Journal of
Bat Research & Conservation

OPEN ACCESS
www.secemu.org
DOI: 10.14709/BarbJ.14.1.2021.16

ORIGINAL ARTICLE

New bat records in south-eastern Bosnia and Herzegovina with additional confirmation of *Myotis alcathoe* and *Myotis brandtii*

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DOI: https://doi.org/10.14709/BarbJ.14.1.2021.16

Keywords: Balkans, bat inventory, Čajniče, Cicelj Nature Reserve, Chiroptera, *Myotis mystacinus* morphogroup

received: February, 1st 2021
accepted: December, 19th 2021

ABSTRACT

The Balkans represent one of the regions with the highest bat diversity in Europe. Thus far, 31 bat species have been recorded in Bosnia and Herzegovina (BiH). The bat fauna of this Balkan country is one of the least studied in Europe. In order to reduce this knowledge gap, bat research was carried out during a ten-day inventory in the vicinity of Čajniče in south-eastern BiH (Republic of Srpska). Mist net surveys resulted in the capture of 15 bat species: *Rhinolophus ferrumequinum*, *R. hipposideros*, *Myotis brandtii*, *M. mystacinus sensu lato*, *M. alcathoe*, *M. emarginatus*, *M. nattereri*, *M. bechsteinii*, *M. blythii*, *Nyctalus leisleri*, *Eptesicus serotinus*, *Vespertilio murinus*, *Pipistrellus pipistrellus*, *P. pygmaeus*, and *Plecotus auritus*. Acoustic surveys resulted in recording one additional species: *Barbastella barbastellus*. Roost surveys yielded in the finding of three maternity colonies of *R. hipposideros*. The genetic analysis resulted in the first molecularly confirmed *M. brandtii* and *M. alcathoe* records for BiH. Capture of several post-lactating females, sexually active males and multiple juveniles is proof for reproducing populations of these species in the country. During this brief study, we found more than half of all known bat species in BiH in an area of circa 75 km^2.

INTRODUCTION

The Balkans is considered to be one of the biodiversity hotspots in Europe (Gaston & David 1994). It is a region in south-eastern Europe that comprises several countries and is situated roughly between the Black and Mediterranean Seas. The exceptional species richness of this region is also expressed in mammalian fauna (Temple & Terry 2007), including bats. Most European bat species are present in this region (Dietz & Kiefer 2016). Compared to central and northern European regions, the mammalian fauna of the Balkans is less extensively studied (Kryštufek 2004). This particularly applies to bat fauna, as countries such as Albania (Théou & Bego 2018), North Macedonia (Micevski et al. 2014), Montenegro (Presetnik et al. 2014), and Bosnia and Herzegovina further referred to as BiH (Karapandža et al. 2014) are relatively poorly studied. Even though interest in bat research in the Balkans has increased recently (e.g., see the journal Hypsugo), more published data is necessary to improve bat conservation in this region (Zamolo et al. 2018).

Currently, there are 31 bat species recorded in BiH, with *Myotis alcathoe* being the latest addition to this list (Babić et al. 2018). There are extensive knowledge gaps about bat fauna in the country. Hence, we studied the bat fauna in the area of Čajniče in south-eastern BiH. The study site partially overlapped Cicelj Nature Reserve, in which, as far as known, no bat research was conducted previously. The survey was part of a mammal inventory in general (Miteva et al. 2020), organised by the Field Study Group of the Dutch Mammal Society. Our goal was to describe the bat species richness and, if possible, confirm cryptic species using genetic research. The results of this study contribute to the general knowledge about the distribution of several bat species and their roost sites.

MATERIAL AND METHODS

Study site

Data on bats were gathered between 30 July and 9 August 2019 at 21 sites in the area of Čajniče (43.557185°N, 19.071691°E, 825 m) in south-eastern BiH (Fig. 1). Čajniče is a municipality that is situated in the Dinaric Alps. The area's mountainous landscape is diverse and interspersed with extensively exploited deciduous and pine forests, pastures, and orchards. Mountain sides and peaks mostly consist of mixed spruce forests, while beech (*Fagus sylvatica*) becomes more dominant in lower areas. Several streams flow through the area. The study area partially included Cicelj Nature Reserve (Brujić et al. 2011). This protected area of

approximately 250 ha consists of forests with mixed spruce and European silver fir (*Abies albis*), extensively grazed meadows, and steep rocky slopes. Despite the known plant diversity (Brujić et al. 2011), hardly any data is available about the fauna of the reserve (Miteva et al. 2020).

Field methods

A combination of techniques, including mist net surveys, acoustic surveys, and roost surveys, was deployed following Flaquer et al. (2007). Each method is subsequently described, including sample sites. Bats were caught and sampled under the license 07/1/625-479/19 issued by the Republic Institute for the Protection of Cultural, Historical and Natural Heritage. All data on bat observations were deposited to www.observado.org (Observado 2020).

Mist net surveys were conducted at eleven sites in the area of Čajniče (Fig. 1). The altitude of the surveyed sites ranged from 530-1180 m a.s.l. All surveys were conducted between sunset, and 01:30 am. Depending on the conditions at each site, 1-6 mist nets were set up in suitable habitats, such as forest lanes (n = 3), over ponds and streams (n = 7), and in front of a cave entrance (n = 1). Mist nets varied in length from 3-18 m, all with a mesh size of 16 mm. Captured bats were weighed using a Pesola 30-60 (±0.25-0.50) g spring balance, and external diagnostic measurements were determined using Mitutoyo 0-150 (±0.02) mm steel callipers, in accordance with Haarsma (2008). Species identification was carried out as fast as possible using an identification key of Dietz & Kiefer (2016). To support species identification, most bats were photographed. Subsequently, bats were released at the site of capture.

Measurements included body mass and forearm length. The age of bats was classified as either adult or juvenile (young of the year) depending on the closure of the cartilaginous epiphysis in finger joints (Brunet-Rossinni & Wilkinson 2009). The colouration of the chin-spot was used as an indicative characteristic for the age classification of species in the *Myotis* genus (Richardson 1994). The reproductive status of females was classified by nipple condition, baldness, colouration, and size (Haarsma 2008). Male bats were classified as sexually active based on the size and shape of the testes and the colouration of the epididymis (Haarsma 2008).

