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DNA polymorphism within *Sorex araneus* from European Russia as inferred from mtDNA cytochrome *b* sequences

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ABSTRACT. Genetic variation in the common shrew (*Sorex araneus*) in European Russia was studied using cytochrome *b* gene sequences. The genetic diversity, based on nucleotide substitutions (Kimura 2-parameter d=0.015±0.003, h=0.933), and the number of mtDNA haplotypes, was three times higher than described previously. However, levels of molecular divergence are often in contradiction with karyological data. While there are more than 20 karyotypic races in European Russia, only one clear phylogenetic group is revealed for cytochrome *b* (the North-eastern Group). The relationship of this group to other European *S. araneus* haplotypes is not clear. Over the main part of the European range of the common shrew geographic subdivision between haplotypes is lacking.

KEY WORDS: common shrew, *Sorex araneus*, cytochrome *b*, phylogeography, mtDNA polymorphism.

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Полиморфизм обыкновенной бурозубки *Sorex araneus* Европейской России по гену цитохрома *b* мтДНК

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РЕЗЮМЕ. В работе проведена оценка генетического разнообразия внутри вида *Sorex araneus* на территории Европейской части России, на основе нуклеотидной последовательности гена цитохрома *b*. Выявленный уровень генетического разнообразия, как по количеству нуклеотидных замен, так и по количеству и пространственному распределению митохондриальных гаплотипов в несколько раз превышает ранее известный. При этом особенности молекулярной дивергенции часто находятся в противоречии с кариологическими особенностями. Выявлена лишь одна филогенетическая группировка (Северо-восточная Группа), проявляющая черты явного генетического своеобразия. На большей части европейского ареала вида между гаплотипами не обнаруживается географической разобщённости. Последнее указывает на отсутствие заметной генетической подразделённости на этом участке ареала вида.

КЛЮЧЕВЫЕ СЛОВА: обыкновенная бурозубка, *Sorex araneus*, цитохром *b*, филогеография, мтДНК-полиморфизм.

Introduction

Studies of chromosome variation in the common shrew (*Sorex araneus*) were started about 50 years ago. Currently, a clear geographic subdivision of this species into dozens of chromosomal races has been demonstrated by analysis over the whole species range (Zima *et al*., 1996; Searle & Wójcik, 1998; Brünner *et al*., 2002a, b). It might be expected that this high level of chromosomal variability would be matched by other genetic markers. The earliest studies of genic variation in common shrews involved allozymes. These generated average heterozygosities that varied from 0.02 (George, 1988) to 0.065 (Catzeflis *et al*., 1988; Wójcik & Wójcik, 1994) for different local populations. This is within the heterozygosity limits for the genus *Sorex* (0.01–0.054: Catzeflis *et al*., 1988; George, 1988) and mammals in general (0.008–0.085: Nevo, 1978).

The issue of genetic isolation of karyologically different populations led to detailed investigations of hybrid zones between chromosome races of the common shrew. Initially such investigations were based on chromosomal data only. Later, hybrid zone analysis extended to allozymes (Frykman *et al*., 1983; Frykman & Bengtsson, 1984; Frykman & Simonsen, 1984; Neet & Hausser, 1991; Wójcik & Wójcik 1994; Ratkiewicz *et al*., 1994, 1996, 2003; Brünner & Hausser, 1996; Wójcik *et al*., 2002). Recently, investigations have included AFLP-analysis (Bannikova *et al.*, 2003) and studies of cytochrome *b* sequences and microsatellites (Taberlet *et al.*, 1991, 1994; Lugon-Moulin *et al.*, 1996, 1999a, b; Wyttenbach & Hausser, 1996; Wyttenbach *et al*., 1999).

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However, the pattern of genic differentiation discovered proved to be different from the pattern of distribution of chromosomal races. Ratkiewicz *et al.* (2002) have found that the distribution of mitochondrial haplotypes of cytochrome *b* within common shrews in Poland by no means connected with their racial affiliation to the West or East European Karyotypic Groups. Moreover, any traces of recent insularity or bottlenecking during the evolution history of these populations are lacking. These findings have impacted on current views on chromosomal raciation in this species. At the same time other studies (Taberlet *et al.*, 1991; Lugon-Moulin *et al.*, 1996, 1999a, b; Wyttenbach & Hausser, 1996; Wyttenbach *et al*., 1999) showed that the distribution of microsatellite markers among neighbouring local populations of shrews inhabiting Switzerland, Northern Italy, France and Germany aligns with the chromosome races. Bannikova *et al.* (2003) also obtained relevant data from an AFLP-marker investigation of some populations in European Russia which showed one group of markers (MIRs-repeats) matched to the chromosome variation (although with very low bootstrapping values), whereas other markers (SOR-elements) did not. The existence of such a contradiction in data obtained from karyological and molecular genetic investigations encouraged us to carry out a further molecular genetic study in *Sorex araneus* using mitochondrial cytochrome *b* sequences for samples from European Russia simultaneously subject to chromosomal analysis.

