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The Voles Genus *Alexandromys* (Rodentia, Arvicolinae) of the Middle Amur Lowland and Description of Four New Karyotype Variants of *Alexandromys maximowiczii* (Rodentia, Arvicolinae)

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Abstract—Previously, only *Alexandromys fortis* had been found to occur in the northeastern part of the Middle Amur Lowland. Karyotype studies on voles at the northernmost locality of the lowland, near the village of Elban, reveal a second species, *A. maximowiczii*, shifting its eastern range limit 200 km east of the nearest known one. Four karyotype variants found in individuals from the Middle Amur Lowland are described for the first time for Maximowicz's vole, which shows multiple chromosomal polymorphism. Two karyotype variants are found to predominate: $2n = 40$, $NF = 58$ and $2n = 41$a, $NF = 60$, and two that are rare: $2n = 41$b, $NF = 59$ and $2n = 41$c, $NF = 59$. According to the $2n$ and NF numbers, these variants correspond to the chromosomal form "C." The variability of the chromosome number in this species is due to the tandem fusion of metacentric chromosomes no. 3 and no. 4 to form a large metacentric no. 3/4. Centric fusion of acrocentric chromosomes no. 11 and no. 20 to form a medium-sized metacentric chromosome no. 11.20 is generally stabilized, with the exception of one individual of 54 examined. A sharp decrease in heterozygotes for tandem fusion is noted for individuals in the middle part of the lowland, compared to the northeastern part where it was high. The number of chromosomes being 39 is excluded from the chromosomal form "C" as unsubstantiated. Rare variants have one pair of autosomes in the heterozygous state (SM/A). Such variability seems to be associated with a shift in the centromere in chromosome no. 10 in variant 41b and a pericentric inversion in chromosome no. 16 in variant 41c. Based on the previously published data of genetic, allozyme, chromosomal, and molecular–genetic analyses for both species involved, *A. maximowiczii* and *A. fortis,* the chromosomal characteristics and their habitats, including syntopic ones, are presented.

Keywords: habitat, chromosomes, variability, Far Eastern vole, Maximowicz's vole, Amur region, *Alexandromys fortis*

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INTRODUCTION

Many types of gray (*Microtus* Schrank 1798) and East Asian (*Alexandromys* Ognev 1914) voles have weak morphological differences, but unique chromosomal characteristics allow them to be clearly diagnosed (Orlov, 1974; Zagorodnyuk, 1992; Meyer et al., 1996; Orlov et al., 2023). In a number of taxonomic summaries on rodents, data on the number of chromosomes are included in the keys for identifying modern species of voles (Gromov and Polyakov, 1977; Gromov and Erbaeva, 1995; Kostenko, 2000). In the southern regions of the Russian Far East, there are two species of East Asian voles: the Far Eastern vole (*Alexandromys fortis* (Büchner 1889)) (Fig. 1a) and Maximowicz's vole (*Alexandromys maximowiczii* (Schrenck 1859)) (Fig. 1b), which have variable differentiating features, which complicates their diagnosis and, as was shown in the study of vole skull collections (Lisovsky et al., 2018), can lead to erroneous species identification. These species differ clearly in the number and morphology of chromosomes. *A. fortis* has a stable diploid number ($2n = 52$) and small chromosomes. *A. maximowiczii* has a variable number of chromosomes ($2n = 36$–44), the sizes of which vary from large to small. In populations of the southern Russian Far East, this number varies from 39 to 42 (Kartavtseva et al., 2008). Maximowicz's vole has proven to be a difficult object for elucidating intraspecific karyotype variability, since it has multiple chromosomal variability, including tandem, centric (Robertsonian) fusions, inversions, and shift of centromere (Meyer et al., 1996). For the first time, the multiple chromosomal polymorphism of *A. maximowiczii* was discovered for the populations of Transbaikalia (Buryatia), from where thirteen karyotype variants were described (Kovalskaya, 1977) and three chromosomal forms were isolated "A", "B", and "V" (Kovalskaya et al., 1980). The description of karyotype variants was made without differential staining, which led to incorrect identification of chromosomal rearrangements and,

accordingly, karyotype variants (Meyer et al., 1996). An analysis of chromosomal characteristics was carried out for 242 Maximowicz's voles from Transbaikalia, the Russian Far East, and Mongolia. From this sample, six chromosomal forms "A", "B", "V", "D", "C" and "I" were isolated from 74 new individuals from 12 previously unstudied local populations of Transbaikalia and the Russian Far East. The first three chromosomal forms were previously isolated for voles in Transbaikalia (Kovalskaya et al., 1980), then another chromosomal form ("D") was isolated for this region (Kartavtseva et al., 2008). For populations of the southern Russian Far East, two new chromosomal forms were identified: "C" and "I" (Kartavtseva et al., 2008). Later, voles of the chromosome form "I" (subspecies *Microtus maximowiczii gromovi* Vorontsov 1988) were removed from the composition of *M. maximowiczii* and are now identified as Gromov's vole (*Alexandromys shantaricus* Ognev 1929 (Sheremetyeva, 2023)).

It should be noted that, in these two works, the chromosomal forms differed only in the values 2*n* and *NF*, without indicating the nature of chromosomal rearrangements and description of chromosomal variants of the karyotype. The use of the method of differential G-staining of chromosomes of voles from three populations from Buryatia, Zabaikals, and Khabarovsk krais made it possible to describe seven chromosomal rearrangements for the species, leading to a change in the number and morphology of chromosomes (Meyer et al., 1996). Three of the seven rearrangements were structural: one was a tandem fusion and two were centromeric fusions. In this work, the nature of chromosomal rearrangements in three populations of Maximowicz's vole (two populations from Transbaikalia and one population from the Russian Far East) was identified. The number of individuals studied was small, which did not allow the authors to describe karyotype variants of chromosomal forms.

Data on the number of chromosomes in the karyotype of the Maximowicz's vole from the Middle Amur Lowland were published in three works, where the method of differential staining was used without specifying the numbers of chromosome pairs (Meyer et al., 1996; Kartavtseva et al., 2008; Frisman et al., 2011). The sequence of chromosomes and their groups (two-armed and one-armed) differed in these studies. In the first work, the karyotype ($2n = 39–41$) is represented by two morphological groups of chromosomes: two-armed (metacentric, submeta-, and subtelocentric) and one-armed (acrocentric). In the second work, the karyotype ($2n = 40–41$) is represented by three groups of chromosomes: two groups with two-armed (metacentric and submeta-subtelocentric) chromosomes and one group with one-armed (acrocentric) chromosomes. In the third work, the karyotype ($2n = 40–41$) is represented by four groups of chromosomes: metacentric, submetacentric, subtelocentric, and acrocentric. The same numbers and groups of chromosomes as in the last work, without differential staining, are given for the Khabarovsk population (Kartavtseva et al., 2017).

