

A new species of *Grandinenia* Minato & Chen, 1984 (Gastropoda, Stylommatophora, Clausiliidae, Garnieriinae) from Guangxi, China

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Abstract

A molecular phylogenetic study was conducted on genus *Grandinenia*, based on *COI* and *16S* sequences. A total of eight out of 26 species in the genus, as well as three unidentified species were sequenced. Phylogenetic results supported the monophyly of *Grandinenia* and the validity of all sampled species and subspecies. A new species, *Grandinenia jiangjilini* Chen, Lin, Wu & Ouyang, **sp. nov.**, from Guangxi, southern China is identified and described, based on morphological comparison and molecular phylogeny. The discovery indicates that the inflated-fusiform shell is not isolated in genus *Grandinenia* and the species diversity of the genus still remains to be explored.

Key Words

Door snails, karst landscape, phylogeny, taxonomy

Introduction

The Guangxi Zhuang Autonomous Region is situated in southern China and is renowned for its distinctive landscape and rich biodiversity. The karst landscape in this region provide a suitable habitat for land snails. The well-developed and exposed limestone have nurtured diverse rock-dwelling gastropod groups, with genus *Grandinenia* Minato & Chen, 1984 of subfamily Garnieriinae Boettger, 1926 being the most diverse and widespread in the region (Nordsieck 2012a, 2012b, 2012c, 2016; Lin and Lin 2022).

The subfamily Garnieriinae Boettger, 1926 is a group of medium to large-sized door snails distributed from Myanmar to southern China. It is characterised by a furrowed neck, projected and unattached, so-called apostrophic peristome and a lunella-type lunellar region (Nordsieck 2007). There are few molecular phylogenetic studies on the Garnieriinae and the limited studies suggest its close relationship with Synprophyminae Nordsieck, 2007 and Phaedusinae Wagner, 1922 (Uit de Weerd and Gittenberger 2013; Mamos et al. 2021; Uit de Weerd et al. 2023). Currently, Garnieriinae consist of seven genera, three of which are recorded in China: *Garnieria*

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Bourguignat, 1877, *Grandinenia* Minato & Chen, 1984 and *Tropidauchenia* Lindholm, 1924 (Nordsieck 2012a, 2012b, 2012c). The genus *Grandinenia* is defined by the furrowed neck, the distinctly expanded and unattached peristome, the lunella-type lunellar and the inferior lamella separated from the superior lamella (Nordsieck 2007, 2012a). It is distributed in Laos, Vietnam and southern China and consists of 26 known species (Nordsieck 2012a, 2012c, 2016; Grego et al. 2014; Lin and Lin 2022). Guangxi is the centre of diversity of the genus, with a total of 17 species recorded (Nordsieck 2012a, 2012c, 2016; Lin and Lin 2022). Most of these species were described in recent years, indicating that the species diversity has been poorly known in the past. Currently, the taxonomy of genus *Grandinenia* mainly rely on shell morphology, only two out of 26 species have been sequenced, but without detailed molecular phylogenetic studies.

In this study, we conducted the molecular phylogenetic analysis of genus *Grandinenia*, based on partial *COI* and *16S* sequences and described a new species with a peculiar morphology from Guangxi, southern China. The discovery of this new taxon further increases the species diversity of land snails in Chinese karst landforms.

Materials and methods

Samples were collected from Guangxi and Guangdong, China from 2022 to 2023. Living specimens were initially frozen at -20 °C for 12 hours and subsequently thawed at room temperature for 12 hours to extract the soft parts. The soft parts were then fixed in 70% ethanol. Empty shells were cleaned, dried and preserved at 4 °C. All specimens were deposited in the School of Life Sciences, Nanchang University (Nanchang, Jiangxi, China). Photographs were taken by a Sony® Alpha a6500 Digital Camera and edited in Adobe Photoshop CC 2015 (Adobe, San Jose, US). Maps were made in ArcGIS Pro (Esri, Redlands, US).

Genomic DNA was extracted from foot tissues preserved in 70% ethanol using a TIANamp Marine Animals DNA Kit (Tiangen Biotech, China). The quality and concentration of the DNA were checked on 1% agarose gel electrophoresis and NanoDrop 2000 (Thermo Scientific, USA). Partial cytochrome c oxidase subunit I (*COI*) and partial 16S ribosomal RNA (*16S*) gene segments were amplified and sequenced for molecular phylogenetic analyses. Polymerase chain reaction (PCR) systems, conditions and primer pairs are listed in Table 1. Sequences were aligned using MEGA v. 6.0 (Tamura et al. 2013) and

checked manually. The accession numbers of newly-obtained sequences and other species are given in Table 2.

Phylogenies were reconstructed by the dataset combined *COI* and *16S* genes using Maximum Likelihood (ML) and Bayesian Inference (BI). Five clausiliid species were used as outgroups for rooting the tree. ML analyses were performed in IQ-TREE v. 1.6.12 (Minh et al. 2013) using the Ultrafast fast bootstrap approach (Minh et al. 2013) with 10000 reiterations. The most appropriate model of sequence evolution (GTR+I+G for *COI*, GTR+G for *16S*) was selected under PartitionFinder2 v. 1.1 (Robert et al. 2017). Bayesian Inference (BI) was conducted in MrBayes v. 3.2.6 (Ronquist et al. 2012). The most appropriate model of sequence evolution (GTR+I+G) was selected under ModelFinder (Subha et al. 2017). Four simultaneous runs with four independent Markov Chain Monte Carlo (MCMC) were implemented for 10 million generations and trees were sampled every 1000 generations with a burn-in of 25%. The convergence was checked with the average standard deviation of split frequencies < 0.01 and the potential scale reduction factor (PSRF) ~ 1. Trees were visualised in FigTree v.1.4.3.

Abbreviations

NCU_XPWU Laboratory of Xiao-Ping Wu, Nanchang University (Nanchang, Jiangxi, China); **cp** clausilium plate; **il** inferior lamella; **lu** lunella; **pp** principal plica; **sc** subcolumellar lamella; **sl** superior lamella; **sp** spiral lamella; **At** atrium; **BC** bursa copulatrix; **BCD** bursa copulatrix duct; **D** diverticulum; **Ep** epiphallus; **FO** free oviduct; **P** penis; **PC** penial caecum; **PR** penial retractor muscle; **V** vagina; **VD** vas deferens.

