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# Phylogeography of the gray red-backed vole *Craseomys rufocanus* (Rodentia: Cricetidae) across the distribution range inferred from nonrecombining molecular markers

**Natalia I. Abramson, Tatyana V. Petrova, Nikolai E. Dokuchaev, Ekaterina V. Obolenskaya & Andrey A. Lissovsky***

ABSTRACT. A range-wide phylogeographic study of the gray red-backed vole (*Craseomys rufocanus*), was carried out using cytochrome *b* and cytochrome oxidase subunit I of mtDNA and a fragment of the Y-chromosome SRY gene. The results, based on 271 specimens from 81 localities over the majority of the species distribution, demonstrate that there are four main mitochondrial lineages with partly sympatric distribution. Spatial distribution of SRY haplotypes generally coincides with the mitochondrial distribution, being different mainly in details. However one of the mitochondrial lineages is not reflected in Y-chromosome based data. Most of the genetic diversity of *C. rufocanus* is allocated within the south-eastern part of the range, where representatives of all discovered mtDNA lineages and most of the SRY haplogroups have been found. The observed genetic structure could be explained by repeated glacial fragmentation of the species range together with following dispersion from several refugia.

KEY WORDS: Gray Red-backed Vole, *Craseomys rufocanus*, phylogeography, cytochrome *b*, SRY gene.

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# Филогеография красно-серой полёвки *Craseomys rufocanus* (Rodentia: Cricetidae) в пределах области распространения по данным анализа нерекомбинантных молекулярных маркеров

**Н.И. Абрамсон, Т.В. Петрова, Н.Е. Докучаев, Е.В. Оболенская, А.А. Лисовский**

РЕЗЮМЕ. Исследование филогеографии красно-серой полёвки (*Craseomys rufocanus*), выполнено на всем ареале с использованием митохондриальных генов цитохрома *b* и первой субъединицы цитохром оксидазы С, а также фрагмента гена SRY, расположенного в Y-хромосоме. Результаты, основанные на анализе 271 экземпляра из 81 точки с большей части ареала вида, показывают наличие четырёх основных митохондриальных линий с частично симпатричным распространением. Пространственное распределение гаплотипов SRY в целом соответствует митохондриальному распределению, отличаясь лишь в деталях. Однако, одна из митохондриальных линий не прослеживается в данных SRY. Большая часть генетического разнообразия *C. rufocanus* сосредоточена в юго-восточной части ареала, где встречены представители всех митохондриальных линий и большинство SRY гаплотипов. Наблюдаемая генетическая структура может быть объяснена неоднократной фрагментацией ареала, вызванной периодическими оледенениями в плейстоцене, и последующими расселениями полёвок из нескольких таёжных рефугиумов.

КЛЮЧЕВЫЕ СЛОВА: красно-серая полёвка, *Craseomys rufocanus*, филогеография, цитохром *b*, ген SRY.


Figure 1. Sampling localities for *Craseomys rufocanus* used in the study. Locality numbers correspond to Appendix 1. The northern and southern limits of *C. rufocanus* distribution are shown with a dotted line according to Shenbrot & Krasnov (2005).

## Introduction

It is well known that Quaternary climate oscillations had a strong impact on the geographical distribution and demographic history of extant species (Bennett, 1997). Especially dramatic changes took place in Earth's biota at the transition between the Pleistocene and Holocene, particularly in the Northern Hemisphere (Markova *et al*., 2008). Past isolation in separate refugia and differing routes of postglacial colonization are often reflected in the geographical patterns of intraspecific genetic variation (Hewitt, 1996). Populations that have been effectively separated for several glacial cycles show divergence through the accumulation of neutral and possibly selected DNA changes (Hewitt, 2004). Paleoecological evidence suggests that most of northern Eurasia and North America was not covered by trees during several glacial periods (West, 2000) as most forest species had contracted into refugial areas. Thus it implies that refugial and colonization history of species that closely associate with boreal forests in Eurasia will largely reflect the Quaternary environmental history of the taiga (Kulik, 1972).

The gray red-backed vole (*Craseomys rufocanus* Sundevall, 1846; the name is according to Abramson & Lissovsky (2012)) is a wide-ranging species closely associated with Eurasian taiga (Gromov & Erbajeva, 1995). It is distributed from Scandinavia to Pacific coast and inhabiting the taiga mountain forests of Siberia, Kamchatka, Sakhalin, northern (Paramushir) and southern (Kunashir, Shikotan, Zeleniy) Kuril Islands, Hokkaido Island, mountain regions of northern Mongolia and China (Shenbrot & Krasnov, 2005). The gray red-backed vole is one of the several species, which constitute the modern core of Eurasian taiga fauna (Kulik, 1972). In spite of quite high abundance of typical mammal taiga species, there are only a few publications on phylogeography of these species (Iwasa *et al*., 2000; Ohdachi *et al*., 2001; Iwasa *et al*., 2002; Fedorov *et al*., 2008; Lee *et al*., 2008). The major part of them describes only eastern part of the range, while vast territory covered with taiga remains poorly studied. The gray red-backed vole is not exception. Up to date the phylogeographical study of this species was performed only within limited part of the species range (Far East of Russia, Sakhalin, Kamchatka and Hokkaido) (Iwasa *et al*., 2000).

Thus, here we for the first time present the phylogeographic investigation of typical Eurasian taiga mammal species over its entire distribution range.

## Material and methods

### Sampling and DNA extraction

We examined 271 specimens of *C. rufocanus* from 81 localities, including 37 cytochrome *b* (*cyt b*) sequences retrieved from GenBank (Appendix 1, Fig. 1). In addition, 97 specimens were sequenced for cyto-

chrome oxidase subunit I (*COI*). To compare phylogeographic patterns of maternal and paternal lines, we analyzed 77 SRY sequences, 62 of them obtained for the current study. Voucher specimens of most individuals sequenced in this study are deposited in collection of Zoological Museum of Moscow State University and the Zoological Institute of the Russian Academy of Sciences in Saint Petersburg. The voucher specimens from the territory of Eurasian North-East are deposited in the Institute of Biological Problems of the North Far Eastern Branch of the Russian Academy of Sciences in Magadan.

Sequences were deposited in GenBank under the accession numbers: JF693294 – JF693314, JF903067 – JF903285 (*COI*), JF713462 – JF713681 (*cyt b*) and JF693315, JF693316 (SRY) (Appendix 1).

Total genomic DNA was extracted from muscles stored in 96% ethanol using salt extraction protocol (Miller *et al*., 1988).

## PCR amplification and sequencing

A segment (960 bp) of the *cyt b* gene was amplified by polymerase chain reaction (PCR) and automatically sequenced using primers described elsewhere (Abramson *et al*., 2009). The partial (657 bp) *COI* was amplified using VF1_t1/ VR1_t1 developed in the Canadian Centre for DNA Barcoding (Ivanova *et al*., 2007) or slightly modified (VUTF 5' TGTAAAACGACGGCCAGTTCTCAACCAAYCAYAARGAYATYGG 3' and VUTR 5' CAGGAAACAGCTATGACTARACTTCTGGRTGKCCRAARAAYCA 3'). The fragment of SRY gene was amplified with the use of primers SRY286 – HMG777 (Sinclair *et al*., 1990; Suzuki *et al*., 1997). In some cases in order to increase the yield of the PCR product we performed nested PCR with external primers SRY286 – HMG777 and internal primers R-SRY306 – U-HMG597 (Sinclair *et al*., 1990; Suzuki *et al*., 1997). The final SRY fragment was approximately 350 bp. All PCR reactions included negative controls to check for contamination. PCR products were visualized on 1.5% agarose gel and purified on the columns of an Omnix Gel Extraction Kit. Both strands were directly sequenced using the ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction Kit on an ABI PRISM 3130 (Applied Biosystems Inc.). Sequences were aligned with Clustal W algorithm (Thompson *et al*., 1994) using the program Bioedit 7.0 (Hall, 1999) and manually edited.

## Phylogenetic analyses

Tree reconstructions were performed separately for the *cyt b* alignment (271 *C. rufocanus* specimens and 3 specimens as an outgroup) and for the concatenated *cyt b* and *COI* dataset (97 and 3 specimens accordingly).

Maximum parsimony tree and bootstrap support estimation based on 500 replicates, with a constraint of a maximum 1000000 rearrangements per replicate, were carried out in PAUP*, beta-test version 4.0b10 (Swofford, 1998). Unweighted maximum parsimony analyses were conducted using a heuristic search algorithm with simple stepwise sequence addition and nearest neighbor interchange branch swapping.

Maximum likelihood analyses, including selection of the optimal model of molecular evolution, reconstruction of the ML tree and bootstrapping, were performed using Treefinder (Jobb, 2008). The AIC criterion was used to choose the best model of molecular evolution in Propose Model dialog. Independent models for each gene were used while analyzing the combined matrix. Bootstrap analysis was conducted using 500 replicates. For the *cyt b* data, the best fitting model was Tamura-Nei with some proportion of invariable sites and a gamma distribution of rates across sites (Tamura & Nei, 1993). For *COI*, the model chosen was Hasegawa *et al*. (1985) with a gamma-distributed rates across sites.

Bayesian analysis was performed in MrBayes 3.1.2 (Ronquist & Huelsenbeck, 2003) with 5000000 generations; 5 independent chains with a heating parameter of 0.07, 1000000 burn in, sample frequency of 1 000 and the GTR + I + Γ model.

To root the *cyt b* tree the following sequences were used: *C. rex* (FJ792793), *Myodes glareolus* (AJ639662), *M. rutilus* (AB072224). The concatenated tree was rooted with *C. rex* (*cyt b* FJ792793, *COI* JF693314), *M. glareolus* (*cyt b* EU035703, *COI* JF903067) and *M. rutilus* (*cyt b* JF713681, *COI* JF903285).

All phylogeographic analyses were performed with *cyt b* data only. A maximum parsimony haplotype network was constructed with Network 4.5 (Bandelt *et al*., 1999). Haplotype (h) and nucleotide (p) diversities and their standard deviations within samples were calculated using the program DnaSP version 5.0 (Librado & Rozas, 2009). To test for evidence of recent population growth from a low-diversity founder population, two methods from the program Arlequin ver. 3.1 (Excoffier *et al*., 2006) were used. First, Fu's *Fs* test (Fu, 1997) was performed to test for an excess of rare alleles, which would be indicative of a recent expansion. Second, pairwise mismatch distributions among individuals were plotted and tested for goodness-of-fit to a model of sudden expansion using parametric bootstrapping (1000 replicates).

