Phylogeny of some gastropod mollusks derived from 18S rDNA sequences with emphasis on the Euthyneura

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ABSTRACT

The phylogenetic relationships among gastropod subgroups, with emphasis on the Euthyneura, were investigated through the analyses of nearly complete 18S rDNA sequences of 29 representative gastropods. Neighbor-joining, maximum-likelihood, and maximum-parsimony methods were used in the construction of phylogenetic trees. The 18S rDNA data support the monophyly of Vetigastropoda, the vetigastropod clade Trochoidea, and Caenogastropoda. However, the monophylies of two caenogastropod subgroups, Neotaenioglossa and Neogastropoda, are not supported. The basal position of Neritopsina is confirmed. Within the Euthyneura, the Stylommatophora and the Systellommatophora are monophyletic, but the Opisthobranchia, the Pulmonata, and the Basommatophora are not. The present study supports the inclusion of Succineidae within Stylommatophora. However the phylogenetic position of Systellommatophora within Gastropoda remains unresolved.

Additional key words: Mollusca, molecular phylogeny, Apogastropoda, Caenogastropoda, Opisthobranchia, Pulmonata, Vetigastropoda, Stylommatophora, Basommatophora, Systellommatophora, Archaeopulmonata.

INTRODUCTION

Of the molluscan classes, Gastropoda is the most diverse and the most ubiquitous group. It has successfully adapted to most habitats, including marine, freshwater, and terrestrial environments.

Many comparative studies were published based on morpho-anatomical characters, including those of shell, pallial complex, and nervous, reproductive, and digestive systems (for recent reviews, see Haszprunar, 1988a; Bieler, 1992; Ponder and Lindberg, 1997). However, the status of knowledge of the phylogenetic relationships among and within the gastropod subgroups is still controversial (e.g., Golikov and Starobogatov, 1975; Graham, 1985; Haszprunar, 1988a; Bieler, 1992; Ponder and Lindberg, 1996; 1997). This uncertainty is largely due to the lack of informative morphological characters common to the different taxa and the presence of the high level of phenotypic diversity observed in the Gastropoda.

In addition to morphological characters, molecular sequences have proven to be very useful for in phylogenetic reconstructions. 18S rDNA sequences are amongst the most informative molecular characters along a broad range of taxa within the Mollusca (e.g., Steiner and Müller, 1996; Winnepenninckx *et al.*, 1996; Winnepenninckx *et al.*, 1998a; Winnepenninckx *et al.*, 1998b; Adamkewicz *et al.*, 1997; Bargues and Coma, 1997; Canapa *et al.*, 1999) and other animal phyla. Several studies were published on the molecular phylogeny of Gastropoda based on the sequence data of 28S rDNA (Rosenberg *et al.*, 1994; 1997; Tillier *et al.*, 1994; Tillier *et al.*, 1996) and 18S rDNA (Winnepenninckx *et al.*, 1996; Winnepenninckx *et al.*, 1998a; Harasewych *et al.*, 1997a; b; 1998). Winnepenninckx *et al.* recently (1998a) investigated the phylogeny of gastropod groups below the class rank using the complete 18S rDNA sequences from 18 gastropod species.

To further address gastropod phylogeny with emphasis on Euthyneura (Opisthobranchia + Pulmonata), a group that has not been examined or discussed in detail from 18S rDNA data, we determined the complete 18S rDNA sequences for five representative gastropods. They include the first complete sequence data from Cephalaspidea (within Opisthobranchia) and Archaeopulmonata (within Pulmonata), and additional sequence data from Vetigastropoda and Stylommatophora (this latter within Pulmonata). These sequences were analyzed in conjunction with previously published sequences of 24 other gastropods. We focus on testing the monophyly of each of the euthyneuran subgroups, the Opisthobranchia (e.g., Boettger, 1955; Ghiselin, 1965; Gosliner, 1981; 1985; 1991; Gosliner and Ghiselin, 1984; Poulicek *et al.*, 1991; Ponder and Lindberg, 1997), the Basommatophora (e.g., Tillier, 1984; Haszprunar and Huber, 1990; Nordsieck, 1992), the Stylommatophora (e.g., Nordsieck, 1992), and the Systellommatophora (Salvini-Plawen, 1980; Climo, 1980; Tillier, 1984; Haszprunar and Huber, 1990; Nordsieck, 1992). We also discuss the phy-

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logenetic position of the Succineidae in the Stylommatophora (Rigby, 1965; Solem, 1978; Tillier, 1989; Nordsieck, 1992). In addition, we examine the monophylies of the Vetigastropoda (Salvini-Plawen, 1980; Salvini-Plawen and Haszprunar, 1987; Haszprunar, 1988a; b; Ponder and Lindberg, 1996) and the vetigastropod clade Trochoidea (Haszprunar, 1988a).

