

Deciphering “cryptic” nature of European rock-dwelling

***Pyramidula* snails (Gastropoda: Stylommatophora)**

Veronika Horsáková | ORCID: 0000-0002-3264-7728

Department of Botany and Zoology, Faculty of Science, Masaryk University, Brno
61137, Czech Republic

veronika.horsakova@seznam.cz

Eva Líznařová

Department of Botany and Zoology, Faculty of Science, Masaryk University, Brno
61137, Czech Republic

Oihana Razkin

Department of Zoology and Animal Cell Biology, University of the Basque Country
(UPV/EHU), 01006 Vitoria-Gasteiz, Spain

Jeffrey C. Nekola

Department of Botany and Zoology, Faculty of Science, Masaryk University, Brno
61137, Czech Republic

Michal Horsák

Department of Botany and Zoology, Faculty of Science, Masaryk University, Brno
61137, Czech Republic

Supplementary material

Table S1. Summary of populations used for phylogeny reconstruction and morphometrics. Taxon name, location information, sample code in phylogeny reconstruction, and GenBank accession number for each of the 64 analyzed *Pyramidula* specimens. Number of individuals used in morphometric analyses per each population is also shown. Specimens from populations used in previous studies of Razkin et al. (2016, 2017) are marked by asterisks; n.a. = DNA sequence not available.

Taxon/	No of ind. used in		GenBank accession number					
Country	morphometric analyses	Settlement	Latitude °N	Longitude °E	Sample code	COI	ITS1	ITS2
<i>Pyramidula jaenensis</i>								
France*	3	Toulon	43.15418	5.84244	P355	ON331879	ON352456	ON352514
Italy	5	Gaeta	41.23934	13.49248	P210	ON331856	n.a.	n.a.
San Marino	5	San Marino	43.93475	12.44975	P207	ON331853	ON352434	ON352492
Spain*	4	Alaior	39.93988	4.13302	P358	ON331882	ON352458	ON352516
Spain*	2	Jijona	38.53664	-0.53306	P368	ON331892	ON352467	ON352525
Spain*	4	Cazorla	38.09167	-2.82894	P357	ON331881	ON352457	ON352515
Spain*	6	Venta del Moro	39.47704	-1.36065	P376	ON331900	ON352475	ON352533
<i>Pyramidula pusilla</i>								
Austria	4	Johnsbach	47.55353	14.65342	P192; P196	ON331842; ON331846	ON352423; ON352427	ON352481; ON352485
Austria	9	Spital am Pyhm	47.64950	14.29350	P193; P194	ON331843; ON331844	ON352424; ON352425	ON352482; ON352483

Taxon/	No of ind. used in					GenBank accession number		
Country	morphometric analyses	Settlement	Latitude °N	Longitude °E	Sample code	COI	ITS1	ITS2
Czech Republic	5	Pavlov	48.87727	16.66346	P211	ON331857	ON352437	ON352495
Czech Republic	11	Štramberk	49.58482	18.11407	P212	ON331858	ON352438	ON352496
Italy	3	Gimillan	45.63864	7.391889	P190	ON331840	ON352421	ON352479
Slovakia	0	Blatnica	48.95800	18.99467	P223; P353	ON331866;	ON352445;	ON352503;
						ON331877	ON352455	ON352513
Slovakia	1	Bytča	49.18614	18.60758	P201	ON331847	ON352428	ON352486
Slovakia	4	Zádiel	48.62542	20.83496	P227; P191	ON331869;	ON352448;	ON352506;
						ON331841	ON352422	ON352480
Slovakia	1	Dolný Harmanec	48.81890	19.04440	P364; P365	ON331888;	ON352463;	ON352521;
						ON331889	ON352464	ON352522
						ON331849;	ON352430;	ON352488;
Slovakia	1	Horný Jelenec	48.86428	19.14786	P203; P221; P218;	ON331864;	ON352443;	ON352501;
					P219	ON331861;	n.a.;	n.a.;
						ON331862	ON352441	ON352499
Slovakia	6	Valentová	48.86533	19.12228	P205	ON331851	ON352432	ON352490
Slovakia	2	Liptovské Revúce	48.88180	19.16260	P369; P370	ON331893;	ON352468;	ON352526;
						ON331894	ON352469	ON352527
Slovakia	8	Tatranská Kotlina	49.22003	20.26206	P213	ON331859	ON352439	ON352497

