THE ROYAL SOCIETY

doi 10.1098/rspb.2000.1372

A phylogeny of the land snails (Gastropoda: Pulmonata)

Christopher M. Wade[1*], **Peter B. Mordan**[2] **and Bryan Clarke**[1]

[1] *Institute of Genetics, University of Nottingham, Queen's Medical Centre, Clifton Boulevard, Nottingham NG7 2UH, UK*
[2] *Department of Zoology, The Natural History Museum, Cromwell Road, London SW7 5BD, UK*

We have undertaken the first large-scale molecular phylogenetic analysis of the Stylommatophora. Sequences of the ribosomal RNA gene-cluster were examined in 104 species of snails and slugs from 50 families, encompassing all the currently recognized major groups. It allows an independent test of the present classification based on morphology. At the level of families our molecular phylogeny closely supports the current taxonomy, but the deep branches within the tree do not. Surprisingly, a single assemblage including the families Achatinidae, Subulinidae and Streptaxidae lies near the base of the tree, forming a sister group to all remaining stylommatophorans. This primary division into 'achatinoid' and 'non-achatinoid' taxa is unexpected, and demands a radical reinterpretation of early stylommatophoran evolution. In particular, the Orthurethra appear to be relatively advanced within the 'non-achatinoid clade', and broadly equivalent to other super-familial clusters. This indicates that supposedly primitive features such as the orthurethran kidney are derived. The molecular tree also suggests that the origin of the Stylommatophora is much earlier than the main period of their diversification.

Keywords: Stylommatophora; ribosomal RNA genes; molecular phylogeny; Orthurethra; Helicoidea; Achatinoidea

1. INTRODUCTION

Land snails and slugs represent one of the largest invasions of the land, comprising some 30 000–35 000 species (Solem 1984). They have become models for studies on the mechanisms of evolution, and have proved particularly valuable in examining the effects of ecology on evolutionary change (Crampton 1932; Cain & Sheppard 1950; Cain & Currey 1963; Cowie 1992; Johnson *et al.* 1993). Their low vagility also makes them suitable as indicators for biogeographical studies of early tectonic events (Solem 1981). Nonetheless, their evolutionary interrelationships remain largely unresolved.

Four-fifths of land snails belong to the pulmonate (sub)order Stylommatophora. The basis of their present classification was established by Pilsbry (1900*a*) who defined three primary divisions (infraorders), the Orthurethra, Heterurethra and Sigmurethra, based on the anatomy of the excretory system. A fourth division, the Mesurethra, was added by Baker (1955). Of these, only the Orthurethra, the members of which are united by a synapomorphy, have remained universally recognized. Morphological studies have given conflicting results (Tillier 1989; Nordsieck 1986; Shileyko 1979), having been hampered by a long evolutionary history, by relatively rapid radiations, and by convergences.

When morphological features fail to give clear information about relationships, molecular techniques have proved invaluable. There have been some molecular studies of gastropod phylogeny (Emberton *et al.* 1990; Rosenberg *et al.* 1994; Tillier *et al.* 1996; Thollesson 1999) but they have used relatively short sequences and few (< 15) stylommatophoran taxa. Here we present the first comprehensive molecular phylogeny for the Stylommatophora, incorporating over 100 species across a wide range of families, and based on an analysis of 1460 nucleotides of the ribosomal (r) RNA gene-cluster. For the first time, this permits an independent test of the current taxonomy.

2. MATERIAL AND METHODS

(a) *Biological material*

One hundred and four species of 94 genera in 50 stylommatophoran families have been examined. Six non-stylommatophoran pulmonate genera, from the orders Eupulmonata, Basommatophora and Systellommatophora, were incorporated as outgroups. Details of the specimens, the sampling localities, and the collectors are given in table 1.

(b) *Extraction, amplification and sequencing of DNA*

Methods for the extraction of DNA, its amplification by the polymerase chain reaction, and the automated sequencing of approximately 1460 nucleotides from the rRNA gene-cluster, have been described in Wade & Mordan (2000). The sequenced region includes about 80 nucleotides at the 3′-end of the 5.8S gene, the complete internal transcribed spacer 2 (ITS-2) region, and approximately 840 nucleotides at the 5′-end of the large subunit (28S) gene.

(c) *Sequence analysis*

Sequences were assembled using the STADEN package (Staden 1993) and then aligned manually within v. 2.2 of the Genetic Data Environment package (Smith *et al.* 1994). Phylogenetic analyses were performed using PAUP* (v. 4.0d65) (Swofford 1998) and were based on 843 unambiguously aligned nucleotide sites. Evolutionary trees of all 110 taxa were constructed using the neighbour-joining (NJ) (Saitou & Nei 1987) and Fitch–Margoliash (FM) (Fitch & Margoliash 1967)

---

[*] Author and address for correspondence: Department of Zoology, The Natural History Museum, Cromwell Road, London SW7 5BD, UK (c.wade@nhm.ac.uk).

Proc. R. Soc. Lond. B (2001) **268**, 413–422
Received 17 August 2000 *Accepted* 24 October 2000

Table 1. *Samples, collectors and localities*

(The classification broadly follows Vaught (1989).)

