Molecular phylogeny of the Orthurethra (Panpulmonata: Stylommatophora)

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Received 16 May 2020; revised 27 September 2020; accepted for publication 6 November 2020

We have undertaken a molecular analysis of the Orthurethra, one of the major groups of stylommatophoran land snails and slugs. Approximately 4000 nucleotides of the rRNA gene cluster [5.8S, internal transcribed spacer 2 (ITS2) and almost the full-length large subunit (LSU; 28S) gene] were sequenced for 40 orthurethran genera belonging to 19 families. Our phylogeny recovers three well-supported clades within the Orthurethra; the Azecidae, Chondrinidae + Truncatellinidae, and a main clade comprising all remaining orthurethran families. The first division in the Orthurethra separates the Azecidae from all other orthurethran taxa. Of those families represented by more than one genus, the Achatinellidae, Azecidae, Cerastidae, Partulidae and Vertiginidae are recovered as strongly supported monophyletic units, whereas the Chondrinidae, Enidae, Pupillidae and Valloniidae are unsupported in the tree. Although there is relatively little support for the deep-level relationships among the main orthurethran groups, some groupings are strongly supported. The sister-group relationship of the Cochlicopidae with the Amastridae is strongly supported in our molecular analyses, and there is also some support for the grouping of the Orculidae with the Pyramidulidae, and the Draparnaudiidae with the Gastrocoptidae. The findings of our molecular analyses support dividing the Orthurethra into three superfamilies: the Azecoidea, Chondrinoidea and Pupilloidea.

ADDITIONAL KEYWORDS: classification – molluscs – ribosomal RNA – snails.

NTRODUCTION

The Orthurethra comprise approximately 6000 species, assigned to some 200 genera. The anatomy of the excretory system formed the basis of the classic Pilsbry–Baker system for the Stylommatophora (Pilsbry, 1900; Baker, 1955), with the Orthurethra characterized by having a simple, straight ureter that runs from the anterior end of the kidney towards the pneumostomal area of the lung, and opens as a backward-pointing pore. In most cases, a secondary structure is developed that varies from a short groove in many species, a short tube (e.g. *Acanthinula* Beck, 1847), a longer tube running most of the length of the kidney (e.g. *Abida* Turton, 1831) or, additionally, back along the rectum (the so-called pseudosigmurethrous condition found in *Amimopina* Solem, 1964). In this traditional classification, the Orthurethra are one of four primary divisions of the Stylommatophora. Pilsbry (1900) considered the Orthurethra to be a primitive group and ancestral to all other Stylommatophora. This was based on the belief that the orthurethran pallial system with its long kidney and direct ureter most closely resembled that of the supposedly more ancient Basommatophora, and this view has generally persisted (e.g. Schileyko, 1979; Nordsieck, 1986; Pokryszko, 1994). The shape of the ureter does not allow water reabsorption, which was suggested by Solem (1978) as the reason the Orthurethra does not contain any slugs.

The Orthurethra range from Europe and regions bordering the Mediterranean to Japan and have reached most of Africa, India, much of Asia, both North and South America, the islands of the Pacific and Australia. The four orthurethran families Achatinellidae, Amastridae, Draparnaudiidae and Partulidae are endemic to the islands of the Pacific. Many of these Pacific species have narrow distributional ranges and are under severe threat from habitat destruction and introduced predators; amastrids, which are endemic to Hawaii, have almost all become extinct, and the loss of numerous achatinellid and partulid taxa has been well documented (see: Cowie, 1992; Coote & Loeve, 2003; Holland & Hadfield, 2004; Régnier *et al*., 2015).

In his classic paper dealing with the affinities of the Pacific genera *Achatinella* Swainson, 1828 and *Partula* Férussac, 1821, Pilsbry (1900) recognized five families within his new group Orthurethra: the Achatinellidae, Partulidae, Pupidae (= Pupillidae) and, questionably, the Valloniidae and Cochlicopidae. Later he published a provisional list of subfamilies in his Pupillidae as follows: the Gastrocoptinae, Pupillinae, Pagodininae, Acanthulinae, Vertigininae, Orculinae and Strobilopsinae (Pilsbry, 1916–18). Subsequently, he added the Pagodulininae for two genera, *Spelaeodiscus* Brusina, 1886 and *Pagodulina* Clessin, 1876, which would not fit conveniently elsewhere (Pilsbry, 1922– 26). His work formed the basis of the Orthurethra as we know them today. At an early stage, Pilsbry (1916–18) admitted that his Pupillidae had been given wide limits, but Watson (1920) was to go much further

in suggesting that all but a few genera of orthurethrans should be combined into a single broad concept of the family Pupillidae.

In contrast, the detailed anatomical studies of Steenberg (1925) led him to recognize a total of 16 families in the Orthurethra as a whole. However, the limits of the group were not changed significantly, since most of these additional families resulted from the splitting of existing orthurethran groups, principally the Pupillidae *s.l.*. Anatomical support for the various family-group taxa within the Orthurethra has always been poor. Baker (in: Pilsbry, 1927–35) gave a comprehensive review of the limited anatomical information available at the time, but concluded that in respect of the Pupilloidea: 'any deductions based on the animal alone would be weak and this would be especially true of a group in which the shell characters... appear manifest while those of the soft parts are difficult to study and more so to evaluate'. As Nordsieck (1986) pointed out, the relationships within the Orthurethra established by Pilsbry and Baker (Pilsbry, 1927–35) are essentially based on conchological characters, which he believed overvalued the importance of the shell. This situation changed little until the work of Tillier (1989) who surveyed a fresh suite of characters of the pallial, nervous and alimentary systems. Tillier (1989) and Barker (2001) represent the only two attempts at a morphological phylogenetic analysis of the Orthurethra. Tillier considered the Orthurethra to be monophyletic and the sister-group of all other Stylommatophora (Tillier, 1989; Emberton & Tillier, 1995). Although Tillier's tree divided the Orthurethra into two clades, his classification recognized three superfamilies: the Pupilloidea, Chondrinoidea and Partuloidea. However, Tillier (1989) considered his own classification of the Orthurethra to be unsatisfactory. In Barker's unconstrained analysis of the Stylommatophora (including 12 orthurethran families) based on 57 mainly morphological characters, the orthurethrans fell into two distinct clades, each showing relationship with non-orthurethran families: one of these clades included the Sphincterocheilidae and Urocoptidae, the second included the Corillidae and Acavidae. These results appear to be even less satisfactory than Tillier's classification. In the classification of Bouchet & Rocroi (2005), the Orthurethra are considered as an informal group and subclade of the Stylommatophora, with the Orthurethra partitioned into five superfamilies: the Partuloidea, Achatinelloidea, Cochilicopoidea, Pupilloidea and Enoidea, and 20 families, one of which, the Cylindrellinidae, is extinct. Most recently, Bouchet *et al*. (2017) revised the earlier Bouchet & Rocroi (2005) classification, recognizing a total of 26 families placed within a single superfamily, the Pupilloidea '(making Orthurethra and Pupilloidea synomyms)'. Several molecular studies of the Orthurethra have been conducted. Most of them focused on the phylogeography of a particular genus (Goodacre & Wade, 2001a; Goodacre, 2002; Tongkerd *et al*., 2004; Ketmaier *et al*., 2006; Sischo & Hadfield, 2017) or within a family (Goodacre & Wade, 2001b; Holland & Hadfield, 2004; Lee *et al*., 2009, 2014; Slapcinsky & Kraus, 2016; Köhler *et al*., 2017). The first comprehensive molecular studies of the Orthurethra aimed to solve both the relationships within the Orthurethra and to place them within the stylommatophoran phylogeny as a whole (Wade *et al*., 2001, 2006). However, these phylogenies

