

## RESEARCH NOTES

### Molecular phylogeny of the helicoid land snails (Pulmonata: Stylommatophora: Helicoidea), with special emphasis on the Camaenidae

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The superfamily Helicoidea is a large and diverse group of land snails belonging to the Pulmonate suborder Stylommatophora. It has an almost worldwide distribution, absent from only sub-Saharan Africa, southern South America, New Zealand and some Pacific islands (Scott, 1996, 1997). The composition of the superfamily has remained somewhat controversial. Morphological studies have suggested the inclusion of between three and nine families, with only the Helicidae and Bradybaenidae common to all classifications (Thiele, 1931; Zilch, 1959–1960; Taylor & Sohl, 1962; Shileyko, 1979; Solem, 1979; Boss, 1982; Nordsieck, 1985, 1986; Tillier, 1989; Vaught, 1989; Scott, 1997; Bouchet & Rocroi, 2005). Our recent molecular study of the Stylommatophora has demonstrated the monophyly of a 'Helicoidea' comprising the Helicidae, Bradybaenidae, Helminthoglyptidae, Hygromiidae, Camaenidae, Polygyridae and Sagdidae (Wade, Mordan & Naggs, 2006). In this study, we apply molecular phylogenetic techniques to investigate specifically the evolutionary relationships within this 'helicoid' group.

Of particular interest are the relationships among members of the helicoid family Camaenidae. The camaenids have an unusual bihemispheric disjunct distribution in Asia and Australasia (from Sri Lanka and India through Myanmar and southeast Asia to Japan, the Philippines, New Guinea to Australia and some Pacific islands) and Central and South America (from Costa Rica, the Greater and Lesser Antilles to northern Argentina) (Scott, 1996; Cuzzo, 2003). The Camaenidae are defined by the absence of a dart sac and related glands on the female genitalia (Pilsbry, 1939), but there is no unique synapomorphy that is characteristic of the group (Scott, 1996) and morphological studies are contradictory with regards to the monophyly or para/polyphyly of the group (Scott, 1996; Cuzzo, 2003). We use molecular data to specifically investigate this issue here.

We have previously sequenced 48 helicoid species for a partial fragment of the rRNA gene cluster (Table 1, Wade, Mordan & Clarke, 2001; Wade *et al.*, 2006). Here we undertake extensive new phylogenetic analyses of the Helicoidea using these sequences, in order to focus specifically on the evolutionary relationships within the helicoid group. By restricting our analyses to the Helicoidea we are able to recruit new sites into our analyses and we present phylogenies based on 915 (for all Helicoidea) and 1,012 (for a subset of the Helicoidea) unambiguously aligned nucleotide sites. Evolutionary trees were constructed using the neighbour-joining (NJ; Saitou & Nei, 1987) and Fitch-Margoliash (FM; Fitch & Margoliash, 1967) distance methods, maximum-likelihood (ML; Felsenstein, 1981), Bayesian Inference (BI; Larget & Simon, 1999) and maximum parsimony (MP; Fitch, 1971). NJ, FM, ML and

MP analyses were performed using PAUP\* (version 4.0d65; Swofford, 1998). For the NJ, FM and ML methods, multiple hits were accounted for using the general time-reversible (GTR) model (Lanave *et al.*, 1984) with between-site rate heterogeneity accounted for by incorporating a proportion of invariant sites (I) and gamma-distributed rates (G) into the model (Gu, Fu & Li, 1995). The rate matrix, base frequencies, proportion of invariant sites (pinvar) and shape parameter ( $\alpha$ ) of the gamma distribution (based on 16 rate categories) were estimated using likelihood by iteration from an initial neighbour-joining tree. For FM, ML and MP methods, tree searching used a heuristic procedure with tree-bisection-reconnection branch swapping. Bootstrap resampling (Felsenstein, 1985) (NJ only with 1,000 bootstrap replicates) was employed to assign support to particular branches within the tree. BI analysis was performed using the MrBayes (version 3.1.2) package (Ronquist & Huelsenbeck, 2003). A GTR+G model was used and the tree space was explored using four chains of a Markov Chain Monte Carlo algorithm for 5 million generations, sampling every 100 generations. In order to ensure adequate chain swapping, the heating parameter was set to 0.05. A consensus tree was built using the last 1,000 trees (burnin = 49,001 samples). Alternative phylogenetic hypotheses were evaluated by likelihood using a Kishino–Hasegawa RELL test (Kishino, Miyata & Hasegawa, 1990) as implemented in PAUP\*.

