

Phylogenetic reconstruction and taxonomy of *Retinella* land snails: congruence and conflicts across morphological, mitochondrial, and nuclear datasets

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Abstract

Robust phylogenetic inference requires character data that reliably reflect evolutionary change, yet not all data streams are equally informative. We address this issue through a comparative analysis of the land snail genera *Aegopinella* and *Retinella*. Using all extant species, we contrast genital and shell morphology with three DNA datasets: mitochondrial markers, the multicopy nuclear ribosomal internal transcribed spacer (ITS) region, and two newly developed single-copy nuclear (SCN) loci. All datasets recovered well-supported, reciprocally monophyletic species-level clades, but their ability to resolve deeper relationships differed markedly. SCN data provided the most consistently resolved topology and showed the greatest congruence with independent morphological and fossil evidence. Mitochondrial data recovered a similar topology but with weaker support for deeper nodes and unresolved relationships. In contrast, ITS failed to resolve key subgeneric relationships and produce implausible divergence time estimates. Morphological traits also varied in phylogenetic utility. Genitalic characters were highly informative at the species level, whereas most shell traits were strongly homoplasious. Shell microsculpture, however, retained signal across deeper timescales. Our results support synonymizing the two genera under *Retinella* and the description of a new species, *Retinella bodoni* Bichain, Nardi & Ryelandt sp. nov., while highlighting the utility of targeted SCN loci for genus-to-species-level phylogenetic reconstruction in morphologically complex land snails.

Keywords Gastropoda, phylogeny, morphology, genetics, homoplasy

Introduction

Inference of evolutionary relationships depends strongly on the properties and limitations of the character data being analysed. Early cladistic analyses were limited to observable macro-scale character states selected through expert judgment, but these were subjective and prone to homoplasy. For

instance, in land snails, initial efforts extending back to the 1800s relied exclusively on shell morphology, yet most shell traits evolve rapidly under strong selection and rarely represent synapomorphies. Analyses based on 14 shell characters with 71 states in North American polygyrids show greatly reduced resolution and major topological conflicts compared

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to datasets including anatomy and behaviour (Emberton 1995). Similarly, even though all *Vertigo* species can be reliably identified via shell characters, homoplasy prevents the use of these traits in diagnosing subgenera (Nekola *et al.* 2018). Moreover, traditional, easily-observed shell characters may reflect intraspecific variation rather than species- or genus-level taxonomic differences (Teshima *et al.* 2003, Hirano *et al.* 2014, Nekola *et al.* 2026).

Anatomical characters, incorporated by snail workers since the early 1900s, have proven similarly problematic. Radular traits can vary with ontogeny (Bertsch 1976) and are also often under strong ecological selection pressure (Kesler *et al.* 1986). Genital traits are likewise limited in their inferential ability: unlike arthropods, snails lack a hard exoskeleton, so lock-and-key mechanisms are not possible. In addition, genital morphology can change across ontogeny (Emberton 1985) and is prone to observational error due to variation in specimen preparation (Emberton 1989). Anatomical traits thus also often fail to reflect genetic relationships; for instance, some genitalic characters assumed to define subgenera have turned out to only represent intraspecific variability (Roth *et al.* 2013). Consequently, up to 50% of currently accepted species delimitations based on traditional shell and anatomical criteria do not correspond to genetically supported groupings (Nekola and Horsák 2022).

The advent of DNA sequencing has transformed phylogenetics by providing large amounts of reproducible character data that can be used to infer evolutionary relationships. However, commonly used mtDNA markers (e.g. 16S, *COI*, *CytB*) are limited by high saturation of substitutions, mitochondrial introgression, and strictly matrilineal inheritance, which can obscure deeper relationships and generate geographically structured but taxonomically uninformative clades (Nekola *et al.* 2015, 2018). Nuclear DNA use has been largely limited to components of the rRNA gene cassette, which possesses hundreds of copies distributed across all chromosomes (Long and Dawid 1980). Unfortunately, its 18S and 28S regions are so highly conserved that they represent Ultra Conserved Regions (Bejerano *et al.* 2004) that provide sufficient signal only for resolution of generic and deeper relationships (e.g. Nekola and Coles 2016). Although the greater variability of concatenated *ITS1* + *ITS2* (Wade and Mordan 2000, Armbruster and Bernhard 2000) can enable species-level resolution (Nekola *et al.* 2018, Horsáková *et al.* 2020, Horsák *et al.* 2026), accurate and phylogenetically relevant indel alignment is limited to closely related taxa (Horsák *et al.* 2026). Sequences may also demonstrate variation between copies within a single individual, likely reflecting different cassette histories (Nekola *et al.* 2018). These factors limit the ability of *ITS1* + *ITS2* to reliably reconstruct phylogeny and resolve taxonomic groups.

Single-copy, protein-coding nuclear genes provide a promising alternative, as reading-frame constraints are maintained while potential copy-related ambiguities are eliminated. However, their use in land snails has been hindered by a genome-wide duplication event in a common ancestor during the Cretaceous (Au *et al.* 2025), which has generated multiple paralogues for most genes. Combined with the relatively rapid evolutionary

rates for most nuclear loci, until now there has been no way to access their information short of conducting whole-genome next-generation sequencing (NGS) analyses. Recent work has identified dozens of single-copy, protein-coding loci across all linkage groups/chromosomes which can be amplified across a diverse range of land snail taxa via PCR (Nekola *et al.* in review). This enables, for the first time, direct comparison of the phylogenetic patterns and resolution provided by mtDNA, multi-copy nDNA, and single-copy nDNA loci. These data can also be used to assess the evolutionary relevance of traditional shell and genitalic traits, something that has only rarely been conducted (e.g. Collin 2003) in gastropods.

We undertake this analysis by focusing on the currently recognized species of *Aegopinella* and *Retinella*, two related land snail genera restricted to western Eurasia and adjacent Macaronesian archipelagos. Using two mitochondrial, two multi-copy nuclear, and two single-copy nuclear markers, we separately reconstruct their phylogenies, allowing us to document their relative robustness. We also consider traditionally-used conchological and genitalic character states, with character-state transformations mapped onto the molecular phylogenetic hypotheses. From this we conduct maximum-parsimony analyses to assess which morphological character patterns, if any, are congruent with the molecular phylogenetic reconstructions.

Materials and methods

Study system and sample collection

Two closely related terrestrial gastropod genera, *Aegopinella* Lindholm, 1927 and *Retinella* P. Fischer, 1877 (e.g. Ehrmann 1933, Ložek 1955), were selected as our model systems. Their shells exhibit highly similar general morphology, being flattened, possessing a distinct umbilicus and rapidly expanding final whorl, while lacking internal barriers (Welter-Schultes 2012). Shell diameter, microsculpture, colour, and genital anatomy have been primarily used to diagnose species (Forcart 1957, 1959, Riedel 1980, 1983, Kerney *et al.* 1983, Giusti *et al.* 1986, Horsák *et al.* 2013). While most species are easily separable, some are not (Alonso *et al.* 2013, Horsák *et al.* 2013). We obtained tissue samples from all extant nominal species listed by MolluscaBase (2025) from both genera except for *Retinella osoriensis* (Wollaston, 1878) and *Retinella rochebruni* (J. Mabille, 1882) from the Canary Islands. We could not locate tissue samples for these taxa, and strongly suspect, based on the other three Canarian taxa, that they represent junior synonyms of *Retinella circumscissa* (Shuttleworth, 1852). We have not attempted to obtain tissue samples from the Galapagos *Retinella actinophora* (Dall, 1900) as its upper shell surface microsculpture is characteristic of *Lapa*, *Nesovitrea*, and *Perpolita* (Saito *et al.* 2025). Given its geographical distribution, this is likely the third species of the *Lapa* lineage (Saito *et al.* 2025). *Hyalina* (*Mesomphix*) *semisculpta* Mousson, 1876, from the Persian shore of the Caspian Sea, is currently listed in MolluscaBase (2025) with uncertain placement in *Retinella*. However, the shell shape and, in particular, the characteristic reticulate microsculpture described for this species render its

assignment to *Retinella* unlikely. Given that several sympatric species of the related genus *Oxychilus* exhibit comparable reticulated sculpture on the upper shell surface, placement in *Oxychilus* appears more plausible, although confirmation requires anatomical or molecular data. We selected four species within the closely related genus *Perpolita* H.B. Baker, 1928 (Saito *et al.* 2025) to function as an outgroup.

DNA isolation and amplification

Individuals chosen for DNA extraction were sourced from across the geographic and ecological range of each species (Fig. 1), while attempting to include material from at or near ($\sim < 150$ km radius) each type locality (Supplementary Table S1). DNA was extracted from ~ 2 mm³ samples of naturally air-dried soft body (i.e. mummified) or ethanol-fixed foot

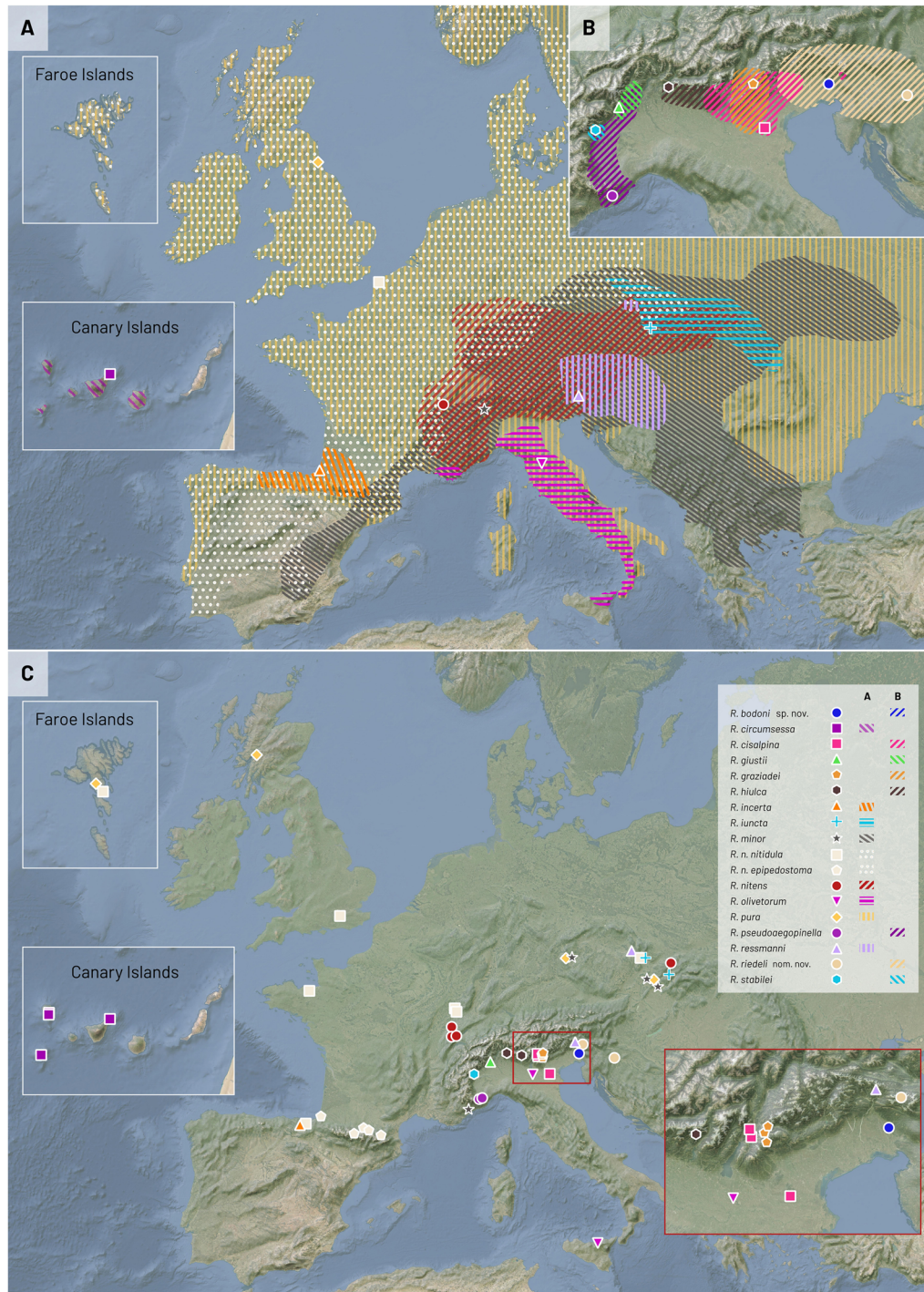


Figure 1 Schematic distribution of the studied *Retinella* taxa, showing the type localities of taxa defined by the revision (A, B) and the locations of 51 *Retinella* individuals included in the phylogenetic analyses (C). Panel B shows the distribution and type localities of Alpine species with restricted ranges. Species distributions were compiled from the available literature and from our field records beyond those represented by the samples included in the analyses. Made with Natural Earth (naturalearthdata.com).

Table 1 Forward (F) and reverse (R) primer sequences used for nested PCR of newly designed single-copy nuclear (SCN) loci in this study, with anneal temperatures and extensions: (Fo) forward primer sequence for outer steps of nested PCR; (Ro) reverse primer sequence for outer steps; (Fi) forward primer sequence for inner steps; (Ri) reverse primer sequence for inner steps. Asterisk: used for sequencing.

Region	Sequence	Anneal (°C)	Extension (s)
KYP1 (Fo)	5'-TTYGTCATYAAAWTRARRGC-3'	41	60
KYP1 (Fi)*	5'-TTTATYCAGACHCGDGGHGA-3'	50	45
KYP1 (Ri)*	5'-ACDGCRTAWAGTCCYTTVCC-3'	50	45
KYP1 (Ro)	5'-TACTGWAGYARYGAYGARTA-3'	41	60
LOC8662 (Fo)	5'-TAYCCDTCHATCTACGKTC-3'	45	60
LOC8662 (Fi)*	5'-ATYTACGKTCTCYAGYTCBGG-3'	45	60
LOC8662 (Ri)*	5'-TCCATYTTBCCRTTRCADA-3'	45	60
LOC8662 (Ro)	5'-TCRTCRAADCCRCGYTCCAT-3'	45	60

tissue using the E.Z.N.A. Mollusc DNA Kit (Omega BioTek). Extractions were stored at -80°C .

PCR amplification and Sanger sequencing were performed on two mitochondrial (16S, *COI*), two multi-copy nuclear (*ITS1*, *ITS2*), and two single-copy [*Kyphoscoliosis Peptidase 1* (*KYP1*) and *LOC106068662* (*LOC8662*)] nuclear loci. *KYP1* is located on Linkage Group 1 of *Achatina immaculata* and is involved in the function, maturation, and stabilization of the neuromuscular junction and may be required for normal muscle growth. *LOC8662* is resident on Linkage Group 5 of *A. immaculata*, and codes for an unidentified protein. These loci were selected from the 60 available due to their amplification success in *Aegopinella* (i.e. the crown group of *Politenella*, see below) combined with high interspecific base pair variation (4–40 bases; Nekola *et al.* in review).