Acoustic surveys were carried out using several types of bat detectors. Sound recordings were made with Pettersson D240x bat detectors and Elekon M batloggers. Most acoustic surveys were combined with mist netting surveys (Fig. 1). Analysis of recordings was carried out using BatExplorer (version 2.1) and BatSound® (version 4.4), if possible, up to species level. Recordings were manually analysed by the first three authors by comparing spectrograms and measured pulse characteristics with Barataud (2015). Recordings on which no consensus was reached were discarded from further analysis or assigned to the level of genus (e.g., *Myotis* spp.) or species pairs (e.g., *P. nathusii/kuhlii*).

Finally, during daytime, ten potential bat roosts were inspected (Fig. 1). Suitable sites such as attics, barns, caves, and cottages were surveyed using flashlights.

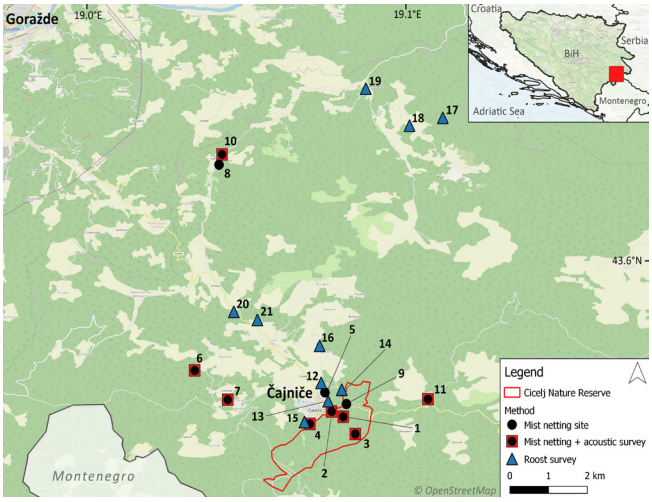

Fig. 1 - Sites of mist net, acoustic and roost surveys in the area of Čajniče in south-eastern Bosnia and Herzegovina. The red line denotes boundaries of Cicelj Nature Reserve. Site numbers correspond with Table 1 and Supplementary Material.

Tissue sample collection and analysis

Tissue samples were taken from one preliminary identified *M. brandtii* female and one *M. alcathoe* female. Samples were collected from the uropatagium using a sterilised 3 mm Miltex® biopsy punch. This is a standard method for collecting samples since the uropatagium regenerates quickly (Faure et al. 2009). Tissue samples were preserved in an Eppendorf-tube filled with 70% ethanol and stored in a fridge at 7°C.

Datura Molecular Solutions BV performed the analysis of both samples by qPCR-sequencing using a COI primer set pair SFF_145f/SFF_351r (Walker et al. 2016). The degree of similarity between the obtained sequences and sequences of *M. brandtii* and *M. alcathoe* available from GenBank was determined by performing a BLASTN analysis (NCBI 2020). Sequence alignment was performed using Clustal Omega (EMBL 2020). Phylogenetic analysis was performed to display genetic similarity within the amplified COI fragments of samples of *M. brandtii* (Boston et al. 2010, Kruskop et al. 2012) and *M. alcathoe* (Galimberti et al. 2012, De Pasquale & Galimberti 2014) obtained from GenBank. Three reference samples of *M. mystacinus* (Kruskop et al. 2012, Hussain 2018, Isaksen 2019) were included to compare the phylogeny. The phylogenetic analysis was performed with the software package MEGA 7.0.14 (Tamura et al. 2011), using the maximum likelihood method with partial deletion (site coverage cut-off 95%) and 1000 bootstrap replications. The obtained sequences of *M. alcathoe* and *M. brandtii* were deposited in GenBank under accession numbers MT946701 and MT946702, respectively.

RESULTS AND DISCUSSION

Accumulative species diversity

In total, 16 bat species and one species pair were recorded. Eight bat species and one species pair were confirmed for Cicelj Nature Reserve.

The following 15 species were captured: *Rhinolophus ferrumequinum* (1), *R. hipposideros* (2), *Myotis brandtii* (4), *M. mystacinus s.l.* (4), *M. alcathoe* (6), *M. emarginatus* (2), *M. nattereri* (1), *M. bechsteinii* (3), *M. blythii* (1), *Nyctalus leisleri* (2), *Eptesicus serotinus* (1), *Vespertilio murinus* (2), *Pipistrellus pipistrellus* (8), *P. pygmaeus* (6), and *Plecotus auritus* (1) (Table 1). Morphometric data of captured bats are included in Table 1 in Supplementary Material.

Barbastella barbastellus was solely recorded acoustically. Furthermore, acoustic surveys resulted in recordings of *P. nathusii/kuhlii* species pair (Table 1).

Roost surveys resulted in finding one occupied site by *R. ferrumequinum* and nine roost sites of *R. hipposideros* (Table 2 in Supplementary Material).

For the observed species, according to the IUCN Red List (Temple & Terry 2007), *M. alcathoe* is categorised as "Data Deficient", ten species as "Least Concern", three as "Near Threatened" (*R. ferrumequinum, R. hipposideros*, and *M. blythii*), and two as "Vulnerable" (*M. bechsteinii* and *B. barbastellus*).

More detailed information per species or species pair is described below in taxonomical order (Dietz & Kiefer 2016). Subsequently, the results are discussed.

Species accounts

***Rhinolophus ferrumequinum* (Schreber, 1774)**

One adult *R. ferrumequinum* male was caught while emerging from a small cave south-west of Čajniče (Table 1). The capture site was situated at an altitude of 1000 m. A visual inspection of the cave previous to the catching rendered no observations of this species. Nevertheless, the flight direction during the capture indicated that this cave was a roost.

In BiH, various sites of *R. ferrumequinum* are known (e.g., Pašić et al. 2013) and the species is considered rather common.

