	37	66
Favia fragum	Florida	Tavernier	1307	71	37	66
Favia fragum	**Florida**	**Tavernier**	**1308**	**73**	**37**	**66**
Favia fragum	Florida	Tavernier	1309	73	37	66
Favia fragum	Florida	Tavernier	1310	71	37	66
Favia fragum	Florida	Tavernier	1311	73	37	66
Favia fragum	Florida	Tavernier	1312	71	37	66
Favia fragum	Florida	Tavernier	1313	73	37	66
Favia fragum	Florida	Tavernier	1314	73	37	66
Favia fragum	Florida	Tavernier	1315	73	37	66
Favia fragum	Florida	Tavernier	1316	73	37	66
Favia fragum	Florida	Tavernier	1317	71	37	66
Favia fragum	Florida	Tavernier	1318	73	37	66
Favia fragum	Florida	Tavernier	1332	73	37	66
Favia fragum	St. Croix	Green Cay	1367		35	
Favia fragum	St. Croix	Green Cay	1368		37	
Favia fragum	**St. Croix**	**Green Cay**	**1369**	**70**	**36**	**66**
Favia fragum	St. Croix	Green Cay	1370	71		66
Favia fragum	St. Croix	Green Cay	1371		37	
Favia fragum	St. Croix	Green Cay	1372	70	36	66
Favia fragum	St. Croix	Green Cay	1373		35	
Favia fragum	**St. Croix**	**Green Cay**	**1374**	**70**	**37**	**66**
Favia fragum	St. Croix	Green Cay	1375	70		
Favia fragum	St. Croix	Green Cay	1376	71		66
Favia fragum	St. Croix	Green Cay	1377			66
Favia fragum	**St. Croix**	**Green Cay**	**1378**	**71**	**36**	**66**
Favia fragum	St. Croix	Green Cay	1379	70	36	66
Favia fragum	St. Croix	Tague Bay	1380	75		66
Favia fragum	St. Croix	Tague Bay	1381	71		
Favia fragum	**St. Croix**	**Tague Bay**	**1382**	**72**	**37**	**66**
