Conserving the European ground squirrel (*Spermophilus citellus*, Rodentia: Scuridae)—microhabitat requirements, distribution and population status in the Deliblato Sands region, Serbia

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Contents

Abstract

The European ground squirrel (*Spermophilus citellus*) is listed as a vulnerable species by IUCN and is currently in decline across most of its European range. The species is recognized as an important prey for many IUCN vulnerable and endangered species, including the Eastern imperial eagle and saker falcon. In Serbia's stronghold area of the Deliblato Sands, Europe's largest sand-steppe area, populations have halved since 1950 due to pastoral abandonment, grazing intensity reduction and habitat transformation or loss. This study established current *S. citellus* population status and distribution in relation to species microhabitat requirements in the Special Nature Reserve "Kraljevac" and developed general guidelines for species conservation and habitat management across the Deliblato Sands region. *S. citellus* population status and distribution were established by recording the location and number of burrows in active use. Population density was 56 individuals/ha, one of the highest recorded in the Deliblato Sands region, suggesting that the studied population was viable. The population distribution proved to be affected by the sheep grazing intensity and the distance to the water source, the flatness of the ground, and the thickness and height of vegetation cover. *S. citellus* preferred species-rich steppe and steppe-grassland and avoided areas dominated by tall, exotic and invasive weeds, such as *Onopordum acanthium* and *Ailanthus altissima*. To ensure the continued survival of the species in the Deliblato Sands region and to mitigate any further declines, it is recommended to intensify grazing with mixed herds of sheep and goats, supplemented where necessary by mowing and the mechanical and chemical control of weeds. Also, sheep enclosures should be translocated from areas occupied by *S. citellus* to prevent soil compaction and overfertilization. Furthermore, non-invasive monitoring of future population success and increasing public awareness of the conservation value and ecological role of *S. citellus* in steppe-grassland habitats is imperative to the continuation of the species.

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Introduction and Aims

In 1994, Caughley (1994, p. 227) wrote in his seminal work, "Directions in Conservation Biology,"

> The dominant tenet of the declining-population paradigm is distilled easily enough: the contraction of the range of a species and the decline in the number of its members has a tangible cause which with skill may be identified and defeated.

In 2013, in response to recent serious declines of the European ground squirrel (*Spermophilus cietellus*) populations across most of their range, the European Species Action Plan was prepared and supported by the European Commission (Janák, Marhoul & Matějů, 2013). The Commision's stated objectives included the following: resisting the decline in *S. citellus* by ensuring necessary habitat management at sites where populations exist currently, re-establishing meta-populations through habitat restoration, and developing connectivity across the entire species range by creating corridors and stepping stones for dispersal and recolonization. It was recommended that within this framework national and regional plans should be developed to prevent further loss of the species and its habitats.

Hence, the main aims of this study were as follows:

- to establish *S. citellus* microhabitat requirements, distribution, and population status in the Deliblato sands region; and
- to develop general guidelines for *S. citellus* conservation and habitat management.

Literature Review and Research Questions

S. citellus is a small (170–450 g) diurnal ground dwelling rodent from the family Sciuridae endemic to central and south-eastern Europe (**Fig. 1** and **Fig. 2**). It is an obligate hibernator (September–February) and has a largely herbivorous diet, including roots, shoots, flowers, seeds, fruits, and some animal material (Janák, Marhoul & Matějů, 2013). Although *S. citellus* lives in colonies it cannot be regarded as a truly social species; animals occupy separate burrow systems and sexes come together only for a 2–3 week mating period in April (Millesi et al., 1999). The species is associated with short-grass steppe and natural grasslands and similar anthropogenic habitats (pastures, lawns, sports fields, golf courses) on light, well-drained soils where it can excavate its burrows and easily sight predators (Kryštufek, 1999).

The ecological role of *S. citellus* is poorly understood, unlike other burrowing, social, and herbivorous mammals (e.g., prairie dogs, marmots and pikas) which are known to transform grassland habitats through their burrowing and foraging activity (Davidson, Detling & Brown, 2012). *S. citellus* is known to serve as an important prey for many regional predators, including the large whip snake (*Coluber jugularis*), the weasel (*Mustela nivalis*), the common polecat (*Mustela putorius*), and the steppe polecat (*Mustela eversmanni*; Hut & Scharff, 1998; Janák, Marhoul & Matějů, 2013). Furthermore, recent declines in birds of prey inhabiting steppe grasslands, such as the Eastern imperial eagle (*Aquila heliaca*) and the saker falcon (*Falco herrug*), and the associated changes in their foraging patterns have been attributed to the disappearance or low numbers of *S. citellus*—a key prey type (Chavko et al. 2007; Chavko, 2010).

S. citellus populations are currently in decline due to habitat fragmentation, the conversion of grasslands and pastures to cultivated fields or forests, and the abandonment of traditional grazing regimes which results in the reversion to unsuitable tall-grass and scrubby habitats (Janák, Marhoul & Matějů, 2013). The species has been listed as vulnerable in the 2008 IUCN Red List ver. 3.1 and in Appendix II of the Bern Convention and Annexes II and IV of the EU Habitats and Species Directive (Coroiu et al., 2008). Its populations have become geographically isolated and extinctions have occurred in peripheral parts of its range in Germany (where it was extirpated c.1985 because of forestry; Feiler, 1988) and Poland (where the last definite autochthonous records date from the 1970s, although the species has recently been successfully reintroduced (Matějů et al., 2010)). Furthermore, population declines have been observed in the north and north-western parts of its range, including Austria, Czech Republic, and Bulgaria (Hoffmann et al., 2003; Koshev, 2008; Matějů, 2008). Overall, declines are suspected to be more than 30% over the last ten years.

Figure 1 European ground squirrel (*Spermophilus citellus*, Rodentia: Scuridae) and its active burrow with running tracks.

Figure 2 Distribution of *S. citellus* in Europe (distribution data from www.iucnredlist.org, modified by K. Petrović).

In Serbia, at the southern-most part of its range, *S. citellus* once occupied the vast area of the Vojvodina Province (**Fig. 3**; Ružić-Petrov, 1950). Today, the species occurs in three stronghold areas, along the Tisza River in Bačka, in Srem's Fruška Gora Mountains, and the Deliblato Sands region, Europe's largest sand-steppe area (**Fig. 4**; Ćosić et al., 2013). Within the Deliblato Sands region three isolated colonies were identified within the Special Nature Reserve "Deliblatska peščara" with remaining colonies occurring outside the reserve (Ham, 2011; **Fig. 5**). During 2013 investigations of *S. citellus* population dynamics in the Deliblato sands, only a few individuals were recorded in the former stronghold areas of Hatarica and Mali Pesak with the Special Nature Reserve "Kraljevac" sustaining the highest population density (Ćosić, personal communication, May 2014) .

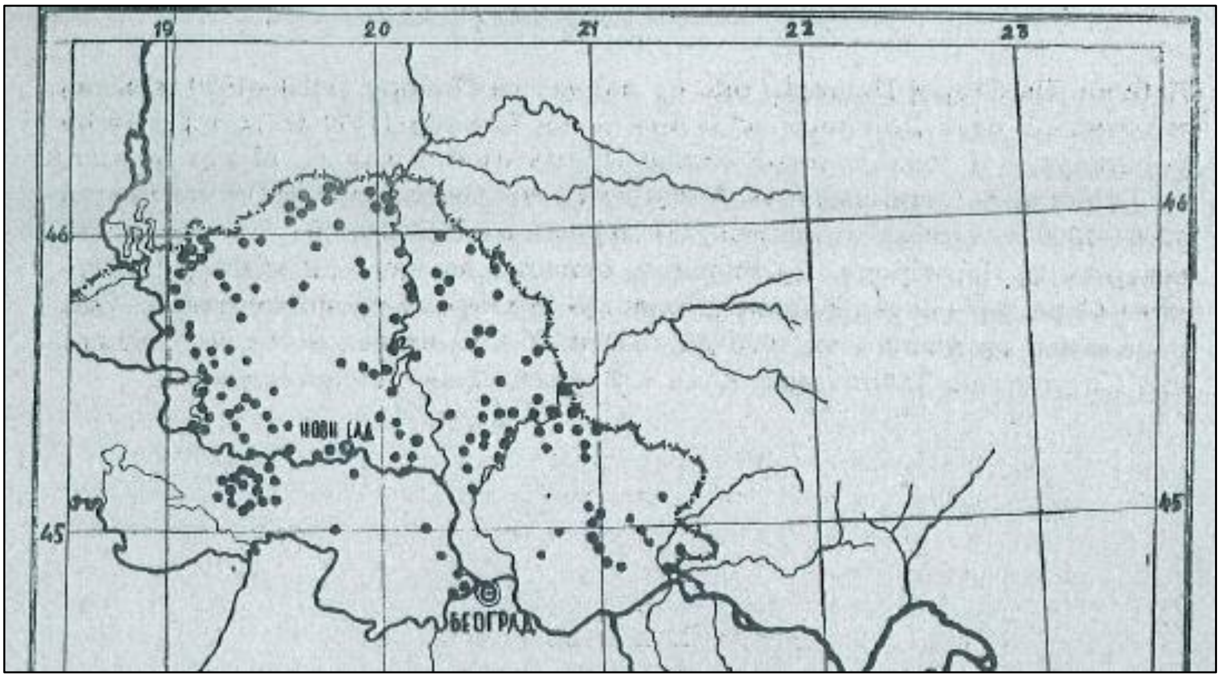

Figure 3 Past distribution of *S. citellus* in the Vojvodina Province, Serbia (Ružić-Petrov, 1950).