family	species	collection or location	collector
stylommatophoran pulmonates (phylum Mollusca: class Gastropoda: subclass Pulmonata: order Eupulomonata: suborder Stylommatophora)			
infraorder Orthurethra			
Cochlicopidae	*Cochlicopa lubrica* (Müller, 1774)	Box Hill, Dorking, UK	P. Mordan and E. Platts
	Cochlicopa lubricella (Porro, 1838)	San Miguel, Azores	P. Mordan
Amastridae	*Leptachatina lepida* Cooke, 1910	Hawaii Island, Hawaii	P. Mordan and R. Cowie
Pupillidae	*Lauria cylindracea* (da Costa, 1778)	Mullaghmore, Co. Sligo, Ireland	E. Platts
	Lauria fasciolata (Morelet, 1860)	San Miguel, Azores	P. Mordan
Valloniidae	*Vallonia costata* (Müller, 1774)	San Miguel, Azores	P. Mordan
	Vallonia eccentrica Sterki, 1892	San Miguel, Azores	P. Mordan
Vertiginidae	*Vertigo antivergo* (Draparnaud, 1801)	Chuett, Arnoldstein, Austria	P. Miltner
Orculidae	*Orcula austriaca* Zimmerman, 1932	Kuhberg, Austria	P. Miltner
Pyramidulidae	*Pyramidula rupestris* (Draparnaud, 1801)	Mullaghmore, Co. Sligo, Ireland	E. Platts
Chondrinidae	*Chondrina avenacea* (Bruguiere, 1792)	Verdon Gorge, France	H. Selvadaruai
	Chondrina clienta (Westerlund, 1883)	Villach, Austria	P. Miltner
	Solatopupa similis (Bruguiere, 1792)	Verdon Gorge, France	A. Davison
Buliminidae	*Buliminus labrosus* (Olivier, 1804)	Saladin's Castle, Syria	P. Mordan
	Pene sidonensis (Férussac, 1821)	Saladin's Castle, Syria	P. Mordan
	Macaronapaeus vulgaris (Morelet & Drouet, 1857)	San Miguel, Azores	P. Mordan
	Mastus pupa (Bruguiere, 1792)	Sicily	A. Davison
Cerastidae	*Cerastus schweinfurthii* (Martens, 1895)	Al-Mahuit, N. Yemen	P. Mordan
	Pachnodus silhouettanus Van Mol & Coppois, 1980	Silhouette Island, Seychelles	J. Gerlach
Partulidae	*Partula suturalis* Pfeiffer, 1855	Moorea	B. Clarke
	Samoana conica (Gould, 1848)	Samoa	R. Cowie
	Eua zebrina (Gould, 1848)	Samoa	R. Cowie
infraorder Mesurethra			
Clausiliidae	*Cochlodina laminata* (Montagu, 1803)	South Downs, East Sussex, UK	B. Clarke
	Albinaria xantostoma (Boettger, 1883)	Crete	D. Thomaz
	Papillifera papillaris (Müller, 1774)	Sicily	A. Davison
	Clausilia bidentata (Ström, 1765)	Kirkdale, Derbyshire, UK	C. Wade
	Macrogastra rolphii (Turton, 1826)	South Downs, East Sussex, UK	B. Clarke
	Stereophaedusa japonica (Crosse, 1871)	Yamaguchi City, Japan	P. Callomon
	Mundiphaedusa decapitata (Pilsbry, 1902)	Osaka City, Japan	P. Callomon
infraorder Elasmognatha			
Succineidae	*Succinea putris* (L., 1758)	Southampton, UK	C. MacDonald
Athoracophoridae	*Athoracophorus bitentaculatus* (Quoy & Gaimard, 1832)	Mere Mere, New Zealand	G. Barker
infraorder Sigmurethra			
Bulimulidae	*Placostylus ambagiosus* Suter, 1906	Manaaki Whenua, New Zealand	D. Gleeson
Cerionidae	*Cerion incanum* (Binney, 1851)	Florida Keys, USA	J. Taylor
Subulinidae	*Subulina striatella* (Rang, 1831)	Kew Gardens, UK (introduced)	F. Naggs
	Bocageia sp.	Sao Thomé	A. Gascoigne
	Glessula ceylanica (Pfeiffer, 1845)	Colombo, Sri Lanka	P. Karunaratne
	Rumina decollata (L., 1758)	Sicily	A. Davison
	Xerocerastus sp.	Otjiwarongo, Namibia	W. Sirgel
	Zootecus insularis (Ehrenberg, 1831)	Dubai, United Arab Emirates	S. Green
Achatinidae	*Achatina fulica* Bowdich, 1822	unknown (Zool. Soc. Lond. colln)	P. Pearce-Kelly
	Archachatina marginata (Swainson, 1821)	Nigeria (NHM collection)	unknown
	Limicolaria sp.	Somalia (NHM collection)	M. Leng
Coeliaxidae	*Pyrgina umbilicata* Greeff, 1882	Sao Thomé	A. Gascoigne
Spiraxidae	*Euglandina rosea* (Férussac, 1821)	Moorea (Zool. Soc. Lond. colln)	P. Pearce-Kelly
Testacellidae	*Testacella scutulum* Sowerby, 1821	North London, UK	R. Hurst
Streptaxidae	*Gonaxis quadrilateralis* Preston, 1910	Reunion	O. Griffiths
	Gonospira sp.	Mauritius	O. Griffiths
Megalobulimidae	*Megalobulimus oblongus* (Müller, 1774)	Antigua (Zool. Soc. Lond. colln)	P. Pearce-Kelly
Dorcasiidae	*Dorcasia alexandri* Gray, 1938	Windhoek, Namibia	C. Boix-Hinzen
	Trigonephrus globulus (Müller, 1774)	Natal, South Africa	W. F. Sirgel
Acavidae	*Acavus phoenix* (Pfeiffer, 1854)	Kitugula, Sri Lanka	P. Karunaratne
	Leucotaenius proctori (Sowerby, 1894)	Beheloa, Madagascar	O. Griffiths

(*Cont.*)

Table 1. (*Cont.*)

family	species	collection or location	collector
Caryodidae	*Caryodes dufresnii* Leach, 1815	Mt Wellington, Hobart, Tasmania	B. Smith
Rhytididae	*Rhytida stephenensis* Powell, 1930	Manaaki Whenua, New Zealand	D. Gleeson
	Schizoglossa sp.	Kaikarangi, New Zealand	G. Barker
Chlamydephoridae	*Chlamydephorus burnupi* (Smith, 1892)	Pevensey, Natal	D. Herbert
Haplotrematidae	*Haplotrema vancouverense* (Lea, 1839)	Eugene, Oregon	D. Taylor
Corillidae	*Corilla adamsi* Gude, 1914	Sri Lanka	D. Raheem
Punctidae	*Laoma* sp.	Mannacau Harbour, New Zealand	P. Mordan
Charopidae	*Suteria ide* (Gray, 1850)	Waitomo, New Zealand	P. Mordan
Otoconchidae	*Otoconcha dimidiata* (Pfeiffer, 1853)	Waitakere New Zealand	P. Mordan
Discidae	*Discus rotundatus* (Müller, 1774)	Kirkdale, Derbyshire, UK	C. Wade
Euconulidae	*Euconulus fulvus* (Müller, 1774)	New Forest, Hampshire, UK	P. Mordan
Helicarionidae	*Fastosarion brazieri* (Cox, 1873)	Mossman, Queensland, Australia	J. Stanisic
	Rhysotina hepatzion (Gould, 1848)	Sao Thomé	A. Gasgoine
	Harmogenanina argentea (Reeve, 1852)	Reunion	O. Griffiths
	Louisia barclayi (Benson, 1850)	Mauritius	O. Griffiths
	Plegma caelatura (Férussac, 1821)	Reunion	O. Griffiths
	Hiona sp.	Moorea	P. Pearce-Kelly
Ariophantidae	*Cryptozona bistrialis* (Beck, 1837)	Sri Lanka	D. Raheem
	Asperitas inquinata (v.d. Busch, 1842)	Java	J. Reynolds
Trochomorphidae	*Trochomorpha pallens* Pease, 1870	Faatoai Valley, Moorea	unknown
Vitrinidae	*Vitrina pellucida* (Müller, 1774)	Kirkdale, Derbyshire, UK	C. Wade
	Plutonia laxata (Morelet, 1860)	San Miguel, Azores	P. Mordan
Vitreidae	*Vitrea crystallina* (Müller, 1774)	New Forest, Hampshire, UK	P. Mordan
Zonitidae	*Oxychilus alliarius* (Miller, 1822)	Deepdale, Derbyshire, UK	C. Wade
	Oxychilus helveticus (Blum, 1881)	Kirkdale, Derbyshire, UK	C. Wade
	Oxychilus cellarius (Müller, 1774)	Co. Kerry, Ireland	unknown
Milacidae	*Milax budapestensis* (Hazay, 1881)	Kirkdale, Derbyshire, UK	C. Wade
Limacidae	*Deroceras reticulatum* (Müller, 1774)	Kirkdale, Derbyshire, UK	C. Wade
Polygyridae	*Vespericola columbiana* (Lea, 1838)	Eugene, Oregon, USA	D. Taylor
Camaenidae	*Polydontes undulata* (Férussac, 1821)	Dominican Republic	G. Seal
	Satsuma japonica (Pfeiffer, 1847)	Osaka City, Japan	P. Callomon
	Craterodiscus pricei McMichael, 1959	Ravenshoe, NE Qld, Australia	J. Stanisic
Hygromiidae	*Trichia striolata* (Pfeiffer, 1828)	Deepdale, Derbyshire, UK	C. Wade
	Trichia hispida (L., 1758)	Deepdale, Derbyshire, UK	C. Wade
Helicellidae	*Cochlicella acuta* (Müller, 1774)	Porthcurnick, Cornwall, UK	E. Bailes
	Cernuella virgata (Da Costa, 1778)	Porthcurnick, Cornwall, UK	E. Bailes
Helicidae	*Cantareus aspersa* (Müller, 1774) (= *Helix aspersa*)	Kettering, Northants, UK	C. Wade
	Cantareus apertus (Born, 1778)	Sicily	A. Davison
	Cepaea nemoralis (L., 1758)	Marlborough Downs, Wiltshire, UK	A. Davison
	Cepaea hortensis (Müller, 1774)	Marlborough Downs, Wiltshire, UK	A. Davison
	Marmorana scabriuscula (Deshayes, 1830)	Sicily	A. Davison
	Theba pisana (Müller, 1774)	Sicily	A. Davison
	Arianta arbustorum (L., 1758)	Deepdale, Derbyshire, UK	C. Wade
	Helicigona lapicida (L., 1758)	Deepdale, Derbyshire, UK	C. Wade
Bradybaenidae	*Bradybaena similaris* (Férussac, 1821)	Sri Lanka	P. Karunaratne
	Aegista vulgivaga (Schumacher & Boettger, 1890)	Osaka City, Japan	P. Callomon
	Euhadra amaliae (Kobelt, 1875)	Osaka City, Japan	P. Callomon
	Euhadra sandai Pilsbry, 1928	Osaka City, Japan	P. Callomon
Helminthoglyptidae	*Monadenia fidelis* (Gray, 1834)	Oregon	D. Taylor
Arionidae	*Arion hortensis* Férussac, 1819	Kirkdale, Derbyshire, UK	C. Wade
	Arion ater (L., 1758)	Kirk Ireton, Derbyshire, UK	C. Wade
	Geomalacus maculosus Allman, 1843	unknown	P. Wisniewsky
non-stylommatophoran pulmonates (phylum Mollusca: class Gastropoda: subclass Pulmonata)			
order Eupulmonata			
Ellobiidae	*Melampus luteus* (Quoy & Gaimard, 1832)	Souilla, Mauritius	O. Griffiths
	Laemodonta sp.	Suralaya, W. Java	B. Dharma
Carychiidae	*Carychium tridentatum* (Risso, 1826)	Abelheira, San Miguel, Azores	P. Mordan
order Basommatophora			
Siphonariidae	*Siphonaria pectinata* (L., 1758)	Zamara Los Atunes, Spain	S. Hawkins
order Systellommatophora			
Veronicellidae	*Laevicaulis alte* (Férussac, 1823)	Dubai, United Arab Emirates	A. Green
Rathouisiidae	*Atopos australis* (Heynemann, 1876)	Malanda, Queensland, Australia	J. Stanisic

