

A molecular phylogeny of Rissoidae (Caenogastropoda: Rissooidea) allows testing the diagnostic utility of morphological traits

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The Rissooidea is an evolutionarily ancient and mega-diverse group of marine micro-gastropods that occur from intertidal to deep waters at all latitudes. Their current systematics is predominantly based on phenetic grounds and there has been no comprehensive molecular phylogeny. Based on sequences of mitochondrial and nuclear DNA from the most complete sampling of Rissoidae to date, this work represents the first treatment of the group performed through a phylogenetic approach. The main goals are to clarify the phylogenetic position of the Rissoidae, investigate the relationships within rissoid taxa and test the utility of some diagnostic morphological traits. Our phylogeny indicates that the Rissoidae are one of six distinct family-lineages within the superfamily Rissooidea (along with Barleeiidae, Emblandidae, Lironobidae, Rissoinidae and Zebinidae) whose recognition is supported by several synapomorphies. While most of the characters studied exhibit widespread convergence, some others prove useful in separating genera and broader taxonomic groups. The relationships among rissoid taxa challenge the current systematics, indicating the non-monophyly of some genera with purportedly trans-oceanic distribution and the need of taxonomic revision for some highly diverse genera. Our phylogeny suggests that the Rissoidae originated in shallow seas and independently radiated into bathyal waters at least twice.

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INTRODUCTION

Marine microgastropods are a major component of gastropod diversity and the most poorly known (e.g. Albano, Sabelli & Bouchet, 2011). The caenogastropod family Rissoidae Gray, 1847 (Rissooidea) comprises one of the major families of microgastropods, with about 160 nominal genus-group taxa, 31 of which were treated as valid in the most recent comprehensive review of the group (Ponder, 1985a). Representatives of this family are found world-wide, from polar waters (Warén, 1973; Ponder, 1983b) to the tropics, and from the intertidal to the deep sea. They can be

abundant in the littoral and sublittoral zones, especially in the Mediterranean Sea (e.g. *Rissoa* Desmarest, 1814, *Alvania* Risso, 1826, *Pusillina* Monterosato, 1884, *Setia* H. & A. Adams, 1852) and the North-East Atlantic (e.g. Warén, 1973, 1996a), where their larvae constitute an important source of food for young herrings (Lebour, 1936; Fretter & Shale, 1973). Rissoidae have an extensive fossil record reaching back to at least the Lower Jurassic (e.g. Conti, Monari & Oliverio, 1993; Kaim, 2004; Hikuroa & Kaim, 2007). Rissoids have been examined in studies focusing on their life history (e.g. Lebour, 1934, 1936, 1937; Smidt, 1938; Russo & Patti, 2005), ecology (Wigham, 1975a; Southgate, 1982; Ávila, 2000; Fredriksen, Christie & Bostrom, 2004), reproductive biology (Ávila *et al.*, 2008), biogeography (e.g. Gofas,

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2007; Ávila, Goud & de Frias Martins, 2012), speciation (Kowalke & Harzhauser, 2004; Iljina, 2006; Gofas, 2007), phenotypic plasticity (Wigham, 1975b) and genetics (Colognola *et al.*, 1986; Munksgaard, 1990; Oliverio, 1994; Criscione, Scuderi & Patti, 2009; Criscione & Patti, 2010).

There have been several comprehensive treatments of the family at the generic level but the earlier ones (Thiele, 1929–1935; Wenz, 1938–1944; Coan, 1964) were based almost entirely on shell characters, particularly sculpture. Due to the high degree of convergence in shell characters, these reviews suffered from the inclusion of many genera no longer included in the family. Coan's (1964) literature-based review of the genera was revised by Ponder (1967) who used head-foot, radular and anatomical information and recognized three subfamilies. Ponder (1967, 1985a) also removed many taxa from the Rissoidae, transferring them to other families in the Vetigastropoda, Heterobranchia and Caenogastropoda. In the most comprehensive review of the family to date, Ponder (1985a) recognized two subfamilies, the Rissoinae and Rissoininae. His review included some numerical analyses of shell, opercular, radular and anatomical characters. Since then the phylogeny of the group has not been examined comprehensively. Recent molecular studies have indicated that the Rissoidae may not be monophyletic (Criscione & Ponder, 2013; Wilke *et al.*, 2013; Takano & Kano, 2014). However, these studies included only a few representative rissoid taxa, and no complete molecular phylogeny of the entire family has been presented so far.

Based on newly produced mitochondrial and nuclear DNA sequences from a larger number of species, we are presenting the most comprehensive molecular phylogeny of the Rissoidae to date. Our study aims to test the monophyly of the Rissoidae as presently delineated and to reconstruct the relationships among the main lineages within this group. Furthermore, we employ the molecular phylogeny to investigate the evolution of morphological traits that had been considered to be systematically significant. Using a maximum-likelihood (ML)-based method of ancestral state reconstruction, we identify patterns of convergence and apomorphy in morphological characters to assess their suitability as taxonomic markers. In addition, we investigate the evolutionary origin of the deep-sea taxa. The problem of how the endemic fauna of the bathyal and abyssal depths diverged and evolved has been the focus of much attention (for a review see Williams *et al.*, 2013). Rissoids have represented an important taxon in studies on the evolution and biogeography of deep-sea gastropods (e.g. Rex & Etter, 1990). In the present study, we test whether the phylogeny could explain

the different trends in bathymetric distribution and geographical differentiation between *Benthonella* Dall, 1889 and other deep-sea rissoids (Etter & Rex, 1990).

MATERIAL AND METHODS

TAXON SAMPLING

This study is based on ethanol-preserved samples of 20 of the 31 currently recognized rissoid genera, mainly collected in Australia, the Fiji Islands, Japan, New Zealand and Sicily. Samples of representative species of all other rissoid families and outgroup taxa from additional caenogastropod families were selected based on previous molecular phylogenies of related caenogastropod groups (Criscione & Ponder, 2013; Takano & Kano, 2014). All material studied is deposited in the malacological collections of the Australian Museum, Sydney (AM) and the Atmosphere and Ocean Research Institute, University of Tokyo (AORI). Species examined, classification and voucher details are listed in the Supporting Information, Tables S1 and S2.

MOLECULAR STUDIES

DNA was extracted from whole specimens by use of a Qiagen DNA extraction kit for animal tissue following the standard protocol of the manual. Fragments of one nuclear gene (28S rRNA) and one mitochondrial gene (16S rRNA) were amplified by PCR using the primer pairs 28SDKF (Strong *et al.*, 2011) and LSU 1600R (Williams, Taylor & Glover, 2004), and 16SARis and 16SBRis (Criscione & Ponder, 2013), respectively. Reactions were performed with an annealing step of 60 s at 55 °C for 28S and of 30 s at 52 °C for 16S with elongation times of 120 and 60 s, respectively. Both strands of PCR fragments were purified by using ExoSAP (Affymetrix) and Sanger sequenced using the PCR primers. Chromatograms were manually corrected for misreads, if necessary, and forward and reverse strands were merged into contigs using CodonCode Aligner v.3.6.1 (CodonCode Corporation). Sequences have been deposited in GenBank (Supporting Information, Table S1). Additional 16S and 28S GenBank sequences of rissoid and outgroup taxa were added to the final dataset (Supporting Information, Tables S1 and S2). Sequences were aligned using the online version of MAFFT (Katoh *et al.*, 2005) using the structural alignment option. For the alignment of 16S sequences, areas of uncertain alignment were removed using the following settings in Gblocks (Castresana, 2000): minimum 70% of sequences for a conserved position, minimum 90% of sequences for a

flanking position, maximum three contiguous non-conserved positions, minimum block length of five positions and gaps allowed. Base composition of nucleotide sequences was analysed and nucleotide homogeneity was tested (by χ^2 statistics) with PAUP* (Swofford, 2002). Each DNA fragment was tested for sequence saturation by a test implemented in DAMBE (Xia & Lemey, 2009). Phylogenetic trees were reconstructed using Bayesian inference (BI) and ML methods. Prior to the model-based phylogenetic analyses, the best-fit model of nucleotide substitution was identified for each gene partition separately by means of the Bayesian Information Criterion calculated with MrModeltest (Nylander, 2002). A data partition was applied in the BI analyses of combined datasets, which allowed the model parameters to be estimated independently for each partition. The ML analysis was performed using MEGA 5.0 (Tamura *et al.*, 2011) with Nearest-Neighbour-Interchange (NNI) as heuristic method and automatic generation of the initial tree. One-thousand ML bootstrap replicates (BTSP) were performed to assess topology support. Bayesian posterior probabilities of phylogenetic trees (BPP) were estimated by running a 10^7 generations Metropolis-coupled Markov chain Monte Carlo (MCMC, two runs each with four chains, of which one was heated) as implemented by MrBayes v.3.2 (Ronquist *et al.*, 2013). Sampling rate of the trees was 1000 generations. Stationarity was considered to be reached when the average standard deviation of split frequencies shown in MrBayes was less than 0.01 and the log likelihoods ($\ln L$) of sampled trees reached a stationary distribution. The program Tracer (Rambaut & Drummond, 2007) was used to generate parametric plots. Generations sampled before stationarity were discarded as burn-in, and the remaining trees were used to create a 50% majority-rule consensus tree and to estimate Bayesian posterior probabilities. Alternative topological hypotheses were tested in MrBayes by computing the ratio of their marginal likelihoods (Bayes factor). This ratio was achieved by comparing the harmonic mean estimates of the likelihoods of two models obtained after running two separate 10^5 -generation MCMC analyses, where either positive or negative constraint was enforced. A log difference between the harmonic mean estimates of two alternative models in the range of 3–5 units is considered as strong evidence in favour of the better model, while a difference above 5 is considered very strong evidence (Kass & Raftery, 1995).

