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Filling the gap: the common hamster, *Cricetus cricetus,* phylogeography – a case study of Ukraine as potential refugial area

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Abstract. The phylogeographic analyses of the common hamster revealed the existence of five mitochondrial DNA (mtDNA) lineages. However, the analyses did not include Ukraine and the area outside Carpathian arch located in Romania that is important for the species as presumably refugial territory. We investigated both areas and described phylogeographic relationships of the populations on the basis of a partial cytochrome *b* (cyt*b*, 904 bp) region of the mtDNA. Haplotype network and gene tree analyses did not produce a clear phylogeographic pattern for investigated territories that is typical for refugial populations. The highest diversity was found at the left bank of the River Dnieper and the area was called the Dnieper Lowland Refugial Area. Moreover only three from five described phylogeographic lineages (North, Pannonia and Caucasus) were fully separate on the network and gene trees. The haplotypes of other two lineages described previously from Eastern Europe (*E1* and *E0* lineages) mixed together with haplotypes from Ukraine and Moldovian Plateau. This study highlights the need for a re-examination of the phylogeography divisions of the common hamster.

Key words: oceanic-continental climate gradient, continental adapted species, cytochrome *b*, glacial refugia, phylogenetic, postglacial migration

Introduction

The Quaternary glacial cycles had a significant influence on the genetic diversity of organisms and distribution of different genetic lineages in the northern hemisphere. During unfavorable periods, species retreated from large continental areas to relatively small territories called refugia (Hewitt 1996, 2004). According to Stewart et al. (2010), refugia are small geographical regions that represent the species' maximum contraction in geographical range. Depending on how the species responded to the past climate changes, different types of refugia located in different areas could be identified (Sommer & Nadachowski 2006, Bhagwat & Willis 2008, Stewart et al. 2010, Feliner 2011).

In most phylogeographic studies, the latitude was the dominant dimension that was taken into account during refugia analysis. As it was proposed by Stewart et al. (2010), the longitudinal dimension that in Europe is described by oceanic-continental climate gradient, was also significantly variable during Pleistocene glacial cycles. Species with the "oceanic" adaptations require more humid and less seasonably variable climate, while "continental" adapted species prefer a drier climate with greater seasonal variation (Stewart et al. 2010). Possible refugia for continental-adapted taxa might be found in the Eurasian steppes, with cryptic western refugia in southeastern Balkans and in the Pannonian Basin (Říčanová et al. 2013). Nonetheless, there is still little information about refugia for species reacting with the range shifts in the longitudinal dimension. Therefore, more detailed phylogeographic analyses are needed to assess whether their structure is also as complex as in the case of southern refugia. Study of range-wide phylogeography of the European ground squirrel (*Spermophilus citellus* Linnaeus, 1766) shed some light on the predicted refugia for continental climate adapted species. As the authors claimed, the areas with the highest genetic diversity of the

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European ground squirrel populations corresponded to biogeographically ancestral areas located exactly in Pannonian refugium (Říčanová et al. 2013).

Furthermore, the analysis of the common vole (*Microtus arvalis*, Pallas 1779) and field vole (*Microtus agrestis*, Linnaeus 1761) populations in Central Europe suggested that voles of both species belonging to the Eastern lineages could be better adapted to the colder continental climate. On the other hand, the Western lineages of these species were associated with a milder climate. This study provided evidence that oceanic-continental climate gradient affected the distribution of different genetic lineages of the common and field vole (Stojak et al. 2018).

Another good example of typically continental climate adapted species, but with larger European range, is the common hamster (*Cricetus cricetus* Linnaeus, 1758). The distribution area of this rodent extends from the River Yenisei in Russia to Western Europe where it forms isolated populations in Belgium, the Netherlands, and France (Mitchell-Jones et al. 1999). The natural habitat for common hamsters is considered to be steppe and forest-steppe zone. Despite the lack of natural steppe areas in Central and Western Europe, the common hamster was able to survive by adapting to agriculture landscapes. Nowadays, species is associated almost exclusively with agricultural habitats, especially in the westernmost parts of its range. As a continental adapted taxon, common hamster reacts with the range shifts to the oceanic-continental climate gradient. The species is adapted to high summer temperatures, low precipitations and high differences between summer and winter temperatures (Nechay 2000). Moreover the common hamster attracted attention as during the last 40 years, the decline of its populations was noticed (Surov et al. 2016) and the species became endangered in most areas of its occurrence. Considering the fact that populations suffering from a decline quickly lose their genetic variation, it becomes important to investigate refugial areas that are usually characterized by high genetic diversity and could serve in conservation management as reservoirs of the variability. Furthermore, potential exchange of individuals between populations for genetic rescue should be performed within lineages and rather not between them. Therefore, it is necessary to have a detailed knowledge of the phylogeographic history of the species.

Until now, the phylogeography of the common hamster was described on the basis of three partial sequences of the mitochondrial DNA (mtDNA): control region (*ctr*), cytochrome *b* (cyt*b*) and 16SrRNA (*16S*) (Neumann et al. 2005, Banaszek et al. 2010, Hegyeli et al. 2015, Melosik et al. 2017) or partial cyt*b* only (Feoktistova et al. 2017). As a result, several ancient mtDNA lineages were identified i.e. north in France, Belgium, Netherlands, Germany and western Poland (Neumann et al. 2005, Melosik et al. 2017), Pannonia in the Pannonian Basin and southern Poland (Neumann et al. 2005, Banaszek et al. 2010, Hegyeli et al. 2015), E1 in eastern Poland (Banaszek et al. 2010), E0 in Central Russia, Ural region, Northern Kazakhstan and Crimea and Caucasus in Ciscaucasian region (Feoktistova et al. 2017). The maximum range contraction of the species could be roughly located within the Saalian glaciation and the Eemian interglacial. The hamsters survived in eastern steppe refugia from which they migrated westwards through northern European plains or through the southern route around Carpathians to Central and Western Europe (Neumann et al. 2005, Banaszek et al. 2010). Once more the species range was severely reduced during the Last Glacial Maximum (LGM). This time the Pannonian lineage was able to survive the LGM in the Pannonian Basin but two other lineages North and E1 had to retreat again from northern European plains to eastern or southern refugia (Neumann et al. 2005, Banaszek et al. 2010, Feoktistova et al. 2017). The precise localization of these refugia is not known, but it was suggested that again the Ukrainian and Russian steppe belt could serve as such area (Neumann et al. 2005, Feoktistova et al. 2017). Paleontological data also confirmed the continuous presence of this species in areas of Eastern Europe (Markova et al. 1995). Furthermore, according to Feoktistova et al. (2017) the North lineage might survive the LGM in a refugium located in southern France and from this refugium the Caucasus lineage could also originate. Additionally, Feoktistova et al. (2017) suggested that Ural region could also serve as a refugium. Summing up, it is still debatable where the source populations for mtDNA lineages were located and which areas were used as main corridors for migration.