MATERIALS AND METHODS

Specimens Analyzed

The 18S rDNA sequences of two vetigastropods (*Nordotis discus*, 1858 base pairs, from Cheju Island and *Batillus cornutus*, 1859 base pairs, from Mara Island), one opisthobranch (*Bullacta exarata*, 1849 base pairs, from Inchon), and two pulmonates (*Ellobium chinensis*, 1845 base pairs, from Tamjin River, and *Acusta despecta sieboldiana*, 1847 base pairs, from the Campus of Seoul National University). The material above was collected in Korea, and their sequences are described for the first time in the present study. The sequences of two neogastropods (*Rapana venosa* and *Reishia bronni*), one pulmonate (*Anthosiphonaria sirius*), and one chiton (*Lepidozona* (*Lepidozona*) *coreanica*) were reported in our previous study (Yoon *et al.*, 1996) and the sequences of the remaining 21 other gastropods and 2 bivalves were obtained from GenBank.

The nearly complete 18S rDNA sequences were analyzed for the 29 representative gastropods (one neritoid, three vetigastropods, nine caenogastropods, two opisthobranchs, and 14 pulmonates), two bivalves, and one chiton species. Of these, the polyplacophoran *Lepidozona* (*Lepidozona*) *coreanica* was used as an outgroup, as the class Polyplacophora (included in the Aculifera) is currently accepted as the stem group of the classes Gastropoda and Bivalvia (included in Conchifera), from studies based on morphological characters (see Salvini-Plawen, 1980; 1990; Haas, 1981; Runnegar and Pojeta, 1985; Brusca and Brusca, 1990; Ponder and Lindberg, 1996) as well as molecular results (Adamkewicz *et al.*, 1997; Bargues and Mas-Coma, 1997; Harasewych *et al.*, 1997b). Table 1 lists the studied taxa and GenBank accession numbers for the respective sequences. The baseline classification used in this work follows Haszprunar (1988a) for Streptoneura, Vaught (1989) for Opisthobranchia, and Boss (1982) for Pulmonata.

DNA Extraction, PCR Amplification, and Sequencing

Total nucleic acids were extracted from foot muscle of live-collected and ethanol-preserved snails by modifications of standard procedure of Sambrook *et al.* (1989). The 18S rDNAs were amplified using the polymerase chain reaction (PCR) with two oligonucleotide primers corresponding to conserved sequences proximal to 5′ and 3′ termini of metazoans (Nelles *et al.*, 1984: 1–19, 5′-CCTGGTTGATCCTGCCAG-3′; 1848–1868, 5′-TAATGATCCTTCCGCAGGTTA-3′: the numbers cor-

Table 1. Gastropod species used in the present study, with GenBank accession numbers for sequences.

NERITOPSINA
NERITOIDEA
Neritidae
Nerita albicilla X91971
VETIGASTROPODA
HALIOTOIDEA
Haliotidae
**Nordotis discus* AF082177
TROCHOIDEA
Trochidae
Monodonta labio X94271
Turbinidae
**Batillus cornutus* AF165311
CAENOGASTROPODA
NEOTAENIOGLOSSA
LITTORINOIDEA
Littorinidae
Littorina littorea X91970
Nodilittorina punctata Y11755
CALYPTRAEOIDEA
Calyptraeidae
Crepidula aduncа X94277
TONNOIDEA
Bursidae
Bursa rana X94269
NEOGASTROPODA
MURICOIDEA
Muricidae
Reishia bronni X98827
Rapana venosa X98826
Buccinidae
Pisania striata X94272
Nassariidae
Nassarius singuijorensis X94273
Fasciolariidae
Fasciolaria lignaria X94275
EUTHYNEURA
OPISTHOBRANCHIA
CEPHALASPIDEA
PHILINOIDEA
Hamineidae
**Bullacta exarta* AF188675
APLYSIOMORPHA
APLYSIOIDEA
Aplysiidae
Aplysia sp. X94268
PULMONATA
ARCHAEOPULMONATA
ELLOBIOIDEA
Ellobiidae
**Ellobium chinensis* AF190452
BASOMMATOPHORA
SIPHONARIOIDEA
Siphonariidae
Anthosiphonaria sirius X98828
Siphonaria algesirae X91973
LYMNAEOIDEA
Lymnaeidae
Lymnaea glabra Z73982
Bakerilymnaea cubensis Z83831
STYLOMMATOPHORA
MEASURETHRA
CLAUSILIOIDEA
Clausiliidae
Balea biplicata X94278
HETERURETHRA
Succineidae
Oxyloma sp. X94276
Omalonyx matheroni AF047199
Athoracophoridae
Athoracophorus bitentaculatus AF047198
SIGMURETHRA
ACHATINOIDEA
Achatinidae
Limicolaria kambeul X66374
HELICOIDEA
Bradybaenidae
**Acusta despecta sieboldiana* AF190453
Helicidae
Helix aspersa X91976
SYSTELLOMMATOPHORA
ONCHIDIIOIDEA
Onchidiidae
Onchidella celtica X70211
VERONICELLOIDEA
Veronicellidae
Laevicaulis alte X94270