Results

Phylogenetic analyses

A dataset consisting of 39 *COI* and 42 *16S* sequences from 11 species of *Grandinenia*, along with five outgroup taxa, was employed for phylogenetic analyses (Table 2). The aligned lengths of *COI* and *16S* genes were 669 and 484 nucleotides. Within these sequences, 236 and 233 were revealed as variable sites, while 232 and 229 were designated as parsimony informative sites. Phylogenetic analyses generated ML and BI trees with congruent topologies (Fig. 1). Genus *Grandinenia* forms a monophyly and further clustered into 12 dis-

Table 1. Primer pairs and PCR conditions used in the analyses of the *COI* and *16S* rRNA genes of *Grandinenia*.

Genes	Primer pairs	Reaction systems	Cycling conditions	Reference
<i>COI</i>	LC01490:	12.5 µl 2 × Taq Plus Master Mix II (Vazyme, Nanjing, China),	94 °C: 2 min; 94 °C: 10 s, 50 °C:	Folmer et al. (1994)
	GGTCAACAATCATAAAGATATTGG	1 µl template DNA, 1 µl of each pair of primers, 9.5 µl ddH ₂ O	60 s, 72 °C: 1 min, 35 cycles; 72 °C:	
	HCO2198:		10 min	
	TAAACTTCAGGTTGACCAAAAAATCA			
<i>16S</i>	16SA: CGGCCGCCTGTTTATCAAAACAT	12.5 µl 2 × Taq Plus Master Mix II (Vazyme, Nanjing, China),	94 °C: 2 min; 94 °C: 10 s, 50 °C:	Pál-Gergely et al. (2019)
	16SB: GGAGCTCCGGTTTGAACCTCAGATC	1 µl template DNA, 1 µl of each pair of primers, 9.5 µl ddH ₂ O	60 s, 72 °C: 1 min, 35 cycles; 72 °C:	
			10 min	

Table 2. GenBank accession numbers of the sequences for this study.

Species	Locality	CO1	16S	References
<i>Grandinenia mirifica</i>	Lianggu, Qintang, Guigang, Guangxi, China (type locality), 23°19'1"N, 109°14'34"E	PP473344	PP472576	This study
		PP473345	PP472577	This study
		PP473346	PP472578	This study
		PP473347	PP472579	This study
<i>G. jiangjilini</i> sp. nov.	Yao mountain, Binyang, Nanning, Guangxi, China, 23°26'12"N, 108°51'49"E	PP473375	PP472607	This study
		PP473376	PP472608	This study
		PP473377	PP472609	This study
		PP473378	PP472610	This study
		PP473379	PP472611	This study
		PP473380	PP472612	This study
<i>G. ookuboi pulchricosta</i>	Shanglin, Nanning, Guangxi, China, 23°27'9"N, 108°45'52"E	PP473381	PP472613	This study
		PP473382	PP472614	This study
		PP473383	PP472615	This study
		PP473384	PP472616	This study
		PP473369	PP472601	This study
		PP473370	PP472602	This study
<i>G. rex</i>	Chenghuang, Xingye, Yulin, Guangxi, China (type locality), 22°36'36"N, 109°46'19"E	PP473371	PP472603	This study
		PP473366	PP472598	This study
		PP473367	PP472599	This study
		PP473368	PP472600	This study
<i>G. cf. rutila</i>	Binyang, Nanning, Guangxi, China, 23°12'14"N, 109°8'16"E	PP473361	PP472593	This study
		PP473362	PP472594	This study
<i>G. fuchsi</i>	Guilin, Guangxi, China, 25°18'35"N, 110°16'19"E	PP473351	PP472583	This study
		PP473352	PP472584	This study
		PP473353	PP472585	This study

Species	Locality	CO1	16S	References
<i>G. gastrum gastrum</i>	Lianggu, Qintang, Guigang, Guangxi, China (type locality), 23°18'51"N, 109°15'49"E	PP473348	PP472580	This study
		PP473349	PP472581	This study
		PP473350	PP472582	This study
<i>G. gastrum laticosta</i>	Qintang, Guigang, Guangxi, China, 23°18'55"N, 109°16'30"E	PP473354	PP472586	This study
		PP473355	PP472587	This study
		PP473356	PP472588	This study
<i>G. ignea</i>	Zhongshan, Hezhou, Guangxi, China (type locality), 24°27'48"N, 111°10'35"E		PP472617	This study
			PP472618	This study
			PP472619	This study
<i>G. magnilabris</i>	Guzhai, Mashan, Nanning, Guangxi, China (type locality), 23°41'7"N, 108°19'11"E	PP473363	PP472595	This study
		PP473364	PP472596	This study
		PP473365	PP472597	This study
<i>G. sp. 1</i>	Shanglin, Nanning, Guangxi, China, 23°26'42"N, 108°44'33"E	PP473372	PP472604	This study
		PP473373	PP472605	This study
		PP473374	PP472606	This study
<i>G. sp. 2</i>	Menggong, Qintang, Guigang, Guangxi, China, 23°10'52"N, 109°22'11"E	PP473357	PP472589	This study
		PP473358	PP472590	This study
<i>Tropidauchenia yanghaoi</i>	Huajji, Zhaoqing, Guangxi, China (type locality), 23°55'18"N, 112°9'59"E		PP472620	This study
			PP472621	This study
			PP472622	This study
<i>T. orientalis</i>	Chongzuo, Guangxi, China, 22°16'29"N, 107°4'14"E	PP473359	PP472591	This study
		PP473360	PP472592	This study
<i>Agathylla goldi</i>	Europe	KC756080	KF601271	Fehér et al. (2013b), Parmakelis et al. (2013)
<i>Alopi mariae</i>	Europe	JQ911821		Fehér et al. (2013a)
<i>Isabellaria praestans</i>	Europe	AY425575		Uit de Weerd et al. (2004)

tinct lineages. The phylogenetic relationships did not reflect a significant geographical correlation. *Grandinenia magnilabris* Nordsieck, 2012 from the middle northern Guangxi is the earliest diverging lineage. *Grandinenia jiangjilini* sp. nov. formed a distinct lineage, but its relationship within the genus was not well resolved (bootstrap supports = 55, posterior probabilities = 0.88). The genetic distances of *COI* sequences between *Grandinenia jiangjilini* sp. nov. and other congeneric species ranged from 9.1% to 19.3% (Table 3).

Taxonomy

Family Clausiliidae Gray, 1855

Subfamily Garnieriinae Boettger, 1926

Genus *Grandinenia* Minato & Chen, 1984

Type species. *Steatonenia mirifica* Chen & Gao, 1982, by original designation.

Table 3. Genetic distances of *COI* sequences computed by MEGA 6 of *Grandinenia*.

