WORLDWIDE PHYLOGEOGRAPHY OF LIMPETS OF THE ORDER PATELLOGASTROPODA: MOLECULAR, MORPHOLOGICAL AND PALAEONTOLOGICAL EVIDENCE

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ABSTRACT

A molecular phylogeny, based on sequence data from three mitochondrial (12S rRNA, 16S rRNA and COI) genes, is presented for the Patellogastropoda, including representatives of almost all genera belonging to the Acmaeidae, Lepetidae, Lottiidae, Nacellidae and Patellidae. For comparison, a smaller dataset of sequences from two nuclear (18S rRNA and 28S rRNA) genes is presented. The mitochondrial gene phylogeny shows considerable disagreement with earlier hypotheses derived from morphological data. The Patellidae, Nacellidae and Lepetidae are monophyletic, but the Acmaeidae and Lottiidae are polyphyletic. The family Acmaeidae is divided into two clades corresponding to the subfamilies Acmaeinae and Pectinodontinae, but these two do not form a clade. The Acmaeinae are synonymized with the Lottiidae, and the Pectinodontinae are elevated to familial rank. Our results suggest that the *Patelloida profunda* group (formerly assigned to the Lottiidae) is the most basal group within the Patellogastropoda. We assign this group to a new genus, *Eoacmaea*, in the new family Eoacmaeidae. We used a Bayesian Markov-Chain Monte Carlo approach together with the fossil record to estimate divergence times from the combined DNA sequence data. The lineage of extant Patellogastropoda is estimated to have originated as long ago as Late Jurassic. The phylogeny also suggests that the principal clades and antitropical distribution pattern of the Patellogastropoda formed during the Mesozoic to early Cenozoic, in association with the disruption of Pangea and following the establishment and decline of the circumglobal equatorial current.

INTRODUCTION

The limpets belonging to the Patellogastropoda are of particular evolutionary interest, since this is the most primitive group of living Gastropoda (Golikov & Starobogatov, 1975; Wingstrand, 1985; Haszprunar, 1988a; Harasewych *et al*., 1997; Colgan, Ponder & Eggler, 2000) and is the sister taxon of all other gastropods (Ponder & Lindberg, 1997). The oldest fossils date back to the Middle Ordovician (Yochelson, 1988, 1994). The monophyly of these limpets is supported by a number of synapomorphic characters distinguishing them from other gastropods (e.g. secondarily uncoiled shells, two pairs of outer lateral radular teeth, subpallial sensory streaks, shell microstructure including foliated and conical crossed-lamellar layers, the presence of pallial gills, rotation of the pericardium) (Lindberg, 1998b). The extant members of Patellogastropoda are currently classified in five families: Patellidae, Nacellidae, Acmaeidae, Lottiidae and Lepetidae.

Limpets are common and familiar inhabitants of seashores throughout the world oceans from tropical to polar regions. Most members of this group occur on intertidal rocky shores, and they play an important role in intertidal marine ecosystems (Branch, 1985a, b). They have also colonized other littoral habitats and can be found on *Zostera* (*Lottia*: Lindberg, 1979, 1981; Carlton *et al*., 1991; *Discurria*: Lindberg, 1988; *Notoacmea*: Powell, 1979), on limestone in the supratidal zone (*Patelloida profunda* group: Lindberg & Vermeij, 1985; Kirkendale & Meyer, 2004), on coralline algae (*Yayoiacmea*: Sasaki & Okutani, 1993b; *Patelloida*: Sasaki, 2000), on wood (*Pectinodonta*: Marshall, 1985; *Potamacmaea*: Lindberg, 1990), on the shells of other molluscs (*Patelloida*: Morton, 1980; Nakano & Ozawa, 2005) and in association with vents or seeps (*Bathyacmaea*: Sasaki, Okutani & Fujikura, 2003). Since they are so widespread, adapted to many substrata and readily accessible, they have been widely used as models in evolutionary studies (Giesel, 1970; Lindberg & Wright, 1985; Hocky, Bosman & Ryan, 1987; Byers, 1989; Espoz & Castilla, 2000). Furthermore, the Patellogastropoda have provided examples of adaptive radiation and historical biogeography (Koufopanou *et al*., 1999; Nakano & Ozawa, 2004).

Historically, taxonomic studies of limpets have used external shell morphology, but the high degree of variability of shells has led to taxonomic confusion and the failure to understand species complexes (*Cellana radiata*: Powell, 1973; *Lottia strigatella*: Simison & Lindberg, 2003; *Notoacmea fascicularis*: Simison & Lindberg, 1999; *P. saccharina*: Sasaki, 1999; Kirkendale & Meyer, 2004; *P. pygmaea*: Morton, 1980; Nakano & Ozawa, 2005). It has been recognized that radular morphology is a useful character not only for familial and generic differentiation, but also for species-level distinctions (Pilsbry, 1891; Thiele, 1929; Powell, 1973; Moskalev, 1977; Lindberg, 1998a, b; Sasaki, 1998). Other characters that have been successfully used to differentiate species are coloration of the foot and pallial tentacles (Evans, 1947; Bowman, 1981), coloration of the egg (Habe, 1944), jaw morphology (Sasaki & Okutani, 1994), gill morphology (Lindberg, 1988), configuration of the radular sac (Sasaki & Okutani, 1993a), sperm ultrastructure (Koike, 1985; Healy, 1988; Hodgson & Bernard, 1988; Hodgson *et al*., 1996) and karyotype (Nakamura, 1987; Cervella *et al*., 1988).

Both morphological characters and molecular data have been used for phylogenetic analyses. Cladistic analyses based on morphological characters have been employed for familial relationships (Lindberg, 1998b; Sasaki, 1998), and for generic-level analyses of the Patellidae (Jamieson, Hodgson & Bernard,

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1991), Lottiidae and Acmaeidae (Lindberg, 1988) and Patellidae and Nacellidae (Ridgway, 1994; Ridgway *et al*., 1998). A few molecular analyses have been based on allozyme frequencies (Sella, Robotti & Biglione, 1993; Cretella *et al*., 1994; Weber *et al*., 1997). More recently, DNA-sequence data have been used. Koufopanou *et al*., 1999 used mitochondrial genes in a study of Patellidae and Nacellidae, and Goldstien, Schiel & Gemmell, 2006 examined Nacellidae. Nakano & Ozawa, 2004 constructed molecular phylogenetic using mitochondrial 12S rRNA and 16S rRNA, in order to clarify the relationships among the patellogastropods, with particular reference to the Lottiidae of the northern Pacific. Despite numerous studies on the taxonomy of the Patellogastropoda, the phylogeny is still unclear and there has been no molecular test of phylogenetic hypotheses for the entire order.

Many authors have discussed gastropod evolution by combining phylogenetic trees with morphological or habitat characters (e.g. Ridgway *et al*., 1998; Simison & Lindberg, 1999, 2003; Nakano & Ozawa, 2005; Williams & Ozawa, 2006). Furthermore, phylogenetic trees can be used to produce hypotheses of historical biogeography. Molecular techniques provide both reconstruction of the relationships between living species and estimates of their approximate divergence time. These data with known geographical distributions produce hypotheses of historical speciation events (Williams, 2000; Lessios, Kessing & Pearse, 2001; Kooistra, Coppejans & Payri, 2002; Meyer, 2003). This methodology is particularly important for the molluscs found on intertidal rocky shores, where fossils are seldom preserved in the high-energy environment. Even if a fossil limpet is preserved, it is often difficult to make generic and even familial assignments based on shell morphology. However, the pioneering work of MacClintock (1967) demonstrated an extraordinary diversity of microstructure and mineralogy in the patellogastropod shell, laying the foundation for a classification based on characters that are preserved in fossils. Thus, the diagnostic characters of shell microstructure provide reliable palaeontological evidence for estimating divergence times of clades (Lindberg & Hickman, 1986; Lindberg, 1988; Hedegaard, Lindberg & Bandel, 1997).

The historical biogeography of patellid, nacellid and northern lottiid limpets is relatively well understood, being connected with tectonic and climatic changes following the disruption of the Pangean supercontinent (Nakano & Ozawa, 2004). However, comparable studies are lacking for southern Pacific species of Lottiidae, for Lepetidae and for the deep-water fauna. Among the lottiids there are radiations not only in the northern Pacific, but also in the southern oceans (*Lottia*, *Patelloida*, *Notoacmea* in Australia; *Notoacmea*, *Patelloida*, *Radiacmea*, *Atalacmea* in New Zealand; *Scurria* in western South America). This distribution suggests the possibility of antitropical patterns, as are known among many marine organisms (Ekman, 1953; Raven, 1963; Pielou, 1979). Only a few antitropical patterns have been analysed based on molecular data, and some results suggest that these distributions were established by transtropical migration during glacial periods of the Plio-Pleistocene (e.g. Hilbish *et al*., 2000; Grant & Leslie, 2001). However, Koufopanou *et al*. (1999) and Nakano & Ozawa (2004) showed much older antitropical patterns of limpets dating back to the Mesozoic.

The purpose of this study is to examine the phylogeny, historical biogeography and timing of radiations in the Patellogastropoda and to study the resulting patterns of global species richness and antitropical distribution. In this paper we present mtDNA data for all major lineages of the Patellogastropoda, including approximately 80% of the recognized species and an additional 12 ESUs (evolutionary significant units; Moritz, 1994) discovered by intensive worldwide sampling. We combine molecular data and a limited fossil record to estimate a time scale. We show that general patterns of distribution and diversity are the result of historical vicariance and dispersal in association with the disruption of the Pangean supercontinent.

MATERIAL AND METHODS

Sampling and identification

Table 1 lists the species and collection localities of specimens used in this study. Generic assignments were made following Powell (1973, 1979), Ponder & Creese (1980), Lindberg & Vermeij (1985), Lindberg (1986), Daniel (2000), Sasaki, 2000, Kirkendale & Meyer, 2004 and Nakano & Ozawa (2004), with minor amendments. If not already recognized as species or subspecies, distinct lineages are given ESU numbers (e.g. *Lottia* ESU1). In total, 87 species and an additional 12 ESUs were newly sequenced, and combined with published sequences for 12S rRNA and 16S rRNA of 13 species (Koufopanou *et al*., 1999) and 41 species (Nakano & Ozawa, 2004). *Emarginula foveolata fijitai* and *Emarginula variegata* (Fissurellidae) and three species of the Cocculinidae were used as outgroup taxa. As a result of the phylogenetic analysis it is necessary to revise the generic classification of the Patellogastropoda; this revised classification is used in Table 1 and in the following text. All voucher specimens are deposited in the Laboratory of Geobiology, Department of Earth and Planetary Sciences, Nagoya University, Japan, excepting loans from museums.

Selection of markers

We have chosen here three mitochondrial genes, 12S rRNA and 16S rRNA (in order to add sequences from previous studies: Koufopanou *et al*., 1999; Nakano & Ozawa, 2004), and COI. For comparison, we also analysed portions of the nuclear genes 18S rRNA and 28S rRNA from representatives of all families. Harasewych & McArthur (2000) suggested that 18S rRNA diverges at widely differing rates among patellogastropod lineages. We confirmed that rates in the Nacellidae are significantly different from other Patellogastropoda in both 18S rRNA and 28S rRNA genes, and that these genes are highly conserved within the other lineages (Fig. 1). Therefore the phylogeny based on the nuclear ribosomal genes was not robust, as also pointed out by Harasewych & McArthur (2000). Furthermore, the incongruence length difference (ILD) test detected incongruence between nuclear and mitochondrial genes. For these reasons we use the three mitochondrial genes to reconstruct the phylogeny of Patellogastropoda.

DNA extraction, PCR amplification and DNA sequencing

Living specimens were preserved in 95% ethanol. Total DNA was extracted from a fragment of the mantle or foot muscle tissue. Extraction was performed using High Pure PCR Template Preparation Kit (Roche) or a standard phenol/chloroform extraction method. In the latter procedure, a small amount of tissue was treated with 200 μl of TEN buffer (10 mM Tris, ph 8.0, 10 mM EDTA, 10 mM NaCl), 20 μl of 10% SDS and 40 μl of proteinase K (20 mg/ml) at 37°C for 1–2 h with gentle rotation, followed by phenol/chloroform and chloroform extractions, and ethanol precipitation.

The mitochondrial small-subunit ribosomal RNA (12S rRNA), large-subunit ribosomal RNA (16S rRNA), cytochrome-*c* oxidase I (COI) genes, nuclear 18S rRNA and 28S rRNA were amplified and sequenced. Polymerase chain reactions (PCR) were used to amplify approximately 300 to 400 bp of 12S rRNA, 480 to 700 bp of 16S, 1000 bp of 18S, 1500 bp of 28S and 658 bp (for Eoacmaeidae, Patellidae, Nacellidae, Pectinodontidae, Lepetidae, *Erginus*, *Acmaea*,

Table 1. Specimens and localities sequenced in this study.