Taxon/ Country	No of ind. used in morphometric analyses	Settlement	Latitude °N	Longitude °E	Sample code	GenBank accession number		
						COI	ITS1	ITS2
<i>Pyramidula rupestris</i>								
France*	5	Arles	43.73339	4.76423	P359; P360	ON331883; ON331884	n.a.; ON352459	n.a.; ON352517
France*	2	Meyreuil	43.50368	5.51049	P373	ON331897	ON352472	ON352530
France*	1	Ollioules	43.15418	5.84244	P354	ON331878	n.a.	n.a.
Spain*	1	Alcoi	38.53664	-0.53306	P377	ON331901	ON352476	ON352534
Spain*	1	Xàtiva	38.96346	-0.49775	P374	ON331898	ON352473	ON352531
Spain*	5	Encío	42.67138	-3.09526	P366	ON331890	ON352465	ON352523
Spain*	3	Mura	41.67806	2.00308	P375	ON331899	ON352474	ON352532
Spain*	2	Portell de Morella	40.51984	-0.28498	P367	ON331891	ON352466	ON352524
Spain*	0	Ricote	38.15271	-1.37932	P356	ON331880	n.a.	n.a.
Switzerland	8	Twann	47.09369	7.15219	P202; P372	ON331848; ON331896	ON352429; ON352471	ON352487; ON352529
<i>Pyramidula saxatilis</i>								
Austria	11	Hochschwab	47.61158	15.18642	P189	ON331839	ON352420	ON352478
Austria	0	Johnsbach	47.55353	14.65342	P352	ON331876	ON352454	ON352512
Austria	12	Lunz am See	47.83821	15.06553	P230; P231	ON331871; ON331872	ON352450; ON352451	ON352508; ON352509

Taxon/ Country	No of ind. used in					GenBank accession number		
	morphometric analyses	Settlement	Latitude °N	Longitude °E	Sample code	COI	ITS1	ITS2
Austria	1	Mürzsteg	47.70510	15.51060	P362	ON331886	ON352461	ON352519
Austria	5	Schladming	47.28108	13.62303	P206	ON331852	ON352433	ON352491
Austria	2	Spital am Pyhrn	47.64950	14.29350	P195	ON331845	ON352426	ON352484
Italy	2	Selva di Valgardena	46.57183	11.76861	P224	ON331902	ON352477	ON352535
Italy	1	Selva di Valgardena	46.56011	11.81628	P248; P249	ON331874;	n.a.;	n.a.;
						ON331875	ON352453	ON352511
San Marino	1	San Marino	43.93475	12.44975	P209	ON331855	ON352436	ON352494
Slovakia	0	Bytča	49.18614	18.60758	P222	ON331865	ON352444	ON352502
Slovakia	0	Dolný Harmanec	48.81890	19.04440	P371	ON331895	ON352470	ON352528
Slovakia	0	Horný Jelenec	48.864278	19.14786	P204; P217; P220	ON331850;	ON352431;	ON352489;
						ON331860;	ON352440;	ON352498;
Slovakia	0	Motyčky	48.86480	19.16360	P361; P363	ON331863	ON352442	ON352500
						ON331885;	ON352460;	ON352518;
Slovenia	2	Kamnik	46.30281	14.62844	P232	ON331887	ON352462	ON352520
Switzerland	8	Nods	47.11420	7.04240	P229	ON331873	ON352452	ON352510
Switzerland	0	Tavannes	47.20940	7.19830	P225; P226	ON331870	ON352449	ON352507
						ON331867;	ON352446;	ON352504;
						ON331868	ON352447	ON352505

Taxon/ Country	No of ind. used in		Latitude °N	Longitude °E	Sample code	GenBank accession number		
	morphometric analyses	Settlement				COI	ITS1	ITS2
Switzerland	5	Villeret	47.131083	7.02881	P208	ON331854	ON352435	ON352493

References

- Razkin, O., Sonet, G., Breugelmans, K., Madeira, M.J., Gómez-Moliner, B.J. & Backeljau, T. (2016) Species limits, interspecific hybridization and phylogeny in the cryptic land snail complex *Pyramidula*: the power of RADseq data. *Mol. Phylogenet. Evol.*, 101, 267–278.
- Razkin, O., Gómez-Moliner, B.J., Vardinoyannis, K., Martínez-Ortí, A. & Madeira, M.J. (2017) Species delimitation for cryptic species complexes: case study of *Pyramidula* (Gastropoda, Pulmonata). *Zool. Scr.*, 46, 55–72.