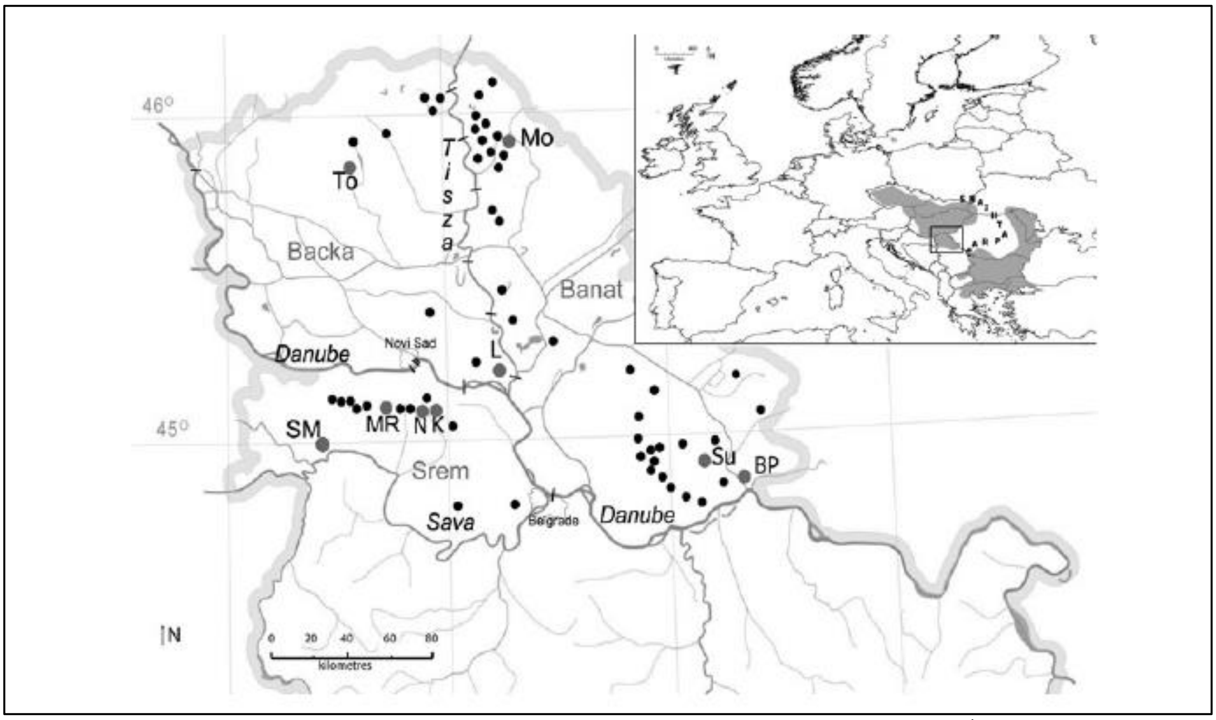

Figure 4 Current distribution of *S. citellus* in the Vojvodina Province, Serbia (Ćosić et al., 2013).

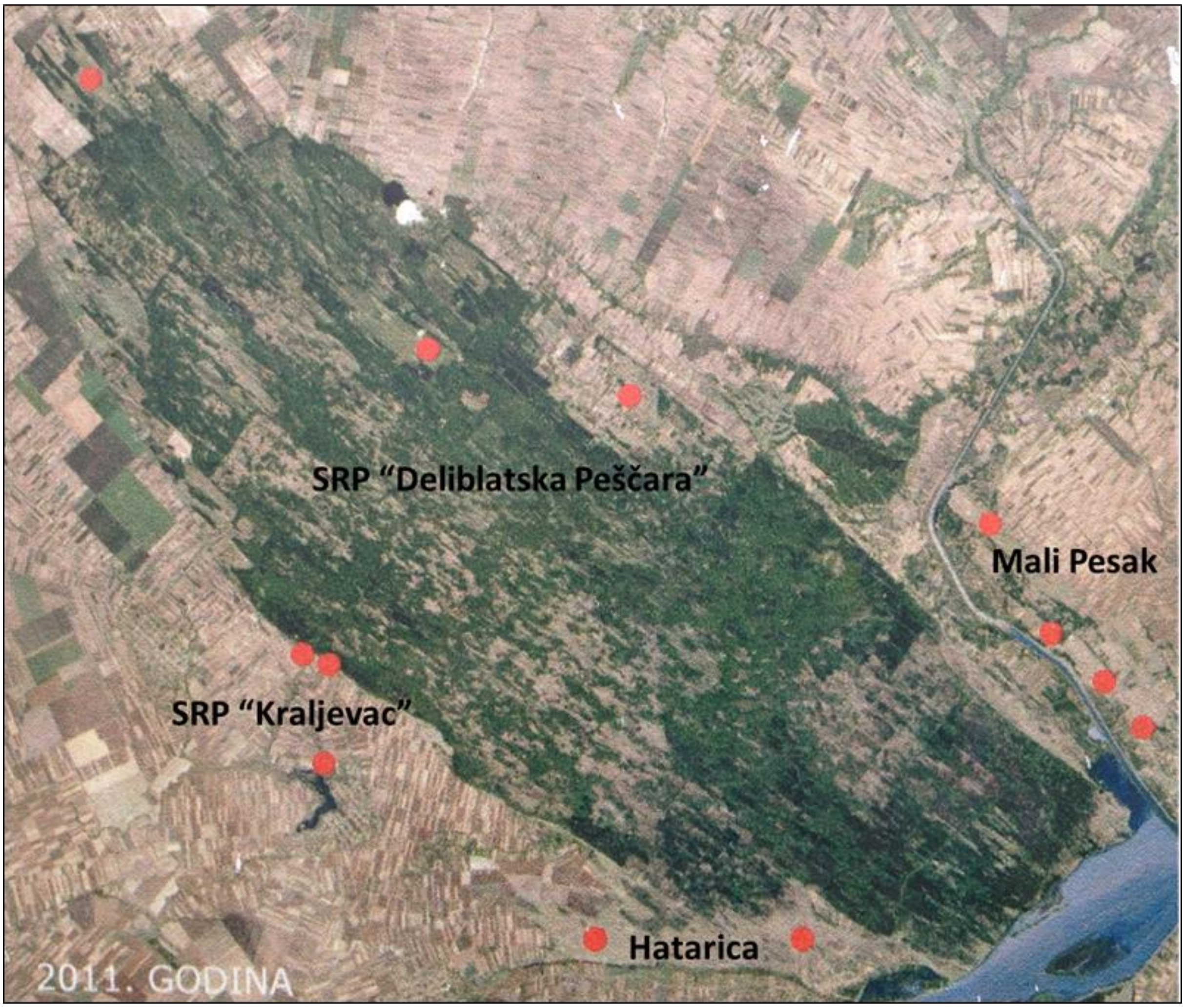

Figure 5 Location of *S. citellus* colonies in the Deliblato sands region (Ham, 2011; modified by K. Petrović).

The underlying causes of *S. citellus* decline in the Deliblato sands region are unknown. Pastoral abandonment, reduction in grazing intensity, transformation or loss of habitat, use of fertilizers, herbicides and pesticides, as well as isolation of populations and within-population processes could all contribute to low population numbers (Ćirović, personal communication, May 2014). Therefore, a detailed study of *S. citellus* microhabitat requirements, distribution and population status in its stronghold area, the Special Nature Reserve "Kraljevac," can provide valuable insights into the persistence of the species in the Deliblato sands region.

Specifically, the present study aimed to address the following research questions:

What are the *S. citellus* microhabitats' vegetation composition and structure?
What is the *S. citellus* colony's distribution?
What is the *S. citellus* colony's size?
What is the *S. citellus* colony's age and sex structure?

Methods

Study area

The Deliblato Sands is situated in Serbia, about 70 km northeast of Belgrade between the Danube river and the Carpathian Mountains' south-western slopes, and represents a part of the Pannonian Plain (**Fig. 6**). The Deliblato Sands is characterised by dune habitat with steppe-grassland plains, pastures, steppe-forest, and a few remaining desert and semi-desert areas. A temperate-continental climate with big diurnal and annual fluctuations of temperature (-30 and +42°C), relatively low annual precipitation (633 mm), absence of surface water-courses, and sandy soil resulted in a special vegetation-geographical area called *Deliblaticum* that gave rise to unique biocoenoses (Stjepanović-Veseličić, 1979). Within the boundaries of the Deliblato Sands there are two nature reserves, the Special Nature Reserve "Deliblatska Peščara" and the Special Nature Reserve "Kraljevac."

The Special Nature Reserve "Kraljevac" (44° 50' 48" N 21° 01' 23" E) is situated in the south-western part of the Deliblato Sands (**Fig. 6**) and includes a 20 ha steppe-grassland pasture on loess terrace (**Fig. 7**), a 53 ha forest corridor connecting the reserve with the Special Nature Reserve "Deliblatska Peščara," and the Lake Kraljevac 192 ha in size. The reserve is a part of NATURA2000 network and the pasture is classified as a category II nature conservation area ("Sl. glasnik RS", No. 135/92) and IUCN protected areas category IV, which requires regular and active management interventions to ensure habitat and species survival (Habijan-Mikeš, 2005). The area is grazed by a native breed of sheep, Cigaja (100–300 individuals), from April until November and occasionally mowed (**Fig. 7**).

The Special Nature Reserve "Kraljevac" represents an important habitat for the European ground squirrel (*S. citellus*) and the European mole (*Talpa europea*), a breeding site for the European bee-eater (*Merops apiaster*) and the sand martin (*Riparia riparia*), and a foraging site for the saker falcon (*Falco herrug*), a globally endangered species. The dominant vegetation community is a dry steppe *Festucetum-Potentilletum arenarie* in different stages of degradation and grassland dominated by *Cynodon dactylon* and different herbs from Fabaceae family (Stjepanović-Veseličić, 1979). In areas with soil disturbed by sheep defecation, the weedy and nitrophilous species, *Onopordum acanthium*, *Xanthium spinosum* and *Urtica dioca,* are present. Woody vegetation is composed of the exotic and invasive species, *Ailanthus altissima*.