Using the method of molecular cytogenetics, fluorescent in situ hybridization (FISH) of probes of whole vole chromosomes of *Microtus agrestis* (Linnaeus 1761) on metaphase chromosomes of Maximowicz's vole, made it possible to identify species-specific conservative chromosomal segments. Differential G-staining made it possible to determine the numbers of chromosome pairs in the karyotype of *A. maximowiczii* (from $2n = 41$, *NF*a = 54) and show two structural rearrangements—tandem (no. 3/4) and centromeric (no. 11.20) chromosome fusions (Lemskaya et al., 2010). The chromosome numbers in this work were based on the maximum number of chromosomes ($2n = 44$), which do not have structural rearrangements, which made it convenient to use such numbering for further study of chromosomal variability of the species and identification of chromosomal rearrangements. In this work, the point of capture of one female Maximowicz's vole is not indicated, but the oral communication of F.N. Golenishchev, who provided this specimen for karyotyping, suggests that the vole was caught in the Transbaikal territory and possibly corresponds to the chromosomal form "A". In order to understand which chromosome numbers accompanied the structural rearrangements of the Maximowicz's vole of the chromosomal form "C", the karyotypes of individuals from the population of the Norskii Reserve in the Amur Region were studied using the FISH and G-chromosome staining methods (Kartavtseva et al., 2013). Moreover, the karyotypes of these individuals were previously studied and included in the work (Kartavtseva et al., 2008) devoted to the isolation of chromosomal forms. According to the data presented, the same two structural rearrangements are characteristic of this karyotype of individuals of this population and for the karyotype of an individual of chromosomal form "A" from Transbaikalia. No studies have been conducted on the presence of other chromosomal rearrangements for the population of the Norskii Reserve. The intra- and interpopulation karyotypic variability of five chromosomal forms of *A. maximowiczii* (four in Transbaikalia and one in the Russian Far East) is still unknown.

In addition to chromosomal methods (Meyer et al., 1996; Kartavtseva et al., 2009; Kartavtseva et al., 2008), for diagnostics of the *A. fortis* and *A. maximowiczii* allozyme (Frisman et al., 2009, 2011, 2016), molecular genetic methods (Sheremetyeva et al., 2015, 2022; Wang et al., 2014; Sheremetyeva et al., 2024) were used to study the southern part of the Russian Far East. These studies made it possible to identify reliably the habitat of two species on the territory of the Middle Amur Lowland. However, a complete picture of their distribution is missing. *A. maximowiczii* and *A. fortis* can also be differentiated by the shape of the sperm head (Meyer et al., 1996). This method is the

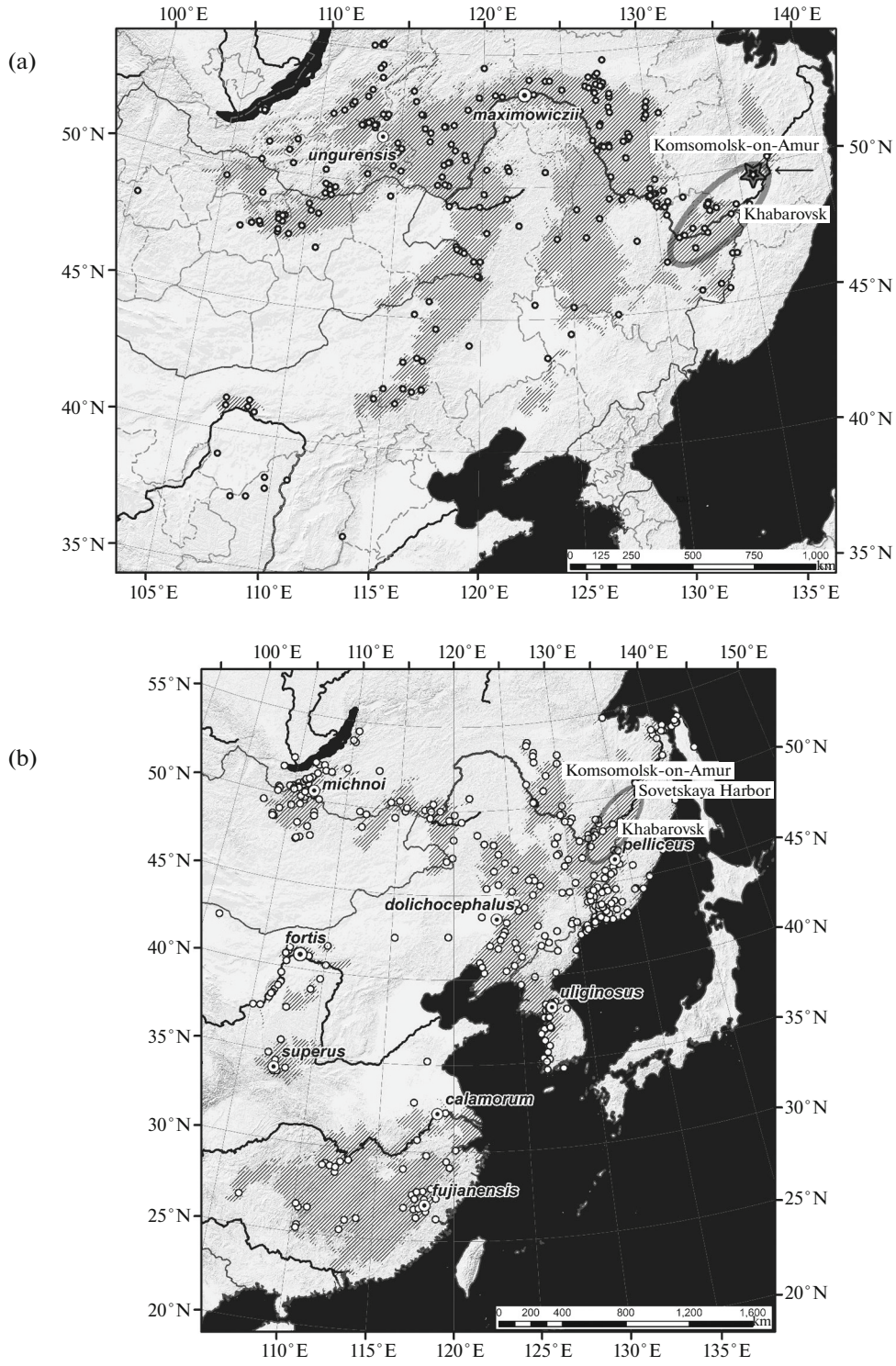

Fig. 1. The ranges of two species of East Asian voles of the genus *Alexandromys*: (a) *Alexandromys maximowiczii*, (b) *Alexandromys fortis* (by Kryštufek and Shenbrot, 2022). The ellipse highlights the Middle Amur Lowland, the hatching indicates the supposed range of the species, small dots indicate the places where voles were caught, and large dots indicate terra typica locations and the names of subspecies. The asterisk and arrow mark a new find of the karyotyped Maximowicz's vole.

simplest, as it allows species to be identified in the field, but it is only applicable to sexually mature males and is not used by zoologists.

Before the start of genetic studies of voles in the Middle Amur Lowland, zoologists believed that only one species was widespread in this area, *A. fortis*. As

early as 1991, two strains of hantavirus, "Khabarovsk" (HBRV) and "Vladivostok" (VlAV), were discovered in a population of Far Eastern voles living near the city of Khabarovsk (Dzagurova et al., 1995; Hörling et al., 1996), which was unusual, since a certain strain of the virus corresponds to a certain host species (Kariwa et al., 1999). It was only in 2008 that the results of genetic research in China (species were identified using *cytb* mtDNA gene analysis) showed that the carrier of the HBRV genotype, previously described for the Khabarovsk population of *A. fortis*, is another kind, *A. maximowiczii* (Zou et al., 2008). Based on the results of this work, it was concluded that in the Khabarovsk population the HBRV strain also belongs to *A. maximowiczii*, and the VlAV strain, to *A. fortis* (Yashina et al., 2008). Chromosomal (Kartavtseva et al., 2009) and molecular genetic data (Sheremetyeva et al., 2022; Sheremetyeva et al., 2024) of voles in this population confirmed the cohabitation of the two species *A. fortis* and *A. maximowiczii*.

