Molluscan Research 25(2): 85–98
http://www.mapress.com/mr/

ISSN 1323-5818
Magnolia Press

The molecular phylogeny of the Buccinidae (Caenogastropoda: Neogastropoda) as inferred from the complete mitochondrial 16S rRNA gene sequences of selected representatives

SEIJI HAYASHI
Department of Earth and Planetary Sciences, Graduate School of Environmental Studies, Nagoya University, Furo-cho, Chikusa-ku, Nagoya 464-8602, Japan. E-mail:seijih@geobio.eps.nagoya-u.ac.jp

Abstract

Complete mitochondrial 16S rRNA gene sequences from 35 caenogastropods were obtained to evaluate the phylogenetic relationship of the family Buccinidae at the intra-supra familial level. With respect to intergeneric relationships within the family, the molecular phylogeny supported three clades with statistical significance: *Buccinum* + *Neptunea*, *Engina* + *Pisania* + *Pollia* and *Penion* + *Kelletia*. These groupings are generally consistent with the morphological and paleontological evidence. In particular, it is noteworthy that the monophyly of *Penion* and *Kelletia* was established. Their close relationship as antitropical genera is confirmed for the first time. *Babylonia* was positioned remotely from the other buccinids confirming recent morphological and molecular work. The monophyly of the current concept of Buccinidae was violated by intercalations of nassariids and fasciolariids.

Key words: Buccinoidea, Buccinulinae, Pisaniinae, fossil record, antitropical distribution

Introduction

As generally interpreted, the Buccinidae is one of the most diverse families of caenogastropods. It ranges from the poles to the equator and inhabits a wide variety of mainly marine environments (from fresh water to abyssal). Fossil records of this family, the Fasciolariidae, Melongenidae and Cancellariidae date back to the early Cretaceous, whereas other neogastropod families appeared between the late Cretaceous to early Paleogene (Tracey *et al.* 1993 and references therein), suggesting that the former families represent first offshoots of neogastropods. However, Bandel (1993) claimed that some Cretaceous neogastropod fossils need confirmation and it is still unresolved which systematic units of the Neogastropoda appeared first.

Ponder (1974) considered the Buccinidae to be closely related to the Nassariidae, Fasciolariidae and Melongenidae because of the anatomical similarities of these families. Ponder and Warén (1988) downgraded these four families into subfamilies under a single inclusive Buccinidae. Hereinafter, I will use the term 'Buccinidae *s. l.*' to refer to this definition of Buccinidae for convenience in discussion. Later, Ponder and Lindberg (1997) regarded the lack of accessory salivary glands and an anal (rectal) gland as plesiomorphic conditions of the Neogastropoda rather than a secondary loss in the Buccinoidea as suggested previously (e.g., Ponder 1974, etc.). In their cladogram (Ponder and Lindberg 1997), the Buccinidae (+ Nassariidae) clade was depicted as the sister group to the remaining neogastropods, the Conoidea + Muricoidea. Kantor (1996) emphasized the close affinity among the Buccinidae, Nassariidae, Fasciolariidae and Columbellidae based on their shared characteristics (e.g., a long or very long proboscis, the loss of glandular dorsal folds and a tendency for reduction of the gland of Leiblein). In contrast to Ponder and Lindberg's (1997) scheme, Kantor regarded the 'loss of accessory salivary glands' as being a shared character of the Buccinoidea and some other families (e.g., Harpidae and Mitridae, see Fig. 19.7 in Kantor (1996)). Furthermore, Kantor (2002) disputed the polarization of character states in the cladistic analysis of Ponder and Lindberg (1997), and claimed that the Buccinidae cannot be the most primitive family of neogastropods, based on their advanced proboscis structure. Riedel (2000) proposed Buccinoidea (= Buccinidae, Melongenidae and Nassariidae) and Columbelloidea (= Fasciolariidae and Columbellidae) and grouped them under the new infraorder Buccinina, suggesting that Buccinidae and Fasciolariidae may be paraphyletic.

The phylogenetic relationships among the members of the family also remain quite ambiguous. There is no consensus as to the limits of the family, or of the relationships of the more than 200 included genera and subgenera (Harasewych 1998). Some subfamilial assignments for the Buccinidae have been published, based mainly on shell and radula characters, with and without justification (Table 1). However, the radula may not be particularly suited as a diagnostic tool below the family level (e.g., Cernohorsky 1975).

Recently, Harasewych and Kantor (2002) showed that the genus *Babylonia* is more closely related to the volutoideans than to any other buccinoideans, in both morphological and molecular phylogenies. With his in-depth inspection of the buccinoidean stomach, Kantor (2003) succeeded in discriminating all of the families of the Buccinoidea, except for the closely related Buccinidae and Buccinulidae, and questioned the current familial position of the genera *Clea*, *Busycon* and *Nassaria* from evidence provided by stomach anatomy.

COPYRIGHT © 2005 MALACOLOGICAL SOCIETY OF AUSTRALASIA

TABLE 1. Representative (sub) familial designation for analyzed buccinids.

	Powell (1929, 1951)	Kuroda *et al.* (1971)	Vaught (1989)	Higo *et al.* (1999)
Buccinum	Buccinidae	Buccininae	Buccininae	Buccininae
Neptunea	Neptuneidae	Neptuneinae	Buccininae	Neptuneinae
Japeuthria	-	Photinae	Buccininae	Pisaniinae
Phos	Cominellinae[a, b]	Photinae	Photinae	Photinae
Nassaria	-	Photinae	Photinae	Photinae
Siphonalia	Neptuneidae	Photinae	Buccininae	Siphonaliinae
Pollia	-	Photinae	Pisaniinae	Pisaniinae
Engina	-	-	Pisaniinae	Pisaniinae
Pisania	-	-	Pisaniinae	Pisaniinae
Cantharus	-	-	Pisaniinae	Pisaniinae
Burnupena	Buccinidae	-	Buccininae	-
Kelletia	Buccinulinae[a]	Photinae	Buccininae	Siphonaliinae
Penion	Buccinulinae[a]	-	Buccininae	-
Buccinulum	Buccinulinae[a]	-	Buccininae	-
Cominella	Cominellinae[a, b]	-	Photinae	-

[a]as a subfamily under the Buccinulidae [b]a synonym of Photinae

Despite the recent increase in nucleotide sequence data for Gastropoda, the data for buccinids are still limited, having mostly been obtained in the analysis of higher categories: the Neogastropoda or Gastropoda (e.g., Harasewych *et al*. 1997; McArthur and Koop 1999; Riedel 2000; Tillier *et al*. 1992; Winnepenninckx *et al.* 1998). To address the phylogeny of the Buccinidae, and mainly to elucidate the intergeneric relationships within the family, the sequences of the entire mitochondrial 16S rRNA gene were determined for 35 species of Caenogastropoda: 17 buccinids, three nassariids, two fasciolariids, one melongenid, one columbellid (24 buccinoideans in total), two muricoideans, two conoideans, two 'volutoideans', one cancellarioidean, two tonnoideans, one ficoidean and one littorinid.

Materials and Methods

DNA extraction

The sampling locations, voucher numbers and GenBank accession numbers for DNA sequences of the material used in this study are listed in Table 2. All the voucher specimens sequenced in this study are housed in the Geobiology lab, Department of Earth and Planetary Sciences, Graduate School of Environmental Studies, Nagoya University. Total DNA was extracted from 10–50 mg of the foot/mantle tissue of the snails using either the standard proteinase K/SDS/phenol procedure of Sambrook *et al*. (1989) followed by ethanol precipitation or by using a PCR template purification kit (Roche Diagnostics), followed by recovery in 300 µl of TE (pH = 8.0). The DNA extracts were diluted 10–100 fold prior to their use in the PCR process.

PCR

Three DNA fragments that encompassed the entire mitochondrial 16S rRNA gene were amplified by PCR using three pairs of oligonucleotide primers: 12SA-L–DY 16S748R, 16sar-L–16sbr-H and DY16S779F–CGLeu^{UUR}R. The primer sequences were as follows: 12SA-L: 5'-AAACTGGGATTAGATACCCCACTAT-3' (Palumbi *et al*. 1991), DY16S748R: 5'-GGCAAATGATTATGCTACCTTT GCACGGTCAG-3', 16sar-L: 5'-CGCCTGTTTATCAAAAA CAT-3' (Palumbi *et al*. 1991), 16sbr-H: 5'-CCGGTCTGAAC TCAGATCACGT-3' (Palumbi *et al*. 1991), DY16S779F: 5'-CTGACCGTGCAAAGGTAGCATAATCATTTGCC-3' and CGLeu^{UUR}R: 5'-TATTTAGGGCTTAAACCTAATGCAC-3'.

The design of the DY16S748R and DY16S779F sequences was based on a consensus of the sequences of the 16sar-L–16sbr-H fragments of the majority of examined taxa. The sequence of CGLeu^{UUR}R was developed using an alignment of the partial sequences of the DY16S779F–MoND1R1 (5'-TCAGAYTCYCCYTCWGCA AA-3') fragment taken from selected taxa. The latter primer was designed from a consensus sequence taken from published ND1 gene sequences: *Albinaria coerulea* (Hatzoglou *et al.* 1995; X83390), *Cepaea nemoralis* (Terrett *et al*. 1996; U23045), *Katharina tunicata* (Boore and Brown 1994; U09810) and *Lumbricus terrestris* (Boore and Brown 1995; U24570).

After initial heating to 94 °C for 3 min, template DNA (1 µl) was subjected to 30 cycles of PCR amplification (40 s at 94 °C for denaturation, 60 s at 45–50 °C for annealing and 60 s at 72 °C for extension) in 25 µl of reaction mixture (0.5 U Ex *Taq* (Takara), 1X Ex *Taq* buffer, 0.2 µM of each primer, 50 µM each dNTP and 400 µg/ml BSA (Sigma)) followed by an extension at 72 °C for 5 min. The PCR products were purified using a High Pure PCR product purification kit (Roche Diagnostics). The sequencing reactions were

performed using the cyclic reaction termination method employing fluorescence-labelled ddNTP (Du Pont) and Thermosequenase (Amersham-pharmacia) following the method of Takumi *et al*. (1997) or BigDye Terminator Cycle Sequencing Kit (ABI PRISM, Perkin-Elmer Biosystems). Electrophoresis and data collection were run on either a Shimadzu 2000L (for the former reaction products) or an ABI 377XL automated sequencer (for the latter ones).