PCR amplifications of 16S, *COI*, *ITS1*, and *ITS2* were conducted using standard protocols (e.g. Saito *et al.* 2025). However, a two-step nested PCR amplification approach (van Pelt-Verkuil *et al.* 2008) was used for both single-copy nuclear loci using primer designs and cycling conditions elaborated in Table 1. Nested PCR was used because of the greatly reduced concentration of target DNA in genomic extractions, as compared to multi-copy mtDNA and nDNA loci, which renders PCR chain reaction initiation unpredictable. Secondary amplification of this initial product using an interior primer set, however, makes generation of target sequence amplicons very likely while at the same time filtering out off-target material.

All PCR products were cleaned and sequenced by SEQme s.r.o., Dobříš, Czech Republic (see Horsáková *et al.* 2019). Forward and reverse strands were assembled in ChromasPro 2.1.8 (Technelysium Pty Ltd.) and checked by eye for potential errors. Sequence alignment was done with MAFFT v.7 using the Q-INS-i algorithm (Katoh and Toh 2008). All alignments were inspected for potential errors and corrected by eye. Amino acid translations of *COI*, *KYP1*, and *LOC8662* products were used to ensure that the target sequence amplified, and erroneous stop codons were absent. GenBank accession numbers for all sequences used in phylogenetic analysis are provided in Supplementary Table S1.

Molecular phylogenetic analysis

Initial maximum-likelihood trees were run separately on each locus and visually inspected to ensure that their topologies were

concordant. Phylogenetic reconstructions were then separately generated for concatenated mitochondrial (16S + *COI*; referred to as 'MT'), multi-copy nuclear (*ITS1* + *ITS2*; 'ITS'), and single copy nuclear (*KYP1* + *LOC8662*; 'SCN') loci. Indel size and placement information in the ITS construct was formally coded following Simmons and Ochoterena (2000), but we also ran the analyses without indel consideration to explore their influence on the resulting phylogenetic reconstructions. All three alignments were subjected to maximum likelihood (ML), Bayesian inference (BI), and maximum parsimony (MP) analyses. ML analysis was carried out in RAxML v.8.2 (Stamatakis 2014), using 500 search replicates. The GTR + G nucleotide substitution model was used based on jModelTest 2 (Darriba *et al.* 2012). Internal node support was assessed via 1000 non-parametric bootstrap replicates (Felsenstein 1985). BI was conducted using a Metropolis-coupled Markov chain Monte Carlo algorithm, with four parallel runs, each with four concurrent chains (one cold and three hot) over 5 000 000 generations; performed using MrBayes v.3.2.6 (Huelsenbeck and Ronquist 2001). Trees were sampled every 100 generations, and the first 25% of trees were discarded as 'burn-in' after confirming the standard deviation split frequency dropped below 0.01. The convergence of evolutionary model parameters was verified with Tracer v.1.7.1 (Rambaut *et al.* 2018). MP analysis was conducted in PAUP* v.4.0a168 (Swofford 2002), with support values being calculated via 1000 bootstrap replicates. A given node was considered to exist when it possessed support ≥ 95 in BI, and > 70 in ML and MP. Genetic support for morphologically delimited species was considered strong when the corresponding individuals formed highly supported species-level clades across all three molecular trees. Because ML and BI returned almost identical topologies for supported nodes, we chose ML to visualize trees, flipping/swapping resultant SCN, ITS, and MT subtrees to generate maximum visual correspondence.

Divergence time estimates

We used both SCN and ITS constructs for the divergence time estimation. BEAST2 (Bouckaert *et al.* 2014), using the same evolutionary models as BI (see above), was conducted with the optimized relaxed clock model (Douglas *et al.* 2021) and four fossil calibrations. The oldest shell with characteristics comparable to modern *Retinella/Aegopinella* (calibration 1) appears to be *Retinella* (*Lyrodiscus*) *subcellaria* (Thomä, 1845) from the

Oligocene of the Mainz Basin (Hochheim). It has subsequently also been reported from the Paris Basin, including Fontainebleau, where it occurs in the Calcaire d'Étampes deposits (Wenz 1914). These strata are assigned to the Rupelian stage of the Oligocene (~29.8 Mya; Harzhauser and Neubauer 2021). A second calibration point (**calibration 2**) is the Early Miocene *Aegopinella denudata* (Reuss in Reuss & Meyer 1849), from the Tann assemblage (Rhön, Germany). This is assigned to the Early to Middle Burdigalian (~20.0 Mya; Harzhauser et al. 2014, Harzhauser and Neubauer 2021). We place *A. denudata* within the subgenus *Politenella* because of its open umbilicus, rapidly expanding last whorl, wide and elongate-ovoid aperture, delicate microscopic spiral striation, and shell diameter exceeding 10 mm (Harzhauser et al. 2014). *Aegopinella vetusta* (Klika, 1891), from the Turoňice assemblage (Most Basin, Czech Republic), may represent an additional *Politenella* fossil species. This deposit correlates with European Mammal Zone MN3b (Early Burdigalian; 18.4 Mya). The lectotype of *Aegopinella subnitens* (Klein, 1853) was used as the third calibration point (**calibration 3**). It was recovered from the Silvana-Beds of Zwiefalten (Germany) from Late Burdigalian–Early Langhian times (~15.9 Mya; Harzhauser and Neubauer 2021). This taxon is assigned to *Aegopinella* based on its relatively deep suture, moderately expanding last whorl, wide umbilicus, and small shell size (diameter = 5.6 mm; Salvador et al. 2016). Finally, *Retinella (Lyrodiscus) jourdani* (Michaud, 1862), originally described from Rhône Valley (France) sediments, was used to constrain the initial appearance of subgenus *Lyrodiscus* to ~5 Mya. This taxon possesses the diagnostic microsculpture of the subgenus consisting of alternating fine and stronger spiral threads producing a punctate surface. Because older fossils assigned to this subgenus [e.g. *Retinella (Lyrodiscus) circumscisa* (Gottschick, 1920)] lack this sculpture, we do not consider them to actually represent this subgenus. The MIS 11 (~400 kya) occurrence of *Retinella (Lyrodiscus) elephantium* (Bourguignat, 1869) from northern France (Limondin-Lozouet and Antoine 2006) provides the most recent occurrence of the subgenus from continental Europe (**calibration 4**).

For calibrations 1–3, lognormal priors were set with mean in real space (M) = 2 and log-space standard deviation (S) = 1. For offset settings, we used the age of the oldest fossils: 29.8 Mya, 20.0 Mya, 15.9 Mya (i.e. calibration 1–3). For calibration 4, because colonization of *Lyrodiscus* in the Canary Islands could have occurred anytime between the subgenus initial European mainland occurrence and ultimate extinction, and with timing of archipelago colonization being unknown, we assumed a uniform probability distribution ranging from 5.0–0.4 Mya.

Anatomical trait matrix

Genital anatomy was assessed for all species distinguished in the molecular analyses, using the same individuals or those originating from a sequenced population. *Perpolita hammonis* (Strøm, 1765) was chosen as the outgroup. While terminology is largely based on previous studies (particularly Forcart 1957, 1959, Riedel 1957, 1980, 1983, 1998, Hudec 1964, Giusti et al. 1986, and Alonso et al. 2013) in these works, multiple terms are sometimes used for the same or closely comparable

structures. We thus standardized trait nomenclature, using Giusti et al. (1986) and Gómez (2001) as principal sources. Because of this we use 'proximal' to indicate a position near the gonad, and 'distal' a position farther away.

Dissections were performed under a stereomicroscope using 0.06 mm forceps in a 60:30:10% water:ethanol:glycerine solution. Reproductive systems were removed, photographed, and converted into line drawings. Other illustrations were produced from photographs or literature, as appropriate. For each species, from 5 to 20 individuals were examined when sufficient material was available. When fewer specimens were available, observations were supplemented by original descriptions, subsequent anatomical redescriptions, and published illustrations. Character states were scored based on consistent differences among specimens, with observed intraspecific variation coded as polymorphism.

The final genital trait matrix comprised 17 species and 31 characters (Supplementary Table S2). Female traits include presence/absence of glandular tissue and the relative length and shape of the vagina, free oviduct, bursa copulatrix, and bursa copulatrix duct. Male traits include position of the penial retractor muscle, the external morphology of the penis, and the structure of the vas deferens–epiphallus transition. Additionally, the internal structure of the penis was examined to identify subdivisions and distinctive characters, such as folds of the inner wall. Each anatomical character was classified into up to four states defined from comparative observations and coded as categorical responses. Ordered characters represent states occurring along a biologically meaningful gradient or sequence, such as ratios, size differences, degrees of elongation, or increasing structural complexity. Unordered characters represent qualitatively different states with no assumed evolutionary sequence. In these cases, intermediate values do not exist and transitions have equal cost. Polymorphisms were noted when more than one state was observed within a species. Inapplicable conditions were coded as missing data.

Conchological trait matrix

A representative set of 35 shells from genetically validated samples, mostly the same set as that used for genitalic anatomy observations, were imaged in top, bottom, and front views with an Olympus SZX10 microscope, Olympus C-7070 Wide Zoom camera, and QuickPHOTO MICRO software at c. 20×. For *Retinella bodoni*, a Leica Z16 APO macroscope with a Leica K3C camera and Leica Application Suite X (LAS X) software was used. Seven conchological characters: adult median shell width and height, number of whorls, presence of a shoulder on shell periphery, and three upper shell microsculpture features: lustre, presence/absence of microsculptural spiral striae ('sil-lons') cut into the upper shell surface, and periostracal ridges were recorded. Character states were coded as either ordered or unordered categorical responses, with polymorphism being allowed (Supplementary Table S3). When their states represented a biologically meaningful gradient or sequential transformation, characters were coded as ordered, whereas those representing qualitatively distinct states without an inherent sequence were treated as unordered.

Maximum parsimony, ancestral characters, and phylogenetic congruence

PAUP* v.4.0a168 (Swofford 2002) was used to conduct maximum-parsimony reconstructions using anatomical character states. Support values were calculated via bootstrapping with 1000 replicates. Mesquite v.4.02 (Maddison and Maddison 2025) was used to help reconstruct ancestral states by mapping character states onto the ML and divergence time-estimated phylogenetic SCN reconstructions. All character changes were estimated via parsimony, with the number of steps required to achieve the ancestral state being calculated. Parsimony was selected because it allowed ordered characters to be constrained to biologically meaningful transition pathways.

Topological differences between each phylogeny were calculated using Robinson–Foulds and quartet distances (Robinson and Foulds 1981, Estabrook *et al.* 1985) based on 10 000 replicates from each 1000-replicate bootstrap ML or MP tree. All distances were standardized and graphed using ‘ggplot2’ (Wickham 2016) in R (R Core Team 2024).

Morphological differentiation among species was explored using principal coordinates analysis (PCoA) using a Gower dissimilarity matrix, run separately on anatomical and shell characters. All analyses and graphics were performed in R (R Core Team 2024) using the ‘readr’ (Wickham *et al.* 2024), ‘dplyr’ (Wickham *et al.* 2023), ‘cluster’ (Maechler *et al.* 2023), ‘ape’ (Paradis and Schliep 2019), ‘vegan’ (Oksanen *et al.* 2024), ‘ggplot2’ (Wickham 2016), and ‘ggrepel’ (Slowikowski 2023) packages.

Results

Phylogenetic reconstruction

DNA sequences were retrieved from 55 individuals, including four outgroup samples (Supplementary Table S1), with 42 providing sequences across all six markers. The SCN, ITS, and MT constructs were 1001 bp, 1823 bp, and 1157 bp in length, and possessed 251, 549, and 453 variable bases within *Aegopinella*/*Retinella*, respectively. To obtain comparable phylogenetic trees, each analysis included 46 focal-group individuals and two outgroup individuals, with individual sampling largely overlapping among datasets. While ML, MP, and BI reconstructions highly supported *Aegopinella*/*Retinella* as monophyletic, traditional species assignments into *Aegopinella* and *Retinella* generated genus-scale polyphyly (Fig. 2; Supplementary Fig. S1). Complete congruence was noted in species-level clades, however, with each being strongly supported across all three trees (Fig. 3A–C). Fourteen of the seventeen documented species-level clades aligned with traditional species assignments (see Taxonomy for differences and Table 2). No cases of mitochondrial introgression, incomplete lineage sorting, hybridization, or other forms of horizontal gene transfer were noted (Fig. 2). While SCN and MT trees shared congruent shallow and deep topologies based on both Robinson–Foulds and quartet distances (Fig. 4A, B), the latter differed by possessing considerably weaker deep node support (Fig. 2) and in the rooting of *Aegopinella pura* and *Retinella incerta*. The ITS tree differed from both the SCN and MT trees (Supplementary Fig. S1) principally by being unable to resolve

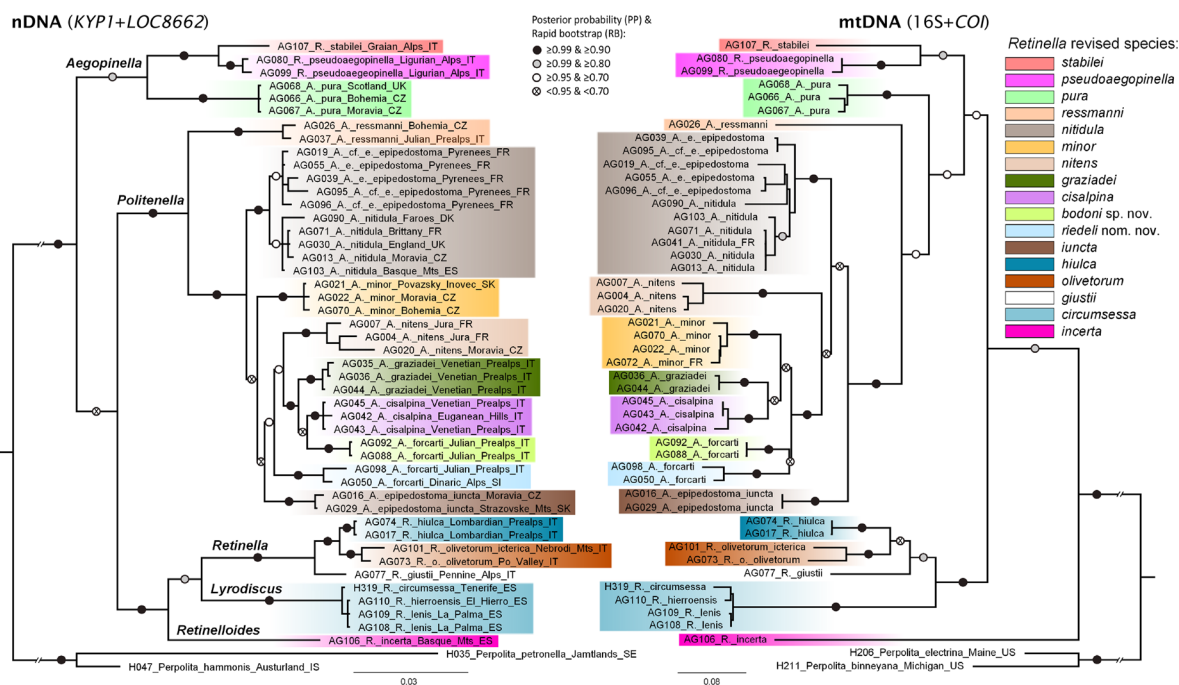


Figure 2 Maximum likelihood (ML) phylogenetic reconstruction of all extant *Retinella* and *Aegopinella* species based on single-copy nuclear markers (left) and mitochondrial markers (right). Node support is indicated by symbols corresponding to Bayesian posterior probabilities and ML rapid bootstrap values. For maximum parsimony bootstrap support, see Figure 3. Tip labels include DNA isolate codes (see Supplementary Table S1), original morphology-based identifications, regional locations, and two-letter country codes. Samples shared between both trees are shown without country codes in the mtDNA phylogeny, whereas samples unique to the mtDNA dataset retain country codes.

