SPECIATION AND DIVERSIFICATION IN THE INDO-WEST PACIFIC: INFERENCES FROM THE MOLECULAR SYSTEMATICS OF REEF-ASSOCIATED CRUSTACEANS

By

MARIA CELIA DEFRANCE MALAY

A DISSERTATION PRESENTED TO THE GRADUATE SCHOOL
OF THE UNIVERSITY OF FLORIDA IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

UNIVERSITY OF FLORIDA

2010

To my extraordinary grandparents, Marie Leprêtre Defrance, Léonce Defrance, Paula Carolina Santos Malay, and Armando de Jesus Malay

ACKNOWLEDGMENTS

Acknowledgments for Chapter 2: We are deeply grateful to Joseph Poupin for his advice, encouragement, specimens, and for kind permission to use the images and information posted on his excellent website on *Calcinus*. Yoyo Rahayu kindly lent us a paratype specimen, and Regis Cléva facilitated access to the MNHN collections. We thank Patsy McLaughlin and Joseph Poupin for help with some of the identifications. We wish to acknowledge the many people who contributed specimens to this study: D. Eernise, M. Frey, J. Hooper, L. Kirkendale, J. O'Donnell, B. Olaivar, C. Pittman, J. Poupin, L. Rocha, W. Sterrer, and P. Wirtz. Photo credits: J. Poupin (*C. californiensis, explorator, imperialis, inconspicuus, mclaughlinae, orchidae, pascuensis, aff. sirius*), L. Albenga (*albengai, aff. albengai*), J. Hoover (*hakahau*), W. Sterrer (*verrilli*), C. d'Udekem d'Acoz (*tubularis*), P. Wirtz (*talismani*), J. Okuno (*anani*), and D.L. Felder (*obscurus*). All other photos are by G. Paulay and M. Malay. We are also grateful to Mike Hellberg and 3 anonymous reviewers for their helpful comments on the manuscript.

Acknowledgements for Chapters 3 and 4: I am grateful to Bill Newman, Arnold Ross (1936-2006), Roger Portell, Yair Achituv, Andrew Hosie, Fabio Pitombo, and Benny Chan for patiently answering my questions on barnacle taxonomy, morphology, and evolution. For contributing samples, I thank Patricia Cabezas-Padilla, Camilla Floros, François Michonneau, Seabird McKeon, Lisa Kirkendale, John Starmer, Gustav Paulay, Helmut Zibrowius, Yair Achituv, and Andrew Hosie. For contributing sequences and for logistical support in sequencing, I thank Marcos Pérez-Losada, Maegan Finley, Chris Meyer, and Maggie Fagan. I also wish to acknowledge help from Rebecca Kimball, Ed Braun, Mike Miyamoto, and Fan Qiu for advice on topological tests, and

Pam Soltis for advice on how to deal with missing data. For logistical and/or administrative support in field work, I am very grateful to Annete Juinio-Meñez, Perry Aliño, Edgardo Gomez, Wilfredo Licuanan, the University of the Philippines Marine Science Institute, Keryea Soong, Minghui Chen, Michel Claereboudt, Esther Emmanuelli, Vincent Hollevout, the École Pratique des Hautes Études (EPHE), the Centre des Recherches Insulaires et Observatoire de l'Environnement (CRIOBE) in Mo'orea, the Gump Station in Mo'orea, Mireille Guillaume, Henrich Bruggemann, the Université de la Réunion, Philippe Bouchet, Victor Bonito, Miri Tabaiwalu and everyone in the village of Votua, Gerald McCormack, and Teina and Jackie Rongo. Jada-Simone White generously allowed me to use her larval culturing setup at the Gump Station in Mo'orea. I thank my two student volunteers, Karina Concha and Austin Perlmutter, for all their hard working help in dissecting barnacles and setting up PCRs.

I am grateful to the governments of the Republic of the Philippines, the Gouvernement de la Polynésie Française, the Republic of the Fiji Islands, the Government of the Cook Islands, Taiwan/the Republic of China, the Sultanate of Oman, Terres Australes et Antartiques Françaises (TAAF), and the Republic of France, for granting permission to sample in their territories.

I am exceedingly grateful for financial support I have received through the years from the University of Florida Alumni Fellowship, the Department of Zoology (now Biology), the Brian Riewald Memorial Fund Research Grant, the Leila and William Brayfield Scholarship in Invertebrate Paleontology, the Lerner-Gray Grant at the American Museum of Natural History, the FACE program (Ocean Bridges: A Florida-France Training & Research Cooperative in Coral Reef Conservation and Biodiversity),

the Kuroshio Research Fellowship at the National Sun Yat-Sen University of Taiwan, the American Microscopical Society, the Crustacean Society, and the Carcinological Society of Japan.

The Florida Museum of Natural History and the Department of Biology at the University of Florida have been excellent academic homes throughout my PhD. I am most grateful to the team at the Invertebrate Zoology lab at FLMNH, especially Mandy Bemis, Sarah McPherson, Jenna Moore, Chelsey Campbell, Kim Kemppanion, and John Slapcinsky, for all the curatorial support. I also acknowledge Chris Meyer, Lisa Kirkendale, Jessica Light, François Michonneau, and Nat Evans for advice on phylogenetic programs and analyses; and Matt Gitzendanner and the University of Florida Phyloinformatics Cluster for High Performance Computing in the Life Sciences for computational support. My committee members David Reed, Pam Soltis, Rebecca Kimball, and Mike Miyamoto were there to help me throughout the long process of the PhD, and I wish to thank them for their support. I most gratefully acknowledge my awesome PhD supervisor, Gustav Paulay, for all the intellectual, financial, logistical, emotional support through the years, and for getting me started on these projects in the first place. None of these studies would have been possible without him.

Lastly, I thank my family; my Mama and Papa, my sister Pat and brother Ali, my cousins near and far, aunts and uncles, and my dear departed grandparents; all of whom I love so much and who love me equally back. My fiancé Jimmy Huang gave me all-out love and emotional and moral support through these years, and kept me honest and grounded on reality. Jimmy's family, especially Ping, have been wonderfully

supportive too. Lastly I thank all my friends, old and new (there are too many to mention!) for their love.

TABLE OF CONTENTS

page

LIST OF TABLES

LIST OF FIGURES

Abstract of Dissertation Presented to the Graduate School
of the University of Florida in Partial Fulfillment of the
Requirements for the Degree of Doctor of Philosophy

SPECIATION AND DIVERSIFICATION IN THE INDO-WEST PACIFIC: INFERENCES FROM THE MOLECULAR SYSTEMATICS OF REEF-ASSOCIATED CRUSTACEANS

By

Maria Celia Defrance Malay

August 2010

Chair: Gustav Paulay
Major: Zoology

This study integrates molecular phylogenetics, morphological analysis, and biogeographic data to study speciation, phylogeography, and systematics of two reef-associated crustacean taxa: *Calcinus* hermit crabs and coral dwelling barnacles (Pyrgomatidae). *Calcinus* is a charismatic genus of colorful hermit crabs that is most diverse on coral reefs. On the other hand, the family Pyrgomatidae is a little-known yet remarkably specialized group of coral-dwelling barnacles. I found that speciation is primarily allopatric in both systems; however, the location of speciation events differed greatly. In *Calcinus*, most recent speciation events clustered in remote oceanic archipelagos, while in the pyrgomatid barnacle 'genus' *Trevathana s.l.* recent speciation occurred between the West Indian Ocean and the West Pacific, and between the West Pacific and Central Pacific. From the *Calcinus* data, I found that allopatric sister-species pairs are younger than sympatric ones, suggesting that >2 million years are needed for sympatry to develop. From the *Trevathana s.l.* data, I determined that both geographic isolation and host-switching drive diversification. Younger sister-species are geographically structured while older divergences are structured by host, implying that geographic isolation is a relatively fast driver of speciation while host switching occurs at

a much slower rate. Differences in speciation patterns, together with the fact that neither hermit crabs nor coral barnacles exhibited a biodiversity hotspot in the Indo-Malayan 'coral triangle', imply that patterns of marine speciation result from very diverse processes that probably cannot be explained by only a single model of marine speciation.

In *Calcinus*, differences in color patterns have evolved very rapidly between sister-species; while in *Trevathana s.l.*, sister-species exhibit differences in skeletal morphology. In both systems molecular phylogenetic data were able to uncover numerous species that are new to science; further proof of the utility of molecular data in species discovery. In addition, in a separate study on the systematics of the coral barnacles (Pyrgomatidae), I found that several morphological characters traditionally used in pyrgomatid taxonomy are phylogenetically homoplasious, such that the systematics of the entire family needs to be revisited. Major groupings within the Pyrgomatidae *sensu stricto* did not correspond to the traditional subfamilies, and one supposed 'outgroup' to the pyrgomatids actually fell within the clade. Traditionally, taxonomic limits in the pyrgomatids were defined on the basis of skeletal fusion; however, results from my study indicate that other phenotypic characters related to control of coral overgrowth may be more important in delineating subgroups within the Pyrgomatidae.

CHAPTER 1
INTRODUCTION

Introduction to Indo-West Pacific Marine Biogeography

The tropical Indo-West Pacific (IWP) is the single largest marine biogeographic region in the world, stretching half the globe from the eastern coast of Africa to the central Pacific. IWP biodiversity peaks in the comparatively small triangle formed by Indonesia, the Philippines, and New Guinea. This well-known 'Indo-Malayan biodiversity triangle' or 'coral triangle' is the center of species richness for a wide variety of marine taxa, including reef fishes, corals, gastropods, and crustaceans. Species- and genus-level diversity progressively diminishes as one moves away from this central region (reviewed in Paulay 1997).

The location of the Indo-Malayan biodiversity hotspot is correlated with the amount of habitat area: the Indo-Malayan region has very extensive tracts of shallow-water reef habitat, which may in turn sustain elevated levels of biodiversity (Bellwood and Hughes 2001, Karlson et al. 2004, Bellwood et al. 2005). Yet despite its fame, there is still no consensus on the question of how the hotspot originated. Why is species diversity so high in the Indo-Malayan triangle, and what mechanism(s) of species origination gave rise to current-day patterns of species distribution in the region? Aside from species-area relationships and mid-domain effects (e.g., Bellwood and Hughes 2001, Bellwood et al. 2005), many hypotheses incorporating ideas on species origination have been proposed (see review in Rosen 1988). While they all propose allopatric or peripatric models of speciation, these theories fall into 3 main categories that disagree about whether new species form (a) in remote islands at the peripheries of the IWP (e.g., Center of Accumulation theory); (b) in the complex sub-basins of the Indo-Malayan

triangle (Center of Origin theory); or (c) whether high diversity is simply due to the overlap of two separate biogeographic provinces, the Western Pacific and Indian Ocean (Center of Overlap theory; reviewed in Palumbi 1997, Paulay 1997).

The issue is made more confusing by the fact that these 3 main hypotheses can each be further subdivided into dispersal- and vicariance-based explanations, which differ in their predictions on the timing of speciation across different taxa. For instance, one variant of the Center of Overlap theory may predict that faunas on the W Pacific and Indian Ocean diverged simultaneously because of a single major vicariant event, while another variant of the theory would argue that the pattern resulted from an accumulation of chance migrations across a current-driven dispersal barrier during periods of low sea levels (Paulay 1997). A Center of Overlap scenario could also have arisen as a result of divergence across an oceanic- vs. continental reef ecological gradient. Similarly, the Center of Accumulation scenario could have resulted from rare founder speciation events in remote islands of the central Pacific, or from the fragmentation of previously more extensive species ranges followed by reinvasion of the central IWP by the newly-formed species (Paulay and Meyer 2002). Finally, some proponents of the Center of Origin theory posit synchronous patterns of speciation in different faunal groups during periods of reduced connectivity in the Indo-Malayan triangle (e.g., during Plio-Pleistocene low sea level stands; Barber and Bellwood 2005). However, it is also possible that some *in situ* speciation events occurred as a result of the escalating complexity of ecological interactions in the Indo-Malayan diversity hotspot (i.e., existing diversity begetting even more diversity; Emerson and Kolm 2005).

Evaluating the different hypotheses is very difficult because different evolutionary scenarios may result in identical biogeographic patterns (Palumbi 1997, Paulay 1997, Kirkendale and Meyer 2004). Patterns of speciation are overlaid by subsequent migrations and local extinctions, such that present-day species distributions alone may not be sufficient to establish where speciation took place. While some studies make the assumption that present-day centers of species ranges are equivalent to the region of species origination (Mora et al. 2003), such assumptions may very likely be erroneous (e.g., Barber and Bellwood 2005). For instance, while the remote central Pacific islands have surprisingly high numbers of endemic fore-reef species, fossils of these species from the Indo-Malayan region prove that this pattern is a result of reliction rather than speciation, presumably because the relict species are unable to cope with escalating ecological pressures in the more diverse regions of the IWP. Conversely, the depauperate inner-reef fauna in the central Pacific has resulted from localized extinctions of lagoonar specialists from remote Pacific atolls as a result of fluctuating sea levels (Paulay 1990, 1996). These conclusions were made on the basis of detailed examinations of bivalve fossil records; however, good records are lacking for many IWP marine taxa, thus the use of fossils is taxonomically limited. Another important consideration is that similar distributional patterns are not necessarily the result of identical speciation mechanisms - different processes may give rise to concordant patterns in different taxa. Neither can one rule out the possibility that more than one speciation mechanism is operating within a single taxonomic group (Meyer et al. 2005, Paulay 1997, Palumbi 1997, Randall 1998). Thus it does not seem likely that marine biogeographers will soon agree on a single unambigous explanation for the existence of

the Indo-Malayan biodiversity hotspot; and I believe there should be no reason to expect a single, simple explanation for this complex pattern.

Molecular phylogenetics and phylogeography are powerful tools for inferring the evolutionary histories of species and clades. The application of phylogenetic techniques to marine systems has already caused major shifts in our understanding of marine biogeography, despite the fact that the entire field of phylogeography is a little over two decades old (Avise et al. 1987). The overall picture emerging from studies of IWP phylogeography is that both species diversity and population-level diversity are much greater than previously thought (e.g., Knowlton 1993, Knowlton 2000, Meyer et al. 2005). Moreover, populations and species exhibit more geographic structuring than previously thought (e.g., Williams and Reid 2004, Meyer et al. 2005, Barber et al. 2006). These new realizations challenge the old paradigm that marine biota comprise “open” systems (compared to terrestrial environments), with very few barriers to dispersal and thus less opportunities for allopatric diversification. In reality, we are only now starting to appreciate how diverse, and how geographically structured, the IWP truly is.

Aside from revising our understanding of diversification, what other insights have molecular phylogenetics provided? Can we now use phylogenetic information to infer the mechanisms by which IWP diversity was generated? Different studies do not agree on a single mechanism of diversification. There have been studies claiming support for the Center of Overlap theory (e.g., in organisms such as starfish, snapping shrimp, coconut crabs, patelloid limpets; Williams 2000, Williams et al. 2002, Lavery et al. 1996, Kirkendale and Meyer 2004); yet there are also studies that favor a Center of Origin (in reef fish, Briggs 1999 and Mora et al. 2003) or a Center of Accumulation scenario (e.g.,

in *Echinometra oblonga* sea urchins, Landry et al. 2003; and reef fish, Bellwood and Wainwright 2002, Hughes et al. 2002). In several cases, a combination of different processes have been proposed to operate in a single taxonomic system (in turbinid gastropods, Meyer et al. 2005; wrasses, Barber and Bellwood 2005; and cowries, Meyer 2003). Different geological, evolutionary, and ecological processes have interacted in complex manners to produce the patterns we observe today. Thus, the best way to understand what has transpired (and is transpiring) in the IWP is by applying the time-honored comparative method: studying the phylogenetics and phylogeography of a wide variety of taxonomic groups, and hopefully gaining an understanding of the overall picture of diversification in the IWP through an inductive process.

Phylogenetic studies in the IWP have so far focused mainly on geographic isolation (i.e., allopatry through dispersal and vicariance) as a mechanism of species origination; few have given more than a passing mention to the possible influence of ecological adaptations on the process of speciation (see Paulay 1996 for discussion of adaptations to different reef habitats; Landry et al. 2003 on presence or absence of congeneric species), and very few have directly investigated such a hypothesis (but see Duffy 1996; Faucci et al. 2007). Divergent ecological pressures can give rise to speciation in populations connected by gene flow, or through reinforcement of reproductive barriers following secondary overlap of allopatric populations (Rice and Hostert 1993, Coyne and Orr 2004). There is also theoretical (Diekmann and Doebeli 1999, Gavrilets 2000) and limited empirical evidence supporting speciation in wholly sympatric situations (e.g., Schliewen et al. 1994, Eastman and McCune 2000, Turelli et

al. 2001, Via 2001). In particular, *in situ* speciation through host-race formation has been receiving much attention recently (e.g., Feder et al. 1994, Abrahamson et al. 2002, Emelianov et al. 2004). Such a speciation mechanism would be expected in commensals, parasites, and mutualists with limited mobility and highly evolved interactions with specific hosts – ecological guilds that are particularly species-rich in coral reefs, as in all hyperdiverse communities. However, as of present there have been extremely few phylogenetic studies of obligately symbiotic reef-associated organisms.

Overall Goals

For my dissertation, I studied the phylogenetics and phylogeography of two representative reef-associated crustacean taxa: hermit crabs in the genus *Calcinus*, and coral-dwelling barnacles in the family Pyrgomatidae. One objective was to evaluate what new data can tell us about existing biogeographic hypotheses about diversification in the IWP. In the case of the Pyrgomatidae, another major objective was to re-evaluate the systematics of the family and analyze the evolution of character traits, including traits related to adaptation to a symbiotic coral-dwelling lifestyle. Towards these goals, in Chapter 2 I discuss global speciation patterns in my first study system, the hermit crab *Calcinus*. In Chapter 3 I tackle the systematics of the pyrgomatid coral-dwelling barnacles. Then in Chapter 4 I focus on one of the phylogenetically well supported clades, the *Trevathana sensu lato* group, and analyze the patterns of distribution and speciation of all members of the clade. These patterns are summarized and synthesized in the last chapter, Chapter 5.

The overarching goal of this work is to contribute to ongoing efforts to understand mechanisms of species diversification in the sea, with a special emphasis on Indo-West

Pacific coral reef crustacean taxa. Just as research on phytophagous insects has helped terrestrial biologists to understand the diversity of tropical rainforests (reviewed in Coyne and Orr 2004), I employed my work on crustacean speciation to make inferences on the origins of species in coral reefs, the “rainforests of the sea”.

CHAPTER 2
PERIPATRIC SPECIATION DRIVES DIVERSIFICATION AND DISTRIBUTIONAL PATTERN OF REEF HERMIT CRABS (DECAPODA: DIOGENIDAE: *Calcinus*)

Introduction

The marine tropics can be divided into four broad regions defined by largely endemic biotas: the East Atlantic (EA; West African tropical coastline and offshore islands, Mediterranean), West Atlantic (WA; East American tropical coastline, Caribbean, and offshore islands including Bermuda), East Pacific (EP; West American tropical coastline to offshore islands including Galapagos & Clipperton), and Indo-West Pacific (IWP; from East Africa to Easter Island) regions (Ekman 1953, Briggs 1974). Diversity is lowest in the EA and highest, by about an order of magnitude, in the IWP (Paulay 1997). Further patterns are evident within the vast IWP, where marine biodiversity peaks in the Indo-Malayan triangle bounded by the Philippines, Indonesia, and New Guinea and decreases in a striking manner toward the central Pacific (Stehli & Wells 1971). Much early work focused on these striking spatial patterns and attempted to find single or at least dominant processes to explain them. While numerous hypotheses have been proposed to explain observed spatial patterns in reef diversity (often focused on the high diversity of Indo-Malaya) (Rosen 1988), three have been most emphasized: the center of origin, center of overlap, and center of accumulation hypotheses. These have attributed the Indo-Malayan diversity peak to *in situ* diversification, overlap in the ranges of Indian and Pacific basin species, and accumulation of species originating elsewhere, respectively. Increasing documentation of variation in spatial diversity patterns as well as modes of speciation have, however, led to the realization that multiple processes must be involved in generating the observed patterns of diversity (Palumbi 1997, Paulay 1997, Williams 2007).

Molecular phylogenetics provides a powerful tool for understanding the origins of observed patterns of species richness. By analyzing diverse taxa we can address questions of diversification from a quantitative, mechanistic perspective: what is the relative importance of different modes of speciation in generating species richness and spatial patterns of diversity? In the past, analyses of spatial patterns of diversity were largely inferential (i.e., top-down): by examining biota-level patterns, researchers inferred likely processes of diversification. In contrast a quantitative phylogenetic approach provides a mechanistic (i.e., bottom-up) perspective: by documenting numerous speciation events, we can investigate how regional-level diversity patterns arise. Such an approach necessitates thorough spatial and taxonomic sampling, so that most or all speciation events in a clade are identified and characterized. Thorough taxonomic coverage is also one of the most important factors in recovering an accurate tree topology, as shown by both empirical and simulation studies (e.g., Graybeal 1998, Zwickl & Hillis 2002, Soltis et al. 2004).

The objectives of this study are to pursue a comprehensive phylogenetic and biogeographic analysis of the reef-associated hermit crab genus *Calcinus*, to: (1) determine spatial and temporal patterns of diversification, (2) evaluate the relative importance of different modes of speciation and how they gave rise to observed patterns of diversity and distribution, and (3) assess the roles of color and ecology in diversification. *Calcinus* are diverse, medium-sized, diurnal, conspicuous, colorful, and abundant diogenids. All known species are tropical or subtropical, most live on coral reefs, and several are facultative coral associates, frequently encountered within branching corals. The genus is circumtropical, with 41 recognized species (Table 2-1):

33 in the IWP and 2-4 in each of the remaining regions (EA, WA, EP). There is substantial variation in ranges among IWP species, with some extending from East Africa to Hawaii, while others are known from single islands or archipelagos (Table 2-1).

Calcinus species are most readily identified from, and a few can only be reliably differentiated based on, their color pattern (e.g., Poupin and McLaughlin 1998, Poupin and Lemaitre 2003). Partly because colors fade in preserved specimens, coloration has been underutilized in crustacean taxonomy in the past. However, more effective field methods, including SCUBA, field photography, increased sampling, and appreciation of color differences, have substantially improved our knowledge of *Calcinus* in recent decades. Alpha taxonomy and geographic distributions are now comparatively well documented (Poupin 2003), making *Calcinus* an excellent focus for evolutionary and biogeographic study.

We constructed a phylogeny of *Calcinus* based on most described species in the genus, including samples from multiple locations spanning the known ranges of most widespread species. Sequence data provide evidence for substantial cryptic diversity in the genus. In some species color pattern appears to have evolved so rapidly that sister species with strikingly different color patterns are only slightly or not genetically differentiated. Most young sister species pairs have allopatric distributions, indicating that allopatric speciation is the main or only mechanism for diversification. Isolation on remote island groups appears to be the most common cause of speciation.

Materials and Methods

Specimens

We sampled 37 of the 43 nominal species of *Calcinus* and 9 additional, undescribed phylogenetic species recognizable on the basis of sequence data (Table 2-

1). The species not sequenced are *Calcinus urabaensis*, known from a single specimen in Colombia, *Calcinus kurozumii*, known only from a single collection on Pagan Island (Marianas), *C. tropidomanus*, known from a single collection in Somalia, and *C. sirius* from Australia. We also did not sample *"Calcinus" paradoxus*, a species based on a single specimen collected in much deeper (500 m) water than any other *Calcinus*, whose generic assignment even its author questioned (Bouvier, 1922); nor the dwarf species *C. revi*, suspected to be the juvenile of more common *Calcinus* species (Poupin, pers. comm.). Much of the material was collected by reef walking, snorkeling, or scuba-diving, fixed in 75-95% ethanol, and deposited in the Invertebrate Collections of the Florida Museum of Natural History, University of Florida (UF; Table 2-1). Additional specimens were borrowed from other institutions (Table 2-1). Whenever possible living animals were photographed to record color pattern. We identified specimens using Poupin's (2003) interactive taxonomic key, the primary taxonomic literature, and in consultation with J. Poupin and P. McLaughlin. Data on geographic ranges and ecology of species were compiled from the taxonomic literature, Poupin's (2003) website on the genus, the UF specimen database, and the authors' field observations. The diogenid hermit crab genera *Ciliopagurus* and *Dardanus* were chosen as the closest outgroup taxa based on a phylogenetic analysis (not shown) of a larger set of hermit crab genera.

Samples for sequencing were selected to span as much of the geographic range of each species as available material permitted (Table 2-1, Figs. 2-4 to 2-15). We collected DNA sequence data from 150 operational taxonomic units (OTUs). All but 1 (*C. talismani*) of the 150 OTUs were sequenced for the cytochrome oxidase I (COI)

mitochondrial gene fragment. We generated phylogenetic trees for the COI-only dataset, and on the basis of these trees we selected a subset of 96 OTUs for further sequencing of 16S rDNA and Histone 3 (H3) genes. The 96-OTU subset was comprised of only the 2 genetically most divergent individuals in each species or genetically distinct putative new species. Thus the full 150-OTU taxon set was utilized for constructing the COI-only tree while a "pruned" subset of 96 OTUs was used for constructing individual gene trees and a concatenated 3-gene tree. The ILD test for data combinability (see below) was also performed on the 96-OTU subset. Lastly, molecular clock analyses (see below) were performed on a further reduced 50-OTU taxon subset, in order to keep computations manageable.

Molecular Methods

DNA was extracted from muscle tissue using DNAzol and proteinase K following the protocol given in Meyer 2003. Sequence data were collected for two mitochondrial DNA markers (COI and 16S) and one nuclear marker (H3). Average length of the amplified fragments and PCR primers used are as follows: COI: ~645 base pairs (bp), primers dgLCO (5'-GGT CAA CAA ATC ATA AAG AYA TYG G-3') and dgHCO (5'-TAA ACT TCA GGG TGA CCA AAR AAY CA-3'; Meyer 2003). 16S: ~550 bp, primers 16SAR (5'-CGC CTG TTT ATC AAA AAC AT-3') and 16SBR (5'-GCC GGT CTG AAC TCA GAT CAC GT-3'; Palumbi 1996). H3: ~350 bp, primers H3af (5'-ATG GCT CGT ACC AAG CAG ACV GC-3') and H3ar (5'-ATA TCC TTR GGC ATR ATR GTG AC-3'; Colgan et al. 1998). PCR thermocycler profiles for COI and 16S were as in Meyer (2003), while the PCR profile for H3 followed Pérez-Losada et al. (2004). PCR products were either (a) cleaned using Wizard PCR Preps (Promega) and sequenced using the ABI Big Dye protocol and a Perkin-Elmer ABI Automated Sequencer; or (b) cleaned

using the exo-sap cleanup protocol and sequenced at the high-throughput sequencing facility of the University of Florida's Interdisciplinary Center for Biotechnology research (ICBR) in a 96-well format using BigDyeTerminator cycle sequencing reactions, employing an ABI-3730-XL for electrophoresis. Initially, mitochondrial DNA sequencing was done along both directions of a DNA fragment, and as our confidence in base calls increased in later stages, only 1 strand was sequenced (unless base ambiguities were noted, in which case the 2nd direction was sequenced). Histone 3 sequencing was always done on both directions.

Sequence Analysis

Chromatograms of the sequences were manually checked and edited using the software Sequencher ver. 4.2 (Gene Codes). Sequence alignment was done by eye using Se-Al v2.0a11 (Rambaut, http://tree.bio.ed.ac.uk/software/seal/). Sequences are available in GenBank (accession nos. FJ620149-FJ620493, EF683559-EF683561). We also included COI data from GenBank for *Calcinus obscurus* (AF436039). In all analyses, all sites were weighted equally, characters were unordered, and gaps were treated as missing data.

We used two approaches to decide whether or not to pool the 3 gene fragments into a single analysis. Firstly, we used the incongruence length difference (ILD) test (Farris *et al*. 1995), a parsimony-based statistical test of data combinability commonly employed in phylogenetic studies. We used PAUP* ver. 4.0b10 (Swofford 2002) to perform the ILD test simultaneously for the 3 data partitions. No significant incongruences were noted among the 3 gene trees. However, the usefulness of the ILD test for evaluating data combinability has been called into question (e.g., Yoder *et al*. 2001, Barker & Lutzoni 2002). To address these concerns, and to explore our data

further, we also visually compared Bayesian tree topologies resulting from independent searches for each of the 3 gene regions. A visual comparison of the gene trees showed no conflict with each other nor with the 3-gene concatenated analysis (data not shown). Based on this evidence, we decided that a combined analysis was appropriate.

We determined the simplest model of evolution that best fit our COI-only dataset as well as our 3-gene dataset using the Akaike Information Criterion (AIC) as implemented by the program Modeltest 3.6 (Posada & Crandall 1998). Phylogenetic relationships were estimated using maximum likelihood (ML), maximum parsimony (MP), and Bayesian statistics (BS). Parsimony analyses were done using PAUP, ML analyses were implemented using both PAUP and GARLI v0.951-1 (Zwickl 2006, http://www.bio.utexas.edu/faculty/antisense/garli/Garli.html), while Bayesian analyses were implemented using MrBayes v3.1.2 (Huelsenbeck & Ronquist 2001; Ronquist & Huelsenbeck 2003). In the MP and ML analyses using PAUP, heuristic searches started with random addition of taxa replicated 10 times using the tree-bisection-reconnection (TBR) branch-swapping algorithm. Branch support in the MP analyses was estimated by bootstrap support values, calculated as above with 1,000 (for the 3-gene tree) or 200 (for the COI tree) replicates. ML branch support values were not calculated using PAUP due to computational constraints. In the ML analyses using GARLI, we used random starting trees and performed 5-7 independent runs to obtain the best tree. Branch support values were estimated in GARLI using 2,200 and 1,300 bootstrap replicates for the COI-only and 3-gene datasets, respectively. In the Bayesian analyses, we ran 2 independent chains for 1 million generations each; each chain was sampled every 100 generations. The MCMC runs reached stationarity in 60k generations or less. We

discarded the initial 25% of the trees as the burn-in phase. Bayesian posterior probabilities were calculated based on the remaining 75% of the trees.

We calculated pairwise COI genetic distances for each sister species pair identified in our phylogenetic trees using PAUP. We used Kimura's (1980) K2P distance metric to facilitate comparison with earlier studies.

Molecular Clock Analysis

We used BEAST 1.4.8 (Drummond & Rambaut 2007) to estimate divergence times of *Calcinus* sister taxa. BEAST employs a Bayesian statistical framework to simultaneously estimate phylogenetic trees and divergence times, thus it is capable of integrating uncertainty in topology in the divergence date estimation. BEAST also allows for incorporation of uncertainty in calibration points. We did a partitioned analysis for all 3 genes (3-nucleotide codon partitions for the coding regions COI and H3, and 1 partition for the non-coding 16S, for a total of 7 partitions) using an uncorrelated log-normal relaxed clock. For each data partition we specified a GTR+I+G model of sequence evolution. We asked BEAST to estimate the time to most recent common ancestor (TMRCA) of each pair of sister species in the phylogeny. We used a Yule tree prior, specified a UPGMA starting tree, and did 2 independent runs of $1x10^7$ generations each. We sampled the posterior distributions of the dates being estimated by sampling the runs every 1,000 generations, after removing the first 10% of the MCMC chain as the burn-in period. Convergence of the results was checked by loading the posterior distributions into the program Tracer. The analysis was calibrated by specifying a prior on the date of divergence of the transisthmian species pair *Calcinus tibicen* and *C. explorator*. The timing of vicariance of transisthmian sister species varies substantially among taxa, with many falling around the time of final severing of the land bridge

around 3.1 my (Coates & Obando 1996), but others are older (cf. Knowlton & Weigt 1998, Lessios 2008). As a preliminary approximation we set a prior with a lognormal distribution with a mean of 3.5 my and a standard deviation of 1.0 (this was approximated by specifying a lognormal mean of 1.21352716 and a lognormal standard deviation of 0.28012786). In our analysis a normal distribution was not appropriate because a transisthmian divergence time of zero would then have a positive probability, which is an unrealistic prior and would cause calculation problems (AJ Drummond, personal communication). Using a lognormal prior ensures that a divergence time of zero is excluded from the analysis, while allowing for a TMRCA substantially older than 3.5 my.

Analysis of Speciation and Biogeography

Our analysis of speciation patterns focuses on recognized species as well as previously unrecognized, but genetically distinct Evolutionary Significant Units (ESUs; *sensu* Moritz 1994). ESUs are defined as reciprocally monophyletic populations for the locus investigated (here 16S and COI mtDNA; H3 was not considered due to low levels of interspecific divergence), that have at least one other independent, defining attribute such as distinct color pattern, structural morphology, distribution, or reciprocal monophyly in another, independent marker. ESUs satisfy the phylogenetic species concept, and are clades with an evolutionary history separate from other ESUs. Some ESUs are as morphologically and genetically distinctive as recognized species; conversely a few recognized species are not reciprocally monophyletic in mtDNA (see below). ESUs are thus species-level units which, unlike biological species, can be defined in allopatric as well as in sympatric settings without experimental tests of interbreeding.

We call the divergence of ESUs from each other Evolutionary Significant Events (ESEs). ESEs are to speciation what ESUs are to species: they are objectively defined diversification events that give rise to ESUs. To quantify the relative importance of different modes of diversification, we enumerated all identifiable ESEs that have given rise to at least one individual ESU (or recognized species). That is, we considered ESEs that have given rise to either two separate ESUs, or led to the separation of one ESU from a clade that subsequently further diversified.

Species occurrence records were mapped in ArcGIS, and species ranges inferred by drawing a polygon around bordering record points. Species were considered allopatric when they had separate ranges; such ranges may end on adjacent islands, but are then separated by open ocean. Species ranges that truly abut, or overlap for <10% of the range of the narrower sister taxon's range, were termed parapatric.

Diversity contour maps were generated from these data by superimposing inferred distributional range of each species. Such diversity contour maps can be biased in that 1) diversity in interior areas can be overestimated when species are actually absent from there but inferred to occur because of peripheral records, and 2) lack of sampling of marginal occurrence will lead to an underestimation of marginal range, but lack of sampling of central occurrence will not lead to an underestimation of central range. As a second method for estimating local diversity, we also assembled species lists for relatively well studied areas and have indicated the number of species known from these on the contour maps. The latter method is prone to the biases of geographically varied sampling methods and efforts.

Results

Sequence Attributes

The COI region sequenced was 609 base pairs (bp) long, with 368 invariable and 238 parsimony-informative sites. Mean base frequencies were: 0.25A, 0.17C, 0.23G, 0.35T, showing an A-T bias of 60%. The model that best fit these data was a GTR+I+G model. The 16S gene fragment contained some regions that could not be confidently aligned across all taxa. We tested the importance of these hypervariable regions by running separate analyses with and without them. Inclusion or exclusion of hypervariable regions did not result in substantial topological differences, thus they were included in the final analyses. The 16S gene fragment was 459 bp long, with 276 bp invariable and 125 bp parsimony-informative sites, and mean base frequencies of 0.32A, 0.18C, 0.13G, 0.36T (A-T bias 68%). The H3 gene fragment was 336 bp long, with 279 bp invariable and 53 bp parsimony informative sites, and mean base frequencies of 0.19A, 0.34C, 0.28G, 0.19T (A-T bias 38%). The best fit models were GTR+I+G for COI, 16S, and the combined 3-gene set, and GTR-I for H3.

Phylogeny Reconstruction and Species Boundaries

The 3 methods of phylogenetic analyses used (MP, ML, BS) gave congruent results, and the topologies generated from the 3-gene and COI-only datasets were likewise congruent (fig. 2-1 a & b). Bootstrap values were higher in the 3-gene trees (particularly at the deeper nodes), as expected. We thus used the 3-gene trees to identify supra-specific clades within *Calcinus*. Ten strongly supported clades were identifiable within the genus. We defined strong phylogenetic support as >60% bootstrap values in the MP and ML trees and >90% posterior probabilities in the BS trees (see clades I-X in fig. 2-1a; the sole exception to our criterion for defining clades

was clade IV, which was supported by both ML and BS analysis, but had no bootstrap support under MP; nonetheless, this grouping was recovered in all methods of analysis used). Relationships of ESUs within these clades were generally well resolved, but the relationships of the clades to each other was generally poorly resolved. Thus these clades served as the basic units for our analyses of speciation patterns.

Because the COI analyses (fig. 2-1b) covered more individuals from more geographic locations, these were used to delineate species and ESUs. Analyses revealed 9 ESUs (22% of the sampled IWP fauna) that do not correspond to previously described species. Eight of these 9 are allopatrically divergent populations of recognized species, while one is co-distributed with its sister-species but has a non-overlapping depth range. Three traditionally recognized species were not reciprocally monophyletic: *Calcinus minutus, C. nitidus*, and *C. rosaceus* are interdigitated in a mostly unresolved species complex. All other nominal species for which multiple individuals were sequenced were recovered as monophyletic units with high bootstrap/posterior probability support values. Thus most named species fulfilled the ESU criterion and phylogenetic species concept (Wheeler and Meier 2000).

Excluding the *C. minutus* complex (see Discussion), intra-specific K2P distances ranged from 0-6% (1.3+/-1.0%), with only one outlier with K2P>4%. Pairwise, interspecific K2P distances within clades ranged from 4-25% (K2P) (Fig. 2-2a). Thus there was no barcoding gap (Hebert et al. 2003, Meyer & Paulay 2005), but also little overlap between intra-specific and inter-specific distances. Including the *C. minutus* complex creates a much larger overlap between intra- and interspecific differences (Fig. 2-2b).

Out of 267 pairwise intraspecific K2P distance comparisons, 10% had values >2.7%. These were within *C. argus, C. pulcher s.s., C. haigae,* and *C. anani*. These species appear to exhibit substantial geographic structuring across their range (see fig. 2-1b): *C. argus* appears to have divergent populations in Reunion Island and Hawaii; *C. pulcher* has a distinct population in the Philippines; *C. haigae* shows divergence in the Tuamotus; and *C. anani* from the Marquesas and Papua New Guinea appear genetically differentiated. Interestingly, we observed distinct color morphs for a *C. anani* specimen from the Philippines (not sequenced) and for juvenile *C. haigae* from the Tuamotus (illustrated in Poupin 2003). However, the distinct groupings were not consistently supported across all methods of analysis and small sample sizes also limit our ability to further investigate differentiation within these species.

Molecular Clock Results

The molecular clock analyses showed that allopatrically-distributed sister species pairs were significantly younger than sympatric sister species (allopatric sister species: mean=2.0 my, range=0.4-6.3 my; sympatric sister species: mean=5.8 my, range=2.2-10.2 my; fig. 2-3; $p>0.05$, t-test) and all young (<2.5 my) divergences were among allopatric sister taxa. There is considerable spread in TMRCAs, particularly for sympatric sister species pairs. There is no temporal gap dividing the ages of strictly allopatric species from sister species that have broadly overlapping geographic ranges.

Distribution of *Calcinus* Species

Our surveys led to numerous new geographic records and substantial improvement in the documentation of the distribution of *Calcinus* species (see http://www.flmnh.ufl.edu/scripts/dbs/malacol_pub.asp for source of records). Figures 2-4 to 2-15 show the presently known geographic range of each species.

Diversity Patterns in *Calcinus*

The species richness of *Calcinus* is highest in the oceanic Pacific, and does not peak in the Indo-Malayan triangle (Fig. 2-16). Both projected and known diversity peak in the Mariana and Tuamotu Islands, at the NW and SE Oceania. Sixteen species have been recorded from the Marianas and 15 from the Tuamotus. Diversity in the Indo-Malayan triangle is substantially lower, with 8 species recorded from the Philippines, 9 from Indonesia, and 10 from all of New Guinea. Only 12 species have been recorded from the entire Indo-Malayan archipelago, compared with 21 species from SE Polynesia.

Speciation

Twenty-four ESEs were identified in the IWP and 4 in other regions. Six of the IWP ESEs separate sympatric sister taxa, others are geographically structured. Of the 22 geographically structured ESEs 20 separate allopatric sister taxa and two split parapatric sisters with narrow areas of distributional overlap. Sympatric sister taxa are generally separated by deeper genetic distances than allopatric or parapatric taxa (see above). All 21 pairs of allopatric or parapatric sister taxa appear to have adjacent ranges as far as current sampling can document.

Geographically-structured ESEs span the globe, but cluster in some areas (Fig. 2-17); most fall in areas previously recognized as potentially important in speciation, as evidenced by a high proportion of endemics. Within the IWP, four ESEs separate ESUs in Hawaii. Three ESEs each separate ESUs between the tropical and subtropical S Pacific, and across the Indian Ocean (although the exact locations of the latter separations are poorly constrained because of sparse sampling in the Indian Ocean). Two ESEs each separate ESUs in the Marquesas, SE Polynesia, and at subtropical

latitudes across Australia. Single ESEs separate ESUs in Arabia and Easter Island (Fig. 2-17). Outside the IWP, 2 ESEs separate ESUs between adjacent regions (EP – WA, and Bermuda – EA), and two separate sister taxa within the EP: along the central American coast, and between Clipperton Island and the central American coast.

Discussion

Species Boundaries

Although there is general correspondence between described species and genetically-defined ESUs, 22% of the taxa examined were not concordant. Three of the named species were not reciprocally monophyletic, while 9 ESUs represent previously undescribed (and mostly unrecognized) forms. Lack of correspondence between named morphological species and ESUs delineated with genetic methods is commonly encountered in genetic surveys of well studied taxa, and can have multiple causes (Funk & Omland 2003, Meyer & Paulay 2005).

Calcinus minutus, C. nitidus, and *C. rosaceus* failed to sort into monophyletic units. Most specimens in this complex form a tight cluster (K2P<2%, with all species combinations represented at K2P≥0.5%), except for one *C. rosaceus* from Reunion in the Mascarene Islands (K2P=10%). These three nominal species have allopatric, abutting ranges, are very similar in structural morphology, with *C. minutus* and *C. nitidus*, in particular, nearly impossible to distinguish except by color (Fig. 2-6 ; Morgan 1991, Poupin and McLaughlin 1998, Poupin 2003). Several factors can cause species-level non-monophyly (Funk & Omland 2003). First, there may be insufficient differences in the marker used to differentiate species. We consider this unlikely because mitochondrial gene regions used cleanly resolve other *Calcinus* species. Second, ancestral polymorphisms may have been retained, because of a slow rate of evolution

or recent speciation. While there is no evidence for a slow-down in the rate of evolution in this lineage, species divergence may have been so recent that ancestral haplotypes have not had sufficient time to sort into monophyletic clades. The virtual lack of morphological differentiation other than color between *C. nitidus* and *C. minutus* is suggestive of recent divergence. Third, mitochondrial haplotypes could have introgressed across species boundaries. The occurrence of a divergent sequence in one *C. rosaceus* specimen, sister to all others in the complex, suggests that introgression is a plausible explanation. Structurally as well as in color pattern *C. rosaceus* is closest to *C. haigae*, the sister taxon to this complex (Poupin 2003; Asakura and Tachikawa 2003). This suggests that the divergent sequence may represent the original *C. rosaceus* genotype, which has largely been replaced by a sweep of *C. minutus* haplotypes. Independent markers could provide a test of this hypothesis. The H3 nuclear sequences show slow rates of evolution and are not variable across this complex, and appear to lack the power to resolve this problem. The distinct color patterns are likely under genetic, nuclear control, thus they represent an independent marker; however, color may be under selection and could thus have evolved more rapidly than potentially neutral mitochondrial markers (see below). Future work with other markers is needed to resolve the status of these species.

In contrast, six previously-recognized species show marked differentiation into 2 or 3 ESUs each. In two (*C. albengai, C. elegans*), the differentiated ESUs show conspicuous and previously noted color forms that have not been taxonomically recognized (Poupin & Lemaitre 2003, Haig & McLaughlin 1984). In the six others (*C. hazletti, C. vachoni* X2*, C. latens* X2, and *C. pulcher*), no color differences were noted

during collection, but are evident in four of the five for which live images were taken. Color differences could not be discerned only in photographs of *C. hazletti* ESUs in Micronesia and the Hawaiian Islands (although color polymorphism has been reported in this species in Japan; Asakura 2004). No images were available for the SE Polynesian *C. vachoni* ESU.

Much of the incongruence between morphology-based species and genetic ESUs result from changing taxonomic traditions, and reflect a lack of systematic revision. Historically, carcinologists hesitated to describe species distinguished solely by color pattern; thus, the strikingly-distinctive Hawaiian color form of *C. elegans* has not been named (Haig & McLaughlin 1984). More recently, workers have tended to recognize such structurally-similar color forms, such as the Marquesan endemic *C. hakahau*, as distinct species (Poupin & McLaughlin 1998). A well-executed revision should rectify alternate species concepts currently in use.

Species boundaries can be defined based on a variety of criteria and characters (e.g., Wheeler & Meier 2000). When taxa are sympatric and co-occurring, species limits are usually straightforward; however, species limits are more subjective for allopatric taxa not subject to potential interbreeding. Genetics, color pattern, structural morphology, and/or geography can all inform taxonomic delineations. We defined ESUs as reciprocally monophyletic taxa in a genetic marker, that are also distinguishable by at least one additional independent character. Three recognized species do not meet this definition, as they are not demonstrably reciprocally monophyletic with the genetic markers used. However, these three forms do have other, independent characters that

correlate: color pattern and geography, implying that they are on independent evolutionary trajectories.

Evolution of Color Patterns

The general correspondence between color forms and ESUs indicates that color patterns are almost always reliable and sufficient for differentiating *Calcinus* species. Color pattern-level differentiation between morphologically-similar sister species is common among Crustacea (e.g., Knowlton 1993; Macpherson and Machordom 2000; Ravago and Juinio-Meñez 2003) as well as in other taxa, such as reef fish (e.g. McMillan et al. 1999, Bowen et al. 2006; reviewed in Knowlton 1993). Among reef fishes, there have also been documented cases of closely-related species that differ strikingly in color and yet show few (if any) structural differences and are not reciprocally monophyletic at the mitochondrial level (e.g., McMillan et al. 1999, Bowen et al. 2006). If the lack of monophyly is not due to introgression, these findings imply that the rate of color pattern evolution can outpace mitochondrial sequence divergence, which suggests that differentiation in coloration may be driven by selection.

In *Calcinus*, coloration is so conspicuous and varied that it can be reasonably assumed to serve a purpose and thus be acted upon by natural selection. For example, it has been demonstrated that the size of the white chelar patch in *Calcinus laevimanus* influences success in interspecific agonistic encounters (Dunham 1978). It is likely that other *Calcinus* species utilize color patterns in adaptive ways. If coloration is involved in conspecific interactions, then strong selection on these visual cues could result in the rapid color evolution, and genetically isolated populations may diverge in these cues over relatively short periods of time. Moreover, if color patterns are used for species recognition, then divergence in color may lead to the development of reproductive

isolation barriers and thus speciation. Color patterns have been shown to serve in species recognition and mate choice in other marine groups, including fiddler crabs (Detto et al. 2006) and fish (McMillan et al. 1999, Puebla et al. 2007, Seehausen et al. 2008).

Geography of Speciation

Speciation appears to be largely or exclusively allopatric in *Calcinus*, as in most animals (Coyne & Orr 2004), and allopatric separation of sister taxa is retained for more than 2 million years (fig. 2-3). The narrowly allopatric to parapatric ranges of all young sister taxa imply either that the geography of the original speciation event has been maintained in these taxa and there has been little post-speciational changes in distribution, or that such changes were reciprocal; i.e., expansion in the range of one ESU was associated with contraction in the range of the other. Although the latter hypothesis is difficult to falsify, the former is much more parsimonious and also more likely because boundaries between sister ESUs tend to fall at recognized zones of transition associated with major dispersal or ecological barriers.

