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NEOGASTROPOD PHYLOGENY: A MOLECULAR PERSPECTIVE

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ABSTRACT

The origin and evolution of the gastropod order Neogastropoda was investigated using an iterative, two gene (18S rDNA and cytochrome *c* oxidase I) approach to phylogeny reconstruction. Partial sequences spanning approximately 450 base pairs near the 5′ end of the 18S rDNA gene confirmed the monophyly of Apogastropoda and its two subclades, the Caenogastropoda (including Neogastropoda and Architaenioglossa) and the Heterobranchia, but were incapable of resolving relationships among neogastropod families, or between Neogastropoda and higher Caenogastropoda. The monophyly of Heterobranchia is additionally supported by the presence within this group of a large insert of variable length in the 18S rDNA gene in the region corresponding to the E-10-1 helix of the RNA molecule. Cytochrome *c* oxidase I sequences were able to resolve fully the relationships among representatives of ten families of Neogastropoda. Maximum parsimony, maximum likelihood and neighbor-joining analyses of these data all revealed that Buccinoidea and Muricoidea [*sensu* Thiele, 1929] each represent a clade, while the families assigned by Thiele and some subsequent authors to the superfamily Volutoidea comprise a grade. Although the two toxoglossan taxa included in our study emerged as a grade rather than a clade, denser taxonomic sampling of this group will be undertaken to investigate further the paraphyly of Conoidea. Based on percent transversions at third codon positions of the CO I gene, differences among neogastropod families as well as those between the neogastropod families and *Cerithium* are comparable to genetic differences between orders of mammals, but are only slightly greater than differences between genera of penaeid shrimp.

INTRODUCTION

The order Neogastropoda encompasses the descendants of a rapid and extremely successful adaptive radiation of predatory marine gastropods that formed part of a major reorganization of benthic marine faunas termed 'the Mesozoic Marine Revolution' by Vermeij (1977). Neogastropods appear abruptly in the fossil record, with approximately one fourth of the 20+ presently recognized families represented in Albian (100 mya) strata and nearly all of the remaining families established in essentially modern form before the end of the Cretaceous (65 mya) (Taylor, Morris & Taylor, 1980; Taylor, Cleevely & Morris, 1983). Because of their distinctive shell morphologies, which generally include an elongated siphonal canal, even the earliest authors were able to segregate many of the component higher taxa of Neogastropoda (*e.g.* Linné, 1757; Rafinesque, 1815; Swainson, 1840; Gray, 1853). By the mid-nineteenth century, two major groups, the Rachiglossa (Gray, 1853) and the Toxoglossa (Troschel, 1847), were recognized on the basis of radular morphology. These were later united into the Stenoglossa (Bouvier, 1887), an earlier name for Neogastropoda (Wenz, 1943). Additional data accumulated from a variety of sources, including anatomical characters (Summarized by Ponder, 1974; Kantor, 1996), chromosome numbers (Patterson, 1969), and cellular DNA content (Hinegardner, 1974), have provided further support for the monophyly of Neogastropoda and/or its rachiglossan and toxoglossan subclades. However, the relationship of Neogastropoda to other gastropods, and especially the relationships among higher taxa included within Neogastropoda have proven to be more difficult to discern.

Many publications still in use incorporate classifications of neogastropods that were proposed by Thiele (1929) and slightly modified by Wenz (1943). These authors divided the order Neogastropoda into four superfamilies, the Muricoidea, the Buccinoidea, the Volu-

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toidea (including Cancellariidae), and the Conoidea (=Toxoglossa) (Fig. 2A). Knight, Batten & Yochelson (1954) proposed the independent derivations of the Muricoidea, Buccinoidea and the extinct Nerineioidea from Jurassic Subulitidae, and hypothesized that the Volutoidea and Conoidea subsequently evolved from the Nerineioidea (Fig. 1A).

In a detailed comparative anatomical study of the Neogastropoda, Ponder (1974) suggested that this group evolved from the Archeogastropoda or a very primitive caenogastropod type rather than from the higher, proboscidiferous caenogastropods as had been believed previously (*e.g.* Amaudrut, 1898; Bouvier, 1887; Graham, 1941). Based on the morphology of the anterior alimentary system, Ponder divided the order Neogastropoda into three groups, comprising the superfamilies Muricoidea [Muricoidea *sensu* Ponder = Muricoidea + Buccinoidea + Volutoidea—Cancellariidae *sensu* Thiele (1929) and Wenz (1943) = Rachiglossa Gray (1853)], Cancellarioidea and Conoidea. Ponder further concluded that, apart from the families previously included in Buccinoidea and the Marginellidae + Volutomitridae, all the rachiglossan families evolved from a common ancestor more or less simultaneously, and that there were no natural higher groupings (Fig. 2B). Other authors (Sheridan, Van Mol & Bouillon, 1973; Golikov & Starabogatov, 1975; Shimek & Kohn, 1981) questioned the monophyly of Neogastropoda and hypothesized an independent origin for the Conoidea. Taylor and Morris (1988) reviewed the anatomical data and surmised the Conoidea were the sister taxon of the Rachiglossa, but regarded the exact relationships of the Cancellarioidea to be less certain (Fig. 1B). These authors further concluded

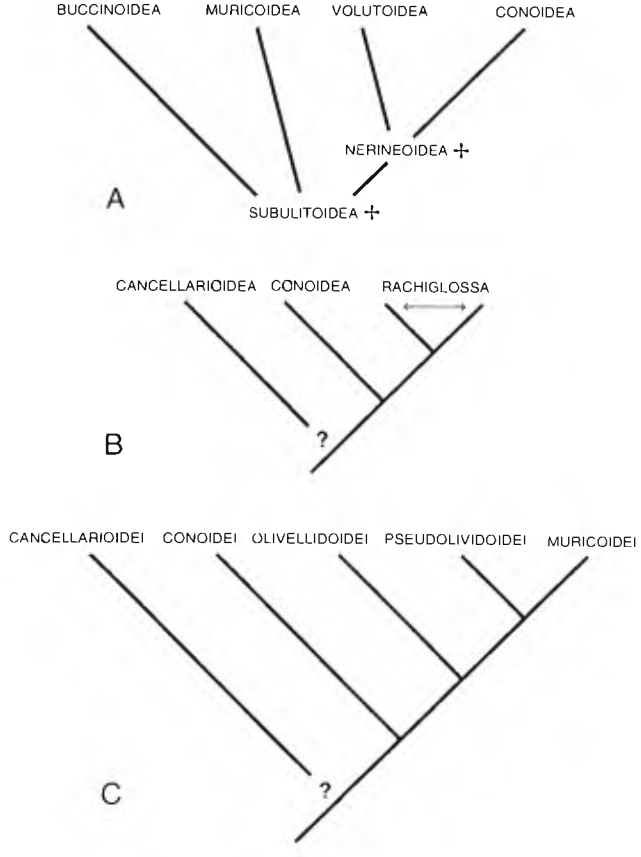

Figure 1. Morphology-based phylogenetic hypotheses of relationships among neogastropod suprafamilial taxa (with nomenclature updated in some cases). **A.** Knight *et al.*, 1954. Taxa followed by crosses (+) indicate extinct, Mesozoic ancestors. **B.** Taylor & Morris, 1988: fig. 6. **C.** Kantor, 1996: fig. 2.

that present evidence favors the derivation of Neogastropoda from higher caenogastropods and suggested the mesozoic Purpurinidae or Columbellinidae as possible ancestors.

Incorporating anatomical data published since Ponder's classic work, Kantor (1966) re-evaluated relationships among neogastropod taxa. He concluded that the order Neogastropoda was monophyletic, and elevated the superfamilies recognized by Ponder to subordinal status (Cancellarioidei, Connoidei, and Muricoidei), while adding the suborders Olivellidoidei and Pseudolividoidei (Fig. 1C). Kantor (1996) also published both cladistic (Fig. 2C) and intuitive (Fig. 2D) phylogenetic hypotheses of relationships within Muricoidei. Most recently, Ponder and Lindberg (1997) proposed a phylogeny of the Gastropoda in which Buccinidae appear as sister group to the remaining neogastropods in their study [Conoidea + Muricidae].

To date, relatively few neogastropods have appeared in DNA/RNA sequence-based phylogenies (Tillier, Masselot, Hevré & Tillier, 1992; Tillier, Masselot, Guerdoux & Tillier, 1994; Rosenberg, Kuncio, Davis &

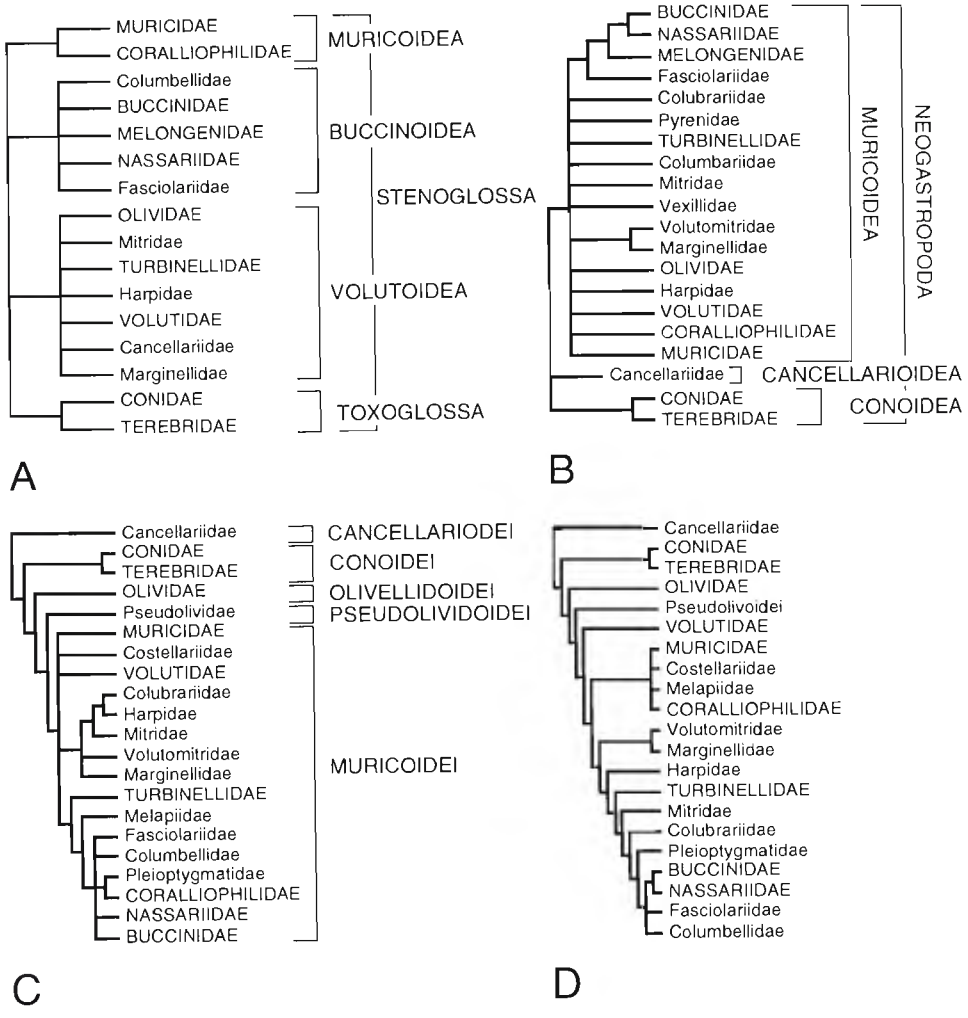

Figure 2. Morphology-based hypotheses of phylogenetic relationships among the families of Neogastropoda. Taxa listed in CAPITALS are represented in the present study (see Table 1). **A.** Based on classification of Thiele, 1929. **B.** Based on Ponder, 1974: fig. 4. **C.** Composite of Kantor, 1996: figs. 2, 4, 6 (cladistic analysis). **D.** Composite of Kantor, 1996: figs. 2, 4, 7 (intuitive tree).

Harasewych, 1992; Rosenberg, Tillier, Tillier, Kuncio, Hanlon, Masselot & Williams, 1997; Harasewych, Adamkewicz, Blake, Saudek, Spriggs & Bult, 1997). While sequence data have invariably placed neogastropods within a monophyletic Caenogastropoda, only twice have neogastropods been resolved as a separate clade (Rosenberg *et al.*, 1992, 1997).

We had earlier provided support for the monophyly of Apogastropoda (*sensu* Ponder & Lindberg, 1997) and its component clades Caenogastropoda and Heterobranchia using partial sequences of the 18S rDNA gene (Harasewych *et al.*, 1997). The primary objectives of the present paper are to discern the major features of neogastropod evolution using sequences derived from the 18S rDNA and cytochrome *c* oxidase subunit I genes, and to compare these results with the prior, morphology-based hypotheses of neogastropod phylogeny.

