Data 2. Taxa used for the phylogenetic study of the Achatinoidea. Taxa marked with (*) are new to this study. The DNA for the remaining taxa was provided by C. Wade from the collections obtained for the Wade et al. (2001, 2006) studies. Family units follow Vaught (1989). Subfamily units within the Subulinidae follow Schileyko (1999) with the addition of Glessulinae based on Vaught (1989).

Family		Species	Collection/Location	Collector/Provider
Achatinidae	*	*Achatina achatina* (Linneaus, 1758)	Unknown (Zool. Soc. Lond. Colln.)	NHM**
		Achatina fulica Bowdich, 1822	Captive bred, unknown origin	NHM**
	*	*Achatina stuhlmanni* von Martens, 1892	Semuliki National Park, Uganda	B. Rowson
	*	*Cochlitoma ustulata* (Lamarck, 1822)	Western Cape Prov., South Africa	A. Moussalli & D. Stuart-Fox
Coeliaxidae		*Coeliaxis blandii* (Pfeiffer, 1852)	New Bradford, South Africa	N. Smith
		Pyrgina umbilicata Greeff, 1882	São Thomé	A. Gascoigne
Ferussaciidae	*	*Cecilioides gokweanus* (Boettger, 1870)	Cape Vida, Bhangazi Hill, Zululand, South Africa	D. Herbert
		Ferussacia folliculus (Gmelin, 1791)	Los Alcornales, Prov Cadiz, Spain	M. Seddon
Subulinidae				
Glessulinae		*Glessula ceylanica* (Pfeiffer, 1845)	Colombo, Sri Lanka	P. Karunaratne
Petriolinae		*Bocageia* sp.	São Thomé	A. Gascoigne
	*	*Subulona* sp.	Ossen Forest, Tupen Hills, Kenya	M. Pickford
Rishetiinae	*	*Eutomopeas layardi* (Benson, 1863)	Koralegama, Sri Lanka	?
	*	*Tortaxis erectus* (Benson, 1842)	Guilin, Guangxi Prov., China	R. Anderson
Rumininae		*Riebeckia* sp.	Samha, Sokotra Archipelago	E. Neubert
		Rumina decollata (Linneaus, 1758)	Sicily	A. Davison
		Xerocerastus sp.	Otjiwarongo, Namibia	W. Sirgel
Subulininae	*	*Allopeas clavulinum* (Potiez & Michaud, 1838)	University of Sao Paulo, Brazil	F. Florens & C. Baider
	*	*Leptinaria lamellata* (Potiez & Michaud, 1838)	Botanical Garden, Rio de Janeiro, Brazil	F. Florens & C. Baider
	*	*Paropeas achatinaceum* (L. Pfeiffer, 1846)	Agra Gajaba's Garden Sri Lanka	D. Raheem
	*	*Subulina octona* (Bruguiere, 1789)	Island of Pulo Anna, Sonsorol, Southwest Islands, Republic of Palau	R. Rundell & A.M. Gawel
		Subulina striatella (Rang, 1831)	Kew Gardens (introduced)	F. Naggs
	*	*Subulina vitrea* (Mousson, 1887)	Gauss, Namibia	?
		Zootecus insularis (Ehrenberg, 1831)	Dubai, United Arab Emirates	S. Green
Thyrophorellidae		*Thyrophorella thomensis* Greeff, 1882	Zampala, São Thomé, West Africa	A. Gascoigne
Streptaxidae (outgroups)	*	*Gibbulinella dewinteri* Bank, Groh & Ripken, 2002	Puntas Coloradas, La Gomera Island	M. Ibañez
		Gonaxis quadrilateralis Preston, 1910	Reunion	O. Griffiths
		Gonospira sp.	Mauritius	O. Griffiths

