

# Distribution and genetic status of brown bears in FYR Macedonia: implications for conservation

Alexandros A. Karamanlidis · Aleksandar Stojanov · Miguel de Gabriel Hernando · Gjorge Ivanov · Ivna Kocijan · Dimche Melovski · Tomaž Skrbinšek · Andreas Zedrosser

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**Abstract** Conservation and management of large carnivores is often hampered by the lack of information of basic biological parameters. This is particularly true for brown bears (*Ursus arctos*) in the Former Yugoslav Republic (FYR) of Macedonia. The bear population in this country is important, as it links bear populations of the central part of the Dinaric–

Pindos population and the endangered population to the south in Greece. The aim of this study was to assess bear presence in FYR Macedonia and to provide the first evaluation of the genetic status of the species in this country. Bear presence was assessed through a questionnaire and sign surveys, while the genetic status of the species was evaluated through non-invasive genetic sampling from power poles and microsatellite analysis. The results of the study indicate the continuous and permanent presence of brown bears in FYR Macedonia from the border to Kosovo in the northwest, along the border to Albania and Greece in the south; bear presence around Mount Kožuf in the south of the country was seasonal. High levels of genetic diversity were recorded, and it appears that this bear population is currently not threatened by low genetic variability. Cross-border movements of bears between FYR Macedonia and Greece were documented, indicating the presence of an interconnected population and outlining the necessity for a coordinated international approach in the monitoring and conservation of the species in south-eastern Europe.

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A. A. Karamanlidis · M. de Gabriel Hernando  
ARCTUROS, Civil Society for the Protection and Management of Wildlife and the Natural Environment, Thessaloniki 54624, Greece

A. Stojanov · G. Ivanov · D. Melovski  
Macedonian Ecological Society, Building of the Institute of Biology, Faculty of Natural Sciences and Mathematics, Skopje 1000, FYR Macedonia

I. Kocijan  
Department of Biology, Faculty of Science, University of Zagreb, HR-10000, Zagreb, Croatia

T. Skrbinšek  
Department of Biology, Biotechnical Faculty, University of Ljubljana, Večna pot 111, 1000, Ljubljana, Slovenia

A. Zedrosser  
Faculty of Arts and Sciences, Department of Environmental and Health Studies, Telemark University College, 3800, Bø i Telemark, Norway

A. A. Karamanlidis (✉) · A. Zedrosser  
Department of Ecology and Natural Resource Management, Norwegian University of Life Sciences, Ås 1432, Norway  
e-mail: akaramanlidis@gmail.com

A. Zedrosser  
Department of Integrative Biology and Biodiversity Research, Institute of Wildlife Biology and Game Management, University of Natural Resources and Applied Life Sciences, Vienna 1180, Austria

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## Introduction

While the brown bear (*Ursus arctos*) is the most widespread bear species in the world, it has disappeared from large parts of its original range in Europe. Centuries of human persecution, as well as fragmentation, degradation, and ultimately loss of habitat have taken their toll on the species (Swenson et al. 2000). This is especially evident in the western and Mediterranean

parts of Europe, where brown bears survive mainly in fragmented populations, and many of the smaller populations (e.g., Spain, France, Italy, and Greece) are considered threatened and in need of assistance (Zedrosser et al. 2001; Swenson et al. 2011). The largest remaining bear population in southeastern Europe is the Dinaric–Pindos population (Linnell et al. 2008), which stretches from Slovenia in the north over the entire Dinaric mountain range and all the way into Greece to the south. Despite its international importance for large-scale bear conservation in Europe (Swenson et al. 2000; Zedrosser et al. 2001), detailed and accurate information about the biological and conservation status of these bears, especially in some former Yugoslav countries, is still scarce.

Bears in the Former Yugoslav Republic (FYR) of Macedonia are of considerable conservation importance, as they constitute the link between the bear populations of the central part of the Dinaric–Pindos population (i.e., in Serbia and Montenegro) and the endangered population to the south in Greece, where brown bears reach their southernmost distribution in Europe. Reliable field data from FYR Macedonia on bear distribution and abundance are currently lacking for most of the country. Questionnaire-based information indicates that brown bears are mainly distributed in the mountainous areas in the western, central, and southern parts of FYR Macedonia, and that there is occasional migration into FYR Macedonia from Bulgaria in the east (Stojanov et al. 2010). The population size in FYR Macedonia is estimated at 160–200 individuals (Stojanov et al. 2010); however, this estimate is not based on systematic genetic or other population monitoring methods. Although brown bears are protected according to Articles 9 and 13 of the Law on Hunting of 2009, they are considered to be endangered due to extensive poaching and habitat loss and fragmentation (Stojanov et al. 2010).