CHARACTER HISTORY RECONSTRUCTION

A data matrix of 17 multistate anatomical characters was compiled for the taxa included in this study

based on available literature data (Warén, 1983; Ponder, 1985a, 1988; Houbriek, 1987; Supporting Information, Table S3). The ML-based criterion according to the Mk1 (Markov k-state 1 parameter) model as implemented in Mesquite v.2.75 (Maddison & Maddison, 2011) was used to map the evolution of each anatomical character on the molecular phylogeny. The ancestral state reconstruction was summarized over a set of representative trees and the probability calculated for each state at each node.

Abbreviations used in text and figures

16S, 16S rRNA gene fragment; 28S, 28S rRNA gene fragment; AM, Australian Museum, Sydney; AORI, Atmosphere and Ocean Research Institute, University of Tokyo; BI, Bayesian inference; BPP, Bayesian posterior probability; BTSP, bootstrap value; MCMC, Metropolis-coupled Markov chain Monte Carlo; ML, maximum likelihood.

RESULTS

MOLECULAR STUDIES

For clarity, results are presented with reference to the taxonomy as revised below (Supporting Information, Table S1). The underlying taxon delimitations resulted from the assessment of the molecular and morphological differentiation and were not foregone conclusions preceding this analysis.

Phylogenetic analyses were based on 52 newly produced 28S sequences and 60 new 16S sequences from rissoid specimens as well as 44 GenBank sequences of rissoids (nine 28S, seven 16S sequences) and outgroup species (14 each gene). After alignment and removal of ambiguously aligned sites, 381 bp remained of 16S (50% of the original alignment) and 1445 bp of the 28S alignment. Xia *et al.*'s (2003) tests indicated no or little saturation in both fragments ($\text{Iss} < \text{Iss.c}$ with $P < 0.01$). For the inconsistent taxon sampling in both DNA datasets, we analysed (1) a dataset containing all available 16S sequences, (2) a dataset containing all available 28S sequences and (3) a concatenated dataset of 28S and 16S, where missing sequence fragments were coded as unknown. For all single-gene and combined datasets, the χ^2 statistic showed no significant heterogeneity in the distribution of bases ($P = 1$) and the incongruence length difference (ILD) test revealed no significant ($P < 0.01$) incongruence between the two gene partitions. In correspondence to the phylogeny shown by Criscione & Ponder (2013), sequences of *Alaba monile* Adams, 1862 (Lithiopidae) were used as outgroup to root the trees.

Likelihood ratio tests using the Bayesian information criterion revealed GTR + G + I as the best-fit

model of sequence evolution in both sequence datasets for both genes. This model was applied in all ML and BI analyses. The first 4000 Bayesian trees were discarded as burn-in. No conflict was observed between the topologies of trees produced for the single-gene datasets and the concatenated analysis. Therefore, only the trees from the combined datasets are shown (Figs 1, 2). Both phylogenies (Figs 1, 2) rejected the monophyly of Rissoidae as presently delimited (Ponder, 1985a) for the inclusion of members of the two other rissoidae families (Barleeiidae Gray, 1857 and Emblandidae Ponder, 1985).

In both BI and ML phylogenies, the ingroup taxa clustered into five well-supported and well-differentiated monophyla, for which family-group names are available: Rissoidae Stimpson, 1865, Zebinidae Coan, 1964, Lironobidae Ponder, 1967, Emblandidae and Rissoidae.

Barleeiidae and Emblandidae are maintained as delineated by Ponder (1983a,b). All these groups are treated herein as distinct families. The relationships of taxa within each family were generally well resolved with a few exceptions (described below). In particular the Rissoidae included well-supported (in terms of nodal support) and well-differentiated (in terms of basal branch lengths) lineages (A–F in Figs 1, 2). The taxonomic status of the above lineages and of the taxa included is evaluated below.

The topology test based on the Bayes factor was applied to two alternative models where positive and negative constraint was applied to the monophyly of the Rissoidae as presently delimited. The difference between the harmonic mean estimates of the latter and the former models (>800 log units) rejected the monophyly of the Rissoidae as currently delimited with statistical significance.

CHARACTER HISTORY RECONSTRUCTION

Anatomical data were obtained from available literature for 30 ingroup and eight outgroup taxa included in the molecular analysis. We used a set containing all >95% log-likelihood trees plus the BI consensus tree in Fig. 1 (607 trees altogether) to reconstruct the evolutionary history of 17 characters of shell, operculum, head-foot and reproductive system. We removed all taxa that lacked anatomical information from the trees and calculated the probability of each character at each node with reference to the BI

consensus tree (Figs 3, 4, Supporting Information, Fig. S1). The value presented for a node for a state was then the average frequency of that state across all the trees possessing that node.

DISCUSSION

EVOLUTION OF KEY MORPHOLOGICAL CHARACTERS

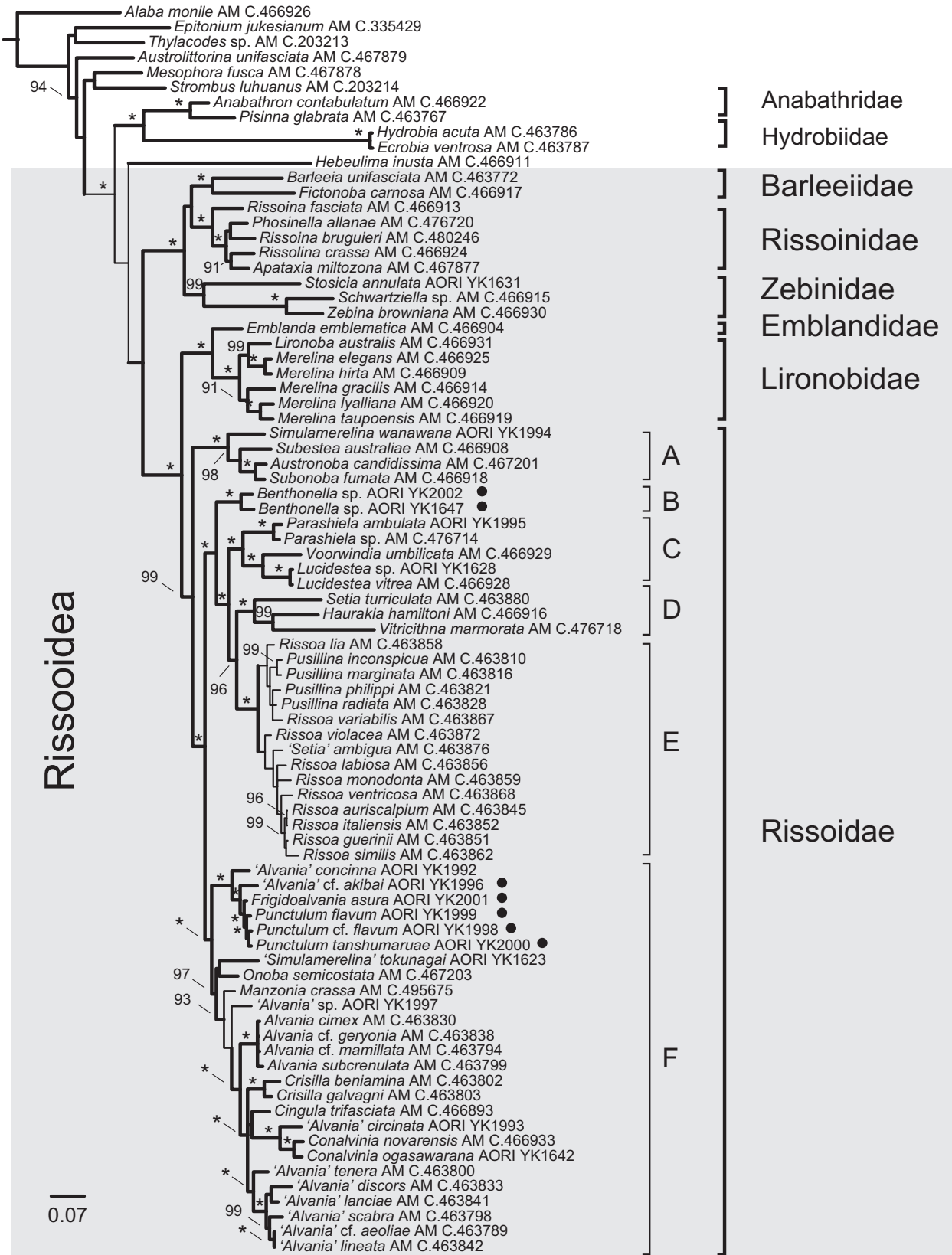
Previous systematic studies of rissoidae relied entirely on analyses of morphological characters. Ponder (1985a) produced a phenogram for the Rissoidae (*s.l.*) based on a combination of discrete and continuous characters, and later (Ponder, 1988) produced a cladogram of the Truncatelloidea (*s.l.*) based on discrete multistate characters only. To assess the usefulness of these multistate characters, we mapped them onto a combined gene tree from which we pruned all taxa for which there is no anatomical data (Figs 3, 4, Supporting Information, Fig. S1). While many of the characters used by Ponder (1985a) are useful in separating genera when used in combination with other features, some were more useful across broader groups of taxa. We selected the following, in which we included the characters considered as particularly diagnostic by Ponder (1985a, 1988).