Narrowly allopatric ranges also imply that localized endemics are the result of speciation rather than reliction. Endemism can be high on peripheral island groups, but endemics can result from either local (typically peripatric) speciation (e.g., neoendemics) or reliction (paleoendemics; see Ladd 1960, Stehli & Wells 1971, Newman & Foster 1987). Reliction refers to the survival of formerly widespread taxa often in remote, biologically less intense, “safe” places (Vermeij 1987). As relicts are generally older taxa that have undergone substantial reduction in their range, they are not expected to be narrowly allopatric with their sister taxa, but to show disjunct or

sympatric ranges. None (except *C. albengai*) of the insular endemics are sympatric or have disjunct distribution with their sister taxa.

The location of geographic speciation events span the globe, but are not randomly distributed. Peripatric speciation on remote islands is most prevalent, while speciation events within the Indo-Malayan triangle or between the Indian and Pacific ocean basins are absent / rare. Speciation across ecological gradients, such as latitude and depth, and between the four tropical regions is also evident.

Isolation on remote islands and archipelagos appears to be the most prevalent cause of speciation in *Calcinus*: 60% of the ESEs have resulted in at least one of the sister taxa becoming restricted to a remote island group. A similar pattern of predominantly peripatric speciation has been found in *Thalassoma* wrasses (Bernardi et al. 2004). Insular endemics have evolved in Hawaii (4), Marquesas (2), SE Polynesia (2), Mascarenes (2), Easter Island (1), Clipperton (1), and Bermuda (1); an additional endemic putatively assigned to the genus (*C. paradoxus*; see above) in the Azores has not been sampled. These are some of the most remote islands in the world, renowned for high endemism (e.g., Briggs 1974, Randall 1998), thus it is not surprising that they also host endemic *Calcinus*. Among fish, the highest levels of endemism in the IWP are encountered in peripheral areas: Easter, Hawaiian, Marquesas, Mascarene islands and the Red Sea (4.4-23% endemics; Randall 1998, Allen 2007). Among these remote island groups, we have sampled the Hawaiian Islands most thoroughly, and have sequenced 9 of 10 species known from there. Of the 9, four (44%) are endemic: *C. laurentae,* and endemic ESUs of the widespread *C. hazletti, C. latens,* and *C. elegans*. *Calcinus isabellae* (known from two Hawaiian records) remains untested. In the

Marquesas, two of four recorded species are endemic, while one of three from Easter Island are. However, the status of widespread species in the Marquesas and Easter remain to be genetically evaluated.

Four clades appear to have given rise to multiple peripheral endemics. In two, peripatric speciation from a widespread form appears to have been the source of these endemics, while in two others, insular endemics appear to have undergone local diversification within a basin. *Calcinus elegans* and *C. latens*, both ranging from East Africa to Polynesia, gave rise to four peripatric endemics: three on remote islands and one on the Arabian peninsula. The wide-ranging ESU is a terminal branch in both clades, implying it was the source of successive peripheral endemics. In contrast the insular sister-endemics *C. laurentae-C. hakahau-C. gouti* and Hawaiian-Micronesian ESUs of *C. hazletti* represent lineages diversifying within the central Pacific, and are only more distantly related to widespread taxa (*C. lineapropodus-pulcher* and *C. minutus*-complex, respectively).

Calcinus also includes several species restricted to relatively cool, subtropical or moderately deep (100-300m) waters. The following species are known only from subtropical latitudes in the southern IWP: *C. sirius, C. aff. sirius, C. albengai, C. aff. albengai-shallow, C. dapsiles* (clade X), *C. spicatus, C. pascuensis* (clade V) and *C. imperialis, C. vanninii* (clade VII). The origin of these taxa is predominantly by *in situ* diversification within the subtropics. Similar latitude-based niche conservatism has been demonstrated in gastropods (Frey & Vermeij 2008, Williams et al. 2003, Williams 2007). Only the last clade has a relatively recent and thus readily identifiable origin in the tropics, sister to the parapatric *C. isabellae*.

All deep water species investigated (*C. anani, C. albengai*-deep, *C.* aff. *sirius*) are members of clade X, suggesting that invasion of deep reef habitats may have occurred only once. Interestingly this clade also includes a large portion of subtropically-restricted *Calcinus*, implying that temperature may be an important factor limiting their distribution. Our field observations show that even the two clade X members known from relatively shallow, tropical waters (*C. argus* and *C. anani*) are rare in those habitats, but also occur in the subtropics or deep water. Sequence and/or morphological data suggest incipient differentiation in four of five recognized species in this clade (*C. anani, C. argus*, *C. sirius, C. albengai*), the only exception being the geographically-restricted W Australian endemic *C. dapsiles*. Moreover, subtropical and deep reef habitats remain substantially undersampled for *Calcinus*, and future explorations will likely result in discovery of numerous new forms and document additional radiation. The small number of samples on hand prevent detailed analysis of speciation in this clade.

In contrast to the abundance of peripheral speciation, *Calcinus* show no diversification within the Indo-Malayan area: no ESEs are identified within the area, and only one species, *C. gaimardii,* is (largely) confined to it. This contrasts with many marine taxa that have numerous endemics in Indo-Malaya, some with substantial *in situ* diversification (e.g., Paulay 1997, Meyer et al. 2005, Barber et al. 2006, Williams & Reid 2004). Overall, speciation along continental shorelines appears to be uncommon in *Calcinus*, with the EP species *C. californiensis* (Gulf of California to El Salvador) and *C. obscurus* (El Salvador to Peru) the only known example.

Calcinus species show little differentiation between the Indian and Pacific Ocean basins. In contrast the restricted seaway between the Indian and Pacific basins is one of

the most important sites of speciation for other marine taxa, with numerous well-known as well as cryptic species-pairs differentiating across the boundary between these great basins (e.g., Randall 1998, Read et al. 2006, Barber et al. 2000). In *Calcinus* only 2 ESEs are known that may fall in this area, i.e., in the *C. pulcher* and *C. vachoni* complexes. However, the location of the boundary between western and eastern ESUs of both species are poorly constrained, as no samples have been genetically tested between the Philippines/Ryukyus and Mascarenes (Figs. 2-7 and 2-14). In contrast none of the other 5 widespread species tested (*C. laevimanus, C. argus, C. elegans, C. guamensis, C. latens*) show much genetic differentiation between populations in the Indian and Pacific Ocean basins. The genetic homogeneity of such wide-ranging species, prevalence of peripatric speciation on remote archipelagos, and diverse *Calcinus* assemblages on the world's most isolated islands imply that these crabs have great powers of dispersal, that has influenced their modes of speciation.

Inter-Regional Comparisons

While the diversity of *Calcinus* in the IWP and EP are largely the result of *in situ* radiation, inter-regional speciation was the source of Atlantic diversity. All non-IWP species studied are in two clades (I and VI). Clade I is comprised of *C. tubularis* (EA) and *C. verrilli* (Bermuda). The eastward relationship of the Bermudan endemic is unusual, as the majority of marine organisms in Bermuda originated from the WA, a result of the Gulf Stream facilitating dispersal (Sterrer 1986, Floeter et al. 2007). Among fishes, only a single Bermudan species appears to be clearly of EA in origin (Smith-Vaniz et al 1999).

Clade VI (Fig. 2-8) is comprised of 4 EP, 1 WA and 1 EA species. Species in this clade are nearly identical in structural morphology, but readily distinguished by color

pattern. The EA *Calcinus talismani* is not represented in the COI-only phylogeny because only the 16S region could be amplified from available specimens. In 16S-only, 2- and 3-gene trees, *C. talismani* is sister to *C. tibicen*. Among the remaining clade VI species, *C. tibicen* (WA) and *C. explorator* (EP) appear to be geminate species isolated by the emergence of the Isthmus of Panama. The other subclade is comprised of EP species only (*C. californiensis, C. obscurus,* and *C. mclaughlinae*). *Calcinus mclaughlinae* is endemic to Clipperton Island, ~1100 km offshore of central America, while *C. californiensis* and *C. obscurus* have parapatric ranges along the central American coast and are absent from EP oceanic islands. Offshore EP islands (Clarion, Socorro, Clipperton, Cocos, Galapagos) mostly harbor *C. explorator*, a species also present in and near the Gulf of California, but not along the continental coast further south (Fig. 2-8).

Ecology

Species distributional boundaries can be set by ecological limitations as well as dispersal barriers (with dispersal barriers themselves a type of ecological limitation). A prevalent form of distributional restriction in the IWP is to “continental” or “oceanic” habitats (Abbott 1960, George 1974, Paulay 1994, Reid et al. 2006). While both can be caused by dispersal as well as ecological limitations, ecological restriction is implied for species that range widely among remote islands, but are absent from nearby continents. Pacific-plate endemism (Springer 1982, Kay 1984) is a well-documented example of such ecological oceanic restriction (Paulay 1997). Continental and oceanic habitats differ in many ways, including levels of primary productivity, terrigenous influence, habitat diversity, and presence/absence of predators and competitors that are restricted to continental shores by dispersal limitations.

Oceanic restriction is prevalent in *Calcinus*. Thus only 7 of 17 species of *Calcinus* recorded from Australian territories are known from the continent, the remainder are recorded only from offshore islands (Morgan 1991). While 12 species are recorded from Cocos Keeling and Christmas Islands, small oceanic islands just SW of Indonesia, only 9 species have been recorded from all of Indonesia, the most diverse marine archipelago in the world. Similar continental avoidance occurs in some Australian brachyurans and the terrestrial hermit crab *Coenobita* (Paulay & Starmer in prep). *Calcinus isabellae* is a classic widespread Pacific-plate endemic (Fig. 2-11), and five other species appear to be regionally-widespread, yet largely confined to islands: *C. sirius* in the South Pacific, *C. argus* and *C. seurati* across the IWP, *C. explorator* in the EP, and *C. talismani* in the EA. An additional 16 species are restricted to one or a few neighboring oceanic island groups, but could be so restricted by dispersal limitation as well as ecology. Conversely, only three species show largely continental restriction: *C. gaimardii* in the IWP and *C. californiensis* and *C. obscurus* in the EP.

There is substantial niche conservatism in *Calcinus*, but also interesting ecological shifts between sister species. As discussed above, several clades are restricted to cool waters. Sister species *C. tubularis* and *C. verrilli* are the only *Calcinus* known with sexually dimorphic behavior, with females commonly adopting a sessile habit, living in tubes of sessile turritellid and vermetid gastropods (Markham 1977; Gherardi 2004). Sister species *C. seurati* and *C. laevimanus* are the only high intertidal/supratidal *Calcinus*, a habitat otherwise occupied by the related (but competitively inferior – Hazlett 1981) diogenid genus *Clibanarius*. However, while *C. laevimanus* lives in the upper intertidal, *C. seurati* is restricted to supratidal splash pools. Two ESUs of *C.*

albengai separate by depth: one ranging from shore to <50 m depths, while the other is exclusively deep-water (50-280 m; Poupin and Lemaitre 2003). The role of ecology vs. geography in the divergence of these species deserves further attention; the latter, with both forms only known from one small island, has potential as sympatric speciation through niche differentiation.

Diversity Patterns

Calcinus species diversity is about an order of magnitude higher in the IWP than other regions, a pattern typical for reef organisms (Paulay 1997). The IWP is home to 40 ESUs, the EP, WA, and EA to 4, 3, and 2 respectively. Local diversity shows similar inter-regional differences, with up to 16 species coexisting in one archipelago (Marianas) in the IWP, but at most 2 in other regions.

Calcinus species richness does not peak within the Indo-Malayan triangle, but is highest in Oceania, peaking at two locations: the Mariana and Tuamotu Islands, with 16 and 15 species (Fig. 2-16). This contrasts with the majority of marine taxa that reach their diversity peak in Indo-Malaya, from where richness decreases in all directions, but most conspicuously across the Pacific basin (Stehli et al. 1967, Briggs 1974, Hoeksema 2007). Nevertheless, diversity patterns, especially the steepness of the diversity increase towards Indo-Malaya varies greatly among taxa, and in large groups it is the composite of different, clade-specific, underlying patterns (see Fig. 4 in Paulay & Meyer 2006). Thus it is not surprising that particular smaller taxa, like *Calcinus*, deviate from the predominant pattern.

We propose that the diversity pattern of *Calcinus* is a reflection of the genus' affinity to oceanic conditions. *Calcinus* species tend to have great dispersal ability, broad ranges, and reach remote islands. The 10 species diversity contour ranges from

the Mascarene to the Hawaiian Islands, and although 13 species are recorded from across all of SE Asia, only 10 are known from any one country in the Indo-Malayan archipelago. As noted above, many species avoid continental habitats, such that diversity is higher on oceanic islands immediately outside this diversity center, than on the more terrigenous and continental settings of Indo-Malaya and Australia. Finally the predominance of peripatric speciation has lead to local diversity hotspots in peripheral locations, like in SE Polynesia, where 15 species are known from the Tuamotus, and 21 when the faunas of immediately adjacent (and biogeographically contiguous) are included.

The high diversity in the Indo-Malayan triangle has been attributed to three different hypotheses: center of origin, center of accumulation, or center of overlap. The center of origin hypothesis posits that species arise preferentially in Indo-Malaya. Such a pattern is clearly seen in some taxa with limited dispersal capacity, with species commonly restricted to, and arising within, the Indo-Malayan triangle (Paulay 1997, Meyer et al. 2005). With at most one Indo-Malayan-restricted species and no *in situ* diversification, this hypothesis fails for *Calcinus*.

The center of overlap hypothesis posits that the high diversity in the Indo-Malayan area is the result of overlapping ranges of Indian and Pacific basin sister species (Woodland 1983). Differentiation between the Indian and Pacific basins is prevalent in marine organisms (e.g., Randall 1998, Read et al. 2006, Reid et al. 2006), and since such sister taxa often show distributional overlap in Indo-Malaya, this hypothesis is supported as an important contributor to the diversity peak in the area (Woodland, 1983). In contrast *Calcinus* shows little speciation between the Indian and Pacific basins

(0-2 examples). Furthermore no sister species are known to have overlapping ranges in the Indo-Malaya, albeit we have very limited sampling in the area.

The center of accumulation hypothesis posits that species predominantly originate in peripheral areas but subsequently accumulate in Indo-Malaya by distributional expansion followed by reliction (Ladd 1960, Jokiel & Martinelli 1992). *Calcinus* includes an abundance of peripheral endemics. Such endemics may follow one of two trajectories: (1) range expansion after establishment of reproductive barriers with their sister species, leading to buildup of regional species diversity, or (2) maintenance of restriction until eventual extinction in their isolated ranges. It is difficult to test the importance of these two alternatives, although the predominance of peripatric speciation in *Calcinus*, combined with high diversity of widespread taxa and substantially greater age of sympatric sister species, suggest that the first trajectory may occur reasonably frequently. In contrast, reliction to Indo-Malaya has not been an important process, as demonstrated by the paucity of Indo-Malayan *Calcinus* endemics.

As noted above, many *Calcinus* species appear to prefer oceanic habitats. With an abundance and local radiations of oceanic taxa, combined with substantial dispersal ability of many *Calcinus* (most IWP-wide species are genetically relatively homogeneous across the two ocean basins), it is not surprising that species richness in *Calcinus* peaks in oceanic parts of the western Pacific.

Conclusions

Our study has uncovered a wealth of unrecognized diversity in a relatively well-known reef dweller, *Calcinus*. The number of ESUs in the IWP was augmented by 22%. This large increase in ESUs was made possible by our approach of exhaustively sampling populations of every accessible species across their entire range. Through

photo-documentation of live specimens, we conclude that differences in coloration correspond to boundaries between ESUs, thus documenting color patterns is very important in species delineations. We show that color pattern differences evolve extremely rapidly, and we hypothesize that coloration may serve an adaptive purpose. Perhaps coloration is important in intraspecific recognition and mate selection. This hypothesis deserves further investigation, for instance by studying the evolution of genes responsible for differences in decapod coloration.

The geographic distributions of *Calcinus* species are now well documented and illustrate several patterns atypical for reef fauna. Non-IWP species all fall in 2 clades. One clade connects a Bermudan endemic to an EA species, a rarely observed pattern. The second non-IWP clade groups together species from EP, WA, and EA (including one geminate species pair). This clade contains the only known instances in *Calcinus* of speciation along a continental margin.

Among IWP species, we show that the center of species diversity is not in the Indo-Malayan triangle, but further east in the Marianas islands. This may be the result of a tendency in *Calcinus* to prefer oceanic habitats. We found no support for either center of origin or center of overlap theories. Instead, our results show a history of generally high dispersal abilities coupled with bouts of peripatric speciation in remote areas. The youngest sister species pairs all have narrowly allopatric distributions, and a substantial amount of time (>2 million years, usually much longer) is needed for sister species to develop sympatric distributions. Contrary to the predictions of the center of accumulation hypothesis, the Indo-Malayan triangle is not the center of *Calcinus* diversity and does not act as a refuge for relictual taxa.

Ecological factors have also played a role in *Calcinus* distribution and speciation. Distributions are shaped in part by restriction of species to oceanic environments (common) or continental environments (rare). Phylogenetic conservatism of ecological niches is common; however, there are also a few cases of large ecological shifts between sister species. In one instance, a shift between a shallow-water and a deep-water morph may have occurred in sympatry.

None of the 3 dominant IWP biogeographic models succeed in fully explaining species diversity patterns in *Calcinus*. This is to be expected, as all these models simplistically propose that the Indo-Malayan diversity hotspot can be explained by a single vicariance- or dispersal-driven mechanism. The reality is much more complex. A diverse range of factors, including both historical and ecological mechanisms, influence species distributions. Given this complexity, biogeographers should expect different taxa to show non-identical biogeographic patterns, reflecting the unique histories and ecological adaptations of the different groups. The overall top-down picture of marine biodiversity is a summation of all these individual histories.

Table 2-1. Known species of *Calcinus* and new ESUs, including their geographic ranges and accession information for all the sequenced specimens (including outgroup specimens).

		Reported geographic range			Specimen info				
ESU count	Species	Region	W	E	Specimen provenance	Specimen no.	Museum Catalog no.	COI seq?	16S seq?	H3 seq?
1	*Calcinus albengai* Poupin & Lemaitre 2003 - deep morph	IWP	Austral Ids.	Austral Ids.	Austral Ids.	H92	MNHN Pg.6378	+	+	+
2	*Calcinus albengai* aff. Poupin & Lemaitre 2003 - shallow morph	IWP	Austral Ids.	Austral Ids.	Austral Ids.	H94	MNHN Pg.6385	+	+	+
3	*Calcinus anani* Poupin & McLaughlin, 1998	IWP	Japan	Tuamotus; Marquesas	Bismarck Arch. (PNG)	H77	UF 4808	+	+	+
4	*Calcinus argus* Wooster, 1984	IWP	Mascarene Ids.	Hawaii	Marquesas	H95	MNHN Pg.6357	+	+	+
					Bismarck Arch. (PNG)	H324	UF 11740	+		
					Mascarene Ids.	H62	UF 5437	+		
					Mascarene Ids.	H114	UF 5446	+		
					N Marianas	H96b	UF 5714	+		
					Hawaii	H146	UF 7364	+	+	+
					Hawaii	H192	UF 8038	+		
					New Caledonia	H203	MNHN	+		
					Mascarene Ids.	H312	UF 12814	+		
					Mascarene Ids.	H313	UF 13022	+	+	+
5	*Calcinus dapsiles* Morgan, 1989	IWP	W Australia	W Australia	W Australia	H101	UF 6297	+	+	+
					W Australia	H133	UF 6297	+	+	+

Table 2-1. Continued

		Reported geographic range			Specimen info					
ESU count	Species	Region	W	E	Specimen provenance	Specimen no.	Museum Catalog no.	COI seq?	16S seq?	H3 seq?
6	*Calcinus elegans* H. Milne Edwards, 1836	IWP	S Africa	Tuamotus; Marquesas	Marianas	H5	UF 325	+	+	+
					Mascarene Ids.	H67	UF 5504	+		
					Tuamotus	IP31	UF 1351	+	+	+
					Line Ids.	H306	UF 11487	+		
					Line Ids.	H317	UF 11204	+		
7	*Calcinus elegans* aff. - Hawaii	IWP	Hawaii	Hawaii	Hawaii	H15	UF 3216	+	+	+
					Hawaii NW Hawaiian Ids.	H113	UF 8350	+	+	+
					NW Hawaiian Ids.	H292	UF 12060	+		
					NW Hawaiian Ids.	H293	UF 12064	+		
					NW Hawaiian Ids.	H294	UF 12064	+		
					NW Hawaiian Ids.	H304	UF 12068	+		
					Hawaii	H307	UF 14838	+		
8	*Calcinus gaimardii* H. Milne Edwards, 1848	IWP	Maldives	Fiji	Palau	H42	UF 3924	+	+	+
					Philippines	H136	UF 6744	+	+	+
9	*Calcinus gouti* Poupin, 1997	IWP	Line Ids.	Tuamotus	Tuamotus	H25	UF 1349	+		
					Tuamotus	IP5	UF 1863	+	+	+
					Line Ids.	H190	UF 8604	+	+	+

Table 2-1. Continued

		Reported geographic range			Specimen info					
ESU count	Species	Region	W	E	Specimen provenance	Specimen no.	Museum Catalog no.	COI seq?	16S seq?	H3 seq?
10	*Calcinus guamensis Wooster, 1984*	IWP	Somalia	Hawaii; Marquesas	Am. Samoa	H23	UF 3224	+		
					Hawaii	H60b	UF 3219	+		
					Marquesas	H49	UF 5171	+	+	+
					Society Ids.	IP44	UF 1888	+		
					Mascarene Ids.	H58	UF 5418	+	+	+
					Hawaii	H142	UF 3219	+		
11	*Calcinus haigae* Wooster, 1984	IWP	Red Sea	Hawaii; Tuamotus	Am. Samoa	H41	UF 3225	+		
					Marianas	H83	UF 5713	+	+	+
					Tuamotus	H82	UF 1744	+		
					Tuamotus	H120	UF 1332	+	+	+
					Tuamotus	H232	UF 9270	+	+	+
					Line Ids.	H175	UF 8372	+		
					Hawaii	H139	UF 8035	+		
					Hawaii	H230	UF 8035	+	+	+
					Line Ids.	H176	UF 8379	+		
					Tuamotus	H231	UF 9269	+		
12	*Calcinus hakahau* Poupin & Mclaughlin, 1998	IWP	Marquesas	Marquesas	Marquesas	H51	UF 5175	+	+	+
					Marquesas	H117	UF 5166	+	+	+
13	*Calcinus hazletti* Haig & McLaughlin, 1984	IWP	Hawaii	Hawaii	Hawaii	H119	UF 8349	+	+	+
					NW Hawaiian Ids.	H295	UF 12157	+	+	+
					NW Hawaiian Ids.	H302	UF 12158	+		

Table 2-1. Continued

ESU count	Species	Reported geographic range: Region	W	E	Specimen info: Specimen provenance	Specimen no.	Museum Catalog no.	COI seq?	16S seq?	H3 seq?
14	*Calcinus hazletti* aff. - Northern Marianas		Japan? / N Marianas	N Marianas	N Marianas	H79	UF 5732	+	+	+
					N Marianas	H90	UF 5728	+	+	+
					Wake Atoll	H191	UF 8438	+		
15	*Calcinus imperialis* Whitelegge, 1901	IWP	E Australia	Easter Is.	Easter Is.	H38	UF 3646	+	+	+
16	*Calcinus inconspicuus* Morgan, 1991	IWP	E Australia	New Caledonia	New Caledonia	H107	MNHN	+	+	+
17	*Calcinus isabellae* Poupin, 1997	IWP	Marianas	Hawaii; Pitcairn	Marianas	IP42	UF 732	+	+	+
					Tuamotus	IP20	UF 1758	+		
					Line Ids.	H174	UF 8371	+		
					Wake Atoll	H199	UF 8449	+	+	+
					Cook Ids.	H228	UF 10354	+		
18	*Calcinus kurozumii* Asakura & Tachikawa, 2000	IWP	N Marianas (Pagan only)	N Marianas (Pagan only)						
19	*Calcinus laevimanus* Randall, 1840	IWP	S Africa	Hawaii; Tuamotus	Hawaii	H75	UF 3221	+		
					Mascarene Ids.	H66	UF 5426	+	+	+
					Marianas Ids.	IP28	UF 601	+		
					Tuamotus	H76	UF 1720	+		
					Hawaii	H13	UF 3221	+		
					Wake Atoll	H198	UF 8445	+	+	+

Table 2-1. Continued

ESU count	Species	Reported geographic range: Region	W	E	Specimen info: Specimen provenance	Specimen no.	Museum Catalog no.	COI seq?	16S seq?	H3 seq?
20	*Calcinus latens* Randall, 1840	IWP	Mozambique; Yemen	Tuamotus	Marianas Ids.	IP39	UF 460	+		
					Mascarene Ids.	H316	UF 12564			
					Mascarene Ids.	H110	UF 5450	+	+	+
					Tuamotus	IP7	UF 1712	+		
					Tuamotus	IP9	UF 1712	+		
					Line Ids.	H322	UF 10805	+		
					Cook Ids.	H297	UF 10339	+		
					Wake Atoll	H320	UF 8440	+		
					Line Ids.	H308	UF 10686	+		
					Cook Ids.	H298	UF 10339	+	+	+
					Wake Atoll	H321	UF 8440	+		
21	*Calcinus latens* aff. - Hawaii	IWP	Hawaii	Hawaii	Hawaii	H16	UF 3217	+	+	+
					Hawaii	H109	UF 3217	+	+	+
					NW Hawaiian Ids.	H299	UF 12066	+		
					NW Hawaiian Ids.	H300	UF 12066	+		
22	*Calcinus latens* aff. - Oman	IWP	Oman	Oman	Oman	H81	UF 5428	+	+	+
					Oman	H314	UF 5416	+	+	+
23	*Calcinus laurentae* Haig & McLaughlin, 1984	IWP	Hawaii	Hawaii	Hawaii	H39	UF 3625	+	+	+
					NW Hawaiian Ids.	H291	UF 12059	+	+	+
					NW Hawaiian Ids.	H303	UF 12278	+		

Table 2-1. Continued

		Reported geographic range			Specimen info					
ESU count	Species	Region	W	E	Specimen provenance	Specimen no.	Museum Catalog no.	COI seq?	16S seq?	H3 seq?
24	*Calcinus lineapropodus* Morgan & Forest, 1991	IWP	Cocos Keeling	Tuamotus	Am. Samoa	H84	UF3255	+		
					Marianas Ids.	IP19	UF 1322	+	+	+
					Ryukyus	H137	UF 6990	+	+	+
					Line Ids.	H177	UF 8600	+		
25	*Calcinus minutus* Buitendijk, 1937	IWP	Cocos Keeling	Samoa	Am. Samoa	H86	UF3263	+	+	+
					Marianas	IP32	UF 1321	+		
					Philippines	H140	UF 6511	+		
					Ryukyus	H149	UF 6982	+	+	+
26	*Calcinus morgani* Rahayu & Forest, 1999	IWP	S Africa	Tuamotus	Am. Samoa	H27	UF 3236	+	+	+
					Society Ids.	IP33	UF 1350	+		
					Marianas Ids.	IP43	UF 652	+	+	+
					Palau	H130	UF 3992	+	+	+
					Ryukyus	H145	UF 6995	+	+	+
					Ryukyus	H147	UF 7237	+		
27	*Calcinus nitidus* Heller, 1865	IWP	Society	Tuamotus	Society Ids.	H26	UF 1334	+	+	+
					Society Ids.	H121	UF 1334	+		
					Society Ids.	H129	UF 6886	+		
					Tuamotus	IP2	UF 1347	+	+	+
28	*Calcinus orchidae* Poupin, 1997	IWP	Marquesas	Marquesas	Marquesas	H50	UF 5177	+	+	+
29	*Calcinus pascuensis* Haig, 1974	IWP	Easter Is.	Easter Is.	Easter Is.	H37	UF 3648	+	+	+

Table 2-1. Continued

		Reported geographic range			Specimen info					
ESU count	Species	Region	W	E	Specimen provenance	Specimen no.	Museum Catalog no.	COI seq?	16S seq?	H3 seq?
30	*Calcinus pulcher* Forest, 1958	IWP	Seychelles	New Caledonia	Palau	H40	UF 3890	+	+	+
					Mascarene Ids.	H59	UF 5430	+	+	+
					Philippines	H135	UF 8357	+	+	+
					Micronesia	H193	UF 5396	+		
					Philippines	H194	UF 6531	+	+	+
					Papua New Guinea (Milne Bay)	H148	UF 5553	+	+	+
31	*Calcinus pulcher* aff. - Mascarenes	IWP	Mascarene Ids.	Mascarene Ids.	Mascarene Ids.	H309	UF 12741	+		
					Mascarene Ids.	H144	UF 5430	+	+	+
32	*Calcinus revi* Poupin & McLaughlin, 1998	IWP	Japan	Tuamotus	Tuamotus					
33	*Calcinus rosaceus* Heller, 1861	IWP	Red Sea	Gulf of Oman; Mauritius	Oman	H63	UF 5427	+	+	+
					Oman	H118b	UF 5435	+	+	+
					Mascarene Ids.	H310	UF 12781	+		
					Mascarene Ids.	H305	UF 12635	+	+	+
34	*Calcinus seurati* Forest, 1951	IWP	Somalia	Hawaii; Tuamotus	Marianas	IP36	UF 562	+	+	+
					Hawaii	H14	UF3223	+	+	+
35	*Calcinus sirius* Morgan, 1991	IWP	W Australia	E Australia						
36	*Calcinus sirius* aff. Poupin 1997	IWP	W Australia	Austral Ids.	Austral Ids.	H93b	MNHN Pg.6395	+	+	+

Table 2-1. Continued

		Reported geographic range			Specimen info					
ESU count	Species	Region	W	E	Specimen provenance	Specimen no.	Museum Catalog no.	COI seq?	16S seq?	H3 seq?
37	*Calcinus spicatus* Forest, 1951	IWP	E Australia	Pitcairn Is.	New Caledonia	H106	MNHN	+	+	+
					Cook Is.	H229	UF 10337	+	+	+
38	*Calcinus tropidomanus* Lewinsohn, 1981	IWP	Somalia	Somalia						
39	*Calcinus vachoni* Forest, 1958	IWP	Mascarene Ids.	Easter	N Marianas	H88	UF 5742	+	+	+
					Ryukyus	H131	UF 6992	+		
					Philippines	H132	UF 6748	+	+	+
40	*Calcinus vachoni* aff. - Cook Islands	IWP	Cook Ids.	Cook Ids.	Cook Ids.	H47	UF 1377	+	+	+
					Cook Ids.	H301	UF 11702	+		
41	*Calcinus vachoni* aff. - Réunion	IWP	Mascarene Ids.	Mascarene Ids.	Mascarene Ids.	H311	UF 12634	+	+	+
					Mascarene Ids.	H318	UF 13011	+		
					Mascarene Ids.	H319	UF 13011	+	+	+
42	*Calcinus vanninii* Gherardi & McLaughlin, 1994	IWP	Mascarene Ids.	Mauritius	Mascarene Ids.	H80	UF 5425	+	+	+
					Mascarene Ids.	H87	UF 5412	+	+	+
43	*Calcinus californiensis* Bouvier, 1898	EP	Baja California	El Salvador	Baja CA Sur	H98	UF 8367	+	+	+
					Baja CA Sur	H99	UF 15221	+	+	+
44	*Calcinus explorator* Boone, 1930	EP	Gulf of CA	Galapagos	Clipperton Atoll	H179	MNHN Pg.7617	+		
					Clipperton	H204	MNHN	+	+	+

Table 2-1. Continued

		Reported geographic range			Specimen info					
ESU count	Species	Region	W	E	Specimen provenance	Specimen no.	Museum Catalog no.	COI seq?	16S seq?	H3 seq?
	Calcinus obscurus		El Salvador	Ecuador; Colombia	Panama					
45	Stimpson, 1859	EP				H105	UF 8359	+	+	+
					Panama	H111	UF 8359	+	+	+
	Calcinus mclaughlinae				Clipperton Atoll		MNHN			
46	Poupin, 2006					H178	Pg.7622	+	+	+
	Calcinus tibicen			Ubatuba	Florida					
47	Herbst, 1791	WA	Belize	a Brazil		H102	UF 8363	+	+	+
					Florida	H103	UF 8364	+		
					Tobago	H124	UF 8358	+	+	+
	Calcinus urabaensis									
	Campos &		Colombia	Colombia						
48	Lemaitre, 1994	WA								
	Calcinus verrilli		Bermuda	Bermuda	Bermuda					
49	Rathbun, 1901	WA				H138	UF 8365	+	+	+
	Calcinus paradoxus									
50	Bouvier, 1922	EA	Azores	Azores						
	Calcinus talismani A. Milne Edwards & Bouvier,		Cape		Cape Verde					
51	1892	EA	Verde	Guinea		H206	MNHN		+	+
			Ascension Is.;		Madeira					
	Calcinus tubularis		Madeira	Lebanon						
52	Linnaeus, 1767	EA				H91	UF 8361	+	+	+
					Madeira	H97	UF 8361	+	+	+
Outgroups										
	Ciliopagurus strigatus				Marianas Ids.					
	(Herbst, 1804)					IP21	UF 1871	+	+	+
	Ciliopagurus tricolor Forest,				Mascarene					
	1995				Ids.	H68	UF 5433	+	+	+
	Ciliopagurus galzini Poupin				Tuamotus					
	& Malay, 2009					H32	UF 1742	+	+	+

Table 2-1. Continued

ESU count	Species	Reported geographic range: Region	W	E	Specimen info: Specimen provenance	Specimen no.	Museum Catalog no.	COI seq?	16S seq?	H3 seq?
	Dardanus lagopodes (Forskal, 1775)				Marianas Ids.	IP15	UF 326	+	+	+
					Tuamotus	IP18	UF 1760	+	+	+
	Dardanus sanguinocarpus Degener, 1925				Hawaii	H45	UF 3507	+	+	+
	Dardanus longior Asakura, 2006				Marquesas	H56	UF 3639	+	+	+

Table 2-2. List of ESUs used in biogeographic analyses, and their geographic distributions relative to each other.

Clade	Distribution	ESU pair
I	allopatric	C. verrili - C. tubularis
II	allopatric	C. latens - C. aff. latens Hawaii
II	allopatric	C. latens - C. aff. latens Oman
		C. hazletti - C. aff. hazletti N
III	allopatric	Marianas
III	allopatric	C. minutus - C. rosaceus
III	allopatric	C. minutus - C. nitidus
		C. haigae - C. minutus/C.
III	sympatric	rosaceus/C. nitidus
III	parapatric / slightly sympatric	C. inconspicuus - rest of clade III
IV	allopatric	C. vachoni - C. aff. vachoni Cooks
		C. vachoni - C. aff. vachoni
IV	allopatric	Mascarenes
V	allopatric	C. spicatus - C. pascuensis
VI	allopatric	C. mclaughlinae - C. obscurus
		C. californiensis - C.
VI	parapatric / slightly sympatric	mclaughlinae/C. obscurus
VI	allopatric	C. tibicen - C. talismani
		C. explorator - C. tibicen/C.
VI	allopatric	talismani
VII	sympatric	C. gaimardii - C. morgani
		C. elegans - C. aff. elegans
VII	allopatric	Hawaii
VII	allopatric	C. imperialis - C. vaninii
		C. isabellae - C. imperialis/C.
VII	parapatric / slightly sympatric	vaninii
VIII	sympatric (depth-separated)	C. laevimanus - C. seurati
		C. pulcher - C. aff. pulcher
IX	allopatric	Mascarenes
IX	allopatric	C. hakahau - C. gouti
IX	allopatric	C. laurentae - C. hakahau/C. gouti
IX	sympatric	C. lineapropodus - rest of clade IX
X	sympatric (depth-separated)	C. albengai - C. aff. albengai deep
X	allopatric	C. dapsiles - C. albengai complex
X	allopatric	C. argus - C. aff. sirius
X	sympatric	C. anani - C. argus/C. sirius

A

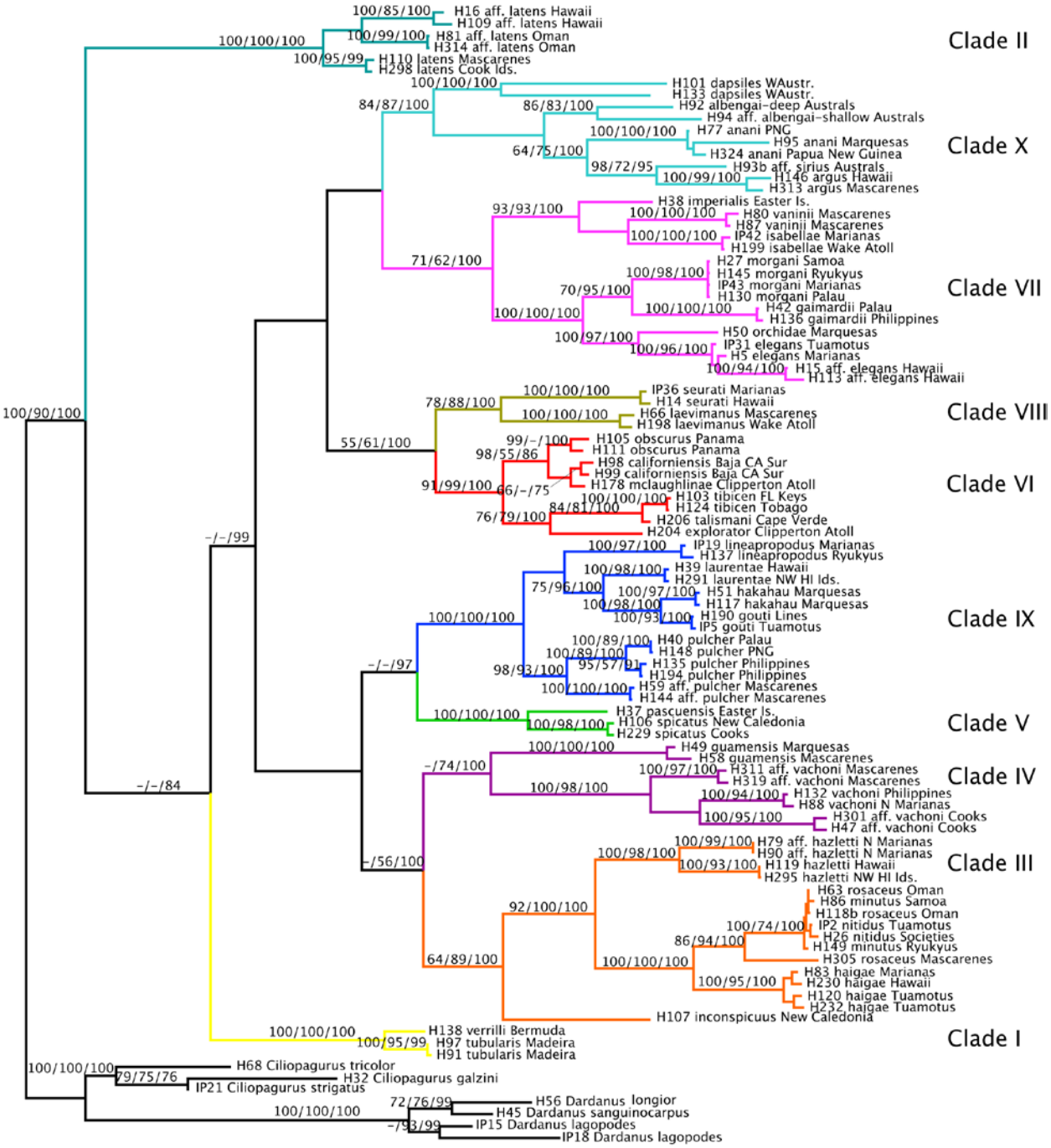

Figure 2-1. Bayesian phylograms constructed using (a)3 concatenated genes and (b)COI only. The values above the branches represent parsimony bootstraps / maximum likelihood bootstraps / Bayesian posterior probabilities, respectively.

B

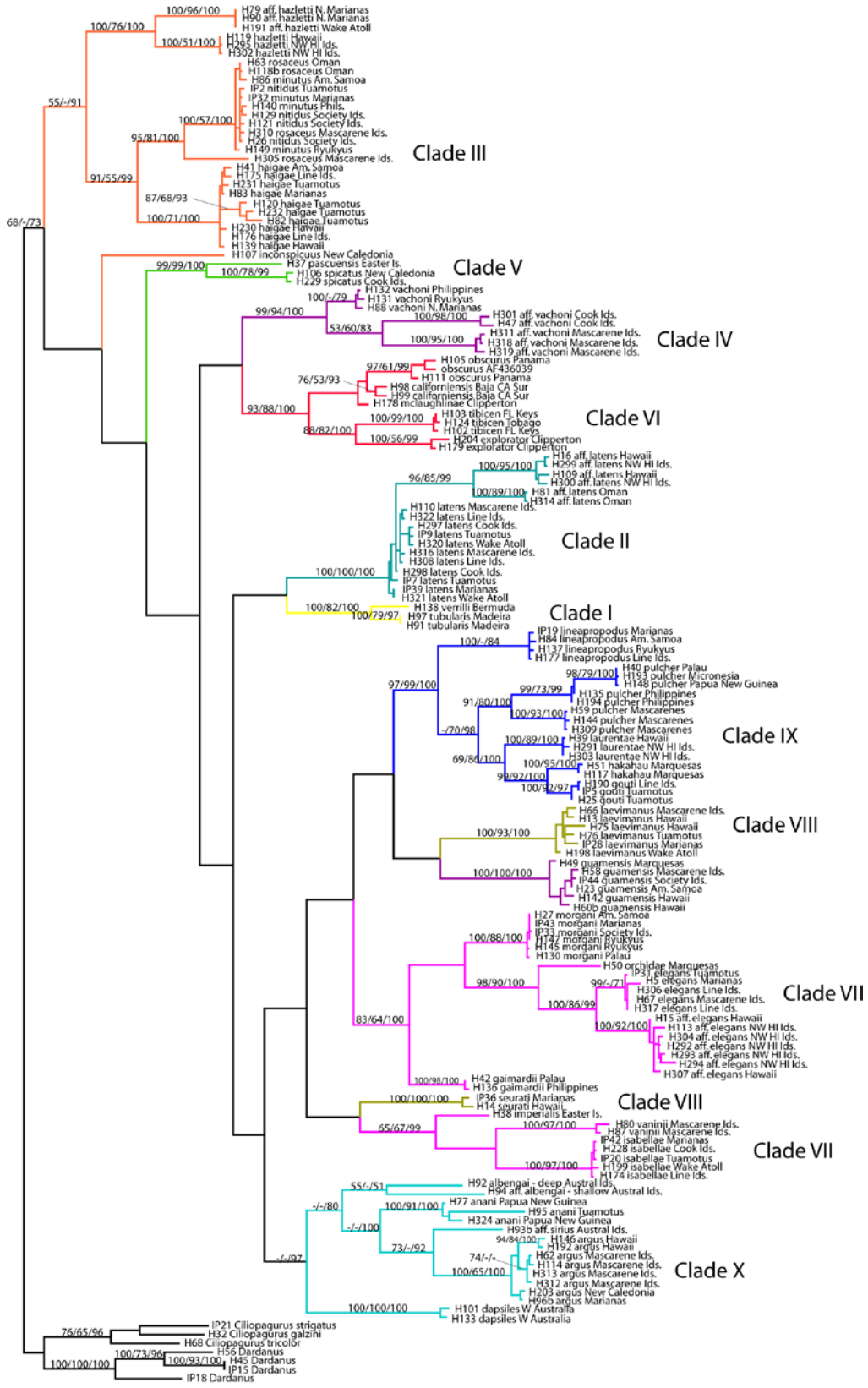

Figure 2-1. Continued

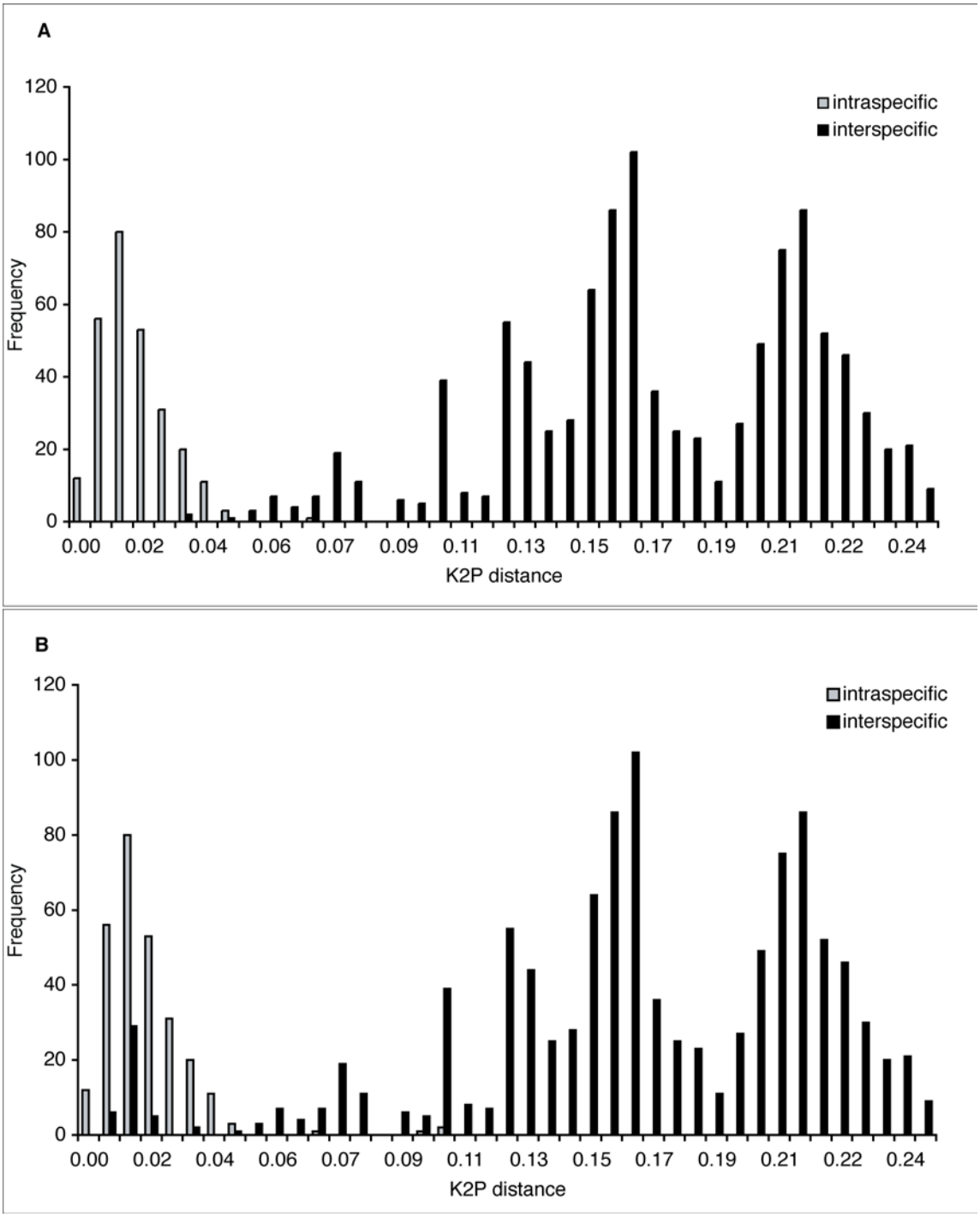

Figure 2-2. Frequency distribution of K2P distances for intraspecific variation and interspecific distances in *Calcinus*, without (a) and with (b) the *C. minutus* complex.