For the conservation and management of bears in FYR Macedonia, but also in the entire region of southeastern Europe, a financially feasible, reliable, and rapidly obtained assessment of the conservation status and biology of the species is essential and has been identified as a priority for the implementation of effective conservation and management actions for brown bears in the country (Stojanov et al. 2010). The main goal of our study was to assess brown bear presence in FYR Macedonia and provide the first evaluation of its genetic status in the country. The results of the study are set in the context of the limited information on the biology of the species and the urgent need for effective management and conservation actions for brown bears in FYR Macedonia.

## Materials and methods

### Study area

The range of brown bears in FYR Macedonia is dominated by rugged topography with high mountain peaks ranging between 2,300 and 2,800 m. Forests at lower altitudes (700–900 m) are dominated by Italian oak (*Quercus frainetto*) and Turkey oak (*Quercus cerris*), and at medium altitudes (900–1,500 m) by oaks (*Quercus* sp.) and beeches (*Fagus* sp.). Forests in the subalpine zone (>1,500 m) are dominated by beeches and conifers (*Pinus* sp.). The local fauna includes all three large carnivores of southern Europe [i.e., brown bears, wolves (*Canis lupus*), and lynx (*Lynx lynx*)] (Melovski et al. 2008). The distribution of brown bears in FYR Macedonia also includes all three National Parks (NP) of the country, NP Mavrovo, NP Galichica, and NP Pelister.

### Assessing the presence of brown bears in FYR Macedonia

The survey design to assess bear presence was based mainly on the natural marking and rubbing behavior of bears on wooden poles of the electricity and telephone network (hereafter power poles) that have been treated with creosote and did not use any lure to attract the animals, which makes the method cost-effective. This method for detecting bear presence has been developed and successfully tested in the adjacent bear populations of Greece (Karamanlidis et al. 2007, 2010b). Signs of bear presence on power poles such as bite and claw marks are the most frequent bear signs in the field. They are also durable and provide, therefore, a cumulative history of the presence of the species at a specific location. As power poles are in fixed and easily approachable locations, their inspection is easy, rapid, and consequently reasonably economical (Karamanlidis et al. 2007, 2010b).

Our research efforts focused on the core range of brown bears in the western, central, and southern parts of FYR Macedonia, where power poles were inspected during the initial exploratory surveys in 2008 and 2009 and evaluated based on predefined suitability criteria, such as power pole visibility, type of habitat, and vegetation density (Karamanlidis 2008). The most suitable power poles were selected to create a network of sampling sites for the direct assessment of bear presence, but also for the noninvasive genetic monitoring of the species, which would cover as much of the study area as possible. Barbed-wire hair traps (Kendall and McKelvey 2008; Karamanlidis et al. 2010b) were placed on the poles and inspected monthly (April–October 2008 and 2009). In addition, while traveling to the power poles, other indirect signs of bear presence (i.e., tracks, scats, feeding signs) were recorded (Karamanlidis et al. 2007).

Because of the low density of power poles and forestry roads in the central part of southern FYR Macedonia (i.e., the areas around Mount Pelister and Mount Kožuf, Fig. 1), we assessed bear presence in these areas with a questionnaire survey. The questionnaire consisted of ten questions aimed at obtaining information about the respondent (i.e., sex, age, place of residency, profession), information about the presence of bears in the area [i.e., presence/absence of bears, type of presence (i.e., permanent vs. seasonal vs. sporadic), records of reproduction], and information about local human–bear interactions [i.e., damages/no damages caused by bears, types of damages, annual damage frequency, records of bears aggressive towards humans, poaching of bears, and the perceptions of bears by the locals (i.e., dangerous vs. not dangerous, harmful vs. not harmful, aggressive vs. not aggressive)] (Table 1). Whenever possible, interviewed people were asked to specify the geographic locations and the dates of bear sightings and interactions with humans. The respondents were randomly selected during visits to villages in the study area.

