

Martin Straka<sup>1</sup>, Jozef Štofík<sup>2</sup>, Ladislav Paule<sup>1</sup>

<sup>1</sup>Lesnícka Fakulta TU vo Zvolene  
T. G. Masaryka 24, 960 53 Zvolen  
straka@vsld.tuzvo.sk, paule@vsld.tuzvo.sk  
<sup>2</sup>Národný Park Poloniny  
ul. Mieru 193, 067 61 Stakčín  
stofik@soprsr.sk

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## INVENTORY OF BROWN BEARS IN THE POLONINY NATIONAL PARK (SLOVAKIA) BY COMBINATION OF SNOW TRACKING AND GENETIC IDENTIFICATION OF INDIVIDUALS

Inwentaryzacja niedźwiedzi brunatnych w Parku Narodowym  
“Połoniny” (Słowacja) w oparciu o kombinację tropienia po śniegu  
oraz genetycznej identyfikacji osobników

**Abstract:** This paper presents preliminary results of study of brown bear *Ursus arctos* population in Slovak part of Eastern Carpathians using faecal and hair samples which were collected in the period from October 2008–March 2009. The combination of snow tracking and non-invasive genotyping provides the reliable identification of bears. The minimum population of 15 individuals (9 males and 6 females) was documented in the area of Eastern Carpathian subpopulation in the Poloniny National Park.

**Key words:** *Ursus arctos*, non-invasive genetic monitoring, Eastern Carpathians.

### Introduction

Information on the presence and abundance of endangered species is basic step for designing conservation plans and understanding the species population ecology (Palomares et al. 2002; McKelvey et al. 2006). Studies based on snow tracking were commonly used for monitoring of large carnivores (Thompson et al. 1989; Halfpenny et al. 1995; Śmietana, Wajda 1997; Beauvais, Buskirk 1999).

Depending on the field conditions, biologists may encounter potential problems with identifying tracks and the likelihood of track misidentification increases as snow conditions deteriorate (Ulizio et al. 2006; McKelvey et al. 2006). Problems with identifying the tracks can be avoided by non-invasive genetic sampling (NGS), which enables the accurate species and individual identification (Flagstad et al. 2004; McKelvey et al. 2006; Adams, Waits 2007; Schwartz et al. 2007).

The hibernation of brown bears can be interrupted in some stages (Jakubiec 2001; Zięba, Zwijacz-Kozica 2010; Štofík 2012; Štofík, Saniga 2012). By

occasional activity during the winter period it is possible to track the bears on snow (Jakubiec 2001; Zięba, Zwijacz-Kozica 2010; Štofík 2012), collect the data on the abundance and structure of the population (Hell, Slamečka 1999; Lushchak 2009; Pčola 2002; Štofík et al. 2010) as well as observe some behavioural aspects (Štofík, Saniga 2012). The method of snow tracking can be used also as complementary to individual identification based on non-invasive genetic sampling (Straka et al. 2009, 2012).

We combined the snow tracking and NGS to study the population of brown bear *Ursus arctos* L. in the Poloniny National Park (Fig. 1). The aim of our study was to acquire a minimum population size estimate and to evaluate the suitability of study methods for monitoring of the population of brown bear in the Poloniny National Park. We took advantage of behaviour response of bears in the Poloniny to local conditions: bears were active throughout the period of our study in winter season.

