

18S Ribosomal DNA Sequences Provide Insight into the Phylogeny of Patellogastropod Limpets (Mollusca: Gastropoda)

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To investigate the phylogeny of Patellogastropoda, the complete 18S rDNA sequences of nine patellogastropod limpets *Cymbula canescens* (Gmelin, 1791), *Helcion dunkeri* (Krauss, 1848), *Patella rustica* Linnaeus, 1758, *Cellana toreuma* (Reeve, 1855), *Cellana nigrolineata* (Reeve, 1854), *Nacella magellanica* Gmelin, 1791, *Nipponacmea concinna* (Lischke, 1870), *Niveotectura pallida* (Gould, 1859), and *Lottia dorsuosa* Gould, 1859 were determined. These sequences were then analyzed along with the published 18S rDNA sequences of 35 gastropods, one bivalve, and one chiton species. Phylogenetic trees were constructed by maximum parsimony, maximum likelihood, and Bayesian inference. The results of our 18S rDNA sequence analysis strongly support the monophyly of Patellogastropoda and the existence of three subgroups. Of these, two subgroups, the Patelloidea and Acmaeoidea, are closely related, with branching patterns that can be summarized as [(*Cymbula* + *Helcion*) + *Patella*] and [(*Nipponacmea* + *Lottia*) + *Niveotectura*]. The remaining subgroup, Nacelloidea, emerges as basal and paraphyletic, while its genus *Cellana* is monophyletic. Our analysis also indicates that the Patellogastropoda have a sister relationship with the order Cocculiniformia within the Gastropoda.

Keywords: 18S rDNA Sequences; Gastropoda; Mollusca; Patellogastropoda; Phylogenetic Tree Reconstruction.

Introduction

18S rDNA has several characteristics that make it useful

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for phylogenetic investigations, including its universality, the presence of many potentially informative sites (generally, about 1,800–1,990 base pairs), high stability, constancy of function, and the presence of both conserved and variable regions (Canapa *et al.*, 1999; Winnepenninckx *et al.*, 1994). Hence 18S rDNA sequences are frequently used for elucidating phylogenetic relationships among the molluscan taxa (Adamkewicz *et al.*, 1997; Barges and Coma, 1997; Canapa *et al.*, 1999; Harasewych and McArthur, 2000; Harasewych *et al.*, 1997; Winnepenninckx *et al.*, 1996; Yoon and Kim, 2000; 2005) as well as other animal groups.

The order Patellogastropoda differs morphologically from other gastropods in terms of shell structure, alimentary system, and radula morphology and action (Lindberg, 1988). Patellogastropod limpets have successfully adapted to all seas from tropical to polar regions, and are commonly found in rocky intertidal habitats. In some regions, the larger intertidal limpets, such as *Cellana* species, are regarded as significant for local fishing economies. As important, abundant organisms, the patellogastropod limpets have been extensively studied in terms of life history, food, behavior, and physiology (Fretter and Graham, 1994).

The extant members of this order are usually divided into three superfamilies (Lindberg, 1998): Patelloidea, Nacelloidea, and Acmaeoidea. However, despite many comparative studies based on morpho-anatomical features (Dall, 1876; Haszprunar, 1988a; 1988b; Lindberg, 1988; 1998; McLean, 1990; Ponder and Lindberg, 1996; 1997; Sasaki, 1998), the phylogeny of the Patellogastropoda is still controversial. This is largely due to the lack of synapomorphic characters, and remarkable phenotypic diversity. To address this problem, several molecular studies have been performed using partial 28S (McArthur and Koop, 1999; Rosenberg *et al.*, 1997; Tillier *et al.*, 1994; 1996) and partial 18S (Harasewych and McArthur, 2000; Harasewych *et al.*, 1997) rDNA sequence data. Recently, Harasewych and McArthur (2000) reviewed earlier work and further examined the phylogeny of Patellogastropoda using partial 18S rDNA sequences of 25 gastropod species. Their analysis

indicated that Patellogastropoda are monophyletic, and very weakly supported a sister group relationship between the Patellogastropoda and the Cocculiniformia within the Gastropoda. However, the monophyly of the Patellogastropod subgroups (the Patelloidea, the Nacelloidea, and the Acmaeidea) was not examined, nor was the relationship among the three subgroups within the patellogastropod clade tested. Therefore, we have analyzed longer sequences from additional representatives to produce a more robust patellogastropod phylogeny.