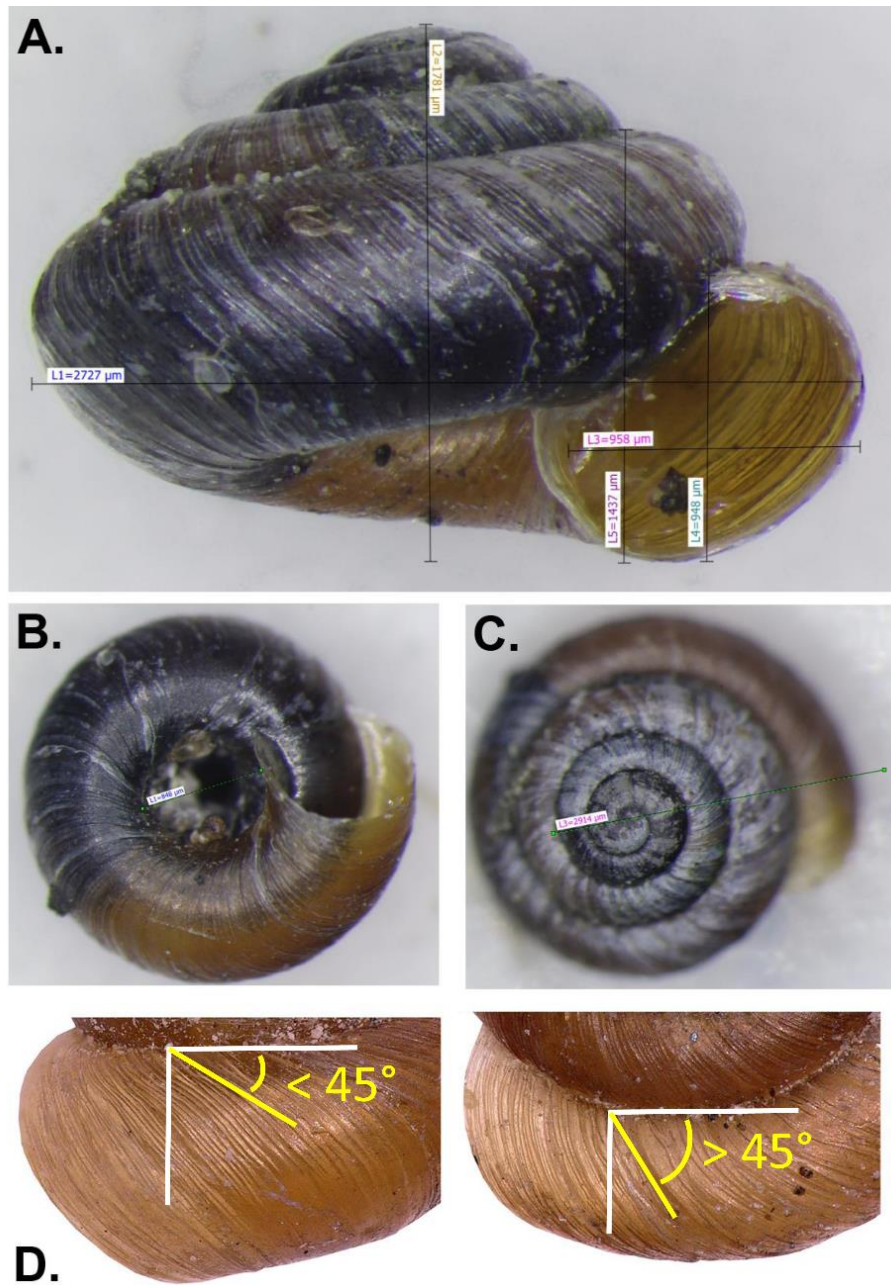


FIGURE S1 Illustration of measurements taken for *Pyramidula* shells. A. Shell height, shell width, body height, aperture height, and aperture width; B. Umbilicus width; C. Number of whorls; D. Angle of growth ridges right after the suture; left – less than 45° angle (*P. jaenensis*, *P. rupestris*), right – more than 45° angle (*P. pusilla*, *P. saxatilis*).