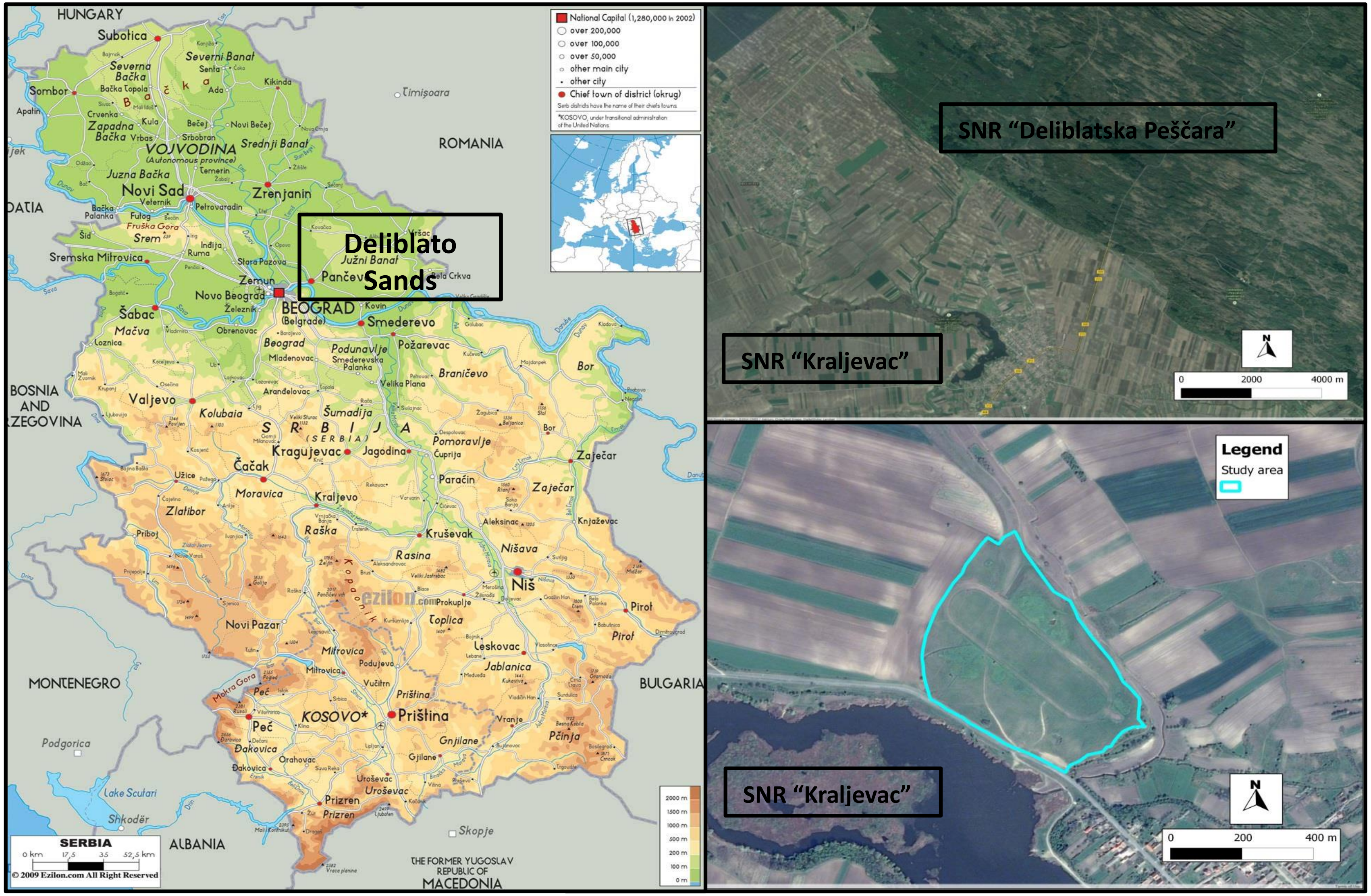

Figure 6 Location of the study area within the Special Nature Reserve "Kraljevac" in the Deliblato Sands region, Serbia.

Figure 7 Sheep grazing at steppe-grassland pasture on loess terrace in the Special Nature Reserve "Kraljevac".

Survey timeline

All field-work was carried out in May when steppe and grassland vegetation was well developed and in July when *S. citellus* juveniles were fully weaned and occupied separate burrows from their mothers (**Tab. 1**).

Table 1 Survey timeline

May 2014	**July 2014**	
4th week	2nd week	3rd week
Vegetation survey Colony distribution survey	Colony sex and age structure survey	Colony size survey

Vegetation composition and structure

To describe and map microhabitats in the Special Nature Reserve "Kraljevac," the vegetation species composition and cover abundance as well as the height of vegetation were measured within 1 x 1 m quadrats set at 25 m intervals along 11 transects (**Fig. 8**). The vegetation cover abundance was estimated using the Braun-Blanquet scale: + (less than 5% cover with few individuals), 1 (cover up to 5%), 2 (5–25% cover), 3 (25–50%), 4 (50–75%), and 5 (75–100%). The height of vegetation was estimated using a 5-point scale: 1 (< 10 cm), 2 (<20 cm), 3 (< 30 cm), 4 (< 40 cm), 5 (< 50 cm and over). The Euro+Med PlantBase was used to check plant nomenclature. Additionally, the following environmental variables were recorded: altitude, slope, aspect, and distance from water. Altitude, aspect, and the exact location of quadrats were recorded using a handheld GPS Garmin *e*Trex 10. Slope was measured using a two-axis inclinometer Theodolite iPhone App (Hunter Research & Technology). Distance from water was measured using a Measure Line feature in Quantum GIS for Desktop 1.8.0. Lisboa.

Figure 8 Sampling design for establishing vegetation composition and structure and distribution of *S. citellus* colony in the study area.

Grazing intensity

Information on the grazing intensity (high, moderate, low) and other management practices in the Special Nature Reserve "Kraljevac" was obtained from a governing body, the USR "Deliblatsko Jezero," Deliblato and local sheep owners.

Distribution of S. citellus *colony*

To establish the distribution of the *S. citellus* colony in the Special Nature Reserve "Kraljevac," the entire study area (14.7 ha) was surveyed walking along 11 line transects (250–400 long) set 50 m apart, and the number and location of all active burrows within 5 x 5 m quadrats, located at 25 m intervals, were recorded using a handheld GPS Garmin *e*Trex 10 (**Fig. 8**). Burrows were considered active or used by *S. citellus* if they had clean and open entrances and were not covered with cobwebs and vegetation or collapsed. Signs of digging, running tracks, and presence of fresh faeces were also used as indicators of burrow use.

Size of S. citellus *colony*

To establish the size of the *S. citellus* colony, three 50 x 50 m experimental plots each divided into four quadrats (20 x 20 m) were set up in the study area to represent low, medium, and high animal burrow densities (**Fig**. **9**). Each adult *S. citellus* occupies one burrow system with a nest, usually with one to five entrances, and several bolt holes used for hiding from predators (**Fig. 10**). Therefore, the number of active burrows within experimental plots was used to calculate the size of the *S. citellus* colony. Previous studies have demonstrated that counting active burrows is adequate for detecting differences in local *S. citellus* densities (Koshev 2008; Męczyński et al., 2010). Within each plot, all entrances of active burrows were closed with dry grass in the evening. In the morning after animals emerged from their burrows, they were rechecked for the presence of intact dry grass. Burrows were considered used by *S. citellus* if dry grass was pushed outside the burrow when animals left their burrows in the morning ("plug") or pushed inside the burrow when animals returned to their burrows during the day ("ring", **Fig. 11**).

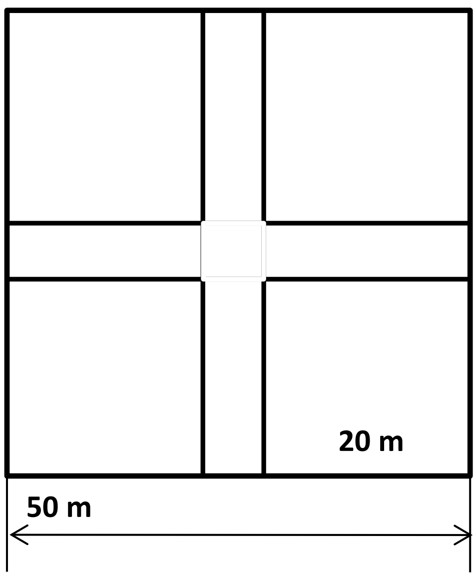

Figure 9 Sampling design for establishing the size of the *S. citellus* colony in the study area.

