Evolutionary Diversification of the Marine Bivalve Clade Galeommatoidea

by

Jingchun Li

A dissertation submitted in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy
(Ecology and Evolutionary Biology)
in the University of Michigan
2014

Doctoral Committee:

Professor Diarmaid Ó Foighil, Chair
Associate Professor Catherine E. Badgley
Professor Tomasz K. Baumiller
Professor L. Lacey Knowles
Assistant Professor Daniel Rabosky

To my beloved husband Paul Richard Shearer, father Legong Li, mother Hongyue Wang and late grandmother Huailin Ji

ACKNOWLEDGEMENTS

My dissertation work benefitted tremendously from the contributions of the extraordinary people and organizations who provided intellectual, emotional, and financial support throughout my graduate study.

My advisor, Professor Diarmaid Ó Foighil, has been a dedicated and supportive mentor, an enthusiastic and inspiring scientist, and a great role model. His previous works on galeommatoidean clams and our shared interests in marine biodiversity directly led to the choice of my dissertation research theme. He taught me the way to be the best kind of scientist – curiosity driven, open-minded and respecting only the facts. He devoted immense time and efforts to my professional development and guided me through critical steps towards the completion of my dissertation. I will always remember the countless versions of manuscripts we worked through before each successful publication. This dissertation would not have been possible without him.

I am extremely fortunate to have a group of brilliant scientists on my doctoral committee from whom I learnt invaluable skills and ideas. I am especially grateful to Professor Catherine Badgley for introducing me to the field of biogeography and revising various manuscripts; Professor Tomasz Baumiller for our discussion on the phylogeography project and thoughtful comments during each committee meeting; Professor L. Lacey Knowles for her incredibly creative, critical and in-depth ideas on statistical phylogeography and lineage diversification, which benefitted not only the dissertation but also my future career development; and Professor Daniel Rabosky, for equipping me with crucial quantitative and programming skills that brought my research to a new level and his inspirational discussions on macroevolution and biodiversity. Drs. Miriam Zelditch and Donald Swiderski, though not on my committee, provided exceptional guidance on the geometric morphometric analyses, as well as conceptual development of my projects. I can not stress enough the importance of the above people and I hope our future paths cross often.

As for any global scale marine project, it is not possible to accomplish anything

without the generous assistance of collaborators. Many people have provided me valuable specimens – the foundation of my entire dissertation. For that I thank Professors/Drs. Philippe Bouchet, Ellen Strong, Gustav Paulay, Takuma Haga, Tan Siong Kiat, Paul Valentich-Scott, Lisa Kirkendale, Bruce Marshall, Paula Mikkelsen, Rüdiger Bieler, John Taylor, Emily Glover, Shin'ichi Sato, Greg Rouse, Gonzalo Giribet, Maria Byrne, Don Colgan, David Cowles, Brian Bingham, and all personnels who helped with processing and loaning the specimens. Another important perspective of my dissertation is the challenging fieldwork. I could not have completed them without the help from Professors/Drs. John Chapman, Peter Middelfart, Don Cadien, Gray Williams and Phil Colman.

The Mollusk Division of the Museum of Zoology has become a second home of mine and the people here my family. My memory of Professor Jack Burch kindly offering his "private lessons on mollusks" is still vivid. Professor Tom Duda, encouraging and generous, has always treated me as one of his own students and offered me tremendous help. Dr. Taehwan Lee is not only the go-to guy for any obstacles I have had, but also a good friend with whom I shared fun conversations about science, culture, and many more. For making my experience here wonderful, I thank past and present students and postdocs: Andrea Walther, Celia Churchill, Dan Chang, Cindy Bick, Paula Teichholtz, Samantha Flowers, Semoya Phillips, Alex Moore, Andrew Woods, Joong-Ki Park, Gang Chen and David Weese. I also thank Drs. Janice Pappas and Dan Miller for adding so much fun to our mollusk journal club. I'm grateful for all undergraduate students who have worked with me and contributed much to my project: Heather Heiser, Elizabeth Yu, Jon Jimenez and Sara Colom; I also thank Dr. Margaret Liu for introducing some of them to me. I deeply appreciate the support from the EEB administrative team: Fritz Paper, Norah Daugherty, Robbin Murrell, Jane Sullivan, Amber Stadler, Cynthia Carl and Deborah Dault.

For the wisdom and friendships that I treasure for life, I gave special thanks to my peers in EEB: Huijie Gan, Qixin He, Lucy Tran, Huateng Huang, Ya Yang, Doug Jackson and Jen-Pen Huang. For all the encouragements that kept me going, I am grateful to my parents Legong Li and Hongyue Wang, my aunts Lehong Li and Xiping Cheng, the Shearer and Sandkamp families and my lifelong friend Jing Yang. Finally, a simple thanks to my husband Paul Shearer because no words are adequate for expressing my deep gratitude to him.

This dissertation was made possible by the generous financial support from EEB, the Rackham Graduate School, NSF, the National Geographic Society, the American Malacological Society and the Malacological Society of Australasia.

TABLE OF CONTENTS

LIST OF FIGURES

Figure

LIST OF TABLES

Table

ABSTRACT

Evolutionary Diversification of the Marine Bivalve Clade Galeommatoidea
by
Jingchun Li

Chair: Diarmaid Ó Foighil

This dissertation investigates the diversification and morphological evolution of a major extant marine invertebrate lineage—the bivalve superfamily Galeommatoidea. It is inspired by the increasing realization among macroevolutionary biologists that the interplay between abiotic and biotic factors has shaped global biodiversity through time and that biotic interactions cannot be ignored if we wish to reconcile theory with natural systems. Evolutionary studies of contemporary marine lineages, in particular, are typically framed within abiotic hypothesis-testing contexts and have collectively lagged behind terrestrial studies in developing an integrated framework that includes a meaningful biotic perspective. I addressed this deficiency using the morphologically and taxonomically diverse Galeommatoidea as a study system. It is a particularly apt group because it contains large numbers of obligate commensal as well as free-living species and is therefore amenable to comparative approaches. I examined the ecological and evolutionary patterns of free-living and commensal galeommatoidean species on three levels: 1) on a microevolutionary level, focusing on commensal species that occupy multiple hosts; 2) on a regional level, for a faunal assemblage of galeommatoidean taxa that span three well-defined biogeographic provinces in southern Australia; 3) on a global level, for the entire superfamily. My ecological synthesis (Ch. 2) suggests that the free-living lifestyle is strongly correlated with living in hard-bottom habitats while the commensal lifestyle is an adaptation for living in sediments. Commensal associations with bioturbating hosts allow the small-bodied clams to attain refuges at depth from predation while remaining oxygenated through

their hosts' bioturbation. A case study on *Neaeromya rugifera* (Ch. 3) indicates that clam populations occupying different hosts differ significantly in shell morphologies, but do not show host-specific genetic structuring. Regional phylogeographic analyses of an endemic Australian galeommatoidean species (Ch. 4) show that the interaction of the Middle Miocene Climate Transition with the specific geography of the southern coastline of Australia was the primary cladogenic driver in this group. Macroevolutionary study of Galeommatoidea (Ch. 5) reveals that commensal/sediment-dwelling is the ancestral lifestyle of the superfamily and free-living/hard-bottom-dwelling is derived. A major free-living clade exhibits higher rates of lineage diversification compared to the commensals, possibly driven by complex ecological interactions in coral reef ecosystems. However, commensal species exhibit higher morphological disparity and intercladal convergence, likely reflecting host-specific morphological adaptations. Taken together, my multi-level study demonstrates that the present diversity of Galeommatoidea is shaped by the inseparable interactions between abiotic and biotic factors.

CHAPTER 1

Introduction

1.1 Biotic Interactions and Evolutionary Diversification

During a visit to the remote Saint Paul Archipelago in the North Atlantic, Charles Darwin was engrossed by the local terrestrial fauna on the rocks. He reported that "Not a single plant...grows on this islet...yet it is inhabited by several insects...a fly (*Olfersia*) living on the booby, and a tick which must have come here as a parasite on the birds; a small brown moth, belonging to a genus that feeds on feathers..." [8]. A great observer, Darwin astutely emphasized partnerships as an essential component of the local community. The survival of the insects on the infertile rocks is strongly dependent on their associations with sea birds. It is therefore essential to realize that the diversity of life is not only composed of the numerous species on earth, but also the entangled interactions among them. Competition, predation, parasitism, mutualism, as well as other types of interactions play important roles in shaping community structures and evolutionary trajectories of species [9].

The evolutionary significance of biotic interactions is best recognized in terrestrial systems where coevolutionary dynamics (*e.g.*, between insects and angiosperms) are well documented [10]. In the >70% of the planet covered by oceans, there is ample evidence for abiotic drivers of diversification, such as major tectonic events [11, 12], nutrient availability [13] and climate/sea level-induced vicariant breakpoints [14–18]. Many marine neontological evolutionary studies use such abiotic drivers to frame their diversification hypotheses, usually in the context of spatial distribution parameters and processes [19–27]. Paleontological studies have implicated biotic factors in post-mass extinction faunal recoveries [28], in adaptive escalations [29–31] and in interaction with abiotic factors [32]. The neontological literature, with some notable exceptions [33–38], tends to engage narrowly with the topic [39–43] due primarily to

understandable sampling issues. In particular, the scope of marine ecological interactions remains poorly understood [44], especially regarding subtle interactions such as facilitation (in which the presence of one species "facilitates" survival of another) that may be very important in nature [44–46].

Biotic interactions are being increasingly recognized as under-appreciated components in empirical and theoretical macroevolutionary studies [28, 44, 47–49]. Given this, it is important that we start to explore how biotic and abiotic factors interactively shape extent marine biodiversity. A logical approach would be to identify a candidate marine lineage that has the following characteristics: 1) a member of one of the two most diverse extant classes, *i.e.*, Gastropoda or Bivalvia [50]; 2) within that class, represents a diverse lineage; 3) exhibits exceptional phenotypic disparity; 4) embodies a clear ecological dichotomy in that many taxa have obligatory biotic associations while the remainder are free-living. As detailed in the following section, the bivalve superfamily Galeommatoidea possesses arguably all of these desired attributes. The major focus of this dissertation is to investigate the relative importance of biotic and abiotic factors in shaping the evolution of this diverse marine group.

1.2 Superfamily Galeommatoidea

Galeommatoidean bivalves are a well known, but poorly studied, marine superfamily with a fossil record extending possibly to the Cretaceous, but unambiguously to the Palaeocene [51–54]. They comprise approximately 100 ([55]) to 140 (Middelfart, unpubl.) genera and an estimated 500 described species [55], although many more species remain undescribed [56, 57]. These bivalves are small-bodied, typically <2 cm in length, range in occurrence from the intertidal to the deep sea [58], and usually occur in small aggregations either in rock/coral crevices or in commensal associations with invertebrate hosts [53, 59–62].

Although a small number of galeommatoidean species can achieve high densities [63, 64], most are rare and poorly studied. In nature, most species are relatively rare [65] and rarity may be critical to the attainment of heightened diversity [66]. Ignorance concerning rare species hinders our ability to attain accurate estimates of fundamental diversity [67]. Diversities of many marine taxa reach global maxima in the Western Pacific Indo-Australian Archipelago (IAA) coral reef ecosystems [21, 68–70], including bivalves [71]. Over the past decade, the application of comprehensive sampling methodologies to IAA coral reef ecosystems in New Caledonia [72] and Guam [57] has catapulted the Galeommatoidea from relative obscurity

to the apex of bivalve biodiversity (Table 1.1). Paulay [57] considered his Guam Galeommatidae *s. l.* (= Galeommatoidea) tally to be a substantial underestimate and that the actual number is likely several times greater than any co-occurring bivalve family. Remarkably similar results [73] were obtained from a Lower Pleistocene Mediterranean fossil assemblage survey (Table 1.1), even though small fragile galeommatoidean shells are less likely to persist in the fossil record. Taken together, these studies reinforce Paulay's [57] conclusion that Galeommatoidea is now a megadiverse group.

Table 1.1: Most speciose bivalve families in three independent surveys. Note that the New Caledonia Galeommatoidea *s. l.* were the most diverse despite their relative rarity.

	Guam [57]	Koumac, New Caledonia [72]		Harokopio, Greece [73]
Family	# of species (%)	# of species (%)	# of individuals	# of species (%)
Galeommatidae *s. l.*	**39 (11%)**	**61 (12%)**	**739**	**11 (13%)**
Tellinidae	38 (11%)	51(10%)	3560	6 (7%)
Cardiidae	29 (9%)	37 (7%)	4316	8 (10%)
Veneridae	28 (8%)	53 (10%)	5041	14 (16%)

Galeommatoideans are known for their extraordinary morphologies and life histories; a taste of which can be gleaned from Figure 1.1. Compared to most other bivalve lineages, they exhibit exceptional morphological innovation, often involving major modifications of the bivalve shell: pronounced reduction and/or internalization [60, 74, 75] (Fig. 1.1A-E, J), held open horizontally to form a limpet-like shield [76, 77](Fig. 1.1I). The mantle can be hypertrophied to cover the shell, either permanently [74, 75, 78, 79] (Fig. 1.1A-E), or facultatively [60, 78](Fig. 1.1F-I); extended into innervated, extendable defensive papillae/tentacles with dynamic display, autotomizing and secretory functions [78, 80–82]; or enlarged to form an expanded brood chamber [83](Fig. 1.1B-D). The foot is modified for crawling, rather than digging [53](Fig. 1.1E-J), for movement within host alimentary tracts [83,84] (Fig. 1.1B-D), attachment to external body walls of hosts [85](Fig. 1.1K-O) or to smooth-walled burrows. [78, 86]. The extent of morphological transformation is such that many species superficially resemble non-bivalve taxa, including nudibranchs (Fig. 1.1E-G), limpets (Fig. 1.1I), and even cnidarians (Fig. 1.1J).

Besides the crevice-dwelling free-living species, Galeommatoidea contains a considerable number of commensal species. Commensal galeommatoideans are generally presumed to suspension feed on the host's bioirrigation current [59], but some are deposit feeders [87–90], others are kleptoparasitic (externally [83], or internally [83, 84, 91, 92]), and one deep-sea species putatively feeds on host body fluids [58].

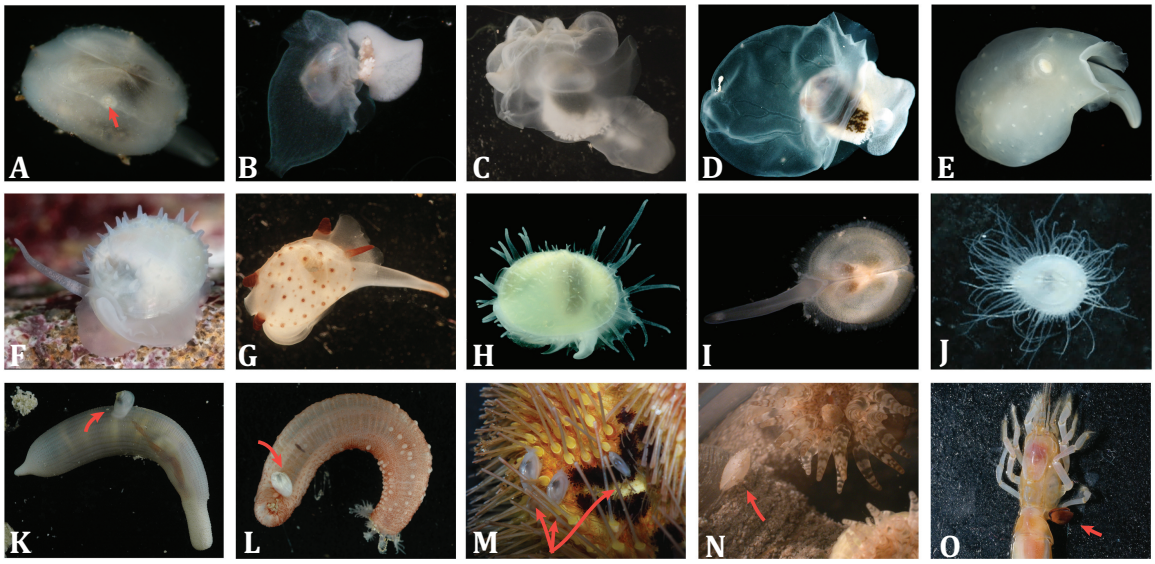

Figure 1.1: Montage of some galeommatoidean species available for this study. All exemplars except E (G. Rouse) and J (P. Middelfart) were collected by Muséum National d'Histoire Naturelle, Paris (photos by P. Maestrati and B. Buge). Arrow in A points to a dwarf male within a specialized mantle pouch of a female. Arrows in K-O respectively point to ectocommensals of sipunculan, holothuroid, echinoid, anemone and crustacean hosts.

The intricate nature of bivalve-host associations has been revealed through experimental demonstration of specific host-taxes by commensal clams [88, 93–95]. But there is considerable variation in host fidelity: some taxa associate with multiple hosts [88, 96, 97], and single host species may be colonized by multiple commensal species [78,86,98]. The commensal species can be associated with a remarkably diverse group of hosts, including polychaetes [1, 93, 96, 99–103]; sipunculans [56, 95, 104, 105]; crustaceans [78, 86, 97, 106–111]; holothuroids [60, 83–85, 91, 92, 112–116]; echinoids [94, 98, 117]; anemomes [118–120]; echiurans [103, 104, 121–123]; brachiopods [124]; chitons [125, 126]; bivalves [127]; ophiuroids [88, 103] and sponges [77].

Doubts regarding the monophyly of the superfamily [59] have been assuaged by the small number of molecular phylogenetic studies of Bivalvia that have utilized galeommatoidean species [128–131]. These studies recovered a robust galeommatoidean clade within Heterodonta that lacked convincing sister lineages but contained a representative of Sportellidae, a small (∼50 species [61]) commensal [123] family traditionally placed in the Cyamioidea [59, 61, 132]. Within-Galeommatoidea phylogenetic studies have been sparse and restricted to one cladistic analysis of Galeommatidae [133], one regional molecular phylogeny [131], and in-depth molecular analyses of the genus *Lasaea* [4, 63, 134–138]. Operational estimates of the number of families range from

1 [53] to 6 [139], although many researchers currently favor either two: Galeommatidae and Lasaeidae [62], or one: Galeommatidae *sensu lato* [53,57]. In reality, there is little consensus regarding supra-specific taxonomic or phylogenetic galeommatoidean relationships which are described as being in constant confusion [56]; ill-defined [53, 140]; poorly understood [57]; controversial [141]; confused [62]; and in need of review using molecular methods [58].

1.3 Chapter Overviews

This dissertation is composed of four self-contained manuscripts (chapters 2-4) that address diversification patterns of free-living and commensal Galeommatoidean taxa on different spatial and taxonomic levels. The studies infer major abiotic and biotic factors that may have played important roles in shaping the present-day gaelommatoidean diversity.

Chapter 2 is an ecological synthesis (based on literature reviews) that addresses the ecological importance of commensalism in Galeommatoidea. It reveals that the formation of commensal associations is robustly correlated with an abiotic environmental setting: living in sediments. Sediment-dwelling bivalves are exposed to intense predation pressure that drops markedly with depth of burial. Commensal galeommatoideans routinely attain refuges from predation at depths many times their body lengths by virtue of their host's burrowing and bioturbation. This study indicates that biotic associations with infaunal bioturbating hosts are essential for the proliferation of Galeommatoidea in soft-bottom habitats.

Chapter 3 is a case study of the northeast Pacific galeommatoidean *Neaeromya rugifera*, which routinely occupies two distinct host species: the blue mud shrimp *Upogebia pugettensis* and the polychaete sea mouse *Aphrodita spp.* This study tests if this host difference has resulted in the formation of host races using shell morphologies and genetic markers (COI). Results show that clam populations from different hosts differ significantly in shell morphology but do not show host-specific genetic structuring, indicating the existence of a panmictic population.

Chapter 4 is a phylogeographic study that aims to identify regional factors that drive the diversification of the free-living galeommatoidean species *Laeasa australis* – arguably the most common bivalve on southern Australian rocky shores. The southern coast of Australia is composed of three distinct biogeographic provinces distinguished primarily by intertidal community composition. Several ecological mechanisms have been proposed to explain their formation and persistence, but no consensus

has been reached. This study examines whether *L. australis* exhibits cryptic genetic structure corresponding to the provinces by assaying variation in two mitochondrial genes (16S and COIII) and one nuclear gene (ITS2). Results shows that *L. australis* is comprised of three cryptic mitochondrial clades, each corresponding almost perfectly to one of the three biogeographic provinces. Divergence time estimates place their cladogenesis in the Neogene. Evidence indicates that the interaction of the Middle Miocene Climate Transition (14.013.7 Ma) with the specific geography of the southern coastline of Australia is likely to be the primary cladogenic driver for this clam lineage.

Chapter 5 is a macroevolutionary study based on global-scale sampling. A multigene, time-calibrated phylogeny is reconstructed using 217 galeommatoidean morphospecies. Shell morphologies are quantified using geometric morphometric methods and ecological information of all morphospecies is documented. Phylogenetic comparative analyses reveal that commensalism/sediment-dwelling is likely to be the ancestral condition of Galeommatoidea and that secondary invasions of hard-bottom habitats is linked with the loss of commensalism. One major radiation of free-living species is detected and it exhibits a higher diversification rate than that of the commensal clades, likely driven by frequent niche partitioning in highly heterogenous yet stable hard-bottom habitats, especially in coral-reef environments. On the other hand, commensal clades show much higher within-clade morphological disparity and intercladal convergence, likely promoted by their intimate associations with diverse hosts. This study points out that clams with different lifestyles exhibit distinct patterns of lineage diversification and morphological evolution; and this lifestyle dichotomy is strongly governed by benthic habitat types.

Finally, Chapter 6 summarizes the major findings of this dissertation and poses new questions stemming from these findings. This chapter discusses limitations of currently employed approaches in answering macroevolutionary questions and proposes possible future research directions.

CHAPTER 2

Ecological Significance of the Commensal Associations in Galeommatoidea

2.1 Introduction

One of the classic questions in biology concerns the mechanisms that control the generation and maintenance of planetary biodiversity [9]. Two broad classes of macroevolutionary drivers are generally recognized. The Red Queen model [142, 143] states that biotic factors play major roles in shaping lineage diversification, while the Court Jester model [144] places more emphasis on abiotic factors. Although both sets of drivers operate on different spatial and temporal scales [144], they clearly play off each other [44] and their relative importance remains an active area of contention in fundamental biodiversity research [32, 47, 145].

The importance of biotic drivers is most evident in terrestrial ecosystems whose dominance by insects and angiosperms is attributed substantially to coevolutionary dynamics [10]. Much of the evidence for biotic drivers of marine diversification is paleontological [28, 30–32] and, with some notable exceptions (*e.g.*, [34, 38]), neontological marine evolutionary studies typically focus on abiotic drivers [11, 12, 22, 27]. This is primarily because the scope of ecological interactions remains poorly characterized for most marine clades, especially regarding subtle effects such as facilitation (presence of one species enhances survival of another) that may be very important in nature [44, 46]. Our ignorance concerning the role of biotic interactions in macroevolutionary processes is being increasingly recognized as a serious deficiency that may underlay the frequent mismatch between empirical data and theoretical

A version of this chapter has been published as: Jingchun Li, Ó Foighil D. and Middelfart P. 2012. The evolutionary ecology of biotic association in a megadiverse bivalve superfamily: sponsorship required for permanent residency in sediment. PLoS ONE 7: e42121

models [32, 44, 47, 146]. Given this, how might one test the relative importance of marine biotic and abiotic diversification drivers in an extant marine clade?

Our approach is comparative and involves targeting an exemplar marine taxon, the marine bivalve superfamily Galeommatoidea. This clade is suitable for addressing our question for two reasons. Firstly, Galeommatoidea is recognized as a "megadiverse" group [57]. Those small-bodied ($<2cm$) bivalves comprise an estimated 500 described species [55], although this is a serious underestimate: a large fraction remains undescribed [56,57]. Recent quantitative biodiversity surveys of Western Pacific coral reefs have found that Galeommatoidea had the highest species diversity among Bivalvia, despite their relatively low abundance [57, 72]. Secondly, Galeommatoidea embodies a clear ecological dichotomy in that some members are free-living while others have obligate biotic associations (mostly commensals) with invertebrate hosts [53,59]. The commensals exhibit specific host-taxes [88, 93–95], although in some cases commensals may associate with multiple hosts [88, 96, 147] and single host species may be colonized by multiple commensals [78, 98].

Our strategic goals are to test the relative importance of free-living and commensal life styles in driving galeommatoidean diversification and to establish the ecological context for evolutionary transitions among the two life styles. The former goal involves constructing comprehensive phylogenetic trees that will allow us to detect the effect of the traits of interest (presence/absence of biotic association) on diversification rates. In this present study, our focus is on the latter goal. If the lifestyle dichotomy is correlated with discrete ecologies, specific hypotheses regarding the role of facilitative biotic associations can be proposed and tested.

Galeommatoidea has significant diversity in the two primary benthic habitats: soft- and hard-bottoms. The two types of habitats differ greatly in terms of physical properties as well as in faunal composition and community structure [148–150]. Adaptation to either habitat requires a certain degree of morphological and behavioral specialization [148]. Previous workers have hypothesized that commensalism in Galeommatoidea is an adaptation to soft-bottom infaunal habitats [59, 64], but this hypothesis has not been formally tested at the superfamily level. We do so here by performing a literature based statistical analysis to test if the evolution of this pronounced lifestyle dichotomy is correlated with the acquisition of discrete benthic ecologies.

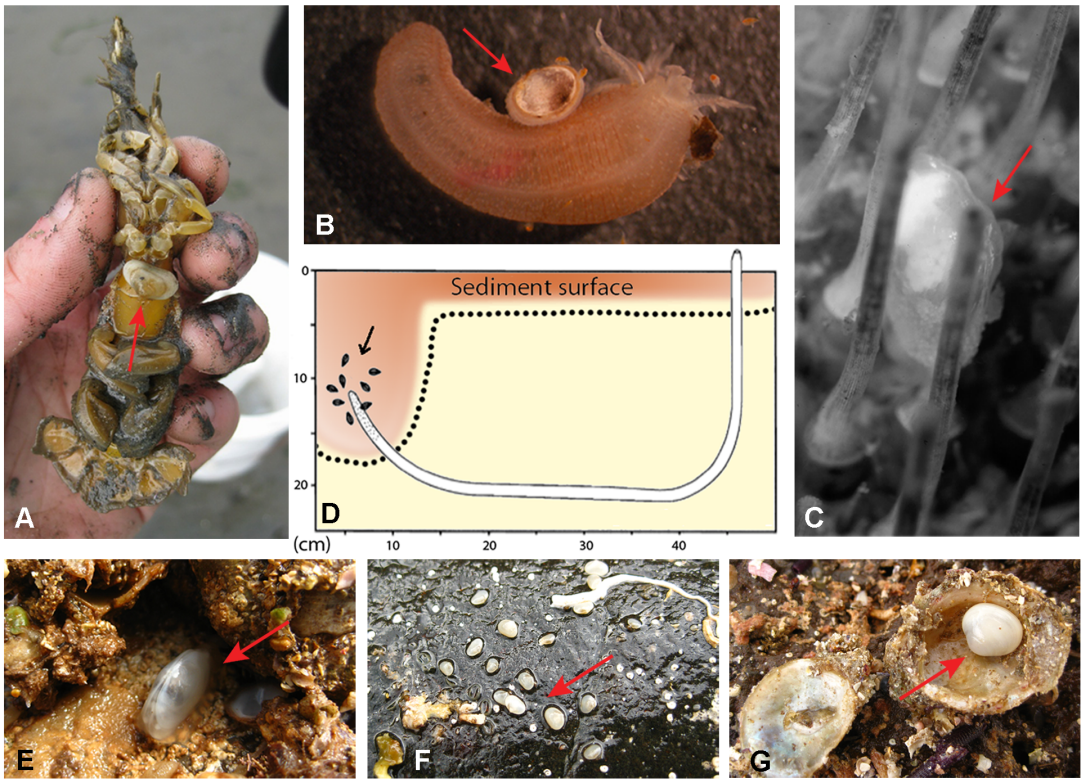

Figure 2.1: A. The commensal clam *Neaeromya rugifera* attached to the ventral side of a mud shrimp *Upogebia pugettensis*. B. The commensal clam *Scintillona bellerophon* attached to its holothuroid host *Leptosynapta clarki*. C. The commensal clam *Waldo sp.* attaching to the surface of its benthic irregular sea urchin host *Brisaster latifrons*. D. Clustering of commensal *Rochfortia* (*Mysella*) *tumida* (arrow), within the exhalent oxic halo of *Mesochaetopterus taylori*. Dotted line separates oxygenated (red) and anoxic (yellow) sediment zones (After [1]). E. The free-living *Scintilla* (*Lactemiles*) *strangei* in its rock crevice. F. Underside of a rock showing several free-living *Borniola lepida* individuals attached by byssal threads. G. A free-living *Kellia sp.* nestled within an empty bivalve shell. (Photo credit: A, E-G: J. Li; B: L. Kirkendale; C: D. Ó Foighil)

2.2 Materials and Methods

To investigate whether commensal life styles in galeommatoidean clams are correlated with specific benthic habitat types, we extracted habitat and lifestyle information for a total of 121 species from 90 source documents, including peer-reviewed journals, book chapters, museum report and personal observations (see all references in supplementary materials). Our data set contains a number of likely sampling biases. Due to limitations in marine sampling methodologies, our species pool is weighted toward taxa from intertidal and shallow subtidal habitats and there is a relatively low representation of deep-sea taxa. However this is unlikely to affect our results because the sampling bias applies to both hard-bottom and soft-bottom deep-sea species. A potentially more serious bias could involve significant differences in sampling free-living versus commensal sediment dwellers. If the former were relatively intractable, it would bias our results in favor of the hypothesis. We consider this unlikely, however, because free-living taxa are easier to sample given their primary location in the shallow surface layers of sediment, rather than in the deep burrows of their commensal hosts.

2.2.1 Searching

The initial literature search was conducted through the ISI Web of Knowledge database using "Galeommatoidea" as a topic keyword. This search resulted in 57 English publications between the years of 1899 and 2011. Because much of the relevant literature on this superfamily is not archived in the ISI web of Knowledge database, we investigated the older literature cited by these 57 publications and elicited additional sources from The Australian Museum Research Library and The University of Michigan Museums Library. These activities yielded an additional 69 publications to give a total of 126.

2.2.2 Selection

Our classification criteria for habitat and lifestyle data were as follows. Benthic habitat was divided into two major categories: soft-bottom and hard-bottom. Soft-bottom includes all benthic substrates composed of unconsolidated sediment, whereas hard-bottom includes all rocky or consolidated substrates, including coral galleries. Lifestyle was classified as either commensal, free-living or (facultatively) both. To obtain a "commensal" designation, taxa had to have identified hosts; a generic assumption of a commensal lifestyle by the reporting authors was insufficient. Host

identification can be relatively straightforward in cases where the commensal galeommatoidean attaches directly to its host (Fig. 2.1A, B, C) and is not dislocated during sampling. In contrast, it can be quite difficult when the commensal remains unattached and locates in the oxygenated envelope surrounding its host's temporary burrow (Fig. 2.1D). In the latter cases, it may require very careful benthic sampling, and/or laboratory behavioral experiments, to identify specific host taxa [64, 88]. We encountered a few cases of galeommatoidean taxa that were initially listed as free-living, prior to subsequent host identification, *e. g. Arthritica bifurca* [93, 99]. In addition, a small number of species were reliably recorded as being both commensal and free-living. These were classified as facultative commensals.

2.2.3 Validity assessment

Critical analysis of these 126 publications found 36 to be deficient in that they contained insufficient information to unambiguously determine habitat ($N = 34$) or lifestyle ($N = 2$) of the species of interest. All 36 were removed from the analysis, resulting in a final working list of 90 publications. Excluding 2 putatively commensal galeommatoidean species with unidentified hosts may have resulted in an underestimation of the relative number of commensal taxa. However, all of these excluded putative commensal occurred in soft-bottom benthic habitats and their exclusion has therefore not contributed to the pronounced correlation of commensalism and sediment-dwelling observed in the 60 commensal taxa analyzed.

2.2.4 Data abstraction

Galeomatoidean habitat type and life style information was extracted, identified and classified manually for a total of 121 species from our final list of 90 publications (see supplementary materials for detailed habitat and lifestyle information for all species included). The numbers of species that belonged to each habitat-lifestyle combination were summarized in a contingency table (Table 2.1).

2.2.5 Quantitative data synthesis

In order to detect possible correlations between habitat preference and lifestyle, Fisher's exact test was performed using R 2.13.1 [151]. Note that a small number of facultative (*i.e.*, both commensal and free-living) species are present in the table, but these were not included in the test because it is inappropriate to classify them discretely as either commensal or free-living.

2.3 Results and Discussion

Habitat and life-style information for 121 galeommatoidean species was extracted from the literature (see supplementary materials for details) and the Materials and Methods section summarizes how case studies were classified as being free-living, commensal or (facultatively) both. Our dataset encompassed representatives from all major ocean basins and from a wide variety of benthic habitats. It contained a total of 57 free-living taxa, *i.e.*, occupying abiotic microhabitats (Fig. 2.1E, F, G) and 60 commensal species. Many of the commensals directly attached to their invertebrate hosts (Fig. 2.1A, B, C), the remainder locating around host tubes/burrows (Fig. 2.1D). We also obtained data on 4 species with facultative lifestyles that were reliably recorded from abiotic as well as biotic microhabitats.

Our main result is presented in Table 2.1: commensal and free-living galeommatoidean taxa exhibited a striking ecological disjunction in benthic habitat type. All but 2 of 57 free-living species were restricted to hard-bottom habitats, typically hidden in rock/coral crevices. In contrast, 56 out of 60 commensal species were infaunal sediment dwellers. Our result establishes that formation of commensal associations by galeommatoidean clams is robustly correlated with living in sediments ($P<0.001$). This clear-cut finding is consistent with the hypothesis that biotic association is primarily an adaptation to living in soft-bottom infaunal habitats [59, 64], but does not, in itself, explain the putative adaptive nature of such associations.

Table 2.1: Numbers of species that belong to each habitat-lifestyle combination.

	Free-living	Commensal	Both	Total
Hard-bottom	55	4	2	61
Soft-bottom	2	56	2	60
Total	57	60	4	121

2.3.1 Soft-bottom taxa

How might we test the adaptive significance of biotic association in sediment-dwelling Galeommatoidea? One approach would be to perform detailed comparative ecological studies of fitness in species that have facultative life styles and contain significant numbers of free-living and commensal individuals. Two of the four facultative life style taxa in our survey occur in sediments: *Kurtiella bidentata* (Montagu, 1803) and *Mysella vitrea* (Laseron, 1956) [88, 90, 152], and the ecology of the former

has been studied in considerable detail. *K. bidentata* is associated with an unusually wide variety of bioturbating invertebrate hosts, most notably with the burrowing ophiuroid *Amphiura filiformis* [88]. Across its range, commensal individuals of *K. bidentata* attain much greater population densities [88, 152] and locate deeper in the sediment [88, 152, 153] (Table 2.2) than do free-living conspecifics. These distinctions have been attributed to two very different processes. One hypothesis states that positioning of commensals within the hosts's oxygenated burrow provides a depth refuge from predation and that the increased commensal population density stems from lower mortality rates [88]. A competing hypothesis views *K. bidentata*'s commensal associations as byproducts of density-dependent competition: high population densities driving individuals deeper into the sediment to form commensal associations [152]. Available evidence strongly favors the predation depth refuge hypothesis: *K. bidentata* exhibits positive host chemotaxis irrespective of clam density and free-living populations do experience much higher mortality rates (and lower fitness) than commensals [88].

Predation is a key factor that affects species survival and community structure in benthic environments [154–156] and bivalves have evolved two general anti-predator strategies: increasing handling time (via armor) or reducing the encounter rate (via avoidance) [157]. Galeommatoideans are small-bodied clams that typically specialize in avoidance rather than armor; indeed many species (in both hard- and soft-bottom substrates) have undergone significant shell reduction and/or internalization [56, 59, 60]. In hard bottom substrates, crevices provide preexisting spatial refuges. Crevices are not available in soft-bottom substrates and the most common avoidance adaptation is to become infaunal [157]. The depth refuge hypothesis for *Kurtiella bidentata* [88] is consistent with extensive experimental evidence that predation pressure on infaunal bivalves drops markedly with depth of burial [157–163].

What about the rest of the soft-bottom Galeommatoidea? Although the data are limited, commensalism is typically associated with deeper burial. For instance, the other facultative species, *Mysella vitrea*, positions significantly deeper in sediments in the presence of its host [90] and recorded depths for most commensals are much deeper than the two known free-living sediment dwellers, the Antarctic species *M. charcoti* and *M. narchii*, which are restricted to the top few millimeters of sediment (Table 2.2). The few data on predation rates includes reports of greatly reduced predation on the deeply buried commensal *Aligena elevata* [100] but heavy predation on the shallowly buried non-commensal *M. charcoti* [164]. *M. charcoti* survives passage through the alimentary tracts of some predatory fishes, and may indeed be dispersed

primarily through this process [165], indicating that in this non-commensal species armor rather than avoidance may be the primary anti-predation strategy. Why this strategy is not more widely adopted by non-Antarctic galeommatoideans is not clear, but may be related to a greater spectrum of shell-crushing/boring/disarticulating predators operating on temperate and tropical sediment-dwellers.

Predator avoidance through deeper burial is not cost-free because the infauna requires contact with the sediment-water interface for basic physiological functions including respiration, and in many cases also feeding, reproduction and defecation [166]. Most infaunal bivalve species engage in a trade-off between access to the interface and lethal predator avoidance by investing in extendable siphons that allow individuals to directly contact the water column while their main body mass remains deeply buried. Burial depth is therefore a function of siphon length and biomass, but the clams are still exposed to sub-lethal predation on exposed siphon tips [159, 167–169]. In contrast, most galeommatoidean bivalves have modest siphons or even lack them completely [53, 59], yet commensal species routinely attain sediment depth refuges many times their body lengths (Table 2.2).

Within-sediment galeommatoidean hosts are bioturbators that construct irrigated tubes/burrows. Bioirrigation and bioturbation processes facilitate nutrient intake from the water column and oxygen penetration into deeper sediment [170, 171]. By locating within the host's oxygenated sediment envelope [1, 64, 88], commensal galeommatoideans in effect use their much larger hosts as giant auto-irrigating siphon substitutes. This enables commensals to decouple burial depth from body size and solve the surface access/predator avoidance trade-off while remaining small-bodied; other benefits such as filter-feeding from respiration or feeding currents of the hosts could also accrue. The scope of depth refuges obtained by commensal galeommatoideans is set by host borrowing parameters and spans that of free-living infaunal bivalves. For instance, the world's largest burrowing clam, recently renamed *Panopea generosa* [172], attains a depth refuge of up to 1 meter below the sediment/water column interface thanks to its enormous siphons [173]. Remarkably, this maximum burial depth is matched by the tiny (∼5mm in body length) facultative commensal *Mysella vitrea* in sediments bioirrigated by its host, the ghost shrimp *Trypaea australiensis* [90].

2.3.2 Hard-bottom taxa

The vast majority of hard-bottom species are free-living (Table 2.1). They nestle in crevices within or underneath rocks, coral heads or encrusting epifauna that are

Table 2.2: Habitat depth of selected soft-bottom galeommatoideans (free-living examplers are indicated).

Species	Habitat depth	Max. shell length	References
Mysella charcoti (free)	Top few millimeters	3.0 mm	[164]
Mysella narchii (free)	Top few millimeters	3.1 mm	[174]
Kurtiella bidentata (host absent)	0-5 cm	3.5 mm	[88, 152]
Kurtiella bidentata (host present)	5-50 cm	3.5 mm	[88, 152]
Mysella vitrea (host present)	15-95 cm	5 mm	[90]
Arthritica bifurca	about 6 cm	4.1 mm	[93, 99]
Brachiomya stigmatica	10-15 cm	3.0 mm	[98]
Divariscintilla maoria	over 15 cm	6.0 mm	[80]
Halcampicola tenacis	15-30 cm	5.0 mm	[119]
Montacuta elevata	up to 17 cm	6.0 mm	[100]
Montacutella echinophila	10-15 cm	7.9 mm	[98]
Nipponomysella subtruncata	5-15 cm	6.8 mm	[175]
Rochfortia (*Mysella*) *tumida*	12-15 cm	3.5 mm	[1]

passively ventilated by ambient water flow [60] and they may show a simple hierarchy of geo-, photo- and thigmotaxes to remain within these microhabitats [176]. Unlike sediments, crevices are common in hard-bottom benthos and afford these minute clams effective abiotic refuges from predators in addition to contact with the water column [60, 177]. With the possible exception of *Pristes oblongus*, a poorly studied species reported to attach to chitons [125], the relatively small number of hard-bottom commensals all associate with infaunal hosts that can form burrows in hard substrates. They include *Arthritica crassiformis* associated with the boring bivalve *Anchomasa similis* [127]; *Ephippodonta lunata* and *Ephippodontana macdougalli* in the burrow of slow shrimp *Strahlaxius plectorhynchus* [178], and the genus *Jousseaumia* associated with sipunculans within corals [179]. Note that *Ephippodonta lunata* and *Ephippodontana macdougalli* are facultative commensals that are also found in rock crevices [178], but we have no data on comparative survival rates of free-living and commensal individuals. It is likely that abiotic crevices in most hard-bottom benthic environments greatly exceed, in number and in spatial heterogeneity, those produced by any actual or potential host species. The overwhelming predominance of free-living galeommatoidean lifestyles in these communities (Table 2.1) suggests that

for this bivalve superfamily, the number of available crevices is more important than crevice spatial uniformity, or biotic association, in promoting lineage diversification in hard-bottom benthic environments.

2.3.3 Biotic association and diversification

Infaunal sediment bioturbators have long been recognized as key ecosystem engineers that alter the physical and chemical properties of the substrate and impact nutrient cycles [180–182]. Their biotic impact on benthic communities is also an active topic area in both paleontological macroevolutionary [44, 182, 183] and neontological microevolutionary [171, 184] studies. It is typically negative for co-occurring taxa that require stable sediments, but positive, over both ecological and evolutionary timescales, for commensal species [44, 171]. This latter effect is robustly evident for galeommatoideans and our data strongly support the hypothesis that formation of commensal relationships with burrowing macroinvertebrates has been a key adaptation in their success in sediments [59, 64]. This is significant because most of the global marine benthos is soft bottom [185,186] and relatively few bivalve lineages (*e.g.*, Mytilidae [187], Pectinidae [188] and Arcoidea [189]) have achieved significant diversity in both hard-bottom and soft-bottom habitats, presumably due to the distinctive functional/morphological constraints imposed by adapting to either habitat [190]. Sediment-dwelling Galeommatoidea have superseded these functional/morphological constraints via behavioral innovation; acquiring many of the necessary functions, including deep burrow construction and irrigation, indirectly through biotic association with larger invertebrate infauna.

