Turkish Journal of Zoology

Volume 41 | Number 5 Article 2

1-1-2017

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Turkish Journal of Zoology

http://journals.tubitak.gov.tr/zoology/

Research Article

Turk J Zool
(2017) 41: 774-782

doi:10.3906/zoo-1603-23

Mitochondrial genetic diversity and structuring of northern white-breasted hedgehogs from the Central Balkans

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Received: 10.03.2016 • **Accepted/Published Online:** 12.02.2017 • **Final Version:** 28.09.2017

Abstract: Even though the phylogeography of hedgehogs has been well studied, information on the genetic variability of the northern white-breasted hedgehog *Erinaceus roumanicus* from the Balkans is lacking, since the previous studies were based on very limited sampling across the Balkans. The aim of this study is to estimate the genetic diversity and population structuring of *E. roumanicus* from the Central Balkans and to complement an already proposed phylogeographic scenario of this species. Tissue samples of 108 road-killed northern white-breasted hedgehogs were collected across the Central Balkan countries of Serbia, Montenegro, Bosnia and Herzegovina, and Macedonia. A partial fragment of the mtDNA control region (CR) was amplified and sequenced. Nine of 13 haplotypes detected in this study have not been previously published. The results indicate a moderate level of haplotype diversity of *E. roumanicus* from the Central Balkans and differentiation into four spatial groups, which are named after the approximate sampling localities as northwestern-central, northeastern, southwestern, and southeastern groups. The observed population structure in the Central Balkans remains less pronounced in further phylogenetic and phylogeographic analyses of the dataset comprising *E. roumanicus* and *E. concolor* mtDNA CR sequences. The central position of Balkan haplotypes in a median-joining network indicated its role as a primary source population for postglacial northward expansion.

Key words: *Erinaceus roumanicus*, Central Balkans, mtDNA diversity, northern white-breasted hedgehog, phylogeography

1. Introduction

In the western Palearctic, the genus *Erinaceus* is currently represented by three species: the west European hedgehog (*E. europaeus*) distributed in western Europe, the northern white-breasted hedgehog (*E. roumanicus*) distributed in eastern Europe and Ponto-Mediterranean regions, and the southern white-breasted hedgehog (*E. concolor*) distributed in Asia Minor and the Levant (Hutterer, 2005; Bolfíková and Hulva, 2012). In the scientific literature, until recently, *E. roumanicus* was listed mostly as *E. concolor* (Sommer, 2007). Studies based on morphological data (Kryštufek, 2002) and mitochondrial and nuclear genetic data (Santucci et al., 1998; Seddon et al., 2001, 2002; Schaschl et al., 2002; Berggren et al., 2005; Bannikova et al., 2014) indicated a deep split between *E. roumanicus* and *E. concolor*, suggesting them as sister species with a divergence time of approximately 1–2 Myr (Bannikova et al., 2014). The phylogeography of hedgehogs is well studied and represents a known example of postglacial colonization routes in the western Palearctic (Hewitt, 2000). A deep split between hedgehogs and their east-west and north-south subdivisions based on genetic data (Santucci et al., 1998; Suchentrunk et al., 1998; Seddon et al., 2001, 2002) suggests the strong effects of climate on the current distribution of their genetic variation and indicates different refugia history and expansion patterns (Berggren et al., 2005). The Balkans has been identified as the most likely refugium of *E. roumanicus* (Seddon et al., 2002; Bolfíková and Hulva, 2012). The northward expansion route of *E. roumanicus* from the Balkans into central Europe followed a pattern of vegetation expansion; however, previous studies found a slightly divergent lineage in Austria, Hungary, and western Russia, which indicated that there may have been more than one route from the Balkans to the north (Seddon et al., 2002). Seddon et al. (2001) indicated that refugia populations existed rather as a series of small isolated populations due to spatial variability in climate, as suggested by pollen data (Huntley, 1999).

The Pleistocene climatic oscillations had a strong influence on the patterns of genetic and geographical

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distribution and demography dynamics of thermophilic species, forcing them into major latitudinal and/or altitudinal range shifts (Hewitt, 1999, 2004; Schmitt, 2007). The glacial survival in the southern refugia (Iberian, Apennine, and Balkan peninsulas), followed by postglacial recolonization of northern Europe, seems to be a general model (Hewitt, 2001), suggesting that populations from different refugia responded individually to habitat availability during the interglacial periods, as well as at the end of the last glaciations, therefore expanding their distribution ranges and different genetic lineages northwards (Taberlet et al., 1998; Hewitt, 1999, 2004). It is noteworthy that recent genetic analyses revealed that typical Mediterranean species could also survive glacial phases in extra-Mediterranean refugia in some climatically favorable but geographically limited areas, such as the Carpathians or even north of the Alps (Schmitt and Varga, 2012). In the Balkan Peninsula, which is recognized as one of the hotspots of biodiversity, genetic studies of several mammal species (wild boars, gray wolves, brown hares) have proved the existence of high genetic diversity and provided signs of population structuring, with southerly biased gene pools (Djan et al., 2014; Veličković et al., 2015). The increase of genetic diversity toward the southern part of the peninsula and phylogeographic analyses, specifically for wild boars and brown hares, support a leading-edge colonization hypothesis (Hewitt, 1999), because recolonization was only based on the gene pool present in the northern areas of each peninsula during the last glacial maximum.

