Faculty of Biology University of Belgrade

Viability increase and recovery of Brown Bear Ursus arctos L. 1758 population in northeastern Serbia

Feasibility study

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Photo: Milan Živković

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1. Goal of the project – feasibility study

In agreement with international directives for translocations and reintroductions of animals (IUCN 1987, 1998), the goal of this project is to establish a viable population of the brown bear (*Ursus arctos* L.) in northeastern Serbia. The tasks of feasibility study should include (Genovesi *et al.* 2000):

- A) Verification of recovery possibilities for the resident surviving population in northeastern Serbia without any translocation activities,
- B) Estimation of the region's capacity to sustain a viable population of brown bears,
- C) Estimation of the scope and acceptability of conflicts that would arise between bears and humans,
- D) Identification of the main factors that may negatively impact the establishment of a viable population in the region.

1.1. Definitions

Present knowledge and estimates of the status of the autochthonous brown bear population in northeastern Serbia are quite sporadic and not based on exact data or samples. However, the occasional appearance of individuals, sometimes quite unexpected in certain parts of the region of eastern Serbia, confirms that brown bears are present. Until recently there were no written official documents recording the presence of bears in the study area and existing observations are almost exclusively based on the assumed range and a rough estimate of population numbers. This includes Derdap National Park, on the northern boundary of the sub-region of northeastern Serbia.

As there has been a generous offer for the donation of 4 brown bear individuals from Tatre National Park in Slovakia, for possible **augmentation** rather that **reintroduction**, the Directorate of Environmental Protection, Ministry of Science and Environment Protection, Republic of Serbia, and the Directorate of Forests, Ministry of Agriculture, Forestry and Water Management, Republic of Serbia, have decided that for such activity first it would be necessary to prepare a feasibility study, which would present documented facts on the status, needs and necessary measures for conservation of the brown bear population in northeastern Serbia. The feasibility study should present data on the current status of the brown bear population, as well as recommendations for increasing its viability and abundance.



2. Introduction

- 2.1. Bionomic characteristics of the brown bear
 - 2.1.1. General characteristics

The brown bear (*Ursus arctos*) has the widest distribution of any bear species: Holarctic in nature, it includes Europe, Asia and North America, stretching from Arctic tundra in the north to dry desert areas in the southern part of the range. The brown bear has a number of specific morphological characteristics: a massive head with elongated snout, small rounded ears, small eyes, short tail, square massive body with pronounced hump (Fig.1). The fur can be various shades of brown. There are five rounded toes on each paw; the toes on the forefeet are longer and equipped with long claws, while the hind feet are longer than the forefeet. The sense of smell is the best developed of all the bear's senses. Males are larger than females (males 130-320 kg, females 100-200 kg). European bears rarely reach extremes of size.



Fig. 1 – Brown bear (Ursus arctos L., 1758). Photo: Đuro Huber.

2.1.2. Diet

The brown bear is an omnivore, as shown by the adaptations of its teeth and digestive system. It has long canine teeth, which may be used for defence, killing prey or tearing



apart carrion, as well as well-developed premolars and molars, adapted to a diet of plant material and invertebrates. The digestive system is basically of the carnivore type, but the gut is somewhat more elongated, probably in order to enable better digestion of plant food. There are no special organs for the digestion of structural plant material (cellulose), but it is able to digest about 50% of plant proteins and carbohydrates (primarily sugars and starch).

The brown bear passes through 3 biochemical and physiological states during the active period from spring to autumn. They start with hypophagia (decreased food intake) in spring and end with hyperphagia (increased food intake) in autumn. In late summer and early autumn it is very important for the bears to consume food with high energy content, in order to accumulate the fatty tissue necessary for hibernation. Green plant parts are consumed during the period of hypophagia, in spring and early summer. When forest fruits begin to ripen (Fig.2), bears redirect their attention to this source of food. Later in autumn, as well as in winter and spring, the bear may consume large quantities of hard-shelled forest fruits (nuts) such as beech mast, acorns, chestnuts and hazelnuts, but also soft fruit (wild plums, wild apples, pears). The northern populations mostly feed on nuts while the southern populations use both nuts and soft fruits.



Fig. 2 – Wild raspberries *Rubus idaeus* and other forest fruits are common in the bear's diet. Photo: Đuro Huber.

Insects, especially Hymenoptera (ants, bees and wasps) may be an important food source for bears, especially in spring. Their high protein content makes insects one of several protein sources in the spring, as well as a source of certain essential amino



acids. It has been recorded that female bears consume a greater quantity of such food than males.

Meat is an important factor in the normal diet of the brown bear; it is consumed both as freshly caught prey and carrion (Fig. 5). Bears are not particularly good hunters of adult ungulates, so they mostly orient their effort on young animals. Livestock (particularly sheep) are sometimes preyed upon in certain parts of Europe, especially in areas where the method of husbandry is to leave livestock to graze unattended or with an insufficient degree of attention.

2.1.3. Reproduction

The brown bear is characterized by a relatively long lifespan (20-30 years), late onset of sexual activity (after the fourth year of life) and a prolonged reproductive cycle. This is a polygamous species, and the mating season lasts from mid-May to early July. Multiple fatherhood in a single litter is a possible and proven phenomenon. After fertilization, the embryo develops until the blastocyst stage and then its development stops until implementation in the uterus in late November. In the period January-March the female gives birth to 1-4 (most often 2) cubs, whose average birth weight is about 0.5 kg (Fig. 3). Cubs become able to live alone when 1.4-2.4 years old.



Fig. 3 – Mother bear with a single cub. Photo: Đuro Huber.



2.1.4. Hibernation

Depending on the geographic position (latitude) and if they have formed enough fat reserves, brown bears become lethargic in late autumn and hibernate for 3-7 months. They dig dens in soil or old anthills (as is usually the case in eastern Scandinavia), or use natural hollows under rocks, tree stumps, fallen trees etc. Some bears in southern populations may stay active throughout the year. Use of dens in most probably an adaptation to seasonal changes in food availability and perhaps to giving birth to fragile cubs unable to maintain their own thermoregulation.

2.1.5. Activity and home range size

Brown bears may be active by day and night, depending on the habitat conditions, availability of food and human activity. Constant disruption by human activities caused European bears to switch to a secluded nocturnal life, which is not the case with, for example, Siberian or North American bears. As in all other large carnivores, density of bear populations in natural conditions is low, while the home ranges of individual bears are relatively large. The density is lowest in the northernmost part of the range and increases toward the south. The size of a bear's home range depends on availability and distribution of food as well as on population density, and is greatest in the north and lowest in the south of the continent.

2.1.6. Social organization and dispersal

The social organization of bears is very poorly known, but the relationship between individuals, particularly adults, greatly depends on available space and reciprocal avoidance, except in the mating season. Dispersal of males has been demonstrated, while females remain in their mother's home range or its immediate vicinity. Overlapping of activity ranges of individual bears has been studied in the northern populations. This phenomenon is less well understood in southern "dense" populations but overlapping of home ranges has been documented by radio-telemetry studies in Croatia (Huber *et* Roth 1993), Romania (Martens *et* Sandor 2000) and Poland (Jakubiec 2001).

2.1.7. Habitat

The European brown bear lives in various habitat types. In conditions of unaltered nature or insignificant anthropogenic impact, bears live not only in forested areas but also in steppe and northern and alpine tundra. Presently humans occupy most of the former bear range, so bears have adapted to forested regions, generally with low human population density. Such areas are now very scarce in Europe.



The key components of habitat may be grouped into three large sets: food, general seclusion (relief and vegetation) and den sites. It may be concluded that brown bears need large continuous habitats, with enough suitable food and shelter.

2.1.8. Relationship with humans

2.1.8.1. Public opinion

In the contact zone of human settlements and natural bear habitats, humans have negative attitudes toward this animal, primarily due to fear and the damage that bears may occasionally cause to livestock and crops.

2.1.8.2. Threat to humans

The size and physical strength of bears enable them to hurt and even kill humans. However, rare attacks on humans are not a result of predatory behaviour, but almost exclusively self-defence or protection of cubs and/or prey from humans (Fig. 4). The presence of a wounded bear in an area is especially dangerous. The main factors of increased bear aggressiveness are (in decreasing order): a) presence of cubs; b) presence of prey – carrion; c) surprised and scared bear; d) presence of a den; e) presence of dogs.



Fig. 4 – Museum taxidermy of a bear in the threat pose. Photo: Đuro Huber.



2.1.8.3. Attacks on livestock

Livestock animals, raised for generations to provide for human needs have lost most or all defence mechanisms against large carnivores. Efficient measures of livestock husbandry and protection, necessary for the coexistence of herds and large carnivorous mammals, are no longer used in much of Europe, partly due to economic, social and political changes, but also as a result of the extermination of large carnivores from most of their original ranges. Individual bears returning to previously vacant habitats easily come into contact with unprotected livestock, which then inevitably becomes prey (Fig. 5). Damage is more often caused to small stock (sheep, goats, calves and steer) than large animals (cows and horses). Bears that kill livestock are usually shot as "rogue killer bears". This has a more destructive impact in areas with small, critically diminished or reintroduced populations, than in areas where bear populations are large and abundant.