were based on only 823 nucleotides of the rRNA gene cluster and the resulting orthurethran tree was poorly resolved and, except between closely related taxa, support for groupings was relatively low. The monophyly of the Orthurethra was recovered and the Orthurethra fell as an apparently derived group within the 'non-achatinoid' clade, and not as a primitive group at the base of the Stylommatophora, as was generally believed (Wade *et al*., 2001, 2006; Mordan & Wade, 2008). Armbruster *et al*. (2005) used histone genes to analyse the relationships of 13 stylommatophoran genera (including nine orthurethran genera). The Orthurethra were not recovered as a monophyletic group in their study, and three orthurethran families, the Cochlicopidae, Vertiginidae and Valloniidae, were paraphyletic in their tree. Madeira *et al*. (2010) examined the phylogenetic position of the orthurethran genera *Azeca* Fleming, 1828, *Cryptazeca* Folin & Bérillon, 1877 and *Hypnophila* Bourguignat, 1858, essentially incorporating these taxa within the Wade *et al.* (2006) rRNA sequence dataset. Their analysis provided strong support for a monophyletic clade comprising *Azeca*, *Cryptazeca* and *Hypnophila*,

which led them to propose a new family, the Azecidae. Nekola & Coles (2016) undertook a molecular analysis aimed at elucidating the supraspecific taxonomy of the Vertiginidae, but that also included a number of other orthurethran sequences, some new and some from earlier studies, principally those of Wade *et al*. (2001, 2006). Their phylogenetic conclusions were essentially based on 28S sequences of some 800 base pairs, and replicated many of the results of Wade *et al*. (2001, 2006); a 'vertiginid' clade was recovered that broadly corresponded with the Vertiginidae as outlined in Bouchet & Rocroi (2005). The overall conclusion was that their results 'strongly suggest that formal reconsideration of supraspecific concepts across the entire infraorder are warranted, based upon DNA sequence data'. *Truncatellina* Lowe, 1852 and *Columella* Westerlund, 1878 were excluded from the Vertiginidae as they cluster with the Chondrinidae. Finally, the molecular study of Harl *et al*. (2017) targeted the family Orculidae, but included many non- orculid taxa; whilst clarifying the monophyly of the orculids, their other main contribution was the erection of two new orthurethran families: the Agardhiellidae and Fauxulidae.

Here we present the results of a molecular analysis that includes the sequences of 40 genera representing 19 families of Orthuretha, for approximately 4000 nucleotides of the ribosomal (r) RNA gene cluster [including part of the 5.8S gene, the complete internal transcribed spacer 2 (ITS2) region and almost the full- length large subunit (LSU; 28S) gene], and discuss the observed patterns of relationship within the group.

MATERIAL AND METHODS

Biological material

A total of 40 genera, representing 19 orthurethran families, were included in this study. Details of the specimens, sampling localities and collectors are given in Table 1.

Table 1. Details of specimens, sampling localities and collectors. Family-level classification of samples follows Bouchet *et al*. (2017).

Family	**Species**	**Collection Location**	**Collector**
Orthurethran Taxa			
Achatinellidae	*Elasmias luakahaense* Pilsbry and Cooke, 1915	Koolau Range, Oahu, Hawaii	R. Rundell & K. Olival
	Partulina proxima (Pease, 1862)	Maui, Hawaii	B. Holland
Cochlicopidae	*Cochlicopa lubricella* (Porro, 1838)	São Miguel, Azores	P. Mordan
Amastridae	*Leptachatina lepida* Cooke, 1910	Hawaii Island, Hawaii	P. Mordan & R. Cowie
Pupillidae	*Pupoides albilabris* (Adams, 1841)	Wilson County, Tennesse, USA	J. Slapcinsky & B. Coles
	Pupilla muscorum (Linnaeus 1758)	Garden, Victoria Grove, London, UK	Ellen Graubart
Lauriidae	*Lauria cylindracea* (da Costa, 1778)	Mullaghmore, Co. Sligo, Ireland	E. Platts
Valloniidae	*Vallonia excentrica* Sterki, 1892	São Miguel, Azores	P. Mordan
	Acanthinula aculeata (Müller, 1774)	Box Hill, Surrey, UK	J. Ablett
Vertiginidae	*Vertigo antivertigo* (Draparnaud, 1801)	Chuett, Arnoldstein, Austria	P. Miltner
	Pronesopupa acanthinula (Ancey, 1892)	Koolau Range, Oahu, Hawaii	R. Rundell & K. Olival
Gastrocoptidae	*Gastrocopta armifera* (Say, 1821)	Wilson County, Tennesse, USA	J. Slapcinsky & B. Coles
Orculidae	*Orcula austriaca* Zimmerman, 1932	Kuhberg, Austria	P. Miltner
Strobilopsidae	*Eostrobilops nipponica* (Pilsbry, 1908)	Osaka, Japan	I. Matsumura
Pyramidulidae	*Pyramidula rupestris* (Draparnaud, 1801)	Mullaghmore, Co. Sligo, Ireland	E. Platts
Chondrinidae	*Chondrina clienta* (Westerlund, 1883)	Villach, Austria	P. Miltner
	Solatopupa similis (Bruguière, 1792)	Verdon Gorge, France	A. Davison
	Abida secale (Draparnaud, 1801)	Pulpit Down, Buckinghamshire, UK	P. Mordan
	Granaria frumentum (Draparnaud, 1801)	Pelsivec Plateau, Slovakia	J. Grego
Enidae	*Buliminus labrosus* (Olivier, 1804)	Saladin's Castle, Syria	P. Mordan
	Pene sidonensis (Férussac, 1821)	Saladin's Castle, Syria	P. Mordan
	Luchuena reticulata (Reeve, 1849)	Kikai Island, Ryukyu, Japan	S. Chiba
	Napaeus pruninus (Gould, 1846)	São Miguel, Azores	A. Polasczek
	Macaronapaeus vulgaris (Morelet & Drouet, 1857)	São Miguel, Azores	P. Mordan
	Ena obscura (Müller, 1774)	Biela voda, Olcnava, Slovakia	J. Steffek

	Mastus pupa (Bruguière, 1792)	Sicily, Italy	A. Davison
	Mirus stalix (Benson, 1863)	Agra, Sri Lanka	D. Raheem
	Chondrula albolimbata (Pfeiffer, 1848)	Spissky tlrad, Slovakia	J. Grego
Cerastidae	*Cerastus schweinfurthi* (Martens, 1895)	Al-Mahuit, N. Yemen	P. Mordan
	Pachnodus silhouettanus van Mol & Coppois, 1980	Silhouette Island, Seychelles	J. Gerlach
	Amimopina macleayi (Brazier, 1876)	Queensland, Australia	P. Mordan
Draparnaudiidae	*Draparnaudia singularis* Reeve, 1854	Mont Koghis, New Caledonia	C. Wade & K. Bowman
Partulidae	*Samoana conica* (Gould, 1847)	Samoa	R. Cowie
	Eua zebrina (Gould, 1847)	Samoa	R. Cowie
	Partula tohiveana Crampton, 1924	Moorea	Unknown
Azecidae	*Azeca goodalli* (A. Férussac, 1821)	Gorges de Kakuetta, Pyrenées-Atlantiques, France	Provided by B. Gomez
	Cryptazeca monodonta (de Folin & Bérillon, 1877)	Gorges de Kakuetta, Pyrenées-Atlantiques, France	Provided by B. Gomez
	Hypnophila boissii (Dupuy, 1851)	Tortella, Garrotxa, Girona, Spain	Provided by B. Gomez
Argnidae	*Argna bielzi* (Rossmässler, 1859)	Lacu Roşu river, Gheorgheni, Harghitaa, Romani, coordinates: 46°47'39.1"N 25°47'46.7"E	Provided by the Natural History Museum Vienna (NHM Vienna), NHMW109000/ AL/00460/7026
Truncatellinidae	*Columella columella* (G. von Martens, 1830)	Niederösterreich Sierningtal, Austria, coordinates: 47°45.421′N, 15°58.99′E	Provided by NHM Vienna, NHMW109000/ AL/00168/5439
Non Orthurethran Taxa			
Ferussaciidae	*Ferussacia folliculus* (Gmelin, 1791)	Los Alcornales, Prov Cadiz, Spain	M. Seddon
Achatinidae	*Lissachatina fulica* (Bowdich, 1822) [= *Achatina fulica*]	Unknown (Zool. Soc. Lond. colln.)	P. Pearce-Kelly
Streptaxidae	*Gonaxis quadrilateralis* (Preston, 1910)	Réunion	O. Griffiths