The aim of this study was to complete the missing knowledge about the distribution of mtDNA lineages of the common hamster in the eastern European part of its range, at the territory of Ukraine and Eastern Romania. This research area is important for phylogeographic study for several reasons. According to Feoktistova et al. (2017) the grasslands of Ukraine might have been an area where E1 lineage arose. Additionally, populations that inhabit the area of Ukraine might have served as source of expansion

into Central Europe as E1 lineage. The migration from steppe refugia could not only be westwards but also southwards to south-eastern Romania. However, in this area ancestral populations for Pannonia lineage could be present and/or populations from the Pannonian Basin might migrate eastwards following the demographic explosion (Neumann et al. 2005). Thus, the characteristic of populations that inhabit south-eastern Romania might be complex. In general obtained information may help to resolve the history of Pannonia and E1 lineages and their relationship with other groups. Additionally, the phylogeographic analysis of Ukrainian and Romanian populations may be important for common hamster conservation management as the study can provide valuable information for reintroduction plans and indicate areas with high conservation value. The particular aims planned to achieve the main goal of this study were: 1) to describe mtDNA cyt*b* diversity among Ukraine and Eastern Romania populations; 2) to describe the phylogeography of the common hamster in Ukraine and Eastern Romania.

Material and Methods

Study area and sampling

The study was conducted in the agricultural sites of Ukraine and Romania (Fig. 1). Non-invasive sampling was performed during field work, using hair traps as described by Reiners et al. (2011). The hair samples of the Ukrainian hamsters were collected after the harvest, between late July and early August in 2009, 2012 and 2013. In total, 106 samples were collected. Additional 14 dried skin samples from the Zoological Museum of Taras Schevchenko National University of Kiev were obtained and included for phylogeographic analysis. The museum's samples that originated from Lugansk, Vilkovo, Bolgrad, Kiev, Vinnitsia, Ulanowka and Lvov (Fig. 1) were collected between 1930 and 1973 (Table S1). Moreover, the samples from Lugansk, Vinnitsia, Vilkovo, and Bolgrad come from areas where the common hamster is currently either extremely rare or even extinct (Rusin et al. 2013). Samples from Romania were also collected after the harvest, in late July and early August 2014. The Romanian study area comprises the eastern (Moldavian Plateau) and south-eastern part of the country (Romanian Plain). Sampling places were chosen according to personal reports of field biologists from the "Milvus Group" Bird and Nature Protection Association. In total, 35 samples from that area were collected (Table S1). Furthermore, previously published sequences from the European species range were used for the phylogeography. The GenBank accession numbers are as follow: AJ633765, AJ633766, AJ633769, AJ633770, AJ633773, AJ633775 (Neumann et al. 2005), EU107523-EU107529, EU107531-EU107535 (Banaszek et al. 2010), KF271752-KF271763, KR706035-KR706041 (Feoktistova et al. 2016), KR010651-KR010664 (A. Banaszek, unpublished data), KT224635-KT224640 (Hegyeli et al. 2015), KY748062-KY748079 (Feoktistova et al. 2017).

DNA extraction, samples verification and microsatellite genotyping

Genomic DNA was isolated from hair bulbs and dried skins (museum samples) with the use of DNeasy Blood & Tissue Kit (Qiagen) accordingly to manufacturer's instructions. All hair samples were checked in a part of mitochondrial DNA (mtDNA) cytochrome *b* (cyt*b* 600 bp, sequenced in one direction, for PCR protocol, see below) to verify whether they belong to the common hamster. Among the 141 hair samples, eight belonged to other species (Table S1). Additionally, three samples (two from Ukraine and one from Romania) were excluded due to poor amplification quality. A set of 17 microsatellite loci were used to identify individuals from collected hair samples to avoid including the same individual twice (Ccrµ3, Ccrµ4, Ccrµ10, Ccrµ11, Ccrµ12, Ccrµ13, Ccrµ19, Ccrµ20, Neumann & Jansman 2004, IPK01, IPK03, IPK05, IPK09, Jacob & Mammen 2006, IPK06, IPK07, IPK12, Ccrµ15, Ccrµ17, Reiners et al. 2013). Analysed loci were not excessively long (all alleles < 230 bp) and were significantly amplified from small amounts of DNA. The PCR profiles for microsatellite and the method of analysis followed the procedures described in Banaszek et al. (2011) and Reiners et al. (2013). The microsatellites were analysed manually using GeneMapper v 4.0 software (Applied Biosystems). The Autobin was used for allele binning based on raw sizes (Gichoux et al. 2011). Identification of individuals by finding matching pairs of genotypes was carried out in Cervus 3.0 (Kalinowski et al. 2007). Identity analysis revealed two pairs of genotypes among 130 hair samples that were identical across 17 loci. As a result, 128 different genotypes were found in analysed hair samples. Therefore, after adding 14 samples from the museum, 142 samples of the common hamster were used for phylogeographic analysis.