#### Evaluation of genetic status

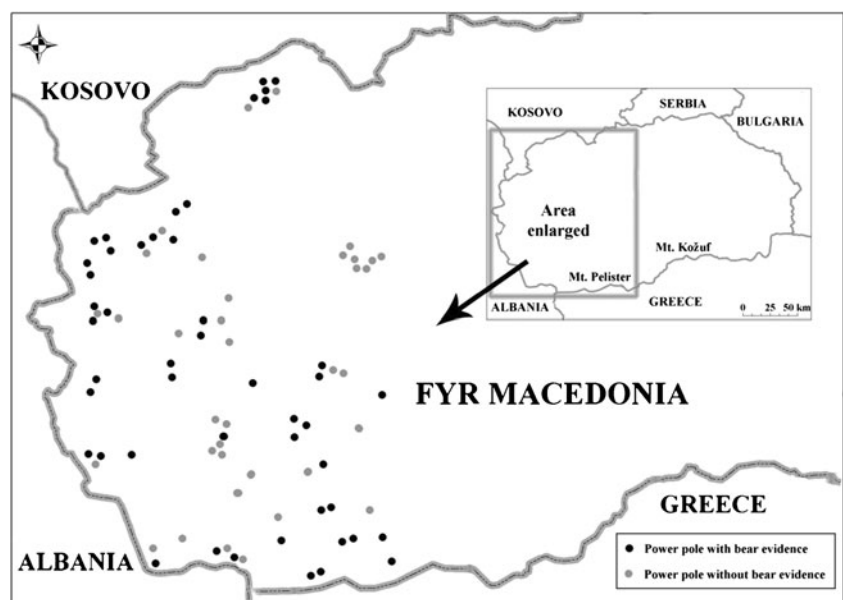
Each tuft of hairs on a spike of the barbed wire was considered a sample and was collected; hair samples were collected without contacting human skin, placed in paper envelopes, and then stored at room temperature in ziplock bags with silica gel (Roon et al. 2003). DNA was extracted using the DNeasy Blood & Tissue kits (QIAGEN, Hilden, Germany), following the manufacturer's instructions. All extractions took place in a facility in which amplified DNA had never been handled before.

Each sample was genotyped at the microsatellite loci G1A, G1D, G10H, G10J, G10L, G10U (Paetkau and

Strobeck 1994; Paetkau et al. 1998), G10C, G10M, G10P (Paetkau et al. 1995), MU23, MU26, MU50, MU51, MU59 (Taberlet et al. 1997), CXX110 (Proctor et al. 2002), Msut-2 (Kitahara et al. 2000), REN144A06, and REN145P07 (Breen et al. 2001). Sex identification was established through the analysis of the amelogenin gene (Ennis and Gallagher 1994). Thermal cycling was performed using an MJ Research PTC100 thermocycler with 96-well “gold” blocks. PCR buffers and conditions were used according to Paetkau et al. (1998), except that markers were not co-amplified, as co-amplification may reduce the success rates for hair samples (Paetkau, personal communication). We used 3  $\mu$ l of DNA extract per PCR reaction, except during error checking, when 5  $\mu$ l was used; in this way, low-quality samples were culled efficiently, and we did not run out of template DNA for samples that required a lot of cleanup. Two millimolars of  $MgCl_2$  was used for all markers except G10J (1.8 mM). Genotyping was performed by the Wildlife Genetics International (Nelson, B.C., Canada). The sizing of the PCR products was performed using capillary electrophoresis. We used an automated sequencer (ABI 310), and genotypes were determined using ABI Genescan and Genotyper version 2.1 software. Error checking and general quality assurance followed the recommendations of Paetkau (2003) and Roon et al. (2003) that have been summarized by Kendall et al. (2008); error rates have been reported according to Kendall et al. (2009). Test for allelic dropout, presence of null alleles, and scoring errors caused by stutter peaks were performed with Micro-Checker version 2.2.3 (van Oosterhout et al. 2004).

The informativeness of the loci in the study for evaluating genetic diversity was assessed by calculating

**Fig. 1** Map of the FYR Macedonia indicating the study area and the locations of 367 power poles inspected (2008–2009) to assess brown bear presence (because of the scale of the map a *single point* may represent more than one pole)



**Table 1** Results of a questionnaire survey of randomly selected people on the distribution of bears and on human–bear interactions carried out in two study areas [southwest ( $N=29$ ), south-central ( $N=13$ )] in southern FYR Macedonia in 2009