We have determined the complete 18S rDNA sequences of nine additional patellogastropod species and have analyzed them along with the previously published 18S rDNA sequences of 35 other gastropods, one bivalve, and one chiton species. The aims of the present study were (1) to test the monophyly of the Patellogastropoda and of its three subgroups; (2) to assess the phylogenetic relationships between these three patellogastropod subgroups; (3) to examine the phylogenetic position of the patellogastropod limpets relative to other gastropod orders.

Materials and Methods

Materials We determined the complete 18S rDNA sequences of nine patellogastropod limpets, including five limpet species (*Cel-lana toreuma*, *Cellana nigrolineata*, *Nipponacmea concinna*, *Niveotectura pallida*, and *Lottia dorsuosa*) collected in Korea and four obtained from colleagues [*Cymbula canescens* from St. Helena (South Atlantic Ocean), *Helcion dunkeri* from Bloubergstrand (South Africa), *Patella rustica* from Madeira (Portugal) and *Nacella magellanica* from Baia Lapataia (Argentina)]. The patellogastropod sequences obtained were analyzed together with comparable sequences from 37 representative molluscs (35 gastropods, one bivalve, and one chiton) selected from GenBank. The polyplacophoran *Lepidozona coreanica* was used as an outgroup because the class Polyplacophora (included in the Aculifera) has been reported to be the sister group of the classes Gastropoda and Bivalvia (included in Conchifera) based on morphological characteristics (see Brusca and Brusca, 1990; Ponder and Lindberg, 1996; Runnegar and Pojeta, 1985; Salvini-Plawen, 1990) and molecular data (Adamkewicz *et al.*, 1997; Bargues and Mas-Coma, 1997; Yoon and Kim, 2000). The taxa utilized in the present study are listed in Table 1, along with their GenBank accession numbers. Voucher specimens have been deposited in the Invertebrate Resources Bank of Korea (IRBK).

DNA extraction, PCR amplification, and sequencing Total genomic DNAs were extracted from the foot muscles of live and ethanol-preserved individuals using a Qiagen DNeasy tissue kit. The 18S rDNA sequences were PCR amplified using oligonucleotide primers derived from the 5' (5'-CCT GGT TGA TCC TGC CAG-3') and the 3' (5'-TAA TGA TCC TTC CGC AGG TTA-3') termini of the gene (Medlin *et al.*, 1988). The PCR amplification conditions consisted of 30 cycles of 94°C for 1 min, 52°C for 2

min, and 72°C for 3 min. The amplified DNA fragments were cloned as reported by Yoon and Kim (2000). The amplicons were sequenced in both the forward and reverse orientations on an ABI 310 automated sequencer (Perkin Elmer Co.) using the previously reported sequencing primers (Moon *et al.*, 1996).

Phylogenetic analysis of 18S rDNA sequences The 18S rDNA sequences (excluding primer sites) were initially aligned with CLUSTAL X version 1.81 (Thompson *et al.*, 1997) and then corrected by visual inspection. Regions of uncertain alignment were eliminated from the final analysis, and alignment gaps were treated as missing data. Accordingly, our analyses were limited to reliably aligned regions comprising a total of 1,711 nucleotide positions. These alignments can be obtained from the authors.