Mitochondrial DNA amplification and sequencing

A partial cytochrome *b* (cyt*b*, 904 bp) region of the mtDNA was analysed. This fragment was

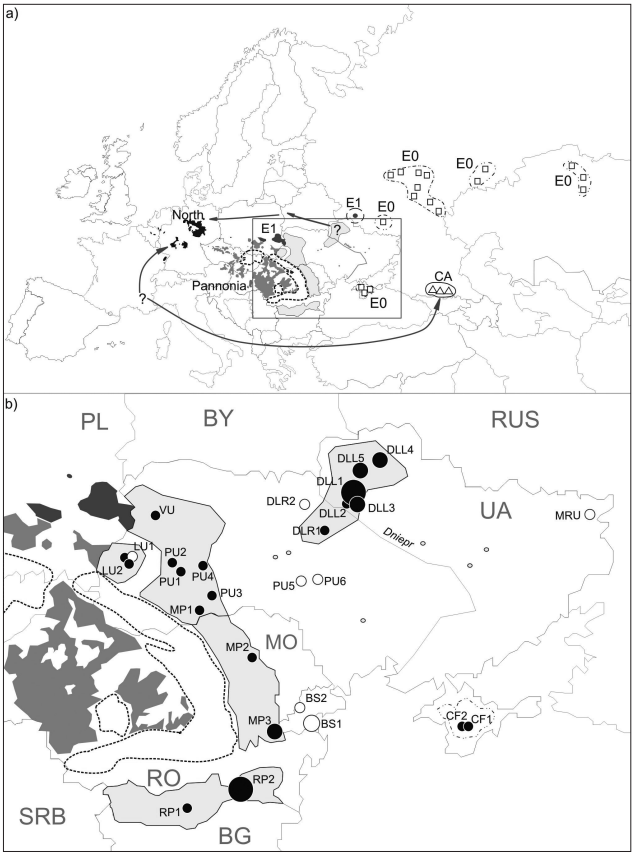

Fig. 1. a) Distribution of the phylogeographic lineages of the common hamster based on Neumann et al. (2005), Banaszek et al. (2010), Hegyeli et al. (2015), Feoktistova et al. (2017) and Melosik et al. (2017). The hypothetical migration ways of the north and Caucasus lineages and potential glacial refugia (question marks) for North lineage are indicated, according to Neumann et al. (2005), Banaszek et al. (2010) and Feoktistova et al. (2017). The Carpathians are marked with a dashed line. Light grey – areas of unknown phylogeographic affiliation. As the distribution of the common hamster in Russia and Kazakhstan is not described precisely, only points described by Feoktistova et al. (2017) were marked in that part of species range. b) Geographic origin of modern and museum samples used in this study (museum samples in white, modern samples in black). The diameter of the circle corresponds to the ratio of the number of haplotypes/number of individuals in a given location (the biggest circle – the ratio higher than 0.65, the middle one – the ratio from 0.64 to 0.34, the smallest one – the ratio lower than 0.33). Samples IDs are in agreement with Table 1.

chosen as it is shared by the data from published studies for other parts of the species range and enables their comparison. For amplification, two novel primers were designed: Ccricyt*b* F (5'-ATCATCAACCATGCGTTCATTG-3') and Ccricyt*b* R (5'-TTATGCTTGCGATTGGTATGA-3'). PCRs were performed in a total volume of 10 µl, consisting of 2 µl DNA, and 8 µl mixture containing Qiagen Multiplex PCR kit, both primers and water. Cycling conditions were 95 °C for 15 min followed by 30 cycles of 94 °C for 60 s, 52 °C for 60 s and 72 °C for 120 s with a final extension step at 72 °C for 10 min. Additionally, for museum samples another two pairs of primers were designed to amplify analysed part of cyt*b*: CriCyt*b*1F (5'-CCCCTCAAATATCTCATCCTGA-3'), CriCyt*b*1R (5'-TGATGATGAAGGGGAGGATAA-3'), CriCyt*b*2F (5'-TCACACGATTCTTCGCATTC-3') and CriCyt*b*2R (5'-TGAAAGGGTATTCTACTGG TTGTC-3'). Each pair of primers was designed to cover a 500 bp fragment as longer fragments usually cannot be amplified from a small amount and low quality DNA. In this case, PCR amplification consisted of 15 min of initial activation step at 95 °C, 35 cycles of 94 °C for 30 s, 58 °C for 90 s and 72 °C for 60 s followed by 60 ºC for 30 min. Amplified products were purified using Exonuclease I (Thermo Scientific) and FastAP Thermosensitive Alkaline Phosphatase (Thermo Scientific). Sequencing reactions were performed using the BigDye™ Terminator Sequencing kit v 3.1 (Applied Biosystems) in both directions. Amplification and sequencing reactions were performed in TProffesional Thermocycler (Biometra) and in SensoQuest Labcycler (Biomedizinische Elektronik). The sequencing reaction products were run on a 3130 Genetic Analyzer (Applied Biosystems).

Statistical analysis

Obtained sequences were aligned in BioEdit v 7.0.5 (Hall 1999) and checked manually in ChromasLite v 2.4 (http://technelysium.com.au/). For alignment reference, the GenBank sequences of the common hamster were used. Identification of the separate haplotypes was performed in DNASP version 5.1 (Librado & Rozas 2009). The nucleotide diversity (π) and haplotype diversity (h) were calculated using Arlequin v 3.5 (Excoffier & Lischer 2010). Phylogenetic relationship among haplotypes was reconstructed using maximum parsimony (MP), minimum evolution (ME) and neighbor-joining (NJ) algorithms implemented in MEGA 6 (Tamura et al. 2013). MP, ME and NJ results were compared for the congruence of tree topologies. As the outgroup, sequences from the related species *Cricetulus griseus* were used. The relative stability of the phylogeographic trees was assessed with bootstrap analysis using 1000 replicates. The topologies inferred with these methods were compared with Bayesian tree performed in MrBayes 3.2 (Ronquist et al. 2012) using Markov chain Monte Carlo (MCMC) method. The data was analysed using an HKY + G + I model as it was previously indicated as the best-fit substitution model by MEGA 6. Two independent runs with four chains (one "cold" and three "hot" chains), starting with random trees, were performed

for three million generations, the sampling frequency of every 1000 generation and a burn-in of 25 %. The consensus tree was drawn in FigTree v1.4.2 (Rambaut 2014). In this study Bayesian phylogeny tree with the corresponding bootstrap values from ME, MP and NJ trees is presented. Additionally, to show a genealogy relationship, a network of haplotypes was constructed using a median-joining (MJ) algorithm based on maximum parsimony implemented in the program PopART (Leigh & Bryant 2015).