Material and methods

Sampling localities. The geographical location of sampling sites, racial identity of populations and GenBank accession numbers for individuals are given in Tab. 1 and Fig. 1. Except for samples from Pinega Nature Reserve and Cymlyansk District which were not karyotyped and samples from Yagry Island (Arkhangelsk Province) for which racial identity is presented here in English for the first time, all other sampling localities have been previously published. All comparable sequences of *Sorex araneus* from GenBank were included in this investigation. These sequences represent the following countries: Poland, Sweden, Switzerland, Italy, France, Bosnia and the Lake Baikal region of Russia.

DNA isolation and amplification. Total DNA was extracted from a 0.1-cm^3 piece of liver or kidney, stored in 96% ethanol. Extraction was carried out using a standard proteinase K-phenol-chloroform method (Kocher

Table 1. List of locations for shrew samples.

Sample names and GenBank accession numbers	Locality, chromosome race	Locality number	Number of specimens	First reference
New sequences				
Pinega 26-39 DQ417697-DQ417701	Russia, Arkhangelsk Province, Pinega, unknown race	1	5	This study
Yagry 11-24 DQ41702- DQ417707	Russia, Arkhangelsk Province, Yagry Island, Yagry race	2	6	
Onega 2-19 DQ417708-DQ417714	Russia, Arkhangelsk Province, Onega, Kirillov race	3	7	
Bizuk 85-90 DQ417715-DQ417719	Russia, Saratov Province, Rovensky District, Sok race	4	5	
Paleh 9 DQ417720	Russia, Ivanovo Province, Paleh, Moscow race	5	1	
PTZ 398 DQ417721	Russia, Moscow Province, Serpukhov, Moscow race	6	1	
Chernogolovka 62 DQ417722	Russia, Moscow Province, Chernogolovka, Moscow race	7	1	
Tver 4 DQ417724	Russia, Tver Province, Staritsa District, Moscow race	8	1	
Cimla 233-235 DQ417725-DQ417727	Russia, Rostov Province, Cymlyansk District, unknown race	9	3	
Kaluga 391 DQ417723	Russia, Kaluga Province, Kaluga, Neroosa race	10	1	
Karelia 385, 386 DQ417728, DQ417733	Russia, Karelia Republic, Ladoga Lake, eastern bank, Ilomantsi race	11	2	
Komi 565-67 DQ417729-DQ417730	Russia, Komi Republic, Pechora-Ilych Nature Reserve, Serov race	12	2	
Pecora 513 DQ417731	Russia, Komi Republic, Pechora-Ilych Nature Reserve, Serov race		1	
Pech-Il 1 DQ417732	Russia, Komi Republic, Pechora-Ilych Nature Reserve, Serov race		1	

Table 1 (continued).

Sample names and GenBank accession numbers	Locality, chromosome race	Locality number	Number of specimens	First reference
Sequences obtained from GenBank				
Haplotypes 1-21 AJ409867-409894	Poland, different races	–	21	Ratkiewicz *et al.*, 2002
Brunner AJ000416	unknown race	–	1	Fumagalli *et al.*, 1999 Brünner *et al.*, 2002b
Brunner AJ000415	unknown race	–	1	
AJ312039	Switzerland	–	1	Taberlet *et al.*, 1994
AJ312036 *S. antinorii*		–	1	
AJ312034 *S. antinorii*		–	1	
AJ312033		–	1	
AJ312035	Northern Italy	–	1	
AJ312037 *S. antinorii*		–	1	
AJ312038		–	1	
AJ312040 *S. antinorii*		–	1	
AJ312032	South-eastern France	–	1	
AJ312031	Bosnia	–	1	
AJ312028	Hungary	–	1	
AJ312030	United Kingdom, Oxford race	–	1	
AJ312029	Russia, Baikal region	–	1	
AJ245893	Sweden	–	1	Mouchaty *et al.*, 2000
AY936837	Sweden, Neptuni ängar, Öland race	–	1	Andersson *et al.*, 2005
AY936836	Sweden, Kalkstadt, Öland race	–	1	
AY936835	Sweden, Uppsala, Uppsala race	–	1	
AY936834	Sweden, Björksjön, Björksjön race	–	1	
AY936833	Sweden, Bjurholm, Abisko race	–	1	
AY936832	Sweden, Odeshog, Hällefors race	–	1	
AJ000419 *S. coronatus*	France	–	1	Fumagalli *et al.*, 1999
AJ000429 *S. samniticus*	Italy	–	1	

et al., 1989). DNA was purified by twofold ethanol precipitation. Amplification of mitochondrial DNA sequences containing part of the cytochrome *b* gene was performed in a 25 µl volume containing 50 µM of each dNTP, 2mM $MgCl_2$, PCR buffer (Sintol), 1mM primers (each), 1 unit *Taq* DNA polymerase and 2.5 µl DNA template per tubes in a Tercik (DNK-Tehnologia) thermal cycler using the following protocol: initial denaturation for 5 min at 95ºC, denaturation for 30 s at 95ºC, annealing for 1 min at 50ºC, and elongation for 30 s at 72ºC. The following primer set was used: L14841, L14724, L15162, H15915 (Irwin *et al*., 1991), H15149 (Kocher *et al*., 1989) and H15573 (Taberlet *et al*., 1991) in different combinations. Amplified DNA was cleaned by twofold ethanol reprecipitation and sequenced using an ABI PRISM 377 automated sequencer in both directions in accordance with the manufacturer's instructions.