Our literature survey returned an approximately equal number of soft- and hard-bottom galeommatoidean species (Table 2.1), although the true ratio is unknown due to the very significant number of undescribed species in both habitats [56,57,60]. Nevertheless, it is clear that commensalism underlies the evolutionary genesis of a major fraction of galeommatoidean diversity and has likely been instrumental in attaining their "megadiverse" status among marine bivalves [57]. Unlike most bivalve lineages, Galeommatoidea does not have a comprehensive fossil record for effectively inferring its long-term diversity dynamics. In fact, less than half of the living genera are known from the fossil record [191]. Therefore, an in-depth understanding of the role that biotic association has played in galeommatoidean diversification requires a detailed molecular phylogenetic framework for the group. This is currently unavailable, but is badly needed as there is very little consensus regarding supra-specific taxonomic relationships in this superfamily [53,56–58,140]. The Red Queen and Court Jester models

provide a simple theoretical framework: do commensal galeommatoideans represent discrete adaptive radiations where speciation is driven by host-shifts (Red Queen) or a polyphyletic melange of evolutionary dead-ends (Court Jester)? We are presently constructing molecular phylogenies to address these questions.

2.4 Conclusions

Evolutionary studies of contemporary marine biotas are typically framed within abiotic hypothesis-testing contexts and have collectively lagged behind terrestrial studies in developing an integrated framework that includes a meaningful biotic/ecological perspective. The strong correlation between lifestyle and habitat preference in Galeommatoidea suggests that the relative importance of the Red Queen model can be greatly influenced by abiotic ecological factors such as benthic substrate type: maximal in soft-bottom and minimal in hard-bottom. Facilitative biotic associations such as commensalism are not rare in marine environments [122], and it is likely that the evolution of many other commensal-rich marine benthic lineages have also been tailored by ambient abiotic factors.

2.5 Supplementary Materials

Available galeommatoidean habitat, lifestyle and (for commensal species) host information, including references. Species names are arranged in alphabetical order

Soft-bottom	S	Commensal	C
Hard-bottom	H	Free-living	F

Species			Hosts	Habitat Details	References
Anisodevonia ohshimai	S	C	*Patinapta ooplax*	Attached to host	Kato, 1998; Kawahara, 1942
Arthritica bifurca	S	C	*Pectinaria australis*	Attached to host tubes	Chanley and Chanley, 1980
					Wear, 1966
Arthritica crassiformis	H	C	*Barnea similis*	Attached to host (rock-boring pholadid)	Chanley and Chanley, 1980; Morton, 1973; Ponder, 1965
Arthritica japonica	S	C	*Xenophthalmus pinnotheroides*	Attached to host	Lützen *et al.*, 2003
Austrodevonia sharnae	S	C	*Taeonigyrus australianus*	Attached to host	Middelfart and Craig, 2004
Barrimysia siphonosomae	S	C	*Siphonosoma cumanense*	In host burrows	Jespersen *et al.*, 2002; Morton and Scott, 1989
Borniola lepida	H	F		Under rocks	Personal observation, Li, 2011
Brachiomya stigmatica	S	C	*Brissus latecarinatus*	Attached to host	Jespersen *et al.*, 2004; Yamamoto and Habe, 1974
Chlamydoconcha orcutti	H	F		Under large stones	Morton, 1981
Curvemysella paula	S	C	*Spiropagurus spiriger; Diogenes edwaedsii*	Within host-occupied shells	Goto *et al.*, 2007
Devonia perrieri	S	C	*Leptosynapta inhaerens*	Attached to host	Clench and Aguayo, 1931
Divariscintilla cordiformis	S	C	*Lysiosquilla scabricauda*	In host burrows	Mikkelsen and Bieler, 1992
Divariscintilla luteocrinita	S	C	*Lysiosquilla scabricauda*	In host burrows	Mikkelsen and Bieler, 1992
Divariscintilla maoria	S	C	*Lysiosquilla spinosa*	In host burrows	Judd, 1971
Divariscintilla octotentaculata	S	C	*Lysiosquilla scabricauda*	In host burrows	Mikkelsen and Bieler, 1992
Divariscintilla troglodytes	S	C	*Lysiosquilla scabricauda*	In host burrows	Mikkelsen and Bieler, 1989
Divariscintilla yoyo	S	C	*Lysiosquilla scabricauda*	In host burrows	Mikkelsen and Bieler, 1989
Duoconclavis piscator	H	F		Under stones	Middelfart, 2005
Entovalva amboinensis	S	C	*Patinapta laevis*	Within host esophagus	Bristow *et al.*, 2010; Spärck, 1931
Entovalva lessonothuriae	S	C	*Holothuria (Lessonothuria) pardalis*	Within host esophagus	Kato, 1998
Entovalva major	S	C	*Holothuria curiosa*	Attached to host	Lützen *et al.*, 2005
Entovalva mirabilis	S	C	*Patinapta crosslandi*	Within host esophagus	Bristow *et al.*, 2010; Voeltzkow, 1890
Entovalva nhatrangensis	S	C	*Holothuria leucospilota; Holothuria spinifera*	Within host esophagus	Bristow *et al.*, 2010
Entovalva semperi	S	C	*Protankyra bidentata*	Attached to host	Morton and Scott, 1989, Ohshima, 1930
Ephippodonta gigas	H	F		Under boulders	Kubo, 1996; Lützen and Nielsen, 2005
Ephippodonta gregaria	H	F		In crevices	Gofas, 1991; Middelfart, 2005
Ephippodonta lunata	H	C	*Strahlaxius plectorhynchus*	In host burrows	Cotton 1938; Middelfart, 2005
Ephippodonta lunata	H	F		In rock crevices	Cotton 1938; Middelfart, 2005
Ephippodontina murakamii	H	F		Attached to deep sea coral	Arakawa, 1960; Middelfart, 2005
Ephippodontina oedipus	H	F		Within coral galleries	Middelfart, 2005
Ephippodontoana macdougalli	H	C	*Strahlaxius plectorhynchus*	In host burrows	Cotton 1938; Middelfart, 2005
Ephippodontoana macdougalli	H	F		In rock crevices	Cotton 1938; Middelfart, 2005
Ephippodontomorpha hirsutus	S	C	*Lysiosquillina maculata or L. tredecimentata*	In host burrows	Middelfart, 2005
Epilepton clarkiae	S	C	*Various sipunculans*	Attached to host	Jespersen *et al.*, 2007
Fronsella ohshimai	S	C	*Sipunculus nudus*	Attached to host	Manning and Morton, 1987
Galeomma ambigua	H	F		Under shale blocks	Lützen and Nielsen, 2005
Galeomma coalita	H	F		Under stones	Gofas, 1991
Galeomma layardi	H	F		Under rocks	Lützen and Nielsen, 2005
Galeomma obockensis	H	F		Under coral blocks	Lützen and Nielsen, 2005
Galeomma phuketi	H	F		Under shale blocks	Lützen and Nielsen, 2005
Galeomma sagenata	H	F		In coral rubble	Oliver and Holmes, 2004
Galeomma takii	H	F		Under rocks	Morton, 1973b
Galeomma turtoni	H	F		In large crevices	Gofass, 1991
Halcampicola tenacis	S	C	*Halcampoides sp.*	Attached to host	Oliver, 1993
Jousseaumlella heterocyathi	H	C	*Aspidosiphon sp.*	Attached to host within coral crevices	Bourne, 1906
Jousseaumlella heteropsammiae	H	C	*Aspidosiphon sp.*	Attached to host within coral crevices	Bourne, 1906
Kellia jacksoniana	H	F		In crevices, within mussel beds	Laseron, 1956
Kellia laperousii	H	F		In crevices, inside empty shells	Keep and Hannibal, 1911
Kellia porculus	H	F		Within coral galleries	Morton and Scott, 1989
Kellia suborbicularis	H	F		In crevices, inside empty shells	Jespersen and Lützen, 2007; Lebour,

					2006
Koreamya arcuata	S	C	*Lingula anatina*	Attached to host	Lützen *et al.*, 2009; Sato *et al.*, 2011
Kurtiella bidentata	S	C	*Amphiura filiformis; Maxmuelleria lankesteri; Nephtys incisa*	In host burrows	Jespersen and Lützen, 2001; Nickell *et al.*, 1994; Ockelmann and Muus, 1978; Prevedelli *et al.* 2001
Kurtiella bidentata	S	F		Upper sediment layer	Prevedelli *et al.* 2001
Kurtiella pellucida	H	F		Within bioclastic gravels	Gofas and Salas, 2008
Kurtiella triangularis	H	F		In rock and algal turf crevices	Gofas and Salas, 2008
Lasaea adansoni	H	F		In rock crevices	Altnöder and Haszprunar, 2008; Crisp and Standen, 1988
Lasaea australis	H	F		In rock and encrusting epifaunal crevices	Ó Foighil & Thiriot-Quievreux, 1999
Lasaea colmani	H	F		In rock and encrusting epifaunal crevices	Ó Foighil & Thiriot-Quievreux, 1999
Lasaea maoria	H	F		In damp crevices and beneath stones	Ponder, 1971b
Lasaea undulata	H	F		In rock and encrusting epifaunal crevices	Iwasaki, 1996
Lepton squamosum	S	C	*Upogebia deltaura; Upogebia stellata*	In host burrows	Kallonas *et al.*, 1999; Norman *et al.*, 1891
Litigiella glabra	S	C	*Sipunculus nudus*	Attached to host	Kallonas *et al.*, 1999; Lamy, 1908
Marikellia solida	H	F		Crevices, within mussel beds	Laseron, 1956
Melliteryx acupuncta	H	F		Under rocks	Personal observation
Montacuta percompressa	S	C	*Leptosynapta tenuis*	Attached to host	Chanley and Chanley, 1970; Fox *et al.*, 2007
Montacuta phascolionis	S	C	*Phascolion strombi*	Within host-occupied shells	Jespersen and Lützen, 2000; Gage, 1979; Gibbs, 1978
Montacuta substriata	S	C	*Spatangus purpureus, Echinocardium flavescens* and other spatangoids	Attached to host	Gage, 1966; Fox *et al.*, 2007; Kallonas *et al.*, 1999
Montacutella echinophila	S	C	*Brissus latecarinatus*	Attached to host	Jespersen *et al.*, 2004
Montacutona ceriantha	S	C	*Cerianthus sp.*	Within host tubes	Ponder, 1971
Montacutona compacta	H	F		Attached to coral heads	Morton, 1980
Montacutona olivacea	S	C	*Cerianthus cf. filiformis*	Within host tubes	Morton, 1980
Mysella charcoti	S	F		Upper sediment layer	Domaneschi *et al.*, 2002; Passos *et al.*, 2005
Mysella cuneata	S	C	*Phascolion strombi*	Within host-occupied shells	Gage, 1979
Mysella gregaria	S	C	*Burowing actinian*	Attached to host	Rotvit *et al.*, 2007
Mysella narchii	S	F		Upper sediment layer	Passos and Domaneschi, 2006
Mysella pedroana	S	C	*Blepharipoda occidentalis; Isocheles pilosus*	Attached to host	Carpenter, 2005; Boyko and Mikkelsen, 2002
Mysella vitrea	S	C	*Trypaea australiensis;*	In host burrows	Kerr and Corfield, 1998;
Mysella vitrea	S	F	*Trypaea australiensis;*	In host burrows	Kerr and Corfield, 1998;
Neaeromya rugifera	S	C	*Upogebia pugettensis; Aphrodita sp.*	Attached to host	Boss, 1965b; Narchi, 1969; Ó Foighil, 1985
Nipponomysella subtruncata	S	C	*Siphonosoma cumanense*	Attached to host	Lützen *et al.*, 2001
Parabornia palliopapillata	S	C	*Lysiosquilla scabricauda*	Attached to host	Simone, 2001
Parabornia squillina	S	C	*Lysiosquilla scabricauda*	Attached to host	Boss, 1965
Peregrinamor ohshimai	S	C	*Upogebia major*	Attached to host	Itani *et al.*, 2002; Kato and Itani, 1995
Phlyctaenachlamys lysiosquillina	S	C	*Lysiosquillina maculata*	In host burrows	Popham, 1939
Pristes oblongus	H	C	Chitons		Kelsey, 1902
Pseudogaleomma japonica	H	F		Under rocks	Lützen and Nielsen, 2005; Ueng and Wang, 1999
Pseudopythina macrophthalmensis	S	C	*Macrophthalmus convexus*	Attached to host	Jespersen et al., 2001; Kosuge and Itani, 1994; Morton and Scott, 1989
Pseudopythina muris	S	C	*Aphtodita japonica*	Within host respiratory cavity	Rosewaer, 1984
Pseudopythina nodosa	S	C	*Sipunculus nudus*	Attached to host	Morton and Scott, 1989
Pseudopythina ochetostomae	S	C	*Ochetostoma erythrogrammon*	In host burrows	Jespersen *et al.*, 2002; Morton and Scott, 1989
Pseudopythina subsinuata	S	C	*Squilla nepa; Squilla raphidea; Oratosquilla oratorio*	Attached to host	Appukuttan 1972; Jespersen *et al.*, 2009; Morton 1972; Morton and Scott, 1989
Pseudopythina tsurumaru	S	C	*Protankyra bidentata*	Attached to host	Lützen *et al.*, 2004; Morton and Scott, 1989
Rochfortia (Mysella) tumida	S	C	*Mesochaetopterus taylori*	Within host's exhalent oxic halo	Sendall *et al.*, 1995
Scacchia oblonga	H	F		Within algal holdfasts	Kallonas *et al.*, 1999
Scintilla agilis	H	F		Under stones, within coral galleries	Lützen and Nielsen, 2005
Scintilla cuvieri	H	F		Under stones, within coral galleries	Lützen and Nielsen, 2005; Morton and Scott, 1989

Scintilla dubia	H	F		Under coral and slate blocks	Lützen and Nielsen, 2005
Scintilla imperatoris	H	F		Under dead coral	Lützen and Nielsen, 2005
Scintilla larcombae	H	F		In coral rubble crevices	Oliver and Holmes, 2004
Scintilla longitentaculata	H	F		Under stones	Lützen and Nielsen, 2005
Scintilla lynchae	H	F		Under coral and volcanic rock blocks	Oliver and Holmes, 2004
Scintilla macrodactylus	H	F		Under coral blocks	Lützen and Nielsen, 2005
Scintilla minor	H	F		Under coral blocks	Lützen and Nielsen, 2005
Scintilla mortoni	H	F		Under coral blocks	Lützen and Nielsen, 2005
Scintilla nitidella	H	F		Under coral, shale or rock blocks	Lützen and Nielsen, 2005
Scintilla nitidella	H	F		Under coral, shale or rock blocks	Lützen and Nielsen, 2005
Scintilla ovalis	H	F		Under rocks	Lützen and Nielsen, 2005
Scintilla ovulina	H	F		In coral galleries	Lützen and Nielsen, 2005
Scintilla papillosa	H	F		Under coral blocks	Lützen and Nielsen, 2005
Scintilla philippinensis	H	F		In crevices, under shale blocks	Lützen and Nielsen, 2005
Scintilla pisum	H	F		In coral rubble crevices	Oliver and Holmes, 2004
Scintilla sannio	H	F		Under rocks	Lützen and Nielsen, 2005
Scintilla (*Lactemiles*) *strangei*	H	F		Under rocks	Personal observation, Li, 2011
Scintilla unicornia	H	F		Under coral blocks	Lützen and Nielsen, 2005
Scintilla verrucosa	H	F		Under rocks	Lützen and Nielsen, 2005
Scintilla violescens	H	F		Attached to gorgonians	Arakawa, 1961; Kuroda and Taki, 1961
Scintilla vitrea	H	F		Under coral and volcanic rock blocks	Oliver and Holmes, 2004
Scintillona bellerophon	S	C	*Leptosynapta clarki*	Attached to host	Ó Foighil and Gibson, 1984
Scintillona brissae	S	C	*Brissus latecarinatus*	Attached to host	Jespersen *et al*., 2004; Morton and Scott, 1989
Scintillona zelandica	S	C	*Trochodota dendyi*	Attached to host	Morton, 1957
Tellimya ferruginosa	S	C	*Echinocardium cordatum* and other spatangoids	In host burrows	Fox *et al.*, 2007; Gage, 1966; Kallonas *et al.*, 1999; Morton, 1962
Tellimya tenella	S	C	*Brissopsis lyrifera*	Attached to host	Fox *et al.*, 2007; Kallonas *et al.*, 1999
Varotoga cryptozoica (Scintilla anomala)	H	F		Undersides of stones, coral galleries	Lützen and Nielsen, 2005
Waldo parasiticus	S	C	*Tripylus sp; Abatus cavernosus; Abatus agassizii; Abatus cordatus; Abatus bidens; Triphylaster philippii; Triphylus excavatus*	Attached to host	Zelaya and Ituarte, 2002
Waldo trapezialis	S	C	Irregular echinoids	Attached to host	Zelaya and Ituarte, 2002

References for Supplementary Materials

[1] Altnöder A, Haszprunar G: **Larval morphology of the brooding clam *Lasaea adansonii* (Gmelin, 1791) (Bivalvia, Heterodonta, Galeommatoidea)**. *Journal of Morphology* 2008, **269**(6):762–774.

[2] Appukuttan K: ***Pseudopythina subsinuata* (Lischke), a commensal bivalve of *Squilla nepa* (Latricelle) and *Squilla raphidea* Fabricius**. *Journal of the Marine Biological Association of India* 1972, **14**:412–415.

[3] Arakawa K: **Ecological observations on an aberrant lamellibranch, *Ephippodonta murakamii***. *Venus* 1960, **21**:50–61.

[4] Arakawa K: **A note on the animal of *Scintilla violescens* collected in Genkai Sea**. *Venus* 1961, **21**:143–146.

[5] Boss K: **Symbiotic erycinacean bivalves**. *Malacologia* 1965, **3**:183–195.

[6] Boss K: **A new mollusk (Bivalvia, Erycinidae) commensal on the stomatopod crustacean *Lysiosquilla***. *American Museum Novitates* 1965, (2215).

[7] Bourne G: **On *Jousseaumia*. A new genus of eulamellibranche commensal with the corals *Heterocyathus* and *Heteropsammia***. *Ceylon Pearl Oyster Fisheries* 1906, **Supplemntary Reports No. XXXVII**:212–266.

[8] Boyko CB, Mikkelsen PM: **Anatomy and biology of *Mysella pedroana* (Mollusca: Bivalvia: Galeommatoidea), and its commensal relationship with *Blepharipoda occidentalis* (Crustacea: Anomura: Albuneidae)**. *Zoologischer Anzeiger - A Journal of Comparative Zoology* 2002, **241**:149–160.

[9] Bristow GA, Berland B, Schander C, Vo DT: **A new endosymbiotic bivalve (Heterodonta: Galeommatoidea), from Pacific holothurians.** *The Journal of Parasitology* 2010, **96**:532–534.

[10] Carpenter S: ***Mysella pedroana*, a commensal bivalve (Lasaeidae) on two decapod crustacean hosts**. *Nautilus* 2005, **119**(3):105–108.

[11] Chanley P, Chanley M: **Larval development of the commensal clam, *Montacuta percompressa* Dall**. *Proceedings of the Malacological Society of London* 1970, **39**:59–67.

[12] Chanley P, Chanley M: **Reproductive biology of *Arthritica crassiformis* and *A. bifurca*, two commensal bivalve molluscs (Leptonacea)**. *New Zealand Journal of Marine and Freshwater Research* 1980, **14**:31–43.

[13] Clench W, Aguayo C: ***Entovalva* (*Devonia*) *perrieri* (Malard) in the western atlantic**. *Occasional papers of the boston Society of Natural History* 1931, **8**:5–8.

[14] Cotton BC: ***Ephippodonta* – South Australia's most perculiar bivalve shell**. *Victorian Naturalist* 1938, **55**:58–61.

[15] Crisp DJ, Standen A: ***Lasaea rubra* (Montagu) (Bivalvia: Erycinacea), an apomictic crevice-living bivalve with clones separate by tidal level preference**. *Journal of Experimental Biology and Ecology* 1988, **117**:27–45.

[16] Domaneschi O, da Silva J, Neto L, Passos F: **New perspectives on the dispersal mechanisms of the Antarctic brooding bivalve *Mysella charcoti* (Lamy, 1906)**. *Polar Biology* 2002, **25**(7):538–541.

[17] Ó Foighil D, Gibson A: **The morphology, reproduction and ecology of the commensal bivalve *Scintillona bellerophon* spec. nov (Galeommatacea)**. *Veliger* 1984, **27**:72–80.

[18] Ó Foighil D, Thiriot-Quievreux C: **Sympatric Australian *Lasaea species* (Mollusca : Bivalvia) differ in their ploidy levels, reproductive modes and developmental modes**. *Zoological Journal of the Linnean Society* 1999, **127**:477–494.

[19] Fox TH, Jespersen Å, Lützen J: **Sperm transfer and reproductive biology in species of hermaphroditic bivalves (Galeommatoidea: Montacutidae)**. *Journal of Morphology* 2007, **268**:936–952.

[20] Gage J: **Experiments with the behaviour of the bivalves *Montacuta substriata* and *M. ferruginosa*, 'commensals' with spatangoids**. *Journal of the Marine Biology Association, U.K.* 1966, **46**:71–88.

[21] Gage J: **Mode of life and behaviour of *Montacuta phascolionis*, a bivalve commensal with the sipunculan *Phascolion strombi***. *Journal of the Marine Biological Association of the United Kingdom* 1979, **59**:635–657.

[22] Gibbs PE: ***Menestho diaphana* (Gastropoda) and *Montacuta Phascolionis* (Lamellibranchia) in association with the sipunculan *Phascolion strombi* in Brsitish Waters**. *Journal of the Marine Biological Association of the United Kingdom* 1978, **58**:683–685.

[23] Gofas S: **The family Galeommatidae (Bivalvia: Leptonacea) in the Eastern Atlantic**. *Veliger* 1991, **34**:344–353.

[24] Gofas S, Salas C: **A review of European '*Mysella*' species (Bivalvia, Montacutidae), with description of *Kurtiella* new genus**. *Journal of Molluscan Studies* 2008, **74**:119–135.

[25] Goto R, Hamamura Y, Kato M: **Obligate commensalism of *Curvemysella paula* (Bivalvia: Galeommatidae) with hermit crabs**. *Marine Biology* 2007, **151**:1615–1622.

[26] Itani G, Kato M, Shirayama Y: **Behaviour of the shrimp ectosymbionts, *Peregrinamor ohshimai* (Mollusca : Bivalvia) and *Phyllodurus sp.* (Crustacea : Isopoda) through host ecdyses**. *Journal of Marine Biological Association Of The United Kingdom* 2002, **82**:69–78.

[27] Iwasaki K: **Seasonal changes in size structure and reproduction of the minute Galeommatacean Bivalve *Lasaea undulata* (Gould) within intertidal Mussel beds**. *The Veliger* 1996, **39**(3):244–249.

[28] Jespersen Å, Lützen J, Oliver PG: **Morphology, biology and systematic position of *Epilepton clarkiae* (Clark, 1852)(Galeommatoidea: Montacutidae) a bivalve commensal with Sipunculans**. *Journal of Conchology* 2007, **39**(4):391–401.

[29] Jespersen Å, Lützen J: **Sex, seminal receptacles, and sperm ultrastructure in the commensal bivalve *Montacuta phascolionis* (Veneroida; Galeommatacea)**. *Acta Zoologica* 2000, **81**:69–75.

[30] Jespersen Å, Lützen J: **Ultrastructure of the seminal receptacle and the dimorphic sperm in the commensal bivalve *Mysella bidentata* (Veneroida; Galeommatoidea; Montacutidae)**. *Acta Zoologica* 2001, **82**:107–115.

[31] Jespersen Å, Kosuge T, Lützen J: **Sperm dimorphism and spermatozeugmata in the commensal bivalve *Pseudopythina macrophthalmensis* (Galeommatoidea, Kelliidae)**. *Zoomorphology* 2001, **120**:177–189.

[32] Jespersen Å, Lützen J, Morton B: **Ultrastructure of dimorphic sperm and seminal receptacle in the hermaphrodites *Barrimysia siphonosomae* and *Pseudopythina ochetostomae* (Bivalvia, Galeommatoidea)**. *Zoomorphology* 2002, **121**:159–172.

[33] Jespersen Å, Lützen J, Nielsen C: **On three species and two new genera (*Montacutella* and *Brachionlya*) of galeommatoid bivalves from the irregular sea urchin *Brissus latecarinatus* with emphasis on their reproduction** . *Zoologischer Anzeiger* 2004, **243**:3–19.

[34] Jespersen Å, Lützen J: **Sperm ultrastructure in *Kellia suborbicularis* (Bivalvia : Galeommatoidea : Kellidae)**. *Acta Zoologica* 2007, **88**:59–63.

[35] Jespersen Å, Lützen J, Itani G: **Sperm structure and sperm transfer in *Pseudopythina subsinuata* (Bivalvia; Galeommatoidea).** *Zoologischer Anzeiger* 2009, **248**:57–67.

[36] Judd W: **The structure and habits of *Divariscintilla maoria* Powell (Bivalvia: Galeommatidae)**. *Journal of Molluscan Studies* 1971, **39**:343–354.

[37] Kallonas M, Zenetos A, Gofas S: **Notes on the ecology and distribution of microbivalvia in Greek waters**. *La Conchiglia* 1999, **291**:11–20.

[38] Kato M, Itani G: **Commensalism of a bivalve, *Peregrinamor Ohshimai*, with a thalassinidean burrowing shrimp, *Upogebia Major***. *Journal Of The Marine Biological Association Of The United Kingdom* 1995, **75**(4):941–947.

[39] Kato M: **Morphological and ecological adaptations in montacutid bivalves endo- and ectosymbiotic with holothurians**. *Canadian Journal of Zoology* 1998, **76**:1403–1410.

[40] Kawahara T: **On *Devonia oshimai sp. nov.*, a commensal bivalve attached to the synaptid *Leptosynapta ooplax***. *Venus* 1942, **11**:153–164.

[41] Keep J, Hannibal HB: *West Coast Shells. A description of the principal marine mollusks living on the west coast of the United States, and of the land shells of the adjacent region*. San Francisco: The Whitaker & Ray-Wiggin Company 1911.

[42] Kerr G, Corfield J: **Association between the ghost shrimp *Trypaea australiensis* Dana 1852 (Crustacea : Decapoda) and a small deposit-feeding bivalve *Mysella vitrea* Laserson 1956 (Mollusca: Leptonidae)**. *Marine And Freshwater Research* 1998, **49**:801–806.

[43] Kosuge T, Itani G: **A record of the crab associated bivalve, *Pseudopythina macrophthalmensis* from Iriomote Island, Okinawa, Japan**. *Venus* 1994, **53**(3):241–244.

[44] Kubo H: ***Ephippodonta gigas* n. sp. (Bivalvia: Galeommatoidea) from Okinawa Island, Southwestern Japan**. *Venus* 1996, **55**:1–5.

[45] Kuroda T, Taki I: **On a new species of *Scintilla* (Galeommatidae) from Japan**. *Venus* 1961, **21**:141–142.

[46] Lamy ME: **Description d'une coquille nouvelle de la Côte Atlantique Française**. *Journal de Conchyliologie* 1908, **56**:35–37.

[47] Laseron CF: **A revision of the New South Wales Leptonidae**. *Records of the Australian Museum* 1956, **24**:7–22.

[48] Lebour M: **The life history of *Kellia suborbicularis***. *Journal of the Marine Biological Association of the United Kingdom* 1938, **22**(2):447–451.

[49] Lützen J, Takahashi T, Yamaguchi T: **Morphology and reproduction of *Nipponomysella subtruncata* (Yokoyama), a galeommatoidean bivalve commensal with the sipunculan *Siphonosoma cumanense* (Keferstein) in Japan**. *Journal of Zoology* 2001, **254**:429–440.

[50] Lützen J, Takahashi T: ***Arthritica japonica, sp. nov.*(Bivalvia: Galeommatoidea: Leptonidae), a commensal with the pinnotherid crab *Xenophthalmus pinnotheroides* White, 1846**. *Yuriyagai* 2003, **9**:11–19.

[51] Lützen J, Jespersen Å, Takahashi T, Kai T: **Morphology, structure of dimorphic sperm, and reproduction in the hermaphroditic commensal bivalve *Pseudopythina tsurumaru* (Galeommatoidea : Kellidae)**. *Journal of Morphology* 2004, **262**:407–420.

[52] Lützen J, Kato M, Kosuge T, Ó Foighil D: **Reproduction involving spermatophores in four bivalve genera of the superfamily Galeommatoidea commensal with holothurians**. *Molluscan Research* 2005, **25**(2):99–112.

[53] Lützen J, Nielsen C: **Galeommatid bivalves from Phuket, Thailand**. *Zoological Journal of the Linnean Society* 2005, **144**:261–308.

[54] Lützen J, Hong J, Yamashita H: ***Koreamya arcuata* (A. Adams, 1856) gen. nov.(Galeommatoidea: Montacutidae), a commensal bivalve associated with the inarticulate brachiopod Lingula anatina**. *Journal of Conchology* 2009, **39**:669.

[55] Manning RB, Morton B: **Pinnotherids (Crustacea: Decapoda) and Leptonaceans (Mollusca: Bivalvia) associated with sipunculan worms in Hong Kong**. *Proceedings of the Biological Society of Washington* 1987, **100**(3):543–551.

[56] Middelfart P, Craig M: **Description of *Austrodevonia sharnae n. gen. n. sp.* (Galeommatidae: Bivalvia), an ectocommensal of *Taeniogyrus australianus* (Stimpson, 1855)(Synaptidae: Holothuroidea)**. *Molluscan Research* 2004, **24**:211–219.

[57] Middelfart P: **Review of *Ephippodonta sensu lato* (Galeommatidae: Bivalvia), with descriptions of new related genera and species from Australia**. *Molluscan Research* 2005, **25**:129–144.

[58] Mikkelsen PM, Bieler R: **Biology and comparative anatomy of *Divariscintilla yoyo* and *D. troglodytes*, two new species of Galeommatidae (Bivalvia) from stomatopod burrows in eastern Florida**. *Malacologia* 1989, **31**:175–195.

[59] Mikkelsen PM, Bieler R: **Biology and comparative anatomy of three new species of commensal Galeommatidae, with a possible case of mating-behavior in bivalves**. *Malacologia* 1992, **34**:1–24.

[60] Morton JE: **The habits of *Scintillona zelandica* (Odhner) 1924 (Lamellibrancma: Galeommatidae)**. *Proceedings of the malacological society* 1957, **32**:185–188.

[61] Morton B: **Habit and orientation in the small commensal bivalve mollusc, *Montacuta ferruginosa***. *Animal Behaviour* 1962, **10**:126–133.

[62] Morton B: **Some aspects of functional morphology and biology of *Pseudopythina subsinuata* (bivalvia: Leptonacea) commensal on stomatopod crustaceans**. *Journal of Zoology* 1972, **166**:79–96.

[63] Morton B: **Some factors affecting location of *Arthritica crassiformis* (Bivalvia: Leptonacea) commensal upon *Anchomasa similis* (Bivalvia: Pholadidae)**. *Journal of Zoology* 1973, **170**:463–473.

[64] Morton B: **Biology and functional morphology of *Galeomma (Paralepida) takii* (Bivalvia: Leptonacea)**. *Journal of Zoology* 1973, **169**:133–150.

[65] Morton B: **Some aspects of the biology and functional morphology (including the presence of a ligamental lithodesma) of *Montacutona compacta* and *Montacutona olivacea* (Bivalvia, Leptonacea) associated with coelenterates in Hong Kong**. *Journal of Zoology* 1980, **192**:431–455.

[66] Morton B: **The biology and functional morphology of *Chlamydoconcha orcutti* with a discussion on the taxonomic status of the Chlamydoconchacea (Mollusca: Bivalvia)**. *Journal of Zoology* 1981, **195**:81–121.

[67] Morton B, Valentich-Scott P: **The Hong Kong Galeommatacea (Mollusca: Bivalvia) and their hosts, with descriptions of new species**. *Asian Marine Biology* 1989, **6**:129–160.

[68] Narchi W: **On *Pseudopythina rugifera* (Carpenter, 1864) (Bivalvia)**. *Veliger* 1969, **12**:43–52.

[69] Nicell L, Atkinson R, Hughes D, Ansell A, Smith C: **Burrow morphology of the echiuran worm *Maxmuelleia Lankesteri* (Echiura, Bonelliidae), and a brief review of burrow structure and related ecology of the echiura**. *Journal Of Natural History* 1995, **29**(4):871–885.

[70] Norman RCA, MA, DCL, FRS, c: **Lepton squamosum, a commensal**. *The Annals and Magazine of Natural history* 1891, **7**:276–278.

[71] Ockelmann KW, Muus K: **The biology, ecology, and behavior of the bivalve *Mysella Bidentata* (Montagu)**. *Ophelia* 1978, **17**:1–93.

[72] Ó Foighil D: **Form, function, and origin of temporary dwarf males in *Pseudopythina rugifera* (Carpenter, 1864)(Bivalvia: Galeommatacea)**. *Veliger* 1985, **27**:72–80.

[73] Ohshima H: **On *Entovalva semperi* Ohshima, an aberrant commensal bivalve**. *Venus* 1931, **2**:161–177.

[74] Oliver PG: **A new commensal bivalve associated with a burrowing sea anemone *Halcampicola tenacis gen. et sp. nov.*, (Galeommatoidea: Montacutidae) on *Halcampoides sp.* (Anthozoa: Antipatharia: Halcampoididae)**. In *Proceedings of the Fifth International Marine Biological Workshop: The Marine Flora and Fauna of Rottnest Island, Western Australia.*. Edited by Wells FE, Walker DI, Kirkman H, Lethbridge R, Perth: Western Australian Museum 1993:634.

[75] Oliver PG, Holmes AM, Killeen IJ, Turner JA: **Marine Bivalve Shells of the British Isles (Mollusca: Bivalvia)** 2010.

[76] Passos F, Domaneschi O, Sartori AF: **Biology and functional morphology of the pallial organs of the Antarctic bivalve *Mysella charcoti* (Lamy, 1906) (Galeommatoidea: Lasaeidae)**. *Polar Biology* 2005, **28**(5):372–380.

[77] Passos FD, Domaneschi O: **A new species of *Mysella Angas*, 1877 (Bivalvia: Galeommatoidea) from Admiralty Bay, King George Island, South Shetlands, Antarctica, with data on its biology and functional anatomy**. *Polar Biology* 2006, **29**(5):389–398.

[78] Ponder WF: **The biology of the genus *Arthritica***. *Transactions of the Royal Society, New Zealand, Zoology,* 1965, **6**:75–86.

[79] Ponder WF: **Some New Zealand and subantarctic bivalves of the cyamiacea and leptonacea with descriptions of new taxa**. *Records of the Dominion Museum* 1971, **7**(13):119–141.

[80] Ponder WF: **"*Montacutona ceriantha*" n.sp., A commensal leptonid bivalve living with 'cerianthus'**. *Journal de Conchyliologie* 1971, **109**:16–25.

[81] Popham ML: **On *Phlyctaenachlamys lysiosquillina gen. et sp. nov*: a lamellibranch commensal in the burrows of *Lysiosquilla maculara***. *British Museum, Natural History, Great Barrier Reef Expedition, 1928-29. Scientific Reports* 1939, **6**:61–84.

[82] Rosewater J: **A new species of Leptonacean bivalve from off northwestern Peru (Heterodonta, Veneroida, Lasaeidae)**. *Veliger* 1984, **27**:81–89.

[83] Rotvit L, Lützen J, Jespersen Å, Fox T: ***Mysella gregaria* new species, a bivalve (Galeommatoidea : Montacutidae) commensal with an intertidal burrowing sea anemone from North Carolina, USA**. *Nautilus* 2007, **121**:191–200.

[84] Sato S, Owada M, Haga T, Hong JS, Lützen J, Yamashita H: **Genus-specific commensalism of the galeommatoid bivalve *Koreamya arcuata* (A. Adams, 1856) associated with lingulid brachiopods**. *Molluscan Research* 2011, **31**(2):95–105.

[85] Simone: **Revisin of the genus *Parabornia* (Bvalvia: Galeommatoidea: Galeommatidae) from the western Atlantic with description of a new species from Brazil**. *Journal Of Conchology* 2001, **37**(2):159–169.

[86] Spärck R: ***Cycladoconcha amboiensis n. gen. n. sp.* A commensalistic lamellibranch. Papers from Dr. Th. Mortensens's Pacific Expedition 1914-16.** *Videnskabelige Meddelelser Dansk Naturhis torisk Forening* 1931, **91**:227–239.

[87] Ueng YT, Wang JP: ***Pseudogaleooma japonica* (Galoemmatidae), a familial and generic record new to Veneroida fauna of Taiwan**. *Journal of Taiwan Museum* 1999, **52**:7–11.

[88] Voeltzkow A: ***Entovalva mirabilis*, eine schmarotzende Muschel aus dem Darm einer Holothurie**. *Zoologische Jahrbücher, Abteilung für Systematik, Ökologi und Geographie der Tiere* 1890, **5**:619–628.

[89] Yamamoto T, Habe T: ***Scintillona stigmatica* new to Japan**. *Venus* 1974, **33**(3):115–116.

[90] Zelaya DG, Ituarte C: **The identity of *Waldo parasiticus* (Dall, 1876) and description of *Waldo trapezialis* new species (Bivalvia : Galeommatoidea)**. *Nautilus* 2002, **116**:109–117.

CHAPTER 3

Host-mediated Morphological Divergence of the Commensal Bivalve *Neaeromya rugifera*

3.1 Introduction

Galeommatoidean clams are a poorly studied superfamily of minute, morphologically diverse bivalves [192]. Their outsized role in marine alpha biodiversity has become apparent over the last decade with the application of comprehensive sampling methodologies that include smaller taxa [72]. Although individual species are typically rare, they collectively exhibit among the highest levels of bivalve alpha diversity in both neontological [57, 72] and paleontological [73] surveys. Consequently, this superfamily is now recognized as a "megadiverse" group [57].

Galeommatoidea is also notable for containing large numbers of commensal species in addition to free-living taxa. The spectrum of host taxa utilized by the commensals includes crustaceans, holothuroids, echinoids, cnidarians and polychaetes, among others [56, 86, 98, 126]. Commensals either attach directly to their hosts, or live in or around host burrows, and individual clam species may associate with single or multiple host species. The prevalence of commensal life histories among galeommatoideans raises the possibility that this life history has contributed to their exceptional species diversity. Specifically, one may ask whether speciation by host shifts occurred frequently in this group and accelerated its diversification.

Host shifts in symbiotic systems provide unique opportunities for ecological divergent selection to occur [193]. In a symbiotic association, the host can be viewed as a microhabitat and the symbiont oftentimes evolves specialized adaptations to a specific

A version of this chapter has been published as: Jingchun Li and Ó Foighil D. 2012. Host-specific morphologies but no host races in the commensal bivalve *Neaeromya rugifera*. Invertebrate Biology 131: 197-203

host [194]. If a host shift occurs, offspring of individuals that utilize different hosts may experience lower fitness on either host species, resulting in ecologically-dependent post-zygotic isolation [195]. Additionally, if the symbiont exhibits high host fidelity, then shifting to a new host can directly cause pre-zygotic barriers between parental and daughter populations [196]. Even though host shifts could significantly reduce gene flow among populations, the process is gradual and may not always lead to complete reproductive isolation. Depending on the levels of gene flow, individuals occupying different host species could represent within species polymorphisms (panmictic), host races (restricted gene flow), or true species (no gene flow) [197,198]. Host races are defined as genetically differentiated sympatric populations that show high levels of host fidelity, but experience at least some gene flow [197]. Their formation is both an intermediate step and a prerequisite for host-mediated speciation [198, 199].

Host races have been reported mostly from parasitic and phytophagous organisms in terrestrial systems [199]. Relatively few studies have been done on marine taxa, even though symbiotic associations such as commensalism are not rare in marine environments [122]. Studies on sponge-dwelling alphid shrimps and bivalve-associated pea crabs have revealed high degrees of host-specific genetic and phenotypic structuring [200, 201], suggesting that host shift-driven diversification in marine commensal species may be relatively common. To test if this ecological speciation mechanism has played a role in galeommatoidean diversification, one possible strategy is to look for evidence of host races formation in commensal species with multiple hosts.

To date, there has been one such study. Sato et al. (2011) [202] attempted to distinguish populations of a commensal galeommatoid, *Koreamya arcuata* (ADAMS, 1856), from two congeneric lingulid brachiopod hosts. They found subtle morphological differences between the two populations but failed to detect host-specific genetic structuring. This is perhaps not very surprising because the two hosts are very similar in their biology and ecology and the commensals are therefore less likely to be under strong divergent selection. A more rigorous test of the host-shift diversification hypothesis would involve commensal species with very different host species, thereby providing more opportunities for divergent selection to occur.

Neaeromya rugifera (CARPENTER, 1864) (Fig. 3.1) is a Northeastern Pacific commensal species distributed from Alaska to Lower California [59]. It is associated with two strikingly different hosts that are sympatrically distributed: the blue mud shrimp *Upogebia pugettensis* (DANA, 1852) and the polychaete worm *Aphrodita spp.*The two hosts are very different in their morphology and ecology. *U. pugettensis* is a thalassinid shrimp that builds deep, permanent Y-shaped burrows in intertidal

mudflats in estuaries [203]. The genus *Aphrodita* is composed of broad-bodied polychaetes, commonly known as the sea mice, that burrow just below the surface of subtidal muddy bottoms [204]. Due to taxonomical uncertainty, it is unsure whether *N. rugifera* is associated with one or multiple *Aphrodita* species (C. Brantley, pers. comm.), thus we refer the sea mouse host as *Aphrodita spp.* in this study. *N. rugifera* attaches to the ventral surface of both hosts by byssal threads (Fig. 3.1A, B), but it also occurs in the respiratory cavity of *Aphrodita spp.* [96, 205] (Fig. 3.1C).