Note: Classification follows Haszprunar (1988a) for Streptoneura, Vaught (1989) for Opisthobranchia, and Boss (1982) for Pulmonata. * New sequences marked with asterisk.

respond to positions of human 18S rDNA). PCR amplifications were performed with Taq DNA polymerase for 30 cycles (94° C for 1 min, 52° C for 2 min, and 72° C for 3 min). The ends of the amplified DNA fragments were modified for blunt-ended ligation using T4 kinase and T4 polymerase. The blunt-ended 18S rDNAs were ligated into pGEM-3zf(-) plasmid vector and transformed into DH5-α cell lines. Sequencing primers used in this study were reported in a previous paper (Moon *et al.*, 1996). 18S rRNA-coding regions were completely sequenced in both directions with complete overlap. The DNA sequencing was performed by the dideoxynucleotide chain-termination method (Sanger *et al.*, 1977) using a Taq-Track kit (Promega Co.), according to the manufacturer's instructions. Electrophoresis of sequencing reaction mixtures was performed on buffer-gradient 6% polyacrylamide gels and examined by autoradiography.

Phylogenetic Analysis of 18S rDNA Sequences

The sequences were initially aligned with the CLUSTAL W multiple-alignment program (Thompson *et al.*, 1994) and the alignment refined manually. A data-set of alignment-stable positions was produced by excluding those positions that differed between alignments (Gatesy *et al.*, 1993). Analyses were limited to reliably aligned regions, which included a total of 1754 nucleotide positions. Phylogenetic reconstructions were performed using the neighbor-joining (NJ), maximum-likelihood (ML), and maximum-parsimony (MP) methods. PHYLIP version 3.572c (Felsenstein, 1995) was used for the neighbor-joining (Saitou and Nei, 1987) analyses. The distance analyses were done using Kimura (1980) and Jukes and Cantor (1969) matrices as input for the neighbor-joining analyses. Maximum-likelihood analyses were performed using the HKY (Hasegawa *et al.*, 1985) model in PAUP 4.0b2 (Swofford, 1999). For the quartet puzzling method (the number of puzzling steps is 1000), empirical nucleotide frequencies, and transition/transversion ratio of 1.5 were estimated. Parsimony analyses were also performed using the computer program PAUP version 4.0b2 with closest stepwise addition options. The analyses employed a heuristic search using TBR branch swapping with random taxon addition. Branch length was optimized according to the ACCTRAN option. Bootstrap analyses (Felsenstein, 1985) of one hundred replicates were performed to examine the confidence of nodes in NJ, ML, and MP analyses.

RESULTS

Figure 1A shows the phylogenetic tree resulting from the neighbor-joining (NJ) analysis using the Kimura (1980) distances of an alignment of complete 18S rDNA sequences of 29 gastropod species. The polyplacophoran *Lepidozona* (*Lepidozona*) *coreanica* was used as outgroup. The same tree topology was also obtained using Jukes and Cantor (1969) distances. The Neritoidea branches off first and the Vetigastropoda (Trochoidea + Haliotoidea) diverges next as an independent clade before the clade Apogastropoda (Caenogastropoda + Euthyneura). The monophyly of the Vetigastropoda and its subclade the Trochoidea (represented by *Monodonta* and *Batillus*) is clearly shown in the tree, with very high boostrap support (100%: 100%). The Caenogastropoda shows a sister group relationship with the Euthyneura with very high bootstrap support (94%). Monophyly of the Caenogastropoda is supported (boostrap value = 100%), though neither the Neotaenioglossa (= Mesogastropoda) nor the Neogastropoda emerged as monophyletic clades.