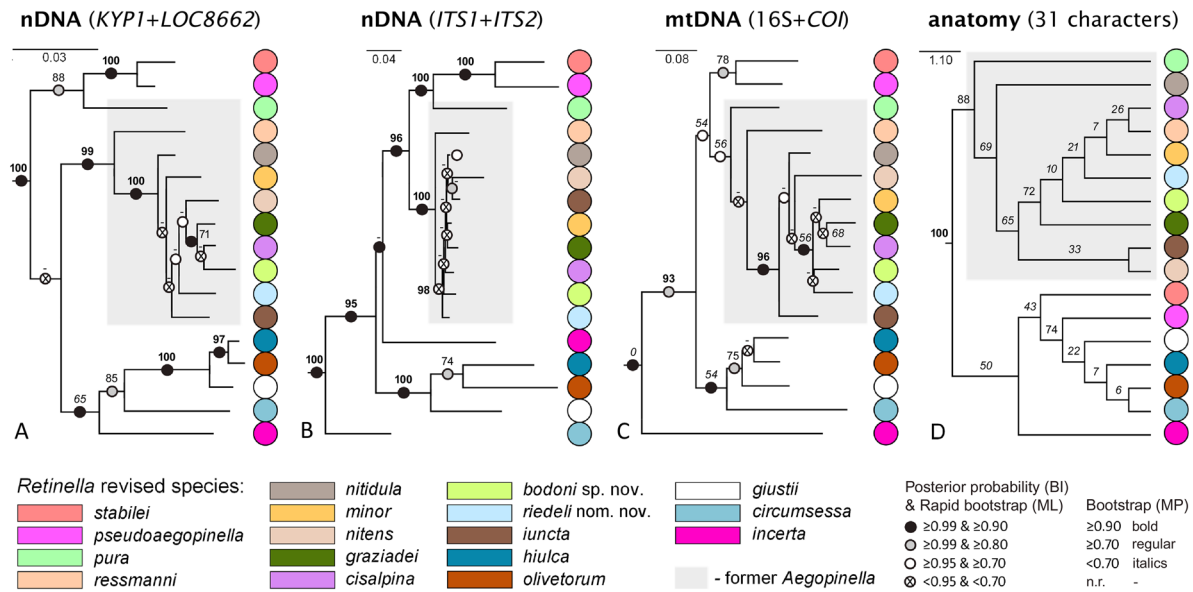


Figure 3 Comparison of phylogenetic reconstructions based on four independent datasets. Phylogenies inferred using Bayesian inference (BI), maximum likelihood (ML) and maximum parsimony (MP) from molecular datasets (A–C), and maximum parsimony from morphological data (D). The analyses are based on: (A) concatenated sequences of two single-copy nuclear genes, (B) two multi-copy nuclear ITS genes, (C) two mitochondrial genes, and (D) 31 anatomical characters of the reproductive system. Terminal branches represent species; molecular phylogenies (A–C) are generally strongly supported, with the exception of *R. nitidula* in the ITS dataset (B). The morphology-based maximum parsimony tree (D) is presented as an isometric cladogram, i.e. branch lengths are not proportional to the amount of evolutionary change. DNA-based phylogenies correspond to those presented in Figure 2 and Supplementary Figure S1.

interspecific phylogenetic structure within *Politenella* and being unable to resolve *Lyrodiscus*, *Retinella*, and *Retinelloides* into a single clade.

Time-calibrated phylogeny

BEAST2 analysis of the SCN tree suggested that the ingroup root was dated to the Oligocene (~31.8 Mya), with principal lineages, corresponding to subgenera-scale clades, originating from the Late Oligocene to Early Miocene (Fig. 5). The *Retinella*/*Retinelloides*/*Lyrodiscus* and *Aegopinella*/*Politenella* clades appeared to date to 23 and 30 Mya, respectively. *Retinelloides* diverged from *Retinella*–*Lyrodiscus* around 23 Mya, followed by the *Retinella*/*Lyrodiscus* split at c. 18 Mya. Radiation within *Aegopinella*, *Lyrodiscus*, *Politenella*, and *Retinella* generally dated from ~8–18 Mya but with *A. ressmanni* diverging from remaining *Politenella* at 21 Mya. Crown diversification for remaining *Politenella* dated to 13–10 Mya, and to 7.5 Mya in *Retinella*. Most of the current species' diversification dated to ~5 Mya during the Late Miocene and Early Pliocene. While this analysis was also conducted on ITS data (Supplementary Fig. S2) the resulting divergence estimates showed very wide 95% highest posterior density intervals for deeper nodes. Therefore, we did not draw evolutionary/ecological conclusions from that tree.

Reconstructions based on anatomical and conchological traits

Maximum parsimony analysis of the anatomical matrix supported the presence of two traditional genera, with only *Aegopinella* receiving moderate support (Fig. 3D). Both distance measures showed some congruence between

morphology and DNA-based reconstructions, with the MT reconstruction having the closest topological overlap (Fig. 4A, B). Copulatory organ morphology split species into four groups, three of them including representatives of at least two subgenera (Fig. 4C). However, although *Politenella* species were the most conchologically homogeneous clade (Figs 4D, 5), each possessed profound genitalic differences especially in terms of penial structures (Fig. 6B, C). In contrast, shell characters were relatively congruent at the subgeneric level (Fig. 4D), particularly with respect to upper shell microsculpture (Fig. 6). Based on ancestral character reconstruction, two anatomical and one conchological traits were found to be the most informative for deeper phylogeny reconstruction (Fig. 6A). Three unambiguous character transitions were detected, two in the time calibrated tree (related to penial cavity and upper shell microsculpture) and one (bursa copulatrix) in the time uncalibrated ML phylogeny (Fig. 6A).

Taxonomy

The main taxonomic implications of these results were (see Table 2): (i) *Aegopinella* and *Retinella* derived from a single evolutionary lineage, with no molecular-based support for their separation. Given priority, all species currently assigned to *Aegopinella* should be transferred to *Retinella*. (ii) Additionally, *Retinella* represented five distinct subgenera. Available names for these are: *Aegopinella*, *Politenella*, *Retinella*, *Lyrodiscus*, and *Retinelloides*, of these the latter two are monotypic. (iii) All analyses consistently supported the existence of a previously overlooked species closely resembling *Retinella riedeli*. While first revealed by molecular data, subsequent anatomical

Table 2 List of all taxa and nomina used in this study. Taxonomy and nomenclature before and after the revision, with taxonomic acts applied, are shown.

Previous classification (MolluscaBase 2025)	Classification proposed in this study	Taxonomic act
<i>Aegopinella pura</i> (Alder, 1830)	<i>Retinella</i> (<i>Aegopinella</i>) <i>pura</i> (Alder, 1830)	comb. nov.
<i>Retinella</i> (<i>Retinelloides</i>) <i>stabilei</i> (Pollonera, 1886)	<i>Retinella</i> (<i>Aegopinella</i>) <i>stabilei</i> (Pollonera, 1886)	comb. nov.
<i>Retinella</i> (<i>Retinella</i> ?) <i>pseudoaegopinella</i> Fo. Giusti, Boato & Bodon, 1986	<i>Retinella</i> (<i>Aegopinella</i>) <i>pseudoaegopinella</i> Fo. Giusti, Boato & Bodon, 1986	comb. nov.
<i>Aegopinella ressmanni</i> (Westerlund, 1883)	<i>Retinella</i> (<i>Politenella</i>) <i>ressmanni</i> (Westerlund, 1883)	comb. nov.
<i>Aegopinella nitidula</i> (Draparnaud, 1805)	<i>Retinella</i> (<i>Politenella</i>) <i>nitidula nitidula</i> (Draparnaud, 1805)	comb. nov.
<i>Aegopinella epipedostoma epipedostoma</i> (Fagot, 1879)	<i>Retinella</i> (<i>Politenella</i>) <i>nitidula epipedostoma</i> (Fagot, 1879)	comb. nov. & stat. nov.
<i>Aegopinella epipedostoma iuncta</i> Hudec, 1964	<i>Retinella</i> (<i>Politenella</i>) <i>iuncta</i> (Hudec, 1964)	comb. nov. & stat. nov.
<i>Aegopinella minor</i> (Stabile, 1864)	<i>Retinella</i> (<i>Politenella</i>) <i>minor</i> (Stabile, 1864)	comb. nov.
<i>Aegopinella nitens</i> (Michaud, 1831)	<i>Retinella</i> (<i>Politenella</i>) <i>nitens</i> (Michaud, 1831)	comb. nov.
<i>Aegopinella cisalpina</i> A. Riedel, 1983	<i>Retinella</i> (<i>Politenella</i>) <i>cisalpina</i> (A. Riedel, 1983)	comb. nov.
<i>Aegopinella graziadei</i> (Boeckel, 1940)	<i>Retinella</i> (<i>Politenella</i>) <i>graziadei</i> (Boeckel, 1940)	comb. nov.
<i>Aegopinella forcarti</i> A. Riedel, 1983	<i>Retinella</i> (<i>Politenella</i>) <i>riedeli</i> Horsák	nom. nov.
<i>Aegopinella forcarti</i> A. Riedel, 1983	<i>Retinella</i> (<i>Politenella</i>) <i>bodoni</i> Bichain, Nardi & Ryelandt	sp. nov.
<i>Retinella</i> (<i>Retinella</i>) <i>giustii</i> A. Riedel, 1998	<i>Retinella</i> (<i>Retinella</i>) <i>giustii</i> A. Riedel, 1998	No
<i>Retinella</i> (<i>Retinella</i>) <i>hiulca</i> (Albers, 1850)	<i>Retinella</i> (<i>Retinella</i>) <i>hiulca</i> (Albers, 1850)	No
<i>Retinella</i> (<i>Retinella</i>) <i>olivetorum</i> (Gmelin, 1791)	<i>Retinella</i> (<i>Retinella</i>) <i>olivetorum</i> (Gmelin, 1791)	No
<i>Retinella</i> (<i>Retinelloides</i>) <i>incerta</i> (Draparnaud, 1805)	<i>Retinella</i> (<i>Retinelloides</i>) <i>incerta</i> (Draparnaud, 1805)	No
<i>Retinella</i> (<i>Lyrodiscus</i>) <i>circumsessa</i> (Shuttleworth, 1852)	<i>Retinella</i> (<i>Lyrodiscus</i>) <i>circumsessa</i> (Shuttleworth, 1852)	No
<i>Retinella</i> (<i>Lyrodiscus</i>) <i>lenis</i> (Shuttleworth, 1852)	<i>Retinella</i> (<i>Lyrodiscus</i>) <i>circumsessa</i> (Shuttleworth, 1852)	syn. nov.
<i>Retinella</i> (<i>Lyrodiscus</i>) <i>hierroensis</i> M.R. Alonso & Ibáñez, 2013	<i>Retinella</i> (<i>Lyrodiscus</i>) <i>circumsessa</i> (Shuttleworth, 1852)	syn. nov.
<i>Retinella</i> (<i>Lyrodiscus</i>) <i>osoriensis</i> (Wollaston, 1878)	<i>Retinella</i> (<i>Lyrodiscus</i>) <i>circumsessa</i> (Shuttleworth, 1852)	syn. nov.
<i>Retinella</i> (<i>Lyrodiscus</i>) <i>rochebruni</i> (Mabille, 1882)	<i>Retinella</i> (<i>Lyrodiscus</i>) <i>circumsessa</i> (Shuttleworth, 1852)	syn. nov.

comparisons documented consistent differences. The description of this species is provided below; the remaining nomenclatural changes are presented afterwards.

Phylum Mollusca

Class Gastropoda Cuvier, 1795

Order Stylommatophora Schmidt, 1855

Family Gastrodontidae Tryon, 1866

Genus *Retinella* P. Fischer, 1877

Subgenus *Politenella* Balashov, 2016

***Retinella bodoni* Bichain, Nardi & Ryelandt sp. nov.**

(Fig. 7)

ZooBank registration: zoobank.org/act:D7E32B3F-926C-48CC-96CF-BFF262655742.

