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Authors: Kaňuch, Peter, Fornůsková, Alena, Bartonička, Tomáš, Bryja, Josef, and Řehák, Zdeněk

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Do two cryptic pipistrelle bat species differ in their autumn and winter roosting strategies within the range of sympatry?

Peter KAŇUCH[1,2]*, Alena FORNŮSKOVÁ[1,3], Tomáš BARTONIČKA[3], Josef BRYJA[1,3] and Zdeněk ŘEHÁK[3]

[1] *Institute of Vertebrate Biology, Academy of Sciences of the Czech Republic, v.v.i., Květná 8, 603 65 Brno, Czech Republic*

[2] *Institute of Forest Ecology, Slovak Academy of Sciences, Štúrova 2, 960 53 Zvolen, Slovakia; e-mail: kanuch@netopiere.sk*

[3] *Department of Botany and Zoology, Masaryk University, Kotlářská 2, 611 37 Brno, Czech Republic*

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Abstract. Large hibernating aggregations and behaviour called late summer or autumn "invasions" when large groups of bats enter buildings are known in pipistrelles. We investigated differences in roosting behaviour between two cryptic species (common pipistrelle, *Pipistrellus pipistrellus*, and soprano pipistrelle, *Pipistrellus pygmaeus*) during autumn and winter periods. In total 463 bats were sampled in both caves and buildings with temporary occurrence during the period of late summer and autumn mating and presumable migrations from late July to September (10 sites), and in all known types of hibernacula from late November to March (34 sites). Sampling sites were located within the Czech Republic, Slovakia, Serbia and Romania in areas where the two species occur sympatrically throughout the summer. Using a DNA-based identification method, all but four individuals were identified as *P. pipistrellus*. It means that winter roosts of *P. pygmaeus* remain largely unknown in the area. Similarly, no *P. pygmaeus* was found in the "invasion" assemblages. Very abundant groups of *P. pipistrellus* in underground hibernacula and its exclusive occurrence in sites of "invasions" suggest that roosting behaviour during this time may be species-specific.

Key words: *Pipistrellus*, ecology, hibernacula, invasions, PCR-identification

Introduction

The sympatric occurrence of morphologically similar animal species can often be explained by differences in ecological requirements and competition (Abrams 1983, Carapelli et al. 1995, Bruna et al. 1996, Trewick 1998). Identifying the mechanisms that permit the coexistence of morphologically similar and sympatric species of bats is further complicated by their nocturnal lifestyle. Differences in species ecology can include roosts, diet, migrating behaviour, etc. However several cases of niche partitioning between cryptic and sympatric bat species have been described. One particularly well studied case is that of the greater, *Myotis myotis* (Borkhausen, 1797), and lesser, *Myotis blythii* (Tomes, 1857), mouse eared bats. *M. myotis* feeds preferentially on carabid beetles inside the forests in contrast to *M. blythii* which prefers bush-crickets in more open sites (Arlettaz et al. 1997, Arlettaz 1999). Also the morphologically similar long-eared bats of the genus *Plecotus* have different foraging and habitat preferences (e.g. Ševčík 2003, Pavlinić & Tvrtković 2004). In two recently discovered, genetically distinct but morphologically cryptic species – common pipistrelle, *Pipistrellus pipistrellus* (Schreber, 1774), and soprano pipistrelle, *Pipistrellus pygmaeus* (Leach, 1825) with sympatric distribution across a large part of Europe (Barratt et al. 1995, 1997, Mayer & von Helversen 2001, Benda et al. 2003, Hulva et al. 2004), species-specific differences in ecological requirements are suggested, including habitat preferences and foraging ecology (e.g. Barlow & Jones 1999, Bartonička & Řehák

* *Corresponding Author*

2004, Davidson-Watts & Jones 2006, Nicholls & Racey 2006a, b, Sattler et al. 2007).

