



Roadkill of Bats (Microchiroptera) in a Biodiversity Hotspot: a Case Study of the Kresna Gorge, Bulgaria

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Abstract: A study on bat casualties was carried out between 2013 and 2016 on the 16-km road stretch passing through the Kresna Gorge – a European biodiversity hotspot with high conservation value. We found 78 road-killed bats belonging to ten species, adding new data on the bat species composition in the gorge. We identified one road section as a roadkill hotspot (a place where bats experienced collisions more frequently). Our analyses showed that more numerous bat casualties occurred on road segments close to bat roosts and on segments with bridges. The construction of a motorway through the Kresna Gorge has been planned for the near future. The consequent loss of large portions of high-quality habitats and the increase of bat road casualties due to the higher velocity and traffic intensity would negatively influence the bat populations in the region. The identified hotspots could be useful for the planning of appropriate mitigation measures against the bat roadkill in this and other regions.

Key words: roadkill, road features, bat roost, Struma River

Introduction

The Kresna Gorge is a biodiversity hotspot with a high conservation value for Bulgaria and the Balkan Peninsula. In the gorge region, many rare, endemic and protected species and habitats are found (HUBENOV 2012). Since the beginning of the 21st century, several road and motorway projects have threatened the ecological condition of this area (SPIRIDONOV et al. 2007). The intense chiropterological research since 1993 has revealed that the Kresna Gorge is an area of great importance for bats, providing habitats for 20 species (PETROV 2001, BENDA et al. 2003) out of the 33 known species in Bulgaria (BENDA et al. 2003, POPOV & LAKOVSKI 2019). There is growing evidence that roads negatively affect bat populations (ALTRINGHAM & KERTH 2016). Theoretical studies have shown that, in some situations, collisions might lead to a population decline (see references in ALTRINGHAM

& KERTH 2016). Identification of the road sections where bats experience collisions more frequently can have strong conservation implications and could be essential for the appropriate planning of mitigation measures. Although bats have been intensely studied in the Kresna Gorge, information related to road mortality has not been published for this region. The purpose of the present study was to identify the road sections where bat collisions were clustered and to check if this clustering was connected to the proximity of road features (presence of bridges and tunnels), facilities (train stations, restaurants) and bat roosts. Additionally, we analysed the influence of the land cover type close to the road on the number of bat casualties.

Materials and Methods

We monitored the high-traffic intensity along a part of the E 79 road passing through the Kresna

Gorge: c. 16-km section between the Krupnik Village (41.847583° N, 23.141739° E) and the Kresna Village (41.729400° N, 23.154594° E). It is a part of the Pan-European corridor IV, providing a direct route through Bulgaria to the Aegean Sea and connecting the biggest towns in the western part of the country. This is the Bulgarian road with the highest traffic in the north-south direction. The average number of vehicles per day was 7969 in 2014, while the maximum in some days at the end of the tourist season (end of August and beginning of September) could reach more than 11,000. The average speed of the vehicles in the section was 70.5 km/h (TRAFFIC MONITORING KRESNA GORGE 2014).

The fieldwork was conducted by a team of four trained people and the team leader was the second author. Between 2013 and 2016, from March to October, roadkilled bats were recorded weekly along the studied route. Each of the field team members explored one section in both directions, with an average length of about 4 km for about 24 hours. All locations of dead animals were recorded with exact geographical coordinates (accuracy ± 3 m, datum WGS84), using handheld Garmin units (GPSMAP 64s, eTrex HCx, eTrex 10). To avoid re-counting, the individuals and the respective location on the road were marked with a colour spray after detection. Only morphologically identifiable carcasses were collected by the second author and were later identified and stored in the laboratory of the Bat Research and Conservation Centre (BRCC) in the National Museum of Natural History at the Bulgarian Academy of Sciences (NMNH-BAS).