TABLE 2. Species included in this study with localities of samples, followed by voucher numbers and GenBank accession numbers (GBAN) for DNA sequences.

Taxon	Locality	Voucher number	GBAN	Source
Caenogastropoda				
Sorbeoconcha				
Cerithioidea				
Cerithiidae				
Cacozeliana lacertina (Gould, 1861)	Long reef, New South Wales, Australia		AF101007	Lydeard *et al*. (2000)
Hypsogastropoda				
Littorinimorpha				
Littorinoidea				
Littorinidae				
Littorina saxatilis (Olivi, 1792)	Gann estuary, Pembrokeshire, Wales		AJ132137	Wilding *et al*. (1999)
Littorina brevicula (Philippi, 1844)	Gamagori, Aichi, central Japan	NUGB-G2039	AB044246	this study
Ficoidea				
Ficidae				
Ficus subintermedia (d'Orbigny, 1852)	Kochi Bay, Kochi, western Japan	NUGB-G2117	AB207900	this study
Tonnoidea				
Ranellidae				
Biplex perca (Perry, 1811)	off Kushimoto, Wakayama, central Japan	NUGB-G2040	AB044247	this study
Tonnidae				
Tonna luteostoma (Küster, 1857)	Mikawa bay, Aichi, central Japan	NUGB-G2075	AB207899	this study
Neogastropoda				
Muricoidea				
Muricidae				
Thais savignyi (Deshayes, 1844)	Nago, Okinawa, Japan	NUGB-G2047	AB044248	this study
Thais clavigera (Küster, 1860)	Gamagori, Aichi, central Japan	NUGB-G2034	AB044249	this study
Buccinoidea				
Nassariidae				
Niotha semisulcata (Rousseau, 1854)	Onna, Okinawa, Japan	NUGB-G2038	AB044250	this study
Reticunassa festiva (Powy, 1835)	Gamagori, Aichi, central Japan	NUGB-G2033	AB044251	this study
Zeuxis siquijorensis (A. Adams, 1852)	Irino Bay, Kochi, western Japan	NUGB-G2025	AB044252	this study
Fasciolariidae				
Fusinus akitai Kuroda and Habe, 1961	off Atsumi, Aichi, Japan	NUGB-G2006	AB044253	this study
Granulifusus niponicus (E.A. Smith, 1879)	off Kushimoto, Wakayama, central Japan	NUGB-G2043	AB044254	this study
Buccinidae				
Buccinum opisoplectum Dall, 1907	unknown	NUGB-G2029	AB044257	this study
Neptunea intersculpta (Sowerby III, 1899)	off Hokkaido, north Japan	NUGB-G2032	AB044265	this study
Japeuthria ferrea (Reeve, 1847)	Suga Island, Ise bay, Mie, central Japan	NUGB-G2017	AB044262	this study
Phos laeve Kuroda and Habe, 1961	off Kushimoto, Wakayama, central Japan	NUGB-G2042	AB044268	this study
Nassaria magnifica Lischke, 1871	off Kushimoto, Wakayama, central Japan	NUGB-G2041	AB044264	this study

..... continued on the next page

TABLE 2 (continued)

Taxon	Locality	Voucher number	GBAN	Source
Siphonalia cassidariaeformis (Reeve, 1846)	off Shizuoka, central Japan	NUGB-G2030	AB044271	this study
Pollia tincta Conrad, 1846	St. Pete Beach, Pinellas, Florida, USA	NUGB-G2035	AB044270	this study
Engina mendicaria (Linnaeus, 1758)	Nago, Okinawa, Japan	NUGB-G2037	AB044261	this study
Pisania pusio (Linnaeus, 1758)	Pelican Shoal, Monroe, Florida, USA	NUGB-G2013	AB044269	this study
Cantharus multangulus (Philippi, 1848)	Tierre Verde, Pinellas, Florida, USA	NUGB-G2036	AB044259	this study
Burnupena cincta (Röding, 1798)	Cape Town, South Africa	NUGB-G2045	AB044258	this study
Kelletia kelletii (Forbes, 1850)	Santa Barbara Island, Los Angeles, USA	NUGB-G2051	AB121037	this study
Kelletia lischkei Kuroda, 1938	Wakasa bay, Fukui, central Japan	NUGB-G2031	AB044263	this study
Penion chathamensis (Powell, 1938)	Chatham Rise, New Zealand	NUGB-G2009	AB044266	this study
Penion sulcatus (Lamarck, 1816)	unknown, New Zealand	NUGB-G2016	AB044267	this study
Buccinulum linea (Martyn, 1784)	Leigh Harbour, New Zealand	NUGB-G2011	AB044256	this study
Cominella adspersa (Bruguière, 1789)	Orewa, New Zealand	NUGB-G2012	AB044260	this study
Melongenidae				
Hemifusus tuba (Gmelin, 1791)	off Shizuoka (Enshu-nada), central Japan	NUGB-G2023	AB044272	this study
Columbellidae				
Mitrella bicincta (Gould, 1860)	Suga Island, Ise bay, Mie, central Japan	NUGB-G2018	AB044273	this study
Volutoidea				
Olividae				
Oliva mustelina Lamarck, 1811	Ise bay, Mie, central Japan	NUGB-G2078	AB121038	this study
Babyloniidae				
Babylonia lutosa (Lamarck, 1822)	East China Sea	NUGB-G2028	AB044255	this study
Conoidea				
Turridae				
Comitas kaderlyi (Lischke, 1872)	off Atsumi, Aichi, central Japan	NUGB-G2021	AB044275	this study
Conidae				
Conus praecellens (A. Adams, 1854)	off Kushimoto, Wakayama, central Japan	NUGB-G2022	AB044276	this study
Cancellarioidea				
Cancellariidae				
Cancellaria sinensis Reeve, 1856	Tosa bay, Kochi, western Japan	NUGB-G2024	AB044274	this study

Sequence comparison and alignment

Sequence differences in terms of the pairwise 'global' alignment were calculated using ALIGN implemented in the GENESTREAM bioinformatics resource server at the Institut de Génétique Humaine, Montpellier, France (http://www2.igh.cnrs.fr/). Multiple sequence alignments for 35 newly determined and two published sequences, *Littorina saxatilis* (Olivi, 1792) (Wilding *et al.* 1999) and *Cacozeliana lacertina* (Gould, 1861) (Lydeard *et al.* 2000) were generated using the Clustal X v.1.8 package (Thompson *et al*. 1997), applying the default settings. Some modification of the alignment was conducted by eye. Regions of poor or uncertain alignment were omitted from any subsequent analysis. The alignment is available online at TreeBASE (http://www.treebase.org/) as the matrix under accession No. M2342.

Phylogenetic analyses

All of the phylogenetic analyses were performed with PAUP*4.0b10 (Swofford 2002). A χ^2 test for homogeneity of the base frequencies across the taxa was performed. Unweighted maximum parsimony (UMP) and the weighted (transversion two times over transition) maximum parsimony (WMP) analyses were conducted using a heuristic search with 100 random additions, tree bisection and reconnection (TBR) branch swapping and the MULTREES option in effect. A neighbour-joining tree (NJ; Saitou and Nei 1987) was constructed using Kimura's two-parameter distance (Kimura 1980) with the missing ambiguous data option being set to 'ignore site for affected pairwise comparison'. The robustness of the internodes was assessed by bootstrapping (Felsenstein 1985) for the NJ and maximum parsimony (MP) trees, with 1,000 and 500 times replication, respectively. Decay analysis was also employed

to evaluate the nodal support in the MP tree using the TreeRot program (Sorenson 1999). In a maximum likelihood (ML) analysis, the best-fit model for DNA substitution and the parameter estimates used for the tree construction were chosen by performing hierarchical likelihood ratio tests (Huelsenbeck and Crandall 1997) with Modeltest 3.06 (Posada and Crandall 1998). Heuristic ML searches were performed with 10 replicates of random sequence addition, TBR branch swapping and the MULTREES option in effect. Bootstrap analysis for likelihood criteria was not performed because of the heavy computational burden involved. In both the MP and ML analyses, a gap was treated as missing. To root the tree, two species of *Littorina* and *Cacozeliana lacertina* were used as outgroups. A Shimodaira-Hasegawa (SH) test (Shimodaira and Hasegawa 1999) was employed to compare the plausibility of the phylogenetic hypothesis. To generate the hypothesized trees, I performed heuristic searches under the likelihood criterion using the same parameters as in the initial ML search with pertinent constrained trees being invoked.

