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Chromosomal forms of *Microtus maximowiczii* (Schrenck, 1859) (Rodentia, Cricetidae): variability in 2n and NF in different geographic regions

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ABSTRACT. Analysis of the chromosomal characteristics of the Maximowicz's vole *Microtus maximowiczii* (242 specimens) from Transbaikalia, Russian Far East, and Mongolia, including new data from 12 local populations, supplemented information about chromosomal polymorphism of the species (2n=36–44; NF=52–62). We describe chromosomal data for the populations which have not been investigated previously, reveal a fixation of the chromosomal reconstructions in the various geographical regions and describe for the first time the population with stabilized karyotype (2n=42) in the Chita Region. The revision of the chromosomal characteristics has been done, that resulted in suggestion to consider five forms in the species composition, three of which (C, D and I) are established herein. Perhaps *M. m. gromovi* exemplified by the chromosome form I should be considered as an independent species. The chromosomal forms differ not only in 2n and NF, but also in number of the biarmed and acrocentric chromosomes.

KEY WORDS: chromosome, karyotype, polymorphism, *Microtus maximowiczii*.

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Хромосомные формы *Microtus maximowiczii* (Schrenk, 1859) (Rodentia, Cricetidae): изменчивость 2n и NF в различных географических регионах

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РЕЗЮМЕ. Анализ хромосомных характеристик 242 полевок Максимовича из Забайкалья, Дальнего Востока России и Монголии, включая собственные новые данные из 12 локальных популяций, позволил дополнить информацию о хромосомном полиморфизме вида (2n=36–44; NF=52–62), описать хромосомные данные для ранее не исследованных популяций, выявить фиксацию хромосомных перестроек в различных географических регионах, впервые описать популяцию со стабилизировавшимся кариотипом (2n=42) в Читинской области. В результате ревизии хромосомных характеристик выделено шесть форм, три из которых (C, D и I) установлены впервые. Подвид *M. m. gromovi*, представленный хромосомной формой I, возможно, следует рассматривать как самостоятельный вид. Показано, что хромосомными характеристиками для каждой формы являются не только 2n и NF, но и числа двуплечих и акроцентрических хромосом.

КЛЮЧЕВЫЕ СЛОВА: хромосома, кариотип, полиморфизм, *Microtus maximowiczii*.

Introduction

The Maximowicz's vole *Microtus maximowiczii* (Schrenck, 1859) is a widely distributed species inhabiting humid biotopes of the forest zone in East Asia (Russia, Mongolia, and North-East China). For the Russian territory two subspecies have been described: *M. m. ungurensis* Kastschenko, 1912 in Transbaikalia and *M. m. maximowiczii* (Schrenck, 1859) in Amur Region. The polymorphism in number and morphology of chromosomes (2n=36–44; NF=52–62) for this species has been reported before for 134 animals from 19 local populations (Kovalskaya, 1977; Kovalskaya *et al.*, 1980; Golenishchev & Radjabli, 1981; Meyer *et al.*, 1996). Three chromosomal forms (A, B, and V) based on 2n and NF was shown for this area (Kovalskaya *et al.*,