Neaeromya rugifera is a protandric hermaphrodite. A large female individual typically houses one or more dwarf males in its mantle cavity, thus mating and fertilization occur only on the host. The female broods fertilized eggs, then releases the larvae, which undergo a planktotrophic development [96]. Given that *N. rugifera* occurs on two dramatically different host species, it seems plausible that populations have developed specialized morphological/behavioral adaptations to each host and genetically distinct host races have been formed in this species. Alternatively, it is also possible that individuals respond to different host types via phenotypic plasticity, thus represent a panmictic population. Here we tested the hypothesis that host races have been formed in this species and its alternative.

3.2 Methods

3.2.1 Experimental approach

Ideally, testing our hypothesis would involve raising veliger larvae from clams associated with the two hosts and then test larvae/juveniles host preference. Host fidelity can be confirmed if larvae/juveniles always prefer host species their parents were associated to. However, such experiments are impractical as it is extremely challenging to raise and track pelagic larvae, as well as to maintain the two host species under artificial environments in the laboratory. Instead we sampled *N. rugifera* specimens from both *Upogebia pugettensis* and *Aphrodita spp.* and tested for host-specific morphologies and genetic structuring.

3.2.2 Specimen collecting

A total of 35 *Neaeromya rugifera* individuals were collected from *Upogebia pugettensis* and 7 individuals from *Aphrodita spp.* The sampling process (over three years) was very challenging due to difficulties in collecting the host species and the low incidence of clams on hosts. *U. pugettensis* is currently experiencing a dramatic popula-

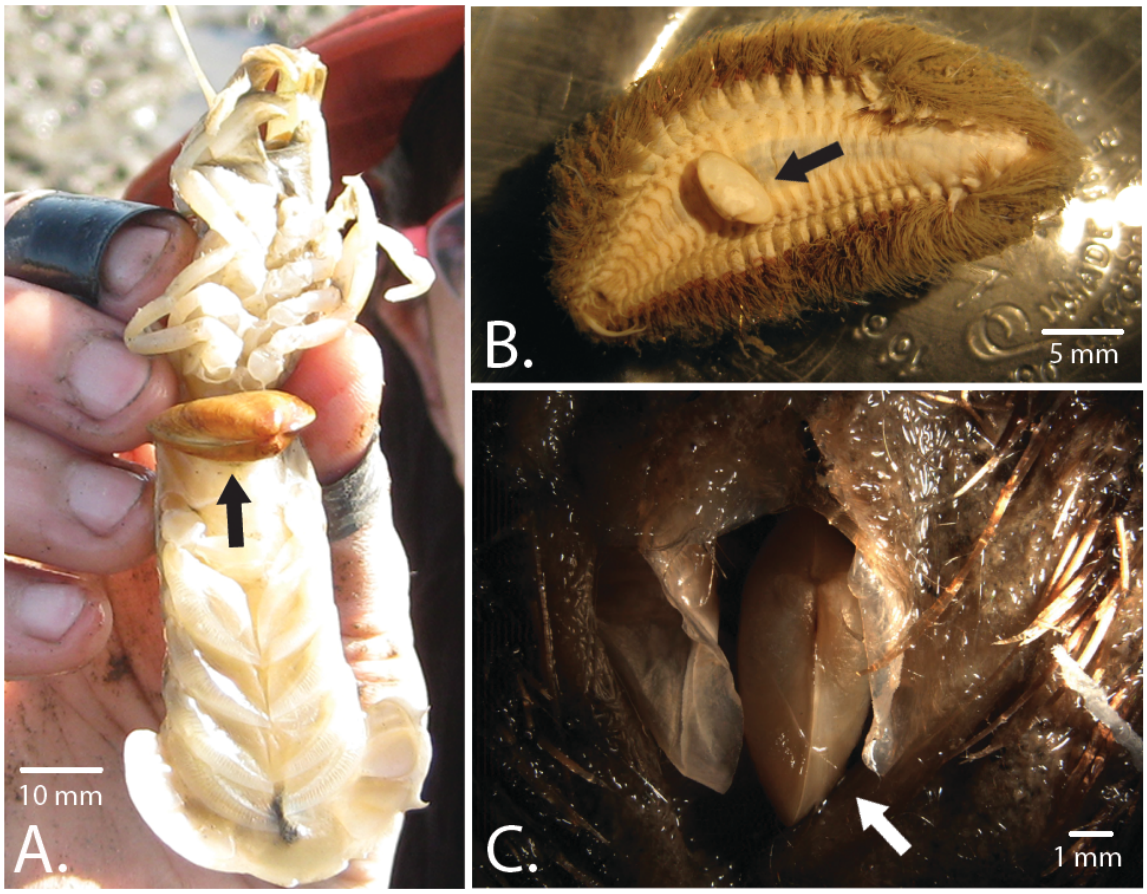

Figure 3.1: The commensal galeommatoidean clam *Neaeromya rugifera* on its hosts. A. A clam (black arrow) attached to the ventral surface of the blue mud shrimp, *Upogebia pugettensis*. B. A clam (black arrow) attached to the ventral surface of the sea mouse *Aphrodita sp.* C. A clam (white arrow) inside the respiratory cavity of the sea mouse *Aphrodita sp.* The clam was revealed by cutting open the dense "felt" covering the dorsal surface of the worm. Photo in C by L. Kirkendale.

tion decline likely due to an introduced isopod parasite; several previously abundant populations from estuaries in California area have been reported as locally extinct and populations in Washington and Oregon are collapsing rapidly [203]. Therefore we were restricted to one sampling location in Yaquina Bay, Newport, Oregon, where the shrimp population was still relatively abundant in 2009. The shrimp were collected from their borrows using a yabbie pump. The clams were detached from the host and deposited in the Museum of Zoology, University of Michigan (UMMZ 302939). The sea mouse commensals were even more difficult to obtain because the host is subtidal and its distribution is scattered. To search for the commensals, the first author joined several dredging trips conducted by the Friday Harbor Laboratories (FHL) on the San Juan Island, Washington and the Sanitation Districts of Los Angeles County (LACSD) in San Pedro, California. However, despite the sampling trips and long-term inquiries of Aphrodita spp. to institutions that perform regular dredging activities, no clams were found on any of the *Aphrodita spp.* ($N = 11$) that were freshly collected. We were restricted to old samples previously collected and preserved by the California Academy of Sciences (CASIZ 85863, collected from Puget sound, Washington, USA, 1924, formalin fixed), the Royal British Columbia Museum (990-00393-008, collected from Moresby island, British Columbia, Canada, 1978, formalin fixed), the Shannon Point Marine Center (UMMZ 302992, collected from Anacortes, Washington, USA, 2009, preserved in 75% ethanol) and LACSD (UMMZ 302993, collected from San Pedro, California, USA, 2008, preserved in 95% ethanol; UMMZ 302994, collected from San Pedro, California, USA, 2000, formalin fixed).

3.2.3 Morphometric analyses

Shell morphologies of the two groups were compared using a geometric morphometric approach [206]. The external lateral view of the right valve of each individual was photographed using a Leica DFC320 digital camera system and processed with Image-Pro Discovery 5.1. Because bivalve shells usually lack informative homologous points for landmark placement, we treated the shell shapes as curves and adopted a semi-landmark [207] approach. One hundred semi-landmarks were evenly placed along the shell outline of each specimen in tpsDig2 [208] to capture the overall shell shape. Semi-landmarks were slid following the minimum bending energy criterion [207] in tpsRelw 1.49 [208] to ensure shape homology among individuals. Shape coordinates of all specimens were then superimposed using the Procrustes method [209] in CoordGen7a [210] to remove variation caused by differences in shell size, position and orientation. Canonical variate analysis (CVA) was performed in CVAgen7b [210] on

the shape coordinates to test whether shell shapes of the two groups are significantly different (PCA reduction was used to account for small sample size). The first canonical variate (CV1) scores were plotted using the software R 2.12 (2011) [151]. A deformation grid presenting vector on landmarks was generated in CVAgen7b [210] to show how general shell shape changes along CV1. A Jackknife grouping test was performed in CVAgen7b [210] to cross validate the grouping procedure. In the test, each specimen was left out in turn and the CVA was done on the remaining n-1 specimens. CV axis derived from the analysis was then used to assign the left-out specimen to one of the groups. This was done for all specimens and a classification table was generated to present the results.

3.2.4 Molecular analyses

The mitochondrial cytochrome oxidase I (COI) gene segment was selected in this study to demonstrate the populations genetic structuring of *Neaeromya rugifera*. A small piece of mantle tissue from each specimen was used for genomic DNA extraction using the Omega Biotek E.Z.N.A. Mollusc DNA Kit. The target gene was amplified from the freshly-sampled shrimp commensals using universal primers LCO1490/HCO2198 [211], following a touchdown PCR protocol. The initial annealing temperature was set to 55°C and was decreased by 2°C/cycle until the final annealing temperature 45°C, then the reaction was maintained for an additional 40 cycles. However, this primer combination did not work for any of the formalin- and ethanol-preserved museum sea mouse commensal specimens, presumably due to suboptimal DNA template quality. To surmount this technical difficulty, a doubly-nested amplification procedure was developed. The first round of PCR was performed as above using the universal primer set to increase template DNA amount. Products from the first PCR were then used as templates for a second round touchdown PCR using a novel internal primer set: 17N2: 5'-CGTTATTGTGACTGCTCATGC-3'; 18N1: 5'-GCATAGTGATAGCACCAGC-3' designed from shrimp commensal sequences. Negative controls (PCR cocktails without DNA templates) were used during every amplification to test for contamination. All PCR products were directly sequenced at the University of Michigan Sequencing Core. Sequences were aligned using ClustalW [212] implemental in CodonCode Aligner 3.1.7 and correctly by eye. COI gene segments amplified from the shrimp commensals had a length of 658 bp, but those from sea mouse commensals were shorter (420 bp) due to the use of internal primers. Comparative analyses among both sets of commensal clams used the homologous 420 bp fragment. Parsimony network of all haplotypes was constructed using TCS 1.21 [213]

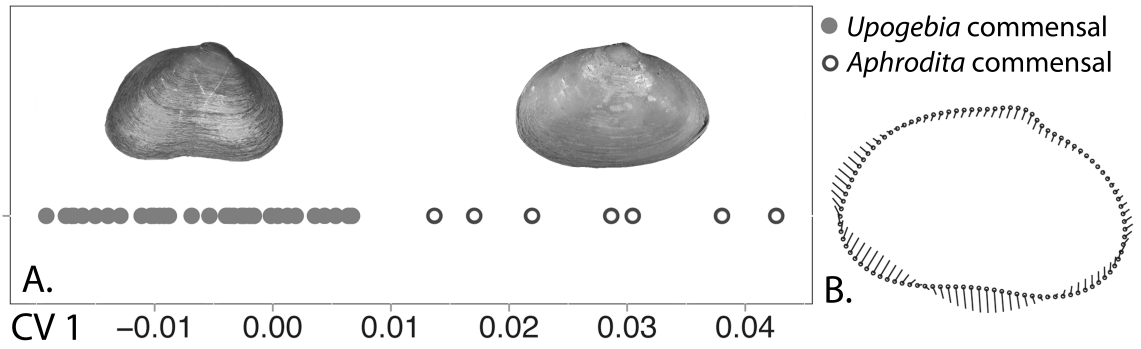

Figure 3.2: Canonical variate analysis (CVA) on shell shape of individuals of *Neaeromya rugifera* from two host species. A. Scatter plot of CV1 scores of all specimens. Solid and hollow circles represent specimens from each host species. B. Vectors on landmarks showing how shell shape changes along CV1, representing how shell shape changes from a shrimp commensal type to a sea mouse commensal type.

to visually represent genetic structuring of the clams.

3.3 Results

The canonical variate analysis on *Neaeromya rugifera* shows that clams from the two hosts represent two distinct groups and the difference is highly significant ($P<0.001$) (Fig. 3.2). The two groups occupy distinct regions in the morphospaces with no overlap. The Jackknifed grouping test shows that 34 out of 35 shrimp commensals and 5 out of 7 sea mouse commensals were placed in the correct CVA group. The vector on landmarks grid indicates that major morphological change along CV1 occurs on the shell ventral margin. Specifically, the ventral margin of individuals on the shrimp host shows a distinctive inward curvature, which is completely lacking on individuals occupying the sea mouse host. We did not identify significant differences among individuals that attached to the ventral surface ($N = 6$) or in the respiratory cavity ($N = 1$) of the sea mouse.

Sequences from 27 shrimp commensals (GenBank accession numbers: JQ712843-69) and 3 sea mouse commensals (GenBank accession numbers: JQ712840-42) were successfully amplified. The low sequence recovery rate from the sea mouse commensals was mainly due to poor template quality. From the haplotype network (Fig. 3.3), we did not detect strong evidence for genetic differentiation (with the caveat that our

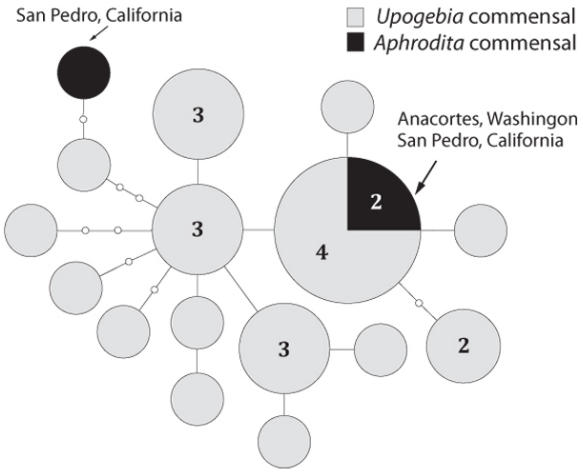

Figure 3.3: COI haplotype network showing genetic structuring of *Neaeromya rugifera* collected from two host species. Each circle represents a unique haplotype. Circle diameter represents how many specimens share the same haplotype, as do numbers in circles (only present if that haplotype was found more than once). Each connection represents one inferred base pair change. All shrimp commensals are from Newport, Oregon. Localities for the sea mouse commensals are indicated by labels.

sea mouse commensal sample size is low and that faster-evolving markers might yield a different result). The same haplotype is the most common in both sets of commensals and haplotypes did not cluster according to either host type or geographic location. Among the 3 sea mouse commensal specimens that were successfully genotyped, two were directly preserved in ethanol and one was formalin fixed. Sequence from one of the ethanol preserved specimen (San Pedro, CA, 2008) represents a unique haplotype that differs from a shrimp commensal haplotype by 2 inferred nucleotide substitutions. The other (Anacortes, WA, 2009), together with the formalin fixed specimen (San Pedro, CA, 2000), exhibited the most common haplotype. Due to the sensitive nature of PCR reactions, there is a risk that the sequence amplified from the formalin fixed individual may actually come from trace contaminations from shrimp commensals, despite the absence of evidence for such in our negative controls. However, even taking this possibility into account, the main pattern of haplotype distribution does not change and the result still holds.

3.4 Discussion

Our results reject the hypothesis that host races have been formed in *Neaeromya rugifera*. Despite the strong morphological distinction, the lack of host-specific genetic structuring suggests that the populations are panmictic and that host fidelity has not yet been established. The host-specific shell morphologies most likely represent ecophenotypic plasticity rather than incipient speciation. Ecophenotypic variation in shell morphology is not rare in bivalves [214]. Because *N. rugifera* attaches to its hosts directly, it is possible that the shell developmental processes are affected by the texture and shape of the attachment surfaces. The shrimp has a relatively hard, smooth exoskeleton, and its ventral abdomen surface is narrow and slightly convex; whereas the sea mouse represents a soft, board and relatively flat attachment surface. Therefore, shrimp commensals may need to produce more/stronger byssal threads to establish a stable association with the host, and a curvature on the ventral margin could form gradually around the attachment point during shell growth. Massive byssal threads production is not necessary for sea mouse commensals to form a stable attachment, especially for the ones that settled inside the host's respiratory cavity, thus their shell growth may be less influenced by the byssal attachment point.

Given the disparity in its host taxa, it is a little surprising that *N. rugifera* lacks host races. Two contributing factors come to mind. Firstly, this species undergoes obligate planktotrophic larval development [96]. For a host-shift to directly impose rapid pre-zygotic isolation, newly metamorphosed juveniles must display fidelity to the new host when re-establishing the benthic commensal association. This critical condition could be hard to meet when organisms exhibit long-range dispersal. Secondly, a generalist strategy may possess selective advantages compared to a specialist one in this group. Because the commensal lifestyle for most galeommatoidean clams is obligate, flexibility in utilizing hosts will protect them from host extinctions events, even though it requires the larvae/juveniles to recognize multiple host species upon metamorphosis. The ongoing collapse of *Upogebia pugettensis* populations is perhaps a vivid example. Without the second host *Aphrodita spp.*, *N. rugifera* will be greatly threatened.

A congeneric species, *Neaeromya compressa* (DALL, 1899), has a distribution that largely overlaps with *N. rugifera* [59]. This species has only been recovered through dredging and although suspected to be a commensal with burrowing invertebrates [59], no confirmed host association has been identified to-date. *N. compressa* is morphologically similar to *N. rugifera* (more to the sea mouse commensals because

it lacks a ventral curvature), but is taxonomically distinguished from the latter by a more flattened and compressed shell form [59, 215]. Given the high degree of phenotypic plasticity displayed by *N. rugifera*, it is possible that shell form of *N. compressa* falls within the shape spectrum of *N. rugifera*, and may not represent a species level diagnostic character. However, to further investigate the relationships of *N. rugifera* and *N. compressa*, one would need to quantitatively examine the shell morphology (particularly inflation) of the two species and to incorporate genetic analyses. In conclusion, although *N. rugifera* occupies two drastically different host species and exhibits distinct host-specific shell phenotypes, we did not detect evidence of host-race formation. Instead, the results indicate that *N. rugifera* possesses a high degree of developmental and behavioral plasticity that enables the larvae/juveniles to recognize (possibly through chemical cues) two distinct benthic host species and form stable physical associations with them. The case study of *N. rugifera* along with previous works [202] show that speciation by host shifts may not be a major diversification mechanism in Galeommatoidea. However, the results need to be further corroborated with additional commensal species associated with distinct hosts.

CHAPTER 4

Phylogeography of the Australian Galeommatoidean Bivalve *Lasaea australis*

4.1 Introduction

Despite a growing body of literature devoted to deciphering the mechanisms of speciation, our knowledge of marine speciation processes remains limited [22,216–218]. The lack of absolute barriers in the marine realm and the prevalence of planktotrophic larval development challenges the classic view that vicariant speciation plays a predominant role in species diversification [216, 219, 220]. Alternative models, such as speciation via ecological divergent selection (*i.e.* ecological speciation) [195,221], have been proposed to explain restricted gene flow and local divergence of taxa with high dispersal potential [217, 222, 223].

The existence of marine biogeographic provinces – regions composed of evolutionary distinct biotas – has been recognized and studied for more than a century [224–228]. Although such provinces are typically delineated based on disjunctions in regional community composition, recent phylogeographic studies have revealed that province boundaries may also resemble genetic break points where latent genetic discontinuities are consistently found in morphotaxa that have continuous distributions across multiple provinces [229–231]. Such concordant genetic disjunctions in regions without absolute barriers often indicate the presence of latent impediments to gene flow [19, 232]. Two major categories of latent barriers have been proposed: historical barriers and contemporary soft (invisible) barriers [216, 226, 233–235]. The former represent absolute barriers formed through past geological events but can no longer

A version of this chapter has been published as: Jingchun Li, Ó Foighil D. and Park J. 2013. Triton's trident: cryptic Neogene divergences in a marine clam (*Lasaea australis*) correspond to Australia's three temperate biogeographic provinces. Molecular Ecology 22: 1933-1946

be observed in modern environments. For example, sea level drops during Pleistocene glacial maxima were known to generate vicariant land bridges that separated marine populations and promoted genetic divergence [236]. The latter represent existing environmental characteristics that reduce the dispersal of certain marine organisms [216]. Such barriers may include the lack of suitable habitats [237], strong current dynamics [238], and steep gradients in sea surface temperature or salinity [239]. Historical barriers promote lineage diversification via the classic allopatric model, while soft barriers can also give rise to ecological speciation [240]. Nonetheless, both mechanisms are likely to interact with each other and to collectively shape regional community compositions.

The high dispersal potential of many marine taxa often makes it difficult to track population diversification processes, as those taxa can sometimes span vast geographic ranges [241]. To better evaluate how historical and/or contemporary barriers affect marine taxa diversification on evolutionary timescales, we need to identify biogeographic breakpoints within a regional biota characterized by a high degree of endemism. The temperate coastal biota of southern Australia represents such a study system because of its well-documented endemicity [238, 242, 243]. In addition, it contains three long-recognized biogeographic provinces along a continuous coastline [2, 238, 244, 245]: Peronia (south-east), Maugea (Tasmania and southern Victoria) and Flindersia (south-west) (Fig. 4.1). These were initially characterized on the basis of qualitative faunal distribution patterns and physical parameters [2,245,246], but have more recently been validated by quantitative biogeographic studies [247, 248]. Cryptic genetic breaks at province boundaries have been detected among a taxonomically heterogeneous subsample of continuously distributed morphospecies [249–253], although many studies have focused on the East/West disjunction only [249, 252, 254, 255].

No general consensus has been reached on what mechanisms drove the population divergences among the provinces, and they are likely to be taxa specific [254]. Both historical and contemporary isolation mechanisms have been proposed. The historical barrier hypothesis states that the emergence of the Bass Isthmus, a land bridge connecting Tasmania to Victoria during glacial maxima [256], promoted East-West allopatric population divergence [14], and this has been supported by phylogeographic patterns of multiple costal taxa [14, 251–253, 257, 258]. However, the land bridge alone cannot explain the existence of a distinct Maugean region, which includes the coastline of Victoria and Tasmania. It has been proposed that unsuitable habitats, such as the Ninety Mile Beach (Fig. 4.2) in southeastern Victoria, may

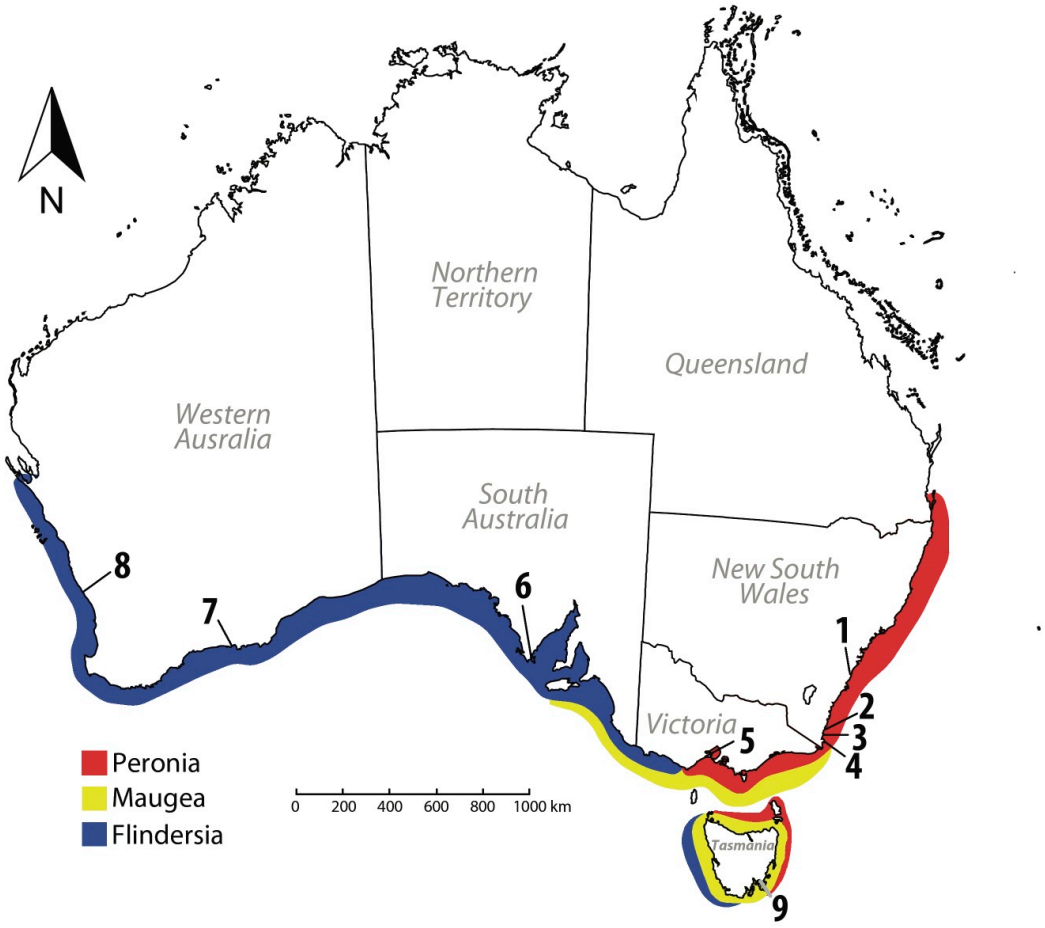

Figure 4.1: Map of Australia showing the three biogeographic provinces and the study sampling locations (biogeographic province placements after [2]). The sampling locations were as following: 1: Sydney; 2: Tura Head; 3: Haycock Headland; 4: Green Cape; 5: Jan Juc Beach; 6: Port Lincoln; 7: Esperance; 8: Guilderton; 9: Tasmania

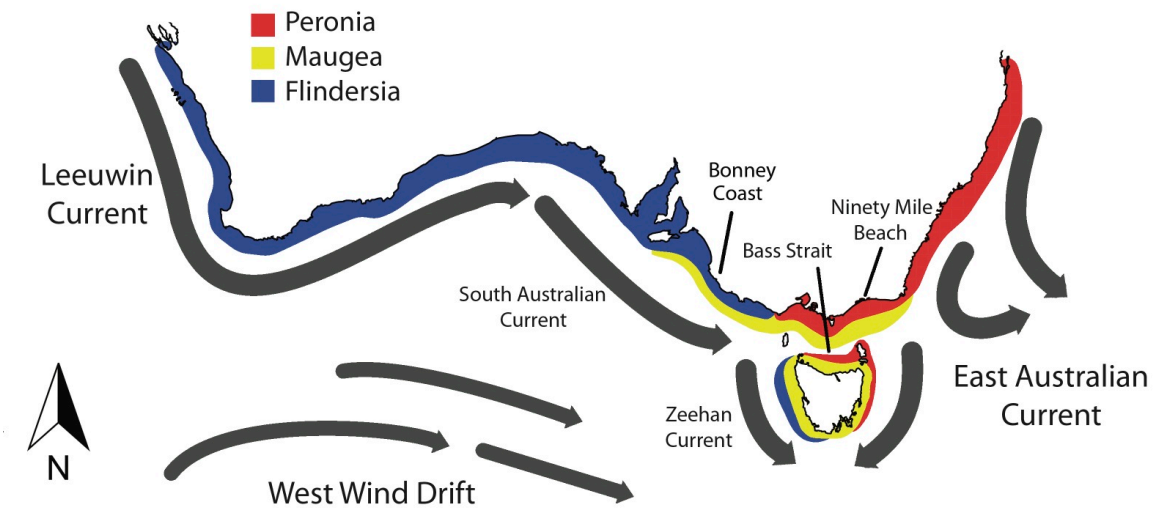

Figure 4.2: Major current systems of the southern Australian coast (after [3]).

block dispersal for some rocky shore species and promote population divergence between the Peronian and Maugean region [237]. Alternatively, it has been argued that oceanographic currents and sea surface temperature gradient may have played roles in the formation and maintenance of genetic disjunctions among the three provinces (Fig. 4.2) [2,238,245,247,252]. On the east coast of southern Australia, the tropical-origin East Australian Current flows south into the Tasman Sea and veers offshore around Sydney, but sometimes can extend further south to Tasmania [238,242,259]. On the west coast, the warm, southward-flowing Leeuwin Current turns eastwards into the Great Australian Bight and connects with the South Australian Current, then is replaced by the cooler southward Zeehan current near Bass Strait and Tasmania [3,242,260,261]. The circumpolar West Wind Drift is responsible for the intrusion of cold water mass into Bass Strait, bringing cool-temperate elements into southern Australia [242]. In addition, extensive coastal upwelling along the west coast of Victoria and east South Australia (Bonney Coast) during the austral summer also brings cooler water towards the ocean surface in the Maugean province [262,263]. A sharp sea surface temperature (SST) drop in the Maugean region has been documented [2,245], possibly resulting from the combination of cold water body influence and the latitudinal temperature gradient. This could potentially act as an isolating factor for taxa that have narrow temperature/salinity tolerance. In fact, there is strong evidence that the Maugean province is mostly composed of typical cool-temperate taxa while the other two provinces harbor warm-temperate taxa [2,245].

The temperate Australian coast harbors a small endemic clam species, *Lasaea australis* (LAMARCK, 1818). It is arguably the most common bivalve in the temperate Australian rocky intertidal fauna [264] and occurs in all three biogeographic provinces, nestled in rock crevices, under coralline algae or among encrusting epifauna [5, 63, 265]. *L. australis* is the only member of the near-cosmopolitan genus *Lasaea* known to have planktotrophic larval development. All others are direct developers that release crawl-away juveniles [5] and they are primarily composed of asexual clonal lineages [63,134–136]. The global collective range of direct developing congeners has been attributed to long distance rafting: asexual clams that release non-pelagic juveniles are more likely to turn a rare rafting event into a successful colonization than are sexual congeners with obligate planktotrophic larval development [4, 5].

Because of its endemicity to temperate Australian shores, its ecological prevalence there, its pelagic larval development and the availability of a global generic phylogenetic framework [136], we consider *Lasaea australis* to be a model exemplar taxon to investigate marine genetic diversification along the southern Australian coast. Our primary questions in this study concern whether *L. australis* exhibits cryptic genetic structuring corresponding to the three regional biogeographic provinces, and if so, what mechanisms may have promoted this diversification.

4.2 Material and Methods

4.2.1 Specimen collecting

Live *Lasaea australis* were sampled from intertidal crevices at 9 locations along the southern coast of Australia and from eastern Tasmania (Fig. 4.1) and preserved in 95% ethanol by a variety of collectors over a 16-year period (see section 4.6.1 for details). Four of these locations were located in the Peronian Province (1-4), two in the Maugean Province (5,9) and three in the Flindersian Province (6-8). Note that the 22 Tasmanian individuals were collected from five localities; but because four out of the five contributed only one individual each, we considered all Tasmanian individuals as representing one Maugean population in population genetic analyses. Two direct developers from Sydney Harbour, *Lasaea colmani* and an unidentified *Lasaea* species, were also collected to be added to the global *Lasaea* phylogeny. Specimens of congeneric direct developers from Hong Kong, that place sister to *L. australis* in molecular phylogenies, were collected from intertidal rocky shores of Shek O, Hong Kong by the first author.

4.2.2 DNA amplification

Genomic DNA was extracted from mantle tissue or from the whole animal using the Omega Biotek E.Z.N.A. Mollusc DNA Kit (Omega tech). Fragments of two ribosomal genes, the mitochondrial (mt) large subunit 16S and the nuclear internal transcribed spacer 2 (ITS2), were used to investigate the population genetic structure of *L. australis*. The target 16S fragment was initially amplified using the universal primer set 16sar/16sbr [266]. For templates that failed to amplify, a *Lasaea* specific primer set 16SLasF (5'-TAGATTAAGGGTTGGGCCTG-3')/16SLasR(5'-GCCTAAATGGTAAGACTGTT-3') was developed and used to increase the success rate. A touchdown PCR protocol was used for both primer sets. The initial annealing temperature was 55°C; it was decreased by 2°C per cycle until the final annealing temperature (48°C) was reached, and then the reaction was continued for an additional 35 cycles. Gene fragments were successfully amplified from 107 *L. australis* individuals and 8 *Lasaea sp.* (Hong Kong) individuals. All PCR products were either sequenced on an ABI 377 automated DNA sequencer (Perkin-Elmer) or at the University of Michigan Sequencing Core facility. GenBank accession numbers of all unique sequences are provided in section 4.6.1. Sequences were aligned using ClustalW [212] implemented in CodonCode Aligner 3.1.7 and corrected by eye. The 16S gene segments amplified using *Lasaea* specific primers (388 bp) were shorter than the ones using universal primers (∼500 bp). The homologous 388 bp fragment was used for further analysis.

The 16S dataset indicated that many individuals shared identical haplotypes; thus only a subset ($N = 29$) of the templates from representative locations was selected for nuclear marker characterization. There was a complication for locations 1, 6 & 9 in that mitochondrial genotyping (16S & COIII) was performed 12 years ago (by the third author). The entire clams were used to prepare DNA templates and the templates were degenerated by accident in later years. Therefore, we no longer have tissue samples or DNA templates from those specimens anymore. In these cases, additional specimens ($N = 17$) from location 1, 6 & 9 (or nearby locations) were used for generating new DNA templates for the subsequent ITS2 PCR. The ITS2 fragment (453 bp) was amplified using the combination of two sets of primers: the universal primers ITS2F /ITS2R [267] and a *Lasaea* specific primer set LasITS2F (5'-CAATGTGGTCTGCAATTCAC-3')/LasITS2R (5'-GGAATCCTAGTTAGTCTC-3'). A standard PCR protocol with an annealing temperature at 52°C was adopted. PCR products were sequenced and aligned as described for the 16S gene.

Another mitochondrial gene, cytochrome oxidase III (CO III, 624bp), was used to put *L. australis* mt lineages from all three provinces into a global phylogenetic framework [4, 136]. *L. australis* ($N = 9$), *L. colmani* ($N = 1$) and the unidentified Sydney Harbor direct developer "LundSdy" ($N = 2$) were genotyped using the primer set COIIILas1/COIIILas2 [134] following a 35-cycle touchdown PCR protocol. The initial annealing temperature was 52°C and was decreased by 2°C per cycle until the final annealing temperature (42°C) was reached. PCR products were sequenced as previously described. Previously published COIII sequences ($N = 29$) for one *L. australis* individual, congeneric *Lasaea* taxa and the outgroup *Kellia laperousi* (see details in [4,136]) were downloaded from Genbank and aligned with *Lasaea* sequences obtained in this study. The quality of the first 12 bp and last 13 bp of the alignment was poor and these parts of the alignment were eliminated from the phylogenetic analysis. The final size of the COIII gene segment used in this study was 598 bp.

4.2.3 Molecular and phylogenetic analyses

We used JModelTest 2.0 [268] to calculate likelihood scores of different substitution models for the mt 16S datasets. Models were ranked according to the Bayesian information criterion (BIC). Specific models used in different analyses were chosen based on their respective BIC rankings and their model availabilities in the software.

A hierarchical Analysis of Molecular Variance (AMOVA) was performed using Arlequin 3.5 [269] on the mt16S dataset to evaluate degrees of genetic variance within and among the three provinces. Individuals from Tura Head, Haycock Headland and Green Cape (Fig. 4.1, locations 2-4) were combined into one population because these locations are geographically close (within 50 km). Populations were then assigned into three groups, representing the three biogeographic provinces respectively. The Tamura 3-parameter model [270] with a gamma distribution (G=0.16) was used to calculate genetic distances between haplotypes. The variance components and Φ statistics were calculated for among provinces, among populations within provinces and within populations respectively. A null distribution of the haplotypes was generated through 10,100 permutations and the probability of obtaining a larger variance component than the observed value by chance (*i.e.*, P value) was obtained for all three sources of variance. The mean genetic distances among and within the three groups were calculated in MEGA 5.0 [271] using the same substitution model.

Both Bayesian and maximum likelihood (ML) inferences were used to reconstruct the within-species phylogeny of *L. australis* using the 16S dataset. Identical haplotypes were removed from the alignment, leaving only unique haplotypes in the dataset.

The Bayesian analysis was performed using MrBayes 3.2.0 [272]. The best-fit model selected by JModelTest using BIC was HKY+G [273]. However, the next best model TPM3uf+G [274] had a very similar log likelihood score. We therefore chose not to set up a prior substitution model, but to use the "mixed" model (+G) implemented in MrBayes 3.2.0, which allows MrBayes to move across different substation schemes as part of its MCMC sampling to account for uncertainty concerning the correct substitution model [275]. We also did the same analysis only using the HKY+G model, so the results can be compared. The Markov chain Monte Carlo (MCMC) was run for two million iterations with trees sampled every 1000 interactions. Two cold and two heated chains were used in each run and two independent runs were performed. The cumulative split frequencies were confirmed to be below 0.01. All parameters were examined in Tracer v1.5 [276] to ensure convergence and proper mixing. The first 500 trees were discarded as burn-in and a 50% majority consensus tree was obtained. The maximum likelihood analyses were conducted with 100 bootstrap replicates using the RAxML (Randomized Axelerated Maximum Likelihood) 7.2.8 [277] online serves hosted at the T-Rex (Tree and Reticulogram Reconstruction, [278]) web server (www.trex.uqam.ca.). The best scoring tree was selected to represent the phylogeny.

Because the ITS2 dataset includes very few phylogenetically informative sites (4/453 bp), we constructed a haplotype network to visually represent the population genetic structure of *L. australis*. The ITS2 sequences of three individuals (out of 46 individuals) genotyped for this marker exhibited heterozygotic profiles in certain sites, which suggest intragenomic variation for the ITS2 gene. It is known that ribosomal genes can have hundreds of copies within a genome (Snchez& Dorado, 2008). There are cases (including marine bivalves) where multiple ITS2 haplotypes were found within one genome [279, 280]. Therefore, to avoid arbitrarily selecting one haplotype over another, all sequences were phased using PHASE 2.1.1 [281]. Note that the above step is only to separate two possible haplotypes from the heterozygotic sequences. The homozygotic sequences were also phased simply to maintain the proper haplotype frequencies. Haplotype network of all phased sequences were subsequently constructed in TCS 1.21 [213].

We used BEAST 1.7.1 [282], together with fossil record and molecular clock calibrations respectively, to estimate the divergence times among the three *L. australis* mt lineages analyzed within the available global generic mt COIII dataset [136]. Substitution models and partition scheme of the COIII gene were simultaneously selected according to BIC using the program PartitionFinder [283]. The TrN+G model [284] was selected for the first and third codon positions and the HKY+G model was

selected for the second codon position.

The marine bivalve fossil record [285] indicates that the divergence time between *Lasaea* and its closely related genus Kellia (used as an outgroup) is approximately ≥51.9 Mya. We therefore applied this calibration point by offsetting the root height of the mt COIII phylogeny by 51.9 Myr [prior distribution=lognormal, mean=3, Log (Stdev)=0.75]. We assumed an uncorrelated lognormal distribution for the molecular clock and the Yule process for the speciation model. Codon partition and substitution models were set according to the scheme selected by PartitionFinder. Default prior distributions were used for other parameters. Two independent MCMC analyses were run for 10 million iterations respectively and sampled every 1000 iterations. Convergence diagnostics were conducted in Tracer v1.5 [276] and reliable ESS values (>200) were ensured. Trees from the two MCMC runs were combined using LogCombiner [282] with the first 1000 trees discarded as burn-in respectively; the maximum credibility tree was generated from the combined trees in TreeAnnotater [282].

There are a number of well-known problems with the fossil calibration approach that may also apply to this study, such as inaccurate fossil record and rate heterogeneity across the phylogeny, etc. To compensate for these uncertainties, we also performed the same analysis using a calibrated molecular clock method. There are currently no available calibrations for the mollusk COIII gene and we applied available calibrations for the mt COI gene of ark clam species [286]. Marko (2002) [286] estimated the sequence divergence rate for COI in three partitions: 1st + 2nd codon positions, 3rd position only and all sites. Because mt COIII allows relatively more amino acid substitutions among closely related species than mt COI gene [134], it exhibits a higher rate of 1st and 2nd codon position changes. We therefore applied two dating strategies: the ark clam mt COI overall divergence rate to the whole *Lasaea* mt COIII dataset; the ark clam COI 3rd codon position rate to a *Lasaea* COIII 3rd codon positions only dataset. Detailed prior settings for the molecular clocks are shown in Table 4.1. Note that sequence divergence rate = substitution rate × 2 [287]. Thus the mean substitution rates defined in our analyses were obtained by dividing the mean sequence divergence rates in Marko (2002) [286] by two. Also, Marko (2002) [286] used three calibration points to calculate the divergence rate respectively for each partition; here we take the mean of the three rates to represent the sequence divergence rate of each partition scheme.

Table 4.1: Two molecular clock calibrations used to estimate the divergence time of the three *Lasaea australis* clades. All units are per site per Myr.

Molecular clock	Sequence divergence rates from Marko (2002) [286]	Substitution rate specified in BEAST, prior distribution = normal
COI third position	Mean = 5% SD = 1.3%	Mean = 2.5% SD = 0.7%
COI all sites	Mean = 1% SD = 0.2%	Mean = 0.5% SD = 0.1%

4.3 Results

4.3.1 Analysis of mt 16S molecular variance

The 107 *Lasaea australis* individuals genotyped for the mt 16S marker yielded 44 unique haplotypes: 11 Peronian, 23 Maugean and 10 Flindersian. No two provinces shared the same haplotype. Results from the hierarchical AMOVA are shown in Table 4.2. Among-province variance accounts for the overwhelming majority (94%) of total genetic variation across the species' range. A modest amount of within-population heterogeneity was also detected, but very little phylogenetic structure was found among populations within the same province. The mean genetic distances (substitutions per site) among the three groups are: 10.3% (Peronian/Maugean), 11.4% (Peronian/Flindersian) and 9.9% (Maugean/Flindersian). And the mean distances within the groups are: 0.4%(Peronian), 0.9%(Maugean) and 0.2% (Flindersia).

Table 4.2: Results of the hierarchical AMOVA for the *Lasaea australis* mt 16S gene. Note that slightly negative variance components are usually considered to be statistical artifacts, can occur when the true value is zero and are generally viewed as indicating a lack of genetic structuring [7].

Source of variation	d.f.	Variance components	% Total variation	Φ	P
Among provinces	2	12.45	94.08	0.94	<0.01
Among populations within provinces	4	-0.02	-0.21	-0.03	0.96
Within populations	98	0.81	6.12	0.94	<0.01

4.3.2 mt 16S phylogeny

Results for the 16S phylogeny are shown in Fig. 4.3a. Both Bayesian and ML analyses yielded congruent topologies. Bayesian analyses using the "mixed" model

and the HKY+G model both showed the same topology. The Baysian consensus tree is shown here (see supplementary Fig. 4.5 for the complete phylograms). Three well-supported clades are observed, each corresponding to one of the biogeographic provinces. *L. australis* individuals from each province form their own monophyletic groups, except for one Maugean clam (Jan Juc Beach origin, Fig. 4.3a, asterisk), whose haplotype placed unambiguously in the Flindersian clade. The Maugean and the Flindersian clades are derived sister lineages in this phylogeny, but the ML bootstrap value (63%) for this node is relatively low, which indicates a possibility of polytomy. No well-supported subclade structuring was recovered within the topology of any of the three provincial clades.