Phylogenetic reconstructions were generated using three different analytical methods, maximum parsimony (MP), maximum-likelihood (ML), and Bayesian inference (BI). MP analyses were conducted using PAUP version 4.0b10 (Swofford, 2003) with the closest stepwise addition options. The analyses employed a heuristic search using TBR branch swapping. Branch length was optimized according to the ACCTRAN option. The ML analyses (Felsenstein, 1981) were performed with the program PUZZLE version 4.0.2 (Strimer and von Haeseler, 1996) with the HKY (Hasegawa *et al.*, 1985) model. For the quartet puzzling method, nucleotide frequencies and transition/transversion ratios were estimated from the data set. The BI was also executed in MrBayes version 3.0b4 (Huelsenbeck and Ronquist, 2001; also Ronquist and Huelsenbeck's, 2003 on-line manual) under the GTR + G + I model as selected using hLRT in Model Test version 3.06 (Posada and Crandall, 1998). The Monte Carlo Markov Chains (MCMC) were simulated for 2,000,000 generations and sampled every 100 generations: four chains were run and 10,000 initial trees were discarded (burn in). Bootstrap values (Felsenstein, 1985), representing the robustness of each node in the MP tree, refer to 1,000 replications. Quartet puzzling (using 10,000 puzzling steps) provided reliability values for the ML analyses (Strimmer and von Haeseler, 1996), and Bayesian posterior probabilities were estimated based on the 50% majority rule consensus of the trees.

Results

The complete 18S rDNA sequences determined in the present study ranged from 2,014 (*Cellana nigrolineata*) to 2,106 (*Nacella magellanica*) base pairs in length (excluding primer sites), which were longer than the sequences previously reported for any other gastropod species (usually ranging from 1,800 to 1,990 bp) (e.g., Bargues and Coma, 1997; Winnepeninckx *et al.*, 1996; Yoon and Kim, 2000, 2005). Accordingly, the newly sequenced rDNA genes had many insertions throughout variable regions V1-V5 and V7-V9 [based on the secondary structure model of the 18S rDNA of *Drosophila melanogaster* (Neefs *et al.*, 1991)]. The V2, V4, V7 and V8 regions were especially variable, with the longest insertions located in V7 and V8

Table 1. Species and sequences used in the present study, along with GenBank accession numbers. New sequences are marked with an asterisk (*).

