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The distribution and genetic diversity of the common hamster *Cricetus cricetus* in Central and Western Romania

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Abstract. The existing literature, museum records, personal reports of field biologists and our own field results were compiled to assess the present distribution of the common hamster within Transylvania and the Pannonian Plain of Romania. Combining available distribution data and the existence of natural barriers we were able to designate five, possibly separate, populations: the Pannonian Plain, the Transylvanian Plateau, the Olt Valley, the Braşov Depression and the Ciuc Depression population. The Pannonian Plain and the Transylvanian Plateau populations showed mass outbreaks in recent years. Twenty three individuals were available for the genetic analyses. The populations belonged to the Pannonia lineage, based on the sequences of *16S*rRNA, *cytb* and *ctr* of mtDNA. In general we found very high diversity in mtDNA and 16 microsatellite loci. Moreover the most common *ctr* haplotypes for the Transylvanian Plateau were also present in the Pannonian Plain population and in populations from Hungary and Slovakia, which indicates recent or even current exchange of individuals. Summing up, recent mass outbreaks and high levels of genetic diversity, with some indication of current or very recent gene flow, showed that Romanian populations are in good state, at least compared to many other European countries. As such, these populations should be of particular interest and placed under protection, as they could serve as the reservoir of the genetic variability for the European Pannonia lineage of the common hamster.

Key words: conservation, endangered species, mtDNA, phylogeography, species range

Introduction

The common hamster (*Cricetus cricetus* L.) is a steppe species with extensive range from the River Yenisei in Asia to Western Europe, where it forms some isolated populations in Belgium, France, the Netherlands and Western Germany (Mitchell-Jones et al. 1999). The species was never very abundant in its original habitat but, in contrast, it benefited greatly from man-made agricultural habitats. Great availability of food, especially in cereal fields and low intensity agricultural areas created perfect habitats for the species. For the first half of the 20th century, the common hamster was very abundant and considered a serious pest in many European countries (Nechay 2000). The species was heavily culled with the use of rodenticides and by specialized hamster trappers. However, in the second half of the 20th century information about the breakdown of the common

hamster populations started to appear (Weinhold 2008). This was first noticed in the populations of western European countries which were isolated from the main range of the species. In the Netherlands the species went extinct by 2002 and the last individuals were collected for a breeding and reintroduction programme. Currently existing populations of the common hamster in the Netherlands originate from breeding centres (Müskens et al. 2003). The populations in France (Alsace), Belgium and western parts of Germany fared better – i.e. they managed to survive. However, they are subject to very intensive protection and augmented constantly by individuals from breeding programmes (Weinhold 2008). It appeared very soon that the shrinkage of the range affects also formerly very strong populations from Central Europe. For example the German population, which up to the 1970s showed mass outbreaks and

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subsequent mass cullings – with numbers of trapped hamsters reaching millions yearly between 1953 and 1966 – was severely fragmented and lost large parts of its inhabited area (Weidling & Stubbe 1988); the current difference between Western and Central German populations is illustrated in Meinig et al. (2014). In Hungary, although the hamster is still a frequent and common species east of the Danube, the western part of the country witnessed a strong fragmentation of the range and remaining populations are endangered (Bihari 2004). Next came the information from Poland. Although Polish populations were never characterized by mass appearances like German ones, still they were relatively strong. The monitoring of the populations performed between 1999 and 2005 showed a dramatic decline of the range to 25 % of the former and also significant fragmentation (Ziomek & Banaszek 2007). Then, it was found that the range of the species in the Czech Republic was also severely reduced and the hamsters retreated to the most suitable habitats in the river valleys (Tkadlec et al. 2012). Still it was supposed that the species was not seriously endangered as it was commonly thought that the eastern European and Asiatic part of the range was unaffected and that the populations were still abundant there. Based on this belief the IUCN status of the common hamster is least concern (Kryštufek et al. 2008). This view was changed once the information appeared that the hamsters went extinct in huge parts of Ukraine and just three areas, probably isolated from each other, were left from the Ukrainian species range (Korbut et al. 2013, Rusin et al. 2013). Moreover, it appeared that the situation of the species in the Asiatic range is also far from stable. The reduction in the inhabited area was reported from the Novosibirsk region in Siberia (Sidorov et al. 2011). All this information leads to the conclusion that shrinkage of local ranges is a problem across the whole species range.

To protect the species it is necessary to recognize the status of all the remaining populations. In Europe, as was already mentioned, many countries have already updated their information, however, there are still areas with no current data on the species' situation, Romania being an example. The hamster was a common species in this country inhabiting lowland and hilly areas up to 600-700 m altitude, with a range covering all parts of the country except for the mountains (Carpathians and Apuseni Mountains), Dobruja and parts of Oltenia (Nechay et al. 1977). Distribution data and more recent maps from the literature (e.g. Murariu 1998, 2005), however, are unsatisfying and often confusing.