	1	2	3	4	5	6	7	8	9	10	11
1 <i>Grandinenia mirifica</i>	0.006										
2 <i>G. jiangjilini</i> sp. nov.	0.106	0.001									
3 <i>G. ookuboi pulchricosta</i>	0.103	0.100	0								
4 <i>G. rex</i>	0.097	0.091	0.087	0.001							
5 <i>G. cf. rutila</i>	0.116	0.101	0.083	0.079	0.001						
6 <i>G. fuchsi</i>	0.119	0.120	0.125	0.115	0.131	0.003					
7 <i>G. gastrum gastrum</i>	0.055	0.119	0.121	0.102	0.132	0.122	0.003				
8 <i>G. gastrum laticosta</i>	0.063	0.127	0.131	0.119	0.135	0.124	0.036	0.004			
9 <i>G. magnilabris</i>	0.198	0.193	0.212	0.183	0.207	0.216	0.207	0.209	0.005		
10 <i>G. sp. 1</i>	0.097	0.100	0.016	0.083	0.088	0.124	0.118	0.128	0.206	0.001	
11 <i>G. sp. 2</i>	0.118	0.142	0.130	0.115	0.130	0.145	0.127	0.145	0.200	0.130	0.001

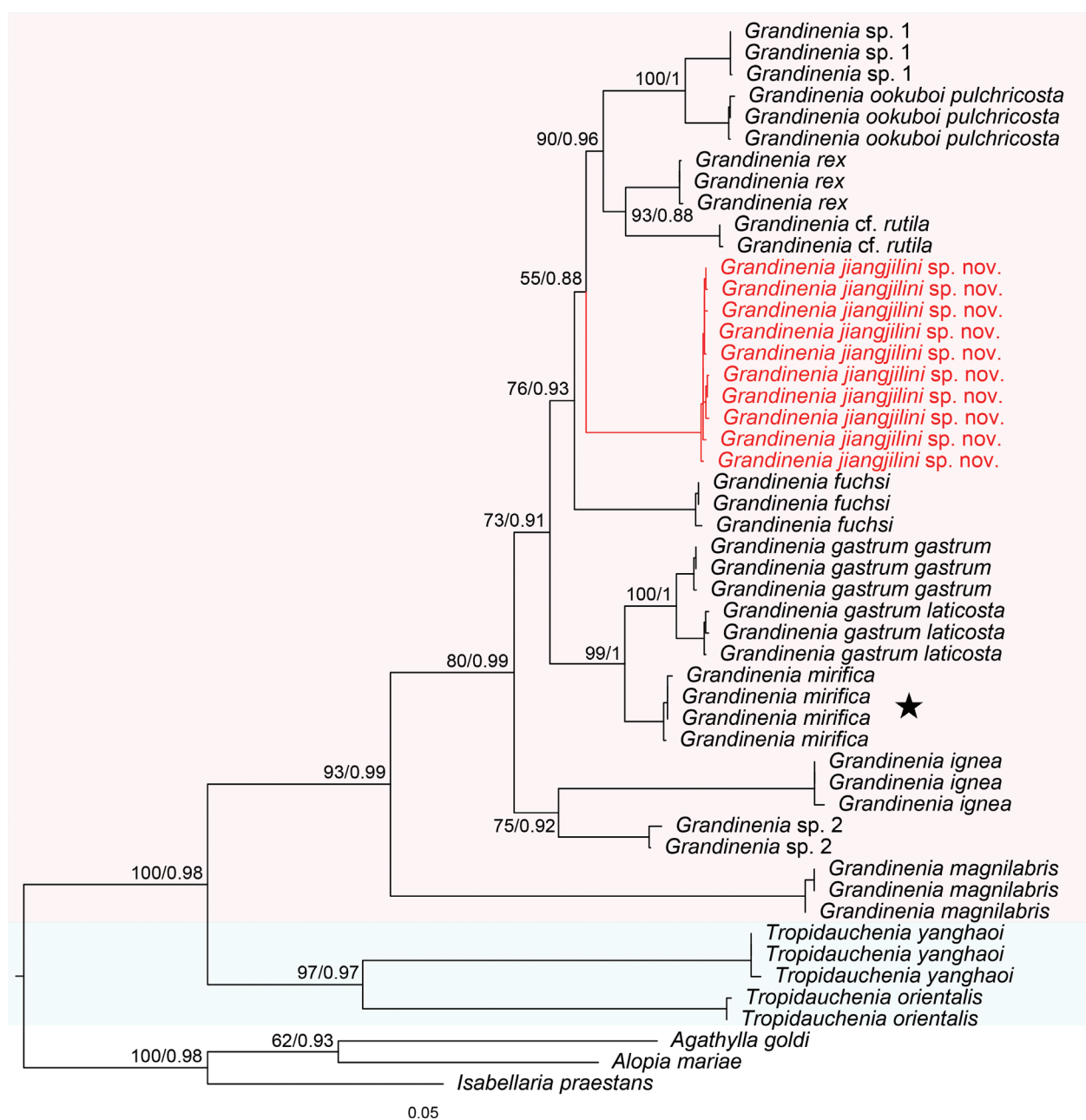


Figure 1. Maximum Likelihood tree and Bayesian Inference tree inferred from *COI* and *16S* gene sequences. Bootstrap supports/posterior probabilities are shown on the left/right of nodes. Star shows the type species of the genus.

***Grandinenia jiangjilini* Chen, Lin, Wu & Ouyang, sp. nov.**

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Figs 2A, 3, 4A, B

Type material. *Holotype*. 23_NCU_XPWU_YG01, Yao Mountain [瑶山], Binyang County [宾阳县], Nanning City [南宁市], Guangxi Zhuang Autonomous Region [广西壮族自治区], China, 23°26'12"N, 108°51'49"E, leg. Zhong-Guang Chen, Ji-Lin Jiang & Guang-Long Xie, September 2023.

Paratypes. 49 specimens, 23_NCU_XPWU_YG02–50, other information same as holotype.

Different diagnosis. Shell entire (vs. decollated in *G. ardouiniana* (Heude, 1885), *G. gabijakabi* Grego &

Szekeres, 2014, *G. gastrum* (Nordsieck, 2005), *G. mirifica* (Chen & Gao, 1982), *G. pallidissima* Nordsieck, 2010, *G. pseudofuchsi* (Nordsieck, 2005), *G. rex* Nordsieck, 2007, *G. rutila* Nordsieck, 2016, *G. schomburgi* (Schmacker & Boettger, 1890), *G. takagii* (Chang, 2004), *G. umbra* (Chang, 2004)), hardly decollated, inflated-fusi-form (vs. slender-fusi-form in all other congeners, except *G. mirifica*), light yellowish-brown, semitranslucent; teleoconch with broad, blunt and sparse wrinkles (ribs) (vs. without or with thin and dense ribs in all other congeners); peristome not reflected; inferior lamella lower in front than within; penial caecum present (vs. absent in *G. fuchsi* (Gredler, 1883), *G. pseudofuchsi*, *G. takagii* and *G. mirifica*).

