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Molecular Investigation of the Evolutionary Origins of Hydrothermal Vent Gastropods

by

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B.Sc., University of Western Ontario, 1991

A Dissertation Submitted in Partial Fulfillment of the Requirements for the Degree of

DOCTOR OF PHILOSOPHY

in the Department of Biology

We accept this dissertation as conforming to the required standard

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ABSTRACT

Hydrothermal vent communities exhibit great taxonomic novelty with 88% of species, 51% of genera, and 21% of families new to science. Given the severe physiological barriers to invasion presented by hydrothermalism and the energetic independence of the community due to *in situ* primary production by chemoautotrophic bacteria, it has been previously proposed that hydrothermal vents may have acted as a refugia for groups of metazoan animals that originated during the Cambrian, Paleozoic, or Mesozoic. The alternate explanation is of rapid change of recent immigrants from the adjacent deep-sea and false taxonomic inflation. Six major groups of hydrothermal vent endemic gastropods exhibit high taxonomic novelty and a lack of known fossils. Discovery of these hydrothermal vent endemic groups has resulted in dramatic changes in how we view the evolution and phylogeny of the Gastropoda, particularly in regards to the novel anatomy of the Neomphalina (Neomphalidae + Peltospiridae). Recent cladistic examinations of gastropod phylogeny using anatomical and morphological characters disagree on the placement and monophyly of the Neomphalina or find few characters supporting their position in the overall gastropod phylogeny. In this dissertation, a molecular systematic investigation of gastropod phylogeny was performed to examine the antiquity of the vent endemic Neomphalina.

Twenty-three new D1 domain and thirty new D6 domain DNA sequences of the 28S ribosomal RNA gene were obtained from fresh-frozen and formalin-ethanol preserved specimens. These were combined with previously published molluscan 28S ribosomal RNA sequences for a total of 159 sequences. Gastropod phylogeny was examined using both parsimony and distance-based analyses. The 28S ribosomal RNA gene exhibited saturation of substitutions beyond 15% divergence between sequences, estimated using Kimura's two-parameter model. Alone, either domain exhibited poor resolution of gastropod phylogeny but together (32 genera only) monophyly of the Neritimorpha, Neomphalina, Vetigastropoda, Patellogastropoda, Caenogastropoda (including *Viviparus*, *Ampullaria*, and *Campanile*), and Heterobranchia (Euthyneura plus *Valvata*) was supported by bootstrap values. Relationships among these groups could not be resolved due to saturation of substitutions. Evidence of elevated evolutionary rates in the Patellogastropoda conformed to previous studies and confounded analyses. Regardless, the hydrothermal vent Neomphalina exhibited divergence values and phylogenetic novelty equivalent to the other early-Paleozoic radiations, supporting its consideration as a vent refugial phylogenetic relic

28S ribosomal RNA sequences cannot resolve Cambrian or early Paleozoic radiations of the Gastropoda and use of diverse specimens limits reliability of sub-ordinal relationships due to long-branch attraction. Sequences of 28S ribosomal RNA are best used to examine within-order gastropod relationships due to saturation of substitutions at higher levels and among-order evolutionary rate variation.

Examiners:

Dr. V. Tunnicliffe, Co-Supervisor (School for Earth and Ocean Sciences)

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CONTENTS

TABLES

FIGURES

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In memory of Howard Bruce and Charles McArthur

INTRODUCTION

Overview

This dissertation covers three major themes: the evolutionary novelty of deep-sea hydrothermal vent communities, the systematics and phylogeny of gastropods (snails, slugs, limpets), and the use of tools from molecular biology to examine both (molecular systematics). The three themes arise from a single question: do the novel animals found at hydrothermal vents represent ancient lineages of animals that have survived refugially in these environments since the Mesozoic or Paleozoic?

Hydrothermal Vent Communities

Spurred on by the acceptance of plate tectonics theory as a general explanation for continental drift, oceanic spreading centres were first examined by submersibles in the 1970s (Lonsdale 1977, Corliss et al. 1979). These locations, where two of the earth's tectonic plates drift apart, are the source of new ocean crust as magma rises from the lithosphere to fill the space between the separating plates. The spreading of tectonic plates and the formation of new ocean crust result in mid-ocean ridges extending up to 2.5 km above and 1000-3000 km along the sea-floor. Roughly two-thirds of the heat released from the Earth's core is released by the formation and cooling of new oceanic crust at mid-ocean spreading centres. This release of heat results in hydrothermalism - the convection of sea-water through the heated lithosphere (Kennish & Lutz 1992, Fornari & Embley 1995). Cold sea-water enters the permeable crust at mid-ocean ridges and heat is transferred to it from the heated subsurface rocks. The heated sea-water then rises through the crust to emerge in the form of hydrothermal venting - strong or diffuse flow of hot water from the newly formed oceanic crust. Temperatures in the venting fluid can reach 400°C and the fluid is rich in hydrogen sulphide and heavy metals acquired from the crust, which precipitate from the sea-water as it cools to ambient temperature (2-4°C). The chemical composition of the venting fluid results in low acidity, variable salinity, variable concentrations of carbon dioxide, and near absent amounts of oxygen, nitrates, and phosphates (Tivey 1991).

The chemical, physical, and thermal extremes occurring at hydrothermal environments excluded the consideration of possible biological communities, but the first explorations found diverse and abundant animal communities living under the influence of hydrothermalism (Lonsdale 1977, Corliss et al. 1979). The biomass of hydrothermal vent communities exceeded that of other known deep-sea communities. Other deep-sea communities are energetically dependent upon primary production by plankton at the ocean's surface but what reaches the sea-floor is low-energy detritus from the food webs above (Tyler 1995). Deep-sea communities are thus low in biomass. In contrast, the hydrothermal vent communities are dependent upon *in situ* primary production by chemoautotrophic bacteria which fix inorganic carbon using energy derived from the oxidation of hydrothermally produced sulphide (Jannasch 1985, Jannasch & Wirsen 1985). Grazing and filter-feeding of chemoautotrophic bacteria forms the basis of the hydrothermal vent food-chain (see reviews by Grassle 1986, Tunnicliffe 1991, Lutz & Kennish 1993) but several groups of animals have evolved complex symbioses with chemoautotrophic bacteria and are thus energetically independent (Fisher 1990, Childress & Fisher 1992). Chemoautotrophy has resulted in a nearly self-contained ecosystem that supports a local high abundance of animals, independent of solar-derived energy. This abundance of life at hydrothermal vents has resulted in the frequent use of an oasis analogy for the hydrothermal environment (eg. Corliss & Ballard 1977, Carney 1994). This unique ecosystem is not completely independent of the surrounding ocean as oxygen, needed for chemosynthesis, must be imported from the surrounding sea-water. Since hydrogen sulphide and oxygen are mutually exclusive in sea-water, most hydrothermal vent animals must exist on the border between the two environments, the distance between which can be a matter of centimetres due to turbulence.

In addition to their abundance, the nature of hydrothermal vent animals is also unique. The community is dominated by animals with tubes or shells (polychaetes, vestimentiferans, bivalves, gastropods, crustaceans) in contrast to the sponges, cnidarians, and echinoderms of other deep-sea communities (Tunnicliffe 1992). Defense against the rain of heavy metal precipitates may be a requirement for successful invasion of the vent environment (Tunnicliffe 1992). Many of the animals exhibit physiological adaptations to the extremes of the environment: low oxygen, hydrogen sulphide poisoning, temperature fluctuations, and high heavy metal concentrations (Childress & Fisher 1992). The majority of species known from hydrothermal vent communities are endemic and new to science (Table 1). Even more strikingly, systematic novelty and endemicity extends to high taxonomic levels (Table 2). Newman (1985) and Tunnicliffe (1992) have both suggested these endemic elements may represent ancient animal lineages that have survived and radiated *in situ* at hydrothermal vents since their extinction elsewhere in the Paleozoic or Mesozoic. Sea-floor spreading has existed throughout the Phanerozoic and globally dispersed hydrothermal vent communities are more similar to each other than they are to neighbouring deep-sea communities, suggesting evolutionary independence of the

Table 1. Endemism of invertebrate animals found at hydrothermal vents. Data from V. Tunnicliffe (University of Victoria, Canada).

Taxon	Total Number	Number Endemic	Percentage Endemic
Species	**366**	**324**	**88.5 %**
Genus	**213**	**108**	**50.7%**
Family	**102**	**20**	**19.6%**
Order	**51**	**3**	**5.9%**
Class	**18**	**1**	**5.6%**
Phylum	**9**	**0**	**nil**

Table 2. Systematics of endemic hydrothermal vent families. Updated from Tunnicliffe (1992). A listing of systematic and anatomical citations for endemic gastropod groups is presented in the Appendix. The highest bolded taxonomic rank represents the highest endemic taxonomic grouping. For example, the annelid family Nautiliniellidae and the gastropod order Neomphalina, containing the families Neomphalidae and Peltospiridae, are endemic. Basibranch pogonophorans are also known from hydrocarbon seep communities, a sulphophilic environment much like hydrothermal vents, allowing their continued inclusion as endemics. Seep basibranch species exist within the family Lamellibrachiidae and the seep endemic family Escarpiidae. McLean (1990a) has additionally reported the discovery of a representative of the Neolepetopsidae at hydrocarbon seeps.

- Phylum Pogonophora
    - **Class Obturata (=Vestimentifera)**
        - **Order Basibranchia**
            - **Family Lamellibrachiidae**
            - **Family Alaysiidae**
            - **Family Ridgeiidae**
            - **Family Tevniidae**
        - **Order Axonobranchia**
            - **Family Riftiidae**

- Phylum Annelida
    - Class Polychaeta
        - Order Terebellida
            - **Family Alvinellidae**
        - Order Phyllodocida
            - **Family Nautiliniellidae**

- Phylum Hemichordata
    - Class Enteropneusta
        - Order Uncertain
            - **Superfamily Uncertain**
                - **Family Saxipendiidae**

- Phylum Arthropoda
    - Class Crustacea
        - Order Sessilia
            - **Suborder Brachylepadomorpha**
                - **Family Brachylepadidae**
            - Suborder Verrucomorpha
                - **Family Neoverrucidae**
        - Order Siphonostomatoida
            - **Family Dirivultidae**
            - **Family Ecbathyriontidae**
        - Order Decapoda
            - **Superfamily Bythograeoidea**
                - **Family Bythograeidae**

- Phylum Mollusca
  - Class Gastropoda
    - Order Patellogastropoda
      - **Suborder Lepetopsina**
        - **Superfamily Lepetopsoidea**
          - **Family Neolepetopsidae**
    - **Order Neomphalina**
      - **Family Neomphalidae**
      - **Family Peltospiridae**
    - Order Vetigastropoda
      - Superfamily Fissurelloidea
        - **Family Clypeosectidae**
      - **Superfamily Lepetodriloidea**
        - **Family Lepetodrilidae**
        - **Family Gorgoleptidae**
    - Order Cocculiniformia
      - Superfamily Lepetellioidea
        - **Family Pyropeltidae**
    - Order Caenogastropoda
      - Superfamily Loxonematoidea
        - **Family Provannidae**

hydrothermal vent communities (Tunnicliffe & Fowler 1996, Tunnicliffe et al. 1996). Novel adaptations and the drastic differences in faunal composition suggest the presence of strong physiological barriers to invasion and thus possible protection from newly evolving competitors and predators throughout the Phanerozoic. Energetic independence in the form of chemoautotrophy could have protected the community during mass extinctions, a suggestion supported by the observation that the distribution of vent communities reflects ancient sea-floor spreading histories and not re-colonization after marine mass extinctions (Tunnicliffe & Fowler 1996).

These assertions of antiquity rely upon an assumed relationship between taxonomic rank and age. Newman (1985) cites an average survival time of animal families and orders as one hundred to three hundred and several hundreds of millions of years, respectively. The high taxonomic ranking of vent endemic groups has been based on their novel baupläne (Fretter et al. 1981, McLean 1981, Fretter 1988, McLean 1988, McLean 1989a, Newman 1989) or their similarity to extinct, ancient forms (McLean 1990a, Newman 1995). For some, like the gastropods, fossil affinities are uncertain and antiquity is inferred from hypothesized scenarios of morphological evolution (eg. McLean 1981, Batten 1984). These same scenarios have been challenged and disrupted by the very discovery of new baupläne at hydrothermal vents (eg. Haszprunar 1988). The severe environment at hydrothermal vents could have favoured rapid evolution and recent convergence to ancient or novel form and thus false taxonomic inflation (Cohen & Haedrich 1983, Hickman 1984). Hydrothermal vent species could be recently derived from species of the adjacent deep-sea.

Antiquity of Hydrothermal Vent Gastropods

The primary objective of this dissertation is to examine if the gastropods endemic to hydrothermal vents represent refugial survivors of Paleozoic or Mesozoic origins. The alternate hypothesis is a rapid change of recent immigrants from the adjacent deep-sea. Many gastropod groups have invaded the hydrothermal vent habitat independently, as represented by many unrelated endemic species and genera derived from non-endemic gastropod families. Six other groups appear to represent more ancient gastropod lineages since they are endemic at the familial level or higher (Table 2). On average, extant marine gastropod families with a fossil record appeared in the late Cretaceous (66-97 MYBP) with less than ten appearing in the Paleozoic (Tracey et al. 1993). All but a few marine gastropod orders appear in the fossil record at the end of the Cambrian or during the early Ordovician (478-523 MYBP, Tracey et al. 1993). The vent endemic, taxonomically unique groups are non-provincial and occur at geographically disjunct hydrothermal vent communities, indicating their relationship with hydrothermal vents probably dates back to at least the Mesozoic (Tunnicliffe 1988, Tunnicliffe & Fowler 1996, Tunnicliffe et al. 1996). One of these groups, the Neomphalina, has a remarkably novel bauplan and may have originated in the early Paleozoic (McLean 1981). Typified by *Neomphalus*, this group presents characteristics plesiomorphic for the Gastropoda and found predominantly in the Archaeogastropoda (rhipidoglossate radula, bipectinate ctendium, eipodial tentacles, and hypoathroid nervous system), uniquely combined with characteristics previously considered synapomorphic for the Meso-Neogastropoda (now Caenogastropoda; loss of right pallial complex, heart with single auricle, loss of right kidney, and glandular gonoducts) (see Appendix for key citations). The Neomphalina is considered to represent one of the major gastropod lineages, equivalent in novelty to other gastropod orders, and its discovery at hydrothermal vents has revolutionized concepts of gastropod phylogeny (Haszprunar 1988, Ponder & Lindberg 1996a).

Fossil affinities of the six endemic hydrothermal vent groups are unclear (McLean 1981, Batten 1984, Haszprunar 1988, McLean 1988), with the exception of the Neolepetopsidae (McLean 1990a), and their systematic position and hypothesized antiquity presently is based upon their novel anatomy and hypotheses of gastropod anatomical evolution (Haszprunar 1988, Ponder & Lindberg 1996a). Citations for their anatomical descriptions are presented in the Appendix. For the neomphalinid *Melanodrymia aurantiaca*, Hickman (1984) cited contrary evidence based upon radula design supporting Late Tertiary or Quaternary origins (< 10 MYBP) from within the vetigastropod family Trochidae. While hypotheses regarding gastropod phylogeny have not supported Hickman's (1984) view (Haszprunar 1989a, McLean 1990b), the strict systematic placement of the Neomphalina has been unclear (Haszprunar 1988) or weakly supported by reductionist evolution of a few anatomical characters (Ponder & Lindberg 1996a).

Gastropod Systematics

Gastropods first appear in the fossil record in the latest Cambrian and radiated into many major groups in the early Paleozoic (Table 3). Many Paleozoic lineages went extinct but the Patellogastropoda, Vetigastropoda, Caenogastropoda, and Neritimorpha are still extant. For over a decade higher gastropod systematics has been in a state of flux due to the discovery of novel new species (including those from hydrothermal vents),

Table 3. Brief description of fossil and extant gastropod orders, with some Paleozoic groups of uncertain affinity excluded. Stratigraphic ranges and familial diversities were compiled from Tracey et al. (1993). Natural history characteristics of the early Paleozoic groups are unclear. Linsley (1978) hypothesized that hyperstrophically coiled groups (versus orthostrophic) may represent descendants of coiled Monoplacophora not of coiled, torted Gastropoda.

Bellerophontina **Late Cambrian - Early Triassic**

One of the first gastropod groups to appear in the fossil record. Marine molluscs with an isostrophically coiled shell (ie. bilateral symmetry). All other gastropod groups have anisostrophic shells (ie. not bilaterally symmetrical) and the Bellerophontina may either actually belong within the Monoplacophora or may represent the first gastropod lineage (implying that anisostrophic shell coiling evolved after torsion) (Linsley 1978). Three families known, two of which arose in the late Cambrian. Last family went extinct during the Scythian extinction event.

Macluritina **Late Cambrian - End Ordovician**

Large, hyperstrophically coiled molluscs, not thought to be torted by Linsley & Kier (1984). One family known (Macluritidae) that went extinct during the Ashgillian (end-Ordovician) mass extinction event.

Hyperstrophina **Late Cambrian - Early Carboniferous**

Hyperstrophically coiled gastropods with clear inhalent and exhalent channels, not thought to be torted by Linsley & Kier (1984). Three families known, two of which arose in the late Cambrian. Two families lost during the Givetian-Frasnian (late Devonian) mass extinction event while one survived to the early Carboniferous.

Vetigastropoda **Late Cambrian - Present**

Marine gastropods encompassing a large part of the traditional Archaeogastropoda. Grazers with shell form varying from limpet-like to coiled. Representatives known from all marine environments. Dominant gastropod lineage for the Paleozoic and early Mesozoic (43 families have a fossil record), until the radiation of the Caenogastropoda that began in the Jurassic. Highest number of families during late Paleozoic followed by a drastic faunal changes associated with the end-Permian and Triassic extinction events and the Mesozoic marine revolutions that resulted from the evolution of new predators (Vermeij 1977). Presently has the second-most number of families of all the marine gastropod groups. Hydrothermal vent endemic species are known from several vetigastropod families. Two vent endemic lineages, Lepetodriloidea and Clypeosectidae, appear to represent older invasions and *in situ* radiations.

Euomphalina **Early Ordovician - End Permian**

Probably filter-feeding gastropods that rested their shell on the sediment instead of balancing it above the body mass (McLean 1981). One family known (Euomphalidae) which went extinct during the end-Permian mass extinction event, although tentative euomphalinid genera are known from the Triassic. McLean (1981) considered that the hydrothermal vent endemic family Neomphalidae could represent a surviving euomphalinid lineage (see Neomphalina).

Caenogastropoda **Early Ordovician - Present**

Supercedes the traditional Mesogastropoda and Neogastropoda to include both groups, with the Mesogastropoda paraphyletic. The largest modern group of marine gastropods containing both filter-feeding and predatory species. Originated in the early Ordovician but did not radiate to the current large number of families until the Jurassic. Predators such as *Buccinum* are associated with hydrothermal vents and the filter-feeding family Provannidae is endemic.

Patellogastropoda **Early Ordovician - Present**

Marine limpets common to coastal inter-tidal zones but species can be found in nearly all marine environments. Limpet-form shell lacks coiling. Grazers consuming primarily marine algae. Considered an early branch of gastropod phylogeny, sister to the rest of the Gastropoda (ie. Patellogastropoda vs. Orthogastropoda *sensu* Ponder & Lindberg 1996a). Extant families appeared in the fossil record during the Triassic and Cretaceous. The hydrothermal vent endemic Neolepetopsidae is considered a living relic of the early Lepetopsina branch of patellogastropod evolution that went extinct during the Triassic (McLean 1990a), but Fretter (1990) questions the reliance of this assertion upon radular characteristics.

Neritimorpha **Late Silurian - Present**

An extremely diverse group with representatives known from marine, brackish, freshwater, and terrestrial environments. Marine representatives include species from hydrothermal vent and hydrocarbon seep communities. Species exist with coiled shells, limpet-form shells, and a lack of shell. Anatomy and morphology variable. Many species are extremely rare.

Cocculiniformia **Middle Paleogene - Present**

Deep-sea limpets commonly found associated with biogenic substrates (wood falls, algal holdfasts, carcasses). Preference for sulphophilic habitats, including hydrothermal vents and hydrocarbon seeps. Fossil record from the mid-Paleogene but hypothesized as one of the major radiations (Paleozoic origins?) by Haszprunar (1988), a contention not supported by Ponder & Lindberg (1996a). Two families known (Cocculinidae and Lepetellidae) but affinities uncertain.

Neomphalina **No Fossil Record**

First described in McLean (1981), this enigmatic group of gastropods is only known from hydrothermal vent communities. The two families (Peltospiridae and Neomphalidae) contain both limpet and coiled forms. They appear to be grazers upon bacterial mats in combination with an ability to filter feed (McLean 1990b). Their internal anatomy exhibits a combination of traditional archaeogastropod and mesogastropod characters and their discovery is in part responsible for the current confusion in prosobranch systematics. The Neomphalidae has been considered to represent an extant member of the Euomphalina (McLean 1981), although opinion remains divided (Batten 1984). Current phylogenetic schemes consider the Neomphalina representative of a major radiation with phylogenetic novelty equivalent to other gastropod orders that originated in the early Paleozoic.

Architaenioglossa **Middle Jurassic - Present**

Earliest branch of the Caenogastropoda according to Ponder & Lindberg (1996a) despite not being known from Paleozoic fossils. Includes freshwater (filter-feeding) and terrestrial (herbivory) families. Nervous system is hypoathroid (archaeogastropod-like) but other features shared with the Caenogastropoda.

Heterobranchia **Devonian - Present**

The crown group of the Gastropoda, united in having a hyperstrophic larval protoconch. Representatives from marine, aquatic, terrestrial, and ectoparasitic habitats. Because of their size, the euthyneuran groups Opisthobranchia and Pulmonata are often treated as orders and the Heterobranchia as supra-ordinal. The non-Euthyneuran heterobranchs are sometimes referred to as Allogastropoda or Heterostropha and appear to be paraphyletic (Haszprunar 1988).

Euthyneura **Early Carboniferous - Present**

Unique in having a euthyneurus (secondarily untorted) nervous system, this group contains two large groups: Opisthobranchia (marine snails and slugs) and Pulmonata (terrestrial snails and slugs). Both groups may be paraphyletic and phylogenetic distinction between the two is uncertain. Euthyneury is a phenomenon of convergence (Haszprunar 1985) but the overall monophyly of the Euthyneura is supported by other neural features (Salvini-Plawen & Steiner 1996, Ponder & Lindberg 1996a).

closer examination of traditional groupings, and the use of new tools (Haszprunar 1988, Bieler 1992, Haszprunar 1993, Ponder & Lindberg 1996a). Descriptions of the major gastropod groups can be found in Parker (1982), Haszprunar (1988), and Ponder & Lindberg (1996b). Classical divisions such as the Prosobranchia, Archaeogastropoda, Mesogastropoda, and Streptoneura have been revealed as phylogenetically meaningless or as grades of organization. The Archaeogastropoda, the first group of gastropods to appear in the fossil record, has been the object of much debate (Hickman 1988, Haszprunar 1993). This renewed interest in gastropod phylogeny has resulted in several new phylogenetic hypotheses. Haszprunar's (1988, see Figure 1) hypothesis of gastropod phylogeny was generated using a flawed "clado-evolutionary" methodology, a cladistic approach hampered by *ad hoc* intuitive modifications of cladistic methodology, inconsistent data presentation, and non-reproducible analyses (Bieler 1990, but see Haszprunar 1990). Regardless of the problems, Haszprunar (1988) built upon earlier attempts to modernize concepts of gastropod evolution (eg. Golikov & Starobogatov 1975, Haszprunar 1985, Salvini-Plawen & Haszprunar 1987). Haszprunar (1988) also clearly indicated gaps in phylogenetic information and helped initiate an aggressive examination of anatomical-histological, ultrastructural, comparative, and biochemical variation within the Gastropoda (reviewed in Bieler 1992, Ponder & Lindberg 1996a). This resulted in the cladistic examinations of Ponder & Lindberg (1996a) and Salvini-Plawen & Steiner (1996). The differences in these two studies outline critical problems in phylogenetic analyses of anatomical data. Each uses different anatomical characters and make different assumptions about character evolution. Salvini-Plawen & Steiner (1996, see Figure 2) expand upon Haszprunar's (1988) data set but do not define character

Figure 1. Haszprunar's (1988) phylogenetic hypothesis for the Gastropoda, based upon flawed methodology (Bieler 1990). Groups strictly endemic to hydrothermal vents and hydrocarbon seeps are boxed. The Neolepetopsidae has been moved to the Patellogastropoda following McLean (1990a) and the Seguenzioidea to the Vetigastropoda following Haszprunar (1996). At the time this hypothesis was presented, very little was known about the hydrothermal vent endemic gastropods (Neolepetopsidae, Neomphalidae, Peltospiridae, Lepetodriloidea). *Melanodrymia* was the only known representative of the Peltospiridae. Monophyly of both the Neomphalina and Architaenioglossa was uncertain as was the position of the Neomphalidae.