Descriptive statistics and tree construction. The sequences obtained were aligned by the BioEdit program (Hall, 1999). An estimation of haplotype (h) and nucleotide (d) diversity was calculated according to the Kimura 2-parameter model (Kimura, 1980). Phylogenetic and molecular evolutionary analyses were carried out using MEGA version 3.1 (Kumar *et al*., 2004). An estimation of relationships among different groups of haplotypes was also carried out. To estimate the phylogeographical pattern of distribution of different haplotypes within the species range, neighbour-joining (NJ), maximal parsimony (MP) and minimal evolution (ME) trees were constructed on the Kimura 2-parameter model. In addition, we performed a neutrality test (Tajima, 1989) and a Z-test for neutrality in accordance with the Kumar model (Kumar *et al*., 1993).

Results and discussion

In this study we detected 37 new cytochrome *b* sequences with a maximal reading frame of 1004 bp. All these sequences represent different haplotypes with the exception of two sequences from Karelia, which

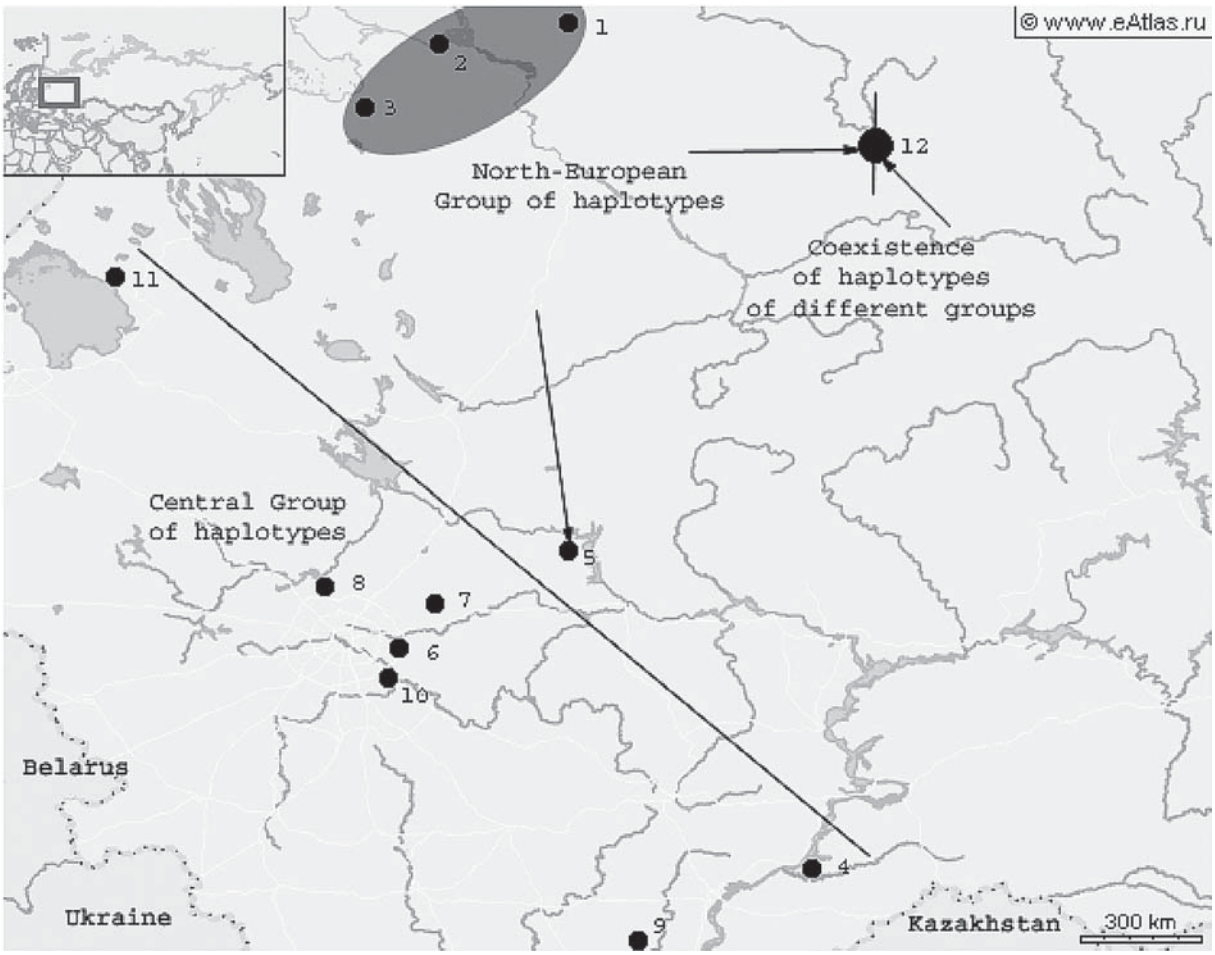


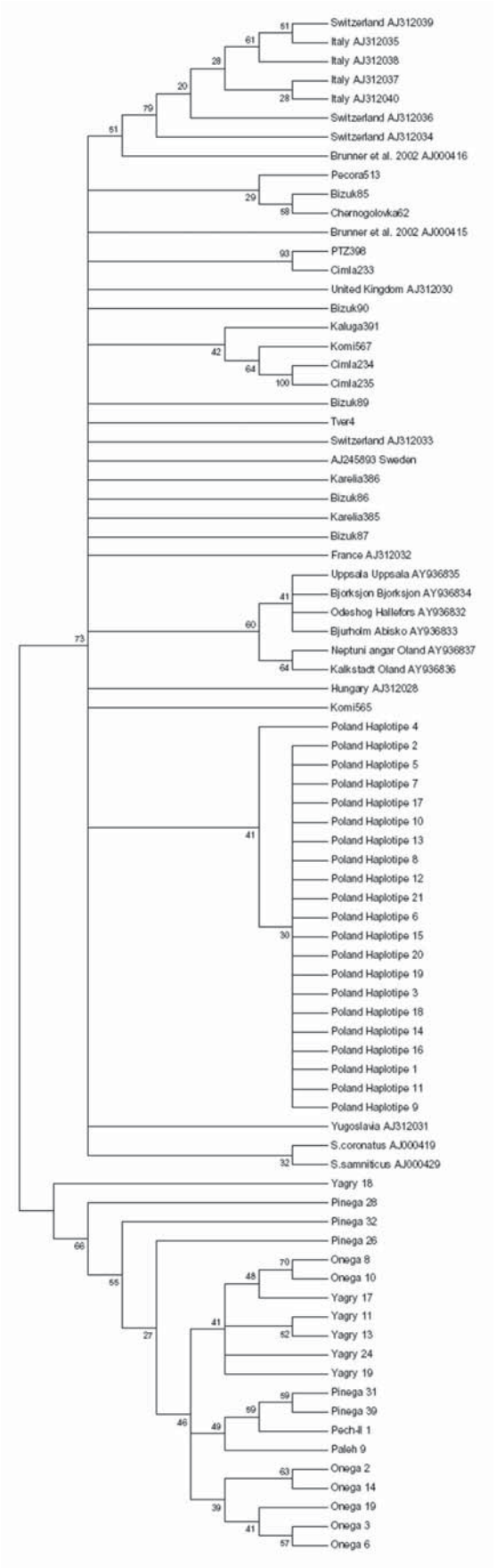
Figure 1. The new sample localities. Numbering follows Tab. 1. The relationships of the groups are explained in the text.