** NHM-Natural History Museum, United Kingdom

DNA extraction

For all new specimens, tissue slices (approximately eight mm^3) from the foot muscle of the snail were obtained and the DNA was extracted using a CTAB DNA extraction method (Goodacre & Wade, 2001) with modifications. Ethanol-preserved tissue slices were soaked in 1 ml TE buffer (10 mM Tris-HCl, 1mM EDTA) for approximately one hour in order to remove excess ethanol to soften the tissue prior to DNA extraction. Tissue was then cut into small pieces, placed into 500 µl of CTAB solution [100mM Tris-HCl pH 8, 20mM EDTA pH 8, 1.4 mM NaCl, CTAB 2% (w/v)] and ground using sterile glass beads and a plastic pestle. 20 µl of Proteinase K (10mg/ml) was added to each tube followed by 10 µl of β-mercaptoethanol. The tubes were vortexed, then incubated at 55^0 C for at least one hour until the tissue slices were completely digested. 500 µl of ice-cold chloroform-isoamyl alcohol (24:1) was added, after which the tubes were inverted several times for 5 minutes. The tubes were then centrifuged for 10 minutes at 13,000 rpm, after which the aqueous phase was transferred to a new tube (~400 µl). 2.5 volumes (~1 ml) of ice-cold 95% ethanol and 1/10 volume (~40 µl) of 3M sodium acetate (NaOAc) were added to the mix followed by overnight incubation at -80^0 C. The tubes were centrifuged for 15 minutes at 13,000 rpm and the supernatant was carefully removed. The remaining pellets were washed with 500 µl of ice-cold 70% ethanol and centrifuged for five minutes at 13,000 rpm, after which excess ethanol was washed off. The remaining pellets were air-dried on a heat block at 45°C for a maximum of 15 minutes. The pellets were then re-suspended in 150 µl TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.5). Each suspension was boiled for 15 min at 100°C. The DNA extracts were stored at -80^0 C until use.

PCR amplification

Listed in Table 3 are the PCR components used and their concentrations. The optimal concentrations varied depending on the size of the gene and the number of copies available per cell. In particular, the concentration of $MgCl_2$ differed between the nuclear (1.5 mM) and the mitochondrial (2.5 mM) genes.

Data 3. PCR components used and their concentrations for the different genes

Components	Initial concentration	Final concentration		Volume (μl)	
Buffer	10X	1X		5.0	
dNTPs	1.25 mM	1^0 PCR for LSU: 300 μM	2^0 PCR for LSU/1^0 & 2^0 PCR for actin/ other genes: 200 μM	1^0 PCR for LSU: 12.0	2^0 PCR for LSU/1^0 & 2^0 PCR for actin/ other genes: 8.0
[a]$MgCl_2$	50 mM	LSU rRNA/ Actin/ Histone3: 1.5 mM	16S/ Cytochrome c oxidase I: 2.5 mM	LSU rRNA/ Actin/ Histone3: 1.5	16S/ Cytochrome c oxidase I: 2.5
Primer 1	10 mM	200 μM		1.0	
Primer 2	10 mM	200 μM		1.0	
[b]Q-solution	5X	1X		10	
Taq	1 unit/μl	0.01 unit/μl		0.5	
Sterile distilled H_2O	--	--		To make final volume of 50 μl	

[a]Some manufacturers provided PCR buffers that already contained $MgCl_2$ (e.g. QIAgen™ with 25 mM concentration), while other manufacturers separated the buffer and the $MgCl_2$ (e.g. Bioline™, which separately provided 50 mM $MgCl_2$). Care was taken in order to guarantee that the recommended final $MgCl_2$ concentration was followed.

[b]Provided by QIAgen™

Data 4. Primers used for PCR amplification.