MATERIALS AND METHODS

All the taxa used in this study were freshly collected, frozen while living, transported to the laboratory on dry ice, and maintained at −80°C until DNA was extracted. A listing of taxa, collection localities, tissues extracted, and voucher specimen information is provided in Table 1. Protocols for DNA extraction, PCR amplification, DNA sequencing and data management, and multiple sequence alignment are identical to those reported in Harasewych *et al.* (1997), as are the primers used for sequencing the 18S rDNA and cytochrome *c* oxidase I gene regions. Individual sequences were submitted to the public sequence database via GenBank and are accessible through any of the standard databases (GSDB, GenBank, EMBL, Entrez, DataBank of Japan, etc.). GenBank sequence accession numbers are provided in Table 1. Aligned sequences are available in nexus format from the corresponding author.

Phylogenetic analyses were conducted using PAUP 4.0.0d40 (Swofford, 1996, ppc beta test version) on a Power Macintosh 6100/66. All characters were treated as unordered. Autapomorphic insertions (present in a single taxon) and regions of uncertain homology, including large insertions of varying length not present in all taxa (Fig. 3A, b), were excluded from the 18S rDNA data set prior to maximum parsimony analysis using the branch and bound search option. Bootstrap and Jackknife analyses (1000 replicates) were performed using the 'fast' step-wise addition option.

Cytochrome *c* oxidase I sequences of 16 neogastropods and *Cerithium* (as outgroup) were aligned using the 'protein assisted' feature of Sutton's multiple sequence alignment algorithm (MSA) (Bult, Adams, White, Sutton, Clayton, Kerlavage, Fields & Venter, 1997) and analyzed using the branch and bound option for determining the most parsimonious trees. The two-parameter model variant for unequal base frequencies (Hasegawa, Kishino & Yano, 1985) was used to determine the maximum likelihood tree using a heuristic search, while Jukes-Cantor distances were used to calculate a tree using the neighbor joining algorithm. Exploratory analyses were also conducted using amino acid sequences, first and second codon positions only, transversions only, and combined CO I and 18S rDNA data sets. These resulted either in poorer resolution (amino acid sequences), or inconsistent groupings of taxa that varied widely depending on data set and tree building algorithm, and are not reported here. Pairwise comparisons of sequences were calculated using MEGA (Kumar, Tamura & Nei, 1993), while the effects of alterations in tree topology on tree length and character indices were determined using MacClade 3.05 (Maddison & Maddison, 1992).

RESULTS

A region spanning approximately 450 base pairs (bp) from near the 5′ end of the 18S rDNA gene was sequenced for 1 architaenioglossan, 15 neogastropod, and 2 heterobranch taxa and aligned against sequences from 3 vetigastropod, 3 neotaenioglossan, 2 neogastropod, and 5 heterobranch taxa from Harasewych *et al.* (1997) and 2 additional heterobranch taxa from GenBank (see Table 1). The portion of the gene utilized in this study corresponds to positions 60 to 515 of the 18S rRNA sequence of *Onchidella celtica* (Cuvier, 1817) as published by Winnepenninckx, Backeljau & De Wachter (1994:101). The length of the gene between these two positions varies among the included taxa, ranging from 430 bp in *Xenophora* to 496 bp in *Rissoella*. The alignment containing the 33 taxa included in this study spans 516 positions (Appendix 1). Most of the length variation is accounted for by two regions of insertions, one near the terminal loop of helix 10 (aligned positions 137-151, Fig. 3 A, b i1), the other near the distal region of helix E-10-1 (aligned positions 193-254, Fig. 3 A, b i2). *Cipangopaludina* is the only taxon in the present study to contain an insertion that spans the first region, while each vetigastropod contains a smaller insertion in this region that is of questionable homology to the *Cipangopaludina* insert. The second and larger region contains insertions of variable length. While vetigastropods and caenogastropods contain 4–8 bp in this region, all heterobranchs contain a 21–61 bp insert. Only the large (61 bp) insert of *Rissoella*

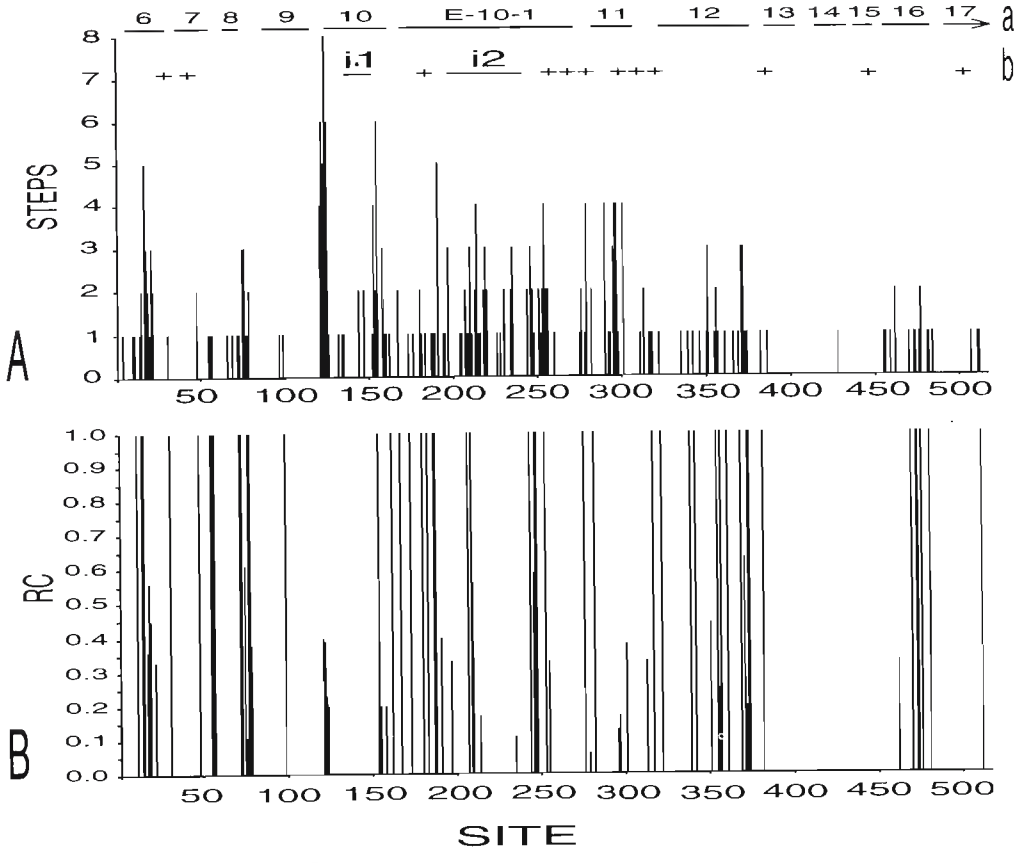

Figure 3. Variation in sites along the portion of the 18S rDNA gene among the 33 taxa in this study (Table 1). **A.** Distribution of variable sites. **a.** Positions of helices (based on Winnepenninckx *et al.*, 1992: fig. 1). **b.** Positions of bases excluded from phylogenetic analyses (+) due to uncertain homology or because they were autapomorphic insertions. **i1** = first large insert (*Cipangopaludina*) **i2** = second large insert (Heterobranchia). **B.** Rescaled consistency indices of variable sites.

includes regions that align both with the small, caenogastropod region and the larger insert of the remaining heterobranchs.

These two large insertion regions were excluded from phylogenetic analyses, as were six autapomorphic, single-nucleotide insertions (aligned positions 25, 176, 298, 387, 442, 507) and two regions (aligned positions 251–253, 296–297) that could not be reliably aligned (Fig. 3 A, b). Of the remaining 443 alignable sites, 327 were constant and 40 were parsimony uninformative. Maximum parsimony analyses of the 76 informative sites yielded 1096 equally parsimonious trees of length 214 (ci = 0.696; ri = 0.821; rc = 0.572). The strict consensus of these trees is shown in Figure 4. Results of bootstrap and jackknife analyses (1000 replicates) appear above and below the branches of the consensus tree. Nodes marked by an asterisk (*) were not supported in a 50% majority rule bootstrap/jackknife consensus tree.

Comparisons of the 443 reliably aligned positions between pairs of taxa are provided in Table 2, with the total number of nucleotide differences tabulated above the diagonal and the number of transversions below the diagonal. This table shows that the divergence rates (measured both in terms of total number of nucleotide differences as well as in terms of transversions) between Vetigastropoda and Caenogastropoda are comparable to those between Vetigastropoda and Heterobranchia. However rates within Caenogastropoda (total = 0–18, transversions only = 0–11) are approximately half those within Heterobranchia (total = 6–37, transversions only = 0–25).

Table 1. Locality data, tissue extracted, voucher specimen information, and sequence accession numbers for taxa used in this study. EMBL = European Molecular Biology Laboratory Data Library; GB = GenBank; GSDB = Genome Sequence Data Bank; USNM = Mollusk collection, National Museum of Natural History, Smithsonian Institution. † = data from Harasewych *et al.* 1997.

TAXON	Collection Locality	Tissue	Voucher material	Sequence Accession Number 18S	Number CO I
VESTIGASTROPODA					
FISSURELLOIDEA					
Diodora cayenensis (Lamarck, 1822)	Sebastian Inlet, FL, USA	Buccal muscle	USNM 888660	†GSDB L78884	
HALIOTOIDEA					
Haliotis rufescens (Swainson, 1822)	Bamfield, B.C., Canada	Buccal muscle	USNM 888642	†GSDB L78885	
TROCHOIDEA					
Astraea caelata (Gmelin, 1791)	Berry Islands, Bahamas	Buccal muscle	USNM 888603	†GSDB L78886	
CAENOGASTROPODA					
ARCHITAENIOGLOSSA					
AMPULLARIOIDEA					
Cipangopaludina japonica (von Martens, 1860)	Yao, Osaka, Japan	Brooded juvenile	USNM 888674	GB U86304	
NEOTAENIOGLOSSA					
CERITHIOIDEA					
Cerithium atratum (Born, 1778)	Sebastian Inlet, FL, USA	Buccal muscle	USNM 888663	†GSDB L78895	†GSDB L78907
XENOPHOROIDEA					
Xenophora exutum Reeve, 1843	Minabe, Japan	Buccal muscle	USNM 888631	†GSDB L78896	
TONNOIDEA					
Fusitriton oregonense (Redfield, 1848)	Dutch Harbor, AK, USA	Buccal muscle	USNM 888634	†GSDB L78897	
NEOGASTROPODA					
BUCCINOIDEA					
Illyanassa obsoleta (Say, 1822)	Cape Henlopen, DE, USA	Buccal muscle	USNM 888636	GB U86305	GB U86322
Busycon carica (Gmelin, 1791)	Cape Henlopen, DE, USA	Buccal muscle	USNM 888705	GB U86306	GB U86323
Busycon sinistrum Hollister, 1958	Ft. Pierce, FL, USA	Buccal muscle	USNM 888706	GB U86307	GB U86324
Busycotypus canaliculatus Linné, 1758	Cape Henlopen, DE, USA	Buccal muscle	USNM 888632	GB U86308	GB U86325
Neptunea polycostata Scarlatto, 1952	Hokaido, Japan	Buccal muscle	USNM 888625	GB U86309	GB U86326
Buccinum oedematum Dall, 1907	Dutch Harbor, AK, USA	Buccal muscle	USNM 888635	GB U86310	GB U86327

MURICOIDEA					
Phylonotus pomum (Gmelin, 1791)	Berry Islands, Bahamas	Buccal muscle	USNM 888601	GB U86311	GB U86328
Murex troscheli Lischke, 1868	Minabe, Japan	Buccal muscle	USNM 888707	GB U86312	GB U86329
Siratus beauii (Fischer & Bernardi, 1857)	Guadaloupe	Buccal muscle	USNM 888708	GB U86313	
Coralliophila abbreviata (Lamarck, 1816)	Berry Islands, Bahamas	Buccal muscle	USNM 888603	GB U86314	GB U86331
Thais haemastomoa canaliculata (Gray, 1839)	Pensacola, FL, USA	Buccal muscle	USNM 888709	GB U86315	GB U86330
'VOLUTOIDEA'					
Turbinella angulata (Lightfoot, 1786)	Berry Islands, Bahamas	Buccal muscle	USNM 888602	GB U86316	GB U86332
Oliva sayana Ravenel, 1834	Ft. Pierce, FL, USA	Buccal muscle	USNM 888605	†GSDB L78898	GB U86333
Arctomelon stearnsii (Dall, 1872)	Unalaska Island, AK, USA	Buccal muscle	USNM 888633	GB U86317	GB U86334
Scaphella junonia (Lamarck, 1804)	St. Petersburg, FL, USA	Buccal muscle	USNM 888615	GB U86318	GB U86335
CONOIDEA					
Hastula cinerea (Born, 1778)	Ft. Pierce, FL, USA	Buccal muscle	USNM 888611	†GSDB L78899	GB U86336
Conus floridanus Gabb, 1868	Sanibel Island, FL, USA	Buccal muscle	USNM 888639	GB U86319	GB U86337
HETEROBRANCHIA					
HETEROSTROPHA					
RISSOELLOIDEA					
Rissoella caribea Rehder, 1943	Fiesta Key, FL, USA	Whole animal	USNM 881221	GB U986320	
PYRAMIDELLOIDEA					
Fargoa bushiana Bartsch, 1909	Sebastian Inlet, FL, USA	Whole animal	USNM 888638	†GSDB L78900	
EUTHYNEURA					
OPISTHOBRANCHIA					
Haminoea antillarum (d'Orbigny, 1841)	Ft. Pierce, FL, USA	Buccal muscle	USNM 888664	†GSDB L78901	
Aplysia dactylomela Rang, 1828	Minabe, Japan	Buccal muscle	USNM 888624	†GSDB L78902	
PULMONATA					
Siphonaria pectinata (Linné, 1758)	Sebastian Inlet, FL, USA	Buccal muscle	USNM 888707	GB U86321	
Onchidella celtica (Cuvier, 1817)	Genbank ex Winnepenninckx *et al.*, 1994		EMBL X70211		
Physa heterostropha Say, 1822	Ithaca, NY, USA	Buccal muscle	USNM 888613	†GSDB L78905	
Limicolaria kambuel (Bruguière, 1792)	Genbank ex Winnepenninckx *et al.*, 1992		EMBL X66374		
Limax maximus Linné, 1758	Silver Spring, MD, USA	Buccal muscle	USNM 888604	†GSDB L78906	

Table 2. Number of nucleotide differences between taxa in the portion of the 18S rDNA gene (443 unambiguously aligned positions) used to construct the maximum parsimony trees reported in Figure 4. Total number of nucleotide differences above the diagonal, transversions only below the diagonal.