Species	Localities	12S	16S	COI	18S	28S	Specimen voucher
Order Patellogastropoda							
Eoacmaeidae							
Eoacmaea albonotata (Smith, 1901)	Park Rynie Beach, Natal, South Africa	•	•	•	–	–	UF295658
E. chamorrorum (Lindberg & Vermeij, 1985)	Pago Bay, Guam, USA	•	•	•	–	–	NUGB-L558
E. conoidalis (Pease, 1868)	Mangaia Island, Cook Islands	•	•	•	•	•	UF296033
E. javanica (Nakano, Aswan & Ozawa, 2005)	Sedekan Beach, Java, Indonesia	•	•	•	–	–	NUGB-L526
E. mauritiana (Pilsbry, 1891)	Mauritius	•	•	•	–	–	UF296014
E. omanensis (Christiaens, 1975)	Al Q'urm, Oman	•	•	•	–	–	UF295654
G *E. profunda* (Deshayes, 1863)	Reunion	•	•	•	–	–	UF295652
Eoacmaea ESU1	Zanzibar, Tanzania	•	•	•	–	–	UF296016
Lepetidae							
Cryptobranchia kuragiensis (Yokoyama, 1920)	Aikappu, Akkeshi, Hokkaido, Japan	•	•	•	•	•	NUGB-L505
Lepeta caeca pacifica Moskalev, 1977	Aikappu, Akkeshi, Hokkaido, Japan	•	•	•	•	•	NUGB-L503
Lottiidae							
G *Acmaea mitra* Rathke, 1833	Boiler Bay, Washington, USA	AB106470*	AB106518*	•	•	•	NUGB-L247
Atalacmea fragilis (Sowerby, 1823)	Hartington Point, Dunedin, South Island, New Zealand	•	•	•	–	–	NUGB-L592
G *Discurria insessa* (Hinds, 1842)	Santa Cruz, California, USA	•	•	–	–	–	NUGB-L572
Erginus sybaritica (Dall, 1871)	Nemuro, Hokkaido, Japan	•	•	•	•	•	NUGB-L500
Lottia antillarum (Sowerby, 1831)	Lucea Bay, Jamaica	•	•	•	–	–	NUGB-L406
L. cassis (Eschscholtz, 1833)	Akkeshi, Hokkaido, Japan	AB106442*	AB106490*	•	–	–	NUGB-L117
L. digitalis (Rathke, 1833)	Cape Alava, WA, USA	•	•	•	–	–	NUGB-L616
L. dorsuosa (Gould, 1859)	Morozaki, Aichi, Japan	AB106454*	AB106502*	–	–	–	NUGB-L125
L. emydia (Dall, 1914)	Aininkappu, Hokkaido, J'apan	AB106449*	AB106497*	–	–	–	NUGB-L282
L. fenestrata (Reeve, 1855)	Strawberry Hill, Washington, USA	AB106459*	AB106507*	–	–	–	NUGB-L262
L. filosa (Carpenter, 1865)	Lunda Bay, Palaos Verdes, Los Angels, USA	•	•	•	–	–	NUGB-L507
G *L. gigantea* Sowerby, 1834	Royal Palms, Los Angeles, USA	AB106450*	AB106498*	•	•	•	NUGB-L288
L. jamaicensis (Gmelin, 1791)	Lucea Bay, Jamaica	•	•	–	–	–	NUGB-L407
L. kogamogai Sasaki & Okutani, 1994	Hiraiso, Ibaraki, Japan	AB106445*	AB106493*	•	–	–	NUGB-L143
L. langfordi (Habe, 1944)	Goshikinohama, Kochi, Japan	AB106458*	AB106506*	•	–	–	NUGB-L244
L. limatula (Carpenter, 1864)	Coal Oil Point, California, USA	AB106455*	AB106503*	•	–	–	NUGB-L291
L. lindbergi Sasaki & Okutani, 1994	Akkeshi, Hokkaido, Japan	AB106446*	AB106494*	•	–	–	NUGB-L161
L. luchuana (Pilsbry, 1901)	Ogimi, Okinawa, Japan	AB106452*	AB106500*	•	–	–	NUGB-L131
L. luchuana (Pilsbry, 1901)	Pelabuan Ratu, Java, Indonesia	•	•	•	–	–	NUGB-L497
L. mesoleuca (Menke, 1851)	Manuel Antonio National Park, Costa Rica	•	•	•	–	–	NUGB-L425
L. onychitis (Menke, 1843)	Burns Rocks, WA, Australia	•	•	•	–	–	NUGB-L637
L. orbigny (Dall, 1909)	Chile	•	•	•	–	–	NUGB-L555
L. pelta (Rathke, 1833)	Cattle Point, Washington, USA	AB106443*	AB106491*	•	–	–	NUGB-L256
L. persona (Rathke, 1833)	Cattle Point, Washington, USA	AB106447*	AB106495*	•	–	–	NUGB-L259
L. scabra (Gould, 1846)	Royal Palms, Los Angeles, USA	AB106456*	AB106504*	–	–	–	NUGB-L287
L. scutum (Rathke, 1833)	Cattle Point, Washington, USA	AB106448*	AB106496*	•	–	–	NUGB-L253
L. septiformis (Quoy & Gaimard, 1834)	Moses Rock, WA, Australia	•	•	•	–	–	NUGB-L618
L. smithi Lindberg & McLean, 1981	Puerto Ayora, Santa Cruz, Galapagos Islands	•	•	•	–	–	NUGB-L408
L. subrotundata (Carpenter, 1865)	Panama	•	•	•	–	–	NUGB-L597
L. tenuisculpta Sasaki & Okutani, 1994	Kaino, Mie, Japan	AB106451*	AB106499*	•	–	–	NUGB-L158
L. testudinalis (Müller, 1776)	Coal Oil Point, California, USA	AB106469*	AB106517*	–	–	–	NUGB-L295
L. testudinalis (Müller, 1776)	Millport, UK	AB107900*	AB107905*	–	–	–	NUGB-L335
Lottia ESU1	Akkeshi, Hokkaido, Japan	AB106441*	AB106489*	•	–	–	NUGB-L119
Lottia ESU2	Osika, Miyagi, Japan	•	•	•	–	–	NUGB-L355
Lottia ESU3	Tangalle, Sri Lanka	•	•	•	–	–	NUGB-L411

Continued

Table 1. *Continued*

Species	Localities	12S	16S	COI	18S	28S	Specimen voucher
Nipponacmea concinna (Lischke, 1870)	Morozaki, Aichi, Japan	AB106463*	AB106511*	•	–	–	NUGB-L185
N. fuscoviridis (Teramachi, 1949)	Akasyouzaki, Fukui, Japan	AB106461*	AB106509*	•	–	–	NUGB-L204
N. gloriosa (Habe, 1944)	Kamo, Shizuoka, Japan	AB106467*	AB106515*	•	–	–	NUGB-L234
N. habei (Saski & Okutani, 1994)	Yamada, Iwate, Japan	AB106465*	AB106513*	•	–	–	NUGB-L183
N. nigrans (Kira, 1961)	Hiraiso, Ibaraki, Japan	AB106468*	AB106516*	•	•	•	NUGB-L220
N. radula (Kira, 1961)	Gesuzima, Kagoshima, Japan	AB106464*	AB106512*	•	–	–	NUGB-L217
G *N. schrenckii* (Lischke, 1868)	Sukari, Mie, Japan	•	•	•	–	–	NIGB-L180
N. teramachii (Kira, 1961)	Owase, Mie, Japan	AB106466*	AB106514*	•	–	–	NUGB-L227
G *Niveotectura pallida* (Gould, 1859)	Aininkappu, Hokkaido, Japan	AB106471*	AB106519*	•	•	•	NUGB-L279
Notoacmea alta Oliver, 1926	Moses Rock, WA, Australia	•	•	•	–	–	NUGB-L620
N. badia (Oliver, 1926)	Kaka Point, Dunedin, South Island, New Zealand	•	•	•	–	–	NUGB-L582
N. elongata (Quoy & Gaimard, 1834)	Hartington Point, Dunedin, South Island, New Zealand	•	•	•	–	–	NUGB-L584
N. flammea (Quoy & Gaimard, 1834)	Albany, WA, Australia	•	•	•	–	–	NUGB-L635
N. helmsi (E.A. Smith, 1894)	Kaikoura, South Island, New Zealand	•	•	•	–	–	NUGB-L588
N. inconspicua (Gray in Dieffenbach, 1843)	Kaikoura, South Island, New Zealand	•	•	•	–	–	NUGB-L596
N. parviconoidea (Suter, 1907)	Kaikoura, South Island, New Zealand	•	•	•	–	–	NUGB-L590
N. petterdi (Tenison Woods, 1876)	Botany Bay, NSW, Australia	•	•	•	–	–	NUGB-L643
G *N. pileopsis* (Quoy & Gaimard, 1834)	St Clair Beach, Dunedin, South Island, New Zealand	•	•	•	–	–	NUGB-L586
Patelloida alticostata (Angas, 1865)	Palau	•	•	•	–	–	NUGB-L417
P. alticostata (Angas, 1865)	Albany, WA, Australia	•	•	•	–	–	NUGB-L633
P. conulus (Dunker, 1861)	Tahara, Aichi, Japan	AB106435*	AB106483*	•	–	–	NUGB-L94
P. corticata (Hutton, 1880)	Omaha Beach, North Island, New Zealand	•	•	•	–	–	NUGB-L594
P. heroldi (Dunker, 1861)	Yukinoura, Mie, Japan	•	•	•	–	–	NUGB-L352
P. insignis (Menke, 1843)	Pambula Beach, NSW, Australia	•	•	–	–	–	NUGB-L650
P. latistrigata (Angas, 1865)	Pambula Beach, NSW, Australia	•	•	–	–	–	NUGB-L649
P. lentiginosa (Reeve, 1855)	Benoki, Okinawa, Japan	•	•	•	–	–	NUGB-L343
P. mimula (Iredale, 1924)	Nullica River, NSW, Australia	•	•	–	–	–	NUGB-L641
P. nigrosulcata (Reeve, 1855)	Albany, WA, Australia	•	•	•	–	–	NUGB-L631
P. pygmaea (Dunker, 1860)	Tahara, Aichi, Japan	AB106436*	AB106484*	•	•	•	NUGB-L89
P. ryukyuensis Nakano & Ozawa, 2005	Hanezi, Okinawa, Japan	•	•	•	–	–	NUGB-L465
P. saccharina lanx (Reeve, 1855)	Oga, Akita, Japan	AB106439*	AB106487*	•	–	–	NUGB-L63
P. saccharina saccharina (Linnaeus, 1758)	Katsuren, Okinawa, Japan	AB106438*	AB106486*	•	–	–	NUGB-L80
P. saccharinoides Habe & Kosuge, 1996	Pelabuan Ratu, Java, Indonesia	•	•	•	–	–	NUGB-L498
P. signata (Pilsbry, 1901)	Hayama, Kanagawa, Japan	•	•	–	–	–	NUGB-L351
P. striata (Quoy & Gaimard, 1834)	Awung, Lombok, Indonesia	•	•	•	–	–	NUGB-L423
Patelloida ESU1	Cape d'Aguilar, Hong Kong	AB106440*	AB106488*	•	–	–	NUGB-L310
Patelloida ESU2	Tanjong Labeh, Johor, West Malaysia	•	•	•	–	–	NUGB-L509
Patelloida ESU3	Carita Beach, Java, Indonesia	•	•	•	–	–	NUGB-L529
Patelloida ESU4	Karang Hawu Beach, Java, Indonesia	•	•	–	–	–	NUGB-L561
Patelloida ESU5	Sulawesi, Indonesia	•	•	•	–	–	NUGB-L424
Patalloida ESU6	Galle Fort, Sri Lanka	•	•	•	–	–	NUGB-L409
Patelloida ESU7	Bohol, Philippines	•	•	•	–	–	UF320075
Scurria bahamondina (Ramirez-Bohme, 1974)	Chile	•	•	•	–	–	NUGB-L567
S. ceciliana ceciliana (d'Orbigny, 1841)	Chile	•	•	•	–	–	NUGB-L552
S. ceciliana magellanica (Strebel, 1907)	Chile	•	•	•	–	–	NUGB-L570
S. chaitena (Ramirez-Bohme, 1974)	Chile	•	•	•	–	–	NUGB-L568
S. dalcahuina (Ramirez-Bohme, 1974)	Chile	•	•	•	–	–	NUGB-L554

