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A Pilot Monitoring Survey of the Lesser Blind Mole-rat *Nannospalax* (superspecies *leucodon*) (Mammalia: Rodentia) in Bulgaria

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Abstract: A pilot monitoring survey of the lesser blind mole-rat *Nannospalax* (superspecies *leucodon*) in Bulgaria was carried out in July–October 2014 for the purposes of the National Biodiversity Monitoring System. A methodological approach for long-term monitoring of the species is proposed and discussed. A total of 122 transects were done in suitable habitats in four large territories representing three biogeographic regions in Bulgaria. Actual distribution data of the species were collected. The occurrence of *N. leucodon* is considered at the transect level, at the monitoring territory level and at the habitat type level.

Key words: *Nannospalax*, lesser blind mole-rat, monitoring, methodology

Introduction

The lesser blind mole-rat *Nannospalax leucodon* (Nordmann, 1840) (mole-rat hereafter) is a phytophagous subterranean rodent with a restricted distribution range (Balkan Peninsula, northwest to Hungary and northeast to Moldova and southern Ukraine). It faces a serious threat by habitat loss and habitat fragmentation (Savić & Soldatović 1978, Németh et al. 2013, Kryštufek & Amori 2017). The mole-rat is an important component of grassland ecosystems. This species is a food resource for a number of bird and mammalian species, some of which are threatened (Nemeth et al. 2016). Due to its obscure taxonomy (Savić & Nevo 1990, Musser & Carleton 2005, Nemeth et al. 2009, Arslan et al. 2016), the global assessment of the lesser blind mole-rat is Data Deficient (Kryštufek & Amori 2017). Recent karyological studies (Nemeth et al. 2009, Kandemir et al. 2014) confirm the assumption that a detailed taxonomic revision would lead to split of the lesser mole-rat into several species, which are included in an aggregate of the rank of superspecies, i.e. *Nannospalax* (superspecies *leucodon*); some of species belonging to it may be threatened with extinction. As a hole, this superspecies is affected by range reduction and population decline (Kryštufek & Amori 2017). Balkan populations of the lesser blind mole-rat are in degradation for the last several decades of XX century (Petrov 1977, Peshev 1981).

The mole-rat is poorly investigated in Bulgaria. The underground lifestyle and the difficult distinction of the visible signs of its presence from those left by other species are among the main reasons for the insufficient knowledge of the species. The lack of monitoring and up-to-date data on its distribution and abundance in the country are reasons for the lack of current knowledge of what its population trends are. Detailed studies are needed on the taxonomy, distribution, ecology and biology of this superspecies complex. A special attention should be paid on the threatening factors for its populations, which effect is not completely elucidated.

The aim of the present study was to develop a methodology for monitoring of the lesser blind mole-rat, including to verify it in practice and to provide recommendations for its further improvement as well as to collect initial data for the future monitoring of this species. Moreover, the conducted field survey made it possible to provide up-to-date data on the distribution and habitat preferences of this rodent species group in Bulgaria. The study was conducted for the purposes of the National Biodiversity Monitoring System (NBMS). The methodological approach is published also as a report (in Bulgarian language) on the website of the Executive Environmental Agency (http://eea.government.bg/bg/bio/nsmbr/praktichesko-rakovodstvo-metodiki-za-monitoring-i-otsenka/Metodika_za_ocenka_Nannospalax_leucodon.pdf).

Materials and Methods

Data collection

Monitoring of fossorial rodents is difficult as it is problematic to correlate visible signs of the species presence with its population density. For this reason, an indirect index method was chosen to estimate the variation of the mole-rat abundance through the observed variation of the index value. The chosen approach was based on an easy-to-use, non-invasive method of registration and consisted in recording the presence of the species through the soil mounds resulted from the digging activity of the animals. The developed methodology was a modification of a method successfully applied for another fossorial small mammal species, the European mole *Talpa europaea* (Morilhat et al. 2007). The survey for signs of mole-rats was conducted over 1000 m transects. Each transect was divided to 100 m intervals and the presence/absence of the species was recorded for each of them. The number of mounds was not recorded as a non-reliable indicator for the species abundance – the size of the individual home ranges and the intensity of the burrowing activity had been found to vary greatly depending on a number of factors (Savic 1973, Zuri & Terkel 1997). The area investigated was 2.5 m on each side of the transect line (it is visible even in dense vegetation). The length of the intervals was conformed with a presumed area of the individual home range of the blind Mole-rat from 194 m² to 1000 m² (Savic 1973). We assumed that a single individual could be detected in the area of one 100 m interval and thus the re-counting of the same individual should be avoided. The habitat type was recorded for every 100 m interval. The chosen approach was based on the assumption that the number of positive intervals correlates with the relative abundance of the mole-rat.