4.3.3 ITS2 halpotype network

A total of 46 individuals was successfully amplified for the ITS2 gene fragment. Because three individuals from the Flindersian province produced heterogeneous sequences, the whole dataset was phased to separate these haplotypes. Ninety-two haplotypes were obtained as a result and 7 unique haplotypes are present in the populations.

Three most common haplotypes were detected (Fig. 4.3b, P, F and FM). Haplotype P is shared exclusively among the 14 Peronian individuals typed for this marker. Haplotype F is shared by 7 Flindersian individuals. Haplotype FM, however, is shared by all 13 Maugean individuals and 10 Flindersian individuals (including 2 heterozygous individuals). Of those, 10 Flindersian individuals, 9 were sampled from Port Lincoln (Eyre Peninsula), a location near the boundary of Maugea and Flindersia (Fig. 4.3, arrow) and one individual was from a more distant location: Guilderton, on the Indian Ocean coast of Western Australia (Fig. 4.1, location 8). Four rare haplotypes were present in the Flindersian populations (F1-F4), three of which were present in heterozygous condition. Haplotype F1 occurred in two heterozygous individuals that also had the common haplotype FM. Haplotypes F2 and F3 were recovered from a third heterozygotic Flindersian individual. Finally, haplotype F4 was from one homozygous Flindersian individual.

4.3.4 Divergence time estimation

Fig. 4.4 shows a global *Lasaea* phylogeny based on mt COIII gene sequence variation with a fossil calibration. The topology is congruent with that previously published by Taylor & Ó Foighil (2000) [136] except that *L. colmani* is now sister to

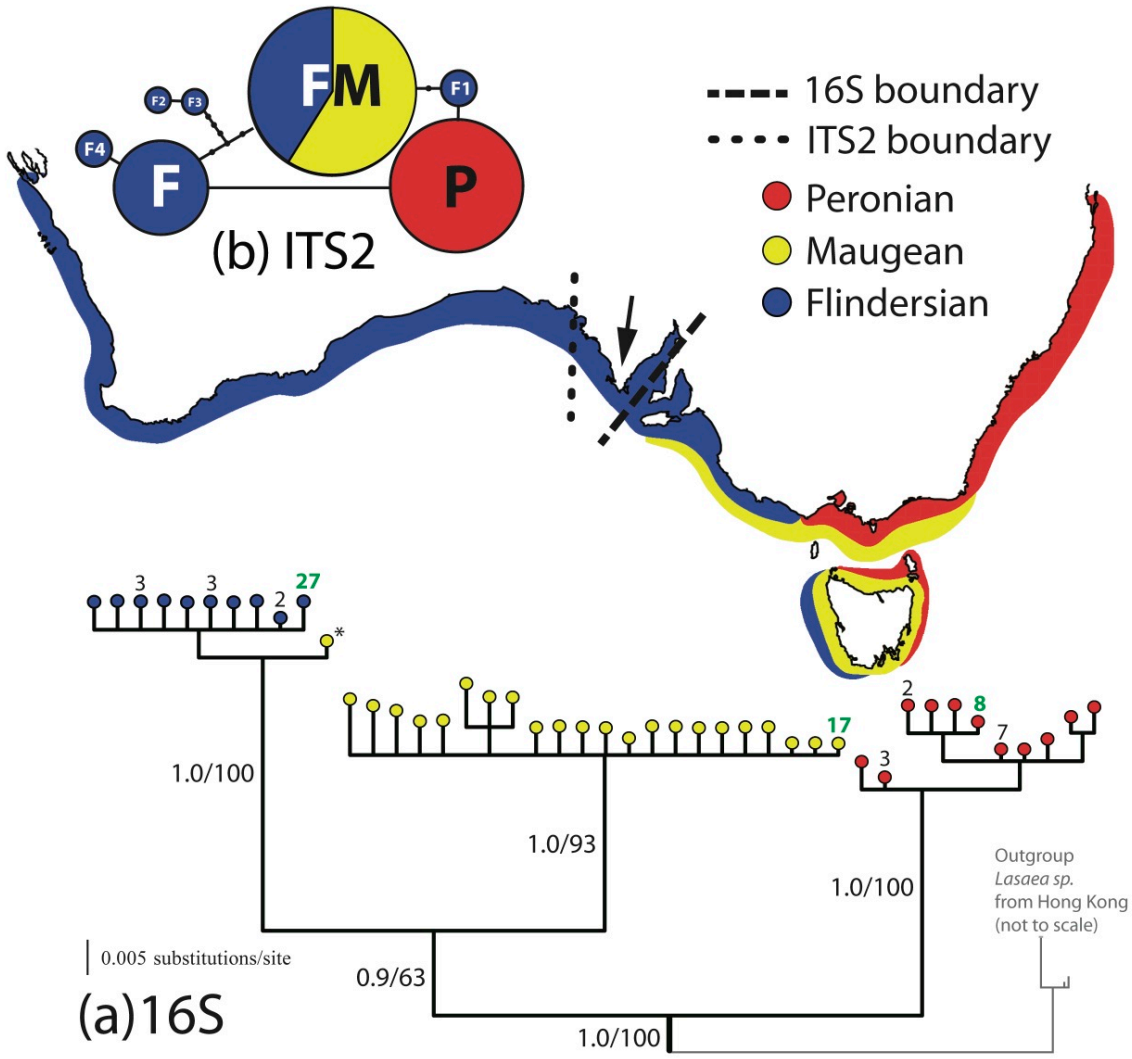

Figure 4.3: (a) Bayesian mt 16S phylogeny of *L. australis*. Clams haplotypes are colour-coded according to their biogeographic provinces of origin. Haplotype frequencies (N >1) were indicated by the accompanying terminal number. Branch labels represent Bayesian posterior probabilities and ML bootstrap values respectively. An ectopic Flindersian clade haplotype, recovered from a Maugean clam, is indicated by an asterisk. (b) ITS2 haplotype network. Each circle represents one unique haplotype. The size of each circle is proportional to numbers of that unique haplotype in the population and haplotypes are colour-coded according biogeographic province. Each black dot represents one inferred base pair change. The arrow on the map points out Port Lincoln on the Eyre Peninsula, where most clams shared the same haplotype as Maugean individuals. The inferred geographic boundaries between the Maugean and Flindersian lineages based on 16S (heavy dashed line) and ITS2 (light dashed line) are shown on the map respectively.

another (undescribed and newly sequenced) Australian direct developer (LundSdy02-03). *L. australis* COIII haplotypes formed a weakly supported derived clade within the genus and they additionally formed robust, province-specific terminal clades. Unlike the mt 16S topology (Fig. 4.3), the Peronian clade is sister to the Maugean clade here (for both fossil and COI third codon calibrations); although the support value for this relationship is quite low (0.6). However, in the phylogeny estimated using the COI all sites substitution rate (not shown), the Peronian clade is sister to the Flindersian clade with a weak support (0.4). Thus, the phylogenetic relationships among the three clades are not congruent, nor well supported, for both mt markers.

Results of the mt COIII divergence time estimations for *Lasaea australis* clades are shown in Table 4.3. Note that the fossil-calibrated nodal age estimates (Table 4.3, Fig. 4.4) are largely congruent with the mt COI-calibrated estimates for third codon positions only. Accordingly, the age estimates for the *L. australis* lineage divergence from the common ancestor of its sister direct-developing congeners are ∼17.3 Mya (fossil calibration) and ∼17.1 Mya (COI third codon calibration). The respective age estimates for the divergence of the Flindersian clade from the common ancestor of the Maugean and Peronian clades are ∼13.4 Mya and ∼13.1 Mya; ages for the Maugean/Peronian split are ∼12.0 Mya and ∼11.7 Mya. Divergence dates estimated based on the COI all sites rate are older. Estimated divergence age for the *L. australis* clade is ∼31.7 Mya; the Maugean clade diverged from the other two around ∼24.9 Mya and the estimated age for the Peronian/Flindersian split is ∼20.1 Mya. The estimated COIII substitution rates for all three calibration methods are also shown in Table 4.3.

Table 4.3: Results of the divergence-time estimates based on COIII sequences for the three *Lasaea australis* clades. Clade names are abbreviated as following: Peronia (P), Maugea (M) and Flindersia (F). Divergence time estimates for the three calibration methods used are reported, each with a 95% highest posterior density (HPD). Estimated substitution rates are also shown. Time units are in Mya and substitution rate units are per site per Myr.

Methods	F/P + M	95% HPD	P/M	95% HPD	*Lasaea australis*	95% HPD	Substitution rate	95% HPD
Fossil calibration	13.4	7.7-19.6	12.0	5.5-19.0	17.3	10.9-24.2	2.0%	1.4-2.6
COI 3rd position	13.1	6.1-22.7	11.7	4.6-20.7	17.1	8.0-28.8	2.7%	1.3-4.0
	M/P + F		P/F					
COI all	24.9	11.1-42.4	20.1	8.3-35.3	31.7	15.0-53.5	0.5%	0.3-0.7

4.4 Discussion

4.4.1 *Lasaea australis* phylogeography

The population genetic analyses and phylogenetic reconstructions based on the mitochondrial genes demonstrate unambiguously that *L. australis* is composed of three distinct clades that correspond with high fidelity to the three temperate biogeographic provinces of southern Australia. Although mean genetic distances among the provincial clades were pronounced, populations within each province showed little evidence of genetic differentiation. Within-provincial clade variation was dominated by one (Flindersia, Maugea) or two (Peronia) common haplotypes with assorted singletons (Fig. 4.3a). The pattern of mt lineage distribution observed is consistent with the presence of sharp cryptic genetic disjunctions between the provincial clades coupled with high levels of within-province connectivity.

The 16S and COIII phylogenetic analyses yielded different sister relationships among the three provincial clades and both reconstructions recovered short internodes among the three clades relative to their respective stem branches – indicating a relatively old and rapid lineage diversification process within *L. australis*. Due to the incongruent 16S and COIII topologies, we conservatively view the three provincial clades as a polytomy until more data can be brought to bear on this issue.

The nuclear gene ITS2 had much less sequence variation among the study populations compared to the mitochondrial genes. This is not surprising as nuclear genes tend to evolve slower than mitochondrial genes [288]. In one study on marine bivalves, it has been estimated that the substitution rate of ITS2 can be 10 times slower than the mitochondrial protein-coding gene COI (Faure et al., 2009). Nonetheless, the ITS2 haplotype network largely corroborated the mt genetic disjunctions among the three provinces. Most of the discrepancy between results from the nuclear gene and mitochondrial genes involved one Flindersian population from Port Lincoln (Fig. 4.3, arrow). In the mitochondrial tree (Fig. 4.3a), all 20 individuals genotyped from this location belonged to the Flindersian clade, but in the ITS2 network, all 9 genotyped Port Lincoln individuals shared the same haplotype (Fig. 4.3, FM) as the Maugean samples. Based on this nuclear marker, the genetic break between the Maugean and Flindersian lineages lies to the west of Port Lincoln; while according to the mitochondrial data, the boundary lies to the east of Port Lincoln as traditionally defined (Fig. 4.3).

Topological discordance between mitochondrial and nuclear genes is not uncommon [289]. They can result from incomplete lineage sorting, introgression, and gene

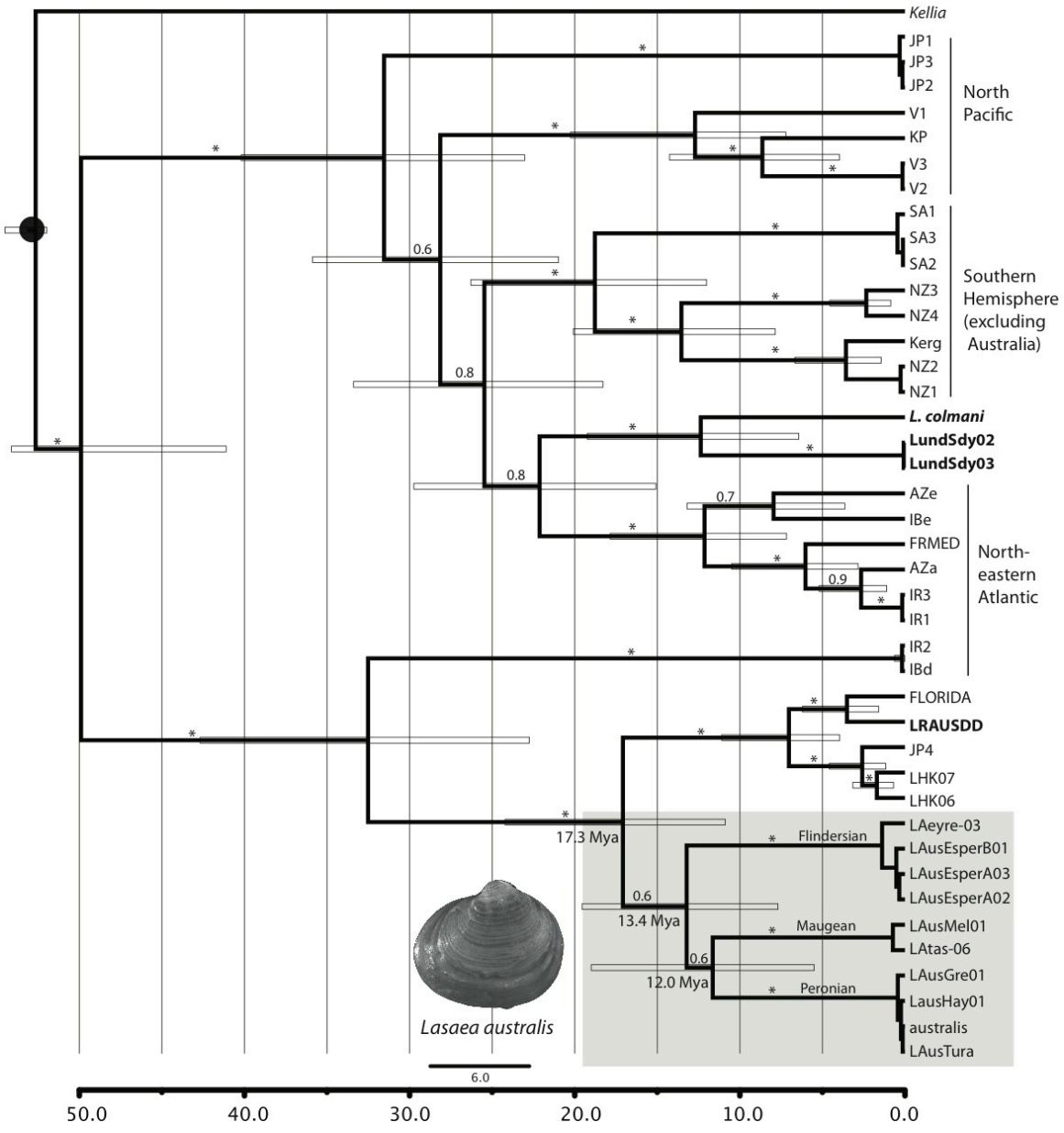

Figure 4.4: BEAST divergence time estimation of global *Lasaea* lineages using a fossil calibration. The *L. australis* clades are highlighted by grey shading and divergence time estimations are labeled under the branches. Posterior probabilities are shown as branch labels (1.0 is indicated by an asterisk). The fossil calibration point is indicated by a black circle. Bars on branch nodes represent 95% Highest Posterior Density age intervals. Name abbreviations of other *Lasaea* species see Ó Foighil & Jozefowicz (1999) [4]. Australian direct-developing *Lasaea* lineages are shown in bold. Time units are Mya. All taxa depicted are direct developers apart from *L. australis* and the outgroup *Kellia laperousi*.

duplication/extinction events [290]. Because the mitochondrial genome is maternally inherited and haploid, it has a smaller effective population size and experiences a faster coalescence time [291]. Therefore, we may observe complete lineage sorting for mitochondrial genes but not for nuclear markers [292]. And this is likely to be the case for *L. australis*. In particular, the presence of intragenomic variation for the ITS2 gene further suggests the possibility of incomplete lineage sorting or even gene introgression. Therefore, our mitochondrial phylogenies may have a higher probability of reflecting the true lineage diversification processes in *L. australis*.

Up to now, *Lasaea australis* has been considered as a single continuously distributed southern Australian species [136]. The mitochondrial phylogenies strongly indicate the possibility of three cryptic species, as the genetic distances among the clades exceed the general threshold (10 times average intraspecific difference) for new species [293]. However, we did not detect any clade-specific morphological characters that can distinguish the three lineages. Thus, we consider it best to view *L. australis* as a cryptic species complex at present – one that requires detailed ecological and genetic study, especially at biogeographic province boundaries.

4.4.2 Diversification mechanisms

Determining divergence times among the three provincial *L. australis* clades is crucial for evaluating potential diversification mechanisms. Estimates vary depending on the calibration method used (Table 4.3). Divergence date estimations using two independent calibration methods, fossil record and the ark clam COI third codon position rate [286], yielded highly consistent results that the divergence time among the three *L. australis* lineages are around 11-14 Mya. Analysis based on the COI all sites substitution rate yielded much older dates (20-24 Mya). However, because the average substitution rate of the COIII gene is higher than the COI gene [134], our molecular clock analysis based on the COI all sites rate is very likely to overestimate the divergence times. In contrast, similar substitution rates can be more reasonably assumed for third codon positions (least exposed to purifying selection) of both mt genes. Thus, our estimation based on the COI third codon position rate is more likely to approach a realistic time range for *L. australis* provincial clade diversification, especially that the results concur with the fossil calibrations.

The divergence times based on the fossil calibration and the COI third codon position rate both date back to the Mid to Late Miocene, a timeframe that is incongruent with some of the hypotheses proposed to explain the genesis of the three biogeographic provinces. This includes the historical barrier hypothesis invoking Pleistocene glacial

maxima vicariance events as diversification drivers (Dartnall, 1974; Waters et al., 2004; Dawson, 2005; Waters et al., 2005). Conservatively, even if considering the earliest age estimates obtained within the 95% highest posterior density (HPD), these Pliocene divergence times, ∼4.6 & 6.1 Mya (Table 4.3), still predate the onset of the Northern Hemisphere Glaciation (∼2.75 Mya, Ravelo et al., 2004) and the emergence of the Bass Strait land bridge. Our divergence estimates also greatly pre-date the proposed dispersal barrier at Ninety Mile Beach area, an extended sandy shore along the northeast coast of Victoria that emerged fairly recently (<6000 years ago) after the post-glacial submergence of the East Gippsland coast [294].

The near shore current systems around southern Australia (Fig. 4.2) are known to affect species dispersal and distributions [238,258,259] and the main current dynamics were established during Miocene [295]. However, among-provinces dispersal of the clams are unlikely to be hindered by the currents because they touch on multiple provinces: the East Australia Current flows southwards into Peronia but can also enter Maugea as far as Tasmania [259] and the Leeuwin Current, South Australian Current and the Zeehan Current collaboratively connect Flindersia and Maugea. In addition, the strength of each current varies seasonally and is affected by relatively complicated local up/down-welling events [259,261,263]. *L. australis* has an irregular spawning pattern with peak summer and autumn recruitment phases, at least in Western Australia [265]. Thus, larvae could persist in the water column through much of the year and, larval dispersal is highly unlikely to be restricted to individual biogeographic provinces.

Despite rejecting several hypotheses, one important ecological factor, the sea surface temperature (SST) gradient, remains a plausible driver for the *L. australis* diversification, especially taking into account paleoclimate condition in southern Australia. At present, Maugea shows a significantly cooler SST than the other two provinces. An abrupt SST gradient (>3°C, [2]) occurs at provincial boundaries, and is associated with a significant turnover in species richness and composition [247]. However, the steep SST gradient was not formed until the Middle Miocene Climate Transition (MMCT, 14.2 -13.8 Mya), which marked one of the major steps in Cenozoic climate evolution [296, 297]. Prior to the MMCT, southern Australian waters during the Early Miocene warm phase and the Miocene Climatic Optimum (MCO, 17 to 14 Myr) exhibited a much warmer and more uniform temperature regime [295]. The MCO ended with a rapid climate transition characterized by major growth of the East Antarctic ice sheets, Antarctic cooling and intensification of Southern Ocean circulation [296–298]. The meridional temperature gradient in southern Australia

was greatly increased and zonality was strengthened [295].

Formation of the SST gradient, and associated cooling of the Maugean region, roughly corresponds to the estimated divergence time among the *L. australis* clades (Fig. 4.4). We propose that this cooling process partitioned the coastline into two warm-temperate zones (the future Flindersia & Peronia) separated by a new cold-temperate zone (the future Maugea). We assume that the ancestral *L. australis* population had a continuous distribution along the coastline and that selective pressure associated with the formation of the cold-temperate zone promoted the evolution of a cold-adapted southern population: the present day Maugean clade. Meanwhile, the two disjunct warm-adapted eastern (Peronian) and western (Flindersian) clades started to diverge due to isolation, yielding the characteristic trident topology (Fig. 4.3). At present, the cooler waters around Tasmania may still act as invisible ecological barriers that prevent the two warmer province lineages from colonizing the Maugean region and the Maugean lineage from expanding northward. Testing the temperature boundary hypothesis would require intensive sampling across provincial boundaries and transplantation experiments among the different *L. australis* clades. This hypothesis also allows us to infer past distributions of these lineages based on the SST paleo-record (See supplementary Fig. 4.6).

From a marine biogeographic perspective, the temperate coastline of Australia is a fascinating nearshore evolutionary setting. Unlike better-studied marine faunal transition points, such as the Gulf-Atlantic disjunction in peninsular Florida [15,232], it contains not one, but two sharp genetic breaks, associated with three biogeographic provinces. The challenge to biogeographers is to provide a plausible general mechanism that explains the formation of these three geographically-proximate distinct provinces along a contiguous continental coastline.

The emergence of the Bass Strait land bridge has been frequently proposed to explain the existence of an "Eastern" and a "Western" clade in various marine invertebrates, such as cnidarians [249, 252], cuttlefish [250], gastropods [255, 299, 300], sea stars [251, 301] and barnacles [253]. However, many of the studies only focused on one or two of the provinces [249, 252, 255, 299]. Thus, the "two" distinct clades could potentially represent a Peronian/Maugean, a Peronian/Flindersian or a Maugean/Flindersian disjunction. Studies that sampled across the entire coastline have typically found three provincial clades that form similar phylogenetic topologies to that of *L. australis* [250, 251, 301]. Although the Bass land bridge may well be responsible for the allopatric diversifications of some regional marine organisms, that particular vicariant process can only produce geminate clades. It is not sufficient to

explain a rapid formation of three distinct lineages in some taxa and the community level differentiation of three biogeographic provinces. In addition, some of the estimated divergence dates predate the emergence of the land bridge (*i.e.*, 5-6 Mya for the snail Nerita [255]; 7-10 Mya for cirrhitod fishes [254]; up to ∼36 Mya for certain limpets in the genus *Siphonaria* [302]). Waters *et al.* (2004) [301] suggested that those deeply divergent lineages could be the result of glacial isolations in central coastal regions, but no supporting evidence has been provided.

4.5 Conclusion

Our case study of *L. australis* highlights the fact that the SST gradient in the southern Australia is formed during the Miocene Climate Transition and that its interaction with the unique geometry of the coastline (the southern protruding landmasses of Victoria and Tasmania) made it possible to have one southern "cold" province separating two northern "warm" provinces. This unique geographical/ecological interaction could potentially be the primary long-term driver for marine fauna diversification in southern Australia, ultimately resulting in the well-documented province-specific community compositions on this coastline [2, 245, 247, 248, 303]. If this hypothesis is correct, we predict that biota-wide endemic radiations in southern Australia marine fauna would frequently be characterized by a trident-like phylogeny, where three distinct lineages diverged within a relatively short period of time. Divergence times among the lineages may be taxon-specific, depending on when the respective lineages became established in southern Australia. We recommend that future studies focusing on marine diversification processes in southern Australia target all three provinces if possible and adopt model-based divergence time estimations to effectively test competing hypotheses. We also call attention to the possibility that contemporary ecological factors (*e.g.*, SST) may sometimes stem from paleoclimatic processes and that their influence on lineage diversification can be long-term.

4.6 Supplementary Materials

4.6.1 Detailed specimen collecting information and GenBank accession numbers

Specimen ID	16S Haplotype ID	ITS Haplotype	Location	Province	Year collected	Collector	16S	ITS2	COIII	Vocher number
LAeyre-01	LAusEsperA02		Port Lincoln, Eyre Peninsula, SA	Flindersian	1999	Maria Byrne	x			UMMZ303932.1
LAeyre-02	LAusEsperA02		Port Lincoln, Eyre Peninsula, SA	Flindersian	1999	Maria Byrne	x			UMMZ303932.2
LAeyre-03	LAusEsperA02		Port Lincoln, Eyre Peninsula, SA	Flindersian	1999	Maria Byrne	x		JX910460	UMMZ303932.3
LAeyre-04	LAeyre3		Port Lincoln, Eyre Peninsula, SA	Flindersian	1999	Maria Byrne	JX910475			UMMZ303932.4
LAeyre-05	LAeyre2		Port Lincoln, Eyre Peninsula, SA	Flindersian	1999	Maria Byrne	JX910473			UMMZ303932.5
LAeyre-06	LAusEsperA03		Port Lincoln, Eyre Peninsula, SA	Flindersian	1999	Maria Byrne	x			UMMZ303932.6
LAeyre-07	LAusEsperA02		Port Lincoln, Eyre Peninsula, SA	Flindersian	1999	Maria Byrne	x			UMMZ303932.7
LAeyre-08	LAusEsperA02		Port Lincoln, Eyre Peninsula, SA	Flindersian	1999	Maria Byrne	x			UMMZ303932.8
LAeyre-09	LAusEsperA02		Port Lincoln, Eyre Peninsula, SA	Flindersian	1999	Maria Byrne	x			UMMZ303932.9
LAeyre-10	LAusEsperA02		Port Lincoln, Eyre Peninsula, SA	Flindersian	1999	Maria Byrne	x			UMMZ303932.10
LAeyre-13	LAeyre5		Port Lincoln, Eyre Peninsula, SA	Flindersian	1999	Maria Byrne	JX910476			UMMZ303932.11
LAeyre-14	LAeyre3		Port Lincoln, Eyre Peninsula, SA	Flindersian	1999	Maria Byrne	x			UMMZ303932.12
LAeyre-15	LAusEsperA02		Port Lincoln, Eyre Peninsula, SA	Flindersian	1999	Maria Byrne	x			UMMZ303932.13
LAeyre-16	LAusEsperA02		Port Lincoln, Eyre Peninsula, SA	Flindersian	1999	Maria Byrne	x			UMMZ303932.14
LAeyre-17	LAusEsperA02		Port Lincoln, Eyre Peninsula, SA	Flindersian	1999	Maria Byrne	x			UMMZ303932.15
LAeyre-20	LAusEsperB01		Port Lincoln, Eyre Peninsula, SA	Flindersian	1999	Maria Byrne	JX910480			UMMZ303932.16
LAeyre-21	LAusEsperA02		Port Lincoln, Eyre Peninsula, SA	Flindersian	1999	Maria Byrne	x			UMMZ303932.17
LAeyre-22	LAusEsperA02		Port Lincoln, Eyre Peninsula, SA	Flindersian	1999	Maria Byrne	x			UMMZ303932.18
LAeyre-23	LAusEsperA02		Port Lincoln, Eyre Peninsula, SA	Flindersian	1999	Maria Byrne	x			UMMZ303932.19
LAeyre-25	LAusEsperA02		Port Lincoln, Eyre Peninsula, SA	Flindersian	1999	Maria Byrne	x			UMMZ303932.20
LAusEsperA02	LAusEsperA02		Esperance, WA	Flindersian	2003	John Tayler, Emily Glover	JX910474		JX910457	UMMZ303933.1
LAusEsperA03	LAusEsperA03	F	Esperance, WA	Flindersian	2003	John Tayler, Emily Glover	JX910477	JX910468	JX910458	UMMZ303933.2
LAusEsperA04	LAusEsperA02	F	Esperance, WA	Flindersian	2003	John Tayler, Emily Glover	x	x		UMMZ303933.3
LAusEsperA05	LAusEsperA05		Esperance, WA	Flindersian	2003	John Tayler, Emily Glover	JX910478			UMMZ303933.4
LAusEsperA06	LAusEsperA02		Esperance, WA	Flindersian	2003	John Tayler, Emily Glover	x			UMMZ303933.5
LAusEsperA07	LAusEsperA02		Esperance, WA	Flindersian	2003	John Tayler, Emily Glover	x			UMMZ303933.6
LAusEsperA08	LAusEsperA02		Esperance, WA	Flindersian	2003	John Tayler, Emily Glover	x			UMMZ303933.7
LAusEsperA09	LAusEsperA03		Esperance, WA	Flindersian	2003	John Tayler, Emily Glover	x			UMMZ303933.8
LAusEsperA10	LAusEsperA10		Esperance, WA	Flindersian	2003	John Tayler, Emily Glover	JX910479			UMMZ303933.9
LAusEsperB01	LAusEsperB01	F4	Esperance, WA	Flindersian	2003	John Tayler, Emily Glover	x	JX910469	JX910459	UMMZ303933.10
LAusEsperB02	LAusEsperA02		Esperance, WA	Flindersian	2003	John Tayler, Emily Glover	x			UMMZ303933.11
LAusEsperB03	LAusEsperA02	F	Esperance, WA	Flindersian	2003	John Tayler, Emily Glover	x	x		UMMZ303933.12
LAusEsperB04	LAusEsperA02		Esperance, WA	Flindersian	2003	John Tayler, Emily Glover	x			UMMZ303933.13
LAusEsperB05	LAusEsperB05		Esperance, WA	Flindersian	2003	John Tayler, Emily Glover	JX910481			UMMZ303933.14
LAusEsperB06	LAusEsperA02	F2 and F3	Esperance, WA	Flindersian	2003	John Tayler, Emily Glover	x	JX910470		UMMZ303933.15
LAusEsperB07	LAusEsperA02	F	Esperance, WA	Flindersian	2003	John Tayler, Emily Glover	x	x		UMMZ303933.16
LAusEsperB08	LAusEsperA02		Esperance, WA	Flindersian	2003	John Tayler, Emily Glover	x			UMMZ303933.17
LAusEsperB09	LAusEsperB01		Esperance, WA	Flindersian	2003	John Tayler, Emily Glover	x			UMMZ303933.18
LAusEsperB10	LAusEsperB10		Esperance, WA	Flindersian	2003	John Tayler, Emily Glover	JX910482			UMMZ303933.19
LAusEyr01	N/A	FM	Port Lincoln, Eyre Peninsula, SA	Flindersian	1999	Maria Byrne		x		UMMZ303932.21
LAusEyr02	N/A	FM	Port Lincoln, Eyre Peninsula, SA	Flindersian	1999	Maria Byrne		x		UMMZ303932.22
LAusEyr04	N/A	FM	Port Lincoln, Eyre Peninsula, SA	Flindersian	1999	Maria Byrne		x		UMMZ303932.24
LAusEyr05	N/A	FM	Port Lincoln, Eyre Peninsula, SA	Flindersian	1999	Maria Byrne		x		UMMZ303932.25
LAusEyr06	N/A	FM	Port Lincoln, Eyre Peninsula, SA	Flindersian	1999	Maria Byrne		x		UMMZ303932.26
LAusEyr07	N/A	FM	Port Lincoln, Eyre Peninsula, SA	Flindersian	1999	Maria Byrne		x		UMMZ303932.27
LAusEyr08	N/A	F1 and FM	Port Lincoln, Eyre Peninsula, SA	Flindersian	1999	Maria Byrne		JX910471		UMMZ303932.28
LAusEyr10	N/A	FM	Port Lincoln, Eyre Peninsula, SA	Flindersian	1999	Maria Byrne		x		UMMZ303932.29
LAusEyr11	N/A	F1 and FM	Port Lincoln, Eyre Peninsula, SA	Flindersian	1999	Maria Byrne		x		UMMZ303932.30
LAusGui01	N/A	FM	Guilderton, WA	Flindersian	2010	Diarmaid O'Foighil		x		WAMS34689, Western Australia Museum
LAusGui02	N/A	F	Guilderton, WA	Flindersian	2010	Diarmaid O'Foighil		x		WAMS34689, Western Australia Museum
LAusGui03	LAusEsperA02	F	Guilderton, WA	Flindersian	2010	Diarmaid O'Foighil	x	x		WAMS34689, Western Australia Museum
LAusGui04	LAusEsperA02	F	Guilderton, WA	Flindersian	2010	Diarmaid O'Foighil	x	x		WAMS34689, Western Australia Museum
C468111	N/A	FM	Bridport, TAS	Maugean	2003	Don Colgan		JX910467		C.468111.001, The Australian Museum
C468113	N/A	FM	Midway Point, TAS	Maugean	2007	Don Colgan		x		C.468113.001, The Australian Museum
C468119	N/A	FM	Bicheno, TAS	Maugean	2003	Don Colgan		x		C.468119.001, The Australian Museum
C468120	N/A	FM	Avalon, TAS	Maugean	2007	Don Colgan		x		C.468120.001, The Australian Museum
LAtas-01	LAtas2		Eaglehawk neck, TAS	Maugean	1996	Maria Byrne	JX910498			UMMZ303931.1
LAtas-02	LAtas5		Eaglehawk neck, TAS	Maugean	1996	Maria Byrne	JX910488			UMMZ303931.2
LAtas-03	LAtas3		Eaglehawk neck, TAS	Maugean	1996	Maria Byrne	JX910487			UMMZ303931.3
LAtas-04	LAtas1		Eaglehawk neck, TAS	Maugean	1996	Maria Byrne	JX910484			UMMZ303931.4
LAtas-06	LAtas1		Eaglehawk neck, TAS	Maugean	1996	Maria Byrne	x		JX910456	UMMZ303931.5
LAtas-07	LAtas1		Eaglehawk neck, TAS	Maugean	1996	Maria Byrne	x			UMMZ303931.6
LAtas-08	LAtas1		Eaglehawk neck, TAS	Maugean	1996	Maria Byrne	x			UMMZ303931.7
LAtas-09	LAtas4		Eaglehawk neck, TAS	Maugean	1996	Maria Byrne	JX910505			UMMZ303931.8
LAtas-11	LAtas1		Eaglehawk neck, TAS	Maugean	1996	Maria Byrne	x			UMMZ303931.9
LAtas-12	LAtas6		Eaglehawk neck, TAS	Maugean	1996	Maria Byrne	JX910501			UMMZ303931.10
LAtas-13	LAtas7		Eaglehawk neck, TAS	Maugean	1996	Maria Byrne	JX910485			UMMZ303931.11
LAtas-14	LAtas1		Eaglehawk neck, TAS	Maugean	1996	Maria Byrne	x			UMMZ303931.12
LAtas-17	LAtas1		Eaglehawk neck, TAS	Maugean	1996	Maria Byrne	x			UMMZ303931.13
LAtas-19	LAtas1		Eaglehawk neck, TAS	Maugean	1996	Maria Byrne	x			UMMZ303931.14
LAtas-20	LAtas8		Eaglehawk neck, TAS	Maugean	1996	Maria Byrne	JX910489			UMMZ303931.15
LAtas-21	LAtas9		Eaglehawk neck, TAS	Maugean	1996	Maria Byrne	JX910500			UMMZ303931.16
LAtas-22	LAtas1		Eaglehawk neck, TAS	Maugean	1996	Maria Byrne	x			UMMZ303931.17
LAtas-23	LAtas1		Eaglehawk neck, TAS	Maugean	1996	Maria Byrne	x			UMMZ303931.18
LAusMel01	N/A	FM	Jan Juc Beach, VIC	Maugean	2010	Diarmaid O'Foighil		x	JX910455	UMMZ303930.1
LAusMel02	LAusMel02	FM	Jan Juc Beach, VIC	Maugean	2010	Diarmaid O'Foighil	JX910490	x		UMMZ303930.2
LAusMel03	LAusMel03	FM	Jan Juc Beach, VIC	Maugean	2010	Diarmaid O'Foighil	JX910491	x		UMMZ303930.3
LAusMel04	LAusMel04	FM	Jan Juc Beach, VIC	Maugean	2010	Diarmaid O'Foighil	JX910492	x		UMMZ303930.4
LAusMel05	LAusMel05	FM	Jan Juc Beach, VIC	Maugean	2010	Diarmaid O'Foighil	JX910493	x		UMMZ303930.5
LAusMel06	LAusMel06	FM	Jan Juc Beach, VIC	Maugean	2010	Diarmaid O'Foighil	JX910483	x		UMMZ303930.6
LAusMel07	LAusMel07	FM	Jan Juc Beach, VIC	Maugean	2010	Diarmaid O'Foighil	JX910499	x		UMMZ303930.7
LAusMel08	LAusMel08	FM	Jan Juc Beach, VIC	Maugean	2010	Diarmaid O'Foighil	JX910494	x		UMMZ303930.8
LAusMel09	LAtas1		Jan Juc Beach, VIC	Maugean	2010	Diarmaid O'Foighil	x			UMMZ303930.9
LAusMel10	LAusMel10	FM	Jan Juc Beach, VIC	Maugean	2010	Diarmaid O'Foighil	x	x		UMMZ303930.10
LAusMel11	LAtas1		Jan Juc Beach, VIC	Maugean	2010	Diarmaid O'Foighil	x			UMMZ303930.11
LAusMel12	LAusMel12		Jan Juc Beach, VIC	Maugean	2010	Diarmaid O'Foighil	JX910495			UMMZ303930.12
LAusMel13	LAtas1		Jan Juc Beach, VIC	Maugean	2010	Diarmaid O'Foighil	x			UMMZ303930.13
LAusMel14	LAusMel14		Jan Juc Beach, VIC	Maugean	2010	Diarmaid O'Foighil	JX910504			UMMZ303930.14
LAusMel15	LAusMel15		Jan Juc Beach, VIC	Maugean	2010	Diarmaid O'Foighil	JX910503			UMMZ303930.15
LAusMel16	LAusMel16		Jan Juc Beach, VIC	Maugean	2010	Diarmaid O'Foighil	JX910502			UMMZ303930.16
LAusMel17	LAtas1		Jan Juc Beach, VIC	Maugean	2010	Diarmaid O'Foighil	x			UMMZ303930.17
LAusMel18	LAusMel18		Jan Juc Beach, VIC	Maugean	2010	Diarmaid O'Foighil	JX910486			UMMZ303930.18
LAusMel19	LAusMel19		Jan Juc Beach, VIC	Maugean	2010	Diarmaid O'Foighil	JX910496			UMMZ303930.19
LAusMel20	LAtas1		Jan Juc Beach, VIC	Maugean	2010	Diarmaid O'Foighil	JX910497			UMMZ303930.20
LAusMel21	LAtas1		Jan Juc Beach, VIC	Maugean	2010	Diarmaid O'Foighil	x			UMMZ303930.21
LAusTas01	LAtas1		Eaglehawk neck, TAS	Maugean	1996	Maria Byrne	x			UMMZ303931.19
LHK01	LHK01		Shek O, Hong Kong	N/A	2010	Jingchun Li	JX910519			UMMZ303937.1
LHK02	LHK02		Shek O, Hong Kong	N/A	2010	Jingchun Li	JX910520			UMMZ303937.2
LHK03	LHK03		Shek O, Hong Kong	N/A	2010	Jingchun Li	JX910524			UMMZ303937.3
LHK04	LHK04		Shek O, Hong Kong	N/A	2010	Jingchun Li	JX910517			UMMZ303937.4
LHK05	LHK05		Shek O, Hong Kong	N/A	2010	Jingchun Li	JX910523			UMMZ303937.5
LHK06	LHK06		Shek O, Hong Kong	N/A	2010	Jingchun Li	JX910518		JX910454	UMMZ303937.6
LHK07	LHK07		Shek O, Hong Kong	N/A	2010	Jingchun Li	JX910521		JX910453	UMMZ303937.7
LHK08	LHK08		Shek O, Hong Kong	N/A	2010	Jingchun Li	JX910522			UMMZ303937.8
Lasaea colmani	N/A		Sydney, NSW	Peronian	1998	Diarmaid O'Foighil			JX910466	UMMZ303935.1
LAsyd-02	LAsyd1		Sydney, NSW	Peronian	1998	Diarmaid O'Foighil	x			UMMZ303929.1
LAsyd-03	LAsyd2		Sydney, NSW	Peronian	1998	Diarmaid O'Foighil	JX910510			UMMZ303929.2
LAsyd-12	LAsyd1		Sydney, NSW	Peronian	1998	Diarmaid O'Foighil	AF215774			UMMZ303929.3
LAsyd-15	LAsyd3		Sydney, NSW	Peronian	1998	Diarmaid O'Foighil	JX910508			UMMZ303929.4
LAsyd-16	LAsyd4		Sydney, NSW	Peronian	1998	Diarmaid O'Foighil	JX910511			UMMZ303929.5
LAsyd-18	LAsyd2		Sydney, NSW	Peronian	1998	Diarmaid O'Foighil	x			UMMZ303929.6
LAsyd-21	LAsyd6		Sydney, NSW	Peronian	1998	Diarmaid O'Foighil	JX910512			UMMZ303929.7
LAsyd-22	LAsyd5		Sydney, NSW	Peronian	1998	Diarmaid O'Foighil	JX910515			UMMZ303929.8
LAsyd-37	LAsyd7		Sydney, NSW	Peronian	1998	Diarmaid O'Foighil	JX910506			UMMZ303929.9
LAsyd-38	LAsyd2		Sydney, NSW	Peronian	1998	Diarmaid O'Foighil	x			UMMZ303929.10
LAsyd-39	LAsyd1		Sydney, NSW	Peronian	1998	Diarmaid O'Foighil	x			UMMZ303929.11
LAsyd-40	LAsyd8		Sydney, NSW	Peronian	1998	Diarmaid O'Foighil	JX910516			UMMZ303929.12
LAsyd-41	LAsyd8		Sydney, NSW	Peronian	1998	Diarmaid O'Foighil	x			UMMZ303929.13
LAsyd-42	LAsyd1		Sydney, NSW	Peronian	1998	Diarmaid O'Foighil	x			UMMZ303929.14
LAsyd-48	LAsyd2		Sydney, NSW	Peronian	1998	Diarmaid O'Foighil	x			N/A
LAsyd-49	LAsyd6		Sydney, NSW	Peronian	1998	Diarmaid O'Foighil	x			N/A
LAsyd-50	LAsyd6		Sydney, NSW	Peronian	1998	Diarmaid O'Foighil	x			N/A
LAsyd-54	LAsyd2		Sydney, NSW	Peronian	1998	Diarmaid O'Foighil	x			N/A
LAusGre01	N/A		Green Cape, NSW	Peronian	2009	Don Colgan, Peter Middelfart			JX910463	C.468118.001, The Australian Museum
LAusGre02	LAsyd2	P	Green Cape, NSW	Peronian	2009	Don Colgan, Peter Middelfart	x	JX910472		C.468118.001, The Australian Museum
LAusGre03	LAsyd3	P	Green Cape, NSW	Peronian	2009	Don Colgan, Peter Middelfart	x	x		C.468118.001, The Australian Museum

LAusGre04	LAsyd2	P	Green Cape, NSW	Peronian	2009	Don Colgan, Peter Middelfart	x	x		C.468118.001, The Australian Museum
LAusGre05	LAsyd2	P	Green Cape, NSW	Peronian	2009	Don Colgan, Peter Middelfart	x	x		C.468118.001, The Australian Museum
LAusGre06	LAusGre06	P	Green Cape, NSW	Peronian	2009	Don Colgan, Peter Middelfart	JX910509	x		C.468118.001, The Australian Museum
LAusGre07	LAsyd3	P	Green Cape, NSW	Peronian	2009	Don Colgan, Peter Middelfart	x	x		C.468118.001, The Australian Museum
LAusGre08	LAusGre08	P	Green Cape, NSW	Peronian	2009	Don Colgan, Peter Middelfart	JX910514	x		C.468118.001, The Australian Museum
LAusHay01	N/A	P	Haycock Headland, NSW	Peronian	2009	Don Colgan, Peter Middelfart		x	JX910461	C.468116.001, The Australian Museum
LAusHay02	LAusHay02	P	Haycock Headland, NSW	Peronian	2009	Don Colgan, Peter Middelfart	JX910507	x		C.468116.001, The Australian Museum
LAusTura01	N/A	P	Tura Head, NSW	Peronian	2009	Don Colgan, Peter Middelfart		x	JX910462	C.468117.001, The Australian Museum
LAusTura02	LAusTura02		Tura Head, NSW	Peronian	2009	Don Colgan, Peter Middelfart	JX910513			C.468117.001, The Australian Museum
LundSdy02	NA		Sydney, NSW	Peronian	1998	Diarmaid O'Foighil			JX910464	UMMZ303936.1
LundSdy03	NA		Sydney, NSW	Peronian	1998	Diarmaid O'Foighil			JX910465	UMMZ303936.2
Sdy1103	NA	P	Long reef, NSW	Peronian	2011	Jingchun Li		x		C.468615.001, The Australian Museum
Sdy1104	NA	P	Long reef, NSW	Peronian	2011	Jingchun Li		x		C.468615.001, The Australian Museum
Sdy1105	NA	P	Long reef, NSW	Peronian	2011	Jingchun Li		x		C.468615.001, The Australian Museum
Sdy1106	NA	P	Long reef, NSW	Peronian	2011	Jingchun Li		x		C.468615.001, The Australian Museum

NSW: New South Wales
VIC: Victoria
TAS: Tasmania
SA: South Australia
WA: Western Australia

Non-L.australis are highlighted
x indicates that the individual has been sequenced for this marker, unique haplotypes were given genbank numbers

4.6.2 Complete 16S phylogenies of *Lasaea australis*

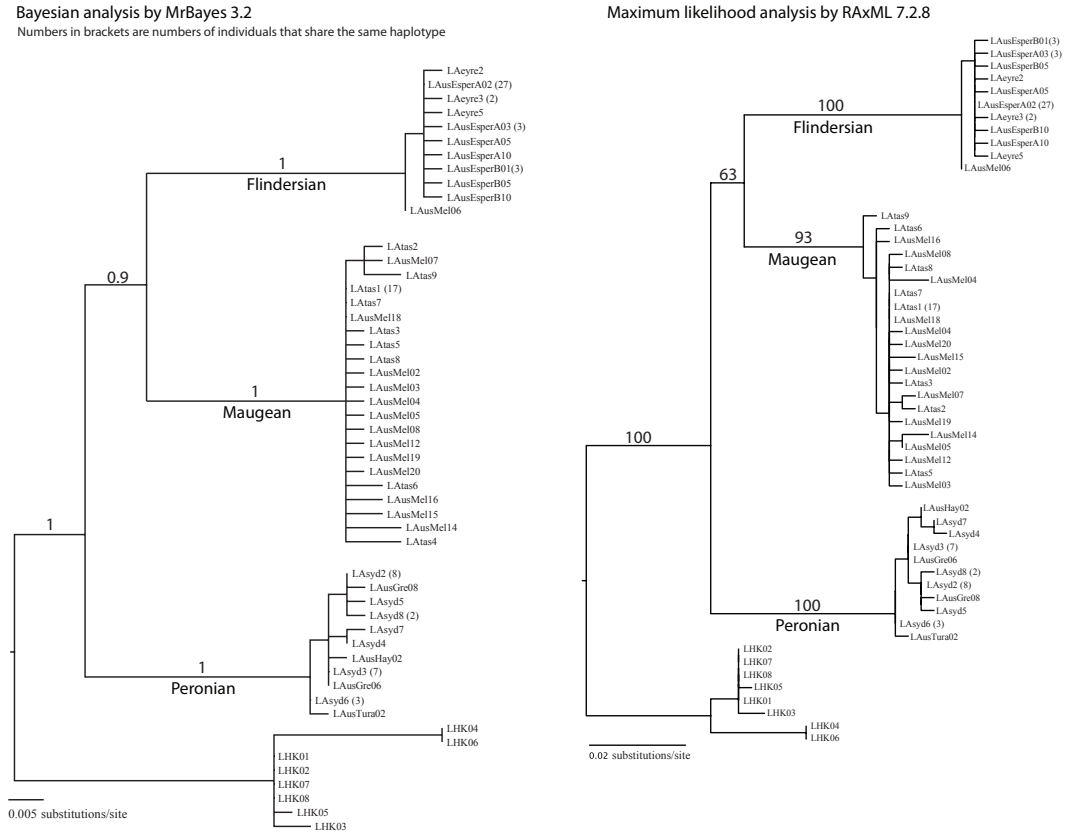

Figure 4.5: Bayesian (left) and ML (right)16S phylogenies of *Lasaea australis*. Posterior probabilities and bootstrap values were listed above major branches respectively.