TABLE 3. Sequence difference (%) based on pairwise 'global' alignment (below the diagonal) and sequence divergence (%) based on multiple alignment corrected by Kimura's 2-parameter method (unambiguously aligned region only, above the diagonal)

	Pca	Psu	Kke	Kli	Bli	Cad	Bci	Bop	Nin	Jfe	Sca	Pla	Nma	Eme	Ppu	Pti	Cmu	Gni
Pca		1.5	3.9	3.3	6.2	5.9	6.5	7.5	7.9	10.5	12.6	10.1	10.9	10.4	11.6	10.4	11.2	9.1
Psu	4.3		4.2	3.2	6.1	6.3	6.6	7.4	8.0	10.7	13.2	9.8	11.0	10.0	11.2	10.0	11.2	8.9
Kke	7.3	8.3		1.3	6.5	7.0	7.0	7.1	7.7	10.1	14.4	8.7	10.7	10.5	11.3	9.2	11.2	8.7
Kli	7.2	8.0	3.1		5.4	6.2	6.4	6.2	7.2	9.5	13.4	8.4	10.5	9.7	11.1	8.6	10.7	8.2
Bli	11.6	12.0	11.5	10.8		7.4	8.3	7.3	8.3	10.2	13.5	9.3	10.8	10.5	11.8	10.3	12.5	9.3
Cad	12.2	12.7	13.4	13.1	14.3		7.9	7.8	8.5	11.0	13.7	9.4	11.7	10.1	10.9	9.6	10.8	9.1
Bci	11.3	12.0	12.2	11.3	13.0	13.4		7.8	9.5	11.2	14.3	9.2	11.4	10.7	11.6	10.6	12.4	10.8
Bop	12.8	13.1	12.6	11.8	12.8	14.2	12.5		5.8	10.1	13.6	9.5	11.9	10.4	11.7	10.8	13.5	10.0
Nin	13.3	13.9	13.6	12.9	13.5	15.1	13.9	9.9		9.9	15.1	10.7	12.4	11.3	12.7	11.8	13.3	10.6
Jfe	15.7	16.5	16.1	15.5	16.6	16.8	16.5	16.2	16.2		12.8	12.2	13.0	13.2	14.4	13.4	16.2	11.6
Sca	18.6	19.0	20.3	19.2	18.8	19.0	19.9	19.4	20.5	18.2		14.8	15.2	16.4	17.5	16.9	19.0	16.5
Pla	16.3	16.5	15.4	15.0	15.8	16.3	15.1	16.6	16.6	18.1	20.9		11.3	11.9	12.6	10.7	14.3	11.2
Nma	18.3	19.0	17.9	17.7	17.6	19.0	17.8	19.1	18.7	19.7	22.3	18.2		12.7	14.4	13.6	14.2	12.2
Eme	16.3	16.4	16.4	15.6	16.3	17.3	16.4	16.8	17.3	19.1	22.5	18.4	19.3		5.8	5.0	12.7	11.1
Ppu	17.0	17.0	17.6	17.3	17.7	17.8	17.1	17.9	18.4	19.4	23.0	19.2	20.9	10.6		5.9	14.0	11.2
Pti	17.4	17.2	16.3	16.0	16.4	17.0	15.9	17.6	17.3	19.1	22.9	17.7	19.9	10.2	10.7		12.4	10.1
Cmu	17.7	17.6	16.4	16.3	18.4	17.8	17.6	17.9	18.3	20.9	23.5	19.7	21.0	18.2	19.1	18.1		13.3
Gni	14.3	13.9	13.6	13.6	14.7	14.5	15.4	15.4	15.8	17.4	20.6	16.8	18.5	16.1	17.0	15.9	18.6	
Fak	17.8	18.0	18.6	17.4	18.5	18.2	18.3	19.1	18.8	20.9	23.7	21.4	21.2	19.0	19.5	20.0	20.3	17.4
Nse	18.8	18.6	18.0	17.5	17.3	18.0	17.5	17.8	17.2	19.2	23.1	17.5	20.5	18.4	19.4	19.0	20.3	17.8
Zsi	19.0	19.0	18.3	17.4	17.7	18.8	18.1	19.0	18.9	19.8	24.4	18.4	21.0	20.3	20.0	19.3	21.4	18.8
Rfe	17.9	18.4	18.0	17.4	18.6	18.1	18.0	17.8	17.5	20.6	22.2	18.6	20.9	19.3	19.0	19.3	19.8	18.5
Htu	21.5	21.6	21.1	20.6	21.9	21.4	21.0	20.6	21.6	23.4	25.7	21.2	22.9	22.0	22.3	21.5	22.5	21.9
Mbi	21.9	21.8	20.9	20.5	20.9	20.9	21.1	21.8	21.6	22.7	25.7	21.8	22.4	23.1	23.3	22.0	24.5	20.9
Tsa	24.1	24.1	23.3	23.8	24.5	23.2	24.2	23.5	24.1	24.9	27.4	24.2	24.8	24.8	24.9	24.8	25.3	24.8
Tcl	24.8	24.7	23.6	23.4	24.2	22.1	23.7	24.1	23.6	24.8	26.9	24.9	24.3	23.5	24.4	24.0	23.6	24.1
Blu	23.2	22.7	22.7	22.9	22.2	22.4	22.4	21.6	22.4	22.9	25.2	24.6	24.0	23.6	23.7	23.6	24.7	22.5
Omu	23.6	23.7	22.9	22.8	23.5	21.5	23.7	23.6	23.0	23.7	27.1	23.1	23.3	23.3	23.9	23.0	24.1	22.3
Cka	21.5	21.8	21.2	21.4	21.2	21.4	21.2	21.2	22.2	22.7	24.9	23.3	22.7	23.2	22.9	22.7	24.1	21.5
Cpr	24.7	24.7	25.3	24.2	24.1	25.1	24.2	24.2	24.5	25.8	25.9	25.5	25.0	24.1	25.1	24.8	26.3	24.3
Csi	25.2	25.1	25.0	24.8	24.4	25.0	24.7	24.2	24.9	25.9	26.2	25.0	25.9	24.0	24.6	24.0	26.2	23.9
Bpe	22.4	22.2	22.2	22.3	22.4	21.5	22.5	22.0	23.3	23.4	24.9	22.2	22.4	22.2	23.4	22.5	23.8	22.2
Tlu	24.6	24.4	23.7	23.9	22.8	23.4	23.6	23.7	23.7	23.8	26.6	22.5	23.0	23.0	24.5	23.3	24.4	23.9
Fsu	24.2	23.8	23.0	22.8	23.1	22.6	22.1	23.1	23.9	24.0	26.2	23.1	23.2	22.0	23.8	23.0	23.7	22.7
Lsa	28.6	28.0	28.3	28.2	27.7	28.7	28.2	27.5	28.5	28.3	29.4	28.5	28.2	27.4	29.4	28.2	29.7	27.6
Lbr	27.7	28.2	27.6	27.4	28.4	28.8	28.3	27.4	27.6	29.1	29.1	29.3	28.5	27.6	29.1	28.0	29.4	28.3
Cla	32.7	32.8	32.1	32.6	31.1	32.4	31.4	32.1	31.3	31.4	32.9	32.4	32.7	31.7	32.0	32.2	32.7	32.2

.....continued on the next page

TABLE 3 (continued)

	Fak	Nse	Zsi	Rfe	Htu	Mbi	Tsa	Tcl	Blu	Omu	Cka	Cpr	Csi	Bpe	Tlu	Fsu	Lsa	Lbr	Cla
Pca	10.6	10.8	10.7	9.5	14.3	15.5	17.4	17.6	16.1	14.7	12.4	17.3	18.2	15.4	17.7	17.2	24.7	23.5	34.9
Psu	11.1	10.0	10.3	9.8	14.4	15.5	18.1	17.3	16.0	15.2	12.6	16.7	18.1	15.3	17.8	17.1	24.2	23.4	34.3
Kke	11.7	10.1	9.6	9.8	13.9	14.3	15.8	16.3	15.9	14.3	12.3	17.7	18.4	15.2	17.2	15.8	24.4	23.8	34.9
Kli	11.1	9.2	8.9	9.2	13.7	14.7	16.4	16.2	15.3	14.6	12.0	16.4	17.6	15.0	17.3	15.8	24.7	24.0	34.9
Bli	11.7	9.6	10.0	10.0	15.7	15.5	17.9	17.1	15.0	14.3	12.4	16.5	18.1	15.3	16.0	16.5	23.5	24.2	34.4
Cad	10.5	10.0	10.3	9.6	13.3	14.9	16.3	16.0	14.6	13.9	12.8	17.4	17.0	14.6	16.2	16.4	25.5	25.3	32.6
Bci	12.8	10.7	11.4	10.7	15.5	15.0	17.5	18.3	14.3	16.1	13.1	17.3	19.2	14.9	17.9	17.4	26.2	25.4	34.3
Bop	12.6	10.0	11.1	10.1	14.2	15.8	17.5	17.5	14.5	15.1	13.0	16.0	18.4	14.5	16.0	17.3	23.7	23.1	34.4
Nin	12.6	11.0	11.6	10.1	15.3	17.3	17.5	19.0	15.4	15.3	14.4	17.4	19.6	16.6	18.1	17.9	24.1	24.1	33.9
Jfe	13.8	11.8	12.7	12.3	17.7	17.6	18.0	18.5	15.9	16.8	14.6	17.9	18.9	16.6	18.7	18.1	25.9	27.0	33.9
Sca	17.7	15.7	16.9	15.0	21.2	22.4	21.7	21.6	19.1	20.3	17.2	19.5	19.2	17.9	19.3	22.2	27.1	26.5	36.0
Pla	14.0	10.2	10.1	10.4	15.5	17.7	17.4	19.1	16.7	15.8	13.7	18.2	17.8	14.9	17.4	16.8	26.7	26.0	36.0
Nma	14.4	12.5	13.0	12.2	15.6	16.8	18.2	18.0	16.6	15.3	14.6	18.4	19.4	15.9	17.1	17.5	26.5	25.9	35.1
Eme	13.1	11.8	12.8	12.3	15.6	17.8	18.3	17.6	16.5	16.0	14.7	16.8	18.1	15.9	17.1	17.8	23.7	23.4	33.2
Ppu	13.0	12.2	12.4	12.3	15.5	19.2	18.3	18.2	17.1	16.4	14.2	17.8	19.8	17.1	17.0	18.9	25.5	24.5	34.3
Pti	12.7	10.9	11.6	11.4	14.8	17.3	18.7	17.8	16.8	15.6	14.3	17.1	17.4	16.3	18.3	17.8	24.4	23.8	33.8
Cmu	15.0	14.1	14.3	13.6	17.7	20.0	20.5	20.2	18.0	17.0	16.7	19.8	21.6	17.7	19.3	18.7	28.7	28.5	31.9
Gni	11.4	11.3	12.0	11.6	16.5	16.7	19.0	18.7	16.0	14.8	13.5	17.4	18.4	15.9	18.2	17.0	24.7	24.4	34.5
Fak		14.4	14.0	13.6	16.7	18.1	19.9	18.8	17.2	17.6	15.3	19.0	18.4	17.4	19.3	18.3	27.8	26.5	34.9
Nse	20.5		4.6	8.1	17.6	15.3	17.8	17.7	15.4	14.7	14.1	16.9	17.1	13.5	14.3	14.3	23.9	23.4	35.0
Zsi	21.2	9.3		8.6	17.2	16.5	18.3	17.9	16.3	15.5	14.0	18.1	18.0	15.5	16.2	15.3	23.7	23.5	33.6
Rfe	20.2	15.4	16.1		16.4	16.3	18.6	17.4	15.6	14.5	13.3	18.5	17.6	14.8	16.3	15.0	25.2	24.2	32.8
Htu	23.9	23.5	23.6	22.5		19.8	19.6	19.5	20.2	18.8	18.0	21.8	19.4	18.2	20.4	19.7	29.8	29.6	34.7
Mbi	22.6	21.5	21.9	21.8	23.9		21.9	20.1	19.0	18.2	17.5	21.7	18.8	17.9	20.1	20.0	26.4	26.5	35.6
Tsa	26.4	24.2	25.7	25.9	27.0	26.3		11.5	20.0	19.3	18.2	24.4	23.1	21.0	22.3	19.3	29.3	29.7	33.3
Tcl	24.6	24.2	24.7	24.5	25.7	24.9	18.5		21.4	19.4	18.2	22.5	20.8	20.1	19.7	19.1	27.9	28.2	34.0
Blu	24.4	23.2	24.1	23.8	26.8	25.5	25.6	26.0		18.5	16.6	20.2	21.1	15.1	18.1	19.6	26.5	26.1	32.9
Omu	24.7	22.7	23.1	22.6	25.6	24.4	25.9	25.4	24.7		14.5	20.0	20.9	18.5	17.1	17.4	25.4	25.3	34.9
Cka	23.5	22.7	22.9	22.8	25.4	24.3	25.2	25.0	24.1	22.4		17.1	18.8	15.3	17.2	16.9	27.0	26.2	34.7
Cpr	26.0	24.6	25.6	26.0	27.9	27.2	29.1	27.9	27.5	27.1	24.9		22.4	18.5	18.3	20.1	27.6	27.9	36.9
Csi	25.3	23.6	24.6	23.9	25.1	24.0	29.0	26.9	28.9	26.4	26.5	28.1		18.9	20.1	19.3	26.3	25.5	34.4
Bpe	23.5	21.7	23.6	23.0	24.4	23.8	26.3	25.1	22.3	25.5	22.3	25.7	25.7		14.9	14.9	23.7	22.9	35.0
Tlu	24.9	21.8	23.5	24.0	25.9	24.6	27.6	25.8	24.2	24.5	23.8	25.6	25.8	21.2		19.3	25.2	24.8	36.0
Fsu	24.3	21.8	22.1	22.9	25.5	23.7	24.9	24.1	25.0	23.4	22.3	24.9	25.5	21.1	22.3		24.5	24.3	38.2
Lsa	30.2	30.2	29.3	29.1	31.5	29.1	30.3	30.8	29.1	27.9	28.9	30.5	29.9	27.6	28.1	27.6		3.4	35.7
Lbr	29.3	28.8	29.2	29.8	31.8	30.1	31.0	31.3	29.4	28.3	29.5	31.2	29.6	28.0	27.7	28.2	6.3		35.6
Cla	31.7	32.3	32.4	32.9	33.1	32.4	32.6	32.2	31.7	33.3	32.4	35.2	32.6	33.5	32.6	33.2	33.7	33.8	