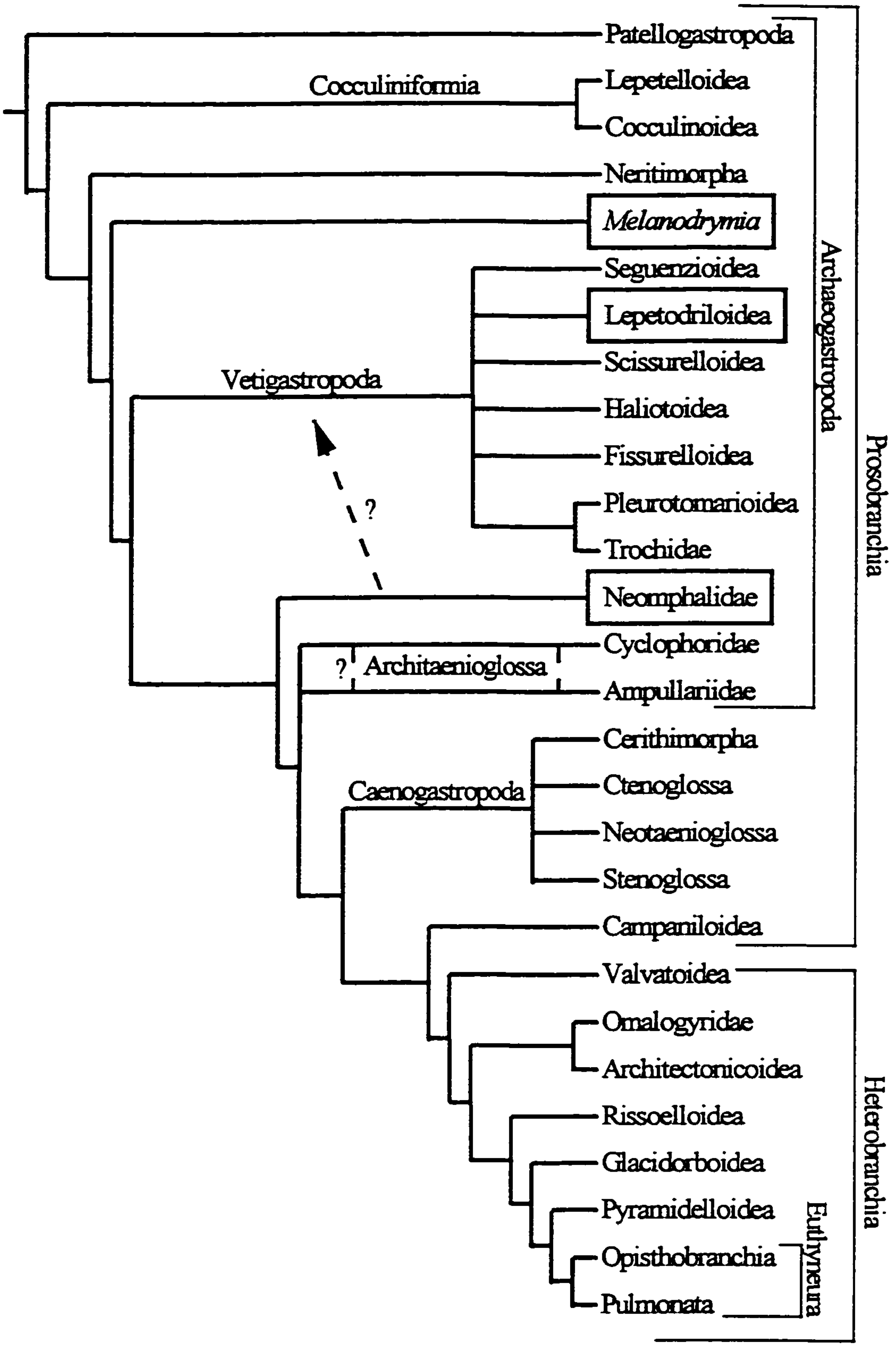
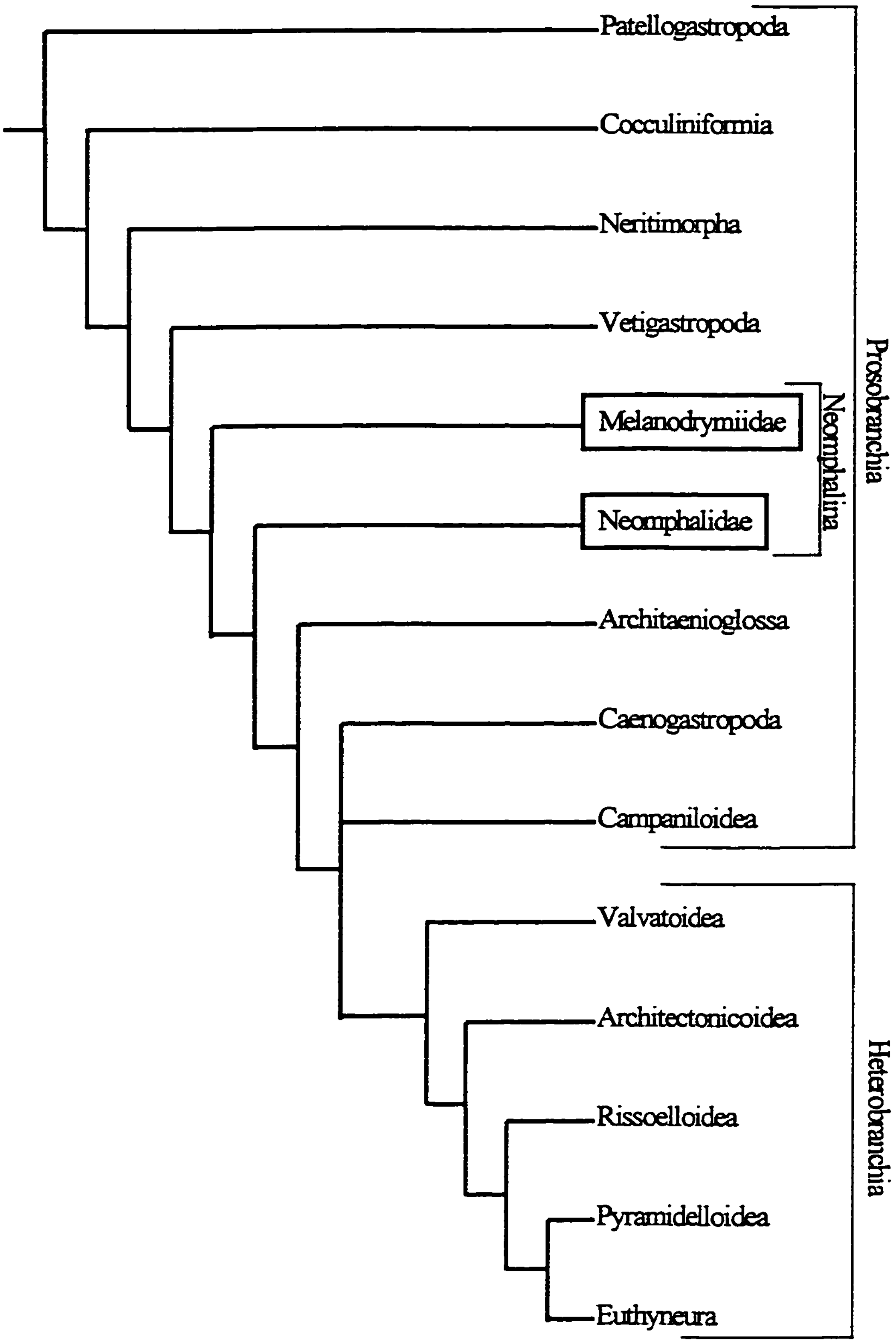

Patellogastropoda
Cocculiniformia
Lepetelloidea
Cocculinoidea
Neritimorpha
Melanodrymia
Seguenzioidea
Lepetodriloidea
Scissurelloidea
Vetigastropoda
Haliotoidea
Fissurelloidea
?
Pleurotomarioidea
Trochidae
Neomphalidae
Cyclophoridae
?
Architaenioglossa
Ampullariidae
Cerithimorpha
Ctenoglossa
Caenogastropoda
Neotaenioglossa
Stenoglossa
Campaniloidea
Valvatoidea
Omalogyridae
Architectonicoidea
Rissoelloidea
Glacidorboidea
Pyramidelloidea
Opisthobranchia
Pulmonata
Archaeogastropoda
Prosobranchia
Heterobranchia
Euthyneura

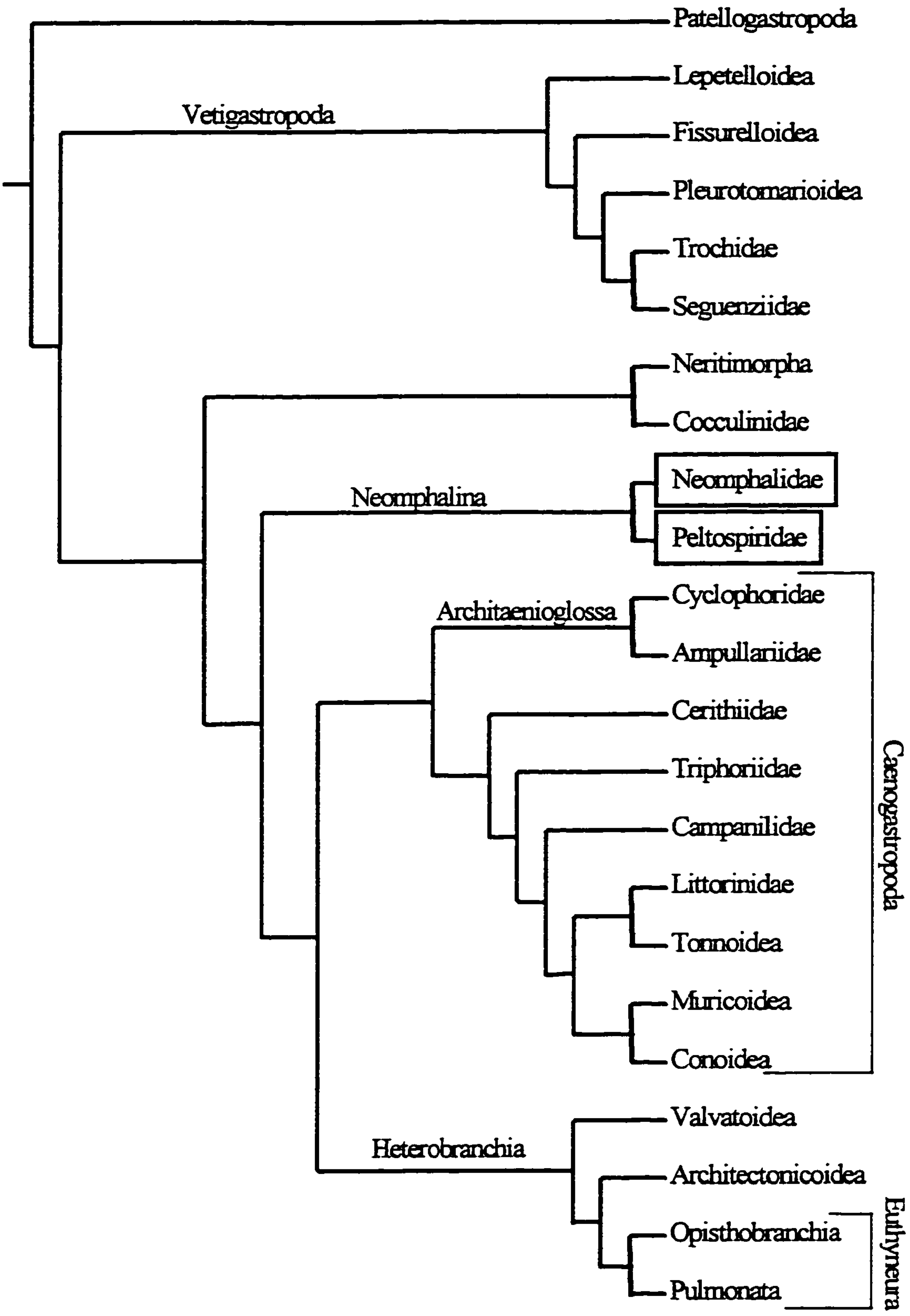
Figure 2. Salvini-Plawen & Steiner's (1996) phylogenetic hypothesis for the Gastropoda, based on strict cladistic analysis of 68 morphological, anatomical, and ultrastructural characters (see critique in introductory text). Groups strictly endemic to hydrothermal vents and hydrocarbon seeps are boxed. Usage of the taxon Melanodrymiidae is uncertain but likely represents just the genus *Melanodrymia*, endemic to hydrothermal vents. Other representatives of the hydrothermal vent endemic Peltospiridae were not included and thus the representation of the Neomphalina was incomplete. They also state that the hypothesized position of the Melanodrymiidae is the victim of poor data and that, with the exception of the exact position of the Campaniloidea, their hypothesis is identical to Haszprunar's (1988).


Patellogastropoda
Cocculiniformia
Neritimorpha
Vetigastropoda
Melanodrymiidae
Neomphalidae
Architaenioglossa
Caenogastropoda
Campaniloidea
Valvatoidea
Architectonicoidea
Rissoelloidea
Pyramidelloidea
Euthyneura
Neomphalina
Prosobranchia
Heterobranchia

polarities or if reversions (convergences) are restricted or unrestricted, although character polarity appears to have been defined by the use of outgroup taxa. This lack of explicitly stated assumptions and methodology renders evaluation of Salvini-Plawen & Steiner's (1996) results impossible. Ponder & Lindberg (1996a, see Figure 3) presented preliminary results based on a yet to be published larger examination of gastropod phylogeny (Ponder & Lindberg 1996b). Unlike Salvini-Plawen & Steiner (1996), they presented explicit assumptions about character evolution, polarity, and overall analysis. Contrasting results could be evaluated based on clear assumptions about character transformations and possible reversions (convergences). Ponder & Lindberg (1996a) also assessed the strength of their results and found that hypotheses sometimes exhibited insufficient or dubious support for major divisions stemming from the limited sample of characters.

In the case of the hydrothermal vent endemic gastropods, a clear understanding of phylogenetic position would allow assessment of age in the absence of known fossils. Although Ponder & Lindberg (1996a) present a more explicit examination of gastropod phylogeny than Salvini-Plawen & Steiner (1996), it is very difficult to evaluate which utilizes more informative or trustworthy characters. Other than clear evidence of frequent homoplasy (convergence), arguments for and against specific suites of morphological characters are generally unresolvable. In addition, assumptions about character evolution and homoplasy may prove to be incorrect, a possibility that can only be explored using independent information. For example, the euthyneurous neural organization in the Heterobranchia is now known to result from several convergent developmental processes (Haszprunar 1985). Both Salvini-Plawen & Steiner (1996) and Ponder & Lindberg (1996a) conclude that the hydrothermal vent Neomphalina represents a major gastropod

Figure 3. Ponder & Lindberg's (1996a) phylogenetic hypothesis for the Gastropoda based on strict cladistic analysis of 95 morphological, anatomical, and ultrastructural characters. Groups strictly endemic to hydrothermal vents and hydrocarbon seeps are boxed. The Cocculiniformia (Lepetelloidea + Cocculinidae) was not hypothesized to be monophyletic while monophyly of the hydrothermal vent Neomphalina was assumed. The vent endemic Lepetodriloidea was not included. Their definition of the Caenogastropoda was expanded to include the Architaenioglossa.


Patellogastropoda
Vetigastropoda
Lepetelloidea
Fissurelloidea
Pleurotomarioidea
Trochidae
Seguenziidae
Neritimorpha
Cocculinidae
Neomphalina
Neomphalidae
Peltospiridae
Architaenioglossa
Cyclophoridae
Ampullariidae
Cerithiidae
Triphoriidae
Campanilidae
Littorinidae
Tonnoidea
Muricoidea
Conoidea
Caenogastropoda
Heterobranchia
Valvatoidea
Architectonicoidea
Opisthobranchia
Pulmonata
Euthyneura

lineage (paraphyletic?) with probable origins during the early Paleozoic based upon assumed non-convergent anatomical organization, but present no conclusions for the other hydrothermal vent endemic gastropod groups. Their results could be spurious if the bauplan of the Neomphalina is the result of rapid evolution and convergence or if some of their assumptions about gastropod evolution are incorrect. Additionally, both Haszprunar (1988) and Salvini-Plawen & Steiner (1996) used incomplete representation of the Neomphalina while Ponder & Lindberg (1996) assumed a monophyletic Neomphalina without clear justification. A molecular systematic approach could resolve gastropod phylogeny robustly where neontological approaches lack resolution or clarity, particularly for the endemic hydrothermal vent groups.

Molecular Systematics

Molecular data can contribute many characters to systematic investigations (ie. nucleotide sequence data). While the neontological data of Ponder & Lindberg (1996a) included 95 anatomical and shell characters, molecular sequences in excess of 1000 characters are available for molluscan systematics (Littlewood 1994, Stiener & Muller 1996), although not all the characters in molecular sequences are informative for parsimony (eg. Stiener & Muller 1996: 1883 characters gave 321 informative for parsimony) nor can molecular and anatomical characters been considered directly comparable. Use of orthologous genes (ie. derived from a common ancestor) found in all the taxa of interest, combined with sequence alignment anchored by conserved sequence motifs and secondary structures, ensures a high confidence of homology (Gould 1986, Olsen & Woese 1993). This homology extends to outgroups, allowing the easy assessment of character polarization. Unlike most morphological evolution, a mechanistic understanding of molecular evolution exists, allowing the use or rejection of empirical models and assumptions in analyses (Hillis et al. 1993). In addition, this mechanistic understanding greatly aids in the detection of homoplasy (ie. convergence) and additional models of molecular evolution exist that compensate for frequent sources of homoplasy. Many molecular sequences also evolve independently of neontological features, allowing an independent examination of phylogeny.

Molecular data are not infallible since the variation examined must be appropriate to the questions posed and is a function of the genes and taxa chosen for examination. Selection of genes exhibiting appropriate variation is one of the most critical steps in molecular systematics. Emberton et al. (1990), Tillier et al. (1992, 1994, 1996), and Rosenberg et al. (1994) have used 28S ribosomal RNA (rRNA) sequences to examine aspects of gastropod phylogeny. Ribosomal RNAs are structurally important to the functioning of ribosomes and homologous genes encoding them are found in all organisms (Hillis & Dixon 1991). Sequences from ribosomal RNA genes have been widely used for molecular systematics given their universality, the ability of different genes or domains within genes to address different time scales, and their relative ease in direct RNA sequencing, DNA amplification, or RFLP analysis (Hillis & Dixon 1991, Olsen & Woese 1993). Hillis & Dixon (1991) reported that the large nuclear subunit (28S in eukaryotes) could be used to examine evolutionary events of the Paleozoic and Mesozoic. Phylogenetic studies of the Gastropoda have utilized the D1 domain (Tillier et al. 1992, 1994, 1996) and D6 domain (Emberton et al. 1990, Rosenberg et al. 1994) of 28S rRNA via direct RNA sequencing. Bootstrap examinations by Tillier et al. (1994) indicated information for the early Paleozoic radiations of the Gastropoda was rare in the D1 domain. Alone, the two domains could be either too short, as proposed by both Rosenberg et al. (1994) and Tillier et al. (1994), or could exhibit variation inappropriate for the robust examination of phylogeny amongst the major gastropod groups (untested). Conservative evaluation of the quality of phylogenetic information best serves systematics. It is possible that domains of 28S rRNA that have been used to examine basal gastropod phylogeny are inappropriate, as evidenced by Tillier et al.'s (1996) restriction to euthyneuran subgroups.

Uneven taxonomic sampling can mislead phylogenetic analyses (LeCointre et al. 1993), in part due to undetected multiple substitutions between sequences from distantly related species (long-branch attraction *sensu* Hendy & Penny 1989). The analyses of Emberton et al. (1990), Tillier et al. (1992, 1994, 1996), and Rosenberg et al. (1994) did not include representatives from endemic hydrothermal vent groups (with one exception for Tillier et al. 1994) nor representatives of many other major prosobranch groups needed to resolve the Paleozoic radiations. Resolution of early gastropod phylogeny using molecular sequences requires an even taxonomic sampling to avoid taxon-specific biasing of the results.

To address the primary objective of this dissertation, a molecular systematic investigation of overall gastropod phylogeny, with emphasis upon the Paleozoic radiations, was undertaken. The work of Tillier et al. (1992, 1994, 1996) and Rosenberg et al. (1994) was expanded to include a more representative sample of overall gastropod phylogeny, including the endemic hydrothermal vent groups, since these studies provided preliminary indications that these gene domains could be appropriate for testing the vent gastropod antiquity question. This molecular systematic approach tests if the bauplāne of hydrothermal vent endemic gastropods are the result of antiquity or recent evolution of novel or convergent form. If these groups truly represent a distinct lineage of gastropod evolution, a resolved gastropod phylogeny including them, coupled with the known gastropod fossil record, would aid in the assessment of their age and origins.

METHODS

Gastropod Specimens and Sequencing Strategy

Sixty-three DNA or RNA sequences from the D1 domain of the gene encoding for 28S ribosomal RNA were compiled for 60 gastropod, one bivalve, and two polyplacophoran species from donated unpublished sequences of Dr. Simon Tillier (Muséum national d'Histoire naturelle, France) and sequences obtained by DNA sequencing. The donated sequences were slightly longer than those previously published for the same species by Tillier et al. (1992, 1994, 1996). Ninety-six DNA or RNA sequences from the D6 domain of the gene encoding for 28S ribosomal RNA were similarly compiled for 62 gastropod, 25 bivalve, 2 polyplacophoran, 6 cephalopod, and one scaphopod species from donated unpublished sequences of Dr. Gary Rosenberg (Academy of Natural Sciences, U.S.A.), previously published sequences, and sequences obtained by DNA sequencing. Of the 159 total sequences obtained, sequences of both domains were obtained for 32 gastropod, one bivalve, and two polyplacophoran genera. Sources for each of the DNA or RNA sequences are listed in Table 4 and a systematic framework for the genera examined is presented in Table 5. In total, 23 new D1 domain and 30 new D6 domain DNA sequences were obtained via polymerase chain reaction amplification and automated DNA sequencing of DNA molecules extracted from 32 molluscan specimens. Table 6 lists the condition, locality, and donor for specimens from which new DNA sequences were obtained.

Table 4. Sources for DNA and RNA sequences of the D1 and D6 domains of the 28S rRNA gene. Sequences denoted by GenBank accession numbers were obtained by DNA sequencing.

Species	D1 Domain Sequences	D6 Domain Sequences
Acanthochitona fascicularis	RNA, Tillier (unpublished)	RNA, Rosenberg (unpublished)
Achatina fulica	RNA, Tillier (unpublished)	none
Acteon tornatilis	RNA, Tillier (unpublished)	none
Amblema plicata	none	RNA, Rosenberg et al. (1994)
Amphibola avellana	RNA, Tillier (unpublished)	none
Ampullaria sp.	U75841	U78643
Aneitea sp.	RNA, Tillier (unpublished)	none
Anguispira alternata	RNA, Tillier (unpublished)	none
Anodonta spp. (3 sequences)	none	RNA, Rosenberg et al. (1994)
Aplysia californica	U75842	U78644
Aplysia depilans	RNA, Tillier (unpublished)	none
Archidoris adhneri	none	U78645
Archidoris tuberculata	RNA, Tillier (unpublished)	none
Bathymargarites symplector	U75843	U78646
Bathynerita naticoides	U75844	U78647
Biompharlaria glabrata	none	RNA, Emberton et al. (1990)
Buccinum sp.	U75845	U78648
Buccinum undatum	RNA, Tillier (unpublished)	none
Bursatella leachii	none	U78649
Busycon carica	none	RNA, Rosenberg et al. (1994)
Calliostoma zizyphinum	RNA, Tillier (unpublished)	none
Calyptraea chinensis	RNA, Tillier (unpublished)	none
Campanile symbolicum	U75846	U78650
Cerastoderma edule	none	RNA, Rosenberg (unpublished)
Cerithidea spp. (2 sequences)	none	RNA, Rosenberg (unpublished)
Clanculus corallinus	U75847	U78651
Cochlodina laminata	RNA, Tillier (unpublished)	none
Cumberlandia monodonta	none	RNA, Rosenberg et al. (1994)
Cyathermia naticoides	U75848	U78652

Species	D1 Domain Sequences	D6 Domain Sequences
Dentalium vulgare	none	RNA, Rosenberg (unpublished)
Depressigyra globulus	U75849	U78653
Diodora aspera	none	U78654
Diodora graeca	RNA, Tillier (unpublished)	none
Elliptio complanata	none	RNA, Rosenberg et al. (1994)
Eulepetopsis vitrea	U75850	U78655
Fusconaia cerina	none	RNA, Rosenberg et al. (1994)
Geomelania spp. (2 sequences)	none	RNA, Rosenberg et al. (1994)
Gibbula umbilicalis	RNA, Tillier (unpublished)	none
Gonaxis montisnimbae	RNA, Tillier (unpublished)	none
Gonidea angulata	none	RNA, Rosenberg et al. (1994)
Haliotis kamtschatkana	none	U78656
Haliotis tuberculata	RNA, Tillier (unpublished)	none
Haplotrema concavum	none	RNA, Emberton et al. (1990)
Helicina orbiculata	none	RNA, Emberton et al. (1990)
Helix aspersa	RNA, Tillier (unpublished)	RNA, Rosenberg (unpublished)
Heleobops sp.	none	RNA, Rosenberg (unpublished)
Katharina tunicata	U75851	U78657
Lampsilis spp. (2 sequences)	none	RNA, Rosenberg et al. (1994)
Lepetodrilus fucensis	U75852	U78658
Limax maximus	RNA, Tillier (unpublished)	none
Lirularia succincta	none	U78659
Littorina sp.	none	U78660
Littorina littorea	RNA, Tillier (unpublished)	none
Loligo forbesi	none	RNA, Rosenberg (unpublished)
Loliolus opalsescens	none	RNA, Rosenberg (unpublished)
Lymnaea stagnalis	RNA, Tillier (unpublished)	none
Mancinella deltoidea	none	RNA, Rosenberg et al. (1994)
Margaritifera spp. (2 sequences)	none	RNA, Rosenberg et al. (1994)

Species	D1 Domain Sequences	D6 Domain Sequences
Megalonaias boykiniana	none	RNA, Rosenberg et al. (1994)
Melanodrymia aurantiaca	U75853	U78661
Melanoides tuberculata	none	RNA, Rosenberg (unpublished)
Mesodon zaletus	RNA, Tillier (unpublished)	none
Mesodon inflectus & M. normalis	none	RNA, Emberton et al. (1990)
Mesomphix latior	none	RNA, Emberton et al. (1990)
Monodonta lineata	RNA, Tillier (unpublished)	RNA, Rosenberg (unpublished)
Mercenaria mercenaria	none	RNA, Rosenberg (unpublished)
Mytilus edulis	RNA, Tillier (unpublished)	RNA, Rosenberg (unpublished)
Nautilus spp. (2 sequences)	none	RNA, Rosenberg (unpublished)
Neohelix albolabris	none	RNA, Emberton et al. (1990)
Nerita costata	U75854	U78662
Nucella lapillus	RNA, Tillier (unpublished)	none
Obliquaria reflexa	none	RNA, Rosenberg et al. (1994)
Ocenebra erinacea	RNA, Tillier (unpublished)	none
Olgasolaris tollmanni	U75855	U78663
Oncomelania hupensis	none	RNA, Emberton et al. (1990)
Ouagapia cf. inaequalis	RNA, Tillier (unpublished)	none
Pararhytida dictyodes	RNA, Tillier (unpublished)	none
Patella sp.	none	U78664
Patella vulgata	RNA, Tillier (unpublished)	RNA, Rosenberg (unpublished)
Patina pellucida	RNA, Tillier (unpublished)	none
Peltospira operculata	U75856	U78665
Perotrochus maureri	none	RNA, Rosenberg et al. (1994)
Physa fontinalis	RNA, Tillier (unpublished)	none
Phytia myosotis	RNA, Tillier (unpublished)	none
Placostylus fibratus	RNA, Tillier (unpublished)	none
Planorbis sp.	none	RNA, Rosenberg (unpublished)
Plectomerus dombeyanus	none	RNA, Rosenberg et al. (1994)

Species	D1 Domain Sequences	D6 Domain Sequences
Pleurobema cordatum	none	RNA, Rosenberg et al. (1994)
Pleurodonte dentiens	RNA, Tillier (unpublished)	none
Pomatias elegans	RNA, Tillier (unpublished)	none
Progabbia cooperi	none	RNA, Rosenberg et al. (1994)
Pseudoveronicella zootoca	RNA, Tillier (unpublished)	none
Quadrula spp. (2 sequences)	none	RNA, Rosenberg et al. (1994)
Radix sp.	none	RNA, Rosenberg (unpublished)
Rhynchopelta concentrica	RNA, Tillier (unpublished)	U78666
Sepia officinalis	none	RNA, Rosenberg (unpublished)
Shinkailepas tufari	U75857	U78667
Siphonaria algesirae	RNA, Tillier (unpublished)	none
Sphaerium sp.	none	RNA, Rosenberg (unpublished)
Succinea putris	RNA, Tillier (unpublished)	none
Tectura scutum	U75858	U78668
Tegula pulligo	U75859	U78669
Temnocinclis euripes	U75860	U78670
Theodoxus sp.	U75861	U78671
Triodopsis hopetonensis	none	RNA, Emberton et al. (1990)
Truncatella spp. (7 sequences)	none	RNA, Rosenberg et al. (1994)
Unio pictorum	none	RNA, Rosenberg et al. (1994)
Uniomerus tetralasmus	none	RNA, Rosenberg et al. (1994)
Uroteuthis edulis	none	RNA, Rosenberg (unpublished)
Valvata sp.	U75862	U78672
Ventridens acerra	RNA, Tillier (unpublished)	none
Ventridens cerinoideus	none	RNA, Emberton et al. (1990)
Venus verrucosa	none	RNA, Rosenberg (unpublished)
Viviparus viviparus	U75863	none
Viviparus sp.	none	RNA, Rosenberg (unpublished)
Zebrina detrita	RNA, Tillier (unpublished)	none

Table 5. Systematics of molluscan species included in the phylogenetic analyses. Genera from hydrothermal vent or hydrocarbon seep habitats are underlined. A listing of systematic citations for endemic gastropod groups is presented in the Appendix

POLYPLACOPHORA
- Acanthochitonina
    - *Acanthochitona*
- Chitonina
    - *Katharina*

SCAPHOPODA
- Dentaliidae
    - *Dentalium*

CEPHALOPODA
- Nautiloida
    - *Nautilus*
- Coleoida
    - Sepiida
        - *Sepia*
    - Teuthoida
        - *Loligo, Loliolus, Uroteuthis*

BIVALVIA
- Paleoheterodonta
    - Unionoidea
        - *Unio, Amblema, Megalonaias, Plectomerus, Quadrula, Elliptio, Fusconaia, Pleurobema, Uniomerus, Gonidea, Lampsilis, Obliquaria, Anodonta, Margaritifera, Cumberlandia*
- Heterodonta
    - Veneroida
        - *Cerastoderma, Sphaerium, Mercenaria, Venus*
- Pteriomorphia
    - Mytiloida
        - *Mytilus*

GASTROPODA
- Patellogastropoda
    - Patellina
        - *Patella, Patina*
    - Nacellina
        - *Tectura*
    - Lepetopsina
        - *Eulepetopsis*

- Vetigastropoda
  - Fissurelloidea
    - *Diodora*
  - Pleurotomarioidea
    - *Haliotis, Perotrochus*
  - Scissurellioidea
    - *Temnocinclis*
  - Trochidae
    - *Calliostoma, Gibbula, Bathymargarites, Clanculus, Monodonta, Tegula, Lirularia*
  - Lepetodriloidea
    - *Lepetodrilus*

- Neritimorpha
  - Neritidae
    - *Nerita, Bathynerita, Theodoxus*
  - Phenacolepadidae
    - *Shinkailepas, Olgasolaris*
  - Helicinidae
    - *Helicina*

- Neomphalina
  - Neomphalidae
    - *Cyathermia*
  - Peltospiridae
    - *Melanodrymia, Rhynchopelta, Peltospira, Depressigyra*

- Caenogastropoda
  - Ampullarioidea
    - *Ampullaria, Viviparus*
  - Cerithioidea
    - *Cerithidea, Melanoides*
  - Campanilioidea
    - *Campanile*
  - Calyptraeoidea
    - *Calyptraea*
  - Littorinioidea
    - *Pomatias, Littorina*
  - Rissooidea
    - *Oncomelania, Truncatella, Goemelania, Heleobops*
  - Cancellarioidea
    - *Progabbia*
  - Muricoidea
    - *Nucella, Ocenebra, Buccinum, Busycon, Mancinella*

- Heterobranchia
  - Valvatioidea
    - *Valvata*
  - Opisthobranchia
    - Cephalaspidea
      - *Acteon*
    - Anaspidea
      - *Aplysia, Bursatella*
    - Nudibranchia
      - *Archidoris*
  - Pulmonata
    - Basommatophora
      - *Biomphalaria, Radix, Planorbis, Siphonaria, Amphibola, Lymnaea, Physa*
    - Non-stylommatophoran Eupulmonata
      - *Phytia, Pseudoveronicella*
    - Stylommatophoran Eupulmonata
      - *Achatina, Aneitea, Anguispira, Cochlodina, Gonaxis, Limax, Ouagapia, Placostylus, Pleurodonte, Succinea, Zebrina, Helix, Mesodon, Ventridens, Haplotrema, Mesomphix, Neohelix, Triodopsis, Pararhytida*

Table 6. Condition, locality, and donor of specimens used to obtained new DNA sequences from the D1 and D6 domains of the 28S rRNA gene. Vouchers of all specimens were placed in the V. Tunnicliffe collection, University of Victoria, Canada (codes other than AGM refer to original codes used by the donors). Et = 12 to 24 hours in formalin followed by storage in 70% ethanol. DNA = extracted DNA stored in water or TE buffer. Fr = frozen whole animal or tissue. J.B. = J.A.M. van den Biggelaar, Univ. Utrecht, The Netherlands, M.M. = M. Medina, University of Miami, Florida, U.S.A., J.H. = J. Holcroft, University of Victoria, Canada, R.L. = R. Lutz, Rutgers University, New Jersey, U.S.A., J.Z. = J. Zande, Louisiana State Univ, Louisiana, U.S.A., V.T. = V. Tunnicliffe, University of Victoria, Canada, W.P. = W. Ponder, The Australian Museum, Sydney, Australia, L.B. = L.A. Beck, Phillips-Universitat Marburg, Germany, A.M. = A.G. McArthur, University of Victoria, Canada, L.P. = L. Page, University of Victoria, Canada, S.L. = S. Leys, University of Victoria, Canada, J.He. = J. Heller, Hebrew University of Jerusalem, Israel.