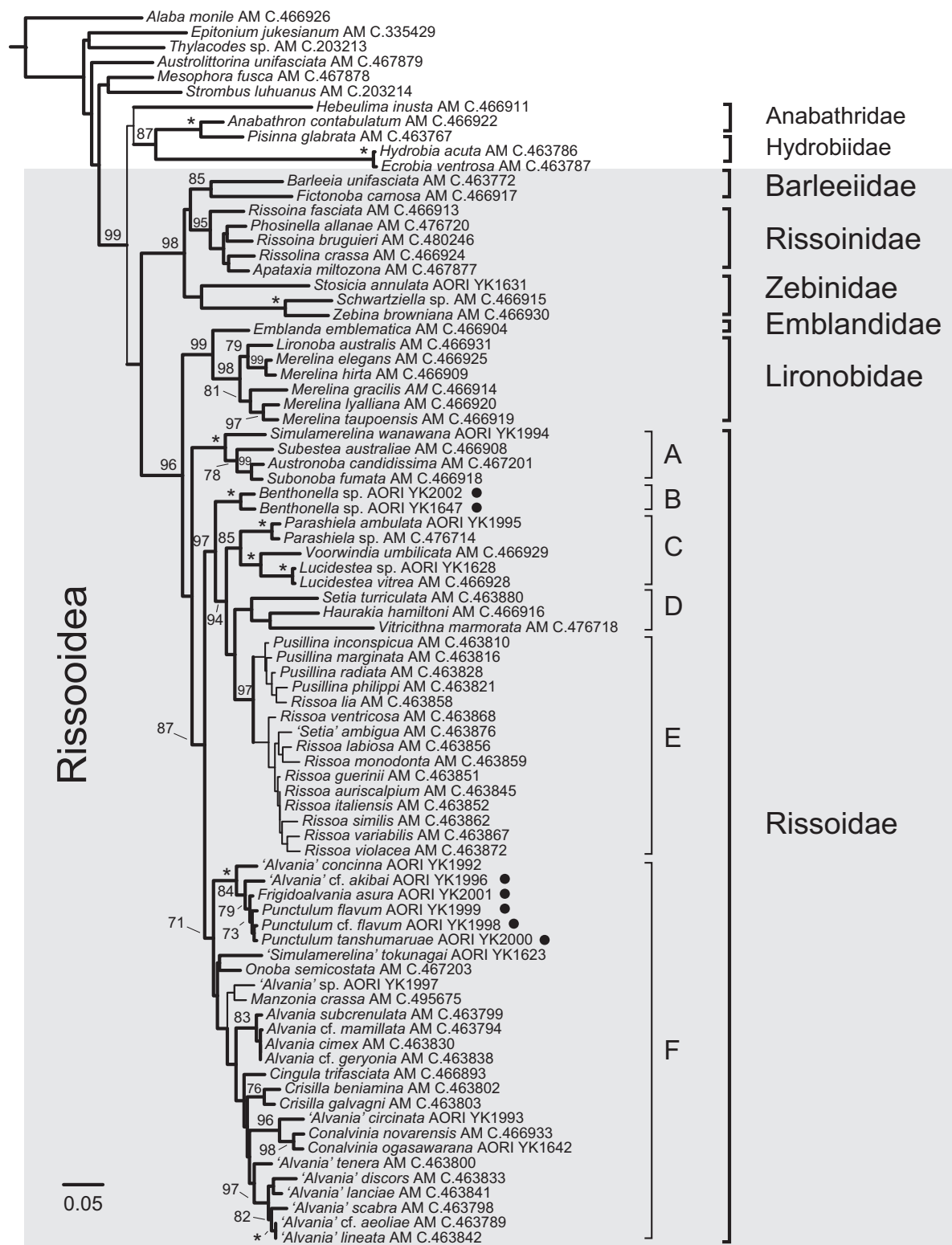
Shell characters

Due to the variety of shell sculptures exhibited by several rissoidae genera, shell sculpture is deemed to be highly convergent (e.g. Ponder, 1985a, 1988) and it was excluded from our analysis.

The presence of an anterior channel and an aperture with a duplicated peristome were considered significant shell characters within the Rissoidae at the generic level or above by Ponder (1985a). Of the taxa included in our analysis, most have a simple, rounded anterior edge to the aperture but an anterior (and posterior) channel-like structure is present in *Rissoina* d'Orbigny, 1840 and related genera (see Fig. 3A), in *Stosicia* Brusina, 1870, and in *Haurakia* Iredale, 1915 and *Vitricithna* Laseron, 1956. It is, however, constructed differently in each of these cases. In most of them, the inner edge of the channel is formed at a sharp angulation at the junction of the inner and outer lips of the aperture. This is particularly pronounced in *Rissoina*, where the inner lip forms a distinct angulation with the most anterior

Figure 1. Bayesian consensus phylogram based on analyses of the concatenated 16S and 28S sequences. Numbers on branches indicate nodal support (%) by Bayesian posterior clade probabilities (BPP; only values $\geq 90\%$ are shown; values of 100% are represented by asterisks). Thick lines mark branches that are consistent with the topology of the ML tree. Taxa included in the Rissoidae are shaded. Taxon names are followed by registration numbers. Names of bathyal taxa are followed by a dot.





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Figure 2. Maximum-likelihood phylogram based on analyses of the concatenated 16S and 28S sequences. Numbers on branches indicate nodal support (%) by ML bootstrap (BTSP; only values $\geq 70\%$ are shown; values of 100% are represented by asterisks). Thick lines mark branches that are consistent with the topology of the BI tree. Taxa included in the Rissoidae are shaded. Taxon names are followed by registration numbers. Names of bathyal taxa are followed by a dot.