Classification	Species	Accession No.
GASTROPODA		
PATELLOGASTROPODA		
PATELLOIDEA		
Patellidae	* <i>Cymbula canescence</i> (Gmelin, 1791)	DQ013350
	* <i>Helicon dunkeri</i> (Krauss, 1848)	DQ013351
	* <i>Patella rustica</i> Linnaeus, 1758	DQ013352
NACELLOIDEA		
Nacellidae	* <i>Cellana toreuma</i> (Reeve, 1855)	AF308646
	* <i>Cellana nigrolineata</i> (Reeve, 1854)	DQ013353
	* <i>Nacella magellanica</i> (Gmelin, 1791)	DQ013349
ACMAEOIDEA		
Lottiidae	* <i>Nipponacmea concinna</i> (Lischke, 1870)	DQ013354
	* <i>Niveotectura pallida</i> (Gould, 1859)	AF308644
	* <i>Lottia dorsuosa</i> Gould, 1859	AF308645
COCCULINIFORMIA		
Cocculinidae	<i>Cocculina messingi</i> McLean & Harasewych, 1995	AY090796
NERITOPSINA		
Neritidae	<i>Nerita albicilla</i> Linnaeus, 1758	X91971
	<i>Theodoxus fluviatilis</i> (Linnaeus, 1758)	AF120515
VETIGASTROPODA		
Pleurotomiidae	<i>Entemnotrochus adansonianus</i> (Crosse & Fischer, 1861)	AF120509
	<i>Perotrochus midas</i> Bayer, 1965	AF120510
Fissurellidae	<i>Macroschisma dilatatum</i> (Adams, 1851)	AF335560
	<i>Tugali gigas</i> (Martens, 1881)	AF335561
Scissurellidae	<i>Sinezona confusa</i> Rolán & Luque, 1994	AF120512
Lepetodrilidae	<i>Lepetodrilus elevatus</i> McLean, 1988	AY145381
Haliotidae	<i>Nordotis discus</i> (Reeve, 1846)	AF082177
	<i>Haliotis tuberculata</i> Linnaeus, 1758	AF120511
Trochidae	<i>Chlorostoma argyrostoma lischkei</i> (Tapparone-Canefri, 1874)	AF335562
Turbinidae	<i>Batillus cornutus</i> (Lightfoot, 1786)	AF165311
CAENOASTROPODA		
Viviparidae	<i>Viviparus georgianus</i> (Lea, 1834)	AF120516
Littorinidae	<i>Littorina littorea</i> (Linnaeus, 1758)	X91970
	<i>Nodilittorina punctata</i> (Gmelin, 1791)	Y11755
Truncatellidae	<i>Truncatella guerinii</i> Villa & Villa, 1841	AF120518
Calyptraeidae	<i>Crepidula adunca</i> Swerby, 1825	X94277
Bursidae	<i>Bursa rana</i> (Linnaeus, 1758)	X94269
Muricidae	<i>Rapana venosa</i> (Valenciennes, 1846)	X98826
Buccinidae	<i>Pisania striata</i> Gmelin, 1791	X94272
Nassariidae	<i>Nassarius sinquijorensis</i> Adams, 1852	X94273
Fascioliidae	<i>Fasciolaria lignaria</i> Linnaeus, 1758	X94275
HETEROBRANCHIA		
Rissoellidae	<i>Rissoella caribaea</i> Rehder, 1943	AY090797
Pyramidellidae	<i>Boonea seminuda</i> (Adams, 1839)	AY145367
OPISTHOBRANCHIA		
CEPHALASPIDEA		
Heminoeidae	<i>Bullacta exarata</i> (Philippi, 1848)	AF188675
SACOGLOSSA		
Elysiidae	<i>Thuridilla bayeri</i> (Marcus, 1965)	AF249220
APLYSIOMORPHA		
Aplysiidae	<i>Aplysia punctata</i> (Lamarck, 1809)	AJ224919

(Continued)

Classification	Species	Accession No.
NOTASPIDEA		
Umbraculidae	<i>Umbraculum umbraculum</i> (Lightfoot, 1786)	AY165753
NUDIBRANCHIA		
Onchidorididae	<i>Acanthodoris pilosa</i> (Abildgaard in Muller, 1789)	AJ224770
PULMONATA		
ARCHAEOPULMONATA		
ELLOBIOIDEA		
Ellobiidae	<i>Ellobium chinensis</i> Pfeiffer, 1854	AF190452
BASOMMATOPHORA		
Siphonariidae	<i>Anthosiphonaria sirius</i> (Pilsbry, 1894)	X98828
Lymnaeidae	<i>Lymnaea glabra</i> (Müller, 1774)	Z73982
STYLOMMATOPHORA		
Helicidae	<i>Helix aspersa</i> (Müller, 1774)	X91976
SYSTELLOMMATOPHORA		
Onchidiidae	<i>Onchidella celtica</i> (Cuvier, 1817)	X70211
Outgroup		
POLYPLACOPHORA	<i>Lepidozona coreanica</i> (Reeve, 1847)	X98829
BIVALVIA	<i>Placopecten magellanicus</i> (Gmelin, 1791)	X53899

(73 base pairs and 74 base pairs, respectively). Our multiple alignment analysis of sequences from the 46 molluscan taxa (44 gastropods, one bivalve, and one chiton) spanned 2,453 positions including insertions and/or deletions. Of these nucleotide positions, 742 sites, which included indel sites and could not be unambiguously aligned, were eliminated from the final analyses. Accordingly, our phylogenetic analyses focused on reliably aligned regions comprising a total 1,711 nucleotide positions. Of these, 895 (52.3%) characters were variable and 690 (40.3%) were parsimony informative.