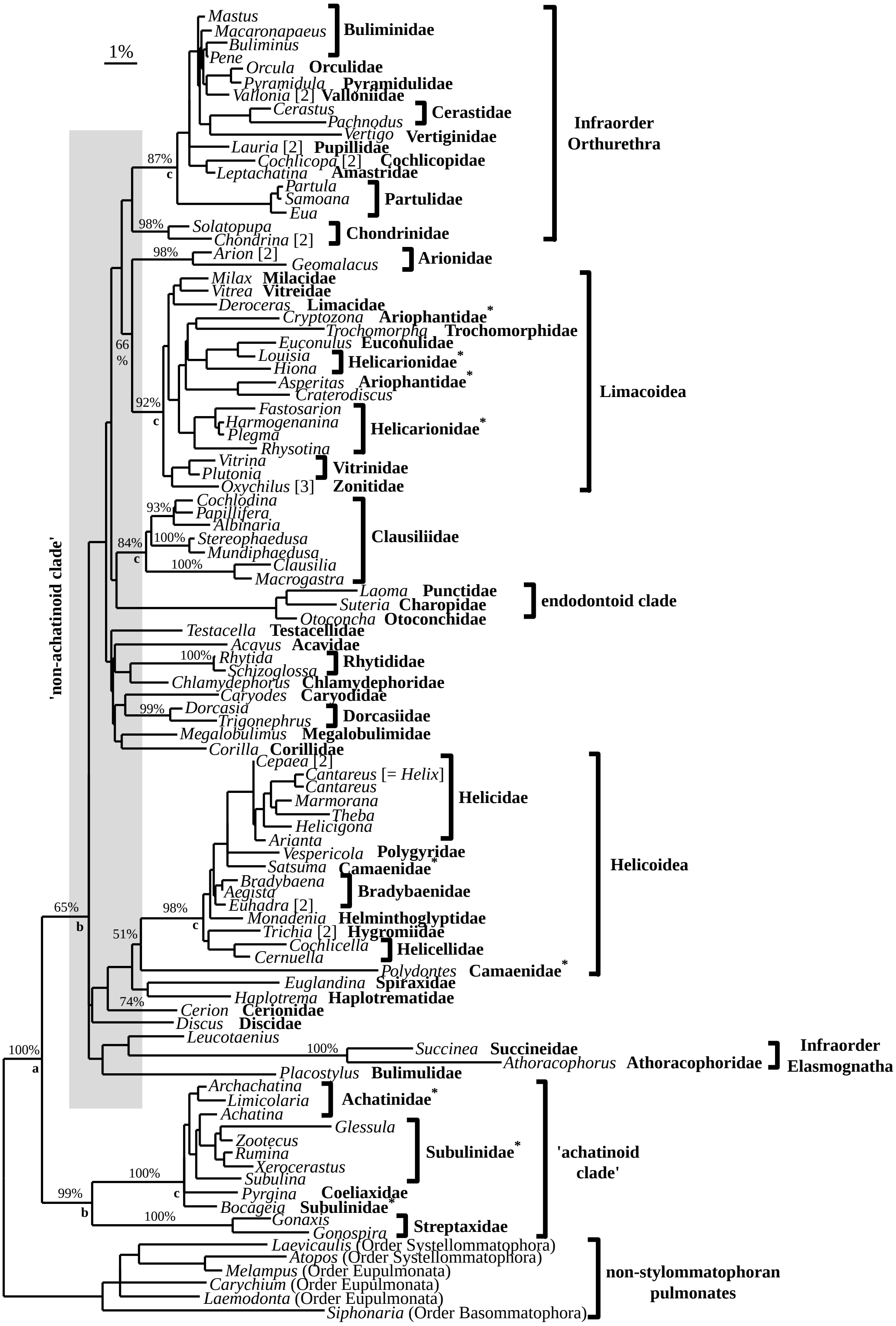

Figure 1. NJ phylogenetic tree for the stylommatophoran pulmonates. The phylogeny is based on 843 nucleotide sites, and genetic distances are estimated using a GTR model incorporating variation in rates between sites (pinvar = 0.290, $\alpha = 0.367$). Bootstrap values indicate the percentage support for individual branches based on 1000 replicates (only bootstrap values over 50% are shown). The scale bar corresponds to one substitutional change per 100 nucleotide positions. The phylogeny is rooted on the non-stylommatophoran pulmonate taxa. (a–c) indicate the major periods of diversification; see §4(d). The asterisks indicate families that do not appear to be monophyletic in the tree. Numbers in brackets after the genus name indicate the number of species sequenced in that genus.