Apl = *Aplysia*, Arc = *Arctomelon*, Ast = *Astraea*, Bca = *Busycon carica*, Bsi = *Busycon sinistrum*, Buc = *Buccinum*, Bus = *Busycotypus*, Cer = *Cerithium*, Cip = *Cipangopaludina*, Con = *Conus*, Cor = *Coralliophila*, Dio = *Diodora*, Far = *Fargoa*, Fus = *Fusitriton*, Hal = *Haliotis*, Ham = *Haminaea*, Has = *Hastula*, Ily = *Ilyanassa*, Lim = *Limicolaria*, Lmx = *Limax*, Mur = *Murex*, Nep = *Neptunea*, Oli = *Oliva*, Onc = *Onchidella*, Psa = *Physa*, Phy = *Phylonotus*, Ris = *Rissoella*, Sca = *Scaphella*, Sip = *Siphonaria*, Sir = *Siratus*, Tha = *Thais*, Tur = *Turbinella*, Xen = *Xenophora*.

Taxa	Dio	Hal	Ast	Cip	Cer	Xen	Fus	Bca	Bsi	Bus	Nep	Buc	Ily	Tur	Arc	Oli	Sca	Phy	Mur	Sir	Tha	Cor	Con	Has	Ris	Far	Ham	Apl	Onc	Sip	Psa	Lim	Lmx
Dio	–	19	20	58	58	52	54	56	54	56	54	55	55	53	54	54	56	52	52	53	54	54	53	53	67	56	56	55	52	54	52	61	57
Hal	8	–	6	48	52	47	48	51	49	51	48	48	50	49	49	49	51	50	50	51	49	49	50	48	59	50	49	49	47	48	47	54	50
Ast	7	1	–	48	48	44	46	49	47	49	46	46	48	47	47	47	48	48	49	49	47	47	48	46	61	50	49	47	45	47	44	52	49
Cip	27	22	21	–	14	14	14	16	14	16	13	14	15	14	17	15	18	16	17	15	16	13	16	13	50	34	35	37	36	36	38	38	35
Cer	25	24	23	8	–	14	10	14	13	14	14	12	13	12	16	13	11	17	18	16	13	13	15	12	50	33	34	35	32	35	33	35	33
Xen	22	20	19	8	9	–	4	5	5	5	5	5	4	3	8	4	7	9	10	10	5	5	7	4	47	26	29	30	29	31	30	30	27
Fus	21	20	19	7	7	3	–	4	4	4	4	2	3	2	7	3	4	9	10	10	4	5	6	4	47	26	29	30	29	31	30	30	27
Bca	22	21	20	8	6	3	1	–	2	0	5	4	3	2	7	3	7	9	10	10	4	4	6	3	49	25	28	29	28	30	29	29	26
Bsi	22	21	20	8	6	3	1	0	–	2	5	4	3	2	5	3	7	7	8	8	4	2	4	1	48	25	28	29	28	30	29	29	26
Bus	22	21	20	8	6	3	1	0	0	–	5	4	3	2	7	3	7	9	10	10	4	4	6	3	49	25	28	29	28	30	29	29	26
Nep	23	20	19	7	9	5	2	3	3	3	–	3	4	3	8	4	7	8	9	9	5	5	7	4	47	24	26	28	26	28	28	29	26
Buc	23	20	19	7	7	4	2	1	1	1	2	–	3	2	7	3	5	7	8	8	4	4	6	3	47	26	26	29	28	30	30	30	27
Ily	23	22	21	9	7	4	2	1	1	1	4	2	–	1	6	2	6	7	8	8	2	3	5	2	48	25	28	29	27	30	28	29	26
Tur	21	21	20	8	6	3	1	0	0	0	3	1	1	–	5	1	5	7	8	8	2	2	4	1	47	24	27	28	27	29	28	28	25
Arc	23	22	21	11	9	6	4	3	3	3	6	4	4	3	–	4	10	10	11	11	5	5	7	4	51	28	29	28	28	30	30	30	27
Oli	22	21	20	8	6	3	1	0	0	0	3	1	1	0	3	–	6	8	9	9	1	3	5	2	48	25	28	27	26	28	28	27	24
Sca	24	23	22	10	8	5	3	2	2	2	5	3	3	2	5	2	–	12	13	13	7	7	8	6	49	28	29	30	28	31	28	30	28
Phy	22	22	21	9	10	7	6	5	5	5	6	4	5	5	8	5	7	–	1	1	7	5	9	6	50	29	30	32	30	33	31	33	30
Mur	22	22	21	9	10	7	6	5	5	5	6	4	5	5	8	5	7	0	–	2	8	6	8	7	51	30	31	33	31	34	30	34	31
Sir	23	23	22	8	9	8	7	6	6	6	7	5	6	6	9	6	8	1	1	–	8	6	10	7	49	30	31	33	31	34	32	34	31
Tha	22	21	20	9	6	4	2	1	1	1	4	2	1	1	4	1	3	4	4	5	–	4	6	3	49	26	29	28	26	29	28	28	25
Cor	23	22	21	7	7	4	2	1	1	1	4	2	2	1	4	1	3	4	4	5	2	–	4	1	48	25	27	29	28	30	29	29	26
Con	22	21	20	9	7	4	2	1	1	1	4	2	2	1	4	1	3	6	6	7	2	2	–	3	50	27	30	31	30	31	28	31	28
Has	22	21	20	8	6	3	1	0	0	0	3	1	1	0	3	0	2	5	5	6	1	1	1	–	47	24	27	28	27	29	28	28	25
Ris	34	28	29	27	26	28	26	27	27	27	26	28	28	27	30	27	29	30	30	29	28	28	28	27	–	35	33	37	36	35	37	37	34
Far	25	24	23	16	15	14	10	11	11	11	10	12	12	11	14	11	13	16	16	17	12	12	12	11	22	–	6	12	12	11	16	12	10
Ham	25	22	23	17	17	16	12	13	13	13	12	14	14	13	14	13	15	17	17	18	14	13	14	13	22	2	–	11	12	9	17	17	13
Apl	24	21	20	16	17	16	12	13	13	13	10	12	14	13	14	13	15	16	16	17	14	14	14	13	25	3	3	–	13	12	19	12	9
Onc	24	23	22	17	13	14	11	10	10	10	11	11	10	10	11	10	12	13	13	14	9	11	11	10	23	5	5	6	–	9	12	14	13
Sip	26	22	23	18	17	16	12	13	13	13	12	14	14	13	14	13	14	18	18	19	14	14	14	13	21	3	2	4	5	–	15	10	8
Psa	24	21	22	20	15	16	14	13	13	13	14	14	13	13	14	13	14	14	14	15	12	14	14	13	25	9	8	10	7	6	–	18	16
Lim	26	24	23	16	15	14	10	11	11	11	10	12	12	11	14	11	13	16	16	17	12	12	12	11	21	1	4	4	5	2	8	–	6
Lmx	26	24	23	16	15	14	10	11	11	11	10	12	12	11	14	11	13	16	16	17	12	12	12	11	21	1	4	4	5	2	8	0	–

Use of the 'protein-assisted' alignment algorithm resulted in an unambiguous alignment of the cytochrome *c* oxidase I sequences (Appendix 2) that corresponds to positions 34 to 625 of the CO I sequence of *Drosophila yakuba* (Folmer, Black, Hoeh, Lutz & Vrijenhoek, 1994: Table 2). Of the 591 nucleotides, 329 were constant and 43 were parsimony uninformative. A maximum parsimony analysis of the 219 informative sites using the branch and bound algorithm resulted in a single most parsimonious tree (L = 1028; ci = 0.422; ri = 0.333; rc = 0.140) illustrated in Figure 6A. Bootstrap and jackknife support values are indicated only for those nodes supported in 50% majority rule consensus trees. The same CO I data set was also subjected to a heuristic search using the maximum likelihood algorithm and the Hasegawa *et al.* (1985) two parameter model for unequal base frequencies. A single tree (score = 5352.0901) was produced (Fig. 6B). Finally, a neighbor-joining tree was calculated based on Jukes-Cantor distances (Fig. 6C). Bootstrap and jackknife proportions are provided only for values exceeding 50%.

Pairwise comparisons of the 591 aligned positions are shown in Table 3. Total nucleotide differences/amino acid differences are given above the diagonal, while differences in the number of transversions/percent transversions at third base codon positions are tabulated below the diagonal. The distribution of variable sites along the studied portion of the CO I gene, the proportion of variation by codon position, and the distribution of the number of steps per character are shown in Figure 5 .

DISCUSSION

Of the taxa included in our earlier studies of gastropod phylogeny based on the identical region of the 18S rDNA gene (Harasewych *et al.*, 1997), the Vetigastropoda were found to be most closely related to the Apogastropoda. Three vetigastropod taxa were selected to serve as outgroups in order to minimize the problems associated with the use of distant outgroups, which may behave as random outgroups, joining the ingroup at the longest branch and rendering root position unreliable (Wheeler, 1990). Pleurotomariids were not included among the outgroup taxa because of their large insertion in the E-10-1 helix that is of questionable homology to the E-10-1 insertion of the Heterobranchia (Harasewych *et al.*, 1997: Fig. 3). A maximum parsimony analysis of the unambiguously aligned portions of the 18S sequences reconfirmed the monophyly of the Apogastropoda, as redefined by Ponder & Lindberg, 1997, and its two constituent subclades, the Caenogastropoda and the Heterobranchia, with each of these three taxa supported by high bootstrap and jackknife proportions (Figure 4).

While the use of a more proximal outgroup and the inclusion of two additional taxa improved resolution of the Heterobranchia and significantly increased bootstrap and jackknife support for at least some of its nodes (compare Figure 4 to Harasewych *et al.*, 1997, Fig. 5), neither the use of a closely related outgroup nor the addition of 16 taxa improved resolution within Caenogastropoda, which, with three exceptions, remained unresolved. The single Architaenioglossan (*Cipangopaludina*) included in this study emerged within the Caenogastropoda (as indicated by Ponder & Lindberg, 1996, 1997) rather than as a sister taxon to the Caenogastropoda + Heterobranchia (as hypothesized by Haszprunar, 1988). While grouped together with *Cerithium*, with which it shares a distinctive euspermatozoan morphology (Healy, 1988) (possibly on the basis of long branch attraction rather than close similarity, see Table 2), *Cipangopaludina* contains a 14 bp insertion in helix 10 (Fig. 3 A, b, i1) that does not occur in any of the other caenogastropods in this study. Further research is required to determine if this insert is present in other architaenioglossans. An additional 2 steps were required to move *Cipangopaludina* to the base of the Caenogastropoda, while placing *Cipangopaludina* as the sister taxon to the Apogastropoda increased tree length by 9 steps.

The remaining two 18S rDNA-based groupings of caenogastropods each circumscribe a Cenozoic divergence within Neogastropoda. The most strongly supported was the grouping of three muricid taxa (*Murex* + *Siratus* + *Phylonotus*) belonging to the subfamily Muricinae and sharing an Oligocene (35 mya) common ancestor (Vokes, 1971: Fig. 4). Members of this group differed from other neogastropods at nearly twice as many sites as was usual between neogastropod taxa (both total differences and transversions only, see Table 2), with most of the differences located immediately adjacent to the insert regions (Figure 3A, i1 and i2).