## Molecular dating

Considering the tendency towards observing decreased mutation rates backwards in time (Ho *et al*., 2005; Peterson & Masel, 2009), we have taken the most recent and most ancient events and calculated corresponding times using the Calibrate Tree algorithm, Local Rates method of Treefinder (Jobb, 2008). We used two calibration points in starting hypothesis for Treefinder. 1) Taking into account the fact that European part of the range began to recover taiga vegetation after the last glacier retreat during Boreal phase of Holocene (8000–9000 YA) (Markova *et al*., 2008; Velichko, 2009) and considering the close connection of gray red-backed vole with taiga, we roughly took the

Table 1. Sequence polymorphism of the SRY gene in *Craseomys rufocanus*.

| | GenBank accession | 50bp | | 125 bp | | | | | | | | 180 bp |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Type I | AB031858 | T* | … | $TG(TC)_2$ | $TG(TC)_2$ | $TG(TC)_2$ | ------ | $TG(TC)_3$ | $TG(TC)_4$ | $TG(TC)_2$ | $TG(TC)_2$ | CC |
| Type II | AB031859 | T | … | $TG(TC)_2$ | $TG(TC)_2$ | ------ | ------ | $TG(TC)_3$ | $TG(TC)_4$ | $TG(TC)_2$ | $TG(TC)_2$ | CC |
| Type III | AB031860 | G | … | $TG(TC)_2$ | $TG(TC)_2$ | $TG(TC)_2$ | ------ | ------ | ------ | $TG(TC)_2$ | $TG(TC)_2$ | CC |
| Type IV | JF693315 | G | … | $TG(TC)_2$ | $TG(TC)_2$ | $TG(TC)_2$ | $TG(TC)_2$ | ------ | ------ | $TG(TC)_2$ | $TG(TC)_2$ | CC |
| Type V | AB031861 | G | … | $TG(TC)_2$ | $TG(TC)_2$ | $TG(TC)_2$ | ------ | ------ | ------ | ------ | $TG(TC)_2$ | CC |
| Type VI | AB031862 | G | … | $TG(TC)_2$ | $TG(TC)_2$ | ------ | ------ | ------ | ------ | ------ | $TG(TC)_2$ | CC |
| Type VII | JF693316 | G | … | $TG(TC)_2$ | ------ | ------ | ------ | ------ | ------ | ------ | $TG(TC)_2$ | CC |

start point of European haplotypes differentiation equal to 8000 YA. Operationally, this date was fixed to the split between two European specimens separated with distance equal to the average distance of European haplotypes from the ubiquitous H1 haplotype. 2) The split of *M. rutilus* / *M. glareolus* at 2.5 MYA, taken from previous publication (Lebedev *et al*., 2007). The confidence intervals for divergence times were evaluated using 500 replicates. The τ values were converted to an absolute time scale using an assumption of constant generation lifetime during investigated period: time in years = τ / double mutation rate per sequence per year. This mutation rate was calculated using τ of European haplotypes and "known" expansion time of European haplotypes equal to 8000 YA as described above.

## Results

### SRY variation

As already described in Iwasa *et al*. (2000), this region is variable within *C. rufocanus*, largely due to the presence of several repeated microsatellite-like elements: TG(TC)n. In addition to 5 types of SRY recognized previously, we found two more types distributed on the continent, hereafter types IV and VII (Table 1). Type IV is found only in the Olekma region of Yakutia, the basin of the Lena River and type VII is restricted only to north-eastern China. Geographical distribution of SRY types is shown on Fig. 2.

### Mitochondrial sequence data

The final alignment of the mitochondrial genes included 817 bp for *cyt b* and 651 bp for *COI*. There were 173 different *cyt b* haplotypes, defined by 184 variable sites among 271 Gray Red-backed Voles; 110 sites were informative for the maximum parsimony median-joining haplotype network. Base composition of *cyt b* was C — 29.8%, T — 27.6%, A — 29.0%, G — 13.6%. There were 60 different *COI* haplotypes defined by 93 variable sites among 97 Gray Red-backed Voles, 58 nucleotide sites were parsimony informative. The base composition was as follows: C — 27.3%, T — 27.8%, A — 27.3%, G — 17.6%.

Most haplotypes were restricted to a single population or particular area. Only two haplotypes, H1 and H44, were shared among several populations found within remote geographical regions (Appendix 1). H1 haplotype was found in 25 animals trapped from Finland and Kola Peninsula at the west to Kamchatka Peninsula at the east and Altai Mts., Hentiyn Nuruu and Transbaikalia at the south. H44 was found in samples from Transbaikalia and Kamchatka Peninsula.

The median-joining network based on *cyt b* revealed four main haplogroups (Fig. 3). We designated these haplogroups as A, B, C and D. The geographical distribution of members of these haplogroups is not strictly allopatric (Fig. 4).

Haplogroup A unites 21 haplotypes from Hokkaido and adjacent islands including Kunashir Island. The haplogroup B is formed by 28 haplotypes, which are divided into two principal subgroups: B1 with 21 haplotypes from Sakhalin Island and B2 with seven haplotypes from Central Siberia. Voles with haplotypes from B2 subgroup were found together with those bearing C1 haplotypes in one locality on the right bank in the middle part of Yenisey River.

The haplogroup C includes 109 haplotypes, which form two principal nodes (C1 and C2), yielding a clear star-like structure with one central haplotype H1. H1 haplotype range is described above. The whole subgroup C1 range coincides with it: extending to Chukchee Peninsula and the south of Primorye. Subgroup C2 is distributed from Olekma basin and eastern Transbaikalia to lower Amur and upper Kolyma basins. The distribution of the most common haplotype H2 is limited to the territory of the continental coast of the Sea of Okhotsk. These subgroups are partly sympatric. A contact zone between C1 and C2 take place in eastern Transbaikalia and Olekma basin. However, one individual belonging to this subgroup was also found in Primorye territory. Other voles from Primorye territory (excluding those composing the own haplogroup D),


Figure 2. Spatial distribution of SRY haplotypes in *Craseomys rufocanus*.

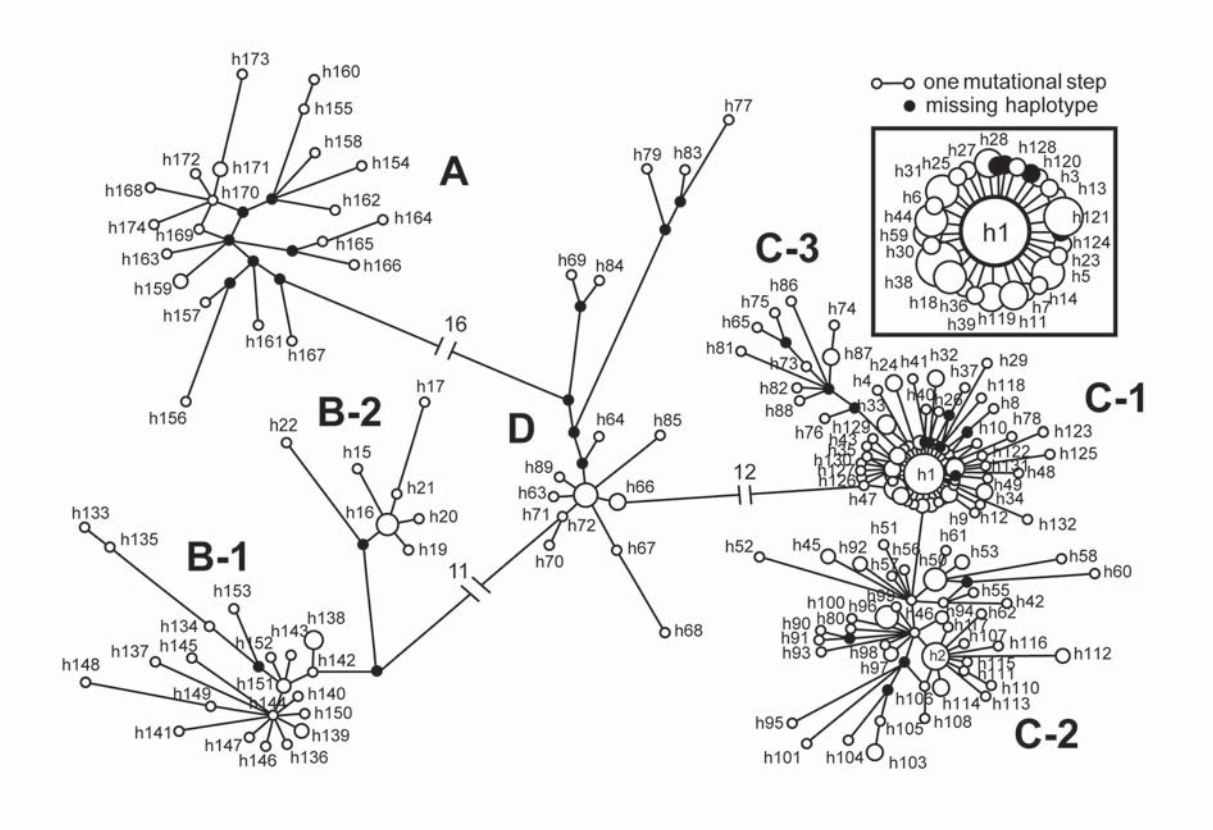



Figure 3. Maximum parsimony network of *Craseomys rufocanus cyt b* haplotypes. Haplotype labels refer to Appendix 1. The number of mutational steps separating the haplotypes are proportional to the branches length or indicated on the interrupted branch. The size of circles is proportional to the haplotype frequency.


Figure 4. Spatial distribution of *cyt b* haplogroups in *Craseomys rufocanus*.

form a small separate branch within the haplogroup C, designated on Figs as C3.

The haplogroup D is formed by 15 haplotypes of voles inhabiting the Sungari and Ussuri river basins, north-eastern China and the coast of the Sea of Japan. The geographical distribution of haplogroup D is partly sympatric with the C haplogroup.

Trees built with different methods (maximum parsimony, maximum likelihood and Bayesian phylogenetic analyses), both from individual genes and the concatenated matrix, exhibit a similar topology but differ in bootstrap support for individual branches. The structure described above for network was also visible in the phylogenetic trees (Figs 5 and 6). A and B haplogroups have high bootstrap support on both datasets. The D haplogroup was poorly supported on the tree based on *cyt b*; however, in the tree based on two genes support increases considerably. There was also a clear tendency towards an association of B and D haplogroups. The C haplogroup was better supported in *cyt b* tree than in the combined dataset. Only C2 and C3 subgroups received support in some analysis variants. The node uniting A+B+D supported in Bayesian analysis based on combined dataset (Fig. 6).