were the same. The overall number of haplotypes was 81 including the GenBank sequence set and a few representatives of *S. antinorii*, *S. coronatus* and *S. samniticus* as outgroups. All comparisons, calculations and estimations were performed for the 954 bp common part of the cytochrome *b* sequence (positions 133-1087) representing 83.7% of the full gene.

We found that 786 (82.4%) of these 954 sites were conservative, 168 (17.6%) were variable, 77 (8%) were parsimony informative and 91 (9.54%) were singletons. The average d-value (Kimura 2P) was 0.015±0.003, which is three times larger than values obtained previously (Taberlet *et al*., 1994, Ratkiewicz *et al*., 2002). Haplotype diversity was also high: h=0.933. The transition/transversion ratio, R=s/v, was equal to 4.25±0.989. Such a level of genetic diversity exceeded data obtained in previous investigations in Poland and Sweden up to 5 times. Samples from the northern part of European Russia were the most diverse. These samples originated mostly from the coast of White Sea and adjacent territories. However, samples from Karelia were monomorphic and obviously different from them.

Based on the nucleotide sequences available, three types of trees (NJ, ME and MP) were constructed. Calculations were based on three different options: taking into account all codon positions, taking into account only the 1st and 2nd positions, and then on the basis of amino acid constitution. The second model was the most reliable and resulted in the tree with the highest bootstrap support.