	Primers	Reference	Fragment Size (bp)
Ribosomal Gene Cluster			
A	LSU-1ii (sense): 5'-CTAGCTGCGAGAATTAATGTGA-3' LSU-3ii (anti-sense): 5'-ACTTTCCCTCACGGTACTTG-3'	Wade & Mordan (2000); Wade et al. (2001); Wade *et al.* (2006)	~900-1200
	LSU-1iii (sense):5'-TGCGAGAATTAATGTGAATTGC-3' LSU-3iii (anti-sense): 5'-ACGGTACTTGTCCGCTATCG-3'	designed by C. Wade	
B	LSU-2ii (sense): 5'-GGGTTGTTTGGGAATGCAGC-3' LSU-5ii (anti-sense): 5'-GTTAGACTCCTTGGTCCGTG-3'	Wade & Mordan (2000); Wade et al. (2001); Wade et al. (2006)	~580
C	LSU-4ii (sense): 5'-GTCGGCATTCCACCCGACC-3' LSU-7 (anti-sense): 5'- GCAGGTGAGTTGTTACACACTC-3' LSU-7i (anti-sense): 5'-GTTGTTACACACTCCTTAGCGG-3'	designed by C. Wade	~700
D	LSU-6 (sense): 5'-AAGGTGCCAAACGCTGACGC-3' LSU-6ii (sense): 5'-GTGCCAAACGCTGACGCTCA-3' LSU-9 (anti-sense): 5'-CAGTCCTCAGAGCCAATCCTT-3' LSU-9ii (anti-sense): 5'-ACCCAGTCCTCAGAGCCAATC-3'	designed by C. Hudelot	~850
E	LSU-8 (sense): 5'-CCATATCCGCAGCAGGTCTC-3' LSU-8ii (sense): 5'-GTGCACAGCCTCTAGTCGATA-3' LSU-11 (anti-sense): 5'-CTGAGCTCGCCTTAGGACAC-3' LSU-11ii (anti-sense): 5'-TCCTCCTGAGCTCGCCTTAG-3'	designed by C. Hudelot	~850
F	LSU-10 (sense): 5'-ATCCGCTCTGAAGACAGTGTC-3' LSU-10i (sense): 5'-GGCCGCGATCCGTCTGAAGA-3' LSU-12 (anti-sense): 5'-TTCTGACTTAGAGGCGTTCAG-3' LSU-12i (anti-sense): 5'-GGCTTCTGACTTAGAGGCGTT-3'	designed by C. Hudelot	~500
Actin			
	ActF1 (sense): 5' –TATGTTGGTGATGAGGCTCAG-3'	Morgan et al. (2002)	~900
	ActF2 (sense): 5' –GGTATGGGTCAGAAGGACAGCTATG-3' ActR1 (anti-sense): 5' –GAAGCATTTCCTGTGGTCAATG-3' ActR2 (anti-sense): 5' –GATCCACATCTGTTGGAAGGT-3'	designed by C. Hudelot	
Histone 3			
	H3aF (sense): 5'-ATGGCTCGTACCAAGCAGACVGC-3' H3aR (anti-sense): 5'-ATATCCTTRGGCATRATRAGTGAC-3'	Colgan et al. (1998)	328
	H3Fm (sense): 5'-ATGGCTCGTACCAAGCAGAC-3' H3Fm1 (sense): 5'-ATGGCTAGAACGAAGCAGAC-3' H3Rm (anti-sense): 5'-TCCTTGGGCATGATGGTGAC-3' H3Rm1 (anti-sense): 5'-CCAACTGAATATCTTTGGGCAT-3'	designed by C. Hudelot	331-340
Cytochrome c oxidase subunit I			
	LCO 1490 (sense): 5'-GGTCAACAAATCATAAAGATATTGG–3' HCO 2198 (anti-sense): 5'-TAAACTTCAGGGTGACCAAAAAATCA–3'	Folmer et al. (1994)	655
	STY_LCOi (sense): 5'-TCAACGAATCATAAGGATATTGG-3' STY_LCOii (sense): 5'–ACGAATCATAAGGATATTGGTAC–3' STY_LCOiii (sense): 5'-TTTGGTATTTGATGTGGGTTAGT-3' STY_HCO (anti-sense): 5'-GAATTAAAATATATACTTCTGGGTG-3'	designed by I. Fontanilla	628-667

Different reaction cycles were employed depending on the size of the expected PCR product. For the rRNA gene cluster, two rounds of PCR were done. The primary PCR reaction made use of primers LSU-1 and LSU-12 or LSU-2 and LSU-12 and had the following running conditions: one round of 96 ^{O}C for two minutes followed by 35 cycles of 94 ^{O}C for 30 sec., 55 ^{O}C for 30 sec., and 72 ^{O}C for 5 mins. Using the PCR products from this round as template, another round of PCR was done for every rRNA gene cluster fragment using the following running conditions: one round of 94 ^{O}C for two minutes followed by 35 cycles of 94 ^{O}C for 30 sec., 45 ^{O}C for 30 sec., and 65 ^{O}C for 1 min. For all other genes, reaction cycles followed those used for the rRNA secondary PCR reactions. The amplified products were visualized on a 1.25% ethidium bromide stained agarose gel and excised using a sterile scalpel blade.

Sequence processing and phylogenetic analyses

Sequences were assembled using the STADEN package version 1.5.3 (Staden et al., 2000) and aligned manually within the Genetic Data Environment (GDE) Version 2.2 (Smith et al., 1994). The nuclear rRNA and the mitochondrial 16S rRNA sequences were aligned following alignments generated by C. Hudelot for the Stylommatophora based on the secondary structure of these genes.