Species	Condition	Locality	Donor	Collection Number
Ampullaria sp.	Et	Unknown (aquaria supply)	J.B.	AGM-01
Aplysia californica	DNA	Unknown	M.M.	AGM-11
Archidoris adhneri	Fr	MacKenzie Bight, British Columbia, Canada	J.H.	AGM-19
Bathymargarites symplector	Fr	East Pacific Rise, Hydrothermal vents	R.L.	A2232
Bathynerita naticoidea	Fr	Gulf of Mexico Hydrocarbon seeps	J. Z.	AGM-16
Buccinum sp.	Fr	Juan de Fuca Ridge, Hydrothermal vents	V.T.	R268-F1
Bursatella leachii	DNA	Unknown	M.M.	AGM-13
Campanile symbolicum	DNA	Southwestern Australia	W. P.	Camp. 1F
Clanculus corallinus	Et	Rhodes Island, Mediterranean Sea	L.B.	002879
Cyathermia naticoides	Fr	East Pacific Rise, Hydrothermal vents	R.L.	A2232
Depressigyra globulus	Fr	Juan de Fuca Ridge, Hydrothermal vents	V.T.	HYS 202
Diodora aspera	Fr	Cattle Point, British Columbia, Canada	A.M.	AGM-18
Eulepetopsis vitrea	Fr	East Pacific Rise, Hydrothermal vents	R.L.	A2224
Haliotis kamtschatkana	Fr	Vancouver Island, British Columbia, Canada	L.P.	AGM-17
Helix aspersa	Fr	Sidney, British Columbia, Canada	A.M.	AGM-30
Katharina tunicata	Fr	Cattle Point, British Columbia, Canada	A.M.	AGM-26
Lepetodrilus fucensis	Fr	Juan de Fuca Ridge, Hydrothermal vents	V.T.	F20-A2413
Lirularia succincta	Fr	Cattle Point, British Columbia, Canada	A.M.	AGM-28
Littorina sp.	Fr	Cattle Point, British Columbia, Canada	A.M.	AGM-20
Melanodrymia aurantiaca	Fr	East Pacific Rise, Hydrothermal vents	R.L.	A2233
Nerita costata	Et	Island of Celebes, Indo-Pacific Ocean	L.B.	003468
Olgasolaris tollmanni	Et	Manus Back-Arc Basin, Hydrothermal vents	L.B.	56GTVA
Patella sp.	Et	The Netherlands	J.B.	AGM-05
Peltospira operculata.	Fr	Galapagos Rift, Hydrothermal vents	R.L.	A2010
Rhynchopelta concentrica	Fr	East Pacific Rise, Hydrothermal vents	R.L.	A2232
Shinkailepas tufari	Et	Manus Back-Arc Basin, Hydrothermal vents	L.B.	56GTVA
Tectura scutum	Fr	Cattle Point, British Columbia, Canada	A.M.	AGM-22
Tegula pulligo	Fr	Bamfield, British Columbia, Canada	S.L.	AGM-25
Temnocinclis euripes	Fr	Juan de Fuca Ridge, Hydrothermal vents	V.T.	A2078-1452
Theodoxus sp.	Et	Nahal David (Ein Gedi), Israel	J.He.	AGM-02
Valvata sp.	Et	Utrecht, The Netherlands	J.B.	AGM-03
Viviparus viviparus	Et	Utrecht, The Netherlands	J.B.	AGM-04

DNA Extraction

DNA was extracted from foot or mantle tissue, except for minute (2-10 mm) specimens for which the entire animal was used. The extraction protocol was based on modifications of Doyle & Doyle (1987) with additional unpublished modifications from the Center for Theoretical and Applied Genetics (Rutgers University, U.S.A). Approximately 1-2 cubic millimeters of tissue were ground in 60°C CTAB isolation buffer (100 mM Tris-HCl, 1.4 M NaCl, 20 mM EDTA, 2% CTAB, 0.2% 2-mercaptoethanol) with the addition of a small amount of sterile quartz sand. The ground tissue was then incubated at 60°C for 30 minutes in the isolation buffer, choloroform-isoamyl alcohol extracted once, and the nucleic acids precipitated with the addition of one tenth volume 3M sodium acetate (pH 5.2) and two volumes of cold 70% ethanol. The isolated DNA was stored in 0.1X TE buffer.

DNA Amplification by the Polymerase Chain Reaction

A region of nuclear DNA encoding for large subunit nuclear ribosomal RNA (28S) homologous to the D1 domain RNA sequences of Tillier et al. (1992, 1994, 1996) was amplified using the polymerase chain reaction (PCR). DNA was amplified using primers D1a, 5'CAGTAACGGCGAGTGAACAG, and D1b, 5'TCGTGCCGGTATTTAGCCTTAGAT. These primers were designed to be complementary to regions conserved in Hassouna et al.'s (1984) alignment of mouse, amphibian, yeast, and slime mold 28S rRNA sequences and to be thermodynamically desirable for PCR as decided by the computer program PRIMEMATE (DNASTAR Inc.). If this primer pair failed or the amplification was weak, DNA was amplified using the D1b primer and primer LSU5b,ACCCGCTGAAYTTAAGCA, which is identical to Littlewood's (1994) LSU5 primer with the removal of the 5' polylinker sites. Primer LSU5b binds 58 bases downstream (5') of primer D1a, resulting in a larger PCR product. If the LSU5b/D1b amplification was weak, the product was secondarily hemi-amplified using the D1a/D1b combination. The sequences obtained were homologous to positions 25 (LSU5b) or 83 (D1a) through 347 (D1b) of the mouse large subunit ribosomal RNA sequence of Hassouna et al. (1984).

A region of nuclear DNA encoding for large subunit nuclear ribosomal RNA (28S) homologous to the D6 domain RNA sequences of Rosenberg et al. (1994) and Emberton et al. (1990) was also amplified using the polymerase chain reaction (PCR). The sequences obtained were homologous to positions 1829 through 2150 of the mouse large subunit ribosomal RNA sequence of Hassouna et al. (1984). DNA was amplified using primers D6a, 5'CAACTAGCCCTGAAAATGGATGG, and D6b, 5'TTCGGCCTTCAAAGTTCTCGTT. These primers were also designed to be complementary to regions conserved in Hassouna et al.'s (1984) alignment of mouse, amphibian, yeast, and slime mold 28S rRNA sequences and to be thermodynamically desirable for PCR as decided by the computer program PRIMEMATE.

All PCR primers were phosphorylated prior to PCR to ensure the presence of a 5' phosphate which was needed for efficient ligations during cloning. The target DNA was amplified in GeneAmp PCR System 9600 (Perkin Elmer Corp.) or PTC-200 DNA Engine (MJ Research, Inc.) using 50 µl reactions containing 1 µl of extracted DNA, 2.5 mM $MgCl_2$, 200 µM each dNTP, 500 nM each primer, 50 mM KCl, 10 mM Tris-HCl (pH 9.0), 0.1% Triton X-100, and 1-2 units *Taq* DNA polymerase. PCR was performed using 4 minutes of denaturation at 94°C, 35 cycles of 30 seconds at 94°C, 45 seconds at 55°C, and 1 minute at 72°C followed by a 7 minute extension step at 72°C. The size of PCR products was confirmed by 3% TAE agarose gel electrophoresis prior to cloning.

Construction and Isolation of Recombinant DNA

PCR products were cloned into dephosphorylated T-vector M13mp18 phage using T4 DNA *Ligase* under the suggested buffer conditions at 16°C overnight. Six microlitres of fresh unpurified PCR products were ligated to 37.5 ng of T-vector M13mp18 in a 10 µl ligation reaction. The T-vector M13mp18 was constructed after Marchuk et al. (1991) using a ten-fold overdigestion of *HincII* restriction endonuclease at 37°C for 1.5 hours to linearize the phage at the insertion site. Each recombinant clone was grown up in the DH5αF'IQ™ strain of *Escherichia coli* (Gibco BRL, Inc.) and purified using PEG/NaCl precipitation, phenol-chloroform purification, and ethanol precipitation (Sambrook et al. 1989). Recombinant clones were screened using PCR with the M13 universal and reverse primers to confirm correct-sized products (insert plus flanking vector) by 3% TAE agarose gel electrophoresis.

DNA Sequencing and Sequence Alignment

DNA sequences were obtained from the recombinant molecules using a model 373 automated DNA sequencer (Applied Biosystems, Inc.) and the M13 universal sequencing primer. Since the use of a T-vector allowed insertion of PCR products into M13mp18 in either orientation, each reported sequence represents the consensus of sequences from a minimum of four recombinant vectors with at least one sequence obtained from sequencing the inserted PCR product in either direction. Consensus sequences were compiled using the computer program SEQMAN (DNASTAR Inc.). The collected DNA and RNA sequences were aligned using the CLUSTAL algorithm of computer program

MEGALIGN (DNASTAR Inc.), with verification and correction by eye in the context of the alignments of Tillier et al. (1994, 1996) and Rosenberg et al. (1994).

Phylogenetic Analyses

Three separate data sets were examined: the D1 domain and D6 domain alone plus a combined domain data set that included only those genera for which sequences of both domains were available. Excluding the polyplacophoran outgroups and *Mytilus,* the D6 domain alignment was the only alignment to include representatives of more than just gastropods (eg. bivalves, cephalopods, and a scaphopod), allowing the examination of gastropod monophyly. Phylogenetic analyses were performed using a pre-release version of the computer program PAUP* (Swofford 1996) using the Polyplacophora (*Acanthochitona* and *Katharina*) as an outgroup (after Salvini-Plawen & Steiner 1996, Runnegar 1996). For analyses of the D1 domain alone and the combined domains, the bivalve *Mytilus* was used as an additional outgroup to the gastropod ingroup.

Parsimony analyses were performed using 300 random-addition replicates with TBR branch-swapping and restriction to a maximum of twenty trees in memory per replicate, with gaps treated as fifth character states and sequence ambiguities (N) treated as missing data. Since the random-addition approach would not find all equally-parsimonious trees, the shortest trees found were subjected to further TBR swapping to find all equally-parsimonious trees. To additionally be sure the random addition approach was finding the most parsimonious trees, an additional TBR swapping of a tree produced by closest-neighbour addition of taxa was performed in which the analysis was allowed to retain 2000 trees in memory during the analysis. To examine the effects of undetected homoplasy, neighbour-joining analyses were performed using the Kimura two parameter model for estimating distances among sequences (Kimura 1980), with an assumed transitions:transversion ratio of 2:1.

A measure of internal consistency for the phylogenetic trees found was obtained by bootstrap analysis of neighbour-joining analyses with Kimura's (1980) two-parameter model predicted distances (100 replicates). Bootstrapping of parsimony analyses could not be performed for the two domains alone due to the high number of taxa involved (prohibitive computing time) but were performed for the combined domain data (100 bootstrap replicates of 10 random addition replicates each).

RESULTS

DNA Sequences

Eleven of thirteen specimens preserved in formalin before transfer to 70% ethanol yielded PCR products for both the D1 and D6 domains of 28S rRNA (<300 base pairs in length). Attempts to amplify larger DNA molecules using Littlewood's (1994) primers for the D1-D3 domains of 28S rRNA (~950 bp), Folmer et al.'s (1994) primers for partial cytochrome oxidase subunit I (710 bp), and O'Foighil & Smith's (1995) primers for partial cytochrome oxidase subunit III (624 bp) failed for nearly all formalin preserved specimens but succeeded for fresh-frozen specimens, indicating that only short DNA fragments could be amplified from formalin preserved specimens. Newly obtained cytochrome oxidase subunit III sequences were deposited in the GenBank database (U78673-U78677).

Specimens from the hydrothermal vent genera *Symmetromphalus* (Neomphalidae) and *Pyropelta* (Cocculiniformia) and the vetigastropod genus *Perotrochus* failed to provide D1 and D6 domain PCR products. DNA samples of *Provanna* (Caenogastropoda) proved to be contaminated with *Depressigyra* (Neomphalina) DNA, based on comparison of caenogastropod and neomphalid sequences, and all *Provanna* sequences were discarded. Sequence identity for previously published D6 domain sequences are discussed in Rosenberg et al. (1994). Of the new DNA sequences, there were two identical pairs for the D6 domain: *Nerita* and *Olgasolaris*, *Shinkailepas* and *Bathynerita*. These pairs belong to the gastropod order Neritimorpha, which was nearly invariant for the D6 domain. For neither of these pairs was DNA extracted nor amplified at the same time. D1 domain sequences obtained from the same DNA extracts were not identical. All new D1 and D6 domain sequences were deposited in the GenBank database (U75841-U75863, U78643-U78672).

Sequence Alignments

The final alignments of the D1 and D6 domains are reported as DNA sequences in Figures 4 and 5, respectively. Three regions of the D1 domain, hereafter referred to as the variable regions, were highly variable and had to be aligned separately (positions 139-159, 179-214, 267-308, inclusive). Similarly, a large portion of the D6 domain variation occurred within the loop region (positions 59 to 101, inclusive) which also had to be aligned separately. These regions aligned well only within some taxonomic subgroups. To avoid bias from attempts to make their alignment more universal, the independent alignments predicted by MEGALIGN for these regions were retained with no alterations. The position of the D6 domain stems was confirmed by examination of complementary base pairing for each sequence. Complementary substitutions within the 5' stem and 3' stem were frequent. D1 domain RNA secondary structural predictions were examined using the computer program MFOLD (D. Gilbert, Indiana University, U.S.A.) but no conserved structures were found that could have aided in D1 domain alignment.

Attempts to include 28S rRNA sequences from the mouse (Hassouna et al. 1984) in the alignments, following Rosenberg et al. (1994), failed for the D1 domain and loop region of the D6 domain. Use of the D6 stem and flank mouse sequences did not change results and they were excluded from all analyses. The final alignments were incomplete for both domains at the 5' and 3' ends as many sequences started and terminated at different positions of the total alignment. The complete alignments were used in phylogenetic analysis with absent 5' and 3' flanks coded as missing data.

Figure 4. Alignment of the D1 domain of the 28S rRNA gene utilized in phylogenetic analyses. All sequences are presented as DNA. Identity to the sequence of *Mytilus edulis* is presented as a dot. Gaps are presented as a dash. Boxed regions were aligned separately.

