

# Bat Diversity in Lednitsata and Forgovskata Dupka Caves: Two Potentially Important Swarming Sites in the Western Rhodopes Mts., Bulgaria

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**Abstract:** The Western Rhodope Mountains contain great bat diversity but the number of studies from the region is still low. This article demonstrates the importance of Lednitsata and Forgovskata Dupka Caves for the forest-dwelling bat species. Moreover, *Myotis alcathoe* was established within the Bulgarian part of the mountain for the first time. Lednitsata Cave was found to be important swarming site, especially for *M. emarginatus* and *Plecotus auritus*. The bat species reported from the Forgovskata Dupka Cave highlight its importance as a day roost and also showed its potential for swarming site.

**Key words:** bats, new records, swarming, caves, Balkans

## Introduction

Extensive studies of bat fauna were performed in the Bulgarian part of the Western Rhodopes in the last few decades (BENDA et al. 2003, HORÁČEK et al. 1971, 1974, PETROV & VON HELVERSEN 2011). A total of 29 bat species have been identified within the territory of the mountains, which accounts for 88% of the bat diversity in Bulgaria. Two of those species are globally endangered (IUCN 2016). The Western Rhodopes are characterised by a high diversity of potential bat habitats such as caves, permanent and seasonal streams, mixed and coniferous forests (PETROV & VON HELVERSEN 2011). Most of the caves in the Western Rhodopes are a part of the European ecological network Natura 2000, under habitat 8310. Due to their role as swarming and hibernaculum sites for different bat species they have high conservation importance. Generally, swarming sites are close to the hibernaculum sites. The swarming sites act as “hot spots” for the gene flow between populations and within them bats exchange information (KERTH et al. 2003). The highest activity there is between August and October and is characterised with male numerical superiority. Suitable caves in the Western Rhodopes are the Lednitsata Cave, located close to

Gela Village, and potentially the Forgovskata Dupka Cave, located close to Kesten Village. The aims of the present study are to characterise bat diversity of the Lednitsata and Forgovskata Dupka Caves and explore their role as potential swarming sites.

## Materials and Methods

Lednitsata Cave (N41.64872 E24.52631) was surveyed over the swarming period on 09-10/08/2013, 24/09/2013, 15/08/2014 and 26/08/2015. Forgovskata Dupka Cave (N41.56062 E24.43753) was surveyed on 06-08/08/2013, 25/09/2013 and 16/08/2014. Bats were captured using mist nets (16 mm mesh size, length of 3 m and 6 m) placed at the cave entrance. All body measurements were taken with plastic calliper and DNA samples were taken using 3 mm punch from the wing membrane. Species identification followed the field guide of DIETZ & VON HELVERSEN (2004). All bats were released on the site of their capture after their identification. Capture and handling of the animals were carried out with permissions № 525/03.06.2013, № 575/10.04.2014 and № 645/13.08.2015 from the

Ministry of Environment and Water of Bulgaria.

Molecular identification of individuals from the whiskered bat group was provided using the MÜLLENBACH et al. (1989) salt – chloroform DNA isolation protocol and ND1 amplification using ER65 forward primer: 5' - CCTCGATGTTGGATCAGG – 3' and ER66 reverse primer: 5' - GTATGGGCCCGTAGCTT- 3' (PETIT et al. 1999). PCRs were performed in 25 µl volumes containing 10x ThermoPol Reaction Buffer, 40µmol dNTPs, 10µM primers and 5000U/ml Taq Polymerase (Biolabs).

## Results

Overall, 11 bat species were identified in the Lednitsata Cave and nine in the Forgovskata dupka Cave (Table 1) in the present study. *Myotis bechsteinii*, *M. alcaethoe* and *Vespertilio murinus* from the Lednitsata Cave, and *M. emarginatus*, *M. bechsteinii*, *M. alcaethoe*, *M. daubentonii* and *Plecotus auritus* from the Forgovskata dupka Cave are newly reported species for the respective sites. Together with the published data, now 15 bat species are known from the Lednitsata Cave (PETROV & HELVERSEN 2011), which makes more than half of the known species from the Rhodopes Mountains. During the research two previously reported species, *Eptesicus serotinus* and *Barbastella barbastellus* (BENDA et al. 2003, HORÁČEK et al. 1971, 1974, NOWOSAD et al. 1987), were not caught.