Continued

Table 1. *Continued*

Species	Localities	12S	16S	COI	18S	28S	Specimen voucher
S. parasitica (d'Orbigny, 1841)	Chile	•	•	•	–	–	NUGB-L569
S. plana (Philippi, 1846)	Chile	•	•	–	–	–	NUGB-L566
G *S. scurra* (Lesson, 1841)	Chile	•	•	•	–	–	NUGB-L550
S. silvana (Ramirez-Bohme, 1974)	Chile	•	•	•	–	–	NUGB-L562
S. variabilis (Sowerby, 1839)	Chile	•	•	–	–	–	NUGB-L553
S. viridula (Lamarck, 1822)	Chile	•	•	•	–	–	NUGB-L563
S. zebrina (Lesson, 1830)	Chile	•	•	•	–	–	NUGB-L565
G *Tectura virginea* (Müller, 1776)	Millport, UK	AB107903*	AB107908*	•	•	•	NUGB-L338
G *Yayoiacmea oyamai* (Habe, 1955)	Omaezaki, Shizuoka, Japan	AB106460*	AB106508*	•	•	•	NUGB-L269
Nacellidae							
Cellana eucosmia (Pilsbry, 1891)	Hurghada, Egypt	•	•	•	–	–	NUGB-L396
C. denticulata (Martyn, 1784)	Kaikoura, South Island, New Zealand	•	•	•	–	–	NUGB-L575
C. flava (Hutton, 1873)	Kaikoura, South Island, New Zealand	•	•	•	–	–	NUGB-L576
C. grata (Gould, 1859)	Kaino, Mie, Japan	AB106428*	AB106476*	•	–	–	NUGB-L54
C. karachiensis (Winckworth, 1930)	Oman	•	•	•	–	–	UF292785
C. nigrolineata (Reeve, 1839)	Kaino, Mie, Japan	AB106429*	AB106477*	•	–	–	NUGB-L30
C. ornata (Dillwyn, 1817)	Omaha Beach, North Island, New Zealand	•	•	•	–	–	NUGB-L578
C. pricei Powell, 1973	O Le Pupu, Upolu, Western Samoa	•	•	•	–	–	NUGB-L397
C. radians (Gmelin, 1791)	Omaha Beach, North Island, New Zealand	•	•	•	–	–	NUGB-L580
C. radiata capensis (Gmelin, 1791)	Cape Vidal, South Africa	•	•	•	–	–	NUGB-L395
C. radiata enneagona (Reeve, 1854)	Madagascar	•	•	•	–	–	NUGB-L490
C. radiata orientalis (Pilsbry, 1891)	Okinawa, Japan	AB106430*	AB106478*	•	•	•	NUGB-L27
C. radiata orientalis (Pilsbry, 1891)	Tanah Lot, Bali, Indonesia	•	•	•	–	–	NUGB-L400
C. radiata orientalis (Pilsbry, 1891)	Pelabuan Ratu, Java, Indonesia	•	•	•	–	–	NUGB-L494
C. radiata radiata (Born, 1778)	Kovalam, Kerala, India	•	•	•	–	–	NUGB-L398
C. radiata radiata (Born, 1778)	Hendala, Sri Lanka	•	•	•	–	–	NUGB-L399
C. radiata radiata (Born, 1778)	Negombo, Sri Lanka	•	•	•	–	–	NUGB-L515
C. radiata radiata (Born, 1778)	Kalbarri, WA, Australia	•	•	•	–	–	NUGB-L625
C. solida (Blainville, 1825)	Orford, Tasmania, Australia	•	•	•	–	–	NUGB-L401
C. taitensis (Röding, 1798)	Taihoae Bay, Nuku Hiva, Marquesas Islands	•	•	•	–	–	NUGB-L402
C. tesutudinaria (Linnaeus, 1758)	Okinawa, Japan	AB106432*	AB106479*	•	–	–	NUGB-L42
C. toreuma (Reeve, 1854)	Oga, Akita, Japan	AB106425*	AB106473*	•	–	–	NUGB-L3
C. toreuma (Reeve, 1854)	Pelabuan Ratu, Java, Indonesia	•	•	•	–	–	NUGB-L496
C. tramoserica (Holten, 1802)	Botany Bay, NSW, Australia	•	•	•	–	–	NUGB-L647
Nacella deaurata (Gmelin, 1791)	Bahia Lapataia, Tierra del Fuego, Argentina	•	•	•	–	–	NUGB-L413
N. magellanica (Gmelin, 1791)	Bahia Lapataia, Tierra del Fuego, Argentina	•	•	•	•	•	NUGB-L414
N. mytilina (Helbling, 1779)	Traiguen Island, Chile	•	•	•	–	–	NUGB-L415
Patellidae							
Cymbula compressa (Linnaeus, 1758)	Kommetjie, Cape Town, South Africa	•	•	•	–	–	NHM
C. miniata (Born, 1778)	Kommetjie, Cape Town, South Africa	•	•	•	–	–	NHM
C. oculus (Born, 1778)	West Bank, East London, South Africa	•	•	•	–	–	NHM
C. safiana (Lamarck, 1819)	Ceuta, Spain	•	•	•	–	–	NUGB-L657
Helcion concolor (Krauss, 1848)	West Bank, East London, South Africa	•	•	•	–	–	NHM
H. dunkeri (Krauss, 1848)	Bloubergstrand, Cape Town, South Africa	•	•	•	–	–	NHM
H. pruinosus (Krauss, 1848)	Dalebrook, Cape Town, South Africa	•	•	•	–	–	NHM
Patella aspera Röding, 1798	Madeira, Portugal	AF058201**	AF058249**	–	–	–	–
P. caerulea Linnaeus, 1758	Ceuta, Spain	•	•	•	–	–	NUGB-L653
P. candei d'Orbigny, 1839	Santa Maria, Azores, Portugal	AF058206**	AF058255**	–	–	–	–
P. depressa Pennant, 1777	Zahara los Atunes, near Tarifa, Spain	AF058208**	AF058257**	–	–	–	–
P. ferruginea Gmelin, 1791	Ceuta, Spain	•	•	•	–	–	NUGB-L655
P. lugubris Gmelin, 1791	Pedra de Lume, Sal Island, Cabo Verde	AF058210**	AF058259**	–	–	–	–

Continued

Table 1. *Continued*

Species	Localities	12S	16S	COI	18S	28S	Specimen voucher
P. pellucida Linnaeus, 1758	Swanage, Dorset, UK	AF058174**	AF058223**	–	–	–	–
P. rustica Linnaeus, 1758	Ceuta, Spain	•	•	•	–	–	NUGB-L651
G *P. vulgata* Linnaeus, 1758	Millport, UK	•	•	•	•	•	NUGB-L340
Scutellastra aphanes (Robson, 1986)	Munster, South Africa	AF058178**	AF058226**	–	–	–	–
S. argenvillei (Krauss, 1848)	Kommetjie, Cape Town, South Africa	AF058179**	AF058227**	–	–	–	–
S. barbara (Linnaeus, 1758)	Kommetjie, Cape Town, South Africa	•	•	•	–	–	NHM
S. chapmani (Tenison-Woods, 1875)	Ulladula, NSW, Australia	AF058181**	AF058229**	–	–	–	–
S. cochlear (Born, 1778)	Kommetjie, Cape Town, South Africa	AF058182**	AF058230**	–	–	–	–
S. exusta (Reeve, 1854)	Madagascar	•	•	•	–	–	NUGB-L491
S. flexuosa (Quoy & Gaimard, 1834)	Tanabe, Wakayama, Japan	AB106433*	AB106481*	•	–	–	NUGB-L274
S. granularis (Linnaeus, 1758)	Kommetjie, Cape Town, South Africa	AF058184**	AF058232**	–	–	–	–
S. laticostata (Blainville, 1825)	Albany, WA, Australia	•	•	•	–	–	NUGB-L629
S. longicosta (Lamarck, 1819)	Kommetjie, Cape Town, South Africa	AF058186**	AF058234**	–	–	–	–
S. miliaris (Philippi, 1848)	Moçâmedes, Angola	AF058195**	AF058243**	–	–	–	–
S. obtecta (Krauss, 1848)	Cape Vidal, South Africa	AF058187**	AF058236**	–	–	–	–
S. optima (Pilsbry, 1904)	Takarazima, Kagoshima, Japan	AB106434*	AB106482*	•	•	•	NUGB-L319
S. peronii (Blainville, 1825)	Little Long Point, via Walpole, WA, Australia	•	•	•	–	–	NUGB-L622
S. ESU1	Madagascar	•	•	•	–	–	NUGB-L492
Pectinodontidae							
G *Bathyacmaea nipponica* Okutani, Tsuchida & Fujikura, 1992	Japan	•	•	•	•	•	NUGB-Ba1
Pectinodonta rhyssa (Dall, 1925)	Off shore, Kiishirahama, Wakayama, Japan	•	•	•	•	•	NUGB-L428
Order Cocculiniformia							
Cocculinidae							
Coccopigya punctoradiata (Kuroda & Habe, 1949)	Kamikawaguchi, Kochi, Japan	•	•	•	•	•	NUGB-L266
Cocculina sp.	Kumanonada, Mie, Japan	•	•	•	•	•	NUGB-L429
Pseudococculina sp.	Tosa Bay, Kochi, Japan	•	•	•	•	•	NUGB-L617
Order Vetigastropoda							
Fissurellidae							
Emarginula foveolata fugitai Habe, 1953	Hiroshima, Seto Inland Sea, Japan	AB106472*	AB106520*	•	•	•	NUGB-L277
E. variegata A. Adams, 1852	Benoki, Okinawa, Japan	•	•	•	•	•	NUGB-L348

Abbreviation: G, type species of genus.
Identification of specimens was based on Powell (1973, 1979), Ponder & Creese (1980), Lindberg & Vermeij (1985), Lindberg (1986), Sasaki (2000), Daniel (2000), Nakano & Ozawa (2004) and Kirkendale & Meyer (2004) with minor amendments. Specimens used to obtain sequence data for a particular gene fragment are marked with a•. *indicates additional sequences from Nakano & Ozawa (2004) and **from Koufopanou *et al.* (1999).

Niveotectura, *Tectura* and most *Nipponacmea*) or 661 bp (for *Lottia*, *Scurria*, *Patelloida*, *Notoacmea*, *Atalacmea* and *Nipponacmea gloriosa*) or 664 bp (for *Yayoiacmea*) of COI. PCR amplification was performed in 25 μl of reaction volume containing 10 mM Tris-HCl ph 8.3, 50 mM KCL, 1.5 mM $MgCl_2$, 200 μM dNTPs, 0.2 μM of a forward and reverse PCR primer (listed in Table 2), 0.5 mg/ml BSA (Sigma), 2 units of Taq polymerase (Takara) and 1 μl of template DNA solution. The cycling parameters for amplification consisted of an initial denaturation for 3 min at 94°C; followed by 30 cycles of denaturation for 45 s at 94°C, annealing for 90 s at a gene-specific annealing temperature (50–56°C for 12S, 50°C for 16S, 45–50°C for COI, 52–54°C for 18S and 28S) and extension for 120 s at 72°C; and ended with a 5 min extension at 72°C. Successful PCR products were purified using High Pure PCR Product Purification Kit (Roche). Direct double-stranded cycle sequencing of 25 to 30 ng of each PCR product was performed in both directions using the Applied Biosystems BigDye v 3 dye terminator cycle sequencing kit. Cycle sequencing was performed using an Applied Biosystems GeneAmp PCR System 9700. The cycling parameters were 25 cycles of 10 s at 96°C, 5 s at 50°C and 4 min at 60°C. Sequencing reaction products were purified using ethanol precipitation and analysed on an ABI PRISM 377 DNA sequencer. Sequences were verified by forward and reverse comparisons. All sequences have been deposited in Gen Bank under accession numbers AB238235-AB238345 (12S rRNA), AB238346-AB238456 (16S rRNA), AB238457-AB238594 (COI), AB282757-AB282777 (18S rRNA) and AB282778-AB282798 (28S rRNA).