Abbreviations of species names are as follows; Pca = *Penion chathamensis*, Psu = *Penion sulcatus*, Kke = *Kelletia kelletii*, Kli = *Kelletia lischkei*, Bli = *Buccinulum linea*, Cad = *Cominella adspersa*, Bci = *Burnupena cincta*, Bop = *Buccinum opisoplectum*, Nin = *Neptunea intersculpta*, Jfe = *Japeurhria ferrea*, Sca = *Siphonalia cassidariaeformis*, Pla = *Phos laeve*, Nma = *Nassaria magnifica*, Eme = *Engina mendicaria*, Ppu = *Pisania pusio*, Pti = *Pollia tincta*, Cmu = *Cantharus multangulus*, Gni = *Granulifusus niponicus*, Fak = *Fusinus akitai*, Nse = *Niotha semisulcata*, Zsi = *Zeuxis siquijorensis*, Rfe = *Reticunassa festiva*, Htu = *Hemifusus tuba*, Mbi = *Mitrella bicincta*, Tsa = *Thais savignyi*, Tcl = *Thais clavigera*, Blu = *Babylonia lutosa*, Omu = *Oliva mustelina*, Cka = *Comitas kaderlyi*, Cpr = *Conus praecellens*, Csi = *Cancellaria sinensis*, Bpe = *Biplex perca*, Tlu = *Tonna luteostoma*, Fsu = *Ficus subintermedia*, Lsa = *Littorina saxatilis*, Lbr = *Littorina brevicula* and Cla = *Cacozeliana lacertina*.

Results

Length variation and sequence difference

The length variation of the mt16S rRNA gene sequences of the buccinids (n = 17, range = 1332–1371 nt, average = 1356.5 nt) was generally equivalent to that of the other taxa examined (n = 20, range = 1341–1397 nt, average = 1366.9 nt). Generally, closely related taxa showed similar lengths. However, this did not serve as a clear-cut diagnostic character at the subfamilial-familial level. The GC content

for all the sequences averaged 27.7%, and varied moderately across the taxa (range = 23.2–36.4%). The sequence difference (uncorrected) based on the pairwise 'global' alignment (Table 3, below the diagonal) among the taxa ranged from 3.1 % between *Kelletia kelletii* (Forbes, 1850) and *K. lischkei* Kuroda, 1938 to 35.2% between *Cacozeliana lacertina* and *Conus praecellens* (A. Adams, 1854). Smaller differences were observed among the following pairs/groups of genera: 7.7% (on average) between *Kelletia* and *Penion*, 9.9% between *Buccinum* and *Neptunea*, 9.3% between *Niotha* and *Zeuxis*, 10.5% (on average) between *Engina*, *Pisania* and *Pollia* and 12.1% (on average) between *Penion*, *Kelletia*, *Burnupena*, *Buccinulum*, *Cominella*, *Buccinum* and *Neptunea*. In a familial comparison within the Buccinidae *s.l.*, the Buccinidae, Fasciolariidae and Nassariidae showed moderate differences between one another (Buccinidae *vs*. Fasciolariidae = 17.7 %, Buccinidae *vs*. Nassariidae = 19.0% and Nassariidae *vs*. Fasciolariidae =19.5%). In contrast, the Melongenidae showed a slightly larger difference to the above three families (21.9% *vs*. Buccinidae, 22.9% vs. Fasciolariidae and 23.2% vs. Nassariidae).

Molecular phylogeny

As a result of the multiple alignment, 1,013 sites were unambiguously aligned in total. Of these, 481 sites were invariable and 422 sites were phylogenetically informative under the parsimony criterion. The profile of pairwise sequence divergence based on multiple alignment (Table 3, above the diagonal) was comparable to that based on pairwise 'global' alignment. The χ^2 test for homogeneity of the base frequencies across the taxa resulted in no significant P values (χ^2 = 95.48, d.f. = 108, P = 0.80), suggesting that compositional bias has no effect on the recovery of the phylogenetic signal.

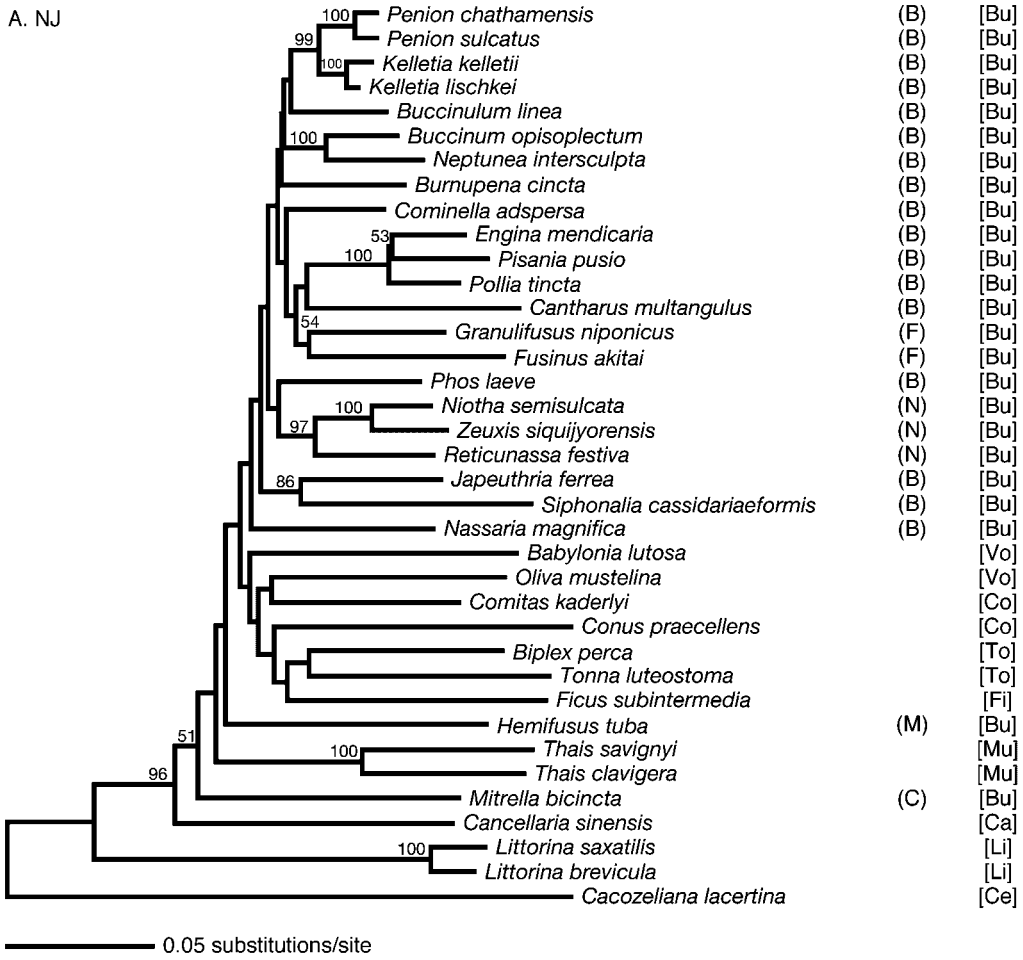
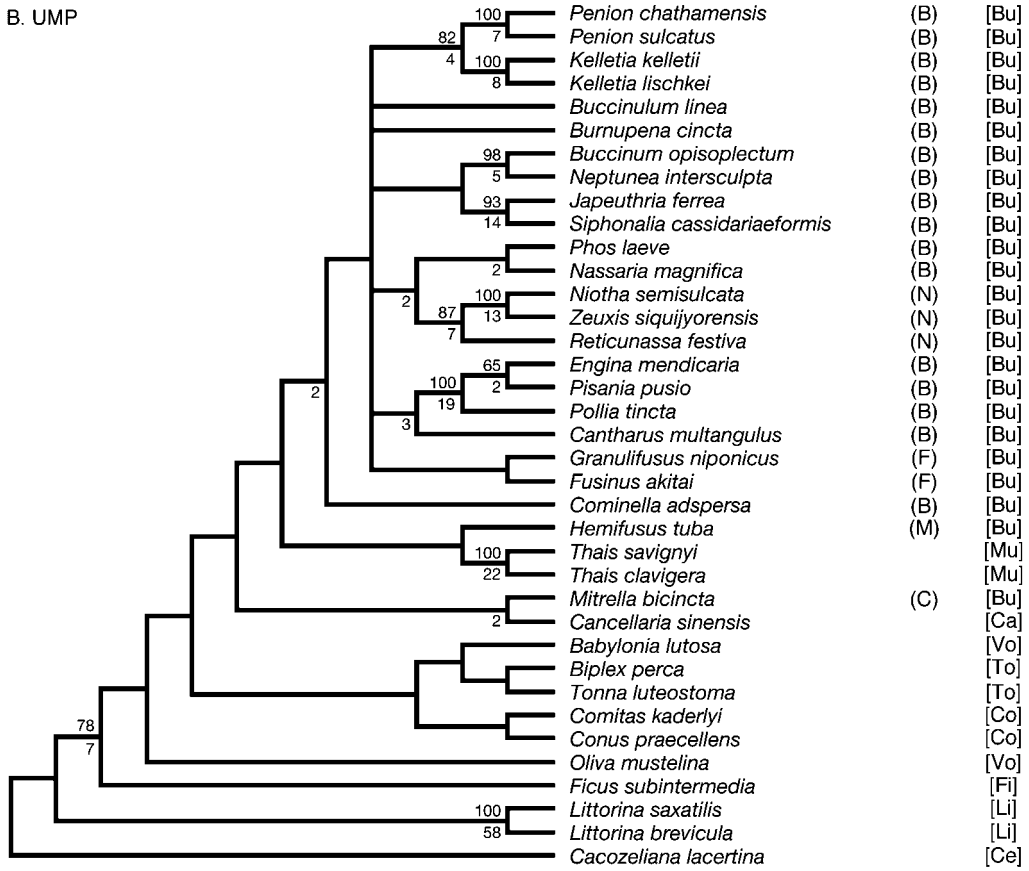