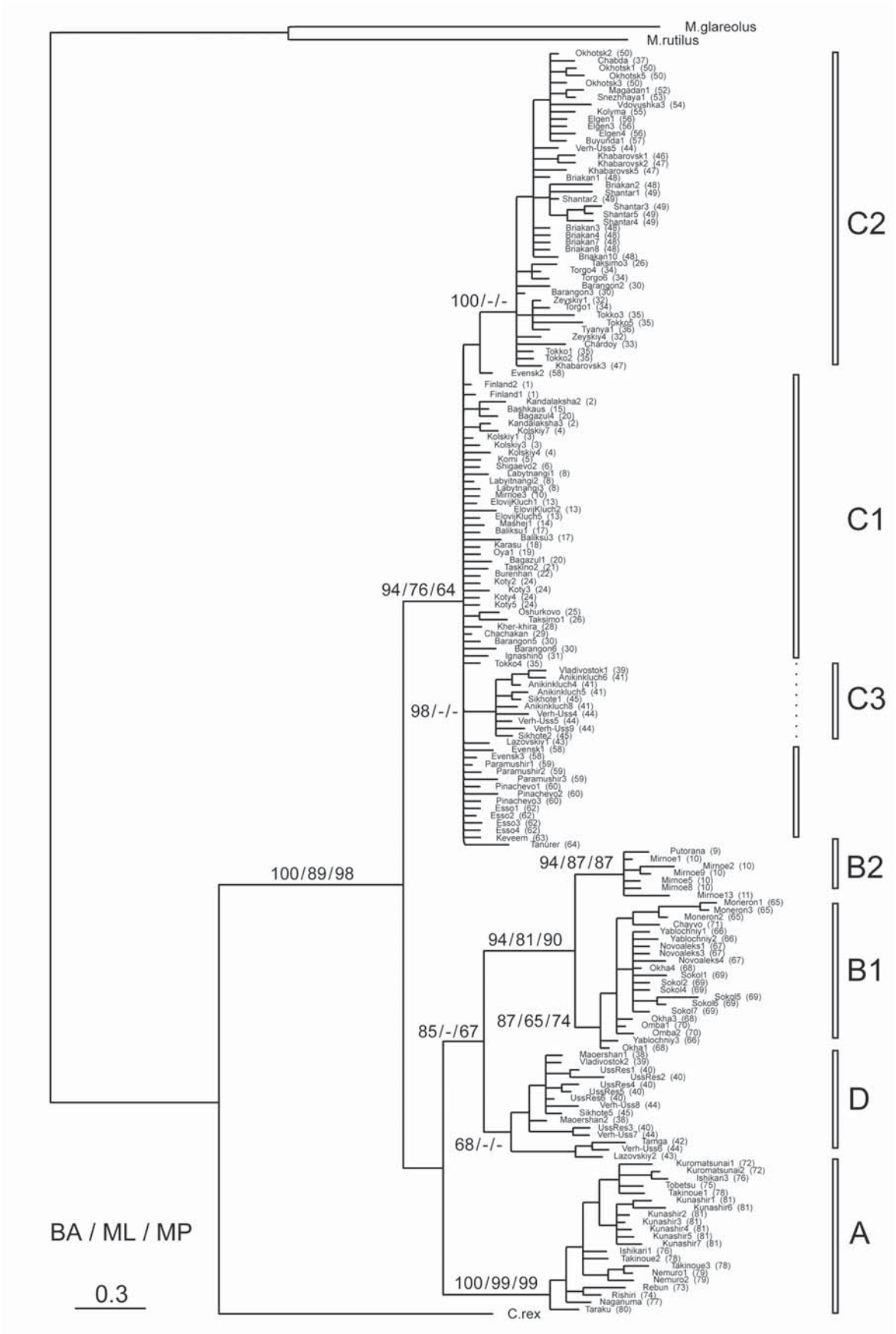
Intrahaplogroup haplotype diversity (Hd) and nucleotide diversity (p) of *cyt b* are shown in Table 2. The level of genetic differentiation (based on ML distances in *cyt b*) between major haplogroups varied from 0.5 to 3.7 % (Table 3).

All main haplogroups and subgroups except B2 demonstrate significant large negative Fu' *Fs* values (Table 4). In cases of the A, C1 and C2 mismatch distributions, SSD analysis failed to reject the model of population expansion (Table 4). Taken together with the result of Fu's *Fs* test (Fu, 1997), we can interpret this as evidence of recent expansion of these 3 groups. SSD analysis of mismatch distribution rejected the model of population expansion for B1 and B2. In the case of B2, this agrees with the results of Fu's *Fs* test, indicating no signs of population expansion or selection. However, the B1 haplogroup exhibited large negative values of Fu's *Fs*, which probably points to some other kind of selectivity then studied model of population expansion. Regarding the D haplogroup, SSD analysis also failed to reject the model of population expansion; however, the small non-significant value for Fu's *Fs* statistic and the wide confident intervals of τ indicate that the probability of expansion scenario for this haplogroup should be studied additionally.

## Time estimation

Relative expansion time estimates (τ) for haplogroups and some populations are given in the Table 4. The τ values given in the Table 4 for the timing of

Figure 5. Maximum likelihood tree for *Craseomys rufocanus* constructed using *cyt b* haplotypes. Numbers on branches indicate Bayesian posterior probabilities and bootstrap support for different kinds of analyses in the following order: BA / ML / MP. Values lesser than 50 are not shown. Specimen codes and location numbers in brackets refer to Appendix 1 and Fig. 1. Mitochondrial haplogroups are highlighted.


M.glareolus
M.rutilus
C2
C1
C3
B2
B1
D
A
100/-/-
94/76/64
98/-/-
100/89/98
94/87/87
94/81/90
87/65/74
85/-/67
68/-/-
100/99/99
BA / ML / MP
0.3
C.rex