Even species action plans (Weinhold 2008) are forced to rely on these poor and sometimes erroneous data, which have not been updated for several decades. The most detailed distribution map is probably the one by Nechay (2000), however, even this is a shaded map not presenting occurrence localities or dates. Hence, current distribution and status of the common hamster is poorly known across the country.

It is very important from a conservation point of view, to recognize the current situation of Romanian populations, especially in light of recent information about the global decrease of the common hamster. The reasons for the dramatic decline of the hamster populations are multiple, and among urbanization, intensification of agriculture practices and use of chemicals, climate change is also mentioned (Nechay 2000, Neumann et al. 2005). If climate change is an important causal factor in shrinking the species range, then it is unreasonable to believe that we can protect the populations on the margins of the species range and the most important conservation task becomes to discover and protect the source populations. The phylogeographic research indicated that the main refuge area for the common hamster during Pleistocene glaciations was the steppe belt in Ukraine and Russia (Neumann et al. 2005). However, once the hamsters colonized the Carpathian Basin, they were able to survive the last glaciation there and the populations were strong enough to expand during and after the last glaciation into southern Poland. All the populations from the Carpathian Basin and southern Poland belong to one phylogeographic lineage called Pannonia (Neumann et al. 2005, Banaszek et al. 2010). The Hungarian populations from the Pannonian plain are still the strongest European populations of the common hamster and they harbour large amounts of the genetic diversity (Neumann et al. 2005). The Romanian populations showed mass appearances even at the end of the 20th century. For example, Nechay (2000) reported that in the 90s of the 20th century Hungarian hamster trappers used to collect hamsters for fur trading in Romania. We suppose that hamster populations in Romania are also genetically rich and they may form a very valuable reservoir of the genetic diversity for the shrinking populations of species.

Concluding, the aim of this work is: 1) to describe the current species range in Central and Western Romania, 2) to describe the levels of genetic diversity in mtDNA sequences and nuclear microsatellites and compare them with other populations of Pannonia lineage.

Material and Methods

Collecting data for the distribution map

Literature data, museum records, personal reports of field biologists and our own field observations were compiled to assess the present-day distribution of the species within the Transylvanian Plateau and the Pannonian Plain of Romania. Literature records include data from scientific publications only from the period after 1990. The literature used in the mapping includes Ardelean (1998), Istrate (1998, 2005), Murariu (1998), Ardelean & Oprea (2000), Daróczy & Nagy (2009), and Sándor & Ionescu (2009). A total of 13 museum collections were inquired for hamster specimens, of which seven provided positive data; of these, only the records dating from the period after 1990 are mapped hereby. Field data comes from a combination of three sources of information: 1) sightings of live or dead hamsters (for example roadkill), 2) prey remains of birds (including pellets) and mammals found in nests

and dens or seen carried by the predators, and 3) burrows (Fig. 1). Although in some regions systematic surveys were also performed, most of the field data was collected using an opportunistic approach, in the 2008-2014 period. Populations were considered as separate based on the natural barriers (mountainous regions, continuous forested areas or narrow river valleys) between them. Accordingly, possible limits of populations were drawn using both distribution data and the above mentioned natural barriers. While all of the mapped records originate from the period after 1990 (Fig. 1), old records were also considered when drawing the potential distribution limits, for the sake of presenting them as accurately as possible (Fig. 2). While acknowledging that the distribution of such a species living in agricultural areas can show a dynamic pattern even within a relatively short period of time, our assumption was that large-scale distribution has not changed dramatically during the