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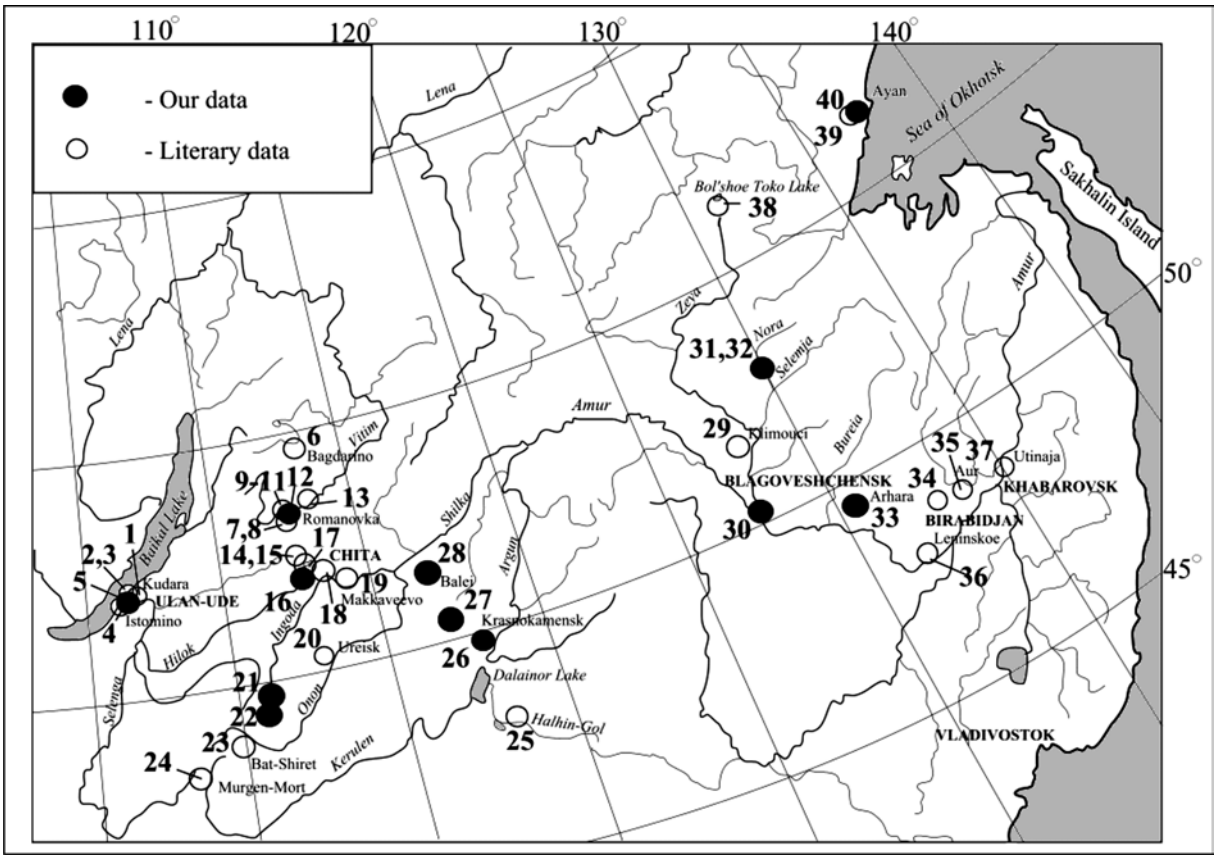

Figure 1. Geographic position of *Microtus maximowiczii* (Schrenck, 1859) samples (numbers correspond to those in tab. 1).

1980). The chromosomal form "A" with high chromosomal numbers (2n=42–44; NF=56–62) includes individuals from the Northern and Central Transbaikalia and Amur area. Voles of the chromosomal form "B" with 2n=38–42 are noted in the lower reaches of Selenga River (2n=39–41; NF=54–57) and in the middle part of the Onon River in the Amur River basin (2n=39–42; NF=54–58). The voles of the chromosomal form "V" with the least numbers of chromosomes (2n=36–38; NF=52–55) are found out on slopes of Hentey Mountains in Mongolia (Kovalskaya *et al.*, 1980). The G-banding of several animals of two chromosomal forms makes possible to show, that chromosomal polymorphism of *M. maximowiczii* is connected with Robertsonian reorganizations, inversions and also tandem fusion of middle metacentrics with formation of large metacentrics (Meyer *et al.*, 1996). According to data of the chromosomal reorganizations proved by using of methods of differential staining of chromosomes, the number of chromosomal rearrangements in various geographic populations should be reconsidered. Therefore nowadays the chromosomal variability of *M. maximowiczii* is mainly still unknown. The purpose of the present work is to investigate variability of chromosomal characteristics: 2n, NF, number of biarmed (meta-, submeta-, and subtelocentrics) and acrocentric chromosomes in karyotype of Maximowicz's vole throughout its area, using both original and published data.