***Rhinolophus hipposideros* (Bechstein, 1800)**

Two not sexually active (1F - 1M) adult *R. hipposideros* were caught at the entrance of the same cave where *R. ferrumequinum* was caught (Table 1). Visual inspection of the cave previous to the moment of capture rendered no indication for the presence of this species. During acoustic surveys, *R. hipposideros* was recorded at one additional site, a deciduous forest habitat at an altitude of 1080 m. Roost surveys resulted in finding nine roosts of this species (Table 2 in Supplementary Material). Three roosts were occupied by maternity colonies and in five roosts one or two individuals were present. The first maternity colony counted 40 individuals (adults and juveniles together) and was present in a small cave at an altitude of 980 m. The second colony counted 30 animals and was found in an abandoned house at an altitude of 710 m. The third maternity colony consisted of 40 individuals and was found in a concrete cottage at an altitude of 490 m.

Dietz et al. (2009) described *R. hipposideros* as most likely having big and stable populations in the Balkans. However, this species is currently threatened here by habitat loss, habitat fragmentation, and pesticide use (Dietz & Kiefer 2016). In BiH, multiple maternity colonies are known (e.g., Matović & Mulaomerović 2019), but no colonies were previously found in caves. The observations in the area of Čajniče indicate that *R. hipposideros* is a common species here.

***Myotis brandtii* (Eversmann, 1845)**

In total, four individuals (1F - 3M) of *M. brandtii* (Fig. 2a) were caught at two sites. The capture of both (adult) sexes, sexually active males and a young of the year, strongly indicates that *M. brandtii* has a reproducing population in the study area. The capture sites were at 750 and 830 m and concerned the same riparian habitats where most *M. alcathoe* individuals were also caught (Table 1). *Myotis alcathoe* and *M. brandtii* often co-occur (Dietz & Kiefer 2016).

Myotis brandtii has been described as resident in BiH since 2007, when one individual was found roosting in a cave approximately 100 km north-west of Čajniče (Mulaomerović 2013). This species' second and third records also originate from approximately 100 km north-west of Čajniče (Presetnik et al. 2017). Due to the lack of additional data, the distribution of *M. brandtii* in parts of south-eastern Europe, including BiH, is unclear (Dietz et al. 2009). However, Dietz & Kiefer (2016) describe its fragmented occurrence in the surroundings of the study site. It is assumed that the distribution of *M. brandtii* in the Balkans is limited to mountainous areas (Benda 2004, Dietz & Kiefer 2016).

qPCR yielded a 202 base pair product, and BLASTN analysis resulted in 100% (E value of 1^{-99}) similarity with known *M. brandtii* COI sequences from Italy (Galimberti et al. 2012), western Russia (Kruskop et al. 2012) and Germany (Boston et al. 2010). The phylogenetic analysis displays minor differences in COI sequence between the obtained sequence and the included *M. brandtii* reference sequence (Fig. 3). This is the first molecular confirmation of *M. brandtii* in BiH.

***Myotis mystacinus sensu lato* (Kuhl, 1817)**

Recently, the historical replacement of *M. mystacinus* by *M. davidii* populations in the Balkans was suggested by Çoraman et al. (2020). Differentiation between both species based on morphological characteristics is not possible, not even with the use of mitochondrial DNA markers (Çoraman et al. 2020). Therefore, we retained the name *M. mystacinus s.l.* for the presented findings. *Myotis mystacinus s.l.* was caught at three sites, with a total of four individuals (2F - 2M). The sites of capture were riparian habitats and over a small pond. The altitude of these sites ranged from 830-950 m. Three juveniles, simultaneously captured shortly after sunset at Kapov Han (Fig. 1, site 8), indicate a maternity colony of *M. mystacinus s.l.* in the vicinity.

In BiH, *M. mystacinus s.l.* is spread widely throughout the country (e.g., Presetnik et al. 2017, 2019), but currently, only one maternity colony is known (Dervović 2015). At

the capture sites in riparian habitat, *M. mystacinus s.l.* was caught together with *M. brandtii* and *M. alcathoe* (Fig. 4). The co-occurrence of these three species in the vicinity of river sites surrounded by thermophilous woodlands has also been documented in former Yugoslavian countries, namely Montenegro (Benda 2004) and Slovenia (Presetnik & Zamolo 2018).

Myotis alcathoe von Helversen & Heller, 2001

In total, six individuals (3F - 3M) of *M. alcathoe* (Fig. 2b) were caught at four different sites (Table 1). The altitude of the capture sites ranged between 530-830 m. Most individuals were caught in a riparian habitat which is considered preferable foraging habitat of this species (Niermann et al. 2007). The capture of adult males, post-lactating females, and a young of the year strongly indicates reproduction and the presence of a population of this species in BiH.

Previously, *M. alcathoe* was observed only once in BiH, when in 2018, one male was caught approximately 60 km north-west of Čajniče (Babić et al. 2018). Additionally, one *M. alcathoe* record is known from Montenegro, about 40 km south-west of the study site (Benda et al. 2012).

qPCR yielded a 202 base pair product, and BLASTN analysis resulted in 98% similarity with an E value of 2^{-92} with known sequences of the COI gene of *M. alcathoe* originating from Italy (De Pasquale & Galimberti 2014), Portugal (Rebelo et al. 2020), and Switzerland (Ruedi et al. 2021). This is the first molecular confirmation of *M. alcathoe* for BiH. Phylogenetic analysis displays three distinct clades, each corresponding to a species of the *M. mystacinus* morphogroup (Fig. 3). The analysis also indicates minor genetic differences between known *M. alcathoe* sequences and the obtained sequence, which is likely caused by a relatively small part of the COI gene that was targeted by the used primers. Because *M. alcathoe* was caught at four sites, this species seems to be relatively common in the area of Čajniče.

Myotis emarginatus (Geoffroy, 1806)

Two *M. emarginatus* (1F - 1 unknown sex) were caught at two different sites (Table 1). The altitude of the capture sites ranged between 540-920 m. Both individuals were caught in riparian habitats alongside streams. One caught female was post-lactating. *Myotis emarginatus* is present throughout the whole Balkans (Dietz & Kiefer 2016) and is common in BiH (Rnjak et al. 2017).

Myotis nattereri (Kuhl, 1817)

One adult male *M. nattereri* was caught at Nurina pećina, a small cave south-west of Čajniče (Fig. 1, site 4). The cave was located at an altitude of 1000 m. The individual was caught entering the cave when light rain occurred, indicating that the cave is used at least temporarily as a roost.