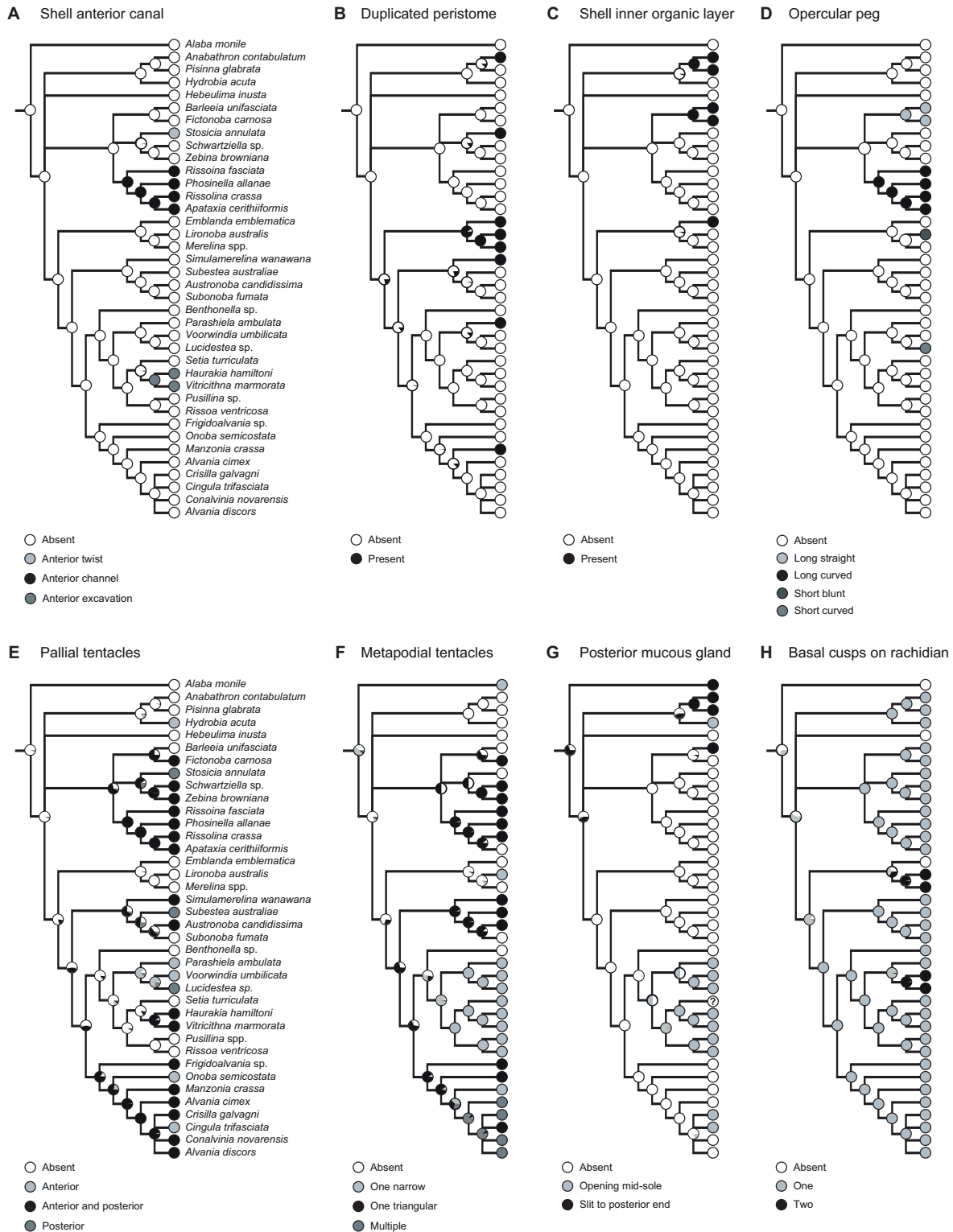


Figure 3. Maximum-likelihood-based ancestral character state reconstruction for anatomical characters mapped on the Bayesian consensus tree of Fig. 1. A, shell anterior canal. B, duplicated peristome. C, shell inner organic layer. D, opercular peg. E, pallial tentacles. F, metapodial tentacles. G, posterior mucous gland. H, basal cusps on rachidian tooth. Taxa for which anatomical information was not available have been excluded from the tree. Branches with BPP support values lower than 90% are shown as collapsed. A likelihood of state at each node lower than 1% is omitted.

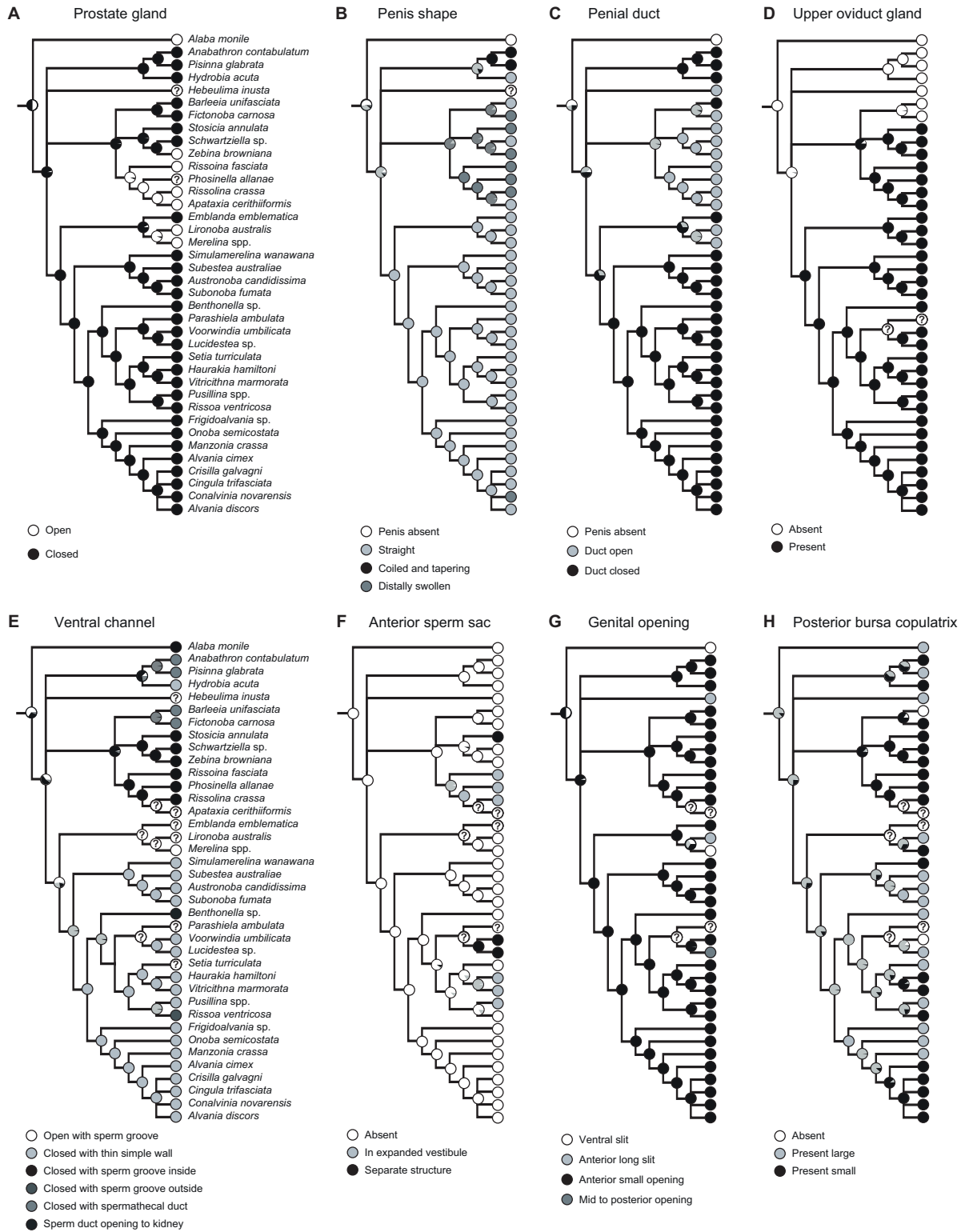


Figure 4. Maximum-likelihood-based ancestral character state reconstruction for anatomical characters mapped on the Bayesian consensus tree of Fig. 1. A, prostate gland. B, penis shape. C, penial duct. D, upper oviduct gland. E, ventral channel. F, anterior sperm sac. G, genital opening. H, posterior bursa copulatrix. Taxa for which anatomical information was not available have been excluded from the tree. Branches with BPP support values lower than 90% are shown as collapsed. Question marks on nodes indicate an unknown character state. A likelihood of state at each node lower than 1% is omitted.

part twisted to form a notch-like structure but without any excavation of the lip. In *Stosicia* there is an excavation in the thickening of the lower-most part of the outer lip with one edge at the point where it meets the inner lip and with a weak thickening delineating its border at about the most anterior part of the lower outer lip. There is a weak form of a similar structure in the figured species of *Schwartziella* G. Nevill, 1881 (Fig. 5H) but many other species of that genus, or most members of the genus *Zebina* H. & A. Adams, 1854, do not show any trace of a channel. In the form of the channel seen in *Stosicia* and some *Schwartziella*, the edge of the outer lip remains intact. However, in *Haurakia* and *Vitricithna*, a shallow channel is formed by an excavation of the edge of the outer lip itself and the embayment is utilized by the head, not the mantle edge tentacles (W.F.P., pers. observ.). Because no such embayment is formed in *Rissoina* or the *Zebina*–*Schwartziella*–*Stosicia* group, we treat the condition seen in *Haurakia* and *Vitricithna* as a separate state (Fig. 3A).

The anterior channel in *Rissoina* houses the anterior pallial tentacle (which is often bilobed in that genus) while the posterior pallial tentacle is housed in the posterior apertural channel (Ponder, 1965). The function of these tentacles is uncertain. They do not have mobile cilia (Ponder, 1965) but, at least in *Schwartziella* (Marcus & Marcus, 1964), both have stiff cilia, a feature in accord with a sensory function.

A duplicated peristome is found in several non-rissooid taxa, including *Anabathron* Frauenfeld, 1867 (Anabathridae), *Stosicia* (Zebinidae) and Lironobidae. Furthermore, the rissoids *Parashiela* Laseron, 1956, *Manzonina* Brusina, 1870 and *Simulamereina* Ponder, 1985 all occur within different clades, indicating the duplicated peristome, while being an uncommon feature of importance at a supra-specific level, is convergent within separate lineages (Fig. 3B).