For a long time without confirmation data, it was considered that in Khabarovsk krai, *A. maximowiczii* lives in the vicinity of the city of Sovetskaya Gavan and nearby villages (Kostenko, 2000). According to other sources (Fig. 1b), *A. fortis* lives here (Shenbrot and Krasnov, 2005; Kryštufek and Shenbrot, 2022), on the basis of which, in the first work, the distribution of the species was indicated throughout the entire territory of Sikhote-Alin, while in the second it was shown along the eastern slopes of the mountain range of the coast of the Sea of Japan to the city of Sovetskaya Gavan. However, karyotyping of voles from the city of Sovetskaya Gavan and surrounding villages (Kartavtseva et al., 2011) made it possible to identify an invasive species, the East European vole (*Microtus rossiameridionalis* Ognev 1924). Molecular genetic analysis (Sheremetyeva et al., 2021) confirmed the presence of the East European vole here, and also its repeated arrival in the city of Khabarovsk. All three species found in Khabarovsk are carriers of species-specific strains of various viral and bacterial infections that are dangerous to humans (Lapin et al., 2015), so knowledge of their distribution is of interest not only to zoologists, but also to epidemiologists.

The Middle Amur Lowland is a flat, heavily swampy area. The main river of the Middle Amur Lowland is the Amur, the riverbed of which is characterized by the presence of numerous channels, branches, backwaters, and lake basins (Kryukova, 1999). The northeastern part of this lowland (Amur–Sungari Lowland) is located in Russia (Khabarovsk krai), and the middle part is on the territory of both Russia (Jewish Autonomous Region (Jewish AO) and Khabarovsk krai, along the valley of the Ussuri River), and in the northeastern part of China (Sanjiang Plain), while the southern part is only in northeastern China. In the middle part of the lowland (before the advent of genetic methods), only one species was noted, *A. fortis*, and the eastern border of the range of *A. maximowiczii* reached the Zeya River in the Amur region (Kostenko, 2000). Chromosomal studies (Meyer et al., 1996) have revealed *A. maximowiczii* on the bank of the Tunguska River, which flows into the Amur near the city of Khabarovsk, and on this basis, have shifted the eastern boundary of the species' range to Khabarovsk (Shenbrot and Krasnov, 2005). Later, the eastern boundary of the range was shifted to the east to the city of Komsomolsk-on-Amur (Kryštufek and Shenbrot, 2022), where a new point of discovery of Maximowicz's vole appeared (near the city of Komsomolsk-on-Amur), without reference to a source describing this find, and further north, to the region of the Evoron-Chukchagir Lowland, also without references to finds (Fig. 1a). According to genetic studies of voles of the genus *Alexandromys*, in the Evoron–Chukchagir lowland, there is only the Evoron vole (*Alexandromys evoronensis* (Kowalskaya et Sokolov 1980)) (Kartavtseva et al., 2022). In the northeastern part of the Middle Amur Lowland between the cities of Khabarovsk and Komsomolsk-on-Amur, there is only the habitation of *A. fortis* (Sheremetyeva et al., 2022).

The absence of geographical barriers for Maximowicz's vole in the Middle Amur Lowland suggests that this species may inhabit humid biotopes in the northeastern part of the lowland. That is why the aim of our study was to analyze the distribution of East Asian voles in a previously unexplored region of the northeastern part of the Middle Amur Lowland using the chromosome method not only for species diagnostics, but also to clarify the nature of chromosomal rearrangements and their role in intrapopulation variability at the cellular level. The use of previously published data on the karyotype of Maximowicz's vole made it possible to determine karyotype variants and chromosomal rearrangements in previously studied populations of the middle part of the Middle Amur Lowland. Based on published data on a comprehensive genetic (chromosomal, molecular genetic, and allozyme) study of Maximowicz's vole and the Far Eastern vole in the Middle Amur Lowland, places of both separate and joint habitation were established.

MATERIALS AND METHODS

As a result of field work in the northeastern part of the Middle Amur Lowland (northern bank of the Amur River), in the vicinity of the village of Elban in Khabarovsk krai (Fig. 1a), we caught voles of the genus *Alexandromys* ($n = 20$). Trapping was carried out using Sherman traps from July 20–23, 2023, at two locations. Point 1 ($n = 18$) was located 4 km west of the village of Elban, located on the right bank of the river of the same name, a tributary of the Amur river (50°06′03″ N, 136°27′39″ E). Point 2 ($n = 2$) was located 10 km southeast of the village, on the left bank of the Elban river (50°02′45″ N, 136°33′43″ E). Both points were located in abandoned agricultural fields (fallow lands) located near small broad-leaved forests

with two types of biotopes: wet (dominated by sedge and reed grass) and dry (dominated by burnet).

Vole chromosome suspensions were prepared in the field using a standard method with a slight modification of the generally accepted method (Ford and Hamerton, 1956; MacGregor and Varley, 1986) from bone marrow cells taken from the femur with preliminary administration of a 0.04% colchicine solution for 25–30 min (not 40 min). Bone marrow from the femur was washed into a test tube using a medical syringe filled with a hypotonic solution (0.56% KCl) and then left to incubate for 20–25 min at room temperature (not 15 min at 37°C). The solution was then centrifuged (800–1000 rpm) for five minutes. After centrifugation, the supernatant was drained, and the cell sediment, without breaking it up, was fixed with a freshly prepared fixative—a mixture of 96% ethanol (not methanol) and glacial acetic acid (in a ratio of 3 : 1) for at least ten minutes. The sediment (consisting of nuclei and chromosomes at different stages of division) was then broken up into a homogeneous suspension. The fixative was changed at least three times, centrifuging the sediment for five minutes. The total fixation time is not less than 40 minutes. In the last portion of the fixative, the cell suspension was left in the refrigerator for storage at a temperature of –20°C.

To analyze chromosome numbers and chromosome morphology, the preparations were stained with 2% orcein dissolved in glacial acetic acid. In field conditions we used a small Longway LW91-06E (China) microscope, with eyepieces: 40/0.65 and 100/1.25. Further processing of chromosomal preparations was carried out in laboratory conditions. For microphotography, we used an Axio Imager 1 microscope, a digital camera, and Metasystems software from Carl Zeiss MicroImaging GmbH (Germany) of the Biotechnology and Genetic Engineering Shared Use Center of the Federal Scientific Center of Biodiversity, Far East Branch, Russian Academy of Sciences (Vladivostok). The karyotype chromosome nomenclature we used was developed earlier for Maximowicz's vole from Transbaikalia (Lemskaya et al., 2010) and applied to the voles of the Amur region (Kartavtseva et al., 2013). The nomenclature was created using FISH and G-staining of chromosomes, which made it possible to characterize the chromosomal form "C," to determine the structural rearrangements and the chromosome numbers involved in these rearrangements. Thus, the metacentric chromosome formed as a result of the centric fusion of chromosome pairs no. 11 and no. 20 is assigned the number 11.20 (the dot indicates a Robertsonian fusion), and another metacentric chromosome formed as a result of the tandem fusion of pairs no. 3 and no. 4 is assigned the number 3/4 (the slash indicates a tandem or telomeric merger). Karyotype variants with the same number of chromosomes, differing in the number of arms (*NF*), were assigned a letter, which was placed after the diploid number, for example $2n = 41a$, $2n = 41b$, etc. This designation of karyotype variants was previously used for the Evoron vole, which has multiple chromosomal rearrangements (Kartavtseva et al., 2021). Option $2n = 41b$, $NF = 59$ is described according to the published chromosome layout (Meyer et al., 1996, insert Fig. 86d), variant $2n = 41c$, $NF = 59$ is described based on previously obtained data (Kartavtseva et al., 2017), where chromosome numbers were not assigned.