	South-west	South-central
Are bears present in your area?	Yes (100 %)/no (0 %)	Yes (46 %)/no (53 %)
Is the presence of bears in the area permanent, seasonal, or sporadic?	Permanent (82 %), seasonal (18 %), sporadic (0 %)	Permanent (0 %), seasonal (67 %), sporadic (33 %)
Have females with cubs been observed in your area?	Yes (27 %)/no (73 %)	Yes (16 %)/no (84 %)
Do bears cause damages to human property in the area?	Yes (65 %)/no (35 %)	Yes (0 %)/no (100 %)
What types of property have been damaged?	Crops (46 %), orchards (27 %), beehives (18 %), livestock (9 %)	None (100 %)
What is your estimate of the mean annual number of damages by bears?	1–5 cases (45 %); 6–10 cases (33 %); >10 cases (22 %)	None (100 %)
Have bears ever been aggressive towards humans?	Yes (7 %), no (93 %)	Yes (0 %), no (100 %)
Have bears been killed by humans in the area?	Yes (0 %)/no (100 %)	Yes (0 %)/no (100 %)
Do you believe that bears are dangerous?	Yes (45 %)/no (55 %)	Yes (31 %)/no (69 %)
Do you believe that bears are harmful?	Yes (49 %)/no (38 %); no opinion (13 %)	Yes (31 %)/no (69 %)
Do you believe that bears are aggressive towards humans?	Yes (14 %)/no (76 %); no opinion (10 %)	Yes (0 %)/no (84 %); no opinion (16 %)

the polymorphism information content (PIC) (Botstein et al. 1980) in the program PowerMarker version 3.25 (Liu and Muse 2005). To evaluate the suitability of the marker set for identifying individuals, the probability of identity among siblings ( $P_{ID-Sib}$ ) (Waits et al. 2001) was calculated using the Gimlet version 1.3.2 software (Valière 2002). The pairs of genotypes that were matched on all but one, two, and three loci (1-MM, 2-MM, and 3-MM pairs) were identified using the program GenAlEx 6 (Peakall and Smouse 2006).

Nuclear DNA diversity was measured as the number of alleles per locus ( $A$ ), the expected ( $H_e$ ) and observed heterozygosity ( $H_o$ ), with the program PowerMarker version 3.25 (Liu and Muse 2005). Deviations from the Hardy–Weinberg equilibrium (HWE) were tested using the exact probability test implemented in the software Genepop version 4.0.10 (Raymond and Rousset 1995); a Markov chain set to 100 batches, with 5,000 iterations per batch and 10,000 steps of dememorization, was used to obtain an unbiased estimate of the exact probability. Pairwise tests for linkage disequilibrium were performed using Fisher’s method (Sokal and Rohlf 1994) with 1,000 batches and 10,000 iterations per batch.  $P$  values were adjusted for multiple comparisons using the sequential Bonferroni correction (Rice 1989). Global HWE tests across all loci for heterozygote deficiency and heterozygote excess were performed using Fisher’s method.

In order to compare genetic diversity of bears in FYR Macedonia with other well-studied bear populations, we used the reference population approach (Skrbinšek et al. 2012a). In this approach, the locus set of the reference and the studied population are reduced to the loci they both have

in common. To correct for unequal sample size, genotypes from the reference population are then resampled with replacement multiple times to the same sample size as that of the studied population, and average allelic richness, expected heterozygosity, and their standard deviations are calculated over all the subsamples. Heterozygosity ratio ( $H_{er}$ ) and allelic richness ratio ( $A_{rt}$ ) indices are then calculated as the ratio between these summary statistics in the studied population and their subsampling-corrected values in the reference population. This provides a comparison of genetic diversity of the studied population with the reference population and all other populations that have had these indices calculated using the same reference population (Skrbinšek et al. 2012b). We used the data by Skrbinešek et al. (2012b) on brown bears in Slovenia as the reference population since this large dataset has already been used as a reference for comparisons of a large number of brown bear populations around the world. We used the ten loci that our study has in common with the reference dataset (i.e., G1A, G10C, G10D, G10J, G10L, G10M, G10P, MU23, MU50, MU51, and MU59). We applied R-scripts provided by Skrbinešek et al. (2012b) to run the subsampling, with 1,000 random subsamples. All analyses were run in R version 2.14.2 (R Development Core Team 2011). We used heterozygosity ratio ( $H_{er}$ ) and allelic richness ratio ( $A_{rt}$ ) to compare the genetic diversity of bears in FYR Macedonia with some of the other populations in the Dinaric–Pindos range and the Carpathian Mountains. Because of multiple resampling in the reference population approach, the errors of  $H_{er}$  and  $A_{rt}$  should be normally distributed, and the  $Z$  test (Sokal and Rohlf 1994) was used to test for statistical significance of the

difference between these indices in bears in FYR Macedonia and other populations.