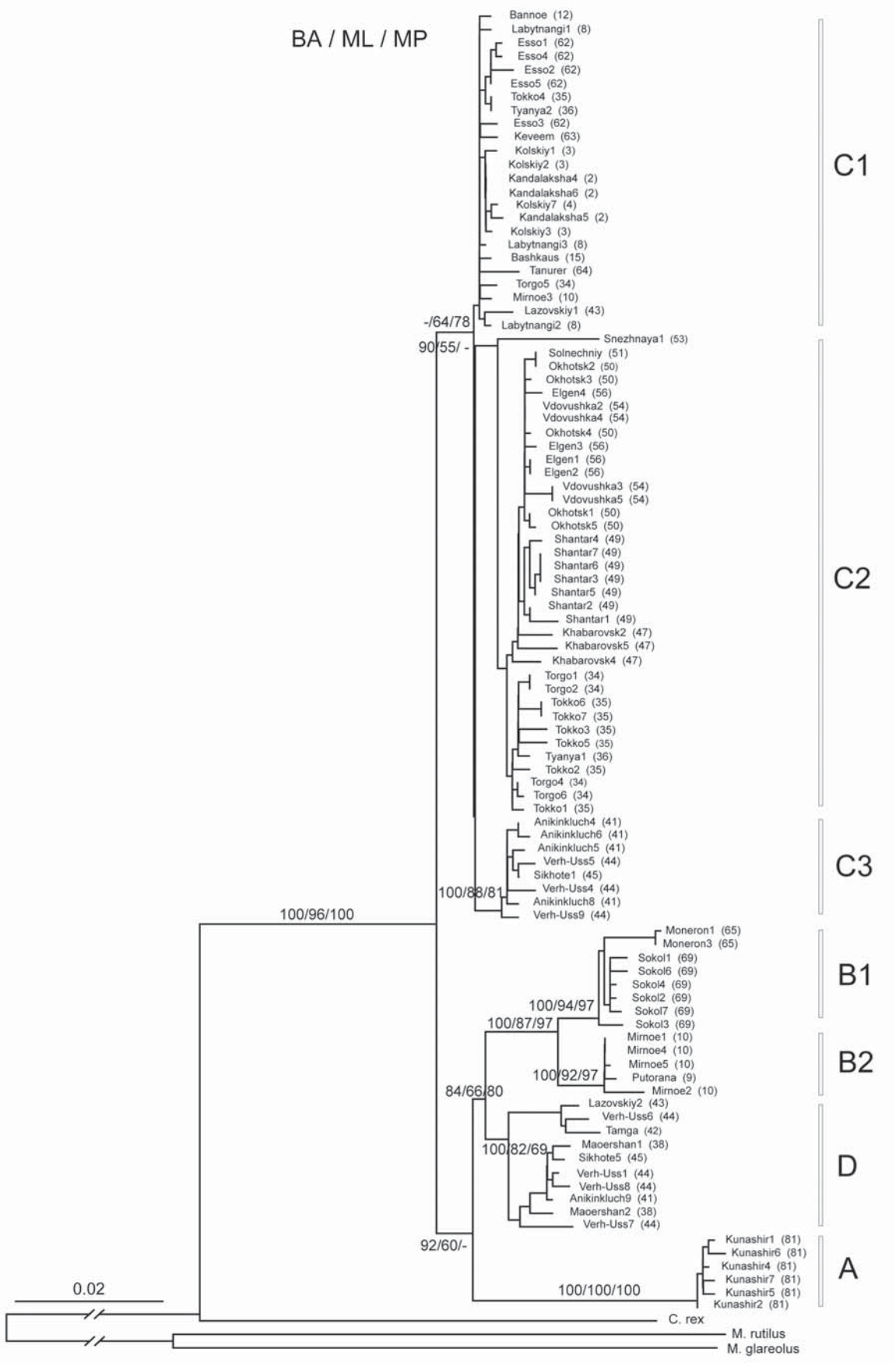

BA / ML / MP
Bannoe (12)
Labytnangi1 (8)
Esso1 (62)
Esso4 (62)
Esso2 (62)
Esso5 (62)
Tokko4 (35)
Tyanya2 (36)
Esso3 (62)
Keveem (63)
Kolskiy1 (3)
Kolskiy2 (3)
Kandalaksha4 (2)
Kandalaksha6 (2)
Kolskiy7 (4)
Kandalaksha5 (2)
Kolskiy3 (3)
Labytnangi3 (8)
Bashkaus (15)
Tanurer (64)
Torgo5 (34)
Mirnoe3 (10)
Lazovskiy1 (43)
Labytnangi2 (8)
-/64/78
90/55/ -
Snezhnaya1 (53)
Solnechniy (51)
Okhotsk2 (50)
Okhotsk3 (50)
Elgen4 (56)
Vdovushka2 (54)
Vdovushka4 (54)
Okhotsk4 (50)
Elgen3 (56)
Elgen1 (56)
Elgen2 (56)
Vdovushka3 (54)
Vdovushka5 (54)
Okhotsk1 (50)
Okhotsk5 (50)
Shantar4 (49)
Shantar7 (49)
Shantar6 (49)
Shantar3 (49)
Shantar5 (49)
Shantar2 (49)
Shantar1 (49)
Khabarovsk2 (47)
Khabarovsk5 (47)
Khabarovsk4 (47)
Torgo1 (34)
Torgo2 (34)
Tokko6 (35)
Tokko7 (35)
Tokko3 (35)
Tokko5 (35)
Tyanya1 (36)
Tokko2 (35)
Torgo4 (34)
Torgo6 (34)
Tokko1 (35)
Anikinkluch4 (41)
Anikinkluch6 (41)
Anikinkluch5 (41)
Verh-Uss5 (44)
Sikhote1 (45)
100/88/81
Verh-Uss4 (44)
Anikinkluch8 (41)
Verh-Uss9 (44)
100/96/100
Moneron1 (65)
Moneron3 (65)
Sokol1 (69)
Sokol6 (69)
Sokol4 (69)
Sokol2 (69)
100/94/97
Sokol7 (69)
100/87/97
Sokol3 (69)
Mirnoe1 (10)
Mirnoe4 (10)
Mirnoe5 (10)
100/92/97
Putorana (9)
84/66/80
Mirnoe2 (10)
Lazovskiy2 (43)
Verh-Uss6 (44)
Tamga (42)
100/82/69
Maoershan1 (38)
Sikhote5 (45)
Verh-Uss1 (44)
Verh-Uss8 (44)
Anikinkluch9 (41)
Maoershan2 (38)
Verh-Uss7 (44)
92/60/-
Kunashir1 (81)
Kunashir6 (81)
Kunashir4 (81)
Kunashir7 (81)
Kunashir5 (81)
Kunashir2 (81)
100/100/100
0.02
C. rex
M. rutilus
M. glareolus
C1
C2
C3
B1
B2
D
A

Table 2. Parameters of genetic diversity within the main haplogroups of *Craseomys rufocanus* based on *cyt b*. Pi — nucleotide diversity, Hd — haplotype diversity, SD — standard deviation.

| Haplogroup / Population | N samples | N haplotypes | Pi ± SD (%) | Hd ± SD |
|---|---|---|---|---|
| A | 23 | 21 | 0.752 ± 0.059 | 0.992 ± 0.015 |
| B | 37 | 28 | 0.883 ± 0.077 | 0.973 ± 0.016 |
| B1 | 26 | 21 | 0.548 ± 0.077 | 0.975 ± 0.021 |
| B2 | 11 | 7 | 0.307 ± 0.100 | 0.818 ± 0.119 |
| C | 189 | 109 | 0.717 ± 0.029 | 0.976 ± 0.006 |
| C1 | 106 | 55 | 0.317 ± 0.025 | 0.938 ± 0.018 |
| C2 | 71 | 44 | 0.533 ± 0.038 | 0.971 ± 0.010 |
| C3 | 12 | 10 | 0.412 ± 0.060 | 0.955 ± 0.057 |
| D | 22 | 15 | 0.704 ± 0.152 | 0.922 ± 0.046 |

Table 3. Genetic differences within and between the major haplogroups based on *cyt b*. Diagonal elements — average number of pairwise differences within haplogroups. Elements below diagonal — maximum likelihood distances between haplogroups.

| | A | B1 | B2 | C1 | C2 | C3 | D |
|---|---|---|---|---|---|---|---|
| A | **0.008** | | | | | | |
| B1 | 0.033 | **0.006** | | | | | |
| B2 | 0.037 | 0.010 | **0.003** | | | | |
| C1 | 0.026 | 0.029 | 0.031 | **0.003** | | | |
| C2 | 0.032 | 0.034 | 0.037 | 0.006 | **0.005** | | |
| C3 | 0.031 | 0.033 | 0.031 | 0.005 | 0.010 | **0.004** | |
| D | 0.024 | 0.017 | 0.019 | 0.016 | 0.022 | 0.020 | **0.007** |

expansion in different parts of the C1 distribution increase in direction from west to east (i.e., along Europe – Altay-Sayan – Kamchatka). The converted absolute times of expansion and splitting events are given in the Table 5.

## Discussion

Our results indicate a complicated genetic structure in *C. rufocanus*. Based on *cyt b* data, we can distinguish four main genetic groups with some subdivision (Figs 3, 5, 6). The results from the SRY gene provided poor data for phylogenetic analysis, due to peculiar pattern of its variation (Table 1); however, they can be successfully used as a marker for Y-chromosome based geographical variation (Fig. 2). The main genetic diversity is allocated within the south-eastern part of the range, while the major part of the range shows genetic homogeneity (Figs 2 and 4).

In general, the spatial distributions of *cyt b* and SRY haplotypes coincide with one another (Figs 2 and 4). Minor differences in the distribution of C1 and C2 *cyt b* haplotypes and Type III and Type V SRY haplotypes could be explained by local differences of dispersal in male and female voles. The local Type IV SRY haplotype (slightly different from Type III), is probably a result of locally inconsistent substitution rates in two genes. The local Type II haplotype is also only slightly

Table 4. Estimated parameters of population expansion of six haplogroups and three gray red-backed vole populations.

| Haplogroup / population | SSD | SSD *p*-value | Raggedness index | Raggedness *p*-value | Tau (τ) with [confidence interval] | Fu' Fs | Fs *p*-value |
|---|---|---|---|---|---|---|---|
| A | 0.009 | 0.204 | 0.024 | 0.25 | 6.459 [4.621–7.963] | –14.70 | 0.00 |
| B1 | 0.072 | 0.002 | 0.015 | 0.99 | 2.320 [1.377–3.350] | –14.33 | 0.00 |
| B2 | 0.265 | 0.007 | 0.038 | 1.00 | 0.424 [0.000–1.205] | –2.18 | 0.07 |
| C1 | 0.001 | 0.60 | 0.022 | 0.61 | 1.719 [0.750–4.324] | –26.20 | 0.00 |
| C2 | 0.0003 | 0.92 | 0.017 | 0.60 | 4.399 [2.526–5.364] | –25.77 | 0.00 |
| D | 0.015 | 0.962 | 0.021 | 0.93 | 0.471 [0.330–17.035] | –4.72 | 0.02 |
| Europe | 0.003 | 0.67 | 0.048 | 0.59 | 1.182 [0.487–3.471] | –27.06 | 0.00 |
| Altay-Sayan | 0.011 | 0.29 | 0.057 | 0.41 | 1.660 [0.636–3.891] | –24.00 | 0.00 |
| Kamchatka | 0.002 | 0.74 | 0.035 | 0.59 | 2.179 [1.083–3.993] | –26.39 | 0.00 |

Figure 6. Maximum likelihood tree for *Craseomys rufocanus* constructed using concatenated *cyt b* and *COI* dataset. Other information is the same with Fig. 5.

Table 5. Time estimates for some splitting and expansion events based on *cyt b*.

| Splitting and expansion events | Mean (ya) | Confidence interval (ya) |
|---|---|---|
| *C. rex* – *C. rufocanus* splitting | 805900 | 473900–1092000 |
| Basal splitting: C – (A+B+D) | 332000 | 228000–470000 |
| A segregation: A – (B+D) | 274200 | 180300–396100 |
| B – D splitting | 215900 | 138700–301200 |
| B1 – B2 splitting | 121000 | 69900–179700 |
| A expansion | 46447 | 33230–57263 |
| C2 expansion | 31634 | 18164–38573 |
| C1 expansion | 12362 | 5393–31094 |

different from neighboring Type I. The major difference between *cyt b* and SRY distributions is a total absence of any own SRY haplotypes in voles bearing *cyt b* B haplotypes (Figs 2 and 4). Thus we can treat B haplogroup as a mitochondrial relict.

As far as *cyt b* haplogroups display discrete phylogenetic structure (Fig. 6), we can suppose their origin is the result of consecutive fragmentation in the species range together with the long-term isolation of separated populations in several refuges. It is very possible that fragmentation and isolation were related to periodical glaciations during Pleistocene, as it was stated in the Introduction. Based on this evidence, the modern phylogeographic structure could be explained by repeated waves of migration from glacial refuges during interglacial periods. Currently, there is no allopatric distribution of any haplogroups in the continental part of the range. The voles with C1 haplotypes, characterized by the most recent expansion (Table 5), occur together with voles bearing all spatially neighboring haplotypes: B2, C2, D. The existence of C1, C2 and C3 haplotypes in Primorye together with its own D, reflects probably three consecutive waves of migration of C bearing voles in this region.

The hypothesis about glacial influence on gray red-backed vole allows us to suppose the probable historical scenario of the formation of the modern genetic structure of this species. If our initial suggestions are correct, the earliest traceable split (Table 5) between *C. rufocanus* populations (C / A+B+D haplogroups) took place roughly at the boundary between Early and Middle Pleistocene (Volkova & Babushkin, 2000). Logically, this event was more probably associated with cold epoch, when disjunction of taiga zone was possible. The information about geographic position of refuges of that time was erased by later colonization events.

The estimated separation of insular haplogroup A from B+D (Table 5) probably occurred within the beginning of Late Middle Pleistocene (Volkova & Babushkin, 2000), while during warm period (e.g. synchronous to Siberian Tobol period) large islands of the southern Far East were separated from the mainland by channels.

The separation of B and D haplogroups (Table 5) was most likely induced by next taiga fragmentation and corresponds according to our calculations to Siberian Samarovo glacial period (Volkova & Babushkin, 2000). The splitting of B1 and B2 subgroups (Table 5) falls into the first warm part of the Late Pleistocene (Kazantsevo period) (Volkova & Babushkin, 2000; Velichko, 2009). It seems realistic because during this time insular B1 could be separated by channel from the mainland. We can suppose that penetration of B1 haplotypes into Sakhalin vole population took place via hybridization and not via colonization of the territory free of voles. Such scenario explains the existence of SRY haplotypes shared between Sakhalin and Hokkaido islands.

The time of disappearance of the SRY haplotypes in B2 voles, as well as the reason of extinction of any B haplotypes in the eastern part of the range could not be resolved using our data. Finally, our data allow us to infer that the first Late Pleistocene cold period (Volkova & Babushkin, 2000; Velichko, 2009) affected Gray Red-backed Voles at the south of the Far East region much more strongly than the second. Two populations: A and C2, experienced the population expansion (Table 5) during the next warm Kargino time period (Volkova & Babushkin, 2000; Velichko, 2009) which means the population bottleneck during first Late Pleistocene cold period. We did not find any signs of population bottleneck during the last cold period in this region.