There is strong bootstrap support (100%) for the monophyly of Euthyneura (Opisthobranchia + Pulmonata), though the monophylies of Opisthobranchia (Cephalaspidea + Anaspioidea) and Pulmonata are not supported. The Basommatophora, consisting of the Siphonarioidea and Lymnaeoidea, did not emerge as a monophyletic group. On the other hand, there is good support for the monophylies of two additional groups in the Euthyneura, the Stylommatophora (boostrap value = 84%) and the Systellommatophora (boostrap value = 87%). Bootstrap values strongly support the position of the Succineidae (*Oxyloma* and *Omalonyx*) within the stylommatophoran clade. However, the position of the Systellommatophora within the Gastropoda was not positively determined in the present study.

The resulting tree from maximum-likelihood (ML) analyses of the same data set is shown in figure 1B. The ML tree confirms all the major results of the NJ tree (figure 1A), with the exception that clades within Euthyneura lack significant bootstrap-support. The new branching order of Systellommatophora (*Onchidella* and *Laevicaulis*), Aplysiomorpha (*Aplysia*), Archaeopulmonata (*Ellobium*)–Siphonarioidea (*Siphonaria* and *Anthosiphonaria*), Cephalaspidea (*Bullacta*), and Stylommatophora in the clade differs from the order shown in the NJ tree (figure 1A), Aplysiomorpha–Systellommatophora–Siphonarioidea and Archaeopulmonata–Cephalaspidea–Stylommatophora.

The maximum parsimony (MP) analyses produced a single tree with minimum length of 950 steps (figure 1C). Generally speaking, MP analyses also yielded similar results except for minor differences in topologies among groups within the caenogastropod and the euthyneuran clades. Maximum parsimony shows topological shifts within the caenogastropod clade, e.g., the new branching order of *Bursa*, *Nassarius*, and *Pisania–Fasciolaria–Crepidula* instead of the order shown in the NJ tree (figure 1A), *Crepidula*, *Bursa*, *Pisania*, *Nassarius*, and *Fasciolaria*. Within the Euthyneura, the MP tree differs from the NJ tree only in those branching points with low bootstrap values. There is no sister-group relationship among the Cephalaspidea (*Bullacta*), Aplysiomorpha (*Aplysia*), Archaeopulmonata (*Ellobium*), Siphonarioidea, Stylommatophora, and Systellommatophora.