Fig.5 – Freely wandering domestic ungulates are the easiest prey for bears. Photo: Đuro Huber.

2.2. Present status of brown bear populations in Serbia

The brown bear is one of 98 species of mammals (Mammalia) and one of 19 species of carnivores (Carnivora) in Serbia. Although its appearance, activity and presence in the Serbian fauna have not gone unnoticed, it may be said with certainty that it is one of the least studied species of mammals. Data on its abundance and distribution in Serbia were, until recently, based only on hunters' statistics, reports and plans, while studies and scientific papers on this species were very scarce (Paunović 2002).



A distribution map of the brown bear in Serbia has been composed according to data collected by the Hunting Association of Serbia, data collected by the authors in the field, as well as through interviewing various segments of the local community. The darkly shaded areas in Fig. 7 represent areas of permanent presence and reproduction (core areas), while the lightly shaded areas are places of occasional recording of individual bears, that is, parts of the range where individuals are recorded at irregular intervals and in transition. In the last 10 years there were over 100 records of brown bears in Serbia (Paunović et al. 2005). A first glance at the distribution map shows that bears are absent in Vojvodina province and in the central parts of Serbia, in the regions of Šumadija and Veliko Pomoravlje, and even in the borderline areas of these and neighbouring regions, as a consequence of significant anthropogenic modification of these areas by timber harvest, agriculture and urbanisation. On the other hand, the marginal parts of Serbia in the west, east and southwest represent areas of medium and high mountains, which form natural units with areas outside Serbia's borders. These areas are usually forested, with sparse human population and extensive agricultural production. These are areas of highest biodiversity and as such in some parts are optimal habitats for brown bear.



Fig. 6 – A subadult individual near a feeding place in Tara NP. Photo: Boris Ivančević.

The marginal high mountain parts of Kosovo-Metohija province, such as Bogićevica Mt., Prokletije Mt., Junićke Planine Mts., Paštrik Mt., Koritnik Mt., Šar-Planina Mt., as well as Hajla and Mokra Gora Mts., are considered optimal areas for the brown bear's survival and the most important parts of the range in Serbia. Almost equally important is the range of Tara Mt. (with a National Park of the same name; Fig. 6), Zvijezda, Šargan Mts. and the Beli Rzav River canyon in western Serbia, where abundance and population density are greatest for so-called Middle Serbia. This is the



second most important core area in Serbia (Fig. 7). Besides these two areas, the mountains Zlatar, Jadovnik, Giljeva and Ozren toward Montenegro, as well as Golija and Čemerno toward the central parts of Serbia, are also important.



Fig. 7 – Brown bear range in Serbia. The number of records during the last 10 years is shown in 20x20 km UTM squares.

Especially important for the survival and conservation of bear population in western and southwestern Serbia is the existence of corridors between the core areas, both in Serbia and in the corresponding parts of the range in neighbouring countries. Besides, the large forest complexes in eastern Serbia, in a relatively narrow zone along the Danube River, in the area of Majdanpečka Domena, Homoljske Planine, Severni



Kučaj, Deli Jovan and part of Južni Kučaj Mt., there are also optimal habitats for bears, but it is obvious that the habitat capacity in these areas is unused (Fig. 7). In one part of Stara Planina Mt., in the direction of Arbinje – Topli Do, there is also evidence of the occasional occurrence of individuals or reproductive groups, which is believed to be a consequence of range expansion of the population in neighbouring Bulgaria. In the last 10 years the presence of at least 8 reproductive groups has been recorded, indicating an ongoing reproductive process in the territory of Serbia. On the other hand, this data set indicates that reproductive groups are relatively rarely seen (Fig. 8), leading to the conclusion that the brown bear really deserves to be placed on the List of Natural Rarities, which has not been the case (Off. Gazette 50/93).





Fig. 8 - Range of permanent and temporary presence of the brown bear in Serbia, with marked areas where reproductive groups were recorded.

The chronology of the deterioration and decline of game animals and hunting areas (habitats) in Serbia, including of the brown bear, which was always considered a game animal, was presented by Živančević (1956). The social-economic and political crises during the second half of 20th Century, especially in the 1990s, caused continued and accelerated devastation of Serbian nature. This was particularly pronounced at the end of this period, which included military activities in the territory of Serbia, especially in the Province of Kosovo-Metohija. There is an absence of data from Kosovo-Metohija beginning in 1998, when international conflict was gaining momentum and becoming more intense, culminating in the events of 1999 and the arrival of forces of the international community.

In 1985, 168 individual bears were recorded in Serbia, including 75 in Kosovo (Krže 1988). According to official statistics, 5 individuals were shot in Serbia in 1986, including 2 in Kosovo (*ibidem*). According to the optimistic data of the Hunting Association of Serbia, population numbers in 1997 and 1998 were not much different than that mentioned above. It was then estimated that about 160 bears lived in Serbia, while according to official data 18 individuals were illegally shot in 1996 (Paunović 2002) (Table 1). According to the most recent estimate (Paunović *et al.* 2005), the population number of brown bears in Serbia is not greater than 53 individuals, but with this estimate we must keep in mind that since 1998 there are no estimates or any other statistical indicators on the population numbers in Kosovo-Metohija province.

Total	Official data by the Hunting Association of Serbia						
surface	Previous population numbers				Present numbers		
area (km ²)	1985	1988	1997	1998	2005		
88.361	168	164	160	70	max. 53		
				Without	data for Kosovo-Metohija province		

Table 1 – Population numbers of brown bear (*Ursus arctos*) in Serbia in the last 20 years, according to official data by the Hunting Association of Serbia (Paunović 2005).

According to the present Hunting Law (Off. Gazette 39/93), the brown bear was placed in the group of game animals protected with a closed season and to shoot a bear it became necessary to obtain a decision and special permit from the relevant Ministry. In 2002, at the suggestion of several non-governmental organizations and the Natural History Museum, Belgrade, but primarily due to increased awareness of the threatening conditions and small number of individuals remaining in the wild, the brown bear was protected by closed season throughout the year by a special Order on Closed Seasons of Game Animals (Off. Gazette 19/02), in reality resulting in a complete ban on hunting any individuals of this mammal species.



Other than this, the brown bear is placed in the category of Vulnerable Species (Savić *et al.* 1995) and is placed on the list of species suggested for the Red Book of Mammals and Vertebrates of Serbia (Vasić *et al.* 1991). However, the present Ordinance on Protection of Natural Rarities (Off. Gazette 50/93) does not include the brown bear. The proposal of the new Ordinance, that at present (February 2006) has still not passed the parliamentary process, includes raising the degree of protection through active population management and permanent monitoring.

2.3. Status of brown bear populations in Europe and neighbouring countries

Although the range of the brown bear in the Balkan Peninsula (Serbia and neighbouring countries) shows significant fragmentation, and even a disjunctive character, it is obvious that it includes complementary trans-boundary components. Therefore, in order to properly understand the status of the population in Serbia, it is necessary to have an insight into the status in neighbouring countries and regions.

The brown bear was originally distributed throughout the European continent, except some small islands (Crete, Sardinia, Corsica etc). The species disappeared from many regions of Europe due to pressure from the growth and spread of the human population, disappearance of appropriate habitats due to deforestation and the spread of agriculture, as well as over-hunting (Smit *et* Vijngaarden 1981, Swenson *et al.* 2000). Today, the total number of bears in Europe is estimated at 50,000, only 13,000-14,000 of which live outside Russia. However, these bears are divided into a number of relatively isolated populations (Table 2).

Population	No. individuals
Northeastern Europe (incl. Russia)	37,500
Carpathian Mts.	8,100
Dinaric Mts. and eastern Alps	2,800
Scandinavia	1,000
Rila-Rhodope Mts.	520
Stara Planina Mt.	200
Western Cantabrian Mts.	50-65
Apennines	70-80
Western Pyrenees	6
Central Pyrenees	5
Southern Alps	3-4
Total in Europe	c. 50,000

Table 2 – List of abundance in various brown bear (*Ursus arctos*) populations in Europe (according to Swenson *et al.* 2000).

The population density is variable and depends on food availability and abundance, hunting rates and current status of bear populations. The highest density was recorded



in Romania (100-200 bears / 1000 km²), and smallest in Finland and Norway (0.5-1.0 bear / 1000 km²).

In contrast to Serbia, the present population number of brown bears in Montenegro has not been precisely estimated recently, but it is assumed that there are about 150-200 individuals. According to data from 1985, there were 244 individuals in Montenegro. The Hunting Association of Montenegro has data on 13 bears shot illegally in the period 1990-1995 (Paunović 2002).

A comparative list of brown bear abundance in Balkan countries is presented in Table 3. Illegal shooting occurs in a number of countries, while legal hunting is recorded and adequately regulated only in Slovenia and recently in Croatia (Dečak *et al.* 2005). The stable population trend in these two countries is a result of constant monitoring, an existing plan and a strategy for active population management (*ibidem*). In most countries the brown bear is protected by a closed season, but the actual status and population numbers, habitat status and weak implementation of the closed season (e.g. permanent poaching) has led the governments of many countries neighbouring Serbia to prescribe total protection (Table 3).

