



ЭКОЛОГИЯ

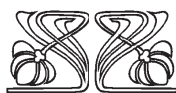
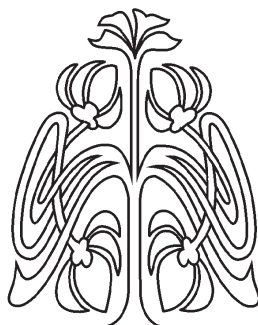
Известия Саратовского университета. Новая серия. Серия: Химия. Биология. Экология. 2024. Т. 24, вып. 3. С. 328–333

Izvestiya of Saratov University. Chemistry. Biology. Ecology, 2024, vol. 24, iss. 3, pp. 328–333

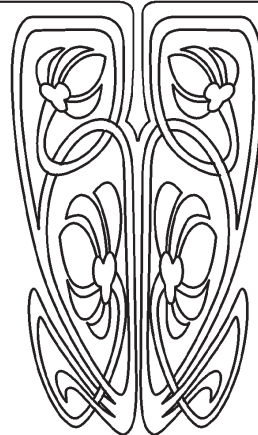
<https://ichbe.sgu.ru>

<https://doi.org/10.18500/1816-9775-2024-24-3-328-333>

EDN: WXNEKL



НАУЧНЫЙ
ОТДЕЛ



Article

The role of unequilibrium dynamics of the number and genetic structure of populations in interspecific interactions of the russet (*Spermophilus major* Pall.) and the speckled (*Spermophilus suslicus* Güld.) ground squirrels in a wide zone of sympatry

N. A. Kartavov , O. V. Chernyshova, S. S. Zaks, S. V. Titov

Penza State University, 40 Krasnaya St., Penza 440026, Russia

Nikita A. Kartavov, nikitakartavov@yandex.ru, <https://orcid.org/0000-0001-9480-7523>

Olga V. Chernyshova, oliarabbit@yandex.ru, <https://orcid.org/0009-0007-8287-9221>

Svetlana S. Zaks, s.s.zaks@yandex.ru, <https://orcid.org/0009-0000-7305-5916>

Sergey V. Titov, svtitov@yandex.ru, <https://orcid.org/0000-0001-9634-1157>

Abstract. The dynamics of the number and genetic structure of populations is one of the leading factors determining the success of the existence of populations in time and space. Based on observations (changes in numbers) and molecular genetic analysis data (*CR* and *cyt b*), the dynamics of the demographic and genetic structure of the populations of russet (*Spermophilus major* Pall.) and speckled (*S. suslicus* Güld.) ground squirrels in the sympatry zone were studied. A similar cyclical nature of the temporal dynamics of the genetic structure of populations in both sympatric species is shown. In the russet ground squirrel, a more ecologically plastic species and prone to migratory activity, population genetic processes occur on a large scale. The explosive demographic situation associated with the passage of populations through the “bottleneck” state is one of the factors leading to interspecific hybridization.

Key words: population dynamics, russet ground squirrel, speckled ground squirrel, hybridization

Acknowledgements. The study was supported by Russian Science Foundation project No. 22-24-00108, <https://rscf.ru/project/22-24-00108/>.

For citation: Kartavov N. A., Chernyshova O. V., Zaks S. S., Titov S. V. The role of unequilibrium dynamics of the number and genetic structure of populations in interspecific interactions of the russet (*Spermophilus major* Pall.) and the speckled (*Spermophilus suslicus* Güld.) ground squirrels in a wide zone of sympatry. *Izvestiya of Saratov University. Chemistry. Biology. Ecology*, 2024, vol. 24, iss. 3, pp. 328–333 (in Russian). <https://doi.org/10.18500/1816-9775-2024-24-3-328-333>, EDN: WXNEKL

This is an open access article distributed under the terms of Creative Commons Attribution 4.0 International License (CC-BY 4.0)