Description. Shell (Figs 2A, 3A, B, 4A, B) (n = 50). Entire, with 8.75–9.5 whorls, hardly decollated,

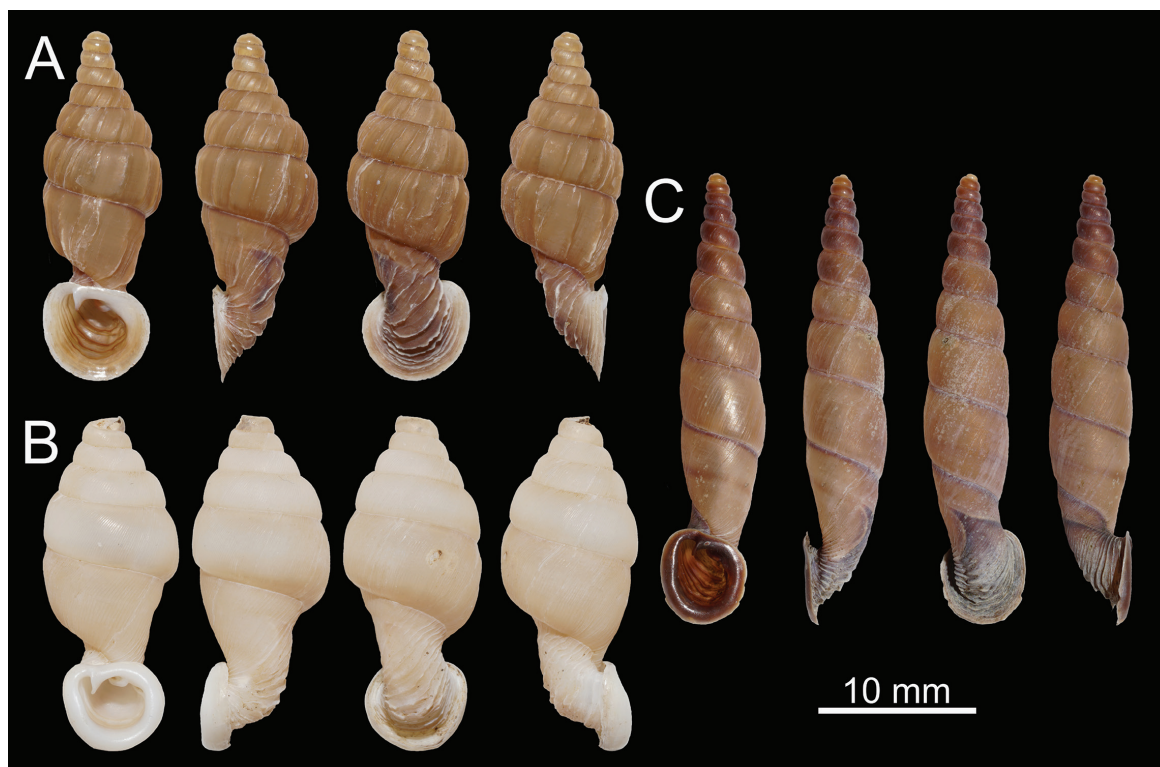


Figure 2. *Grandinenia jiangjilini* sp. nov. and two congeners. **A.** *Grandinenia jiangjilini* sp. nov., holotype (23_NCU_XPWU_YG01); **B.** *G. mirifica*; **C.** *G. ignea*.

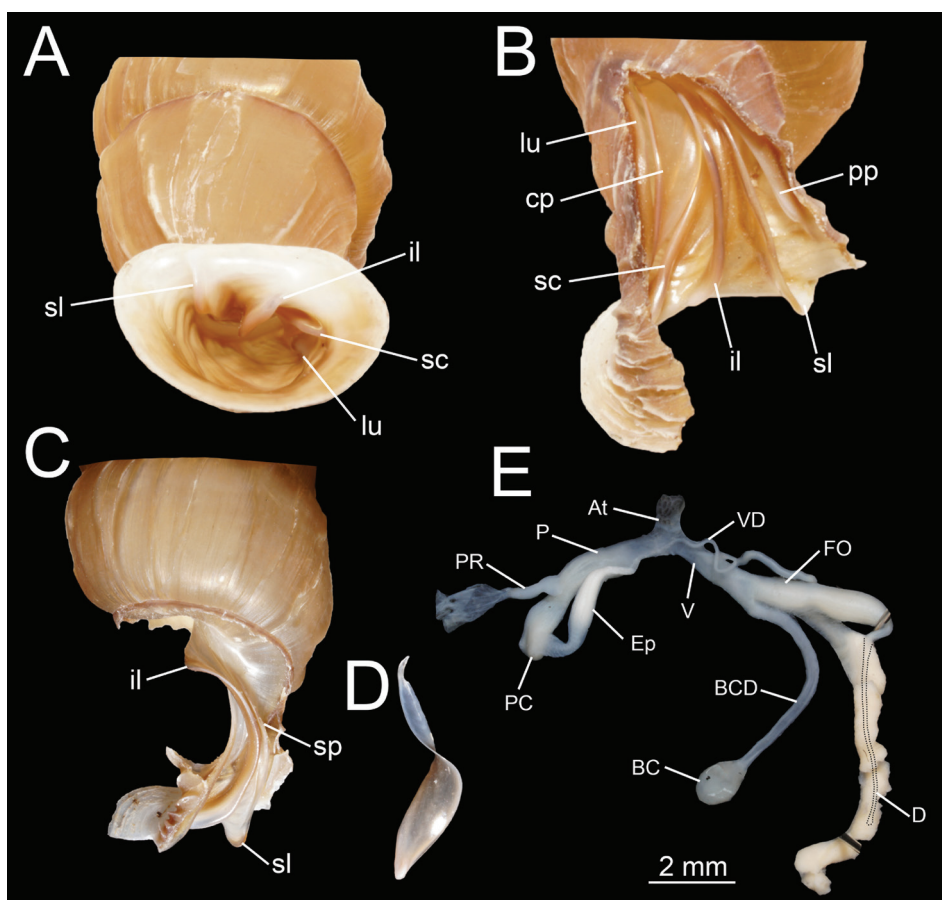


Figure 3. Detailed morphology of *Grandinenia jiangjilini* sp. nov. **A–C.** Shell morphology; **D.** Clausilium plate; **E.** Genital anatomy. Abbreviations: cp clausilium plate; il inferior lamella; lu lunella; pp principal plica; sc subcolumellar lamella; sl superior lamella; sp spiral lamella; At atrium; BC bursa copulatrix; BCD bursa copulatrix duct; D diverticulum; Ep epiphallus; FO free oviduct; P penis; PC penial caecum; PR penial retractor muscle; V vagina; VD vas deferens.