FIGURE 1. Molecular phylogenetic trees based on the 1013 nt unambiguously aligned region of the mitochondrial 16S rRNA gene. A = NJ tree, B = Strict consensus UMP tree generated from the two shortest trees (L = 2502, RI = 0.382 and CI = 0.355), C = Strict consensus WMP tree generated from the two shortest trees (L = 3449, RI = 0.401 and CI = 0.350) and D = ML tree (- ln L = 11507.20261). In the NJ and ML trees, branch lengths are scaled in terms of the estimated number of substitutions per site. Bootstrap values (in the NJ and MP trees) are indicated above the internodes only when the nodes receive more than 50% probability. Numbers below the internodes in MP trees represent decay indices and are shown only when the nodes are supported by more than one step. Characters in parentheses and brackets denote the familial and superfamilial attribution of genera, respectively (B = Buccinidae, F = Fasciolariidae, N = Nassariidae, M = Melongenidae, C = Columbellidae, Bu = Buccinoidea, Mu = Muricoidea, Vo = Volutoidea, Co = Conoidea, Ca = Cancellarioidea, To = Tonnoidea, Fi = Ficoidea, Li = Littorinoidea and Ce = Cerithioidea). Other than Buccinoidea, only the superfamilial allocations are indicated.

.....continued on the next two pages



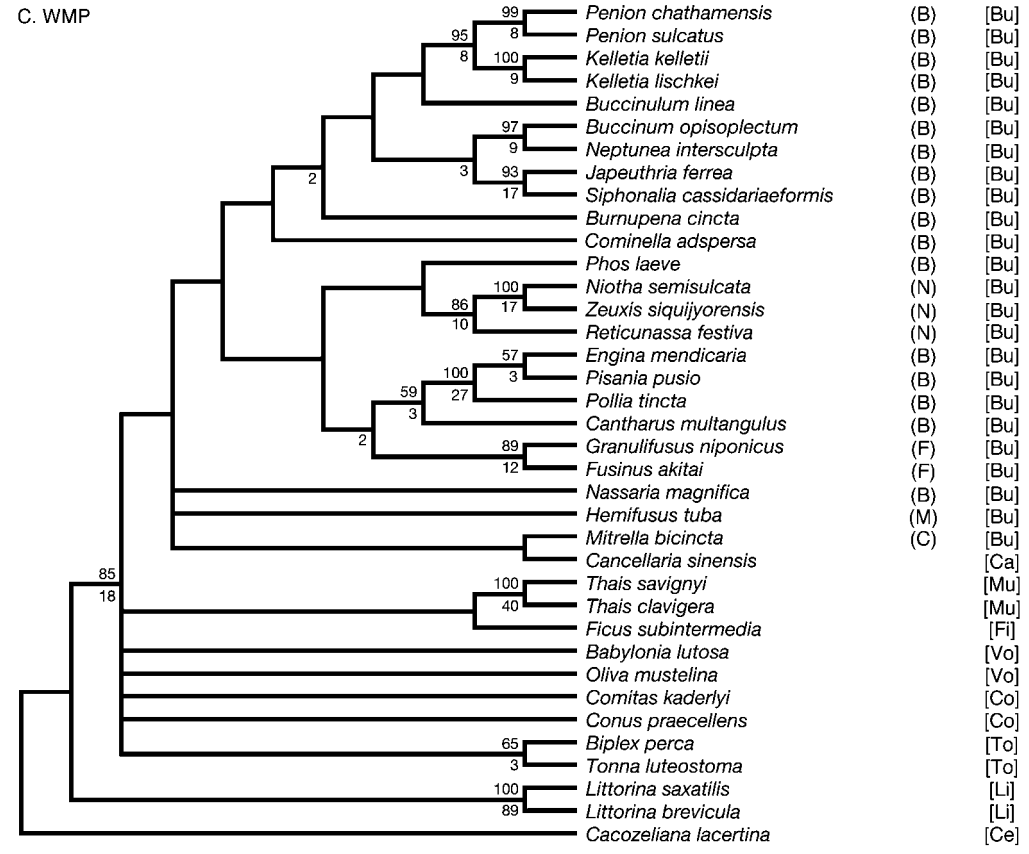


FIGURE 1 (continued)

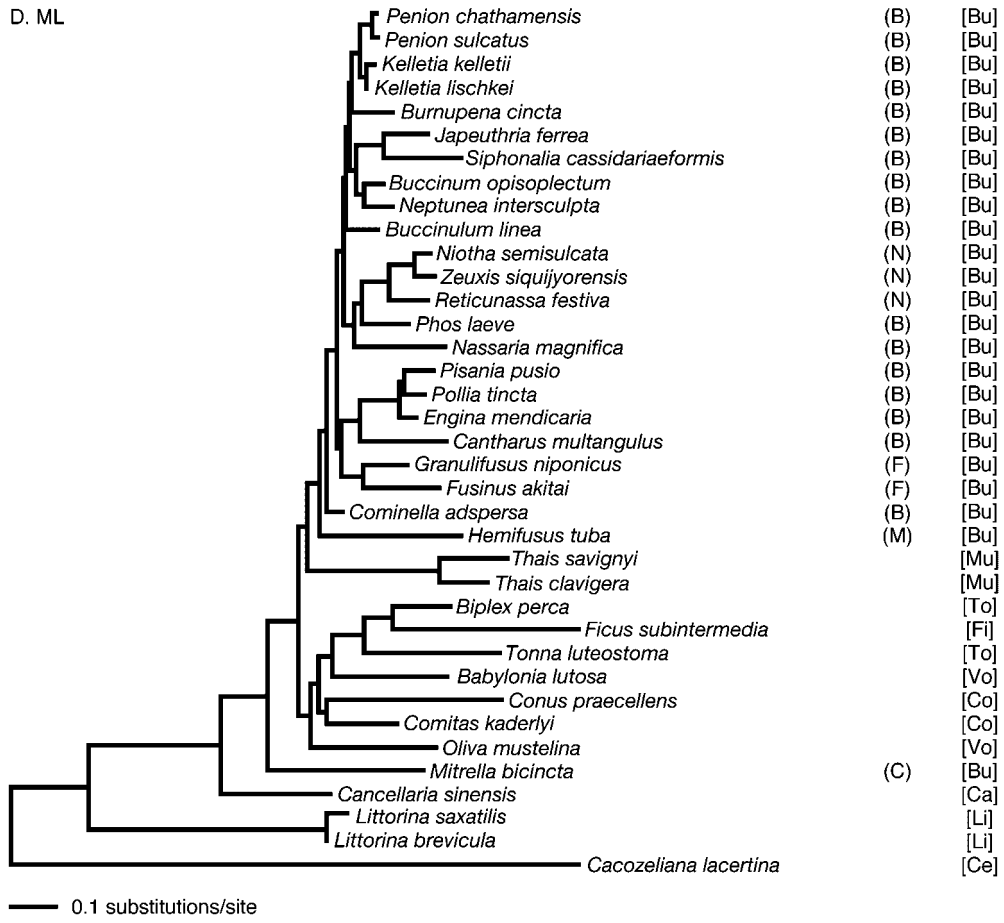


FIGURE 1 (continued)

Hierarchical likelihood ratio tests (Huelsenbeck and Crandall 1997) indicated that the TVM+I+G model with unequal base frequencies was the most appropriate model for subsequent ML analysis. Estimates of the base frequencies and substitution rates under this model were as follows: A = 0.3678, C = 0.1092, G = 0.1324, T = 0.3906, A to C = 1.6094, A to T = 1.5099, C to G = 2.0108, G to T = 1.0000 and transitions (A to G and C to T) = 15.0897. The proportion of invariant sites and the gamma distribution shape parameter were estimated as 0.3436 and 0.4518, respectively.