Научная статья
УДК 591.158.1

Роль неравновесной динамики численности и генетической структуры популяций в межвидовых взаимодействиях большого (*Spermophilus major* Pall.) и крапчатого (*Spermophilus sulicus* Güld.) сусликов в широкой зоне симпатрии

Н. А. Картавов ✉, **О. В. Чернышова**, **С. С. Закс**, **С. В. Титов**

Пензенский государственный университет, Россия, 440026, г. Пенза, ул. Красная, д. 40

Картавов Никита Александрович, аспирант кафедры «Зоология и экология», nikitakartavov@yandex.ru, <https://orcid.org/0000-0001-9480-7523>

Чернышова Ольга Валерьевна, кандидат биологических наук, доцент кафедры «Зоология и экология», oliarabbit@yandex.ru, <https://orcid.org/0009-0007-8287-9221>

Закс Светлана Сергеевна, кандидат биологических наук, доцент кафедры «Зоология и экология», s.s.zaks@yandex.ru, <https://orcid.org/0009-0000-7305-5916>

Титов Сергей Витальевич, доктор биологических наук, профессор, декан факультета физико-математических и естественных наук, заведующий кафедрой "Зоология и экология", svtitov@yandex.ru, <https://orcid.org/0000-0001-9634-1157>

Аннотация. Динамика численности и генетической структуры популяций являются одними из ведущих факторов, определяющих успешность существования популяций во времени и пространстве. На основе наблюдений (изменения численности) и данных молекулярно-генетического анализа (*CR* и *cyt b*) изучена динамика демографической и генетической структуры популяций большого (*Spermophilus major* Pall.) и крапчатого (*S. suslicus* Güld.) сусликов в зоне симпатрии. Показана схожая цикличность временной динамики генетической структуры популяций у обоих симпатрических видов. У большого суслика, как экологически более пластичного вида и склонного к миграционной активности, популяционно-генетические процессы происходят в больших масштабах. Взрывная демографическая ситуация, связанная с прохождением популяций состояния «бутылочного горлышка», является одним из факторов, приводящих к межвидовой гибридизации.

Ключевые слова: динамика популяции, большой суслик, крапчатый суслик, гибридизация

Благодарности. Работа выполнена при финансовой поддержке Российского научного фонда (проект № 22-24-00108, <https://rscf.ru/project/22-24-00108/>).

Для цитирования: Kartavov N. A., Chernyshova O. V., Zaks S. S., Titov S. V. The role of unequilibrium dynamics of the number and genetic structure of populations in interspecific interactions of the russet (*Spermophilus major* Pall.) and the speckled (*Spermophilus suslicus* Güld.) ground squirrels in a wide zone of sympatry [Картавов Н. А., Чернышова О. В., Закс С. С., Титов С. В. Роль неравновесной динамики численности и генетической структуры популяций в межвидовых взаимодействиях большого (*Spermophilus major* Pall.) и крапчатого (*Spermophilus sulicus* Güld.) сусликов в широкой зоне симпатрии] // Известия Саратовского университета. Новая серия. Серия: Химия. Биология. Экология. 2024. Т. 24, вып. 3. С. 328–333. <https://doi.org/10.18500/1816-9775-2024-24-3-328-333>, EDN: WXNEKL

Статья опубликована на условиях лицензии Creative Commons Attribution 4.0 International (CC-BY 4.0)

Introduction

The dynamics of the demographic and genetic structure of populations is one of the leading factors determining the success of the existence of populations over time and the maintenance of their species specificity in conditions of a changing polymorphic profile [1, 2]. Ground squirrels, as edificatory species of steppe biogeocenoses, on the one hand, in recent decades have experienced a strong decline in numbers and associated large-scale restructuring of the structure of their habitats, on the other hand, they are a convenient model for studying population dynamics. At the same time, the ground squirrel species living in the Volga region enter into interspecific interactions at points of contact, up to successful hybridization [3, 4]. The study of ground squirrels species genetic structure of populations in time dynamics in the regions has not yet been carried out. Similar studies have not been carried

out in other parts of the range of the studied species. Only the results of studying the genetic structure of speckled ground squirrel populations are known due to the strong fragmentation of the European part of the range [5]. The purpose of the work was to study the dynamics of the genetic structure of populations of two sympatric species of ground squirrels – russet (*Spermophilus major* Pall.) and speckled (*S. suslicus* Güld.) – in the Middle Volga region using the example of two single-species colonies in the Ulyanovsk region.