4.6.3 Inferred paleodistributions of the three *Lasaea australis* lineages during the Last Glacial Maximum

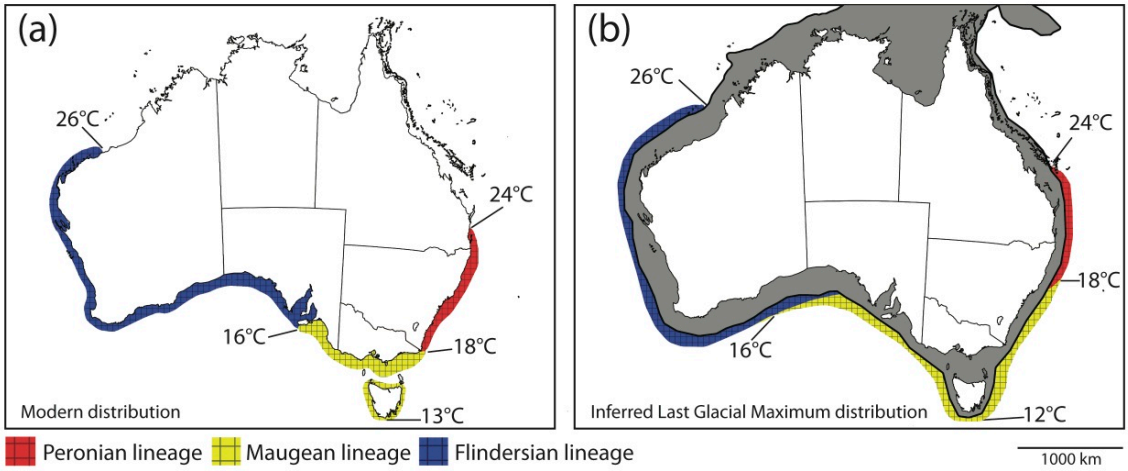

Figure 4.6: (a) Present-day distributions of the three *Lasaea australis* lineages. Northern boundaries of the Peronian and Flindersian lineages are defined based on Museum records [5]. Distribution of the Maugean lineage is inferred based on the range of Maugean province [2]. (b) Inferred paleodistributions of the three lineages during the Last Glacial Maximum based on inferred Sea Surface Temperatures [6].

CHAPTER 5

Molecular Phylogeny and Macroevolution of the Superfamily Galeommatoidea

5.1 Introduction

Abiotic and biotic factors both modulate the long-term evolutionary dynamics of diverse lineages and undoubtably contribute to the generation and maintenance of global biodiversity [9, 32]. The roles of abiotic factors in driving organismal evolution have been relatively well recognized, yet the way biological interactions shape macroevolutionary processes remains a perennial topic of contention in fundamental evolutionary research [145, 304, 305].

The evolutionary consequences of biotic interactions are most apparent in terrestrial biotas, where coevolutionary processes drive the evolution of major clades (*e.g.*, plants and insects [10]). In marine ecosystems, there is ample evidence for abiotic drivers, such as major tectonic events [11], nutrient availability [13] and climate/sea level-induced vicariant breakpoints [18]. However, the evolutionary importance of biotic factors has been best investigated in the paleontological literature, which has implicated biotic factors in post-mass extinction faunal recoveries [28] and in adaptive escalations [30, 31]. Macroevolutionary studies on extant marine taxa generally lack a meaningful biotic perspective (but see [35, 306, 307]), presumably because the nature of marine biological interactions remains poorly understood, especially regarding non-antagonistic interactions (*e.g.*, mutualism, commensalism) that may be prevalent in nature [44, 46].

Failure to incorporate biotic perspectives in macroevolutionary research may underline the frequent mismatch between theoretical models and observed patterns of

This chapter comprises an unpublished manuscript. The authors (in order) are: Jingchun Li, Diarmaid Ó Foighil and Ellen Strong

lineage diversification [28, 44, 49]. Given this, it is necessary to start examining the importance of biotic factors in shaping neontological marine biodiversification and how they interact with abiotic factors. A logical prerequisite would be to identify a representative marine lineage that: 1) exhibits exceptional species diversity and phenotypic disparity to allow statistically inferring patterns of macroevolution; 2) contains both free-living species and taxa with obligate biotic associations, therefore is amenable to comparative approaches; 3) has significant diversity in all major marine benthic habitats. The marine bivalve superfamily Galeommatoidea possesses all of these desired attributes, which enables us to investigate the relative roles of biotic and abiotic factors in shaping its macroevolution.

Galeommatoidean bivalves are a hyperdiverse, but poorly studied marine superfamily with a fossil record extending to the Cretaceous [53, 285]. Over the past decade, the application of comprehensive and quantitative sampling methodologies to marine ecosystems has catapulted Galeommatoidea from relative obscurity to the apex of Bivalvia biodiversity [57, 72]. Galeommatids were found to be the most diverse bivalve family and the sixth most diverse molluscan family at an intensively studied coral reef site in New Caledonia [72]. Paulay [57] similarly found Galeommatidae *s. l.* (= Galeommatoidea) to be the most diverse bivalve group on Guam and speculated that its actual diversity to be several times greater than any co-occurring bivalve family. The superfamily comprises approximately 500 described species [55], although many more species remain undescribed [57]. These bivalves also possess exceptional morphological disparity and innovations (Fig. 5.2), including pronounced shell reduction/internalization [60] and elaborated soft-tissue structures.

Galeommatoidea exhibits a striking ecological dichotomy in that some species are free-living while others have obligate commensal relationships with diverse burrowing invertebrate hosts, including crustaceans, holothuroids, echinoids, and sipunculans, *etc* [131,308]. A recent ecological synthesis [308] revealed that this lifestyle dichotomy is tightly associated with benthic habitat types: free-living species are typically found in hard-bottom habitats, hidden in rock and coral head crevices. In contrast, commensal species are typically restricted to soft-bottoms, where they occur within the oxygenated exnvelope produced by their bioturbating hosts. The associations with infaunal hosts allow the minute clams to attain depth refuges while maintaining access to oxygenated water currents, and may well be a prerequisite for their long-term colonization of soft-bottoms [308].

Symbiotic associations and hard-bottom habitat heterogeneity have both been shown to promote lineage diversification in marine vertebrates [306, 309]. Given the

lifestyle dichotomy among galeommatoidean clams, we are interested in how commensal and free-living lifestyles respectively contribute to the diversification and morphological evolution of this hyperdiverse marine lineage. To address these questions, it is essential to establish the evolutionary relationships between commensal and free-living lineages. To date, there is little consensus regarding the taxonomic and phylogenetic relationships within the superfamily [53, 57]. Molecular phylogenetic studies have been mostly restricted to one recent study focusing on Japanese galeommatoidean fauna (38 species) [131] and molecular analyses of the genus *Lasaea* [310].

Galeommatoidea is globally distributed and individual species tend to have broad distributions [311]. Therefore, a meaningful macroevolutionary study requires a comprehensive phylogenetic framework based on a multi-basin, global sampling strategy. Here, we reconstructed a global-scale molecular phylogeny of Galeommatoidea taking advantage of several large-scale international biodiversity expeditions (Fig. 5.1) as well as museum collections and published sources [131]. We gathered ecological and morphological information of the clams and compared patterns of lineage diversification and trait evolution between commensal and free-living species.

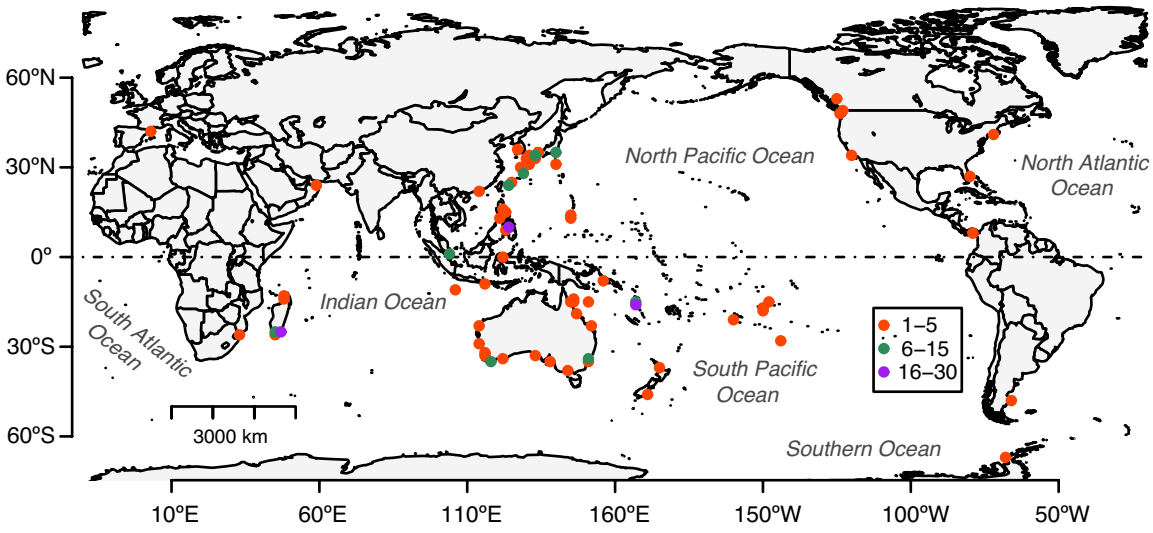

Figure 5.1: Sampling localities of galeommatoidean clams used in this study. Color scales correspond to numbers of species collected at each location

5.2 Materials and Methods

5.2.1 Sampling

The majority of our specimens were collected from extensive biodiversity expeditions in the Philippines (Panglao, Aurora), Vanuatu (Espiritu Santo), Madagascar (Atimo Vatae), and Mozambique. The expeditions were aimed to unbiasedly assess regional marine biodiversity in all possible local habitats; special attention was given to associations between mollusks and invertebrates [312].

Coastal/shallow-water specimens were collected from several "Our Planet Reviewed" expeditions (Santo, Atimo Vatae). Deep-water specimens were collected from cruises of the Tropical Deep-Sea Benthos program aboard the RV Alis in the SW Pacific; the FV DA-BFAR in the Philippines (Panglao, Aurora); and the RV Vizconde de Eza in the Mozambique Channel (Mainbaza).

All specimens from the biodiversity expeditions were deposited in Muséum National d'Histoire Naturelle (MNHN, France). Additional specimens were loaned from the Florida Museum of Natural History, the Raffles Museum of Biodiversity Research (Singapore), the National Museum of Nature and Science (Japan), the Australian Museum, the Western Australian Museum, the South Australian Museum, the Field Museum, the Santa Barbara Museum of Natural History, the Royal British Columbia Museum (Canada), and the University of Michigan Museum of Zoology (supplementary section 5.6.1).

Specimens were identified to distinct morphospecies and were assigned species names whenever possible according to the taxonomic literature (see section 2.5). Due to the large number of undescribed species and the lack of systematic revision in this superfamily, not all morphospecies can be identified to species and were kept as undescribed morphospecies. Commensal lifestyles were identified based on existing species descriptions and field records (Information of all specimens see supplementary section 5.6.1).

5.2.2 Phylogenetic analyses

Genomic DNA was extracted from mantle tissues of the specimen using the E.Z.N.A. Mollusc DNA kit (Omega Biotek). Four gene segments were used to reconstruct the molecular phylogeny: 16S rRNA gene, 28S rRNA gene, histone H3 and adenine nucleotide translocator (ANT). The 16S and 28S fragments were amplified following protocols in [310] and [311] respectively. The H3 gene was amplified following a standard PCR protocol (annealing temperature = 53°C) using a forward

primer [313]: HexAF (5'-ATG GCT CGT ACC AAG CAG ACG GC-3') and a customized reverse primer LasHexA2 (5'-TAG CGC ACA AGT TGG TGT C-3'). The ANT gene was amplified following a touchdown PCR protocol, using a customized forward primer ANTGF1 (5'-GCC AAC TGC ATT CGG TAT TTC CC-3') and a reverse primer ANTR1(5'-TTC ATC AAM GAC ATR AAM CCY TC-3') reported in [314]. For the touchdown PCR, the annealing temperature was decreased from 55°C - 48°C (1°C per cycle) and then continued at 48°C for an additional 35 cycles. The PCR products were gel-isolated and extracted using the QIAquick Gel Extraction Kit (Qiagen). All cleaned PCR products were sequenced at the University of Michigan Sequencing Core facility. 28S and H3 genes of additional galeommatoidean species [131] were downloaded from Genbank. See dataset S1 for GenBank accession numbers of all sequences.

Sequences were aligned using MUSCLE [315] implemented in CodonCode Aligner 3.1.7 and corrected by eye. Final alignment lengths for the gene segments are: 1084 bp (28S), 464 bp (16S), 295 bp (H3) and 580 bp (ANT). Substitution models and partition schemes of the genes were selected using PartitionFinder 1.0.1 [283] based on the Bayesian information criterion (BIC). For both 16S and 28S genes, the GTR+G+I model was selected for the whole gene segment. For the H3 gene, each codon position was selected as an independent partition; GTR+G+I was selected for the first and third codon positions and K80+G+I was selected for the second codon. For the ANT gene, codon partition scheme was selected as (1+2), 3. The SYM+G+I model was selected for the first and second codons and GTR+G+I was selected for the third.

The tree topology and divergent times were estimated simultaneously in BEAST 1.7.3 [282]. Outgroup taxa were selected from several relatively closely related veneroid bivalve families (Gastrochaenidae, Neoleptonidae and Lucinidae), because no clear sister groups to Galeommatoidea have been confidently identified to date [130, 316, 317]. The minimal age offset of the superfamily was set based on the earliest documented appearance of Galeommatoidea in the fossil records (105.6 Mya [285]) and a lognormal prior (mean=2, stdev=1) was applied to this calibration point. We did not include calibrations on internal nodes due to the long-standing taxonomical confusions and poor fossil records for this superfamily – the monophyly of many genera are questionable [131] and it is unclear whether fossil species can be confidently identified to the correct group. A relaxed molecular clock with an uncorrelated lognormal distribution was used and the Yule process was selected as the speciation model. Codon partition schemes and substitution models were manually set in the XML files generated by BEAUTi 1.7.3 [282] according to the PartitionFinder re-

sults, except that the proportion of invariant sites (I) was not applied as it may add unnecessary model complexity [318]. A maximum-likelihood starting tree was generated using RAxML 7.6.6 [319] with partitioned genes and the GTRCAT model. Three independent Markov chain Monte Carlo (MCMC) analyses were run on the Cipres Gateway [320] for 100 million iterations respectively and sampled every 10000 iterations. Convergence diagnostics were conducted in Tracer 1.5 [276] and reliable effective sampling size values (>500) were ensured. The first 1000 trees of each MCMC run were discarded as burn-in. The remaining trees were combined and "thinned" using customized shell scripts, resulting in 9000 posterior trees. A maximum credibility consensus tree was generated from the 9000 trees in TreeAnnotater 1.7.3 [282].

To assess whether the phylogenetic analysis is robust to missing data (missing sequences or gaps), a second dataset was prepared including only species that contain at least three successfully amplified gene markers. In addition, alignments in the 28S and 16S sequences were trimmed using trimAl [321], which removed columns contain gaps in more than 50% of the sequences while retained 80% of the original alignment. This dataset was then used to reconstruct a phylogeny in BEAST using the same settings described above. The main topology estimated from the reduced dataset was consistent with the original reconstruction. Therefore, the consensus tree reconstructed from the full dataset was used for further analyses.

5.2.3 Analyses of lineage diversification

To estimate the phylogenetic signal of the lifestyles (free-living and commensal), Pagel's λ [322] was calculated using the R [151] package PHYTOOLS 0.2.46 [323]. The signal was calculated twice with species with unknown lifestyles treated as commensal and free-living respectively. P values was calculated using a likelihood ratio test, comparing the estimated model to a null model in which λ was fixed to zero.

Ancestral lifestyles of four backbone nodes (Fig. 5.2A) were estimated using the Discrete and MultiState methods [324] implemented in BayesTraits 2.0. The analyses were conducted over 1000 post burn-in trees selected at even intervals throughout the combined BEAST trees to accommodate for phylogenetic uncertainties. The nodes were specified using the addMRCA option and probabilities of the two lifestyles at the ancestral nodes were estimated using a MCMC approach. An exponential prior (mean=10) was used for all parameters. Two independent chains were run for 10 million iterations respectively and sampled every 1000 iterations. Convergence of the two runs was confirmed and results were combined after a 10% burn-in. Posterior probabilities of the ancestral states were visually represented as pie charts on the

phylogeny.

We evaluated diversification rates in free-living and commensal lineages using two approaches: BiSSE (Binary State Speciation and Extinction) [325] and BAMM (Bayesian Analysis of Macroevolutionary Mixtures) [326]. BiSSE [325] analyses were conducted using the R package DIVERSETREE 0.9.3 [327]. Both likelihood and Bayesian approaches were used to estimated the six BiSSE parameters (speciation, extinction and transition rates for both commensal and free-living states). For the likelihood analyses, both constrained (free-living and commensal species have same speciation rates) and unconstrained (all rates allow to vary) models were fitted to the data. Fitness of the two models were compared using a likelihood ratio test. For the Bayesian estimation, an exponential prior with rate 1/2r was used, where r is the diversification rate estimated from the constrained model. Two MCMC chains were run for 10000 iterations each with a 10% burn-in. Results from the two chains were combined and the posterior distribution of the parameter estimations were obtained (Fig. S4). To assess the impact of possible sampling bias, we randomly dropped 10%-90% percent of the free-living taxa from the tree and estimated the parameters again using the likelihood approach for each scenario.

Speciation rates of all branches on the phylogeny were estimated using the software BAMM (http://bamm-project.org) [326]. Two MCMC chains were run for 10 million iterations and sampled every 10000 iterations, assuming an estimated 75% missing taxa and a random taxon sampling. The two chains converged quickly and was combined with a 10% burn-in each. Mean speciation rates of all branches were calculated and used to scale the branch lengths of the original phylogeny.

To test whether the free-living lifestyle is significantly correlated with a tropical distribution, a phylogenetic logistic regression [328] was conducted using the R package PHYLOLM 2.0. Species collected between 24°N and 24°S were considered as having a tropical distribution.

5.2.4 Geometric morphometrics and trait evolution

Among the 217 species included in the phylogeny, 174 have complete shells available for morphological analyses. Shell morphologies of each specimen was captured using a geometric morphometrics approach described in [329]. Multiple individuals (2-7) per species were included whenever possible. The lateral view of the left valve of each specimen was photographed and digitized using the software tpsDig2 [208]. One landmark was placed on the umbo (shell apex) of the shell and 45 semi-landmarks were placed evenly along the shell outline. Shape coordinates of all individuals were

superimposed using the Procrustes method [209] to remove variations caused by differences in size, position, and orientation. During this process, semi-landmarks were also slid following the minimum bending energy criterion [207] to ensure shape homology among individuals. Mean Procrustes coordinates for each species were then calculated from multiple individuals and superimposed again. The final aligned Procrustes shape coordinates were used in the subsequent analyses. Mean log centroid sizes for all species were calculated and used as representations of shell sizes. A Welch's two sample t-test was performed to test whether free-living and commensal taxa differ significantly in shell sizes. All morphometric manipulations were conducted using the R package GEOMORPH 1.1.0.

To assess the standing disparity of Galeommatoidea shell shapes, a principle component analysis (PCA) was performed on the aligned coordinates of all species. For visual representation, scores of the first two PCs were plotted and shell shapes on extreme axis were plotted to show how general shell shape changes along PC1 and PC2. Standing disparities of the free-living and commensal taxa were compared using a multivariate homogeneity test of group dispersions [330] based on the first 20 PCs. PCA was also performed on the free-living and commensal species separately to assess shape variations within each group. The results were plotted respectively and individuals were color-coded based on the subclades they belong to. The subclades were selected so that each subclade includes at least five species and has more than 60% posterior support.

To assess how shell shape disparities evolve along the phylogeny, Disparity Through Time (DTT) analyses [331] for the aligned shape coordinates of free-living and commensal taxa were conducted respectively using the R package GEIGER 1.99.2 [332]. The DTT analysis calculated the ratio between the average within-subclade disparity and the total disparity in the phylogeny (*i.e.*, mean relative disparity) at all nodes in the chronogram. It then compared the observed values to values simulated under a Brownian motion (BM) model. Deviations from the BM simulation were summarized as the morphological disparity index (MDI) [333]. Null distributions of the DTT curve were generated from 100 Brownian simulations.

Lastly, we compared patterns of morphological evolution for commensal and free-living taxa by fitting the BM model (with rate parameter σ^2) and three ecologically-relevant modifications: single peak Ornstein-Uhlenbeck (OU) [334], early burst (EB) [331] and speciational evolution (SE) [335]. The OU model constrains the walk with a central tendency whose strength is proportional to α. In the EB model, the rate of trait evolution decreases over time with rate parameter a. The SE model allocates

a portion of morphological divergence as step changes at speciation events, and the fraction of such changes is represented by ψ. Shell shapes were represented by the first three PCs (96% of total variation) from the PCA analyses on free-living and commensal species respectively. Shell sizes were represented by the mean log centroid sizes. The early burst model for size evolution was evaluated using GEIGER 1.99.2, and the same model for shape evolution (multi-variant) was evaluated using the R function fitContinuousMV written by G. Slater (http://fourdimensionalbiology.com). The rest three models for both size and shape evolution were evaluated using the R package MOTMOT 1.0.1 [336]. Model comparison was conducted based on the multivariate-corrected AIC_c [337]. Phylogenetic signals (Pagel's λ) of both shape and size data were also estimated in MOTMOT 1.0.1 and the significance was evaluated using likelihood ratio tests.

5.3 Results

5.3.1 Phylogenetic relationships

We examined more than 1000 galeommatoidean specimens from biodiversity surveys and museum collections. The final phylogeny includes 97 species currently considered valid and 120 undescribed morphospecies, spanning 39 known genera. Among the total 217 species (*sensu lato*), 67 are commensal, 135 are free-living and 15 have unidentified lifestyles (see supplementary 5.6.1 for information on each species).

Deep phylogenetic relationships within the superfamily were well-resolved with basal clades composed of mostly commensal species (Fig. 5.2A). Five well supported clades were identified (Fig. 5.2A): clades a-d represent four major commensal clades and clade e represents one major free-living clade, although it includes a commensal subclade (Fig. 5.2A, CS7). Seven commensal subclades (Fig. 5.2A, CS1-7) and nine free-living subclades (Fig. 5.2A, FS1-9) were further identified (Supplementary Figs. 5.6 and 5.7).

Ancestral state reconstruction [324] strongly suggests that the ancestral lifestyle of galeommatoideans is commensalism/sediment-dwelling. The distribution of commensal and free-living lifestyles on the phylogeny is highly clustered with a high phylogenetic signal ($\lambda_{sig} = 0.75$ when unknowns are treated as commensal; $\lambda_{sig} = 0.83$ when treated as free-living). Occasional lifestyle transitions occurred within both major commensal and free-living clades.

Degrees of host lineage fidelity vary among different commensal subclades. Species in CS2 and CS7 are strictly restricted to echinoid and stomatopod hosts respectively.

Species in CS1 are almost all holothuroid commensals, except for one that is associated with a sipunculan host. Species in CS3 - 6, however, occupy a diverse spectrum of invertebrate hosts without apparent patterns of large scale host lineage specialization (see dataset S1 for host information).

5.3.2 Lineage diversification

Two BiSSE models were fitted to the phylogeny: the full model allows rates of speciation and extinction to differ between commensal and free-living species; the simpler equal-rates model constrains the rates to be the same. Results shown that the full model fits significantly better than the equal-rates model ($P < 0.001$, Table 5.1) and that the free-living species exhibit a nearly two-fold higher speciation rate than the commensals (0.070 vs. 0.036, Fig. 5.3). Estimated extinction rates for both groups are close to zero and the transition rate from commensal to free-living is slightly higher (0.009 vs. 0.005, Fig. 5.3). To test whether this pattern was driven by possible over-representation of free-living lineages, we randomly removed free-living taxa from the phylogeny in increments from 10% to 90%. The BiSSE results were robust until removing more than 50% of all free-living taxa (Table 5.1).

Although the free-living species collectively show a higher rate of speciation, it is possible that this overall high rate is driven by a few fast-evolving lineages. Therefore, it is important to evaluate diversification rates independent of ecological characters. To do so, we further estimated the speciation rate for every branch in the phylogeny using the BAMM method. This method does not assume any *a priori* classification of taxa and allows shifts of diversification parameters to occur along any branch in the tree. Figure 5.2B shows the Bayesian Galeommatoidea phylogeny with branch length proportional to estimated speciation rate on that branch. It is evident that species belonging to one clade (Fig. 5.2A, labeled by a blue star) exhibit higher speciation rates – estimated rates for each branch are typically 2-4 times higher than the rest of the tree (see all rates in supplementary Figs. 5.6 and 5.7). The star clade includes most free-living species, except for subclades FS1 and FS9 (Fig. 5.2A), which do not show significantly higher speciation rates than the commensal subclades.

Many marine bivalves exhibit a latitudinal biodiversity gradient and this pattern has been partially attributed to elevated diversification rates in tropical regions [71]. In our phylogeny, 48% of the species are tropical and 52% are non-tropical (Fig. 5.8). To ensure that the observed rate difference between free-living and commensal species is not merely a result of latitudinal bias, we tested whether being free-living is significantly correlated with being tropical using a phylogenetic logistic

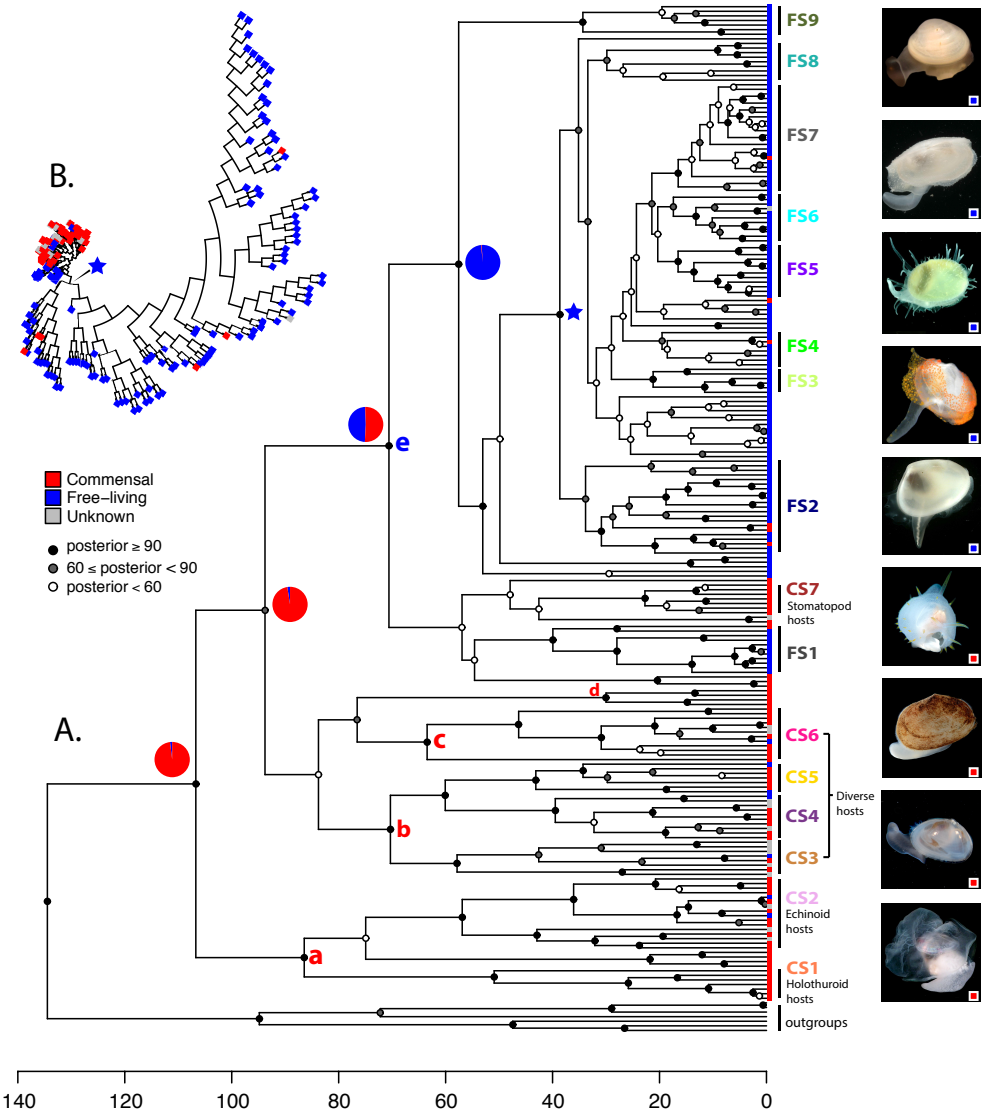

Figure 5.2: A. Time-calibrated molecular phylogeny of Galeommatoidea. Colored tip labels indicate the lifestyle of each morphospecies, node labels indicate the posterior probability of each branching event. Pie charts near the nodes represent the probability of commensal or free-living being the lifestyle at theses nodes. Free-living and commensal subclades are labeled as FS and CS respectively. Photos on the right show exemplars of representative galeommatoidean clams; colored squares at the bottom right indicate the lifestyles of the clams. Host information for the commensal subclades are shown. B. The same topology as A, with branch length proportional to rate of speciation estimated using BAMM. The clade labeled with a blue star corresponds to the star clade in A. (Photo credit: P. Maestrati & A. Anker)

Table 5.1: BiSSE model fitting results for the full phylogeny and five reduced phylogenies with 10-90% of free-living taxa removed. Estimated speciation, extinction and transition rates for the two character states (*i.e.*, free-living and commenal) are represented by λ, μ and q. Likelihood value (lnLik) for each model and P-value (Pr) for each likelihood ratio test are given.

Model	Free-living			Commensal				
Full phylogeny	λ_0	μ_0	q_{10}	λ_1	μ_1	q_{01}	lnLik	Pr
Full model	0.070	0.004	0.009	0.036	0.000	0.005	-936.52	< 0.001
Equal-rates model	0.060	0.000	0.007	0.060	0.030	0.006	-938.62	
10% free-living removed	λ_0	μ_0	q_{10}	λ_1	μ_1	q_{01}	lnLik	Pr
Full model	0.065	0.000	0.009	0.035	0.000	0.006	-899.93	< 0.001
Equal-rates model	0.058	0.000	0.007	0.058	0.027	0.007	-905.16	
30% free-living removed	λ_0	μ_0	q_{10}	λ_1	μ_1	q_{01}	lnLik	Pr
Full model	0.059	0.000	0.010	0.035	0.000	0.006	-817.15	< 0.001
Equal-rates model	0.052	0.0008	0.008	0.052	0.019	0.007	-821.26	
50% free-living removed	λ_0	μ_0	q_{10}	λ_1	μ_1	q_{01}	lnLik	Pr
Full model	0.050	0.000	0.010	0.035	0.000	0.007	-742.85	< 0.05
Equal-rates model	0.044	0.000	0.009	0.044	0.007	0.007	-744.86	
70% free-living removed	λ_0	μ_0	q_{10}	λ_1	μ_1	q_{01}	lnLik	Pr
Full model	0.043	0.000	0.009	0.036	0.000	0.003	-646.45	$= 0.3$
Equal-rates model	0.039	0.000	0.010	0.039	0.000	0.003	-646.96	
90% free-living removed	λ_0	μ_0	q_{10}	λ_1	μ_1	q_{01}	lnLik	Pr
Full model	0.038	0.000	0.010	0.036	0.000	0.007	-555.47	$= 0.8$
Equal-rates model	0.037	0.000	0.010	0.037	0.000	0.007	-555.50	

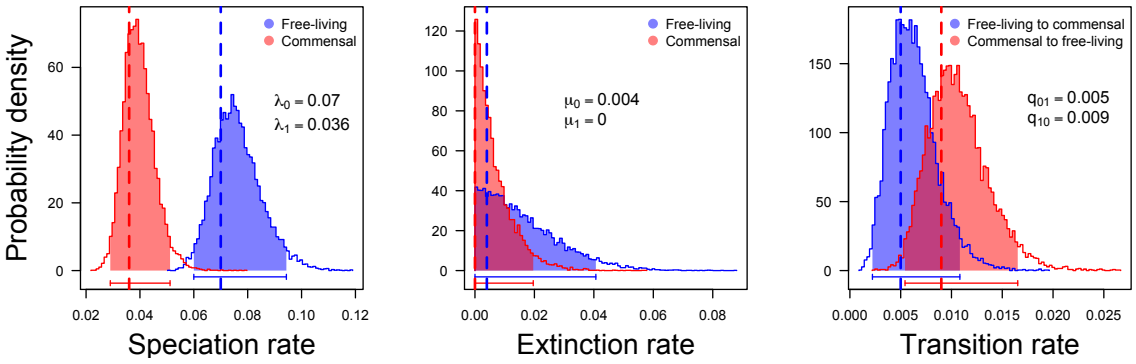

Figure 5.3: Parameter estimations from the BiSSE full model. States 0 and 1 represent free-living and commensal respectively. Estimated speciation, extinction and transition rates for the two character states (*i.e.*, free-living and commenal) are represented by λ, μ and q. Area curves represent probability density distributions of the parameters from MCMC sampling. Dashed lines represent maximum likelihood estimations and the estimated values are labeled in the plot.

regression method [328]. Our results indicated that the two traits are not correlated ($\alpha = 0.006$).

5.3.3 Morphological evolution

Phylogenetic comparative analyses revealed that the commensal lineages exhibit much lower phylogenetic signal and higher within-clade disparity than the free-living taxa.

The principal component analysis (PCA) showed that major shape variations are captured by the first two principal components (PCs), which account for 57% and 35% of the total variations respectively (Table 5.2); Both PCs reflect variations in shell elongation and umbo (*i.e.*, shell apex) position (Fig. 5.5A). Clams with either lower PC1 scores or higher PC2 scores exhibit more elongated shell forms. Species on the positive extremes of PC1 or PC2 possess umbos positioned in the anterior portion of the shell; whereas species on the negative extremes possess more posteriorly-positioned umbos (Fig. 5.5A, umbo positions pointed by arrows). Free-living and commensal species tend to overlap in morphospace and their total shell shape disparities do not differ significantly (P = 0.16). However, most free-living species occupy the top right region where shell umbos are positioned anteriorly, while commensal taxa tend to have posteriorly pointed umbos. Further, shell sizes of free-

Table 5.2: Summary of principal component analyses for shell shapes of all species, free-living taxa only and commensal taxa only.

	All Species				
	PC1	PC2	PC3	PC4	PC5
Standard deviation	0.085	0.067	0.024	0.011	0.011
Proportion of variance	0.569	0.351	0.043	0.011	0.009
Cumulative proportion	0.569	0.920	0.964	0.975	0.984
	Free-living				
	PC1	PC2	PC3	PC4	PC5
Standard deviation	0.084	0.050	0.016	0.009	0.008
Proportion of variance	0.702	0.240	0.027	0.009	0.007
Cumulative proportion	0.702	0.942	0.970	0.978	0.985
	Commensal				
	PC1	PC2	PC3	PC4	PC5
Standard deviation	0.085	0.077	0.036	0.015	0.011
Proportion of variance	0.480	0.397	0.087	0.015	0.008
Cumulative proportion	0.480	0.877	0.964	0.980	0.987

living species are significantly larger than those of commensal species ($P < 0.001$, Fig. 5.4).

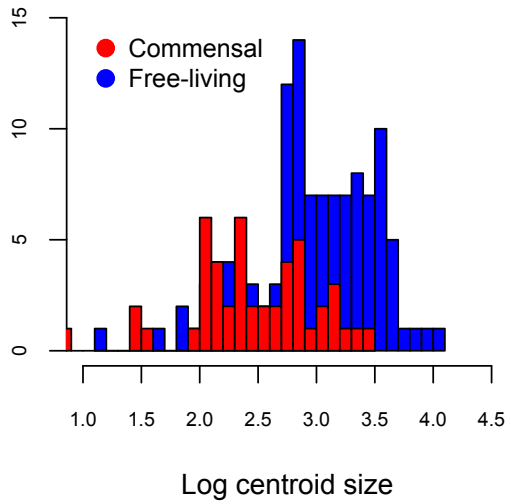

Figure 5.4: Distribution of the log centroid size for free-living and commensal species.