DNA EXTRACTION, POLYMERASE CHAIN REACTION (PCR) AMPLIFICATION AND SEQUENCING

DNA was extracted from a small (1–2 mm^3) tissue sample taken from the foot of the snail or the whole snail (for small specimens) using a CTAB DNA extraction protocol (Goodacre & Wade, 2001b). Approximately 4000 nucleotides of the rRNA gene cluster [including part of the 5.8S gene, the complete

ITS2 region and almost the full-length large subunit (LSU; 28S) gene] were sequenced using a nested PCR approach, with the product of the primary PCR being used as a template for the secondary PCR. The primary PCR was performed using the primers LSU-1ii and LSU-12

followed by secondary PCR of six internal fragments (A, B, C, D, E and F) (see Table 2 for details of primers). Polymerase chain reaction amplification for the primary PCR was performed using the Qiagen Taq DNA polymerase and Q buffer system (1X buffer, 1X Q-solution, 0.3 mM dNTP, 1.5 mM magnesium chloride, 0.2 µM each primer and 1U Taq in a 50 µL final volume). Secondary PCR amplification was identical to the primary PCR, with the exception that a lower 0.2 mM concentration of dNTPs was used. 1 µL of the primary PCR product was used as the template in the secondary PCR. The cycling conditions (with a Perkin Elmer cycler) of the primary PCR were as follows: 96 °C for 2 min, followed by 35 cycles of 94 °C for30s,50°Cfor30s,72°Cfor3minandthena final extension step at 72 °C for 5 min. The secondary PCR cycle conditions were as follows: 96 °C for 2 min, followed by 35 cycles of 94 °C for 30 s, 45 °C for 1 min, 72 °C for 2 min and then a final extension step at 72 °C for 5 min. Amplification products were purified from an agarose gel using a Qiagen gel extraction kit. Both sense and antisense strands were sequenced directly on an Applied Biosystems 3730 DNA sequencer using Big Dye terminator cycle sequencing chemistries at Macrogen Inc.

Table 2. Primers used for PCR amplification.

Fragments	Primers	Reference	Fragment Size (bp)
Primary PCR	LSU-1ii (sense): 5'-CTAGCTGCGAGAATTAATGTGA-3' [Labelled as Primer LSU-1 in Wade *et al*. (2006)]	Wade & Mordan (2000); Wade *et al.* (2001, 2006)	~4000
	LSU-12 (anti-sense): 5'-TTCTGACTTAGAGGCGTTCAG-3'	Fontanilla *et al.* (2017); Saadi & Wade (2019)	
A	LSU-1ii (sense): 5'-CTAGCTGCGAGAATTAATGTGA-3' (Labelled as Primer LSU-1 in Wade et al. (2006)] Or LSU-1iii (sense): 5'-TGCGAGAATTAATGTGAATTGC-3'	Wade & Mordan (2000); Wade *et al.* (2001, 2006) Fontanilla *et al.* (2017); (Saadi *et al*. (2020)	~900-1200
	LSU-3ii (anti-sense): 5'-ACTTTCCCTCACGGTACTTG-3' [Labelled as Primer LSU-3 in Wade et al. (2006)] Or LSU-3iii (anti-sense): 5'-ACGGTACTTGTCCGCTATCG-3'	Wade & Mordan (2000); Wade *et al.* (2001, 2006) Fontanilla *et al.* (2017); (Saadi *et al*. (2020)	
B	LSU-2ii (sense): 5'-GGGTTGTTTGGGAATGCAGC-3' [Labelled as Primer LSU-2 in Wade *et al.* (2006)]	(Wade & Mordan (2000); Wade *et al.* (2001, 2006)	~580
	LSU-5ii (anti-sense): 5'-GTTAGACTCCTTGGTCCGTG-3' [Labelled as Primer LSU-5 in Wade *et al*. (2006)]	Wade & Mordan (2000); Wade *et al.* (2001, 2006)	
C	LSU-4ii (sense): 5'-GTCGGCATTCCACCCGACC-3' Or LSU-4iii (sense): 5'-CGGTGGCGAGTCTGTCGGC-3'	(Fontanilla *et al.* (2017); Saadi & Wade (2019) (Saadi *et al*. (2020)	~700
	LSU-7 (anti-sense): 5'- GCAGGTGAGTTGTTACACACTC-3' LSU-7i (anti-sense): 5'-GTTGTTACACACTCCTTAGCGG-3'	(Fontanilla *et al.* (2017); Saadi & Wade (2019) (Fontanilla *et al*. (2017); (Saadi *et al*. (2020)	

D	LSU-6i (sense): 5'-GTGCCAAACGCTGACGCTCA-3'	Fontanilla *et al.* (2017); Saadi & Wade (2019)	~850
	LSU-9i (anti-sense): 5'-ACCCAGTCCTCAGAGCCAATC-3'	Fontanilla *et al.* (2017); Saadi & Wade (2019)	
E	LSU-8ii (sense): 5'-GTGCACAGCCTCTAGTCGATA-3'	Fontanilla *et al.* (2017); Saadi & Wade (2019)	~850
	LSU-11ii (anti-sense): 5'-TCCTCCTGAGCTCGCCTTAG-3'	Fontanilla *et al.* (2017); Saadi & Wade (2019)	
F	LSU-10i (sense): 5'-GGCCGCGATCCGTCTGAAGA-3'	Fontanilla *et al.* (2017); Saadi & Wade (2019)	~550
	LSU-12i (anti-sense): 5'-GGCTTCTGACTTAGAGGCGTT-3'	Fontanilla *et al.* (2017); Saadi & Wade (2019)	

SEQUENCE PROCESSING AND PHYLOGENETIC ANALYSES

DNA sequences were assembled using the STADEN package v.1.5.3 (Staden *et al*., 2000) and aligned using v.2.2 of the Genetic Data Environment (GDE) package (Smith *et al*., 1994). The sequences were aligned manually using the secondary structure as a guide. GBlocks v.0.91b (Castresana, 2000) with default settings was used as a guide to select the reliably aligned nucleotide sites.

Phylogenetic trees were constructed using maximum likelihood (ML) (Felsenstein, 1981), neighbour-joining (NJ) (Saitou & Nei, 1987) and Bayesian inference (BI) (Larget & Simon, 1999). The general time-reversible model incorporating gamma (GTR+Γ) (Lanave *et al*., 1984; Gu *et al*., 1995) was used to correct for multiple substitutions for all model-based methods (ML, NJ and BI). Maximum likelihood trees were constructed using the PhyML (v.3.0) package (Guindon *et al*., 2010) with tree searching following a heuristic procedure with ten random start trees and best of nearest-neighbour- interchange and subtree-pruning-regrafting branch- swapping. Neighbour-joining analysis was performed using the PAUP* (v.4.0b10) package (Swofford, 2002). For NJ analysis, model parameters were estimated following an iteration process; for each tree, the parameters were estimated and used to build the next tree until there was no further improvement of the likelihood score. Bootstrap resampling (Felsenstein, 1985) with 1000 replicates was undertaken for ML and NJ trees. Bayesian inference analysis was undertaken using the MrBayes (v.3.1.2) package (Ronquist & Huelsenbeck, 2003). Two independent runs with four chains of a Markov chain Monte Carlo (MCMC) algorithm were used to explore the tree space. Bayesian inference analysis was conducted for 5 million generations with tree sampling every 100 generations. To ensure adequate chain-swapping, a range of heating parameters was tested with the optimal parameter used to construct the final trees. Only after the Bayesian MCMC searches had reached a stationary phase (indicating convergence of the chains onto the target distribution) was the run ended. A consensus tree was built using the last 75% of trees (burn-in = 12 501). Alternative phylogenetic hypotheses were evaluated using a Shimodaira–Hasegawa RELL test (Shimodaira & Hasegawa, 1999) as implemented in the PAUP* (v.4.0b10) package (Swofford, 2002). Branches

supported with bootstrap values ≥ 70% and Bayesian posterior probabilities ≥ 0.9 are considered to be well supported.

