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Invasion of *Microtus rossiaemeridionalis* into the Territory of the Russian Far East

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Abstract—In this study, we report the first case of observation of the East European vole *Microtus rossiaemeridionalis* Ognev, 1924 in the south of the Russian Far East, Khabarovsk krai, in urbanized biotopes in the vicinity of the city of Sovetskaya Gavan and two adjacent rural settlements. The species was identified by karyological characteristics—$2n = 54$, NF = 56, C-banding—and morphological characteristics—body, cranium, baculum, and spermatozoa.

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INTRODUCTION

In 2009 and 2010 in the city of Sovetskaya Gavan and its vicinity, small gray voles belonging to the genus *Microtus* Shrank, 1798 were trapped. This genus was previously considered to be represented by only an isolated population of *M. maximowiczii* voles in that habitat (Kostenko, 2000). However, the captured voles differed in physical appearance from the vole species that occur in the south of the Russian Far East and Northeast China. They were not identified as a species whose range was relatively close to Sovetskaya Gavan, which is situated on the coast of the Tatar Channel. The voles were neither the Maximovicz vole nor the Far Eastern vole *M. fortis*, whose nearest habitat is the Amur River valley, nor the Sakhalin vole *M. sachalinensis*, which inhabits Sakhalin Island. Judging by preliminary analysis of the physical appearance and odontologic properties, these voles are the most closely related to the *Microtus arvalis* group, whose primary range is restricted to the European part of the continent. With allowance for this fact as well as the difficulty in identifying the species of the captured voles from the genus *Microtus* owing to poor morphological differentiation, it was necessary to conduct a complex taxonomic study using morphological description and karyotyping that are reliably used to identify species of this genus.

MATERIALS

In 2009 and 2010, a total of 38 voles were captured in the vicinity of the city of Sovetskaya Gavan and in the vicinity of two adjacent rural settlements in the south of Khabarovsk krai in the Russian Far East (Fig. 1). The capture sites were located close to residential buildings. The capture sites, biotope descriptions, and the number of captured voles are given below.

1. Lososin rural settlement, coast of Bukhta Lososin (49°0′24.30″N, 140°19′3.00″E), forb meadow at the bank of the Lososin Creek that was previously cleared away for garage construction. About 50 m from it on one bank, there is a mooring; on the other bank, there are garages and storage structures. The meadow is intersected by a path. The vegetation is dominated by gramineous plants, nosebleed, wormwood, and clover, $n = 7$.

2. Outskirts of Lososin rural settlement (48°59′22.50″N, 140°17′49.50″E), a grass-shrub fallow land around abandoned sanitary warehouses is 10 m away from the highway that leads to the Lososin rural settlement. There are residential buildings and summer cottages intermingled with forest stands of birches, aspens, and larches. This biotope occurred in relation to construction of warehouses and the highway in the past. After the warehouses were dismantled, this area was occasionally used as a dumping site. The vegetation is dominated by gramineous plants, wormwood, thistle, nosebleed, and clover. The prevailing shrub is dogrose, $n = 11$.

3. Sovetskaya Gavan city, a wild grass land within Bunker port (48°58′37.94″N, 140°13′15.90″E); among all biotope studies, this one was the most urbanized and closest to the city (it is 0.8 km from the city in a straight line). Ten meters away is a gatehouse;

