

# *Pilsbrylia*, a dextral-shelled door snail from South America (Gastropoda, Clausiliidae)

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## Abstract

The land snail genus *Pilsbrylia* Hylton Scott, 1952 has been recently shown to not belong to the superfamily to which it was originally assigned (i.e., the Orthalicoidea), instead pointing out a relationship with the Clausilioidea. In this study, we included the type species of the genus in a multi-marker molecular phylogenetic framework to reassess its family-level classification. Our results show that *Pilsbrylia* belongs to family Clausiliidae (known as ‘door snails’) and more specifically, to subfamily Peruniinae. This family is unique among stylommatophorans for consisting almost exclusively of animals with sinistral (left-handed) shells, whilst *Pilsbrylia* has a “typical” dextral shell.

## Key Words

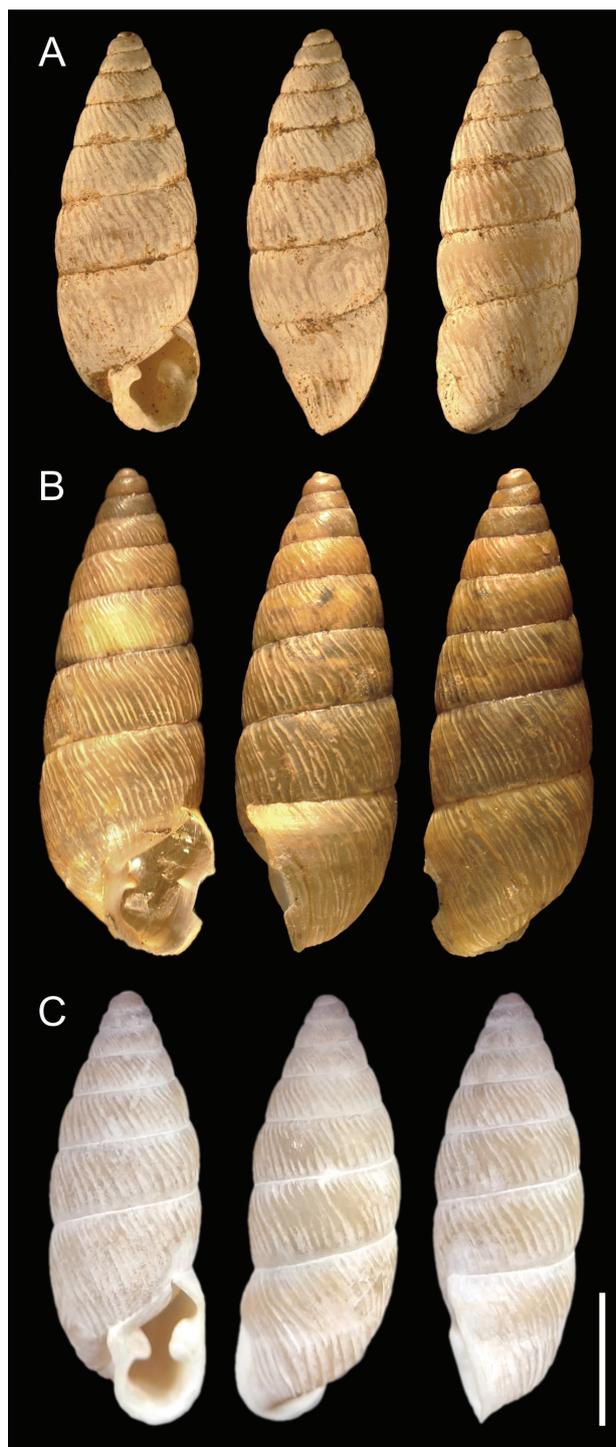
chirality, Eupulmonata, Orthalicoidea, Peruniinae, Stylommatophora

## Introduction

The South American genus *Pilsbrylia* Hylton Scott, 1952 contains three species, which inhabit areas in southern Brazil and northern Argentina (Simone 2018): *Pilsbrylia paradoxa* Hylton Scott, 1952 (the type species), *P. hyltonae* Fernández & Rumi, 1980, and *P. dalli* Simone, 2018 (Fig. 1). Members of this genus have high-spired and narrow shells and the shell aperture bears a number of teeth and lamellae. The shells are morphologically similar to the members of family Cyclodontinidae (which was formerly part of Odontostomidae; see Salvador et al. 2023 for the revised classification within superfamily Orthalicoidea), in particular to members of genera such as *Clessinia* Doering, 1875 and *Cyclodontina* H. Beck, 1837. Thus, since its description, *Pilsbrylia* has been classified in that family (e.g., Hylton Scott 1952; Breure 1974; Fernández and Rumi 1980; Schileyko 1999; Cuezco et al. 2013; Simone 2018).