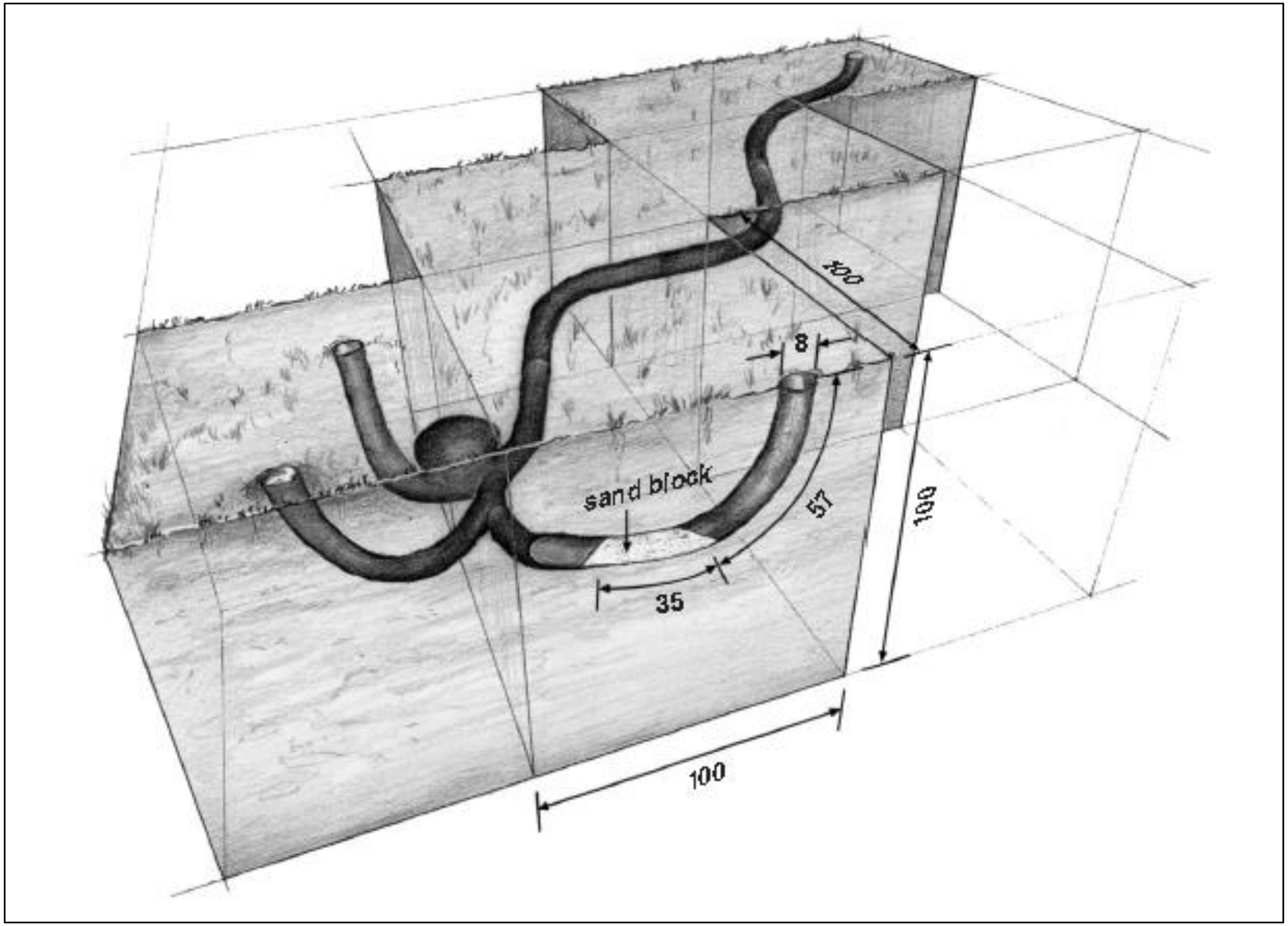

Figure 10 Structure of *S. citellus* burrow (Hut & Scharff, 1998).

Figure 11 Burrows used by *S. citellus* with dry grass pushed outside the burrow ("plug") or pushed inside the burrow ("ring").

Age and sex structure of S. citellus *colony*

To establish the age and sex structure of the *S. citellus* colony, animals were captured following the procedure described by Vaughan et al. (2006). Captured animals were sexed, aged, and weighed to the nearest 0.05 g using a Team Cormoran digital scale. Animals were transferred to wire traps to dry and released at the point of capture at their burrows' entrances. Animal capturing and handling were carried out by the experienced zoologist, Nada Ćosić from the University of Belgrade. No animal health or welfare issues arose during the capture and handling. All capturing and handling of animals was conducted under the auspices of the Animal Care and Ethics Committee of the University of Belgrade and the Ministry of Agriculture and Environmental Protection research permit.

Statistical and spatial analyses

To identify vegetation types present in the study area, the data matrix in Turboveg ver. 2.1.07. was subjected to the Cluster Analysis using the Relative Sørensen distance measure and Ward's group linkage method in PC-ORD ver. 4. The species percentage frequency and modified fidelity index (phi coefficient) were used to identify species specific for the distinguished clusters (**Appendix 1**). The Detrended Correspondence Analysis (DCA) was carried out using the Ellenberg's indicator values to establish environmental requirements (air temperature, climatic continentality, light availability, soil pH, moisture, and soil fertility) of plant species present within the studied vegetation quadrats (n = 124).

All distinguished vegetation types and *S. citellus* burrows were plotted on a Google Satellite map with Quantum GIS for Desktop 1.8.0. Lisboa. The probability density of the *S. citellus* colony based on the number and spatial aggregation of burrows was calculated in ArcGIS for Desktop 3.3. using the Fixed Kernel Home Range function (Worton, 1989).

The distribution of *S. citellus* burrows in regards to slope and aspect, as well as vegetation type, cover, and height, and grazing intensity was analysed using the Chi-squared tests. To explore the distribution of the *S. citellus* colony in regards to all studied environmental, vegetation, and management variables, the Binomial Generalised Linear Models (GLMs) with logit link function were built using the statistical program R ver. 3.0.1. To delimit effects of space autocorrelation, the variable "Transect" (where individual quadrats were nested) was included in all models as an explanatory covariable (after Ricankova et al., 2006). First, single variables were included in the "Null" model and tested for which variable gave significant results using Akaike Information Criterion (AIK) and type-II ANOVA. Since there was no interaction stated between "Transect" and tested variables, in the next computation only the significant variables were used for building more complex models. All variables chosen in the previous step (significant result and lowest AIC) were included in the best model and the procedure was repeated until the model fit was not improved.

The *S. citellus* colony's size was calculated in Excel® by dividing the average number of opened burrows per hectare by the average number of burrows opened by individual animals (taken from Ružić-Petrov, 1950; a study carried out in the same study area). To test if sex ratio in the studied colony was different from expected 1:1 the Chi-squared Goodness of Fit test was used. To establish differences in body mass among individuals depending on sex and age, the Mann-Whitney U-test was used since data departed from normality when analysed using the Shapiro-Wilk test. All values were expressed as means with standard error or medians with range. All calculations were performed using the statistical program R ver. 3.0.1. with a significance level $\alpha = 0.05$.

Results

Vegetation composition and structure

In the study area, six vegetation types (clusters) were identified characterised by several indicator species (**Fig. 12**; **Appendix 1**). Cluster 1 represented a degraded steppe with the encroaching exotic tree, *Ailanthus altissima*. Steppe vegetation was represented by the grasses, *Festuca valesiaca* and *Dactylis glomerata*, and leguminous perennials, *Vicia angustifolia* and *Medicago falcata*. Cluster 2 represented a species-rich steppe from the *Festuceto-Potentilletum arenariae* association and was dominated by *F. valesiaca*, and perennial herbs *Potentilla arenaria*, *Thymus pannonicus* and *T. glabrescens*. Cluster 3 represented a transitional community between steppe and grassland and was dominated by *F. valesiaca*, and leguminous perennials, *Trifolium campestre* and *Medicago minima*. Cluster 4 represented a species-poor steppe and was dominated by the *Festuca* species, most likely *Festuca valesiaca* and *F. pseudovina*. Cluster 5 represented a grassland dominated by the grass *Bromus hordeaceus*, and the annual herb, *Sherardia arvensis*. The other common grassland species included the perennial herb, *Achillea millefolium*, and the annual herb, *Erodium cicutarium*. Finally, cluster 6 represented a degraded grassland with nitrophilous weedy species, *Onopordum acanthium* and *Xanthium spinosum*.

The identified vegetation types had patchy distribution in the study area (**Fig. 13**). The degraded steppe with *Ailanthus altissima* (Cluster 1) occupied the pasture's outermost parts and bordered cultivated fields and road. The species-rich steppe (Cluster 2) occupied the pasture's south-east end and central part, a former horse racing ground, which was intermixed with grasssland vegetation (Clusters 3, 4 and 5). The degraded grassland with *Onopordum acanthium* (Cluster 6) occupied the pasture's north-east side where previously sheep were kept in an enclosure overnight. Most of plant species found in vegetation quadrats within the study area were habitat generalists or had no special environmental requirements (**Fig. 14**). Plant species from the degraded grassland with *Onopordum acanthium* (Cluster 6) were the only exception and were found to have high nutrient and moisture requirements.

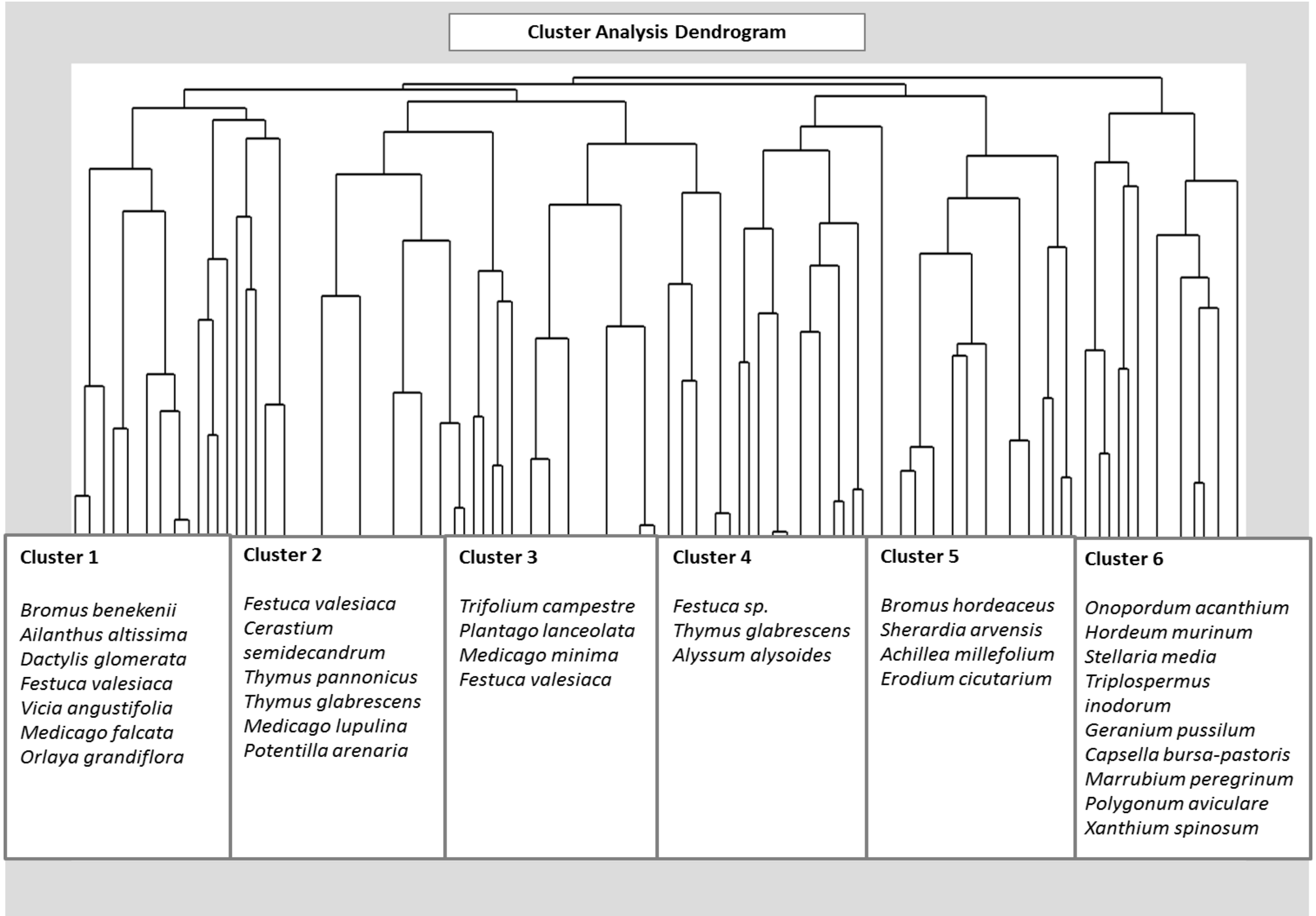

Figure 12 Cluster analysis dendrogram of vegetation types (six clusters) with indicator species in the study area.