## Study area

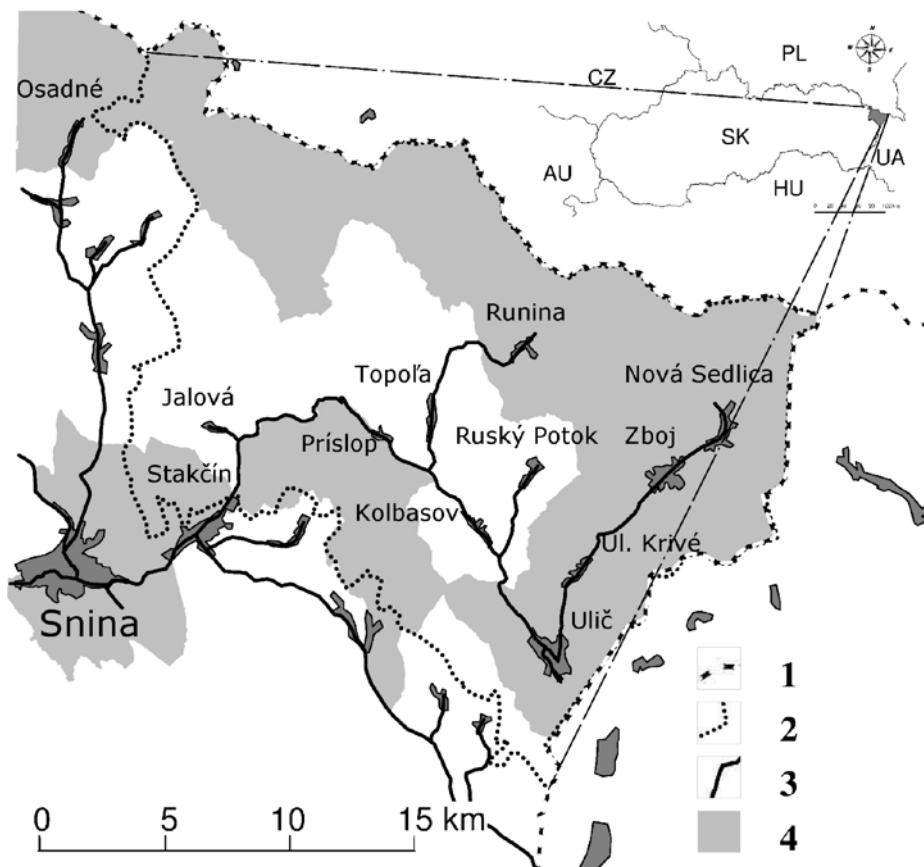
The study area (Fig. 1) is located (48.75°–49.45° N, 21.49°–22.57° E) in the Alpine-Himalayan system, subsystem Carpathians, Eastern Carpathians province, subprovince Outer Eastern Carpathians and the Poloniny region (Mazúr, Lukniš 1986). The northern border represents state boundary with Poland (PL), in the east neighbours with Ukraine (UA). The Poloniny National Park is a part of International Biosphere Reserve "Eastern Carpathians" covering the border area of Slovakia, Poland and Ukraine (UNESCO – MAB Biosphere Reserves Directory 2007), representing the important territory in context of conservation of large carnivores as well other animal species.

IUCN (2001) classifies the brown bear *Ursus arctos* as a low endangered animal – dependent on protection. According to the Act on Nature and Landscape Protection (No. 543/2002) bear is treated as a protected animal, but it allows asking for an exception. Hunting of the brown bear is not allowed in the wider surroundings of the study area, for that reason population of the brown bear have been growing in all three countries (Jakubiec 2001; Delehan et al. 2011; Štofík et al. 2010). Results of DNA analyses from the Carpathians demonstrated that evaluated population is part of Eastern-Carpathian subpopulation which is different as bear population in Romania (Straka et al. 2012).

## Materials and methods

### Samples

Faecal and hair samples were collected in the period from October 2008 to March 2009. Samples were collected at known feeding places visited by brown



**Fig. 1.** Study area in the Poloniny National Park, Eastern Slovakia. 1 – state boundary, 2 – boundary of the Poloniny NP, 3 – roads, 4 – distribution of population of the brown bear in 2008 – data from hunting grounds (Koreň et al. 2011).