Next, we focused on the Euthyneura in separate,

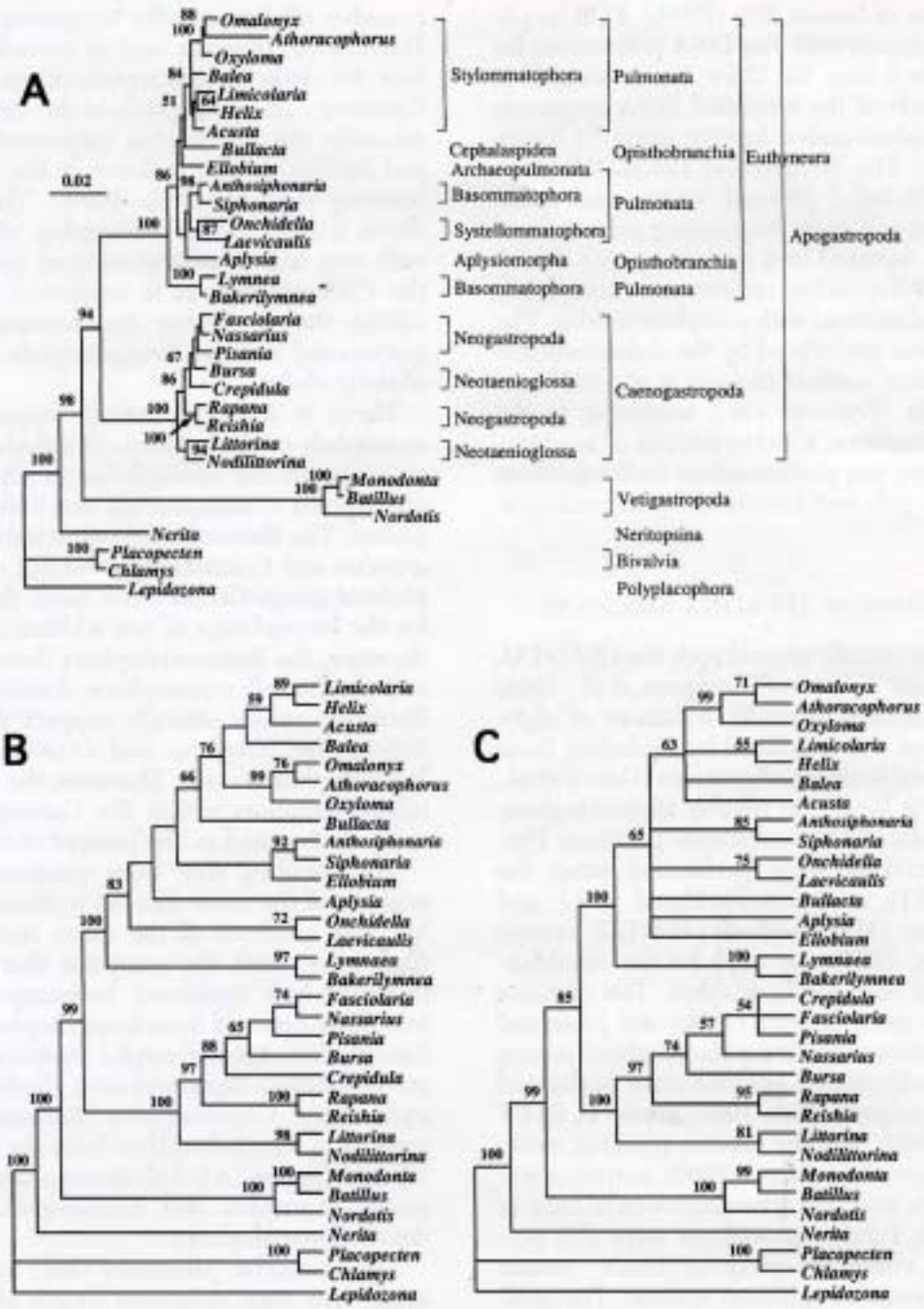

Figure 1. **A.** Neighbor-joining tree determined by an alignment of 29 nearly complete 18S rDNA sequence data for gastropods with *Lepidozona coreanica* (Polyplacophora) as outgroup. Bootstrap percentages are shown above branches supported in at least 50% of 100 replicates. **B.** Strict consensus tree resulting from maximum-likelihood analyses of 29 nearly complete gastropod 18S rDNA sequences. Quartet puzzling method and HKY (Hasegawa *et al.*, 1985) setting model were used. *Lepidozona coreanica* (Polyplacophora) was the outgroup. Bootstrap analysis was performed with 100 replicates; values above 50% are indicated above the nodes. **C.** Strict consensus tree of maximum parsimony analyses based on the 333 informative sites of an alignment of 29 nearly complete gastropod 18S rDNA sequences (length = 950; CI = 0.6611; RI = 0.8418). *Lepidozona coreanica* (Polyplacophora) was the outgroup. Bootstrap values higher than 50% are indicated above the nodes.

mainly because the use of outgroups that are too far removed from the clade in study may give origin to additional homoplasies between ingroup and outgroup species. Figure 2A shows the results of NJ analyses of 16 nearly complete euthyneuran 18S rDNA sequences, with the caenogastropod *Littorina littorea* as outgroup. The resulting tree exhibits the same topology as the complete NJ tree (figure 1A), with the exception that *Ellobium* (Archaeopulmonata) becomes the sister group to the clade Aplysiomorpha *(Aplysia)* + Systellommatophora–Siphonarioidea, instead of clade Cephalaspidea (*Bullacta*) + Stylommatophora, as present in the com-