NUCLEOTIDE SEQUENCE ACCESSION NUMBERS

Nucleotide sequences generated in this study have been given the GenBank accession numbers MT862218– MT862253. Accession numbers of the previously published orthurethran taxa were taken from Davison *et al*. (2016): *Cochlicopa lubricella* (Rossmässler, 1835) = KU341313 and from Saadi & Wade (2019): *Buliminus labrosus* (Olivier, 1804) = MN022658, *Chondrina clienta* (Westerlund, 1883) = N022657 and *Vallonia excentrica* Sterki, 1893 = MN022656. Non orthurethran sequences were taken from Fontanilla *et al*. (2017): *Ferussacia folliculus* (Gmelin, 1791) = MF444871, *Gonaxis quadrilateralis* (Preston, 1910) = MF444893), *Lissachatina fulica* (Bowdich, 1822) = MF444864.

RESULTS

Sequences of ~4000 nucleotides of the rRNA gene cluster were generated for 40 orthurethran genera. Phylogenetic trees for the Orthurethra were constructed using 3370 unambiguously aligned nucleotide sites of the LSU and 5.8S gene. The ITS2 region of the rRNA cluster was removed completely from all analyses as it could not be aligned across all taxa due to its high variability. The 'achatinoid' clade (Wade *et al*., 2001, 2006), represented by *Ferussacia folliculus*, *Gonaxis quadrilateralis* and *Lissachatina fulica*, was used as an outgroup to root the phylogenetic trees.

The Bayesian inference tree of the Orthurethra is shown in Figure 1. Branches supported in ≥ 50% of bootstrap replicates and with Bayesian posterior probabilities (PP) ≥ 0.70 are consistent across all phylogeny reconstruction methods (BI, Fig. 1; ML, Supporting Information, Fig. S1; NJ, Supporting Information, Fig. S2). The Orthurethra form a well-resolved monophyletic group in the tree fully supported in all three analyses (100% ML, 100%

NJ bootstrap replicates and PP = 1.00 BI). They are dividedintothreemaingroups:theAzecidaesupported in 100% ML, 99% NJ bootstraps and PP = 1.00 BI, the Chondrinidae + Truncatellinidae supported in 80% ML, 78% NJ bootstraps and PP = 1.00 BI, and a main clade comprising the remaining orthurethran taxa (shaded area on tree, Fig. 1) fully supported in 100% ML, 100% NJ bootstraps and PP = 1.00 BI. The first division in the Orthurethra separates the Azecidae from all remaining orthurethran families and is well supported in 74% ML, 92% NJ bootstraps and PP = 0.95 BI. The Azecidae is strongly supported as a monophyletic group in the tree (100% ML, 99% NJ bootstraps and PP = 1.00

BI) and within the Azecidae there is strong support for the sister-relationship between *Cryptazeca monodonta* (de Folin & Bérillon, 1877) and *Hypnophila boissii* (Dupuy, 1851) (97% ML, 86% NJ

bootstraps and PP = 1.00 BI). The second division in the Orthurethra separates the Chondrinidae + Truncatellinidae from the main clade (shaded area on tree, Fig. 1). The Chondrinidae + Truncatellinidae form a well-supported group (80% ML, 78% NJ bootstraps and PP = 1.00 BI) within which *Abida secale* (Draparnaud, 1801) and *Chondrina clienta* cluster together as sister-taxa with full support and *Granaria frumentum* (Draparnaud, 1801) and *Solatopupa similis* (Bruguière, 1792) cluster as sister-taxa with strong support (99% ML, 100% NJ bootstraps and PP = 1.00 BI).

The main clade in the orthurethran tree (shaded area on tree, Fig. 1) is fully supported in all three analyses but the phylogenetic relationships within this clade are generally poorly resolved, with just a few strongly supported clades. The Cerastidae is well supported (85% ML, 91% NJ bootstraps and PP = 1.00 BI) and within the Cerastidae there is strong support for the sister-group relationship between *Cerastus schweinfurthi* (Martens, 1895) and *Pachnodus silhouettanus* Van Mol & Coppois, 1980 (94% ML, 90% NJ bootstraps and PP = 1.00 BI). The Partulidae form a fully supported clade in all three analyses and within the Partulidae, the sister-group relationship between *Partula tohiveana* Crampton, 1924 and *Samoana conica* (Gould, 1847) is strongly supported (86% ML, 86% NJ bootstraps and PP = 0.96 BI). The Vertiginidae is strongly supported in the tree (95% ML, 99% NJ and PP = 1.00 BI) and the Achatinellidae is fully supported in all three analyses.

The sister-group relationship between theAmastridae (*Leptachatina lepida* Cooke, 1910) and Cochlicopidae [*Cochlicopa lubricella* (Porro, 1838)] is strongly supported in 99% ML, 98% NJ bootstraps and PP = 1.00 BI. Likewise, the sister-group relationship between *Pupilla muscorum* (Linnaeus, 1758) (Pupillidae) and *Lauria cylindracea* (da Costa, 1778) (Lauriidae) is fully supported in all three analyses. There is also a sister- group relationship between *Gastrocopta armifera* (Say, 1821) (Gastrocoptidae) and *Draparnaudia singularis* Reeve, 1854 (Draparnaurdiidae), although with weak support (53% ML, 65% NJ bootstraps and PP = 0.73 BI). Finally, the sister-group relationship between *Orcula austriaca* Zimmerman, 1932 (Orculidae) and *Pyramidula rupestris* (Draparnaud, 1801) (Pyramidulidae) is also supported in the tree, although in only 55% ML and 69% NJ bootstraps and with a Bayesian posterior probability of 0.99.