From BiH, few records are known of this species, and the records that exist are spread widely throughout the country (e.g., Hodžić et al. 2017, Presetnik et al. 2019).

Fig. 2 - During this study four individuals of *Myotis brandtii* (a) and six individuals of *Myotis alcathoe* (b) were caught. Photos: (a) Jan Buys and (b) Joris Verhees.

Myotis bechsteinii (Kuhl, 1817)

Three individuals (2F - 1M) of *M. bechsteinii* were caught, each at a different site (Table 1). The altitude of the sites of capture ranged between 950-1080 m. One individual was caught entering a cave, one in a beech forest lane and one over a small pond in a semi-open landscape.

Myotis bechsteinii is one of the rarest European bat species and an indicator species for high-quality old-growth broad-leaved forest such as oak (*Quercus* spp.) forests and temperate beech forest zones (Dietz & Kiefer 2016). Even though densities in the Balkans are locally higher than in the rest of Europe (Dietz & Kiefer 2016), there are only four previously known findings of *M. bechsteinii* in BiH (Hodžić et al. 2017). However, this is most likely a consequence of the lack of research effort.

At Viline bukve (Fig. 1, site 6), the forest habitat partly consists of ancient beech trees (Mićić et al. 2018) and other mature deciduous trees, including oak and maple (*Acer* spp.). Here, one post-lactating *M. bechsteinii* was caught. The reproductive status of this female indicates the presence of a maternity colony in the vicinity. This is also indicative of these forests' suitability for *M. bechsteinii* and should be recognised as highly valuable habitat for this species.

Myotis blythii (Tomes, 1857)

One sexually active adult male *M. blythii* was caught in an orchard at an altitude of 750 m (Table 1). *Myotis blythii* occurs throughout BiH (Dietz & Kiefer 2016). Here, the presence of large maternity colonies are known (e.g., Husanović & Mulaomerović 2018).

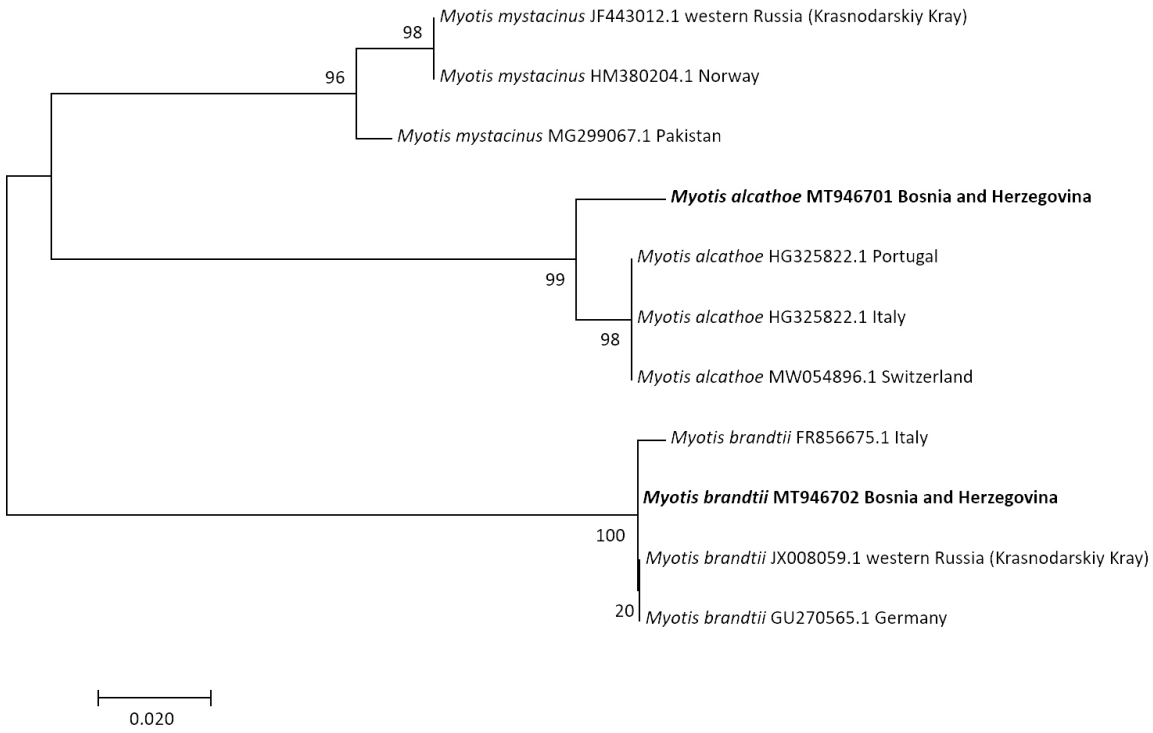

Fig. 3 - Phylogenetic analysis of the obtained sequences (depicted in bold) partial COI gene in relation to known *Myotis brandtii*, *Myotis mystacinus* and *Myotis alcathoe* sequences. For each species, a distinct clade is distinguished. The codes depict GenBank accession numbers and the country of origin is stated at the end of each sequence. The bar represents a sequence divergence of 2%. The evolutionary distances were computed using the maximum likelihood method with pairwise deletion and 1000 bootstrap replications.

Fig. 4 - Riparian habitat of the Janjina stream (Fig. 1, site 9) surrounded by thermophilous woodland where *Myotis brandtii*, *Myotis mystacinus sensu lato*, and *Myotis alcathoe* were captured together. Photo: Jan Buys.

Nyctalus leisleri (Kuhl, 1817)

Two adults, sexually not active, *N. leisleri* males were caught at a single site (Table 1). The capture site is a riparian habitat alongside the Janjina stream in Kapov Han (Fig. 1, site 10) at an altitude of 540 m.

In the Balkans, little data is available about *N. leisleri* and knowledge of breeding populations is lacking (Mirić & Paunović 1997). From BiH, multiple records of this species are described, the closest from approximately 10 km west of Čajniče (e.g., Mulaomerović et al. 2020).

Eptesicus serotinus (Schreber, 1774)

One adult nulliparous *E. serotinus* was caught over a pond and acoustic recordings were made at two sites in riparian habitat (Table 1). The altitude of the capture and the recording sites ranged between 570-920 m.