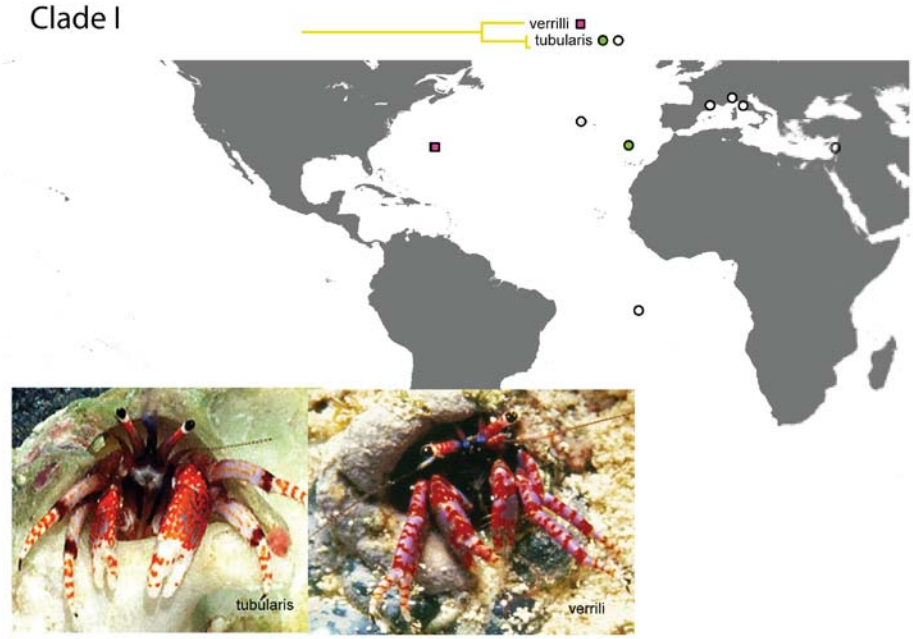

Figure 2-3. Distributions, color patterns, and COI phylogeny of Clade I *Calcinus* species. Colored symbols represent specimens available in the FLMNH collection; unfilled/black symbols represent records derived from the literature.

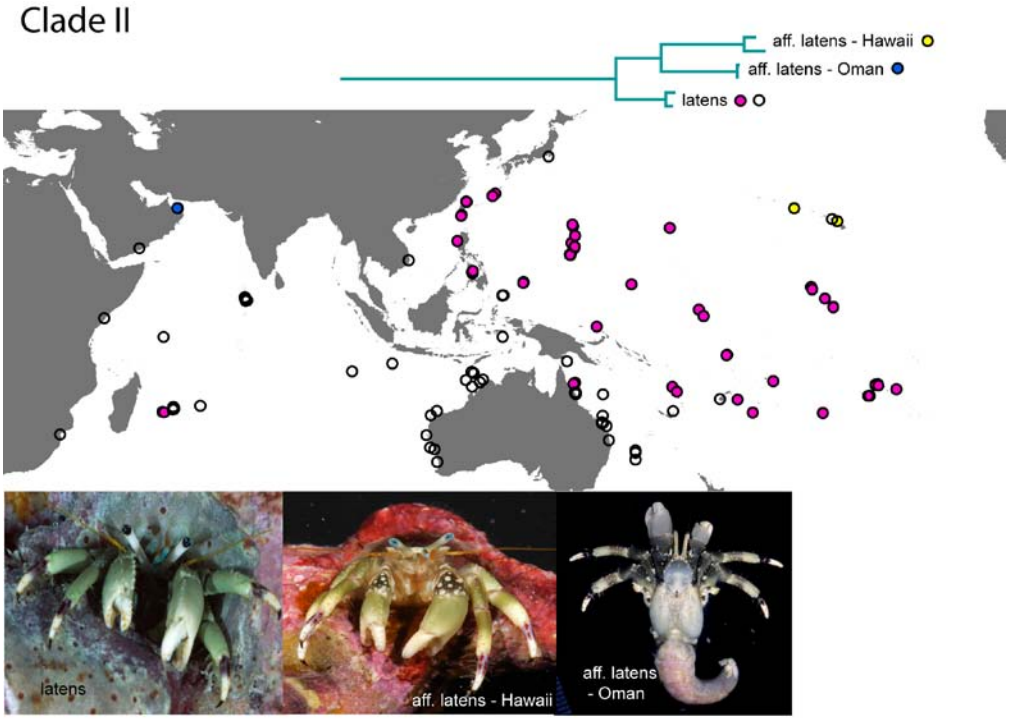

Figure 2-4. Distributions, color patterns, and COI phylogeny of Clade II *Calcinus* species. Symbols follow Fig. 3.

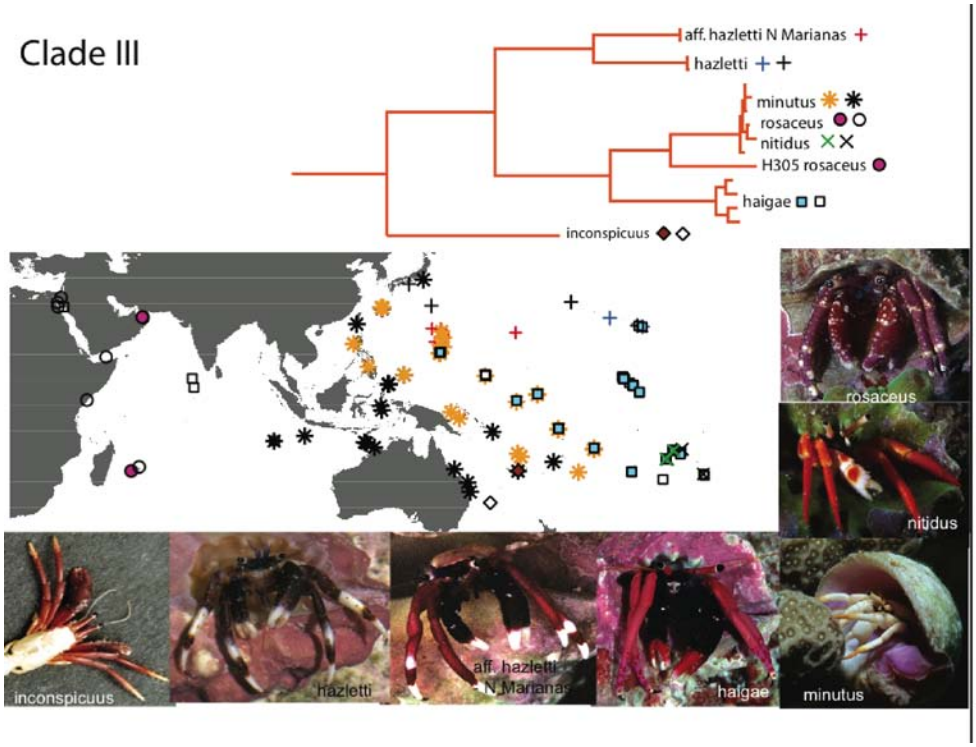

Figure 2-5. Distributions, color patterns, and COI phylogeny of Clade III *Calcinus* species. Symbols follow Fig. 3.

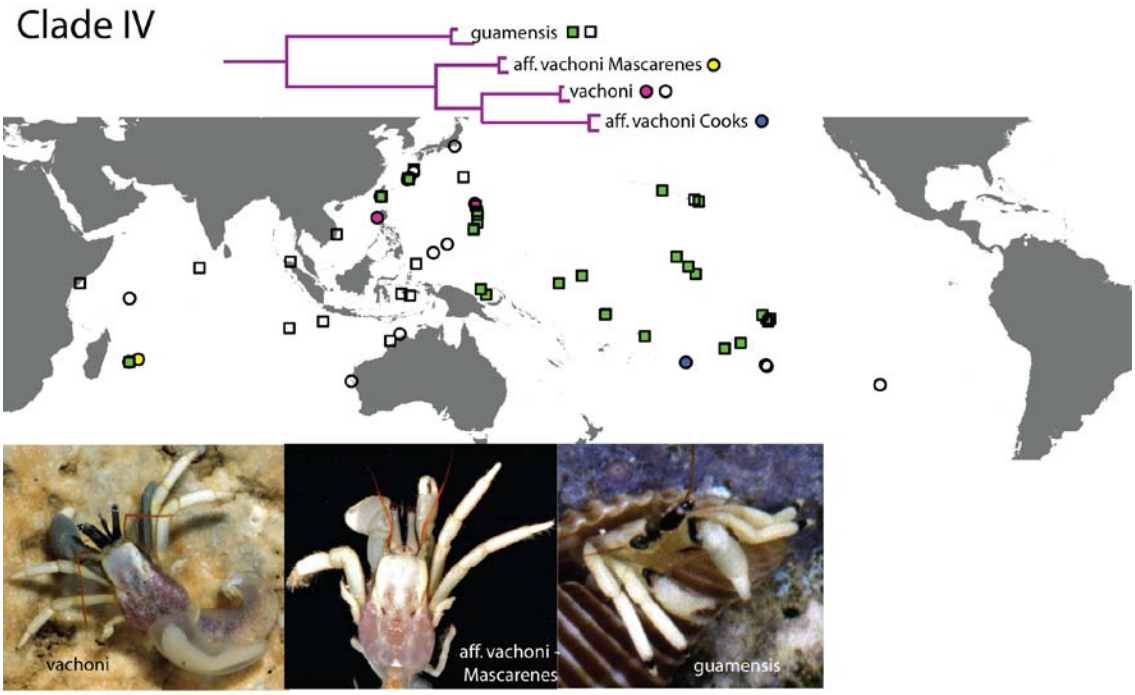

Figure 2-6. Distributions, color patterns, and COI phylogeny of Clade IV *Calcinus* species. Symbols follow Fig. 3.

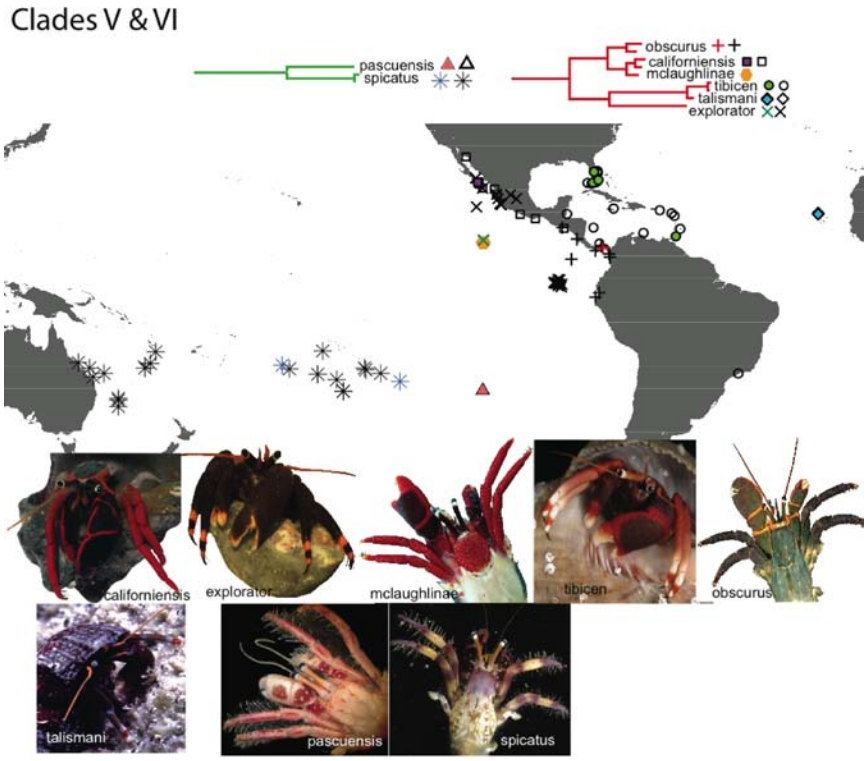

Figure 2-7. Distributions, color patterns, and COI phylogeny of Clades V and VI *Calcinus* species. Symbols follow Fig. 3.

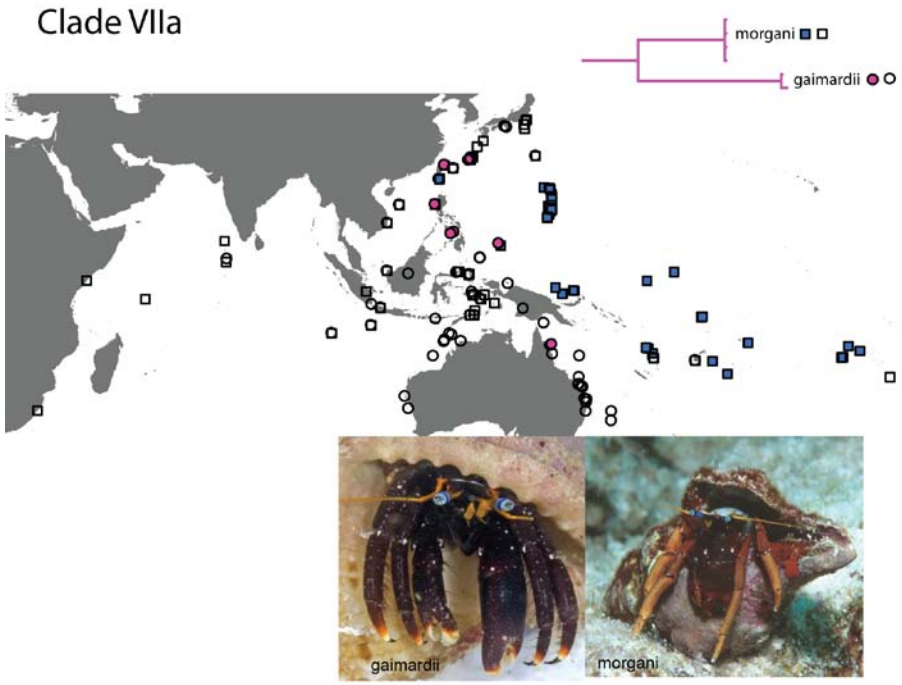

Figure 2-8. Distributions, color patterns, and COI phylogeny of Clade VIIa *Calcinus* species. Symbols follow Fig. 3.

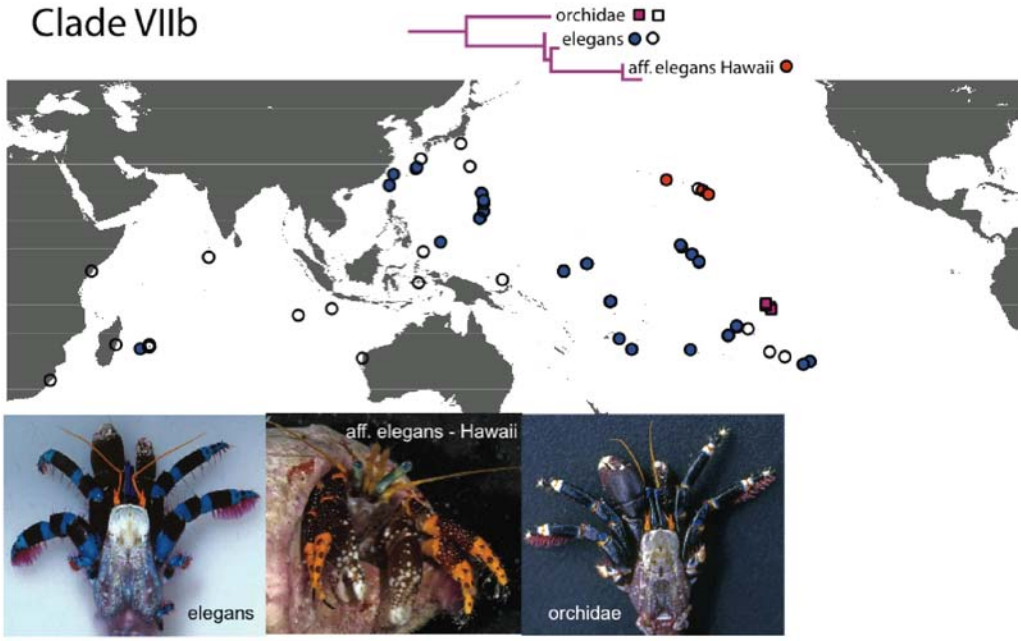

Figure 2-9. Distributions, color patterns, and COI phylogeny of Clade VIIb *Calcinus* species. Symbols follow Fig. 3.

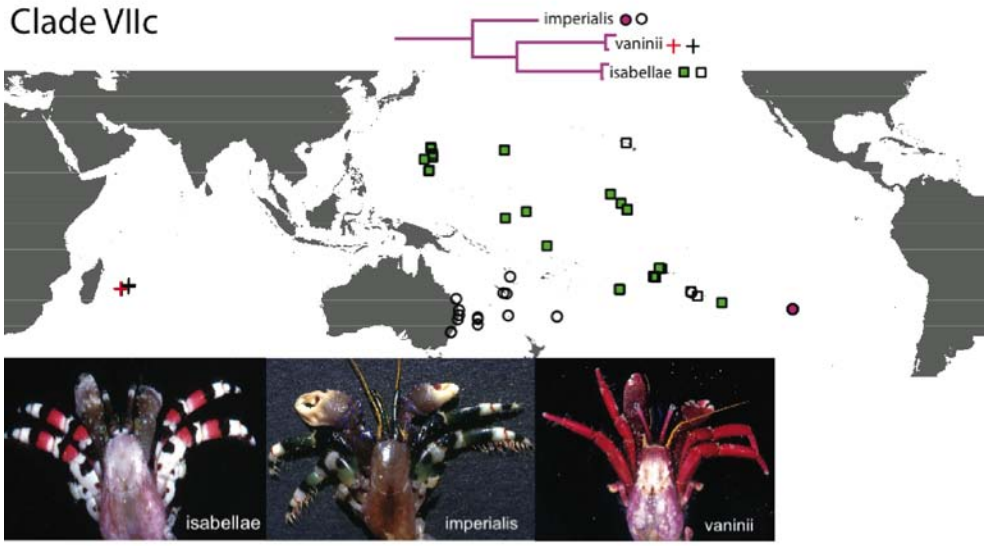

Figure 2-10. Distributions, color patterns, and COI phylogeny of Clade VIIc *Calcinus* species. Symbols follow Fig. 3.

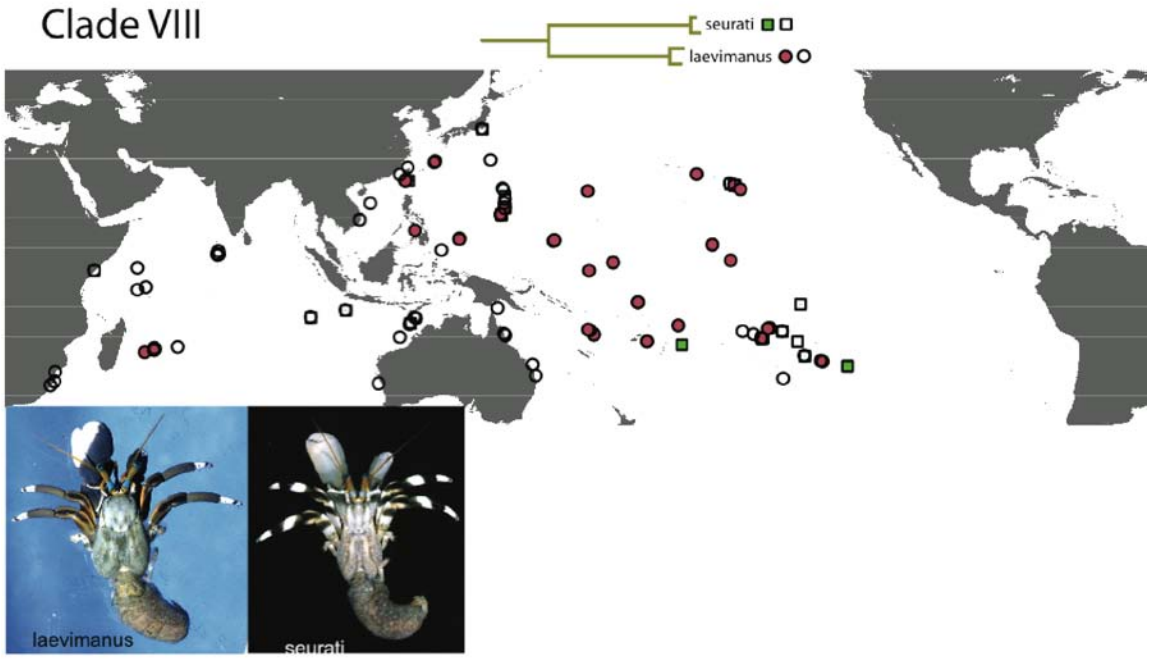

Figure 2-11. Distributions, color patterns, and COI phylogeny of Clade VIII *Calcinus* species. Symbols follow Fig. 3.

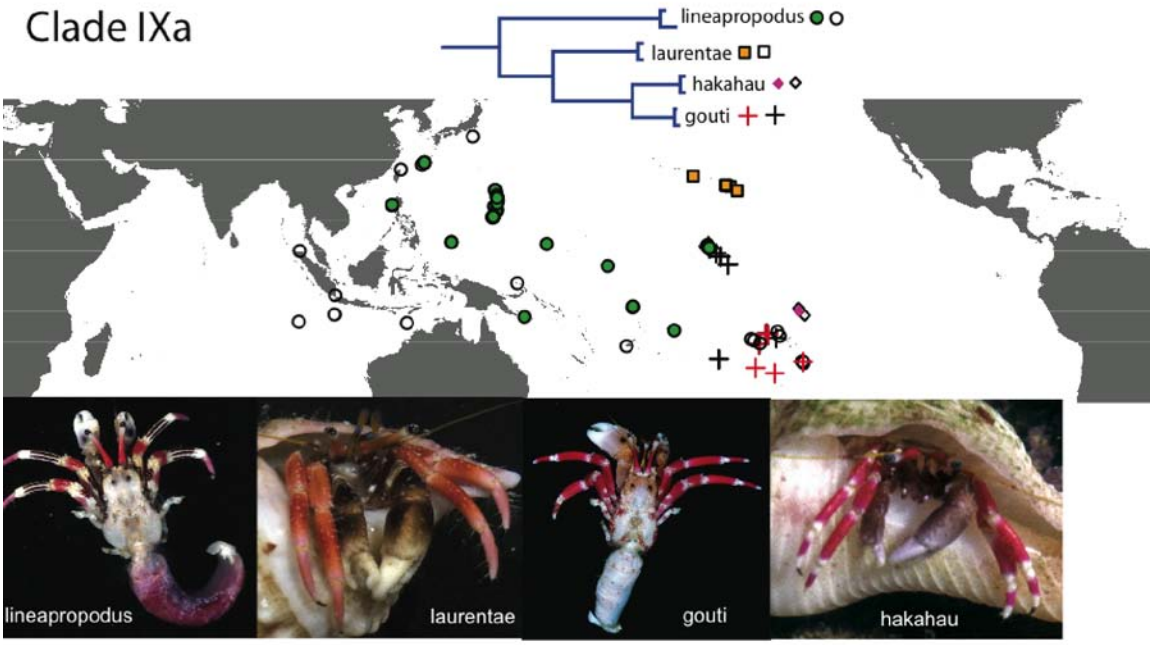

Figure 2-12. Distributions, color patterns, and COI phylogeny of Clade IXa *Calcinus* species. Symbols follow Fig. 3.

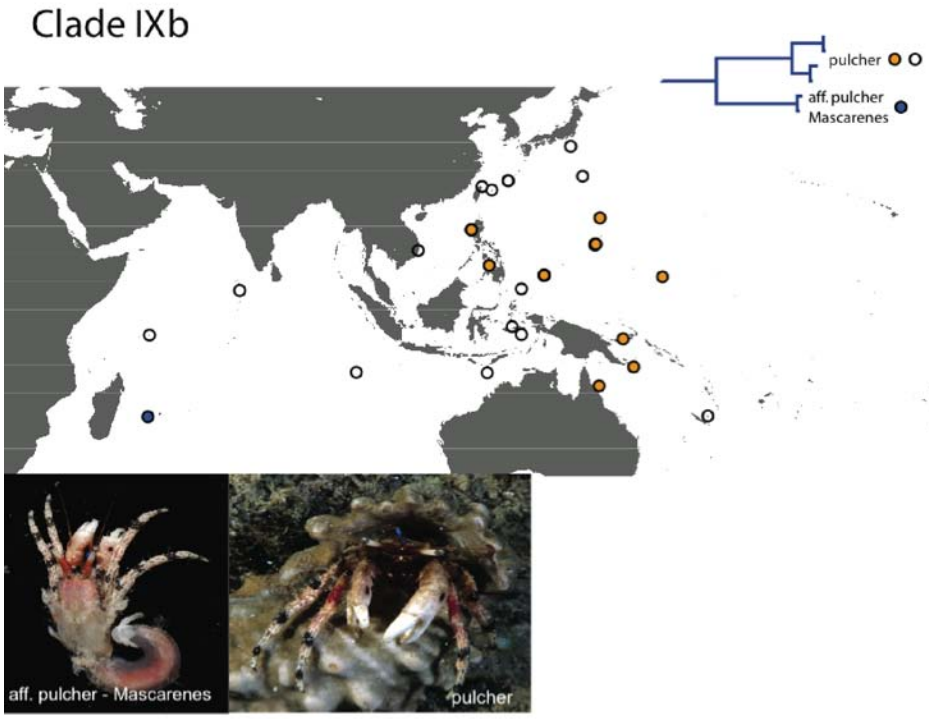

Figure 2-13. Distributions, color patterns, and COI phylogeny of Clade IXb *Calcinus* species. Symbols follow Fig. 3.

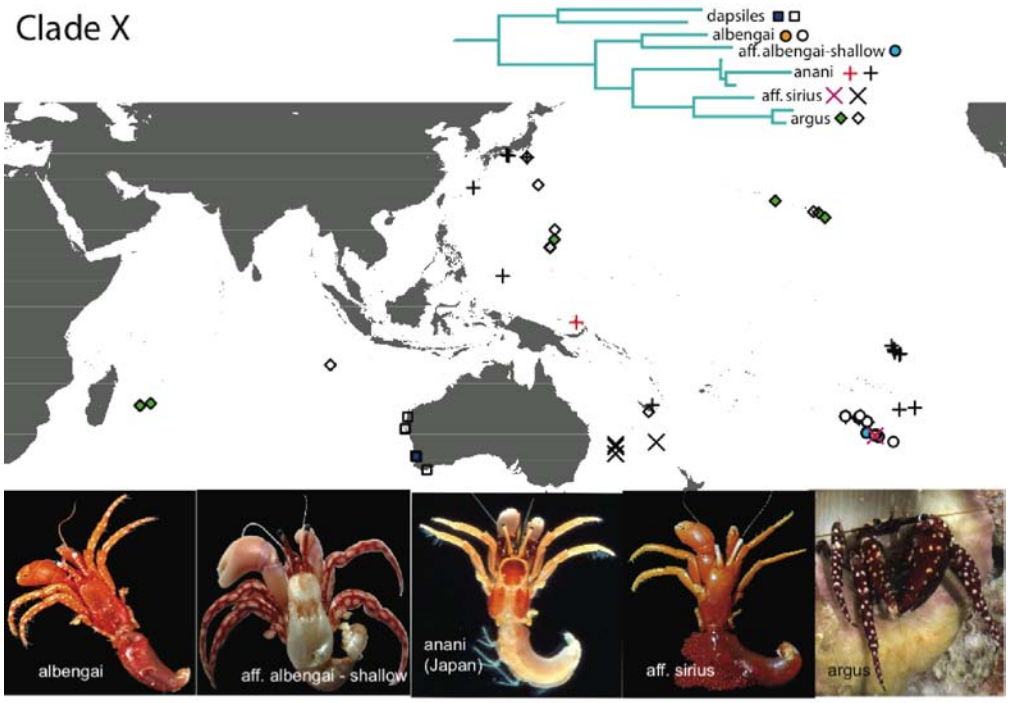

Figure 2-14. Distributions, color patterns, and COI phylogeny of Clade X *Calcinus* species. Symbols follow Fig. 3.

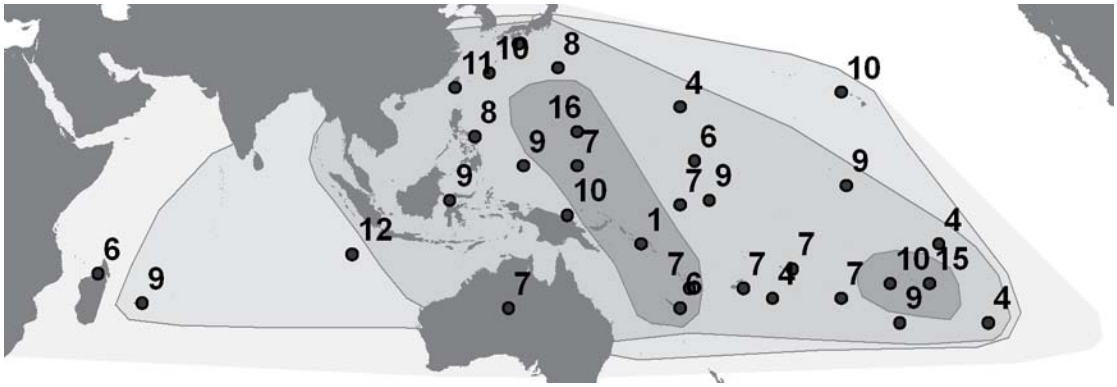

Figure 2-15. Spatial distribution of species richness. Contour lines represent number of species expected in area based on overlay of species ranges (Figs. 3-14, see methods). Contours are drawn for 4, 10, 13, and 17 species in increasingly darker shades. Numbers represent number of documented species within the following regions and archipelagoes: Australia, Austral-Rapa, Caroline, Cocos-Christmas, Cook, Fiji, Gilbert, Hawaii, Indonesia, Japan, Line, Madagascar, Mariana, Marquesa, Marshall, Mascarene, Nauru, New Caledonia, New Guinea, Ogasawara, Palau, Philippine, Pitcairn, Ryukyu, Samoa, Society, Solomon, Taiwan, Tonga, Tuamotu, Vanuatu, and Wake.

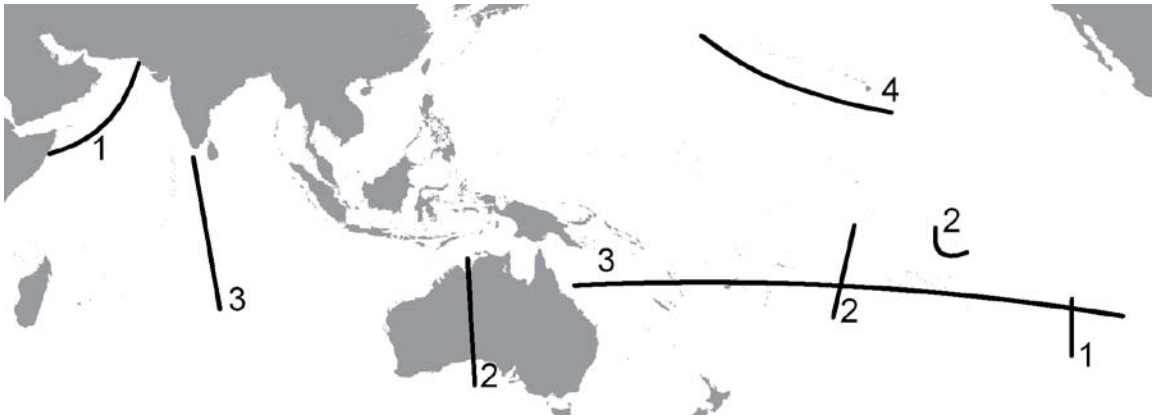

Figure 2-16. Approximate distribution of IWP ESEs. Boundaries separating the ranges of sister taxa -- and thus the location of ESEs -- are drawn as lines. Numbers indicate how many ESEs occur across each of these zones.

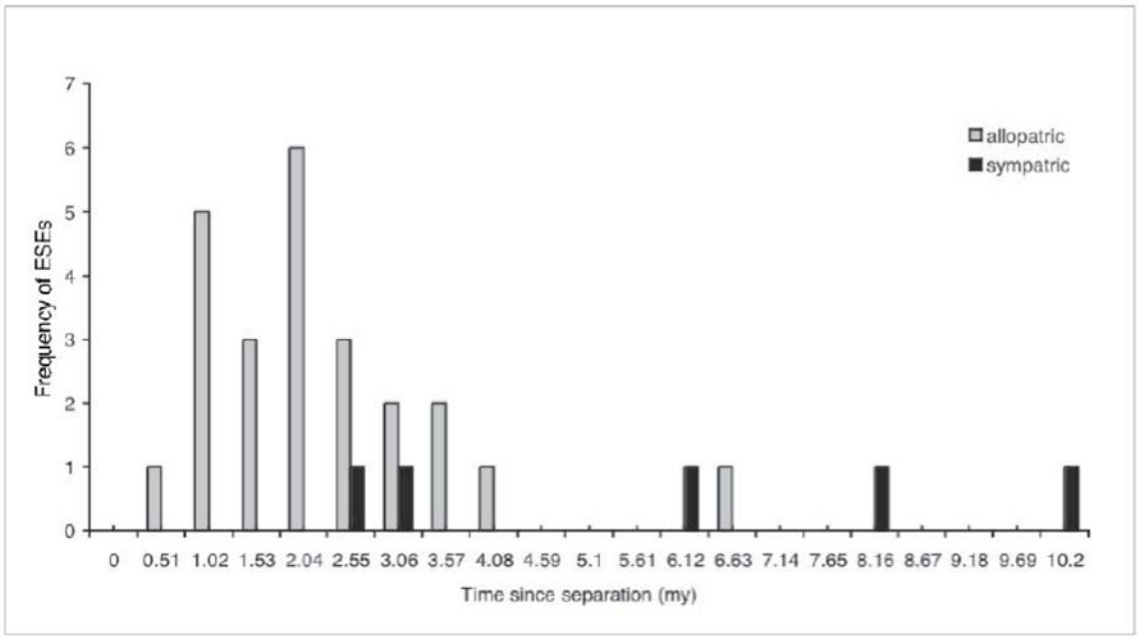

Figure 2-17. Age distribution (in million years, my) of *Calcinus* sister species pairs.

CHAPTER 3
PHYLOGENETIC SYSTEMATICS OF CORAL DWELLING BARNACLES (BALANOMORPHA: PYRGOMATIDAE)

Introduction

The Balanoidea is a large superfamily of both free-living and symbiotic acorn barnacles. Newman and Ross (1976) recognized three balanoid families: the Balanidae, Archaeobalanidae, and Pyrgomatidae. The phylogenetic relationship of these three families are presently not well resolved. Recent molecular and morphological studies (Healy and Anderson 1990; Pérez-Losada et al. 2004; Pérez-Losada et al. 2008) have shown that balanids and archaeobalanids are mutually paraphyletic. The placement and status of the Pyrgomatidae is even less understood, because pyrgomatids were not included in these recent phylogenetic studies.

This study focuses on the phylogenetic systematics within the Pyrgomatidae. The pyrgomatids are the coral-dwelling barnacles; a morphologically and ecologically distinctive group whose members are obligatory symbionts of hard corals (mostly scleractinians as well as a few hydrocorals). Pyrgomatid cyprid larvae settle and metamorphose on the coral surface; however, the details of the settlement process are still unknown. Upon settlement, the barnacle exhibits rapid lateral growth (Utinomi 1943), followed later in life by mostly vertical growth at the margin of the basis. This vertical extension allows the barnacle to keep pace with the vertical growth of the coral host (fig. 3-1). Coral growth over the orifice and wall margins is suppressed through an unknown, possibly chemically-mediated process (Anderson 1992). As the barnacle and its host grow vertically, the barnacle develops a distinctive cup-shaped basis that may extend a considerable length into the coral. Aside from the cup-shaped basis, other features characterizing this family include a tendency towards the development of a

flattened wall and towards the reduction and fusion of skeletal parts. While most balanoids have 6 wall plates, all extant pyrgomatids either have 4-plated walls or a single, fused wall plate (although one extinct pyrgomatid genus, *Eoceratoconcha*, had 6 wall plates). The fused, single wall plate is a character unique to the pyrgomatids. The opercular valves (i.e., scutum and tergum) are also fused in many of the pyrgomatid genera (fig. 3-1). Fusion of skeletal elements has traditionally been one of the most important characters used in delineating pyrgomatid genera and species, and is considered an apomorphic character (e.g., Darwin 1854, Baluk and Radwanski 1967, Ross and Newman 1973).

Taxonomy and Global Biogeography of the Pyrgomatids

The Pyrgomatidae is currently divided into 3 subfamilies: the Ceratoconchinae, Pyrgomatinae, and Megatreminae; each of these groups is discussed in turn below.

The subfamily Ceratoconchinae includes the extinct genus *Eoceratoconcha* and one extant genus, *Ceratoconcha*. *Eoceratoconcha* is known from the early Miocene to the Pliocene in the Caribbean; while *Ceratoconcha* first appeared in the late Oligocene in the Caribbean and attained its peak diversity and geographic range during the Miocene, with species in the tropical Atlantic, eastern Pacific (EP), and Paratethyan region (Ross and Newman 2002). Only 4 *Ceratoconcha* species are extant, and all are limited to the tropical West Atlantic (WA; Table 3-1). The ceratoconchines have never been reported in the present-day Indo-West Pacific (IWP). Morphologically, ceratoconchines are distinguished by a 6-plated (in *Eoceratoconcha*) or 4-plated wall (in *Ceratoconcha*), typically balanoid and unfused opercular valves, and by the common occurrence of a prominent ridge or plate on the carinal segment of the tergum instead of depressor muscle crests (Table 3-2; Ross and Newman 1973).

The Pyrgomatinae is the largest subfamily with 20 genera and 74 extant species (Table 3-1), all found exclusively in the IWP. The pyrgomatines show the highest morphological and ecological diversity among coral-dwelling barnacles, ranging from 4-plated to single-plated taxa, and from typically balanoid opercular valves and cirri to highly derived morphologies (see Table 3-2; reviewed in Ross and Newman 1973; Anderson 1992). Within the pyrgomatines, two tribes were erected for the most distinctive members: Hoekiini (5 genera, 11 species) and Pyrgopsellini (1 genus, 2 species). The hoekines have abandoned planktotrophy in favor of parasitism -- they feed exclusively on coral tissue with enlarged biting mouthparts and have degenerate, non-functional cirri (Ross and Newman 1995). Hoekines are morphologically extremely apomorphic, with highly modified opercular valves, an irregularly-shaped wall, and a partly membranous basis (e.g., the area of the basis directly below the wall is membranous). The pyrgopsellines, on the other hand, largely resemble other pyrgomatines but have an almost entirely membranous basis. Previously thought to be sponge dwellers (Rosell 1975), pyrgopsellines are now known to live suspended in the tissue of scleractinian corals (Achituv and Simon-Blecher 2006). The other 14 pyrgomatine genera have been placed in the catch-all tribe Pyrgomatini, and range from plesiomorphic taxa that largely resemble free-living balanoids in skeletal characters (e.g., *Cantellius*) to more apomorphic genera with fused and modified skeletal parts (e.g., *Trevathana*, *Nobia*, etc.; Table 3-2; fig. 3-1). The earliest fossil records for pyrgomatines date from the late Miocene of the IWP. There are also some Pleistocene and Holocene records; however, fossil records for pyrgomatines are relatively scarce.

All reported fossil pyrgomatines have been classed into extant species (Asami and Yamaguchi 1997; Ross and Newman 2002).

The Megatrematinae is comprised of the tribes Megatrematini and Pyrgominini; each with 2 genera. This small subfamily (6 extant species; Table 3-1) is distributed in the WA, IWP, and east Atlantic (EA), and includes both shallow-water genera on hermatypic corals as well as deeper-water taxa on ahermatypic corals. The megatrematines have a fossil record extending to the Pliocene of the Mediterranean and the Pleistocene of the Caribbean, yet despite their wide distribution have never attained high species diversity (Ross and Newman 2002). All megatrematines possess a fused wall and typically balanoid opercular valves. Members of the tribe Pyrgominini have a tall conical (instead of a flattened) wall, while members of the Megatrematini are distinct in having a trapezoidal beaked tergum (Table 3-2; Ross and Newman 1973; Newman and Ross 1976; Ross and Pitombo 2002).

In summary, the oldest records for the Pyrgomatidae date to the late Oligocene (Newman and Ladd 1974; Ross and Newman 2002). The family has had 2 areas of radiation: one centered in the Caribbean that attained its peak diversity in the Miocene (e.g., the Ceratoconchinae), and a much larger, extant center of diversification in the IWP (e.g., the Pyrgomatinae). The radiation of pyrgomatines appears to have paralleled the radiation of modern scleractinian corals in the tropical IWP; on the other hand, the decline of the ceratoconchines was roughly concomitant with the decrease in numbers of West Atlantic (WA) and East Pacific (EP) corals and the Messinian salinity crisis in the Mediterranean (Veron 1995; Ross and Newman 2002; Ross and Pitombo 2002). In

contrast, the megatrematines have always had a scattered distribution and relatively few species.

The three subfamilies – as well as the tribes, genera, and species constituting them – are mostly defined on the basis of only one to a few morphological characters. Many of these characters relate to skeletal morphology, particularly the fusion and form of the wall and opercular valves. A fused and flattened wall, and fused and modified opercular valves, are considered apomorphic; while 6-plated or 4-plated conical walls, and unfused and typically balanoid opercular valves, are considered plesiomorphic. Given the paucity of characters utilized and diversity of opinion regarding the origins of coral barnacles (see below), it is hardly surprising that the systematics of the pyrgomatids continues to be highly unstable. The numerous new pyrgomatids still being described (22 new species since 2000; table 3-1) is further evidence of our imperfect knowledge of the group.

Phylogenetic Hypotheses Regarding the Pyrgomatids

Earlier barnacle taxonomists essentially espoused non-cladistic views on pyrgomatid evolution, envisioning that extant 'basal' pyrgomatid taxa evolved into other, 'more advanced' forms. For instance, it has been generally accepted that pyrgomatids evolved from other, less morphologically specialized coral-associated balanoids. Several non-pyrgomatid balanoids live obligately on corals (e.g., *Megabalanus ajax* and *M. stultus* (Megabalanidae) on the stinging hydrocoral *Millepora*, Ross 1999; *Tetraclita* sp. (Tetraclitidae) on the blue coral *Heliopora*, Newman and Ladd 1974; *Hexacreusia* spp. (Archaeobalanidae) on *Porites*, Pitombo and Ross 2002; *Armatobalanus* spp. (Archaeobalanidae) on various scleractinians, Pilsbry 1913, Anderson 1992). Of these, the archaeobalanid genus *Armatobalanus* is thought to be closest to the pyrgomatids

(Hiro 1938; Ross and Newman 1973; Healy and Anderson 1990; Anderson 1992; Ross and Newman 2000; Simon-Blecher et al. 2007). *Armatobalanus* is a relatively small taxon (12 species), and not all members of this genus are coral-associated. Characters used to diagnose this genus include 6 wall plates and the presence of teeth on the third or fourth pair of cirri (Pilsbry 1913). Note, however, that at least one *Armatobalanus* species lacks cirral armature (i.e., *A. oryza;* Broch 1931). Moreover, teeth on cirri III or IV are not exclusive to *Armatobalanus* (other examples: the mainly sponge-dwelling family Acastinae, Kolbasov 1993; the gorgonian- or antipatharian-associated archaeobalanid *Conopea cymbiformis*, Pilsbry 1913).

In their review of the Pyrgomatidae, Ross and Newman (1973) suggested that the 3 pyrgomatid subfamilies could represent balanoid lineages that independently colonized corals. They based their argument on the subfamilies' distributions and also highlighted the plesiomorphic appearance of *Ceratoconcha* relative to the pyrgomatines. In contrast, monophyly of the family was supported by Healy and Anderson (1990) and Anderson (1992) on the basis of sperm structure and skeletal and cirral morphology, respectively. Ross and Newman (2002) likewise espoused the hypothesis of monophyly, and proposed that the pyrgomatids may have arisen in the western Tethys during the Palaeogene, thus implying that pyrgomatines and ceratoconchines may both be Tethyan relicts.

It has also been suggested that the morphologically plesiomorphic genus *Cantellius* represents the 'ancestral stock' from which other pyrgomatine genera evolved, and that several extant *Cantellius* species independently gave rise to other pyrgomatine genera (e.g., Ross and Newman 1973; Anderson 1992). While the idea of

extant species giving rise to other Recent lineages no longer conforms to present-day concepts of evolutionary mechanisms, the prevailing belief is that pyrgomatids are derived from a morphologically generalized, 6-plated ancestor, which evolved into a 4-plated form, and then into more morphologically derived forms (e.g., Healy and Anderson 1990, Anderson 1992).

These and other evolutionary hypotheses were recently reviewed and tested by Simon-Blecher et al. (2007). Using 3 genes (16S, 12S, and 18S), Simon-Blecher et al. (2007) recovered a non-monophyletic Pyrgomatidae: one genus (*Wanella*) fell outside of the pyrgomatid clade; while the presumed closest outgroup (*Armatobalanus*) fell within the pyrgomatid clade and sister to the morphologically plesiomorphic genus *Cantellius*. However, these relationships were only weakly supported, and the authors were not able to reject the alternative hypothesis of a monophyletic Pyrgomatidae. Overall, the phylogeny of the family is still not well resolved, and this paper attempts to shed greater light on pyrgomatid evolution.

Materials and Methods

Taxon Selection and Identification

Specimens were collected by scuba diving in reef sites spanning the IWP, as well as at locations in the WA and EA. Table 3-3 lists the samples that were sequenced for this study. I included 64 specimens, including ~16 ingroup genera (80% of all genera), as well as 10 outgroup specimens. I chose outgroups to represent a broad range of both free-living and symbiotic balanoids. The majority of the specimens are deposited in the Invertebrate Zoology collections of the Florida Museum of Natural History, University of Florida (UF). Additional specimens or tissue samples were borrowed from other institutions, and further supplemented by sequences accessed from GenBank (Table 3-

3). Three samples were identified as *Armatobalanus* with high confidence by barnacle experts Yair Achituv (Bar-Ilan University, Israel), Gregory Kolbasov (Moscow State University, Russia), and Andrew Hosie (Western Australian Museum, Australia; Achituv pers. comm.; Hosie pers. comm.), while the *Pyrgopsella youngi* sample was identified by Yair Achituv. I identified most pyrgomatid specimens using the primary taxonomic literature. Because the species level taxonomy of coral-dwelling barnacles is problematic, many samples could not be identified to the species level with a high degree of confidence. Thus for the purposes of this study I conservatively only identified specimens to the genus level, and instead confined the analyses to reciprocally monophyletic clades with strong branch support (strong support is herein arbitrarily set at bootstrap values $\geq$60% and/or posterior probabilities $\geq$90%).

Genetic analysis started by first sequencing a wide range of pyrgomatids (>350 specimens) for the mitochondrial gene cytochrome oxidase I or COI. Based on these initial results, I selected representatives of each genus to cover as much intrageneric genetic diversity as possible. These representative taxa were sequenced for 4 additional genes (mitochondrial genes 16S ribosomal DNA and 12S ribosomal DNA; nuclear genes 18S ribosomal DNA and Histone 3 or H3; see Table 3-3).

Molecular Methods

Two different protocols were employed in two labs for extracting and amplifying DNA. At the Smithsonian Institution's Laboratories of Analytical Biology (LAB), barnacle tissue was first digested overnight in 150 ul M2 buffer and 150 ul M1 + proteinase K buffer at 56.5°C and 50 rpms (revolutions per minute). Extraction was done using an automated phenol-chloroform extraction (Autogen AutoGenprep 965 Automated DNA Isolation System). PCRs were performed on an ABI 2720 Thermal Cycler or MJ

Research PTC-225 Peltier Cycler. The PCR profile used for amplifying COI was as follows: an initial denaturation at 95ºC for 5 minutes, followed by 35 cycles of denaturation at 95ºC for 30 sec, annealing at 48ºC for 30 seconds (sec), elongation at 72ºC for 45 sec, and a terminal elongation at 72ºC for 5 mins. PCR products were cleaned using ExosapIT (from USB). In-house sequencing was done in a 96-well format using ABI BigDyeTerminator cycle sequencing reactions. The reactions were cleaned using Sephadex G-50 (Sigma Aldrich), and run on an ABI-3730-XL DNA analyzer. All PCR products were sequenced along both directions. Only COI was sequenced at the LAB.

