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Pupilla pratensis (Gastropoda: Pupillidae) in the Czech Republic and Slovakia and its distinction from *P. muscorum* and *P. alpicola* based on multidimensional analysis of shell measurements

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Abstract: *Pupilla pratensis* (Clessin, 1871) was recently confirmed as a distinct species based on morphological, ecological and molecular evidence. The main purpose of this study is to publish the first reliable data on the occurrence of *P. pratensis* in the Czech Republic and Slovakia. The second goal is to analyse conchometry of *P. pratensis*, *P. muscorum* (L., 1758), and *P. alpicola* (Charpentier, 1837) to find out whether it is possible to reliably distinguish these species solely based on shell measurements. For multidimensional analysis of shell measurement variation we used principal components analysis (PCA). We documented six populations of *P. pratensis* in the Czech Republic and one in SW Slovakia. The revision of voucher material showed that all previously reported records of *P. alpicola* from the Czech Republic belonged in fact to *P. pratensis*. This requires the exclusion of *P. alpicola* from the list of Czech molluscs. Based on multidimensional analysis of shell measurements it was possible to distinguish *P. pratensis* from *P. muscorum* with no overlapping specimens. *Pupilla alpicola* was almost completely different from *P. muscorum* with only few overlapping specimens, contrary to *P. pratensis* which was mostly impossible to distinguish from *P. alpicola* based on analysed shell measurements. Shell width was the best single shell measurement for distinguishing *P. pratensis* and *P. muscorum*. Shell measurements of two Swedish populations of *P. pratensis* did not differ from shell variation of Czech and Slovak populations. However, Scandinavian populations displayed some differences from central European populations in apertural barriers which are discussed in detail. Czech and Slovak populations of *P. pratensis* occurred in calcium-rich fen meadows which perfectly matches with site characteristics reported from Scandinavia. We assume that the observed morphometric differences between *P. pratensis* and *P. muscorum* can be useful for distinguishing the species also outside the Czech territory and for palaeoecological studies where only empty shells are available. Since these species occupy ecologically different habitats their reliable identification in fossil material can improve the reconstructions of past environments.

Key words: conchometry; taxonomy; autecology; shell variation; identification characters; distribution

Introduction

Recently, von Proschwitz et al. (2009) have suggested that *Pupilla pratensis* (Clessin, 1871) was a distinct species. Previously it was mostly considered as an ecophenotype of *P. muscorum* (L., 1958). The taxonomic identity of these two taxa was suggested based on morphological, ecological, and molecular evidence. *Pupilla muscorum* var. *pratensis* was described from wet peat meadows in Bavaria, Germany (Clessin 1871). Unfortunately, this taxon obtained little attention (e.g., Kerney et al. 1983) until the publication of Jueg (1997). The shell of *P. pratensis* differs from *P. muscorum* by having larger width and height, the number of whorls is usually greater than that of *P. muscorum* (von Proschwitz et al. 2009); *P. pratensis* has also a more darkish, chestnut brown shell colour. There are also considerable ecological differences: *P. pratensis* is an inhabitant of wet meadows contrary to the rather xerophile *P. muscorum*. In Scandinavia, *P. pratensis* is a typical species of open calcareous fens or calcareous wet meadows. As the species was largely overlooked, current data on its distribution are only fragmentary and need further attention. So far, the species was reliably confirmed from Poland, Germany, Denmark, Norway and Sweden (von Proschwitz et al. 2009). There is also an old record published by Clessin (Clessin 1887–1890) from Moravia (Czech Republic) that needs to be revised. Therefore we decided to revise all available voucher material of *P. muscorum*, especially those mentioned from wet meadows, from the Czech Republic and Slovakia.