Table 5.3: Model fitting results and phylogenetic signal for shell shape and size evolution of Galeommatoidea. Models shown are Brownian motion (BM), Ornstein-Uhlenbeck (OU) and speciational evolution (SE). Asterisk indicates statistically significant phylogenetic signal.

		Shell shape				Shell size			
		BM (σ^2)	OU (α)	SE (ψ)	phy. sig.	BM (σ^2)	OU (α)	SE (ψ)	phy. sig.
Free-living	Likelihood	615.4	644.3	693.5	-	-144.4	-85.4	-68.5	-
	ΔAICc	150	98	0	-	90	34	0	-
	Parameter	0.0004	0.03	0.25	0.88*	0.03	0.16	0.56	0.77*
Commensal	Likelihood	167.3	201.3	189.1	-	-58.0	-35.9	-41.7	-
	ΔAICc	61	0	24	-	45	0	12	-
	Parameter	0.0007	0.09	0.68	0.54	0.02	0.21	1	0.23

We repeated the PCA analysis for free-living and commensal taxa separately (Fig.5.5B and C, Tabel 5.2) to compare their subclade distributions in morphospace. For free-living lineages, species in the same subclades tend to cluster with each other and the degree to which subclades overlap reflects phylogenetic relatedness. In contrast, commensal subclades mostly overlap with each other and within-clade disparity can be quite high (*e.g.*, CS7). This discordance in morphospace distribution is confirmed by the high phylogenetic signal ($\lambda_{sig} = 0.88$) for free-living shell shapes and relatively low phylogenetic signal ($\lambda_{sig} = 0.54$) for the commensals (Table 5.3). A similar pattern is observed in the shell size data, where phylogenetic signal is high ($\lambda_{sig} = 0.77$) for the free-living taxa and low ($\lambda_{sig} = 0.23$) for the commensals (Table 5.3).

The observed DTT curve for free-living species (Fig. 5.5D) generally falls within the 95% confidence interval of the BM simulations. Although mean relative disparities tend to exceed the median BM expectations in the early half of the plot, they decrease relatively linearly over time (MDI=0.11). In contrast, the mean relative disparities for commensal species (Fig. 5.5E) remained above the BM 95% confidence interval throughout the phylogeny (MDI=0.35) and peaked near the present. This implies that the within-subclade disparities in the commensal lineages are consistently higher than what would be expected from a random walk.

Models fitting results are shown in Table 5.3). Fitting results for the EB model are not shown because it consistently converged to the simpler BM model (*i.e.*, $a = 0$) during maximum likelihood estimations for all datasets. Among the remaining three models, the SE model is strongly supported for free-living taxa morphological evolution, whereas the OU model is strongly favored for commensal species (both shape and size).

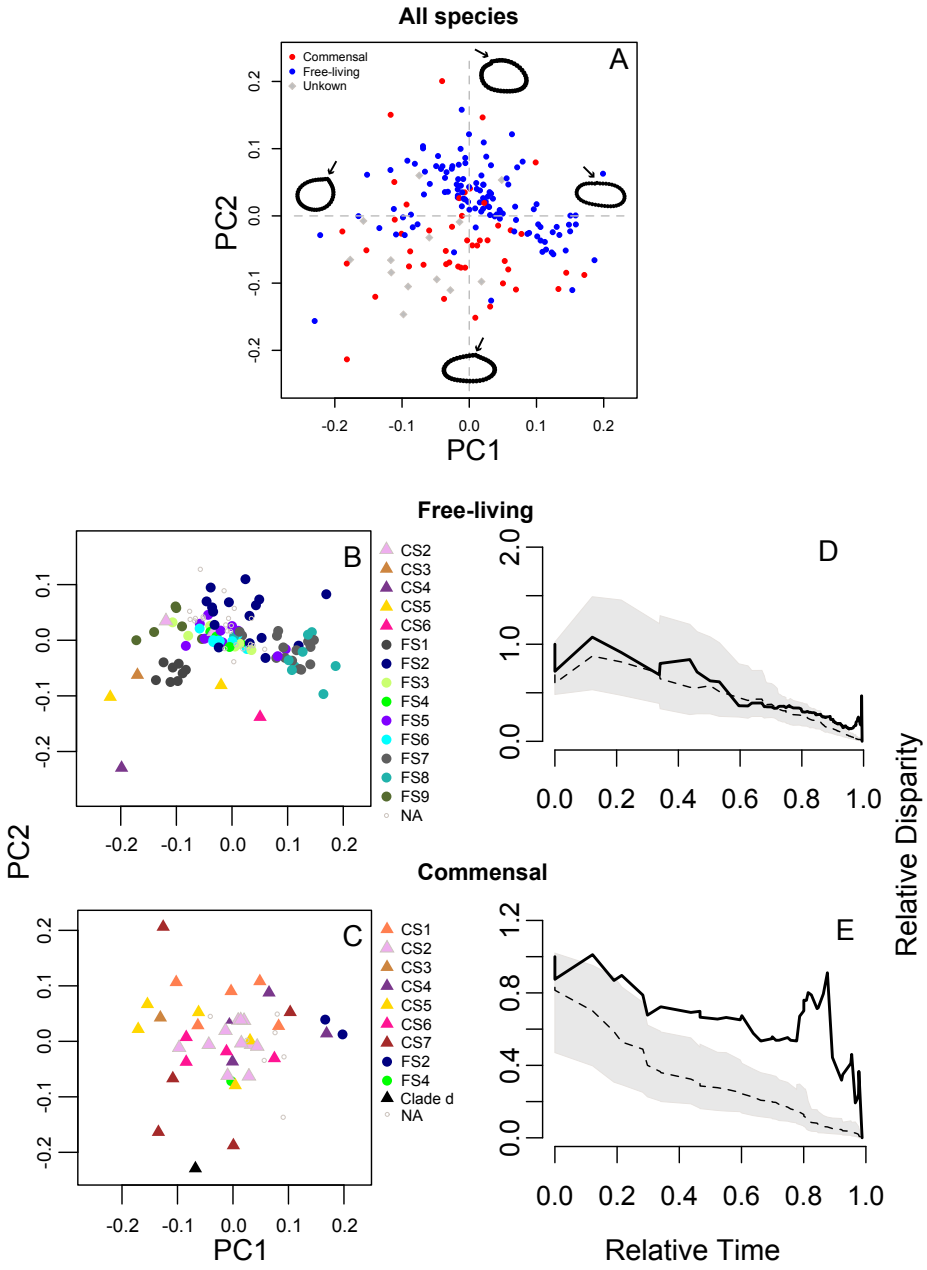

Figure 5.5: A. Scatter plot of the first two PCs of the lateral shell shape (left valve) variance among all species. Lifestyles are color coded. Four deformed grid plots represent shape changes along each axis to aid visualization. B-C. Scatter plot of the first two PCs from PCA analysis of free-living and commensal species respectively. Color code corresponds to the subclade each species belongs to. Triangles and squares represent species belonging to the major commensal and free-living subclades respectively. Species belonging to unresolved clades are colored grey. D-E. Diversity Through Time (DDT) plot for free-living and commensal species. The dashed line represent DDT under a Brownian motion model of trait evolution. 95% confidence intervals are shaded in grey.

5.4 Discussion

5.4.1 Phylogenetic and taxonomic implications

Our study provides the first global phylogenetic framework of Galeommatoidea and is the best representation of galeommatoidean diversity to date. More than half of the taxa sampled are currently undescribed, confirming the perception that the total galeommatoidean diversity must be substantially higher than currently documented and that Galeommatoidea is a "megadiverse" group in Bivalvia.

The five major clades (Fig. 1A, Clade a-e) recovered in our Galeommatoidea phylogeny are largely consistent with results from a regional molecular phylogenetic analysis [131]. The basal position of the commensal Clade a is strongly supported. Phylogenetic relationships within most commensal and free-living subclades are also largely resolved. However, certain among-clades relationships remain unclear. Specifically, internal relationships among three major commensal clades (Clade b, c, d) are unresolved. This is at least partially due to the instability of Clade d (Genus *Basterotia*), a group of rare bivalves which are traditionally placed in a different superfamily (Cyamioidea), because they possess posterior inhalant siphons that other galeommatoideans lack [123]. *Basterotia* and other closely related groups are notably underrepresented in the phylogeny; a more extensive sampling is necessary to further confirm their phylogenetic status.

Within the major free-living Clade e, relationships among different subclades are characterized by poorly-supported short branches, especially for the star clade. This is likely caused by the rapid radiation among different free-living subclades, which requires more genetic markers with appropriate variation levels and the use of coalescent approaches to improve their phylogenetic resolution.

Supraspecific taxonomy in Galeommatoidea has always been poorly understood and controversial [57, 131], largely due to the lack of distinct synapomorphic shell characters, difficulties in quantifying soft-tissue structures and the presence of a large number of undescribed species. Operational estimates of the number of families range from one [53] to six [139], although many currently favor two: Galeommatidae and Lasaeidae [62]. Not surprisingly, our phylogeny does not match any of the family level classification schemes and reveals that most of the common and formally accepted genera (*e.g.*, *Scintilla, Galeomma, Pseudopythina, Mysella*), both free-living and commensal, are not monophyletic.

Relationships between galeommatoidean genera in our dataset and the sixteen phylogenetic subclades are presented here. For the free-living subclades, FS1 is com-

posed of the genera *Lasaea* and *Arthritica*, forming a sister group; FS2 includes species from the genera *Ephippodonta*, *Melliteryx* and *Nesobornia*; FS3 includes one *Borniola* species; FS4-6 include many species that are traditionally placed in the genus *Scintilla*; FS7-8 include species that belong to the genera *Galeomma* and *Pseudogaleomma*; and FS9 is composed of the genus *Kellia*.

For the commensal subclades, CS1 includes the genera *Anisodevonia*, *Devonia*, *Entovalva*, *Austrodevonia* and *Nipponomysella*; CS2 includes *Brachiomya* and *Montacutella*; CS3-5 includes species in *Montacutona*, *Mysella*, *Kurtiella*, *Curvemysella*, *Koreamya*, *Nipponomontacuta*, *Scintillona*, *Nipponomysella* and *Borniola*; CS6 contains the genera *Pseudopythina*, *Barrimysia* and *Peregrinamor*; and CS7 includes *Phlyctaenachlamys*, *Divariscintilla* and *Ephippodontomorpha*. Note that our phylogeny contains a large number of undescribed morphospecies and their taxonomic status are undetermined.

In conclusion, our phylogenetic analysis not only suggests the need for a taxonomical revision of the superfamily based on molecular data, but also calls for the exploration of other phenotypic characters, such as sperm morphologies or reproductive modes, which are potentially more phylogenetically informative than shell morphologies alone [338].

5.4.2 Ecological opportunities for elevated diversification

Our data revealed a striking dichotomy in galeommatoidean diversification. The star clade (Fig. 5.2A), comprising the majority of free-living species, exhibits a much higher speciation rate than the rest of the phylogeny, while no commensal clades/subclades show signs of elevated diversification. The difference is robust to removing up to half of the free-living taxa. High speciation rates in marine lineages have been linked to numerous non-mutually-exclusive environmental and life history factors, such as high temperature, high spatial complexity, intense interspecies competition and non-planktotrophic development, *etc.* [339, 340]. The challenge here is to identify what mechanisms can selectively lead to high speciation in the free-living star clade but not in the commensals. We have already shown that the free-living lifestyle in Galeommatoidea is not significantly correlated with a tropical distribution. In fact, one of the major commensal clades, Clade a, is almost entirely tropical (Supplementary Fig.5.8), yet its estimated speciation rate is no higher than other commensal clades. Therefore, geographical distribution or global temprature gradient alone cannot explain the elevated speciation rate in the free-living star clade.

One important distinction between the two lifestyles is that almost all commen-

sal taxa are found in soft-bottom habitats while the great majority of free-living species are hard-bottom dwellers [308]. Only a few exceptions occur in the commensal clades, where some lineages transitioned to a free-living lifestyle yet remained sediment-dwelling (*e.g.*, *Mysella charcoti* [164]). Our analysis suggests that ancestral galeommatoideans are likely sediment-dwelling commensals and that colonizations of hard-bottom habitats were coupled with losses of commensalism. This habitat transition is ecologically significant as it opens up previously unavailable niches in highly heterogeneous hard-bottom habitats. Geologically and biologically complex hard-bottom habitats, particularly in coral reef ecosystems, have been shown to promote high speciation in multiple marine groups through vicariance processes and niche partitioning driven by species interactions [20, 309, 341]. In Galeommatoidea, many species in the star clade are found in reef-associated habitats, especially in the Indo-Australian Archipelago (IAA) (Fig. 5.1). Interestingly, the diversification of many free-living subclades falls within the timeframe of modern coral reef expansions (40-23 Mya [342]). Further, the two free-living subclades that do not show higher speciation rates, FS1 (genera *Lasaea* and *Arthritica*) and FS9 (genus *Kellia*), are non-reef associated. These observations suggest that coral reef habitats play key roles in driving the rapid divergence among free-living lineages. A comprehensive test of this hypothesis would entail additional taxon sampling from other significant coral reefs (*e.g.*, Caribbean region) and detailed ecological studies of reef-associated galeommatoidean species.

One perhaps surprising result of this study is that commensal lineages diversified more slowly than free-living ones. Generally, host-switching events in symbiotic systems are expected to provide additional opportunities for ecological divergence and promote speciation [223]. In Galeommatoidea, many commensal lineages lack clade-specific host fidelity, implying that host-switching is relatively common [131]. However, such processes do not seem to result in exceptionally high speciation rates. In a recent framework [343], Dynesius & Jansson pointed out three principal controls of speciation: rate of within-species lineage splitting, degree of persistence for split lineages, and time required for such lineages to become full species. Thus, the commensal clams could have high rates of within-species lineage splitting due to host-switching events, but still have low speciation rates if the initially split lineages exhibit low degrees of persistence. Low persistence could be caused by either lineage merging due to increased gene flow or by within-species lineage extinction [343], both of which are probable in Galeommatoidea. Microevolutionary studies on multiple commensal species [202, 344] found no evidence of pronounced genetic differentiation among pop-

ulations occupying different hosts, suggesting high levels of gene flow among these populations. In addition, because many commensal-host associations are obligate, host extinction events [344] can cause co-extinction of specialized commensal populations and thereby reduce rates of speciation. Compared to the commensals, free-living taxa not only have a higher probability of lineage splitting due to the availability of heterogeneous hard-bottom habitats, but may also experience high degrees of lineage persistence owing to the stability of such habitats, resulting in the overall high speciation rate.

In addition to population-level extinction, high host dependence could also result in higher species-level extinction for the commensal taxa. Although the estimated extinction rates are low for both commensal and free-living taxa based on current data (Fig. 5.3), the legitimacy of extracting information about extinction from patterns of molecular phylogenies is still highly controversial [345]. For now, we cannot confidently compare patterns of extinction without an extensive fossil record, which galeommatids do not have; not to mention the difficulty of assigning small, featureless shells to recent clades with any degree of certainty.

Because free-living and commensal lifestyles in Galeommatoidea show high phylogenetic conservatism (*i.e.*, only one major free-living radiation), there is little statistic power (*i.e.*, lack phylogenetic repetition) to demonstrate causal relationships between ecological characters and the higher speciation rates. Besides habitat heterogeneity, other clade-specific life history traits may also contribute to the accelerated speciation in the free-living lineages. For example, the average body size of free-living species is significantly larger than the commensals. Since body size is typically positively related to brood size and fecundity, larger body size can also increase the probability of within-species lineage persistence. Another important trait related to marine speciation is larval development, as it is generally assumed that the wide dispersal of planktotrophic larvae can suppress genetic divergence and reduce speciation rate [339]. While most galeommatoidean species possess planktotrophic larvae (indirect development), some taxa release crawl-away juveniles (direct development) [5]. Both developmental modes have free-living and commensal representatives. However, case studies on galeommatoideans have shown that direct developers can have extensive geographic ranges [5, 311] and that indirect developers can be geographically quite restricted [310]. Therefore, the impact of larval ecology on galeommatoidean speciation could be complex and is unlikely to be the major driver of the observed patterns.

5.4.3 Modes of morphological evolution

This study's multiple assessments of galeommatoidean morphology collectively reveal a consistent discordance between the free-living and commensal lineages. For the free-living species, morphologies (lateral shell shape and size) of closely related species tend to resemble each other and among-clade disparity is higher than within-clade disparity throughout the phylogeny. Among the four trait evolution modes, the speciational evolution (SE) model provides the best fit to the data, indicating that besides the gradual trait evolution occurring along the phylogeny, a fraction ($\psi = 0.25$) of the trait variation is contributed by step changes at speciation events. This is consistent with the notion that ecological niche partitioning driven by structural and biological (*e.g.*, predation) complexity is promoting the diversification of free-living taxa.

In contrast, most of the morphological disparities in the commensal species are explained by within-clade rather than among-clade disparity, indicating that closely related species can be morphologically highly divergent and distantly related species sometimes resemble each other (intercladal convergence). The OU model is strongly favored for the commensal trait evolution. Although the fit of an OU model is regularly interpreted as selection towards a trait optimum, caution is required before making such links because different evolutionary scenarios (*e.g.*, stasis, low phylogenetic conservatism, *etc.*) can produce similar trait distribution patterns that resemble an OU process [331]. What can be inferred from the OU model is that within-clade disparities remain relatively constant through time and different clades overlap in the morphospace. This requires species in each clade to quickly occupy the available morphospace after initial divergence. Such a pattern can be generated under two extreme conditions (or their combination): first, the available morphospace may be tightly constrained, and second, the species may be exploring the morphospace rapidly. Given that the shell shapes of extant commensal species are relatively diverse (*e.g.*, even umbo orientations differ between closely related taxa), it is unlikely that their shell shape morphospace is highly constrained. Therefore, a plausible explanation for the observed pattern is that there is rapid morphological divergence among commensal species regardless of phylogenetic relatedness.

The high level of morphological divergence among commensal species is likely driven by the host-commensal associations. Many commensal species are host-specific and they directly attach to the hosts' body walls or even occupy the hosts' body cavities [83]. Such obligate and specialized associations often require host-specific adaptations and the clam shells are usually shaped to fit the available attachment

spaces. Therefore, attachment mechanisms/positions likely have great influence on the shell morphologies of commensal species. For example, an obligate hermit crab commensal species possesses unique crescent-shaped shells that conform to the hosts' coiled snail shells [111]. Because closely related commensal species sometimes occupy very different host species, it is to be expected that their shell morphologies do not reflect phylogenetic affinity, but rather similarities among the micro-habitats they occupy, which may result in different levels of morphological convergence.

Our analyses of galeommatoidean morphologies are based on shell shapes and sizes. However, many species also posses complex soft tissue structures, such as hypertrophied mantles that facultatively or permanently cover the shells [74, 78, 79]. The mantles can form expanded brood chambers [83], or are further elaborated into innervated, extendable papillae and tentacles. Functions of these soft-tissue structures are poorly understood, but limited studies suggest that they serve autotomizing/secretory functions and are likely associated with defensive behaviors [78, 81, 82]. These structures could be especially important to the free-living species as they may be under much higher predation pressure than the commensals. Therefore, to further understand the impact of lifestyles on galeommatoidean morphological evolution, close examination of the evolution of soft tissue structures is also needed.

5.5 Conclusion

In summary, both free-living and commensal species contribute significantly to the galeommatoidean diversity. However, the evolution of the two groups are influenced by distinct sets of biotic and abiotic factors. Free-living species are likely experiencing more intense interspecific competition and higher predation pressure while commensals are more constrained by their host associations. These biotic interactions are in turn governed by one important abiotic factor: benthic habitat types. Our study demonstrates that large-scale marine diversification processes are likely shaped by the inseparable interactions between abiotic and biotic factors and neither component can be neglected if we wish to fully understand patterns of marine macroevolution. Especially, the inclusion of biotic factors should be more widely applied to studies on neontological marine diversification.

5.6 Supplementary Materials

5.6.1 Detailed specimen information

Species	Phylogeny_Tip_ID	Subclade	Ecology	Host_type	Latitude	Longitude	Source (Voucher)
Anisodevonia ohshimai	Anisodevonia_ohshimai	CS1	commensal	sea cucumber	24.4	124.2	genbank
Arthritica japonica	Arthritica_japonica	FS1	commensal	crab	34.1	133	genbank
Arthritica semen	S82676	FS1	free-living		-31.95	115.9	WAM S82676
Austrodevonia sharnae	AShar	CS1	commensal	sea cucumber	-33.74	151.31	UM304391
Barrimysia cumingii	HPC1324	CS6	free-living		24.39	123.82	UM302916
Barrimysia siphonosomae	BSiph	CS6	commensal	peanut worm	22.2	114.2	UM302941
Basterotia carinata	Basterotia_carinata	clade d	commensal	spoon worm	28.1	129.2	genbank
Basterotia gouldi	Basterotia_gouldi	clade d	commensal	spoon worm	34.3	132.6	genbank
Basterotia sp	Basterotia_sp	clade d	commensal	spoon worm	24.4	124.2	genbank
Basterotia sp1	MN7634	clade d	commensal	unknown	-15.38	167.19	MN7634
Borniola lepida	C468609	CS5	free-living		-33.74	151.31	C468609
Borniola reniformis	BReni	FS3	free-living		-36.6	174.8	UM302927
Brachiomya cf stigmatica	MN7689	CS2	commensal	sea urchin	9.6	123.75	MN7689
Byssobornia deshayesiana	HPC1354	NA	free-living		24.36	124.11	UM302925
Byssobornia yamakawai	Byssobornia_yamakawai	CS6	commensal	spoon worm	24.4	124.2	genbank
Curvemysella paula	HPC2105	CS4	commensal	hermit crab	35.2	139.6	UM302934
Curvemysella sp	MN31676	CS4	commensal	hermit crab	-25.44	44.91	MN31676
Devonia semperi	Devonia_semperi	CS1	commensal	sea cucumber	34.4	132.9	genbank
Divaricella irpex	C448361	outgroup	NA		NA	NA	C448361
Divariscintilla luteocrinita	F318896	CS7	commensal	mantis shrimp	27.46	-80.3	F318896
Divariscintilla sp	M301615	CS7	commensal	mantis shrimp	-45.9	170.7	M301615
Divariscintilla toyohiwakensis	Divariscintilla_toyohiwakensis	CS7	commensal	mantis shrimp	33.6	131.2	genbank
Divariscintilla yoyo	F254	CS7	commensal	mantis shrimp	27.46	-80.3	F254
Entovalva lessonothuriae	Entovalva_lessonothuriae	CS1	commensal	sea cucumber	24.4	124.2	genbank
Entovalva sp1	MN6957	CS1	commensal	sea cucumber	9.57	123.82	MN6957
Entovalva sp2	MN7623	CS1	commensal	sea cucumber	-15.56	167.28	MN7623
Ephippodonta gigas	Ephippodonta_gigas	FS7	commensal	ghost shrimp	28.2	129.3	genbank
Ephippodonta lunata	C432607	FS2	commensal	slow shrimp	-35.08	137.75	C432607
Ephippodontoana macdougalli	MN24133	FS2	commensal	slow shrimp	-33.92	121.91	MN24133
Ephippodontomorpha hirsutus	C452337	CS7	commensal	mantis shrimp	-19.17	146.84	C452337
Galeomma ambigua	PS411	FS7	free-living		1.2	103.8	PS411
Galeomma ambigua	PS412	FS7	free-living		1.2	103.8	PS412
Galeomma sp	Galeomma_sp	FS7	free-living		24.3	123.8	genbank
Galeomma sp1	PS414	FS7	free-living		1.2	103.8	PS414
Galeomma turtoni	Gturtoni	FS8	free-living		42.3	3.2	UM304394
Gastrochaenidae	UF426031	outgroup	NA		NA	NA	UF426031
HPC1188	HPC1188	FS3	free-living		31.25	139.58	UM302937
HPC2111	HPC2111	FS3	free-living		35.25	139.57	UM302923
HPC2113	HPC2113	CS5	commensal	boring bivalve	35.25	139.57	HPC2113
Kellia japonica	HPC2080	FS9	free-living		32.55	130.11	UM302931
Kellia porculus1	HPC154	FS9	free-living		35	139.8	UM302922
Kellia porculus2	HPC2082	FS9	free-living		32.55	130.11	UM302924
Kellia suborbicularis	KLap	FS9	free-living		34.41	-119.89	UM304396
Koreamya arcuata	TUMC111020	CS4	commensal	brachiapods	36.13	126.58	UM302947.1
Lasaea australis F	LAusF	FS1	free-living		-33.9	121.9	UM303933
Lasaea australis M	LAusM	FS1	free-living		-38.3	144.3	UM303930
Lasaea australis P	LAusP	FS1	free-living		-33.8	151.2	UM303929
Lasaea colmani	LCol	FS1	free-living		-33.8	151.2	UM303935
Lasaea rubra	Lasaea_rubra	FS1	free-living		NA	NA	genbank
Lasaea sp	LHK	FS1	free-living		22.2	114.25	UM303937
Lasaea undulata	Lasaea_undulata	FS1	free-living		34.5	133.5	genbank
Litigiella pacifica	Litigiella_pacifica	NA	commensal	peanut worm	24.4	124.2	genbank
Lucinidae gen sp1	MN20039	outgroup	NA		NA	NA	MN20039
Lucinidae gen sp2	MN20044	outgroup	NA		NA	NA	MN20044
Marikellia solida	C468616	NA	free-living		-33.74	151.31	C468616
Melliteryx acupuncta	C468623	NA	free-living		-33.74	151.31	C468623
Melliteryx puncticulata	Melliteryx_puncticulata	FS2	free-living		33	132.6	genbank
MN13196	MN13196	FS7	free-living		-25.55	45.11	MN13196
MN13197	MN13197	FS5	free-living		-25.43	44.94	MN13197
MN16635	MN16635	CS6	unknown		-25.42	47.05	MN16635
MN16643	MN16643	CS2	commensal	sea urchin	14.92	123.2	MN16643
MN16650	MN16650	NA	commensal	shrimp	15.95	121.75	MN16650
MN16661	MN16661	CS2	commensal	sea urchin	-25.04	47	MN16661
MN19370	MN19370	NA	free-living		-25.03	47	MN19370
MN19374	MN19374	NA	free-living		-25.45	44.93	MN19374
MN19377	MN19377	FS2	free-living		-25.03	47	MN19377
MN19380	MN19380	FS9	free-living		-25.03	47	MN19380
MN19389	MN19389	NA	free-living		-25.03	47	MN19389
MN19390	MN19390	FS4	free-living		-25.02	47.01	MN19390
MN19391	MN19391	NA	free-living		-25.02	47.01	MN19391
MN19392	MN19392	NA	free-living		-25.02	47.01	MN19392
MN19395	MN19395	FS7	free-living		-25.03	47	MN19395

MN19396	MN19396	NA	free-living		-25.05	47	MN19396
MN19401	MN19401	FS4	free-living		-25.03	47	MN19401
MN19413	MN19413	FS7	free-living		-25.59	45.14	MN19413
MN19414	MN19414	FS3	free-living		-25.58	45.13	MN19414
MN19416	MN19416	FS4	free-living		-25.06	46.96	MN19416
MN19421	MN19421	FS3	free-living		-25.58	45.13	MN19421
MN19423	MN19423	FS9	free-living		-25.44	44.94	MN19423
MN19436	MN19436	CS4	commensal	sponge	-25.04	47.01	MN19436
MN19439	MN19439	FS2	free-living		-25.03	47	MN19439
MN19440	MN19440	FS4	free-living		-25.02	47.01	MN19440
MN19441	MN19441	FS6	free-living		-25.14	46.8	MN19441
MN19446	MN19446	NA	free-living		-25.58	45.13	MN19446
MN19448	MN19448	FS8	free-living		-25.04	47.01	MN19448
MN19451	MN19451	CS3	unknown		-25.03	47	MN19451
MN19453	MN19453	FS1	free-living		-24.98	47.1	MN19453
MN19459	MN19459	FS5	free-living		-25.48	44.97	MN19459
MN19462	MN19462	NA	free-living		-25.45	44.93	MN19462
MN19466	MN19466	FS5	free-living		-25.03	47	MN19466
MN19470	MN19470	NA	free-living		-25.16	46.75	MN19470
MN19475	MN19475	NA	free-living		-27.6	-144.32	MN19475
MN19487	MN19487	CS2	commensal	sea urchin	-24.99	47.09	MN19487
MN19489	MN19489	CS2	commensal	sea urchin	-24.99	47.09	MN19489
MN20031	MN20031	FS1	free-living		-24.98	47.1	MN20031
MN20045	MN20045	CS2	commensal	sea urchin	16.02	121.9	MN20045
MN24110	MN24110	FS8	free-living		-26.04	32.89	MN24110
MN24116	MN24116	NA	free-living		-35.06	117.95	MN24116
MN24118	MN24118	FS9	free-living		-35.08	117.97	MN24118
MN24134	MN24134	FS5	free-living		-33.92	121.91	MN24134
MN24144	MN24144	FS5	free-living		-34.97	118.18	MN24144
MN24146	MN24146	FS5	free-living		-34.97	118.18	MN24146
MN24147	MN24147	FS5	free-living		-34.97	118.18	MN24147
MN24148	MN24148	FS5	free-living		-34.97	118.18	MN24148
MN37140	MN37140	NA	free-living		-15.48	167.26	MN37140
MN6726	MN6726	FS5	free-living		-15.48	167.26	MN6726
MN6727	MN6727	FS7	free-living		-15.58	167.21	MN6727
MN6740	MN6740	FS2	free-living		-15.48	167.25	MN6740
MN6744	MN6744	CS3	free-living		-15.38	167.2	MN6744
MN6764	MN6764	NA	free-living		9.69	123.85	MN6764
MN6765	MN6765	CS4	unknown		9.69	123.85	MN6765
MN6766	MN6766	FS6	free-living		9.62	123.77	MN6766
MN6767	MN6767	CS2	free-living		9.52	123.69	MN6767
MN6769	MN6769	CS3	unknown		9.64	123.86	MN6769
MN6944	MN6944	CS4	unknown		9.61	123.87	MN6944
MN6949	MN6949	FS6	free-living		9.69	123.85	MN6949
MN6956	MN6956	FS7	free-living		9.69	123.85	MN6956
MN6965	MN6965	CS2	unknown		9.62	123.77	MN6965
MN6974	MN6974	CS6	unknown		-15.44	167.25	MN6974
MN7602	MN7602	NA	commensal	limpets?	-15.56	167.21	MN7602
MN7605	MN7605	FS5	free-living		-15.56	167.21	MN7605
MN7606	MN7606	FS2	free-living		-15.51	167.02	MN7606
MN7609	MN7609	FS2	free-living		-15.56	167.21	MN7609
MN7610	MN7610	CS2	free-living		-15.61	167.02	MN7610
MN7614	MN7614	FS7	free-living		-15.64	167.25	MN7614
MN7616	MN7616	NA	free-living		-15.54	167.28	MN7616
MN7620	MN7620	FS4	free-living		9.52	123.68	MN7620
MN7621	MN7621	FS6	free-living		9.64	123.86	MN7621
MN7625	MN7625	FS7	free-living		-15.55	167.3	MN7625
MN7626	MN7626	FS7	free-living		-15.46	167.26	MN7626
MN7628	MN7628	FS7	free-living		-15.56	167.21	MN7628
MN7631	MN7631	CS2	unknown		-7.72	156.42	MN7631
MN7633	MN7633	NA	free-living		9.63	123.78	MN7633
MN7636	MN7636	FS6	free-living		-15.56	167.21	MN7636
MN7641	MN7641	FS7	free-living		-15.52	167.2	MN7641
MN7642	MN7642	FS4	free-living		-15.58	167.21	MN7642
MN7644	MN7644	FS6	unknown		-15.56	167.28	MN7644
MN7646	MN7646	CS3	unknown		NA	NA	MN7646
MN7658	MN7658	CS2	unknown		8.75	123.3	MN7658
MN7664	MN7664	NA	commensal	siponculan	-15.58	167.21	MN7664
MN7670	MN7670	NA	free-living		9.68	123.85	MN7670
MN7673	MN7673	CS2	commensal	sea urchin	9.5	123.92	MN7673
MN7676	MN7676	NA	commensal	sea cucumber	-7.72	156.42	MN7676
MN7686	MN7686	FS2	commensal	sea urchin	9.56	123.71	MN7686
MN7701	MN7701	CS2	commensal	sea urchin	9.6	123.75	MN7701

Montacutella cf sp1	MN7695	CS2	commensal	sea urchin	9.6	123.75	MN7695
Montacutella cf sp2	Z65120	CS2	commensal	sea urchin	-28.7	114.04	WAM Z65120
Montacutona japonica	HPC1424	CS4	unknown		35.31	139.79	UM302936
Montacutona sp	Montacutona_sp	CS4	commensal	sea anemone	33.3	132.6	genbank
Mysella cf sp	Kurtiella_affbidentata	CS3	unknown		34.2	133.1	genbank
Mysella charcoti	MChar	CS5	free-living		-67.1	-68	UM304397
Mysella pedroana	MPedr	CS3	commensal	hermit crab	34.4	-119.9	UM302940
Mysella planulara	MPlan	CS3	unknown		41.3	-72	UM304392
Mysella sp1	S82677	CS5	commensal	shrimp	-32.56	115.74	WAM S82677
Mysella vitrea	C468618	CS5	commensal	shrimp	-35.26	150.5	C468618
Neolepton sp	C436134	outgroup	NA		NA	NA	C436134
Nesobornia sp	Neso	FS2	free-living		31.29	130.21	UM302938
Nipponomontacuta actinariophila	Nipponomontacuta_actinariophila	CS4	commensal	sea anemone	33	132.6	genbank
Nipponomysella oblongata	HPC2114	CS4	free-living		35.25	139.57	UM302926
Nipponomysella subtruncata	NSubt	CS1	commensal	peanut worm	31.25	130.65	UM302935
Paraborniola matsumotoi	Paraborniola_matsumotoi	FS7	free-living		34.3	132.6	genbank
Peregrinamor gastrochaenans	Peregrinamor_gastrochaenans	CS6	commensal	mud shrimp	28.3	129.4	genbank
Peregrinamor ohshimai	C468611	CS6	commensal	mud shrimp	-33.43	133.4	C468611
Phlyctaenachlamys lysiosquillina	UF436851	CS7	commensal	mantis shrimp	-17.49	-149.91	UF436851
Pseudogaleomma japonica	Pseudogaleomma_japonica	FS7	free-living		NA	NA	genbank
Pseudogaleomma sp	Pseudogaleomma_sp	FS7	free-living		24.3	123.8	genbank
Pseudopythina aff ariake	Pseudopythina_affariake	CS6	commensal	sea cucumber	34.3	132.6	genbank
Pseudopythina aff nodosa	Pseudopythina_affnodosa	CS6	commensal	peanut worm	28.26	129.46	genbank
Pseudopythina macrophthalmensis	Pseudopythina_macrophthalmensis	CS6	commensal	crab	24.4	124.2	genbank
Pseudopythina ochetostomae	POche	CS6	commensal	spoon worm	22.42	114.23	UM302942
Pseudopythina subsinuata	Pseudopythina_subsinuata	CS6	commensal	mantis shrimp	34.3	132.6	genbank
Radobornia sp1	MN6733	NA	free-living		-15.58	167.21	MN6733
Radobornia sp2	HPC2112	NA	free-living		35.25	139.57	UM302933
Rochefortia tumida	RTumi	CS5	commensal	polychaet worm	48.66	-123.45	UM304393
Salpocola philippinensis	Salpocola_philippinensis	NA	commensal	peanut worm	28.3	129.4	genbank
Scintilla aff hydatina	Scintilla_affhydatina	FS4	free-living		24.4	124.2	genbank
Scintilla cf timorensis	HPC2125	NA	free-living		13.42	121.16	UM302913
Scintilla cuvieri	PS420	FS6	free-living		1.2	103.8	PS420
Scintilla ovulina	PS409	FS6	free-living		1.2	103.8	PS409
Scintilla phillipinensis	PS495	NA	free-living		1.2	103.8	PS495
Scintilla rosea	Scintilla_rosea	FS7	free-living		28.1	129.2	genbank
Scintilla sp1	PS421	NA	free-living		1.2	103.8	PS421
Scintilla sp2	ScintiJ	FS7	free-living		24.9	125.28	UM302920
Scintilla sp7	Scintilla_sp7	FS6	free-living		24.3	123.8	genbank
Scintilla sp8	Scintilla_sp8	NA	free-living		24.4	124.2	genbank
Scintilla strangei	SStr	FS5	free-living		-14.65	150.54	MAL72830
Scintilla violescens	HPC910	FS6	free-living		31.25	130.65	UM302913
Scintillona bellerophon	SBell	CS5	commensal	sea cucumber	48.36	-123.72	RBC INVT 011:2001
SpAH	SpAH	NA	commensal	peanut worm	15.88	121.88	MN16649/16651
SpAX	SpAX	FS2	free-living		-15.48	167.26	MN6940
SpBL	SpBL	FS3	free-living		-25.44	44.94	MN19468/19465
SpBQ	SpBQ	FS7	free-living		-15.46	167.26	MN7613/7635
SpBT	SpBT	FS6	free-living		-15.48	167.26	MN6729/7608
SpCF	SpCF	FS7	free-living		-25.03	47	MN19375/19437
SpCG	SpCG	FS8	free-living		-25.04	47.01	MN19376/19404
Spengieria sp	UF450497	outgroup	NA		NA	NA	UF450497
Spengleria rostrata	UF450420	outgroup	NA		NA	NA	UF450420
Tellimya cf sp	Tell	NA	unknown		30.17	127.86	UM302928
UF289017	UF289017	FS2	free-living		13.5	144.8	UF289017
UF291831A	UF291831A	FS2	free-living		-15.13	-148.23	UF291831A
UF292279	UF292279	FS5	free-living		13.5	144.8	UF292279
UF296115	UF296115	FS2	free-living		-10.5	105.7	UF296115
UF296549	UF296549	FS2	free-living		-0.31	121.98	UF296549
UF305072	UF305072	FS2	free-living		13.42	144.79	UF305072
UF337873A	UF337873A	FS2	free-living		-8.7	115.5	UF337873A
UF367967	UF367967	CS3	commensal	sea urchin	23.68	58.5	UF367967
UF372711A	UF372711A	NA	free-living		8.33	-79.11	UF372711A
UF375846	UF375846	FS2	free-living		-14.91	145.49	UF375846
UF375859	UF375859	FS2	free-living		-14.73	145.59	UF375859
UF392522A	UF392522A	FS2	free-living		-21.2	-159.79	UF392522A
UF423581	UF423581	FS8	free-living		-13.58	47.82	UF423581
UF423591	UF423591	CS2	commensal	sea urchin	-13.41	48.29	UF423591
UF423655	UF423655	FS7	free-living		-13.49	47.98	UF423655
UF423947	UF423947	FS7	free-living		-13.3	48.15	UF423947
UF428447A	UF428447A	FS2	free-living		-14.45	145.5	UF428447A
UF434671	UF434671	FS8	free-living		-22.66	113.62	UF434671
UF436141	UF436141	FS8	free-living		-22.61	113.64	UF436141
UF436804	UF436804	CS7	commensal	burrow	-17.49	-149.91	UF436804

UF441374	UF441374	FS8	free-living		-23.47	151.95	UF441374
UF447622	UF447622	FS8	free-living		-17.5	-149.86	UF447622
Varatoga cryptozoica	C468612	FS4	commensal	shrimp	-34.06	151.13	C468612
Waldo arthuri	Waldo	NA	commensal	sea urchin	53.05	-125.14	UM303919
Waldo digitatus	WaldoSpB	NA	commensal	sea urchin	-47.75	-65.87	UM303928
Waldo paucitentaculatus	WaldoSpA	NA	commensal	sea urchin	-47.75	-65.87	UM303927

Voucher Abbriviations	
C/MAL	Australian Museum
F	Field Museum
HPC	National Museum of Nature and Science (Japan)
M	South Australian Museum
MN	Muséum National d'Histoire Naturelle (France)
PS	Project Semakau, Raffles Museum of Biodiversity Research (Singapore)
RBC	Royal British Columbia Museum (Canada)
UF	Florida Museum of Natural History
UM	University of Michigan Museum of Zoology
WAN	Western Australian Museum

5.6.2 Detailed Galeommatoidea phylogeny

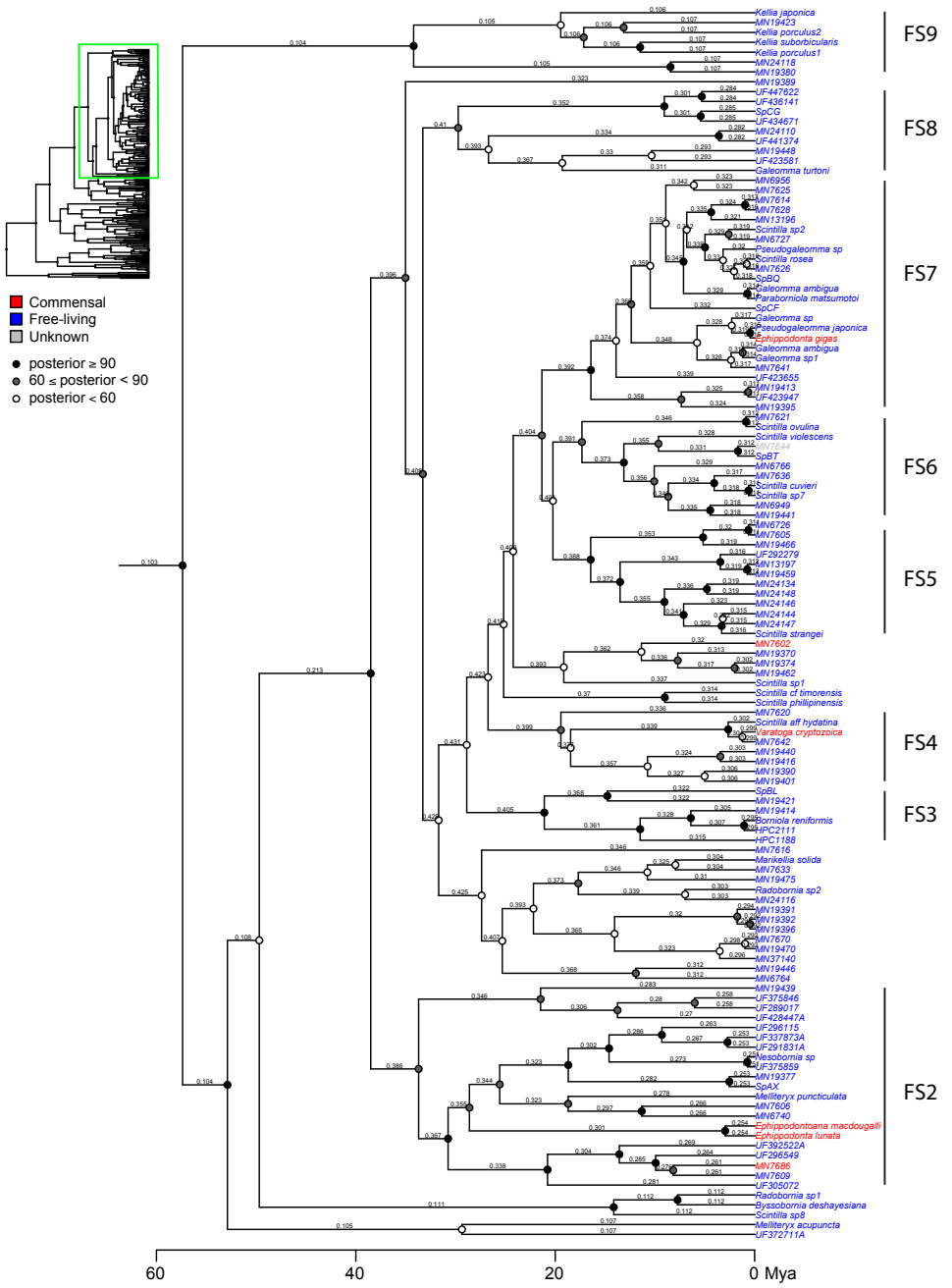

Figure 5.6: Bayesian phylogeny of Galeommatoidea (consistent with Fig. 5.2A), showing details of subclades FS2-9. Numbers above branches represent estimated speciation rates.