Material and methods

To analyze the dynamics of the population structure of the russet and speckled ground squirrels, the results of studies of two isolated model populations of ground squirrels in different time periods of their existence were used – *S. major* (Chirikovo village, Kuzovatovsky district, Uly-



anovsk region; 2010, $n = 7$ and 2018, $n = 10$; 53.7472 N, 47.8277 E) and *S. suslicus* (Trubetchina village, Kuzovatovsky district, Ulyanovsk region; 2016, $n = 7$ and 2022, $n = 6$; 53.6429 N, 47.8695 E). To exclude the influence of environmental (biotopic, seasonal, climatic) factors on the studied populations, closely located colonies (about 11 km) were selected from known settlements of these ground squirrel species in a wide zone of sympatry.

The population dynamics in settlements was studied using the method of total trapping in a designated area. DNA was isolated from tissue samples fixed after a biopsy (a piece of the auricle or fingertips) in ethanol (96%) according to the generally accepted chloroform-phenol method [6, 7]. Manipulations with live animals and collection of biomaterial were carried out using non-invasive methods, taking into account Directive 2010/63/EU of the European Parliament and the Council of the European Union for the Protection of Animals Used for Scientific Purposes (Articles 9 and 42). Material collection methods complied with recommendations of ASAB/ABS, *Guidelines for the treatment of animals in behavioural research and teaching* [8] and were approved by the Local Ethics Committee at Penza State University (protocol No. 7 of 03.23.2023). Squirrels were captured using non-commercial homemade wire cage traps. Each caught animal was immediately removed from the trap, and its sex and age were determined. The animal was weighed, measured and marked with a microchip (Bayer AG, Leverkusen, Germany) and a black paint mark (urzol) in the form of individually recognizable patterns on the fur.

The genetic structure of populations and its dynamics over time were studied by changes in the polymorphism of two mtDNA markers – fragments of the control region (CR) and the *Cyt b* gene. Cloning of mtDNA fragments was carried out according to standard methods using primer systems specific to ground squirrels [9]. Sequencing was carried out on a sequencer ABI 3500 (Applied Biosystems) using a set of reagents BigDye® Terminator v. 3.1 Cycle Sequencing Kits during initial sample preparation in a thermal cycler SimpliAmp™ Thermal Cycler. Nucleotide sequences were read and edited using the program BioEdit 7.1.3.0 [10]. Alignment of nucleotide sequences was carried out according to the algorithm *Clustal W* in the program Mega X with mandatory manual modification [11]. To determine phylogeographic interactions, we used the maximum likelihood (ML) method in the program MEGA X [12]. Haplotype and nucleotide diversity

were studied using the program DnaSP 5.10.01 and PopArt [13, 14]. The obtained sequences of the isolated mtDNA haplotypes (CR and *Cyt b*) were placed in GeneBank NCBI (OP896081–OP896085; OP896086–OP896090). Statistical data processing was carried out in the program *Statistica for Windows 13.3* (StatSoft.® Russia, SN: AXA9081943629FAACD-A). Parametric comparison criteria were used in the analysis.