A maximum-likelihood tree showing the evolutionary relationships among the Helicoidea is presented in Figure 1. The phylogeny is based on the analysis of 915 unambiguously aligned nucleotide sites, of which 221 were variable and 152 were parsimony informative. The tree is rooted on the branch to *Haplotrema* and *Euglandina*, shown to fall as sister taxa to the Helicoidea in the Wade *et al.* (2001, 2006) analyses of stylommatophoran rDNA data. All methods of tree construction employed (ML, BI, NJ, FM and MP) produced trees that were generally consistent, though there were some rearrangements in the relative placement of the basal lineages in the helicoid tree with different methods.

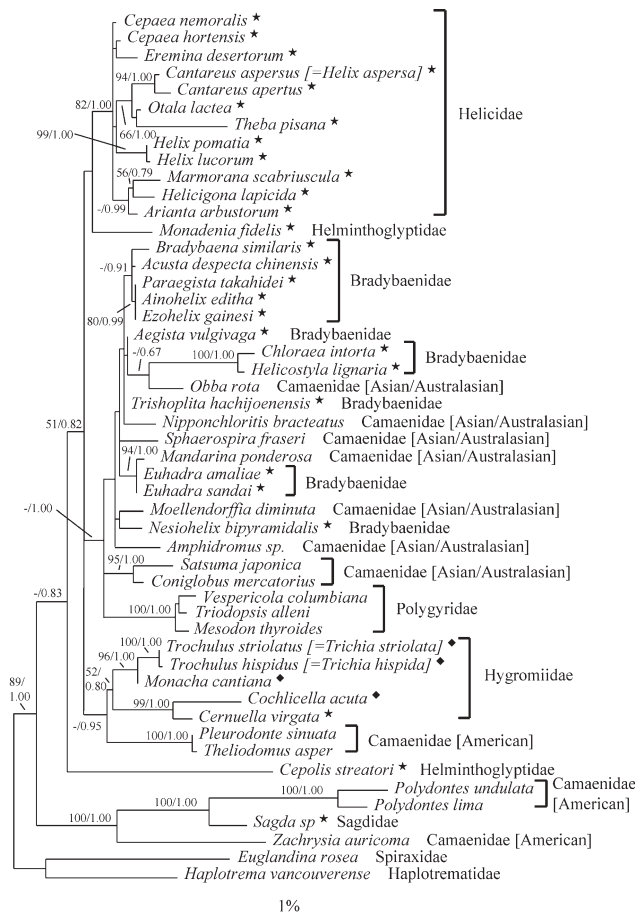
The Helicoidea are consistently resolved as a distinct clade with all tree methods and are supported in 89% of NJ bootstraps ( $P = 1$ , BI) (Fig. 1). Within the Helicoidea, the molecular phylogenies confirm the monophyly of the Helicidae (currently represented by 12 taxa from nine genera; 82%, NJ bootstraps;  $P = 1$ , BI), a group well supported in all molecular studies of the Stylommatophora/Helicoidea to date (Wade *et al.*, 2001; Steinke, Albrecht & Pfenninger, 2004; Koene & Schulenburg, 2005; Manganelli, Salomone & Giusti, 2005; Wade *et al.*, 2006). The Hygromiidae, represented by five taxa from four genera (52%, NJ bootstraps;  $P = 0.8$ , BI), are also monophyletic in our trees, a finding again consistent with other molecular studies (Wade *et al.*, 2001; Steinke *et al.*, 2004; Koene & Schulenburg, 2005; Manganelli *et al.*, 2005; Wade *et al.*, 2006), though

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**Table 1.** Samples, collectors, localities and GenBank accession numbers. The classification broadly follows Vaught, 1989.