Figure 13 Distribution of vegetation types (six clusters) in the study area. Cluster 1 (blue dots) represents degraded steppe with *Ailanthus altissima*, Cluster 2 (purple dots) species-rich steppe, Cluster 3 (yellow dots) steppe-grassland, Cluster 4 (red dots) species-poor steppe, Cluster 5 (green dots) grassland, and Cluster 6 (grey dots) degraded grassland with *Onopordum acanthium*.

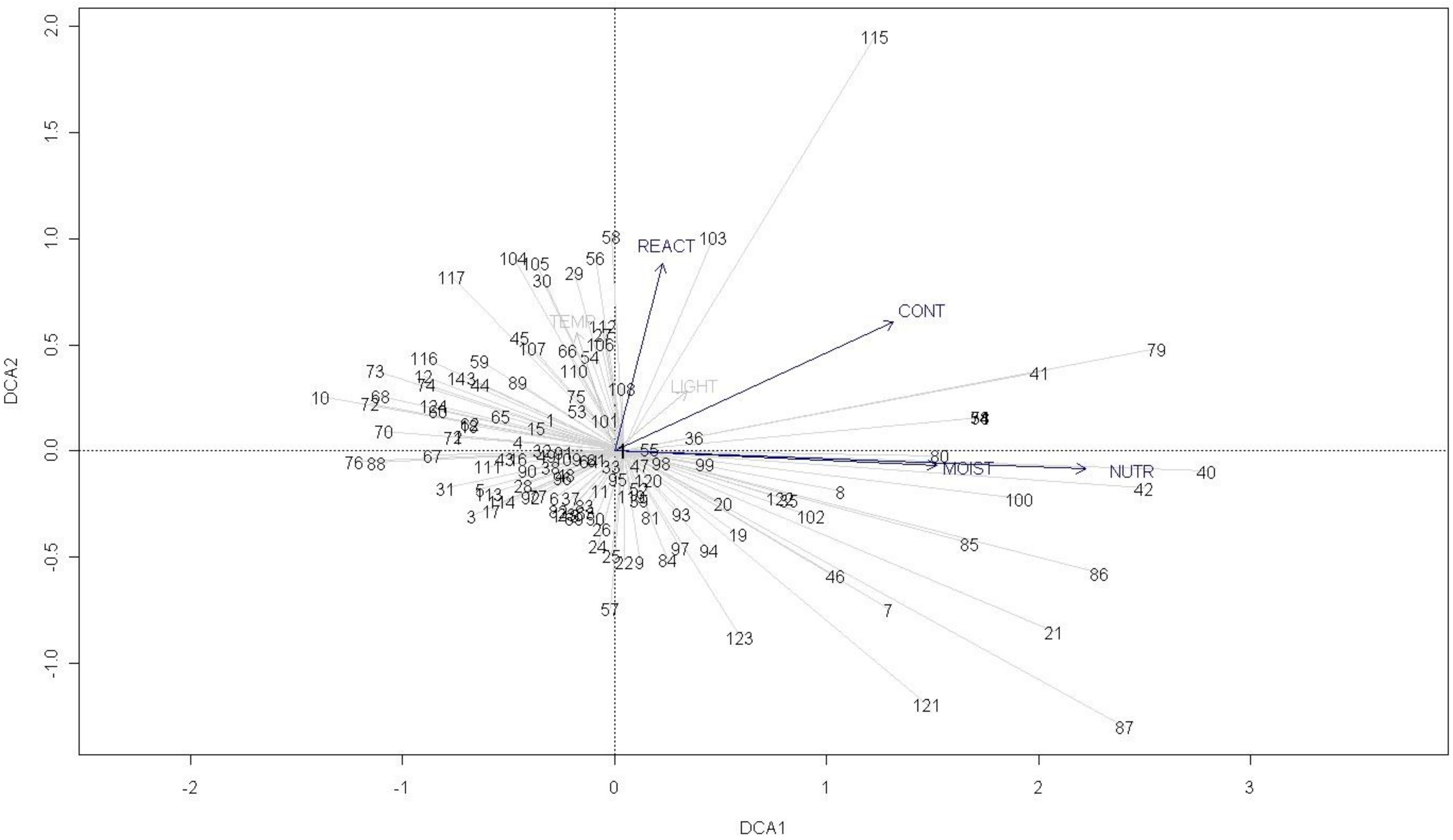

Figure 14 Detrended correspondence analysis (DCA) of environmental requirements of plant species in the study area (numbers represent vegetation quadrats). Abbreviations represent Ellenberg's indicator values: TEMP (air temperature), CONT (climatic continentality), LIGHT (light availability), REACT (soil pH), MOIST (soil moisture), NUTR (soil fertility).

Distribution of S. citellus *colony*

The areal extent of *S. citellus* colony determined from the position of the outermost burrows was 8.6 ha and covered 58.5% of the study area. The studied colony had aggregated distribution with the majority of burrows found in the pasture's south-east end (**Fig. 15**). Burrows were found most often on flat ground (80.5% of cases; $\chi^2 = 43.3659$, df = 2, $P < 0.001$), in areas with a thick vegetation cover exceeding 90% (92.7% of cases; $\chi^2 = 65.3171$, df = 2, $P < 0.001$), and low and medium height vegetation (> 10 cm 24.4% and > 20 cm 70.7% of cases; $\chi^2 = 28.1463$, df = 2, $P < 0.001$), as well as in moderately and intensively grazed areas (78.1 % of cases; $\chi^2 = 3.7073$, df = 2, $P = 0.157$) with species-rich steppe (Cluster 2; 29.7% of cases; **Fig. 16**) and steppe-pasture vegetation (Cluster 3; 31.7% of cases; $\chi^2 = 18.561$, df = 5, $P < 0.05$). Finally, when taking into account all measured environmental, vegetation, and management variables, the Binomial Generalised Linear Model (GLM) indicated that the best variables for predicting the *S. citellus* colony distribution were water distance and grazing intensity ($D^2 = 0.22$, $P < 0.001$; **Fig. 17**, **Tab. 2**).

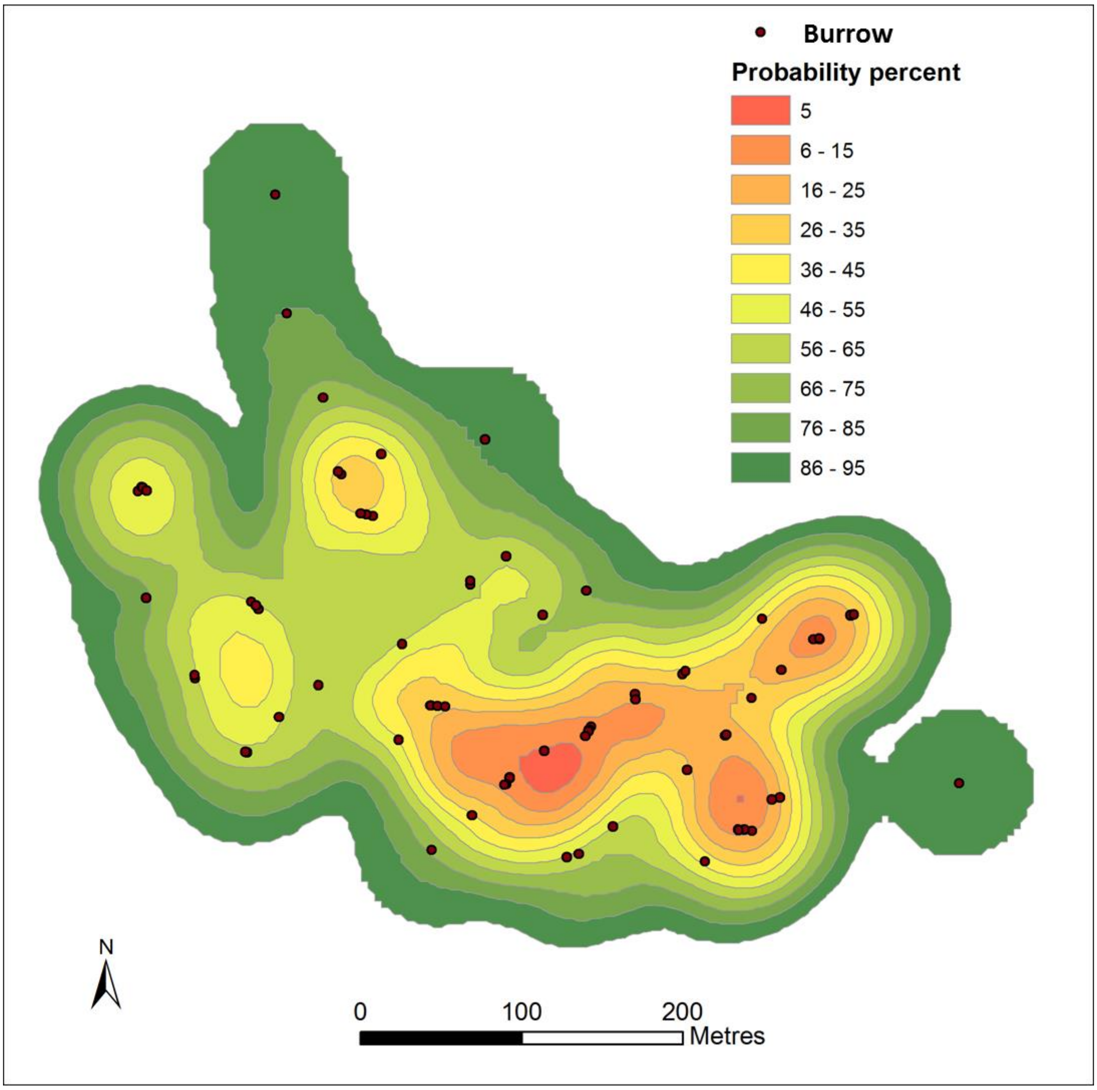

Figure 15 Probability density of *S. citellus* burrows in the study area.