The topologies of the NJ and ME trees are very similar, both showing a clear subdivision into two main groups. The NJ tree, the one with the highest bootstrap values, is shown in Fig. 2. The first group combines the haplotypes from shrews inhabiting the northeastern regions of European Russia (Yagry race, Kirillov race, Pinega population, some individuals from the Serov race and one individual from the most north-eastern site of the Moscow race). Surprisingly, this group separated from the others even earlier than the *S. coronatus* and *S. samniticus* clades. All other haplotypes form a second phylogenetic group in which interrelations between haplotypes are often not clearly resolved because of low bootstrap values (<50%). As a part of this cluster, Swedish, Polish and some of Alpine haplotypes form a clear grouping. The numerous groups of haplotypes from the central part of European Russia do not form any clear subgroups corresponding to racial characteristics or geographic distribution.



A second neighbour-joining analysis was performed after combining haplotypes geographically into five groups of *Sorex araneus* (Central, North-eastern, Polish, Alpine and Swedish) and *S. antinorii* (Fig. 3). Corresponding nucleotide diversities are shown in Tab. 2.

All Polish samples form a single cluster in the neighbour-joining analysis (Fig. 3). One sample from the Komi Republic is close to this cluster. Swedish samples mostly group into a single clade with one unresolved individual. The two alpine groups (*S. araneus* and *S. antinorii*) together form a well-supported branch with a single individual external to this clade. It should be noted that the Central Russian haplotypes (irrespectively to the race) cluster more or less close to Polish or Swedish haplotypes with a small group separated more distantly; these latter haplotypes derive from a variety of geographical locations. The group of haplotypes that originated from north and north-eastern parts of European Russia, which we call the "North-eastern Group" (NE), is the only clade showing a similar branching mode in all trees and forming the most external group with respect to all other haplotypes, and even to *S. coronatus* and *S. samniticus*. This suggests that they diverged very early. The level of differentiation of the population forming this group is visibly higher than in any other group (Tab. 3).

We had to reject the null hypothesis of neutrality in the pooled sampling for all samples by Tajima's test both with respect to nucleotides ($D=-2.06$, $p=0.04$) and amino acids ($D=-7.67$, $p=0.04$). The Kumar's Z-test for selection provides the same result for overall samples, NE, Alpine and Swedish haplotypes. These results suggest recent and substantial population expansions for the common shrew.

It is necessary to emphasise that haplotypes from the same race in many cases are scattered around the phylogeny. For example, the Moscow race is represented by four haplotypes obtained from four individuals caught in four different localities. One of these haplotypes is very similar to the haplotypes that belong to the NE group; others were situated closely to the Scandinavian cluster, while another was near the Alpine cluster. Despite the relatively small number of samples, the tremendous genetic diversity of haplotypes is evident, especially among the NE haplotypes (Tab. 2). Another example of this kind relates to haplotypes obtained from the Pechora-Ilych Nature Reserve, in the south of the Komi Republic. Four individuals were trapped within a large but geographically poorly subdivided area, which was thought to be inhabited by the Serov race described from this locality. Nonetheless, one of these haplotypes grouped with the Central haplotypes, another with Polish haplotypes while the third is found within the NE group. These data on the one hand point to a huge

Figure 2. Neighbour-joining tree, as inferred from 954 bp cytochrome *b* gene sequences for 75 *Sorex araneus* haplotypes and six individuals representing sibling species. Bootstrap values are shown at the branch nodes. Locality affiliations relate to Tab. 1.