Favia fragum	**St. Croix**	**Tague Bay**	**1383**	**70**	**35**	**67**
Favia fragum	St. Croix	Tague Bay	1384		37	66
Favia fragum	St. Croix	Tague Bay	1385		37	66
Favia fragum	St. Croix	Tague Bay	1386	**70**	**35**	**70**
Favia fragum	St. Croix	Tague Bay	1387	**70**	**35**	**66**
Favia fragum	St. Croix	Tague Bay	1388	70		70
Favia fragum	St. Croix	Tague Bay	1389	70	35	67
Favia fragum	**St. Croix**	**Tague Bay**	**1390**	**74**	**37**	**67**
Favia fragum	St. Croix	Tague Bay	1391		35	66

Favia fragum	St. Croix	Tague Bay	1392	70	35	67
Favia fragum	**St. Croix**	**Tague Bay**	**1393**	**72**	**35**	**66**
Favia fragum	St. Croix	Tague Bay	1394			66
Favia fragum	Florida	Tavernier	1395	**73**	**37**	**66**
Favia fragum	Florida	Tavernier	1396	71	37	66
Favia fragum	Florida	Tavernier	1397	73	37	66
Favia fragum	Florida	Tavernier	1398	73		66
Favia fragum	Florida	Tavernier	1399	71	37	66
Favia fragum	Florida	Tavernier	1400	71	37	66
Favia fragum	Panama	STRI Point	1401	71	?	66
Manicina areolata	**Panama**	**Colon**	**982**	**51**	**23/?**	**47/52**
Manicina areolata	Panama	Colon	983	47	24	
Manicina areolata	**Panama**	**Colon**	**984**	**45/51**	**25**	**50/51**
Manicina areolata	**Panama**	**Colon**	**985**	?	**23/26**	**51/52**
Manicina areolata	**Panama**	**Colon**	**986**	**?**	**26**	**47**
Manicina areolata	**Panama**	**Colon**	**987**	**50**	**25**	**49/56**