At UF, DNA was extracted from barnacle muscle tissue using DNAzol and proteinase K following the protocol given in Meyer (2003). DNA extracts were cleaned using QIAGEN cleanup kits. Sequence data was collected for three mitochondrial gene fragments (COI, 16S, and 12S) and two nuclear markers (18S and H3). Primers and PCR protocols followed Pérez-Losada et al. (2004; for H3 and 18S), Meyer (2003; for COI and 16S), and Simon-Blecher et al. (2007; for 12S). PCR products were cleaned using the exo-sap cleanup protocol and sequenced at the high-throughput sequencing facility of the University of Florida's Interdisciplinary Center for Biotechnology Research (ICBR) in a 96-well format using BigDyeTerminator cycle sequencing reactions, employing an ABI-3730-XL for electrophoresis. All PCR products were sequenced along both directions.

Phylogenetic Analyses

Chromatograms were checked and manually edited using the software Geneious Pro 4.9.2 (Drummond et al. 2009). Sequence alignment was done either: (a) completely manually for all gene regions, using Se-Al v2.0a11 (Rambaut,

http://tree.bio.ed.ac.uk/software/seal/); or (b) using MAFFT v.6.717 (Katoh et al. 2002) for the 3 non-coding gene fragments (16S, 18S, and 12S) and manually for the 2 coding genes (COI, H3). In the MAFFT alignments, I used the L-INS-i search strategy and the following parameters: scoring matrix for nucleotide sequences=1PAM/κ=2; gap opening penalty=1.53; offset value=0.1. Phylogenies resulting from both manually-aligned and software-aligned sequences were compared for topological congruence. Since no incongruences were found, and automated alignment is more objective, the MAFFT analysis was used for all downstream analyses. In addition, higher branch support values were obtained from the MAFFT-aligned dataset (data not shown) than from the manual alignment.

In all of the sequence analyses, all sites were weighted equally, characters were unordered, and gaps were treated as missing data. Both maximum likelihood (ML) and Bayesian approaches were used for phylogenetic reconstruction. First I determined the simplest model of evolution that best fit the 5-gene dataset using the Akaike Information Criterion (AIC) as implemented by the program Modeltest 3.6 (for ML analyses; Posada & Crandall 1998). Maximum likelihood analyses were implemented using PAUP* ver. 4.0b10 (Swofford 2002) and RAxML 7.0.4 (Stamatakis 2006). In the PAUP* analyses, heuristic searches started with random addition of taxa replicated 10 times using the tree-bisection-reconnection (TBR) branch-swapping algorithm. For the RAxML analyses, I performed 1,000 rapid bootstrap inferences followed by a thorough ML tree search. A GTRGAMMA model and a random starting tree were utilized.

Bayesian analyses were performed using MrBayes v3.1.2 (Huelsenbeck & Ronquist 2001; Ronquist & Huelsenbeck 2003) using GTR+I+GAMMA models and flat

priors. For the 5-gene concatenated dataset, the dataset was partitioned prior to analysis (the partitions corresponded to the 5 sequenced gene regions), parameters and models of evolution in each partition were unlinked. I ran 2 independent chains for 1 million generations each; each chain was sampled every 100 generations. All runs reached stationarity before 100,000 generations. The initial 25% of the trees was discarded as the burn-in phase, and posterior probabilities were calculated based on the remaining 75% of the trees.

To determine the appropriateness of concatenating the 5 gene regions into a single analysis, I visually compared Bayesian and ML tree topologies from independent searches for each of the 5 gene regions. No strongly supported incongruences were found, and all downstream analyses were performed on the concatenated dataset.

Topological Tests

The highest-scoring ML and Bayesian trees showed uncertainties in the phylogenetic placement of the genus *Wanella*, and in the monophyly of the archaeobalanid *Armatobalanus* (see Results section). In order to further explore these results, I investigated whether the data were consistent with two alternative phylogenetic hypotheses: (1) that *Wanella* is monophyletic with the rest of the Pyrgomatidae (hypothesis “pyrgomatidae_monophyletic”); and (2) that *Armatobalanus* is monophyletic (hypothesis “armato_monophyletic”). To evaluate these alternative hypotheses, I investigated whether the 5-gene concatenated tree with the highest ML score (obtained using PAUP*) was significantly better than topologies constrained to conform with the “pyrgomatidae_monophyletic” and “armatobalanus_monophyletic” hypotheses. This comparison was done using the non-parametric Shimodaira-Hasegawa (S-H) test (Shimodaira and Hasegawa 1999; Goldman et al. 2002). The S-H

test was implemented in PAUP* by comparing the best-scoring ML tree to an ML tree where pyrgomatids were constrained to be monophyletic, as well as to additional best-scoring trees obtained using the maximum parsimony algorithm. The number of nonparametric bootstrap replicates was set to 1,000 and a RELL approximation was used.

I also computed the percentage of the post-stationarity Bayesian trees (from the 5-gene concatenated analysis) that conformed with each of the two topological constraints tested. This percentage, divided by the total number of post-stationarity trees, gives the posterior probability of the hypothesis being tested (following http://insects.oeb.harvard.edu/farrell_lab/techniques/pa_hypothesis.html).

Character Tracing and Morphological Evolution

I analyzed morphological evolution in pyrgomatids by concentrating on phenotypic features related to fusion and simplification of shell structures, as well characters related to the interactions between the barnacle and its coral host. The following seven characters were examined: (1) fusion of opercular valves, (2) number of wall plates, (3) height of wall plates, (4) calcification of basis, (5) presence of teeth on the anterior margin of the 3rd pair of cirri, (6) amount of coral overgrowth on the wall, and (7) mechanical vs. non-mechanical prevention of coral overgrowth. Representatives of the different character states are illustrated in fig. 3-2. While most of the phenotypic characters are straightforward, the two characters related to control of coral overgrowth require some explanation. In most pyrgomatid species, the coral overgrows the barnacle wall all the way to the aperture. Coral overgrowth is typically a thin layer coral tissue (t) as well as bits of calcareous coral material (c). However, in some individuals the area surrounding the barnacle aperture is free of calcareous material, or free of both

coral tissue and calcareous material. There are also pyrgomatid species whose walls are entirely overgrown by coral tissue but do not exhibit any deposition of calcareous coral material (see fig. 3-2).

To prevent the host from overgrowing the aperture, barnacles can mechanically scrape off the coral using their opercular valves and cirri; alternatively, control of overgrowth can be by non-mechanical means. The latter is thought to be common in pyrgomatids (Anderson 1992). To determine the mechanism of overgrowth control, I examined the edge of the barnacle orifice microscopically. When the edge of the coral growth on the aperture was rough, I interpreted that to indicate that coral overgrowth is prevented by the barnacle using mechanical means. When the edge was smooth, or if calcareous deposition by the coral stopped some distance away from the orifice, I interpreted that to indicate that control of coral overgrowth is probably non-mechanical. Potential non-mechanical controls may involve chemically mediated communication or control by the barnacles on the coral margins, as suggested by Anderson (1992), but this remains to be tested.

Specimens were removed from the host coral using a hammer and chisel, pliers, or a blunt dental probe, then dissected using fine tungsten needles under a stereomicroscope. All shell characters were scored under a stereomicroscope. To examine cirral armature, cirrus III was excised, mounted on a slide, and examined under a compound microscope. Whenever possible, I examined both the sequenced individual as well as other conspecific barnacles co-occurring on the same coral colony. When specimens were unavailable, observations were supplemented by reports from the published literature (e.g., Pilsbry 1916, Achituv and Simon-Blecher 2006).

The character history was traced onto the pyrgomatid phylogeny using the software package Mesquite 2.72 (Maddison and Maddison 2009). The parsimony model used to reconstruct the number of wall plates was a weighted step matrix where reversal of wall fusion (i.e., from 1 plate back to 4 plates, and from 4 plates to 6 plates) cost one more step than wall plate fusion. With the exception of wall plates, all other characters were specified as unordered, and character gains and losses were assumed to have equal weights. For wall plate fusion, I decided to apply a step matrix model of evolution because fusion of wall plates is well established as a prevailing and recurring theme in barnacle evolution (e.g., Newman 1987); for instance the Mio-/Pliocene genus *Eoceratoconcha* was 6-plated (Zullo and Portell in litt.). The phylogram used for tracing characters was obtained from the RaxML analysis. Nodes with <80% bootstrap support and also <95% Bayesian posterior probability were collapsed.

To determine whether a phenotypic trait is phylogenetically structured, I calculated the parsimony score (PS; Fitch 1971), and association index (AI, Wang et al. 2001) using the software BaTS v1.0 (Parker et al. 2008). I evaluated the null hypothesis that the trait shows no phylogenetic structure. BaTS explicitly accounts for phylogenetic uncertainty by calculating and averaging phylogeny-trait association statistics across a posterior sample of trees (PST) generated by Bayesian Markov chain Monte Carlo (MCMC) programs. For each trait analyzed, a new set of 5-gene concatenated Bayesian phylogenies were used as input trees; each dataset was first trimmed to exclude specimens with unknown/unrecorded character states. One hundred replicates of state randomizations were used to calculate the null distributions of the statistics.

Results

Sequence Characteristics

The length of the gene fragments, number of parsimony-informative sites, number of invariant sites, and the best likelihood models are presented for each gene fragment in Table 3-4. Parsimony-informative nucleotide sites totaled 173 base pairs (bp) for nuclear genes (H3 and 18S) and 486 bp for mitochondrial genes (COI, 16S, and 12S).

Phylogenetic Relationships within the Pyrgomatidae and Congruence of Gene Trees

Comparison of phylogenies for each of the 5 sequenced markers showed that individual gene trees were largely congruent (Figs. 3-3 to 3-7). The gene trees were also essentially congruent with the combined mitochondrial-only and nuclear-only topologies (Fig. 3-8 to 3-9), and with analyses of the 5 concatenated markers (Fig. 3-10). However, some incongruences were noted, notably with respect to the placement of the fire coral-associated genus *Wanella* and of the archaeobalanid outgroup *Armatobalanus*. Instances of topological incongruences are listed in Table 3-5. The 18S gene tree was responsible for most of the deviations. Note, however, that while high posterior probability values in the 18S tree (Fig. 3-6) indicate strong topological support, in actuality only 4% of the 18S data is informative (Table 3-4).

All analyses identified several well-supported clades ('well supported' herein defined as $\geq$80% BS and/or $\geq$99% PP; Fig. 3-10). Most pyrgomatine genera – including the most apomorphic groups – clustered in Clade I. *Adna* and *Ceratoconcha* (representing the subfamilies Megatrematinae and Ceratoconchinae, respectively), were consistently recovered as sister taxa (Clade II). Clade III was comprised of the plesiomorphic genus *Cantellius*, and in most cases 2 specimens of the archaeobalanid

‘outgroup’ *Armatobalanus*. Lastly, the fire coral-associated genus *Wanella* did not usually cluster with other pyrgomatids in most of the topologies, and was designated Clade IV. Relationships between the different clades were not well resolved. Clades II-IV each contained only one or two genera; whereas Clade I contained nine genera. Many pyrgomatid genera and tribes were recovered as reciprocally monophyletic units with high branch support, but 6 were not: *Pyrgopsella*, Hoekiini, *Neotrevathana*, *Trevathana*, *Galkinia*, and *Hiroa*). Within clade I, the phylogeny recovered a large subclade (herein designated the *Trevathana sensu lato* subclade) comprised of *Trevathana*, *Neotrevathana*, *Pyrgopsella*, and the Hoekiini. The ‘genera’ that make up this subclade were not recovered as reciprocally monophyletic units.

Placement of Outgroup Taxa

Four different sequences identified as *Armatobalanus* were included in the analyses. None of the gene trees nor any of the concatenated analyses, recovered a well-supported monophyletic *Armatobalanus* clade (Figs. 3-3 to 3-10; Table 3-5). “*Armatobalanus allium*” (KACb154) and “*Armatobalanus* sp.” (UF 11887) were often recovered as sister to the pyrgomatid genus *Cantellius*; “*A. allium*” (TAU Ar27835) was resolved as sister to the balanid genus *Megabalanus* in most trees including the 5-gene concatenated analyses; while “*Armatobalanus* sp.” (KACb163) also came out with the outgroup specimens yet did not group with the previous specimen. There are several possible reasons for this unexpected pattern, including DNA contamination, specimen misidentification, or non-monophyly of *Armatobalanus*. I consider contamination to be unlikely because none of the *Armatobalanus* sequences are identical (or even very close to) the other barnacle sequences. Specimen misidentification is possible, particularly since I did not have an opportunity to compare putative *Armatobalanus*

specimens side by side. Polyphyly of *Armatobalanus* is also very possible, particularly since (to my knowledge) there is no publication that comprehensively reviews the genus and compares it to morphologically similar groups, nor am I aware of any synapomorphies that unite the genus. A phylogenetic reappraisal of *Armatobalanus* is beyond the scope of this study.

Balanus glandula (Balanoidea: Balanidae) did not group with *Megabalanus*, the other balanid genus represented in the phylogeny. Instead *B. glandula* was consistently recovered as sister to the archaeobalanid *Semibalanus balanoides*. This agrees with previous phylogenetic studies suggesting that the taxonomic division between the families Balanidae and Archaeobalanidae may not be valid (Healy and Anderson 1990; Pérez-Losada et al. 2004; Pérez-Losada et al. 2008).

Topological Tests

I used topological tests to investigate whether the Pyrgomatidae is monophyletic and also whether the 'outgroup' *Armatobalanus* is monophyletic. While the S-H test rejected the 2 most parsimonious trees (MPTs), it was unable to reject the hypothesis of a monophyletic Pyrgomatidae, nor of a monophyletic *Armatobalanus* (Table 3-6).

In contrast to the S-H test results, I found that of the 15,000 post-stationarity trees obtained from Bayesian analysis, not one of the trees conformed to the hypothesis of a monophyletic Pyrgomatidae. Neither did any of the trees conform to the hypothesis of a monophyletic *Armatobalanus*.

Character Tracing and Phylogeny-Trait Correlation

Figures 3-11 to 3-17 show the parsimony reconstructions of phenotypic traits on the pyrgomatid phylogeny. Assuming symmetrical rates of character gains and losses, fusion of the opercular valves is estimated to have occurred approximately 4-5 times. All

instances of valve fusion were recovered in Clade I, three in the *Trevathana sensu lato* subclade alone. There is at least one instance of reversal from fused opercular valves to unfused valves (i.e., *Hiroa*; fig. 3-11).

Fusion of 4 wall plates to a single plate is estimated to have evolved twice, once in Clade I and another time in Clade IV (*Wanella*). The ancestral state of Clade I was reconstructed as single-plated. Two instances of reversals from 1 plate to 4 wall plates were recovered. In addition, there is one instance of fusion from 6 plates to four plates, in Clade III (fig. 3-12).

The height of the wall (e.g., from high conical to flat) shows multiple state changes within clades, genera, and even single species (fig. 3-13). Nearly all pyrgomatids possess a wholly calcareous basis; however, a partly- to fully-membranous basis appear to have evolved 3-4 times, all in Clade I (fig. 3-14).

Figure 3-15 plots the presence or absence of teeth on the anterior margin of cirrus III. All members of Clades II and III had cirral teeth. This character was absent from all other clades. However, cirrus III teeth were present all specimens identified as *Armatobalanus* spp., including both those included in Clade III and those that fell outside Pyrgomatidae *sensu stricto*.

Coral overgrowth in most of the specimens examined consisted of both coral tissue and calcareous deposition on the barnacle walls. However, in ~5-7 instances coral overgrowth was limited to coral tissue only (i.e., without coral skeletal deposition on the barnacle wall), and in several other instances calcareous and/or tissue deposition stopped before reaching the barnacle aperture. While this character is

variable within genera and even species, all instances of coral suppression occurred in Clades I and II (fig. 3-16).

Figure 3-17 plots the incidence of apparent mechanical and non-mechanical control of coral overgrowth over the barnacle aperture. Clades II and IV showed evidence for mechanical erosion of coral overgrowth, while in all other pyrgomatids control of coral overgrowth was apparently non-mechanical.

The BaTS analyses rejected the null hypothesis of no phylogenetic structure for all of the characters investigated (Table 3-7).

Discussion

Systematics

The phylogeny recovered 3 major pyrgomatid clades (clades I-III) that together clearly form a monophyletic unit, i.e., the Pyrgomatidae sensu stricto. However, the genus *Wanella* (Clade IV) may or may not be the sister taxon of Clades I-III. The new phylogenetic results contradict classical hypotheses of pyrgomatid taxonomy in several fundamental ways. First, the traditional pyrgomatid subfamilies (the Ceratoconchinae, Pyrgomatinae, and Megatrematinae) were not resolved as the basal clades of the family. Second, the division of the Pyrgomatinae into 3 tribes (Pyrgomatini, Pyrgopsellini, and Hoekiini) was likewise not supported by the phylogenetic evidence. The hoekiines and pyrgopsellines, although highly apomorphic morphologically and ecologically, are nested within the large pyrgomatine genus *Trevathana*. Third, the hydrocoral associate *Wanella* was not resolved as being part of the pyrgomatid ingroup, and its affinities to the main clade of pyrgomatids are unstable. Fourth, *Armatobalanus* was not recovered as monophyletic, and although the specimen used in Simon-Blecher et al. (2007) was resolved as an outgroup to the Pyrgomatidae (as expected from the

traditional classification), two other specimens were resolved as sister to *Cantellius* and well within the main pyrgomatid clade.

Some of the shallower portions of the tree correspond with earlier hypotheses. The tree recovers the '*Savignium* group' of Ross and Newman (1973), with the exception of *Wanella*, which was included in the Savignium group by Ross and Newman (1973). While many nominal genera were well resolved phylogenetically, 6 were not (i.e., *Pyrgopsella*, *Hoekiini*, *Neotrevathana*, *Trevathana*, *Galkinia*, and *Hiroa*). All the unresolved genera were in Clade I.

The following groupings were recovered within Clade I: (*Nobia*), (*Pyrgoma*,(*Galkinia*,*Hiroa*),*Darwiniella*), and (*Savignium*, *Trevathana sensu lato*). The topology largely agrees with the results of Simon-Blecher et al. (2007), except in the present study *Hiroa* and *Galkinia* were strongly resolved as sister taxa (*Galkinia* – erroneously called *Creusia* by Simon-Blecher et al. 2007 – was resolved as sister to a *Hiroa-Darwiniella* clade in Simon-Blecher et al. 2007). Within the *Trevathana sensu lato* subclade, *Neotrevathana* was resolved as diphyletic (in contrast to the findings of Simon-Blecher et al. 2007), and the tribes Pyrgopsellini and Hoekiini were both nested within *Trevathana sensu lato* (in agreement with Simon-Blecher et al. 2007).

Clade II unites the only East Atlantic (EA) genus, *Adna* (Megatrematinae), with *Ceratoconcha* (Ceratoconchinae), one of 2 extant WA genera. In the future it will be interesting to sequence the missing megatrematine groups (i.e., the WA/IWP *Megatrema* and the IWP *Memagreta* and *Pyrgomini*) to test whether the megatrematines truly form a monophyletic clade spanning the WA, EA, and IWP. Such a result would imply that diversification occurred across these 3 biogeographic regions.

An alternative hypothesis might be that the two WA genera, *Ceratoconcha* and *Megatrema*, are sister-taxa, implying that diversification occurred within the WA.

Clade III unites *Cantellius* with 2 of the four sequenced *Armatobalanus* outgroups. These results have two implications (assuming samples had not been mis-identified): (1) *Armatobalanus* as presently defined may be polyphyletic; and (2) the Pyrgomatidae needs to be re-circumscribed. There are also indications that Clade III may be sister to Clade II, and although branch support for this relationship is low, a new morphological character supports such a grouping (see next section). Overall, the results show that the systematic relationships within the Pyrgomatidae need to be thoroughly re-examined.

Character State Evolution

The ancestral character state reconstructions suggest that fusion of shell structures (wall and opercular valves) and reduction in basis calcification evolved multiple times in pyrgomatids, with several instances of reversals from more fused to less fused character states. However, these ancestral state reconstructions should be interpreted with some caution, because the model of evolution used in tracing characters may not be biologically realistic. The state reconstructions (excepting wall plate fusion) are based on the assumption that character gains and losses are equally probable. While this might not be a realistic model, given the absence of fossil information on character states of ancestral pyrgomatids, any model of morphological evolution applied to the barnacles would be mostly conjectural. For the number of wall plates, a step matrix model of evolution was deemed appropriate since there is abundant fossil evidence indicating that wall fusion is a recurrent theme in barnacle evolution (e.g., Newman 1987).

Even though ancestral character state reconstructions are equivocal, what emerges clearly from data is that changes in shell characters are phylogenetically structured. A tendency towards fusion and reduction of skeletal elements particularly characterizes Clade I pyrgomatids. All known instances of opercular valve fusion and reduction in basis calcification occurred in Clade I. Passageways or membranous zones in the pyrgomatid basis have been proposed to function in barnacle-coral communication (Ross and Newman 1973; Ross and Newman 2000) or perhaps in the direct uptake of dissolved nutrients from the host (Anderson 1992; Ross and Newman 1995). Alternatively, an incompletely calcified basis could be less energetically costly, and may permit more rapid growth (Ross and Newman 1995). A similar reduction in shell calcification has been noted in sponge barnacles (Acastinae), and in this group splits or 'windows' between wall plates were also suggested to be involved in barnacle-host communication (Kolbasov 1993).

A single wall plate seems to have arisen independently in members of Clade I and in *Wanella* (Clade IV). Another instance of wall fusion was documented in Clade III. Surprisingly, Clade III contains both 4-plated and 6-plated morphs. Previously, the only 6-plated pyrgomatid known was *Eoceratoconcha*, from the Miocene to Pliocene in the WA. The fact that *Cantellius* is sister to another 6-plated coral-dwelling barnacle (the 'archaeobalanid' *Armatobalanus*) implies that the development of 4 wall plates may have occurred more than once. It has been suggested that fusion of wall plates, particularly the development of a single-plated wall, may be an adaptation allowing pyrgomatids to better withstand the lateral pressure exerted by the coral as it grows,

and that the single-plated wall in *Wanella* and in Clade I pyrgomatids is a result of convergent evolution (Simon-Blecher et al. 2007).

Characters related to the control of coral overgrowth are also phylogenetically structured. One surprising result in this study was that all specimens from Clades II and III that were examined bore teeth on the anterior margins of the 3rd pair of cirri, while none of the pyrgomatids in the other clades had cirral armature. Cirral teeth are probably used to rasp away coral overgrowing the barnacle aperture (Anderson 1992). Cirral armature was previously believed to be a feature characteristic of *Armatobalanus*; its presence in Cantellius was considered by Anderson (1992) to be 'vestigial' and limited to only 2 species. Recent taxonomic papers have often included cirral characters, and yet cirral teeth have not been reported in newly-described species of Clade III *Cantellius* (e.g., Achituv and Hoeksema 2003; Achituv et al. 2009). To my knowledge, this is the first report of cirral armature in the Clade II genera *Adna* and *Ceratoconcha*. Interestingly, Clades II and III appear to be sister-groups in most phylogenetic reconstructions; however, branch support for this relationship was low. The fact that members of both clades bear teeth on cirrus 3 suggests that cirral armature may be a synapomorphy uniting these two clades. Note, however, that cirral armature is not limited to clades II and III only: among the outgroups there are 2 additional '*Armatobalanus'* specimens that also bore teeth on cirrus 3; and the acastine specimen also possessed teeth but on the 4th pair of cirri.

Coral overgrowth in most of the pyrgomatids examined consisted of a layer of coral tissue and calcareous material extending from the perimeter of the barnacle wall to the edge of the aperture. However, there were also instances where calcareous

deposition stopped some distance away from the barnacle aperture, such that the aperture rim was covered only by coral tissue, or was completely bare of coral-derived material. I also observed that in some pyrgomatids the walls were only covered by coral tissue without any calcareous deposition. These variations in coral overgrowth seem to represent different ways by which a pyrgomatid suppresses coral deposition on the wall and aperture. All instances of coral overgrowth suppression clustered in Clades I and II, raising the possibility that suppression of overgrowth may have evolved independently in Clades II and III.

Incidences of mechanical control of coral overgrowth clustered in Clades II and IV. This is evidenced by abrasions on the portion of the coral skeleton overgrowing the rim of the aperture. Curiously, this character does not completely mirror the results on cirral armature: while clade III species bear cirral teeth, they apparently exert non-mechanical control on coral overgrowth. Cirral teeth in *Cantellius* (Clade III) may indeed be a vestigial non-functional character, as suggested by Anderson 1992. Conversely, Clade IV (*Wanella*) shows evidence of mechanical control, and yet lacks cirral teeth. It is possible that *Wanella* mechanically abrades coral overgrowth using the occludent margins of the opercular valves instead of cirral teeth. Only members of Clade II exhibit both cirral armature and mechanical control of coral overgrowth.

Evolution of Parasitism

The only known pyrgomatid eu-parasites were resolved as members of Clade I, specifically within the *Trevathana sensu lato* subclade. The tribe Hoekiini are the ‘coral-eating barnacles’, a bizarre and highly host-specific taxon with reduced, nonfunctional cirral nets and large biting mouthparts that are used to feed on the coral tissue that completely covers its wall and orifice (Ross and Newman 1995). Both the soft parts and

shell characters of hoekiines are extremely apomorphic, hence their placement in the midst of *Trevathana sensu lato* was one of the more surprising results from phylogenetic analyses (e.g., Simon-Blecher et al. 2007 and this study). The present study also shows that despite their extreme morphological apomorphy and ecological divergence, hoekiines are not a long branch on the phylogeny, suggesting that the group may actually have evolved quite recently from a *Trevathana*-like ancestor. An alternative explanation for the relatively short hoekine branch may be that the lineage is relatively older, but is somehow evolving at a slower rate than other pyrgomatids; although an evolutionary transition to parasitism is more commonly associated with an increase, rather than a decrease, in the tempo of molecular evolution (e.g., Dowton and Austin 1995).

Conclusions and Recommendations

This study recovered 3 major clades within the Pyrgomatidae sensu stricto: Clade I, containing most genera from the subfamily Pyrgomatinae (excepting *Wanella* and *Cantellius*) is exclusively IWP, tends towards fusion and reduction of skeletal elements, and controls coral overgrowth through non-mechanical means. This group includes the only known pyrgomatid eu-parasite, the 'tribe' Hoekiini, which may be a recently evolved lineage within the large *Trevathana sensu lato* subclade. Clade II is currently known from the WA and EA, possesses unfused opercular valves, 4-plated or 1-plated walls, and uses mechanical means to control coral overgrowth. Clade III is exclusively IWP and possesses unfused opercular valves, 6-plated or 4-plated walls, and appears to exert non-mechanical control on the growth of its coral host. Among the pyrgomatids, only Clades II and III bear teeth on the anterior margins of cirrus 3; although this character has also been noted in other, non-pyrgomatid balanoids.

So what is a pyrgomatid? All pyrgomatids are coral-dwellers, and yet not all balanoids living on corals belong to the family Pyrgomatidae (e.g., non-ingroup '*Armatobalanus'* spp., *Hexacreusia* spp., *Megabalanus* spp., etc.). Some pyrgomatids control coral overgrowth using mechanical means, but most species are able to suppress coral overgrowth through non-mechanical, possibly chemical, mechanism(s). While a single, fused wall is believed to be an exclusively pyrgomatid trait, possible non-monophyly of the 1-plated *Wanella* with the Pyrgomatidae *sensu stricto* suggests that a concrescent wall plate may not be restricted to the pyrgomatids. Previously it was also thought that all of the living pyrgomatid species had no more than 4 wall plates, but the fact that some (but not all) members of the 6-plated archaeobalanid genus *Armatobalanus* are sister to *Cantellius* proves that 6-platedness does occur in the Pyrgomatidae. However, it does appear that fusion of the opercular valves is a character exclusively found in pyrgomatids, in particular among members of Clade I.

In the future it would be very interesting to re-evaluate the status of the genus *Hexacreusia*, an exclusively coral-dwelling archaeobalanid from the Gulf of California. Zullo (in litt. in Newman 1996) suggested that *Hexacreusia* be placed in the Pyrgomatidae; however, Ross and Newman (2000) did not agree because *Hexacreusia* is 6-plated and uses mechanical means to break coral overgrowth – characteristics it shares with *Armatobalanus*. Since (some members of) *Armatobalanus* have now been shown to belong within the Pyrgomatidae, *Hexacreusia* might also very well be pyrgomatids as well. If so, that would mean that the Pyrgomatidae has extant members in the East Pacific as well.

Table 3-1. List of recent pyrgomatid species. Type species are marked with an asterisk.

Family Pyrgomatidae Gray 1825

- Subfamily Ceratoconchinae Newman and Ross 1976
  - *Ceratoconcha* Kramberger-Gorganovic 1889
    - *domingensis* (Des Moulins 1866)
    - *floridana* (Pilsbry 1931)
    - *paucicostata* Young 1989
    - *quarta* (Kolosvary 1947)
- Subfamily Pyrgomatinae (Gray 1825)
  - Tribe Pyrgomatini Gray 1825
    - *Arossella* (Anderson 1993)
      - *lynnae** Ross 2000
    - *Cantellius* Ross and Newman 1973
      - *acutum* (Hiro 1938)
      - *albus* Ren 1986
      - *alphonsei* Achituv 2001
      - *arcuatum* (Hiro 1938)
      - *brevitergum* (Hiro 1938)
      - *cardenae* Achituv and Hoeksema 2003
      - *euspinulosa* (Broch 1931)
      - *gregarius* (Sowerby 1823)
      - *hiroi* Galkin 1982
      - *hoegi* Achituv, Tsang, and Chan 2009
      - *iwayama* (Hiro 1938)
      - *madreporae* (Borradaile 1903)
      - *maldiviensis* Galkin 1982
      - *octavus* Ross and Newman 1973
      - *pallidus* (Broch 1931)
      - *preobrazhenskyi* Galkin 1982
      - *pseudopallium* (Kolosvary 1947)
      - *quintus* Ross and Newman 1973
      - *secundus* (Broch 1931)
      - *septimus* (Hiro 1938)
      - *sextus* (Hiro 1938)
      - *sinensis* Ren 1986
      - *sumbawae* (Hoek 1913)
      - *transversalis** (Nilsson-Cantell 1938)
      - *tredecimus* (Kolosvary 1947)
    - *Cionophorus* Ross and Newman 1999
      - *guillaumae* Achituv and Newman 2002
      - *soongi** Ross and Newman 1999
    - *Creusia*Leach 1817
      - *spinulosa** Leach 1818

Table 3-1. Continued

Family Pyrgomatidae Gray 1825

Darwiniella Anderson 1992
*conjugatum** (Darwin 1854)
Galkinia Ross and Newman 1995
angustiradiata (Broch 1931)
decima (Ross and Newman 1973)
*indica** (Annandale 1924)
supraspinulosa Ogawa 2000
Hiroa Ross and Newman 1973
*stubbingsi** Ross and Newman 1973
Neopyrgoma Ross and Newman 2002
*lobata** (Gray 1825)
*Neotrevathana*Ross 1999
elongatum (Hiro 1931)
Nobia Sowerby 1839
*grandis** Sowerby 1839
halomitrae (Kolosvary 1947)
orbicellae (Hiro 1934)
Pyrgoma Leach 1817
*cancellatum** Leach 1818
japonica Weltner 1897
kuri Hoek 1913
projectum Nilsson-Cantell 1938
sinica (Ren 1986)
Savignium Leach 1825
*crenatum** (Sowerby 1823)
tuamotum Achituv and Langsam 2005
Trevathana Anderson 1992
*dentata** (Darwin 1854)
isfae Achituv and Langsam 2009
margaretae Brickner et al. 2010
jensi Brickner et al. 2010
mizrachae Brickner et al. 2010
niuea Achituv 2004
orientale (Ren 1986)
paulayi Asami and Yamaguchi 2001
sarae Brickner et al. 2010
synthesysae Achituv and Langsam 2009
tureiae Achituv and Langsam 2005
Wanella Anderson in Ross 1999
andersonorum (Ross 1999)
*milleporae** (Darwin 1854)

Table 3-1. Continued

Family Pyrgomatidae Gray 1825

snelliusi (Kolosvary 1950)

Tribe Pyrgopsellini Ross and Newman 1995

Pyrgopsella Zullo 1967

*annandalei** (Gruvel 1907)

youngi Achituv and Simon-Blecher 2006

Tribe Hoekiini Ross and Newman 1995

Ahoekia Ross and Newman 1995

chuangi Ross and Newman 1995

microtrema Ross 2000

*tanabensis** Ross and Newman 1995

Australhoekia Ross and Newman 2000

*cardenae** Ross and Newman 2000

Eohoekia Ross and Newman 1995

*chaos** Ross and Newman 1995

nyx Ross and Newman 1995

Hoekia Ross and Newman 1973

fornix Ross and Newman 1995

*monticulariae** (Gray 1831)

mortensi Ross and Newman 1995

philippinensis Ross 2000

Parahoekia Ross and Newman 1995

*aster** Ross and Newman 1995

Subfamily Megatrematinae Holthuis 1982

Tribe Megatrematini Holthuis 1982

Megatrema Sowerby 1823

*madreporarum** (Bosc 1801)

youngi Ross and Pitombo 2002

Memagreta Ross and Pitombo 2002

*pandorae** Ross and Pitombo 2002

Tribe Pyrgominini Ross and Pitombo 2002

Adna Sowerby 1823

*anglica** Sowerby 1823

Pyrgomina Baluk and Radwanski 1967

djanae Ross and Pitombo 2002

oulastreae (Utinomi 1962)

Table 3-2. Morphological and ecological characters of the genera, as synthesized from the taxonomic literature

	Current biogeog. region	No. wall plates	Wall externally conical (c), low conical (lc), or flat (f)	Operc. valves unfused (uf) or fused (f)	Operc. valves balanoid (b) or modified (m)	Basis fully-calc. (ca), w/ passageways (cp), w/ narrow membranous zone (mz), mostly memb. (me)	Planktotrophic (pk) or parasitic (pr)	Host zooxanthellate scleractinian (zx), azoox. scleractinian (az), or hydrocoral (h)
Family Pyrgomatidae Gray 1825								
Subfamily Ceratoconchinae Newman and Ross 1976								
Ceratoconcha Kramberger-Gorganovic 1889	WA	4	c	uf	b	ca	pk	zx
Subfamily Pyrgomatinae (Gray 1825)								
Tribe Pyrgomatini Gray 1825								
Arossella (Anderson 1993)	IWP	4	f	uf	m	ca	pk	zx
Cantellius Ross and Newman 1973	IWP	4	c / f	uf	m	ca	pk	zx / h
Cionophorus Ross and Newman 1999	IWP	1	f	f	m	ca	pk	zx
*Creusia*Leach 1817	IWP	4	lc	f	b	ca	pk	zx
Darwiniella Anderson 1992	IWP	1	f	f	m	ca	pk	zx
Galkinia Ross and Newman 1995	IWP	4	f	f	b	ca	pk	zx
Hiroa Ross and Newman 1973	IWP	4	lc / f	uf	m	ca	pk	zx
Neopyrgoma Ross and Newman 2002	IWP	1	lc	unk.	unk.	cp	pk	zx
Neotrevathana Ross 1999	IWP	1	lc?	f	m	ca	pk	zx
Nobia Sowerby 1839	IWP	1	f	f	m	ca	pk	zx
Pyrgoma Leach 1817	IWP	1	lc / f	uf	m	cp	pk	zx / az
Savignium Leach 1825	IWP	1	f	uf	m	ca	pk	zx
Trevathana Anderson 1992	IWP	1	f	uf	m	ca	pk	zx
Wanella Anderson in Ross 1999	IWP	1	f	uf	m	ca	pk	h

Table 3-2. Continued

	Current biogeog. region	No. wall plates	Wall externally conical (c), low conical (lc), or flat (f)	Operc. valves unfused (uf) or fused (f)	Operc. valves balanoid (b) or modified (m)	Basis fully-calc. (ca), w/ passageways (cp), w/ narrow membranous zone (mz), mostly memb. (me)	Planktotrophic (pk) or parasitic (pr)	Host zooxanthellate scleractinian (zx), azoox. scleractinian (az), or hydrocoral (h)
Tribe Pyrgopsellini Ross and Newman 1995								
Pyrgopsella Zullo 1967	IWP	1	lc	uf	m	me	pk	zx
Tribe Hoekiini Ross and Newman 1995								
Ahoekia Ross and Newman 1995	IWP	1	f	f	m	mz	pr	zx
Australhoekia Ross and Newman 2000	IWP	1	f	f	m	mz	pr	zx
Eohoekia Ross and Newman 1995	IWP	1	f	f	m	mz	pr	zx
Hoekia Ross and Newman 1973	IWP	1	f	f	m	mz	pr	zx
Parahoekia Ross and Newman 1995	IWP	1	f	f	m	mz	pr	zx
Subfamily Megatrematinae Holthuis 1982								
Tribe Megatrematini Holthuis 1982								
Megatrema Sowerby 1823	WA, IWP	1	f	uf	b	ca	pk	zx
Memagreta Ross and Pitombo 2002	IWP	1	f	uf	b	ca	pk	zx
Tribe Pyrgominini Ross and Pitombo 2002								
Adna Sowerby 1823	EA	1	c	uf	b	ca	pk	zx / az
Pyrgomina Baluk and Radwanski 1967	IWP	1	c	uf	b	ca	pk	zx / az

Table 3-3. List of sequenced specimens.

Count	Extr#	Accession#	Genus	Host	Specimen provenance	H3	COI	16S	18Sa	18Sb	12S s
1	H326		Adna	Oculina patagonica	Spain	1	1	1	1	1	1
2	H329		Adna	Oculina patagonica	Spain	1	1	1	1	1	1
3	H331		Adna	Oculina patagonica	Spain	1	1	1	1	1	1
4	H167	UF 8664	Cantellius	Montipora	Philippines	1	1	1	1	1	1
5	H213		Cantellius	Porites	Philippines	1	1	1	1	1	1
6	H169		Cantellius	Montipora	Philippines	1	1	1	1	1	1
7		UF 6541	Cantellius	Acropora	Philippines	1	1	1	1	1	1
8	H173	UF 8676	Cantellius	Acropora	Philippines	1	1	1	1	1	1
9	H218		Cantellius	Acropora	Philippines	1	1	1	1	1	1
10	H160	UF 8634	Cantellius	Pachyseris rugosa	Philippines	1	1	1	1	1	1
11	H172	UF 8636	Cantellius	Pachyseris rugosa?	Philippines	1	1	1	1	1	1
12	H209		Cantellius	Porites rus	Philippines	1	1	1	1	1	1
13		UF 13225	Ceratoconcha	Siderastrea	Florida, USA	1	1	1	1	1	0
14		UF 13227	Ceratoconcha	Siderastrea	Florida, USA	1	1	1	1	1	0
15		UF 13228	Ceratoconcha	Madracis	Florida, USA	1	1	1	1	1	1
16	H158	UF 13125	Darwiniella	Goniastrea pectinata complex	Philippines	1	1	1	1	1	1
17	H212	UF 8635	Darwiniella	Goniastrea pectinata complex	Philippines	1	1	1	1	1	1
18	H238		Darwiniella	Hydnophora aff microconos	Oman	1	1	1	1	1	1
19		UF 13136	Darwiniella	Hydnophora exesa	Philippines	1	1	1	1	1	1
20	H219		Darwiniella	Montastrea curta	Fiji	1	1	1	1	1	1
21	H161		Galkinia	Leptastrea purpurea?	Philippines	1	1	1	1	1	1
22	H244	UF 7460	Galkinia cf.	Cyphastrea	Oman	1	1	1	1	1	1
23		UF 11796	Galkinia cf.	Cyphastrea	Taiwan	1	1	1	1	1	1
24		UF 8966	Hiroa	Astreopora	Papua New Guinea	1	1	1	1	1	1

Table 3-3. Continued

Count	Extr#	Accession#	Genus	Host	Specimen provenance	H3	COI	16S	18Sa	18Sb	12S s
25		UF 14102	Hiroa	Astreopora	Madagascar	1	1	1	0	1	1
26	H240		Hoekiini	Hydnophora	Philippines	1	1	1	1	1	1
27	H243		Hoekiini	Hydnophora microconos	Philippines	1	1	1	1	1	1
28	H274	UF 9273	Neotrevathana	Cyphastrea	Society Ids.	1	1	1	1	1	1
29	H252	UF 8690	Neotrevathana	Favia	Philippines	1	1	1	1	1	1
30	H265	UF 9277	Neotrevathana	Favia rotumana	Tuamotu Ids.	1	1	1	1	1	1
31	H214		Nobia	Coeloseris mayeri	Philippines	1	1	1	1	1	1
32	H157	UF 10361	Nobia	Galaxea	Philippines	1	1	1	1	1	1
33	H211		Nobia	Goniopora	Philippines	1	1	1	1	1	1
34	H156	UF 10364	Nobia	Euphyllia	Philippines	1	1	1	1	1	1
35	H150	UF 9278	Pyrgoma	Tubastrea	Philippines	1	1	1	1	1	1
36		UF 13133	Pyrgoma	Turbinaria	Philippines	1	1	1	1	1	1
37	h ORI-11A	from GenBank	Pyrgoma	Turbinaria	South Africa	1	1	1	1	1	1
38			Pyrgopsella	Symphyllia radians	Indonesia*	1	1	1	1	1	1
39	H171		Savignium	Echinophyllia aspera	Philippines	1	1	1	1	1	1
40	H237		Savignium	Acanthastrea lordhowensis	Oman	1	1	1	1	1	1
41	H202		Savignium	Oxypora lacera	Philippines	1	1	1	1	1	1
42	H181	UF 1327	Trevathana	Acanthastrea echinata	Tuamotu Ids.	1	1	1	1	1	1
43	H255	UF 9271	Trevathana	Acanthastrea echinata	Society Ids.	1	1	1	1	1	1
44	H253		Trevathana	Echinopora lamellosa	Philippines	1	1	1	1	1	1
45	H227		Trevathana	Favia stelligera	Cook Ids.	1	1	1	1	1	1

Table 3-3. Continued

Count	Extr#	Accession#	Genus	Host	Specimen provenance	H3	COI	16S	18Sa	18Sb	12S s
46	H225	UF 15304	Trevathana	Favia stelligera	Fiji	1	1	1	1	1	1
47		UF 10408a	Trevathana	Favia stelligera	Guam	1	1	1	1	1	1
48	H221	UF15303	Trevathana	Goniastrea	Fiji	1	1	1	1	1	1
49	H226	UF 10353	Trevathana	Montastrea curta branching	Cook Ids.	1	1	1	1	1	1
50	H166	UF 8645	Wanella	Millepora	Philippines	1	1	1	1	1	1
51		9099A	Wanella	Millepora	Papua New Guinea	1	1	1	1	1	1
52		12617A	Wanella	Millepora	Mascarene Ids.	1	1	1	1	1	1
53	H184	UF 8030	Wanella	Millepora	Vanuatu	1	1	1	1	1	1
54		UF 13193	Acastinae		Philippines	1	1	1	1	1	1
55	KACb154		Armatobalanus			1	0	1	1	1	1
56		TAU Ar27835	Armatobalanus			0	0	1	1	1	1
57	KACb163		Armatobalanus			1	0	1	1	1	1
58		UF 11887	Armatobalanus	Montipora	Taiwan	1	1	1	1	1	1
59	H241		Tetraclita	Heliopora	Palau	1	1	1	1	1	1
60	H159		unknown	Leptogorgia	Florida, USA	1	1	1	1	1	1
61		UF 11767	Conopea	Eunicea flexuosa	Panama	1	1	1	1	1	1
62		UF 13338	Megabalanus		Panama	1	1	1	1	1	1
63		from GenBank	Semibalanus			1	1	1	1	1	1
64		from GenBank	Balanus			1	1	1	1	1	1

Table 3-4. Sequence attributes for the 5 gene fragments.

Gene	Sequence length (bp)	#Parsimony-informative sites (bp)	#Invariable sites (bp)	A-T bias	Best fit model
COI	599	228	360	66%	GTR+I+G
16S	458	126	296	72%	TVM+I+G
12S	361	132	193	69%	TVM+I+G
18S	1766	74	1633	47%	GTR+I+G
H3	324	96	212	37%	TrN+I+G

GTR=general time-reversible model; TVM=transversional model; TrN=Tamura-Nei model; I=invariant sites; G=gamma shape parameter for rate variation among sites.

Table 3-5. Topological incongruencies in ML (computed using RAxML) and Bayesian (computed using MrBayes) gene trees. Strongly supported (arbitrarily set at >60% BS and >90% PP) incongruencies with the 5-gene concatenated analysis & with majority of the gene trees are italicized

	COI		16S		12S		18S	
	ML	Bayesian	ML	Bayesian	ML	Bayesian	ML	Bayesian
UF 11887 Armatobalanus	unresolved	unresolved	sister to Cantellius (76% BS)	sister to Cantellius (95% PP)	unresolved	w/ Cantellius (93% PP)	w/ main (non-Wanella) pyrgomatid clade (90% BS)	*w/ clades I & II + 2 Cantellius sequences (93% PP)*
KACb154 Armatobalanus allium	no data	no data	unresolved	unresolved	unresolved	unresolved	*w/ TAU Ar27835 (81% BS), w/in main (non-Wanella) pyrgomatid clade (90% BS)*	*sister to TAU Ar27835 (94% PP) w/ clades I & II (93% PP)*
TAU Ar27835 Armatobalanus allium	no data	no data	w/ Megabalanus (97% BS)	w/ Megabalanus (100% PP)	w/ Megabalanus (99% BS)	sister to Megabalanus (99% PP)	*w/ KACb154 (81%), w/in main (non-Wanella) pyrgomatid clade (90%)*	*sister to KACb154 (94% PP) w/ clades I & II (93% PP)*
KACb163 Armatobalanus sp.	no data	no data	unresolved	unresolved	unresolved	w/ H159"Conopea" (93% PP)	w/ H159"Conopea" (77% BS)	w/ H159"Conopea" (99% PP)
Wanella clade	no sister clade	no sister clade	no sister clade	no sister clade	no sister clade	no sister clade	w/ outgroup seqs Acastinae+Conopea+Megabalanus (61% BS)	w/ outgroup seqs Acastinae+Conopea+Megabalanus (96% BS)
Others								*UF 11887+2 Cantellius sequences not monophyletic w/ rest of Cantellius (93% PP)*

Table 3-5. Continued

	H3		MT		NUC		5GENE - PART	
	ML	Bayesian	ML	Bayesian	ML	Bayesian	ML	Bayesian
UF 11887 Armatobalanus	unresolved	unresolved	sister to Cantellius (94% BS)	sister to Cantellius (99% PP)	unresolved w/in main (non-Wanella) pyrgomatid clade (88% BS)	unresolved w/in main (non-Wanella) pyrgomatid clade (100% PP)	sister to Cantellius (91% BS)	sister to Cantellius (100% PP)
KACb154 Armatobalanus allium	unresolved	unresolved	sister to 11887 and Cantellius (76% BS)	sister to 11887 and Cantellius (99% PP)	*w/ TAU Ar27835 (89% BS), w/in main (non-Wanella) pyrgomatid clade (88% BS)*	*sister to TAU Ar27835 (97% PP) w/ clades I, II, & III (100% PP)*	sister to UF11887 and Cantellius (80% BS)	sister to UF11887 and Cantellius (99% PP)
TAU Ar27835 Armatobalanus allium	no data	no data	w/ Megabalanus (100% BS)	w/ Megabalanus (100% PP)	*w/ KACb154 (89% BS), w/in main (non-Wanella) pyrgomatid clade (88% BS)*	*sister to KACb154 (97% PP) w/ clades I, II, & III (100% PP)*	w/ Megabalanus (69% BS)	w/ Megabalanus (100% PP)
KACb163 Armatobalanus sp.	unresolved	unresolved	w/ H159"Conopea" (91% BS)	unresolved	w/ H159"Conopea" (91% BS)	w/ H159"Conopea" (100% PP)	w/ H159"Conopea" (93% BS)	w/ H159"Conopea" (100% PP)
Wanella clade	no sister clade	no sister clade	no sister clade	no sister clade	no sister clade	no sister clade	no sister clade	no sister clade

Table 3-6. P-values obtained from topological tests.