**Ryc. 1.** Teren badań w Parku Narodowym „Połoniny“, wschodnia Słowacja. 1 – granice państwowe, 2 – granica PN Połoniny, 3 – drogi, 4 – aktualne rozmieszczenie populacji niedźwiedzia brunatnego w 2008 roku – dane z obwodów łowieckich (Koreň i in. 2011).

bears, nearby roads and at transects in the study area, which were regularly checked. Samples were collected also opportunistically based on the observations of local people. For each bear faeces we measured the diameter, identified the food type and samples were taken for DNA analysis. Faecal samples were stored in 96% ethanol in cold place. Hairs were stored in paper envelopes at room temperature. During the snow season, bear tracks were followed in opposite direction of the bear movement to avoid disturbing of the animal. We collected spatially referenced data on snow tracks (ST) and the locations of non-invasive samples and behavioural activities with a Global Positioning System device (Garmin eTrex Vista H). Length and width of the footprint was recorded if possible on the snow or mud.

### Data analysis

The procedure of DNA isolation, genetic typing and reliability of typing of non-invasive samples dealing with genotyping errors as well as estimating of probability of identity was described in article of Straka et al. (2012).

Bears were assigned to age categories according to the front paw width (PW  $\pm 0.5$  cm, Hell, Slamečka 1999). Five age categories were defined: cubs (PW < 9cm), yearlings and young bears until the age of 3 years ( $9.1 < PW < 12$ ), subadults from 3 to 11 years old ( $12.1 < PW < 15$ ), adults older than 12 years ( $15.1 < PW < 18$ ) and large adults (PW > 18cm). Bear tracks were assigned to the individuals according to genetic identification of individuals.

CONVERT program (Glaubitz 2004) was used to prepare input file for other genetic programs. Samples belonging to the same individuals were regrouped in GIMLET software (Valière 2002). Population size estimate was calculated by rarefaction indices of Eggert et al. (2003) and Chessel (Valière 2003), as well as by CAPWIRE software (Miller et al. 2005).

## Results

### Reliability of typing of non-invasive samples and individual identification

In total, 41 faecal samples were collected. Three hair samples were collected on the daybeds and one was found on the ST. The genotyping was successful in 27 out of 41 faecal samples and 2 out of 4 hair samples; this represents the genotyping success rate of 66% for faeces and 50% for hair.

Fifteen different individuals were identified out of 29 genotyped samples. There were 9 males and 6 females identified (Tab. 1).

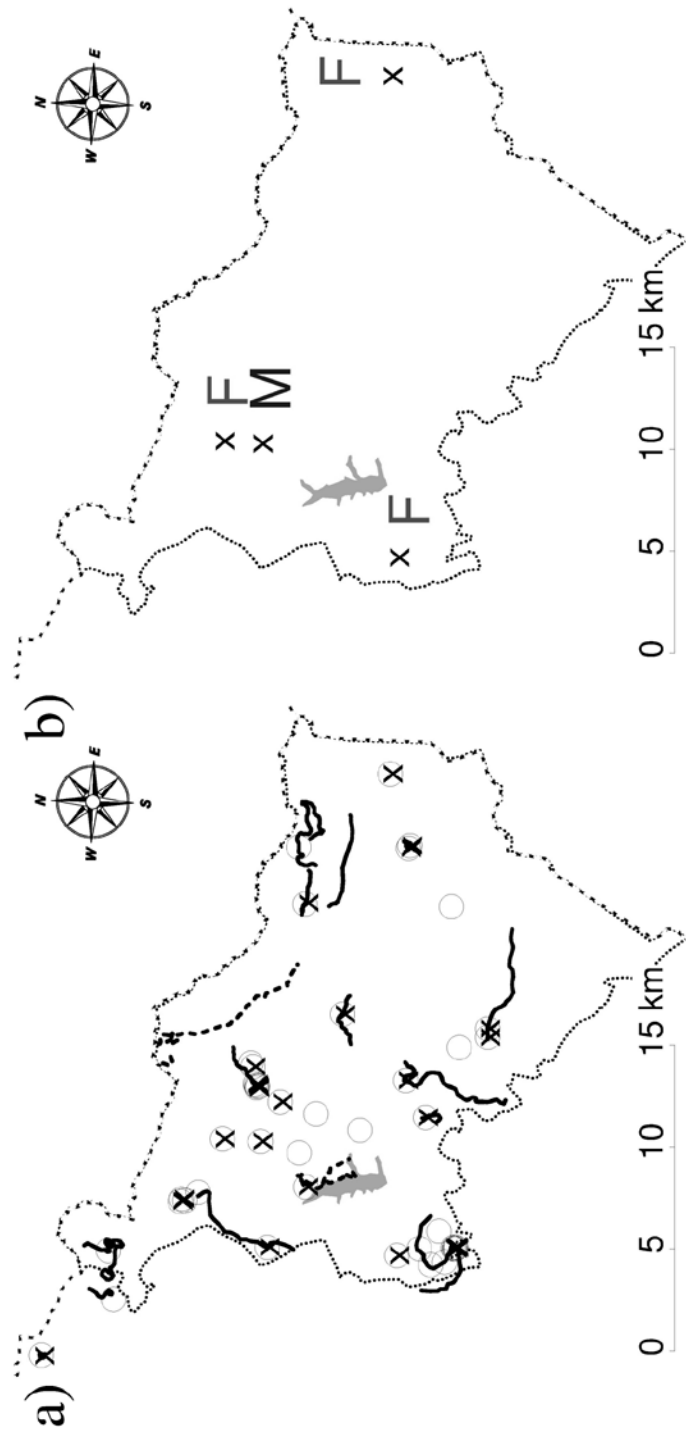
The trajectories of ST and the localization of non-invasive samples are shown on Fig. 2a.