Manicina areolata	**Panama**	**Colon**	**988**	**51**	**23/24**	**52/65**
Manicina areolata	**Panama**	**Colon**	**989**	**?**	**26**	**57**
Manicina areolata	**Panama**	**Colon**	**990**	**54/55**	**26**	**52/61**
Manicina areolata	**Panama**	**Colon**	**991**	**45**	**23/?**	**51/64**
Manicina areolata	**Panama**	**Colon**	**992**	**51**	**23/26**	**58**
Manicina areolata	**Panama**	**Colon**	**993**	**51/55**	**26**	**47/60**
Manicina areolata	Florida	Tavernier	1319	?		45
Manicina areolata	**Florida**	**Tavernier**	**1320**	**45**	**27**	**45**
Manicina areolata	**Florida**	**Tavernier**	**1321**	**45**	**20/?**	**?**
Manicina areolata	**Florida**	**Tavernier**	**1322**	**45**	**20**	**?**
Manicina areolata	**Florida**	**Tavernier**	**1323**	**45**	**?**	**45**
Manicina areolata	**Florida**	**Tavernier**	**1324**	**45/46**	**?**	**?**
Manicina areolata	**Florida**	**Tavernier**	**1325**	**45**	**?**	**45**
Manicina areolata	Florida	Tavernier	1326		21	46
Manicina areolata	**Florida**	**Tavernier**	**1327**	**45**	**20**	**45**
Manicina areolata	**Florida**	**Tavernier**	**1328**	**?**	**23**	**?**
Manicina areolata	Florida	Tavernier	1329	45	20	45
Manicina areolata	**Florida**	**Tavernier**	**1330**	**45**	**21**	**?**
Manicina areolata	Florida	Matecombe	1333			45
Manicina areolata	**Florida**	**Matecombe**	**1334**	**45**	**22**	**45**
Manicina areolata	**Florida**	**Matecombe**	**1335**	**45**	**?**	**46**