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                             10        20        30        40        50        60        70        80       90
                             |         |         |         |         |         |         |         |         |
Mytilus              CGAC-CTCA-GATCAGA--CGAGAGTA--CCCGCTGAATTTAAGC-ATATCACTAA-GCGGAGGAAAAGAAACTAACTAGGATTCCCCT
Acanthochitona                 ..............C....................................................N.........G.
Katharina                                                               .....................A........A.........T.
Patella             GN.G.....G.....GAG.......T.............C..................................CN..CG........TC
Patina                .G.T...G.....GAG.......T..........A.C..A.GCA.-..........................C...CG........TC
Tectura                                                                 ..........................C...CG........TC
Eulepetopsis                                                            ..........................C...CG........TC
Bathynerita                                                             ...........G..................A..........C
Nerita                                                                  ...........G..................A..........C
Olgasolaris                                                             ...........G..................A..........C
Shinkailepas                                                            ...........G..................A..........C
Theodoxus                                                               ...........G..................A...........
Cyathermia                                                              ..............................A.........TC
Rhynchopelta           .-.......T............CC...................................................A.........TC
Depressigyra                                                            ..............................A.........TC
Melanodrymia                                                            ..............................A.........TC
Peltospira                                                              ..............................A.........TC
Diodora                 ..........N..........T...........................G........................A.........TC
Temnocinclis                                                            .....G..............-.........C.........TC
Haliotis                  ...................C.............C.............G........................A.........TC
Lepetodrilus
Clanculus
Calliostoma             .....................C...........................G........................A.........TC
Gibbula                    ..................C...........................G........................A.........T.
Monodonta               .....................C...........................G........................A.........T.
Tegula                                                                  .....G........................A.........T.
Bathymargarites                                                         ........................................TC
Ampullaria                                                              ..............................C.........TC
Viviparus                                                               ..............................A.........TC
Campanile                                                               .....T........................A.........TC
Calyptraea             G...........G..TCT....T....................................................A.........TC
Littorina            .N.N..........G.N.......T....................................................NN........TC
Pomatias                                                                       .......................C.........TC
Nucella                 ......G....G.........T....................................................A.........TC
Ocenebra             ..............G.........T....................................................A.........TC
Buccinum (DNA)                                                          ..............................A.........TC
Buccinum (RNA)                                                                  ............-......N.NN.........TC
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                              10        20        30        40        50        60        70        80        90
                              |         |         |         |         |         |         |         |         |
Mytilus               CGAC-CTCA-GATCAGA--CGAGAGTA--CCCGCTGAATTTAAGC-ATATCACTAA-GCGGAGGAAAAGAAACTAACTAGGATTCCCCT
Valvata                                                              ...T..................................G.........TC
Actaeon                    .........G.........TG........................A.................................T...C
Aplysia (RNA)          .N...........G.........T.........................A..........................A.C....T....
Aplysia (DNA)                                                        ...A..........................A......T...C
Archidoris                   .N......G.........TC........................A..........................C..........C
Physa                    .T.N........G.G.......T.........................A..........................A.........AC
Amphibola                ..........TG.........T.........................A...N......................N......T...C
Siphonaria            ..............G.C.......T.........................A..........................N......T...C
Lymnaea                .N...........G.........T.........................A..........................A......T...C
Phytia                 .............G.N...N..NT.........................A..........................N......T...C
Pseudoveronicella       G...........G.N.......T..........-..............A.......................N..A......T...C
Achatina               .............G.........T.........................A..........................A......T...C
Aneitea                 ......N.....G.N.......T.............C............T..........................A......T...C
Anguispira              .NN.........G.........T.........................A..........................A......T...C
Cochlodina                 .........G.N.......T................NN...N.N.N...N...................N..N......T...C
Gonaxis                                   ....T....................G....A...N.A....................A..........C
Helix                 ..............G.........T.........................T..........................A.........TC
Limax                  .N...........G.........T.........................A..........................A......T...C
Mesodon                     .-......G.N..N....T.........................A..........................N.........TC
Ouagapia              ..............G.........T.........................A..........................A......T...C
Pararhytida                 ........G.N.......T.........................A..........................A......T...C
Placostylus                .........G.N..N....T.........................T..........................A......T...C
Pleurodonte             ............G.T....N.NT.........................A..................N.......A..N.......C
Succinea                             .........T..TT.....................T..........................A......T...C
Ventridens             .............G.T......GT.........................A..........................A......T...C
Zebrina                                                                                                   T...C
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                          100       110       120       130       140       150       160       170       180
                           |         |         |         |         |         |         |         |         |
Mytilus           AGT-AATGG-CGAATGAAGCGGGAAGAGCTC-A--GCACCGAATCCCGCA-GCCT-T-GCGCTGC--AGGGAACTGTGGTGTTTGGGACG
Acanthochitona    ......C.N.-..G...............C.......G.........A.-.--GGC.C.TAG.CGCT-...........C..A.A....A
Katharina         ......C......G...............C.......G.........AS-.--GGG.C.TA..CGTT-...........C..A.A....A
Patella           .....GC.A....GC.......N......C...TC..CGA.......C.GCT.GGACCT..GCCGTTC.....T.....CN.AA...T.-
Patina            .....GC......GC..............C...TC..C--.......C.G.T.GGACCT..GC.GTTC.....T.....C..AA...T.-
Tectura           .....GC......GC..........A...C...TC..C--.......C.G.T.GGACCT..GC.GTTT...........C..AA...T.-
Eulepetopsis      .....GC......GC..............C...TC..C--.......C.G.T.GGACCA..GC.GTT.....C......C..AA...T.-
Bathynerita       ......C......G.....T.........C...................GC...........C........................C.A....
Nerita            ......C......G...............C.................T.GGC..C.G.CT.YC.A..C...............C.A....
Olgasolaris       ......C......G...............C.................C.GGC....G.CG..C.G..C...............C.A.T..
Shinkailepas      ......C......G.....T.........C...................GC...........C........................C.A....
Theodoxus         ......C......G...............C...................GGC.-C.A....TC....C...............C.A.T..
Cyathermia        ......C......G...............C...................................C....C..........A......
Rhynchopelta      ......C......G...............C.......................G..C..........C.......N.......G......
Depressigyra      ......C......G...............C...................................C....C.........CG......
Melanodrymia      ......C......G...............C.................T.................C....C..........A......
Peltospira        ......C......G...............C...................................C....C..........A......
Diodora           ......C...--.G...........C...C.................C..G.GGAAACT.--..G.CG.....................
Temnocinclis      ......C......G........-......C.................C.GGC.-..C.....C.G..C.....................
Haliotis          ......C.A....G...............C.................CTGGTT..GC.--N.C.G..C..............G.A.--..
Lepetodrilus                           ....A...C.................C.GGTTT.GC.--..C.G..C.....................
Clanculus                              ........C.................CTGGTT..GC.--...AG..C.....T................
Calliostoma       ......C......G...............C.................CTGGTT..GC.--..CAG..C....GT.N......N......-.
Gibbula           ......C......G...............C.................CTGGTT..GC.--...AG..C.....T.............N.-
Monodonta         ...T..C......G........T......C.................CTGGTT..GC.--...AG..C.....T.............-..
Tegula            ......C......G...............C.................CTGGTT..GC.--..CAG..C.....................
Bathymargarites   ......C......G...........A...C.................CTGGT.G.GC.TT..CAG........A................
Ampullaria        ......C......G...............C.................T.GGC.-..G.T...C.A..C..............G.......
Viviparus         ......C......G..........CA...C.................C.GGCA-..G.CA..C.G..T.....A........GC......
Campanile         ......C......G...............C.................C.GGC.-..G.T..TC.G..C..............A.......
Calyptraea        ......C......G........T.TC...C.................C.....A..C.T.....G..C...CGA........A.......
Littorina         ......C...N..G..........TC...C.................C....T.....TG....G..C..N...........A.......
Pomatias          ..G.N.C......G........N......C.................C....T.G.A.CG....G..N..T....T......A..A....
Nucella           ......C......G..........CC...C.................C.....A..C.T.....G..C..............A.......
Ocenebra          ..N...C.N....G..........CC...C.................C.....A..C.T.....G..C..............A.......
Buccinum (DNA)    ......C......G..........T....C.................C.....A..G.T.....G..C..............A.......
Buccinum (RNA)    ....N.C......G..........TC...C.................C.....A..G.T.....G..C..............A.......
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                           100       110       120       130       140       150       160       170       180
                            |         |         |         |         |         |         |         |         |
Mytilus            AGT-AATGG-CGAATGAAGCGGGAAGAGCTC-A--GCACCGAATCCCGCA-GCCT-T-GCGCTGC--AGGGAACTGTGGTGTTTGGGACG
Valvata            ......C......G..........T....C.................T.CGT.G-.CCA..GC.GA.C....C.........G....G.A
Actaeon            ......C......G...............C.................T.GGCG-..C.T..TC.A..C..............G.......
Aplysia (RNA)      ......C......G........A......C.................C....TG..A.AT....G..C..............G.......
Aplysia (DNA)      ......C......G...............C.................C....TG..C.A.....G..C..............G.......
Archidoris         ......C......G........T......C.................C.GGC.-C...C..TC.G..C..T...........GC......
Physa              ......C......G........T..A...C.................C....TG..C.AT....G..C..............G.......
Amphibola          ....N.C......G........N......C.................C.GGTG-..G.CA..C.G..C..............G.......
Siphonaria         ......C......G........A.T....C.................T....TG..G.A.....A..C..............G....N..
Lymnaea            ......C......G...............C.................C....TG..C.A.....G..C..............G.......
Phytia             ......C......G...............C.................T....TG..C.AT....A..C..............G.......
Pseudoveronicella  .N....C......G........A..T...C.................T....TG..C.......A..C..............G.......
Achatina           ......C......G........N..T...C.................T....TG..C.A.....A..C.......N......G.......
Aneitea            ......CNN....G.N......N..A...C.................C.G..TG..C.A...C.G..C..............NA......
Anguispira         ......C......G........N..A...C..N..............T....TG..C.N.....A..C.......N......G.......
Cochlodina         ....N.C......G........NN.A...C.C...............T....TG..C.A.....A..C..N...........G....N..
Gonaxis            ......CT.....G........N..AG..C.................T....TG..C.......A..C..............G.......
Helix              ......C......G........T..T...C.................T....TG..C.AT....A..C..............G.......
Limax              ......C......G...............C.................T....TG..C.A.....A..C..............G.......
Mesodon            ......C......G........N..T...C.................T....TG..C.AT....A..C..............G.......
Ouagapia           ......C......G........A......C.................T....TG..C.A.....A..C..............G.......
Pararhytida        ......C...N..G.....A..A..T...C...T.............C....TG..A.A.....A.................G.......
Placostylus        ......C......G........N......C.................T....TG..C.AT....A..C..N...........G.......
Pleurodonte        ......C......G........N..T...C.................T....TG..C.AT....A..C........N.....G.......
Succinea           ......C......G........T..A...C.................C....TG..C.A.....G..C..............G.......
Ventridens         ......C......G........T..T...C.................T....TG..C.A.....A..C..............G.......
Zebrina            ....N........G........N......C.................T....TG..C.A.....A..C..N....N......GC......
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                        190       200       210       220       230       240       250       260       270
                         |         |         |         |         |         |         |         |         |
Mytilus          T---CTATT-GGC---GCGATGTCCGGGTGCCTAGGTCCTCCTGATCGGGGCC-TCTCCC-AG-AGCGGGTGTCAGG-CCTTT-A--CCG
Acanthochitona   G....C-.CT.-T.....CGC..T........CGA....................T.................T.......C......A.
Katharina        G....C-.CT.CT.....CGC..A...TC..TCGA....................T......T..........T.......C......TA
Patella          CGACTCG.CA.--.GC.TC-..A...TCC.AAC.A....CG.G.GA.A..TGT.G...........T......G...........GTT..
Patina           CGACTCG.CA.--.GC.TC-..A...TCC.AAC.A....CG.G.GA.A..TGT.G...........T......G...........GTT..
Tectura          CGACTCG.CG.--.GC.TC-..A...TCC.AAC.A....CG.G.GA.A..TGT.G...........T......G...........GTT..
Eulepetopsis     CTACTCG.CG.--.GC.TC-..A...TCC.AAC.A....CG.G.GA.A..TGT.G...........T......G...........GTT..
Bathynerita      CGAGA.GC-C.T.CTC.-----.G.CA.C...C.A....................T..........T.......................
Nerita           .GAGT.G.-C.A.CTC.-----CG.-ATC...C.A......TC...T........T..........T.......................
Olgasolaris      .GAGT.G.-C.A.CTC.-----C..-.TC...C.A......TC...T........T..........T.......................
Shinkailepas     CGAGA.GC-C.T.CTC.-----.G.CA.C...C.A....................T..................................
Theodoxus        .GAGA.GC-C.A.CCC.-----.G..AAC...C.A.......C............T..........T.....CT..............T.
Cyathermia       CCAA..G.C..T.GAGTT-----TTCT.C...A.A...........................C.G.T.....................T.
Rhynchopelta     CCAA.CG.C..A.GGGT.CT-CGTT.C.C--.C.A...........................C.G......................G..
Depressigyra     CCAA.CG.C..T.GGGT.CT-A.T..C.C...C.A...........................C.G.T....................G..
Melanodrymia     CCGA..G.C..T.GGGTTC.----.AC.C...C.A...T.........T.....A.....C.C.G.T..................CA.T.
Peltospira       CCAA..G.C..T.GGGTTCC---T.TT.C...C.A...........................T.G.........................
Diodora          C...A.-.C........GTCGC..TCT.C...C.A.....T..............T.......G..T.........C.G.....G..G..
Temnocinclis     C....G.CC........TTCG....CT.....C.A.....T..............T..........T.................G..G..
Haliotis         NAAC.CG..C...ATC.ACGCACT.CAN....C.A.................-.--............................N..G..
Lepetodrilus     C....GGCC........TAC.....CT.....C.A.....T.....T........T......T...T....................G..
Clanculus        C.......C.........TG.....CT.....C.A.....T..............T......T...T....................G..
Calliostoma      C....GG.C.........C......CT.....C.A.....T.....N........T......T...T....................G..
Gibbula          -.......C.........TG.....CT.....C.A.....T..............T......T...T....................G..
Monodonta        C...-...C.........TG.....CT.....C.A.....T..............T......T...T....................G..
Tegula           C....GG.C.........TG.....CT.....C.A..T..T..............T......T...T....................G..
Bathymargarites  C....G..CC.......TCTG....T.....TC.A.....T.....T.......AT..........T.....G...........G..G..
Ampullaria       CCAA..G.C.C..GGT..CG-.CAG-----..G.A....C..........................T.................G.....
Viviparus        CCAA..G.C..T.CGC.TTG-.AGA-----..G.A...................AA......C.........................-T
Campanile        CCAA..GCC.CA.GGC.TT.-..GG-----..G.A.....................................................A-
Calyptraea       CCAA..G.C..T.CGCA.CG-..GG-----..G.A...............................T.....................-T
Littorina        CCAA..G.C..T.CGA-.CG-.GGA-----..G.A...........................C.........................-T
Pomatias         .CAA..G.C..T.CGAT.CG-..GG-----..G.A..............N................T............N.........T
Nucella          CCAA..G.C..T.TCC..CG-..GG-----..G.A.....................................................-T
Ocenebra         CCAA..G.C..T.TCC-.CG-..GG-----..G.A.....................................................-T
Buccinum (DNA)   CCAC..G.C..AATGCA.CG-..GT-----..G.A...............................T.....................A-
Buccinum (RNA)   .CAC..G.C..AATGCA.CG-..NT-----..G.A...............................T.....................A-
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                          190       200       210       220       230       240       250       260       270
                           |         |         |         |         |         |         |         |         |
Mytilus           T---CTATT-GGC---GCGATGTCCGGGTGCCTAGGTCCTCCTGATCGGGGCC-TCTCCC-AG-AGCGGGTGTCAGG-CCTTT-A--CCG
Valvata           CCAC.AG.C..--..C.G..-ACGG--CC...G.A.............A...T....................A.........TC..-G.
Actaeon           CCAA..G.C..AGCGC-GCG-.GG.C----..C.A.................TT.....-..T..........A..........C..-G.
Aplysia (RNA)     CCAC.AG.C..--..C...T-.CGA--.CA..G.A.............A...T...A................A..........G...T.
Aplysia (DNA)     CCAC.AG.C..--..C...T-.CGA--.CA..G.A.............A...T...A................A..........G...T.
Archidoris        CCAC.AGCC..--..C....-.CGG--.C...G.A.............A.............C.G.T......A..........G...T.
Physa             CCAC.AG.C..--..CATT.-.CGG--.C...G.A.............A...T..TA................A..........G...A.
Amphibola         CCAC.AG.C..--..C..AT-.C.G--A....C.A.............A...T...A................A.............--.
Siphonaria        CCAC.AG.C..--..TATC.-.CGG--ACA..G.A.............A...T...A.............N..A..........G...A.
Lymnaea           CCAC.AG.C..--..CATC.-.CGG--.CA..G.A.............A...T...A................A..........G...G.
Phytia            CCAC.AG.C..--..CATC.-.CGG--.C...G.A.............A...T...A.........T......A..........G...A.
Pseudoveronicella CCAC.AG.C..--..CATC.-..GG--.CA..G.A.............A...T...A................A..........G...A.
Achatina          CCAC.AG.C..--..CATC.-.AGG--.C.T.G.A.................T...A................A...G......G...A.
Aneitea           CCAC.AG.C..--..C..CG-.AGG--.....G.A.................T...A....G...........A..........G...T.
Anguispira        CCAC.AG.C..--..CATC.-.AGG--.C...G.A.................T...A................N..........G...G.
Cochlodina        .CNT.NG.C..--..C.TC.-.AGG--.C...G.A...........N.....T...A.............N..A..........G...N.
Gonaxis           CCAC.AG.C..--..CATC.-.AGG--.C.T.G.A.................T...A.............N..A..........G...A.
Helix             CCAC.AG.C..--..CATC.-.AGG--.C...G.A.................T...A................A..........G...G.
Limax             CCAC.AG.C..--..CATC.-.AGG--.C...G.A.................T...A................A..........G...A.
Mesodon           CCAC.AG.C..--..CATC.-.AGG--.N...G.A.................T.N.A.............N..A..........G...G.
Ouagapia          CCAC.AG.C..--..CATC.-.AGG--.....G.A.................T...A................A.............A.
Pararhytida       CCAC.AG.C..--..CATAC-.AGG--.N...G.A.................T...A.............N..A..........G...A.
Placostylus       CCAC.AG.C..--..CATCG-.AGG--.....G.A.................T...A.............N..A..........G...A.
Pleurodonte       CCAC.AGCC..--..CATC.-.AGG--.C...G.A.................T...A........N.......A..........G...G.
Succinea          CCAC.AG.C..--..C..CG-.AGG--.....G.A.................T...A................A..........G...T.
Ventridens        CCAC.AG.C..--..CATC.-.AGG--.C...G.A.................T...A.........N......A..........G...A.
Zebrina           CCAC.AG.C..--..CA.C.-.AGG--..A..G.A...........NN....T...A................A..........G...T.
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                          280       290       300       310       320       330       340       350
                           |         |         |         |         |         |         |         |
Mytilus           GCACC-TG-G-CGTCGTGCC-TCAG--AGCGTCCTTGGAGTCGGGTTGTTTGAGAATGCAGCCCAAAGCGGGTGGTAAACTCC
Acanthochitona    ...T.....A...CG..AG....T...........CA...............G................A........
Katharina         ..GA..C..T...CG..AG....T...........CA...............G................T.............
Patella           .T-.TGG.TCTTC.GCCCGGCG...TCG.TA-...GC......A....C...G..TC..N..T..........
Patina            .T-.TGG.TCTTC.GCCCGGCG...TCG.TA-...GC......A....C...G..TC..N..T........
Tectura           .T-.TGG.TCTTC.GC.CGGCG...TCG.TA-...GA......A....C...G..TC.....T.....T.........G....
Eulepetopsis      .T-.TGG.TCTTC.GTCCGGCG...TCG.TA-..CGA......A....C...G..TC.....T...............G....
Bathynerita       ..GA-G-...C..GG.C.G....C.........TCAA...............G..............................
Nerita            ..G..A...TG..GGTC.G....C....T....TCAA...............G..............................
Olgasolaris       ..G..AC...G..GGTC.G....C....T..A.TCAA...............G..............................
Shinkailepas      ..GA-G-...C..GG.C.G....C.........TCAA...............G..............................
Theodoxus         ..GA-TCA..C..GG.C.G....C.........TCAA...............G...............T..............
Cyathermia        ..G-.G-..AA.TCGTC.A....C..........CAA...............................T..............
Rhynchopelta      ..G-.G-...A.TCGTC.G....C..........CAA..................................
Depressigyra      ..G-.G-...A.TCGTC.A....C.........TCGA..............................................
Melanodrymia      ..G-.G-...A.TCG.C.G...AC..........CAC...........................T...T..............
Peltospira        ..G-.G-..AA.TCG.C.G....C..........CAC...............................T..............
Diodora           ..G-.G-...AGC.TCC.T.CCTC...........C................G......
Temnocinclis      ...-.G-...A.CGT.C.T.T.-C...........A................G...........C...T..............
Haliotis          ...-.G-...A..AGAC.T.T.GC...-.......C................G...............TN.
Lepetodrilus      ...-.G-A..A..GT.C.T.C.-C...........A................G...........T..................
Clanculus         ...-.G-...A.TAA.C.T.C.T-................................G...........T...T..............
Calliostoma       ...-.G-..-A.TAG.C.T.C.TC...........C.....N..........GN.....N..T.TN..T.....-.
Gibbula           ...-.G-...A.TAG.C.T.C.T-................................G......N.............
Monodonta         ...-.G-...A.TAG.C.T.C.T-................................G..........T-.........
Tegula            ...-.G-...A.TAA.C.T.C.TC................................G...........T...T..............
Bathymargarites   ...-.G-...A.CGG.C.T.T.TC...........A................G...............T..............
Ampullaria        .-CTTGCTC.GTCCGCG.G.TG.-.........TCA................G...............T..............
Viviparus         .-GT.TCT..G.CGG.C.G.TG.-.........TCA................G..............................
Campanile         .-G..ACC..G.CCGTG.G.TG.-.........TCC................G...........T..................
Calyptraea        .-G.TGCT..GTCGGTC.G.TG.-N.........CC................G......N.......
Littorina         .-GTGCCT..GTCGGTC.G.TG.-.........TC.C...............G...........T...A......
Pomatias          .-G.NGCT..GTCGGTC.G.TG.-.........TCC..N...T.........G....................--.T
Nucella           .-G.TGCT..G..GGTC.G.TG.-.........TCC................G......
Ocenebra          .---.GCT..G..GGTC.G.TG.-.........TCC................G......N................
Buccinum (DNA)    .-G..GCT..GT.CGTC.G.TG.-.........TCC................G...........T..................
Buccinum (RNA)    .-G..GCT..GT.CGTC.G.TG.-.........TCC................G...........TN.........
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                           280       290       300       310       320       330       340       350
                           |         |         |         |         |         |         |         |
Mytilus           GCACC-TG-G-CGTCGTGCC-TCAG--AGCGTCCTTGGAGTCGGGTTGTTTGAGAATGCAGCCCAAAGCGGGTGGTAAACTCC
Valvata           ..G.GCC.TCC..---C.G.CG.-.....T.C.TCC................G..............................
Actaeon           .AG.-.--C-NN.CGT.CG.CG.-..........CA................G......N........A..N.
Aplysia (RNA)     .TG.T.-T..CT.CG-C.G.CG.-.........TCA.................................
Aplysia (DNA)     .TG.T.-TC.CT.CG-C.G.CG.-.........TCA................G...............T..............
Archidoris        -.G-GCC.GCG..CG-C.G.CG.-..........CA........N.......G.......................
Physa             .TG.-.CTC.CT..G-C.G.CG.-.........TCA................G......T....
Amphibola         CTGGTACTC.G.-.GCGCG.CG.-..........CA................G................
Siphonaria        .TG.-.CTGTCT..G-C.G.CG.-N........TC......N..........G......N..
Lymnaea           .TG.TCC-..CT..G-C.G.CGA-.........TCN................G......N.......-..
Phytia            .TG.-.CTC.CT..G-C.G..G.-.........TCA................G..........N.....
Pseudoveronicella .TG-TCCTA-CT..G-C.G.CG.-.........TCA................G...................
Achatina          ..G.-.CTCTCT..G-C.G.CG.-.........TCA................G......N.........
Aneitea           ....-.CTCTC..CG-C.G.CG.C..C......TCN................G......N.........N.
Anguispira        .TG.-.CTCTCT..G-C.A.CG.-.........TCN................G.....................
Cochlodina        .TG.-.CTCTCT.CG-C.G.NGA-.........TCN................G......T............
Gonaxis           ..G.TCCTCTCT..G-C.G.CG.-.........TCA................G...................N.
Helix             .TG.-.CTCTCT..G-C.G.CG.-.........TCA................G...........................
Limax             .TG.-.CTCTCT..G-C.G.CG.-.........TCA................G..............N...
Mesodon           .TG.-.CTCTCT..G-C.G.CG.-.........TCN................G.....................
Ouagapia          .TG.-.CACTCT..G-C.G.CG.-.........TCA................G...................
Pararhytida       .TG.-.CTCTCT..G-C.G.NG.-.........TCA................G..................
Placostylus       .TG.-..TCTC...G-C.G.CG.-.........TCA................GN..............
Pleurodonte       .TG.-.CTCTCT..G-C.G.CG.-.........TCA.NNNN..........NNNN..............
Succinea          ....-.CTCTC..CG-C.G.CG.-.........TCN................G.....N............T..
Ventridens        .TG.-.CTCTCT..G-C.G.CG.-.........TCA................G...
Zebrina           .T..-.CTCTCT..G-C.G.TG.-.........TCA.....N..........G..............NNN.N.--.
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Figure 5. Alignment of the D6 domain of the 28S rRNA gene utilized in phylogenetic analyses. All sequences are presented as DNA. Identity to the sequence of *Acanthochitona* is presented as a dot, except for the last 37 bases of the alignment which are plotted against *Katharina*. Gaps are presented as a dash. Boxed regions were aligned separately. The stem regions flanking the variable loop region are indicated by asterisk and underlining. *Ma.* = *Margaritifera*, *Na.* = *Nautilus*, *Ce.* = *Cerithidea*.

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                            10        20        30        40        50        60        70        80        90
                            |         |         |         |       **|*********|         |         |         |
Acanthochitona     CCCTGAAAATNGATGGCGCT-AG-AGCGTCGTGCCTAT-ACCGCA-CCG-TCGC-CGCATCG-CGT-----GCGCCCCG-----------
Katharina                          ...........................................-....T............A............
Mytilus            ....T.....G....................GA..........G........AA.G...ANN.NNA.....C.A...T............
Amblema            ..........G...................NA..........AGG..............CTA...A.......C................
Anodonta grandis   ..........G...................NA..........AGG..............CTA...A.......C................
A. cataracta       ..........G...................AA..........AGG..............CTA...A.......C................
A. imbecilis       ..........G...................NA..........AGG..............CTA...A.......C................
Cumberlandia       ..........G..............N...NNA..........ANG..........AN.NATA...A.......C................
Elliptio           ..........G...................AA..........AGG..............CTA...A.......C................
Fusconaia          ..........G..................NNA..........AGG..N...........CTA...A.......C................
Gonidea            ..........G...................AA..........AGG..............ATA...A.......C................
Lampsilis teres    ..........G..................NNA..........AGN.NN...........CTA...A.......C................
L. claibornensis   ..........G..................NNA..........AGN.NN...........CTA...A.......C................
Ma. falcata        ..........G...................NA..........ANG..........A...ATA..T........TAA.AT...........
Ma. margaritifera  ..........G...................NA..........ANG..........A...ATA..A........C................
Megalonaias        ..........G..................NNA..........AGG..............CTA...A.......C................
Obliquaria         ..........G..................NNA..........AGG..............CTA...A.......C................
Plectomerus        ..........G..................NNA..........AGG.NN...........CTA...A.......C................
Pleurobema         ..........G...................NA..........ANN.NNN..........CTA...A.......C................
Quadrula quadrula  ..........G..................NNA..........AGG..N...........CTA...A.......C................
Q. cylindrica      ..........G..................NNA..........AGN..............CTA...A.......C................
Unio               ..........G...................NA..........AGG..............CTA...A.......C................
Uniomerus          ..........G...................NA..........AGG..............CTA...A.......C................
Cerastodermia      ..........G..........G..T....T.G...........G...............---.---T....A.T.----..AT.......
Mercenaria         ..........G..........GA........A...........GG..............ATA...A.......C................
Sphaerium          ..........G..........GA......T.A...........AG..............ATA.TA........T...A............
Venus              ..........G..........GN....N...A...........GN.N............ATA...A.......CN..A............
Dentalium          ..........G..........GA....N.T.G...........G.........GGG...GTN...A........G..--.GCTT.....G
Na. macromphalus   ..........G........NT.A........G...G...G...G.........T.....AAC.---..GAA.TT..TNAAACC.......
Na. pompilius      ..-.......G...........A........G-..G...G..NNN........T.....AAC.---..GAA.TT..TAAAACC.......
Loligo             ...-......G..........G...N-.A..GA..G...G..NN...........G..GAG..---..AGC.AATATAAACGC.......
Loliolus           ...-......G..........G...N-.A..GA..G...G..NN...........G..GAG..---..AGC.AATANNNNNTC.......
Sepia                                                                                     .-------.CGC.......
Uroteuthis         ...-......G..........G...N-.A..GA..G...G..NN...........G...AG..---..AGC.AATNTAAACTC.......
Patella (RNA)       .........G..........GT....N.GCA.....C....CNN.N.....T..G...ATTC.--...GCCT...---.....GCGGGG
Patella (DNA)                      .....GT......GCA.....C....CGG.......T..G...ATTC.TCGCGGCCT...---.....GCGGGG
Tectura                            .....GT......GCA.....C....CGG.......T..G...ATTTTC.CCGGCCT...---.....GGTAGA
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                          10        20        30        40        50        60        70        80        90
                           |         |         |         |       **|*********|         |         |         |
Acanthochitona    CCCTGAAAATNGATGGCGCT-AG-AGCGTCGTGCCTAT-ACCGCA-CCG-TCGC-CGCATCG-CGT-----GCGCCCCG-----------
Eulepetopsis                      .....GT......GCA.....C....CGG.......T..GA..ATTT.TCTCGCCT.T.TTT.TGTAAGGCAGG
Bathynerita                       .....T.........AA.........CG...........A...GTA...A.......A................
Nerita                            .....T.........AA.........CG...........A...CTA...A.......A................
Olgasolaris                       .....T.........AA.........CG...........A...CTA...A.......A................
Shinkailepas                      .....T.........AA.........CG...........A...GTA...A.......A................
Theodoxus                         .....T.........AA.........CG...........A...ATA...A.......A................
Helicina          ..........G..........C....-....A..........CN...........A...CTA...A.......A.....C..........
Cyathermia                        ...............G...........G........TG.G...ATA...A.......A....C...........
Rhynchopelta                      ...............G...........GG.......CG......ATA...A...........AC...........
Depressigyra                      ...............G.....C.....G........TG.GA..ATCG...CGTTT..CGTT.ACGCGGTTTTCG
Melanodrymia                      ...............G...........A........TG.G...ATA...A.......A...TT...........
Peltospira                        ...............G...........A........TG.G...ATA...A.......A....C...........
Diodora                           ...............G...........G...........G...CTAAA.A.......C.GTTTTG.........
Temnocinclis                      ...............G...........G...........G...CTAAA.A.......C..GGTT.CCG.....G
Haliotis                          ...............G...........G.......T...G...ATAAA.A.....A.C..--TC...G.....G
Perotrochus
Lepetodrilus                      ...............G...........G.......T...G...CTAAA.A.......C.TGTTCGTCA.....G
Clanculus                         ...............GA..........G.......T..TG...ATAAA.A.....A.C..--TC...G.....G
Monodonta         ..........G.......N..N.........GA....N.N...G.......T...G...AGNAA.A.....A....--TC...G.....G
Tegula                            ...............GA..........G.......T..TG...CTAAA.A.....A.C..--TC...G.....G
Lirularia                         ...............GA..........G.......T...G...ATAAA.A.....A.C..--TC...G.....G
Bathymargarites                   ..............CG...G.......A.......T..CG...GTAAA.A.....A.C..--TT.CGG.....G
Ampullaria                        .....G........AGA..........G........T..G...A...A.AAT..C..T.GGTCAAACG.....A
Viviparus         ..........G..........G.........GA..........GG..........G....GAG.TC.....T.C..TTTGCGGG.....A
Ce. costata       ..........G..........G.........GA..........G........T..G...AGAG.--.....--C..TTTC.GGG.....G
Ce. scaliformis   ..........G..........G.........GA..C.......G........T..G...AGAG.--.....-.C..TTTCGGGG.....G
Melanoides        ..........G..........G.........GA..........G........T..G...--T..AGTTGGG..C.TT......G.....G
Campanile                         .....G........AGA..C.......G........T..G...---.A.A.......C.TG.TGG.........
Littorina                         .....G........AGA..C.......GG....C.TC-.G...ATT.---..ATT.T..TGTAAAGT.......
Heleobops         ..........G..........G........CCA.T....T...G.....C.T.-.G...AT..---.AGAGCTT-----...........
Geomelania sp.    ..........G..........G........CCA.T....T...G.....C.T.-.G...A.-...A.....CTCT-.--...........
Geomelania typica ..........G..........G.......NCCA.T....T...G.....C.T.-.G...A.-...A.....CTCTT.--...........
Oncomelania       ..........G..........G....N.NNCCA.T....T...G.....T.T.-.G...AT..---..GT.---.T.TAAAG........
Truncatella sp.   ..........G..........G........CCA.T....T...G.....C.T.-.G...GT-.-TG.....-TTTT.AAAAA.A.....A
T. caribaeensis   ..........G..........G........CCA.T....T...G.....C.T.-.G...--...AAT..GGA.C..A-A....A.....A
T. clathrus       ..........G..........G........CCA.T....T...GN....C.T.-.G...AT..---..GT.....GG.CA..........
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                           10        20        30        40        50        60        70        80        90
                           |         |         |         |       **|*********|         |         |         |
Acanthochitona    CCCTGAAAATNGATGGCGCT-AG-AGCGTCGTGCCTAT-ACCGCA-CCG-TCGC-CGCATCG-CGT-----GCGCCCCG-----------
T. pulchella      ..........G..........G.......NCCA.T....T...G.....C.T.-.G...GTT.GTG.TC..-.TT.T..CGGAG.....G
T. reclusa        ..........G..........G........CCA.T....T...G.....C.T.-.G...GT-.GTG.....-.TTG.GAAAGCA.....A
T. scalaris         ........G..........GN..N.N.NCCA.T....T...GN....C.T.-.G...GT..---..GC..-..GG.CNNA........
T. subcylindrica  ..........G..........G........CCA.T....T...GN....C.T.-.G..CCA..---..GA.AGC.GGAAACG........
Progabbia         ..........G..........G.......TNNA..C..T...NG.....C.TC-.G...AG...-AT.....TT.----..ATA.....T
Buccinum                          .....G........AGA..C.......G.....C.TC-.G...C....C.TCG..-...AATTGCGCG.....G
Mancinella        ..........G..........G.T.....TNNA..C..T....GN....C.TC-.G...AG-.GCNNNC..-.T.GTA.GGGN.......
Busycon           ..........G........-.G.A.....NN-A..C.......G.T...C.TC-.G...---.---...TT..CGTG.-...........
Valvata                           ...............CA..C.......GG........G...G.A.....AGAGGC.TCTAGTCCT.CG.....A
Aplysia                           .....T-........GA..C.......GG.......T..G...--..TC-TTGG.-------A....A.....A
Archidoris                        .....G.........GA..C.......GG.......T..G...AG..---.AGGACTTAAA.TCCCCG.....A
Bursatella                        .....T-........GA..C.......GG.......T..G...---.---......TCT----...........
Radix             G.........G.....T.T.C..........GA..........GG.....A.T..G...A--.---.T...TTT.----..AT.......
Biomphalaria      ..........G...............T...NGA..........-G.......T..G...AT-...AGT...-.TGGAA-.GACA......
Planorbis         ..........G....................GA..........GG.......T..G...ATC.-TG.TT..-------ATAACG.....A
Helix             ..........G......A...T..T....A.GA....A.....TG.......T..G...AT-...G.TCA.-.T.GTAAGGACA.....A
Mesodon inflectus ..........G...................NGA..C.......-G.......T..G...AT-...G.TCA.-.T.GAAAGGACA.....A
Mesodon normalis  ..........G...................NGA..C.......-G.......T..G...AT-...G.TCA.-.T.GAAAGGACA.....A
Ventridens        ..........G...................NNA..C.......-G.NN....T..G...AT-...G.TAA.-.T.GAAAAGGCG.....A
Haplotrema         .........G...................NNA..C.......-G.NN....T..G...AT-...A.TCA.-.T.GAAAAGGCA.....A
Mesomphix         ..........G..................TNNA..C.......-G.......T..G...AT-...A.TCA.-.T.GAAAAGGCG.....A
Neohelix          ..........G...................NGA..C.......-G.......T..G...AT-...G.TCA.-.T.GAAAGGACA.....A
Triodopsis        ..........G...................NGA..C.......-G.......T..G...AT-...G.TCA.-.T.GAAAGGACA.....A
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```
                          100       110       120       130       140       150       160       170       180
                           | ********|*        |         |         |         |         |         |         |
Acanthochitona    ---GGTGCCAAGCGGCGA-CG-AGTAGG-AGGGC-CGCT---GCGGTgAGCACTGAAGCC-TC-GGG-CGCGAGC-CTGGGTGGAGCcgc
Katharina         ...............................................G......................T................CGC
Mytilus           .............TTT....C..C.N..A.....C...GCCGC.....T..GTC....T...G......NT.........A......
Amblema           ..........T....NN.....................C....................T..T...........................
Anodonta grandis  ..........T....NN.....................C....................T............................N.
A. cataracta      ..........T.....C.....................C.........N..........T..............................
A. imbecilils     ..........T....NN.....................C....................T..............................
Cumberlandia      .....A....T...TNN..................................GTC.................N......A......NT
Elliptio          ..........T.....C.....................C....................T..T...........................
Fusconaia         ..........T....NN..N..................C....................T..T...........................
Gonidea           .........NT.....C..NN.................C....................T..T............C............N.
Lampsilis teres   ..........T....NN.....................C....................T..............................
L. claibornensis  ..........T....NN.....................C....................T..............................
Ma. falcata       ..G...A...T..T..N..................................GTC........T.......T................T..
Ma. margaritifera ..........T..T..N..................................GTC........T.......NN..........N....C
Megalonaias       ..........T....NN.....................C....................T.......................
Obliquaria        .......N..T....NN.....................C....................T..............................
Plectomerus       ..........T....NN.....................C....................T..T...........................
Pleurobema        ..........T.....C.....................C....................T..T...........................
Quadrula quadrula ..........T....NN.....................C....................T..............................
Q. cylindrica     ..........T....NN.....................C....................T..............................
Unio              ..........T....NN.....................C....................T..T..........................
Uniomerus         ..........T....NN.....................C....................T..............................
Cerastodermia     .CNNAAA.G.T...................A..-...................A.....-....T.C.........GC.AC..C.N.
Mercenaria        .....A....T..........................TC.........-..G.A....T..CG.......T.........A.........
Sphaerium         .....A.G..T......................T.................GTG........G..C....T.....GC............
Venus             .....A....T..........................TC....T.......GTA.......CG..C..A.T..T..GC.
Dentalium         G...CNC...T..CT........N.........T.................G.C.....T.CT..............C.A...-.
Na. macromphalus  GAG.AAC.-.G...T........N.........-....C.........C..TTA...A..A.TC..........T......C.....C
Na. pompilius     GAG.AAC.-.G...T..................-....C.........C..TTA...A..A.TC.......
Loligo            GCCTAAC.G.T..C...................-.N..C.........T....G........A........N.....C
Loliolus          GCCTAAC.G.T..C.......
Sepia             GCCTAAC.G.T..C...................-.N..C.........C....G........A........N.....C.....
Uroteuthis        GCCTAAC.G.T..C.......
Patella (RNA)     GACT.AC.G....C.A.....G...........T....C..G.....C...N..........T.A.A.......T........T...
Patella (DNA)     .ACT.AC.G....C.A.................T....C........C...G..........T.A.A.......T........T...G..
Tectura           GACT.AC.G....C.A.................T....C........C...G..........T.A.A.......T........T...G..
```