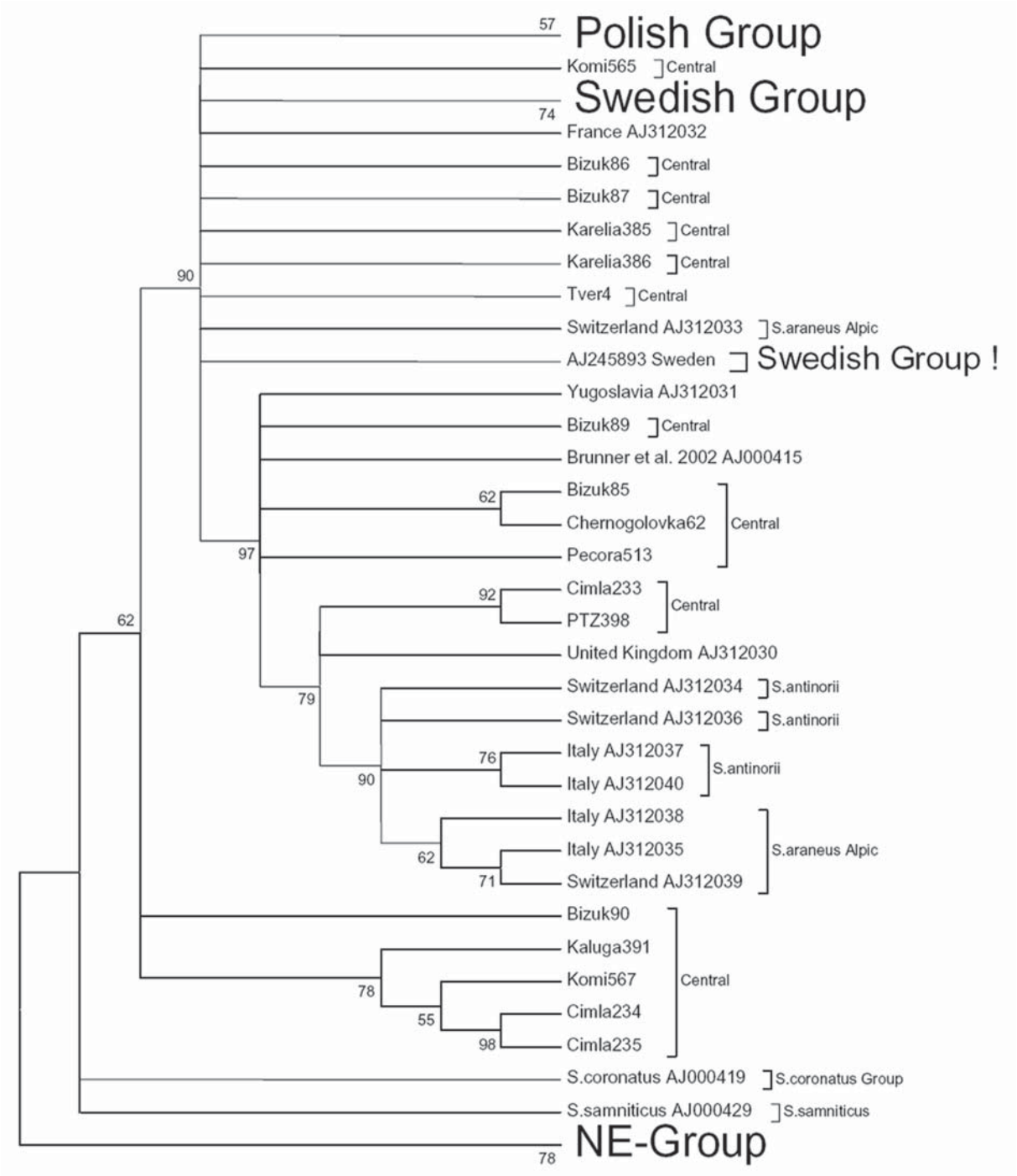


Figure 3. Neighbour-joining tree of 954 bp cytochrome *b* gene haplotypes of *S. araneus*, after grouping haplotypes geographically. Bootstrap values are shown at the branch nodes. Locality affiliations relate to Tab. 1.

genetic heterogeneity of this geographic population, and on the other hand allow us to doubt the accuracy of the racial affiliation. However, as mentioned above, the Moscow race confirms that a high level of genetic divergence can be found within one race.

In view of the distinctiveness of the NE group of haplotypes, particular attention should be focused on these samples. The cytochrome *b* data suggest that a special status should be assigned to these populations as has been done for *S. antinorii*. However, this approach has to be rejected on the basis of karyology and morphology; they undoubtedly should be classified as *S. araneus*.

Thus, we believe that there is only one geographic population of common shrew within European Russia that shows signs of genetic isolation. This is the above-

Table 2. Nucleotide diversities of five geographic groups of *Sorex araneus* (including *S. antinorii*).

Group	*N*	D±S.E. ($1^{st}+2^{nd}+3^{rd}$ codon position)	D±S.E. ($1^{st}+2^{nd}$ codon position)	D±S.E. (amino acid constitution)
S. araneus Central	17	0.012±0.002	0.008±0.001	0.018±0.004
S. araneus North-eastern	19	0.015±0.002	0.012±0.002	0.023±0.004
S. araneus Polish	18	0.004±0.001	0.001±0.000	0.001±0.000
S. antinorii	4	0.009±0.002	0.001±0.001	0.001±0.001
S. araneus Alpine	4	0.013±0.002	0.004±0.002	0.004±0.002
S. araneus Swedish	6	0.004±0,001	0.002±0.001	0.003±0.002
Entire *S. araneus* sample	75	0.015±0.002	0.010±0.002	0.015±0.003

Table 3. Mean Kimura 2-parameter distances between groups of *Sorex araneus* (including *S. antinorii*, D±S.E.). D values in lower left, S.E. values in upper right.

Group	Group number	1	2	3	4	5	6
S. araneus Central	1		0.004	0.002	0.004	0.003	0.002
S. araneus North-eastern	2	0.024		0.004	0.005	0.005	0.004
S. araneus Polish	3	0.011	0.020		0.004	0.003	0.002
S. antinorii	4	0.028	0.039	0.024		0.002	0.004
S. araneus Alpine	5	0.023	0.033	0.019	0.011		0.003
S. araneus Swedish	6	0.010	0.020	0.006	0.024	0.019	

mentioned NE group with a range from the coast of White Sea eastwards to the Pechora River basin at least. Precise limits of this phylogenetic group and especially the location and character of the contact zone with other European Russia groups of haplotypes are unknown and require further investigation.