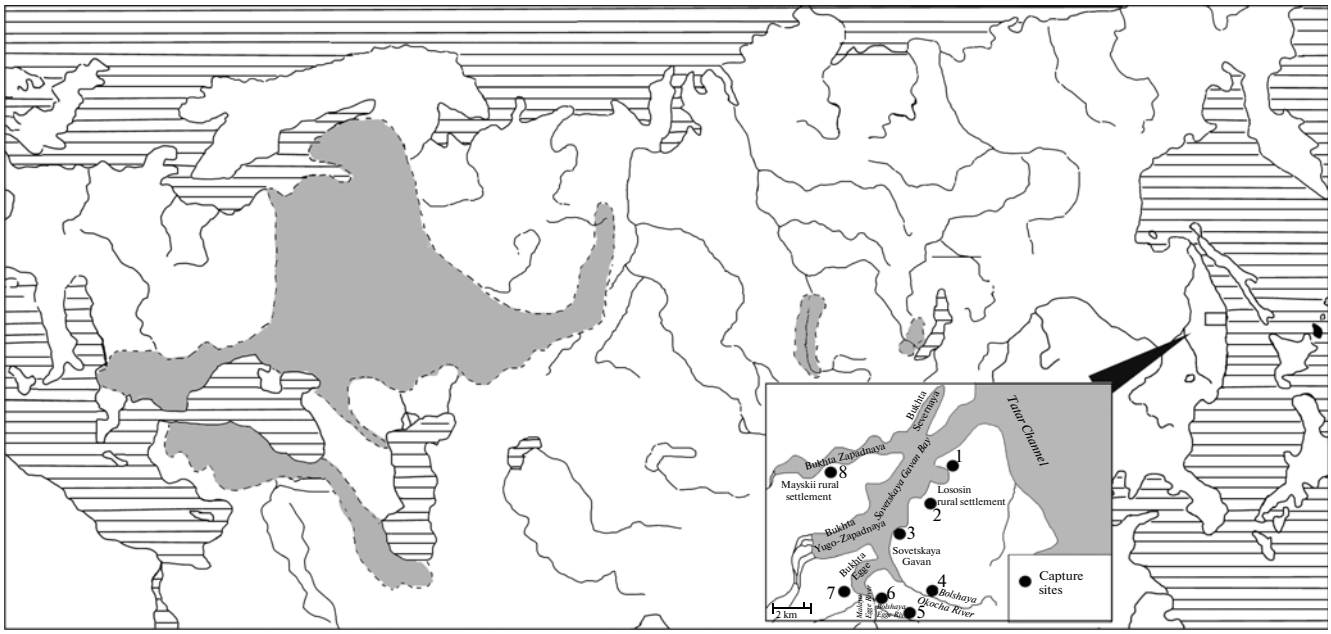

Fig. 1. The range of *Microtus rossiaemeridionalis* Ognev, 1924 (according to Shenbrot and Krasnov, 2005) and capture sites in the Russian Far East: (1) Lososin rural settlement; (2) outskirts of Lososin rural settlement; (3) Sovetskaya Gavan city, Bunker port territory; (4) Bolshaya Okocha River; (5) Malaya Egge River; (6) left bank of lower reaches of the Bolshaya Egge River; (7) bank of Bukhta Egge; (8) northeast outskirts of Mayskii rural settlement.

20 m away and farther, there are other frequently visited buildings. Edificators are sedge grasses, wormwood, and reeds, $n = 3$.

4. A forb meadow close to the Bolshaya Okocha River (48°57′15.40″N, 140°18′9.50″E) is located opposite to abandoned summer cottages and garages and close to wood residential buildings of the Okocha rural settlement. The biotope replaced a forest logged for construction. This forest is currently borders on the biotope. The meadow and adjacent areas are grazed. The vegetation is dominated by sedge grasses, gramineous plants, and clover, $n = 3$.

5. 48°56′36.20″N, 140°17′5.20″E. A fallow land near the Malaya Egge River, which used to be a garden and is close to the railroad tracks and a forest road. It is surrounded by channels, willow stands, and mixed forest. The vegetation is represented by sedge grasses, reed, clover, nosebleed, and wormwood, $n = 9$.

6. A forb meadow in the lower reach of the left bank of the Bolshaya Egge River (48°56′50.60″N, 140°15′31.50″E) formed as a consequence of incomplete construction. On one side opposite to it, there are half-constructed garages, a wood residential building, and forest; on the other side, there are railroad and road bridges and fishing and recreation sites. The meadow is crossed by footpaths and overgrown unpaved back roads. The edificators are gramineous plants, sedge grasses, nosebleed, and clover. Some places have dog rose, $n = 1$.

7. A fallow land within the littoral area of the Bukhta Egge (48°57′5.50″N, 140°14′34.40″E) used to be a garden. There are torn-down military barracks close by, which currently serve as dumping sites. About 200–300 m away from the biotope, there are wood residential buildings and grazing areas. The vegetation is dominated by gramineous plants, nosebleed, clover, wormwood, and thistle, $n = 3$.

8. A forb meadow in the vicinity of the Mayskii rural settlement is a hay field (49°0′6.90″N, 140°13′41.20″E). Opposite to it, there is a fallow land and abandoned gardens across the road. The biotope is surrounded by a coniferous small-leaved forest and dissected by three large furrows. Residential buildings are located 100–200 away from this site. The vegetation is dominated by gramineous plants, thistle, clover, and sedge grasses, $n = 1$.

METHODS

Standard morphological characteristics were studied in 25 adult and immature individuals (12♀♀, 13♂♂): L = body length, C = tail length, Au = ear length, Pl = foot length. The morphology of the first (M_1) lower and third upper (M^3) cheek teeth were studied in 22 individuals.