Manicina areolata	Florida	Matecombe	1336	45	23	?
Manicina areolata	**Florida**	**Matecombe**	**1337**	**45**	**?**	**45**
Manicina mayori	**Panama**	**Cayo Agua**	**1020**	**?**	**31**	**56**
Manicina mayori	**Panama**	**Isla Popa**	**1034**	**?**	**25/31**	**56**
Manicina mayori	**Panama**	**Isla Popa**	**1035**	**?**	**26/34**	**56**
Manicina mayori	**Panama**	**Isla Popa**	**1036**	**50/51**	**26**	**56**
Manicina mayori	**Panama**	**Isla Popa**	**1037**	**51/61**	**23/26**	**56**

Appendix 2. Coral Fossil Stratigraphy Data

Compiled first (FO) and last occurrence (LO) data, references and notes for all Caribbean fossil faviid species**.**

Species	FO (Ma)	LO (Ma)	FO notes	FO Ref.	LO notes	LO Ref
Colpophyllia amaranthus	2.9-3.2	living	Costa Rica - Buenos Aires	[1, 2]		
Colpophyllia breviserialis	3-5.6	living	Curacao - Ridges	[3]		
Colpophyllia duncani	40.4-48.6	36.6-40	Jamaica - Yellow Lm	[4]	Panama - Gatuncillo	[5]
Colpophyllia elegans	36.6-40	36.6-40	Panama - Gatuncillo	[5]	Panama - Gatuncillo	[5]
Colpophyllia mexicanum	20.4-23	20.4-23	Mexico - Chiapas (LaQuinta)	[6]	Mexico - Chiapas (LaQuinta)	[6]
Colpophyllia natans	5.1-5.3	living	Dom. Rep. - cana5	[7]		
Colpophyllia sp.A	3-5.6	living	Curacao - Ridges	[3]		[3]
Colpophyllia willoughbiensis	36.6-40	23-28.4	Panama - Gatuncillo	[5]	Antigua, Chiapas, Puerto Rico	[6, 8, 9]
Diploria antiguensis	27.3-29.4	27.3-29.4	Antigua	[9, 10]	Antigua	[9, 10]