Results and Discussion

Long-term (2000–2023) tracking of the model populations of the russet and speckled ground squirrel in the sympatric zone showed that all of them are subject to more or less large-scale changes in numbers [4]. The russet ground squirrel is characterized by an 8-year cycle (2010–2018), associated with almost identical maximums of population density (10 ind/ha and 11 ind/ha, respectively) and a minimum (2015) of 1 ind/ha (Chirikovo village). The speckled ground squirrel has a less extended 6-year cycle (2016–2022), which is characterized by unequal maximums (10 ind/ha and 14 ind/ha, respectively) and minimum (2018) population density of 2 ind/ha (Trubetchina village).

To study the dynamics of the genetic structure of the populations of the russet and spotted ground squirrels, a phylogenetic analysis (ML-model) of the relationships of the identified haplotypes was carried out. In general, similar results were obtained for the two mitochondrial markers analyzed (CR, *cyt b*). To illustrate this, we present an ML tree of relations between haplotypes of the control region (CR) (Fig. 1). The dendrogram shows that specimens of the population samples of *S. major* and *S. suslicus*, characterizing the population in different years, are clustered differently. If the russet ground squirrel has a clearly expressed grouping of samples by year, then in *S. suslicus* clusters are formed with a mixture of samples from samples taken at different times.

The results of the analysis of genetic diversity and genetic differentiation of the population of ground squirrels colonies by year are presented in the table. Testing of sequences (*Tajima's D* and *Fu's Fs*) of two time samples of the russet ground squirrel population according to the degree of their differentiation indicates the action of stabilizing selection against the background of rapid population growth after the population passed through the “bottleneck” state. The analyzed temporary populations of the speckled ground squirrel are poorly differentiated

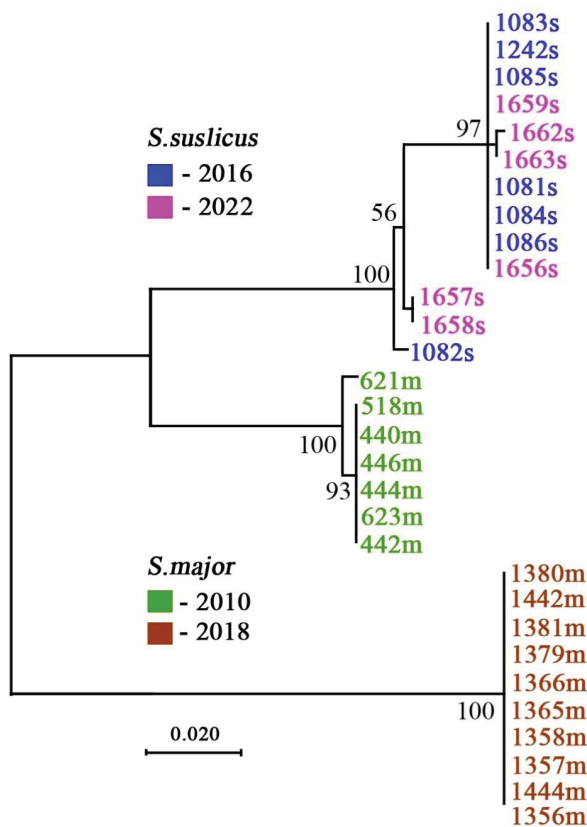


Fig. 1. Results of ML analysis (model HKY + G, BIC = 5033.41, InL = -2196.43) of nucleotide sequences ($n = 30$) of the mtDNA CR region (1036 bp), showing phylogenetic interactions between haplotypes for mtDNA characterizing model populations of a russet (Chirikovo village – Ch1, 2010; Ch2, 2018) and speckled (Trubetchina village – Tr1, 2016, Tr2, 2022) ground squirrels. The nodes of the tree show the results of bootstrap analysis (1000 replicas), the scale shows genetic distances between haplotypes (color online)

and hardly differ from each other, except for their haplotype structure. At the same time, this species has a cyclical change in population size with a low rate of restructuring of the genetic structure.