Fig. 1 (A–D) shows the molecular phylogenetic trees generated by the NJ, UMP, WMP and ML analyses. The putative family-level groupings *Reticunassa* + *Niotha* + *Zeuxis* (Nassariidae) and *Thais savignyi* + *Thais clavigera* (Muricidae) were supported by high bootstrap probabilities (BP) and decay indices (DI) (For the Nassariidae, BP = 97% in NJ, 87% in UMP and 86% in WMP; DI = 7 in UMP and 10 in WMP. For the Muricidae, BP = 100% in NJ, UMP and WMP; DI = 22 in UMP and 40 in WMP). In contrast, statistical support for *Granulifusus* + *Fusinus* (Fasciolariidae) was less robust (BP = 54% in NJ, less than 50 % in UMP and 89% in WMP; DI = 1 in UMP and 12 in WMP). The following buccinid clades received high bootstrap support: *Buccinum* + *Neptunea* (BP = 100% in NJ, 98% in UMP and 97% in WMP; DI = 5 in UMP and 9 in WMP), *Engina* + *Pisania* + *Pollia* (BP = 100% in NJ, UMP and WMP; DI = 19 in UMP and 27 in WMP) and *Penion* + *Kelletia* (BP = 99% in NJ, 82% in UMP and 95% in WMP; DI = 4 in UMP and 8 in WMP) . *Siphonalia* + *Japeuthria* was also recovered with a high statistical support (BP = 86% in NJ, 93% both in UMP and WMP; DI = 14 in UMP and 17 in WMP). *Babylonia* was placed remotely from the other buccinids, agreeing with the findings of Harasewych and Kantor (2002). The monophyly of the current concept of Buccinidae was violated by intercalations of nassariids and fasciolariids. In addition, most of the currently accepted restricted suprafamilial groupings were poorly resolved.

Discussion

Buccinum and *Neptunea*

Some workers have regarded *Buccinum* and *Neptunea* as belonging to distinct subfamilies (e.g., Powell 1929) based on the differences in the features of their operculum and radula, especially the former (*Buccinum* = ovate operculum with a median submarginal nucleus, *Neptunea* = leaf-shaped operculum with terminal nucleus). Anatomically, however, they are very similar and differ only by the extent of the development of the gland of Leiblein and the presence of an oesophageal caecum (Harasewych and Kantor 2002). Their

fossil records begin at approximately the same time and within the same geographic area (late Eocene–early Oligocene of the northwest Pacific, Amano 1997; Titova 1994). These lines of evidence are consistent with their robust clustering in the present analysis. Calibrating the molecular clock with the fossil record and the evolutionary rate along the lineage led to the estimate of 0.15% / million years (myr). The bootstrap value for this clade in the partial COI tree (Harasewych and Kantor 2002) was less than 50%, due, possibly, to the dearth of synapomorphic mutations or saturation in this part of the gene.

Based on similarities in radula dentition, Powell (1951) argued for the close affinity of *Buccinum* and *Burnupena* and considered that the latter genus originated in the northern hemisphere and then invaded the southern hemisphere. In the present analysis, *Burnupena* is closer to the southern ocean genera than to *Buccinum*. *Burnupena*'s fossil record is very poor and geologically recent (from the Pliocene onwards, R. N. Kilburn, personal communication). However, the split is relatively deep, based on the genetic divergence, which is almost equivalent to that between *Buccinum* and *Neptunea*. The discordance between the age rank and the clade rank may be due to the lack of a fossil record or the dearth of more closely related genera in the present analysis.

Engina + *Pisania* + *Pollia* (Pisaniinae)

The Pisaniinae comprise three morphological groups: those around the genera *Engina, Pisania* and *Cantharus* (Vermeij 2001). In this subfamily, the generic and subgeneric allocations have largely depended on shell morphology and in particular on features of the aperture (Cernohorsky 1975). In the analysis, the monophyly of *Engina* + *Pisania* + *Pollia* is well supported, but the branching order of the three is rather unstable. This clade further clusters with *Cantharus multangulus* (Philippi, 1848), however, statistical support for overall pisaniine monophyly is somewhat weak (BP = less than 50% in NJ and UMP and 59% in WMP; DI = 3 in both UMP and WMP). Although denser taxonomic sampling is required, a basal paraphyly of the *Cantharus* group with respect to the other two groups is suggested. This is consistent with the fact that Palaeogene pisaniines consist mostly of the *Cantharus* group (Vermeij 2001).

Cernohorsky (1971) referred to the possibility of a pisaniine affinity of *Phos*. However, such a relationship was not detected in the analysis. The generic assignment for pisaniine species should be assessed by multiple sequence sampling per genus, because the diagnostic features of genera (e.g., sculpture and apertural ornamentation) do not seem to be very stable.

Penion and *Kelletia*

This group exhibits higher genetic proximity than any other pair of buccinid genera, in spite of the disjunction of current distribution. It is concordant with both morphological (anatomical and shell) and paleontological evidence. Powell (1929) noted close shell and radula characters, Wenz (1943) treated *Penion* as a subgenus of *Kelletia* and Ponder (1973) stated that there are no major anatomical differences between these two taxa. The oldest fossil record of *Penion* is in the early Palaeocene of New Zealand (*Penion proavitus* (Finlay & Marwick, 1937)) and the genus probably reached South America in the late Oligocene–early Miocene (*P. subreflexus* (Sowerby, 1846) and *P. subrectus* (von Ihering, 1899)) by virtue of enhancement of the Antarctic circumpolar current (Beu *et al.* 1997). The first credible *Kelletia* in North America is *K. posoensis* (Anderson and Martin, 1914) in the early Miocene, as the Paleogene species of *Kelletia* reported in Ruth (1942) are highly dubious (L. Groves, personal communication). In the 16S tree, no significant rate difference was detected between the *Buccinum* + *Neptunea* and the *Penion* + *Kelletia* lineages. Applying the substitution rate in the *Buccinum* + *Neptunea* lineage (0.15% / myr), the divergence date of *Penion* and *Kelletia* is estimated to be about 24 Ma. An integration of paleontological and molecular evidence suggest the possibility that extant *Kelletia* may have been derived from the New Zealand *Penion via* South America, during the late Oligocene–early Miocene. Using the same substitution rate as above, the divergence date between the North American and Japanese *Kelletia* is estimated to be about 8.6 Ma. This value agrees approximately with the first record of fossil *Kelletia* in Japan (*K. brevis* (Ozaki, 1954), late Miocene, Tomida 1996), although recent examination of available fossil material seems to extend this date further back (Y. Kurihara, pers. com.). These lines of evidence suggest that the genus *Kelletia* may have had the same migration history as *Littorina, Nucella* and *Lirabuccinum*, i.e., moving from the eastern to the western Pacific (e.g., Amano and Vermeij 2003).

Buccinulinae and the 'southern ocean genera'

Ponder (1973) stated that the stomach of *Buccinulum* is very like that of *Penion*. The monophyly of Buccinulinae (*sensu* Powell 1951) was recovered in the NJ and WMP tree, although statistical support was rather weak. In the ML tree, another southern genus, *Burnupena* replaced *Buccinulum* as sister to the *Penion* + *Kelletia* clade. However, the likelihood of a constrained tree enforcing the monophyly of the Buccinulinae was nearly as good as the best tree (difference in - log likelihood (- ln L) = 2.20365 and the P value of SH test = 0.9660; Table 4). According to Cernohorsky (1971), the rachidian of juvenile *Burnupena* specimens is similar to that of *Buccinulum* and may provide evidence for a relationship between *Burnupena* and buccinulines. A detailed anatomical study of *Burnupena* could also shed light on the relationships of this genus. The constrained tree enforcing the monophyly of the Buccinulidae (*sensu* Powell 1951) was inferior to the best tree (difference in - ln L = 21.99485 and the P value of SH test = 0.3695; Table 4). However, excluding *Phos* from the latter constraint improved the likelihood score (difference in - ln L = 10.12716 and the P value of SH test = 0.6261; Table 4).

Removal of ambiguous parts of the alignments is an issue that must be faced with RNA data, even though information is clearly lost. For example, although not so robust in the phylogenetic trees, the affinity of a buccinulids + *Burnupena* + boreal taxa (*Neptunea* and *Buccinum*) was

suggested by the relatively low sequence differences and unambiguously aligned sequences for these groups resulted in about 1,300 bp lengths (data not shown). The excision of the ambiguously aligned regions, when a broad array of neogastropod taxa is aligned, results in the exclusion of synapomorphic sites for subordinate groups, such as the latter taxa. Therefore, their affinity may be obscured in a standard phylogenetic analysis.

Kantor (2003) successfully discriminated most families of Buccinoidea by stomach characters, but failed to separate the Buccinulidae from some boreal buccinids, *Colus gracilis* (da Costa, 1778) and *Siphonorbis danielsseni* (Friele, 1879). Unfortunately, these latter species were not included in this study. However, these lines of evidence, both from morphological and molecular aspects, are suggestive of a southern-boreal kinship at some level.

Remarks on other genera

Siphonalia + *Japeuthria* received fairly high bootstrap support, as shown in the results. However, preliminary analysis of the complete mitochondrial 12S rRNA gene sequences (including most of OTUs in the present analysis) did not recover their grouping, whereas all of the remnant clades supported in the 16S trees were also highly supported in the 12S trees. With the exclusion of *Siphonalia*, *Japeuthria* directly clustered with the clade *Buccinum* and *Neptunea* (BP = 55% in NJ, less than 50% in both UMP and WMP). It should be noted that the radula of *Japeuthria* has hexa-cuspid central and tricuspid lateral teeth, which are similar to those of *Burnupena* and *Buccinum* (Cernohorsky 1971). In contrast, with the exclusion of *Japeuthria*, *Siphonalia* moved to a more basal position with *Nassaria* (no significant statistical support for the clustering of these taxa). Finlay (1928) included *Siphonalia* in his Buccinulidae under the subfamily Siphonaliinae. However, no sign of their relatedness was obtained through the analyses or in the preliminary 12S trees.