Skulls, skins, chromosome preparations, and chromosome suspensions are stored in the laboratory of evolutionary zoology and genetics of the Federal Scientific Center of Biodiversity, Far East Branch, Russian Academy of Sciences.

For species diagnostics of two species of voles (Far Eastern and Maximowicz's vole) in field conditions, the morphology of the sperm head of adult males was also analyzed. In Maximowicz's vole and the Evoron vole, the sperm head is round, while in the Far Eastern vole, it is hooked (Meyer et al., 1996). The contents of the epididymis of males (without saline solution and fixative) were applied to a glass slide in a thin layer and viewed under a field microscope at a magnification of ×40, without staining or fixation. Next, the epididymis was fixed with the same fixative as the chromosome suspensions.

The locations of Maximowicz's vole and Far Eastern vole finds in the Middle Amur Lowland were determined based on data on the localities of voles obtained during molecular, chromosomal, and allozyme studies (Tables 1, 2).

The distribution of two species of the genus *Alexandromy*s in the Middle Amur Lowland was established using previously published data from genetic analysis (chromosomal, allozyme, and molecular genetic) (see Tables 1 and 2). Sometimes two or three of the genetic methods mentioned above were applied to one individual, so it is difficult to reconstruct the number of voles captured in a locality; therefore, in the tables the number of individuals and the number of localities indicate the use of a specific method in the study. Karyotyping of voles in these works was carried out by I.V. Kartavtseva; therefore, in this work for the previously identified numerical chromosomal characteristics and the chromosomal layouts available to us, karyotype variants were determined, which made it possible to identify the frequency of occurrence of variants in previously studied voles from populations in the Middle Amur Lowland. The capture of voles in the Jewish Autonomous Region was carried out by I.V. Kartavtseva together with I.N. Sheremetyeva and L.V. Frisman, as well L.V. Frisman and K.V. Korobitsyna. In the vicinity of Khabarovsk, the capture was carried out by zoologists from the Khabarovsk Anti-Plague Station of Rospotrebnadzor—A.V. Adnagulova and N.P. Vysochina. On the left bank of the Bikin River, in the vicinity of the village of Orenburgskoe, the catch was carried out by I.V. Kartavtseva.

Table 1. Places of genetic research, chromosomal, molecular genetic, and allozyme, of Maximowicz's vole (*Alexandromys maximowiczii*) on the territory of the Middle Amur Lowland

No.	Locality	Methods of study						Source
		molecular	chromosomal				allozyme	
			$2n = 40$a	$2n = 41$				
				a	b	c		
Khabarovsk krai								
left bank of the Amur								
1	Elban		10	8				Our data
right bank of the Amur								
2	Kukan		3				13	Frisman et al., 2011
3	Railway junction Utinaya, Tunguska River (eastern coast)		4		1			Meyer et al., 1996
4	Galkino, southern bank of the Amur River	10						Sheremetyeva et al., 2015; Sheremetyeva et al., 2024
	Galkino, southern bank of the Amur River		8			1		Kartavtseva et al., 2017
5	Bolshoi Ussuriiskii Island (China)	11						Sheremetyeva et al., 2024; Wang et al., 2014
6	Orenburgskoe, left bank of the Bikin River	2						Sheremetyeva et al., 2015, Sheremetyeva et al., 2024
Jewish Autonomous Region								
left bank of the Amur								
7	Kul'dur						4	Frisman et al., 2011, 2019
8	Izvestkovyi						17	Frisman et al., 2011, 2019
9	Pashkovo						1	Frisman et al., 2019
10	Radde						3	Frisman et al., 2011
11	Amurzet	3						Sheremetyeva et al., 2015; Sheremetyeva et al., 2024
	Amurzet		2					Frisman et al., 2011
12	Sadovoe	9						Sheremetyeva et al., 2015; Sheremetyeva et al., 2024
13	Leninskoe	4						Sheremetyeva et al., 2015
	Leninskoe		6	1				Kartavtseva et al., 2008
	Leninskoe		3				3	Frisman et al., 2011
	Leninskoe						5	Frisman et al., 2009
14	Yellow Yar, Bir River	1						Sheremetyeva et al., 2024
15	Birobidzhan	5						Sheremetyeva et al., 2024
	Birobidzhan		1					Kartavtseva et al., 2008
16	13 km south of Birobidzhan		1					Frisman et al., 2011
17	Bastak Nature Reserve (Main Cluster)	4						Sheremetyeva et al., 2024

Table 1. (Contd.)

No.	Locality	Methods of study						Source
		molecular	chromosomal				allozyme	
			$2n = 40$a	$2n = 41$				
				a	b	c		
17	Bastak Nature Reserve (Main Cluster)		3				8	Frisman et al., 2011
	Bastak Nature Reserve (Main Cluster)						4	Frisman et al., 2019
18	Aur			1				Frisman et al., 2009
	Aur		1					Kartavtseva et al., 2008
	Aur						2	Frisman et al., 2009
Number of individuals		49	42	10	1	1	60	
Number of localities		9	10	3	1	1	7	

RESULTS

Sperm of all adult males examined ($n = 8$) had a rounded head, which corresponded to the characteristics of Maximowicz's vole and the Evoron vole.

Karyotype 18 studied in voles from two points in the vicinity of the village of Elban was variable in chromosome number, with diploid numbers of 40 and 41. The frequency of voles falling into traps at these points was 20%. At point 1, nine voles had 40 chromosomes: males no. 4836, no. 4841, no. 4856, no. 4866, no. 4867, no. 4868, and females no. 4835, no. 4865, no. 4839 and seven voles had 41 chromosomes: males no. 4837, no. 4838, no. 4840, no. 4849, no. 4852, no. 4855, and female no. 4850. At point 2, female no. 4854 had 40 chromosomes and female no. 4853 had 41 chromosomes. In the karyotype, the X chromosome is acrocentric, medium-sized, and the Y chromosome is acrocentric, small in size. This karyotype corresponded to *A. maximowiczii*. Based on the variability of the number and morphology of chromosomes of the karyotype of individuals from the studied population, two karyotype variants were identified. Analysis of our own and published data allowed us to identify two more karyotype variants of Maximowicz's vole from the Middle Amur Lowland.

Chromosomal Characteristics of A. maximowiczii in the Vicinity of Elban in the Northeastern Part of the Middle Amur Lowland

Option $2n = 40$a, $NF = 58$ (Fig. 2a), chromosome set: one pair of large metacentrics (no. 3/4), formed by telomeric fusion of medium-sized metacentric chromosomes (no. 3 and no. 4); two pairs of large and medium-sized subtelocentrics (no. 1 and no. 2); three pairs of medium-sized and approximately identical metacentrics (nos. 5, 6, and 7); a medium-sized metacentric (no. 11.20), formed by the fusion of the centromeres of acrocentric chromosomes (no. 11 and no. 20); two pairs of small subtelocentric chromosomes (nos. 12 and 16), and ten pairs acrocentrics (nos. 8–10, 13–15, 17–19, and 21). The X chromosome was medium sized acrocentric, and the Y chromosome is small acrocentric.