Country	Surface area (km ²)	Previous data on population numbers ¹	Present number	Population trend	Protection ²
Serbia	88,361	168 (1985)	53 (Kosovo?)	Decreasing	CSWY
Montenegro	13,812	244 (1985)	150-200 (?)	Decreasing (?)	CS
Macedonia	75,713	85 (1985)	90	Stable	Р
Bosnia and Herzegovina	51,129	1,332 (1985)	1,200	Decreasing (?)	CS
Croatia	56,538	648 (1985)	400	Stable	CS
Slovenia	20,254	280 (1985)	300	Stable	CS
Greece	131,957	40 (1979)	110-145	Decreasing	Р
Bulgaria	110,912	40 (1979)	720	Decreasing	Р
Romania	237,500	5,700 (1979)	6,600	Decreasing	CS
Albania	28,748	20-30 (1979)	250	Stable	Р

 1 – official data by Krže (1988) and CIC (1979); the year of the estimate is given in parentheses.

 2 - CS = protected by closed season, CSWY = closed season all year, P = complete, permanent protection.

Table 3 – Comparative list of certain indicators of previous and present status of brown bear (*Ursus arctos*) populations in Serbia, Montenegro and other Balkan countries.



2.4. Status and population trend in the study area

The oldest literature source citing actual localities of bear presence in eastern Serbia is the paper "On bears" by Dr. Sima Trojanović (Trojanović 1899). This author cites localities in Južni Kučaj Mt. that are presently also active, but all his records are based on stories by local folk and he wrote them in the past tense. He mentions the occasional presence of a so-called "carrion bear" which killed livestock on Kučaj Mt., and cites damage of 40 cows killed at the locality of Bele Vode (presently in the territory of Boljevac municipality). Besides Bele Vode, he also cites other localities in Južni Kučaj that used to be inhabited by bears, such as Kose, Lomčić, Risova Poljana, Koprivno Brdo, Gajine Mlake, and states that "… presently … there are some at Nekudovo, 7 hours on horseback going north from Jablanica village…" Trojanović (1899) also cited a note by N. Đ. Milićević, a writer, who mentioned "… richness in beasts … of which wolves and bears draw most attention upon themselves … in the spacious mountains from Rtanj Mt. to the Danube River and from the villages in the Morava Valley to the Timok Valley…". It also includes a description of hunting a bear in a den, in the vicinity of Gornjani village (30 km north from the town of Bor).



Fig. 9 – Print of the right fore paw of a brown bear in Tara NP. Photo: Duško Ćirović.

While we were preparing this study, we tried to assess present status mainly in the last 10 years, although the data collected cover the period of the last 30 years. Many data were collected by interviewing the local community, mostly forest rangers, forestry technicians, hunters and local farmers, as well as through immediate fieldwork in



collecting data on the presence or activities of the brown bear. These methods yielded the following data: 17 records of paw prints and tracks in substrate (Fig. 9) and 17 direct observations of bears, of which 4 included the death of the animal due to poaching. Data on poaching were difficult to check, but they are partially documented with samples of skin, hair or tissue, which were later used for genetic profiling (see 3.3.). Reproductive groups (females with cubs) were recorded on four occasions. A den was found on only one occasion. During this period only four cases were verified of attacks on livestock, including sheep and cows. However, in the last ten years, a considerable amount of damage to livestock, crops, orchards and beehives was recorded.

Keeping this in mind, it is clear that in eastern Serbia there is a relatively isolated population fragment in the Carpathian sub-region, accounting for almost all the records. In the Balkan sub-region, there is one record of bear being shot in the last ten years and one occurrence of a reproductive group at Stara Planina Mt. These are most probably marginal individuals from the population of bears in neighbouring Bulgaria.

Data from the last 30 years may lead to the conclusion that the bear population in eastern Serbia and its Carpathian sub-region is declining. It had a more extensive range in the 1980s and early 1990s, while presently the greatest concentration (of evidence data) is in Derdap National Park and a narrow zone around it. According to our data, the dense old beech forests of the Severni Kučaj Mts. on Šomrda, Liškovac and Zlatica are particularly important. Although there are no official estimates, the bear population number in eastern Serbia ranges from a pessimistic 3-5 individuals to a maximum of 10 individuals. It is apparent that there is some poaching and that on average at least one individual is killed illegally each year. There are no credible data on immigration from the neighbouring parts of Romania, although it is very much possible that the Danube River does not present an impenetrable barrier in the area of Upper and even Lower Derdap, as the water flow is slowed down by the Derdap 1 dam. In comparing haplotypes in genetic profiles determined for bears in eastern Serbia with those known from the literature, a particular similarity to bears from Bulgaria is evident (see 3.3.3., Figs. 22, 23), pointing to a potential immigration direction from the east or southeast.

2.5. Reintroductions and translocations of bears – experiences and recommendations

Reintroduction of living organisms is defined as an attempt to re-establish a species or lower taxonomic unit in an area that used to be part of its historic range, but from where it was exterminated or became extinct (IUCN 1987, 1998). This definition is overly general for practical use, and may cause confusion in a large number of cases, as they differ in their causes and factors. For example, in the case of this study, the autochthonous native individuals are still present in the territory (although in very small numbers), so this kind of reintroduction is better termed supplementation, augmentation or fortifying (*ibidem*). However, all introductions necessarily include re-establishment of populations and not relocation of individuals.



An overview, a review of previous bear reintroductions and analysis of their success and basic postulates and directions are given by Clark *et al.* (2002). According to their findings, reintroductions of bears are recently especially numerous worldwide, as a consequence of attempts to overcome and prevent the dramatic reduction of population numbers and distribution, habitat fragmentation and loss, as well as overhunting (Servheen 1990, *fide* Clark *et al.* 2002). In recent times, some progress is evident in maintaining and developing awareness of the necessity to conserve and protect remaining individuals and fragments of their habitats, as well as the development and implementation of legal measures necessary to prevent poaching and over-hunting. Many habitats previously lost have now returned to their previous form, optimal for the survival of bears.

It should be noted that a special problem for most bears, including the brown bear, is the slow process of natural re-colonisation, due to a low reproduction rate and weak dispersal capability. The great dispersal capabilities documented for males are not characteristic of all age and sex classes; dispersal capabilities are weak (almost nonexistent) in immature females that after separation from their mother settle in her territory or its immediate vicinity.

Reintroductions are expensive long-term projects that have resulted in viable populations in only 11% of cases (Beck *et al.* 1994 *fide* Clark *et al.* 2002). Their success depends on a number of conditions, the most important being a large number of reintroduced individuals, stability and optimal character of habitat, availability and presence of shelter, high and consistent rate of population growth and low intraspecific competition (Griffith *et al.* 1989 *fide* Clark *et al.* 2002). For the brown bear, all these conditions are negatively correlated with a pronounced so-called "*homing*" instinct, the return to the natal territory, where distances covered successfully may be measured in hundreds of kilometres.

Reintroductions undertaken to date have been performed in two main ways: "hard" and "soft" release. Hard release involves capturing the animal, transporting and releasing it into a new habitat without an acclimatization period, while soft release includes an acclimatization period of variable length, sometimes involving making dens and hibernation in an enclosed section of the new habitat. The application of these methods depends on a number of circumstances, but it is clear that the so-called hard release has been more successful so far (Fig. 10).





Fig. 10 – An anaesthetized brown bear in a transport container before "hard" release into a new habitat. Photo: Đuro Huber.

Experience in the realisation of reintroductions has shown that for their success it is necessary to solve a number of problems simultaneously and adopt a multidisciplinary approach. In order to *prevent the homing effect* it is necessary to perform reintroduction in spring or early summer when food is relatively common and easily available. If that is not the case, artificial provision of food may have the desired effect of homing minimization. Presence of barriers for homing may also be effective, although it is usually difficult to achieve. The presence of roads and other anthropogenic infrastructure, aside from its negative impacts, can be very helpful in this case. Therefore, the homing effect is closely connected to increased mortality of reintroduced individuals in road accidents, and is highest within a year of the moment of reintroduction. Translocation distances should be sufficiently large. According to present experience, this means that the distance between the capture and release sites should be no less than 400 km. In the case of augmentation, greatest success should be ensured by introducing sub-adult individuals (Clark *et al.* 2002).

The demographic characteristics of the introduced bears, and possibly also of bears in the population to be augmented, must be constantly checked through permanent *monitoring* lasting several years (Fig. 11). In practice, the costs of such monitoring are often neglected during planning, so after they appear in later phases of reintroduction such costs are usually reduced by complete lack of monitoring. This is counterproductive and may have pronounced negative consequences on the success of the planned reintroduction.





Fig 11 – Detailed processing of a bear for monitoring in Nopikoski, Sweden. Photo: Sven Brunberg.

The implementation of reintroduction *without genetic profiling* of a previous or existing small population may have the *effect of genetic genocide* (Griffiths *et al.* 1996 *fide* Clark *et al.* 2002). Whenever possible, the reintroduced individuals should belong to the same or phylogenetically closest population or, in an extreme case, to the same subspecies (IUCN 1998).

