学位論文

Evolution of parasitic strategies and morphological diversification in eulimid and pyramidellid gastropods

（ハナゴウナ科およびトウガタガイ科腹足類における寄生戦略の進化と形態多様化）

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東京大学大学院理学系研究科

生物科学専攻

髙野　剛史

Abstract

Parasitism is one of the commonest and most successful modes of life on Earth. Parasites have played a significant role in the evolution of other, non-parasitic organisms and hence contributed to the overall biodiversity. Furthermore, they can alter the physiology and behavior of the hosts that have a significant role in systems, which in turn modifies community structure. Illuminating current status and evolutionary transitions of host-parasite interaction is therefore crucial to understand the origin and maintenance mechanisms of biodiversity. Diversification processes of parasites have indeed been investigated using molecular methods for various lineages in several phyla including Arthropoda, Nematoda, Platyhelminthes and Acanthocephala. However, quite little is known about the timing of their ecological transitions, morphological evolution and species diversification, making it difficult to reveal a more complete picture of parasite evolution. This scarcity of knowledge is attributable to the extremely rare fossil record for small and soft-bodied parasites.

The class Gastropoda offers an unmatched advantage for studying the evolution of parasites with its abundant fossil record. Among parasitic gastropods, the Eulimidae and Pyramidellidae have achieved significant diversification during their Cenozoic radiation that resulted in thousands of extant species in each family. Interestingly, ecological and morphological traits are quite different between the two groups. Eulimids are exclusive parasites of echinoderms and exhibit rich varieties of parasitic strategies (temporary, ecto- and endoparasitism) and shell shapes (slender, globose and capuliform). Pyramidellids in contrast parasitize on annelids and other mollusks, mostly as temporary parasites with rather uniformly high-spired shells. Despite being such fascinating targets for studies on parasite evolution, their ingroup relationships have been poorly understood due to the lack of comprehensive molecular phylogenies. Here in this dissertation, the evolutionary histories and diversification

patterns are first illuminated and compared between these two largest families of parasites in Gastropoda.

The relationships of the Eulimidae among non-parasitic taxa are not well understood, while such knowledge is essential for the inference of the ancestral states and evolutionary transition in a parasitic lineage. In the Chapter 1 of this thesis, Bayesian and maximum likelihood phylograms are reconstructed to explore the phylogenetic position of Eulimidae within its parent taxon Hypsogastropoda, based on the nucleotide sequences of five genes (nuclear 18S/28S rRNA and Histone H3 and mitochondrial 16S rRNA and COI) from 58 species in 38 hypsogastropod families and from five cerithioideans as the outgroup. The phylogenetic trees suggest Vanikoridae as the sister group of Eulimidae; the two families are collectively placed in the newly redefined superfamily Vanikoroidea, with Truncatelloidea and Rissooidea as its closest relatives. Vanikorids are protandrous hermaphrodites as are many eulimids and are essentially carnivorous, differing from the mostly gonochoristic and herbivorous or detritivorous Truncatelloidea and Rissooidea. The parasitic lifestyle in the Eulimidae was probably derived from carnivorous mode of feeding as in the case of many other parasitic organisms.

The internal phylogeny of the Eulimidae and their evolutionary consequences are examined in the Chapter 2 by molecular phylogenetic reconstruction and morphometric analysis of shells. Phylogenetic trees are inferred from six-gene sequences (a total of 4.7 kb) from 101 eulimid species belonging to over 50 genera as well as three vanikorids for outgroup comparison. Reconstruction of ancestral character states and divergence time estimates based on the tree topology reveal that (1) eulimids exploiting each of the five echinoderm classes belong to two or three phyletic groups, (2) each of the teleoconch and radula has been lost more than once in the evolution of eulimids, and (3) globose to capuliform shells as well as endoparasitism have evolved independently and rapidly in several of the lineages. In addition, the

principal component analysis based on seven measurements of eulimid shells reveals a strong correlation between shell morphology and parasitic strategy. These results indicate that the evolution of the Eulimidae involves the process of repeated adaptive radiation. Respective radiations have started from temporary parasitic ancestors bearing a slender shell and ended in permanent ectoparasites and endoparasites with globose to patelliform shells or without a shell. These radiations involving the adhesion and infiltration to the host of a particular echinoderm class thus have a strong deterministic component, as has shown in the replicated adaptive radiation by other organismal lineages on islands and in lakes. Fossil records suggest that the repeated radiation has occurred throughout the evolutionary history of Eulimidae, since well before and more frequently than it can be traced by the ancestral state reconstruction based on phylogenetic relationships among extant species and distribution of their ecological traits.

The Chapter 3 is devoted to illuminate evolutionary relationships and diversification process in the Pyramidellidae. A molecular phylogeny of the family is reconstructed based on six-gene sequences (5.1 kbp); also estimated are the ancestral conditions of the shell shapes and habitats. This phylogenetic analysis includes 59 pyramidellid species in more than 40 genera as well as 14 related taxa for comparison. The resulting trees reject the monophyly of the Pyramidellidae and all of its four subfamilies as currently defined based almost solely on shell morphology. Although many species of the family apparently exhibit low host specificity, which may decrease the diversity of accessible niches for colonization, they probably have achieved the great diversification through frequent shifts among different environments while often retaining dependence to a particular lineage of hosts, ranging from a single species to various taxa in a phylum. The reasons why pyramidelloids have not specialized to give rise endoparasites or why they have achieved a permanent ectoparasitic lifestyle

only once are discussed in comparison with the repeated adaptive radiation of the Eulimidae.

Summing up, the diversification processes greatly differ in the two most speciose groups of parasitic gastropods, Eulimidae and Pyramidellidae: Recurrent specialization to the permanent parasitic lifestyle has enhanced the diversification in the former, while frequent habitat shifts among disjunct marine environments have contributed to the species richness of the latter. The present study on eulimid diversification provides perhaps the most complete and dynamic picture of parasite evolution in terms of the large number of parallel specialization events. This study also indicates that the fossil records of the Gastropoda can provide unmatched knowledge on the evolution of host-parasite interaction, particularly if a number of conchological characters are properly evaluated and only truly unique conditions are used to diagnose monophyletic groups. Further investigations on the evolutionary history of parasitic gastropod lineages, each of which exhibits different ecological and morphological conditions but unanimously benefits from the rich fossil record, would elucidate diversification of parasitic organisms in time and space.

Contents

General Introduction

Parasitism is one of the commonest and most successful modes of life on Earth (Thompson, 1994; Windsor, 1998; Poulin & Morand, 2004). Parasites are a critical component of biodiversity by representing roughly half the known animals on Earth, if parasitism is defined broadly as "obligate feeding on a living organism without death to the host" (Poulin & Morand, 2000, 2004). This lifestyle has been acquired more than 60 times in ten phyla of the animal kingdom and several of these parasitic lineages have achieved great diversification over time (Poulin & Morand, 2000; Summers et al., 2003). For example, an exclusively parasitic clade in the phylum Platyhelminthes includes more than 40,000 species; the Acari of the phylum Arthropoda (mites and ticks) represent another lineage with a remarkable species richness of parasites, comprising over 30,000 species in at least two parasitic clades (Poulin & Morand, 2004).

Parasites have also played a significant role in the evolution of other, non-parasitic organisms and hence contributed to the overall biodiversity: host-parasite interactions can accelerate genetic and phenotypic evolution of the both (Schulte et al., 2010) or speciation of the host (Buckling & Rainey, 2002). Such a rapid evolution of hosts and parasites is generated by a co-evolutionary process that imposes selection on both (the Red Queen's Race). For example, co-evolution experiments using nematode hosts and microbial parasites by Schulte et al. (2010) have shown higher genetic diversity in microsatellite loci of the hosts and toxin genes of the parasites, which results in both higher virulence of the parasites and resistance of the hosts. The increase of genetic diversity enhances speciation of not only parasites but also hosts.

Recent studies on "ecosystem parasitology" have also highlighted the importance of parasites in biological communities and material cycles (Hatcher & Dunn, 2011; Tompkins et al., 2011). Parasites can alter the physiology and behavior of the hosts that have a significant role in systems, which in turn modifies community

structure (Wood et al., 2007). The inclusion of parasites in the analyses of ecosystem functions therefore provides more diverse and complex pictures of food-web structure (Byers, 2008; Lafferty et al., 2008; Dunne et al., 2013; Lafferty, 2013). To conclude, illuminating current status and evolutionary transitions of host-parasite interaction is crucial to understand the origin and maintenance mechanisms of biodiversity.

Phylogenetic reconstruction of parasite evolution

The evolutionary pathways of parasitic organisms had traditionally been discussed based on phylogenetic relationships that were inferred from morphological characteristics (e.g. Morris & Crompton, 1982; Brooks et al., 1985; Littlewood et al., 1999). However, molecular phylogenetic studies that paid attention to phenotypic diversification have revealed striking incongruence between the traditional, morphology-based classification and true evolutionary history and relationships among taxa (e.g. Littlewood et al., 2001; Perkins et al., 2009; Johnson et al., 2012). This inconsistency is most plausibly attributable to convergent evolution—where nearly identical phenotypes evolve in unrelated organisms as adaptive consequences of similar selective pressures—as such pressures would be particularly intense in the host-parasite interaction (Johnson et al., 2012). The reconstruction of phylogenetic relationships among parasites should therefore be based on characters that are less affected by the selection, to better elucidate their evolutionary history including adaptation and diversification. Nucleotide sequences of mitochondrial and nuclear rRNA genes, on the other hand, are probably among the most useful and informative characters to infer the relationships of parasites plausibly with a negligible degree of convergence (e.g. Kunz, 2002).

Molecular phylogenies have indeed been applied to answer a variety of questions on the evolution and diversification of parasites. The origin of parasitism is

naturally a primary area of interest (Herlyn et al., 2003; Lanterbecq et al., 2006; Rees et al., 2014). Many other works have focused on ecological events after developing the parasitic mode of life. These include host switching (e.g. Blaxter et al., 1998; Whitfield, 1998; Littlewood, 2006; Huys et al., 2007), microhabitat specialization or habitat selection in the host (Šimková et al., 2004; Ketmaier et al., 2008; Johnson et al., 2012) and evolution of host specificity (Mendlová & Šimková, 2014), many of which have greatly contributed to the diversification of parasites (see below).

Although parasites in various animal groups including the Arthropoda, Nematoda and Acanthocephala have been the focuses of molecular phylogenetic reconstruction, the phylum Platyhelminthes represents the most thoroughly investigated lineage in this context. The flatworm provides valuable opportunities to study the evolution of parasites by exhibiting a wide range of ecological adaptations on/in various hosts, high species richness, and both simple and complex life cycles (Poulin & Morand, 2004). Molecular phylogenetic analyses at various taxonomic levels from within-genus to phylum-wide have revealed the evolutionary transitions of life cycle traits (Park et al., 2007), hosts (Zietara & Lumme, 2002; Littlewood, 2006; Webster et al., 2006; Park et al., 2007) and infection sites (Ketmaier et al., 2008; Mladineo et al., 2010). For example, Park et al. (2007) suggested based on mitochondrial genomes across the phylum that endoparasitic flatworms with a complex lifecycle constitute a monophyletic group (Cestoda + Trematoda) with an ectoparasitic common ancestor on a vertebrate host.

Despite many such phylogenetic studies on parasites, quite little is known about the timing of their ecological transitions, morphological evolution and species diversification. This scarcity of knowledge is attributable to the extremely rare fossil record for small and soft-bodied parasites (Morris, 1981; Leung, in press). A limited number of divergence time estimates have been made based on molecular phylogenetic reconstruction and fossil records as calibration points for wasps (Hymenoptera) and

barnacles (Crustacea); their chitinous exoskeleton and calcareous shell plates allow fossilization to take place and identification possible (Whitfield, 2002; Glenner & Hebsgaard, 2006; Murphy et al., 2008; Rees et al., 2014). On the other hand, the age of geological events and/or divergence of host organisms have been used to indirectly estimate a time scale of evolution in the soft-bodied Platyhelminthes (Verneau et al., 2002; Knapp et al., 2011; Héritier et al., 2015). The fossil record can provide not only a solid basis for time calibration but also the only direct evidence of past parasites (De Baets & Littlewood, 2015). Combined evidence from molecular, morphological and paleontological data would reveal a more complete picture of parasite evolution (Leung, in press).

Mechanisms of species diversification

The extraordinary high species richness of parasites is often tied to their close interaction with the host, small body size, short generation time and small effective population size (e.g. Huyse et al., 2005; Emelianov, 2007). Adaptive speciation, a subtype of sympatric speciation, has been considered the main process in their diversification (Poulin & Morand, 2004; Emelianov, 2007; Rascalou & Gourbière, 2014). The mechanism of adaptive speciation in parasites involves the colonization of novel hosts or infection sites, which is triggered by resource limitation and followed by frequency-dependent competition for survival or for mating opportunity (Emelianov, 2007). In other words, ecological interactions among parasite species or between parasites and hosts play an important role in the parasite diversification.

Species richness of parasites therefore seems to largely depend on accessible opportunities for the adaptive diversification or the diversity of colonization niches provided from the host group (Coyne & Orr, 2004; Poulin & Morand, 2004; Emelianov, 2007). The available niche diversity for parasites may be greater in a host group with

a wider range of habitats, more complex body structure, and most importantly, a higher number of species (Eichler's rule; see Vas et al., 2012). Besides the obvious contribution of a speciose host group in various environments to the niche diversity for parasites, the complex body plan of hosts has played an important role in generating the present diversity of, for example, flatworms. Several species of *Dactylogyrus* in the subclass Digenea are considered to have diverged from a common ancestor through changing infection sites in the gill of the same host fish species (Ketmaier et al., 2008); the diversification of the Didymozoidae has been driven by the exploitation of different organs of bluefin tunas, including the gill, tongue and nerves, as attachment sites (Mladineo et al., 2010). Life cycle traits of parasites also affect the opportunities for their niche (hence species) diversification. Parasites with a complex life cycle involving host changes during their ontogeny tend to be more diversified than those with a simple, one-host life cycle, as a result of increased opportunities for the adaptive diversification (Poulin & Morand, 2004).

Naturally, Platyhelminthes, Nematoda and Acari represent the three most species-rich taxonomic groups of metazoan parasites. They fulfill the presumed requirements of increased diversification, by inhabiting various environments from marine to freshwater to terrestrial realms, by exhibiting a complex life cycle (except for the Acari), and by exploiting a broad range of hosts including vertebrates that have complex structures of the body (Table I). However, some parasitic lineages have achieved significant diversification without having such ecological characteristics. For example, the parasitic lice of the order Phthiraptera are comprised of more than 4,000 species that inhabit only on land with a simple lifestyle (Poulin & Morand, 2004). Similar cases exist in marine environments. Eulimidae and Pyramidellidae are among the most speciose families of Gastropoda (Mollusca) and each consists of thousands of parasitic species, regardless of their dependence on a single type of invertebrate hosts throughout ontogeny (Table I; Warén & Gittenberger, 1993; Schander et al., 1999a).

What kind of ecological traits or phylogenetic constraints, such as body size and generation time, have served as the driving force behind the increased diversification has yet to be resolved for the latter parasitic groups in the sea.

Parasites belonging to the molluscan class Gastropoda

The class Gastropoda offers an unmatched advantage for studying the evolution of parasites with its abundant fossil record. As noted above, recent authors have highlighted the importance of such direct paleontological data for the understanding of history of host-parasite interactions (De Baets & Littlewood, 2015; Leung, in press). Numerous fossils of parasitic gastropods and their non-parasitic relatives have been recovered from various geologic ages across broad geographic regions (e.g. Cossmann & Pissarro, 1904–06; Yokoyama, 1922; Laseron, 1959; Lozouet, 2014). These fossils often retain taxonomic characters that enable reliable inference of their phylogenetic positions thorough comparison with Recent taxa (Bieler, 1988). Moreover, at least eight lineages of the class have independently developed the parasitic mode of life, which subordinate only to the numbers in two arthropod classes, Copepoda and Malacostraca (Poulin & Morand, 2000). Parasitic gastropods have been successful in terms of host diversity, while each lineage has a relatively restricted selection of hosts: Eulimidae on echinoderms; Pyramidellidae on other molluscan groups as well as on annelids; Coralliophilinae (Muricidae) mainly on reef-building scleractinian corals, Ovulidae on alcyonacean soft corals, and Epitoniidae and Architectonicidae on actinarians and zoanthid anemones, respectively (see Okutani, 2000a for datails). Utilization of diverse host groups theoretically enables niche differentiation among these parasitic gastropods and contributes to the overall increase of diversity in the phylum (see Vas et al., 2012).

Of these, the Eulimidae and Pyramidellidae have achieved significant

diversification which may be comparable to that of nematodes and flatworms (see above): they are among the “Big Five” families of Gastropoda and each occupies approximately 5% diversity of the phylum Mollusca in tropical coastal environments (Bouchet et al., 2002). The two families may therefore comprise a total of 20,000 species among the estimated 200,000 living species of molluscs (Ponder & Lindberg, 2008). However, the ingroup relationships of two families were poorly understood due to the lack of comprehensive molecular phylogenies.

The Eulimidae (Fig. I-1) belong to Caenogastropoda and are exclusive parasites of echinoderms including all five classes of the phylum, namely Echinoidea (sea urchins), Holothuroidea (sea cucumbers), Asteroidea (sea stars), Ophiuroidea (brittle stars) and Crinoidea (sea lilies and feather stars; Warén, 1984). Many eulimids lack a radula and feed on the dermal tissue and body fluid of hosts using a specialized proboscis (e.g. Lützen, 1976; Warén, 1980a; Fretter & Graham, 1982). This family constitutes probably one of the most beautiful materials to study the evolution of parasitism. Eulimids exhibit a rich variety of parasitic strategy: many species are temporary ectoparasites, crawling from host to host, while some others are highly modified endoparasites (e.g. Warén, 1980a; Fretter and Graham, 1982). The family also shows a broad range of sexual strategies ranging from hermaphroditism to gonochorism and from environmental to genetic sex determination, as well as may different types of shell forms including slender, conical, globose and capuliform ones (Warén, 1984). Presence of a rich fossil record makes this taxon further attractive and advantageous. The first occurrence of eulimid shell dates back to the Late Cretaceous (Sohl, 1964); the oldest putative trace fossil of the group (drill holes on sea urchin tests) also comes from the same epoch (Neumnn & Wisshak, 2009). Cenozoic deposits have yielded much more numerous species including over a hundred from the European lower Oligocene to upper Miocene faunas (Lozouet, 2014). Unfortunately, despite its unmatched potential for the study on the evolution of parasitism, there is limited

knowledge about either evolutionary relationships within the Eulimidae or the phylogenetic position of the family (see Colgan et al., 2007; Dgebuadze et al., 2012; Criscione & Ponder, 2013).

The Pyramidellidae (Fig. I-2A–C, E, F) represent another speciose parasitic group in Gastropoda and infest annelid worms and other molluscan groups. Compared to eulimids, pyramidellids exhibit relatively little ecological and morphological diversity. Most of them are temporary parasites with a high-spired shell and all are simultaneous hermaphrodites (Ponder & de Keyzer, 1998b). Although pyramidellid specimens are very often collected as free-living or empty shells with no host information (e.g. Hori, 2000b), a parasitic mode of life is assumed for all species based on such morphological characteristics as the lack of a radula and the presence of a long acrembolic proboscis and a buccal pump to suck out body fluids of the host (Wise, 1996; Ponder & de Keyzer, 1998b). They seem to have originated at nearly the same time with eulimids. The oldest known species dates back to the Cretaceous and more recent fossils are ubiquitous in almost all ages and areas of the Cenozoic (e.g. Laseron, 1959; Kiel & Bandel, 2001). Phylogenetic relationships of pyramidellids have been investigated using morphological (Wise, 1996; Schander et al., 1999b) and molecular data (Schander et al., 2003; Dinapoli et al., 2011), while these studies have dealt with only a limited number of taxa with focuses on the classification of genera and subfamilies. Another uncertainty comes from the absence of any molecular data for the Amathinidae (Fig. I-2D), which is the only other family of Pyramidelloidea and a putative sister to Pyramidellidae according to an anatomical study by Ponder (1987). The position of the superfamily within Heterobranchia have also been contentious; previous reconstruction variously recovered them as a sister clade of Glacidorboidea (Dinapoli & Klussmann-Kolb, 2010; Dinapoli et al., 2011) or Amphiboloidea (Jörger et al., 2010) or Lymnaeoidea (Dayrat et al., 2011).

Aims of this dissertation

The goals of this thesis are to first reveal and compare the evolutionary histories and diversification patterns of the two largest parasitic gastropod families, Eulimidae and Pyramidellidae. For this purpose, phylogenetic reconstruction was conducted based on nucleotide sequences from the mitochondrial and nuclear rRNA genes to uncover their evolutionary histories and to elucidate what kinds of driving force are responsible for their enormous diversification. Information on phenotypic and ecological traits is derived from the literature and my own observations on specimens and habitats and is incorporated to the phylogenetic reconstruction of ancestral states. Combined with their rich fossil record, this study aims to provide a more complete picture of parasite evolution than previous attempts based on soft-bodied flatworms and nematodes.

The Chapter 1 is dedicated to exploring the phylogenetic position of Eulimidae within its parent taxon Hypsogastropoda and to evaluating ancestral states from which the eulimid parasitic mode of life and their modified phenotypes have been derived. Fifty-eight species from 38 hypsogastropod families were analyzed together with five cerithioideans as outgroup to make an assessment of their relationships. The Chapter 2 explores the internal phylogeny of the Eulimidae and their evolutionary concequences based on 101 ingroup species belonging to more than 50 genera along with three species from the newly determined sister family Vanikoridae. Morphometric analyses were also conducted to investigate the correlation between parasitic strategies and phenotypes (shell shapes) in the Eulimidae. Finally, the Chapter 3 deals with the phylogeny of the Pyramidelloidea by incorporating 68 ingroup species that represent over 40 genera along with all the three putative sister taxa of the superfamily. The specimens used for this dissertation originate from all over the world (Fig. I-3), from the Equator to the poles and from intertidal to abyssal (5,300 m) depths, to maximize the ecological,

phenotypic and phylogenetic diversity in the analyses.

Definitions of parasitism

Parasites set themselves apart from commensals and mutualists in causing negative effects to the host (Rohde, 2005). However, the difference between parasitism and predation is much less clear; predators in general kill their prey almost immediately while parasites live in or on their hosts for an extended period of time. The broadest definition of parasitism may therefore be described as "obligate feeding on a living organism without death to the host" (Poulin & Morand, 2000), while another use of the term refers to "a close association of two organisms, in which one—the parasite—depends on the other—the host—deriving some benefit from it" (Rohde, 2005). The former, broader definition of parasites is used throughout this dissertation, because how "closely associated" to the host has not been documented or investigated for a vast majority of eulimid and pyramidellid species. Some eulimids and many pyramidellids are collected as free-living (autonomous) in soft sediment or among calcareous algae, away from any potential host animal (e.g. Bouchet & Warén, 1986; Hori, 2000b). However, their total dependence to particular host groups as food resource is warranted by the highly specialized alimentary system in all species (Warén, 1984; Ponder & de Keyzer, 1998b). The low vagility of snails in general also seems to justify an assumption that eulimids and pyramidellids attach to and suck the blood of the host for "an extended period of time."

In this regard, parasites can be divided into two subtypes based on the presence or absence of autonomous periods to search and feed on multiple hosts—namely temporary (or periodic) and permanent parasites (see Rohde, 2005 for details). Permanently parasitic gastropods often lack a functional foot to move from host to host (Warén, 1980a, 1980b, 1981a). Another categorization of parasites concerns their

positions relative to the host: ectoparasites and endoparasites live outside and inside the host, respectively (Rohde, 2005). In this dissertation, eulimid gastropods are classified into three categories by emphasizing different degrees of their dependence on and adaptation to (a single individual of) a single host species: (1) temporary parasites, (2) ectoparasites and (3) endoparasites, the latter two of which include permanent parasites only. Among the study taxa of Eulimidae, *"Hypermastus" lacteus* alone represents an endoparasitic species that is herein classified as a temporary parasite (based on the presence of autonomous periods and a functional foot; see Chapter 2). All pyramidelloids fall into the category of temporary parasites except the amathinid genera *Amathina* and *Cyclothyca*, which are treated as ectoparasites (Chapter 3).

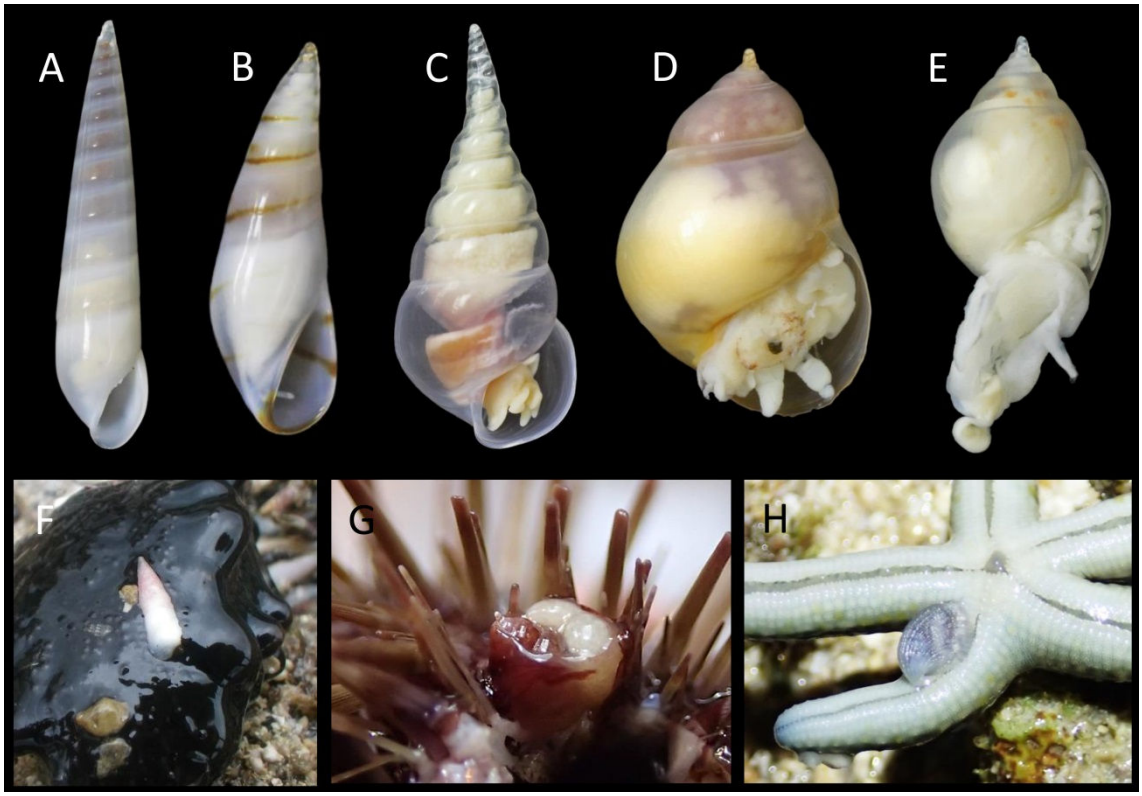

Figure I-1. Specimens and in situ images of Eulimidae. **A**: *Melanella* sp. J (shell height: 11.4 mm, YK#1588); **B**: *Hemiliostraca peasei* (5.0 mm, YK#1518); **C**: *Scalenostoma subulatum* (7.7 mm, YK#2551); **D**: *Pelseneeria* sp. A (6.1 mm, YK#1587); **E**: *Stilifer utinomi* (4.2 mm, YK#1608); **F**: *Melanella* sp. on the black knobby sea cucumber *Stichopus chloronotus*; **G**: *Monogamus entopodia* on a rocky-shore sea urchin, *Echinometra mathaei*; **H**: *Thyca crystallina* on a sea star *Linckia multifora*. The YK numbers refer to DNA samples at the Benthos Laboratory, Atmosphere and Ocean Research Institute, the University of Tokyo (AORI).

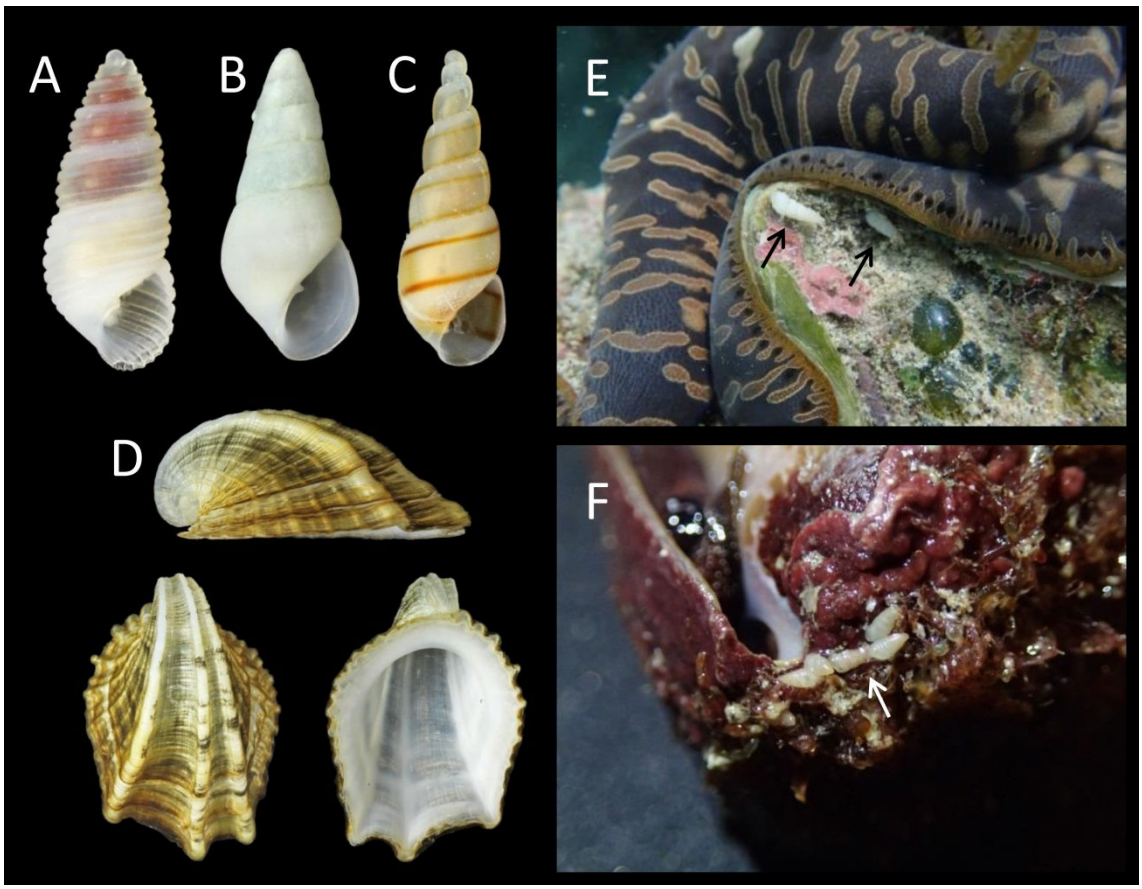

Figure I-2. Pyramidellidae and Amathinidae. **A**: *Odetta lirata* (shell height: 2.6 mm, YK#908); **B**: *Odostomia desimana* (5.4 mm, YK#894); **C**: *Bacteridium vittatum* (3.9 mm, YK#893); **D**: *Amathina tricarinata* (shell length: 6.9 mm, YK#811); **E**: *Turbonilla cummingi* near the mantle margin of the small giant clam *Tridacna maxima* (arrows); **F**: *Miralda scopulorum* attaching to the shell aperture of the conid snail *Conus lividus* (arrow).

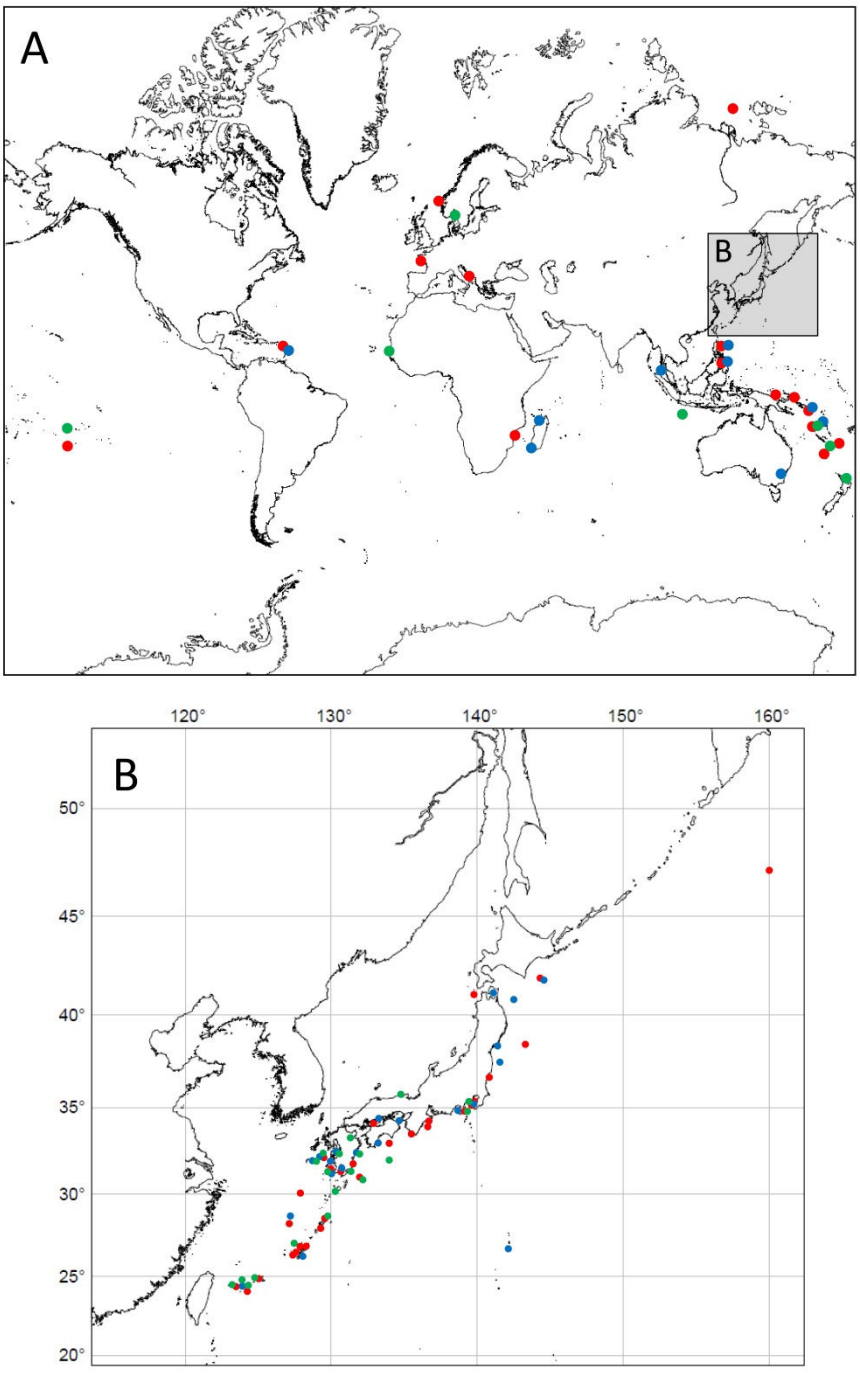

Figure I-3. Sampling localities in the world (**A**) and around Japan (**B**). Red and green dots denote sampling sites of eulimids (Chapter 2) and other hypsogastropods (Chapter 1), respectively; blue dots represent pyramidellids and their relatives (Chapter 3). Maps drawn by PanMap (available at http://www.pangaea.de/software/PanMap/).

Table I. Number of species, habitats, life cycle and date of origin for five highly diversified lineages of parasitic animals, with comparison to two gastropod families Eulimidae and Pyramidellidae. Note that parasitic species of Nematoda and Acari are polyphyletic (Poulin & Morand, 2000) and Trematoda are possibly paraphyletic (Park et al., 2007). Data modified from Poulin & Morand (2004).

Taxon	Estimated *n* of species	Habitat[*1]	Life cycle[*2]	Host	Estimated date of origin (Ma[*3])
Nematoda	> 10,500	T, FW, M	C, S	Animals, plants	Early Devonian? (> 396?)[*4]
Platyhelminthes					
Monogenea	> 20,000	FW, M	S	Vertebrates	Early Devonian (> 410)[*5]
Trematoda	> 15,000	T, FW, M	C	Vertebrates, molluscs	Early Devonian (> 410)[*5]
Cestoda	> 5,000	T, FW, M	C	Vertebrates, crustaceans	Early Devonian (> 410)[*5]
Arthropoda					
Acari	> 30,000	T, FW, M	S	Vertebrates, invertebrates	Early Carboniferous (c. 336)[*6]
Mollusca					
Eulimidae	> 4,000[*7]	M	S	Echinoderms	Late Cretaceous (> 72)[*9]
Pyramidellidae	> 5,000[*8]	M	S	Annelids, molluscs	Middle Cretaceous (> 107)[*10]

[*1]T: terrestrial, FW: freshwater, M: marine. [*2]C: complex, S: simple. [*3]Million years ago. [*4]Poinar et al. (2008). [*5]Littlewood et al. (1999). [*6]Jeyaprakash & Hoy (2009). [*7]Warén & Gittenberger (1993). [*8]Schander et al. (1999a). [*9]Neumann & Wisshak (2009). [*10]Jörger et al. (2010).

Chapter 1

Phylogenetic position of the Eulimdiae within Hypsogastropoda

1-1. Introduction

The class Gastropoda is one of the most successful animal lineages as parasites and has acquired parasitism at least eight times, fewer only than the numbers in two arthropod classes, Copepoda and Malacostraca (Poulin & Morand, 2000). With the great impact on the global evolution of animals and plants, the origins of parasitic lineages and their evolutionary histories of ecological and morphological traits have attracted much attention from phylogenetic systematists (e.g. Whitfild, 1998; Herlyn et al., 2003; Littlewood, 2006). However, while the phylogenetic position of the parasites among non-parasitic taxa is not necessarily well understood, such knowledge is essential for the inference of the ancestral states and evolutionary transition in the parasitic lineage. Among the parasitic groups of Gastropoda, phylogenetic position has been investigated for the Coralliophilinae (Barco et al., 2010), Pediculariinae (Meyer, 2003, 2004; Schiaparelli et al., 2005) and Pyramidellidae (Dinapoli & Klussmann-Kolb, 2010; Jörger et al., 2010; Dayrat et al., 2011; Dinapoli et al., 2011). These studies have provided interesting insights that parasitic snails often constitute a clade with carnivorous taxa, which might represent the prerequisite condition for parasitism. Coralliophilinae is one of the terminal subfamilies of the large carnivorous family Muricidae (Barco et al., 2010). This family also includes *Vitularia*, which parasitizes molluscan hosts (Herbert et al., 2009) and represents either the sister clade of Coralliophilinae or another terminal lineage among carnivorous genera (Barco et al., 2010). Pediculariinae belongs to the monophyletic, otherwise carnivorous Ovulidae (Schiaparelli et al., 2005), whose putative sister taxa also comprise predators on sponges and tunicates (Cypraeidae, Velutinidae & Triviidae; Wilson, 1998a, 1998b).

Pyramidellidae represents a possible sister clade of Glacidorbidae (Dinapoli & Klussmann-Kolb, 2010; Dinapoli et al., 2011), Amphiboloidea (Jörger et al., 2010) or Lymnaeoidea (Dayrat et al., 2011). The species of Glacidorbidae feed on the tissue of wounded invertebrates (Ponder, 1986). On the other hand, amphiboloids and lymnaeoids are deposit feeders and omnivores strongly oriented to animal food, respectively (Bovbjerg, 1968; Roach & Lim, 2000).

Eulimidae and its phylogenetic position

The family Eulimidae represents one of the most diverse groups of parasitic molluscs in terms of not only the number of extant species but also the existence of the widest range of parasitic strategies. These parasites exhibit a large variety of parasitic modes (e.g. endoparasitism, ectoparasitism and gall forming), sexual strategies (hermaphroditic, gonochoristic and environmental sex determination) and shell shapes (slender, conical, globose and capuliform; Warén, 1984). The Eulimidae are exclusive parasites of echinoderm hosts including all five classes, i.e. Echinoidea, Holothuroidea, Asteroidea, Ophiuroidea and Crinoidea (Warén, 1984), while the Late Cretaceous origin of this gastropod family clearly post-dates the Paleozoic divergence of the echinoderm clades (Neumann & Wisshak, 2009).

The phylogenetic position of the family has not been established within the Gastropoda. Eulimids had been placed in Ptenoglossa, which originally included a number of families that share a comb-like or "ptenoglossate" radula (Gray, 1853). Ptenoglossa was later confined to Eulimoidea, Epitonioidea and Triphoroidea based on the common presence of an acrembolic proboscis and two pairs of salivary glands in the three superfamilies (see Ponder et al., 2008). However, this group was found to be paraphyletic or polyphyletic in a cladistic analysis using morphological characters (Ponder & Lindberg, 1997) and therefore treated as an informal group in the working

classification by Bouchet & Rocroi (2005). In particular, eulimids differ from other ptenoglossans in lacking the distinctive parasperm (Healy, 1988). Molecular phylogenetic studies also support the polyphyly of the Ptenoglossa among the Hypsogastropoda (Colgan et al. 2000, 2007; Churchill et al., 2011a; Criscione & Ponder, 2013).

Hypsogastropoda represents the largest clade among the superorder Caenogastropoda with Cerithioidea as a possible sister taxon and consists of three provisional subgroups, i.e. Littorinimorpha, Neogastropoda and Ptenoglossa (Ponder & Lindberg, 1997; Bouchet & Rocroi 2005; Ponder et al., 2008). Of these, Neogastropoda constitutes a robust clade (Ponder & Lindberg, 1997; Zou et al., 2011) that is only remotely related to eulimids (Colgan et al., 2007). Previous phylogenetic studies have identified the Rissoinidae of the Littorinimorpha as the sister clade of Eulimidae (Colgan et al., 2007; Churchill et al., 2011a; Criscione & Ponder, 2013). However, this relationship remains inconclusive due to insufficient taxon sampling. Littorinimorpha and Ptenoglossa comprise a total of 65 families in 18 superfamilies (Bouchet & Rocroi, 2005), only less than half of which were included in those phylogenies, and the closest relative of Eulimidae may be found among other neglected taxa. Also the microalgal and bacterial feeding of rissoinids (Ponder & de Keyzer, 1998a) is at variance with the generally suggested position of parasitic lineages among carnivorous relatives.

In this study, 58 species from 38 hypsogastropod families were analyzed along with five outgroup species from Cerithioidea, with a special emphasis on littorinimorph and ptenoglossan taxa. Our goals are to determine the phylogenetic position of Eulimidae and to verify the monophyletic nature of the family in order to unravel the ancestral states from which parasitic life has derived.

1-2. Materials and Methods

Taxonomic sampling

Fifty-two littorinimorph and ptenoglossan species belonging to 32 families were collected and selected for the present molecular analysis to increase the total phylogenetic diversity of operational taxonomic units (OTUs; Table 1-1). Special emphasis was placed on Rissooidea and Truncatelloidea, which have been identified as possible close relatives of Eulimidae in previous studies (Colgan et al., 2007; Criscione & Ponder, 2013). Also included in the analysis was the type species of *Aclis* in the family Aclididae. Bouchet & Rocroi (2005) remarked that the Aclididae share certain morphological conditions with the Eulimidae and classified the two families as the exclusive members of Eulimoidea. However, a molecular phylogeny transferred the family to the superorder Heterobranchia based on sequences from *Larochella*, but not from the type genus *Aclis* (Dinapoli & Klussmann-Kolb, 2010; see also Warén, 2013). Nine eulimid species were also included in our phylogenetic reconstruction to cover the widest ranges of morphology and host diversity of the family as possible (Table 1-2). Most live snails were boiled in 70–90 ºC water for 0.1–1 min and the animals were extracted from the shells and preserved in pure ethanol. Voucher material has been deposited at Atmosphere and Ocean Research Institute, The University of Tokyo, unless otherwise noted in Table 1-1. All shell, operculum, radula and cephalic part of the animal were kept undamaged in most specimens for future taxonomic studies.

For outgroup comparisons, published cerithioid sequences were retrieved from the DDBJ/EMBL/Genbank (e.g. Zou et al., 2011), along with other sequences from five littorinimorph and one neogastropod species (Kameda & Kato, 2011). Neogastropoda was also represented by new sequences of *Chauvetia tenuisculpta* (Buccinidae), which is plausibly a parasite on echinoderms (Oliver & Rolan, 2008; Wirtz, 2011).

DNA extraction, PCR amplification and sequencing

Total DNA was extracted from the foot tissue using DNeasy Blood and Tissue Kit (Qiagen) and purified by GeneReleaser (Bioventures) following the manufacturer's recommendations. Portions of the mitochondrial and nuclear genes were amplified using the primer sets LCO1490-HCO2198 (for mitochondrial cytochrome *c* oxidase subunit 1, COI), 16SarL-16SbrH (16S rRNA), LSU5-LSU1600R and 1100F-na2 (nuclear 28S rRNA), 18A1-1800r (18S rRNA) and H3MF-H3MR (Histone H3; see Appendix 1). PCR reactions were conducted in a total volume ca. 25 µl: 17.5 µl DDW, 0.13 µl *TaKaRa Ex Taq* Hot Start Version (TaKaRa Bio Inc.), 2.5 µl *Ex Taq* Buffer (10x), 2.0 µl dNTP mixture (2.5 mM each), 0.3 µl forward and reverse primers (20 µM each) and 2.5 µl genomic DNA. After an initial denaturation for 2 min at 94 ºC, the reaction solution was run for 35 cycles with the following parameters: denaturation for 30 sec at 94 ºC, annealing for 40 sec at 50 ºC and extension for 60 sec at 72 ºC, followed by the final extension at 72 °C for 4 min; an annealing temperature at 42 ºC was used instead for the COI amplification. If amplification was unsuccessful under these conditions, either or both of the primers were replaced by others listed in Table S1-1. Amplicons were purified by ExoSAP-IT (Affymetrix) following the described protocol. Purified PCR products were sequenced with the amplification and/or internal primers; sequencing reactions were prepared using a Big Dye Terminator Cycle Sequence Kit ver. 3.1 (Applied Biosystems) following the manufacturer's protocol. The reaction mixtures were analyzed on an ABI PRISM 3130xl sequencer after purification with a Big Dye XTerminator Purification Kit (Applied Biosystems).

Phylogenetic analyses

I generated two datasets based on different combinations of genes and OTUs. The first

dataset comprised partial sequences of the 28S (spanning domains D1–D5; see Michot et al., 1984) and COI genes representing 60 species and 40 families from the whole Hypsogastropoda and its outgroup taxa. The second, five-gene dataset was made to reconstruct a more detailed phylogeny for Eulimidae and its related taxa, which were illustrated by the two-gene analyses. This dataset consisted of longer 28S fragments (D1–D7b), entire 18S and partial H3, COI and 16S sequences from 30 species and 15 families. For each dataset, the sequences of the three rRNA and one coding (COI) genes were aligned individually by ProAlign 0.5 alpha 1 (Löytynoja & Milinkovitch, 2003) with the band-width set to 1,200; the COI fragments were aligned as deduced amino acid sequences. The H3 sequences had no indels and were aligned by eye in MEGA 5 (Tamura et al., 2011). Each aligned dataset was masked to remove alignment ambiguous sites by ProAlign and Gblocks 0.91b (Castresana, 2000), resulting in four alignments (2gPA, 2gGB, 5gPA and 5gGB). For the 2gPA and 5gPA alignments, regions with posterior probabilities below 50% in the ProAlign analyses were excluded in the succeeding phylogenetic reconstruction. The 2gGB and 5gGB alignments were masked with the default parameters of Gblocks except that the "Minimum number of sequences for a conserved position" was set to 60% of OTUs, "Minimum number of sequences for a flank position" to 80% of OTUs and "Allowed gap positions" to "With half."

Phylogenetic trees were reconstructed from the four alignments using the Bayesian inference and Maximum Likelihood (ML) methods. In the Bayesian analyses performed with MrBayes 3.1.2 (Ronquist & Huelsenbeck, 2003), the general time-reversible model was used for all the datasets with invariant site frequency and gamma-shaped parameters estimated from the data (GTR + Γ + I), which was selected as the best-fit model by the Akaike information criterion in MEGA 5. The shape, proportion of invariant sites, state frequency and substitution rate parameters were estimated for each codon position separately in the amino acid coding COI and H3

genes. Each gene was allowed to have different parameters, hence the two-gene and five-gene alignments had four and nine partitions, respectively. Two parallel runs were made for 20,000,000 generations (with a sample frequency of 100), using the default value of four Markov chains. The first 100,000 trees for each run were discarded to make sure the four chains reached stationarity by referring to the average standard deviation of split frequencies (Ronquist & Huelsenbeck, 2003). The consensus tree and posterior probabilities (PP) were computed from the remaining 200,000 trees (100,000 trees, two runs). Posterior probabilities equal to or above 0.95 were considered meaningful support. The ML analyses were performed using the Pthreads version of RAxML v7.2.6 (Stamatakis, 2006) with the same partitions as the Bayesian analyses and the following commands: a rapid bootstrap analysis and search for the best-scoring ML tree in one single program run (-f a) and 1,000 bootstrap replicates (-# 1000) under the GTR + Γ + I substitution model (-m GTRGAMMAI). Bootstrap probabilities (BP) equal to or above 70% were considered meaningful support. Bayesian analyses were also performed for individual genes with 5,000,000 generations and burn-in value setting at 25,000 to compare evolutionary rates and to eliminate possible contamination and erroneous sequences. All trees were edited by FigTree v1.3.1 (http://tree.bio.ed.ac.uk/software/figtree/).

1-3. Results

Sequence data

The numbers of total, excluded, variable, and parsimony-informative sites are shown for the four alignments in Table 1-3. *Stenothyra thermaecola* and *Tubbreva* sp. were found to have extremely high evolutionary rates of the 28S gene and were therefore excluded from the multi-gene alignments; *Aclis minor* was also excluded due to

difficulties in amplifying gene fragments except H3. The two-gene dataset had 2,235 sites, of which 309 and 382 were masked in the 2gPA and 2gGB alignments, respectively. The five-gene dataset had 5,616 sites and 610 and 646 were excluded in the respective 5gPA and 5gGB analyses. Gblocks tended to exclude more sites of 18S and 28S than ProAlign did, whereas the 16S alignments showed the opposite pattern. The proportion of variable sites varied from 9.6% in the 18S gene of the 5gGB alignment to 60.6% in the COI of the 2gGB alignment. Parsimony-informative sites varied from 4.8% in the 18S of the 5gGB to 50.2 % in the COI of the 2gPA (Table 1-3). There were two 3-bp deletions in the COI matrix at the positions 95–97 (*Vanikoro helicoidea*) and 296–298 (*Caecum globellum* and *Iravadia sakaguchii*).

Phylogenetic analyses of the combined datasets

Bayesian and likelihood analyses yielded the same results for all four alignments in terms of clades with meaningful support values. I therefore show only Bayesian trees with posterior probabilities and ML bootstrap values on branches (Figs. 1-1, 1-2).

The two-gene dataset recovered the Eulimidae as a robust monophyletic clade in the analyses of both 2gPA and 2gGB alignments (PP = 1.00, BP ≥ 98%; Fig. 1-1, see Appendix 1 for the 2gGB tree). The family consisted of two subclades, reflecting the presence or absence of the radula (1.00, ≥ 89%; Table 1-2). The monophyletic Vanikoridae (*Vanikoro* + *Macromphalus*: 1.00, 100%) constituted a well-supported clade with Eulimidae as the newly redefined superfamily Vanikoroidea (1.00, 100%). *Lyocyclus*, a genus previously assigned to Vanikoridae or its own family Lyocyclidae, was found to be distant from the type genus *Vanikoro* and formed a moderately supported clade with *Macrocypraea* (Cypraeidae) in the 2gGB analysis (0.96, < 50%). The previously suggested affinity of Hipponicidae to Vanikoridae (as a member of Vanikoroidea; e.g. Ponder & Warén 1988; Bouchet & Rocroi 2005) was clearly rejected

in all analyses. The superfamily Rissooidea (Rissoidae, Rissoinidae and Barleeiidae) was paraphyletic to the Vanikoroidea albeit with insignificant support values (≤ 0.91, ≤ 68%). The two superfamilies constituted a robust clade with the Truncatelloidea (1.00, 89%). Twenty other suprageneric nodes received meaningful PP and BP values in both analyses: *Niso* + *Pyramidelloides* + *Hemiliostraca* (1.00, ≥ 95%), *Monogamus* + *Vitreolina* + *Stilifer* + *Thyca* (1.00, 100%), *Monogamus* + *Vitreolina* (≥ 0.97, ≥ 83%), *Stilifer* + *Thyca* (1.00, 100%), Rissoidae (1.00, 100%), *Benthonella* + *Lucidestea* (1.00, ≥ 90%), Rissoinidae (1.00, 100%), Rissoinidae + Barleeiidae (1.00, ≥ 92%), Truncatelloidea (1.00, 100%), *Assiminea* + *Truncatella* + *Cecina* + *Falsicingula* + *Potamopyrgus* + *Amphithalamus* (≥ 0.97, ≥ 71%), *Assiminea* + *Truncatella* + *Cecina* + *Falsicingula* (1.00, ≥ 99%), *Teniostoma* + *Iravadia* (≥ 0.99, ≥ 72%), Hipponicidae (1.00, 100%), Epitonioidea (1.00, ≥ 91%), Janthinidae + *Alexania* + *Epitonium* (1.00, 100%), Janthinidae + *Alexania* (≥ 0.95, ≥ 72%), Nystiellidae + *Opalia* (1.00, ≥ 96%), Pterotracheoidea (1.00, ≥ 87%), Neogastropoda (≥ 0.99, ≥ 78%), Cerithioidea + Pickworthiidae (1.00, ≥ 99%), *Pelycidion* + *Microliotia* (≥ 0.99, ≥ 75%). The Tornidae and Epitoniidae *sensu* Bouchet & Rocroi (2005) were recovered as non-monophyletic groups in our analyses. The monophyly of Cerithioidea + Pickworthiidae was confirmed by a separate two-gene analysis with *Campanile symbolicum* (Campaniloidea) and three heterobranch species as outgroup taxa (see Appendix 1).

The five-gene dataset recovered the relationships among and within the Vanikoroidea, Truncatelloidea and Rissooidea with higher posterior and bootstrap values (Figs. 1-2, see Appendix 1 for the 5gGB tree). The sister relationship between the redefined Vanikoroidea and Truncatelloidea was supported in both 5gGB (1.00, 64%) and 5gPA (0.95, 62%) analyses. The superfamily Rissooidea, here represented by Rissoidae and Rissoinidae, was supported in the Bayesian analysis of the 5gGB alignment (0.97; ML: < 50%) but not in the 5gPA analyses (0.88, < 50%). The relationships among eulimid genera in the 5gGB trees were not concordant with those

recovered in the two-gene and 5gPA analyses: *Hemiaclis* was the basal-most offshoot of the family in the 5gGB analyses (1.00, 75%) while it constituted a clade with *Niso* + *Pyramidelloides* + *Hemiliostraca* with lower support indices in the 5gPA analyses (0.96, 68%). The two ophiuroid parasites included in the dataset formed a robust clade in both analyses (*Pyramidelloides* + *Hemiliostraca*; 1.00, ≥ 92%). On the other hand, the asteroid parasites *Stilifer* and *Thyca* were distantly related to *Niso*, another group exploiting sea stars (1.00, 100%).

Independent gene analyses

Most of the 13 Bayesian analyses for independent gene sequences resulted in poorly resolved trees (see Appendix 1), while the monophyly of the Eulimidae was unambiguously supported in 28S, COI and 16S trees (PP = 1.00). Other clades with meaningful posterior probabilities (≥ 0.95) include: all four eulimids without the radula (supported by 18S, 28S and COI), Vanikoridae (18S, 28S, H3 and 16S), Vanikoroidea (28S), Rissoidae (18S and 28S), Rissoinidae (18S, 28S and COI), Hipponicidae (28S and COI), Nystiellidae + *Opalia* (28S, H3 and 16S), Epitonioidea (18S and 28S). There were a few contradictory clades with meaningful support values in the independent gene trees, particularly between nuclear rRNA and mitochondrial COI topologies with regard to the positions of Vanikoridae, possibly reflecting excessive evolutionary rates of the latter gene and long-branch attraction.

The shorter fragments of the 28S gene (D1–D5) confirmed the truncatelloid affinity of *Stenothyra thermaecola* (PP = 1.00), while *Tubbreva* sp. of Cingulopsidae appeared in a large, basal polytomy (Appendix 1; see also Criscione & Ponder, 2013). The phylogenetic position of *Aclis minor*, the type of the family Aclididae, could not be resolved with the available H3 sequences. However, this H3 sequence showed the smallest uncorrected distances to *Schwartziella subulata* (5.2%) and *Macromphalus* sp.

(6.2%; Appendix 1), which suggests a position of the family among the Vanikoroidea, Rissooidea and Truncatelloidea, and corroborates with the classification by Fretter & Graham (1982), Bouchet & Rocroi (2005) and Warén (2013).

1-4. Discussion

Phylogenetic position and ancestral states of the Eulimidae

The most significant finding of the present study is the robust sister relationship of the Eulimidae and Vanikoridae (Figs. 1-1, 1-2) and we propose that the two families constitute a newly redefined Vanikoroidea Grey, 1840, which has nomenclatural precedence over Eulimoidea Philippi, 1853. Earlier molecular phylogenies that suggested that the closest relationship of Eulimidae is with Rissoinidae (Colgan et al., 2007; Churchill et al., 2011a; Criscione & Ponder, 2013) did not include vanikorids. The gastropod classification by Bouchet & Rocroi (2005) assigned Vanikoridae along with Hipponicidae and Haloceratidae into Vanikoroidea, and Eulimidae and Aclididae in Eulimoidea, based on shared, but plausibly symplesiomorphic, conditions of the early ontogeny and feeding ecology (see Ponder, 1998). The Hipponicidae and Vanikoridae have been analyzed in a molecular phylogeny that showed their distant relationship (Collin, 2003; see also Ponder et al., 2008), but again Eulimidae was not included.

The Vanikoridae are globose to conical, small- to medium-sized, non-parasitic snails living in shallow intertidal waters as well as at subtidal, shelf and bathyal depths (Warén & Bouchet, 1988; Ponder, 1998). There seems to be no clear synapomorphy among described conchological or anatomical conditions to support the monophyletic group comprising Eulimidae and Vanikoridae. However, limited anatomical information available for vanikorids has been obtained mainly from the large, possibly autapomorphic genus *Vanikoro* (e.g. Simone, 2002) and little is known for the various

genera from deeper waters; one of the few shared anatomical features of the family is the presence of the epipodial flap on each side of the foot, which is lacking in Eulimidae (Warén & Bouchet, 1988).

Interestingly, the two families share some reproductive and ecological conditions. Most hypsogastropod species are dioecious (Heller, 1993), while many eulimids are sequential hermaphrodites (Warén, 1984; Bouchet & Warén, 1986) as are vanikorids (Ponder, 1998). In addition, Goto et al. (2011) have found a vanikorid, *Macromphalus tornatilis*, in the burrows of echiuran worms and suggested a certain association between them. Although the feeding ecology of the Vanikoridae has not been adequately studied, sponge spicules, foraminifers and diatoms have been found in the stomach contents of *Vanikoro cancellata* (Golding et al., 2009). Indeed, species of *Vanikoro* are almost always found attached on/near sponges on the underside of deep-buried coral rubble (Y. Kano, personal observation; Appendix 1), suggesting omnivorous or carnivorous feeding habits for the family. If this is the case, the common ancestor of Eulimidae and Vanikoridae might have depended on animal flesh for its nutrient requirement and differentiated from the detritivorous modes in the Rissooidea and Truncatelloidea, which represent possible sister clades of Vanikoroidea (Fig. 1-1). The parasitic mode of life in eulimids has therefore likely originated from a predatory ancestor as in the cases of some other gastropod (Schiaparelli et al., 2005; Barco et al., 2010).

Vanikoroidea potentially includes two other extant families, namely Aclididae and Haloceratidae. Aclidids are small animals imperfectly known both in morphology and way of life, because of their rarity and sublittoral habitats. The species of the type genus *Aclis* are almost certainly carnivores, which have an acrembolic proboscis and small ptenoglossan radula (Fretter & Graham, 1982). They most closely resemble the Eulimidae among the polyphyletic ptenoglossan families in that they share similar anatomical conditions and protoconch morphology, although the tumid teleoconch

whorls and the lack of a penis differentiate the former from the latter (Fretter & Graham, 1982; Bouchet & Rocroi, 2005). The presence of a large epipodial fold on each side of the foot in *Aclis* (Bouchet and Warén, 1986; Gofas et al., 2011) and vanikorids (Warén & Bouchet, 1988; Ponder 1998) may further suggest the affinity of Aclididae to Vanikoroidea. The available specimen of the type species (*A. minor*) yielded only a H3 sequence that did not clearly show a phylogenetic position in the Bayesian analysis for this gene, while the comparison of genetic distances supported the vanikoroid affinity but not a relationship to the Epitoniidae, another possible candidate as the closest relative of Aclididae (Bouchet & Warén, 1986). A previous molecular phylogeny transferred Aclididae to the superorder Heterobranchia based on sequences from *Larochella* (Dinapoli & Klussmann-Kolb, 2010; see also Warén, 2013). However, so-called aclidids contain many polyphyletic genera with small and slender shells but with a fundamentally different anatomy, and *Larochella* actually belongs to an unrelated heterobranch family, Graphididae (Warén, 2013), or its possible senior synonym Tofanellidae (Gründel & Nützel, 2013). A future analysis with a better-preserved specimen of *A. minor* is needed to determine the precise phylogenetic position of Aclididae.

The deep-sea family Haloceratidae represents another rare and poorly studied group with an uncertain affinity in Hypsogastropoda. Warén & Bouchet (1991) noted in the description of the family that haloceratids are probably sedentary carnivorous animals with sequential hermaphroditism (see also Warén, 1993). These characteristics may suggest their close affinity to the Vanikoridae (Ponder 1998) as well as to the Eulimidae and the predatory mode of life as the ancestral condition for the latter family. Haloceratids are also similar to vanikorids in sharing a characteristic foot that is divided into two functionally different parts, although other morphological conditions instead suggest their affinity to either the Capulidae (Capuloidea) or the Laubierinidae (Tonnoidea; Warén & Bouchet 1991). The Haloceratidae may represent

another important group in future phylogenies to shed light on the evolution of the parasitic mode of life in Vanikoroidea.

Convergent evolution and superficial resemblance to Vanikoroidea

The present study reveals that some taxa that have been included in Vanikoroidea or assigned close to or within Vanikoridae are distantly related and have independently acquired morphological resemblance. Simone (2002, 2011) showed that the Vanikoridae have certain similarities to the Hipponicidae, Calyptraeidae and Capulidae in conchological and anatomical characters. Of these, Hipponicidae has been considered a member of Vanikoroidea, while each of Calyptraeidae and Capulidae represents an independent superfamily in many of the current classifications (e.g. Bouchet & Rocroi, 2005). All four families have been included in a molecular phylogenetic analysis (Collin, 2003) that showed distant relationships among the Hipponicidae, Vanikoridae and Calyptraeidae + Capulidae. Based on the present and previous molecular phylogenies, Hipponicidae is provisionally transferred from Vanikoroidea to its own monotypic superfamily Hipponicoidea Troschel, 1861. Convergence is also apparent within the Vanikoridae. There are little-known genera from the deep sea, for example *Lyocyclus*, which have been classified into this family based on similarities in external anatomy and radular morphology, regardless of their rather unusual shell shapes (Warén & Bouchet, 1988; Warén, 1989). *Lyocyclus* is found to be very distant from *Vanikoro* + *Macromphalus* and represents its independent family Lyocyclidae Thiele, 1925 (Fig. 1-1). There might be more heterogeneous taxa in Vanikoridae that deserve independent familial status or belong to other hypsogastropod families.