Option $2n = 41$a, $NF = 60$ (Fig. 2b), chromosome set: large metacentric no. 3/4; two metacentrics no. 3 and no. 4; two pairs subtelocentrics (no. 1 and no. 2); three pairs of medium-sized metacentrics, relatively equal in size (no. 5, no. 6, and no. 7); pairs metacentrics of medium size (no. 11.20); two pairs of small subtelocentric chromosomes (no. 12 and no. 16) and ten pairs acrocentrics (nos. 8–10, nos. 13–15, nos. 17–19, and no. 21). The X chromosome is medium-sized acrocentric, and the Y chromosome is small acrocentric.

Chromosomal Characteristics of A. maximowiczii in the Middle Part of the Middle Amur Lowland

According to previously published (Meyer et al., 1996; Kartavtseva et al., 2017) chromosomal data ($2n$ and *NF*) on Maximovich's voles from the Middle Amur Lowland (Table 1), we describe two more variants of the karyotype with **$2n = 41$**.

Option $2n = 41$b, $NF = 59$, the chromosome set is characterized by a heterozygous state of three chromosomes: no. 11 and no. 20 (acrocentrics) and no. 11.20 (metacentric). One of the pairs of autosomes is of medium size, approximately equal to pair no. 10 in the studied karyotype variants (Fig. 2). The description of the karyotype is given according to the published layout (Meyer et al., 1996). The variant was found for a vole from the Tunguska River valley (locality no. 3, Table 1). The remaining individuals in this population

Table 2. Places of genetic research, chromosomal, molecular genetic, and allozyme of the Far Eastern vole (*Alexandromys fortis*) on the territory of the Middle Amur Lowland

No.	Locality	Methods of Study			Source
		molecular	chromosomal	allozyme	
	Khabarovsk krai, left bank of the Amur				
1	Komsomolsk-on-Amur	3	8		Sheremetyeva et al., 2006, 2022
2	Pivan		2		Meyer et al., 1996
	right bank of the Amur				
3	Tomskoe		2	6	Frisman et al., 2011
4	Galkino, southern bank of the Amur River	14			Sheremetyeva et al., 2015, 2022
	Galkino, southern bank of the Amur River		2		Kartavtseva et al., 2009
5	Bikin, right bank of the Ussuri River	8			Sheremetyeva et al., 2015, 2022
	Jewish Autonomous Region left bank of the Amur				
6	Danilovka, Tunguska River (west coast)	2			Sheremetyeva et al., 2015, 2022
7	Yellow Yar, Bir River	2			Sheremetyeva et al., 2022
8	Birobidzhan			1	Frisman et al., 2009
	Birobidzhan		2	15	Frisman et al., 2011
	Birobidzhan	1			Sheremetyeva et al., 2022
9	13 km south of Birobidzhan		1	1	Frisman et al., 2011
10	Bastak Nature Reserve, Zabelovskii area, Amur River floodplain	2			Frisman et al., 2013 (based on Sheremetyeva); Sheremetyeva et al., 2015, 2022
11	Kuldur			1	Frisman et al., 2011, 2013, 2019
12	Obluchye			6	Frisman et al., 2019
	Obluchye	5			Sheremetyeva et al., 2022
13	Pashkovo		2	2	Frisman et al., 2019
14	Pillar			11	Frisman et al., 2019
15	Amurzet		2	5	Frisman et al., 2011
16	Bidjan	8			Sheremetyeva et al., 2022
17	Leninskoe		2	2	Frisman et al., 2009
	Leninskoe	2			Sheremetyeva et al., 2022
Number of individuals		47	23	50	
Number of localities		10	9	11	

had the variant **$2n = 40a$**. Sex chromosomes are acrocentric.

Option $2n = 41c$, $NF = 59$ (Fig. 3), the chromosome set is similar to the variant **$2n = 41a$**, but differs from it in the heterozygous state of small pair no. 16, which is represented by an acrocentric (A) and a metacentric (M). Variability in the morphology of a small pair of chromosomes was discovered earlier in Khabarovsk krai, in the vicinity of the village of Galkino (Kartavtseva et al., 2017). The karyotype variant was not described in that work, and the chromosome pairs did not have numbers. Of the nine individuals of this population previously studied, only one male was found to have variability in the morphology of a small pair of autosomes. The rest of the voles had **$2n = 40$.**

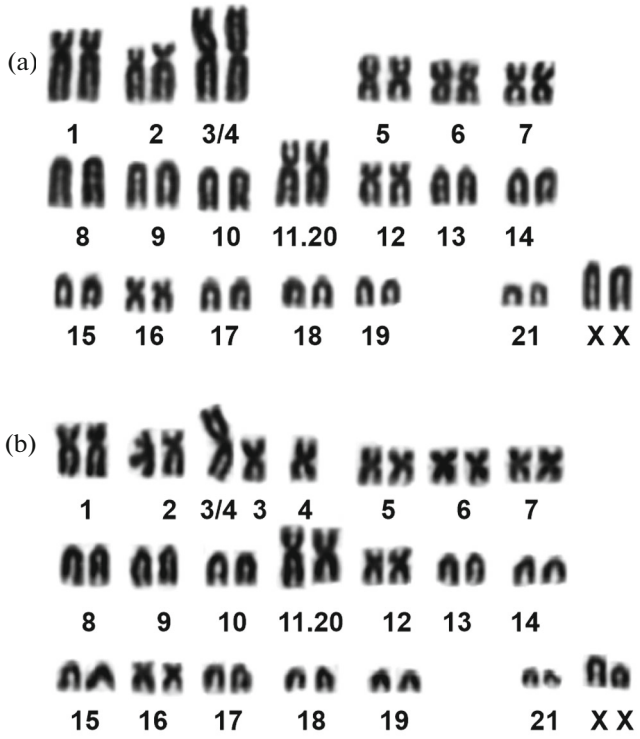

Fig. 2. Chromosomes of Maximowicz's vole *Alexandromys maximowiczii* from the vicinity of the village of Elban, Khabarovsk krai: (a) female no. 4854 from point 2 (option 2) n = 40a, NF = 58), (b) male no. 4838 from point 1 (option $2n$ = 41a, NF = 60). The fraction in the number 3/4 indicates tandem fusion of chromosomes, and the dot in the number 11.20 indicates acrocentric fusion. In the karyotype, pairs of autosomes no. 12 and no. 16 are submetacentric. The numbers of chromosome pairs correspond to those for voles of the Transbaikal krai, given in the publication by Lemskaya et al. (2010).

Analysis of Variability in the Frequencies of Karyotype Variants and Chromosomal Rearrangements in Populations of A. maximowiczii from Various Parts of the Middle Amur Lowland

If in the Elban population (two local points) studied in this work, the frequencies of the two described karyotype variants (**$2n$ = 40a, NF = 58** nd **$2n$ = 41a, NF = 60)** are relatively equal at 55.6 and 44.4% respectively, then in the populations of the middle part of the Middle Amur Lowland the variant **$2n$ = 40a, NF = 58** prevailed (87.5%). Only two voles from localities no. 8 and no. 12 in the Jewish Autonomous Region (Table 1) the option to have **$2n$ = 41a, NF = 60** (5.55%). Rare variants **$2n$ = 41b, NF = 59** (3.5%) and **$2n$ = 41c, NF = 59** (3.5%) were found in voles of Khabarovsk krai in localities no. 3 and no. 4.