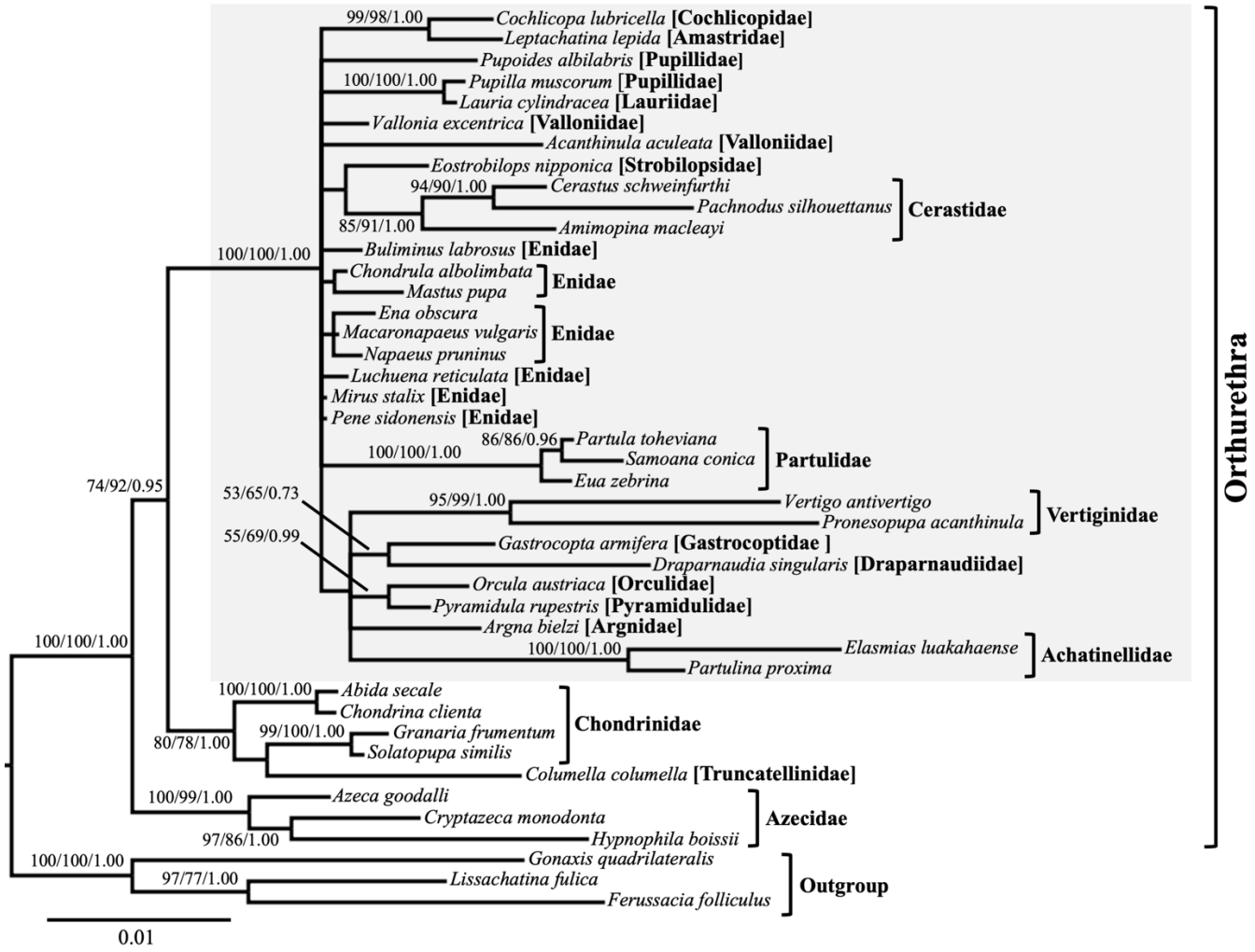

Figure 1. Bayesian phylogenetic tree of the Orthurethra based on 3370 unambiguously aligned nucleotide sites of the LSU rRNA (and 5.8S) gene. Values on nodes represent bootstrap support for maximum likelihood and neighbour-joining (1000 replicates) and posterior probabilities for Bayesian inference (based on the last 75% of trees), respectively. Bootstrap support values < 50% and posterior probabilities < 0.7 are not shown. The scale bar represents one substitutional change per 100 nucleotide positions. The shaded area represents the main clade.

DISCUSSION

Within the orthurethran tree presented here, the phylogenetic signal is strong where it exists: the resolved groupings are strongly supported, and the unresolved ones are hardly supported at all. These areas of the tree lacking resolution reflect the extreme difficulty that morphological taxonomists have encountered over the years in classifying and understanding relationships within the Orthurethra. Our phylogeny recovers three well-supported clades within the Orthurethra: the Azecidae, the Chondrinidae

+ Truncatellinidae, and a main clade comprising the remaining orthurethran families. These findings are consistent with those of Madeira *et al*. (2010), who also recovered three clades within the Orthurethra: the Azecidae, Chondrinidae and a main clade comprising the remaining orthurethran taxa; but the relationships between these clades were unresolved in Madeira's tree. In our study, we found a principal division between the Azecidae and all other orthurethran taxa followed by a subsequent division within this latter clade between the Chondrinidae + Truncatellinidae and the remaining orthurethran families. Wade *et al*. (2001, 2006) did not include the Azecidae and Truncatellinidae in their analyses but showed a principal division within the Orthurethra between the Chondrinidae and the remaining orthurethran taxa consistent with the results of our study. Likewise, Nekola & Coles (2016) and Harl *et al*. (2017) showed a principal division within the Orthurethra between the Chondrinidae + Truncatellinidae and the remaining orthurethran taxa. Again their findings are consistent with the results of this study in respect of the Chondrinidae and Truncatellinidae, but neither Nekola & Coles (2016) nor Harl *et al*. (2017) included the Azecidae in their analyses.

Of the 26 families recognized in the classification of Bouchet *et al*. (2017), 19 are represented in our tree, more than in any other molecular analysis. A number of interesting points have emerged and are discussed below.

AZECIDAE

The Azecidae have a distribution centred on southern Europe, with only a few species extending into northern Europe and north Africa. There is a clear sister-group relationship between the Azecidae on one hand, and a clade comprising all remaining orthurethran taxa on the other, in our orthurethran tree. Based on morphological studies, the taxonomic position of the azecid taxa, *Azeca*, *Cryptazeca* and *Hypnophila*, remain unclear. *Azeca*, *Cryptazeca* and *Hypnophila* were originally included in the family Ferussaciidae (Pilsbry, 1908). Steenberg (1925) placed *Azeca*, *Hypnophila* and *Cochlicopa* within the family Cochlicopidae, with this later adopted in the taxonomy of Zilch (1959–60). Schileyko (1976) placed *Cryptazeca* within the family Ferussaciidae. Gomez & Angulo (1987) proposed a new taxonomic position for *Cryptazeca* based on the anatomy of the excretory system in which *Cryptazeca* was placed within the Cochlicopidae alongside *Azeca*, *Hypnophila* and *Cochlicopa*, with this later adopted by Vaught (1989) and Bouchet & Rocroi (2005). In the molecular study of Armbruster *et al*. (2005), a single azecid taxon [*Azeca goodalli* (A. Férussac, 1821)] was included, and this was shown to be sister to a clade comprising *Truncatellina* and *Columella*, though without strong support. Madeira *et al*. (2010) included three azecid taxa (*Azeca goodalli*, *Cryptazeca monodonta* and *Hypnophila boissii*), which formed a clear monophyletic group consistent with our results, though their phylogenetic position in relation to other orthurethran taxa was unresolved. Our analysis provides strong support for the sister-

group relationship between *Cryptazeca monodonta* and *Hypnophila boissii*, consistent with the finding of Madeira *et al*. (2010), who showed a sister-group relationship between these taxa.

CHONDRINIDAE/TRUNCATELLINIDAE

The Chondrinidae consist of small (shell < 10 mm) snails, mainly restricted to the Iberian Peninsula, the south of France, Italy and the Balkans. The Chondrinidae are not recovered as a monophyletic group in our BI and ML trees due to the clustering of *Columella columella* (G. von Martens, 1830) from

the Truncatellinidae within the Chondrinidae. This is consistent with the findings of Nekola & Coles (2016) who also showed that the Truncatellinidae cluster within the Chondrinidae. Harl *et al*. (2017) showed a sister-group relationship between the Chondrinidae and Truncatellinidae, consistent with our NJ analysis in which *Columella columella* falls as a sister-taxon to the Chondrinidae, though their taxon sampling for these two families was limited as they included a single genus only from each family (*Granaria* and *Columella*).

The sister-group relationship of the Chondrinidae + Truncatellinidae relative to the main group of orthurethran families in our tree (shaded area in Fig. 1) is strongly supported and consistent with the results of Nekola & Coles (2016) and Harl *et al*. (2017). Steenberg (1925) was the first to propose the Chondrinidae as a family in its own right. Suvorov (1993) concluded that the most primitive pattern of orthurethran dentition (i.e. that most similar to the Ellobiidae) is to be found in members of the Chondrinidae. Pilsbry (1916–18) grouped the chondrinids and gastrocoptids on conchological characters, whilst Baker (in Pilsbry, 1927–35) later added several anatomical characters: short spermatheca, convoluted uterus, prostate as long as uterus, long and thin oviducal cul-de-sac and simple penis. Nordsieck (1986) excluded the gastrocoptines from the chondrinids, whilst Tillier (1989) united his Chondrinidae (including the gastrocoptids) in a clade with the Enidae, Partulidae, Vertiginidae, Orculidae and Cochlicopidae/Amastridae, based on a single nervous system character (the length of the cerebro- pedal connectives). The relationships proposed by Pilsbry and Tillier are not supported in our molecular trees.

ACHATINELLIDAE

The Achatinellidae are widely distributed on the islands of the Pacific, but reach their highest diversity in the Hawaiian Islands. The Achatinellidae are represented in the tree by *Partulina* and *Elasmias* Pilsbry, 1910, which were long treated as belonging in separate families, respectively, the Achatinellidae and Tornatinellidae (Pilsbry & Cooke, 1912–14; Steenberg, 1925). Later, Cooke & Kondo (1960) studied the soft anatomy of these two groups and concluded that they should be united.

The molecular analyses support Cook and Kondo's morphological interpretations with the Achatinellidae resolved as a strongly supported monophyletic family in our molecular trees (Fig.1).

CERASTIDAE

The Cerastidae were first recognized as a distinct group of orthurethran land snails by Watson (1920). The family consists of approximately 15 genera, the largest exceeding 40 mm in shell height. They reach their greatest diversity in the Afrotropical zone and they also extend eastwards into the Indian subcontinent, with one genus, *Amimopina*, found in Australia and a probable cerastid, *Bulimus subangulatus* L. Pfeiffer, 1863 recorded from Cambodia (Mordan, 1984, 1992; Nordsieck, 1986; Solem, 1978). The monophyly of the Cerastidae is well supported in our molecular analysis (Fig. 1). Phylogenetic relationships within the Cerastidae were analysed by Mordan (1992) based on a morphological cladistic analysis. The family is characterized by what Solem (1978), in a discussion of the anatomy of *Amimopina*, has called a 'pseudosigmurethrous' pallial system, in which the ureter initially runs straight towards the pneumostome from the anterior tip of the kidney, then extends back along the edge of the kidney and then returns towards the pneumostome along the side of the rectum. This structure takes the form of a groove or fully enclosed tube throughout its length. It is a synapomorphic character of the family, and distinct from the true sigmurethrous condition found in most Stylommatophora.