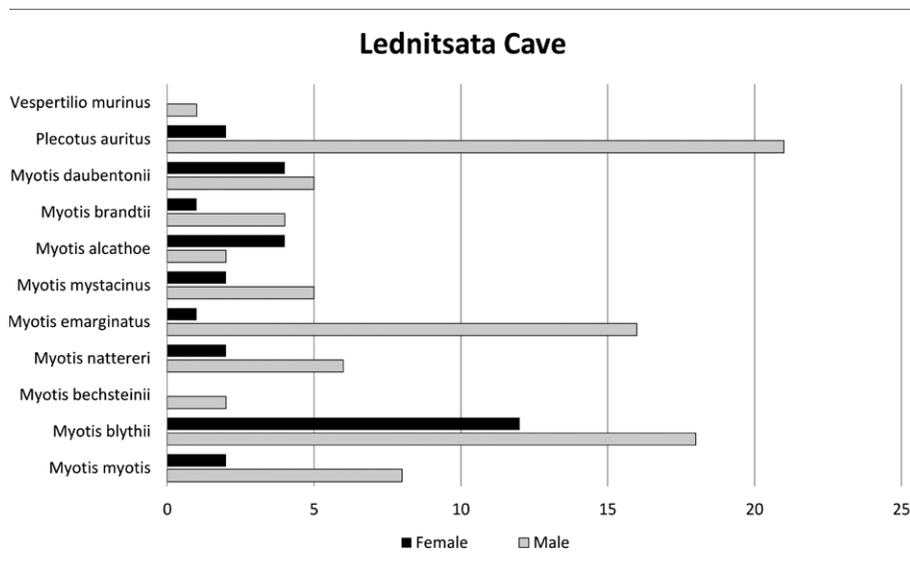
## Discussion

Molecular confirmation was possible for three species belonging to the whiskered bat group (*M. al-*

*cathoe*, *M. mystacinus* s.l. and *M. brandtii*) from the Lednitsata Cave and for one (*M. alcaethoe*) from the Forgovskata dupka Cave based on the mitochondrial ND1. Thus, the presence of *M. alcaethoe* in the Bulgarian part of the Western Rhodopes was reported for the first time in both caves. In Bulgaria this species was molecularly identified from the Vodnite dupki Cave in the Central Balkan Mountains (SCHUNGER et al. 2004), while only unclear forms sharing morphological features between *M. alcaethoe* and *M. mystacinus* were reported from the Bulgarian part of the Rhodopes Mountains (PETROV & VON HELVERSEN 2011). Bats of the whiskered bat group (*M. mystacinus* s.l.) cannot be identified unambiguously at the species level by the ND1-gene due to genetic introgression (Dundarova, unpubl.). Morphologically they can be assigned to *M. davidii*. Further genetic studies are necessary to support this preliminary field identification. The presence of *M. brandtii* is confirmed based on morphological and ND1 identification, as well.

*Myotis blythii*, *M. emarginatus* and *P. auritus* were the predominant species in the Lednitsata Cave (Fig. 1). Lower numbers of *M. myotis*, *M. nattereri*, *M. mystacinus*, *M. alcaethoe*, *M. brandtii*, *M. daubentonii* and single individuals of *M. bechsteinii* and *Vespertilio murinus* were also caught.

In the Forgovskata dupka Cave no obvious predominant species were established, only low numbers of *Rhinolophus ferrumequinum*, *R. hipposideros*, *M. myotis*, *M. blythii*, *M. bechsteinii*, *M. emarginatus*, *M. alcaethoe*, *M. daubentonii* and *P. auritus* were captured (Fig. 2). Prevalence of male individuals was established in both caves (Figs. 1 and 2). At least two species (*M. emarginatus* and *P. auri-*



**Fig.1.** Composition of bat species in Lednitsata Cave.

*tus*) displayed swarming behaviour in the Lednitsata Cave. The third predominant species was *M. blythii*. Field identification of *M. blythii* and *M. myotis* was provided based on lengths of forearm (FA) and the shape of face and ears. Both species occurred in sympatry and displayed different mating strategies. *Myotis myotis* uses the caves as a swarming site. However, molecular identification of both species is recommended because of the unclear morphometric pattern of some individuals; the existence of hybrids is not excluded (AFONSO et al. 2017).

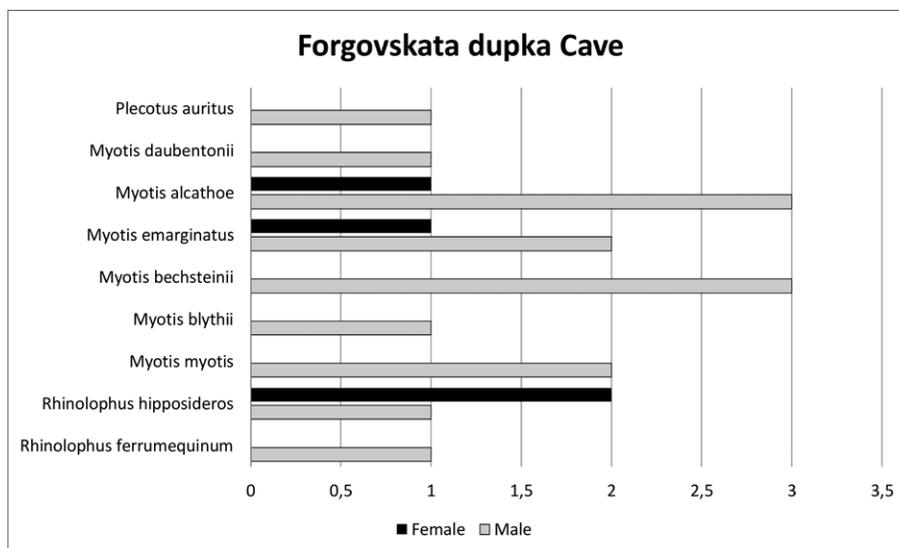
*Myotis nattereri* and *M. daubentonii* form big swarm colonies in North-western Europe (PARSONS et al. 2003a) while in the Lednitsata Cave both species were present in low numbers. A possible reason

could be altitude preference since the cave is located at 1613 m a.s.l. A similar result from Bulgaria is reported by SCHUNGER et al. (2004) from the Vodnite dupki Cave (1400 m a.s.l.). For comparison, in the Netherlands such sites do not exceed 105 m in altitude (VAN SCHAIK et al. 2015). However, the maximum abundance of both species is around September – October (PARSONS et al. 2003a, VAN SCHAIK et al. 2015) while the study period in Bulgaria was at the beginning of August and the end of September 2013, middle of August 2014 and the end of August 2015.