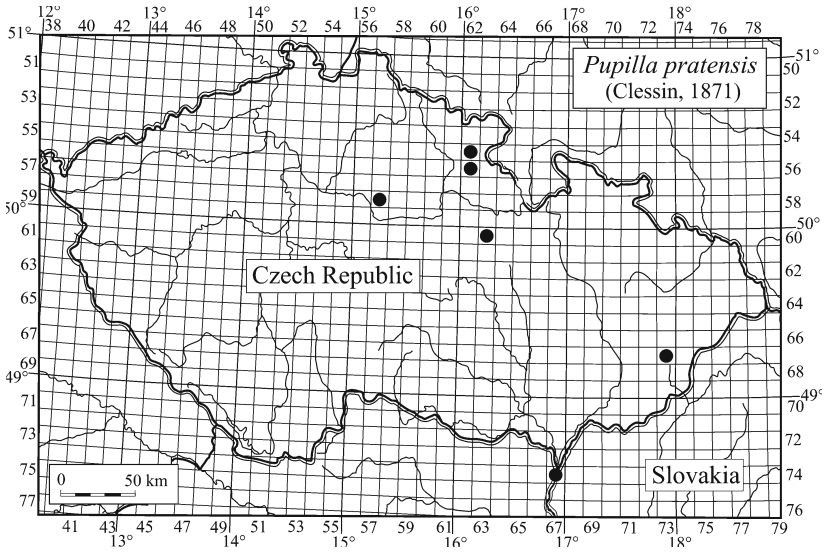

Fig. 1. Localities of first known populations of *Pupilla pratensis* in the Czech Republic and Slovakia. For details see Appendix 1.

Table 1. Spearman correlations among shell measurements of all studied *Pupilla* species and specimens' scores on the first three PCA ordination axes. Values of correlation coefficient (r_S) and their significances (P) are shown. Significant correlations are in bold.

$n = 495$	Axis 1		Axis 2		Axis 3	
	r_S	P	r_S	P	r_S	P
Shell height	**–0.93**	< 0.001	**0.32**	< 0.001	**0.13**	0.003
Shell width	**–0.58**	< 0.001	**0.27**	< 0.001	**–0.66**	< 0.001
Body whorl height	**–0.53**	< 0.001	**0.61**	< 0.001	**0.29**	< 0.001
Mouth height	**–0.71**	< 0.001	**0.39**	< 0.001	0.07	0.147
Mouth width	**–0.60**	< 0.001	**0.44**	< 0.001	**–0.12**	0.008
No. of whorls	**–0.97**	< 0.001	**–0.19**	< 0.001	0.07	0.112

The main purpose of this study is to publish distributional data of *P. pratensis* from the Czech Republic and Slovakia. The second goal is to analyse conchometry of *P. pratensis*, *P. muscorum* and *P. alpicola* to find out whether it is possible to reliably distinguish these species solely based on shell measurements. These data should be useful in palaeoecological studies since these species occupy ecologically different habitats. We are also briefly describing habitats and ecological requirements of *P. pratensis* and comparing them with those reported from Scandinavia.

Material and methods

We revised available voucher material from personal collections of the authors and from the collection of the National Museum in Prague. Samples were collected mostly qualitatively by hand picking or by wet sieving method (Horsák 2003). All adult shells with a completely developed mouth were picked out for shell measurements.

Shells were measured under a stereoscopic dissecting microscope and the following six parameters were measured: shell height and width, body whorl height, mouth height and width, and number of whorls. In total, five Czech, one Slovak and two Swedish populations of *P. pratensis* were measured (for details see Appendix 1). In order to compare shell variation of this species with the conchologically highly similar two species of the genus; six Czech populations of *P. muscorum* and 14 Slovak populations of *P. alpicola* were measured and analysed (Appendix 1).

For multidimensional analysis of shell measurement variation we used principal components analysis (PCA) on correlation matrix in the CANOCO programme (ter Braak & Šmilauer 2002). Measured shell characters were correlated among each other using Spearman correlations (r_S) and with the specimens' scores on the first three PCA axes to detect most differing characters among these three species.

Results

So far, we documented six populations of *P. pratensis* in the Czech Republic (five in E Bohemia and one in SE Moravia) and one in SW Slovakia (Fig. 1, Appendix 1). The sites inhabited by this species were calcium-rich treeless wetlands, mostly calcareous fen meadows of low altitude (median = 275 m, range = 146–500 m a.s.l.).

PCA of shell measurements revealed three major directions of shell measurement variation which were significantly associated with at least one of measured parameters (Table 1). The first PCA axis was highly correlated with shell height and the number of whorls, which were variables that expressed the highest level of correlation from all measured shell dimensions (Table 2). The second axis expressed mainly variation in body whorl height and the third axis significantly cor-