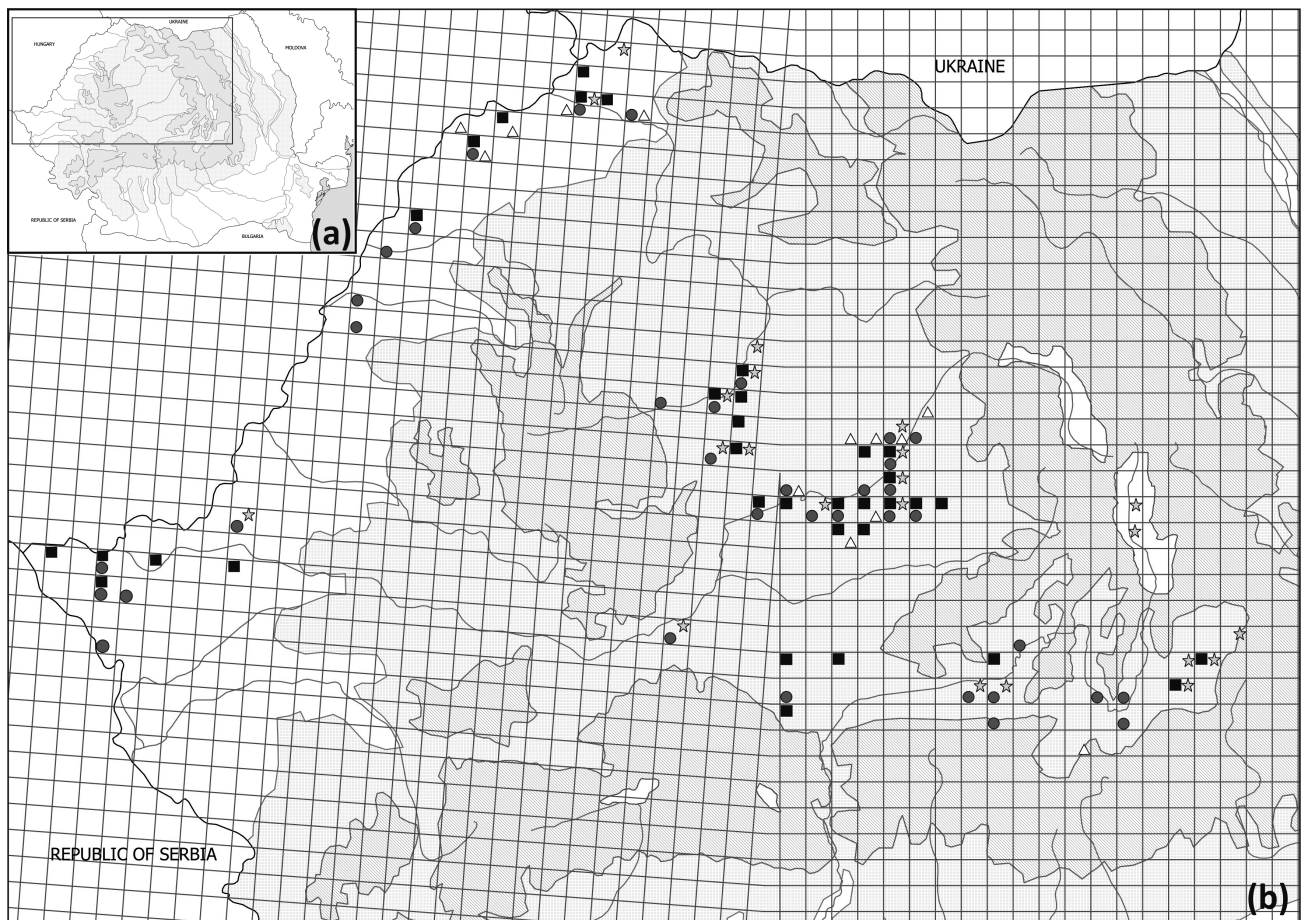


Fig. 1. The map of hamster localities in Central and Western Romania recorded after 1990. The area under research (a) and localities (b). The results of our own research and personal communications from field biologists (most records from 2008-2014) are indicated as follows: circles for the sightings of live or dead hamsters, stars for prey remains and squares for burrows. The literature data and museum collections information on the occurrence of the common hamster since 1990 are shown as triangles. The UTM grid of 10×10 kilometers was superimposed on the map and the points were arranged within UTM squares i.e. one square contains a single point of one kind of information. In result, the points might cover a few localities, hence the number of points on the map does not coincide with the number of localities given in the text.