In contrast to the southern part of the Far East, the northern part as well as the major part of Siberia (except for the range of B2 haplogroup) retained only traces of the Holocene expansion of the C1 haplogroup. The putative origin of the C1 expansion is of special interest. The $\tau$ values (Table 4) decreases from east to west, leading us to conclude that postglacial colonization also proceeded in this direction. The largest $\tau$ value corresponds to Kamchatka Peninsula, indicating that wave of dispersal was the oldest there. It is known, that the center of Kamchatka Peninsula remained a forest refugium throughout the Pleistocene (Skiba, 1975), so it is possible to suppose that *C. rufocanus* survived there during the last glaciation. Later, in Holocene, Kamchatka could be the source of wide colonization.

It is of special interest to test our hypothetic scenario with palaeontological data. However fossil data on *C. rufocanus* are scarce. The first fossils of clearly *rufocanus*-like voles are known only from early Late Middle Pleistocene of the West-Siberian lowland (Zazhigin, 1980; Borodin, 1988). This date seems to support our scenario, if we suggest it was the first time of

widescale expansion of this species. It is possible, as it was proposed above, that a range expansion before Late Middle Pleistocene led to colonization of the major part of the modern range, with subsequent separation of C haplogroup. During the Late Middle Pleistocene the fossils of *rufocanus*-like voles are known not only from Siberia and Baikal region, but also from Japanese Isles (Kowalski & Hasegawa, 1976; Galkina, 1977; Galkina & Foronova, 1980; Agadjanian & Serdyuk, 2005). The fossil records from Late Pleistocene are known already from the vast territory approaching to the area of the modern range (Agadjanian & Serdyuk, 2005; Markova *et al*., 2008). The most continuous study of fossils from western Altai (Agadjanian & Serdyuk, 2005) shows that remains of *C. rufocanus* are scarce in the beds corresponding to Late Middle and Late Pleistocene. At the same time, the findings of *C. rufocanus* are discontinuous through time. This phenomenon could be explained by periodical contraction and expansion of the species range. Thus fossil data contain no contradictions with the suggested scenario for formation of the modern genetic variation.

Since our results contain information from both mitochondrial and nuclear genes, they could be used in the future taxonomic revision of the species. There was only one complete taxonomical revision of *C. rufocanus* from the whole range (Ognev, 1964). The later study completely copied the previous work in part of taxonomy of this species (Gromov & Erbajeva, 1995). The study recognized 4 subspecies based mainly on the data on general body size and coat coloration: European *C. r. rufocanus*, *C. r. irkutensis* from Siberia and southern Far East mainland, *C. r. smithi* from insular southern Far East, and *C. r. wosnessenskii* from Asian North-East. Such subdivision needs thorough revision even without our data — the taxon *smithii* with another taxonomic limits is separated as the species now (Musser & Carleton, 2005). Our results contradict to the scheme mentioned above. Both mitochondrial and nuclear variation don't support separation of European and Siberian voles into different subspecies. On the contrary, the taxonomic structure of the Far Eastern voles seems to be more complicated than it was described in the Ognev system. The study of morphological variation based on the vast available museum collections as well as modern methods is needed to complete a taxonomical intraspecific revision of *C. rufocanus*.

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**Appendix 1.** Map references, geographical locations, sample abbreviations and GenBank Accession, vouchers and tissue reference numbers of *Myodes rufocanus* haplotypes used in this study.

| Map | Collecting locality | Tree code | Cyt b haplotype (haplogroup) | SRY type | Cytb GenBank accession | Co1 GenBank accession | Lab tissue number | Voucher specimen number |
|---|---|---|---|---|---|---|---|---|
| 1 | Finland, Kilpisjarvi 63.5º N; 27.3º E | Finland1 | h3 (C-1) | | AY309413* | | | AF3153* |
| | | Finland2 | h1 (C-1) | | AY309414* | | | AF3157* |
| 2 | Russia, Murmansk Region, Kandalaksha Nature Reserve 66.54º N; 33.29º E | Kandalaksha 1 | h1 (C-1) | V | JF713462 | | 1031 | |
| | | Kandalaksha 2 | h4 (C-1) | | JF713463 | | 1033 | ZIN 97742 |
| | | Kandalaksha 3 | h5 (C-1) | | JF713464 | | 1034 | ZIN 97743 |
| | | Kandalaksha 4 | h1 (C-1) | | JF713465 | JF903219 | 1035 | |
| | | Kandalaksha 5 | h5 (C-1) | | JF713466 | JF903225 | 1036 | |
| | | Kandalaksha 6 | h1 (C-1) | | JF713467 | JF903233 | 1038 | |
| | | Kandalaksha 7 | h5 (C-1) | | JF713468 | | 1357 | |
| 3 | Russia, Murmansk Region, research station of RAS 68.99º N; 35.76º E | Kolskiy1 | h6 (C-1) | V | JF713469 | JF903246 | 673 | |
| | | Kolskiy2 | h1 (C-1) | | JF713470 | JF903116 | 676 | |
| | | Kolskiy3 | h7 (C-1) | | JF713471 | JF903166 | 710 | ZIN 86356 |
| 4 | Russia, Murmansk Region, Ponoy River 67.8º N; 35.97º E | Kolskiy4 | h8 (C-1) | | AY309415* | | | AF15465* |
| | | Kolskiy5 | h1 (C-1) | | AF272640* | | | AF15469* |
| | | Kolskiy6 | h1 (C-1) | | JF713472 | | 677 | ZIN 82180 |
| | | Kolskiy7 | h9 (C-1) | | JF713473 | JF903120 | 679 | ZIN 86355 |
| 5 | Russia, Republic of Komi, Pechora-Ilych Nature Reserve 62.52º N; 56.73º E | Komi | h10 (C-1) | V | JF713474 | | 358 | |
| 6 | Russia, Sverdlovsk Region, Shigaevo 57.35º N; 58.70º E | Shigaevo1 | h1 (C-1) | | JF713475 | | 480 | IPAE C9563 |
| | | Shigaevo2 | h11 (C-1) | V | JF713476 | | 534 | IPAE C9584 |
| 7 | Russia, Sverdlovsk Region, Khomutovka 56.83º N; 59.85º E | Khomutovka 1 | h11 (C-1) | | JF713477 | | 1131 | IPAE C4242 |
| | | Khomutovka 2 | h1 (C-1) | V | JF713478 | | 1135 | IPAE C4307 |
| 8 | Russia, Yamal-Nenets Autonomous District, Labytnangi 66.9º N; 65.73º E | Labytnangi1 | h12 (C-1) | | JF713479 | JF903180 | 1167 | ZIN 98068 |
| | | Labytnangi2 | h13 (C-1) | V | JF713480 | JF903185 | 1168 | ZIN 98069 |
| | | Labytnangi3 | h14 (C-1) | | JF713481 | JF903188 | 1169 | ZIN 98070 |

**Appendix 1** (continued).

| Map | Collecting locality | Tree code | Cyt b haplotype (haplo-group) | SRY type | Cytb GenBank accession | Co1 GenBank accession | Lab tissue num-ber | Voucher specimen number |
|---|---|---|---|---|---|---|---|---|
| 9 | Russia, Krasnoyarsk Territory, Putorana Plateau 68.3º N; 92.85º E | Putorana | h15 (B-2) | | JF713482 | JF903281 | 497 | ZMMU s181729 |
| 10 | Russia, Krasnoyarsk Territory, Mirnoe, the right bank of Yenisei River 62.33º N; 89.0º E | Mirnoe1 | h16 (B-2) | | JF713483 | JF903175 | 1276 | ZMMU s184923 |
| | | Mirnoe2 | h17 (B-2) | | JF713484 | JF903184 | 1293 | ZMMU s184950 |
| | | Mirnoe3 | h18 (C-1) | | JF713485 | JF903192 | 1295 | ZMMU s184924 |
| | | Mirnoe4 | h16 (B-2) | V | JF713486 | JF903237 | 1306 | ZMMU s184939 |
| | | Mirnoe5 | h19 (B-2) | V | JF713487 | JF903251 | 1310 | ZMMU s184937 |
| | | Mirnoe6 | h16 (B-2) | | JF713488 | | 1932 | ZMMU s183613 |
| | | Mirnoe7 | h18 (C-1) | | JF713489 | | 1937 | ZMMU s183637 |
| | | Mirnoe8 | h20 (B-2) | | JF713490 | | 1940 | ZMMU s183638 |
| | | Mirnoe9 | h21 (B-2) | | JF713491 | | 1951 | ZMMU s183601 |
| | | Mirnoe10 | h18 (C-1) | | JF713492 | | 1955 | ZMMU s183600 |
| | | Mirnoe11 | h16 (B-2) | | JF713493 | | 1956 | ZMMU s183621 |
| | | Mirnoe12 | h16 (B-2) | | JF713494 | | 1959 | ZMMU s183607 |
| | | | | V | | | 1948 | ZMMU s183630 |
| 11 | Russia, Krasnoyarsk Territory, Mirnoe, the left bank of Yenisei River 62.27º N; 89.09º E | Mirnoe13 | h22 (B-2) | | JF713495 | | 1958 | ZMMU s183627 |
| 12 | Russia, Altai Republic, Bannoe 50.30º N; 84.76º E | Bannoe | h1 (C-1) | | JF713496 | JF903247 | 1 | ZIN 91130 |
| 13 | Russia, Altai Republic, Eloviy Klyuch River 50.95º N; 85.74º E | ElovijKluch 1 | h23 (C-1) | V | JF713497 | | 1967 | ZMMU s183499 |
| | | ElovijKluch 2 | h24 (C-1) | | JF713498 | | 1968 | ZMMU s183500 |
| | | ElovijKluch 3 | h24 (C-1) | | JF713499 | | 1970 | ZMMU s183501 |
| | | ElovijKluch 4 | h24 (C-1) | | JF713500 | | 1972 | ZMMU s183502 |
| | | ElovijKluch 5 | h25 (C-1) | V | JF713501 | | 1978 | ZMMU s183503 |
| 14 | Russia, Altai Republic, Mashey River 50.23º N; 87.62º E | Mashej1 | h26 (C-1) | | JF713502 | | 2632 | |
| | | Mashej2 | h1 (C-1) | | JF713503 | | 2633 | ZIN 99290 |
| | | Mashej3 | h1 (C-1) | | JF713504 | | 2634 | ZIN 99291 |
| 15 | Russia, Altai Republic, Bashkaus River 50.63º N; 87.93º E | Bashkaus | h27 (C-1) | | JF713505 | JF903212 | 42 | |
| 16 | Russia, Altai Republic, Tydtugem River 50.19º N; 88.16º E | Tidtugem | h1 (C-1) | V | JF713506 | | 1963 | ZMMU s183498 |
| 17 | Russia, Republic of Khakassia, Balyksa 53.43º N; 89.17º E | Baliksu1 | h28 (C-1) | | JF713507 | | 1980 | ZMMU s183504 |
| | | Baliksu2 | h28 (C-1) | | JF713508 | | 1981 | ZMMU s183505 |
| | | Baliksu3 | h29 (C-1) | | JF713509 | | 1984 | ZMMU s183506 |
| 18 | Russia, Republic of Tuva, Karasu 50.42º N; 90.0º E | Karasu | h30 (C-1) | | JF713510 | | 2547 | ZIN 99289 |