Vespertilio murinus Linnaeus, 1758

Two adults *V. murinus* males, were caught at two sites in riparian habitats (Table 1). Both males were sexually active. The altitude of the sites of capture were at 540 and 830 m.

Because of seasonal occurrence due to migrating individuals and sex-related segregation, the distribution of *V. murinus* in south-eastern Europe is complicated (Dietz & Kiefer 2016). In the Balkans most observations are known from mountainous areas, where this species is present in low densities (Dietz et al. 2009). Few records originate from BiH, scattered throughout the country (Tiganj & Mulaomerović 2017).

Pipistrellus pipistrellus (Schreber, 1774)

Eight individuals (7F - 1M) of *P. pipistrellus* were caught at three sites. During acoustic surveys, this species was recorded at four additional sites (Table 1), and several single observations with bat detectors were done in the town of Čajniče. This species was observed in riparian habitats, forest lanes, over a pond, and in urban areas. The altitude of the capture and recording sites ranged between 540-1160 m. Captured females were predominantly post-lactating, and several individuals were classified as young of the year. Additionally, the moment of capture shortly after sunset and

the frequent observations in the town indicate the presence of a maternity colony in Čajniče.

Pipistrellus pygmaeus (Leach, 1825)

In total, six individuals (4F - 2M) *P. pygmaeus* were caught at three sites. During acoustic surveys, this species was recorded at two additional sites. Capture and recording sites consisted of riparian habitats at an altitude of 540-920 m (Table 1). All captured females were post-lactating, and both males were sexually active. Because of the reproductive status of the caught individuals, presence of a maternity colony of this species in the study area is likely.

From south-eastern BiH few *P. pygmaeus* records are known, but it is likely that the fragmented distribution of this species is a result of the low intensity of bat surveys in this region (Karapandža et al. 2014).

Pipistrellus nathusii (Keyserling & Blasius, 1839) / *Pipistrellus kuhlii* (Kuhl, 1817)

Pipistrellus nathusii/kuhlii species pair was recorded at three sites (Table 1), and several observations with bat detectors were done in the town of Čajniče. Recordings did not contain social calls, which might have led to certain species identification. Distinguishing both species solely based on echolocation is not possible (Barataud 2015). Apart from the town of Čajniče, all recording sites concerned riparian habitats alongside streams, at an altitude ranging from 540-920 m. *Pipistrellus nathusii* and *P. kuhlii* are both known to be present in BiH (Karapandža et al. 2014). However, breeding areas of *P. nathusii* are particularly situated in north-eastern Europe (Dietz & Kiefer 2016). In contrast, *P. kuhlii* is widely distributed throughout the Mediterranean Region and adjacent areas (Dietz et al. 2009). Given the recording dates and the (seasonal) European distribution, it is most likely that the recordings are of *P. kuhlii*.

Barbastella barbastellus (Schreber, 1774)

This species was recorded acoustically at two sites in deciduous and pine forests and three times in riparian habitat (Table 1). The altitude of these sites ranged between 540-1080 m. The fact that we recorded *B. barbastellus* in five out of eleven sites suggests that this species is present commonly around Čajniče. In the surrounding forests, presence of a population is likely.

Barbastella barbastellus inhabits a wide variety of forest habitats where it primarily roosts under loose bark (Dietz & Kiefer 2016). It is assumed that *B. barbastellus* is not rare in BiH because of the many suitable forest habitats (Pašić & Napotnik 2014). This also applies to the direct area of Čajniče, where land is covered up to 80% with forests (Glück et al. 2011).

Table 1 - Documented bat species and species pairs in the area of Čajniče in south-eastern Bosnia and Herzegovina in 2019. Site numbers correspond with Fig. 1. Species recorded acoustically are displayed as (r). *: site in Cicelj Nature Reserve.

Site number	Survey date	Survey site and local name	Species	Number of species
1*	30 July 2019	Janjina stream (Prašumski rezervat Borica)	*P. pipistrellus* (r), *P. nathusii/kuhlii* (r), *B. barbastellus* (r)	3
2*	31 July 2019	Janjina stream and over pool (Trim staza u Cicelju)	*M. emarginatus* (1)	1
3*	1 August 2019	Coniferous forest lane (Kladni do)	*P. pipistrellus* (r), *B. barbastellus* (r)	2
4	2 August 2019	Small cave entrance (Nurina pećina)	*R. ferrumequinum* (1), *R. hipposideros* (2), *M. nattereri* (1), *M. bechsteinii* (1)	4
5	3 August 2019	Janjina stream, orchard and forest edge (Podkamen)	*M. brandtii* (3), *M. alcathoe* (3), *M. blythii* (1), *P. pipistrellus* (2), *P. pygmaeus* (1)	5
6	4 August 2019	Beech forest (Viline bukve)	*R. hipposideros* (r), *M. bechsteinii* (1), *N. leisleri* (r), *P. pygmaeus* (r), *B. barbastellus* (r)	5
7	5 August 2019	Over small pool at Baha (Studenac)	*M. mystacinus s.l.* (1), *M. bechsteinii* (1), *N. leisleri* (r), *E. serotinus* (1), *P. pipistrellus* (r), *B. barbastellus* (r), *P. auritus* (1)	7
8	5 August 2019	Next to Janjina stream (Kapov Han)	*M. alcathoe* (1)	1
9*	6 August 2019	Over and alongside Janjina stream (Pomol)	*M. brandtii* (1), *M. mystacinus s.l.* (3), *M. alcathoe* (1), *V. murinus* (1), *P. pipistrellus* (5), *P. pygmaeus* (4)	6
10	7 August 2019	Over and alongside Janjina stream (Kapov Han)	*M. emarginatus* (1), *M. alcathoe* (1), *N. leisleri* (2), *E. serotinus* (r), *V. murinus* (1), *P. pipistrellus* (r), *P. pygmaeus* (r), *P. nathusii/kuhlii* (r), *B. barbastellus* (r)	9
11	8 August 2019	Over and alongside Altima and Janjina stream (Brezovice)	*N. leisleri* (r), *E. serotinus* (r), *P. pipistrellus* (1), *P. pygmaeus* (1), *P. nathusii/kuhlii* (r)	5

***Plecotus auritus* (Linnaeus, 1758)**

One adult male *P. auritus* was captured over a small pond. The capture site was located at 950 m and surrounded by mixed forest habitat.