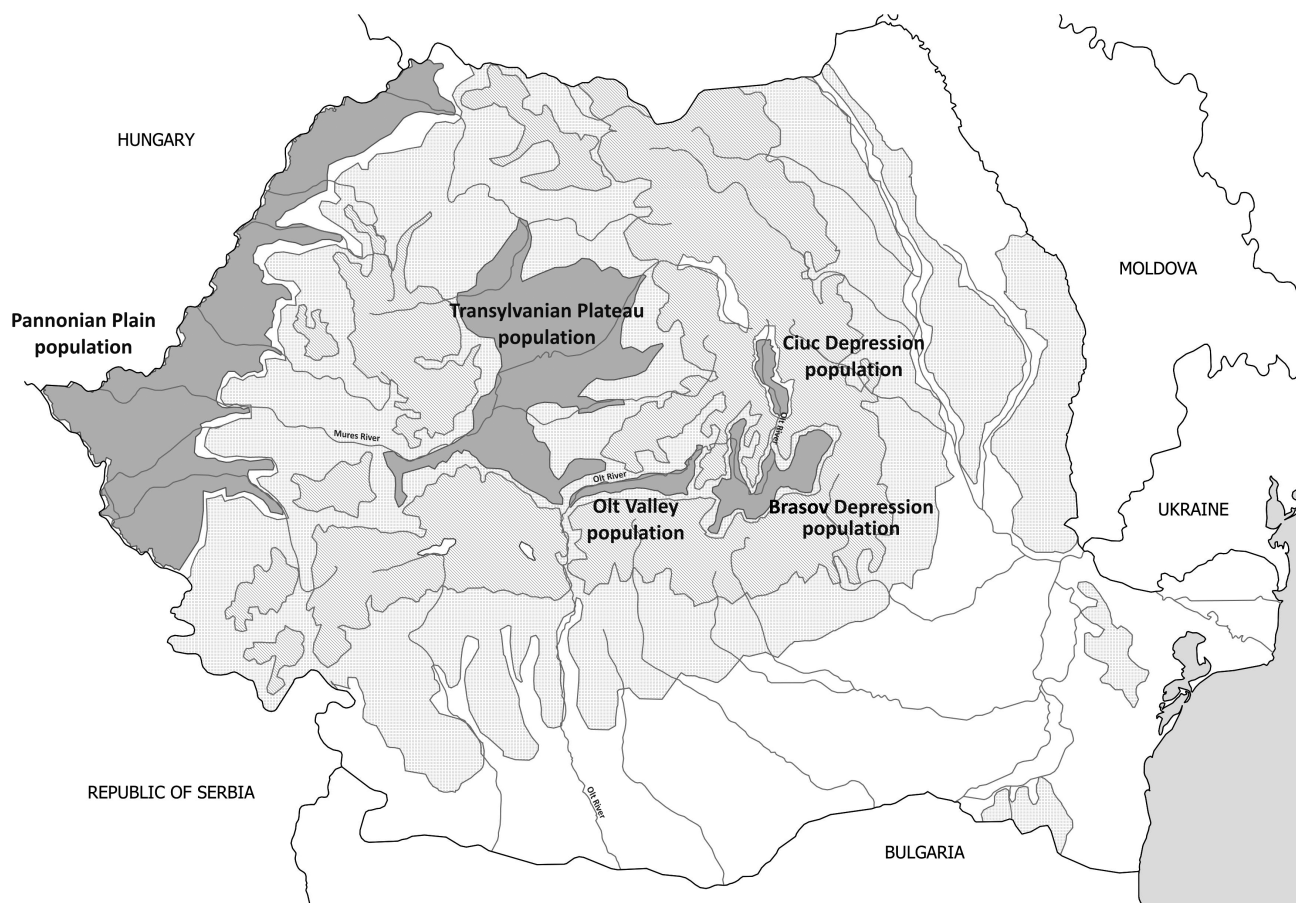


Fig. 2. The current range of the common hamster with the distribution of five putative populations in Central and Western Romania shown as grey areas. The River Mureş and the River Olt which probably serve as important migration corridors are indicated. The Carpathians (mountain regions) are hatched and higher uplands are dotted.

past decades, apart from potential local extinctions, which, however, have not been proven so far.

DNA analysis

The samples were taken from dead animals found in fields or on the roads. DNA isolation using Dneasy Blood and Tissue kit (Qiagen) was performed from dried pieces of skin or fingers. The samples were taken from 23 animals (Table 1) and isolation was successful in all of them. In the cases when the carcass was not totally disintegrated the age and sex of the animal was determined (Table 1).

The 337bp of the mitochondrial control region (*ctr*) were sequenced for all animals. We managed to sequence 906bp of cytochrome *b* (*cytb*) for 20 animals. We were not able to get clear sequences for the next three ones, most probably because of the disintegration of longer DNA sequences in the samples from dead animals. For three animals we also sequenced 486bp of 16S rRNA (*16S*). Seventeen hamsters from the Transylvanian Plateau, which could be treated as one population, were genotyped in 16 microsatellite loci,

which worked well for the Pannonian populations (Banaszek et al. 2011b). The loci were the following: Ccr μ 4, 10, 11, 12, 13, 15, 17, 19 and 20 (Neumann & Jansmann 2004; AJ532554, AJ532556-AJ532563) and CriCriIPK-01, 03, 05, 06, 07, 09 and 12 (Jacob & Mammen 2006; AM167541, AM167543-AM167548). The PCR profiles for microsatellite and mtDNA sequences amplification, the method of microsatellite analysis and sequencing reactions for *ctr*, *cytb* and *16S* were performed as described previously (Banaszek et al. 2009, 2010, 2011a, b). The basic indices of genetic variability (mean number of alleles and heterozygosities for microsatellite loci, haplotype and nucleotide diversity for the sequences) were calculated in ARLEQUIN 3.5.1.3 (Excoffier & Lischer 2010). Fis (inbreeding coefficient) and its statistical significance were checked in FSTAT 2.9.3 (Goudet 1995). The Hardy-Weinberg equilibrium was calculated in GENEPOP (Raymond & Rousset 1995). The translation of *cytb* sequence into aminoacids was performed in MEGA 3 (Kumar et al. 2004) using the vertebrate mitochondrial code.