distance methods and maximum parsimony (MP) (Fitch 1971). In addition, maximum-likelihood (ML) (Felsenstein 1981) trees were constructed based on a smaller set of 22 taxa spread widely across the NJ tree. For the NJ, FM and ML methods, we corrected for multiple hits using the general time-reversible (GTR) model. The rate matrix, base frequencies, proportion of invariant sites (pinvar) and gamma shape parameter (α) of the gamma distribution (based on 16 rate categories) were estimated using likelihood by iteration from an initial NJ tree. Parameters estimated from the initial tree were used to make a new NJ tree. The parameters were then re-estimated, and the process repeated until there was no further improvement in likelihood. Tree searching for FM, ML and MP methods used a heuristic procedure with tree-bisection–reconnection branch swapping. Bootstrap resampling (Felsenstein 1985) assigned support to particular branches within the tree. Alternative phylogenetic hypotheses were tested using the Kishino–Hasegawa likelihood-ratio test (Kishino & Hasegawa 1989) in PAUP. Alternative trees were generated under particular topological constraints, allowing the optimization of a tree by rearrangement of the unconstrained taxa. The likelihood of the optimal tree generated under a specific constraint was then compared with that of the actual (i.e. best unconstrained) tree. Principal coordinates analysis was performed using the PCOORD program (Higgins 1992).

(d) *Nucleotide sequence accession numbers*

Nucleotide sequences in this study have been given the GenBank accession numbers AY014018–AY014163.

3. RESULTS

(a) *Variation in rDNA sequences*

The sequenced region includes a combination of conserved, variable and highly variable sections (see Wade & Mordan 2000). The ITS-2 region showed extremely high variability between taxa and most of its sites could not be aligned across all the taxa. These sites were excluded from the phylogenetic analyses. There were 843 nucleotide sites that could be aligned, and phylogenetic comparisons were based on this subset.

DNA base frequencies were highly consistent, ranging from 59 to 63% GC. ML estimates of the pinvar and the α were 0.290 and 0.367, respectively. Thus about 30% of sites seemed to be invariant. The remainder followed a gamma distribution in which the majority seemed to be evolving slowly, but a few were more variable. Genetic distances between taxa ranged from 0 to 24.5% when the non-stylommatophoran pulmonate taxa were included, and from 0 to 22.1% within the Stylommatophora. Genetic distances between taxa ranged from 0 to 10.2% between genera within a family, and from 0 to 1.8% between species within a genus.

(b) *Evolutionary relationships among the stylommatophoran pulmonates*

An NJ phylogeny of all 94 stylommatophoran genera is given in figure 1. The tree is rooted on the non-stylommatophoran pulmonate taxa *Melampus*, *Laemodonta*, *Carychium*, *Siphonaria*, *Laevicaulis* and *Atopos* (in the orders Eupulmonata, Basommatophora and Systellommatophora, according to the taxonomy of Haszprunar & Huber (1990)). These taxa cluster outside the Stylommatophora in other molecular analyses (Wade & Mordan 2000).

(c) *Phylogenetic relationships above the family level*

The molecular phylogeny clearly supports the monophyly of the Stylommatophora (figure 1). The clade is consistently resolved by all the methods of constructing trees, and supported in 100% of bootstrap replicates. At the base of the Stylommatophora there is a single dichotomy giving rise to two major clades. The first includes the families Achatinidae, Subulinidae (including the Coeliaxidae) and Streptaxidae, and is strongly supported in 99% of replicates. It will henceforth be called the 'achatinoid' clade. The remaining Stylommatophora, including representatives of all four infraorders, appear in a second clade. This 'non-achatinoid' grouping is more weakly supported (65%). Nonetheless, it is consistently resolved in all the trees (NJ, FM and MP based on 110 taxa, and ML based on 22 taxa). Moreover, the primary division of the Stylommatophora into 'achatinoid' and 'non-achatinoid' groups is reinforced by a principal-coordinates analysis of distances, in which the 'achatinoid'–'non-achatinoid' split forms the major distinction, encompassing 25% of the total variation (figure 2).

The 'achatinoid clade' (figure 1) shows two distinct lineages, one including the Streptaxidae, and the other the Achatinidae and Subulinidae (incorporating the coeliaxid *Pyrgina*). Both lineages are supported in 100% of bootstraps. The branching at the base of the 'non-achatinoid clade' is not resolved, having little evident structure (figure 1, shaded area). Nevertheless, several well-supported clades correspond to currently accepted familial or superfamilial groups. Two of Pilsbry's (1900*a*) primary stylommatophoran divisions, the Orthurethra and Heterurethra (or more inclusively the Elasmognatha), are retained, and appear to be monophyletic.

The Heterurethra, when first described, included only the Succineidae, but they are now normally regarded as also embracing the Athoracophoridae. These two families (together termed the Elasmognatha) form a monophyletic group which is supported in 100% of bootstraps and resolved by all methods of constructing trees. While the monophyly of the Elasmognatha is convincing, their phylogenetic placement is less certain, as both taxa exhibit long branches. Long-branch taxa tend erroneously to fall at the base of trees (Felsenstein 1978; Philippe & Laurent 1998).

The Orthurethra (figures 1 and 3*a*), represented by 18 genera from 11 families, consistently form a monophyletic group. However, when the Chondrinidae are included the clade is only weakly supported (30% of bootstrap replicates). When they are excluded, the branch to the remaining orthurethrans is supported in 87% of bootstraps. Thus the more restricted group (including the families Partulidae, Amastridae, Cerastidae, Buliminidae, Vertiginidae, Orculidae, Pyramidulidae, Cochlicopidae and Valloniidae) appears to be monophyletic.

The Limacoidea *sensu lato* (Hausdorf 1998), represented by 17 genera from the families Milacidae, Vitreidae, Limacidae, Ariophantidae, Trochomorphidae, Euconulidae, Helicarionidae, Vitrinidae and Zonitidae, form a distinct monophyletic group, supported in 92% of bootstraps (figures 1 and 3*b*). Interestingly, they associate

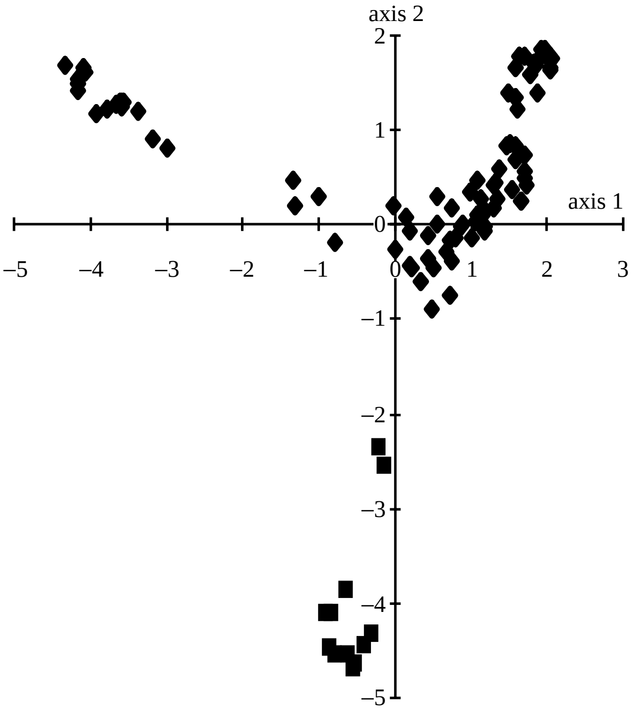

Figure 2. Principle coordinate analysis using PCOORD (Higgins 1992). Symbols: solid diamonds, 'non-achatinoid' taxa; solid squares, 'achatinoid' taxa. Only the first and second axes, explaining 14 and 11%, respectively, of the variation, are shown. The next two axes explain 7 and 5% of the variation. The remaining axes each explain less than 5%. The 'non-achatinoid' and 'achatinoid' taxa are separated along axis 2. Additionally, the helicoid taxa are separated from other 'non-achatinoid' taxa along axis 1.

with the Arionidae (figure 1), a relationship supported in 66% of bootstrap replicates, and by all tree methods. The Helicoidea (figures 1 and 3*c*), represented by 17 genera from the families Helicidae, Polygyridae, Bradybaenidae, Helminthoglyptidae, Hygromiidae, Helicellidae and Camaenidae, are also monophyletic, although the New World camaenid *Polydontes* is weakly related to the others. The main helicoid clade excluding *Polydontes* is supported in 98% of bootstrap replicates; with *Polydontes* the support falls to 51%.

