

Intraspecific Variation of Brown Rat *Rattus norvegicus* in Russia by D-Loop mtDNA Data

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Abstract—For the first time in Russia, the intraspecific structure of the brown rat *Rattus norvegicus* was studied using the mitochondrial control region (D-loop) as a molecular marker. The mtDNA sequence was determined in brown rats from eight regions of European and Asian Russia. Three rat clades were identified, including one in European Russia and two in Asian Russia. The synanthropic subspecies *R. n. norvegicus* was found to have two, European and Asian, lineages. The European lineage included haplotypes from central and southern regions of Russia, and the Asian lineage included haplotypes from Eastern Siberia and the Russian Far East. The exoanthropic subspecies *R. n. caraco* from the Russian Far East formed a separate genetic lineage.

Keywords: brown rat, intraspecific structure, mtDNA D-loop, *Rattus norvegicus*, *R. n. norvegicus*, *R. n. caraco*

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The brown rat *Rattus norvegicus* Berkenhout, 1769 is a true synanthropic rodent species and has spread with humans worldwide, including Russia [1, 2]. Brown rats commonly pose a substantial threat to human health by carrying agents of dangerous infections, cause damage to infrastructures (communications, etc.), and are classed with the most dangerous invasive species in Russia [3]. Northeastern plains of China were earlier thought to give origin to the species [4]. However, paleontological finds of ancestral brown rat forms have recently been collected in Southwestern China and dated to 1.2–1.6 million years ago [5], indicating that the species originates from Southern China. This origin is supported by molecular genetic studies of nuclear DNA and mtDNA [6, 7]. In the territory of modern Russia, ancient local areas inhabited by the exoanthropic form *R. n. caraco* formed in the

Far East and Amur region, which border China. The synanthropic brown rat form arose in Western Europe in the 16th century and entered European Russia from there [8, 9]. In spite of the vast species range, the variation is relatively low in the brown rat compared with other true synanthropic species belonging to the superspecies complex *Mus musculus* s.l. Based on the ecological and morphological data, four brown rat subspecies are now recognized in the area of the primary species range: *R. norvegicus norvegicus*, *R. n. caraco*, *R. n. humiliatus*, and *R. n. soccer* [10–12]. Of these, *R. n. norvegicus* and *R. n. caraco* live in Russia [13, 14]. The intraspecific structure of the brown rat has been studied in Central Asia, Western Europe, North America, and Africa [6, 7, 9, 15, 16]. However, the *R. norvegicus* intraspecific structure in Russia has not been studied by molecular genetic methods, and only single specimens collected from European and Asian Russia have been examined [9, 16]. Vigorov's book [14] is noteworthy among special studies of morphological polymorphism, providing a detailed study of the variation in exterior and craniological characters of rats in Eurasia by methods available at that time. According to Vigorov's data, brown rats from Eastern Siberia and the Russian Far East considerably differ in several diagnostic traits from European brown rats. Molecular genetic studies of the brown rat in Russia are essential for understanding the phylogenetic structure of the species in the Palearctic in full. The objective of this work was therefore to study the intraspecific structure of *R. norvegicus* with data on the variation of the mtDNA control region (D-loop).

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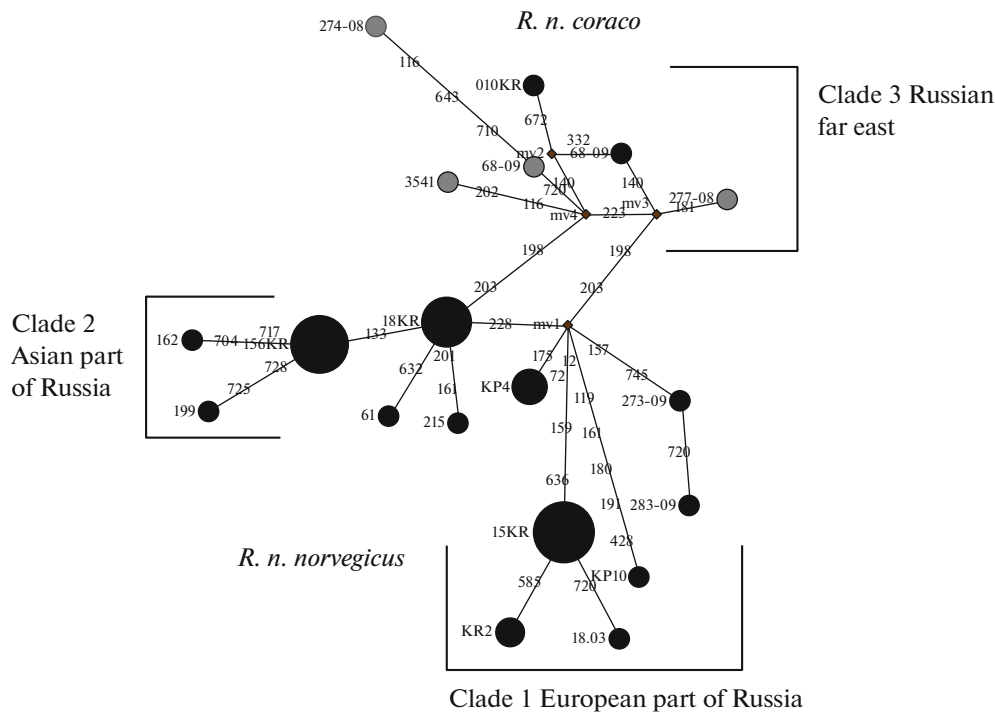


Fig. 1. Phylogenetic analysis of brown rat haplotypes.