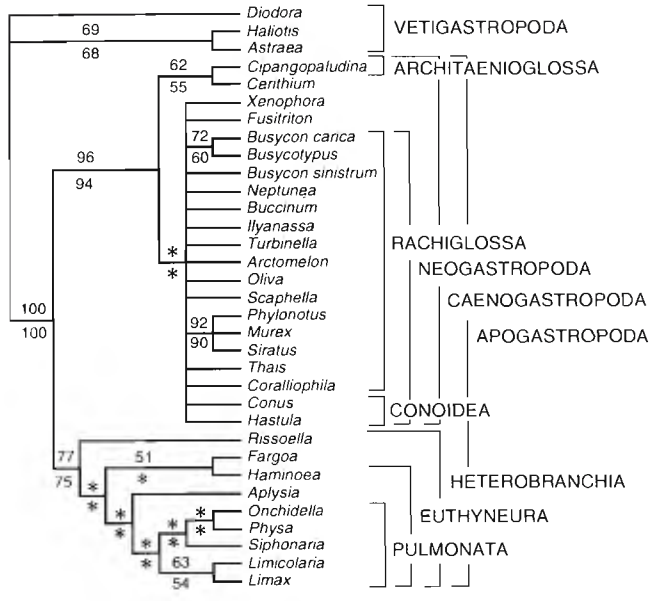

Figure 4. Phylogenetic relationships among the Apogastropoda [*sensu* Ponder & Lindberg, 1997 = Caenogastropoda + Heterobranchia] based on ≈ 450 bp of 18s rDNA sequence from which autapomorphic insertions, two large insertions, and regions of uncertain homology have been excluded. Selection of vetigastropods as outgroup is based on Harasewych *et al.*, 1997. Tree is a strict consensus of 1096 shortest trees (L = 214; ci = 0.696; ri = 0.821; rc = 0.572) resulting from a branch and bound search. Bootstrap proportions given in % above the node and jackknife proportions given in % below the node. An asterisk (*) indicates that the node was not supported in a 50% majority rule bootstrap/jackknife consensus tree.

By contrast, the grouping of two of the three busyconine taxa was based on their identical sequences. The divergence of *Busycotypus* and *Busycon sensu stricto*, the latter represented by *Busycon carica*, occurred during the middle Miocene (20 mya), yet their 18S rDNA sequences are identical. Sinistral *Busycon*, referred to the subgenus *Sinistrofulgur* and represented in this study by *Busycon sinistrum*, first appeared during the early Pliocene (5 mya), yet this species differs from both *Busycon s.s.* and *Busycotypus* by two base substitutions (transitions).

Although partial 18S sequences are insufficiently informative to resolve relationships among neogastropod families or between Neogastropoda and higher caenogastropods, they contradict the derivation of Neogastropoda from 'Archeogastropoda' or lower caneogastropods hypothesized by Ponder (1974) and subsequently refuted by Haszprunar (1988) and Healy (1988).

Harasewych *et al.* (1997) noted that relationships of taxa containing insertions in their 18S rDNA genes tended to be better resolved than those of taxa lacking insertions, even when the insertions were excluded from phylogenetic analyses. Their prediction that the 18S rDNA gene would better resolve relationships within Heterobranchia, which contain a large insertion, than within Caenogastropoda, which lack insertions, is corroborated. Substitution rates within Heterobranchia were found to be approximately twice those within Caenogastropoda (Table 2).

In a study of the evolutionary rates of the 18S rDNA gene, Philippe, Chenuil & Adoutte (1994) concluded that rapid adaptive radiations spanning less than 40 million years are generally beyond the limits of resolution of the entire 18S gene. As the radiation of neogastropod higher taxa was rapid, spanning approximately 35 million years (Taylor *et al.*, 1980), the general lack of resolution of these taxa using 18S rDNA sequence data is entirely concordant with the predictions of Philippe *et al.* (1994). Phylogenetic resolution was further hampered by the low proportion of nucleotide differences (p = 0.0–2.9%) among neogastropod taxa. As sequence data for the remainder of the 18S rDNA gene was, in our opinion, unlikely to provide significantly improved

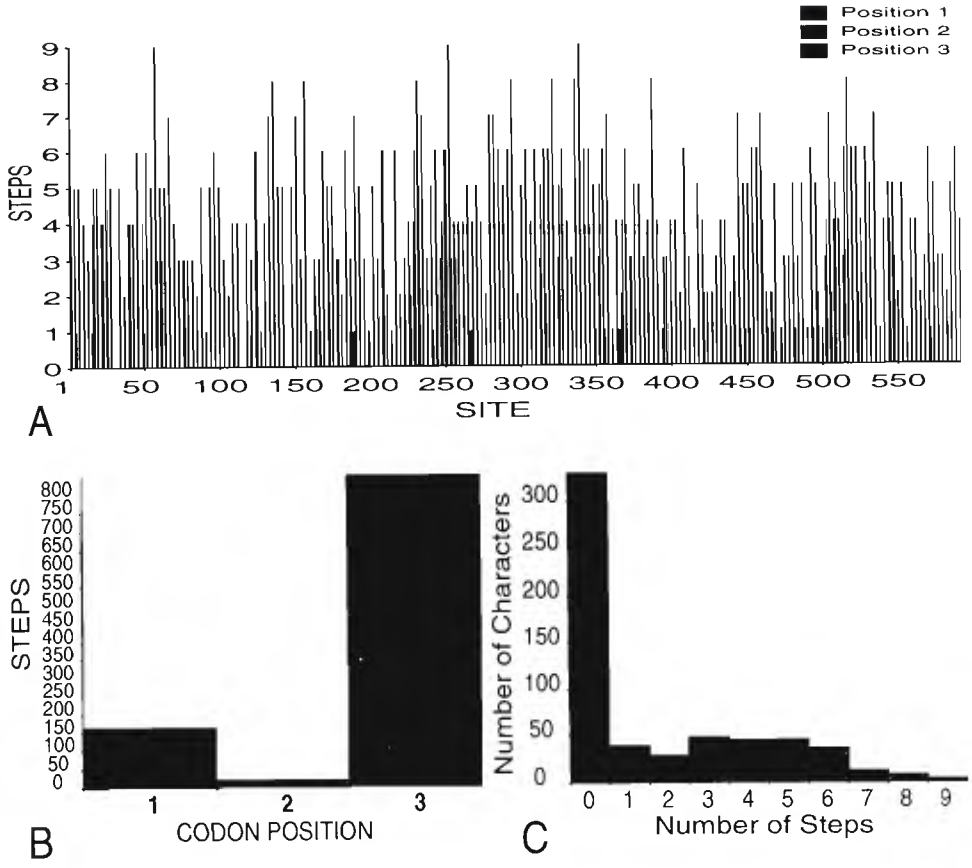

Figure 5. Variation in sites along the cytochrome *c* oxidase I gene among the 17 taxa shown in Figure 6. **A.** Distribution of variable sites along the 591 bp segment of the gene corresponding to positions 34 to 625 of the CO I sequence of *Drosophila yakuba* (Folmer *et al.*, 1994: Table 2). **B.** Histogram illustrating the proportion of variation by codon position. **C.** Histogram illustrating the distribution of the number of steps per character.

resolution of neogastropod phylogeny, a more rapidly evolving gene was sought to investigate further the evolutionary history of the Neogastropoda.

While evolutionary rates of the mitochondrial genome are more rapid than those of the nuclear genome (Miyata, Hayashida, Kikuno, Hasegawa, Kobayashi & Koike, 1982), the cytochrome *c* oxidase I (CO I) gene is the most conservative mitochondrial protein-coding gene (Jacobs, Elliott, Math & Farquharson, 1988). Kumazawa & Nishida (1993) compared the utility of mitochondrial protein-coding genes (cytochrome *c* oxidase I and cytochrome b) with those of mitochondrial tRNA genes for phylogenetic inference and concluded that the protein-coding genes did not perform as well in resolving divergences older than 300 million years. Because significant differences in mitochondrial gene order among molluscan classes (Hoffman, Boore & Brown, 1992; Boore & Brown, 1994; Terrett, Miles & Thomas, 1994), as well as within Gastropoda (Ueshima, Nishizaki & Kurabayashi, 1995) present methodological problems in sequencing tRNA genes that are not encountered among vertebrates, these genes were precluded from further study. Kumazawa & Nishida (1993: Fig. 4B) showed that the cytochrome *c* oxidase I gene is best suited for resolving divergences younger than 100 million years, making this gene a particularly promising candidate for studies of neogastropod evolution.

Analyses of a 591 bp portion of the CO I gene using maximum parsimony (MP) (Fig. 6A), maximum likelihood (ML) (Fig. 6B), and

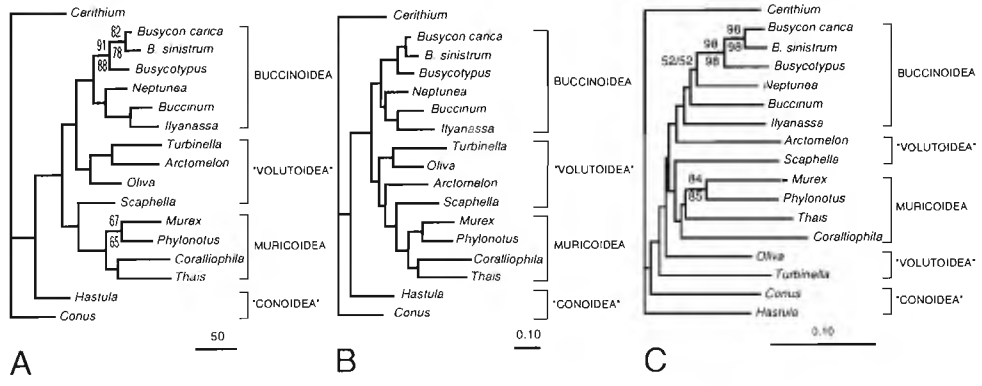

Figure 6. Phylogenetic relationships among Neogastropoda based on 591 bp of cytochrome *c* oxidase I sequence. *Cerithium* was selected as the outgroup based on the 18S rDNA tree (Figure 4). **A.** The single most parsimonious tree resulting from a branch and bound search using maximum parsimony (L = 1028; ci = 0.422; ri = 0.333; rc = 0.140). Bootstrap and jackknife proportions given in % only for those nodes supported in 50% majority rule consensus trees. **B.** The single tree (score = 5352.0901) resulting from a maximum likelihood analysis using the Hasegawa *et al.* (1985) two parameter model for unequal base frequencies. **C.** Neighbor-joining tree based on Jukes-Cantor distances. Bootstrap and jackknife proportions given in % only for those nodes supported in 50% majority rule consensus trees.

the neighbor-joining (NJ) (Fig. 6C) algorithms each produced unique trees. All divided the Neogastropoda into a monophyletic Stenoglossa and a paraphyletic Toxoglossa, with 7 additional steps required to join the two toxoglossan taxa on the maximum parsimony tree. Within Stenoglossa, both maximum parsimony and maximum likelihood algorithms grouped the buccinoidean and muricoidean taxa into monophyletic clades with identical topologies. 'Volutoidean' taxa emerged as a grade between Buccinoidea and Muricoidea (MP & ML), with the shortest possible maximum parsimony solution yielding a monophyletic Volutoidea (emerging as sister taxon to Muricoidea) requiring 7 additional steps. By contrast, 9 additional steps are required to unite *Scaphella* and *Arctomelon* in a monophyletic Volutidae. The NJ tree also produced monophyletic Buccinoidea and Muricoidea, but ladderized their basal taxa while distributing 'volutoidean' taxa more widely. A reanalysis of the data with *Cerithium* deleted and *Conus* specified as outgroup produced a maximum parsimony tree with buccinoidean and muricoidean taxa emerging as previously, but with the 'volutoidean' taxa distributed as in the NJ tree.

Within the Caenogastropoda and Neogastropoda, the cytochrome *c* oxidase I gene is strongly conserved in terms of amino acid sequence, but highly variable at silent sites (Table 3). Transition/transversion ratios range from 12.0 among congeners (*Busycon carica/B. sinistrum*) to less than 1.0 among the most distantly related Neogastropods. Substitution rates are high (to 23.2%) within Neogastropoda. The majority of substitutions occur in the third-base positions of codons (Fig. 5B) and are homoplasous (Fig. 5C, steps > 3). Consequently, trees based on these data (Fig. 6), while representing unique solutions, have low consistency and retention indices. As with the 18S rDNA data, only those taxa sharing a Cenozoic ancestor where supported by bootstrap and jackknife support values in excess of 50%.

Quantitative comparisons of the sequence-based maximum parsimony tree with prevailing phylogenetic hypotheses (Table 4) are difficult, since values based on trees that are less than fully resolved are skewed and should be interpreted with caution. All morphological classifications agree in segregating stenoglossan and toxoglossan taxa on the basis of distinctive radular morphology, the morphology and derivation of the proboscis, and the presence in Toxoglossa of a poison gland and muscular bulb. While the monophyly of the Stenoglossa is supported by CO I sequence data, the toxoglossan (Conoidean) taxa emerge as a grade in the molecular trees. As this group is represented in our data set by only two distantly related taxa, the addition of other conoidean taxa may alter this outcome.