Figure 4. Living specimens of *Grandinenia*. **A, B.** *Grandinenia jiangjilini* sp. nov.; **C.** *G. gastrum*; **D.** *G. mirifica*.

inflated-fusiform, thin, fragile, semi-translucent, light yellowish-brown, with distinct darkish-red ribbon beneath the suture (fades quickly after fixing); dark seam along principal plica and lunella, body whorl in front of lunella darker; apical part conical to strongly attenuated. Suture deep. Protoconch smooth with 2.0–2.5 whorls. Wrinkles (ribs) on the teleoconch broad and blunt, most of them extending across the whole whorl, rather evenly distributed and widely spaced; on the neck, riblets white, thinner, stronger, more widely spaced and undulate. Aperture vastly extended, oval. Peristome expanded, not reflected. Superior lamella continuous with spiral lamella without a curve. Inferior lamella visible in front view of the aperture, steeply ascending, moderately low to high within, it ends deeper than the end of spiral lamella. Subcolumellar lamella strong, bent, visible or not in front view of the aperture, ending less deeply than the end of inferior lamella. Lunella vertical, in oblique view, partly visible through the aperture. Principal plica short, initiates ventrolaterally and extending laterally, not reaching peristome. Clausilium plate in oblique view nearly fully invisible, semi-translucent; overall slender; stalk thin; plate relatively broad.

Genitalia (Fig. 3C) ($n = 10$). Atrium short and relatively broad. Penis almost cylindrical and shortly narrower at transition to epiphallus. Penial caecum present. Epiphallus slender, shorter than penis and smaller diameter. Penial retractor relatively thick and short, inserted at the middle part of penis. Vas deferens relatively slender and

short. Vagina thick, cylindrical, slightly longer than free oviduct. Basal part of diverticulum thick, rapid thinning to apical part and attached to spermoviduct. Spermoviduct thick and long. Pedunculus of bursa copulatrix slender and long. Bursa copulatrix large, oval.

Measurements. Holotype: shell height 24.5 mm, width 8.3 mm; aperture height 7.0 mm, width 7.7 mm. Paratypes: shell height 21.9–28.5 mm, width 7.4–8.4 mm; aperture height 5.9–7.3 mm, width 6.9–8.0 mm ($n = 49$).

Etymology. The species is named after Mr Ji-Lin Jiang who first discovered the new species and assisted in the field survey.

Vernacular name. 江氏斜管螺 (Pinyin: jiāng shì xié guǎn luó).

Distribution and ecology. *Grandinenia jiangjilini* sp. nov. is found from the Yao Mountain only (Figs 5–7). No other localities were found during the detailed survey conducted in 2022–2023 of the surrounding hills. It inhabits the vertical limestone cliff together with *Papilliphaedusa porphyrea* (Möllerndorff, 1882) (Fig. 4A, B).

Discussion

The placement of the new species within *Grandinenia* is supported by both morphology (inferior lamella separated from superior lamella) and molecular phylogeny. The absence of a comprehensive description of the genitalia,

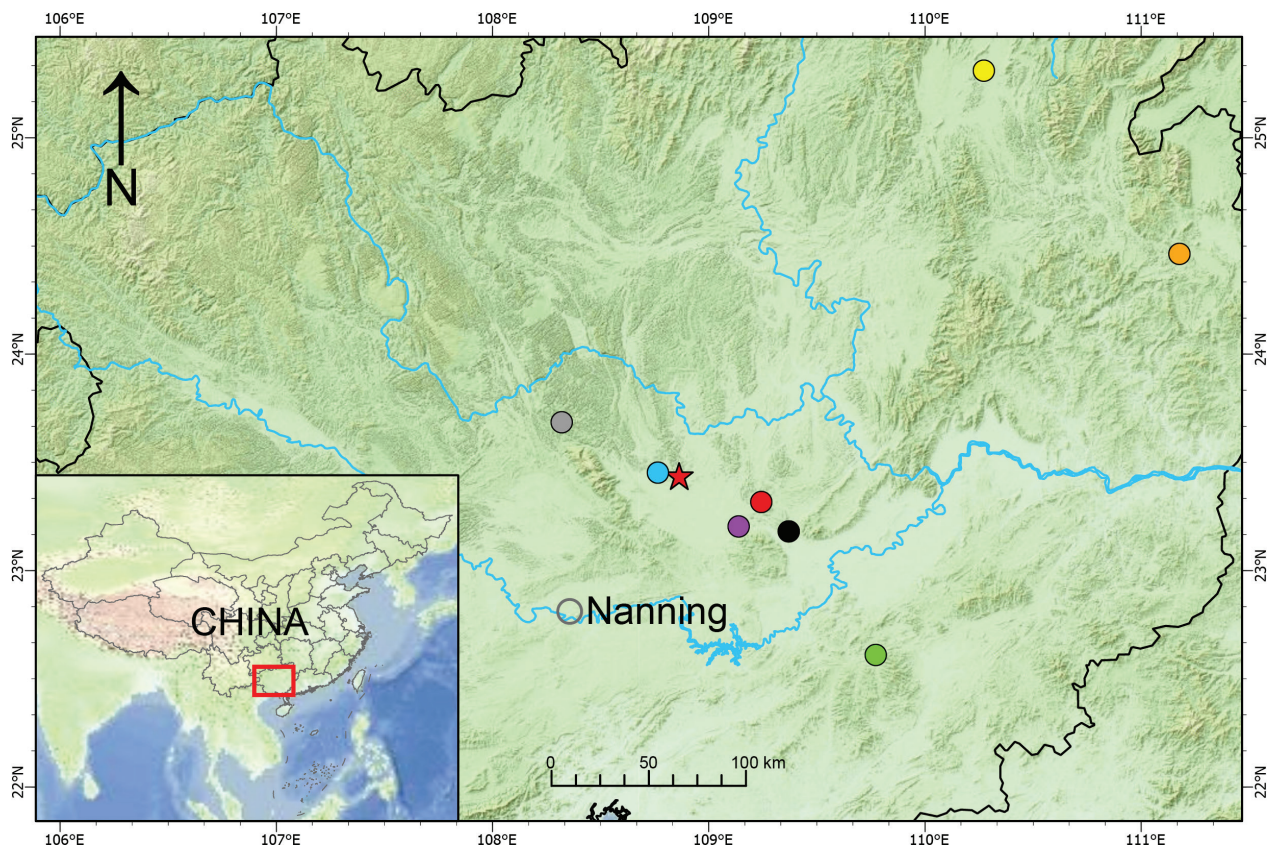


Figure 5. Sample localities of *Grandinenia* used in this study. **Star.** *Grandinenia jiangjilini* sp. nov.; **red point.** *G. mirifica*, *G. gastrum gastrum* and *G. gastrum laticosta*; **blue point.** *G. ookuboi pulchricosta* and *G. sp. 1*; **green point.** *G. rex*; **purple point.** *G. cf. rutila*; **yellow point.** *G. fuchsi*; **orange point.** *G. ignea*; **grey point.** *G. magnilabris*; **black point.** *G. sp. 2*.