While the phylogeography of hedgehogs has been well studied (Santucci et al., 1998; Seddon et al., 2001, 2002; Berggren et al., 2005), information on the genetic variability of *E. roumanicus* is scarce. Previous studies were mostly based on wide samples from central and eastern Europe (Seddon et al., 2001, 2002; Bolfíková and Hulva, 2012), and even though these studies did include some samples from the Balkan countries, there were no hedgehog specimens from the Central Balkan region. In response to ever-increasing anthropogenic changes to natural ecosystems, genetic monitoring through the usage of different molecular markers is the best estimator of natural populations' sustainability, since genetic variability underpins populations' long-term potential for survival and adaptation (Palsbøll et al., 2007; Schwartz et al., 2007). Mitochondrial DNA is one of the most extensively used molecular markers in determining molecular diversity and phylogeography of many species (e.g., *Castor fiber* (Durka et al., 2005), *Cervus elaphus* (Zachos and Hartl, 2011), *Sus scrofa* (Alexandri et al., 2012; Veličković et al., 2015), *Ursus actros* (Hirata et al., 2013)), given the high evolutionary rate and lack of recombination (Avise, 2004). Even though analyses based solely on mtDNA have their own limitations due to mtDNA being a single locus marker with an effective population size of one-fourth of nuclear autosomal sequences, it is still a choice in preliminary analyses of genetic variability of wild populations. The main aim of this study is to estimate the genetic diversity and structuring of *E. roumanicus* individuals from the Central Balkans based on the variability of mtDNA control region sequences, but also to complement an already proposed phylogeographic scenario of this species with more comprehensive sampling across the Central Balkans.

2. Materials and methods

Tissue samples of 108 road-killed northern white-breasted hedgehogs were collected across Central Balkan countries: Serbia (74), Montenegro (9), Bosnia and Herzegovina (9), and Macedonia (16) (Figure 1).

Total DNA was extracted using a slightly modified approach as published by Kocher et al. (1989). The 5' segment of the mitochondrial control region was amplified following the procedure published by Seddon et al. (2001) with the primer pair Prol-He and DLH-He. The PCR products were purified following the ExoSAP protocol (Thermo Fisher Scientific, Waltham, MA, USA), and sequencing was conducted using the forward primer given above.

Sequences were aligned using the Clustal W algorithm (Thompson et al., 1994) implemented in BioEdit 7.0.9.0. (Hall, 1999), and final adjustments were done by eye. The dataset consisted of 108 sequences with the full length of alignment of 420 bp (419 bp excluding sites with gaps).

DNA polymorphism parameters for all sequences (h - haplotype diversity, π - nucleotide diversity, k - mean number of pairwise differences) were calculated in DnaSP v.5 (Librado and Rozas, 2009), not considering sites with gaps. The neutrality tests (Fu's Fs and Tajima's D test), as well as the mismatch analysis, were done in ARLEQUIN 3.5.1.2 (Excoffier and Lischer, 2010), also not considering sites with gaps. The significance of the fit of the observed mismatch distribution to the expected was estimated by means of the sum of the squared deviations (SSD). In order to avoid any possible bias in prior sample groupings and loss of real structure patterns, spatial clustering of individuals was performed using Geneland 3.0 (Guillot et al., 2005). The model based on the multinomial distribution of genotypes conditionally based on allele frequencies, population memberships, and linkage equilibrium was used with a total of 5,000,000 iterations in 5 independent runs and number of clusters (k) from 1 to 10, with sampling every 100 steps and discarding the first 20% as burn-in.

DNA polymorphism parameters, neutrality tests, and mismatch analysis were also calculated for each detected spatial group using DnaSP and ARLEQUIN. The analysis of molecular variance (AMOVA) among and within detected groups, as well as calculation of F_{ST} pairwise differences, was done using ARLEQUIN.

Poor sampling and/or missing information in previously published studies did not allow us to include all available control region mtDNA sequences of hedgehogs from the Central Balkans. One sequence from Serbia was submitted to GenBank (Acc. No. AF379754), but the exact sampling locality was not available in the original reference (Seddon et al., 2001); it is marked in the figure map somewhere between the border of Croatia and Slovenia. In addition, one sequence was published from the Bulgarian city of Asenovgrad (Acc. No. HM462028), located in the central southern part of Bulgaria, but we did not include it since it is not a clear representation of the Central Balkan region. Finally, five sequences were available from Greece, two of which were represented by samples from the European part of Turkey and Crete, and we did not include those either.