```
                          100       110       120       130       140       150       160       170       180
                           | ********|*        |         |         |         |         |         |         |
Acanthochitona    ---GGTGCCAAGCGGCGA-CG-AGTAGG-AGGGC-CGCT---GCGGTgAGCACTGAAGCC-TC-GGG-CGCGAGC-CTGGGTGGAGCagc
Eulepetopsis      GACC.AC.G...TC.A..................T....C........C...G..........T.A.A.......T........T...G..
Bathynerita       .....C.......T..........................C.........-...TC.......CG..A..........T.............
Nerita            .....C....C..T..........................C.........-...T.........G..A..........T.............
Olgasolaris       .....C....C..T..........................C.........-...T.........G..A..........T.............
Shinkailepas      .....C.......T..........................C.........-...TC.......CG..A..........T.............
Theodoxus         .....C....T..T..........................C.........-...TC........G..A..........T.............
Helicina          ....NC....T..TC.N.......................C.........-...TC........G..A..........T...........
Cyathermia        ......A...T..CCA...................T....C....T........TA.......CG.A.....T...................
Rhynchopelta      ......A...T...CG...................T....C....T...............T..TC.............C............
Depressigyra      A.C..CA...C.TCCA...................T....C............GTC.....T.CG.A..........................
Melanodrymia      ......A...T..CCA...................T....C....T........TC.......CG.A..........................
Peltospira        ......A...T..CCA...................T....C....T........TA.......CG.A.....T...................
Diodora           G....A.T.....C.....................T....C....A.......G.G.....T..G................A..........
Temnocinclis      G....AAT..C..C.....................T....C....A........TG.....T..G.......A........A..........
Haliotis          G....A.T..C..C..A..................T....C....A.......GTG.....T..G................A..........
Perotrochus         T-.A....G..T.AAG..............NTN.NN...............G.C.N......N...A.......T........
Lepetodrilus      G....A.T..C..C..A..................T....C....A.......GTG.....T..G................A..........
Clanculus         G....A.T..C..C..A..................T....C....A.......GTG.....T..G................A..........
Monodonta         G....A.T.NC..C..A.......NN.........N....C....A.......GTG.....T..G............N...A........
Tegula            G....A.T..C..C..A..................T....C....A.......GTG.....T..G................A..........
Lirularia         G....A.T..C..C..A..................T....C....A.......GTG.....T..G................A..........
Bathymargarites   G....A.T..C..C..A..................T....C....A.......GT......T..G.......T...................
Ampullaria        GCGA.A....C..T.A...................................G.C.........G...A.......T...............
Viviparus         G.........................A.............C..........G.C.........G...A.......T...............
Ce. costata       G...AA....T....A...................................GTA.....T..G...........................
Ce. scaliformis   G...AA....T....A...................................GTA.....T..G...........................
Melanoides        GCC-.A.......C.A........................C..........G.C.........G...........................
Campanile         G...CA.........A........................C.........T..G.C.........G...A.......T...............
Littorina         ACTTTG.......T.-A.G................................GTC.....T..G...A...T...T...AA..........
Heleobops         ...-----....NCC-A.G.....................C....TT.....G.C.........G...A...T...T...............
Geomelania sp.    ...-.A.......TC-A.G...........N..........T.......GTC.........G...A...T...T.....
Geomelania typica ...-.A.......TC-A.G......................T.......GTC.........G...A...T...T.......
Oncomelania       ...----......TC-A.A......................T.......GTC.........G.A.A...T...T...........
Truncatella sp.   G..-AA.......TC-A.G......................T.......GTC.........G...A.......T.....
T. caribaeensis   GCC-AA.......TC-A.G......................T.......GTC.........G...A.......T......
T. clathrus       ...-AA.......CC-A.G......................T.......GTC.........G...A...
```

```
                           100       110       120       130       140       150       160       170     180
                           | ********|*        |         |         |         |         |         |         |
Acanthochitona    ---GGTGCCAAGCGGCGA-CG-AGTAGG-AGGGC-CGCT---GCGGTgAGCACTGAAGCC-TC-GGG-CGCGAGC-CTGGGTGGAGCcgc
T. pulchella      A..-AA.......TC-A.G.............N..........T.......GTC........G...A
T. reclusa        C..-AA.......TC-A.G........................T.......GTC........G...A.......T.....
T. scalaris       GCC-AA.......CC-N.G........................T.......GTC........G...A
T. subcylindrica  GCT.AA.A.....TC-A.G........................T.......GTC........G...A.......T......
Progabbia         GCG-A..A.....C.-A.G..............N.N..C.........G..G.C........G...A.......T............NN.
Buccinum          A....A.......T.-A.G................................G.C........G...A...T...T...............
Mancinella        ...-NA.......T.-A.G..............N.N..C............G.C........G...A.......T............A.
Busycon           ...-AA...N...T.-A.G................................G.C........G...A.......T...............
Valvata           GCC-.AA...G..CC..................T....C....G..A....GTC....T...T...A.A....TT..C..A.......T.
Aplysia           G..--A.......C.A.....................TC....G.......GTG........G.....A....T................
Archidoris        GC.----...G..C.A......................C....G.......G.G........G.....A....T........T.......
Bursatella        .CT-.AT......C.A.....................TC....G.......GTG........G.....A....T................
Radix             .TGAAAA-.....C.A...........A...............G.......GTC........G...A.A....TT...............
Biomphalaria      G..----------T.AC..........................G.......GTG........G...A.A....TT.............
Planorbis         A..-.A.--....T.A...................................CT.........
Helix             G..----------C.A.....................TC....G.......GTG........G.....A.T..T......-..-.
Mesodon inflectus G..----------T.AC....................TC....G.......GTG........G.....A.T..T..............
Mesodon normalis  G..----------T.AC....................TC....G.......GTG........G.....A.TN.T........N.....
Ventridens        G..----------C.AC....................TC....G.......GTG........G.....A....T............
Haplotrema        G..----------C.AC....................TC....G.......GTG........G.....A....T.......
Mesomphix         G..----------C.AC....................TC....A.......GTN........G.....A....T............
Neohelix          G..----------T.AC....................TC....G.......GTG........G.....A.T..T........N.....
Triodopsis        G..----------T.AC....................TC....G.......GTG........G.....A.T..T..............
```

