

Molecular phylogeny of basal gastropods (Vetigastropoda) shows stochastic colonization of chemosynthetic habitats at least from the mid Triassic

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Abstract: A molecular phylogeny of 40 basal gastropod species representing 14 families (including eight species in four families from chemosynthetic habitats) was constructed, based on 3038 characters (Histone 3, COI, 18S). Unambiguous fossils indicated minimum ages of four major lineages. It suggests that chemosynthetic habitats were colonized several times independently and date back to at least the mid Triassic.

Keywords: Gastropods • Hydrothermal vent • Molecular phylogeny • Colonization • Triassic

Introduction

Determining the age of taxa living at chemosynthetic habitats (hydrothermal vents, cold seeps, whale and wood falls) is an interesting question. Several groups of organisms are found in more than one chemosynthetic habitat type, but are not known from either shallow water or the general deep sea. Limited fossil data on chemosynthetic habitats from the Tertiary led us to investigate the question using molecular phylogeny in conjunction with fossil data from shallow water forms to infer minimum ages of chemosynthetic lineages. We chose basal marine gastropods (Vetigastropoda) as our study group, as it contains several chemosynthetic as well as non-chemosyn-

thetic lineages. We can analyze their relationships despite the fact that the membership of Vetigastropoda is not yet fully agreed upon. There is a broad consensus that Haliotidae (abalone), Scissurellidae (little slit shells), Fissurellidae (key hole limpets), Pleurotomariidae (slit shells) and Trochoidea (top snails, turban snails) belong in Vetigastropoda. Some of the chemosynthetic groups (Lepetodrilidae, Peltospiridae, Neomphalidae) and Seguenziidae are now usually included in Vetigastropoda but have also been recognized at a higher level as distinct lineages (e.g., Ponder, 1998). A broad array of Vetigastropoda was included in this study, for which four fragments of three genes were sequenced as detailed in Geiger & Thacker (2005) and shown in Figure 1.