Datasets

Eight datasets were used for constructing trees. The dataset based on nuclear genes, 18S rRNA and 28S rRNA, included 21 species (Fig. 1). The large taxon set included 166 individuals for which 12S rRNA and 16S rRNA genes had been sequenced (Fig. 2). The large combined data set included 138 individuals for which all three genes had been sequenced (Fig. 3). Among these are included 21 new sequences for 18S rRNA and 28S rRNA, 112 for 12S rRNA, 111 for 16S rRNA and

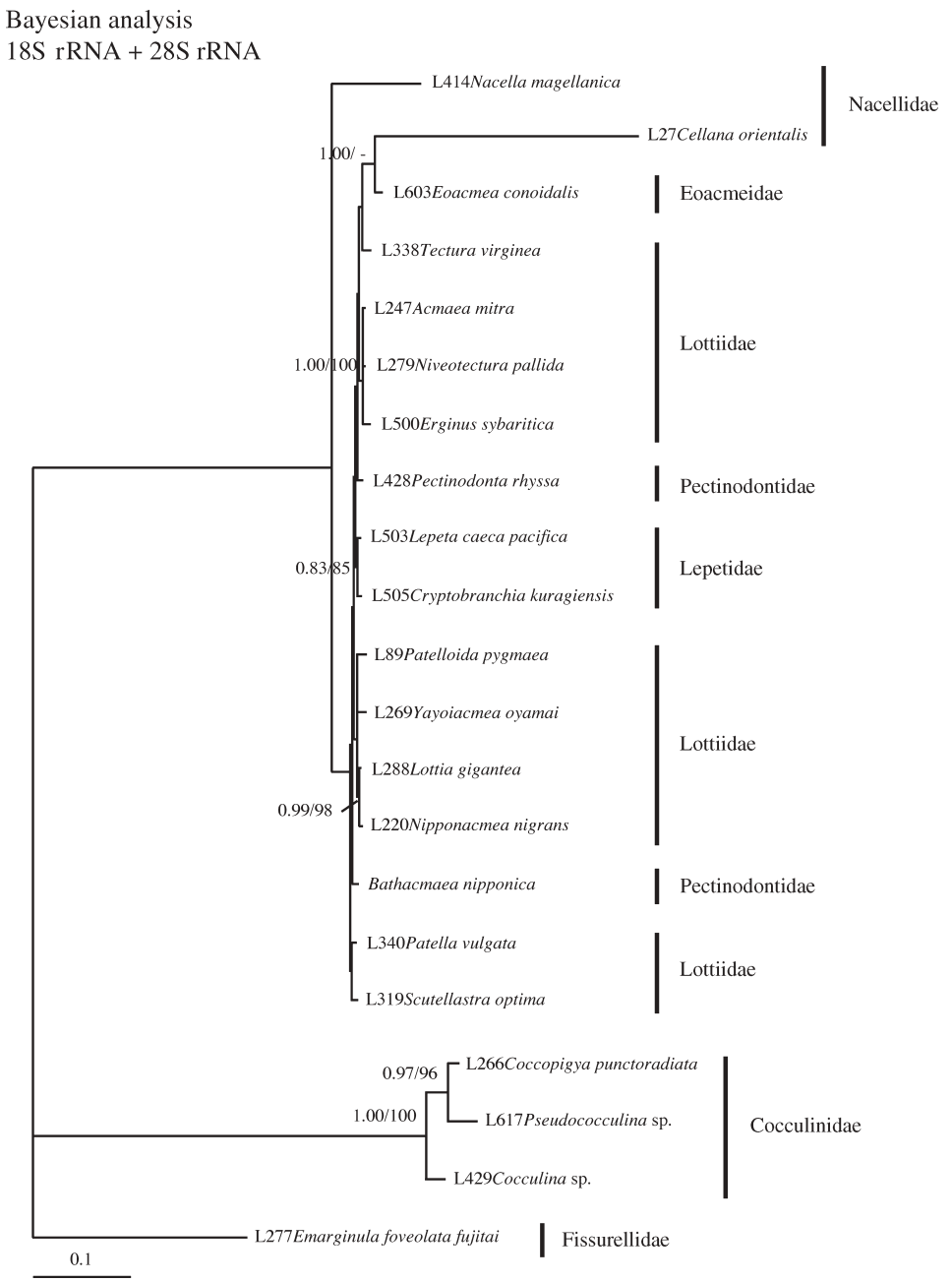

Figure 1. Molecular phylogeny of Patellogastropoda produced by Bayesian analysis of nuclear gene dataset (concatenated sequences from 18S rRNA and 28S rRNA). Support values are posterior probabilities and unweighted parsimony bootstrap values: < 80% (PP) or < 50% (BS) are not shown.

138 for COI (Table 1). Phylogenetic trees were also constructed separately for each gene, but the results are not reported here.

Sequence analysis and phylogeny reconstruction

Sequences were assembled and edited using Sequencher (version 4.1, Gene Codes Corporation). Sequences of ribosomal genes were aligned using Clustal X (Thompson *et al*., 1997). Small adjustments were made by eye, referring to secondary structure models for 12S (Hickson *et al*., 1996) and 16S (Gutell, 1994) ribosomal RNAs. The ambiguous regions of the alignments were excluded from the analyses using Gblocks 0.19b (Castresana, 2000). Sequences of COI were aligned using MacClade 4.03 (Maddison & Maddison, 2002), referring to translated amino acid sequences.

All five genes were tested for congruence of phylogenetic signal using the ILD test of Farris *et al*. (1995), as implemented by the partition homogeneity test in PAUP 4.0 version b10 (Swofford, 2002) (100 replicates).

Codon positions within COI were also tested using the ILD test to examine the incongruence of third positions in the COI gene. Sequence data were divided into two portions, first and second codon positions together, and third codon positions only.

The appropriate models to be used in phylogenetic analyses were determined with MrModelTest v3.06 (a variation of ModelTest by Posada & Crandall, 1998). The substitution models chosen were TrN+G for the 12S rRNA and COI genes, GTR + I+G for the 16S rRNA, GTR+G for the 18S rRNA and TIM+G for the 28S rRNA gene.

Table 2. Forward (F) and reverse (R) PCR primers used to amplify and sequence three genes.

Gene	Primer name	Sequence 5′–3′	Source
12S	12Sma (F)	CTG GGA TTA GAT ACC CTG TTA T	Koufopanou *et al.* (1999)
	12S97L (F)	AAC YCA AAG RAC TTG GCG GT	Nakano & Ozawa (2004)
	12Smb (R)	CAG AGA GTG ACG GGC GAT TTG T	Koufopanou *et al.* (1999)
16S	16LRN13398 (F)	CGC CTG TTT AAC AAA AAC AT	Koufopanou *et al.* (1999)
	16SRHTB (R)	ACG CCG GTT TGA ACT CAG ATC	Koufopanou *et al.* (1999)
18S	18S-5' (F)	CTGGTTGATYCTGCCAGT	Winnepenninckx *et al.* (1998)
	18S1100 (R)	CTTCGAACCTCTGACTTTCG	Williams *et al.* (2003)
28S	LSU5 (F)	TAGGTCGACCCGCTGAAYTTAAGCA	Littlewood *et al.* (2000)
	LSU1600 (R)	AGCGCCATCCATTTTCAGG	Williams *et al.* (2003)
COI	LCO1490 (F)	GGT CAA CAA ATC ATA AAG ATA TTG G	Folmer *et al.* (1994)
	HCO2198 (R)	TAA ACT TCA GGG TGA CCA AAA AAT CA	Folmer *et al.* (1994)

Phylogenetic trees were constructed for combined datasets in which two or three gene sequences were concatenated (Figs 1–3), using a Bayesian approach (MrBayes v3.1.1, Ronquist & Huelsenbeck, 2003). In the Baysian analysis, base frequencies were estimated, four chains were used (the default parameter) and the starting tree was random. The analysis was run for 2,500,000 generations, with a sample frequency of 100. The first 5000 trees were discarded as 'burn-in'. The posterior probabilities (PP) supporting the nodes were calculated by MrBayes. For comparison, analyses of both large taxon and large data sets were also performed with PAUP version1b10 (Swofford, 2002), using maximum parsimony (MP, with both unweighted and user-specified 3:1 transversion: transition ratios, estimated by maximum likelihood on the tree found by an equally weighted MP search). The parsimony analyses were done with 10 random-addition sequence replicates and TBR branch swapping (1000 replicates).

Estimating divergence times

Since our data were rejected by a likelihood-ratio tests (Felsenstein, 1981) ($P < 0.001$), we used the relaxed Bayesian method for multilocus data (Thorne, Kishino & Painter, 1998; Thorne & Kishino, 2002) as implemented in the software package Multidistribute (available from J. Thorne's website: http://statgen.ncsu.edu/thorne/multidivtime.html). The Bayesian dating method uses a probabilistic model to describe the change in evolutionary rate over time and uses the Markov-Chain Monte Carlo (MCMC) procedure to derive the posterior distribution of rates and times. The manual of Rutchmann (2004) was followed for using multidivtime. The mean of the prior distribution for the time separating the ingroup root from the present (rttm) and the standard deviation (SD) of the prior distribution (rttmsd) were set to 4.5 (450 Ma). After inspecting the branch lengths estimated by estbranches for each gene, the evolutionary rate of the root node was given a gamma prior distribution with mean (rtrate) and SD (rtratesd) both equal to 0.3 substitutions at the average site per 100 Ma. We chose this prior to obtain a distribution for the root that was simultaneously reasonable and relatively diffuse. The rtrate and rtratesd were estimated as suggested in the multidivtime manual. Prior distributions approximated under the MCMC approach involved an initial burn-in 10,000 cycles, after which the Markov chain was sampled 1000 times every 100 cycles. Default options were chosen for all the other parameters of the prior distribution and the MCMC procedure.

Divergence time estimation from molecular sequence data is improved by calibration from external dating information provided by the fossil record, geological events or other sources. Individual node times can be constrained so as to be either earlier or later than some specific date. In the present study, five reliable fossil records were used as prior information about the minimum age of some patellogastropod lineages. They were chosen based on reliability of identification, and on whether they were informative in setting lower bounds on prior ages in the Bayesian analyses. All fossils used in our analyses have been examined for shell microstructure. The time used for the divergence of *Nacella* and *Cellana* followed Nakano & Ozawa (2004).

The earliest fossils of *Eoacmaea* have been found in the Upper Cretaceous (Albian, about 100 Ma) of England (Akpan, Farrow & Morris, 1982; Lindberg, 1988), and fossil *Patelloida* dating to the Late Cretaceous (Campanian, about 75 Ma) occur in California (MacClintock, 1967; Lindberg, 1983). We took 100 Ma and 75 Ma as the minimum ages of *Eoacmaea* and *Patelloida*, respectively. The earliest known fossil of *Cellana* is *Cellana ampla* from the Upper Eocene of Oregon (Lindberg & Hickman, 1986; Lindberg, 1988). We set the divergence between *Nacella* and *Cellana* at a minimum of 38 Ma (see discussion in Nakano & Ozawa, 2004). Fossils of *Niveotectura* are found in the Miocene to Pleistocene sediments in the northern Pacific, ranging from Alaska (Lindberg & Marincovich, 1988) to northern Japan (Hase, 1965; Kaseno & Matsuura, 1965). Therefore, 15 Ma was used as the minimum age of *Niveotectura*. *Pectinodonta palaeoxyloida* from the Late Oligocene of Washington State (Lindberg & Hedegaard, 1996) implies that stem *Pectinodonta* are at least 25 Ma. Although a reliable fossil record of *Scutellastra* has been recognized from the Cretaceous of Hokkaido, Japan, we did not use it as a lower limit of its clade since *Scutellastra* has been revealed as a paraphyletic group (Koufopanou *et al*., 1999; Nakano & Ozawa, 2004; this study). The reliable fossil records are summarized in Table 3.

RESULTS

Sequence

The lengths of the alignment for the 12S rRNA, 16S rRNA, 18S rRNA and 28S rRNA fragments were 443 bp, 770 bp, 1109 bp and 1610 bp, respectively. After removal of ambiguous regions 207 bp, 402 bp, 979 bp and 1216 bp were retained for these genes respectively. However, unfortunately only about 400 bp of 28S rRNA were sequenced for *E. conoidalis*, *S. optima*, *B. nipponica*, *Cocculina* sp. and *Pseudococculina* sp. because of the difficulty of sequencing this gene. Thus we used 397 bp of 28S rRNA to construct trees. Third codon positions of COI sequences were excluded in all analyses, since ILD test showed that the phylogeny estimated from first and second positions was significantly