The mtDNA control region was used as a mitochondrial molecular marker. In total, 47 brown rats were captured for the molecular genetic study in eight Russian regions: 12 in Moscow, 1 in Moscow Oblast (village Lesnoi, Pushkino region), 2 in Tula Oblast (town Plavsk), 8 in Rostov Oblast (Rostov-on-Don), 12 in Zabaikal'skii Krai (Chita), 8 in Primorsky Krai (5 in Vladivostok, 2 in a village of Rubinovka, and 1 in Komsomolsk-on-Amur), 2 in Khabarovsk Krai (village Lososina, Gavanskii district), and 2 in the Jewish Autonomous Region (village Leninskoe). Of the 12 rats captured for the study in the Russian Far East, four (two from Leninskoe, Jewish Autonomous Region and two from Rubinovka, Primorsky Krai) were identified as *R. n. caraco* by morphological characters [12]. The rats were captured from natural habitats and were distinguished by a smaller size and a bicolored hairy tail.

The study was carried out at the Collective Use Center "Instrumental Methods in Ecology" (Severtsov Institute of Ecology and Evolution). DNA was isolated using a DNeasy Blood & Tissue kit (Qiagen) and a Tissue kit for DNA isolation from animal tissues (Raissol, Sesana). PCR was run on a SimpliAmp thermal cycler (Applied Biosystems). A HotStarTaq Plus Master Mix kit (Qiagen) was used. The reaction mixture (25 μ L) included a polymerase mix (HS-Taq and Pfu), a dNTP mix, a PCR buffer, Mg^{2+} , and other reagents 1–2 ng of template DNA, deionized water, and 0.1–800 nM forward and reverse primers). Amplification included 94°C for 3 min; 25 cycles of

94°C for 30 s, 58°C for 30 s, and 72°C for 1 min 30 s; and final elongation at 72°C for 10 min. Primers specific to the mtDNA control region (D-loop) were used in PCR with total brown rat DNA [6]. The sequence under study was 746 bp after sequence alignment.

Multiple sequence alignment was performed using Bioedit v. 7.0.5.3. [17]. A Median-Joining phylogenetic network was constructed using the Network program [18]. The p-distance between phylogenetic groups was calculated using the Mega X program [19].

In total, 23 brown rat haplotypes were identified in the phylogenetic analysis. The haplotypes were represented by the two subspecies, *R. n. norvegicus* and *R. n. caraco*, in the Median-Joining network (Fig. 1). The subspecies *R. n. norvegicus* was separated into two clades, from European and Asian parts of Russia. The exoanthropic subspecies *R. n. caraco* from the Russian Far East formed a separate clade.

Clade 1 included mostly haplotypes from European Russia: Rostov-on-Don, Moscow, and Tula Oblast (Plavsk) (Fig. 1). Two haplotypes from Khabarovsk Krai (Lososina) were exceptions. Clade 2 from Asian Russia included the haplotypes from Eastern Siberia (Chita), the Russian Far East (Vladivostok), and Moscow. The genetic distance (p-distance) between clades 1 and 2 was calculated to be 0.006397. Clade 3 of the Median-Joining network included the haplotypes of the rats identified as *R. n. caraco* by exterior characters (from Primorsky Krai and the Jewish Autonomous Region) along with the haplotypes of rats identified as *R. n. norvegicus* by the same charac-

ters (from Komsomolsk-on-Amur and Moscow Oblast (Lesnoi)) (Fig. 1). The p-distances between the haplotypes of *R. n. coraco* and those of the European and Asian *R. n. norvegicus* clades were 0.008945 and 0.005962, respectively.

The presence of brown rat haplotypes from Moscow and Moscow Oblast in haplogroups of Asian Russia and vice versa suggests continuous long-distance migrations with humans for the species, as has been noted in many studies [2]. Rats could be brought from the Russian Far East to Central Russia and backward through major transport routes, such as Trans-Siberian and Baikal–Amur railways. This is possible to infer from the haplotype similarity between the rats from Moscow (18KP) and Vladivostok in the Asian clade. Hybridization is possible between exoanthropic and synanthropic brown rat subspecies. For example, hybridization between other related *R. norvegicus* forms has been observed in Northern China [12]. Vigorov [14], who studied *R. n. caraco* in Eastern Siberia and the Russian Far East, has also not excluded that matings between rats of the exoanthropic subspecies *R. n. caraco* and the synanthropic subspecies *R. n. norvegicus* can occur in nature. Putative hybrids were similarly detected in our study, belonging to *R. n. coraco* clade 3 (Fig. 1, 010KP, 328-09).

Two, European and Asian, genetic lineages of brown rat haplotypes with several clusters possible in each lineage have similarly been observed in other studies based on nuclear DNA and mtDNA analyses [6, 7, 9]. The origin of brown rats from Russia is still poorly understood. Scarce data are only available and point to a common origin of brown rats from Central Russia (Moscow and Tver') and rats from Scandinavia [9].

To conclude, although the territory of Russia has relatively recently been colonized by brown rats, their intraspecific structure is nonhomogeneous and is characterized by a subdivision into two subspecies, *R. n. norvegicus* and *R. n. caraco*. The synanthropic subspecies *R. n. norvegicus* inhabits most of Russia and has European and Asian haplogroups. The exoanthropic subspecies *R. n. caraco* inhabits mostly the Russian Far East and hybridizes with the nominative synanthropic form. Adaptation of particular brown rat forms to different environmental conditions and a transition to a synanthropic lifestyle might facilitate differentiation to the subspecies level.

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ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Captured animals were kept and sacrificed in accordance with the guidelines of the European Convention for

the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes. Animal studies were approved by the Ethics Committee of the Severtsov Institute of Ecology and Evolution, protocol no. 39 dated July 27, 2020.

CONFLICT OF INTEREST

The authors of this work declare that they have no conflicts of interest.

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