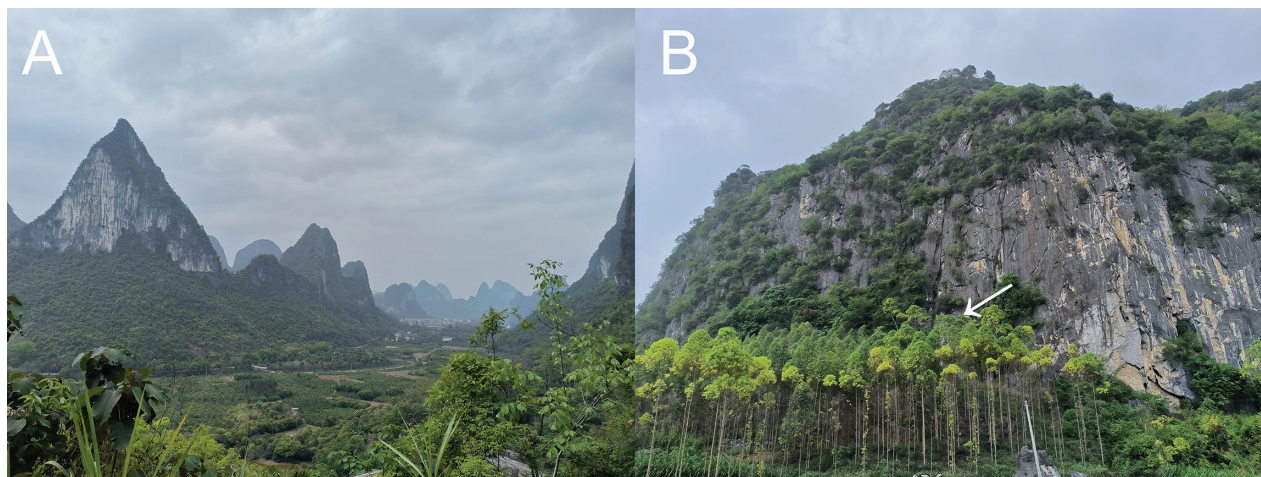


Figure 6. Sampling locality. **A.** Karst hills surrounding the type locality; **B.** Type locality of *Grandinenia jiangjilini* sp. nov. Arrow shows the sampling locality.

as well as the dearth of illustrations of lamellae and genitalia in the most original descriptions of *Grandinenia* species, precludes the possibility of detailed comparison of the new species with most other congeners for these two characters. The comparison of the shell morphology of the new species with that of eight congeners collected in this study revealed that the lamellae of them are highly similar. In contrast to *Tropidauchenia*, no variation in the fusion or separation of lamellae was identified between

Grandinenia species. The new species is preliminarily distinguished from *G. fuchsi*, *G. pseudofuchsi*, *G. takagii* and *G. mirifica* by the presence of penial caecum. However, the shell appearance of the new species is sufficiently distinctive that it can be readily distinguished from all other congeners through a simple comparison. *Grandinenia jiangjilini* sp. nov. can be easily distinguished from all other congeners by the teleoconch with broad, blunt and sparse wrinkles (ribs) (vs. without or with thin and dense ribs).