Table 1. The mtDNA cytochrome *b* (cyt*b*) variability analysis. Haplotype diversity (h) and nucleotide diversity (π) are calculated for the main geographic regions of the common hamster occurrence in Ukraine and Romania.

Geographic region	Locality	Sample ID	N	N of haplotypes	Frequency of haplotypes	h	π (%)
Dnieper Lowland Dnieper left bank	Baryshivka	DLL1	6	11	Cbu4 (0.13) Cbu5 (0.22) Cbu6 (0.044) Cbu7 (0.044) Cbu8 (0.044) Cbu9 (0.13) Cbu10 (0.13) Cbu11 (0.044) Cbu12 (0.044) Cbu13 (0.087) Cbu16 (0.087)	0.92 ± 0.03	0.59 ± 0.33
	Berezan	DLL2	1				
	Yahotyn	DLL3	12				
	Baturyn	DLL4	2				
	Nizhyn	DLL5	2				
Dnieper Lowland Dnieper right bank	Velykopolovetske	DLR1	14	3	Cbu14 (0.176) Cbu15 (0.647) Cbu22 (0.176)	0.55 ± 0.11	0.14 ± 0.10
	Kiev, Ivankov	DLR2	3				
Middle Russian Upland	Lugansk, Melovoe	MRU	5	1	Cbu23 (1.0)	-	-
Podolian Upland	Halych	PU1	3	4	Cbu1 (0.6) Cbu15 (0.267) Cbu20 (0.067) Cbu26 (0.067)	0.60 ± 0.11	0.12 ± 0.09
	Rohatyn	PU2	3				
	Kamienec Podolski	PU3	1				
	Hrymailiv	PU4	6				
	Vinnitsia	PU5	1				
	Ulanowka	PU6	1				
Volyn Upland	Lutsk	VU	7	1	Cbu3 (1.0)	-	-
Lublin-Lvov Upland	Lvov	LU1	9	3	CbP3 (0.438) Cbu19 (0.188) Cbu21 (0.375)	0.68 ± 0.06	0.48 ± 0.28
	Sambir and Old Sambir	LU2	7				
Crimean Foothills	Simferopol Botanic Park	CF1	10	2	Cbu17 (0.545) Cbu18 (0.455)	0.52 ± 0.4	0.06 ± 0.06
	Simferopol Sewastopolska Street	CF2	12				
Black Sea Lowland	Vilkovo	BS1	2	3	Cbr11 (0.333) Cbu24 (0.333) Cbu25 (0.333)	1.0 ± 0.27	0.22 ± 0.21
	Bolgrad	BS2	1				
Moldavian Plateau	Chernovtsy	MP1	8	8	Cbu2 (0.368) Cbr8 (0.263) Cbr9 (0.053) Cbr10 (0.053) Cbr11 (0.105) Cbr12 (0.053) Cbr18 (0.053) Cbr19 (0.053)	0.81 ± 0.07	0.25 ± 0.16
	Husi	MP2	1				
	Braila	MP3	10				
Romanian Plain	Alexandria	RP1	7	6	Cbr7 (0.466) Cbr13 (0.2) Cbr14 (0.067) Cbr15 (0.067) Cbr16 (0.067) Cbr17 (0.133)	0.76 ± 0.10	0.61 ± 0.35
	Oltenita	RP2	8				