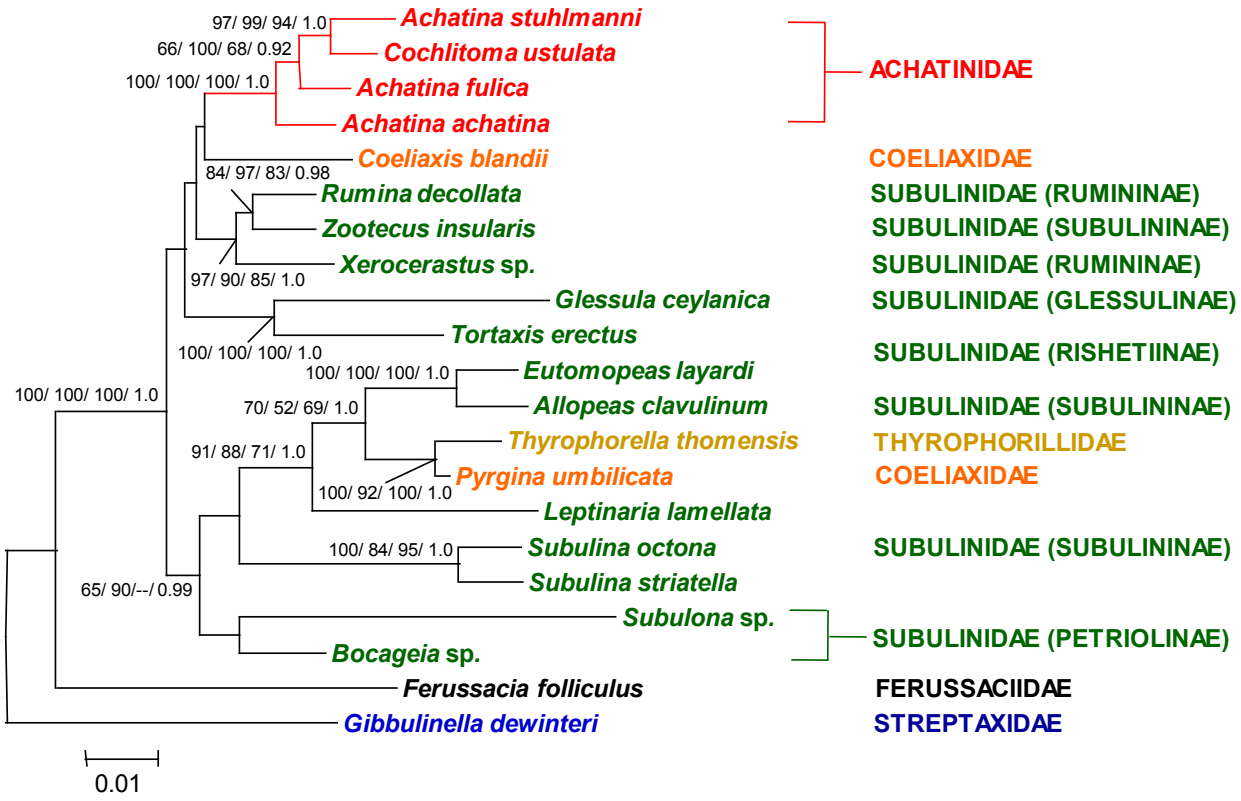
For each gene fragment, the numbers of variable and parsimony-informative sites were determined in PAUP* Version 4.0b10 package (Swofford, 2002). Corrected distances were computed after determination of the optimal model for DNA sequence evolution; the models evaluated were the JC69 (Jukes & Cantor, 1969), F81 (Felsenstein, 1981), K2P (Kimura, 1980), HKY85 (Hasegawa *et al.*, 1985), TN93 (Tamura & Nei, 1993) and the GTR (Rodriguez et al., 1990) as well as their variant that incorporated gamma distributed rates (Γ) (Yang, 1993). Their

likelihood scores were estimated in PAUP*, after which they were compared for significant differences using the Likelihood Ratio Test (LRT) (Huelsenbeck & Crandall, 1997).

Each gene fragment was also checked for oversaturation using the Xia Test in DAMBE (Xia et al., 2003). The genes were then tested to determine if they could be combined and analysed as a single dataset of concatenated sequences using a partition-homogeneity test (Cunningham, 1997) carried out within PAUP* (Swofford, 2002).