The families Punctidae, Charopidae and Otoconchidae, each represented by a single taxon, form an 'endodontoid' clade which has 100% bootstrap support. A fourth supposedly endodontoid family, the Discidae, is excluded (figure 1). Several other poorly supported clusters of families occur in the tree. Perhaps the most interesting is a 'southern' group comprising the 'acavoid' (*sensu* Zilch 1959–1960) families Acavidae, Caryodidae, Dorcasiidae and Megalobulimidae, as well as the Rhytididae, Chlamydephoridae and Corillidae. A second 'southern' grouping unites the elasmognaths with *Placostylus* (Bulimulidae) and *Leucotaenius*, transferred from the Achatinidae to the Acavidae by Mead (1985).

(d) *Family-level groupings*

Fifty of the currently recognized stylommatophoran families are represented in the tree. When they include more than one taxon, the monophyly of the family is usually supported, as in the cases of the Cerastidae (96% of bootstrap replicates), Partulidae (100%), Chondrinidae (98%), Arionidae (98%), Clausiliidae (84%), Rhytididae (100%), Dorcasiidae (99%), Helicidae (93%), Helicellidae (96%) and Streptaxiidae (100%) (figures 1 and 3). Some groups, such as the Buliminidae (Orthurethra, figure 3*a*), the subulinid–achatinid complex (achatinoid clade, figure 1), and the bradybaenid–polygyrid complex (helicoid clade, figure 3*c*), show no clear evidence for or against monophyly.

In two families, the Ariophantidae and the Helicarionidae (figure 3*b*), there is good support for diphyletic or polyphyletic origins. In Kishino–Hasegawa tests, for the ariophantids, the best tree is one that indicates a polyphyletic origin (−ln likelihood (L) 9291.77550) and it is significantly better than any tree that assumes a monophyletic origin (−ln L 9320.95260, s.d. = 11.68919, $p < 0.05$). Similarly, for the Helicarionids, the best tree indicates polyphyly (−ln L 9291.77550) and is significantly better than any tree assuming monophyly (−ln L 9332.04913, s.d. = 12.53077, $p < 0.01$). It is surprising that the helicarionid *Louisia* strongly clusters with the Euconulidae (figure 3*b*). There is evidence that the Camaenidae, represented by *Polydontes* and *Satsuma* may be diphyletic. Although phylogenies constructed under the constraint that the camaenids are monophyletic are poorer than the optimal tree, in which they are diphyletic, the difference is not significant. *Polydontes* has a relatively long branch, which may lead to problems in its placement.

4. DISCUSSION

This study represents the most comprehensive molecular analysis of land snail phylogeny, dealing with almost 100 genera of snails and slugs. We have already demonstrated the monophyletic nature of the Stylommatophora (Wade & Mordan 2000), confirming the findings of Emberton *et al.* (1990) and Tillier *et al.* (1996). The evidence for their monophyly is even more convincing in the present analysis.

(a) *Evolutionary relationships at the level of the family*

One striking aspect of the phylogeny is its agreement at the family level with the currently accepted taxonomy. The families Cerastidae, Partulidae, Chondrinidae, Arionidae, Vitrinidae, Clausiliidae, Rhytididae, Dorcasiidae, Helicidae, Bradybaenidae, Helicellidae and Streptaxidae, each represented by two or more genera, form well-defined monophyletic groups with good bootstrap support. Several major superfamilies, such as the Limacoidea, Achatinoidea and Helicoidea, are also shown to be monophyletic.

The molecular tree resolves several areas of contention. The endodontoid families fall into two distinct groups: the Pacific Charopidae, Punctidae and Otochonchidae, and the strictly Holarctic Discidae. The sister-group relationship between the Achatinidae and the Subulinidae is supported, endorsing Fischer's (1887) original unification. The enigmatic Australian snail *Craterodiscus* (previously thought to be either a corillid (Tillier 1989) or a camaenid (Smith 1992)) is clearly placed as a sister group of the ariophantid *Asperitas* in the Limacoidea. Finally,