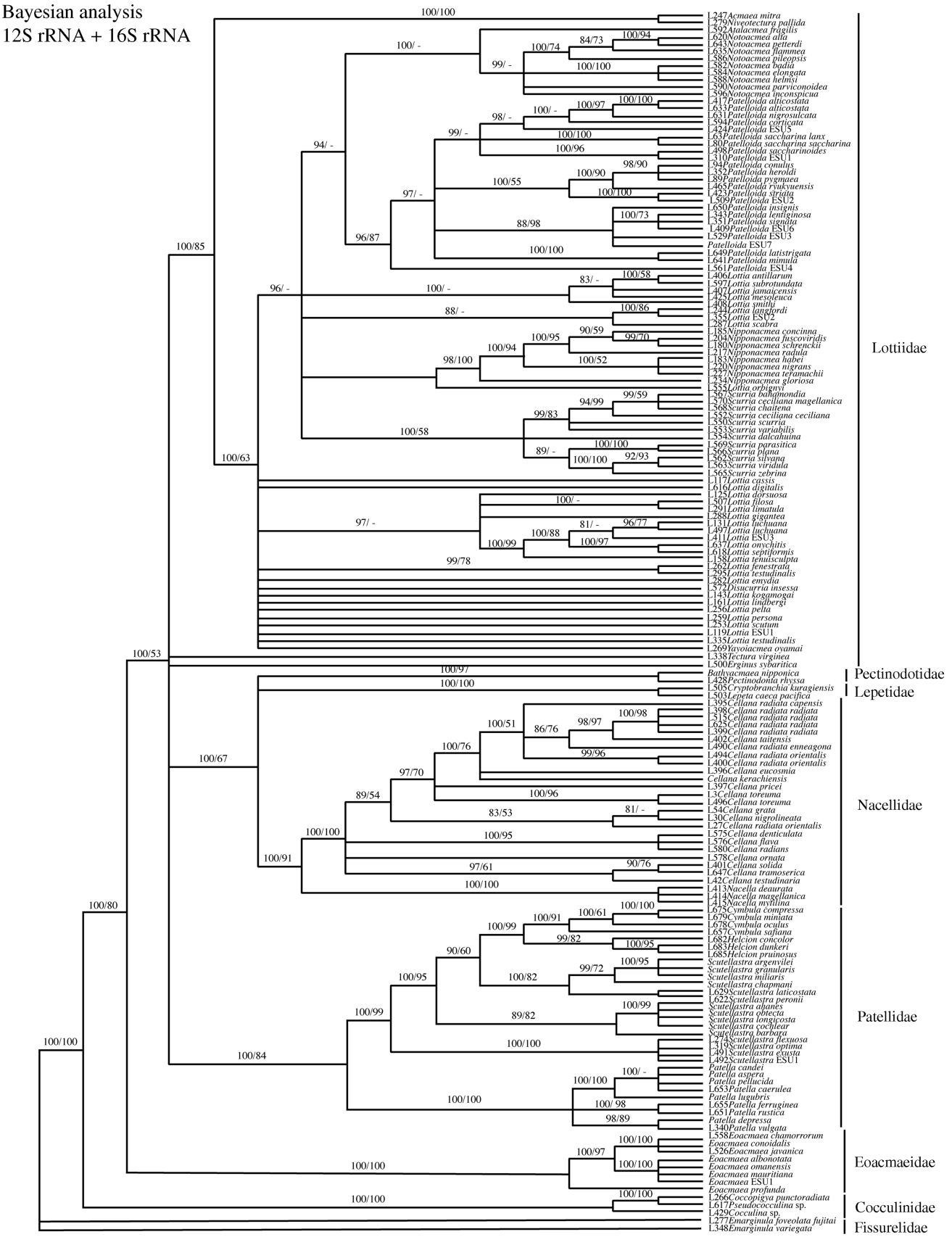

Figure 2. Molecular phylogeny of Patellogastropoda produced by Bayesian analysis of the large taxon set (concatenated sequences from 12S rRNA + 16S rRNA). Branches with less than 80% support have been collapsed (branch lengths have no meaning). Support values are posterior probabilities and weighted parsimony bootstrap. All trees were rooted using members of the family Fissurellidae as outgroup.

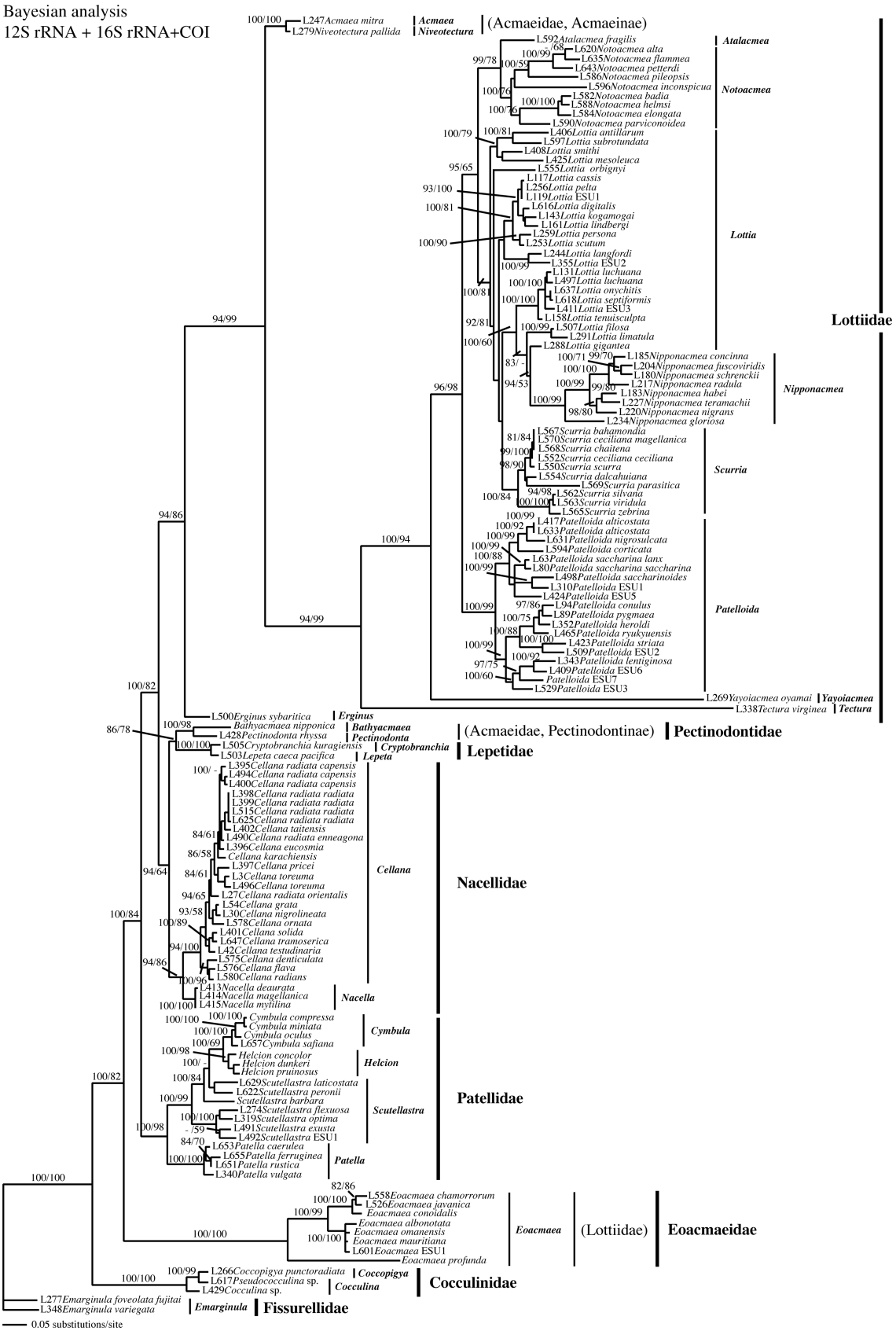

Figure 3. Molecular phylogeny of Patellogastropoda produced by Bayesian analysis of the large dataset (concatenated sequences from 12S rRNA + 16S rRNA + COI). Third codon positions of COI were excluded from the analysis. Support values are posterior probabilities and weighted parsimony bootstrap, a dash indicates <80% (PP) or <50% (BS) support. All trees were rooted using members of the family Fissurellidae as outgroup. Family names (in bold) for each clade are based on the results of this study.

Table 3. Summary of the reliable fossil records of Patellogastropoda.

Taxonomy	Geological time	Locality	Reference
Eoacmaea	Late Cretaceous	England	Akpan *et al.* (1982)
Patellidae	Late Triassic	Italy	Hedegaard *et al.* (1997)
Patella	Middle Eocene	France	Koufopanou *et al.* (1999)
Scutellastra	Late Cretaceous	Japan	Kase & Shigeta (1996)
Helcion	Late Miocene	South Africa	Vermeij (1992)
Cymbula	Late Pliocene	South Africa	Ridgway *et al.* (1998)
Cellana	Late Eocene	Oregon	Lindberg & Hickman (1986)
	Late Eocene	Antarctica	Stillwell & Zinsmeister (1992)
Cryptobranchia	Pliocene	Japan	Taki & Oyama (1954)
Pectinodonta	Oligocene	America	Lindberg & Hedegaard (1996)
Niveotectura	Miocene	Alaska	Lindberg & Marincovich (1998)
Patelloida	Late Cretaceous	California	Lindberg (1983)
Lottia	Late Pliocene	California	Lindberg (1988)
Nipponacmea	Pliocene	Japan	Noda (1973)

different from that obtained from third positions ($P = 0.01$). Thus, 442 bp were retained for COI.

Although the partition homogeneity test detected no significant incongruity among the mitochondrial gene partitions ($P = 0.10$) and between nuclear genes ($P = 0.66$), both nuclear genes are significantly different from the mitochondrial genes ($P = 0.01$). The sequences of 12S rRNA, 16S rRNA and COI were therefore combined and consequently a total of 1051 bp were used for constructing trees. The combined dataset from 18S rRNA and 28S rRNA gave a total of 1376 bp.

The trees produced by Bayesian analysis of the nuclear datasets (18S rRNA and 28S rRNA; 21 taxa, 1376 bp), large taxon set (12S rRNA and 16S rRNA; 153 taxa, 609 bp) and large dataset (12S rRNA, 16S rRNA and COI; 124 taxa, 1051 bp) are shown in Figures 1, 2 and 3 respectively. The unweighted and weighted parsimony trees for these datasets are in basic agreement with the Bayesian trees, excluding species-level groupings supported by low bootstrap values. Parsimony bootstrap values are sometimes lower than pp for equivalent nodes in the Bayesian tree, but this is to be expected, as the parsimony algorithm has not been optimized to allow for an appropriate evolutionary model as it was in the Bayesian analysis (Simmons & Pickett, 2004). Although the analyses of the combination of 12S rRNA, 16S rRNA and COI are well resolved (Fig. 3), some nodes are not supported (pp $<80\%$, bootstrap values $<50\%$) in the phylogenetic trees based on the 12S rRNA and 16S rRNA (Fig. 2). These incongruences may be due to short sequence length (609 bp) in the large taxon set. However, the nodes that are well supported are congruent in both large taxon and large datasets. There are significant differences in the position of Nacellidae, which has a long branch, between the trees based on nuclear and mitochondrial genes (Figs 1–3). We focused on the results based on the mitochondrial genes, since these are likely to be more informative for the phylogeny of Patellogastropoda.

Molecular phylogeny of Patellogastropoda

The monophyly of the Patellogastropoda is strongly supported (PP = 100%, BS = 100%) in the both combined dataset trees (Figs 2, 3). Although the members of the Patellidae, Nacellidae and Lepetidae form monophyletic groups (PP $\geq 94\%$, BS $\geq 86\%$), the Acmaeidae and Lottiidae are polyphyletic (Fig. 3). The Acmaeidae are traditionally divided into two subfamilies, the Acmaeinae (PP = 100%, BS = 100%) and Pectindontinae (PP = 100%, BS = 100%), but these two clades do not cluster together. In turn, the Lottiidae are divided into three groups, *Eoacmaea* (which contains species of the *profunda* group formerly assigned to *Patelloida*; PP = 100%, BS = 100%), *Erginus* (PP = 94%, BS = 86%) and the remainder of Lottiidae (PP = 94%, BS = 99%).

At the generic level, 20 monophyletic clades were well supported (PP $\geq 94\%$, BS $\geq 76\%$), comprising species classified in the following genera: *Acmaea*, *Niveotectura*, *Atalacmea*, *Notoacmea*, *Nipponacmea*, *Scurria*, *Patelloida*, *Yayoiacmea*, *Tectura*, *Erginus*, *Bathyacmaea*, *Pectinodonta*, *Cryptobranchia*, *Lepeta*, *Cellana*, *Nacella*, *Cymbula*, *Helcion*, *Patella* and *Eoacmaea* (Fig. 3). The phylogenies revealed *Lottia* and *Scutellastra* to be paraphyletic. The monophyly of *Discurria* is not supported (PP $< 80\%$, BS $< 50\%$) in the large taxon dataset (Fig. 2), but *Discurria* is nested in *Lottia* on the NJ tree (Saito & Nei, 1987; Kimura's 2 parameter, Kimura, 1980) based on same datasets (BS = 82%) (result not shown).

At the species level, *Cellana radiata* is classified into five subgroups, which comprise *C. radiata capensis*, *C. radiata enneagona*, *C. radiata radiata*, *C. radiata orientalis* (Japan) and *C. radiata orientalis* (Indo-Pacific). *Cellana radiata orientalis* individuals sampled from Japan are genetically distinct from the individuals from the Indo-Pacific region, suggesting that *C. radiata orientalis* is a species complex. Molecular results suggest that *Patelloida saccharina* is also a species complex, with genetically and morphologically distinct geographically structured lineages (Fig. 3); these were labelled *Patelloida* ESU1 and 5 (Table 1).

DISCUSSION

Family-level phylogeny of Patellogastropoda

Since Lindberg (1986) first defined the Patellogastropoda, both morphological and molecular studies have attempted to resolve the relationships within this group (e.g. Dall, 1871, 1876; Lindberg, 1988, 1998b; Sasaki, 1998; Koufopanou *et al.*, 1999; Harasewych & McArthur, 2000; Nakano & Ozawa, 2004; summarized in Fig. 4). However, there is incongruence between the morphological and molecular analyses, and also among authors. The phylogeny and even the composition of this group are still debated. This disagreement is particularly striking among morphological studies, since the radular, gill and shell characters used in cladistic analyses are convergent among limpets. No previous molecular studies have examined the phylogeny of all patellogastropods, and so have suffered from limited taxon sampling within the group. In this study, we analysed at least two species of every family within the Patellogastropoda and used longer sequences than previous studies. The present study therefore adds considerable resolution to the molecular phylogeny of the Patellogastropoda.