Conclusion

Our data in the context of previous findings provide strong evidence for considerable genetic exchange between chromosomal races and suggest a lack of bottlenecking in the evolution of *Sorex araneus* populations. Ratkiewicz *et al*. (2002) showed the same for Polish populations. Our data suggest that the nucleotide and haplotype diversity within *Sorex araneus* in Europe is much higher than previously estimated and that this diversity is independent on the karyological diversity. At the same time we discovered a very different population to the north of the Russian Plains (the NE group). It is necessary to specify the taxonomical status of this group. We do not believe that it should be regarded as a separate species, though there are some reasons for such a consideration. These animals belong to typical *S. araneus* morphologically and karyologically. The range of distribution of the NE haplotypes and the extent to which their occurrence reflects geographical isolation is unknown and will be considered further in Orlov *et al*. (2007).

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References

Andersson A.-C., Alström-Rapaport C. & Fredga K. 2005. Lack of mitochondrial DNA divergence between chromosome races of the common shrew, *Sorex araneus,* in Sweden. Implication for interpreting chromosomal evolution and colonization history // Molecular Ecology. Vol.14. P.2703–2716.

Bannikova A.A., Bulatova N.S., Krysanov E.Y. & Kramerov D.A. 2003. DNA polymorphism within *Sorex araneus* and two congeneric species as inferred from inter-SINE-PCR // Mammalia. Vol.67. P.263–274.

Brünner H., Lugon-Moulin N., Balloux F., Fumagalli L. & Hausser J. 2002a. A taxonomical revaluation of the chromosome race Valais of the common shrew *Sorex araneus* (Insectivora: Soricidae) // Acta Theriologica. Vol. 47. P. 245–275.

Brünner H., Turni H., Kapischke H.-J., Stubbe M. & Vogel P. 2002b. New *Sorex araneus* karyotypes from Germany and the postglacial recolonization of central Europe // Acta Theriologica. Vol.47. P.277–293.

Brünner H. & Hausser J. 1996. Genetic and karyotypic structure of a hybrid zone between the chromosomal races Cordon and Valais in the common shrew, *Sorex araneus* // Hereditas. Vol.125. P.147–158.

Catzeflis F., Graf J.D., Hausser J. & Vogel. P. 1988. Comparaison biochimique des musaraignes du genre *Sorex* en

Europe occidentale (Soricidae, Mammalia) // Zeitschrift für Zoologische Systematik und Evolutionsforschung. Vol.20. P.223–233.

Frykman I. & Bengtsson B.O. 1984. Genetic differentiation in *Sorex*. III. Electrophoretic analysis of a hybrid zone between two karyotypic races of *Sorex araneus* // Hereditas. Vol.100. P.259–270.

Frykman I. & Simonsen V. 1984. Genetic differentiation in *Sorex*. II. An electrophoretic comparison between *Sorex araneus* and three other shrew species // Hereditas. Vol.100. P.155–160.

Frykman I., Simonsen V. & Bengtsson B.O. 1983. Genetic differentiation in *Sorex*. I. Electrophoretic analysis of the karyotypic races of *Sorex araneus* in Sweden // Hereditas. Vol.99. P.279–292.

Fumagalli L., Taberlet P., Stewart D.T., Gielly L., Hausser J. & Vogel P. 1999. Molecular phylogeny and evolution of *Sorex* shrews (Soricidae: Insectivora) inferred from mitochondrial DNA sequence data // Molecular Phylogenetics and Evolution. Vol.11. P.222–235.

George S.B. 1988. Systematics, historical biogeography and evolution of the genus *Sorex* // Journal of Mammalogy. Vol.69. P.443–461.

Hall T.A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT // Nuclear Acids Symposium Series. Vol.41 P.95–98.

Irwin D., Kocher T.D. & Wilson A.C. 1991. Evolution of the cytochrome *b* gene of mammals // Journal of Molecular Evolution. Vol.32. P.128–144.

Kimura M. 1980.A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences // Journal of Molecular Evolution. Vol.16. P.111–120.

Kocher T.D., Thomas W.K., Meyer A., Edwards S.V., Pääbo S., Villablanca F. & Wilson A. 1989. Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers // Proceedings of the National Academy of Sciences of the USA. Vol.86. P.6196–6200.

Kumar S., Tamura K. & Nei M. 1993. MEGA: Molecular Evolutionary Genetics Analyses. Ver.1.02. Pennsylvania State University, University Park, PA.

Kumar S., Tamura K. & Nei M. 2004. MEGA3: Integrated software for Molecular Evolutionary Genetics Analysis and sequence alignment // Briefings in Bioinformatics. Vol.5. P.150–163.

Lugon-Moulin N., Wyttenbach A., Brünner H., Goudet G. & Hausser J. 1996. Study of gene flow through a hybrid zone in the common shrew (*Sorex araneus*) using microsatellites // Hereditas. Vol.125. P.159–168.

