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Genetic differentiation in *Cricetulus migratorius* Pallas, 1773 (Rodentia, Cricetidae)

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Abstract

The grey hamster *Cricetulus migratorius* is the most widespread and ecologically opportunistic species among Palearctic hamsters. Genetic diversity across most of its range was examined by using cytochrome b (*cytb*) and cytochrome oxidase I (*coI*) gene sequences. Phylogenetic analyses revealed three well-differentiated allopatric lineages. The western lineage is distributed in Ukraine, Central and South Russia, Caucasus and Anatolia; the eastern lineage occupies Kazakhstan, Turan, Mongolia, West China and some mountain areas in western Central Asia. The Lower Volga valley may act as the barrier between these two phylogroups. The third lineage is found only in the Qurama Mountains (Uzbekistan). Mitochondrial data are in good agreement with the results of previous craniometric and allozyme studies, suggesting a subdivision into *migratorius* (eastern) and *phaeus* (western) subspecies groups.

Keywords: phylogeography, mtDNA, Palearctic hamsters, steppe fauna, Pleistocene

Phylogeographic studies of wide-range species can provide important insights into the tempo and mode of evolutionary diversification, the range dynamics through major climatic shifts and the nature of biogeographical boundaries. The Quaternary history of species associated with temperate forest zone has been well studied (Schmitt, 2007); however, the impact of ice ages on the species inhabiting the Eurasian steppe and desert zones remains insufficiently known (see review Kajtoch et al., 2016).

The grey hamster *Cricetulus migratorius* (Pallas 1773) is one of the most widespread Palearctic rodent species. Its range covers a vast area from Greece to south-eastern Mongolia and from Central Russia to Belujistan (Gromov, Erbajeva, 1995; Kryštufek, Vohralík, 2009, Lebedev, 2012). This hamster occurs in different open habitats including steppe, forest-steppe, semidesert and desert; it is found both in lowlands and mountains, reaching altitudes of more than 4000 masl. Ecological plasticity is complemented by extensive morphological variation. Based on external traits (coloration and body size) up to 27 nominal subspecies were described, which were joined into 12-14 subspecies in a revision by Argyropulo (1932). The results of a range-wide craniometric study (Lebedev, 2000) suggested subdivision into at least five allopatric morphogroups treated as subspecies groups. Only two of them have wide distribution ranges, which, in combination, cover most of the lowland area from Ukraine to Mongolia. These two subspecies groups (western *phaeus* and eastern *migratorius*) are separated by the lower Volga. Genetic data on *C. migratorius* are still scarce and are based on geographically restricted samples (Lebedev et al., 2000; İbiş, et al., 2017). The aim of the present study is to examine the mtDNA variation across most of the range, primarily focusing on the *phaeus* – *migratorius* relationships.

Sequences of cytochrome b gene (*cytb*) were examined in 94 exemplars of *C. migratorius* from 44 localities including five sequences from the GenBank (Fig. 1, Supplementary 1). Six sequences of other hamster species were used as outgroups in the phylogenetic analysis (Supplementary 1). In addition, we analysed an alignment consisting of 37 sequences of cytochrome oxidase I (*CoI*, 657 bp), which were obtained from online projects housed by Barcode of Life Data System (BOLD; www.boldsystem.org). Process IDs are presented in the Supplementary 1.

The details of DNA extracting, amplification and sequencing of the entire *cytb* (1140 bp) follow those described in a previous study (Poplavskaya et al., 2012). Four primers were developed specifically for the present study: polymerase chain reaction (PCR) was performed with the forward/reverse primers L_Cric_1 (5'-AATGACATGAAAAATCATCGTTGTAAT-3') or L_Cric_2a (AYCTATGACCAATGACATGAAAAATC) / H_Cric_2a (TTATGTACAGGAAGTAGTTTARCAAGAA) or H_Cric_1b (5'-GGTTTACAARACCAAKGTAATTTTTATACTA-3'); sequencing was conducted using the internal primers L466 (5'-ATCGGAACAACCTTAGTAGAATGAAT-3') and H741 (5'-AGTGTAATTATCTGGGTCTCCGA-3'). All new haplotypes of *C. migratorius* were deposited into GenBank with the Accession Numbers /// - ///.