The Patellogastropoda are genetically distinct from Cocculiniformia (PP = 100%, BS = 100%) (Figs 2, 3). This separation is also supported by morphological characters (e.g. Golikov & Storabogatov, 1975; Haszprunar, 1988a; Ponder & Lindberg, 1997), and by nuclear genes (e.g. Tillier *et al.*, 1994; Colgan *et al.*, 2000). In general, cocculiniform limpets are characterized by having very fragile and thin shells, generally white, and with weak sculpture (Haszprunar, 1988b, c, d). The shell structure has not yet been studied extensively (Hedegaard, 1990), but in general crossed-lamellar aragonite is present, whereas nacre is lacking. Monophyly of the Cocculinidae is also supported by cladistic analysis using morphological characters (Strong, Harasewych & Haszprunar, 2003).

One of the most striking findings of this study is that *Eoacmaea* is the most basal group within the Patellogastropoda (Fig. 3). Lottiidae are not monophyletic, but divide into three groups,

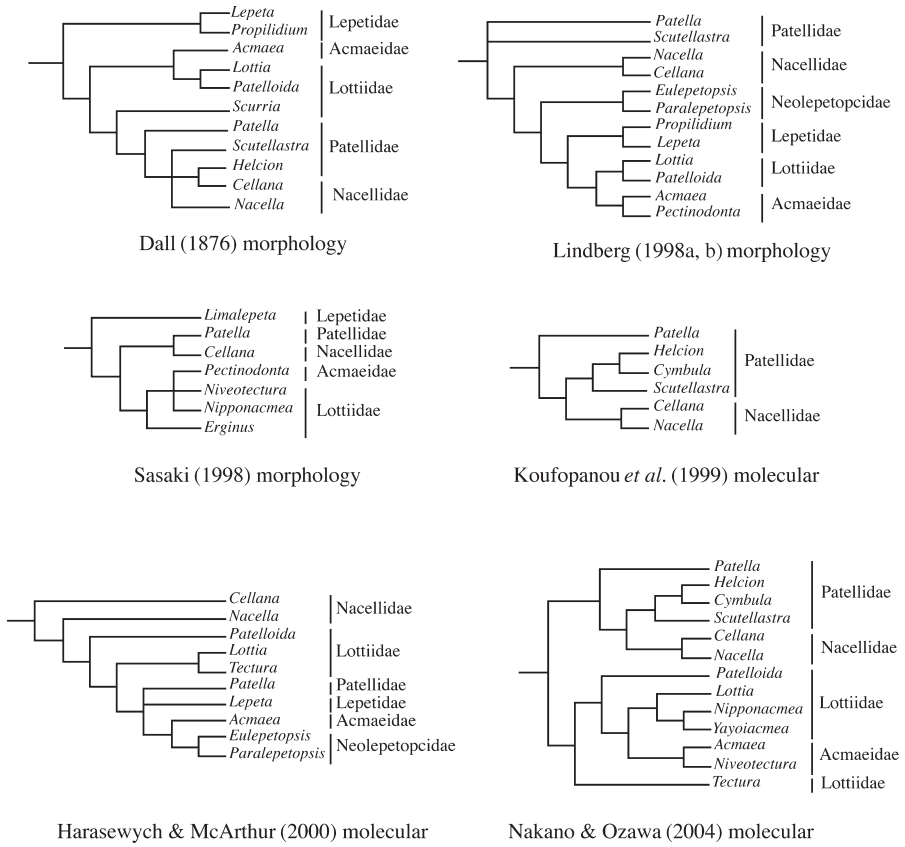

Figure 4. Summary of phylogenies of Patellogastropoda produced by various authors using either morphological characters or molecular data.

Eoacmaea, *Erginus* and the remainder of Lottiidae. The Lottiidae are traditionally clearly distinguished from Patellidae and Nacellidae by lack of calcitic foliated shell structures, having a single left ctenidium, solid radular teeth and simple basal plates; short, wide lateral teeth and rachidian teeth are lacking (Lindberg, 1998b).

The molecular data suggest that Acmaeidae are also not monophyletic (Fig. 3). The Acmaeidae are characterized by the co-occurrence of foliated and fibrillar prismatic shell microstructures, robust and simple radular segments with associated basal tooth plates arranged in chevrons, and lack of uncini (Lindberg, 1998b). This group is traditionally divided into two subfamilies, Acmaeinae and Pectinodontinae (Lindberg, 1998b). However, the Acmaeinae are nested within the Lottiidae and the Pectinodontinae are closely related to Lepetidae (Fig. 3). Previous workers have suggested that Acmaeidae are more related to the Lottiidae than the Patellidae, Nacellidae and Lepetidae (Lindberg, 1998b; Sasaki, 1998; Nakano & Ozawa, 2004; Fig. 4). A reconsideration of the status of the Acmaeidae is clearly needed (see discussion below).

Our results suggest that Patellidae and Nacellidae are both monophyletic (Fig. 3). Although there have been molecular studies that have suggested the possibility of a paraphyletic Patellidae (Koufopanou *et al*., 1999; Nakano & Ozawa, 2004), there is morphological support for a monophyletic Patellidae (Sasaki, 1998). For example, monophyly of Patellidae is supported by the occurrence of one pair of salivary glands each with two pairs of ducts, a foliated outer shell layer and five pairs of radular cartilages (Powell, 1973; Lindberg, 1988, 1998b; Sasaki, 1998). In the Nacellidae, the species lack crossed-lamellar structures above the myostracum, and show an increase in the degree of fusion of the outer lateral teeth and the development of tooth plate structures (Lindberg, 1988, 1998b). The Nacellidae have been grouped in a clade with Patellidae by earlier authors (e.g. Dall, 1876) and, more recently, by Sasaki (1998) on the basis of numerous shared characters, including pallial sensory organs, radular musculature and odontophoral cartilages (Fig. 4). Lindberg (1988, 1998b), however, regarded the Nacellidae as the sister taxon of Acmaeidae and Lottiidae, arguing that similarities with Patellidae were shared primitive characters. In contrast to phylogenies based on morphological and mitochondrial DNA data, nuclear genetic sequence data place the Nacellidae as a paraphyletic grade at the base of Patellogastropoda (Harasewych & McArthur, 2000; Fig. 4). However, these authors suggested that the region of the 18S rDNA used in their study diverges at widely differing rates (notably the Nacellidae are significantly different from other Patellogastropoda at about 5 to 15% of positions), and therefore did not provide meaningful resolution of the relationships among patellogastropod taxa. Our results also confirmed elevated rates of both 18S rRNA and 28S rRNA in Nacellidae (Fig. 1). Thus, the mitochondrial genes seem likely to be more informative about relationships within Patellogastropoda.

This is the first molecular study of the family Lepetidae using mitochondrial genes. Members of the Lepetidae are characterized by the fusion of the inner lateral teeth, the loss of the outer lateral tooth field, ctendium, osphradial ganglia and osphradia and the reduction of mantle tentacles (Sasaki, 1998; Lindberg, 1988, 1998b). They also have only a single pair of salivary glands and the eyes lack pigment (Angerer & Haszprunar, 1996). Based primarily on features of the radula, Dall (1876) and Sasaki (1998) consider Lepetidae to be the basal clade of Patellogastropoda (Fig. 4). Lindberg & Hedegaard, (1996)

and Angerer & Haszprunar (1996) concluded that Lepetidae are more closely related to Lottiidae than to Acmaeidae, while Lindberg (1998b) placed the Lepetidae as sister taxon to Acmaeidae plus Lottiidae (Fig. 4). Partial 18S sequences were unable to resolve their relationships with Acmaeidae and Lottiidae (Harasewych & McArthur, 2000). Our results suggest that Lepetidae are more closely related to Pectinodontidae and share a common ancestor with Nacellidae.

Revised classification of the families Acmaeidae and Lottiidae

Lindberg (1986) reinstated the senior synonym Lottiidae for almost all taxa previously assigned to the Acmaeidae, and restricted the usage of Acmaeidae to a small monophyletic group of limpets that share their most recent common ancestor with the northeastern Pacific species *Acmaea mitra*. According to Lindberg (1986), *A. mitra* can be distinguished at the familial level from all other putative intertidal Lottiidae in being subtidal, having three pairs of uniform lateral teeth, lacking uncini and belonging to the shell structure group 15 (of MacClintock, 1967). *Acmaea mitra* is the only included species of *Acmaea*.

Recently, *Niveotectura pallida* has been transferred from the Lottiidae to the Acmaeidae, based on molecular data and similarity of the radula (Nakano & Ozawa, 2004). Although the monophyly of *A. mitra* and *N. pallida* was strongly supported by molecular analyses (Nakano & Ozawa, 2004; Figs 2, 3), *N. pallida* belongs to shell structure group 1, which includes many members of Lottiidae (MacClintock, 1967). These results suggest that shell microstructure may be convergent among some groups of Patellogastropoda. In turn, three pairs of uniform lateral teeth and lack of uncini are commonly found in the subtidal lottiids (e.g. *Tectura virginea*, *Yayoiacmea oyamai* and *Erginus sybaritica*: Sasaki & Okutani, 1993b; Sasaki, 1998; Nakano & Ozawa, 2004). The presence of a ctendium also suggests *A. mitra* is more closely related to Lottiidae. Our results show that *Acmaea* and *Niveotectura* cluster within a larger clade of Lottiidae (including the basal *Erginus*), so we propose that *Acmaea* and *Niveotectura* are included within Lottiidae. Thus, Acmaeidae becomes a synonym of Lottiidae.

Although *Pectinodonta* (usually classified as subfamily Pectinodontinae within Acmaeidae) is also the member of the shell structure group 15 (Lindberg, 1981), the radular tooth morphology is quite distinct from that of *A. mitra* and is unique among patellogastropods. The inner lateral tooth is unicuspidate, the second lateral tooth is uni- or bicuspidate, and the outer lateral tooth is multicuspidate with between seven and twelve cusps per tooth (Lindberg, 1998b). The subfamily Pectinodontinae should be elevated to familial rank as the Pectinodontidae, since it is clearly unrelated to *A. mitra* and the Lottiidae. The Pectinodontidae are closely related to the Lepetidae, belonging to the same shell structure group 15 (Fig. 3), but they are distinguished from each other by radular morphology.

In the Lottiidae, Nakano & Ozawa (2004) recognized seven monophyletic clades corresponding to the genera *Patelloida*, *Lottia*, *Nipponacmea*, *Yayoiacmea*, *Acmaea*, *Niveotectura* and *Tectura*. In this study, seven genera *Erginus*, *Notoacmea*, *Atalacmea*, *Patelloida profunda* group, *Scurria* and *Discurria* were newly sequenced, giving new insights into the phylogeny of Lottiidae.

According to Lindberg & Vermeij (1985) *Patelloida* consists of at least two subclades. One subgroup includes species characterized by low to medium shell profiles, strong radial ribs or many fine riblets, reduced third lateral teeth, and habitats including various substrata. The other subclade, which has been called the *P. profunda* group by Christiaens (1975) and Lindberg & Vermeij (1985), consists of species characterized by medium to high shell profiles, many ribs, equal-sized lateral teeth, and is restricted to calcareous substrata. Below, we assign the *P. profunda* group to a new genus *Eoacmaea*, since it is not only genetically but also morphologically distinct from the other species of *Patelloida* (Figs 2, 3). Although *Eoacmaea* shares similar morphological characters with members of the Lottiidae, *Eoacmaea* is distant from the other lineages (Figs 2, 3). We propose here a new family Eoacmaeidae for *Eoacmaea*.

The genus *Erginus* is the most basal group within the Lottiidae (Fig. 3). *Erginus* can be distinguished from the other members of Lottiidae since they are the only brooders within Patellogastropoda (Lindberg, 1983; Sasaki, 1998). The species included in this genus are characterized by light coloured shells and association with coralline algae (Nakano & Ozawa, 2006).

The genus *Notoacmea* has been believed to exhibit a bipolar distribution in temperate waters in the southern and northern Pacific. Lindberg (1986) first pointed out that *Notoacmea* is restricted to New Zealand and Australia, and transferred North American species from *Notoacmea* to *Tectura* on the basis of the difference in shell structure. Later, the genus *Nipponacmea* was proposed for the Japanese lottiid limpets previously assigned to *Notoacmea*, based on shell structure, anatomical characters and endemism to the Japanese zoogeographical province (Sasaki & Okutani, 1993a). Recently, Nakano & Ozawa (2004) transferred the species of *Tectura* from the northern Pacific to *Lottia*, which shares similar radular teeth and shows genetic affinity. The genus *Tectura* is now restricted to one northeastern Atlantic species, *T. virginea*. As the result of revision of these genera, the usage of *Notoacmea* is now restricted species from New Zealand and Australia. Molecular results confirmed that the integrity of *Notoacmea*, *Tectura* and *Nipponacmea* (Fig. 3).