Hypothesis tested	S-H test
Pyrgomatidae (incl. *Wanella*) monophyletic	0.187
Armatobalanus monophyletic	0.057
MPT 1	9.038*
MPT 2	0.007*

*Significant at $P \leq 0.05$

Table 3-7. Results of BaTS analyses of seven phenotypic characters.

	Observed value (95% CI)	Expected value (95% CI)	Obs.& exp. overlap?	P-value	
Opercular valve fusion					
AI	0.246 (0.153-0.351)	2.778 (1.871-3.651)	n	0.000	*
PS	6.360 (6.000-7.000)	15.122 (12.997-16.998)	n	0.000	*
No. wall plates					
AI	0.189 (0.123-0.269)	4.428 (3.573-5.381)	n	0.000	*
PS	7.000 (7.000-7.000)	26.499 (23.033-29.230)	n	0.000	*
Wall height					
AI	0.7541 (0.6967-0.8832)	3.3272 (2.4736-4.2729)	n	0.000	*
PS	11.8032 (11.000-12.000)	19.1661 (17.0089-20.9992)	n	0.000	*
Basis calcareousness					
AI	0.3204 (0.2958-0.3823)	1.5993 (1.1363-2.1020)	n	0.000	*
PS	5.0083 (5.000-5.000)	7.9273 (7.9365-8.000)	n	0.000	*
Cirrus III teeth					
AI	0.2776 (0.2501-0.5001)	1.3096 (0.6223-1.900)	n	0.000	*
PS	2.6542 (2.000-3.000)	7.5975 (5.0933-9.4393)	n	0.000	*
Coral overgrowth					
AI	1.0293 (0.9756-1.1005)	2.0907 (1.4787-2.6407)	n	0.000	*
PS	8.7616 (8.000-9.000)	10.3453 (9.0595-11.000)	n	0.050	*
Overgrowth control					
AI	0.3003 (0.2500-0.3650)	2.6583 (1.9177-3.4918)	y	0.000	*
PS	4.2861 (3.000-5.000)	12.8256 (11.3960-14.000)	n	0.000	*

* Significant at $P \leq 0.05$

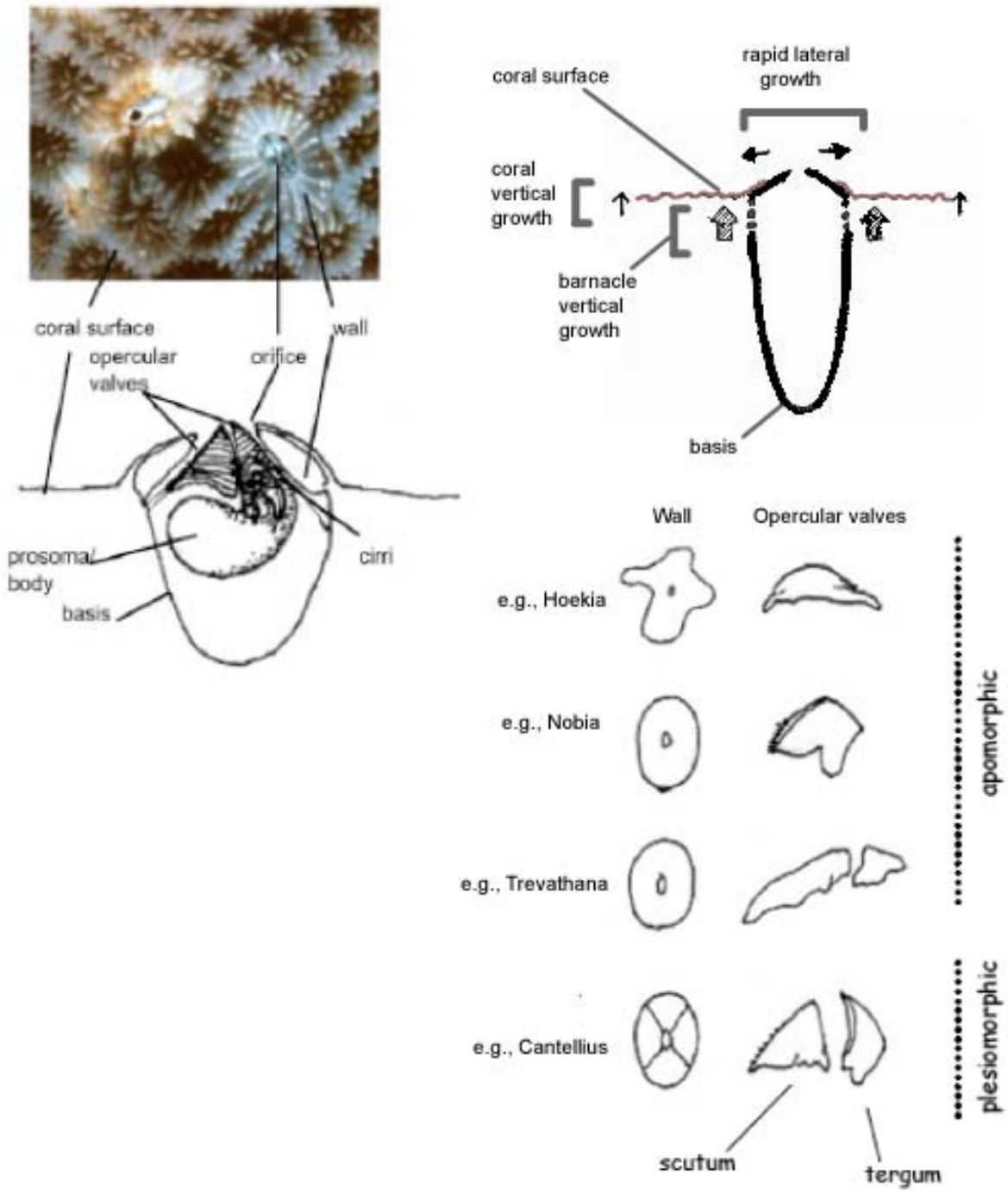

Figure 3-1. Schematic illustrations of pyrgomatid anatomy and growth process, and diversity in shell morphology.

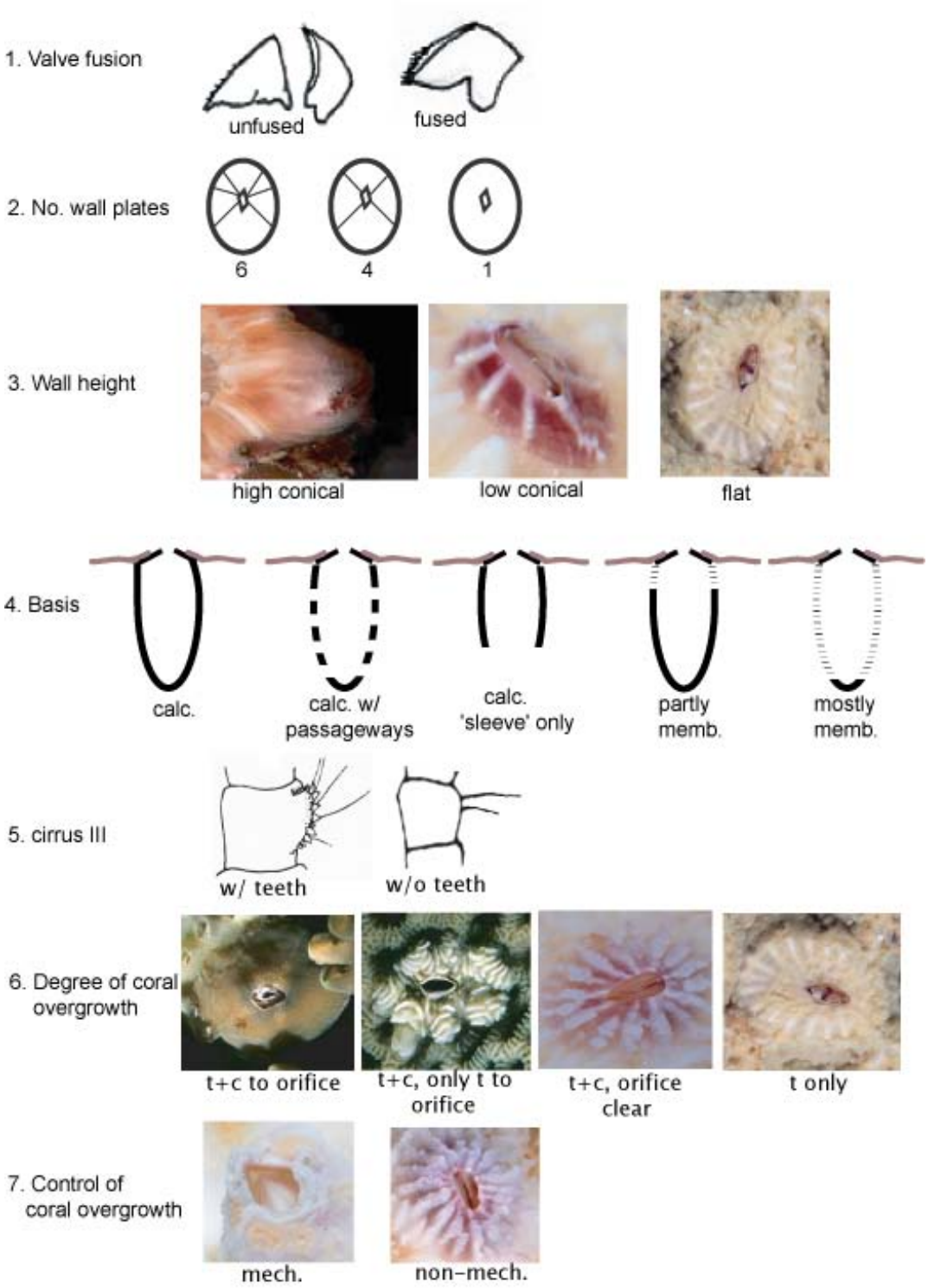

Figure 3-2. Phenotypic characters traced onto the pyrgomatid phylogeny, with the various character states illustrated.

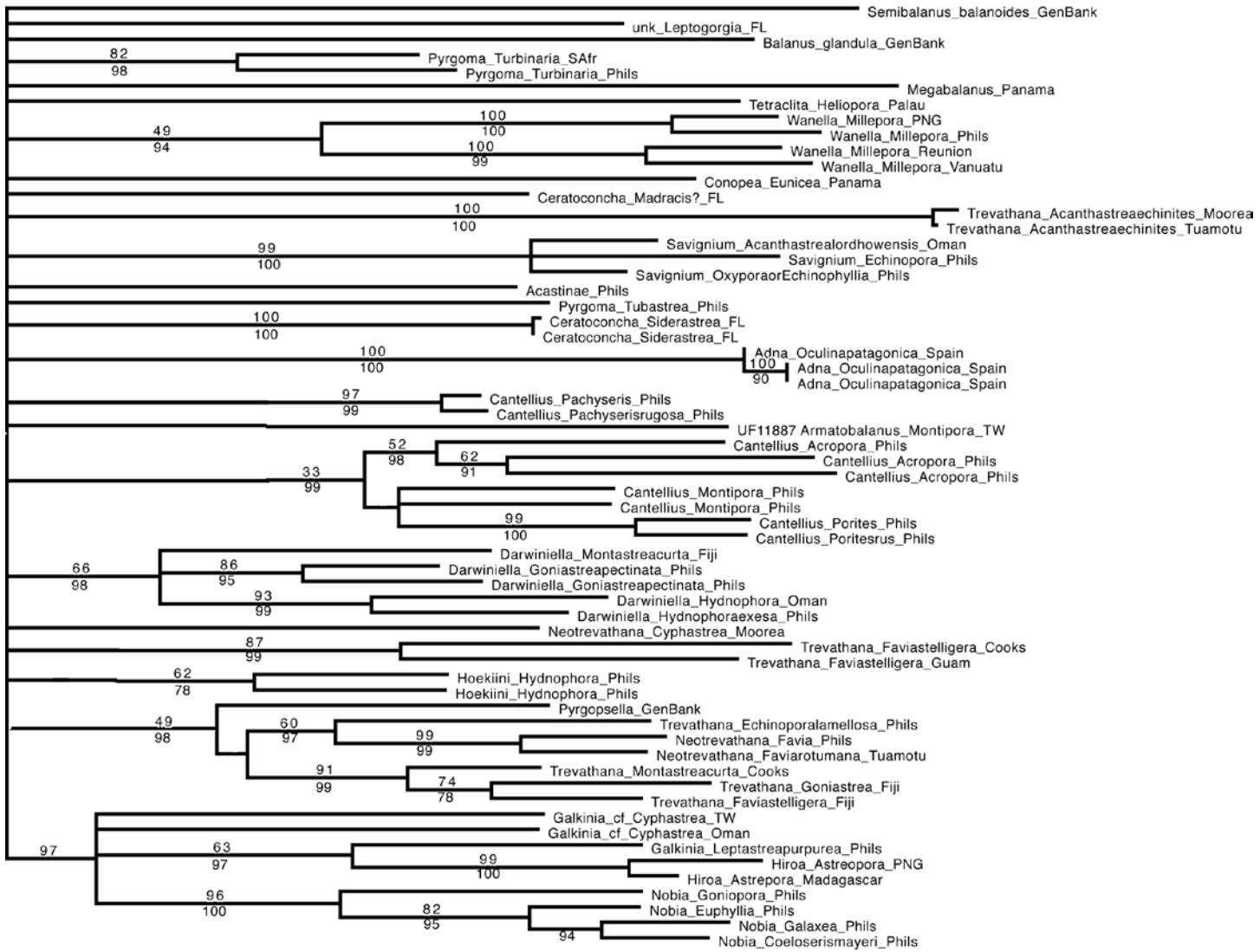

Figure 3-3. Maximum likelihood phylogram for COI computed using RAxML. Values above the branches are bootstrap support values (RAxML), values below the branches are Bayesian posterior probabilities (MrBayes).

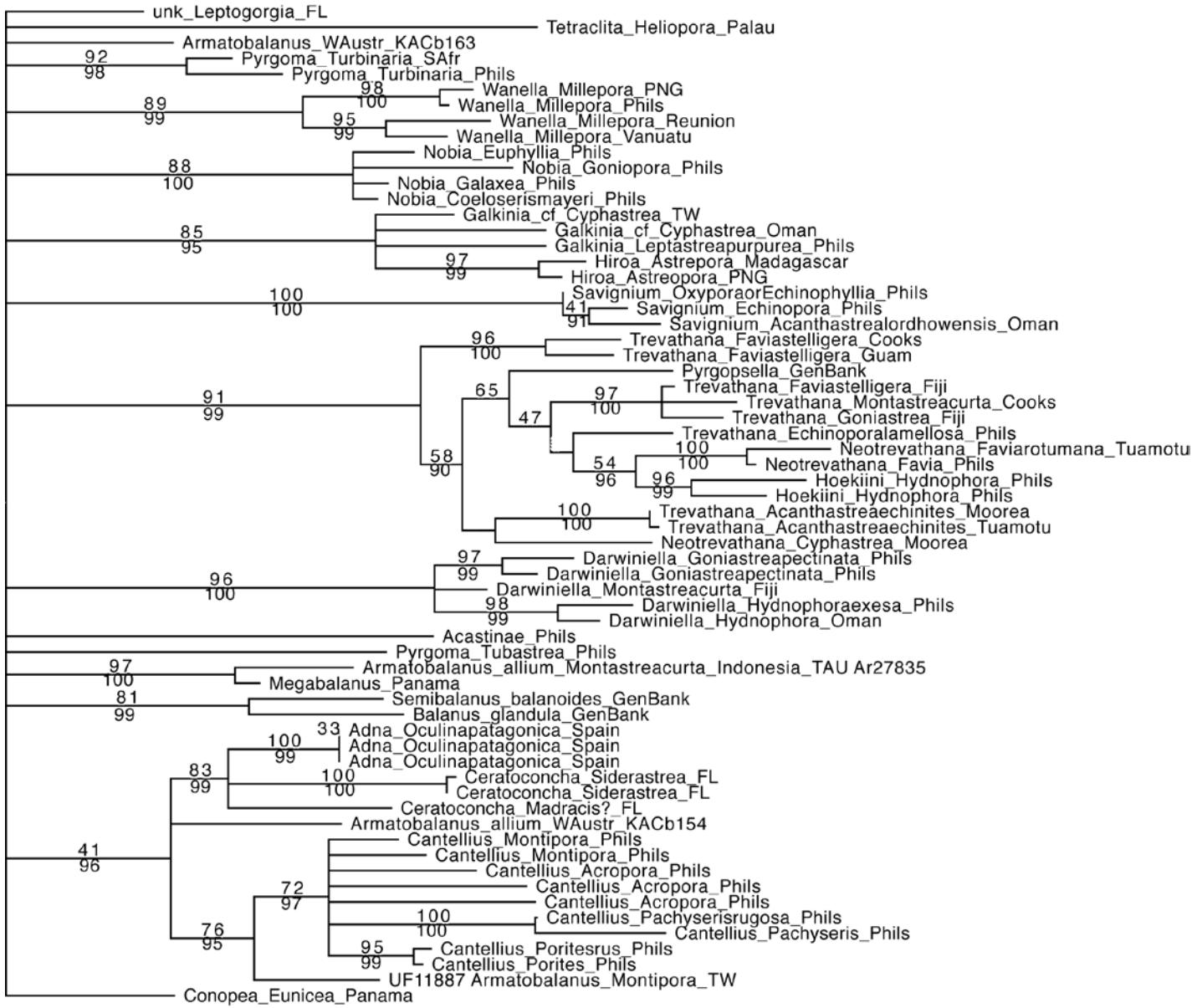

Figure 3-4. Maximum likelihood phylogram for 16S computed using RAxML. Notation follows fig. 3-3.

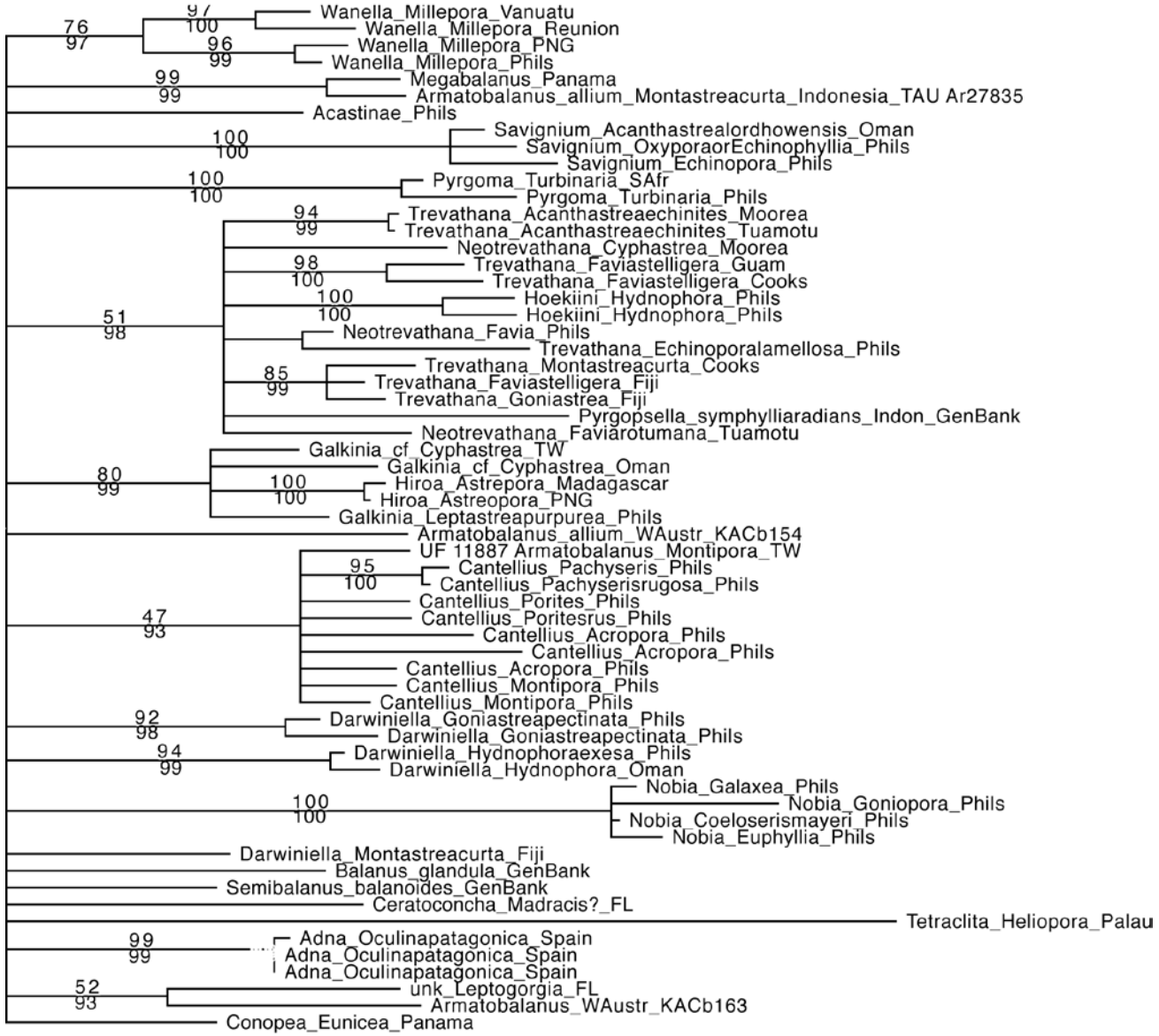

Figure 3-5. Maximum likelihood phylogram for 12S computed using RAxML. Notation follows fig. 3-3.

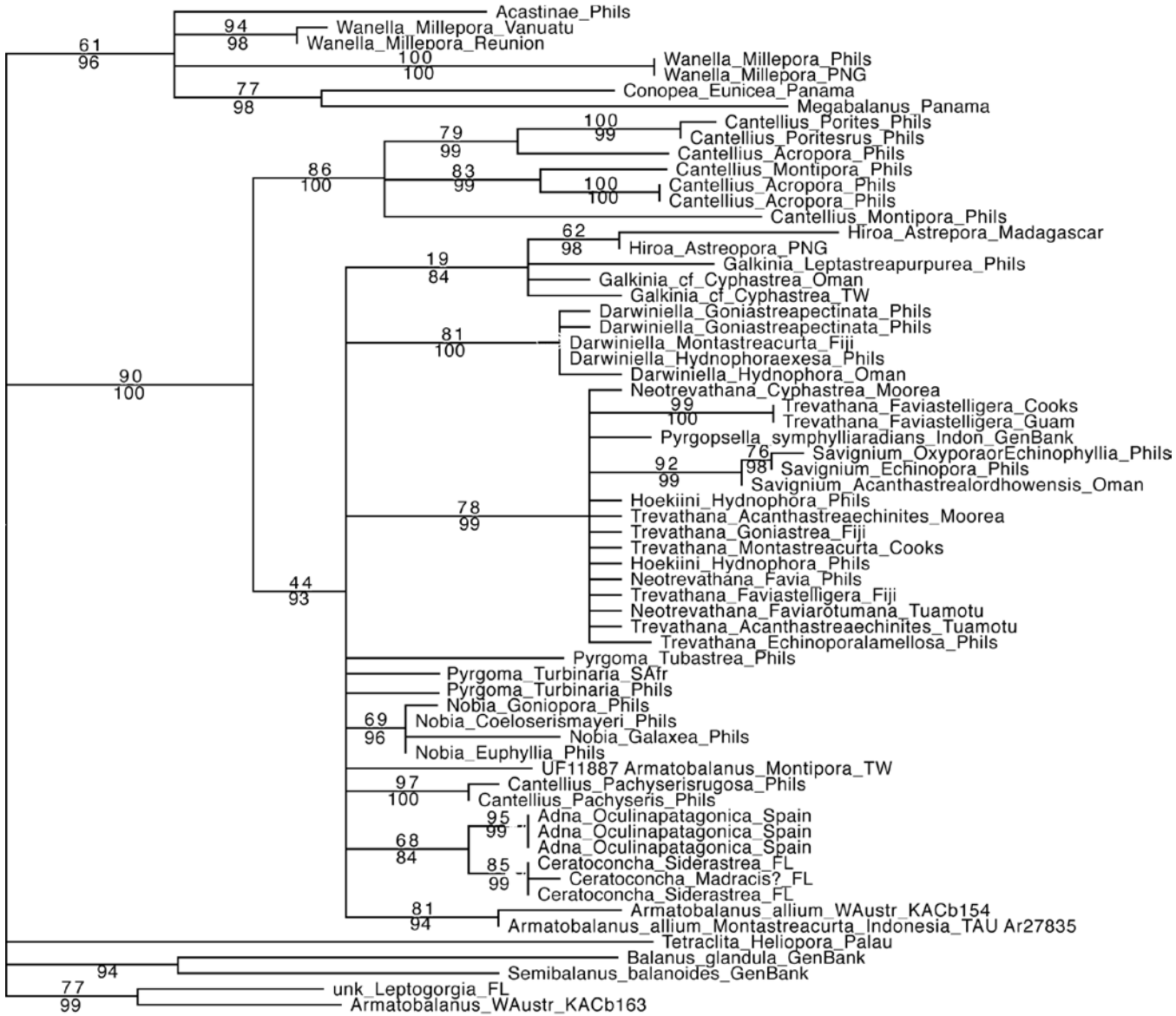

Figure 3-6. Maximum likelihood phylogram for 12S computed using RAxML. Notation follows fig. 3-3.

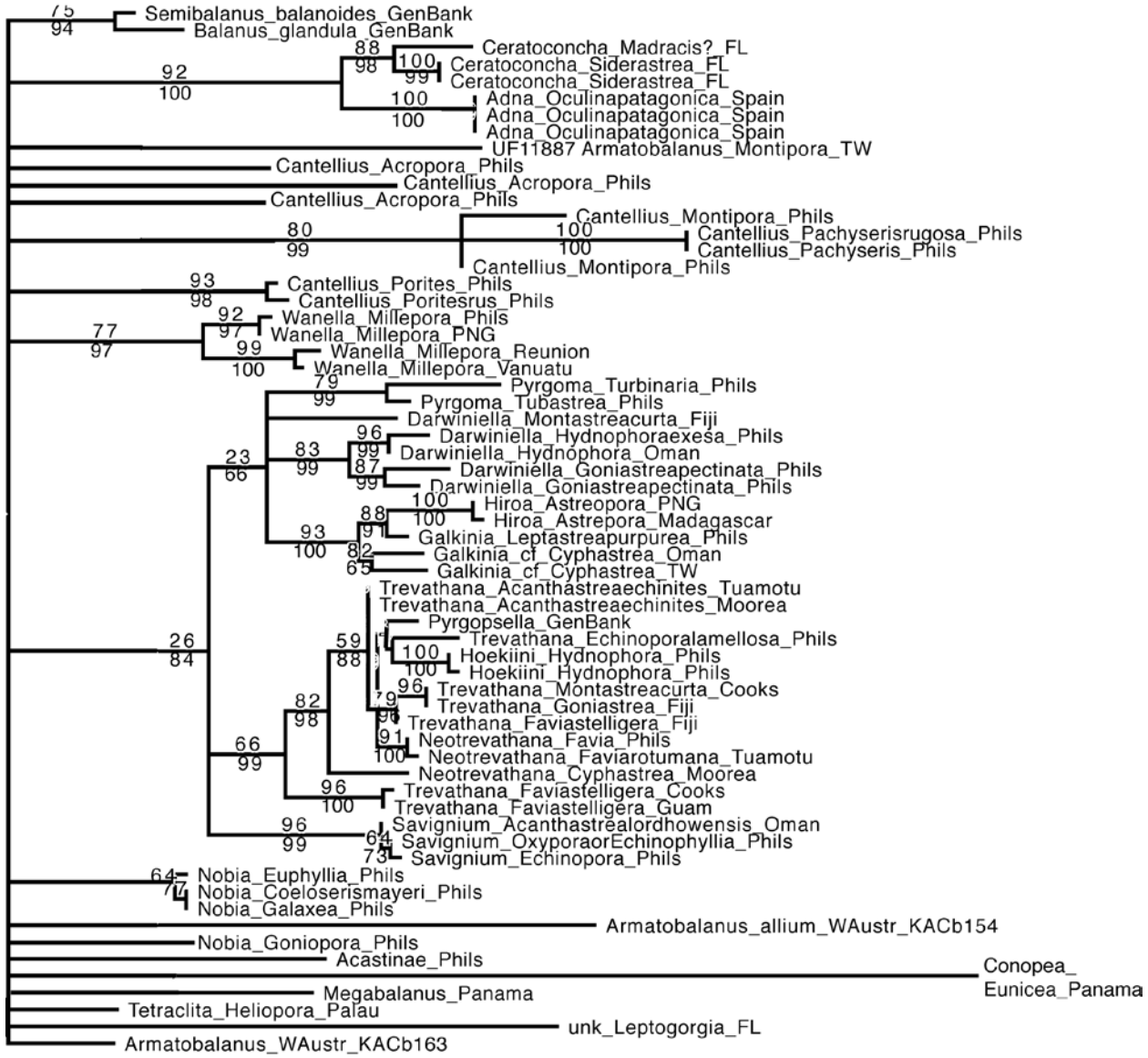

Figure 3-7. Maximum likelihood phylogram for H3 computed using RAxML. Notation follows fig. 3-3.

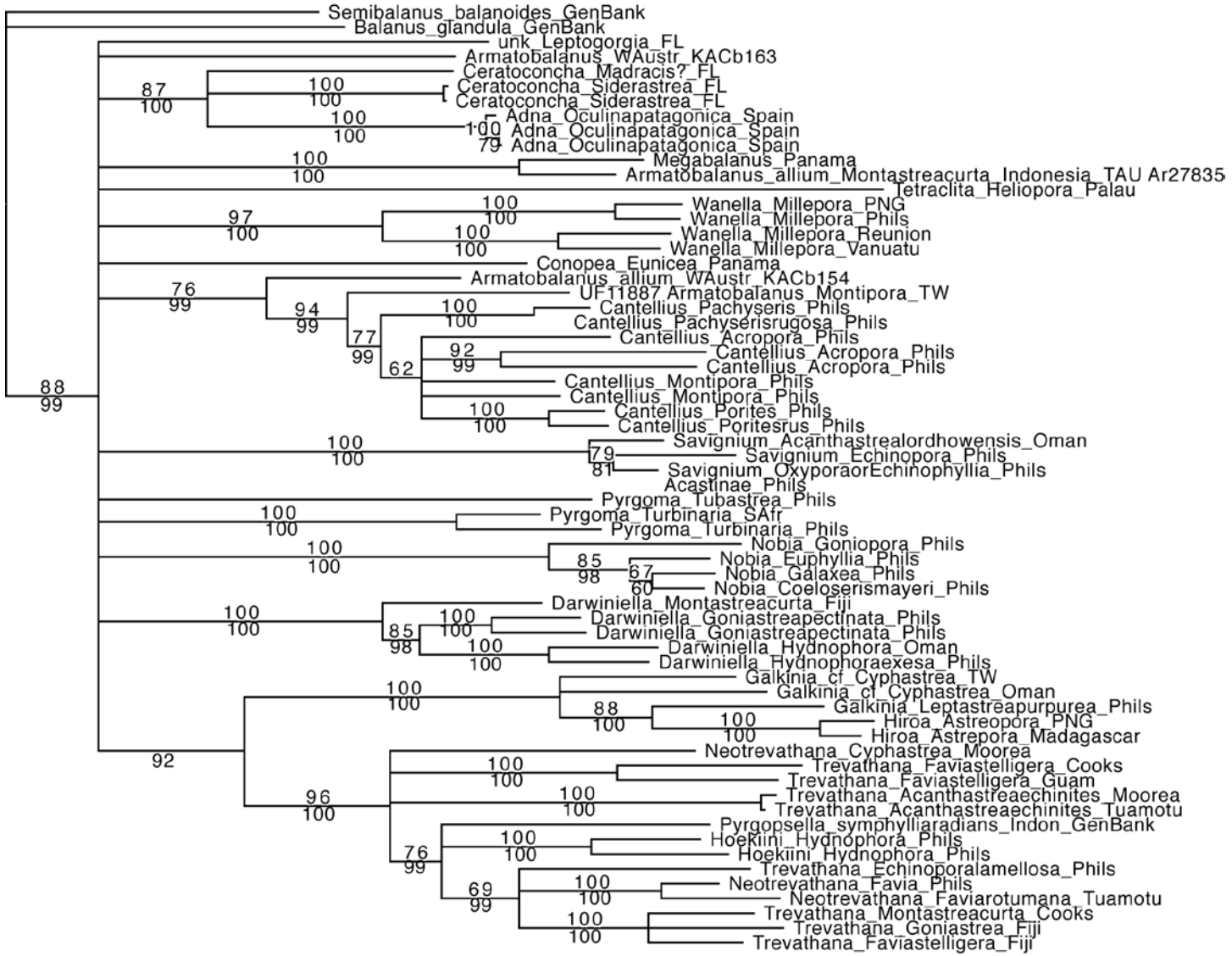

Figure 3-8. Maximum likelihood phylogram for all mt genes computed using RAxML. Notation follows fig. 3-3.

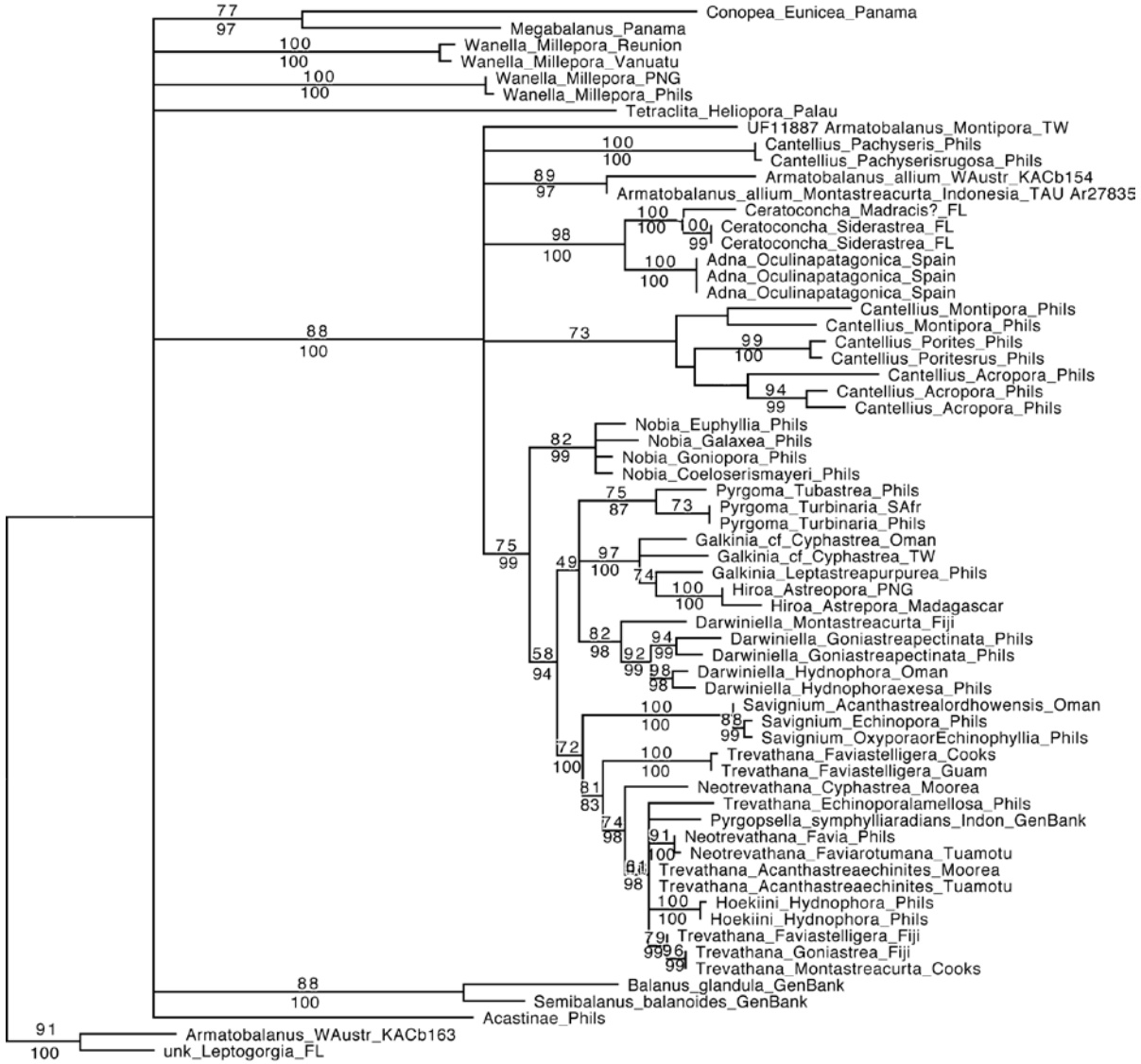

Figure 3-9. Maximum likelihood phylogram for all nuc genes computed using RAxML. Notation follows fig. 3-3.

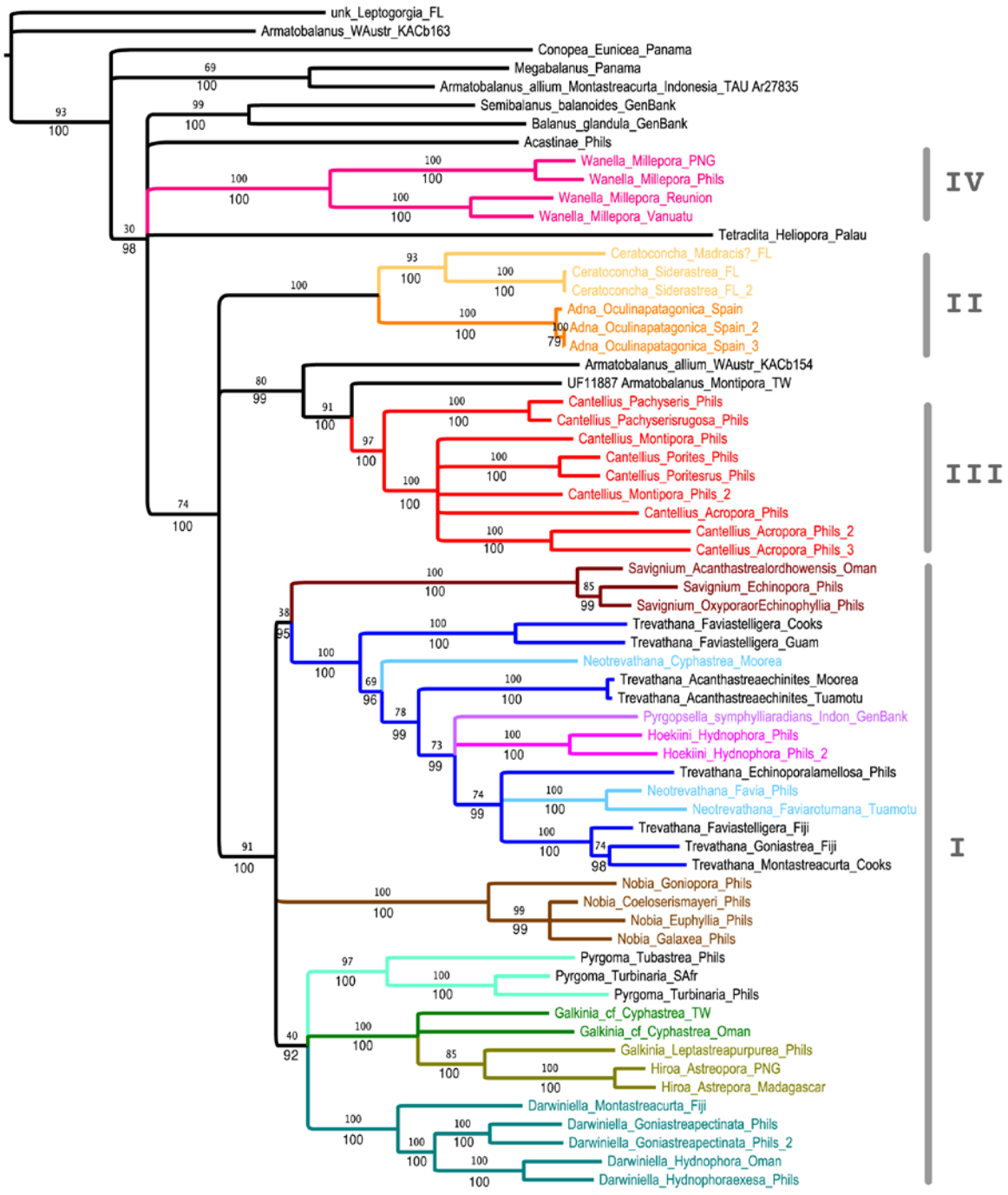

Figure 3-10. Maximum likelihood phylogram for all 5 sequenced genes computed using RAxML. Nodes with <60% bootstrap support and <90% posterior probability were collapsed. Taxa and branches are colored according to their different nominal genera; taxa in black represent non-pyrgomatid samples. Values above the branches are bootstrap support values (RAxML), values below the branches are Bayesian posterior probabilities (MrBayes).

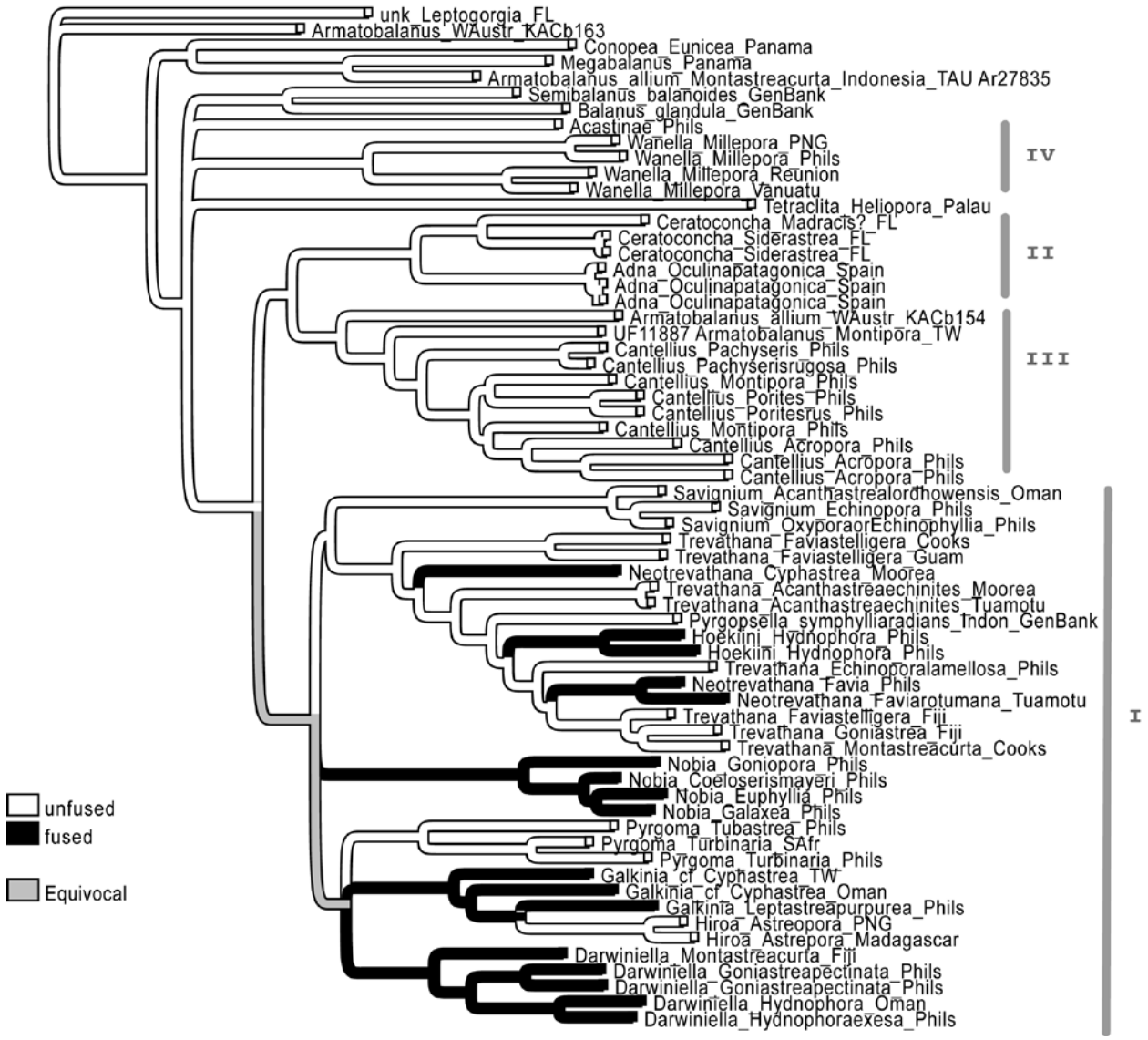

Figure 3-11. Reconstruction of ancestral character states for opercular valve fusion. The topology follows fig. 3-10.

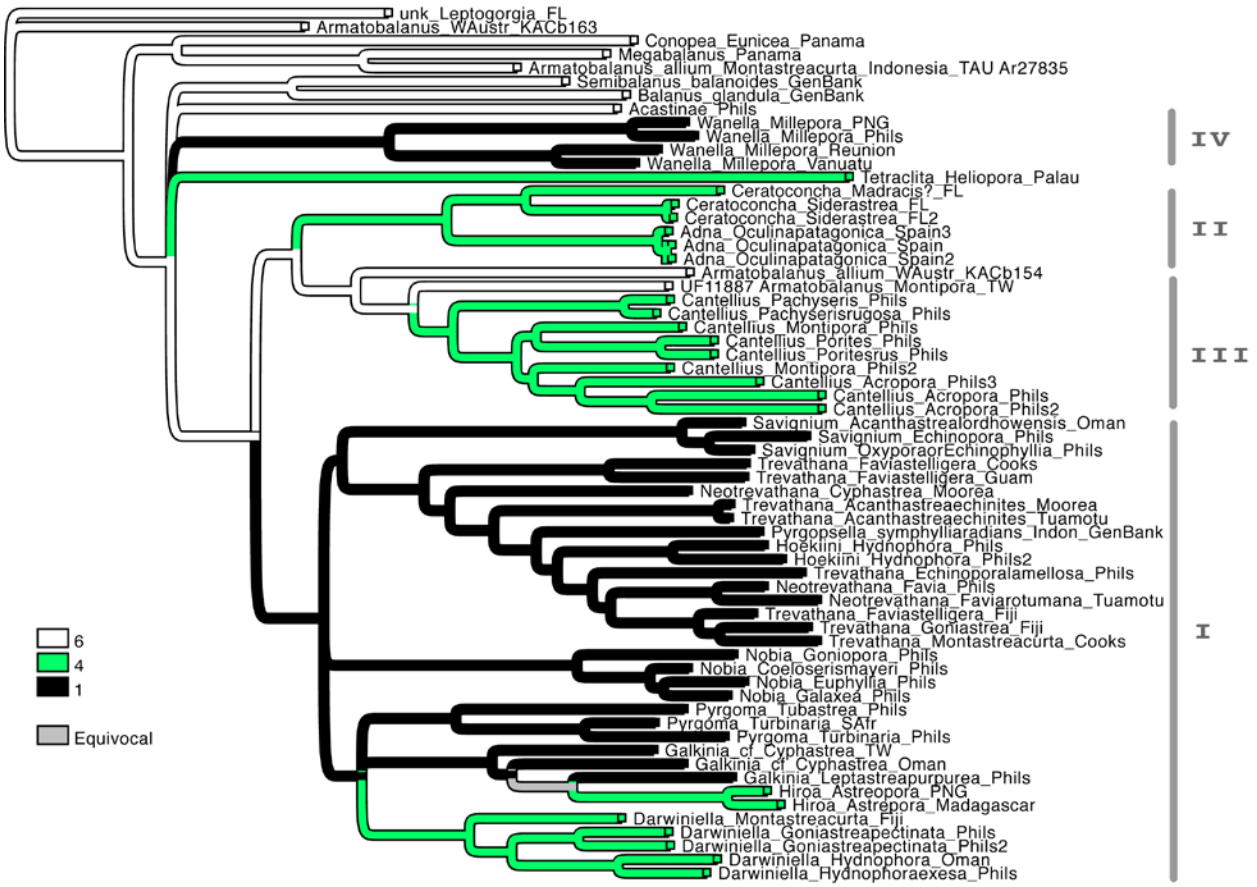

Figure 3-12. Reconstruction of ancestral character states for number of wall plates. The topology follows fig. 3-10.