Polyphyly of the informal group Ptenoglossa was reaffirmed (see Bouchet & Rocroi, 2005; Colgan et al., 2007; Churchill et al., 2011a). Ptenoglossate radulae have

been acquired independently in Vanikoroidea, Epitonioidea and Triphoroidea as well as in many other, totally distant gastropod groups, e.g. some of Trochaclididae, Pseudococculinidae (both Vetigastropoda) and Architectonicidae (Heterobranchia), probably to serve similar feeding ecologies (Warén, 1984; Warén & Gofas, 1996). Also, parasitism on echinoderms has probably evolved more than once in Hypsogastropoda. *Chauvetia tenuisculpta* apparently parasitize echinoids and asteroids (Oliver & Rolan, 2008; Wirtz, 2011), while the present trees confirm its position within Neogastropoda (Buccinidae) and distant from Eulimidae (Fig. 1-1).

Ecological radiation and morphological differentiation in the Eulimidae

The present phylogeny demonstrates that the family Eulimidae constitutes a robust clade (Figs. 1-1, 1-2), although the nine genera included in the analysis have considerably different morphologies, hosts and parasitic strategies (Table 1-2). Adams and Adams (1853) established a separate family Styliferidae for *Stilifer* that bears a broader and more globose shell than that of *Eulima*, the type genus of Eulimidae. Succeeding authors had placed several other eulimid genera with similarly broad shells in Styliferidae (e.g. Laseron, 1955). These conchological differences, however, have been shown to be specializations connected with the degree of parasitism; the inflated shells are presumably apomorphic and acquired in multiple genera where parasites permanently attach to their hosts (Warén, 1984). The distant relationship between *Stilifer* and another globose genus *Monogamus* in the present molecular trees verifies the plasticity of the shell shape in the evolution of the Eulimidae. Further support of this plasticity is indicated by the terminal position of the limpet-shaped genus *Thyca*, which shows an even more derived condition from *Stilifer*. This apparently represents morphological adaptation for stronger attachment to the host with a larger sole of the

foot, as suggested for multiple lineages of rocky-shore limpets to substrates (Vermeij, 1993).

The Eulimidae are exclusive parasites of echinoderms including all five classes. Warén (1984) noted that each class of the host seemed to be infected by a single lineage of eulimids, with a possible exception by the genus *Vitreolina* that includes ophiuroid and echinoid parasites. However, the present phylogeny demonstrates at least one more exceptional case where a host class is parasitized by multiple eulimid clades. The asteroid parasites *Stilifer* and *Thyca* are distantly related to *Niso*, another group exploiting sea stars (Warén, 1984). Regardless, the evolutionary history of host associations cannot be dealt with precisely without including additional taxa. There are more than 1,250 described species and over 90 genera in the family which has a global distribution from the equator to the poles and occupy a wide range of depths, from intertidal to abyssal waters (Warén, 1984; Bouchet & Warén, 1986). The polarity of evolutionary transitions among sexual (gonochoristic and protandric/simultaneous hermaphroditic) strategies is even more difficult to evaluate due to the rarity of properly preserved specimens that represent various ontogenetic stages.

One of the few morphological or ecological characters that accord well with our tree topology is the presence or absence of the radula. Radula-less species always constitute a robust monophyletic clade, while snails with the radula (*Hemiaclis*, *Niso*, *Pyramidelloides* and *Hemiliostraca*) were either monophyletic or paraphyletic in the two-gene and five-gene reconstructions, respectively (Figs. 1-1, 1-2; Table 1-2). The Eulimidae have acquired the ptenoglossate radula in parallel to those of Epitonioidea and Triphoroidea (see above) and one of the ancestral lineages of the family has apparently lost this digestive apparatus, which may have a limited use in their blood-sucking mode of feeding (Warén, 1984). A more detailed ingroup phylogeny would provide further insights on the loss of the radula and transitions of other morphological and ecological traits.

Rissooidea and Truncatelloidea

Relationships among Vanikoroidea, Rissooidea and Truncatelloidea were not clearly resolved in our trees. The sister relationship between Vanikoroidea and Truncatelloidea was supported by the highest Bayesian posterior probability but insignificant ML bootstrap values in the 5gPA tree (Fig. 1-2, see also Appendix 1). This topology differs from that of a previously published phylogeny (Criscione & Ponder, 2013), which places a eulimid species within the Rissooidea with high posterior and bootstrap support (PP = 1.00, BP = 93%) based on two of the five markers used in the present analyses (28S and 16S, a total of *ca*. 2.2 kbp). Possible explanations for the incongruence include differences in the numbers of markers and OTUs and the method of sequence alignment (see also Fig. 1-1). On the other hand, Barleeiidae and Rissoinidae consistently form a robust clade within Rissooidea, both in the present and previous (Criscione & Ponder, 2013) phylogenies. These two families share a pegged operculum, which is lacking in the type family Rissoidae (Ponder, 1985).

Our phylogenetic reconstruction reveals more insights on the internal relationship of the Truncatelloidea. The analyzed ten families belong to one of two major clades: Anabathridae + Hydrobiidae + Assimineidae + Truncatellidae + Pomatiopsidae + Falsicingulidae, and Elachisinidae + Caecidae + Iravadiidae + Tornidae (Figs. 1-1, 1-2). The former clade comprises all marine, freshwater and terrestrial taxa, while the species of the latter clade inhabit only the marine environment including brackish estuaries and mangrove swamps (see Ponder & de Keyzer, 1998a). A subclade of the former clade (Hydrobiidae + Assimineidae + Truncatellidae + Pomatiopsidae + Falsicingulidae) has already been recovered with the highest PP value in Criscione & Ponder (2013), while its sister relationship to Anabathridae is first resolved here (Fig. 1-2). The monophyletic nature of the Tornidae (= Vitrinellidae; Bouchet & Rocroi, 2005) is clearly rejected by the sister relationship between *Vitrinella*

and *Iravadia*, confirming the previous suspicion that this family comprises heterogeneous groups (Ponder & de Keyzer, 1998a).

Other hypsogastropod clades

The present phylogeny provides further information on the suprageneric classification of Hypsogastropoda and other caenogastropod taxa. Nystiellidae of the superfamily Epitonioidea (*Opaliopsis* sp.) is included for the first time in a molecular analysis and is found to occupy a terminal position within the Epitoniidae. Nystiellidae was originally established as a subfamily of Epitoniidae (Bouchet & Warén, 1986) and later given a distinct familial status based almost solely on the presence of dense axial ribs in the protoconch (Nützel, 1998; Bouchet & Rocroi, 2005). However, nystiellids have general shell shapes that are very similar to those of some typical epitoniids with a smooth protoconch (e.g. *Opalia*; Bouchet & Warén, 1986). The present tree indeed shows a close relationship between *Opalia* and *Opaliopsis* (Fig. 1-1); the protoconch ornamentation has possibly been acquired as an apomorphy in the latter lineage. The neustonic Janthinidae represents another terminal clade within the Epitoniidae as has already been discussed by Churchill et al. (2011a). Interestingly, *Alexania* represents the closest benthic relative of Janthinidae in our trees with meaningful nodal support values (Fig. 1-1). The broad, smooth and brown shell of *Alexania* differs noticeably from the tall, ribbed white shells of other epitoniids and closely resembles that of the plesiomorphic janthinid genus *Recluzia* (Robertson & Habe, 1965; Churchill et al., 2011a, b). Unfortunately, our knowledge of their anatomy is insufficient to verify their close kinship and to infer morphological differentiation and adaptation that have accompanied the radical habitat transition from the benthic to neustonic mode of life.

A further, significant finding concerns the position of the little-known, mainly cavernicolous family Pickworthiidae. Only a few snails of the family have been

collected alive from submarine caves and similar cryptic voids in the shallow subtidal waters of the tropics and subtropics (Table 1-1; Bouchet & Le Renard, 1998; Kase, 1998). The Pickworthiidae have been tentatively assigned to Littorinoidea based on protoconch morphology alone (Bouchet & Le Renard, 1998; Bouchet & Rocroi, 2005), while the same morphological character also implies a relationship to Cerithioidea, a possible sister clade of Hypsogastropoda (Ponder & Lindberg, 1997; Colgan et al., 2007; Ponder et al., 2008). Our molecular data recover three pickworthiid genera as the sister clade of, or paraphyletic to, the Cerithioidea (Fig. 1-1). The genera *Pelycidion* and *Microliotia* are clustered with high support values, whereas the former has been classified in an independent family (Pelycidiidae) with a unique combination of the tall, minute shell and rhipidoglossate-like radula (Ponder & Hall, 1983; Bouchet & Le Renard, 1998) or later a subfamily of Pickworthiidae (Bouchet & Rocroi, 2005). The paraphyletic nature of Pickworthiinae (here represented by *Microliotia* and *Mareleptopoma*), however, suggests that the morphologies unique to *Pelycidion* are apomorphic, derived conditions within the family. Cerithioid anatomy has been examined in detail (e.g. Houbrick, 1988; Strong et al., 2011), but the Pickworthiidae are neglected due to the inaccessibility of live animals (Bouchet and Le Renard, 1998; Kase, 1998). In summary, integrated molecular, morphological and ecological investigations, covering taxa from the deep sea and other inaccessible habitats, are essential to reveal hypsogastropod relationships and evolution of various life history strategies including parasitism.

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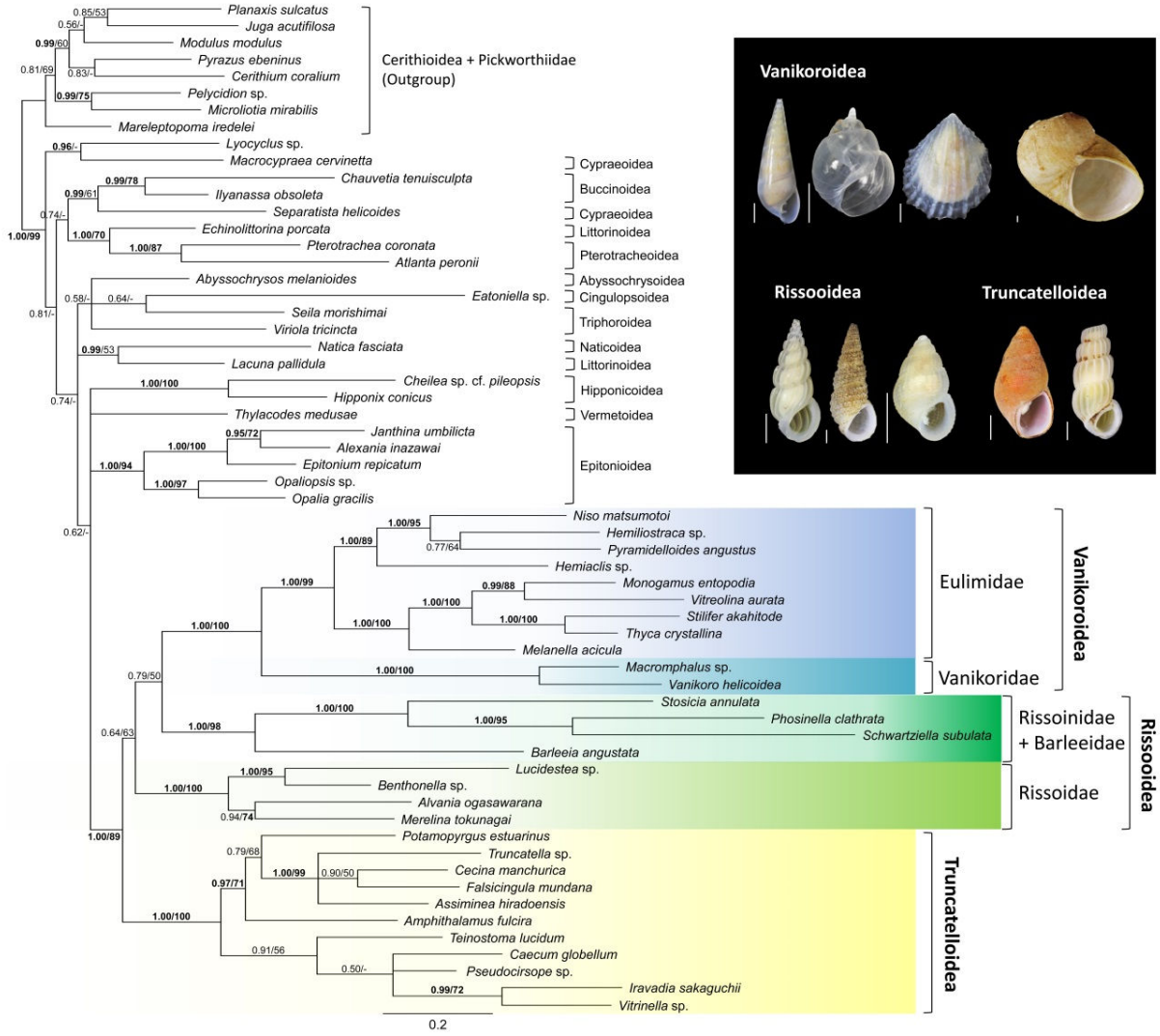

Figure 1-1. Bayesian phylogeny of Hypsogastropoda inferred from 2gGB alignment of 28S (D1–D5) and COI genes (1,853 sites in total). Numerals on branches denote posterior probabilities (PP, left) and likelihood-based bootstrap values shown as percentages (BS, right); significant support in bold (PP ≥ 95%, BS ≥ 70%). Shells from upper left to lower right: *N. matsumotoi*, *M. acicula*, *M. entopodia*, *T. crystallina*, *V. helicoidea*, *S. subulata*, *R. clathrata*, *M. tokunagai*, *A. ogasawarana*, *I. sakaguchii*, *Truncatella* sp. and *C. globella* (scale bars: 1 mm).

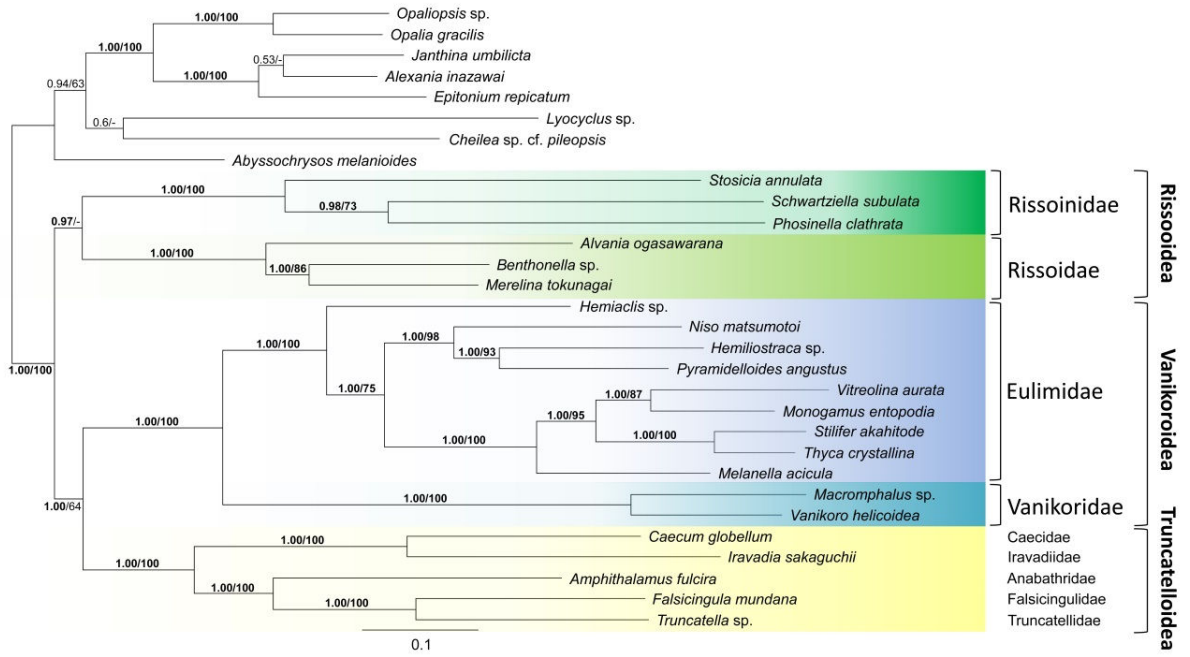

Figure 1-2. Bayesian phylogeny of Vanikoroidea, Truncatelloidea and Rissooidea inferred from 5gGB alignment of 28S (D1–D7b), 18S, H3, 16S and COI genes (4,969 sites in total). Numerals on branches denote posterior probabilities (PP, left) and likelihood-based bootstrap values shown as percentages (BS, right); significant support in bold (PP ≥ 95%, BS ≥ 70%).

Table 1-1. Species used in present analyses with DDBJ/EMBL/GenBank accession numbers and collection sites and habitats of specimens. Accession numbers of newly obtained sequences are given in bold. Suprageneric classification reflects topology of new trees.

Classification	Species	28S D1–5	28S D6–7b	18S	H3	16S	COI	DNA/Voucher	Coordinates	Locality; habitat and depth
Vanikoroidea[*1]										
Eulimidae	*Hemiaclis* sp.	**AB930331**	**AB930331**	**AB930382**	**AB930436**	**AB930409**	**AB930465**	YK#1580	31°07'N, 131°39'E	KT-11-12 (T-10-2), off Cape Toi, Miyazaki, Japan; 1063–1082 m
	Hemiliostraca sp.	**AB930332**	**AB930332**	**AB930383**	**AB930437**	**AB930410**	**AB930466**	YK#1584	31°44'N, 131°28'E	Nojima, Miyazaki, Japan; intertidal rocky shore
	Melanella acicula	**AB930330**	**AB930330**	**AB930381**	**AB930435**	**AB930408**	**AB930464**	YK#1571	28°09'N, 129°21'E	Isu, Amami Is., Japan; intertidal, on *Stichopus chloronotus*
	Monogamus entopodia	**AB930324**	**AB930324**	**AB930375**	**AB930429**	**AB930402**	**AB930458**	YK#1481	28°07'N, 129°21'E	Yadorihama, Amami Is., Japan; intertidal, on *Echinometra mathaei*
	Niso matsumotoi	**AB930335**	-	**AB930385**	**AB930440**	**AB930413**	**AB930469**	YK#1594	34°26'N, 136°55'E	Off Toba, Mie, Japan; 10–25 m
	Pyramidelloides angustus	**AB930336**	**AB930336**	**AB930386**	**AB930441**	**AB930414**	**AB930470**	YK#1601	26°52'N, 128°16'E	Cape Hedo, Okinawa Is., Japan; intertidal, on *Ophiocoma scolopendrina*
	Stilifer akahitode	**AB930327**	**AB930327**	**AB930378**	**AB930432**	**AB930405**	**AB930461**	YK#1541	32°33'N, 130°06'E	Tsuji Is., Amakusa, Japan; intertidal, in *Certonardoa semiregularis*
	Thyca crystallina	**AB930326**	**AB930326**	**AB930377**	**AB930431**	**AB930404**	**AB930460**	YK#1519	09°49'N, 123°22'E	Moalboal, Cebu Is., Philippines; on *Linckia laevigata*
	Vitreolina aurata	**AB930323**	**AB930323**	**AB930374**	**AB930428**	**AB930401**	**AB930457**	YK#1475	34°40'N, 138°59'E	Shimoda, Izu, Japan; intertidal, on *Hemicentrotus pulcherrimus*
Vanikoridae	*Macromphalus* sp.	**AB930369**	**AB930369**	**AB930399**	**AB930453**	**AB930425**	-	YK#1655	09°29'N, 123°41'E	Off Panglao Is., Bohol, Philippines; ca. 300 m, on sunken wood
	Vanikoro helicoidea	**AB930359**	**AB930359**	**AB930395**	**AB930450**	**AB930421**	**AB930487**	YK#1643	24°27'N, 124°08'E	Kabira, Ishigaki Is., Japan; tidal flat
Rissooidea										
Barleeiidae	*Barleeia angustata*	**AB930348**	-	-	-	-	**AB930479**	YK#1630	34°40'N, 138°59'E	Shimoda, Izu, Japan; intertidal rocky shore
Rissoidae	*Alvania ogasawarana*	**AB930358**	**AB930358**	**AB930394**	**AB930449**	-	**AB930486**	YK#1642	24°29'N, 124°17'E	Tamatorizaki, Ishigaki Is., Japan; intertidal rocky shore
	Benthonella sp.	**AB930363**	**AB930363**	**AB930396**	-	**AB930422**	**AB930489**	YK#1647	31°07'N, 131°39'E	KT-11-12 (T-10-2), off Cape Toi, Miyazaki, Japan; 1063–1082 m
	Lucidestea sp.	**AB930347**	-	-	-	-	-	YK#1628	34°40'N, 138°59'E	Shimoda, Izu, Japan; intertidal rocky shore
	Merelina tokunagai	**AB930344**	**AB930344**	**AB930389**	**AB930443**	**AB930416**	**AB930476**	YK#1623	34°40'N, 138°59'E	Shimoda, Izu, Japan; intertidal rocky shore
Rissoinidae	*Phosinella clathrata*	**AB930351**	**AB930351**	**AB930392**	**AB930446**	**AB930419**	-	YK#1633	24°21'N, 123°45'E	Shirahama, Iriomote Is., Japan; tidal flat
	Schwartziella subulata	**AB930341**	**AB930341**	**AB930388**	**AB930442**	-	**AB930474**	YK#1618	31°24'N, 130°11'E	Koura, Kagoshima, Japan; intertidal rocky shore
	Stosicia annulata	**AB930349**	**AB930349**	**AB930391**	**AB930445**	**AB930418**	**AB930480**	YK#1631	32°30'N, 131°43'E	Nobeoka, Miyazaki, Japan; intertidal rocky shore
Truncatelloidea										
Anabathridae	*Amphithalamus fulcira*	**AB930345**	**AB930345**	**AB930390**	**AB930444**	**AB930417**	**AB930477**	YK#1624	34°40'N, 138°59'E	Shimoda, Izu, Japan; intertidal rocky shore
Assimineidae	*Assiminea hiradoensis*	AB611805	-	-	-	-	AB611807			
Caecidae	*Caecum globellum*	**AB930352**	**AB930352**	**AB930393**	**AB930447**	-	**AB930481**	YK#1634	34°40'N, 138°59'E	Shimoda, Izu, Japan; intertidal rocky shore
Elachisinidae	*Pseudocirsope* sp.	**AB930360**	-	-	-	-	-	YK#1644	30°16'N, 130°25'E	Kurio, Yakushima Is., Japan; intertidal rocky shore
Falsicingulidae	*Falsicingula mundana*	**AB930366**	**AB930366**	**AB930398**	**AB930452**	**AB930424**	**AB930492**	YK#1651	35°42'N, 135°03'E	Kotobikihama, Kyoto, Japan; intertidal rocky shore
Hydrobiidae	*Potamopyrgus estuarinus*	**AB930357**	-	-	-	-	**AB930485**	YK#1640	36°34'S, 174°41'E	Orewa, N of Auckland, New Zealand; estuary

Iravadiidae	*Iravadia sakaguchii*	**AB930339**	-	**AB930387**	-	**AB930415**	**AB930473**	YK#1616	31°25'N, 131°15'E	Honjo R., Kushima, Miyazaki, Japan; estuary
Pomatiopsidae	*Cecina manchurica*	AB611741	-	-	-	-	AB611743			
Stenothyridae	*Stenothyra thermaecola*	**AB930355**	-	-	-	-	-	YK#1638	33°16'N, 131°22'E	Yufuin, Oita, Japan; hot spring
Tornidae	*Teinostoma lucidum*	**AB930343**	-	-	-	-	-	YK#1621	35°15'N, 139°35'E	Hayama, Kanagawa, Japan; intertidal rocky shore
	Vitrinella sp.	**AB930362**	-	-	-	-	-	YK#1646	35°15'N, 139°35'E	Hayama, Kanagawa, Japan; intertidal rocky shore
Truncatellidae	*Truncatella* sp.	**AB930353**	**AB930353**	-	**AB930448**	**AB930420**	**AB930482**	YK#1635	24°47'N, 125°16'E	Hisamatsu, Miyako Is., Japan; stream mouth
Cingulopsoidea										
Cingulopsidae	*Tubbreva* sp.	**AB930370**	-	-	-	-	-	YK#1656	32°33'N, 130°06'E	Tsuji Is., Amakusa, Japan; intertidal rocky shore
Eatoniellidae	*Eatoniella* sp.	**AB930346**	-	-	-	-	**AB930478**	YK#1626	34°40'N, 138°59'E	Shimoda, Izu, Japan; intertidal rocky shore
Hipponicoidea[*1]										
Hipponicidae	*Cheilea* sp. cf. *pileopsis*	**AB930365**	-	**AB930397**	**AB930451**	**AB930423**	**AB930491**	YK#1650	24°28'N, 123°49'E	Hatoma Is., Okinawa, Japan; tidal flat
	Hipponix conicus	**AB930364**	-	-	-	-	**AB930490**	YK#1649	31°44'N, 131°28'E	Nojima, Miyazaki, Japan; intertidal rocky shore
Epitonioidea										
Epitoniidae	*Alexania inazawai*	**AB930329**	**AB930329**	**AB930380**	**AB930434**	**AB930407**	**AB930463**	YK#1552	28°26'N, 129°40'E	Tekebu, Amami Is., Japan; tidal flat
	Epitonium replicatum	**AB930328**	**AB930328**	**AB930379**	**AB930433**	**AB930406**	**AB930462**	YK#1551	32°29'N, 131°41'E	Kadokawa, Miyazaki, Japan; intertidal seagrass bed
	Opalia gracilis	**AB930334**	**AB930334**	**AB930384**	**AB930439**	**AB930412**	**AB930468**	YK#1591	17°29'S, 149°50'W	Phaena, Moorea Is., French Polynesia; intertidal seagrass bed
Janthinidae	*Janthina umbilicata*	**AB930333**	**AB930333**	-	**AB930438**	**AB930411**	**AB930467**	YK#1590	36°50'S, 174°26'E	Muriwai, W of Auckland, New Zealand; beach drift
Nystiellidae	*Opaliopsis* sp.	**AB930373**	-	-	**AB930456**	**AB930427**	-	YK#1775[*2]	18°33'S, 164°20'E	Récif Pétrie, New Caledonia; 580–703 m
Capuloidea										
Capulidae	*Separatista helicoides*	**AB930338**	-	-	-	-	**AB930472**	YK#1615	31°18'N, 130°12'E	Marukihama, Kagoshima, Japan; intertidal, on *Sabellastarte* tube
Vermetoidea										
Vermetidae	*Thylacodes medusae*	**AB930337**	-	-	-	-	**AB930471**	YK#1614	35°09'N, 139°35'E	Off Misaki, Kanagawa, Japan; 80 m
Cypraeoidea										
Cypraeidae	*Macrocypraea cervinetta*	FM999134	-	-	-	-	DQ207132			
Naticoidea										
Naticidae	*Natica fasciata*	**AB930361**	-	-	-	-	**AB930488**	YK#1645	24°27'N, 124°08'E	Kabira, Ishigaki Is., Japan; tidal flat
Pterotracheoidea										
Atlantidae	*Atlanta peronii*	**AB930340**	-	-	-	-	-	YK#1617	32°15'N, 129°29'E	N295 (B), SW of Nagasaki, Japan
Pterotracheidae	*Pterotrachea coronata*	**AB930356**	-	-	-	-	**AB930484**	YK#1639	32°12'N, 128°58'E	N295 (O), off Fukue Is., Nagasaki, Japan
Triphoroidea										
Cerithiopsidae	*Seila morishimai*	**AB930354**	-	-	-	-	**AB930483**	YK#1637	32°35'N, 130°23'E	Nogama Is., Amakusa, Japan; intertidal rocky shore
Triphoridae	*Viriola tricincta*	**AB930342**	-	-	-	-	**AB930475**	YK#1620	31°14'N, 130°39'E	Ibusuki, Kagoshima, Japan; tidal flat

Abyssochrysoidea										
Abyssochrysidae	*Abyssochrysos melanioides*	**AB930325**	**AB930325**	**AB930376**	**AB930430**	**AB930403**	**AB930459**	YK#1482	32°07'N, 133°42'E	KT-11-12 (K), SE of Cape Ashizuri, Kochi, Japan; 2107–2168 m
Littorinoidea										
Lacunidae	*Lacuna pallidula*	AJ488645	-	-	-	-	AJ488604			
Littorinidae	*Echinolittorina porcata*	AJ623186	-	-	-	-	AJ622969			
Buccinoidea										
Buccinidae	*Chauvetia tenuisculpta*	**AB930371**	-	-	-	-	-	YK#1730[*3]	14°45'N, 17°30'W	Cape Verde, Dakar, Senegal; 16 m, on *Paracentrotus lividus*
Nassariidae	*Ilyanassa obsoleta*	AY145411	-	-	-	-	DQ238598			
Superfamily unknown										
Aclididae	*Aclis minor*	-	-	-	**AB930455**	-	-	YK#1734[*4]	58°52'N, 11°05'E	Kostergrund, Bohuslän, Sweden, Skagerrak; fine silt, 20–40 m
Lyocyclidae	*Lyocyclus* sp.	**AB930372**	**AB930372**	**AB930400**	**AB930454**	**AB930426**	-	YK#1732[*5]	22°54'S, 169°27'E	Banc Ellet, New Caledonia; 159–756 m
(Outgroup taxa)										
Cerithioidea										
Cerithiidae	*Cerithium coralium*	AM932695	-	-	-	-	AM932754			
Modulidae	*Modulus modulus*	FJ606987	-	-	-	-	AM932757			
Planaxidae	*Planaxis sulcatus*	FJ606988	-	-	-	-	HQ834106			
Pleuroceridae	*Juga acutifilosa*	DQ256748	-	-	-	-	EF586920			
Potamididae	*Pyrazus ebeninus*	FJ606989	-	-	-	-	AM932752			
Superfamily unknown										
Pickworthiidae	*Pelycidion* sp.	**AB930350**	-	-	-	-	-	YK#1632	34°40'N, 138°59'E	Shimoda, Izu, Japan; intertidal rocky shore
	Mareleptopoma iredalei	**AB930367**	-	-	-	-	-	YK#1652	26°43'N, 127°50'E	Daidokutsu, Ie Is., Okinawa, Japan; submarine cave, 20 m
	Microliotia mirabilis	**AB930368**	-	-	-	-	**AB930493**	YK#1654	10°28'S, 105°36'E	Thunder Dome, Christmas Is., Australia; submarine cave, 8–10 m

[*1]Redefined superfamilies. [*2]Muséum National d'Histoire Naturelle, Paris (MNHN) IM-2009-24069; [*3]Swedish Museum of Natural History (SMNH) 109316; [*4]SMNH-123661; [*5]MNHN IM-2009-24193.

Table 1-2. Ecological and morphological characteristics of eulimid species included in the present phylogeny. Specimens of *Niso matsumotoi* and *Hemiliostraca* sp. were collected as free-living while the two genera are known to parasitize Asteroidea and Ophiuroidea, respectively (Warén, 1984); no information available for *Hemiaclis*. Morphological conditions after Warén (1984) and Bouchet and Warén (1986).

Species	Host class	Mode of life	Shell shape	Radula
Hemiaclis sp.	unknown	Temp	conical	present
Hemiliostraca sp.	Ophiuroidea	Temp	slender	present
Melanella acicula	Holothuroidea	Temp	slender	absent
Monogamus entopodia	Echinoidea	Ecto	globose	absent
Niso matsumotoi	Asteroidea	Temp	conical	present
Pyramidelloides angusta	Ophiuroidea	Temp	slender	present
Stilifer akahitode	Asteroidea	Endo	globose	absent
Vitreolina auratus	Echinoidea	Temp	slender	absent

Table 1-3. Summary of four sequence alignments.

	Alignment length	Excluded sites	Variable sites	Parsimony informative
2gGB				
28S D1–D5	1,605	382	331	224
COI	630	0	382	316
Total	2,235	382	713	540
2gPA				
28S D1–D5	1,605	306	384	268
COI	630	3	380	315
Total	2,235	309	764	583
5gGB				
28S D1–D7b	2,352	397	375	274
18S	1,795	60	167	83
H3	314	0	110	90
16S	525	189	205	173
COI	630	0	375	303
Total	5,616	646	1,232	923
5gPA				
28S D1–D7b	2,352	337	399	287
18S	1,795	50	174	84
H3	314	0	110	90
16S	525	220	172	141
COI	630	3	373	302
Total	5,616	610	1,228	904

Chapter 2

Elucidating the evolutionary history of parasitism in eulimid gastropods: gradual specialization to permanent endoparasites or repeated adaptive radiation?

2-1. Introduction

Adaptive radiation is a key mechanism in the diversification of many organismal lineages. The strict concept of adaptive radiation is described as "the evolution of ecological and phenotypic diversity within a rapidly multiplying lineage" (Schluter, 2000), while in a more general sense, the definition can be broadened to "a pattern of species diversification in which a lineage of species occupies a diversity of ecological roles" (Randell & Price, 2009; see also Givnish, 1997). In this process, the morphological and ecological diversification can be repeated within entire radiation (Rundell & Price, 2009; Schluter, 2009). However, the repeated pathways of evolutionary diversification often result in disparate outcomes even if primal conditions are similar, as Gould (1989) employed the metaphor "replay the tape of life" (see also Wiens, 1989; Ricklefs & Schluter, 1993; Losos et al., 1998; Losos, 2010). The difference in the outcomes is probably attributable to unique evolutionary events or faint environmental differences among distinct areas or clade-specific responses to similar selective factors (Ricklefs & Schluter, 1993). On the other hand, some repeated diversifications show morphological convergence in species of different lineages that live under similar ecological conditions ("repeated adaptive radiation"; Schluter, 2000; Losos, 2010). In other words, different clades may respond in similar ways to common selective pressures (Schluter, 2009).

Repeated adaptive radiations can occur in both sympatric (Kozak et al, 2009; Frédérich et al., 2013; Ingram & Kai, 2014) and allopatric (Schluter, 2000; Losos, 2010) lineages and provide strong evidence for natural selection, especially in allopatric cases.

Such allopatric radiations are specifically termed “replicated adaptive radiation” (Losos, 2010; Ingram & Kai, 2014), where the diversifications are predicted to be affected essentially by site-specific environmental factors. Replicated adaptive radiations are considered to be rare and are typically found in closed systems, such as islands or lakes (Losos, 2010, but see Melville et al., 2006), for instance *Anolis* lizards on Caribbean islands (e.g. Losos et al., 1998), *Tetragnatha* spiders in Hawaii, (Gillespie, 2004), *Mandarina* snails on Bonin Islands (Chiba, 2004) and cichlid fishes in African rift lakes (Meyer et al., 1990). These organisms have diversified into the same set of ecomorphs, i.e. specialists for particular microhabitats, on/in respective islands or lakes and each ecomorph of different sites exhibits morphological convergence.

Host-parasite systems have been identified as island systems (Janzen, 1968; Kuris et al., 1980) by distinct host species or groups probably working as isolated “islands” for parasites. Repeated adaptive radiations in parasites therefore imply that the radiations have a powerful deterministic component as are the case with replicated adaptive radiations (Schluter, 2000). Evolutionary diversification of parasites on different hosts may result in disparate outcomes due to interactions with host-specific physiological and/or behavioral features. Avian feather lice represent an example of repeated adaptive divergence in parasites (Johnson et al., 2012). The lice have diversified into four ecomorphs to adapt to host’s preening behavior; however, a molecular phylogenetic reconstruction intriguingly revealed seven sister relationships between two different ecomorphs of the lice that attach to the same host group (Johnson et al., 2012). Repeated adaptive divergence—or perhaps better called replicated adaptive radiation—might be found occasionally or even commonly in host-parasite systems, if molecular phylogenetic investigations are made for other parasitic lineages with ecological and morphological diversities.

Previous hypotheses for the evolution of eulimid gastropods

The parasitic gastropod family Eulimidae presents "the most beautiful example of a series of progressively adaptive stages to a new environment that is known in the entire field of evolution" (Combes, 2005). The Eulimidae parasitize echinoderms including all five classes, i.e. Echinoidea, Holothuroidea, Asteroidea, Ophiuroidea and Crinoidea, and sequential stages of specialization to the parasitic mode of life have been found in association with each host class (Warén, 1984). While uniformly feeding on the dermal tissues and body fluids, eulimids include both temporary parasites that may crawl from host to host and highly modified ecto- and endoparasites that permanently attach to the host (e.g. Lützen, 1976; Warén, 1980a; Fretter & Graham, 1982). The modification in the family finally results in a clear "sacculinization" that means the loss of sense organs and other structures, such as the shell, a twisted visceral mass and even the anus (e.g., Baer, 1952; Lützen, 1968; Combes, 2005; Poulin, 2007). Previous workers have suggested evolutionary escalation for eulimids from crawling, temporary parasitic ancestors to more modified taxa that first buried their body in the host tissue for further specilization as endoparasites (Fretter & Graham, 1982; Warén, 1984; Combes, 2005).

Eulimid gastropods were classified historically into two distinct families based on the profile of the shell: slender species in Eulimidae s.s. and globose ones in Styliferidae (Adams & Adams, 1853; Habe, 1952, 1976; Laseron, 1955). Interestingly, most temporary parasites have slender shells while endoparasites tend to bear thin, globose shells (Warén, 1984; Vermeij, 1993). The traditional classification therefore implies that fat styliferids evolved through early specialization to the permanent parasitic life with a subsequent radiation across the echinoderm classes. On the other hand, Warén (1984) has synonymized Styliferidae and some other younger names for highly specialized lineages (e.g. Pelseneeridae, Asterophilidae and Enteroxenidae)

under the older Eulimidae based on his extensive investigation on the anatomy and shell morphology. This new classification scheme of Eulimidae (s.l.) has been widely used since then (e.g. Bouchet & Warén, 1986; Hori, 2000a; Bouchet & Rocroi, 2005) and is followed here. He also proposed an entirely different hypothesis that assumes ecological and morphological convergence in multiple clades of Eulimidae. That is to say, endoparasitism as well as globular shells might have evolved in parallel after the specialization of ancestral lineages to different echinoderm classes (Warén, 1984). Under this scenario, the Eulimidae potentially represent a beautiful lineage of parasites where diversification took place via the process of repeated adaptive radiation. However, as noted by Combes (2005), it remains totally unclear as to how many such specialization processes had occurred independently (Warén, 1984) or this escalation involved a single, linear process with multiple host shifts between different classes of echinoderms (Adams & Adams, 1853; Laseron, 1955). A robust phylogenetic reconstruction and precise assessment of ecological and morphological conditions are badly needed here for a more detailed and sophisticated argument.

The aim of this chapter is to illuminate the diversification process of the Eulimidae or to test the alternative hypotheses: an early specialization to the permanent parasitic life followed by radiation across different echinoderm groups, or repeated adaptive radiation involving independent specialization in each host class. For this purpose, phylogenetic and morphometric approaches were conducted for the family. Phylogenetic trees were inferred from partial sequences of three nuclear (18S, 28S and H3) and three mitochondrial (12S, 16S and COI) genes, c. 4.7 kbp in total, from 101 species belonging to more than 50 genera that represent parasites of all five echinoderm classes. Correlation between the parasitic strategies and shell shape was investigated by principal component analyses based on seven shell measurements. Ancestral conditions of the ecological traits (host class and parasitic strategy) and morphological characters (shell shape and presence/absence of the radula, pseudopallium and pedal

folds) were reconstructed based on the inferred phylogenetic relationships and character states in the Recent species. Furthermore, divergence time analyses were performed using the occurrence of three fossil species as calibration points to estimate the absolute ages of the evolutionary transitions.

2-2. Materials and Methods

Taxonomic sampling

Ninety eulimid species belonging to more than 50 genera were newly collected from all over the world (Fig. I-3; Table 2-1). Nine eulimids from Chapter 1 were also included in the present analyses; note that "*Hemiaclis* sp." and "*Hemiliostraca* sp." have been renamed to "sp. A" of each genus in the present chapter. Species identification and generic assignment of specimens followed Warén (1980a, 1980b, 1981a, 1981b), Bouchet & Warén (1986) and Hori (2000a). The study species cover the widest range of ecological diversity for the family, namely different hosts, habitats, depths and parasitic strategies; morphological diversity is also very widely represented for example by various shell shapes and the presence or absence of the radula and pseudopallium (see Warén, 1984). Two additional species, *Aclis thesauraria* and *Kimberia loveniana*, were also included in the present analyses despite their current positions in another family, Aclididae, which is nested within Eulimidae (Takano, pers. obs.). Three vanikorids were used as outgroup taxa based on the results from Chapter 1. One of them, *Macromphalus tornatilis*, is known to have a certain association with echiuran worms (Goto et al., 2011) and was therefore newly sequenced to assess its possible implications for the origin of parasitism in Eulimidae.

Most live snails were boiled in 70–90ºC water for 0.1–1 min; the animals were then extracted from their shells, and preserved in pure ethanol. Voucher material has

been deposited in the Atmosphere and Ocean Research Institute, The University of Tokyo, Japan (AORI), Muséum National d'Histoire Naturelle, Paris, France (MNHN), Swedish Museum of Natural History, Stockholm, Sweden (SMNH) or National Museum of Nature and Science, Tsukuba, Japan (NSMT).

Phylogenetic reconstruction

Sequences of three nuclear (18S, 28S and H3) and three mitochondrial (12S, 16S and COI) genes were amplified and sequenced for each species using various primers listed in Table S1-1 of Appendix 1. DNA extraction, PCR, and sequencing reaction were carried out in accordance with the methods described in Chapter 1. Two datasets were generated with different combinations of taxa. The first, full dataset, includes all species listed in Table 2-1. *Entocolax olgae*, *Enteroxenos oestergreni* and *Enteroxenos* sp. were found to have extremely high evolutionary rates of the nuclear genes (see Results) and were therefore excluded from the second dataset, which is hereafter referred to as the limited dataset.

The sequences of the four rRNA genes were aligned individually by MAFFT 7.047b (Katoh & Standley, 2013) with the "--auto" algorithm and ambiguous sites were removed by Gblocks 0.91b (Castresana, 2000) with the default parameters except for the "Allowed gap positions" to "With half." Protein-coding COI and H3 sequences were aligned by eye in MEGA 5 (Tamura et al., 2011). The best-fit nucleotide substitution model for each rRNA locus or each codon position of the protein-coding genes was determined with jModelTest 2 (Guindon & Gascuel, 2003; Darriba et al., 2012) from 24 models according to the Akaike information criterion (AIC, see Table 2-2).

Phylogenetic trees were reconstructed using Bayesian and ML methods. Bayesian analysis was performed with MrBayes 3.1.2 (Ronquist & Huelsenbeck, 2003)

setting respective substitution models for each of the ten partitions. Two parallel Markov chain Monte Carlo (MCMC) sampling were made for 50,000,000 generations (four chains) with a sample frequency of 1,000. A consensus tree and posterior probability (PP) of each branch were computed from 75,000 trees (i.e. the burn-in value set at 12,500). Convergence of MCMC was verified by referring the average standard deviation of split frequencies (ASDSF) was less than 0.01. ML analysis was performed using RAxML 7.2.6 (Stamatakis, 2006) with 1,000 bootstrap replicates. The dataset was divided into the same ten partitions as the Bayesian analyses. The GTR + G + I model was applied to all partitions in the full-dataset, while the GTR + G model was used in the limited-dataset because the first model resulted in an application error. In the present chapter, clades with both PP $\geq$ 0.95 and bootstrap probabilities (BS) $\geq$ 75% were considered as significant. Bayesian analyses were also performed for individual genes with 5,000,000 generations (a sample frequency set to 1,000) and burn-in value setting at 1,250 to compare evolutionary rates and to eliminate possible erroneous sequences. All trees were edited by FigTree 1.3.1 (available at http://tree.bio.ed.ac.uk/software/figtree/).

Morphometric analyses

To examine differences of shell profiles, principal component analyses (PCAs) were performed in R 3.2.2 (R Core Team, 2015) for Recent specimens and Miocene–Paleocene fossil material. Two datasets, representing only the Recent taxa, and both Recent and fossil taxa, were analyzed. Eight measurement positions selected by referring to Wada et al. (2013) were taken from digital images or figures in published journal articles (e.g. Wrigley, 1944; Warén, 1980a; Lozouet, 1999) or books (e.g. Cossmann & Pissarro, 1904–06; Hori, 2000a; Severns, 2011) for a total of 168 Recent and 44 fossil species (see Appendix 2 for details). The measurement positions

included the shell height (H), shell width (D), height of the spire (SH), width of the spire (SW), length from the apex to the uppermost point of the aperture (PAL), height of the aperture (AH), width of the aperture (AW), and roundness of the whorl (CV; see Fig. 2-1). For PCAs, the ratio to H was calculated for all other measurements but CV. Five other measurement positions used in Wada et al. (2013) were not applicable for many eulimids due to their near-straight inner/basal/outer lip of the aperture. For specimens with a broken apex, the lost part was complemented by imaginary lines tangent to the spire and by the protoconch shape of conspecies or congeners. Females were measured for eulimid species with a large degree of sexual dimorphism as far as possible, since they are considered protandrous hermaphrodites (Warén, 1984). The species of *Thyca* with capuliform shells, too young, or seriously damaged specimens were excluded from the analyses.

The k-means clustering was applied to PC scores obtained from the PCA for Recent species to investigate the correlation between the shell shape and host class or parasitic strategy (see below). The number of species with each of the three shell types (distinguished by the clustering, i.e. k = 3) was counted for each ecological condition.

Definition of ecological conditions

Based on their parasitic habits, eulimid species can be classified into three groups, namely temporary parasites, ectoparasites and endoparasites (see General Introduction and Appendix 2 for details). In the present chapter, endoparasites are defined as species that permanently live in the host body or where most of the body is protected by host tissue. Gall-forming snails (e.g. *Stilifer* and *Tropiometricola*; see Warén, 1984) were thus treated as endoparasites. Ectoparasites represent species attaching to the body surface of the host and exhibit low or no mobility due to a reduced foot or a large, non-retractile proboscis (e.g. *Echineulima*, *Stilapex* and *Thyca*). Although species of

Pelseneeria and *Pulicicochlea* found on sea urchins have a functional foot and crawl on the test of the host, they have never been found as free living (Ponder & Gooding, 1978; Warén, 1984) and were considered exceptionally as ectoparasites. Others, including species collected as free living, were labeled as temporary parasites, which can crawl among host individuals. "*Hypermastus*" *lacteus* alone represents an endoparasitic species that is herein classified as a temporary parasite based on the presence of autonomous periods and a functional foot (R. Mukai, pers. comm.; Takano, pers. obs). Only three genera showed two different parasitic strategies: *Peasistilifer* either temporary parasitic or ectoparasitic, *Monogamus* and *Trochostilifer* ectoparasitic or endoparasitic.

Parasitic strategy could not be determined for Cf. *Crinolamia* spp. A and B, Cf. *Mucronalia* sp., "*Stilapex*" *koyamai* and "*S.*" *teramachii* (Appendix 2). Cf. *Crinolamia* spp. were found as free living in deep-sea trawl hauls (Table 2-1) where numerous sea cucumbers were collected as their possible hosts. These deep-sea eulimids resemble *Crinolamia* species in having a thin shell with convex whorls and lacking pigmented eyes (see Bouchet & Warén, 1986). *Crinolamia* permanently attach to their hosts (Warén, 1984). The unidentified snails thus possibly detached from the host in the trawl hauls, although their mode of life was not determined for the analysis. Cf. *Mucronalia* sp. has a more globular shell than the typical *Mucronalia*; unfortunately, no anatomical or ecological information was available for this species except its association with Ophiuroidea (A. Warén, pers. comm.). Lastly, "*Stilapex*" *koyamai* and "*S.*" *teramachii* attach to the sea cucumber *Stichopus* sp. (Hori, 2000a), while the type and most other species of *Stilapex* parasitize Ophiuroidea (Warén, 1981a), hence casting doubts as to the generic assignment of the former two species. Warén (1980b) actually argued a possibility of their affinity to *Peasistilifer*, but future anatomical and ecological investigation is required for a rigorous taxonomic treatment for the two species.

The class of host echinoderms is another important ecological trait for eulimid parasites. This information is readily available for specimens associated with their hosts. Although many specimens of *Eulima* and *Melanella* were collected as free living, they could safely be assumed as ophiuroid and holothuroid parasites, respectively, based on Warén (1983, 1984). On the other hand, two distinct groups of *Niso* with different phylogenetic backgrounds (see Results) have a more complicated situation. Species belonging to the two lineages were easily distinguished from each other by examining the color of the apex. Those with a white apex, which seem to represent the real *Niso*, have been recorded from sea stars (Poppe, 2008; Takano, pers. obs.) and thus were treated as asteroid parasites, while no host information is available for other species with a brown apex. Other free-living species were coded as parasites of a particular echinoderm class only when these could be assigned safely to genera where hosts are known and invariable (Warén, 1984).

Ancestral state reconstruction

The ancestral states of ecological and morphological traits were reconstructed using Mesquite 3.04 (Maddison & Maddison, 2015) under the Markov one-parameter (Mk1) model of the ML method. Eulimid gastropods exploit the five classes of echinoderms, Asteroidea, Crinoidea, Echinoidea, Holothuroidea and Ophiuroidea, and show the widest range of parasitic modes, i.e. temporary-, ecto- and endoparasitic. These ecological features were mapped on the Bayesian tree inferred from the full dataset.

Three types of shell morphologies were recognized for eulimid snails by the k-means clustering (see below), besides the capuliform and shell-less states. The condition of the shell was therefore coded as one of the five states for each study species. In addition, the presence or absence of the radula as well as the pseudopallium (sac-like structure derived from the snout) or the pedal folds were mapped on the tree and

reconstructed for ancestral nodes. The latter two structures, with a similar appearance but supposedly with different ontogenetic origins, may have evolved independently to reduce defensive actions by the host (Warén, 1984). Morphological conditions were derived from previous literatures including Warén (1984) and Bouchet and Warén (1986), or were newly observed (Takano, unpublished).

Divergence time estimation

The divergence dates among eulimids were calculated using a lognormal relaxed clock model in BEAST 1.8.0 (Drummond et al., 2012). For this analysis, the "limited" dataset was used to avoid the negative impact of extremely long branches to three holothurian endoparasites (see above). The respective substitution models (Table 2-2) were applied to the ten partitions with the exception of SYM for the 2nd codon position of H3 (JC was unselectable; delta AIC = 2.57). The speciation model of Yule process was used as the tree prior. Four groups were constrained as monophyletic to designate outgroup taxa and to set the time calibration points: Vanikoridae, Eulimidae, *Pyramidelloides* + *Palisadia*, and *Costaclis* + *Niso* with a white apex.

Branch lengths were time-calibrated by setting priors based on the ages of three clades. Firstly, the time to the most recent common ancestor (TMRCA) of the Eulimidae was assumed to be 72 million years ago (Ma). This family appeared as body fossils since the Late Campanian to Early Maastrichtian age of the Late Cretaceous (e.g. Sohl, 1964; see Warén, 1984 for review). The occurrence of trace fossils on sea-urchin tests supports the Campanian origin of eulimids (Neumann & Wisshak, 2009). In addition, the slender shell of early eulimids (e.g. *Subularia*) is congruent with the result of the ancestral state estimation (see below). These pieces of fossil evidence were used to set an exponential distribution with an offset of 72 Ma with a "Mean" value of 4.0, resulting in a 95% highest probability density (HPD) interval of

72–84 Ma. Secondly, the earliest occurrence of *Pyramidelloides* from the Bartonian of the middle Eocene (Lozouet & Dockery, 2001) was used for the TMRCA of *Pyramidelloides* + *Palisadia* (exponential distribution with an offset value of 37.8 Ma). "Mean" value was set to 1.3, resulting in a 95% HPD of 37.8–42 Ma, which covers the Bartonian stage. The two genera share strong axial ribs of the shell, while *Palisadia* differs from *Pyramidelloides* in having symmetrical wing-like varices as its autapomorphy. Lastly, the TMRCA of *Costaclis* + the real *Niso* (those with a white apex) was set to the Ypresian of the lower Eocene (exponential; offset: 47.8 Ma). The species of *Niso* with a white apex are also characterized by the presence of a strong keel that encircles the umbilicus of the shell (e.g. Hori, 2000a). Such a condition of the umbilicus can be seen in several species of *Niso* from the early Eocene to Miocene faunas of France and the United Kingdom (Cossmann & Pissarro, 1904–06; Cossmann & Peyrot, 1917–18; Wrigley; 1944). For this calibration point, the "Mean" value was set to 2.5 to make the HPD 100–115% of the offset value (47.8–55.3 Ma). This range is broader than the ranges used in Jörger et al. (2010; 100–110%), due to low support values for this clade in the topology reconstruction (PP = 0.72, BP = 58%; see Fig. 2-2).

The MCMC was run for 100,000,000 generations with a sample frequency of 1,000, resulting in 100,000 estimates. The convergence of chains was assessed by TRACER 1.5.0 (available at http://tree.bio.ed.ac.uk/software/tracer/) and the first 50,000 estimates were discarded as a burn-in.

2-3. Results

Sequence data

Table 2-2 shows the estimated model of substitution, length of aligned sequences after the masking of alignment ambiguous sites and numbers of variable and

parsimony-informative sites for each partition of the sequence data. The full and limited datasets had 4,743 and 4,627 sites, respectively, after trimming alignment-ambiguous sites. The same substitution model was selected for both datasets except for the 1st codon position of the H3 gene and 3rd of the COI gene. The proportions of variable and parsimony informative sites were lowest in the 18S gene of the limited dataset (12.3 and 6.6%), while both were 100% in the 3rd codon positions of COI (Table 2-2). COI sequences of vanikorid species had a 3-bp deletion at the positions 95–97.

Phylogenetic relationship within Eulimidae

Bayesian and likelihood analyses yielded the same results for the two datasets in terms of clades with meaningful support values. Therefore, only the Bayesian trees are shown with posterior probabilities and ML bootstrap values on branches (Fig. 2-2). Finding extremely high evolutionary rates in three shell-less endoparasites, *Entocolax olgae*, *Enteroxenos oestergreni* and *Enteroxenos* sp. (Appendix 2), branch lengths were modified for these species in Figure 2-2.

In the tree reconstructed from the full dataset, three clades with meaningful support values (PP = 1.00, BP > 78%) were found within the Eulimidae (Clades I–III in Fig. 2-2). These three clades were further divided into the "segmentalized groups" (e.g. U1–3, H1–3; capitals represent initials of host groups) based on the ancestral state reconstruction as detailed later (see below). To avoid redundancy, the distribution of these groups in the best Bayesian tree along with their nodal support indices is introduced under this subheading. The Clade I consisted of *Hemiaclis*, Eulimidae gen. sp. "Bullet," *Thaleia* and *Niso* with the brown apex, which collectively constituted the group U1. Relationships within the Clade I were unambiguously resolved (1.00, ≥ 99%) except among *Thaleia* and two species of *Niso*.

The Clade II included three groups, U2, A1 and O1, with U2 is paraphyletic to A1. The monophyly of each A1 and A1 + U2 were meaningfully supported (1.00, 99% and 0.99, 82%, respectively), whereas relationships among *Asterophila*, *Costaclis* and the real *Niso* were unclear. O1 was a robust clade (1.00, 100%) of several genera parasitic to ophiuroids (e.g. *Eulima*, *Hemiliostraca* and *Ersilia*), but their internal relationships were not sufficiently resolved. Clades with meaningful support values in O1 included *Eulima* (1.00, 93%), aclidids (1.00, 100%), *Hemiliostraca* + *Sticteulima* + *Arcuella* + Cf. *Mucronalia* sp. + Cf. *Fusceulima* sp. (1.00, 100%), and *Pyramidelloides* + *Palisadia* + Cf. *Oceanida* sp. (1.00, 100%). *Hemiliostraca* and *Sticteulima* were polyphyletic. "*Haliella*" spp. B–D were distantly related to each other in the Clade II.

The Clade III comprised 66 species belonging to ten groups besides the solitary and basal-most *Haliella* sp. A. The monophyly of the following groups was well supported (> 0.97, > 77%): H2, C1, U3, H2, C2, O2, E2, A2, U2 + H2, E2 + H3 + A2, O2 + E2 + H3 + A2, and U2 + H2 + C2 + O2 + E2 + H3 + A2 . Two other clades, E1 and H2 + C1 + U2 + H2 + C2 + O2 + E2 + H3 + A2, received significant PP but insufficient BP values (0.99, 59% and 1.00, 74%, respectively). E1 consisted of two clades, "*Melanella*" *areosomae* + "*Strombiformis*" *langforgi* + "*Vitreolina*" *akauni* (1.00, 78%) and *Pulicicochlea* + *Pelseneeria* (1.00, 100%), and H2 was divided into shell-bearing (Eulimidae gen. spp.; 1.00, 100%) and shell-less (*Entocolax* + *Enteroxenos*; 1.00, 96%) clades. U2 included four undescribed species, Cf. *Melanella* sp. H, Eulimidae gen. sp. "KH" and Cf. *Crinolamia* spp. A and B. Of these, two Cf. *Crinolamia* species formed a monophyletic group (1.00, 100%). Eleven of 14 species of *Melanella* plus "*Hypermastus*" *lacteus* formed H2 with six well-supported subclades: *M. kuronamako* + *M. acicula* (1.00, 94%), *Melanella* sp. A + *M. kuronamako* + *M. acicula* + *M.* sp. cf. *tortuosa* (1.00, 100%), *M. teinostoma* + *Melanella* sp. A + *M. kuronamako* + *M. acicula* + *M.* sp. cf. *tortuosa* (1.00, 99%), *Melanella* sp. C + *Melanella* sp. I (1.00, 92%), *Melanella* sp. D + "*H.*" *lacteus* (1.00, 100%), and

Melanella sp. C + *Melanella* sp. I + *Melanella* sp. D + "*H.*" *lacteus* (1.00, 93%). C2 was composed of *Crinophtheiros*, *Annulobalcis* and *Goodingia*, of which the latter two were closely related (1.00, 100%). O2 included *Vitreolina incurva* and three *Stilapex* species (1.00, 100%), while E2 contained 11 echinoid parasites in seven genera (*Vitreolina*, *Hypermastus*, *Echineulima*, *Scalenostoma*, *Robillardia*, *Sabinella* and *Monogamus*). Each of *Scalenostoma* and *Monogamus* was monophyletic (1.00, 100%) whereas *Vitreolina* and *Hypermastus* were polyphyletic within the group E2 and in such other groups as E1, H2 and O2. H3 was paraphyletic to A2 and its ingroup relationships were poorly resolved. The monophyly of each *Megadenus* and *Peasistilifer* was supported with the highest values (1.00, 100%). Finally, A2 consisted of 11 asteroid parasites. *Apicalia* was its most basal offshoot (1.00, 100%) and the others were divided into two clades: *Thyca* + *Stilifer* sp. aff. *pisum* (1.00, 76%) and *Parvioris* + "*Apicalia*" + two other species of *Stilifer* (1.00, 82%). The relationships within the latter clade were resolved with high credibility (1.00, > 83%). The tree topology inferred from the limited dataset differed to some extent from that of the full dataset. However, both datasets yielded the same results in terms of clades with meaningful support values (see Appendix 2).

Twelve Bayesian analyses for independent gene sequences resulted in poorly resolved trees (Appendix 2). The long branches of *Entocolax* and *Enteroxenos* had little influence on the tree topologies, and hence the following description is based on the trees with a full set of taxa. The monophyly of the Eulimidae was unambiguously supported in all trees (> 0.99). Clades with meaningful posterior probabilities (≥ 0.95) in more than one tree include: A1 (in 16S, 12S, COI and H3 trees), A2 (28S and H3), O1 (28S and 12S), O2 (18S, 28S, 16S and H3), C1 (18S and 28S), C2 (16S and12S), H2 (18S, 12S and COI), O2 + E2 + H3 + A2 (18S and 12S), *Hemiaclis* (all but H3), aclidids (all), *Pelseneeria* (all but H3), *Enteroxenos* (28S, 16S, 12S and COI), *Annulobalcis* + *Goodingia* (all but 18S), *Megadenus* (all but 16S). There were some contradictory

clades with meaningful support values in the independent gene trees, for example the position of two *Megadenus* species in the 18S and 28S trees, possibly because there were too few informative characters for shallow nodes in the former gene.

Correlation between shell shape and parasitic strategy

In the PCA for the Recent eulimids, the first two principal components (PC1 and 2) explained roughly 85% of the total variance (Table 2-3). Although the eigenvalue of PC2 was less than one, shell morphology was evaluated by two components on the basis of the cumulative proportion > 80%. PC1 indicates slenderness of the shell (positive SH and PAL with negative D) and PC2 represents the roundness of the whorls (positive CV). In the scatter plot of PC1 vs. PC2, eulimids were clustered by parasitic strategies with significantly different PC scores among the three categories (temporary parasites, ectoparasites and endoparasites; Steel-Dwass test, $p < 0.001$; Figs 2-3, 2-4). PC1 scores were the largest in the temporary parasites, which means they bear slender shells, while lowest values were observed for globose endoparasites (Fig. 2-4). The shells of ectoparasites representing the largest PC2 scores are characterized by their more strongly inflated whorls than those of other groups (Fig. 2-4). Thus, eulimids can be classified conchologically into three ecomorphs, namely “temporary,” “ecto” and “endo.” On the other hand, these shells were indistinguishable by host classes (Fig. 2-3B).

The k-means clustering recognized three types of shells (types A, B and C in Fig. 2-3C). The clustering largely reflected the PC1 scores, thus A, B and C types corresponded to slender, intermediate and globose shapes, respectively. A strong correlation was found between the k-means clustering of the shells (A, B and C) and the above classification into the three types of ecomorphs (Cramer's coefficient of association with Yates’ correction, $V_{Yates} = 0.70$; Fisher’s exact test, $p < 0.001$; Table 2-4).

The PCA for both Recent and fossil eulimids yielded a similar result (Table 2-3). Most Paleocene and Eocene fossils were morphologically similar to temporary parasites, whereas three Oligocene species and an Eocene species, "*Stylifer*" *pellucidus,* were plotted near ectoparasites (Fig. 2-3D). A Miocene species, "*Pelseneeria*?" *senuti* was also plotted far from the cluster of temporary parasites and closer to either the typical ectoparasites or endoparasites.

Ancestral states

Ancestral states were reconstructed for ecological and morphological traits as shown in Figure 2-5. The common ancestor of the Eulimidae was estimated to be a temporary parasite (a proportional likelihood value of 0.99) that had a slender shell (0.99) and a radula (0.95), but its host was unclear with the largest PL of 0.51 for Echinoidea. Parasites of each echinoderm class were not clustered as a monophylum (Fig. 2-5A). Eleven groups comprising parasites of particular host classes were recognized (PL > 0.94): A1 and A2 with asteroid parasites, O1 and O2 with ophiuroid parasites, E1 and E2 with echinoid parasites, C1 and C2 with crinoid parasites and H1 to H3 with holothuroid parasites. Of these, H3 was possibly paraphyletic to A2 regardless of datasets used, while E1 was paraphyletic only in the tree inferred from the limited-taxon dataset (see Appendix 2). The common ancestor of each group was estimated to be a temporary parasite (> 0.94) with a slender shell (> 0.95 except for 0.91 in A2). In addition, three groups were recognized for species with unknown hosts (U1 to U3); of these, U2 was paraphyletic to A1. The temporary parasitic mode and slender shell were suggested for the common ancestors of U1 and U2, while parasitic strategy was unclear for that of U3. Permanent ecto- and endoparasitism have evolved parallelly in seven and six groups, respectively (Fig. 2-5B). Likewise, both intermediate and globose shells were acquired independently in eight groups (Fig. 2-5C).

The loss of each of the teleoconch and radula has occurred more than once in eulimid evolution. Shell-less species were included in the groups A1 (*Asterophila japonica*) and H1 (*Entocolax olgae*, *Enteroxenos oestergreni* and *Enteroxenos* sp.; Fig. 2-5D); note that the estimated multiple losses of the shell in the group H1 probably erroneously came from the extremely long branches of the three species. The radula has been lost twice. The first occasion was very early in the history of Eulimidae—right after the first split among extant members of the family. The second loss occurred much later in the direct ancestor of *A. japonica* (Fig. 2-5D). The pseudopallium and pedal folds have also been acquired multiple times during the eulimid radiation: pseudopallium mostly by the endoparasites of holothuroids and asteroids, and pedal folds entirely by the permanent parasites of echinoids and crinoids (Fig. 2-5E).