Two previously reported species, *Eptesicus serotinus* and *Barbastella barbastellus* (BENDA et al 2003, HORÁČEK et al. 1971, 1974, NOWOSAD et al. 1987), were not captured. A possible explanation for

**Table 1.** List of bat species caught in both cave during the research period 2013-2015.

STUDIED CAVES Research period Species	LEDNITSATA CAVE					FORGOVSKATA DUPKA CAVE				
	09.08.2013	10.08.2013	24.09.2013	15.08.2014	26.08.2015	06.08.2013	07.08.2013	08.08.2013	25.09.2013	16.08.2014
<i>Rhinolophus ferrumequinum</i> (Schreber, 1774)								+		
<i>Rhinolophus hipposideros</i> (Borkhausen, 1797)									+	
<i>Myotis myotis</i> (Borkhausen, 1797)	+	+	+		+	+	+			
<i>Myotis blythii</i> (Tomes, 1857)		+			+		+			
<i>Myotis bechsteinii</i> (Kuhl, 1817)			+	+		+			+	
<i>Myotis nattereri</i> (Kuhl, 1817)					+					
<i>Myotis emarginatus</i> (Geoffroy, 1806)	+			+	+	+				
<i>Myotis cf. mystacinus</i>	+	+			+					
<i>Myotis alcathoe</i> von Helversen et Heller, 2001	+			+	+		+	+		+
<i>Myotis brandtii</i> (Eversmann, 1845)	+	+								
<i>Myotis daubentonii</i> (Kuhl, 1817)	+	+	+		+			+		
<i>Plecotus auritus</i> (Linnaeus, 1758)	+	+	+	+	+				+	
<i>Vespertilio murinus</i> Linnaeus, 1758					+					



**Fig. 2.** Composition of bat species in Forgovskata dupka Cave.

the absence might be seasonal preferences not coinciding with the study period.

In Bulgaria, *P. auritus* is a widespread species in mountain areas and mostly swarms at caves located at high altitudes. The highest known cave is in the Pirin Mountains, located at 2347 m a.s.l. (Dundarova & Petrov pers. obs.). According to the literature (PARSONS et al. 2003b, VEITH et al. 2004, RIVERS et al. 2006), swarming sites are localised close to the hibernaculum sites or the same sites are used for both swarming and hibernation. The prevalence of *P. auritus* in the Lednitsata Cave and the swarming behaviour of all individuals might be explained by the proximity to the possible hibernaculum, or the cave is used for both according to the skeletons found by HORÁČEK et al. (1971, 1974) in the cave deposits.

## Conclusions

Swarming caves have an important role for the gene flow between bat populations from one species, which plays a key role for their conservation. The Lednitsata Cave is an important swarming site in the Western Rhodope Mountains. The establishment of forest-dwelling species like *M. bechsteinii*, *M. daubentonii*,

*M. alcaethoe* and *P. auritus* and cave-dwelling species *Rhinolophus ferrumequinum*, and *Rh. hipposideros* in the Forgovskata dupka Cave shows that the cave is used for day roost and potential swarming site. Still, many ambiguities regarding swarming-hibernaculum behaviour of forest-dwelling species, such as the cryptic whiskered bats *M. mystacinus* s.l., *M. alcaethoe*, *M. brandtii* and *Plecotus* spp., remain open on the Balkan Peninsula. Therefore, the strategic location of both caves in the Western Rhodope Mountains could reveal further details for the behavioural aspects and distribution of the aforementioned species. Future studies regarding forest-dwelling bats in the Western Rhodopes, respectively seeking for more prospective sites in Bulgaria, would benefit from additional studies on the life of the forest-dwelling bat species on the Balkan Peninsula.

**Acknowledgments:** I would like to thank Chrisitan Dietz (Biologische Gutachten Dietz), Frieder Mayer (Naturkundemuseum Berlin), Nikolay Simov (NMNHS), Ivan Pandourski (IBER – BAS) and Roumiana Metcheva (IBER – BAS) for the remarks and useful comments on the early version of the manuscript, also to Elena Stoeva (Green Balkans), Krustio Dimitrov (University of Sofia “St. Kliment Ohridski”) and Jan Zukal (IVB AV ČR) for the help during the field work in 2013, 2014 and 2015. The English of the final draft was kindly corrected by Simeon Lukanov (IBER – BAS).

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Received: 14.08.2017

Accepted: 23.01.2018