Having in mind the recent problematic taxonomic situation of *E. concolor* vs. *E. roumanicus*, but also in order to complement an already proposed phylogeographic scenario for *E. roumanicus*, obtained sequences in this study were combined with all available *E. roumanicus* and *E. concolor* sequences present in GenBank (Seddon et al., 2001, 2002; Bolfíková and Hulva, 2012) (see Supplementary Table S1). For each of those downloaded haplotype sequences, respective numbers of individuals per haplotype were taken from the original reference. *Erinaceus concolor* haplotype sequences obtained from GenBank were regarded as *E. roumanicus* if the locality information in the original references was in accordance with the revised distribution ranges of these species. The combined set consisted of 225 sequences with a full alignment length of 385 bp (further analyses were done considering sites with gaps). A median-joining (MJ) network (Bandelt et al., 1999) was calculated using Network v.4.6.1.3 (available at http://www.fluxus-engineering.com/sharenet.htm), applying the default settings (ε = 0 and the variable sites weighted equally = 10), with additional postprocessing with the maximum parsimony (MP) option. The relationships among haplotypes were also assessed by Bayesian inference using MrBayes v.3.2.2 (Ronquist et al., 2012) via CIPRES Science Gateway v.3.3 (Miller et al., 2010). Four Markov chains (one cold and three heated) were run simultaneously for 30 million generations, with trees sampling every 100 generations. The HKY model was used, following the best nucleotide evolution model determined by MEGA6 (Tamura et al., 2013). The first 30% of sampled trees were discarded as a burn-in, while the remaining trees were used to build a 50% majority-rule consensus tree rooted using the sequence of *Erinaceus europaeus* (Acc. No.: X88898.2) as an outgroup.

3. Results

In the dataset of 108 control region mtDNA sequences from the hedgehogs in the Central Balkans, 13 different haplotypes were detected (Table 1). The total number of polymorphic sites was 13; 12 of them were parsimoniously informative transitions, with 1 singleton. Haplotype

Table 1. List of control region mtDNA haplotypes of *E. roumanicus* revealed in the present study.

	f	Locality	Accession number
ErB1	5	MNE (7, 9); SR (21, 25, 26)	KY366248
ErB2	3	MNE (13); SR (38, 42)	KY366249
ErB3	35	BH (1, 2); MNE (8, 13); SR (14, 16, 17, 18, 19 (7), 20 (3), 23, 28, 32, 33, 34(4), 38, 41, 46); MAC (49, 50, 51, 52, 53, 58)	KY366250
ErB4	12	BH (3); MNE (11, 12); SR (19 (3), 20, 31, 34 (2), 37, 39)	KY366251
ErB5	14	BH (2); MNE (10); SR (15, 19 (2), 20, 22, 24, 33 (2), 34 (2), 36 (2))	KY366252
ErB6	4	SR (15, 35, 37 (2))	KY366253
ErB7	7	SR (15, 19, 20 (2), 28, 32, 40)	KY366254
ErB8	3	SR (27, 29, 30)	KY366255
ErB9	5	BH (6); SR (37, 45, 48), MAC (54)	KY366256
ErB10	4	SR (43, 44), MAC (53, 58)	KY366257
ErB11	1	SR (25)	KY366258
ErB12	8	BH (4, 5, 6 (2)); MNE (9); SR (32, 34, 47)	KY366259
ErB13	7	MAC (55, 56 (2), 57, 58 (3))	KY366260

Locality numbers of individuals sampled in this study correspond to those in Figure 1 (if more than one individual was sampled per locality, its number is denoted in parentheses following locality number). Sampling countries are denoted as SR (Serbia), Bosnia and Herzegovina (BH), Montenegro (MNE), and Macedonia (MAC).

diversity value was 0.851 ± 0.023, while nucleotide diversity (π) was 0.00443 and the average number of nucleotide differences (k) was 1.855. Nine of 13 haplotypes detected in this study were new and not present in the GenBank.

The majority of haplotypes in the total sample of 108 individuals were represented with low frequencies, ranking from 2.77% to 6.48%, while only one haplotype (ErB11) was unique and represented with one individual. The most common haplotype was ErB3, occurring in 32.4% of analyzed individuals (Table 1).

Geneland analysis revealed the presence of four spatial groups, which are named after the approximate sampling localities: northwestern-central (NWC), northeastern (NE), southwestern (SW), and southeastern (SE) groups (Figures 1 and 2; Figure S1). The observed differentiation in the four groups is further supported by significant pairwise Φ_{ST} values (Supplementary Table S2). Analysis of molecular variance between defined groups also supported a genetic differentiation with a statistically significant Φ_{ST} value (0.210; $P < 0.001$), even though most of the genetic variability was due to differences among individuals within groups (78.98%).

The haplotype diversity was highest in the NE group, while nucleotide diversity and average number of nucleotide differences was highest in the SE group (Table 2).