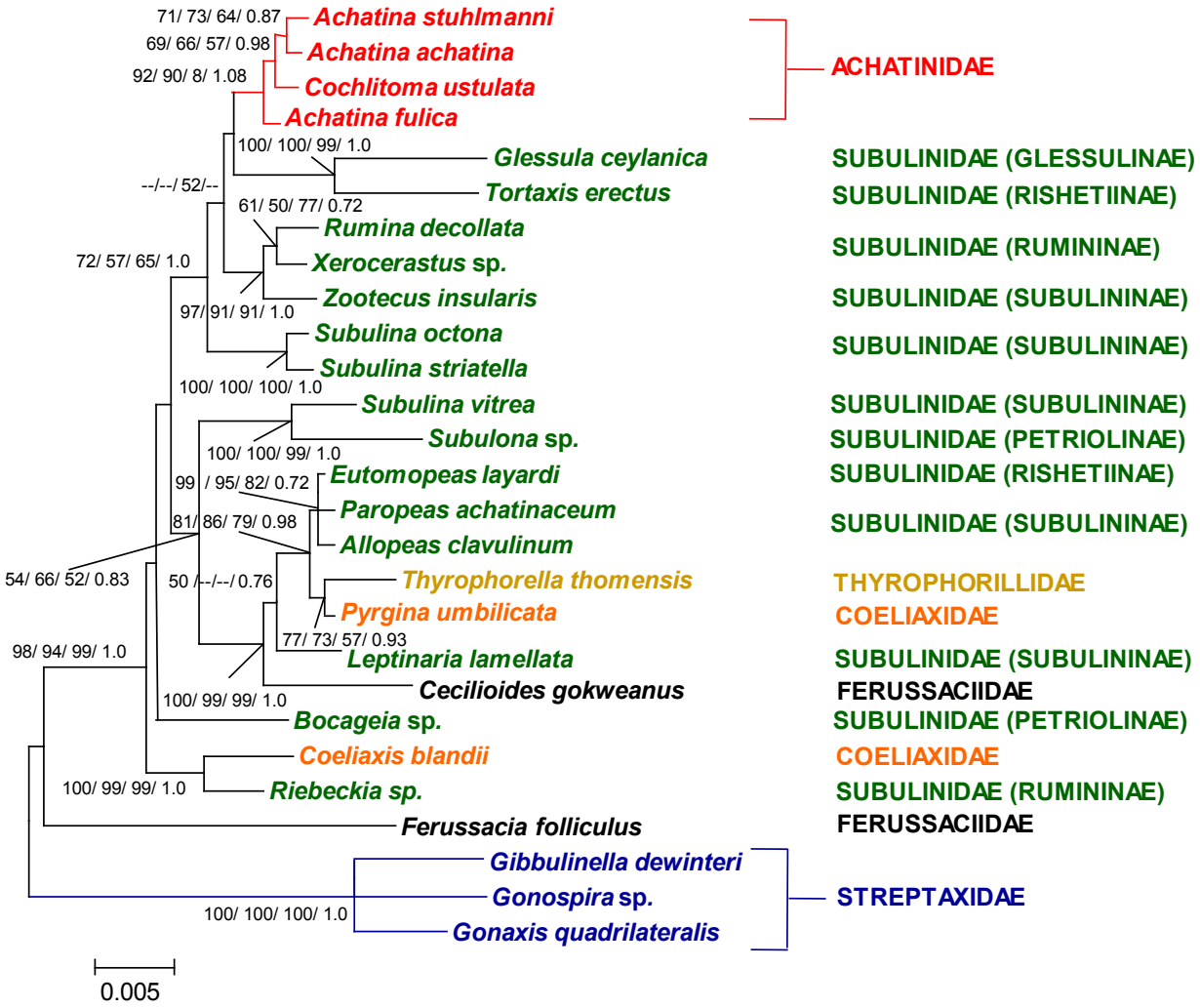
Phylogenetic trees of the concatenated dataset were generated using the model based maximum likelihood (ML, Felsenstein, 1981), Bayesian inference (BI, Rannala and Yang, 1996) and neighbor-joining (NJ, Saitou & Nei, 1987) methods and the non-model based maximum parsimony (MP, Fitch, 1977) method. The trees for NJ and MP were generated in PAUP* while that of ML was generated using PHYML Version 2.4.4 (Guindon et al., 2005). Bootstrap resampling (Felsenstein, 1985) with 1000 replicates for MP and NJ (using PAUP*) and 1000 replicates for ML (using PHYML) were also carried out. Bayesian inference (BI) was performed using the MrBayes (Version 3.1.2) package (Ronquist & Huelsenbeck, 2003) using four chains of a Markov Chain Monte Carlo algorithm and two million generations set on a heating temperature of 0.125 for the combined gene analysis. A consensus tree was constructed using the last 1000 trees.