Family	Species	Collection/location	Collector	GenBank Accessions	
Helicoidea:					
Helicidae	<i>Helix pomatia</i> L., 1758	Pulpit Down, Buckinghamshire, UK	P. Mordan	AY841333	
	<i>Helix lucorum</i> L., 1758	Unknown	Unknown	AY841334	
	<i>Cantareus aspersus</i> (Müller, 1774) [= <i>Helix aspersa</i> ]	Kettering, Northants, UK	C. Wade	AY014128	
	<i>Cantareus apertus</i> (Born, 1778)	Sicily	A. Davison	AY014129	
	<i>Cepaea nemoralis</i> (L., 1758)	Marlborough Downs, Wiltshire, UK	A. Davison	AY014130	
	<i>Cepaea hortensis</i> (Müller, 1774)	Marlborough Downs, Wiltshire, UK	A. Davison	AY014131	
	<i>Eremina desertorum</i> (Forskål, 1775)	Unknown	Unknown	AY841335	
	<i>Marmorana scabriuscula</i> (Deshayes, 1830)	Sicily	A. Davison	AY014132 & AY014133	
	<i>Otala lactea</i> (Müller, 1774)	Unknown	Unknown	AY841336	
	<i>Theba pisana</i> (Müller, 1774)	Sicily	A. Davison	AY014134 & AY014135	
	<i>Arianta arbustorum</i> (L., 1758)	Deepdale, Derbyshire, UK	C. Wade	AY014136	
	<i>Helicigona lapicida</i> (L., 1758)	Deepdale, Derbyshire, UK	C. Wade	AY014137	
	Polygyridae	<i>Mesodon thyroides</i> (Say, 1816)	York Co. Pennsylvania, USA	F. Thompson	AY841315
		<i>Triodopsis alleni</i> (Wetherby, 1883)	Williams Creek, Iowa, USA	R. Cameron	AY841316
<i>Vespericola columbiana</i> (Lea, 1838)		Eugene, Oregon, USA	D. Taylor	AY014120	
Hygromiidae	<i>Trochulus striolatus</i> (Pfeiffer, 1828) [= <i>Trichia striolata</i> ]	Deepdale, Derbyshire, UK	C. Wade	AY014124	
	<i>Trochulus hispidus</i> (L., 1758) [= <i>Trichia hispida</i> ]	Deepdale, Derbyshire, UK	C. Wade	AY014125	
	<i>Cochlicella acuta</i> (Müller, 1774)	Porthcurnick, Cornwall, UK	E. Bailes	AY014126	
	<i>Cernuella virgata</i> (Da Costa, 1778)	Porthcurnick, Cornwall, UK	E. Bailes	AY014127	
	<i>Monacha cantiana</i> (Montagu, 1803)	Pulpit Down, Buckinghamshire, UK	P. Mordan	AY841331 & AY841332	
	Helminthoglyptidae	<i>Monadenia fidelis</i> (Gray, 1834)	Oregon	D. Taylor	AY014142
		<i>Cepolis streatoris</i> (Pilsbry, 1889)	Grand Cayman	S. Chiba	AY841346
	Bradybaenidae	<i>Bradybaena similis</i> (Férussac, 1821)	Sri Lanka	P. Karunaratne	AY014138
		<i>Acusta despecta chinensis</i> (Sowerby, 1839)	Japan	S. Chiba	AY841337
		<i>Ainohelix editha</i> (A. Adams, 1868)	Shimamaki, Hokkaido, Japan	S. Chiba	AY841338