In some European countries (e.g. in Germany, France, Czech Republic, Slovakia and Romania) large hibernating aggregations of hundreds up to thousands of pipistrelles are found in natural caves, mines or buildings (Lustrat & Julien 1993, Murariu 1995, Kretzschmar & Heinz 1995, Pčola 1997, Matis 2000, Sendor et al. 2000, Matis et al. 2002, Nagy & Szántó 2003, Sachteleben & von Helversen 2006, Anděra & Hanák 2007). Moreover, pipistrelles are also known for their typical behaviour called "invasions" where temporary groups of tens up to hundreds of bats fly into the buildings in towns and shelter often in curious sites (Palášthy & Gaisler 1965, Gaisler et al. 1990, Smit-Viergutz & Simon 2000). These phenomena and the recently proposed theory about long-migratory behaviour of *P. pipistrellus* and *P. pygmaeus* (Bryja et al. 2009), raised questions of similarities or divergences in their behaviour. The aim of the present study was to find differences in roosting behaviour between two cryptic pipistrelle species during autumn and winter periods.

Material and Methods

Our study area included central and southern Europe where sympatric occurrence of the two species is assumed (cf. Mayer & von Helversen 2001) and common occurrence of them during summer is well known (e.g. Gaisler et al. 2002, Bartonička & Řehák 2004, Anděra & Hanák 2007, Petrášová 2008, Bryja et al. 2009). We used already sampled (museum specimens) or live individuals which were found in caves as well as in buildings with temporary occurrence during the period of late summer and autumn mating and presumable migrations from late July to September (10 sites, Table 1) and in all known types of pipistrelles hibernacula from late November to March (34 sites, Table 1). Altogether 463 specimens of pipistrelle bats, collected between 1966 and 2008 in the area of the Czech Republic, Slovakia, Serbia and Romania were analysed. Because the bats were sampled mostly during hibernation when it was not possible to identify species on the basis of their echolocation calls, a DNA-based identification method was used in this study. Following DNA extraction from wing membrane (3 mm biopsy punches from individuals alive or museum ethanol-preserved specimens) by quick HotShot method (Truett et al. 2000), we used a simple species-identification test which consists of multiplex PCR and agarose gel electrophoresis (Kaňuch et al. 2007). Positive controls, i.e. one specimen of each *P. pipistrellus* and *P. pygmaeus* identified by sequencing of 320 bp fragment of cyt *b* gene (Kaňuch et al. 2007) were always included in the test.

Results and Discussion

During the winter period all but four individuals were determined as *P. pipistrellus* (99%, n = 463). Three individuals of *P. pygmaeus* were admixed within a large colony of *P. pipistrellus* hibernating in a castle cellar at Nový Hrádek near Znojmo (Czech Republic). Additionally, one individual of *P. pygmaeus* was found in the hall of a small family house at Samotíšky near Olomouc (Czech Republic, Table 1). It was a flightless adult female with a serious wing membrane injury. Since the analysed material was collected in underground (caves, gallery, cellar) or urban above ground shelters (town buildings, church, castle) and sites (some starving individuals were found directly on the town streets) there is also a need to check other possible roost types. We found no individuals of soprano pipistrelles in the buildings of mass invasions of common pipistrelles (Table 1).

Our brief results could also indicate differences in the mating system and social behaviour of these species. In *P. pipistrellus* mating sites (even with densely packed territories) have been found around winter roosts in urban environments (Sendor & Simon 2003, Sachtelleben & von Helversen 2006). On the other hand, dispersed mating territories in *P. pygmaeus* were usually situated very near to lowland forest nursery colonies (Bartonička & Řehák 2004). It corresponds well with our results where only common pipistrelles were mist-netted at the entrance to large hibernacula (caves) during the mating time (Table 1).