Lugon-Moulin N., Brünner H., Balloux F., Hausser J. & Goudet G. 1999a. Do riverine barriers, history or introgression shape the genetic structuring of the common shrew (*Sorex araneus*) population? // Heredity. Vol.83. P.155–161.

Lugon-Moulin N., Brünner H., Wyttenbach A., Hausser J. & Goudet G. 1999b. Hierarchical analysis of genetic differentiation in a hybrid zone of *Sorex araneus* (Insectivora, Soricidae) // Molecular Ecology. Vol.8. P.419–432.

Mouchaty S.K., Gullberg A., Janke A. & Arnason U. 2000.The phylogenetic position of the Talpidae within Eutheria based on analysis of complete mitochondrial sequences // Molecular Biology and Evolution. Vol.17. P.60–67.

Neet C.R. & Hausser J. 1991. Biochemical analysis and determination of living individual of the Alpine karyotypic races and species of the *Sorex araneus* group // Mémoires Société Vaudoises Sciences Naturelles. Vol.19. P.97–106.

Nevo E. 1978.Genetic variation in natural populations: patterns and theory // Theory of Population Biology. Vol.13. P.121–177.

Orlov V.N., Kozlovsky A.I., Okulova N.M. & Balakirev A.E. 2007. Postglacial recolonisation of European Russia by the common shrew *Sorex araneus* // Russian Journal of Theriology. Vol.6. P.97–104.

Ratkiewicz M., Banaszek A. & Łobodzińska J. 1994. Isoenzyme variation in the common shrew (*Sorex araneus*) from the hybrid zone between the chromosomal races Drużno and Łęgucki Młyn: preliminary results // Folia Zoologica. Vol.43. Suppl.1. P.21–28.

Ratkiewicz M., Banaszek A., Jadwiszczak K., Chętnicki W. & Fedyk S. 2003. Genetic diversity, stability of population structure and barriers to gene flow in a hybrid zone between two *Sorex araneus* chromosome races // Mammalia. Vol.67. P.275–283.

Ratkiewicz M., Fedyk S., Banaszek A., Chętnicki W., Szałaj K., Gielly L. & Taberlet P. 2002. The evolutionary history of the two karyotypic groups of the common shrew, *Sorex araneus*, in Poland // Heredity. Vol.88. P.235–242

Ratkiewicz M., Wójcik J., Lukačova L. & Macholan M. 1996. Nomenclature of electrophoretically studied loci and allozymes in the common shrew (*Sorex araneus* L.) // Abstracts of 4th ISACC Meeting. Uppsala, August 22–27, 1996. P.34–35.

Searle J.B. & Wójcik J.M. 1998. Chromosomal evolution: the case of *Sorex araneus* // Wójcik J.M. & Wolsan M. (eds.). Evolution of Shrews. Białowieża: Mammal Research Institute, Polish Academy of Sciences. P.219-268.

Taberlet P., Fumagalli L. & Hausser J. 1991. MtDNA comparison of Alpine chromosomal races and species of the *Sorex araneus* group: preliminary results // Mémoires Société Vaudoises Sciences Naturelles. Vol.19. P.107–118.

Taberlet P., Fumagalli L. & Hausser J. 1994. Chromosomal versus mitochondrial DNA evolution: tracking the evolutionary history of the southwestern European populations of the *Sorex araneus* group (Mammalia, Insectivora) // Evolution. Vol.43. P.623–636.

Tajima F. 1989. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism // Genetics. Vol.123. P.585–595.

Wójcik J.M. 1993. Chromosome races of the common shrew *Sorex araneus* in Poland: a model of karyotype evolution // Acta Theriologica. Vol.38. P.315–338.

Wójcik J.M. & Wójcik A.M. 1994. Protein variation in the common shrew (*Sorex araneus* L.) in Poland, in relation to karyotype // Folia Zoologica. Vol.43. Suppl.1. P.53–61.

Wójcik J.M., Ratkiewicz M. & Searle J.B. 2002. Evolution of the common shrew *Sorex araneus*: chromosomal and molecular aspects // Acta Theriologica. Vol.47. Suppl.1. P.139–167.

Wyttenbach A. & Hausser J. 1996. The fixation of metacentric chromosomes during the chromosomal evolution in the common shrew (*Sorex araneus*, Insectivora) // Hereditas. Vol.125. P.209–217.

Wyttenbach A., Narain Y. & Fredga K. 1999.Genetic structuring and gene flow in a hybrid zone between two chromosome races of the common shrew (*Sorex araneus*, Insectivora) revealed by microsatellites // Heredity. Vol.82. P.79–88.

Zima J., Fedyk S., Fredga K., Hausser J., Mishta A., Searle J.B., Volobouev V.T. & Wójcik J.M. 1996. The list of the chromosome races of the common shrew (*Sorex araneus*) // Hereditas. Vol.125. P.97–107.