For the identification of the sections with the higher number of roadkills than expected by chance (hotspots), we applied the following approach: a line feature representing the central line of the studied road stretch (15,972 m) was drawn using satellite imagery in Google Earth Pro 7.3.3.7786. For the rest of the steps, we used QGIS 3.6.0-Noosa. The line feature was divided into 32 sections with a length of 500 m. We created a 40-m buffer on both sides of each section broader than the road. Its size was chosen for visualisation purposes. The number of roadkills in each section was counted with the function “Count points in polygons”. Roadkill hotspots were identified following Malo’s method, as it seems to perform better than a number of other methods in detecting fatality hotspots (GOMES et al. 2009). The method is based on “comparing the spatial pattern of collisions with that expected in a random situation, in which case the likelihood of col-

lisions for each road section would show a Poisson distribution” (MALO et al. 2004). Using the mean number of collisions per section (with length 500 m instead of the one-kilometre sections, used in the cited paper) λ , the probability of any road section, having x number of collisions was:

$$p(x) = \lambda^x / (x!e^\lambda)$$

A graph with the probability values ($p(x)$) versus the number of roadkills (x), as described in SILLERO et al. (2019), was used for the hotspots identification: hotspots were the road sections, where the number of roadkilled bats was equal or higher than the values situated in the graph close to the asymptote.

Data for the land-cover categories in the region were extracted from a shapefile part of the digital land-cover and land-use dataset RIPARIAN ZONES 2012 – LAND USE LAND COVER. We created a polygon layer by applying a 250-m buffer on both sides of the lines representing each 500-m-long road section. The file with the land cover polygons was intersected with the buffered road sections layer. Using the attributes of the polygons in the intersection layer, the percent of the area occupied by each land cover type was calculated for the buffer around each of the road sections. The rest of the analysis was performed in R 3.6.3 (R CORE TEAM 2020). We tested for correlations between the land cover variables and the number of bat remains found on each section, using Spearman’s correlation coefficient (R_{sp}). Further, we included the land cover variables that correlated significantly (or bordering on significance) with the number of the bat remains in generalized linear models (GLM) with a Poisson error structure. The amount of deviance accounted for by the models was calculated using the “Dsquared” function from the package “modEVA” (BARBOSA 2013).

We followed all ethical requirements for working with bats. The research was carried under permits by the Bulgarian Biodiversity Act (No 554/20.01.2014, No 696/19.01.2017).

Results

We recorded 78 dead bats on the road, belonging to ten species (Table 1). Our study increased the number of the known species in the Kresna Gorge to 22. *Myotis brandtii* (Eversmann, 1845) was recorded for the first time in the Struma Valley south of Blagoevgrad, including Kresna Gorge, Sandanski-Petrich Valley and neighbouring mountains. We confirmed the presence of *Nyctalus leisleri* (Kuhl, 1817) and *Miniopterus schreibersii* (Kuhl, 1817), previously with single or doubtful records (PETROV

Table 1. Records of dead bats on the road in the Kresna Gorge, 2013–2016. When the species identification was not possible, the specimens are marked as “Chiroptera”.