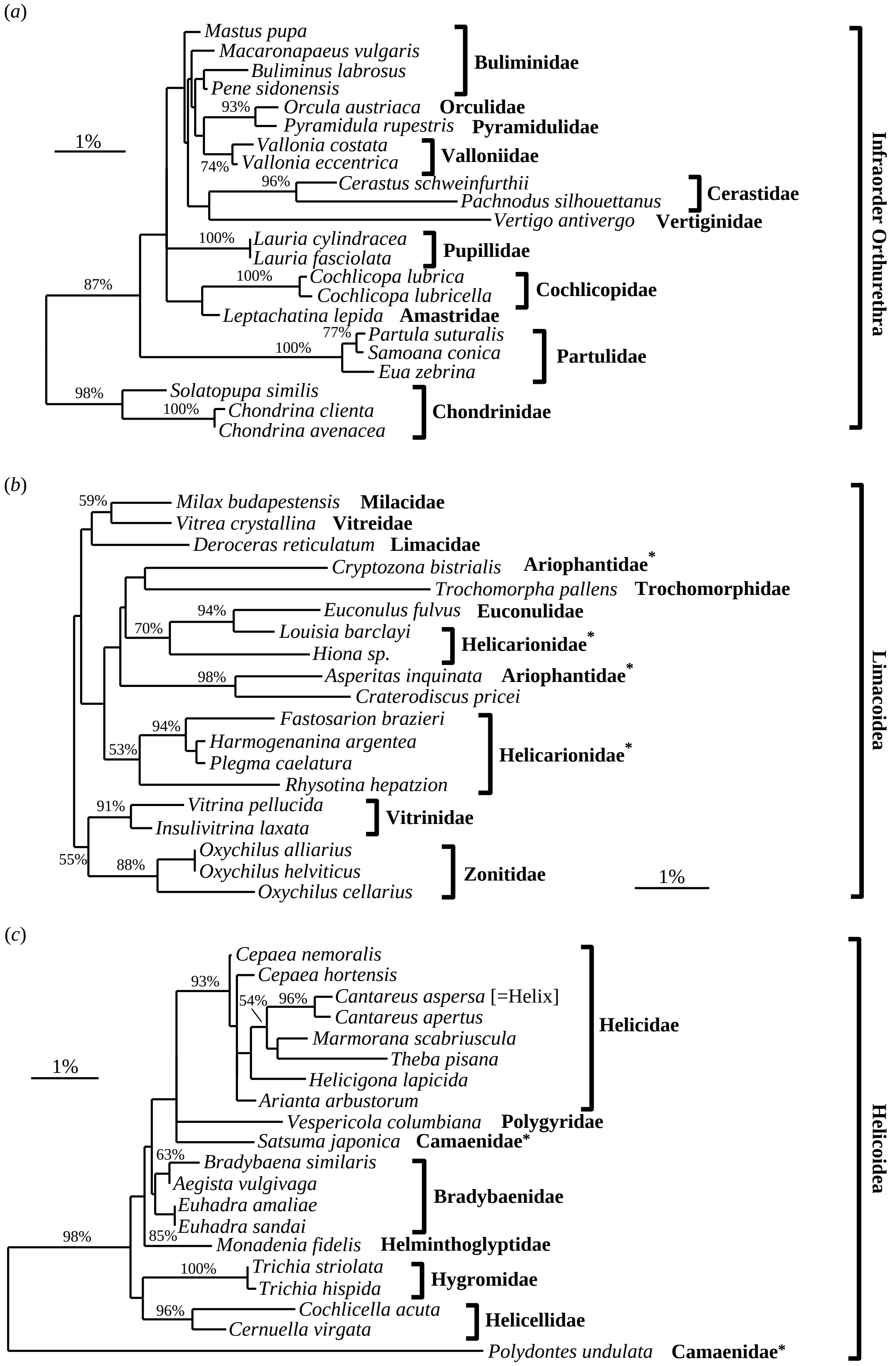

Figure 3. Phylogenetic trees for (*a*) the Orthurethra, (*b*) Limacoidea and (*c*) Helicoidea. Each tree is taken from the main NJ phylogeny and is constructed under the same constraints. Bootstrap values indicate the percentage support for individual branches based on 1000 replicates (only bootstrap values over 50% are shown). The scale bar corresponds to one substitutional change per 100 nucleotide positions. The asterisks indicate families which do not appear to be monophyletic in the tree.

the distance between *Polydontes* and *Satsuma* in the tree supports Scott's (1996) argument that the New and Old World Camaenidae are not sister groups.

(b) *Polyphyletic origins of slugs*

Seven families of slugs are represented in the tree, and each appears to be independently derived. Although this polyphyly is reflected in morphological taxonomies (Tillier 1989; Vaught 1989; South 1992) our tree establishes sister-group relationships for some controversial groups: the South African slug family Chlamydephoridae appears to come from rhytidid snails, and the Pacific athoracophorid slugs appear to come from succineid snails. Less well supported are the pairings of the Milacidae with the Vitreidae, and the Vitrinidae with the Zonitidae. The arionid slugs appear as a sister group of the Limacoidea, but the relationships of testacellid slugs are not resolved. It is interesting that slugs are absent from several major clades such as the Orthurethra, Helicoidea and Achatinoidea. Apart from the pairing of the Haplotrematidae with the Spiraxidae, predatory snail families are also polyphyletic.

(c) *Deep-level evolutionary relationships: the primary divisions in stylommatophoran taxonomy*

While the upper levels of the molecular tree conform remarkably well to current orthodoxy, the deeper levels disagree fundamentally with all morphological taxonomies, which are themselves inconsistent. The primary divisions in the Stylommatophora were laid down by Pilsbry (1900*a*), and subsequently modified by Baker (1955). Four infraorders make up the so-called 'Pilsbry–Baker' system, based on the morphology of the kidney and ureter: the Sigmurethra, Orthurethra, Heterurethra and Mesurethra. The subsequent history of this system has been complex (see Emberton *et al.* 1990), but although variously modified it still forms the basis of most modern classifications.

Pilsbry used the name Orthurethra for a small group of families possessing a straight ureter leading directly from the kidney to near the pneumostome. Although orthurethrans closely resemble other Stylommatophora in their renal histology, they show differences such as the lack of cilia in the elongated ureter (Delhaye & Bouillon 1972*a*,*b*). Pilsbry's concept of the Orthurethra, including the Partulidae, which are excluded by Shileyko (1979) and Nordsieck (1986), is supported by the molecular phylogeny. However, the family Chondrinidae falls at the base of the clade. This considerably reduces the bootstrap support for the group as a whole, although when they are considered separately the chondrinid clade and the remaining Orthurethra both have strong support. There are no obvious morphological or biogeographical reasons for separating the Chondrinids from the remaining Orthurethra. Note that the Pacific Partulidae and Amastridae appear to have arisen independently, as previously supposed from their anatomy, following the separation of the Chondrinidae.

The infraorder Mesurethra was introduced by Baker (1955) for a group characterized by a relatively short, triangular kidney and generally with a lateral pore but no ureter. It included the families Cerionidae, Dorcasiidae, Corillidae and Strophocheilidae (including the Megalobulimidae (Leme 1973)). Later Baker (1961) enlarged the group to include the Clausiliidae. All five 'mesurethran' families are represented in the molecular tree, but there is no support for their monophyly. This reflects current opinion. Most of the recent classifications reject the Mesurethra entirely (Shileyko 1979; Nordsieck 1986; Tillier 1989) or reduce it to the single family Clausiliidae (Vaught 1989).

The Heterurethra originally contained only the succineids, and Pilsbry (1900*a*) specifically excluded the athoracophorid slugs. Baker (1955) added the Aillidae and the Athoracophoridae. Several morphological similarities between the succineids and the athoracophorids, as in the mechanism of tentacular retraction (Burch & Patterson 1969), have caused many workers to unite them as the Elasmognatha (Shileyko 1979; Nordsieck 1986). Our molecular tree strongly supports this relationship.

Implicit in Pilsbry's original description of the infraorder Sigmurethra was the view that they are a monophyletic assemblage of families possessing a 'sigmoid' ureter (Pilsbry 1900*a*,*b*, text and figure on p. 570). The group has persisted in most morphological classifications, but has become larger with the incorporation of the Mesurethra and/or Heterurethra. Our molecular analysis shows that the Sigmurethra are not monophyletic. Rather they appear as a paraphyletic stem within which both the monophyletic Orthurethra and Elasmognatha, and the polyphyletic 'mesurethran' groups evolved. Central to this interpretation is the early separation of the fully supported 'achatinoid' clade from the remainder (i.e. the 'non-achatinoid' clade).

If this dichotomy did indeed precede the principal stylommatophoran radiation, then it is parsimonious to suggest that the stylommatophoran excretory system was primitively sigmurethrous, and the 'mesurethran' grade, with the heterurethran and orthurethran clades, were derived from it. This is a direct contradiction of Pilsbry's (1900*a*,*b*) belief that the orthurethran kidney was primitive and ancestral, a view that is still widely accepted (Shileyko 1978; Nordsieck 1985; Tillier 1989; Pokryszko 1994). Our phylogeny shows that the Orthurethra are an advanced group, occupying a position equivalent to that of several other recently evolved superfamilies. Histological studies by Delhaye & Bouillon (1972*a*,*b*) have already suggested that the similarity between the orthurethran and basommatophoran excretory systems is a consequence of convergence. Principal coordinates analysis and Kishino–Hasegawa testing confirm the position of the Orthurethra in our tree: PCOORD analysis places the Orthurethra with the other 'non-achatinoid' taxa and Kishino–Hasegawa testing shows that a phylogeny in which the Orthurethra are advanced is significantly better than one assuming that they are basal and ancestral ($-\ln L$ 9291.77550 and $-\ln L$ 9314.79238, respectively, s.d. = 11.45053, $p < 0.05$).