Divergence time estimation

Figure 2-6 shows a chronogram inferred from the taxon-limited dataset and three time-calibration priors based on fossil records. The TMRCA of Eulimidae was calculated to have existed at 106.6 Ma (middle Cretaceous) with a 95% HPD of 88.4–125.5 Ma (spanning from the Barremian to the Coniacian). The calculated mean at 106.6 Ma was older than the upper limit of 95% HPD of the calibration prior for this node. The same set of fossil-based priors yielded TMRCAs of the Clades I, II and III at 63.5 Ma (95% HPD: 44.2–82.5 Ma), 83.5 (70.5–96.7) Ma and 94.9 (77.5–112.3) Ma, respectively. The estimated ages for TMRCAs of the segmentalized groups varied from 83.4 Ma (E2) to 26.3 Ma (U3), seven of which fell into the Eocene period (A1, C1, C2, O2, H2, H3 and E2: 39.3–52.6 Ma). The origin of A2 was calculated at 31.6 Ma in the Oligocene; two splits into the endoparasite and ectoparasite within this group,

namely *Thyca lactea* versus *Stilifer* sp. aff. *pisum,* and "*Apicalia*" *palmipedis* versus *Stilifer* spp., were estimated to have occurred at 16.2 and 13.1 Ma, respectively.

2-4. Discussion

Diversification dynamics of the Eulimidae

The present molecular phylogenetic and morphometric analyses revealed that the Eulimidae have diversified through repeated adaptive radiation. On each class of echinoderms, a similar set of specialists with particular parasitic strategies (ecomorphs) has occurred, each of which shows morphological convergence across the family despite their independent evolutionary origins (Figs. 2-2, 2-3, 2-5). This contrasts with the argument by Gould (1989) who emphasized historical contingencies that would lead evolutionary radiations to different paths and disparate outcomes, even with identical starting conditions. The vagaries are attributable to unique historical events and subtle environmental factors that include host-specific characteristics for parasites. The present study indicates that, however, such events and factors do not necessarily lead to different evolutionary pathways in the host-parasite interaction, where ecological constraints seem to often be more significant. Respective radiations in the Eulimidae have started from temporary parasitic ancestors bearing a slender shell and ended in permanent ectoparasites and endoparasites with globose to capuliform shells or without a shell (Fig. 2-5). These radiations involving the adhesion and infiltration to the host thus have a strong deterministic component, as has shown in the replicated adaptive radiation on islands and in lakes (Losos et al., 1998; Chiba, 2002; Gillespie, 2004).

An example of repeated adaptive diversification in parasitic organisms has been reported in avian feather lice. The lice attach to the different parts of body surface of hosts and have diversified into four ecomorphs, including specialists on the

head, wing and body, and a generalist that can be found over most part of the bird's body (Johnson et al., 2012). In this group of lice, seven radiations to particular host groups are represented by two of the four ecomorphs that are not always the same set; the ancestral condition is unclear, with the generalist tends to occur on terminal branches (Johnson et al., 2012). The present study provides a more comprehensive, solid and dynamic picture of repeated adaptive diversification in the evolution of parasites. Eulimid snails have diversified into one or two ecomorphs (herein referred to as "ecto" and/or "endo") from similar starting conditions ("temporary") in each of more than ten independent radiations.

Such an evolutionary process of the Eulimidae fits more comfortably into Warén's (1984) hypothesis than the alternative scenario. The former assumes that the permanent ectoparasites and endoparasites as well as their globular shells have independent origins in each of different echinoderm classes (Warén, 1984). The alternative hypothesis, as exemplified by the dichotomous classification of eulimids into the slender Eulimidae (s.s.) and fat Styliferidae (Adams & Adams, 1853; Laseron, 1955), postulates a single, early specialization event to the permanent parasitic life with a subsequent radiation across the Echinodermata. The eulimid radiation, however, has repeated more numerously than presumed by Warén (1984). Although he considered that eulimids exploiting particular host classes constitute respective monophyletic clades, each "island" of echinoderm classes has actually been invaded twice or three times by this group of snails (Fig. 2-5).

Both ancestral and specialized parasitic strategies seem to present in the fossil record of eulimids as illustrated by the morphometric analysis of the shell (Fig. 2-3D). Most species from the Paleocene and Eocene periods had a slender shell and thus presumably a temporary parasitic lifestyle (e.g. Lauridsen & Schnetler, 2014). The predominance of slender species early in the history of Eulimidae, which started in the Late Cretaceous (Sohl, 1964; Warén, 1984), is congruent with the result from the

ancestral state estimation where such a shell was recovered as plesiomophic (Fig. 2-5). Globular species as presumed permanent parasites occurred first in the Eocene of France and became more common since the Oligocene time (e.g. Lozouet, 1999). Here it is interesting to note that, based on resembling shell morphology, the olderst Eocene species "*Stylifer*" *pellucidus* (see Cossmann & Pissarro, 1904–06) has been placed in the Recent genus for endoparasites in starfish arms. However, the divergence time of this terminal genus was estimated to be as late as the Miocene (Fig. 2-6) so that the much older Eocene species most probably represents an entirely different lineage with a convergent shell shape.

Trace fossils further corroborate the past, hidden radiation of the Eulimidae. The ichnospecies *Oichnus halo*, attachment and feeding traces on the sea-urchin tests from the Campanian of the Cretaceous period (Neumann & Wisshak, 2009), is surprisingly similar to scars made by the species of *Thyca*, which are globose to capuliform ectoparasites on sea stars in the Recent seas. The Eulimidae appeared as body fossils also since the Campanian (e.g. Sohl, 1964; see Warén, 1984 for review), while the origin of the family was estimated to be somewhat older in the present divergence time chronogram (Fig. 2-6). Although their eulimid affinity seems to be convincing, we cannot ascribe the Cretaceous traces to an ancestral species of *Thyca*, as the original authors did (Neumann & Wisshak, 2009). As in the case of *Stilifer*, this terminal clade of ectoparasites appeared much later than the convergently similar fossil species (late Oligocene; Fig. 2-6). To sum up, the repeated adaptive radiation has occurred throughout the evolutionary history of the family, since well before and more frequently than it can be traced by the ancestral state reconstruction based on phylogenetic relationships among extant species and distribution of their ecological traits (Fig. 2-5).

Previous studies on replicated adaptive radiation have suggested that the process of shaping communities involves both radiation within an area and dispersal

between areas (Losos et al., 1998; Gillespie, 2004). In Hawaiian spiny-leg spiders, for example, two or three ecomorphs exist on each island, while some apomorphic ecomorphs of each community probably have migrated from other islands (Gillespie, 2004). Although eulimids exploiting a particular echinoderm class have multiple origins, the "migrations" or interclass host switching has always been achieved by the plesiomorphic "temporary" ecomorph (Fig. 2-5). The other ecomorphs would have great difficulties in interclass host switching, probably due to the loss of mobility after the settlement and metamorphosis from the swimming larva, and/or specialization to the physiological and other biological characteristics of a particular host group. These causes of impediment seem to be less important or non-existent for some other parasitic and commensal molluscan groups including the pyramidellid gastropods (see Chapter 3) and galeommatoid bivalves (Goto et al., 2012), where specialization and dependence to a single host lineage have yet to be developed. Interestingly, host switching between different families in a single echinoderm class seems to have been frequent in the evolutionary history of Eulimidae (Table 2-1, Fig. 2-2, see also Warén, 1980a for the hosts of *Stilifer*). Such traits as the composition of body fluid, structure of the epidermis and detection and rejection mechanisms against parasites may be similar enough within the same class of echinoderms to allow such host switching by eulimids.

Adaptive significance and evolution of morphology

The shell shapes of the three ecomorphs seem to be beneficial and adapative in respective lifestyles. The slender shells of "temporary" species are favorable for crawling in soft sediment, whereas the more globose to capuliform shells of the "ecto" species would enable stronger attachment to the host with a larger surface of the foot sole, as suggested for multiple lineages of rocky-shore limpets that adhere themselves to hard substrates (Vermeij, 1993). The large aperture and foot in both the "ecto" species

of eulimids and rocky-shore limpets may have a common advantage in avoiding dislodgement by wave action and/or predators. The inflated shells in "endo" species have presumably been acquired to reduce the cost of shell construction. This presumption is supported by the fact that a globose shell maximizes the internal shell volume per unit of shell mass (Kemp & Bertness, 1984) and that most shells of endoparasitic eulimids are very thin (Warén, 1984). Through the eulimid radiation, the slender shell has been conserved for tens of millions of years in many lineages, but it has radically changed to globose in the group A2 since the Miocene period (Figs. 2-5C, 2-6). The adaptive diversification of eulimids may have been driven by strong selective pressures that enhanced the rapid modification of the shell. A similar correlation between the parasitic mode and shell shape is found in Coralliophilinae (Muricidae). In species with a close association to host corals, including *Coralliophila neritoides* and *C. madreporaria*, shells exhibit globular to near-patelliform shapes; species of *Rapa* and *Magilus* that bury their shells in soft or hard corals bear more inflated and thinner shells than their relatives with an ectoparasitic mode of life (Tsuchiya, 2000).

Another remarkable evolutionary trend in eulimid diversification is the complete loss of the teleoconch that has resulted in several worm-like or naked ball-like endoparasitic species. Evidently, effective protection inside the host body as a shelter has made the shell needless against predators or other disturbances in the lineages leading to these highly specialized parasites. The presence or absence of a shell in endoparasitic eulimids might possibly reflect the difference of host classes: present in Asteroidea and Holothuroidea while absent in others. However, there might exist many unsampled taxa, not only the species of *Molpadicola*, *Entoconcha* and *Diacolax* that are all in sea cucumbers (Warén, 1984). Regardless, the shell tends to become thin and globular and then, sometimes, completely lost in a linage of eulimids with an endoparasitic lifestyle (see Combes, 2005).

Parallel evolution in Eulimidae is not confined to the shell characteristics but also occurs in anatomical traits. Both pseudopallium and pedal fold have been acquired independently in several groups (Fig. 2-5E), presumably to cope with the defensive activities of the host (Warén, 1984). These structures, especially the pseudopallium, are capable of avoiding contact between the host body and the shell and/or foot of the parasites. Interestingly, pedal folds have evolved in crinoid and echinoid parasites only, and pseudopallia are possessed mostly by holothuroid and asteroid parasites (Fig. 2-5A, E). This might suggest that defensive mechanisms of the hosts are similar in the two respective classes. The latest phylogeny and classification for the Echinodermata (Telford et al., 2014), however, recognize such three subclades or subphyla as Asteroidea + Ophiuroidea (Asterozoa), Echinoidea + Holothuroidea (Echinozoa) and Crinoidea (Crinozoa), and hence do not support the idea that the acquisition of the epseudopallium or the pedal fold was differential responses to phylogenetically inherited differences in certain biological traits of the host.

Finally, the radula has been lost at least twice in eulimid evolution (Fig. 2-5D), most probably due to the limited use of this digestive apparatus in their blood-sucking mode of feeding (Warén, 1984). The radula is also absent in other parasitic gastropod lineages, Pyramidellidae and Coralliophilinae (Ponder & de Keyzer, 1998b; Barco et al., 2010).

Evolution of endoparasitism

The morphological convergence in endoparasitic eulimids strongly suggests that each lineage has similarly responded to unversal selective pressures. In other words, interactions between parasites and host-specific traits have not greatly affected the evolution of their shell profiles. A possible and uncommon effect of host-specific factors relates to the shell size. The species of *Tropiometricola* that live inside crinoids

are all small (up to 3 mm; see Warén, 1984) most probably due to a spatial constraint of the thin-armed Crinoidea.

Euzet & Combes (1998) introduced three factors as the driving force of the evolution of endoparasitism in Monogenea (Platyhelminthes): (1) competition with other ectoparasites, (2) access to the internal tissue of the host as probably better resources, and (3) predation. They concluded that, among these, predation pressure was the most important driving agency for their flatworm group, as is most probably applicable to the cause of endoparasitism in the Eulimidae. Temporary and ectoparasitic species of the latter family may be exposed to considerable predation pressure. They have actually been found in the stomach of sea stars (Habe, 1965; Warén, 1984) and fishes (Carpentieri et al., 2015). Predation by crabs and naticid snails has also been observed (Warén, 1984). Our results, as particularly beautifully exemplified in the group A2 where the endoparasitic *Stilifer* was shown to have formed from the temporary parasitic ancestor of *Parvioris* + "*Apicalia*" in a time period as short as 8 Ma (Fig. 2-6), suggest that endoparasitism can evolve repeatedly and rapidly as a consequence of adaptation to predation pressure. On the other hand, the two other factors proposed for the Monogenea, competition and access to better resources, are most unlikely in eulimid evolution. Although many echinoderm parasites are known in other animal clades (Jangoux, 1987; Lester & Sewell, 1989), ectoparasites are not abundant and therefore the body surface would be rarely saturated as habitats. In addition, many ectoparasitic eulimids possess a long proboscis to feed on body fluids (Lützen, 1976; Warén, 1984). They can thus access to the internal host tissue without achieving the endoparasitic condition.

The polyphyletic nature of endoparasites (or gall/cyst-forming parasites) has been indicated also in wasps (Whitfield, 1998) and myzostomid annelids (Lanterbecq et al., 2006; Summers & Rouse, 2014). Phylogenies of the Hymenoptera have indicated that evolutionary transitions of parasitic strategies have occurred in both directions:

endoparasites were derived from an ectoparasitic ancestor in Ichneumonoidea, while ectoparasitic species evolved from an endoparasitic ancestor in Chalcidoidea (Whitfield, 1998). Endoparasitic annelids of Myzostomida are generally apomorphic (Summers & Rouse, 2014; Fig. 2-5B) as it is always the direction for endoparasitic eulimids. The same, one-way evolution from ectoparasites to endoparasites has been suggested for flatworms (Euzet & Combes, 1998; Park et al., 2007; but see Littlewood et al., 1999). Such a difference in the evolutionary tendency may probably be attributable to whether the endoparasitic lifestyle is limited to juvenilehood (as in wasps) or the trait of reproductive adults (annelids, flatworms and eulimid snails).

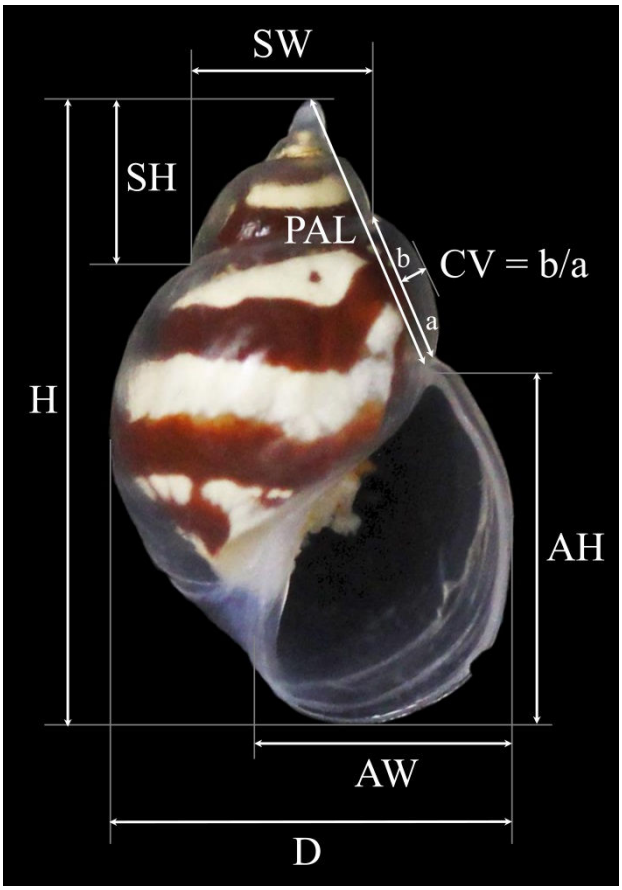

Figure 2-1. Measurements of the shell used in the principal component analyses. H: shell height, D: shell width, SH: height of the spire, SW: width of the spire, PAL: length from the apex to the uppermost point of the aperture, AH: height of the aperture, AW: width of the aperture, CV: roundness of the whorl (photo: *Goodingia varicosa*; YK#1968).

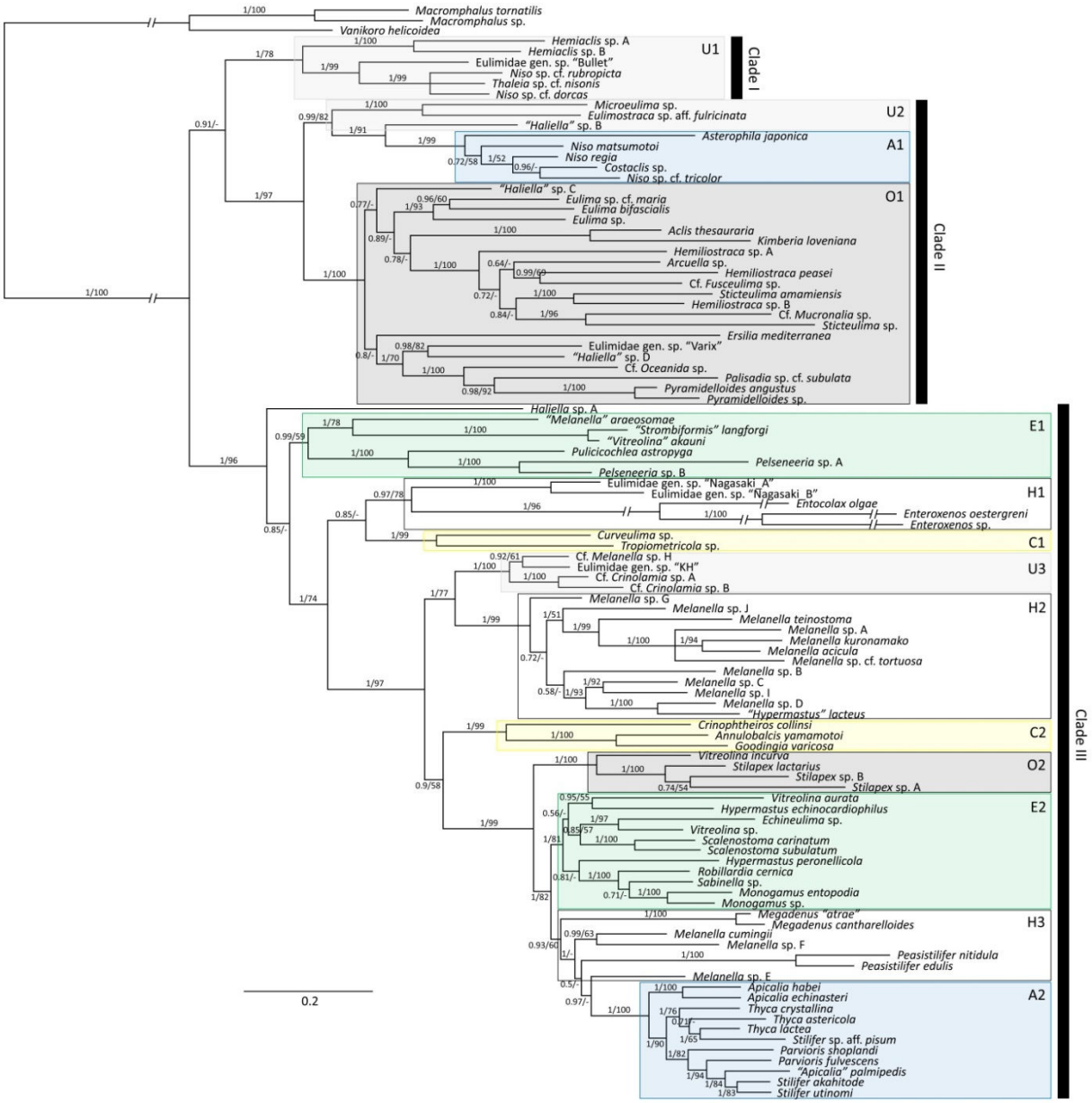

Figure 2-2. Bayesian phylogeny of the family Eulimidae inferred from the full dataset of 18S, 28S, H3, 12S, 16S and COI genes (4,743 sites in total). Numerals on branches denote posterior probabilities (PP, left) and likelihood-based bootstrap values shown as percentages (BS, right). Branch lengths were modified for a clade in the group H1 (as "//"); the original tree is shown in Fig. S2-2 in Appendix 2. See text and Fig. 2-4A for colored groups.

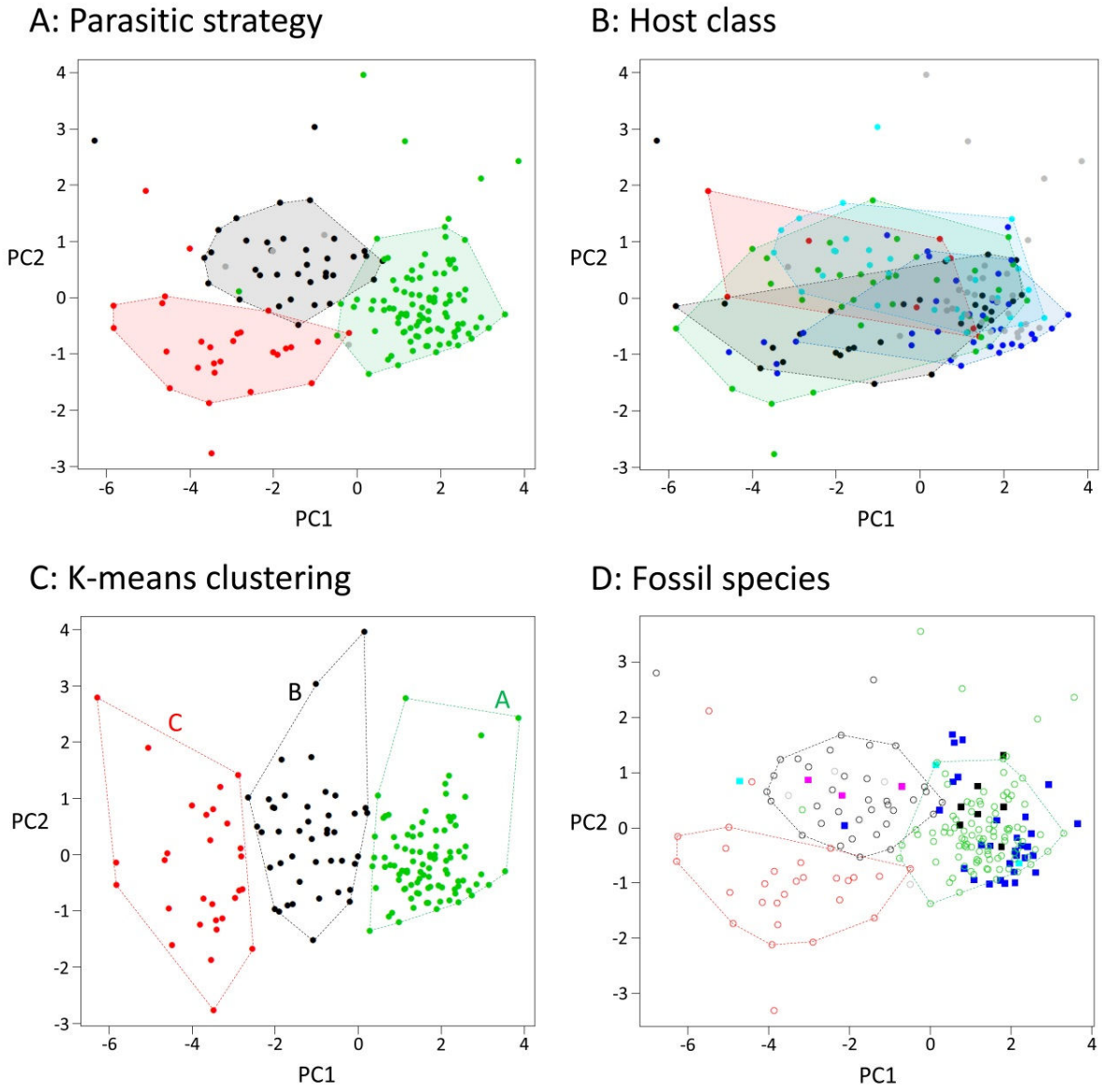

Figure 2-3. Scatter plots of the scores on PC1 and PC2 for eulimid species, both Recent (A–C) and fossil (D). (A**)** Morphological differences among temporary (green), ecto- (black) and endoparasites (red). (B**)** Differences among parasites of asteroids (black), crinoids (red), echinoids (green), holothuroids (blue) and ophiuroids (light blue). Each group was masked except outliers in Fig. 2-4. (C**)** Three types (type A, B and C; corresponded to slender, intermediate and globose shapes in text and Fig. 2-5, respectively) divided using k-means clustering based on PC scores. (D) Plot of Recent species (open circles) and fossils (solid squares) of Paleocene (black), Eocene (blue), Oligocene (purple) and Miocene (light blue). Recent species colored by parasitic strategies. Grey symbols in A, B and D represent ecology/host unknown. Eulimids are morphologically distinguishable by their parasitic strategies but not by host classes.

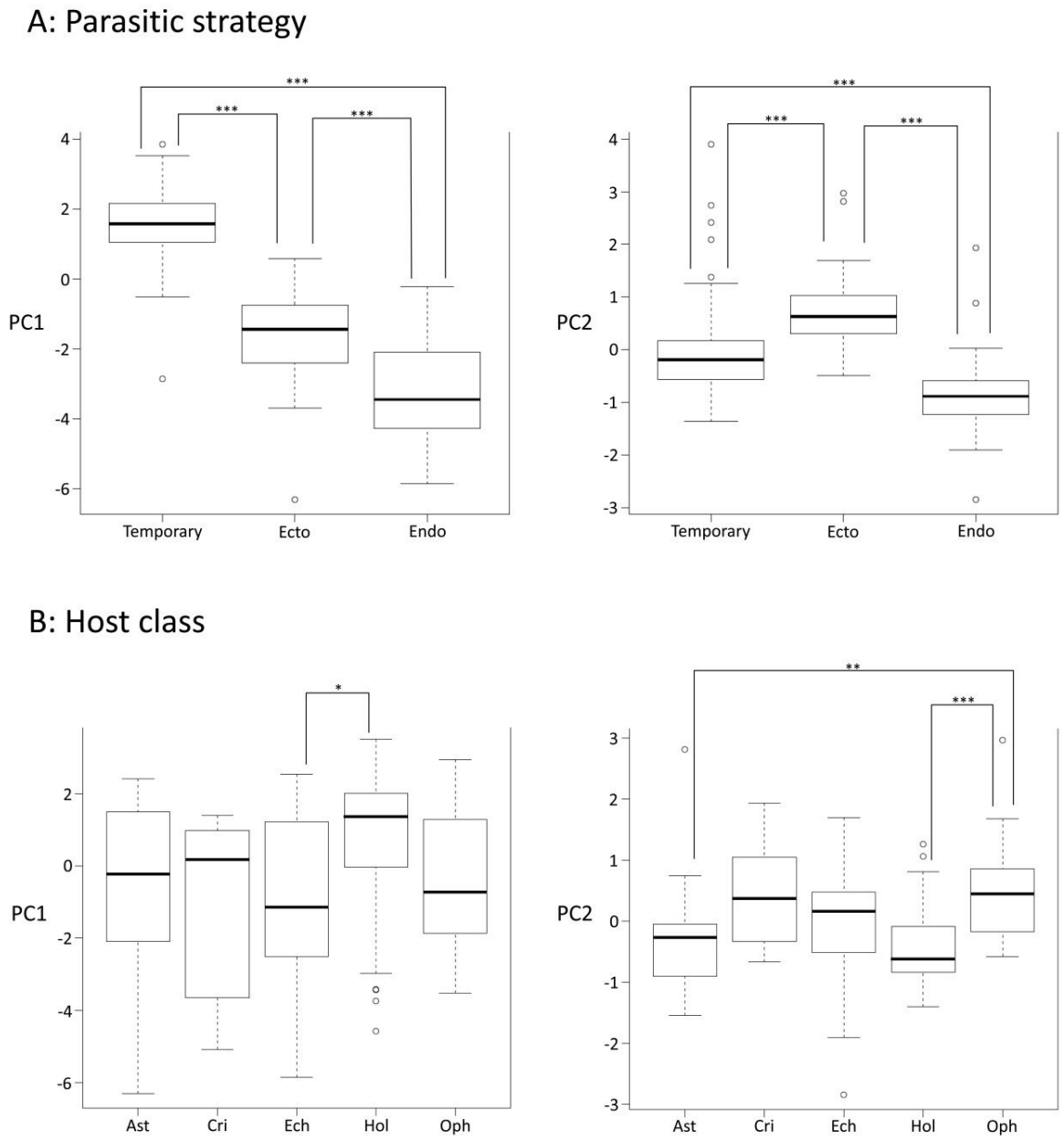

Figure 2-4. Boxplots of the PC scores for Recent eulimids. PC1 and PC2 represent slenderness of the shell and roundness of the whorls. Data are divided into three groups by parasitic strategies (A) and five groups by host classes (B) to identify morphological differences and outliers. The PC1 and PC2 scores were the largest in the temporary parasites and ectoparasites, respectively, indicating that the species of the former group bear slender shells and shells of the latter have a relatively rounded spire. Ast: Asteroidea, Cri: Crinoidea, Ech: Echinoidea, Hol: Holothuroidea, Oph: Ophiuroidea. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ (Steel-Dwass test).

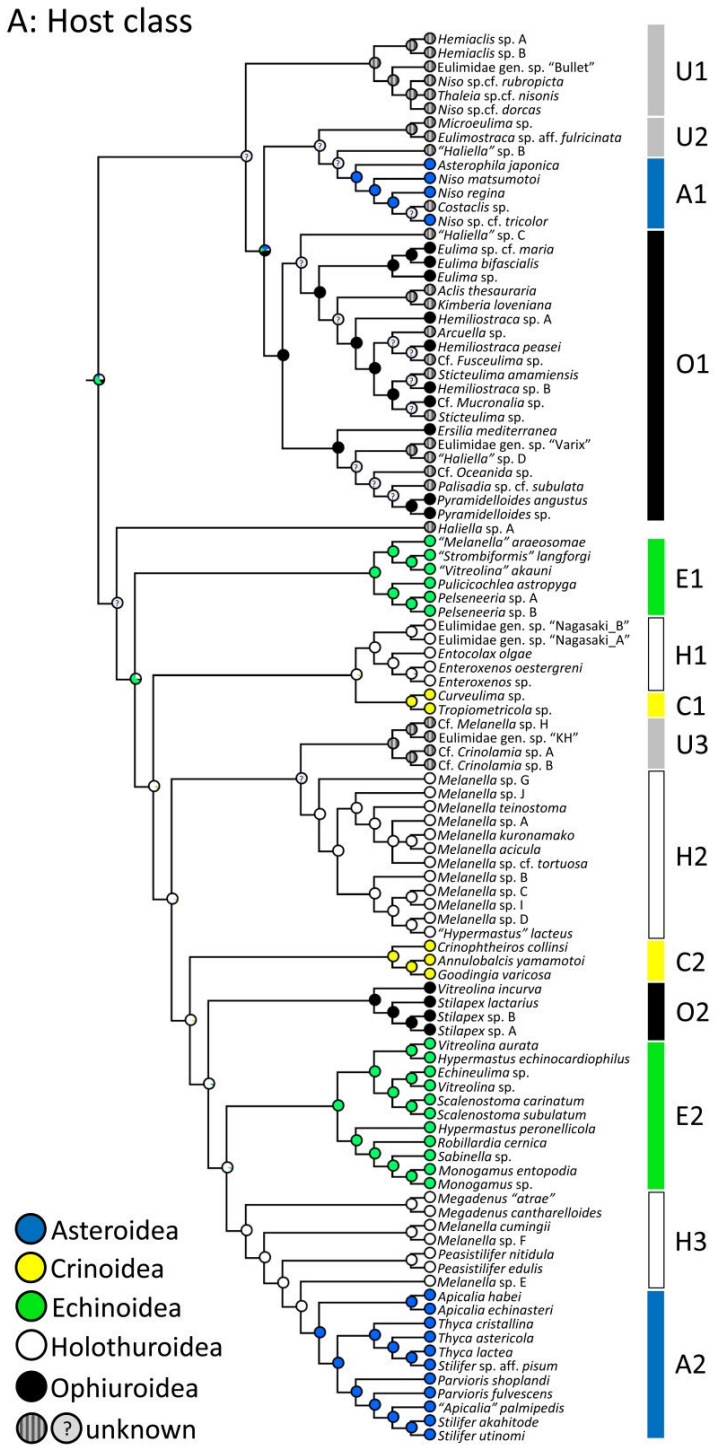

Figure 2-5. Ancestral state reconstruction of Eulimidae for the host class (A), parasitic strategy (B), shell shape (C), the presence or absence of a radula (D), and of a pseudopallium/pedal folds (E). Tree topology was inferred from a Bayesian analysis of the full-dataset. Pie charts at nodes indicate the proportion of each state. Parasites of each echinoderm class were not monophyletic: eleven groups comprising parasites of particular host classes, as well as three unknown-host groups were recognized.

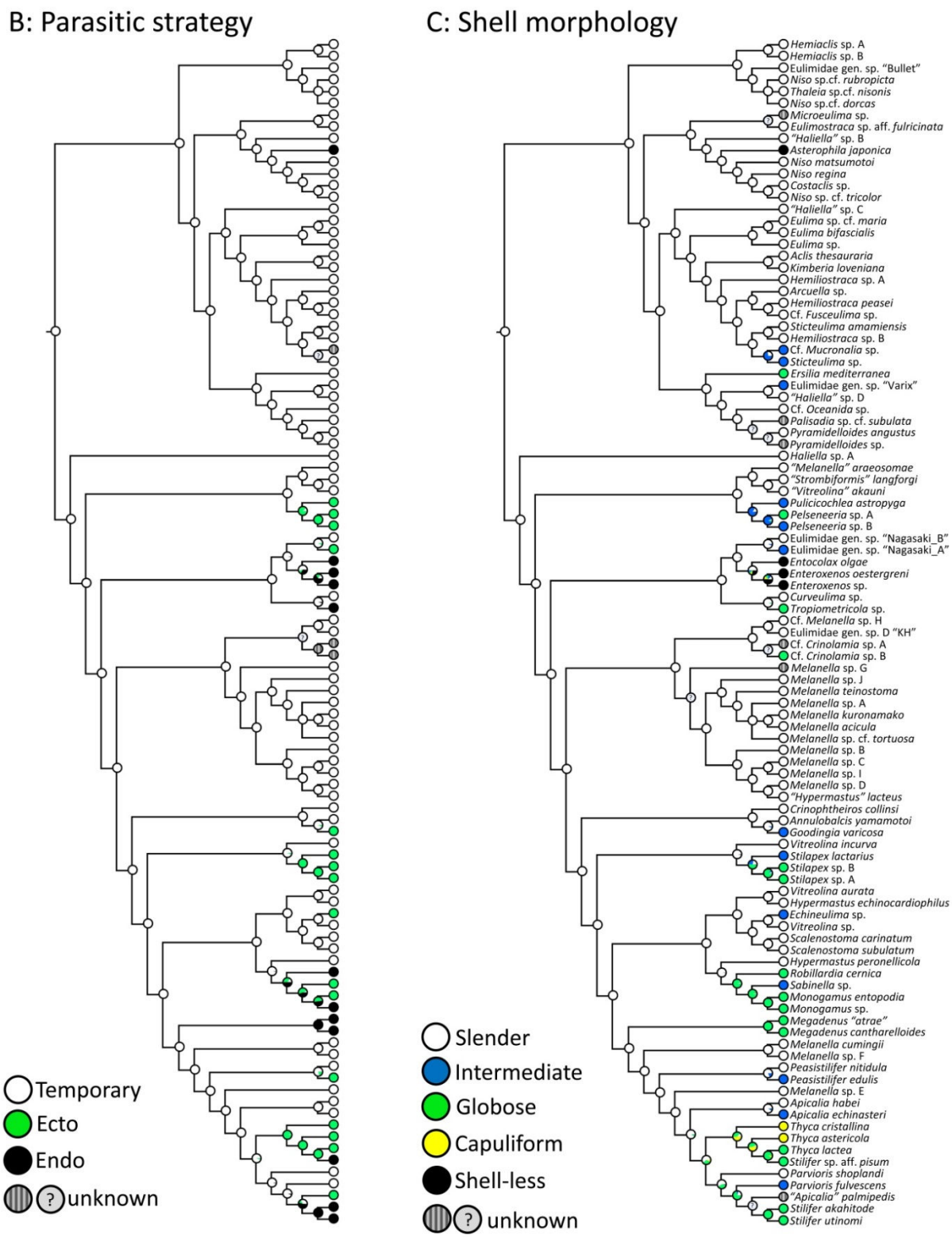

Figure 2-5. Continued. Permanent ecto- and endoparasitism have evolved independently in seven and six groups, respectively. The loss of the teleoconch occurred at least twice in Eulimidae.

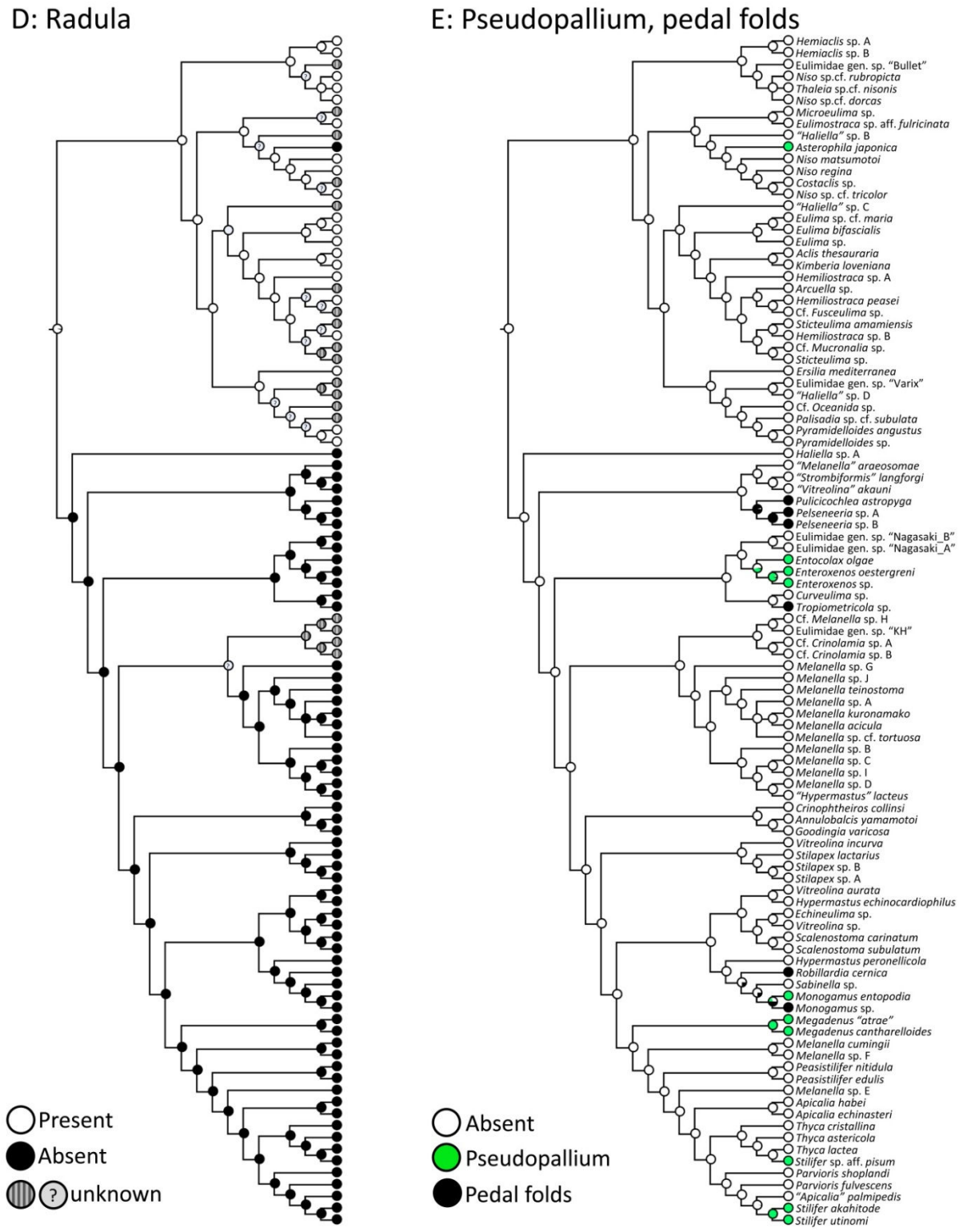

Figure 2-5. Continued. The loss of a radula and acquisition of a pseudopallium and pedal folds have clearly occurred more than once in Eulimidae.

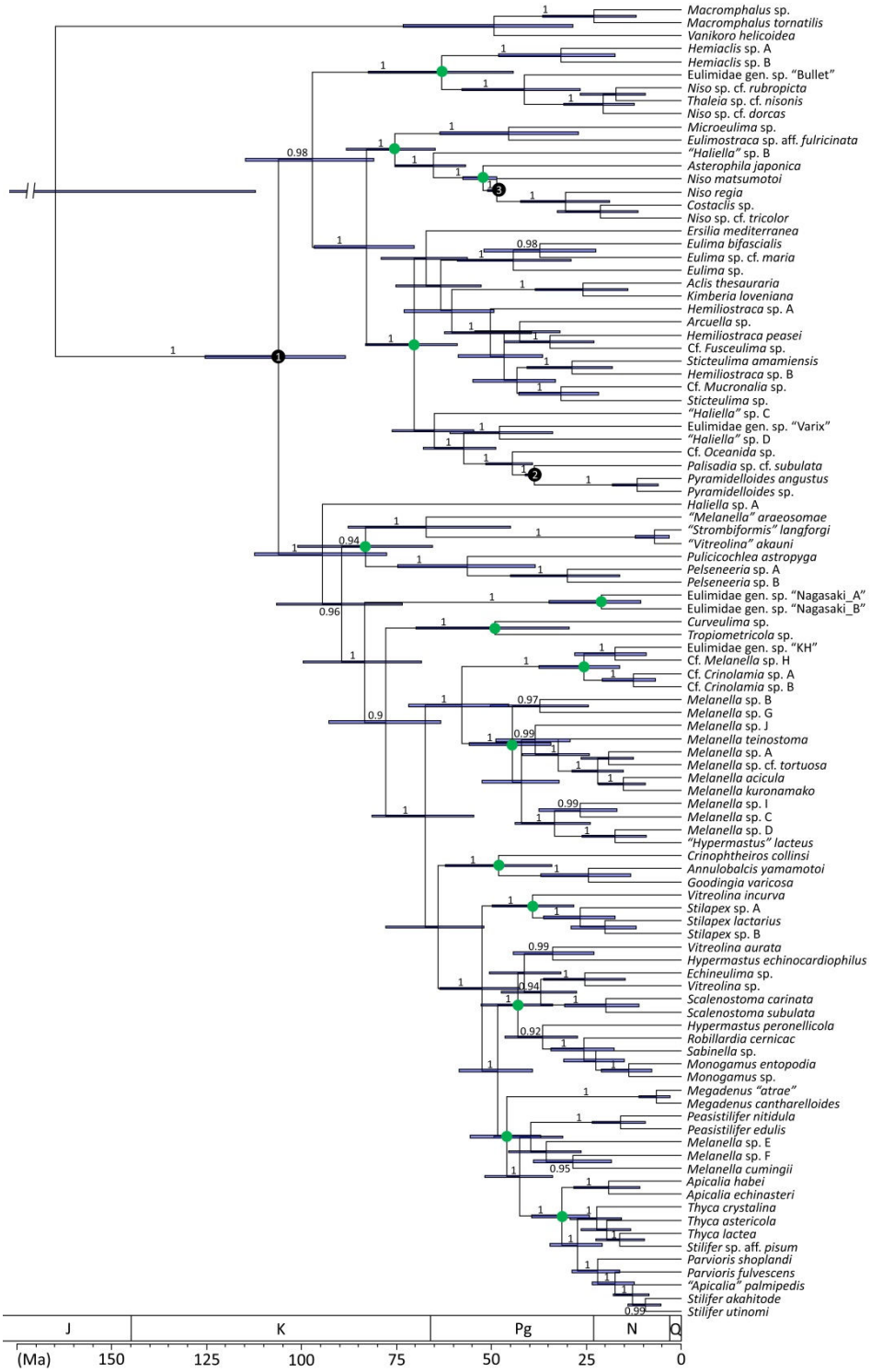

Figure 2-6. Divergence time chronogram of Eulimidae inferred from the limited dataset. Three calibration points at nodes 1–3 were set as priors based on fossil records. Posterior probabilities above 0.9 are shown on branches. Horizontal bars represent 95% HPD intervals of node ages. Green circles indicate nodes of common ancestors of each group (see Fig. 2-5A).

Table 2-1. Coordinates, localities and habitats of specimens newly sequenced in this chapter.

Species	DNA	Coordinates	Locality; habitat and depth
Eulimidae			
Aclis thesauraria	YK#2537	33°59'N, 135°03'E	Off Shirasaki, Yura, Hidaka, Wakayama, Japan; 40 m
Annulobalcis yamamotoi	YK#1479	35°08'N, 139°49'E	Off Hota, Kyonan, Chiba, Japan; 20–40 m, on *Tripiometra afra macrodiscus*
Apicalia echinasteri	YK#2293	5°10'S, 145°50'E	Tab Is., Papua New Guinea (Expédition PAPUA NIUGINI, Stn. PR39); inner slope, on *Echinaster luzonicus*
Apicalia habei	YK#1477	34°40'N, 138°59'E	Shimoda, Izu, Shizuoka, Japan; intertidal rocky shore, on *Coscinasterias acutispina*
"Apicalia" palmipedis	YK#2078	26°20'N, 127°38'E	N of Keise Is., Okinawa, Japan; 209 m, on *Anseropoda rosacea*
Arcuella sp.	YK#2648[*1]	5°11'S, 145°50'E	N of Kranket Is., Papua New Guinea (Expédition PAPUA NIUGINI, Stn. PB47); 5 m
Asterophila japonica	-	41°2'N, 140°7'E	Off Gosyogawara, Aomori, Japan; in *Leptychaster anomalus*
Costaclis sp.	YK#2659[*2]	9°28'N, 123°38'E	Bohol Sea, Philippines (N.O. "DA-BFAR", PANGLAO 2005, Stn. CP2389); 784–786 m
Cf. *Crinolamia* sp. A	YK#1824	42°11'N, 144°11'E	Off Tokachi, Hokkaido, Japan (R/V Tansei-maru KT-12-18, Stn. H3); 1210–1248 m
Cf. *Crinolamia* sp. B	YK#1825	38°33'N, 143°24'E	Off Kesennuma, Miyagi, Japan (R/V Tansei-maru KT-12-18, Stn. 12); 2477–2448 m
Crinophtheiros collinsi	YK#2059[*3]	47°28'N, 3°05'W	Pointe du Conguel, Quiberon, France; intertidal rocky shore, on *Antedon bifida*
Curveulima sp.	YK#2552	2°45'S, 150°44'E	Kavieng Lagoon, Papua New Guinea (Expédition KAVIENG 2014, Stn. KR46); 2–41 m, on unidentified Comatulida
Echineulima sp.	YK#1478	28°07'N, 129°21'E	Yadorihama, Amami Is., Kagoshima, Japan; intertidal reef flat, on *Echinometra mathaei*
Enteroxenos oestergreni	YK#1653[*4]	58°9'N, 10°9'E	Koster area, Bohuslän, Sweden; in *Stichopus tremulus*
Enteroxenos sp.	YK#2495	2°38'S, 150°38'E	New Ireland Lemus, Papua New Guinea (Expédition KAVIENG 2014, Stn. KM24); 0–1 m, in *Holothuria pervicax*
Entocolax olgae	YK#2650	75°12'N, 128°28'E	Eastern Lapterv Sea, Russia; silty-sandy bottom, 36 m, in *Myriotrochus rinkii*
Ersilia mediterranea	YK#2056	43°49'N, 15°36'E	Betina, Murter Is., Croatia; 2–4 m, on *Ophioderma longicauda*
Eulima bifascialis	YK#2547	34°59'N, 139°49'E	Kouyatsu, Tateyama, Chiba, Japan (R/V Seaster); seagrass bed, 10 m
Eulima sp. cf. *maria*	YK#1589	28°39'N, 127°04'E	Off Amami Is., Kagoshima, Japan (T/V Nagasaki-maru N295, Stn. R-2[2]); 632–704 m
Eulima sp.	YK#2061	16°08'N, 61°47'W	N of Baie de Bouillante, Guadeloupe (Campagne KARUBENTHOS 2012, Stn. GD10); 54 m
Eulimostraca sp. aff. *fulricinata*	YK#2661[*5]	16°23'N, 61°46'W	Têre à I'Anglais (Campagne KARUBENTHOS 2012, Stn. GB06); 23 m
Cf. *Fusceulima* sp.	YK#1613	34°40'N, 138°59'E	Shimoda, Izu, Shizuoka, Japan; intertidal rocky shore
Goodingia varicosa	YK#1968	5°06'S, 145°49'E	S of Sek Is., Papua New Guinea (Expédition PAPUA NIUGINI, Stn. PR35); inner slope, on unidentified Comatulida

Haliella sp. A	YK#1520	33º05'N, 134º03'E	SW of Cape Muroto, Kochi, Japan (R/V Tansei-maru KT-11-12, Stn. K6-2); 728–746 m
"Haliella" sp. B	YK#2656[*6]	2º14'S, 147º16'E	E of Manus Is., Papua New Guinea (N.O. "Alis", Campagne BIOPAPUA, Stn. CP3690); 611–618 m
"Haliella" sp. C	YK#2658[*7]	8º48'S, 159º41'E	Off Santa Isabel Is., Salomon Isls. (N.O. "Alis", Campagne SALOMON 2, Stn. CP2180); 708–828 m
"Haliella" sp. D	YK#2660[*8]	15º57'N, 121º50'E	Off Baler Bay, Philippines (N.O. "DA-BFAR", Campagne AURORA 2007, Stn. 2749); 473 m
Hemiaclis sp. B	YK#2653[*9]	21º46'S, 36º25'E	Bazaruto, Mozambique Channel (N.O. "Vizconde de Eza", Campagne MAINBAZA, Stn. CC3157); 1410–1416 m
Hemiliostraca peasei	YK#1518	28º09'N, 129º21'E	Isu, Amami Is., Kagoshima, Japan; intertidal rocky shore, on *Ophiocoma dentata*
Hemiliostraca sp. B	YK#1602	26º52'N, 128º16'E	Cape Hedo, Okinawa Is., Japan; intertidal reef flat, on *Ophiocoma scolopendrina*
Hypermastus echinocardiophilus	YK#1820	34º12'N, 133º06'E	Hakata, Ehime, Japan; tidal flat, on *Echinocardium cordatum*
Hypermastus peronellicola	YK#1597[*10]	35º10'N, 139º37'E	Off Miura, Kanagawa, Japan; 15 m, on *Peronella japonica*
"Hypermastus" lacteus	YK#1625	34º20'N, 136º41'E	Hunakoshi, Mie, Japan; tidal flat, in *Patinapta ooplax*
Kimberia loveniana	YK#2536[*11]	34º39'N, 138º56'E	Off Kisami, Shimoda, Izu, Shizuoka, Japan (R/V Tsukuba, TB-13-01); 34–37 m
Megadenus cantharelloides	-	28º26'N, 129º39'E	Itton, Amami Is., Kagoshima, Japan; intertidal rocky shore, in *Stichopus chloronotus*
Megadenus "atrae"	YK#1821	24º14'N, 123º59'E	Nakamoto, Kuroshima Is., Okinawa, Japan; intertidal reef flat, in *Holothuria atra*
Melanella cumingii	YK#1470	31º44'N, 131º28'E	Nojima, Miyazaki, Japan; intertidal rocky shore
Melanella kuronamako	YK#1548	28º24'N, 129º40'E	Tsuchihama, Amami Is., Kagoshima, Japan; intertidal reef flat, on *Holothuria leucospilota*
Melanella teinostoma	YK#1604	26º31'N, 127º52'E	Serakaki, Okinawa Is., Japan; reef flat, on *Bohadschia* sp.
Melanella sp. cf. *tortuosa*	YK#1573	31º14'N, 130º39'E	Ibusuki, Kagoshima, Japan; estuary
Melanella sp. A	YK#1523	32º10'N, 129º31'E	SW of Nagasaki, Japan (T/V Nagasaki-maru N226, Dredge A); 470–487 m, on unidentified Holothuroidea
Melanella sp. B	YK#1569	28º09'N, 129º21'E	Isu, Amami Is., Kagoshima, Japan; intertidal rocky shore, on *Holothuria nobilis*
Melanella sp. C	YK#1572	35º09'N, 139º35'E	Off Misaki, Miura, Kanagawa, Japan; 80 m
Melanella sp. D	YK#1574	28º21'N, 129º19'E	Hienhama, Amami Is., Kagoshima, Japan; intertidal reef flat
Melanella sp. E	YK#1575	31º24'N, 130º11'E	Koura, Kasasa, Kagoshima, Japan; intertidal rocky shore
Melanella sp. F	YK#1577	28º06'N, 129º21'E	Ankyaba, Kakeroma Is., Kagoshima, Japan; intertidal rocky shore
Melanella sp.G	YK#1579	31º07'N, 131º39'E	Off Cape Toi, Miyazaki, Japan (R/V Tansei-maru KT-11-12, Stn. T10-2); 1063–1082 m
Cf. *Melanella* sp. H	YK#1581	31º07'N, 131º39'E	Off Cape Toi, Miyazaki, Japan (R/V Tansei-maru KT-11-12, Stn. T10-2); 1063–1082 m
Melanella sp. I	YK#1585	31º44'N, 131º28'E	Nojima, Miyazaki, Japan; intertidal rocky shore
Melanella sp. J	YK#1588	32º06'N, 129º29'E	SW of Nagasaki, Japan (T/V Nagasaki-maru N319, Stn. B6-1); 606–610 m
"Melanella" araeosomae	YK#2657[*12]	2º15'S, 150º16'E	W of New Hanover Is., Papua New Guinea (N.O. "Alis", Campagne BIOPAPUA, Stn. CP3655); 402–440 m

Microeulima sp.	YK#2554	2°37’S, 150°32’E	NW of Nubils Is., Papua New Guinea (Expédition KAVIENG 2014, Stn. KS39); ledge flat, 20 m
Monogamus sp.	YK#1570	28°09’N, 129°21’E	Isu, Amami Is., Kagoshima, Japan; intertidal rocky shore, in *Echinometra* sp. *“tsumajiro”*
Cf. *Mucronalia* sp.	YK#2060	5°N, 145°E	Collected during Expédition PAPUA NIUGINI 2012, Station unknown; on *Ophiothela danae*
Niso regia	YK#2645*13	3°16’S, 144°05’E	SE of Vokeo Is., Papua New Guinea (N.O. “Alis”, Expédition PAPUA NIUGINI, Stn. DW4074); 460–605 m
Niso sp. cf. *dorcas*	YK#1967*14	25°16’S, 168°56’E	Banc Athos, SE of New Caledonia (N.O. “Alis”, Campagne NORFOLK 2, Stn. DW2067); 614–690 m
Niso sp. cf. *rubropicta*	YK#2646*15	6°04’S, 148°09’E	SE of Tuam Is., Papua New Guinea (N.O. “Alis”, Campagne PAPUA NIUGINI, Stn. CP4007); 460–528 m
Niso sp. cf. *tricolor*	YK#2655*16	2°34’S, 150°47’E	Off Feni Isls., Papua New Guinea (N.O. “Alis”, Campagne BIOPAPUA, Stn. CP3760); 613–660 m
Cf. *Oceanida* sp.	YK#2494	2°34’S, 150°47’E	Kavieng Lagoon, Papua New Guinea (Expédition KAVIENG 2014, Stn. KS13); sandy platform, 13 m
Palisadia sp. cf. *subulata*	YK#2493	2°35’S, 150°46’E	Kavieng Lagoon, Papua New Guinea (Expédition KAVIENG 2014, Stn. KB16); oceanfront reef, 13–14 m
Parvioris fulvescens	YK#1543	9°49’N, 123°22’E	Matutinao, Badian, Cebu, Philippines; intertidal, on *Archaster typicus*
Parvioris shoplandi	YK#1549	24°21’N, 123°45’E	Shirahama, Iriomote Is., Okinawa, Japan; tidal flat, on *Archaster typicus*
Peasistilifer edulis	YK#1607	24°47’N, 125°14’E	Off Hisamatsu, Miyako Is., Okinawa, Japan; subtidal, on *Holothuria edulis*
Peasistilifer nitidula	YK#1546	28°26’N, 129°40’E	Tekebu, Kasari, Amami Is., Kagoshima, Japan; tidal flat, on *Holothuria atra*
Pelseneeria sp. A	YK#1587	32°10’N, 129°31’E	SW of Nagasaki, Japan (T/V Nagasaki-maru N226, Dredge A); 470–487 m, on unidentified Echinoidea
Pelseneeria sp. B	YK#1862*17	23°21’S, 150°43’W	Banc Arago, Tupua’i Is., French Polynesia (N.O. “Alis”, Campagne BENTHAUS, Stn. DW1981), 650–1150 m, on *Aspidodiadema* sp.
Pulicicochlea astropyga	YK#2647*18	5°09’S, 145°48’W	Jais Aben Resort, Papua New Guinea (Expédition PAPUA NIUGINI, Stn. PR226); on *Diadema* sp.
Pyramidelloides sp.	YK#1610	26°28’N, 127°49’E	Onna, Okinawa Is., Japan; subtidal reef, on unidentified Ophiuroidea
Robillardia cernica	YK#1629	26°30’N, 127°51’E	Manzamo, Okinawa Is., Japan; intertidal, in *Echinomentra oblonga*
Sabinella sp.	YK#1522	09°29'N, 123°41’E	S of Balicasag Is. Bohol, Philippines; ca. 300 m, on Cidaridae sp.
Scalenostoma carinatum	YK#1627	26°29’N, 127°50’E	Onna, Okinawa Is., Japan; reef slope, in dead coral
Scalenostoma subulatum	YK#2551	2°38’S, 150°27’E	Kavieng Lagoon, Papua New Guinea (Expédition KAVIENG 2014, Stn. KB42); rubble slope, 6–12 m
Sticteulima amamiensis	YK#1586	31°11’N, 130°30’E	Hanasezaki, Ibusuki, Kagoshima, Japan; intertidal rocky shore
Sticteulima sp.	YK#2294	5°12’S, 145°49’E	Kranket Is., Papua New Guinea (Expédition PAPUA NIUGINI, Stn. PB12); 7–15 m
Stilapex lactarius	YK#1596	35°10'N, 139°46’E	Off Kanaya, Futtsu, Chiba, Japan; 200–260 m, on *Ophiothrix panchyendyta*
Stilapex sp. A	YK#1600	36°50'N, 140°48’E	Izura, Kitaibaraki, Ibaraki, Japan; intertidal, on *Ophiothrix* sp.
Stilapex sp. B	YK#1605	34°7–10’N, 136°24–31’E	Off Owase, Mie, Japan (T/V Seisui-maru #1126, Stn. D-3, 4); 123–138 m, on *Ophiothrix* sp.
Stilifer utinomi	YK#1608	26°28’N, 127°49’E	Onna, Okinawa Is., Japan; subtidal reef, in *Linckia multifora*
Stilifer sp. aff. *pisum*	YK#1476	32°10’N, 129°28’E	SW of Nagasaki, Japan (T/V Nagasaki-maru N275, Stn. A); 497 m, in *Brisinga andamanica*

"Strombiformis" langforgi	YK#1542	33º36'N, 135º23'E	Shirahama, Wakayama, Japan; intertidal rocky shore, on *Anthocidaris crassispina*
Thaleia sp. cf. *nisonis*	YK#2662*[19]	22º24'S, 171º45'E	E of Matthew, New Caledonia (N.O. "Alis", Campagne EXBODI, Stn. DW3894); 843–845 m
Thyca astericola	YK#2496	2º36'S, 150º46'E	W side of Nago Is., Papua New Guinea (Expédition KAVIENG 2014, Stn. KM01); 0–1 m, on *Protoreaster nodosus*
Thyca lactea	YK#1472	32º10'N, 129º28'E	SW of Nagasaki, Japan (T/V Nagasaki-maru N275, Stn. A); 497 m, on Goniasteridae sp.
Tropiometricola sp.	YK#2651*[20]	19º05'S, 163º28'E	N of Île Pott, New Caledonia (N.O. "Alis", Campagne CONCALIS, Stn. 2936); 245–258 m, in unidentified Comatulida
"Vitreolina" akauni	YK#1611	34º40'N, 138º59'E	Shimoda, Izu, Shizuoka, Japan; intertidal rocky shore
Vitreolina incurva	YK#2024	43º49'N, 15º36'E	Betina, Murter Is., Croatia; 1–4 m, on *Ophioderma longicauda*
Vitreolina sp.	YK#1545	28º21'N, 129º19'E	Hienhama, Amami Is., Kagoshima, Japan; intertidal reef flat, on *Tripneustes pileolus*
Eulimidae gen. sp. "Varix"	YK#2553	2º37'S, 150º32'E	NW of Nubils Is., Papua New Guinea (Expédition KAVIENG 2014, Stn. KS39); ledge flat, 20 m
Eulimidae gen. sp. "Nagasaki_A"	YK#1480	32º09'N, 129º31'E	SW of Nagasaki, Japan (T/V Nagasaki-maru N295, Stn.A-1); 495–500 m, on Molpadida sp.
Eulimidae gen. sp. "Nagasaki_B"	YK#1544	30º10'N, 127º51'E	W of Tokara Isls., Kagoshima, Japan (T/V Nagasaki-maru N275, Stn. U); 342 m, on Molpadida sp.?*
Eulimidae gen. sp. "KH"	YK#2550	47º00'N, 160º02'E	Off Kamchatka Pen., on the high-seas (R/V Hakuho-maru KH-14-02, Stn. NBD-1); 5223–5179 m
Eulimidae gen. sp. "Bullet"	YK#2652*[21]	23º33'S, 35º55'E	Inhanbane, Mozambique Channel (N.O. "Vizconde de Eza", Campagne MAINBAZA, Stn. CC3141); 684–698 m
(Outgroup taxon)			
Vanikoridae			
Macromphalus tornatilis	-	34º12'N, 132º40'E	Kure, Hiroshima, Japan; intertidal, in the burrow of *Ikedosoma gogoshimaense*

*[1]Muséum National d'Histoire Naturelle, Paris (MNHN) IM-2013-53102; *[2]MNHN IM-2013-53110; *[3]Swedish Museum of Natural History (SMNH) 127075; *[4]SMNH-23414; *[5]MNHN IM-2013-53106; *[6]MNHN IM-2013-53121; *[7]MNHN IM-2013-53108; *[8]MNHN IM-2013-53111; *[9]MNHN IM-2013-53118; *[10]Misaki Marine Biological Station (MMBS), The University of Tokyo Mo-084; *[11]National Museum of Nature and Science, Tsukuba (NSMT), Mo78793; *[12]MNHN IM-2013-53122; *[13]MNHN IM-2013-53096; *[14]MNHN IM-2009-12193; *[15]MNHN IM-2013-9720; *[16]MNHN IM-2013-53119; *[17]SMNH-56020; *[18]MNHN IM-2013-53100; *[19]MNHN IM-2013-53132; *[20]MNHN IM-2013-53124; *[21]MNHN IM-2013-53117.

Table 2-2. Summary of sequence partitions of the full dataset including 104 OTUs. Values of the limited dataset (101 OTUs) are shown in brackets if it differs from the value in the full dataset.