The presence of an inner organic ('chitinous') layer was the only shell character used by Ponder (1988)

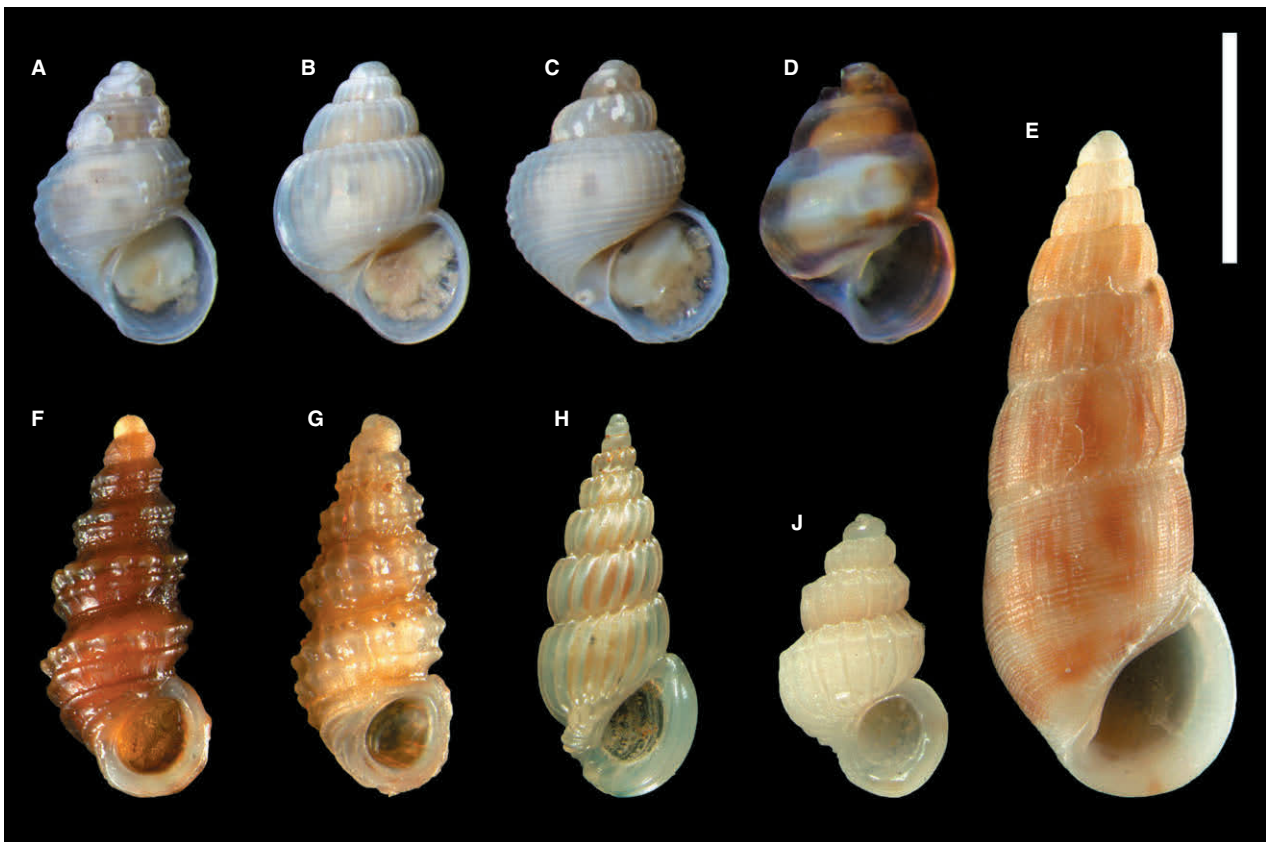


Figure 5. Shells of rissoid taxa included in the analysis and not figured elsewhere. A, '*Alvania*' cf. *akibai* (Yokoyama, 1926) (AORI YK1996). B, '*Alvania*' sp. (AORI YK1997). C, *Punctulum* cf. *flavum* (Okutani, 1964) (AORI YK1998). D, *Lucidestea* sp. (AORI YK1628). E, *Rissoina fasciata* Adams, 1852 (voucher AM C. 466913). F, *Merelina lyalliana* (Suter, 1898) (voucher AM C.466920). G, *Merelina taupoensis* Powell, 1938 (voucher AM C.466919). H, *Schwartziella* sp. (voucher AM C.466915). J, *Parashiela* sp. (voucher AM C.476714). Scalebar = 2mm. [Correction added on 14 June 2016, after first online publication: A scale bar has since been added on the top right of Figure 5.]

and was considered to have significant phylogenetic utility, being present in barleeiids and anabathrids, as well as in *Emblanda* Iredale, 1955 (Fig. 3C), although it is weakly developed in the last-named. Although an apparently identical layer is present in members of the Cingulopsoidea (e.g. Ponder, 1988), other caenogastropods lack this feature. Further detailed investigation is necessary to ascertain the homology of this structure.

Opercular characters

All of the taxa included have a horny, coiled operculum. While coiling and shape of the operculum were not considered important characters by Ponder (1988), he included two other opercular characters in his analysis, while a third (presence of calcareous deposits) is not relevant for the taxa included here. A peg on the inner surface of the operculum has originated independently in at least three lineages in the taxa included in the present analysis, the Barleeiidae, Rissoinidae and the Lironobidae [in *Lironoba australis* (Tenison-Woods, 1877) only, where it is rudimentary] and there is a small peg in *Lucidestea* Laseron, 1956 of Rissoidae (Fig. 3D). The morphology of the peg differs among the four clades, supporting its multiple origins. The barleeiids and rissoinids are of particular interest in that the peg is straight in the former family while its proximal end coils into the opercular nucleus in the latter family. In both groups the peg is large and extends well beyond the edge of the operculum. In rissoinids the operculum is yellow and paucispiral while in barleeiids it is red and concentric.

An operculum with two layers is restricted to the Anabathridae and Emblandidae (Supporting Information, Fig. S1A).

Mantle edge tentacle characters

The mantle edge is plesiomorphically simple but a pallial tentacle can occur on its anterior or posterior corner or on both (Fig. 3E). While there is some variation in the distribution of this character (Fig. 3E), it has some phylogenetic utility as two tentacles are present in Rissoinidae, most Zebinidae and in several taxa in the Rissoidae. Single anterior or posterior tentacles are also found in a few rissoids. Such mantle tentacles are found in several unrelated groups of caenogastropods so we do not treat them as having significant phylogenetic utility. However, the anterior tentacles, and rarely the posterior tentacles in Rissoinidae are often double or bilobed, a character not seen in other family-group taxa.

Head-foot characters

The shape of the cephalic tentacles is slender and either tapering or parallel-sided in all rissoidean

families, while the anabathrids have diagnostic paddle-shaped tentacles with conspicuous stationary cilia (Supporting Information, Fig. S1B).

Metapodial tentacles occur in some rissoideans and can be manifested as one broadly triangular tentacle, one single narrow tentacle (probably plesiomorphic) or multiple narrow tentacles (Fig. 3F). Broad triangular tentacles are seen in Rissoinidae and Zebinidae, and in the barleeiid *Fictonoba* Ponder, 1967 as well as in some rissoids (Ponder, 1985a, 1988). A single narrow tentacle is seen in rissoids of clade E and in *Manzonina* (clade F). At least some species of *Rissoa* (Ponder, 1985a, fig. 9C) have a metapodial tentacle with a broad base that tapers into a narrow tentacle. A narrow tentacle emerges from the foot behind the opercular lobe in *Parashiela*, *Voorwindia* Ponder, 1985 and *Lucidestea*. We are uncertain as whether this latter condition is present in some taxa of the rissoid clade E, so we have scored it for this clade as the narrow state as seen in the type species of *Rissoa* and *Pusillina*. Multiple metapodial tentacles are diagnostic of the clade including *Alvania*, *Crisilla* Monterosato, 1917, *Conalvinia* Ponder, 1967 and the Mediterranean '*Alvania*', with a reversal in *Cingula* Fleming, 1818.

A posterior mucous gland with a narrow opening in the middle of the foot sole is an apomorphy found only in Rissoidae of clades C, D and E, while anabathrids and *Barleeia* W. Clark, 1853 have a large metapodial gland with a long, slit-like furrow that runs from the middle of the sole to the posterior edge (Fig. 3G). A similar metapodial gland and furrow is seen in many cingulopsoideans and a number of other small caenogastropods but this condition is assumed to be apomorphic and convergent as most caenogastropods lack a distinct metapodial gland. The metapodial mucus gland is a structure that is usually correlated with living on or amongst algae, whereas, in the intertidal zone at least, small gastropod taxa that lack this structure are usually found under stones where they may live attached to the under surface or in the sediments beneath.

Radular characters

Most of the taxa included here have a single pair of basal cusps on the central (rachidian) radular tooth (Fig. 3H). These are duplicated in some lironobids (*Merelina* Iredale, 1915 and *Lironoba* Iredale, 1915) and in two rissoid genera in the analysis (*Lucidestea* and *Voorwindia*). Basal cusps are lost in the reduced central tooth of *Emblanda* which, very unusually for littorinimorph caenogastropods, also has only three teeth in each row, while all the other taxa included have a typical taenioglossan radula (seven teeth in each row).

Male genital system characters

An open prostate gland (Fig. 4A) is plesiomorphic (e.g. Ponder, 1988; Ponder & Lindberg, 1997). This condition is seen in the cerithioidian outgroup, in *Zebina* and in all the Rissoinidae. *Schwartziella* is scored as closed, but this is questionable (Ponder, 1985a). Lironobids (*Lironoba* + *Merelina*) also have open prostate glands.