All the trees resulting from MP (Fig. 1A), ML (Fig. 1B), and BI (Fig. 1C) yielded consistent findings in terms of the phylogeny of Patellogastropoda. The MP analyses produced a single most-parsimonious tree [length = 2,693 steps, consistency index (CI) = 0.5596, retention index = 0.8300]. The topologies of the MP, ML, and BI trees indicated that the Patellogastropoda itself (100% bootstrap values/quartet puzzling reliability values/Bayesian posterior probabilities, in all analyses) and two of its subclades, the Patelloidea (92%, 100%, 100%) and the Acmaeidea (93%, 100%, 100%), were monophyletic. In terms of superfamilies, the Patelloidea and Acmaeidea had branching orders of ((*Cymbula* + *Helcion*) + *Patella*) and ((*Nipponacmea* + *Lottia*) + *Niveotectura*), respectively (99%, 100%, 100%; 87%, 99%, 100%). In contrast, the superfamily Nacelloidea was found to be paraphyletic. Within the Patellogastropoda, the Nacelloidea branched off first and the Patelloidea and the Acmaeidea appeared as sister groups to each other with 100% node confidence values in all analyses. The phylogenetic trees also strongly supported the clade Patellogastropoda as the sister group to order Cocculiniformia (100%, 98%, 94%) within the Gastropoda.

Next, we focused separately on the Patellogastropoda, mainly because the use of outgroups that are too far removed from the clade under study may produce additional homoplasies between ingroup and outgroup species. We used the Cocculiniform limpet, *Cocculina messingi*, as the outgroup for this analysis. A single concordant topology was obtained from MP, ML, and BI of the newly characterized 18S rRNA gene sequences from nine patellogastropods (Fig. 2). The MP analyses yielded a single most-parsimonious tree (length = 882 steps, CI = 0.9218, RI = 0.7971). The patellogastropod trees exhibited the same topology as the complete trees (Fig. 1) and strongly supported all the identified phylogenetic relationships within the patellogastropod limpets. This analysis also confirmed the monophyly of the Patelloidea (87%, 100%, 100%) and Acmaeidea (93%, 100%, 100%), and further showed the previously identified branching patterns {[(*Cymbula* + *Helcion*) + *Patella*] and [(*Nipponacmea* + *Lottia*) + *Niveotectura*]: 99%, 100%, 100%; 88%, 97%, 100%}. Within the patellogastropod clade, the paraphyletic Nacelloidea diverged first, and the Acmaeidea and Patelloidea were strongly supported as sister taxa (100%, 100%, 100%).

Discussion

The monophyly of Patellogastropoda has been studied in recent years from both morphological and molecular standpoints. Haszprunar (1988a; 1988b) suggested that the patellogastropod limpets are synapomorphically united by the occurrence of duct-dense salivary glands, reduced hypobranchial glands, and distinctive osphradia localized close to the 'wart-organ'. Ponder and Lindberg (1996) noted that Patel-