Several attempts have been made to split the Sigmurethra *sensu lato* into primary subdivisions but none agrees with the basal 'achatinoid'–'non-achatinoid' dichotomy shown in our molecular tree. Pilsbry (1896, 1900*a*) recognized two primary divisions based on pedal morphology: the Holopoda and the Aulacopoda. The 'achatinoid' clade includes only holopod taxa, but holopods also occur in the 'non-achatinoid' clade with the aulacopod groups (Limacoidea, Arionoidea and Endodontoidea). It is therefore parsimonious to suggest that the holopod foot is plesiomorphic. Thus the primitive condition of the Stylommatophora was probably sigmurethrous and holopod.

(d) *Origins and diversification of the Stylommatophora*

Following the initial divergence of the Stylommatophora there appear to have been three principal periods of diversification (a–c, figure 1).

After the division into 'achatinoid' and 'non-achatinoid' lineages (a, figure 1), each lineage shows further diversification (b, figure 1). In the 'non-achatinoid' clade this first cladogenesis gives rise to the progenitors of the Orthurethra and Heturethra, the various 'mesurethran' families and a major subset of the Sigmurethra (figure 1, shaded area). The branching at this level is not resolved, and it could reflect either a period of explosive cladogenesis or a limitation in the resolving power of the sequence. At the equivalent stage in the 'achatinoid' clade there is a simple dichotomy between the streptaxids on the one hand and the achatinids and subulinids on the other.

Both the 'non-achatinoid' and 'achatinoid' clades include groups of biogeographical interest, since their present distributions suggest origins in the southern Mesozoic supercontinent Gondwanaland. In the 'non-achatinoid' clade, the most notable are the 'acavoid' families Acavidae, Caryodidae, Megalobulimidae and Dorcasiidae (Zilch 1959–1960), as well as the Rhytididae, Corillidae and Bulimulidae, which appear to branch more or less directly from the base of this clade. These taxa show high levels of endemicity and disjunct southerly continental distributions. Because of their large size and tendency to lay small numbers of large eggs (Peake 1978) their distributions are unlikely to have arisen by transoceanic dispersal. They can reasonably be assumed to have had a wide distribution in Gondwanaland before its fragmentation in the Mesozoic around 150 million years before present (Myr BP) (Peake 1978). The 'achatinoid' clade similarly includes both the Subulinidae and Streptaxidae, which, although they are dominant Afrotropical families, show endemic radiations in South America and India, again suggesting a wide distribution in Gondwanaland (Zilch 1959–1960; Van Bruggen 1997). These considerations favour a Mesozoic date for diversifications within both the 'non-achatinoid' and 'achatinoid' clades, perhaps correlated with the break up of Gondwanaland. This would indicate that the initial separation of the two clades (a, figure 1) took place even earlier. Unambiguous fossil evidence about the timing of this split does not exist, but there are records of fossil terrestrial pulmonates in the Upper Carboniferous coal forests (*ca.* 300 Myr BP). Solem & Yochelson (1979) regarded them as the earliest stylommatophoran land snails, although some have been re-interpreted as non-stylommatophoran eupulmontes (Bandel 1991, 1997). A Mesozoic diversification of the 'non-achatinoid' and 'achatinoid' clades is consistent with the existence of Stylommatophora in the Palaeozoic. This would push back their divergence and early diversification to a date well before that suggested by Tillier *et al.* (1996) on the basis of a 'molecular clock' approach (90–60 Myr BP) and by Bandel (1991, 1997) on the basis of fossils (130 Myr BP).

Radiations higher in the tree, such as those of the Orthurethra, Limacoidea, Helicoidea, Clausilioidea and Achatinidae–Subulinidae (c, figure 1), probably took place much later, possibly in the Late Cretaceous or Early Tertiary, when a diverse fossil stylommatophoran fauna first appears (Tracy *et al.* 1993). This would coincide with the early diversification of the flowering plants (Crane *et al.* 1995), an event likely to have been significant in land snail evolution.

In conclusion, while the study gives strong support to current taxonomy at the familial and superfamilial levels, it poses fundamental questions about the deep phylogeny of the land snails. This has not been satisfactorily resolved by classical methods, and the new data present strong challenges to the morphologist.

We are specially indebted to Fred Naggs for his help and encouragement throughout the project and for his valuable discussions and comments on the manuscript. We also thank Sara Goodacre, Angus Davison and Vivien Frame for their help and assistance. We are grateful to all the collectors, listed in table 1, who have provided specimens. The research was supported by the Natural Environment Research Council molecular taxonomy initiative (GST/02/1338) and the Leverhulme Trust (F/696/L).

REFERENCES

Baker, H. B. 1955 Heterurethrous and Aulacopod. *Nautilus* **58**, 109–112.

Baker, H. B. 1961 Puerto Rican pupillids and clausilioids. *Nautilus* **75**, 33–36.

Bandel, K. 1991 Gastropods from brackish and fresh water of the Jurassic–Cretaceous transition (a systematic evaluation). *Berliner Geowiss. Abh.* A **134**, 9–55.

Bandel, K. 1997 Higher classification and pattern of evolution of the Gastropoda. *Cour. Forsch.-Inst. Senkenberg* **201**, 57–81.

Burch, J. B. & Patterson, C. M. 1969 The systematic position of the Athoracophoridae (Gastropoda: Euthyneura). *Malacologia* **9**, 259–260.

Cain, A. J. & Currey, J. D. 1963 Area effects in *Cepaea*. *Phil. Trans. R. Soc. Lond.* B **246**, 1–181.

Cain, A. J. & Sheppard, P. M. 1950 Selection in the polymorphic land snail *Cepaea nemoralis*. *Heredity* **4**, 275–294.

Cowie, R. H. 1992 Evolution and extinction of Partulidae, endemic Pacific island land snails. *Phil. Trans R. Soc. Lond.* B **335**, 167–191.

Crampton, H. E. 1932 Studies on the variation, distribution, and evolution of the genus *Partula*. The species inhabiting Moorea. *Carnegie Inst. Wash. Pub.* **410**, 1–335.

Crane, P. R., Friis, E. M. & Pederson, K. R. 1995 The origin and early diversification of angiosperms. *Nature* **374**, 27–33.

Delhaye, W. & Bouillon, J. 1972*a* L'evolution et l'adaptation de l'organe excréteur chez les mollusques gastéropodes pulmonés. II. Histophysiologie comparée du rein des stylommatophores. *Bull. Biol.* **106**, 123–142.

Delhaye, W. & Bouillon, J. 1972*b* L'evolution et l'adaptation de l'organe excréteur chez les mollusques gastéropodes pulmonés. III. Histophysiologie comparée du rein chez les soleoliferes et conclusions generales pour tous les pulmones. *Bull. Biol.* **106**, 295–314.

Emberton, K. C., Kunico, G. S., Davis, G. M., Philips, S. M., Monderewicz, K. M. & Guo, Y. H. 1990 Comparison of recent classifications of stylommatophoran land-snail families, and evaluation of large-ribosomal-RNA sequencing for their phylogenetics. *Malacologia* **31**, 327–352.

Felsenstein, J. 1978 Cases in which parsimony and compatibility methods will be positively misleading. *Syst. Zool.* **27**, 401–410.

Felsenstein, J. 1981 Evolutionary trees from DNA sequences: a maximum likelihood approach. *J. Mol. Evol.* **17**, 368–376.