Type material: Holotype. Italy • subadult, shell and soft parts; Friuli-Venezia Giulia, Province of Udine, Municipality of Premariacco, left bank of the Natisone River, opposite Paderno; latitude/longitude: 46.04694N/13.39861E; elevation: 96 m asl; 21-October-2023; G. Nardi & A. Braccia leg.; deposited at the Musée d'Histoire naturelle et d'Ethnographie de Colmar (France) under the collection number

MHNEC_MOLL-2026-03_15.5 (labelled specimen AG092 in this study). Paratypes. Italy • 8 subadults, shells and soft parts; type locality; 21-October-2023; G. Nardi & A. Braccia leg.; deposited with holotype, MHNEC_MOLL-2026-03_14.1 to 14.5, and MHNEC_MOLL-2026-03_15.1 to 15.4. • 4 adults, shells only; type locality; 14-July-2023; M. Horsák & V. Horsáková leg.; deposited with holotype, MHNEC_MOLL-2026-04_01. • 2 adults (shells only; P6M044161), 2 subadults (shell with dry tissue; P6M044162), 4 subadults (shells and soft parts fixed in 70% ethanol, for dissection; P6M044162), and 4 subadults (shells and soft parts fixed in 96% ethanol, for DNA; P6M044163, specimen AG088 in this study originated from the same sample); same data as for the type locality; all deposited at the National Museum (Prague, Czechia). • 3 subadults: 1 empty shell and 2 ethanol-preserved specimens (shell and soft parts); type locality; 21-October-2023; G. Nardi & A. Braccia leg.; deposited at the Museo di Storia Naturale, University of Florence, Zoological Section 'La Specola', Italy (MZUF), under the collection number MZUF GC/74710. • 7 subadults, 1 empty shell and 6 alcohol-preserved specimens (dissected); type locality; 21-October-2023; G. Nardi & A. Braccia leg.; deposited at the G. Nardi collection (Italy), under the collection number 5953. Italy • 4 subadults, shell and soft parts; Friuli-Venezia

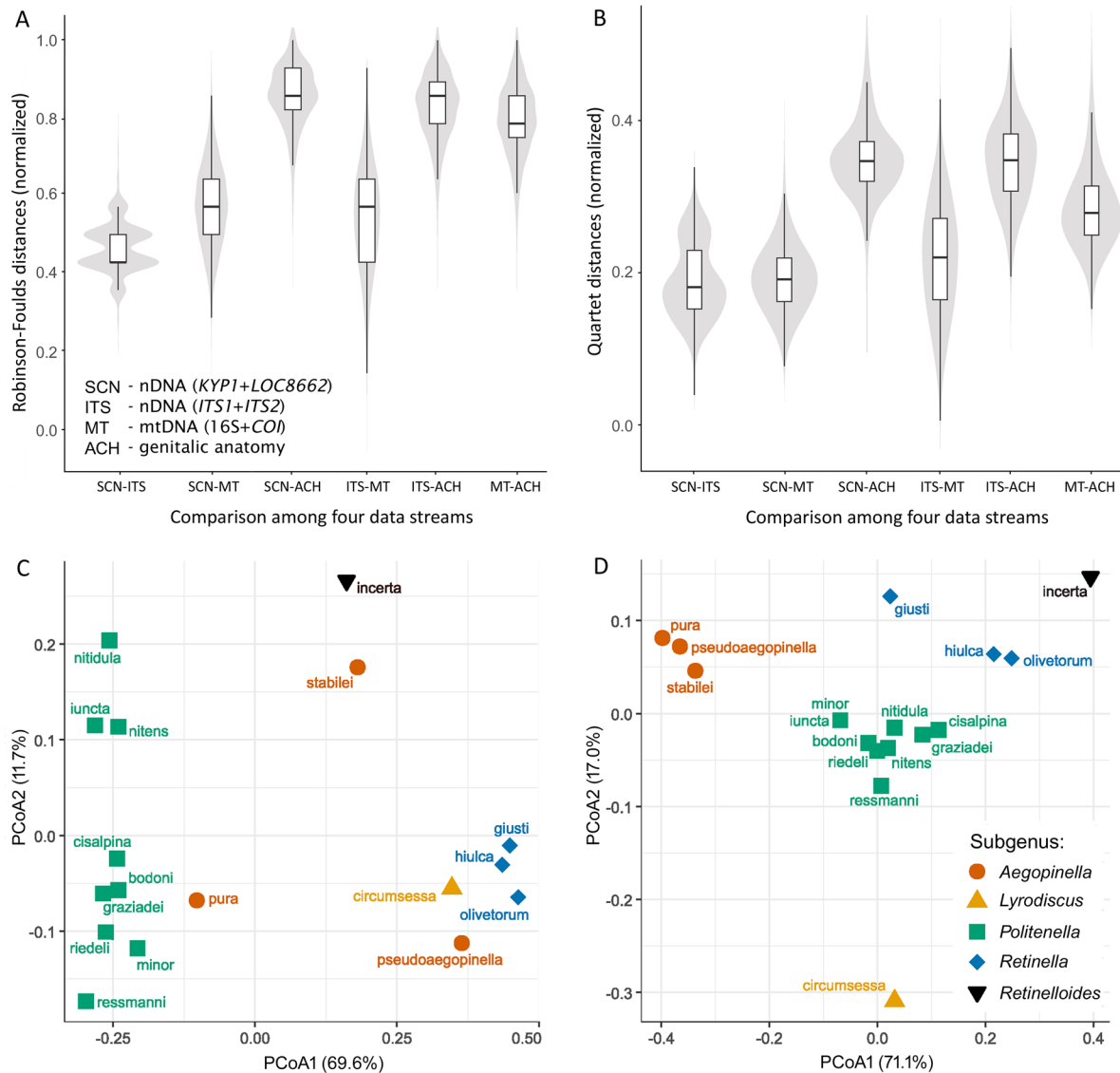


Figure 4 Congruence among four phylogenetic reconstructions (A–B; see Fig. 3) and comparison of similarities among 17 revised species based on genitalia anatomy and shell morphology (C–D, respectively). A–B, pairwise congruence among phylogenies inferred from four data sets: SCN (single-copy nuclear markers), ITS (multi-copy nuclear markers), MT (mitochondrial markers), and ACH (genitalia morphology based on 31 characters). Distributions show normalized Robinson–Foulds distances (A) and normalized quartet distances (B) for all pairwise tree comparisons. Violin plots represent the density of distances with embedded boxplots indicating medians and interquartile ranges; lower values indicate greater topological similarity among trees. C–D, principal coordinate analysis based on Gower dissimilarities using 31 anatomical characters of the reproductive system (C) and seven shell characters describing size and microsculpture (D). Percentages on axes indicate variation explained by each ordination axis. Points represent taxa; colours and symbols correspond to subgenera classification.

Giulia, Province of Udine, Municipality of Premariacco, right bank of the Natisone River, downstream of the little church of Santi Ilario e Taziano, village of Paderno; latitude/longitude: 46.04806/13.39805; elevation: 83 m asl; 21-October-2023; G. Nardi & A. Braccia leg.; deposited at the Musée d'Histoire naturelle et d'Ethnographie de Colmar (France) under the collection numbers MHNEC_MOLL-2026-03_16.1 to 16.3 and MHNEC_MOLL-2026-03_17.

Etymology: This new species is named after Marco Bodon (Genoa, Italy), in recognition of his important contributions to the study of Italian non-marine molluscs.

Description: Shell—Strongly depressed, with a low spire and rapidly increasing whorls; the last whorl is large, slightly widened near the aperture. Aperture oblique, oval; umbilicus moderately wide. Holotype with shell width 8.49 mm, shell height 4.83 mm, umbilicus width 1.71 mm, and 4 whorls. In the type series ($n = 9$ subadults specimens collected along with holotype), excluding the single juvenile specimen, shell width ranges from 8.38 to 9.13 mm (mean 8.81 mm), shell height from 4.27 to 4.83 mm (mean 4.56 mm), and umbilicus width from 1.55 to 1.79 mm (mean 1.73 mm); all specimens have 4 whorls. Other paratypes

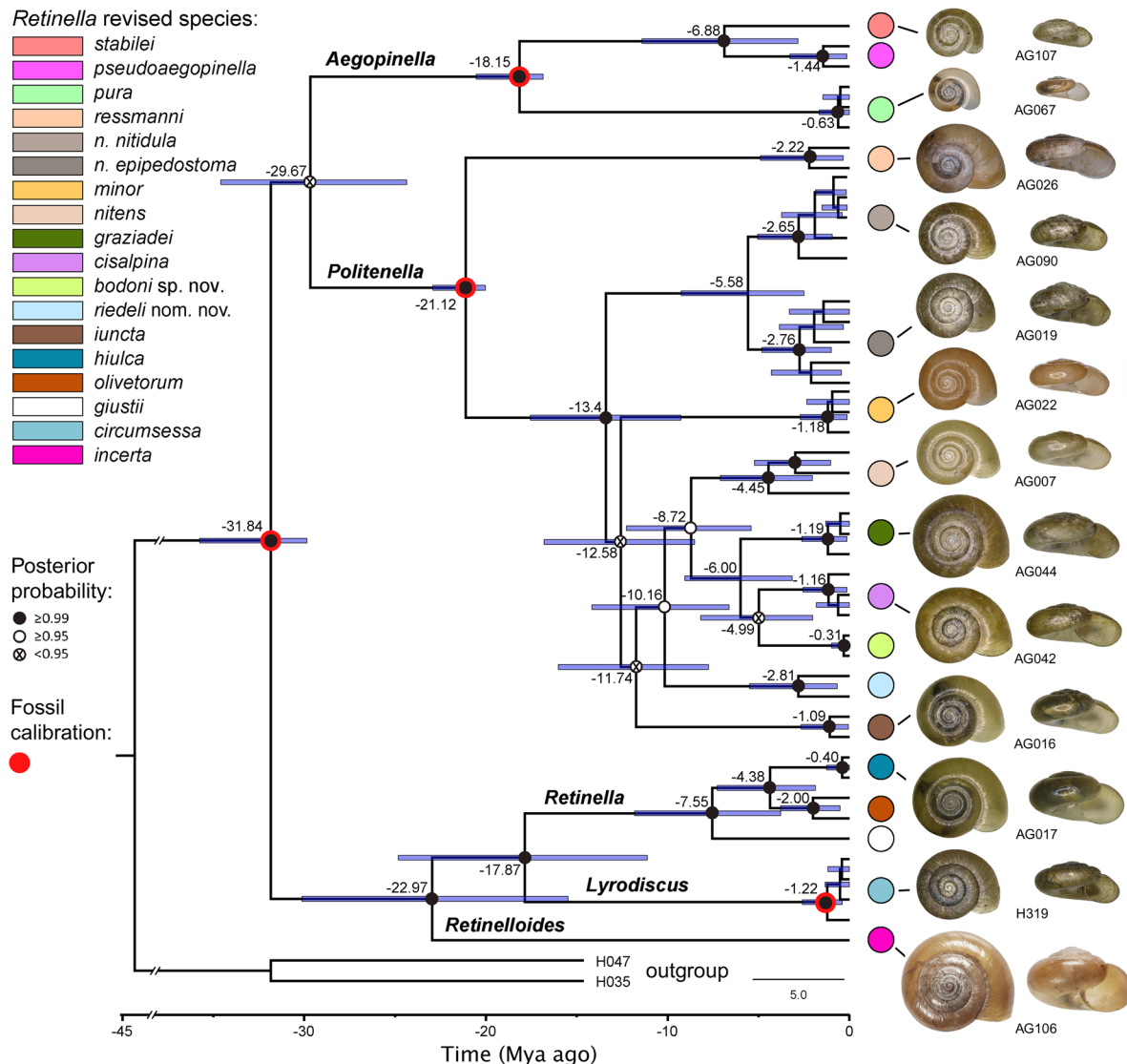


Figure 5 Divergence time estimation of the genus *Retinella* on the maximum clade credibility tree based on SCN (*KYP1* + *LOC8662*, single-copy nuclear markers). The red circle indicates the node calibrated by a fossil record. The blue bars at each node indicate 95% Bayesian credible interval estimated with BEAST2. Note that imaged shells are from the same populations as the respective DNA isolates (see [Supplementary Table S1](#) for locality information), but represent different individuals than those sequenced.

($n = 6$ adult specimens collected from type locality), shell width ranges from 9.84 to 11.73 mm (mean 10.73 mm), shell height from 4.51 to 5.74 mm (mean 5.07 mm), umbilicus width from 1.95 to 2.42 mm (mean 2.20 mm), and whorl counts from 4.30 to 4.65 (mean 4.48).

Reproductive system—Atrial or vaginal gland absent. Vagina (VG) clearly developed, short to long, without distinct distal dilatation, and shorter than the free oviduct (FO) when fully extended. Bursa copulatrix (BC) ovoid to pyriform; bursal duct (BCD) shorter than to subequal in length to the bursa. Bursa–duct complex (BC + BCD) not reaching the spermoviduct (SpOv) when the free oviduct is fully extended. Penis usually longer than the free oviduct, with irregular diameter and several marked constrictions. Internally, the penis is divided into distal and proximal cavities connected by a short, more or less sinuous canal formed by folds of the inner penial wall. The

distal cavity bears a strongly developed fold, the sarcobelum (SA), occupying most of the lumen. The proximal cavity consists of convoluted internal folds delimiting a tortuous canal extending proximally towards the epiphallus (EP). Externally, the boundary between distal and proximal penile portions is not always distinctly delimited but is usually indicated by a constriction of variable depth. Distal penis (DP) cylindrical to elongate. Proximal penis (PP) composed of two successive regions: a globular distal portion (PP [dp]) and a proximal duct-like portion (PP [pp]) of variable diameter. The latter is abruptly folded back onto the distal portion, initially coiled upon itself, then expanded into a cushion-shaped swelling adherent to the penis by connective tissue; penial retractor muscle (PRM) inserting on this swelling. The initial section of the proximal duct-like portion has approximately the same diameter as the epiphallus and/or vas deferens. Epiphallus short and not clearly distinguishable externally, scarcely differentiated

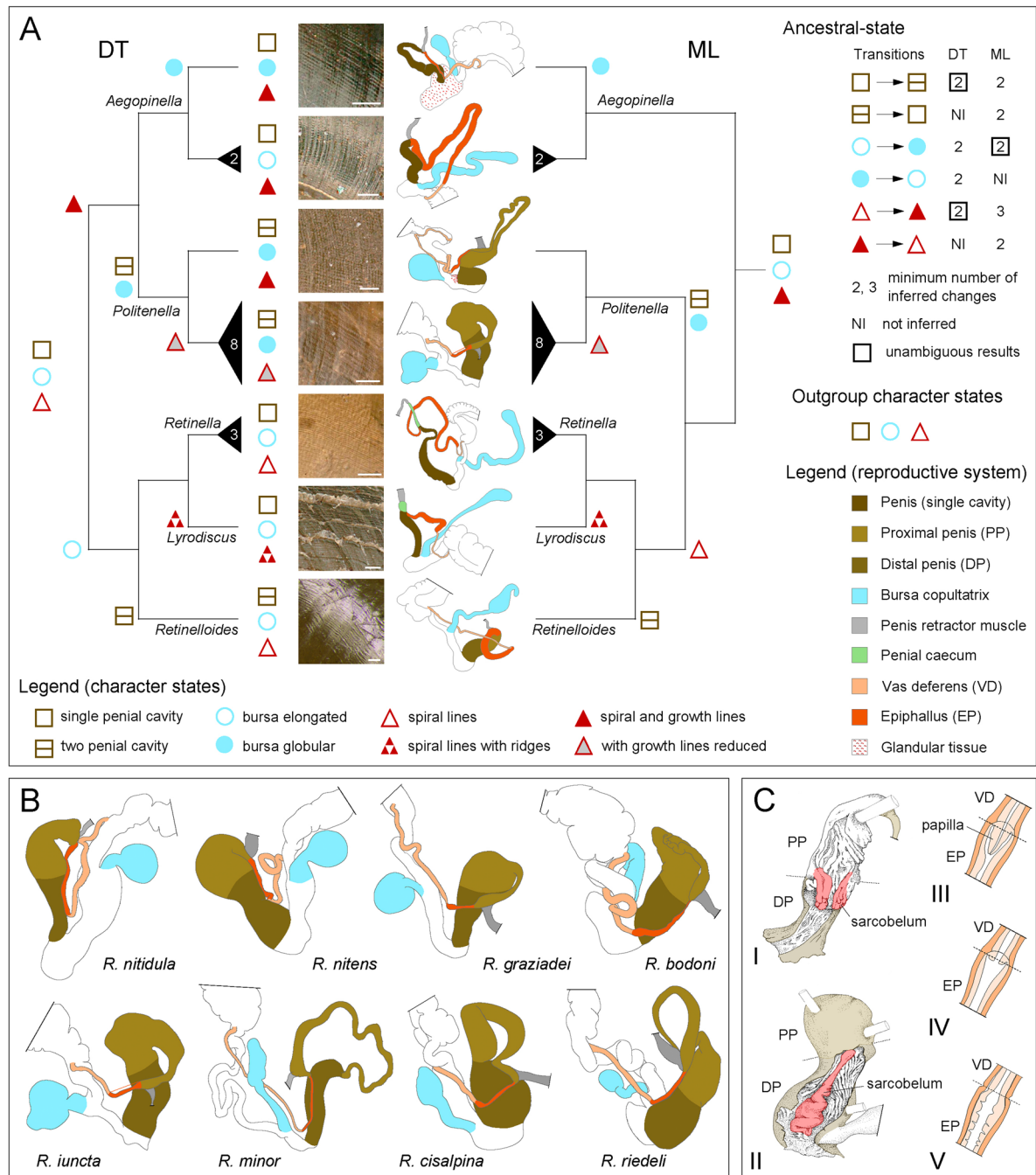


Figure 6 Morphological variation and character evolution in *Retinella*. **A**, two alternative phylogenetic scenarios using single-copy nuclear markers based on the divergence time-estimated phylogeny (DT) and maximum likelihood phylogeny (ML), showing the distribution and inferred transitions of the three characters with the strongest phylogenetic signal: penial cavity type, shape of the bursa copulatrix, and upper shell microsculpture (scale bar = 0.1 mm). The right-hand panel summarizes the number of transitions reconstructed for each character under the two phylogenetic hypotheses using ancestral character reconstruction analysis. Numbers within black triangles indicate the number of species represented by the corresponding collapsed tip. **B**, schematic drawings of the penis and associated genital structures in eight species of the subgenus *Politenella*. **C**, detail of variation in penial anatomy within *Politenella*, showing two sarcobelum types (I, *R. nitidula*; II, *R. nitens*) and two modes of vas deferens insertion into the epiphallus (III, *R. nitens*; IV, *R. minor*) in comparison with that in the subgenus *Aegopinella* (V, *R. stabilei*). For abbreviations see the legend in (A).

from the terminal vas deferens (VD) or apical penial region (PP [pp]). It is here defined as the segment extending from the insertion of the penial retractor muscle, marking the transition to the proximal penis, to the internal papilla (PA) marking the transition to the vas deferens. This papilla is well developed internally. Externally, its position is not always clearly visible,

but may appear as a bulbous area adherent to the basal part of the penis, near the emergence of the vagina.