<i>Ezohelix gainesi</i> (Pilsbry, 1900)		Sapporo, Hokkaido, Japan	S. Chiba	AY841339	
<i>Aegista vulgivaga</i> (Schumacher & Boettger, 1890)		Osaka City, Japan	P. Callomon	AY014139	
<i>Paraegista takahidei</i> Kuroda & Azuma, 1951		Hokkaido, Japan	S. Chiba	AY841340	
<i>Euhadra amaliae</i> (Kobelt, 1875)		Osaka City, Japan	P. Callomon	AY014140	
<i>Euhadra sandai</i> Pilsbry, 1928		Osaka City, Japan	P. Callomon	AY014141	
<i>Nesiohelix bipyramidalis</i> Kuroda & Emura, 1943		Ryukyu, Japan	S. Chiba	AY841341	
<i>Helicostyla lignaria</i> (Pfeiffer, 1842)		Bohol Island, Philippines	S. Chiba	AY841342 & AY841343	
<i>Chloraea intorta</i> (Sowerby, 1840)		Bohol Island, Philippines	S. Chiba	AY841344	
<i>Trishoplita hachijoensis</i> (Pilsbry, 1902)		Niijima Island, Izu Islands, Japan	S. Chiba	AY841345	
Camaenidae		<i>Amphidromus</i> sp.	Unknown	D. Reid	AY841317 & AY841318
		<i>Nipponchloritis bracteatus</i> (Pilsbry, 1902)	Sendai, Japan	S. Chiba	AY841319
		<i>Mandarina ponderosa</i> (Pilsbry, 1901)	Hahajima, Bonin Islands, Japan	S. Chiba & A. Davison	AY841320
		<i>Theliodomus asper</i> (Férussac, 1821)	Windsor, Jamaica	S. Chiba	AY841321
	<i>Pleurodonte sinuata</i> (Müller, 1773)	Green Grot Cave, Jamaica	S. Chiba	AY841322	
	<i>Polydontes undulata</i> (Férussac, 1821)	Dominican Republic	G. Seal	AY014121	
	<i>Polydontes lima</i> (Férussac, 1821)	Dorado, Puerto Rico	A. Davison	AY841323	
	<i>Satsuma japonica</i> (Pfeiffer, 1847)	Osaka City, Japan	P. Callomon	AY014122	
	<i>Coniglobus mercatorius</i> (Pfeiffer, 1854)	Kikai Island, Ryukyu, Japan	S. Chiba	AY841324	
	<i>Sphaerospira fraseri</i> (Griffith & Pigeon, 1833)	Brisbane, Queensland, Australia	J. Stanisic	AY841325	
	<i>Zachrysis auricoma</i> (Férussac, 1821)	Nr. Dorado, Puerto Rico	A. Davison	AY841326	
	<i>Obba rota</i> (Broderip, 1841)	Bohol Island, Philippines	S. Chiba	AY841327 & AY841328	
	<i>Moellendorffia diminuta</i> Pilsbry & Hirase, 1905	Ryukyu, Japan	S. Chiba	AY841329 & AY841330	
Sagdidae	<i>Sagda</i> sp.	Windsor, Jamaica	S. Chiba	AY841347	
Outgroups:					
Spiraxidae	<i>Euglandina rosea</i> (Férussac, 1821)	Moorea (Zool. Soc. Lond. colln.)	P. Pearce-Kelly	AY014074	
Haplotrematidae	<i>Haplotrema vancouverense</i> (Lea, 1839)	Eugene, Oregon, USA	D. Taylor	AY014090	