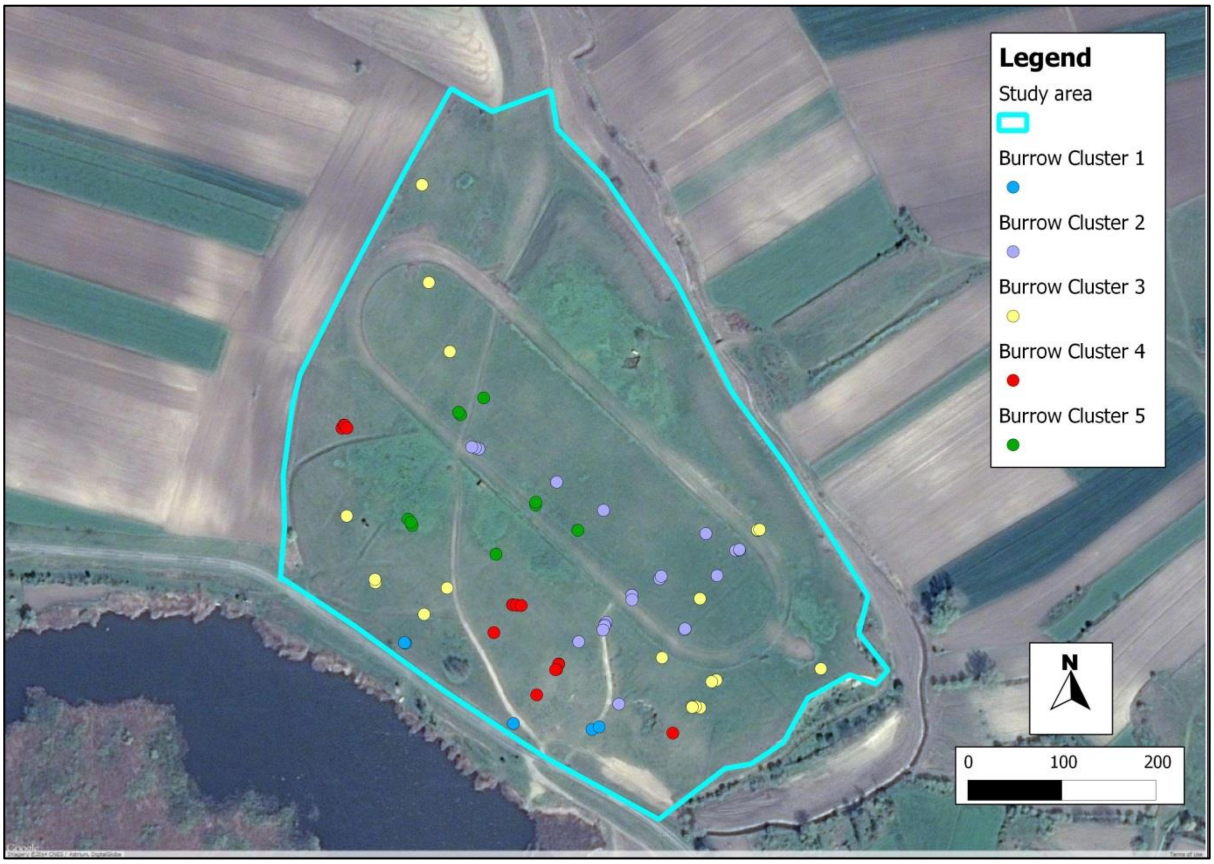

Figure 16 Distribution of the *S. citellus* colony in relation to vegetation types (six clusters). Cluster 1 (blue dots) represents degraded steppe with *Ailanthus altissima*, Cluster 2 (purple dots) species-rich steppe, Cluster 3 (yellow dots) steppe-grassland, Cluster 4 (red dots) species-poor steppe, Cluster 5 (green dots) grassland.

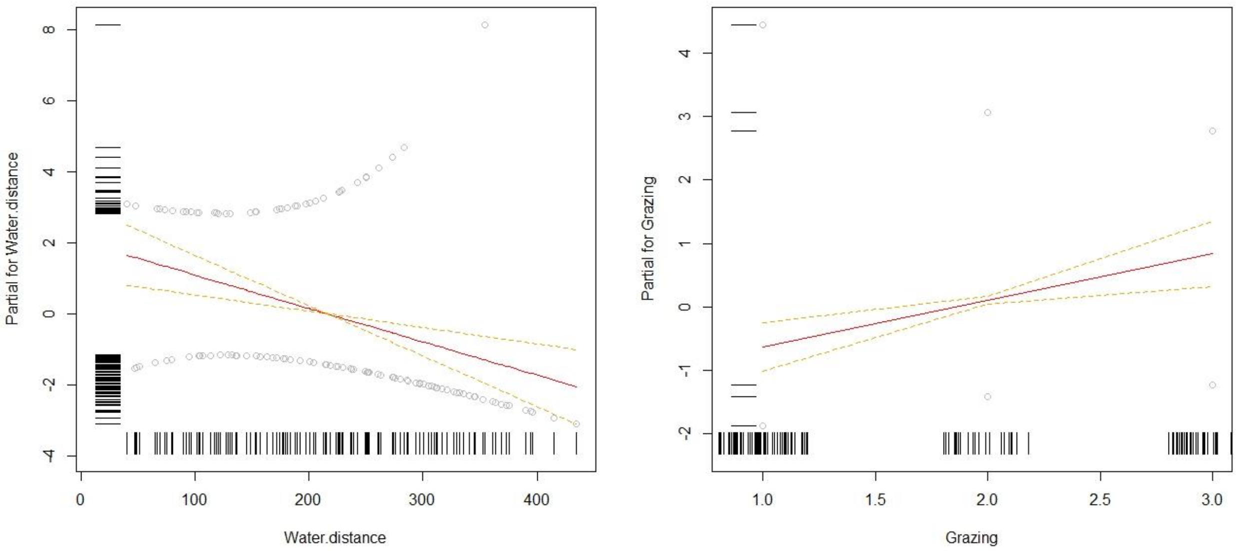

Figure 17 Effect of water distance and grazing on the distribution of the *S. citellus* colony. The broken lines represent 95% confidence intervals.

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Table 2 Effects of single variables and the best Binomial Generalised Model on the distribution of the *S. citellus* colony. The asterisk indicates significance at P < 0.05 (*), P < 0.01 (**), and P < 0.001 (***) levels. Abbreviation NS stands for not significant and AIC, Akaike Information Criterion

Model	**Res. Dev.**	**Res. df**	**AIC**	**P**
Null	157.39	123	159.39	
Null + Transect	151.58	122	155.58	*
Environment				
Aspect	156.84	122	157.88	NS
Slope	156.84	122	157.88	NS
Altitude	155.22	122	159.13	NS
Water distance	138.99	122	143.57	***
Vegetation				
Cover	157.33	122	159.48	NS
Height	152.85	122	153.85	*
Type	155.99	122	156.41	NS
Management				
Grazing	146.14	122	149.62	***
Best model-combined variables				
Water distance + Grazing	122.18	121	128.18	***

Size of S. citellus *colony*

In the burrow blocking experiment, designed to establish the *S. citellus* colony size, out of 323 burrows closed with dry grass in the evening, 252 burrows or 78% were found to have been opened by animals the next day (**Fig. 18**). In 44.8% of the cases, dry grass was pushed outside the burrow ("plug") by the emerging animals, and in 45.2% of the cases dry grass was pushed inside the burrow ("ring") by animals returning to their burrows. The average number of opened burrows per sampling plot (20 x 20 m) was 21 ± 1.6 or 525 ± 38.8 per hectare. Since one individual can on average open 9.3 blocked burrows (Ružić-Petrov, 1950), it was estimated that in the study area there were 56 individuals per hectare. It is important to stress here that during the experiment 30 burrows were missed when blocking the entrances introducing a 9.3% sampling error.