Table 1. Location of the samples taken from dead *Cricetus cricetus* individuals. Age and sex of the hamster was determined if possible.

Geographic region	N	Locality	County	Age	Sex
Pannonian Plain	2	Curtici	Arad	Ad	-
		Sânnicolau Mare	Timiș	-	-
		Juc Herghelie	Cluj	Ad	-
		Juc Herghelie	Cluj	Ad	m
		Iernut	Mureș	Ad	m
		Glodeni	Mureș	Ad	-
		Corunca	Mureș	Ad	-
		Corunca	Mureș	-	-
		Corunca	Mureș	-	-
Transylvanian Plain	17	Târgu Mureș	Mureș	-	-
		Târgu Mureș	Mureș	Juv	-
		Târgu Mureș	Mureș	Ad	m
		Târgu Mureș	Mureș	Juv	-
		Târgu Mureș	Mureș	Ad	m
		Târgu Mureș	Mureș	Ad	m
		Crăciunești	Mureș	-	-
		Glodeni	Mureș	-	-
		Petelea	Mureș	Ad	f
		Acățari	Mureș	Ad	m
Olt Valley	2	Șercaia	Brașov	-	-
		Făgăraș	Brașov	-	-
Brașov Basin	2	Zăbala	Covasna	-	-
		Zăbala	Covasna	Ad	-

The diversity of mtDNA sequences was used to test for demographic changes in the Romanian populations. We calculated Tajima's D and Fu's Fs values and performed the mismatch analysis under the demographic expansion model and spatial expansion model using ARLEQUIN. We used a divergence rate of 7.5-13 % per my originally used for voles (Galbreath & Cook 2004) to calculate the molecular dating of potential demographic or spatial expansion. The BOTTLENECK (Cornuet & Luikart 1996) was used to test for recent reductions in population size using microsatellites variability. Both methods implemented in the program were used: the heterozygosity excess and the distribution of allele frequency. Three mutation models available in the program were used: the IAM, SMM and TPM models. The departures from the mutation-drift equilibrium were tested by the Wilcoxon test.

Results

Current distribution

Two hundred ninety-eight records attest present-day (post 1990) occurrence of hamsters in Central and

Western Romania, covering 71 UTM grids of 10 × 10 km (Fig. 1). Of these, 281 records (94.3 % of records) have been obtained during our investigations. A number of 17 recent literature and museum records have been collected. Individuals were assembled to at least five potentially independent populations based on the natural barriers separating them (Fig. 2):

Pannonian Plain population – the distribution of this large population covers most plain areas from Timiș, Arad and Bihor counties (Banat region, Mureș Plain and Crișurilor Plain), and, at least in the south, it is interconnected with the population of the Great Hungarian Plain from Hungary and Vojvodina, Serbia. While it is likely that the population from northern Bihor County and Satu Mare County (Ier, Barcău and Someș river valleys) is included here, in neighbouring Hungary these two populations seem to have lost connection in recent decades (Bihari 2004). Signs of an outbreak were locally felt in 2012 in the Someș Valley (Satu Mare County). In the southern part of the region population outbreaks were observed in 2007-2008 in the Romanian part (Duma 2010) and in 1998-2000 and 2009 in the Hungarian part (Csathó

& Csathó 2009). News reports exist from the same period about illegal hamster trapping in the Banat region.

While not very likely, some connection between the Pannonian and Transylvanian populations might occur via the Lower Mureş Valley, or at least could have occurred in the recent past. Recent changes in agriculture practices in the arid regions preferred by the species, leading to disappearance of small-scale arable land, parcel edges and fallow lands, might exercise a serious pressure on this population, and it is likely that there are already several separated populations here that do not communicate with each other.

Transylvanian Plateau population – this is a seemingly large population, distributed mostly (though not entirely) in the river valleys of central Transylvania, in a moderately to severely arid region where small-parcel agriculture still dominates. Mass outbreaks were observed in 2008-2009 (Someşul Mic Valley in Cluj County), 2011-2012 (Mureş Valley in Mureş and Alba counties), in the latter case hamsters also entering urban environments in Târgu Mureş town (Zs. Hegyeli, unpublished), as well as in 2013-2014 (Târnava Mică Valley in Mureş County). Illegal hamster trapping activity is also known to have taken place here in recent years (news reports and personal communications by farmers), which also suggests a good population, or at least the presence of outbreaks. However, the drastic changes which occurred in land-use practices since Romania joined the EU in 2007, represent a serious threat to small-scale agriculture in Transylvania.