Species	Total count	Date(s), counts	Coordinates
<i>Hypsugo savii</i> (Bonaparte, 1837)	4	6/04/2014(2)	41.75457° N, 23.1524° E; 41.75158° N, 23.15283° E
		1/06/2015(1)	41.81506° N, 23.15698° E
		13/07/2015(1)	41.7763° N, 23.15394° E
<i>Miniopterus schreibersii</i> (Kuhl, 1819)	3	20/04/2016(1)	41.79702° N, 23.15767° E
		20/09/2016(1)	41.81038° N, 23.16247° E
		29/09/2016(1)	41.7899° N, 23.15598° E
<i>Myotis bechsteinii</i> (Kuhl, 1818)	2	25/04/2013(1)	41.82733° N, 23.1519° E
		21/04/2016(1)	41.82387° N, 23.15773° E
<i>Myotis brandtii</i> (Eversmann, 1845)	1	6/07/2016(1)	41.83083° N, 23.15348° E
<i>Myotis emarginatus</i> (Geoffroy, 1806)	3	13/07/2015(2)	41.79454° N, 23.15904° E; 41.78998° N, 23.15748° E
		4/09/2015(1)	41.74551° N, 23.16385° E
<i>Myotis mystacinus</i> (Kuhl, 1819)	3	5/06/2014(1)	41.76367° N, 23.15303° E
		9/09/2014(1)	41.8326° N, 23.15427° E
		19/06/2015(1)	41.77642° N, 23.15408° E
<i>Nyctalus leisleri</i> (Kuhl, 1818)	1	17/04/2015(1)	41.77171° N, 23.15475° E
<i>Pipistrellus pipistrellus</i> (Schreber, 1774)	5	5/09/2013(2)	41.76968° N, 23.15392° E; 41.76075° N, 23.15238° E
		25/04/2014(1)	41.83097° N, 23.15387° E
		12/09/2015(1)	41.83784° N, 23.14987° E
		14/10/2015(1)	41.82938° N, 23.15295° E
<i>Rhinolophus ferrumequinum</i> (Schreber, 1774)	3	5/07/2015(1)	41.78987° N, 23.15607° E
		13/07/2015(1)	41.74262° N, 23.16088° E
		10/09/2015(1)	41.8219° N, 23.15858° E
<i>Rhinolophus hipposideros</i> (Bechstein, 1800)	4	5/09/2013(2)	41.83088° N, 23.15375° E; 1.82538° N, 23.15217° E
		6/06/2016(1)	41.83888° N, 23.15008° E
		11/09/2016(1)	41.77967° N, 23.1549° E
		18/09/2013(1)	41.84073° N, 23.1504° E
Chiroptera	49	9/07/2014 (3)	41.82909° N, 23.1525° E; 41.79563° N, 23.15807° E; 41.79563° N, 23.15807° E
		10/07/2014(1)	41.79563° N, 23.15807° E
		11/07/2014(1)	41.76778° N, 23.15295° E
		12/07/2014(2)	41.7948° N, 23.1588° E ; 41.7794° N, 23.15458° E
		13/07/2014(4)	41.81818° N, 23.15753° E; 41.79422° N, 23.15923° E; 41.77892° N, 23.15432° E; 41.74906° N, 23.15472° E
		8/09/2014(1)	41.76273° N, 23.15285° E
		10/09/2014(2)	41.79355° N, 23.15958° E; 41.76198° N, 23.15273° E
		11/09/2014(1)	41.75302° N, 23.1522° E
		12/09/2014(1)	41.77802° N, 23.15457° E
		17/09/2014(1)	41.83355° N, 23.15442° E
		2/10/2014(1)	41.74835° N, 23.16115° E
		17/04/2015(1)	41.78003° N, 23.15461° E
		18/04/2015(1)	41.76856° N, 23.15311° E
		7/05/2015(1)	41.7634° N, 23.15292° E
		28/05/2015(1)	41.76283° N, 23.15294° E
		24/06/2015(2)	41.75443° N, 23.15235° E; 41.75127° N, 23.15287° E
		3/07/2015(2)	41.83935° N, 23.15026° E; 41.80419° N, 23.1623° E
		5/07/2015(1)	41.80065° N, 23.15924° E
		4/09/2015(2)	41.8078° N, 23.1626° E; 41.76832° N, 23.15308° E
		8/09/2015(1)	41.72985° N, 23.15662° E
		9/09/2015(1)	41.78543° N, 23.15288° E
		10/09/2015(2)	41.77861° N, 23.15444° E; 41.73448° N, 23.15911° E
		18/04/2016(1)	41.82465° N, 23.15424° E
		19/04/2016(1)	41.74042° N, 23.15902° E
		20/04/2016(1)	41.84441° N, 23.14687° E
		20/06/2016(1)	41.77893° N, 23.15436° E
		4/07/2016(2)	41.80638° N, 23.16223° E; 41.77987° N, 23.15472° E
6/07/2016(2)	41.78887° N, 23.15406° E; 41.78003° N, 23.1547° E		
15/07/2016(3)	41.83455° N, 23.15432° E; 41.7338° N, 23.15897° E; 41.73742° N, 23.15877° E		
10/09/2016(1)	41.78733° N, 23.15285° E		
11/09/2016(3)	41.83497° N, 23.15457° E; 41.80997° E, 23.16297° E; 41.73619° N, 23.15861° E		