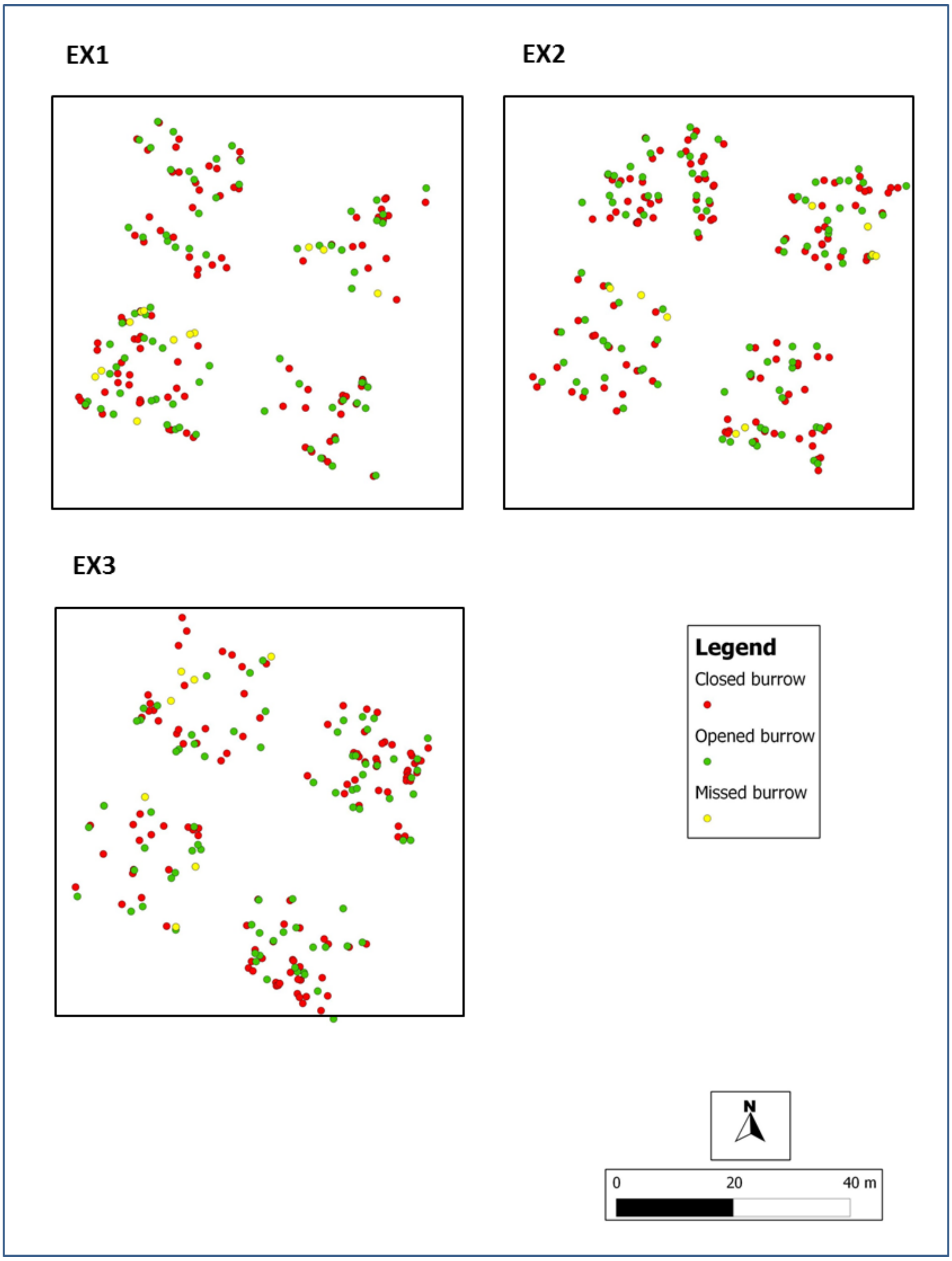

Figure 18 Distribution of *S. citellus* burrows in three experimental plots. Red dots represent burrows that were closed with dry grass, green dots represent burrows that were opened by animals, and yellow dots represent burrows that were missed in the experiment.

Out of 31 individuals trapped in the study area, 13 were adults and 18 were juveniles (**Tab. 3**). The sex ratio (the proportion of males to females) in adults was slightly female biased (0.46; χ^2 = 0.0769, df = 1, P = 0.782) and among juveniles it was male biased (0.67; χ^2 = 2, df = 1, P = 0.157). Body mass varied among individuals depending on sex and age (**Tab. 3**).Yet, no significant difference was observed in body mass among adult males and females (adult males, median 324 g, range 2 74–414 g, versus adult females, median 354 g, range 254–354 g; Mann-Whitney U-test = 24.5, P = 0.663). Juvenile females were slightly heavier than juvenile males (juvenile females, median 234 g, range 214–254 g, versus juvenile males, median 214 g, range 174–254), but the result was not significant (Mann-Whitney U-test, P = 0.113).

Table 3 Summary statistics of age and sex structure and body mass (g) of *S. citellus*

Sex	**Age**	**Body mass (g)**					**n**
		Mean	SE	Median	Range	CV	
Male	Adult	344.0	23.5	324	274–414	16.8	6
Male	Juvenile	219.0	7.3	214	174–254	11.6	12
Female	Adult	319.7	16.7	354	254–354	13.9	7
Female	Juvenile	237.3	6.2	234	214–254	6.3	6

Discussion

Microhabitat requirements and distribution of S. citellus *colony*

The distribution of the *S. citellus* colony in the Special Nature Reserve "Kraljevac" was determined mainly by grazing intensity and distance to the water source as well as flatness of the ground, thickness and height of vegetation cover, and vegetation type. *S. citellus* preferred species-rich steppe and steppe-grassland and avoided areas dominated by weedy species.

In the study area, *S. citellus* avoided excavating burrows on steep terrain, reaching 70 degrees in the north-east end of the pasture, probably due to difficult burrow construction and lower water permeability of the soil surface. The flat terrain with better drainage and a higher proportion of coarse sandy particles requires less energy expenditure during excavation, prevents the collapse of tunnels, and allows the achievement of sufficient depth below ground (reaching two meters in some parts of the Deliblato Sands; Ružić-Petrov, 1950) to create stable environmental conditions for raising young and for hibernation. Similarly, other burrowing animals, such as badgers, foxes, and Arctic lynx, have been found to select their den sites based on the angle of slope, soil type, and vegetation cover (Smith et al. 1992; Slough, 1999; Macdonald et al., 2004).

Gedeon et al. (2012) recognized the presence of short-stalk grass cover, which allows easy detection of predators (> 20 cm), as the main factor in determining *S. citellus* occurrence. *S. citellus* relies on its sight for predator detection and just like meerkats (*Suricata suricatta*), another colonial and burrowing species, emit predator-specific alarm calls to warn colony members of potential danger (Manser, 2001; Koshev & Pandourski, 2008). Furthermore, Janák, Marhoul, and Matějů (2013) stated that vegetation cover and height are more important for *S. citellus* than specific vegetation types or plant species due its broad diet and high adaptability to local conditions. The current study strengthens this view by showing that *S. citellus* occurred in all vegetation types with different frequency, yet avoided degraded grassland with two-meter-high *Onopordum acanthium* and highly toxic *Ailanthus altissima* (**Fig. 19**).

Figure 19 Degraded grassland with *Onopordum acanthium* and *Ailanthus altissima*.

Vegetation height is closely linked with grazing intensity; the current study demonstrated that *S. citellus* was most often found in moderately and intensively grazed parts of the pasture. Notably, intensively grazed parts of the pasture did not experience vegetation degradation or species loss due to overgrazing and trampling, suggesting that the current number of sheep is optimal (100 sheep/ 6 ha). Yet, other parts of pasture were overgrown and invaded by *Onopordum acanthium, Xanthium spinosum*, and *Ailanthus altissima* due to the abandonment of traditional grazing resulting in the disappearance of suitable microhabitats for *S. citellus*. Because of an increase of grass height in natural grasslands, especially in the European steppe of Russia, two species of ground squirrels, *S. citellus* and *S. pygmaeus*, almost completely disappeared (Galushin, Moseikin & Sanin, 2000).

Finally, distance to the lake was one of the key factors determining the distribution of the *S. citellus* colony. The species current distribution in the Vojvodina Province with most of the populations found along the Danube and Tisza Rivers (Ćosić et al., 2013) aligns with this finding. Yet, the species is known to be negatively affected by flooding and high water table levels (Katona, Váczi & Altbäcker, 2002; Koshev, 2008; Baltag et al., 2014) possibly suggesting a negative colonization process in the study area. Namely, animals first started excavating burrows in the most optimal habitat at the elevated plateau (old horserace ground) and, as population density increased, moved gradually to lower, suboptimal habitats close to the lake and road. As a result, *S. citellus* dispersal success might be decreased due to the incidence of roadkill and the lake as a gene flow barrier.

Population status

In the current study, the *S. citellus* population density was 56 individuals/ ha, similar to Ruzić-Petrov's 1948 density record (48 individuals/ ha) at the experimental plot close to the study area. Similarly, Ćirović, Ćosić and Penezić (2008) noted that the mean density in the Vojvodina Province was 41.6 individuals/ ha. The current findings suggest that the studied population is viable and survival conditions in the Special Nature Reserve "Kraljevac" area are favourable. Viability was also reflected in the sex ratio of adults only slightly deviating from the expected 1:1. The juvenile sex ratio was male biased indicating low mortality rates probably due to the lack of long-distance dispersal movements and low predation rates. Yet, the lack of significant differences in body mass between sexes in both age categories was surprising and could be explained by a small sample size and require further investigation.

Conservation and management guidelines

To improve the conservation status and management of *S. citellus* habitats in the Special Nature Reserve "Kraljevac" and the Deliblato Sands region the following measures are recommended:

- intensification of grazing, evenly spread across *S. citellus* occupied areas, through the introduction of additional mixed sheep and goat herds (not more than 100 individuals/6 ha);
- additional mowing throughout the vegetation season (May—July) of areas with inadequate grazing to maintain short-stalk grass cover (> 20 cm high);
- mechanical removal of weeds, *Onopordum acanthium* and *Xanthium spinosum*, before their inflorescence and seed production;
- Chemical control of *Ailanthus altissima* with systemic low toxicity herbicides, such as Garlon 4 (Burch & Zedaker, 2003);
- translocation of sheep enclosures from pastures to areas unsuitable for *S. citellus* colonization and outside of their colony range;
- predator control and removal of stray dogs observed destroying *S. citellus* burrows in the study area;
- monitoring of *S. citellus* population fluctuations using the non-invasive method of burrow blocking;
- increasing public awareness about the conservation value of *S. citellus* as important prey for critically endangered raptors, the Eastern imperial eagle and the saker falcon; and
- securing government, European Union, and NGO funding for promoting traditional grazing practices and implementing conservation measures.

Conclusions

The current study demonstrated that *S. citellus* has complex microhabitat requirements with grazing intensity being the most important factor for the survival of this vulnerable species. While *S. citellus* population is viable in the Special Nature Reserve "Kraljevac," its habitat has been degraded and reduced in size due to inadequate management practices. Lack of sufficient grazing or its complete abandonment in some parts of the pasture, unsuitable position of sheep enclosures resulting in soil nitrification, and the growth and spread of invasive weeds were identified as major threats. Future management actions should focus on addressing these threats if the Special Nature Reserve "Kraljevac" is to be used as a role model for the proper management of *S. citellus* in the whole Deliblato Sands region.