Partition	Substitution model	Final length	Variable sites	Parsimony informative
18S	SYM + G + I	1762 (1764)	261 (217)	129 (117)
28S	GTR + G + I	1378 (1358)	485 (311)	349 (221)
H3 1st codon	GTR + I (GTR + G)	103	19 (18)	12
H3 2nd codon	JC	102	2	1
H3 3rd codon	GTR + G + I	102	89 (87)	85 (83)
12S	GTR + G + I	348 (299)	295 (250)	264 (214)
16S	GTR + G + I	312 (333)	210 (228)	185 (202)
COI 1st codon	GTR + G + I	212	123 (109)	104 (90)
COI 2nd codon	GTR + G + I	212	71 (57)	43 (38)
COI 3rd codon	HKY + G (GTR + G + I)	212	212	212

Table2-3. Variable loadings, eigenvalues and cumulative proportions (%) in the first two principal components for Recent eulimids (left), and Recent and fossil taxa (right). See text for abbreviations.

	Recent		Recent and fossil	
Variables	PC1	PC2	PC1	PC2
SH	0.427	-0.025	0.424	-0.061
D	-0.421	-0.087	-0.420	-0.151
SW	-0.285	-0.378	-0.280	-0.492
AW	-0.423	-0.094	-0.423	-0.122
AH	-0.423	0.005	-0.422	0.046
PAL	0.409	-0.003	0.405	-0.074
CV	-0.190	0.917	-0.210	0.842
Eigenvalue	5.070	0.869	5.081	0.821
Cumulative proportion	72.44	84.85	72.61	84.34

Table 2-4. Correlation between parasitic strategies and shell types of eulimid snails inferred from the k-means clustering. Numbers of species are shown in each column. All shell types have occurred on each host class. *P*-value was calculated by the Fisher's exact test.

	Slender	Intermediate	Globose
Parasitic strategy ($p < 0.001$)			
Temporary	94	5	1
Ecto	2	27	7
Endo	0	8	20
Host			
Asteroidea	13	9	7
Crinoidea	4	2	2
Echinoidea	12	14	9
Holothuroidea	25	5	6
Ophiuroidea	14	11	4

Chapter 3

Evolutionary relationships and diversification pattern in Pyramidellidae

3-1. Introduction

The Pyramidellidae—parasites of annelids and other mollusks—consist of at very least of 5,000 species that belong to approximately 350 genera and subgenera (Schander et al., 1999a; Bouchet & Rocroi, 2005). They are the major constituents of the superfamily Pyramidelloidea that also includes Amathinidae as the other exclusive family (Ponder, 1987; Bouchet & Rocroi, 2005). Regardless of their high species richness, the Pyramidellidae exhibit rather restricted ecological and morphological diversity. Host specificity is fairly low with each species often exploiting multiple animal groups as hosts, which are sometimes distantly related to each other (Ponder & de Keyzer, 1998b; Hori, 2000b). Most pyramidellids are temporary parasites and bear a functional foot and a high-spired shell that may facilitate efficient crawling in soft sediment (Vermeij, 1993). Actually, they are collected often as free-living or empty shells without information on their preference to specific hosts (e.g. Hori, 2000b). Unlike many other parasitic taxa, sexual dimorphism is absent in Pyramidelloidea. They are all simultaneous hermaphrodites as a phylogenetic constraint of Heterobranchia to which the superfamily belongs (Ponder & de Keyzer, 1998b; Jörger et al., 2010; Dinapoli et al., 2011; Zapata et al., 2014). Anatomical traits in their digestive system, however, seem to warrant a parasitic mode of life for all pyramidelloid species. These traits include the absence of a radula and the presence of a long acrembolic proboscis and a buccal pump to suck out body fluids of the host (Wise, 1996; Ponder & de Keyzer, 1998b). Some species can have negative impacts on such bivalve hosts as giant clams and oysters, thus negatively affecting fishery resources as harmful pests (e.g. Cumming & Alford, 1994; Carroll & Finelli, 2015). For example, *Boonea impressa* is known as the

"oyster mosquito" and downregulate the growth rate of juvenile oysters (Carroll & Finelli, 2015).

The Amathinidae represent a more specialized group of parasites on bivalve mollusks than their presumed sister group Pyramidellidae does (Ponder, 1987). They also differ morphologically from pyramidellids in having a generally lower spire and a higher expansion rate of the aperture that result in globose to limpet-like (patelliform) appearances of the shell (Ponder & de Keyzer, 1998b). Such shell shapes are tied to their closer association with the host, including the sedentary mode of life on the host shell in the patelliform *Amathina* and *Cyclothyca*, than the entirely autonomous pyramidellids with more slender shells (Ponder & de Keyzer, 1998b). Amathinids are also characterized anatomically in having diffuse salivary glands and a secondary gill situated on the left side of the pallial cavity and in lacking the hypobranchial gland, stylet or buccal bulb (Ponder, 1987; Hori & Tsuchida, 1995).

Systematic studies on the Pyramidelloidea have been largely based on shell morphology (e.g. Wise, 1996; Høisæter, 2014; see also Schander et al., 2002 and references therein). Schander et al. (1999a) proposed a higher classification for the group where he recognized six distinct families including Pyramidellidae, Odostomiidae, Syrnolidae, Turbonillidae, Anisocyclidae and Amathinidae by compiling previous conchological studies. In their review of gastropod classification, Bouchet & Rocroi (2005) largely adopted the Schander et al.'s (1999a) scheme for Pyramidelloidea, while they downgraded Pyramidellidae, Odostomiidae, Syrnolidae and Turbonillidae to subfamilies of Pyramidellidae (s.l.) and synonymized Anisocyclidae under Turbonillinae; Schander et al.'s (1999a) subfamilies for Pyramidellidae (s.l.) have also downgraded to 11 tribes in Pyramidellinae, Odostomiinae, Syrnolinae and Turbonillinae. Unfortunately, this widely accepted classification scheme (Bouchet & Rocroi, 2005) does not reflect true phylogenetic relationships of the group or fragmented results from previous phylogenetic analyses. Both morphology (Schander et al., 1999b) and

molecular-based phylogenetic reconstructions (Schander et al., 2002) have suggested the non-monophyly of Odostomiinae and Turbonillinae *sensu* Bouchet & Rocroi (2005). Dinapoli et al. (2011) on the contrary corroborated the monophyly of both subfamilies based on more extensive sampling of genes, but from a more limited number of taxa that did not include the other two subfamilies of Pyramidellidae.

Without sufficient information on phylogenetic relationships, the evolutionary transitions of morphology and ecology in Pyramidelloidea are essentially unknown and rarely discussed in previous papers. An exception is the phylogenetic reconstruction of 13 species by Wise (1996), who concluded from conchological and anatomical traits that the Odostomiinae (*sensu* Bouchet & Rocroi, 2005) represent the plesiomorphic conditions of Pyramidellidae as its basal most lineage. However, this phylogenetic relationship was inferred on the premise that the Amathinidae and Pyramidellidae are reciprocal sister groups in the superfamily and from similarities in the conditions of the alimentary tract between the Odostomiinae and Amathinidae (Wise, 1996). The sister relationship of the two families however has not been tested and thus the evolutionary direction remains to be investigated, ideally based on molecular data that are independent from morphological adaptation. Overall, our limited knowledge on their phylogeny and parasitic ecology combined makes it difficult to reconstruct the history of radiation and speciation of the Pyramidelloidea.

The position of the Pyramidelloidea has also been contentious, while its inclusion in the Panpulmonata of Heterobranchia is widely accepted by recent molecular phylogenies (e.g. Jörger et al., 2010; Zapata et al., 2014). Heterobranchia was coined by Haszpruner (1985) and now it is broadly accepted as a monophyletic clade and a sister group to Caenogastropoda (Ponder & Lindberg, 1997; Kocot et al., 2011; Smith et al., 2011). The original concept of Heterobranchia includes Allogastropoda (= "Lower-Heterobranchia") and Pentaganglionata (= Euthyneura). Pyramidelloidea was first assigned to the former group along with Architectonicoidea

based on the presence of a special secondary gill and with the extinct Nerineoidea based on a heterostrophic protoconch and columellar lamellae of the teleoconch (Haszpruner, 1985). Bouchet & Rocroi (2005) followed this classification and placed pyramidelloids in the "Lower-Heterobranchia," together with such superfamilies as Architectonicoidea, Omalogyroidea, Rissoelloidea and Ringiculoidea. However, recent molecular phylogenies of Heterobranchia unanimously and strongly indicate the panpulmonate affinity of Pyramidelloidea within Euthyneura (Dinapoli & Klussmann-Kolb, 2010; Jörger et al., 2010; Dayrat et al., 2011; Dinapoli et al., 2011; Zapata et al., 2014). These molecular studies, however, suffer from the lack of resolution as to the exact position of Pyramidelloidea. Their possible sister clades include Glacidorboidea (Dinapoli & Klussmann-Kolb, 2010; Dinapoli et al., 2011), Amphiboloidea (Jörger et al., 2010) and Lymnaeoidea (Dayrat et al., 2011). The feeding ecology of non-parasitic ancestor of Pyramidelloidea is therefore unclear: glacidorboids feed on the tissue of wounded invertebrates (Ponder, 1986), while amphiboloids are deposit feeders and lymnaeoids are omnivores with more animal oriented diets (Bovbjerg, 1968; Roach & Lim, 2000).

The lack of a resolved phylogeny for Pyramidelloidea also precludes rigorous inference of their diversification pattern and driving force behind the enormous species richness. The temporary parasitic mode and low host specificity in most pyramidellids suggest that their diversification process might not have involved repeated adaptive radiation as in the case of eulimids (see Chapter 2). In the absence of knowledge on relationships among taxa, however, Dinapoli et al. (2011) have proposed a hypothesis that the extraordinary richness of Pyramidellidae might be attributable to their adaptive radiation triggered by the switch to the carnivorous from an herbivorous ancestor that open for them newly accessible resources. This hypothesis can be elaborated by assuming that the species richness has increased by numerous accessible opportunities for adaptive diversification provided from the host groups: annelids and mollusks

consist of numerous species (c. 230,000 in total; Chapman, 2009) and live in various habitats across wide ranges of depths and geographic areas (see General Introduction). In other words, pyramidellids might perhaps have diversified by frequently changing their host groups and habitats.

In this chapter, phylogenetic relationships within the superfamily and its position relative to the presumed sister taxa are investigated to illuminate the adaptation process and morphological diversification, as well as higher taxonomy, of the parasitic pyramidelloids. To this aim, a molecular phylogeny of the group was inferred from concatenated sequences of three nuclear (18S, 28S and H3) and three mitochondrial (COI, trnV and 16S) genes (c. 5.1 kbp in total) and the ancestral states of host utilization, habitat and shell shape were estimated based on the obtained tree topology. This chapter also aims to test the hypothesis that the Pyramidellidae have diversified through frequent host switches and habitat shifts into different marine environments. If the hypothesis is true, closely related pyramidellids are expected to have a tendency to utilize different groups of the host in disjunct environments.

3-2. Materials and methods

Taxonomic sampling

Fifty-one pyramidellid species were collected and analyzed along with published sequences from eight confamilial species (e.g. Dinapoli et al., 2011; Tables 3-1, 3-2). These species represent ten of the 11 tribes and all four subfamilies that were recognized by Bouchet & Rocroi (2005). Species identification and generic assignment of the study specimens followed Warén (1991) and Hori (2000b). Published sequences from *Miralda* sp. "EED-Phy-918" were not included since *M. scopulorum* (YK#2742) yielded similar and a more complete set of sequences. The

study specimens cover nearly the whole ranges of habitats and hosts for the family (see above). Five species of unique shell morphologies and undetermined subfamilial positions were also collected and analyzed for the present molecular phylogeny; these include the planispiral snail *Moerchinella* sp., patelliform "Pyramidellidae gen. sp. Limpet", two open-coiling snails "Pyramidellidae gen. spp. Neji_S and Neji_F", and Pyramidellidae gen. sp. A. The last species somewhat resembles the species of *Orinella* and *Tiberia* in having a relatively broad shell with a brown spiral band on slightly inflated whorls, but its bathyal occurrence does not accord with the known habitats of the latter genera (Hori, 2000b; see Appendix 3).

In addition, the first molecular data for Aamathinidae were obtained from species of *Leucotina*, *Amathina* and *Cyclothyca* to test their sister-group relationship to the Pyramidellidae (Wise, 1996). The sister group of the superfamily Pyramidelloidea was explored by obtaining new sequences from *Glacidorbis hedleyi* (Glacidorbidae) and by including published data from another glacidorbid *G. rusticus* as well as from *Salinator* spp. (Amphibolidae) and *Radix auricularia* (Lymnaeidae; Table 3-2). Trees were nested by the species of *Peronia* (Eupulmonata; Onchidiidae) based on recent molecular phylogenies of Heterobranchia (e.g. Jörger et al., 2010).

Most live snails and limpets were boiled in 70–90ºC water for 0.1–1 min and the animals were extracted from the shells and preserved in pure ethanol. Voucher material has been deposited in the Atmosphere and Ocean Research Institute, The University of Tokyo, Japan (AORI), Okayama University, Japan, or Muséum National d'Histoire Naturelle, Paris, France (MNHN).

DNA sequencing, phylogenetic analyses and ancestral state reconstruction

Sequences of three nuclear genes and one mitochondrial fragment spanning three genes were amplified for each species using the primer sets listed in Table S1-1. The three

nuclear fragments include the nearly entire 18S gene, domains D1–D3 of the 28S gene and a short segment of the H3 gene. In most heterobranchs, the mitochondrial COI and 16S genes are neighboring with a short trnV sequence between them, so that the three genes can be sequenced by the primer set LCO1490-16SbrH (Grande et al., 2002, 2004). DNA extraction, PCR and sequencing reaction were done in accordance with the Chapter 1 except setting different annealing temperatures for the primer sets 28SC1-28SD3 (52ºC) and het3-D3m (58ºC) for the amplification of 28S gene.

Phylogenetic trees were reconstructed using the Bayesian and ML methods from a concatenated dataset of the six genes. The dataset was divided into nine partitions as shown in Table 3-3; note that the short trnV sequence was treated as a part of the 16S gene fragment. In Bayesian analyses, four Markov chains were run for 30,000,000 generations with a sample frequency of 1,000, and the first 7,500 trees for each run were discarded as a burn-in (see Chapter 2 for more details on phylogenetic analyses).

The ancestral states of the habitat and shell morphology were reconstructed using Mesquite 3.04 (Maddison & Maddison, 2015) under the Markov one-parameter (Mk1) model of the ML method. Pyramidellid gastropods occupy a wide range of marine habitats; the analyzed species can be categorized into the rocky shore, tidal flat, upper subtidal (1–27 m), continental shelf (70–200 m) and bathyal water (> 343 m, see Table 3-1 for more details). This partitioning of the depth level was based on the traditional concept that the shelf break at approximately 200 m marks the boundary between the continental shelf and deep-sea faunas (Gage & Tyler, 1991); the upper subtidal is treated as a further different environment in terms of the abiotic stresses: organisms in this habitat may be exposed to relatively strong wave action and solar radiation that are a part of the major environmental stresses for intertidal species (Moran, 1999). The sunken-wood habitat was coded as a different state from the shelf or the deep sea and was applied to Pyramidellidae gen. spp. “Neji_S” and “Neji_F”. This

separate coding is based on recent studies on the gastropod faunas of sunken wood that have revealed its unique composition of genera and species, regardless of depths (e.g. Hasegawa, 1997; Warén, 2011). The environment of the sampling localities was presumed as the typical habitat of each species and was mapped onto the Bayesian consensus tree. Shell morphology was coded as a binary character, i.e. coiled or patelliform, and mapped onto the tree.

Unfortunately, the lack of host information for a majority of species prevented the reconstruction of ancestral states for this character. The known distribution of annelid and molluscan parasites among study taxa was instead shown in the Bayesian consensus tree. Similarly, although abundant fossils of pyramidellids are known from the Cretaceous and Cenozoic faunas, their indeterminable positions in the molecular tree made attempts to estimate divergence times not feasible by using such records as calibration points.

3-3. Results

Sequence data

Table 3-3 shows the estimated model of substitution, length of aligned sequences after the masking of alignment ambiguous sites and numbers of variable and parsimony-informative sites for each partition of the sequence data. The concatenated dataset had 5,055 sites and the GTR + G + I model was selected for all partitions except the 2nd codon of H3 gene. Among the rRNA genes, the proportions of variable and parsimony informative sites were lowest in the 18S gene (14.5 and 8.0%, respectively) and highest in the 16S gene (70.0 and 64.0%: Table 3-3). Both proportions were highest in the 3rd codon of COI gene (99.8 and 99.4%) among amino-acid coding regions. The raw-data matrix of COI sequences had 3-bp and 6-bp deletions at the

positions 98–100 (all species but *Eulimella ventricosa*), 353–355 (*Iolaea neofelixoides*), 362–364 (Cf. *Chrysallida* sp., *Megastomia tenera*, *Megastomia* sp. A, *Megastomia* sp. B, *Odetta lirata*, Pyramidellidae gen. spp. "Neji_S" and "Neji_F", *Pyrgisculus* sp., *Pyrgulina casta* and *Turriodostomia* sp.), 944–949 (all but *Quirella suprafila*), 1403–1408 (*Ondina* sp.), 1409–1414 (*Iolaea neofelixoides*, *Miralda gemma*, *Miralda scopulorum* and *Odostomia desimana*), 1418–1423 (*Pyrgolampros fulvizonata*, Pyramidellidae gen. sp. A and Pyramidellidae gen. sp. "Limpet") and 1442–1444 (all but *Megastomia tenera*, *Megastomia* sp. A, *Megastomia* sp. B and *Odetta lirata*).

Phylogenetic relationships

Bayesian and ML analyses yielded the same result in terms of clades with significant support values, and therefore only the Bayesian tree is shown with posterior probabilities (PP) and ML bootstrap percentages (BS) on branches (Fig. 3-1).

The members of the superfamily Pyramidelloidea were recovered as a robust clade (PP = 1.00, BS = 100%), while the Pyramidellidae *sensu* Bouchet & Rocroi (2005) were non-monophyletic with the Amathinidae nested within the former family (1.00, 93%). The Pyramidelloidea could be divided into five groups based on the tree topology (Groups 1–5; Fig. 3-1). Two of them, Groups 1 and 4, were reciprocally monophyletic with a meaningful support value in both Bayesian and ML trees (1.00, > 88%), while others received insufficient bootstrap and/or posterior support and only provisionally assigned to these groups for the sake of reference. The relationships among the groups were unclear except the monophyly of the Group 4 + Group 5 (1.00, 93%).

The Group 1 included species belonging to five genera of Turbonillinae, namely *Cingulina*, *Eulimella*, *Parasingulina*, *Pyrgolampros* and *Turbonilla*. Relationships within this clade were relatively well resolved: five of seven nodes

received the highest PP (1.00) and significant BS scores (>89%). The genus *Cingulina* was paraphyletic to *Parasingulina* (1.00, 100%) and *Turbonilla* potentially polyphyletic (0.99, 63%) or at least paraphyletic within the group (1.00, 89%).

The Group 2 consisted of three robust clades (each with 1.00, 100%): these include Sayellini sp. "Nukarumi" + Cf. *Aartsenia* sp., *Otopleura* + *Longchaeus* + *Orinella* + *Tiberia* + Cf. *Tiberia* sp. + Cf. *Eulimella* sp., and *Styloptygma* + *Bacteridium* + *Pyrgiscus*. Four other nodes received meaningful PP and BP values in the Group 2: *Orinella* + *Tiberia* (1.00, 89%), Cf. *Eulimella* sp. + *Orinella* + *Tiberia* (1.00, 90%), *Pyrgiscus* (1.00, 100%) and *Bacteridium* + *Pyrgiscus* (1.00, 100%).

Paramormula scrobiculata was only ambiguously included in the Group 3 as its basal-most offshoot (0.91, <50%). The remaining species of this group formed a robust clade (1.00, 98%), which contained five well-supported nodes: Cf. *Turbonilla* sp. + Pyramidellidae gen. sp. A + *Moerchinella* sp. (0.97, 90%), Pyramidellidae gen. sp. "Limpet" + Cf. *Turbonilla* sp. + Pyramidellidae gen. sp. A + *Moerchinella* sp. (1.00, 98%), *Herviera* + *Tibersyrnola* + *Syrnola* + *Paramormula* sp. cf. *speciosa* + *Colsyrnola* (1.00, 100%), *Eulimella* + Cf. *Turbonilla* sp. "Orange" (1.00, 82%), and *Herviera* + *Tibersyrnola* + *Syrnola* + *Paramormula* sp. cf. *speciosa* + *Colsyrnola* + *Eulimella* + Cf. *Turbonilla* sp. "Orange" (1.00, 77%).

The Group 4 comprised eight species, seven of which were previously classified in Amathinidae. Two clades were recognized in this group: one with three patelliform species (1.00, 98%) and the other with seven species of conical snails (1.00, 77%). Within the former clade, the Indo-West Pacific genus *Amathina* was paraphyletic to the Caribbean *Cyclothyca* (0.99, 98%). The relationships within the latter snail clade were well resolved except *Leucotina* sp. aff. *niphonensis* + Cf. *Leucotina* sp. "N295" (BS = 68%) and *Leucotina dianae* + *Monotigma* sp. + *Leucotina* sp. aff. *niphonensis* + Cf. *Leucotina* sp. "N295" (BS = 54%). The terminal position of

Monotigma renders the former members of Amathinidae (Schander, 1999a) paraphyletic within this group (1.00, 82%).

The Group 5 represented the largest clade (24 species) in the present phylogeny. Its subclades were supported mostly with significant values; these include *Sinuatodostomia* + *Hinemoa* (0.99, 79%), *Boonea* + *Ondina* + *Iolaea* (1.00, 78%), *Odostomia* + *Miralda* (1.00, 80%), Cf. *Liostomia* + Cf. *Aartsenia* (1.00, 100%), *Odetta* + *Megastomia* + *Turriodostomia* (1.00, 100%), *Quinella* + *Chrysallida* + *Pyrgulina* + *Pyrgisculus* + Pyramidellidae gen spp. "Neji_S" + "Neji_F" (0.99, 77%). *Odostomia* was found to be paraphyletic to *Miralda* (1.00, 100%); *Megastomia* was polyphyletic with *Odetta* and *Turriodostomia* branched off from it (1.00, > 99%).

Although host information is lacking for a majority of study species (Fig. 3-1), parasites of annelids were revealed as phylogenetically close to those on mollusks in a subclade of the Group 5 (*Boonea* and *Miralda* on annelids; *Iolaea* and *Odostomia* on molluks). The Group 1 also contained both annelid (*Cingulina*, *Numaegilina*) and molluscan (*Turbonilla cummingi*) parasites. On the other hand, the Group 4 seemed to exclusively contain molluscan parasites (e.g. *Amathina*, *Cyclothyca* and *Leucotina*).

The sister group of Pyramidelloidea could not be rigorously determined. Among the candidate sister taxa, a moderately supported clade of *Salinator* + *Radix* (0.99, 61%) represented the closest lineage to Pyramidelloidea, albeit with insignificant PP and BP values (0.94, < 50%).

Five Bayesian analyses based on independent gene sequences resulted in poorly resolved trees (Appendix 3). Clades with meaningful posterior probabilities (≥ 0.95) include: Pyramidellidae (in 18S, H3 and COI trees), *Cingulina* + *Parasingulina* (18S, H3 and 16S), *Bacteridium* + *Pyrgiscus* (H3, 16S and COI), *Longchaeus* + *Orinella* + *Otopleura* + *Tiberia* + Cf. *Tiberia* sp. + Cf. *Eulimella* sp. (H3 and 16S), amathinid snails + *Monotigma* (18S, 16S and COI), *Odetta* + *Megastomia* + *Turriodostomia* (28S, H3, 16S and COI). There were a few contradictory clades with

such meaningful support values in the independent gene trees. *Turbonilla cummingi* showed a very high evolutionary rate of mitochondrial genes that might have resulted in its inconsistent positions in the nuclear and mitochondrial gene trees (see Appendix 3).

Comparison with the current classification

The present molecular phylogeny refuted the familial status of Amathinidae Ponder, 1987, which was recovered as a terminal group of Pyramidellidae Gray, 1840 (Fig. 3-1). The former, younger name is therefore treated as a junior synonym of the latter name in the following lines. Monophyly was also rejected for all four subfamilies of Pyramidellidae *sensu* Bouchet & Rocroi (2005). Although the members of Turbonillinae mainly belonged to the Group 1, two genera of the same subfamily (*Bacteridium* and *Pyrgiscus*) were nested within the Group 2 and another genus *Paramormula* represented a basal lineage of the Group 3. Similarly, the subfamily Syrnolinae was polyphyletic and scattered across three groups: *Orinella*, *Styloptygma* and *Tiberia* in the Group 2 and *Colsyrnola*, *Syrnola* and *Tibersyrnola* in the Group 3. Pyramidellinae, represented herein by Sayellini sp., *Otopleura* and *Longchaeus* in the Group 2, were polyphyletic or at least paraphyletic depending on the generic assignment of unidentified species. Most study species of Odostomiinae were included in the Group 5, but *Herviera* and *Monotigma* were recovered within the Groups 3 and 4, respectively. The terminal position of *Monotigma* within the Group 4 also rendered Amathinidae non-monophyletic.

Likewise, at least five tribes (Bouchet & Rocroi, 2005) did not constitute respective monophyletic clades. The present phylogenetic reconstruction showed distant relationships among *Turbonilla*, *Pyrgiscus* and *Palamormula* (Turbonillini), among *Orinella* and *Syrnola* (Syrnolini), among *Ondina*, *Odostomia* and *Megastomia* (Odostomiini), among *Numaegilina*, *Iolaea* and *Chrysallida* (Chrysallidini), and

between *Eulimella* and *Bacteridium* (Eulimellini). The polyphyletic nature of Pyramidellini (*Otopleura* and *Longchaeus*) was also likely (PP > 0.95). The monophyly of Cingulinini (*Cingulina* and *Paracingulina*) was well supported in the Group 1 (1.00, 99%).

Ancestral state reconstruction

The reconstruction of ancestral states is shown for the shell shape and habitat in Fig. 3-2. The common ancestor of Pyramidellidae plausibly had a coiled, conical shell with a proportional likelihood value (PL) of 0.99 and inhabited the tidal flat (0.99). Transition to the patelliform shell occurred independently in the lineages leading to Pyramidellidae gen. sp. "Limpet" and the clade *Amathina* + *Cyclothyca* (Fig. 3-2A). The distribution of each habitat type was intermingled in the tree (Fig. 3-2B), indicating frequent evolutionary shifts between different bathymetric zones and types of sediment. The habitat shifts occurred mainly in the direction from the tidal flat (the ancestral condition) to other environments (derived conditions). Under the premise that ancestral species lived in a marine environment of PL > 0.5, habitat shifts to each of the rocky shore and bathyal water have occurred six or seven times. Similarly, upper subtidal and continental shelf species have multiple origins. On the other hand, a single origin was estimated for the two sunken-wood species (Fig. 3-2B).

3-4. Discussion

Phylogeny and systematics of Pyramidelloidea

The present phylogeny reveals that none of the four subfamilies of Pyramidellidae (*sensu* Bouchet & Rocroi, 2005) can be justified as a natural, monophyletic group.

Several tribes were also rejected as non-monophyletic groups. This current classification of Pyramidellidae into suprageneric taxa is based on such conchological characters as the profile of the teleoconch, surface sculpture, number of columellar teeth and shape of the protoconch (e.g. Laseron, 1959; Høisæter, 2014), but these do not seem to represent sufficient criteria to uncover deep evolutionary relationships within the family. The conditions of such shell characters may well be plastic and amendable to change in an evolutionary timescale. In this context, Wise (1996) has rightly pointed out that the protoconch configuration of pyramidellids seems to reflect more of different modes of early development, or the presence or absence of a planktotrophic larval period. Likewise, slender shells typically seen in Turbonillinae are common in the Groups 1–3 that also contain members of other subfamilies. Under the principle of parsimony, this condition of the shell shape can be regarded as plesiomorphic for the entire Pyramidelloidea (see Appendix 3). Short conical to ovate shells are therefore a derived condition that has appeared more than once in the polyphyletic Odostomiinae (e.g. *Herviera*, *Numaegilina* and *Quirella*) and also in the lineage leading to the genus *Leucotina*. Dinapoli et al. (2011) on the contrary recovered both Turboniellinae and Odostomiinae as natural monophyletic groups and thus implied that the pyramidellid shell morphology reflects ture phylogenetic relationships, i.e. the short shells evolved only once. This implication with no doubt resulted from a limited number of taxa used in their phylogeny, which lacked any of the Pyramidellinae or Syrnolinae or Amathinidae.

The terminal phylogenetic position of “Amathinidae” within the newly defined Pyramidellidae means that apomorphic conditions have been used to identify the former taxon and plesiomophic ones the latter. Amathinidae was characterized by the secondary gill situated to the left of the dorsal ciliated ridge in the mantle cavity, presence of diffuse salivary glands, and absence of the hypobranchial gland, stylet or buccal bulb (Ponder, 1987; Hori & Tsuchida, 1995; Ponder & de Keyzer, 1998b).

Some of these conditions are shared by the members of Odostomiinae (Wise, 1996), in good concordance with the unambiguous sister relationship of the Groups 4 and 5 (Fig. 3-1). Unfortunately, the only previous phylogenetic analysis with the "Amathinidae" (Wise, 1996) treated the latter as an outgroup for Pyramidellidae based on a priori justification; there, the polarity of these anatomical characters was reversed and the Odostomiinae were recovered as the basal-most group of the latter family.

The Group 4, a strongly supported clade, comprises not only these "amathinids" but also a species of *Monotygma*. This genus has been assigned in the traditional Pyramidellidae (Schander et al., 1999a), apparently based on its slender shell alone (Appendix 3). However, this shell shares inflated whorls and axial threads in rather deep spiral grooves with that of the "amathinid" genus *Leucotina* (see Hori, 2000b). The present molecular phylogeny robustly groups *Monotygma* with *L. dianae*, one of broader species in the latter genus, and thus dismisses the usefulness of height/width ratio of the shell for their generic or familial classification (see below).

The usefulness of anatomical characters for the higher classification of pyramidelloids, on the other hand, is clearly demonstrated in the present molecular analysis. The above-mentioned characteristics seen in the alimentary tract of *Amathina, Leucotina* and *Odostomia* (Hori & Tsuchida, 1995; Wise, 1996) would probably represent synapormorphies for the Groups 4 and 5. Similarly, anatomical peculiarities of *Herviera* among the former Odostomiinae as pointed out by Schander et al. (1999b) are found to be consistent with the present topology, where the genus is nested within the Group 3 instead of the Group 5 (Fig. 3-1). Other relationships shown in their phylogenetic reconstruction based on combined anatomical and conchological data (Schander et al., 1999b: fig. 10) are also largely congruent with those in the molecular tree here, except the erroneous rooting in the former reconstruction. However, the current lack of data regarding soft part morphology for more than 80% of named genera and subgenera (Peñas & Rolán, 2010) and a similarly limited number of

study taxa in the present molecular study hinder establishing a new familial and subfamilial classification of Pyramidelloidea. Further accumulation of anatomical and molecular data is badly needed to reconstruct a more complete phylogeny and to define each taxon with morphological synapomorphies.

Adaptive significance of shell forms

The slender shell, which is presumably a plesiomorphic condition for pyramidelloids, is generally considered to be suited for burrowing and crawling in sand and mud bottoms (Vermeij, 1993). This general trend for gastropods is concordant with the tidal-flat habitat herein estimated for the common ancestor of the superfamily (Fig. 3-2B). On the other hand, the relatively broad shell typically found in the Odostomiinae and *Leucotina* of Amathinidae *sensu* Bouchet & Rocroi (2005) might have been adaptive in preventing dislodgement from hard substrates by wave action with an increased size of attachment area of the foot, or simply resulted from liberation from the ecological constraint of the shell shape for burrowing, in these less mobile snails with a closer connection to the host (Ponder & de Keyzer, 1998b). The planispiral shell of *Moerchinella* may also represent an apomorphic condition (Fig. 3-1). Unfortunately, the study specimen (YK#2737) yielded only a short H3 sequence and anatomical or ecological information has not been published for the genus. Further investigation is needed to confirm the phylogenetic position of *Moerchinella* in the Group 3 and to explore its history of morphological and ecological transistions.

The patelliform shell evolved twice during the course of pyramidellid diversification (Fig. 3-2A). The habitats of the two patelliform lineages differ greatly: species of the clade *Amathina* + *Cyclothyca* permanently attach to the surface of large bivalve shells, while Pyramidellidae gen. sp. “Limpet” lives inside annelid tubes (Table 3-1). The limpet shell of the former clade is probably an adaptive consequence of the

requirement of stronger attachment to the host by a very large foot, as previously suggested for multiple lineages of rocky-shore limpets and parasitic snails (Vermeij, 1993; see also Chapter 2). The entirely different adaptive significance for the latter species seems to rest upon a very flat body that allows the limpet to crawl inside the annelid tube without preventing the move of the host. A similar consequence of adaptation is found in the concave shell of the calyptraeid limpet *Ergaea walshi*. This limpet attaches exclusively to the interior of the shells that are occupied by hermit crabs (Okutani, 2000b).

Interestingly, the two open-coiled species Pyramidellidae gen. spp. "Neji_S" and "Neji_F" also live in tubular voids—empty tunnels made by isopods and boring bivalves—in deep-sea sunken wood. An open-coiling shell can attains a more slender form than a normally coiled shell by retaining the same volume of the soft part and has been acquired in *Nozeba* (Caenogastropoda: Iravadiidae), an entirely different lineage of sunken-wood snails (see Warén, 2011). These fundamentally dissimilar shells of the unnamed pyramidellid species ("Limpet" and "Neji_S and F") show intricate and different adaptive response by gastropods to similar environmental conditions.

Diversification mechanisms in pyramidellid gastropods

The present phylogenetic reconstruction reveals that closely related species of Pyramidellidae (s.l.) tend to occupy different marine environments (Fig. 3-2B). This suggests pyramidellid diversification via frequent shifts between habitats with different abiotic conditions, which probably also accompanies host switching. In this context, Emelianov (2007) argues that species richness of parasites is proportional to available opportunities for adaptive diversification. Annelids and mollusks can indeed offer numerous colonization niches to pyramidellids by inhabiting various marine habitats and by representing extraordinary high numbers of species (Chapman, 2009).

Although many species of Pyramidellidae apparently exhibit low host specificity (Ponder & de Keyzer, 1998b), which may decrease the diversity of accessible niches for colonization, they probably have achieved the great diversification through frequent shifts among different environments while often retaining dependence to a particular lineage of hosts, ranging from a single species to various taxa in a phylum (Ponder & de Keyzer, 1998b; Hori, 2000b). Multiple occasions of interphylum host switching are clearly seen in the present reconstruction where annelid and molluscan parasites did not constitute reciprocal monophyletic groups (Fig. 3-1). However, such host switches seem to have been less frequent events than habitat shifts, as exemplified by the apparently bivalve-specific Group 3. The planktonic larval phase in many pyramidellid species perhaps encourage them to colonize new environments more frequently than to adapt to new host groups with different morphological and physiological characteristics.

The reconstructed shallow-water origin of pyramidellid gastropods is congruent with the freshwater to estuarine habitats of the possible sister taxa, including the Glacdorboidea, Lymnaeoidea and Amphiboloidea (Goulding et al., 2007; Strong et al., 2008; Dinapoli et al., 2011). As noted above, the phylogenetic position of the Pyramidelloidea remains unclear relative to these superfamilies in Panpulmonata (Fig. 3-1). Regardless, Dinapoli et al. (2011) proposed that the adaptive radiation of Pyramidellidae might have been triggered by the switch of feeding ecology from herbivorous to carnivorous. The Glacidorbidae feed on the tissue of wounded invertebrates (Ponder, 1986), while amphiboloids are deposit feeders and lymnaeoids are omnivores with a preference for animal food (Bovbjerg, 1968; Roach & Lim, 2000). The feeding modes of these related lineages, which are at least not herbivorous, therefore suggest that the common ancestor of the Pyramidelloidea and its sister taxon already had a (predominantly) carnivorous diet. The much fewer numbers of known species in Glacidorboidea (ca. 20), Lymnaeoidea (180) and Amphiboloidea (15;

Goulding et al., 2007; Strong et al., 2008) than in the remarkably species-rich Pyramidelloidea corroborate the idea that the acquisition of the parasitic lifestyle and the succeeding colonization of various marine environments are more important agents of diversification in the latter group.

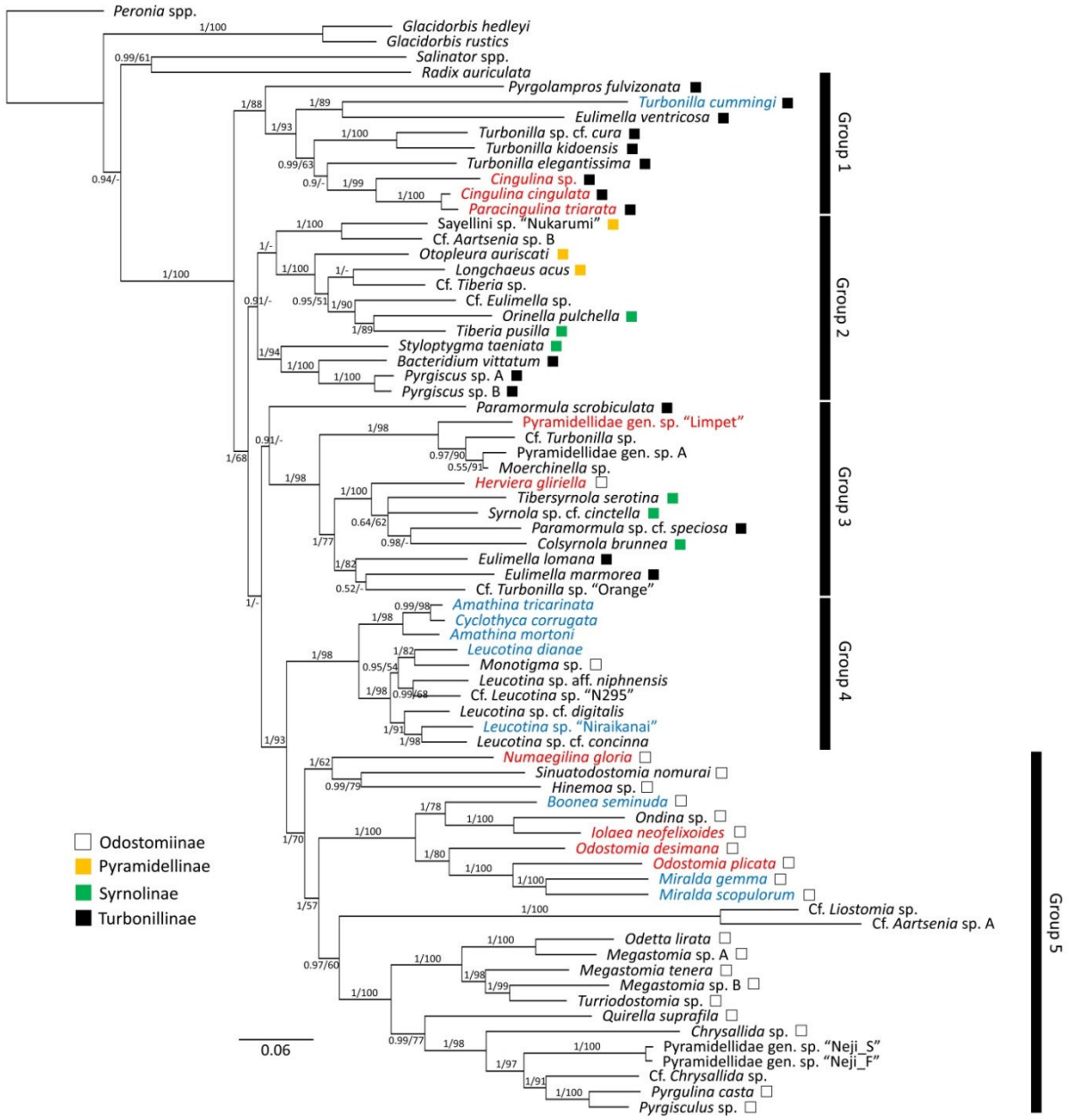

Figure 3-1. Bayesian phylogeny of Pyramidelloidea inferred from concatenated sequences of the nuclear 18S, 28S and H3 and mitochondrial COI, trnV and 16S genes (5,055 sites in total). Pyramidelloidea could be divided into five groups based on the tree topology. Numerals on branches denote posterior probabilities (PP, left) and likelihood-based bootstrap values shown as percentages (BS, right). Colors of OTU names represent their host information: Mollusca (Blue), Annelida (Red) or unknown (Black). Squares near OTU names show subfamilial positions in Bouchet & Rocroi (2005).

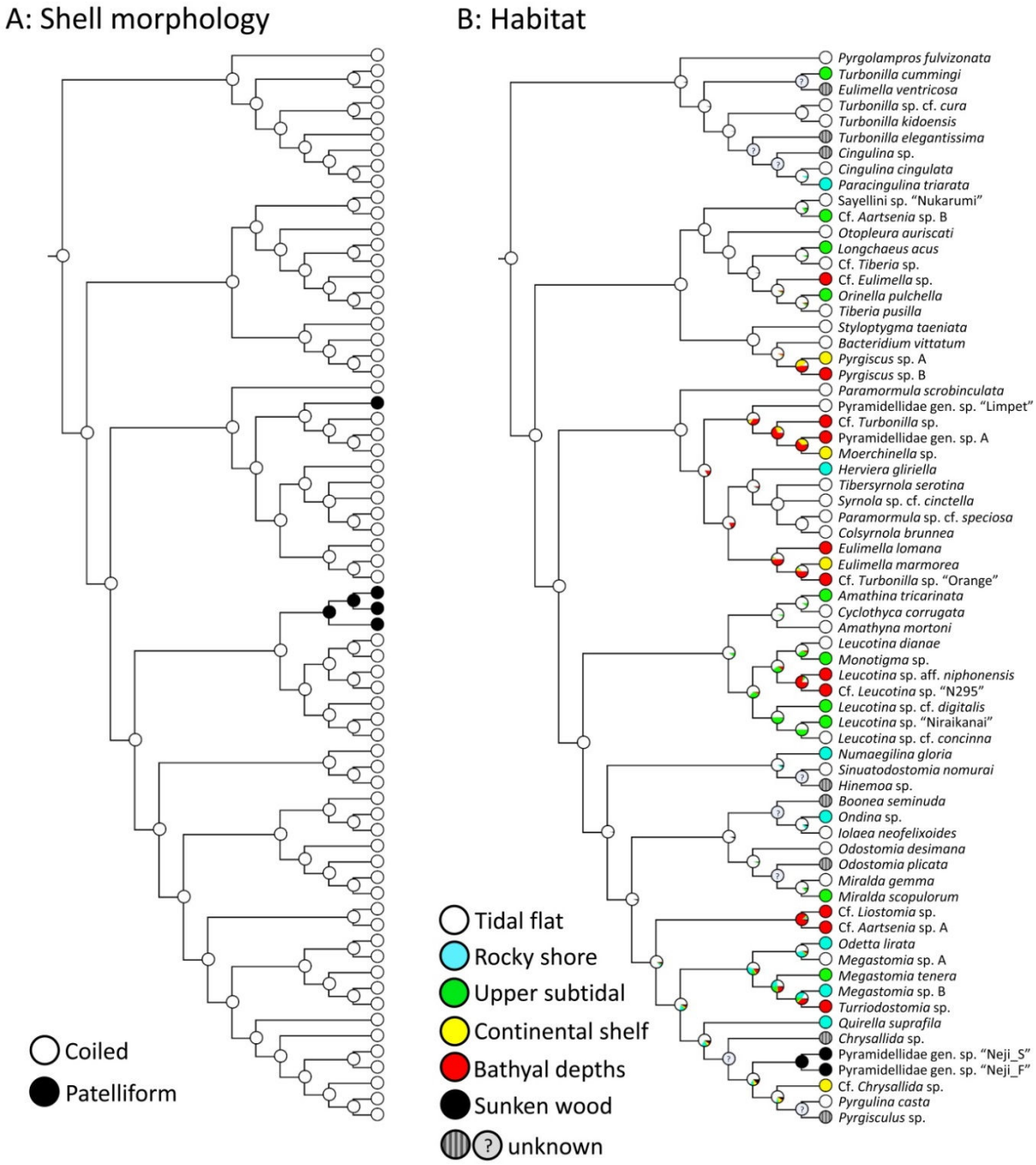

Figure 3-2. Ancestral state reconstruction for the shell shape (A) and habitat (B) of the Pyramidelloidea. Tree topology was inferred from Bayesian analysis of concatenated five-segment dataset. Pie charts at nodes indicate proportion of each state. Patelliform shape has evolved twice and habitat shifts occurred mainly in the direction from the tidal flat to other environments.

Table 3-1. Species names, coordinates, localities and habitats of specimens used for the phylogenetic reconstruction of Pyramidelloidea.

Species	DNA	Coordinates	Locality; habitat and depth
Pyramidellidae			
Pyramidellinae			
Pyramidellini			
Longchaeus acus	YK#875	34°49'N, 138°46'E	Arari, Izu, Shizuoka, Japan; 8–10 m
Otopleura auriscati	YK#876	24°24'N, 140°51'E	Nagura, Ishigaki Is., Okinawa, Japan
Sayellini			
Sayellini sp. "Nukarumi"	YK#835[*1]	41°17'N, 141°11'E	Minatomachi, Mutsu, Aomori, Japan; tidal flat
Odostomiinae			
Chrysallidini			
Iolaea neofelixoides	YK#820	32°31'N, 130°25'E	Aitsu, Amakusa, Kumamoto, Japan; tidal flat, on Terebellidae sp.
Miralda scopulorum	YK#2742	27°05'N, 142°12'E	Sakaiura, Chichijima Is., Ogasawara, Japan; 3 m, on *Conus lividus*
Miralda gemma	YK#957	31°14'N, 130°39'E	Ibusuki, Kagoshima, Japan; tidal flat
Monotygma sp.	YK#2732	9°42'N, 123°51'E	Dakabayan, Bohol Is., Philippines (PANGLAO 2004, Stn. T19); 10–26 m
Numaegilina gloria	YK#818	32°30'N, 131°43'E	Kashi, Akamizu, Nobeoka, Miyazaki, Japan; intertidal rocky shore
Pyrgulina casta	YK#895	32°31'N, 130°25'E	Aitsu, Amakusa, Kumamoto, Japan; intertidal sand bottom
Quirella suprafila	YK#907	31°44'N, 131°28'E	Nojima, Miyazaki, Japan; intertidal rocky shore
Odostomellini			
Herviera gliriella	YK#947	31°18'N, 130°12'E	Marukihama, Bonotsu, Kagoshima, Japan; intertidal
Odostomiini			
Megastomia tenera	YK#874	35°09'N, 139°35'E	Off Misaki, Miura, Kanagawa, Japan (R/V Rinkai, Stn. 1); subtidal

Megastomia sp. A	YK#911	31°14’N, 130°39’E	Ibusuki, Kagoshima, Japan; tidal flat
Megastomia sp. B	YK#910	32°30’N, 131°43’E	Kashi, Akamizu, Nobeoka, Miyazaki, Japan; intertidal rocky shore
Odetta lirata	YK#908	32°30’N, 131°43’E	Kashi, Akamizu, Nobeoka, Miyazaki, Japan; intertidal rocky shore
Odostomia desimana	YK#894	32°35’N, 130°23’E	Nogama Is. Amakusa, Kumamoto, Japan; intertidal seagrass bed
Ondina sp.	YK#809	31°44’N, 131°28’E	Nojima, Miyazaki, Japan; intertidal rocky shore
Sinuatodostomia nomurai	YK#873	32°31’N, 130°25’E	Aitsu, Amakusa, Kumamoto, Japan; tidal flat
Turriodostomia sp.	YK#1203	32°14’N, 129°27’E	SW of Nagasaki, Japan (T/V Nagasaki-maru N275, Stn. B); 420 m
Syrnolinae			
Syrnolini			
Colsyrnola brunnea	YK#899	31°14’N, 130°39’E	Ibusuki, Kagoshima, Japan; tidal flat
Orinella pulchella	YK#871	34°59’N, 139°49’E	Kouyatsu, Tateyama, Chiba, Japan (R/V Seaster); seagrass bed, 5–16 m
Styloptygma taeniata	YK#953	32°05’N, 130°31’E	Wakimoto, Akune, Kumamoto, Japan; tidal flat
Syrnola sp. cf. *cinctella*	YK#903	31°14’N, 130°39’E	Ibusuki, Kagoshima, Japan; tidal flat
Tibersyrnola serotina	YK#952	32°05’N, 130°31’E	Wakimoto, Akune, Kumamoto, Japan; tidal flat
Tiberiini			
Tiberia pusilla	YK#954	32°05’N, 130°31’E	Wakimoto, Akune, Kumamoto, Japan; tidal flat
Turbonillinae			
Cingulinini			
Cingulina cingulata	YK#816	32°30’N, 131°41’E	Totoro, Nobeoka, Miyazaki, Japan; tidal flat
Paracingulina triarata	YK#956	35°15’N, 139°35’E	Hayama, Kanagawa, Japan; intertidal rocky shore
Eulimellini			
Bacteridium vittatum	YK#893	32°35’N, 130°23’E	Nogama Is. Amakusa, Kumamoto, Japan; intertidal seagrass bed
Eulimella lomana	YK#2052	27°35’N, 111°28’W	Sonora Margin, Gulf of California, Mexico (Nautile Dive 1760, Stn. BIG14, ASPI3); 1561 m, cold seep

Eulimella marmorea	YK#2741	37º31'N, 141º30'E	Off Namie, Fukushima, Japan (R/V Shinsei-maru KS-14-06, Stn. NO1); 143 m
Turbonillini			
Paramormula scrobiculata	YK#950	32º05'N, 130º31'E	Wakimoto, Akune, Kumamoto, Japan; tidal flat
Paramormula sp. cf. *speciosa*	YK#897	32º35'N, 130º23'E	Nogama Is. Amakusa, Kumamoto, Japan; intertidal seagrass bed
Pyrgiscus sp. A	YK#870	35º00'N, 139º48'E	NW of Okinoshima, Tateyama, Chiba, Japan; 80 m
Pyrgiscus sp. B	YK#1202	32º13'N, 128º58'E	Off Fukue Is., Nagasaki, Japan (T/V Nagasaki-maru N275, Stn. O-1); 392 m
Pyrgolampros fulvizonata	YK#900	31º14'N, 130º39'E	Ibusuki, Kagoshima, Japan; tidal flat
Turbonilla cummingi	YK#2747	27º05'N, 142º12'E	Sakaiura, Chichijima Is., Ogasawara, Japan; 1 m, on *Tridacna maxima*
Turbonilla kidoensis	YK#951	32º05'N, 130º31'E	Wakimoto, Akune, Kumamoto, Japan; tidal flat
Turbonilla sp. cf. *cura*	YK#872	32º31'N, 130º25'E	Aitsu, Amakusa, Kumamoto, Japan; intertidal
Subfamily undetermined			
Cf. *Aartsenia* sp. A	YK#2751	42º11'N, 144º11'E	Off Tokachi, Hokkaido, Japan (R/V Tansei-maru KT-12-18, Stn. H3); 1210–1248 m
Cf. *Aartsenia* sp. B	YK#948	35º01'N, 139º50'E	Off Nakohunakata, Tateyama, Chiba, Japan; 12–20 m
Cf. *Chrysallida* sp.	YK#2749	37º00'N, 141º05'E	Off Iwaki, Fukushima, Japan (R/V Shinsei-maru KS-14-01, Stn. 1); 96–101 m
Cf. *Eulimella* sp.	YK#1819	28º33'N, 127º02'E	W of Amami Is., Kagoshima, Japan (T/V Nagasaki-maru N342, Stn. J6-6); 634–635 m
Cf. *Liostomia* sp.	YK#2750	40º58'N, 141º46'E	Off Hachinohe, Aomori, Japan (R/V Tansei-maru KT-12-18, Stn. A1); 498–459 m
Moerchinella sp.	YK#2737	9º36'N, 123º45'E	N of Panglao Is., Philippines (PANGLAO 2004, Stn. P1); 90–200 m
Cf. *Tiberia* sp.	YK#2739	15º29'S, 167º16'E	Palikulo Pen., Espiritu Santo Is., Vanuatu (SANTO 2006, Stn. VM12); intertidal soft bottom
Cf. *Turbonilla* sp. "Orange"	YK#2761	28º33'N, 127º02'E	W of Amami Is., Kagoshima, Japan (T/V Nagasaki-maru N319, Stn. J6-1); 605–611 m
Cf. *Turbonilla* sp.	YK#2735[*2]	8º53'S, 159º23'E	New Georgia Sound, Salomon Isles. (N.O. "Alis", Campagne SALOMONBOA 3, Stn. CP2783); 1501–1545 m
Pyramidellidae gen. sp. A	YK#2734	15º27'N, 121º36'E	Off Dingalan, Luzon Is., Philippines (N.O. "DA-BFAR", Campagne AURORA 2007, Stn. CP2732); 556 m
Pyramidellidae gen. sp. "Limpet"	YK#2743[*3]	25º28'S, 44º58'E	Ambatobe, Bavarama, Madagascar (Expédition ATIMO VATAE, Stn. BM06); 0–1 m
Pyramidellidae gen. sp. "Neji_S"	YK#1279	33º00'N, 133º02'E	Off Irino, Tosa, Kochi, Japan; 70–110 m, on sunken wood
Pyramidellidae gen. sp. "Neji_F"	YK#1276	34.5ºN, 137.2ºE	Off Atsumi Pen., Aichi, Japan; 150–200 m, on sunken wood

"Amathinidae"			
Amathina mortoni	YK#2740	8º11'N, 98º19'E	Dan Yit, Phuket Is., Thailand; mangrove swamp, on Ostreidae sp.
Amathina tricarinata	YK#811	33º42'N, 134º31'E	Minami, Kaifu, Tokushima, Japan; pearl farm, on *Pinctada fucata martensii*
Cyclothyca corrugata	YK#2745	16º13'N, 61º32'W	S of Silos, Guadeloupe (Campagne KARUBENTHOS 2012, Stn. GM01); intertidal, on *Isognomon* sp.
Leucotina dianae	YK#810[*4]	34º22'N, 133º07'E	Hosonosu, Mihara, Hiroshima, Japan; intertidal seagrass bed, on *Pinna bicolor*
Leucotina sp. cf. *concinna*	YK#1278	38º24'N, 141º24'E	Mangokuura, Miyagi, Japan; tidal flat
Leucotina sp. cf. *digitalis*	YK#2738	15º30'S, 46º06'E	Off Majunga, Madagascar (Campagne MIRIKY, Stn. CP3272); 22–27 m
Leucotina sp. aff. *"niphonensis"*	YK#2736	15º58'N, 121º49'E	Off Dipaculao, Luzon Is., Philippines (N.O. "DA-BFAR", Campagne AURORA 2007, Stn. CP2658); 422–431 m
Leucotina sp. "Niraikanai"	YK#808	26º19'N, 127º51'E	Awase, Okinawa Is., Japan; subtidal sand bottom, on *Glycymeris reevei*
Cf. *Leucotina* sp. "N295"	YK#2748	28º32'N, 126º58'E	Off Amami Is., Kagoshima, Japan (T/V Nagasaki-maru N295, Stn. R-1); 343–345 m
(Outgroup taxon)			
Glacidorbidae			
Glacidorbis hedleyi	YK#2746	33º53'S, 150º02'E	Boyd River Crossing, Kanangra Road, NSW, Australia

[*1]Okayama University, Japan OKCAB M17271; [*2]Muséum National d'Histoire Naturelle, Paris (MNHN) IM-2009-7067; [*3]MNHN IM-2009-10889; [*4]OKCAB M10892.

Table 3-2. DDBJ/EMBL/GenBank accession numbers of published sequences used for the phylogenetic reconstruction of Pyramidelloidea.

Species	18S	28S	H3	16S–COI
Pyramidellidae				
Boonea seminuda	AY145367	AY145395	-	AF355163
Cingulina sp.	GU331940	GU331930	-	GU331959, GU331950
Chrysallida sp.	GU331935	GU331925	-	GU331945
Eulimella ventricosa	FJ917213	FJ917235	-	FJ917274, FJ917255
Hinemoa sp.	GU331936	GU331926	-	GU331955, GU331946
Odostomia plicata	GU331938	GU331928	-	GU331957, GU331948
Pyrgisculus sp.	GU331939	GU331929	-	GU331958, GU331949
Turbonilla elegantissima	GU331941	GU331931	-	GU331960, GU331951
(Outgroup taxa)				
Glacidorbidae				
Glacidorbis rusticus	FJ917211	FJ917227	-	FJ917284, FJ917264
Amphibolidae				
Salinator spp.	HQ659937	JQ228464	DQ093510	JN620539
Lymnaeidae				
Radix auricularia	FR797818	AY465067	-	KP098540
Onchidiidae				
Peronia spp.	HQ659975	GU252157	-	JN619346

Table 3-3. Summary of sequences for pyramidelloid phylogeny (shown for each partition).

Partition	Substitution model	Final length	Variable sites	Parsimony informative
18S	GTR + G + I	1778	257	142
28S	GTR + G + I	895	337	208
H3 1st codon	GTR + G + I	109	20	17
H3 2nd codon	JC	109	2	2
H3 3rd codon	GTR + G + I	110	104	97
trnV + 16S	GTR + G + I	589	412	377
COI 1st codon	GTR + G + I	488	261	232
COI 2nd codon	GTR + G + I	488	171	140
COI 3rd codon	GTR + G + I	489	488	486

General Discussion

This thesis is devoted to elucidate and compare the evolutionary histories of the two most speciose groups of parasitic gastropods, Eulimidae and Pyramidellidae. Both groups comprise thousands of species (Table I; Warén & Gittenberger, 1993; Schander et al., 1999a), while at the macroevolutionary level, their diversification processes differed: recurrent specialization to the permanent parasitic lifestyle has enhanced the diversification in the Eulimidae (Chapter 2), and frequent habitat shifts among disjunct marine environments have contributed to the species richness of the Pyramidellidae (Chapter 3).

Contrasting driving forces behind the eulimid and pyramidellid diversifications

By combining comprehensive molecular phylogenetic reconstruction, morphometric analyses of the shell and examination of fossil records, the present study first reveals that the Eulimidae have diversified through repeated adaptive radiation, which involves parallel specialization—including endoparasitism—throughout the evolutionary history of the family since the Late Cretaceous. This dynamic diversification process in eulimid gastropods suggests that, as a general rule, the parallel transition of parasitic strategies and morphological modification can occur within several close lineages and contribute substantially to high species richness in a relatively short geologically timescale. In terms of the large number of parallel specialization events with reference to the timescale, the present study on eulimid diversification provides perhaps the most complete and dynamic picture of parasite evolution.

The diversification of the parasitic lice (Arthropoda: Phthiraptera), which are comprised of more than 4,000 species that inhabit only on land and have a simple life cycle, involves a similar process (Johnson et al., 2012). The class Copepoda

(Arthropoda: Crustacea) is a group comparable to eulimids in having a series of adaptive stages to the parasitic mode of life (Combes, 2005), which potentially involve multiple pathways of specialization. Although there exist studies on the host switching and morphological diversification of copepods (e.g. Huys et al., 2006, 2007; Anton & Schrödl, 2013), previous phylogenetic studies have mostly focused on their systematics (e.g. Bucklin et al., 2003; Huys et al., 2006; Song et al., 2008) and how many specialization processes had occurred independently has not been investigated for the entire radiation of the class. Revealing the evolutionary history of parasitic copepods and comparing it with the herein elucidated eulimid evolution will contribute greatly towards understanding not only the general diversification process of parasites but also organisms' adaptation to a new environment.

In constrast, the diversification pattern of the Pyramidellidae (including the former Amathinidae; see Chapter 3) may support the prediction that species richness in parasites is proportional to the diversity of colonization niches provided from the host group (Emelianov, 2007). The high diversity of accessible niches may also have resulted in the large numbers of species in other parasitic animals such as nematodes, flatworms, and mites (see General Introduction). According to the prediction, species richness increases when each parasitic species inhabits a specific host or infection site. In theory, high host specificity has been considered an important factor that promotes speciation at the microevolutionary level (Futuyma & Moreno, 1988), although Šimková et al. (2006) indicated that strict specificity does not promote diversification in the Monogenea (Platyhelminthes). The Pyramidellidae also have achieved great diversification despite their low host specificity (Ponder & de Keyzer, 1998b) that may decrease accessible niche diversity. The present study supports the Šimková et al.'s outcome that host specificity does not always affect parasite diversification and contributes to elucidate the diversification pattern of generalists.

Common drivers and shared ancestral conditions

In the microevolutionary process of parasitic organisms, factors such as small body size, small effective population size, high host specificity, aggregated distribution among hosts, short generation time of parasites, and the low mobility of hosts are considered to enhance the fragmentation of populations followed by increasing genetic structure and ultimately speciation (Huyse et al., 2005; Emelianov, 2007). The Eulimidae and Pyramidellidae fulfill some of the presumed requirements such as exploiting host groups with low locomotion ability (echinoderms, annelids, and mollusks) and their small shell size (many species do not attain 10 mm in shell length; see Hori, 2000a, 2000b). In addition, in some cases, the Pyramidellidae show a highly aggregated distribution, a short generation time and a small population size (Cumming, 1993; see also Fig. I-2F). For example, *Turbonilla cummingi* has a generation time of approximately 72 days (Cumming & Alford, 1994). Although our knowledge on the life history of eulimids is more limited, reported cases indicate longer generation times: 15 months in *Parvioris* spp. (Morton, 1979) and more than 20 months in *Hypermastus tokunagai* (Matsuda et al., 2013). Additional ecological studies will be most beneficial to further illustrate the driving force behind the increased diversification of parasitic gastropods.

Although the diversification pathways of the two gastropod families are different, they probably had similar ancestral conditions that allowed the acquisition of parasitism. Poulin (2007) suggested three typical origins of the parasitic mode of life: specialized parasites can evolve from (1) opportunistic foragers that are capable of remaining on the host for certain periods of time, (2) phoretic organisms, which use other organisms for transport or shelter, and (3) prey of larger animals. The temporary parasitic species as the plausible ancestral condition for the two groups suggests that they evolved from opportunistic foragers, which is also supported by the carnivorous or

animal-oriented omnivorous mode of feeding in their related families (see Chapters 1 and 3). The evolution of parasitic species from a carnivorous opportunistic visitor is also probable in such other lineages as isopods and flat worms. Isopods that parasitize fishes and crustaceans (e.g., Corallanidae, Cymothoidae and Bopyridae) collectively constitute a clade sister to the predatory and scavenging family Cirolanidae (Dreyer & Wägele, 2001). Among the Platyhelminthes, the Turbellaria that are paraphyletic to a clade of exclusive parasitic flatworms (Neodermata) are largely carnivorous and several turbellarian lineages exhibit close association with other animals (Littlewood et al., 2001).

Eulimids and pyramidellids share not only similar ancestral conditions but also a common evolutionary trend where permanent parasites are derived by specialization from temporary parasites (Fig. 2-5B). The above-mentioned isopods show a similar trend; the basal Corallanidae and Aegidae are temporary parasites of fishes, while terminal groups, Cymothoidae and Bopyridae, bear reduced feet and permanently attach to fishes and crustaceans, respectively (Dreyer & Wägele, 2001). Interestingly, the evolutionary direction of sexual strategies that protandrous hermaphroditic species have derived from gonochoristic ancestors is probably also shared by isopods and eulimid gastropods (Warén, 1984; Dreyer & Wägele, 2001). Acanthocephalans and parasitic flatworms represent two more examples of similar evolutionary pathways. The endoparasitic lifestyle of acanthocephalans presumably evolved from the epizoic mode of life in the Seisonidea, a class of the potentially paraphyletic phylum Rotifera (Herlyn et al., 2003). Similarly, endoparasitic flatworms seem to have originated more than ones from ectoparasitic ancestors (Euzet & Combes, 1998; Park et al., 2007). Furthermore, parasitic plants belonging to the family Orobanchaceae (euasterids I: Lamiales) are plesiomorphically facultative hemiparasites (e.g. *Triphysaria*), while derived ones include obligate hemiparasites (*Striga*) and holoparasites with no ability of photosynthesis (*Orobanche*; Westwood et al., 2010).

Why pyramidellids have not specialized to give rise endoparasites?