Options **$2n$ = 40a, NF = 58, $2n$ = 41a, NF = 60** are characterized by two structural changes that are present simultaneously. For the option **$2n$ = 40a, NF = 58,** a homozygous state of two structural rearrangements is characteristic: *centromeric* metacentric fusion no. 11.20; *tandem* fusion of two medium-sized metacentrics to form metacentric no. 3/4. The karyotype with 41 chromosomes has three variants: the first is a heterozygous state *tandem* mergers **($2n$ = 41a, NF = 60)**; the second is a heterozygous state *Robertson's* restructuring and centromere displacement **($2n$ = 41b, NF = 59);** and third is heterozygous state *tandem* mergers and *pericentric inversion* **($2n$ = 41c, NF = 59)**. The karyotype **$2n$ = 42** chromosome form "C," previously discovered from the Amur–Zeya Plain (Meyer, 1968; Meyer et al., 1967; Kartavtseva et al., 2008) in the Amur region (karyotype variant not described), was not found in the Middle Amur Lowland.

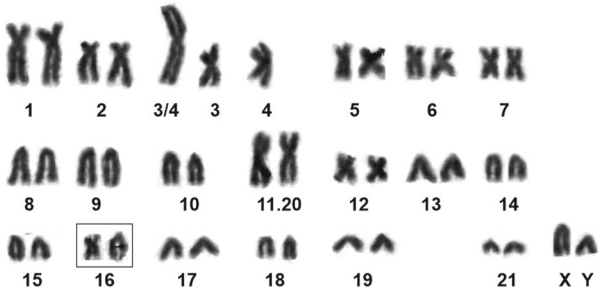

Fig. 3. Karyotype of male no. 694 of Maximowicz's vole (*Alexandromys maximowiczii*), option $2n$ = 41c, NF = 59 (Khabarovsk population) in the vicinity of the village of Galkino (by Kartavtseva et al., 2017, with altered position of chromosome pairs). In the frame is heteromorphic (M/A) pair no. 16.

DISCUSSION

Chromosomal Variability of Maximowicz's Vole (A. maximowiczii)

Previously, for species diagnostics of Maximowicz's vole of the Middle Amur Lowland (Table 2), only data $2n$ and NF were used without description of the karyotype variants. The use of the nomenclature of the karyotype of Maximowicz's vole based on our own and published data made it possible to assign numbers to the chromosomes involved in the rearrangements, as well as to identify and describe four variants of the karyotype.

After assigning numbers to pairs of chromosomes in the karyotype of Maximowicz's vole (Lemskaya et al., 2010), two structural rearrangements were identified for the chromosomal form "C" (*tandem* no. 3/4 and *centromeric* no. 11.20 chromosome fusion) for voles from the Amur–Zeya Plain (Kartavtseva et al., 2013) and the Middle Amur Lowland (this work). However, the karyotype with **$2n$ = 41** from Transbaikalia differed from the variant **$2n$ = 41b** from the Middle Amur Lowland, which we found in one individual, in that it had different chromosomal characteristics (a different number of small acrocentric chromosomes) and, consequently, a different karyotype variant with **$2n$ = 41.** Karyotype variants of Maximowicz's

vole from the Amur–Zeya Plain and Transbaikalia have not yet been described.

Frequencies of Variants Associated with Tandem Chromosome Fusion

For voles in the vicinity of the village of Elban, frequency variants **2*n* = 40a, *NF* = 58** and **2*n* = 41a, *NF* = 60** accounted for 55.6 and 44.4%, respectively. For voles in the middle part of the Middle Amur Lowland, these figures were different at 88.9% **(2*n* = 40a)** and 11.1% **(2*n* = 41a)** (Table 2), which indicates the process of karyotype stabilization **2*n* = 40a, *NF* = 58.** It is possible that stabilization of the chromosomal rearrangement *tandem* as a type of chromosome fusion is due to intrapopulation inbreeding of the studied populations, which was previously shown in the analysis of allozyme variability of Maximowicz's vole in the Jewish Autonomous Region (Frisman et al., 2016). In addition, according to the results of the study of the control region of mitochondrial DNA, each individual sample in the Jewish Autonomous Region had a significant decrease in nucleotide diversity (more than 1.5 times), for the species as a whole the decrease was less pronounced (Sheremetyeva et al., 2024). An exception was the sample from the vicinity of the village of Leninskoe (sample no. 13, Table 1), where the nucleotide diversity was more than two times higher than in the other samples studied. The authors explain the decrease in nucleotide diversity in individual samples by the biology of the species, which is characterized by deep population depressions. Expansion of nucleotide diversity within the sample of the environs of the village of Leninskoe is explained by the authors by the discovery of two subclades here, "Amur" and "Khab," of the phylogenetic group "Amur." No chromosomal differences were detected between these two subclades.

According to the G-staining data of the chromosomes of Maximowicz's vole from Transbaikalia (location unknown) (Lemskaya et al., 2010) and the southern part of the Russian Far East in the Amur Region, Zeya-Bureya Plain (Kartavtseva et al., 2013), it was shown that the large metacentric chromosome no. 3/4 formed as a result *tandem* fusion of biarmed chromosomes. The similarity of G-stained large biarmed chromosomes of voles from Transbaikalia (Meyer et al., 1996), the Amur Region (Kartavtseva et al., 2008), the Jewish Autonomous Region (Frisman et al., 2011), and Khabarovsk Krai (Meyer et al., 1996; Kartavtseva et al., 2017) indicates that in these populations the formation of this chromosome occurs as a result of *tandem* fusion of the same biarmed autosomes. A large two-armed chromosome was previously noted in other populations of Transbaikalia (Kovalskaya et al., 1980). Since this restructuring occurs throughout the entire range of the species from Transbaikalia to the south of the Russian Far East, it is probably not harmful to the species. The high frequency of this chromosome in the heterozygous state in the populations of voles of three chromosomal forms ("A," 46%; "B," 42.8% (Kovalskaya et al., 1980) of Transbaikalia and the chromosomal form "C" from the northeastern part of the Middle Amur Lowland that we studied, 44.4%), according to the interpretation of the Hardy–Weinberg model, indicates a normal distribution of heterozygotes. On the contrary, the decrease in the frequency of the heterozygous state of this chromosome in voles of Transbaikalia of the chromosomal forms "B" 14.7% (Kovalskaya et al., 1980), "D" 10% (Kartavtseva et al., 2008), and voles of the central part of the Middle Amur Lowland at 11.1% (Table 1) indicates that inbreeding occurs in populations, stabilizing structural reorganization (*tandem* fusion) of chromosomes.

All previously studied chromosomal forms of Maximowicz's vole in Buryatia were characterized by a large chromosome, which was recorded in both homozygous and heterozygous states (Kovalskaya et al., 1980) with varying frequencies. Moreover, only for one individual in one population in Transbaikalia (chromosomal form "A") was a homozygous state (!) of chromosomes no. 3 and no. 4, participating in tandem fusion, detected. In all other populations studied, the homozygous state of chromosomes no. 3 and no. 4 was not detected. Probably, in one of these pairs a harmful mutation occurred (in Transbaikalia), incompatible with life. Since chromosome no. 3/4 is always present in the karyotypes of Maximowicz's vole from Transbaikalia to the Russian Far East, it can be assumed that the fusion of chromosomes no. 3 and no. 4 occurred before the harmful mutation appeared in Transbaikalia and spread in four chromosomal forms ("B", "C", "D," and "V"), where the original karyotype with **2*n* = 44** was not found.