Analysis of haplotype and nucleotide diversity of mtDNA (CR) species samples gave the following results. In the russet ground squirrel sample, the haplotype diversity (Hd) was 0.5588, and 3 haplotypes were identified with a nucleotide diversity (π) of 0.0557 and an average number of nucleotide substitutions (K) 56.24. Moreover, for the 2010 sample, 2 haplotypes were identified ($n = 7$), and for the 2016 sample, only one haplotype ($n = 10$). *Tajima's D* and *Fu's Fs* tests revealed low levels of low- and high-frequency polymorphisms, indicating the action of balancing selection and stabilization of numbers after rapid growth (*Tajima's D* = 3.041, $p < 0.001$; *Fu's Fs* = 30.995, $p < 0.001$). For the speckled ground squirrel sample, significantly lower genetic indicators were obtained ($Hd = 0.5256$; $\pi = 0.0037$; $K = 3.7692$) and 4 haplotypes were identified – two each for 2016 and 2022. Tests by *Tajima's D* and *Fu's Fs* revealed that the number of identified haplotypes corresponded to the number of segregating sites, which indicates a possible genetic equilibrium of the population.

To identify the relationship between the detected mtDNA haplotypes (CR) and microevolutionary events, a median network was constructed (Fig. 2). The network is built on the basis of 148 segregating and 89 parsimony-informative sites. The nucleotide diversity (π) of the sequences included in the network was 0.4694. The coefficient of differentiation (F_{ST})

Indicators of genetic diversity (π , K , h , Hd) and differentiation (*Tajima's D* and *Fu's Fs*) populations of russet (Chirikovo village – Ch1, 2010; Ch2, 2018) and speckled (Trubetchina village – Tr1, 2016, Tr2, 2022) ground squirrels (control region, CR and *Cyt b* gene fragment)

Pop	π		K		h		Hd			
	Dloop	Cytb	Dloop	Cytb	Dloop	Cytb	Dloop	Cytb		
<i>Spermophilus major</i>										
Ch1	0.049	0.047	49.64	53.82	5 из 8	0.625	4 out of 9	0.444	0.714	0.573
Ch2	0.029	0.025	29.37	28.67	4 из 8	0.500	6 out of 9	0.667	0.423	0.571
<i>Tajima's D</i>	1.430		3.991		<i>Fu's Fs</i>		21.865		29.836	
p	$p > 0.10$		$p < 0.001$		Strobeck's S statistic (p)		$p < 0.001$		$p < 0.001$	
<i>Spermophilus suslicus</i>										
Tr1	0.003	0.003	3.14	3.90	2 из 5	0.400	4 out of 5	0.800	0.286	0.714
Tr2	0.006	0.003	5.67	3.43	4 из 5	0.800	3 out of 5	0.600	0.867	0.524
<i>Tajima's D</i>	0.192		-1.028		<i>Fu's Fs</i>		2.040		1.589	
p	$p > 0.10$		$p > 0.10$		Strobeck's S statistic (p)		$p > 0.10$		$p > 0.10$	

Note. π – nucleotide diversity, K – average number of nucleotide differences, h – number of haplotypes, Hd – haplotype diversity, *Tajima's D* – Tajima test, *Fu's Fs* – Fu test.