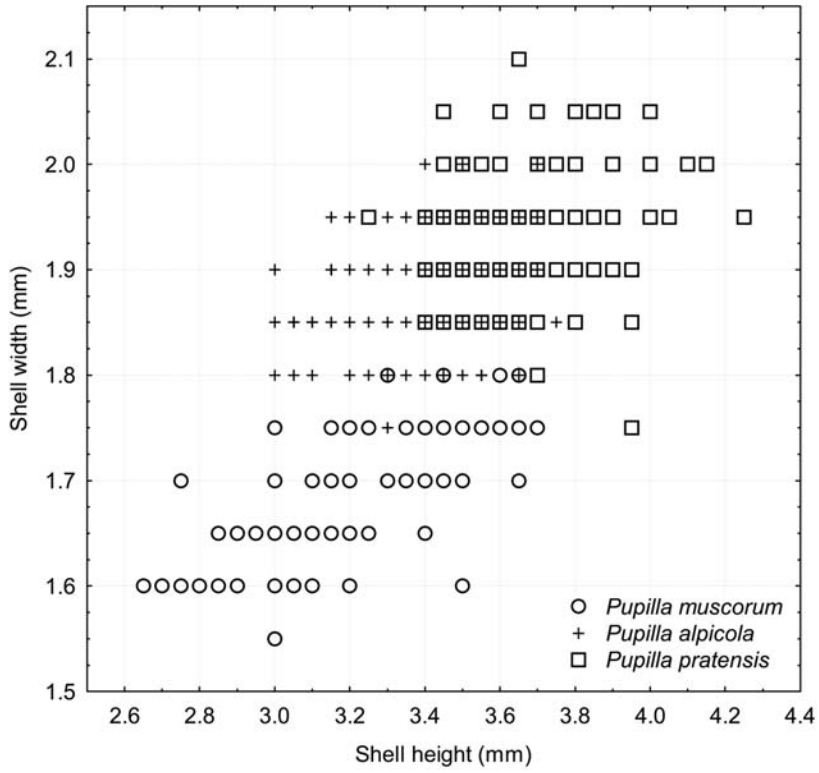

Fig. 2. Relationship between shell height and shell width of three conchologically similar *Pupilla* species; *P. pratensis* ($n = 127$), *P. alpicola* ($n = 259$) and *P. muscorum* ($n = 109$).

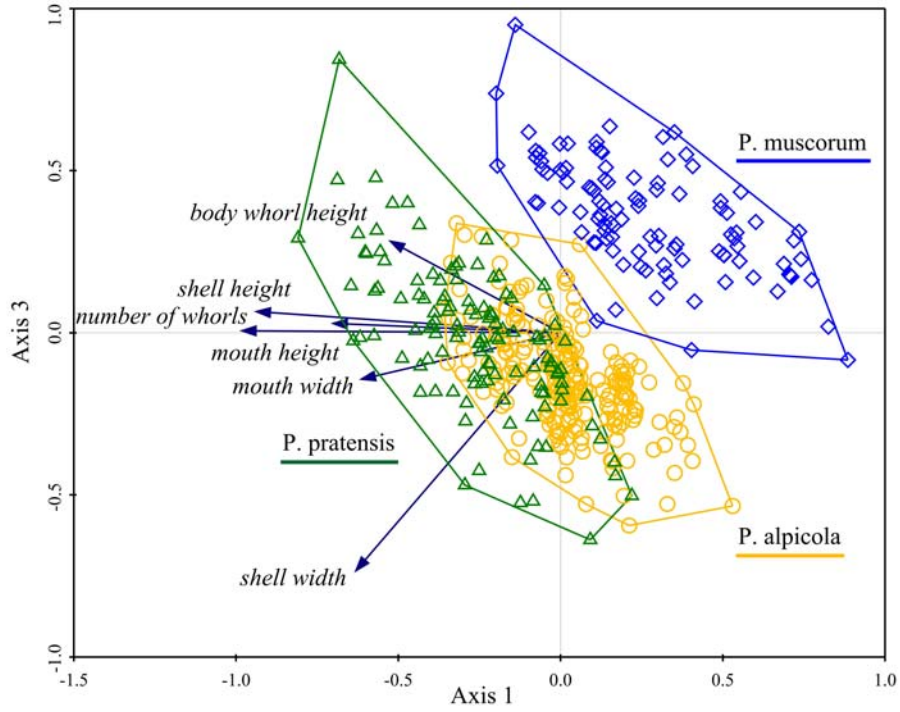

Fig. 3. PCA diagram of 495 measured shells of *Pupilla pratensis* ($n = 127$), *P. alpicola* ($n = 259$) and *P. muscorum* ($n = 109$) showing their position along the first and third ordination axis based on six measured shell dimensions (arrows). Correlations among these measured characters and PCA axis are given in Table 1.