The photine radula consists of tricuspid central teeth with a loop-like basal extension and bicuspid lateral teeth (Cernohorsky 1971). As far as the present data show, no affinity of the two photine genera (*Phos* and *Cominella*) was detected. *Nassaria* has a unique rachidian (rectangular, much wider than high, 7–10 cusps) for the Buccinidae, but is often assigned to the Photinae, probably due to a shared shell feature and simple bicuspid lateral teeth (Cernohorsky 1981). Anatomically, Kantor (2003) claimed that the stomach of *Nassaria* resembles that of the Nassariidae as well as that of *Clea*. In the analysis, *Phos* and nassariids formed a clade in the NJ and WMP tree. *Phos* and *Nassaria* clustered in the UMP tree as a sister group of nassariids. These two genera appeared in the ML tree as a paraphyletic grade with respect to the nassariid clade. Thus, the affinity of *Phos*, *Nassaria* and the Nassariidae were suggestive although statistical support was rather low in the present analysis.

Relationships at the interfamilial level

The Buccinidae (especially Buccinulinae) occupy a more derived position than other Neogastropoda in all the tree-making methods, supporting the view of Kantor (2002), where he stressed the advanced features of the Buccinidae. All the topologies generated by the three methodologies showed a more or less paraphyletic Buccinidae, whereas the monophyly of the Buccinidae *s. l.* was recovered in the ML tree.

Babylonia and *Oliva* did not cluster directly (exclusively) in any of the tree-making methods as demonstrated by Harasewych and Kantor (2002), however, *Babylonia* showed closer affinity to volutoidean than to other Buccinidae and a constrained tree imposing the monophyly of *Babylonia* and *Oliva* was almost as good as the best tree (difference in - ln L = 0.87165 and the P value of SH test = 0.9555; Table 4).

TABLE 4. Shimodaira-Hasegawa (SH) test for resultant and hypothesized trees.

	- ln L	differences in - ln L	P value of SH test
Resultant trees			
ML	11507.20261	(best)	
UMP	11524.16047	16.95786	0.5115
WMP	11527.12336	19.92076	0.4211
NJ	11528.28123	21.07862	0.4085
Hypothesized trees			
Buccinidae *s. s.*	11511.68723	4.48462	0.8720
Buccinoidea	11513.10886	5.90626	0.8166
Buccinulinae	11509.40625	2.20365	0.9660
Buccinulidae	11529.19746	21.99485	0.3695
Buccinulidae (without *Phos*)	11517.32976	10.12716	0.6261
Riedel	11532.61301	25.41041	0.2765
Neogastropoda	11513.14054	5.93794	0.8313
(Neogastropoda + Ficoidea) + Tonnoidea	11515.33438	8.13177	0.7801
Babylonia + *Oliva*	11508.07425	0.87165	0.9555

The monophyly of Neogastropoda was interrupted by the intervention of Tonnoidea/Ficoidea in all the phylogenies as is seen in some molecular phylogenetic trees in Riedel (2000) and Colgan *et al.* (2003). However, a ML tree enforcing the monophyly of Neogastropoda was slightly inferior to the ML tree (difference in - ln L = 5.93794 and the P value of SH test = 0.8313; Table 4). Riedel (1994, 2000) claimed that Ficoidea was closer to Neogastropoda than to Tonnoidea. A hypothesized tree incorporating that scenario was as good as the latter hypothesized tree (difference in - ln L = 8.13177 and the P value of SH test = 0.7801; Table 4). In contrast, a ML tree constrained to be consistent with cladograms in Riedel (2000) (Fig. 2) was the most inferior amongst hypothesized trees tested in the analysis (difference in - ln L = 25.41041 and the P value of SH test = 0.2765; Table 4). Judging from a relatively better likelihood score of the hypothesized tree for the relationship of ((Neogastropoda + Ficoidea) + Tonnoidea), a cluster of the Columbellidae and Fasciolariidae seems to be responsible for the lower likelihood score of Riedel's (2000) tree.

Thus, branching order was unstable at the interfamilial level and the low differences in likelihood among the alternative hypothesized trees suggest limited resolving power of the present data at this phylogenetic depth. This needs re-examination with an expanded dataset (in both the number of genes and taxonomic sampling density) to clarify the origin of the Buccinidae and the relationship among its constituent genera.

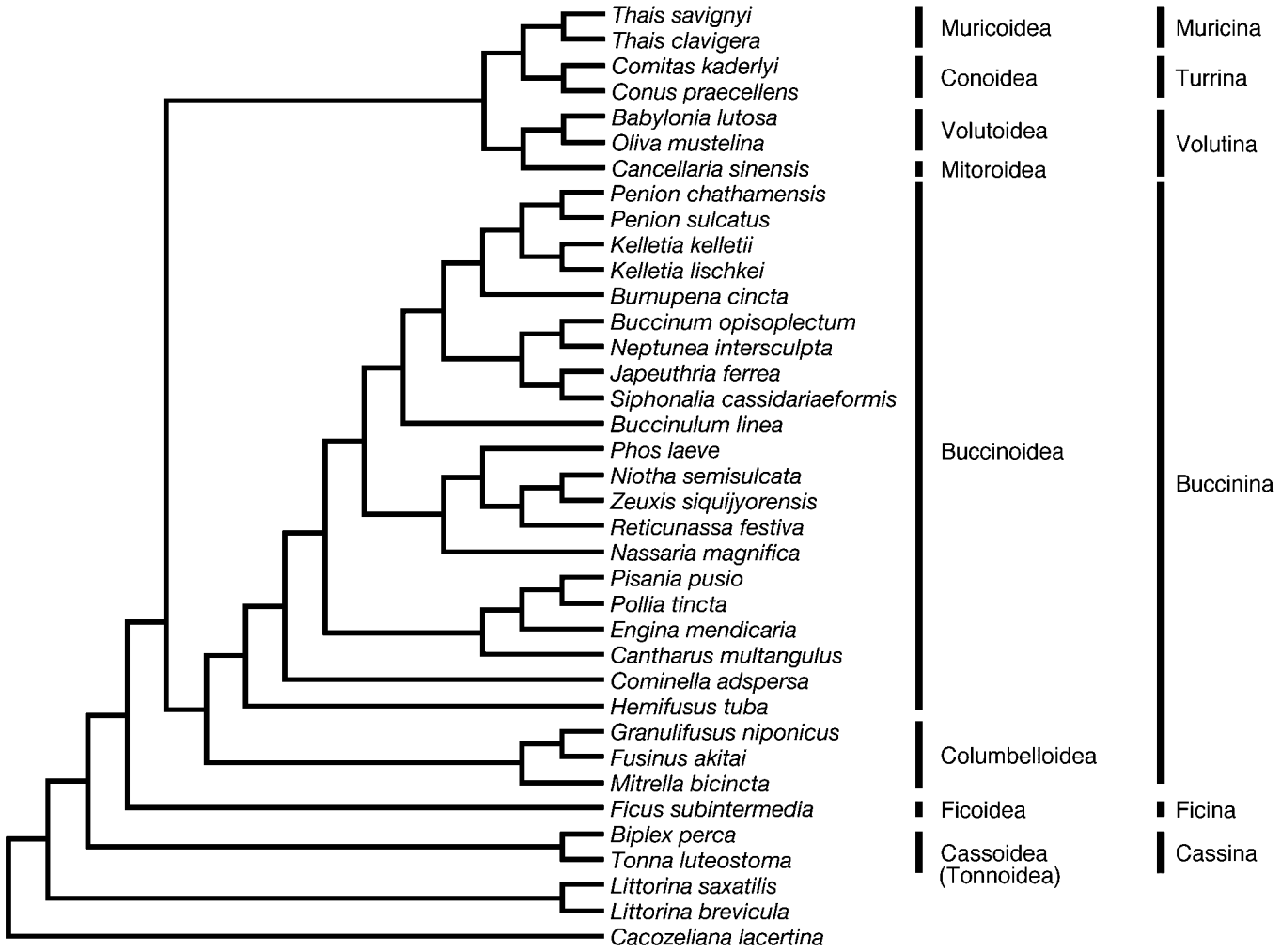


FIGURE 2. A constrained ML tree that is consistent with cladograms in Riedel (2000) with his taxonomic scheme beside the tree.

Acknowledgements

I am grateful to Seiji Nagai (Kushimoto, Wakayama, Japan), Shoichi Kimura (Miya Fishery High School), Hideyuki Kurono (Kanesu, Co., Ltd.), Yoshiki Inagaki (Isshiki, Aichi, Japan), Tsutomu Isobe (Isshiki, Aichi, Japan), Kenya Okumoto (Ohgata, Kochi, Japan), Hiroshi Nagano (Kochi, Japan), Philip Poland (Florida), Norman B. Potter (Shell Study Center, New Zealand), Brian Hayes (Port Elizabeth, South Africa) and Kathleen & Joseph Kalohi (Los Angeles) for donating the specimens used in this work. Thanks are also due to Hirofumi Kubo (Yaeyama Branch, Okinawa Prefectural Fisheries Experiment Station) for providing sampling tips on Okinawa Island and and to Hiromi and Dan Yoshimoto for offering information on buccinids of the western coast of the USA. I am greatly indebted to Geerat P. Vermeij (University of California at Davis), Richard N. Kilburn (Natal Museum, South Africa), Lindsey Groves

(Natural History Museum of Los Angeles County), Alan Beu (Institute of Geological and Nuclear Sciences, New Zealand), Yukito Kurihara (Saitama Museum of Natural History), Kazutaka Amano (Joetsu University of Education) for invaluable information on the fossil records and to Paul Callomon (Academy of Natural Science of Philadelphia) for providing relevant help with nomenclature. I would like to express my gratitude to Tomowo Ozawa (Nagoya University) for numerous discussions and valuable advice. Winston Ponder (Australian Museum) and an anonymous reviewer are thanked for their suggestions and improvements recommended to the manuscript. This work was supported by the Grant-in-aid for JSPS Fellows (No. 90002052) and for Scientific Research [Wakate B] (No. 16740292) to S.H. from the Ministry of Education, Culture, Sports, Science and Technology (MEXT) of Japan.