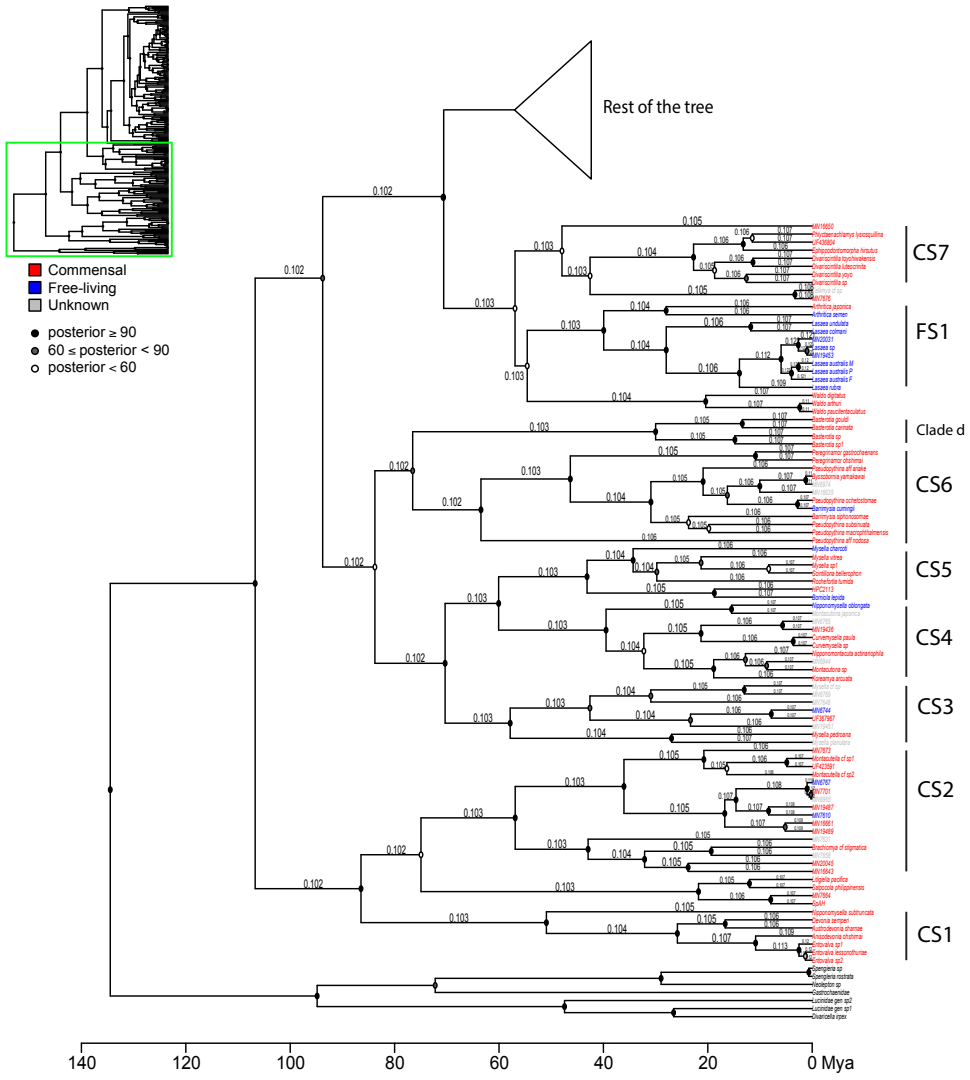

Figure 5.7: Bayesian phylogeny of Galeommatoidea (consistent with Fig. 5.2), showing details of subclades CS1-7 and FS1. Numbers above branches represent estimated speciation rates.

5.6.3 Latitudinal distributions mapped on phylogeny

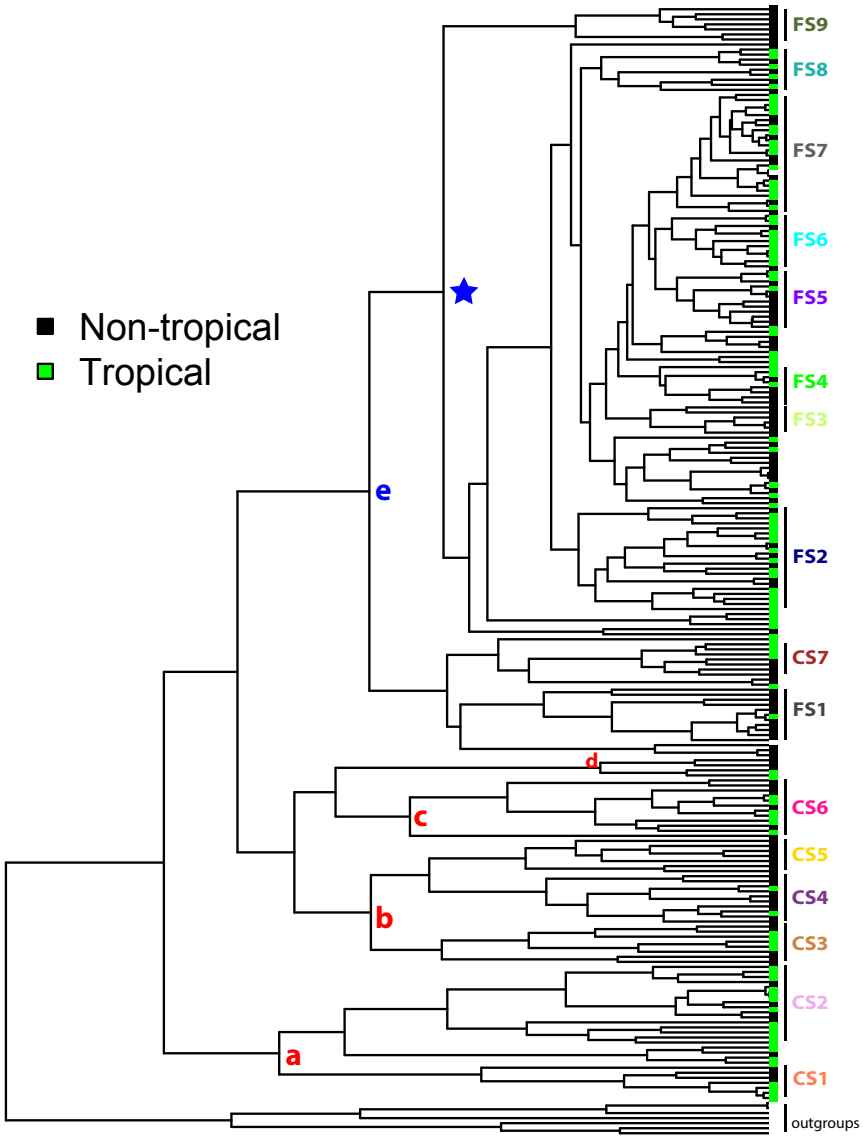

Figure 5.8: Time-calibrated molecular phylogeny of Galeommatoidea. Tip labels indicate the geographical origin (tropical vs. non-tropical) of each terminal. Clade and subclade labels are as for Fig. 5.2. A phylogenetic logistic regression analysis indicates that the free-living lifestyle is not significantly correlated with tropical distributions.

CHAPTER 6

Conclusions and Future Directions

Using the superfamily Galeommatoidea as a study system, this dissertation only begins to unveil the complex interactions between abiotic and biotic factors in shaping neontological marine diversity. In Galeommatoidea, the commensal lifestyle not only represents an adaptation for sediment-dwelling (Ch. 2), but also an important driver for rapid morphological diversification (Ch. 3 and 5). On the other hand, one major transition from soft-bottom to hard-bottom habitats is coupled with a fast radiation of crevice-dwelling, free-living lineages (Ch. 4 and 5). Therefore, both habitat types and biotic associations are playing important roles in shaping the evolution of Galeommatoidea; while many other potentially important ecological factors (*e.g.*, sexual selection, developmental mode, predation, *etc.*) remain unexplored and certainly require further examination.

Although the dissertation is focused on galeommatoidean clams, its general implications are likely applicable to other systems. For example, one important abiotic factor discussed in chapters 2 and 5 – benthic substrate type – likely acts as a strong selection pressure for the evolution of other marine symbiotic associations, such as animal-microbe chemosymbioses that evolved independently in various marine invertebrates [346]. Such associations, in turn, can provide novel mechanisms of lineage diversification (*e.g.*, symbiont-driven habitat partitioning [347]) and further change the evolutionary trajectory of participating taxa [348]. So far, such themes have not been systematically studied and call for thorough examinations across diverse taxa.

Recent development of sampling and methodological approaches allow testing of evolutionary hypotheses on relatively large scales. However, macroevolutionary analyses tend to detect patterns rather than the underlying biological process. For example, it is possible to show that the evolution of certain traits (*e.g.*, biotic associations) is correlated with changes of diversification patterns [309, 348], but it cannot im-

mediately be concluded that such traits actually drove the observed changes (*i.e.*, causal relationships). Although several general macroevolutionary models have been developed for both lineage diversification (*e.g.*, early burst [331]) and morphological evolution (*e.g.*, Ornstein-Uhlenbeck models [334]), each model can correspond to multiple biological processes which are difficult to distinguish. Unaided, these models cannot statistically detect the effect of biotic interactions on macroevolutionary patterns.

Given this, several approaches can be taken in order to better understand the way biotic interactions influence marine biodiversification. First of all, it is desirable to gather independent lines of evidence that support macroevolutionary hypotheses. Besides phylogenetic information, other aspects of ecological interactions between targeted groups need to be addressed, including behavior, physiology, biochemistry and functional genomics, *etc.* In addition, development of new model organisms [349] for studying marine biotic interactions at cellular levels may facilitate the merging of organismal biology and macroevolution. Another important aspect that calls for more attention is the link between microevolutionary and macroevolutionary processes. As microevolutionary processes may directly influence macroevolutionary patterns [343], new sampling strategies (*e.g.*, collecting population level data for each species) and methodologies (*e.g.*, incorporating coalescent processes [350]) need to be employed. Lastly, statistical approaches that allow testing of complex ecological scenarios (*e.g.*, Approximate Bayesian Computation [351]) should be given more consideration, as this could allow researchers to incorporate biotic interactions into existing macroevolutionary models and generate reasonable theoretical expectations.

BIBLIOGRAPHY

[1] Sendall K.A., Fontaine A.R., and Ó Foighil D., 1995. Tube torphology and activity patterns related to feeding and tube building in the polychaete *Mesochaetopterus taylori* Potts. *Canadian Journal of Zoology*, 73:509–517.

[2] Bennett I. and Pope E.C., 1953. Intertidal zonation of the exposed rocky shores of Victoria, together with a rearrangement of the biogeographical provinces of temperate Australian shores. *Marine and Freshwater Research*, 4:105–159.

[3] James N.P. and Bone Y., 2010. *Neritic carbonate sediments in a temperate realm: Southern Australia*. Springer, Dordrecht.

[4] Ó Foighil D. and Jozefowicz C., 1999. Amphi-Atlantic phylogeography of direct-developing lineages of *Lasaea*, a genus of brooding bivalves. *Marine Biology*, 135:115–122.

[5] Ó Foighil D., 1989. Planktotrophic larval development is associated with a restricted geographic range in *Lasaea*, a genus of brooding, hermaphroditic bivalves. *Marine biology*, 103:349–358.

[6] Barrows T.T. and Juggins S., 2005. Sea-surface temperatures around the Australian margin and Indian Ocean during the Last Glacial Maximum. *Quaternary Science Reviews*, 24:1017–1047.

[7] Caterino M.S. and Chatzimanolis S., 2009. Conservation genetics of three flightless beetle species in southern California. *Conservation genetics*, 10:203–216.

[8] Darwin C., 1859. *The Origin of Species by Means of Natural Selection, or the Preservation of Favoured Races In the Struggle for Life*. John Murray, London.

[9] Benton M.J., 2009. The Red Queen and the Court Jester: species diversity and the role of biotic and abiotic factors through time. *Science*, 323:728–732.

[10] Futuyma D.J. and Agrawal A.A., 2009. Macroevolution and the biological diversity of plants and herbivores. *Proceedings of the National Academy of Sciences USA*, 106:18054–18061.

[11] Lessios H.A., 2008. The great American schism: divergence of marine organisms after the rise of the Central American Isthmus. *Annual Review of Ecology, Evolution, and Systematics*, 39:63–91.

[12] Renema W., Bellwood D.R., Braga J.C., Bromfield K., Hall R., Johnson K.G., Lunt P., Meyer C.P., McMonagle L.B., Morley R.J., O'Dea A., Todd J.A., Wesselingh F.P., Wilson M.E.J., and Pandolfi J.M., 2008. Hopping hotspots: global shifts in marine biodiversity. *Science*, 321:654–657.

[13] Cárdenas A.L. and Harries P.J., 2010. Effect of nutrient availability on marine origination rates throughout the Phanerozoic eon. *Nature Geoscience*, 3:430–434.

[14] Dartnall A., 1974. Littoral biogeography. In W. Williams, editor, *Biogeography and Ecology in Tasmania*, pages 171–194. Junk, The Hague.

[15] Avise J.C., 2000. *Phylogeography: the History and Formation of Species*. Harvard University Press, Cambridge.

[16] Barber P.H., Palumbi S.R., Erdmann M.V., and Moosa M.K., 2000. Biogeography: A marine Wallace's line? *Nature*, 406:692–693.

[17] Riginos C., 2005. Cryptic vicariance in Gulf of California fishes parallels vicariant patterns found in Baja California mammals and reptiles. *Evolution*, 59:2678–2690.

[18] Shen K.N., Jamandre B.W., Hsu C.C., Tzeng W.N., and Durand J.D., 2011. Plio-Pleistocene sea level and temperature fluctuations in the northwestern Pacific promoted speciation in the globally-distributed flathead mullet *Mugil cephalus*. *BMC Evolutionary Biology*, 11:83.

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

[20] Williams S.T. and Duda T.F., 2008. Did tectonic activity stimulate oligo-miocene speciation in the Indo-West Pacific? *Evolution*, 62:1618–1634.

[21] Reaka M.L., Rodgers P.J., and Kudla A.U., 2008. Colloquium Paper: Patterns of biodiversity and endemism on Indo-West Pacific coral reefs. *Proceedings of the National Academy of Sciences USA*, 105:11474–11481.

[22] Barber P.H., 2009. The challenge of understanding the Coral Triangle biodiversity hotspot. *Journal of Biogeography*, 36:1845–1846.

[23] Bellwood D.R. and Meyer C.P., 2009. Endemism and evolution in the Coral Triangle: a call for clarity. *Journal of Biogeography*, 36:210–212.

[24] Briggs J.C., 2009. Diversity, endemism and evolution in the Coral Triangle. *Journal of Biogeography*, 36:2008–2010.

[25] Marko P.B., Hoffman J.M., Emme S.A., Mcgovern T.M., Keever C.C., and Cox L.N., 2010. The 'Expansion-Contraction' model of Pleistocene biogeography: rocky shores suffer a sea change? *Molecular Ecology*, 19:146–169.

[26] Ilves K.L., Huang W., Wares J.P., and Hickerson M.J., 2010. Colonization and/or mitochondrial selective sweeps across the North Atlantic intertidal assemblage revealed by multi-taxa approximate Bayesian computation. *Molecular Ecology*, 19:4505–4519.

[27] Kelly R.P. and Palumbi S.R., 2010. Genetic structure among 50 species of the northeastern Pacific rocky intertidal community. *PLoS ONE*, 5:e8594.

[28] Alroy J., 2008. Dynamics of origination and extinction in the marine fossil record. *Proceedings of the National Academy of Sciences USA*, 366:11536–11542.

[29] Vermeij G.J., 1977. The Mesozoic marine revolution: evidence from snails, predators and grazers. *Paleobiology*, 3:245–258.

[30] Vermeij G.J., 2008. Escalation and its role in Jurassic biotic history. *Palaeogeogr Palaeoclimatol Palaeoecol*, 263:3–8.

[31] Kosnik M.A., Alroy J., Behrensmeyer A.K., Fürsich F.T., Gastaldo R.A., Kidwell S.M., Kowalewski M., Plotnick R.E., Rogers R.R., and Wagner P.J., 2011. Changes in shell durability of common marine taxa through the Phanerozoic: evidence for biological rather than taphonomic drivers. *Paleobiology*, 37:303–331.

[32] Ezard T.H.G., Aze T., Pearson P.N., and Purvis A., 2011. Interplay between changing climate and species' ecology drives macroevolutionary dynamics. *Science*, 332:349–351.

[33] Reid D.G., Dyal P., Lozouet P., Glaubrecht M., and Williams S.T., 2008. Mudwhelks and mangroves: the evolutionary history of an ecological association (Gastropoda: Potamididae). *Molecular Phylogenetics and Evolution*, 47:680–699.

[34] Lanterbecq D., Rouse G.W., and Eeckhaut I., 2010. Evidence for co-speciation events in the host-symbiont system involving crinoids (Echinodermata) and their obligate associates, the myzostomids (Myzostomida, Annelida). *Molecular Phylogenetics and Evolution*, 54:357–371.

[35] Frey M.A., 2010. The relative importance of geography and ecology in species diversification: evidence from a tropical marine intertidal snail (*Nerita*). *Journal of Biogeography*, 37:1515–1528.

[36] Hultgren K.M. and Duffy J.E., 2011. Multi-locus phylogeny of sponge-dwelling snapping shrimp (Caridea: Alpheidae: *Synalpheus*) supports morphology-based species concepts. *Journal of Crustacean Biology*, 31:352–360.

[37] Lanterbecq D., Rouse G., and Eeckhaut I., 2009. Bodyplan diversification in crinoid-associated myzostomes (Myzostomida, Protostomia). *Invertebrate Biology*, 128:283–301.

[38] Hultgren K. and Duffy J., 2010. Sponge host characteristics shape the community structure of their shrimp associates. *Marine Ecology Progress Series*, 407:1–12.

[39] Thompson A.R., Thacker C.E., and Shaw E.Y., 2005. Phylogeography of marine mutualists: parallel patterns of genetic structure between obligate goby and shrimp partners. *Molecular Ecology*, 14:3557–3572.

[40] Obst M., Funch P., and Giribet G., 2005. Hidden diversity and host specificity in cycliophorans: a phylogeographic analysis along the North Atlantic and Mediterranean Sea. *Molecular Ecology*, 14:4427–4440.

[41] Richards V.P., Thomas J.D., Stanhope M.J., and Shivji M.S., 2007. Genetic connectivity in the Florida reef system: comparative phylogeography of commensal invertebrates with contrasting reproductive strategies. *Molecular Ecology*, 16:139–157.

[42] Crandall E.D., Jones M.E., Muñoz M.M., Akinronbi B., Erdmann M.V., and Barber P.H., 2008. Comparative phylogeography of two seastars and their ectosymbionts within the Coral Triangle. *Molecular Ecology*, 17:5276–5290.

[43] Kochzius M., Seidel C., Hauschild J., Kirchhoff S., Mester P., Meyer-Wachsmuth I., Nuryanto A., and Timm J., 2009. Genetic population structures of the blue starfish *Linckia laevigata* and its gastropod ectoparasite *Thyca crystallina*. *Marine Ecology Progress Series*, 396:211–219.

[44] Jablonski D., 2008. Biotic interactions and macroevolution: extensions and mismatches across scales and levels. *Evolution*, 62:715–739.

[45] Valiente-Banuet A., Rumebe A.V., Verdú M., and Callaway R.M., 2006. Modern Quaternary plant lineages promote diversity through facilitation of ancient Tertiary lineages. *Proceedings of the National Academy of Sciences USA*, 103:16812–16817.

[46] Bronstein J.L., 2009. The evolution of facilitation and mutualism. *Journal of Ecology*, 97:1160–1170.

[47] McInnes L., Baker W.J., Barraclough T.G., Dasmahapatra K.K., Goswami A., Harmon L.J., Morlon H., Purvis A., Rosindell J., Thomas G.H., Turvey S.T., and Phillimore A.B., 2011. Integrating ecology into macroevolutionary research. *Biology Letters*, pages rsbl.2011.0358v1–rsbl20110358.

[48] Phillimore A.B. and Price T.D., 2008. Density-dependent cladogenesis in birds. *PLoS Biology*, 6:483–489.

[49] Rabosky D.L., 2009. Ecological limits and diversification rate: alternative paradigms to explain the variation in species richness among clades and regions. *Ecology Letters*, 12:735–743.

[50] Alroy J., 2010. The shifting balance of diversity among major marine animal groups. *Science*, 329:1191–1194.

[51] Chavan A., 1969. Superfamily Leptonacea Gray, 1847. In R.C. Moore, editor, *Treatise on Invertebrate Paleontology, Part N, Mollusca 6 (Bivalvia)*, pages 518–537. University of Kansas Press, Kansas.

[52] Pohlo R., 1982. Evolution of the Tellinacea (Bivalvia). *Journal of Molluscan Studies*, 48:245–256.

[53] Ponder W.F., 1998. Superfamily Galeommatoidea. In P.L. Beesley, G.J.B. Ross, and A. Wells, editors, *Mollusca: The Southern Synthesis*, pages 316–318. CSIRO Publishing, Melbourne.

[54] Cossmann M. and Pissarro G., 1904. *Iconographie Complète des Coquilles Fossiles de l'Éocène des Environs de Paris*. H. Bouillants, Paris.

[55] Huber M., 2010. *Compendium of Bivalves*. A Full-color Guide to 3300 of the World's Marine Bivalves. A Status on Bivalvia after 250 Years of Research. ConchBooks, Hackenheim.

[56] Morton B. and Valentich-Scott P., 1989. The Hong Kong Galeommatacea (Mollusca: Bivalvia) and their hosts, with descriptions of new species. *Asian Marine Biology*, 6:129–160.

[57] Paulay G., 2003. Marine Bivalvia (Mollusca) of Guam. *Micronesica*, 35-36:218–243.

[58] Oliver P.G. and Lützen J., 2011. An anatomically bizarre, fluid-feeding, galeommatoidean bivalve: *Draculamya Porobranchiata* gen. *et* sp. nov. (Mollusca: Bivalvia). *Journal of Conchology*, 40:365–392.

[59] Coan E.V., Valentich-Scott P., and Bernard F.R., 2000. *Bivalve Seashells of Western North America*. Santa Barbara Museum of Natural History, Santa Barbara.

[60] Lützen J. and Nielsen C., 2005. Galeommatid bivalves from Phuket, Thailand. *Zoological Journal of the Linnean Society*, 144:261–308.

[61] Mikkelsen P.M. and Bieler R., 2008. *Seashells of Southern Florida: Living Marine Mollusks of the Florida Keys and Adjacent Regions, Bivalves*. Princeton University Press, Princeton.

[62] Tunnell J.W., Andrews J., Barrera N.C., and Moertzsohn F., 2010. *Encyclopedia of Texas Seashells: Identification, Ecology, Distribution, and History*. Texas A&M University Press, College Station.

[63] Ó Foighil D. and Thiriot-Quiévreux C., 1999. Sympatric Australian *Lasaea species* (Mollusca: Bivalvia) differ in their ploidy levels, reproductive modes and developmental modes. *Zoological Journal of the Linnean Society*, 127:477–494.

[64] Jespersen Å. and Lützen J., 2009. Structure of sperm, spermatozeugmata and 'lateral organs' in the bivalve *Arthritica* (Galeommatoidea: Leptonidae). *Acta Zoologica*, 90:51–67.

[65] Lyons K.G., Brigham C.A., Traut B.H., and Schwartz M.W., 2005. Rare species and ecosystem functioning. *Conservation Biology*, 19:1019–1024.

[66] Vermeij G.J. and Grosberg R.K., 2010. The great divergence: when did diversity on land exceed that in the sea? *Integrative and Comparative Biology*, 50:675–682.

[67] Lim G.S., Balke M., and Meier R., 2011. Determining species boundaries in a world full of rarity: singletons, species delimitation methods. *Systematic Biology*, 60:4.

[68] Briggs J.C., 1999. Coincident biogeographic patterns: Indo-West Pacific ocean. *Evolution*, 53:326–335.

[69] Roberts C.M., McClean C.J., Veron J.E.N., Hawkins J.P., Allen G.R., McAllister D.E., Mittermeier C.G., Schueler F.W., Spalding M., Wells F., Vynne C., and Werner T.B., 2002. Marine biodiversity hotspots and conservation priorities for tropical reefs. *Science*, 295:1280–1284.

[70] Bellwood D.R., Hughes T.P., Connolly S.R., and Tanner J., 2005. Environmental and geometric constraints on IndoPacific coral reef biodiversity. *Ecology Letters*, 8:643–651.

[71] Crame J.A., 2000. Evolution of taxonomic diversity gradients in the marine realm: evidence from the composition of Recent bivalve faunas. *Paleobiology*, 26:188–214.

[72] Bouchet P., Lozouet P., Maestrati P., and Heros V., 2002. Assessing the magnitude of species richness in tropical marine environments: exceptionally high numbers of molluscs at a New Caledonia site. *Biological Journal of the Linnean Society*, 75:421–436.

[73] Schneider S. and Hochleitner R., 2006. Great diversity in small space - a remarkable bivalve association from the Lower Pliocene of Harokopio (SW Peloponnesus, Greece). In N. Malchus and J.M. Pons, editors, *International Congress on Bivalvia (23.07.-27.07.2006) - Scientific program and abstracts*, page 72. Universitat Autònoma de Barcelona., Barcelona.

[74] Morton B., 1981. The biology and functional morphology of *Chlamydoconcha orcutti* with a discussion on the taxonomic status of the Chlamydoconchacea (Mollusca: Bivalvia). *Journal of Zoology*, 195:81–121.

[75] Vermeij G.J., 2005. Shells inside out: the architecture, evolution and function of shell envelopment in molluscs. In D.E.G. Briggs, editor, *Evolving Form and Function: Fossils and Development: Proceedings of a Symposium Honoring Adolf Seilacher for His Contributions to Paleontology, in Celebration of His 80th Birthday*, pages 197–221. Peabody Museum of Natural History, Yale University, New Haven.

[76] Arakawa K.Y., 1960. Ecological observations on an aberrant lamellibranch, *Ephippodonta murakamii*. *Venus*, 21:50–61.

[77] Middelfart P., 2005. Review of *Ephippodonta sensu lato* (Galeommatidae: Bivalvia), with descriptions of new related genera and species from Australia. *Molluscan Research*, 25:129–144.

[78] Mikkelsen P.M. and Bieler R., 1992. Biology and comparative anatomy of three new species of commensal Galeommatidae, with a possible case of mating-behavior in bivalves. *Malacologia*, 34:1–24.

[79] Simone L.R.L., 2008. A new species of *Chlamydoconcha* Dall, 1884, from southeastern Brazil (Bivalvia: Chlamydoconchidae). *Nautilus*, 122:252–258.

[80] Judd W., 1971. The structure and habits of *Divariscintilla maoria* Powell (Bivalvia: Galeommatidae). *Journal of Molluscan Studies*, 39:343–354.

[81] Morton B., 1975. Dymantic display in *Galeomma polita* Deshayes (Bivalvia: Leptonacea). *Journal of Conchology*, 28:365–369.

[82] Morton B., 1976. Secondary brooding of temporary dwarf males in *Ephippodonta (Ephippodontina) oedipus* sp. nov.(Bivalvia: Leptonacea). *Journal of Conchology*, 29:31–39.

[83] Kato M., 1998. Morphological and ecological adaptations in montacutid bivalves endo- and ecto-symbiotic with holothurians. *Canadian Journal of Zoology*, 76:1403–1410.

[84] Bristow G.A., Berland B., Schander C., and Vo D.T., 2010. A new endosymbiotic bivalve (Heterodonta: Galeommatoidea), from Pacific holothurians. *Journal of Parasitology*, 96:532–534.

[85] Ó Foighil D. and Gibson A., 1984. The morphology, reproduction and ecology of the commensal bivalve *Scintillona bellerophon* spec. nov (Galeommatacea). *Veliger*, 27:72–80.

[86] Mikkelsen P.M. and Bieler R., 1989. Biology and comparative anatomy of *Divariscintilla yoyo* and *D. troglodytes*, two new species of Galeommatidae (Bivalvia) from stomatopod burrows in eastern Florida. *Malacologia*, 31:175–195.

[87] Yonge C.M. and Thompson T.E., 1976. *Living Marine Molluscs*. Collins, London.

[88] Ockelmann K.W. and Muus K., 1978. The biology, ecology, and behavior of the bivalve *Mysella bidentata* (Montagu). *Ophelia*, 17:1–93.

[89] Reid R.G.B., McMahon R., Ó Foighil D., and Finnegan R., 1992. Pedal-palp feeding in juvenile and adult bivalve molluscs. *The Veliger*, 35:93–104.

[90] Kerr G. and Corfield J., 1998. Association between the ghost shrimp *Trypaea australiensis* Dana 1852 (Crustacea : Decapoda) and a small deposit-feeding bivalve *Mysella vitrea* Laserson 1956 (Mollusca: Leptonidae). *Marine and Freshwater Research*, 49:801–806.

[91] Voeltzkow A., 1890. *Entovalva mirabilis*, eine schmarotzende Muschel aus dem Darm einer Holothurie. *Zoologische Jahrbücher, Abteilung für Systematik, Ökologi und Geographie der Tiere*, 5:619–628.

[92] Bruun A.F., 1938. A new endocommensalistic bivalve, *Entovalva major n.sp.*, from the Red Sea . *Videnskabelige Meddelelser Dansk Naturhis- torisk Forening*, 102:163–167.

[93] Wear R.G., 1966. Physiological and ecological studies on the bivalve *Arthritica bifurca* (Webster, 1908) living commensally with the tubiculous polychaete *Pectinaria australis* Ehlers, 1905. *The Biological Bulletin*, 130:141–149.

[94] Gage J., 1966. Experiments with the behaviour of the bivalves *Montacuta substriata* and *M. ferruginosa*, 'commensals' with spatangoids. *Journal of the Marine Biological Association of the United Kingdom*, 46:71–88.

[95] Gage J., 1979. Mode of life and behaviour of *Montacuta phascolionis*, a bivalve commensal with the sipunculan *Phascolion strombi*. *Journal of the Marine Biological Association of the United Kingdom*, 59:635–657.

[96] Ó Foighil D., 1985. Form, function, and origin of temporary dwarf males in *Pseudopythina rugifera* (Carpenter, 1864)(Bivalvia: Galeommatacea). *Veliger*, 27:72–80.

[97] Boyko C.B. and Mikkelsen P.M., 2002. Anatomy and Biology of *Mysella pedroana* (Mollusca: Bivalvia: Galeommatoidea), and its Commensal Relationship with *Blepharipoda occidentalis* (Crustacea: Anomura: Albuneidae). *Zoologischer Anzeiger*, 241:149–160.

[98] Jespersen Å., Lützen J., and Nielsen C., 2004. On three species and two new genera (*Montacutella* and *Brachionlya*) of galeommatoid bivalves from the irregular sea urchin *Brissus latecarinatus* with emphasis on their reproduction . *Zoologischer Anzeiger*, 243:3–19.

[99] Ponder W.F., 1965. The biology of the genus *Arthritica*. *Transactions of the Royal Society of New Zealand*, 6:75–86.

[100] Gage J., 1968. Mode of life of *Montacuta elevata* a bivalve commensal with *Clymenlla torquata* (Polychaeta). *Canadian Journal of Zoology*, 46(5):877–892.

[101] Rosewater J., 1984. A new species of Leptonacean bivalve from off northwestern Peru (Heterodonta, Veneroida, Lasaeidae). *Veliger*, 27:81–89.

[102] Ó Foighil D., 1985. Sperm transfer and storage in the brooding bivalve *Mysella tumida*. *Biological Bulletin*, 169:602–614.

[103] Ruppert E.E., 1988. *Seashore Animals of the Southeast: A Guide to Common Shallow-Water Invertebrates of the Southeastern Atlantic Coast*. University of South Carolina Press, Columbia.

[104] Jespersen Å., Lützen J., and Morton B., 2002. Ultrastructure of dimorphic sperm and seminal receptacle in the hermaphrodites *Barrimysia siphonosomae* and *Pseudopythina ochetostomae* (Bivalvia, Galeommatoidea). *Zoomorphology*, 121:159–172.

[105] Jespersen Å., Lützen J., and Oliver P.G., 2007. Morphology, biology and systematic position of *Epilepton clarkiae* (Clark, 1852)(Galeommatoidea: Montacutidae) a bivalve commensal with Sipunculans. *Journal of Conchology*, 39:391.

[106] Popham M.L., 1939. On *Phlyctaenachlamys lysiosquillina gen. et sp. nov*: a lamellibranch commensal in the burrows of *Lysiosquilla maculara*. *British Museum, Natural History, Great Barrier Reef Expedition, 1928-29. Scientific Reports*, 6:61–84.

[107] Morton B., 1972. Some aspects of functional morphology and biology of *Pseudopythina subsinuata* (bivalvia: Leptonacea) commensal on stomatopod crustaceans. *Journal of Zoology*, 166:79–96.

[108] Lützen J., Sakamoto H., Taguchi A., and Takahashi T., 2001. Reproduction, dwarf males, sperm dimorphism, and life cycle in the commensal bivalve *Peregrinamor ohshimai* Shoji (Heterodonta : Galeommatoidea : Montacutidae). *Malacologia*, 43:313–325.

[109] Jespersen Å., Kosuge T., and Lützen J., 2001. Sperm dimorphism and spermatozeugmata in the commensal bivalve *Pseudopythina macrophthalmensis* (Galeommatoidea, Kelliidae). *Zoomorphology*, 120:177–189.

[110] Lützen J. and Takahashi T., 2003. *Arthritica japonica, sp. nov.*(Bivalvia: Galeommatoidea: Leptonidae), a commensal with the pinnotherid crab *Xenophthalmus pinnotheroides* White, 1846. *Yuriyagai*, 9:11–19.

[111] Goto R., Hamamura Y., and Kato M., 2007. Obligate commensalism of Curvemysella paula (Bivalvia: Galeommatidae) with hermit crabs. *Marine Biology*, 151(5):1615–1622.

[112] Spärck R., 1931. *Cycladoconcha amboiensis n. gen. n. sp.* A commensalistic lamellibranch. Papers from Dr. Th. Mortensens's Pacific Expedition 1914-16. *Videnskabelige Meddelelser Dansk Naturhis torisk Forening*, 91:227–239.

[113] Kawahara T., 1942. On *Devonia oshimai sp. nov.*, a commensal bivalve attached to the synaptid *Leptosynapta ooplax*. *Venus*, 11:153–164.

[114] Morton J.E., 1957. The habits of *Scintillona zelandica* (Odhner) 1924 (Lamellibranchia: Galeommatidae). *Journal of Molluscan Studies*, 32:185–188.

[115] Middelfart P. and Craig M., 2004. Description of *Austrodevonia sharnae n. gen. n. sp.* (Galeommatidae: Bivalvia), an ectocommensal of *Taeniogyrus australianus* (Stimpson, 1855)(Synaptidae: Holothuroidea). *Molluscan Research*, 24:211–219.

[116] Fox T.H., Jespersen Å., and Lützen J., 2007. Sperm transfer and reproductive biology in species of hermaphroditic bivalves (Galeommatoidea: Montacutidae). *Journal of Morphology*, 268:936–952.

[117] Zelaya D.G. and Ituarte C., 2002. The identity of *Waldo parasiticus* (Dall, 1876) and description of *Waldo trapezialis* new species (Bivalvia : Galeommatoidea). *Nautilus*, 116:109–117.

[118] Morton B., 1980. Some aspects of the biology and functional morphology (including the presence of a ligamental lithodesma) of *Montacutona compacta* and *Montacutona olivacea* (Bivalvia, Leptonacea) associated with coelenterates in Hong Kong. *Journal of Zoology*, 192:431–455.

[119] Oliver P.G., 1993. A new commensal bivalve associated with a burrowing sea anemone *Halcampicola tenacis* gen. *et* sp. nov., (Galeommatoidea: Montacutidae) on *Halcampoides sp.* (Anthozoa: Antipatharia: Halcampoididae). In F.E. Wells, D.I. Walker, H. Kirkman, and R. Lethbridge, editors, *Proceedings of the Fifth International Marine Biological Workshop: The Marine Flora and Fauna of Rottnest Island, Western Australia.*, page 634. Western Australian Museum, Perth.

[120] Rotvit L., Lützen J., Jespersen Å., and Fox T., 2007. *Mysella gregaria* new species, a bivalve (Galeommatoidea : Montacutidae) commensal with an intertidal burrowing sea anemone from North Carolina, USA. *Nautilus*, 121:191–200.

[121] Habe T., 1962. *Achasmea thalassemicola sp. nov.*, a new commensal bivalve found in an echiuroid, *Thalassema mucosum* Ikeda. *Venus*, 22:117–119.

[122] Anker A., Murina G.V., Lira C., Vera Caripe J.A., Palmer A.R., and Jeng M.S., 2005. Macrofauna associated with echiuran burrows: a review with new observations of the innkeeper worm, *Ochetostoma erythrogrammon* Leuckart and Rüppel, in Venezuela. *Zoological Studies*, 44:157–190.

[123] Goto R., Hamamura Y., and Kato M., 2011. Morphological and ecological adaptation of Basterotia bivalves (Galeommatoidea: Sportellidae) to symbiotic association with burrowing echiuran worms. *Zoological Science*, 28(3):225–234.

[124] Lützen J., Hong J., and Yamashita H., 2009. *Koreamya arcuata* (A. Adams, 1856) gen. nov.(Galeommatoidea: Montacutidae), a commensal bivalve associated with the inarticulate brachiopod Lingula anatina. *Journal of Conchology*, 39:669.

[125] Kelsey F.W., 1902. General Notes. *Nautilus*, 15:144.

[126] Boss K.J., 1965. Symbiotic erycinacean bivalves. *Malacologia*, 3:183–195.

[127] Morton B., 1973. Some factors affecting location of *Arthritica crassiformis* (Bivalvia: Leptonacea) commensal upon *Anchomasa similis* (Bivalvia: Pholadidae). *Journal of Zoology*, 170:463–473.

[128] Campbell D.C., 2000. Molecular evidence on the evolution of the Bivalvia. In E. Harper, J.D. Taylor, and J.A. Crame, editors, *The Evolutionary Biology of the Bivalvia*, pages 31–46. Geological Society of London Special Publication, London.

[129] Giribet G. and Distel D.L., 2003. Bivalve Phylogeny and Molecular Data. In C. Lydeard and D. Lindberg, editors, *Molecular Systematics and Phylogeography of Mollusks*. Smithsonian Institution Press, Washington, D.C.

[130] Taylor J.D., Williams S.T., Glover E.A., and Dyal P., 2007. A molecular phylogeny of heterodont bivalves (Mollusca: Bivalvia: Heterodonta): new analyses of 18S and 28S rRNA genes. *Zoologica Scripta*, 36(6):587–606.

[131] Goto R., Kawakita A., Ishikawa H., Hamamura Y., and Kato M., 2012. Molecular phylogeny of the bivalve superfamily Galeommatoidea (Heterodonta, Veneroida) reveals dynamic evolution of symbiotic lifestyle and interphylum host switching. *BMC Evolutionary Biology*, 12(172).

[132] Ponder W.F. and de Keyzer R.G., 1998. Superfamily Cyamioidea. In P.L. Beesley, G.J.B. Ross, and A. Wells, editors, *Mollusca: The Southern Synthesis*, pages 318–322. CSIRO Publishing, Melbourne.

[133] Bieler R. and Mikkelsen P.M., 1992. Preliminary phylogenetic analysis of the bivalve family Galeommatidae. *American Malacological Bulletin*, 9:157– 164.

[134] Ó Foighil D. and Smith M.J., 1995. Evolution of asexuality in the cosmopolitan marine clam *Lasaea*. *Evolution*, 49:140–150.

[135] Ó Foighil D. and Smith M.J., 1996. Phylogeography of an asexual marine clam complex, *Lasaea*, in the northeastern Pacific based on cytochrome oxidase iii sequence variationaea, in the northeastern pacific based on cytochrome oxidase III sequence variation. *Molecular phylogenetics and evolution*, 6:134–142.

[136] Taylor D.J. and Ó Foighil D., 2000. Transglobal comparisons of nuclear and mitochondrial genetic structure in a marine polyploid clam (*Lasaea*, Lasaeidae). *Heredity*, 84:321–330.

[137] Park J.K. and Ó Foighil D., 2000. Genetic diversity of oceanic island *Lasaea* (Mollusca : Bivalvia) lineages exceeds that of continental populations in the northwestern Atlantic. *Biological Bulletin*, 198:396–403.

[138] Ó Foighil D., Jennings R., Park J.K., and Merriwether D.A., 2001. Phylogenetic relationships of mid-oceanic ridge and continental lineages of *Lasaea spp.* (Mollusca : Bivalvia) in the northeastern Atlantic. *Marine Ecology Progress Series*, 213:165–175.