A cephalic penis is present in all included ingroup taxa, and the penial morphology falls into three main categories: markedly swollen distally, straight and evenly tapering or parallel-sided, and tapering and coiled (Fig. 4B). The last condition is found only in one of the outgroups (Anabathridae). Distally swollen penes are found in the clade including Barleeiidae, Zebinidae and Rissoinidae with the exception of *Barleeia* and *Apataxia* Laseron, 1956 (Ponder, 1983a, 1985a). The only rissoid known with a distally swollen penis is *Conalvinia novarensis* (Frauenfeld, 1867) (Ponder, 1985a). Detailed histological comparisons are needed to further refine the morphology of these structures which we do not distinguish here.

The penial duct is either open (plesiomorphic – Ponder, 1988) or closed (Fig. 4C). The former condition largely tracks the open prostate with the exception of *Fictonoba*, *Stosicia* and *Schwartziella* having open ducts and closed prostate glands (Ponder, 1983a, 1985a).

Female genital system characters

As noted below, the upper oviduct gland is a key character used by Ponder (1985a, 1988) to define the Rissoidae (*s.l.*), and it occurs in all members of the Rissoidae, Rissoinidae, Lironobidae and Zebinidae (Fig. 4D). It is absent in Barleeiidae and is unknown in any other caenogastropod taxa so is clearly an apomorphic state. The function of this glandular structure is unknown and given its presence in rissoinids and rissoids a detailed investigation is needed to test its homology.

The ventral channel is a structure usually employed for the transfer of sperm to the posterior sperm sacs, and for the transport of fertilized eggs to the exterior. It can be in the form of a simple open groove (the plesiomorphic condition; e.g. Ponder, 1988), a closed channel with or without a distinct sperm groove, or the sperm groove is closed over to form a separate duct (the so-called spermathecal duct) (Fig. 4E). Convergence in this latter condition is seen in *Fictonoba* and *Anabathron* where the spermathecal duct is joined to the anterior vestibule while in *Barleeia* and *Pisinna* Monterosato, 1878 the short sperm duct opens to the posterior end of the mantle cavity. In *Rissoa* the sperm groove lies on the outside of the ventral channel, but it is inside the ventral channel in *Pusillina* (as it is in other

rissoids), indicating that this groove has closed in different ways in these two lineages at least. A major departure is observed in the enigmatic deep-water genus *Benthonella*, where the sperm duct opens to the kidney, and renal copulation presumably occurs – a phenomenon seen, probably convergently, in truncatellids, falscingulids and a few pomatiopsids (Ponder, 1988).

An anterior sperm sac is plesiomorphically absent (Ponder, 1988) but is convergently developed in some taxa (Fig. 4F). It can be formed from an expanded vestibule (anterior part of ventral channel) as in *Rissoina* and related taxa and, convergently, in some taxa similar to *Rissoa* (*Pusillina*, *Haurakia* and *Vitricithna*). The anterior sperm sac forms a separate structure in *Stosicia* (Zebinidae) and convergently in *Voorwindia* and *Lucidestea* (Rissoidae). Barleeiids lack an anterior sperm sac but *Barleeia* differs from all other taxa in the analysis in having a sperm sac formed from an anteriorly projecting arm of the albumen gland. In *Fictonoba* there is a posterior sperm sac (bursa copulatrix) that opens to the spermathecal tube (Ponder, 1983a).

The capsule gland opens to the exterior by a small anterior or subterminal opening in most taxa, but in Lironobidae it is either a long slit (as in *Lironoba*) or the capsule gland is open ventrally (as in *Merelina*) and the opening is more posterior in *Lucidestea* (Fig. 4G).

The bursa copulatrix is relatively large in many rissoids but tends to be smaller in rissoinids and zebinids. It is convergently absent in *Barleeia* and in *Voorwindia* (Fig. 4H).

TAXONOMIC IMPLICATIONS

Our phylogeny (Figs 1, 2) indicates that, in order to maintain monophyletic taxa, splitting the Rissoidae *s.l.* is inevitable unless well-established and morphologically highly distinct families are synonymized. Accordingly, the families Barleeiidae and Emblandiidae are maintained. The Rissoidae is restricted to the former Rissoinae (*sensu* Ponder, 1985a) with the exclusion of the genera *Merelina* and *Lironoba*. These two genera form a clade recognized here as a separate family for which the name Lironobidae Ponder, 1967 is available. Two distinct families are here recognized for the former Rissoininae (*sensu* Ponder, 1985a): Rissoinidae and Zebinidae.

Although all these family-level lineages are generally well supported by our molecular analyses (Figs 1, 2), some of them are separated by relatively short basal branches. In addition, our character history reconstruction (Figs 3, 4, Supporting Information, Fig. S1) reveals a substantial lack of clear-cut autapomorphies for some of these families. Based on

these results, the recognition of Rissoinidae, Barleeiidae and Zebinidae and also of Emblandidae and Lironobidae as separate families may appear questionable. However, our results show that each of those five family-group taxa exhibit unique combinations of characters (discussed below), which reflect a substantial amount of morphological differentiation and indicate their putatively long separate evolutionary histories. Given this morphological distinctiveness and its evolutionary significance we recognize all five as separate families, as summarized below.

FAMILY BARLEEIIDAE GRAY, 1857

A long straight opercular peg (Fig. 3D) and the presence of a spermathecal duct (Fig. 4E) are the two autapomorphies that clearly define this small family. Barleeiids are also the only rissooideans exhibiting a plesiomorphic lack of the upper oviduct gland (Fig. 4D). The Barleeiidae is represented in this analysis by two of the five recognized genera (Ponder, 1983a). In our tree, barleeiids are nested between Rissoinidae and Zebinidae from which they are morphologically distinct. Barleeiidae differ from rissoinids and zebinids by their generally smaller shells with simple peristomes, distinctly pitted protoconch, an inner organic shell layer and, usually, a posterior pedal gland with a slit reaching the posterior end of the foot (absent in *Fictonoba* only). Most lack metapodial and pallial tentacles (simple right pallial tentacle and short, triangular metapodial tentacle present in *Fictonoba*). Barleeiid opercula are distinctive in having on their inner sides a prominent straight peg and a thick longitudinal ridge. It is dark red in all but one taxon (*Protobarleeia* Ponder, 1983) and its growth pattern is subconcentric rather than spiral. While there are some similarities between *Fictonoba* and some rissoinids and zebinids, *Fictonoba* exhibits the distinctive apomorphies of Barleeiidae detailed above. *Fictonoba* also differs from rissoinids and zebinids in features such as the pallial bursa copulatrix [instead of a posterior (visceral) bursa], the lack of an upper oviduct gland and the ventral channel stripped from much of the capsule gland as a sperm tube (instead of being a simple ventral channel attached and open to the capsule gland). This sperm tube opens at the posterior end of the mantle cavity in *Barleeia*. A simple ventral channel is seen in one barleeiid genus, *Lirobarleeia* Ponder, 1983, which is not included in our analysis, but all other key features of that genus are typically barleeiid.

FAMILY RISSOINIDAE STIMPSON, 1865

Treated as a subfamily of Rissoidae by Ponder (1985a), this group was previously treated either as

a separate family (e.g. Laseron, 1956; Coan, 1964) or considered a junior synonym of the Rissoidae (e.g. Wenz, 1938–1944). In our analysis, it contains five species representing three genera. Rissoinids are characterized by two distinct autapomorphies: an anterior notch in the inner lip that forms a small channel without an embayment or excavation (Fig. 3A), and a long curved opercular peg (Fig. 3D). In particular, the opercular peg differs from that of the sister group Barleeiidae in having a spiral inner end and the operculum is paucispiral and yellow, not concentric and red as in the latter group. Rissoinids and barleeiids differ by a number of additional characters which are described above. The Rissoinidae differ from the Zebinidae in having a strongly pegged, thick yellowish operculum and a differently formed anterior channel (as described above) or this latter structure is absent in Zebinidae. The posterior chamber of the stomach is very long (Ponder, 1965, 1985a) (Supporting Information, Fig. S1C) and at least some rissoinids feed on forams (Ponder, 1965).

FAMILY ZEBINIDAE COAN, 1964

This name has been treated as a synonym of Rissoinidae ever since its introduction. They are similar to rissoinids in general shell morphology and anatomy but differ in having a shorter posterior stomach chamber (Supporting Information, Fig. S1C) and in having a thin operculum which lacks an opercular peg (Fig. 3D). Their shell aperture differs in having the inner lip lacking an anterior notch and has a simple, rounded anterior end or, as seen most noticeably in species of the genus *Stosicia*, an indistinct to distinct anterior canal may be present (e.g. Ponder, 1985a, fig. 142A, E) that is formed differently from the angulation in rissoinids.

In contrast to rissoinids, there is no evidence for anything other than a microphagous diet in zebinids, with all the examined taxa having a short stomach (Marcus & Marcus, 1964; Ponder, 1985a).

Zebinids and rissoinids have had long independent histories as there are records of both groups from the Jurassic (Ponder, 1985a; Conti *et al.*, 1993; Kaim, 2004).