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

Fischer, P. 1887 *Manuel de conchyliologie*. Paris: F. Savy.

Fitch, W. M. 1971 Toward defining the course of evolution: minimum change for a specified tree topology. *Syst. Zool.* **20**, 406–416.

Fitch, W. M. & Margoliash, E. 1967 Construction of phylogenetic trees. A method based on mutation distances as estimated from cytochrome *c* sequences is of general applicability. *Science* **155**, 279–284.

Haszprunar, G. & Huber, G. 1990 On the central nervous system of Smeagolidae and Rhodopidae, two families questionably allied with the Gymnomorpha (Gastropoda: Euthyneura). *J. Zool.* **220**, 185–199.

Hausdorf, B. H. 1998 Phylogeny of the Limacoidea *sensu lato* (Gastropoda: Stylommatophora). *J. Moll. Stud.* **64**, 35–66.

Higgins, D. G. 1992 Sequence ordinations: a multivariate analysis approach to analysing large sequence datasets. *Comput. Appl. Biosci.* **8**, 15–22.

Johnson, M. S., Murray, J. & Clarke, B. 1993 The ecological genetics and adaptive radiation of *Partula* on Moorea. *Oxf. Surv. Evol. Biol.* **9**, 167–238.

Kishino, H. & Hasegawa, M. 1989 Evaluation of the maximum likelihood estimate of the evolutionary tree topologies from DNA sequence data, and the branching order in Hominoidea. *J. Mol. Evol.* **29**, 1170–1179.

Leme, J. L. M. 1973 Anatomy and systematics of the Neotropical Strophocheiloidea (Gastropoda, Pulmonata) with the description of a new family. *Arquivos de Zoologia* **23**, 295–337.

Mead, A. R. 1985 Anatomical studies transfer Leucotaenius from Achatinidae to Acavidae (Pulmonata: Sigmurethra). *Arch. Moll.* **116**, 137–155.

Nordsieck, H. 1985 The system of the stylommatophora (Gastropoda), with special regard to the systematic position of the Clausiliidae. I. Importance of the excretory and genital systems. *Arch. Moll.* **116**, 1–24.

Nordsieck, H. 1986 The system of the stylommatophora (Gastropoda), with special regard to the systematic position of the Clausiliidae. II. Importance of the shell and distribution. *Arch. Moll.* **117**, 93–116.

Peake, J. F. 1978 Distribution and ecology of the Stylommatophora. In *Pulmonates. 2A. Systematics, evolution and ecology* (ed. V. Fretter & J. F. Peake), pp. 427–534. London and New York: Academic Press.

Philippe, H. & Laurent, J. 1998 How good are deep phylogenetic trees? *Curr. Opin. Gen. Dev.* **8**, 616–623.

Pilsbry, H. A. 1896 The Aulacopoda: a primary division of the monotremate land Pulmonata. *Nautilus* **9**, 109–111.

Pilsbry, H. A. 1900*a* On the zoological position of *Achatinella* and *Partula*. *Proc. Acad. Nat. Sci. Philadelphia* **52**, 561–567.

Pilsbry, H. A. 1900*b* The genesis of mid-Pacific faunas. *Proc. Acad. Nat. Sci. Philadelphia* **52**, 568–575.

Pokryszko, B. 1994 On the mono(?)phyly and classification of the Orthurethra/Pupilloidea (Gastropoda: Pulmonata: Stylommatophora). *Genus* **5**, 371–390.

Rosenberg, G., Kunico, G. S., Davis, G. M. & Haraswewych, M. G. 1994 Preliminary ribosomal RNA phylogeny of gastropod and unioidean bivalve mollusks. *Nautilus* Suppl. 2, 111–121.

Saitou, N. & Nei, M. 1987 The neighbor-joining method: a new method for reconstructing evolutionary trees. *Mol. Biol. Evol.* **4**, 406–425.

Scott, B. 1996 Phylogenetic relationships of the Camaenidae (Pulmonata: Stylommatophora: Helicoidea). *J. Moll. Stud.* **62**, 65–74.

Shileyko, A. A. 1978 Peculiarities of the structure of the excretory system of the pulmonata in relation to subclass classification. *Malac. Rev.* **11**, 68–70.

Shileyko, A. A. 1979 The system of the order Geophila (= Helicida) (Gastropoda: Pulmonata). *Proc. Zool. Inst. Acad. Sci. USSR* **80**, 44–69.

Smith, B. J. 1992 Non-marine Mollusca. *Zool. Catalog. Austr.* **8**, 1–399.

Smith, S. W., Overbeek, R., Woese, C. R., Gilbert, W. & Gillevet, P. M. 1994 The genetic data environment, an expandable GUI for multiple sequence analysis. *Comput. Appl. Biosci.* **10**, 671–675.

Solem, A. 1981 Land-snail biogeography: a true snail's pace of change. In *Vicariance biogeography: a critique* (ed. G. Nelson & D. E. Rosen), pp. 197–221. New York: Columbia University Press.

Solem, A. 1984 A world model of land snail diversity and abundance. In *World-wide snails: biogeographical studies on non-marine Mollusca* (ed. A. Solem & A. C. van Bruggen), pp. 6–22. Leiden, The Netherlands: Brill.

Solem, A. & Yochelson, E. L. 1979 North American Paleozoic land snails, with a summary of other Paleozoic nonmarine snails. *US Geol. Surv. Prof. Pap.* **1072**, 1–42, plates 1–10.

South, A. 1992 *Terrestrial slugs. Biology, ecology and control*. London: Chapman & Hall.

Staden, R. 1993 STADEN package update. *Genome News* **13**, 12–13.

Swofford, D. L. 1998 *PAUP**: *phylogenetic analysis using parsimony* (**and other methods*), v. 4. Sunderland, MA: Sinauer Associates.

Thollesson, M. 1999 Phylogenetic analysis of Euthyneura (Gastropoda) by means of the 16S rRNA gene: use of a 'fast' gene for 'higher-level' phylogenies. *Proc. R. Soc. Lond.* B **266**, 75–83.

Tillier, S. 1989 Comparative morphology, phylogeny and classification of land slugs and snails (Gastropoda: Pulmonata: Stylommatophora). *Malacologia* **30**, 1–303.

Tillier, S., Masselot, M. & Tillier, A. 1996 Phylogenetic relationships of the pulmonate gastropods from rRNA sequences, and tempo and age of the stylommatophoran radiation. In *Origin and evolutionary radiation of the Mollusca* (ed. J. D. Taylor), pp. 267–284. Oxford University Press.

Tracey, S., Todd, J. & Erwin, D. H. 1993 Mollusca: Gastropoda. In *The fossil record 2* (ed. M. J. Benton), pp. 131–167. London: Chapman & Hall.

Van Bruggen, A. C. 1997 *Achatina* and *Helix*, a view from Gondwanaland: aspects of the relations of land molluscs of the Afrotropical and Palaearctic regions. *Heldia* **4**, 70.

Vaught, K. C. 1989 *A classification of the living Mollusca.* Melbourne, FL: American Malacologists, Inc.

Wade, C. M. & Mordan, P. B. 2000 Evolution within the gastropod molluscs: using the ribosomal RNA gene cluster as an indicator of phylogenetic relationships. *J. Moll. Stud.* **66**, 565–570.

Zilch, A. 1959–60 Gastropoda: Euthyneura. *Handb. Palaeozool.* **6**, 1–834.

As this paper exceeds the maximum length normally permitted, the authors have agreed to contribute to production costs.