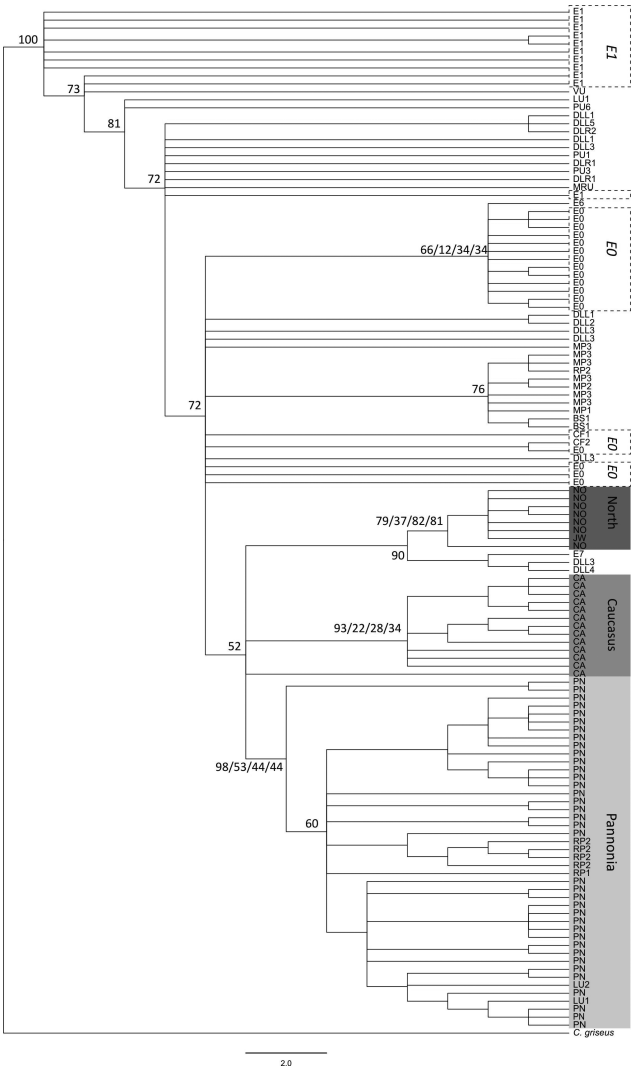

Fig. 2. Bayesian phylogeny tree based on 128 cyt*b* (904 bp) mtDNA haplotypes. Numbers on branches correspond to posterior probability and bootstrap support (MrBayes/MP//NJ/ME, 1000 replicates). Haplotype of related hamster species *Cricetulus griseus* served as an outgroup. Samples Ids follow Table 1 and Fig. 1. All GenBank haplotypes are named as haplogroups: PN – Pannonia, CA – Caucasus, NO – North, E1 – *E1*, E0 – *E0*, single haplotype from Ural – E6 and Novosibirsk – E7 (Neumann et al. 2005, Banaszek et al. 2010, Hegyeli et al. 2015, Feoktistova et al. 2017, Melosik et al. 2017).

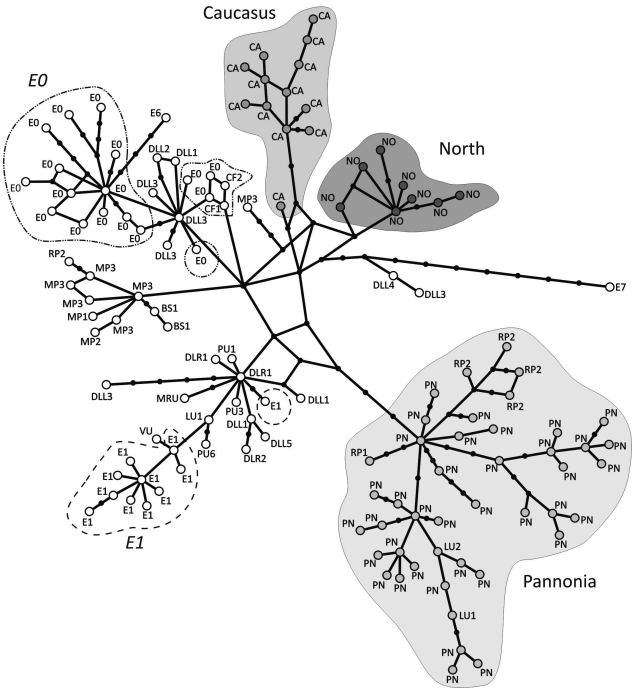

Fig. 3. Median-joining network based on 128 cyt*b* (904 bp) mtDNA haplotypes. Each line between black dots represents a mutation. Separate phylogeographic lineages are marked with a solid line, groups of haplotypes included to presumable *E1* and *E0* lineages are marked with a dashed line. Samples Ids are the same as in Bayesian phylogeny tree, Fig. 2.

Results

Fourty cyt*b* haplotypes were found in 142 individuals taken into account. Nearly all haplotypes were new (39, accession no. MH444207-MH444245) and only one haplotype was previously reported by Banaszek et al. (2010, EU107525). Fifty observed substitutions were transitions and seven were transversions. Fourty seven out of fifty seven variable sites were informative under parsimony.

Results for cyt*b* variability analysis showed that both Romanian and Ukrainian populations were polymorphic. One haplotype reported before (CbP3) was found exclusively in Lublin-Lvov Upland. All remaining cyt*b* haplotypes were new and they were specific to each region (Table 1). Nucleotide diversity in most of Ukrainian and Moldavian Plateau populations was low and only populations from Romanian Plain and Dnieper Lowland (the Dnieper left bank) had higher values. Haplotype diversity reached high values in populations from Romanian Plain, Dnieper Lowland (the Dnieper left bank) and Moldavian Plateau (Table 1). Populations from Dnieper Lowland and Moldavian Plateau showed also the highest ratio values of the haplotypes' number to the number of individuals in a given location (Fig. 1).

For phylogeographic analysis, 128 different haplotypes were used. Haplotypes network, as well as phylogenetic trees, revealed that samples from Eastern Europe do not create any separate lineage (Figs. 2 and 3). On the Bayesian tree, three from five described so far phylogeographic lineages were found. One group was created by samples belonging to Pannonia lineage. Another group was formed by haplotypes characteristic for North lineage and the third one contained exclusively haplotypes belonging to Caucasus lineage. The phylogroups were supported by high probability values (Fig. 2). However, on the trees constructed using MP, NJ, ME algorithms Pannonia and Caucasus groups had no bootstrap

support. Only North lineage on the NJ and ME trees had statistical support but not very high (Fig. 2). On the other hand, the E0 and E1 lineages did not create here any separate clusters. Therefore, from now on the E1 and E0 lineages names will be written in italics. Haplotypes supposed to be characteristic for both lineages were nested among samples from Ukraine (Fig. 2). Among populations from Dnieper Lowland (DLL, DLR), Podolian Upland (PU), Volyn Upland (VU), Middle Russian Upland (MRU) and Crimea (CF) it was not possible to find any phylogeographic structure (Figs. 2 and 3). Samples from Moldavian Plateau (MP) were grouped together with museum samples from Black Sea Lowland (BS), but with not high statistical support. Other samples from Romania, collected in Romanian Plain (RP), linked on the phylogenetic trees with samples from Pannonian Basin, belonging to Pannonia lineage. Within this lineage, samples from Lublin – Lvov Upland (LU) (Figs. 2 and 3) were also nested.