There are also lottiid limpets belonging to the genera *Atalacmea* and *Radiacmea* in New Zealand waters (Powell, 1979). *Atalacmea* formed a clade with *Notoacmea*, but *Radiacmea* was nested within *Notoacmea* (Fig. 3). *Radiacmea* differs slightly from *Notoacmea* by having blunt-pointed lateral teeth (Powell, 1979). However, blunt cusps are commonly found in the species associated with coralline algae or the shells of other molluscs (Sasaki & Okutani, 1993a, b). Therefore, *Radiacmea* is herein subsumed within *Notoacmea*. Although *Atalacmea* has similar radular morphology to *Notoacmea*, further work is needed to clarify the relationships between *Atalacmea* and *Notoacmea*.

Based on the shell structure, Lindberg (1988) divided the subfamily Lottiinae into two tribes, Lottiini including *Lottia*, and Scurrini including *Scurria* and *Discurria*. Although the monophyly of *Lottia* was strongly supported by a previous molecular analysis (Nakano & Ozawa, 2004), the present results revealed *Lottia* as a paraphyletic group (Fig. 3). However, *Lottia* is distinguished from *Scurria*, which forms a monophyletic clade (Fig. 3). Unfortunately, COI sequences could not be obtained from *Discurria insessa* so that *Discurria* was excluded in the analysis of the large dataset. Although the monophyly of *Discurria* is not supported in the large taxon datasets based on Bayesian analysis and both weighted and unweighted MP analyses (Fig. 2), *Discurria* is in *Lottia* on the NJ tree (Kimura's 2-parameter method; Kimura, 1980) based on the same datasets (result not shown). Furthermore, the monophyly of *Scurria* was supported, but *Discurria* did not form a sister group to *Scurria* (Fig. 2). *Discurria* was proposed by Lindberg (1988) for the species having radular teeth with broad, straight cutting edges, laterally compressed shell and similarities of shell morphlogy to *Scurria*. However, the morphologies of radular teeth and shell are highly variable in limpets, because they are related to the diet and habitat (e.g. Lindberg, 1979; Nakano & Ozawa, 2005). The straight cutting edges of lateral teeth and laterally compressed shell are commonly found in limpets inhabiting seagrass (Lindberg, 1979, 1981). Thus, the generic status of *Discurria* is suspect.

Yayoiacmea oyamai, formerly assigned to *Lottia*, was established as the type species of the genus *Yayoiacmea*, based on radular morphology (Sasaki & Okutani, 1993b). *Yayoiacmea* is shown here to be genetically distinct from *Lottia* (Fig. 3).

Genus- and species-level phylogeny of Patellidae and Nacellidae

The relationships within Patellidae and Nacellidae are similar to those reported by Koufopanou *et al*. (1999) and Nakano & Ozawa (2004). Based on morphological analysis, Ridgway *et al*. (1998) recognized four principal clades in the Patellidae corresponding to the genera *Scutellastra*, *Cymbula*, *Helcion* and *Patella*. Molecular phylogenetic analysis, however, revealed that *Scutellastra* is a paraphyletic group (Koufopanou *et al*., 1999; Nakano & Ozawa, 2004; this study).

The Nacellidae include two genera, *Cellana* and *Nacella*, which can be distinguished by radular morphology and shell structure (Powell, 1973; Lindberg, 1998b). According to Powell (1973), *Cellana radiata* consists of four subspecies, *C. radiata radiata* (Born, 1778), *C. radiata capensis* (Gmelin, 1791), *C. radiata enneagona* (Reeve, 1854) and *C. radiata orientalis* (Pilsbry, 1891). Molecular results recognized five genetically distinct lineages corresponding to four subspecies and one other (Fig. 3). *Cellana radiata orientalis* sampled from Japan is genetically distinguished from the individuals of this subspecies from the Indo-Pacific region. Further work is needed to clarify the relationships with nominal *C. radiata*, but these lineages may deserve specific recognition.

Estimation of divergence times within Patellogastropoda

The fossil record of Patellogastropoda is sparse, because limpets frequently inhabit wave-swept rocky shores where conditions for preservation are poor. Consequently, the divergence time of clades must be estimated from genetic distances and calibrations based on a small number of fossil records. This method can result in an underestimate or overestimate of the divergence time, and depends critically on correct identification of fossils. If a limpet fossil is preserved, it is often difficult to make generic and even familial assignments based on the shell morphology. However, MacClintock (1967) showed that shell microstructures were often sufficiently well preserved in fossil limpet shells that they could be used for generic and familial classification. The diagnostic characters of shell microstructure can thus provide reliable palaeontological evidence for estimating divergence times of clades (e.g. Lindberg & Hickman, 1986; Lindberg, 1988). However, fossils must be used with care, since the shell microstructures may be convergent among some groups of Patellogastropoda, as indicated above.

Bayesian inference of divergence times is a suitable method for taxa that have a fragmentary fossil record. The Bayesian approach allows anchorpoints (e.g. fossil records, geological events) to be concomitantly evaluated as suitable calibrations. Most importantly, Bayesian methods can be used without knowing the rates of DNA substitution. Figure 5 and Table 4 summarize the results of the Bayesian inference of divergence times and show the 95% credibility intervals. Our discussion is based on these credibility intervals, as this is a more appropriate approach when the pp distribution of divergence time is not normally distributed, and cladogenesis is a time-continuous process that should not be measured as single point estimates.

This method indicates that diversification within Patellogastopoda started at least in the Early Cretaceous between 143 and 279 Ma. Although this range overlaps that of 132–154 Ma calculated by Nakano & Ozawa (2004), the latter lies near the lower limit of the 95% credibility interval. The credibility intervals obtained in this study may be criticized as too broad and our estimates of divergence times therefore thought to be unreliable. However, the Bayesian credibility intervals are expected to be broader than the analogous confidence intervals obtained by a frequentist approach, because only the former accounts for all the uncertainties associated with estimating divergence times (Porter, Pérez-Losada & Crandall, 2005; Brammer & Von Dohlen, in press). Our estimation of divergence time used more fossil records than the previous estimation by Nakano & Ozawa (2004), perhaps explaining the lower estimate in the previous work.

Biogeography of the Patellogastropoda

Hypotheses of historical biogeography can be constructed from the combination of species-level phylogeny, divergence time and recent geographical distributions (e.g. Reid, Rumbak & Thomas, 1996; Koufopanou *et al*, 1999; Williams, Reid & Littlewood, 2003; Nakano & Ozawa, 2004; Williams & Reid, 2004). In the present study, the stem of the extant Patellogastropoda may be traced to at least the Late Jurassic and they began to diversify from the Jurassic to the Cretaceous (Fig. 5, Table 4). The supercontinent of Pangea existed from the close of the Palaeozoic into the Mesozoic, and on the east side the Tethys Sea was open to the Pacific Ocean. Pangea began to split into Laurasia in the Northern Hemisphere and Gondwana in the South during the Triassic. Crame (1993) recognized three principal historic phases of antitropical distribution. The oldest phase was linked to vicariant events following the breakup of the Pangean supercontinent in the Jurassic and Cretaceous. In the second phase, the cool-water fauna was likely interrupted by a period of global warming (early Miocene). The latest phase was explained by dispersal across the tropics during the Plio-Pleistocene glaciations. The origin of the extant Patellogastropoda is estimated at 143–279 Ma, so that their worldwide distribution and antitropical pattern have potentially been affected by all these three phases during their evolutionary history.

The palaeogeography of this period, together with the basal placement of *Eoacmaea* and the worldwide distribution of its Recent members from South Africa to the Caribbean (Christiaens, 1975; Ponder & Creese, 1980; Lindberg & Vermeij, 1985), suggest that the Patellogastropoda may have been widespread in the Tethys Sea around the Early Jurassic to Middle Cretaceous.

Antitropicality of the Patellidae in the Atlantic

The divergence of the Patellidae from the remaining four families is estimated at 128–272 Ma. The distribution of the recent patellids is mostly antitropical; the genus *Patella* is restricted to Europe, and the remainder of the Patellidae are mostly distributed in southern Africa and Australasia, with a few Indo-West Pacific species. The *Patella* clade shows an early divergence (121–231 Ma) from the rest of the Patellidae. Similarly, previous authors have also estimated its origin at 69–169 Ma (Koufopanou *et al*., 1999) and 91–110 Ma (Nakano & Ozawa, 2004). These estimates argue against a recent (Plio-Pleistocene) dispersal event from southern Africa, as hypothesized by Vermeij (1992) and Ridgway (1994). *Patella* may have been present in the northern Atlantic since the Mesozoic, although the oldest fossil of *Patella* in Europe is only of Middle Eocene age (Koufopanou *et al*., 1999). Based on the timing of the origin of this family, palaeogeography and the distribution of its recent members, the Patellidae may have been the first patellogastropods to diversify in the Atlantic following its opening in the Jurassic. Consistent with this suggestion, the oldest patellid fossil is from the Triassic of Italy (Hedegaard *et al*., 1997).

Scutellastra is a widespread and paraphyletic group, which consists of three clades with distinct geographical distributions, in the Indo-Pacific, southern Africa and southern Australia. Nakano & Ozawa (2004) suggested that the basal Indo-Pacific branch extended its distribution westwards from the Atlantic to the Pacific in the Cretaceous by rafting on ammonites

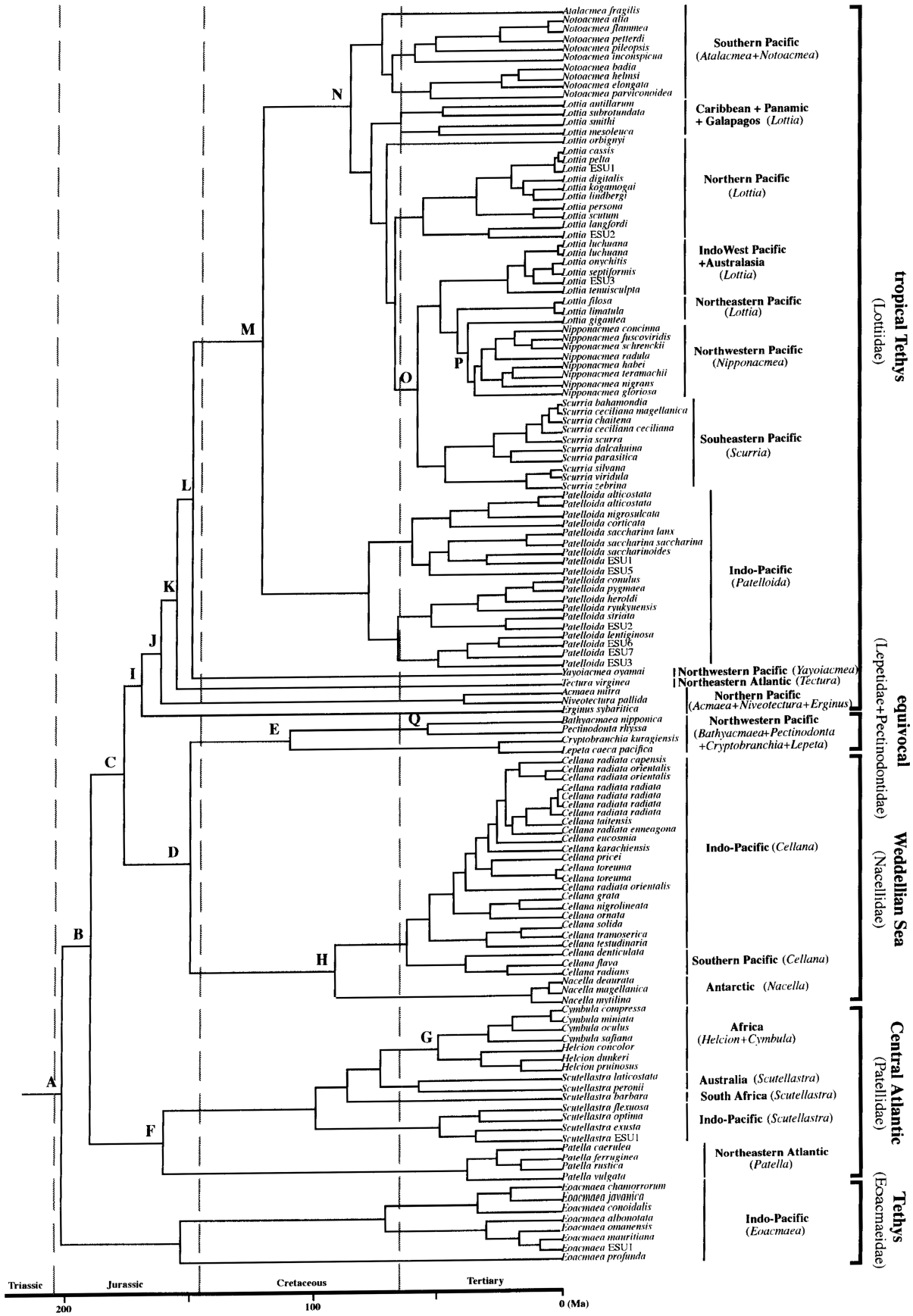

Figure 5. Chronogram showing divergence times of Patellogastropoda estimated using topology of Figure 3. Letters are node names as in Table 4. Scale at the bottom represents geological time. The recent geographical distribution of the clades and possibly origin of the families are shown.