Common roosting of both species in the same maternity roost has not been recorded which was originally one of the reasons to consider them as separate species (Park et al. 1996). A temporary colony consisting of both species (echolocation calls recorded) was found during the time of spring migrations in central Slovakia (Ceľuch et al. 2006). Up to present only one anecdotal record (identification performed on the basis of morphological features) of a mixed winter colony was made in Poland (Wojtaszyn et al. 2004). The rare finding of a few *P. pygmaeus* individuals in the castle cellar at Nový Hrádek together with more numerous *P. pipistrellus* (Table 1) was therefore an accidental event rather than a rule, due to the frequent summer occurrence of the two species in the area (Reiter et al. 2003).

***Table 1.** List of the* Pipistrellus pipistrellus *and* P. pygmaeus *(in parentheses) records during late summer and autumn (cave, chasm = swarming sites, building = invasion sites) and winter periods (different types of hibernacula and winter records) in the Czech Republic (CZ), Slovakia (SK), Serbia (SB) and Romania (RO).*

Locality name	Site	Geographic coordinates	Date	Adults		Young		Age unknown			Total
				M	F	M	F	M	F	Ind.	
Autumn records											
Adamov, CZ	building	49° 17´, 16° 39´	20.8.2003				1				1
Aš, CZ	building	50° 13´, 12° 11´	31.8.2006							3	3
Brno, Technical University, CZ	building	49° 12´, 16° 35´	23.7.2003	1							1
			5.8.2003							1	1
			18.8.2003	1		26	26				53
			20.8.2004	1							1
Drienovecká jaskyňa, SK	cave	48° 37´, 20° 57´	9.8.2006		1						1
Erňa, jaskyňa, SK	cave	48° 36´, 20° 50´	25.9.2007	7	20	13	1				41
Hranická propast, CZ	chasm	49° 32´, 17° 45´	21.8.2001	2	2						4
Kateřinská jeskyně, CZ	cave	49° 22´, 16° 43´	4.9.2004							2	2
Košice, Bulharská, SK	building	48° 42´, 21° 15´	7.9.2007	1	2	9	6				18
Lazareva Pećina, SB	cave	44° 02´, 21° 58´	22.8.1994					5	4		9
Pezinok, SK	building	48° 17´, 17° 16´	1.8.2006					6	15		21
Winter records											
Bítov, hrad, CZ	cellar	48° 56´, 15° 42´	7.3.2008	15	6						21
Brno, Čápkova, CZ	building	49° 12´, 16° 35´	28.1.2003						1		1
Brno, Ibsenova, CZ	town	49° 13´, 16° 37´	23.11.2007		1					1	2
Brno, Kolejní, CZ	building	49° 13´, 16° 34´	17.3.2006	1							1
			28.1.2007	2							2
Brno, kostel sv. Tomáše, CZ	church	49° 11´, 16° 36´	3.12.2007	1	1						2
			2.1.2008	1							1
			8.1.2008		1						1
Brno, Královo Pole, CZ	building	49° 13´, 16° 35´	28.3.2001		1						1
Brno, Lidická , CZ	building	49° 12´, 16° 36´	20.2.2006							1	1
Brno, Lipová, CZ	building	49° 12´, 16° 35´	19.3.2003		1						1
Brno, Merhautova, CZ	building	49° 12´, 16° 37´	1.1.2006							1	1
Brno, Nové sady, CZ	building	49° 11´, 16° 36´	3.12.2003				1				1
Brno, Tábor, CZ	building	49° 12´, 16° 35´	1.3.2005							1	1
Brno, Tučkova, CZ	town	49° 12´, 16° 35´	26.2.2003	1	4						5
Brno, Vranovská, CZ	building	49° 12´, 16° 37´	19.2.2002						1		1
Brno, Zábrdovice, CZ	building	49° 12´, 16° 37´	12.2.2003		1						1
			26.1.2006				1				1
			7.3.2008	1						1	2
Dielik, SK	tunnel	48° 43´, 19° 57´	3.3.2008	3	2						5
Drienovecká jaskyňa, SK	cave	48° 37´, 20° 57´	1.1.2007					2			2
			28.3.2007	1						1	2
Erňa, jaskyňa, SK	cave	48° 36´, 20° 50´	25.1.2008	7	8						15
Harmanecká jaskyňa, SK	cave	48° 49´, 19° 02´	6.3.2008	2	9						11
Havířov, CZ	building	49° 46´, 18° 27´	10.3.2008							1	1
Horšovský Týn, CZ	cellar	49° 31´, 12° 56´	1.1.2007							2	2
Huda lui Papara, RO	cave	46° 23´, 23° 17´	10.3.2008							23	23
Jablonec nad Nisou, CZ	building	50° 44´, 15° 09´	14.1.2003		1						1
Jaskyňa pod sokolom, SK	cave	48° 45´, 19° 03´	6.3.2008	3							3
Liberec, CZ	building	50° 45´, 15° 02´	1.2.2006							6	6
Nový Hrádek, CZ	cellar	48° 50´, 15° 54´	7.3.2008	11 (1	14 (2)						28
Olomouc, Samotišky, CZ	building	49° 37´, 17° 19´	22.12.2007		(1)						1
Plzeň, Bolevec, CZ	building	49° 48´, 13° 23´	13.1.2008	1							1
Stariná, SK	gallery	49° 02´, 22° 15´	4.2.2008	21	4						25