FAMILY EMBLANDIDAE PONDER, 1985

This monotypic group was erected as a family by Ponder (1985b). In our analysis it is the basal member of a clade that also contains *Merelina* and *Lironoba* (Lironobidae) and together they are the sister taxon of the Rissoidae. It is distinguished from all other rissooideans in having a double-layered operculum (a character otherwise known from the

Anabathridae; Supporting Information, Fig. S1A), a radula that has only central and lateral teeth (i.e. three rows rather than seven as in all other rissoidaeans and truncatelloideans; Fig. 3H), and the jaws are lacking, a character unknown in other rissoidaeans. Additional autapomorphic characters include: a very narrow propodium; a very thick hypobranchial gland; a hypobranchial gland sheath for the penis; short anterior salivary glands; and tiny style sac (much shorter than in all other related taxa). The posterior chamber of the stomach is rather longer than in most rissoidaeans, although much shorter than in Rissoidae, so we have scored it as 'short' in Supporting Information, Fig. S1C. The female genital system was not studied in full by Ponder (1985b) and it is unknown whether the upper oviduct gland is present or absent.

FAMILY LIRONOBIDAE PONDER, 1967

This clade is made up of *Merelina* and one species attributed to *Lironoba*. Regrettably only shell characters are known for the New Zealand type species of *Lironoba*, *L. suteri* (Hedley, 1904), and it is by no means certain that the species attributed to *Lironoba* in this analysis (*L. australis*) is congeneric (Ponder, 1985a). If *L. suteri* should prove to belong to a different group, the family name Merelinidae Golikov & Starobogatov, 1975 is available. Species of *Merelina* and *Lironoba australis* share some unusual anatomical characters, including a penis with an open seminal groove (Fig. 4C) and a long slit-like opening in the capsule gland (Fig. 4G), features not known in Rissoidae as here recognized.

In most other anatomical features the group resembles the Rissoidae to which they were assigned (in the subfamily Rissoidae by Ponder (1985a)). Shell features in the group include strong spiral cords, sometimes also with axial ribs, as in *Merelina*, and usually the protoconch is sculptured with one or more spiral ridges that are gemmate in *Merelina*. The radula of members of this group has two pairs of basal cusps on the central tooth whereas most rissoids and rissoidaeans have only one pair.

Another poorly known Australasian genus, *Attenuata* Hedley, 1918, may be related to, or included in, this taxon. It shares the gemmate protoconch spirals with *Merelina* but has a very distinctive radula (Ponder, 1967, 1985a) with a very wide central tooth lacking basal cusps. It is unknown anatomically.

FAMILY RISSOIDAE GRAY, 1847

The taxa included in this analysis are a broad representation of this diverse, entirely marine family. Six main clades can be delimited in the phylogeny (A–F

in Figs 1, 2), and some genera have been found not to be monophyletic, as briefly discussed below.

Clade A contains four genera. One species, *Simulameralina* cf. *wanawana* (Kay, 1979), is attributed to *Simulameralina* following Hasegawa (2000) as it is rather similar to the type species, *S. corruga* (Laserson, 1956). This taxon was previously treated as a subgenus of *Alvinia* Monterosato, 1884 [type species *A. weinkauffi* (Weinkauff, 1868)] by Ponder (1985a). However, both taxa are found not to be intimately related. Consequently, we tentatively treat *Simulameralina* as a distinct genus, although acknowledging that examination of the type species is required to confirm this. Also included in this clade are the type species of *Subonoba* Iredale, 1915, *Austronoba* Powell, 1927 and *Subestea* Cotton, 1944. These taxa were included in *Onoba* H & A. Adams, 1852 (type species *O. semicostata* Montagu, 1803) 'somewhat tentatively' by Ponder (1985a), the first two as synonyms of *Onoba* s.s., and the third as a subgenus. The general shell morphology and anatomy of the taxa Ponder (1985a) attributed to *Onoba* are rather similar with their conical to elongate-conical shells bearing spiral threads and, sometimes, weak axial ridges. Their head-foot, radulae, opercula and anatomy are also all rather similar and these features may be plesiomorphic. For example, none has a posterior pedal gland and if a metapodial tentacle is present it is short and triangular. The type species of *Onoba* was included in our analysis but is widely separated and is the basal member of clade F (containing *Alvania* and related taxa). On the basis of these results we treat *Subonoba* and *Subestea* as distinct genera. Whether *Austronoba* should be maintained as a genus, a subgenus of *Subonoba* or a synonym will have to await further study. The only substantive difference is that *Austronoba* has a more slender shell with axial ridges.

Clade B is basal to a monophyletic group that includes clades C and D + E. It consists of two unidentified Japanese species (Hasegawa, 2005, fig. 5I, L) attributed to the deep-water genus *Benthonella* (type species *B. tenella* Jeffreys, 1869). The anatomy of the type species of *Benthonella* was described by Ponder (1985a) and is particularly unusual in that it appears to practise renal copulation. This unusual means of transferring sperm is not unique as it is also known, as noted above, in a few members of three other truncatelloidean families (Ponder, 1988).

Clade C is sister to the D + E clade and contains three unusual genera, all of the type species of which were included in the analysis: *Lucidestea* (type species *L. vitrea* Laserson, 1956), *Parashiela* (type species *P. ambulata* Laserson, 1956) and *Voorwindia* (type species *V. umbilicata* Ponder, 1985). These

taxa are all characterized by small, conical shells that range from being sculptured with axial and spiral ridges to fine spiral threads or being smooth. *Lucidestea* species, uniquely for Rissoidae, have a small peg on the inner side of the operculum. The central tooth of the radula of *Lucidestea* and *Voorwindia* has two pairs of cusps while that of *Parashiela* has only a single pair, as in most other rissoids. The radula of the European genus *Obtusella* Cossmann, 1921 is similar to that of *Lucidestea* but that taxon was not included in our analysis. These three included genera share a single short, narrow metapodial tentacle that emerges from the foot behind the opercular lobe. *Lucidestea*, *Voorwindia* and *Obtusella* also have a large anterior sperm sac, another unique feature within Rissoidae. The female anatomy of *Parashiela* has not been studied.

Clade D includes *Setia turriculata* Monterosato, 1884, *Haurakia hamiltoni* Suter, 1898 and *Vitricithna marmorata* (Hedley, 1907); the latter two species represent the type species of *Haurakia* and *Vitricithna*, respectively. Ponder (1985a) treated *Vitricithna* as a synonym of *Haurakia*, which itself was given subgeneric status within *Pusillina* [type species *P. dolium* (Nyst, 1843)]. This treatment is not supported in the molecular analysis of this study (Figs 1, 2) and *Haurakia* and *Vitricithna* are here treated as distinct genera. Pending more taxon sampling, we tentatively treat *S. turriculata* as being more typical of *Setia* (type species *S. pulcherrima* Jeffreys, 1848) than the other species in our analysis, '*S. ambigua*' (Brugnone, 1873), which is also usually included in that genus.

Clade E contains several species attributed to *Rissoa* (type species *R. ventricosa* Desmarest, 1814) and *Pusillina*, as well as '*Setia ambigua*'. There are two groups within this subclade in the BI tree; one includes *Rissoa lia* Monterosato, 1884 (the type species of *Liavenustia* Nordsieck, 1972), synonymized with *Rissoa* by Ponder (1985a) and *R. variabilis* Megerle von Mühlfeld, 1824 and four species attributed to *Pusillina*, none of which is the type species. *Pusillina inconspicua* is the type species of *Mutiturbocella* Nordsieck, 1972, and *P. radiata* is the type species of *Radiata* Nordsieck, 1972, both of which were treated as synonyms of *Pusillina* by Ponder (1985a). In the ML tree, *R. variabilis* is included in the second group with the other species of *Rissoa*.

The second group includes seven species attributed to *Rissoa*, including the type species and the types of seven other taxon names included in the synonymy of *Rissoa* by Ponder (1985a), and '*Setia ambigua*'. This latter species has a smooth, transparent, elongately conical simple shell that resembles a few other species included in *Setia* but is rather different from the type species of that genus, as indicated above.

Because our analysis did not include the type species either of *Pusillina* or of *Setia*, it is not possible to make definitive comments on the validity of these taxon names. We recommend leaving the status quo given that our results are not clear cut, but with the clear realization that the *Rissoa*-group of taxa needs revision.

Clade F contains several subclades which are consistent in both of our analyses. A basal branch, which is the sister to the rest of the clade, contains several taxa from Japanese waters, some of which are unidentified. These include the shallow-water '*Alvania concinna*' (A. Adams, 1861) and several deep-water species that were recently reviewed by Hasegawa (2014). These latter are '*Alvania akibai*' (Yokoyama, 1926) (Fig. 5A), *Frigidoalvania asura* (Yokoyama, 1926), *Punctulum flavum* (Okutani, 1964), *Punctulum* cf. *flavum* (Fig. 5C) and *P. tanshumaruae* Hasegawa, 2014. These taxa are all rather similar in having broad shells, most have axial and/or spiral ribs and some have a thick periostracum. Given that they are all very similar in our analysis, we suggest that they should all be referred to *Punctulum* Jeffreys, 1884 which, on the basis of this result, might include *Frigidoalvania* Warén, 1974 (type species *Rissoa janmayeni* Friele, 1878) as a synonym. There are, however, some differences between the type species of these two genera, for example some details of the anatomy and the metapodial tentacles (see Ponder, 1985a) so we do not formally synonymize them here.