Five voles (3♀♀ and 2♂♂) were karyotyped by light microscopy using bone marrow cells of the astragalus as described in Ford and Hamerton (Ford and Hamerton, 1956). Subcutaneous administration of baker's yeast solution was employed to stimulate mitotic division 24 h before sacrifice (0.5 mL per 25 g

of animal weight) (Lee and Elder, 1980). Thirty minutes before sacrifice, the animals received a colchicine solution intraperitoneally (Merck, 1 mL of 0.04% solution per 100 g of animal weight). The bone marrow from the astragalus was washed into a centrifuge tube using a syringe with a hypotonic solution (0.56% potassium chloride) and incubated at room temperature for 20–25 min. After hypotony, the cells were fixed by a solution of 96% ethanol and glacial acetic acid (3 : 1). Preparations were pipetted onto cooled premoisterized slides. Dried preparations were stained with acetoorseine or 2% azure eosin (Giemsa stain, Merck, Germany). C-band staining of chromosome preparations was done as described in Sumner (Sumner, 1972). For two individuals, chromosome suspensions were prepared using a short-term culture of bone marrow cells (Grafodatskii and Radzhabili, 1988).

Stained chromosome preparations were examined using an Axioplan-2 imaging microscope (Zeiss, Germany). Micrographs were captured and processed by a Metasystems digital camera and software (Carl Zeiss MicroImaging GmbH, Germany).

RESULTS

During zoological-epidemiological field studies by the Khabarovsk plague control station in the vicinity of Sovetskaya Gavan and two adjacent rural settlements in September–October 2009 and 2010, a total of 38 *Microtus* gray voles were trapped. Together with the gray voles, the habitat was also shared by two forest rodent species typical of this site, the Korean field mouse *Apodemus peninsulae* Thomas 1906 and the gray red-backed vole *Myodes* (=*Clethrionomys*) *rufocanus* Sundervall, 1846.

The color of the back of trapped adult and immature gray voles was gray with a light chestnut coloring (mottled). The venter was light gray with a silvery coloring. The borderline between the back and venter is light-chestnut. The tail was two-colored: dark gray on top with a darkish coloring; gray on bottom with light chestnut coloring. The foot had 6 plantar protuberances. The body was 81–114 (91.5) mm long, the tail was 26–43 (32.6) mm long, the foot was 13–18 (14.8) mm long, and the ear was 9–13 (10.6) mm long.

Among the M^3 morphotypes, the dominating ones are "simplex" and "typical." M_1 has five inner and four outer protruding angles; the grinding surface has six or seven closed dentinal-enamel spaces.

The sperm head length and head width were 7.2 and 4.2 μm, respectively. There was a pronounced dark spot at the base of the sperm head, separated by a bright line from the other part of the sperm's nucleus (Fig. 2).

The os penis length (rodent baculum) was 3.0 mm. The base was spade-shaped with the highest width at the anterior end (Fig. 3).

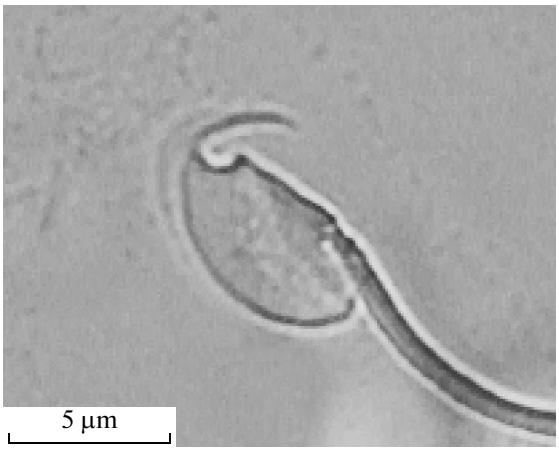

Fig. 2. Micrograph of a sperm of the East Asian vole in the vicinity of Sovetskaya Gavan.

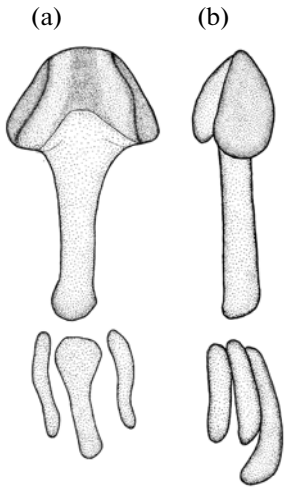

Fig. 3. The structure of the os penis (baculum) of the East Asian vole in the vicinity of Sovietskaya Gavan city. Side view: (a) ventral side, (b) lateral side.

The number of diploid chromosomes is 54. The number of arms is 56 (Fig. 4a). Autosomes are nearly completely acrocentric, eventually decreasing in size. The last pair of chromosomes is metacentric. The X chromosome is the largest acrocentric chromosome in the karyotype. The Y chromosome is acrocentric, the same as the first autosome pair. Staining of structural heterochromatin detected C-bands in centromere regions of all chromosomes. The X chromosome has bright staining from the middle of the arm to the telomere; the Y chromosome is entirely heterochromatic (Fig. 4b).