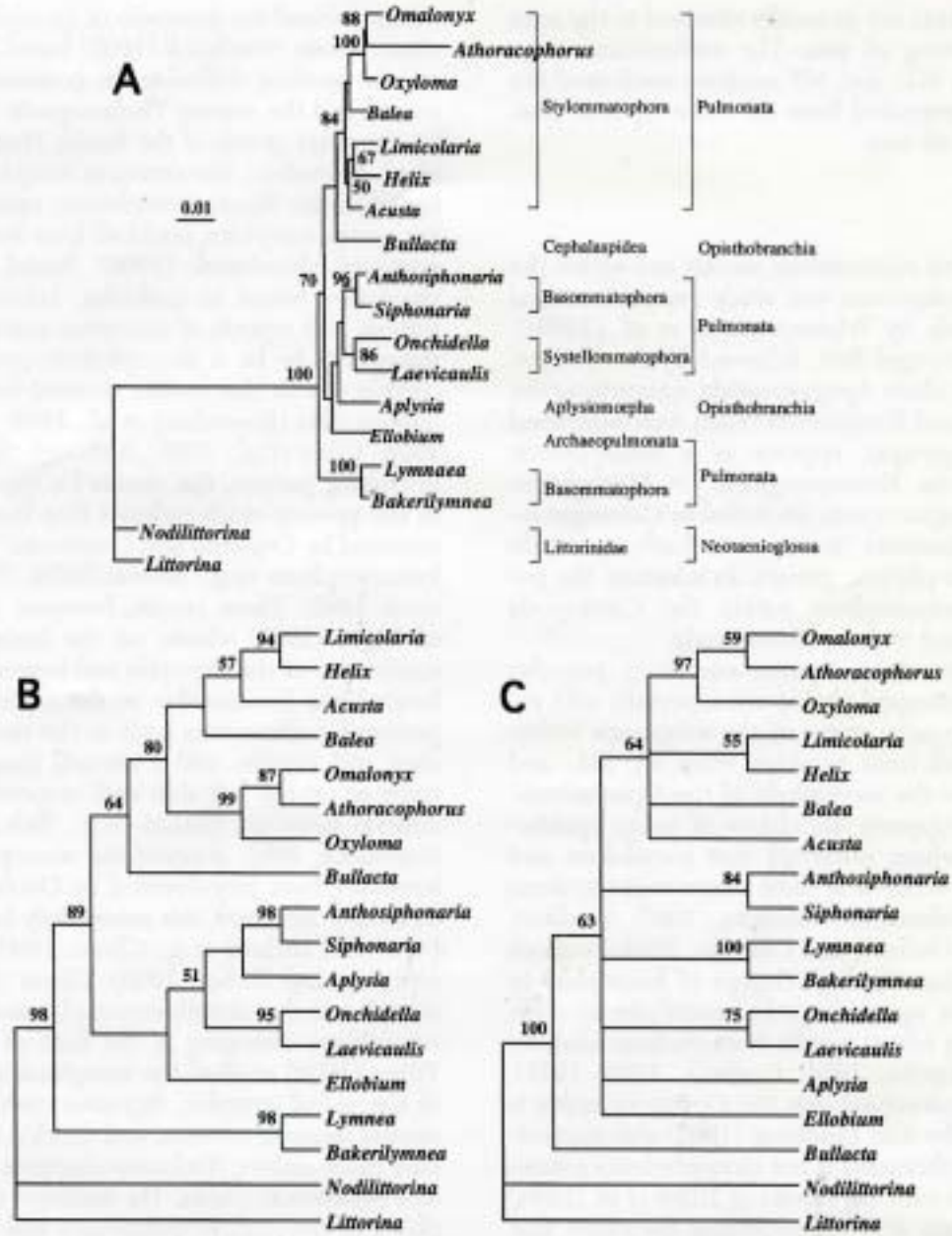

Figure 2. **A.** Euthyneuran neighbor-joining tree determined by an alignment of 16 nearly complete euthyneuran 18S rDNA sequences, using *Littorina littorea* (Caenogastropoda: Littorinidae) as outgroup. Numbers at a node indicate bootstrap values higher than 50%. **B.** Euthyneuran maximum-likelihood tree determined by an alignment of 16 nearly complete euthyneuran 18S rDNA sequences, using *Littorina littorea* (Caenogastropoda: Littorinidae) as outgroup. Quartet puzzling method and HKY (Hasegawa *et al.*, 1985) setting model were used. Bootstrap values above 50% are indicated above the nodes. **C.** Euthyneuran maximum parsimony tree calculated from the 149 informative sites of an alignment of 16 nearly complete euthyneuran 18S rDNA sequences, using *Littorina littorea* (Caenogastropoda: Littorinidae) as outgroup (length = 415; CI = 0.7494; RI = 0.6750). Only bootstrap values higher than 50% are indicated.