Discussion

The phylogeography of the common hamster in Ukraine

A large number of common hamster studies focused entirely on Western and Central Europe, often omitting such an important for the species area as Eastern Europe. Recent work of Feoktistiva et al. (2017) concerned, among others, Eastern European populations. However, the study did not include hamsters from Ukraine, an area that provided an important link between Eastern and Central Europe. Furthermore, according to Stewart et al. (2010) and Neumann et al. (2005) the main refugial area for continental climate adapted taxa, like the common hamster, comprises the steppe habitats of the Ukraine, Russia and Central Asia. Therefore, our study fills this gap and provides new insight into the phylogeography of *C. cricetus*. Results of Rusin et al. (2013) study in Ukraine showed that the common hamster disappeared from vast parts of the country and currently occurs only in three larger areas such as Crimean peninsula, West, and North-Eastern Ukraine. In this study, all regions described by Rusin et al. (2013) were analysed. Additionally, the museum's samples from regions where the common hamster is no longer present were used. Obtained results showed a great haplotype differentiation in cyt*b* sequences among the Ukrainian populations. Moreover, network and gene tree analyses did not produce a clear phylogeographic pattern that is typical for refugial populations (Hewitt 1996). It is in agreement with previous assumptions that the territory of Ukraine may be the source area for several lineages of that species (Neumann et al. 2005). The populations from the Ukrainian part of Lublin – Lvov Upland constitute an exception as they clearly belong to the Pannonia lineage. These populations are characterized by low haplotype and nucleotide diversity values. Low genetic diversity in Ukrainian Pannonia populations may characterize edge populations of a former migration wave from the Pannonian Basin. The Moravian Gate probably served as a corridor for population extension into southern Poland and then eastwards to the Polish Sandomierz Basin (Banaszek & Ziomek 2012) as well as Lvov and Sambir region in Ukraine. This finding shows that the Pannonia lineage almost encircled the Carpathians.

Other analysed Ukrainian samples did not form any phylogroups. The greatest haplotype diversity occurred in an eastern part of the analysed area, the Dnieper Lowland (DLL samples). Such pattern of variability is characteristic for refugial area populations (Hewitt 1996). Therefore, we will refer to this territory as the Dnieper Lowland Refugial area. Haplotypes from that region did not cluster together on the phylogenetic network but they showed connections with haplotypes from different parts of the eastern species range. Populations from this region were probably the source for *E1* and *E0* common hamster lineages. Moving westwards, a considerable decline in the haplotype variety was observed, through to Volyn Upland, where all collected individuals had the same haplotype. That finding suggests that hamsters inhabiting Volyn Upland may comprise the edge of a migration wave from the east. Moreover, these populations are closely located to populations belonging to *E1* genetic lineage described by Banaszek et al. (2010) in Poland. According to Banaszek et al. (2010), the *E1* lineage in Poland showed signs of being the edge population. There was a very low genetic variation among Polish populations of that lineage and it showed a star-like haplotype topology in the network (Banaszek et al. 2009, 2010). Thus, our research confirmed earlier assumptions that Polish populations represent a western edge population of Ukrainian hamsters.

The phylogeography of the common hamster in Romania

So far, there was only one mtDNA lineage described for Romania. According to Hegyeli et al. (2015) hamsters in the area of Transylvanian Plateau and Pannonian Plain i.e. inside the Carpathians arch belong to the Pannonia lineage. The only single hamster sample from the Romanian Plain (Craiova)

that was analysed by Neumann et al. (2005) did not cluster with Pannonian haplotypes. Therefore, this study focused on the populations located outside the Carpathians arch. Firstly, the presence of the common hamster in Moldavian and Romanian plains was confirmed, as no population monitoring was performed in that area. Secondly, it has been found that the individuals from Romanian Plain (RP) linked on the phylogenetic trees and network directly with other samples from Pannonia lineage. Such a result may indicate that these populations could be the wave of migration after demographic expansion in Pannonian Basin (Neumann et al. 2005). On the other hand, individuals from Moldavian Plateau (MP) showed no clear affiliation to any of the phylogeographic lineages described so far. As phylogenetic trees and haplotype network revealed, these populations linked together with population from Ukrainian part of Moldavian Plateau and three museum samples from Black Sea Lowland (BS) among eastern samples. Moreover, one sample from Oltenita (RP2) was also located in this group. However, we are not able to decide with the use of a single cyt*b* fragment if this haplotype group forms a distinct phylogeographic lineage. Further research with the use of other sequences is necessary to check the relationship of this group with Pannonia, *E1* and *E0* lineages. Furthermore, as the populations from Moldavian Plateau are characterised by high values of haplotype diversity and the ratio of the number of haplotypes/number of individuals it is possible that they could be ancestral populations forming an ancient link between Pannonia and other lineages.

The future of phylogeography of the common hamster

In the available literature, there are five phylogeographic lineages of the common hamster (Neumann et al. 2005, Banaszek et al. 2010, Feoktistova et al. 2017) and three of them were described in the eastern part of species' range. However, our study revealed that only the lineages North, Pannonia and Caucasus form truly separated and statistically supported haplotype groups. Banaszek et al. (2010) and Feoktistova et al. (2017) assumed the presence of other two phylogeographic groups in the eastern European range of the common hamster – *E1* and *E0* lineages. Nevertheless, in this research, a lack of structure among the mtDNA haplotypes assigned to these lineages was found. On the phylogenetic trees and haplotype network, the haplotypes included so far in *E0* and *E1* lineages mixed together with samples from Ukraine. In contrast, Feoktistova et al. (2017) presented a clear division between *E0* and *E1* lineage that was supported by high probability values. In our opinion, the clustering differences among the studies may be the result of using haplotypes of *E1* lineage derived from one region in eastern Poland, which is quite specific in genetic structure as it is an edge of the migration wave with decreased haplotype diversity (Banaszek et al. 2010). Considering this, there is no certainty to distinguish *E1* and *E0* as separate phylogeographic lineages. On the other hand, on the phylogenetic tree constructed using the Bayesian method, most of the haplotypes characteristic for *E0* lineage were grouped together, but with low probability values. It is possible that both groups are the result of two waves of migration. *E1* lineage might be the result of migration from the Dnieper Lowland Refuge area to the west and *E0* linage would be the result of migration to the east. Perhaps, additional analysis of the nuclear sequences or 16SrRNA region of mtDNA, that well separates phylogeographic lineages in the common hamster, would allow distinguishing the phylogroups.