Diploria bowersi	6.3-6.5	6.3-6.5	California - Imperial Formation	[11, 12]	California - Imperial Formation	[11, 12]
Diploria clivosa	4.6-5.9	living	Curacao - SalinaTop	[3]		
Diploria dumblei	23-28.4	23-28.4	Mexico - Tamaulipas (San Rafael)	[13]	Mexico - Tamaulipas (San Rafael)	[13]
Diploria labyrinthiformis	2.9-3.2	living	Costa Rica - Buenos Aires	[1, 2]		
Diploria portoriciensis	23-28.4	23-28.4	Puerto Rico - Lares	[8, 13]	Puerto Rico - Lares	[8, 13]
Diploria sarasotana	3-3.5	1.5-2.5	Florida - Pinecrest	[14, 15]	Panama - lagruta	[16]
Diploria strigosa	4.6-5.9	living	Curacao - Salina Top	[3]		
Diploria zambensis	11.7-16.9	1.5-2.5	Trinidad - Tamana	[10, 17]	Panama - lagruta	[16]

Favia aff. domincensis	13.1-17.3	13.1-17.3	Dom. Rep. - yaque3	[15,18,19]	Dom. Rep. - yaque3	[15,18,19]
Favia dominicensis	20.4-23	13.1-17.3	Mexico - Chiapas (LaQuinta)	[6]	Dom. Rep. - yaque2	[15,18,19]
Favia favioides	65.5-43	65.5-43	Barbados - Scotland	[20, 21]	Barbados - Scotland	[20, 21]
Favia fragum	3-5.6	living	Curacao - Ridges	[3]		
Favia gravida	none	living				
Favia gregoryi	40.4-48.6	40.4-48.6	Jamaica - Yellow Lm	[4]	Jamaica - Yellow Lm	[4]
Favia macdonaldi	27.3-29.4	23-28.4	Antigua	[9, 10]	Puerto Rico - Lares	[8, 13]
Favia maoadentrensis	6.25-6.4	3.5-3.6	Dom. Rep. - cana1	[7]	Dom. Rep. - cana11	[7]
Favia vokesae	6.25-6.4	1.8-1.9	Dom. Rep. - cana1	[7]	Bahamas - U3	[22]
Favia weisbordi	40.4-48.6	36.6-40	Cuba - Camaguey (Nuevitas)	[23]	Panama - Gatuncillo	[5]