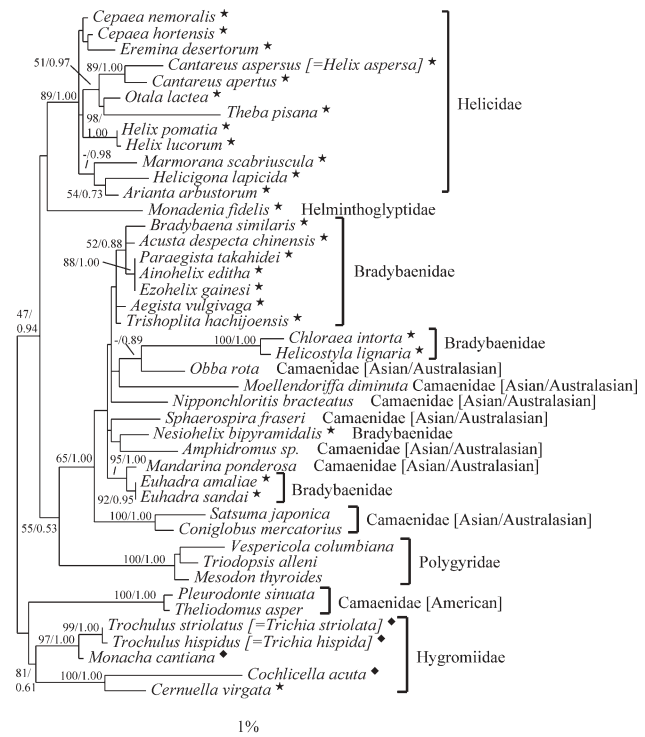


**Figure 1.** Maximum-likelihood phylogenetic tree showing the evolutionary relationships among the Helicoidea. The phylogeny is based on 915 unambiguously aligned nucleotide sites and the tree is rooted on *Euglandina* and *Haplotrema*, which have previously been shown to form the sister clade to the Helicoidea (Wade *et al.*, 2001, 2006). Correction for multiple hits was employed using a GTR + I+G model (proportion of invariant sites,  $\text{pinvar} = 0.335$ , and gamma distribution,  $\alpha = 0.304$ ). The scale bar corresponds to 1 substitutional change per 100 nucleotide positions. Bootstrap values (1,000 NJ bootstraps expressed as a percentage) and Bayesian posterior probabilities indicating support for individual branches are shown on the tree (NJ bootstraps/BI posterior probabilities). Individual taxa marked with a ★ have love darts. ◆ denotes taxa that do not have love darts but do have a dart sac.

within the Hygromiidae the subfamily Helicellinae (*Ceruellu*, *Cochlicella* and *Monacha*) appears to be paraphyletic in that it includes the Trochulinae (= Trichiinae, type genus *Trichia*; *Trochulus* is a replacement name for *Trichia* ICZN ruling, Opinion 2079, 2004). The Polygyroidea, represented by three genera, are also clearly monophyletic (100%, NJ bootstraps;  $P = 1$ , BI) within the molecular trees.

The helminthoglyptids *sensu* Zilch (1959–60), represented in our trees by the genera *Cepolis* and *Monadenia*, are clearly not monophyletic. However, both taxa were excluded by Roth (1996) from his restricted Helminthoglyptidae and allocated to a Bradybaenidae/Xanthonychidae clade. Clearly a wider taxonomic coverage is required in order to resolve this.

The Camaenidae are of particular interest, with the molecular tree providing strong support for the division of the Camaenidae into three groups (Fig. 1). The first includes the American taxa *Polydantes* and *Zachrysia* (and also *Sagda* from the family Sagdidae) and is supported in 100% of NJ bootstraps ( $P = 1$ , BI)



**Figure 2.** Maximum-likelihood phylogenetic tree showing the evolutionary relationships among a subset of the Helicoidea. The exclusion of the divergent helicoid taxa, *Zachrysia*, *Sagda*, *Polydantes* and *Cepolis* and the outgroups permits an additional 97 sites to be recruited into analyses and the phylogeny is based on 1012 unambiguously aligned nucleotide sites. Correction for multiple hits was employed using a GTR + I+G model (proportion of invariant sites,  $\text{pinvar} = 0.395$ , and gamma distribution,  $\alpha = 0.312$ ). Bootstrap values (1,000 NJ bootstraps expressed as a percentage) and Bayesian posterior probabilities indicating support for individual branches are shown on the tree (NJ bootstraps/BI posterior probabilities). The scale bar corresponds to 1 substitutional change per 100 nucleotide positions. Individual taxa marked with a ★ have love darts. ◆ denotes taxa that do not have love darts but do have a dart sac.

and with all methods of tree construction. The second includes the American taxa *Pleurodonte* and *Theliodomus* and is again resolved with all tree methods and supported in 100% of NJ bootstraps ( $P = 1$ , BI). The remaining Asian and Australasian camaenid taxa appear to form a complex with the Bradybaenidae, with camaenid and bradybaenid taxa interspersed in the molecular tree. The phylogenetic placement of the Polygyridae in relation to the bradybaenids and Asian/Australasian camaenids remains unclear.

In order to elucidate more clearly the evolutionary relationships among the Asian/Australasian camaenids and the bradybaenids, supplementary phylogenetic analyses were undertaken in which the outgroups and the divergent (long-branch) helicoid taxa, *Zachrysia*, *Sagda*, *Polydantes* and *Cepolis*, were excluded. This permitted an additional 97 sites to be recruited into the phylogenetic analyses thereby providing greater resolution within the helicoid tree (Fig. 2). In these analyses the Bradybaenidae and the Asian/Australasian Camaenidae consistently cluster together with all methods of phylogeny reconstruction, to form a complex which is supported in 65% of NJ bootstraps ( $P = 1$ , BI).