Habitat evaluation of the area where reintroduction is planned is also one of the key factors for the success of the task. It presupposes the estimation and evaluation of presence and production of food sources, degree of seclusion in the habitat, including presence of roads and other anthropogenic infrastructure, scope and intensity of timber exploitation, hunting and poaching etc. The goal of all evaluations is to understand the reason for the demise of the autochthonous population. If these causes are not removed and the habitat is not revitalized, the reintroduction should not be performed.

For successful reintroduction and revitalization of a population in a given area it is crucially important to introduce individuals that behave like *normal wild animals*. Any deviance from normal behaviour (Fig. 12) causes dependence of individuals on anthropogenic sources of food and greater exposure to humans, leading to reduced survival. Introduction of bears with problematic behaviour results in a lack of acceptance by the local community and removal from the reintroduction process.





Fig. 12 – A bear in a garbage container – typical behaviour of problematic individuals. Photo: Đuro Huber.

Finally, the *socio-economic and political aspects* of reintroduction are connected to the latter observation. Although they are commonly neglected, their importance is so great that without acceptance of the whole project by the local community and sufficient preparation, the reintroduction may experience failure. It is in fact known in practice that the traditionally positive perception of bears as animals is usually followed by a revolt of local public toward reintroduction and/or an increase in population number. The reasons for this phenomenon should be sought in the belief that such projects decrease the degree of freedom in the use of natural resources, justifiable concern that an increase in the number of bears may lead to an increased number of cases of damage, and even fear of attacks and injuries to people caused by bears. Therefore, *research of public opinion and education* through mass media must be the basis of every bear reintroduction, in order to draw positive opinions from the public, to prevent fear and mysticism and to convey ecologically and biologically based facts about these large mammals.



3. Feasibility of bear introductions into northeastern Serbia

3.1. Study area

For the needs of certain sections of this study (distribution range, collection of samples and data on presence) we considered the whole territory of Serbia as one unit, that is, areas of recent distribution of the brown bear. In this context, we studied the areas of western Serbia, southwestern and southern Serbia with the Province Kosovo-Metohija, as well as eastern and southeastern Serbia. However, during preparation of this study and due to activities planned for the future, we focused on the region of eastern Serbia. Therefore eastern Serbia and its constituent parts (Carpathian and Balkan sub-regions) are presented in additional detail because the geological substrate, climate, vegetation, flora and fauna, industrial and demographic characteristics, may have a crucial impact on the study and subsequent activities.

Eastern Serbia

This region of mountains and valleys stretches between the Danube River in the north, the Bulgarian border in the east, the divide toward the Vlasina and Južna Morava Rivers in the south and the valleys of the Velika Morava, Mlava and Pek Rivers in the west (Marković 1980). It includes $13,607 \text{ km}^2$, about 500,000 inhabitants and an average human population density of 36 people per 1 km². It is characterized by the presence of younger fold mountains, complex geotectonics, a great variety and mosaic distribution of geological formations, dominated by limestone of various types and ages. The relief is very diverse, dominated by hilly and hilly-mountainous areas. Large river valleys are situated at the periphery of the region and its centre has smaller watercourses. The climate is very complex, of temperate-continental type with pronounced extreme values of certain components in certain seasons. The region has a very diverse flora and fauna, and for certain groups (mammals, birds) it represents a geographical centre of biodiversity (Stevanović *et* Vasić 1995).

The demographics are very specific. Human settlements are concentrated in the north of the region, population growth is the lowest of all the regions of Serbia, while the multiethnic character is dominated by Serbs, followed by Valachians and Bulgarians. Valachians are mostly present in the northern part of eastern Serbia and Bulgarians are in the borderland area of the southern part of eastern Serbia. Depopulation is great in intensity and especially pronounced in rural areas. Industry includes mining and traditional (extensive) agriculture. Forestry and timber exploitation are highly developed, while the forest complexes are richest in the northern part of the region (Fig. 13).





Fig. 13 – Satellite picture of eastern Serbia. Green areas represent forest complexes. The intensity of the colour indicates the density (structure) of the forest.

3.1.1. Carpathian Serbia (Carpathian sub-region of eastern Serbia)

This sub-region of mountains and valleys is named after the southern slopes of the Carpathians that continue from neighbouring Romania into the northern part of eastern Serbia. The alternative name of the sub-region is northeastern Serbia. The southern boundary of the sub-region is the line of mountains formed by Ražanj-Rtanj-Tupižnica-Stara Planina (Marković 1980). Its micro-regions are Derdap, Zvižd, Homolje, Resava, Crnorečki Kraj, Donjetimočki Basen, Negotinska Krajina, Poreč and Ključ (Fig. 14). The total surface area is 8,423 km², with about 350,000 inhabitants and an average human population density of about 41 people per km².

The geological substrate is characterized by extraordinary diversity and a mosaic distribution of various formations: crystal-like schist, red sandstones, Mesozoic limestone, Paleogene and Neogene lacustrine sediments, Quaternary and Aeolian river sediments, igneous rocks etc. The relief is polygenous and of very diverse forms: paleoabrasive, riverine, karst, Aeolian. It is dominated by numerous small, low mountains separated by small valleys. Numerous riverbeds have formed gorges and canyon valleys.





Fig. 14 – Map of the Carpathian sub-region of eastern Serbia, from Marković (1980).

Limestone formations encompass almost half Carpathian Serbia, mostly its central and northern parts, resulting in the presence of several hundred caves, ledges, pits and other speleological formations. Due to the specific mountain-valley relief, the climate of the Carpathian sub-region is influenced by the climatic factors in neighbouring, relatively homogenous areas: steppe-continental (Banat), temperate-continental (central Serbia) and typical continental climate (Valachian Plain). The mountains usually have subalpine climatic characteristics. The winters are cold and sharp with a lot of precipitation, while the summers are very warm with sparse precipitation. The watercourses are numerous but small, and their network is a result of previous moister climatic phases. There are numerous strong river springs, but also underground rivers. There are no large natural lakes, but there are some artificial ones, the largest being Đerdapsko Lake on the Danube. The Carpathian sub-region is known since ancient times for its richness in forests. These forests are still present, but they are visibly devastated and diminished. They have remained well preserved in mountain areas where exploitation was more difficult, and sometimes they even have the character of primeval forest. The largest continuous complexes are in the north of the sub-region,



in the area of Majdanpečka Domena, Đerdap National Park and Severni Kučaj Mt. (Fig. 15). The fauna is very rich and diverse. For certain classes of vertebrates, the Carpathian sub-region represents the centre of taxonomic diversity (Stevanović *et* Vasić 1995). Such a situation is present primarily in mammals, of which 67 species were recorded in the mid 1990s (Savić *et al.* 1995), while the latest results include 76 species of mammal (unpublished data by a group of authors).



Fig. 15. The Largest continuous forest complexes are present in the area of Majdanpečka Domena, Đerdap NP and Severni Kučaj Mt.. *Google* satellite shot.

3.1.2. Balkan Serbia (Balkan sub-region of eastern Serbia)

This sub-region is named after the mountain range of the Balkan Mountains starting in Serbia with Stara Planina Mt., which continues into central Bulgaria. It represents the southern half of eastern Serbia (Marković 1980). It is composed of numerous mountain, valley and lowland microregions, such as Sokobanjski, Svrljiški and Staroplaninski Kraj, Ponišavlje, Koritnica, Jelašnica, Zaplanje etc. (Fig. 16). It includes 5,184 km² with about 150,000 inhabitants and an average human population density of about 29 people per km².





Fig. 16 – Map of the Balkan sub-region of eastern Serbia, from Marković (1980).

The geological structure of this sub-region is very diverse. The valleys are filled with lacustrine and river sediments, while the mountains are mostly built of chalky limestone, although there are also some sandstones, schist, andesite eruptives and gabbro. The mountains originated during the Alpine orogenesis and stretch in a latitudinal direction, in the north of the sub-region coming into contact with the meridian-positioned Carpathians. The largest and best-known mountains are Stara Planina and Suva Planina, Svrljiške Mts., Ozren, Devica, Tupižnica, Tresibaba and Belava.

Karst relief is very well distributed in Balkan Serbia, with numerous speleological formations and the only karst field in eastern Serbia, Odorovačko Polje, to the south of Pirot. The climate is very similar to the climate of the Carpathian sub-region. The limestone mountains are dry; small and large springs appear in their foothills. The Balkan sub-region lags behind Carpathian Serbia in industrial development. It is more an agricultural than a mining-industrial area, with extensively developed, traditional agriculture, primarily in hill and mountain areas. Scarce precipitation, combined with strong erosion and denudation, are serious problems in the sub-region, causing presence of denuded areas and less forest than in the Carpathian sub-region. The largest continuous forest complexes are present in part of Stara Planina Mt., on Mt.