Hadrophyllia saundersi	5.5-5.6	1-1.8	Dom. Rep. - gurabo6	[7]	Jamaica - hopegate2	[24]
Manicina aff. mayori	6.25-6.45	5.1-5.3	Dom. Rep. - Bellaco	[7]	Dom. Rep. - cana5	[7]
Manicina areolata	3-5.6	living	Curacao - Ridges	[3]		
Manicina geisteri	6.25-6.45	3-3.3	Dom. Rep. - Bellaco	[7]	Jamaica - bowden2	[24]
Manicina grandis	6.25-6.4	3-3.3	Dom. Rep. - cana1	[7]	Jamaica - bowden2	[24]
Manicina jungi	5.75-5.9	3-3.3	Dom. Rep. - gurabo3	[7]	Jamaica - bowden2	[24]
Manicina mayori	2.9-3.2	living	Costa Rica - Buenos Aires	[1, 2]		
Manicina pliocenica	5.1-5.3	1.8-2	Dom. Rep. - cana5	[7]	Jamaica - oldpera2	[24]
Manicina puntgordensis	4.6-5.9	1.5-1.9	Curacao - Salina Top	[3]	Costa Rica - Lomas del Mar	[1, 2]

Manicina sp.E	7.8-10.3	2-2.6	Curacao - Salina Base	[3]	Curacao - SeaCliff	[3]
Thysanus corbicula	15-18	1.8-2	Florida - Chipola	[15, 25]	Jamaica - oldpera2	[24]
Thysanus excentricus	6.15-6.25	2-3	Dom. Rep. - gurabo1	[7]	Jamaica - oldpera1	[24]
Thysanus navicula	5.6-5.75	1.5-2.5	Dom. Rep. - gurabo5	[7]	Panama - grcreek	[16]
Thysanus sp. A	3.2-3.5	1.9-2.9	Costa Rica - Qchoco	[1, 2]	Costa Rica - Lomas del Mar	[1, 2]

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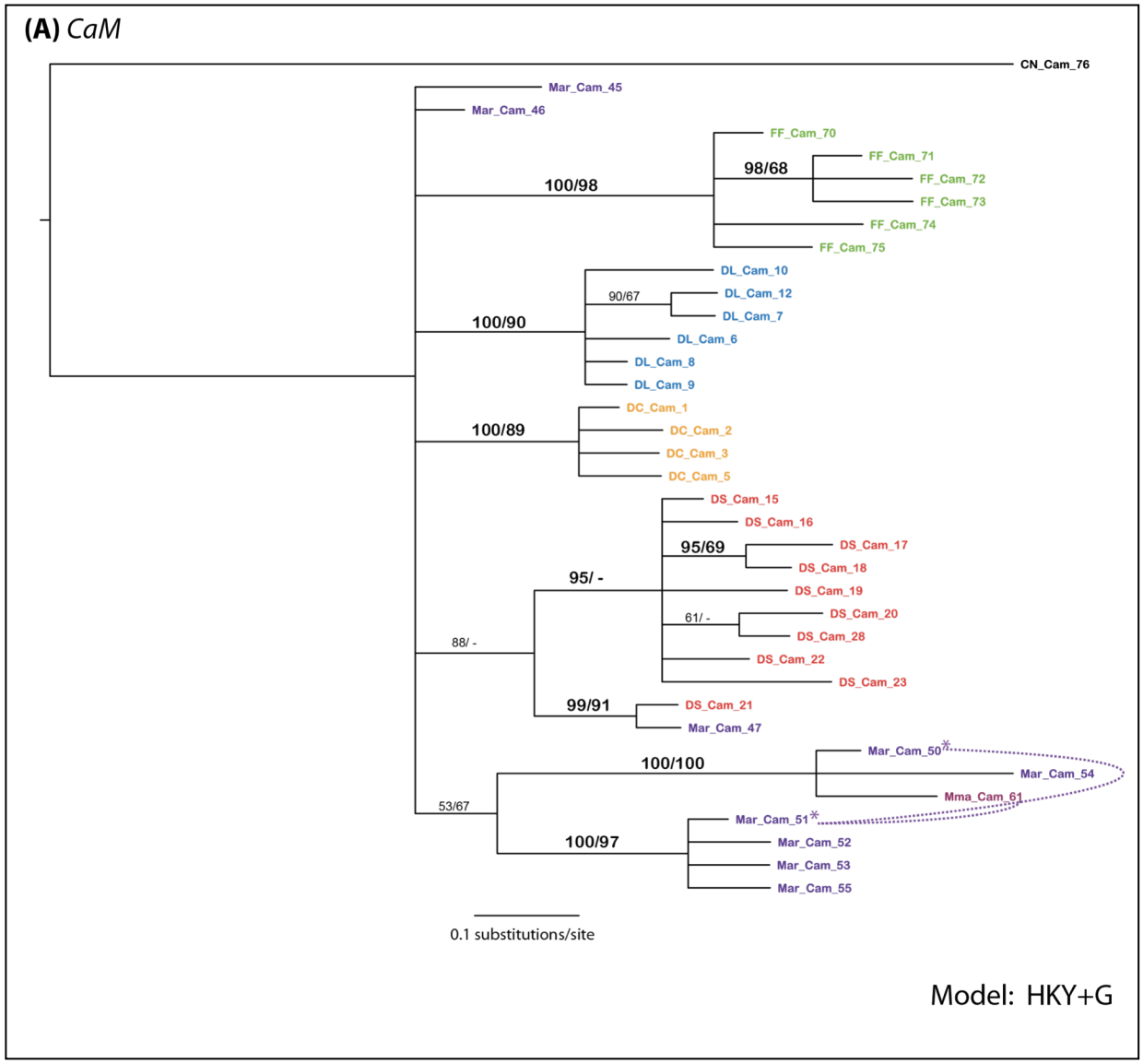
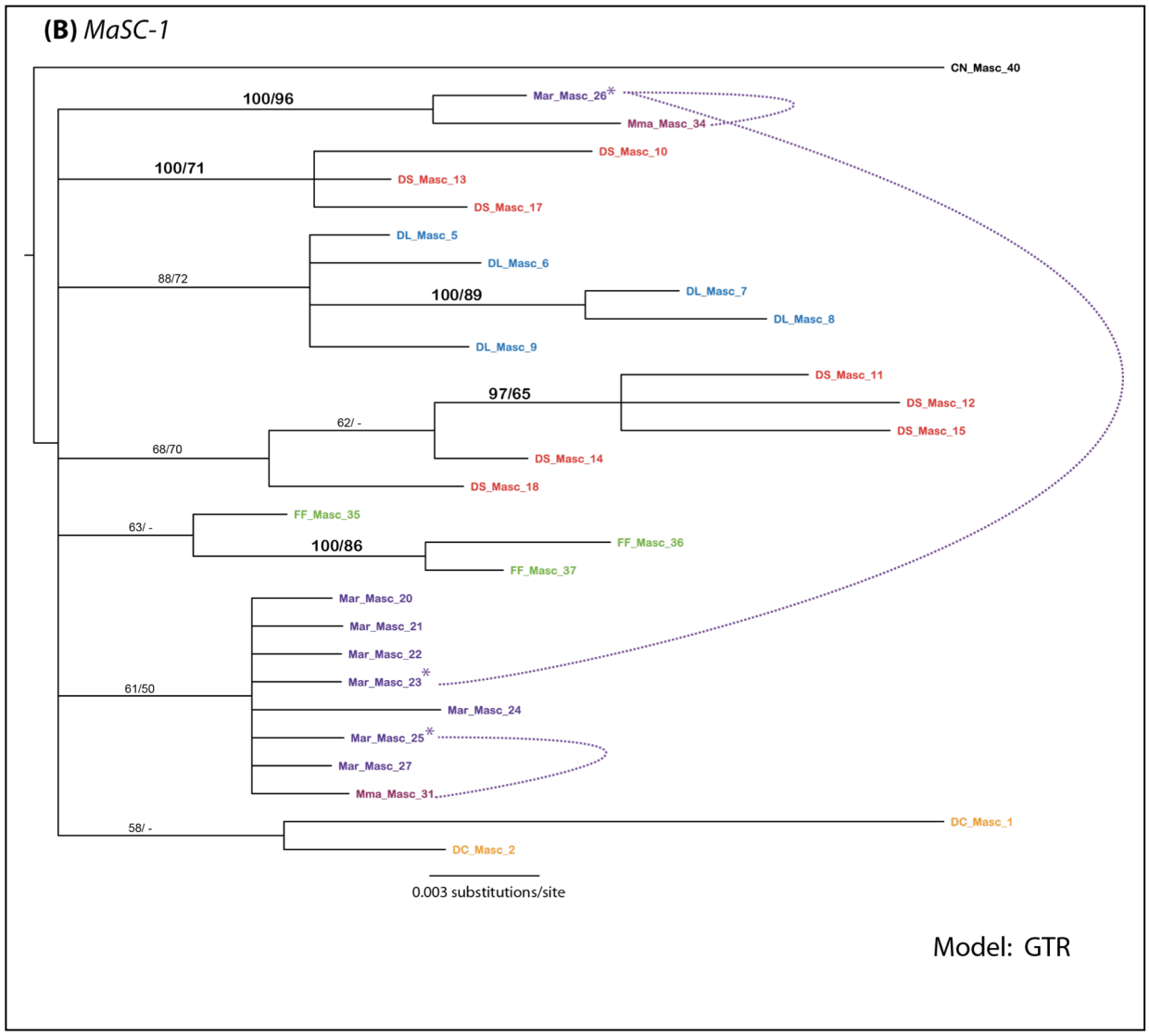
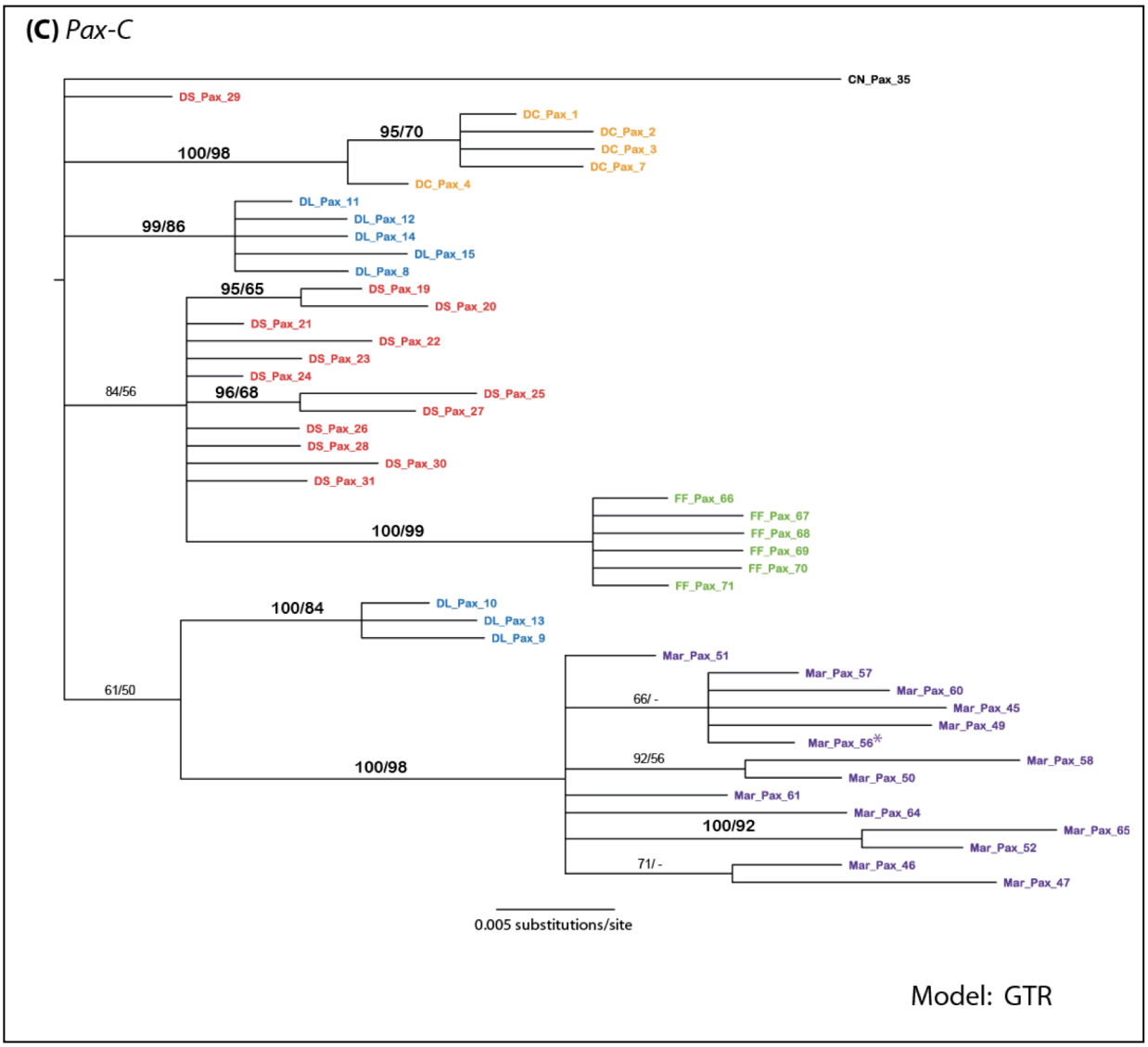
Appendix 3. Coral gene genealogies for (A) *CaM*, (B) *MaSC-1*, and (C) *Pax-C*.