Where taxonomic groups expected to be monophyletic based on current taxonomy did not cluster together in the optimal trees, their monophyly was tested using the Shimodaira-Hasegawa (1999) test in PAUP*.

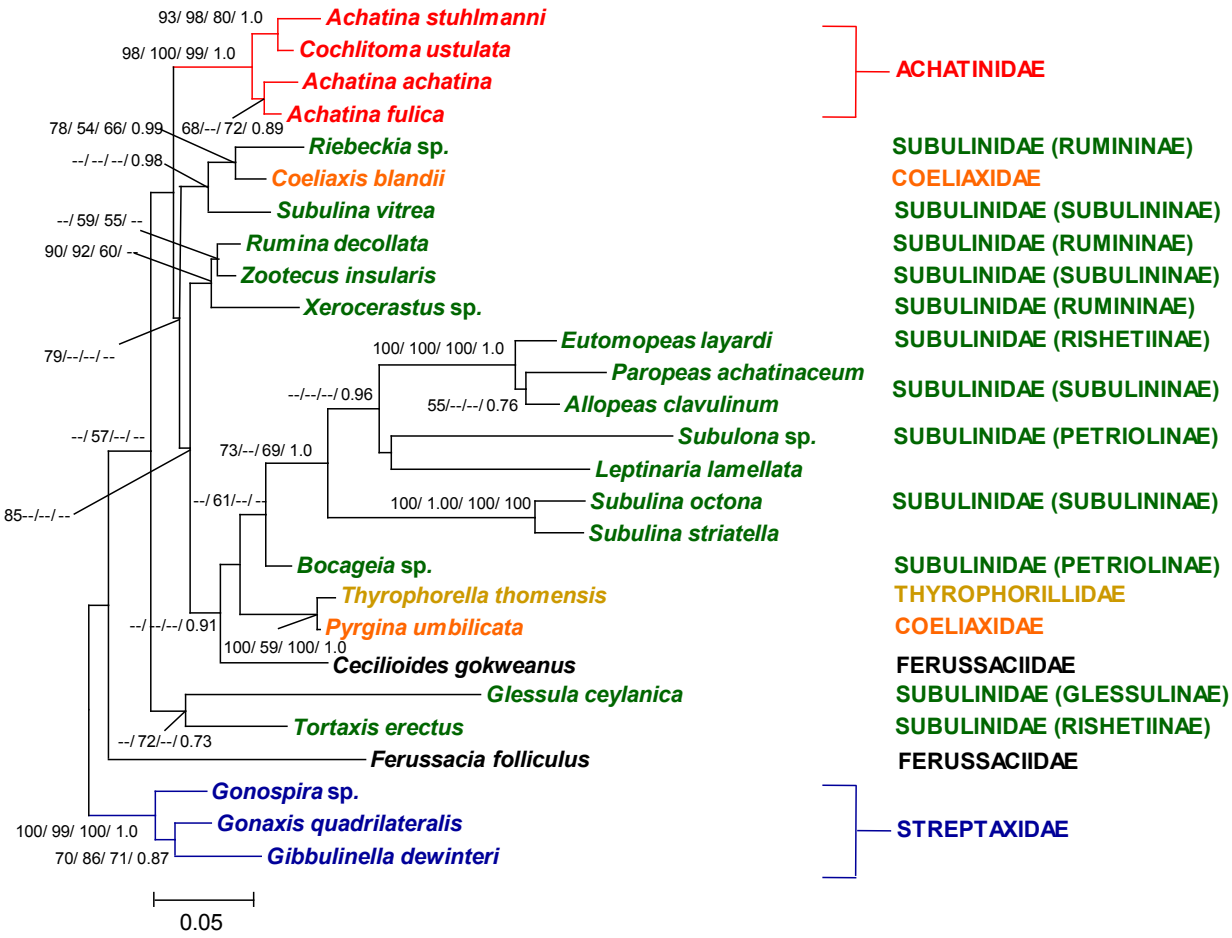


Data 5. Maximum likelihood phylogeny of the Achatinoidea based on a concatenated sequence of 5028 nucleotides from the combined dataset of the rRNA cluster, actin and H3 genes and the 1st and 2nd codon positions of the COI gene and including only those taxa with complete DNA sequence data. The phylogeny is rooted on the streptaxid *Gibbulinella dewinteri*. Values on nodes represent bootstrap support (1000 replicates) for ML, NJ and MP and posterior probabilities (based on last 1000 trees) for BI, respectively. Bootstrap support less than 50% and posterior probabilities less than 0.7 are not shown. For BI, the optimized number of generations to explore the tree space was 2,000,000 while the optimized temperature was 0.125. The scale bar represents 1 substitutional change per 100 nucleotides.