PARTULIDAE

The Partulidae consist of medium-sized, ovoviviparous (live-bearing) tree snails endemic to the high islands of the Pacific Ocean (Cowie, 1992) from Palau and the Marianas to the Marquesas and Society Islands, but absent from Hawaii. Extensive evolutionary studies of the partulids have been undertaken to understand how these species colonized the different islands of the Pacific and how the speciation processes occurred (Cowie, 1992; Goodacre & Wade, 2001a, 2001b; Goodacre, 2002; Lee *et al*., 2009, 2014; Sischo & Hadfield, 2017). Although we now have a better understanding of partulid evolution, we still do not know where they came from. The anatomy of the Partulidae is characterized by a relatively short, triangular kidney with a shortened ureter, a penis lacking an appendix and a spermatheca with a greatly thickened stalk, on which the narrow oviduct inserts laterally (Pilsbry, 1909–10). Schileyko (1979) also examined the anatomy of *Partula,* interpreting the group to be one of the most primitive within the Stylommatophora. This conclusion was based on the anatomy of the kidney, foot and reproductive system. Nordsieck (1985) excluded the Partulidae from the Orthurethra and included them in his 'achatinoid Sigmurethra' on the basis of the pallial system, as well as aspects of

the male reproductive anatomy, which he considered to resemble more closely those of the Orthalicidae. The synthetic classification of Vaught (1989) follows Nordsieck in this respect, while Tillier

(1989) followed Solem (1978) in uniting the Partulidae with the Enidae because of similarities in the length of the cerebral commissure. On the basis of similarities in both the nervous and reproductive systems, Tillier & Mordan (1995) suggested that the Draparnaudiidae might be the sister-group of the Partulidae. In the molecular tree (Fig.1), the partulids appear as a strong monophyletic family falling within the main orthurethran group.

PUPILLIDAE/LAURIIDAE

The Pupillidae and Lauriidae are generally minute snails (< 4 mm in length) living in both terrestrial and arboreal habitats (Solem, 1978). The taxonomy of the Pupillidae was mainly developed by Pilsbry (1916–18), Steenberg (1925) and Baker (1935), based on the shell morphology and several anatomical features. The Pupillidae are not resolved as a monophyletic group in the molecular tree (Fig.1) and their monophyly is strongly refuted in Shimodaira–Hasegawa likelihood testing [-ln Likelihood (L) 8902.507 29 (best tree shown in Fig. 1) versus -ln L = 8993.780 51 (Pupillidae monophyletic), $P < 0.05$]. The Lauriidae are distributed in Europe, Caucasus and Africa (Herbert, 2010). The family is represented by just one taxon, *Lauria cylindracea*, in our tree, which has a close sister-group relationship with *Pupilla muscorum* (Pupillidae) with full support, with *Lauria* and *Pupilla* separated by short genetic distances in the tree (Fig. 1). The systematic position of the Lauriidae has been interpreted variously. Bouchet & Rocroi (2005), for example, treated them as a family within the Pupilloidea and the molecular tree offers no justification for excluding them. However, in the H3/H4 tree of Harl *et al*. (2017), *Pupilla muscorum* does not cluster with the putative lauriid genus *Leiostyla*, suggesting that the position of the latter in the Lauriidae should be questioned.

VERTIGINIDAE

The Vertiginidae are a large family of small snails that are widespread in the Northern Hemisphere. In the present analysis, the family is represented by two taxa, *Vertigo antivertigo* (Draparnaud, 1801) and *Pronesopupa acanthinula* (Ancey, 1892), which together form a monophyletic group with strong support (Fig. 1). This agrees with the finding of Nekola & Coles (2016).

COCHLICOPIDAE/AMASTRIDAE

The Cochlicopidae are a small family of minute (shell < 10 mm) snails found across the Palaearctic but predominantly in Western Europe, while the Amastridae are a more diverse family endemic to the Hawaiian Islands. Although these two groups have always been considered to be distinct families, their

sister-group relationship has long been recognized. Pilsbry (1907–08) remarked that: 'no character of importance separates *Cochlicopa* from *Leptachatina*'. This led Watson (1920) to the conclusion that there was no justification in placing these genera in different families. Confirmation of the sister-group relationship of the Amastridae and Cochlicopidae has been obtained in subsequent morphological studies (e.g. Tillier, 1989) and they have been grouped in the Cochlicopoidea. The molecular data (Fig. 1) support these morphological analyses, with the Cochlicopidae and Amastridae being strongly supported as sister-taxa in our trees.

ENIDAE

The Enidae are a large family of medium-sized snails, with a geographical range extending throughout much of the Palaearctic and North Africa to Japan, with a high diversity in the Far and Middle East and around the Mediterranean Sea. In Macaronesia, they are represented by extensive endemic radiations in the Canaries and Azores (*Napaeus* Albers, 1850), but are absent from Madeira and Cape Verde. Although relatively abundant in the north of the Indian subcontinent, only low diversity is found in central and southern India (*Mirus*). The monophyly of this family has not been a particular point of debate and it is, therefore, surprising that the molecular data fail to recover it as a monophyletic clade. We note, however, that the monophyly of Enidae is not refuted in the Shimodaira–Hasegawa likelihood testing [-ln Likelihood (L) 8902.507 29 (best tree shown in Fig. 1) versus -ln L = 8905.507 29 (Enidae monophyletic), P = 0.676].

ORCULIDAE/PYRAMIDULIDAE

The Orculidae are a family of small (shell < 10mm) snails with a main distribution in Europe. They extend in the western Palaearctic from Spain in the west to the Pamir Mountains in the East. They occur also in North Africa and have their highest diversity in the Near East (Hausdorf, 1996). The Pyramidulidae comprise a single genus *Pyramidula*, represented in the tree by its type species *Pyramidula rupestris*, a minute ovoviviparous (live-bearing) snail that lives in rocky areas in the southern Palaearctic from Western Europe to Japan. The grouping of the Orculidae and Pyramidulidae has weak support (Fig. 1). Both are generally included in the Pupillidae *s.l.*, though the possibility that they might be sister-taxa was not recognized until the molecular analyses of Wade *et al*. (2001, 2006). Harl *et al*. (2017) also confirmed the sister- grouping of the Orculidae with the Pyramidulidae,

though using several orculid taxa and a single, but different, species of *Pyramidula*. Baker (in: Pilsbry, 1927–35) placed Orculidae and Pyramidulidae in separate subfamilies (the Pupillinae and Valloniinae, respectively). Steenberg (1925) included *Pyramidula* in the Valloniidae and the orculids in a distinct family of their own. Watson (1920) examined the anatomy of *Pyramidula* in detail and interpreted it as a paedomorphic member (Gould, 1977) of the Pupillidae, developed by progenesis (sexual maturity

attained at a larval or juvenile stage). In the phylogenetic analysis of Tillier (1989), which used a different suite of anatomical characters, the Pyramidulidae and Orculidae were not sister-groups. It is difficult to establish any particular anatomical justification for uniting Orculidae and Pyramidulidae; certainly many of the reproductive characters differ, but these may have become modified in the latter as a result of ovoviviparity. The shell of *Pyramidula* lacks any internal lamellae, but whilst orculids are characterized by lamellae in the post- embryonic shell (Pilsbry, 1922–26), it appears that they are absent from the embryonic shell of *Orcula* (Reinhardt, 1877), which would be consistent with a progenetic origin of the Pyramidulidae.