[139] Oliver P.G., Holmes A.M., Killeen I.J., and Turner J.A., 2010. *Marine Bivalve Shells of the British Isles (Mollusca: Bivalvia).* Amgueddfa Cymru - National Museum Wales.

[140] Jespersen Å. and Lützen J., 2006. Reproduction and sperm structure in Galeommatidae (Bivalvia, Galeommatoidea). *Zoomorphology*, 125:157–173.

[141] Bieler R. and Mikkelsen P.M., 2006. Bivalvia–a look at the branches. *Zoological Journal of the Linnean Society*, 148:223–235.

[142] Van Valen L., 1973. A new evolutionary law. *Evolutionary Theory*, 1:1–30.

[143] Stenseth N.C. and Smith J.M., 1984. Coevolution in ecosystems: Red Queen evolution or stasis? *Evolution*, 38:870–880.

[144] Barnosky A.D., 2001. Distinguishing the effects of the Red Queen and Court Jester on Miocene mammal evolution in the northern Rocky Mountains. *Journal of Vertebrate Paleontology*, 21:172–185.

[145] Venditti C., Meade A., and Pagel M., 2010. Phylogenies reveal new interpretations of speciation and the Red Queen. *Nature*, 463:349–352.

[146] Aberhan M., Kiessling W., and Fürsich F.T., 2006. Testing the role of biological interactions in the evolution of mid-Mesozoic marine benthic ecosystems. *Paleobiology*, 32:259–277.

[147] Carpenter S., 2005. *Mysella pedroana*, a commensal bivalve (Lasaeidae) on two decapod crustacean hosts. *Nautilus*, 119:105–108.

[148] Cowles A. and Hewitt J., 2009. Density, biomass and productivity of small mobile invertebrates in a wide range of coastal habitats. *Marine Ecology Progress Series*, 384:175–185.

[149] Danovaro R. and Fraschetti S., 2002. Meiofaunal vertical zonation on hardbottoms: comparison with soft-bottom meiofauna. *Mar Ecol Prog Ser*, 230:159–169.

[150] Jones N.S., 1950. Marine bottom communities. *Biological Reviews*, 25(3):283–313.

[151] R Development Core Team, Vienna, Austria, 2011. *R: A language and environment for statistical computing*.

[152] Prevedelli D., Simonini R., and Ansaloni I., 2001. Relationship of non-specific commensalism in the colonization of the deep layers of sediment. *J Mar Biol Assoc U K*, 81(06):897–901.

[153] Nicell L., Atkinson R., Hughes D., Ansell A., and Smith C., 1995. Burrow morphology of the echiuran worm *Maxmuelleia Lankesteri* (Echiura, Bonelliidae), and a brief review of burrow structure and related ecology of the Echiura. *Journal of Natural History*, 29:871–885.

[154] Coma R., Serrano E., Linares C., Ribes M., Díaz D., and Ballesteros E., 2011. Sea urchins predation facilitates coral invasion in a marine reserve. *PLoS ONE*, 6:e22017.

[155] Osman R. and Whitlatch R.B., 2004. The control of the development of a marine benthic community by predation on recruits. *Journal of Experimental Marine Biology and Ecology*, 311:117–145.

[156] Russ G., 1980. Effects of predation by fishes, competition, and structural complexity of the substratum on the establishment of a marine epifaunal community. *J Exp Mar Biol Ecol*, 42(1):55–69.

[157] Seitz R.D., Lipcius R.N., Hines A.H., and Eggleston D.B., 2001. Density-dependent predation, habitat variation, and the persistence of marine bivalve prey. *Ecology*, 82:2435–2451.

[158] Blundon J.A. and Kennedy V.S., 1982. Refuges for infaunal bivalves from blue crab, *Callinectes sapidus* (Rathbun), predation in Chesapeake Bay. *Journal of Experimental Marine Biology and Ecology*, 65:67–81.

[159] Cledón M. and Nuñez J., 2010. Siphon nipping facilitates lethal predation in the clam *Mesodesma mactroides* (Reeve, 1854)(Mollusca: Bivalvia). *Marine Biology*, 157:737–745.

[160] Smith T.E., Ydenberg R.C., and Elner R.W., 1999. Foraging behaviour of an excavating predator, the red rock crab (*Cancer productus* Randall) on soft-shell clam (*Mya arenaria* L.). *Journal of Experimental Marine Biology and Ecology*, 238:185–197.

[161] Virnstein R.W., 1977. The importance of predation by crabs and fishes on benthic infauna in Chesapeake Bay. *Ecology*, 58:1199–1217.

[162] Whitlow W.L., Rice N.A., and Sweeney C., 2003. Native species vulnerability to introduced predators: testing an inducible defense and a refuge from predation. *Biological Invasions*, 5:23–31.

[163] Zaklan S.D. and Ydenberg R., 1997. The body size-burial depth relationship in the infaunal clam *Mya arenaria*. *Journal of Experimental Marine Biology and Ecology*, 215:1–17.

[164] Passos F.V.D., Domaneschi O., and Sartori A.F., 2005. Biology and functional morphology of the pallial organs of the Antarctic bivalve *Mysella charcoti* (Lamy, 1906) (Galeommatoidea: Lasaeidae). *Polar Biology*, 28(5):372–380.

[165] Domaneschi O., da Silva J., Neto L., and Passos F., 2002. New perspectives on the dispersal mechanisms of the Antarctic brooding bivalve *Mysella charcoti* (Lamy, 1906). *Polar Biology*, 25:538–541.

[166] Kamermans P. and Huitemab H., 1994. Shrimp (*Crangon crangon* L.) browsing upon siphon tips inhibits feeding and growth in the bivalve *Macoma balthica* (L.). *Journal of Experimental Marine Biology and Ecology*, 175:59–75.

[167] de Goeij P., Luttikhuizen, P. C., van der Meer J., and Piersma T., 2001. Facilitation on an intertidal mudflat: the effect of siphon nipping by flatfish on burying depth of the bivalve *Macoma balthica*. *Oecologia*, 126:500–506.

[168] Zwarts L. and Wanink J., 1989. Siphon size and burying depth in deposit and suspension feeding benthic bivalves. *Marine Biology*, 100:227–240.

[169] Zwarts L., Blomert A.M., Spaak P., and de Vries B., 1994. Feeding radius, burying depth and siphon size of *Macoma balthica* and *Scrobicularia plana*. *Journal of Experimental Marine Biology and Ecology*, 183:193–212.

[170] Gallagher E.D., Jumars P.A., and Trueblood D.D., 1983. Facilitation of soft-bottom benthic succession by tube builders. *Ecology*, 64:1200–1216.

[171] Pillay D., 2010. Expanding the envelope: linking invertebrate bioturbators with micro-evolutionary change. *Marine Ecology Progress Series*, 409:301–303.

[172] Vadopalas B., Pietsch T., and Friedman C., 2010. The proper name for the geoduck: resurrection of *Panopea generosa* Gould, 1850, from the synonymy of *Panopea abrupta* (Conrad, 1849) (Bivalvia: Myoida: Hiatellidae). *Malacologia*, 52:169–173.

[173] Campbell A., Dovey G., Yeung C., and Zhang Z., 2004. Population biology of the Pacific geoduck clam, *Panopea abrupta*, in experimental plots, southern British Columbia, Canada. *Journal of Shellfish Research*, 23:661–673.

[174] Passos F.D. and Domaneschi O., 2006. A new species of *Mysella Angas*, 1877 (Bivalvia: Galeommatoidea) from Admiralty Bay, King George Island, South Shetlands, Antarctica, with data on its biology and functional anatomy. *Polar Biology*, 29:389–398.

[175] Lützen J., Takahashi T., and Yamaguchi T., 2001. Morphology and reproduction of *Nipponomysella subtruncata* (Yokoyama), a galeommatoidean bivalve commensal with the sipunculan *Siphonosoma cumanense* (Keferstein) in Japan. *Journal of Zoology*, 254:429–440.

[176] Morton J.E., 1960. The responses and orientation of the bivalve *Lasaea rubra* Montagu. *Journal of the Marine Biological Association of the United Kingdom*, 39:5–26.

[177] Ó Foighil D., 1988. Random mating and planktotrophic larval development in the brooding hermaphroditic clam *Lasaea australis* (Lamarck, 1818). *Veliger*, 31:205–214.

[178] Cotton B.C., 1938. *Ephippodonta* – South Australia's most perculiar bivalve shell. *The Victorian Naturalist*, 55:58–61.

[179] Bourne G., 1906. On *Jousseaumia*. A new genus of eulamellibranchs commensal with the corals *Heterocyathus* and *Heteropsammia*. *Ceylon Pearl Oyster Fisheries*, Supplementary Reports No. XXXVII:212–266.

[180] Bertics V.K., Sohm J.A., Treude T., Chow C.E.T., Capone D.G., Fuhrman J.D., and Ziebis W., 2010. Burrowing deeper into benthic nitrogen cycling: the impact of bioturbation on nitrogen fixation coupled to sulphate reduction. *Marine Ecology Progress Series*, 409:1–15.

[181] Krantzberg G., 1985. The influence of bioturbation on physical, chemical and biological parameters in aquatic environments - a review. *Environmental Pollution*, 39:99–122.

[182] Meysman F., Middelburg J., and Heip C., 2006. Bioturbation: a fresh look at Darwin's last idea. *Trends in Ecology & Evolution*, 21:688–695.

[183] Bottjer D., Hagadorn J., and Dornbos S.Q., 2000. The Cambrian substrate revolution. *GSA Today*, 10:1–7.

[184] Pillay D. and Branch G.M., 2011. Bioengineering effects of burrowing thalassinidean shrimps on marine soft-bottom ecosystems. *Oceanography and Marine Biology - An Annual Review*, 49:137–191.

[185] Levin L.A., Etter R.J., Rex M.A., Gooday A.J., Smith C.R., Pineda J., Stuart C.T., Hessler R.R., and Pawson D., 2001. Environmental influences on regional deep-sea species diversity. *Annual Review of Ecology, Evolution, and Systematics*, 32:51–93.

[186] McClain C.R. and Hardy S.M., 2010. The dynamics of biogeographic ranges in the deep sea. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 277:3533–3546.

[187] Buschbaum C., Dittmann S., Hong J.S., Hwang I.S., Strasser M., Thiel M., Valdivia N., Yoon S.P., and Reise K., 2009. Mytilid mussels: global habitat engineers in coastal sediments. *Helgoland Marine Research*, 63:47–58.

[188] Alejandrino A., Puslednik L., and Serb J.M., 2011. Convergent and parallel evolution in life habit of the scallops (Bivalvia: Pectinidae). *BMC Evolutionary Biology*, 11(1):164.

[189] Oliver P.G. and Holmes A.M., 2006. The Arcoidea (Mollusca: Bivalvia): a review of the current phenetic-based systematics. *Zoological Journal of the Linnean Society*, 148:237–251.

[190] Seilacher A., 1984. Constructional morphology of bivalves - Evolutionary pathways in primary versus secondary soft-bottom dwellers. *Palaeontology*, 27:207–237.

[191] Valentine J.W., Jablonski D., Kidwell S., and Roy K., 2006. Assessing the fidelity of the fossil record by using marine bivalves. *Proceedings of the National Academy of Sciences USA*, 103:6599–6604.

[192] Coan E. and Valentich-Scott P., 2012. *Bivalve Seashells of Tropical West America – Marine Bivalve Mollusks from Baja California to Northern Peru*. Santa Barbara Museum of Natural History Press, Santa Barbara, California.

[193] Simon J.C., Carre S., Boutin M., Prunier-Leterme N., Sabater-Muñoz B., Latorre A., and Bournoville R., 2003. Host–based divergence in populations of the pea aphid: insights from nuclear markers and the prevalence of facultative symbionts. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 270:1703–1712.

[194] Reznick D.N. and Ghalambor C.K., 2001. The population ecology of contemporary adaptations: what empirical studies reveal about the conditions that promote adaptive evolution. *Genetica*, 112-113:183–198.

[195] Rundle H.D. and Nosil P., 2005. Ecological speciation. *Ecology letters*, 8:336–352.

[196] Feder J.L., Opp S.B., Wlazlo B., Reynolds K., Go W., and Spisak S., 1994. Host fidelity is an effective premating barrier between sympatric races of the apple

maggot fly. *Proceedings of the National Academy of Sciences USA*, 91:7990–7994.

[197] Drès M. and Mallet J., 2002. Host races in plant-feeding insects and their importance in sympatric speciation. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences*, 357:471–492.

[198] Peccoud J., Ollivier A., Plantegenest M., and Simon J.C., 2009. A continuum of genetic divergence from sympatric host races to species in the pea aphid complex. *Proceedings of the National Academy of Sciences USA*, 106:7495–7500.

[199] Berlocher S.H. and Feder J.L., 2002. Sympatric speciation in phytophagous insects: moving beyond controversy? *Annual Review of Entomology*, 47:773–815.

[200] Stevens P., 1990. A genetic analysis of the pea crabs (Decapoda: Pinnotheridae) of New Zealand. I. Patterns of spatial and host-associated genetic structuring in Pinnotheres novaezelandiae Filhol. *Journal of Experimental Marine Biology and Ecology*, 141:195–212.

[201] Duffy J.E., 1996. Species boundaries, specialization, and the radiation of sponge-dwelling alpheid shrimp. *Biological Journal of the Linnean Society*, 58:307–324.

[202] Sato S., Owada M., Haga T., Hong J., Lützen J., and Yamashita J., 2011. Genus-specific commensalism of the galeommatoid bivalve *Koreamya arcuata* (A. Adams, 1856) associated with lingulid brachiopods. *Molluscan Research*, 31:95–105.

[203] Dumbauld B.R., Chapman J.W., Torchin M.E., and Kuris A.M., 2011. Is the collapse of mud shrimp (*Upogebia pugettensis*) populations along the Pacific coast of North America caused by outbreaks of a previously unknown bopyrid isopod parasite (Orthione griffenis)? *Estuaries and coasts*, 34:336–350.

[204] Carlton J., editor, 2007. *The Light & Smith Manual: Intertidal Invertebrates from Central California to Oregon, 4th Edition.* University of California Press, Berkeley.

[205] Narchi W., 1969. On *Pseudopythina rugifera* (Carpenter, 1864) (Bivalvia). *Veliger*, 12:43–52.

[206] Zelditch M., Swiderski D., Sheets H., and Fink W., 2004. *Geometric Morphometrics for Biologists: a Primer.* Elsevier Press, New York.

[207] Bookstein F.L., 1997. Landmark methods for forms without landmarks: morphometrics of group differences in outline shape. *Medical Image Analysis*, 1:225–243.

[208] Rohlf F., 2010. *TPS Series Software for Morphometric Analysis*. Department of Ecology and Evolution, State University of New York at Stony Brook, Stony Brook.

[209] Rohlf F.J. and Slice D., 1990. Extensions of the Procrustes method for the optimal superimposition of landmarks. *Systematic Biology*, 39:40–59.

[210] Sheets H., 2011. *Integrated Morphometrics Package*. Department of Physics, Canisius College, Buffalo.

[211] Folmer O., Black M., Hoeh W., Lutz R., and Vrijenhoek R., 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology*, 3:294–299.

[212] Thompson J., Higgins D., and Gibson T., 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignments through sequence weighting, position specific gap penalties and weight matrix choice. *Nucleic Acids Research*, 22:4673–4680.

[213] Clement M., Posada D., and Crandall K., 2000. TCS: a computer program to estimate gene genealogies. *Moleculor Ecology*, 9:1657–1660.

[214] Valladares A., Manríquez G., and Suárez-Isla B., 2010. Shell shape variation in populations of *Mytilus chilensis* (Hupe 1854) from southern Chile: a geometric morphometric approach. *Marine Biology*, 157:2731–2738.

[215] Dall W.H., 1899. Synopsis of the recent and tertiary Leptonacea of North America and the West Indies. *Proceedings of the United States National Museum*, 21:873–897.

[216] Palumbi S.R., 1994. Genetic divergence, reproductive isolation, and marine speciation. *Annual Review of Ecology and Systematics*, 25:547–572.

[217] Puebla O., 2009. Ecological speciation in marine vs. freshwater fishes. *Journal of Fish Biology*, 75:960–996.

[218] Miglietta M.P., Faucci A., and Santini F., 2011. Speciation in the sea: overview of the symposium and discussion of future directions. *Integrative and Comparative Biology*, 51:449–455.

[219] Knowlton N., 1993. Sibling species in the sea. *Annual Review of Ecology and Systematics*, 24:189–216.

[220] Mikkelsen P.M., 2011. Speciation in modern marine bivalves (Mollusca: Bivalvia): insights from the published record*. *American Malacological Bulletin*, 29:217–245.

[221] Schluter D., 2001. Ecology and the origin of species. *Trends in Ecology & Evolution*, 16:372–380.

[222] Appelbaum L., Achituv Y., and Mokady O., 2002. Speciation and the establishment of zonation in an intertidal barnacle: specific settlement vs. selection*. *Molecular Ecology*, 11:1731–1737.

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

[224] Forbes E., 1856. Map of the distribution of marine life. In A. Johnston, editor, *The Physical Atlas of Natural Phenomena*, pages 99–102. William Blackwood and Sons, Edinburgh.

[225] Ekman S. and Palmer E., 1967. *Zoogeography of the Sea*. Sidgwick and Jackson London.

[226] Briggs J.C., 1974. Operation of zoogeographic barriers. *Systematic Biology*, 23:248–256.

[227] Spalding M.D., Fox H.E., Allen G.R., Davidson N., Ferdaña Z.A., Finlayson M., Halpern B.S., Jorge M.A., Lombana A., Lourie S.A., *et al.*, 2007. Marine ecoregions of the world: a bioregionalization of coastal and shelf areas. *BioScience*, 57:573–583.

[228] Briggs J.C. and Bowen B.W., 2012. A realignment of marine biogeographic provinces with particular reference to fish distributions. *Journal of Biogeography*, 39:12–30.

[229] Riginos C. and Nachman M., 2001. Population subdivision in marine environments: the contributions of biogeography, geographical distance and discontinuous habitat to genetic differentiation in a blennioid fish, *Axoclinus nigricaudus*. *Molecular Ecology*, 10:1439–1453.

[230] Barber P., Palumbi S., Erdmann M., and Moosa M., 2002. Sharp genetic breaks among populations of *Haptosquilla pulchella* (Stomatopoda) indicate limits to larval transport: patterns, causes, and consequences. *Molecular Ecology*, 11:659–674.

[231] Teske P., Winker H., McQuaid C., and Barker N., 2009. A tropical/subtropical biogeographic disjunction in southeastern Africa separates two Evolutionarily Significant Units of an estuarine prawn. *Marine Biology*, 156:1265–1275.

[232] Avise J.C., 1992. Molecular population structure and the biogeographic history of a regional fauna: a case history with lessons for conservation biology. *Oikos*, 63:62–76.

[233] Schopf T., 1979. The role of biogeographic provinces in regulating marine faunal diversity through geologic time. In J. Gray and A. Boucot, editors, *Historical Biogeography, Plate Tectonics, and the Changing Environment*, pages 449–457. Oregon State University Press, Corvallis.

[234] Liu J.X., Gao T.X., Wu S.F., and Zhang Y.P., 2007. Pleistocene isolation in the Northwestern Pacific marginal seas and limited dispersal in a marine fish, Chelon haematocheilus (Temminck & Schlegel, 1845). *Molecular Ecology*, 16:275–288.

[235] Luiz O.J., Madin J.S., Robertson D.R., Rocha L.A., Wirtz P., and Floeter S.R., 2012. Ecological traits influencing range expansion across large oceanic dispersal barriers: insights from tropical Atlantic reef fishes. *Proceedings of the Royal Society B: Biological Sciences*, 279:1033–1040.

[236] Reeb C.A. and Avise J., 1990. A genetic discontinuity in a continuously distributed species: mitochondrial DNA in the American oyster, *Crassostrea virginica*. *Genetics*, 124:397–406.

[237] Hidas E.Z., Costa T.L., Ayre D.J., and Minchinton T.E., 2007. Is the species composition of rocky intertidal invertebrates across a biogeographic barrier in south-eastern Australia related to their potential for dispersal? *Marine and Freshwater Research*, 58:835–842.

[238] Knox G., 1963. The biogeography and intertidal ecology of the Australasian coasts. *Oceanography and Marine Biology Annual Review*, 1:341–404.

[239] Hutchins L.W., 1947. The bases for temperature zonation in geographical distribution. *Ecological Monographs*, pages 325–335.

[240] Teske P.R., Papadopoulos I., Mmonwa K.L., Matumba T., Mcquaid C.D., Barker N.P., and Beheregaray L.B., 2011. Climate-driven genetic divergence of limpets with different life histories across a southeast African marine biogeographic disjunction: different processes, same outcome. *Molecular Ecology*, 20:5025–5041.

[241] Duda Jr T. and Lessios H., 2009. Connectivity of populations within and between major biogeographic regions of the tropical Pacific in *Conus ebraeus*, a widespread marine gastropod. *Coral Reefs*, 28:651–659.

[242] Wilson B. and Allen G., 1987. Major components and distribution of marine fauna. In G. Dyne and D. Walton, editors, *Fauna of Australia*, pages 43–68. Australian Government Publishing Service, Canberra.

[243] McNeill S., 1994. The selection and design of marine protected areas: Australia as a case study. *Biodiversity & Conservation*, 3:586–605.

[244] Hedley C., 1904. The effect of the Bassian Isthmus upon the existing marine fauna: A study in ancient geography. In *Proceedings of the Linnean Society of New South Wales*, pages 876–883. Linnean Society of New South Wales.

[245] Bennett I. and Pope E.C., 1960. Intertidal zonation of the exposed rocky shores of Tasmania and its relationship with the rest of Australia. *Marine and Freshwater Research*, 11:182–221.

[246] Rowe F. and Vail L., 1982. The distribution of Tasmanian echinoderms in relation to southern Australian biogeographic provinces. In J. Lawrence, editor, *Echinoderms, Proceedings of the International Conference, Tampa Bay*, pages 219–225. Balkema, Rotterdam.

[247] O'Hara T.D. and Poore G.C., 2000. Patterns of distribution for southern Australian marine echinoderms and decapods. *Journal of Biogeography*, 27:1321–1335.

[248] Waters J.M., Wernberg T., Connell S.D., Thomsen M.S., Zuccarello G.C., Kraft G.T., Sanderson J.C., West J.A., and Gurgel C.F., 2010. Australia's marine biogeography revisited: Back to the future? *Austral Ecology*, 35:988–992.

[249] Billingham M. and Ayre D., 1996. Genetic subdivision in the subtidal, clonal sea anemone *Anthothoe albocincta*. *Marine Biology*, 125:153–163.

[250] Kassahn K.S., Donnellan S.C., Fowler A.J., Hall K.C., Adams M., and Shaw P.W., 2003. Molecular and morphological analyses of the cuttlefish *Sepia apama* indicate a complex population structure. *Marine Biology*, 143:947–962.

[251] Waters J.M. and Roy M.S., 2003. Marine biogeography of southern Australia: phylogeographical structure in a temperate sea-star. *Journal of Biogeography*, 30:1787–1796.

[252] Dawson M.N., 2005. Incipient speciation of *Catostylus mosaicus* (Scyphozoa, Rhizostomeae, Catostylidae), comparative phylogeography and biogeography in south-east Australia. *Journal of Biogeography*, 32:515–533.

[253] York K.L., Blacket M.J., and Appleton B.R., 2008. The Bassian Isthmus and the major ocean currents of southeast Australia influence the phylogeography and population structure of a southern Australian intertidal barnacle Catomerus polymerus (Darwin). *Molecular ecology*, 17:1948–1961.

[254] Burridge C.P., 2000. Biogeographic history of geminate cirrhitoids (Perciformes: Cirrhitoidea) with east–west allopatric distributions across southern Australia, based on molecular data. *Global Ecology and Biogeography*, 9:517–525.

[255] Waters J., King T., O'Loughlin P., and Spencer H., 2005. Phylogeographical disjunction in abundant high-dispersal littoral gastropods. *Molecular Ecology*, 14:2789–2802.

[256] Lambeck K. and Chappell J., 2001. Sea level change through the last glacial cycle. *Science*, 292:679–686.

[257] Knox G.A., 1980. Plate tectonics and the evolution of intertidal and shallow-water benthic biotic distribution patterns of the southwest Pacific. *Palaeogeography, Palaeoclimatology, Palaeoecology*, 31:267–297.

[258] Waters J.M., 2008. Marine biogeographical disjunction in temperate Australia: historical landbridge, contemporary currents, or both? *Diversity and Distributions*, 14:692–700.

[259] Ridgway K H.K., 2009. The East Australian Current. In R.A. Poloczanska ES Hobday AJ, editor, *A Marine Climate Change Impacts and Adaptation Report Card for Australia 2009*, 1-16. National Climate Change Adaptation Research Facility, Gold Coast, Australia.

[260] Baines P., Edwards R., and Fandry C., 1983. Observations of a new baroclinic current along the western continental slope of Bass Strait. *Marine and Freshwater Research*, 34:155–157.

[261] Rochford D., 1986. Seasonal changes in the distribution of Leeuwin Current waters of Southern Australia. *Marine and Freshwater Research*, 37:1–10.

[262] Kämpf J., Doubell M., Griffin D., Matthews R.L., and Ward T.M., 2004. Evidence of a large seasonal coastal upwelling system along the southern shelf of Australia. *Geophysical Research Letters*, 31:L09310.

[263] Middleton J.F. and Bye J.A., 2007. A review of the shelf-slope circulation along Australia's southern shelves: Cape Leeuwin to Portland. *Progress in Oceanography*, 75:1–41.

[264] Tong L., 1990. *The Population Ecology of the Intertidal Bivalve Lasaea australis*. Ph.D. thesis, The University of Sydney, Sydney.

[265] Roberts D., 1984. A comparative study of *Lasaea australis*, *Vulsella spongiarum*, *Pinna bicolor* and *Donacilla cuneata* (Mollusca; Bivalvia) from Princess Royal Harbour, western Australia. *Journal of Molluscan Studies*, 50:129–136.

[266] Kessing B., Croom H., Martin A., McIntosh C., Mcmillan W.O., and Palumbi S., 1989. *The simple fool's guide to PCR*. University of Hawaii, Honolulu.

[267] Xu Z., Guo X., Gaffney P., and Pierce J., 2001. Chromosomal location of the major ribosomal RNA genes in *Crassostrea virginica* and *Crassostrea gigas*. *Veliger*, 44:79–83.

[268] Darriba D., Taboada G.L., Doallo R., and Posada D., 2012. jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods*, 9:772–772.

[269] Excoffier L. and Lischer H.E., 2010. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. *MOLECULAR ECOLOGY RESOURCES*, 10:564–567.

[270] Tamura K., 1992. Estimation of the number of nucleotide substitutions when there are strong transition-transversion and G+ C-content biases. *Molecular Biology and Evolution*, 9:678–687.

[271] Tamura K., Peterson D., Peterson N., Stecher G., Nei M., and Kumar S., 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution*, 28:2731–2739.

[272] Ronquist F. and Huelsenbeck J.P., 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics*, 19:1572–1574.

[273] Hasegawa M., Kishino H., and Yano T.a., 1985. Dating of the human-ape splitting by a molecular clock of mitochondrial DNA. *Journal of Molecular Evolution*, 22:160–174.

[274] Posada D., 2008. jModelTest: phylogenetic model averaging. *Molecular biology and evolution*, 25:1253–1256.

[275] Ronquist F., Teslenko M., van der Mark P., Ayres D.L., Darling A., Höhna S., Larget B., Liu L., Suchard M.A., and Huelsenbeck J.P., 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology*, 61:539–542.

[276] Rambaut A D.A., 2009. Tracer v. 1.5. Computer program and documentation distributed by the authors.

[277] Stamatakis A., Hoover P., and Rougemont J., 2008. A rapid bootstrap algorithm for the RAxML web servers. *Systematic Biology*, 57:758–771.

[278] Boc A., Makarenkov V., *et al.*, 2012. T-REX: a web server for inferring, validating and visualizing phylogenetic trees and networks. *Nucleic Acids Research*, 40:W573–W579.

[279] Sánchez JA D.D., 2008. Intragenomic ITS2 variation in Caribbean seafans. In *Proceedings of the 11th International Coral Reef Symposium*, vol. 2, pages 1383–1387. Ft. Lauderdale, Florida.

[280] Civetta A., Vierna J., Martínez-Lage A., and González-Tizón A.M., 2009. Analysis of ITS1 and ITS2 sequences in Ensis razor shells: suitability as molecular markers at the population and species levels, and evolution of these ribosomal DNA spacers. *Genome*, 53:23–34.

[281] Stephens M., Smith N.J., and Donnelly P., 2001. A new statistical method for haplotype reconstruction from population data. *The American Journal of Human Genetics*, 68:978–989.

[282] Drummond A.J., Suchard M.A., Xie D., and Rambaut A., 2012. Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Molecular Biology and Evolution*, 29:1969–1973.

[283] Lanfear R., Calcott B., Ho S.Y., and Guindon S., 2012. PartitionFinder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. *Molecular Biology and Evolution*, 29:1695–1701.

[284] Tamura K. and Nei M., 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Molecular Biology and Evolution*, 10:512–526.

[285] Jablonski D., Roy K., Valentine J.W., Price R.M., and Anderson P.S., 2003. The impact of the pull of the recent on the history of marine diversity. *Science*, 300:1133–1135.

[286] Marko P.B., 2002. Fossil calibration of molecular clocks and the divergence times of geminate species pairs separated by the Isthmus of Panama. *Molecular Biology and Evolution*, 19:2005–2021.

[287] Wilke T., Schultheiß R., and Albrecht C., 2009. As time goes by: a simple fool's guide to molecular clock approaches in invertebrates*. *American Malacological Bulletin*, 27:25–45.

[288] Brown W.M., George M., and Wilson A.C., 1979. Rapid evolution of animal mitochondrial DNA. *Proceedings of the National Academy of Sciences USA*, 76:1967–1971.

[289] Wiens J.J., Kuczynski C.A., and Stephens P.R., 2010. Discordant mitochondrial and nuclear gene phylogenies in emydid turtles: implications for speciation and conservation. *Biological Journal of the Linnean Society*, 99:445–461.

[290] Maddison W.P., 1997. Gene trees in species trees. *Systematic Biology*, 46:523–536.

[291] Moore W.S. *et al.*, 1995. Inferring phylogenies from mtDNA variation: mitochondrial-gene trees versus nuclear-gene trees. *Evolution*, 49:718–726.

[292] Zink R.M. and Barrowclough G.F., 2008. Mitochondrial DNA under siege in avian phylogeography. *Molecular Ecology*, 17:2107–2121.

[293] Hebert P.D., Stoeckle M.Y., Zemlak T.S., and Francis C.M., 2004. Identification of birds through DNA barcodes. *PLoS Biology*, 2:e312.

[294] Bird E., 1961. The coastal barriers of east Gippsland, Australia. *Geographical Journal*, 127:460–468.

[295] Gallagher S., Smith A., Jonasson K., Wallace M., Holdgate G., Daniels J., and Taylor D., 2001. The Miocene palaeoenvironmental and palaeoceanographic evolution of the Gippsland Basin, Southeast Australia: a record of Southern Ocean change. *Palaeogeography, Palaeoclimatology, Palaeoecology*, 172:53–80.

[296] Flower B.P. and Kennett J.P., 1994. The middle Miocene climatic transition: East Antarctic ice sheet development, deep ocean circulation and global carbon cycling. *Palaeogeography, Palaeoclimatology, Palaeoecology*, 108:537–555.

[297] Shevenell A.E., Kennett J.P., and Lea D.W., 2004. Middle Miocene southern ocean cooling and Antarctic cryosphere expansion. *Science*, 305:1766–1770.

[298] Holbourn A., Kuhnt W., Simo J., and Li Q., 2004. Middle Miocene isotope stratigraphy and paleoceanographic evolution of the northwest and southwest Australian margins (Wombat Plateau and Great Australian Bight). *Palaeogeography, Palaeoclimatology, Palaeoecology*, 208:1–22.

[299] Brown L.D., 1991. Genetic variation and population stucture in the blacklip abalone, *Haliotis rubra*. *Marine and Freshwater Research*, 42:77–90.

[300] Colgan D. and Schreiter S., 2011. Extrinsic and intrinsic influences on the phylogeography of the *Austrocochlea constricta* species group. *Journal of Experimental Marine Biology and Ecology*, 397:44–51.

[301] Waters J.M., O'Loughlin P.M., and Roy M.S., 2004. Cladogenesis in a starfish species complex from southern Australia: evidence for vicariant speciation? *Molecular Phylogenetics and Evolution*, 32:236–245.

[302] Colgan D. and da Costa P., 2013. Possible drivers of biodiversity generation in the *Siphonaria* of southeastern Australia. *Marine Biodiversity*, 43:73–85.

[303] Connell S.D. and Irving A.D., 2008. Integrating ecology with biogeography using landscape characteristics: a case study of subtidal habitat across continental Australia. *Journal of Biogeography*, 35:1608–1621.

[304] Liow L., Van Valen L., and Stenseth N., 2011. Red Queen: from populations to taxa and communities. *Trends in Ecology & Evolution*, 26:349–358.

[305] Vermeij G. and Roopnarine P., 2013. Reining in the Red Queen: the dynamics of adaptation and extinction reexamined. *Paleobiology*, 39:560–575.

[306] Litsios G., Sims C., Wüest R., Pearman P., Zimmermann N., and N. S., 2012. Mutualism with sea anemones triggered the adaptive radiation of clownfishes. *BMC Evolutionary Biology*, 12:212.

[307] Simpson C., 2013. Species selection and the macroevolution of coral coloniality and photosymbiosis. *Evolution*, 67:1607–1621.

[308] Li J., Ó Foighil D., and Middelfart P., 2012. The evolutionary ecology of biotic association in a megadiverse bivalve superfamily: sponsorship required for permanent residency in sediment. *PLoS ONE*, 7:e42121.

[309] Alfaro M.E., Santini F., and Brock C.D., 2007. Do reefs drive diversification in marine teleosts? Evidence from the pufferfishes and their allies (Order Tetraodontiformes). *Evolution*, 61:2104–2126.

[310] Li J., Ó Foighil D., and Park J.K., 2013. Triton's trident: cryptic Neogene divergences in a marine clam (*Lasaea australis*) correspond to Australia's three temperate biogeographic provinces. *Molecular Ecology*, 22:1933–1946.

[311] Valentich-Scott P., O'Foighil D., and Li J., 2013. Where's Waldo? A new commensal species, *Waldo arthuri* (Mollusca, Bivalvia, Galeommatidae), from the Northeastern Pacific Ocean. *ZooKeys*, 316:67–80.

[312] Bouchet P., Ng P.K.L., Largo D., and Tan S.H., 2009. PANGLAO 2004: Investigations of the marine species richness in the Philippines. *Raffles Bulletin of Zoology*, 20:1–19.

[313] Ogden T. and Whiting M.F., 2003. The problem with "the Paleoptera Problem:" sense and sensitivity. *Cladistics*, 19:432–442.

[314] Audzijonyte A. and Vrijenhoek R.C., 2010. Three nuclear genes for phylogenetic, SNP and population genetic studies of molluscs and other invertebrates. *Molecular Ecology Resources*, 10:200–204.

[315] Edgar R.C., 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research*, 32:1792–1797.

[316] Bieler R., Mikkelsen P.M., Collins T.M., Glover E.A., González V.L., Graf D.L., Harper E.M., Healy J., Kawauchi G.Y., Sharma P.P., Staubach S., Strong E.E., Taylor J.D., Tëmkin I., Zardus J.D., Clark S., Guzmán A., McIntyre E., Sharp P., and Giribet G., 2014. Investigating the Bivalve Tree of Life –an exemplar-based approach combining molecular and novel morphological characters. *Invertebrate Systematics*, 28:32–115.

[317] Sharma P.P., González V.L., Kawauchi G.Y., Andrade S.C., Guzmán A., Collins T.M., Glover E.A., Harper E.M., Healy J.M., Mikkelsen P.M., Taylor J.D., Bieler R., and Giribet G., 2012. Phylogenetic analysis of four nuclear protein-encoding genes largely corroborates the traditional classification of Bivalvia (Mollusca). *Molecular Phylogenetics and Evolution*, 65:64 – 74.

[318] Allman E A.C. and J R., 2008. Identifiability of a Markovian model of molecular evolution with gamma-distributed rates. *Advances in Applied Probability*, 40:229–249.

[319] Stamatakis A., 2006. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics*, 22:2688–2690.

[320] Miller M., Pfeiffer W., and Schwartz T., 2010. Creating the CIPRES Science Gateway for inference of large phylogenetic trees. In *Proceedings of the Gateway Computing Environments Workshop (GCE)*, pages 1 – 8. Proceedings of the Gateway Computing Environments Workshop (GCE), New Orleans, LA.

[321] Capella-Gutierrez S., Silla-Martinez J.M., and Gabaldon T., 2009. trimAl: a tool for automated alignment trimming in large-scale phylogenetic analyses. *Bioinformatics*, 25:1972–1973.

[322] Pagel M., 1999. Inferring the historical patterns of biological evolution. *Nature*, 401:877–884.

[323] Revell L.J., 2012. phytools: an R package for phylogenetic comparative biology (and other things). *Methods in Ecology and Evolution*, 3:217–223.

[324] Pagel M., Meade A., and Barker D., 2004. Bayesian estimation of ancestral character states on phylogenies. *Systematic Biology*, 53:673–684.

[325] Maddison W.P., Midford P.E., and Otto S.P., 2007. Estimating a binary character's effect on speciation and extinction. *Systematic Biology*, 56:701–710.

[326] Rabosky D.L., 2014. Automatic Detection of Key Innovations, Rate Shifts, and Diversity-Dependence on Phylogenetic Trees. *PLoS ONE*, 9:e89543.

[327] FitzJohn R.G., 2012. Diversitree: comparative phylogenetic analyses of diversification in R. *Methods in Ecology and Evolution*, 3:1084–1092.

[328] Ives A.R. and Garland T., 2010. Phylogenetic logistic regression for binary dependent variables. *Systematic Biology*, 59:9–26.

[329] Zelditch M.L., Swiderski D.L., and Sheets H.D., 2012. *Geometric Morphometrics for Biologists: A Primer*. Elsevier Science.

[330] Anderson M.J., 2006. Distance-based tests for homogeneity of multivariate dispersions. *Biometrics*, 62:245–253.

[331] Harmon L.J., Losos J.B., Jonathan Davies T., Gillespie R.G., Gittleman J.L., Bryan Jennings W., Kozak K.H., McPeek M.A., Moreno-Roark F., Near T.J., Purvis A., Ricklefs R.E., Schluter D., Schulte Ii J.A., Seehausen O., Sidlauskas B.L., Torres-Carvajal O., Weir J.T., and Mooers A.O., 2010. Early bursts of body size and shape evolution are rare in comparative data. *Evolution*, 64:2385–2396.

[332] Harmon L., Weir J., Brock C., Glor R., and Challenger W., 2008. GEIGER: investigating evolutionary radiations. *Bioinformatics*, 24:129–131.

[333] Harmon L.J., Schulte J.A., Larson A., and Losos J.B., 2003. Tempo and mode of evolutionary radiation in iguanian lizards. *Science*, 301:961–964.

[334] Butler M.A. and King A.A., 2004. Phylogenetic comparative analysis: a modeling approach for adaptive evolution. *The American Naturalist*, 164:683–695.

[335] Ingram T., 2011. Speciation along a depth gradient in a marine adaptive radiation. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 278:613–618.

[336] Thomas G.H. and Freckleton R.P., 2012. MOTMOT: models of trait macroevolution on trees. *Methods in Ecology and Evolution*, 3:145–151.

[337] Bedrick E.J. and Tsai C.L., 1994. Model selection for multivariate regression in small samples. *Biometrics*, 50:226–231.

[338] Lützen J., Kato M., Kosuge T., and Ó Foighil D., 2005. Reproduction involving spermatophores in four bivalve genera of the superfamily Galeommatoidea commensal with holothurians. *Molluscan Research*, 25:99–112.

[339] Jablonski D. and Lutz R.A., 1983. Larval ecology of marine benthic invertebrates: paleobiological implications. *Biological Reviews*, 58:21–89.

[340] Vermeij G.J., 2005. From phenomenology to first principles: toward a theory of diversity. *Proceedings-California Academy of Sciences*, 56:12–23.

[341] Bowen B.W., Rocha L.A., Toonen R.J., and Karl S.A., 2013. The origins of tropical marine biodiversity. *Trends in Ecology & Evolution*, 28:359 – 366.

[342] Wood R., 1999. *Reef Evolution*. Oxford University Press, Oxford.

[343] Dynesius M. and Jansson R., 2014. Persistence of within-species lineages: a neglected control of speciation rates. *Evolution*, 68:923–934.

[344] Li J. and Ó Foighil D., 2012. Host-specific morphologies but no host races in the commensal bivalve *Neaeromya rugifera*. *Invertebrate Biology*, 131:197–203.

[345] Rabosky D.L., 2010. Extinction rates should not be estimated from molecular phylogenies. *Evolution*, 64:1816–1824.

[346] Dubilier N., Bergin C., and Lott C., 2008. Symbiotic diversity in marine animals: the art of harnessing chemosynthesis. *Nature Reviews Microbiology*, 6:725–740.

[347] Beinart R.A., Sanders J.G., Faure B., Sylva S.P., Lee R.W., Becker E.L., Gartman A., Luther G.W., Seewald J.S., Fisher C.R., *et al.*, 2012. Evidence for the role of endosymbionts in regional-scale habitat partitioning by hydrothermal vent symbioses. *Proceedings of the National Academy of Sciences USA*, 109:E3241–E3250.

[348] Lorion J., Kiel S., Faure B., Kawato M., Ho S.Y., Marshall B., Tsuchida S., Miyazaki J.I., and Fujiwara Y., 2013. Adaptive radiation of chemosymbiotic deep-sea mussels. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 280:20131243.

[349] Lehnert E.M., Burriesci M.S., and Pringle J.R., 2012. Developing the anemone *Aiptasia* as a tractable model for cnidarian-dinoflagellate symbiosis: the transcriptome of aposymbiotic *A. pallida*. *BMC genomics*, 13:271.

[350] Morlon H., Potts M.D., and Plotkin J.B., 2010. Inferring the dynamics of diversification: a coalescent approach. *PLoS Biology*, 8:e1000493.

[351] Csilléry K., Blum M.G., Gaggiotti O.E., and François O., 2010. Approximate Bayesian computation (ABC) in practice. *Trends in Ecology & Evolution*, 25:410–418.