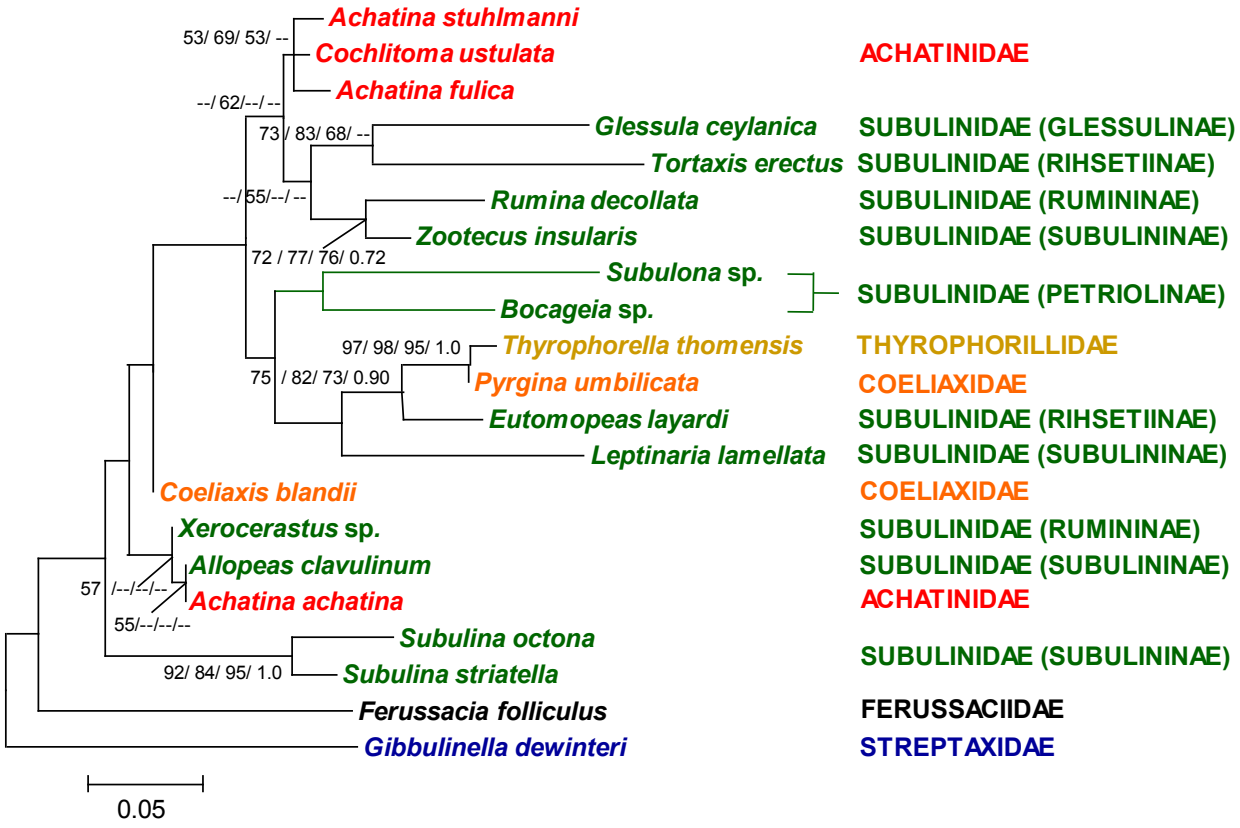
Data 6: (See next four pages). Maximum likelihood phylogenetic trees of the Achatinoidea based on (**a**) the rRNA cluster (3435 nucleotides); (**b**) actin gene (861 nucleotides); (**c**) the histone 3 gene (328 nucleotides); and (**d**) the 1st and 2nd codon positions of the COI gene (404 nucleotides). The phylogenies were rooted on the streptaxids *Gibbulinella dewinteri*, *Gonaxis quadrilateralis* and *Gonospira* sp for the rRNA and actin datasets and *Gibulinella dewinteri* for the histone 3 and COI datasets. Values on the nodes represent bootstrap support (1000 replicates) for ML, NJ and MP, and posterior probabilities (based on the last 1000 trees) for BI, respectively. Bootstrap support less than 50% and posterior probabilities less than 0.7 are not shown. For BI, the optimized number of generations to explore the tree space was 2,000,000 for all the genes; the optimized heating temperatures for each gene were as follows rRNA cluster-0.1; actin-0.1; histone 3-0.1; 1st and 2nd codon positions of COI-0.075. The scale bar for (**1**) represents 5 substitutional changes per 1000 nucleotide positions; those of (**2**), (**3**) and (**4**) represent 5 substitutional changes per 100 nucleotide positions.