Table 3. Nucleotide and amino acid differences between taxa in the portion of the cytochrome *c* oxidase I gene (591 aligned positions) used in this study. Total number of nucleotide differences/number of amino acid differences are shown above the diagonal, transversions only/percent transversions at third codon positions are tabulated below the diagonal.
Arc = *Arctomelon*, Bca = *Busycon carica*, Bsi = *Busycon sinistrum*, Buc = *Buccinum*, Bus = *Busycotypus*, Cer = *Cerithium*, Con = *Conus*, Cor = *Coralliophila*, Has = *Hastula*, Ily = *Ilyanassa*, Mur = *Murex*, Nep = *Neptunea*, Oli = *Oliva*, Phy = *Phylonotus*, Sca = *Scaphella*, Tha = *Thais*, Tur = *Turbinella*.

Taxa	Cer	Bca	Bsi	Bus	Nep	Buc	Ily	Tur	Oli	Arc	Sca	Mur	Phy	Cor	Tha	Con	Has
Cer	–	127/4	129/4	122/5	126/1	77/2	122/4	126/7	120/2	132/7	136/8	126/2	126/2	140/9	136/2	116/9	119/5
Bca	57/28	–	24/2	39/1	67/3	54/5	93/6	119/9	96/4	101/5	116/8	110/4	113/4	140/11	122/4	106/11	117/7
Bsi	55/27	2/1	–	52/3	73/3	51/3	89/6	119/9	101/4	107/5	111/8	108/4	119/4	138/11	114/4	116/11	115/7
Bus	57/31	3/2	5/3	–	73/4	56/5	86/7	109/10	93/5	101/5	101/9	106/5	101/5	127/12	112/5	101/12	110/7
Nep	55/28	25/12	25/12	27/14	–	56/2	83/3	120/6	100/1	95/6	105/7	108/1	109/1	124/8	112/1	115/8	112/6
Buc	34/24	15/10	15/10	17/11	18/13	–	60/2	71/7	68/3	70/6	80/5	87/2	80/2	88/6	77/3	83/8	84/6
Ily	52/26	47/22	45/21	45/22	38/18	24/16	–	118/8	100/4	106/9	115/10	116/4	112/4	123/7	113/4	116/11	104/9
Tur	53/26	58/28	58/28	55/29	59/29	36/26	57/28	–	110/7	120/12	127/13	137/7	132/7	137/11	130/7	115/13	130/12
Oli	55/27	44/21	42/20	38/20	53/26	30/21	51/24	51/25	–	112/7	105/8	113/2	108/2	131/9	113/2	109/9	120/7
Arc	56/27	45/22	45/22	42/22	50/24	33/23	52/24	64/30	47/22	–	116/9	118/5	124/5	132/14	127/7	133/14	122/10
Sca	59/29	48/23	46/22	43/22	55/27	32/23	59/28	58/28	40/19	51/24	–	112/6	111/6	124/15	128/8	127/15	121/11
Mur	57/28	51/25	49/24	47/24	51/25	44/32	59/29	61/30	49/24	54/26	50/25	–	86/0	123/99	111/2	116/9	129/7
Phy	56/28	51/25	51/25	47/25	50/25	37/27	59/29	57/28	49/24	56/27	51/25	19/10	–	116/9	110/2	105/9	121/7
Cor	64/29	69/31	69/31	65/32	62/28	48/33	61/29	70/32	67/30	66/29	63/28	52/24	56/25	–	129/9	125/12	135/11
Tha	68/35	49/24	49/24	47/25	46/23	34/24	49/24	63/32	51/25	60/29	59/29	49/24	54/27	62/28	–	117/9	135/7
Con	53/25	59/28	59/28	54/26	56/27	40/26	59/27	57/28	58/28	62/69	64/30	52/24	45/21	63/28	55/27	–	116/11
Has	48/24	48/23	46/22	44/23	53/26	32/22	49/23	55/27	54/26	53/25	56/27	55/27	57/28	63/28	65/32	47/22	–

Table 4. Comparisons of the cytochrome *c* oxidase I sequence-based, maximum parsimony phylogeny of the Order Neogastropoda with contemporary, morphology-based hypotheses. For purposes of comparison, trees were pruned to contain the same sets of taxa. When families were represented in the molecular data set by multiple species, a single representative was selected that had the smallest number of missing bases or ambiguous base assignments. Melongenidae is therefore represented by *Busycon carica*, Muricidae by *Phyllonotus pomum* and Volutidae by *Scaphella junonia*. *Cerithium atratum* served as the outgroup in all analyses. Comparisons are based on taxa identified by UPPER CASE in Figure 2.

Phylogenetic Hypothesis	Tree length	CI	RI	RC	No. neogastropod families
Thiele, 1929 (Figure 2A)	699+	0.55	0.37	0.20	10
Best fully resolved tree*	750	0.51	0.26	0.13	10
CO I tree	735	0.53	0.29	0.15	10
Ponder, 1974 (Figure 2B)	714+	0.63	0.54	0.34	10
Best fully resolved tree*	748	0.52	0.27	0.14	10
CO I tree	733	0.53	0.29	0.15	10
Kantor, 1996 (Figure 2C)	690+	0.54	0.27	0.15	9
Best fully resolved tree*	702	0.53	0.24	0.13	9
CO I tree	678	0.55	0.30	0.17	9
Kantor, 1996 (Figure 2D)	693	0.54	0.27	0.14	9
CO I tree	678	0.55	0.30	0.17	9

*–Polytomies in original tree resolved manually to produce the shortest tree. Relationships among taxa thus resolved generally conform to those predicted by the CO I tree.

Cancellarioideans are not represented in the molecular data matrix reported here, a situation we hope to remedy within the year. The single cancellariid (*Progabbia cooperi*) included in the 28S rRNA data set analyzed by Rosenberg *et al.* (1994) emerged within Stenoglossa, sharing a more recent common ancestor with Muricidae than with Buccinidae. These authors regarded the Cancellariidae to be a highly derived group within Stenoglossa, where it had been referred by Thiele (1929) (Fig. 2A) and Wenz (1943), but not in more recent classifications (Fig. 2B–D).

Morphology-based classifications also agree in uniting Buccinoidean taxa as a monophyletic lineage, but differ in the rank accorded to some of the included groups (*e.g.* Thiele, 1929; Wenz, 1938; Powell, 1951; Habe & Sato, 1972). In their listing of apogastropod taxa at or above the family level, Ponder & Warén (1988) treated the Nassariidae, Melongenidae, and Fasciolariidae as subfamilies of Buccinidae, while retaining Columbellidae as a family. Subsequent authors generally concurred with Ponder (1974), who regarded the lack of rectal and accessory salivary glands among Buccinoidean taxa to be due to a synapomorphic loss of these organs, which are present in other neogastropod groups. More recently, Ponder & Lindberg (1997) placed buccinoideans at the base of Neogastropoda, regarding the rectal and accessory salivary glands to be uniquely derived in the ancestor of the remaining neogastropods after their divergence from Buccinoidea. The latter relationship is contradicted by the CO I sequence data.

Most previous authors have either united the families Muricidae and Coralliophilidae in a clade (Fig. 2A, D), a conclusion supported by the molecular data, or speculated on their possible close relationships (*e.g.* Ponder, 1974: 329; Boss, 1982: 1014) while treating them as distinct families (Fig. 2B). Kantor (1996; Fig. 2D) regarded the families Costellariidae and Melapiidae to be closely related to Muricidae, but these two families are not represented in our study.

The remaining stenoglossan families had generally been relegated to the superfamily Volutoidea on the basis of conchological similarity (presence of often pronounced columellar folds) and the reduction or loss of the operculum (Thiele, 1929; Wenz, 1938), although some authors regarded the Mitridae to be members of the Toxoglossa (Risbec, 1955; Taylor & Sohl, 1962). Ponder (1974) noted the absence of unifying anatomical

similarities (synapomorphies) among these families and recognized that they do not form a 'natural higher grouping'. Subsequently, Kantor (1996) promoted the Olividae and Pseudolividae to the suborders Olivellidoidei and Pseudolivellidoidei, regarding them to be basal to the remaining stenoglossans (Fig. 2C, D). Phylogenies constructed using CO I sequences indicate that the 'volutoidean' families constitute a grade (maximum parsimony and maximum likelihood) or grades (neighbor-joining) rather than a clade. Only the neighbor-joining algorithm places Olividae near the base of the Stenoglossa, as hypothesized by Kantor (1996).

Despite research attention spanning several decades, relationships within the Order Neogastropoda have proven to be among the most refractory within Gastropoda to traditional methods of phylogenetic inference. As noted by Ponder (1974), the adaptive radiation of neogastropod taxa was extremely rapid, with subsequent diversification of the resulting lineages marked by tendencies to modify organ systems in parallel fashions. As a consequence, only a very few morphological characters that originated during the initial adaptive radiation are presently recognized. These unite small subsets of higher taxa, while the remaining families are unresolved. The absence of congruent patterns in the evolution of the majority of morphological characters have frustrated attempts at phylogenetic inference using strict cladistic methodology (Kantor, 1996, see Fig. 2C). Most arrangements of neogastropod higher taxa are based instead on evolutionary scenarios in which the evolution of a single organ, organ system or character is heavily weighted (*e.g.* radular morphology, site of elongation of the proboscis, position of the buccal mass within the proboscis, passage of odontophoral retractor muscle through the nerve ring). While the cytochrome *c* oxidase I sequence data produce single trees that fully resolve the relationships between neogastropod suborders and families, these trees are characterized by low character indices and a high degree of homoplasy (Fig. 5C). This is concordant with the hypothesis of a rapid initial diversification of Neogastropoda, in which few diagnostic synapomorphies were accumulated in the rapidly anastomosing clades, followed by a longer period on rapid evolution within each lineage that only obscured the original phylogenetic signal. Irwin, Kocher & Wilson (1991) noted that transversion substitutions at silent sites (third base codon positions) accumulate linearly in mitochondrial DNA, require little correction for multiple hits, and may be used to compare genetic divergence between taxa. Based on percent transversions at third codon positions, Palumbi & Benzie (1991) estimated that genetic differences among genera of penaeid shrimp are greater than those among orders of placental mammals. Using the same measure (Table 3), we found differences among neogastropod families to be, on average, only slightly lower than those between neogastropod families and *Cerithrium*, comparable to genetic differences between orders of mammals, yet only on the order of intergeneric differences among penaeid shrimp.

As with the 18S rDNA data, only those groupings sharing common ancestors in the Cenozoic emerged with significant bootstrap/jackknife support. This suggests that, within Neogastropoda, the CO I gene may be more useful for resolving phylogenetic relationships of younger divergences. It should be noted that only approximately half of the families included within Neogastropoda are sampled in this study, with the 'volutoidean' and toxoglossan taxa being particularly underrepresented. We expect that relationships of these taxa will be more clearly and robustly resolved as the density of taxonomic sampling increases. Contrary to our earlier suggestion (Harasewych *et al.*, 1997) the combination of 18S rDNA and CO I sequence data has not proven fruitful in resolving phylogenetic relationships within Neogastropoda, as the 18S data appear to contribute more noise than signal. Data from a gene that evolves more slowly than CO I, either singly or in combination with CO I will likely shed more light on neogastropod phylogeny.

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Appendix I. Aligned partial sequences of the gastropod 18S rDNA gene corresponding to positions 60–515 of the 18S rRNA of *Onchidella celtica* as reported by Winnepenninckx *et al.* (1994: 101). All sequences confirmed by at least two sequencing reactions in one direction and a third sequencing in the reverse direction. Ambiguous base assignments are noted using IUPAC symbols. Dots (·) represent nucleotides identical to those of *Diodora*, dashes (–) represent missing data, and quotes (") represent gaps inserted during alignment.