Material and methods

The karyotypes of 242 animals from 40 local populations based on original and published data were investigated (Fig. 1; Tab. 1). We present here new chromosomal data of 94 voles from 12 localities: 76 Maximowicz's voles from ten new localities (16, 21, 22, 26–28, 30, 31–32, and 33) and 18 voles from two localities (5 and No. 12), from which chromosomal data were known earlier, have been trapped in 2003–2008. The coordinates of localities were provided only for our data (Tab. 1). Chromosomes were obtained from bone marrow cells. After colchicine (0.04%) treatment and hypotonic treatment with KCl-solution (0.56%), the cells were fixed with acetic-methanol (1:3), then air or flame-dried and stained with 4% Giemsa. The preparations were processed in the field with exception of a few animals. The diploid number (2n) and total number of chromosomal arms (fundamental number, NF) were determined after examination about 20 metaphases per animal. The chromosomal forms are given in accordance to the characteristics proposed by Kovalskaya (1977).

Table. Chromosomal data of *Microtus maximowiczii* (Schrenk, 1859) from different regions

Locality and specimen numbers (##)		2n	Chromosomes		NF	Reference
			Biarmed M+SM+ST	A		
Microtus maximowiczii ungurensis Kastschenko, 1912 Form B						
1	Buryatia, Kabansk District, Baikalo-Kudara (n=5)					1
	#48	38	16	22	54	
	#47, #503	40	14	26	54	
	#385	40	15	25	55	
	#226, #227	41	15	26	56	
2	Buryatia, Selenga River delta, Sotnikovskii Island (n=3)					
	#78	40	15	25	55	
	#186	41	?	?	55	
	#182	41	16	25	57	
3	Buryatia, Selenga River delta, Kolesovskogo Island (n=7)					
	#159, #158	38	17	21	56	
	#81, #79, #80	39	16	23	55	
	#196	40	15	25	55	
	#187	40	16	24	56	
4	Buryatia, Kabansk District, Istomino (n=7)					
	#362, #596, #615	39	16	23	55	
	#361	41	14	27	55	
	#572, #571, #529	40	16	24	56	
5	Ibidem (2006), N 52008' 26.00", E 106017' 57.85" (n=5)					our data
	#2023	38	19	19	57	
	#2016	39	17	22	56	
	#2031	40	16	24	56	
	#2017, #2030	40	14	26	54	
	#2024	42	14	28	56	
Total		38–42	14–19	19–28	54–57	
Form A						
6	Buryatia, Bauntovsk District, Bagdarino (n=6)					1
	#503	40	14	26	54	
	#569	42	14	28	56	
	#625	41	16	26	58	
	#606	41	17	25	58	
	#598	41	15	26	57	
	#595	43	16	27	59	
7	Buryatia, Bauntovsk District, Romanovka (n=2)					2
	no specimen number	42	16	26	58	
	no specimen number	43	17	26	60	
8	Ibidem (n=2)					3
	no specimen number	42	14	28	56	
	no specimen number	43	15	28	58	
9	Ibidem (n=2)					1
	#407, #433	42	16	26	58	
10	Ibidem (n=1)					4
	no specimen number	42	16	26	58	
11	Ibidem (n=4)					1
	#566	44	18	26	62	
	#415	42	16	26	58	
	#504	42	15	27	57	
	#221	42	14	28	56	
	4 hybrids (#433 x #566: 2n=42 x 2n=44)	43	17	26	60	

Table (continued).