## Results

### Presence of brown bears in FYR Macedonia

A total of 367 power poles were evaluated during the initial exploratory surveys (2008,  $N=278$ ; 2009,  $N=89$ ). Eighty power poles in the study area showed signs of bear marking and rubbing activity; no signs of bear presence were found on power poles in central FYR Macedonia (Fig. 1). From the 80 power poles with signs of bear activity, 45 fitted the suitability criteria by Karamanlidis (2008) and were selected to create a network of stations for assessing bear presence through time and for the noninvasive collection of genetic samples (Fig. 2). Overall, 340 inspections of these 45 hair traps were carried out (hair traps were not inspected in July–September 2008 due to widespread forest fires in the study area), and bear presence was recorded on 85 occasions, mainly in north- and southwestern FYR Macedonia. While traveling for the inspection of the power poles, 11 additional signs of brown bear presence were recorded along forestry roads (six scats, one track, two livestock deprecations, one dead bear, and one visual observation).

Forty-two questionnaires were conducted in 2009, 29 in the western part of southern FYR Macedonia (Mount Pelister area), and 13 in the central part (Mount Kožuf area) (Fig. 1, Table 1). The majority of people interviewed were males (93 %) with a mean age of 54 years. Respondents were involved in a variety of outdoor-related activities (i.e., 11 % farmers, 11 % hunters, 10 % livestock breeders, 5 % foresters, 53 % other). Eighty-two percent of people

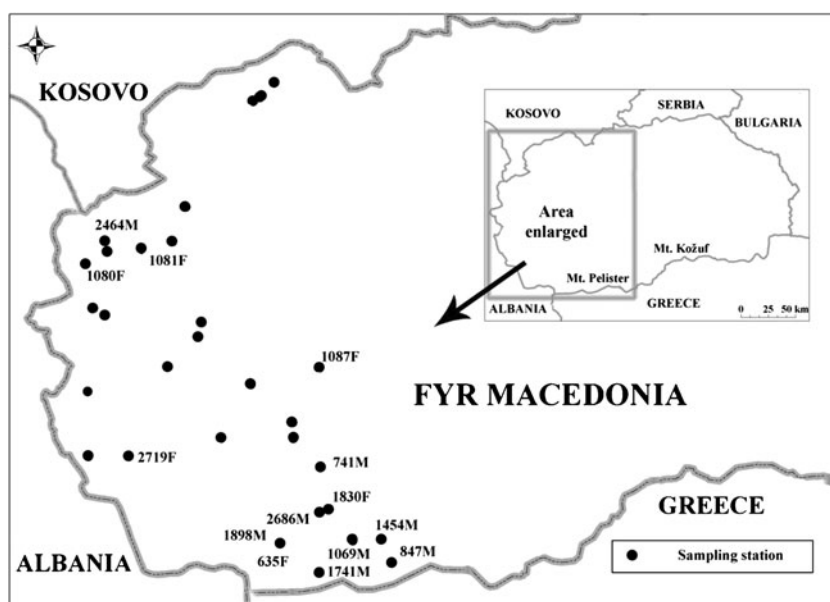
interviewed in southwestern FYR Macedonia believed that bear presence in their area was permanent, while the majority of people (67 %) in the Mount Kožuf area believed that bear presence was seasonal.

### Genetic status of brown bears

The hair traps were inspected 340 times, and 106 hair samples were collected (Table 2). Forty-eight samples were culled prior to the first stage of analysis because they did not contain any follicles; we analyzed 58 samples, obtained a full 18-locus genotype for 20 (34 %) of these samples, and identified 14 unique genotypes [six females (individuals “635,” “1080,” “1081,” “1087,” “1830,” and “2719”) and eight males (individuals “741,” “847,” “1069,” “1454,” “1741,” “1898,” “2464,” and “2686”)] (Fig. 2). Twelve of the animals were identified in or near NP Mavrovo and NP Pelister, and six individuals were identified on multiple occasions during the study period.