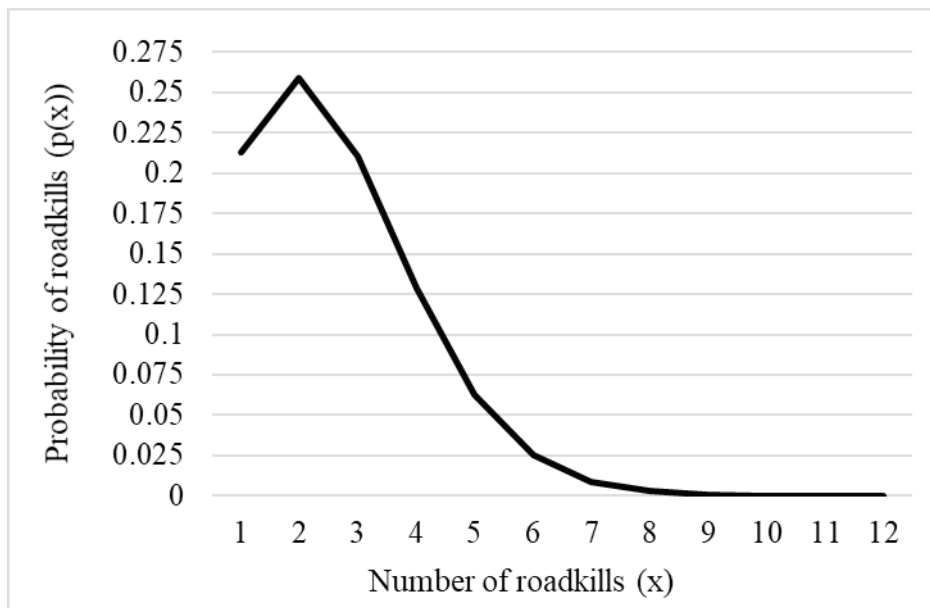


Fig. 1. Probability values ($p(x)$) versus the number of roadkills (x), obtained after the application of Malo's method: hotspots are identified as sections with ≥ 7 total roadkills during the period of the study.

Table 2. Correlation between the land cover variables and the number of bat casualties. The two segments identified as hotspots were excluded from the correlation analysis as the high number of fatalities on them was most likely connected to the proximity of bat roosts.

Land cover category	R_{sp}	p
Industrial, Commercial and Military Units	-0.13697	0.454756
Road Networks and Associated Land	-0.0354	0.847479
Railways and associated land	-0.32843	0.066471
Arable Irrigated and Non-Irrigated Land	-0.0527	0.774525
Land Principally occupied by Agriculture	-0.02009	0.913085
Natural & Semi-Natural Broadleaved Forest	-0.12475	0.496342
Natural & Semi-Natural Coniferous Forest	0.337484	0.058894
Natural & Semi-Natural Mixed Forest	0.030468	0.868524
Semi-Natural Grassland	-0.04959	0.787524
River Banks	0.034098	0.85302
Interconnected Water Courses	0.087265	0.634848

2001, BENDA et al. 2003).

The recorded bat mortality rate was 1.2 bats/km/year. As hotspots were identified sections with ≥ 7 total roadkills (Fig. 1). There was only one such hotspot and it was relatively close to two bat roosts and a bridge (Fig. 2). Additionally, all the sections that had 5–6 total roadkills, a relatively high number for the studied road stretch, included bridges and/or tunnel exits (Fig. 2).

Amongst the land cover variables, only the percent of the coniferous forest was bordering on significance ($p = 0.058$) with the total bat road casu-

alties per segment (Table 2). The model ($\beta = 0.016$, $p = 0.048$) built using this variable accounted for 9.6% of the deviance in the fatalities.

Discussion

About half of the species of the bat fauna in the Kresna Gorge have been identified as road casualties. The recorded bat mortality rate is close to that reported (1.5 bats/km/year) in a study with a similar weekly monitoring scheme (LESIŃSKI 2007). Nevertheless, the short persistence of bat remains on the road makes the count of all casualties unachievable (ALTRINGHAM & KERTH 2016). Therefore, the actual rate of the bat roadkill along the studied stretch is most likely much higher than the estimate given here.