	Locality and specimen numbers (##)	2n	Chromosomes		NF	Reference
			Biarmed M+SM+ST	A		
Microtus maximowiczii ungurensis Kastschenko, 1912 Form B						
12	Ibidem (n=13)					our data
	#2350	41	16	25	57	
	#2375, #2335, #2336, #2349, #2353, #2373	42	15	27	57	
	#2373	43	17	26	60	
	#2374, #2333	43	16	27	59	
	#2334	43	13	30	56	
	#2345	40	13	27	53	
	#2352	44	18	26	62	
13	Buryatia, 50 km from Romanovka, Endodinka River (n=2)					1
	no specimen number	42	14	28	56	
	no specimen number	43	15	28	58	
14	Transbaikalia, Ivan Lake (n=2)					2
	no specimen number	43	17	26	60	
	no specimen number	44	16	28	60	
15	Transbaikalia, Ivan Lake (n=3)					3
	no specimen number	42	14	28	56	
	no specimen number	44	16	28	60	
16	Transbaikalia, Malyi Undugun Lake (n=17)					our data*
	#1754	41	15	26	56	
	#1737, #1744 , #1745, #1750, #1752, #1739	42	15	27	57	
	#1749, #1759	42	13	29	55	
	#1738, #1740, #1741, #1743, #1747, #1751	43	15	28	58	
	#1742	43	16	27	59	
	#1748	43	14	29	57	
17	Transbaikalia, Beklemishevo (n=5)					5
	no specimen numbers	42–43	□	□	55–59	
18	Transbaikalia, near Chita city (n=3)					2
	no specimen numbers	41–42	15–16	26	52–56	
19	Transbaikalia, Makkaveevo (n=21)					5
	no specimen numbers	42–43	□	□	56–59	
	one animal from this sample without number	42	16	26	58	
20	Transbaikalia, Akshinsk District, Ureisk (n=15)					
	no specimen numbers	39–42	13–18	21–29	54–58	
	one animal from this sample without number	42	17	24	58	
	Total	39–44	13–18	21–30	53–60	
Form V						
21	Transbaikalia, Sokhondinskii Reserve, Bukukun River, N 49027' 40", E 111007' 11.36" (n=7)*					our data*
	#1668, #1678, #1682	38	16	22	54	
	#1667, #1688, #1694	39	16	23	55	
	#1669	40	16	24	56	
22	Ibidem, Enda River, N 49027' 36.74" E 1110021' 22.10" (n=12)					
	#1671	36	16	20	52	
	#1672	37	15	22	52	
	#1665, #1666, #1685,	38	16	22	54	
	#1662, #1676, #1697, #1692	39	17	22	56	
	#1663, #1664	40	16	24	56	
	#1696	40	□	□	□	
23	Mongolia, Hentei Aimak, Bat-Shiret, Onon River (n=13)					5
	no specimen numbers	36–38	14–18	18–24	52–55	
24	Mongolia, Central Aimak Dzynbyrx and Borxon rivers (n=21)					
	no specimen numbers	36–37	15–18	18–22	52–54	
	one animal from this sample without number	36	17	19	53	
	Total	36–40	15–18	18–24	52–56	

Table (continued).

Locality and specimen numbers (##)		2n	Chromosomes		NF	Reference
			Biarmed M+SM+ST	A		
Microtus maximowiczii ungurensis Kastschenko, 1912 Form D						
25	Mongolia, East Aimak, Halhin-Gol River (n=3)					5
	no specimen numbers	42–43	14–15	28	56, 58	
26	Transbaikalia, Krasnokamenskii Reserve, near Krasnokamensk (n=2)					our data
	#2120	40	14	26	54	
	#2121	43	15	28	58	
27	Transbaikalia, Kovyli (n=1)					
	#2343	42	14	28	56	
28	Transbaikalia, Baleiskii reserve, Podoinicino, N 51040' 26.77", E 116047' 28.86" (n=8)					
	#2326-2328, #2330-2332, #2347-2348	42	14	28	56	
	Total	40–43	14–15	26–28	54–58	
Microtus maximowiczii maximowiczii (Schrenck, 1859) Form C						
29	Amur Region, Klimoutci (n=1)					6
	no specimen number	42	20	22	62	
30	Amur Region, near Blagoveshchensk, N 50015' 22.13", E 127034' 41.58V (n=1)					our data
	# 1659	42	16	26	58	
31	Amur Region, Norskii reserve (2001), N 52029.019', E130000.948' (n=18)					our data**
	#1518–1522, #1526, #1528, #1531–1533, #1537, #1539	40	18	22	58	
	#1527, #1529–1530, #1536, #1538	41	19	22	60	
32	Ibidem (2006), N 52029.019', E 130000.948' (n=8)					
	#2060, #2064–2066	40	18	22	58	
	#2059, #2061–2063	41	19	22	60	
33	Amur Region, Arhara, N 49006' 11.46", E 130006' 11.41" (n=1)					our data
	#2058	41	19	22	60	
34	Evreiskii Avtonomnyi Region, near Birabidjan (n=1)					7
	no specimen number	40	18	22	58	
35	Evreiskii AR, Aur (n=1)					
	no specimen number	41	18	22	58	
36	Evreiskii AR, Leninskoe (n=4)					
	no specimen numbers	40	18–19	22	58–60	
	no specimen number	41	19	22	60	
37	Khabarovsk Territory, Utinaya station (n=5)					2
	no specimen numbers	39–41	16–18	22–23	55–59	
	one animal from this sample without number	41	18	23	59	
	Total	39–42	16–19	22–63	55–60	
Microtus maximowiczii gromovi Vorontsov, Boeskorov, Lyapunova & Revin, 1988 Form I						
38	Yakutia, Bolshoe Toko Lake (n=1)					8
	# S-140238	44	16	28	60	
39	Khabarovsk Territory, Ayano-Maisk District, Ayan (2002; n=3)					9
	##1453–1455	44	16	28	60	
40	Ibidem (2008; n= 1)					our data
	#2383	44	16	28	60	
	Total	44	16	28	60	