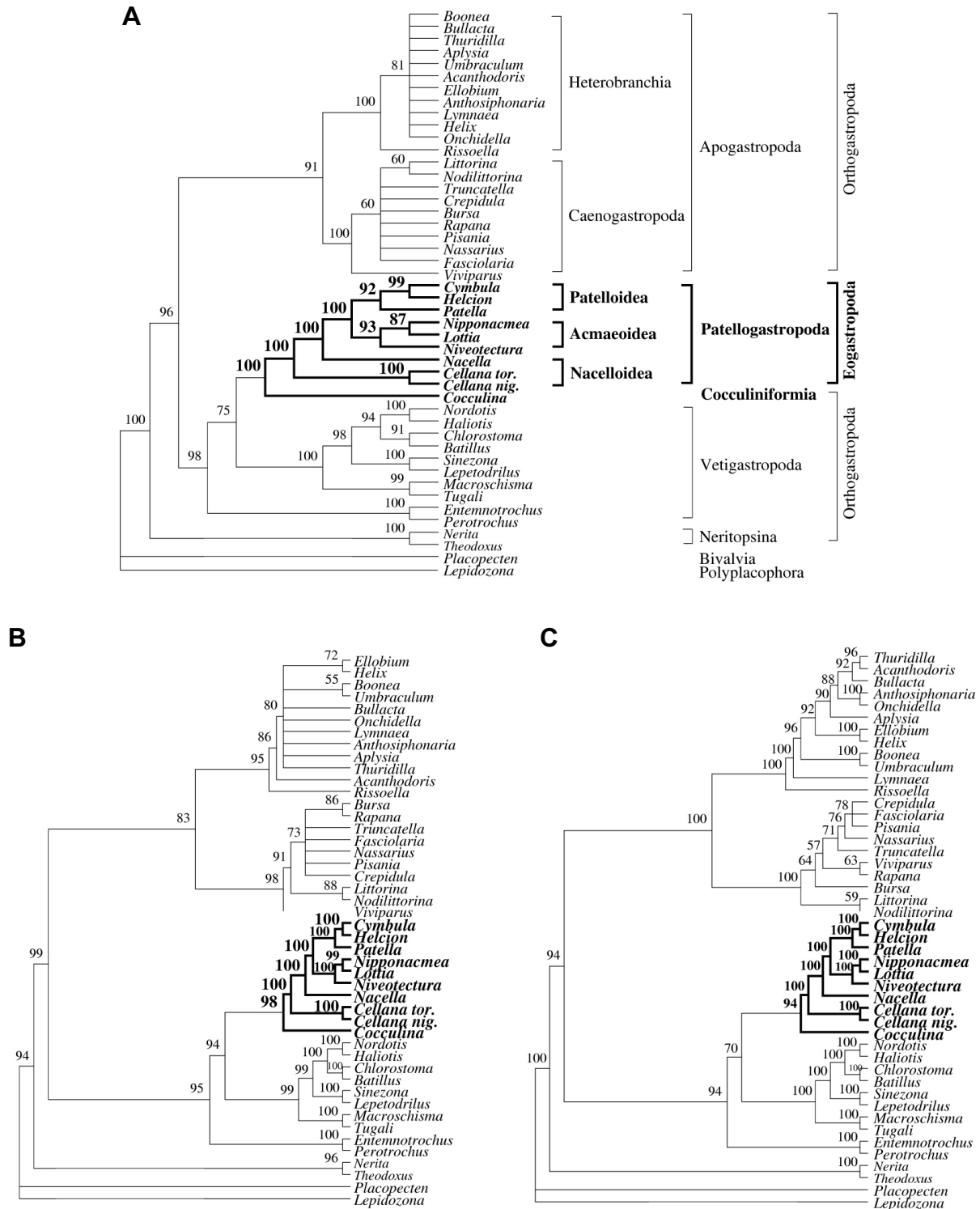


Fig. 1. **A.** Majority-rule consensus tree derived from maximum parsimony analyses on the basis of the alignment of complete 18S rDNA sequences for 44 gastropods, with *Lepidozona coreanica* (Polyplacophora) as the outgroup (length = 2,693; CI = 0.5596; RI = 0.8300). Bootstrap values higher than 50% of 1,000 replicates are indicated above the branches. **B.** Maximum likelihood tree based on the alignment of complete 18S rDNA sequences for 44 gastropods, with *Lepidozona coreanica* (Polyplacophora) as the outgroup. The number of utilized puzzling steps was 10,000, and the maximum likelihood quartet puzzling reliability values are indicated above the nodes. **C.** Majority-rule consensus tree based on Bayesian inference from an alignment of the complete 18S rDNA sequences of 44 gastropod species with *Lepidozona coreanica* (Polyplacophora) as the outgroup. Numbers above internal branches indicate Bayesian posterior probabilities (shown as percentages).