Overall mismatch distribution was unimodal, and SSD values were not statistically significant, confirming the hypothesis of sudden expansion. None of the performed neutrality tests were significant, but Tajima's D was negative for the total sample and the NWC group, while Fu's Fs value was only positive in the SE group.

The dataset comprising *E. roumanicus* and *E. concolor* sequences contained 30 different haplotypes (Table S1). Results of MJ network and Bayesian inference analyses (Figure 3) clearly indicated the existence of two main clades that correspond to the abovementioned species. All previously published *concolor* sequences from Europe clustered together with *E. roumanicus*. The *E. concolor* cluster in the Bayesian tree showed further subdivision into "eastern" and "western" Asia Minor groups. Only forward sequencing used in this study resulted in shorter alignment and caused some of the previously published haplotypes to collide together, but the general phylogeographic scenario was not affected. In fact, after the alignment with the sequences obtained in this study, some of the previously published haplotypes collided together, but the majority of them were haplotypes from the same geographic region. In the constructed MJ network, haplotypes from Vojvodina (a northern Serbian province) were marked as being from the Central European group according to Vojvodina's

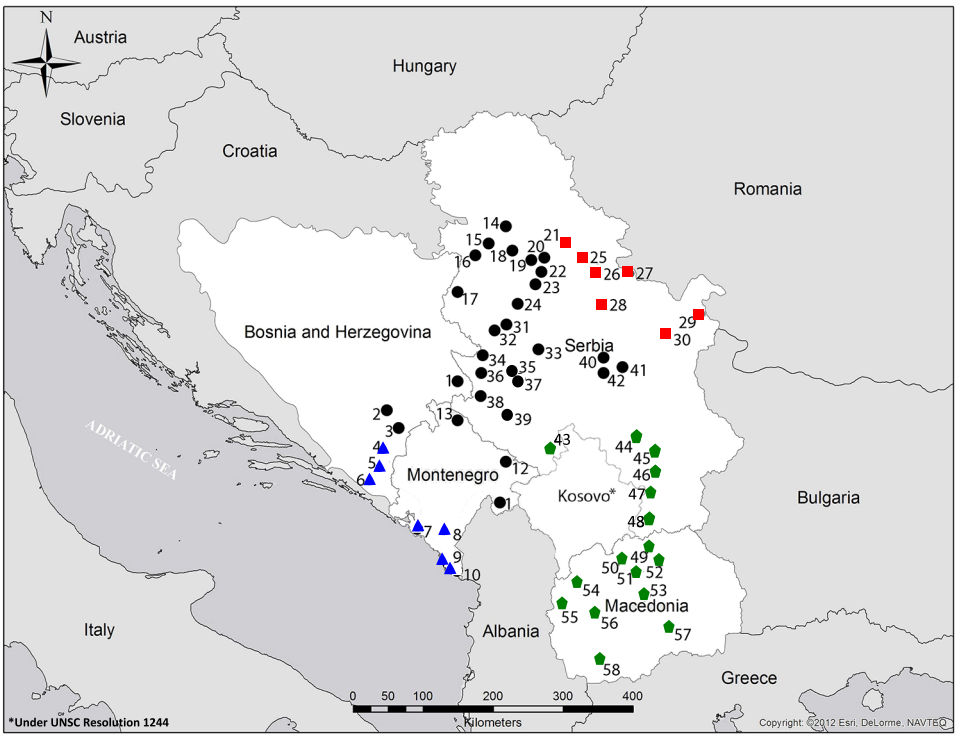

Figure 1. Geographic position of sampled localities of *E. roumanicus* from the Central Balkans in this study. Numbers of localities correspond to those shown in Table 1, where the number of individuals sampled for each locality is also given. Localities were organized in four groups as suggested by Geneland analysis (NWC – black circles; NE – red squares; SE – green polygons; SW – blue triangles).