Diagnosis: No conchological characters allow for its reliable separation from most crown *Politenella* species. However, its penial anatomy is distinctive, especially compared to

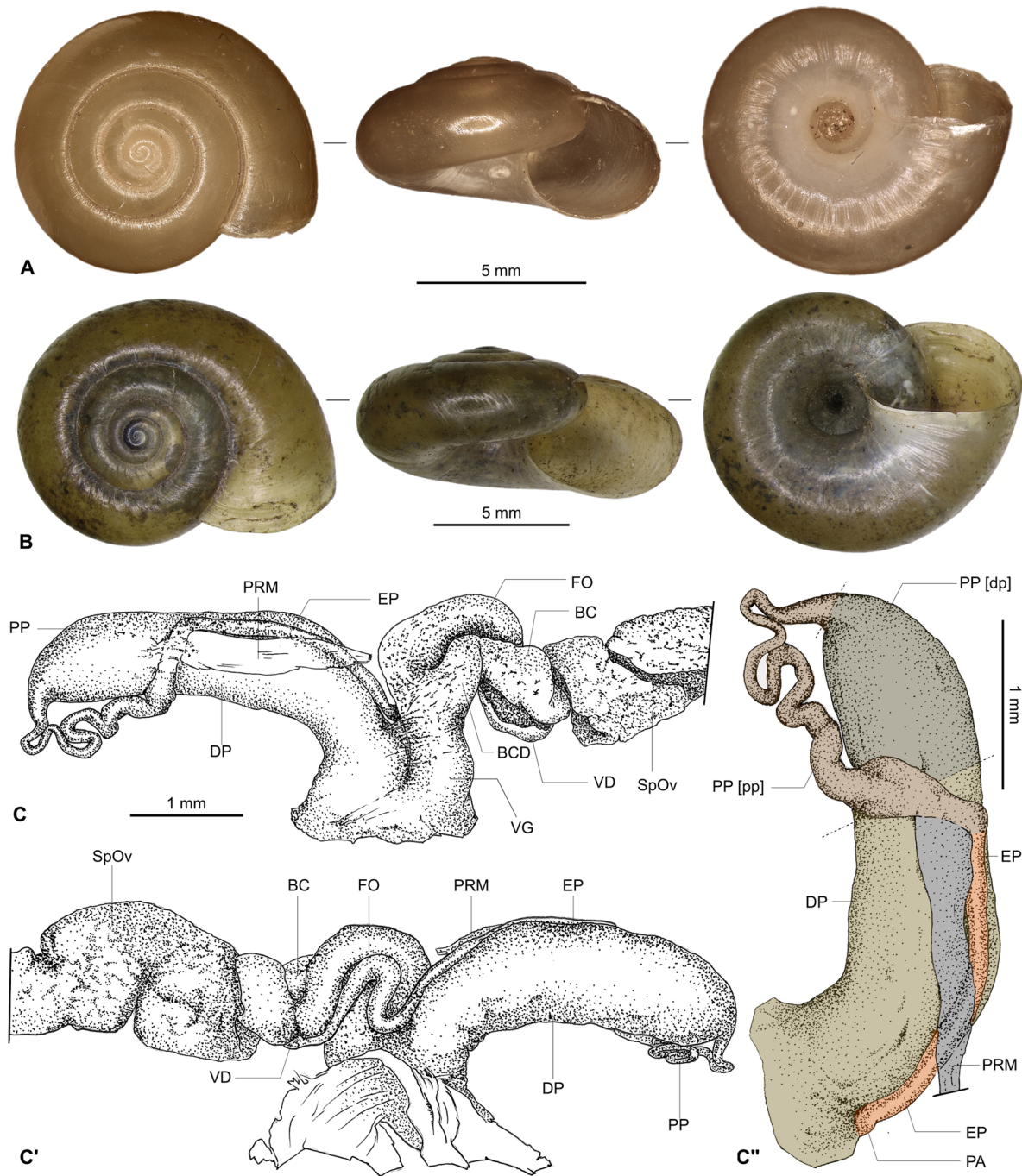


Figure 7 Shell images and anatomy of *Retinella bodoni*. A, holotype (AG092). B, an adult shell from the type locality. C to C'', distal part of the reproductive system of the holotype. Abbreviations: BC, bursa copulatrix; BCD, bursa copulatrix duct; DP, distal part of penis; EP, epiphallus; FO, free oviduct; PA, papilla; PP, proximal part of penis; PP [dp], distal portion of the proximal part of penis; PP [pp], proximal portion of the proximal part of penis; PRM, penis retractor muscle; SpOv, spermiduct; VD, vas deferens; VG, vagina. Colours are applied in C'' to highlight the different external penile structures.

geographically-proximate taxa (see Fig. 6B). *Retinella bodoni* is the most similar to *R. riedeli*, from which it can be distinguished by its external proximal penis structure which is differentiated into a globular distal region and a proximal duct-like region which is abruptly folded back, coiled and expanded into a cushion-shaped swelling bearing the insertion of the penial retractor muscle. In *R. riedeli*, the proximal duct-like portion of

the penis is initially very slender, consistently narrower than the epiphallus and/or vas deferens, and is followed by a longer and widened zone where the retractor muscle inserts distally. Although *Retinella cisalpina* may occur sympatrically with *R. bodoni*, it can be distinguished by its thick, elongated proximal penis, which curves into an almost loop-like inverted 'U' towards the distinctly bulbous distal region.

Distribution and ecology: Known only from the type locality in Friuli-Venezia Giulia, north-eastern Italy, in the surroundings of Premariacco, on both the left and right banks of the Natisone River, at 83–96 m asl. Some previous records of *R. riedeli* from the Udine region and nearby areas may represent *R. bodoni*. At the type locality, it inhabits dense limestone talus forest on steep river valley slopes.

***Retinella riedeli* Horsák nom. nov.**

Replaced name: *Aegopinella forcatti* A. Riedel, 1983.

Remarks: The transfer of *Aegopinella forcatti* A. Riedel, 1983 to *Retinella* creates a nomenclatural conflict, as the resulting combination becomes a junior secondary homonym of the fossil species *Retinella forcatti* Schlickum & Strauch, 1979. We therefore propose *Retinella (Politenella) riedeli* Horsák, nom. nov., as a replacement *nomen* for this entity. While a fossil *Perpolita riedeli* Schlickum & Strauch, 1975 exists, its shell microsculpture clearly distinguishes it from *Retinella* (Saito *et al.* 2025; this study), thus greatly reducing the risk for future secondary homonymy.

***Retinella nitidula epipedostoma* (Fagot, 1879)
comb. nov. & stat. nov.**

Previous combination: *Aegopinella epipedostoma* (Fagot, 1879).

Remarks: Pyrenean populations traditionally assigned to *Aegopinella epipedostoma* showed limited genetic divergence and actually represent a geographically-isolated population of *R. nitidula*. This is consistent with differentiation following long-term isolation in peripheral refugia. The Pyrenees are known for high endemism at multiple evolutionary scales, including haplotypes (e.g. Segarra-Moragues *et al.* 2007, Horsák *et al.* 2026).

***Retinella iuncta* (Hudec, 1964) comb. nov. &
stat. nov.**

Previous combination: *Aegopinella epipedostoma iuncta* Hudec, 1964.

Remarks: The Carpathian endemic *Retinella iuncta*, previously treated as a subspecies of *R. epipedostoma*, must be elevated to a full species as it is genetically quite distinct despite its general conchological similarity.

***Retinella (Lyrodiscus) circumsesta*
(Shuttleworth, 1852)**

Retinella (Lyrodiscus) lenis (Shuttleworth, 1852) syn. nov.
Retinella (Lyrodiscus) osoriensis (Wollaston, 1878) syn. nov.
Retinella (Lyrodiscus) rochebruni (Mabille, 1882) syn. nov.
Retinella (Lyrodiscus) hierroensis M.R. Alonso & Ibáñez, 2013 syn. nov.

Remarks: Despite minor conchological and genital differences, at least three of the putative five subgenus *Lyrodiscus* species endemic to the Canary Islands (*R. circumsesta*, *R. hierroensis*, *R. lenis*; Alonso *et al.* 2013) are conspecific, possessing very low genetic divergence. There is no morphological or geological evidence to suggest that the remaining two

unanalysed taxa (*R. osoriensis*, *R. rochebruni*) are not the same: not only are their distinguishing morphological characters of no taxonomic value in the subgenus, but both putative taxa occur on islands roughly the same geological age as Tenerife. Divergence time estimates suggest *R. circumsesta* s.l. represents a recent (~1.2 Mya) colonization from the mainland, with populations on geologically younger islands (La Palma, El Hierro) being less divergent from each other than those on Tenerife. Recent arrival is furthermore supported by shell evolution, with *Lyrodiscus*-type microsculpture appearing in continental Europe only from the Pliocene (~5 Mya) onwards.

Discussion

While the four principal data streams (MT, ITS, and SCN DNA sequences, and morphological traits) demonstrate strong congruence at the species level, substantial conflict is present in reconstruction of deeper nodes. This lack of congruence highlights fundamental data differences and underscores the importance of considering the inferential limits and biases present within each. Taken together, these results emphasize that robust phylogenetic inference across evolutionary scales requires integration of multiple complementary data sources rather than reliance on any single marker system (Dayrat 2005, Padial *et al.* 2010).

Use and reliability of morphological characters in evolutionary reconstruction

Although morphological characters frequently provide misleading or poorly resolved reconstructions of land snail evolutionary relationships (e.g. Emberton 1995), as seen in this study, such signals can nevertheless exist. The critical challenge lies in identifying those characters that reliably reflect phylogenetic relationships. As with species delimitation, where relevant diagnostic traits may not be readily apparent from a human perspective (e.g. Wiese *et al.* 2022, Nekola *et al.* 2026), determination of ancestral vs. derived character states should not rely solely on expert judgment. Rather, their phylogenetic relevance needs to be evaluated against independent sources of evidence, including molecular data, within an integrative framework (Dayrat 2005). Without this integration, there is an increased risk of misinterpreting character evolution and, consequently, of drawing erroneous phylogenetic conclusions.

In *Retinella*, comparison of genital morphology with the SCN phylogeny shows that some features not only diagnose extant species accurately but also illuminate deeper evolutionary patterns. This is likely due to their role in generating prezygotic isolation, which can lead to rapid divergence even among closely related taxa (Hollander *et al.* 2013). While some features appear to have arisen independently multiple times, giving rise to the erroneous splitting of the group into two traditional genera, ancestral character mapping demonstrates that other traits (such as penile and bursa copulatrix features) also inform deeper phylogenetic relationships. As a result, the reconstruction of their evolution closely corresponds to SCN and MT DNA sequence trees. This congruence is also reflected in specific cases, such as *Retinella incerta*,

which retains ancestral microsculptural features and occupies a basal position in both phylogenies.

Shell shape morphology was less useful for providing evolutionary and taxonomic signal. We observed multiple cases where conchologically similar taxa were anatomically and genetically clearly distinct (e.g. *R. minor* vs. *R. nitens*), as well as instances where anatomically similar species exhibited pronounced shell morphology differences (e.g. *R. stabilei* vs. *R. incerta*). These instances are likely related to a combination of convergence, phenotypic plasticity, and strong ecological selection pressures acting on many shell traits (Knowlton 2000, Castelin *et al.* 2017).

However, shell microsculpture, which may be more neutral, performed remarkably well in deciphering evolutionary history. This follows a number of recent works (Nekola *et al.* 2015, 2025, Horsák *et al.* 2022, Adamcová *et al.* 2024, Saito *et al.* 2024) showing that shell microsculpture can provide robust taxonomic and phylogenetic signal. In *Retinella*, the differentiation of closely related subgenera *Retinelloides*, *Retinella*, and *Lyrodiscus* can be accomplished by observing upper shell microsculpture. While there is a progressive reduction and eventual loss of these structures in crown *Politenella* species, in *Lyrodiscus* comparison of modern to fossil taxa (Rousseau and Puisségur 1990) indicates a reverse trajectory, with these features becoming more pronounced over time. It should also be remembered that *Lyrodiscus* diversified on the continental mainland well before its colonization of the Canary Islands ~1.2 Mya, but the timing of this is impossible to document in this DNA sequence tree because those species are extinct.