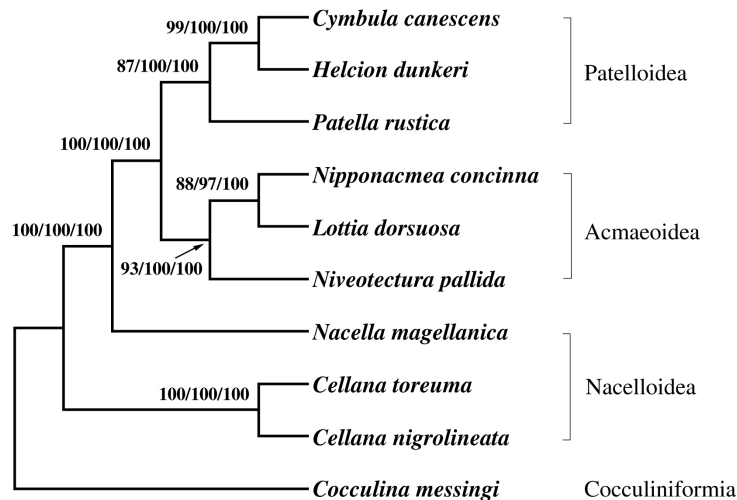


Fig. 2. Phylogenetic relationships of the patellogastropod limpets based on the 18S rDNA sequences for nine patellogastropod limpets. *Cocculina messingi* (Cocculiniformia) was used as the outgroup. The phylogenetic tree was constructed by the maximum parsimony analyses. Numbers at nodes represent (left to right): bootstrap values for maximum parsimony tree (length = 882, CI = 0.9218, RI = 0.7971); quartet puzzling reliability values for maximum likelihood tree; and posterior probabilities for Bayesian inference tree.

logastropoda are characterized by osphradia positioned on the pallial floor, and then the monophyly of Patellogastropoda was further supported by other synapomorphic characters, such as the presence of retractile circumpallial tentacles, transverse labial muscles, dorsal protractors of the odontophore, an anterior jaw band, a muscular bulbous aorta (Sasaki, 1998), secondarily flattened shells, shell microstructures including foliated and cone crossed-lamellar layers, two pairs of outer lateral teeth, pallial gills, 'Spengel's' or 'wart' organs, and the loss of the style and gastric shield (Lindberg, 1998). Molecular studies using partial 28S (Tillier *et al.*, 1994) and partial 18S (Harasewych *et al.*, 1997; Harasewych and McArthur, 2000) rDNA sequences supported the monophyly of Patellogastropoda. In the present work we used complete 18S rDNA sequences rather than the previous partial ones and added nine new patellogastropod limpet sequences. Our results strongly support the monophyletic origin of the patellogastropod limpets.

Our findings further indicate that two of the patellogastropod subgroups, the Patelloidea and the Acmaeoidea, are each monophyletic and that the remaining subgroup Nacelloidea is paraphyletic. Some morphologists have based the monophyly of the Patelloidea on the occurrence of one pair of salivary glands, each with two pairs of ducts, an esophagus that is rotated $> 230^\circ$, a foliated outer shell layer, and five pairs of radula cartilages (Lindberg, 1988; 1998; Powell, 1973; Sasaki, 1998). Several authors, however, have claimed that the Patelloidea are not monophyletic, on the basis of morphological characteristics (Dall, 1876) as well as mitochondrial 12S and 16S rRNA sequences (Koufopanou *et al.*, 1999; Nakano and Ozawa, 2004). Within the Patelloidea, *Cymbula* and *Helcion* are more closely related to each other than *Patella* in our trees, a configuration that