The largest range was recorded for adult male and covered up to 100 sq. km (Fig. 3a). The largest bear footprint was observed in the north-western part of the National Park (long dash lines from winter 2007/2008 on Fig. 3b). Several faecal samples with diameter of 8.5 cm (with large diameter extraordinary large for whole study area – faeces containing the beech nuts) were obtained for these ST. However, no DNA profile was obtained from the sample. However, analysis of the scat with the same diameter provided the DNA profile; therefore we can only assume that this faecal sample belongs to this bear with largest width diameter of the tracks. Another large male (Fig. 3b) occupied the north-eastern part of the Poloniny National Park. Male of age category III was tracked in the easternmost part of the Poloniny National Park near village of Zboj (Fig. 3b). The rest of the individuals ( $n = 4$ ) were identified based only on DNA sample as no ST were available (Fig. 2b).

**Table 1.** Number and sex of individual bears by age categories (Hell, Slamečka 1999).**Tabela 1.** Liczba i płeć osobników niedźwiedzia w danej kategorii wiekowej (Hell, Slamečka 1999).

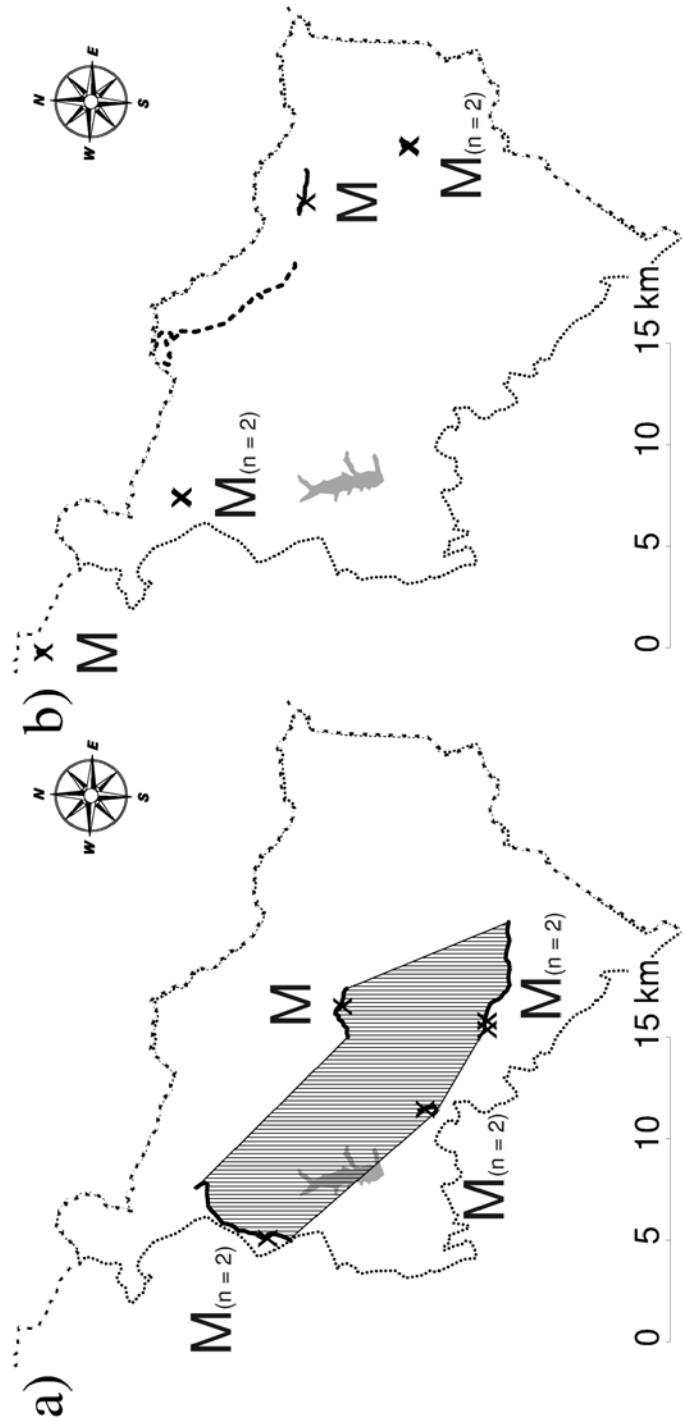