Olt Valley population – distributed in the plain region of southern Transylvania in the Olt River Valley (Braşov and probably Sibiu counties). Its possible connection to the Transylvanian Plateau population can be assumed on geographic grounds. Generally, we have very little information about this population, and thus no data about eventual population outbreaks are available.

Braşov Depression population – this population is distributed in most parts of the Braşov Depression from south-east Transylvania (Braşov and Covasna counties) in the plain areas of Olt and Râul Negru River Valleys. This depression is more or less isolated from the Transylvanian Plateau by forested areas and narrow river valleys. This and the Olt Valley population are not believed to be interconnected, perhaps apart from outbreak years. Although we have insufficient information about this population, signs of an outbreak are known to have been last felt in the 1980s in Covasna County (based on personal communication of local people).

Ciuc Depression population – the common hamster has only recently been identified in this depression with a cold climate, situated in the Eastern Carpathians. First records were based on observations of a few individuals (Sz. Sugár, pers. comm.), after which it was also found as bird prey. No genetic sampling has yet taken place here, and there is similarly almost no information on the species' distribution. However, based on claims of local people the potential distribution area of the population has been drawn to include the entire agricultural area of the Ciuc Depression, which nevertheless would still make it the smallest population geographically. This

Table 2. The mtDNA control region (*ctr*) and cytochrome *b* (*cytb*) variability analysis. Haplotype diversity (H) and nucleotide diversity (π) are calculated for the putative populations of the common hamster occurrence in Romania. ¹ – the haplotypes described by Neumann et al. (2005) from Hungary, ² – the haplotype described by Banaszek et al. (2009) from Poland.

Population	Sequence	N	N of halotypes	Frequency of halotypes	H	π
Hungarian Plain	<i>ctr</i>	2	2	Ccd129 ¹ (0.5) Ccd128 ¹ (0.5)	1.0 ± 0.5	0.318 ± 0.289
	<i>cytb</i>	2	2	Cbr3 (0.5) Cbr5 (0.5)	1.0 ± 0.5	0.993 ± 0.497
Transylvanian Plateau	<i>ctr</i>	17	5	Ccd129 ¹ (0.235) Ru1 (0.176) Ccd128 ¹ (0.471) Ru2 (0.06) Po5 ² (0.06)	0.728 ± 0.083	0.345 ± 0.262
	<i>cytb</i>	16	4	Cbr1 (0.19) Cbr2 (0.50) Cbr3 (0.25) Cbr4 (0.06)	0.692 ± 0.086	0.298 ± 0.051
Olt Valley	<i>ctr</i>	2	2	Ccd127 ¹ (0.5) Ru4 (0.5)	1.0 ± 0.5	0.297 ± 0.420
	<i>cytb</i>	1	1	Cbr6	-	-
Braşov Depression	<i>ctr</i>	2	2	Ru3 (0.5) Ru2 (0.5)	1.0 ± 0.5	0.594 ± 0.727
	<i>cytb</i>	1	1	Cbr4	-	-
Total	<i>ctr</i>	23	8		0.818 ± 0.059	0.515 ± 0.348
	<i>cytb</i>	20	6		0.758 ± 0.077	0.387 ± 0.073

region is separated from suitable hamster habitats in the Braşov Depression by the narrow, forested valley of the River Olt.

The populations from the Olt Valley and the Ciuc Depression are newly recorded ones; as to our knowledge no published information has been available about hamsters from these two regions.

Genetic diversity

The *ctr* was sequenced for 23 individuals and eight haplotypes were found, four of them already published (Ccdl 27, Ccdl 28, Ccdl 29 AJ 633729-31, Neumann et al. 2005, Po5 Eu 016110, Banaszek et al. 2009), and four new ones (GenBank No: KT224631-T224634) (Table 2). There were six polymorphic sites, all of them transitions in a sequence of 337 bp long. The *cytb* was sequenced for 20 hamsters and six haplotypes were found, all of them new CbR1-CbR6 (GenBank accession no: KT224635-KT224640) (Table 2). There were 14 variable sites (six singletons, eight parsimony informative) in a sequence of 906 nucleotides. The substitutions were 13 transitions and one transversion. The sequence could be translated into 301 aminoacids with one non-synonymous substitution. The Tajima D was non-significant ($D = -0.40413$) and ns for synonymous and non-synonymous regions. The mutation on protein level was characteristic for CbR4 haplotype present in the Transylvanian Plateau and Braşov Depression populations. Three individuals checked for *I6S* had the same haplotype already published as characteristic for Pannonia lineage (AJ 633 754 Neumann et al. 2005). As Pannonia lineage does not show variability in this sequence (Neumann et al. 2005), we did not try to sequence more individuals. For mtDNA *ctr* the Romanian populations are polymorphic with high values of haplotype and nucleotide diversity. The haplotypes Ccdl28 and Ccdl29, which are the most common in the Transylvanian Plateau population occur also in the Pannonian Plain hamsters. On the other hand, the Olt Valley and Braşov Depression hamsters do not have these haplotypes. The Braşov Depression population has one haplotype in common with the Transylvanian Plateau population (Table 2). For *cytb* sequence variability indices are also high. The Transylvanian Plateau population has the haplotype CbR3 in common with the Pannonian Plain population and CbR4 with Braşov Depression hamsters (Table 2).