Leskovik and on Svrljiške Planine. The fauna is very rich and diverse. For some classes of vertebrates the Balkan sub-region represents a centre of taxonomic diversity (Stevanović *et* Vasić 1995). Such a situation primarily affects mammals. In the mid-1990s 50 mammal species were recorded (Savić *et al.* 1995), while the latest results include 55 species (unpublished data by a group of authors). These data show that biodiversity in Balkan Serbia is high, although lower than in the neighbouring northern sub-region.

3.2. List of habitats and analysis of their appropriateness for bear survival

The habitat of an animal species includes the total of all physical and biotic factors, such as the climate, geological substrate, geomorphologic forms of relief and vegetation of an area. As the brown bear is primarily a silvicolous mammal species, which assumes the presence of continuous forest complexes, vegetation as a very important component of habitat is given a special place in this chapter. The other habitat factors for the area of northeastern Serbia are given in basic form in chapter 3.1.

Forests are important for several reasons, the dominant one being their role in providing the trophic base, either as a direct source of plant food or as a source of food for numerous species of herbivores partially included in the bear's diet. Forests are also important from the aspect of seclusion, as they present safety and shelter, as well as a large choice of sites for dens. The larger forest habitats are also poorly inhabited or uninhabited by humans, providing the necessary seclusion and absence of disruption and harassment for bears. The latter factor is positively correlated with undisrupted reproduction and a regular and normal lifecycle of all forest animals, especially the bear.

3.2.1. Vegetation of eastern Serbia

The vegetation of eastern Serbia is characterized by richness, complexity and diversity of plant associations, due to very complex geological and geomorphologic development, a complex of diverse climatic conditions as well as the very specific developmental history of the flora. The plant associations in this area are often highly contrasting from an ecological viewpoint, even in spatially very close areas (Mišić 1981). Generally, the region is significantly dominated by beech forest, although due to the dominant temperate-continental climate with some traces of arid, steppe climate it would be easy to expect domination by climatogenous forests of Italian and Turkey oak. Beech forest occupies the central parts of the region, while the peripheral eastern parts are dominated by thermophilous oak forests, making a huge contrast by their appearance. Their existence might be explained by the above-mentioned climatic factors. The most important vegetation complexes are composed as follows:



Forests of Italian oak *Quercetum frainetto* Forests of Italian and Turkey oak *Quercetum frainetto-cerris* Forests of Sessile oak sensu lato *Quercetum petraeae, sensu lato* Upland beech forests *Fagetum submontanum* Montane beech forests *Fagetum montanum*

Forests of Turkey oak *Quercetum cerris* Forests of Turkey oak and oriental hornbeam *Quercetum cerris carpinettosum orientalis* Forests of oriental hornbeam *Carpinetum orientalis* Low forests and thickets of lilac *Syrino-Carpinetum orientalis*, *Cotino-Syringetum*

Beech-fir forests *Abieto-Fagetum* Spruce forests *Piceetum excelsae* Spruce-fir forests *Abieto-Piceetum*

Sub-alpine shrubby associations of low juniper, bilberry and sub-alpine spruce *Vaccinio-Junipero-Piceetum*

The position of these forest associations in the list was determined according to their vertical zonal distribution. Willow-poplar and ash-pedunculate oak forests, relict glacial association of Silesian willow and green alder and associations of beech and Greek maple are not included in the list due to their minimal presence as well as due to their low relevance as components of potential habitat of the brown bear. This is also true for the highest parts of dominant mountains such as Stara and Suva Planina Mts., where on the highest peaks there are some associations of dwarf pine and pasture vegetation, and for lowland and valley areas dominated by forest-steppe or steppe formations which are mostly anthropogenously changed and cultivated.

3.2.2. Vegetation of northeastern Serbia

The contrast in groups of plant communities (in relatively close areas in this subregion) is the most pronounced and unique in Serbia. Before presenting a detailed list of plant associations in this sub-region, it is necessary to explain some characteristics of the relief, as they play a significant role when explaining the specifics of the vegetation. Two rows of low and medium-height mountains stretch parallel to each other in north-south direction: in the western parts of northeastern Serbia one row is composed of Mts. Šomrda, Homoljske Planine, Kučaj, Rtanj, followed by Ozren and Devica, which according to Marković (1981) are outside the sub-region. The somewhat more eastern row starts in the north with Miroč Mt., continues to Veliki Greben, Veliki and Mali Krš, Stol, and with interruption to Tupižnica, all the way to Stara Planina, which represents the largest mountain of the neighbouring Balkan subregion of Serbia. These two elongated lines of mountains play a role as barriers that cause the specific mesoclimatic and other characteristics of this upland sub-region. The vegetation in this area is rich, lush and diverse, with the ability to regenerate and



develop quickly, due to favourable climatic conditions. The temperate-continental climate is modified by the presence of the Valachian Lowland, the Carpathian Mountains and the Danube River basin, causing increased precipitation, increased relative air humidity, decreased wind impact, decreased variation in temperature and humidity. These favourable climatic modifications are particularly important for vegetation, especially forest, with the result that forest complexes are increasingly luxuriant toward Danube River in the north (Fig. 17).

Diverse and well-developed vegetation, and especially its mosaic distribution, are also caused by the diversity and mosaic distribution of geological substrate. The most important is andesite, represented in the substrate from Majdanpek and Debeli Lug to Vlaole and Bor, including the great Basin of Bor (Mišić 1981).



Fig. 17 – Satellite picture of the northern part of eastern Serbia, showing occurrence and density gradation of forest complexes toward the Danube shore. *Google* satellite shot.

Other important minerals are crystal schists, granodiorite and diabase. However, especially important for the development and presence of various types of vegetation such as polidominant forests with beech, maples, ashes and lilac, as well as several poor relict associations with witch hazel, walnut, Mahaleb cherry etc, is the presence of limestone substrate and rocks, with the common appearance of typical karst.

The low altitudes of the mountains in northeastern Serbia (up to 1,000m a.s.l.) and their small altitudinal range (less than 700m) have caused the character of the



vegetation's altitudinal zonation, with the highest peaks ending in beech forest but the lower boundary of the zone of montane beech forest *Fagetum montanum* being situated at 500-600m a.s.l. Terrains that gravitate toward the Danube (Šomrda, Homoljske Planine) have some widely distributed forests of beech and silver lime (Fig. 18) and beech-maple forests of mixed structure. The next widest distributed is sessile oak forest, represented with several types. Besides, the edapho-climatogenous type of Turkey oak forests and Turkey oak / oriental hornbeam forests is also widely distributed in this part of northeastern Serbia.



Fig. 18 – Forest of beech and silver lime on the northeastern slopes of Šomrda Mt. Photo: Milan Paunović.

In Negotinska Krajina, on the easternmost slopes of the mountains and hills of northeastern Serbia, there are just a few areas of forest vegetation, and such a situation is particularly pronounced in lowland and low-hill areas. Negotinska Krajina is dominated by Italian oak forest, while the area of Ključ has Italian and Turkey oak forest. On the other hand, the western parts of northeastern Serbia, have many features in common with the eastern parts of this sub-region, in a vegetation sense, due to the presence of former lake terraces and Holocene alluvial plains along the larger rivers. At the lowest altitudes, the dominant associations are light willow, poplar, pedunculate oak and ash forests, followed by fragments of forests of various types and beech forests in the valleys, while beech forests dominate from 500m upwards at Homoljske Planine Mts. In the limestone terrain the dominant associations are forests of Turkey



oak and oriental hornbeam, oriental hornbeam forests on steep terrain and low forests and thickets of lilac and oriental hornbeam.

The northeastern sub-region and the whole of eastern Serbia are very rich in various types of gorges and canyons on limestone substrate, have very specific and very rich flora and vegetation that often has features of sub-Mediterranean refugia, where the concentration of thermophilous floristic elements and vegetation associations is very pronounced.

3.2.3. Importance of vegetation in northeastern Serbia for the presence and survival of brown bears

Although the brown bear, according to its taxonomic position and body shape is classified among the Carnivora, it fulfils about 95% of its trophic needs with plant material. The remaining 5% or so of animal food is mostly composed of invertebrates and vertebrate carrion. Therefore, forest vegetation provides the bear with seclusion, shelter and den sites, and to a large extent also supports its trophic needs. Besides the direct use of various kinds of forest fruits and other plant parts, the forest vegetation indirectly provides the bear with first order consumers, primarily the young of various even-toed ungulates (Artiodactyla).



Fig. 19 – Lower stratus herbaceous vegetation in the forest of beech and silver lime at Šomrda Mt. In the foreground is wild raspberry *Rubus idaeus*. Photo: Milan Paunović.



In certain phases of the annual cycle, the diet of brown bears is dominated by different types of food (see 2.1.2.). This phenomenon is mostly caused by the availability of food types during various yearly phenophases. Although the upper forest strata enable feeding on fruit of forest trees during the autumn period, during the remaining part of the year when the bear is active, the lower strata of vegetation and layer of herbaceous plants gain importance (Fig. 19). The phenological order of consumption of various forest plants and their fruits is presented in Table 4.