The similarities in the evolutionary patterns prompt the question as to why endoparasitism evolved in the Eulimidae but not in the Pyramidellidae. In addition, permanent ectoparasites are common in the former family and rare in the latter (restricted to a single subclade or the former Amathinidae). Possible causes of these differences can be hypothesized that (1) the segmented or stiff body of hosts prevents endoparasites from becoming established on/in the body wall and deeper tissues, (2) parasite snails cannot move efficiently and fast enough, or hold their body, on the mucous-rich skin of host mollusks, (3) shell and tube of molluscan and annelid hosts provide them a further protection from parasites, (4) permanent parasites on/in the host are not meaningfully more adaptive than their ancestral, temporary parasites, and/or (5) competition with other organisms is too intense to establish a close interaction as parmanent parasites.

The first hypothesis is connected to the body plans of host annelids and mollusks as well as those of parasitic gastropods. Compared to most echinoderms, polychaete worms have smaller and more slender bodies that are separated by septa into a number of segments (Glasby et al., 2000). The body of polychaetes may therefore not have adequate space for endoparasitic snails or other animals (but see Suárez-Morales et al., 2014 for an exceptional case of the copepod parasite *Monstrilla*). Polychaetes, and mollusks as well, also generally lack or have only a reduced internal body space or the coelom under the body wall. The absence of endoparasitic pyramidellids might be attributable to such a lack of space inside the host body.

Meanwhile, large clams and snails are known to have pea crabs, copepods and flat worms in their mantle cavity (e.g. Haines et al., 1994; Littlewood et al., 1999) that can potentially provide sufficient space also for pyramidellids. It seems that, however,

the inability of snails to move efficiently and fast enough against the attempt of the host to clear its mantle cavity is probably the most reasonable explanation for the total absence of gastropods, not only the Pyramidellidae but also other groups, as the endoparasite of mollusks (Warén, 1984; Lorenz, 2005). The gastropod foot is functional in principal by the movement of cilia on the sole of the foot (Fretter & Graham, 1994). This largely differs from the multiple, segmented and pointed feet of arthropods, which seem to allow crabs and copepods stay inside the mantle cavity of mollusks. Furthermore, the arthropod appendages are often modified to specialized hooks to facilitate a stable connection to the body of the host (Anton & Schrödl, 2013). Eulimids have developed their probosces not only to suck the host blood but also to firmly attach themselves to the echinoderm hosts as permanent parasites (Warén, 1984), although the pyramidellid probosces do not provide the latter function on the molluscan host. An entirely different but effective way of attachment to the host is practiced by the glochidial larvae of unionid mussels that bite into fish gills with their shell hooks (Kat, 1984).

The shell as a protector of course offers another explanation for the absence of gastropod endoparasites inside mollusks. The endoparasitic eulimid of the genus *Entocolax* bore the body tissue of holothuroids after settling on the host's surface as larvae (Heding & Mandahl-Barth, 1938; Altnöder et al., 2007). In this process, the position of settlement can probably be arbitrary with no specific site suitable for penetration. Pyramidellids, with no ability to drill the shell of host mollusks, however cannot access the edible part except from the shell margin or aperture, which is as it stand its reason protected securely from predators and other disturbance. Annelids that live in tubes must also benefit from protection against various extrinsic interferences.

The "non-adaptive" hypothesis fits comfortably into parasites on annelids. Annelids play a critical role in the marine benthic food chain as prey items for various organisms such as flatfishes, cods, mollusks, crustaceans, and other polychaetes (De

Vlas, 1979; Hutchings, 1998; Glasby et al., 2000). Parasites on polychaetes can therefore be too frequently eaten by the predators of hosts. In contrast to eulimids that are securely protected by the echinoderm hosts (see Warén, 1984), the transition to a more specialized, permanent parasitic life may thus offer little advantage to pyramidellids on polychaetes. Temporary parasites that can actively leave the host are probably more adaptive under a high probability of predation on the host, as exemplified by such eulimids as *Vitreolina* and *Eulima* (Warén, 1984). These parasites of brittle stars fall off at slight disturbance from the host, which are primal prey items of fishes (Witman & Sebens, 1992). Pyramidellids except the former amathinids are similarly sensitive to disturbance and easily detach from the host.

Lastly, the absence of endoparasitic pyramidellids might perhaps be attributable also to competition with other organisms. Endoparasitic copepods and digeneans (Platyhelminthes: Trematoda) exploit the same set of host groups as the Pyramidellidae, including annelid worms, gastropods and bivalves (Littlewood et al., 2001), and in some cases they cause a high rate of infection (Suárez-Morales et al., 2014). However, it is difficult to evaluate the effect of possible competition without sufficient knowledge on the ecology of pyramidellids or these endoparasites. To conclude, these intrinsic and extrinsic factors are probably complexly intertwined with each other, resulting in the present ecological states of the Pyramidellidae.

Fossil record of parasitic gastropods: utility and difficulty

The present reconstuction of the evolution of host-parasite interaction for eulimids (Chapter 2) is greatly benefited from the divergence time analysis using fossil records as calibration points for the estimation of the timing of their ecological transitions, morphological evolution, and species diversification. The resulting time-caribrated tree suggests that endoparasitism as well as globose shells can evolve repeatedly and

rapidly as a consequence of adaptation to predation pressure and/or dislodging by wave action. In addition, fossil species including "*Stylifer*" *pellucidus* and "*Pelseneeria*?" *senuti* from the Eocene and Miocene are plotted near the Recent, supposedly phylogenetically unrelated permanent parasites in the PCA analysis (Fig. 2-5D). This result beautifully illustrates that the repeated adaptive radiation has occurred throughout the evolutionary history of the family, since well before and more frequently than it can be traced by the ancestral state reconstruction based on phylogenetic relationships among extant species and distribution of their ecological traits (Fig. 2-5). In other words, combining information from both living and extinct taxa provide knowledge on the diversification process that remains unknown without the fossil evidence. Studies on soft-bodied parasites such as flatworms and nematodes have suffered from the lack of suitable fossils, whereas gastropod shells retain taxonomic characters for millions of years and enable inference of their phylogenetic positions thorough comparison with the Recent taxa.

The present phylogenies, however, also suggested the difficulty in using the fossil records of parasitic gastropods as time calibration points in divergence time analyses. In both families, the slender shell, which most probably represents the ancestral state, has been conserved for a long period of geological time in multiple unrelated lineages, sometimes without noticeable phenotypic changes under similar ecological constraints as temporary parasites. Furthermore, the globose shells as apomorphic conditions in eulimid evolution are also convergently very similar to each other despite their independent origins (Figs. 2-3A, 2-5C). Such morphological stasis and convergence often make fossil records difficult to interpret for dating a particular node. However, the fact remains that the fossil records of the Gastropoda can provide unmatched knowledge on the evolution of host-parasite interaction—particularly if a number of conchological characters are properly evaluated and only truly unique conditions are used to diagnose monophyletic groups.

Future perspectives

New knowledge on phylogenetic relationships contributes to substantiate and accelerate various branches of evolutionary and ecological studies as well as practical sciences. For example, previous studies have indicated that several eulimids and pyramidellids exert negative impacts on host species, some of which are important in coastal fisheries (e.g. Musashi & Habe, 1991; Cumming & Alford, 1994; Carroll & Finelli, 2015). *Pelseneeria castanea* (Eulimidae) parasitizes young individuals of edible sea urchins and may induce mortality before the infected hosts grow to adults (Kawai & Nagasawa, 2006). This observation implies that its congeners, and likely also the species of the closely related genus *Pulicicochlea*, can seriously harm their hosts including other commercially important species. Understanding the effect on the host is even more important to determine the function and importance of these snails in the food webs. However, host organisms have not been identified for many, if not most, species of Eulimidae and Pyramidellidae (see Chapters 2 and 3). In this context, recent authors have argued that parasitic species should be included in food web analyses (Byers, 2013; Lafferty, 2013), but it is not easily practicable partly due to their uncertain trophic levels as they often utilize more than one host species (Lafferty et al., 2008). The present phylogenies provide information about candidate hosts, especially in the case of eulimids. Such information can be used as the basis for further field observations and for determining the positions of parasitic snails in the biological community structure.

The knowledge of evolutionary relationships also aids in understanding the genetic basis that enables a parasitic life on/in certain host groups. Recently, next generation transcriptome sequencing technologies provide great opportunities to gain a better perspective of the molecular biology of parasitic plants (Yang et al., 2015). The Parasitic Plant Genome Project (PPGP; http://ppgp.huck.psu.edu/) has been established

to “discover the genome-wide changes that led to the establishment of the parasitic lifestyle and the changes that resulted as a consequence of adoption of the parasitic life-style.” A major focus of the project is to identify “parasitism genes” that relate to the initiation and development of the parasitic-plant specific attachment organ, called the haustorium (Yang et al., 2015). In this project, comparative transcriptome sequencing was performed for the Orobanchaceae, a plant family with the haustorium and the widest range of parasitic ecologies (Westwood et al., 2010), resulting in the identification of 1,809 candidate “parasitism genes” that were assigned to 298 orthogroups. Among parasitic gastropods, the Eulimidae show the most diverse and specialized parasitic strategies that involves the acquisition of a new organ for attachment to echinoderm hosts as endoparasites, called the pseudopallium (Warén, 1984). The comprehensive eulimid phylogeny (Chapter 2) revealed independent origins of both endoparasitism and pseudopallia (Fig. 2-5E). Genetic approaches similar to those for the parasitic plants perhaps identify core genes for specialization to the parasitic life in eulimids. Likewise, comparative transcriptome sequencing of three parasite species on/in the black sea-cucumber *Holothuria atra*, i.e. *Melanella kuronamako*, *Peasistilifer nitidula* and *Megaenus* “*atrae*”, may shed light on their host identification mechanisms. These eulimid species are phylogenetically distant from each other (Fig. 2-2), but it is predicted that genes for host recognition resemble due to convergent evolution by sharing the same host species.

Finally, this study would contribute greatly to the elucidation of the evolution of parasitism in Gastropoda. The class Gastropoda includes several other parasitic lineages such as Coralliophilinae, Epitoniidae, Marginellidae and Ovulidae (Warén, 1984; Lorenz, 2005), while phylogenetic relationships within these groups are only partly resolved (e.g. Schiaparelli et al., 2005; Barco et al., 2010; Churchill et al., 2011a). Most of them are temporary parasites or ectoparasites, while they have a relatively restricted selection of hosts: Coralliophilinae (Muricidae) mainly on reef-building

scleractinian corals, Epitoniidae on actinarians anemones, Ovulidae on alcyonacean soft corals, and Marginellidae on fishes. Further investigations on the evolutionary history of parasitic gastropod lineages, each of which exhibits different ecological and morphological conditions but unanimously benefits from the rich fossil record, would elucidate diversification of parasitic organisms in time and space. Ultimately, the long succession of evolutionary studies of parasites will aid in understanding the origin and maintenance mechanisms of biodiversity.

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References

Adams, H., Adams, A., 1853. From Eulimidae to Styliferidae. *In*: *The Genera of Recent Mollusca; Arranged According to Their Organization, Vol. 1*. van Voorst, London, pp. 235–239.

Altnöder, A., Bohn, J.M., Rückert, I.-M., Schwabe, E., 2007. The presumed shelled juvenile of the parasitic gastropod *Entocolax schiemenzii* Voigt, 1901 and its holothurian host *Chiridota pisanii* Ludwig, 1886. *Spixiana* 30: 187–199.

Anton, R.F., Schrödl, M., 2013. The gastropod-crustacean connection: towards the phylogeny and evolution of the parasitic copepod family Splanchnotrophidae. *Zoological Journal of the Linnean Society* **167**: 501–530.

Baer, J.D., 1952. *Ecology of Animal Parasites*. University of Illinois Press, Urbana.

De Baets, K., Littlewood, D.T.J., 2015. The importance of fossils in understanding the evolution of parasites and their vectors. *Advances in Parasitology* **90**: 1–49.

Barco, A., Claremont, M., Reid, D.G., Houart, R., Bouchet, P., Williams, S.T., Cruaud, C., Couloux, A., Oliverio, M., 2010. A molecular phylogenetic framework for the Muricidae, a diverse family of carnivorous gastropods. *Molecular Phylogenetics and Evolution* **56**: 1025–1039.

Bieler, R., 1988. Phylogenetic relationships in the gastropod family Architectonicidae, with notes on the family Mathildidae (Allogastropoda). *Malacological Review, Supplement* **4**: 205–240.

Blaxter, M.L., de Ley, P., Garey, J.R., Liu, L.X., Scheldeman, P., Vierstraete, A., Vanfleteren, J.R., Mackey, L.Y., Dorris, M., Frisse, L.M., Vida, J.T., Thomas, W.K., 1998. A molecular evolutionary framework for the phylum Nematoda. *Nature* **392**: 71–75.

Bouchet, P., Warén, A., 1986. Revision of the northeast Atlantic bathyal and abyssal Aclididae, Eulimidae, Epitoniidae (Mollusca, Gastropoda). *Bollettino Malacologico, Supplement* **2**: 299–576.

Bouchet, P., Le Renard, J., 1998. Family Pickworthiidae, *In*: Beesley, P.L., Ross, G.J.B., Wells, A. (Eds.), *Fauna of Australia, Vol. 5, Mollusca: The Southern Synthesis*. CSIRO Publishing, Melbourne, pp. 739–741.

Bouchet, P., Lozouet, P., Maestrati, P., Héros, 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.

Bouchet, P., Rocroi, J.P., 2005. Classification and nomenclator of Gastropod families. *Malacologia* **47**: 1–397.

Bovbjerg, R.V., 1968. Responses to food in lymnaeid snails. *Physiological Zoology* **41**: 412–423.

Brooks, D.R., Ó Grady R.T., Glen, D.R., 1985. Phylogenetic analysis of the Digenea (Platyhelminthes: Cercomeria) with comments on their adaptive radiation. *Canadian Journal of Zoology* **63**: 411–443.

Bucklin, A., Frost, B.W., Bradford-Grieve, J., Allen, L.D., Copley, N.J., 2003. Molecular systematic and phylogenetic assessment of 34 calanoid copepod species of the Calanidae and Clausocalanidae. *Marine Biology* **142**: 333–343.

Buckling, A., Rainey, P.B., 2002. The role of parasites in sympatric and allopatric host diversification. *Nature* **420**: 496–499.

Byers, J.E., 2008. Including parasites in food webs. *Trends in Parasitology* **25**: 55–57

Carpentieri, P., Serpetti, N., Colloca, F., Criscoli, A., Ardizzone, G., 2015. Food preferences and rhythms of feeding activity of two co-existing demersal fish, the longspine snipefish, *Macroramphosus scolopax* (Linnaeus, 1758) and the boarfish

Capros aper (Linnaeus, 1758), on the Medditerranean deep shelf. *Marine Ecology* **2015**: 1–13.

Carroll, J.M., Finelli, C.M., 2015. Impacts of the ectoparasitic snail *Boonea impressa* on growth of postset juvenile oysters. *Journal of Molluscan Studies* **81**: 161–163.

Castresana, J., 2000. Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. *Molecular Biology and Evolution* **17**: 540–552.

Chapman, A.D., 2009. *Numbers of Living Species in Australia and the World, 2nd edition*. Australian Biological Resource Study, Canberra.

Chiba, S., 2004. Ecological and morphological patterns in communities of land snails of the genus *Mandarina* from the Bonin Islands. *Journal of Evolutionary Biology* **17**: 131–143.

Churchill, C.K.C., Ó Foighil, D., Strong, E.E., Gittenberger, A., 2011a. Females floated first in bubble-rafting snails. *Current Biology* **21**: 802–803.

Churchill, C.K.C., Strong, E.E., Ó Foighil, D., 2011b. Hitchhiking juveniles in the rare neustonic gastropod *Recluzia* cf. *jehennei* (Janthinidae). *Journal of Molluscan Studies* **77**: 441–444.

Colgan, D.J., Ponder, W.F., Eggler, P.E., 2000. Gastropod evolutionary rates and phylogenetic relationships assessed using partial 28S rDNA and histone H3 sequences. *Zoologica Scripta* **29**: 29–63.

Colgan, D.J., Ponder, W.F., Beacham, E., Macaranas, J., 2007. Molecular phylogenetics of Caenogastropoda (Gastropoda: Mollusca). *Molecular Phylogenetics and Evolution* **42**: 717–737.

Combes, C., 2005. *The Art of Being a Parasite*. The University of Chicago Press, Chicago and London.

Collin, R., 2003. Phylogenetic relationships among calyptraeid gastropods and their implications for the biogeography of marine speciation. *Systematic Biology* **52**: 618–640.

Cossmann, M., Pissarro, G., 1904–06. *Iconographie Complète des Coquilles Fossiles de l'Éocène des Environs de Paris*. H. Bouillant, Paris.

Cossmann, M., Peyrot, A., 1917–18. Conchologie néogénique de l'Aquitanie. *Actes de la Société Linnéenne de Bordeaux* **70**: 1–491.

Coyne, J.A., Orr, H.A., 2004. *Speciation*. Sinaure Associates, Sunderland.

Criscione, F., Ponder, W.F., 2013. A phylogenetic analysis of rissooidean and cingulopsoidean families (Gastropoda: Caenogastropoda). *Molecular Phylogenetics and Evolution* **66**: 1075–1082.

Cumming, R.L., 1993. Reproduction and variable larval development of an ectoparasitic snail, *Turbonilla* sp. (Pyramidellidae, Opisthobranchia), on cultured giant clams. *Bulletin of Marine Science* **52**: 760–771.

Cumming, R.L., Alford, R.A., 1994. Population dynamics of *Turbonilla* sp. (Pyramidellidae, Opisthobranchia), an ectoparasite of giant clams in mariculture. *Journal of Experimental Marine Biology and Ecology* **183**: 91–111.

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

Dayrat, B., Tillier, A., Lecointre, G., Tillier, S., 2001. New clades of euthyneuran gastropods (Mollusca) from 28S rRNA sequences. *Molecular Phylogenetics and Evolution* **19**: 225–235.

Dayrat, B., Conrad, M., Balayan, S., White, T.R., Albrecht, C., Golding, R., Gomes, S.R., Harasewych, M.G., de Frias Martins, A.M., 2011. Phylogenetic relationships and evolution of pulmonate gastropods (Mollusca): new insights from increased taxon sampling. *Molecular Phylogenetics and Evolution* **59**: 425–437.

Dgebuadze, P.Y., Fedosov, A.E., Kantor Y.I., 2012. Host specificity of parasitic gastropods of the genus *Annulobalcis* Habe, 1965 (Mollusca, Gastropoda, Eulimidae) from crinoids in Vietnam, with descriptions of four new species. *Zoosystema* **34**: 139–155.

Dinapoli, A., Klussmann-Kolb, A., 2010. The long way to diversity – phylogeny and evolution of Heterobranchia (Mollusca: Gastropoda). *Molecular Phylogenetics and Evolution* **55**: 60–76.

Dinapoli, A., Zinssmeister, C., Klussmann-Kolb, A., 2011. New insights into the phylogeny of the Pyramidellidae (Gastropoda). *Journal of Molluscan Studies* **77**, 1–7.

Dreyer, H., Wägele, J.W., 2001. Parasites of crustaceans (Isopoda: Bopyridae) evolved from fish parasites: molecular and morphological evidence. *Zoology* **103**: 157–178.

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

Dunne, J.A., Lafferty, K.D., Dobson, A.P., Hechinger, R.F., Kuris, A.M., Martinez, N.D., McLaughlin, J.P., Mouritsen, K.N., Poulin, R., Reise, K., Stouffer, D.B., Thieltges, D.W., Williams, R.J., Zander, C.D., 2013. Parasites affect food web structure primarily through increased diversity and complexity. *PLoS Biology* **11**: e1001579.

Emelianov, I., 2007. How adaptive is parasite species diversity? *International Journal of Parasitology* **37**: 851–860.

Euzet L., Combes, C., 1998. The selection of habitats among the monogenean. *International Journal for Parasitology* **28**: 1645–1652.

Folmer, O., Black, M., Hoeh, W., Luts, R., 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.

Frédérich, B., Sorenson, L., Santini, F., Slater, G.J., Alfaro, M.E., 2013. Iterative ecological radiation and convergence during the evolutionary history of damselfishes (Pomacentridae). *American Naturalist* **181**: 94–113.

Fretter, V., Graham, A., 1982. The prosobranch molluscs of Britain and Denmark, Part 7. 'Heterogastropoda'. *Journal of Molluscan Studies, Supplement* **11**: 363–434.

Fretter, V., Graham, A., 1994. *Britich Prosobranch Molluscs. Their Functional Anatomy and Ecology, Revised and Updated Edition.* The Ray Society, London.

Futuyma, D.J., Moreno, G., 1988. The evolution of ecological specialization. *Annual Review of Ecology and Systematics* **19**: 207–233.

Gage, J.D., Tyler, P.A., 1991. *Deep-Sea Biology: A natural history of organisms at the deep-sea floor*. Cambridge University Press, Cambridge.

Gillespie, R., 2004. Community assembly through adaptive radiation in Hawaiian spiders. *Science* **303**: 356–359.

Givnish, T.J., 1997. Adaptive radiation and molecular systematics: issues and approaches, *In*: Givnish, T.J., Sytsma, K.J. (Eds.), *Molecular Evolution and Adaptive Radiation*. Cambridge University Press, Cambridge, pp. 1–54.

Glasby, C.J., Hutchings, P.A., Fauchald, K., Paxton, H., Rouse, G.W., Russell, C.W., Wilson, R.S., 2000. Class Polychaeta, *In*: Beesley, P.L., Ross, G.J.B., Glasby, C.J. (Eds.), *Fauna of Australia, Vol. 4A, Polychaetes & Allies: The Southern Synthesis*. CSIRO Publishing, Melbourne, pp. 1–296.

Glenner, H., Hebsgaard, M.B., 2006. Phylogeny and evolution of life history strategies of the parasitic barnacles (Crustacea, Cirripedia, Rhizocephala). *Molecular Phylogenetics and Evolution* **41**: 528–538.

Gofas, S., Moreno, D., Salas, C., 2011. *Moluscos marinos de Andalcía. Vol. I*. Servicio de Publicaciones e Intercambio Científico, Universidad de Málaga, Málaga.

Gould, S.J., 1989. Wonderful life: the Burgess Shale and the nature of history. Norton, New York.

Golding, R.E., Ponder, W.F., Byrne, M., 2007. Taxonomy and anatomy of Amphiboloidea (Gastropoda: Heterobranchia: Archaeopulmonata). *Zootaxa* **1476**: 1–50.

Golding, R.E., Ponder, W.F., Byrne, M., 2009. The evolutionary and biomechanical implications of snout and proboscis morphology in Caenogastropoda (Mollusca: Gastropoda). *Journal of Natural History* **43**: 2723–2763.

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

Goto, R., Kawakita, A., Ishikawa, H., Hamamura, Y., 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.

Gougerot, L., Le Renard, J., 1977. Nouvelles espèces de petits gastéropodes marins de l'Éocène des Bassins de Paris, de Nantes et du Cotentin. *Bulletin d'Information des Geologues du Bassin de Paris* **14**: 3–33.

Grande, C., Templado, J., Cervera, J.L., Zardoya, R., 2002. The complete mitochondrial genome of the nudibranch *Roboastra europaea* (Mollusca: Gastropoda) supports the monophyly of opisthobranchs. *Molecular Biology and Evolution* **19**: 1672–1685.

Grande, C., Templado, J., Cervera, J.L., Zardoya, R., 2004. Molecular phylogeny of Euthyneura (Mollusca: Gastropoda). *Molecular Biology and Evolution* **21**: 303–313.

Gray, J.E., 1853. On the division of ctenobranchous gasteropodous Mollusca into larger groups and families. *Proceedings of the Zoological Society of London* **21**: 32–44.

Guindon, S., Gascuel, O., 2003. A simple, fast and accurate method to estimate large phylogenies by maximum-likelihood. *Systematic Biology* **52**: 696–704.

Habe, T., 1952. Parasitic gastropods found in echinoderms from Japan. *Publications of the Seto Marine Biological Laboratory* **2**: 73–85.

Habe, T., 1965. Description of *Annulobalcis shimazui* n. gen. et sp. (Eulimidae). *Venus* **24**: 106–107.

Habe, T., 1976. Parasitic gastropods from echinoderms of Japan. *Bulletin of the Natural Science Museum, ser. A, Zoology* **2**: 157–168.

Haines, C.M.C., Edmunds, M., Pewsey, A.R., 1994. The pea crab, *Pinnotheres pisum* (Linnaeus, 1767), and its association with the common mussel, *Mytilus edulis* (Linnaeus, 1758), in the Solent (UK). *Journal of Shellfish Research* **13**: 5–10.

Hasegawa, K., 1997. Sunken wood-associated gastropods collected from Suruga Bay, Pacific side of the Central Honshu, Japan, with descriptions of 12 new species. *National Science Museum Monographs* **12**: 59–123.

Haszprunar, G., 1985. The Heterobranchia—a new concept of the phylogeny of the higher Gastropoda. *Zeitschrift für Zoologie, Systematik und Evolutionsforschung* **23**: 15–37.

Hatcher, M.J., Dunn, A.M., 2011. *Parasites in Ecological Communities: From Interactions to Ecosystems.* Cambridge University Press, Cambridge.

Healy, J.M., 1988. Sperm morphology and its systematic importance in the Gastropoda. *Malacological Review, Supplement* **4**: 251–266.

Heding, S.G., Mandahl-Barth, G., 1938. Investigations on the anatomy and systematic position of the parasitic snail *Entocolax* Voigt. *Meddelelser om Grønland* **108**: 1–40.

Heller, J., 1993. Hermaphroditism in molluscs. *Biological Journal of the Linnean Society* **48**: 19–42.

Herbert, G.S., Dietl, G.P., Fortunato, H., Simone, L.R.L., Sliko, J., 2009. Extremely slow feeding in a tropical drilling ectoparasite, *Vitularia salebrosa* (King and Broderip, 1832) (Gastropoda: Muricidae), on molluscan hosts from Pacific Panama. *Nautilus* **123**: 121–136.

Héritier, L., Badets, M., du Preez, L.H., Aisien, M.S.O., Lixian, F., Combes, C., Verneau, O., 2015. Evolutionary processes involved in the diversification of chelonian and mammal polystomatid parasites (Platyhelminthes, Monogenea, Polystomatidae) revealed by palaeoecology of their hosts. *Molecular Phylogenetics and Evolution* **92**: 1–10.

Herlyn, H., Piskurek, O., Schmitz, J., Ehlers, U., Zischler, H., 2003. The syndermatan phylogeny and the evolution of acanthocephalan endoparasitism as inferred from 18S rDNA sequences. *Molecular Phylogenetics and Evolution* **26**: 155–164.

Høisæter, T., 2014. The Pyramidellidae (Gastropoda, Heterobranchia) of Norway and adjacent waters. A taxonomic review. *Fauna norvegica* **34**: 7–78.

Hori, S., 2000a. Familiy Eulimidae, *In*: Okutani, T. (Ed.), *Marine Mollusks in Japan*. Tokai University Press, Tokyo, pp. 344–363.

Hori, S., 2000b. Familiy Pyramidellidae, *In*: Okutani, T. (Ed.), *Marine Mollusks in Japan*. Tokai University Press, Tokyo, pp. 702–731.

Hori, S., Tsuchida, E., 1995. A revision of systematic position of genus *Leucotina* (Gastropoda: Heterobranchia). *Venus* **54**: 279–293.

Houbrick, R.S., 1988. Cerithioidean phylogeny. *Malacological Review, Supplement* **4**: 88–128.

Hutchings, P., 1998. Biodiversity and functioning of polychaetes in benthic sediments. *Biodiversity and Conservation* **7**: 1133–1145.

Huys, R., Llewellyn-Hughes, J., Olson, P.D., Nagasawa, K., 2006. Small subunit rDNA and Bayesian inference reveal *Pectenophilus ornatus* (Copepoda *incertar sedis*) as highly transformed Mytilicolidae, and support assignment of Chondracanthidae and Xarifiidae to Lichomolgidae (Cyclopioda). *Biological Journal of the Linnean Society* **87**: 403–425.

Huys, R., Llewellyn-Hughes, J., Conroy-Dalton, S., Olson, P.D., Spinks, J.N., Johnston, D.A., 2007. Extraordinary host switching in siphonostomatoid copepods and the

demise of the Monstrilloida: integrating molecular data, ontogeny and antennulary morphology. *Molecular Phylogenetics and Evolution* **43**: 368–378.

Huyse, T., Poulin, R., Théron, A., 2005. Speciation in parasites: a population genetics approach. *Trends in Parasitology* **21**: 469–475.

Ingram, T., Kai, Y., 2014. The geography of morphological convergence in the radiations of Pacific *Sebastes* rockfishes. *American Naturalist* **184**: E115–E131.

Jangoux, M., 1987. Diseases of Echinodermata. II. Agents metazoans (Mesozoa to Bryozoa). *Diseases of Aquatic Organisms* **2**: 205–234.

Janzen, D., 1968. Host plants as islands in evolutionary and contemporary time. *American Naturalist* **102**: 592–595.

Jeyaprakash, A., Hoy, M.A., 2009. First divergence time estimate of spiders, scorpions, mites and ticks (subphylum: Chelicerata) inferred from mitochondrial phylogeny. *Experimental and Applied Acarology* **47**: 1–18.

Johnson, K.P., Shreve, S.M., Smith, V.S., 2012. Repeated adaptive divergence of microhabitat specialization in avian feather lice. *BMC Biology* **10**: 52.

Jörger, K.M., Stöger, I., Kano, Y., Fukuda, H., Knebelsberger, T., Schrödl, M., 2010. On the origin of Acochlidia and other enigmatic euthyneurian gastropods, with implications for the systematics of Heterobranchia. *BMC Evolutionary Biology* **10**: 323.

Kameda, Y., Kato, M., 2011. Terrestrial invasion of pomatiopsid gastropods in the heavy-snow region of the Japanese Archipelago. *BMC Evolutionary Biology* **11**: 118.

Kano, Y., 2008. Vetigastropod phylogeny and a new concept of Seguenzioidea: independent evolution of copulatory organs in the deep-sea habitats. *Zoologica Scripta* **37**: 1–21.

Kano, Y., Chiba, S., Kase, T., 2002. Major adaptive radiation in neritopsine gastropods estimated from 28S rRNA sequences and fossil records. *Proceedings of the Royal Society B* **269**: 2457–2465.

Kano, Y., Chikyu, E., Warén, A., 2009. Morphological, ecological and molecular characterization of the enigmatic planispiral snail genus *Adeuomphalus* (Vetigastropoda: Seguenzioidea). *Journal of Molluscan Studies* **75**: 397–418.

Kase, T. 1998. The family Pickworthiidae (Gastropoda : Caenogastropoda) from tropical Pacific submarine caves : four new species of *Sansonia*. *Venus* **57**: 161–172.

Kat, P.W., 1984. Parasitism and the Unionacea (Bivalvia). *Biological Reviews* **59**: 189–207.

Katoh, K., Standley, D.M., 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular Biology and Evolution* **30**: 772–780.

Kawai, T., Nagasawa, K., 2006. Host body size, prevalence and breeding season of the microparasitic snail *Pelseneeria castanea* on the sea urchin *Strongylocentrotus nudus* in castal waters of the Sea of Japan off Western Hokkaido, Japan. *Japanese Journal of Benthology* **61**: 16–20.

Kemp, P., Bertness, M.D., 1984. Snail shape and growth rates: evidence for plastic shell allometry in *Littorina littorea*. *Proceedings of the National Academy of Sciences* **81**: 811–813.

Ketmaier, V., Joyce, D.A., Horton, T., Mariani, S., 2008. A molecular phylogenetic framework for the evolution of parasitic strategies in cymothoid isopods (Crustacea). *Journal of Zoological Systematics and Evolutionary Research* **46**: 19–23.

Kiel, S., Bandel, K., 2001. About Heterostropha (Gastropoda) of the Campanian of Torallola, Spain. *Journal of the Czech Geological Society* **46**: 319–334.

Knapp, J., Nakao, M., Yanagida, T., Okamoto, M., Saarma, U., Lavikainen, A., Ito, A., 2011. Phylogenetic relationships within *Echinococcus* and *Taenia* tapeworms (Cestoda: Taeniidae): an inference from nuclear protein-coding genes. *Molecular Phylogenetics and Evolution* **61**: 628–638.

Kocot, K.M., Cannon, J.T., Todt, C., Citarella, M.R., Kohn, A.B., Meyer, A., Santos, S.R., Schander, C., Moroz, L.L., Lieb, B., Halanych, K.M., 2011. Phylogenomics reveals deep molluscan relationships. *Nature* **477**: 452–456.

Kozak, K.H., Mendyk, R.W., Wiens, J.J., 2009. Can parallel diversification occur in sympatry? Repeated patterns of body-size evolution in coexisting clades of North American salamanders. *Evolution* **63**: 1769–1784.

Kunz, W., 2002. When is a parasite species a species? *Trends in Parasitology* **18**: 121–124.

Kuris, A.M., Blaustein, A.R., Javeir Alió, J., 1980. Hosts as islands. *American Naturalist* **116**: 570–586.

Lafferty, K.D., 2013. Parasites in marine food webs. *Bulletin of Marine Science* **89**: 123–134.

Lafferty, K.D., Allesina, S., Arim, M., Briggs, C.J., Leo, G.D., Dobson, A.P., Dunne, J.A., Johnson, P.T.J., Kuris, A.M., Marcogliese, D.J., Martinez, N.D., Memmott, J., Marquet, P.A., McLaughlin, J.P., Mordecai, E.A., Pascual, M., Poulin, R., Thieltges, D.W., 2008. Parasites in food webs: the ultimate missing links. *Ecology Letters* **11**: 533–546.

Lanterbecq, D., Rouse, G.W., Milinkovitch, M.C., Eeckhaut, I., 2006. Molecular phylogenetic analyses indicate multiple independent emergences of parasitism in Myzostomida (Protostomia). *Systematic Biology* **55**: 208–227.

Laseron, C.F. 1955. Revision of the New South Wales eulimoid shells. *Australian Zoologist* **12**: 83–107.

Laseron, C.F., 1959. The family Pyramidellidae (Mollusca) from Northern Australia. *Marine & Freshwater Research* **10**: 177–268.

Lauridsen, B.W., Schnetler, K.I., 2014. A catalogue of Danian gastropods from the Baunekule facies, Faxe Formation, Denmark. *Geological Survey of Denmark and Greenland Bulletin* **32**: 1–117.

Lester, R.J.G., Sewell, K.B., 1989. Checklist of parasites from Heron Island, Great Barrier Reef. *Australian Journal of Zoology* **37**: 101–128.

Leung, T.L.F., in press. Fossil of parasites: what can the fossil record tell us about the evolution of parasitism? *Biological Reviews.*

Littlewood, D.T.J., 2006. The evolution of parasitism in flatworms, *In*: Maule, A.G., Marks, N.J. (Eds.), *Parasitic Flatworms: Molecular Biology, Biochemistry, Immunology and Phylogeny*. CABI, Wallingford, pp. 1–36.

Littlewood, D.T.J., Rohde, K., Bray, R.A., Herniou, E.A., 1999. Phylogeny of the Platyhelminthes and the evolution of parasitism. *Biological Journal of the Linnean Society* **68**: 257–287.

Littlewood, D.T.J., Curini-Galletti, M., Herniou, A., 2000. The interrelationships of Proseriata (Platyhelminthes: Seriata) tested with molecules and morphology. *Molecular Phylogenetics and Evolution* 16: 449–466.

Lockyer, A.E., Olson, P.D., Østergaard, P., Rollinson, D., Johnston, D.A., Attwood, S.W., Southgate, V.R., Horak, P., Snyder, S.D., Le, T.H., Agatsuma, T., McManus, D.P., Carmichael, A.C., Naem, S., Littlewood, D.T.J., 2003. The phylogeny of the Schistosomatidae based on three genes with emphasis on the interrelationships of *Schistosoma* Weinland, 1858. *Parasitology* **126**: 203–224.

Lorentz, F., 2005. Mollusca (molluscs), *In*: Rohde, K. (Ed.), *Marine Parasitology*. CABI Publishing, Wallingford, pp. 240–245.

Löytynoja, A., Milinkovitch, M.C., 2003. A hidden Markov model for progressive multiple alignment. *Bioinformatics* **19**: 1505–1513.

Losos, J.B., 2010. Adaptive radiation, ecological opportunity, and evolutionary determinism. *American Naturalist* **175**: 623–639.

Losos, J.B., Jackman, T.R., Larson, A., de Queiroz, K, Rodrígues-Schettino, L., 1998. Contingency and determinism in replicated adaptive radiations of island lizards. *Science* **279**: 2115–2118.

Lozouet, P., 1999. New species of gastropods (Mollusca: Gastropoda) from Oligocene and Lower Miocene of Aquitaine (Southwestern France). Part 2. *Cossmanniana* **6**: 1–68.

Lozouet, P., Dockery, D.T., 2001. First fossil record of the genus *Pyramidelloides* (Mollusca, Gastropoda, Eulimidae) discovered in the Eocene deposits of Alabama. *Comptes Rendus de l'Académie des Sciences, ser. IIA, Earth and Planetary Science* **333**: 351–356.

Lützen, J., 1968. Unisexuality in the parasitic family Entoconchidae (Gastropoda: Prosobranchia). *Malacologia* **7**: 7–15.

Lützen, J., 1976. On a new genus and two new species of Prosobranchia (Mollusca), parasitic on the tropical sea urchin *Echinometra mathaei*. *Israel Journal of Zoology* **25**: 38–51.

Maddison, W.P., Maddison, D.R., 2015. *Mesquite: a modular system for evolutionary analysis, version 3.04.* Available at http://mesquiteproject.org

Matsuda, H., Hamano, T., Nagasawa, K., 2013. Growth and reproductive cycle of *Hypermastus tokunagai* (Caenogastropoda: Eulimidae), an ectoparasite of the sand dollar *Scaphechinus mirabilis* (Clypeasteroida: Scutellidae) in the Seto Inland Sea, Japan. *Journal of the Marine Biological Association of the United Kingdom* **93**: 1041–1051.

Melville, J., Harmon, L.J., Losos, J.B., 2006. Intercontinental community convergence of ecology and morphology in desert lizards. *Proceedings of the Royal Society B* **273**: 557–563.

Mendlová, M., Šimková, A., 2014. Evolution of host specificity in monogeneans parasitizing African cichlid fish. *Parasites & Vectors* **7**: 69.

Meyer, C.P., 2003. Molecular systematics of cowries (Gastropoda: Cypraeidae) and diversification patterns in the tropics. *Biological Journal of the Linnean Society* **79**: 401–459.

Meyer, A., Kocher, T.D., Basasibawaki, P., Wilson, A.C., 1990. Monophyletic origin of Lake Victoria cichlid fishes. *Nature* 347: 550–553.

Meyer, C.P., 2004. Toward comprehensiveness: increased molecular sampling within Cypraeidae and its phylogenetic implications. *Malacologia* **46**: 127–156.

Michot, B., Hassouna, N., Bachellerie, J.P., 1984. Secondary structure of mouse 28S rRNA and general model for the folding of the large rRNA in eukaryotes. *Nucleic Acids Research* **10**: 4259–4279.

Mladineo, I., Bott, N.J., Nowak, B.F., Block, B.A., 2010. Multilocus phylogenetic analyses reveal that habitat selection drives the speciation of Didymozoidae (Digenea) parasitizing Pacific and Atlantic bluefin tunas. *Parasitology* **137**: 1013–1025.

Moran, A.L., 1999. Size and performance of juvenile marine invertebrates: potential contrasts between intertidal and subtidal benthic habitats. *American Zoologist* **39**: 304–312.

Morris, S.C., 1981. Parasites and the fossil record. *Parasitology* **82**: 489–509.

Morris, S.C., Crompton, D.W.T., 1982. The origins and evolution of the Acanthocephala. *Biological Reviews* **57**: 85–115.

Morton, B., 1979. The population dynamics and expression of sexuality in *Balcis shaplandi* and *Mucronalia fulvescens* (Mollusca: Gastropoda: Aglossa) parasitic upon *Archaster typicus* (Echinodermata: Asteroidea). *Malacologia* **18**: 327–346.

Murphy, N., Banks, J.C., Whitfield, J.B., Austin, A.D., 2008. Phylogeny of the parasitic microgastroid subfamilies (Hymenoptera: Braconidae) based on sequence data from

seven genes, with an improved time estimate of the origin of the lineage. *Molecular Phylogenetics and Evolution* **47**: 378–395.

Musashi, T., Habe, T., 1991. Notes on the distribution and host of *Pelseneeria castanea* (Dall, 1925), a parasitic snail. *Chiribotan* **22**: 69–71.

Nakamura, K., Kano, Y., Suzuki, N., Namatame, T., Kosaku, A., 2007. 18S rRNA phylogeny of sea spiders with emphasis on the position of Rhynchothoracidae. *Marine Biology* **153**: 213–223.

Neumann, C., Wisshak, M., 2009. Gastropod parasitism on late Cretaceous to early Paleocene holasteroid echinoids—evidence from *Oichnus* isp. n. *Palaeogeography, Palaeoclimatology, Palaeoecology* **284**: 115–119.

Nützel, A., 1998. Über die stammesgeschichte der Ptenoglossa (Gastropoda). *Berliner Geowissenschaftliche Abhandlungen, ser. E* **26**: 1–229.

Okutani, T. (Ed.), 2000a. *Marine Mollusks in Japan*. Tokai University Press, Tokyo.

Okutani, T., 2000b. Familiy Calyptraeidae, *In*: Okutani, T. (Ed.), *Marine Mollusks in Japan*. Tokai University Press, Tokyo, pp. 200–201.

Oliver, J.D., Rolan, E., 2008. Las especies del género *Chauvetia* del área de Dakar, Senegal, África occidental, con la descripción de diez especies nuevas. *Iberus* **26**: 133–175.

Palumbi, S., Martin, A., Romano, S., McMillan, W.O., Stice, L., Grabowski, G., 1991. *The Simple Fool's Guide to PCR, Version 2.0*. Department of Zoology and Kewalo Marine Laboratory, University of Hawaii, Honolulu.

Park, J.K., Kim, K.H., Kang, S., Kim, W., Eom, K.S., Littlewood, D.T.J., 2007. A common origin of complex life cycles in parasitic flatworms: evidence from the complete mitochondrial genome of *Microcotyle sebastis* (Monogenea: Platyhelminthes). *BMC Evolutionary Biology* **7**: 11.

Peñas, A., Rolán, E., 2010. Deep water Pyramidelloidea of the Tropical South Pacific: *Turbonilla* and related genera. *Mémoires du Muséum national d'Histoire naturelle* **200**: 1–436.

Perkins, E.M., Donnellan, S.C., Bertozzi, T., Chisholm, L.A., Whittington, I.D., 2009. Looks can deceive: molecular phylogeny of a family of flatworm ectoparasites (Monogenea: Capsalidae) does not reflect current morphological classification. *Molecular Phylogenetics and Evolution* 52: 705–714.

Poinar, G., Kerp, H., Hass, H., 2008. *Palaeonema phyticum* gen. n., sp. n. (Nematoda: Palaeonematidae fam. n.), a Devonian nematode associated with early land plants. *Nematology* **10**: 9–14.

Ponder, W.F., 1985. A review of the genera of the Rissoidae (Mollusca: Mesogastropoda: Rissoacea). *Records of the Australian Museum, Supplement* **4**: 1–221.

Ponder, W.F., 1986. Glacidorbidae (Glacidorbacea: Basommatophora), a new family and superfamily of operculate freshwater gastropods. *Zoological Journal of the Linnean Society* **87**: 53–83.

Ponder, W.F., 1987. The anatomy and relationships of the pyramidellacean limpet *Amathina tricarinata* (Mollusca: Gastropoda). *Asian Marine Biology* **4**: 1–34.

Ponder, W.F., 1998. Superfamily Vanikoroidea, *In*: Beesley, P.L., Ross, G.J.B., Wells, A. (Eds.), *Fauna of Australia, Vol. 5, Mollusca: The Southern Synthesis.* CSIRO Publishing, Melbourne, pp. 769–772.

Ponder, W.F., Gooding, R.U., 1978. Four new eulimid gastropods associated with shallow-water diadematid echinoids in the Western Pacific. *Pacific Science* **32**: 157–181.

Ponder, W.F., Hall, S.J., 1983. Pelycidiidae, a new family of Archaeogastropod molluscs. *Nautilus* **97**: 30–35.

Ponder, W.F., Warén, A., 1988. Classification of the Caenogastropoda and Heterostropha—a list of the family-group names and higher taxa. *Malacological Review, Supplement* **4**: 288–326.

Ponder, W.F., Lindberg, D.R., 1997. Towards a phylogeny of gastropod molluscs—an analysis using morphological characters. *Zoological Journal of the Linnean Society* **19**: 83–265.

Ponder, W.F., de Keyzer, R.G., 1998a. Superfamily Rissooidea, *In*: Beesley, P.L., Ross, G.J.B., Wells, A. (Eds.), *Fauna of Australia, Vol. 5, Mollusca: The Southern Synthesis.* CSIRO Publishing, Melbourne, pp. 745–766.

Ponder, W.F., de Keyzer, R.G., 1998b. Superfamily Pyramidelloidea, *In*: Beesley, P.L., Ross, G.J.B., Wells, A. (Eds.), *Fauna of Australia, Vol. 5, Mollusca: The Southern Synthesis.* CSIRO Publishing, Melbourne, pp. 865–869.

Ponder, W.F., Colgan, D.J., Healy, J.M., Nützel, A., Simone, L.R.L., Strong, E.E., 2008. Caenogastropoda, *In*: Ponder, W.F., Lindberg, D.R. (Eds.), *Phylogeny and Evolution of the Mollusca.* University of California Press, Berkeley, pp. 331–383.

Ponder, W.F., Lindberg, D.R., 2008. Molluscan evolution and phylogeny, *In*: Ponder, W.F., Lindberg, D.R. (Eds.), *Phylogeny and Evolution of the Mollusca.* University of California Press, Berkeley, pp. 1–17.

Poppe, G.T., 2008. *Philippine Marine Mollusks. Vol. 1, Gastropoda-Part 1.* ConchBooks, Hackenheim.

Poulin, R., 2007. *Evolutionary Ecology of Parasites, 2nd edition.* Princeton University Press, Princeton and Oxford.

Poulin, R., Morand, S., 2000. The diversity of parasites. *The Quarterly Review of Biology* **75**: 277–293.

Poulin, R., Morand, S., 2004. *Parasite Biodiversity.* Smithsonian Institution Press, Washington, D.C.

Pras, S., 2013. Correct type designation for two mollusc genera: *Polygireulima* Sacco 1892 (Gastropoda: Eulimidae) and *Coripia* de Gregorio 1885 (Bivalvia: Carditidae). *Cossmanniana* **15**: 99–103.

Rascalou, G., Gourbière, S., 2014. Competition, virulence, host body mass and the diversification of macro-parasites. *Journal of the Royal Society Interface* **11**: 20131108.

R Core Team, 2015. *R: A language and environment for statistical computing, version 3.2.2.* R Foundation for Statistical Computing, Vienna, Austria. Available at http://www.r-project.org/

Rees, D.J., Noever, C., Høeg, J.T., Ommundsen, A., Glenner, H., 2014. On the origin of a novel parasitic-feeding mode within suspension-feeding barnacles. *Current Biology* **24**: 1429–1434.

Ricklefs, R.E., Schluter, D. (Eds.), 1993. *Species Diversity in Ecological Communities: Historical and Geographical Perspective.* University of Chicago Press, Chicago.

Roach, A.C., Lim, R.P., 2000. Variation in the population dynamics of the intertidal pulmonate gastropod *Salinator solida* Martens (Gastropoda: Amphibolidae) at Towra Point, NSW, Australia. *Wetlands Ecology and Management* **8**: 53–69.

Robertson, R., Habe, T., 1965. *Alexania* replaces *Habea* (Epitoniidae). *Nautilus* **78**: 140–141.

Rohde, K., 2005. Definitions, and adaptations to a parasitic way of life, *In*: Rohde, K. (Ed.), *Marine Parasitology*. CABI Publishing, Wallingford, pp. 1–6.

Ronquist, F., Huelsenbeck, J.P., 2003. MrBayes3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* **19**: 1572–1574.

Rundell, R.J., Price, T.D., 2009. Adaptive radiation, nonadaptive radiation, ecological speciation and nonecological speciation. *Trends in Ecology and Evolution* **24**: 394–399.

Schander, C., van Aartsen, J.J., Corgan, J.X., 1999a. Families and genera of the Pyramidelloidea (Mollusca: Gastropoda). *Bollettino Malacologico* 34: 145–166.

Schander, C., Hori, S., Lundberg, J., 1999b. Anatomy, phylogeny and biology of *Odostomella* and *Herviera*, with the description of a new species of *Odostomella*. *Ophelia* **51**: 39–76.

Schander, C., Halanych, K.M., Dahlgren, T., Sundberg, P., 2003. Test of the monophyly of Odostomiinae and Turbonilliinae (Gastropoda, Heterobranchia, Pyramidellidae) based on 16S mtDNA sequences. *Zoologica Scripta* **32**: 243–254.

Schiaparelli, S., Barucca, M., Olmo, E., Boyer, M., Canapa, A., 2005. Phylogenetic relationships within Ovulidae (Gastropoda: Cypraeoidea) based on molecular data from the 16S rRNA gene. *Marine Biology* **147**: 411–420.

Schluter, D., 2000. *The Ecology of Adaptive Radiation.* Oxford University Press, Oxford.

Schluter, D., 2009. Evidence for ecological speciation and its alternative. *Science* **323**: 737–741.

Schulte, R.D., Makus, C., Hasert, B., Michiels, N.K., Schulenburg, H., 2010. Multiple reciprocal adaptations and rapid genetic change upon experimental coevolution of an animal host and its microbial parasite. *Proceedings of the National Academy of Sciences* **107**: 7359–7364.

Severns, M., 2011. *Shells of the Hawaiian Islands*. IKAN Unterwasser-Archiv, Frankfurt.

Šimková, A., Morand, S., Jobet, E., Gelnar, M., Verneau, O., 2004. Molecular phylogeny of congeneric monogenean parasites (*Dactylogyrus*): a case of intrahost speciation. *Evolution* **58**: 1001–1018.

Šimková, A., Verneau, O., Gelnar, M., Morand, S., 2006. Specificity and specialization of congeneric monogeneans parasitizing cyprinid fish. *Evolution* **60**: 1023–1037.

Simone, L.R.L., 2002. Comparative morphological study and phylogeny of

representatives of the superfamily Calyptraeoidea (including Hipponicoidea) (Mollusca, Caenogastropoda). *Biota Neotropica* **2(2)**: 1–137.

Simone, L.R.L., 2011. Phylogeny of the Caenogastropoda (Mollusca), based on comparative morphology. *Arquivos de Zoologia* **42**: 83–323.

Smith, S.A., Wilson, N.G., Goetz, F.E., Feehery, C., Andrade, S.C.S, Rouse, G.W., Giribet, G., Dunn, C.W., 2011. Resolving the evolutionary relationships of molluscs with phylogenomic tools. *Nature* **480**: 364–367.

Sohl, N.F., 1964. Neogastropoda, Opisthobranchia and Basommatophora from the Ripley, Owl Creek and Praire Bluff Formations. *Professional Papers of the U.S. Geological Survey* **331B**: 153–344.

Song, Y., Wang, G.T., Yao, W.J., Gao, Q., Nie, P., 2008. Phylogeny of freshwater parasitic copepods in the Ergasilidae (Copepoda: Poecilostomatoida) based on 18S and 28S rDNA sequences. *Parasitology Research* **102**: 299–306.

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

Steiner, G., Dreyer, H., 2003. Molecular phylogeny of Scaphopoda (Mollusca) inferred from 18S rRNA sequences—support for a Scaphopoda–Cephalopoda clade. *Zoologica Scripta* **32**: 343–356.

Strong, E.E., Gargominy, O., Ponder, W.F., Bouchet, P., 2008. Global diversity of gastropods (Gastropoda; Mollusca) in fleshwater. *Hydrobiologia* **595**: 149–166.

Strong, E.E., Colgan, D.J., Healy, J.M., Lydeard, C., Ponder, W.F., Glaubrecht, M., 2011. Phylogeny of the gastropod superfamily Cerithioidea using morphology and molecules. *Zoological Journal of the Linnean Society* **162**: 43–89.

Suárez-Morales, E., Harris, L.H., Ferrari, F.D., Gasca, R., 2014. Late postnaupliar development of *Monstrilla* sp. (Copepoda: Monstrilloida), a protelean endoparasite of benthic polychaetes. *Invertebrate Reproduction & Development* **58**: 60–73.

Summers, K., McKeon, S., Sellars, J., Keusenkothen, M., Morris, J., Gloeckner, D., Pressley, C., Price, B., Snow, H., 2003. Parasitic exploitation as an engine of diversity. *Biological Reviews* **78**: 639–675.

Summers, M.M., Rouse, G.W., 2014. Phylogeny of Myzostomida (Annelida) and their relationships with echinoderm hosts. *BMC Evolutionary Biology* **14**: 170.

Takano, T., Kano, Y., 2014. Molecular phylogenetic investigations of the relationships of the echinoderm-parasite family Eulimidae within Hypsogastropoda (Mollusca). *Molecular Phylogenetics and Evolution* **79**: 258–269.

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

Telford, M.J., Lowe, C.J., Cameron, C.B., Ortega-Martinez, O., Aronowicz, J., Oliveri, P., Copley, R.R., 2014. Phylogenomic analysis of echinoderm class relationships supports Asterozoa. *Proceedings of the Royal Society B* **281**: 20140479.

Thompson, J.N., 1994. *The coevolutionary process.* University of Chicago Press, Chicago.

Tompkins, D., Dunn, A.M., Smith, M.J., Telfer, S., 2011. Wildlife diseases: from individuals to ecosystems. *Journal of Animal Ecology* **80**: 19–38.

Tsuchiya, K., 2000. Family Muricidae, *In*: Okutani, T. (Ed.), *Marine Mollusks in Japan*. Tokai University Press, Tokyo, pp. 364–421.

Vas, Z., Csorba, G., Rózsa L., 2012. Evolutionary co-variation of host and parasite diversity—the first test of Eichler's rule using parasitic lice (Insecta: Phthiraptera). *Parasitology Research* **111**: 393–401.

Vermeij, D.J., 1993. *A Natural History of Shells*. Princeton Univ. Press, Princeton.

Verneau, O., Bentz, S., Sinnappah, N.D., du Preez, L., Whittington, I., Combes, C., 2002. A view of early vertebrate evolution inferred from the phylogeny of

polystome parasites (Monogenea: Polystomatidae). *Proceedings of the Royal Society B* **269**: 535–543.

De Vlas, J., 1979. Annual food intake by plaice and flounder in a tidal flat area in the Dutch Wadden Sea, with special reference to consumption of regenerating part of microbenthic prey. *Netherlands Journal of Sea Research* **13**: 117–153.

Vonnemann, V., Schrödl, M., Klussmann-Kolb, A., Wägele, H., 2005. Reconstruction of the phylogeny of the Opisthobranchia (Mollusca: Gastropoda) by means of 18S and 28S rRNA gene sequences. *Journal of Molluscan Studies* **71**: 113–125.

Wada, S., Kameda, Y., Chiba, S., 2013. Long-term stasis and short-term divergence in the phenotypes of microsnails on oceanic islands. *Molecular Ecology* **22**: 4801–4810.

Warén, A., 1980a. Revision of the genera *Thyca*, *Stilifer*, *Scalenostoma*, *Mucronalia* and *Echineulima* (Mollusca, Prosobranchia, Eulimidae). *Zoologica Scripta* **9**: 187–210.

Warén, A., 1980b. Descriptions of new taxa of Eulimidae (Mollusca, Prosobranchia), with notes on some previously described genera. *Zoologica Scripta* **9**: 283–306.

Warén, A., 1981a. Revision of the genera *Apicalia* A. Adams and *Stilapex* lredale and description of two new genera (Mollusca, Prosobranchia, Eulimidae). *Zoologica Scripta* **10**: 133–154.

Warén, A., 1981b. Eulimid gastropods parasitic on echinoderms in the New Zealand region. *New Zealand Journal of Zoology* **8**: 313–324.

Warén, A., 1983. An anatomical description of *Eulima bilineata* Alder with remarks on and a revision of *Pyramidelloides* Nevill (Mollusca, Prosobranchia, Eulimidae). *Zoologica Scripta* **12**: 273–294.

Warén, A., 1984. A generic revision of the family Eulimidae (Gastropoda, Prosobranchia). *Journal of Molluscan Studies, Supplement* **13**: 1–96.

Warén, A., 1989. New and little known Mollusca from Iceland. *Sarsia* **74**: 1–28.

Warén, A. 1991. New and little known Mollusca from Iceland and Scandinavia. Sarsia 76: 53–124.

Warén, A., 1993. New and little known Mollusca from Iceland and Scandinavia. Part 2. *Sarsia* **78**: 159–201.

Warén, A. 2011. Molluscs on biogenic substrates, *In*: Bouchet, P., Le Guyader, H., Pascal, O. (Eds.), *The Natural History of Santo*. Muséum national d'Histoire naturelle, Paris; IRD Marseille; Pro-Natura international, Paris, pp. 438–448.

Warén, A., 2013. Murchisonellidae: who are they, where are they and what are they doing? (Gastropoda, lowermost Heterobranchia). *Vita Malacologica* **11**: 1–14.

Warén, A., Sibuet, M., 1981. *Ophieulima* (Mollusca, Prosobranchia), a new genus of ophiuroid parasites. *Sarsia* **66**: 103–107.

Warén, A., Bouchet, P., 1988. A new species of Vanikoridae from the western Mediterranean, with remarks on the northeast Atlantic species of the family. *Bollettino Malacologico* **24**: 73–100.

Warén, A., Bouchet, P., 1991. Mollusca Gastropoda: systematic position and revision of *Haloceras* Dall, 1889 (Caenogastropoda, Haloceratidae fam. nov.). *Mémoires du Muséum National d'Histoire Naturelle, Paris, Ser. A* **150**: 111–161.

Warén, A., Gittenberger, E., 1993. *Turbo politus* Linnaeus, 1758 (currently *Melanella polita*; Mollusca, Gastropoda): proposed conservation of usage of the specific name, so conserving the specific name of *Buccinum acicula* Müller, 1774 (currently *Cecilioides acicula*). *Bulletin of Zoological Nomenclature* **50**: 107–110.

Warén, A., Gofas, S., 1996. *Kaiparapelta askewi* McLean & Harasewych, 1995 (Gastropoda: Pseudococculinidae): a spongivorous cocculiniform limpet and a case of remarkable convergence in radular morphology. *Haliotis* **25**: 107–116.

Webster, B.L., Southgate, V.R., Littlewood, D.T.J., 2006. A revision of the interrelationships of *Schistosoma* including the recently described *Schistosoma guineensis*. *International Journal for Parasitology* **36**: 947–955.

Westwood, J.H., Yoder, J.I., Timko, M.P., dePamphilis C.W., 2010. The evolution of parasitism in plants. *Trends in Plant Science* **15**: 227–235.

White, T.J., Bruns, T., Lee, S., Taylor, J.W., 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics, *In*: Innis, M.A., Gelfand, D.H., Sninsky, J.J., White, T.J. (Eds.), *PCR Protocols: a Guide to Methods and Applications.* Academic Press, New York, pp. 315–322.

Whitfield, J.B., 1998. Phylogeny and evolution of host-parasitoid interactions in Hymenoptera. *Annual Review of Entomology* **43**: 129–151.

Whitfield, J.B., 2002. Estimating the age of the polydnavirus/braconid wasp symbiosis. *Proceedings of the National Academy of Sciences* **99**: 7508–7513.

Wiens, J.A., 1989. *The Ecology of Bird Communities*. Cambridge University Press, Cambridge.

Williams, S.T., Reid, D.G., Littlewood, D.T.J., 2003. A molecular phylogeny of the Littorininae (Gastropoda: Littorinidae): unequal evolutionary rates, morphological parallelism, and biogeography of the southern ocean. *Molecular Phylogenetics and Evolution* **28**: 60–86.

Williams, S.T., Ozawa, T., 2006. Molecular phylogeny suggests polyphyly of both the turban shells (family Turbinidae) and the superfamily Trochoidea (Mollusca: Vetigastropoda). *Molecular Phylogenetics and Evolution* **39**: 33–51.

Wilson, B., 1998a. Superfamily Cypraeoidea, *In*: Beesley, P.L., Ross, G.J.B., Wells, A. (Eds.), *Fauna of Australia, Vol. 5, Mollusca: The Southern Synthesis.* CSIRO Publishing, Melbourne, pp. 780–786.

Wilson, B., 1998b. Superfamily Velutinoidea, *In*: Beesley, P.L., Ross, G.J.B., Wells, A. (Eds.), *Fauna of Australia, Vol. 5, Mollusca: The Southern Synthesis.* CSIRO Publishing, Melbourne, pp. 786–790.

Windsor, D.A., 1998. Most of the species on Earth are parasites. *International Journal for Parasitology* **28**: 1939–1941.

Wirtz, P., 2011. New records of marine invertebrates from the coast of Senegal. *Arquipelago - Life and Marine Sciences* **28**: 7–9.

Wise, J.B., 1996. Morphology and phylogenetic relationships of certain pyramidellid taxa (Heterobranchia). *Malacologia* **37**: 443–511.

Witman, J.D., Sebens, K.P., 1992. Regional variation in fish predation intensity: a historical perspective in the Gulf of Maine. *Oecologia* **90**: 305–315.

Wood, C.L., Byers, J.E., Cottingham, K.L., Altman, I., Donahue, M.J., Blakeslee, A.M.H., 2007. Parasites alter community structure. *Proceedings of the National Academy of Sciences* **104**: 9335–9339.

Wrigley, A., 1944. English Eocene Eulimidae with notes on the torsion of *Eulima* and on Charlesworth's illustrations. *Journal of Molluscan Studies* **26**: 47–62.

Yang, Z., Wafula, E.K., Honaas, L.A., Zhang, H., Das, M., Fernandez-Aparicio, M., Huang, K., Bandaranayake, P.C.G., Wu, B., Der, J.P., Clarke, C.R., Ralph, P.E., Landherr, L., Altman, N.S., Timko, M.P., Yoder, J.I., Westwood, J.H., dePamphilis, C.W., 2015. Comparative transcriptome analyses reveal core parasitism genes and suggest gene duplication and repurposing as sources of structural novelty. *Molecular Biology and Evolution* **32**: 767–790.

Yokoyama, M. 1922. Fossils from the Upper Musashino of Kazusa and Shimosa. *Journal of the Collage of Science, Tokyo Imperial University* **44**: 1–200.

Zapata, F., Wilson, N.G., Howison, M., Andrade, S.C.S., Jörger, K.M., Schrödl, M., Goetz, F.E., Giribet, G., Dunn, C.W., 2014. Phylogenetic analyses of deep gastropod relationships reject Orthogastropoda. *Proceedings of the Royal Society B* **281**: 20141739.

Zietara, M.S., Lumme, J., 2002. Speciation by host switch and adaptive radiation in a fish parasite genus *Gyrodactylus* (Monogenea, Gyrodactylidae). *Evolution* **56**: 2445–2458.

Zou, S., Li, Q., Kong, L., 2011. Additional gene data and increased sampling give new insights into the phylogenetic relationships of Neogastropoda, within the caenogastropod phylogenetic framework. *Molecular Phylogenetics and Evolution* **61**: 425–435.

Appendix 1.