The piles of soil resulting from the digging activity of the lesser blind mole-rat are very similar to those from other subterranean mammals (Gusztáv 2010). In Bulgaria, the mole-rat mounds could be mistaken most often for those of the European mole. For this reason, the presence of mole-rats was recorded only for the intervals where the piles of soil were relatively large, fresh and arranged in a row (typical for the mole-rat).

Study areas

The location and the extent of the study areas were selected on the basis of three criteria. First, they should comprise open habitats suitable for the lesser mole-rats (for habitat types, see Table 4). Second, it was desirable to cover the territory of the country as much as possible because the available information for the current distribution of the species in Bulgaria was insufficient and a large-scale survey was needed to serve as baseline data for future monitoring. Third, the study areas should cover territories, which were representative for the three biogeographic regions where the species was recorded in Bulgaria (Continental, Black Sea and Alpine).

A total of 122 transects were made in four monitoring territories (Fig. 1): 1. West Central Bulgaria (29 transects). 2. Thrace (30 transects). 3. Danube Plain (30 transects). 4. Northern Black Sea coast (33 transects).

Data analysis

The occurrence parameter (F=n/N, i.e. the frequency of positive sampling units) was chosen as main parameter giving indirect information about the species presence and abundance. Three levels of analysis were chosen:

1. Transect level: The occurrence was calculated as the number of intervals with mound presence per transect (n) to the total number of sectors in a transect (N). It gave information about the changes in the species status in time at the local level.

2. Monitoring territory: Number of transects with mound presence (n) to the total number of transects on the territory (N). It gave more general infor-