Season of the year	Plant species			
	Allium ursinum			
	Arum maculatum			
Spring	<i>Gramminae</i> sp.			
	<i>Trifolium</i> sp.			
	<i>Rumex</i> sp.			
	Angelica silvestris			
	Aposeris foetida			
	<i>Fragaria</i> sp.			
Summer	Rubus idaeus			
	Rubus fruticosus			
	Rhamnus cathartica			
	Vaccinium myrtillus			
	Fagus sylvatica			
	Malus sylvestris			
	Pyrus communis			
Autumn	Corylus avellana			
	Sorbus aucuparia			
	Cornus mas			
	<i>Quercus</i> sp.			

Table 4 – List of consumed plant food by phenophases, according to Dečak *et al.* (2005), based on data of brown bear diet in Croatia.

The vegetation of northeastern or the Carpathian sub-region of Serbia completely fulfils the survival needs of the brown bear and enables the unaffected progress of all phases of the lifecycle.

3.2.4. Other characteristics of brown bear habitat in northeastern Serbia

All other natural characteristics and habitat parameters cited in the introductory part of this chapter (3.2.) obviously suit the existence of the brown bear population in eastern Serbia. This is supported by historical and recent data on numerous records of brown bears in this area. However, when discussing habitat characteristics of northeastern Serbia, it is impossible to ignore one of the key factors of protection and conservation of nature in general: the human factor.





Fig. 20 – Position of present and potential pollutants in the area of northeastern Serbia. *Google* satellite picture, with modifications.

In fact, although the cited characteristics show relatively well-preserved, high-quality habitats and their suitability for the life and survival of brown bears, it must also be mentioned that the area of northeastern Serbia includes two large mining basins, chemical industry plants and several military bases. While the chemical industry plants in Prahovo and the mining basin in Bor are basically situated at the fringes of the sub-region, attention must be called to the position of the mining basin in Majdanpek and at least one military base in the central part of habitat suitable for bears. Further, new announcements on the beginning of research on mining ores at Crni Vrh Mt. near Bor and some other localities near Majdanpek may additionally increase the anthropogenic pressure on these areas (Fig. 20). On the other hand, in most of the area there are definite signs of significant depopulation, presence of the so-called "white plague", additionally increasing the tranquillity of the habitat, enabling regeneration of forests, overgrowing of orchards, fields, pastures and other cultivated surfaces, increasing the abundance and density of autochthonous vertebrate species.

3.2.5. Habitat suitability of temporary shelter – quarantine for reintroduced individuals

After a recommendation by the Directorate of Forests of the Ministry of Agriculture, Forestry and Water Management, Republic of Serbia, we visited the locality of Valkaluci–Nekudovo, situated at Južni Kučaj Mt., including the 48th, 49th, 66th, 67th



and 69^{th} departments of Despotovac Forest Enterprise. This locality has a surface area of about 7-15 ha; its altitude is about 900m a.s.l. and it is dominated by montane beech forest *Fagetum montanum*. There are also some artificial (planted) associations of spruce, maple and black pine, as well as several pasture areas and one area under rocks. In the forest association there is usually no ground vegetation or it is very sparse. There is one permanent watercourse, the Nekudovo stream, flowing in a westward direction. This area has a well-developed network of forest roads. According to all characteristics it completely matches the needs for building a temporary shelter – quarantine for reintroduced individuals. The only negative remark might be the proximity of a military base at about 10 km direct line distance.

3.3. Phylogenetic relationships of bears in the study area, in Slovakia's Tatre Mountains and from neighbouring populations

As stressed in chapter 1.1, one of the major tasks in this study was the genetic profiling of brown bears in the study area and in the Tatre Mountains of Slovakia, as well as an analysis of genetic distance between populations from various parts of the range in Serbia. The goal was to determine the interconnections of individuals recorded from relatively isolated areas in Serbia and their relationships with ranges in neighbouring countries. This important task was performed by Milica Keckarević-Marković, MSc., from the PCR Centre at the Faculty of Biology, University of Belgrade. Here we include her report from the successfully completed research. The isolation and processing of genetic material, as well as analysis of the results obtained, were done in the PCR laboratory of the National Institute for Wild Fauna in Bologna, Italy, under the guidance and supervision of Professor Etore Randi.

3.3.1. Introduction

The determination of the genetic structure of a population and of the genetic diversity within it enables the indirect determination of its size (Avise *et al.* 1984) as well as the expected rise of inbreeding in each successive generation (Wright 1931), which could endanger the population by "inbreeding-induced depression", resulting in extinction of the population. On the other hand, determination of genetic diversity among populations should enable reconstruction of phylogeographic relationships among populations (Avise *et al.* 1987) and identification of various microevolution lines, which could be the basis for a conservation strategy based on augmentation of threatened, isolated populations with bears from other populations.

Up to now it has been determined that within the European population of brown bears (*Ursus arctos* L.) there are two lineages (eastern and western) and, according to the determined genetic distance of mitochondrial DNA (mtDNA), which is 7.13%, it is assumed that they separated about 850,000 years ago (Taberlet *et al.* 1994). The eastern lineage includes the brown bears of Russia, Romania and Estonia (Taberlet *et al.* 1994). It is assumed that brown bears in Slovakia also belong to this lineage. On



the other hand, the western lineage has two subgroups. One includes the brown bears of the Iberian Peninsula as well as bears in southern Sweden and Norway, while the other subgroup includes brown bears in the Balkan Peninsula (so far analyses have been performed on populations in Croatia, Slovenia, Greece and Bulgaria) and Italy (the Apennines and Alps) (Taberlet *et al.* 1994, Randi *et al.* 1994). According to the geographic distribution as well as mtDNA genetic distance in bears of the western European lineage, it is assumed that the two subgroups became separated during the last Ice Age. According to geographic distribution, it was assumed that bears from Tara (western Serbia), Bosnia, Šara Mt. (Kosovo) and eastern Serbia (Carpathians-Homoljske Planine Mts.) belong to the western lineage of the European brown bear.

In order to determine the genetic diversity within and among the analysed brown bear populations, biological material collected by non-invasive methods was used. This included: faeces, hairs collected with traps and skin samples of shot bears in private hunters' collections. Use of non-invasive methods (non-invasive genetic sampling) enabled better monitoring and genetic analyses of animals difficult to capture, dangerous animals, as well as better monitoring of threatened populations.

Within this research, the analysis was performed on the sequence of mitochondrial DNA. This has several advantages over other types of analyses, such as analysis of microsatellites, especially in cases of non-invasive genetics, as DNA isolated from faeces is degraded, full of contaminants from the faeces and present in very small quantities. MtDNA is present in 100-1,000 copies in each cell, in contrast to nuclear DNA, of which 2 copies are present in each cell. Vertebrate mtDNA accumulates point mutations about 5-10 times more rapidly than genome DNA, so the genetic analysis of short sequences (the method of choice in non-invasive genetics) may yield information on genetic diversity within and among populations even in cases when a small number of samples is analysed, such as in this case. In addition, mtDNA is not susceptible to recombination and is inherited down maternal lines.

3.3.2. Material and methods

DNA was isolated from the following biological material: hair (7 samples), skin (4 samples) and faeces (19 samples). A total of 30 samples were analysed. Table 5 summarises the biological material from which DNA was extracted and the further analyses that were performed.

Collection of material:

Faeces were collected at locations on Tara Mt, in eastern Bosnia and Šara Mt (Fig. 21), while no faeces were found in eastern Serbia, due to the small size of the bear population and a lack of permanent feeding sites. When collected, faeces were estimated to be 1-15 days old. After collection they were placed in a freezer at -20°C and were later transferred to 96% ethanol for storage. Samples from Slovakia (from localities in Tatry National Park and Nízke Tatry National Park) were kindly provided



Sample number	Collector's number	Type of sample	Locality
1	79	Hair	Slovakia
2	80	Hair	Slovakia
3	81	Hair	Slovakia
4	82	Hair	Slovakia
5	83	Hair	Slovakia
6	84	Hair	Slovakia
7	85	Hair	Slovakia
9	1	Skin	Eastern Serbia
10	2	Skin	Eastern Serbia
11	4	Skin	Eastern Serbia
12	93	Skin	Šara Mt. – Kosovo-Metohija
13	10	Faeces	Tara
14	11	Faeces	Tara
15	21	Faeces	Tara
16	24	Faeces	Tara
17	50	Faeces	Tara
18	58	Faeces	Tara
19	61	Faeces	Tara
20	65	Faeces	Tara
21	70	Faeces	Tara
22	74	Faeces	Tara
23	30	Faeces	Bosnia
24	55	Faeces	Bosnia
25	54	Faeces	Bosnia
26	75	Faeces	Bosnia
27	76	Faeces	Bosnia
28	86	Faeces	Šara Mt. – Kosovo-Metohija
29	87	Faeces	Šara Mt. – Kosovo-Metohija
30	88	Faeces	Šara Mt. – Kosovo-Metohija
31	89	Faeces	Šara Mt. – Kosovo-Metohija

by Robin Rigg, MSc., of the Slovak Wildlife Society, Liptovský Hrádok, Slovak Republic.

Table 5 – Number, type and origin of samples from which DNA was extracted.





Fig. 21 – Geographic position of collecting sites and number of collected samples in Serbia and Bosnia-Herzegovina.