None of the loci used in the study showed evidence of frequency distortion through large allele dropouts or stutter bands or the presence of null alleles. The overall mean of all markers used in the study had a PIC value higher than the recommended value of 0.6 (Buchanan et al. 1993), suggesting a high degree of informativeness of these markers in evaluating genetic diversity (Table 3). The accumulated probability of identity among siblings ( $P_{ID-Sib}$ ) for 18 loci was  $1.53E-07$ . A minimum of five most informative loci (G10P, Msut-2, CXX10, G10L, and G10C; accumulated  $P_{ID-Sib}=7.14E-03$ ) were needed to reach the threshold value of 0.01 recommended for correctly discriminating individuals if the data are to be used for population size estimation (Waits et al. 2001) (Table 3). No samples matching at all but one, two, or three loci were found in

**Fig. 2** Map of the FYR Macedonia indicating the locations of 45 sampling stations for the noninvasive, genetic monitoring of brown bears (2008–2009) and of 14 individuals identified [identification number and sex (female: *F*; male: *M*) is shown for each bear]; because of the scale of the map, a *single point* may represent more than one sampling station



**Table 2** Number of hair traps inspected, how often bear sign at a hair trap was recorded, and number of hair samples collected during a noninvasive genetic survey of brown bears in FYR Macedonia in 2008–2009

	2008				2009						
	Apr	May	Jun	Oct	Apr	May	Jun	Jul	Aug	Sep	Oct
Hair traps inspected	33	33	33	33	28	33	33	33	45	16	16
Bear sign recorded	17	12	12	7	12	9	5	4	5	2	0
Hair samples collected	7	22	12	7	15	19	8	4	5	7	0

our sample set. These results indicate that we did not over- or underestimate the number of individual bears in our study and that the microsatellite loci selected are suitable for the noninvasive genetic study on bears in the country.

All loci used in the study were polymorphic, with the number of alleles per locus ranging between 3 and 9, with a mean of 5.7 (Table 3). The unbiased expected heterozygosity was 0.72, and the mean observed heterozygosity was 0.74. Global tests across loci showed no deviation from HWE ( $P=0.649$ ), while no locus had a significant deficiency in heterozygotes at the  $P<0.05$  level (Table 3). Statistical tests for linkage disequilibrium were computed for all pairs of loci, and four pairs of loci were found to be in linkage disequilibrium [ $P(\text{MU26 and Msut-2})=0.003$ ;  $P(\text{G1D and CXX110})=0.016$ ;  $P(\text{MU59 and P07})=0.027$ ;  $P(\text{G10J and G1A})=0.048$ ], but after adjustment of  $P$  values for multiple comparisons using the sequential Bonferroni correction, none of the tests ( $n=153$ ) revealed statistically significant results at  $\alpha=0.05$  (Table 3).

**Table 3** Descriptive statistics at 18 polymorphic loci of wild brown bears in FYR Macedonia, including the number of alleles ( $A$ ), unbiased expected ( $H_e$ ), and observed ( $H_o$ ) heterozygosity, within-population inbreeding estimate (FIS), the polymorphism information content (PIC), the probability of identity amongst siblings ( $P_{\text{ID-Sib}}$ ), and the multi-locus probability of identity amongst siblings (Prod.  $P_{\text{ID-Sib}}$ )

Marker	$A$	$H_e$	$H_o$	FIS	PIC	$P_{\text{ID-Sib}}$	Prod. $P_{\text{ID-Sib}}$
G10J	6	0.760	0.928	-0.185	0.724	3.93E-01	3.93E-01
G1A	5	0.630	0.846	-0.306	0.560	4.87E-01	1.91E-01
G10C	7	0.790	0.857	-0.046	0.759	3.73E-01	7.14E-02
G1D	5	0.704	0.642	0.123	0.665	4.30E-01	3.07E-02
G10H	7	0.635	0.500	0.247	0.609	4.72E-01	1.45E-02
MU23	4	0.683	0.846	-0.2	0.632	4.46E-01	6.46E-03
MU50	6	0.775	0.785	0.023	0.741	3.83E-01	2.48E-03
MU59	9	0.744	0.714	0.078	0.726	3.98E-01	9.87E-04
G10P	7	0.793	0.785	0.046	0.764	3.71E-01	3.66E-04
G10M	3	0.615	0.769	-0.212	0.535	4.99E-01	1.83E-04
MU26	6	0.674	0.538	0.239	0.634	4.49E-01	8.22E-05
G10U	5	0.688	0.714	-2.09E-17	0.636	4.43E-01	3.64E-05
G10L	6	0.790	0.714	0.133	0.760	3.73E-01	1.36E-05
CXX110	6	0.793	1	-0.225	0.762	3.72E-01	5.05E-06
REN145P07	5	0.739	0.769	0	0.697	4.08E-01	2.06E-06
MU51	5	0.673	0.636	0.102	0.620	4.53E-01	9.33E-07
Msut-2	7	0.792	0.846	-0.027	0.764	3.71E-01	3.46E-07
REN144A06	5	0.692	0.538	0.259	0.635	4.42E-01	1.53E-07
Mean	5.777	0.721	0.746	0.003	0.679		

## Discussion

Assessing distribution is an integral part of monitoring of endangered wildlife populations (Mattson and Merrill 2002; Posillico et al. 2004; Ratnayeke et al. 2007). In the absence of reliable, hard-to-obtain population size estimates of endangered species, changes in distribution can be used to monitor population trends in species of conservation concern (Pollock 2006).