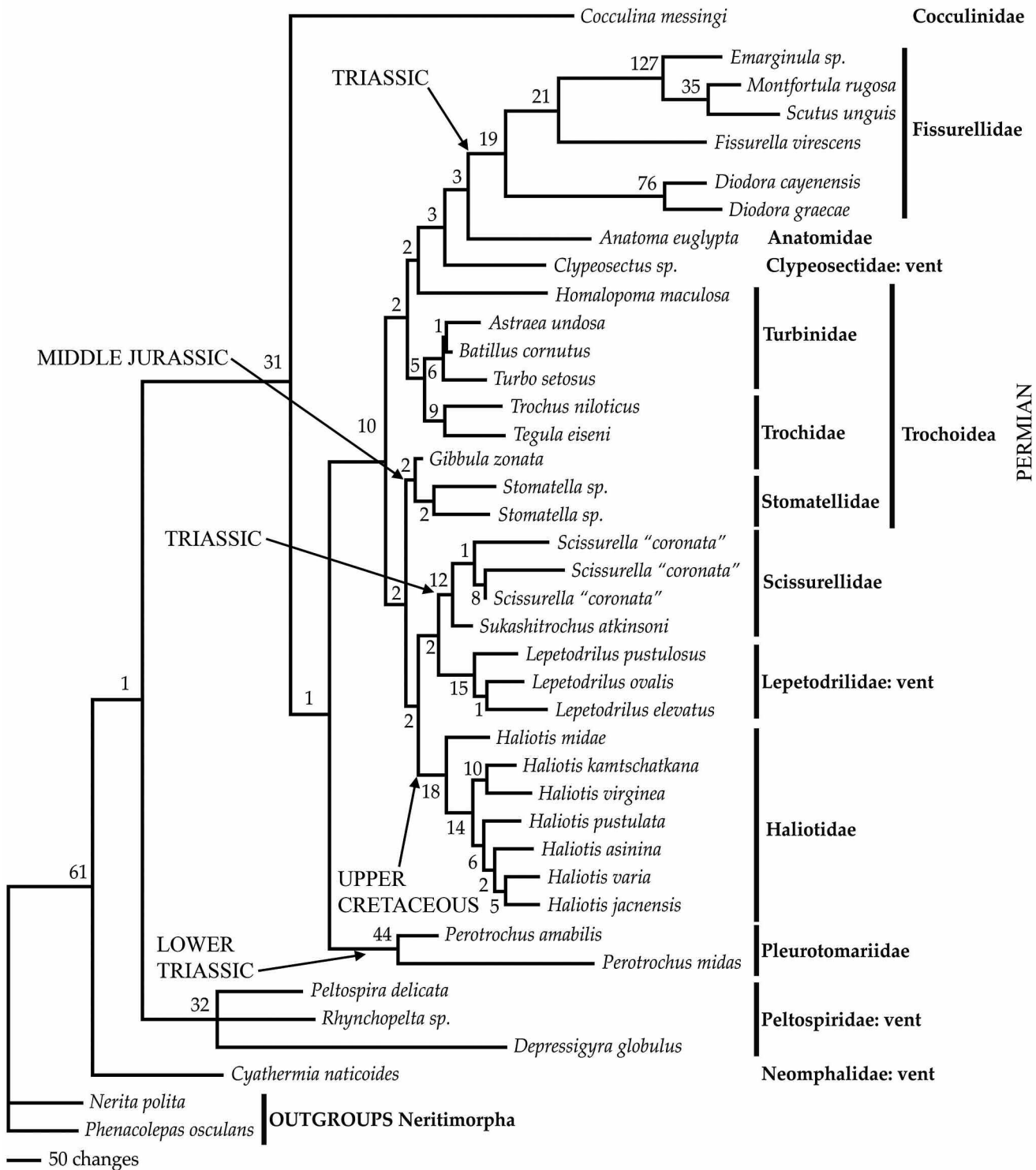


Figure 1. Strict consensus tree of two most parsimonious topologies, shown as a phylogram. Tree length = 6853 steps, ci = 0.489, ri = 0.579, rc = 0.283. Numbers on nodes are Bremer indices. The arrows indicate minimum ages of lineages as supported by the fossil record.

Figure 1. Arbre de consensus strict des deux topologies les plus parcimonieuses, représentées sous la forme d'un phylogramme. Longueur de l'arbre = 6853 pas, ci = 0,489, ri = 0,579, rc = 0,283. Les valeurs sur les nœuds représentent l'indice de Bremer. Les flèches indiquent l'âge minimum des lignées supporté par des fossiles récoltés.

Materials and Methods

Ethanol-preserved tissues for DNA sequencing were obtained from several sources (Table 1). Sequences have been deposited in GenBank under accession numbers AY923870-AY923982. Standard PCR and sequencing protocols were followed. The primers of Colgan et al. (2000) for histone 3, of Folmer et al. (1994: most taxa) and Burton (1998: for Haliotidae) for cytochrome oxidase c subunit I (COI), and those of Giribet et al. (1996) and Whiting et al. (1997) for 18S ribosomal RNA (18S) were used.

Both strands were sequenced separately for each PCR fragment; all fragments of each species were sequenced from the same individual. The resultant chromatograms for both strands were reconciled in Sequencher (version 4.1.2. Gene Codes Corp., Ann Arbor, MI) to check basecalling. Histone 3 and COI were aligned by eye and no ambiguities or gaps in the alignment were found; 18S was automatically aligned with Sequencher. Missing data were coded as ? and gaps were treated as new state (fifth base). Aligned nucleotide sequences were exported from Sequencher as NEXUS files.

All parsimony analyses were performed using PAUP*, version 4.0b4a (Swofford, 1998). One thousand replications of a heuristic search were run, using TBR branch swapping. The data were designated as equally weighted. Decay indices (Bremer, 1988) were calculated with PAUP* and TreeRot v.2 (Sorenson, 1999). Two neritimorphs, *Nerita polita* Linnaeus, 1758 (Neritidae) and *Phenacolepas osculans* Adams, 1852 (Phenacolepadidae: identification kindly confirmed by Takenori Sasaki), were designated outgroup taxa to root the tree and for character polarization. Consistency index (ci), retention index (ri), and rescaled consistency index (rc) were calculated without un-informative characters.

More detailed methodologies have been provided elsewhere (Geiger & Thacker, 2005).