Age category <i>Klasa wiekowa</i>		Front paw width <i>Szerokość przedniej łapy (cm)</i>	Sex <i>Płeć osobnika</i>	Month <i>Miesiąc</i>	Fig. <i>Ryc.</i>
<b>I</b>	cubs / <i>młode</i>	–	–	–	–
<b>II</b>	yearlings and less than 3 years old <i>jednoroczne i poniżej 3 roku życia</i>	9.5	♀	I	4b
		9.5	♂	I	4b
		9.5	♂	XI, I	4a
		9.5	♂	XII	4a
<b>III</b>	subadults and less than 11 years old <i>subadulty i poniżej 11 roku życia</i>	12	♀	I	4a
		13	♀	I	4b
		13.5	♂	XI	3b
<b>IV</b>	12 years and older <i>12-to letnie i starsze</i>	15.5	♂	XI	3b
		15.5	♂	III	3b
		16	♂	XII, I	3a
<b>V</b>	the oldest individuals <i>najstarsze osobniki</i>	18?	♂	X	3b
	individuals without recorded track <i>osobniki których tropy nie zostały odnalezione</i>	?	♀	X	2b
		?	♀	II	2b
		?	♀	XI	2b
		?	♂	III	2b
<b>Total / Razem</b>			♂9 and ♀6 <b>(15)</b>	X–III	2a

Figure 4a shows the movement of female with 2 male yearlings born in winter season 2007/2008 (range 80 km<sup>2</sup>). Another female was tracked in the beginning of 2009 (Fig. 4b). DNA profile was obtained for this female and 2 of her yearlings (female and male). However, based on the ST, female had 3 offsprings in total, but there was no faecal sample obtained for the third yearling.