Figure 3-13. Reconstruction of ancestral character states for wall height. The topology follows fig. 3-10.

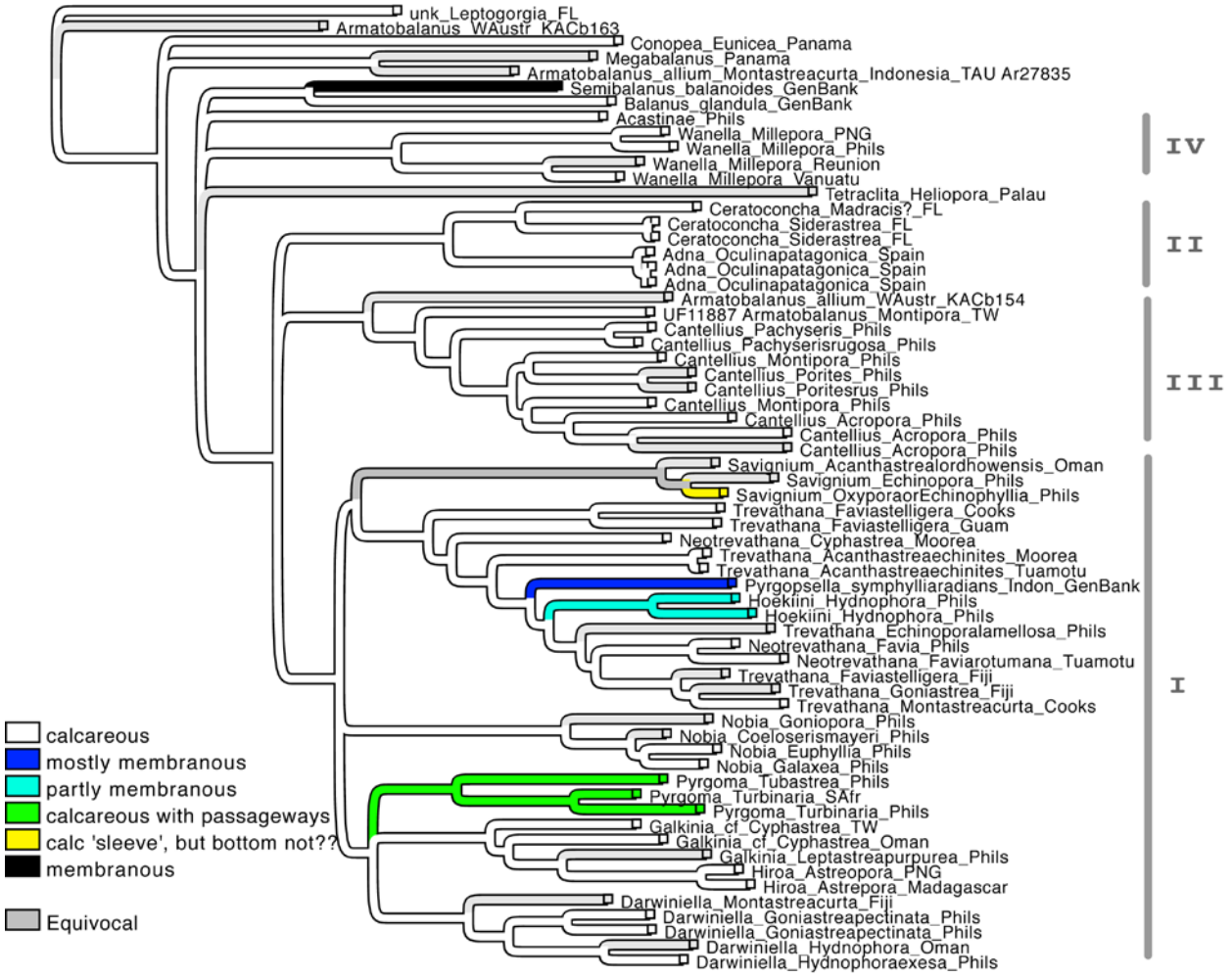

Figure 3-14. Reconstruction of ancestral character states for basis calcareousness. The topology follows fig. 3-10.

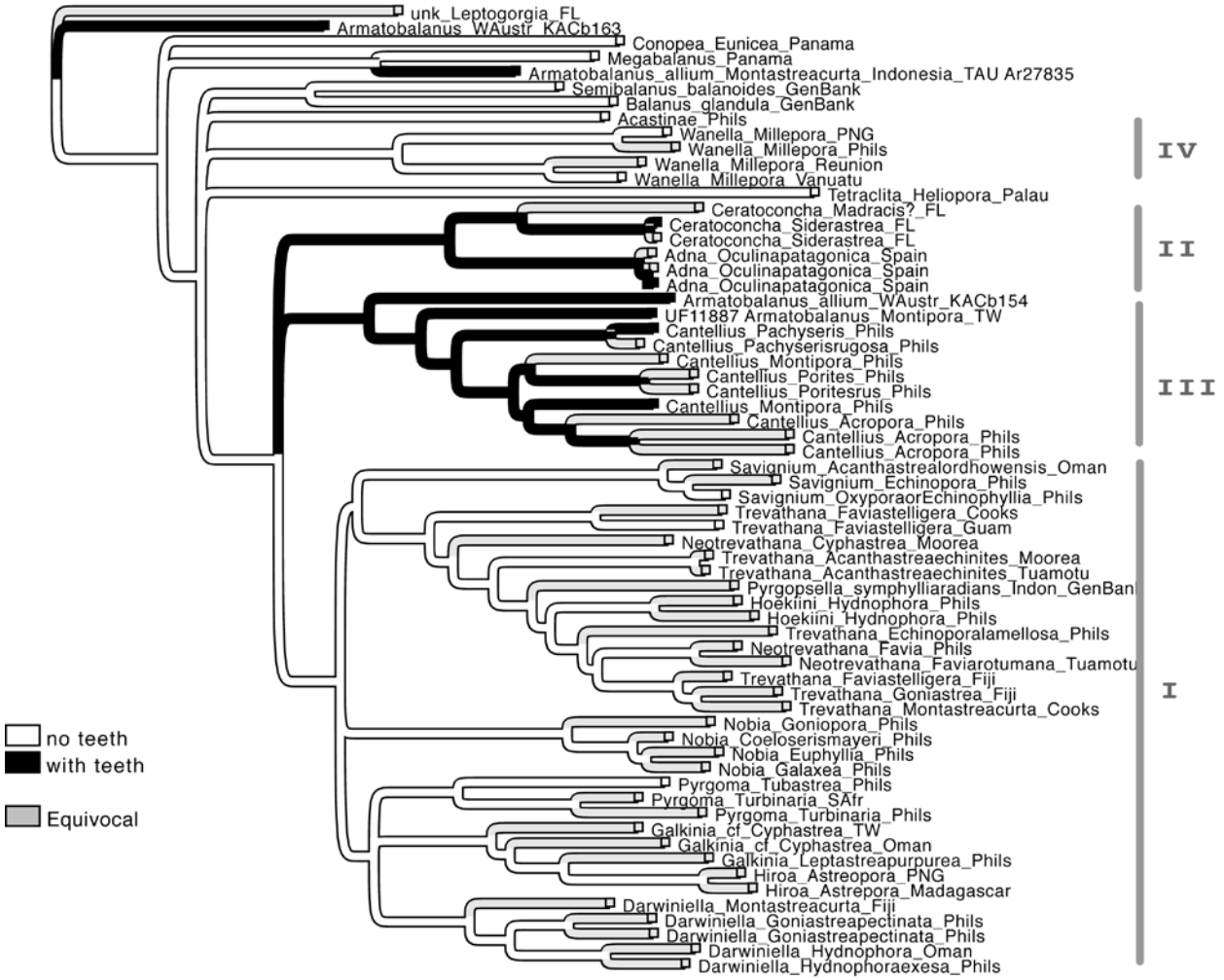

Figure 3-15. Reconstruction of ancestral character states for cirrus 3 armature. The topology follows fig. 3-10.

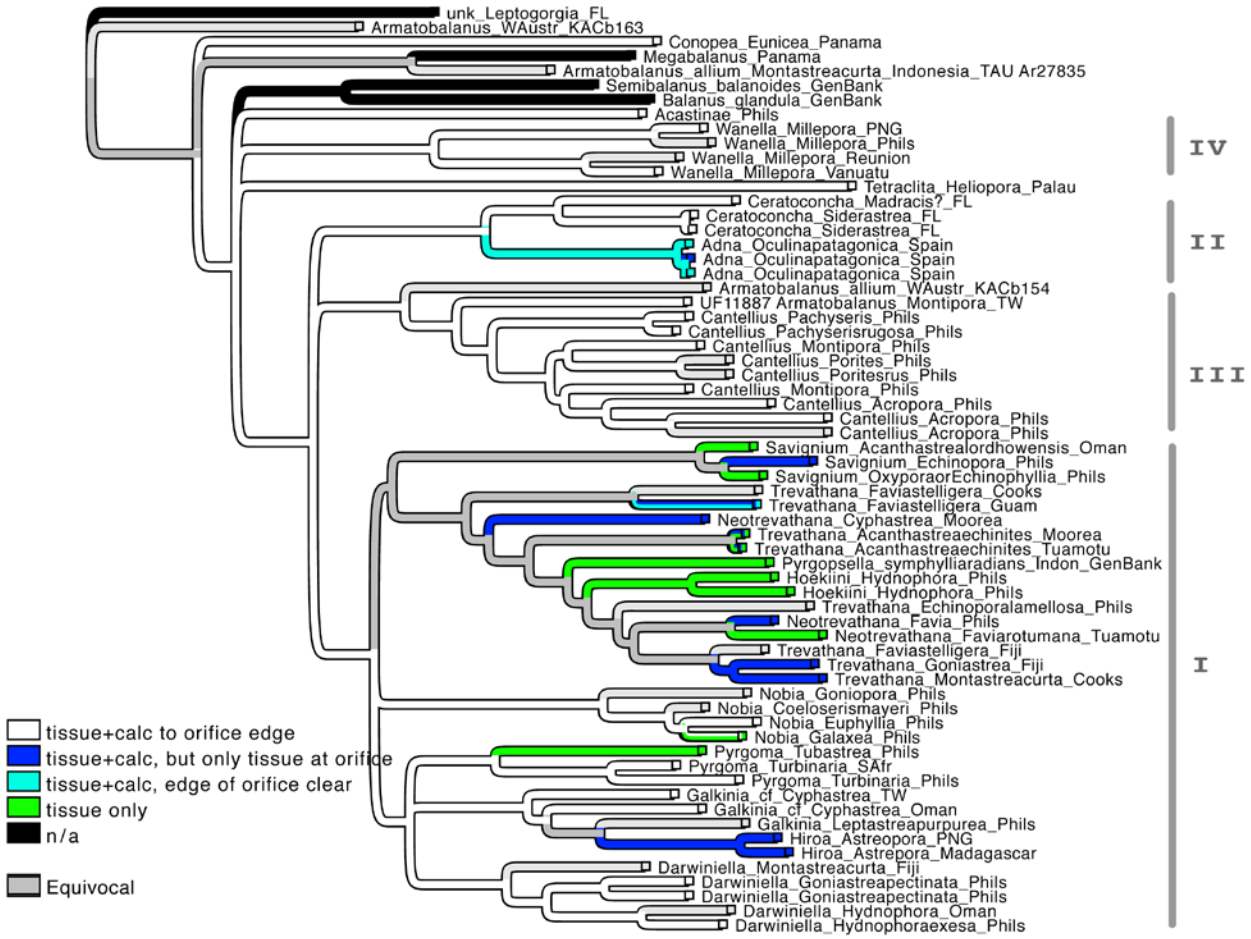

Figure 3-16. Reconstruction of ancestral character states for degree of coral overgrowth. The topology follows fig. 3-10.

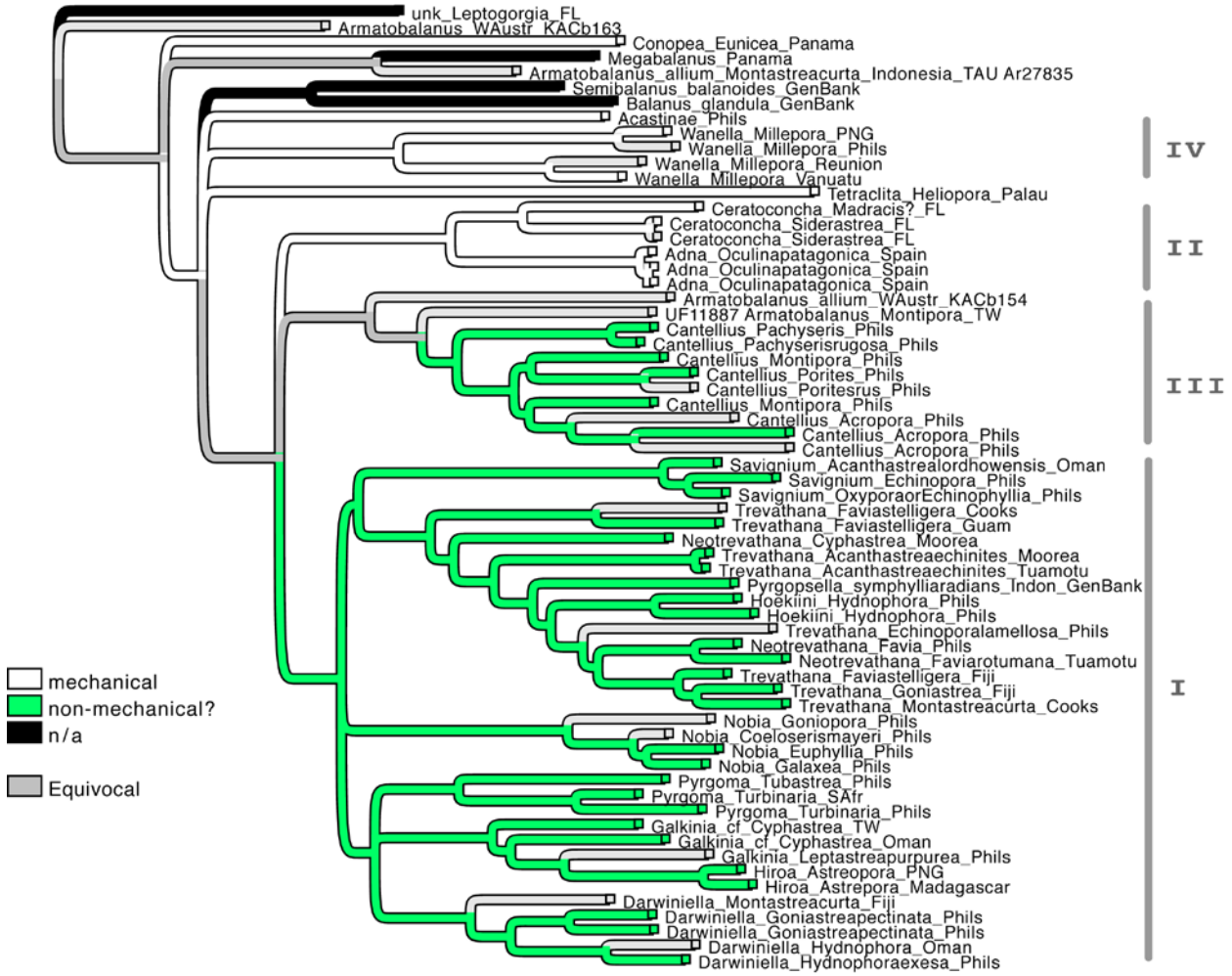

Figure 3-17. Reconstruction of ancestral character states for mechanism of overgrowth suppression. The topology follows fig. 3-10.

CHAPTER 4
GEOGRAPHY AND HOST-SPECIFICITY BOTH INFLUENCE SPECIATION OF CORAL BARNACLES IN THE CLADE *Trevathana SENSU LATO*

Introduction

Speciation in the sea is still not well understood. While recent phylogeographic work has revealed that the marine realm is far more geographically structured than previously thought (e.g., Lessios et al. 2001, Meyer et al. 2005, Barber et al. 2006), other potential mechanisms of speciation have not yet been fully evaluated. For instance, while the role of host-specificity has been well studied in several terrestrial organisms (e.g., in the apple maggot fly, Berlocher and Feder 2002; pea aphids, Hawthorne and Via 2001; fig wasps, Weiblen and Bush 2002), similar systems in the sea have only begun to be explored (e.g., in alpheid shrimp, Duffy 1996; coral-dwelling gobies, Munday et al. 2004; phestillid nudibranchs, Faucci et al. 2006; coral-dwelling barnacles, Mokady et al. 1999, Mokady and Brickner 2001, Tsang et al. 2009). The limited studies available have shown that cryptic or incipient species can be highly specialized on different hosts, suggesting that host shifts are involved in reproductive isolation and speciation. Such a mechanism of speciation may be especially important in marine systems characterized by widespread symbiotic relationships, such as coral reefs. In other words, symbioses could be one reason for high species diversity in reefs: new taxa could arise through ecological speciation (Schluter 2001) and species diversity itself may beget more species (Emerson & Kolm 2005) through the mechanism of host-race speciation.

Studies of host shifts in marine systems have so far had limited geographic coverage, making it impossible to assess the relative contributions of host specificity and geographic isolation to genetic structuring. In this chapter I am interested in

superimposing two layers of information, e.g., geographic structure and host specificity, onto phylogenetic data, in order to evaluate their interactions in the diversification and speciation of marine organisms. My study organisms are the coral-dwelling pyrgomatid barnacles in the clade *Trevathana sensu lato* (s.l.).

Pyrgomatids are a common but often overlooked group of balanomorph barnacles that obligately dwell on living corals. The genus *Trevathana* Anderson 1992 is the second largest genus of coral-dwelling barnacles. Together with its sister-group *Savignium*, *Trevathana* has the widest geographic range among the pyrgomatids, extending from the coast of E Africa to the Tuamotus and Marquesas in the central Pacific. In Chapter 3 I showed that *Neotrevathana*, *Pyrgopsella*, and the parasitic Hoekiini are nested within a paraphyletic *Trevathana*. I call this entire assemblage *Trevathana s. l.*. The 25 nominal species of this clade, their reported hosts, and the known geographic occurrences are listed in Table 4-1.

Earlier studies have shown highly host-differentiated species complexes in *Trevathana* from the Red Sea and Great Barrier Reef (GBR; Mokady et al. 1999; Brickner et al. 2010). Results from this chapter extend earlier results, and demonstrate that both host specialization as well as geographic isolation contribute to the diversity of the clade, albeit at different timescales.

Materials and Methods

Specimens and Morphological Examinations

Specimens were collected by scuba diving on reefs across the Indo-West Pacific (Table 4-2). The following areas were ‘well sampled’ for pyrgomatid barnacles: the Red Sea, Oman, Taiwan, NW Philippine Ids., Guam, Fiji, Rarotonga in the Cook Ids., Mo’orea in the Society Ids., and the Tuamotu Ids. I considered localities to be well

sampled when they were visited specifically to find pyrgomatid barnacles, and where divers spent a minimum of 2 weeks in low coral diversity localities, and 4 weeks in high coral diversity localities, searching for barnacles. Additional locations were sampled less thoroughly as opportunities permitted.

Coral specimens infested by barnacles were collected, and the barnacles removed from the coral using pliers or dental tools. Anatomical dissections were done under a dissecting microscope using fine tungsten needles. A tissue subsample was also dissected out for genetic analyses. Skeletal structures (wall and opercular valves) were briefly soaked in a weak bleach solution to remove adhering membranes, dried, and mounted on stubs. The skeletal structures were sputter-coated and imaged using a Field Emission-Scanning Election Microscope (SEM) at the University of Florida's Interdisciplinary Center for Biotechnology Research (ICBR).

All specimens are housed in the Division of Invertebrate Zoology at the Florida Museum of Natural History (FLMNH). A total of 81 FLMNH specimens were sequenced. Identifications of coral hosts were done by G. Paulay of the FLMNH.

Molecular Methods

All specimens were sequenced for the mitochondrial 'barcoding gene' COI. DNA was extracted from the tissue subsamples using DNAzol and proteinase K, following the protocol given in Meyer (2003). DNA extracts were purified using QIAGEN cleanup kits. The PCR primers and amplification profile followed protocols in Meyer (2003). PCR products were cleaned and sequenced at UF's high-throughput sequencing facility (at the ICBR), following protocols detailed in Chapter 3. All PCR products were sequenced along both directions. Some of the tissue subsamples were extracted and sequenced at the Smithsonian Institution's Laboratories of Analytical Biology, following the same

methodology as in Chapter 3.The 81 newly generated sequences were supplemented with published *Trevathana* sequences originating from specimens collected in the Red Sea and a few other localities, for a total of 120 sequences (Table 4-2).

Data Analyses

Chromatograms were checked, manually edited, and assembled into contigs using Geneious Pro 4.9.2 (Drummond et al. 2009). All sequences lacked insertions and deletions, thus sequence alignment was a trivial task. Codon positions were verified using Macclade 4.08 (Maddison and Maddison 2005). The best-fit model of sequence evolution was determined using the Akaike Information Criterion (Akaike 1974) as implemented in Modeltest 3.7 (Posada and Crandall 1998). The data were analyzed using RAxML 7.0.4 (Stamatakis 2006) using a GTR+GAMMA model and a random starting tree, and with the analysis partitioned into the 3 codon positions. A thousand bootstrap replicates were also performed. The tree was rooted on the distantly-related genera *Cantellius,* and *Armatobalanus*.

I also used a Bayesian approach to analyze the data, using MrBayes v3.1.2 (Huelsenbeck & Ronquist 2001; Ronquist & Huelsenbeck 2003). Flat priors and a GTR+I+GAMMA model were employed. I ran 2 independent chains for 1 million generations each; each chain was sampled every 100 generations. All runs reached stationarity before 100,000 generations. The initial 25% of the trees was discarded as the burn-in phase, and posterior probabilities were calculated based on the remaining 75% of the trees. The sequences were run either (1) unpartitioned, or (2) divided into the 3 codon positions, with all parameters unlinked between partitions. Prior to partitioning, the appropriate model of evolution was selected for each codon position using Modeltest.

In analyzing the topologies, I focused on clades with high support values (arbitrarily set at ≥60% bootstrap values and ≥90% posterior probabilities). These well supported clades were then used to define Evolutionary Significant Units (ESUs; *sensu* Moritz 1994). ESUs are reciprocally monophyletic populations that have at least one other independent character such as a distinct morphology, distribution, ecological niche (e.g., host occupancy), or that show reciprocal monophyly in another, independent marker. ESUs are clades with an evolutionary history separate from other ESUs. ESUs are thus species-level units (phylogenetic species) which can be readily defined in allopatric as well as in sympatric settings, unlike biological species.

ESUs proved difficult to define in instances where only a single sequence was available for a particular locality and host. In those cases, I defined 'putative ESUs' as singletons with a distinct locality or host, and having >2% sequence divergence from the nearest ESU.

I analyzed pairs of sister-ESUs in order to study patterns among the most recent evolutionary divergences. I conservatively defined pairs of sister-ESUs according to the following criteria: (1) each ESU must have robust branch support, (2) only ESUs with >1 sequence were considered (i.e., 'putative ESUs' were excluded), (3) when there was more than one potential sister-ESU, the selected sister-ESU should not have a disjoint geographic distribution. I called the divergence of sister ESUs evolutionarily significant events or ESEs (following Chapter 2).

Results

Figure 4-1 presents the results from the ML and Bayesian analyses. Twelve strongly supported clades were identified, each with between one to approximately 9 ESUs (Table 4-3). Some presumed groups contained only a single specimen, and thus

were assigned putative ESU status. The phylogeny is structured both by host and geography. These 2 factors are discussed in turn below.

Host-Specificity Patterns

Most (but not all) *Trevathana* species inhabit the "traditional" coral family Faviidae, a tangled paraphyletic/polyphyletic set of lineages, most members of which form a well defined clade together with several other "traditional" coral families (Mussidae, Pectiniidae, Merulinidae, Trachyphyliidae; Fukami et al. 2004; Fukami et al. 2008). Most *Trevathana s.l.* species live on corals of Fukami et al.'s (2007) Clade XVII in the latest scleractinian phylogeny, with a few on related corals outside this focal clade: *Leptastrea*, *Plesiastrea*, and *Acanthastrea*. Thus *Trevathana s.l.* appears to be predominantly specialized to a single coral clade.

Of the 10 *Trevathana s.l.* clades represented by more than 1 sequence, eight (80%) are restricted to a single coral genus (Fig. 4-1; Table 4-3). The two exceptions (clades IX and XII) were both found on the genera *Cyphastrea* and *Plesiastrea*. However, closer examination showed clear morphological differences between the "*Cyphastrea*" colonies hosting the 2 different barnacle clades. It seems likely that the 2 morphs (both potentially undescribed species) are not actually members of *Cyphastrea*, but rather derived forms of *Plesiastrea* (Paulay, pers. comm.). Regardless of the status of Clades IX and XII, the overall pattern shows that *Trevathana s.l.* clades are specific to a single coral genus.

Host specificity is also clear at the ESU level. Of 23 ESUs containing >1 sequenced specimen, at least eleven (or up to 15, depending on incomplete host identifications; i.e., about 50%) were found on a single host species (Fig. 4-1; Table 4-3).

Eight evolutionarily significant events or ESEs were identified (Table 4-4), of which five appeared to involve a switch to a different coral host species, while 3 did not involve host-switching. However, since the same host coral species were not consistently sampled across the entire range of *Trevathana s.l.*, it is also possible that apparent instances of host switching are artefacts of incomplete coral sampling.

While most coral genera were inhabited by a single *Trevathana s.l.* clade, three coral genera hosted more than one clade, i.e., *Favia* (occupied by clades I, IV, VIII, and XI), *Plesiastrea* (clades IX and XII), and *Cyphastrea* (clades VI, IX and XII; but see above for discussion of *Cyphastrea* identification problems). However, *Favia* is a polyphyletic genus (Fukami et al. 2008), while the phylogenetic status of *Plesiastrea* is presently not known because only 1 species has been sequenced for that genus. Therefore, in general a single coral taxon only hosts a single barnacle clade. An apparent exception is the well-defined species *Favia stelligera*, which is present in 3 clades (IV, VIII, and XI). Two of the ESUs inhabiting *F. stelligera* are sympatric (VIII-A and XI-B, on Guam). These 2 clades showed no obvious differences in skeletal characters (not illustrated). The two clades may represent cryptic species, or the genetic difference may be due to DNA contamination or the presence of pseudogenes. Increasing the number of sequenced specimens will hopefully resolve this question.

Biogeographic Patterns

The geographic distributions of *Trevathana s.l.* clades are shown in figures 4-2 to 4-11. Clades I and IV range across the entire IWP, encompassing three subregions, i.e., the Western Indian Ocean (WIO; including Madagascar, the Iles Eparses surrounding Madagascar, the Seychelles, Oman, and the Red Sea), Western Pacific (WP; including the Philippines, Indonesia, Taiwan, Palau, Guam, the Northern Marianas/CNMI, Papua

New Guinea/PNG, and Australia), and Central Pacific (CP; including Fiji, Vanuatu, the Cook Ids., Society Ids., and Tuamotus). Clades II, III , VI, and VII extend from the WIO to the WP; while Clades X and XI span the WP to CP. Clade VIII was only found in Oman, Clade XII is known only from the WIO, and Clade IX is only known from the CP. Two clades are only known from single specimens: Clade V from Indonesia, and Clade VIII from Guam (not illustrated).

Most clades are divided into multiple ESUs following the subregional boundaries, and show genetic cohesiveness within each subregion. ESUs are generally restricted to a single subregion, with three singleton exceptions, i.e., ESU IV-C (all from CP except 1 Great Barrier Reef/GBR sequence; fig. 4-5), IV-D (all from the WIO except 1 GBR sample; fig. 4-5), and VI-A (all from the WIO except 1 Philippine sample; fig. 4-7).

Most clades have only 1 ESU per subregion. The exceptions are Clades III, IV, and VII. Clade III may have 2 ESUs in the WIO (tentative assignments; see fig. 4-4). Clade VII has 2 sympatric ESUs in one locality in the Philippines (fig. 4-8). Perhaps the most atypical patterns are exhibited by Clade IV (fig. 4-5). This clade contains many more ESUs than all the other clades (at least 5 and possibly up to 9 ESUs). Several localities (e.g., the Red Sea, Guam, Great Barrier Reef, Fiji, and the Tuamotus) harbor 2 sympatric Clade IV ESUs.

Of the eight ESEs (Table 4-4), 7 were geographically structured and one was not. Among the geographically structured ESEs, four were located between the WIO and WP, and 3 were between the WP and CP. The single non-geographically structured ESE involved Clade VII in the Philippines. Note, however, that this ESE would actually be geographically structured if the putative ESU VII-B (from the WIO) were included in

the analysis. ESEs were difficult to determine for the Clade IV ESUs because of a lack of branch support, thus this clade was excluded pending additional genetic data.

Discussion

Host Specificity

Researchers have been interested in patterns of host specificity in pyrgomatid barnacles for over 70 years (e.g., Hiro 1935). However, studies of host specificity in pyrgomatids have been hampered by a number of factors: (1) some pyrgomatid material in collections lack host records; (2) barnacle taxonomists typically lack expertise in coral taxonomy, thus coral mis-identifications are relatively common in published records; and (3) corals are themselves highly polyphyletic at multiple levels (e.g., Romano and Cairns 2000; Fukami et al. 2008).

The results show that *Trevathana s.l.* is host specific at multiple levels. The entire group is a specialist on 'faviids' or Clade XVII (sensu Fukami et al. 2008) corals. At the clade level, 80-100% occupy only 1 host coral genus; while at the ESU level, about half are known from only single coral host species. For the most part, each coral group hosts only 1 clade of barnacles. The only coral 'species' hosting more than one *Trevathana s.l.* clade is *Favia stelligera* (Fig. 4-1; Table 4-3). The results imply that *Trevathana s.l.* shows phylogenetic conservatism in host use. How host specificity is accomplished is not yet known, but may involve specificity at the larval settlement stage.

Biogeography

In *Trevathana s.l.*, the ESEs (corresponding to speciation events) are located between the WIO and WP, and between the WP and CP. The exact location of the speciation events cannot yet be ascertained because of the disjunct distribution of

sampling localities. In general ESUs are restricted to a single geographic region. Genetic structuring of sister-ESUs largely corresponds with geographic separation, and all of the sister-ESUs are allopatrically distributed.

Temporal Difference Between Host Shifts and Geographical Isolation

Both geography and host specificity have affected the diversification of *Trevathana s.l*. The deeper, clade-level divergences are structured by host (80-100% of all clade-level splits), implying that early diversification of the group was accomplished by radiating over a range of coral hosts. The more recent, ESU-level divergences are structured by geography (in seven out of 8 ESEs). This implies that while both factors are acting on diversification, they operate on different timescales. Geographic separation works more quickly in creating genetic diversity than does host switching; however, given time the geographic signal is lost, probably as a result of shifts and extensions of geographic ranges. A shift in host use is a slower evolutionary process, thus its signature is retained in the phylogeny for a longer period of time. This rate difference between the two evolutionary forces is compatible with the idea that ecological niche, e.g. host choice, tends to be conserved over evolutionary time (i.e., phylogenetic niche conservatism; Peterson et al. 1999; Wiens and Graham 2005).

Species Diversity Patterns

The ESU richness in *Trevathana s.l.* does not peak in any one area (Fig. 4-12). Among the well sampled localities, diversity was highest in the Southwest Indian Ocean (SWIO; n=4-6; data combined from the Iles Eparses, Seychelles, and Madagascar), Red Sea (n=6 ESUs), Guam (n=5-7), and the Tuamotus (n=6). This is followed by the Philippines (n=5) and the Society Ids. (n=4), while ESU richness was relatively low in Oman, Taiwan, Fiji, and the Cook Ids. (all n=2 ESUs). Surprisingly, diversity in

Trevathana s.l. does *not* parallel the pattern for scleractinian corals, which show a diversity hotspot in the Indo-Malayan region and progressively decreasing diversity in all directions, especially towards the eastern Pacific (Stehli et al. 1967). Diversity at the edges of the distribution (e.g., the Red Sea, SWIO, Tuamotus) is just as high as in the Indo-Malayan coral triangle, if not slightly higher.

While *Trevathana s.l.* does not exhibit a biodiversity 'hotspot', the overall diversity across all pyrgomatid barnacles may still peak in the Indo-Malayan 'coral triangle'. What could account for this disparity in patterns? As with *Calcinus* hermit crabs (discussed in Chapter 2), the overall hotspot may result from the accumulation of different clade-specific patterns, each reflecting a separate evolutionary history. The diversity pattern in an individual clade may not necessarily parallel the overall pattern at a higher taxonomic level.

What Are Species?

In an earlier section I explained that ESUs satisfy the conditions of the phylogenetic species concept (see materials and methods). However, some may argue that the clades themselves could be considered species, and that the ESUs merely represent geographic variation within a species. Since sister-ESUs are all allopatrically distributed, it is not possible to determine whether sister-ESU pairs have intrinsic barriers to reproduction (i.e., the biological species concept cannot be applied). Given the absence of information on reproductive isolation, the decision of where to draw the line between different species is dependent on how much variation (genetic, morphological, ecological, etc.) the taxonomist is willing to include within a species. Nonetheless, the 'species question' is not merely academic, because the assignment of a unique species name to a phylogenetic unit influences our perception of whether

species are geographically restricted or wide-ranging. In the case of *Trevathana s.l.*, assigning a clade only one species name means that a single species can range from the Red Sea to the Tuamotus, in accordance with early views that marine species are able to disperse across vast ocean distances. On the other hand, assigning separate species names to each ESU restricts the range of species to a single geographic region; i.e., the WIO, WP, or CP. Moreover, in the case of *Trevathana s.l.,* lumping multiple ESUs under a single species name will give the impression that 'species' are not highly host specific, when clearly host specificity is highly prevalent in these barnacles. Restricting the geographic range and host range of species by delimiting smaller species units may have management and conservation implications as well, since biodiversity is (typically) managed and conserved at the species level.

There are also clear morphological differences between the different ESUs, particularly in the tergal region (see fig. 4-13). Indeed, previous to this study, Brickner et al. (2010) assigned separate species names to two ESUs in a single clade (e.g., *T. jensi* and *T. dentata*, both in Clade IV). This is further evidence that taxonomists recognize the ESUs as natural units. For all these reasons, I believe that the ESUs recovered in these studies all need to be described as separate species. New species descriptions will be forthcoming in a future paper.

Status of *Neotrevathana*

The genus *Neotrevathana* Ross 1999 currently contains a single species, *N. elongatum* (see Table 4-1), described by Hiro (1931) from Seto, Honshu Is., Japan; from *Madrepora* (the host identification is very likely incorrect, as the name *Madrepora* was formerly applied to the staghorn coral *Acropora*, which does not host *Trevathana s.l.*). Hiro (1931) assigned his new species to the genus *Pyrgoma* Leach 1817, which at

that time encompassed all pyrgomatids with a single wall plate, following the precedent set by Darwin (1854). *Pyrgoma* was divided into multiple genera in the revision of Ross and Newman (1973). Anderson (1992) later transferred *P. elongatum* to *Newmania* n. gen., together with '*Newmania' milleporae*. Later recognizing that *Newmania* was preoccupied, Anderson (1993) proposed the genus *Wanella* for these 2 species. However, Ross (1999) noted that based on illustrations, the specimen described by Anderson (1992) could not be the same species that Hiro (1931) described. Ross (1999) concluded that the specimen described by Hiro (1931) was not closely related to *W. milleporae*, and he correctly recognized the affinity of *elongatum* to *Trevathana dentata* (Darwin 1854). At that time *Trevathana* was a monospecific genus. However, instead of including *elongatum* in *Trevathana*, Ross erected a new genus, *Neotrevathana*, to accommodate it.

In comparing *N. elongatum* to *T. dentata,* Ross (1999, p. 835) stated that "*Neotrevathana* differs from *Trevathana* in having broad, low ridges on the shell surface, coalescent opercular plates, a broad occludent ledge…, and by lacking a depression for insertion of the lateral depressor muscle. The tergal spur in *Neotrevathana* is reduced to a knob-like projection, whereas the tergal tooth in *Trevathana* appears to be an elaboration of the tergal spur". Of these characters, the fusion of the opercular valves is the most distinct and readily recognizable character. The absence of the lateral depressor muscle insertion is merely a secondary condition related to valve fusion, as the muscle insertion is located on the basi-tergal angle of the scutum and would be obliterated by the fusion of the scutum and tergum. The other differences between *Neotrevathana* and *Trevathana* have disappeared with the discovery of additional

Trevathana species. For instance, “widely spaced, low radiating ridges” on the external surface of the wall have since been observed in the newly described species *T. mizrachae, T. jensi*, and *T. margaretae* (see illustrations in Brickner et al. 2010). Likewise, the broad occludent ledge and appearance of the tergal spur are no longer unique to *Neotrevathana*. The only remaining character differentiating *Neotrevathana* is fusion of the scutal and tergal valves.

I found 5 different ESUs with fused (thus *Neotrevathana*-like) opercular valves in *Trevathana s.l.* (fig. 4-13). In addition, clade VII (the parasitic hoekiines) also possesses fused valves. Fusion of opercular valves may have occurred at least 3 times in the evolution of *Trevathana s.l.* (fig. 4-13). Moreover, I found that valve fusion can even vary *within* a single ESU, notably in II-A. Mokady et al. (1999) suggested that fusion of valves develops ontogenetically in *Neotrevathana* and that the “articular ridge” on the fused valve represents the site of valve fusion. Indeed, a superficial suture on the external surface of the valves (more rarely on the internal surface) is visible in most of the *Trevathana s.l.* ESUs with fused valves. However, in clade X, there is a complete absence of a suture demarcating the scutal and tergal regions, and it is difficult to imagine valve fusion occurring ontogenetically in this ESU, nor in the highly apomorphic parasitic hoekiines (Clade VII), which also lack a demarcating suture. Clearly, valve fusion is a highly labile character, and cannot be sufficient basis for differentiating genera. Therefore I propose that *Neotrevathana* Ross 1999 be synonymized with *Trevathana* Anderson 1992.

Which of the five ESUs with fused opercular valves corresponds to the *N. elongatum* of Hiro (1931)? Unfortunately, the type specimen appears to have been lost

and no neotype has been designated (Ross 1999), thus we only have the illustrations of Hiro (1931) to refer to. Two other published illustrations for *N. elongata*, by Ross (1999) and Mokady et al. (1999), appear to correspond to different taxa. The specimen illustrated in Ross (1999; fig.1) was collected from *Goniastrea aspera* in the Ryukyu Ids.; while the specimen illustrated by Mokady et al. (fig. 3c in Mokady et al. 1999; also ESU II-A in fig. 4-1) was collected from the Red Sea on *Echinopora gemmacea*. Given the high host specificity and geographic structuring of *Trevathana s.l.*, the fact that host corals and geographic locations were different is already a strong indication that the two studies dealt with 2 different species. The specimen of Mokady et al. (1999; ESU II-A in fig. 4-13) is most likely not the same as the original *N. elongatum*, because the former specimen has a pronounced tergal tooth not visible in the latter. In the illustration of Ross (1999), the tergal tooth is unfortunately not clearly visible, thus it is difficult to determine if his specimen was the same as Hiro's.

Hiro's specimen most closely resembles specimens from Clade I (fig. 4-13). There are 3 ESUs in Clade I, one each in the WIO, WP, and CP. Logically I would assume that Hiro's specimen from Japan would fall in the WP ESU, I-B. Indeed the opercular valve appears to closely match Hiro's illustration. However, given that geographical structuring and substantial cryptic differentiation are rampant in this group, Hiro's (1931) specimen could also represent a different species at the northern limits of pyrgomatid distribution. Only by sampling in Hiro's original collection in Seto can this puzzle be resolved completely.

Conclusions

Speciation in these highly specialized coral symbionts is influenced both by geographic separation and by host switching; however, these two factors operate at

different timescales. Geographic structuring occurs relatively rapidly, thus the most recently diverged ESUs show allopatric distributions. Host switching is an evolutionarily slower process, thus more anciently diverged lineages inhabit different host coral genera. Despite the extensive evolutionary diversification of *Trevathana s.l.* in the IWP, there was no observable gradient in diversity across its range. This surprising result does not seem to be evident at higher taxonomic scales, because large-scale patterns of species richness reflect the accumulation of many different biodiversity patterns resulting from different evolutionary histories. Thus, larger patterns can obscure biologically relevant processes. In order to unravel patterns that are biologically meaningful, we need to look at phylogenetically cohesive units of diversity, that is, reciprocally monophyletic groups. This bottom-up approach offers an interesting contrast to the patterns obtained through more traditional top-down studies of biodiversity.

My results also show that the genus *Neotrevathana* is polyphyletic, and needs to be synonymized with *Trevathana*. Multiple new ESUs were discovered in the course of this study, all of which will need to be taxonomically described. Molecular phylogenetic data is facilitating both revisionary systematics and species discovery in pyrgomatid barnacles.

Table 4-1. All nominal species within the *Trevathana sensu lato* subclade. No attempt was made to re-evaluate barnacle and coral identifications.

Count		Localities	Host(s)	References
Tribe Pyrgomatini Gray 1825				
Trevathana Anderson 1992				
1	*dentata* (Darwin 1854)	Red Sea, Mauritius, Gulf of Thailand, Persian Gulf, Andaman Sea, Bay of Bengal, Singapore, Japan, Hongkong, Taiwan, Indonesia, Philippines, Guam, New Guinea, Great Barrier Reef, Palau, Fiji, Gambiers, Niue, French Polynesia, Tuamotus. Also known from Pleistocene deposits in Japan.	*Goniastrea sp., G. retiformis, G. edwardsi, Meandrina spongiosa, Favia russelli, F. stelligera, F. cf. laxa, F. favus, Cyphastrea sp. C. serailia, C. microphthalma, , Echinophyllia lamellosa, Montastrea sp., M. curta, M. valenciennesi, Plesiastrea versipora, Favites sp., F. cf. abdita, F. russelli, Platygyra lamellina, Leptastrea transversa*	Ross and Newman 1973; Newman and Ross 1976; Foster 1980; Mimoto 1991; Ogawa et al. 1998; Ogawa 2000; Asami and Yamaguchi 2001; Achituv 2004; Achituv and Langsam 2005; Brickner et al. 2010
2	*isfae* Achituv and Langsam 2009	Gambiers, Tuamotus	*Favia stelligera*	Achituv and Langsam 2009
3	*margaretae* Brickner et al. 2010	Red Sea	*Favia favus*	Brickner et al. 2010
4	*jensi* Brickner et al. 2010	Red Sea	*Favites abdita*	Brickner et al. 2010
5	*mizrachae* Brickner et al. 2010	Red Sea	*Platygyra lamellina*	Brickner et al. 2010
6	*niuea* Achituv 2004	Niue Id.	*Goniopora* sp.	Achituv 2004
7	*orientale* (Ren 1986)	Japan, Guangdong, China	*Favia stelligera, Cyphastrea serailia, Goniastrea sp.*	Ren 1986; Asami and Yamaguchi 1997
8	*paulayi* Asami and Yamaguchi 2001	Guam, Gambiers	*Acanthastrea sp., A. echinata*	Asami and Yamaguchi 2001; Achituv and Langsam 2005
9	*sarae* Brickner et al. 2010	Seychelles, Red Sea	*Cyphastrea chalcidium*	Brickner et al. 2010
10	*synthesysae* Achituv and Langsam 2009	Reunion Is.	*Plesiastrea versipora*	Achituv and Langsam 2009
11	*tureiae* Achituv and Langsam 2005	Tuamotu Ids.	*Goniastrea* sp.	Achituv and Langsam 2005

Table 4-1. Continued.

Count		Localities	Host(s)	References
Neotrevathana Ross 1999				
12	*elongatum* (Hiro 1931)	Mauritius, Red Sea, Gulf of Thailand, Japan, Hongkong, Timor Sea, Palau, Guam, Heron Is., Tuamotus	*Echinopora sp., E. lamellosa, E. gemmacea, Madrepora sp., Favia mathaii/pallida, Goniastrea sp., G. aspera*	Hiro 1931; Newman and Ross 1976; Foster 1980; Galkin 1983; Ogawa et al. 1998; Ogawa 2000; Mokady et al. 1999; Achituv & Langsam 2005; Simon-Blecher et al. 2007
Tribe Pyrgopsellini Ross and Newman 1995				
Pyrgopsella Zullo 1967				
13	*annandalei* (Gruvel 1907)	Andaman Ids.	?	Gruvel 1907
14	*youngi* Achituv and Simon-Blecher 2006	Sulawesi, Indonesia	*Symphyllia radians*	Achituv and Simon-Blecher 2006
Tribe Hoekiini Ross and Newman 1995				
Ahoekia Ross and Newman 1995				
15	*chuangi* Ross and Newman 1995	Java Sea, Indonesia	*Hydnophora rigida*	Ross and Newman 1995
16	*microtrema* Ross 2000		*Hydnophora*	
17	*tanabensis* Ross and Newman 1995	Japan	*Hydnophora ?exesa, H. bonsai*	Ross and Newman 1995
Australhoekia Ross and Newman 2000				
18	*cardenae* Ross and Newman 2000		*Hydnophora*	
Eohoekia Ross and Newman 1995				
19	*chaos* Ross and Newman 1995	Red Sea	*Hydnophora* sp.	Ross and Newman 1995
20	*nyx* Ross and Newman 1995	Red Sea	*Hydnophora exesa*	Ross and Newman 1995
Hoekia Ross and Newman 1973				
21	*fornix* Ross and Newman 1995	Moluccas, Indonesia	*Hydnophora exesa*	Ross and Newman 1995
22	*monticulariae* (Gray 1831)	Singapore	*Hydnophora exesa*	Ross and Newman 1995
23	*mortensi* Ross and Newman 1995	Mauritius	*Hydnophora exesa*	Ross and Newman 1995
24	*philippinensis* Ross 2000		*Hydnophora*	
Parahoekia Ross and Newman 1995				
25	*aster* Ross and Newman 1995	New Caledonia	*Hydnophora microconos*	Ross and Newman 1995

Table 4-2. List of sequenced specimens

Accession#	Extraction#	ID (if available)	ESU	Host ID	Provenance
UF 18608			I-A	Favia sp.	Eparses
UF 18651			I-A	F. mathaei	Eparses
	Red1	T. margaretae	I-A	Favia favus	Red Sea
	Red2	T. margaretae	I-A	F. favus	Red Sea
	Red21	T. margaretae	I-A	F. favus	Red Sea
	Red22a	T. margaretae	I-A	F. favus	Red Sea
	Red22b	T. margaretae	I-A	F. favus	Red Sea
	Red22c	T. margaretae	I-A	F. favus	Red Sea
	Red23	T. margaretae	I-A	F. favus	Red Sea
	Red3	T. margaretae	I-A	F. favus	Red Sea
	Red4	T. margaretae	I-A	F. favus	Red Sea
	Red5	T. margaretae	I-A	F. favus	Red Sea
UF 11834			I-B	Favia sp.	Taiwan
UF 8690	H235		I-B	Favia sp.	Philippines
UF 8690	H252		I-B	Favia sp.	Philippines
UF 9274	H266		I-C	Favia rotumana	Tuamotus
UF 9274	H267		I-C	F. rotumana	Tuamotus
UF 9274	H268		I-C	F. rotumana	Tuamotus
UF 9277	H265		I-C	F. rotumana	Tuamotus
UF 18657			II-A	Echinopora gemmacea	Eparses
UF 18657			II-A	E. gemmacea	Eparses
UF 18658			II-A	Echinopora hirsutissima	Eparses
UF 18658			II-A	E. hirsutissima	Eparses
	Red15	"N. elongata"	II-A	Echinopora sp.	Red Sea
UF 11868			II-B	Echinopora sp.	Taiwan
UF 8632			II-B	Favia?	Philippines
UF 8633	H162		II-B	Echinopora lamellosa	Philippines
UF 8633	H253		II-B	E. lamellosa	Philippines
UF 8633	H254		II-B	E. lamellosa	Philippines
MOM5-4a			III-A	?	Oman

Table 4-2. Continued.