<u>Isolation of DNA:</u> Skin samples were cut into small pieces and soaked in water for 60 minutes until they rehydrated.

DNA material from hair (about 10 hairs) and skin (2 mm³) was isolated in TNE (Tris-NaCl-EDTA) buffer for cell lysis with the addition of SDS and proteinase K, after which it was extracted by use of silica and the addition of guanidium thiocyanate (GTC).



About 25 mg of faeces was dried of its alcohol content and soaked into the buffer for cell lysis with GTC, and then DNA was extracted with the use of silica.

<u>Analysis of DNA:</u> With specific primers, the PCR method was used to multiply the sequence of mitochondrial DNA from the sample. The following primers were used:-

- 1. Primers for amplification of part of a control region of mtDNA (D-loop), 88 base pairs (bp) in size (Hoss *et al.* 1992);
- 2. Primers for amplification of part of a control region of mtDNA, 269 bp in size (Taberlet *et al.* 1994).

Amplification of DNA was in both cases performed in an ABI (Applied BioSystem, Foster, USA) 9700 PCR machine, using the following program: 94°C for 15 seconds, 50°C for 15 seconds, 72°C for 30 seconds; 40 amplification cycles.

Success of PCR was checked in 2% agar gel coloured with etidium-bromide.

The PCR products were cleared of surplus primers and NTP by exonuclease and phosphatase, incubated at 37°C for 30 minutes, and which the enzymes were inactivated at 80°C for 15 minutes.

The cleared PCR products were then sequenced using an ABI Dye-Terminator Kit and ABI 3100 automatic sequencer.

The resulting sequences were then arranged in the Seqscape (ABI) program and levelled in the BioEdit program. The MEGA 3.1 computer program (Kumar *et al.* 2004) was used to analyse the final data, where the gathered sequences were compared to previously published referent sequences (Hoss *et al.* 1992, Randi *et al.* 1994, Taberlet *et al.* 1994), that is, determination of genetic distance among the analysed samples and construction of a phylogenetic tree of the brown bear.

3.3.3. Results and discussion

Sequencing the mtDNA region of 88 bp was successful in 21 samples out of 30 samples analysed (70%), while sequencing the region with 269 bp was successful in 4 out of 15 samples (27%). Of the 7 hair samples from Slovakia, 5 samples were successfully analysed for the region of 88 bp; all these samples had the same haplotype (haplotype SLOV88). One of these samples was successfully analysed for 269 bp (haplotype SLOV269). From eastern Serbia, 2 out of 3 samples were successfully analysed for the region of 88 bp (haplotype IS88) and then 1 of these samples was successfully analysed for 269 bp (haplotype IS269). From Tara, 9 out of 10 samples were successfully analysed for 269 bp (haplotype IS269). From Tara, 9 out of 10 samples were successfully analysed for the region of 88 bp, and there were two distinct haplotypes: haplotype TARA1-88 and TARA2-88. One sample from each of these two haplotypes was then analysed for 269 bp (haplotype TARA1-269 and TARA2-269). Out of 5 samples from eastern Bosnia, 3 were successfully analysed, with the same sequence as the samples from Tara (haplotype TARA1-88 and TARA2-88). Out of 5 samples from Šara, 4 were analysed for 88 bp (haplotype ŠARA88). Table 6 shows the sequenced samples, their origin, resulting haplotypes and success.



Sample number	88 bp haplotype	269 bp haplotype	Locality
1	SLOV88	SLOV269	Slovakia
2	/*	**	Slovakia
3	/		Slovakia
4	SLOV88	/	Slovakia
5	SLOV88	/	Slovakia
6	SLOV88	/	Slovakia
7	SLOV88		Slovakia
9	IS88	IS269	Eastern Serbia
10	IS88	/	Eastern Serbia
11	/		Eastern Serbia
12	/		Šara-Kos
13	TARA2-88	TARA2-269	Tara
14	TARA1-88		Tara
15	TARA2-88	TARA2-269	Tara
16	TARA2-88		Tara
17	/		Tara
18	TARA1-88		Tara
19	TARA1-88		Tara
20	TARA1-88		Tara
21	TARA1-88	/	Tara
22	/		Tara
23	TARA2-88	/	Bosnia
24	TARA1-88	TARA1-269	Bosnia
25	TARA1-88		Bosnia
26	/		Bosnia
27	/	/	Bosnia
28	ŠARA88		Šara-Kos
29	ŠARA88	/	Šara-Kos
30	ŠARA88	/	Šara-Kos
31	ŠARA88	/	Šara-Kos

* Unsuccessful analysis of the sample

** Sample not analysed

Table 6 – List of sequenced samples, their origin and success in isolating the two haplotypes.

According to the sequence of part of the control region of mtDNA in brown bear samples analysed within this project, as well as previously published sequences (Taberlet *et al.* 1994, Randi *et al.* 1994), a phylogenetic tree was constructed. This is presented in Figures 22 (88 bp) and 23 (269 bp).



Viability increase and recovery of brown bear population in northeastern Serbia - a feasibility study



Fig. 22 – Phylogenetic tree of brown bears, based on sequence of mtDNA; control region of DNA with 88 bp (Uar-*Ursus arctos*; Uam-*Ursus americanus*; Origin of haplotypes: TRE-Trento, CRO-Croatia; ABR-Abruzzo; SLO-Slovenia; BUL-Bulgaria; GRE-Greece; RUS-Russia; EST-Estonia; RO-Romania; AME-America; NOR-Norway; DAL-Dalarno





(Sweden), CAN-Cantabria, PYR-Pyrenees; Samples processed within this project are marked as: Uar_number of sample_number of haplotype (as in Table 6).

0.01

Fig. 23 – Phylogenetic tree of brown bears, based on sequence of mtDNA; control region of DNA with 269 bp (Uar-*Ursus arctos*; Uam-*Ursus americanus*; Origin of haplotypes: TRE-Trento, CRO-Croatia; ABR-Abruzzo; SLO-Slovenia; BUL-Bulgaria; GRE-Greece; RUS-Russia; EST-Estonia; RO-Romania; AME-America; NOR-Norway; DAL-Dalarno (Sweden), CAN-Cantabria, PYR-Pyrenees; Samples processed within this project are marked as: Uar_number of sample_number of haplotype (as in Table 6).

Figure 22 does not show a clear divergence into two lineages (western and eastern) due to the small size of the analysed sequence (88 bp), while from Figure 23 we may infer that brown bears from localities in Serbia and eastern Bosnia clearly belong to the western European lineage whereas brown bears from Slovakia belong to the eastern European lineage. According to the previously determined genetic distance between the western and eastern European lineages, it is assumed that they separated about 850,000 years ago. Based on recommendations by Taberlet *et al.* (1994), that the eastern and western European lineages should be treated as separate conservation units, due to the large genetic distance between them, the strategy of choice for the conservation and recovery of bear populations in eastern Bosnia, or simply better protection of populations in eastern Serbia (prevention of poaching and the



creation of feeding places), but not introduction of bears from Slovakia. The arrival of bears from Slovakia in eastern Serbia would lead to loss of genetic fund in this population, which is already threatened by low population numbers.

3.4. Estimates of minimum viable populations

According to the available data, collected during 6 months of this project, the existence of reproductive groups was recorded in northeastern Serbia and the population size was estimated to range from a pessimistic 3-5 individuals to an optimistic 10 individuals. Also recorded were a lack of organized hunting and the permanent presence of poaching. The only data on legal hunting in northeastern Serbia is from 1984, when one male was shot at the locality Valka Luci (Hadži Pavlović in verb., Paunović et al. 2005). According to data collected in eastern Serbia over the last few years, we estimate that the poaching rate is at least one bear per year. The results of a study on population dynamics of brown bears showed that the minimum viable population may be estimated at about 25-30 individuals (Saether et al. 1998). As the identified area of current brown bear range in the Carpathian sub-region of eastern Serbia does not exceed 300 km², the population density is 3-4 specimens per 100 km². It is estimated that in this area there is an additional 200 km² of suitable habitat, but that there is also a discontinuity between such habitats. Overall, it seems very possible that the viability of this population is supported by the immigration of individuals from neighbouring areas. This particularly applies to the Bulgarian bear populations whose marginal parts reach the western slopes of Stara Planina (Swenson et al. 2000). This phylogenetic proximity is also proven by genetic profiling (see 3.3.). On the other hand, immigration of individuals is also very possible from neighbouring Romania, as the Danube River should not represent a difficult barrier for bears. This hypothesis has not been proven yet, as we could not collect samples from the neighbouring areas of Romania. Putting this information together, it seems likely that the bear population in the Carpathian sub-region represents a relatively isolated population, which occasionally "adds" specimens from the large, viable populations of neighbouring Bulgaria and the Carpathians, keeping population numbers constant but small. Therefore, due to the regular recording of reproductive groups, it is very possible that this immigration is naturally preventing inbreeding.