Supplementary data for Chapter 1

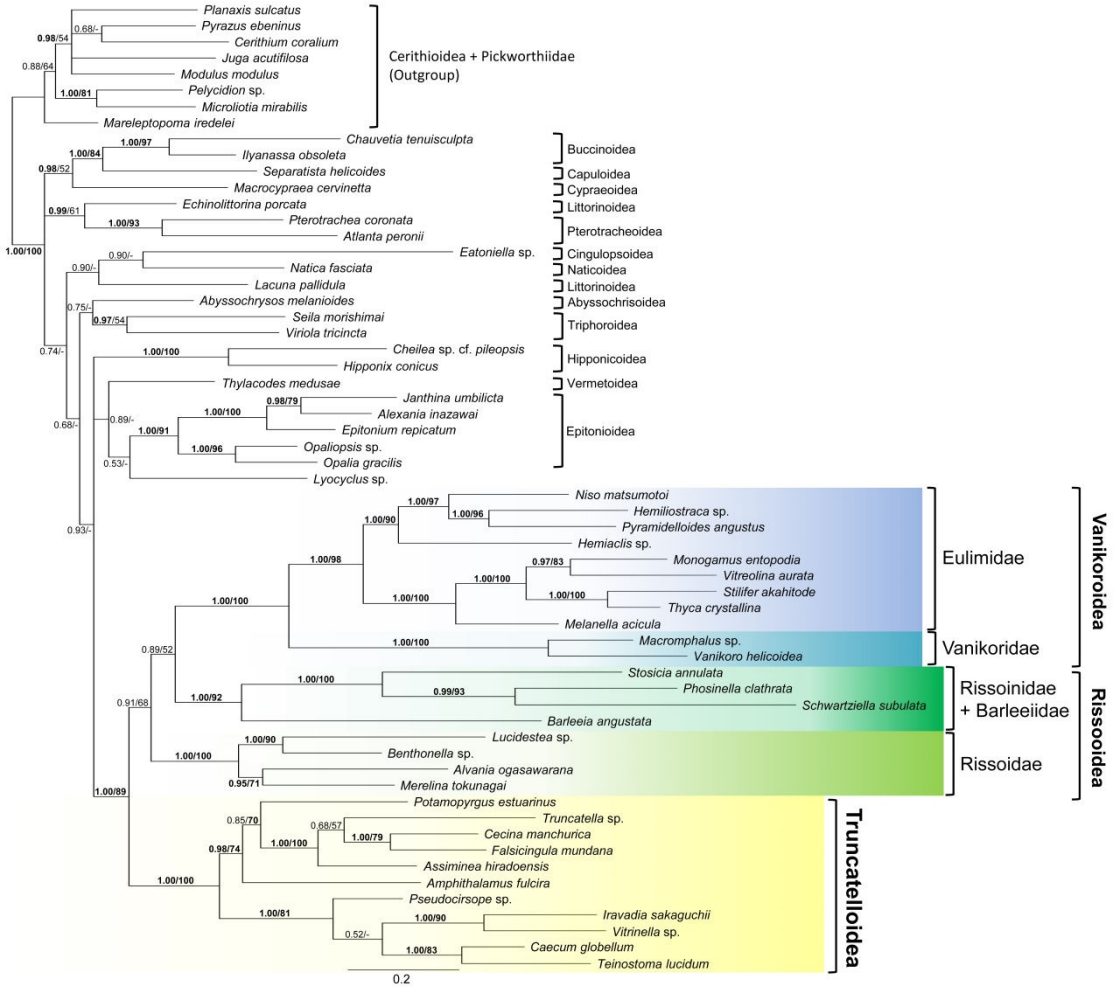

Figure S1-1. Bayesian phylogeny of Hypsogastropoda inferred from 2gPA alignment of 28S (D1–D5) and COI genes (1,926 sites in total). Numerals on branches denote posterior probabilities (PP, left) and likelihood-based bootstrap values shown as percentages (BS, right); significant support in bold (PP ≥ 0.95, BS ≥ 70%).

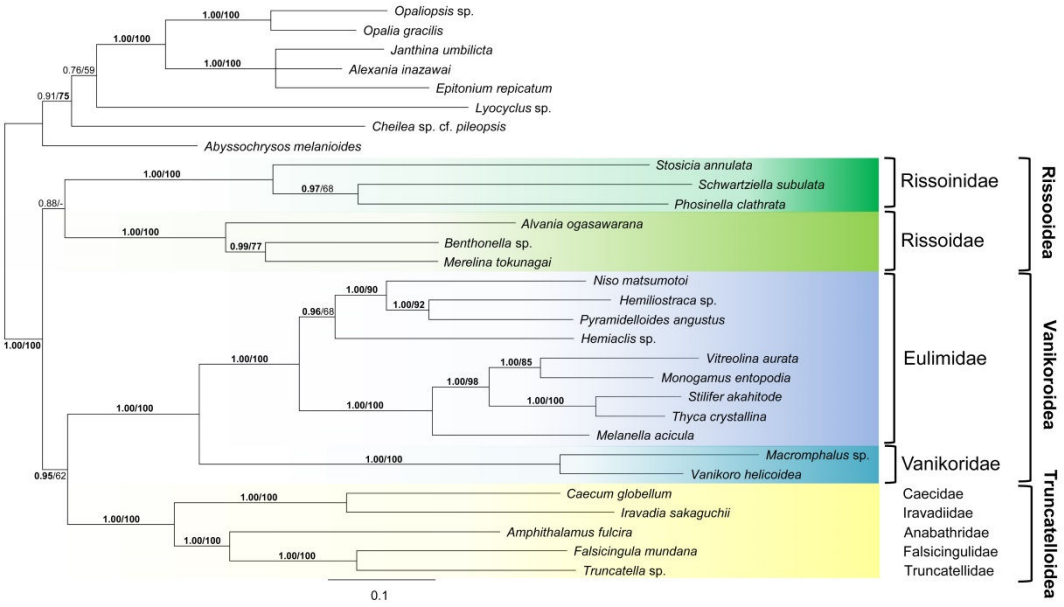

Figure S1-2. Bayesian phylogeny of Vanikoroidea, Truncatelloidea and Rissooidea inferred from 5gPA alignment of 28S (D1–D7b), 18S, H3, 16S and COI genes (5,006 sites in total). Numerals on branches denote posterior probabilities (PP, left) and likelihood-based bootstrap values shown as percentages (BS, right); significant support in bold (PP ≥ 0.95, BS ≥ 70%).

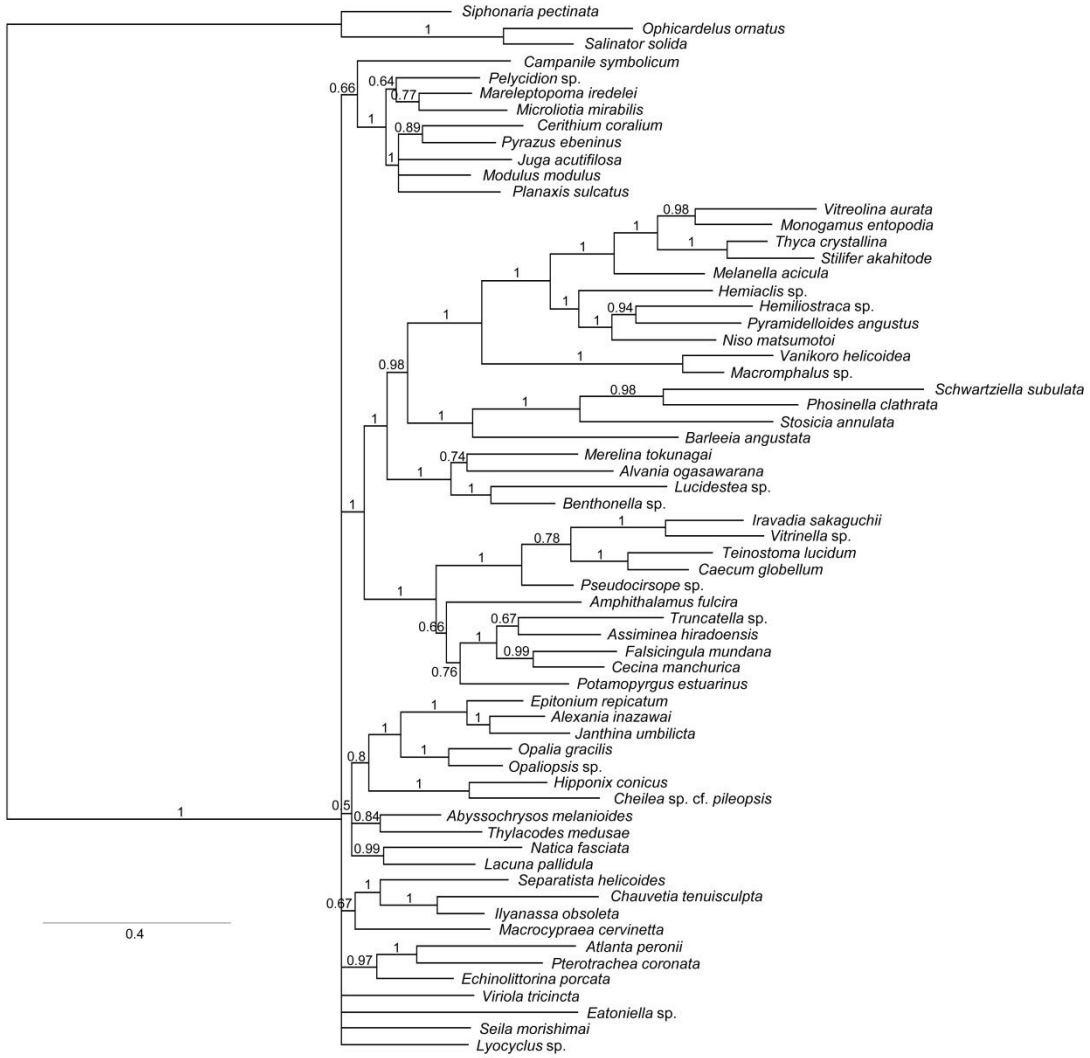

Figure S1-3. Bayesian tree inferred from 28S D1–D5 and COI sequences with *Ophicardelus ornatus*, *Salinator solida*, *Siphonaria pectinata* (Heterobranchia) and *Campanile symbolicum* (Campaniloidea) as outgroup taxa.

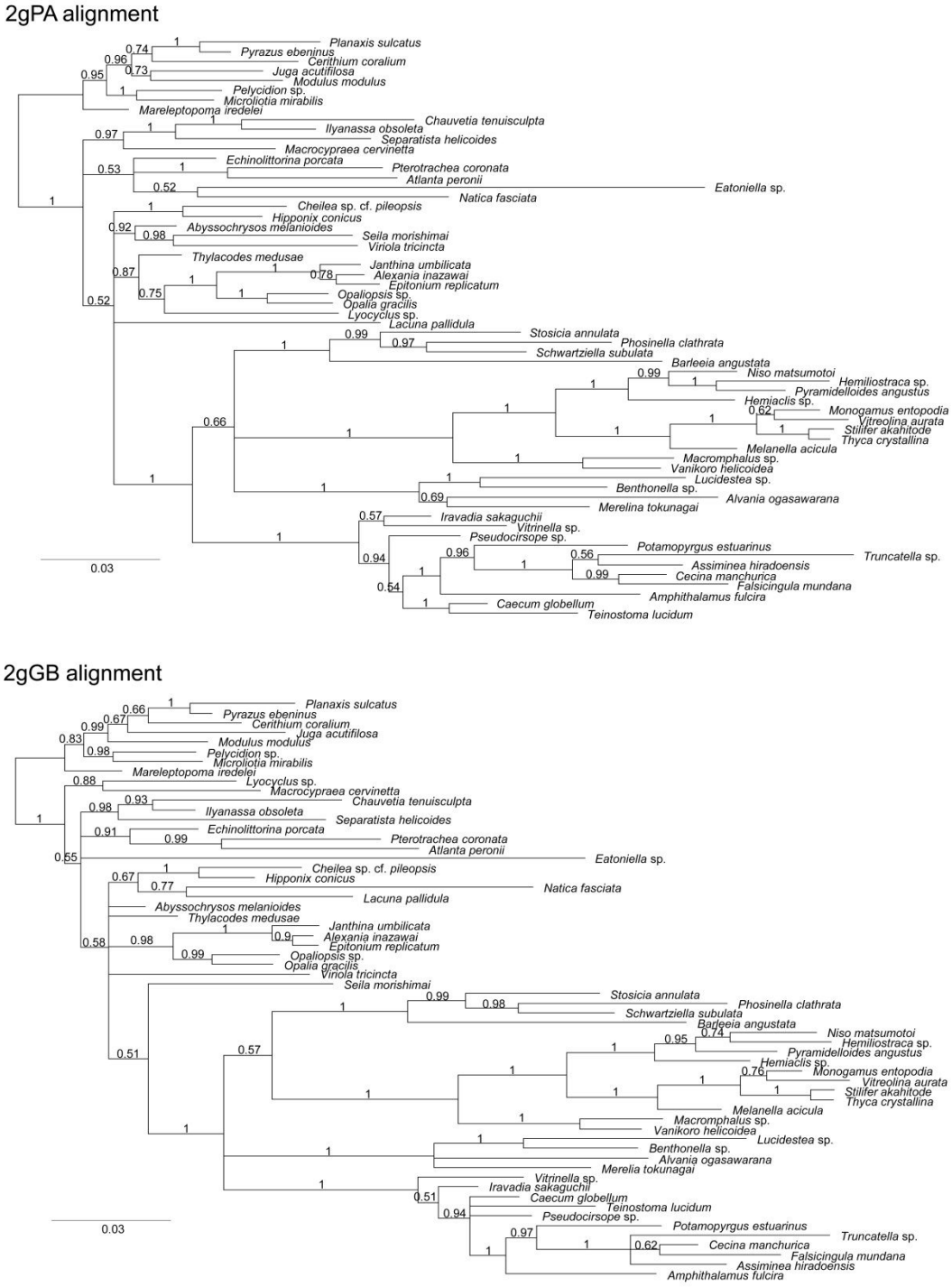

Figure S1-4. Bayesian trees inferred from 28S (D1–D5) gene sequences for two-gene dataset.

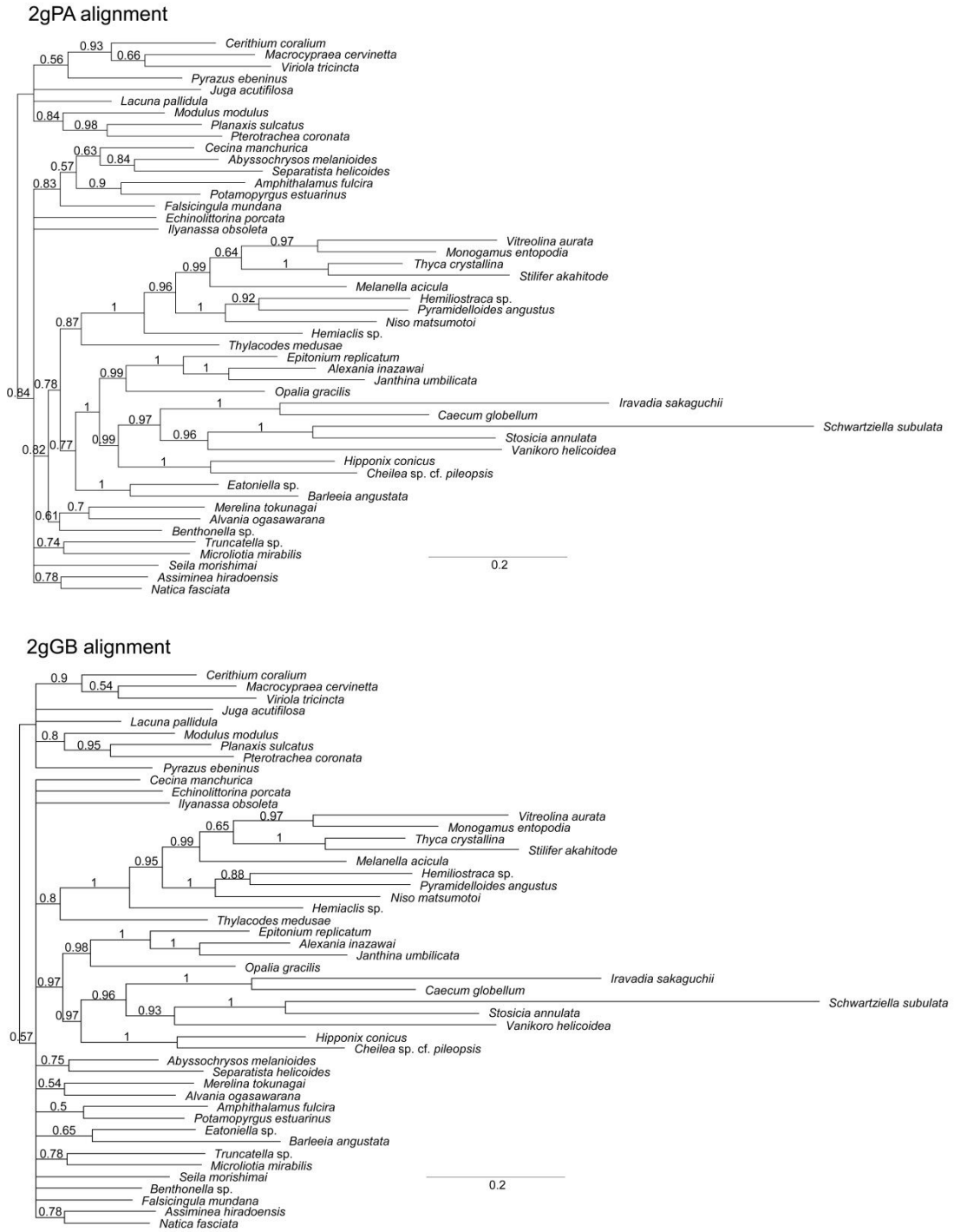

Figure S1-5. Bayesian trees inferred from COI gene sequences for two-gene dataset.

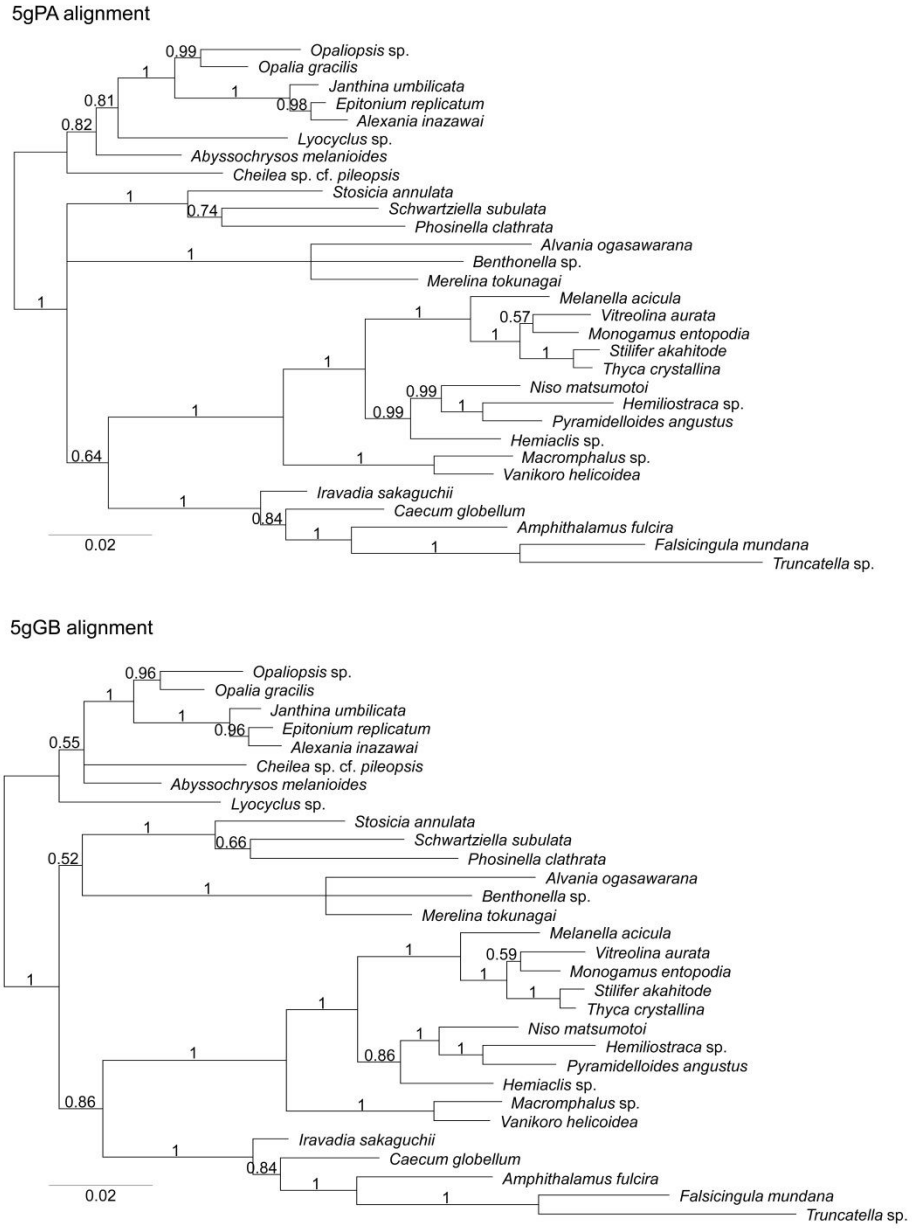

Figure S1-6. Bayesian trees inferred from 28S (D1–D7b) gene sequences for five-gene dataset.

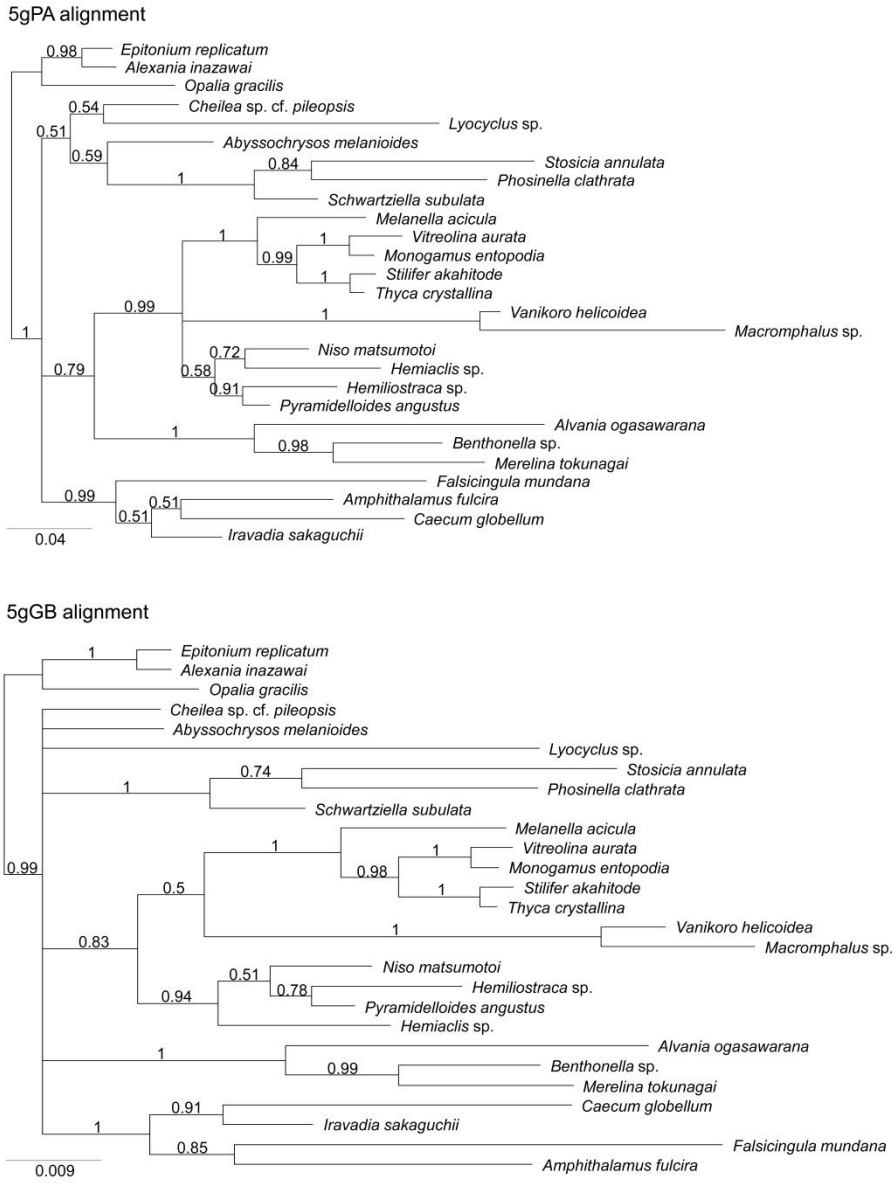

Figure S1-7. Bayesian trees inferred from 18S gene sequences for five-gene dataset.

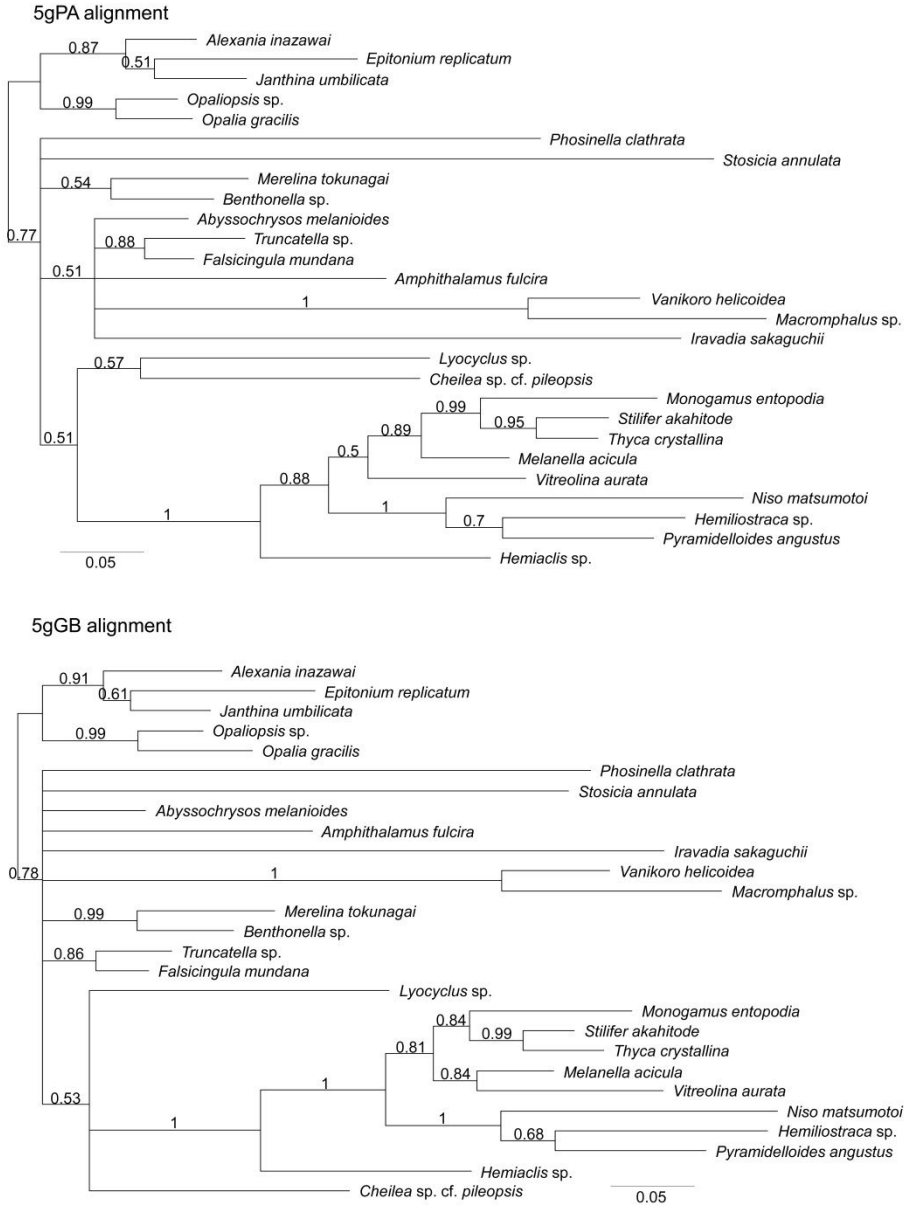

Figure S1-8. Bayesian trees inferred from 16S gene sequences for five-gene dataset.

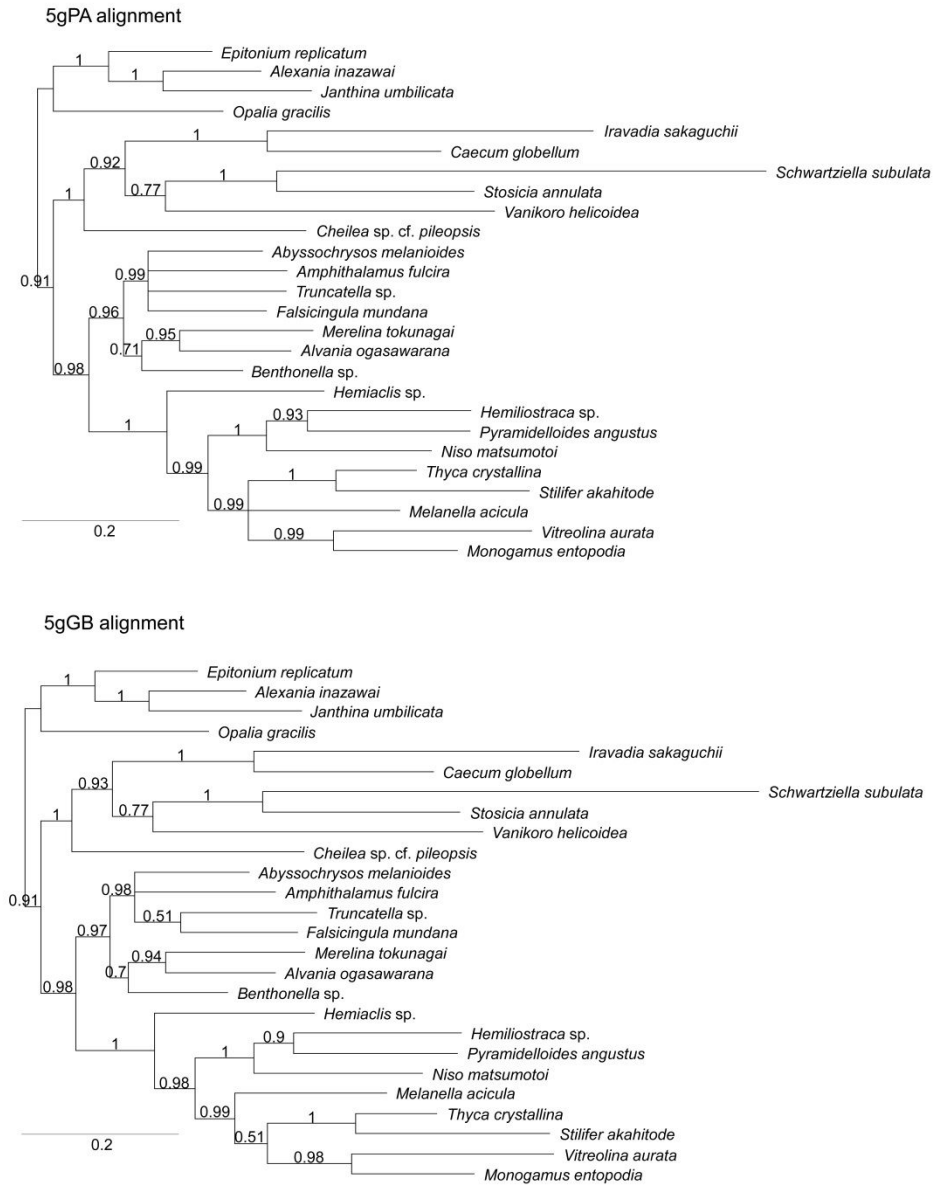

Figure S1-9. Bayesian trees inferred from COI gene sequences for five-gene dataset.

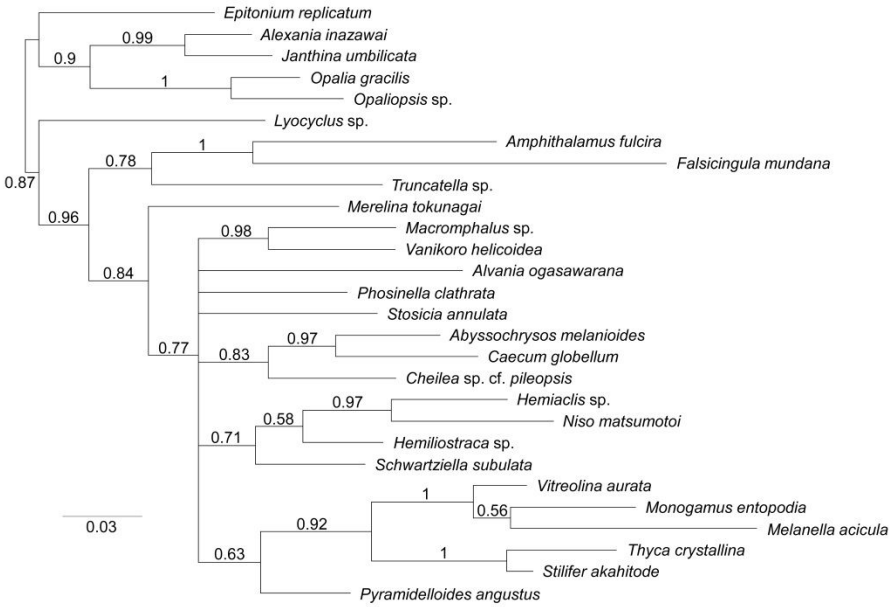

Figure S1-10. Bayesian tree inferred from H3 gene sequences for five-gene dataset.

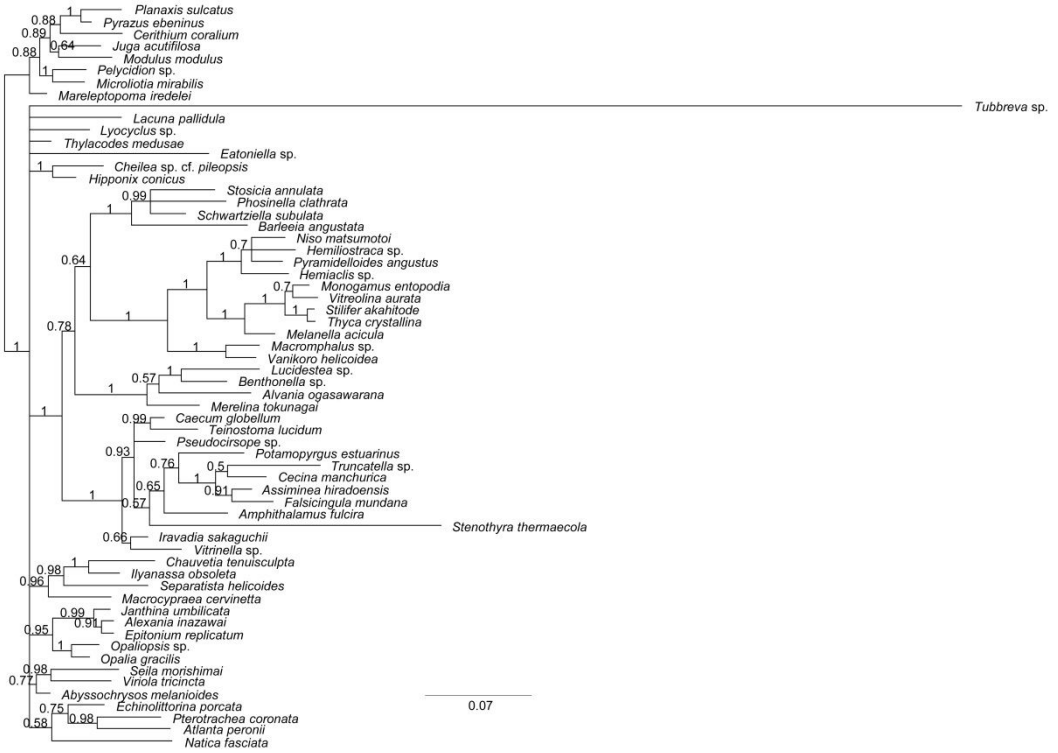

Figure S1-11. Bayesian tree inferred from 28S (D1–D5) gene sequences with *Tubbreva* sp. and *Stenothyra thermaecola*.

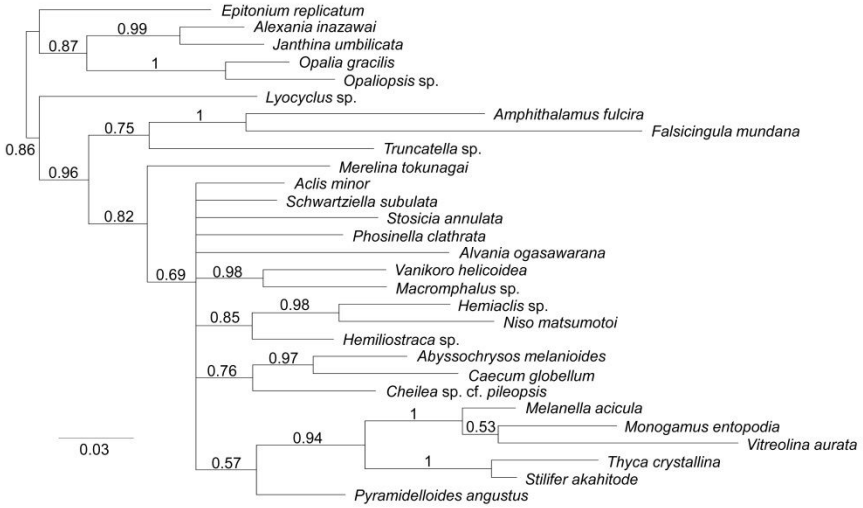

Figure S1-12. Bayesian tree inferred from H3 gene sequences with *Aclis minor*.

Figure S1-13. Live-taken photographs of *Vanikoro* snails, which are almost always found attached on/near sponges on the underside of deep-buried coral rubble. Left: *Vanikoro helicoidea* in Kakeroma Island, Amami, Japan, courtesy of R. Goto. Right: *Vanikoro* sp. cf. *plicata* in Aore Island, Santo, Vanuatu. Note that the shape and arrangement of the greenish egg capsules differ between the two species.

Table S1-1. Nucleotide sequences of primers. Circles represent primers used in each chapter.

Locus	Primer	Sequence	Direction	Chapter 1	Chapter 2	Chapter 3	Reference
28S	28SC1	ACCCGCTGAATTTAAGCAT	Forward			○	Dayrat et al. (2001)
	LSU5	TAGGTCGACCCGCTGAAYTTAAGCA	Forward	○	○	○	Littlewood et al. (2000)
	het3	CCCCAGTAACGGCGAGTGAAGC	Forward			○	Kano et al. (in prep)
	28S_400F	ACTCCATCTAAGGCTA	Forward			○	Kano et al. (in prep)
	28S_500R	CGGTTTCACGTACTCT	Reverse			○	Kano et al. (in prep)
	900F	CCGTCTTGAAACACGGACCAAG	Forward	○	○		Lockyer et al. (2003)
	ECD2S	CTTGGTCCGTGTTTCAAGACGG	Reverse	○	○	○	Williams & Ozawa (2006)
	1100F	GGACCCGAAAGATGGTGAACTATGC	Forward	○	○		Takano & Kano (2014)
	D3m	GACGATCGATTTGCACGTCAGAAT	Reverse	○	○	○	Takano & Kano (2014)
	28SD3	GACGATCGATTTGCACGTCA	Reverse			○	Vonnemann et al. (2005)
	LSU1600R	AGCGCCATCCATTTTCAGG	Reverse	○	○		Williams et al. (2003)
	FL	AAGTGGAGAAGGGTTCCATGT	Forward	○			Takano & Kano (2014)
	hetR	TATCTCCGGGCAAGCCGATTC	Reverse	○			Takano & Kano (2014)
	na2	AGCCAATCCTTATCCCGAAG	Reverse	○			Kano et al. (2002)
18S	18A1	CCTACCTGGTTGATCCTGCCAG	Forward	○	○	○	Steiner & Dreyer (2003)
	NS2	GGCTGCTGGCACCAGACTTGC	Reverse		○	○	White et al. (1990)
	188f	GGATCTATTGGAGGGCAAGT	Forward	○	○	○	Nakamura et al. (2007)
	NS4	CTTCCGTCAATTCCTTTAAG	Reverse		○		White et al. (1990)
	189r	TCGGAATTAACCAGACAAATC	Reverse	○	○	○	Nakamura et al. (2007)
	NS5	AACTTAAAGGAATTGACGGAAG	Forward	○	○	○	White et al. (1990)
	1800r	ATGATCCTTCCGCAGGTTCACC	Reverse	○	○	○	Steiner & Dreyer (2003)

H3	H3MF	ATGGCTCGTACCAAGCAGACTGC	Forward	○	○	○	Kano (2008)
	H3MRI	GGCATGATTGTTACACGCTTGGCGTG	Reverse	○	○	○	Kano et al. (2009)
	H3MR	TGGATGTCCTTGGGCATGATTGTTAC	Reverse	○	○	○	Kano (2008)
16S	Opis A-Rm	ACCCTTATACAARAGG	Reverse			○	This study, modified from Grande et al. (2004)
	16Sar-L	CGCCTGTTTATCAAAAACAT	Forward	○	○	○	Palumbi et al. (1991)
	16Sar-veti	GCCTGTTTAGCAAAAACA	Forward	○	○	○	Kano et al. (2009)
	Opis 1-R	ATTAYGCTACCTTAGCACRGTCA	Reverse			○	Grande et al. (2002)
	16Sab-veti	GATCACGTAAGATTTTAATGGTCG	Reverse	○	○	○	Kano et al. (2009)
	16Sbr-H	CCGGTCTGAACTCAGATCACGT	Reverse	○	○	○	Palumbi et al. (1991)
12S	12S1	GTGCCAGCAGTCGCGGTTAXA	Forward		○		Kano et al. (in prep)
	12S2v	CGAGAGYGACGGGCGA	Reverse		○		Kano et al. (in prep)
	12S2	TACCCCTACTATGTTACGACT	Reverse		○		Kano et al. (in prep)
COI	LCO1490	GGTCAACAAATCATAAAGATATTGG	Forward	○	○	○	Folmer et al. (1994)
	Opis A-Fm	GGRGCARTTAATTTTATTAC	Forward			○	This study, modified from Grande et al. (2004)
	LCOmod	TCTACTAATCATAAGGAYATYGGNAC	Forward	○	○		Kano (2008)
	HCOmod	ACTTCTGGGTGTCCRAARAAYCARAA	Reverse	○	○		Kano (2008)
	HCO2198	TAAACTTCAGGGTGACCAAAAAATCA	Reverse	○	○	○	Folmer et al. (1994)
	Opis COI-Fm	ACTTTTTTTCCTCAACATTTYTT	Forward			○	Kano et al. (in prep), modified from Grande et al. (2004)

Table S1-2: Pairwise p-distance matrix of H3 sequences. *S. subulata* and *Macromphalus* sp. show the lowest distances for *A. minor* (red-letters).

Aclis minor																															
Vanikoro helicoidea	0.088																														
Macromphalus sp.	**0.062**	0.075																													
Vitreolina auratus	0.104	0.091	0.094																												
Monogamus sp.	0.097	0.107	0.081	0.055																											
Thyca cristallina	0.097	0.101	0.110	0.101	0.081																										
Stilifer akahitode	0.091	0.097	0.097	0.084	0.071	0.042																									
Melanella acicula	0.110	0.097	0.097	0.088	0.088	0.130	0.123																								
Hemiaclis sp.	0.094	0.107	0.104	0.114	0.123	0.127	0.120	0.127																							
Hemiliostraca sp.	0.071	0.081	0.075	0.101	0.104	0.123	0.107	0.110	0.071																						
Niso matsumotoi	0.081	0.104	0.084	0.110	0.107	0.127	0.120	0.101	0.071	0.078																					
Pyramidelloides angusta	0.071	0.094	0.071	0.091	0.101	0.101	0.091	0.104	0.091	0.068	0.075																				
Epitonium replicatum	0.146	0.140	0.130	0.149	0.166	0.162	0.166	0.159	0.127	0.136	0.133	0.117																			
Alexania inazawai	0.088	0.110	0.104	0.123	0.127	0.133	0.123	0.136	0.075	0.084	0.088	0.084	0.101																		
Opalia gracilis	0.114	0.143	0.149	0.156	0.175	0.156	0.140	0.175	0.133	0.143	0.146	0.127	0.120	0.094																	
Janthina umbricata	0.084	0.114	0.097	0.107	0.123	0.127	0.120	0.133	0.078	0.091	0.101	0.088	0.107	0.042	0.104																
Abyssochrysos melanoides	0.075	0.088	0.094	0.110	0.101	0.114	0.114	0.117	0.094	0.075	0.097	0.094	0.143	0.104	0.146	0.097															
Schwartziella subulata	**0.052**	0.097	0.081	0.104	0.110	0.117	0.117	0.107	0.068	0.062	0.088	0.084	0.136	0.091	0.123	0.071	0.075														
Manzonia tokunagai	0.088	0.107	0.084	0.120	0.114	0.127	0.117	0.117	0.078	0.088	0.084	0.097	0.127	0.088	0.130	0.084	0.101	0.091													
Amphithalamus fulcira	0.149	0.143	0.153	0.182	0.188	0.156	0.162	0.188	0.127	0.140	0.149	0.136	0.159	0.133	0.153	0.149	0.143	0.143	0.149												
Stosicia annulata	0.078	0.101	0.081	0.114	0.114	0.123	0.101	0.123	0.104	0.101	0.097	0.097	0.143	0.107	0.127	0.110	0.097	0.094	0.107	0.136											
Rissoina clathrata	0.084	0.088	0.088	0.097	0.101	0.127	0.104	0.101	0.107	0.094	0.104	0.091	0.133	0.110	0.133	0.104	0.110	0.081	0.097	0.169	0.088										
Caecum globella	0.088	0.101	0.107	0.110	0.130	0.104	0.104	0.133	0.101	0.094	0.107	0.091	0.136	0.091	0.130	0.081	0.065	0.084	0.114	0.156	0.091	0.123									
Truncatella sp.	0.114	0.114	0.110	0.146	0.146	0.153	0.143	0.143	0.101	0.107	0.110	0.097	0.127	0.084	0.136	0.101	0.123	0.107	0.114	0.127	0.123	0.143	0.127								
Alvania ogasawarana	0.114	0.097	0.088	0.127	0.120	0.136	0.127	0.136	0.104	0.114	0.117	0.104	0.143	0.114	0.166	0.110	0.123	0.114	0.107	0.156	0.104	0.104	0.123	0.117							
Cheilea cf. *pileopsis*	0.071	0.104	0.088	0.127	0.110	0.117	0.120	0.136	0.107	0.084	0.104	0.101	0.130	0.084	0.120	0.088	0.068	0.081	0.104	0.143	0.091	0.097	0.091	0.107	0.104						
Falsicingula mundana	0.136	0.175	0.159	0.198	0.198	0.205	0.195	0.211	0.153	0.140	0.172	0.146	0.172	0.162	0.166	0.172	0.172	0.159	0.156	0.159	0.146	0.146	0.159	0.159	0.153	0.162					
cf. *Opaliopsis* sp.	0.107	0.130	0.130	0.143	0.159	0.140	0.130	0.172	0.117	0.127	0.120	0.120	0.123	0.091	0.058	0.088	0.133	0.120	0.127	0.153	0.130	0.130	0.130	0.127	0.133	0.110	0.162				
Lyocyclus sp.	0.149	0.159	0.162	0.166	0.179	0.153	0.172	0.195	0.127	0.146	0.149	0.130	0.123	0.114	0.140	0.117	0.149	0.136	0.136	0.156	0.159	0.149	0.130	0.140	0.156	0.133	0.169	0.143			

Appendix 2.

Supplementary data for Chapter 2

Plates 2-1–2-9. Selected photos of study species.

Figure S2-1. Bayesian tree inferred from a separate dataset with 101 species.

Figure S2-2. Bayesian tree inferred from a full-dataset with 104 species.

Figures S2-3–S2-14. Independent-gene trees.

Table S2-1. Shell measurements for recent species

Table S2-2. Shell measurements for fossil species

Plate 2-1.
Eulimids used for molecular phylogenetic reconstruction with reference to the specimen number and shell size (A–F, group U1; G–I, group U2; J–M, group A1).

A. *Hemiaclis* sp. A (YK#1580); shell height = 5.5 mm

B. *Hemiaclis* sp. B (YK#2653); 6.7 mm

C. Eulimidae gen. sp. "Bullet" (YK#2652); 16.5 mm

D. *Niso* sp. cf. *rubropicta* (YK#2646); 13.4 mm

E. *Thaleia* sp. cf. *nisonis* (YK#2662); 7.0 mm

F. *Niso* sp. cf. *dorcas* (YK#1967); 20.0 mm

G. *Microeulima* sp. (YK#2554); 2.5 mm

H. *Eulimostraca* sp. aff. *fulricinata* (YK#2661); 4.3 mm

I. *"Haliella"* sp. B (YK#2656); 12.5 mm

J. *Niso matsumotoi* (YK#1594); 9.7 mm

K. *Niso regia* (the same species of YK#2645); 20.6 mm

L. *Costaclis* sp. (YK#2659); 28.8 mm

M. *Niso* sp. cf. *tricolor* (YK#2655); 25.1 mm

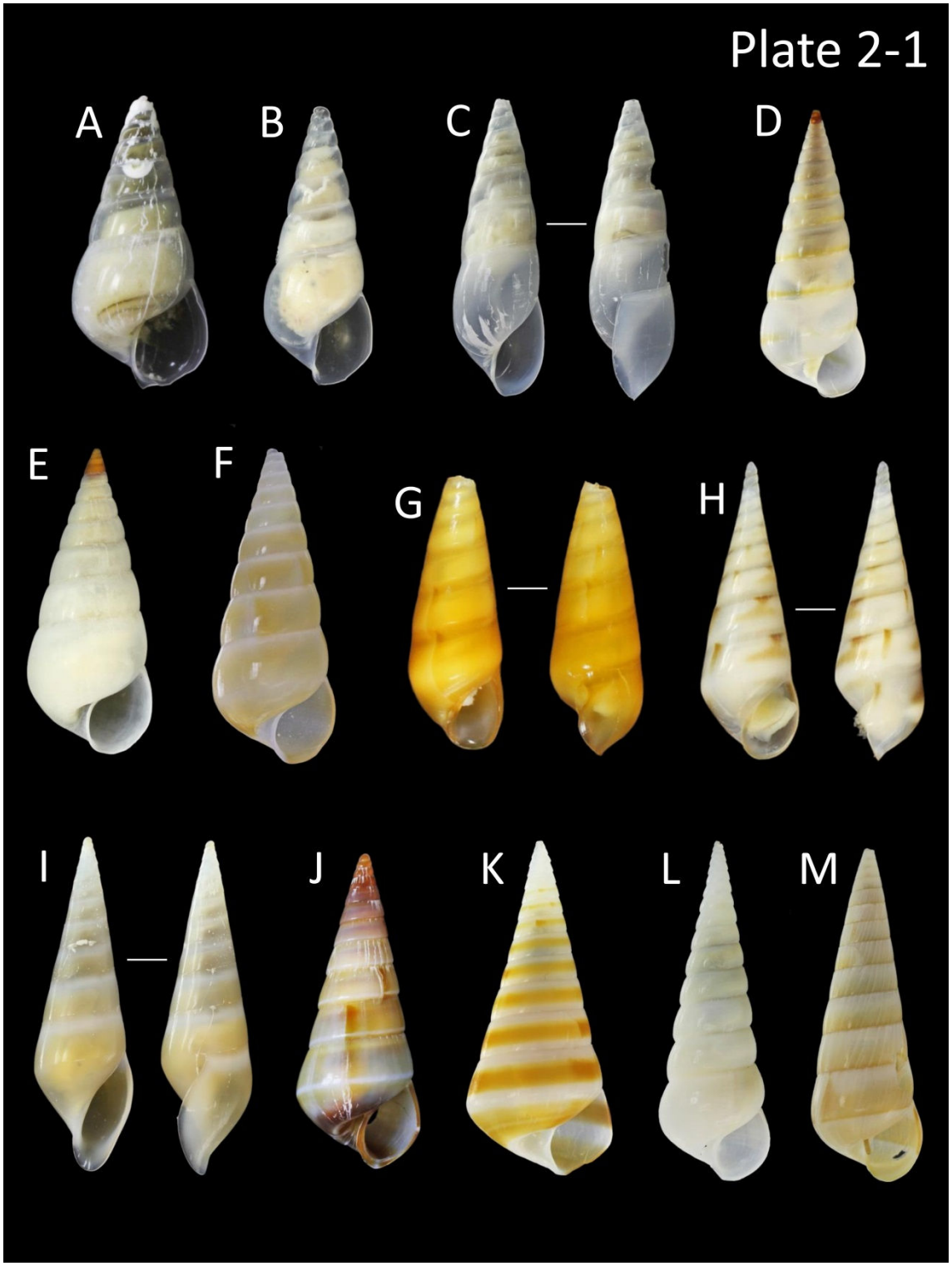
Plate 2-1
A
B
C
D
E
F
G
H
I
J
K
L
M

Plate 2-2.
Eulimids used for molecular phylogenetic reconstruction with reference to the specimen number and shell size (group O1).

A. *"Haliella"* sp. C (YK#2658); shell height = 8.0 mm

B. *Eulima* sp. cf. *maria* (YK#1589); 5.8 mm (broken)

C. *Eulima bifascialis* (YK#2547); 11.8 mm

D. *Eulima* sp. (YK#2061); 5.0 mm

E. *Aclis thesauraria* (YK#2537); 2.2 mm

F. *Hemiliostraca* sp. A (YK#1584); 2.2 mm

G. *Arcuella* sp. (YK#2648); 5.5 mm

H. *Hemiliostraca peasei* (YK#1518); 5.0 mm

I. Cf. *Fusceulima* sp. (YK#1613); 2.0 mm

J. *Sticteulima amamiensis* (YK#1586); 2.7 mm (broken)

K. Cf. *Mucronalia* sp. (YK#2060); 1.7 mm

L. *Sticteulima* sp. (YK#2294); 5.0 mm

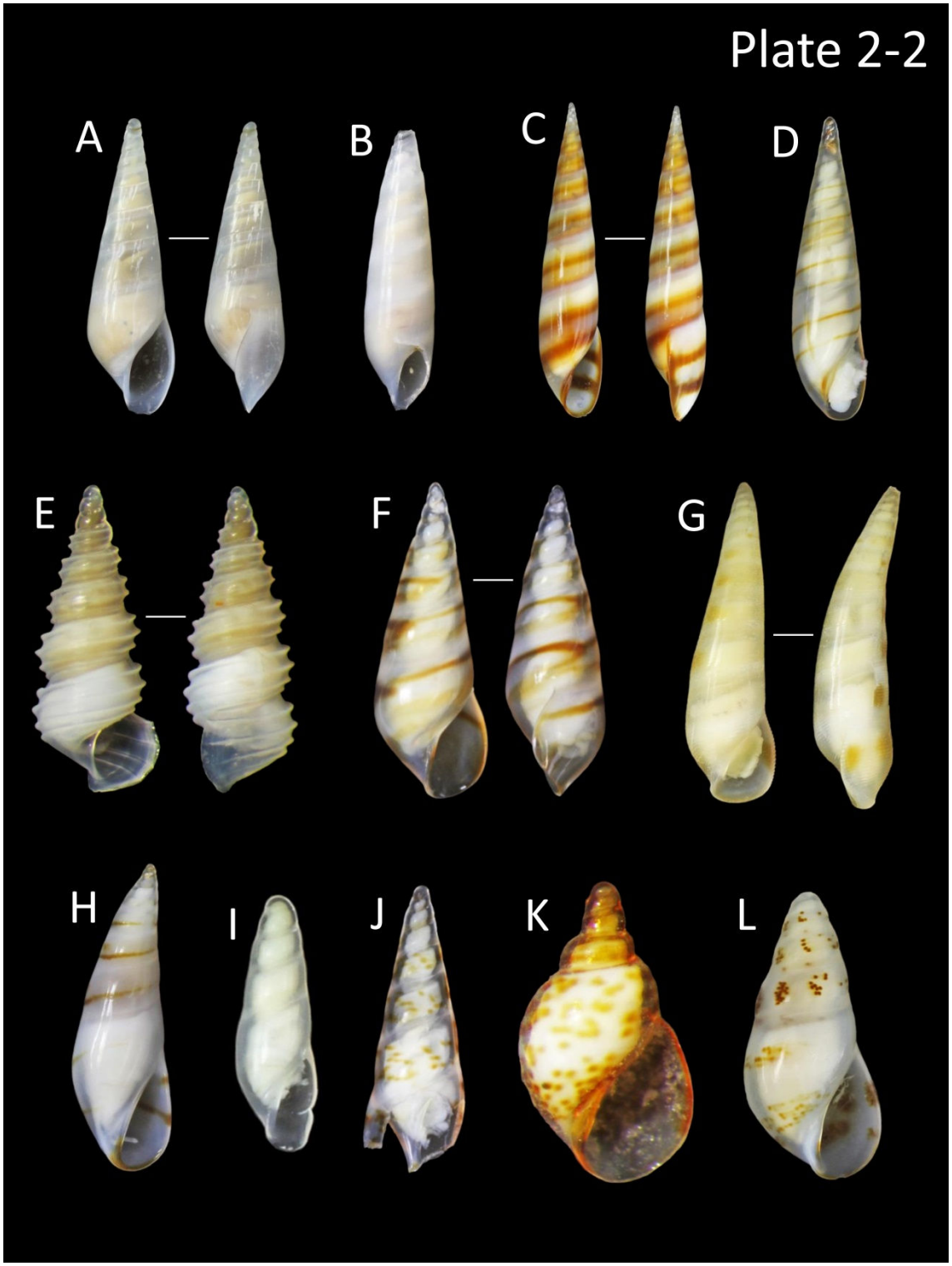
Plate 2-2
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Plate 2-3.
Eulimids used for molecular phylogenetic reconstruction with reference to the specimen number and shell size (A–G, group O1; I–K, group E1; J–M, group A1).

A. *Erisilia mediterranea* (YK#2056); shell height = 1.6 mm

B. Eulimidae gen. sp. "Varix" (YK#2553); 1.8 mm

C. *"Haliella"* sp. D (YK#2660); 5.4 mm

D. Cf. *Oceanida* sp. (YK#2494); 3.5 mm

E. *Palisadia* sp. cf. *subulata* (YK#2493); c. 1 mm (broken)

F. *Pyramidelloides angustus* (YK#1601); 2.5 mm

G. *Pyramidelloides* sp. (YK#1610); 2.7 mm

H. *Haliella* sp. (YK#1520); 7.1 mm

I. *"Melanella" araeosomae* (YK#2657); 24.3 mm

J. *"Strombiformis" langforgi* (YK#1542); 3.4 mm

K. *"Vitreolina" akauni* (YK#1611); 4.4 mm

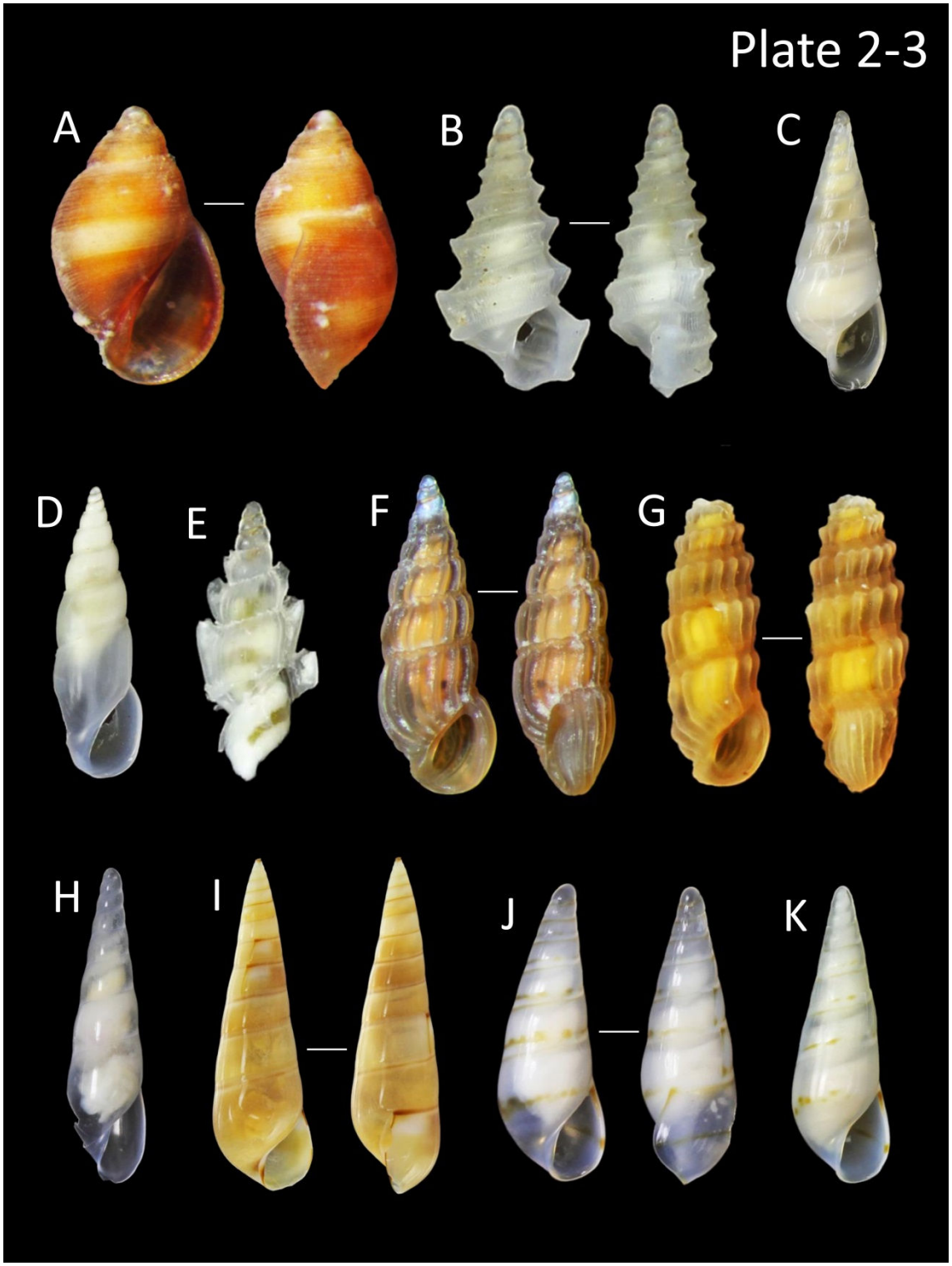
Plate 2-3
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Plate 2-4.
Eulimids used for molecular phylogenetic reconstruction with reference to the specimen number and shell size (A–C, group E1; D–F, group H1; G and H, group C1).