The molecular phylogenetic study of Breure and Romero (2012) showed that *Pilsbrylia* did not belong in Cyclodontinidae and those authors proposed the genus was instead the sister taxon to all other Orthalicoidea. A more recent phylogenetic study (Salvador et al. 2023), showed that *Pilsbrylia* did not belong in Orthalicoidea at all, being instead related to the Clausiliidae, or door snails. That was a surprising result, considering that the door snails typically have sinistral shells (i.e., a shell that “coils” counter-clockwise or, when seen with its aperture facing the observer and the spire top pointing upwards, a shell whose aperture is on the left-hand side).

Furthermore, the two branches of Clausiliidae present in the Americas are restricted to the Caribbean (subfamily Neniinae) and northwest South America (subfamily Peruniinae) (Uit de Weerd and Gittenberger 2013). Thus, the genus *Pilsbrylia* is rather geographically removed from the family’s range.



**Figure 1.** Shells of the three known species of *Pilsbrylia* in apertural, lateral, and dorsal views. **A.** *P. paradoxa*, holotype MLP-Ma 11337 (Museo de La Plata, Argentina); **B.** *P. hyltonae*, lectotype MLP-Ma 3991-1; **C.** *P. dalli*, holotype MZSP 133161 (Museu de Zoologia da Universidade de São Paulo, Brazil). Scale bar: 5 mm.

In the present study we include *Pilsbrylia* in a phylogenetic framework of the Clausiliidae to test if it really belongs to this family and, if so, assess how it is related to other South American door snails.

## Materials and methods

DNA sequences of *Pilsbrylia paradoxa* used in previous Orthalicoidae-focused studies (Breure and Romero 2012; Salvador et al. 2023) were used for the present analysis. They are available on GenBank under accession numbers [JF514745](#) (28S) and [JF514687](#) (H3) and stem from a voucher specimen previously housed in the collection of the Instituto Miguel Lillo, Tucumán, Argentina (registration number IFML-MOLL BD316, collected in northern Argentina, Salta province, km 1650 of Salta-Jujuy highway) but now housed in the Instituto de Biodiversidad Neotropical (IBN) of the Universidad Nacional de Tucumán, Argentina. No further specimen of *Pilsbrylia* spp. preserved in ethanol and suitable for DNA extraction could be found in the present study.

The sequences of *P. paradoxa* were included in the phylogenetic framework for the family Clausiliidae established in the study of Uit de Weerd and Gittenberger (2013). That study included a reasonable sample of South American (subfamily Neniinae) taxa across two distinct clades (tribes), which enables us to test the phylogenetic position of *Pilsbrylia*. Later molecular phylogenetic studies with Clausiliidae focused on Eurasian taxa (e.g., Hausdorf and Neiber 2022) and data from them was not included here.

A total of 67 species of Clausiliidae, belonging to all subfamilies and almost all tribes, was part of the analysis. Five species were selected as outgroup representing the families Cerionidae, Chondrinidae, Enidae, Rhytididae, and Urocopitidae (data from Uit de Weerd 2008; Uit de Weerd and Gittenberger 2013; Saadi and Wade 2019). A complete list of the species used in the analysis, with locality data and GenBank accession numbers is given in the Suppl. material 1.

Data from three nuclear markers were used in the present phylogenetic analysis, following Uit de Weerd and Gittenberger (2013): partial 28S rRNA gene (ca. 1700 bp), partial H3 (histone 3) gene (ca. 270 bp), and partial H4 (histone 4) gene (ca. 260 bp). Information on primers and PCR protocols can be found in Uit de Weerd and Gittenberger (2013); for *Pilsbrylia*, see Breure and Romero (2012).