Feoktistova et al. (2017) indicated a phylogenetic relationship between North and Caucasus lineage, suggesting a connection between populations inhabiting these areas in the past. In our research, these lineages were also grouped together but with some haplotypes from Dnieper Lowland (DLL) and Siberia (E7, Neumann et al. 2005). This finding may indicate that the hypothesis of the continuous range of the common hamster in the northern Mediterranean that extended from the southern parts of modern France to the Caucasus in the Late Pleistocene, may be too far-reaching. In our opinion, the hypothesis of the migration of North phylogroup through the European Plain should not be rejected yet. So far, there was only one population of North lineage described in Poland (Melosik et al. 2017). It is an isolated population located in the southwestern part of the country and it is very probably an edge population of the wave of migration from German populations of this lineage. Although there are no other North lineage populations located east of Germany that could prove the migration through the European Plain, two potential explanations could be assumed. As it was suggested by Banaszek et al. (2010) the lack of North-type haplotypes in northern Poland may result from several rapid expansion and extinction events that could wiped out the hamsters of North lineage from that area. On the other hand, North haplotypes could have been present in northern located populations that became extinct, as the common hamster lost most of its range in Poland during the last 40 years. In this

case, the analysis of samples from the territory of Belarus might be helpful.

Considering all obtained results, more intensive analyses should be conducted using other sequences (e.g. nuclear genome data) in order to resolve the phylogenetic pattern of the eastern common hamster populations. As it was shown in this study, a single partial cyt*b* analysis is not sufficient to describe the phylogeographic pattern among Eastern European populations of that species. Combined data from other sequences, as it was previously done by Neumann et al. (2005) or Banaszek et al. (2010) should enhance the power of phylogenetic statistical analyses and may reveal important phylogeographic signatures for the common hamster populations. Moreover, all phylogeographic analyses were done only on the basis of mtDNA sequences, while the history of genes is sometimes discordant with the history of species. For that reason, a wider variety of molecular markers should be used to obtain a reliable phylogeographical history (Toews & Brelsford 2012). Our study provides also valuable information for the conservation management of the common hamster as we indicated the areas of high genetic variation. As it was showed in this study the most precious for conservation is the area of Dnieper Lowland and Moldavian Plateau with the highest values of haplotype diversity. Such regions could serve in the future as reservoirs of the variability. As the common hamster populations are highly fragmented, they quickly lose their genetic variation. In this case a translocation of individuals from i.e. Dnieper Lowland could help to restore genetic variability. However, the translocation should be carried out rather within the studied phylogeographic lineages to avoid potentially detrimental consequences of outbreeding depression. Only carefully planned conservation actions that are based on all available knowledge will provide a long-term survival of the common hamster populations.

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Literature

Banaszek A., Jadwiszczak K.A., Ratkiewicz M. et al. 2010: Population structure, colonization processes and barriers for dispersal in the common hamster *Cricetus cricetus* (L.) populations in Poland. *J. Zool. Syst. Evol. Res. 48: 151–158.*

Banaszek A., Jadwiszczak K.A., Ratkiewicz M. & Ziomek J. 2009: Low genetic diversity and significant structuring of the common hamster populations *Cricetus cricetus* in Poland revealed by the mtDNA control region sequence variation. *Acta Theriol. 54: 289–295.*

Banaszek A., Jadwiszczak K.A. & Ziomek J. 2011: Genetic variability and differentiation in the Polish common hamster (*Cricetus cricetus* L.): genetic consequences of agricultural habitat fragmentation. *Mamm. Biol. 76: 665–671.*

Banaszek A. & Ziomek J. 2012: Genetic variation and effective population size in an isolated population of the common hamster, *Cricetus cricetus*. *Folia Zool. 61: 34–43.*

Bhagwat S.A. & Willis K.J. 2008: Species persistence in northerly glacial refugia of Europe: a matter of chance or biogeographical traits? *J. Biogeogr. 35: 464–482.*

Excoffier L. & Lischer H.E. 2010: Arlequin suite ver. 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. *Mol. Ecol. Resour. 10: 564–567.*

Feliner G.N. 2011: Southern European glacial refugia: a tale of tales. *Taxon 60: 365–372.*

Feoktistova N.Y., Meschersky I.G., Bogomolov P.L. et al. 2017: Phylogeographic structure of the common hamster (*Cricetus cricetus* L.): Late Pleistocene connections between Caucasus and Western European populations. *PLOS ONE 12: e0187527.*

Feoktistova N.Y., Meschersky I.G., Surov A.V. et al. 2016: Genetic structure of urban population of the common hamster (*Cricetus cricetus*). *Russ. J. Genet. 52: 194–203.*

Guichoux E., Lagache L., Wagner S. et al. 2011: Current trends in microsatellite genotyping. *Mol. Ecol. Resour. 11: 591–611.*

Hall T.A. 1999: BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleid Acids Symp. Ser. 41: 95–98.*

Hegyeli Z., Kecskes A., Korbut Z. & Banaszek A. 2015: The distribution and genetic diversity of the common hamster *Cricetus cricetus* in Central and Western Romania. *Folia Zool. 64: 173–182.*

Hewitt G.M. 1996: Some genetic consequences of ice ages, and their role in divergence and speciation. *Biol. J. Linn. Soc. 58: 247–276.*

Hewitt G.M. 2004: Genetic consequences of climatic oscillations in the Quaternary. *Philos. Trans. R. Soc. Lond. B 359: 183–195.*

Jakob S.S. & Mammen K. 2006: Eight new polymorphic microsatellite loci for genetic analyses in the endangered common hamster (*Cricetus cricetus* L.). *Mol. Ecol. Resour. 6: 511–513.*