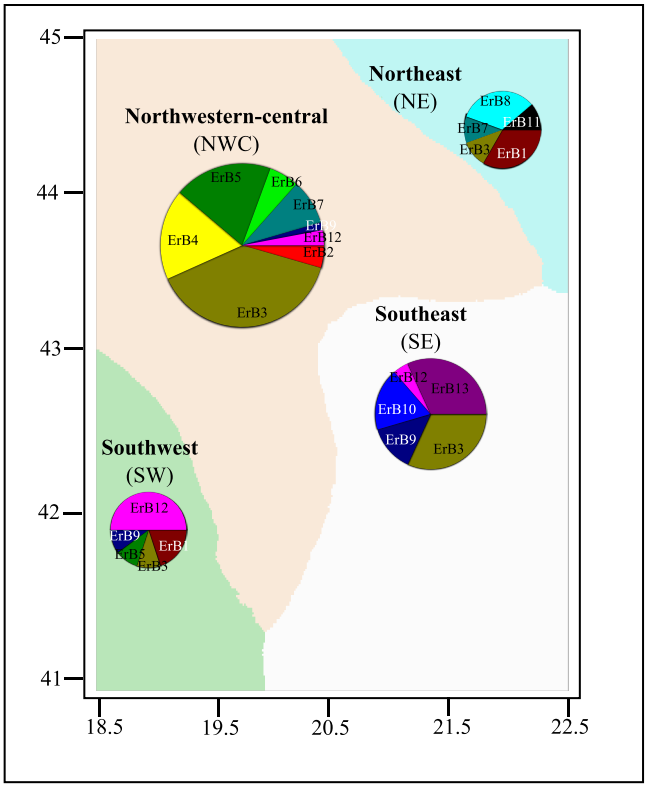

Figure 2. Distribution of haplotype frequencies in four detected groups of *E. roumanicus* from the Central Balkans. The size of pie charts is proportional to sample size. The numbers on the x and y axes correspond to longitude and latitude decimal degrees.

Table 2. Molecular diversity indices and neutrality tests in four detected Northern white-breasted hedgehogs groups in the Central Balkans.

	NWC (northwest-central)	NE (northeast)	SW (southwest)	SE (southeast)	Total
n	67	9	10	22	108
h	8	5	5	5	13
S	7	4	4	7	13
Tr	7	3	4	6	11
Tv	0	1	0	1	2
Hd	0.777 ± 0.033	0.833 ± 0.098	0.755 ± 0.129	0.779 ± 0.046	0.851 ± 0.023
π	0.00342	0.00358	0.00419	0.00518	0.00443
k	1.431	1.500	1.755	2.169	1.855
SSD (P)	0.002 (0.33)	0.034 (0.19)	0.074 (0.20)	0.040 (0.19)	0.002 (0.36)
Tajima's D (P)	–0.238 (0.47)	0.078 (0.56)	0.927 (0.82)	0.414 (0.70)	–0.668 (0.26)
Fu's Fs (P)	–1.202 (0.30)	–1.505 (0.07)	–0.901 (0.20)	1.079 (0.75)	–3.187 (0.11)

n – Number of individuals; h – number of haplotypes; S – number of polymorphic sites; Tr – number of transitions; Tv – number of transversions, Hd – haplotype diversity; π – nucleotide diversity; k – average number of nucleotide differences.

geographic position north of the Sava and Danube river streams (upper boundary of the Balkan Peninsula), while sequences of individuals south of the Danube and Sava river streams were considered as the Balkans group (Figure 3).

4. Discussion

The Balkan Peninsula, through the variety of its regions, complex geological history, and interactions between populations, species, and ecosystems, represents a region with remarkable genetic diversity, shaped by historical as well as contemporary evolutionary forces (Kryštufek and Reed, 2004; Savić, 2008). Keeping in mind the limited sampling in previous studies and the absence of information on the genetic structure of *E. roumanicus* in the Balkans, we used control region mtDNA sequences in the present study with the main aim of exploring genetic diversity and structuring of this species in the Central Balkans.

The results of this study indicate a moderate level of haplotype diversity of northern white-breasted hedgehogs from the Balkans. The nucleotide diversity revealed in this study was similar to that determined in the previous study by Seddon et al. (2001), which was based on the analysis of mitotypes consisting of a partial control region and cytochrome b sequences of 22 individuals from Turkish Thrace and Greece, northward through Austria and Hungary to Estonia. In comparison with the genetic diversity of central Europe (Bolfíková and Hulva, 2012), *E. roumanicus* from the Balkans showed higher genetic diversity indices. In the study of *E. roumanicus* individuals predominantly from the Czech and Slovak republics, haplotype and nucleotide diversity were 0.289 ± 0.077 and 0.00182, respectively (Bolfíková and Hulva, 2012). The first analysis of genetic variability in *E. roumanicus* from a limited sampling region in Serbia indicated a high level of genetic diversity (Stefanović et al., 2016), while broader sampling from the Balkans in this study confirmed higher genetic diversity as compared to that of central Europe.