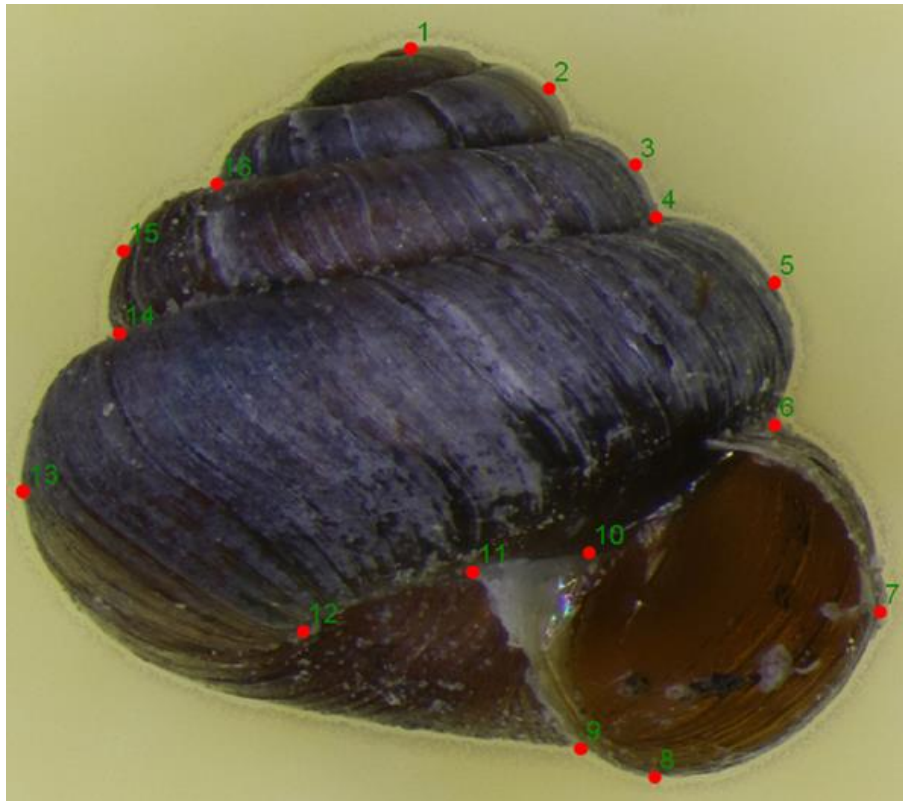


FIGURE S2 Position of 16 landmarks describing *Pyramidula* shell shape in geometric morphometrics.

Tree scale: 0.05

mtDNA (COI)