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                    10         20         30         40         50         60         70         80         90         100
                    .          .          .          .          .          .          .          .          .          .
Diodora             TAAG"TACAA ACTCTCGCCC AGTG"AAACT GCGAA"TGGC TCATTACATC AGTTATGGTT CCTTGGACGA TACCA"TCC" TACTTGGATA ACTGTGGTAA
Haliotis            ....."..... .....A..A. ....."..... ....."..... ......G... .......... ....A..T.. ...A."..." .......... ..........
Astraea             ....."..... .....A..A. ....."..... ....."..... ......G... .......... ....A..T.. ...A."..." .......... ..........
Cipangopaludina     ....T."..C ..C....TA. G..."....C ....."..... ......A... ...CGA.... ....A..T.. .C.ATT.."" .......... ..........
Cerithium           ....T....C ..C..T.TA. G..."....C ....."..... ......A... ...CGA.... ....A..T.. .C.ATT.."" .......... ..........
Xenophora           ....T.".T" ".C....TA. G..."....C ....."..... .."...A... "..CGA.... ....A..T.. .C."A.T"" .......... ..........
Fusitriton          ....T."..C ..C..T.TA. G..."....C ....."..... ......A... ...CGA.... ....A..T.. .C.A.A.W"" .......... ..........
Busycon carica      ....T."..C ..C....TA. G..."....C ....."..... ......A... ...CGA.... ....A..T.. .C.A.ACT"" .......... ..........
Busycon sinistrum   ....T."..C ..C....TA. G..."....C ....."..... ......A... ...CGA.... ....A..T.. .C.A.A.T"" .......... ..........
Busycotypus         ....T."..C ..C....TA. G..."....C ....."..... ......A... ...CGA.... ....A..T.. .C.A.ACT"" .......... ..........
Neptunea            ....T."..C ..C....TA. G..."....C ....."..... ......A... ...CGA.... ....A..T.. .C.A.A.T"" .......... ..........
Buccinum            ....T."..C ..C..T.TA. G..."....C ....."..... ......A... ...CGA.... ....A..T.. .C.A.A.T"" .......... ..........
Ilyanassa           ....T."..C ..C....TA. G..."....C ....."..... ......A... ...CGA.... ....A..T.. .C.A.A.T"" .......... ..........
Turbinella          ....T."..C ..C....T". G..."....C ....."..... ......A... ...CGA.... ....A..T.. .C.A.A.T"" .......... ..........
Oliva               ....T."..C ..C....TA. G..."....C ....."..... ......A... ...CGA.... ....A..T.. .C.A.A.T"" .......... ..........
Arctomelon          ....T."..C ..C....TA. G..."....C ....."..... ......A... ...CGA.... ....A..T.. .C.A.A.T"" .......... ..........
Scaphella           ....T."..C ..C..T.TA. G..."....C ....."..... ......A... ...CGA.... ....A..T.. .C.A.A.T"" .......... ..........
Murex               ....T."..C ..C....TAT G..."....C ....."..... ......A... ...CGA.... ....A..T.. .C...A.T"" .......... ..........
Siratus             ....T."..C ..C....TA. G..."....C ....."..... ......A... ...CGA.... ....A..T.. .C..TA.T"" .......... ..........
Phylonotus          ....T."..C ..C....TA. G..."....C .....A.... ......A... ...CGA.... ....A..T.. .C...A.T"" .......... ..........
Coralliophila       ....T."..C ..C....TA. G..."....C .....A.... ......A... ...CGA.... ....A..T.. .C.A.A.T"" .......... ..........
Thais               ....T."..C ..C....TA. G..."....C ....."..... ......A... ...CGA.... ....A..T.. .C.A.A.T"" .......... ..........
Conus               ....T."..C ..C....TAT G..."....C ....."..... ......A... ...CGA.... ....A..T.. .C.A.A.T"" .......... ..........
Hastula             ....T."..C ..C....TA. G..."....C ....."..... ......A... ...CGA.... ....A..T.. .C.A.A.T"" .......... ..........
Rissoella           ..T."."..C ...T.G.TA. ....."....C ....."..... ......A... ...CGA.... ....A..T.. C..GTT.T.C .......... .......C..
Fargoa              ....T."..C ...G..T.A. G..."....C ....."..... ......A... ...CGA.... ....A..T.. C..G.""T.C .......... .....A.C..
Haminoea            ....T."..C ...G.TT.A. G..."....C ....."..... ......A... ...CGA.... ....A..T.. C..G.""T.C .......... .......C..
Aplysia             ....T."..." ...G..T.A. G...T....C ....."..." ......A... ...CGA.... ....A..T.. C..G.""T.C .......... .......C..
Onchidella          ....T."..C ...G..T.A. G..."....C ....."..... ......A... ...CGA.... ....A..T.. C..G.""T.C .......... .......C..
Siphonaria          ....T."..C ...G..T.A. G..."....C ....."..... ......A... ...CGA.... ....A..T.. C..G.""T.C .......... .......C..
Physa               ....T."..C ...G..C.AT G..."..."C ....."..... ......A... ...CGA.... ....A..T.. CW.G.""T.C .......... .......C..
Limicolaria         ....T."..C ...G..T.A. G..."....C ....."..... ......A... ...CGA.... ....A..T.. C..G.""T.C .......... .......C..
Limax               ....T."..C ...G..C.A. G..."....C ....."..... ......A... ...CGA.... ....A..T.. C..G.""T.C .......... .......C..