Among the species found during the study, *M. schreibersii* is of particular interest. It is a typical cave-dwelling bat; neither caves nor underground roosts with colonies of the above-mentioned species have been recorded in the region (BENDA et al. 2003, IVANOVA 2005). Only one important bat underground habitat has been registered in the region (IVANOVA 2005), an artificial military bunker inhabited by a mixed colony of *M. emarginatus*, *Rhinolophus ferumequinum* (Schreber, 1774) and *Rh. euryale* Blasius, 1853. The abandoned building close to Kresnensko Hanche is a known roost of a small colony of *Rh. ferumequinum* (BENDA et al. 2003). *Miniopterus schreibersii* was recorded only in autumn. This supports the hypothesis of the importance of the Kresna Gorge as a migratory corridor for the bats as

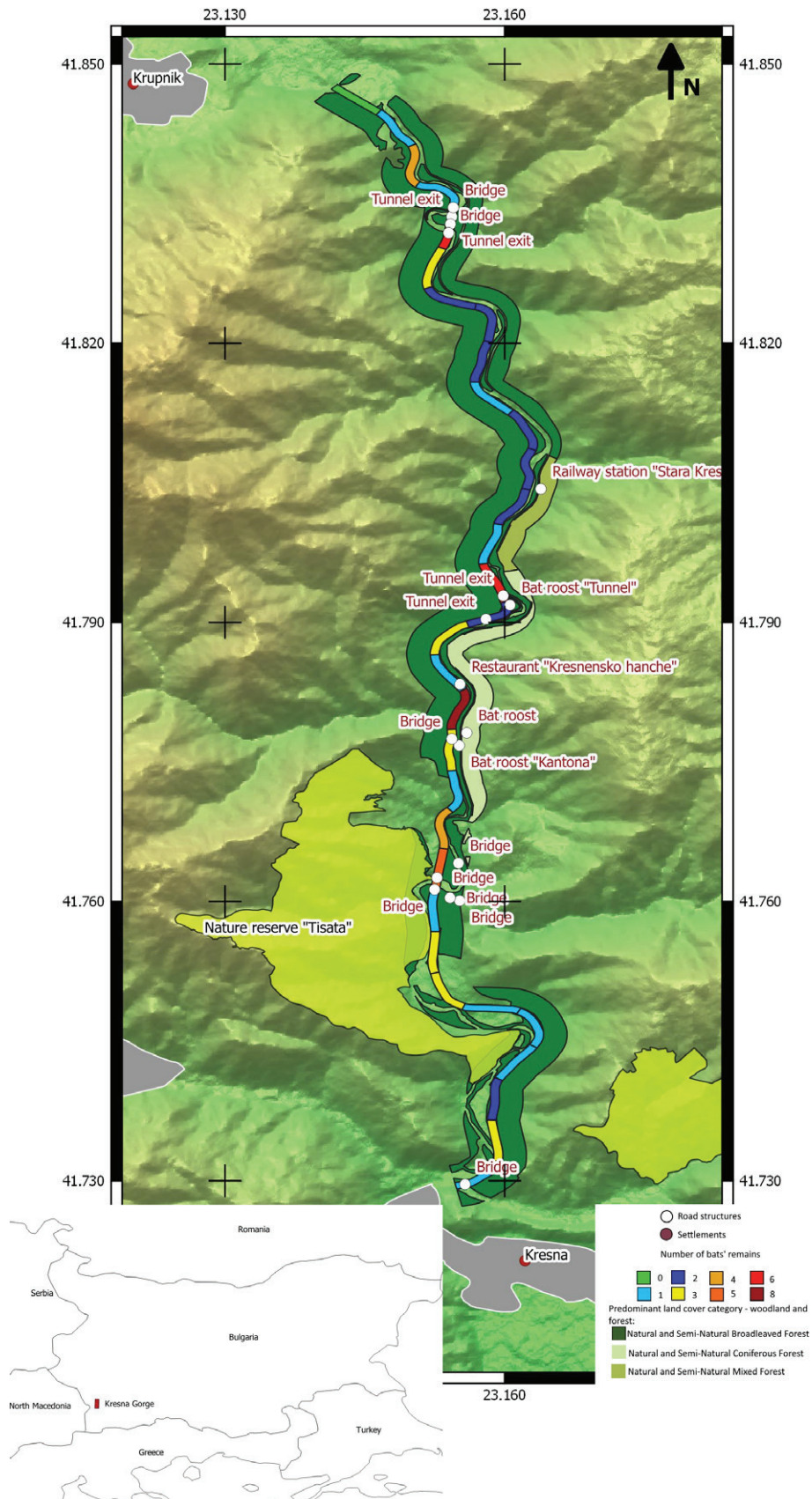


Fig. 2. Number of roadkills registered and predominant land cover in each of the studied road sections. The hotspots are marked with black and red. For the visualisation of the relief, we used a digital elevation model part of dataset ALOS PALSAR available at: <https://search.asf.alaska.edu/>

it is for other groups of animals (SPASSOV & STOEV 2001, SPIRIDONOV et al. 2007).

Similarly to other studies (MEDINAS et al. 2013), our results show that there is a higher number of bat roadkill accidents on road segments closer to bat roosts than on the remaining segments. Bridges could provide roosting places for some bat species (KEELEY & TUTTLE 1999, FRICK et al. 2019); these are also places where riparian corridors, used as bat flyways (ABBOTT et al. 2012), cross the road. These facts could explain the high incidence of roadkills reported here in road segments with bridges. Further studies on the influence of bridges on bat road fatalities are needed as suggested also by MEDINAS et al. (2013). According to previous studies, casualties occur mainly on segments crossing high-quality habitats, including watercourses with riparian vegetation (MEDINAS et al. 2013) and forested areas (LESIŃSKI 2007, MEDINAS et al. 2013) as compared to densely built-up areas (LESIŃSKI 2007). In contrast, SECCO et al. (2017) found a negative relation between roadkill occurrence and the presence of marginal pastures and forests. In the present study, among the forest land cover variables only the area occupied by coniferous forest is bordering on significance with the total bat road casualties per