is in agreement with results derived from morphological analyses (Ridgway *et al.*, 1998) and mitochondrial 12S and 16S rRNA gene sequences (Koufopanou *et al.*, 1999; Nakano and Ozawa, 2004). Monophyly of the Acmaeoidea was also confirmed in the present work. The Acmaeoidea are synapomorphically united by the loss of wart organs, the presence of left ctenidia, lickers with smooth medial ridges, two pairs of functional uncini, and a chromosome number of $2n=10$ (Linderberg, 1998). The monophyly of this group was confirmed by previous molecular studies based on mitochondrial 12S and 16S rDNA sequences (Nakano and Ozawa, 2004), but was disputed by claims that the Acmaeoidea are paraphyletic based on morphological characteristics (Dall, 1876; Sasaki, 1998). In addition, we found that within the Acmaeoidea, *Nipponacmea* and *Lottia* form closely related taxa. In terms of the Nacelloidea, previous molecular findings using partial 18S rDNA (Harasewych and McArthur, 2000) and mitochondrial 12S and 16S (Koufopanou *et al.*, 1999) rDNA sequences indicated that this subgroup was paraphyletic, while other studies identified this subgroup as monophyletic based on mitochondrial 12S and 16S rDNA sequences (Nakano and Ozawa, 2004) and synapomorphic characteristics such as the lack of crossed-lamellar structures above the myostracum (Lindberg, 1988; 1998). Our analysis supports the notion that the Nacelloidea are paraphyletic and suggests that this group should be considered a grade rather than a clade.

Our results clearly indicate that the Patelloidea and Acmaeoidea are sister group taxa. However, previous reports have disagreed on this matter. Morphologists such as Dall (1876), Powell (1973), and Sasaki (1998) reported that the Patelloidea have stronger affinities to the Nacelloidea than to the Acmaeoidea, based on common morphological

characteristics such as nine pairs of chromosomes, and the presence of pallial gills, pallial sensory organs, radula with central teeth, radular musculature, and odontophoral cartilages. These findings were confirmed by molecular analyses of mitochondrial 12S and 16S rDNA sequences (Nakano and Ozawa, 2004). In contrast, McLean (1990) and Lindberg (1998) suggested that the Acmaeioidea resemble the Nacelloidea more strongly than the Patelloidea, on the basis of lack of rachidian teeth and presence of two pairs of salivary glands.

The position of the patellogastropods within the Gastropoda is also a matter of some debate. Haszprunar (1988a) argued that the patellogastropod and caudofoveate limpets should not be considered sister group taxa because they differed in their radula morphologies. Patellogastropod limpets (as well as Polyplacophora and Monoplacophora) have a stereoglossate radula, while the caudofoveate limpets have a rhipidoglossate radula, as do Neritopsina and Vetigastropoda. Similarly, other morphologists (e.g., Boss, 1982; Lindberg, 1981; Seed, 1983) treated patellogastropod limpets as a part of the Archaeogastropoda, which also contain the Vetigastropoda and Neritopsina. Tillier *et al.* (1994) proposed that the Patellogastropoda have a sister group relationship with the order Vetigastropoda, on the grounds of partial 28S rDNA sequences. However, an opposing view based on morphology asserts that the patellogastropod clade holds the most basal position within the Gastropoda (Haszprunar, 1988a; Ponder and Lindberg, 1996; 1997; Salvini-Plawen, 1981; Salvini-Plawen and Haszprunar, 1987; Sasaki, 1998). This proposal has been supported by 28S (McArthur and Koop, 1999; Rosenberg *et al.*, 1997; Tillier *et al.*, 1994) and partial 18S rDNA sequences (Harasewych and McArthur, 2000; Harasewych *et al.*, 1997). Our present results clearly indicate that the Patellogastropoda are most closely related to the order Caudofoveata.

In sum, we have deduced the phylogeny of patellogastropod limpets from complete 18S ribosomal RNA gene sequences. We report new sequences for nine patellogastropod limpets, and use these sequences in conjunction with 35 known gastropod sequences to generate robust bootstrap values using three different phylogenetic analytical methods. Our strong results indicate that 18S ribosomal RNA gene is very useful for reconstructing patellogastropod phylogeny from genus to order. Further work aimed at resolving the phylogenetic relationships at genus level should consider using a more comprehensive taxon.

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