A. *Pulicicochlea astropyga* (YK#2647); shell height = 1.4 mm

B. *Pelseneeria* sp. A (YK#1587); 6.1 mm

C. *Pelseneeria* sp. B (YK#1862); 3.2 mm

D. Eulimidae gen. sp. “Nagasaki_A” (YK#1480); 5.1 mm

E. Eulimdiae gen. sp. “Nagasaki_B” (YK#1544); 3.3 mm (broken)

F. *Enteroxenos oestergreni* (YK#1653); c. 50 mm

G. *Curveulima* sp. (YK#2552); 3.7 mm

H1. *Tropiometricola* sp. (female; YK#2651); 2.1 mm

H2. *Tropiometricola* sp. (male); 1.3 mm

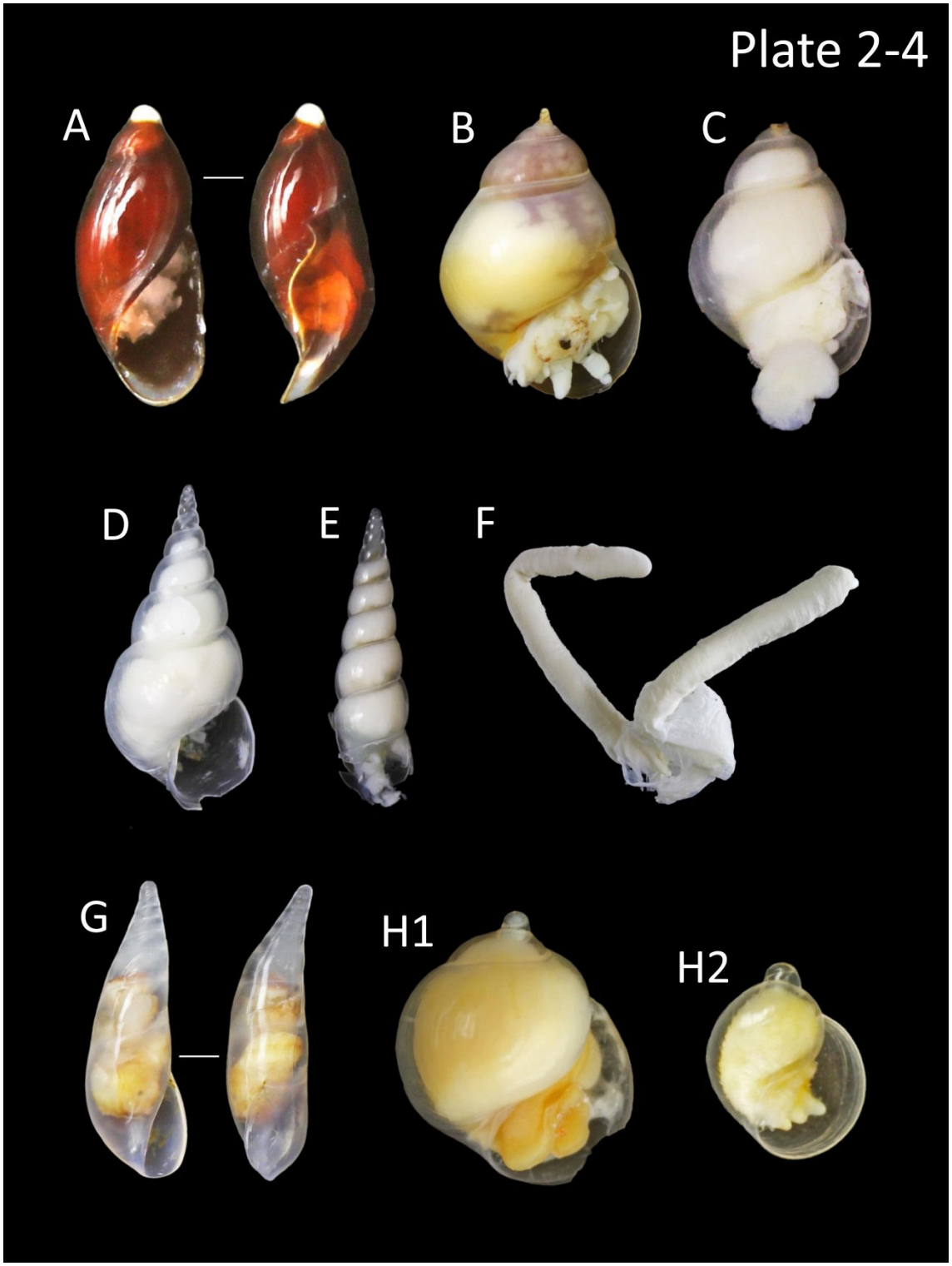
Plate 2-4
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H1
H2

Plate 2-5.
Eulimids used for molecular phylogenetic reconstruction with reference to the specimen number and shell size (A–D, group U3; E–O, group H2).

A. Cf. *Melanella* sp. H (YK#1581); shell height = 3.8 mm

B. Eulimidae gen. sp. "KH" (YK#2550); 3.6 mm

C. Cf. *Crinolamia* sp. A (YK#1824); 7.0 mm

D. Cf. *Crinolamia* sp. B (YK#1825); 5.7 mm

E. *Melanella* sp. G (YK#1579); 4.3 mm (broken)

F. *Melanella* sp. J (YK#1588); 11.4 mm

G. *Melanella* sp. A (YK#1523); 8.4 mm

H. *Melanella kuronamako* (YK#1548); 5.3 mm

I. *Melanella acicula* (YK#1571); 6.3 mm

J. *Melanella* sp. cf. *tortuosa* (YK#1573); 6.8 mm

K. *Melanella* sp. B (YK#1569); 7.0 mm

L. *Melanella* sp. C (YK#1572); 14.2 mm

M. *Melanella* sp. I (YK#1585); 3.0 mm

N. *Melanella* sp. D (YK#1574); 4.7 mm

O. *"Hypermastus" lacteus* (YK#1625); 2.1 mm

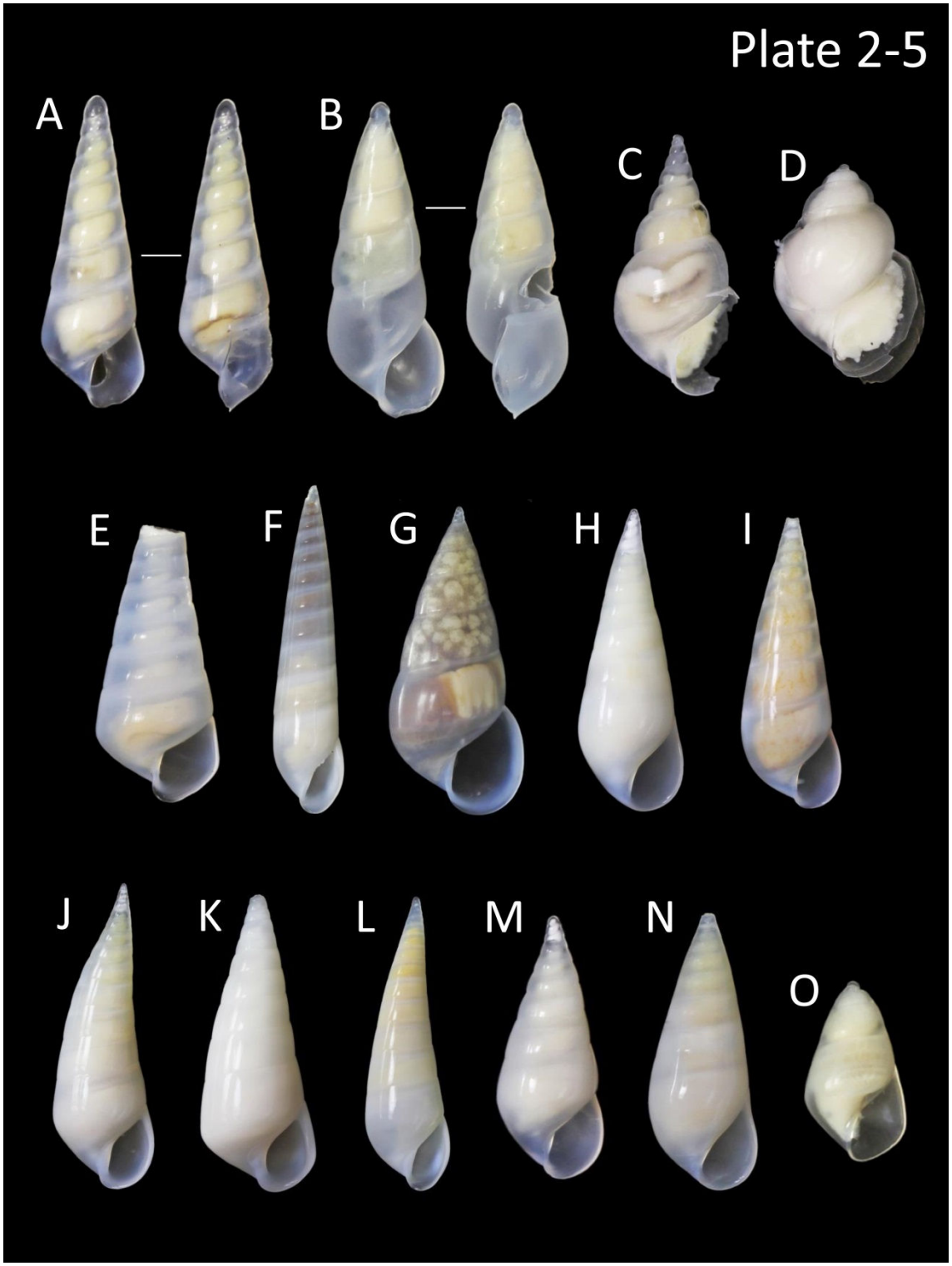
Plate 2-5
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Plate 2-6.
Eulimids used for molecular phylogenetic reconstruction with reference to the specimen number and shell size (A–C, group C2; D–G, group O2; H–K, group E1).

A. *Crinophtheiros collinsi* (YK#2059); shell height = 3.2 mm

B. *Annulobalcis yamamotoi* (YK#1822, the same species of YK#1479); 13.9 mm

C. *Goodingia varicosa* (YK#1968); 3.8 mm

D. *Vitreolina incurva* (YK#2024); 2.4 mm

E. *Stilapex lactarius* (YK1471, the same species of YK#1596); 8.8 mm

F. *Stilapex* sp. B (YK#1605); 2.8 mm

G. *Stilapex* sp. A (YK#1600); 1.8 mm

H. *Vitreolina aurata* (YK#1475); 4.2 mm

I. *Hypermastus echinocardiophilus* (YK#1820); 13.1 mm

J. *Echineulima* sp. (YK#1478); 7.2 mm

K. *Scalenostoma subulatum* (YK#2551); 7.7 mm

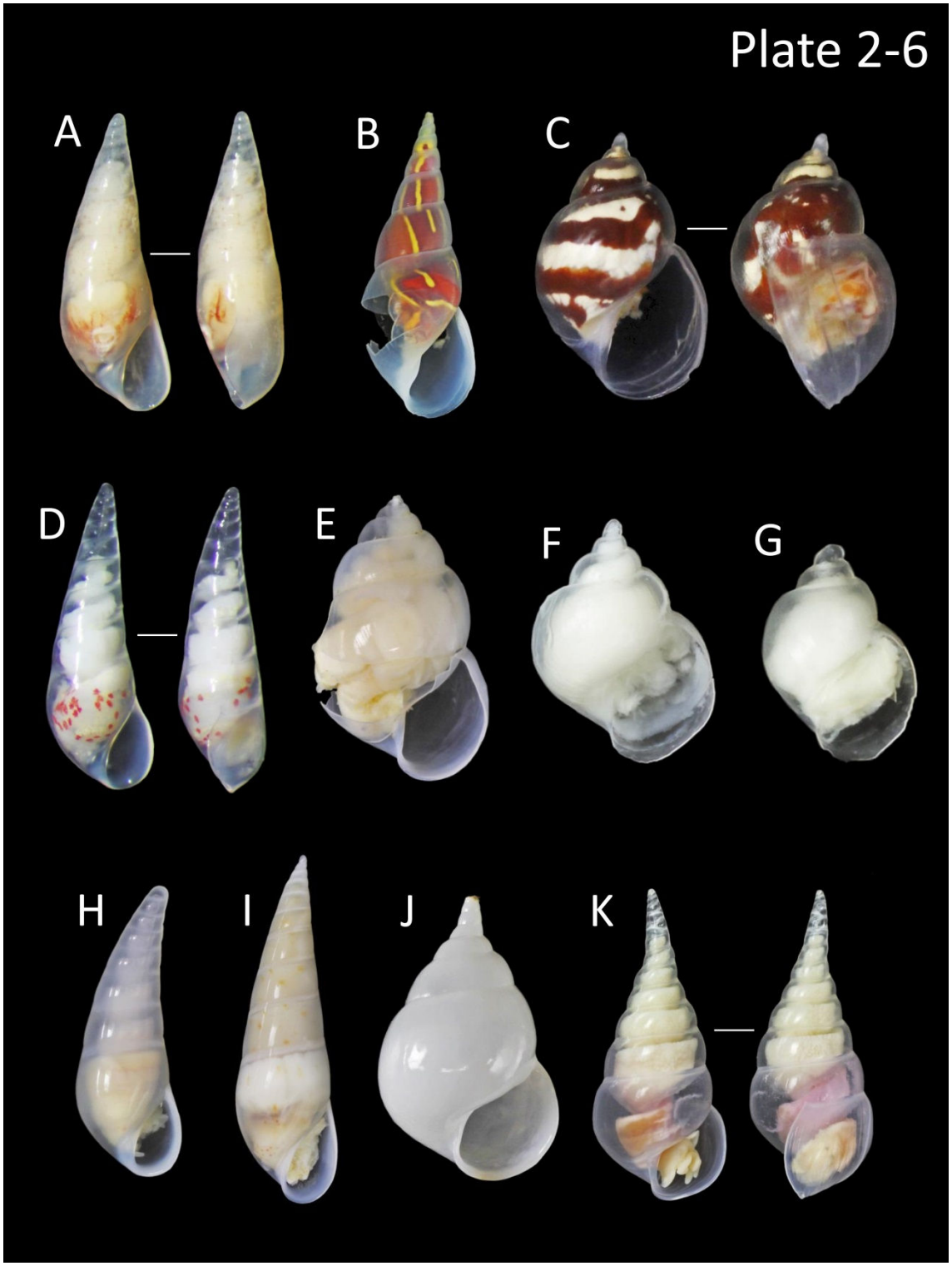
Plate 2-6
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Plate 2-7.
Eulimids used for molecular phylogenetic reconstruction with reference to the specimen number and shell size (group E2).

A. *Hypermastus peronellicola* (YK#1597); shell height = 3.9 mm

B1. *Robillardia cernica* (female; the same species of YK#1629); 4.0 mm

B2. *Robillardia cernica* (male); soft-part length = 4.4 mm

C. *Sabinella* sp. (YK#1522); 7.4 mm

D1. *Monogamus entopodia* (female; YK#1481); 2.7 mm

D2. *Monogamus entopodia* (male); 1.8 mm

E1. *Monogamus* sp. (female; YK#1570); 3.1 mm

E2. *Monogamus* sp. (male); 1.7 mm

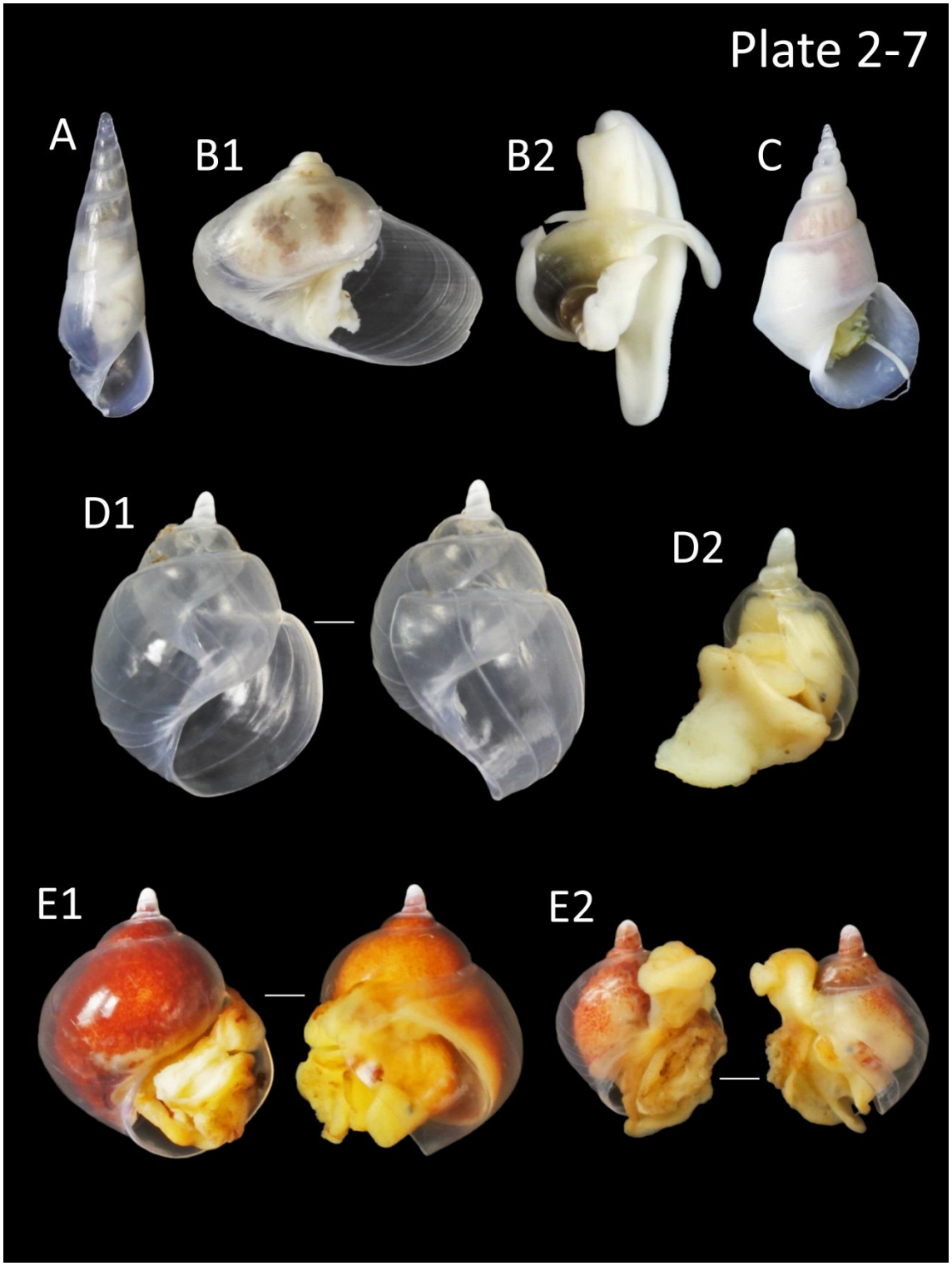
Plate 2-7
A
B1
B2
C
D1
D2
E1
E2

Plate 2-8.
Eulimids used for molecular phylogenetic reconstruction with reference to the specimen number and shell size (A–F, group H3; G–J, group A2).

A. *Melanella cumingii* (YK#1470); shell height = 23.2 mm

B. *Melanella* sp. F (YK#1577); 9.9 mm

C. *Melanella* sp. E (YK#1575); 3.4 mm

D. *Peasistilifer nitidula* (YK#1546); 4.2 mm

E. *Peasistilifer edulis* (YK#1607); 7.9 mm

F. *Megadenus "atrae"* (YK#1821); 3.5 mm

G. *Apicalia habei* (YK#1477); 4.8 mm

H. *Apicalia echinasteri* (YK#2293); 6.8 mm

I. *Parvioris shoplandi* (YK#1549); 3.3 mm

J. *Parvioris fulvescens* (YK#1543); 2.8 mm

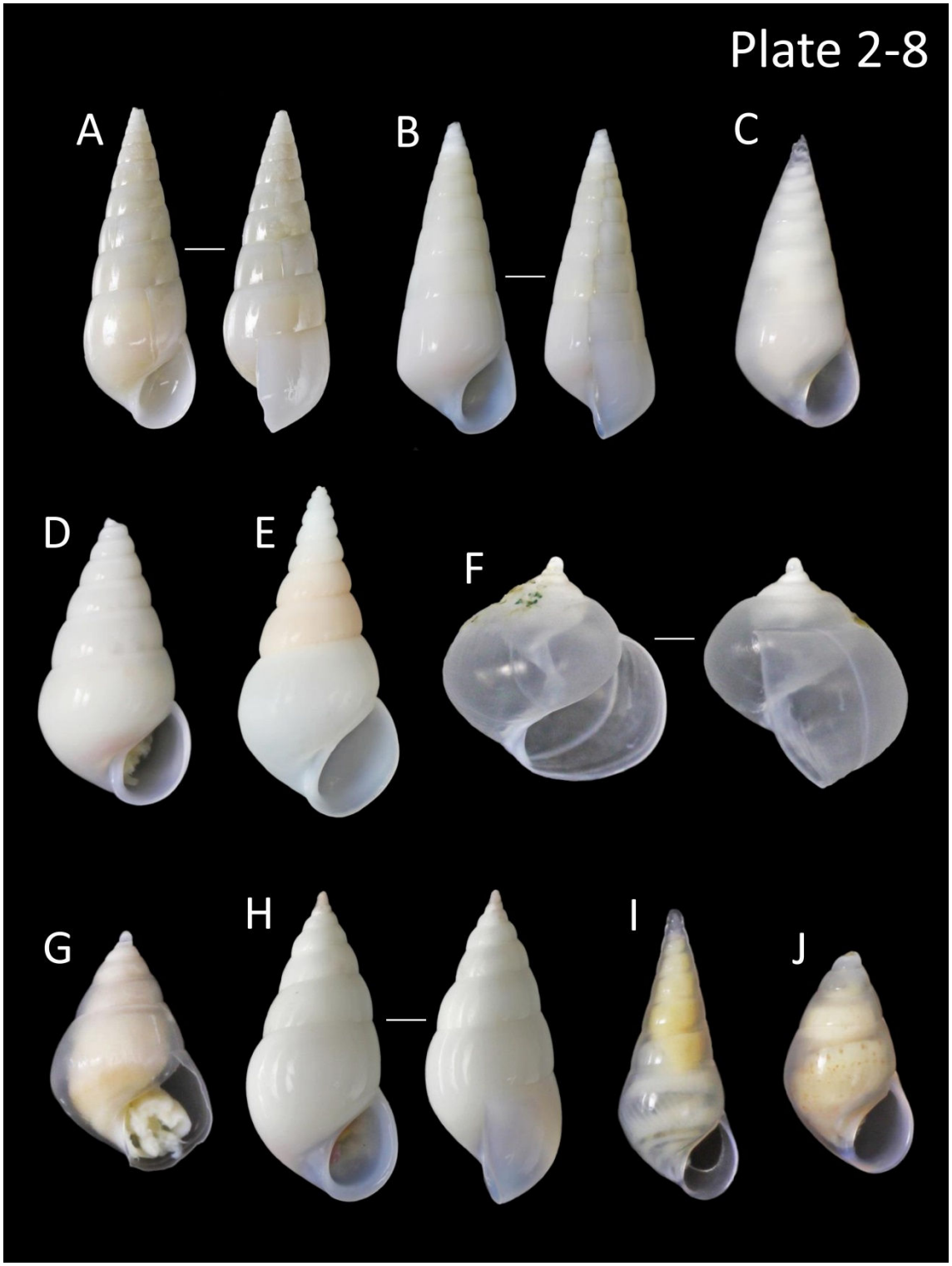
Plate 2-8
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Plate 2-9.
Eulimids used for molecular phylogenetic reconstruction with reference to the specimen number and shell size (group A2).

A. *"Apicalia" palmipedis* (YK#2078); shell height = 3.2 mm

B. *Stilifer akahitode* (YK#1541); 10.2 mm

C. *Stilifer utinomi* (YK#1608); 4.2 mm

D. *Thyca astericola* (YK#2496); height = 8.1 mm

E. *Thyca crystallina* (YK#1519); shell length = 4.9 mm

F. *Thyca lactea* (YK#1472); 6.9 mm

G. *Stilifer* sp. aff. *pisum* (YK#1476); 8.7 mm

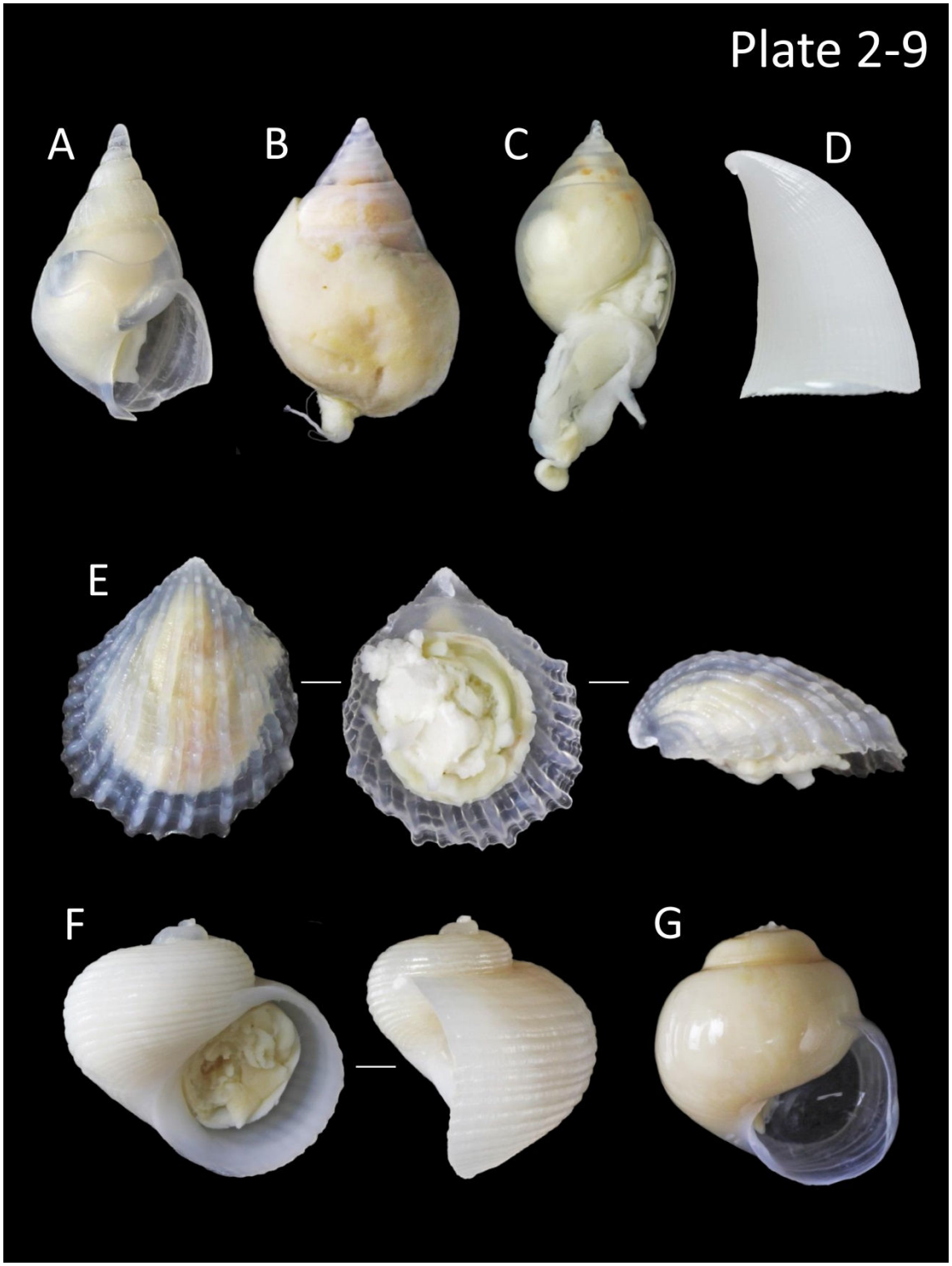
Plate 2-9
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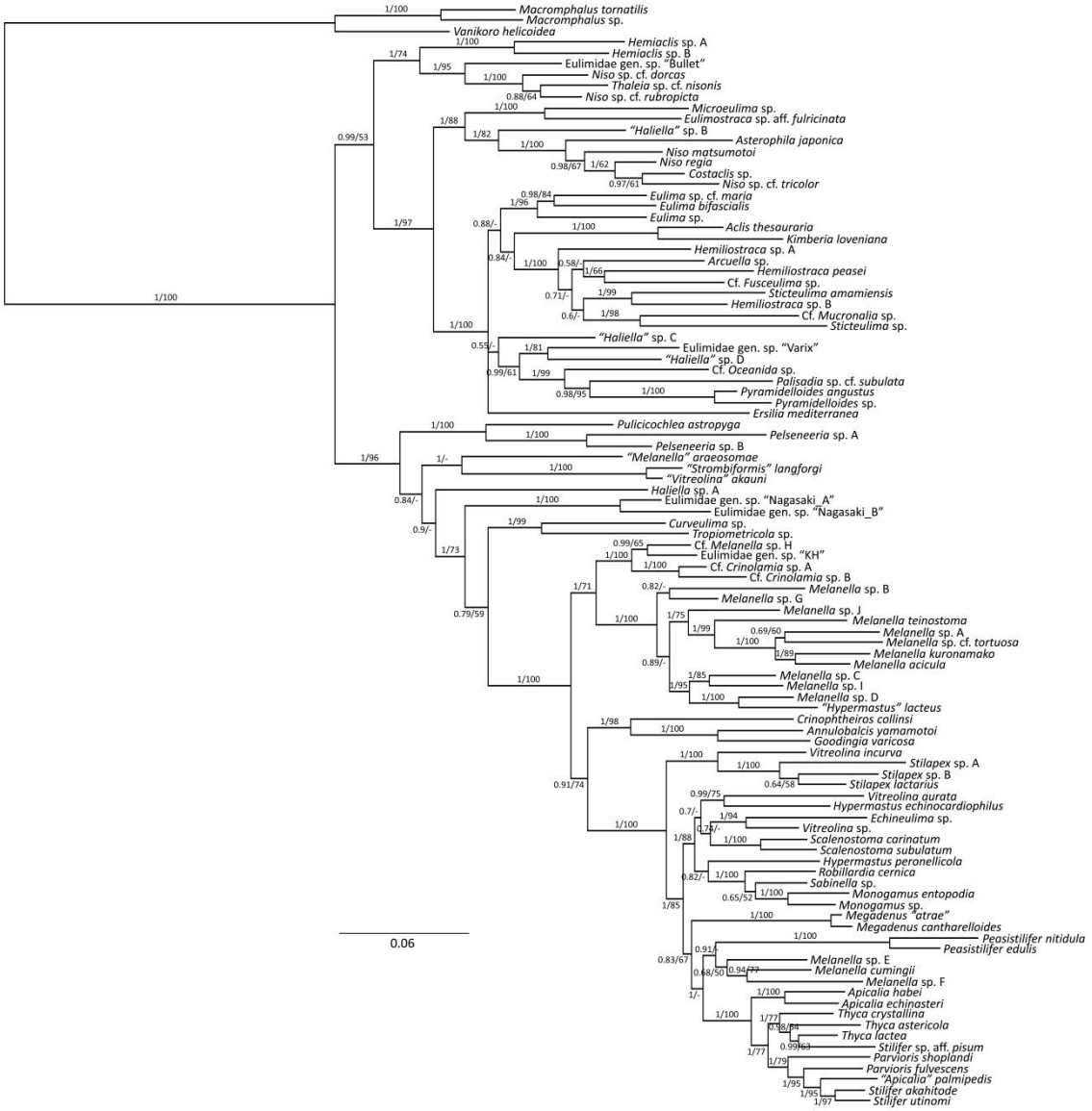

Figure S2-1. Bayesian phylogeny of Eulimidae inferred from the limited-dataset of 18S, 28S, H3, 16S, 12S and COI genes (4,697 sites in total) from 101 species. Numerals on branches denote posterior probabilities (PP, left) and likelihood-based bootstrap values shown as percentages (BS, right).

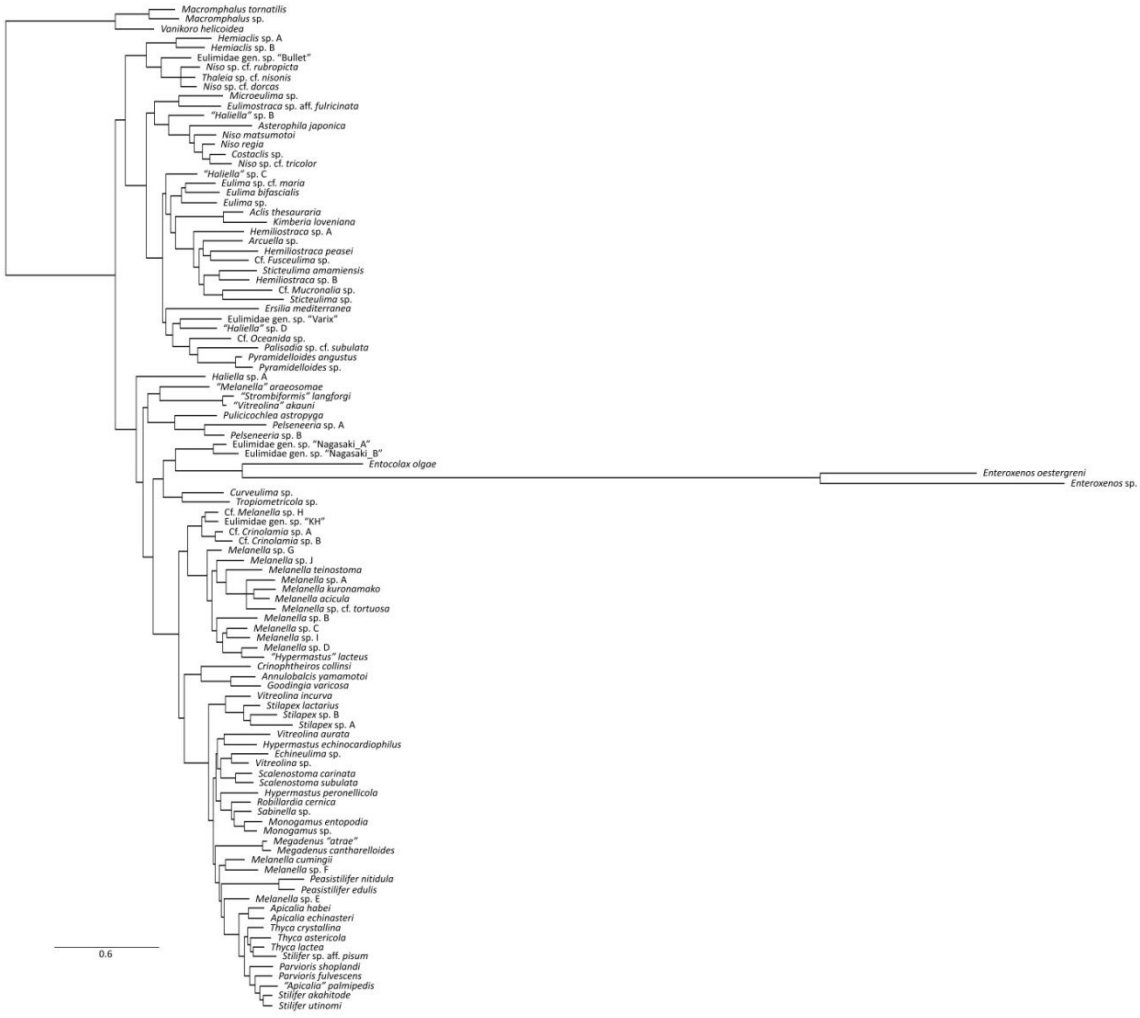

Figure S2-2. Bayesian tree inferred from the full-dataset of 18S, 28S, H3, 16S, 12S and COI genes (4,743 sites in total) from 104 species. See Fig. 2-2 for posterior probabilities.

Figure S2-3. Bayesian tree inferred from 28S gene sequences for full-dataset.

Figure S2-4. Bayesian tree inferred from 18S gene sequences for full-dataset.

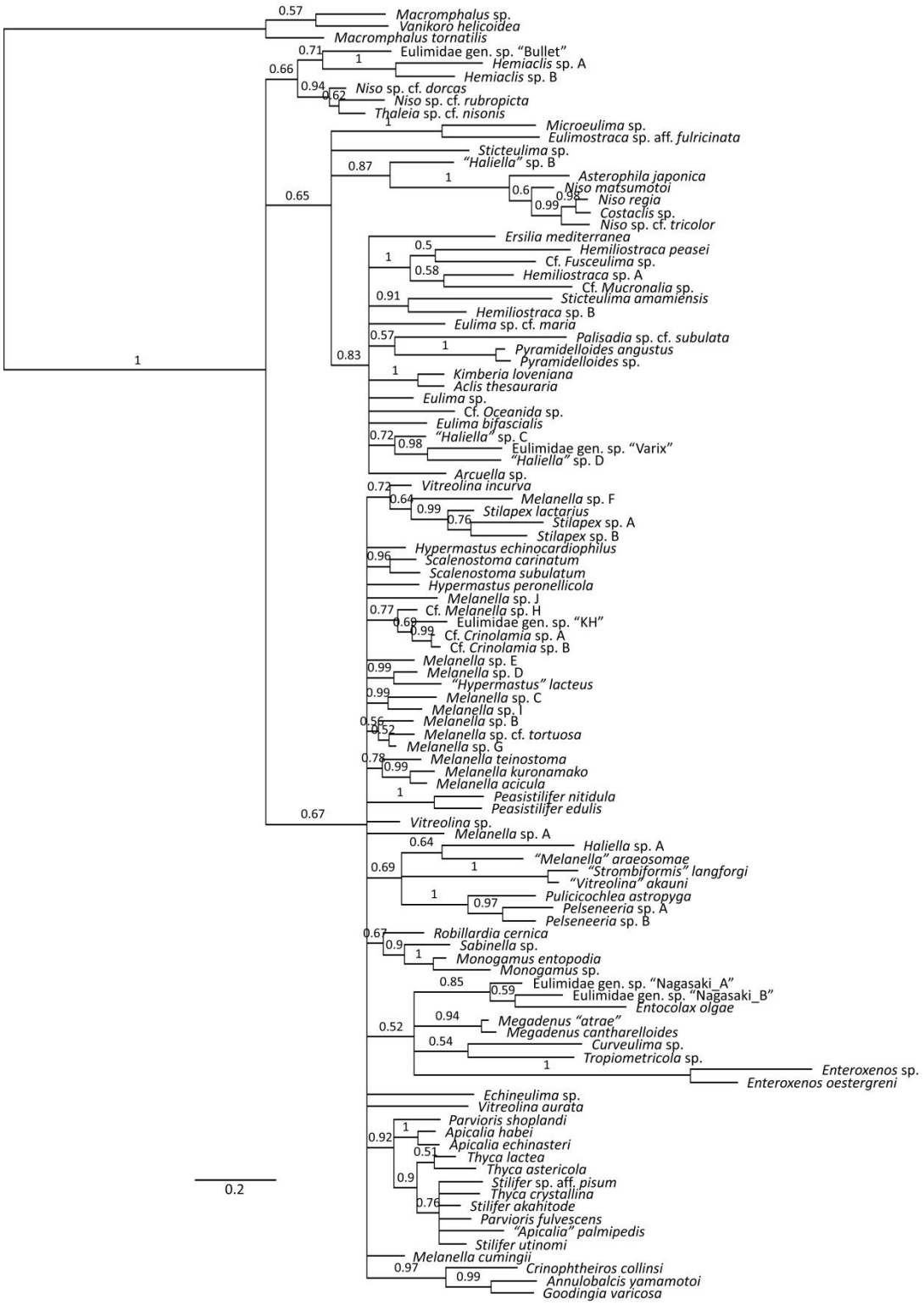

Figure S2-5. Bayesian tree inferred from 16S gene sequences for full-dataset.

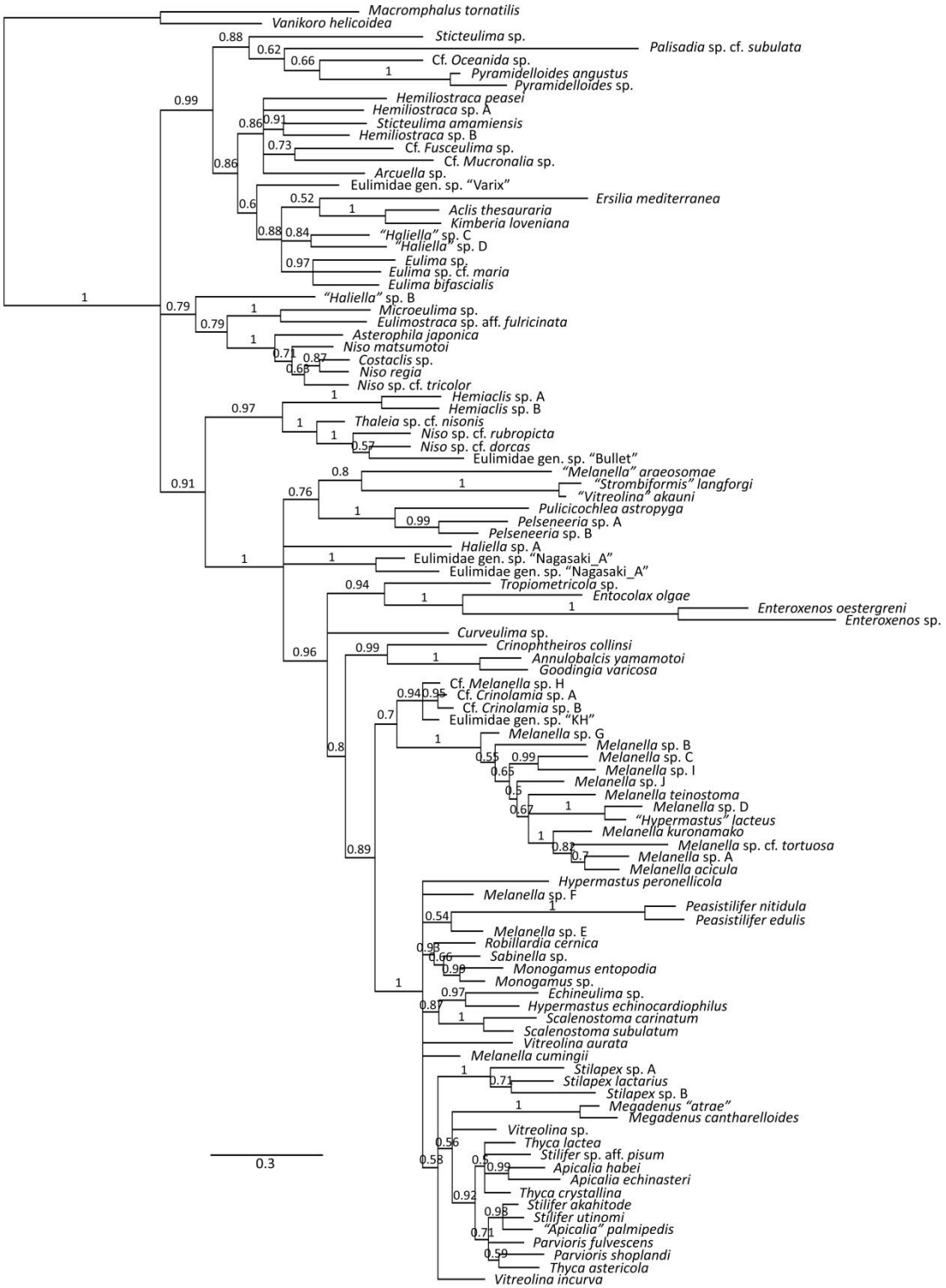

Figure S2-6. Bayesian tree inferred from 12S gene sequences for full-dataset.

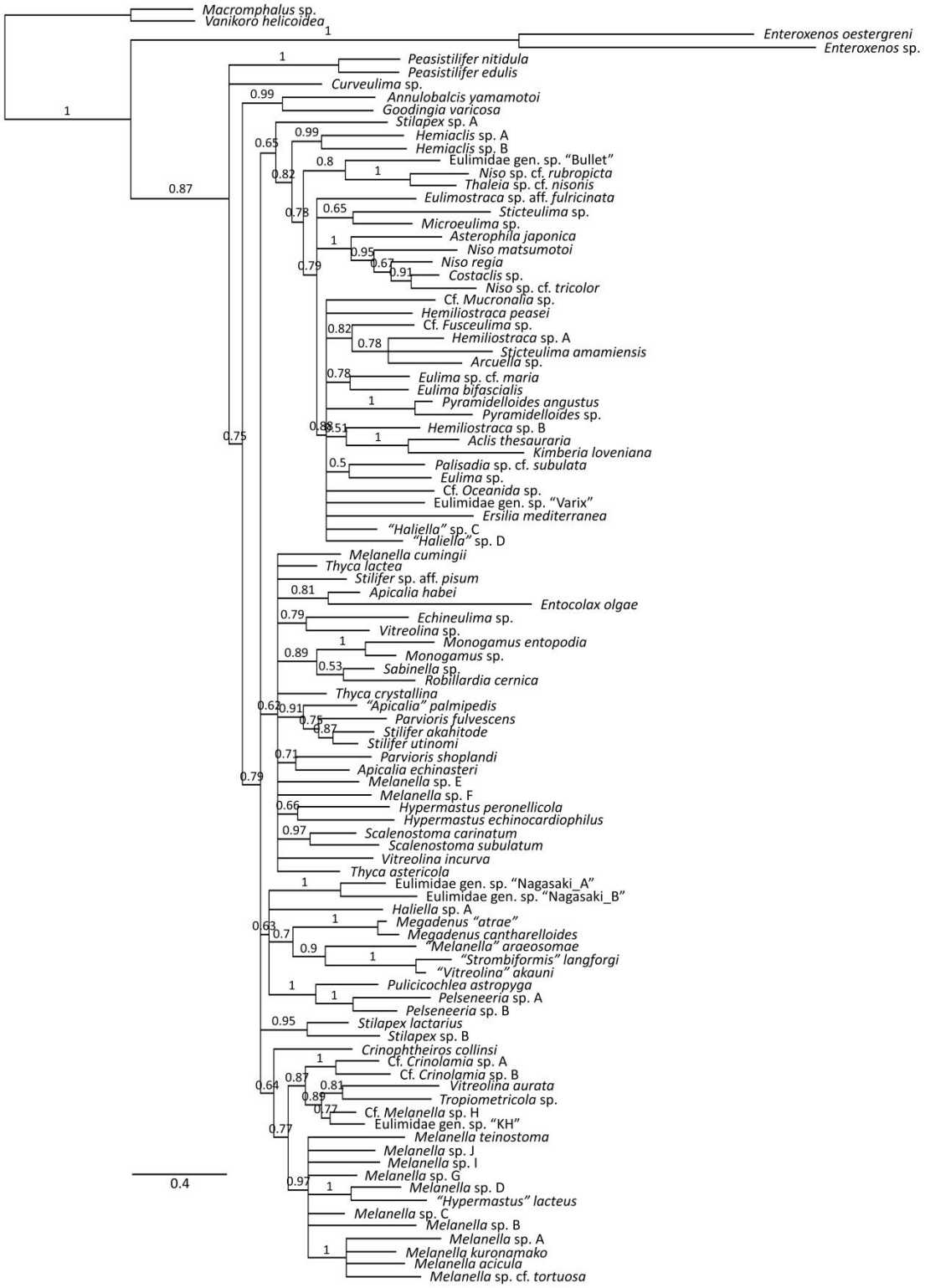

Figure S2-7. Bayesian tree inferred from COI gene sequences for full-dataset.

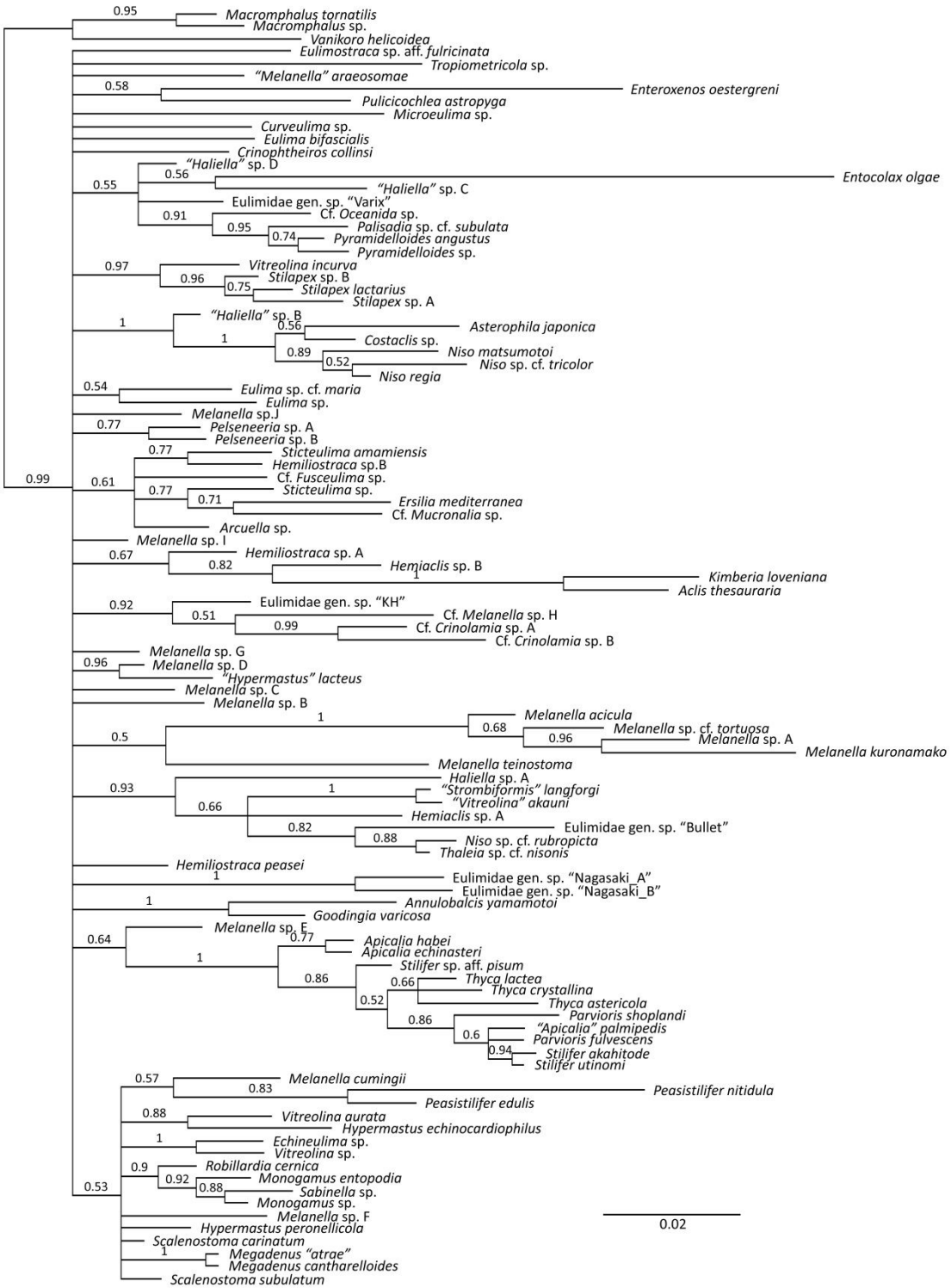

Figure S2-8. Bayesian tree inferred from H3 gene sequences for full-dataset.

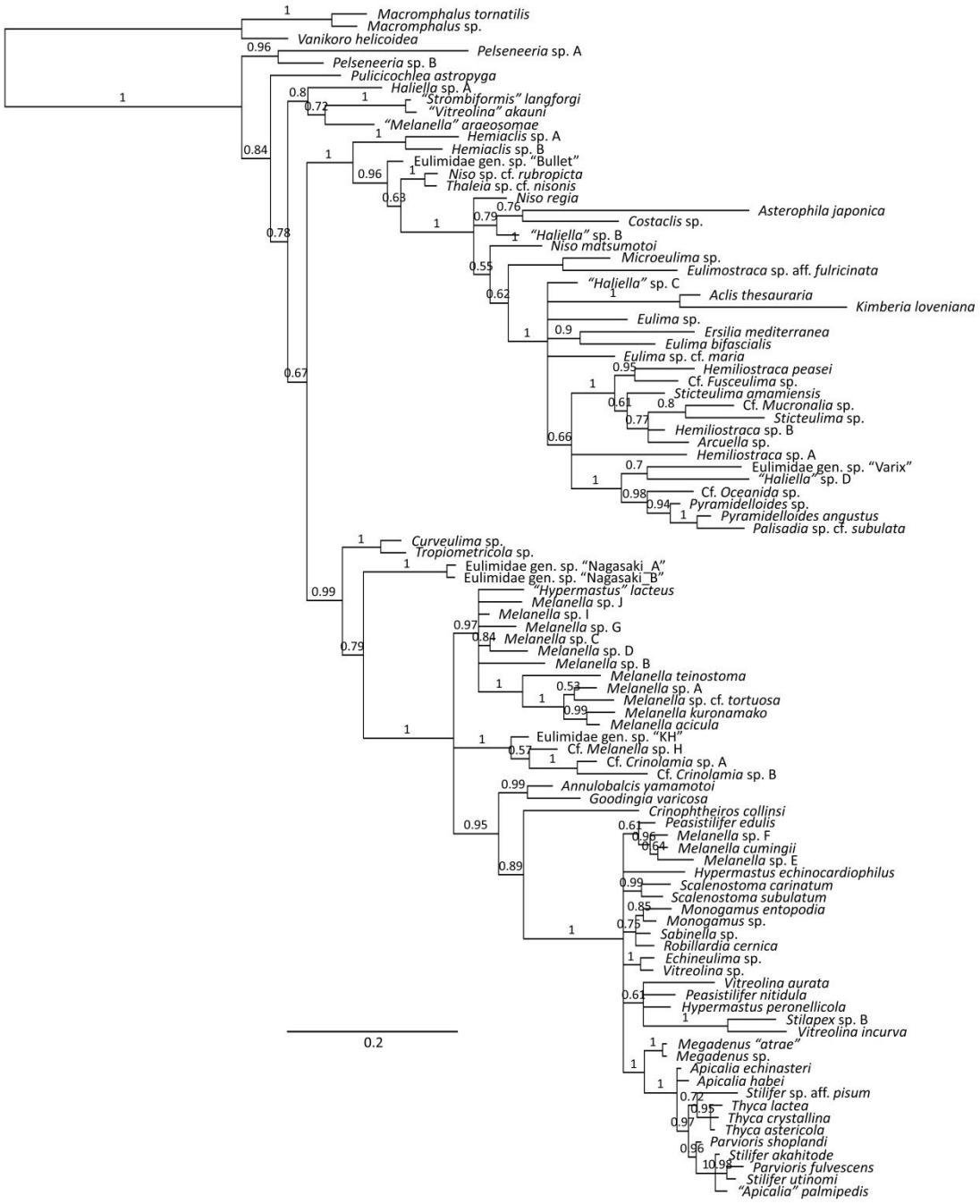

Figure S2-9. Bayesian tree inferred from 28S gene sequences for limited-dataset.

Figure S2-10. Bayesian tree inferred from 18S gene sequences for limited-dataset.

Figure S2-11. Bayesian tree inferred from 16S gene sequences for limited-dataset.

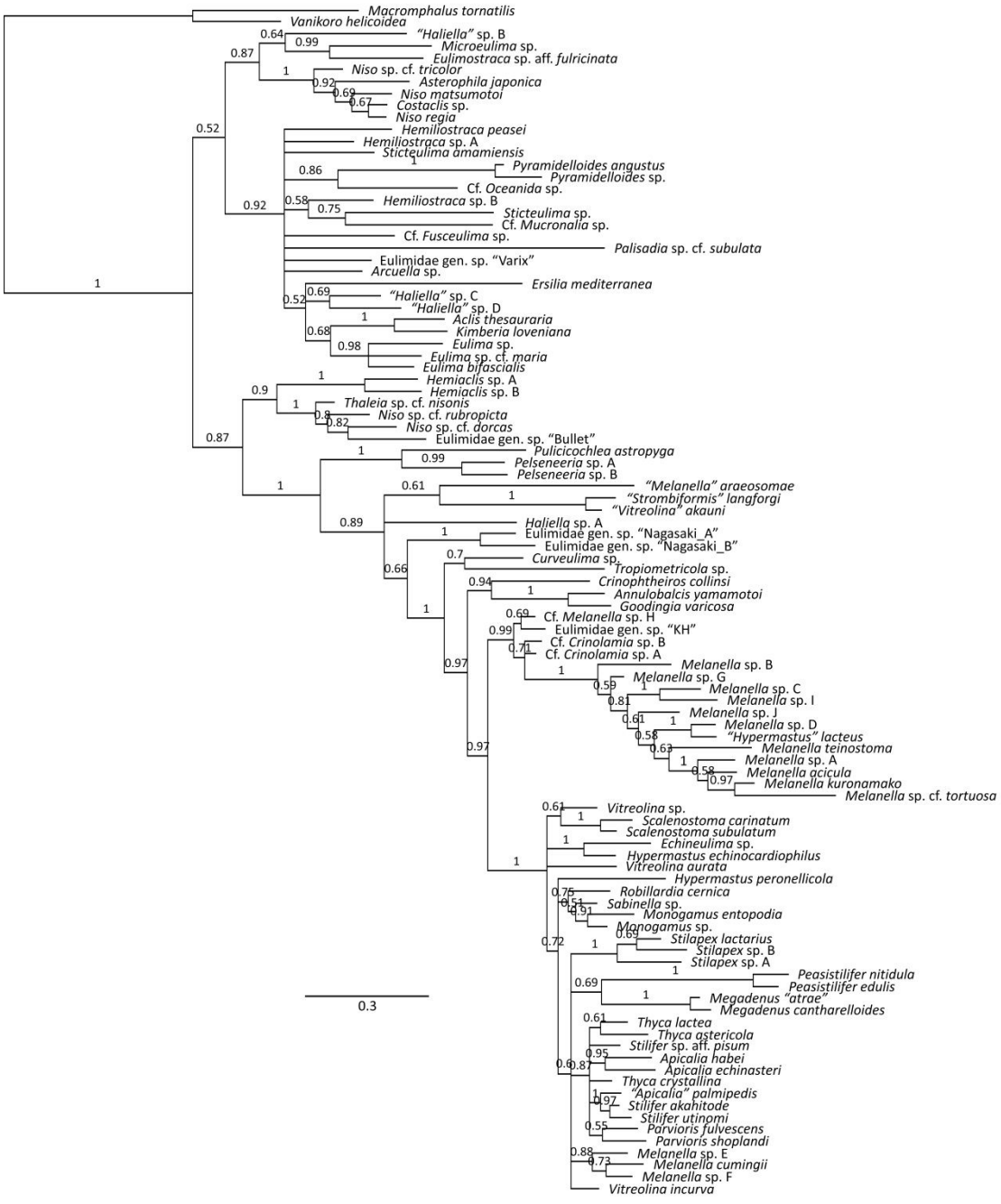

Figure S2-12. Bayesian tree inferred from 12S gene sequences for limited-dataset.

Figure S2-13. Bayesian tree inferred from COI gene sequences for limited-dataset.

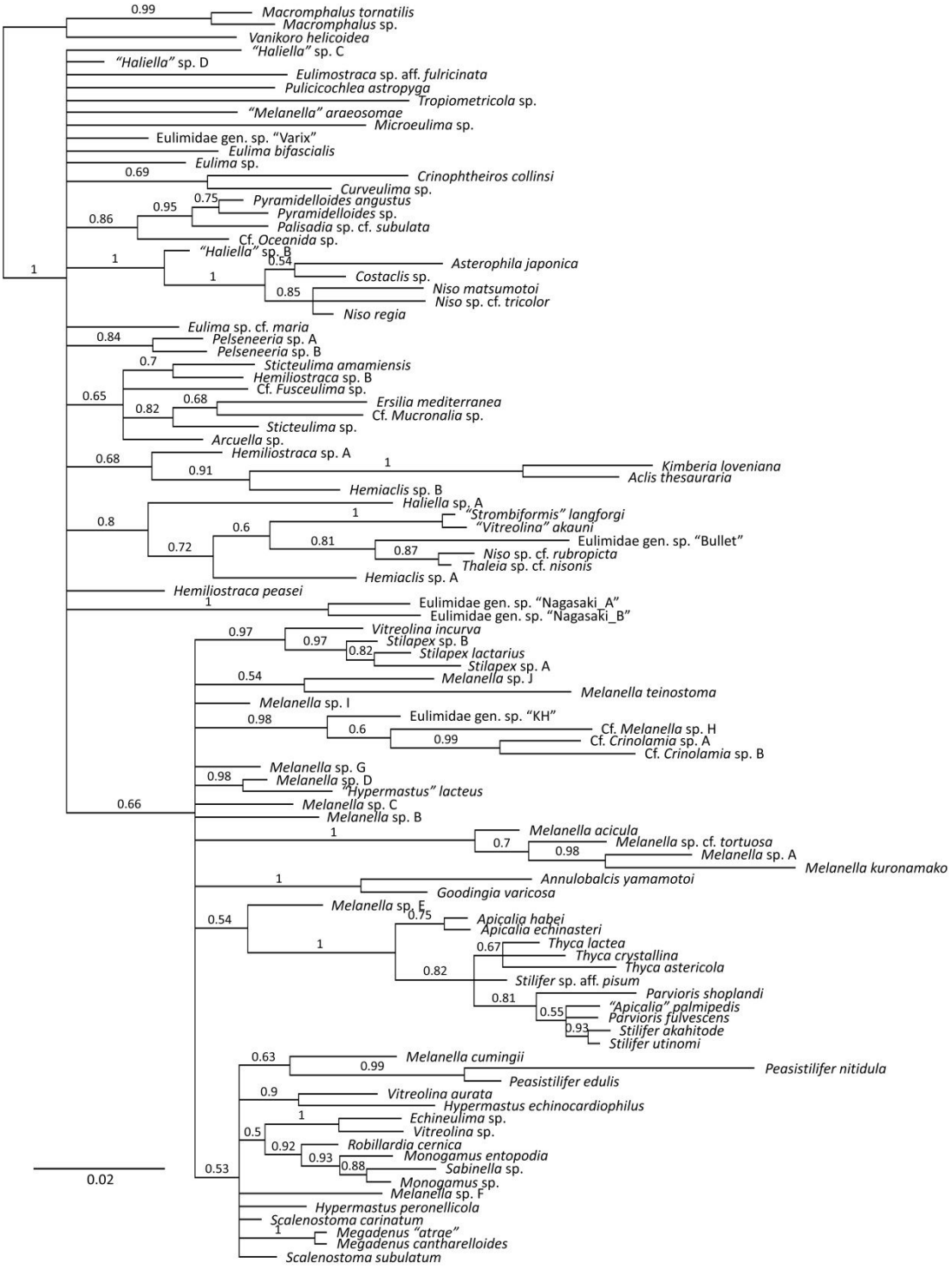

Figure S2-14. Bayesian tree inferred from H3 gene sequences for limited-dataset.

Table S2-1. Recent species used for morphological analyses. Information on the parasitic strategy and host obtained from Hori (2000), Warén (1980a, 1980b, 1981a, 1981b, 1983, 1984), Bouchet & Warén (1986) and Warén & Sibuet (1981).