Problems with mtDNA signal

Mitochondrial markers have been repeatedly shown to possess limitations for phylogenetic inference at some evolutionary scales (e.g. Morii *et al.* 2015, Nekola *et al.* 2015, 2025, Razkin *et al.* 2016), with a common issue in land snails being their susceptibility to mitochondrial introgression, varying from absent [this study, and *Vertigo* (*Vertilla*); Nekola *et al.* 2018] to rare (*Pyramidula*; Horsák *et al.* 2026) and relatively frequent (*Pupilla*; Nekola *et al.* 2015 and *Ezohelix*; Morii *et al.* 2015). Incomplete lineage sorting may also be present (e.g. Böckers *et al.* 2016). Additionally, matrilineal-only heredity frequently generates spurious geographically-restricted branch-tip clades that may reflect geographic population structure rather than species boundaries (e.g. Nekola *et al.* 2015, 2018). Another general limitation of mtDNA is its relatively high rate of substitutional saturation, particularly at deeper phylogenetic levels. Although mitochondrial sequences may remain informative, repeated substitutions at the same sites increase the probability that shared nucleotides reflect homoplasy rather than common ancestry, thereby reducing their reliability relative to more slowly evolving nuclear loci (e.g. Stöger and Schrödl 2013, Fontanilla *et al.* 2017). For these reasons, it is not uncommon for phylogenetic reconstructions based on mtDNA to disperse occurrences of a single species across a tree (e.g. *Euconulus*, Horsáková *et al.* 2019; *Ezohelix*, Morii *et al.* 2015; *Paralaoma*, Nekola *et al.* 2025; *Pupilla*, Nekola *et al.* 2015; *Vertigo*, Nekola *et al.* 2018), while at the same time

possessing low deep node resolution and support (e.g. Nekola and Coles 2016).

While researchers have attempted to address these issues by concatenating mtDNA with nDNA sequence from very slowly evolving nuclear ribosomal regions, such as 18S and 28S regions in the rRNA gene cassette to mtDNA, this approach is largely problematic. First, there are usually 2–100 × more variable sites in mtDNA than in highly conserved nDNA regions (e.g. Duda 2021). Even when a sequence is partitioned to allow for different evolutionary rates, this imbalance tends to drive phylogenetic inference, resulting in topologies that closely resemble those of mtDNA-only trees. Second, combining loci inherited through fundamentally different pathways may obscure rather than clarify phylogenetic patterns (Fisher-Reid and Wiens 2011). Thus, mitochondrial and nuclear datasets should not only be initially analysed separately but also visualized independently, allowing their variability and degree of congruence or incongruence to be directly assessed by readers.

Problems with ITS signal

Concatenated *ITS1* and *ITS2* regions of nuclear rRNA often show variation comparable to that of mitochondrial 16S in land snails, and thus can provide strong signal for delimiting species-scale crown clades (e.g. Nekola *et al.* 2018, Horsáková *et al.* 2019). However, these markers have inherent limitations. In particular, as non-coding regions, they are prone to frequent insertions and deletions. While sequence alignment is relatively straightforward among closely related species (Horsák *et al.* 2026), it becomes ambiguous or even impossible between genera (e.g. *Vertigo* vs. *Nesopupa*; Nekola and Coles 2016). Although alignment algorithms necessarily impose gap placement, their accuracy in identifying homologous indels and distinguishing homoplasy remains uncertain. Consequently, indel treatment can introduce substantial noise into tree reconstructions, even when gaps are formally coded as character states (Simmons and Ochoterena 2000). This issue is especially pronounced at deeper phylogenetic levels, where indel homology is increasingly uncertain and convergence more frequent (Dessimoz and Gil 2010, Redelings *et al.* 2024). However, the phylogenetic impact of indel coding is likely to vary among taxonomic groups because indel frequency, length, and homology differ across lineages. In *Pyramidula*, inclusion of indel characters notably altered the inferred relationships (Horsák *et al.* 2026), whereas in the present study analyses with and without indel coding produced the same topology and only negligible differences in branch lengths and support values. A second major concern is the presence of hundreds of copies of the rRNA gene cassette spread across all chromosomes (Wang *et al.* 2023), with a significant intercopy variability, especially in *ITS1* and *ITS2* (Armbruster and Korte 2006, Feliner and Rosselló 2007). For instance, seven of 10 cloned *ITS1* amplicons generated from a single PCR of a single DNA extraction of *Haplotrema keepi* yielded seven unique haplotypes varying by 7–41 bases in length, with the mean number of base-pair substitutions ranging from 9–23, with the resulting tree replicating the topology of the entire genus (Nekola *et al.* 2023). This variability may result in Sanger sequence traces being unreadable, but even

when a single dominant haplotype amplifies, it may exhibit highly divergent and phylogenetically uninformative patterns (Nekola *et al.* 2018).

The limited ability of ITS sequences to generate plausible phylogenetic hypotheses across multiple scales was clearly evident in *Retinella*. Although we did not encounter cases in which multiple haplotypes generated unreadable sequence traces, issues related to indel placement and/or non-representative haplotype amplification prevented ITS from resolving phylogenetic relationships within the subgenus *Politenella*. The resultant polytomy was not present in either MT or SCN trees. At the same time, the ITS tree was unable to resolve the sister relationships among the subgenera *Lyrodiscus*, *Retinella*, and *Retinelloides*, and produced very poor temporal constraints in molecular clock analyses that were incongruent with the fossil record.

Advantages of SCN sequences in the present dataset

Our results suggest that evolutionary signal obtained from the SCNs can produce trees with high support from crown to root, that are more internally consistent and that demonstrate better agreement with independent lines of evidence than either MT or ITS trees. In *Retinella*, divergence time estimates based on SCN data align better with the fossil record, documenting the recent emergence of diagnostic *Lyrodiscus*-type shell microsculpture. They also produce trees that identify multiple synapomorphies in genitalic character states.

There are likely multiple reasons, but the most important are probably: (i) the presence of a single gene copy, which reduces ambiguity arising from paralogy and may increase congruence with organismal evolutionary history; (ii) the protein-coding nature of both SCNs, limiting indel variation and evolutionary pathways; and (iii) their lower base-pair variability relative to ITS and MT, reducing saturation and homoplasy. When concordant patterns are recovered across independently sorting SCN loci, confidence that the topology is not driven by locus-specific history is increased. This is likely to explain the consistently high node support across the tree. As such, targeted single-copy nuclear loci provide a useful source of phylogenetic information across multiple evolutionary scales. Of course, part of this success stems from prior knowledge that the *KYP1* and *LOC8662* loci provided sufficient interspecific variation to distinguish closely related *Politenella* species, irrespective of the phylogenetic topology they recovered. If we had chosen slower-evolving ultraconserved element (UCE) loci, our results would likely not have resolved crown topologies, whereas less conserved regions would likely fail to resolve deeper nodes and would hinder universal PCR amplification.

Identifying suitable SCNs for phylogenetic reconstruction thus requires balancing levels of sequence conservation and variability. Recently, a set of 60 UCE-derived SCN loci identified by Nekola *et al.* (in review) has undergone empirical evaluation and appears to provide phylogenetic signal across multiple evolutionary depths. Suitable loci can then be selected through pilot analyses that compare shallow and deep sequence divergence among candidate loci within the focal group of taxa. The

process is thus akin to seeking out appropriate porridge and beds in the Three Bear's house: they must be 'just right'.

More data or better data? Targeted loci in the genomic era

In the context of selecting appropriate nuclear loci, one might argue that a more powerful approach is to access thousands of loci using genomic approaches such as RADseq (Andrews *et al.* 2016) or UCE enrichment (Faircloth *et al.* 2015). However, more data do not necessarily translate into greater phylogenetic resolution. Genome-scale datasets contain a mixture of phylogenetically informative and uninformative signals (McLean *et al.* 2019, Koch 2021), including evolutionary rate heterogeneity (Koch 2021), regions with complex indel patterns or gappy alignments that hinder alignment, and noise arising from paralogy or artefactual orthologues (Zhou *et al.* 2022, Frost *et al.* 2024). Although such issues can often be mitigated bioinformatically, they require substantial filtering, and only a small fraction of loci may ultimately prove useful at shallow evolutionary scales.

For example, of the 28 000 UCE loci initially considered by Nekola *et al.* (in review), only a small subset (~1.5%) met the criteria for single-copy status, alignability, and sufficient variability across sampled eupulmonate lineages. An even smaller fraction, 60 loci (0.2%), demonstrated both the ability to be PCR-amplified via universal primers and provided diagnostic variation at the species level. The loci used here were therefore not randomly selected, but instead represent markers known a priori to capture relevant evolutionary signal in land snails, having been specifically selected for *Retinella* based on previous research and testing on representative of *Politenella* subgenus. By focusing on such targeted loci, we reduce sequencing costs and can instead increase sampling across individuals. This is particularly important given the complex and often reticulate nature of evolutionary processes. Moreover, a key advantage of PCR-based approaches is that they allow inexpensive expansion of individual and geographic sampling after initial analyses, enabling the stability of inferred groupings and geographic structure to be evaluated with denser sampling. In this context, targeted sequencing and genomic approaches should be viewed as complementary rather than competing strategies. More broadly, our results demonstrate that combining targeted molecular markers with carefully selected morphological traits and fossil evidence provides a robust framework for reconstructing evolutionary history in morphologically complex groups such as land snails.

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Author contributions

All authors collected the samples and/or generated data; M.H., J.-M.B., and J.R. conceived the idea; M.H. led the data curation;

J.-M.B., G.N., and J.R. conducted the anatomical investigations; T.S., F.N., and S.K. ran all the phylogenetic analyses; M.H., J.-M.B., and J.C.N. led the writing; all authors reviewed and approved the final version of the manuscript.

Supplementary material

Supplementary material is available at *Zoological Journal of the Linnean Society* online.

Conflicts of interest

None declared.

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Data availability

All data generated during this study are included in this published article or its supplementary information data, or are deposited in GenBank.

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Supporting Information for:

Phylogenetic reconstruction and taxonomy of *Retinella* land snails: congruence and conflicts across morphological, mitochondrial, and nuclear datasets

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Figures S1–S2.

Figure S1. Maximum Likelihood phylogenetic reconstruction of all extant *Retinella* and *Aegopinella* species based on two multi-copy nuclear markers (*ITS1* + *ITS2*). Node support is indicated by symbols corresponding to Bayesian posterior probabilities and maximum likelihood Rapid bootstrap values. Tip labels include DNA isolate codes (see Table S1), original morphology-based identifications, regional locations and two-letter country codes.

Figure S2. Divergence time estimation of the genus *Retinella* on the maximum clade credibility tree based on multi-copy nuclear *ITS1* + *ITS2* genes. The red circle indicates the node calibrated by a fossil record. The blue bars at each node indicate 95% Bayesian credible interval estimated with BEAST2.

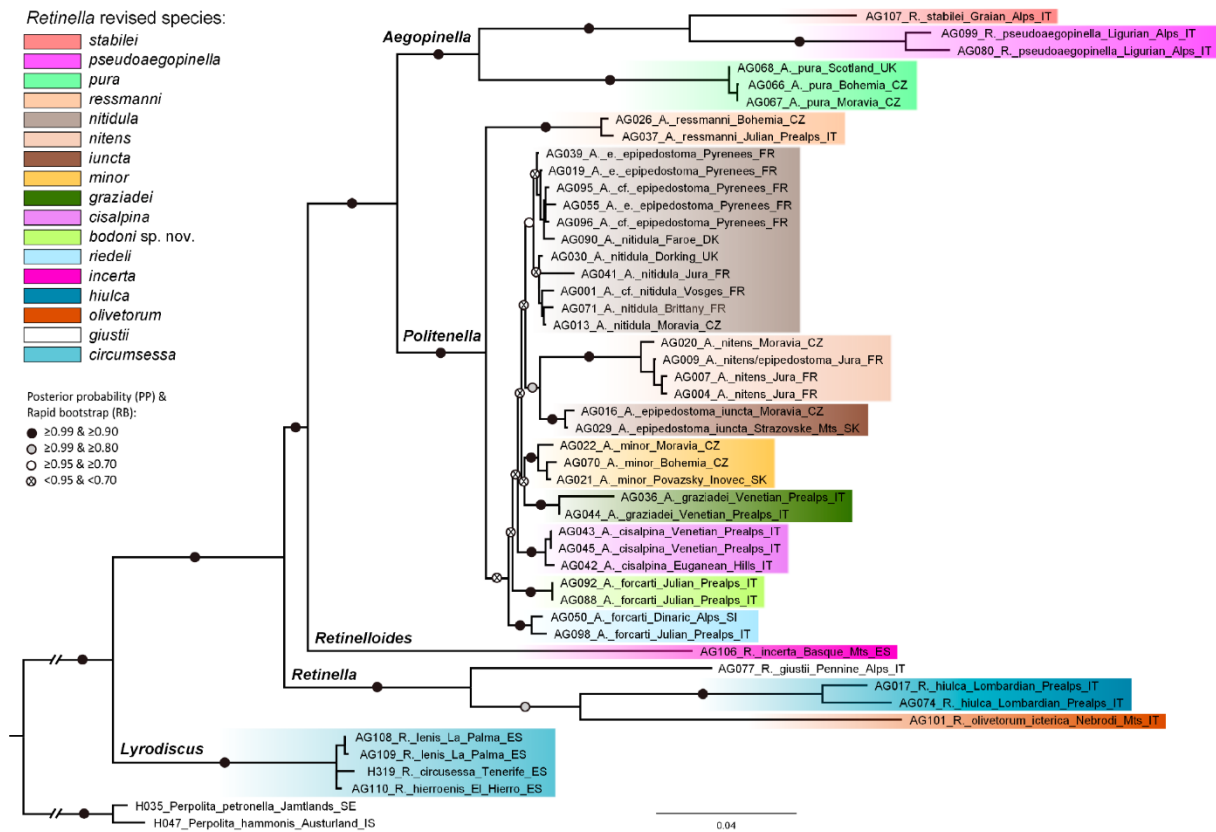


Figure S1. Maximum Likelihood phylogenetic reconstruction of all extant *Retinella* and *Aegopinella* species based on two multi-copy nuclear markers (*ITS1* + *ITS2*). Node support is indicated by symbols corresponding to Bayesian posterior probabilities and maximum likelihood Rapid bootstrap values. Tip labels include DNA isolate codes (see Table S1), original morphology-based identifications, regional locations and two-letter country codes.