The data collected during the genetic, sign, and questionnaire surveys indicate a permanent and continuous presence of brown bears in FYR Macedonia, from the Šar Planina Mountains at the border to Kosovo in the northwest, along the border to Albania until Mount Baba in the south, and along the border to Greece until Mount Kožuf in the east. Considering the lack of bear damages and the other information obtained from the questionnaires, the presence of bears at Mount Kožuf in the central part of southern FYR Macedonia appears to

be seasonal or sporadic. This is in accordance with results of previous studies based on questionnaires (Stojanov et al. 2010), and data on bear distribution in adjacent areas of Greece (Karamanlidis unpublished data); Mount Kožuf has likely just recently been recolonized by the species. Despite reports of bear presence in central FYR Macedonia (Stojanov et al. 2010), we were unable to find support for this while monitoring the power poles, which would suggest a low population density of bears in this area (Karamanlidis et al. 2007, 2010b; Karamanlidis 2008).

Obtaining measures of genetic diversity of populations with known recent demographic history and conservation status provides a useful approach for comparing diversity levels of populations with unknown history and status and can provide valuable insights into the consequences of rarity, which can be critical for conservation planning (Johnson et al. 2009). When compared with other populations using the reference population approach, the genetic diversity of brown bears in FYR Macedonia was high, which is comparable with the genetic diversity of other bears in the Dinaric–Pindos area and significantly higher than in the endangered bear population in the Apennine Mountains (Table 4). This is difficult to imagine without recent gene flow between FYR Macedonia and at least some other parts of the Dinaric–Pindos bear population, and allows for speculations of a favorable genetic conservation status of brown bears in FYR Macedonia. The small sample size, however, limits the power of between-population comparisons, precludes stronger conclusions, and warrants caution in interpretation of these results. Further research is necessary to confirm these conclusions and further understand the connectivity along the Dinaric–Pindos bear population, which would be important for future population-wide management and conservation.

The results of the questionnaires on human–bear interactions in southern FYR Macedonia generally indicate low levels of conflicts and an overall favorable image of the species. Few incidents of bear damages were recorded by the questionnaire survey, and bears were generally not considered to be dangerous to people. Seven percent (two persons) responded to have heard about aggressive bears; however, the respective incidents occurred 20 and 30 years before the current study. People in the area with bear damages viewed bears as more harmful than people in the area without damages. In general, the results of the questionnaire survey are in accordance with the results of a similar study in the northern part of the country (Lescureux and Linnell 2010) but are in contrast to the situation in neighboring Albania (Karamanlidis et al. 2010c) and Greece (Karamanlidis et al. 2011), where high levels of livestock depredation and a generally negative

perception of bears are prevalent. This suggests a more favorable conservation situation for bears in FYR Macedonia; however, this also points out the importance of keeping human–bear conflict levels low through carefully planned management actions.

No indications for poaching were recorded during the questionnaire survey; however, this result must be viewed with caution as it may be related to the secretive and illegal nature of the issue and the resulting wariness of respondents to talk about it, or alternatively that the survey was carried out in areas with low bear densities and few illegal killings.

#### Conservation and management recommendations

The noninvasive genetic sampling efforts enabled the identification of 14 different bears in the western and southwestern part of FYR Macedonia. This number represents roughly 10 % of the population estimated in the country, which is the population identification limit that has been proposed for studies assessing the genetic status of bear populations in the region (Karamanlidis et al. 2010a). The use of a power pole sampling network seems to be applicable as a financially bearable and rapid method for assessing the presence and genetic status of brown bears in the FYR Macedonia, but it is necessary to expand the area covered by the sampling network, while at the same time increasing the sampling frequency. In the areas with known or suspected low bear densities, the power pole sampling method should be augmented with questionnaire surveys carried out in regular intervals. Also the use of multiple sources of genetic sampling [e.g. scats (Bellemain and Taberlet 2004; Skrbinišek et al. 2010)] should be considered (Boulanger et al. 2008). For genetic monitoring purposes, using less than 18 loci would facilitate genotyping of noninvasive samples with low-quantity or low-quality DNA, such as scat or hair with only few follicles.