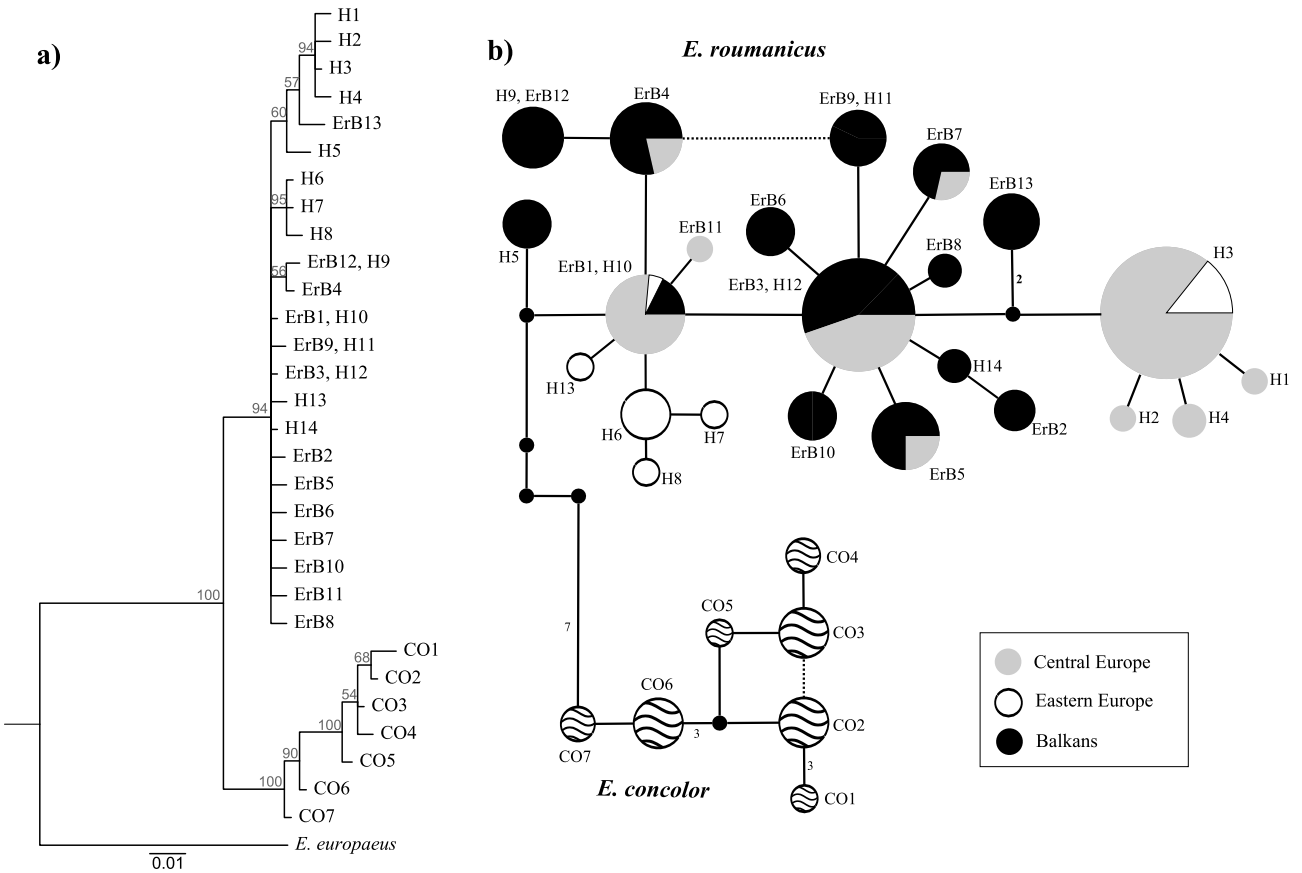

Figure 3. a) Median-joining network of the mtDNA control region haplotypes of *E. roumanicus* and *E. concolor*. Circle sizes are proportional to haplotype frequencies, while circle colors correspond to defined regions as shown in the legend. Each branch represents one mutational step; if more than one mutation step is present, it is denoted by the numbers. The MJ network is presented as the shortest tree, while lost connections between haplotypes after MP processing are shown with dashed lines. Black circles represent median vectors (unsampled or ancestral haplotypes). b) Bayesian phylogenetic tree showing relationships among 30 haplotypes of *E. roumanicus* and *E. concolor*. The trees were rooted using *E. europaeus* as an out-group. Haplotype names correspond to those given in Table 1 and Table S1.

Even though the variety of landscapes and ecological factors across the Balkans could indicate a strong population structure, our results point to a rather moderate genetic structuring of hedgehogs from the Central Balkans, based on mtDNA control region sequences. Spatial analysis indicated the presence of four significantly genetically differentiated groups. Since genetic structuring of populations is not obligatory based on the members' geographic vicinity, an a priori definition of populations can overestimate or hide the real structuring pattern. Spatially explicit Bayesian clustering models can help in delimiting individuals based on genetic information into three possible major types of genetic structuring patterns (genetic clusters, clines, and patterns of isolation-by-distance), possibly at different geographical scales (François and Durand, 2010). Even though we used an mtDNA control region, which is considered a selectively neutral molecular marker, the observed spatial gradients in molecular diversity parameters in hedgehogs from the Central Balkans could be a consequence of adaptations along an environmental gradient or a consequence of secondary contact of groups being separated by climatic or geological changes. Kryštufek et al. (2009) showed that pattern of size variation in *E. roumanicus* across Europe is a smooth cline along a latitudinal gradient, correlated positively with seasonality. On the other hand, the observed patterns of genetic variability somewhat support the previously postulated general pattern of south-north decrease of genetic variability parameters in the Balkans as a consequence of past expansions during postglacial recolonization. Although we found a lower value of haplotype diversity in the defined SE group, its nucleotide diversity and average number of nucleotide differences were higher than those of any other group.