DISCUSSION

All morphological and chromosome characteristics of gray voles studied, captured in the south of Khabarovsk krai, completely fit those of the Eastern European vole *M. rossiaemeridionalis* Ognev, 1924 (Meyer et al., 1996). The Eastern European vole *M. rossiaeme-*

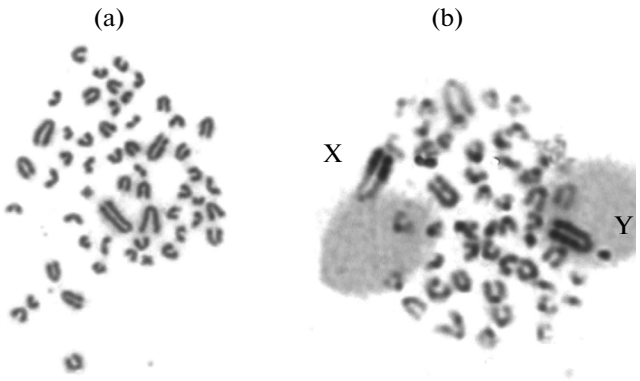

Fig. 4. Male chromosome metaphase plates of a male *Microtus rossiaemeridionalis* Ognev, 1924 (no. 2850) trapped in the vicinity of Sovetskaya Gavan. (a) Azure-eosin staining by Romanovskii, (b) C-staining.

ridionalis was first described as a look-alike species of the common vole *Microtus arvalis* Ondrias, 1966 (synonyms: *Microtus subarvalis*, *M. epirroticus*). These species truly differ only in the number and morphological characteristics of chromosomes. *M. rossiaemeridionalis* has a karyotype of $2n = 54$, NF = 56 (Meyer et al., 1972; 1996), and *M. arvalis* has a karyotype of $2n = 46$ (Malygin, 1983).

Most of the range of the Eastern European vole lies between 30° and 60°E and 60° and 40°N (Fig. 1) and overlaps with the central part of the range of the look-alike species (*Obyknovennaya polevka...*, 1994). Isolated populations have been documented in the south of Krasnoyarsk krai, Khakassia, and Irkutsk oblast. They may have been imported there by man (Kovalskaya and Malygin, 1985). Toward the east from Lake Baikal to the shores of the Pacific Ocean, this species has never been recorded, and it was even believed that further range expansion would hardly be likely (Bobrov et al., 2008). From Irkutsk to Sovetskaya Gavan, which is the terminal of the Baikal-Amur Mainline, is 4200 km by rail.

Overall, it was discovered that to date the Eastern European vole has established a limited and isolated range in the Russian Far East. It inhabits forb meadows, fallow lands, and urbanized areas of the coast of Sovetskaya Gavan Bay of the Tatar Channel of the Sea of Okhotsk. Interestingly, one of the samplings was obtained close to the inshore of the port in the Lososin rural settlement. In the European part of its range, the Eastern European vole carries pathogens affecting humans and therefore merits attention since across the European part of the range it readily invades man-made structures. In rural settlements and undeveloped lands in small and large cities, the species could be high in numbers (Tikhonov et al., 1992, 1998; Karaseva et al., 1994; Tikhonov and Tikhonova, 1997; Tikhonova et al., 1997, 2001). In connection with this, there arises a need to strictly control the population of the vole in rural settlements and cities.

A young pair that mated in November 2010 produced offspring of two individuals (male and female) in late December 2010; in February, it gave birth to seven individuals. Rapid reproduction of voles indicates the potential for year-round reproduction once settled in man-made structures. Such a pattern of year-round reproduction has been shown for the Eastern European vole in the European part of its range (Tikhonov and Tikhonova, 1994). Under laboratory reproduction, the animals preferred carrots in contrast to cereals and legumes. They are not demanding on water resources. When moist food was provided, the drinking troughs were not visited.

In May 2011 in the vicinity of Sovietskaya Gavan, 500 Gero traps were mounted at the capture sites. Unfortunately, the population of all rodent species proved to be zero. Possibly, because of a sharp decline in the ambient temperature on capture days, the rodents were not active.

The routes by which the vole penetrated into the territory of the southern part of Khabarovsk krai, adjacent territory, and Sovetskaya Gavan itself still remain unknown. The species may have been imported together with cereals by rail when the Baikal-Amur Mainline was under construction in the 1950s. The time of introduction is difficult to determine, but regular train service between the Vanino station close to Sovietskaya Gavan and Komsomolsk-on-Amur began July 20, 1945. Judging by the occurrence of the smallest-sized species in Yekaterinburg oblast (Panteleev et al., 1990), which resembles the voles we identified (body length from 90 to 115 mm), we suggest that it is an invader from the Southern Urals.

In summary, the introduction of the Eastern European vole into the Russian Far East is reported for the first time.

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