Based on the molecular results, '*Alvania concinna*' is clearly not a member of the genus *Alvania* Risso, 1826 and there is no generic name that is suitable for it. *Alvania rudis* (Philippi, 1844), the type species of *Thapsiella* Fischer, 1885, has a somewhat similar, tall shell but it does not agree well in other shell characters so we do not include it there.

At the base of the branch including the remaining taxa in clade F are: a species attributed to *Simulamerelina*, '*S. tokunagai*' (Yokoyama, 1927), *Onoba semicostata* (Montagu, 1803), the type species of *Onoba*, *Manzonina crassa* (Kanmacher in J. Adams, 1798) the type species of *Manzonina* Brusina, 1870 and '*Alvania*' sp. (Fig. 5B), which does not fit any named genus.

Many of the remaining taxa are currently placed within *Alvania* as recognized by Ponder (1985a) but this genus is rendered polyphyletic by the inclusion of other clearly distinct lineages. The results are detailed below but final taxonomic decisions must await better taxon sampling, and in particular the inclusion of type species of key genus-group names.

The next clade contains four species of *Alvania*, including the type species, *A. cimex* (Linnaeus, 1758). The next branch is *Cingula trifasciata*

(J. Adams, 1800) (the type species of *Cingula* Fleming, 1818) and then two species attributed to *Crisilla*, but not the type species, *C. semistriata* (Montagu, 1808). *Crisilla* was treated as a subgenus of *Alvania* by Ponder (1985a). The next clade contains three species, '*Alvania*' *circinata* A. Adams, 1861, a spirally ribbed species lacking axial sculpture. It resembles *Alvania hedleyi* Thiele, 1930 from Western Australia but no generic name is available for these taxa. The taxon *Conalvinia* is available for the remaining two taxa, the type species *Conalvinia novarensis* and *C. ogasawarana* (Pilsbry, 1904).

'*Alvania*' *tenera* (Philippi, 1844) is then sister to the final clade which includes five species currently attributed to *Alvania*. '*Alvania*' *tenera* is a broad species with a conical shell sculptured with spiral ribs and weaker axial threads. The remaining five species include '*A.*' *discors* (Allan, 1818), '*A.*' *lanciae* (Calcara, 1845), '*A.*' *scabra* (Philippi, 1844), '*A.*' *aeoliae* Palazzi, 1988 and '*A.*' *lineata* Risso, 1826. Of those, all have conical shells with strong axial ribs with weaker spirals, with '*A.*' *scabra* being distinctive in having a more pagodiform outline. It is the type species of *Alvaniella* Sacco, 1895, which is the earliest genus-group name that could be used for this subclade. '*Alvania*' *lineata* is the type species of *Alvanolira* Nordsieck, 1972.

ORIGIN OF DEEP-SEA RISSOIDS

Our molecular phylogeny suggests that the Rissoidae originated in the shallow sea and independently radiated into bathyal waters at least twice (Figs 1, 2). Bathyal rissoids have been assigned to several genera including *Benthonella*, *Frigidoalvania* Warén, 1974, *Onoba*, *Powellisetia* Ponder, 1965, *Punctulum* and *Pusillina*, as well as to the polyphyletic *Alvania* (Warén, 1974, 1996b; Ponder, 1983b; Hasegawa, 2005, 2014). The seven bathyal species studied herein (459–1919 m; Supporting Information, Table S1) constitute clade B (*Benthonella*) and a subclade of clade F ('*Alvania*', *Frigidoalvania* and *Punctulum*; Figs 1, 2). One of the two studied species of *Benthonella* had previously been placed in *Alvania* (Hasegawa, 2005, fig. 5I) suggesting that the latter genus contains distantly related lineages. Repeated invasion of the bathyal zone has been documented for other gastropod families of shallow water origin (e.g. Williams *et al.*, 2013).

The species of *Benthonella* have a relatively large, thin shell with a simple outer lip of the aperture (Ponder, 1985a). This contrasts with the smaller and more solid shells with a more or less thickened outer lip that characterize *Punctulum*, *Frigidoalvania* and '*Alvania*' in clade F (Fig. 5A; Hasegawa, 2014, figs 2–48). The two deep-sea clades also differ in their

biogeographical and bathymetric distributions. *Benthonella* species have been reported from low- to high-latitude seas (Ponder, 1985a; Warén, 1996b; Lozouet, 2014) with their depth ranges extending to the lower abyssal plain (Rex & Etter, 1990). On the other hand, the bathyal species in clade F are components of more nutrient-rich waters off northern Japan under the influence of the south-flowing Kuril (Oyashio) Current. Most bathyal rissoids in the North Atlantic fall into the latter clade (Warén, 1974, 1996b).

The lack of pigmented eyes is among the best-documented morphological features of deep-sea gastropods. Apomorphic loss of the retinal pigmentation may occur in rather short periods of time (i.e. a few million years; Williams *et al.*, 2013). All species of *Benthonella*, *Punctulum* and *Frigidoalvania* so far investigated lack pigmented eyes (Ponder, 1985a; Hasegawa, 2014) but '*Alvania*' cf. *akibai* retains eye pigmentation (Hasegawa, 2014), despite its co-occurrence with *F. asura* with unpigmented eyes in the same sample (Supporting Information, Table S1). The phylogenetic position of '*A.*' cf. *akibai* basal to *Punctulum* and *Frigidoalvania*, accompanied by small genetic distances among these taxa, implies that the apomorphic loss of the pigmentation occurred only once, and rather recently, in this bathyal subclade of clade F (Figs 1, 2).

MONOPHYLY OF RISSOIDEA

Addressing the question of the monophyly of the Rissoidae was not the main focus of this study. However, given that our molecular analyses are based on the most complete sampling available for this superfamily, and as some results are in conflict with those of other studies, this work may shed light on this yet controversial issue. The superfamily Rissoidae was maintained by Criscione & Ponder (2013, fig. 2) with *Hebeulima* (Vanikoroidea: Eulimidae) as a sister to the rissoid–barleeiid clade. A rissoid–eulimid clade was retrieved in nearly all previous caenogastropod phylogenies (Colgan *et al.*, 2007; Ponder *et al.*, 2008). However, those studies included no other rissoid family. Subsets of the rissoid families were included as outgroups in two molecular studies of other caenogastropod groups (Wilke *et al.*, 2013; Takano & Kano, 2014). In a phylogeny of the 'hydrobioids' (Truncatelloidea), *Barleeia* and Rissoidae were found to be only distantly related (Wilke *et al.*, 2013). In a molecular phylogeny of the Eulimidae, rissoidaeans clustered together in a monophyletic group when five genes were used (Takano & Kano, 2014, fig. 2). However, a tree based on two genes did not support a close relationship of the rissoids with rissoidids, zebinids and barleeiids (Takano & Kano, 2014, fig. 1); the latter three families were

more closely related to Eulimidae and Vanikoridae, albeit without including Emblandidae or Lironobidae in the analysis. Although with relatively weak support, our phylogenies (Figs 1, 2) suggest a monophyletic Risssooidea that is only distantly related to Eulimidae. However, the monophyly of this diverse superfamily can only be investigated with a more comprehensive sampling of its component genera, by including several critical outgroup taxa (e.g. Eulimidae and Vanikoridae), and ideally by combining multi-gene phylogenies with an assessment of key morphological traits. Accordingly, we maintain the tentative recognition of this superfamily as distinct from the Eulimoidea, in accordance with Criscione & Ponder (2013).

CONCLUDING REMARKS

This study represents the first attempt to investigate the relationships within the Risssooidea in a cladistic phylogenetic framework. By producing a phylogeny based on molecular data from the largest number of risssooidean samples to date, we unearthed considerable amounts of previously undetected diversity within the Risssooidea, challenging the current, exclusively phenetic, systematics of the group. Our work is only a glimpse of the evolution of one of the oldest, most widespread megadiverse groups within the Caenogastropoda, which still remains largely neglected. Further studies, based on better taxon sampling and larger amounts of molecular data than the present one, are required to improve the understanding of the risssooidean systematics and evolution.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article:

Figure S1. Maximum-likelihood-based ancestral character state reconstruction for anatomical characters mapped on the Bayesian consensus tree of Figure 1.

Table S1. Museum registration numbers, voucher status and GenBank accession numbers of rissooidean samples included in the molecular analysis. For details of non-rissooidean samples see Criscione & Ponder (2013).

Table S2. Museum registration numbers, voucher status and GenBank accession numbers of non-rissooidean samples included in the molecular analysis.

Table S3. Matrix of anatomical characters used in the Maximum-likelihood-based ancestral character state reconstruction illustrated in Figs 3–4, S1.