It is noteworthy to mention that single marker usage limits in-depth population structure analysis; therefore, further genetic studies should use more informative molecular markers (e.g., microsatellites, SNPs). In the landscape analysis of nuclear and mtDNA data in individuals from central Europe, similar patterns were revealed between the markers used, where two subpopulations with an abrupt transition zone were detected (Bolfíková and Hulva, 2012).

Among all the observed spatial groups, the SW group showed the highest genetic differentiation (Table S2) and the lowest number of migrants interchanged with any other group, even compared to gene flow between all the other groups. Even though the existence of physical landscape barriers in northern Montenegro could have resulted in reduced gene flow, it is noteworthy to mention that the SW group mainly consisted of samples from the coastal region of the Adriatic Sea. This group is therefore under different ecological and climatic influences, which could have resulted in differentiation of this locally adapted group.

Demographic analyses suggested a recent population expansion, which has also been demonstrated for *E. roumanicus* in central Europe (Bolfíková and Hulva, 2012). Furthermore, the expansion signal is stronger in the groups occurring in northern latitudes, while the Fu's Fs and Tajima D values were positive, although not significant, in the SE group. This pattern further supports the existence of older and more stable groups in the south, while a leading bottleneck was more observable along the colonization routes toward the north.

The observed population structure in the Central Balkans remains less pronounced in analyses of the dataset comprising *E. roumanicus* and *E. concolor* sequences. The MJ network and Bayesian phylogenetic tree display two clusters that clearly correspond to *E. roumanicus* and *E. concolor*. None of the sequences obtained in this study had an unexpected phylogenetic position, which completely supported the previously suggested split between *roumanicus* and *concolor* sequence types. Furthermore, the *E. concolor* cluster showed a slight subdivision into two geographically different groups, which corresponds to the previous findings of Seddon et al. (2002). The central position of the *E. roumanicus* haplotypes from the Balkans in the MJ network supports a proposed role of this population as the source for the postglacial northward expansion. Seddon et al. (2001) showed the existence of two higher level clades in the Balkans, based on mtDNA data, one with a northern extension to Poland (Clade 3 – 3) and one to Austria (Clade 3 – 2). The position of central European and eastern European haplotypes in the network supports the proposed phylogeographic scenario (Santucci et al., 1998; Hewitt, 1999, 2000; Seddon et al., 2001, 2002) and may indicate the existence of two different mtDNA lineages originating from the Balkans. In order to completely support the present phylogeographic routes, wider sampling is necessary, specifically in eastern Europe and the region north of the Black Sea. This proposed wider sampling would also contribute to a better understanding of the influence of extra-Mediterranean refugia on phylogeographic patterns of hedgehogs, especially those in the Carpathian Basin, since recent studies have indicated its importance in ice age survival of European temperate species (Schmitt and Varga, 2012).

In conclusion, a moderate level of genetic diversity in northern white-breasted hedgehogs from the Central Balkans is revealed, with clinal latitudinal structuring of genetic variability in the south-north direction. The central position of Balkan haplotypes indicates their role as a primary source during postglacial recolonization of central and eastern Europe. The subsequent expansions from the northern parts of the Balkans may have acted as a bottleneck, leading to the observed decrease of genetic diversity towards central and eastern Europe, and might support the leading edge recolonization pattern proposed for other mammalian species in the Balkans (Veličković et al., 2015).

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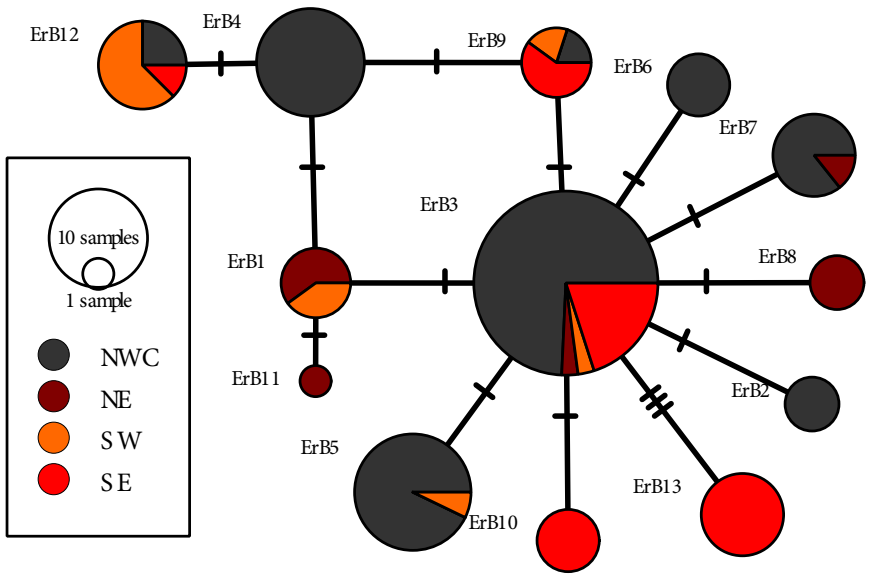

Figure S1. Median-joining network shows the distribution of 13 *Erinaceus roumanicus* haplotypes from the Central Balkans, with pie chart area proportional to haplotype frequencies in relation to the four detected subpopulations.