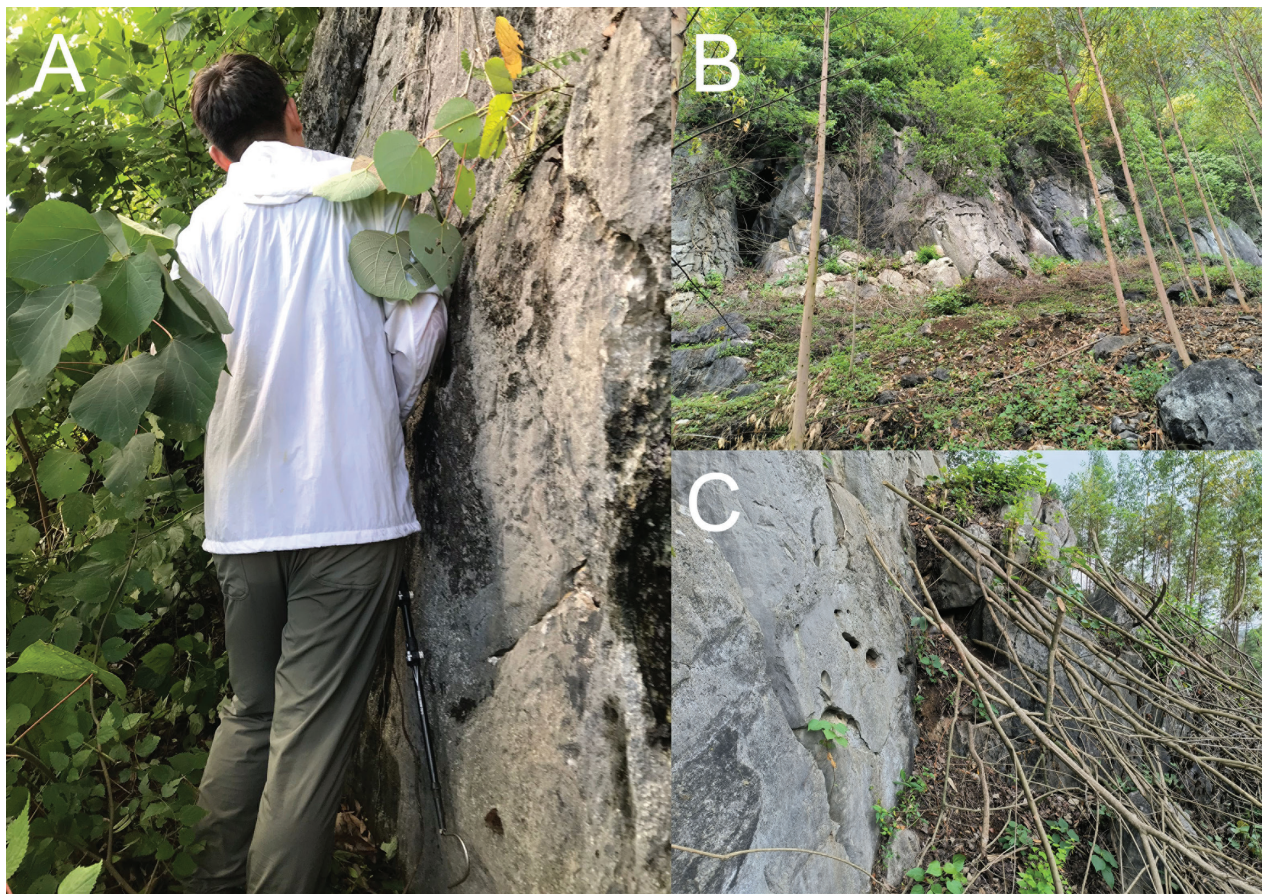


Figure 7. Comparison of type locality in 2023 and 2024. **A.** September 2023; **B, C.** April 2024.

Furthermore, except for *G. mirifica*, the remaining 25 species of *Grandinenia* exhibit a relatively slender shell (Fig. 2C). *Grandinenia jiangjilini* sp. nov. can be easily distinguished from them by the different shell shape (inflated-fusiform vs. slender-fusiform). *Grandinenia jiangjilini* sp. nov. is most similar to *G. mirifica* by similar inflated-fusiform shell (Fig. 2A, B), but differs by the broad and sparse wrinkles (ribs) on teleoconch (vs. thin and dense), peristome not reflected (vs. reflected), protoconch preserved (vs. decollated), shell semi-translucent and fragile (vs. opaque and solid) and the different shell colour (yellowish-brown vs. yellowish-white). *Grandinenia jiangjilini* sp. nov. is also somewhat similar to *G. dautzenbergi* (Morlet, 1892) and *G. yulinensis* Nordsieck, 2012, but differs by the more inflated shell, the stronger ribs on neck, the deeper suture, the thinner-walled and more fragile shell and the broad, blunt and sparse wrinkles (ribs) on teleoconch (vs. with very weak ribs to even smooth).

The validity of *Grandinenia jiangjilini* sp. nov. was also supported by the molecular phylogeny. It forms a distinct lineage and has a distant relationship with *G. mirifica*. The molecular phylogenetic relationships of genus *Grandinenia* do not correspond to the morphological similarities. The important characters of shell including shell shape, integrity, ribs, thickness and spiral ribbon, have homoplasiously evolved more than once. The species like *G. rex*, *G. fuchsi* and *G. ignea* with clearly similar smooth, thin, fragile and semi-translucent shells with spi-

ral ribbons, do not form a monophyletic group. The same phenomenon also occurs in species with similar thick and ribbed shells. The result shows that the shell appearance of *Grandinenia* is an effective means of distinguishing species, but does not reflect the interspecies affinities. The phylogenetic relationship also did not demonstrate a clear geographical correlation overall. *Grandinenia magnilabris* from the middle northern Guangxi (the westernmost distribution of the sampled species) is the earliest diverging lineage. *Grandinenia ignea* from north-eastern Guangxi sistered with *G. sp. 2* from the central region. In addition, *G. fuchsi* from north-eastern Guangxi was sistered with the clade, which consists of four species from the central region and one from the south-eastern region. The distribution pattern may be attributed to multiple independent diffusions from west to east in history. Only two lineages exhibited a certain geographical correlation, *G. sp. 1* and *G. ookuboi pulchricosta* from Shanglin and *G. mirifica*, *G. gastrum gastrum* and *G. gastrum laticosta* from Guigang, which formed monophyletic lineages, respectively. Due to the limited species included in this study, extensive and continuous sampling in future studies may help further analysis of the phylogeny and elucidation of the reasons for its distribution pattern formation.

The variation of shell morphology is frequently selected by environmental factors (Chiba 2004; Rolán-Alvarez 2007; Giokas et al. 2014). The shell characters of land snails play a pivotal role in regulating the water and

heat budget, thereby preventing desiccation (Cowie and Jones 1985; Pfenninger et al. 2005; Giokas et al. 2014). The discovery of the new species shows that the special inflated-fusiform shells are not isolated in genus *Grandinenia*. Phylogenetic result indicates that the new species and *G. mirifica* do not form a monophyletic group, suggesting that the similar shell shape between them may be the result of convergent evolution. Nordsieck (2012a) proposed that the inflated-fusiform shell of *G. mirifica* is an adaptation to a special habitat, but did not specify what this habitat is. For *Grandinenia*, it is probable that the inflated-fusiform shells have increased resistance to desiccation and ultraviolet radiation compared to the slender-fusiform shells, although the precise mechanism of action remains unclear. Through field observation, it was found that the two shell shapes of *Grandinenia* correspond to two life strategies. The majority of *Grandinenia* species with slender-fusiform shells burrow into crevices to hibernate during the dry season (Fig. 4C). It is challenging to find them during the dry season, but a considerable number of individuals can be observed during the rainy season in the same area. In contrast, the two species with inflated-fusiform shells primarily hibernate on rock surfaces which can be found throughout the year, regardless of precipitation patterns (Fig. 4A, B, D).

The discovery of new taxon or just new species indicates that the species diversity of *Grandinenia* in Guangxi still remains to be explored. Nine species of *Grandinenia* have been recoded within a few dozen kilometres of the new species' type locality (Nordsieck 2012a, 2012c, 2016; Lin and Lin 2022) and several specimens which may represent other undescribed species were found during the field survey. Extensive exploration of land snails in Guangxi should be strengthened in the future which may lead to the discovery of yet-to-be-described species. In addition, the protection of *Grandinenia* should be a priority. As a rock-dwelling land snail, the survival of *Grandinenia* depends on the exposed rock environment under the forest. Large-scale mining of limestone and agricultural reclamation in Guangxi pose a threat to it. It was even found that some peaks that used to be rich in *Grandinenia* have been completely blasted and disappeared. The field survey conducted in 2024 revealed that the native shrubs in the type locality of *Grandinenia jiangjilini* sp. nov. had been completely cut down (Fig. 7). This has resulted in the environment of the rock walls becoming more exposed and dry and has led to a significant reduction in the population size of *Grandinenia jiangjilini* sp. nov. It is imperative that further protection measures are implemented without delay, otherwise a significant number of unique species may be lost.