* Published previously in Korobitsyna *et al*., 2005.

** Published previously in Kartavtseva *et al*., 2007.

References: 1) Kovalskaya, 1977; 2) Meyer *et al*., 1996; 3) Golenishchev & Radjabli, 1981; 4) Meyer, 1978; 5) Kovalskaya *et al*., 1980; 6) Meyer *et al*., 1967; 7) Frisman *et al*., in press; 8) Vorontsov *et al*., 1988; 9) Sheremetyeva *et al*., 2008.

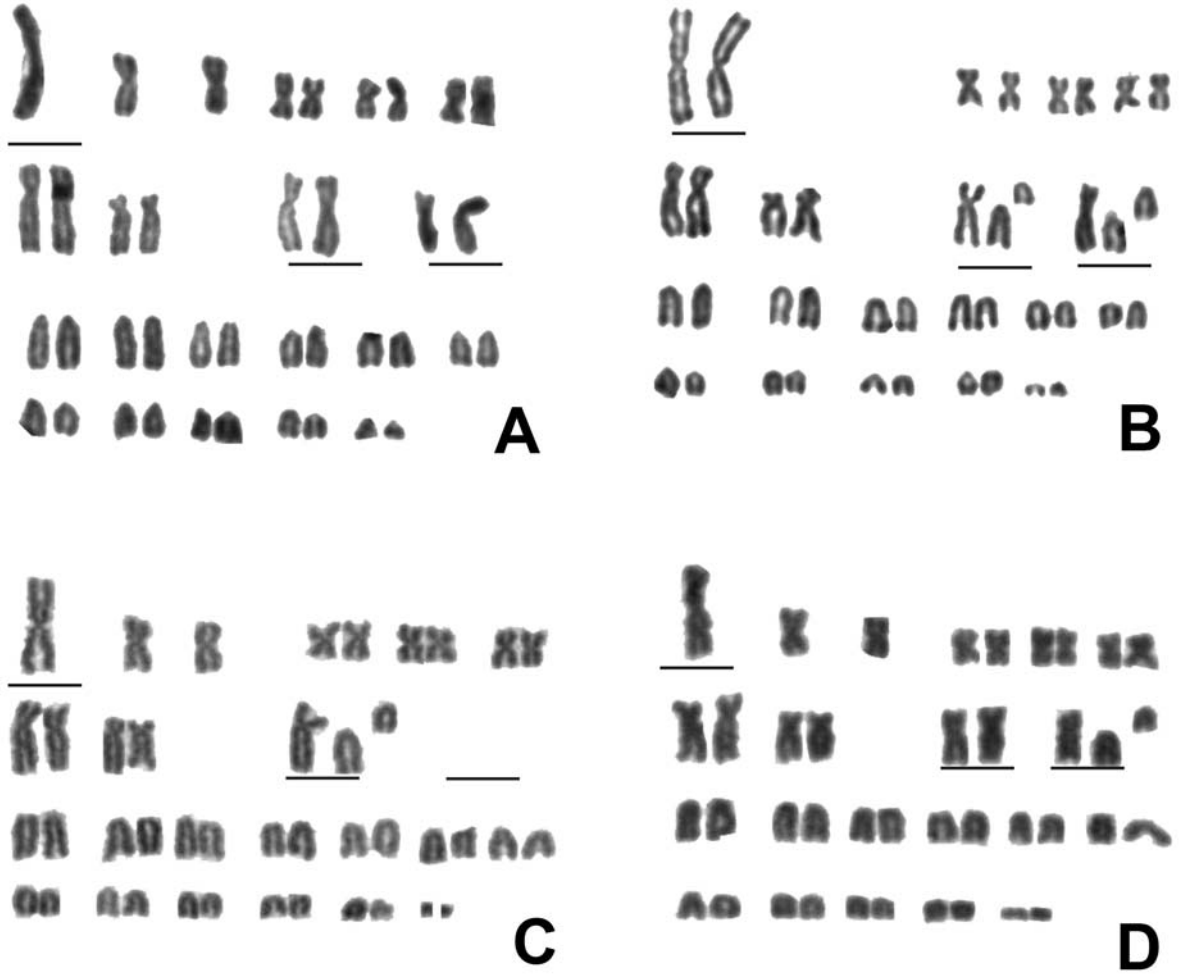

Figure 2. Karyotypes of *Microtus maximowiczii ungurensis* Kastschenko, 1912 from Istomino village, Buryatia. The united chromosomes are underlined.
A — #2016, 2n=39; B — #2017, 2n=42; C — #2024, 2n=42; D — #2031, 2n=40.

Results and discussion

New chromosomal data (2n and NF) of 94 voles from 12 localities and literature data of three *M. maximowiczii* subspecies investigated from Transbaikalia and the Russian Far East is presented in the Tab. 1 and Fig. 1.

Microtus maximowiczii ungurensis Kastschenko, 1912

Form B. Buryatia, Baikal Lake shore, near delta of Selenga River (localities 1–5). For animals from Istomino village (locality 5) we can see polymorphism connected with three variants of chromosomal rearrangement of the sixth pair of chromosomes: two metacentrics and four acrocentrics with two Robertsonian reorganizations and one fusion of metacentrics with formation of large metacentrics (Fig. 2).

Six animals from this sample have 2n=38–40, 42 chromosomes with NF=54–56–57: two animals (#2017 and #2030) have the same chromosomal data (2n=40, NF=54) (Fig. 2A) and four — different: #2016 — 2n=39, NF=56 (Fig. B); #2024 — 2n=42, NF=56 (Fig. 2C); #2031 — 2n=40, NF=56 (Fig. 2D) and #2023 — 2n=38, NF=57.