VALLONIIDAE

The Valloniidae are a group of small land snails with diverse shell forms, principally distributed in North America, Europe, North Africa, Central Asia and Japan, comprising approximately 25 species (Barker, 1985). Two genera, *Vallonia* and *Acanthinula*, are represented in our tree but no clear sister-group relationships emerged. Acanthinulids were earlier recognized as a subfamily, either within the Pupillidae (Pilsbry, 1916–18) or Valloniidae (Steenberg, 1925), and there remains disagreement as to their placement; Tillier (1989), Bouchet & Rocroi (2005) and Bouchet *et al*. (2017) favoured Steenberg, classifying them in the Valloniidae. However, no sister-group relationship between *Acanthinula* and *Vallonia*, nor between either with any other group, was demonstrated in the analysis. The monophyly of the Valloniidae is not rejected in the Shimodaira–Hasegawa likelihood testing [-ln Likelihood (L) 8902.507 29 (best tree shown in Fig. 1) versus -ln L = 8912.61913 (Valloniidae monophyletic), P = 1.00].

DRAPARNAUDIIDAE/GASTROCOPTIDAE

The Draparnaudiidae are a homogenous group of 13 recognized species endemic to the continental Pacific island of New Caledonia (Tillier & Mordan, 1995). The systematic position of the Draparnaudiidae is uncertain, although Tillier and Mordan suggested they appeared anatomically closest to the Partulidae. Likewise, the relationship of the Gastrocoptidae to other orthurethran taxa is unclear (Wade *et al*., 2006; Nekola & Coles, 2016). In the current analysis, *Draparnaudia singularis* has a sister-group relationship to *Gastrocopta armifera*, though the support for this relationship is equivocal. *Gastrocopta* has been generally considered as a vertiginid (Schileyko, 1984; Nordsieck, 1986; Bouchet & Rocroi, 2005) or chondrinid (Pokryszko *et al*., 2009), but it was excluded by Nekola & Coles (2016) as it showed no clear affinity with either the vertiginids or the chondrinids.

STROBILOPSIDAE

The Strobilopsidae are defined by the peculiar structure of the last whorl of the shell, but it lacks sufficient anatomical characters to distinguish it from other families, such as the Pupillidae or Valloniidae. The Strobilopsidae have a wide geographical distribution (Americas, China, Japan, Philippines and New Guinea) with fossil species that date back to the Early Tertiary of Europe (Manganelli *et al*., 1989). Strobilopsids are represented by a single taxon, *Eostrobilops nipponica* (Pilsbry, 1908), in the molecular tree (Fig. 1), the position of which remains unresolved.

ARGNIDAE

The Argnidae are distributed in the southern and eastern Alps, the Carpathians and the Iberian Peninsula (Harl *et al*., 2017). The systematic position of the family is uncertain. Hausdorf (1996) considered the Argnidae as the potential sister-group of the Orculidae. Harl *et al*. (2017) included two genera of the family Argnidae (*Argna* and *Agardhiella*), though their results showed that *Argna* and *Agardhiella* were not sister-taxa and neither of them is closely related to the Orculidae. Therefore, Harl *et al*. (2017) retained *Argna* in the Argnidae and placed *Agardhiella* in a new family Agardhiellidae Harl & Páll-Gergely, 2017. In the present analysis, the family is represented by one genus, *Argna bielzi* (Rossmässler, 1859), which falls within the main orthurethran group (Fig. 1).

SUPERFAMILIES

Bouchet *et al*. (2017) pointed out that none of the previous suggestions to divide the Orthurethra into superfamilies is compatible with the results of recent molecular studies (Madeira *et al*., 2010; Nekola & Coles, 2016). They, therefore, proposed two solutions for dividing the Orthurethra into superfamilies: (1) include all the orthurethran groups in the Pupilloidea '(making Orthurethra and Pupilloidea synomyms)' or (2) retain the Orthurethra but divide into three newly defined superfamilies: Azecoidea, Chondrinoidea and Pupilloidea. Bouchet *et al*. (2017) adopted the first solution as the previous phylogenies of the Orthurethra were not well resolved. The findings of our molecular analyses support the second solution and we, therefore, propose the Orthurethra should be divided into three superfamilies: the Azecoidea, which include the Azecidae, the Chondrinoidea, which include the Chondrinidae + Truncatellinidae, and the Pupilloidea, which include all remaining orthurethran families.

BIOGEOGRAPHY

On the basis of our analysis and the distribution patterns of the included taxa, it is possible to offer some speculative hypotheses on the biogeographic history of the Orthurethra. The greatest level of orthurethran family diversity is to be found in the Palaearctic and, in particular, the western Palaearctic, where there is relatively speciose representation of the Azecidae, Chondrinidae, Truncatellinidae,

Lauriidae, Pupillidae, Vertiginidae, Cochlicopidae, Orculidae, Pyramidulidae, Valloniidae and Argnidae. Several of these families, typically those showing relatively smaller shell size, such as Truncatellinidae, Vertiginidae, Valloniidae, Gastrocoptidae and Lauriidae, do extend to the North and, to a lesser extent, South America and even Australia, but exhibit only relatively low species diversity there. Many also extend around the Mediterranean into North Africa and the islands of the North Atlantic. The Enidae are found in Europe and extend into the Indian subcontinent and the Middle East, but reach their greatest diversity throughout Asia. By contrast, and with the possible exception of the Cerastidae, there is neither family-level endemism, nor high levels of species diversity to be found in southern 'Gondwanan' continental regions of South America, Australia, Africa and India, or indeed in the Nearctic. Such a pattern of geographical distribution and diversity points towards a Laurasian, possibly Palaearctic, origin of the Orthurethra with subsequent limited dispersal of these families.

In our tree, the Azecidae, a European group with only two species extending to North Africa, fall as a sister-group to all the remaining Orthurethra. Within this larger clade, the Chondrinidae, another essentially European family, is sister to a fully supported clade containing all the remaining taxa but which is unresolved at its base. Thus, only European taxa are represented in all three resolved primary clades, which lends further support for a Palaearctic origin.

Two groups of relatively large-shelled species require special consideration. The Cerastidae reach their highest level of species diversity in Africa, but are also found at much lower diversity in Arabia and India, with one genus, *Amimopina*, restricted to South-East Asia and along the north-west coast of Australia. Such

a pattern of distribution might superficially suggest a Gondwanan origin, but the position of the cerastid taxa in the molecular tree does not support this.

The Orthurethra of the Pacific represent an especially interesting case. Four families of relatively large-shelled, predominantly tree-dwelling snails are essentially restricted to the islands of the Pacific: the Achatinellidae, Partulidae, Amastridae and Drapanaudiidae. Partulids are widely distributed on Pacific islands but do not extend beyond, and the same is true of the achatinellids, which reach by far their greatest diversity on the Hawaiian Islands. The other two families are endemic to particular island groups: the Amastridae to the Hawaiian archipelago and Draparnaudidae to New Caledonia. All four families fall within the main clade (shaded area on tree, Fig. 1), which is unresolved at its base. The tree offers no evidence to suggest that any of these individual families is other than monophyletic or, equally, that there is any close relationship between them. The only supported sister- group pairing is that between the Amastridae and Cochlicopidae, the latter with a Holarctic range centred on Western Europe. The tree, therefore, does not refute a view that the Pacific Orthurethra originated as several

distinct invasions, which must have been effected in almost all cases by passive dispersal, since the islands are almost all oceanic in origin.

ACKNOWLEDGEMENTS

We are extremely grateful to all collectors listed in Table 1, who have provided specimens. The authors also acknowledge the financial support of the Higher Committee for Education Development in Iraq (HCED-Iraq) for the PhD studies of Ahmed J. Saadi. We would also like to thank Dr Cendrine Hudelot for her assistance.