The genetic sequences were aligned through the MUSCLE plugin (Edgar 2004) in Geneious Prime (v.2023.0.4, Biomatters Ltd.), using default settings (i.e., optimised for accuracy). The resulting alignments were visually proofed for inconsistencies. The alignment of the 28S marker was run through Gblocks (Talavera and Castresana 2007), using the least restrictive settings, in order to eliminate poorly-aligned or data-deficient positions that could introduce noise into the analysis. The alignments were then concatenated for a single phylogenetic analysis, with each marker being treated as an individual partition.

A Bayesian inference phylogenetic analysis was performed through MrBayes (v.3.2.7; Ronquist et al. 2012) via the CIPRES Science Gateway (v.3.3; Miller et al. 2015). Two concurrent analyses, each with 4 Markov chains of 80 million generations (the first 20% discarded as ‘burn-in’), were run with the default priors,  $nst = 6$ ,

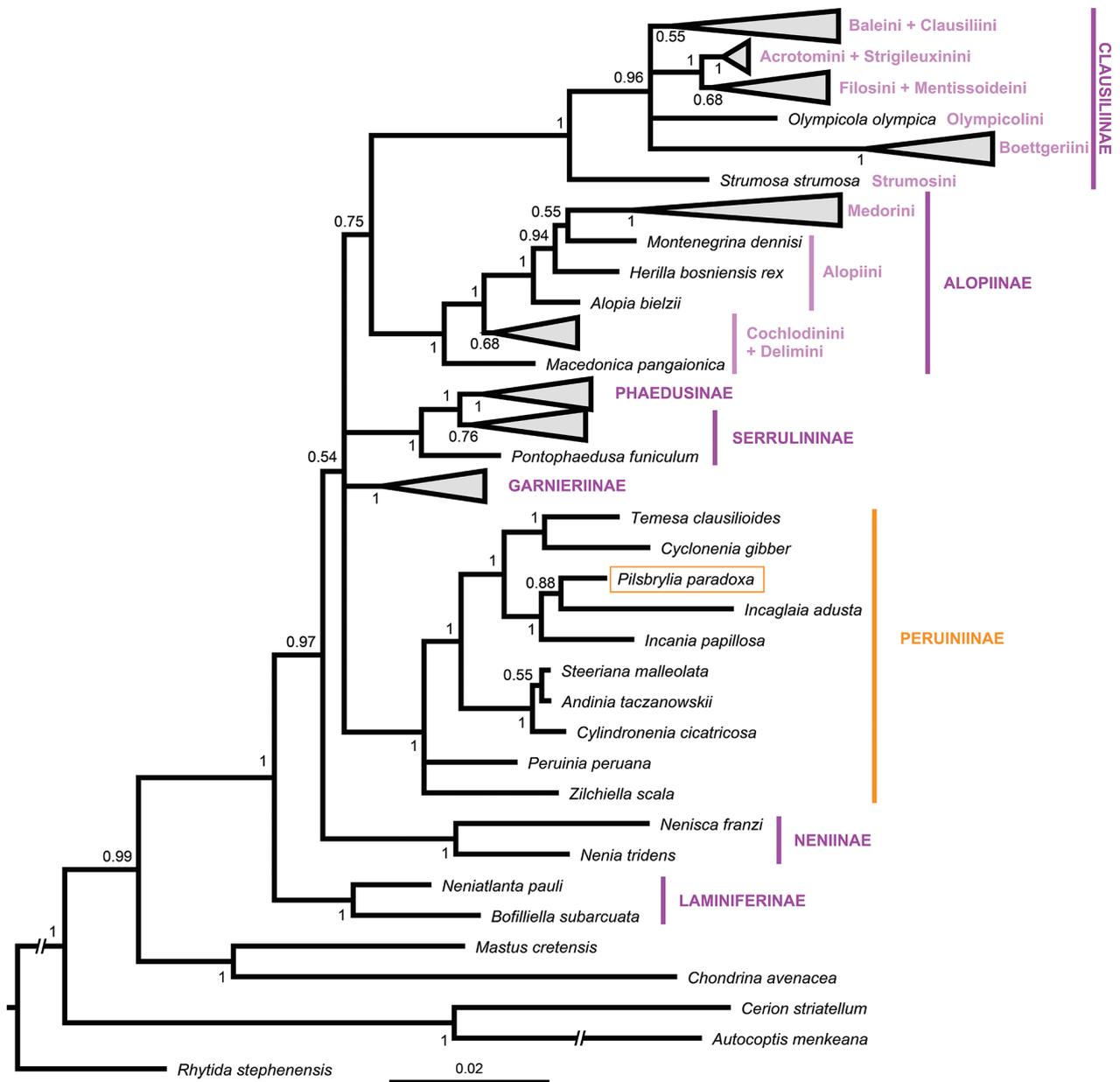
rates = invgamma, temperature parameter = 0.1, sampling every 1,000 generations. Substitution model parameters were unlinked across the markers (28S, H3, and H4). MCMC convergence was assessed using the standard deviation of split frequencies (<0.01) and the potential scale reduction factor PSRF (~1.0), as well as by examining the trace plots (Ronquist et al. 2009).