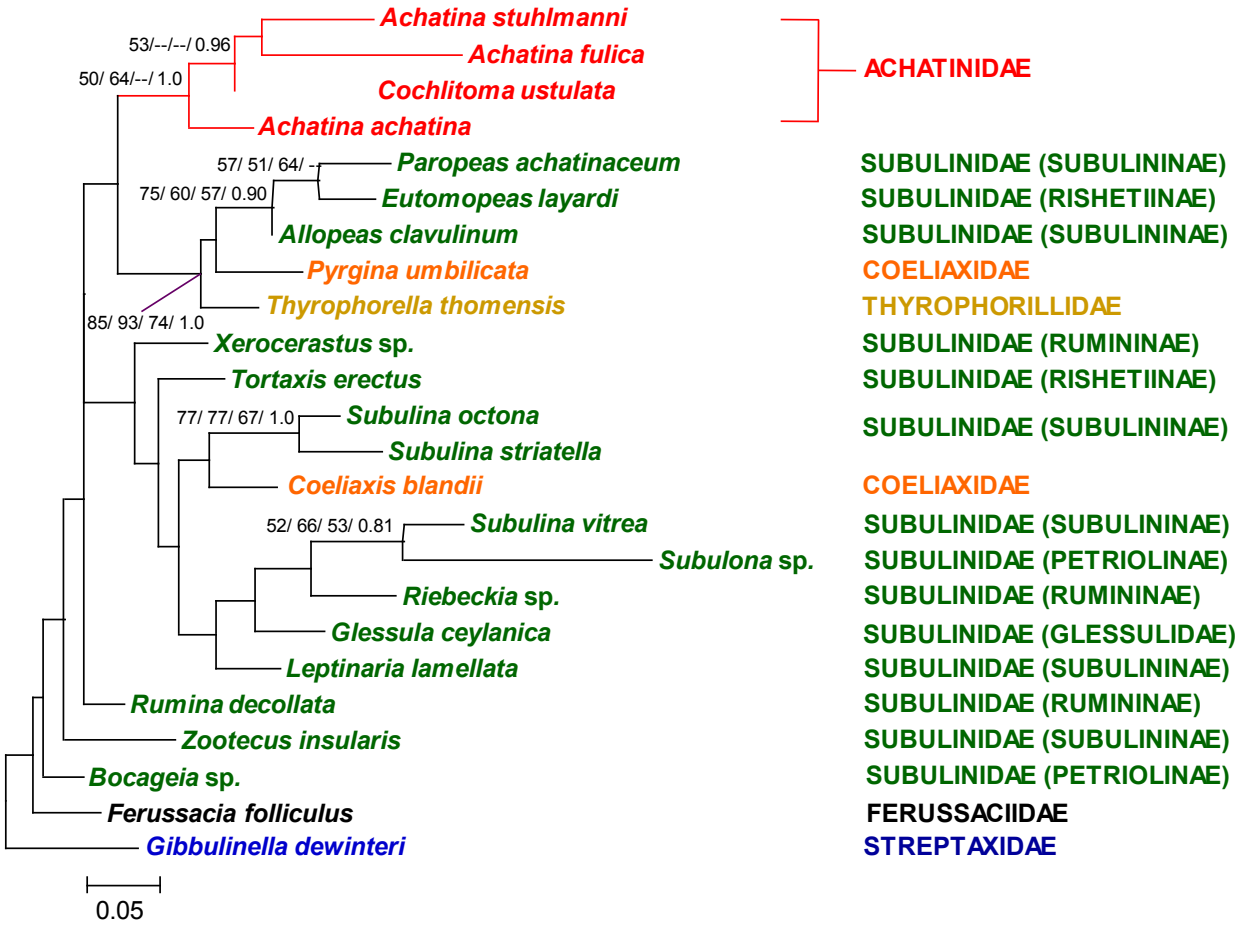
Data 6a. rRNA gene cluster phylogeny.



Data 6b. Actin gene phylogeny.



Data 6c. Histone 3 gene phylogeny.



Data 6d. COI 1st and 2nd codon positons phylogeny.

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