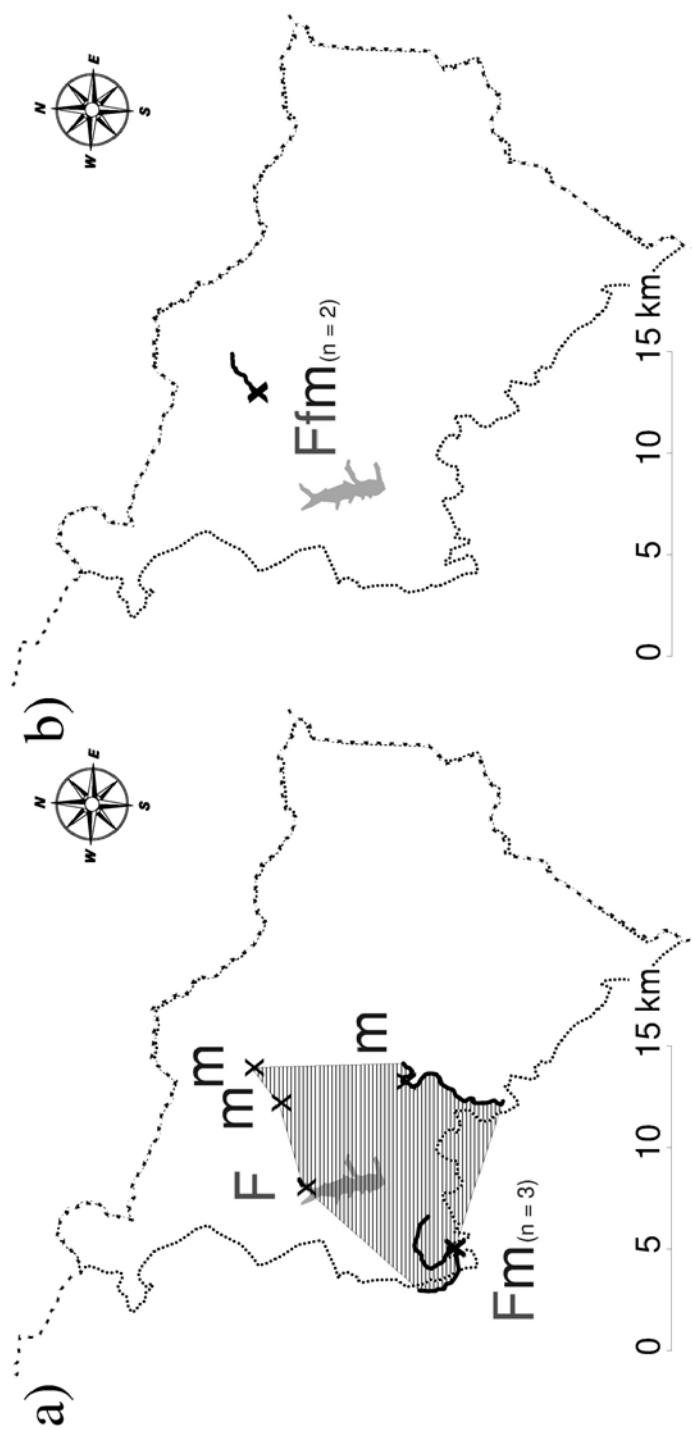
**Fig. 2.** Tracks of bear movement obtained by snow tracking and localization of non-invasive samples (2008–2009) in the Poloniny National Park (a), individuals identified only on the base of genotypic success samples (b). Solid and long dash lines – tracks of bears,  $\circ$  – non-invasive samples,  $\times$  – genotyping success samples, F – females, M – males.

**Ryc. 2.** Trasy przemieszczania się niedźwiedzi, określone na podstawie tropień po śniegu i lokalizacji nieinwazyjnych prób w PN Poloniny (linie ciągłe i przerywane – trasy niedźwiedzi,  $\circ$  – nieinwazyjne próby,  $\times$  – oznaczone próbki genetyczne, F – samice, M – samce).



**Fig. 3.** Tracks and range of a movement of adult bear male obtained by snow tracking and non-invasive samples (a), adult males identified by snow tracking and non-invasive samples (b). Solid and long dash lines – tracks of bears, pattern vertical – range, x – genotypic success samples, M – males.