                    110        120        130        140        150        160        170        180        190        200
                    .          .          .          .          .          .          .          .          .          .
Diodora             TTCTAGAGCT AATACATGCA CTTCG"GCTC CGACCC"""" "TTTCCC""" AA"GGGGAAG AGCGCATTTA TCAGCCTCGA AGCCAGCCGG "G"""""""" .
Haliotis            .......... .......... ..AAA"...." ......"""" "....T"G"" ."".....G.... ....T"...." ......M..A ".""""""""" .
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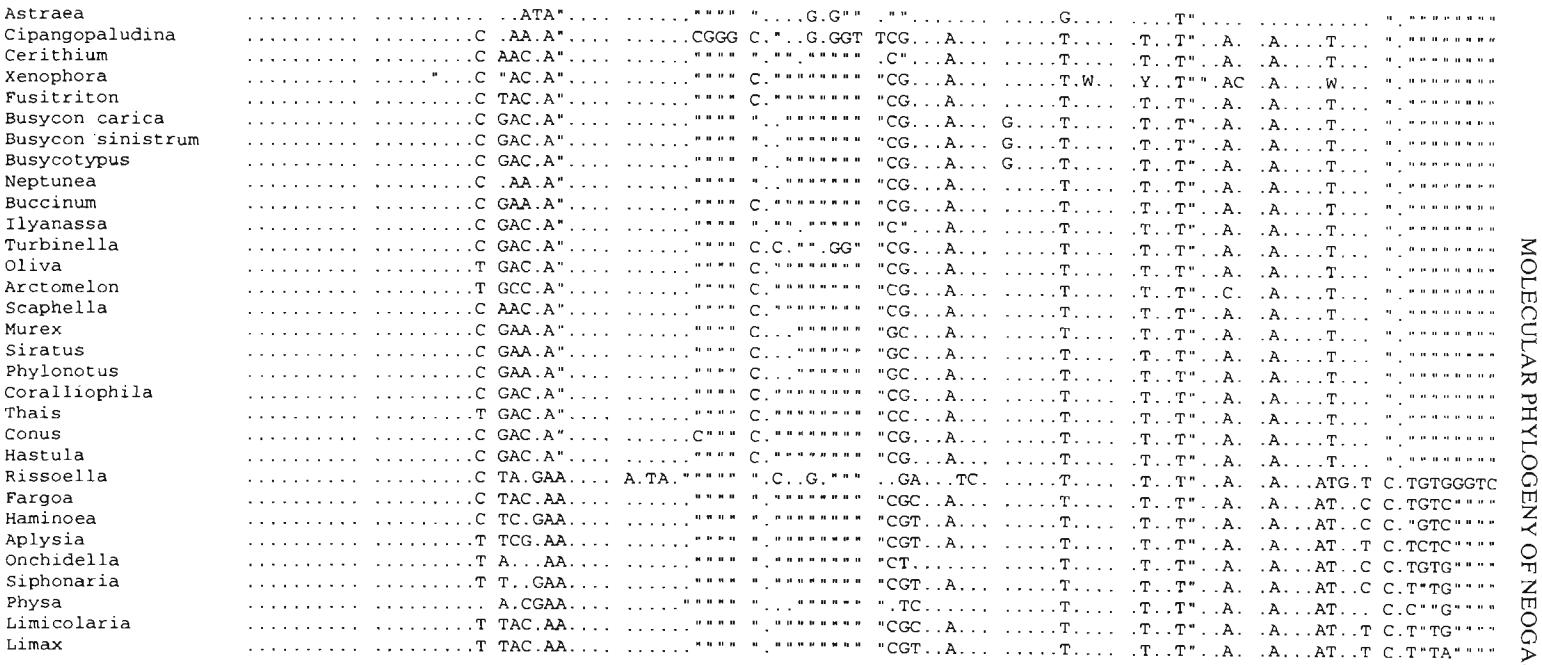
Astraea
Cipangopaludina
Cerithium
Xenophora
Fusitriton
Busycon carica
Busycon sinistrum
Busycotypus
Neptunea
Buccinum
Ilyanassa
Turbinella
Oliva
Arctomelon
Scaphella
Murex
Siratus
Phylonotus
Coralliophila
Thais
Conus
Hastula
Rissoella
Fargoa
Haminoea
Aplysia
Onchidella
Siphonaria
Physa
Limicolaria
Limax
210
220
230
240
250
260
270
280
290
300

```
                    210        220        230        240        250        260        270        280        290        300
                     .          .          .          .          .          .          .          .          .          .
Buccinum            """""T..C" """""""""" """""""""" """""""""" ""...."....  ."""....... .......... ....T.G... C.......T. ...T."""..
Ilyanassa           """""T..T" """""""""" """""""""" """""""""" ""...."....  ."""....... .......... ....T.G... C.......T. ...T."""..
Turbinella          """""T..T" """""""""" """""""""" """""""""" ""...."....  ."""....... .......... ....T.G... C.......T. ...T."""..
Oliva               """""T..T" """""""""" """""""""" """""""""" ""...."....  ."""....... .......... ....T.G... C.......T. ...T."""..
Arctomelon          """""T..'C G""""""""" """""""""" """""""""" ""...."....  ."""....... .......... ...GT.G... C.......T. ...T."""..
Scaphella           """""T..T" """""""""" """""""""" """""""""" ""...."....  ."""....... .......... ....T.G... C.......G. ...GT"T"..
Murex               """""A.TT" """""""""" """""""""" """""""""" ""T.."....  ."""....... .......... ....T.G... C.......T. ...T."""..
Siratus             """""A.TT" """""""""" """""""""" """""""""" ""T.."....  ."""....... .......... ....T.G... C.......T. ...T."""..
Phylonotus          """""A.TT" """""""""" """""""""" """""""""" ""T.."....  ."""....... .......... ....T.G... C.......T. ...T."""..
Coralliophila       """""""".CC G""""""""" """""""""" """""""""" ""T.."....  ."""....... .......... ....T.G... C.......T. ...T."""..
Thais               """""T..T" """""""""" """""""""" """""""""" ""...."....  ."""....... .......... ....T.G... C.......T. ...T."""..
Conus               """""T..T" """""""""" """""""""" """""""""" ""...."....  ."""....... .......... ....T.T... C.......T. ....."""..
Hastula             """""T..T" """""""""" """""""""" """""""""" ""...."....  ."""....... .......... ....T.G... C.......T. ...T."""..
Rissoella           TTCTTT..TC GCTCTTCGG. CGGG..GGGT TGGTCTGTTT AA..GG...T GTT"....A. .......... ....T.GA.. T.......... CA.T.GT".T
Fargoa              """""""""" "TGCTTTCG" """""GGT" "GGC""""""" ""..GGT... .CA"...... .......... ....T.G... T.......T. ...T.G"".C
Haminoea            """""""""" "GTCTTCG." """""GGC" "GGC""""""" """.GGT... .C"...... .......... ....T.G... T.......T. ...T.T"".C
Aplysia             """""""""" GCCGTTTT"" CGGGG.G""" """""""""" ""..GG.... .CA..... .......... ....T.G... T.......T. ...TTTT".C
Onchidella          """""""""" "CTCTTCC." """""GGG" "GCC""""""" "".GG.... .C.."..... .......... ....T.G... T.......T. ...TTTT".C
Siphonaria          """""""""" "CCCTTCG.. """"..GGG" "GGT""""""" ""..GG.... .C.AC.... .......... ....T.G... T.......T. ...""CT".C
Physa               """""""""" ""GCCTCG.. """"..GG"" "GTT""""""" ""..GT.... ."A..... .......... ....T.G... T.......T. ...'TC"".C
Limicolaria         """""""""" "CCCTTC""" """"..CG"" "GGC""""""" ""..GG.... ."AAC"... .......... ....T.G... T.......T. ...TTCT".T
Limax               """""""""" "CCCTTCG." """""""GG" "GGT""""""" ""..GG.... .C.AC"... .......... ....T.G... T.......T. ...T"CAC.T

                    310        320        330        340        350        360        370        380        390        400
                     .          .          .          .          .          .          .          .          .          .
Diodora             GCCGGCGACG CGTCCATCAA ATGTCTGCCC TATCAGACTG TCGATGGTAA GTGCTATGCT TACCATGGTG ATAACGGGTA ACGGGG"AAT CAGGGTTCGA
Haliotis            .......... .A..T..... G......... .......... .......... .......... .......... .......... ......"... ..........
Astraea             .......... .A..T..... G......... .......... .......... .......... .......... .......... ......"... ..........
Cipangopaludina     .......... .A..TT.... .......... .....".A.. A...C....C ...A.C...C ....G..T.. .CT..... G......"... ..........
Cerithium           .......... .A..TT.... .......... .....".A.. A.......CG ...A.C...C .....T.T .CT..... G......"... ..........
Xenophora           .......... .A..TT..". .......... .....".A.. A........C ...A.C...C .....T.A GC..... G......"... ..........
Fusitriton          .......... .A..TT.... .......... .....".A.. A........C ...A.C...C .....T.A GC..... G......"... ..........
Busycon carica      .......... .A..TT.... .......... .....".A.. A........C ...A.C...C .....T.A GC..... G......"... ..........
Busycon sinistrum   .......... .A..TT.... .......... .....".A.. A........C ...A.C...C .....T.. GC..... G......"... ..........
Busycotypus         .......... .A..TT.... .......... .....".A.. A........C ...A.C...C .....T.A GC..... G......"... ..........
Neptunea            .......... .A..TT.... .......... .....".A.. A........C ...A.C...C .....T.A GC..... G......"... ..........
Buccinum            .......... .A..TT.... .......... .....".A.. A........C ...A.C...C .....T.A GC..... G......"... ..........
Ilyanassa           .......... .A..TT.... .......... .....".A.. A........C ...A.C...C .....T.A GC..... G...T."... ..........
Turbinella          .......... .A..TT.... .......... .....".A.. A........C ...A.C...C .....T.A GC..... G......"... ..........
Oliva               .......... .A..TT.... .......... .....".A.. A........C ...A.C...C .....T.A GC..... G......"... ..........
Arctomelon          .......... .A..TT.... .......... .....".A.. A........C ...A.C...C .....T.. GC..... G......"... ..........
Scaphella           .......... .A..TT.... .......... .....".A.. A........C ...A.C...C .....T.A GC..... G......"... ..........
Murex               .......... ....TT.... .......... .....".A.. A........C ...A.C...C .....T.. GC..... G......"... ..........
Siratus             .......... ....TT.... .......... .....".A.. A........C ...A.C...C .....T.. GC..... G......"... ..........
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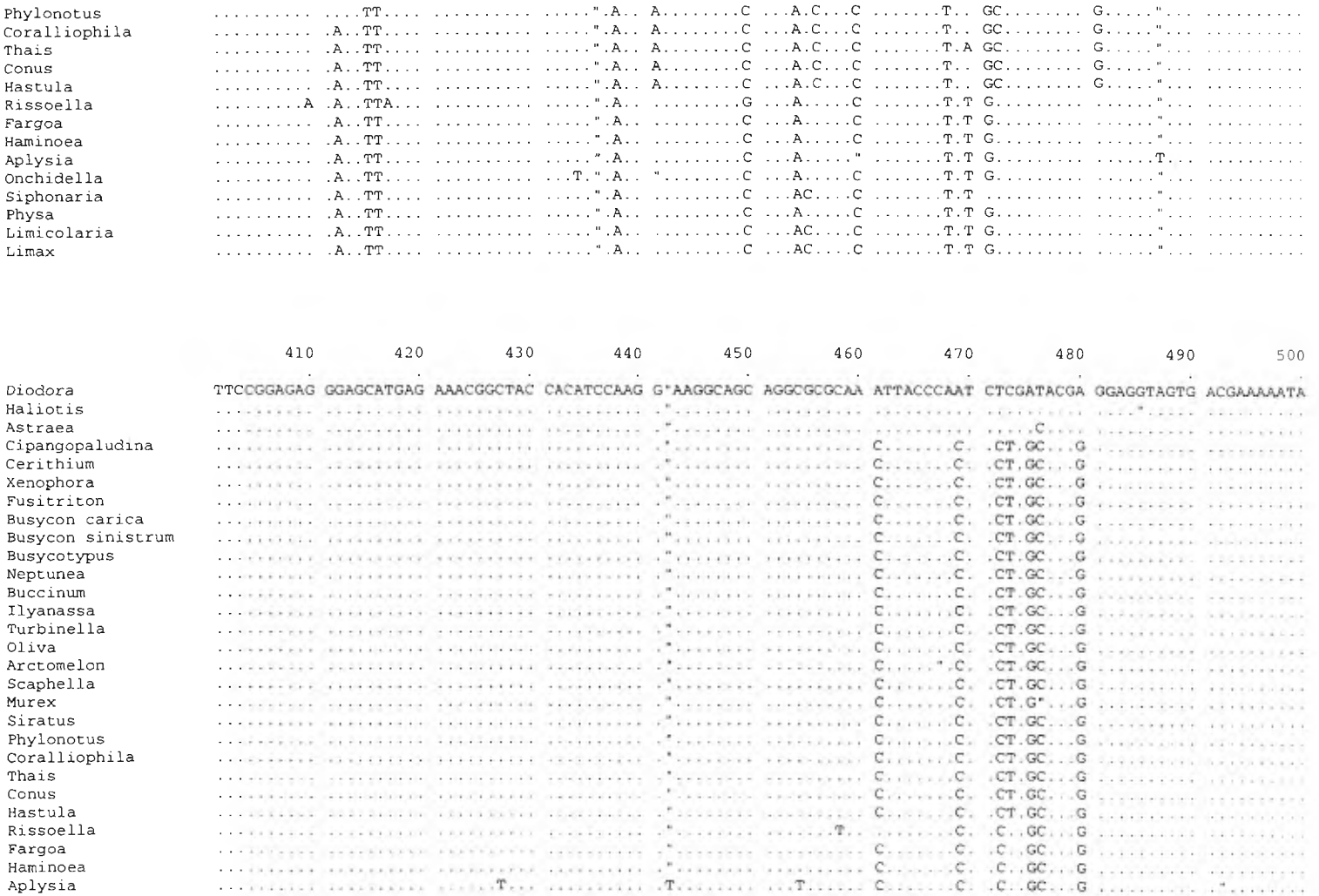
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Haliotis
Astraea
Cipangopaludina
Cerithium
Xenophora
Fusitriton
Busycon carica
Busycon sinistrum
Busycotypus
Neptunea
Buccinum
Ilyanassa
Turbinella
Oliva
Arctomelon
Scaphella
Murex
Siratus
Phylonotus
Coralliophila
Thais
Conus
Hastula
Rissoella
Fargoa
Haminoea
Aplysia
Onchidella
Siphonaria
Physa
Limicolaria
Limax

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                    410        420        430        440        450        460        470        480        490        500
                     .          .          .          .          .          .          .          .          .          .
Onchidella          .......... .......... .......... .......... ."........ .......... C.......C. .C..GC...G .......... ..........
Siphonaria          .......... .......... .......... .......... ."........ .......... C.......C. .C..GC...G .......... ..........
Physa               .......... .......... .......... .......... ."........ .......... C.......C. .C..GC...G ....".... ..........
Limicolaria         .......... .......... .......... .......... ."........ ....A""... C.......C. .C".GC...G A.G....... ..........
Limax               .......... .......... .......... .......... ."........ .......... C.......C. .C..GC...G .......... ..........
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                    510
                     .
Diodora             ACAATA"CGG GACTCT   [449]
Haliotis            ......"... ......   [444]
Astraea             ......"... ......   [446]
Cipangopaludina     ......"... A.....   [455]
Cerithium           ......"..A A.....   [439]
Xenophora           ......"... A.....   [430]
Fusitriton          ......"... A.....   [438]
Busycon carica      ......"... A.....   [438]
Busycon sinistrum   ......"... A.....   [438]
Busycotypus         ......"... A.....   [438]
Neptunea            ......"... A.....   [438]
Buccinum            ......"... A.....   [438]
Ilyanassa           ......"... A.....   [437]
Turbinella          ......"... A.....   [442]
Oliva               ......"... A.....   [438]
Arctomelon          ......"... A.....   [438]
Scaphella           ......"... A.....   [439]
Murex               ......"... A.....   [439]
Siratus             ......"... A.....   [440]
Phylonotus          ......"... A.....   [441]
Coralliophila       ......"... A.....   [439]
Thais               ......"... A.....   [438]
Conus               ......"... A.....   [439]
Hastula             ......"... A.....   [438]
Rissoella           .....C"... ......   [496]
Fargoa              ......"... ......   [457]
Haminoea            ......"... ......   [455]
Aplysia             ......A... ......   [460]
Onchidella          ......"... ......   [456]
Siphonaria          ......"... ......   [458]
Physa               ......"... ......   [454]
Limicolaria         ......"... ......   [452]
Limax               ......"... ......   [456]
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Appendix II. Aligned partial sequences of the gastropod mitochondrial cytochrome *c* oxidase I gene corresponding to positions 34–625 of the CO I sequence of *Drosophila yakuba* as reported by Folmer *et al.* (1994: Table 2). All sequences confirmed by at least two sequencing reactions with each primer. Ambiguous base assignments are noted using IUPAC symbols. Dots (·) represent nucleotides identical to those of *Cerithium*, dashes (–) represent missing data, and quotes (") represent gaps inserted during alignment.

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                    10        20        30        40        50        60        70        80        90       100
                    .         .         .         .         .         .         .         .         .         .
Cerithium           TTGGTTGGAA CTGCTCTCAG TCTATTAATT CG"GCCGAAT TAGGACAGCC TGGGGCCCTT CTTGGTGATG ATCAGCTATA TAATGTAATT GTAACGGCAC
Busycon carica      .........T ....CT.A.. C..TC.T... ..A..T.... .......... A..A..TT.G .......... ....A..T.. ...C...... .....A....
Busycon sinistrum   .........T .....T.A.. ...TC.T... ..G..T.... ....G..A.. A..A..TT.G .......... ....A..T.. ...C...... ..........
Busycotypus         ..A..C..T. ....CT.A.. ...TC.T... ..A..T.... .......... A..A..AT.G .....A..C. ....A..T.. ...C..G..C .....A....
Neptunea            ..A.....T. .......A.. ...TC.T... ..T..T.... .......A.. A.....TT.A .......... ....A..T.. ...C...... .....A....
Buccinum            ---------- ---------- ---------- ---------- ---------- -..A..T..A .......... .C..A..T.. ...C..G... ..G.......
Ilyanassa           ---------- -----T.A.. A..TC.T... ..T..T...C .T.....A.. ...A..A... .........C .C..A..T.. ...C..G... ..G..A..T.
Turbinella          C.AT.A..T. .A...T.A.. ....CC..... ..A..T...C ....T..A.. A..A..... ..A..A.... ....AT.... .......... ..C.....C.
Oliva               C.A..A.... ........T.. GT.G..... ..A..T.... .......A.. A..A..AT.A T.A..A.... ....A..T.. .......... .....A..T.
Arctomelon          ..A.....T. .......... ....C.T... ..G..T...C .T.....A.. C.....T..A .....G.... ....A..... ...C...... .....A..T.
Scaphella           ..A..G..C. .A.....T.. AT..... ..A..T.... .G..C..A.. A.....GT.A T.A..G.... ....AT.... .......... .....A..T.
Murex               ..A.....T. .......G.. ...TC.T... ..A..... ........A. ......TT.A ..G..G..C. ....AT.... ......T... .....T....
Phylonotus          C.T.....T. ....CT.A.. ...TC.T... ..A..T..G. .......... C.....TT.A T.G..A.... .....T.... ......T... .....T....
Coralliophila       ..T"..... .G.GA..A.. .T..C..... ..A..T...C .C..T..A.. A..A...T.A ..C.....C. ....A..... C.....G... ..T.......
Thais               C....A.... .G...T.A.. ...TC.C... ..G..T..GC .C..G..A.. C.....TT.A .....A.... ....AT.... .......... ..G.....