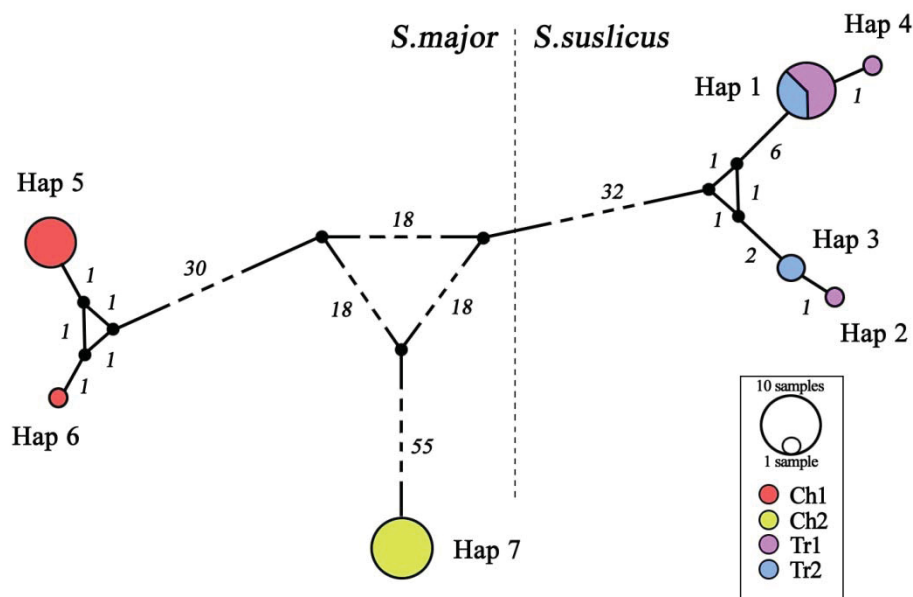


Fig. 2. Median haplotype networks constructed based on sequences of the mtDNA control region (CR, 1036 bp). The numbers next to the branches show the number of mutation events. Colors indicate samples of different species and the time period of existence of the populations of russet (Chirikovo village – Ch1, 2010; Ch2, 2018) and speckled (Trubetchina village – Tr1, 2016, Tr2, 2022) ground squirrels. The size of the circle is proportional to the number of individuals, and the shares are proportional to the sampling frequencies per haplotype (color online)

was equal to 0.998 ($p < 0.0010$), and the Tajima test revealed low levels of low- and high-frequency polymorphisms, i.e. unbalanced ratio of frequencies of mtDNA haplotypes, which indicates a decrease in the number of colonies and population size, as well as the simultaneous or alternative action of balancing selection (*Tajima's D* = 3.312, $p < 0.001$).

The results of the median analysis confirm the data obtained from ML analysis of sequences of the mtDNA control region. However, there is a possibility that the obtained genetic indicators are more related to the divergence of species haplogroups in the discussed median network. To level out this effect, additional analysis of species samples (*S. major* and *S. suslicus*) was carried out using the same algorithm for constructing a median network (MJN). In general, the topographically median networks of species samples correspond to two species branches of the general network (see Fig. 2). Differences were identified only in genetic indicators that describe the relationships between the detected haplotypes. The nucleotide diversity (π) of the *S. major* mtDNA sequences included in the network turned out to be higher than the total and amounted to 0.5713 with 112 segregating sites. The coefficient of differentiation (F_{ST}) was the same – 0.998 ($p < 0.0010$), and the *Tajima's D* indicator was almost equal to the indicator obtained from the analysis of the two-species

sample (3.312, $p < 0.0001$). In contrast, the genetic parameters of the *S. suslicus* sample had lower values. Nucleotide diversity (π) was only 0.3373 with 13 segregating and 10 parsimony-informative sites. The coefficient of differentiation (F_{ST}) had a very low value and was unreliable (0.0305, $p = 0.3550$). The unreliable ($p = 4182$) but close to “0” value of the *Tajima's D* indicator (0.192) indicates that the number of identified haplotypes corresponds to the number of segregating sites, i.e. the expected and observed frequencies of haplotypes correspond to each other, and indicates a possible genetic balance of the population and the absence of signs of selection.

The results of genetic analysis of species samples revealed both similar and specific features of population dynamics for the studied ground squirrel species. Both species show dynamic changes in population structures. The scope and period of cyclicity are greater in *S. major*. At the same time, based on the values of the *Tajima's D* test and qualitative changes in the haplotype structure in samples from different times, it is obvious that the factors leading to population changes in the studied species are different. If for the russet ground squirrel such a factor is migratory activity, then for *S. suslicus* this factor is not of particular importance and is replaced by the direct dynamics of restoration of the number of genetic lines preserved after the depression.