From BiH, few records of *P. auritus* are known, scattered throughout the country (e.g., Karapandža 2014, Presetnik et al. 2016).

CONCLUSIONS

To our knowledge, previous to our ten-day inventory, there were no published bat records in the area of Čajniče. In this area, we documented 16 bat species and one additional bat species pair that could not be identified up to species level by its echolocation calls. This is approximately 55% of all known bat species (31) in BiH. Because of the relatively small study area and short periodical duration of the research, it can be concluded that the area of Čajniče hosts a diverse bat fauna.

Our study also comprises the first bat research in the protected Cicelj Nature Reserve. In this area, we documented the bat species *M. brandtii*, *M. mystacinus s.l.*, *M. alcathoe*, *M. emarginatus*, *V. murinus*, *P. pipistrellus*, *P. pygmaeus*, *B. barbastellus*, and additionally *P. nathusii/kuhlii* that could not be distinguished based on recorded echolocation calls. Just outside the boundaries of Cicelj Nature Reserve we observed *R. ferrumequinum*, *R. hipposideros*, *M. nattereri*, and *M. bechsteinii*.

The observed species *M. alcathoe*, *M. bechsteinii*, and *B. barbastellus* are considered tree roosting species and depend on varied well-structured forests, dead and decaying trees, an abundance of tree cavities, and both densely structured and open areas. It can be presumed that forests in the area of Čajniče offer these conditions and are of high importance for these bat species. Therefore, specific conservation measures should target forest structure and composition and preserve (old) trees that provide roost possibilities such as cavities and loose bark.

The presence of *M. brandtii* and *M. alcathoe* at multiple sites in the area of Čajniče is an important result of this study, wherein we present the first molecularly confirmed records of both species for the country. Based on our results, it can be assumed that reproducing populations of *M. brandtii* and *M. alcathoe* are present in BiH. Also, the relatively numerous observations of roosts of *R. hipposideros* indicate that this species is still widespread in the area.

This study contributes to the knowledge of bat fauna and its spatial distribution in the area of Čajniče in south-eastern BiH. Currently, this country remains one of the most poorly investigated in Europe regarding bat fauna. Further research is needed to acquire better knowledge of species distributions and long-term population trends. Herewith, bat conservation strategies can be prepared for BiH specifically.

ACKNOWLEDGEMENTS

We would like to thank the many local people who supported this study by helping to search for bat roosts. In particular, we would like to thank Njegoš Tupeša and Branko Ćopić (Ekocentre Čajniče) for their hospitality, sharing their excellent terrain knowledge, and their support during the fieldwork. Dejan Radošević helped with local organisation and logistics. We thank the Republic Institute for the Protection of Cultural, Historical and Natural Heritage for issuing the research permit (07/1/625-479/19).

We are grateful to Ivana Budinski and three anonymous reviewers for their valuable comments and constructive feedback that significantly improved earlier drafts of the manuscript. Our appreciation goes out to Tamara Richardson for the English proofreading.

This study is a result of the pleasant collaboration between international camp participants, who are all thanked for their help during the fieldwork. Finally, we are grateful to the Field Study Group for financial support to perform the genetic analysis.

REFERENCES

BABIĆ, N., NICEVIĆ, M. & ŠAFHAUZER, M. (2018). Prvi nalaz patuljastog brkatog šišmiša (*Myotis alcathoe*) u Bosni i Hercegovini i preliminarni spisak faune šišmiša na Zaštićenom pejzažu Bentbaša (Bosna i Hercegovina). *Hypsugo*, 3(2): 4-12.

BARATAUD, M. (2015). Acoustic ecology of European bats. Species identification, study of their habitats and foraging behaviour. ed.: Biotope - Muséum national d'Histoire naturelle. Paris, France, 340 pp.

BENDA, P. (2004). First record of *Myotis aurascens* and second record of *Myotis brandtii* in Montenegro. *Lynx*, 35: 13-18.

BENDA, P., FAIZOLÂHI, K., ANDREAS, M., OBUCH, J., REITER, A., ŠEVČÍK, M., UHRIN, M., VALLO, P. & ASHRAFI, S. (2012). Bats (Mammalia: Chiroptera) of the Eastern Mediterranean and Middle East. Part 10. Bat fauna of Iran. *Acta Soc Zool Bohem*, 76: 163-582.

BOSTON, E. S. M., BUCKLEY, D. J., BEKAERT, M., GAGER, Y., LUNDY, M. G., SCOTT, D. D., PRODÖHL, P. A., MONTGOMERY, W. I., MARNELL, F. & TEELING, E. C. (2010). The status of the cryptic bat species, *Myotis mystacinus* and *Myotis brandtii* in Ireland. *Acta Chiropterol*, 12(2): 457-461. https://doi.org/10.3161/150811010x538025

BRUJIĆ, J., MILANOVIĆ, Đ., TUPEŠA, N. J., ĆUK, B., ĆEHA, M., VESELINOVIĆ, D., RISTANOVIĆ, J., KUVELJA, A., VUKOVIĆ, S., TUPEŠA, D. et al. (2011). Biodiverzitet područja cicelj. ed.: Arbor Magna. Banja Luka, Bosnia and Herzegovina, 105 pp.

BRUNET-ROSSINNI, A. K. & WILKINSON, G. S. (2009). Methods for age estimation and the study of senescence in bats. In: Ecological and behavioral methods for the study of bats. ed.: The Johns Hopkins University Press. Baltimore, USA, p.315-325.

ÇORAMAN, E., DUNDAROVA, H., DIETZ, C. & MAYER, F. (2020). Patterns of mtDNA introgression suggest population replacement in Palaearctic whiskered bat species. *Roy Soc Open Sci*, 7: 191805. https://doi.org/10.1098/rsos.191805

DE PASQUALE, P. P. & GALIMBERTI, A. (2014). New records of the Alcathoe bat, *Myotis alcathoe* (Vespertilionidae) for Italy. *Barbastella*, 7(1): 3-5. https://doi.org/10.14709/BarbJ.7.1.2014.01

DERVOVIĆ, T. (2015). Zimsko istraživanje šišmiša na području grada Visokog i prvi nalaz mogućeg porodiljskog skloništa brkatog šišmiša (*Myotis mystacinus*). *Naš krš*, 48(1): 17-20.