Future population monitoring efforts should aim at assessing the distribution of bears throughout the entire country to create a distribution map, which should be used as a baseline for the future trend monitoring of bears in the FYR Macedonia (Liu et al. 2009). Population monitoring efforts need also to be coordinated with adjacent countries. Three individuals identified in the study have since been identified also in adjacent Greece (Karamanlidis unpublished data), which indicates the presence of a single interconnected population between the two countries and outlines the necessity for a coordinated international approach in the monitoring and conservation of the species in southeastern Europe. The establishment of a regional genetic bear register (Karamanlidis et al. 2010a) would ensure comparability of genetic data between countries and populations and greatly support cross-border monitoring efforts.

**Table 4** Comparison of genetic diversity of brown bears, measured at microsatellite loci, in the Dinaric–Pindos (DP) region, Carpathians and Apennines with genetic diversity of bears in FYR Macedonia using the reference population approach

Population	<i>N</i>	Reference	<i>A</i> (s.e.)	<i>H<sub>e</sub></i> (s.e.)	<i>A</i> (s.e.)	<i>H<sub>e</sub></i> (s.e.)	<i>A<sub>T</sub></i> (s.e.)	<i>H<sub>er</sub></i> (s.e.)	<i>p</i> ( <i>A<sub>T</sub></i> )	<i>p</i> ( <i>H<sub>er</sub></i> )
Romania (Carpathians)	16	A	7.78 (0.81)	0.81 (0.010)	5.15 (0.56)	0.70 (0.030)	1.51 (0.23)	1.16 (0.05)	0.112	0.033
Romania (Carpathians)	109	B	8.46 (0.57)	0.80 (0.014)	6.33 (0.54)	0.73 (0.023)	1.34 (0.15)	1.09 (0.04)	0.219	0.174
FYR Macedonia (DP)	14		5.90 (0.50)	0.72 (0.021)	5.03 (0.51)	0.70 (0.028)	1.17 (0.16)	1.03 (0.05)		
Croatia (DP northwest)	156	D	7.58 (0.54)	0.74 (0.028)	6.48 (0.60)	0.73 (0.025)	1.17 (0.14)	1.01 (0.05)	0.500	0.389
Slovenia (DP northwest—reference population)	513	E	6.68 (0.41)	0.73 (0.020)	—	—	1.00 (0.06)	1.00 (0.03)	0.160	0.303
Greece (DP south)	49	F	6.33 (0.42)	0.76 (0.020)	6.55 (0.52)	0.77 (0.023)	0.97 (0.10)	0.99 (0.04)	0.145	0.266
Northern Slovakia (Carpathians)	71	B	6.08 (0.29)	0.71 (0.025)	6.20 (0.54)	0.73 (0.023)	0.98 (0.10)	0.97 (0.05)	0.157	0.198
Serbia (DP, central)	10	C	5.25 (1.42)	0.67 (0.162)	4.88 (0.21)	0.70 (0.015)	0.96 (0.07)	1.08 (0.12)	0.115	0.350
Central Slovakia (Carpathians)	96	B	6.00 (0.25)	0.70 (0.031)	6.30 (0.54)	0.73 (0.023)	0.95 (0.09)	0.95 (0.05)	0.115	0.129
Eastern Slovakia (Carpathians)	16	B	5.23 (0.22)	0.65 (0.028)	5.47 (0.49)	0.72 (0.025)	0.96 (0.09)	0.91 (0.05)	0.126	0.045
Italy (Apennines)	17	A	2.44 (0.24)	0.44 (0.069)	5.20 (0.21)	0.70 (0.014)	0.63 (0.10)	0.47 (0.05)	0.002	<0.001

References: A: Zachos et al. (2008); B: Straka et al. (2012); C: Karamanlidis et al. (2012); D: Kocijan et al. (2011); E: Skrbinišek et al. (2012a); F: Karamanlidis et al. (2010b)

*N* sample size, *A* mean number of alleles per locus, *H<sub>e</sub>* expected heterozygosity, *A<sub>T</sub>* allelic diversity ratio, *H<sub>er</sub>* expected heterozygosity ratio, *p*(*A<sub>T</sub>*) and *p*(*H<sub>er</sub>*) *p* values of tests comparing allelic diversity and heterozygosity ratios of bears in FYR Macedonia with other populations



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