```
                         190       200       210                               190       200       210
                          |         |         |                                 |         |         |
Katharina        CGCRGGTGCAGATCTTGGTGGTAGTAGCAAATATTCA Katharina       CGCRGGTGCAGATCTTGGTGGTAGTAGCAAATATTCA
Amblema          ...                                   Melanodrymia    .A.G.................................
A. grandis       .                                     Peltospira      .ATG.................................
A. cataracta     ..                                    Diodora         .T.G.................................
A. imbecilis     .                                     Temnocinclis    .T.G.................................
Fusconaia        ...                                   Haliotis        .T.G.................................
Gonidea          .                                     Lepetodrilus    .T.G.................................
Lampsilis teres  ...                                   Clanculus       .T.G.................................
L. claibornen    ...                                   Tegula          .T.G.................................
Obliquaria       ...                                   Lirularia       .T.G.................................
Plectomerus      ...                                   Bathymargarites .T.G.................................
Pleurobema       .                                     Ampullaria      ...A..C..............................
Q. quadrula      ...                                   Viviparus       ...G
Uniomerus        ..                                    Ce. costata     ...A
Mercenaria       ...G                                  Ce. scaliformis ...A
Sphaerium        ...G                                  Melanoides      ...G
Patella (DNA)    ...G.....C..........................G Campanile       ...G..C..............................
Tectura          ...G.....C..........................G Littorina       ...A................................
Eulepetopsis     ...G.....C..........................G Heleobops       .A.G
Bathynerita      ...G................................. Progabbia       .
Nerita           ...G................................. Buccinum        ...A..C..............................
Olgasolaris      ...G................................. Busycon         ...A
Shinkailepas     ...G................................. Valvata         .C.G.................................
Theodoxus        ...G................................. Aplysia         .C.G.................................
Cyathermia       .ATG................................. Archidoris      .C.G.................................
Rhynchopelta     .A.G................................. Bursatella      .C.G.................................
Depressigyra     ...G................................. Radix           .C.G
```

Phylogenetic Signal

Phylogenetic signal and the number of positions informative for parsimony increased with length of sequence (Table 7). Signal also increased with the exclusion of the D6 domain loop and D1 domain variable regions. Rates of evolution varied widely for the D6 domain sequences (Figure 6A) and distances between the Cephalopoda and Gastropoda (0.50-0.80) exceeded the distances of either from the outgroup Polyplacophora (maximum distance 0.40). Evolutionary rates were uniform for the D1 domain and the combined domains, with the exception of roughly two-fold higher rate of evolution for the Patellogastropoda (Figure 6B & 6C). This elevated evolutionary rate for the Patellogastropoda resulted in it being more distant from other gastropods than from the outgroup taxa. These observed patterns of rate variation in both domains remained unchanged after removal of the D1 domain variable regions and D6 domain loop. Saturation of transitions occurred between pairs of sequences with Kimura's two-parameter predicted distance of 0.15 or greater between them for the D1 domain and combined domains (Figure 7), equivalent to the supra-ordinal level of the Gastropoda. Base substitutions between more closely related sequences revealed a slight difference between rate of accumulation of transitions and transversions, indicating a transitions-first model of accumulation of base substitutions was inappropriate. Saturation of all substitutions occurred at the ordinal level for the Gastropoda (Table 8).

Table 7. Information content of the phylogenetic data sets analyzed. The number of positions within the alignment that were constant, uninformative for parsimony, and informative for parsimony are presented. Skewness of the frequency distribution of one hundred thousand random trees is also presented (outgrouping enforced). Greater negative skew indicates that the most parsimonious trees were rarer than expected from a normal distribution, a measure of greater phylogenetic signal for parsimony. Information is presented for the entire alignment, the entire alignment less the D1 domain variable regions (VR) or the D6 domain loop, and the entire alignment less transitions.

Data	Taxa	Constant	Uninformative	Informative	Total	Skew (g_1)
D1 Domain						
Total	**63**	**130**	**55**	**169**	**354**	**-0.465871**
VR excluded	**63**	**128**	**53**	**74**	**255**	**-0.644902**
Transversion only	**63**	**181**	**51**	**122**	**354**	**-0.402051**
D6 Domain						
Total	**96**	**75**	**31**	**111**	**217**	**-0.383356**
Loop Excluded	**96**	**75**	**31**	**68**	**174**	**-0.381620**
Transversion only	**96**	**101**	**31**	**85**	**217**	**-0.400136**
Both Domains						
Total	**35**	**267**	**48**	**256**	**571**	**-0.814014**
VR & LP Excl.	**35**	**263**	**46**	**120**	**429**	**-1.170783**
Transversion only	**35**	**341**	**48**	**182**	**571**	**-0.743392**

Figure 6. Variation in evolutionary rates for the sequences examined. Estimated Kimura's two-parameter distance of each sequence from outgroup sequences is plotted (Polyplacophora = *Acanthochitona* and *Katharina*). The Patellogastropoda exhibits roughly two-fold higher rate of evolution for the D1 and combined domains. **A**: D1 domain, **B**: D6 domain, **C**: combined domains.

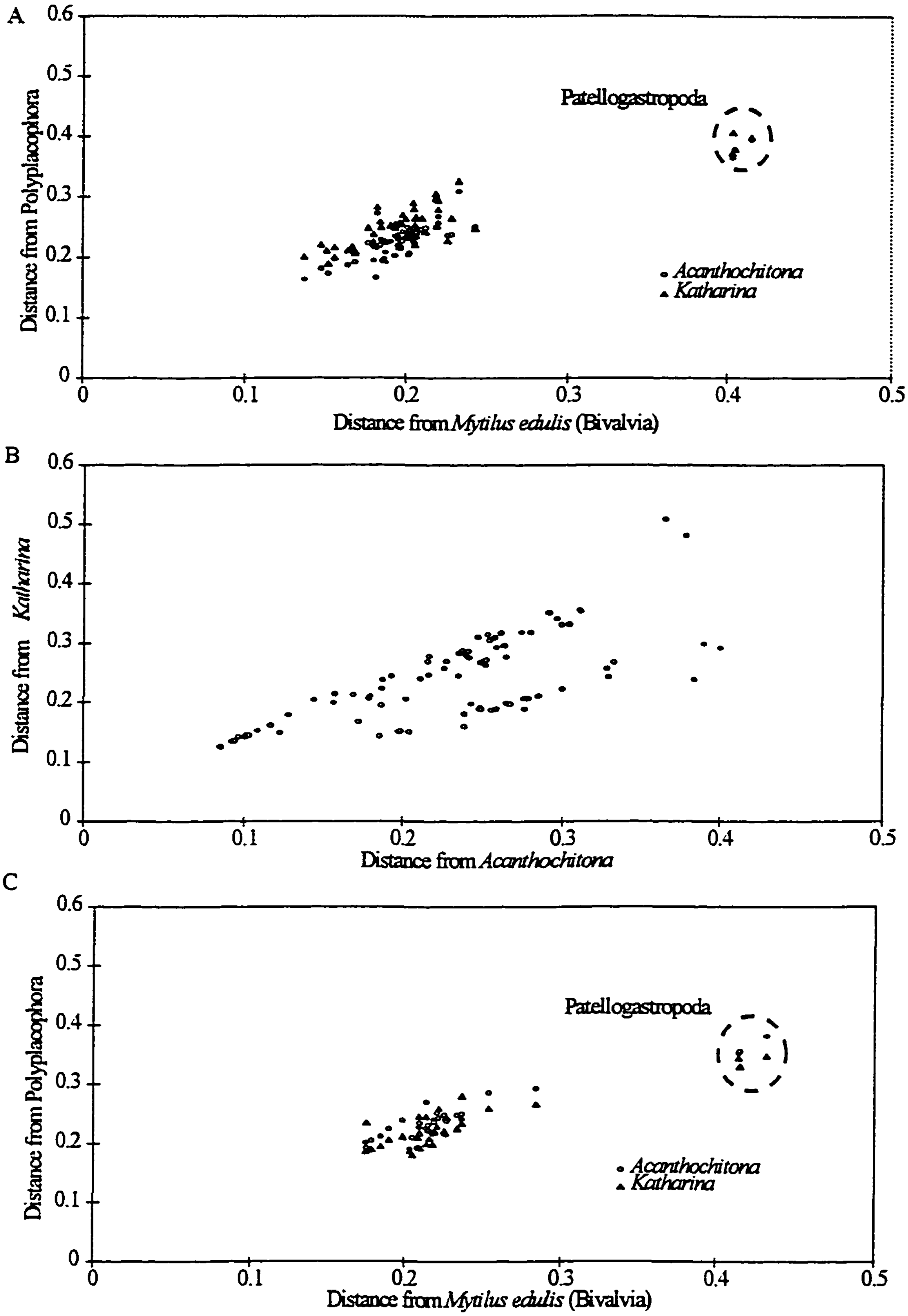
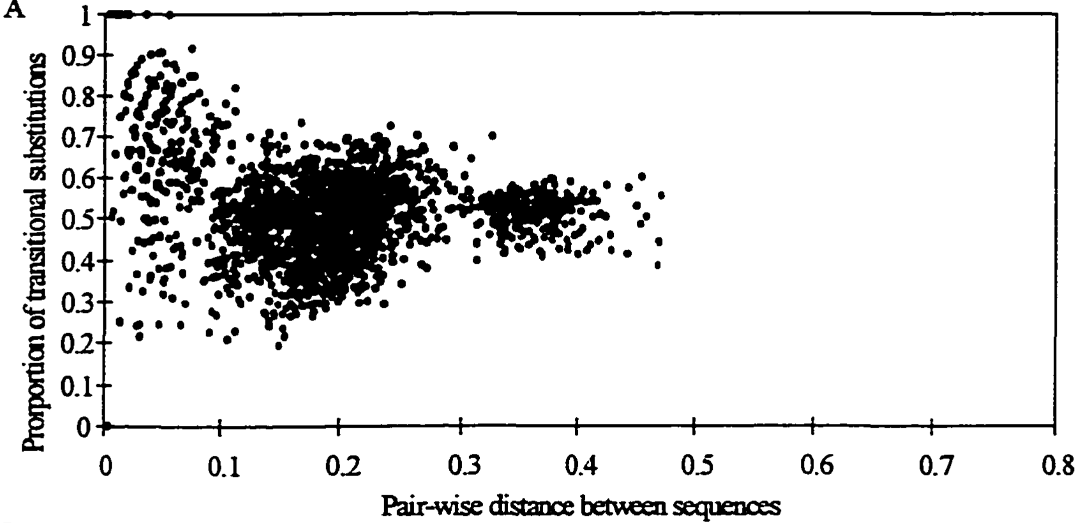
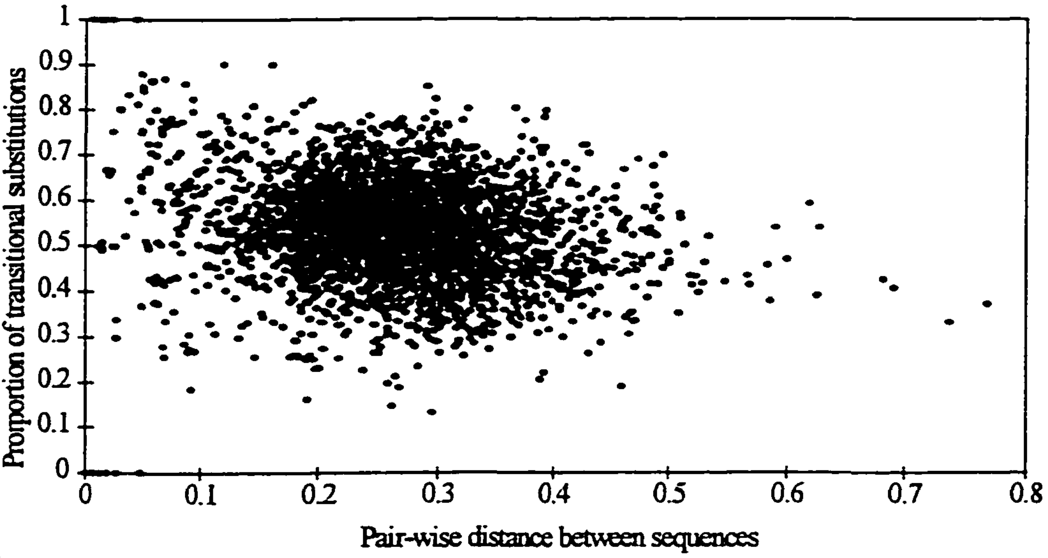

A
0.6
0.5
0.4
0.3
0.2
0.1
0
Distance from Polyplacophora
Patellogastropoda
Acanthochitona
Katharina
0
0.1
0.2
0.3
0.4
0.5
Distance from Mytilus edulis (Bivalvia)
B
Distance from Katharina
Distance from Acanthochitona
C
Distance from Polyplacophora
Patellogastropoda
Acanthochitona
Katharina
Distance from Mytilus edulis (Bivalvia)

Figure 7. Accumulation of transitions among sequences. Sequences are saturated at roughly 15% divergence for the D1 domain and the combined domains. **A**: D1 domain, **B**: D6 domain, **C**: combined domains.

A


Proportion of transitional substitutions
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
Pair-wise distance between sequences
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8

B


Proportion of transitional substitutions
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
Pair-wise distance between sequences
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8

C

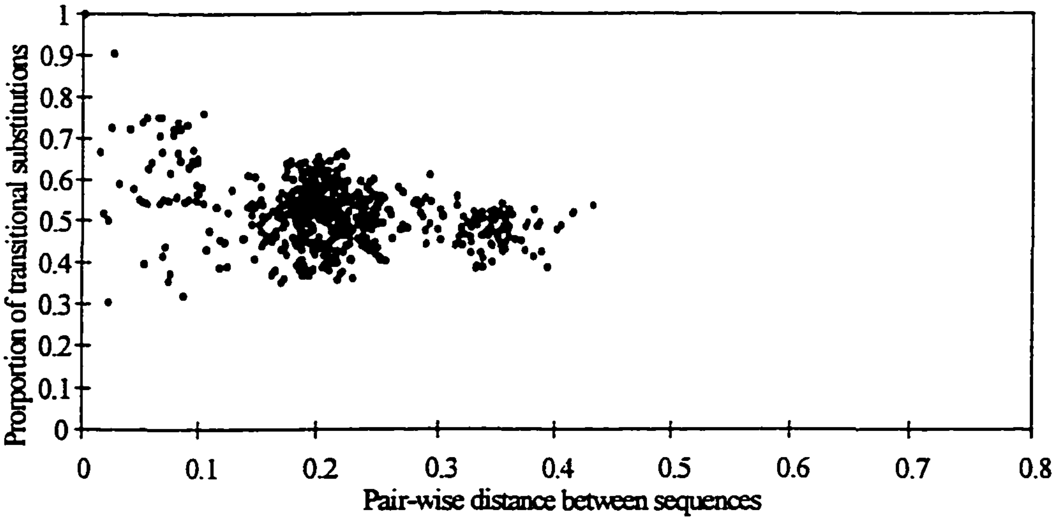

Proportion of transitional substitutions
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
Pair-wise distance between sequences
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8

Table 8. Distance of *Tegula pulligo* sequence from other sequences, estimated using Kimura's two-parameter model. The partial D6 domain sequence of *Perotrochus maureri* was excluded. Distances are presented relative to increasing taxonomic units. Distances among gastropod orders are equivalent to distances from outgroups, indicating saturation of substitutions beyond the ordinal level. The Patellogastropoda was more distant from *T. pulligo* than sequences from the outgroups, indicating an elevated rate of molecular evolution in this gastropod lineage. Overall, the pattern was the same regardless of which reference species was chosen.

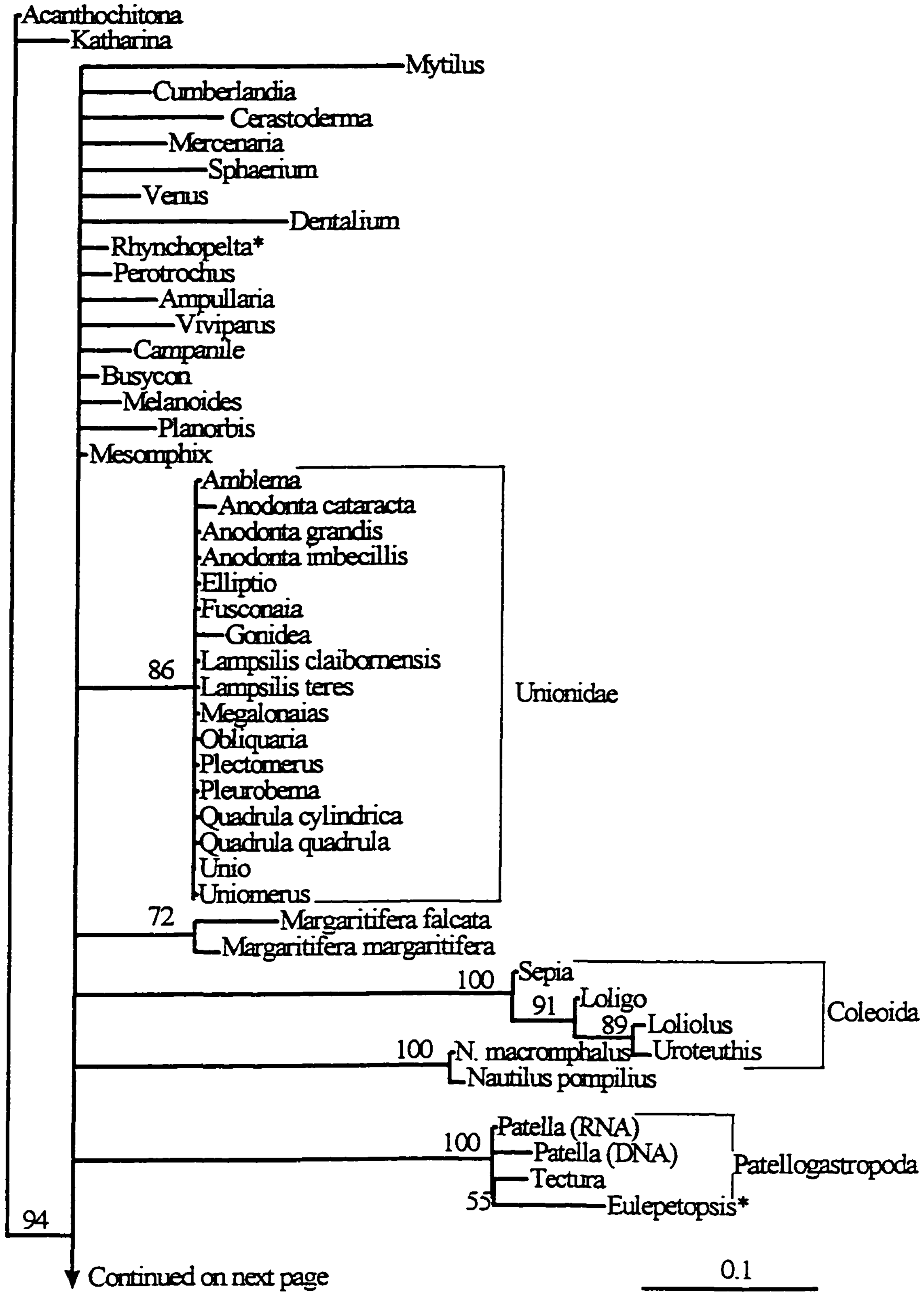
Distances from *Tegula pulligo*	D1 Domain	D6 Domain	Both Domains
Other Genera of Trochidae	**0.0224 - 0.1100**	**0.0060 - 0.0622**	**0.0060 - 0.0622**
Other Vetigastropoda Families	**0.0787 - 0.1591**	**0.0120 - 0.0500**	**0.0120 - 0.0500**
Other Orthogastropod Orders	**0.1693 - 0.2857**	**0.1532 - 0.3292**	**0.1532 - 0.3242**
The Patellogastropoda	**0.3533 - 0.3832**	**0.3126 - 0.3794**	**0.3126 - 0.3204**
Other Molluscan Classes	**0.1793 - 0.2255**	**0.1910 - 0.4890**	**0.2059 - 0.2905**

Phylogenetic Hypotheses

The D6 domain lacked resolution, as measured by bootstrap values, beyond relationships within specific sub-groups of the Gastropoda, Cephalopoda, and Bivalvia (Figure 8). With this lack of resolution, bootstrap analyses neither supported nor rejected monophyly of the molluscan classes. Exclusion of the more variable loop region gave the same results. The groups supported by bootstrap values were the bivalve family Unionidae, the Coleoida, Patellogastropoda, Neritimorpha, Neomphalina (excluding *Rhynchopelta*), Vetigastropoda (excluding *Perotrochus*), the Rissooidea, the holopod pulmonates, and separately the genera *Nautilus*, *Margaritifera*, and *Cerithidea*. A sister relation between *Valvata* and the Opisthobranchia was also weakly supported. Beyond the bootstrap supported groups, the most parsimonious trees exhibited polyphyletic molluscan classes and confused within-class phylogenies (not shown). Forced monophyly of the molluscan classes and exclusion of the loop region or transitions did not improve phylogenetic resolution, as supported by bootstrap values.

Phylogenetic resolution obtained using the D1 domain was superior to that of the D6 domain as bootstrap analyses additionally supported the monophyly of Neomphalina (including *Rhynchopelta*), Caenogastropoda (excluding the Architaenioglossa and *Campanile*), and Euthyneura (with the exception of the opisthobranchs *Actaeon* and *Archidoris* and the pulmonate *Amphibola*) (Figure 9). As for the D6 domain, resolution of older relationships was not supported by bootstrap analyses. Outside of the bootstrap supported groupings, parsimony (Figure 10) and neighbour-joining (Figure 11) analyses additionally supported a Caenogastropoda / Heterobranchia / Patellogastropoda clade.

Figure 8. Bootstrapping of neighbour-joining analyses of the complete D6 domain alignment, using the Polyplacophora as an outgroup. Genera strictly endemic to hydrothermal vents and hydrocarbon seeps are labeled with an asterisk. Nodes with less than 50% bootstrap support are collapsed.



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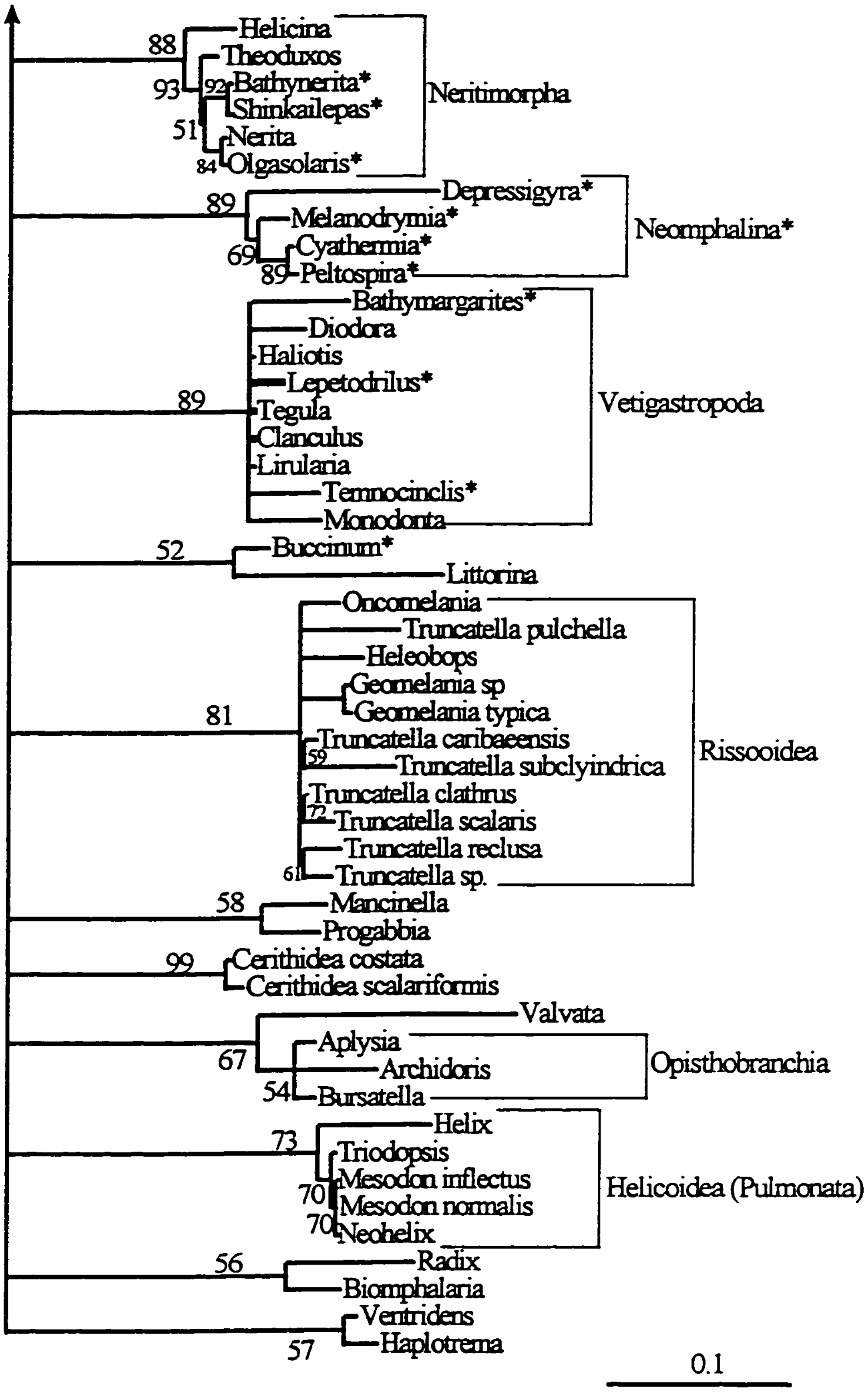

Helicina
Theodoxos
Bathynerita*
Shinkailepas*
Nerita
Olgasolaris*
Neritimorpha
88
93
92
51
84
Depressigyra*
Melanodrymia*
Cyathermia*
Peltospira*
Neomphalina*
89
69
89
Bathymargarites*
Diodora
Haliotis
Lepetodrilus*
Tegula
Clanculus
Lirularia
Temnocinclis*
Monodonta
Vetigastropoda
89
Buccinum*
Littorina
52
Oncomelania
Truncatella pulchella
Heleobops
Geomelania sp
Geomelania typica
Truncatella caribaeensis
Truncatella subclyindrica
Truncatella clathrus
Truncatella scalaris
Truncatella reclusa
Truncatella sp.
Rissooidea
81
59
72
61
Mancinella
Progabbia
58
Cerithidea costata
Cerithidea scalariformis
99
Valvata
Aplysia
Archidoris
Bursatella
Opisthobranchia
67
54
Helix
Triodopsis
Mesodon inflectus
Mesodon normalis
Neohelix
Helicoidea (Pulmonata)
73
70
70
Radix
Biomphalaria
56
Ventridens
Haplotrema
57
0.1

Figure 9. Bootstrapping of neighbour-joining analyses of the complete D1 domain alignment, using the Polyplacophora and *Mytilus* as outgroups. Genera strictly endemic to hydrothermal vents and hydrocarbon seeps are labeled with an asterisk. Nodes with less than 50% bootstrap support are collapsed.

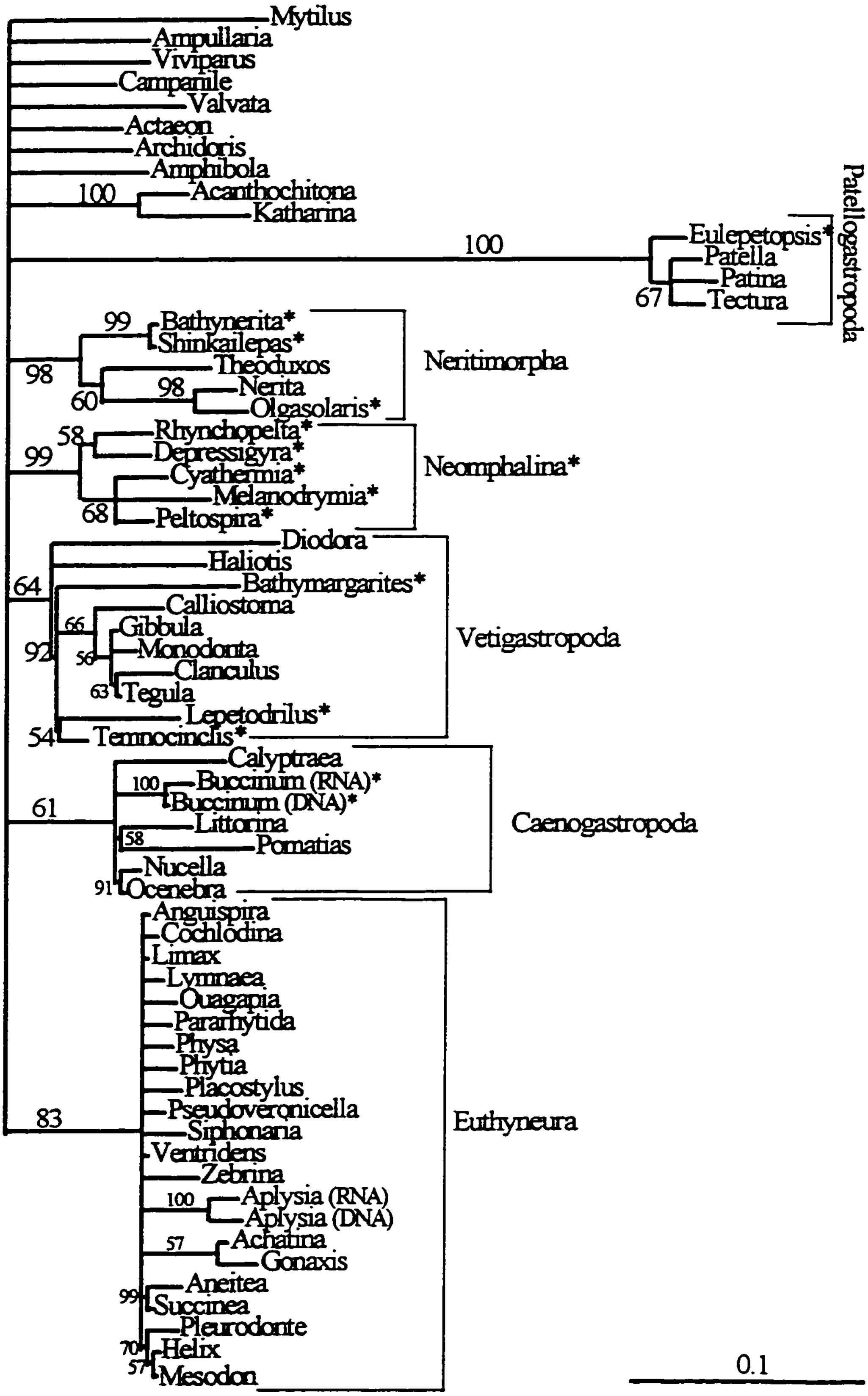

Mytilus
Ampullaria
Viviparus
Campanile
Valvata
Actaeon
Archidoris
Amphibola
100
Acanthochitona
Katharina
100
Eulepetopsis*
Patella
Patina
67
Tectura
Patellogastropoda
99
Bathynerita*
Shinkailepas*
Theodoxos
98
60
98
Nerita
Olgasolaris*
Neritimorpha
58
Rhynchopelta*
Depressigyra*
99
Cyathermia*
Melanodrymia*
68
Peltospira*
Neomphalina*
Diodora
Haliotis
64
Bathymargarites*
Calliostoma
66
Gibbula
92
56
Monodonta
Clanculus
63
Tegula
Lepetodrilus*
54
Temnocinclis*
Vetigastropoda
Calyptraea
100
Buccinum (RNA)*
Buccinum (DNA)*
61
Littorina
58
Pomatias
Nucella
91
Ocenebra
Caenogastropoda
Anguispira
Cochlodina
Limax
Lymnaea
Ouagapia
Pararhytida
Physa
Phytia
Placostylus
83
Pseudoveronicella
Siphonaria
Ventridens
Zebrina
100
Aplysia (RNA)
Aplysia (DNA)
57
Achatina
Gonaxis
Aneitea
99
Succinea
Pleurodonte
70
Helix
57
Mesodon
Euthyneura
0.1

Figure 10. Consensus of the 516 most parsimonious trees from the D1 domain alignment (length of 961 steps). Genera strictly endemic to hydrothermal vents and hydrocarbon seeps are labeled with an asterisk. All nodes were found in 100% of trees unless otherwise indicated. Nodes found in less than 50% of trees are collapsed. The Patellogastropoda branches from within the Heterobranchia. The Opisthobranchia and Basommatophora were not monophyletic and the Stylommatophora was monophyletic except for the inclusion of *Pseudoveronicella*.

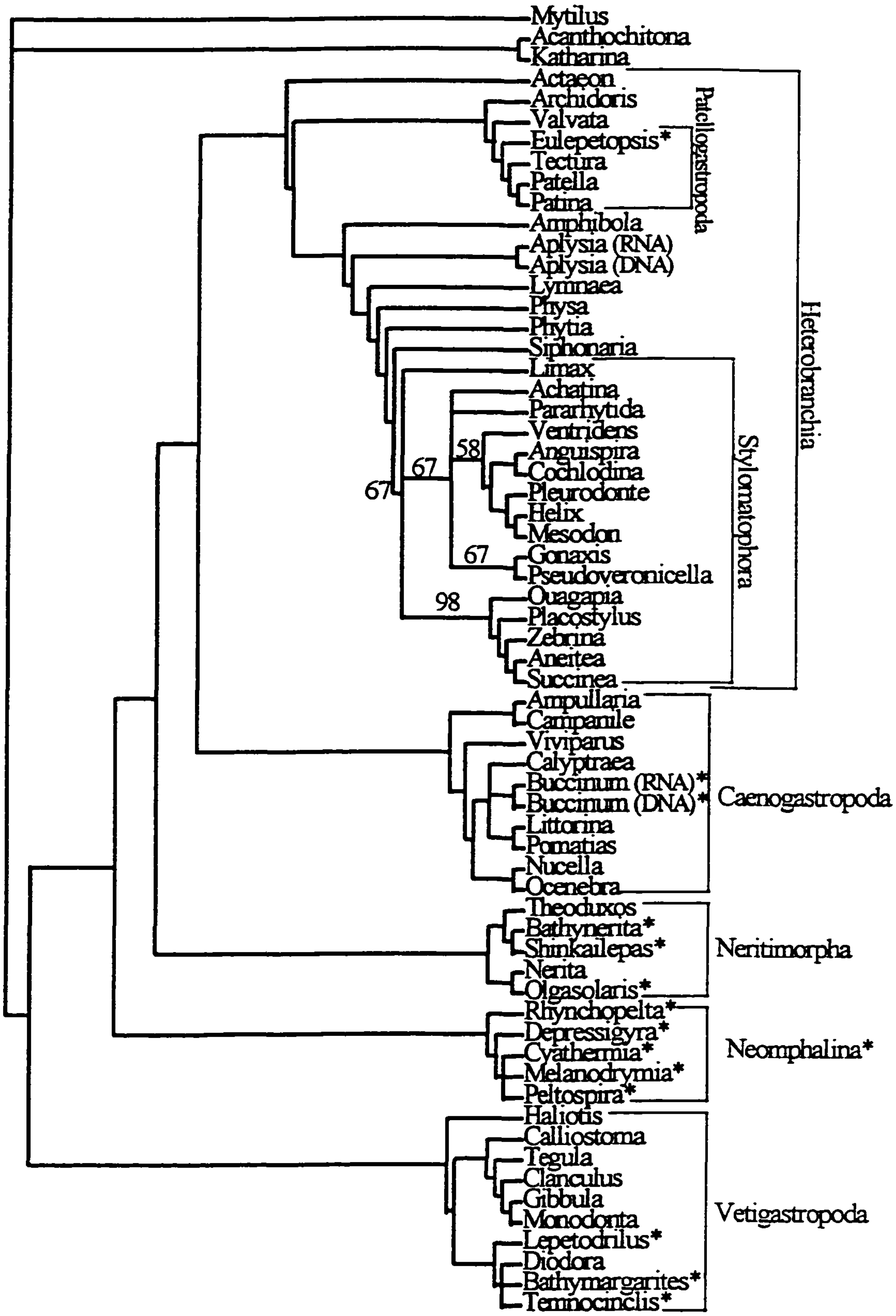
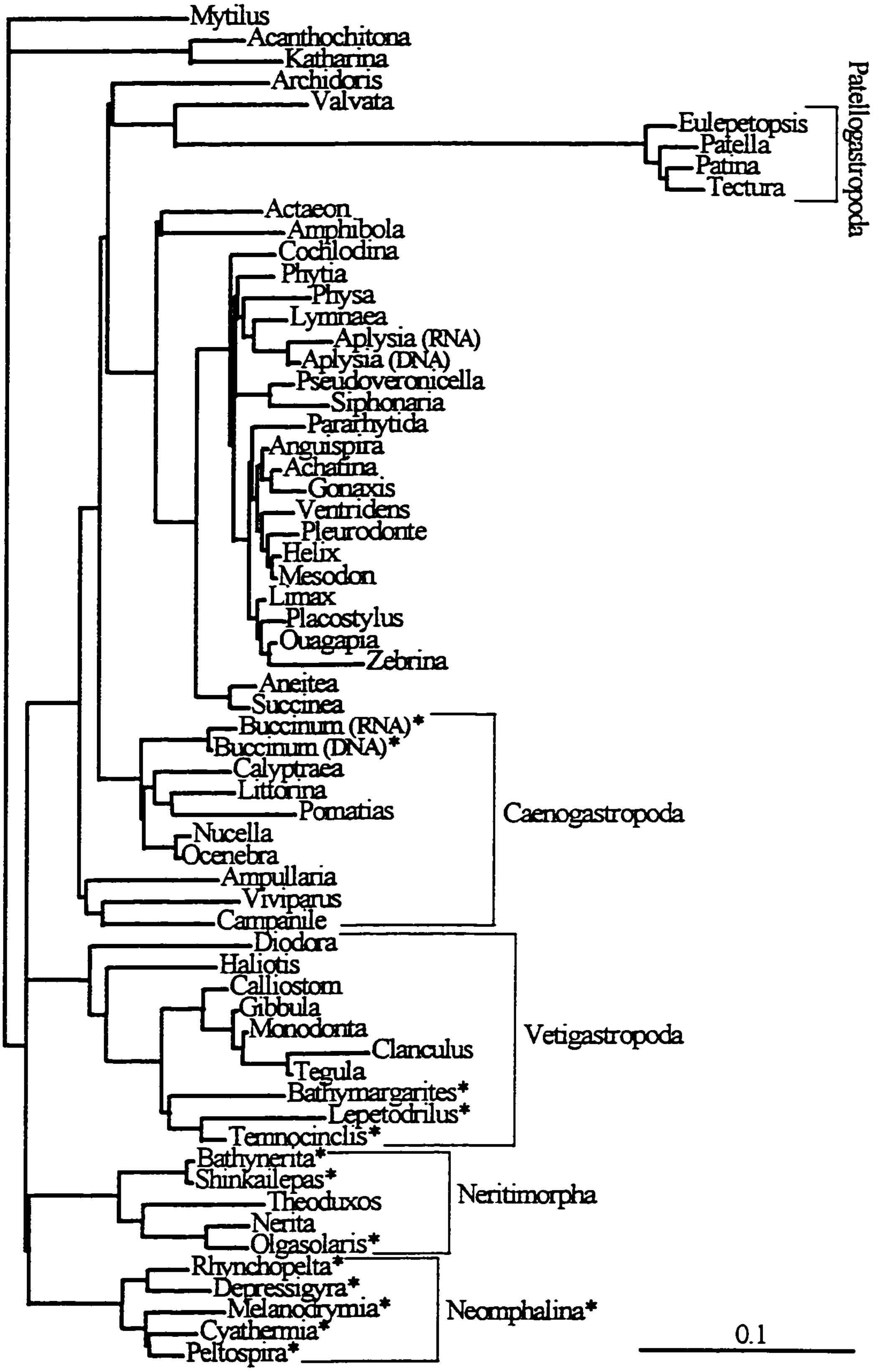

Mytilus
Acanthochitona
Katharina
Actaeon
Archidoris
Valvata
Eulepetopsis*
Tectura
Patella
Patina
Amphibola
Aplysia (RNA)
Aplysia (DNA)
Lymnaea
Physa
Phytia
Siphonaria
Limax
Achatina
Pararhytida
Ventridens
Anguispira
Cochlodina
Pleurodonte
Helix
Mesodon
Gonaxis
Pseudoveronicella
Ouagapia
Placostylus
Zebrina
Aneitea
Succinea
Ampullaria
Campanile
Viviparus
Calyptraea
Buccinum (RNA)*
Buccinum (DNA)*
Littorina
Pomatias
Nucella
Ocenebra
Theodoxus
Bathynerita*
Shinkailepas*
Nerita
Olgasolaris*
Rhynchopelta*
Depressigyra*
Cyathermia*
Melanodrymia*
Peltospira*
Haliotis
Calliostoma
Tegula
Clanculus
Gibbula
Monodonta
Lepetodrilus*
Diodora
Bathymargarites*
Temnocinclis*
58
67
67
67
98
Patellogastropoda
Heterobranchia
Stylommatophora
Caenogastropoda
Neritimorpha
Neomphalina*
Vetigastropoda

Figure 11. Phylogenetic tree produced by neighbour-joining sequences of the D1 domain. Genera strictly endemic to hydrothermal vents and hydrocarbon seeps are labeled with an asterisk. The Caenogastropoda was polyphyletic with the separate origins of a Architaenioglossa - *Campanile* branch. The Patellogastropoda originated from within the Heterobranchia and none of the heterobranch sub-groups were monophyletic.


Mytilus
Acanthochitona
Katharina
Archidoris
Valvata
Eulepetopsis
Patella
Patina
Tectura
Patellogastropoda
Actaeon
Amphibola
Cochlodina
Phytia
Physa
Lymnaea
Aplysia (RNA)
Aplysia (DNA)
Pseudoveronicella
Siphonaria
Pararhytida
Anguispira
Achatina
Gonaxis
Ventridens
Pleurodonte
Helix
Mesodon
Limax
Placostylus
Ouagapia
Zebrina
Aneitea
Succinea
Buccinum (RNA)*
Buccinum (DNA)*
Calyptraea
Littorina
Pomatias
Nucella
Ocenebra
Ampullaria
Viviparus
Campanile
Caenogastropoda
Diodora
Haliotis
Calliostom
Gibbula
Monodonta
Clanculus
Tegula
Bathymargarites*
Lepetodrilus*
Temnocinclis*
Vetigastropoda
Bathynerita*
Shinkailepas*
Theodoxos
Nerita
Olgasolaris*
Neritimorpha
Rhynchopelta*
Depressigyra*
Melanodrymia*
Cyathermia*
Peltospira*
Neomphalina*
0.1

Forced re-positionings of the Patellogastropoda or exclusion of the variable regions did not improve phylogenetic resolution, as supported by bootstrap values.

The longer 28S rRNA sequences acquired by combined analysis of the two domains resulted in a much more complete resolution of gastropod phylogeny. Bootstrap analyses supported the monophyly of the Neritimorpha, Neomphalina, Vetigastropoda, Patellogastropoda, Caenogastropoda (including *Viviparus*, *Ampullaria*, and *Campanile*), and Heterobranchia (Euthyneura plus *Valvata*) (Figures 12 & 13). The basal archaeogastropod relationships remained unresolved while a Caenogastropoda / Heterobranchia / Patellogastropoda grouping was supported. Ponder & Lindberg (1996a) and Haszprunar (1988) have suggested that the Patellogastropoda is a sister group to the rest of the Gastropoda and the results above could have been the result of long-branch attraction given the elevated evolutionary rate of the Patellogastropoda; therefore the combined domain analyses were performed a second time with the Patellogastropoda constrained as sister to the rest of the Gastropoda (hereafter called the Eogastropoda assumption, after Ponder & Lindberg 1996a). Fully resolved trees were supported by bootstrapping of parsimony but not by bootstrapping of neighbour-joining analyses (Figure 14 & 15). Removal of the Patellogastropoda from analyses using parsimony or neighbour-joining resulted in similar strong bootstrap support for monophyly the gastropod orders but no resolution among them except for strong support of the sister relationship between the Caenogastropoda and Heterobranchia (not shown). Close examination revealed that resolution of gastropod phylogeny beyond the strongly supported gastropod orders was primarily dependent upon the poorly aligned D1 domain variable regions and D6 domain loop for which supra-ordinal homology was uncertain and

Figure 12. Bootstrapping of parsimony analyses of the combined D1 and D6 domains, using the Polyplacophora and *Mytilus* as outgroups. Genera strictly endemic to hydrothermal vents and hydrocarbon seeps are labeled with an asterisk. Nodes with less than 50% bootstrap support are collapsed.

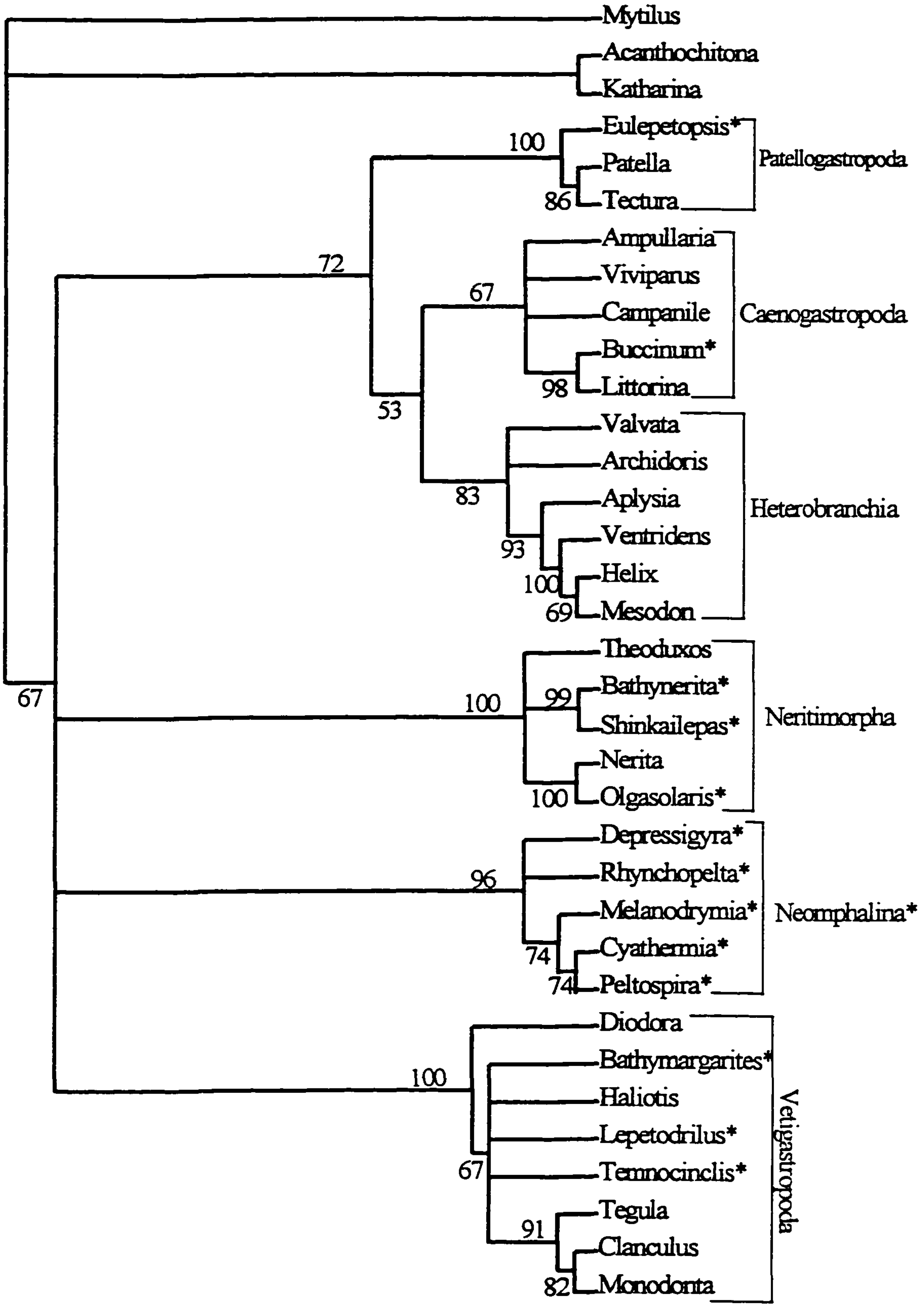

Mytilus
Acanthochitona
Katharina
Eulepetopsis*
Patella
Tectura
Patellogastropoda
100
86
72
Ampullaria
Viviparus
Campanile
Buccinum*
Littorina
Caenogastropoda
67
98
53
Valvata
Archidoris
Aplysia
Ventridens
Helix
Mesodon
Heterobranchia
83
93
100
69
67
Theoduxos
Bathynerita*
Shinkailepas*
Nerita
Olgasolaris*
Neritimorpha
100
99
100
Depressigyra*
Rhynchopelta*
Melanodrymia*
Cyathermia*
Peltospira*
Neomphalina*
96
74
74
Diodora
Bathymargarites*
Haliotis
Lepetodrilus*
Temnocinclis*
Tegula
Clanculus
Monodonta
Vetigastropoda
100
67
91
82

Figure 13. Bootstrapping of neighbour-joining analyses of the combined D1 and D6 domains, using the Polyplacophora and *Mytilus* as outgroups. Genera strictly endemic to hydrothermal vents and hydrocarbon seeps are labeled with an asterisk. Nodes with less than 50% bootstrap support are collapsed. Monophyly of the Caenogastropoda was found in 70% of the bootstrap replicates.

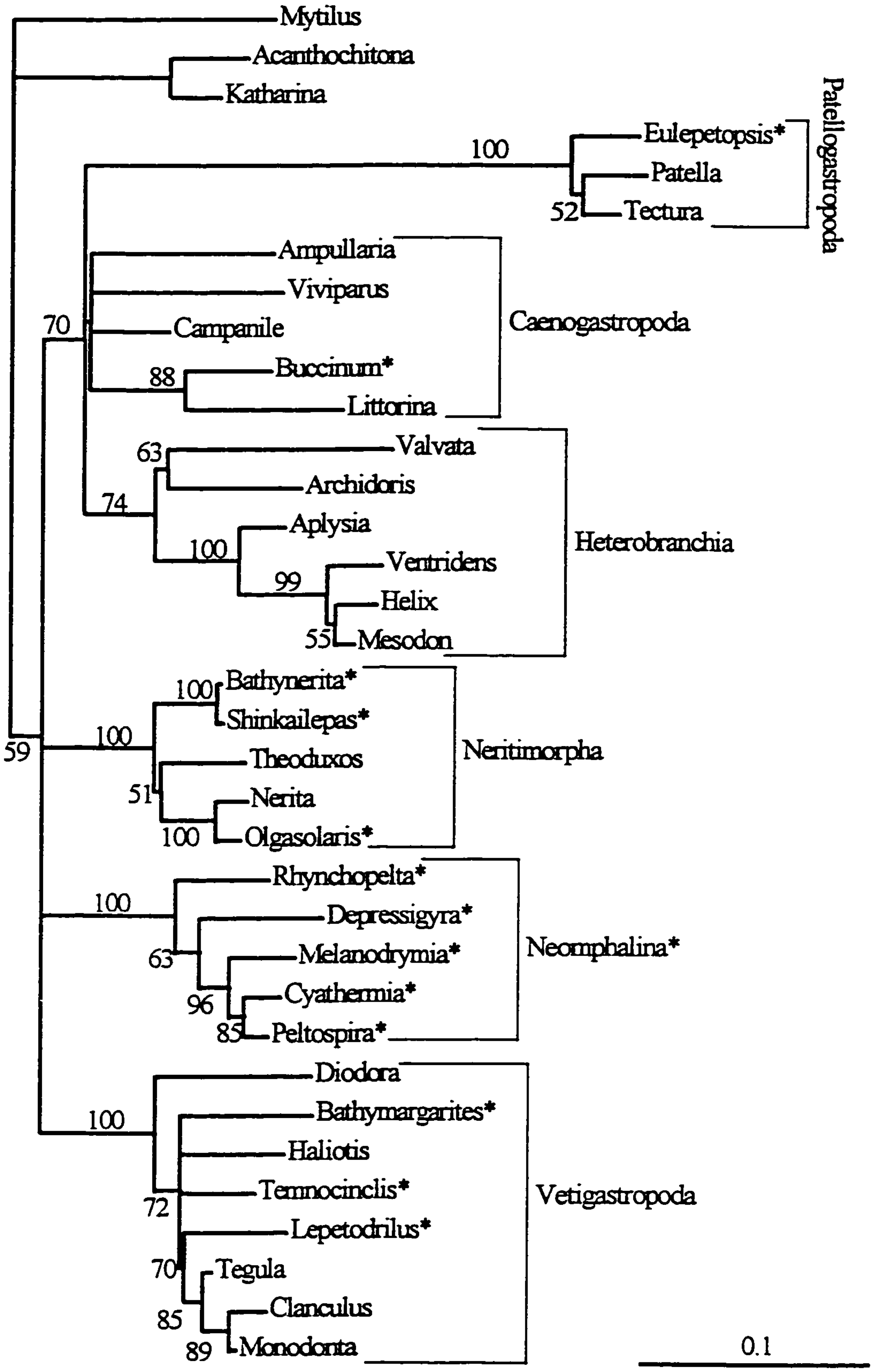

Mytilus
Acanthochitona
Katharina
100
Eulepetopsis*
Patella
52
Tectura
Patellogastropoda
Ampullaria
Viviparus
70
Campanile
88
Buccinum*
Littorina
Caenogastropoda
63
Valvata
Archidoris
74
Aplysia
100
Ventridens
99
Helix
55
Mesodon
Heterobranchia
100
Bathynerita*
Shinkailepas*
100
59
Theodoxos
51
Nerita
100
Olgasolaris*
Neritimorpha
Rhynchopelta*
100
Depressigyra*
63
Melanodrymia*
96
Cyathermia*
85
Peltospira*
Neomphalina*
Diodora
100
Bathymargarites*
Haliotis
Temnocinclis*
72
Lepetodrilus*
70
Tegula
85
Clanculus
89
Monodonta
Vetigastropoda
0.1

Figure 14. Bootstrapping of parsimony analyses of the combined D1 and D6 domains, with the Eogastropoda assumption enforced and the Polyplacophora and *Mytilus* as outgroups. Genera strictly endemic to hydrothermal vents and hydrocarbon seeps are labeled with an asterisk. Nodes with less than 50% bootstrap support are collapsed.

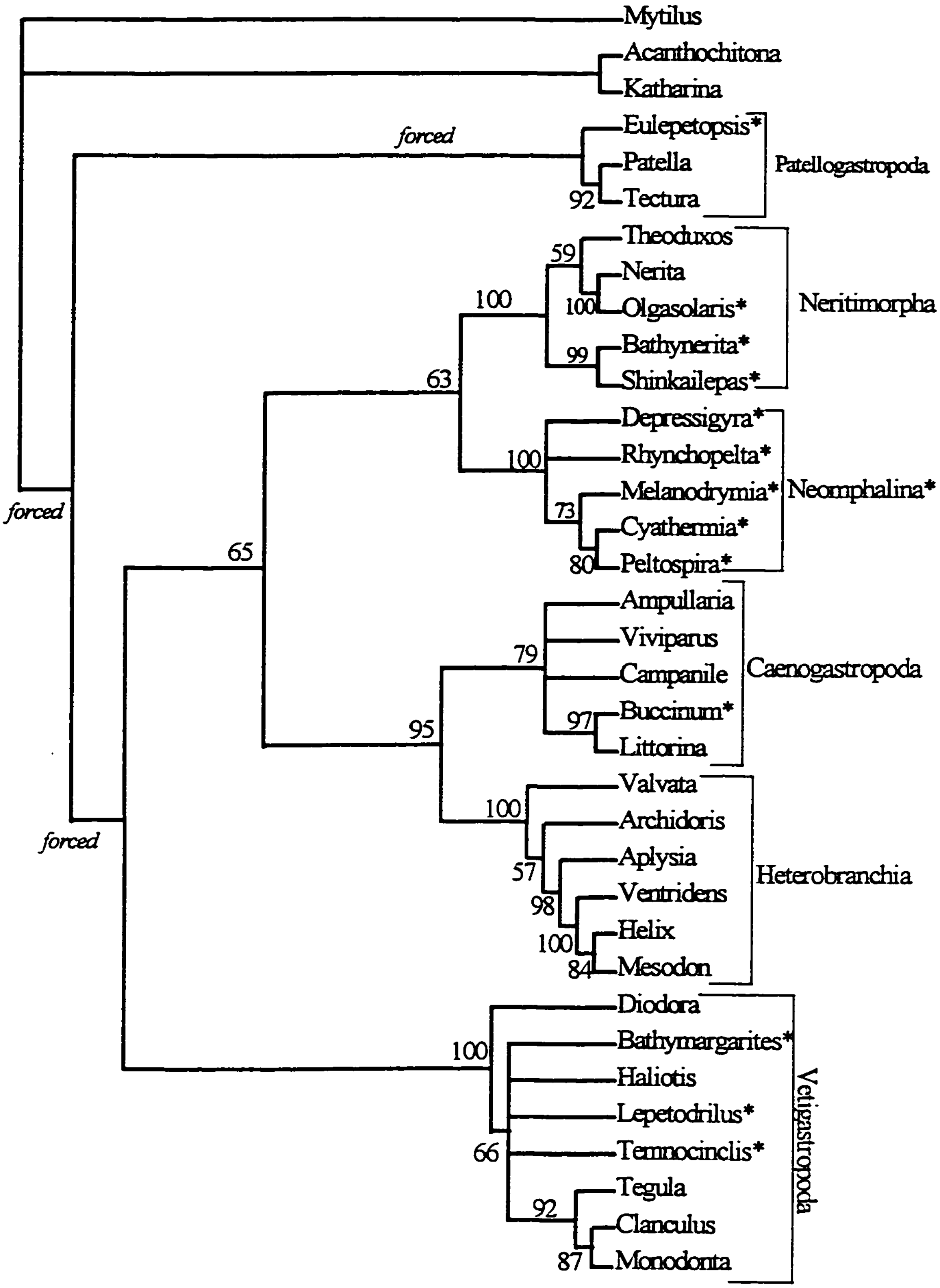