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Appendix 1 Synoptic table with species percentage frequency and modified fidelity index (phi coefficient) for six distinguished clusters

Cluster No.	**1**	**2**	**3**	**4**	**5**	**6**
No. of relevés	24	23	23	17	19	18
Achillea millefolium	**88** ·	**70** ·	**91** $^{10.9}$	**82** $^{1.9}$	**95** $^{\mathbf{19.0}}$	33 ·
Achillea pannonica	26 $^{18.2}$	4 ·	6 ·	.	17 $^{4.0}$	.
Agrimonia eupatoria	4 $^{9.1}$	.	.	.	.	.
Ailanthus altissima	17 $^{\mathbf{31.1}}$	4 ·	.	.	.	.
Alyssum alyssoides	8 ·	.	.	18 $^{\mathbf{20.1}}$	5 ·	.
Alyssum desertorum	4 ·	9 $^{1.6}$	4 ·	12 $^{8.5}$	.	.
Ambrosia artemisiifolia	.	.	.	.	.	6 $^{10.5}$
Anthriscus cerefolium	4 ·	.	4 $^{1.2}$	12 $^{10.0}$	.	.
Aphanes arvensis	.	.	9 $^{7.5}$	.	11 $^{7.4}$	.
Arenaria serpyllifolia	21 ·	52 $^{6.6}$	**78** $^{8.8}$	47 $^{1.0}$	63 $^{6.3}$	17 ·
Ballota nigra	.	.	.	.	.	17 $^{15.8}$
Berteroa incana	12 ·	22 $^{7.1}$	17 $^{3.1}$	6 ·	11 ·	.
Bromus benekenii	50 $^{\mathbf{33.6}}$	4 ·	4 ·	6 ·	11 ·	.
Bromus hordeaceus	17 ·	9 ·	13 ·	12 ·	**89** $^{\mathbf{53.2}}$	6 ·
Bromus sterilis	.	.	13 $^{12.1}$	6 ·	5 $^{6.7}$	6 ·
Bromus tectorum	21 ·	22 ·	52 $^{5.9}$	47 $^{2.7}$	53 $^{4.1}$	**78** $^{14.5}$
Capsella bursa-pastoris	.	.	4 ·	.	11 ·	39 $^{\mathbf{25.0}}$
Carduus nutans	12 $^{8.3}$	.	4 ·	6 ·	5 ·	17 $^{5.5}$
Carex stenophylla	.	.	4 $^{4.4}$	6 $^{7.0}$	.	.
Carlina vulgaris	.	4 ·	4 ·	6 $^{2.3}$	11 $^{5.3}$	.
Cerastium holosteoides	4 $^{4.2}$	.	.	6 $^{7.1}$	.	.
Cerastium semidecandrum	17 ·	**74** $^{\mathbf{23.3}}$	48 ·	41 $^{1.1}$	53 $^{4.3}$	.
Chenopodium album	.	4 $^{2.5}$	.	.	.	6 $^{7.8}$
Cirsium arvense	8 $^{7.9}$	.	4 $^{4.0}$	.	.	.
Convolvulus arvensis	21 $^{6.7}$	.	.	6 ·	5 ·	33 $^{15.1}$
Cruciata pedemontana	21 $^{4.8}$	4 ·	13 ·	12 ·	32 $^{11.4}$	.
Dactylis glomerata	38 $^{\mathbf{28.6}}$	.	.	12 ·	.	.
Descurainia sophia	.	.	.	6 $^{2.4}$	.	17 $^{14.6}$
Elymus repens	29 ·	**83** $^{9.3}$	**78** $^{5.3}$	**82** $^{1.8}$	63 ·	**83** $^{16.7}$
Erigeron annuus	.	.	.	.	.	6 $^{10.5}$
Erodium cicutarium	8 ·	26 ·	48 $^{5.0}$	29 ·	**74** $^{\mathbf{13.0}}$	28 ·
Eryngium campestre	46 $^{6.3}$	48 $^{9.0}$	13 ·	35 $^{8.8}$	32 ·	.
Euphorbia cyparissias	33 $^{12.0}$	4 ·	4 ·	12 $^{8.4}$	.	.

Cluster No.	1	2	3	4	5	6
No. of relevés	24	23	23	17	19	18
Euphorbia helioscopia	$4^{6.2}$	$4^{6.7}$	4 ·	.	.	.
Euphorbia seguieriana	$4^{4.2}$	.	.	$6^{7.1}$	.	.
Falcaria vulgaris	.	.	$4^{4.4}$	$6^{7.0}$	.	.
Festuca sp.	8 ·	9 ·	.	**82** $^{\mathbf{61.9}}$	.	6 ·
Festuca valesiaca	**83** $^{\mathbf{27.0}}$	**100** $^{\mathbf{36.1}}$	**87** $^{\mathbf{17.2}}$	.	53 ·	6 ·
Galium aparine	$4^{2.4}$	.	.	.	$11^{11.3}$	.
Galium verum	.	.	.	6 $^{\mathbf{16.6}}$	.	.
Geranium pusillum	38 ·	26 ·	**78** ·	47 ·	**84** $^{5.1}$	**83** $^{\mathbf{25.7}}$
Glechoma hederacea	$4^{9.9}$	.	.	.	.	$6^{4.1}$
Hordeum murinum	12 ·	13 ·	9 ·	12 ·	$32^{3.7}$	**94** $^{\mathbf{52.2}}$
Lactuca serriola	$4^{4.5}$	.	.	.	$5^{6.4}$	.
Lepidium campestre	$4^{13.9}$	.	.	.	.	.
Lolium perenne	.	.	.	.	$5^{3.3}$	$6^{7.3}$
Malva neglecta	.	.	.	.	$5^{10.2}$	.
Marrubium peregrinum	8 ·	.	4 ·	6 ·	.	33 $^{\mathbf{22.6}}$
Medicago falcata	21 $^{\mathbf{24.9}}$	.	4 ·	.	.	.
Medicago lupulina	$25^{4.5}$	39 $^{\mathbf{13.3}}$	9 ·	12 ·	11 ·	$44^{6.1}$
Medicago minima	33 ·	**70** $^{3.8}$	**96** $^{\mathbf{24.3}}$	**76** $^{8.1}$	$63^{4.6}$	11 ·
Mentha longifolia	$4^{13.9}$	.	.	.	.	.
Onopordum acanthium	4 ·	.	.	6 ·	16 ·	**78** $^{\mathbf{54.5}}$
Orlaya grandiflora	17 $^{\mathbf{23.6}}$	.	.	.	.	.
Pimpinella saxifraga	$12^{4.7}$	$13^{3.8}$	.	$18^{9.0}$	.	.
Plantago lanceolata	46 ·	39 ·	**96** $^{\mathbf{25.2}}$	$47^{1.3}$	32 ·	22 ·
Poa angustifolia	$25^{11.1}$	$22^{5.8}$	13 ·	12 ·	21 ·	22 ·
Poa annua	$4^{8.5}$	.	4 ·	.	.	$6^{3.2}$
Poa compressa	.	.	.	.	.	$6^{10.5}$
Polygonum aviculare	.	.	.	.	.	17 $^{\mathbf{22.0}}$
Potentilla arenaria	$12^{7.7}$	9 $^{\mathbf{9.5}}$	4 ·	6 ·	.	.
Potentilla reptans	$4^{10.6}$	.	$4^{2.9}$	.	.	.
Ranunculus bulbosus	$17^{3.8}$	$17^{4.3}$	9 ·	$18^{4.5}$	5 ·	.
Ranunculus sardous	8 ·	$17^{9.4}$	.	$12^{1.4}$	$16^{4.2}$	.
Rumex conglomeratus	4 ·	4 ·	4 ·	.	$21^{6.4}$	$28^{12.5}$
Rumex crispus	.	.	.	.	.	$6^{8.4}$
Scleranthus annuus	$13^{5.1}$	$13^{10.0}$	$12^{2.2}$	.	.	.
Sherardia arvensis	**83** ·	**78** ·	**70** ·	**76** $^{2.8}$	**95** $^{\mathbf{29.2}}$	6 ·
Sisymbrium officinale	$4^{1.3}$	.	.	.	.	$17^{14.2}$

Cluster No.	**1**	**2**	**3**	**4**	**5**	**6**
No. of relevés	24	23	23	17	19	18
Sisymbrium orientale	8 $^{1.1}$	.	17 $^{8.7}$	.	11 $^{3.0}$	6 $^{\cdot}$
Sonchus arvensis	4 $^{9.1}$	.	.	.	.	.
Stachys germanica	8 $^{7.9}$	.	4 $^{4.0}$	.	.	.
Stellaria media	.	.	.	.	.	28 $^{\mathbf{31.9}}$
Taraxacum officinale	8 $^{5.0}$	.	.	6 $^{2.2}$	.	11 $^{5.8}$
Thymus glabrescens	58 $^{\cdot}$	**87** $^{\mathbf{15.9}}$	43 $^{\cdot}$	**76** $^{\mathbf{20.9}}$	47 $^{\cdot}$	.
Thymus pannonicus	21 $^{\cdot}$	35 $^{\mathbf{18.6}}$	26 $^{10.2}$	12 $^{\cdot}$	.	.
Trifolium campestre	25 $^{\cdot}$	61 $^{\cdot}$	**91** $^{\mathbf{28.0}}$	53 $^{\cdot}$	58 $^{4.1}$	22 $^{\cdot}$
Trifolium repens	17 $^{7.9}$	4 $^{\cdot}$	9 $^{\cdot}$	6 $^{\cdot}$	16 $^{3.0}$	17 $^{4.8}$
Trifolium striatum	.	.	4 $^{9.3}$	.	.	.
Trigonella monspeliaca	.	22 $^{13.7}$	9 $^{\cdot}$	18 $^{6.4}$	.	.
Tripleurospermum inodorum	.	.	.	.	.	22 $^{\mathbf{31.8}}$
Valerianella locusta	4 $^{9.1}$	.	.	.	.	.
Veronica arvensis	21 $^{\cdot}$	48 $^{4.6}$	30 $^{\cdot}$	29 $^{\cdot}$	32 $^{1.1}$	50 $^{5.4}$
Veronica opaca	.	4 $^{2.4}$	.	.	.	11 $^{11.6}$
Veronica persica	.	.	.	.	.	17 $^{\mathbf{18.3}}$
Veronica praecox	4 $^{4.2}$	.	.	6 $^{7.1}$	.	.
Vicia angustifolia	**79** $^{\mathbf{25.6}}$	26 $^{4.2}$	9 $^{\cdot}$	24 $^{\cdot}$	.	.
Vicia lathyroides	.	4 $^{3.9}$	9 $^{8.2}$	.	.	.
Xanthium spinosum	4 $^{\cdot}$	.	.	.	.	6 $^{\mathbf{19.0}}$