Kalinowski S.T., Taper M.L. & Marshall T.C. 2007: Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. *Mol. Ecol. 16: 1099–1106.*

Leigh J.W. & Bryant D. 2015: PopART: full-feature software for haplotype network construction. *Methods Ecol. Evol. 6: 1110–1116.*
Librado P. & Rozas J. 2009: DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics 25: 1451–1452.*
Markova A.K., Smirnov N.G., Kozharinov A.V. et al. 1995: Late Pleistocene distribution and diversity of mammals in northern Eurasia (paleofauna database). *Paleontol. Evol. 28–29: 5–143.*
Melosik I., Ziomek J., Winnicka K. et al. 2017: The genetic characterization of an isolated remnant population of an endangered rodent (*Cricetus cricetus* L.) using comparative data: implications for conservation. *Conserv. Genet. 18: 759–775.*
Mitchell-Jones A.J., Amori G., Bogdanowicz W. et al. 1999: The atlas of European mammals. *Academic Press, London.*
Nechay G. 2000: Status of hamsters: *Cricetus cricetus*, *Cricetulus migratorius*, *Mesocricetus newtoni* and other hamster species in Europe. *Nature and Environment series 106, Council of Europe Publishing, Strasbourg.*
Neumann K. & Jansman H. 2004: Polymorphic microsatellites for the analysis of endangered common hamster populations (*Cricetus cricetus* L.). *Conserv. Genet. 5: 127–130.*
Neumann K., Michaux J.R., Maak S. et al. 2005: Genetic spatial structure of European common hamsters – a result of repeated range expansion and demographic bottlenecks. *Mol. Ecol. 14: 1473–1483.*
Rambaut A. 2014: FigTree v1. 4.2. *University of Edinburgh, Edinburgh.*
Reiners T.E., Eidenschenk J., Neumann K. & Nowak C. 2013: Preservation of genetic diversity in a wild and captive population of a rapidly declining mammal, the common hamster of the French Alsace region. *Mamm. Biol. 79: 240–246.*
Reiners T.E., Encarnação J.A. & Wolters W. 2011: An optimized hair trap for non-invasive genetic studies of small cryptic mammals. *Eur. J. Wildlife Res. 57: 991–995.*
Ronquist F., Teslenko M., van der Mark P. et al. 2012: MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Syst. Biol. 61: 539–542.*
Rusin M., Banaszek A. & Mishta A.V. 2013: The common hamster (*Cricetus cricetus*) in Ukraine: evidence for population decline. *Folia Zool. 62: 207–213.*
Říčanová Š., Koshev Y., Říčan O. et al. 2013: Multilocus phylogeography of the European ground squirrel: cryptic interglacial refugia of continental climate in Europe. *Mol. Ecol. 22: 4256–4269.*
Sommer R.S. & Nadachowski A. 2006: Glacial refugia of mammals in Europe: evidence from fossil records. *Mammal Rev. 36: 251–265.*
Stewart J.R., Lister A.M., Barnes I. & Dalén L. 2010: Refugia revisited: individualistic responses of species in space and time. *Philos. Trans. R. Soc. Lond. B 277: 661–671.*
Stojak J., Borowik T., Górny M. et al. 2018: Climatic influences on the genetic structure and distribution of the common vole and field vole in Europe. *Mammal Res. 64: 19–29.*
Surov A., Banaszek A., Bogomolov P. et al. 2016: Dramatic global decrease in the range and reproduction rate of the European hamster *Cricetus cricetus*. *Endanger. Species Res. 31: 119–145.*
Tamura K., Stecher G., Peterson D. et al. 2013: MEGA6: molecular evolutionary genetics analysis version 6.0. *Mol. Biol. Evol. 30: 2725–2729.*
Toews D.P. & Brelsford A. 2012: The biogeography of mitochondrial and nuclear discordance in animals. *Mol. Ecol. 21: 3907–3930.*

Table S1. Numbers, year and type of samples collected in particular localities of Ukraine and Romania.

Geographic region	Localities (Museum voucher codes)		Year	Number of samples	Type of sample
Dnieper Lowland Dnieper left bank	Baryshivka		2009	6	Hairs
	Berezan		2009	1	Hairs
	Yahotyn		2009	12	Hairs
	Baturyn		2012	3	Hairs
	Nizhyn		2012	2	Hairs
Dnieper Lowland Dnieper right bank	Velykopolovetske		2012	14	Hairs
	Kiev, Ivankov	(4572, 3936)	1955	2	Dried skin
		(3937)	1956	1	Dried skin
Middle Russian Upland	Lugansk, Melovoe	(4339-4343)	1953	5	Dried skin
Podolian Upland	Halych		2009	3	Hairs
	Rohatyn		2013	3	Hairs
	Kamienec Podolski		2013	1	Hairs
	Hrymailiv		2013	7	Hairs
	Vinnytsia	(4827)	1955	1	Dried skin
	Ulanowka	(3937)	1973	1	Dried skin
Volyn Upland	Lutsk		2013	7	Hairs
Lublin-Lvov Upland	Lvov	(2305)	2013	8	Hairs
	Sambir and Old Sambir		2013	8 (1*)	Hairs
Crimean Foothills	Simferopol Botanic Park		2012	10	Hairs
	Simferopol Sewastopolska Street		2012	13	Hairs
Black Sea Lowland	Vilkovo	(625, 626)	1946	2	Dried skin
	Bolgrad	(3889)	1956	1	Dried skin
Moldavian Plateau	Chernovtsy		2013	8	Hairs
	Husi		2014	3 (2*, 1**)	Hairs
	Braila		2014	11 (2**)	Hairs
Romanian Plain	Alexandria		2014	9 (1*)	Hairs
	Oltenita		2014	9 (1**)	Hairs
	Calafat		2014	3 (2*, 1**)	Hairs
Total				141	

*Samples belonging to *Microtus* sp. **Samples belonging to *Spermophilus citellus*.