DIETZ, C., VON HELVERSEN, O. & NILL, D. (2009). Bats of Britain, Europe and Northwest Africa. ed.: Black Publishers. London, UK, 400 pp.

DIETZ, C. & KIEFER, A. (2016). Bats of Britain and Europe. ed.: Bloomsbury. London, UK, 398 pp.

EMBL. (2020). European Bioinformatics Institute. Accessed: 05 March 2020. https://www.ebi.ac.uk/Tools/msa/clustalo/

FAURE, P. A., RE, D. E. & CLARE, E. L. (2009). Wound healing in the flight membranes of big brown bats. *J Mammal*, 90(5): 1148-1156. https://doi.org/10.1644/08-MAMM-A-332.1

FLAQUER, C., TORRE, I. & ARRIZABALAGA, A. (2007). comparison of sampling methods for inventory of bat communities. *J Mammal*, 88(2): 526-533. https://doi.org/10.1644/06-MAMM-A-135R1.1

GALIMBERTI, A., SPADA, M., RUSSO, D., MUCEDDA, M., AGNELLI, P., CROTTINI, A., FERRI, E., MARTINOLI, A. & CASIRAGHI, M. (2012). Integrated Operational Taxonomic Units (IOTUs) in echolocating bats: a bridge between molecular and traditional taxonomy. *Plos One* 7(6): e40122. https://doi.org/10.1371/journal.pone.0040122

GASTON, K. J. & DAVID, R. (1994). Hotspots across Europe. *Biodivers Lett*, 2(4): 108-116. https://doi.org/10.2307/2999714

GLÜCK, P., AVDIBEGOVIĆ, M., ČABARAVDIĆ, A., NONIĆ, D., PETROVIĆ, N., POSAVEC, S. & STOJANOVSKA, M. (2011). Private forest owners in the western balkans - Ready for the formation of interest associations. ed.: EFI Research Report 25. eBook, 230 pp.

HAARSMA, A-J. (2008). Manual for assessment of reproductive status, age and health in European Vespertilionid bats. Version 1. eBook, 62 pp.

HODŽIĆ, M., BABIĆ, N., LOGO, A., NICEVIĆ, M., ALIBAŠIĆ, Z., DOČKAL, I., GOLETIĆ, S. & KALOPER, A. E. (2017). Rezultati istraživanja faune šišmiša na VII. Internacionalnom biološkom kampu, Rujište 2017 (Bosna i Hercegovina). *Hypsugo*, 2(2): 31-40.

HUSANOVIĆ, M. & MULAOMEROVIĆ, J. (2018). Maternity colony *Myotis myotis/oxygnatus*, 28.6.2018, vodena pećina cave, resanovci, Bosnia and Herzegovina. *Hypsugo*, 3(1): 44-45.

HUSSAIN, I. (2018). BOLD Student Data Portal Data Release.

ISAKSEN, K. (2019). iBOL Data Release. Unpublished data.

KARAPANDŽA, B. (2014). Detalji o prvim istraživanjima slepih miševa (Chiroptera, Mammalia) okoline planine Vitoroga i prvim nalazima vrsta *Myotis oxygnathus* Monticelli, 1885, *Myotis bechsteinii* (Kuhl, 1817) i *Barbastella barbastellus* (Schreber, 1774) u Bosni i Hercegovini. *Naš krš*, 47(1): 3-10.

KARAPANDŽA, B., MULAOMEROVIĆ, J., PAUNOVIĆ, M., PAŠIĆ, J., PRESETNIK, P. & ZAGMAJSTER, M. (2014, 1-5 September). The overview of bat fauna (Chiroptera) of Bosnia and Herzegovina with first record of *Pipistrellus nathusii*. XIII European Bat Research Symposium, Šibenik, Croatia.

KRUSKOP, S. V., BORISENKO, A. V., IVANOVA, N. V., LIM, B. K. & EGER, J. L. (2012). Genetic diversity of northeastern Palaearctic bats as revealed by DNA barcodes. *Acta Chiropterol*, 14(1): 1-14. https://doi.org/10.3161/150811012X654222

KRYŠTUFEK, B. (2004). A quantitative assessment of Balkan mammal diversity. In: Balkan biodiversity. Pattern and Process in the European Hotspot. ed.: Springer, Dordrecht. Dordrecht, Netherlands, p.79-108. https://doi.org/10.1007/978-1-4020-2854-0_6

MATOVIĆ, O. & MULAOMEROVIĆ, J. (2019). *Rhinolophus hipposideros* - porodiljska kolonija, 24.7.2018, 2.5.2019, Tjentište, Bosna i Hercegovina. *Hypsugo*, 4(1): 46-47.

MIĆIĆ, N., TRAVAR, N., GIDAS, J. D., BODRUŽIĆ, S., ILIĆ, P., HAJDER, D. & ĐURIĆ, G. (2018). Inheritance of type of tree habit in vilina bukva beech population from Čajnice. *Agro-know J*, 19(4): 299-307. https://doi.org/10.7251/AGREN1804299M

MICEVSKI, N., PRESETNIK, P., MICEVSKI, B. & CEĽUCH, M. (2014). Contribution to the knowledge of the Macedonian bat fauna. *Vespertilio*, 17: 103-114.

MIRIĆ, Đ. & PAUNOVIĆ, M. (1997). New data on the Leisler's bat *Nyctalus leisleri* (Kuhl, 1817) (Vespertilionidae, Chiroptera) from the Balkan peninsula, with a review of the Balkan range. *Myotis*, 35: 67-75.

MITEVA, S., HOWS, M., VAN DEN TEMPEL, C., DAVIES, J., ELENBAAS, P., TWISK, P., VERHEES, J., BUYS, J. & PEEREBOOM, J. (2020). Mammal survey 2019. Cicelj Nature Reserve, Čajniče, Bosnia and Herzegovina. Field Study Group. ed.: Dutch Mammal Society. 54 pp.