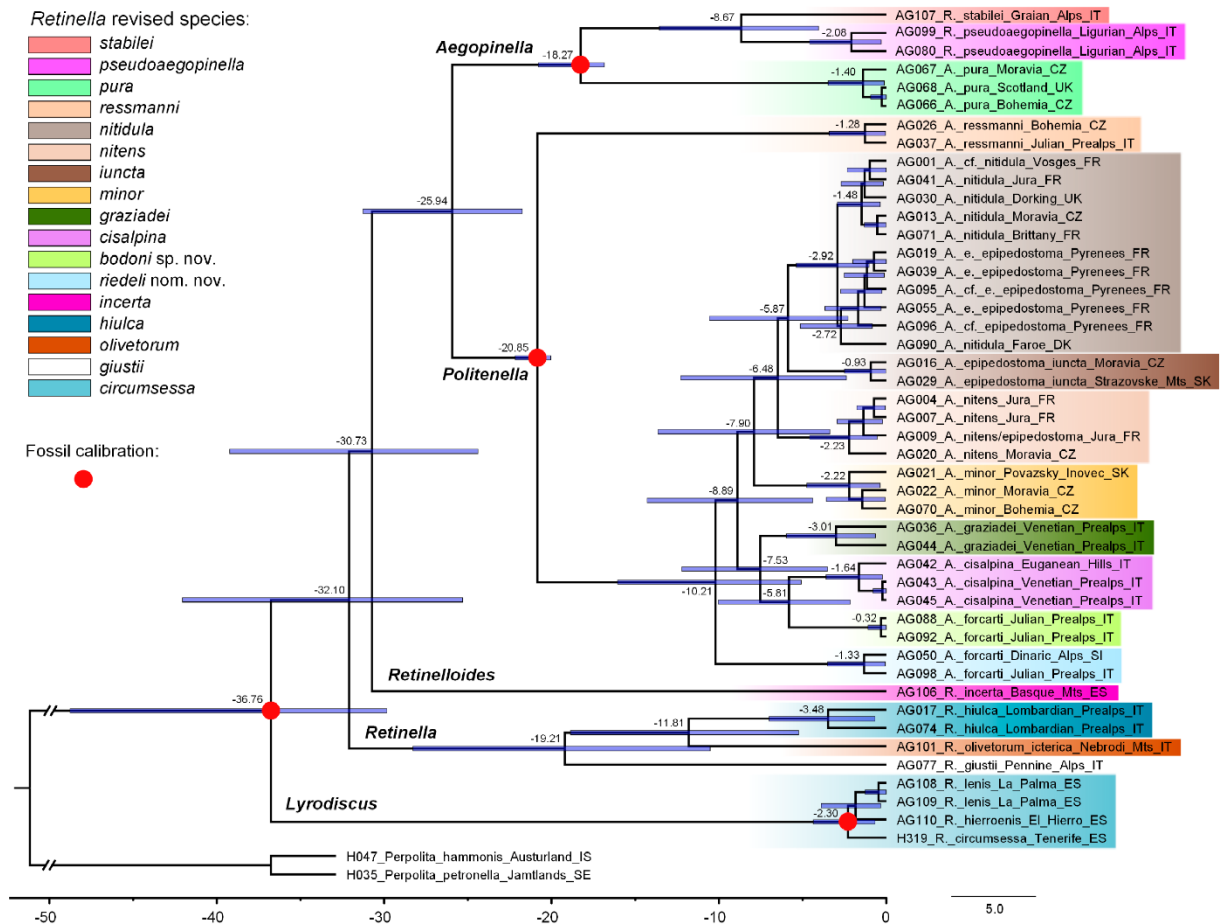


Figure S2. Divergence time estimation of the genus *Retinella* on the maximum clade credibility tree based on multi-copy nuclear *ITS1* + *ITS2* genes. The red circle indicates the node calibrated by a fossil record. The blue bars at each node indicate 95% Bayesian credible interval estimated with BEAST2.

Table S1. Samples included in the genetic analyses, with associated locality information.

GenBank accession number is provided for sequence used in this study; x, sequence unavailable or not used. For “type region” samples, the reported distance in brackets is the geographical distance between the sampling locality and the type locality.

Table S1 · Part 1 of 2 — DNA accessions and identifications

DNA codes	16S	COI	ITS1	ITS2	KY1	LOC8662	DNA ID	morpho ID
AG088	PZ349305	PZ351314	PZ357095	PZ394637	PZ411329	PZ411370	<i>Retinella bodoni</i> sp. nov.	<i>Aegopinella forcarti</i>
AG092	PZ349308	PZ351317	PZ357097	PZ394639	PZ411330	PZ411372	<i>Retinella bodoni</i> sp. nov.	<i>Aegopinella forcarti</i>
AG108	PZ349317	PZ351326	PZ357104	PZ394647	PZ411339	PZ411381	<i>Retinella circumsesta</i>	<i>Retinella lenis</i>
AG109	PZ349318	PZ351327	PZ357105	PZ394648	PZ411340	PZ411382	<i>Retinella circumsesta</i>	<i>Retinella lenis</i>
AG110	PZ349319	PZ351328	PZ357106	PZ394649	PZ411341	PZ411383	<i>Retinella circumsesta</i>	<i>Retinella hierroensis</i>
H319	PZ349320	PZ351329	PZ357107	PZ394650	PZ411342	PZ411384	<i>Retinella circumsesta</i>	<i>Retinella circumsesta</i>
AG042	PZ349290	PZ351299	PZ357081	PZ394623	PZ411316	PZ411358	<i>Retinella cisalpina</i>	<i>Aegopinella cisalpina</i>
AG043	PZ349291	PZ351300	PZ357082	PZ394624	PZ411317	PZ411359	<i>Retinella cisalpina</i>	<i>Aegopinella graziadei</i>
AG045	PZ349293	PZ351302	PZ357084	PZ394626	PZ411319	PX832790	<i>Retinella cisalpina</i>	<i>Aegopinella graziadei</i>
AG077	PZ349303	PZ351312	PZ357093	PZ394635	PZ411327	PZ411368	<i>Retinella giustii</i>	<i>Retinella giustii</i>
AG035	x	x	x	x	PZ411312	PZ411354	<i>Retinella graziadei</i>	<i>Aegopinella graziadei</i>
AG036	PZ349287	PZ351296	PZ357077	PZ394619	PZ411313	PZ411355	<i>Retinella graziadei</i>	<i>Aegopinella graziadei</i>
AG044	PZ349292	PZ351301	PZ357083	PZ394625	PZ411318	PZ411360	<i>Retinella graziadei</i>	<i>Aegopinella graziadei</i>
AG017	PZ349279	PZ351288	PZ357069	PZ394611	PZ411305	PZ411347	<i>Retinella hiulca</i>	<i>Retinella hiulca</i>
AG074	PZ349302	PZ351311	PZ357092	PZ394634	PZ411326	PZ411367	<i>Retinella hiulca</i>	<i>Retinella hiulca</i>
AG106	PZ349315	PZ351324	PZ357102	PZ394645	PZ411337	PZ411379	<i>Retinella incerta</i>	<i>Retinella incerta</i>
AG016	PZ349278	PZ351287	PZ357068	PZ394610	PX832749	PX832793	<i>Retinella iuncta</i>	<i>Aegopinella epipedostoma iuncta</i>
AG029	PZ349285	PZ351294	PZ357075	PZ394617	PZ411310	PZ411352	<i>Retinella iuncta</i>	<i>Aegopinella epipedostoma iuncta</i>
AG021	PZ349282	PZ351291	PZ357072	PZ394614	PZ411308	PZ411350	<i>Retinella minor</i>	<i>Aegopinella minor</i>
AG022	PZ349283	PZ351292	PZ357073	PZ394615	PZ411309	PZ411351	<i>Retinella minor</i>	<i>Aegopinella minor</i>
AG070	PZ349298	PZ351307	PZ357090	PZ394632	PX832747	PX832792	<i>Retinella minor</i>	<i>Aegopinella minor</i>
AG072	PZ349300	PZ351309	x	x	x	x	<i>Retinella minor</i>	<i>Aegopinella minor</i>
AG019	PZ349280	PZ351289	PZ357070	PZ394612	PZ411306	PZ411348	<i>Retinella n. epipedostoma</i>	<i>Aegopinella e. epipedostoma</i>
AG039	PZ349288	PZ351297	PZ357079	PZ394621	PZ411315	PZ411357	<i>Retinella n. epipedostoma</i>	<i>Aegopinella epipedostoma</i>
AG055	PZ349295	PZ351304	PZ357086	PZ394628	PZ411320	PZ411361	<i>Retinella n. epipedostoma</i>	<i>Aegopinella epipedostoma</i>
AG095	PZ349309	PZ351318	PZ357098	PZ394640	PZ411331	PZ411373	<i>Retinella n. epipedostoma</i>	<i>Aegopinella cf. epipedostoma</i>
AG096	PZ349310	PZ351319	PZ357099	PZ394641	PZ411332	PZ411374	<i>Retinella n. epipedostoma</i>	<i>Aegopinella cf. epipedostoma</i>
AG001	x	x	PZ357063	PZ394605	x	x	<i>Retinella n. nitidula</i>	<i>Aegopinella cf. nitidula</i>
AG013	PZ349277	PZ351286	PZ357067	PZ394609	PZ411304	PZ411346	<i>Retinella n. nitidula</i>	<i>Aegopinella nitidula</i>
AG030	PZ349286	PZ351295	PZ357076	PZ394618	PZ411311	PZ411353	<i>Retinella n. nitidula</i>	<i>Aegopinella nitidula</i>
AG041	PZ349289	PZ351298	PZ357080	PZ394622	x	x	<i>Retinella n. nitidula</i>	<i>Aegopinella nitidula</i>

DNA codes	16S	COI	ITS1	ITS2	KY1	LOC8662	DNA ID	morpho ID
AG071	PZ349299	PZ351308	PZ357091	PZ394633	PZ411324	PZ411365	Retinella n. nitidula	Aegopinella nitidula
AG090	PZ349306	PZ351315	PZ357096	PZ394638	PX832748	PZ411371	Retinella n. nitidula	Aegopinella nitidula
AG103	PZ349314	PZ351323	x	x	PZ411336	PZ411378	Retinella n. nitidula	Aegopinella nitidula
AG004	PZ349275	PZ351284	PZ357064	PZ394606	PX832745	PX832789	Retinella nitens	Aegopinella nitens
AG007	PZ349276	PZ351285	PZ357065	PZ394607	PZ411303	PZ411345	Retinella nitens	Aegopinella nitens
AG009	x	x	PZ357066	PZ394608	x	x	Retinella nitens	Aegopinella nitens/epipedostoma
AG020	PZ349281	PZ351290	PZ357071	PZ394613	PZ411307	PZ411349	Retinella nitens	Aegopinella nitens
AG101	PZ349313	PZ351322	x	PZ394644	PZ411335	PZ411377	Retinella o. icterica	Retinella olivetorum icterica
AG073	PZ349301	PZ351310	x	x	PZ411325	PZ411366	Retinella o. olivetorum	Retinella olivetorum olivetorum
AG080	PZ349304	PZ351313	PZ357094	PZ394636	PZ411328	PZ411369	Retinella pseudoaegopinella	Retinella pseudoaegopinella
AG099	PZ349312	PZ351321	PZ357101	PZ394643	PZ411334	PZ411376	Retinella pseudoaegopinella	Retinella pseudoaegopinella
AG066	x	x	PZ357087	PZ394629	PZ411321	PZ411362	Retinella pura	Aegopinella pura
AG067	PZ349296	PZ351305	PZ357088	PZ394630	PZ411322	PZ411363	Retinella pura	Aegopinella pura
AG068	PZ349297	PZ351306	PZ357089	PZ394631	PZ411323	PZ411364	Retinella pura	Aegopinella pura
AG091	PZ349307	PZ351316	x	x	x	x	Retinella pura	Aegopinella pura
AG026	PZ349284	PZ351293	PZ357074	PZ394616	PX832751	PX832795	Retinella ressmanni	Aegopinella ressmanni
AG037	x	x	PZ357078	PZ394620	PZ411314	PZ411356	Retinella ressmanni	Aegopinella ressmanni
AG050	PZ349294	PZ351303	PZ357085	PZ394627	PX832746	PX832791	Retinella riedeli	Aegopinella forcarti
AG098	PZ349311	PZ351320	PZ357100	PZ394642	PZ411333	PZ411375	Retinella riedeli	Aegopinella forcarti
AG107	PZ349316	PZ351325	PZ357103	PZ394646	PZ411338	PZ411380	Retinella stabilei	Retinella stabilei
H035	x	x	PP565257	PZ394651	PZ411343	PZ411385	Perpolita petronella	Perpolita petronella
H047	x	x	PP565277	PZ394652	PZ411344	PZ411386	Perpolita hammonis	Perpolita hammonis
H206	PZ349321	PV123939	x	x	x	x	Perpolita electrina	Perpolita electrina
H211	PZ349322	PZ351330	x	x	x	x	Perpolita binneyana	Perpolita binneyana

Table S1. Samples included in the genetic analyses, with associated locality information.

GenBank accession number is provided for sequence used in this study; x, sequence unavailable or not used. For “type region” samples, the reported distance in brackets is the geographical distance between the sampling locality and the type locality.

Table S1 · Part 2 of 2 — Geographic and collection information (DNA codes repeated)

DNA code s	Geographical region	WSG84 (Lat)	WSG84 (Long)	Country	Region/Area	Settlement Municipali ty (Province)	Location/Habitat	Collector(s)	Date	Type locality / region
AG088	Julian_Prealps_IT	46.04694	13.39861	Italy	Friuli-Venezia Giulia	Premariacco (Udine)	Paderno, Premariacco, slope forest above Natisone river	M. Horsák, V. Horsáková	2023-07-14	type locality
AG092	Julian_Prealps_IT	46.04694	13.39861	Italy	Friuli-Venezia Giulia	Premariacco (Udine)	Premariacco, Left bank of Natisone river, front of Paderno	G. Nardi et A. Braccia	2023-10-21	type locality
AG108	La_Palma_ES	28.6694	-17.8183	Spain	Canary Isl, La Palma	Barranco de Laja	laurisilva in wooded barranco	P. Tatterfield	2025-10-17	
AG109	La_Palma_ES	28.6291	-17.8274	Spain	Canary Isl, La Palma	Refugio de Pilar	laurisilva on steep east slope	P. Tatterfield	2025-10-17	
AG110	El_Hierro_ES	27.73	-18.016	Spain	Canary Isl, El Hierro	La Frontera	Camino de los Llanillos, laurisilva Hoya el Creal	P. Tatterfield	2025-10-17	
H319	Tenerife_ES	28.5324	-16.2807	Spain	Canary Isl, Tenerife	Cruz del Carmen	cloud Laurisilva forest	M. Horsák, V. Horsáková	2022-04-19	type locality
AG042	Euganean_Hills_IT	45.26094	11.74527	Italy	Veneto	Monselice (Padova)	NE slope of Monte Ricco hill, dry shrubby forest with screes	M. Horsák, V. Horsáková	2022-07-18	type locality
AG043	Venetian_Prealps_IT	45.96364	11.23783	Italy	Trentino Alto Adige	Caldonazzo (Trento)	Val di Centa, Case More, alluvial forest between mill ruins, under fallen wood	M. Horsák, V. Horsáková	2022-07-19	
AG045	Venetian_Prealps_IT	45.98243	11.20299	Italy	Trentino Alto Adige	Caldonazzo (Trento)	Doss del Bue, beech forest on limestone	M. Horsák, V. Horsáková	2022-07-20	
AG077	Pennine_Alps_IT	45.71015	8.31073	Italy	Piemonte	Borgosesia (Vercelli)	Presso il Buco della Bondaccia, M. Fenera	M. Bodon, E. Bodon & G. Nardi	2012-10-21	type region (30 km)
AG035	Venetian_Prealps_IT	45.92691	11.26399	Italy	Trentino Alto Adige	Lavarone (Trento)	Grotta Covelo di Rio Malo, i Piccoli, Lavarone	M. Bodon	1990-09-16	
AG036	Venetian_Prealps_IT	45.95027	11.24254	Italy	Trentino Alto Adige	Lavarone (Trento)	Comando Austroungarico Virti, a est di Lavarone	M. Bodon & E. Bodon	2009-09-10	
AG044	Venetian_Prealps_IT	45.97188	11.24402	Italy	Trentino Alto Adige	Caldonazzo (Trento)	Val di Centa, river alluvium by parking lot	M. Horsák, V. Horsáková	2022-07-19	type locality
AG017	Lombardian_Prealps_IT	46.01181	9.10783	Italy	Lombardia	Claino con Osteno (Como)	limestone forest scree by the Santa Giulia waterfall	M. Horsák	2019-07-09	
AG074	Lombardian_Prealps_IT	45.96975	10.1032	Italy	Lombardia	Colere (Bergamo)	Colere, Bergamo, Frazione Grana	G. Nardi (Bs)	2017-06-25	type region (60 km)
AG106	Basque_Mts_ES	43.17034	-2.30148	Spain	Euskadi	Azkoitia	alluvial forest along the river	M. Horsák, V. Horsáková	2024-08-10	type region (100 km)
AG016	Moravia_CZ	49.70994	17.03654	Czechia	Moravia	Mladeč	NPR Vrapač, severozápadní část u smuhy Malá voda	M. Horsák et J. Vašítko	2001-10-11	type region (70 km)
AG029	Strazovske_Mts_SK	49.10056	18.56111	Slovakia	Strážovské vrchy	Bodina	Biela Voda, clacareous spring fen SW Vysoký vrch	M. Horsák	2001-09-15	type region (80 km)
AG021	Povazsky_Inovec_SK	48.63787	17.92776	Slovakia	Považský Inovec Mts.	Stará Lehota	forest in the valley of Modrovský potok	M. Horsák	2001-09-10	
AG022	Moravia_CZ	48.84146	17.58347	Czechia	Moravia	Javorník	deciduous forest at Nardský kopec	M. Horsák et V. Ložek	2000-08-09	
AG070	Bohemia_CZ	49.67951	12.66642	Czechia	Bohemia	Přimda	Přimda NR, forest below the Přimda castle ruin	M. Horsák	2002-05-01	
AG072	Maritime_Alps_FR	43.77106	7.06319	France	Maritime Alps	Coursegoules		O. Gerrier	2022-11-04	type region (250 km)