Alleles are designated with a species abbreviation, locus, and allele number. Allele numbers can be cross-referenced with **Appendix 1** for genotype information. Labels indicate Bayesian posterior probabilities for adjacent node. All trees produced in MrBayes v3.1 (generations= 5,000,000, nruns=2, nchains=4.) Dotted lines between *Manicina* alleles indicate genotypes of individuals that contain alleles shared between the two *Manicina* species.




(B) MaSC-1
CN_Masc_40
100/96
Mar_Masc_26*
Mma_Masc_34
DS_Masc_10
100/71
DS_Masc_13
DS_Masc_17
DL_Masc_5
DL_Masc_6
88/72
100/89
DL_Masc_7
DL_Masc_8
DL_Masc_9
DS_Masc_11
97/65
DS_Masc_12
62/ -
DS_Masc_15
68/70
DS_Masc_14
DS_Masc_18
FF_Masc_35
63/ -
100/86
FF_Masc_36
FF_Masc_37
Mar_Masc_20
Mar_Masc_21
Mar_Masc_22
Mar_Masc_23*
61/50
Mar_Masc_24
Mar_Masc_25*
Mar_Masc_27
Mma_Masc_31
58/ -
DC_Masc_1
DC_Masc_2
0.003 substitutions/site
Model: GTR


(C) Pax-C
CN_Pax_35
DS_Pax_29
DC_Pax_1
DC_Pax_2
DC_Pax_3
DC_Pax_7
DC_Pax_4
95/70
100/98
DL_Pax_11
DL_Pax_12
DL_Pax_14
DL_Pax_15
DL_Pax_8
99/86
95/65
DS_Pax_19
DS_Pax_20
DS_Pax_21
DS_Pax_22
DS_Pax_23
DS_Pax_24
84/56
96/68
DS_Pax_25
DS_Pax_27
DS_Pax_26
DS_Pax_28
DS_Pax_30
DS_Pax_31
FF_Pax_66
FF_Pax_67
FF_Pax_68
FF_Pax_69
FF_Pax_70
FF_Pax_71
100/99
100/84
DL_Pax_10
DL_Pax_13
DL_Pax_9
61/50
Mar_Pax_51
Mar_Pax_57
Mar_Pax_60
66/ -
Mar_Pax_45
Mar_Pax_49
Mar_Pax_56*
92/56
Mar_Pax_58
Mar_Pax_50
100/98
Mar_Pax_61
Mar_Pax_64
100/92
Mar_Pax_65
Mar_Pax_52
71/ -
Mar_Pax_46
Mar_Pax_47
0.005 substitutions/site
Model: GTR

Appendix 4. Parrotfish gene trees for A) *16S* and B) the control region

Trees generated in MrBayes v3.1. Maximum likelihood trees generated in RaxML had non-conflicting clade topologies. Numbers on branches are Bayesian posterior/ML bootstrap support. Clades corresponding to those identified in **Figure 1** are indicated by color**. A)** Branches are *16S* haplotypes. Frequencies > 1 for a haplotype are indicated in parentheses. **B)** For the control region locus**,** structure above the clade level was collapsed. Colored triangles represent all haplotypes within those clades. Black clades represent haplotypes from the outgroup, *Scarus rubroviolaceus.*