The mean number of alleles for microsatellite loci in the Transylvanian Plateau population is very high and equals $A = 12.62 \pm 3.88$. The observed ($H_o = 0.78 \pm 0.09$) and expected ($H_e = 0.83 \pm 0.08$) heterozygosities

do not differ significantly and Fis value (-0.05) is insignificant which indicates random mating. The Transylvanian population is in HW equilibrium ($p = 0.109$, exact tests). The Transylvanian Plateau population was bottlenecked if the IAM mutation model was used (the Wilcoxon test $p = 0.00011$). However, with TPM and SMM mutation models the population did not show the heterozygosity excess and the distribution of allele frequency classes was L-shaped as expected of a stable population.

For phylogeographic and demographic analyses we combined the *ctr* and *cytb* sequences, which resulted in six haplotypes. The Tajima D (0.329 , ns) and Fu's F (2.604 , ns) were non-significant, which indicates constant-size population. The goodness of fit testing of the observed mismatch to that expected under sudden demographic expansion model was performed (1000 bootstrapping) using the SSD statistic (the sum of square deviations). A significant SSD value ($p = 0.04$) indicated a departure from the demographic expansion model. Moreover, the values of the raggedness index (0.21) indicated a rather stationary population. Small values of the raggedness index, which is not the case here, may indicate demographic expansion. Mismatch analysis under the spatial expansion model was used to test for the range changes. The goodness of fit test was non-significant ($p = 0.43$) and number of migrants larger than one ($M = 3.093$), which is usually taken as an evidence of spatial expansion. In the model of the spatial expansion factor tau ($\tau = 6.374$) is estimated, which allows for the calculation of the time at which the range extension took place. The molecular dating for the spatial expansion is 28-49 ka (95 % CI: 12-72 ka).

Discussion

Considering the alarming data of hamster distribution shrinkage and population extinctions in Europe during the past decades, population declines are expected to occur in the near future in at least some areas of Romania (if they have not already occurred). Possible reasons for such declines are the expansion of monoculture crops (which quickly wipe out parcel edges and strips of grassland as well as fallow land), the gradual disappearance of forage crops such as alfalfa (as a result of a decrease in cattle numbers) as well as the building of highways and express roads, which brings about serious habitat fragmentation countrywide. Another serious threat for the survival of the species is that the common hamster is still legally trapped – by quota – in Romania, despite wider legal protection and lack of knowledge on the current distribution and demographic trends. In 2006

a governmental decree was proposed which would have allowed the harvesting of 95000 individuals in three counties. While it was rejected because of the lack of scientific data, a similar decree was accepted in 2009 permitting the harvesting of 110 000 hamsters in two counties (Ministerul Mediului 2009), although no scientific data supporting this has been published since the previous decree.

Even though the available data shows five possibly distinct populations, it is possible that in reality there are more (due to fragmentation) or fewer populations (due to unidentified connections), which could also have a dynamic nature in time. Hence, there is an urgent need to check such areas of possible connection (e.g. the lower Mureş Valley) for hamster presence in order to improve our knowledge about population limits. We consider the populations from the Olt Valley, the Braşov Depression and the Ciuc Depression to be the most vulnerable, due to their reduced size and probable isolation, as well as the lack of data about population outbreaks.

The Transylvanian Plateau is one of the few regions of Romania (and even Europe) which still abound in small-scale agriculture and grasslands. Probably due to this preservation of the traditional landscape, rare steppe rodent species like the Hungarian birch mouse (*Sicista (subtilis) trizona*), the Transylvanian blind mole-rat (*Nannospalax (leucodon) transsylvanicus*) and the Méhely's blind mole-rat (*Spalax antiquus*) are still present here (Németh et al. 2009, Cserkés et al. 2015, Csorba et al. 2015). Alfalfa crops are also still common due to the relatively large number of cattle in Transylvania. The present, seemingly well-preserved conservation status of the common hamster population could, thus, be attributed to these favourable habitat conditions, but also to a strong gene flow from the Pannonian population, which might have lasted for a long period of time or which still occurs periodically. The genetic results shown here are preliminary, as they are performed on a rather small sample, however they show one very important trait of Romanian populations, that they are highly diverse. The nucleotide diversity for the *ctr* and *cytb* sequences is quite similar as in populations from Eastern and Western Slovakia (π for *ctr* 0.29, 0.56 and for *cytb* 0.73, 0.38 respectively) and decidedly higher than for Pannonian populations from southern Poland. In southern Poland two sublineages of Pannonia (PPI and PPII) were described, which differed in predominating *cytb* and *ctr* haplotypes. Nucleotide diversity for PPI is 0.045 *ctr*, 0.19 *cytb* and for PPII is 0.086 *ctr*, 0.21 *cytb*. Both Polish