DNA code s	Geographical region	WSG84 (Lat)	WSG84 (Long)	Country	Region/Area	Settlement Municipali ty (Province)	Location/Habitat	Collector(s)	Date	Type locality / region
AG019	Pyrenees_FR	42.76187	0.49393	France	Pyrenees	Les Granges d’Astau	beech forest on limestone rocks	M. Horsák et V. Horsáková	2014-09-08	
AG039	Pyrenees_FR	42.6898	2.03728	France	Pyrénées	Artigues	(approximate GPS coordinates = municipality centroid)	A. Bertrand	00.06.2021	
AG055	Pyrenees_FR	42.99819	1.03527	France	Pyrénées	Cazavet	(approximate GPS coordinates = municipality centroid)	A. Bertrand	00.06.2021	
AG095	Pyrenees_FR	42.89912	1.33585	France	Ariège	Bousсенac, Abela		A. Bertrand	2021-05-01	subspecies type region (25 km)
AG096	Pyrenees_FR	43.48206	-1.41138	France	Pyrénées	Lahonce		S. Gansoinat	2024-01-24	
AG001	Vosges_FR	47.69636	6.36267	France	Haute-Saône (70)	Adelans-et-le-Val-de-Bithaine	mixed lowland forest on alluvial deposits	J. Ryelandt	2021-05-05	
AG013	Moravia_CZ	49.70994	17.03654	Czechia	Moravia	Mladeč	Vrapač NNR, floodplain forest in NW part by Malá voda	M. Horsák et J. Vašítko	2001-10-11	
AG030	Chalk_Hills_UK	51.25214	-0.31724	United Kingdom	England	Dorking	forest in the vicinity of Box Hill	M. Horsák	2004-11-06	type region (150 km)
AG041	Jura_FR	47.6821	6.36975	France	Franche-Comté	Genevrevuille	mixed lowland forest on marl substrate	J. Ryelandt	2021-05-05	
AG071	Brittany_FR	48.48419	-2.07416	France	Brittany	Taden	lowland riparian forest on granitic substrate	B. Lecaplain	2022-05-17	
AG090	Faroes_DK	61.95048	-6.78397	Denmark	Faroe Islands	Kirkjubøur	vegetated rock ledges on seepage	M. Horsák, V. Horsáková	2023-08-06	
AG103	Basque_Mts_ES	43.17034	-2.30148	Spain	Euskadi	Azkoitia	alluvial forest along the river	M. Horsák, V. Horsáková	2024-08-10	
AG004	Jura_FR	47.09195	6.09394	France	Doubs (25)	Cléron	fresh deciduous forest on limestone slopes	J. Ryelandt	2021-02-23	
AG007	Jura_FR	46.70805	6.09116	France	Jura (39)	Arsure-Arsurette	deciduous forest on limestone slopes	J. Ryelandt	2021-05-26	type region (70 km)
AG009	Jura_FR	46.73987	6.3612	France	Doubs (25)	Jougne	fresh deciduous forest on limestone slopes	J. Ryelandt	2021-05-14	
AG020	Moravia_CZ	49.52773	18.66983	Czechia	Moravia	Horní Lomná	Mionší NNR, forest in valley of the Menšího potoka	M. Horsák	1998-08-29	
AG101	Nebrodi_Mts_IT	37.97611	14.47638	Italy	Sicilia	Caronia (Messina)	Caronia, Castello dell'Impallaccionata, Nebrodi Mountains	I. Sparacio	2023-10-22	
AG073	Po_Valley_IT	45.20441	10.73992	Italy	Lombardia	Marmirolo (Mantova)	Marmirolo, Mantova, Roggia Begotta, area meridionale del Bosco Fontana	G. Nardi (Bs)	2012-06-17	type region (150 km)
AG080	Ligurian_Alps_IT	44.19815	7.671	Italy	Piemonte	Chiusa di Pesio (Cuneo)	Vallone del Salto, Val Pesio, 1350 m	M. Bodon & G. Vezzani	2017-06-02	type region (25 km)
AG099	Ligurian_Alps_IT	44.24167	7.84249	Italy	Piemonte	Frabosa Soprana (Cuneo)	Right bank of the stream Corsaglia, front of Grotta di Bossea (Cave of Bossea)	G. Nardi et A. Braccia	2023-10-09	type region (25 km)
AG066	Bohemia_CZ	49.67951	12.66642	Czechia	Bohemia	Přimda	Přimda NR, forest below the Přimda castle ruin	M. Horsák	2002-05-01	
AG067	Moravia_CZ	48.85818	17.67157	Czechia	Moravia	Vápenky	Javořina NNR, forest near Velička spring	M. Horsák	2001-07-03	
AG068	Highlands_UK	56.67953	-5.09561	United Kingdom	Scotland	Glencoe	wet birch forest SE below the city	M. Horsák	2018-07-13	type region (300 km)
AG091	Faroes_DK	62.01628	-6.9075	Denmark	Faroe Islands	Syðradalur	vegetated rock ledges	M. Horsák, V. Horsáková	2023-08-06	
AG026	Bohemia_CZ	49.98389	16.41425	Czechia	Bohemia	Ústí nad Orlicí	Oldřichovice, alluvial forest along Tiché Orlice river	J. Myšák	2007-09-24	

DNA code s	Geographical region	WSG84 (Lat)	WSG84 (Long)	Country	Region/Area	Settlement Municipali ty (Province)	Location/Habitat	Collector(s)	Date	Type locality / region
AG037	Julian_Prealps_IT	46.55106	13.48214	Italy	Friuli-Venezia Giulia	Malborgehtto Valbruna (Udine)	Rif. Nordio	E. Bodon	1991-08-01	type region (150 km)
AG050	Dinaric_Alps_SI	45.8689	15.4293	Slovenia	Krško Hills	Kostanjevica na Krki	Konstanjeviška jama, old floodplain forest	M. Horsák, V. Horsáková	2022-09-05	type locality
AG098	Julian_Prealps_IT	46.41889	13.5725	Italy	Friuli-Venezia Giulia	Tarvisio (Udine)	Tarvision, Km 82,000 of the road SS54 from Predil Lake to to Predil Pass (near the 2nd bend)	G. Nardi et A. Braccia	2023-05-06	
AG107	Graian_Alps_IT	45.21682	7.37698	Italy	Piemonte	Col San Giovanni, brook N of village	beech forest by the brook	M. Horsák, V. Horsáková, L. Salvadori	2025-10-17	type locality
H035	Jamtlands_SE	63.49822	15.39256	Sweden	Jämtlands	Viken	Hagamarken, alkaline fen	M. Horsák	2006-08-15	
H047	Austurland_IS	64.02667	-16.9765	Iceland	Austurland	Skaftafell NP	shrubby tundra	M. Horsák	2016-08-01	
H206	Maine_US	44.8188	-68.7226	United States	Maine	Brewer Boat Ramp		J. Nekola	2002-10-13	
H211	Michigan_US	46.0397	-84.7134	United States	Michigan	ESE-6904/6905, Pole 623		J. Nekola	2009-09-27	

Table S2. Matrix of 31 anatomical characters (C1-C31) used for the maximum parsimony and PCoA analysis.

Characters are coded for 17 nominal *Retinella* species included in the phylogenetic revision, with *Perpolita hammonis* as the outgroup. For character descriptions, see the sheet “List of all characters.”

Table S2 · Part 1 of 3 — Anatomical characters C1-C11 (taxon columns repeated)

			glandular tissue	glandular tissue	vagina	vagina	vagina	bursa copulatr ix	bursa copulatr ix	bursa copulatr ix	penis	penis	penis
	species name	subgenus	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11
1	pseudoaegopinella	Aegopinella	1	0	1	0	0	1	1	3	1	0	0
2	pura	Aegopinella	1	1	0	na	0	1	0	2	2	0	0
3	stabilei	Aegopinella	0	na	1	0	1	1	1	3	1	0	0
4	circumsessa	Lyrodiscus	0	na	0	0	0	2	1	3	1	0	0
5	bodoni	Politenella	0	na	1	0	0	1	0	0	1	1	1
6	cisalpina	Politenella	0	na	1	0	0	0.25	0	0.25	1	1	1
7	riedeli	Politenella	0	na	1	0	0	0.25	0	0	1	1	1
8	graziadei	Politenella	0	na	1	0	0.5	0.5	0	0	1	1	1
9	iuncta	Politenella	0	na	1	0.5	0.75	0.75	0	2	0.25	1	1
10	minor	Politenella	0	na	1	0	0	1.75	0	0	2	1	1
11	nitens	Politenella	0	na	1	0	1	1	0	2	1	1	1
12	nitidula	Politenella	0	na	1	0.75	1	1	0	2	0.25	1	1
13	ressmanni	Politenella	1	0	1	0	0	0	0	0	1	1	1
14	giustii	Retinella	0	na	0	na	0	2	1	3	1	0	0
15	hiulca	Retinella	0	na	0	na	0	2	1	3	1	0	0
16	olivetorum	Retinella	0	na	0.75	0	0	2	1	3	2	0	0
17	incerta	Retinelloides	0	na	1	0	1	2	1	3	1	0	1
outgroup	Perpolita hammonis	na	1	1	1	0	2	1	1	3	0	0	0

Table S2. Matrix of 31 anatomical characters (C1-C31) used for the maximum parsimony and PCoA analysis.

Characters are coded for 17 nominal *Retinella* species included in the phylogenetic revision, with *Perpolita hammonis* as the outgroup. For character descriptions, see the sheet "List of all characters."

Table S2 · Part 2 of 3 — Anatomical characters C12-C22 (taxon columns repeated)

[illegible]

Table S2. Matrix of 31 anatomical characters (C1-C31) used for the maximum parsimony and PCoA analysis.

Characters are coded for 17 nominal *Retinella* species included in the phylogenetic revision, with *Perpolita hammonis* as the outgroup. For character descriptions, see the sheet “List of all characters.”

Table S2 · Part 3 of 3 — Anatomical characters C23-C31 (taxon columns repeated)

			vas deferens	epiphallus	epiphallus	epiphallus	epiphallus	penial caecum	penial caecum	penial caecum	penial caecum
	species name	subgenus	C23	C24	C25	C26	C27	C28	C29	C30	C31
1	pseudoaegopinella	Aegopinella	0	1	0	0	1	1	0	0	2
2	pura	Aegopinella	2	0	1	0	0	0	na	na	na
3	stabilei	Aegopinella	1	1	1	0	1	0	na	na	na
4	circumsessa	Lyrodiscus	1	1	0	0	0	1	1	0	0
5	bodoni	Politenella	2	0	1	0	0	0	na	na	na
6	cisalpina	Politenella	2	0	1	0	0	0	na	na	na
7	riedeli	Politenella	2	0	1	0	0	0	na	na	na
8	graziadei	Politenella	2	0	1	0	0	0	na	na	na
9	iuncta	Politenella	2	0	1	0	0	0	na	na	na
10	minor	Politenella	2	0	1	0	0	0	na	na	na
11	nitens	Politenella	2	0	1	0	0	0	na	na	na
12	nitidula	Politenella	2	0	1	0	0	0	na	na	na
13	ressmanni	Politenella	2	0	1	0	0	0	na	na	na
14	giustii	Retinella	0	1	0	0	1	1	0	2	2
15	hiulca	Retinella	0	1	0	0	1	1	0	0	1
16	olivetorum	Retinella	0	1	0	0	1	1	0	1	0
17	incerta	Retinelloides	2	1	1	1	1	0	na	na	na
outgroup	Perpolita hammonis	na	2	1	1	0	0	0	na	na	na

Table S3. Matrix of 7 shell morphology characters (C32-C38) used for the PCoA analysis.

Characters are coded for 17 nominal *Retinella* species included in the phylogenetic revision, with *Perpolita hammonis* as the outgroup. For character descriptions, see the sheet “List of all characters.”

Table S3 — Shell morphology characters C32-C38

			width	height	whorl	shoulder	lustre	sillons	ridges
	species name	subgenus	C32	C33	C34	C35	C36	C37	C38
1	pseudoaegopinella	Aegopinella	2	1	2	1	2	3	0
2	pura	Aegopinella	1	2	1	1	2	3	0
3	stabilei	Aegopinella	2	1	3	1	2	3	0
4	circumsessa	Lyrodiscus	4	6	3	0	1	1	1
5	bodoni	Politenella	4	6	3	0	2	2	0
6	cisalpina	Politenella	6	9	4	0	2	2	0
7	riedeli	Politenella	5	6	3	0	2	2	0
8	graziadei	Politenella	6	7	4	0	2	2	0
9	iuncta	Politenella	4	5	3	0	2	2	0
10	minor	Politenella	3	3	3	0	2	2	0
11	nitidula	Politenella	5	5	3	0	2	3	0
12	nitens	Politenella	5	6	3	0	2	2	0
13	ressmanni	Politenella	6	8	3	0	2	3	0
14	giustii	Retinella	3	4	2	0	3	1	0
15	hiulca	Retinella	6	8	4	0	3	1	0
16	olivetorum	Retinella	7	10	5	0	3	1	0
17	incerta	Retinelloides	8	10	6	0	4	1	0
outgroup	Perpolita hammonis	na	1	1	1	0	4	1	0