**Appendix 1** (continued).

| Map | Collecting locality | Tree code | Cyt b haplotype (haplo-group) | SRY type | Cytb GenBank accession | Co1 GenBank accession | Lab tissue num-ber | Voucher specimen number |
|---|---|---|---|---|---|---|---|---|
| 19 | Russia, Krasnoyarsk Territory, Oya River 52.80º N; 93.14º E | Oya1 | h31 (C-1) | | JF713511 | | 1990 | ZMMU s183507 |
| | | Oya2 | h31 (C-1) | | JF713512 | | 1991 | ZMMU s183508 |
| | | Oya3 | h31 (C-1) | V | JF713513 | | 1998 | ZMMU s183509 |
| | | Oya4 | h1 (C-1) | | JF713514 | | 2688 | ZIN 99317 |
| 20 | Russia, Krasnoyarsk Territory, Bagazul River 52.99º N; 93.21º E | Bagazul1 | h32 (C-1) | | JF713515 | | 2675 | ZIN 99304 |
| | | Bagazul2 | h32 (C-1) | | JF713516 | | 2676 | ZIN 99305 |
| | | Bagazul3 | h1 (C-1) | | JF713517 | | 2679 | ZIN 99309 |
| | | Bagazul4 | h33 (C-1) | | JF713518 | | 2680 | ZIN 99310 |
| | | Bagazul5 | h33 (C-1) | | JF713519 | | 2684 | ZIN 99313 |
| | | Bagazul6 | h33 (C-1) | | JF713520 | | 2677 | ZIN 99306 |
| | | Bagazul7 | h1 (C-1) | | JF713521 | | 2678 | ZIN 99307 |
| | | Bagazul8 | h33 (C-1) | | JF713522 | | 2681 | ZIN 99308 |
| | | Bagazul9 | h1 (C-1) | | JF713523 | | 2685 | ZIN 99314 |
| 21 | Russia, Krasnoyarsk Territory, Bereg-Taskino 56.79º N; 93.52º E | Taskino1 | h34 (C-1) | V | JF713524 | | 1910 | |
| | | Taskino2 | h34 (C-1) | | JF713525 | | 1911 | |
| | | Taskino3 | h34 (C-1) | V | JF713526 | | 1914 | |
| 22 | Mongolia, Khövsgöl Province, Burenhan 49.5º N; 98.9º E | Burenhan | h35 (C-1) | | JF713527 | | 55 | ZMMU s179098 |
| 23 | Mongolia, Hentiyn Nuruu 49.07º N; 107.25º E | Hentej | h1 (C-1) | | JF713528 | | 570 | |
| 24 | Russia, Irkutsk Region, Bolshiye Koty 51.91º N; 105.07º E | Koty1 | h1 (C-1) | V | JF713529 | | 1052 | ZMMU s181346 |
| | | Koty2 | h36 (C-1) | | JF713530 | | 1053 | ZMMU s181336 |
| | | Koty3 | h37 (C-1) | | JF713531 | | 1054 | ZMMU s181369 |
| | | Koty4 | h38 (C-1) | | JF713532 | | 1055 | ZMMU s181347 |
| | | Koty5 | h39 (C-1) | V | JF713533 | | 1056 | ZMMU s181361 |
| | | | | V | | | 1057 | ZMMU s181364 |
| 25 | Russia, Republic of Buryatia, Oshurkovo 51.96ºN; 107.46ºE | Oshurkovo | h40 (C-1) | | JF713534 | | 2435 | IBSS 1990 |
| 26 | Russia, Republic of Buryatia, Taksimo 56.34º N; 114.89º E | Taksimo1 | h41 (C-1) | | JF713535 | | 2371 | IBSS 2251 |
| | | Taksimo2 | h38 (C-1) | | JF713536 | | 2372 | IBSS 2258 |
| | | Taksimo3 | h42 (C-2) | | JF713537 | | 2374 | IBSS 2260 |
| 27 | Russia, Zabaikalskiy Territory, Erman Range 50.0º N; 113.08º E | Ermana1 | h1 (C-1) | | JF713538 | | 495 | ZMMU s180430 |
| | | Ermana2 | h1 (C-1) | | JF713539 | | 496 | ZMMU s180431 |
| 28 | Russia, Zabaikalskiy Territory, Kher-khira River 50.41º N; 118.11º E | Kher-khira | h43 (C-1) | | JF713540 | | 59 | ZMMU s178575 |
| 29 | Russia, Zabaikalskiy Territory, Chachakan River 52.32º N; 118.21º E | Chachakan | h44 (C-1) | | JF713541 | | 56 | ZMMU s178577 |
| 30 | Russia, Zabaikalskiy Territory, Barangon River 53.42º N; 121.18º E | Barangon1 | h44 (C-1) | | JF713542 | | 1063 | ZMMU s182012 |
| | | Barangon2 | h45 (C-2) | III | JF713543 | | 1064 | ZMMU s182013 |
| | | Barangon3 | h46 (C-2) | | JF713544 | | 1065 | ZMMU s182014 |
| | | Barangon4 | h45 (C-2) | | JF713545 | | 1066 | ZMMU s182015 |

**Appendix 1** (continued).

| Map | Collecting locality | Tree code | Cyt b haplotype (haplo-group) | SRY type | Cytb GenBank accession | Co1 GenBank accession | Lab tissue num-ber | Voucher specimen number |
|---|---|---|---|---|---|---|---|---|
| 30 | Russia, Zabaikalskiy Territory, Barangon River 53.42º N; 121.18º E | Barangon5 | h47 (C-1) | | JF713546 | | 1068 | ZMMU s182016 |
| | | Barangon6 | h48 (C-1) | | JF713547 | | 1069 | ZMMU s182017 |
| | | | | III | | | 1073 | ZMMU s182020 |
| | | | | III | | | 1075 | ZMMU s182022 |
| 31 | Russia, Amur Region, Ignashino 53.46º N; 122.40º E | Ignashino | h49 (C-1) | III | JF713548 | | 1078 | ZMMU s182024 |
| 32 | Russia, Amur Region, Zeyskiy Nature Reserve 54.28º N; 126.83º E | Zeyskiy1 | h50 (C-2) | | JF713549 | | 2659 | ZMMU s186392 |
| | | Zeyskiy2 | h50 (C-2) | | JF713550 | | 2660 | ZMMU s186394 |
| | | Zeyskiy3 | h50 (C-2) | | JF713551 | | 2661 | ZMMU s186393 |
| | | Zeyskiy4 | h51 (C-2) | | JF713552 | | 2663 | ZMMU s186395 |
| 33 | Russia, Amur Region, Stanovoy Range, Chardoy River 55.71º N; 129.68º E | Chardoy | h52 (C-2) | | JF713553 | | 2665 | ZMMU s187679 |
| 34 | Russia, Republic of Yakutia-Sakha, Torgo River 58.2º N; 199.25º E | Torgo1 | h53 (C-2) | V | JF713554 | JF903144 | 780 | ZIN 97142 |
| | | Torgo2 | h53 (C-2) | | JF713555 | JF903150 | 782 | ZIN 97143 |
| | | Torgo3 | h38 (C-1) | | JF713556 | | 787 | |
| | | Torgo4 | h54 (C-2) | | JF713557 | JF903154 | 783 | |
| | | Torgo5 | h38 (C-1) | V | JF713558 | JF903156 | 784 | |
| | | Torgo6 | h55 (C-2) | | JF713559 | JF903162 | 785 | ZIN 97144 |
| | | Torgo7 | h38 (C-1) | | JF713560 | | 786 | |
| 35 | Russia, Republic of Yakutia-Sakha, Tokko River 58.5º N; 119.93º E | Tokko1 | h56 (C-2) | IV | JF713561 | JF903210 | 802 | ZIN 97137 |
| | | Tokko2 | h57 (C-2) | | JF713562 | JF903191 | 797 | ZIN 97373 |
| | | Tokko3 | h58 (C-2) | | JF713563 | JF903195 | 798 | ZIN 97134 |
| | | Tokko4 | h59 (C-1) | | JF713564 | JF903198 | 799 | ZIN 97135 |
| | | Tokko5 | h60 (C-2) | | JF713565 | JF903201 | 800 | ZIN 97136 |
| | | Tokko6 | h50 (C-2) | | JF713566 | JF903224 | 805 | ZIN 97139 |
| | | Tokko7 | h50 (C-2) | | JF713567 | JF903229 | 806 | |
| 36 | Russia, Republic of Yakutia-Sakha, Tyanya River 59.6º N; 120.23º E | Tyanya1 | h61 (C-2) | | JF713568 | JF903236 | 808 | ZIN 97140 |
| | | Tyanya2 | h59 (C-1) | | JF713569 | JF903135 | 820 | ZIN 97141 |
| 37 | Russia, Republic of Yakutia-Sakha, Chabda River 59.78º N; 134.81º E | Chabda | h62 (C-2) | V | JF713570 | | 2001 | ZMMU s183404 |
| 38 | China, Heilongjiang Province, Maoershan, Liaoyeling 45.4º N; 127.67º E | Maoershan1 | h63 (D) | III | JF713571 | JF903270 | 1342 | ROM 112851 |
| | | Maoershan2 | h64 (D) | VII | JF713572 | JF903267 | 1343 | ROM 112880 |
| | | | | VI | | | 1344 | ROM 112888 |
| 39 | Russia, Primorye Territory, Vladivostok 43.13º N; 131.9º E | Vladivostok 1 | h65 (C-3) | | AB031571* | | | HS943* |
| | | Vladivostok 2 | h66 (D) | | AB031572* | | | HS944* |
| 40 | Russia, Primorye Territory, Ussuriyskiy Nature Reserve 43.8º N; 131.98º E | UssRes1 | h67 (D) | | AB031565* | | | IK115-96* |
| | | UssRes2 | h68 (D) | | AB031566* | | | IK116-96* |
| | | UssRes3 | h69 (D) | | AB031567* | | | IK120-96* |
| | | UssRes4 | h70 (D) | | AB031568* | | | IK122-96* |
| | | UssRes5 | h71 (D) | | AB031569* | | | IK123-96* |
| | | UssRes6 | h72 (D) | VI | AB031570* | | | IK124-96* |
| 41 | Russia, Primorye Territory, Ussuriyskiy Nature Reserve, Anikin klyuch 43.66º N; 132.50º E | Anikinkluch 1 | h72 (D) | | JF713573 | | 1547 | |
| | | Anikinkluch 2 | h72 (D) | | JF713574 | | 1548 | |
| | | Anikinkluch 3 | h72 (D) | III | JF713575 | | 1549 | |