Karyotypes of eight animals from this sample have been described before (Tab. 1, locality 4) with 2n=39–41 and NF=55–57, but with different variants of the sets with three Robertsonian rearrangement and one pericentric inversion (Kovalskaya, 1977). We have confirmed the chromosome fusion with formation of three pairs of metacentric. But Kovalskaya (1977) presumed formation of all metacentrics by acrocentric chromosomes. The same chromosomal variant with 2n=40, NF=54–56 has been found in Buryatia: Kabansk District, Baikalo-Kudara village, locality 1 and Bauntovsk District, Bagdarino village, locality 6 (2n=40, NF=54–59; Tab. 1). In other samples (localities 2 and 3) from delta of Selenga River we presume the same variants of rearrangements and stabilization of karyotype in re-

spect of two large metacentrics in Kolesovskogo Island (locality 3) (Kovalskaya, 1977).

Form A. Central Transbaikalia, Buryatia, near Romanovka village (locality 12). We investigated chromosomal sets of 13 animals with 2n=40–44 and NF=52–62. From this locality, eight animals have been described before (7–11) with 2n=42–44 and NF=57–62 (Kovalskaya, 1977; Meyer, 1978; Golenishchev & Radjabli, 1981; Meyer *et al.*, 1996). The karyotype of *M. maximowiczii* with 2n=44 was described twice: from this place and from sample 14. We have found out one more animal with 2n=44 in the sampling 12. The form A has 2n=42–44 according to Kovalskaya (1977), but we have trapped animal also with 2n=40.

Transbaikalia, Malyi Undugun Lake (locality 16). Chromosomal sets of 18 animals were: 2n=41–43, NF=57–59 from which five animals have 2n=42, NF=57 and six animals 2n=43, NF=58. Preliminary chromosomal data with 2n=41–43, NF=55–59 from this locality has been published earlier (Korobitsyna *et al.*, 2005). We assume a similarity of chromosomal characteristics of animals from two samples from Ivan Lake (localities 14–15) (Golenishchev & Radjabli, 1981; Meyer *et al.*, 1996) which is located close to the Malyi Undugun Lake.