We have not found examples of such chromosome variability in mammalian populations, and such an event is unique and interesting for further research into the role of chromosomal rearrangements in speciation processes. Maximowicz's vole breeds well in captivity and can serve as a laboratory species for genetic research. Tandem fusion polymorphism of chromosomes in mammalian populations is generally considered rare and harmful (King, 1993; Dobigny et al., 2017); however, in populations of Maximowicz's vole, *tandem* mergers are not a rare event. We have also previously shown the absence of harmful effects of two types of *tandem* chromosome fusions in two chromosomal races of the Evoron vole (Kartavtseva et al., 2021; Kartavtseva et al., 2021, 2023). Option **2*n* = 41b,** caused by a heterozygous state of centromeric fusion (no. 11.20, no. 11, no. 20) are rare for populations of the Middle Amur Lowland. Of the 54 karyotyped individuals of Maximowicz's vole from the Middle Amur Lowland (Table 2), in only one local population (no. 3, Table 1) was a Robertsonian rearrangement (11.20) detected in the heterozygous state. Variability in the number of chromosomes associated with cen-

tromeric fusion of chromosomes is a common event (Orlov et al., 2023).

For individuals of population no. 3 of Maximowicz's vole, the diploid chromosome numbers are 40 and 41 (Meyer et al., 1996). However, Table 29, which presents the research material, provides other chromosome numbers for this population—39, 40. The authors also pointed out chromosomal rearrangements in the karyotype **2*n* = 41**. The number 39 must have been associated with another Robertsonian reconstruction that was not described. From this we conclude that there is a typo in the table, since for these voles the number of chromosomes, judging by the nature of the described chromosomal rearrangements of this population, should be only 40 and 41. However, the unusual number of chromosomes (39) was included in the chromosomal characteristics of the "C" chromosomal form in a number of publications (Frisman et al., 2009; Kartavtseva et al., 2008; Kryštufek and Shenbrot, 2022; Sheremetyeva et al., 2024). We believe that this number (39) cannot be used in the future for chromosome numbers of the chromosome form "C" and for it the numbers 40, 41, and 42 (2*n* = 42—should be left as found in voles of the Amur region). Karyotype variants of Maximowicz's vole of the Amur region are not described.

A rare event is the acrocentric (A) state of one of the homologues of autosomes of pair 16 in the heterozygote variant **2*n* = 41c, *NF* = 59** (Fig. 3). In all studied local populations of the Middle Amur Lowland, this pair of chromosomes has a metacentric (M) morphology. According to the results of differential staining, such chromosome rearrangement is associated with pericentric inversion (Kartavtseva et al., 2017). The variability of the morphology of the small four pairs of autosomes of Maximowicz's vole from Transbaikalia, coupled with pericentric inversion, was pointed out by both Kovalskaya (1977), who did not use the method of differential chromosome staining, and Rajabli and Sablina (Meyer et al., 1996), who used the method of differential chromosome staining.

In the variant **2*n* = 41b,** one of the medium-sized autosomes also has a heterozygous state—submetacentric/acrocentric (SM/A). According to Rajabli and Sablina (Meyer et al., 1996), both chromosomes have the same G-block pattern, which serves as evidence for the presence of centromere displacement. Centromere repositioning is observed in chromosomes that were previously formed as a result of the tandem fusion of two or more chromosomes. Centromere sliding may be a fairly common phenomenon in mammals, but studies are limited (Dobigny et al., 2017).

Intraspecific variability in mammalian chromosome morphology as a result of pericentric inversions and centromere displacement has been noted for a number of mammalian species (Dobigny et al., 2017). Of the 12 living species in the genus *Alexandromys,* one can specify five, where for two types, *A. maximowiczii* (Kovalskaya, 1977; Meyer et al., 1996; this work) and *A. mujanensis* Orlov et Kovalskaja 1978 (Kartavtseva et al., 2019), both types of rearrangements were found in four pairs of chromosomes (the frequency of each is difficult to indicate), for two species, *A. fortis* and *A. middendorffi*, inversions in only one of the pairs of autosomes. Thus, variability of autosome morphology (subtelocentric and acrocentric, ST and A), associated with pericentric inversion, was found as a rare event in *A. fortis* populations of the southern Russian Far East, where only four individuals out of 130 studied had an acrocentric morphology of the seventh pair of autosomes (Sheremetyeva et al., 2006). For *A. m. middendorffii* and *A. m. hyperboreus,* pericentric inversion (ST and A) was discovered in the largest pair of autosomes (Gileva, 1972). The heterozygote frequency for *A. m. middendorffii* in this work slightly exceeded the expected frequency according to the Hardy–Weinberg formula in a study of 24 natural and 38 laboratory individuals from southern Yamal.

All four chromosomal variants of the karyotype of Maximowicz's vole have structural rearrangements: *tandem* fusion of metacentric chromosomes (no. 3 and no. 4) and *centromeric* fusion of acrocentric chromosomes (no. 11 and no. 20), and two of these variants also have intrachromosomal rearrangements—*centromere displacement* and *inversion*. Tandem fusion of metacentric chromosomes with the formation of a large two-armed chromosome was noted for all studied individuals of the Middle Amur Lowland. We have shown a decrease in the frequency of heterozygotes for this rearrangement (as a rule, this is a karyotype with **2*n* = 41**) in previously studied populations of Maximowicz's vole, which indicates inbreeding in these populations and confirms the idea of intrapopulation inbreeding of voles (Frisman et al., 2016).

Since for the chromosomal form "C" a different chromosomal number is known (**2*n* = 42**) from the Amur–Zeya Plain in the Amur Region, we plan to continue research into the chromosome sets of voles from various regions of the Russian Far East and to describe new karyotype variants indicating the nature of chromosome rearrangements.

Chromosomal Variability of the Far Eastern Vole (A. fortis)

On the territory of the Middle Amur Lowland, the Far Eastern vole has a unique variability in the number and localization of heterochromatic blocks in the centromeric and telomeric regions of autosomes and sex chromosomes with a stable diploid number (2*n* = 52, *NF*a = 62–64) (Kovalskaya et al., 1991; Sheremetyeva et al., 2006). The uniqueness lies in the variability of the number and localization of heterochromatic material in telomeric regions, which has not been identified for other types of gray and East Asian voles.

This variability is similar to the variability of the Far Eastern vole in Primorskii krai. The subtelocentric (ST) morphology of the 7th pair of autosomes was found in seven voles out of eight studied near the city of Komsomolsk-on-Amur (left bank of the Amur), while acrocentric (A) was found in two voles from the vicinity of the village of Pivan, Komsomolskii district (right bank of the Amur). For the Far Eastern vole from various local populations of the Jewish Autonomous Region, variability in the morphology of this pair of chromosomes has been noted, but the frequency of variability has not been described (Frisman et al., 2011). The short arms of this chromosome pair are often so small that, when the chromosomes are strongly coiled, it is difficult to determine its morphology (A or ST).

Thus, new data on the karyotypes of Maximowicz's vole and the analysis of previously conducted genetic studies made it possible to differentiate the two morphologically similar species of East Asian voles and make an assumption about the distribution of the two species throughout the Middle Amur Lowland of the Russian Far East. Two species can live sympatrically not only in the same territory, but also in the same locality. For Maximowicz's vole, the eastern boundary of the range in Khabarovsk krai has been shifted by us from the previously known one by 200 km towards the city of Komsomolsk-on-Amur.