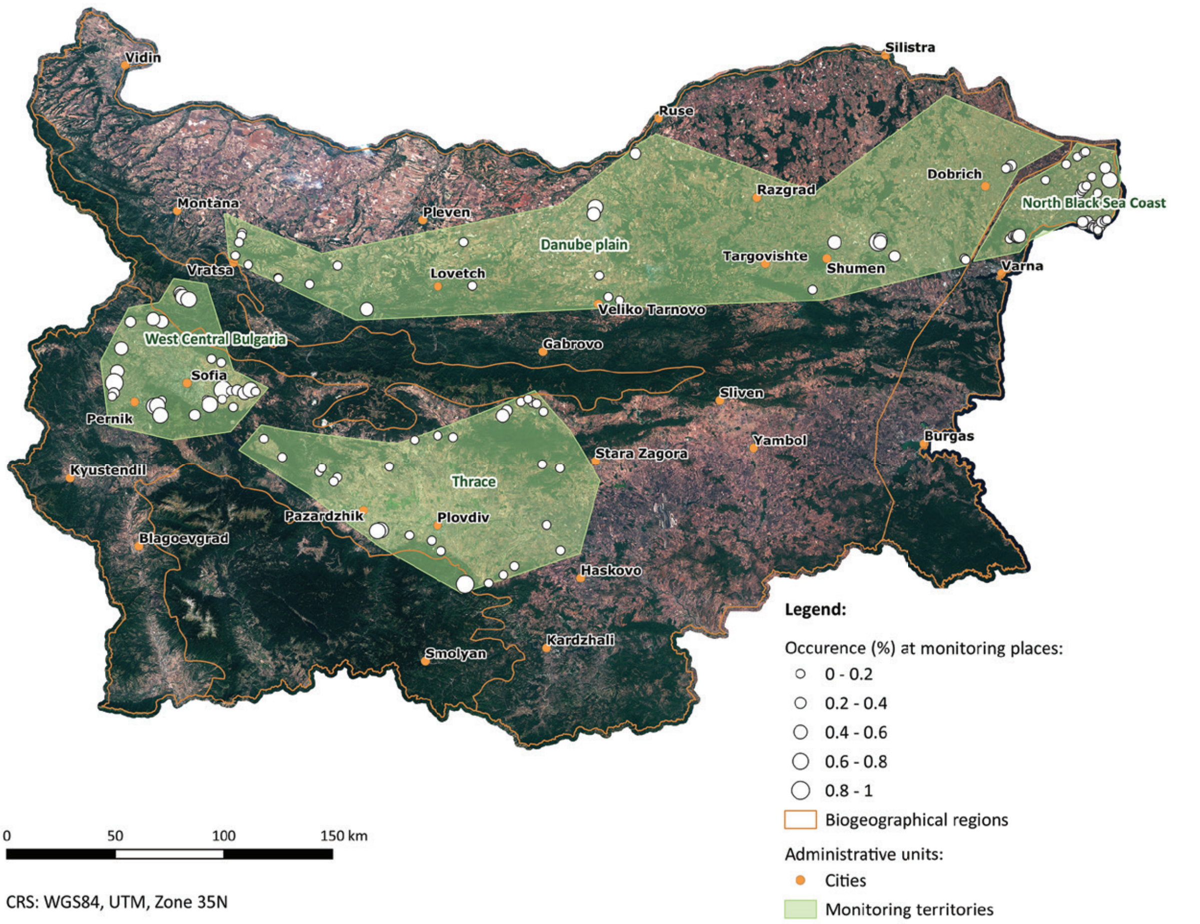

Fig. 1. A map of the occurrences of *Nannospalax leucodon* in the four monitoring territories based on a satellite map of Bulgaria from Sentinel 2, created by Geopolymorphic Cloud. Satellite imagery source: Copernicus.eu.

mation about changes in species status in time on the monitoring territory.

3. Habitat type: The occurrence was calculated as the number of intervals with mound presence in a habitat type (n) to the total number of intervals in that habitat (N). It gave information about the species habitat preferences.

As the samples were relatively small, the Exact Method of Clopper-Pearson for calculation the confidence intervals of data with binomial distribution was used. To test the significance of the differences between species occurrence in the monitoring territories, z-test for proportions was used.

Time period

The monitoring data were collected from the end of July to mid-October 2014. The choice of that period of the year was determined by the annual cycle of the species: the newly-born individuals had already become independent (Savic 1973), i.e. no distortion of results due to the specificity of the breeding period or the dispersal period of the young individuals was expected.

Results

The mole-rat was recorded in 73 out of 122 transects (Fig. 1, Table 1). The occurrence of the species was highest in West Central Bulgaria and lowest in Trace (Table 2). The results of z-test showed significant differences in *N. leucodon* occurrence between Trace and West Central Bulgaria as well between Trace and North Black Sea Coats (Table 3). We were treating the non-significant differences with reserves since the power of performed tests was very low – below 0.5. According to the collected data, the mole-rat showed preference to meadows, vineyards and shrubs (Table 4). It should be noted that the sample of shrubs is too small, so it probably distorted the results. The species was scarce in pasture, steppe and wasteland habitats.

Discussion

The implemented pilot monitoring investigation on the lesser blind mole-rat has allowed considerable amount of data on the distribution and habitat pref-

Table 1. Occurrence of *Nannospalax leucodon* at the transect level: WCB – West Central Bulgaria, T – Thrace, DP – Danube Plain, NBS – Northern Black Sea cost; n/N – occurrence, CI – confidence intervals.