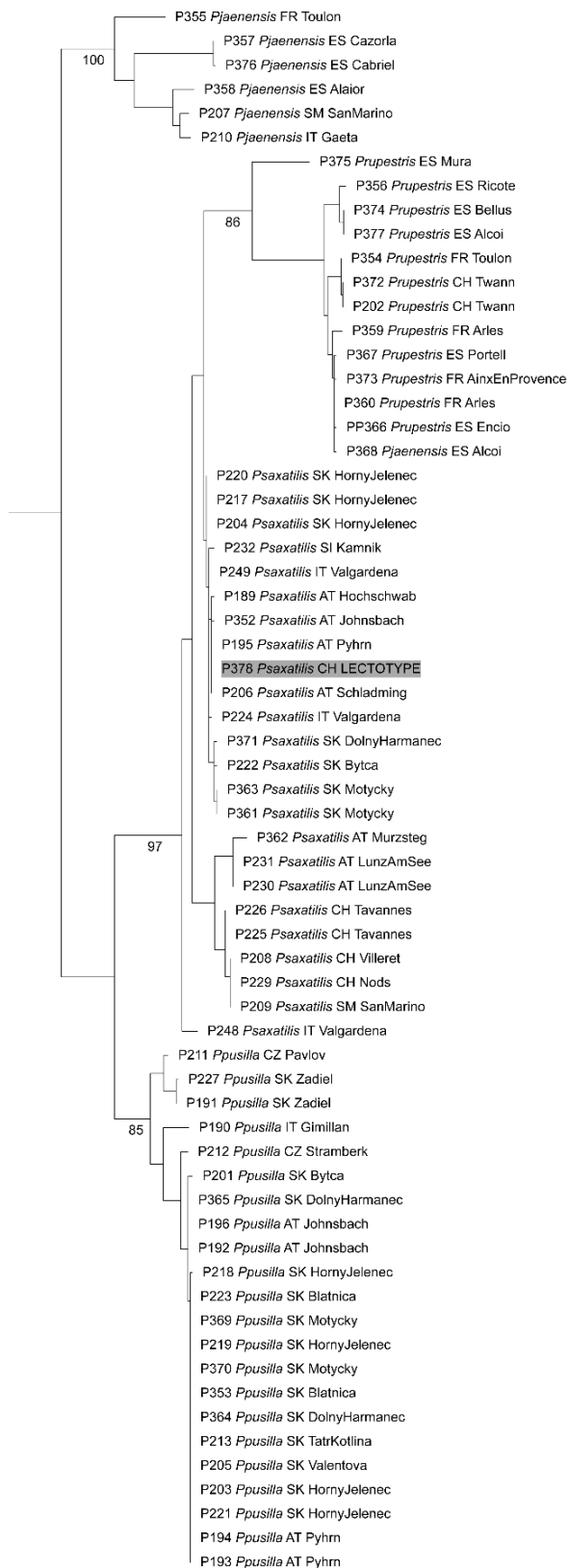
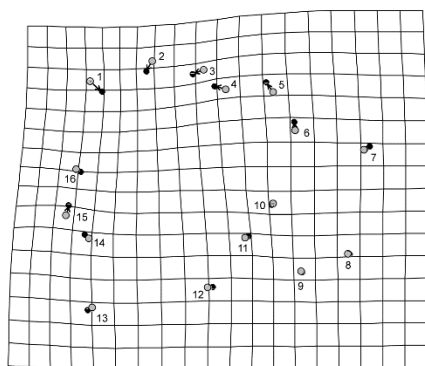
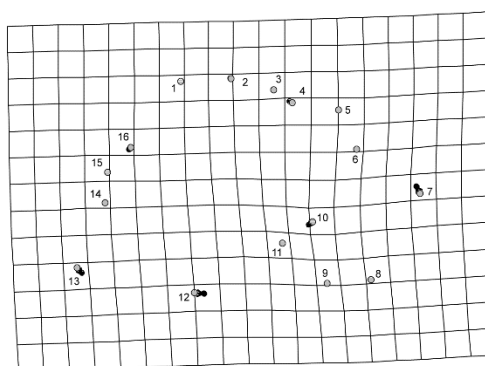


FIGURE S3 Maximum likelihood (ML) phylogenetic reconstruction based on 603 bp mitochondrial DNA (COI) fragment. One representative specimen from > 170 years old museum lot no. M562 (type series, collection of J. D. W. Hartmann, Natural History Museum St. Gallen, Switzerland) fell among the individuals of *Pyramidula saxatilis*. Support values > 70% (1000 bootstrap replicates) are shown next to the corresponding nodes.