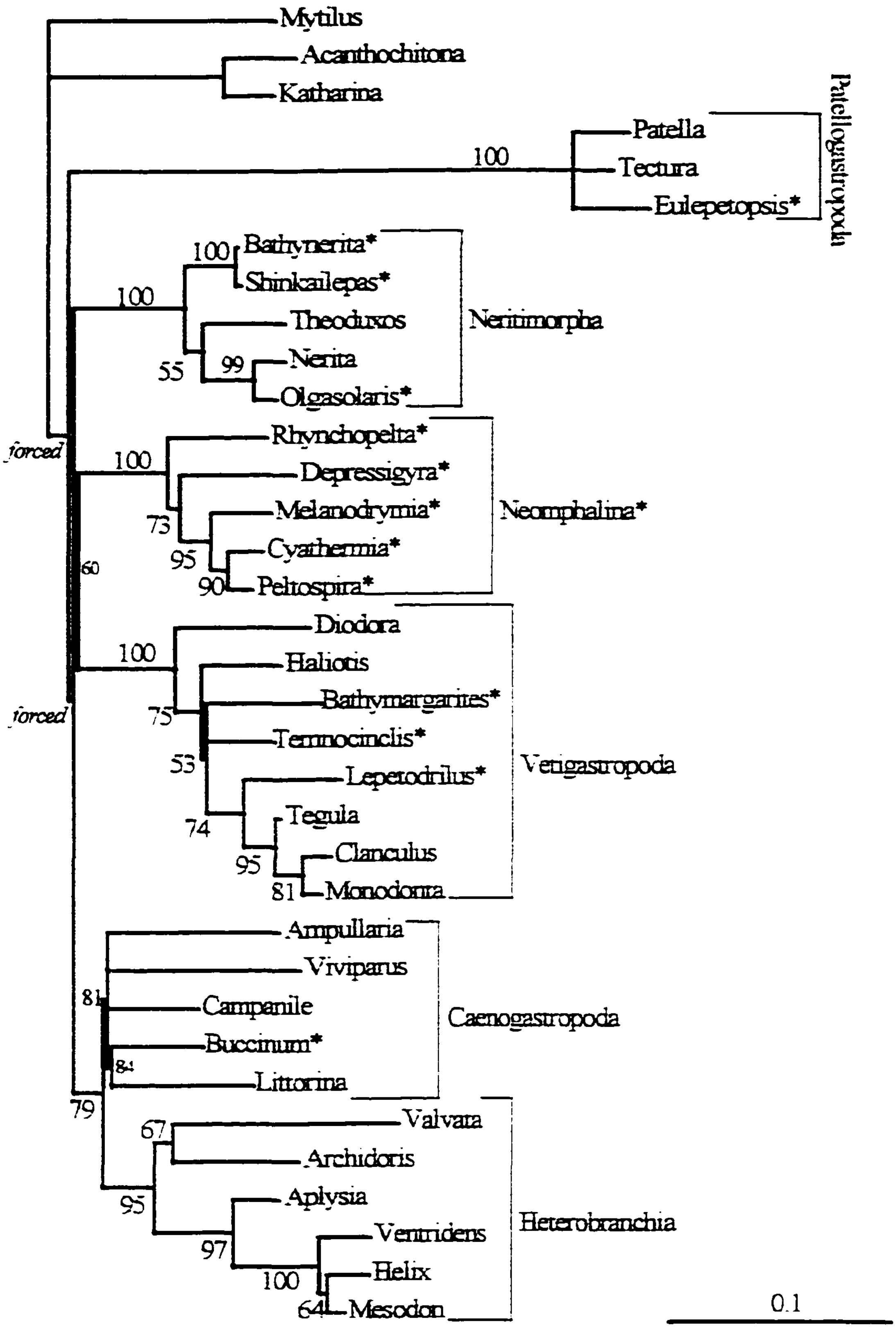

Mytilus
Acanthochitona
Katharina
forced
Eulepetopsis*
Patella
92
Tectura
Patellogastropoda
Theodoxos
59
Nerita
100
Olgasolaris*
Neritimorpha
100
99
Bathynerita*
Shinkailepas*
63
Depressigyra*
Rhynchopelta*
100
Melanodrymia*
Neomphalina*
73
Cyathermia*
80
Peltospira*
forced
65
Ampullaria
Viviparus
79
Campanile
Caenogastropoda
97
Buccinum*
Littorina
95
Valvata
100
Archidoris
Aplysia
57
Heterobranchia
Ventridens
98
Helix
100
84
Mesodon
forced
Diodora
100
Bathymargarites*
Haliotis
Lepetodrilus*
66
Temnocinclis*
Vetigastropoda
Tegula
92
Clanculus
87
Monodonta

Figure 15. Bootstrapping of neighbour-joining analyses of the combined D1 and D6 domains, with the Eogastropoda assumption enforced and using the Polyplacophora and *Mytilus* as outgroups. Genera strictly endemic to hydrothermal vents and hydrocarbon seeps are labeled with an asterisk. Nodes with less than 50% bootstrap support are collapsed.


Mytilus
Acanthochitona
Katharina
100
Patella
Tectura
Eulepetopsis*
Patellogastropoda
100
Bathynerita*
Shinkailepas*
100
Theodoxus
55
99
Nerita
Olgasolaris*
Neritimorpha
forced
Rhynchopelta*
100
Depressigyra*
73
Melanodrymia*
95
Cyathermia*
60
90
Peltospira*
Neomphalina*
Diodora
100
Haliotis
Bathymargarites*
forced
75
Temnocinclis*
53
Lepetodrilus*
Vetigastropoda
74
Tegula
95
Clanculus
81
Monodonta
Ampullaria
Viviparus
81
Campanile
Caenogastropoda
Buccinum*
84
Littorina
79
Valvata
67
Archidoris
95
Aplysia
Ventridens
Heterobranchia
97
Helix
100
64
Mesodon
0.1

homoplasy likely. Additional analyses with these regions excluded yielded bootstrap support only for the gastropod orders, with the exception of *Viviparus* in the parsimony analysis (Figure 16 & 17). The resulting neighbour-joining tree was only five steps longer than the tree obtained by parsimony despite differing in the placement of the Heterobranchia and *Viviparus*.

Figure 16. Majority-rule consensus of 27 equally parsimonious trees (length of 397 steps) found from the analysis of the combined domains, with the D6 domain loop and D1 domain variable regions excluded, the Eogastropoda assumption enforced, and the Polyplacophora and *Mytilus* as outgroups. Genera strictly endemic to hydrothermal vents and hydrocarbon seeps are labeled with an asterisk. The 27 equally parsimonious trees only differed within the *Tegula - Clanculus - Monodonta* trichotomy and the numbers represent bootstrap support for individual nodes.

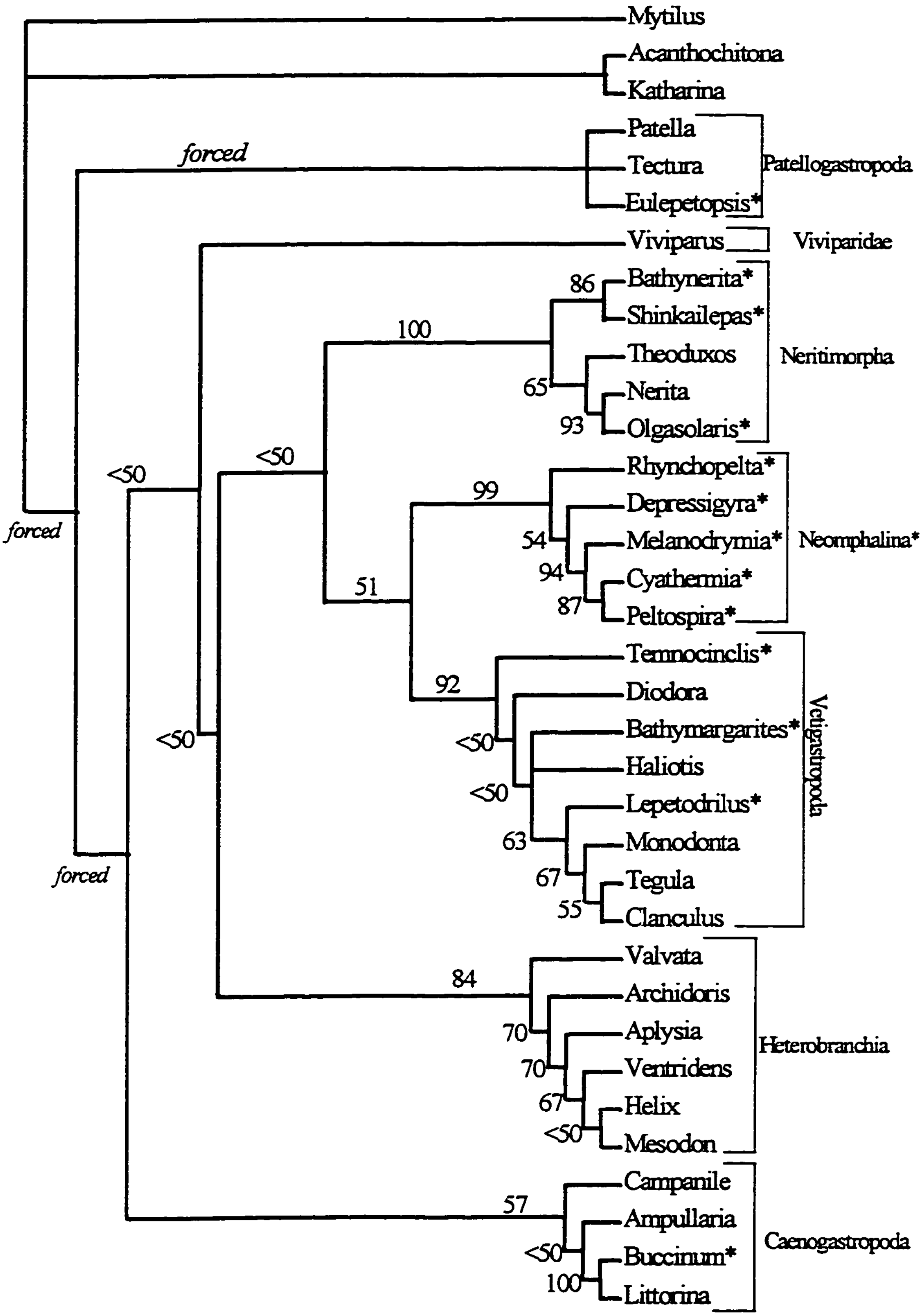
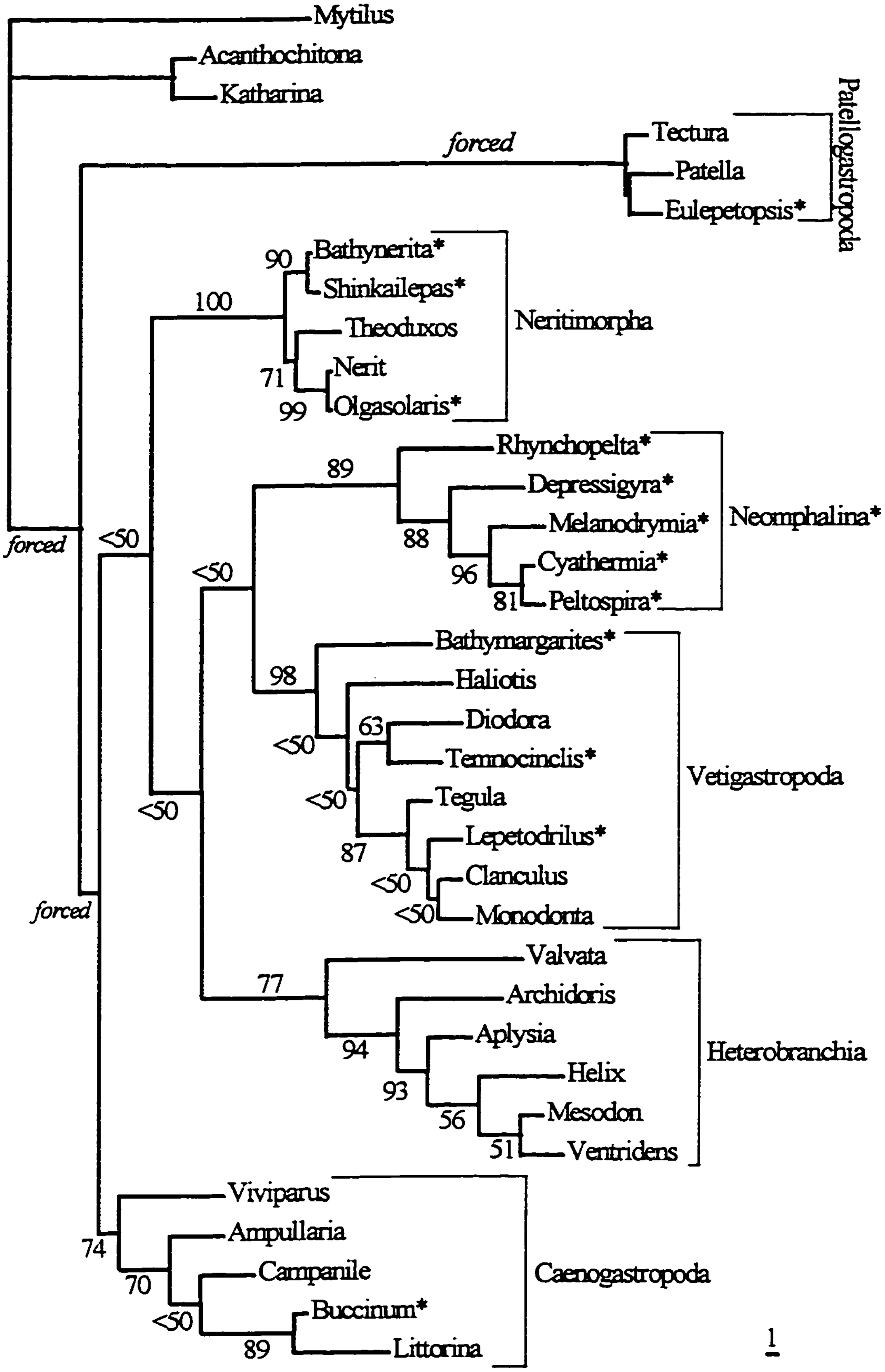

Mytilus
Acanthochitona
Katharina
forced
Patella
Tectura
Eulepetopsis*
Patellogastropoda
Viviparus
Viviparidae
86
Bathynerita*
Shinkailepas*
100
Theoduxos
65
Nerita
93
Olgasolaris*
Neritimorpha
<50
<50
Rhynchopelta*
99
Depressigyra*
forced
54
Melanodrymia*
Neomphalina*
94
Cyathermia*
51
87
Peltospira*
Temnocinclis*
92
Diodora
<50
<50
Bathymargarites*
Haliotis
<50
Vetigastropoda
Lepetodrilus*
63
Monodonta
forced
67
Tegula
55
Clanculus
Valvata
84
Archidoris
70
Aplysia
70
Ventridens
67
Helix
<50
Mesodon
Heterobranchia
Campanile
57
Ampullaria
<50
Buccinum*
100
Littorina
Caenogastropoda

Figure 17. Neighbour-joining tree produced from the analysis of the combined domains, with the D6 domain loop and D1 domain variable regions excluded, the Eogastropoda assumption enforced, and the Polyplacophora and *Mytilus* as outgroups. Genera strictly endemic to hydrothermal vents and hydrocarbon seeps are labeled with an asterisk. Numbers represent bootstrap support (100 replicates) for individual nodes.


Mytilus
Acanthochitona
Katharina
forced
Tectura
Patella
Eulepetopsis*
Patellogastropoda
90
Bathynerita*
Shinkailepas*
100
Theoduxos
Neritimorpha
71
Nerit
99
Olgasolaris*
89
Rhynchopelta*
Depressigyra*
Neomphalina*
88
Melanodrymia*
forced
<50
<50
96
Cyathermia*
81
Peltospira*
Bathymargarites*
98
Haliotis
63
Diodora
<50
Temnocinclis*
Vetigastropoda
<50
Tegula
<50
87
Lepetodrilus*
<50
Clanculus
forced
<50
Monodonta
Valvata
77
Archidoris
94
Aplysia
Heterobranchia
93
Helix
56
Mesodon
51
Ventridens
Viviparus
74
Ampullaria
70
Campanile
Caenogastropoda
<50
Buccinum*
89
Littorina
1

DISCUSSION

Phylogenetic Resolution

Longer sequences of 28S rRNA led to greater resolution of gastropod phylogeny (as per Tillier 1994, 1996). The longer the sequences used, the more inclusive were the taxonomic groupings supported by bootstrap values. However, phylogenetic information for resolving gastropod evolutionary radiations of the early Paleozoic appears to be rare in the 28S rRNA domains examined, making resolution sensitive to homoplasy in these short sequences. Homoplasy in the 28S rRNA data had several sources. The equivalent distances of polyplacophoran and bivalve outgroups from the Gastropoda strongly suggests that saturation of evolutionary substitutions has occurred between the gastropods and the outgroups, as the Bivalvia is presumed to have a closer evolutionary relationship to the Gastropoda (Salvini-Plawen & Steiner 1996, Ponder & Lindberg 1996a). This suggestion is further supported by the observations that some distances within the gastropod ingroup, particularly among orders, were equivalent to the ingroup-outgroup distances and that substitutions appeared to be saturated beyond the ordinal level. Homoplasies were thus likely to be frequent within the early Paleozoic radiations.

In protein encoding genes, transitions are generally saturated before transversions since many transitional substitutions do not result in amino-acid replacement. Exclusion of transitions in such cases can improve resolution as the remaining transversions may not be saturated (Hillis et al. 1993). Evolution of 28S rRNA sequences did not follow this pattern of transitions-first saturation, presumably since the RNA itself is the functional product and its secondary structure is influenced by each base in the sequence. Exclusion of transitions from analyses reduced phylogenetic resolution as supported by bootstrap values and skew statistics. Tillier's (1996) exclusion of D1 domain 28S rRNA transitions based upon assumed transitions-first saturation may have been inappropriate and does not appear to have a theoretical justification.

In addition to possible exclusion of transitions, it is also common in molecular systematics to exclude difficult to align, highly variable, or more rapidly evolving regions within sequence alignments on the grounds that homology is uncertain and homoplasy more likely. The D6 domain loop and variable regions of the D1 domain were thus likely candidates for exclusion from analyses and their exclusion resulted in higher phylogenetic signal and reduction of sequence distances to below Hillis & Dixon's (1991) suggested 30%. Unfortunately, their exclusion greatly reduced the length of available sequence data (25% drop in total number of characters, 53% drop in number of characters informative for parsimony). Analyses using the reduced data set lacked resolution beyond the gastropod orders. Resolution beyond this depth of the phylogeny may not exist for 28S rRNA. The remaining characters from the well aligned regions were either too few in number or were saturated beyond ordinal level, to result in full bootstrap-supported resolution of the phylogeny.

It is an assumption of parsimony analyses that each sequence evolves at approximately the same rate (Felsenstein 1978). Since parsimony assumes minimal evolution, a higher rate of evolution in one sequence results in undetected base substitutions between it and other sequences and the subsequent homoplasies create long-branch attraction to incorrect locations within the phylogeny (Hendy & Penny 1989). The Patellogastropoda consistently exhibited elevated rates of molecular evolution in the 28S rRNA domains examined. The Patellogastropoda originated during the early Paleozoic radiations and is generally hypothesized to represent the first branch of gastropod evolution, sister to the remaining gastropods (Haszprunar 1988, Lindberg 1988, Salvini-Plawen & Steiner 1996, Ponder & Lindberg 1996a). An origin for the Patellogastropoda within the caenogastropod-heterobranch radiations is unlikely in light of the anatomical and paleontological evidence (Salvini-Plawen & Steiner 1996, Ponder & Lindberg 1996a) and the elevated evolutionary rate of patellogastropod 28S rRNA sequences brings into question this result (Figures 6A & 6C). Use of phylogenetic methods that account for undetected base substitutions using models of molecular evolution (Kimura's two-parameter distance, maximum likelihood) did not alter the hypothesized origins of the Patellogastropoda, most likely due to overall saturation of substitutions. It is unlikely that further examination of 28S rRNA will unequivocally resolve patellogastropod origins.

Homoplasy due to variation in evolutionary rates also occurred within the D6 domain. Evolutionary rates varied widely among sequences for the loop, stems, and flanks for this domain. This agrees with Hassouna et al.'s (1984) finding that the D6 domain is dynamic for changes in both size and sequence, even within rat and mouse comparisons. Rate variation in the D6 domain was lineage specific with elevated rates of molecular evolution for the Cephalopoda and Gastropoda relative to the Bivalvia. Steiner & Müller (1996) found similar differences in evolutionary rates between the Gastropoda and Bivalvia in their assessment of 18S rRNA variation, indicating that lineage-specific rate variation may be a consistent problem when using nuclear ribosomal RNA sequences to investigate some aspects of molluscan phylogeny. Between the two 28S rRNA domains examined, rate variation within the Gastropoda (excluding the Patellogastropoda) was less of a problem for the D1 domain. The D6 domain may be useful for more restricted phylogenetic questions, such as relationships within the Rissooidea.

Supra-ordinal resolution of gastropod phylogeny using the D1 and D6 domains of 28S rRNA was not possible due to saturation of substitutions. Sequences from 28S rRNA resolve modern representatives of gastropod lineages that originated in the late Cambrian and early Ordovician but cannot resolve the basal radiations of the Gastropoda that presumably occurred during or just prior this period. Support of ordinal groupings was consistent and became more inclusive with longer sequences, allowing robust identification of the Patellogastropoda, Neritimorpha, Neomphalina, Vetigastropoda, Heterobranchia, and (less-convincingly) the Caenogastropoda. However, due to saturation of substitutions, hypothesized inner relationships of the gastropod orders were likely to be influenced by the homoplasies introduced by the use of overly distant outgroups and sister-orders, particularly for neighbour-joining analyses (Swofford et al. 1996). Domains of 28S rRNA would be excellent tools for examining within-order relationships but only if taxonomic sampling is restricted to the use of closely-related outgroup taxa. Within the taxonomic scope examined, the conservative conclusion is that 28S rRNA can identify major gastropod radiations of early Paleozoic origins, equivalent to the ordinal level of classification. The domains of 28S rRNA examined are saturated for more basal relationships. Additionally, the intra-ordinal relationships presented here should be considered unreliable due to poor intra-order taxonomic sampling and the misleading influence of homoplasies from overly distant outgroup taxa.

The Major Gastropod Groups

The inclusion of the hydrothermal vent limpet *Eulepetopsis* within the Patellogastropoda conforms to McLean (1990a) and Fretter's (1990) assessment of their radula and anatomy. While more representatives of non-vent Patellina and other neolepetopsoideans (ie. *Paralepetopsis, Neolepetopsis*) are needed for a robust test, McLean's (1990a) assertion that the Neolepetopsidae represents a living representative of the Lepetopsina, which last appeared in the fossil record during the Triassic, has not been rejected, although Fretter (1990) questions the reliance of this hypothesis upon radular characteristics. *Eulepetopsis* branches externally to representatives of the Patellina (*Patella, Patina*) and Nacellina (*Tectura*) when the locally-aligned D6 domain loop and D1 domain variable regions are included.

Monophyly of the Vetigastropoda was consistently supported but hypothesized inner relationships differed between analyses, most likely due to use of overly-distant non-vetigastropod sequences. The placement of the Fissurellidae (*Diodora*) external to the other vetigastropod genera in most analyses agrees with Ponder & Lindberg (1996a). *Diodora* (Fissurellidae) and *Temnocinclis* (Scissurellidae) appear to be paraphyletic. Exact resolution within the remainder of the represented vetigastropods is non-existent except for strong support for monophyly of the Trochidae excluding the hydrothermal vent genus *Bathymargarites*. The distance of *Bathymargarites* suggests that it belongs to an older branch of the Trochidae or has independent origins. The inclusion of the Lepetodriloidea (represented by *Lepetodrilus*) within the Vetigastropoda agrees with McLean (1988) and Haszprunar (1988) although the poor resolution makes it difficult to address its antiquity. It does not appear closely related to any of the genera examined.

Broader taxonomic sampling within the Vetigastropoda is need to more clearly resolve inner relationships.

Ponder & Lindberg's (1990a) examination of gastropod phylogeny recorded the anatomical and morphological features of the Neomphalina as a composite of Neomphalidae, Peltospiridae, and the divergent *Melanodrymia*, but Haszprunar (1988) and Salvini-Plawen & Steiner (1996) treated *Melanodrymia* separately from the Neomphalidae. Salvini-Plawen & Steiner (1996) did not include the Peltospiridae and their use of Melanodrymiidae is unclear. Haszprunar (1988), McLean (1990b), and Salvini-Plawen & Steiner (1996) hypothesize independent origins for *Melanodrymia*, primarily due to differing views on *Melanodrymia*'s lack of skeletal rods in the ctenidia (gill). However, the monophyly of the hydrothermal vent endemic Neomphalina (Neomphalidae and Peltospiridae, including *Melanodrymia*) is strongly supported by the 28S rRNA sequences, a suggestion that has only been made by Warén & Bouchet (1989). Little has been hypothesized on the inner relationships of the Neomphalina beyond the division between the Neomphalidae and Peltospiridae (McLean 1990b). The Peltospiridae appears paraphyletic given the placing of the neomphalinid *Cyathermia*. Closer examination with a broader taxonomic sampling would aid in examining neomphalid systematics and hydrothermal vent faunal history.

Newman's (1985) and Tunnicliffe's (1992) hypothesis that the hydrothermal vent fauna represents a refugial fauna of some antiquity is supported by the Neomphalina. It has phylogenetic novelty equivalent to the other major gastropod groups. The Neomphalina likely originated in the early Paleozoic since the first fossil representatives of the other major groups occurred at this time: Neritimorpha (mid-Silurian), Patellogastropoda (earliest Ordovician), Caenogastropoda (earliest Ordovician), Vetigastropoda (late Cambrian) (Tracey et al. 1993). No fossil relatives of the Neomphalina are known, the closest being the questionable Euomphalina of the early Ordovician to end-Permian (McLean 1981), and the entire group is only known from hydrothermal vents. Lacking fossil information, no estimates can be made for when this gastropod lineage invaded this unique habitat nor when it became extinct in all other habitats.

The Neritimorpha is an extremely diverse group with representatives known from marine, freshwater, and terrestrial habitats. Three families, the Neritidae, Helicinidae, and Phenacolepadidae, are represented in the phylogenetic trees obtained but the pattern does not confirm to familial designation since the two phenacolepadids (*Shinkailepas*, *Olgasolaris*) are strongly paired with neritids. Overall variation in the 28S rRNA domains examined is low for the Neritimorpha, so very little information is supporting the predicted relationships. Use of overly-distant non-neritimorph sequences probably also biased results. However, three of the five species are from deep-sea sulphophilic habitats: *Shinkailepas* and *Olgasolaris* from hydrothermal vents and *Bathynerita* from hydrocarbon seeps. Given the evolutionary plasticity of the Neritimorpha, a broader taxonomic sampling from more habitats is required to examine neritimorph systematics.

Bootstrap support for a monophyletic Caenogastropoda that included the Architaenioglossa was moderate. *Ampullaria* and *Viviparus* were paraphyletic and the monophyly of the Architaenioglossa should be examined more closely in future studies. *Ampullaria*, *Viviparus*, and *Campanile* formed a paraphyletic sister group to the remaining Caenogastropoda, monophyly of which was generally supported. Resolution of overall caenogastropod phylogeny was dependent upon the length of sequence used and the method of analysis. Examination of caenogastropod phylogeny using 28S rRNA will require both a broader, more even taxonomic sampling and the addition of sequences from other domains.

Other than the problem of rapid evolution in the Patellogastropoda, the monophyly of the Heterobranchia (*Valvata* + Euthyneura) was supported. The sampled opisthobranch and basommatophoran genera were paraphyletic, as found by Tillier et al. (1994, 1996). Combined domain analyses strongly supported the monophyly of the Stylommatophora, as represented by *Helix*, *Mesodon*, and *Ventridens*. Parsimony analysis of the D1 domain alone additionally supported stylommatophoran monophyly although use of overly distant non-heterobranch sequences in analyses brings into question the reliability of within-Heterobranchia results. The restricted examination of euthyneuran phylogenetics performed by Tillier et al. (1996) that used the same genera with D1 and D2 domain 28S rRNA sequences should be considered more reliable.

Improving Resolution

While Hillis & Dixon (1991) suggested that 28S rRNA could resolve relationships within the Paleozoic, information on the Cambrian and early Paleozoic radiations was rare for gastropod phylogeny in the domains examined. Addition of sequences from other domains could conceivably overcome the existing homoplasies but the effort required to obtain these sequences may not be worth the gain. 28S rRNA domains are separated by long stretches of uninformative, conserved sequences. Separate PCR amplification of domains is both laborious and expensive. Single amplification of multiple domains is possible (eg. Littlewood 1994) but such amplifications are unlikely to work in formalin preserved material, still the source of DNA for most molecular systematic investigations in the Mollusca. Future molecular systematic efforts would best benefit from examining genes more informative for the Cambrian and early Paleozoic radiations, such as 18S rRNA or elongation factor 1α (Hillis & Dixon 1991, Kojima et al. 1993, Friedlander et al. 1994, Steiner & Müller 1996). While it will remain difficult to PCR amplify long stretches of DNA from formalin-preserved material, 300-500 base pair stretches of these more conserved genes may yield more information on the early gastropod radiations than laborious separate amplification of multiple 28S rRNA domains. Systematic effort is additionally diluted when examining 28S rRNA domains since it is necessary to discard sequences from internal, difficult to align loops and variable regions.