Phylogenetic trees were reconstructed based on concatenated alignment of two mitochondrial genes (*Cytb* and *CoI*) using maximum parsimony with 1000 bootstrap replicates (PAUP* 4.0b10, Swofford 2003) and Bayesian inference (MrBayes 3.2; Ronquist et al., 2011). The results of the likelihood ratio test for a molecular clock, which was conducted using PAML ver 4.7 (Yang, 2007) based on the NJ topology, indicate no significant departure from H0 of rate constancy (P>0.25). Therefore, the

Bayesian analyses were performed under a strict clock model. The data were partitioned into two subsets, including 1st+2nd and 3rd codon positions. The analysis included two independent runs with the chain length set at 10 million generations. Convergence was checked using Tracer 1.6 (Rambaut et al., 2014). In addition, to ensure that the phylogenies of the two genes are congruent neighbor-joining trees were reconstructed for each of the separate gene alignments in PAUP*. Relationships among *cytb* haplotypes were examined using median-joining networks in NETWORK, version 4.5.0.0 (Bandelt et al., 1999) under the default options. Divergence times were assessed by using the *cytb* substitution rate of 10.7% per site per Mya as estimated for *Cricetulus barabensis* by Poplavskaya et al. (submitted). This estimate was calculated via regression (non-linear) of *cytb* divergence vs. secondary calibration ages taken from a phylogenetic study of Cricetinae (Lebedev et al., 2018). Ultrametric tree reconstruction was performed based on *cytb* alignment in BEAST v1.84 (Drummond et al., 2012). Measures of molecular diversity, neutrality statistics (Tajima, 1989; Fu, 1997), AMOVA (Excoffier et al., 1992) and mismatch distribution test (Harpending, 1994; Rogers, Harpending, 1992) were calculated by using ARLEQUIN 3.5.1.2 (Excoffier, Lischer, 2010). R2 statistics were estimated in DNASP 5.10 (Librado, Rosas, 2009). Genetic distances (K2p) were calculated in Mega ver.6 (Tamura et al., 2013).

The trees inferred from the mtDNA data clearly demonstrate that all examined haplotypes cluster into three main allopatric groups, the relationships among which appear as a trichotomy (Fig. 1, 2, Suppl. 2 & 3). The distances between these clusters (2.5–2.8%; *cytb*, K2p; Suppl.4) fall within the range corresponding to both intraspecific and interspecific divergence according to Bradley and Baker (2001); however, these values have a low probability of being indicative of distinct species.

Two of these lineages are widespread in the eastern and western parts of the species range. The western (West) lineage is found from the north-western Black Sea shore and Anatolia in the west to the Volga River and Transcaucasia in the east. The eastern (East) lineage occurs in Kazakhstan, Central Asia, Iran, Pakistan, Mongolia and West China. The two lineages are separated by the Lower Volga valley (Fig. 1B). Similar examples of East/West divergence are presented by *Spermophilus pygmaeus* (Ermakov et al., 2006) and *Ellobius talpinus* (Bogdanov et al., 2015). These results highlight the importance of the Volga as a barrier for separation between sibling species or subspecies of steppe and semidesert mammals. The pattern of distribution of the West and East lineages is consistent with the predictions of the craniometric study (Lebedev, 2000), thus supporting the *phaeus* - *migratorius* dichotomy. The presence of two genetic lineages in southern Russia – western Kazakhstan was also demonstrated in a preliminary allozyme study (Lebedev et al, 2000), which identified two putative diagnostic loci out of 24. Allozyme data by Mezzherin (2001), which produced a similar level of divergence between samples from East Ukraine and Turkmenistan, are also consistent with the mtDNA pattern.