## Results

The concatenated sequences of the three markers (after trimming the 28S marker using Gblocks) contained 2172 bp. The total-evidence tree resulting from the Bayesian analysis contained 73 species (including

*Pilsbrylia paradoxa* and the outgroup) and is shown here in simplified format, with the branches of non-immediate interest collapsed (Fig. 2). The full tree can be seen in the Suppl. material 2.

All subfamilies of Clausiliidae are strongly supported (posterior probability PP=1), except for Serrulininae, which is paraphyletic (Fig. 2). As expected in a clade inflated by family-level names, many of the supposed tribes were recovered as para- or polyphyletic (e.g., Cochlodini, Delimini; Suppl. material 2), as already noted by Uit de Weerd and Gittenberger (2013). When also considering the existence of several monotypic tribes (e.g., Garnieriini, Strumosini), the Clausiliidae would benefit from a clean-up of names on the tribe level, as many could easily be synonymized if supported by further research.



**Figure 2.** Bayesian inference tree of the Clausilioidea. The crown group is collapsed to facilitate visualization (see Suppl. material 2 for a full view). Posterior probabilities are shown on nodes. Scale bar is substitutions per site.

Considering that our ingroup was identical to that of Uit de Weerd and Gittenberger (2013) – barred the inclusion of *Pilsbrylia paradoxa* – our tree is largely similar to the one presented by those authors. However, a major difference can be observed: while Uit de Weerd and Gittenberger (2013) had the Neniinae as the sister group to all other clausiliids (though unsupported), our tree places the Laminiferinae in that position (Fig. 2). The Laminiferinae are sister to the remaining clausiliids (PP=0.97) and, inside the latter clade, the Neniinae are sister to all others, which are joined in an unsupported polytomy (PP=0.54). This polytomy includes three strongly supported branches (PP=1: Peruniinae, Garnieriinae, and a clade formed by Phaesusinae and a paraphyletic Serrulininae) and an unsupported branch (PP=0.75) containing the Alopiinae and the Clausiliinae (each PP=1) as sister clades (Fig. 2).

Our taxon of interest, *Pilsbrylia paradoxa*, is included in the Peruniinae, in a derived position (Fig. 2). It was recovered as the sister taxon to the Peruvian *Incaglaia adusta* (O. Boettger, 1880), although with low support (PP=0.88). Both species are the sister clade to *Incania papillosa* Neubert & Nordsieck, 2005 (PP=1), also from Peru. The relationships within Peruniinae recovered by our analysis are largely the same as those in Uit de Weerd and Gittenberger (2013), with a single exception: those authors recovered *Zilchiella scala* Neubert & Nordsieck, 2005 and *Perunia peruana* (Troschel, 1847) as sister taxa (although unsupported), while in the present tree they form a trichotomy with the remaining Peruniinae (Fig. 2).

## Discussion

The results of the present phylogenetic analysis allow the conclusion that *Pilsbrylia* in fact belongs in the family Clausiliidae, more specifically to the South American subfamily Peruniinae, as expected from a biogeographical perspective.

*Pilsbrylia*, however, has a much different shell morphology from other members of the Peruniinae. First of all, the shell is dextral. Dextral shells are rare in Clausiliidae, but are more frequent in subfamily Alopiinae (Nordsieck 2007; Páll-Gergely et al. 2019). Within the genera *Alopiia* Adams & Adams, 1855 and *Albinaria* Vest, 1867, enantiomorph taxon pairs are known, and dextral lineages have evolved from sinistral ancestors multiple times independently (Fehér et al. 2013; Kornilios et al. 2015). While those shells are usually near mirror images of their congeners or conspecifics, *Pilsbrylia*, on the other hand, presents a shell morphology that is more similar to Cyclodontinidae than to most Clausiliidae, notably by not having a complete peristome (Fig. 1), which justified its previous classification.