The division of the Camaenidae into American and Asian/Australasian groups is in agreement with Scott's (1996) argument that the American and Asian/Australasian camaenids are not sister groups and is consistent with our earlier molecular analyses of the Stylommatophora (Wade *et al.*, 2001, 2006). The molecular

data provide conclusive evidence for the separation of the American Camaenidae from the Asian/Australasian Camaenidae, with the monophyly of the Camaenidae strongly refuted in Kishino–Hasegawa likelihood tests [ $-\ln$  Likelihood (L) 4124.60869 (best tree shown in Fig. 1) versus  $-\ln$  L 4221.26103 (Camaenidae monophyletic),  $P < 0.01$ ]. Our results strongly contradict those of Cuzzo (2003), who concluded from a morphological analysis that the camaenids are monophyletic. Moreover, the Asian/Australasian camaenids are interspersed with the Bradybaenidae in our molecular tree, providing additional support for Scott's (1996) suggestions that the Asian/Australasian Camaenidae and the Bradybaenidae are confamilial. In Kishino–Hasegawa tests, the best tree is one that indicates that the Asian/Australasian camaenids are interspersed with the bradybaenids [ $-\ln$  L 4124.60869 (Fig. 1)], but this is not significantly better than any tree that assumes that the Asian/Australasian camaenids are monophyletic [ $-\ln$  L 4145.20601,  $P = 0.075$ ]. The separation of the American camaenids into two groups (with *Polydotes* and *Zachrysia* falling in one group, and *Pleurodonte* and *Theliodoma* in the other) is a novel finding that is of considerable interest. A broader survey of the American camaenids is now required to determine the full picture of diversity within the group. Interestingly, the enigmatic taxon *Sagda* falls with the American camaenids *Polydotes* and *Zachrysia*. Within the Bradybaenidae, *Chloroea* and *Helicostyla* cluster strongly (100%, NJ bootstraps;  $P = 1$ , BI), consistent with their classification within the subfamily Helicostylinae.

The absence of a comprehensive and consistent set of taxa across all molecular studies of the Helicoidea (Steinke *et al.*, 2004; Koene & Schulenburg, 2005; Manganelli *et al.*, 2005) makes it very difficult to make direct comparisons between our molecular phylogeny and those of other groups. However, our phylogeny appears to disagree with that of Steinke *et al.* (2004), where the helicids and hygromids appeared as sister taxa, with *Bradybaena* falling basal to this clade.

While it was not possible to include helicoid sequences from other molecular studies in our main phylogenetic analyses [due either to the sequencing of nonhomologous gene regions (Steinke *et al.*, 2004; Manganelli *et al.*, 2005) or the sequencing of a substantially shorter fragment of the rRNA gene (Koene & Schulenburg, 2005)], we undertook a supplementary analysis of the Helicoidea in which we incorporated the homologous but substantially shorter rRNA sequences of novel genera sequenced by Koene & Schulenburg (2005). This analysis (based on only 712 sites) permitted the inclusion of two new helicid genera (*Chilostoma* and *Eobania*), nine new hygromiid genera (*Pseudotrichia*, *Leptaxis*, *Xerotrichia*, *Helicella*, *Xeromunda*, *Xerosecta*, *Perforatella*, *Monachoides* and *Hygromia*), one new bradybaenid (*Fruticola*) and four new helminthoglyptid genera (*Helminthoglypta*, *Humboldtiana*, *Xerarionta* and *Polymita*). Resolution and support for clades were considerably reduced in this supplementary analysis (data not shown). However, despite the inclusion of new genera, the Helicoidea, Hygromiidae and Polygyridae remained resolved as monophyletic units. Interestingly, the helminthoglyptid genus *Polymita* clustered with *Cepolis* in this analysis, with the other helminthoglyptids falling with *Monadenia*.

Elsewhere, although the molecular phylogeny provides little resolution of the relationships within the Helicidae, one issue of contention is resolved. The brown garden snail *Helix aspersa* clusters with *Cantareus apertus* in the molecular tree (94%/89%, NJ bootstraps;  $P = 1/P = 1$ , BI, Figs. 1 and 2) and not with representatives of the genus *Helix*. This supports the study of Giusti, Manganelli & Schembri (1995) in which *Helix aspersa* was placed in the genus *Cantareus*.