segment. This can be due to the close proximity of two of the segments with the highest number of roadkills to areas occupied mainly by *Pinus nigra* (FORESTRY GIS BASED PLATFORM OF WWF). The same two segments are close to bat roosts and bridges or tunnels, as well. The proximity of these structures could provide a better explanation for the high numbers of casualties registered there. The lack of significant correlation between the forest land cover variables and the road casualties per segment in the present study is most likely due to the prevalence of the forest land cover type on both sides of the studied stretch (Fig. 2). The relationship between the land cover and the roadkill occurrence needs more detailed study.

A big investment project for motorway construction through the Kresna Gorge is to be implemented in the near future (Road Infrastructure Agency 2019; MTITC 2020). The loss of high-quality habitats and the increased bat road casualties due to the higher speed and intense traffic are expected to become a more serious imminence (SPIRIDONOV et al. 2007, MALINOV 2018). Roads and road constructions have a negative impact on bats in several ways. Besides direct mortality, noise, light and active traffic can also elicit a behavioural pattern of avoidance, interfering with bats commuting, foraging and migration (ALTRINGHAM & KERTH 2016, KITZES & MERENLENDER 2014). This barrier effect can lead to a

dramatic decline of total bat activity and diversity of recorded species when increasing proximity to a motorway (BERTHINUSSEN & ALTRINGHAM 2012, KITZES & MERENLENDER 2014). Thus, the bat populations in the region could be seriously threatened in the next few years. There are measures that could mitigate the negative influence of the traffic on the bats living in the studied region. E.g., tree and hedge planting near the road and modified lighting schemes could guide bats towards crossing points or deter bats from crossing at dangerous locations (ALTRINGHAM & KERTH 2016, ELMEROS et al. 2016). Underpasses at pre-existing flyways could be also useful (ALTRINGHAM & KERTH 2016, ELMEROS et al. 2016). On the other hand, motorway construction outside the Kresna Gorge – a solution supported by the Bulgarian nature protection organizations (BSPB Position paper 18/09/2017), remains the best option for the conservation of this biodiversity hotspot in the future.

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