3.5. Public attitude

Conservation of bears is closely connected to their interaction with humans. Bears can cause damage to personal property and in some cases pose a direct danger to people. On the other hand, poaching is the main risk for conservation of this species. Public attitude is a key factor for success of reintroduction projects (see 2.5.). Therefore, part of this study includes research on public opinion on many aspects of life and presence of bears in northeastern Serbia. Here we will present two of these aspects: the general opinion on bears of various target groups and the opinions of various segments of the



public toward a potential project for bear population augmentation in northeastern Serbia.

The survey was performed with the help of volunteers from the local community and students from the Faculty of Biology, University of Belgrade, who were first trained on the issue of reintroduction as well as on methods and techniques of interviewing. The questions in these forms were previously used to research public opinion on carnivorous species of mammals (Paunović 2001), with certain modifications for this study.

The survey was performed in northeastern (so-called Carpathian) Serbia (see 3.2.1.), the region where the bear reintroduction is planned, in 5 municipalities: Majdanpek, Žagubica, Bor, Boljevac and Negotin. Particular attention was given to the municipality of Majdanpek, for several reasons: besides the town of Majdanpek, this municipality also includes Donji Milanovac, most of Đerdap National Park and its fringe forest complexes where in the last 10 years there have been the most records of bears in the whole of eastern Serbia.

The survey had the following target groups: high school students, college students, forest rangers, hunters and the local community. The complete sample was also divided into rural and urban population. While examining perceptions of the bear we focused on two important questions: is the bear dangerous and is it destructive? Out of 363 interviewed persons, 61% believed that the bear is dangerous and 29% believed that it is not; 47% considered bears destructive and 41% beneficial (Table 7, Fig. 24).

		Opinion on brown bear				
Target group	Number of persons interviewed	Dangerous	Not dangerous	Destructive	Beneficial	
Local community	90	66	21	54	27	
Students	78	57	21	33	45	
Forest rangers	36	15	18	3	27	
Hunters	75	57	18	60	15	
High school students	84	57	24	21	60	
Rural population	231	153	72	138	78	
Urban population	132	69	33	33	69	
Total	363	222	105	171	147	

Table 7 – List of answers of interviewed target groups about personal perception of bears.



If the total group of participants is divided according to the residential status into rural and urban, the same ratio is found in both groups as in the total sample on the question of whether bears are dangerous, but there are completely opposite results for the question of whether bears are destructive. The rural population believed, in a ratio of 2:1, that bears are destructive, whereas the urban population believed, in the same ratio, that bears are beneficial. The local population from various backgrounds but living within bear range in northeastern Serbia had the same perceptions as the rural population and the same trend is evident in the target group hunters. On the other hand, high school and college students also believed that bears are dangerous, but they thought them to be beneficial. Perception by forest rangers was different: over half of them believed bears are not dangerous, but they were obviously deeply convinced of their beneficial status.

















Fig. 24 – Comparative representation of perception of bears by various target groups.

When the 363 respondents (Table 8) were asked if they would support a project to augment or increase the population number of bears in their area, 37% gave a positive answer, 39% negative and 24% neutral. The group most opposed to bear reintroduction is the local community, regardless of background, that lives within recent bear range (73% opposed), followed by hunters (52% opposed) and the rural population (46%). The greatest percentage of positive answers was given by high school students (57% in favour), followed by forest rangers (55%) and the urban population (50%), while college students supported such a project in 38% of cases. Also important is the percentage ratio of neutral answers, indicating a relative lack of interest in increasing the bear population. This was most prominent in the target group college students (43% neutral), followed by high school students (36%), while in the range 25-23% followed the rural population, hunters and the urban population, respectively (Fig. 25).

		Opinion on bear introduction project				
Target group	Number of persons interviewed	Positive	Negative	Neutral	No answer	
Local community	90	21	63	3	3	
Students	78	30	15	33	0	
Forest rangers	36	18	12	3	3	
Hunters	75	18	39	18	0	
High school students	84	48	6	30	0	
Rural population	231	66	102	57	6	
Urban population	132	66	36	30	0	
Total	363	132	138	87	6	

Table 8 – List of answers of interviewed target groups about the bear augmentation project.





Fig. 25 – Comparative representation of opinion of various target groups on the bear introduction project.



The results presented clearly show that the most negative perception of bears and the potential project for their introduction is present in the population in most direct contact with nature and the one that has had contact with these mammals. These are primarily the rural and local community, which show resistance toward bears due to direct exposure to damage, but also fear of direct contact with these animals. They are closely followed by hunters, who feel aversion primarily due to belief that a bear can cause considerable damage to local populations of game animals (which are managed by hunters as part of their activities). Of course, a good part of these perceptions is due to insufficient knowledge of bear biology. However, this should not be a surprise, as these people could not have a different opinion on the bear, because it is based exclusively on old folk beliefs and rare cases of direct experience. This way of thinking is also certainly supported with the bear's status as one of the least studied mammal species in Serbia. The relatively positive opinion of bears among forest rangers should be understood in light of their professional effort toward improvement and use of forest ecosystems, as well as the wish for hunting management of this "game" species. The experiences of forestry institutions and organisations of neighbouring countries and countries of the former Yugoslavia in profitable hunting management of brown bears give hope to this target group that this will eventually also "take root" in Serbia.

On the other hand, the urban population and high school and college students are better informed on the biology and ecology of bears and their position and role in nature. This is supported by constant and common exposure to media, especially that of foreign production. Their positive opinions and significant indifference concerning the project of bear population augmentation in their area are the consequence of a lack of direct contact with nature.



4. Conclusions

The brown bear is one of 98 species of mammal (Mammalia) and one of 19 species of carnivore (Carnivora) in Serbia. It is one of the country's least studied and least known mammal species.

There are over 100 records of bears in Serbia from the last ten years. Optimal bear habitats are mostly in the hilly-mountainous parts of western, southwestern, eastern and southern Serbia. While the range and habitats are significantly fragmented, there are connections and corridors with the corresponding parts of the populations in neighbouring countries.

The presence of reproductive groups was recorded regularly but in a small number of cases, indicating low population density and abundance of brown bears in Serbia.

According to the latest estimates, the number of bears in Serbia does not exceed 53 individuals, but this number does not include individuals from the Kosovo-Metohija Province, as no credible data are available from this area since 1998.

The brown bear is protected by a closed season throughout the year. It is classified as Vulnerable, while in the new Ordinance on Protection of Natural Rarities, under preparation, its proposed status is that of a protected species under active management. Due to its status, it is proposed for inclusion in the Red List of Mammals of Serbia, which is presently in preparation.

The presence of bears in northeastern Serbia has been known for a long time and there are many written records. In the last 30 years the population size of this species has been declining; current estimates vary from 3-5 to 10 individuals. Throughout Serbia, including the northeastern sub-region, poaching is constantly present and has significant negative consequences for the state of this population.

Analysis of habitat conditions and capacity has shown that the area of northeastern Serbia has optimal conditions for the development and increase of brown bear populations. This is supported by abiotic and biotic factors, primarily vegetation, availability and diversity of trophic resources, as well as demographic changes.

The analysis of phylogenetic relationships, performed for this project and for the first time in Serbia, gave undisputable answers to the question of which other populations are closest to individuals present in northeastern Serbia. These are primarily members of Bulgarian populations, which live in central Bulgaria and the Balkan sub-region of Serbia. On the other hand, only a small genetic distance was determined to members of the Dinaric population, represented in our country in western, southwestern and southern mountain areas, as well as neighbouring Bosnia-Herzegovina. Bears from the Carpathian population in the Slovak Tatre Mts., kindly offered by the relevant Slovak ministry, belong to a completely different, eastern, phylogenetic lineage of brown bears, which has been separated from the western lineage (that includes all brown bear populations in Serbia) for the last 850,000 years. The introduction of the autochthonous genetic fund.



Analysis of the results of a survey of public opinion indicated that various target groups show variability in perception of brown bears and acceptance of a project to increase the bear population in northeastern Serbia. The results show that pronounced negative opinion on bears and resistance to increasing their numbers is present in the local community, especially the rural population and hunters, that is, the part of the community in direct contact with nature and that which has already had experience and contact with bears. Positive opinions are present in the urban population and participants in younger age cohorts (high school and college students), as well as forest rangers, who see the bear as a game species, which could be managed and that would significantly improve hunting industry and forestry.

Reintroduction, in this case augmentation, is one possible way to revitalization and increase the size of the brown bear population in northeastern Serbia. Projects of this type are becoming increasingly common globally, including in Europe, but their success depends on applying and respecting numerous conditions, explained in detail in this study. Augmentation is an expensive, difficult and risky method for increasing the viability of a population and must never be one-way and uncontrolled. Individuals used for augmentation should be sub-adult, mostly or exclusively females, and the augmentation should in this case be performed by so-called *hard* release, without prolonged presence of bears in the immediate vicinity of humans. Introduction of habituated, problematic individuals must be avoided completely. The area of Južni Kučaj (Valkaluci-Nekudovo) is considered one of the most suitable for augmentation.

Another way to increase viability (including population density and abundance) in northeastern Serbia would be to facilitate natural regeneration. This should include complete control of hunting, prevention of poaching, a ban on any disturbance and persecution, if needed also directed and well-planned construction of feeding places, as well as constant monitoring with the use of modern, non-invasive methods.



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