related with shell width. This is in accordance with the results of correlations among shell dimensions, where shell height and shell width were only weakly correlated and thus their variances were more or less independent (Fig. 2). Based on multidimensional analysis of shell measurements it was possible to distinguish *P. pratensis* from *P. muscorum* with no overlapping specimens (Fig. 3). *Pupilla alpicola* was almost completely differ-

Table 2. Spearman correlations among six shell measurements of all studied specimens of *Pupilla pratensis*. Values of correlation coefficient (r_S; upper right) and their significances (P; down left) are shown. Significant correlations are in bold.

$n = 127$	Shell height	Mouth height	Body whorl height	Shell width	Mouth width	No. of whorls
Shell height		**0.62**	**0.58**	**0.27**	**0.42**	**0.75**
Mouth height	< 0.001		**0.56**	**0.44**	**0.55**	**0.39**
Body whorl height	< 0.001	< 0.001		**0.43**	**0.35**	**0.20**
Shell width	0.002	< 0.001	< 0.001		**0.53**	–0.12
Mouth width	< 0.001	< 0.001	< 0.001	< 0.001		**0.24**
Number of whorls	< 0.001	< 0.001	0.026	0.185	0.006	

Table 3. Descriptive statistics of shell measurements (mm) of selected populations of *Pupilla pratensis* (9 populations, 127 specimens), *P. muscorum* (6 populations, 109 specimens), and *P. alpicola* (14 populations, 259 specimens). For details see Appendix 1.

	Median	Mean	Standard deviation	Minimum	Lower quartile	Upper quartile	Maximum
P. pratensis							
Shell height	3.65	3.68	0.19	3.25	3.55	3.80	4.25
Mouth height	1.10	1.09	0.06	0.95	1.05	1.10	1.25
Body whorl height	1.65	1.65	0.06	1.50	1.60	1.70	1.80
Shell width	1.95	1.93	0.06	1.75	1.90	1.95	2.10
Mouth width	1.10	1.11	0.04	1.00	1.10	1.15	1.20
No. of whorls	5.25	5.31	0.30	4.50	5.00	5.50	6.00
P. muscorum							
Shell height	3.20	3.23	0.25	2.65	3.05	3.45	3.70
Mouth height	1.00	1.00	0.07	0.75	0.95	1.05	1.15
Body whorl height	1.60	1.59	0.09	1.35	1.55	1.65	1.75
Shell width	1.70	1.69	0.06	1.55	1.65	1.75	1.80
Mouth width	1.00	1.02	0.05	0.90	1.00	1.05	1.15
No. of whorls	4.75	4.64	0.33	3.75	4.50	4.75	5.25
P. alpicola							
Shell height	3.40	3.38	0.14	3.00	3.30	3.50	3.75
Mouth height	1.00	1.02	0.05	0.85	1.00	1.05	1.15
Body whorl height	1.55	1.56	0.05	1.45	1.50	1.60	1.70
Shell width	1.90	1.88	0.04	1.75	1.85	1.90	2.00
Mouth width	1.05	1.04	0.04	0.90	1.00	1.05	1.15
No. of whorls	5.00	4.99	0.23	4.25	4.75	5.00	5.50

ent from *P. muscorum* with only few overlapping specimens, contrary to *P. pratensis* which was mostly impossible to distinguish from *P. alpicola* based on analysed shell measurements. These two species had a very similar variation of shell dimensions, however, *P. alpicola* tended to be smaller and thinner (Table 3). Similar results were achieved when plotting only shell height against shell width (Fig. 2); however there were some overlapping specimens among *P. pratensis* and *P. muscorum*, especially with respect to shell height.

Shell width was the best single shell measurement for distinguishing *P. pratensis* and *P. muscorum* (Fig. 4). Except two specimens from 127 studied shells, *P. pratensis* was always wider than 1.85 mm, in contrast to *P. muscorum* reaching at maximum only 1.80 mm.