plete NJ tree. These two clades are not supported by bootstrap analysis of the euthyneuran clade. When the euthyneuran ML tree (figure 2B) is compared to the entire ML tree (figure 1B), small topological shifts are discernible. The Siphonarioidea shows a sister group relationship with Aplysiomorpha (*Aplysia*) instead of with Archaeopulmonata (*Ellobium*), and this Siphonarioidea–Aplysiomorpha cluster appears as sister group of the Systellommatophora instead of Cephalaspidea–Stylommatophora. However, bootstrap values that support these nodes are very low. Nevertheless, the euthyneuran ML tree strongly supported most of the major nodes found in the entire ML tree, with generally higher bootstrap values. Maximum parsimony analyses based on the 149 phylogenetically informative characters of the alignment of 16 euthyneuran species produced a single tree with minimum length of 415 steps (figure 2C). In the euthyneuran MP tree, the first branching member is Cephalaspidea (*Bullacta*) rather than Lymnaeoidea (Basommatophora) found in the entire MP tree. The topologies

for the remaining taxa are generally identical to the ones in the tree containing all taxa. The euthyneuran trees resulting from NJ, ML, and MP analyses confirmed the topology of trees generated from the same types of analyses but based on all taxa.

DISCUSSION

For the phylogenetic relationships among and within the gastropod major subgroups, our study supports several aspects of the study by Winnepennickx *et al.* (1998a). The Neritoidea diverged first, followed by the Vetigastropoda. Next, the clade Apogastropoda, comprising the Caenogastropoda and Euthyneura (each well supported as monophyletic groups), appears as a monophyletic group. However, the Neotaenioglossa (= Mesogastropoda) and the Neogastropoda (included in Caenogastropoda) and the Pulmonata (included in Euthyneura) do not appear as monophyletic groups. In addition, the position of Systellommatophora within the Gastropoda could not be defined in the present study.

In contrast to previous reports, our study provides more details on gastropod phylogeny, especially with regard to the phylogenetic status of the subgroups within the Euthyneura. All trees resulting from NJ, ML, and MP analyses refute the monophyly of the Opisthobranchia. This result supports the claims of many opisthobranch workers, whom observed that parallelism and convergence have occured in most major organ systems within the opisthobranchs (Ghiselin, 1965; Gosliner, 1981; 1985; 1991; Gosliner and Ghiselin, 1984; Poulicek *et al.*, 1991) and that the high degree of homoplasy in many characters in opisthobranchs contribute to difficulties in obtaining robust results from cladistic analyses (Gosliner and Ghiselin, 1984; Gosliner, 1985; 1991). Boettger (1955) maintained that the Opisthobranchia is paraphyletic. Ponder and Lindberg (1997) also suggested that the Opisthobranchia is not monophyletic, a viewpoint in agreement with the results of Tillier *et al.* (1994) from 28S rDNA data. It is not surprising, therefore, that many contradictory phylogenies and classification schemes have been suggested for the Opisthobranchia (e.g., Boettger, 1955; Taylor and Sohl, 1962; Ghiselin, 1965).