The published data about chromosomal sets of animals from the localities in Chita Region (localities 17–20) allow presuming the same variants of chromosomal rearrangements like in sample 16. Our specimens can be referred to Form A.

Form V. Transbaikalia, Sokhondinskii Reserve (localities 21–22). Two local populations isolated from each other by a large forest and mountain range have been investigated near southern border of the Sokhondinskii Reserve. The first population is localized in the lower reach of the Bukukun River (locality 21). From seven individuals studied, three had 2n=38, NF=54, three 2n=39, NF= 55, and one 2n=40, NF=56. The total chromosomal data for this population is 2n=38–40, NF=54–56.

The second population is localized in the lower reach of the Enda River (locality 22). The total chromosomal data in a small population, with the greater spectrum of chromosomal variability was found (2n=36–40, NF=52, 54, 56). Low value of chromosomal number (2n=36) in this population is similar to those found in the three localities from Mongolia (2n=36–37, NF=52–54, locality 23 and 2n=36–37, NF=52–54, locality 24; Tab. 1). Our data coincides with chromosomal characters of the form V.

Form D. South-East Transbaikalia (localities 26–28). From this region we investigated chromosomal sets of grey voles from three local populations:

1) Chromosomal sets of two animals from locality near Krasnokamensk city (locality 26) were 2n=40, NF=54 and 2n=43, NF=58.

2) One animal, trapped near Kovyli village (locality 27), had 2n=42, NF=56.

3) Near Podoinicino village (locality 28) all animals (not only from one family) had 2n=42, NF=56: 6 SM-ST, 2 M (M1/M1), 6 middle M and 26 A, X — middle A, Y — small A (Fig. 3A). This kind of karyotype was typical for many animals from Chita Region (localities 14–19) and Halhin-Gol in Mongolia (locality 25).

Hitherto it has not been described any population of *M. maximowiczii* with stable karyotype. This means that in the given population (locality 28) it occurred a stabilization of the karyotype described above. Such variant of karyotype is noted also in the population from South-East Mongolia, East Aimak, Halhin-Gol River (locality 25). We do not exclude an opportunities of karyotype stabilization in populations in future. Just this chromosomal variant in the Chita Region populations confirms that the similar chromosome pairs participate in the reconstruction in these regions. This set of the chromosomal rearrangements has not been found in the voles of form A (our data), therefore we propose for this the new Form D.

Microtus maximowiczii maximowiczii (Schrenck, 1859)

Form C. Amur area (localities 30–33). In the Amur area we investigated chromosomal sets of 28 animals from three local populations:

1) One animal of *M. maximowiczii* with 2n=42, NF=58 has been trapped inside the *M. fortis* colony near Blagoveshchensk city (locality 30).

2) Twenty six animals from the Norskii Reserve, the left coast of the Nora River, had 2n=40–41, NF=58–60. We trapped animals during two years, 2001 (locality 31) and 2006 (locality 32). This population is characterized by polymorphism: only by one rearrangement: fusion of two middle sized pairs of metacentrics up to formation large metacentric.

3) One animal from Arhara urban village (locality 33) had 2n=41, NF=60. This variant of karyotype is similar with karyotypes of voles from the Norskii Reserve. The G-banding of chromosomes was applied for voles from the Norskii Reserve and Arhara urban village that permitted to determine the pairs involved in the rearrangements mentioned above (Fig. 3B). In this geographical region we deal with chromosomal polymorphism resulted from three reconstruction: fusion of the metacentric chromosomes with formation of large metacentric chromosome, fusion of the acrocentric chromosomes from two pairs with formation a submetacentric chromosome of the middle size, and pericentric inversion of the small acrocentric with formation of small subtelocentrics. Fixation of the chromosomal rearrangements in the vole populations of this region should be studied additionally. This combination of the chromosomal rearrangements has not been found in the voles of form A (our data), so they are attributed to the new Form C.