The third main lineage is represented by the sample collected in the Qurama Mountains (Uzbekistan). A previous study (Brandler 1999) showed that the karyotype of these hamsters consists of 24 chromosomes in contrast to all other examined *C. migratorius* (2n=22). The craniometric data (Lebedev, 2000) suggests that mountain areas of western Central Asia harbour two endemic morphologically distinct groups, which are distributed in Pamir-Tianshan (*coerulescens* subspecies group) and Gissar-Alai region (undescribed subspecies). Additionally, other mountain ranges (Kopetdag, Dzungaar Alatau, and Tarbagatai) are populated by hamsters belonging to the *migratorius* subspecies group. Unfortunately, the morphological material for the hamsters from Qurama range is lacking, and hence, it is unclear whether Qurama hamsters belong to any of the Central Asian morphological groups or represent another undescribed restricted-range subspecies. To illuminate the pattern of genetic variation in the mountain areas of western Central Asia, additional sampling and multi-locus analysis is required. However, the fact that the isolated position of the Qurama lineage is supported by both examined markers (Suppl. 2 & 3) confirms that this result can hardly be attributed to an artefact (e.g., numt).

Throughout most of the East lineage range, minimal structure is observed, the average among-haplotype distance is relatively low and no geographically distinct groupings are evident. Similar haplotypes can be found at the opposite edges of the range - e.g., “E01” from the left bank of Volga (locality 20) and “E23” from Govi-Altai Province in Mongolia (locality 42) are separated by geographic distances of 3700 km and only by two nucleotide changes (Fig. 1A-C, Suppl. 1). The only exception is a divergent haplotype “E03” from the North-West Frontier province of Pakistan. Conversely, the West group is pronouncedly structured and consists of several allopatric sublineages (West I – West IV; see Figs. 1, 2). Clade West I is widespread across steppe and forest-steppe belts of Eastern Europe, while West II is found only in Ciscaucasia southwards from the Lower Don valley. Clades West III and West IV are found in Anatolia and Armenia, respectively, thus apparently corresponding to morphologically distinct clusters (Lebedev, 2000; Lebedev unpubl.).

The presence of two divergent lineages of grey hamster in western Central Asia suggests that the centre of origin of the species was located in this area. The time of divergence among the three main lineages can be tentatively estimated as 130-160 ky. This range refers to the end of Middle Pleistocene and may correspond to a glacial maximum or to glacial-interglacial transition. One can hypothesize that, at that time, the grey hamsters colonized (or re-colonized) a large part of its recent range via dispersal from an ancestral area in southern Central Asia to Western Asia and South-Eastern Europe. Most of the fossils attributed to *C. migratorius* from Europe and Anatolia refer to Middle and Late Pleistocene (e.g., Storch, 2004; Montuire et al., 1994; Royer et al., 2013); however, several Early Pleistocene findings have been reported from these areas (e.g. Hoek Ostende et al., 2015). Notably, the identification of *C. migratorius* can be problematic because of its similarity with the smaller *Allocricetus* species (Hir, 1993; Cuenca-Bescos, 2003). Thus, our understanding of Pleistocene range dynamics in the grey hamster is far from complete.

The time of separation of the West I - IV lineages is estimated as ca. 80 ky, corresponding to the early cold phase of the Late Pleistocene. The grey hamster is considered a rare component of the Late Pleistocene cold steppe fauna (Markova et al., 2008, 2009); during the glacial phase, its range extended westwards well beyond the present-time limits (Bogicevic et al., 2011; Royer et al., 2013). However, compared to the typical representatives of the pleniglacial faunal complex (*Dicrostonyx, Microtus gregalis*) *C. migratorius* is more xerophilic and less cold tolerant; hence, it was apparently more widespread during warmer interstadial events (Horáček, Ložek, 1988). Accordingly, if we accept that the severe conditions of the Last Glacial Maximum period were unfavourable for this species, then it is reasonable to suppose that during this time, the West I and West II sublineages were associated with separate xeric steppe refugial areas (Ciscaucasia and northern Pontic region).

The reconstructions of demographic history using mismatch distribution analysis (Rogers, Harpending, 1992) produced contrasting results for the two main lineages. The combined sample of the West lineage showed no signature of recent expansion (Suppl. 4), nor did West I lineage separately. Conversely, the demographic analysis of the East lineage indicates a recent demographic expansion ca. 25 ky (Suppl. 4). The latter pattern can be explained by fast dispersal from a single restricted source region in Central Asia in the end of the Late Pleistocene.

In conclusion, it is evident that *C. migratorius* is, in fact, a complex of lineages, some of which represent the initial stages of speciation. The taxonomy of grey hamsters requires careful revision. The past population history of grey hamsters appears complex; thus, further studies are required to provide a detailed scenario of the range dynamics.