Accession#	Extraction#	ID (if available)	ESU	Host ID	Provenance
UF 20402			III-A	Platygyra sp.	Oman
	Red1	T. mizrachae	III-A	Platygyra lamellina	Red Sea
	Red11	T. mizrachae	III-A	P. lamellina	Red Sea
	Red17	T. mizrachae	III-A	P. lamellina	Red Sea
	Red25	T. mizrachae	III-A	P. lamellina	Red Sea
UF 10396			III-B	Platygyra pini	Guam
UF 10397			III-B	P. pini	Guam
UF 10398			III-B	P. pini	Guam
UF 14544			III-C?	?	Madagascar
UF 10331			IV-A	Montastrea curta	Cook Ids.
UF 10350			IV-A	M. curta	Cook Ids.
UF 1324	H182		IV-A	M. curta	Tuamotus
UF 15302	H224		IV-A	M. curta	Fiji
UF 15305	H223		IV-A	M. aff. curta	Fiji
UF 9428			IV-A	M. curta	Society Ids.
UF 9431			IV-A	M. curta	Society Ids.
UF 9458			IV-A	M. curta	Tuamotus
UF 9522			IV-A	M. curta	Society Ids.
UF 10399			IV-B?	Favites russelli	Guam
UF 15304	H225		IV-C	Favia stelligera?	Fiji
UF 15503	H221		IV-C	Goniastrea sp.	Fiji
UF 9459			IV-C	F. stelligera	Tuamotus
	GBR14c	"T. dentata"	IV-C	Leptastrea sp.	GBR
	GBR28	T. jensi	IV-D	Favites halicora	GBR
	Red abd16	T. jensi	IV-D	Favites abdita	Red Sea
	Red2	T. jensi	IV-D	F. abdita	Red Sea
	Red21	T. jensi	IV-D	F. abdita	Red Sea
	Red2b	T. jensi	IV-D	F. abdita	Red Sea
	Red3	T. jensi	IV-D	F. abdita	Red Sea
	Red5	T. jensi	IV-D	F. abdita	Red Sea
	Red71	T. jensi	IV-D	F. abdita	Red Sea

Table 4-2. Continued.

Accession#	Extraction#	ID (if available)	ESU	Host ID	Provenance
	Red72	T. jensi	IV-D	F. abdita	Red Sea
	Red1	"T. dentata"	IV-E	Leptastrea transversa	Red Sea
	Red3	"T. dentata"	IV-E	L. transversa	Red Sea
	Red4	"T. dentata"	IV-E	L. transversa	Red Sea
UF 6053			IV-F?	Goniastrea edwardsi	CNMI
UF 8536			IV-G?	F. halicora	Vanuatu
UF 10395			IV-H	G. edwardsi	Guam
UF 6055			IV-H	G. edwardsi	CNMI
UF 8978			IV-I?	G. edwardsi	Palau
TAU Ar27804		P. youngi	V-A	Symphyllia radians	Indonesia
UF 6601			VI-A	Cyphastrea serailia	Philippines
	Red13a	T. sarae	VI-A	Cyphastrea chalcidium	Red Sea
	Red13c	T. sarae	VI-A	C. serailia	Red Sea
	RedB1	T. sarae	VI-A	C. chalcidium	Red Sea
	RedB2	T. sarae	VI-A	C. serailia	Red Sea
	RedB3	T. sarae	VI-A	C. chalcidium	Red Sea
	RedE	T. sarae	VI-A	C. chalcidium	Red Sea
	Sey1	T. sarae	VI-A	Cyphastrea sp.	Seychelles
	Sey2	T. sarae	VI-A	Cyphastrea sp.	Seychelles
	Sey3	T. sarae	VI-A	Cyphastrea sp.	Seychelles
UF 10406			VI-B	C. serailia	Guam
UF 13693			VI-B	Cyphastrea sp.	Guam
UF 10423		Hoekiini	VII-A	Hydnophora microconos	Philippines
UF 8679	H243	Hoekiini	VII-A	H. microconos	Philippines
	H234	Hoekiini	VII-A	H. microconos	Philippines
UF 18617		Hoekiini	VII-B?	Hydnophora sp.	Eparses
UF 6564	H240	Hoekiini	VII-C	Hydnophora exesa	Philippines
UF 6564		Hoekiini	VII-C	H. exesa	Philippines
UF 10400			VIII	F. stelligera	Guam
UF 9268	H269		IX-A	Plesiastrea sp.	Tuamotus
UF 9268	H270		IX-A	Plesiastrea sp.	Tuamotus

Table 4-2. Continued.

Accession#	Extraction#	ID (if available)	ESU	Host ID	Provenance
UF 9273	H273		IX-A	aff. Cyphastrea sp. 1	Society Ids.
UF 9273	H274		IX-A	aff. Cyphastrea sp. 1	Society Ids.
UF 9276	H271		IX-A	aff. Cyphastrea sp. 1	Tuamotus
UF 9276	H272		IX-A	aff. Cyphastrea sp. 1	Tuamotus
UF 10919		T. aff. paulayi	X-A	Acanthastrea echinata	Tuamotus
UF 1327	H181	T. aff. paulayi	X-A	A. echinata	Tuamotus
UF 9260		T. aff. paulayi	X-A	A. echinata	Tuamotus
UF 9265	H258	T. aff. paulayi	X-A	A. echinata	Society Ids.
UF 9271	H255	T. aff. paulayi	X-A	A. echinata	Society Ids.
UF 10381		T. paulayi	X-B	A. echinata	Guam
UF 10384		T. paulayi	X-B	A. echinata	Guam
UF 10359	H227		XI-A	F. stelligera	Cook Ids.
UF 10912			XI-A	F. stelligera	Cook Ids.
UF 9267	H262		XI-A	F. stelligera	Society Ids.
UF 9275	H263		XI-A	F. stelligera	Tuamotus
UF 9275	H264		XI-A	F. stelligera	Tuamotus
UF 10402			XI-B	F. stelligera	Guam
UF 6052			XI-B	F. stelligera	CNMI
UF 18634			XII-A	aff. Cyphastrea sp. 2	Eparses
UF 20280			XII-A	Plesiastrea versipora	Oman
UF 20280			XII-A	P. versipora	Oman
UF 20351			XII-A	P. versipora	Oman
UF 20351			XII-A	P. versipora	Oman
UF 20425			XII-A	P. versipora	Oman
UF 20425			XII-A	P. versipora	Oman
TAUAr27836		C. pallidus	outgroup	Porites sp.	Thailand
UF 11887		Armatobalanus	outgroup	Montipora sp.	Taiwan
UF 8664	H167	Cantellius sp.	outgroup	Montipora sp.	Philippines

Table 4-3. List of ESUs. Putative ESUs are marked with a “?”.

Clade	ESU	Barnacle ID (if available)	Localities	Host IDs
I	A	*T. margaretae*	Red Sea; Iles Eparses	*Favia sp., F. favus, F. mathaei*
	B		Philippines; Taiwan	*Favia sp.*
	C		Tuamotus	*F. rotumana*
II	A	*"Neotrevathana elongata"*	Red Sea; Iles Eparses	*Echinopora gemmacea, E. hirsutissima*
	B		Philippines; Taiwan	*Echinopora sp., E. lamellosa*
III	A	*T. mizrachae*	Red Sea; Oman	*Platygyra sp., P. lamellina*
	B		Guam	*P. pini*
	C?		Madagascar	*Platygyra sp.*
IV	A		Fiji; Cook Ids.; Society Ids.; Tuamotus	*Montastrea curta*
	B?		Guam	*Favites russelli*
	C	*"T. dentata"*	GBR; Fiji; Tuamotus	*Favia stelligera?, Leptastrea, Goniastrea*
	D	*T. jensi*	Red Sea; GBR	*Favites abdita; F. halicora*
	E	*"T. dentata"*	Red Sea	*Leptastrea transversa*
	F?		CNMI	*Goniastrea edwardsi*
	G?		Vanuatu	*F. halicora*
	H		Guam; CNMI	*G. edwardsi*
	I?		Palau	*G. edwardsi*
V	A?	*Pyrgopsella youngi*	Indonesia	*Symphyllia radians*
VI	A	*T. sarae*	Red Sea; Seychelles; Philippines	*Cyphastrea sp., C. serailia, C. chalcidia*
	B		Guam	*Cyphastrea sp., C. serailia,*
VII	A	Hoekiini	Philippines	*Hydnophora microconos*
	B?	Hoekiini	Iles Eparses	*Hydnophora sp.*
	C	Hoekiini	Philippines	*Hydnophora exesa*
VIII	A?		Guam	*F. stelligera*
IX	A		Society Ids.; Tuamotus	*"Cyphastrea sp."; Plesiastrea sp.*
X	A		Society Ids.; Tuamotus	*Acanthastrea echinata*
	B	*T. paulayi*	Guam	*A. echinata*
XI	A		Cook Ids.; Society Ids.; Tuamotus	*F. stelligera*
	B		Guam; CNMI	*F. stelligera*
XII	A		Iles Eparses; Oman	*Plesiastrea sp., P. versipora, "Cyphastrea sp."*

Table 4-4. List of analyzed ESEs.

Clade	ESU1	ESU2	Geog. break	Host switch?
I	I-A	I-B	WIO-WP	y
I	I-B	I-C	WP-CP	y
II	II-A	II-B	WIO-WP	y
III	III-A	III-B	WIO-WP	y
VI	VI-A	VI-B	WIO*-WP	n
VII	VII-A	VII-C	NA	y
X	X-A	X-B	WP-CP	n
XI	XI-A	XI-B	WP-CP	n

*VI-A predominantly from the WIO except for 1 Philippine sample

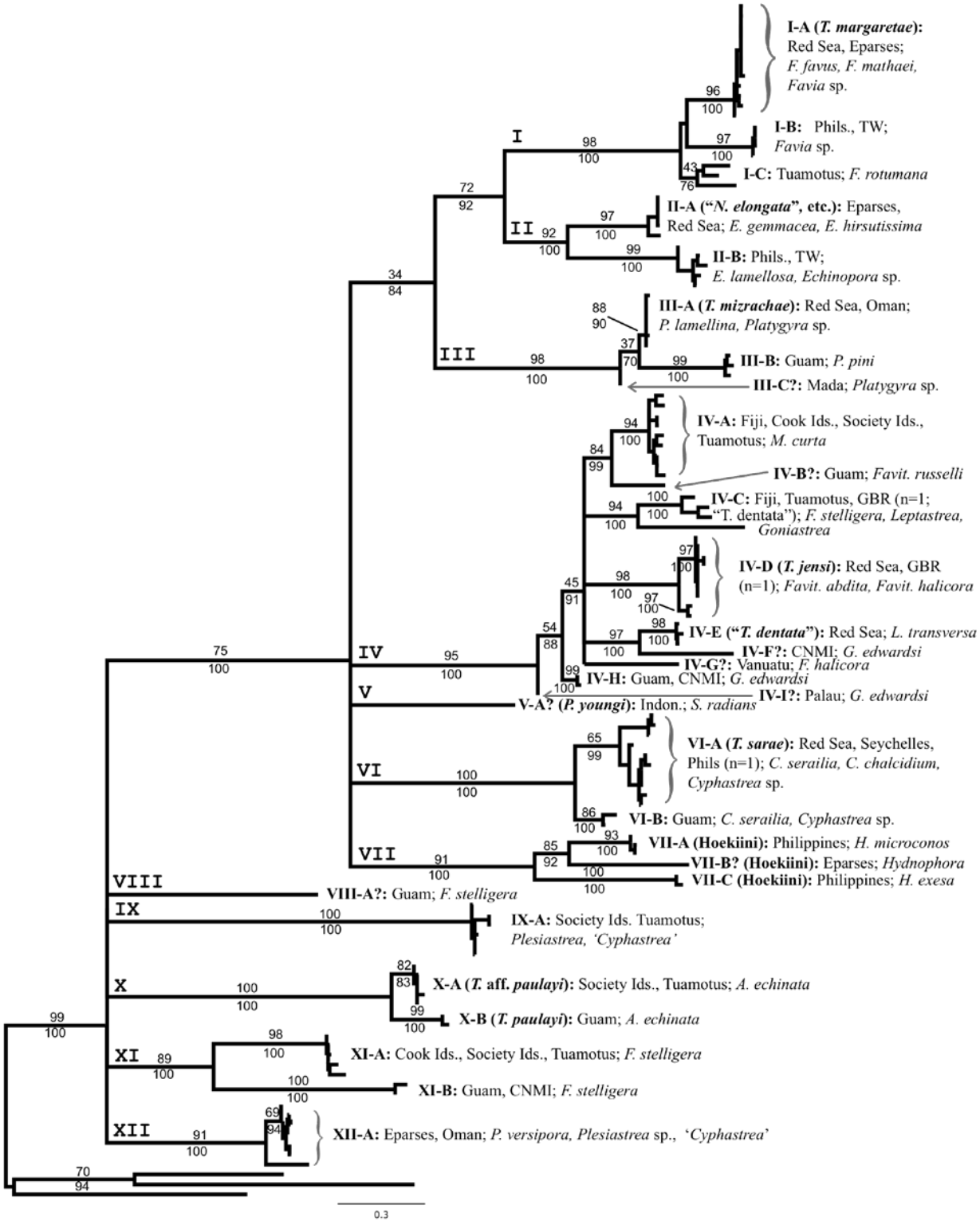

Figure 4-1. RAxML phylogram for Trevathana s.l. Values above branches are ML bootstraps, values below branches are Bayesian posterior probabilities.

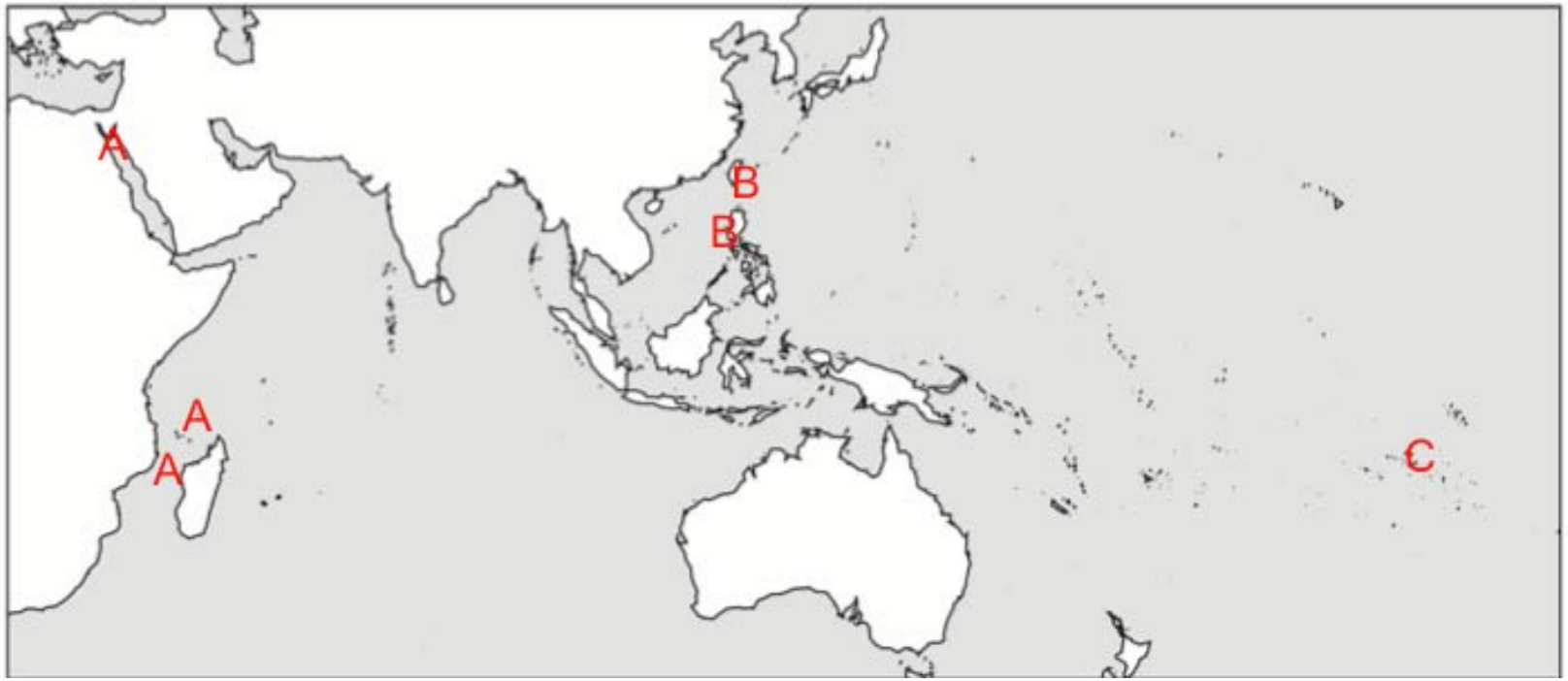

Fig. 4-2. Geographic distribution of Clade I ESUs.

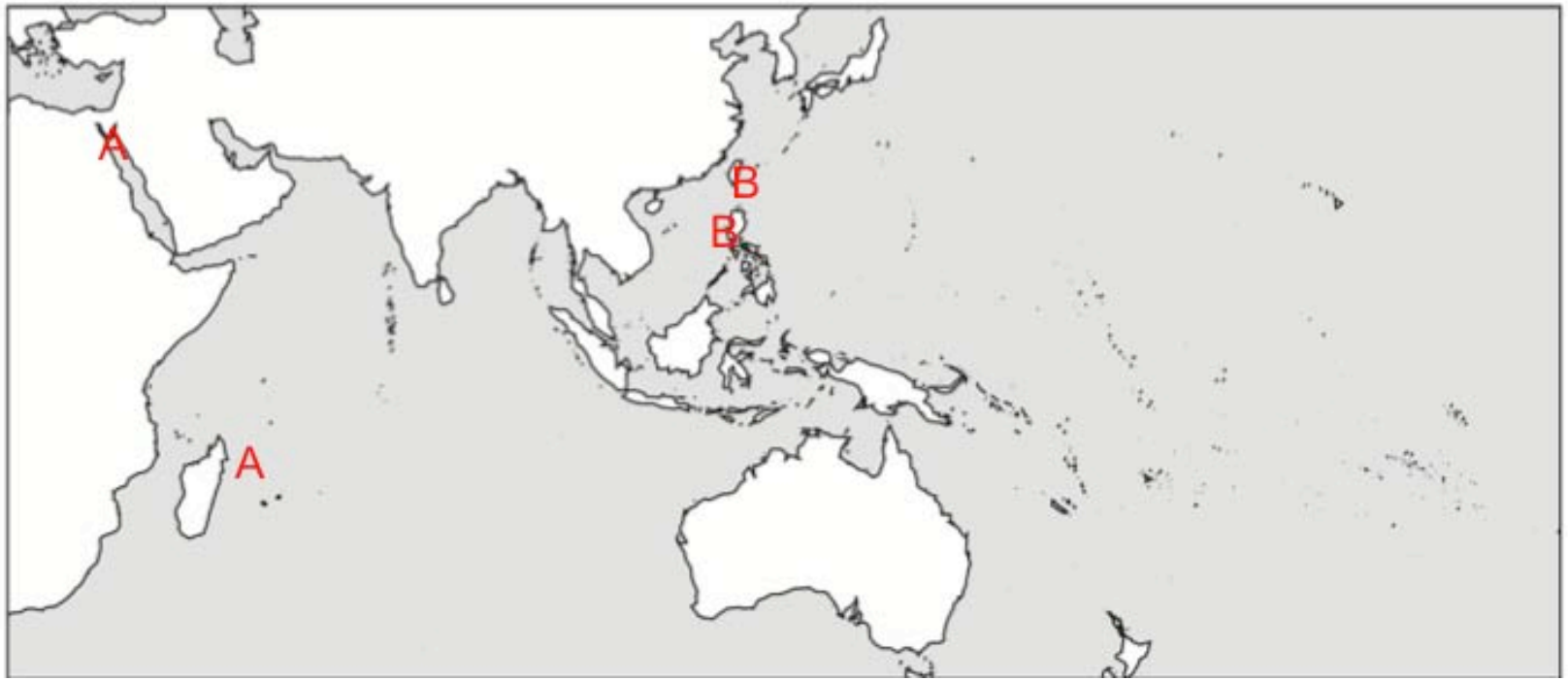

Fig. 4-3. Geographic distribution of Clade II ESUs.

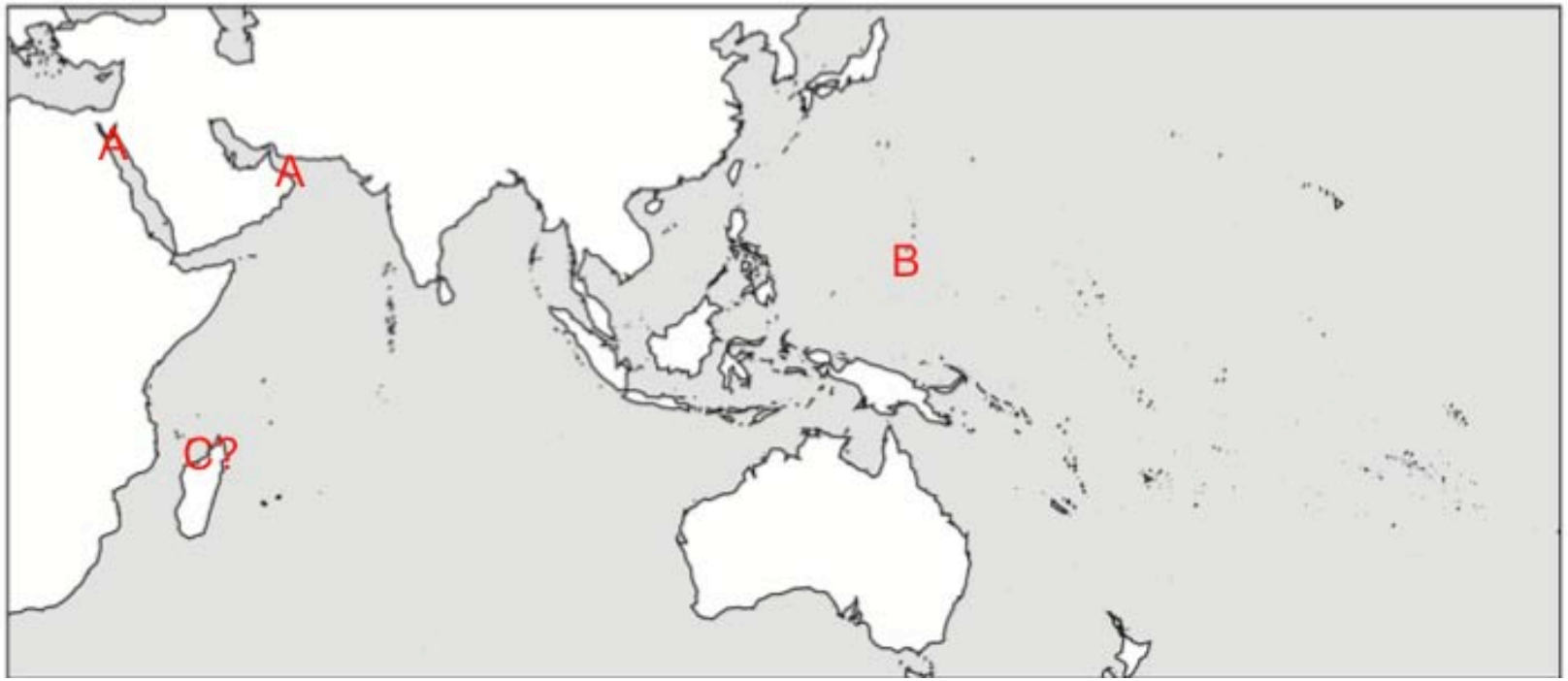

Fig. 4-4. Geographic distribution of Clade III ESUs.

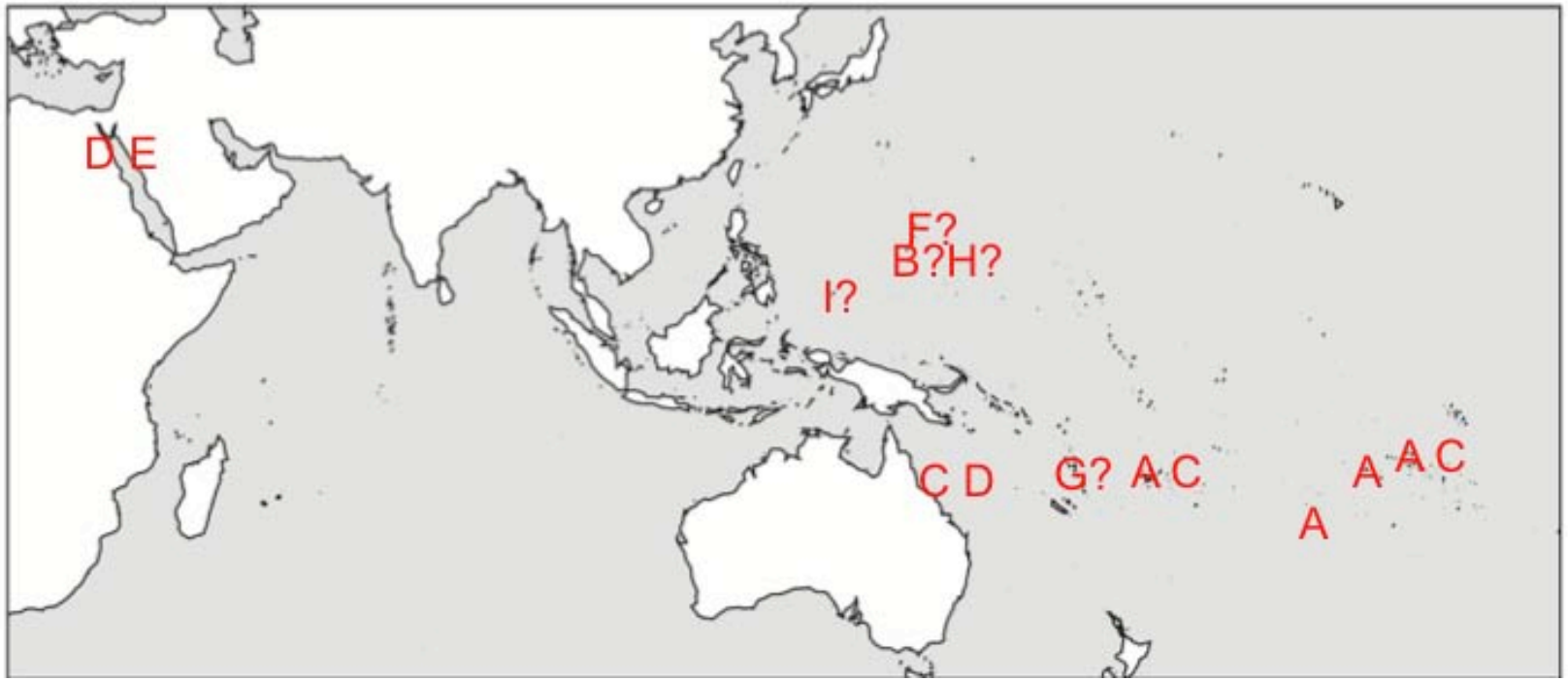

Fig. 4-5. Geographic distribution of Clade IV ESUs.

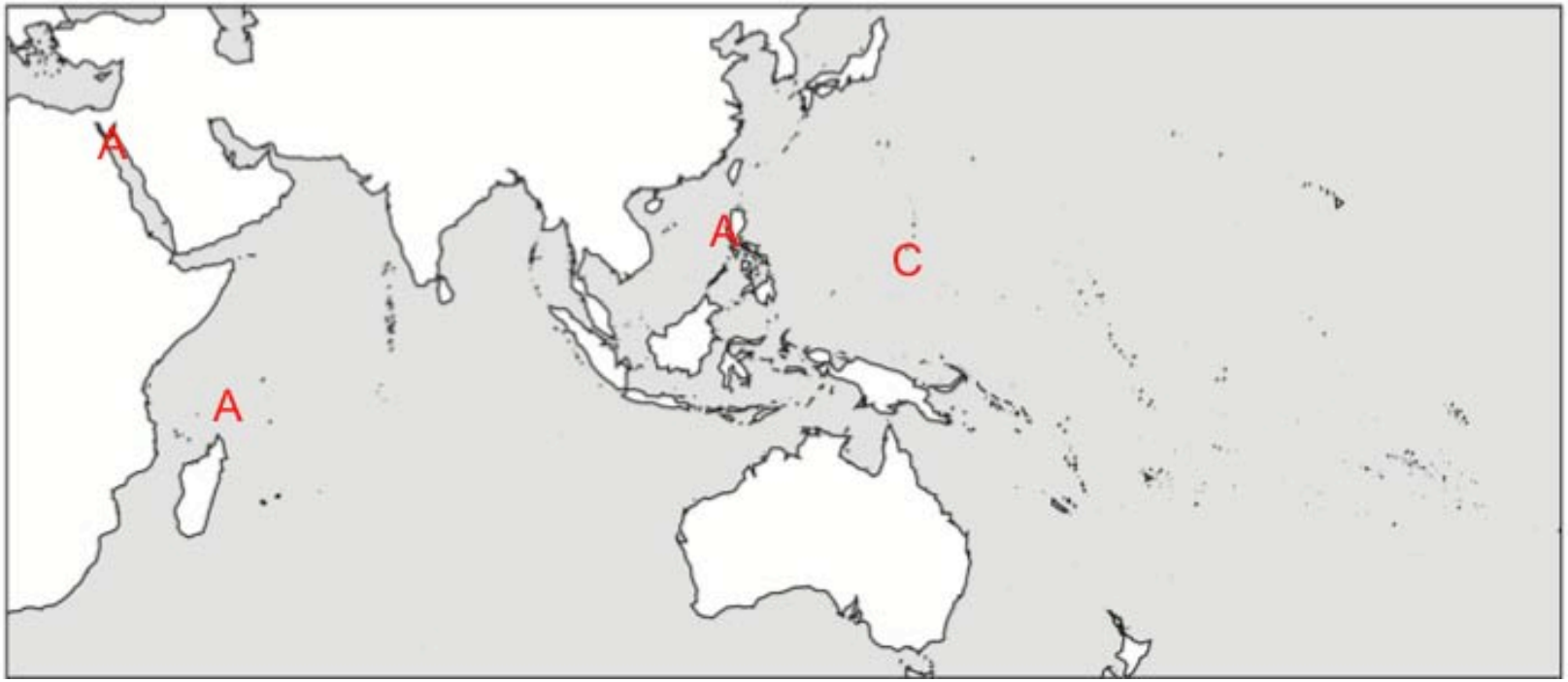

Fig. 4-6. Geographic distribution of Clade VI ESUs.

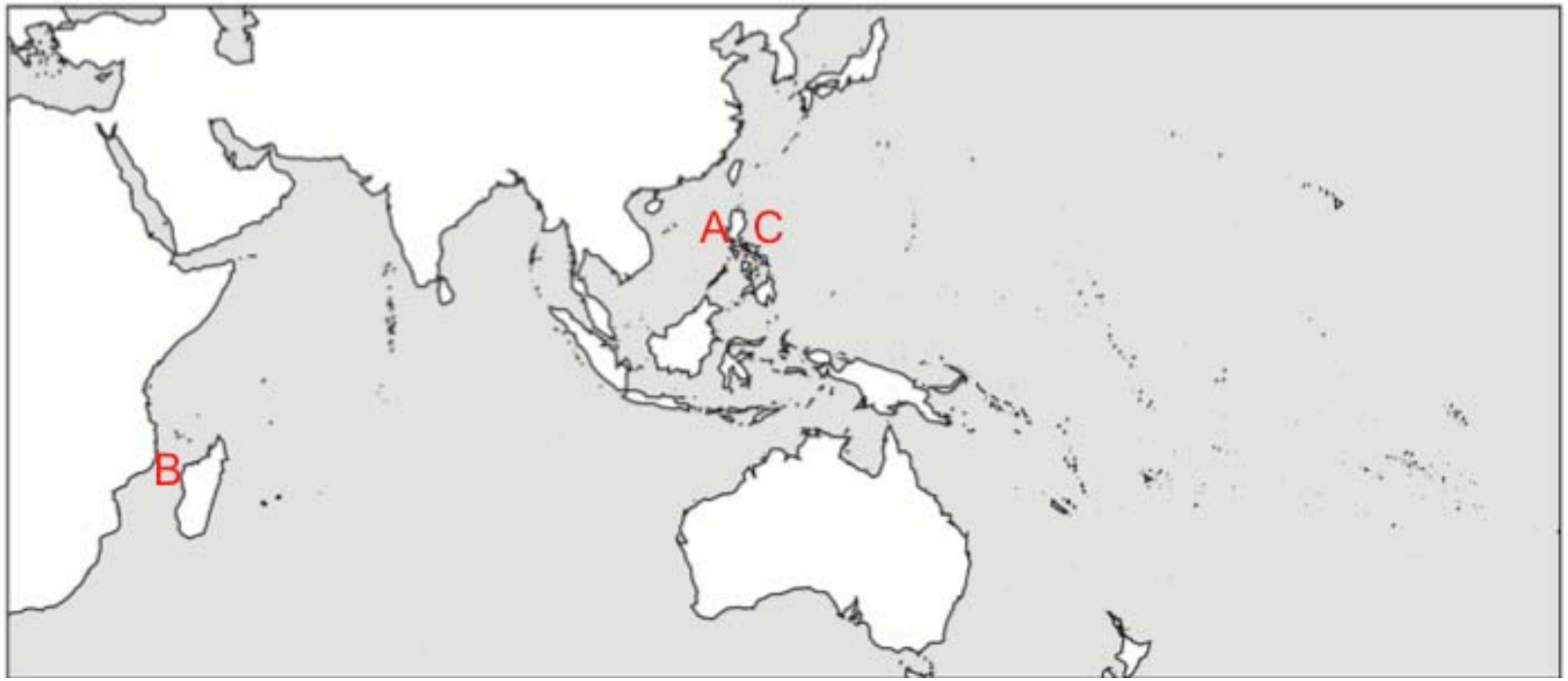

Fig. 4-7. Geographic distribution of Clade VII ESUs.

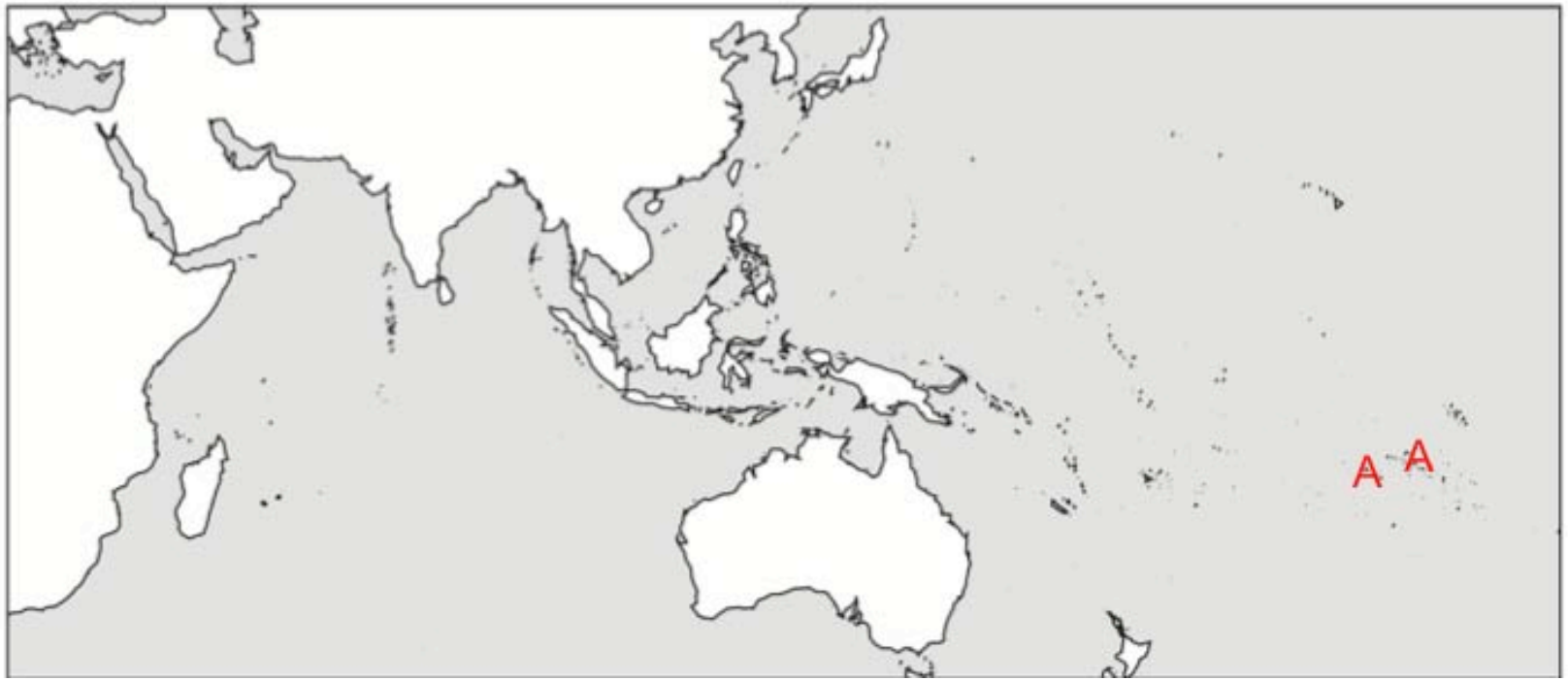

Fig. 4-8. Geographic distribution of Clade IX ESUs.

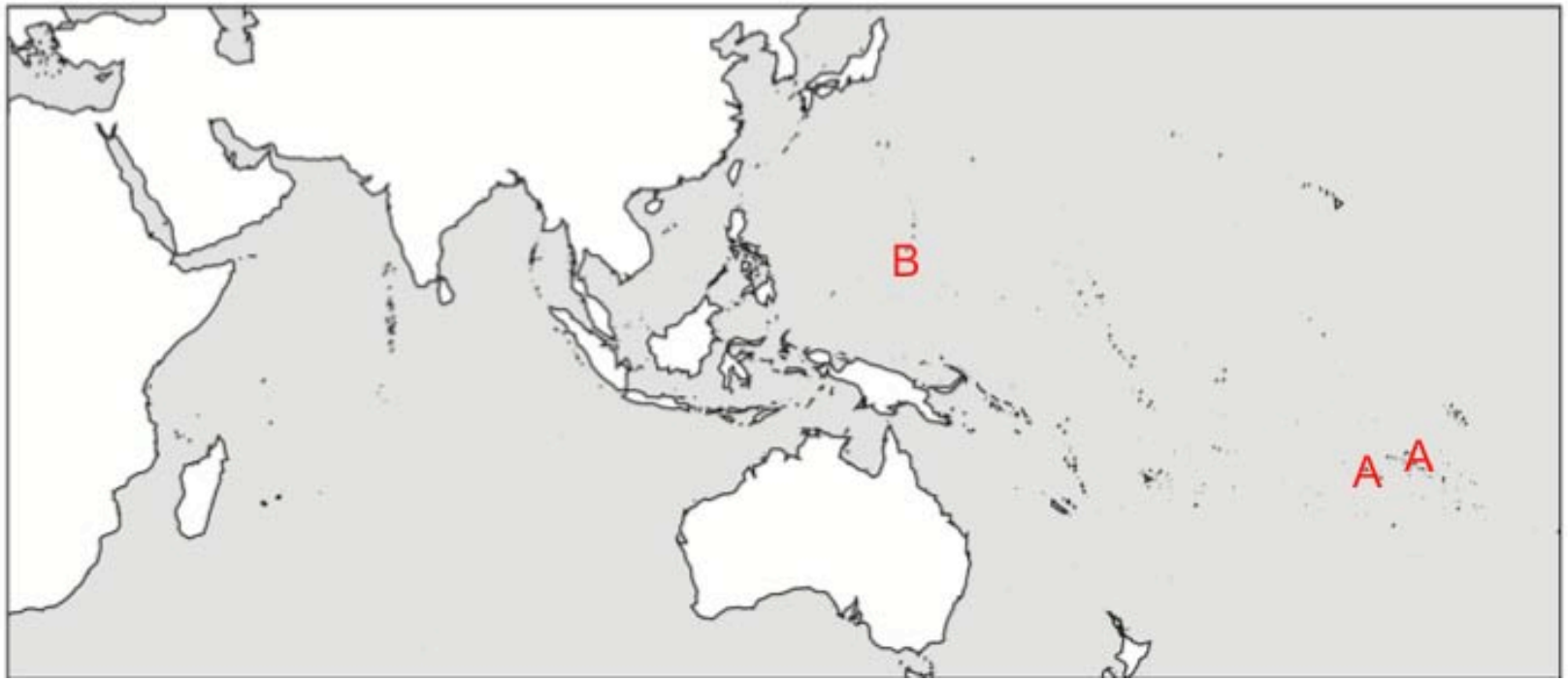

Fig. 4-9. Geographic distribution of Clade X ESUs.

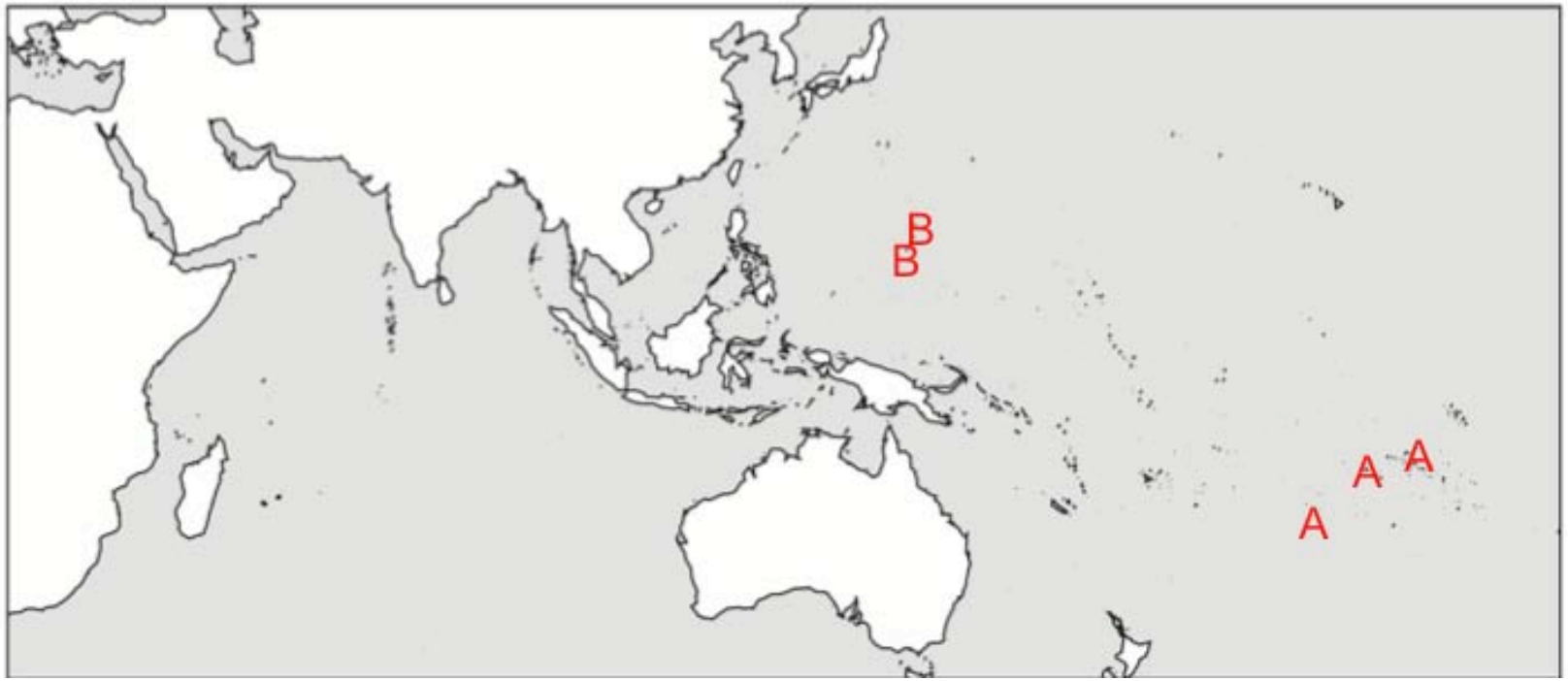

Fig. 4-10. Geographic distribution of Clade XI ESUs.

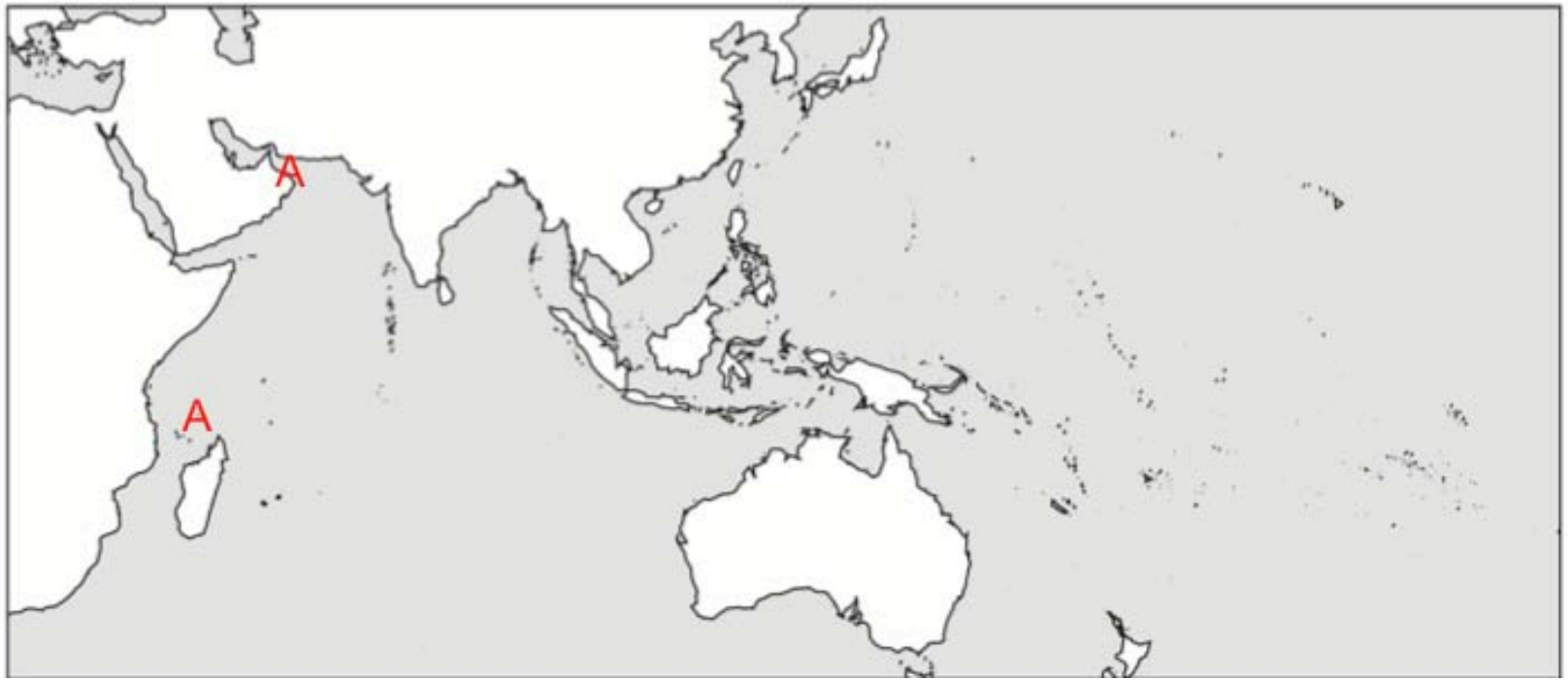

Fig. 4-11. Geographic distribution of Clade XII ESUs.