Microtus maximowiczii gromovi Vorontsov, Boeskorov, Lyapunova & Revin, 1988

Form I. Khabarovsk Territory, Ayano-Maisk District (localities 39 and 40). The karyotype of *M. m. gromovi* has no any big metacentrics and differs from

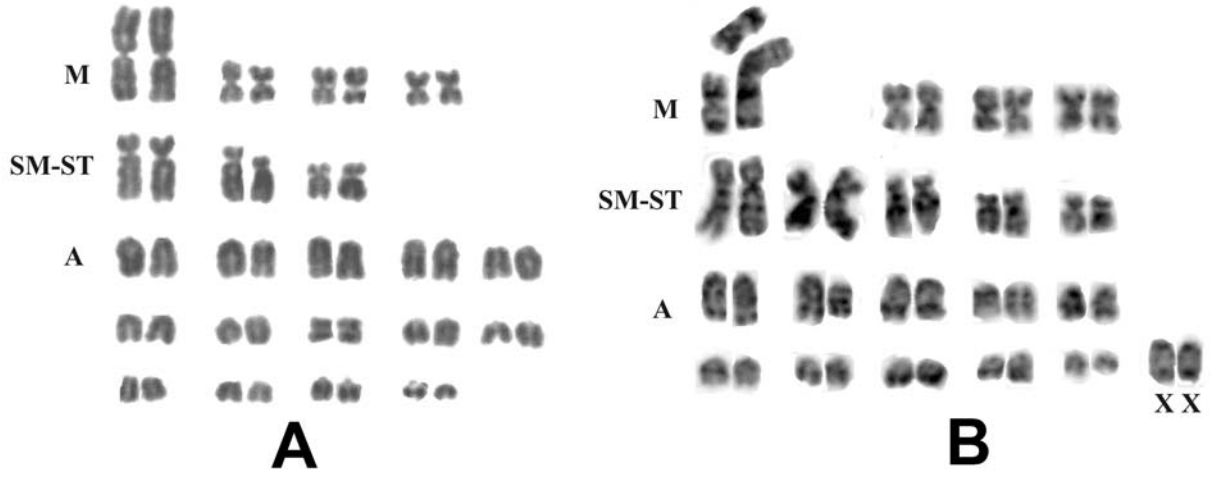

Figure 3. A — Chromosomal set of *M. maximowiczii ungurensis* Kastschenko, 1912 (male #2327) from Podoinicino village, 2n=42. B — G-banding of chromosomes of *M. maximowiczii maximowiczii* (Schrenck, 1859) (female #2058) from Arhara village.

the simple chromosomal variant (2n=44, NF=60) by pericentric inversions on three St pairs of chromosomes including one large St pair (G banding was applied), which in *M. m. maximowiczii* and *M. m. ungurensis* we always meet in constant size and morphology, while in *M. m. gromovi* this pair is presented by a large acrocentric. The data on the morphological and chromosomal analyses enables to consider *M. m. gromovi* (form I) as an independent species (Kartavtseva *et al.*, 2008; Sheremetyeva *et al.*, 2008).

Conclusion

The published and presented here data confirms a high level of chromosomal polymorphism in *M. maximowiczii* (2n=36–44; NF=52–62). There are no less than five chromosomal forms but not three as it has been supposed before. These forms are characterized by various combinations of chromosomal rearrangements with different number of biarmed and acrocentric chromosomes. Characteristics for chromosomal forms are: Form A: 2n=39–44, NF=53–60 (D=13–18, A=21–30); Form B: 2n=38–42, NF=54–57 (D=14–19, A=19–28); Form C: 2n=39–42, NF=55–60 (D=16–19, A=22–26); Form V: 2n=36–40, NF=52–56 (D=15–18, A=18–24); Form D: 2n=40–43, NF=54–58 (D=14–15, A=26–28); Form I: 2n=44, NF=60 (D=16, A=28). It seems, in each geographic region peculiar chromosomal rearrangements and their combinations tend to be fixed. The using of methods of differential staining and ZooFISH of chromosomes will allow defining distinctly the characters of these rearrangements in the chromosomal forms. Clear chromosomal differentiation of *M. m. maximowiczii* from *M. m. ungurensis* is shown. We also confirm species status of subspecies *M. m. gromovi.*

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