**Appendix 1** (continued).

| Map | Collecting locality | Tree code | Cyt b haplotype (haplo-group) | SRY type | Cytb GenBank accession | Co1 GenBank accession | Lab tissue num-ber | Voucher specimen number |
|---|---|---|---|---|---|---|---|---|
| 41 | Russia, Primorye Territory, Ussuriyskiy Nature Reserve, Anikin klyuch 43.66º N; 132.50º E | Anikinkluch 4 | h73 (C-3) | V | JF713576 | JF693294 | 1550 | |
| | | Anikinkluch 5 | h74 (C-3) | | JF713577 | JF693295 | 1552 | |
| | | Anikinkluch 6 | h75 (C-3) | VI | JF713578 | JF693296 | 1553 | |
| | | Anikinkluch 7 | h72 (D) | | JF713579 | | 1554 | |
| | | Anikinkluch 8 | h76 (C-3) | | JF713580 | JF693297 | 1555 | |
| | | Anikinkluch 9 | h72 (D) | VI | JF713581 | JF693298 | 1557 | |
| 42 | Russia, Primorye Territory, Tamga River 45.57º N; 133.61º E | Tamga | h77 (D) | | JF713582 | JF693299 | 2668 | ZMMU s186396 |
| 43 | Russia, Primorye Territory, Lazovskiy Nature Reserve 43.28º N; 134.16º E | Lazovskiy1 | h78 (C-1) | | JF713583 | JF903182 | 864 | MSB 147867 |
| | | Lazovskiy2 | h79 (D) | III | JF713584 | JF903183 | 865 | MSB 147817 |
| 44 | Russia, Primorye Territory, Ussuriyskiy Nature Reserve, Verhne-Ussuriyskiy research station 43.84º N; 134.21º E | Verh-Uss1 | h66 (D) | | JF713585 | JF693300 | 1558 | |
| | | Verh-Uss2 | h80 (C-2) | | JF713586 | | 1559 | |
| | | Verh-Uss3 | h66 (D) | | JF713587 | | 1561 | |
| | | Verh-Uss4 | h81 (C-3) | VI | JF713588 | JF693301 | 1562 | |
| | | Verh-Uss5 | h82 (C-3) | | JF713589 | JF693302 | 1563 | |
| | | Verh-Uss6 | h83 (D) | III | JF713590 | JF693303 | 1564 | |
| | | Verh-Uss7 | h84 (D) | | JF713591 | JF693304 | 1565 | |
| | | Verh-Uss8 | h85 (D) | | JF713592 | JF693305 | 1566 | |
| | | Verh-Uss9 | h86 (C-3) | | JF713593 | JF693306 | 1567 | |
| 45 | Russia, Primorye Territory, Sikhote-Alinskiy Nature Reserve 44.91º N; 136.53º E | Sikhote1 | h87 (C-3) | | JF713594 | JF903112 | 2004 | ZMMU s184228 |
| | | Sikhote2 | h88 (C-3) | | JF713595 | | 2006 | ZMMU s184226 |
| | | Sikhote3 | h87 (C-3) | | JF713596 | | 2009 | ZMMU s184227 |
| | | Sikhote4 | h87 (C-3) | | JF713597 | | 2010 | ZMMU s184222 |
| | | Sikhote5 | h89 (D) | | JF713598 | JF693307 | 2658 | ZMMU s186397 |
| 46 | Russia, Khabarovsk Territory, Khabarovsk 48.28º N; 135.05º E | Khabarovsk1 | h90 (C-2) | | AF429816* | | | CRF890* |
| 47 | Russia, Khabarovsk Territory, Khabarovsk 48.58º N; 135.38º E | Khabarovsk2 | h91 (C-2) | | JF713599 | JF903187 | 866 | |
| | | Khabarovsk3 | h92 (C-2) | | JF713600 | | 868 | |
| | | Khabarovsk4 | h92 (C-2) | | JF713601 | JF903194 | 869 | |
| | | Khabarovsk5 | h93 (C-2) | V | JF713602 | JF903197 | 870 | MSB 153619 |
| 48 | Russia, Khabarovsk Territory, Briakan 52.30º N; 135.56º E | Briakan1 | h94 (C-2) | | JF713603 | | 2649 | ZMMU s186390 |
| | | Briakan2 | h95 (C-2) | | JF713604 | | 2650 | ZMMU s186389 |
| | | Briakan3 | h96 (C-2) | | JF713605 | | 2651 | ZMMU s186388 |
| | | Briakan4 | h97 (C-2) | | JF713606 | | 2652 | ZMMU s186387 |
| | | Briakan5 | h94 (C-2) | | JF713607 | | 2653 | ZMMU s186386 |
| | | Briakan6 | h96 (C-2) | | JF713608 | | 2654 | ZMMU s186385 |
| | | Briakan7 | h98 (C-2) | | JF713609 | | 2655 | ZMMU s186384 |
| | | Briakan8 | h99 (C-2) | | JF713610 | | 2656 | ZMMU s186383 |

**Appendix 1** (continued).

| Map | Collecting locality | Tree code | Cyt b haplotype (haplo-group) | SRY type | Cytb GenBank accession | Co1 GenBank accession | Lab tissue num-ber | Voucher specimen number |
|---|---|---|---|---|---|---|---|---|
| 48 | Russia, Khabarovsk Territory, Briakan 52.30º N; 135.56º E | Briakan9 | h96 (C-2) | | JF713611 | | 2657 | ZMMU s186382 |
| | | Briakan10 | h100 (C-2) | | JF713612 | | 2662 | ZMMU s186377 |
| | | Briakan11 | h97 (C-2) | | JF713613 | | 2697 | ZMMU s186379 |
| | | Briakan12 | h96 (C-2) | | JF713614 | | 2698 | ZMMU s186380 |
| | | Briakan13 | h96 (C-2) | | JF713615 | | 2699 | ZMMU s186381 |
| | | Briakan14 | h96 (C-2) | | JF713616 | | 2700 | ZMMU s186378 |
| 49 | Russia, Khabarovsk Territory, Bolshoy Shantar Island 54.87º N; 137.49º E | Shantar1 | h101 (C-2) | III | JF713617 | JF903252 | 516 | IBPN 6088 |
| | | Shantar2 | h102 (C-2) | | JF713618 | JF903244 | 515 | IBPN 6087 |
| | | Shantar3 | h103 (C-2) | | JF713619 | JF903114 | 517 | IBPN 6089 |
| | | Shantar4 | h104 (C-2) | | JF713620 | JF903221 | 509 | IBPN 6082 |
| | | Shantar5 | h105 (C-2) | | JF713621 | JF903149 | 897 | IBPN 6084 |
| | | Shantar6 | h103 (C-2) | | JF713622 | JF903155 | 899 | IBPN 6095 |
| | | Shantar7 | h103 (C-2) | | JF713623 | JF903238 | 513 | IBPN 6085 |
| 50 | Russia, Khabarovsk Territory, Okhotsk 59.37º N; 143.35º E | Okhotsk1 | h106 (C-2) | | JF713624 | JF903161 | 543 | MSB 148021 |
| | | Okhotsk2 | h2 (C-2) | V | JF713625 | JF903235 | 879 | MSB 148030 |
| | | Okhotsk3 | h107 (C-2) | V | JF713626 | JF903240 | 880 | MSB 148022 |
| | | Okhotsk4 | h2 (C-2) | | JF713627 | JF903243 | 881 | MSB 148031 |
| | | Okhotsk5 | h108 (C-2) | | JF713628 | JF903248 | 882 | MSB 148025 |
| 51 | Russia, Magadan Region, Solnechniy 59.63º N; 150.78º E | Solnechniy | h2 (C-2) | | JF713629 | JF903169 | 249 | MSB 193206 |
| 52 | Russia, Magadan Region, Magadan 59.57º N; 150.8º E | Magadan1 | h110 (C-2) | | AY309412* | | | AF6590* |
| | | Magadan2 | h2 (C-2) | V | AB031580* | | | DK29-97* |
| 53 | Russia, Magadan Region, Snezhnaya dolina 59.73º N; 150.87º E | Snezhnaya1 | h111 (C-2) | | JF713630 | JF903208 | 87 | MSB 192928 |
| | | Snezhnaya2 | h2 (C-2) | | JF713631 | | 89 | MSB 193010 |
| | | Snezhnaya3 | h2 (C-2) | V | JF713632 | | 244 | MSB 193011 |
| 54 | Russia, Magadan Region, Vdovushka Island 59.50º N; 150.92º E | Vdovushka1 | h2 (C-2) | | JF713633 | | 889 | IBPN 6119 |
| | | Vdovushka2 | h2 (C-2) | | JF713634 | JF903127 | 890 | IBPN 6120 |
| | | Vdovushka3 | h112 (C-2) | | JF713635 | JF903131 | 891 | IBPN 6121 |
| | | Vdovushka4 | h2 (C-2) | | JF713636 | JF903134 | 892 | IBPN 6122 |
| | | Vdovushka5 | h112 (C-2) | | JF713637 | JF903137 | 893 | IBPN 6123 |
| 55 | Russia, Magadan Region, upper Kolyma River 62.37º N; 151.9º E | Kolyma | h113 (C-2) | V | JF713638 | | 1377 | IBPN 6133 |
| 56 | Russia, Magadan Region, Elgen River 62.86º N; 152.4º E | Elgen1 | h114 (C-2) | | JF713639 | JF903140 | 527 | |
| | | Elgen2 | h114 (C-2) | | JF713640 | JF903119 | 886 | |
| | | Elgen3 | h115 (C-2) | | JF713641 | JF903121 | 887 | |
| | | Elgen4 | h116 (C-2) | | JF713642 | JF903123 | 888 | |
| 57 | Russia, Magadan Region, Verhnyaya Buyunda 62.5º N; 153.27º E | Buyunda1 | h117 (C-2) | | JF713643 | | 1368 | IBPN 6131 |
| | | Buyunda2 | h114 (C-2) | | JF713644 | | 1386 | IBPN 6132 |
| 58 | Russia, Magadan Region, Evensk 61.92º N; 159.27º E | Evensk1 | h118 (C-1) | | JF713645 | | 1378 | IBPN 6142 |
| | | Evensk2 | h119 (C-1) | | JF713646 | | 1374 | IBPN 6139 |
| | | Evensk3 | h120 (C-1) | | JF713647 | | 1381 | IBPN 6145 |
| | | Evensk4 | h119 (C-1) | | JF713648 | | 1384 | IBPN 6148 |
| | | | | V | | | 1372 | IBPN 6137 |
| | | | | V | | | 1376 | IBPN 6141 |
| 59 | Russia, Sakhalin Region, Kuril Islands, Paramushir Island 50.42º N; 155.81º E | Paramushir1 | h121 (C-1) | V | JF713649 | | 2020 | IBPN 6127 |
| | | Paramushir2 | h122 (C-1) | V | JF713650 | | 2021 | IBPN 6128 |
| | | Paramushir3 | h123 (C-1) | V | JF713651 | | 2022 | IBPN 6129 |