Love darts are present in all taxonomic groups in the tree except for the Camaenidae and Polygyridae (Fig. 1; Tompa, 1980; Baur, 1998; Roth, 1996; Davison *et al.*, 2005). Indeed, the Camaenidae are at least in part defined by the absence of

a dart-sac and other secondary sexual characters (Pilsbry, 1939). In the Hygromiidae, *Cermuella* possess love darts, *Trochulus* [= *Trichia*] forms only unconsolidated granules in the dart sacs and lacks darts; both *Monacha* and *Cochlicella* lack darts but have a dart sac. The absence of love darts in both the Polygyridae and the polyphyletic Camaenidae (which are scattered across the helicoid tree) provides strong evidence that darts have been lost multiple times in helicoid evolution. Kishino–Hasegawa tests demonstrate that phylogenies in which groups with darts or with darts/dart sacs are constrained to be monophyletic are significantly inferior to the best tree shown in Figure 1 [ $-\ln$  L 4124.60869 (best tree) versus  $-\ln$  L 4257.97267 (darts monophyletic),  $P < 0.01$ , or  $-\ln$  L 4221.41890 (darts/dart sacs monophyletic),  $P < 0.01$ ]. Darts are also absent from the spiraxid and haplotrematid outgroups, the best candidates for sister taxa of the Helicoidea *sensu lato* (Wade *et al.*, 2001, 2006).

One issue of concern relates to the relatively long branches of the deeper lineages within the Helicoidea. Long-branch attraction is a well-known problem in phylogeny reconstruction (Philippe & Laurent, 1998) and the occurrence of relatively long branches at the base of the helicoid tree could be due to errors in the process of phylogeny construction. Notably, the removal of these sequences (with concomitant increase in sequence length) led to an improvement of bootstrap support values elsewhere in the tree. There is an immediate need to obtain data for new genes (as well as more sequence data for the rRNA) to clarify relationships within the helicoid tree. Nevertheless, the molecular data are beginning to illuminate the evolutionary relationships within the helicoid group.

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## REFERENCES

- BAUR, B. 1998. Sperm competition in molluscs. In: *Sperm competition and sexual selection* (T. Birkhead & A.P. Møller, eds), 255–305. Academic Press, London.
- BOSS, K. 1982. Mollusca. In: *Synopsis and classification of living organisms* (S.P. Parker, ed), 945–1166. McGraw-Hill, New York.
- BOUCHET, P. & ROCROI, J.-P. 2005. Classification and nomenclator of gastropod families. *Malacologia*, **47**: 1–397.
- CUEZZO, M.G. 2003. Phylogenetic analysis of the Camaenidae (Mollusca: Stylommatophora) with special emphasis on the American taxa. *Zoological Journal of the Linnean Society*, **138**: 449–476.
- DAVISON, A., WADE, C.M., MORDAN, P.B. & CHIBA, S. 2005. Sex and darts in slugs and snails (Mollusca: Gastropoda: Stylommatophora). *Journal of Zoology*, **267**: 329–338.
- FELSENSTEIN, J. 1981. Evolutionary trees from DNA sequences: a maximum likelihood approach. *Journal of Molecular Evolution*, **17**: 368–376.
- FELSENSTEIN, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution*, **39**: 783–791.
- FITCH, W.M. 1971. Toward defining the course of evolution: minimum change for a specified tree topology. *Systematic Zoology*, **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.
- GIUSTI, F., MANGANELLI, G. & SCHEMBRI, P.J. 1995. The non-marine molluscs of the Maltese Islands. *Monografie Museo Regionale di Scienze Naturali (Torino)*, **15**: 1–607.