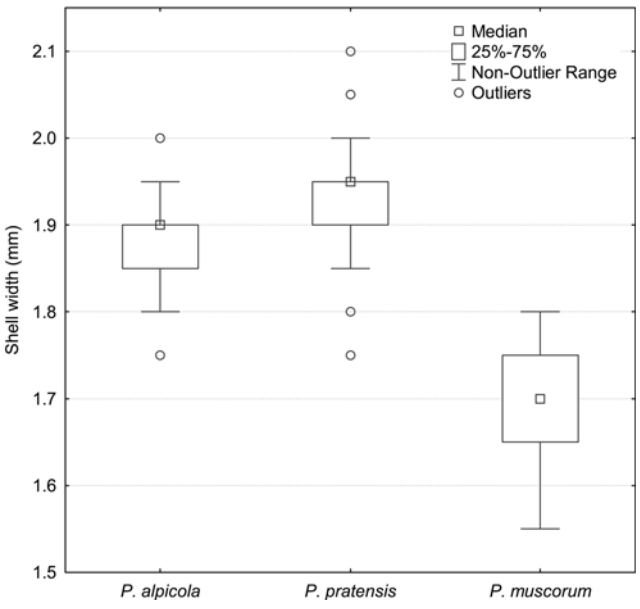

Fig. 4. Variation of shell width within and among studied species: *Pupilla alpicola* ($n = 259$), *P. pratensis* ($n = 127$) and *P. muscorum* ($n = 109$).

Discussion

The occurrence of *P. pratensis* has been reliably confirmed for the first time in the Czech Republic and Slovakia. Even though, the occurrence of *P. muscorum* var. *pratensis* has already been mentioned from the Czech Republic (e.g., Clessin 1886–1887; Ložek 1956; Rafa-