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Conflict of interest: The authors declare that they have no competing interests.

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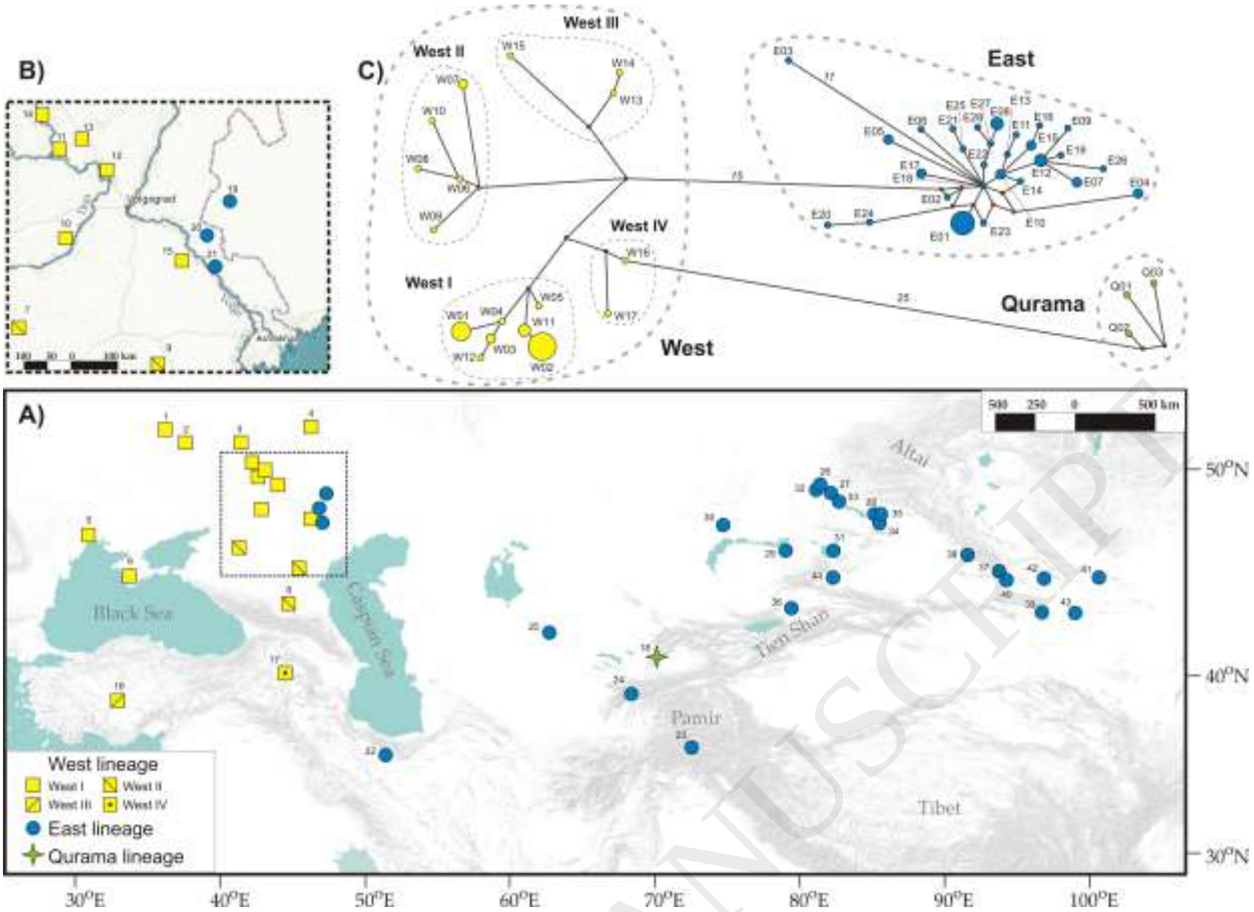

Fig. 1. Geographical variation in mitochondrial DNA of *C. migratorius*. A: distribution of genetic lineages over the range; B: detailed map of the Lower Volga region; C: median-joining network of 253 *cytb* haplotypes; the number of substitutions (>10) is shown above the network branches.