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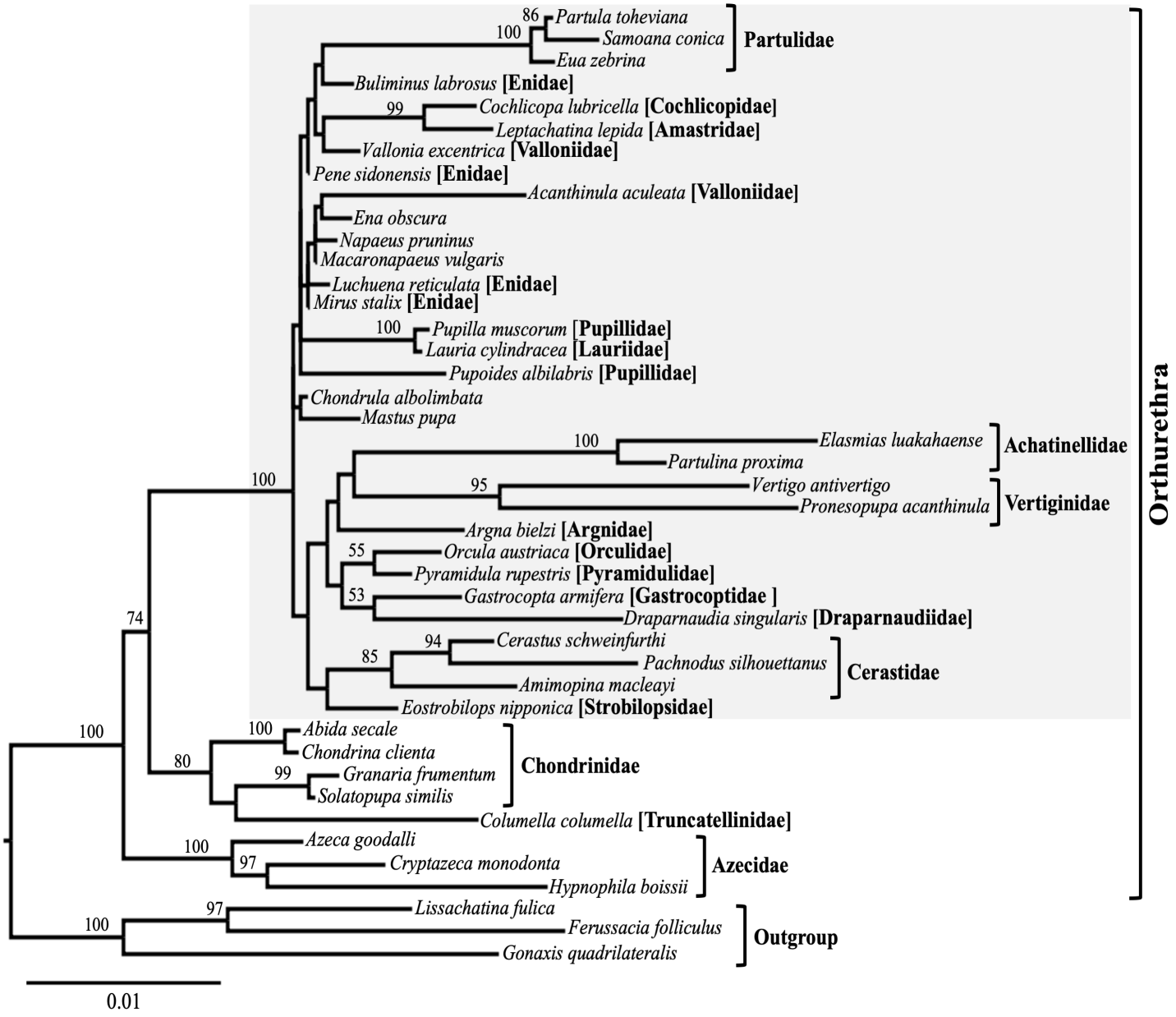
SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site.

Figure S1. Maximum likelihood phylogenetic tree of the Orthurethra based on 3370 unambiguously aligned nucleotide sites of the LSU rRNA (and 5.8S) gene. Values on nodes represent bootstrap support (1000 replicates). Bootstrap support values less than 50% are not shown. The optimal model GTR+Γ was used. The phylogeny is rooted on the 'achatinoid' clade (Wade *et al*., 2001, 2006), represented by *Ferussacia folliculus*, *Gonaxis quadrilateralis* and *Lissachatina fulica.* The scale bar represents 1 substitutional change per 100 nucleotide positions. Classification follows Bouchet *et al*. (2017). The shaded area represents the main clade.

Figure S2. Neighbour-joining phylogenetic tree of the Orthurethra based on 3370 unambiguously aligned nucleotide sites of the LSU rRNA (and 5.8S) gene. Values on nodes represent bootstrap support (1000 replicates). Bootstrap support values less than 50% are not shown. The optimal model GTR+Γ was used. The phylogeny is rooted on the 'achatinoid' clade (Wade *et al*., 2001, 2006), represented by *Ferussacia folliculus*, *Gonaxis quadrilateralis* and *Lissachatina fulica*. The scale bar represents one substitutional change per 100 nucleotide positions. Classification follows Bouchet *et al*. (2017). The shaded area represents the main clade.



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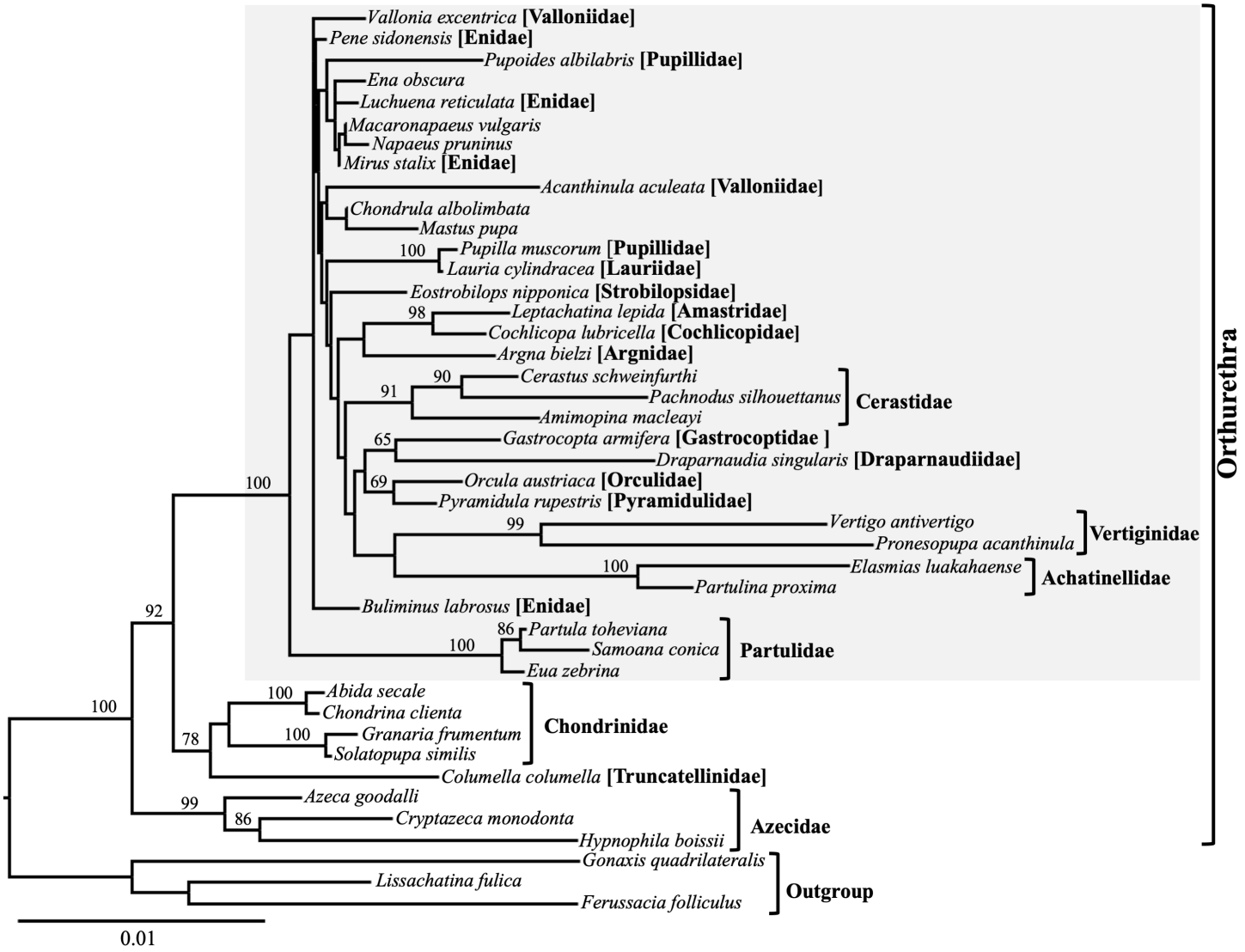

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