Table 1.continued

Šternberk, hrad, CZ	castle	49° 44′, 17° 18′	14.3.1967	1							1
Šternberk, kostel, CZ	church	49° 44′, 17° 18′	16.2.1966		1						1
			14.3.1967	1	1						2
			11.2.1970	2	2						4
			17.2.1971	1	4						5
			7.2.1973		1						1
			5.2.1975		1						1
			3.2.1976	2							2
			6.2.1979	6	1						7
Trutnov, CZ	building	50° 33′, 15° 55′	22.2.2005							1	1
Valeč, CZ	cellar	50° 10′, 13° 14′	30.1.2008	2							2
Vernjikica, SB	cave	44° 01′, 21° 57′	20.12.1998					2	3		5
			26.11.2002					1	2		3
			26.3.2003						1		1
			1.3.2008	2	7						9
Zvíkov, CZ	cellar	48° 58′, 14° 36′	16.3.2008	6	4						10

Since the conspicuous late summer and autumn "invasions" were only confirmed in *P. pipistrellus* (see also Smit-Viergutz & Simon 2000), the presumption was raised that mass-grouping would be species-specific. While *P. pipistrellus,* which can form very large groups, up to several thousands (Nagy & Szántó 2003), occupies usually typical underground hibernacula (mainly caves), winter roosts of the soprano pipistrelle remain largely unknown. It is surprising since the latter is one of the commonest species in lowland areas in central Europe during summer (Bartonička & Řehák 2004, Petrášová 2008). It can be speculated that *P. pygmaeus* may hibernate in smaller, less insulated or obscure sites as tree-hollows, rock crevices, etc. Regular hibernation of small groups in a bat box or under wooden cladding and window sills from buildings is known in UK (S. Phillips, personal communication). Such a pattern would be in accordance with theories of different ecological requirements and competition between cryptic sympatric species (cf. Abrams 1983, Carapelli et al. 1995, Bruna et al. 1996, Trewick 1998). Although the genetic structure of the two species is similar in the study area, with very intense gene flow on long distances suggesting migrations related with mating (Bryja et al. 2009), their roosting behaviour is different. Moreover, the possibility of long distance migration can also indicate different hibernating areas for these species. Then the absence of *P. pygmaeus* may simply reflect geographical differences in their selection of hibernacula rather than actual differences in site selection. Potential hot-spots of gene flow at large hibernacula during autumn swarming seem to exist in *P. pipistrellus* only. Further research is needed to verify the presented hypothesis.

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