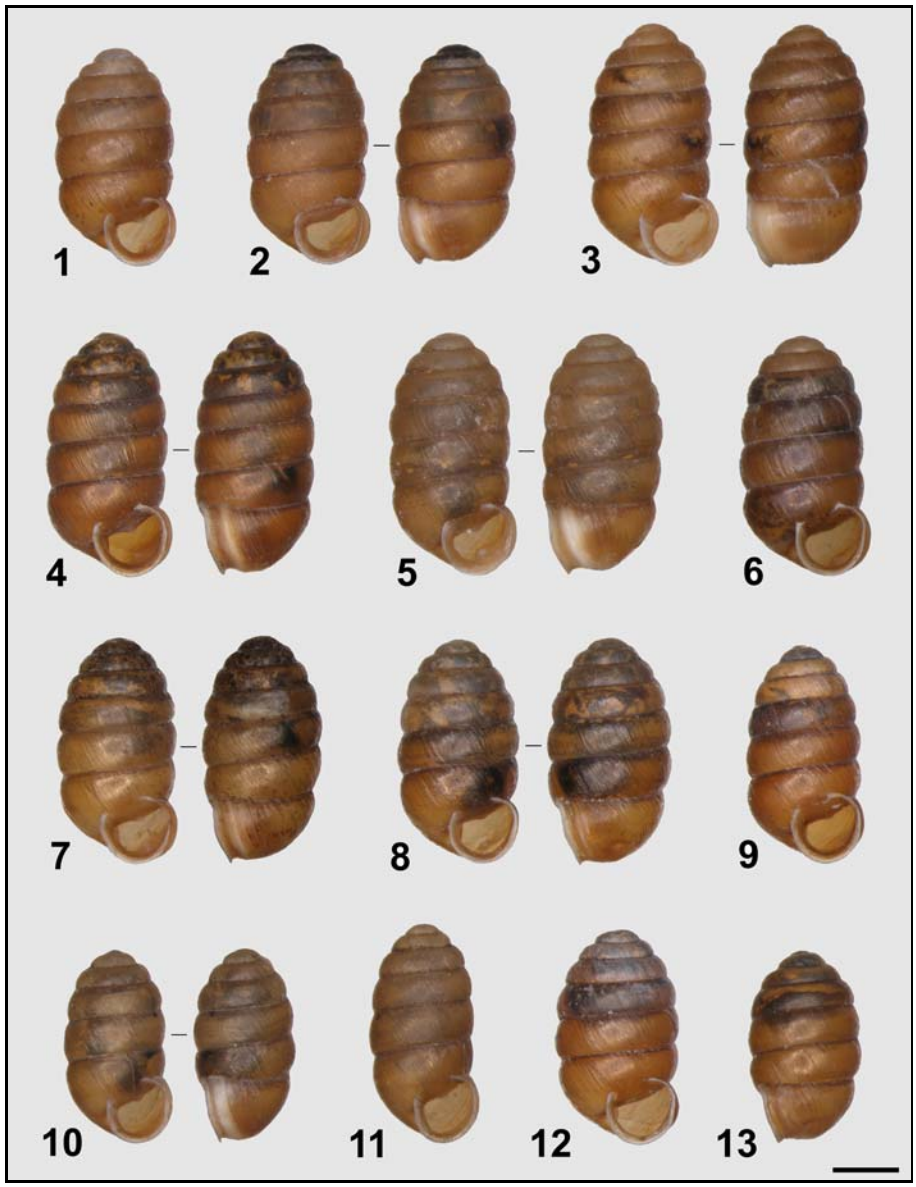

Fig. 5. Shell variation of *Pupilla pratensis* (1–9), *P. muscorum* (10, 11), and *P. alpicola* (12, 13). The numbers of localities match with those given in lists of measured populations; shell measurements in millimetres (height/width). *P. pratensis*: 1–3, Loc. no. 2, 3.5/1.9, 3.6/2.1, 3.9/2.1; 4, Loc. no. 3, 4.0/2.1; 5, Loc. no. 4, 4.0/2.0; 6, Loc. no. 1, 3.9/2.2; 7, Loc. no. 6, 3.7/2.0; 8, Loc. no. 9, 3.5/2.0; 9, Loc. no. 8, 3.6/1.9; *P. muscorum*: 10, Loc. no. 1, 3.2/1.7; 11, Loc. no. 4, 3.6/1.7; *P. alpicola*: 12, Loc. no. 6, 3.5/1.9; 13, Loc. no. 3, 3.2/1.8. Scale 1 mm. Photos by M. Horsák.

jová 2002), a revision based on new taxonomic knowledge (von Proschwitz et al. 2009) was needed. It is not possible to use these older records without revision of voucher material. For data published by Rafajová (2002) only a part of voucher material was available and this turned out to be *P. muscorum*. So far, *P. pratensis* was reliably confirmed from only few sites in the Czech and Slovak Republics, however its more frequent occurrence in some lowland regions is probable. We expect further records particularly in East Bohemia mainly due to a frequent occurrence of suitable habitats – calcium-rich fen meadows with communities of *Caricion davallianae* alliance (Sádlo 2000). Observed, although still rather rough, habitat requirements perfectly match with those reported from Scandinavia (von Proschwitz et al. 2009). Scandinavian populations are also associated with calcareous fens and fen meadows.

Not only ecological requirements seem to be stable in distinct parts of the distributional range. Shell measurements of two Swedish populations did not differ from shell variation of Czech and Slovak populations. Thus, the observed differences between *P. pratensis* and *P. muscorum* can probably be useful outside the Czech territory as well. The plausibility of revealed conchometrical characters is important especially for palaeoecological studies as fossil material do not provide molecular and other data which are easily available from live material. For example, the species differ also in shell colour (*P. pratensis* is more dark brown), but also the colour is mostly lost in fossil material. Since these snails differ in ecological requirements (*P. muscorum* lives mainly in xeric habitats) the possibility of their iden-

tification in dead assemblages can have an important implication in reconstruction of past environments.

There are also differences in apertural barriers; however, our observations from Czech and Slovak populations are somewhat different from those published by von Proschwitz et al. (2009). From Scandinavian populations they stated that *P. pratensis* has a rather weakly developed apertural lip compared to *P. muscorum*. Most of Czech and Slovak specimens have distinctly developed lips (Fig. 5), similarly or even strongly than *P. muscorum*. However, images of shells from German and Polish populations published by von Proschwitz et al. (2009) suggest that the weakly developed lip probably might be limited only to Scandinavian populations. This conclusion is consistent with the observation of two analysed Swedish populations which also have, on average, a weakly developed lip (Fig. 5). Central European populations of *P. pratensis* also have a bit differently developed teeth than mentioned by von Proschwitz et al. (2009) for Scandinavian populations. They concluded that *P. pratensis* has weakly developed apertural teeth (parietal weak, palatal indicated) or it is rather often toothless. In central European material most specimens have both teeth well developed. The palatal gutter, characteristic of *P. alpicola*, was always visible or at least indicated in the Czech and Slovak populations; however it was always weakly developed compared to that of *P. alpicola*. These differences from Scandinavian populations indicate that central European populations have on average more developed apertural structures.