Table 4. Bayesian posterior distribution of divergence time (and 95% credibility intervals) for major lineages of Patellogastropoda.

Node	Diversification	Age (Ma)
	Patellogastropoda vs Cocculinidae	217 (156–297)
A	*Eoacmaea*	203 (143–279)
B	Patellidae	191 (128–272)
C	Nacellidae + Lepetidae + Pectinodontidae vs Lottiidae	177 (121–261)
D	Nacellidae vs Lepetidae + Pectinodontidae	151 (98–216)
E	Lepetidae vs Pectinodontidae	118 (74–177)
F	*Patella*	163 (121–231)
G	*Cymbula* vs *Helcion*	53 (24–91)
H	*Nacella* vs *Cellana*	93 (44–143)
I	*Erginus*	170 (103–227)
J	*Acmaea* + *Niveotectura*	163 (98–216)
K	*Tectura*	156 (88–208)
L	*Yayoiacmea*	149 (87–198)
M	*Patelloida*	121 (82–171)
N	*Atalacmea* + *Notoacmea*	87 (52–148)
O	*Scurria*	59 (30–103)
P	*Nipponacmea*	39 (17–68)
Q	*Bathyacmaea* vs *Pectinodonta*	56 (31–98)

(Kase, Shigeta & Futakami, 1994). *Scutellastra* fossils from the Upper Cretaceous of northern Japan (Kase & Shigeta, 1996; Ridgway *et al*., 1998) suggest that *Scutellastra* reached Japan from the Atlantic during the Cretaceous. Our estimates show that the Australian and southern African clades of *Scutellastra* had also diverged by the Late Cretaceous (Fig. 5). *Cymbula* and *Helcion* are African clades; the single exception is *C. depsta*, which may have reached the southern Indian Ocean by rafting on algae (Ridgway *et al*., 1998).

Southern origin of the Nacellidae

The Nacellidae split from the Lepetidae plus Pectinodontidae between 98–216 Ma, a period that included the fracturing of Gondwana into western and eastern portions in the late Jurassic (Lawver, Gahagan & Coffin, 1992; Crame, 1999). Our results are consistent with a southern origin for the Nacellidae in the Cretaceous, followed by the radiation of *Nacella* in eastern Gondwana (Antarctica and South America), and of *Cellana* in Antarctica, South America, Australia, New Zealand and the Indo-Pacific. During the Cretaceous, the southern Pacific margin was isolated by the geography of Gondwana and prevailing oceanic circulation, and the Weddellian Province supported a distinctive molluscan fauna (Zinsmeister, 1982; Crame, 1996). Nacellid-like fossils have been found in the Antarctic Peninsula (Stilwell & Zinsmeister, 1992) and New Zealand (Beu, Maxwell & Brazier, 1990), but these records are only from the Upper Eocene. *Cellana carpentaria* is present in lower Cretaceous deposits in Australia (Powell, 1973), but this fossil has been not been investigated for shell microstructure so its familial placement is unclear. Between the Upper Cretaceous and the early Cenozoic, the temperate Weddellian molluscan fauna extended from Patagonia to Australia (Stilwell & Zinsmeister, 1992, 2000; Zinsmeister, 1982). The basal placement of the New Zealand *Cellana* also implies that it likely had a palaeoaustral origin. All the available evidence suggests that *Cellana* accomplished its radiation in the tropical Indo-Pacific during the Neogene.

Colonization of deep-water habitats

The Pectinodontidae, including the vent taxon *Bathyacmaea*, are the sister group of Lepetidae. Pectinodontid species are found worldwide, at bathymetric ranges from about 300–6000 m. The highest species diversity is found in the equatorial region, with fewer species occurring in temperate regions (Lindberg, 1998b). Similarly, lepetids are strictly subtidal, but are restricted to cold waters. They can occur just below the low water mark in the Northern Hemisphere, but extend to depths in excess of 5000 m towards the equator (Moskalev, 1977). The origin of these two families adapted to anoxic condition is estimated at 98–216 Ma, and the divergence between *Bathyacmaea* and *Pectinodonta* at 31–98 Ma. The hydrothermal vent habitat has attracted much interest, including questions about the origin of its fauna. Recent molecular studies of several ecologically dominant vent and seep taxa, including vestimentiferan tube worms, vesicomyid clams and bathymodioline mussels (reviewed by Little & Vrijenhoek, 2003), estimated their origins to be in the later Mesozoic to Cenozoic. Our estimation of the divergence of *Bathyacmaea* supports these hypotheses about the timing of origination of the vents. However, Geiger & Thacker (2005) have shown that hydrothermal vents were colonized by at least four different vetigastropod lineages (Neomphalidae, Peltospiridae, Lepetodrilidae and Clypeosectidae), during at least three different time periods. Perhaps some of the extant vent taxa invaded vents repeatedly through geological time. In the case of patellogastropods, our results reject the hypothesis that the extant vent taxa are Palaeozoic relics and suggest that the ancestors of the deep-water fauna, including vent taxa, have been elements of the coastal fauna. Nevertheless, the detailed biogeography of the Lepetidae and Pectinodontidae is not clear, because of the limited sampling in the present study. The ecology of these families is poorly known, but *Pectinodonta* attaches to sunken wood, which may have aided its distribution, since deep-water circulation may have been highly variable in the Cretaceous (Saltzman & Barron, 1982). The larvae of vent taxa are likely to be transported in hydrothermal plumes (Tyler & Young, 2003). Further research is needed to provide information about the origin and evolution of lepetids and pectinodontids.

Antitropicality of the Lottiidae in the Pacific

The origin of the Lottiidae is estimated at 121–261 Ma. A tropical Tethyan origin of Lottiidae was suggested by Nakano & Ozawa (2004). Within the Lottiidae, the species now restricted to the cold waters of the North Pacific are basal. The divergences of *Erginus* and the clade of *Acmaea* and *Niveotectura* are estimated at 103–227 Ma and 98–216 Ma, respectively. During the Middle to Late Jurassic, the bivalves of the East Asian Province were distinct from those of the Tethyan fauna (Hayami, 1987). Similarly, the ammonoid fauna from the boreal Pacific became distinctive in the Middle Jurassic, and was isolated from others by the Late Jurassic (Westermann, 1980). *Erginus*, *Acmaea* and *Niveotectura* likely migrated northeastwards along the coastlines of Laurasia to the Pacific, and adapted to cool water. *Erginus* is unique among patellogastropods as it is the only genus to brood its larvae. *Erginus* may be a Mesozoic relic in the northern Pacific, since it has a low dispersal capability.

Patelloida extended its distribution westwards from its likely origin in the tropical Tethys Sea (Nakano & Ozawa, 2004). The earliest fossils of this genus have been found in the Late Cretaceous of California (Lindberg, 1983). Furthermore, *Patelloida* species commonly occurred in nearshore sediments in North America and Europe during the Tertiary (MacClintock, 1967; Lindberg, 1983; Lindberg & Hickman, 1986). These fossil records support the argument that *Patelloida* reached Europe and North America during the Late Cretaceous. However, this

genus disappeared in Europe and North America following the onset of a cool period (Lindberg, 1988).

The divergence of the *Atalacmea* plus *Notoacmea* clade, now restricted to Australia and New Zealand, is estimated at 52–148 Ma. *Notoacmea* may have reached New Zealand after it became isolated from other continents during the Cretaceous at 60–84 Ma, via dispersal in the current turning south from the palaeoequatorial current. Various marine organisms including Foraminifera, fish and crabs are believed to have colonized New Zealand from the tropics by this means, called the Malayo-Pacific route by Fleming (1962). More recently, a member of *Notoacmea* extended its distribution to Australia, since the Australian *Notoacmea alta*, *N. flammea* and *N. petterdi* are derived from the New Zealand *Notoacmea*. Fossils of *Notoacmea* have not been recognized, but it is expected that their fossil record will extend back to the Upper Cretaceous.

The origin of the remainder of the Lottiidae (*Lottia*, *Nipponacmea* and *Scurria*) may have been tropical America, which is believed to have been a centre of origin for many marine organisms (e.g. Briggs, 1974; White, 1986; Teske, Cherry & Matthee, 2004; Williams & Reid, 2004). This argument is supported by the basal placement of the *Lottia* species from the Caribbean, Panama and the Galapagos. From tropical America *Lottia* may have reached the northwestern Pacific by migration along the northwestern American coast and the Aleutian-Commander Island arc (Vermeij, Palmer & Lindberg, 1990). Patellogastropod larvae have only a short pelagic phase (Hatch, 1977), so migration along coastlines and island chains is likely to be important. This route of dispersal is demonstrated by the close relationships between northwestern Pacific (*Lottia kogamogai*, *L. lindbergi*) and northeastern Pacific species (*L. digitalis*), as already discussed by Nakano & Ozawa (2004). As noted by Lindberg (1988), the distribution of *Lottia* is restricted to continental margins and it is unknown from any major oceanic island groups in the Pacific and Indian Oceans. However, *Lottia* has recently been found on Java (Nakano, Aswan & Ozawa, 2005) and Sri Lanka (this study). The topology of our phylogeny, with the Japanese *L. tenuisculpta* lying basally to the Indian (*L. luchuana* and *Lottia* ESU3) and Australian (*L. onychitis* and *L. septiformis*) species may represent the westward and southward radiation of *Lottia* from northwestern Pacific (30–103 Ma) via islands in the Indo-West Pacific. *Nipponacmea* diverged at 17–68 Ma in the northwestern Pacific and remains there.

There is another antitropical distribution of patellogastropods in the northeastern (*Lottia*, *Discurria*) and southeastern (*Scurria*) Pacific Ocean (Lindberg, 1988). Lindberg (1988, 1991) suggested that faunal exchange between the Californian and Peruvian Provinces took place by crossing the tropical eastern Pacific during the Plio-Pleistocene, and may have been associated with the formation of the Isthmus of Panama in the late Pliocene. However, the divergence of *Scurria* is likely much older (30–103 Ma). The circumequatorial current began to decrease following the collision of India with Asia in the Eocene (50–53 Ma) (Haq, 1984) and finally ceased with the closure the Tethys seaway in the Middle Miocene (Adams *et al*., 1977). Perhaps *Scurria* migrated to South America with the disappearance of the circumequatorial current which formerly prevented the southward dispersal of the Lottiidae. Although lottiids dominate the intertidal fauna of Chile, fossil records of Lottiidae are not known from the region.

The relationships between the New Zealand and South American marine faunas can usually be explained by recent (Plio-Pleistocene) dispersal in the West Wind Drift. Long-distance eastward rafting has been proposed for marine organisms including topshells (Donald, Kennedy & Spencer, 2005), oysters (OFoighil *et al*., 1999) and sea stars (Waters & Roy, 2004). However, among lottiid limpets there are different and much older evolutionary patterns, as also found in some littorinid snails (Williams *et al*., 2003).

Nevertheless, some relatively recent geological events may have influenced the modern distributions of the Patellogastropoda, for example the closing of the Panamic portal about 3.5 Ma (Keigwin, 1978), and opening of the Bering Strait during the Pliocene. In particular, 295 molluscan species took part in the trans-Arctic interchange between the North Pacific and Arctic-Atlantic basins, following the opening of the Bering Strait (Vermeij, 1991). Two Pacific species of *Lottia* apparently migrated into the Atlantic at this time (Carlton *et al*., 1991; Vermeij, 1991), a suggestion supported by molecular data (Nakano & Ozawa, 2004).

Even with a reliable fossil record, scenarios of historical biogeography are speculative. The fossil record of intertidal gastropods will never be complete, but it can be hoped that new discoveries of these rare fossils may resolve uncertainties in the dating of divergences. The further study of larval biology and dispersal abilities will also contribute to an understanding of biogeographic history.

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APPENDIX: NEW CLASSIFICATION OF '*PATELLOIDA PROFUNDA* GROUP'

Family Eoacmaeidae new family

Genus *Eoacmaea* new genus

Type species Patella profunda Deshayes, 1863.

Diagnosis: Light-coloured shell medium to high in profile. All slopes typically straight with rounded radial ribs; brown radial markings common. Ventral plates of radula complex, with strong lateral processes and sutures; lateral teeth typically of equal size and shape. Habitats limited to calcareous substrata in the high intertidal to supratidal zone.

Included species: *E. profunda*, *E. pustulata*, *E. omanensis*, *E. albonotata*, *E. mauritiana*, *E. javanica*, *E. conoidalis*, *E. chamorrorum*, *E. ivani*, *E. calamus*, *E. ceylanica*, and *E. semirubida*.

Etymology: An early (Eo) *Acmaea*. This genus is the most basal branch in the phylogenetic tree of the Patellogastropoda.

Distribution: South Africa, tropical Indo-Pacific, Caribbean.