MULAOMEROVIĆ, J. (2013). Prvi nalaz Brandtovog šišmiša *Myotis brandti* u Bosni i Hercegovini. *Naš krš*, 46(1): 14-22.

MULAOMEROVIĆ, J., DŽEVLAN, A., ČOLPA, F., NAPOTNIK, I., PAŠIĆ, J. & PRESETNIK, P. (2020). *Nyctalus leisleri*, 22.4.2016, Vrbaska rijeka, Kozara planina, 5.7.2019, selo Tušila, Visočica planina (Bosna i Hercegovina). *Hypsugo*, 5(1): 38-40.

NCBI. (2020, March 5). Basic Local Alignment Search Tool. https://blast.ncbi.nlm.nih.gov/Blast.cgi

NIERMANN, I., BIEDERMANN, M., BOGDANOWICZ, W., BRINKMANN, R., BRIS, Y. L., CIECHANOWSKI, M., DIETZ, C., DIETZ, I., ESTÓK, P., VON HELVERSEN, O. et al. (2007). Biogeography of the recently described *Myotis alcathoe* von Helversen and Heller, 2001. *Acta Chiropterol*, 9(2): 361-378. https://doi.org/10.3161/150811007783527988

OBSERVADO (2020, 19 September). www.observado.org.

PAŠIĆ, J., MULAOMEROVIĆ, J. & PRESETNIK, P. (2013). Rezultati pregleda potencijalnih zimskih skloništa šišmiša u Bosni i Hercegovini u zimu 2012/13. *Naš krš*, 46(1): 23-34.

PAŠIĆ, J. & NAPOTNIK, I. (2014). Širokouhi šišmiš (*Barbastella barbastellus*) nađen među drvima u selu Aleksići (s Bosna i Hercegovina). *Naš krš*, 46(1): 35-36.

PRESETNIK, P., PAUNOVIĆ, M., KARAPANDŽA, B., ĐUROVIĆ, M., IVANOVIĆ, C., ŽDRALEVIĆ, M., BENDA, P. & BUDINSKI, I. (2014). Distribution of bats (Chiroptera) in Montenegro. *Vespertilio*, 17: 129-156.

PRESETNIK, P., MULAOMEROVIĆ, J., PAŠIĆ, J., NAPOTNIK, I., MILANOLO, S., BUDINSKI, I. & PEJIĆ, B. (2016). Rezultati pregleda potencijalnih zimskih skloništa šišmiša u Bosni i Hercegovini u zimu 2015/16. *Hypsugo*, 1(2): 34-48.

PRESETNIK, P., MULAOMEROVIĆ, J., PAŠIĆ, J., HODŽIĆ, M., NAPOTNIK, I., MILANOLO S. & HUSANOVIĆ, M. (2017). Ezultati pregleda potencijalnih zimskih skloništa šišmiša u Bosni i Hercegovini u zimu 2016/17. *Hypsugo*, 2(1): 27-41.

PRESETNIK, P. & ZAMOLO, A. (2018). *Myotis alcathoe*, *M. brandtii, M. mystacinus*, 23. 8. 2017, reka Reka v okolici Vodarne Draga, Slovenija. *Hypsugo*, 3(1): 38-40.

PRESETNIK, P., MULAOMEROVIĆ, J., HUSANOVIĆ, M., DERVOVIĆ, T., HODŽIĆ, M. & NAPOTNIK, I. (2019). Rezultati pregleda potencijalnih zimskih skloništa šišmiša u Bosni i Hercegovini, zima 2018./19. *Hypsugo*, 4(1): 15-28.

REBELO, H., FERREIRA, S., AMORIM, F., HORTA, P., RAPOSEIRA, H., SANTOS, H., BEJA, P. & MATA, V. A. (2020). Hidden in our pockets: building of a DNA barcode library unveils the first record of *Myotis alcathoe* for Portugal. *Biodivers Data J*, 8: e54479. https://doi.org/10.3897/bdj.8.e54479

RICHARDSON, P. W. (1994). A new method of distinguishing Daubenton's bats (*Myotis daubentonii*) up to one-year-old from adults. *J Zool*, 233(2): 307-309. https://doi.org/10.1111/j.1469-7998.1994.tb08590.x

RNJAK, D., RNJAK, G., HANŽEK, N. & ZRNČIĆ, V. (2017). Istraživanje faune šišmiša u podnožju planine velež (Bosna i Hercegovina) 2014. *Hypsugo*, 2(2): 11-30.

RUEDI, M., SAIKIA, U., THABAH, A., GÖRFÖL, T., THAPA, S. & CSORBA, G. (2021). Molecular and morphological revision of small Myotinae from the Himalayas shed new light on the poorly known genus *Submyotodon* (Chiroptera: Vespertilionidae). *Mamm Biol*, 101: 465-480. https://doi.org/10.1007/s42991-020-00081-3

TAMURA, K., PETERSON, D., PETERSON, N,. STECHER, G., NEI, M. & KUMAR, S. (2011). MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol Biol Evol*, 28(10): 2731-2739. https://doi.org/10.1093/molbev/msr121

TEMPLE, H. J. & TERRY, A. (2007). The status and distribution of European mammals. ed.: IUCN - The World Conservation Union. Almaty, Kazakhstan, 45 pp.

THÉOU, P. & BEGO, F. (2018). Bats of Albania. ed.: NaturAl project. eBook, 124 pp.

TIGANJ, E. & MULAOMEROVIĆ, J. (2017). V*espertilio murinus*, 1.10.2017., Bjelašnica Planina, Bosna i Hercegovina. *Hypsugo*, 2(2): 68-70.

WALKER, F. M., WILLIAMSON, C. H. D., SANCHEZ, D. E., SOBEK, C. J. & CHAMBERS, C. L. (2016). Species from feces: order-wide identification of Chiroptera from guano and other non-invasive genetic samples. *Plos One*, 11(9): e0162342. https://doi.org/10.1371/journal.pone.0162342

ZAMOLO, A., BORKO, Š., ŠKUFCA, D. & DELIĆ, T. (2018). Prilog k poznavanju podzemnih skloništa šišmiša (Chiroptera) na području Crne gore, Bosne i Hercegovine te Hrvatske. *Hypsugo*, 3(1): 27-37.