- GU, X., FU, Y.-X. & LI, W.-H. 1995. Maximum likelihood estimation of the heterogeneity of substitution rate among nucleotide sites. *Molecular Biology and Evolution*, **12**: 546–557.
- KISHINO, H., MIYATA, T. & HASEGAWA, M. 1990. Maximum likelihood inference of protein phylogeny and the origin of chloroplasts. *Journal of Molecular Evolution*, **31**: 151–160.
- KOENE, J.M. & SCHULENBURG, H. 2005. Shooting darts: co-evolution and counteradaptation in hermaphroditic snails. *BMC Evolutionary Biology*, **5**: 25.
- LANAVE, C., PREPARATA, G., SACCONI, C. & SERIO, G. 1984. A new method for calculating evolutionary substitution rates. *Journal of Molecular Evolution*, **20**: 86–93.
- LARGET, B. & SIMON, D.L. 1999. Markov chain monte carlo algorithms for the Bayesian analysis of phylogenetic trees. *Molecular Biology and Evolution*, **16**: 750–759.
- MANGANELLI, G., SALOMONE, N. & GIUSTI, F. 2005. A molecular approach to the phylogenetic relationships of the western Palaearctic Helicoidea (Gastropoda: Stylommatophora). *Biological Journal of the Linnean Society*, **85**: 501–512.
- 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. *Archiv für Molluskenkunde*, **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. *Archiv für Molluskenkunde*, **117**: 93–116.
- PHILIPPE, H. & LAURENT, J. 1998. How good are deep phylogenetic trees? *Current Opinion in Genetics and Development*, **8**: 616–623.
- PILSBRY, H.A. 1939. The land Mollusca of North America, north of Mexico. part 1. *Proceedings of the National Academy of Natural Sciences of Philadelphia, Monograph*, **3**: 1–573.
- RONQUIST, F. & HUELSENBECK, J.P. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics*, **19**: 1572–1574.
- ROTH, B. 1996. Homoplastic loss of dart apparatus, phylogeny of the genera, and a phylogenetic taxonomy of the Helminthoglyptidae (Gastropoda: Pulmonata). *Veliger*, **39**: 18–42.
- SAITOU, N. & NEI, M. 1987. The neighbor-joining method: a new method for reconstructing evolutionary trees. *Molecular Biology and Evolution*, **4**: 406–425.
- SCOTT, B. 1996. Phylogenetic relationships of the Camaenidae (Pulmonata: Stylommatophora: Helicoidea). *Journal of Molluscan Studies*, **62**: 65–74.
- SCOTT, B. 1997. Biogeography of the Helicoidea (Mollusca: Gastropoda: Pulmonata): land snails with a Pangean distribution. *Journal of Biogeography*, **24**: 399–407.
- SHILEYKO, A.A. 1979. The system of the order Geophila (=Helicida) (Gastropoda Pulmonata). *Proceedings of the Zoological Institute, Academy of Sciences of the USSR*, **80**: 44–69.
- SOLEM, A. 1979. Biogeographic significance of land snails, Palaeozoic to Recent. In: *Historical biogeography, plate tectonics, and the changing environment* (J. Gray. & A.J. Boucot, eds), 277–287. Oregon State University Press, Oregon.
- STEINKE, D., ALBRECHT, C. & PFENNINGER, M. 2004. Molecular phylogeny and character evolution in the Western Palearctic Helicidae *s.l.* (Gastropoda: Stylommatophora). *Molecular Phylogenetics and Evolution*, **32**: 724–734.
- SWOFFORD, D.L. 1998. PAUP\* *Phylogenetic analysis using parsimony (\*and other methods)*. Version 4. Sinauer Associates, Sunderland, Massachusetts.
- TAYLOR, D.W. & SOHL, N.F. 1962. An outline of gastropod classification. *Malacologia*, **1**: 7–32.
- THIELE, J. 1931. *Handbuch der Systematischen Weichtierkunde*, 377–778. Gustav Fischer, Jena.
- TILLIER, S. 1989. Comparative morphology, phylogeny and classification of land slugs and snails (Gastropoda: Pulmonata: Stylommatophora). *Malacologia*, **30**: 1–303.
- TOMPA, A.S. 1980. The ultrastructure and mineralogy of the dart from *Philomycus carolinianus* (Pulmonata: Gastropoda) with a brief survey of the occurrence of darts in land snails. *Veliger*, **23**: 35–42.
- VAUGHT, K.C. 1989. *A classification of the living Mollusca*. American Malacologists Inc., Melbourne, Florida.
- WADE, C.M., MORDAN, P.B. & CLARKE, B.C. 2001. A phylogeny of the land snails (Gastropoda: Pulmonata). *Proceedings of the Royal Society of London: Biological Sciences*, **268**: 413–422.
- WADE, C.M., MORDAN, P.B. & NAGGS, F. 2006. Evolutionary relationships among the Pulmonate land snails and slugs (Pulmonata, Stylommatophora). *Biological Journal of the Linnean Society*, **87**: 593–610.
- ZILCH, A. 1959–1960. Euthyneura. In: *Handbuch der Paläozoologie* (Schindewolf, O.H., ed.), Bd 6.II.2, 1–834. Bornträger, Berlin.

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