References

Amano, K. (1997) Biogeography of the genus *Neptunea* (Gastropoda: Buccinidae) from the Pliocene and the lower Pleistocene of the Japan Sea borderland. *Paleontological Research*, 1, 274–284.

Amano, K. & Vermeij, G.J. (2003) Evolutionary adaptation and geographic spread of the Cenozoic buccinid genus *Lirabuccinum. Journal of Paleontology*, 77, 863–872.

Bandel, K. (1993) Caenogastropoda during Mesozoic times. *Scripta Geologica Special Issue*, 2, 7–56.

Beu, A.G., Griffin, M. & Maxwell, P.A. (1997) Opening of Drake passage gateway and late Miocene to Pleistocene cooling reflected in Southern ocean molluscan dispersal: evidence from New Zealand and Argentina. *Tectonophysics*, 281, 83–97.

Boore, J.L. & Brown, W.M. (1994) Complete DNA sequence of the mitochondrial genome of the black chiton, *Katharina tunicata*. *Genetics*, 138, 422–443.

Boore, J.L. & Brown, W.M. (1995) Complete sequence of the mitochondrial DNA of the annelid worm *Lumbricus terrestris*. *Genetics*, 141, 305–319.

Cernohorsky, W.O. (1971) Indo-Pacific Pisaniinae (Mollusca: Gastropoda) and related buccinid genera. *Records of the Auckland Institute and Museum*, 8, 137–167.

Cernohorsky, W.O. (1975) Supplementary notes on the taxonomy of buccinid species of the subfamily Pisaniinae. *Records of the Auckland Institute and Museum*, 12, 175–211.

Cernohorsky, W.O. (1981) The family Buccinidae. Part 1. The genera *Nassaria*, *Trajana*, and *Neoteron*. Monographs of Marine Mollusca, No.2, 1–52.

Colgan, D.J., Ponder W.F., Becham, E. & Macaranas, J.M. (2003) Gastropod phylogeny based on six segments form four genes representing coding or non-coding and mitochondrial or nuclear DNA. *Molluscan Research*, 23, 123–148.

Felsenstein, J. (1985) Confidence limits on phylogenies: An approach using the bootstrap. *Evolution*, 39, 783–791.

Finlay, H.J. (1928) The recent mollusca of the Chatham Islands. *Transactions and Proceedings of the New Zealand Institute*, 59, 232–286.

Harasewych, M.G. (1998) Family Buccinidae. *In*: Beesley, P.L., Ross, G.J.B. & Wells, A. (Eds.), *Mollusca: The Southern Synthesis*. *Fauna of Australia* vol.5, part B, CSIRO Publishing, Melbourne, pp. 825–827.

Harasewych, M.G., Adamkewicz, S.L., Blake, J.A., Saudek, D., Spriggs, T. & Bult, C.J. (1997) Neogastropod phylogeny: A molecular perspective. *Journal of Molluscan Studies*, 63, 327–351.

Harasewych, M.G. & Kantor, Y.I. (2002) On the morphology and taxonomic position of *Babylonia* (Neogastropoda: Babyloniidae). *Bollettino Malacologico Supplemento*, 4, 19–36.

Hatzoglou, E., Rodakis, G.C. & Lecanidou, R. (1995) Complete sequence and gene organization of the mitochondrial of the land snail *Albinaria coerulea*. *Genetics*, 140, 1353–1366.

Higo, S., Callomon, P. & Goto, Y. (1999) *Catalogue and Bibliography of the Marine Shell-bearing Mollusca of Japan*. Elle Scientific Publication, Osaka, Japan, 749 pp.

Huelsenbeck, J.P. & Crandall, K.A. (1997) Phylogeny estimation and hypothesis testing using maximum likelihood. *Annual Review of Ecology and Systematics*, 28, 437–466.

Kantor, Y.I. (1996) Phylogeny and relationships of Neogastropoda. In: Taylor, J.D. (Ed.), *Origin and Evolutionary Radiation of the Mollusca*. Oxford University Press, Oxford, pp. 221–230.

Kantor, Y.I. (2002) Morphological prerequisites for understanding neogastropod phylogeny. *Bollettino Malacologico Supplemento*, 4, 161–174.

Kantor, Y.I. (2003) Comparative anatomy of the stomach of Buccinoidea (Neogastropoda). *Journal of Molluscan Studies*, 69, 203–220.

Kimura, M. (1980) A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution*, 16, 111–120.

Kuroda, T., Habe, T. & Oyama, K. (1971) *The Sea Shells of Sagami Bay*. Maruzen, Tokyo, 1297 pp.

Lydeard, C., Holznagel, W.E., Schnare, M.N. & Gutell, R.R. (2000) Phylogenetic analysis of molluscan mitochondrial LSU rDNA sequences and secondary structures. *Molecular Phylogenetics and Evolution*, 15, 83–102.

McArthur A.G. & Koop, B.F. (1999) Partial 28S rDNA sequences and the antiquity of hydrothermal vent endemic gastropods. *Molecular Phylogenetics and Evolution*, 13, 255–274.

Palumbi, S., Martin, A., Romano, S. & McMillan, W.O. (1991) *The Simple Fool's Guide to PCR Version 2.0*. Department of Zoology and Kewalo Marine Laboratory, University of Hawaii, 47 pp.

Ponder, W.F. (1973) A review of the Australian species of *Penion* Fischer (Neogastropoda: Buccinidae). *Journal of Malacological Society of Australia*, 2, 401–428.

Ponder, W.F. (1974) The origin and evolution of the Neogastropoda. *Malacologia*, 12, 295–338.

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

Ponder, W.F. & Lindberg, D.L. (1997) Towards a phylogeny of gastropod molluscs: An analysis using morphological characters. *Zoological Journal of the Linnean Society*, 119, 83–265.

Posada, D. & Crandall, K.A. (1998) Modeltest: testing the model of DNA substitution. *Bioinformatics*, 14, 817–818.

Powell, A.W.B. (1929) The recent and Tertiary species of the genus *Buccinulum* in New Zealand, with a review of related genera. *Transactions and Proceedings of the New Zealand Institute*, 60, 57–101.

Powell, A.W.B. (1951) Antarctic and subantarctic mollusca Pelecypoda and Gastropoda. *Discovery Reports*, 26, 47–196.

Riedel, F. (1994) Recognition of the superfamily Ficoidea Meek, 1864 and definition of the Thalassocynidae fam. nov. (Gastropoda). *Zoologische Jahrbücher Abteilung für Systematik, Ökologie und Geographie der Tiere*, 121, 457–474.

Riedel, F. (2000) Ursprung und evolution der “höheren” Caenogastropoda. *Berliner Geowissenschaftliche Abhhandlungen Reihe E: Paläobiologie*, 32, 1–240.

Ruth, J.W. (1942) The molluscan genus *Siphonalia* of the Pacific coast Tertiary. *University of California Publications in*

geological sciences, 26, 287–305.

Saitou, N. & Nei, M. (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution*, 4, 406–425.

Sambrook, J., Fritsch, E.F. & Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual*. *vol. 2*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, pp. 9.16–9.23.

Shimodaira, H. & Hasegawa, M. (1999) Multiple comparisons of log-likelihoods with applications to phylogenetic inference. *Molecular Biology and Evolution*, 16, 1114–1116.

Sorenson, M.D. (1999) TreeRot, version 2. Available on line at http://people.bu.edu/msoren/TreeRot.html. [Accessed on 31 October 2002.]

Swofford, D.L. (2002) PAUP*. Phylogenetic Analysis Using Parsimony (* and Other Methods). Version 4.0b10. Sinauer, Sunderland, MA.

Takumi, T., Fujiwake, H. & Kurachi, Y. (1997) A dye terminator method for automated DNA sequencing using four fluorescent dideoxynucleosides and thermal cycling. *Analytical Science*, 13, 735–739.

Terrett, J.A., Miles, S. & Thomas, R.H. (1996) Complete DNA sequence of the mitochondrial genome of *Cepaea nemoralis* (Gastropoda: Plumonata). *Journal of Molecular Evolution*, 42, 160–168.

Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F. & Higgins, D.G. (1997) The CLUSTAL X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research*, 25, 4876–4882.

Tillier, S., Masselot, M., Philippe, H. & Tillier, A. (1992) Phylogénie moléculaire des Gastropoda (Mollusca) fondée sur le séquençage partiel de l'ARN ribosomique 28S. *Comptes Rendus Academie de Sciences Série III*, 314, 79–85.

Titova, L.V. (1994) Cenozoic history of Turritelloida and Buccinoidea (Mollusca: Gastropoda) in the North Pacific. *Palaeogeography, Palaeoclimatology, Palaeoecology*, 108, 319–334.

Tomida, S. (1996) Late Neogene molluscan faunas from the South Fossa-Magna region. *Bulletin of Mizunami Fossil Museum*, 23, 89–140.

Tracey, S., Todd, J.A. & Erwin, D.H. (1993) Mollusca: Gastropoda. In: Benton, M.J. (Ed.), *The Fossil Record 2*. Chapman and Hall, London, pp. 131–167.

Vaught, K.C. (1989) *A Classification of Living Mollusca*. American Malacologists, Inc., Melbourne, FL, 195 pp.

Vermeij, G.J. (2001) Taxonomy, distribution, and characters of Pre-Oligocene members of the *Cantharus* group of Pisaniinae (Neogastropoda: Buccinoidea). *Journal of Paleontology*, 75, 295–309.

Wenz, W. (1943) Familia Buccinidae. In: Schindewolf, O.H. (Ed.), *Gastropoda Teil 1: Allgemeiner Teil und Prosobranchia*, *Handbuch der Paläozoologie*. *Band 6*, Borntraeger, Berlin, pp. 1151–1210.

Wilding, C.S., Mill, P.J. & Grahame, J. (1999) Partial sequence of the mitochondrial genome of *Littorina saxatilis*: relevance to gastropod phylogenetics. *Journal of Molecular Evolution* 48, 348–359.

Winnepenninckx, B., Steiner, G., Backeljau, T. & De Wachter, R. (1998) Details of gastropod phylogeny inferred from 18S rRNA sequences. *Molecular Phylogenetics and Evolution*, 9, 55–63.