Transect	n/N	95% CI	Transect	n/N	95% CI	Transect	n/N	95% CI	Transect	n/N	95% CI
NBSC01	0.6	0.26÷0.88	DP01	0	0	T01	0.1	0.003÷0.45	WCB01	0.6	0.26÷0.88
NBSC02	0.1	0.003÷0.45	DP02	0	0	T02	0	0	WCB02	0.4	0.12÷0.74
NBSC03	0.3	0.07÷0.65	DP03	0	0	T03	0	0	WCB03	0.7	0.35÷0.93
NBSC04	0.2	0.03÷0.56	DP04	0	0	T04	0	0	WCB04	0.7	0.35÷0.93
NBSC05	0.1	0.003÷0.45	DP05	0	0	T05	0	0	WCB05	0.5	0.19÷0.82
NBSC06	0	0	DP06	0	0	T06	0	0	WCB06	0.5	0.19÷0.82
NBSC07	0	0	DP07	0.1	0.003÷0.45	T07	0	0	WCB07	0.3	0.07÷0.65
NBSC08	0.2	0.03÷0.56	DP08	0.1	0.003÷0.45	T08	0	0	WCB08	0.5	0.19÷0.82
NBSC09	0.3	0.07÷0.65	DP09	0	0	T09	0.1	0.003÷0.45	WCB09	0.5	0.19÷0.82
NBSC10	0	0	DP10	0	0	T10	0	0	WCB10	1	0.69÷1
NBSC11	0.4	0.12÷0.74	DP11	0	0	T11	0.2	0.03÷0.56	WCB11	0.3	0.07÷0.65
NBSC12	0.3	0.07÷0.65	DP12	0.8	0.44÷0.97	T12	0.4	0.12÷0.74	WCB12	0	0
NBSC13	0	0	DP13	0.6	0.26÷0.88	T13	0.5	0.19÷0.81	WCB13	0.1	0.003÷0.45
NBSC14	0	0	DP14	0	0	T14	0	0	WCB14	0.8	0.44÷0.97
NBSC15	0	0	DP15	0.2	0.03÷0.56	T15	0	0	WCB15	0.7	0.35÷0.93
NBSC16	0	0	DP16	0	0	T16	0	0	WCB16	0.7	0.35÷0.93
NBSC17	0.3	0.07÷0.65	DP17	0.4	0.12÷0.74	T17	0	0	WCB17	0.3	0.07÷0.65
NBSC18	0.2	0.03÷0.56	DP18	0.2	0.03÷0.56	T18	0	0	WCB18	0	0
NBSC19	0	0	DP19	0.5	0.19÷0.81	T19	0	0	WCB19	0.8	0.44÷0.97
NBSC20	0.7	0.35÷0.93	DP20	0.1	0.003÷0.45	T20	0	0	WCB20	0.9	0.55÷1
NBSC21	0.1	0.003÷0.45	DP21	1	0.69÷1	T21	0	0	WCB21	0	0
NBSC22	0.4	0.12÷0.74	DP22	0.5	0.19÷0.81	T22	0	0	WCB22	0	0
NBSC23	0	0	DP23	0.6	0.26÷0.88	T23	1	0.69÷1	WCB23	0.4	0.12÷0.74
NBSC24	0.1	0.003÷0.45	DP24	0.4	0.12÷0.74	T24	0	0	WCB24	0.6	0.26÷0.88
NBSC25	0.1	0.003÷0.45	DP25	0.5	0.19÷0.81	T25	0	0	WCB25	0.7	0.35÷0.93
NBSC26	0.2	0.03÷0.56	DP26	0.4	0.12÷0.74	T26	0	0	WCB26	0	0
NBSC27	0	0	DP27	0	0	T27	0.8	0.44÷0.97	WCB27	0.2	0.03÷0.56
NBSC28	0	0	DP28	0	0	T28	0.7	0.35÷0.93	WCB28	0	0
NBSC29	0.2	0.03÷0.56	DP29	0	0	T29	0	0	WCB29	0	0
NBSC30	0.6	0.26÷0.88	DP30	0	0	T30	0	0			
NBSC31	0.3	0.07÷0.65									
NBSC32	0.2	0.03÷0.56									
NBSC33	0.5	0.19÷0.81									

Table 2. Occurrence of *Nannospalax leucodon* at the monitoring territory level.

Monitoring territory	Occurrence (n/N)	95% Confidence intervals
West Central Bulgaria	0.76	0.56÷0.90
Thrace	0.27	0.12÷0.46
Danubian Plain	0.50	0.31÷0.69
North Black Sea Coast	0.67	0.48÷0.82

Table 3. Z-test results of testing *Nannospalax leucodon* occurrence differences between monitoring territories (Yates correction is applied): d – difference, z – test score, p – calculated probability.