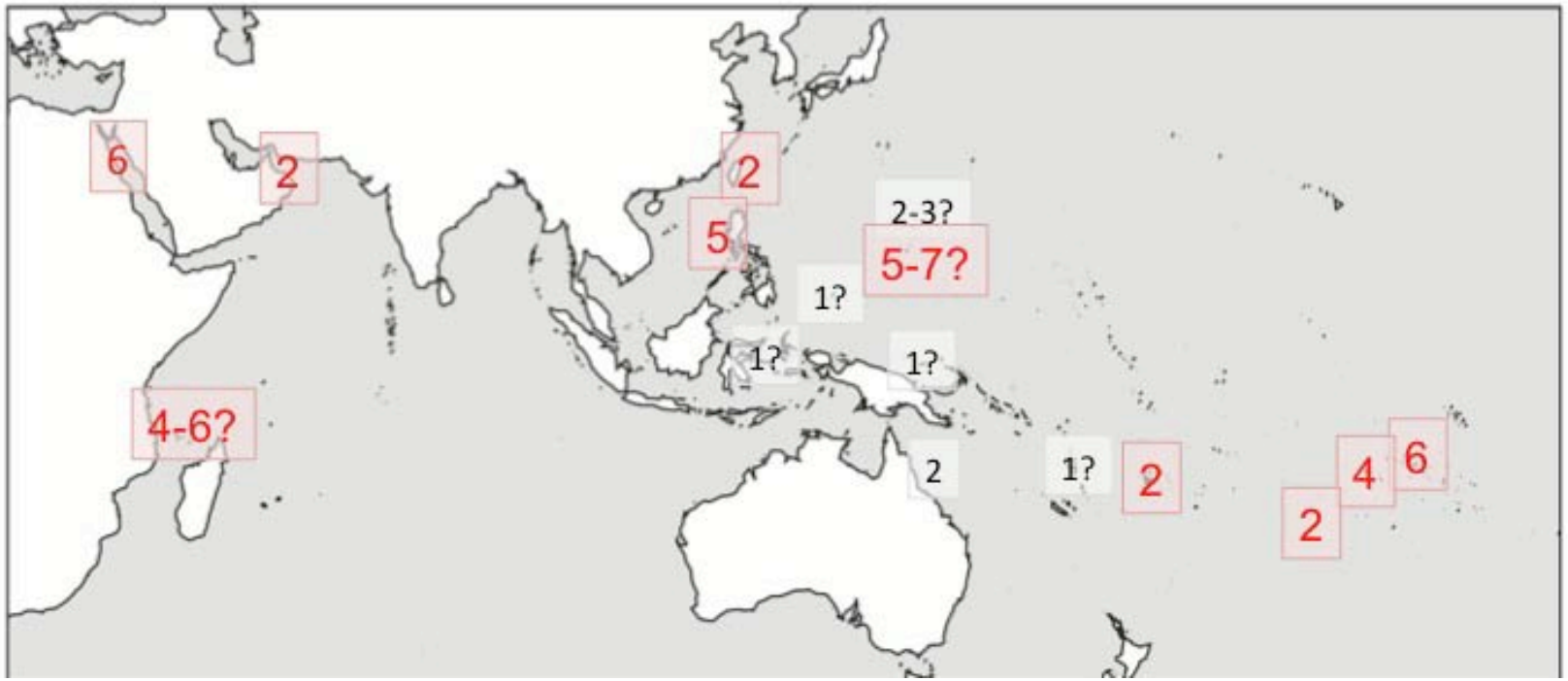

Fig. 4-12. ESU richness in sampled localities. Well sampled locales are in red.

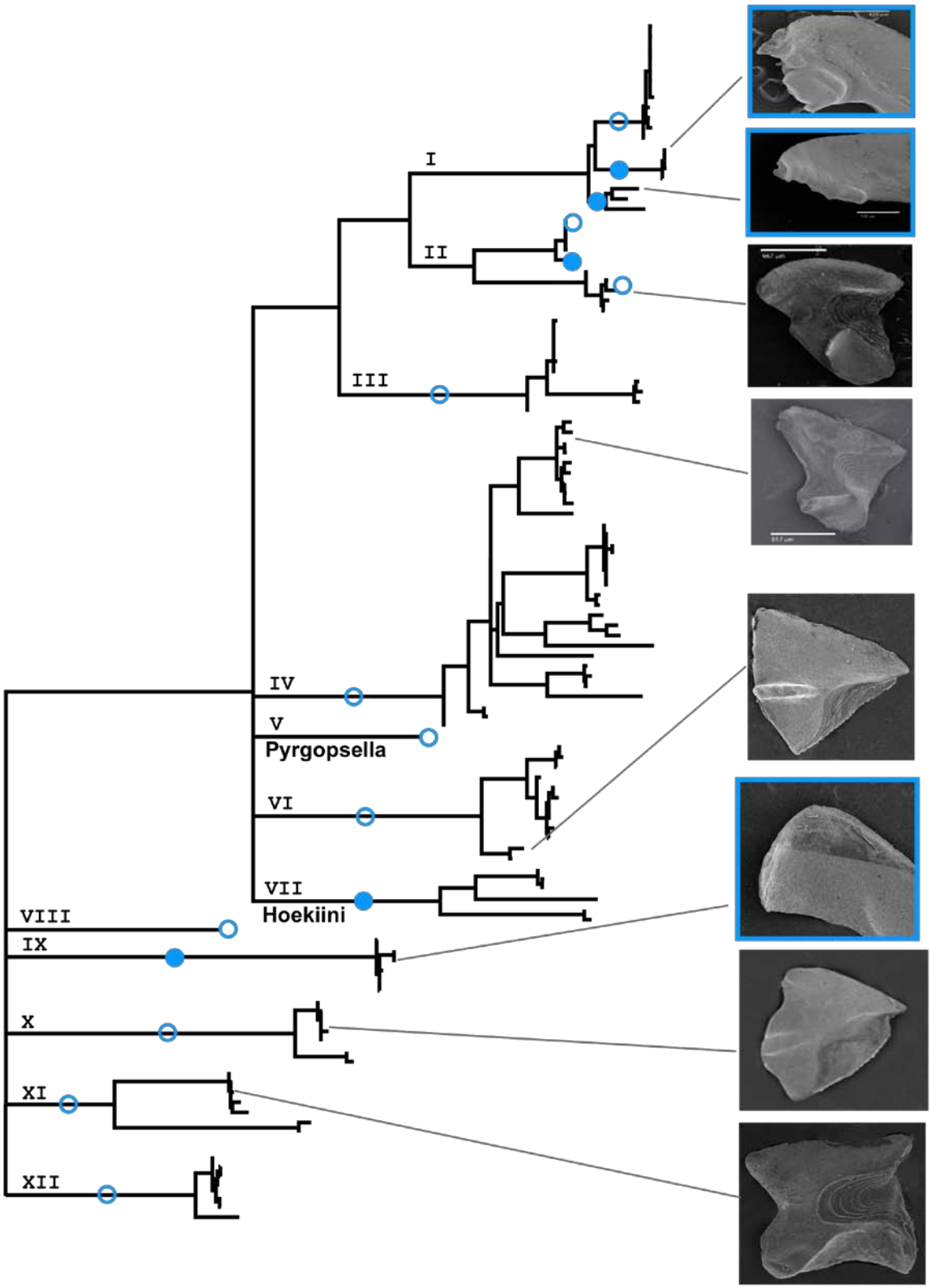

Fig. 4-13. Occurrences of unfused (open circle) and fused (filled circle) opercular valves in the phylogeny. SEMs depict the inner surface of the tergum.

CHAPTER 5
CONCLUSIONS

The species richness of coral reefs has long captivated observers and has focused attention on the sources of this diversity. My dissertation was motivated by a desire to use a molecular phylogenetic and phylogeographic approach to better understand factors promoting diversification in IWP coral reefs.

In order for this approach to be successful, two conditions are necessary: (1) the systematics of the group of interest must be well known, so that all lineages are represented in the study; and (2) sampling must be as comprehensive as possible across all lineages and across the entire geographic range of the group, so that boundaries between sister-ESUs can be accurately identified. These conditions were quite feasible for the first study (Chapter 2), because *Calcinus* is a taxonomically well studied group with relatively well delineated species boundaries; in addition, these hermit crabs are often conspicuous and locally abundant and hence easy to sample. However, applying the same approach to coral-dwelling pyrgomatid barnacles proved much more of a challenge since the taxonomy of the group is still in a state of confusion and many species remain undescribed; moreover, pyrgomatids are cryptic and more difficult to sample. Thus it was necessary to first understand the phylogenetic relationships of the family Pyrgomatidae before attempting a phylogeographic study.

The systematics of the Pyrgomatidae were tackled in Chapter 3, and this in itself proved to be a very fruitful study. I discovered that morphological characters traditionally used to define the family and to delimit genera and species within the family (e.g., number of wall plates, opercular valve fusion) are highly homoplasious and therefore misleading indicators of phylogenetic relationships. I found that other morphological

characters that need to be considered in pyrgomatid evolution include the presence (or absence) of teeth on the anterior margins of the cirri, and the means by which the barnacle controls coral overgrowth. The pyrgomatids *sensu stricto* fell into three major clades that do not correspond to the traditional taxonomic groupings; one genus (*Wanella*) fell outside of the pyrgomatids, and a non-pyrgomatid (the 'archaeobalanid' *Armatobalanus*) was resolved in Clade III of the Pyrgomatidae *sensu stricto*. Notably, the genus I was studying phylogeographically (*Trevathana*) was not recovered as reciprocally monophyletic, and instead included genera with membranous bases (*Pyrgopsella*, Hoekiini) and even eu-parasitic and highly apomorphic genera (Hoekiini). Had I not conducted this study, taxon sampling for the *Trevathana* study (Chapter 4) would have been less complete, and therefore the conclusions less robust.

Studying the speciation patterns of *Calcinus* hermit crabs (Chapter 2) and *Trevathana sensu lato* pyrgomatids (Chapter 4) offered interesting parallels and contrasts. In both systems, the mode of speciation was overwhelmingly allopatric (an allopatric mode of speciation was deemed most likely in all except one of the *Calcinus* ESEs, and in all of the *Trevathana s.l.* ESEs). Young pairs of sister-ESUs are allopatric (shown by *Calcinus* and *Trevathana s.l.* data), and it appears that older sister-ESUs regain sympatry only after >2 my (shown by the *Calcinus* data). This supports the idea that speciation is mostly through geographic isolation, and secondary sympatry can only develop once sufficient time has passed for incipient species to develop barriers to interbreeding.

The location of speciation events differed in the two systems. In *Calcinus*, most of the recent speciation events occurred in remote CP archipelagos (Hawaii, Marquesas,

etc.), or (less frequently) at the tropical-subtropical latitudinal boundary. The IO – WP boundary was a relatively unimportant region for speciation in *Calcinus*. In contrast, in *Trevathana s.l.* all speciation events occurred somewhere between the WIO and WP, or between the WP and the CP. Thus ESU boundaries were completely non-concordant in the two systems studied. The reasons for this difference are currently unknown, but may be related to factors such as dispersal ability (the planktonic larval duration of *Calcinus* is not known; while in *Trevathana s.l.* the planktonic phase lasts approximately 2 weeks in the Society Ids., Malay unpub. data), ecological niches (*Calcinus* appears to prefer oceanic reefs over continental settings; no such preference was observed in *Trevathana s.l.*), the timing of speciation events (may not be concordant in the 2 systems), etc.

Studying speciation in pyrgomatid barnacles allowed the examination of an extra layer of information: the role of host-switching in diversification. In *Trevathana s.l.*, I found that more recent divergences are structured geographically, while older divergences are structured by host use. This suggests that both host-switching and geographic isolation cause diversification, but that these 2 forces act at different rates. Geographic isolation is a relatively rapid engine of diversification, thus recently diverged pairs of sister-ESUs bear a geographic signature; however, this signature becomes erased through evolutionary time as species ranges change. In contrast host-switching is a much slower engine of speciation; switching events occur relatively rarely and thus the host signature is retained in the phylogeny for a longer time.

It is a well known fact that the center of tropical marine biodiversity is located in the so-called Indo-Malayan ‘coral triangle’, bordered by Indonesia, the Philippines and New Guinea. In multiple taxa (reef fish, scleractinian corals, gastropods, seagrasses, etc.;

reviewed in Hoeksema 2007) diversity steeply declines in all directions away from this hotspot. And yet I did not find a diversity hotspot in the coral triangle for either *Calcinus* nor *Trevathana s.l.* This shows that the coral triangle biodiversity peak is not consistent across all groups or across all taxonomic scales. I propose that if one were to compile species occurrence data for all diogenid hermit crabs, or for all pyrgomatid barnacles, one may still observe a hotspot in the coral triangle (such data are not yet available for those groups). Such an observation would be further demonstration of heterogeneity in patterns of diversification in the IWP. Overall, studies are showing that divergence patterns of individual lineages do not match well-worn models put forth to explain the origin of Indo-West Pacific diversity (such as those discussed in Chapter 1). Rather, the overall patterns are simply summations of disparate evolutionary histories, and serve to underscore the complexity of marine diversification.

LIST OF REFERENCES

Abbott, R.T. 1960. The genus *Strombus* in the Indo-Pacific. Indo-Pacific Mollusca 1:33-146.

Abrahamson, W.G., M.D. Eubanks, C.P. Blair, and A.V. Whipple. 2001. Gall flies, inquilines, and goldenrods: a model for host-race formation and sympatric speciation. Amer. Zool. 41: 928-938.

Achituv, Y. and N. Simon-Blecher. 2006. *Pyrgopsella* (Cirripedia: Balanomorpha: Pyrgomatidae) is not a sponge-inhabiting barnacle. Zootaxa 1319: 29-42.

Achituv, Y., L.M. Tsang, and B.K.K. Chan. 2009. A new species of *Cantellius* and a redescription of *C. sextus* (Hiro, 1938) (Cirripedia, Balanomorpha, Pyrgomatidae) from the elephant skin coral, *Pachyseris speciosa* (Dana, 1846) (Scleractinia, Agariciidae) from Taiwan. Zootaxa 2022: 15-28.

Akaike, H. 1974. A new look at the statistical model identification. IEEE Trans. Autom. Contr. 19: 716-723.

Allen, G.R. 2007. Conservation hotspots of biodiversity and endemism for Indo-Pacific coral reef fishes. Aquatic Conserv.: Mar. Freshw. Ecosyst. 18: 541-556.

Anderson, D.T. 1992. Structure, function, and phylogeny of coral-inhabiting barnacles (Cirripedia, Balanoidea). Zool. J. Linn. Soc. 106: 277-339.

Anderson, D.T. 1993. Addendum/Corrigendum. Zoological Journal of the Linnean Society 105:337.

Anderson, J., N. Goldman, and A. Rodrigo. 2000, Internet. Guidelines for performing the SOWH test. <http://www.ebi.ac.uk/goldman/tests/ SOWHinstr.html>.

Asakura, A. 2004. Hermit crabs of the genus *Calcinus* Dana, 1851 from Japan and adjacent waters (Decapoda, Anomura, Diogenidae): a colour variant of *C. hazletti* Haig & McLaughlin. Crustaceana 76: 1237-1241.

Asakura, A. and H. Tachikawa. 2003. Hermit crabs of the genus *Calcinus* Dana, 1851 from Japan and adjacent waters (Decapoda, Anomura, Diogenidae): *C. hazletti* Haig & McLaughlin, 1984 and *C. haigae* Wooster, 1984. Crustaceana 76: 717-739.

Asami, K. and T. Yamaguchi. 1997. Distribution of living and fossil coral barnacles (Cirripedia; Pyrgomatidae) in Japan. Sessile Organisms 14: 9-16.

Avise, J.C., J. Arnold, R.M. Ball, E. Bermingham, T. Lamb, J.E. Neigel, C.A. Reeb, and N.C. Saunders. 1987. Intraspecific phylogeography: the mitochondrial DNA bridge between population genetics and systematics. Ann. Rev. Ecol. Syst. 18:489-522.

Baluk, W. and A. Radwanski. 1967. Miocene cirripeds domiciled in corals. Acta Palaeo/ Polonica 12: 457-521.

Barber, P.H. and D.R. Bellwood. 2005. Biodiversity hotspots: evolutionary origins of biodiversity in wrasses(*Halichoeres*: Labridae) in the Indo-Pacific and new world tropics. Mol. Phylogen. Evol. 35: 235-253.

Barber, P.H., M.V. Erdmann, and S.R. Palumbi. 2006. Comparative phylogeography of three codistributed stomatopods: origins and timing of regional lineage diversification in the coral triangle. Evolution 60:1825-1839.

Barber, P.H., S.R. Palumbi, M.V. Erdmann, and M.K. Moosa. 2000. A marine Wallace's line? Nature 406: 693-694.

Barker, F.K., and F.M. Lutzoni. 2002. The utility of the incongruence length difference test. Syst. Biol. 51:625-637.

Bellwood, D.R. and P.C. Wainwright. 2002. The history and biogeography of fishes on coral reefs. In: P.F. Sale, ed. Coral reef fishes: dynamics and diversity in a complex ecosystem. Academic Press, London, p. 5-32.

Bellwood, D.R. and T.P. Hughes. 2001. Regional-scale assembly rules and biodiversity of coral reefs. Science 292: 1532-1534.

Bellwood, D.R., T.P. Hughes, S.R. Connolly, and J. Tanner. 2005. Environmental and geometric constraints on Indo-Pacific coral reef biodiversity. Ecol. Lett. 8: 643-651.

Berlocher, S.H. and J.L. Feder. 2002. Sympatric speciation in phyotphagous insects: Moving beyond controversy? Ann. Rev. Entomol. 47L 773-815.

Bowen, B.W., A. Muss, L.A. Rocha, and W.S. Grant. 2006. Shallow mtDNA coalescence in Atlantic pygmy angelfishes (genus *Centropyge*) indicates a recent invasion from the Indian Ocean. J. Heredity 97:1-12.

Brickner, I., N. Simon-Blecher, and Y. Achituv. 2010. Darwin's *Pyrgoma* (Cirripedia) revisited: revision of the *Savignium* group, molecular analysis and description of new species. J. Crust. Biol. 30: 266-291.

Briggs, J.C. 1974. Marine Zoogeography. McGraw-Hill Book Co., London.

Briggs, J.C. 1999. Coincident biogeographic patterns: Indo-West Pacific Ocean. Evol. 53: 326-335.

Broch, H. 1931. Papers from Th. Mortensen's Pacific expedition 1914-1916 LVI Indomalayan Cirripedia. Vidensk. Medd. Dansk. Naturh. Kbh. 91: 1-146.

Coates, A. G., and J. A. Obando. 1996. The geologic evolution of the Central American isthmus. In: J. B. C. Jackson, A. F. Budd and A. G. Coates, eds. Evolution and environments in tropical America. University of Chicago Press, Chicago, p. 21-56.

Colgan, D.J., A. McLauchlan, G.D.F. Wilson, S.P. Livingston, G.D. Edgecombe, J. Macaranas, M.R. Gray, and G. Cassis. 1998. Histone H3 and U2 snRNA DNA sequences and arthropod molecular evolution. Aust. J. Zool. 46:419-437.

Coyne, J.A., and H.A. Orr. 2004. Speciation. Sinauer Associates Inc., Sunderland MA.

Darwin, C. 1854. A monograph on the Sub-Class Cirripedia: Balanidae, Verrucidae, etc. Ray Society, London.

Detto, T., P.R.Y. Backwell, J.M. Hemmi, and J. Zeil. 2006. Visually mediated species and neighbour recognition in fiddler crabs (*Uca mjoebergi* and *Uca capricornis*). Proc. R. Soc. Lond. B 273:1661-1666.

Dieckmann, U and M Doebeli (1999) On the origin of species by sympatric speciation. Nature 400: 354-357.

Dowton, M. and A.D. Austin. 1995. Increased genetic diversity in mitochondrial genes is correlated with the evolution of parasitism in the Hymenoptera. J. Mol. Evol. 41: 958-965.

Drummond, A. and A. Rambaut. 2007. BEAST: Bayesian evolutionary analysis by sampling trees. BMC Evol. Biol. 7: 214.

Drummond, AJ, B Ashton, M Cheung, J Heled, M Kearse, R Moir, S Stones-Havas, T Thierer, and A Wilson. 2009, Internet. Geneious v4.7. <http://www.geneious.com/>.

Duffy, J.E. 1996. Species boundaries, specialization, and the radiation of sponge-dwelling alpheid shrimp. Biol. J. Linn. Soc. 58: 307-324.

Dunham, D.W. 1978. Effect of chela white on agonistic success in a diogenid crab (*Calcinus laevimanus*). Marine Behav. Physiol. 5: 137-144.

Eastman, J.T. and A.R. McCune. 2000. Fishes on the Antarctic continental shelf: evolution of a marine species flock? J. Fish Biol. 57: 84-102.

Ekman, S. 1953. Zoogeography of the Sea. Sidgwick & Jackson, London.

Emelianov, I., F. Marec, and J. Mallet. 2004. Genomic evidence for divergence with gene flow in host races of the larch budmoth. Proc. R. Soc. Biol. Sci. Ser. B 271: 97-105.

Emerson, B.C. and N. Kolm. 2005. Species diversity can drive speciation. Nature 434: 1015-1017.

Faucci, A., R.J. Toonen, and M. Hadfield. 2007. Host shift and speciation in a coral-feeding nudibranch. Proc R. Soc. Lond. Ser. B 274: 111-119.

Farris, J.S., M. Kallersjo, A.G. Kluge, and C. Bult. 1995. Constructing a significance test for incongruence. Syst. Biol. 44:570-572.

Feder, J.L., S. Opp, B. Wlazlo, K. Reynolds, W. Go, and S. Spisa. 1994. Host fidelity is an effective pre-mating barrier between sympatric races of the apple maggot fly. Proc. Nat. Acad. Sci. USA 91: 7990-7994.

Fitch, W.M. 1971. Toward defining the course of evolution: minimal change for a specific tree topology. Syst. Zool. 20: 406-416.

Floeter, S. R., and D. R. R. L. A. Rocha, J. C. Joyeux, W. F. Smith-Vaniz, P. Wirtz, A. J. Edwards, J. P. Barreiros, C. E. L. Ferreira, J. L. Gasparini, A. Brito, J. M. Falcón, B. W. Bowen, G. Bernardi. 2008. Atlantic reef fish biogeography and evolution. J. Biogeog. 35:22-47.

Frey, M. A., and G. J. Vermeij. 2008. Molecular phylogenies and historical biogeography of a circumtropical group of gastropods (Genus: Nerita): Implications for regional diversity patterns in the marine tropics. Mol. Phylogen. Evol. 48:1067-1086.

Fukami, H., A.F. Budd, G. Paulay, A. Solé-Cava, A.C. Chen, K. Iwao, and N. Knowlton. 2004. Conventional taxonomy obscures deep divergence between Pacific and Atlantic corals. Nature 427: 832-835.

Fukami, H., C.A.Chen, A.F. Budd. A. Collins, C. Wallace, Y.-Y. Chuang, C. Chen, C.-F. Dai, K Iwao, C. Sheppard, and N. Knowlton. 2008. Mitochondrial and nuclear genes suggest that stony corals are monophyletic but most families of stony corals are not (Order Scleractinia, Class Anthozoa, Phylum Cnidaria). PloS One 3: e32222-

Funk, D.J., and K.E. Omland. 2003. Species-level paraphyly and polyphyly: frequency, causes, and consequences, with sights from animal mitochondrial DNA. Annu. Rev. Ecol. Evol. Syst. 34:397-423.

Gavrilets, S. 2000/ A dynamical theory of speciation on holey adaptive landscapes. Am. Nat. 154: 1-22.

George, R. W. 1974. Coral reefs and rock lobster ecology in the Indo-West Pacific region. Proceedings of the Second International Coral Reef Symposium, Brisbane 1:321-325.

Gherardi, F. 2004. Resource partitioning between sexes in the "unconventional" hermit crab, *Calcinus tubularis*. Behav. Ecol. 15:742-747.

Graybeal, A. 1998. Is it better to add taxa or characters to a difficult phylogenetic problem? Syst Biol 47: 9-17.

Hawthorne, D.J. and S. Via. 2001. Genetic linkage of ecological specialization and reproductive isolation in pea aphids. Nature 412: 904-907.

Haig, J. and P.A. Mclaughlin. 1984. New Calcinus species (Decapoda: Anomura: Diogenidae) from Hawaii, with a key to the local species. Micronesica 19: 107-121.

Hazlett, B. A. 1981. The behavioural ecology of hermit crabs. Ann. Rev. Ecol. Syst. 12: 1-22.

Healy, J. M., and D. T. Anderson. 1990. Sperm ultrastructure in the Cirripedia and its phylogenetic significance. Rec. Aust. Mus. 42: 1–26.

Hebert, P. D. N., Cywinska, A., Ball, S. L., deWaard, J. R. 2003. Biological Identifications through DNA barcodes. Proc. R. Soc. Lond. B 270:313-322.

Hiro, F., 1935. A study of cirripeds associated with corals occurring in Tanabe Bay. Res. Ocean. Wks. Jpn. 7, 1–28.

Hiro, F. 1938. Studies on animals inhabiting reef corals II. Cirripeds of the genera *Creusia* and *Pyrgoma*. Palao Trop. Biol. Stat. Stud. 3: 391-416.

Hoeksema, B. W. 2007. Delineation of the Indo-Malayan centre of maximum marine biodiversity: the coral triangle. Topics in Geobiology 29: 117-178.

Huelsenbeck, J.P. and F. Ronquist. 2001. MrBAYES: Bayesian inference of phylogenetic trees. Bioinformatics 17:754-755.

Hughes, T.P., D.R. Bellwood, and S.R. Connolly. 2002. Biodiversity hotspots, centres of endemicity, and the conservation of coral reefs. Ecol. Lett. 5: 775-784.

Jokiel, P., and F. J. Martinelli. 1992. The vortex model of coral reef biogeography. J. Biogeog. 19:449-458.

Karlson R.H., H.V. Cornell, and T.P. Hughes. 2004. Coral communities are regionally enriched along an oceanic biodiversity gradient. Nature 429: 867-870.

Katoh, K., K. Misawa, K. Kuma, and T. Miyata. 2002. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. Nucl. Acids Res. 30: 3059-3066.

Kay, E.A. 1984. Patterns of speciation in the Indo-west Pacific. Bernice P. Bishop Museum Special Publication 72:15-31.

Kimura, M. 1980. A simple method for estimating evolutionary rate of base substitution through comparative studies of nucleotide sequences. J. Mol. Evol. 16: 111-120.

Kirkendale, L.A. and C.P. Meyer. 2004. Phylogeography of the *Patelloida profunda* group (Gastropoda: Lottidae): diversification in a dispersal-driven marine system. Mol. Ecol. 13: 2749-2762.

Knowlton, N. 1986. Cryptic and sibling species among the decapod crustacea. J. Crust. Biol. 6: 356-363.

Knowlton, N. 1993. Sibling species in the sea. Ann. Rev. Ecol. Syst. 24: 189-216.

Knowlton, N. 2000. Molecular genetic analyses of species boundaries in the sea. Hydrobiol. 420: 73-90.

Knowlton, N. and L.A. Weigt. 1998. New dates and new rates for divergence across the Isthmus of Panama. Proc. R. Soc. Lond. B 265: 2257-2263.

Kolbasov, G.A. 1993. Revision of the genus *Acasta* Leach (Cirripedia: Balanoidea). Zool. J. Linn. Soc. 109: 395-427.

Ladd, H. S. 1960. Origin of the Pacific island molluscan fauna. Am. J. Sci. 258A:137-150.

Landry, C., L.B. Geyer, Y. Arakaki, T. Uehara, and S.R. Palumbi. 2003. Recent speciation in the Indo-West Pacific: rapid evolution of gamete recognition and sperm morphology in cryptic species of sea urchin. Proc. R. Soc. Lond. B 270: 1839-1847.

Lavery, S., C. Moritz, and D.R. Fielder. 1996. Indo-Pacific population structure and evolutionary history of the coconut crab *Birgus latro*. Mol. Ecol. 5: 557-570.

Lessios, H. A. 2008. The Great American Schism: Divergence of Marine Organisms After the Rise of the Central American Isthmus. Ann. Rev. Ecol. Evol. Syst. 39: 63-91.

Lessios, H.A., B.D. Kessing, and J.S. Pearse. 2001. Population structure and speciation in tropical seas: global phylogeography of the sea urchin *Diadema*. Evol. 55: 955-975.

Macpherson, E. and A. Machordom. 2001. Phylogenetic relationships of species of *Raymunida* (Decapoda: Galatheidae) based on morphology and mitochondrial cytochrome oxidase sequences, with the recognition of four new species. J. Crust. Biol. 21: 696-714.

Maddison, W.P. and D.R. Maddison. 2009, Internet. Mesquite: A modular system for evolutionary analysis. Ver. 2.72. <http://mesquiteproject.org>.

Maddison D, Maddison W. MacClade 4.08: Analysis of Phylogeny and Character Evolution (2005) Sunderland (MA): Sinauer Associates.

Markham, J. C. 1977. Preliminary note on the ecology of *Calcinus verrilli*, an endemic Bermuda hermit crab occupying attached vermetid shells. J. Zool. Lond. 181: 131-136.

McMillan, W.O., L.A. Weigt, and S.R. Palumbi. 1999. Color pattern evolution, assortative mating, and genetic differentiation in brightly colored butterflyfishes (Chaetodontidae). Evolution 53:247-260.

Meyer, C.P. 2003. Molecular systematics of cowries (Gastropoda: Cypraeidae) and diversification patterns in the tropics. Biol. J. Linn. Soc. 79: 401-459.

Meyer, C.P., and G. Paulay. 2005. DNA barcoding: error rates based on comprehensive sampling. PLoS Biology 3:2229-2238.

Meyer, C.P., J.B. Geller, and G. Paulay. 2005. Fine scale endemism on coral reefs: archipelagic differentiation in turbinid gastropods. Evolution 59: 113-125.

Mokady, O. and I. Brickner. 2001. Host-associated speciation in a coral-inhabiting barnacle. Mol. Biol. Evol. 18: 975-981.

Mokady, O., Y. Loya, Y. Achituv, E. Geffen, D. Graur, S. Rozenblatt, and I. Brickner. 1999. Speciation versus phenotypic plasticity in coral inhabiting barnacles: Darwin's observations in an ecological context. J. Mol. Evol. 49: 367-375.

Mora, C., P.M. Chittaro, P.F. Sale, J.P. Kritzer, and S.A. Ludsin. 2003. Patterns and processes in reef fish diversity. Nature 421: 933-936.

Morgan, G. J. 1991. A review of the hermit crab genus Calcin*us* Dana (Crustacea: Decapoda: Diogenidae) from Australia, with descriptions of two new species. Invertebrate Taxonomy 5: 869–913.

Moritz, C. 1994. Defining "evolutionarily significant units" for conservation. Trends Ecol. Evol. 9: 373-375.

Munday, P.L., L. van Herwerden, and C.L. Dudgeon. 2004. Evidence for sympatric speciation by host shift in the sea. Current Biol. 14: 1498–1504.

Newman, W.A. 1987. Evolution of cirripedes and their major groups. In: A.J. Southward, ed. Crustacean Issues 5: Barnacle Biology. Balkema, Rotterdam, p. 3-42.

Newman, W. A., and B. A. Foster. 1987. Southern hemisphere endemism among the barnacles: explained in part by extinction of amphitropical taxa? Bull. Mar. Sci. 41:361-377.

Newman and Ladd 1974 Newman, W.A. and H.S. Ladd. 1974.Origin of coral inhabiting balanids (Cirripedia, Thoracica). Verhandl. Naturf. Ges. Basel 84: 381-396.

Newman, W.A. and A. Ross. 1976. Revision of the balanomorph barnacles; including a catalog of the species. Memoirs of the San Diego Society of Natural History 9: 1-108.

Palumbi, S.R. 1996. Nucleic Acids II: the polymerase chain reaction. In: D.M. Hillis, C. Moritz, and B.K. Mable, eds. Molecular Systematics, 2nd ed. Sinauer and Associates, Inc., Sunderland MA, p. 205-246.

Palumbi, S.R. 1997. Molecular biogeography of the Pacific. Coral Reefs 16, Suppl.: S47-S52.

Parker, J, A. Rambaut, and O.G. Pybus. 2008. Correlating viral phenotypes with phylogeny: Accounting for phylogenetic uncertainty. Infection, Genetics, and Evol. 8: 239-246.

Paulay, G. 1990. Effects of late Cenozoic sea level fluctuations on the bivalve faunas of tropical oceanic islands. Paleobiol. 16: 415-434.

Paulay, G. 1994. Biodiversity on oceanic islands: its origin and extinction. Am. Zool. 34:134-144.

Paulay, G. 1996. Dynamic clams: changes in the bivalve fauna of Pacific islands as a result of sea-level fluctuations. Am. Malacol. Bull. 12: 45-57.

Paulay, G. 1997. Diversity and distribution of reef organisms. Pp. 298-353 *in* C.E. Birkeland, ed. Life and death of coral reefs. Chapman and Hall, New York.

Paulay, G. and C. Meyer. 2002. Diversification in the tropical Pacific: comparisons between marine and terrestrial systems and the importance of founder speciation. Integr. Comp. Biol. 42: 922-934.

Paulay, G., and C. Meyer. 2006. Dispersal and divergence across the greatest ocean region: do larvae matter? Integr. Comp. Biol. 46:269-281.

Peterson, A.T., J. Soberon, and V. Sanchez-Cordero. 1999. Conservatism of ecological niches in evolutionary time. Science 285: 1265-1267.

Romano and Cairns. 2000.Perez-Losada, M., J.T. Hoeg, and K.A. Crandall. 2004. Unravelling the evolutionary radiation of the thoracican barnacles using molecular and morphological evidence: a comparison of several divergence time estimation approaches. Syst. Biol. 53:244-264.

Perez-Losada, M., M. Harp, J.T. Hoeg, Y. Achituv, D. Jones, H. Watanabe, and K.A. Crandall .2008. The tempo and mode of barnacle evolution. Mol. Phylogen. Evol. 46: 328-346.

Pilsbry 1913

Pitombo, F.B. and A. Ross. 2002. A review of the Hexacreusia species complex: Eastern Pacific coral barnacles (Cirripedia, Balanomorpha). Arquivos do Museu Nacional, Rio de Janeiro 60: 89-94.

Posada, D. and K.A. Crandall. 1998. Modeltest: testing the model of DNA substitution. Bioinformatics 14: 817-818.

Poupin, J. 2003, Internet. Hermit crabs *Calcinus* of the world (Anomura, Diogenidae). Descriptions, Illustrations, Identification, and Information Retrieval, using the DELTA format. <http://biomar.free.fr/calcinus/>.

Poupin, J. 2007. Les pagures du genre *Calcinus* en Polynésie française, avec le description de trois nouvelles espèces (Crustacea, Decapoda, Diogenidae). Zoosystema 19: 683-719.

Poupin, J. and J.M. Bouchard. 2006. The eastern Pacific species of the genus *Calcinus* Dana, 1851, with description of a new species from Clipperton Island (Decapoda, Anomura, Diogenidae). Zoosystema 28: 465-486.

Poupin, J. and P.A. McLaughlin. 1998. Additional records of *Calcinus* species (Decapoda: Anomura: Diogenidae) from French Polynesia with description of three new species and a key to Indo-West Pacific species of the genus. Crust. Res. 27: 9-27

Poupin, J. and R. Lemaitre. 2003. Hermit crabs of the genus *Calcinus* Dana, 1851 (Decapoda: Anomura: Diogenidae) from the Austral Islands, French Polynesia, with description of a new species. Zootaxa 391: 1-20.

Puebla, O., E. Bermingham, F. Guichard, and E. Whiteman. 2007. Colour pattern as a single trait driving speciation in *Hypoplectrus* coral reef fishes? Proc. R. Soc. Lond. B 274:1265-1271.

Randall, J. E. 1998. Zoogeography of shore fishes of the Indo-Pacific region. Zool. Stud. 37:227-268.

Ravago, R.G. and M.A. Juinio-Meñez. 2003. Phylogenetic position of the striped-legged forms of *Panulirus longipes* (A Milne-Edwards, 1868) (Decapoda, Palinuridae) inferred from mitochondrial DNA sequences. Crustaceana 75: 1047-59.

Read, C. I., D. R. Bellwood, and L. van Herwerden. 2006. Ancient origins of Indo-Pacific coral reef fish biodiversity: A case study of the leopard wrasses (Labridae: *Macropharyngodon*). Mol. Phylogen. Evol. 38:808-819.

Reid, D. G., K. Lal, J. Mackenzie-Dodds, F. Kaligis, D. T. J. Littlewood, and S. T. Williams. 2006. Comparative phylogeography and species boundaries in *Echinolittorina* snails in the central Indo-West Pacific. J. Biogeog. 33: 990-1006.

Rice W.R. and E.E. Hostert. 1993. Laboratory experiments on speciation: what have we learned in 40 years? Evolution 47: 1637-1653.

Romano, S.L. and S.D. Cairns. 2000. Molecular phylogenetic hypotheses for the evolution of scleractinian corals. Bull. Mar. Sci. 67: 1043-1068.

Ronquist, F. and J. P. Huelsenbeck. 2003. MRBAYES 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19:1572-1574.

Rosell, N.C. 1975. On two noteworth balanids (Cirripedia Thoracica) from the Sulu Archipelago, Philippines. Univ. Phils. Nat. Sci. Res. Center Tech. Report 4: 1-12.

Rosen, B. R. 1988. Progress, problems and patterns in the biogeography of reef corals and other tropical marine organisms. Helgoländer Meeresuntersuchungen 42:269-301.

Ross, A. 1999. On the occurrence of *Megabalanus stultus* (Darwin), 1854 (Cirripedia: Balanomorpha) in Taiwan: a reappraisal. Zool. Stud. 38: 275-278.

Ross, A. and W.A. Newman. 1973. Revision of the coral-inhabiting barnacles (Cirripedia, Balanidae). Trans. San Diego Soc. Nat. Hist. 17: 137-174.

Ross, A. and W.A. Newman. 1995. A coral-eating barnacle, revisited (Cirripedia, Pyrgomatidae). Contrib. Zool. 65: 129-175.

Ross, A. and W.A. Newman. 2000. *Pyrgoma kuri* Hoek, 1913: a case study in morphology and systematics of a symbiotic barnacle (Cirripedia: Balanomorpha). Contrib. Zool. 68: 65-80.

Ross, A. and W.A. Newman. 2002. Coral barnacles: Cenozoic decline and extinction in the Atlantic/East Pacific versus diversification in the Indo-West Pacific. Proc. 9th Int. Coral Reef Symp., Bali, Indonesia. Vol. I: 135-139.

Ross, A. and F.B. Pitombo. 2002. Notes on the coral-inhabiting Megatreminae and the description of a new tribe, new genus and three new species (Cirripedia: Sessilia: Pyrgomatidae). Sessile Organisms 19: 57-68.

Schliewen, U.K., D. Tautz, and S. Pääbo. 1994. Sympatric speciation suggested by monophyly of crater lake cichlids. Nature 368: 629-632.

Schluter, D. 2001. Ecology and the origin of species. Trends Ecol Evol 16: 372-380.

Seehausen, O., G. Takimoto, D. Roy, and J. Jokela. 2008. Speciation reversal and biodiversity dynamics with hybridization in changing environments. Mol. Ecol. 17:30-44.

Shimodaira, H. and M. Hasegawa. 1999. Multiple comparisons of log-likelihoods with applications to phylogenetic inference. Mol. Biol. Evol. 16: 1114-1116.

Simon-Blecher, N., D. Huchon, and Y. Achituv. 2007. Phylogeny of coral-inhabiting barnacles (Cirripedia; Thoracica; Pyrgomatidae) based on 12S, 16S and 18S rDNA analysis. Mol. Phylogen. Evol. 44: 1333-1341.

Smith-Vaniz, W. F., B. B. Collette, and B. E. Luckhurst. 1999. Fishes of Bermuda: history, zoogeography, annotated checklist, and identification keys. American Society of Ichthyologists and Herpetologists Special Publication 4:i-x, 1-424.

Soltis, D.E., V.A. Albert, V. Savoilanen, K. Hilu, Y.-L. Qiu, M.W .Chase, J.S. Farris, S. Stefanovic, D.W. Rice, J.D. Palmer, and P.S. Soltis. 2004. Genome-scale data, angiosperm relationships, and 'ending incongruence': a cautionary tale in phylogenetics. Trends Plant Sci. 9: 477-483.

Springer, V.G. 1982. Pacific plate biogeography, with special reference to shorefishes. Smithsonian Contributions to Zoology:1-182.

Stamatakis, A. 2006. RAxML-VI-HPC: Maximum Likelihood-based Phylogenetic Analyses with Thousands of Taxa and Mixed Models. Bioinformatics 22: 2688–2690.

Stehli, F. G., A. L. McAlester, and C. E. Helsley. 1967. Taxonomic diversity of Recent bivalves and some implications for geology. Geol. Soc. Am. Bull. 78:455-466.

Stehli, F.G., and J.W. Wells. 1971. Diversity and age patterns in hermatypic corals. Syst. Zool. 20:115-126.

Sterrer, W. 1986. Marine fauna and flora of Bermuda: a systematic guide to the identification of marine organisms. Wiley, New York.

Swofford, D.L. 2002. Phylogenetic Analysis Using Parsimony (*and Other Methods), version 4. Sinauer Associates, Inc., Sunderland, MA.

Swofford, D.L., G.J. Olsen, P.J. Waddell, and D.M. Hillis. 1996. Phylogenetic Inference. In: D.M. Hillis, C. Moritz, and B.K. Mable, eds. Molecular Systematics. Sinauer, Sunderland Massachusetts, p. 407-514.

Tsang, L.M, B.K.K. Chan, F.-L. Shih, K.H. Chu, and C.A. Chen. 2009. Host-associated speciation in the coral barnacle Wanella milleporae (Cirripedia: Pyrgomatidae) inhabiting the Millepora coral. Mol. Ecol. 18: 1463-1475.

Turelli, M., N.H. Barton, and J.A. Coyne. 2001. Theory and speciation. Trends Ecol. Evol. 16: 330-343.

Vermeij, G. J. 1987. Evolution and escalation, an ecological history of life. Princeton University Press, Princeton NJ.

Veron, J. E. N. 1995. Corals in space and time. The biogeography and evolution of the Scleractinia. University of New South Wales Press, Sydney, Australia.

Via, S. 2001. Sympatric speciation in animals: the ugly duckling grows up. Trends Ecol. Evol. 16: 381-390.

Wang, T.H., Y.K. Donaldson, R.P. Brettle, J.E. Bell, P. Simmonds. 2001. Identification of shared populations of human immunodeficiency Virus Type I infecting microglia and tissue macrophages outside the central nervous system. J. Virol. 75: 11686-11699.

Wheeler, Q.D .and R. Meier, eds. 2000. Species Concepts and Phylogenetic Theory. Columbia University Press, NY.

Weiblen, G.D. and G.L. Bush. 2002. Speciation in fig pollinators and parasites. Mol. Ecol. 11: 1573-1578.

Wiens, J.J. 2004. Speciation and ecology revisited: phylogenetic niche conservatism and the origin of species. Evol. 58: 193-197.

Wiens, J.J. and C.H. Graham. 2005. Niche conservatism: integrating evolution, ecology, and conservation biology. Annu. Rev. Ecol. Syst. 36: 519-539.

Williams, S.T. 2000. Species boundaries in the starfish genus *Linckia*. Mar. Biol. 136, 137-148.

Williams, S.T. 2007. Origins and diversification of Indo-West Pacific marine fauna: evolutionary history and biogeography of turban shells (Gastropoda, Turbinidae). Biol. J. Linn. Soc. 92: 573-592.

Williams, S.T., and D.G. Reid. 2004. Speciation and diversity on tropical rocky shores: A global phylogeny of snails of the genus *Echinolittorina*. Evolution 58:2227-2251.

Williams, S.T., D.G. Reid, and D.T.J. Littlewood. 2003. A molecular phylogeny of the Littorininae (Gastropoda: Littorinidae): unequal evolutionary rates, morphological parallelism, and biogeography of the Southern Ocean. Mol. Phylogen. Evol. 28:60-86.

Williams, S.T., J. Jara, E. Gomez, and N. Knowlton. 2002. The marine Indo-West Pacific break: contrasting the resolving power of mitochondrial and nuclear genes. Integr. Comp. Biol. 42: 941-952.

Woodland, D.J. 1983. Zoogeography of the Siganidae (Pisces): an interpretation of distribution and richness pattern. Bull. Mar. Sci. 33:713-717.

Yoder, A.D., J.A. Irwin, and B.A. Payseur. 2001. Failure of the ILD to determine data combinability for slow loris phylogeny. Syst. Biol. 50:408-424.

Zullo, V.A. and R.W. Portell. In litt. Classification and phylogeny of coral-inhabiting barnacles (Cirripedia: Archaeobalanidae and Pyrgomatidae), with emphasis on the late Cenozoic fauna of the Americas and western Tethys. Unpublished ms.

Zwickl, D. J., 2006. Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion. Ph.D. dissertation, The University of Texas at Austin.

Zwickl, D. J., 2006. Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion. Ph.D. dissertation, The University of Texas at Austin

Zwickl, D.J. and D.M. Hillis. 2002. Increased taxon sampling greatly reduces phylogenetic error. Syst. Biol. 51: 588-598.

BIOGRAPHICAL SKETCH

Maria Celia (Machel) Defrance Malay was born and (mostly raised) in the Philippines, to her French mother, Odile Defrance, and her Filipino father, Badi Malay. She is the youngest of three children. Fascinated with nature from an early age, Machel completed a Bachelor of Science in biology from the University of the Philippines – Diliman in 1998. At that point she knew she wanted to pursue research in the biological sciences, but still had no clue what particular field to specialize in. However, an opportunity to take scuba-diving lessons opened her eyes to the beauties and mysteries of life in the oceans, and she quickly decided to take the plunge into marine biology. Machel worked as a research assistant at the University of the Philippines – Marine Science Institute for 3 happy years, an experience that let her to discover the astounding diversity and heterogeneity of coastal ecosystems in the Philippines. Eventually, this led her to undertake a PhD in Zoology with Dr. Gustav Paulay at the Florida Museum of Natural History, specializing in the systematics, speciation, biodiversity, and biogeography of coral reef-associated invertebrates. Machel completed her doctorate in August 2010.