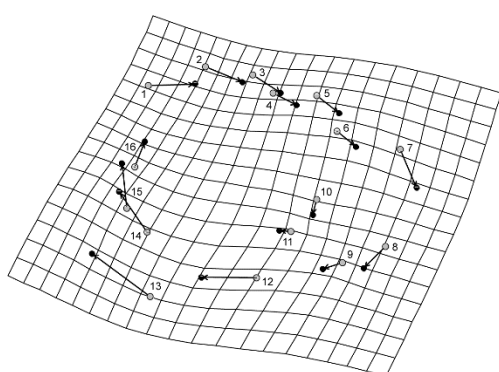
		Shell	Shell	Body	Aperture	Aperture	Umbilicus	Shell	Shell	Body	Aperture	Aperture	Aperture	Shell		
		height	width	height	height	width		height	width	height	height	width	width	height	Umbilicus	
		Shell	Shell	Body	Aperture	Aperture	Umbilicus	/No of	/No of	/Shell	/Body	/Shell	/Shell	/Shell	width /No	No of
		height	width	height	height	width	width	whorls	whorls	height	height	height	width	width	of whorls	whorls
	3rd Quart	2.00	2.66	1.52	0.99	1.02	0.80	0.48	0.64	0.78	0.66	0.54	0.40	0.78	0.19	4.2
	MAX	2.30	2.82	1.78	1.16	1.10	0.92	0.54	0.70	0.83	0.73	0.59	0.45	0.89	0.22	4.9
<i>Pyramidula</i>																
<i>rupestris</i>	MIN	1.71	2.08	1.24	0.78	0.81	0.33	0.44	0.46	0.71	0.55	0.36	0.37	0.79	0.07	3.8
	1st Quart	1.81	2.11	1.34	0.84	0.88	0.41	0.45	0.53	0.73	0.61	0.43	0.41	0.84	0.11	3.9
	MEDIAN	2.05	2.16	1.52	0.95	0.91	0.48	0.50	0.55	0.74	0.62	0.47	0.42	0.90	0.12	4.0
	MEAN	2.03	2.18	1.50	0.94	0.93	0.47	0.51	0.55	0.74	0.63	0.46	0.43	0.93	0.12	4.0
	3rd Quart	2.20	2.20	1.64	1.04	0.99	0.53	0.55	0.56	0.75	0.64	0.49	0.44	1.02	0.13	4.1
	MAX	2.65	2.47	1.96	1.20	1.10	0.60	0.65	0.60	0.77	0.69	0.57	0.51	1.11	0.16	4.8
<i>Pyramidula</i>																
<i>jaenensis</i>	MIN	1.95	1.93	1.33	0.76	0.82	0.39	0.47	0.46	0.62	0.56	0.36	0.36	0.81	0.09	3.9
	1st Quart	2.06	2.16	1.44	0.90	0.88	0.50	0.50	0.49	0.65	0.60	0.40	0.40	0.89	0.11	4.0
	MEDIAN	2.18	2.26	1.51	0.94	0.97	0.55	0.52	0.53	0.69	0.63	0.44	0.43	0.99	0.13	4.2
	MEAN	2.23	2.28	1.52	0.94	0.97	0.55	0.52	0.54	0.68	0.62	0.44	0.43	0.98	0.13	4.3
	3rd Quart	2.35	2.43	1.55	0.98	1.05	0.60	0.54	0.58	0.72	0.64	0.48	0.44	1.08	0.14	4.5
	MAX	2.81	2.73	1.81	1.08	1.18	0.81	0.60	0.68	0.75	0.68	0.55	0.54	1.18	0.19	4.8



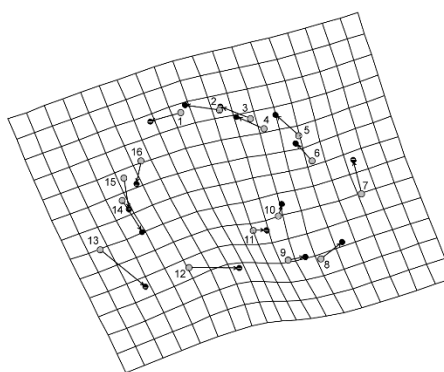
P. jaenensis - *P. rupestris*



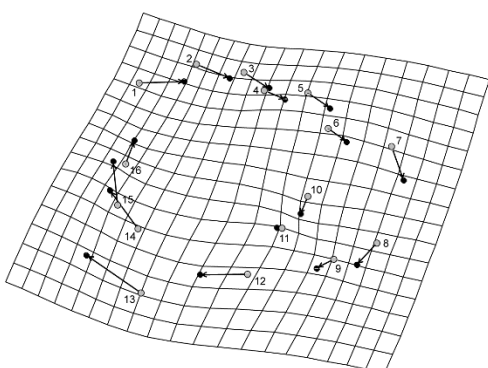
P. pusilla - *P. saxatilis*



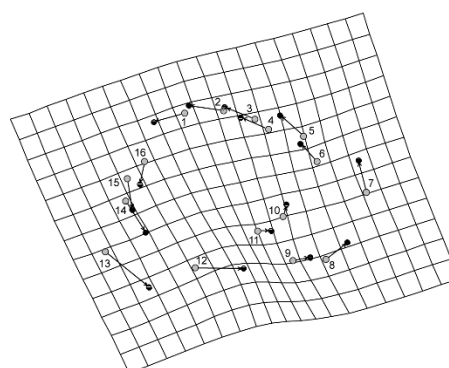
P. jaenensis - *P. pusilla*



P. pusilla - *P. rupestris*



P. jaenensis - *P. saxatilis*



P. saxatilis - *P. rupestris*

FIGURE S4 Thin-plate splines, illustrating the transitions in shape (i.e., landmark vector shifts) between all pairs of four *Pyramidula* species.