**Appendix 1** (continued).

| Map | Collecting locality | Tree code | Cyt b haplotype (haplo-group) | SRY type | Cytb GenBank accession | Co1 GenBank accession | Lab tissue num-ber | Voucher specimen number |
|---|---|---|---|---|---|---|---|---|
| 59 | Russia, Sakhalin Region, Kuril Islands, Paramushir Island 50.42º N; 155.81º E | Paramushir4 | h121 (C-1) | V | JF713652 | | 2023 | IBPN 6130 |
| | | Paramushir5 | h121 (C-1) | V | JF713653 | | 2024 | |
| | | Paramushir6 | h121 (C-1) | | JF713654 | | 2025 | |
| 60 | Russia, Kamchatka Territory, Pinachevo 53.31º N; 158.38º E | Pinachevo1 | h124 (C-1) | | JF713655 | | 2030 | |
| | | Pinachevo2 | h125 (C-1) | | JF713656 | | 2032 | |
| | | Pinachevo3 | h126 (C-1) | V | JF713657 | | 2033 | |
| | | Pinachevo4 | h1 (C-1) | | JF713658 | | 2034 | |
| 61 | Russia, Kamchatka Territory, Milkovo 54.82º N; 158.55º E | Milkovo1 | h1 (C-1) | V | AB031577* | | | IK100-97* |
| | | Milkovo2 | h1 (C-1) | V | AB031578* | | | IK101-97* |
| | | | | V | | | | IK110-97* |
| | | | | III | | | | IK128-97* |
| | | Milkovo3 | h44 (C-1) | | AB031579* | | | IK103-97* |
| 62 | Russia, Kamchatka Territory, Esso 55.92º N; 158.68º E | Esso1 | h127 (C-1) | | JF713659 | JF903153 | 531 | |
| | | Esso2 | h128 (C-1) | III | JF713660 | JF903200 | 871 | |
| | | Esso3 | h129 (C-1) | III | JF713661 | JF903209 | 873 | |
| | | Esso4 | h130 (C-1) | | JF713662 | JF903214 | 874 | |
| | | Esso5 | h1 (C-1) | | JF713663 | JF903218 | 875 | |
| | | | | V | | | 877 | |
| 63 | Russia, Chukot Autonomous District, Keveem River 68.97º N; 173.7º E | Keveem | h131 (C-1) | V | JF713664 | JF903226 | 924 | MSB 150021 |
| 64 | Russia, Chukot Autonomous District, Tanurer River 66.16º N; 175.76º E | Tanurer | h132 (C-1) | V | JF713665 | JF903157 | 328 | ZIN 93394 |
| 65 | Russia, Sakhalin Region, Moneron Island 46.27º N; 141.24º E | Moneron1 | h133 (B-1) | | FJ792779 | JF903227 | 91 | |
| | | Moneron2 | h134 (B-1) | | FJ792780 | | 93 | |
| | | Moneron3 | h135 (B-1) | | FJ792781 | JF903145 | 241 | |
| | | | | I | | | 237 | |
| | | | | I | | | 239 | |
| 66 | Russia, Sakhalin Region, Sakhalin Island, Yablochniy 47.16º N; 142.10º E | Yablochniy1 | h136 (B-1) | I | FJ792788 | | 1318 | |
| | | Yablochniy2 | h137 (B-1) | | JF713666 | | 1321 | ZIN 98125 |
| | | Yablochniy3 | h138 (B-1) | | JF713667 | | 1319 | ZIN 98124 |
| | | Yablochniy4 | h138 (B-1) | | JF713668 | | 1320 | |
| 67 | Russia, Sakhalin Region, Sakhalin Island, Novoaleksandrovsk 47.03º N; 142.72º E | Novoaleks1 | h139 (B-1) | | FJ792790 | | 1314 | ZIIN 98123 |
| | | Novoaleks2 | h139 (B-1) | | FJ792791 | | 1317 | |
| | | Novoaleks3 | h140 (B-1) | | JF713669 | | 1315 | |
| | | Novoaleks4 | h141 (B-1) | | JF713670 | | 1316 | |
| 68 | Russia, Sakhalin Region, Sakhalin Island, Okha 53.97º N; 142.75º E | Okha1 | h142 (B-1) | | AB031573* | | | IK61-97* |
| | | Okha2 | h138 (B-1) | I | AB031574* | | | IK64-97* |
| | | Okha3 | h143 (B-1) | I | AB031575* | | | IK65-97* |
| | | Okha4 | h144 (B-1) | | AB031576* | | | HS639* |
| 69 | Russia, Sakhalin Region, Sakhalin Island, Sokol research station 47.24º N; 142.78º E | Sokol1 | h145 (B-1) | | JF713671 | JF903125 | 235 | MSB 193015 |
| | | Sokol2 | h146 (B-1) | | FJ792776 | JF903217 | 921 | MSB 193018 |
| | | Sokol3 | h138 (B-1) | | FJ792777 | JF903222 | 922 | MSB 192932 |
| | | Sokol4 | h147 (B-1) | I | FJ792778 | JF903213 | 920 | MSB 193184 |
| | | Sokol5 | h148 (B-1) | | FJ792792 | | 1322 | |
| | | Sokol6 | h149 (B-1) | | JF713672 | JF903165 | 247 | MSB 193029 |
| | | Sokol7 | h150 (B-1) | | JF713673 | JF903168 | 248 | MSB 192974 |
| 70 | Russia, Sakhalin Region, Sakhalin Island, Omba River 53.87º N; 142.83º E | Omba1 | h151 (B-1) | | JF713674 | | 2367 | IBSS 2209 |
| | | Omba2 | h152 (B-1) | | JF713675 | | 2368 | IBSS 2213 |
| | | Omba3 | h151 (B-1) | | JF713676 | | 2369 | IBSS 2214 |
| 71 | Russia, Sakhalin Region, Sakhalin Island, Chayvo Bay 52.53º N; 143.06º E | Chayvo | h153 (B-1) | I | JF713677 | | 2002 | ZMMU s187176 |
| 72 | Japan, Hokkaido, Kuromatsunai 42.65º N; 140.3º E | Kuroma-tsunai1 | h154 (A) | | AB031557* | | | KN98107* |
| | | Kuroma-tsunai2 | h155 (A) | | AB031558* | | | KN98108* |

**Appendix 1** (continued).

| Map | Collecting locality | Tree code | Cyt b haplotype (haplo-group) | SRY type | Cytb GenBank accession | Co1 GenBank accession | Lab tissue num-ber | Voucher specimen number |
|---|---|---|---|---|---|---|---|---|
| 73 | Japan, Hokkaido, Rebun Island 45.37º N; 141.02º E | Rebun | h156 (A) | | AB031554* | | | HEGRB-2* |
| 74 | Japan, Hokkaido, Rishiri Island 45.18º N; 141.23º E | Rishiri | h157 (A) | II | AB031553* | | | HS227*<br>HS228* |
| 75 | Japan, Hokkaido, Tobetsu 43.2º N; 141.5º E | Tobetsu | h158 (A) | | AB031560* | | | HEG1-97* |
| 76 | Japan, Hokkaido, Ishikari-shi 43.57º N; 142.82º E | Ishikari1<br>Ishikari2<br>Ishikari3 | h159 (A)<br>h159 (A)<br>h160 (A) | | AY309416*<br>AY309417*<br>AY309418* | | | IF5700*<br>IF5701*<br>IF5702* |
| 77 | Japan, Hokkaido, Naganuma 42.67º N; 142.93º E | Naganuma | h161 (A) | I | AB031559* | | | HS231*<br>HS234* |
| 78 | Japan, Hokkaido, Takinoue 44.18º N; 143.07º E | Takinoue1<br>Takinoue2<br>Takinoue3 | h162 (A)<br>h163 (A)<br>h164 (A) | <br><br>I | AB031561*<br>AB031562*<br>AB031563* | | | HEG117*<br>HEG118*<br>HEG121* |
| 79 | Japan, Hokkaido, Nemuro 43.33º N; 145.58º E | Nemuro1<br>Nemuro2 | h165 (A)<br>h166 (A) | I<br>I<br>I | AB031555*<br>AB031556* | | | HEG141*<br>HEG149*<br>HEG140* |
| 80 | Russia, Sakhalin Region, Kuril Islands, Taraku (Polonskogo) Island 44.35º N; 146.43º E | Taraku | h167 (A) | | AB031564* | | | IN28-98* |
| 81 | Russia, Sakhalin Region, Kuril Islands, Kunashir Island 44.19º N; 146.02º E | Kunashir1<br>Kunashir2<br>Kunashir3<br>Kunashir4<br>Kunashir5<br>Kunashir6<br>Kunashir7<br>Kunashir8 | h168 (A)<br>h169 (A)<br>h170 (A)<br>h171 (A)<br>h172 (A)<br>h173 (A)<br>h174 (A)<br>h171 (A) | I<br><br><br>I<br><br><br><br> | FJ792782<br>FJ792783<br>FJ792784<br>FJ792785<br>FJ792786<br>JF713678<br>JF713679<br>JF713680 | JF693308<br>JF693309<br><br>JF693310<br>JF693311<br>JF693312<br>JF693313<br> | 1325<br>1326<br>1329<br>1331<br>1333<br>1328<br>1327<br>1330 | ZIN 98127<br>ZIN 98128<br>ZIN 98131<br>ZIN 98133<br>ZIN 98135<br>ZIN 98130<br>ZIN 98129<br>ZIN 98132 |

* — sequences from GenBank

**Voucher specimen numbers**
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IBSS — Institute of Biology and Soil Sciences FEB RAS, Vladivostok, Russia;
IPAE — Institute of Plant and Animal Ecology, Ural Department, Russian Academy of Sciences, Yekaterinburg, Russia;
MSB — Museum of Southwestern Biology, University of New Mexico, USA;
ROM — Royal Ontario Museum, Toronto, Canada;
ZIN — Zoological Institute of the Russian Academy of Sciences, St. Petersburg, Russia;
ZMMU — Zoological Museum of Moscow State University, Moscow, Russia.