Spreading of A. fortis and A. maximowiczii in the Middle Amur Lowland

Maximowicz's vole. According to genetic analysis, molecular genetic, chromosomal, and allozyme, in the Middle Amur Lowland, this species was found in 18 localities (Table 1, Fig. 4a). For many populations, one method of species identification or another was used, and only for two populations in the Jewish Autonomous Region, no. 8 and no. 11, were all three methods applied. Figure 4 shows the diagrams of the contribution of each of the three genetic research methods to the identification of two species in three zones of the Middle Amur Lowland. The first two zones are the right and left banks of the Amur in the middle part of the lowland, and the third zone is in the northeastern part of the lowland. Differentially stained chromosomes were studied for two local populations of Khabarovsk krai (no. 3 and no. 4) and one local population of the Jewish Autonomous Region (no. 11), which made it possible to determine not only the species affiliation of the voles, but also to identify pairs of chromosomes and confirm their assignment to the chromosomal form "C".

Far Eastern vole. According to the data of genetic analysis conducted (see Table 2) using three methods, 17 localities are indicated on the map for the Far Eastern vole of the Middle Amur Lowland on the left bank of the Amur (Fig. 4b). The contribution of each of the three genetic methods to the diagnosis of the species is shown by diagrams for three zones of the Middle Amur Lowland.

For two types of local populations, the Jewish Autonomous Region received a greater contribution from allozyme analysis, while Khabarovsk krai received a greater contribution from molecular genetic and chromosomal analysis (Fig. 4).

Cohabitation of two species. In two local populations, two species of voles were caught simultaneously: in the vicinity of the village of Galkino, located near the city of Khabarovsk, locality no. 4 (Tables 1, 2) and the surrounding area of the village of Leninskoye, locality no. 13 for Maximowicz's vole (Table 1) and locality no. 17 for the Far Eastern vole (Table 2). In Fig. 4, the numbers of localities of cohabitation of the Far Eastern vole and Maximowicz's vole are indicated by circles.

Replacement of one species by another in one local population when caught in different years was noted in four local populations of the Jewish Autonomous Region (Figs. 4a, 4b, populations are outlined by a triangle) in two local populations (years of capture are not indicated) near the city of Birobidzhan (Frisman et al., 2009, 2011) and in two populations in the vicinity of the village of Kuldur and the surrounding area of the village of Pashkovo, where Maximowicz's vole was caught in 2010, and the Far Eastern vole in 2011 (Frisman et al., 2016).

Of interest are the findings of two species at the mouths of two rivers (Bikin and Tunguska) in Khabarovsk krai. So, if on the left bank of the Bikin River, the vicinity of the village of Orenburgskoye (population No. 6, Table 1), Maximowicz's vole was discovered, then on the right bank of the Bikin River, in the vicinity of the city of Bikin (population no. 5, Table 2), the Far Eastern vole was. At the mouth of the Tunguska River on the east bank, near Utinaya railway crossing (locality no. 3, Table 1), Maximovich's vole was found on the western bank, while near the village of Danilovka, Jewish Autonomous Region the Far Eastern vole was (locality no. 6, Table 2).

The discovery of Maximowicz's vole in the northeastern part of the Middle Amur Lowland made it possible to change the eastern boundary of its range and clarify the distribution of the species in the study area. Comparison of the results of chromosome analysis revealed four karyotype variants with $2n = 40$ and $2n = 41$. The karyotype is characterized by two structural rearrangements—*tandem* fusion of metacentric chromosomes no. 3 and no. 4 and *centromeric* by merging acrocentric pairs no. 11 and no. 20. *Centromeric* chromosome fusion in all local populations studied is stabilized, with the exception of one case found in the Tunguska River valley, point no. 4 (Table 2). Variability in the number of chromosomes in other cases is associated with tandem fusion of autosomes.

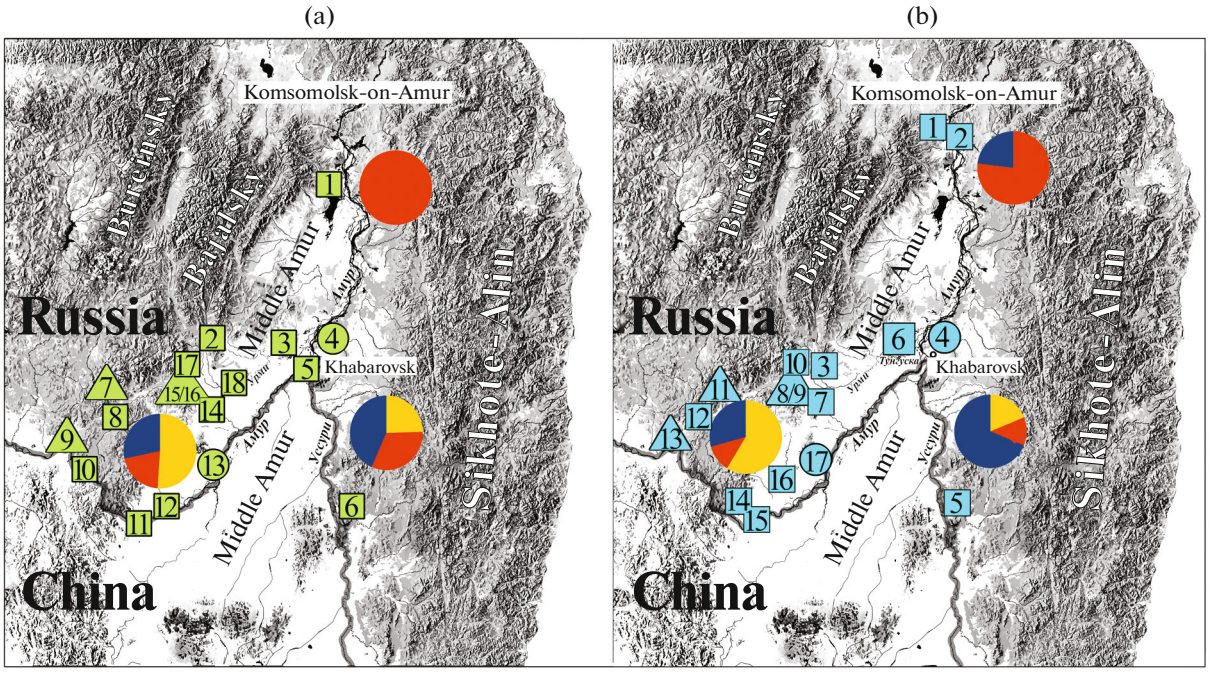

Fig. 4. Local populations of (a) *Alexandromys maximowiczii* and (b) *Alexandromys fortis* on the territory of the Middle Amur Lowland. The samples are grouped into geographical zones of the Middle Amur Lowland: (1) Jewish Autonomous Region, left bank of the Amur; (2) Khabarovsk krai, right bank of the Amur; (3) Khabarovsk krai, left bank of the Amur. The proportion of individuals examined by the three methods is shown in colous: chromosomal (red); allozyme (yellow); and mtDNA control region (blue). The numbers in the square indicate the numbers of the local population in which one species was discovered. In the circle, two species were discovered simultaneously (no. 4 and no. 13), and in the triangle, a change in species was discovered in different years of the study (nos. 7, 9, 15, 16). Local population numbers correspond to those for *Alexandromys maximowiczii* in Table 1 and for *Alexandromys fortis* in Table 2.

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ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study was conducted in accordance with the approved national guidelines for the care and use of laboratory animals and was approved by the Ethics Committee for the Care and Use of Animals of the Federal Research Center for Biodiversity of Terrestrial Bioresources of East Asia (Protocol no. 3 dated February 21, 2023).

CONFLICT OF INTEREST

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

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