Conus               ---------- ---------- ----...... ..T....G. .........A ...T..AT.A .....A.... .C..A..... C.....C... .....A....
Hastula             ........T. .......A.. .T.GC.T... ..T..T..GC ....G...". ......GT.G .....A.... .C.....G.. C.......... ..T..A..T.
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                    110       120       130       140       150       160       170       180       190       200
                    .         .         .         .         .         .         .         .         .         .
Cerithium           ATGCCTTCGT AATAATTTTC TTTTTAGTAA TGCCAATAAT AATTGGTGGA TTTGGCAATT GATTGGTTCC CCTTATGTTG GGAGCTCCTG ACATAGCTTT
Busycon carica      ....T..G.. ......C..T .........T. ....T..... G.....A... .....G..C. .G..A..... TT.A..A..A .........A. .T.......
Busycon sinistrum   ....T..... ......C..T .........T. .......... G.....A... .....G..C. .G..A..... TT.A..A..A .....C..A. .T.....C..
Busycotypus         ....T..G.. ......C..T .........T. .A..C..... G.....A... .....A..C. ....A..... TT.A..A..A .........A. .T.......
Neptunea            ....T..T.. ..........T ..CC.G.... .A..C..... ......G... .....G.... ....A..... TT.A..A..A ..G.....A. .T.......
Buccinum            ....T..... ..........." ...C...... ....T..... ......A..T .....G.... ....A..... TT.A..A..A ..G.....C. .T.......
Ilyanassa           .C..T..... .........AT ..C....... .A........ G.....A..G .....T.... ....A..... TT.A..AC.A .......... .T..G.....
Turbinella          ....T..TA. T.........T ..CC....T. .A..T..... ......A..T ..C..A.... ....A..C.. AT.A.....A .....C..C. ..........
Oliva               ....T..T.. T.........T ..C.....T. .......... G.........T .....A.... ....A..... .T.G.....A ..G.....A. .T.......
Arctomelon          ....T..T.. T.........T .......... .A..T..G.. ......A... ..G..T.... ..C.A..G.. TT.A..A..A ..G.....A. .T.......
Scaphella           ....T..T.. C.........T ...C....T. .A........ .........C .....T.... .....A.... TT.A..A..A ......TTA. ..........
Murex               ....T..T.. T.....C... ........T. .......... ......A... .....T.... ....A..G.. T.....AC.T ..T.....A. .T..G.....
Phylonotus          .C.....T.. T......... ........T. ....T..G.. .........T .....A.... ....A..G.. T.....A..A ..G.....G. ..........
Coralliophila       .C..T..T.. T.........T ..CC..... ....C..T.. .......... .....T.... .G.....A.. T.....A..A ..T.....C. ..........
Thais               ....T..T.. ......C... ........T. .A..C..G.. G.....A..G .......... ..C....... ...G..A..A ..T..A..A. .T.......
Conus               ....T..T.. T.....C..T ...A...... .A..T..... G.......... .....A.... .G.....A.. "G.G.....A ....A..A.. .T....T...
Hastula             ....T..T.. T.........T ..C.....T. .......G.. .......... ..C..A.... ....A..A.. AT.A...C.A .......... .T..G.....
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                    210        220        230        240        250        260        270        280        290        300
                    .          .          .          .          .          .          .          .          .          .
Cerithium           CCCTCGATTA AATAATATAA GTTTCTGACT TTTACCTCCA GCTCTTCTCC TCCTTTTATC TTCAGCAGCA GTAGAGAGGG GTGTTGGAAC TGGCTGAACT
Busycon carica      ......T... .......... .A..TG.GT. A..G..C..T ..CT.AT.AT .GT.AC.T.. A.....T... .....A..T. ....A..T.. ...A......
Busycon sinistrum   T.....T... .......... AA..TG."T. A..G.....T ...CT.AT.AT .GT.AC.T.. A.....T... .....A..T. ....A..T.. ...G......
Busycotypus         ......"C.. .......... .A...G..T. A.........T ..."T.AT.G. .AT.GC.T.. A.....T... .....A..T. ....G..T.. ...A......
Neptunea            .......... .......... .A........ .C.T..C..T ...T.A..TT .AT.AC.T.. A..T..T... ..C..A..T. ....C..... G..A.....A
Buccinum            T.....CC.. .....C..R. AA........ A.........T ...T.A.... .GT.AC.T.. A.....T... ..T..A..A. ....A..G.. "..A......
Ilyanassa           T.......... .......... .A......T. GC.T.....T ...T.A..T. .TT.A..G.. ......C... ..T..A..A. .A........ ...G......
Turbinella          T.......... .....A.... ....T...T. A.........  ..AT.AT.A. .T...C.T.. A.....T... ..T..A..A. .G........ A..T......
Oliva               .......C.. .....C.... .A..T...T. GC.T.....C T..T.A..AT .AT.AC.T.. A.....T... ..G..A..C. .G.....T.. ...T......
Arctomelon          ...A...... ..C....... ....TG.G.. AC.G.....T ...T.A..AT .GT.AC.T.. A.....G... .....A..C. .A.....G.. ..........
Scaphella           T..A..TC.T ........G. .A..T..... .C.......T ...T.A..TT .AT.AC.T.. ...G..C..G .....A..T. .......... A..T......
Murex               T.....GC.. .......... .A..T..GT. AC.T.....G ..A.....T .AT.A..G.. ...T..T..G .....A..T. .G.....G.. C..A.....G
Phylonotus          T.......... ........G. .A.....G.. GC.T..... ..A.....T. .TT.A..... ...T..T... .....A..A. .A..G..G.. ...G.....A
Coralliophila       T......C.T ........G. ....T..... CC.T..C..T ..A..AT.G. .G...C.T.. ...T.GT..T .....A..... .......G.. ......G..A
Thais               T.....T... .....C..G. .A..T..... GC.T.....T ..G.....T. .AT.AC.T.. C.....T..C .....A..T. .A.....G.. A..A......
Conus               .......... .....C.... ........GT. GC.C.....T ..A...T.A. .T..".....  .....T... .....A..A. .A..G..... A..A..G..A
Hastula             T..C..T... .....C.... .......... .........T ..AT.A..AT .A........ A..GT.T..G ........A. .......... ...A......
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                    310        320        330        340        350        360        370        380        390        400
                    .          .          .          .          .          .          .          .          .          .
Cerithium           GTTTATCCTC CTTTAGCAGG GAATCTAGCT CACGCAGGGG GATCAGTCGA CTTAGCTATT TTCTCTCTTC ACCTAGCTGG GGTTTCTTCT ATTTTAGGGG
Busycon carica      ..A.....C. .......... A...T..... ..T..C..A. .T..T..A.. ......A... .....T.A.. .T..T..A.. A.C...A..G ........A.
Busycon sinistrum   ..A.....C. .......... A...T.G... ..T..C..A. .T..T..A.. ......G... .....T.A.. .T..T..A.. A.C...G..A ........A.
Busycotypus         ..A....... .......... A..CT....C ..T..T..A. .T..T..A.. ...G..A... ..T...T.A. ....T..A.. A.C...A..A ........A.
Neptunea            ..A....C.. ..C....... T...T..... ..T....... .G.....A.. .C....A... ..T...T.A. .T..T..A.. T..A.....A ........A.
Buccinum            ..A..C.... .....T.... A..C..G... .....C..A. .C.....T.. TC.T..A... .....T.A. .T..T..A.. T.....A..A ..........
Ilyanassa           ........A. .....T.T.. T...T..... ..T..T..T. .T.....T.. .......... ..T...T.G. .T..T..A.. T..G.....A ...C....A.
Turbinella          .....C..A. .CC.T..C.. A..C..G... ........A. .G..T..... .C..A.A... .......... .T........ T.....C..A ........T.
Oliva               .......... .G.....T.. A...T..... .....C..T. .T..G..T.. ...G..A... ..T....... .TT....A.. T.....A... ........T.
Arctomelon          .......... .......G.. T...T.G... .....C..T. .T..G..T.. .C........ ..T..CT.G. .T..T..... T.CC..A... ........T.
Scaphella           .....C.... ..C.T..T.. A...T....A ..T..T.... .T..T..A.. T......... ..T.....A. .T..T..G.. A.C......A ........A.
Murex               ..A....... ..C.T..C.. ....T.G..A ..T..T..T. .G..T..A.. TC........ ..T...T.A. .TT....... T.......... ...C.T..T.
Phylonotus          .......... ..C.T..T.. A...T....G ..T..T..A. .G..T..A.. T.....A... ..T...T.A. ..T....... T.....C..... ...C....T.
Coralliophila       ..G.....A. ..C.CT.T.. T...G.T..A ..T..C..T. .C..T..A.. TC.G.....C ..TAGC.... .T..G..... T..A..C..... ...C....T.
Thais               .....C.... .A..G..T.. ......T..G ..T..T..A. .T.....AA. TC.T..A... ..T.....G. .TT....A.. T..A..C..... ...C..G..T.
Conus               ..A....... ..C.T..... A.....T... ..T..C..A. ....T..A.. T.....A... ..T....... ....T..... T.....C..... ........A.
Hastula             ..A.....A. ....G..T.. ....T..... ..T..T..T. .T.....G.. ...G...... ..T...T.A. .T..T..A.. T.C....... .....G..A.
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                410        420        430        440        450        460        470        480        490        500
                .          .          .          .          .          .          .          .          .          .
Cerithium       CTGTAAACTT CATTACCACA ATTATTAACA TGCGATGACG AGGAATACAG TTCGAGCGGC TTCCTCTTTT TGTGTGGTCA GTAAAAATCA CTGCCGTTCT
Busycon carica  .......... T.....A..T ........T. .A........ ...T..G... .....A..A. .....T.A.. ...A..A... .........T. .A..TA..T.
Busycon sinistrum .......... T.....A..T ........T. .......... ...T..G..A ..T.....A. .....T.A.. ...A..A... .........T. .A..TA..T.
Busycotypus     .......... T.....A..T ........T. .A........ ...T...... .....A..A. .....T.A.. A..A..A... .........T. .A..TA..T.
Neptunea        .......T.. T..C..A..T ........T. .A..G..... ...T..G..A ........A. .....T.A.. ...A..A..T .........T. .A..AA..T.
Buccinum        .......... T.....A..T ........T. .A........ ......G..A ..T.------ ---------- ---------- ---------- ----------
Ilyanassa       .......T.. T.....A..T ........T. AA........ ......G..A ..T.....T. .....T.A.. ...A..A..T .........T. ....AA..T.
Turbinella      .......T.. T.....T..T G.C.....T. .A........ ....G.T... .....A..A. .......... ...A..A... .........T. ....AA..T.
Oliva           .......T.. T.....A..T ........T. ....T..... ......G... ..T..A.... .....T.A.. ...A..A... .....G..T. .A...A..T.
Arctomelon      .A..T..... T.....A..T ..C..A..T. .A..G..... ...T...... ........CG ....C..C.. ...A..A..T .....G..T. .G..TA..T.
Scaphella       .A..T..T.. T.....G..C ........T. .A........ .......... .......A ..T.....AG .....T.A.. .A.T..A... C....G..T. .AA.TA..T.
Murex           .......... T.....G..C .......... .......... G..G..... ........AG .C...T.A.. ...T...... ..T.....T. .G..TA..T.
Phylonotus      .......T.. ......A..T ........T. .A........ ...G.....A ..T..A..TG .C...T.G.. ...T..A..T ..T..G..T. .A..TA..T.
Coralliophila   .......... T.....A..T G....C..T. .A.......A G..C.....A ..T....... .....T.A.. ...T.....T .....G..T. ....TA..T.
Thais           .A........ T.....A... .......... .A..T..G.. T..T.....A ..T....... .....T.A.. ...A.....T ..G.....T. ....AA..T.
Conus           .G.....T.. T.....T... ..C.....T. .A.......A ......GA.. ..T..A..TT .AT.CT.A.. ...T...... .........T. ....TA..T.
Hastula         .......T.. ......T... ........T. .A.....G.A ...G..G..A C.T.....T. .....T.G.. ...T..A..G ..G..G..T. ....T...T.

                510        520        530        540        550        560        570        580        590
                .          .          .          .          .          .          .          .          .
Cerithium       TTTACTTCTT TCCCTGCCTG TACTTGCAGG TGCTATTACT ATGCTTCTTA CTGACCGGAA CTTTAATACA GCTTTCTTCG ATCCTGCTGG A    [590]
Busycon carica  A..G...T.A ..T.....A. .CT.A..C.. A........A ..A...T.A. ....T..A.. T.....C... ..C..T.... ....A..A.. .    [591]
Busycon sinistrum A..G...T.A ..T.....A. .CT.A..C.. A..C.....A ..A...T.A. ....T..A.. T.....C... ..C..T..T. ....A..A.. .    [590]
Busycotypus     A......T.A ..TT.A..A. .TT.G..T.. A..C.....A ..A...T.A- ---------- ---------- ---------- ---------- -    [547]
Neptunea        A.....CT.A ..T..T..G. .CT.A..T.. A......... ..A...T.A. ....T..A.. T.....C... ..C..T..". .C..A..A.. G    [590]
Buccinum        ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- -    [401]
Ilyanassa       A..G...T.A ..TT...... ..T.A..T.. G........A ........A. .A..T..A.. T.....T ....T..T. ....A..A.. .    [576]
Turbinella      AC.TT.A... ..'T.A.... .TT.A..G.. A..A..C..A ......T.A. ....T..A.. ........." ....T.... .C..A..... T    [589]
Oliva           GC.T...T.A ..T..... .CT.A..... A......... ...T.AT.A. ....T..... .........T ..A..T..T. .....C.. .    [591]
Arctomelon      AC..T.A..A ..TT.A..A. .TT.A..T.. .......... .....G..A. ....T..A.. T..C.....T T.C..T.... .C..A..... G    [591]
Scaphella       A.......... ..AT.A..A. .TT.A..T.. A..C..... ..AT.GT.A. ....T..A.. T........T ..A..T..T. .......... .    [591]
Murex           GC.G...T.A ..T..... .TT.A..T.. G..A..... .....GT.G. ....T..A.. T.....C..G ..A..T..T. .....C.. .    [590]
Phylonotus      G......T.A ..TT.A..A. .TT.A..... A......... ..AT.GT.A. .C..T..A.. T......... ..A.....T. .....C.. .    [591]
Coralliophila   A.....C..A ..TT.A..A. .GT.G..T.. G......... ...T.GT.A. .A..T..T.. .......... ..A..T..T. ....C..C.. .    [590]
Thais           AC.C...T.A ..T..T.... .TT.A..T.. A..A.....A .....AT.A. .G..T..A.. .......... ..A.....T. ....A..A.. G    [591]
Conus           AC.T...T.G ..T..T.... ..T.G..G.. ...A..... ..A..AT.A. .G..T..A.. T.........C .....T..T. ....A..A.. .    [565]
Hastula         A...T.A..A ..AT.A.... .T........ .......... ......T.A. ....T..T.. T..A.....G ..C..T..T. ....A..A.. .    [590]
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