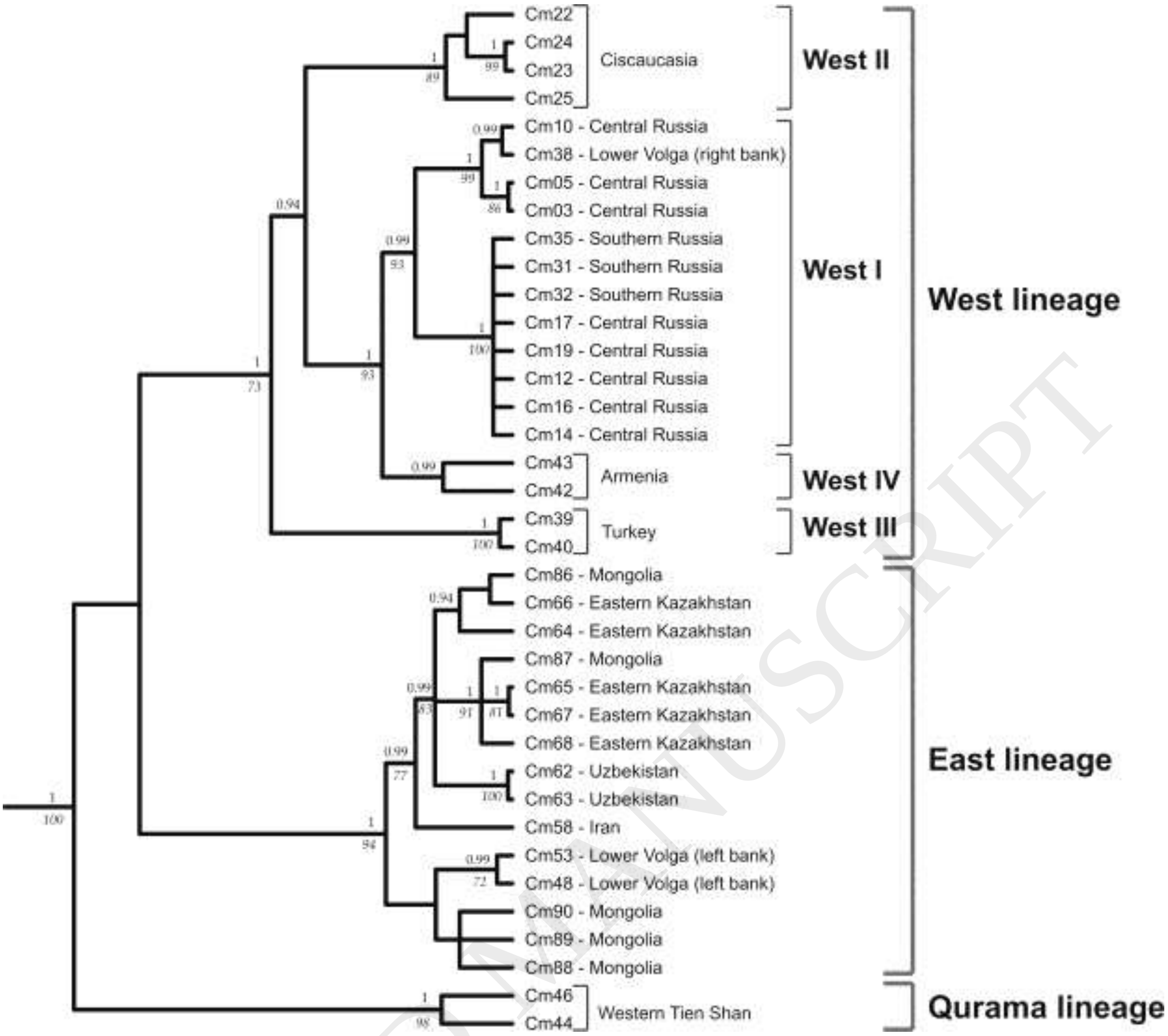

Fig. 2. The phylogenetic tree reconstructed from the concatenated alignment of *cytb* and *coI* in MrBayes. The numbers above and below the branches correspond to Bayesian posterior probabilities (>0.90) and bootstrap values in maximum parsimony analysis (>70%), respectively.