**Ryc. 3.** Trasy i obszar przemieszczania się dorosłego samca, określone na podstawie tropień po śniegu i nieinwazyjnych prób (a), dorosłych samców, określone na podstawie tropień po śniegu i lokalizacji nieinwazyjnych prób (b). Linie ciągłe i przerywane – trasy niedźwiedzi; obszar zakreślony pionowo – zasięg przemieszczania się, x – oznaczone próbki genetyczne, M – samce.



**Fig. 4.** Tracks and range of adult female bear with 2 yearlings movement obtained by snow tracking and non-invasive samples (a), another adult female bear with 2 yearlings movement obtained by snow tracking and non-invasive samples (b). Solid lines – tracks of bears, pattern horizontal – range, x – genotypic success samples, F and f – females, m – males.

**Ryc. 4.** Trasy i obszar przemieszczania się dorosłej samicy z dwójką rocznych młodych określone na podstawie tropień po śniegu i nieinwazyjnych prób (a), innej dorosłej samicy z dwójką młodych również określone na podstawie tropień po śniegu i nieinwazyjnych prób (b). Linie ciągłe i przerywane – trasy niedźwiedzi; obszar zakresowany poziomo – zasięg przemieszczania się, x – oznaczone próbki genetyczne, F oraz f – samice, m – samce.



### Evaluation of snow tracking (ST)

In the study period we tracked 15 bear ST with total length of 61.3 km (average 4.1; SD 2.9 km). For one of the ST (4.9 km) there was no faeces found and for other 3 samples (16.5 km) the DNA isolation failed. The average length of ST necessary to obtain faecal sample ( $n = 45$ ) was 1.4 km and to obtain a sample that was successfully genotyped ( $n = 29$ ) was 2.1 km.

### Population size estimate

The mean value of population size estimate obtained by rarefaction method of Chessel was 17 individuals with confidence interval (CI 95%) ranging from 10.79 to 25.65. Eggert method and CAPWIRE method provided the same estimate of 26 individuals. However, the 95% CI for Eggert method was from 12 to 635. The 95% CI for CAPWIRE was ranging from 16 to 43 individuals.

## Discussion

Based on historical literature sources, the Eastern Carpathian subpopulation was originally part of the continuous population including Western and Eastern Carpathians until the beginning of 20<sup>th</sup> century (Hartl, Hell 1994; Find'o et al. 2007). First reference on the presence of brown bears in region of Slovak part of Eastern Carpathians was written by Bel (1684–1749) in which he mentioned the numerous occurrences of brown bears in that area. At the turn of 18<sup>th</sup> and 19<sup>th</sup> centuries, several villages (Medvedie, Krásna Poruba, Stužica, Ruský Hrabovec, Runina and Valaškovce) included the image of brown bear into the municipal seals (Štofík et al. 2010), suggesting that bears were commonly present in the area. Culled bears were reported from the area of Eastern Slovakia at the turn of 19<sup>th</sup> and 20<sup>th</sup> century (Jamnický 1993). Extensive hunting pressure led to the split of the continuous Carpathian population into populations in Western and Eastern Carpathians (Hartl, Hell 1994; Find'o et al. 2007). The number of bears in Slovakia began to increase since the protection law has been implemented in 1932 (Feriancová 1955). Bear presence in the Eastern Carpathians were documented by several authors (Turček 1949; Feriancová 1955; Škultéty 1970; Randík 1971). According to the recent estimates of population size it was 16 individuals in 1997 and 18 in 2001 (Pčola 2002). The area of Poloniny National Park plays important role for brown bear population due to trans-boundary migration of bears between countries of Ukraine, Poland and Slovakia as well as due to re-establishment of connectivity between populations of Eastern and Western Carpathians.

The genotype was obtained from 66% of faecal and 50% of hair samples. The overall genotyping success rate was higher in comparison to the pilot study performed in Poľana and Veporské vrchy (Straka et al. 2009). This could be attributed to better preservation of DNA in samples which were collected on snow (Flagstad et al. 2004).