Pupilla pratensis can also be confused with *P. alpicola*, most probably due to similar habitat preferences, shell colour and size, which was also the case of all published Czech records of *P. alpicola* (Ložek 1982, 1992; Horsák 2005). Voucher specimens from these sites (sites no. 1, 4, 5 in Appendix 1) were revised and based on characters in mouth (distinctly developed lip, presence of teeth and weakly developed gutter) these populations clearly belonged to *P. pratensis*. Thus, the occurrence of *P. alpicola* in the Czech Republic was not confirmed and this species should be excluded from national Check Lists (Juřičková et al. 2001, 2008) as well as the Red List (Beran et al. 2005). These two species differ in several characters (see von Proschwitz et al. 2009). The main differences are in the development of apertural teeth and lip. *Pupilla alpicola* is often toothless (rarely with weak parietal teeth), but with a prominent palatal gutter (Fig. 5). Apertural lip of *P. alpicola* is also very weak and flat, at the most slightly paler than the shell colour. Based on current data it seems that also distributional ranges of these two species are parapatric in our territory: *P. alpicola* living in calcareous fens in the Western Carpathians (central Slovakia with small extension to Poland in the Pieniny Mts region) and *P. pratensis* living in the Czech Republic with extension to southwest Slovakia.

Since *P. pratensis* seems to be an exclusive inhabitant of calcium-rich fen meadows mainly in large lowlands, the habitats are currently threatened by human degradation and destruction, we suggest classification of this species as Endangered (EN) for both countries. However, further research is needed for obtaining representative data about present distribution and conservation status.

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Appendix 1. Survey of sites with occurrences of *Pupilla pratensis* and lists of measured populations of P. *muscorum* and *P. alpicola*. Data in the lists are as follows: number of the site (bold), geographical co-ordinates (N, E), state, code of the mapping grid for faunistic mapping according to Ehrendorfer & Hamann (1965), nearest settlement, description of the site, altitude (m a.s.l., approximately), date of investigation, number of specimens (collected/measured), name abbreviation of data collector (JB – Jaroslav Brabenec, JM – Jan Myšák, JŠ – Jana Škodová, MH – Michal Horsák, NČ – Nicole Černohorská, TČ – Tomáš Čejka, VL – Vojen Ložek), and citation of literature source for published records. Voucher specimens are deposited in personal collections of particular investigators or in the collection of National Museum in Prague (NMPC).

Pupilla pratensis (686/127)

1 – 49°13′40", 17°59′14", CZ, Moravia, 6773, Pozděchov, a calcareous spring fen on the left bank of the Trubiska brook near Brhel settlement, 500 m, 27.IX.2000, (8/0), MH, originally published as *P. alpicola* (Horsák 2005), 29.IX.2006, (8/0), NČ, 1.VIII.2009, (253/40), JŠ; **2** – 49°57′41″, 16°11′26″, CZ, Bohemia, 6063, Vysoké Mýto, U Vinic Nature Monument (NM) – a fen meadow, 270 m, 16.VI.2006, (39/25), MH, 30.VII.2009, (61/9), JM; **3** – 49°59′19″, 16°11′52″, CZ, Bohemia, 6063, Choceň, Vstavačová louka NR – a fen meadow, 280 m, 26.VI.2009, (210/27), JM; **4** – 50°24′17″, 16°04′09″, CZ, Bohemia, 5562, Česká Skalice, Dubno Nature Reserve (NR) – a calcareous wet meadow, 290 m, 20.VI.1959, (9/9), JB, originally published as *P. muscorum* (Brabenec 1970); **5** – 50°17′48″, 16°04′03″, CZ, Bohemia, 5662, Opočno, Zbytka NR – a calcareous wet meadow, 255 m, 5.VI.1996, (4/3), VL; **6** – 48°32′01″, 16°59′22″, Slovakia, 7467, Závod, Abrod NR – a calcareous wet meadow, 147 m, 24.V.1999, (35/8), TČ; **7** – 48°31′59″, 16°59′46″, Slovakia, 7467, Závod, Abrod NR – a calcareous wet meadow, 146 m, 24.V.1999, (12/3), TČ; **8** – 63°20′11″, 14°26′09″, Sweden, Jämtland, Krokom, Storflon-Andresflon Nature site – a calcareous fen, 308 m, 16.VIII.2006, (17/1), MH; **9** – 55°31′35″, 13°54′03″, Sweden, Skåne, Benestad, Benestads Backar NR – a calcareous fen, 124 m, 13.VIII.2006, (29/2), MH; **10** – 50°10′06″, 15°16′03″, CZ, Bohemia, 5857, Dlouhopolsko, Dlouhopolský rybník – a calcareous fen meadow, 240 m, 17.VI.2006, (1/0), MH.