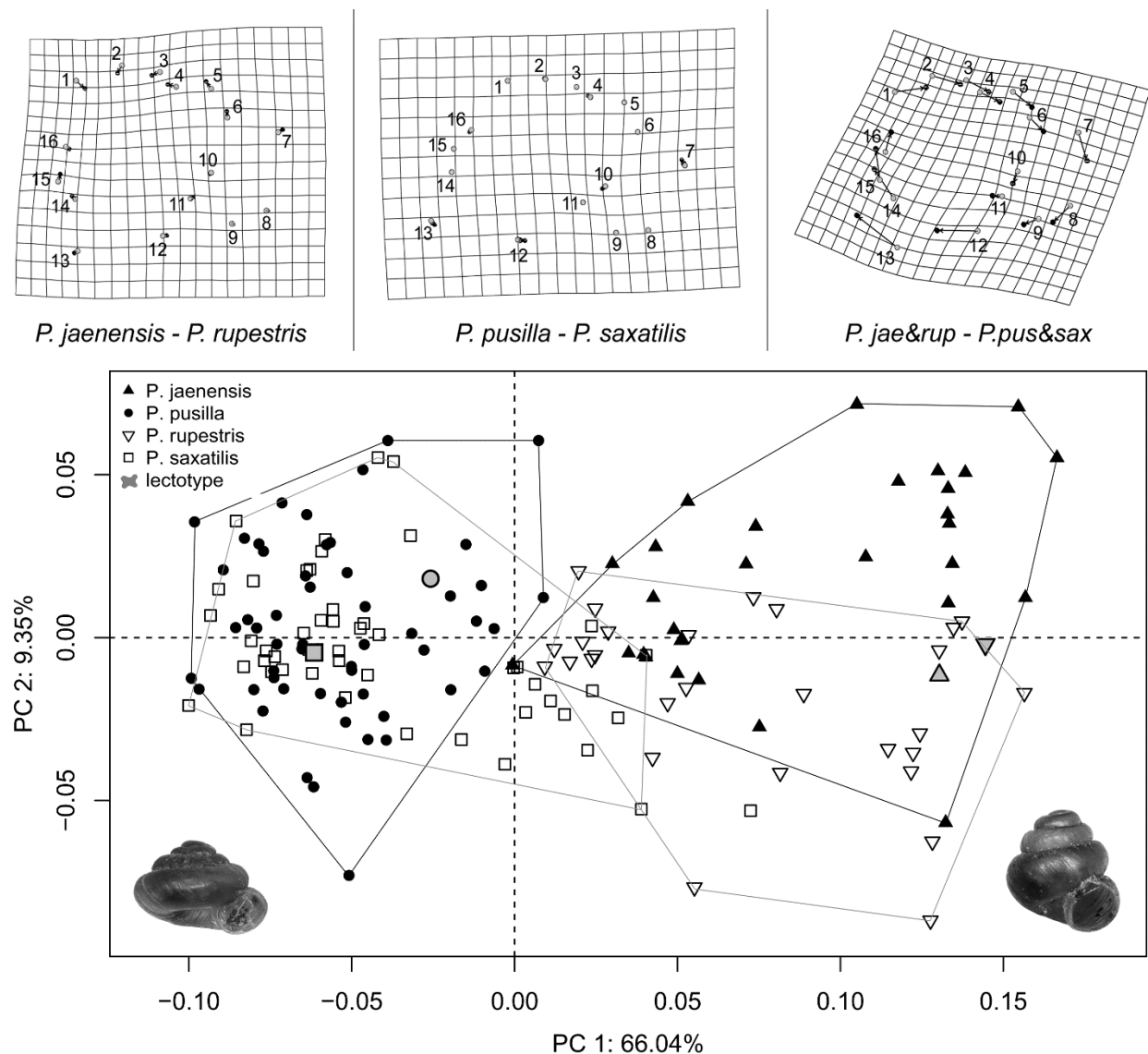


FIGURE S5 Result of geometric morphometrics using sixteen landmarks on the frontal shell view (supplementary fig. S2). Upper part: thin-plate splines, illustrating transitions in shape between pairs of species *P. jaenensis* and *P. rupestris*, and *P. pusilla* and *P. saxatilis*, and between the high-spired (former pair) and low-spired (latter pair) species combined. Lower part: Position of shells along the first two axes of the Principal Component Analysis (PC1, PC2) based on Procrustes shape coordinates of the landmark data. Convex polygons were added to the diagram to highlight the distinction between the four species.

Type specimens of the respective species were also used in the analysis (see fig. 7 for details), and are shown in grey color.