Monitoring territories	Thrace	Danubian Plain	North Black Sea Coast
West Central Bulgaria	d=0.49 z=3.50 p<0.001	d=0.26 z=1.80 p=0.072	d=0.09 z=0.500 p=0.617
Thrace	-	d=0.23 z=1.565 p=0.118	d=0.4 z=2.921 p=0.003
Danubian Plain		-	d=0.17 z=1.11 p=0.266

Table 4. Occurrence of *Nannospalax leucodon* at the habitat-type level.

Habitat type	Number of 100 m sectors	Occurrence (n/N)	95% Confidence intervals
Pasture	283	0.18	0.13÷0.23
Steppe	98	0.16	0.10÷0.25
Meadow	233	0.42	0.36÷0.49
Wasteland	349	0.17	0.13÷0.21
Vineyards	37	0.32	0.18÷0.50
Orchard	5	0.2	0÷0.72
Arable land	58	0.21	0.11÷0.33
Shrub	9	0.33	0.07÷0.70

erences of the mole-rat in Bulgaria to be collected. These data afford an opportunity to improve the quite insufficient knowledge on the species distribution in the country and to specify a methodological approach for its future monitoring. The mole-rat is unevenly distributed throughout the country, almost missing at the core of the Thracian lowland and quite frequent in West Central Bulgaria. The habitat distribution of the mole-rat in the investigated area reveals the importance of meadows and vineyards for the conservation of the species nowadays.

The advantage of the applied monitoring approach is the simple, inexpensive and non-invasive method. However, there are some aspects which could be improved. As a result of the pilot study, the following recommendations took shape:

1. The period for the field survey should be confined to the winter-spring months (December–March for the lowlands and March–April for the highlands) as the burrowing activity of the mole-rats (i.e., formation of mounds) is lowest in summer (Rado et al. 1993; recent field observations of the authors for West Central Bulgaria). It is found that the digging activity of the related mole-rat species is dependent much more from the soil moisture than from the season (Zuri & Terkel 1997). However, summer months are usually characterised by low precipitation in Bulgaria. This makes this period of the year inappropriate for collection of data on the mole-rat presence. Although the period of field work that has previously been chosen looked like it is the optimal period, the field observations in summer confirm the lower digging activity and show that the presence of fresh mounds and, consequently, the mole-rat occurrence could be underestimated in such conditions. The monitoring scheme should be in conformity with this and the field work should be carried out in winter and spring in days without snow cover.

2. Distinguishing mole-rat soil mounds from those made by other fossorial mammals by means of the classical concepts is not always certain. However, recent investigation on the mound morphology of the subterranean mammals in Hungary revealed that the presence of sharply bitten in acute angle remains of roots and stalks and tunnel diameter of more than 5 cm are the most reliable features of the mole-rats' mounds (Gusztáv 2010). This criterion should be used with priority when recording mole-rats' presence.

3. The data collected according the presented methodology give the possibility for correct statistical analysis in time. Since a shift of the individual territories of the mole-rats is possible between the monitoring periods, and, moreover, the life cycle of the species is relatively short, it is probable its presence not to be detected in the given 100 m interval of the transect. By this reason, to detect the occurrence changes between two periods of monitoring at transect and habitat type levels, the data should be treated and compared as independent variables. Therefore, the z-test (testing differences between proportions) would be applicable to test the differences of the lesser blind mole-rat occurrence between two years of monitoring at transect and habitat type levels. However, at the monitoring territories level, the data from two periods of monitor-

ing should be treated as paired data of presence/absence type in a given transect as a whole. Therefore, McNemar test (testing differences between paired nominal data) will be applicable to test the occurrence differences at this level.

With sufficient number of replications of the monitoring program, data will be gathered to justify a biologically significant difference in occurrence changes of *N. leucodon*. Such an assessment could be made by regression models when comparable data for five or more years will be available.

The collected data on the occurrence of the mole-rat at the transect level could be used as reference data for the future monitoring of the species. It should be borne in mind that the results from fresh mounds from the field survey in summer and autumn months in 2018 are probably slightly understated due to the inappropriate season.

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