LeCointre et al. (1993) stressed the importance of even taxonomic sampling for phylogenetic analyses. Tillier et al. (1994) had problems when using D1 domain sequence data and single representatives of the Patellogastropoda and Neomphalina because phylogenetic placement of the single representatives was dependent upon the composition of both the ingroup and outgroup. Similarly, Rosenberg et al. (1994) proposed that their result of a single neritimorph sequence, from *Helicina*, being placed within the Bivalvia was an artifact of poor representation of the Neritimorpha. The broader sampling used in this study helped confirm the monophyly of these groups but the data collected remained unable to robustly resolve their phylogenetic position. The more balanced representation of the major prosobranch lineages did not result in splitting of long branches (Swofford et al. 1996) and resolution of gastropod phylogeny, presumably due to saturation of substitutions in this region of the phylogeny. The poor resolution of the Cambrian to early Paleozoic gastropod radiations with 28S rRNA does not appear to be an artifact of uneven taxonomic sampling and future inclusion of 28S rRNA sequences from other major groups, particularly from the Cocculiniformia, would not improve resolution of the early gastropod radiations as well as use of more conserved genes.

Phylogenetic information for resolving evolutionary radiations can also be rare in molecular sequences if the radiations occurred quickly. To resolve radiations that occurred during the Cambrian or early Paleozoic requires slowly evolving genes that have not lost information due to multiple substitution of bases. Slowly evolving genes will acquire very little diagnostic information during a short radiation and the poor resolution amongst the major gastropod groups may additionally be a result of rapid radiation. The first appearance of gastropods in the fossil record occurred at the end of the Cambrian with the rapid radiation of five major designs: the Hyperstrophina, Macluritina, Bellerophontina, Vetigastropoda, and Helicotomidae (Tracey et al. 1993). Within roughly the first 30 million years of the Paleozoic, the gastropods had diversified to 19 families representing nine major designs with the addition of the Patellogastropoda, Murchisoniidae, Euomphalina, and Caenogastropoda (Tracey et al. 1993). Molecular systematics would have to resolve separate radiations of roughly 20 million years in duration that occurred over 478 million years ago. In the best theoretical case, with uniform evolutionary rates and the suggested maximal divergence of 30% between present-day sequences (Hillis & Dixon 1991), only 1.2% divergence between sequences would be acquired during a radiation of 20 million years in duration. Even in the case of the 28S rRNA domains examined, where distances between the gastropod orders ranged from 35% to 45%, the divergence gained would be less than 2%. Saturation of substitutions for this region of the phylogeny would erase much of this 2%. Longer sequence lengths may not resolve rapid radiations (Steiner & Müller 1996). Rapidity may also account for the poor resolution of the Caenogastropoda as rapid radiations resulting in new superfamilies occurred in the mid-Triassic, mid-Jurassic, and mid-Cretaceous. Five of the eight caenogastropod superfamilies examined appeared during a 35.5 million year period in the mid-Cretaceous (Tracey et al. 1993). It should alternatively be noted that rapid diversification in the fossil record may not reflect actual evolutionary history. Recent geological approaches restrict the Cambrian "explosion" of animal life to 20-40 million years in duration (Bowring et al. 1993), but molecular calibrations suggest that phylogenetic diversification of animal phyla gradually occurred over the 500 million years prior to the Cambrian (Wray et al. 1996).

The Patellogastropoda presents a particular problem for improving molecular resolution of gastropod phylogeny. A higher rate of molecular evolution of this gastropod lineage was observed for both domains of 28S rRNA examined. While the use of slower evolving 18S rRNA may improve resolution of gastropod phylogeny, the 18S and 28S rRNA genes do not evolve independently but instead form, with 5.8S rRNA, a group of adjacent genes in chromosomal DNA that are transcribed as a unit followed by post-transcriptional RNA cleavage to form independent cellular 5.8S, 18S, and 28S rRNA. This transcriptional unit of rRNA is tandemly repeated in the genome of animals and the repeats exhibit concerted evolution - the homogenizing of repeats during DNA replication. While the elevated rate of molecular evolution in patellogastropod 28S rRNA may be solely a function of substitutional processes within patellogastropod 28S rRNA, it could also be the result of patellogastropod specific dynamics of concerted evolution that would additionally affect the evolution of patellogastropod 18S rRNA. Similar rate differences among the Cephalopoda, Gastropoda, and Bivalvia for the D6 domain of 28S rRNA and total 18S rRNA (Steiner & Müller 1996) suggest that lineage-specific differences in evolutionary rate may encompass the entire rRNA transcriptional unit. Unrelated genes may be required to resolve the position of the Patellogastropoda.

Evolutionary Implications for Hydrothermal Vent Communities

Hydrothermalism has existed since the Archaean so the hydrothermal vent habitat has been available for colonization since the origination of the Metazoa in the Cambrian. Six certain fossil communities are known, ranging from the late Silurian (~414 MYBP) to the late Eocene (~40 MYBP) (Table 9). Representation is poor for all the sites examined. Vestimentiferan and polychaete tubes worms are the only animals common to all deposits. The oldest known assemblage at Yaman Kasy, Russia (late Silurian, Little et al. 1996) illustrates that hydrothermal vent communities have changed throughout the Phanaerozoic due to the presence of monoplacophoran molluscs and inarticulate brachiopods which are absent from modern hydrothermal vent communities. Waren (personal communication) reports the discovery of a monoplacophoran near at hydrothermal vents of the Mid-Atlantic Ridge, although its degree of association with hydrothermalism is uncertain. This discovery does not decrease the novelty of the late-Silurian monoplacophorans, which were discovered in high abundance. Monoplacophora are extremely rare in modern oceans. Non-vent marine communities, the ultimate source of vent lineages, have evolved over evolutionary time and the replacement of brachiopods by bivalves and monoplacophorans by archaeogastropods suggested by Little et al. (1996) reflects the patterns of change in Paleozoic marine communities in general (Sepkoski & Miller 1985), a suggestion already made for fossil hydrothermal vent and hydrocarbon seep brachiopods

Table 9. Known hydrothermal vent fossil assemblages from volcanogenic massive sulphide deposits. Putative low-temperature hydrothermal vent barite deposits cited in Gaillard et al. (1992) and Campbell & Bottjer (1995) were excluded, as per Little et al. (1996).

Assemblage	Epoch	Fossils
Zambales Ophiolite, Philippines (Boirat & Fouquet 1986)	Late Eocene	alvinellid-like polychaete tube worms
Azema Ophiolite, New Caledonia (Oudin et al. 1985)	Late Cretaceous to Early Paleocene	tube worms (polychaete or vestimentiferan?)
Troodos Ophiolite, Cyprus (Oudin & Constantinou 1984)	Late Cretaceous	tube worms (polychaete or vestimentiferan?)
Samail Ophiolite, Oman (Haymon et al. 1984)	Late Cretaceous	tube worms (polychaete or vestimentiferan?)
Sibay, Urals, Russia (Kuznetsov et al. 1990)	Middle Devonian	vestimentiferan tube worms, vesciomyid-like bivalves
Yaman Kasy, Urals, Russia (Little et al. 1996)	Late Silurian	vestimentiferan tube worms, polychaete tube worms, monoplacophorans, inarticulate brachiopods

(Campbell & Bottjer 1995). Endemism at hydrothermal vent communities is remarkable but these communities have been subject to continuous invasion throughout their history as evidenced by the mosaic phylogenetic origins of endemic species, genera, and families. Strong evidence also exists that hydrothermal vents have had a historical relationship with hydrocarbon seep communities (Craddock et al. 1995, Tunnicliffe et al. 1996). However, Little et al. (1996) cite the absence of monoplacophorans and brachiopods in modern hydrothermal vents and the likewise absence of extant "relic" archaeogastropods and barnacles in the Yaman Kasy assemblage as evidence that hydrothermal vents are not refugial in nature. The molecular systematic investigations of this dissertation support the early Paleozoic origins of the vent endemic Neomphalina and give preliminary support for the phylogenetic novelty of the Neolepetopsidae. These gastropod groups are not known outside of the hydrothermal vent community. Their existence at hydrothermal vents is evidence of the refugial nature of the habitat. The absence of archaeogastropods from the Yaman Kasy deposit may be due to the small size of the assemblage, but the suggestion that their invasion of hydrothermal vents may have been post-Silurian is an acceptable alternate explanation. Like for other marine communities, they may have replaced monoplacophorans (and brachiopods?) with time. Lineage antiquity and time of invasion of hydrothermal vents are two separate issues and archaeogastropod lineages of early Paleozoic origins may not have invaded vents until later in the Paleozoic.

Other evidence for the refugial nature of hydrothermal vents exists. The recent discovery of *Neobrachylepas relica* in the Lau Basin represents the only extant representative of the earliest sessile barnacles, the Brachylepadomorpha, which were present in the fossil record from the Jurassic to the Miocene (Newman & Yamaguchi

1995). The Yaman Kasy assemblage contains putative representatives of the Vestimentifera, evidence of their antiquity. Hydrothermal vent communities are more similar to each other, even when comparing different oceans, than they are to adjacent deep-sea communities, and their biogeography reflects plate tectonic relationships pre-dating the Cretaceous (Tunnicliffe & Fowler 1996, Tunnicliffe et al. 1996). However, antiquity for many of the hydrothermal vent endemic groups has not been investigated beyond their initial descriptions.

The deep-sea has been proposed to be a refuge for elements of ancient evolutionary faunas that have been displaced to the deep-sea after evolution of novel competitors and predators, mass extinction, and faunal replacement on continental slopes and shelves (Sepkoski & Miller 1985, Conway Morris 1989, Sepkoski 1991). Physiological constraints to invasion of the deep-sea allowed only a limited subset of the slope faunas to successfully enter deep-sea habitats (Robertson 1989, Childress 1995, Tyler 1995). The net result of faunal replacement on continental shelves was displacement and restriction to the deep-sea of elements of older faunas (Conway Morris 1989, Sepkoski 1991). Endemicity was supported by physiological constraints to invasion of some competitors and predators and many deep-sea groups appear to represent phylogenetic relics (Conway Morris 1989). Physiological constraints at hydrothermal vents present an additional barrier to invasion of newly evolved predators and competitors (Tunnicliffe 1992). The hydrothermal vent communities reflect a sub-sample of possible invaders from the adjacent deep-sea exhibiting a mosaic of evolutionary origins. Exclusion of certain predators and competitors has allowed refugial survival of ancient lineages such as the endemic vestimentiferans, archaeogastropods, barnacles, and polychaetes, whereas openness to others has resulted in recent evolution of vent endemic species and genera.

Mass extinction has also been a fundamental force in shaping marine faunas. Marine communities have faced five major mass extinctions (late Ordovician, late Devonian, late Permian, late Triassic, end-Cretaceous) and numerous lesser extinction events (Sepkoski 1986, Benton 1995). The impact of mass extinction upon deep-sea communities is unclear but hydrothermal vent communities may not have responded at all because of their energetic independence based on local chemoautotrophy (Tunnicliffe 1992). The only external requirement is oxygen and the record of deep-sea anoxia is unclear (Tunnicliffe 1992), except for evidence of deep-sea anoxia during the Eocene (~ 57 MYBP; Kennett & Stott 1991).

CONCLUSIONS

A molecular systematic investigation of the evolutionary origins of hydrothermal vent endemic gastropods revealed the early to mid-Paleozoic origins of the hydrothermal vent endemic Neomphalina. Hydrothermal vent communities exhibit high endemicity, endemic animal groups with high taxonomic novelty, severe physiological barriers to invasion, and independent *in situ* primary production. Newman (1985) and Tunnicliffe (1992) hypothesized that animal groups at hydrothermal vents with high taxonomic novelty represent ancient lineages of Mesozoic or Paleozoic origins that have survived in refugia at hydrothermal vents and thus avoided mass extinctions and the origination of new predators and competitors throughout the Phanaerozoic. The alternate explanation is of rapid change of recent immigrants from the adjacent deep-sea and false taxonomic inflation (Cohen & Haedrich 1983, Hickman 1984). Endemic gastropod groups with high taxonomic novelty lack clear fossil affinities, with the possible exception of the Neolepetopsidae (McLean 1990a, but see Fretter 1990). Interpretations of gastropod evolutionary novelty using anatomical and morphological features are hampered by unclear evolutionary scenarios of anatomical evolution, contradictory or weakly supported phylogenetic hypotheses, and the possibility that hydrothermal vent endemic animals are the result of rapid evolution and convergence.

This dissertation illustrated the utility of molecular systematics for independently testing evolutionary hypotheses dependent upon neontological investigations. Twenty-three new D1 domain and thirty new D6 domain DNA sequences of the 28S ribosomal RNA gene were obtained from fresh-frozen and formalin-ethanol preserved specimens. These were combined with previously published molluscan 28S ribosomal RNA sequences for a total of 159 sequences. Alone, either domain exhibited poor resolution of gastropod phylogeny but combined sequences resulted in resolution of gastropod orders that originated in the late-Cambrian and early Paleozoic. The hydrothermal vent Neomphalina exhibited divergence values and phylogenetic novelty equivalent to the other early-Paleozoic radiations, supporting its consideration as a vent refugial phylogenetic relic.

Newman (1985) and Tunnicliffe's (1992) antiquity hypothesis for hydrothermal vent endemic animals is supported by the endemic Neomphalina. The lack of fossil record for the Neomphalina leaves its evolutionary history unclear but the abundance of monoplacophoran molluscs in a Silurian hydrothermal vent fossil community leaves open the possibility that neomphalinid gastropods replaced monoplacophorans in hydrothermal vents in the latter Paleozoic just as other archaeogastropods replaced monoplacophorans in other marine communities at the same time (Sepkoski & Miller 1985, Little et al. 1996). Presumably physiological barriers to invasion excluded competitors or predators that caused the extinction of the Neomphalina in other marine habitats.

Sequences of 28S ribosomal RNA could not resolve supra-ordinal relationships within the Gastropoda due to saturation of substitutions. Among the gastropod orders, the Patellogastropoda exhibited elevated evolutionary rates for the 28S rRNA domains examined, severely hampering phylogenetic analyses. Sequences of 28S ribosomal RNA would be best used to examine within-order gastropod relationships due to saturation of substitutions at higher levels and among-order evolutionary rate variation. More conserved genes, such as 18S ribosomal RNA or elongation factor 1α, should be used to robustly examine the entire phylogeny of the Gastropoda. In addition, this dissertation illustrated that formalin-ethanol preserved specimens can be useful for molecular systematic investigations, opening new avenues of research for paleontological, evolutionary, and systematic investigations.

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Appendix. Systematic citations for hydrothermal vent endemic gastropods. The highest bolded taxonomic rank represents the highest endemic taxonomic grouping. Sources are given for definition of taxon names and description of anatomical and morphological features. Only those genera and species examined in this dissertation are listed, while all supra-generic endemic taxa are detailed.

Taxon	Systematic Definition	Anato-Morphological Description
Order Patellogastropoda		
Suborder Lepetopsina	McLean (1990a)	McLean (1990a), Fretter (1990)
Superfamily Lepetopsoidea	McLean (1990a)	McLean (1990a), Fretter (1990)
Family Neolepetopsidae	McLean (1990a)	McLean (1990a), Fretter (1990)
Eulepetopsis vitrea	McLean (1990a)	McLean (1990a), Fretter (1990)
Order Neomphalina	Warén & Bouchet (1993)[1]	citations below
Superfamily Neomphaloidea	McLean (1981)	McLean (1981), Fretter et al. (1981)
Family Cyathermiidae[2]	McLean (1990b)	McLean (1990b)
Cyathermia naticoides	Warén & Bouchet (1989)	Warén & Bouchet (1989)
Family Neomphalidae	McLean (1981)	Fretter et al. (1981), McLean (1981)
Superfamily Peltospiroidea[3]	McLean (1989a)	McLean (1989a), Fretter (1989)
FamilyPeltospiridae	McLean (1989a)	McLean (1989a), Fretter (1989)
Depressigyra globulus	Warén & Bouchet (1989)	Warén & Bouchet (1989)
Melanodrymia aurantiaca	Hickman (1984)	Hickman (1984), Haszprunar (1989a)
Peltospira operculata	McLean (1989a)	McLean (1989a), Fretter (1989)
Rhynchopelta concentrica	McLean (1989a)	McLean (1989a), Fretter (1989)
Order Vetigastropoda		
Superfamily Fissurelloidea		
Family Clypeosectidae	McLean (1989b)	Haszprunar (1989b)
Superfamily Lepetodriloidea	McLean (1988)	Fretter (1988)
Family Lepetodrilidae	McLean (1988)	Fretter (1988)
Lepetodrilus fucensis	McLean (1988)	Fretter (1988)
Family Gorgoleptidae	McLean (1988)	Fretter (1988)

Taxon	Systematic Definition	Anato-Morphological Description
Order Cocculiniformia		
Superfamily Lepetelloidea		
Family Pyropeltidae	McLean & Haszprunar (1987)	McLean & Haszprunar (1987)
Order Caenogastropoda		
Superfamily Loxonematoidea		
Family Provannidae	Warén & Ponder (1991)	Warén & Ponder (1991)

[1] Also see McLean (1990b) for an earlier discussion of the relationship between Peltospiroidea and Neomphaloidea

[2] The splitting of this family from the Neomphalidae was not followed in this dissertation.

[3] Warén & Bouchet (1993) place the Peltospiridae within Neomphaloidea.

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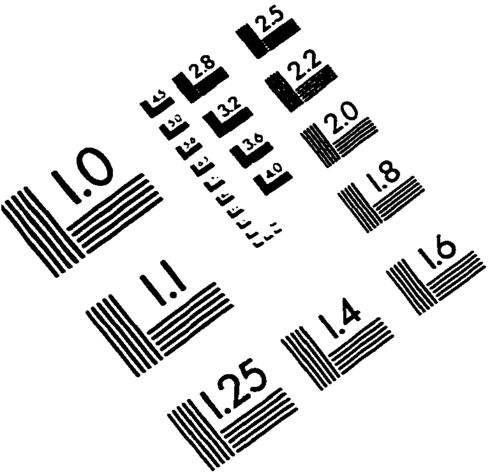

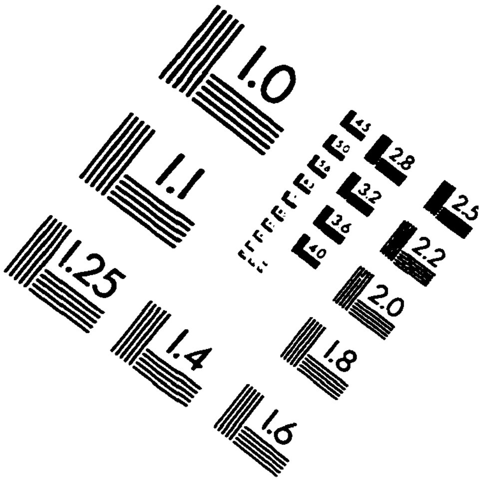

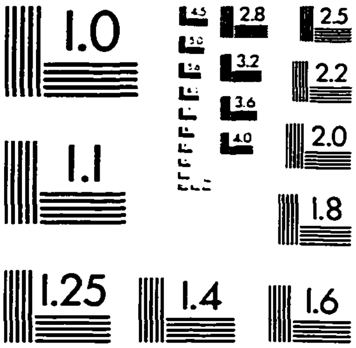

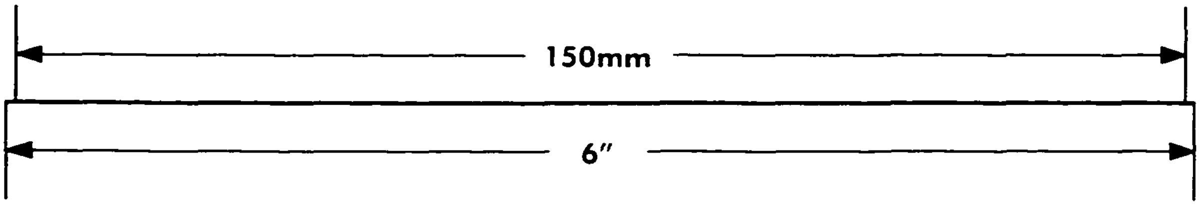

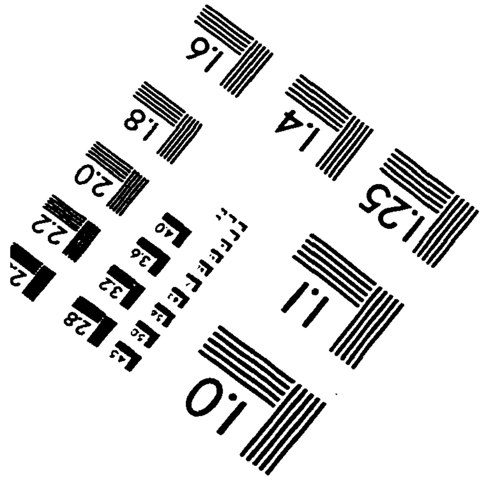

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