In relation to the phylogeny of the Pulmonata, we have mentioned that the Basommatophora (*sensu* Haszprunar and Huber, 1990, Siphonarioidea + Lymnaeoidea), is not monophyletic. Tillier (1984) considered that only Lymnaeoidea belongs to Basommatophora and Siphonarioidea to Archaeopulmonata. He suggested that pulmonates radiated into freshwater habitats as Basommatophora and into marine habitats as Archaeopulmonata. Tillier *et al.* (1996), based on 28S rDNA data, showed that the Basommatophora is not monophyletic. However, Haszprunar and Huber (1990) suggested that both Siphonarioidea and Lymnaeoidea could be allocated in Basommatophora due to the presence of common morphological characters such as a procerebrum comprising only large cells, the lack of a contractile pneumostome, and the presence of an osphradium and pallial ciliary tracts. Nordsieck (1992) based on the presence of an anal opening shifted to the posterior mantle lobe, also considered the marine Thalassophila (Siphonarioidea) to be the sister group of the limnic Hygrophila (Lymnaeoidea). Therefore, the common morphological characters found in the Basommatophoran, rather than representing synapomorphies, could all have been derived by convergence. Nordsieck (1992), based on morphological characters found in tentacles, kidney, central nervous system, and aspects of ontogeny, considered the Stylommatophora to be a monophyletic group, which is concordance with the results derived from 28S rDNA sequence data (Rosenberg *et al.*, 1994; 1997; Tillier *et al.*, 1994; Tillier *et al.*, 1996). Although there is instability of branching pattern, the results for the euthyneuran clade in the present study indicate that the Succineidae (represented by *Oxyloma* and *Omalonyx*) belongs to the Stylommatophora (e.g., Solem, 1978; Tillier, 1989; Nordsieck, 1992). These results, however, contradict the view of Rigby (1965) whom, on the basis of morphological similarities of the digestive and reproductive systems, allocated the Succineidae in the Opisthobranchia. Synapomorphic characters such as the more or less reduced shell and mantle, and a visceral ganglion situated centrally or on the left side with respect to the axis of the central nervous system (e.g., Salvini-Plawen, 1980; Nordsieck, 1992) support the monophyly of the Systellommatophora (represented by Onchidiidae and Veronicellidae). However, this monophyly has been questioned by several authors (e.g., Climo, 1980; Tillier, 1984; Haszprunar and Huber, 1990). Climo (1980) in particular considered the systellommatophorans as a polyphyletic assemblage diverging at the base of the euthyneurans. Tillier (1984) studied the morpho-anatomical characters of the pallial complex, digestive tract, reproductive, and central nervous systems, and divided the Pulmonata into only three orders; Archaeopulmonata, Basommatophora, and Stylommatophora. He included the Systellommatophora in the Archaeopulmonata and suggested that the Onchidiidae (within Systellommatophora) is more closely related to the Ellobiidae (within Archaeopulmonata) than to the Veronicellidae (within Systellommatophora). However, our present result supports the monophyly of Systellommatophora.

Since the erection of the Vetigastropoda by Salvini-Plawen (1980), the presence of synapomorphic characters such as ctenidial sense organs, the epipodial sense organs, and the special structure of the esophagus have generally supported the monophyly of this clade (Salvini-Plawen and Haszprunar, 1987; Haszprunar, 1988a; b; Ponder and Lindberg, 1996; 1997). Other vetigastropod features include the dominant presence of the right dorsoventral retractor muscle, the right excretory organ, and bilamellate ctenidia with skeletal rods. Previous molecular data using partial 18S rDNA (Harasewych *et al.*, 1997a; b) and the 28S rDNA (Tillier *et al.*, 1994) sequences also supported the monophyly of the Vetigastropoda, which is also supported in the present study.

Monophyly of the Trochoidea (represented by *Monodonta* and *Batillus*) is also confirmed herein. The Trochoidea is defined by synapomorphies such as loss of the right ctenidium in relation to the loss of the shell slit (Haszprunar, 1988a; b) and the monophyly of the group is also in concordance with the study based on 28S rDNA sequences by Tillier *et al.* (1994).

In conclusion, the 18S rDNA data strongly support the monophyly of the following higher gastropod clades: Vetigastropoda, Trochoidea (within Vetigastropoda), Apogastropoda, and the two included clades Caenogastropoda and Euthyneura. Within the euthyneuran clade, both the Stylommatophora and the Systellommatophora are monophyletic. However, our 18S rDNA data failed to support monophyly of the Neotaenioglossa and the Neogastropoda (within Caenogastropoda), Opisthobranchia, Pulmonata, and the pulmonate Basommatophora. These non-monophyletic subgroups, therefore, at present can be considered as grades rather than clades. The basal position of Neritopsina is confirmed in this study. In addition, the Succineidae is included in Stylommatophora. Still, the Systellommatophoran position within the Gastropoda, that is, its immediate relationship to either Opisthobranchia or Pulmonata, or to any other group for that matter, could not be defined. The instability of topology and short branch lengths within the Caenogastropoda and the Euthyneura may be due to the fact that the mollusks, including gastropods, apparently radiated in an "explosive" fashion during a relatively short period of time. Most extant major groups of mollusks appeared around a relatively short time at the Precambrian/Cambrian boundary (e.g., Runnegar and Pojeta, 1985; Winnepenninckx *et al.*, 1996; Adamkewicz *et al.*, 1997; Harasewych *et al.*, 1997a). Future studies attempting to define phylogenetic relationships at these levels may take into consideration other molecules such as cytochrome *c* oxidase I and/or 16S rDNA. Such molecules evolve more rapidly than 18S rDNA, and seem more likely to contain information needed to solve phylogenetic relationships within these clades.

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