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References

- Chiba S (2004) Ecological and morphological patterns in communities of land snails of the genus *Mandarina* from the Bonin Islands. *Journal of Evolutionary Biology* 17(1): 131–143. <https://doi.org/10.1046/j.1420-9101.2004.00639.x>
- Cowie RH, Jones JS (1985) Climatic selection on body color in *Cepaea*. *Heredity* 55(2): 261–267. <https://doi.org/10.1038/hdy.1985.100>
- Fehér Z, Németh L, Nicoară A, Szekeres M (2013a) Molecular phylogeny of the land snail genus *Alopi* (Gastropoda: Clausiliidae) reveals multiple inversions of chirality. *Zoological Journal of the Linnean Society* 167(2): 259–272. <https://doi.org/10.1111/zoj.12002>
- Fehér Z, Parmakelis A, Koutalianou M, Mourikis T, Eröss ZP, Krízssik V (2013b) A contribution to the phylogeny of Albanian *Agathylla* (Gastropoda, Clausiliidae): Insights using morphological data and three mitochondrial markers. *The Journal of Molluscan Studies* 80(1): 24–34. <https://doi.org/10.1093/mollus/eyt039>
- Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology* 3: 294–299.
- Giokas S, Páll-Gergely B, Mettouri O (2014) Nonrandom variation of morphological traits across environmental gradients in a land snail. *Evolutionary Ecology* 28(2): 323–340. <https://doi.org/10.1007/s10682-013-9676-5>
- Grego J, Van Luong H, Van Pham S, Szekeres M (2014) Vietnamese clausiliidae (Gastropoda: Pulmonata): New taxa and novel distribution data. *Journal of Conchology* 41(6): 749–757.
- Lin LW, Lin RX (2022) A review of subfamily Garnieriinae C. R. Boettger, 1926. [in Chinese] https://mp.weixin.qq.com/s/?__biz=MzU2MzAyMTI2Mg==&mid=2247484089&idx=1&sn=202e28f8d489c9c3e7b92fb713ea0ce9&chksm=fc61d618cb165f0efad99983465336918e4a3124bfd14c2cd163061c745415e79f8d118eedea&mpshare=1&scene=23&srcid=03128t81MCrF5aG-9GI20jfjn&shareinfo=fd8f3a4ab07b45a317b71d8a05a3f78b&shareinfo_first=fd8f3a4ab07b45a317b71d8a05a3f78b#rd [access on April 21, 2024]
- Mamos T, Uit de Weerd DR, von Oheimb PV, Sulikowska-Drozd A (2021) Evolution of reproductive strategies in the species-rich land snail subfamily Phaedusinae (Stylommatophora: Clausiliidae). *Molecular Phylogenetics and Evolution* 158: 107060. <https://doi.org/10.1016/j.ympev.2020.107060>
- Minh BQ, Nguyen MAT, von Haeseler A (2013) Ultrafast approximation for phylogenetic bootstrap. *Molecular Biology and Evolution* 30(5): 1188–1195. <https://doi.org/10.1093/molbev/mst024>
- Nordsieck H (2007) *Worldwide Door Snails (Clausiliidae), recent and fossil*. ConchBooks, Germany, 214 pp.

- Nordsieck H (2012a) Clausiliidae of Guangxi, southern China (Gastropoda, Pulmonata, Stylommatophora). *Acta Conchyliorum* 12: 3–56.
- Nordsieck H (2012b) Note on Garnieriini (Gastropoda, Stylommatophora, Clausiliidae, Garnieriinae). *Acta Conchyliorum* 12: 57–62.
- Nordsieck H (2012c) Check-list of the Clausiliidae of mainland China (Gastropoda, Stylommatophora). *Acta Conchyliorum* 12: 63–73.
- Nordsieck H (2016) New species taxa of Clausiliidae (Gastropoda, Stylommatophora) from China and Vietnam. *Conchylia* 47: 37–57.
- Páll-Gergely B, Hunyadi A, Chen ZY, Lyu ZT (2019) A review of the genus *Coccoglypta* Pilsbry, 1895 (Gastropoda: Pulmonata: Camaenidae). *Zoosystema* 41(29): 595–608. <https://doi.org/10.5252/zoosystema2019v41a29>
- Parmakelis A, Kotsakiozi P, Rand DM (2013) Animal Mitochondria, Positive Selection and Cyto-Nuclear Coevolution: Insights from Pulmonates. *PLOS ONE* 8(4): e61970. <https://doi.org/10.1371/journal.pone.0061970>
- Pfenninger M, Hrabakova M, Steinke D, Depraz A (2005) Why do snails have hairs? A Bayesian inference of character evolution. *BMC Evolutionary Biology* 5(1): 59. <https://doi.org/10.1186/1471-2148-5-59>
- Robert L, Paul BF, April MW, Tereza S, Brett C (2017) Partitionfinder 2: New methods for selecting partitioned models of evolution for molecular and morphological phylogenetic analyses. *Molecular Biology and Evolution* 34: 772–773. <https://doi.org/10.1093/molbev/msw260>
- Rolán-Alvarez E (2007) Sympatric speciation as a by-product of ecological adaptation in the Galicia *Littorina saxatilis* hybrid zone. *The Journal of Molluscan Studies* 73(1): 1–10. <https://doi.org/10.1093/mollus/eyl023>
- Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck J (2012) MrBayes 3.2: Efficient bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* 61(3): 539–542. <https://doi.org/10.1093/sysbio/sys029>
- Subha K, Bui Quang M, Wong TKF (2017) Modelfinder: Fast model selection for accurate phylogenetic estimates. *Nature Methods* 14(6): 587–589. <https://doi.org/10.1038/nmeth.4285>
- Tamura K, Stecher G, Peterson D, Filipowski A, Kumar S (2013) MEGA6: Molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution* 30(12): 2725–2729. <https://doi.org/10.1093/molbev/mst197>
- Uit de Weerd DR, Gittenberger E (2013) Phylogeny of the land snail family Clausiliidae (Gastropoda: Pulmonata). *Molecular Phylogenetics and Evolution* 67(1): 201–216. <https://doi.org/10.1016/j.ympev.2013.01.011>
- Uit de Weerd DR, Piel WH, Gittenberger E (2004) Widespread polyphyly among Alopinae snail genera: When phylogeny mirrors biogeography more closely than morphology. *Molecular Phylogenetics and Evolution* 33(3): 533–548. <https://doi.org/10.1016/j.ympev.2004.07.010>
- Uit de Weerd DR, Gittenberger E, Mamos T, Sulikowska-Drozdz A (2023) The phylogenetic position of *Synprosymma* A.J. Wagner, 1920 within Clausiliidae: Biogeographic and taxonomic implications. *Archiv für Molluskenkunde* 152(2): 257–267. <https://doi.org/10.1127/arch.moll/152/257-267>

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