Thus, a similar nature of the temporal dynamics of the genetic structure of populations in both sympatric species is noted. At the same time, in the russet ground squirrel, as the most ecologically plastic species and prone to migratory activity, population genetic processes occur on a large scale. As our studies have shown, it is precisely in those points of the range where there is an explosive demographic situation associated with the passage of populations through the “bottleneck” state and restoration of numbers, including as a result of migration, in the presence of a sympatric species, and cases of inter-specific hybridization are recorded [4].

References

1. Altukhov Yu. P. *Genetic processes in populations*. Moscow, Akademkniga, 2003. 431 p. (in Russian).
2. Hanski I. Habitat connectivity, habitat continuity, and metapopulations in dynamic landscapes. *Environmental Sciences. Oikos*, 1999, November, pp. 209–219. <https://doi.org/10.2307/35467>
3. Titov S. V., Kuzmin A. A., Naumov R. V. *Dynamics of habitats and the current state of settlements of terrestrial squirrels in the right bank regions of the Volga region*. Penza, PSU Publishing House, 2015. 124 p. (in Russian).
4. Titov S. V., Kuzmin A. A., Chernyshova O. V., Kartavov N. A., Simakov M. D. Spatio-temporal and genetic features for a hybrid zone structure arisen over a broad sympatric zone of russet (*Spermophilus major* Pallas, 1778) and speckled (*Spermophilus suslicus* Gldenstaedt, 1770) ground squirrels. *Biology Bulletin*, 2023, vol. 50, no. 3, pp. 400–415. <https://doi.org/10.1134/S106235902260324X>
5. Biedrzycka A., Radwan J. Population fragmentation and major histocompatibility complex variation in the spotted suslik *Spermophilus suslicu*. *Molecular Ecology*, 2008, vol. 17, pp. 4801–4811. <https://doi.org/10.1111/j.1365-294X.2008.03955.x>
6. Arrigi F. E., Bergendahl G., Mandel M. Isolation and characterization of DNA from fixed cells and tissues. *Exp. Cell. Res.*, 1968, no. 50, pp. 47–53.
7. Sambrook J., Fritsch E. F., Maniatis T. *Molecular cloning: A laboratory manual*. New York, Cold Spring Harbor Laboratory Press, 1989. URL: www.cshlpress.com
8. Buchanan K., Burt de Perera T., Carere C., Carter T., Hailey A. Guidelines for the treatment of animals in behavioural research and teaching. *Anim. Behav.*, 2012, vol. 83, pp. 301–309.
9. Titov S. V. *Population and genetic mechanisms of inter-specific hybridization of mammals (using the example of the genus Spermophilus)*. Thesis. Diss. Dr. Sci. (Biol.). Moscow, 2009. 48 p. (in Russian).
10. Hall T. A. BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series*, 1999, vol. 41, pp. 95–98. <https://bioedit.software.informer.com>.
11. *Algorithm Clustal W*. Available at: <http://www.ebi.ac.uk/clustalw/> (accessed March 15, 2023).
12. Kumar S., Stecher G., Li M., Knyaz C., Tamura K. MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. *Molecular Biology and Evolution*, 2018, vol. 35, pp. 1547–1549.
13. Librado P., Rozas J. DnaSP v5: A software for comprehensive analysis of DNA polymorphism data. *Bioinformatics*, 2009, vol. 25, pp. 1451–1452.
14. Leigh J. W., Bryant D., Nakagawa S. POPART: Full feature software for haplotype network construction. *Methods in Ecology and Evolution*, 2015, vol. 6, no. 9, pp. 1110–1116. <https://doi.org/10.1111/2041-210x.12410>

Поступила в редакцию: 15.12.2023; одобрена после рецензирования 09.04.2024; принята к публикации 28.04.2024; опубликована 30.09.2024

The article was submitted 15.12.2023; approved after reviewing 09.04.2024; accepted for publication 28.04.2024; published 30.09.2024