Hylton Scott (1952) described the radular and genital anatomy of *P. paradoxa*, the only species in the genus for which anatomical data is available. Notably, that author did not mention a clausilium, which would be a reasonably obvious structure if present (unless it was present as a reduced structure). The single columellar lamella of *Pilsbrylia* spp. only extends about half whorl inside the shell along the columella (Hylton Scott 1952; Simone 2018), which

could indicate a reduced or absent clausilium. Clausiliidae have two columellar lamellae that stretch throughout the entire body whorl (Nordsieck 2007, 2015).

Species of the sister genus to *Pilsbrylia* in the present phylogeny, *Incaglaia* Pilsbry, 1949 (Fig. 2), have a typical clausiliid shell morphology. However, members of the next closest genus, *Incania* Poliński, 1922, can exhibit a simplified peristome, reduced lamellae and reduced clausilial apparatus (Neubert and Nordsieck 2005). Notably, some fossil species of European *Ryllia* Munier-Chalmas, 1883 and *Rillyarex* Nordsieck, 1985 have reduced or absent lamellae and a peristome with similar configuration to *Pilsbrylia* spp. (Nordsieck 2015).

Thus, the epithet '*paradoxa*' given to the first species described in this genus reaffirms itself as a very appropriate name: genetically, it is a Clausiliidae, but it does not possess the two typical traits of the family, i.e., a sinistral shell and a clausilial apparatus (or might present it in a reduced state). Nevertheless, the genetic similarity of *Pilsbrylia* with clausiliids is observed across all markers analysed and thus, a fortuitous similarity of a single marker biasing the phylogenetic analysis is unlikely. In view of the present evidence, we conclude that *Pilsbrylia* is a member of Peruniinae defined by its dextral shell and hypothesize that the simplification of the dextral shell (i.e., aperture and lamellae) is related to the reduction or loss of the clausilium.

The Clausilioidea are considered to have arisen in Europe, as both the extinct Filholiidae and Palaeostoidae, as well as one of the first branches of Clausiliidae (i.e., Laminiferinae; Fig. 2) are from that continent (Nordsieck 2007, 2015; Uit de Weerd and Gittenberger 2013). The Palaeostoidae date back to the Late Cretaceous of western Europe and so does Clausiliidae thanks to the extinct tribe Rillyini Nordsieck, 1985 (Nordsieck 2015).

Still, Clausiliidae has a clear Laurasian component, with Neniinae in the Caribbean and Peruniinae in South America (Fig. 2) and an origin in the latter cannot be entirely excluded (Uit de Weerd and Gittenberger 2013). It has since become known that the presence of the family Clausiliidae (and purportedly of subfamily Peruniinae) in South America is equally old. Namely, there is a fragmentary fossil from the Late Cretaceous of Uruguay classified as Clausiliidae indet. (Salvador et al. 2018; Cabrera et al. 2020). The next oldest fossil is *Temesa? magalhaesi* (Trinidad, 1953) from the Early Eocene of Rio de Janeiro, Brazil (Salvador and Simone 2013; Salvador et al. 2018). The latter species was provisionally assigned to a recent genus, but it likely belongs to a different (and still undescribed) genus, potentially close to the basal node of Peruniinae.

## Conclusion

Considering the present findings, we propose a revised classification of the genus *Pilsbrylia* Hylton Scott, 1952, placing it inside subfamily Peruniinae of the Clausiliidae. Thus, this extends the known range of this subfamily in South America to the south (Argentina and Uruguay) and to the east (Brazil, Minas Gerais state).

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## Supplementary material 1

### **Table listing all species used in the present analysis, including information on their locality of origin and GenBank accession numbers**

Authors: Rodrigo B. Salvador, Abraham S. H. Breure

Data type: xlsx

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Link: <https://doi.org/10.3897/zse.100.110105.suppl1>

## Supplementary material 2

### **Bayesian inference tree of the Clausilioidea showing the complete set of terminal taxa**

Authors: Rodrigo B. Salvador, Abraham S. H. Breure

Data type: png

Explanation note: Posterior probabilities are shown on nodes. Scale bar is substitutions per site.

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