Results and Discussion

Phylogeny

The data matrix with 40 taxa including two designated outgroups consisted of 3038 characters (histone 3: 376, COI: 682, 18S: 1980 aligned), of which 1119 were informative. Analyses of the individual fragments did not indicate any problems with contamination or paralogs (not shown), and topologies are very similar to that of the total evidence analysis. Two most parsimonious trees of 6853 steps (ci = 0.489, ri = 0.579, rc = 0.283) were found. All 1000 replications rapidly found those two trees, indicating a strong signal in the data, which is concordant with the rather high ci and ri values given the size of the data matrix. The strict consensus tree is shown in Figure 1 as a phylogram.

Colonization of chemosynthetic habitats

We assumed that the snail lineages occurring in chemosynthetic environments retained their habitat preference, i.e., did not switch their habitat to the general deep sea or the shallow water over evolutionary time. Our present taxon sampling is restricted to shallow water (0-150 m) and hydrothermal vents; taxa from wood falls (e.g., *Leptogyra*) or seeps (e.g., *Depressigyra*) were not available. We can then place a minimum age on these chemosynthetic taxa, using minimum ages of sistergroups supported by the fossil record. Fossil dates to establish minimum ages of various lineages were obtained from the literature (Haliotidae: Geiger & Groves, 1999; Pleurotomariidae: Harasewych, 2002; Trochoidea: Hickman & McLean, 1990; Scissurellidae, Fissurellidae: Bandel, 1998; Nützel & Geiger, in press). As the systematic affinities of taxa recovered from fossil chemosynthetic sites are debatable, we did not rely on those ambiguous records. For instance, the putative neomphalid of Kiel & Campbell (2005) may also represent a trichotropid or a laubierinid among various possibilities.

Some branches within some families appear longer than average (e.g., Fissurellidae vs. Haliotidae), which can be explained by sampling density (Fissurellidae: 6 of 600 species. Haliotidae: 7 of 56 species). Any effects on the accuracy of our phylogeny do not affect the overall conclusion (Triassic colonization of chemosynthetic habitats), because the minimum ages are applied at the family level.

Chemosynthetic habitats were colonized by at least four different vetigastropod lineages (Neomphalidae, Peltospiridae, Lepetodrilidae, Clypeosectidae) and at minimally three different time periods. Our count is limited by some chemosynthetic groups (Temnocinclinae, Sutilizoninae, Trochoidea) that could not be included in the present study. The absence of a chemosynthetic-gastropod clade shows that the chemosynthetic habitats were colonized repeatedly within vetigastropods.

The present study strongly suggests a colonization of the chemosynthetic environment dating back to at least the mid Triassic. *Clypeosectus* as well as the two chemosynthetic families Neomphalidae (*Cyathermia*) and Peltospiridae (*Peltospira*, *Rhynchopelta*, *Depressigyra*) are basal to the non-chemosynthetic Fissurellidae and Pleurotomariidae; the latter has a fossil record reaching back to at least the Triassic (Bandel, 1998; Harasewych, 2002: Fig. 1). Given that these chemosynthetic taxa are basal to Fissurellidae, they must have originated by at least the Triassic (245-208 million years). Similarly, the non-chemosynthetic Scissurellidae sensu stricto with a fossil record reaching back to the Triassic are sistergroup to the chemosynthetic Lepetodrilidae, which also implies a Triassic age for the chemosynthetic snails. Even assuming some fairly significant rearrangements on the phylogeny presented here, a

Mesozoic colonization of chemosynthetic habitats is extremely likely. More subtle habitat shifts between vents, cold seeps and whale falls within families remain to be explored with the addition of relevant taxa.

The age indication presented here are *minimum* ages as implied by the incomplete fossil record. Triassic minimum ages at the base of Scissurellidae, as well as at the base of Pleurotomariidae, which is more basal to Scissurellidae, do not contradict one another. It suggests that older fossils from certain lineages have not been found yet; some stem lines are currently represented by what paleontologists call 'ghost lineages', time lines implied by sistergroup relationships, but not documented in the fossil record.

Systematic implications

Several consequences for the classification of Vetigastropoda have been discussed elsewhere (Geiger & Thacker, 2005). These include the non-monophyly of "Scissurellidae" (Scissurellinae & Anatominae) and Fissurelloidea (Fissurellidae & Clypeosectidae), as well as the position of cocculiniform limpets.

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