Pupilla muscorum (470/109)

1 – 50°26′23″, 14°34′37″, CZ, Bohemia, 5553, Kokořín, Kokořínsko NR – the Kokořín castle, 330 m, 10.VIII.1998, (126/30), MH; **2** – 50°37′09″, 14°24′53″, CZ, Bohemia, 5352, Blíževedly, a ruin of the Ronov castle, 550 m, 30.V.2000, (22/10), MH; **3** – 49°11′26″, 16°40′29″, CZ, Moravia, 6866, Brno-Slatina, Stránská skála NR – a calcareous steppe, 300 m, 9.VIII.1996, (17/5), MH; **4** – 49°11′17″, 16°35′30″, CZ, Moravia, 6865, Brno-střed, Na Pořící 3 – a meadow in a city garden, 220 m, 12.XI.1997, (66/30), MH; **5** – 49°28′53″, 16°39′43″, CZ, Moravia, 6565, Boskovice, a ruin of the Boskovický hrad castle, 435 m, 30.IV.1999, (10/4), MH; **6** – 48°46′32″, 16°41′59″, CZ, Moravia, 7266, Sedlec u Mikulova, Slanisko u Nesytu NR (east part), 170 m, 25.IX.1999, (229/30), MH.

Pupilla alpicola (1202/259)

1 – 49°09′11″, 19°09′18″, SK, Slovakia, 6880, Stankovany, Močiar NR – a calcareous spring fen, 437 m, 18.VI.2002, (39/13), MH; **2** – 48°49′34″, 20°09′49″, SK, Slovakia, 7186, Telgárt, Meandry Hrona NR – a calcareous fen, 820 m, 27.V.2004, (32/7), MH; **3** – 49°04′06″, 19°59′57″, SK, Slovakia, 6985, Važec, Krivošova lúka – a calcareous fen 800 m E from the end of the Važec settlement , 815 m, 26.V.2004, (124/35), MH; **4** – 48°52′45″, 18°53′24″, SK, Slovakia, 7179, Rakša, Rakšianske rašelinisko NR – a calcareous fen, 512 m, 16.IX.2001, (159/31), MH; **5** – 49°07′31″, 19°12′59″, SK, Slovakia, 6881, Švošov – a calcareous fen at the end of the Komjatnianská valley, 475 m, 18.VI.2002, (34/8), MH; **6** – 48°55′00″, 18°56′51″, SK, Slovakia, 7079, Blatnica, Blatnická dolina valley – a calcareous fen 2.5 km of the village, 579 m, 16.IX.2001, (18/8), MH; **7** – 49°02′42″, 20°20′02″, SK, Slovakia, 6988, Hozelec, a calcareous fen 1.5 km W from Hozelec, 680 m, 29.V.2004, (144/18), MH; **8** – 49°07′52″, 19°15′48″, SK, Slovakia, 6881, Studničná, a calcareous spring fen 450 m S of the village, 780 m, 18.VI.2002, (70/11), MH; **9** – 49°08′55″, 19°09′20″, SK, Slovakia, 6880, Stankovany, Rojkovské rašelinisko NR – a calcareous spring fen, 433 m, 16.IX.2001, (62/19), MH; **10** – 48°53′29″, 18°58′38″, SK, Slovakia, 7179, Blatnica, Rakytovská Valley – a calcareous spring fen 350 m of the end of the valley, 733 m, 16.IX.2001, (65/18), MH; **11** – 49°02′39″, 20°14′39″, SK, Slovakia, 6987, Spišská Teplica, a brown moss rich fen on SW margin of the village, 700 m, 27.V.2003, (61/14), MH; **12** – 48°53′20″, 18°58′41″, SK, Slovakia, 7179, Blatnica, Rakytovská dolina Valley – a calcareous spring fen at the end of the valley, 736 m, 17.IX.2001, (38/17), MH; **13** – 49°06′25″, 19°15′16″, SK, Slovakia, 6881, Hrboltová, a calcareous fen NE of the village, 587 m, 28.V.2004, (120/20), MH; **14** – 49°11′36″, 19°31′20″, SK, Slovakia, 6883, Veľké Borové, a calcareous fen near Borovinka brook and S of the village, 824 m, 19.VI.2002, (236/40), MH.