We identified 15 different individuals: 9 males and 6 females. The reproduction of bears in the area was documented by the presence of 4 yearlings. Another yearling was recorded based on the ST. The documented presence of females with cubs indicates that the area of the Poloniny NP is the place of brown bear reproduction.

The population size estimates varied due to different methods used. According to the estimate given by Chessel's method it was 17 individuals in the study area. This value can be considered as underestimate as we identified 15 different individuals by DNA analysis and another one by ST. Two other methods gave estimates of 26 individuals. The CAPWIRE method seemed to overperform the Eggert's rarefaction method as the confidence interval of the latter was unreasonable. The confidence interval (CI 95%) for CAPWIRE was in range from 16 to 43 individuals. According to literature sources, bear numbers in the area of Poloniny in 20<sup>th</sup> century were very low with only several transient individuals were present (Turček 1949; Feriancová 1955; Škultéty 1970; Randík 1971). The most recent estimates of population size were 16 (1997) and 18 (2001) individuals (Pčola 2002). These estimates were based on information from hunters, foresters and other locals as well as on the bear tracks. Comparison of previous studies (Feriancová 1955; Chovanec 1967; Škultéty 1970; Randík 1971; Sabadoš, Šimiak 1981; Janík et al. 1986) and our study suggest, that bear population in Poloniny NP is growing.

According to Štofík et al. (2010), there are several possible causes of this population growth: (a) displacement of the people in adjacent area of Bieszczady in Poland after World War II; (b) long-term protection of the animals in the Bieszczady National Park and in whole Poland (c) displacement of 7 villages in Poloniny due to construction of water dam in 1986; (d) negative demographic trend of people living in the study area (ÚKE SAV 2004); (e) increase of forest cover in the area from 65.1% in 1949 to 85.4% in 2003 (ÚKE SAV 2004); (f) increase of artificial feeding of ungulates at the end of 20<sup>th</sup> century, which may have effect on brown bear behaviour and reproduction success as can be seen in large omnivorous species of wild boar *Sus scrofa* (Geisser, Reyer 2004; 2005; Bieber, Ruf 2005; Tsachalidis, Hadjisterkotis 2008; Keuling et al. 2010) caused by supplementary feeding (Geisser, Reyer 2004), which were evaluated in food of bears in Slovakia at the beginning of 21<sup>st</sup> century (Rigg, Gorman 2005); (g) strict protection of bears in the area of Poloniny and surrounding area with the aim of reconnection of Western and Eastern Carpathian subpopulations.

Thanks to strict protection of the brown bear in Poland the population size (Jakubiec 2001) as well as the area of distribution (Šmietana et al. 2012) also in eastern part of Polish Carpathians is increasing.

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## Streszczenie

Artykuł przedstawia pilotażowe badania szacowania wielkości populacji niedźwiedzia brunatnego w słowackiej części Karpat Wschodnich, która była ściśle chroniona przez ostatnich 20 lat.

Połączenie tropień po śniegu i nieinwazyjnego genotypowania posiada wiele zalet w porównaniu z innymi powszechnie stosowanymi sposobami: pozwala dokładniej oszacować minimalną wielkość populacji, dostarcza dokładniejszych danych o strukturze populacji, nie wpływa na zachowanie zwierząt. Korzystając z nieinwazyjnej metody genotypowania zidentyfikowano 15 osobników: 9 samców i 6 samic. Cztery z nich były to osobniki młode 1–3-letnie.

Przyszłe badania powinny objąć również sąsiednie obszary w Polsce i na Ukrainie, aby lepiej poznać transgraniczną populację niedźwiedzia brunatnego. Większa skala oraz dokładne nieinwazyjne badania genetyczne mogą dostarczyć więcej informacji na temat struktury wiekowej, przestrzennej oraz dynamiki populacji niedźwiedzia brunatnego.