Species	Source[*2]	Strategy[*3]	Host	SH	D	SW	AW	AH	PAL	CV	Shell type
Aclis thesauraria	1	Temp	?	0.629	0.413	0.260	0.222	0.258	0.751	0.237	A
"Aclis" angulifera	2	Temp	?	0.729	0.331	0.195	0.136	0.169	0.839	0.167	A
"Aclis" loveniana	2	Temp	?	0.764	0.193	0.150	0.100	0.129	0.864	0.167	A
Amamibalcis kawamurai[*1]	2	Temp	?	0.656	0.292	0.240	0.162	0.273	0.727	0.000	A
Annulobalcis shimazui	2	Temp	Crinoidea	0.508	0.379	0.227	0.212	0.379	0.606	0.111	A
Annulobalcis yamamotoi[*1]	1	Temp	Crinoidea	0.512	0.370	0.240	0.233	0.348	0.653	0.094	A
Apicalia echinasteri	1	Temp	Asteroidea	0.466	0.491	0.343	0.232	0.346	0.652	0.084	B
Apicalia habei	2	Temp	Asteroidea	0.566	0.434	0.336	0.230	0.311	0.705	0.125	A
Arcuella sp.[*1]	1	Temp	?	0.617	0.264	0.217	0.167	0.247	0.717	0.000	A
Costaclis sp.[*1]	1	Temp	?	0.716	0.321	0.247	0.164	0.194	0.796	0.115	A
Cf. *Crinolamia* sp.B	1	?	?	0.243	0.668	0.342	0.390	0.587	0.463	0.131	C
Crinophtheiros collinsi	1	Temp	Crinoidea	0.588	0.375	0.269	0.167	0.309	0.654	0.012	A
Curveulima komaii	2	Temp	Crinoidea	0.464	0.400	0.309	0.173	0.436	0.564	0.059	A
Curveulima sp.	1	Temp	Crinoidea	0.500	0.333	0.257	0.135	0.339	0.649	0.014	A
Echineulima dubia[*1]	4	Ecto	Echinoidea	0.355	0.692	0.370	0.404	0.425	0.596	0.097	B
Echineulima leucophaes[*1]	4	Ecto	Echinoidea	0.449	0.542	0.418	0.291	0.347	0.666	0.108	B
Echineulima mittrei	2	Ecto	Echinoidea	0.496	0.487	0.354	0.265	0.336	0.681	0.138	B

Echineulima philippinarum	4	Ecto	Echinoidea	0.433	0.534	0.416	0.276	0.339	0.651	0.137	B
Echineulima robusta	2	Ecto	Echinoidea	0.389	0.574	0.417	0.315	0.398	0.620	0.143	B
Echineulima thanuumi	3	Ecto	Echinoidea	0.361	0.636	0.358	0.361	0.435	0.538	0.126	B
Echineulima tokii	2	Ecto	Echinoidea	0.469	0.586	0.328	0336	0.406	0.625	0.200	B
Echineulima sp.	1	Ecto	Echinoidea	0.363	0.623	0.379	0.361	0.413	0.598	0.104	B
Ersilia mediterranea	1	Temp	Ophiuroidea	0.223	0.624	0.288	0.389	0.579	0.435	0.083	C
Eulima bifascialis	1	Temp	Ophiuroidea	0.590	0.205	0.169	0.108	0.287	0.710	0.025	A
Eulima lacca	2	Temp	Ophiuroidea	0.571	0.202	0.168	0.134	0.303	0.714	0.050	A
Eulima maria	2	Temp	Ophiuroidea	0.658	0.201	0.188	0.121	0.255	0.738	0.000	A
Eulima unilineata	2	Temp	Ophiuroidea	0.560	0.220	0.183	0.147	0.330	0.688	0.000	A
Eulima sp.	1	Temp	Ophiuroidea	0.624	0.241	0.208	0.124	0.281	0.686	0.000	A
“Eulimitra” kawamurai	2	Temp	Ophiuroidea	0.581	0.376	0.299	0.205	0.342	0.667	0.045	A
Eulimostraca sp. aff. *fulricinata*	1	Temp	?	0.622	0.329	0.255	0.188	0.257	0.721	0.014	A
Cf. *Fusceulima* sp.	1	Temp	?	0.467	0.319	0.251	0.192	0.368	0.582	0.034	A
Goodingia varicosa	1	Ecto	Crinoidea	0.266	0.640	0.291	0.368	0.538	0.458	0.142	B
Haliella sp.A	1	Temp	?	0.585	0.250	0.208	0.147	0.286	0.704	0.059	A
“Haliella” sp.B	1	Tamp	?	0.548	0.298	0.225	0.176	0.348	0.654	0.012	A
“Haliella” sp.C	1	Temp	?	0.556	0.293	0.225	0.159	0.323	0.655	0.000	A
“Haliella” sp.D	1	Temp	?	0.541	0.355	0.276	0.199	0.321	0.667	0.040	A
Hemiaclis sp.A[*1]	1	Temp	?	0.540	0.432	0.293	0.246	0.334	0.669	0.065	A
Hemiaclis sp.B	1	Temp	?	0.546	0.387	0.284	0.217	0.322	0.681	0.073	A
Hemiliostraca “vincta”	2	Temp	Ophiuroidea	0.504	0.323	0.228	0.157	0.394	0.622	0.000	A

Hemiliostraca metcalfei	2	Temp	Ophiuroidea	0.454	0.319	0.199	0.163	0.411	0.589	0.074	A
Hemiliostraca peasei	1	Temp	Ophiuroidea	0.501	0.325	0.262	0.163	0.359	0.604	0.039	A
Hemiliostraca sp.A	1	Temp	Ophiuroidea	0.521	0.362	0.271	0.187	0.324	0.654	0.038	A
Hypermastus echinocardiophilus	1	Temp	Echinoidea	0.577	0.296	0.236	0.156	0.277	0.704	0.052	A
Hypermastus pelonellicola	1	Temp	Echinoidea	0.530	0.311	0.239	0.184	0.287	0.674	0.021	A
"Hypermastus" lacteus	2	Temp	Holothuroidea	0.511	0.367	0.311	0.200	0.400	0.667	0.000	A
"Hypermastus" philippianus	2	Temp	?	0.603	0.228	0.206	0.140	0.265	0.721	0.000	A
Megadenus cantharelloides	1	Endo	Holothuroidea	0.280	0.844	0.519	0.498	0.478	0.587	0.078	C
Megadenus sp. *"atrae"*	1	Endo	Holothuroidea	0.238	0.791	0.462	0.480	0.552	0.511	0.092	C
Megadenus sp. *"bohadschiae"*	1	Endo	Holothuroidea	0.235	0.839	0.433	0.494	0.505	0.530	0.059	C
Megadenus sp. *"hillae"*[*1]	1	Endo	Holothuroidea	0.297	0.731	0.464	0.406	0.464	0.554	0.099	C
Megadenus sp. *"leucospilotae"*	1	Endo	Holothuroidea	0.245	0.750	0.441	0.416	0.482	0.541	0.082	C
Megadenus sp. *"solida"*	1	Endo	Holothuroidea	0.188	0.965	0.424	0.612	0.581	0.501	0.078	C
Melanella acicula[*1]	1	Temp	Holothuroidea	0.663	0.317	0.268	0.154	0.243	0.750	0.000	A
Melanella bovicornu[*1]	2	Temp	Holothuroidea	0.606	0.356	0.258	0.182	0.288	0.727	0.050	A
Melanella cumingii[*1]	1	Temp	Holothuroidea	0.599	0.330	0.272	0.178	0.253	0.727	0.052	A
Melanella flexuosa[*1]	2	Temp	Holothuroidea	0.650	0.350	0.266	0.168	0.266	0.734	0.083	A
Melanella grandis	2	Temp	Holothuroidea	0.676	0.351	0.277	0.182	0.243	0.757	0.053	A
Melanella kuronamako	1	Temp	Holothuroidea	0.581	0.350	0.268	0.176	0.289	0.695	0.000	A
Melanella major[*1]	2	Temp	Holothuroidea	0.649	0.318	0.245	0.166	0.258	0.775	0.091	A
Melanella martini	2	Temp	Holothuroidea	0.640	0.387	0.320	0.213	0.267	0.733	0.056	A
Melanella ogasawarana[*1]	2	Temp	Holothuroidea	0.612	0.333	0.288	0.189	0.273	0.727	0.000	A

Melanella persimilis	2	Temp	Holothuroidea	0.699	0.233	0.219	0.130	0.219	0.781	0.000	A
Melanella teramachii	2	Temp	Holothuroidea	0.600	0.339	0.261	0.191	0.261	0.730	0.000	A
Melanella tortuosa[*1]	2	Temp	Holothuroidea	0.669	0.286	0.247	0.156	0.227	0.779	0.000	A
Melanella yamazii[*1]	2	Temp	Holothuroidea	0.540	0.407	0.310	0.195	0.292	0.726	0.053	A
Melanella sp. cf. *tortuosa*	1	Temp	Holothuroidea	0.627	0.338	0.260	0.168	0.243	0.733	0.000	A
Melanella sp.A	1	Temp	Holothuroidea	0.517	0.433	0.327	0.252	0.342	0.658	0.042	A
Melanella sp.B[*1]	1	Temp	Holothuroidea	0.647	0.361	0.284	0.164	0.269	0.729	0.026	A
Melanella sp.C	1	Temp	Holothuroidea	0.659	0.286	0.235	0.130	0.241	0.748	0.000	A
Melanella sp.D[*1]	1	Temp	Holothuroidea	0.537	0.383	0.330	0.197	0.313	0.661	0.000	A
Melanella sp.E[*1]	1	Temp	Holothuroidea	0.571	0.410	0.310	0.187	0.284	0.691	0.021	A
Melanella sp.F[*1]	1	Temp	Holothuroidea	0.607	0.369	0.283	0.190	0.269	0.729	0.025	A
Cf. *Melanella* sp.H	1	Temp	?	0.663	0.330	0.255	0.177	0.264	0.730	0.034	A
Melanella sp.I	1	Temp	Holothuroidea	0.542	0.414	0.324	0.161	0.317	0.690	0.095	A
Melanella sp.J[*1]	1	Temp	Holothuroidea	0.771	0.206	0.175	0.111	0.196	0.779	0.000	A
“Melanella” araeosomae[*1]	1	Temp	Echinoidea	0.678	0.292	0.241	0.157	0.233	0.765	0.042	A
“Melanella” clypeastericola[*1]	2	Temp	Echinoidea	0.631	0.323	0.262	0.154	0.231	0.785	0.091	A
“Melanella” inflexa	2	Temp	Asteroidea	0.516	0.516	0.344	0.237	0.366	0.656	0.000	A
Monogamus entopodia	1	Ecto	Echinoidea	0.264	0.759	0.348	0.475	0.594	0.459	0.149	C
Monogamus parasaleniae	5	Ecto	Echinoidea	0.465	0.470	0.279	0.319	0.288	0.703	0.088	A
Monogamus sp.	1	Endo	Echinoidea	0.215	0.871	0.304	0.544	0.598	0.478	0.151	C
Mucronalia bicincta	2	Temp	?	0.519	0.333	0.287	0.204	0.361	0.639	0.050	A
Mucronalia exilis	2	Temp	Ophiuroidea	0.469	0.388	0.286	0.214	0.388	0.622	0.050	A

Cf. *Mucronalia* sp.	1	?	Ophiuroidea	0.290	0.578	0.240	0.363	0.534	0.488	0.111	B
Niso brunnea	2	Temp	Asteroidea	0.643	0.388	0.279	0.186	0.264	0.744	0.111	A
Niso goniostoma	2	Temp	Asteroidea	0.569	0.446	0.331	0.177	0.315	0.700	0.050	A
Niso hizenensis	2	Temp	Asteroidea	0.643	0.386	0.307	0.186	0.271	0.743	0.045	A
Niso matsumotoi	1	Temp	Asteroidea	0.604	0.393	0.296	0.192	0.265	0.728	0.070	A
Niso regina[*1]	1	Temp	Asteroidea	0.655	0.425	0.327	0.184	0.244	0.766	0.063	A
Niso rubropicta[*1]	2	Temp	?	0.625	0.383	0.300	0.183	0.258	0.742	0.105	A
Niso stenomphala[*1]	2	Temp	Asteroidea	0.607	0.420	0.339	0.179	0.277	0750	0.050	A
Niso tetsuakii[*1]	2	Temp	Asteroidea	0.679	0.333	0.256	0.161	0.226	0.774	0.095	A
Niso yokoyamai	2	Temp	Asteroidea	0.621	0.403	0.298	0.177	0.266	0.718	0.056	A
Niso sp. cf. *dorcas*[*1]	1	Temp	?	0.640	0.367	0.295	0.183	0.248	0.753	0.062	A
Niso sp. cf. *rubropicta*[*1]	1	Temp	?	0.677	0.352	0.283	0.160	0.232	0.770	0.064	A
Niso sp. cf. *tricolor*[*1]	1	Temp	Asteroidea	0.688	0.322	0.263	0.156	0.224	0.766	0.057	A
Oceanida sp.	1	Temp	?	0.548	0.309	0.230	0.172	0.309	0.677	0.064	A
Ophieulima fuscoapicata	7	Ecto	Ophiuroidea	0.379	0.561	0.266	0.262	0.435	0.571	0.122	B
Ophieulima sp.	7	Ecto	Ophiuroidea	0.380	0.520	0.275	0.329	0.398	0.599	0.096	B
Parvioris fulvescens	2	Temp	Asteroidea	0.484	0.538	0.385	0.253	0.363	0.648	0.095	B
Parvioris shoplandi	1	Temp	Asteroidea	0.546	0.409	0.284	0.188	0.267	0.707	0.055	A
Peasistilifer edulis[*1]	1	Ecto	Holothuroidea	0.523	0.479	0.297	0.246	0.351	0.661	0.119	B
Peasistilifer nitidula[*1]	1	Temp	Holothuroidea	0.555	0.495	0.360	0.231	0.312	0.684	0.089	A
Pelseneeria hawaiiensis	3	Ecto	Echinoidea	0.353	0.620	0.341	0.362	0.440	0.601	0.062	B
Pelseneeria sp.	7	Ecto	Echinoidea	0.102	0.727	0.250	0.504	0.567	0.428	0.086	C

Pelseneeria sp.A	1	Ecto	Echinoidea	0.274	0.685	0.382	0.418	0.511	0.514	0.108	C
Pelseneeria ap.B[*1]	1	Ecto	Echinoidea	0.307	0.672	0.348	0.431	0.472	0.544	0.132	B
Pictobalcis articulata[*1]	2	Temp	?	0.672	0.259	0.213	0.149	0.241	0.764	0.000	A
Prostilifer subpellucida	5	Endo	Holothuroidea	0.485	0.483	0.364	0.301	0.344	0.636	0.055	B
Pulicicochlea astropyga	1	Ecto	Echinoidea	0.172	0.429	0.212	0.301	0.581	0.416	0.104	B
Pulicicochlea calamaris	3	Ecto	Echinoidea	0.244	0.341	0.164	0.221	0.475	0.514	0.087	B
Punctifera ophiomoerae	7	Ecto	Ophiuroidea	0.337	0.706	0.425	0.397	0.425	0.594	0.151	B
Pyramidelloides angustus	1	Temp	Ophiuroidea	0.571	0.370	0.271	0.212	0.288	0.712	0.079	A
Pyramidelloides tosaensis[*1]	2	Temp	Ophiuroidea	0.640	0.344	0.240	0.160	0.176	0.752	0.136	A
Robillardia cernica[*1]	1	Endo	Echinoidea	0.195	1.282	0.293	0.794	0.687	0.441	0.078	C
Sabinella sp.	1	Ecto	Echinoidea	0.402	0.575	0.267	0.394	0.430	0.581	0.087	B
Scalenostoma carinata	2	Temp	Echinoidea	0.662	0.308	0.248	0.173	0.233	0.752	0.118	A
Scalenostoma subulata	1	Temp	Echinoidea	0.606	0.427	0.321	0.239	0.298	0.718	0.039	A
Sticteulima amamiensis	2	Temp	?	0.564	0.317	0.248	0.178	0.347	0.653	0.071	A
Sticteulima lentiginosa	2	Temp	?	0.521	0.372	0.298	0.202	0.362	0.638	0.000	A
Sticteulima sp.	1	Temp	?	0.428	0.462	0.320	0.227	0.429	0.544	0.033	B
Stilapex cocckeana	6	Ecto	Ophiuroidea	0.336	0.448	0.360	0.264	0.420	0.564	0.082	B
Stilapex lactarius	2	Ecto	Ophiuroidea	0.388	0.592	0.340	0.350	0.466	0.563	0.160	B
Stilapex montrouzieri	6	Ecto	Ophiuroidea	0.294	0.472	0.359	0.270	0.399	0.582	0.128	B
Stilapex ophiurophila	2	Ecto	Ophiuroidea	0.458	0.530	0.349	0.265	0.373	0.639	0.286	B
Stilapex parva	6	Ecto	Ophiuroidea	0.426	0.540	0.396	0.247	0.357	0.653	0.150	B
Stilapex suzuki[*1]	2	Ecto	Ophiuroidea	0.337	0.547	0.291	0.337	0.477	0.523	0.182	B

Stilapex thielei	6	Ecto	Ophiuroidea	0.231	0.683	0.367	0.367	0.504	0.533	0.194	C
Stilapex zebra	2	Ecto	Ophiuroidea	0.355	0.671	0.342	0.382	0.474	0.579	0.150	B
"Stilapex" koyamai	2	?	Holothuroidea	0.484	0.463	0.411	0.274	0.358	0.663	0.056	B
"Stilapex" teramachii	2	?	Holothuroidea	0.451	0.549	0.373	0.294	0.373	0.667	0.172	B
Stilapex sp.A	1	Ecto	Ophiuroidea	0.195	0.699	0.282	0.458	0.581	0.411	0.130	C
Stilapex sp.B	1	Ecto	Ophiuroidea	0.281	0.747	0.289	0.450	0.574	0.440	0.160	C
Stilifer akahitode	2	Endo	Asteroidea	0.306	0.750	0.407	0.472	0.593	0.500	0.050	C
Stilifer astericola	4	Endo	Asteroidea	0.250	0.763	0.455	0.453	0.600	0.452	0.058	C
Stilifer birtsi	4	Endo	Asteroidea	0.412	0.552	0.423	0.314	0.412	0.592	0.019	B
Stilifer concaves[*1]	4	Endo	Asteroidea	0.420	0.738	0.430	0.425	0.438	0.628	0.064	B
Stilifer inflatus[*1]	4	Endo	Asteroidea	0.257	0.730	0.358	0.470	0.605	0.419	0.049	C
Stilifer kawamurai[*1]	2	Endo	Asteroidea	0.414	0.633	0.362	0.414	0.466	0.560	0.045	B
Stilifer kochianus[*1]	4	Endo	Asteroidea	0.362	0.608	0.360	0.360	0.516	0.498	0.037	B
Stilifer linkiae	3	Endo	Asteroidea	0.376	0.586	0.376	0.328	0.471	0.546	0.046	B
Stilifer ovoideus[*1]	4	Endo	Asteroidea	0.349	0.642	0.387	0.399	0.462	0.555	0.096	B
Stilifer pisum	2	Endo	Asteroidea	0.130	1.043	0.359	0.630	0.739	0.370	0.111	C
Stilifer quadrasi[*1]	4	Endo	Asteroidea	0.455	0.579	0.365	0.350	0.409	0.607	0.050	B
Stilifer utinomi	4	Endo	Asteroidea	0.286	0.678	0.366	0.447	0.531	0.478	0.065	C
Stilifer sp. aff. *pisum*	1	Endo	Asteroidea	0.175	0.902	0.457	0.524	0.612	0.494	0.139	C
"Strombiformis" langforgi	1	Temp	Echinoidea	0.572	0.361	0.283	0.191	0.303	0.654	0.000	A
Thaleia sp. cf. *nisonis*	1	Temp	?	0.583	0.413	0.329	0.210	0.266	0.722	0.086	A
Thyca lactea	1	Ecto	Asteroidea	0.093	1.075	0.222	0.731	0.737	0.359	0.260	C

Trochostilifer domes	5	Endo	Echinoidea	0.380	0.886	0.478	0.485	0.414	0.625	0.044	C
Trochostilifer entospinea	3	Endo	Echinoidea	0.381	1.119	0.517	0.661	0.395	0.630	0.000	C
Trochostilifer hawaiiensis	3	Endo	Echinoidea	0.251	1.056	0.378	0.674	0.559	0.465	0.026	C
Trochostilifer mortenseni	5	Endo	Echinoidea	0.275	0.971	0.425	0.588	0.508	0.576	0.019	C
Trochostilifer striatus	5	Ecto	Echinoidea	0.422	0.547	0.304	0.338	0.348	0.628	0.104	B
Tropiometricola sphaeroconcha	2	Endo	Crinoidea	0.043	0.886	0.229	0.486	0.743	0.371	0.118	C
Tropiometricola sp.	1	Endo	Crinoidea	0.191	0.872	0.363	0.514	0.664	0.406	0.114	C
Vitreobalcis temnopleuricola	2	Temp	Echinoidea	0.592	0.408	0.272	0.192	0.288	0.712	0.091	A
"Vitreobalcis" astropectenicola	2	Temp	Asteroidea	0.638	0.329	0.243	0.158	0.257	0.750	0.043	A
Vitreolina aurata	1	Temp	Echinoidea	0.555	0.365	0.273	0.168	0.295	0.676	0.012	A
Vitreolina incurva	1	Temp	Ophiuroidea	0.626	0.358	0.251	0.146	0.277	0.698	0.049	A
Vitreolina punctozonata[*1]	2	Temp	?	0.622	0.269	0.231	0.141	0.282	0.718	0.000	A
"Vitreolina" akauni	1	Temp	Echinoidea	0.592	0.332	0.271	0.167	0.300	0.674	0.000	A
Vitreolina sp.	1	Temp	Echinoidea	0.552	0.389	0.284	0.196	0.303	0.683	0.042	A
Eulimidae gen. sp. "Varix"	1	Temp	?	0.562	0.532	0.295	0.190	0.274	0.702	0.326	B
Eulimidae gen. sp. "Nagasaki_A"	1	Ecto	Holothuroidea	0.483	0.458	0.293	0.260	0.340	0.669	0.123	B
Eulimidae gen. sp. "Nagasaki_B"	1	Temp	Holothuroidea	0.624	0.302	0.221	0.164	0.245	0.749	0.120	A
Eulimidae gen. sp. "KH"	1	Temp	?	0.580	0.368	0.268	0.210	0.304	0.697	0.061	A
Eulimidae gen. sp. "Bullet" [*1]	1	Temp	?	0.561	0.289	0.235	0.159	0.304	0.689	0.042	A

[*1]Lost part was complemented. [*2]Representing reference of each species. 1: This study, 2: Hori (2000), 3: Severns (2011), 4–7: Warén (1980a, 1980b, 1981a, 1981b). [*3]Temp: temporary parasitic, Ecto: ectoparasitic, Endo: endoparasitic, ?: unknown.

Table S2-2. Fossil specimens used for morphological analyses. Species names adopted from the references and not correspond to the present phylogeny.

Species	Source[*1]	Geological age[*2]	SH	D	SW	AW	AH	PAL	CV
Chileutomia paulensis	4	Upper Oligocene	0.413	0.446	0.264	0.281	0.397	0.587	0.103
Ersilia oligocaenica	4	Upper Oligocene	0.342	0.547	0.299	0.376	0.462	0.487	0.111
Eulima (*Balcis*) *churchilli*	2	Eocene	0.553	0.316	0.211	0.184	0.316	0.684	0.000
Eulima (*Margineulima*) *fallax*	1	Eocene (Lut.)	0.581	0.258	0.226	0.161	0.226	0.710	0.000
Eulima (*Margineulima*) *parisieusis*	1	Eocene (Ypre., Lut.)	0.618	0.265	0.176	0.147	0.235	0.735	0.000
Eulima (Polygireulima) sp.1	7	Paleocene (Da.)	0.528	0.321	0.245	0.198	0.340	0.642	0.063
Eulima (Polygireulima) sp.1	7	Paleocene (Da.)	0.611	0.278	0.204	0.139	0.269	0.722	0.111
Eulima (Polygireulima) sp.2	7	Paleocene (Da.)	0.519	0.346	0.250	0.173	0.221	0.644	0.059
Eulima (Polygireulima) sp.3	7	Paleocene (Da.)	0.579	0.342	0.272	0.158	0.316	0.570	0.050
Eulima (Polygireulima) sp.4	7	Paleocene (Da.)	0.627	0.373	0.255	0.157	0.284	0.667	0.100
Eulima (Polygireulima) danica	7	Paleocene (Da.)	0.602	0.285	0.220	0.146	0.301	0.667	0.000
Eulima (Polygyreulima) parisiensis	3	Eocene (Lut.)	0.646	0.313	0.232	0.141	0.242	0.758	0.000
Eulima (*Polygyreulima*) *polygyra*	2	Eocene (Bart.)	0.693	0.291	0.244	0.173	0.213	0.787	0.062
Eulima (*Polygyreulima*) *sororcula*	2	Eocene	0.620	0.304	0.228	0.165	0.228	0.722	0.000
Eulima (*Polygyreulima*) *sublabrosa*	2	Eocene	0.500	0.375	0.271	0.188	0.313	0.646	0.000
Eulima (*Polygyreulima*) *sulculata*	2	Eocene	0.614	0.298	0.246	0.193	0.246	0.737	0.000
Eulima (*Polygyreulima*) *truncata*	2	Eocene	0.571	0.405	0.286	0.214	0.262	0.667	0.000

Eulima (*Subularia*) *concinua*	1	Eocene (Lut.)	0.417	0.278	0.194	0.194	0.333	0.639	0.125
Eulima (*Subularia*) *goniophora*	1	Eocene (Lut.)	0.634	0.293	0.220	0.171	0.195	0.780	0.000
Eulima (*Subularia*) *spinula*	1	Eocene (Lut.)	0.697	0.182	0.152	0.091	0.152	0.788	0.000
Eulima (*Subularia*) *subnitida*	1	Eocene (Lut., Bart.)	0.571	0.257	0.200	0.114	0.286	0.714	0.000
Eulima barreti	1	Eocene (Bart.)	0.500	0.346	0.231	0.231	0.327	0.692	0.100
Eulima munda	2	Eocene (Bart.)	0.667	0.208	0.167	0.108	0.208	0.767	0.056
Eulima munda	2	Eocene (Bart.)	0.574	0.294	0.206	0.147	0.235	0.750	0.000
Eulima regalis	2	Eocene	0.565	0.315	0.234	0.161	0.266	0.718	0.045
Eulima subemarginata	1	Eocene (Lut., Bart.)	0.600	0.320	0.200	0.160	0.200	0.760	0.000
Eulima venairix	2	Eocene	0.633	0.317	0.267	0.167	0.233	0.750	0.000
Eulima? *subnitida*	2	Eocene	0.484	0.359	0.219	0.125	0.313	0.656	0.000
Hoplopteron cirsophorum	3	Eocene (Lut.)	0.562	0.404	0.225	0.213	0.303	0.652	0.143
Hoplopteropsis pontileviensis	4	Middle Miocene	0.448	0.350	0.217	0.23	0.364	0.615	0.103
Melanella sp.	7	Paleocene (Da.)	0.630	0.310	0.250	0.150	0.240	0.720	0.071
Niso angusta	1	Eocene (Lut.)	0.684	0.316	0.263	0.140	0.193	0.789	0.000
Niso constricta	1	Eocene (Ypre., Lut.)	0.629	0.371	0.286	0.171	0.229	0.714	0.000
Niso micromphala	2	Eocene	0.618	0.382	0.291	0.164	0.218	0.745	0.000
Niso terebellata	2	Eocene (Lut.)	0.613	0.400	0.288	0.213	0.250	0.735	0.000
Niso terebellata	1	Eocene (Lut., Bart.)	0.653	0.367	0.286	0.163	0.224	0.776	0.000
Ophieulima antecessor	4	Upper Oligocene	0.230	0.706	0.304	0.392	0.505	0.520	0.140
Pelseneeria? *senuti*	4	Lower Miocene	0.128	0.744	0.175	0.512	0.744	0.293	0.067
Polygireulima spina	6	Lower Miocene	0.640	0.297	0.248	0.158	0.234	0.739	0.000

Pyramidelloides dolini	5	Eocene (Bart.)	0.537	0.373	0.224	0.179	0.313	0.687	0.143
Rostreulima eocenica	2	Eocene	0.422	0.391	0.250	0.250	0.344	0.672	0.067
Stylifer pellucidus	1	Eocene (Lut.)	0.239	0.563	0.169	0.338	0.577	0.422	0.000
Stylifer propinguus	1	Eocene (Lut.)	0.486	0.378	0.270	0.189	0.297	0.676	0.111

[*1]1: Cossmann & Pissarro, 1904–06, 2: Wrigley, 1944, 3: Gougerot & Le Renard, 1978, 4: Lozouet, 1999, 5: Lozouet & Dockery, 2001, 6: Pras, 2013, 7: Lauridsen & Schnetler, 2014. [*2]Bart.: Bartnian, Lut.: Lutenian, Ypre.: Ypresian, Da.: Danian.

Appendix 3.

Supplementary data for Chapter 3

Plates 3-1–3-5. Selected photos of study species.

Figures S3-1–S3-5. Independent-gene trees.

Plate 3-1.
Pyramidellids used for molecular phylogenetic reconstruction with reference to the specimen number and shell size (A–E, Group 1; F–N, Group 2).

A. *Turbonilla cummingi* (YK#2747); shell height = 5.4 mm

B. *Turbonilla* sp. cf. *cura* (YK#872); 3.9 mm

C. *Turbonilla kidoensis* (YK#951); 8.5 mm

D. *Cingulina cingulata* (YK#816); c. 9 mm

E. *Paracingulina triarata* (YK#956); 8.2 mm

F. Cf. *Aartsenia* sp. B (YK#948); 2.3 mm

G. Cf. *Tiberia* sp. (YK#2739); 6.8 mm

H. Cf. *Eulimella* sp. (YK# 1819); 8.9 mm (broken)

I. *Orinella pulchella* (YK#871); 10.0 mm

J. *Tiberia pusilla* (YK#954); 6.6 mm

K. *Styloptygma taeniata* (YK#953); 4.1 mm

L. *Bacteridium vittatum* (YK#893); 3.9 mm

M. *Pyrgiscus* sp. A (YK#870); 5.2 mm

N. *Pyrgiscus* sp. B (YK# 1202); 11.9 mm

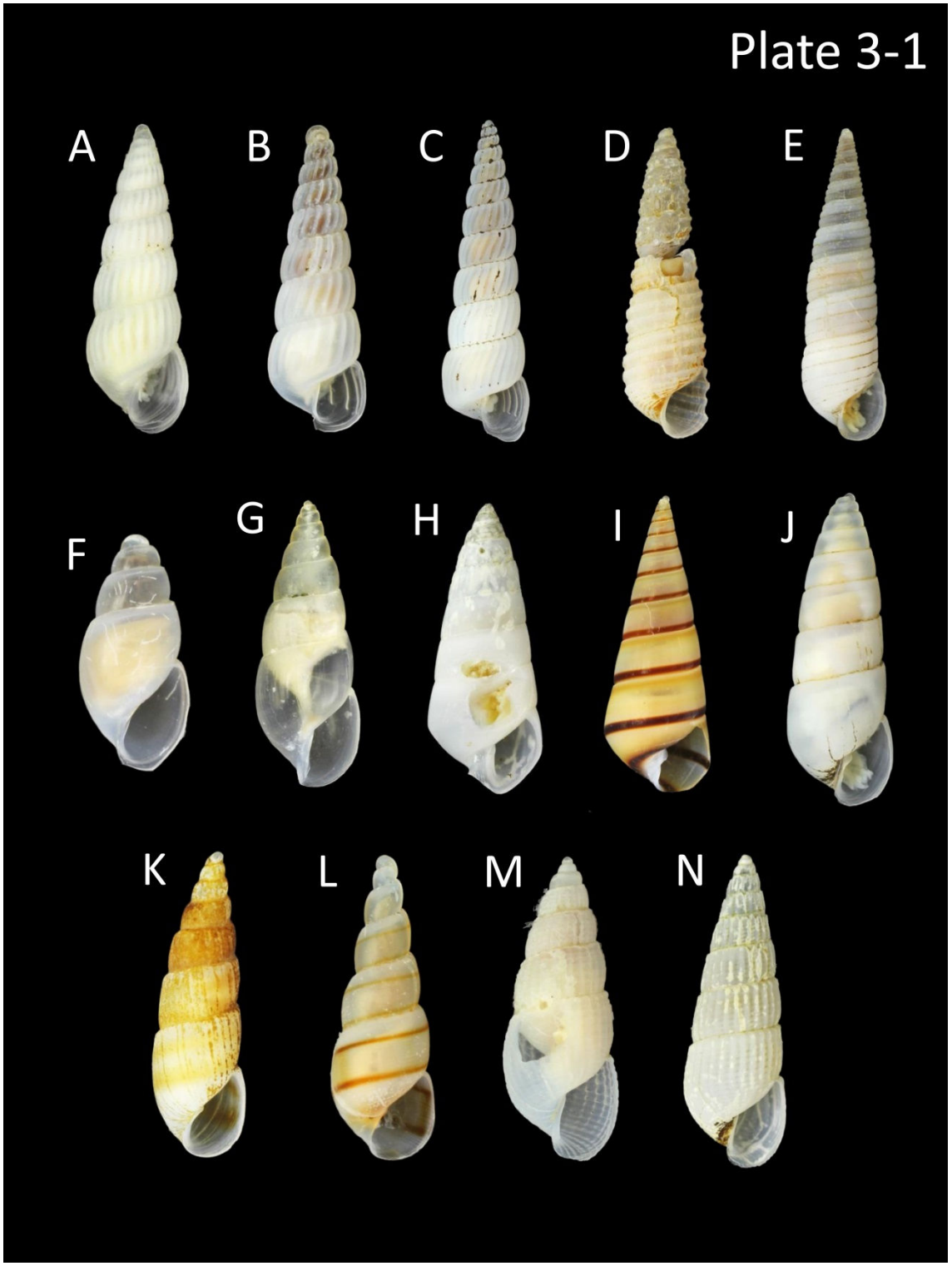
Plate 3-1
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Plate 3-2.
Pyramidellids used for molecular phylogenetic reconstruction with reference to the specimen number and shell size (Group 3).

A. *Paramormula scrobiculata* (YK#950); shell height = 8.4 mm

B. Pyramidellidae gen. sp. “Limpet” (YK#2743); shell length = 3.8 mm

C. Cf. *Turbonilla* sp. (YK#2735); 13.2 mm

D. Pyramidellidae gen. sp. A (YK#2734); 8.4 mm (broken)

E. *Moerchinella* sp. (YK#2737); diameter = 3.1 mm

F. *Herviera gliriella* (YK#947); 2.5 mm

G. *Tibersyrnola serotina* (YK#952); 4.3 mm

H. *Syrnola* sp. cf. *cinctella* (YK#903); 4.8 mm

I. *Paramormula* sp. cf. *speciosa* (YK#897); 5.0 mm

J. *Colsyrnola brunnea* (YK#899); 9.8 mm

K. *Eulimella marmorea* (YK#2741); 10.4 mm

L. Cf. *Turbonilla* sp. “Orange” (YK#2761); 15.0 mm

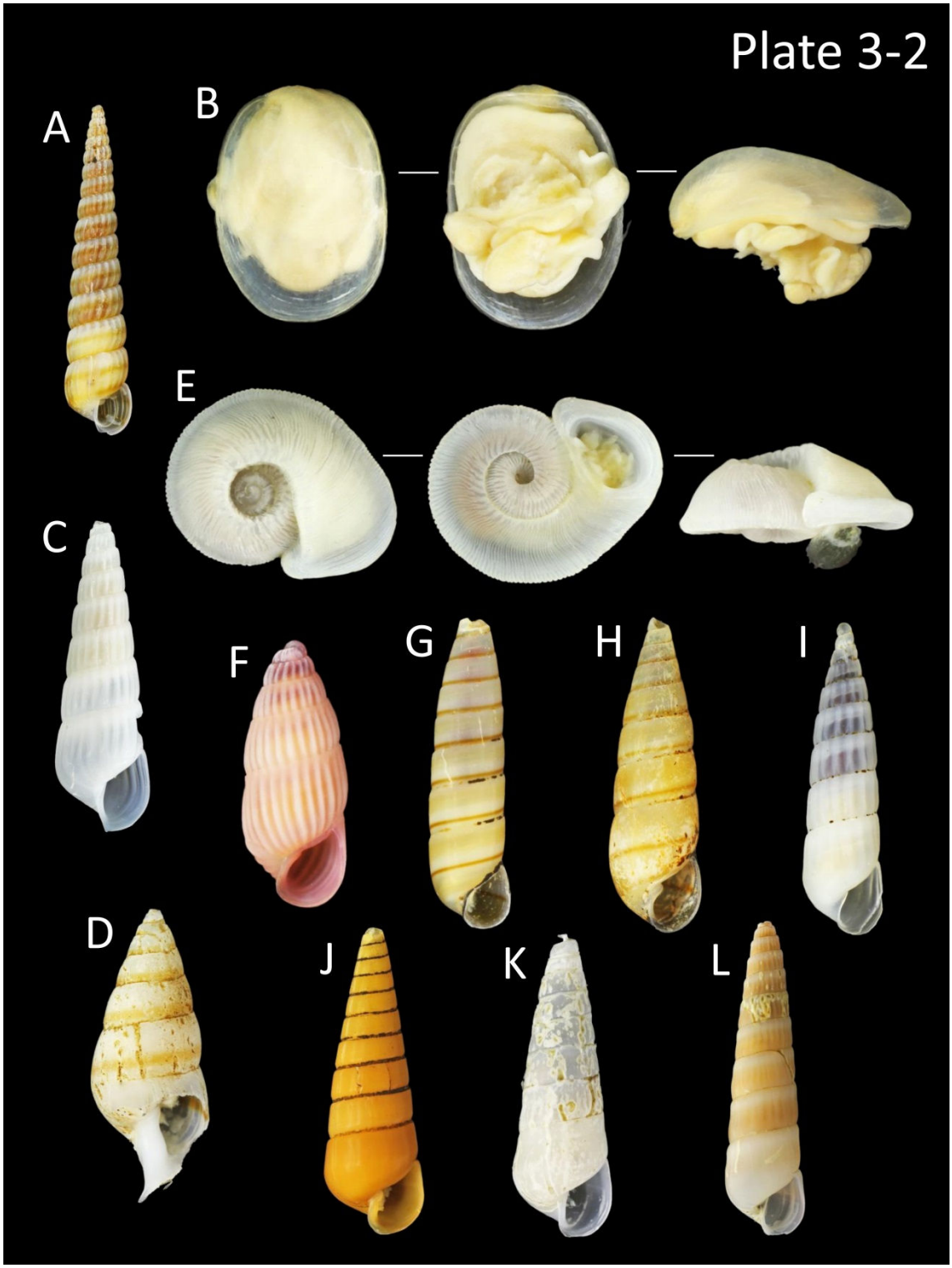
Plate 3-2
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Plate 3-3.
"Amathinid" limpets and a glacidorbid used for molecular phylogenetic reconstruction with reference to the specimen number and shell size (A–C, Group 4; D, outgroup).

A. *Amathina tricarinata* (YK#811); shell length = 6.9 mm

B. *Cyclothyca corrugata* (YK#2745); 7.2 mm

C. *Amathina mortoni* (YK#2740); 8.7 mm

D. *Glacidorbis hedleyi* (YK#2746); diameter = 1.3 mm

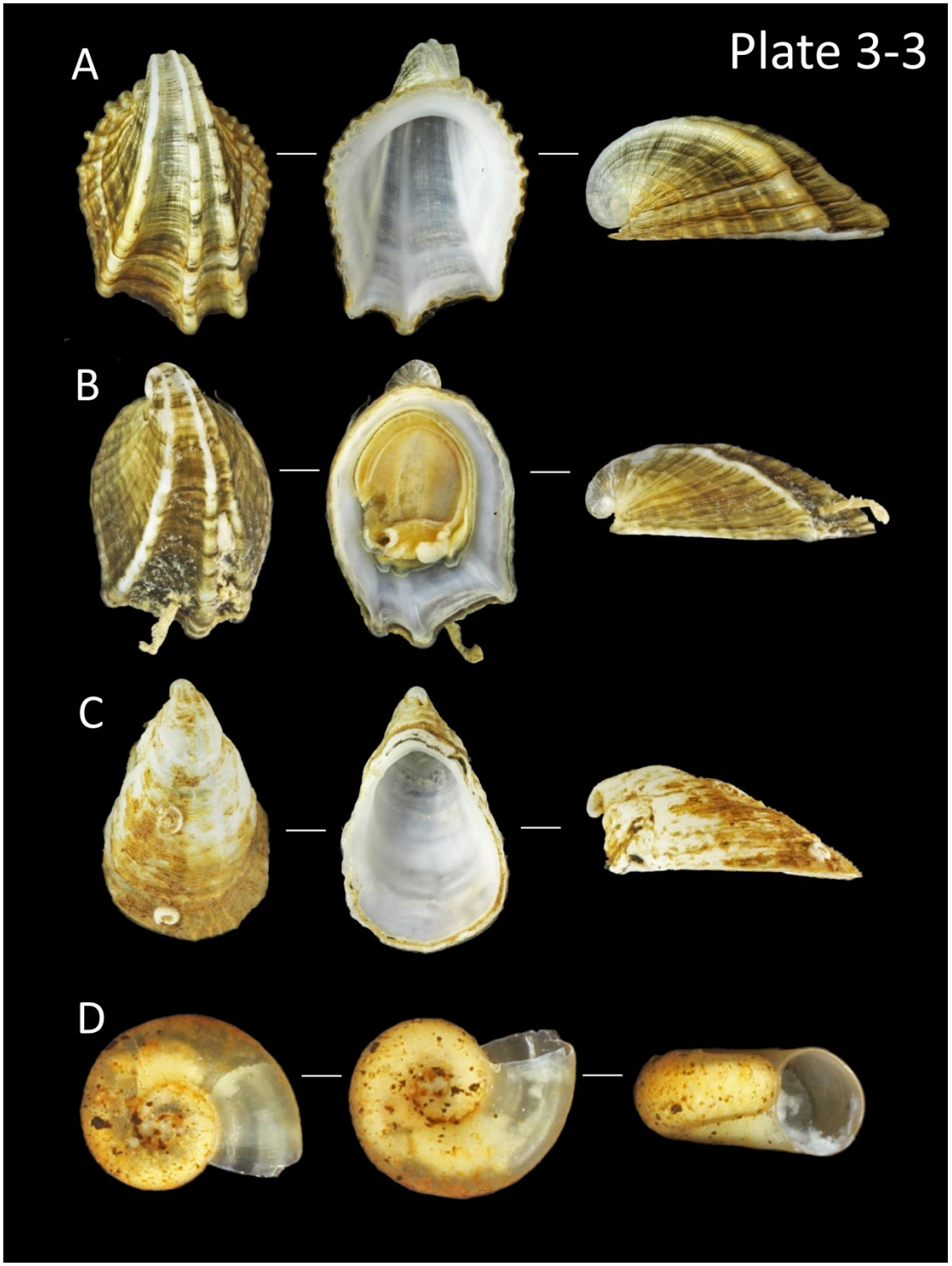
Plate 3-3
A
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D

Plate 3-4.
Pyramidellids and “amathinids” used for molecular phylogenetic reconstruction with reference to the specimen number and shell size (A–F, Group 4; G–O, Group 5).

A. *Monotygma* sp. (YK#2732); shell height = 7.0 mm

B. *Leucotina* sp. aff. *niphonensis* (YK#2736); 7.3 mm

C. Cf. *Leucotina* sp. “N295” (YK#2748); 2.0 mm

D. *Leucotina* sp. cf. *digitalis* (YK#2738); 8.6 mm

E. *Leucotina* sp. “Niraikanai” (YK#808); 3.2 mm

F. *Leucotina* sp. cf. *concinna* (YK#1278); 14.1 mm

G. *Numaegilina gloria* (YK#818); 3.5 mm

H. *Sinuatodostomia nomurai* (YK#873); 3.2 mm

I. *Ondina* sp. (YK#809); 1.4 mm

J. *Iolaea neofelixoides* (YK#820); 2.1 mm

K. *Odostomia desimana* (YK#894); 5.4 mm

L. *Miralda gemma* (YK#957); 3.2 mm

M. *Miralda scopulorum* (YK#2742); 1.9 mm

N. Cf. *Liostomia* sp. (YK#2750); 3.2 mm

O. Cf. *Aartsenia* sp. A (YK#2751); 4.1 mm

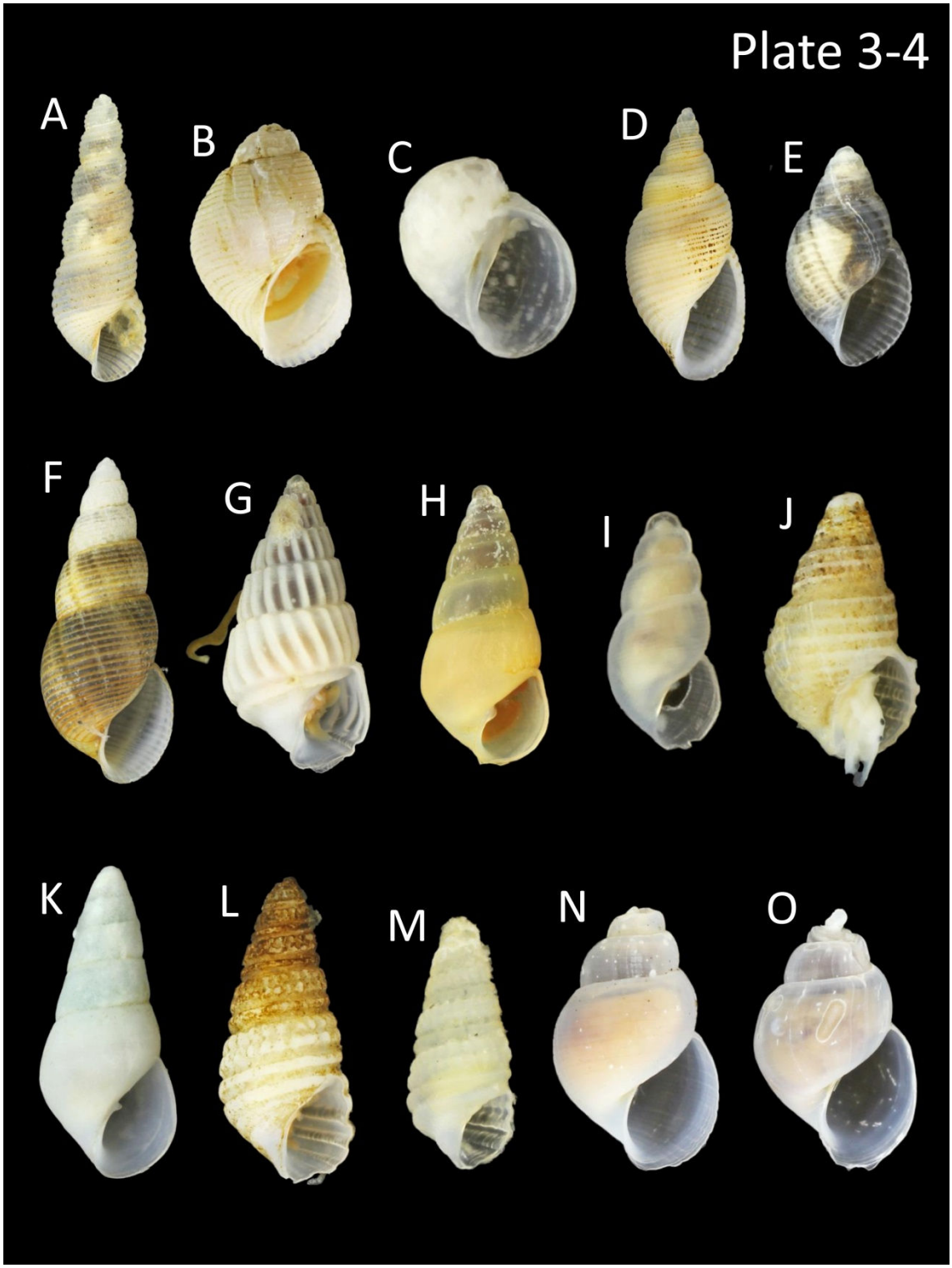
Plate 3-4
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Plate 3-5.
Pyramidellids used for molecular phylogenetic reconstruction with reference to the specimen number and shell size (Group 5).

A. *Odetta lirata* (YK#908); shell height = 2.6 mm

B. *Megastomia* sp. A (YK#911); 3.2 mm

C. *Megastomia tenera* (YK#874); 5.7 mm

D. *Megastomia* sp. B (YK#910); 4.0 mm

E. *Turriodostomia* sp. (YK#1203); 6.4 mm

F. *Quirella suprafila* (YK#907); 2.8 mm

G. Cf. *Chrysallida* sp. (YK#2749); 3.6 mm

H. *Pyrgulina casta* (YK#895); 2.5 mm

I. Pyramidellidae gen. sp. “Neji_F” (YK#1276); 3.2 mm

J. Pyramidellidae gen. sp. “Neji_S” (YK#1279); 2.4 mm

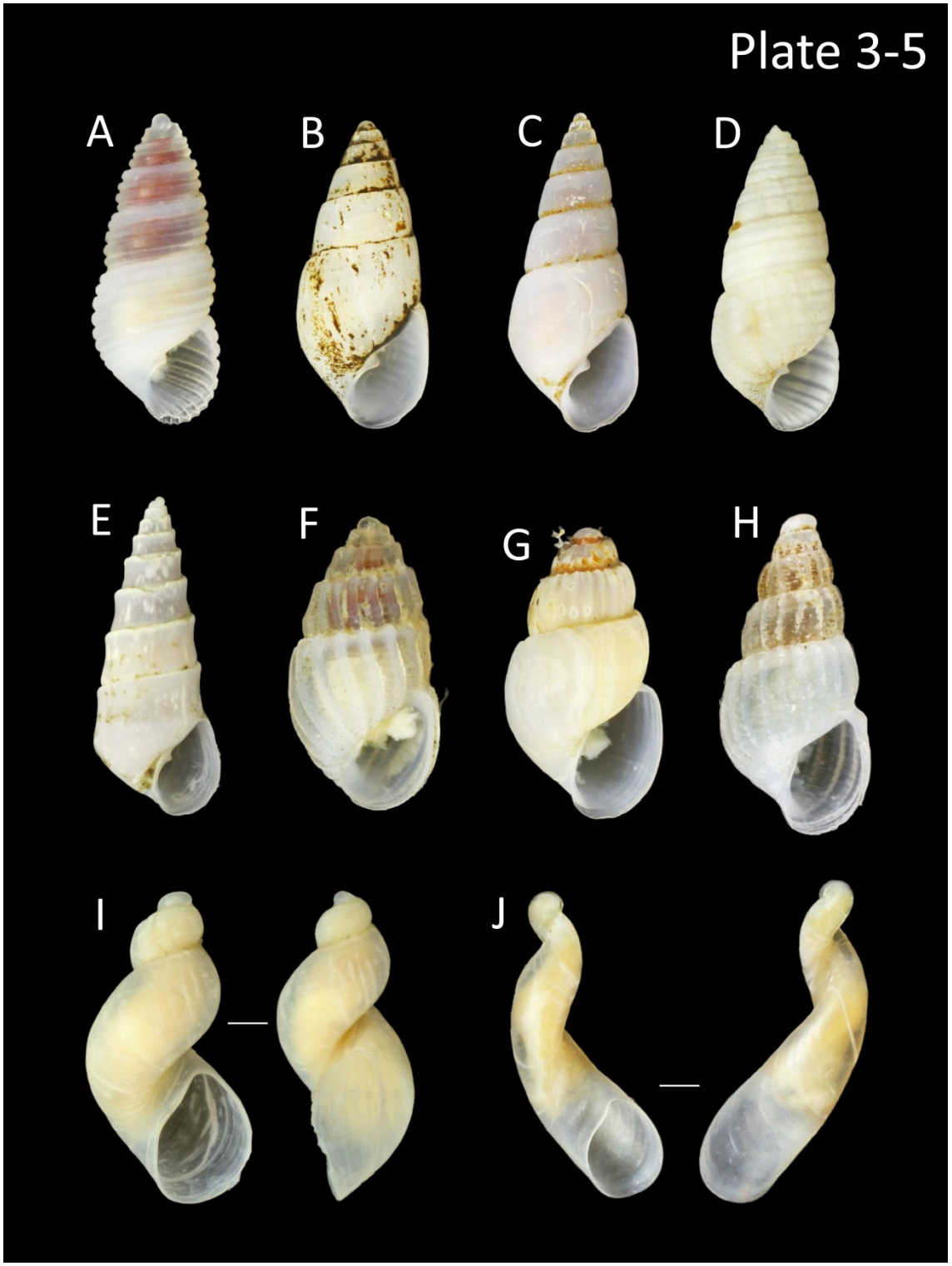
Plate 3-5
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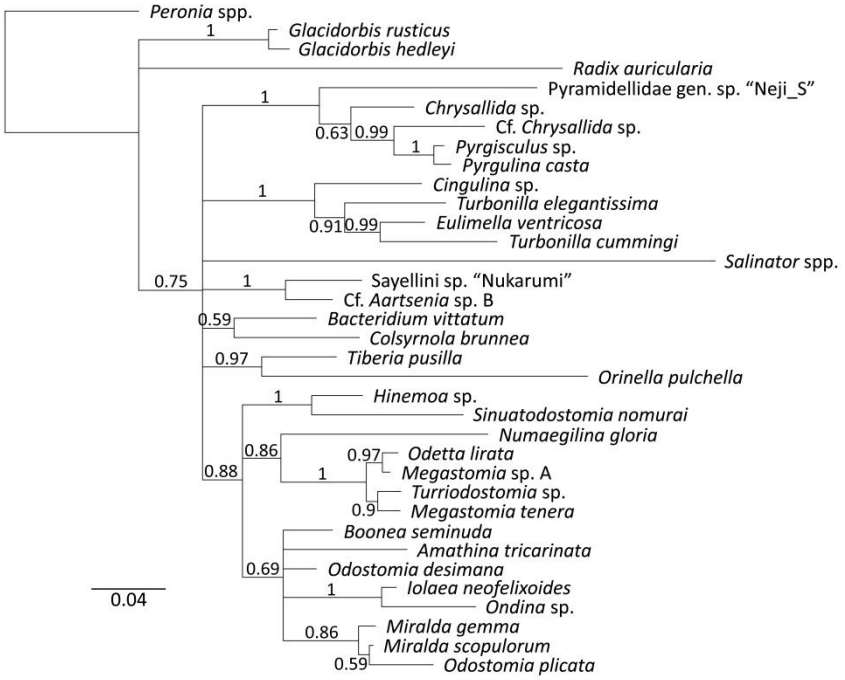

Figure S3-1. Bayesian tree inferred from 28S (D1–D3) gene sequences.

Figure S3-2. Bayesian tree inferred from 18S gene sequences.

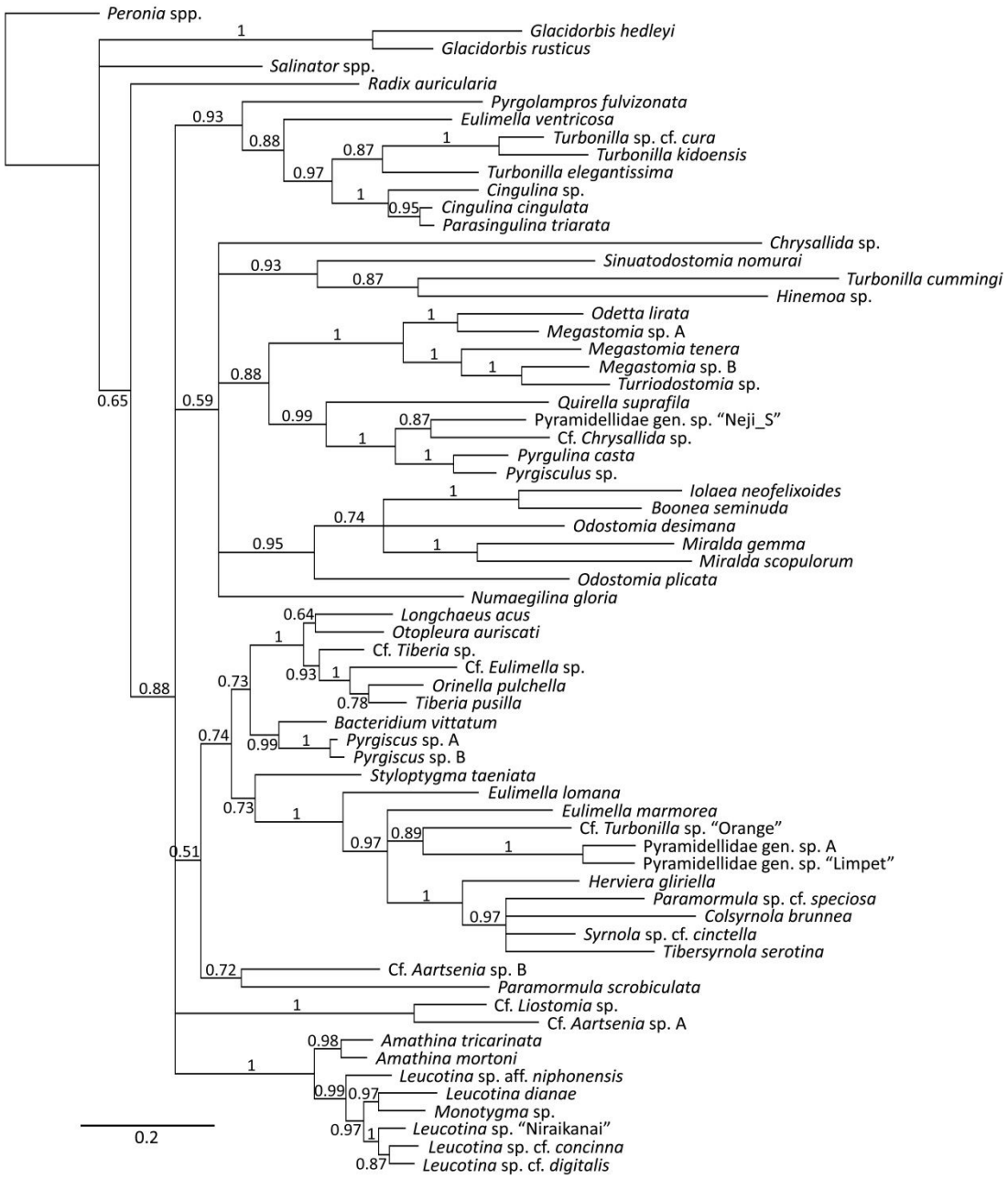

Figure S3-3. Bayesian tree inferred from trnV + 16S gene sequences.

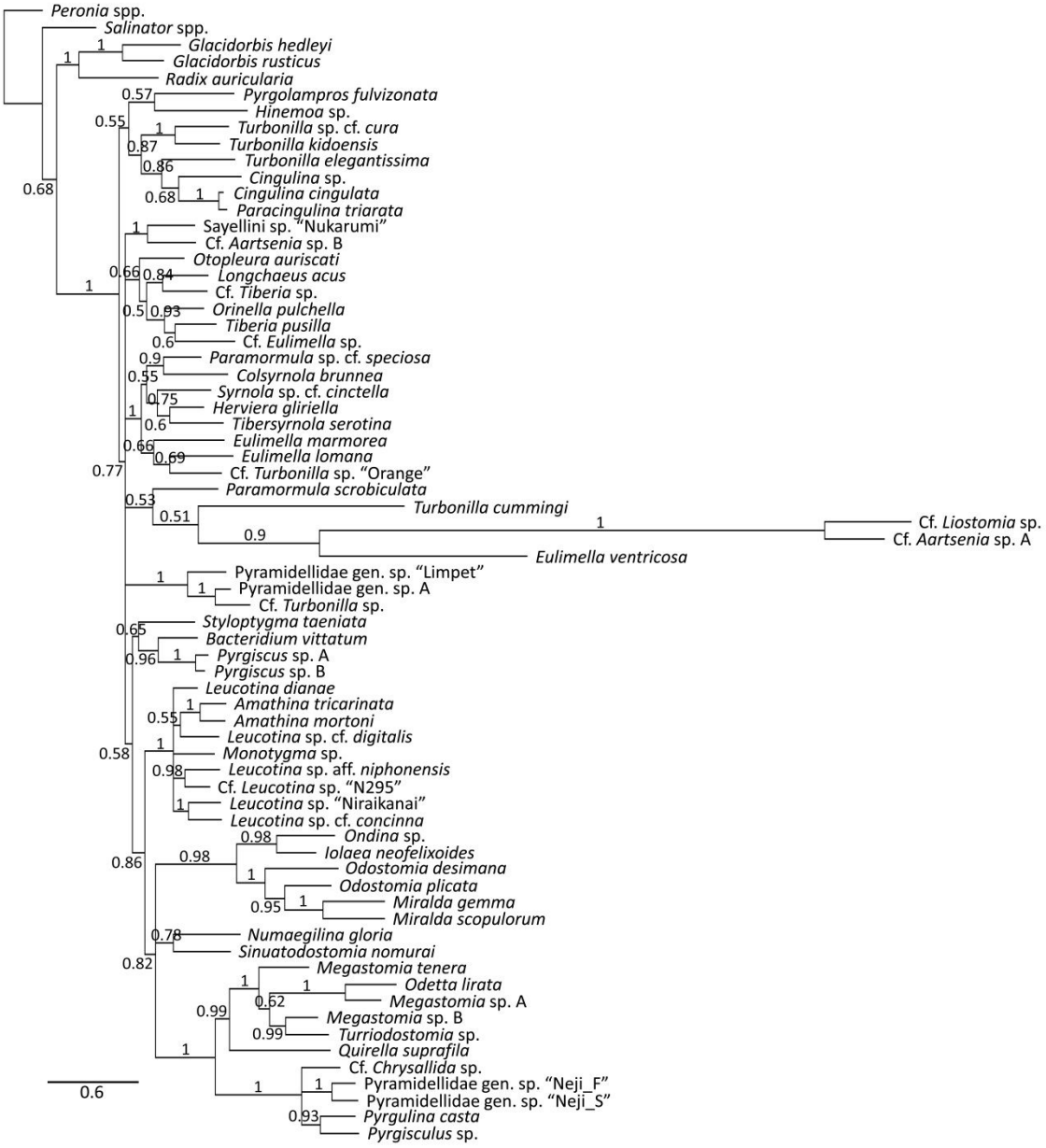

Figure S3-4. Bayesian tree inferred from COI gene sequences.

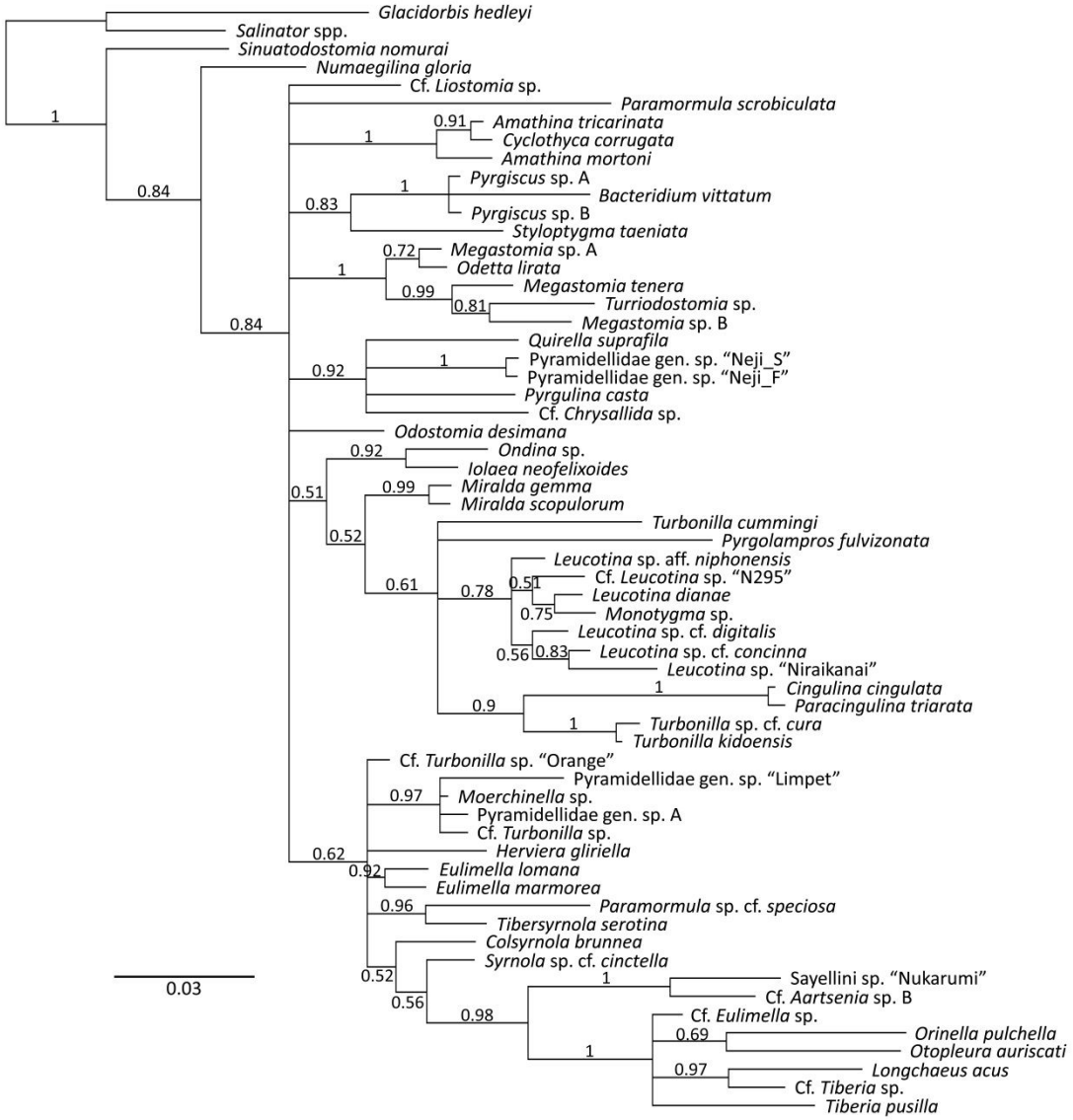

Figure S3-5. Bayesian tree inferred from H3 gene sequences.