Table S1. List of control region mtDNA haplotypes of *E. concolor* and *E. roumanicus* downloaded from GenBank and used in this study.

Original name of haplotype	N	Locality	Region of Europe in MJ network*	Abbreviation in MJ network and Bayesian tree	Reference	AN
C1 - 1	7	Russia, Estonia	EE	H3	Seddon et al., 2001	AF379750
C1 - 2	2	Hungary	CE	H3	Seddon et al., 2001	AF379751
C1 - 3	2	Austria	CE	H4	Seddon et al., 2001	AF379752
C1 - 4	1	Austria	CE	H3	Seddon et al., 2001	AF379753
C1 - 5	6	Austria, Serbia, Hungary	CE	H12	Seddon et al., 2001	AF379754
C1 - 6	1	Greece	BL	H14	Seddon et al., 2001	AF379755
C1 - 7	4	Italy	CE	H12	Seddon et al., 2001	AF379756
C1 - 8	1	Greece	BL	H14	Seddon et al., 2001	AF379757
C1 - 9	1	Greece	BL	H11	Seddon et al., 2001	AF379758
C1 - 10	4	Austria, Hungary	CE	H10	Seddon et al., 2001	AF379759
C1 - 11	1	Poland	CE	H10	Seddon et al., 2001	AF379760
C1 - 12	1	Croatia	BL	H9	Seddon et al., 2001	AF379761
C1 - 13	4	Greece, Turkey	BL	H5	Seddon et al., 2001	AF379762
C2 - 1	4	Turkey		CO2	Seddon et al., 2001	AF379763
C2 - 2	3	Israel		CO3	Seddon et al., 2001	AF379764
C2 - 3	2	Israel		CO4	Seddon et al., 2001	AF379765
C2 - 4	1	Turkey		CO1	Seddon et al., 2001	AF379766
C1	1	Moscow, Russia	EE	H6	Seddon et al., 2002	AF481501
C2	1	Riazan, Russia	EE	H6	Seddon et al., 2002	AF481502
C3	1	Briansk, Russia	EE	H3	Seddon et al., 2002	AF481503
C4	1	Belgorod region, Russia	EE	H10	Seddon et al., 2002	AF481504
C5	1	Belgorod region, Russia	EE	H13	Seddon et al., 2002	AF481505
C6	1	Keherson region, Ukraine	EE	H8	Seddon et al., 2002	AF481506
C7	1	Stavropol, Russia	EE	H6	Seddon et al., 2002	AF481507
C8	1	Stavropol, Russia	EE	H7	Seddon et al., 2002	AF481508
C9	1	Dagestan, Russia	EE	H6	Seddon et al., 2002	AF481509
C10	1	Abkhazia, Georgia		CO6	Seddon et al., 2002	AF481510
C11	1	Abkhazia, Georgia		CO6	Seddon et al., 2002	AF481511
C12	1	Abkhazia, Georgia		CO6	Seddon et al., 2002	AF481512
C13	1	Abkhazia, Georgia		CO6	Seddon et al., 2002	AF481513
C14	1	Khosrov reservation, Armenia		CO7	Seddon et al., 2002	AF481514
C15	1	Karabah, Azerbaijan		CO7	Seddon et al., 2002	AF481515
ER1	45	Slovakia, Poland, Czech Republic	CE	H3	Bolfíková and Hulva, 2012	HM462024
ER2	1	Czech Republic	CE	H2	Bolfíková and Hulva, 2012	HM462025
ER3	1	Czech Republic	CE	H1	Bolfíková and Hulva, 2012	HM462026
ER4	1	Czech Republic	CE	H12	Bolfíková and Hulva, 2012	HM462027
ER5	1	Bulgaria	BL	H11	Bolfíková and Hulva, 2012	HM462028
ER6	3	Slovakia	CE	H10	Bolfíková and Hulva, 2012	HM462029
ER7	3	Slovakia, Czech Republic	CE	H10	Bolfíková and Hulva, 2012	HM462030
EC1	1	Lebanon		CO3	Bolfíková and Hulva, 2012	HM462031
EC2	1	Turkey		CO5	Bolfíková and Hulva, 2012	HM462032

*CE – Central Europe; EE – eastern Europe; BL – Balkans.

Table S2. Pairwise Φ_{ST} values between four detected spatial groups in northern white-breasted hedgehogs from the Central Balkans below the diagonal and its corresponding P-value above the diagonal.

	NWC	NE	SW	SE
NWC		0.01802	0.00000	0.00000
NE	0.11284		0.01802	0.00901
SW	0.26924	0.28396		0.00000
SE	0.18620	0.20496	0.33018	

NWC – Northwest-central; NE – northeast; SW – southwest; SE – southeast.