sublineages had to migrate northwards through the Moravian Gate which resulted in historical bottleneck and loss of variability (Banaszek et al. 2010, 2011b). On the other hand, the Pannonian populations described by Neumann et al. (2005) showed significantly higher nucleotide diversity for *ctr* 1.1 and 0.6 for *cytb*. However, it is worth to notice that the result was given for a pooled sample from several locations in Hungary and one from Moravia (Czech Republic), so the diversity is overestimated through the connection of different populations. The high diversity of Romanian populations is also shown by microsatellite analysis of the Transylvanian Plateau population. The mean number of alleles and heterozygosities is very high and comparable with the Pannonian Plain population from Hungary (Neumann et al. 2005). Moreover, there are no signs of recent bottleneck, save the result for the IAM model. As the microsatellites most probably do not evolve through this model, and the SMM or TPM are more probable ways of the microsatellite evolution, we consider the Romanian population stable in the recent times. The sequence diversity analysis, which is suitable for historical demography also showed the stationary population (non-significant neutrality indices). The Romanian populations do not show the signs of sudden demographic expansion, but on the other hand the signs of spatial expansion were found. The molecular dating for this range extension is 28-49 ka (95 % CI: 12-72 ka) and the estimate very closely coincides with the estimation of spatial expansion of Pannonian populations into southern Poland (29-50 ka; 95 % CI: 14-93 ka, Banaszek et al. 2010). It is worth to mention, that in Poland this estimate is in agreement with paleontological data, which showed the reappearance of the hamsters in southern Poland during the second stadial of Vistulian (Kowalski 2001). Fossil data suggest that hamsters survived the last glaciation in the Pannonian Plain (Jánossy 1986) and molecular dating revealed the sudden demographic expansion of Hungarian populations (Neumann et al. 2005) in the same time frame as spatial expansion of Polish (Banaszek et al. 2010) and Romanian populations. It is quite reasonable to consider that overcrowded populations expanded and settled the lower valleys in Romania and at the same time were able to move into southern Poland.

The Romanian populations share mtDNA haplotypes with other Pannonian populations. The *ctr* haplotypes Ccd127, Ccd128 and Ccd129 are present in Hungarian (Neumann et al. 2005) and eastern Slovakian samples (Banaszek et al. 2011b), while Po5 haplotype is

characteristic for western Slovakia samples and PPII from southern Poland (Banaszek et al. 2010, 2011b). The sharing of haplotypes may indicate the present day gene flow and/or common ancestry of populations. The same conclusion may be drawn for the populations inside Romania. Based on the natural barriers to hamster dispersal, we delineated five putative populations of the common hamster: the Pannonian Plain, Transylvanian Plateau, Braşov Depression, Olt Valley and Ciuc Depression population, the last unfortunately not represented in genetic sampling. The microsatellites, which would be the best marker for genetic clusters and levels of gene flow analysis were checked for Transylvanian Plateau population only, as for other areas we had at disposal only a couple of individuals. Whereas, based on sequences diversity, we may only say that the populations share *cytb* and *ctr* haplotypes, which may be the evidence for gene flow and/or common ancestry.

Summing up, hamster populations from Central and Western Romania seem to be strong and genetically diverse, however they are expected to decline, primarily due to the fast changes in land use practices. A strong gene flow between the Pannonian and Transylvanian Plateau populations might help explain their viability, as for now the Romanian and in general the Pannonian populations are in much better demographic state compared to northern and western populations, and hence the survival of the species in Europe is more likely here.

In this survey we were able to sample the common hamster populations within the Carpathians arch,

which without doubt belong to the Pannonia lineage. Further research is necessary to verify the presence and distribution of the common hamster outside the Carpathians, i.e. in the Moldavian and Wallachian Plains. This region formed the southern migration route from refugial areas in the Ukrainian steppe belt to the plains and valleys within the Carpathians. So far, there was only one hamster available for phylogeographic analysis from Romania outside the Carpathians (Oltenia – Craiova), which formed a link between northern (North lineage) and southern (Pannonia lineage) hamsters (Neumann et al. 2005). Extending our knowledge on the distribution and genetic characteristic of hamsters from eastern and southern Romania will help to resolve the historical expansion routes of the species and relationships between so far described lineages.

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